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EVOLVE AND RESEQUENCING (E & R) OF *TOXOPLASMA GONDII* DURING LAB-ADAPTATION TO IDENTIFY VIRULENCE FACTORS

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Abstract

Evolve and Resequencing (E&R) of Toxoplasma gondii during lab-adaptation to identify virulence factors

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The two type I genotype *T. gondii* strains, RH, a lab-adapted strain, and GT1, a non-lab-adapted strain, have a genetic difference of only 0.002%, but show remarkable phenotypic differences *in vitro.* For example, it has long been known that RH's *in vitro* virulence (i.e. plaquing capacity) and extracellular survival is far superior to that of GT1, likely due to several decades of adaptation to the *in vitro* environment (i.e. lab-adaptation). The genetic basis of these phenotypes, however, remains largely unknown despite previous allele-swapping experiments, thus inspiring two hypotheses: 1) epistatic interactions between two or more alleles and/or 2) gene regulatory mechanisms are responsible for lab-adaptive phenotypes. Uncovering the molecular basis underlying lab-adaptive phenotypes will support our growing understanding of *T. gondii* virulence and suggest therapeutic targets that affect the parasites lytic cycle in a host-independent manner.

To answer this question, we applied Evolve and Resequencing (E&R) of GT1 during the first 1500 generations of its lab-adaptation in order to chronologically identify emerging genotype-phenotype correlations. Indeed, lab-adaptation augmented GT1's *in vitro* virulence by improving its extracellular survival and reinvasion capabilities- both extracellular phenotypes of the lytic cycle. DNA-sequencing of parallel GT1 populations

at multiple evolutionary timepoints (i.e. passages) identified a polymorphic phospholipid flippase gene whose gene expression is critical for *in vitro* virulence but, unfortunately, the evolved mutations could not be functionally characterized due to technical limitations. RNA-seq of both intracellular and extracellular parasites across several passages identified hundreds of “pro-tachyzoite” differentially expressed genes (DEGs), but only in extracellular parasites, paralleling our phenotypic observations. Interestingly, several upregulated DEGs are connected to fatty acid biosynthesis. Lastly, genetic KO of five seemingly non-related DEGs indicates that GT1’s lab-adaptive *in vitro* virulence is a complex and polygenic phenotype that is largely controlled by mechanisms independent of genomic mutations.

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List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
AMA1	Apical membrane antigen 1
AP2	Apetala 2 transcription factor
ApiAP2	Apicomplexa apetala 2
ATP	Adenosine triphosphate
BFD1	Bradyzoite-deficient factor 1
Ca^{2+}	Calcium
CD40	Cluster of differentiation 40
CDC	The Center for Disease Control
CDS	Coding sequence
DEG	Differentially expressed gene
DNA	Deoxyribonucleic acid
E&R	Evolve and Resequencing
eEF2	Eukaryotic elongation factor 2
eIF2α	Eukaryotic initiation factor-2 alpha
ER	Endoplasmic reticulum
FA	Fatty Acid
FASII	Type II fatty acid biosynthesis
G1	Gap1 phase
G2	Gap 2 phase
GAP	Glideosome associated protein
GPI	Glycosylphosphatidylinositol
GRA	Dense granule antigen
HAT	Histone acetyltransferases
HIV	Human immunodeficiency virus
HMT	Histone methyltransferase
IFN	Interferon
iKD	Inducible knockdown
IMC	Inner membrane complex
Indel	Insertion/deletion
IP	Immunoprecipitation
i.p.	Intraperitoneal
IRGs	Immunity Related GTPases
IST1	Inhibitor of STAT1 transcriptional activity
K^+	Potassium
KO	Knock out
LD_{50}	Lethal dosage to kill 50% of test subjects
LT_{50}	Lethal time to kill 50% of test subjects
LTEE	Long-Term Experimental Evolution
M	Mitosis
MAPK	Mitogen-activated protein kinase
MIC	Microneme
MJ	Moving junction
MLC	Myosin light chain

MyoA	Myosin A
ncRNA	Non-coding RNA
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGS	Next Generation Sequencing
PA	Phosphatidic acid
PHD	Prolyl 4-hydroxylase
PLP1	Perforin-like protein 1
PM	Plasma membrane
pO_2	Partial pressure of oxygen
PTM	Post-translational modification
PV	Parasitophorous vacuole
PVM	Parasitophorous vacuole membrane
RB	Residual body
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RON	Rhoptry neck
ROP	Rhoptry
S	DNA synthesis phase
SAG	Surface Antigen Glycoprotein
SCF	Skp/Cullin/F-Box complex
SKP1	S-phase kinase-associated protein 1
SNP	Single nucleotide polymorphism
SRS	SAG-Related Sequence
STAT	Signal transducer and activator of transcription
TNFα	Tumor necrosis factor alpha
WGS	Whole genome sequencing

CHAPTER 1: INTRODUCTION

1.1. Introduction

Toxoplasma gondii was co-discovered in 1908, being isolated from *Ctenodactylus gundi*, an African rodent, by Charles Nicolle and Louis Manceaux at the Pasteur Institute in Tunisia [1], and from the tissues of a rabbit by Alfonso Splendore in Brazil [2]. Throughout the rest of the early 20th century, several suspected human cases of toxoplasmosis were reported worldwide, with the first confirmed case being reported in 1939 by Wolf *et al* in New York, US [3]. Several reviews excellently summarize these historical cases [4, 5]. In 1970, taxonomy classified *T. gondii* as a member of the Apicomplexa, a phylum consisting of over 5,000 unicellular, spore-forming, obligate parasitic species [6, 7]. Seven Apicomplexa genera are responsible for human diseases, for which immunocompromised or prenatal humans are the most vulnerable: *Toxoplasma* (Toxoplasmosis), *Plasmodium* (malaria), *Cryptosporidium* (Cryptosporidiosis), *Cyclospora* (Cyclosporiasis), *Isospora* (Isosporiasis), *Sarcosystis* (Sarcocystosis), and, more rarely, *Babesia* (Babesiosis). Due to its ease of culture and genetic manipulation, *T. gondii* is regarded as a model system for studying Apicomplexan biology [8], which has advanced our ability to combat human and veterinary diseases, and not to mention the annual multi-billion-dollar economic burden on the US [9].

1.2. Toxoplasma gondii's life cycle

T. gondii has evolved to be one of the world's most ubiquitous parasites, capable of infecting any nucleated cell of any warm-blooded organism. Members of the Felidae family (i.e. the cat) are considered the definitive host, where sexual reproduction takes place, while all other animals are considered intermediate hosts permitting only asexual

reproduction [10]. For decades, many scientists have asked, “why the cat”? Well, the reason behind this was recently discovered to be the parasites ability to sense linoleic acid, which is particularly rich in the serum of cats [11]. As a result, *T. gondii* have developed a complex life cycle comprised of disparate reproductive and parasitic stages. Herein, we begin *T. gondii’s* typical life cycle starting with its definitive host, the cat.

Infection of the cat commonly begins via oral consumption of chronically infected prey, such as a mouse or bird, containing infectious tissue cysts (Figure 1.1); tissue cysts are a durable and immunologically-protective vacuolar structure containing up to 3,000 quasi-dormant parasites called bradyzoites [12, 13]. After their consumption, tissue cysts are broken down by digestive enzymes, thereby releasing bradyzoites which subsequently invade the enterocytes of the digestive tract [14, 15]. Upon invasion, bradyzoites convert into merozoites, a fast and replicative form of *T. gondii* within the cat that undergo limited rounds of asexual reproduction (i.e. endopolygeny) [16, 17]. After this brief parasite expansion, merozoites convert into macro- and micro-gametes (i.e. gametogony), marking the sexual cycle for *T. gondii* [16-18]. After gamete fusion and formation of the zygote (i.e. fertilization), the oocyst is formed, an environmentally-protective spore containing a rigid outer cyst wall. Millions of unsporulated oocysts are shed in the cat’s feces and, over a few days, develops into a sporulated oocyst (i.e. sporogony) containing eight sporozoites, a dormant form of *T. gondii* that resides inside the oocyst [17].

Shed oocysts, particularly on farm land, can contaminate grasslands as well as the surface of fruits and vegetables; therefore, infection of an intermediate host commonly occurs with the consumption of oocyst-contaminated grasslands or produce (Figure 1.1) [19]. Once ingested, sporulated oocysts are broken down by digestive enzymes, thereby

releasing the sporozoites [14, 15]. Subsequently, sporozoites invade enterocytes of the digestive tract and convert into tachyzoites, a fast and replicative form of *T. gondii* within intermediate hosts that undergo many rounds of asexual reproduction (i.e. endodyogeny) [16]. Tachyzoites quickly disseminate to all areas of the body and cause tissue damage via its lytic cycle (Figure 1.2a.; see Chapter 1.5) [20-22]. This period of acute infection lasts roughly a week, upon which the immune system starts suppressing the infection by forcing tachyzoites to convert back into bradyzoites, the quasi-dormant form of *T. gondii* that form tissue cysts, preferentially within muscle and neuronal tissue [13]. This period of chronic infection is largely believed to last for the lifetime of the host, albeit parasite clearance has been recently observed in mice 20 months post-infection, suggesting clearance is eventually possible [23].

Continued transmission can occur amongst carnivorous intermediates by the consumption of rare or undercooked meat containing bradyzoite tissue cysts (e.g. predator-prey interactions or steak tartare) (Figure 1.1) [24]. The consumption of tissue cysts will release bradyzoites, which subsequently invade the enterocytes of the digestive tract. Upon invasion, bradyzoites convert back into tachyzoites, rapidly replicate and disseminate throughout the host, and then convert back into the tissue cyst-forming bradyzoites. The life cycle of *T. gondii* comes full circle when tissue cysts from an intermediate host (e.g. mouse or bird) are consumed by a cat (Figure 1.1) [13].

1.3. Population structure and epidemiology

Since the early 1990's, >1000 *T. gondii* isolates from infected human and animals have been genotyped by various molecular phylogenetic techniques. Restriction

Fragment Length Polymorphism (RFLP) and, more recently, DNA-sequencing have identified four clonal ("typical") genotypes of *T. gondii* worldwide, termed type I, type II, type III, and, more recently, type 12 [25, 26]. Studies estimate the genomic sequence of Type I, II, and III to differ only 1-3% [27-29]. More recent studies find all four strain to have highly similar multi-locus genotypes, high levels of linkage disequilibrium, and infrequent recombination events [26]. Such worldwide clonality appears to be the result of a selective sweep that occurred around 10,000 years ago, driven by the emergence of a monomorphic chromosome (Chr1a) which enabled *T. gondii* to infect hosts via oral transmission [30, 31]. Consequently, *T. gondii* can now bypass sexual reproduction in the cat and undergo asexual transmission between a myriad of intermediate hosts. Furthermore, since the asexual stages are haploid, it is only when multiple genotypes infect a single cat that genetic recombination of alleles can actually occur [28]. Taken together, this added complexity in the parasites life cycle resulted in the global asexual expansion of *T. gondii* that we see today.

All four types appear in North America while South America contains many more divergent "atypical" strains, categorized into several haplogroups, which include type 12 [26, 32]. Type I, II, and III are found in Europe [25], whereas Asia appears to be dominated by haplogroup 13 [33, 34]. Africa [35], Australia [36, 37], and possibly Antarctica [38] also encompass a mixture of typical and atypical genotypes. Severity of infection and prevalence of *T. gondii* genotypes can vary drastically. Type II strains tend to dominate the majority of human cases [25, 39-43] while type I and atypical strains tend to be more severe pathologically (e.g. ocular toxoplasmosis) [44-46]. Globally, it is estimated that 30-50% of the human population is infected with *T. gondii,* but depending on geography,

cultural habits, food preparation, and hygiene, prevalence can vary [47, 48]; for example, studies report 95% seroprevalence in France [49], 22.5% in US [50], and only 6.7% in Korea [51]. Typically, human infection is acquired through the consumption of undercooked meat contaminated with tissue cysts or the consumption of unwashed fruits and vegetables contaminated with small traces of cat feces containing infectious oocysts (Figure 1.1) [52-56]; although rare, oocyst-contaminated water can also be a source of infection [57, 58]. Additionally, infection can be acquired congenitally from an immunologically naïve mother, or during a patient's blood transfusion [59] or organ transplant (Figure 1.1) [60].

1.4. Toxoplasmosis

Toxoplasmosis is the second leading cause of death from foodborne illness in the United States (US). The Center for Disease Control (CDC) has named toxoplasmosis as one of five neglected parasitic infections due to the number of people infected, severity of the disease, and limitations on treatment. It is estimated that 30-50% of the global human population is infected with *T. gondii* [47, 48]. While most individuals are asymptomatic, those with compromised immune systems, such as those with AIDS or those undergoing chemotherapy, latent or newly acquired toxoplasmosis can be deadly. For example, 25% of AIDS patients show cases of *Toxoplasma*-related encephalitis, with about 84% of cases leading to death [61]. *T. gondii* is capable of migrating through biological barriers, such as the blood brain barrier, where it can infect the brain and retina, leading to complications in vision (ocular toxoplasmosis) but not cognitive ability [62-65]. It is estimated that ~5,000 cases of ocular toxoplasmosis cases occur annually in the US

[66]. Furthermore, congenital transmission of the disease, via migration through the placental barrier, can cause perinatal mortality or severe birth defects such as blindness or mental disability [67]. In the US, an estimated 400-4,000 congenital cases occur every year [68]. Currently, there is no vaccine to combat infections; treatment is limited only to acute stages of the disease with the co-treatment of pyrimethamine and sulfadiazine, although resistance appears to be on the rise in South America [69]. Pathogenesis of the disease is the direct result of the tissue damage caused by the tachyzoite's unchecked lytic cycle (Figure 1.2a.), which is discussed in detail next.

1.5. The tachyzoite lytic cycle and its associated virulence traits

Acute toxoplasmosis is marked by repeated rounds of the parasite's lytic cycle which causes tissue damage to the host and, if left unchecked, can lead to severe medical complications and even death. The lytic cycle consists of several key steps: replication, egress, extracellular survival, migration, attachment, and invasion (Figure 1.2a.). An impediment or enhancement at any one of these steps can, respectively, stop or speed up the parasites lytic cycle; therefore, these steps are viewed as virulence traits (i.e. parasitic behaviors critical to pathogenesis). Moreover, throughout its lytic cycle, *T. gondii* encounters both intracellular and extracellular milieu's, thus these virulence traits can be sorted into two categories: extracellular virulence traits and intracellular virulence traits.

1.5.1. Extracellular virulence traits of the lytic cycle: Extracellular survival, motility, attachment, and invasion

Extracellular survival:

Extracellular survival is a virulence trait important for local and systemic dissemination within a host, although it is still debated whether extracellular parasites in the bloodstream can actually disseminate to the peripheral tissues [70-73]. Nevertheless, histology shows ~20% of tachyzoites in an infected tissue are extracellular, indicating that *T. gondii* must endure the stress of an extracellular environment during their lytic cycle [73]. Previous studies have shown that 4-hour extracellular tachyzoites respond to stress by reducing amino acid uptake and inhibiting eukaryotic initiation factor-2α (eIF2α) by phosphorylation [74]. Such reduced protein translation, especially by phosphorylation of eIF2α, is commonly observed in other parasites, including *Plasmodium* and Trypanosomes [75-78]. Extracellular triggers of stress are largely unknown, but oxygen tension appears to be the lead suspect. The partial pressure of oxygen (pO_2) can vary dramatically between intracellular and extracellular environments (~0.5-20%), as well as various tissues (~1-10%), and cell lines (~0.5-17%), and therefore it is considered a major cue for environmental sensing by protozoans [79, 80]. Prolyl 4-hydroxylases (PHDs) are crucial oxidoreductase enzymes used for O_2 sensing [81, 82], and two PHDs, PHYa and PHYb, have been described so far in *T. gondii*: TgPHYa is important for tachyzoite growth at low (0.5%) pO_2 [83], whereas TgPHYb is important at high (21%) pO_2 [84]. Tachyzoite growth supported by TgPHYa is also associated with the hydroxylation of S-phase kinase-associated protein 1 (SKP1) which leads to downstream glycosylation and activation of the Skp/Cullin, F-Box containing complex (SCF complex) [85], an E3 ubiquitin ligase that marks proteins for proteasomal degradation and regulates protist growth [83, 86, 87]. Tachyzoite growth supported by TgPHYb is associated with reduced protein translation via the phosphorylation and inhibition of eukaryotic elongation factor 2 (eEF2) [84]. Taken

together, these studies indicate oxygen tension as a trigger of extracellular stress response pathways that ultimately regulate the proteome of the tachyzoite, which is a general stress response mechanism common to eukaryotic cells [88, 89].

Motility:

During the extracellular state of the lytic cycle, tachyzoites are highly motile as they are in search for a host cell to invade. Motility, often described as gliding motility, is powered by the glideosome, an actomyosin motor complex tethered to the inner membrane complex (IMC) [90, 91]; the IMC is a membrane/cytoskeletal cortex made up of multiple flattened alveoli located around the entire periphery of the parasite, just underneath the plasma membrane (PM). The glideosome is assembled between the IMC and PM (Figure 1.2b.) and consists of a myosin A (MyoA) and myosin light chain (MLC) complex immobilized to a set of glideosome associated proteins (GAPs) that anchor MyoA/MLC to the IMC [92-94]. The head domain of MyoA binds to the F-actin of the cytoskeletal cortex, which has been immobilized to the PM via the glideosome-associated connector (GAC) protein [95]. Upon ATP hydrolysis, the IMC-immobilized MyoA motor begins to “walk” down the PM-immobilized F-actin, resulting in a “tread-milling” effect of the PM, driving the parasite toward the apical direction in a substrate-dependent manner. Continued motility is dependent on constitutively-secreted microneme (MIC) proteins that are apically-released onto the parasite’s exoplasmic surface and assure strong adherence to the substrate (e.g. receptors of a host cell). Notably, constitutive MIC secretion and glideosome motility is also crucial for the process of invasion and egress

and is dependent on the concentration of intracellular calcium (Ca^{2+}), as discussed below [91].

Attachment:

Essential to *T. gondii's* attachment of host cells are the GPI-anchored Surface Antigen Glycoproteins (SAGs), otherwise known as SAG-Related Sequence (SRS), which coat the surface of parasites and recognize sulfated proteoglycans on the surface of host cells [96-99]. There are 161 member of the SRS superfamily that range in sequence diversity and gene expression in a genotypically- and developmentally-specific fashion [100]. Upon SRS attachment to the host cell, *T. gondii* re-orients its apical end perpendicular to the surface of the host cell and apically-secretes MIC and rhoptry neck (RON) proteins through the conoid (Figure 1.2b.) in order to form the moving junction (MJ) [101-103]. The MJ is a site of tight parasite-host interaction mediated by the MIC protein apical membrane antigen 1 (AMA1) [102], which is anchored to the parasite, and the RON proteins RON2 [104], 4 [105], 4_{L1} [106], 5 [103], and 8 [107], which are anchored in complex to the host cell's PM and cortex [108-110]. Exactly how secreted RON proteins become so embedded into the host cell remains enigmatic. After formation of the MJ, *T. gondii* can initiate invasion (Figure 1.2a.).

Invasion:

Invasion of *T. gondii,* often described as active penetration, is guided by the MJ and powered by the glideosome [90, 91, 111]. During active penetration, which occurs in a matter of seconds, the parasite continuously pushes forward into the host cell causing

the PM to invaginate around the parasite until the host cells pinches off and forms a parasitophorous vacuole (PV) membrane (PVM) around the parasite [112]; at this point, the parasite resides inside a PV which too is inside the host cell (Figure 1.2a.) [113]. Inside the host cell, the PVM does not fuse with any of the host's membrane trafficking pathways or organelles, or back again with the PM from which it came, protecting the parasite from autophagy or exocytosis and promoting a milieu for intracellular replication [114, 115].

1.5.2. Intracellular virulence traits of the lytic cycle: Replication, host manipulation, bradyzoite differentiation, and egress

Replication:

Asexual replication inside the PV occurs via an internal budding mechanism termed endodyogeny–that is, two daughter cells grow inside of the mother cell [114]. Concluding cell division, the mother cell has been completely disassembled, with many of its proteins, lipids, and organelles being recycled into the two new daughter cells. Residuals of the mother cell, such as some mitochondria and acidocalcisomes, are usually found in the residual body (RB), a small basal compartment that connects the cytoplasm of emerged cells, allowing for recycling, cell-cell communication, and synchronized endodyogeny [116, 117].

The cell cycle of type I tachyzoite endodyogeny will undergo G1 (~3 hours) and S phase (~2 hours), then skip the classical G2 phase and go right into M phase (~1 hour), which happens to dramatically overlap with cytokinesis, totaling a 6 hours cell division cycle [118, 119]. Avirulent type II and type III parasites have slightly longer cell cycles of

8-10 hours [119]. During G1 phase, the parasite duplicates the centrosome and Golgi apparatus and begins elongation of the apicoplast [120-122]. Continued apicoplast elongation and chromosomal duplication marks the S-phase of the cell cycle. Late in S-phase (1.8N), spindles emerge from two centrosomes and initiate a closed M-phase while, simultaneously, the cytoskeletal IMC of the daughter buds appear around this scaffold [119, 123, 124]. As M-phase progresses, IMC buds continue to grow and incorporate the Golgi, mitochondrion, endoplasmic reticulum (ER), nucleus, and other organelles inside the developing bud [125-127]. Organelles such as MICs, rhoptries (ROP), and dense granules (GRA) are largely created *de novo* after the daughter buds form [125]. After completion of the daughter cytoskeleton, cytokinesis is completed when the PM of the mother cell starts to disassemble and recycles itself onto the emerging daughter cells [128]; newly synthesized lipids are produced for the PM as well via the apicoplast's fatty acid synthesis II (FASII) pathway [129]. Upon the dissemination of the mother cell and formation of the daughter cell's PM, the division cycle is completed.

Transcriptomic analysis identified two signature subtranscriptomes of *T. gondii's* cell cycle: the G1 and the S/M transcriptome [130]. Combined, these two subtranscriptomes represent nearly one third of the parasite's genome. During the G1 phase, tachyzoites express many genes involved with biosynthesis and metabolism, including RNA polymerases, splicing enzymes, ribosomal proteins, translation factors, chromatin modifiers, and a few transcription factors [130]. Additionally, G1 expresses metabolic genes associated with the apicoplast (e.g. FASII) and mitochondria (e.g. TCA cycle) [130]. During the S/M phase, tachyzoites express many genes involved with the

formation of daughter cells, such as DNA polymerases, IMC's and other cytoskeletal proteins, motor proteins, secretory proteins, SRS's, and transcription factors [130].

Host manipulation:

Within an *in vivo context,* parasitic infection naturally triggers a host immune response, for which *T. gondii* has developed sophisticated defensive mechanism to foster its continuity. Two major innate cytokine responses for parasite clearance are the interferon gamma (IFNγ) [131] and tumor necrosis factor alpha (TNFα) [132, 133] pathways. IFNγ-mediated clearance works in several ways: degradation of the PV by up-regulating autophagy-inducing Immunity Related GTPases (IRGs) [134-136], degradation of essential growth nutrients such as tryptophan [137], and activation of other clearance pathways via phosphorylation of STAT1 [138, 139]. TNFα-mediated clearance also aims to degrade the PV but by up-regulating autophagy-inducing CD40 instead [140]. *T. gondii* has evolved several evasive mechanisms to resist these immune responses *in vivo* by secreting GRA effector proteins that modulate the host cell's signaling and transcription activity [141]. Interestingly, several of these responses contain strain- and host-specific virulence factors which are discussed further in Chapter 1.6.2.

Bradyzoite differentiation:

Within an *in vivo* context, or under specific laboratory conditions, conversion from tachyzoites into bradyzoites is stress induced (e.g. IFNγ effectors, alkaline pH, heat shock, nutrient deprivation) [142, 143] and appears to be controlled at the transcriptional and translational level [144]. Stress-induced phosphorylation of eIF2α diminishes protein

translation and promotes bradyzoite differentiation [74, 145] while pharmacological inhibition of eIF2α-specific phosphatases can also induce bradyzoite differentiation [145], indicating that phosphorylation of eIF2α is a major determinant of differentiation [144]. Moreover, phosphorylation of eIF2α is important for the viability of extracellular parasites [74], indicating that halted translation is part of a stress response pathway that likely facilitates the conservation of ATP and reprogramming of its transcriptome.

Microarray and RNA-seq studies have identified hundreds-to-thousands of differentially expressed genes (DEGs) between tachyzoites and bradyzoites, illustrating the major reprogramming that underpins bradyzoite differentiation [146]. Many of these genes include cyst wall, metabolic, and SRS proteins. Several Apetala 2 (AP2) transcription factors have been identified as activators (AP2IV-3 [147], AP2XI-4 [148], and AP2Ib-1 [147]) or repressors (AP2IV-4 [149], AP2IX-4 [150], and AP2IX-9 [147, 151]) of bradyzoite-associated gene regulation and/or bradyzoite differentiation. Recent progress has also identified a Myb-like transcription factor, bradyzoite-deficient factor 1 (BFD1), as a master regulator of bradyzoite differentiation [152]. While genetic disruption of AP2 activators and repressors partially influenced bradyzoite differentiation, disruption of BFD1 completely abrogated differentiation, suggesting it as an upstream activator of bradyzoite differentiation [152]. Furthermore, conditional BFD1 expression was found to recapitulate the transcriptomic profiles of stress-induced bradyzoites while chromatin profiling identified BFD1 binding to canonical bradyzoite promoters, including AP2IX-9 [152].

Egress:

After several rounds of replication, or recurrence of latent infection, *T. gondii* will exit from the host cell (i.e. egress) (Figure 1.2a.). Several environmental cues can trigger egress: a dro*p* in host cytoplasmic K^+ (as seen during host apoptosis) [153], a rise in phosphatidic acid (PA) on the parasite surface [154], and a decrease in intravacuolar pH [155]. While the topography of these cues and their downstream targets are quite complex and still developing, they converge onto two crucial intracellular secondary messengers: cyclic guanosine monophosphate (cGMP) and Ca^{2+} [156]. The production of cGMP by guanyl cyclase (GC) activates protein kinase G (PKG), a key mediator in the mobilization of intracellular Ca^{2+}. The triggered release of Ca^{2+} from intracellular stores (e.g. acidocalcisome, ER) [157, 158] simultaneously activates glideosome motility and MIC secretion of perforin-like protein 1 (PLP1) [159-162]. At low pH, PLP1 forms pores within the PVM and host cell PM, allowing motile parasites to break free from surrounding membranes and become extracellular once again [161, 162]. Upon invasion of a new host cell, motility and secretion is turned off by cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) which activates cyclic nucleotide phosphodiesterases (PDE) to degrade cGMP [163]. As a result, PKG activity and intracellular Ca^{2+} levels are reduced, thereby promoting parasite division and thereby completing the parasite's lytic cycle [164].

1.6. *T. gondii* virulence

1.6.1 Virulence phenotypes *in vivo* and *in vitro*

Virulence, from an *in vivo* perspective, is defined as the relative capacity of a pathogen to cause damage within its host and is often expressed quantitatively as the

lethal dose (LD_{50}) or lethal time (LT_{50}) required to kill 50% of test subjects [165, 166]. For *T. gondii* research, test subjects are mouse models, for which the degree of virulence can vary depending on the parasite's genotype. The type I genotype is by far the most virulent, with a LD_{100} of just 1 parasite intraperitoneally (i.p.) injected into healthy mice [167, 168]. The type II and type III genotypes are considerably less virulent with a LD_{50} of ~10^3 and ~10^5, respectively [167]. Type 12 virulence in mice has not been assessed yet. Virulence traits such as replication rate [119, 169, 170], immune evasion [171], bradyzoite conversion [172], motility [173], and extracellular survival [167] are measurably different amongst typical genotypes. Comparatively, type I genotypes display the most enhanced virulence traits, resulting in a deadly lytic cycle in mice [174-178]. Notably, virulence should be considered not only in the context of parasite genotype, but host species as well because *T. gondii's* evasion mechanisms (see Chapter 1.6.2) can be host-specific. In other words, a virulent genotype in one host may be benign in another. For example, i.p. injection of a single type I parasite can kill a healthy mouse but it takes ~10^4-10^7 type I parasites to kill a healthy rat [177, 179-181]. Fortunately, healthy humans (postnatal) are much more resilient to infection as well. Virulence in congenital infections (prenatal) can also vary between species, as seen with pregnant sheep [182].

Virulence, from an *in vitro* perspective, is the degree of damage caused to a cell cultured host (a cell monolayer), which is commonly visualized by plaque assay and quantitatively expressed as plaque size (e.g. mm^2). Plaque size of type I, II, and III parasites reveal an *in vitro* virulence that reflects observed *in vivo* virulence. Interestingly, plaque size of eight type I strains (RH, GT1, GIL, FAJI, VEL, ENT, MOR, and PT) all show relatively similar levels (~1- to 2-fold) of *in vitro* virulence with the exception of RH which

displays ~5- to 10-fold greater *in vitro* virulence [168]. The reasons behind RH's dramatically enhanced *in vitro* virulence has long been a topic of research but the mechanism behind it has remained obscure [168, 183].

1.6.2. Genetic virulence factors

Experimental crosses and QTL analysis of virulent and avirulent strains have determined genetic loci and candidate genes that are associated with virulence in mice. These mainly consist of ROP- and GRA- secreted proteins that aid parasite virulence by modulating the host's defense mechanisms. Polymorphic ROP16 kinase can phosphorylate STAT3 and STAT6 transcription factors and subvert the induction of IFNγ-mediate defense responses such as host apoptosis [184-186]. Specific ROP5/17 or ROP5/18 alleles work in concert to inactivate IFNγ-stimulated IRG-mediated disruption of the PV [187-189]. Polymorphic alleles of GRA15, even found within avirulent isolates [183], can differentially modulate a host's NFκB response [190]. Type II alleles of GRA24 [191] and GRA25 [192] were found to modulate cytokine production and MAPK responses, respectively. Other studies aimed at identifying secreted effector proteins have identified GRA16 [193], GRA24 [191], and IST1 [194, 195] as direct interactors of host cell regulators within the host cell's nucleus, resulting in the misregulation of the host's p53, MAPK, and IFNγ/β response pathways, respectively. In short, *T. gondii* has successfully evolved many effector proteins for targeting host cell signaling and transcription, resulting in successful evasion and established infection [141]. The significance of these virulence factors *in vitro*, however, appear insignificant, suggesting they do not interfere with the acute lytic cycle–the driver of disease pathogenesis.

Interestingly, evolution of the host-pathogen interaction has resulted in host-dependent mechanisms of *T. gondii's* evasion. For example, both of the type I ROP5/17 and the ROP5/18 alleles, which are virulent in mice by blocking IRG-mediated clearance, are not virulent in humans because we lack many of the ROP-targeted IRGs [187-189, 196]. Additionally, a host's defense to *T. gondii* infection can result in divergent defensive mechanisms as well. For example, a family of interferon-inducible GTPases, guanylate binding proteins (GBPs), promote parasite clearance in mice by targeting the PV for destruction, whereas GBPs in humans do not target the PV and alternatively operate to delay the onset of parasite replication [197]. Taken together, these data indicate that the evolution of *T. gondii's* polymorphic virulence factors can result in host-specific offense and defensive mechanisms [196]. Given this diversity in host-pathogen interactions *in vivo*, identifying host-independent virulence factors in *T. gondii* (such as from an *in vitro* context) could be of great value to human and veterinary medicine. To date, the presence of any host-independent polymorphic virulence factors remains unknown.

1.6.3. RH and GT1 at a glance

Previous studies have elucidated virulence factors involved with *in vivo* host defense modulation, but do not explain the differences in virulence traits observed *in vitro*. Because their *in vitro* virulence is dramatically different and, yet, their genomes are very similar (and readily available), RH and GT1 comparative analysis' have been the traditional approach for uncovering the genetic basis responsible for phenotypic differences *in vitro*.

The type I isolate, RH, was first isolated in 1939 from a deceased child with toxoplasmosis [198]. It was immediately propagated and maintained by mouse passages *in vivo* until 1977, upon which it was propogated to *in vitro* cell culture conditions (Figure 1.3) [198, 199]. The RH strain is the strain of choice used by most laboratories, including our own, to understand *T. gondii* and other apicomplexan biology [8]. As a result of being the first isolate available to research, it has experienced several decades of lab-adaptation [168]. Notably, earlier frozen isolates of RH (RH-JSR), which were passaged only *in vivo,* show remarkably reduced *in vitro* virulence, similar to that of GT1, indicating prolonged lab-adaptation as the driver of augmented *in vitro* virulence [168]. The other type I isolate, GT1, was isolated from an infected goat in 1979. Since then, it has been limitedly passaged *in vitro* and has remained largely cryopreserved until this study (Figure 1.3).

Whole genome sequencing (WGS) between RH and GT1 identified 1,394 single nucleotide polymorphisms (SNPs), of which 133 encoded nonsynonymous amino acid changes and 54 were insertions/deletions (indels) within predicted a coding sequence (CDS) (Figure 1.3) [183]. Of these, two SNPs (found in GRA2 and GRA15) were individually found to differentially modulate host defense response pathways, but the degree to which these two polymorphisms have on *in vitro* virulence appears minor [183]. With relatively few polymorphisms between RH and GT1 and failure to connect them with virulent traits of the acute lytic cycle, it is hypothesized that epistatic interactions- the specific combination of alleles necessary to bring about a phenotype- may contribute to the observed differences in *in vitro* virulence [183]. For example, the presence of the

virulent type I ROP18 allele can only prevent IRG-mediated clearance when it is also in the presence of the virulent type I ROP5 allele [187].

Epigenetic differences altering gene expression is another plausible hypothesis for RH's superior *in vitro* virulence. Microarray analysis of RH, RH-JSR, and GT1 has provided 113 candidate genes (>2 fold-change) that are differentially expressed between RH and GT1/RH-JSR, including several ABC transporters [168] which play a role in parasite lipid trafficking [200]; however, none have been validated as differentially expressed *in vitro* virulence factors. Therefore, it is likely that gene regulation may be an additional mechanism underlying the difference in observed *in vitro* virulence traits. Altogether, the approach of comparative genomics between lab-adapted, RH, and non-lab-adapted, GT1, parasites has comprehensively characterized their molecular differences, but largely failed to explain the genetic basis of acquired *in vitro* virulence that stems from prolonged lab-adaptation.

1.7. The epigenome

1.7.1 Epigenetics

Epigenetics refers to heritable changes in gene expression or phenotype without alterations in the DNA sequence. Post-translational modifications (PTM) of genomic DNA and PTM of histones, by histone acetyltransferases (HAT) and histone methyltransferase (HMT), and the expression of non-coding RNAs (ncRNA), have all been observed in *T. gondii*, although the degree to which these epigenetic mechanisms facilitate parasite biology are still being uncovered [201-204]. For example, functional analysis of a *T. gondii* HAT and HMT (GCN5 and CARM1, respectively), uncovered an epigenetic control over

life cycle stage differentiation [205]. Furthermore, histone acetylation was found to regulate chromatin structure and virulence factors in *Plasmodium* [206, 207]. Similarly, transgenerational epigenetic variation in a variety of other organisms (algae, fungi, and insects) has been found to be the basis of adaptive responses [208-212].

1.7.2 The apetala 2 (AP2) transcription factors (TF)

The apetala 2 (AP2) proteins are a family of transcription factors (TF) that were first described back in 1998 in *Arabidopsis thaliana* and since thought to be plant-specific [213]. It wasn't until 2005 when Balaji *et al* identified a novel series of Apicomplexa AP2 (ApiAP2) TF's in the parasitic protozoan, *Plasmodium falciparum* [214]. Since then, numerous ApiAP2 TF's have been identified and characterized across apicomplexan organisms, including *Plasmodium, Eimeria, Sarcocystis, Cryptosporidium,* and *Toxoplasma*. In *T. gondii*, divergent roles for ApiAP2's have been identified such as: association with chromatin remodeling complexes [215] and RNA-induced silencing complexes (RISC) [204]; binding DNA in a sequence-specific manner in order to activate or repress genes critical for parasite virulence and invasion [216, 217]; immune evasion and bradyzoite differentiation [147-151]; and promotion of asexual cell cycle progression [218-220].

While ApiAP2 proteins vary in size (~70-9000 amino acids) across the entire apicomplexan phylum, one thing they all have in common is the presence of at least one 60 aa AP2 DNA-binding domain consisting of 3 β-strands and 1 stabilizing α-helix [214, 221]. Other domains that may found in an ApiAP2 TF include: zinc fingers, AT-hooks, ACDC (AP2-coincident domain mainly at the C-terminus), and pentapeptide

repeat-like domains [222]. These other domains likely aid the ApiAP2 TF in binding of specific DNA loci. Some ApiAP2 proteins are known to dimerize once bound to DNA. For example, X-ray crystallography of a *P. falciparum* ApiAP2 (PF14_0633) identified a domain-swapping mechanism in which the α-helix of one dimer stabilizes the 3 β-strands of its partnering dimer [221]. It is thought that dimerization of ApiAP2's could be influencing gene expression, as is the case with other eukaryotic TFs [221].

Growing evidence indicates that protein-protein interactions with ApiAP2's play key roles in regulating *T. gondii* gene expression [215, 216]. For example, immuno-precipitation (IP) of TgAP2XI-5 followed by mass spectrometry (MS) identified TgAP2X-5 as a sole binding partner. Genetic knockout (KO) of TgAP2X-5 decreased binding of TgAP2XI-5 to specific promoter regions, many of which being ROP gene promoters, which led to decreased invasion efficiency and a complete loss of virulence in mice [216]. Thus, a number of virulence-related genes in *T. gondii* are dependent on the cooperative binding of TgAP2XI-5 and TgAP2X-5. In a different study with similar proteomic approaches, TgGCN5b, a lysine acetyltransferase, was found to associate with four ApiAP2's: TgAP2IX-7, TgAP2X-8, TgAP2XI-2, and TgAP2XII-4 [216]. Interestingly, only one of these ApiAP2's, TgAP2X-8, has an acetylation site, indicating that the other TgAP2's may be responsible for recruiting TgGCN5b to specific DNA loci, thereby allowing for histone H3K9/K14 acetylation and resulting gene upregulation [223]. TgGCN5b is essential for *T. gondii* and an inducible knockdown (iKD) results in a reversible arrest in cell replication, likely due to the misregulation of gene expression [216]. Genetic manipulation of TgGCN5b's four interacting AP2 partners has not been evaluated, but will likely also prove essential for the parasite given their low fitness scores

(< -2.3) [224]. Taken together, AP2 TF's, and their potential cofactors, are emerging as prominent expression regulatory factors controlling many aspects of parasite biology.

1.8 Evolve and Resequence (E&R)

Evolve and Resequencing (E&R) is a recent technique that expands upon an old approach commonly known as experimental evolution. Experimental evolution, is a research approach designed to observe evolution under a controlled experimental condition, generally by altering an organism's environment (e.g. nutrient availability). While experimental evolution can be applied to multicellular organisms [225-228], it is often performed with unicellular organisms because of their ease to culture and manipulate, as well as their relatively fast reproductive and mutation rate [229]. Typically, in experimental evolution, microbes (bacteria, yeast, virus, etc.) are cultured in well-defined growth conditions and monitored over time (e.g. passages, generations) for changes in phenotypes (e.g. plaque size) or overall fitness (e.g. growth rate) [229].

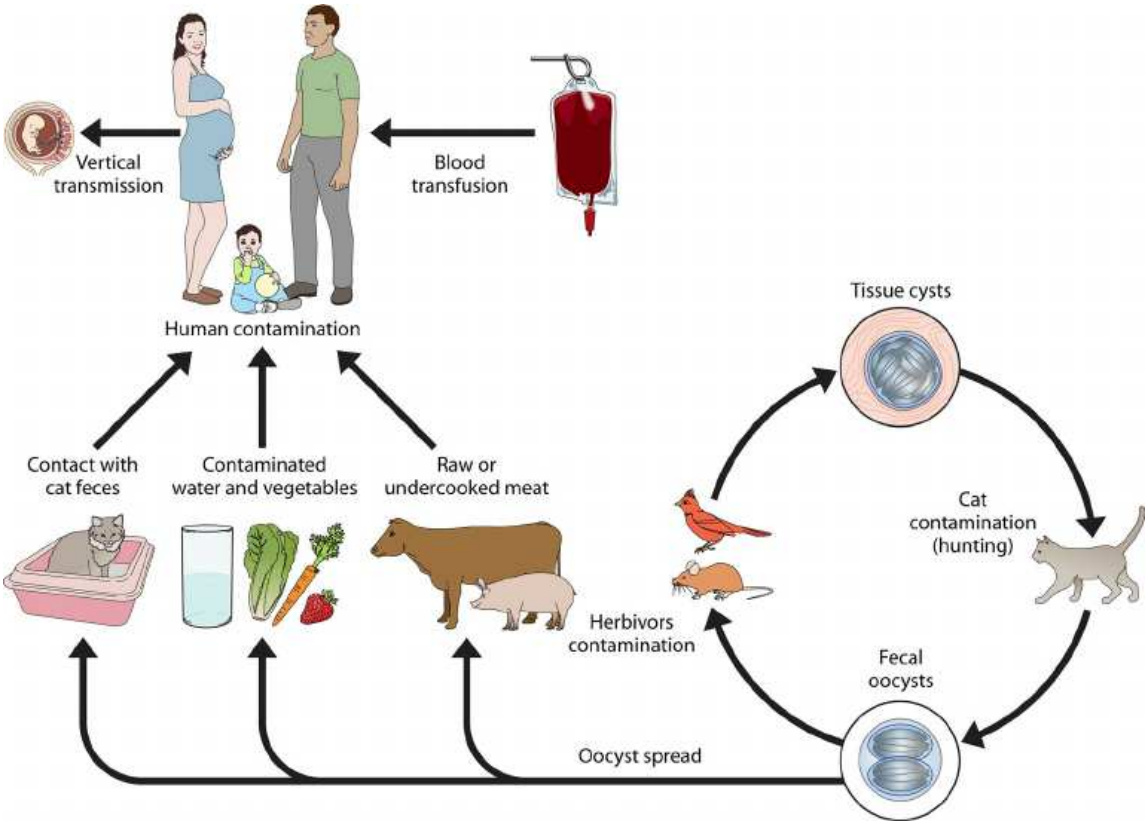
Experimental evolution dates as far back as the late 19th century, with pioneers such as William Dallinger and Charlton Batisian who observed gradual adaptation and ultimate survival of bacteria under superheated conditions [230, 231]. Experimental evolution's popularity as a research approach began skyrocketing at the beginning of the 21st century due to the emergence of high-throughput Next Generation Sequencing (NGS) technology, which now allowed researchers to investigate, in real time, the genetic factors underlying evolution. This synergistic coupling of experimental evolution and NGS sparked the term Evolve and Resequence (E&R) by Turner *et al* in 2011, although experimental evolution and E&R are still often used interchangeably [226].

The most famous and longstanding experimental evolution study is Dr. Lenski's Long-Term Experimental Evolution (LTEE) experiment. Starting in 1988, the Lenski lab has monitored the adaptation of *Escherichia coli* under low glucose conditions for over 66,000 generations (as of 2016) [232]. With the coming age of E&R, the Lenski lab performed NGS and expression profiling on several parallel evolving lines resulting in the identification adaptive alleles, expression profiles, and mechanisms behind *E. coli*'s adaptation to low glucose [233-237]. Moreover, their findings have provided tremendous insight into factors behind microbial population structure, adaptations rates, and genome evolution [235, 236, 238-240]. Across a variety of organisms, other E&R studies have examined adaptation to extreme temperature [241], unbalanced nutrition [228, 242], hypoxia [225], pathogen resistance [227, 243], drug resistance [244, 245], competition [246, 247], aging [248], and multicellularity [249, 250]. Although never applied to *T. gondii,* experimental evolution/E&R (*in vivo* and *in vitro*) has been successfully applied to other Apicomplexa in order to understand parasite virulence [251, 252] and the genetic basis for drug resistance [244, 245]. For example, the apicomplexan parasite *Plasmodium chabaudi*_evolved enhanced virulence when serially passaged in immunized mice, suggesting that strong in-host selection pressures can select for higher virulence. Interestingly, in a separate study, *P. chabaudi* was found to evolve into a more virulent strain when serially passaged in immune-suppressed mice, suggesting that immune-suppressed hosts can too provide an environment that promotes the evolution of virulence factors [252]. Thus, these E&R studies in *Plasmodium* reveal important insights into the evolution of apicomplexan virulence *in vivo.* A list of the above and other noteworthy experimental evolution and E&R publications are listed in Table 1.1.

1.9. Open questions and synopsis of dissertation

The two type I genotype *T. gondii* stains, RH, a lab-adapted strain, and GT1, a non-lab-adapted strain, have a genetic difference of only 0.002%, but show remarkable phenotypic differences *in vitro.* For example, it has long been known that RH's plaquing capacity and extracellular survival is far superior to that of GT1, likely due to prolonged lab-adaptation. The genetic basis of these phenotypes, however, remains largely unknown despite previous efforts interrogating the functionality of individual SNPs. The emerging hypothesis of previous investigations deliberate epistatic interactions as the genetic basis of differential type I phenotypes. Uncovering such epistasis, such as in the polymorphic ROP18/5 example, will support our growing understanding of *T. gondii* virulence. Furthermore, doing so *in vitro* will enable the discovery of more universal virulence factors that can be targeted for therapeutics. To answer this question, E&R is applied on non-lab-adapted GT1 through the *in vitro* lab-adaptation process for roughly 1500 generations (~ 2 years) (Figure 1.4). In short, lab-adaptation results in GT1's augmented *in vitro* virulence; resequencing of genomic DNA at various timepoints identified one candidate polymorphic gene associated with lab-adaptation, suggesting that epistatic interactions are not driving adaptation; resequencing of mRNA identified several hundred candidate genes that are differentially expressed (DE) and functional analysis suggests that epigenetic regulation of many genes are more likely driving GT1's adaptation; furthermore, fatty acid biosynthesis and lipid homeostasis are suggested as key biological processes for GT1's adapted virulence.

Figure 1.1. The life cycle of *Toxoplasma gondii*



Esch *et al.*, 2013

Figure 1.1. The life cycle of *Toxoplasma gondii*

Fecal oocysts shed from Felidaes (e.g. the cat) contaminate soil, water, grass, fruits and vegetables. The consumption of unwashed produce or grasslands contaminated with fecal oocysts infects intermediate hosts, such as humans, cows, mice, etc. Raw or undercooked consumption of infected intermediate hosts can transmit infection in the form of tissue cyst-containing bradyzoites. The consumption of bradyzoites or oocysts during pregnancy can transmit infection vertically to the fetus. Furthermore, organ transplant or blood transfusions from an infected host could transmit infection to recipients. A cat's consumption of infected intermediates hosts, such as bird or mice, completes the parasites life cycle. Figure 1.1 reprinted by permission from American Society for Microbiology (ASM).

Figure 1.2. The acute lytic cycle of *T. gondii*

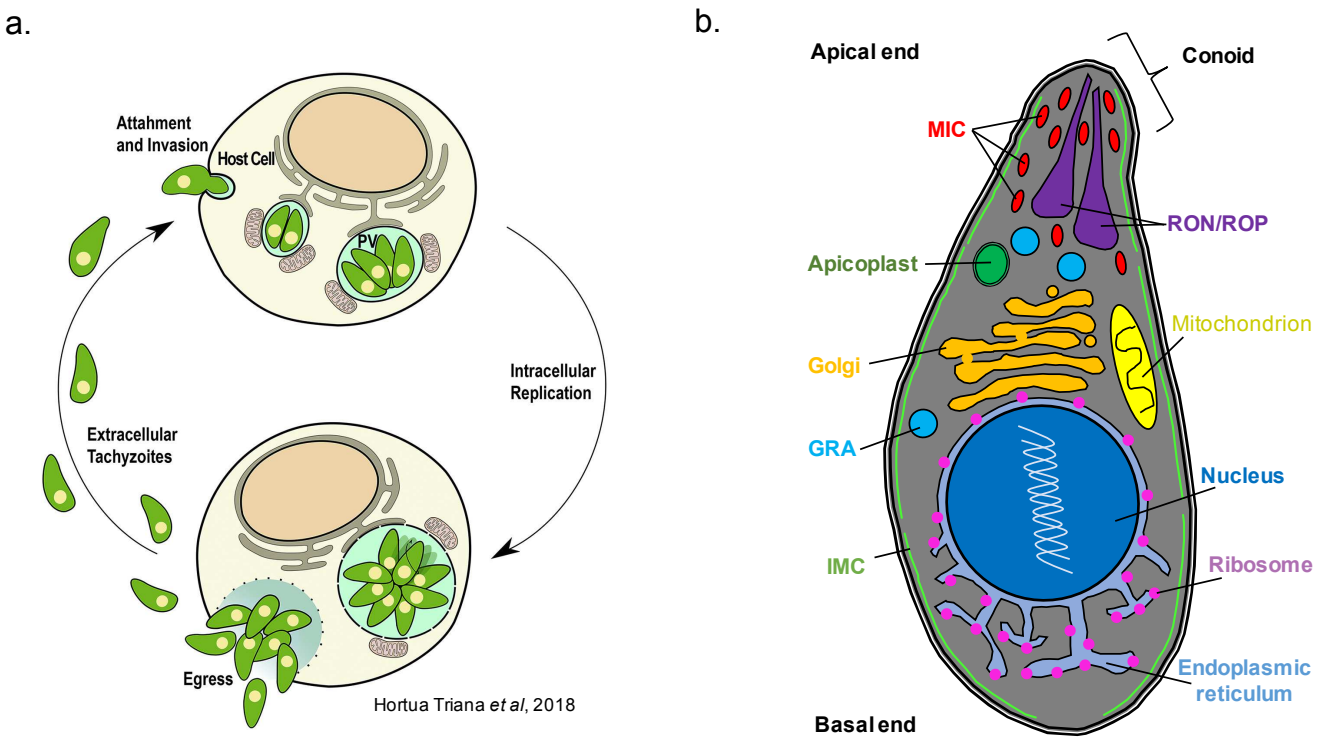


Figure 1.2. The acute lytic cycle of *T. gondii*

a. Highly motile extracellular tachyzoites attach to and orient their apical end toward the host cell before active invasion of the host. Successful invasion of the host cell results in the formation of a protective parasitophorous vacuole (PV) around the parasite. Intracellular parasites replicate by endodyogeny, until internal signals induce

parasite egress, thereby completing the lytic cycle. Figure 1.2a. reprinted by permission from Biochimica et Biophysica Acta (BBA) - Molecular Cell Research (order 4839040152222). **b.** Ultrastructure of *T. gondii*; being a member of the Apicomplexa, *T. gondii* contain unique structures such as the conoid and organelles, such as micronemes (MIC), rhoptries (RON/ROP), and dense granules (GRA).

Figure 1.3. Comparison of RH and GT1 isolates

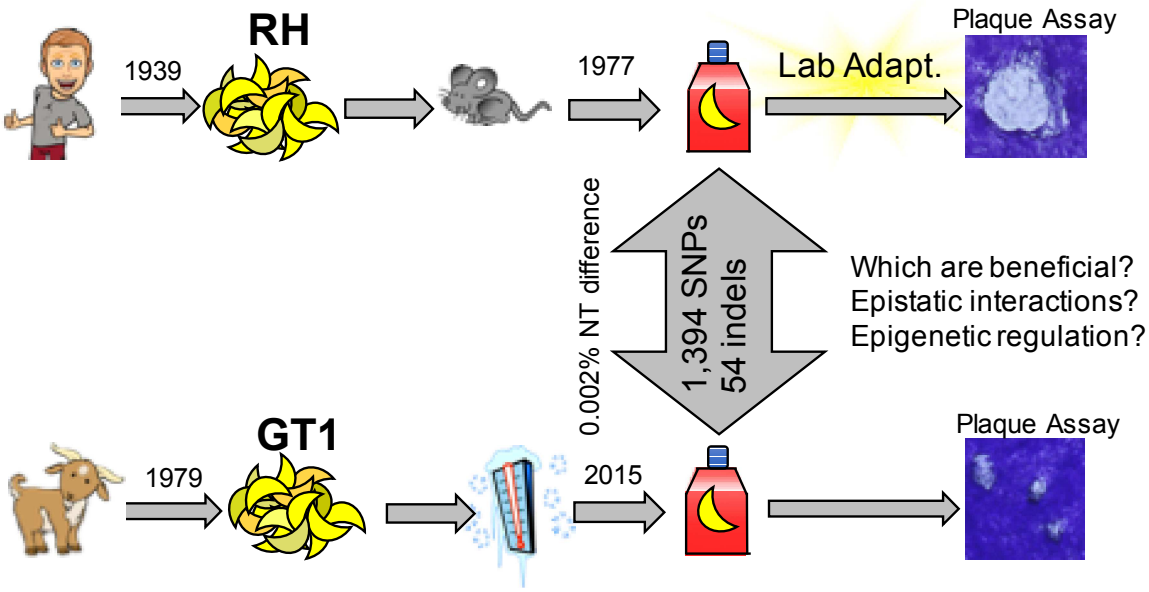


Figure 1.3. Comparison of RH and GT1 isolates

The RH strain was isolated in 1939 by Albert Sabin from a fatal human case of toxoplasmic encephalitis. The RH strain was propagated in mice until 1977 when Elmer Pfefferkorn cloned and propagated the strain into laboratory culture. Because it was the first available isolate, RH has been widely distributed and has undergone several decades of lab-adaptation, resulting in considerable *in vitro* virulence, as measured by plaque assay. The GT1 strain was isolated from a goat in 1979 and expanded briefly *in vitro* for the purpose of mass cryogenic freezing and depositing at BEI Resources Repository. The GT1 strain was thawed and cultured in our laboratory since 2015. Plaque assay analysis revealed significantly reduced *in vitro* virulence of GT1, relative to RH, even though both type I strains are genetically similar. Comparative genomic studies between RH and GT1 identified 1,394 SNPs resulting in 133 nonsynonymous changes to a proteins CDS and 54 CDS indels; however, identifying beneficial and/or epistatic mutations between the two strains has proved challenging.

Figure 1.4. E&R timeline of GT1 populations

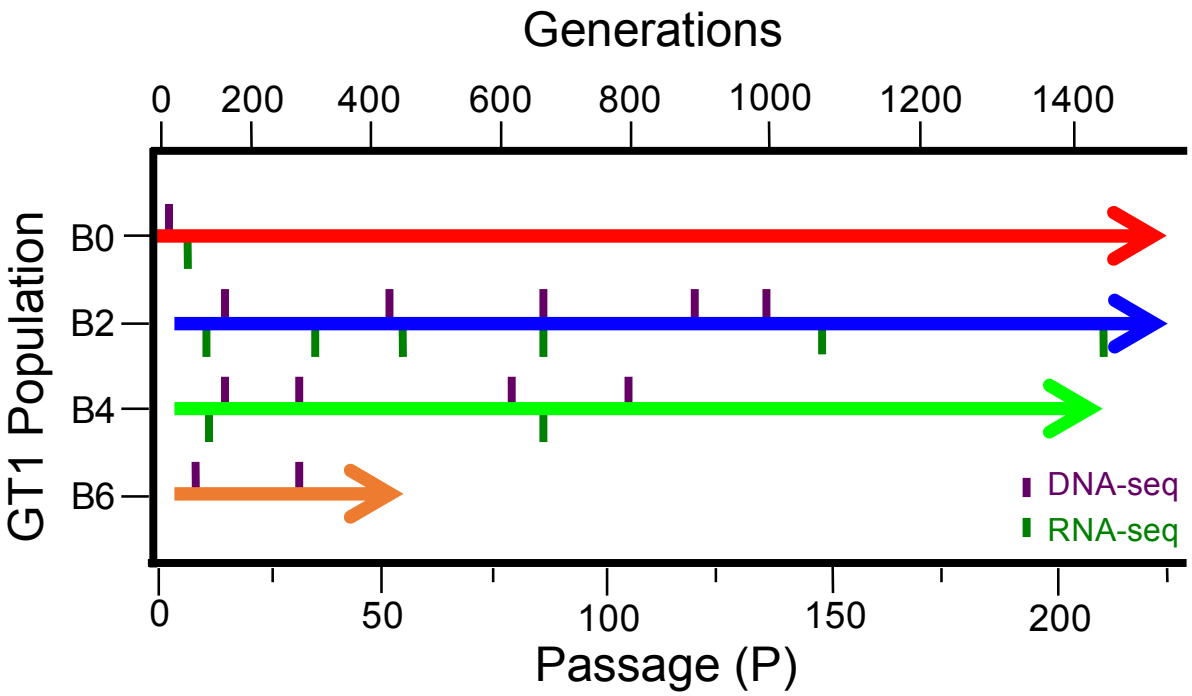


Figure 1.4. E&R timeline of GT1 populations

One un-cloned (B0) and three monoclonal (B2, B4, and B6) starting GT1 populations were introduced to standard *in vitro* culture conditions. Continued lab-adaptation involved serial passaging (1:20) of parasites for up to ~1500 generations, or ~220 passages (P). Evolving parasite populations were continually resequenced by DNA-sequencing and/or mRNA-sequencing at the indicated timepoints. Figure is drawn to scale; colors represent their respective GT1 population in other figures.

Table 1.1. List of key experimental evolution and E&R publications

Author	Year	PMID	Species	Time	Variable	Notes
Jalvingh, K.M., et al.	2014	24500162	Asobara tabida	5 host generations	Pathogen resistance	Strong selection likely acts on standing genetic variation within the population in a short time frame
Haas, J.W.	1887	11624308	Bacteria	500,000 generations	Temperature	Early example of evolved adaptation to stress
Bastian, H.C.	1907	20897082	Bacteria	3 months (est.)	Temperature and salinity	Early example of evolved adaptation to stress
Rechavi, O., G. Minevich, and O. Hobert	2011	22119442	Caenorhabditis elegans	10 generations	Viral replication	RNAi-dependent viral silencing is an epigenetic response
Kronholm, I., et al.	2017	28535256	Chlamydomonas	200 generations	salt stress, phosphate starvation, and high CO_2	Genetic and epigenetic mechanisms (DNA methylation) promote adaptive evolution to various stresses
terHorst, C.P.	2011	20964780	Colpoda	100 generations	Competition	Interspecific competition selected for populations drives phenotypes and increased fitness
Kolss, M., et al.	2009	19473389	Drosophila melanogaster	64 generations	Malnutrition	Larval adaptations to malnutrition results in fitness trade-offs in adult flies, but not larva
Zhou, D., et al.	2011	21262834	Drosophila melanogaster	200 generations	Hypoxia	Regulatory mutations increased regulon expression which confers tolerance to hypoxic conditions
Turner, T.L., et al.	2011	21437274	Drosophila melanogaster	100 generations	Fly size	Fly size is the result of polygenic inheritance
Remolina, S.C., et al.	2012	23106705	Drosophila melanogaster	50 generations	Aging and fecundity	Mutations and gene expression patterns are associated with aging and fecundity
Stern, S., et al.	2012	22832276	Drosophila melanogaster	6 generations	Developmental stress	Epigenetic adaption to developmental stress is indeed heritable, even when stress in removed
Bennett, A.F. and R.E. Lenski	1993	28568084	Escherichia coli	2,000 generations	Temperature	Temperature adapation results in optimal fitness without any fitness tradeoffs at different temperatures (32-42°C)
Lenski, R.E. and M. Travisano	1994	8041701	Escherichia coli	10000 generations	Limited glucose	Parallel-evolving populations display similar phenotypic changes that are initially rapid and then approach stasis
Cooper, T.F., D.E. Rozen, and R.E. Lenski	2003	16702438	Escherichia coli	20,000 generations	Limited glucose	Regulatory mutations increased regulon expression resulting in similar fitness effects of parallel-evolving populations
Woods, R., et al.	2006	16751270	Escherichia coli	20,000 generations	Limited glucose	The same candidate genes are mutated in parallel-evolving populations; observing the same mutations is rare
Cooper, T.F., et al.	2008	18282111	Escherichia coli	20,000 generations	Limited glucose	Epstatic interactions between mutations contribute to adaptive evolution
Blount, Z.D., C.Z. Borland, and R.E. Lenski	2008	18524956	Escherichia coli	40,000 generations	Limited glucose	Adaptation is dependent on the genetic background, suggesting epistatic interactions
Barrick, J.E., et al.	2009	19838166	Escherichia coli	40,000 generations	Limited glucose	Mutations accumulate at a steady state while finess rapidly increase and approaches stasis
Cooper, T.F. and R.E. Lenski	2010	20070898	Escherichia coli	2,000 generations	Fluctuating sugar availability	Genetic diversity within a population increases under fluctuating environmental conditions
Woods, R.J., et al.	2011	21415350	Escherichia coli	883 generations	Fitness competiton	Epistatic interactions between mutations affects fitness landscape and future evolability
Maddamsetti, R., et al.	2017	28379360	Escherichia coli	50,000 generations	Limited glucose	The core genome of E. coli evolves faster under positive selection experiments than they would in nature
Kram, K.E., et al.	2017	28289732	Escherichia coli	300 generations	Delayed serial passaging	Adaptation results in swift selection of genomic changes to the same genes in parallel-evolving populations
Mukherjee, K., et al.	2019	30733453	Galleria mellonella	6 generations	Pathogen resistance	Epigenetic mechanisms (DNA methylation, histone modification, and miRNA) enable adaptive fitness
O'Driscoll, L., et al.	2006	17170223	MIN6 (pancreas) cell line	40 passages	Prolonged serial passaging	Long-term passaging results in phenotypic and gene expression changes
Zbinden, M., C.R. Haag, and D. Ebert	2008	18462312	Octosporea bayeri	15 host generations	Pathogen resistance	Evolution of pathogen tolerance did not result in host fitness trade-offs under prevailing conditions
Mackinnon, M.J. and A.F. Read	2004	15221031	Plasmodium chabaudi	20 passages in host	Immunization	Host immunity promotes evolution of pathogen virulence
Hunt, P., et al.	2010	20846421	Plasmodium chabaudi	N/A	Drug resistance	Drug resistance in Plasmodium is genetic
Martinelli, A., et al.	2011	20858498	Plasmodium chabaudi	N/A	Drug resistance	Drug resistance in Plasmodium is genetic
Barclay, V.C., et al.	2014	25061677	Plasmodium chabaudi	3 passages in host	Immune-suppression	Immune-supression promotes evolution of pathogen virulence
Ariey, F., et al.	2014	24352242	Plasmodium falciparum	5 years	Drug resistance	Drug resistance in Plasmodium is genetic
Rogers, D.W. and D. Greig	2009	18842545	S. cerevisiae	13 mating cycles	Sexual competition	Beneficial alleles are naturally selected for under strong selection pressures, but not weak selection pressures
Araya, C., et al.	2010	20128923	Saccharomyces cerevisiae	188 generations	Sulfate limitation	SNPs and copy number variations are asscoiated with adaptation
Dhar, R., et al.	2011	21375649	Saccharomyces Cerevisiae	300 generations	Salinity	Salt adaptation is associated with DNA ploidy and gene expression and not genomic mutations
Dhar, R., et al.	2013	23125229	Saccharomyces Cerevisiae	300 generations	Alternating salt & oxidative stress	Adaptation to cycling stress is associated with cross-protection and anticipatory gene regulation
Ratcliff, W.C., et al.	2015	25600558	Saccharomyces cerevisiae	227 days	Settling time	Multicellularity and evolvability of snowflake yeast is genetic
Rosenberg, A., et al.	2019	31806760	Toxoplasma gondii	1376 generations	Drug resistance	Drug resistance in Toxoplasma is genetic
Grubaugh, N.D., et al.	2015	25993022	West Nile Virus	5 passages in host	Host species	Strong selection pressures result in less genetic diversity and repeat mutations in parallel experiments
Versace, E., et al.	2014	24387805	Wolbachia	57 host generations	Temperature	The genotype of an endosymbiont reproducibly contributes to fly fitness in cold, but not hot, temperatures

Chapter 2: Lab-adaptation of GT1 results in enhanced *in vitro* virulence traits of the extracellular milieu

2.1. Introduction

Genetic analysis by multi-locus RFL*P* and DNA-sequencing have identified four clonal lineages of *Toxoplasma gondii* termed type I, type II, type III, and type 12 [25, 26]. Phenotypic characterization of type I, II, and III virulence traits are quite comprehensive, while type 12 is limited due to its more recent discovery. Nevertheless, type I genotypes display faster replication [119], enhanced transepithelial migration [173], and greater extracellular viability [167]. Such superior virulence traits enable type I parasites to have a faster lytic cycle and, consequently, greater virulence both *in vitro* and *in vivo*. Comparative genomics aimed at uncovering the genetic basis of these phenotypes have identified many polymorphic virulence factors that aid in parasite virulence *in vivo,* the mechanisms of which are designed to modulate the host's immune response. Unfortunately, the genetic basis of type I *in vitro* virulence and its associated virulence traits (e.g. extracellular survival, invasion, replication, egress), have remained poorly understood.

Within the type I lineage, two particular strains of interest, RH (a lab-adapted strain) and GT1 (a non-lab-adapted strain), show remarkable phenotypic differences. The lab-adapted RH strain displays an exceptionally enhanced *in vitro* virulence, as determined by plaque size, relative to GT1 and six other non-lab-adapted type I isolates [168]. Furthermore, RH's replication rate and extracellular viability are superior to GT1, which likely contribute to the observed differences in *in vitro* virulence [168]. Interestingly, the most ancestral RH population, RH-JSR, that was passaged only in mice shows significantly smaller plaques, similar to that of GT1, indicating that *in vitro* lab-adaptation of RH had resulted in augmentation of its *in vitro* virulence [168].

Interestingly, despite these phenotypic differences, RH and GT1, both of the type I genotype, are genetically similar. Previous comparative genomic studies by Yang *et al.* identified 1,394 single nucleotide polymorphisms (SNPs) between RH and GT1, of which 133 caused nonsynonymous amino acid changes and 54 were insertions/deletions (indels) within predicted coding regions [183]. It was hypothesized that some of these SNPs are responsible for the phenotypic differences observed between RH and GT1. Unfortunately, efforts to uncover the genetic basis of these phenotypes have had limited success. In brief, the authors identified two mutually exclusive SNPs found in GRA2 and GRA15 that differentially modulate host IFNγ and NFκB immune response pathways, respectively; the degree these two polymorphisms have on *in vitro* virulence, however, appears insignificant [183]. The authors concluded that the genetic basis of RH's superior *in vitro* virulence and associated virulence traits are likely a combination of alleles (i.e. epistasis) or gene expression patterns, rather than individual genes [183]. Uncovering epistatic interactions from a list containing 1,394 polymorphisms, however, would generate an unwieldy matrix ($<10^6$) of allele possibilities that will be difficult to experimentally untangle. To overcome this problem, we reasoned that lab-adaptation of the GT1 strain should result in enhanced *in vitro* virulence that coincides with the accumulation of mutations, and, by chronologically identifying emerging mutations during lab-adaptation, we would be able to establish genotype-phenotype correlations that can be experimentally validated. This particular approach to uncover the genetic basis of evolutionary traits is referred to as evolve and resequencing (E&R).

To evolve GT1's phenotypes, we first lab-adapted several GT1 populations by *in vitro* serial passaging and measured phenotypic changes during the first 2-years of lab-

adaptation (~220 passages, ~1500 generations) (Figure 1.4). The RH strain was utilized as a proxy for GT1's lab-adaptation. Indeed, we observe a significant increase in GT1's *in vitro* virulence, albeit still significantly inferior to RH. Additionally, we observe a significant increase in GT1's reinvasion efficiency and extracellular survival- both virulence traits within the extracellular milieu of the lytic cycle. Replication rate and egress, both virulence traits within the intracellular milieu of the lytic cycle, are unaffected.

2.2. Results

2.2.1. Phenotypic comparison of RH (lab-adapted) and GT1 (non-lab-adapted) parasites

To verify familiar, and identify novel differential phenotypes between lab-adapted and non-lab-adapted strains, we first measured the following phenotypes of the acute lytic cycle in both RH and low passage (≤P16) GT1: *in vitro* virulence (plaque size), extracellular survival, invasion, replication, and egress. Plaque size analysis confirmed the dramatic 4.32- to 5.94-fold difference (p-value $\leq 10^{-4}$) in *in* vitro virulence between RH and GT1, as previously reported [168] (Figure 2.1). Temporal extracellular survival assays also confirm the dramatic difference previously reported between RH and GT1 [168], with RH and GT1's extracellular LT_{50} to be 5.5hrs and 2hrs, respectively (p-value ≤ 0.01) (Figure 2.2a). Measuring the area under these survival curves indicated that the overall viability of RH is 1.89-fold higher (p-value ≤ 0.01) than GT1 (Figure 2.2b). Reinvasion efficiency was assessed by measuring the number of plaques, relative to inoculum size, immediately following mechanical release from host cells, and reveals that RH parasites are 3.40- to 3.65-fold (p-value ≤ 0.001) more efficient at reinvading a host cell than GT1 parasites (Figure 2.3). Replication assays confirm previous observations that RH has a faster replication cycle than GT1 [168], with RH having significantly fewer 1- and 4-parasite vacuoles and more vacuoles containing 8 parasites than GT1 (p-value ≤ 0.05) at 24hrs post invasion (Figure 2.4a-c). Lastly, egress assays with 30-hour intracellular parasites reveal that RH has similar egress capabilities to that of GT1 (Figure 2.4d). Taken together, these results reveal that both intracellular and extracellular virulence traits of the lytic cycle are phenotypically different between RH and GT1, which likely

explain in the overall *in vitro* virulence difference observed between these two type I strains.

2.2.2. Lab-adaptation of GT1 results in enhanced *in vitro* virulence

To ascertain a homogenous starting population, we first isolated several GT1 clones from the cryopreserved strain originally used for establishing *T. gondii*'s reference genome [253]. Next, we propagated the original un-cloned GT1 strain (designated B0) as well as three monoclonal GT1 population (designated B2, B4, and B6) into culture for up to 223 serial passages (~2 years, ~1500 generations), with approximately 5% of the culture being serially passed every 2-3 days (Figure 1.4). Parasite populations at various time points along the adaptation process were cryogenically stored for downstream analysis and permit future studies to connect phenotype and genotype.

We monitored lab-adaptation by measuring GT1's *in vitro* virulence, as determined by plaque size, over time; indeed, all GT1 populations showed a steady increase in plaque size (Figure 2.1). The GT1 population B0 displayed a 2.30-fold increase (p-value ≤ 0.001) in plaque size by B0-P218, relative to B0-P15 (Figure 2.1a); the GT1 population B2 displayed 2.19-fold increase (p-value ≤ 0.001) in plaque size by B2-P223, relative to B2-P12 (Figure 2.1b); and the GT1 population B4 displayed 1.86-fold increase (p-value ≤ 0.001) in plaque size by B4-P204, relative to B4-P11 (Figure 2.1c). These data support our initial hypothesis that phenotypic augmentation in *T. gondii* can be induced by lab-adaptation. However, plaque size of RH remains 2.31- to 2.62-fold larger (p-value $\leq 10^{-4}$) than all >P200 GT1 populations, suggesting that GT1 is not as evolved as RH after 2-years of lab-adaptation (Figure 2.1).

Because RH and GT1 strains were isolated from different species (human and goat, respectively) we next tested whether the difference in plaque forming capacity was associated with the host of origin. We therefore measured *in vitro* virulence of RH, B2-P22 and B2-P177 in both human foreskin fibroblasts (HFF) and goat skeletal fibroblasts (GSF) as host cells. Plaque size of RH remained superior to GT1, regardless of host species (Figure 2.1d). Furthermore, plaque size of B2-P177 remained 2.5-fold bigger than B2-P22, regardless of host species (Figure 2.1d). Taken together, these results illustrate that lab-adaptation of *T. gondii* positively affects their *in vitro* virulence in a host-independent manner.

2.2.3. Lab-adaptation of GT1 results in enhanced extracellular survival capacity

Because we observed augmented *in vitro* virulence, we next set out to determine which virulence traits of the acute lytic cycle had evolved. We focused these efforts primarily on GT1 population B2 while analyzing changes in B4 or polyclonal line B0 on an *ad hoc* basis. We first tested the extracellular viability of GT1 during the course of lab-adaptation by subjecting B2 parasites to extracellular conditions for 0-10 hours and assessing viability hourly by plaque assay (Figure 2.2a). These temporal survival curves revealed a LT_{50} of only 2hrs for B2-P9 but a significant increase to 5.5hrs by B2-P211 (p-value <0.05), which is identical to the lab-adapted RH strain (Figure 2.2a). Measuring the area under these survival curves indicated that GT1's overall extracellular viability significantly increases 2.21-fold (p-value ≤0.01) by B2-P211, relative to B2-P13 (Figure 2.2b). Thus, extracellular survival is a major selective pressure intrinsic to laboratory conditions and is thereby a lab-adaptive trait in GT1 parasites.

Given this fast adaptation of extracellular survival capacity, we wondered when during lab-adaptation could such a strong selection pressure have been exerted onto the parasites. We hypothesized that while serial passaging from flask to flask, the inoculated parasites might spend significant time extracellularly while settling by gravity on the new host monolayer. To test this, we determined the kinetics of this settling process by plaque assay using RH parasites. Indeed, only ~50% of RH parasites settled onto a new T25 host cell monolayer 8hrs after passing (Figure 2.2c). Moreover, even after 24 hrs not all parasites that have plaque forming capacity had settled yet (Figure 2.2c). Thus, standard laboratory cultivation instills a significant selection pressure on non-lab-adapted extracellular parasites, resulting in *T. gondii's* evolved extracellular capabilities.

2.2.4. Lab-adaptation of GT1 results in enhanced invasion efficiency

The second virulence trait we tested for adaptability is invasion efficiency. We determined invasion efficiency by measuring the number of plaques, relative to inoculum size, immediately following mechanical release from host cells (as to avoid any bias of extracellular stress upon natural egress). All GT1 populations measured showed a steady increase in reinvasion efficiency over time (Figure 2.3). B0 displayed a 2.76-fold increase (p-value ≤ 0.05) in reinvasion efficiency by B0-P219, relative to B0-P15 (Figure 2.3a); B2 displayed 2.24-fold increase (p-value ≤ 0.01) in reinvasion efficiency by B2-P223, relative to B2-P12 (Figure 2.3b); and B4 displayed 2.27-fold increase in reinvasion efficiency by B4-P204, relative to B4-P10 (Figure 2.3c; n=2 so statistics was not performed). Interestingly, reinvasion efficiency of RH remains 2.24- to 2.77-fold larger than all >P200 GT1 populations, suggesting that longer adaptation is needed for GT1 to be comparable

to RH in this regard (Figure 2.3). Nevertheless, these data indicate that reinvasion efficiency is also a highly lab-adaptive virulence trait in *T. gondii*.

2.2.5. Lab-adaptation of GT1 does not result in altered replication efficiency

To test the contribution of enhanced replication rate during lab-adaptation, we enumerated the number of parasites per vacuole at 24hrs following synchronized invasion for 10 mins. During the course of lab-adaptation, GT1's replication rate shows an extremely modest, at best, change in replication rate by having slightly fewer 4-parasite and slightly more 8-parasite-containing vacuoles at B2-P157, and more 8-parasite-containing vacuoles at B2-P268, relative to B2-P16 (p-value ≤ 0.05) (Figure 2.4a). Expressed as the number of doubling events after 24hrs of replication revealed no significant changes in B2's replication during the course of lab-adaptation (Figure 2.4b). Similar results were observed for GT1 population B4, with little changes in the number of parasites per vacuole after 24hrs of invasion, albeit a small yet significant decrease in the number of vacuoles containing 16 parasites at B4-P260 relative to B4-P16 (p-value ≤ 0.05) (Figure 2.4c). Overall, these data show little phenotypic enhancement, indicating intracellular replication as a very weak lab-adaptive trait in our GT1 study.

2.2.6. Lab-adaptation of GT1 does not result in altered egress efficiency

Lastly, to complete the lytic cycle, we performed egress assays on 30-hour intracellular B2-P12, B2-P83, and B2-P211 parasites using the Ca^{2+} ionophore, A23187, or ethanol (EtOH) to stimulate egress for five minutes (Figure 2.4d). Counting the number of egressed vs non-egressed vacuoles by IFA reveals no significant differences in GT1's

ability to egress during its two years of lab-adaptation (Figure 2.4d). Therefore, it appears that egress efficiency is another intracellular virulence trait that is not lab-adaptive under standard laboratory conditions.

2.3. Discussion

We utilized RH as the golden standard for lab-adaptation because it has been cultured the longest and, moreover, plaque assays of three cryogenically frozen RH populations over a 32-year period show no major change in RH's *in vitro* virulence, indicating that RH has maximized its adaptation to an *in vitro* niche [168]. Indeed, after 2 years of lab-adaptation (~1500 generations), GT1 displayed significant enhancement in various phenotypes. Overall, GT1's *in vitro* virulence increased 1.86- to 2.3-fold, indicating that the parasite was capable of optimizing its lytic cycle to the cell culture conditions. Notably, this was observed in both human and goat cell fibroblasts, indicating that *in vitro* adaptation had proceeded in a host-independent manner and outside the context of an *in vivo* immune response. Paralleling our observations, increased *in vivo* virulence has been observed in *Plasmodium* when serially passaged in immune-suppressed mice, suggesting that immune-deficient milieus can facilitate the evolution of virulence [252].

Interestingly, it appears that extracellular virulence traits were the most affected while intracellular virulence traits were largely unaffected. Extracellular survival is probably the least studied trait of the lytic cycle, likely because it is still debated whether or not extracellular parasites can actually disseminate through the body via the bloodstream. Circulating extracellular tachyzoites have been found in the blood stream of infected mice, rats, and humans, suggesting the possibility of extracellular dissemination [254-256]. However, it is well known that tachyzoites disseminate to the peripheral tissues by invading and hijacking the driver's seat of dendritic cells, macrophages, leukocytes, and monocytes (i.e. phagocytes) and induce hypermotility [15, 257-260]. This "trojan

horse" mode of dissemination appears to be preferential for type II and III parasites, as type I (e.g. RH) do not invade as many phagocytes or induce as robust of a hypermotility phenotype [261]. Furthermore, *ex vivo* experiments reveal that extracellular RH tachyzoites have a superior migratory capacity compared to type II and III parasites, with demonstrated abilities to transmigrate across mouse intestines and penetrate the vascular endothelium [173]. Additionally, *in vitro* microfluidic analysis shows that shear forces, which mimic blood flow, enhance the ability of extracellular RH tachyzoites to glide across and invade endothelial cells [72]. Lastly, following i.p. injection in mice, RH exhibits a more dramatic expansion of parasite biomass in more distant organs compared to type II tachyzoites [262]. Taken together, it would appear that extracellular dissemination via the bloodstream for RH is possible. Our studies have revealed that extracellular viability is a lab-adaptive trait in *T. gondii* which leads us to hypothesize that lab-adaptation of RH has resulted in prolonged extracellular viability, thereby supporting RH's presumed extracellular dissemination to peripheral tissue via the bloodstream. This hypothesis is supported by the fact that type II tachyzoites, which have severely reduced extracellular viability, disseminate to the peripheral tissues of mice almost exclusively through intracellular modes [70]. While further experiments with non-lab-adapted type I parasites are needed to support this hypothesis, it would surely indicate that this overlooked phenotype is a major influence of parasite virulence both *in vitro* and *in vivo.*

Reinvasion efficiency was GT1's fastest lab-adaptive trait, with GT1 being statistically similar to RH by B0-P219 and B2-P163, and, although there is a slight reduction in the phenotype at B2 >P200, the phenotype remains more than 2.2-fold higher than its non-lab-adapted ancestor. It is likely that slightly longer lab-adaptation will result

in phenotypically stable population akin to that of RH. We aimed to reduce the effects of extracellular stress during our reinvasion assays by synchronously needle lysing parasites from their hosts rather than letting parasites egress in a naturally asynchronous way. We also tried to centrifuge parasites onto monolayers, however, this resulted in uneven distribution of parasite settling and resulting plaque formation. Therefore, we let parasites settle by gravity, as performed with serial passaging, albeit in less depth of media than during serial passaging as to reduce the settling time.

Interestingly, intracellular virulence traits of the lytic cycle were relatively static during lab-adaptation. Since egress efficiency was no different between RH and non-lab-adapted GT1, it was expected to see no evolution of GT1's egress efficiency during lab-adaptation. However, considering RH has a faster replication rate than non-lab-adaptive GT1, we were surprised to observe no major shift in GT1's replication rate during lab-adaptation. This suggests that the selection pressure for faster replication is limited under our laboratory conditions possibly due to competition with other phenotypically enhanced populations or to purge selection during the selective extracellular milieu that is intrinsic to serial passaging. As the population adapts to extracellular selection, the purge would be mitigated and natural selection will favor the evolution of other directional phenotypes like replication rate. Thus we hypothesize that replication rate of GT1 will eventually evolve toward RH now that extracellular viability of GT1 has evolved.

Figure 2.1. Lab-adaptation of GT1 results in enhanced *in vitro* virulence in a host-independent manner

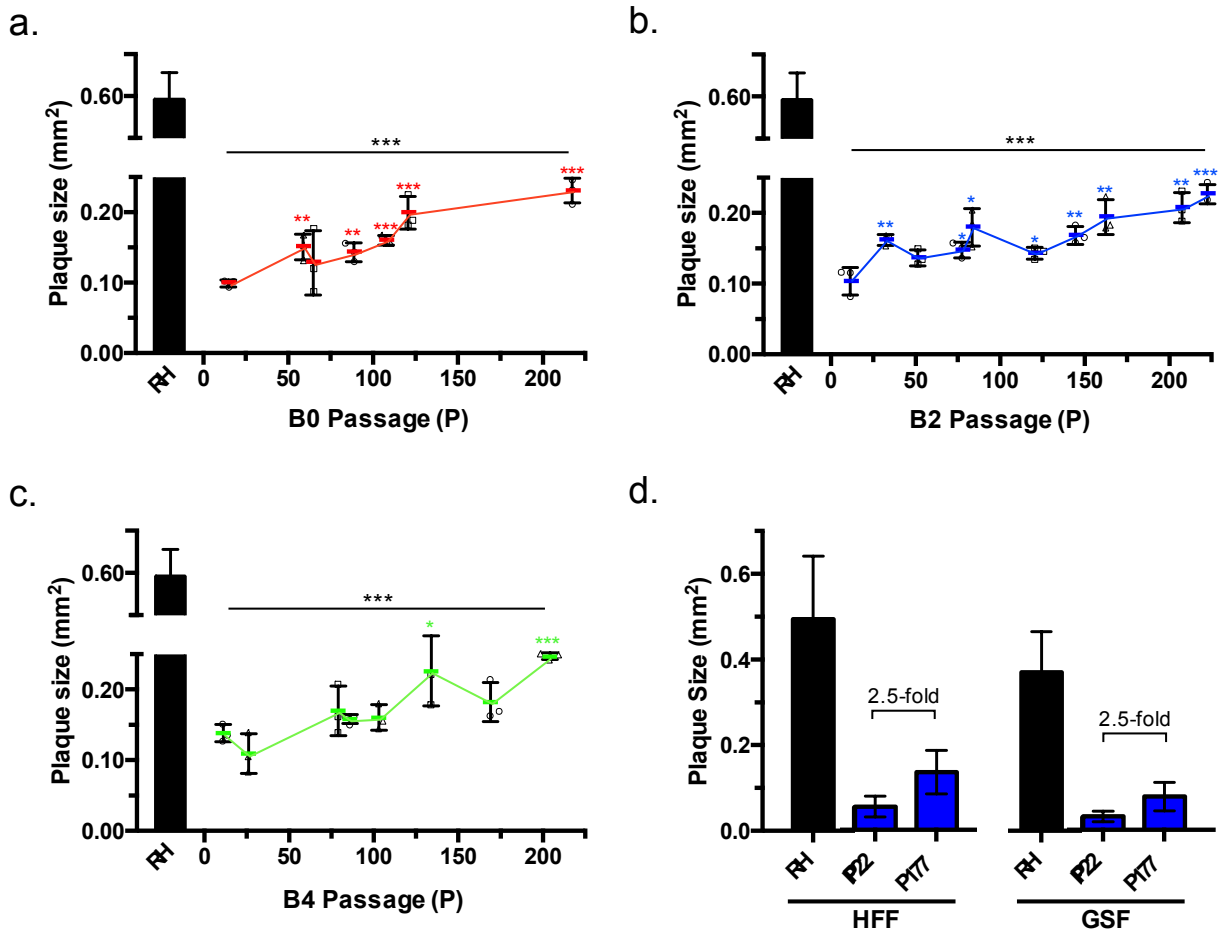


Figure 2.1. Lab-adaptation of GT1 results in enhanced *in vitro* virulence in a host-independent manner

Plaque size was quantified after 11-day plaque assays in order to assess the *in vitro* virulence of RH and of parallel-evolving **a.)** B0, **b.)** B2, and **c.)** B4 GT1 populations over the course of lab-adaptation. Black asterisks (*) indicate the *p*-value of the indicated GT1 passages relative to RH; colored asterisks (*, *, *) indicates the *p*-value of the indicated GT1 passage relative to the respective population's earliest passage. * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. Error bars indicate standard deviation of biological replicates. Colored block indicates mean of biological replicates. Number of biological replicates are indicated in the plot by symbols. One biological replicate is the mean quantification of ≥25 plaques. **d.)** Plaque size was quantified after 11-day plaque assays in order to assess the *in vitro* virulence of RH and B2 in the context human foreskin fibroblasts (HFF) or goat skeletal fibroblast (GSF) host cells. Error bars indicate standard deviation of ≥30 plaques within one biological replicate.

Figure 2.2. Lab-adaptation of GT1 results in enhanced extracellular survival

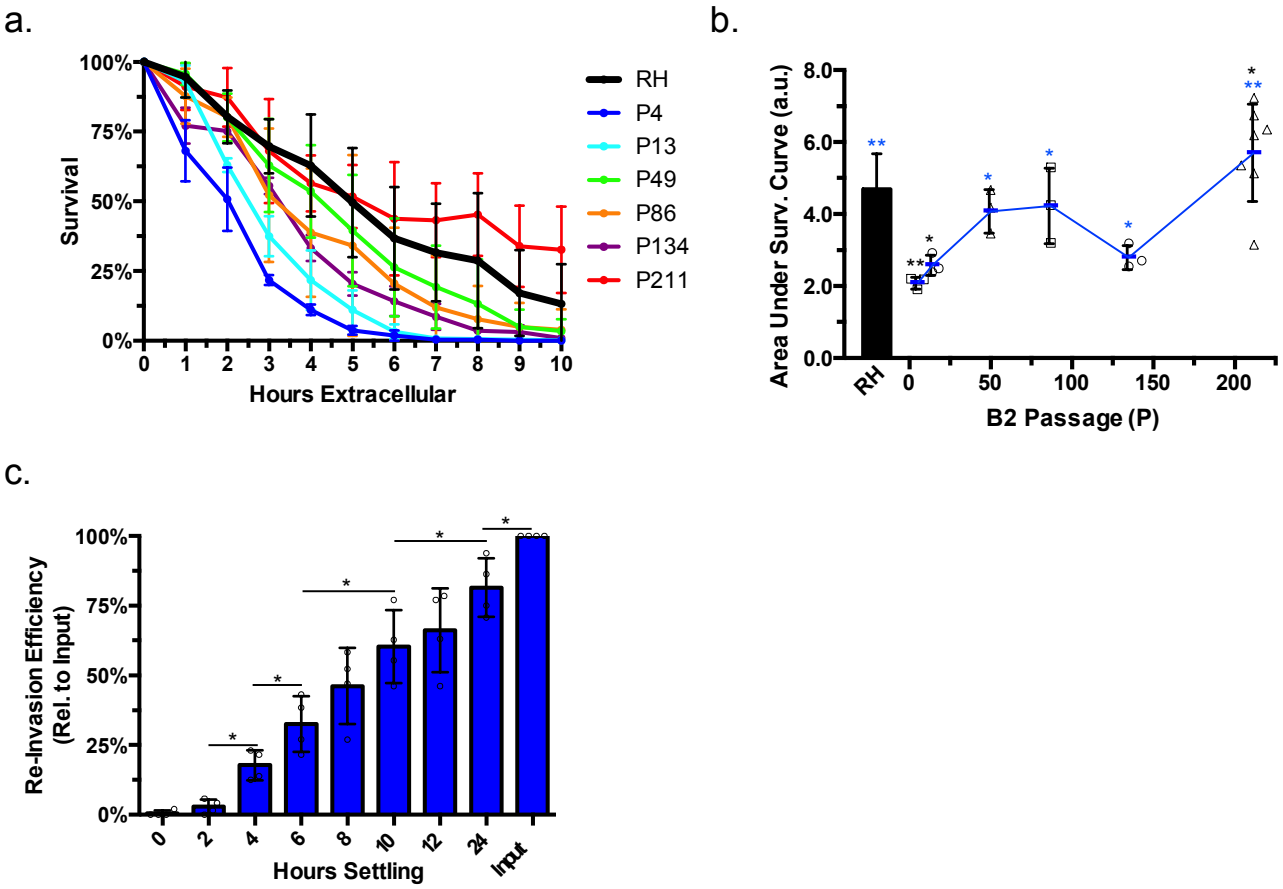


Figure 2.2. Lab-adaptation of GT1 results in enhanced extracellular survival

Parasites mechanically released from their host cells were incubated without a host for 0-10hrs following an 11-day plaque assay to determine parasite viability. **a.)** The number of plaques quantified at the given hour, relative to 0hr, determined the percent survival. **b.)** The area under the survival curves in **a.** was quantified to determine the overall relative viability of parasites during the entire 10hr assay. a.u. = arbitrary units. **c.)** Mechanically egressed parasites were immediately inoculated into T25 flasks containing host cell monolayers and allowed to settle onto monolayers for the given times, upon which flasks were vigorously washed to remove unsettled parasites and incubated for 11-day plaque assays. The number of plaques, relative to the unwashed "input", determined the number of parasites that strongly attached and/or invaded the host cell monolayer. Error bars indicate standard deviation of ≥3 biological replicates, containing ≥2 technical replicates; number of biological replicates are indicated in the plots by symbols.

Figure 2.3. Lab-adaptation of GT1 results in enhanced reinvasion efficiency

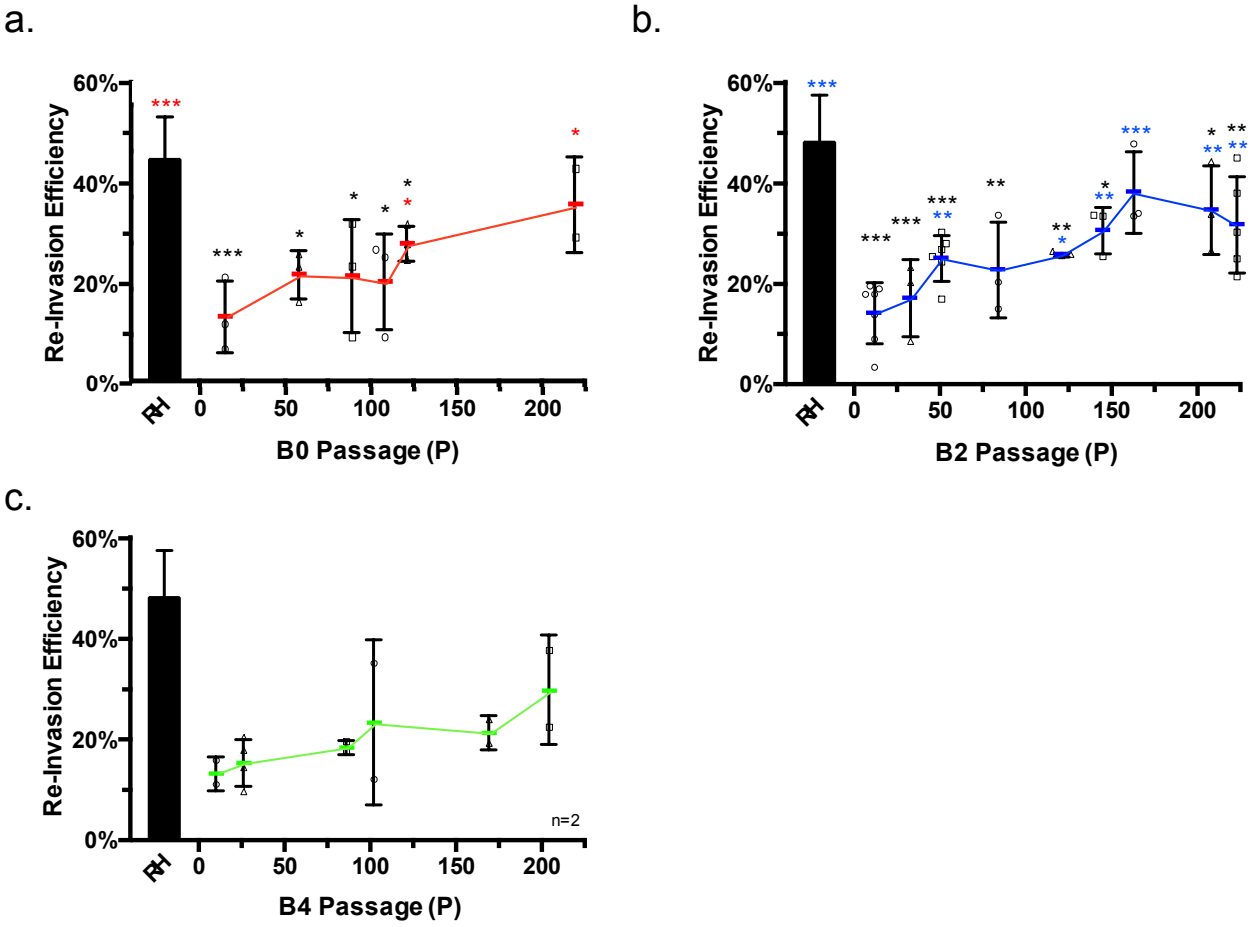


Figure 2.3. Lab-adaptation of GT1 results in enhanced reinvasion efficiency

A known number (input) of mechanically released RH and parallel-evolving **a.)** B0, **b.)** B2, and **c.)** B4 GT1 parasites were immediately inoculated onto host cell monolayers for reinvasion followed by 11-day plaque assay. The number of formed plaques, relative to input, was quantified in order to yield the parasite's reinvasion efficiency. Black asterisks (*) indicate the *p*-value of the indicated GT1 passage relative to RH; colored asterisks (*, *, *) indicates the *p*-value of the indicated GT1 passage relative to the respective population's earliest passage; * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$; error bars indicate standard deviation of ≥3 biological replicates; number of biological replicates are indicated in the plot by symbols, each containing ≥2 technical replicates.

Figure 2.4. Intracellular virulence traits are not enhanced during lab-adaptation

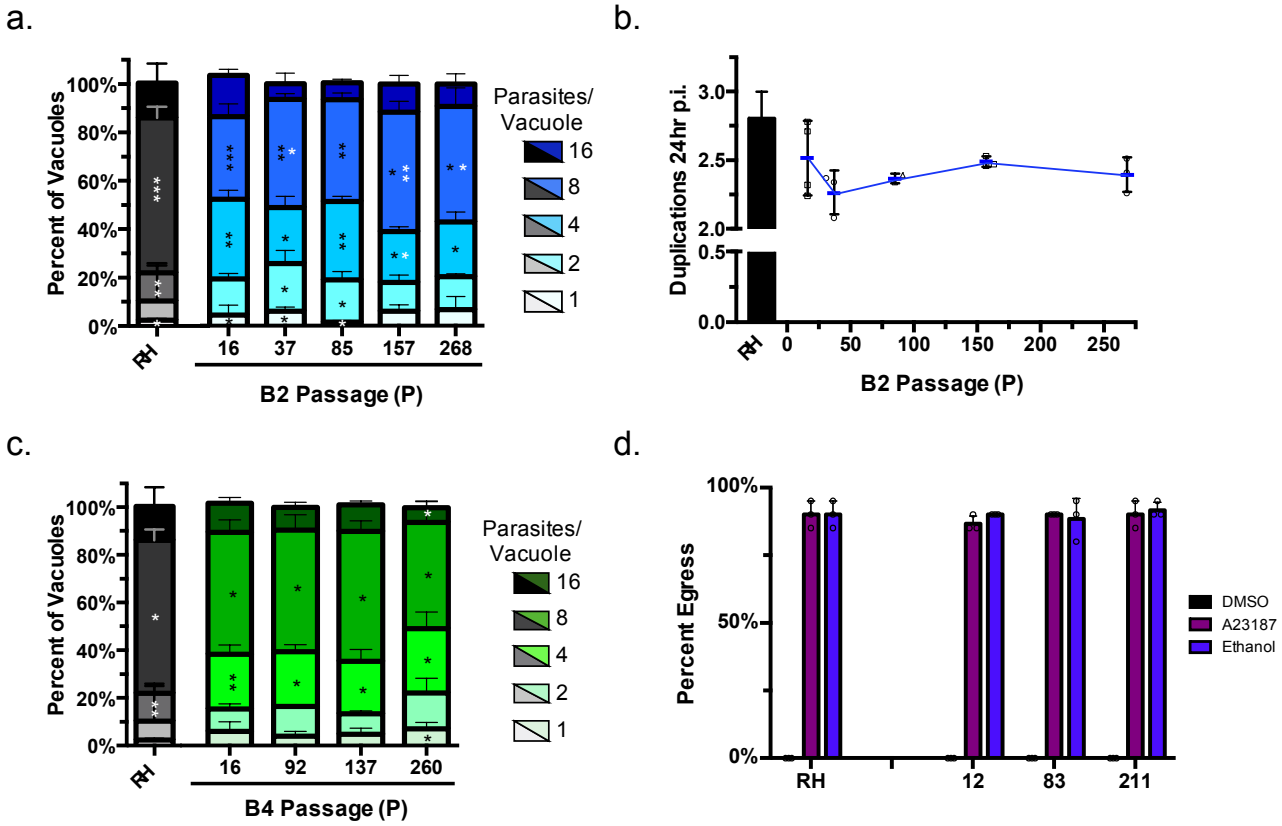


Figure 2.4. Intracellular virulence traits are not enhanced during lab-adaptation

a.) RH and GT1-B2 parasites mechanically released from their host cells were allowed to invade host cells for 10 mins following 24hrs of incubation. The number of parasites per vacuole were observed by IFA and quantified in 100 vacuoles at random fields. **b.)** From the same dataset as **a.**, the average number of duplication events was calculated for RH and GT1-B2. Biological replicates are indicated by symbols. **c.)** Mechanically egressed RH and GT1-B4 parasites were allowed to invade host cells for 10 mins following 24hrs of incubation. The number of parasites per vacuole were observed by IFA and quantified in 100 vacuoles at random fields. **d.)** 30-hr intracellular parasites were treated for five minutes with either DMSO (control), calcium ionophore (A23187), or Ethanol (EtOH). The number of egressed and non-egressed vacuoles was enumerated by IFA; error bars indicate standard deviation of ≥3 biological replicates, each containing ≥2 technical replicates.

Chapter 3: Whole genome resequencing of GT1 during lab-adaptation identified few genomic mutations

3.1. Introduction

To identify the genetic basis underlying GT1's observed phenotypic changes during lab-adaptation (Chapter 2), we performed 150bp paired-end WGS on RH and several GT1 lines (designated B0, B2, and B4) over time (i.e. passages). Previous WGS studies identified 1,394 SNPs/indels between RH and GT1 using reference-guided sequence alignment and analysis [183]. However, these short-read sequencing data rely heavily on an accurately assembled reference genome, for which the GT1 reference genome, comprising 14 chromosomes, is incomplete and assembled into 302 contigs. Therefore, to circumvent the limitations of an incomplete reference genome, we utilized a reference-free variant discovery tool, RUFUS, developed by the Marth lab (https://github.com/jandrewrfarrell/RUFUS) [263]. RUFUS directly compares the k-mer reads from two samples without the need to align reads to a reference genome, thereby eliminating reference biases such as misaligned k-mers, poor reference coverage, poor scaffold assembly, and false positive variants, all without sacrificing sensitivity [263].

Surprisingly, we identified little accumulation of fixed genomic mutations in the evolving populations. This suggests that the basis of phenotypic changes, in contrast to our hypothesis, is not buttressed by changes in CDS or epistatic interactions. Sequencing individual clones from a population half way through the lab-adaptation experiment uncovered several genomic mutations, indicating that random genomic evolution does occur, but they did not fix in the population within 1000 generations. Only a single gene (TGGT1_245510), a P4 phospholipid-translocating P-type ATPase (P4-flippase), independently accumulated two different non-synonymous SNPs that fixed in the population: an L270R in the B2 evolving line and A477D in the B6 evolving line. In both

populations the mutations became fixed in the population before P33 (~200 generations), suggesting they provided a key benefit in the early stages of lab-adaptation. Attempts to directly test the impact of this mutation on GT1 fitness using CRISPR/Cas9-mediated genetic engineering were unable to generate recombinant parasites with the desired SNP, hampering our ability to verify its effect on lab-adaptation. However, we did recover a P4-flippase frameshift mutant resulting in a premature stop codon. This frameshift mutant (ΔP4-Flippase) displayed a dramatic reduction in *in vitro* virulence, suggesting that this gene is critical to the parasite and that the observed mutations are likely beneficial to GT1 parasites during lab-adaption.

3.2. Results

3.2.1. Genetic comparison of RH (lab-adapted) and GT1 (non-lab-adapted) parasites

First, we compared WGS data of all low passaged (≤P15) GT1 samples to RH using RUFUS in order to establish the baseline of population complexity and clone-to-clone variation. In total, 207 SNPs/indels were identified, with 69 causing changes to a genes CDS (Table 3.1). Of those mutations that caused protein coding changes, 55 were non-synonymous and 14 were synonymous. Furthermore, several of these mutations are in common with previous analysis, including the polymorphic virulence factor GRA2 that is associated with immune evasion [183]. Additionally, 15 previously identified mutations were confirmed in our strains by PCR-sequencing (Table 3.2) [183]. Taken together, these results refine previous comparative analyses and identify significantly fewer, higher confidence mutations that may explain the phenotypic differences observed between the RH (lab-adapted) and GT1 (non-lab-adapted) strains.

3.2.2. Parallel resequencing identified two mutations within a P4-flippase

Resequencing was performed with Illumina's Next Generation Sequencing (NGS) platform at several time points amongst three parallel-evolving GT1 populations (B0, B2, and B4), next to RH as control (Figure 1.4). RUFUS-based WGS analysis across the evolving GT1 populations provided the identity and allele frequency of high-quality polymorphisms within the entire genome. To asses clonality of our starting GT1 populations, we first compared the starting population (B0-P4) and three clonal populations (B2-P15, B4-P15, and B6-P10) by RUFUS. Remarkably, no mutational

differences were identified between any these samples, indicating strong clonality within all of our GT1 populations at the beginning of the experiment (data not shown).

The evolving B4 population was sequenced at four passages, P15, P32, P79, and P105 (~802 generations), for which we observed two mutations fixing in the population (Figure 3.1a). The first mutation mapped to the 13th intron of TGGT1_203135 (annotated as a dynein heavy chain) and fixed within the population by P32. The second mutation is in the same intron and also fixed by P32. Interestingly, one of these intronic mutations displayed a dynamic evolution by disappearing at B4-P79 and remerging by B4-P105 (Figure 3.1a).

The evolving GT1 B2 population was sequenced at passages P15, P52, P86, P120, and P135, spanning about 1000 generations. Surprisingly, only one non-synonymous mutation, L270R, emerged within the 2nd exon of TGGT1_245510, a phospholipid-translocating P-type ATPase (P4-flippase) gene, and remained fixed within the entire population by B2-P52 (Figure 3.1b).

The evolving GT1 B6 population was sequenced at only two passages, P10 and P33, spanning ~305 generations, for which one non-synonymous mutation, A477D, emerged within the same P4-flippase gene and fixed within the population by B6-P33 (Figure 3.1c). Interestingly, the A477D mutation identified in population B6 was just 620bp downstream of the L270R mutation identified in population B2. To finer map when and how fast these mutations occurred in the population, we PCR amplified the L270R and A477D genomic regions by PCR and Sanger sequenced the amplicons at additional passages recovered from cryopreserved samples. The chromatogram revealed that the L270R mutation emerged as early as B2-P20, when we observe a 50:50 distribution, and

fixed within the population by B2-P32 (Figure 3.2a). Similar resolution of the A477D mutation was confirmed by B6-P33 (Figure 3.2b), revealing that both mutations emerged within ~200 generations of lab-adaptation.

In order to identify additional mutations, estimate the mutation rate for GT1, and gauge the genetic complexity of GT1 during lab-adaptation, we performed WGS with five clones derived from B2-P86 (Figure 3.1b). As expected, the L270R P4-flippase mutation was found in all sequenced clones (Figure 3.1b). Additionally, 17 mutations were identified amongst the five clones, illustrating the presence of a complex population structure during lab-adaptation and supporting genomic evolution in GT1; however, none of these additional mutations fixed within the population by P135 (Figure 3.1b). Taken together, as expected, we see evidence of random mutations occurring in individual parasites and generating a complex population, however, only one mutation fixed in the B2 or B6 population suggesting that, germane to fitness, most mutations are near neutral or deleterious or hindered by genetic drift.

3.2.3. ΔP4-flippase significantly reduced *in vitro* virulence

Discovering two different non-synonymous mutations in the same gene around the same time across two parallel evolving lines strongly suggest that these mutations confer critical fitness benefits for GT1 during lab-adaptation [237, 264]. In addition, it highlights this gene's candidacy as a novel polymorphic *in vitro* virulence factor. To test whether this gene is a driver of lab-adaptation, we aimed to revert the L270R mutation back to its wild type allele (270L) in GT1 population B2-P86 clone 1 using CRISPR/Cas9-mediated genetic engineering. Briefly, two plasmids containing a Cas9-mNeonGreen fusion

targeting either the upstream or downstream regions of the SNP were co-transfected along with a 2,258bp synthesized cDNA repair template (spanning 4 exons) containing the wild type allele and approximately 600bp arms complementary to the site of double stranded breaks (DSB) in order to allow for homologous recombination (Figure 3.3a). FACS sorting was utilized to enrich mNeonGreen+ (transfected) and mNeonGreen- (untransfected) parasites (Figure 3.3b). Unfortunately, subsequent single cell cloning and screening by PCR-sequencing identified no recombinant parasites with the allele of interest. We did, however, inadvertently generate a clonal frameshift mutant resulting in two 5' premature stop codons (ΔP4-flippase) (Figure 3.4). Serendipitously, we observed severe growth defects in ΔP4-flippase, resulting in a 0.12-fold decrease in GT1's *in vitro* virulence (Figure 3.4). While we were unsuccessful in characterizing the functionality of our candidate SNPs, our ΔP4-flippase frameshift mutant suggests, at the very least, that our candidate mutations do not result in a complete loss-of-function of the gene, thereby supporting our hypothesis that R270L and A477D are beneficial mutations for low passage GT1. Future experiments will be needed to validate this hypothesis (see Chapter 5.2.1.).

3.2.4 Estimating GT1's mutation rate

By sequencing five clonal populations at two timepoints 520 generations (71 passages) apart (B2-P15 and B2-P86) (Figure 32.b), we determined the mutation rate of GT1 to vary between 6.01 $x10^{-11}$ and 2.71 $x10^{-10}$, with an average mutation rate of 1.14 $x10^{-10}$ mutations/bp/generation. Recently, estimations of RH's average mutation rate has been estimated to be ~5.8 $x10^{-11}$ mutations/bp/generation, which is within the

range of our observed mutation rate with GT1 [265]. To put RH and GT1's average mutation rate into context, this is similar to observing roughly 2-4% of a *T. gondii* population accumulating a single mutation within a single passage (2-3 days) under standard laboratory conditions (assuming a 64 Mb genome and our calculated of 5.8 generations per passage).

3.3. Discussion

Our data on the P4-flippase gene suggest its expression is critical for *in vitro* parasite fitness. Typically, P4-flippase proteins are membrane transporters of cations, heavy metals, and particularly, phospholipids across lipid bilayer leaflets in order to maintain lipid homeostasis [266]. Phospholipid flipping activity is of great biological importance for the biogenesis of vesicles [267, 268], creating a fusion-competent bilayer [269], maintaining membrane stability [270, 271], and generating signaling cues [272-274]. These typical functions indicate that the changes in phospholipid flippase activity could contribute access to exogenous phospholipids that confer a competitive edge over parasites missing this mutation. Predictive structural analysis (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index) suggests that the L270R mutation is located within a loop that connects two transduction domains and the mutant allele results in a 72 Å decrease in local cavity space. Similar analysis with the A477D mutation predicts its location to be within the alpha helix of an ATPase transmembrane domain. The significant reduction in plaque size of our frameshift mutant suggest that loss-of-function of P4-flippase will severely reduce parasite fitness; previous KO of P4-flippase in a genome-wide CRISPR study suggests the same essentiality [224]. With this in mind, and because we observe an early fixation of both mutations (~200 generations), these mutations could possibly bestow beneficial functional changes to the flippase activity, likely as an adaptation in response to some limited factor in the *in vitro* environment (e.g. lipids).

Additional functional information can be gained from the subcellular localization of the P4-flippase. Several attempts to tag this protein by insertion of a C-terminal tag in the

genomic locus were unsuccessful (data not shown). However, localization insights can be gleaned from a recent Hyperplexed Localization of Organelle Proteins by Isotope Tagging (hyper-LOPIT) study, which assigned P4-flippase to the Golgi apparatus and plasma membrane fractions [275], suggesting that access to environmental lipids is a putative mechanism on how the mutations could confer competitive advantage.

Resequencing of GT1 population B4 also identified 1 gene of interest that evolved two mutations that are 15 bp away from each other within the 13th intron of a dynein heavy chain family protein (TGGT1_203135). While these intronic mutations might indicate splice variants, we decided not to investigate them due to the extremely low expression (FPKM <0.60) of this gene in all of our samples, and the more than >30-fold increased expression of this gene observed during late merogony in the cat [276], which taken together indicate this gene is likely biologically irrelevant during the tachyzoite stage. Moreover, the high 87% A-T-richness surrounding this mutation make its identification and allele frequency calling unreliable (Figure 3.1b).

Sequencing of clonal B2-P15, B4-P15, and B6-P10 GT1 populations reveals no genetic differences between these three strains (data not shown), indicating strong clonality when we started our lab-adaptation experiment. Roughly mid-way through our study, we resequenced five clones derived from B2-P86 in order to assess genetic complexity of the evolving B2 population. Interestingly, we identify several low-level mutations that are found within clones 3, 4, and 5, but not detected in the polyclonal population, consistent with the notion that mutation accumulation leads to an array of very low-level sub-populations that must overcome genetic drift and clonal interference in order to emerge from natural selection and fix within the entire population [229]. In the

case of clones 3, 4, and 5, they remain largely undetected in the population by B2-P135, indicating these mutations as non-beneficial or were lost to genetic drift. Clone 1 appears to have emerged as the dominant population, with clone 2 making up ~20% of the population by B2-P135, indicating some of these mutations as potentially beneficial. Considering that the B2-P86 clones evolved anywhere from one to nine mutations during 71 passages (~520 generations), relative to the starting clone (B2-P15), we estimated *T. gondii's* mutation rate to be between 6.01 $x10^{-11}$ and 2.71 $x10^{-10}$, with an average mutation rate of 1.14 $x10^{-10}$ mutations/bp/generation. This mutation rate is similar to that of other single-celled organisms [277], such as *S. cerevisiae* (3.3 $x10^{-10}$) [278] and *S. pombe* (2.0 $x10^{-10}$) [279] and *E. coli* (1.6 $x10^{-10}$) [235]. Interestingly, this mutation rate is about 15- to 30-fold lower than what has been calculated for *P. falciparum* (1.7 $x10^{-9}$) [280], suggesting that there is likely a range of *in vitro* selection pressures and adaptive mechanisms for various apicomplexan parasites in culture.

Although E&R has been successfully applied to a variety of organisms, including *Plasmodium* and *Toxoplasma*, to identify the genetic basis of drug resistance [244, 245, 265], our experiments shows that gradual and relatively fast changes in phenotype do not completely correlate with the fixation of genomic mutations in parasites. Hence, the molecular basis of the lab-adaptive phenotypes identified in our experiments must stem from other mechanisms not yet explored (e.g. epigenetic).

Figure 3.1. WGS identifies few genomic mutations in GT1 during lab-adaptation

a.

~802 generations

Gene ID	Chrom.	Position	Codon/NT	Amino Acid	RH	GT1 B4				Product Description
						P15	P32	P79	P105	
TGGT1_203135	VIIa	2687693	CAG -> TTT	Intronic	1.00	0.00	1.00	1.00	1.00	dynein heavy chain family protein
TGGT1_203135	VIIa	2687677	G -> A	Intronic	1.00	0.00	1.00	0.00	1.00	dynein heavy chain family protein

ATAAATTTTTTTTTAAAAAATATAATATTAATTACGCTAAATTATTATTAACAAATTCAAATTTTTGTAATATCATTATAGATTTAAACTTAAAACAAAA
TTTTGTTTTAAGTTTAAATCTATAATGATATTACAAAAAGACAAATTTGTTAATAATGTTTTAGCGTAATTAATATTATATTTTTAAAAAAAAATTTAT

b.

~673 generations ... | GT1 B2-P86 clones | ... 1000 generations

Gene ID	Chrom.	Position	Codon/NT	Amino Acid	RH	GT1 B2			Clone 1	Clone 2	Clone 3	Clone 4	Clone 5	GT1 B2		Product Description
						P15	P52	P86						P120	P135	
TGGT1_245510	XII	2277419	CTG -> CGG	L270R	0.00	0.00	1.00	0.97	0.99	1.00	1.00	0.99	1.00	1.00	0.99	phospholipid-translocating P-type ATPase
TGGT1_234250	X	4144128	TCC -> TCT	S813S	0.00	0.00	0.00	0.02	0.00	1.00	0.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_265870A	IX	1218247	GGG -> GAG	G441E	0.00	0.00	0.00	0.47	0.39	1.00	0.00	0.00	0.00	0.12	0.27	pantoate-beta-alanine ligase
TGGT1_321410	Ib	1842756	TCT -> TGT	S442C	0.00	0.00	0.00	0.38	0.00	0.99	0.00	0.00	0.00	0.15	0.25	hypothetical protein
TGGT1_410050	V	1970981	G -> C	N/A	0.00	0.00	0.00	0.45	0.00	1.00	0.00	0.00	0.00	0.14	0.18	tRNA-Pro
TGGT1_253615	III	1043595	A -> G	Intronic	0.00	0.00	0.00	0.40	0.02	1.00	0.00	0.00	0.00	0.17	0.16	hypothetical protein
TGGT1_320000	IV	549609	G -> A	Intronic	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	SCY kinase (incomplete catalytic triad)
Intergenic	Ib	661732	G -> C	Intergenic	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	N/A
Intergenic	V	1825065	C -> T	Intergenic	0.00	0.00	0.00	0.40	0.36	1.00	0.00	0.00	0.00	0.15	0.29	N/A
TGGT1_215700	X	7003044	GTA -> GCA	V1134A	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	phosphatidylinositol 3- and 4-kinase
TGGT1_228070	X	528213	ACG -> ATG	T1220M	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_206550	VIIa	863356	C -> G	Intronic	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_237890	IV	2493525	T -> C	Intronic	0.01	0.00	0.06	0.02	0.03	0.00	0.99	0.03	0.00	0.00	0.04	calcium-dependent protein kinase CDPK4
Intergenic	XII	2972581	A -> C	Intergenic	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00	N/A
TGGT1_309760	XI	754634	CAG -> TAG	Q169*	0.00	0.00	0.04	0.33	0.00	0.00	0.00	1.00	0.00	0.35	0.04	hypothetical protein
Intergenic	XII	1269841	G -> G	Intergenic	0.00	0.00	0.00	0.00	0.00	0.02	0.00	1.00	0.00	0.00	0.00	N/A
Intergenic	V	2426180	A -> G	Intergenic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	N/A
Intergenic	XII	4886211	G -> C	Intergenic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	N/A

c.

~305 generations

Gene ID	Chrom.	Position	Codon/NT	Amino Acid	RH	GT1 B6		Product Description
						P10	P33	
TGGT1_245510	XII	2276797	GCC -> GAT	A477D	0.00	0.00	1.00	phospholipid-translocating P-type ATPase

Allele Frequency
0.00 0.50 1.00

Figure 3.1. WGS identifies few genomic mutations in GT1 during lab-adaptation

WGS was performed on RH and GT1 populations **a.)** B4, **b.)** B2, and **c.)** B6, alongside with five clones derived from B2-P86, at the various timepoints indicated. Low genetic complexity of the both B4 intronic mutations is shown in panel **a**. Allele frequencies, the percentage of reads with the indicated allele, are represented by the blue/yellow heat map. N/A indicates the gene does not result in a protein product.

Figure 3.2. Validation of P4-flippase mutations in GT1

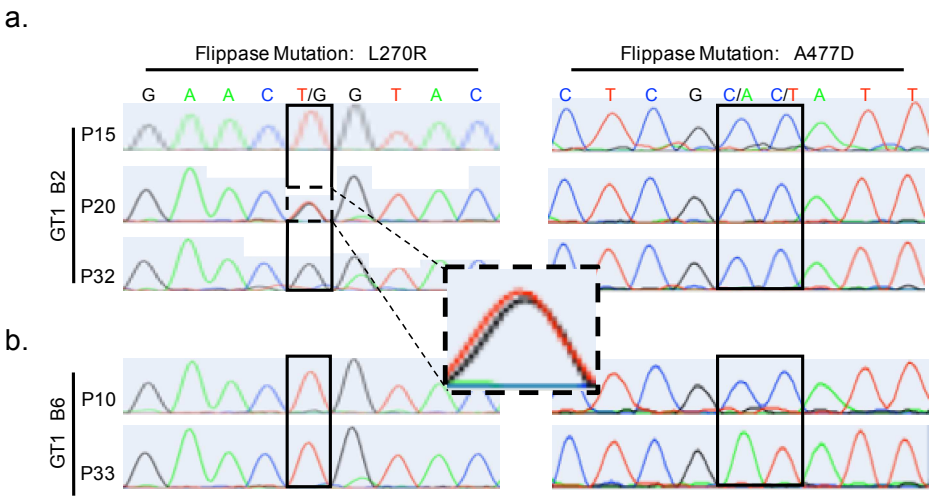


Figure 3.2. Validation of P4-flippase mutations in GT1

PCR-sequencing confirms the presence of the **a.)** L270R mutation in B2 GT1 population. The emergence of two alleles in the evolving population can be observed by P20, zoomed in panel. **b.)** PCR-sequencing confirms the presence of the A477D mutation in the B6 GT1 population.

Figure 3.3. P4-flippase allele swap strategy

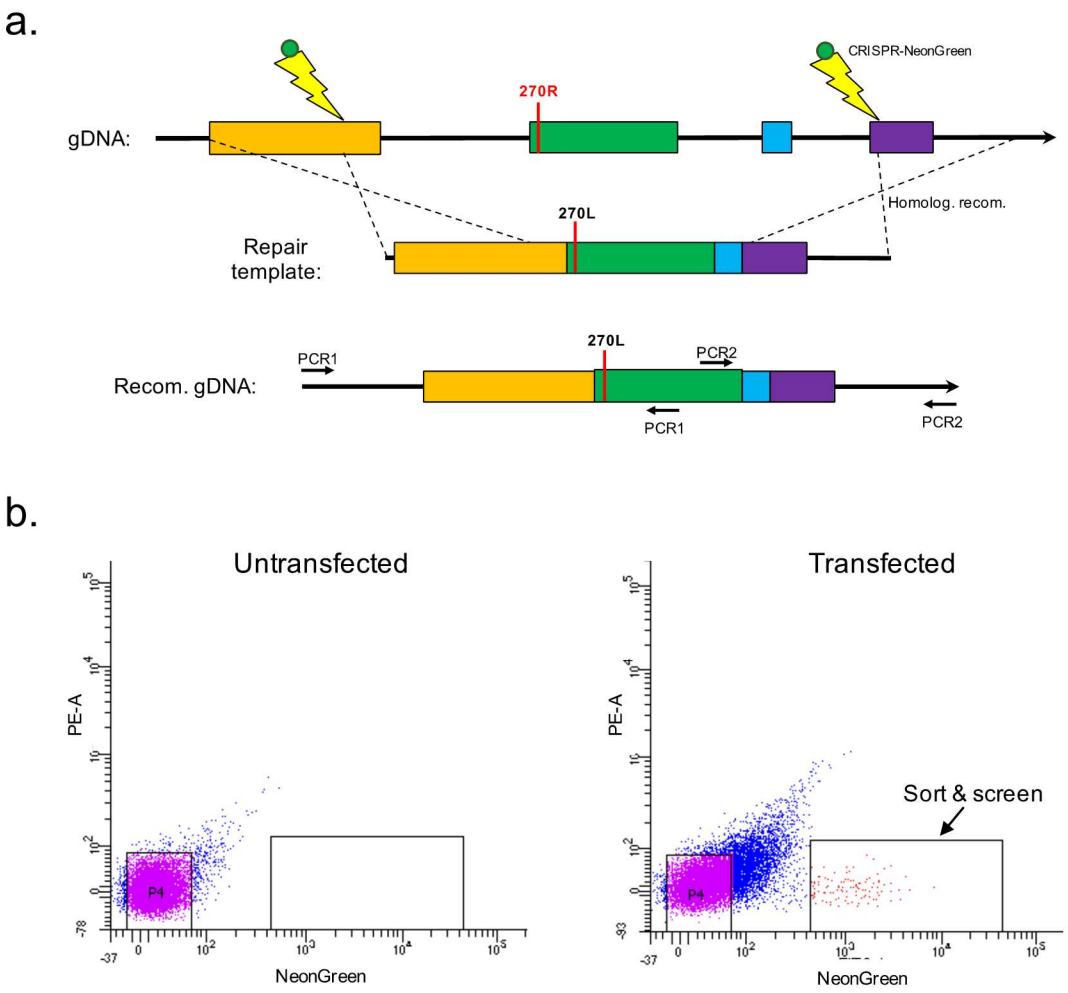


Figure 3.3. P4-flippase allele swap strategy

a.) Transfection of two Cas9-mNeonGreen plasmids encoding two different protospacers and a synthesized cDNA repair template containing the WT allele of the P4-flippase. Successful homologous recombination of the repair template will result in an allele swap in the recombinant parasites. Colored boxes represent exons; black line represents introns. **b.)** FACS-sorting of untransfected and transfected parasites in order to select for mNeonGreen+ parasites for subsequent genotyping.

Figure 3.4. P4-flippase frameshift mutant results in reduced *in vitro* virulence

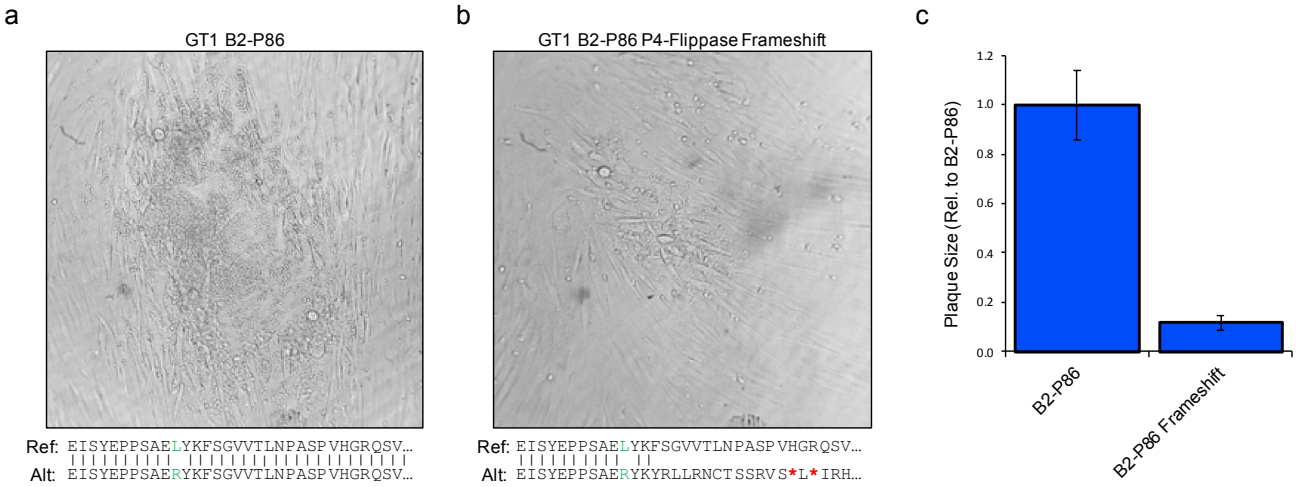


Figure 3.4. P4-flippase frameshift mutant results in reduced *in vitro* virulence

a.) Untransfected and **b.)** transfected B2-P86 parasites were screened for homologous recombination of the repair template but only resulted in the identification of a frameshift mutant containing two premature stop codons. **c.)** Quantification of plaque size reveals a dramatic reduction of *in vitro* virulence in the frameshift mutant.

Chapter 4: GT1 lab-adaptation results in modified transcriptional programs during the extracellular milieu

4.1. Introduction

Since genomic changes do not fully track with the phenotypic changes seen during GT1's lab-adaptation, we reasoned that the mechanistic basis might be due changes in the expression level of particular genes, which has been previously suggested [168]. Similar mechanisms have been observed in yeast adapting to salt stress [281, 282], *E. coli* adapting to limited glucose [236], and mammalian cells lab-adapting to culture conditions [283]. Interestingly, previous microarray analysis of RH, RH-JSR, and GT1 have identified many differences in gene expression, suggesting differential expression (DE) of genes (DEGs) as a molecular basis for phenotypic differences within *T. gondii's* type I lineage [168, 183].

To test this hypothesis, we performed mRNA-sequencing (RNA-seq) across several time points of the GT1 populations (Figure 1.4). Since the lytic cycle consists of intracellular and extracellular milieus, we sampled both intracellular and 6-hour extracellular parasites. Complementary to our phenotypic data (Chapter 2), most changes in gene expression occurred in extracellular parasites. Advanced analysis of these data identified 988 DEGs that trend up or down during 1500 generations of lab-adaptation, of which 319 strongly correlate in time with our phenotype data. Interestingly, the dataset largely reflects a pro-tachyzoite transcriptomic profile as many tachyzoite-related genes are upregulated and other zoite-related genes are down-regulated. We selected five upregulated DEGs for experimental validation, of which four DEGs demonstrated a significant impact on GT1's *in vitro* virulence. Furthermore, we utilized gene set enrichment analysis (GSEA), gene ontology enrichment analysis (GOEA), and metabolic pathway enrichment analysis (MPEA) to gather insights into some of the biological

processes that may be affected by lab-adaptation. The biological pathways displaying the most dramatic changes in gene expression pointed toward acetyl-CoA and *de novo* fatty acid (FA) biosynthesis within the apicoplast as key biological processes upregulated during lab-adaptation. Based on these data, we conclude that lab-adaptation of GT1 is likely anchored in epigenetic changes that promote tachyzoite virulence likely by enhanced access to FAs.

4.2. Results

4.2.1. Transcriptomic comparison of RH (lab-adapted) and GT1 (non-lab-adapted) parasites

To discern the differences in gene expression between lab-adapted and non-lab-adapted parasites, RNA-seq was first performed on asynchronously replicating, 30-hour intracellular and 6-hour extracellular RH and cloned B2-P11 and B4-P11 GT1 populations. Differential expression analysis (DEA) was used to identify DEGs between RH and low passage (<P12) GT1 populations. Genes were considered to be DE if the fold change (FC) was ≥2 and if the *q*-value was ≤0.05. DEA of intracellular parasites identified 545 DEGs between RH and B2-P11, of which 331 DEGs were upregulated and 214 DEGs were downregulated, relative to RH (Table 4.1). Similarly, DEA of intracellular parasites identified 764 DEGs between RH and B4-P11, of which 526 DEGs were upregulated and 238 DEGs were downregulated (Table 4.1). With both GT1 populations combined, intracellular B2-P11 and B4-P11 share 498/811 (~62%) DEGs with similar expression, relative to RH (Table 4.1).

DEA of extracellular parasites identified 927 DEGs between RH and B2-P11, of which 476 DEGs were upregulated and 451 DEGs were downregulated (Table 4.2). Similarly, DEA of extracellular parasites identified 955 DEGs between RH and B4-P11, of which 380 DEGs were upregulated and 575 DEGs were downregulated (Table 4.2). With both GT1 populations combined, extracellular B2-P11 and B4-P11 share 692/1190 (~58%) DEGs with similar expression, relative to RH (Table 4.2).

With a vast number of DEGs observed across the two type I strains, RH and GT1, we also aimed to assess the clonality of gene expression within the GT1 strain. Similar to

our DNA-seq analysis of clones, RNA-seq analysis indicates our starting GT1 population was highly clonal in its gene expression patterns because no significant DEGs were identified amongst B0-P7, B2-P11, and B4-P11 GT1 populations in neither intracellular nor extracellular samples (data not shown). Taken together, the above comparative data reveal that RH and GT1 are very different in terms of their gene expression patterns, both intracellularly and extracellularly, dramatically expanding upon our current knowledge of the transcriptomic differences previously characterized between the two isolates [168, 183], and supporting a hypothesis for differential gene expression as a molecular basis for phenotypic differences observed across *T. gondii*'s type I lineage.

4.2.2. Few transcriptomic changes are observed in intracellular parasites during GT1's lab-adaptation

To uncover changes in gene expression that could be responsible for lab-adaptive changes to GT1's phenotypes, RNA-seq was first performed on asynchronously replicating intracellular GT1-B2 parasites at three passages: P11, P84, and P148. DEA relative to B2-P11 only identified 12 DEGs, with many (9/12) being annotated as hypothetical (Table 4.3). These data indicate that the intracellular state of GT1 is likely not affected by lab-adaptation, or that major differences are within cell-cycle genes, of which the noise of an asynchronized population would obscure. Given that we did not observe any major differences in intracellular virulence traits (Figure 2.4), but did observe major difference in extracellular virulence traits (Figure 2.2 & 2.3), we next hypothesized that the extracellular transcriptomic state of GT1 likely changes during lab-adaptation. Indeed, we observed >80-fold more DEGs in our extracellular data set, and because we

largely observed augmentation of extracellular virulence traits (Chapter 2), we pursued further RNA-seq analysis with only our extracellular transcriptomic data sets.

4.2.3. Time course sequencing analysis of extracellular GT1 identified 988 DEGs that trend toward a tachyzoite-like profile during lab-adaptation

To uncover changes in gene expression that could be responsible for changes in GT1's phenotypes, RNA-seq was performed on extracellular GT1-B2 at several passages: P11, P35, P55, P85, P148, and P210. Because a vast number of DEGs were identified across several passages, we applied a novel RNA-seq analysis pipeline to process and interpret the temporal data (Figure 4.1, see methods). Following DEA, two subsequent analyses, 1) time course sequencing data analysis (TC-seq) and 2) regression analysis (RA), were utilized to interpret the expression data and identify candidate DE virulence factors (Figure 4.1). TC-seq identified 988 DEGs whose gene regulation showed a directional trend (Figure 4.2), with 437 DEGs trending up and 551 DEGs trending down over time (Table 4.4). Of those trending, nearly 55% are genes of unknown function, limiting our ability to characterize the biology of the entire data set. However, other comparative RNA-seq analyses of *T. gondii* at different life stages have dramatically expanded our repertoire of expression data, and allowed us to characterize gene sets in the context of life cycle stages (i.e. tachyzoite, bradyzoite, merozoite, and sporozoite) regardless if those genes are annotated or not. In other words, we can, for example, quantify, or score, how bradyzoite-like a particular list of genes are based off how these genes are differentially expressed in bradyzoites, relative to the three other life stages (Figure 4.3a). This novel method (see methods) of "stage scoring" data sets was

first validated by scoring previously published tachyzoite-, bradyzoite-, and sporozoite-associated lists of genes. Indeed, each list only scored significantly (*p*-value ≤0.05, bootstrap analysis) to their respective life stage, validating our scoring approach (Figure 4.3b-d). Stage scoring was applied to the 988 trending DEGs found during lab-adaptation in 6-hour extracellular GT1 parasites. Of the 437 DEGs that trend up, a significant number were found to be more tachyzoite- and bradyzoite-like (*p*-value ≤0.05, bootstrap analysis); and of the 551 DEGs that trend down, a significant number were found to be bradyzoite-, merozoite-, and sporozoite-like (Figure 4.4) (*p*-value ≤0.05, bootstrap analysis). These data suggest that lab-adaptation results in increased expression of tachyzoite-associated genes and repression of genes associated with other life stages during the extracellular milieu.

4.2.4. Enrichment analysis identified FA synthesis as a lab-adaptive biological process

With so many DEGs to interpret, and with 50% of our DEGs annotated as having an unknown function, we employed three types of statistical enrichment analyses (GSEA, GOEA, MPEA) in order to uncover biological insights into GT1's lab-adaptation. GSEA utilizes previously established lists of *T. gondii* genes that are associated with a particular biological state (G1, bradyzoites, etc.), process (e.g. invasion, metabolism, etc.) or organelle localization (ER, nucleus, etc.); GOEA utilizes a hierarchal classification system of genes (or gene products) that are organized into terms that describe the gene's molecular function (ribosome, Ca^{2+} binding, etc.) biological process (phosphorylation, translation, etc.), and cellular component (proteasome complex, nucleus, etc.); and

MPEA utilizes established molecular interaction/reaction/relation networks (glycolysis, TCA cycle, etc.) in order to contextualize a researcher's dataset.

GSEA identified the upregulation of genes associated with: the apicoplast, saturated FA biosynthesis, bradyzoites, GRA proteins, and more. GOEA identified the upregulation of genes associated with: the apicoplast, FA biosynthesis, oxidoreductase activity, ribosomal structure, FA biosynthesis, metabolic process, and many more. MPEA identified the upregulation of genes associated with: glycolysis, FA biosynthesis, pyruvate to acetyl-CoA biosynthesis, and many more (Figure 4.6).

Even though ~55% of our DEGs are annotated as hypothetical genes with unknown functions, one biological insight was distinct: FA biosynthesis. All three enrichment analyses of our upregulated RNA-seq data set highlight FA biosynthesis as an upregulated biological process in extracellular GT1 parasites during lab-adaptation; moreover, down regulation of FA degradation is also observed (Figure 4.7), supporting FA acquisition as a lab-adaptive biological process. Upon examining the genes involved in the fatty acid synthesis II (FASII) pathway, which is responsible for *de novo* FA synthesis in the apicoplast, we observed the upregulation of many genes over time (Figure 4.8a). Interestingly, many of these upregulated genes are involved with transporting and converting phosphoenolpyruvate (PEP), a product of glycolysis, all the way into FAs (Figure 4.8b). The carbon chains of FA's can be further elongated in the ER, for which we observe a modest upregulation of the FA elongation pathway as well (4.8c-d). Taken together, these data suggest that increased *de novo* FA synthesis was selected for during the extracellular milieu of GT1's lab-adaptation.

4.2.5. Enhanced phenotypes during lab-adaptation are a multifactorial effect of gene expression

The upregulation of genes involved in FA metabolism represent just a small subset of all the DEGs, leaving the functional role of many other genes for *T. gondii* virulence in question. To choose the strongest candidates for functional analysis we performed regression analysis (RA) on our 988 DEGs in order to determine the strongest correlations between gene expression and phenotypic changes over time. RA identified 319 DEGs (at least one time point = FC ≥2, *q*-value ≤0.05) that are highly expressed (CPM ≥50%) and strongly correlated (R_2 ≥0.70) with at least one phenotype (plaque size, reinvasion efficiency, extracellular survival) (Table 4.5). Of those, 153 were upregulated, 166 were downregulated, 192 correlated with plaque size, 275 correlated with reinvasion efficiency, and 52 correlated with extracellular survival (Figure 4.9a).

Of course, correlation does not equal causation, so we sought validation by knocking out five upregulated genes of interest using a CRISPR/Cas9-mediated genetic engineering approach (Figure 4.9b). We generated KO's with B2-P239 as this population had higher fitness and, therefore, likelihood of generating a recombinant parasite (which is already difficult given *Toxoplasma*'s efficient NHEJ DNA-repair pathway). The five candidate genes that were chosen to be validated as DE *in vitro* virulence factors were based off several variables: Log_2FC, R_2 value, phenotype score on ToxoDB (i.e. a modest fitness score to avoid picking genes essential for the lytic cycle [224]), and biological interests. We chose the following three genes from the 90^{th} percentile for Log_2FC and had the strongest R_2 value for plaque size, extracellular survival and/or reinvasion efficiency: TGGT1_262590 and TGGT1_264240 are both hypotheticals genes of unknown function

with the highest R_2 values for extracellular survival and reinvasion efficiency/plaque size, respectively (Figure 4.10a); and TGGT1_315885, a putative glycosyltransferase (Gnt1) also with the highest R_2 value for reinvasion efficiency and plaque size (Figure 4.10a). We also chose the two following genes based on biological interest: TGGT1_230980, a myosin gene (MyoI), and TGGT1_260190, a microneme gene (MIC13). MyoI resides in the RB and ensures synchronicity of division between intravacuolar parasites [116], a process not intuitively associated with the extracellular milieu, whereas MIC13 was recently connected with oxidative stress survival through an unknown mechanism [284], which therefore serves as our positive control.

Diagnostic PCR of pyrimethamine resistant clones confirmed homologous recombination of the DHFR selection cassette into the 5' and/or 3' locus of our gene of interest (Figure 4.9c); while DHFR did not undergo 5' integration in two genes of interest (TGGT1_264240 & TGGT1_230980), 3' integration of DHFR in all genes was confirmed and, moreover, qPCR of clonal populations confirmed ablation of mRNA expression in all transgenic lines (Figure 4.9d). Next, 11-day plaque assays were performed with our five KO's, along with the parental GT1 line, B2-P239, and B2-P18 (Figure 4.10a-b). As previously observed, plaque size and reinvasion efficiency of high passage parasites (B2-P239) is 3.14- and 3.82-fold superior to that of low passage parasites (B2-P18), respectively (Figure 4.10b-d). Relative to B2-P239, four KO lines show a significant (*p*-value $\leq$0.01) 0.51- to 0.76-fold reduction in plaque size while three lines show a significant (*p*-value $\leq$0.05) 0.65- to 0.77-fold reduced reinvasion efficiency (Figure 4.10b-d). Interestingly, 4/5 of our candidate gene KO parasites display phenotypic characteristics that were predicted by the genes calculated R_2 value, indicating a high degree of accuracy

in phenotype prediction from our RA. Moreover, these results indicate that our list of candidate genes likely contains many DE *in vitro* virulence factors that aid in GT1's lab-adaptive phenotypes and overall *in vitro* virulence.

4.3. Discussion

As observed with our phenotypic data (Chapter 2), our transcriptomic data reveals that most lab-adaptive changes occur with extracellular parasites. With so many DEGs and, moreover, many of unknown function, it is difficult to untangle biological insights associated with GT1's lab-adaptation; therefore, we employed multiple enrichment analysis in order to gain biological insights. GSEA, GOEA, and MPEA analysis of GT1's extracellular transcriptomic data revealed the expected downregulation of several related processes such as organelle fission, reproductive process, and cell cycle, which only occur intracellularly, thereby validating this approach. As for upregulated DEGs, MPEA identified glycolysis, acetyl-CoA biosynthesis, and pyruvate metabolism, all of which are intersecting pathways feeding into FA biosynthesis. Interestingly, FA biosynthesis was the only biological insight in common between MPEA, GSEA, and GOEA. Moreover, FA degradation was found to be downregulated by MPEA, supporting FA procurement as a biological mechanism for GT1's lab-adaptation.

Within the cytosol of the parasite, glycolysis converts glucose into phosphoenolpyruvate (PEP), which is then translocated into the apicoplast by apicoplast triosephosphate translocator 1 (APT1) [285]. Within the apicoplast, PEP is converted into pyruvate by pyruvate kinase 2 (PYK2) [286, 287]. The pyruvate dehydrogenase (PDH) complex is activated by lipoylation and then converts pyruvate into acetyl-CoA, the metabolite precursor for the fatty acid synthase II (FASII) pathway [288-290]. The FASII pathway begins with the conversion of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase 1 (ACCase1) [291]. Next, malonyl-CoA and acyl-carrier-protein (ACP) are processed by FabD and FabH to form β-ketoacyl-ACP (i.e. FASII initiation), which is then

processed through a repeated series of reduction and dehydration reactions by FabG, FabZ, and ENR, (i.e. FASII elongation) to elongate the FA chain up to C:16 in length (Figure 4.8b) [292]. Further elongation or saturation of apicoplast-derived FAs can occur in the ER (Figure 4.8d) [293]. Additionally, glycerol 3-phosphate acetyltransferase 1 (ATS1) can convert FAs into lysophosphatidic acid (LPA) [294], while ATS2 can convert LPA into phosphatidic acid (PA) [295], a central precursor for the *de novo* synthesis of all phospholipids (PL). Interestingly, many of these enzymes that feed into the production of short chain FAs are significantly upregulated in extracellular GT1 during lab-adaptation (Figure 4.8). Recent lipidomic studies reveal metabolic plasticity in *T. gondii's* ability to not only scavenge lipids from host cells but create lipids *de novo* via the FasII pathway, supporting our observation of a shifted FASII expression profile [295]. Even under 0% FBS (lipid-starved conditions), lipidomic analysis reveals that RH is able to maintain its superior *in vitro* virulence by upregulating its *de novo* FA/lipid synthesis up by 15% [295]. In our study, we cultured GT1 in 1% FBS, which likely explains the observed natural selection of GT1 parasites with increased FASII expression and thus greater *in vitro* virulence; however future lipidomic experiments are needed to connect the upregulation of this pathway to augmented *in vitro* virulence and associated virulence traits in GT1 (see Chapter 5.2.2.).

Further biological insights were gained from our life-stage analysis, which characterized our trending up- and down-regulated DEGs as tachy-, brady-, mero and/or sporozoite. This transcriptomic analysis revealed that extracellular tachyzoites are becoming more tachyzoite-like during lab-adaptation, which is not surprising given RH is very lab-adapted and exclusively exhibits pro-tachyzoite tendencies. For example, RH

has the fastest asexual lytic of all *T. gondii* strains and no longer readily differentiates into other life stages. Similarly, in *P. falciparum*, it has been known that gametocyte (sexual life stage) production is lost upon prolonged asexual lab-adaptation [296]; interestingly, this is due to loss-of-function mutations in the ApiAP2-G TF that normally controls the expression of several associated gametocyte genes [297]. In RH, however, the genetic basis responsible for the loss of cell differentiation remains unknown. While we cannot rule out a mutation-based mechanism for RH, our study in GT1 suggest that the evolution of pro-tachyzoite tendencies could be, at least in part, due to epigenetic control. Supporting this notion, ApiAP2-G is suggested to be epigenetically controlled in *P. falciparum [297-299]* and its ablation provides an *in vitro* growth advantage [297]. Thus, we hypothesize that epigenetic control of TFs (e.g. AP2 or Myb-domain containing proteins) could be promoting GT1's pro-tachyzoite transcriptomic shift and resulting in augmented *in vitro* virulence during lab-adaptation. Several potential TF's of interest are listed in Table 4.6, of which two have been shown to be bradyzoite activators (AP2IV-3 & AP2Ib-1) [147] and one has been shown to be a bradyzoite repressor (AP2IX-9) [147, 151]- others remain enigmatic.

One mitigating factor in interpreting RNA-seq data is that the differential expression of mRNA does not always correlate with expression at the protein level due to post-transcriptional regulatory mechanisms. In mammalian cells, many mRNAs associated with development and stress response are post-transcriptionally regulated [300]. In apicomplexans, translational control of mRNA transcripts is emerging as a significant regulator, especially of parasitic latency (e.g. bradyzoites, sporozoites), which can often be stress induced [301-303]. Recently, comparative RNA-seq and ribosome/polysome

profiling (Ribo-seq) identified little translational control amongst intracellular *T. gondii* tachyzoites [304]; however, upon extracellular conditions (<1hr estimated) 834 genes (~12% of *T. gondii's* detected translatome) are differentially controlled at the translational level [303], likely as a mechanism of stress response [301]. Of those translationally controlled genes, 93 overlap with our TC-seq-derived (trending) DEG's, and 37 overlap with our RA-derived (phenotypically-correlated) DEGs. Thus, roughly 10% of our list of DE *in vitro* virulence factors are likely to be translationally controlled and may not present a phenotype when manipulated transcriptionally.

To validate our list of DEGs, gene KO lines for five candidate genes trending strongly with phenotypic features were used to functionally validate their association with *in vitro* virulence and virulence traits. To our success, the correlation coefficient, R_2, of phenotypes was highly accurate with 4/5 of our KO lines displaying the expected phenotype. For example, gene expression of MyoI and Gnt1 strongly correlated with plaque size and reinvasion efficiency during lab-adaptation, and KO of these genes validated their influence on those phenotypes. Alternatively, gene expression of TGGT1_262590 did not show strong correlation with plaque size and reinvasion efficiency during lab-adaptation, and phenotypic evaluation of this KO validated that relationship too. However, two unexpected outcomes were observed: TGGT1_264240 displayed reduced reinvasion efficiency of ~17% as expected by RA (R_2 = 1.0), but this did not reach statistical significance (*p*-value = 0.24) due to the sizable standard deviation in our biological replicates; and MIC13 displayed a significant ~35% reduction in plaque size, which was much more dramatic than we expected given its R_2 value of only 0.49. Overall, the majority of our predictions made by RA were accurate in identifying DE genes that

have significant influence on GT1's phenotypes. Notably, no single gene KO was able to reduce the *in vitro* virulence of GT1-P239 down to the level of GT1-P18, indicating that *in vitro* virulence is a polygenic trait.

Exactly how and why some of these genes aid in GT1's lab-adaptation is unknown, but with the three annotated genes we functionally investigated, MIC13, Gnt1, and MyoI, some speculations can be made. First, it is possible that upregulation of MIC13 is part of a stress response to the extracellular environment as recent studies have identified MIC13 as an important factor of RH growth under stressed conditions [284]. In our GT1 study, however, MIC13 appears significant for growth under standard "unstressed" laboratory conditions (Figure 4.10b-c), suggesting that our laboratory conditions are actually perceived as stressful in GT1 parasites or that MIC13 functions differently in RH and GT1. Second, in the case of Gnt1, previous studies have shown this glycosyltransferase to incorporate GlcNAc onto Skp1, an adaptor protein, which then promotes the formation of the Skp1/Cullin-1/F-box protein (SCF) complex, an E3-ubiquitin ligase [87, 305]. The SCF complex will polyubiquitinate specific regulatory and non-regulatory proteins, marking them for degradation by the 26S proteasome. Moreover, the SCF complex was found to be O_2 regulated in *Dictyostelium* [83], indicating its role in maintaining redox homeostasis in the cell. Interestingly, during GT1's lab-adaptation, six genes of the proteasome core complex were upregulated over time, suggesting that lab-adaptation, results in increased protein turnover within extracellular parasites. Exactly how this might aid in GT1's enhanced reinvasion efficiency or *in vitro* virulence requires further investigation but it possible to be a starvation response mechanism aimed at recycling amino acids for new proteins [306] or to combat damage induced by reactive

oxygen species (ROS). After all, lab-adaptation involves the parasite's frequent exposure to atmospheric O_2 levels (21%) which is dramatically higher than what is experienced *in vivo*. Lastly, the identification of MyoI is quite peculiar as it is a class XXIV motor protein that is known to localize to the RB of intracellular parasites [116]. Functional analysis has shown that MyoI is required to establish the basal RB connection that maintains parasite-to-parasite communication and cell synchronicity of dividing parasites [116]. In our study, there is an increased expression of MyoI in extracellular parasites, indicating perhaps a novel extracellular function of MyoI. Indeed, the basal complex of the parasites display several features of an import/export port, and, in intracellular parasites, have been shown to be a likely site of host vesicle uptake (reviewed in [307]). Thus, we hypothesize that the basal complex might serve as lipid uptake port in extracellular parasites.

In conclusion, 8/10 phenotype predictions across 4/5 gene KO's we examined were confirmed in our phenotypic validation experiments, indicating our list of candidate DEGs as likely containing many genes responsible for *in vitro* virulence in our adapted GT1 strain. This gene set therefore provides a rich resource for discovering and assigning new gene-function associations. Furthermore, our DNA- and RNA-seq results, taken together, suggest that epigenetic mechanisms are likely responsible for much of GT1's evolved *in vitro* virulence during lab-adaptation rather than an accumulation of mutations. Future work characterizing the dynamics of chromatin accessibility during lab-adaptation, coupled with our transcriptomic data, will evaluate our hypothesis of epigenetically controlled transcriptional programs during lab-adaptation (see Chapter 5.2.3).

Figure 4.1. Experimental workflow and RNA-seq analysis pipeline

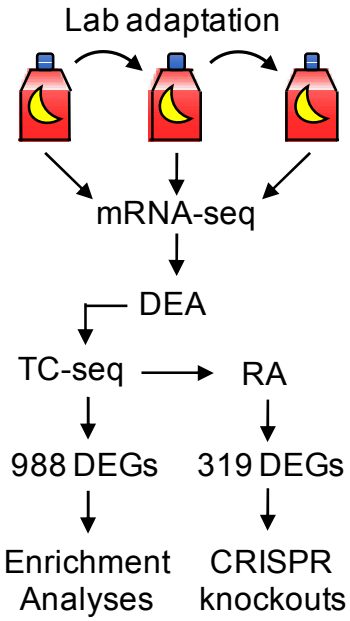


Figure 4.1. Experimental workflow and RNA-seq analysis pipeline

Following lab-adaptation of GT1, mRNA-sequencing (RNA-seq) was performed at several evolutionary time points (i.e. passages) and data was analyzed by differential expression analysis (DEA). To identify expression patterns within the data set, time-course sequencing (TC-seq) was utilized and identified 988 differentially expressed genes (DEGs) that trend up or down over time. Life stage analysis, gene set enrichment analysis (GSEA), gene ontology enrichment analysis (GOEA), and metabolic pathway enrichment analysis (MPEA) were utilized on TC-seq data set to identify biological insights into lab-adaptation. The TC-seq data set was further filtered by regression analysis (RA) to identify 319 DEGs that strongly correlated with lab-adaptive phenotypes. Functional analysis of several DEGs was then performed by CRISPR/Cas9-mediated gene KOs.

Figure 4.2. Time-course sequencing analysis identified 8 expression profiles that largely trend upward or downward during lab-adaptation

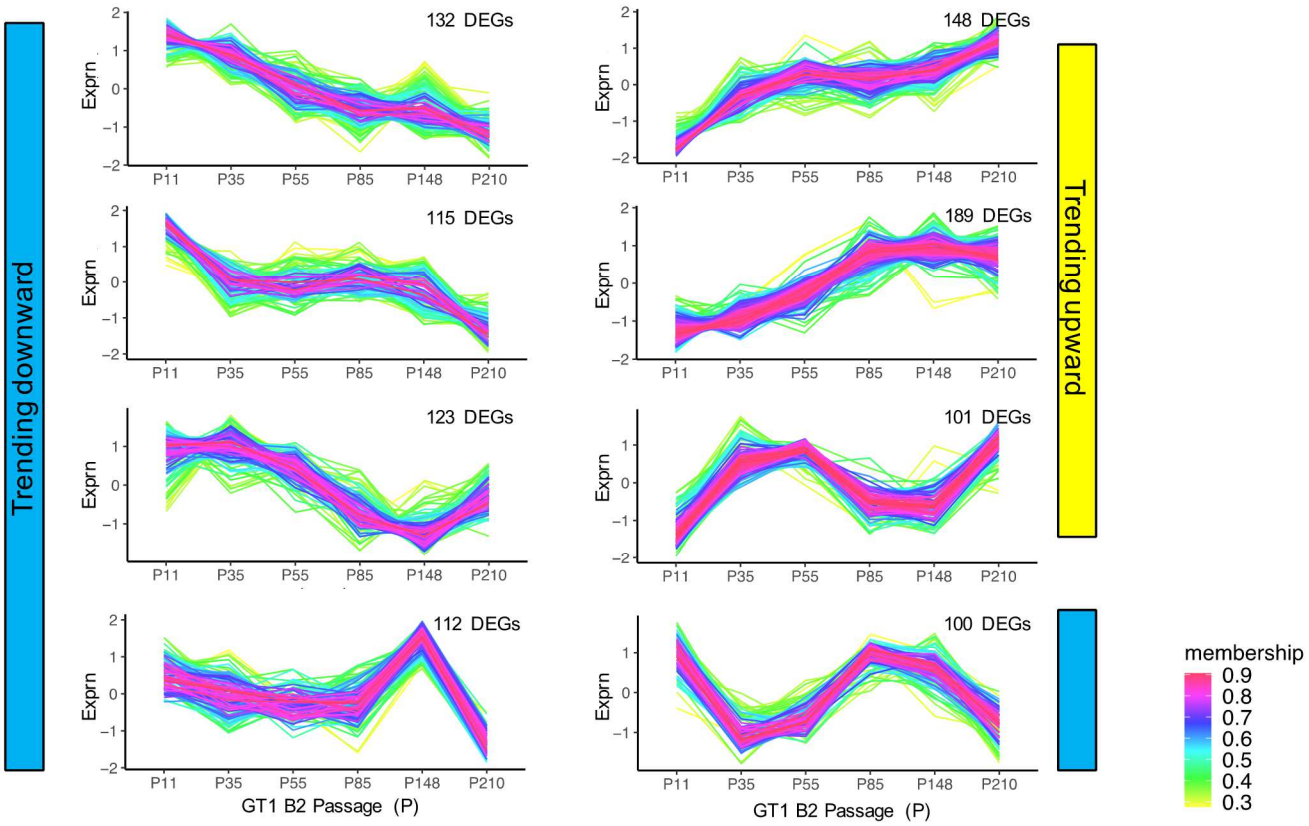


Figure 4.2. Time-course sequencing analysis identified 8 expression profiles that largely trend upward or downward during lab-adaptation

Following RNA-seq of 6-hour extracellular GT1 parasites, TC-seq analysis identified eight prominent expression patterns amongst significant DEGs. DEGs were considered significant if the absolute FC ≥2 and *q*-value ≤0.05 in at least one time point (i.e. passage). Five expression patterns show a general downward trend while three expression patterns show a general upward trend in expression during lab-adaptation. The number of genes within each trend is indicated in each plot. Expression at each timepoint is normalized to the average expression across all timepoints.

Figure 4.3. Developing and validating *T. gondii's* life stage score analysis

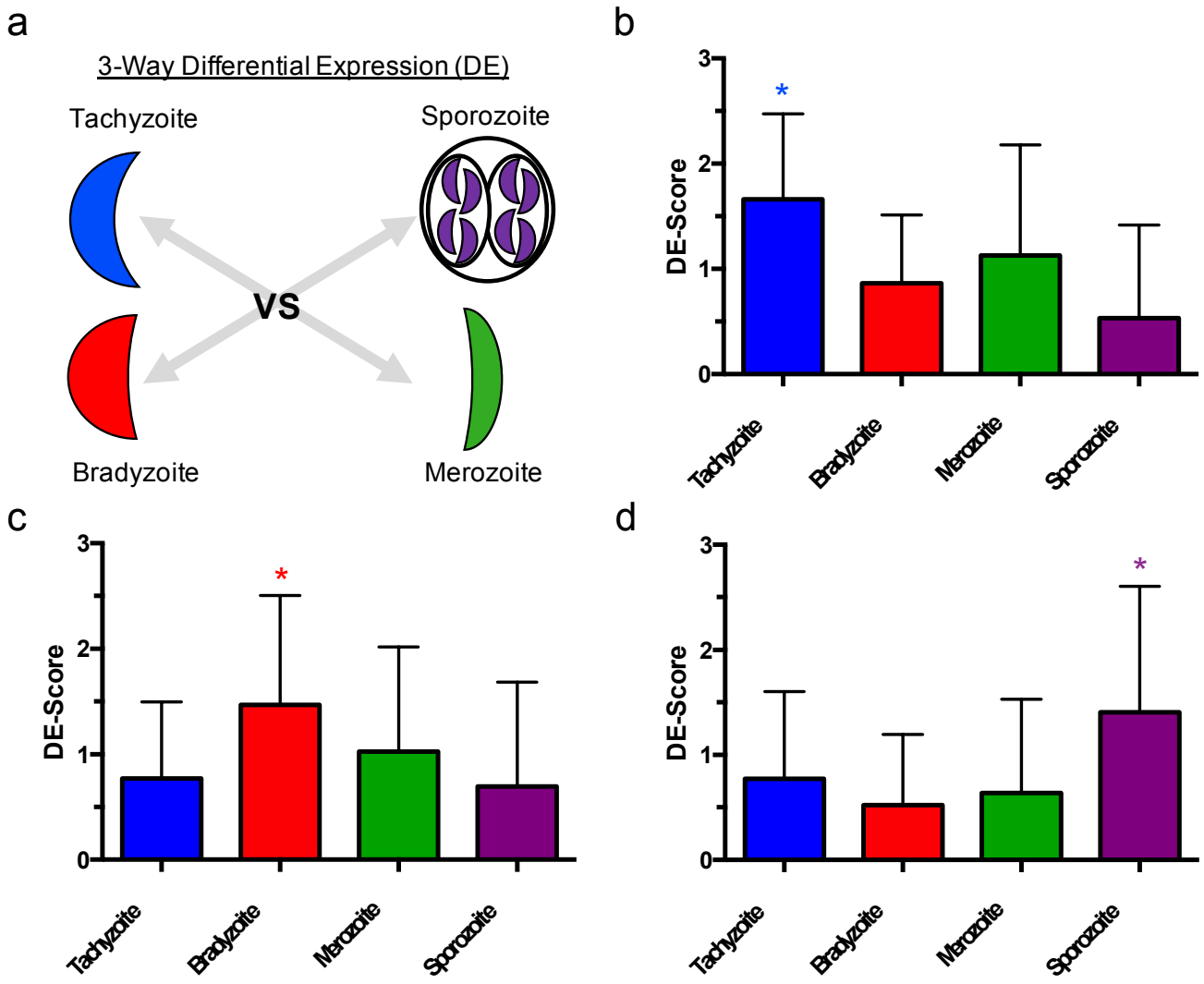


Figure 4.3. Developing and validating *T. gondii's* life stage score analysis

a.) Available tachyzoite, bradyzoite, merozoite, and sporozoite RNA-seq data sets (ToxoDB.org, see methods) were utilized for differential expression analysis (DEA). Each stage was compared to the 3 other stages (e.g. tachyzoite vs. bradyzoite; tachyzoite vs. sporozoite; tachyzoite vs. merozoite). The number of times each gene was significantly upregulated in this 3-way DEA was enumerated to yield a max score of 3 or min score of 0 for each gene. The average enumeration of previously published lists of genes was calculated for **b.)** tachyzoite-, **c.)** bradyzoite-, and **d.)** sporozoite-associated genes. Color-coded asterisks (*) indicate *p*-value ≤0.05, as determined by an independent bootstrap analysis (n= 1000 random sampling) for each individual life stage.

Figure 4.4. Life stage scoring identified pro-tachyzoite gene expression in lab-adapting GT1 parasites

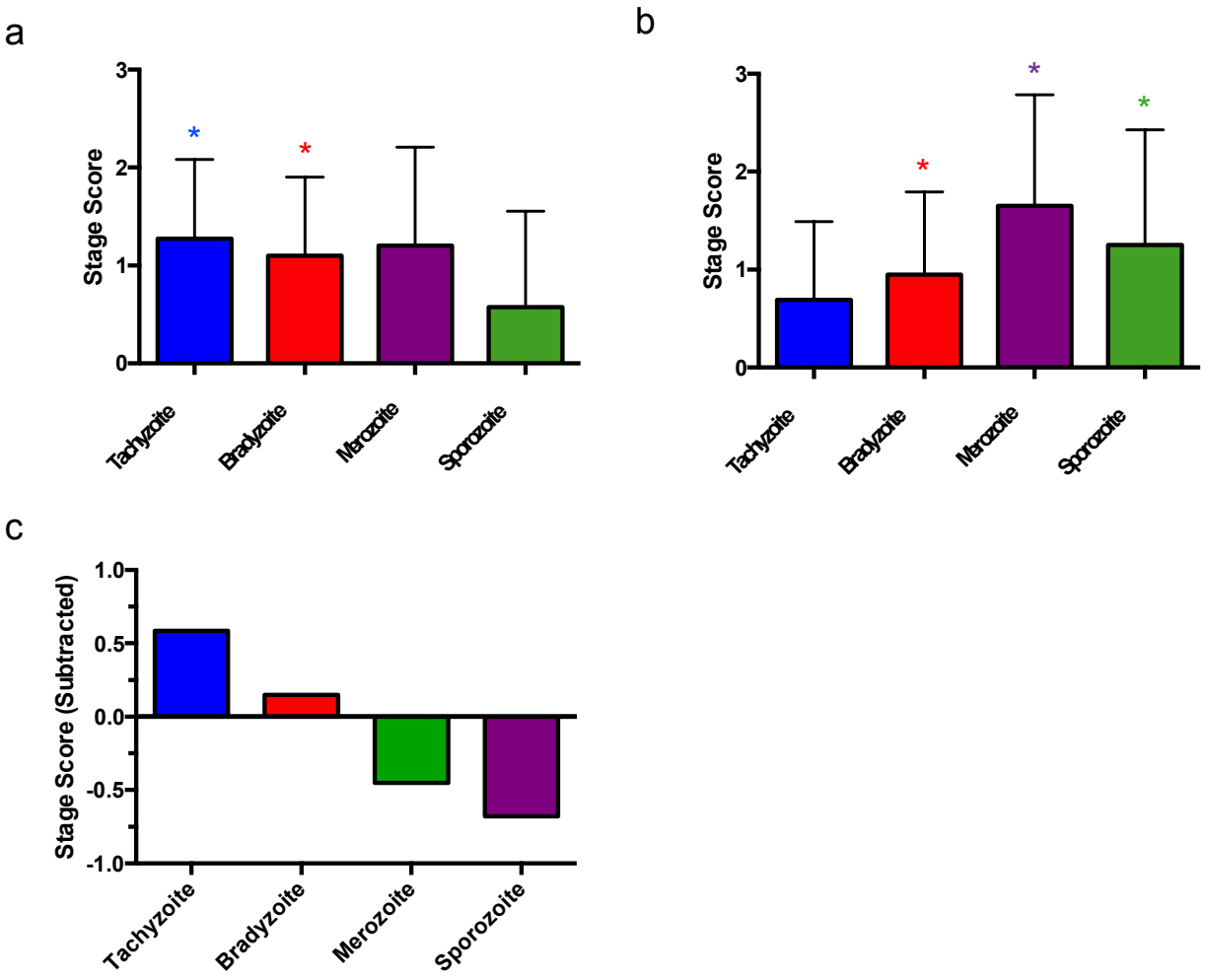


Figure 4.4. Life stage scoring identified pro-tachyzoite gene expression in lab-adapting GT1 parasites

a.) Life stage scoring was performed on 437 DEGs identified by TC-seq as trending upward or **b.)** 551 DEGs identified by TC-seq as trending downward during lab-adaptation. Color-coded asterisks (*) indicate *p*-value ≤0.05, as determined by an independent bootstrap analysis (n= 1000 random sampling) for each individual life stage. **c.)** The downregulated stage score is subtracted from the upregulated stage score to highlight the dominant expression profiles.

Figure 4.5. Gene set enrichment analysis (GSEA) of trending genes

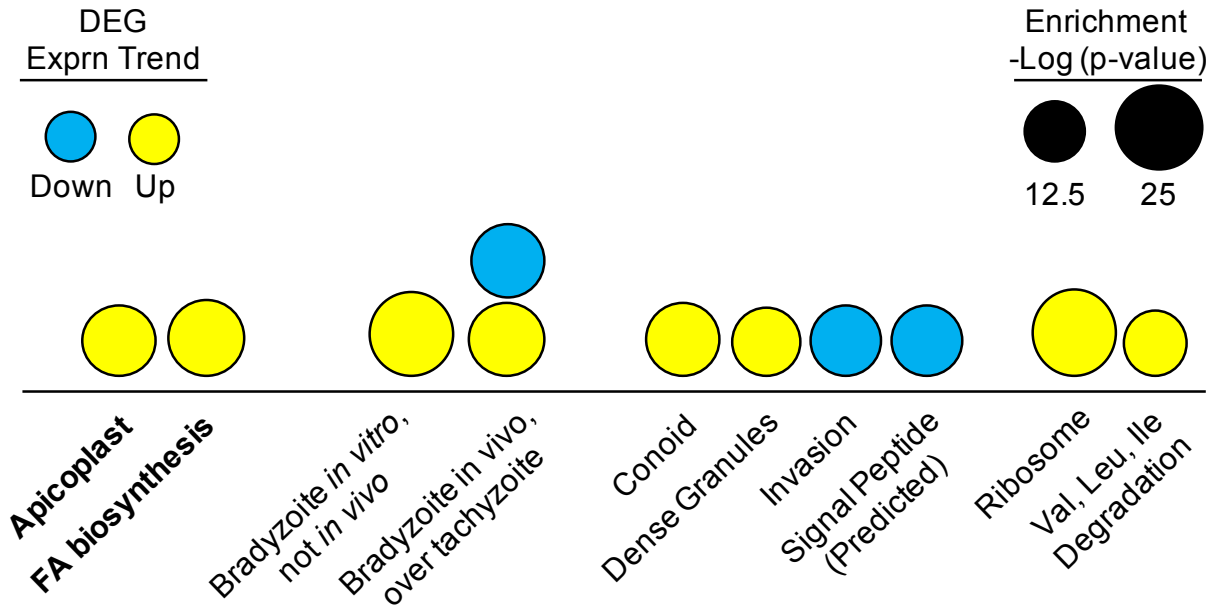


Figure 4.5. Gene set enrichment analysis (GSEA) of trending genes

Up- and down-regulated DEGs identified by TC-seq were subject to GSEA, which utilizes previously published gene sets that describe organelle-associated genes, metabolic genes, life stage genes, and genes involved with different biological processes. The significance of overlap between our set of genes with these previously published gene sets are indicated by the size of the circle.

Figure 4.6. Gene ontology enrichment analysis (GOEA) of trending genes

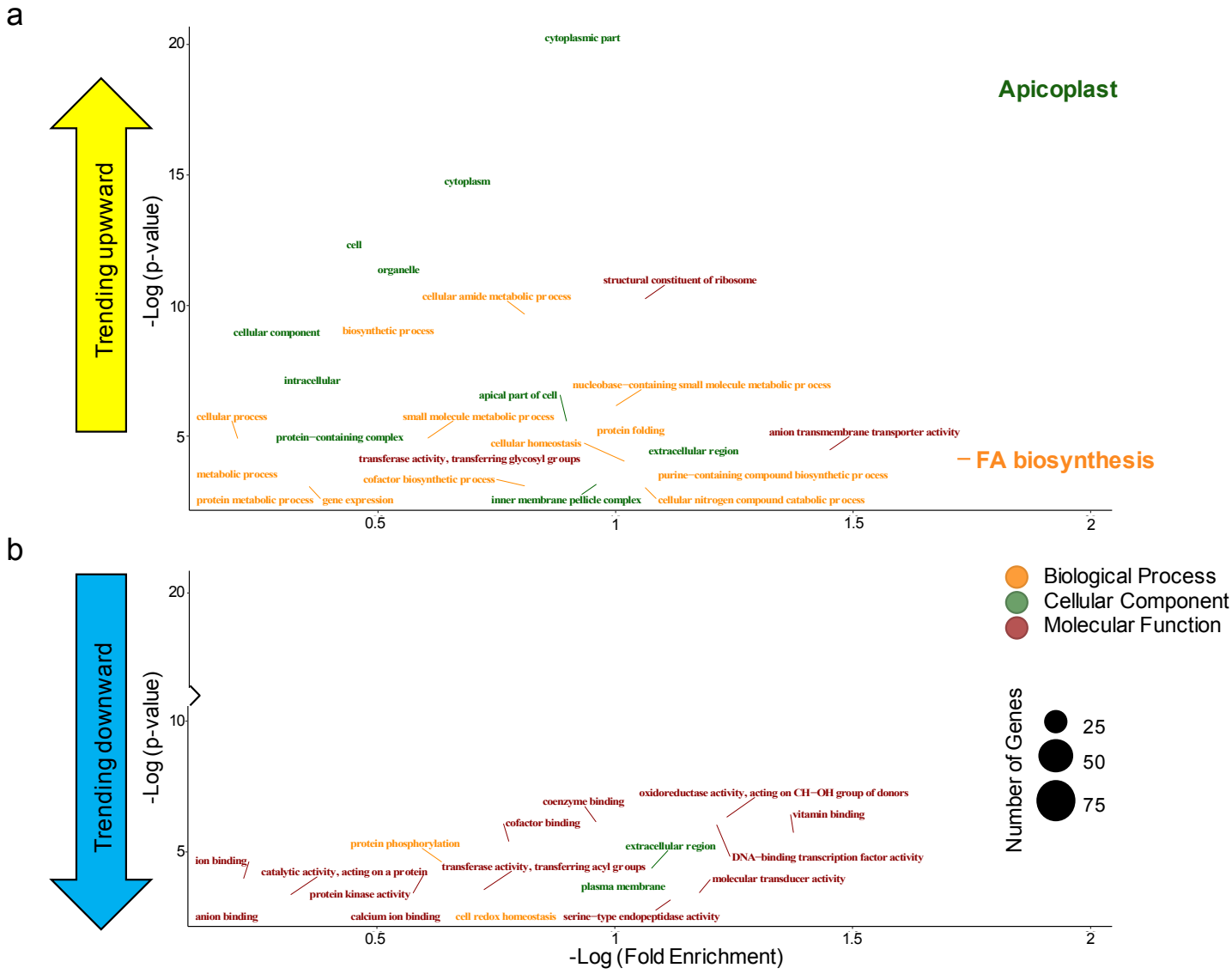


Figure 4.6. Gene ontology enrichment analysis (GOEA) of trending genes

a.) Up- and **b.)** down-regulated DEGs identified by TC-seq were subject to GOEA on ToxoDB.org to identify GO-terms associated with biological processes, molecular functions, and cellular components. Redundant GO terms were removed by REVIGO analysis available on ToxoDB.org (see methods).

Figure 4.7. Metabolic pathway enrichment analysis (MPEA) of trending genes

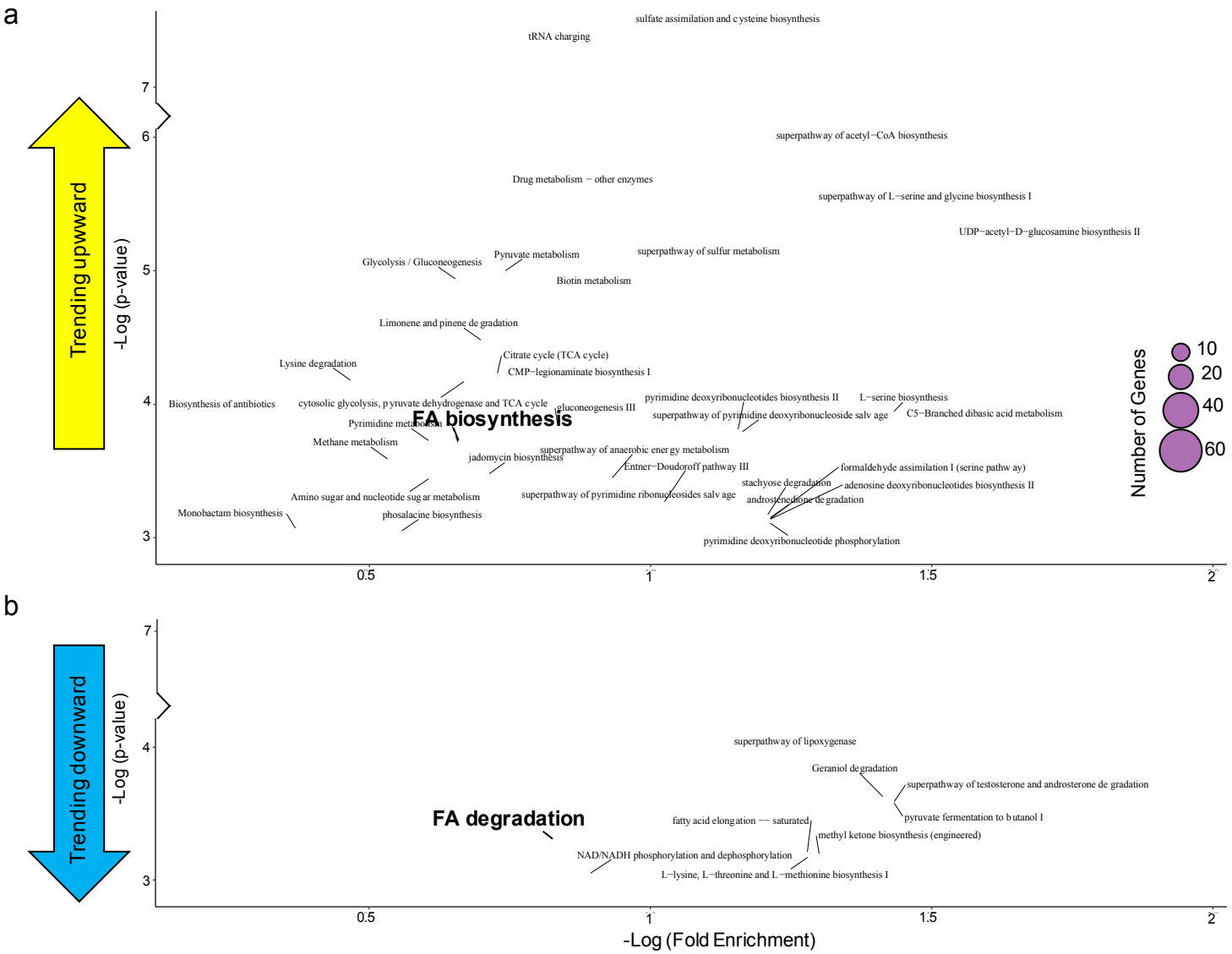


Figure 4.7. Metabolic pathway enrichment analysis (MPEA) of trending genes

a.) Up- and **b.)** down-regulated DEGs identified by TC-seq were subject to MPEA on ToxoDB.org to identify associated metabolic pathways. Several identified terms have been manually removed due to redundancy (see methods).

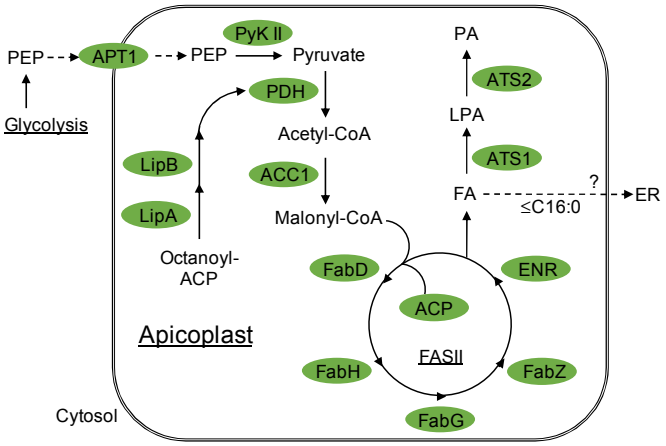
Figure 4.8. Evolution of the FASII pathway and elongation pathway in extracellular GT1 during lab-adaptation

a

Gene ID	Log$_2$ FC (relative to GT1 B2-P11)							Name
	RH	P11	P35	P55	P85	P148	P210	
TGGT1_261070			-0.07	-0.12	1.28	1.23	0.75	APT1
TGGT1_299070	-0.72		-0.18	0.05	0.19	0.26	0.44	PyKII
TGGT1_245670	-0.11		-0.49	-0.08	2.33	1.55	1.50	PDH-E1α
TGGT1_272290	-0.23			-0.18	0.45			PDH-E1β
TGGT1_206610	-0.17		0.45	-0.16	1.14	0.54	0.42	PDH-E2
TGGT1_305980	0.19		0.31	0.90	1.88	1.47	1.65	PDH-E3I
TGGT1_221320	-2.01		0.11	0.22	0.69	0.92	0.95	ACC1
TGGT1_264080	-0.48		0.08	0.40	1.50	1.30	1.21	ACP
TGGT1_225990	-0.16		0.06	-0.26	1.09	0.51	0.40	FabD
TGGT1_231890	-0.03		0.18	0.66	1.34	1.41	1.16	FabH
TGGT1_321570	0.55		0.46	0.66	0.94	1.10	0.73	FabZ
TGGT1_251930	0.36		-0.23	-0.16	2.05	1.52	0.95	ENR
TGGT1_226400	-0.29		-0.22	-0.14	-0.21	-0.10	-0.09	LipA
TGGT1_315640	0.67		0.07	0.42	0.29	0.43	0.53	LipB
TGGT1_270910	-0.78		0.50	0.15	-0.48	-0.48	-0.21	ATS1
TGGT1_297640	-0.29		0.26	0.06	-0.25	-0.41	-0.07	ATS2

Log$_2$ FC
-1 0 1

b



c

Gene ID	Log$_2$ FC (relative to GT1-B2-P11							Name
	RH	P11	P35	P55	P85	P148	P210	
TGGT1_243880	N/A	N/A	N/A	N/A	N/A	N/A	N/A	ELO-A
TGGT1_242380	0.56		0.20	0.48	0.34	0.28	0.48	ELO-B
TGGT1_205350	0.31		0.22	0.35	0.09		0.47	ELO-C
TGGT1_271888	0.05		-0.30	-0.48	-0.71		-0.71	KCR
TGGT1_311290	0.43		0.04	0.29	0.72	0.66	0.62	DEH
TGGT1_285240	0.58		0.06	0.09	0.52	0.50	0.30	ECR

Log$_2$ FC
-1 0 1

d

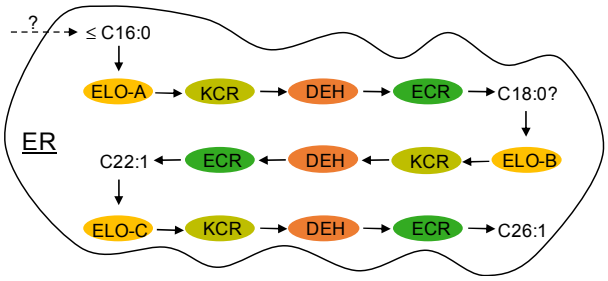


Figure 4.8. Evolution of the FASII pathway and elongation pathway in extracellular GT1 during lab-adaptation

a.) Expression of several genes involved in the multi-step process of *de novo* fatty acid synthesis within the apicoplast. **b.)** Glycolysis produces PEP, which is transported into the apicoplast by APT1 and converted into pyruvate by PyKII. Once lipoylated by LipA/B, the PDH complex converts pyruvate into acetyl-CoA, which is then metabolized to generate malonyl-CoA, the precursor metabolite required for the FASII pathway and FA synthesis. **c.)** Expression of several genes involved in fatty acid elongation within the ER. **d.)** Medium chain FA are translocated from the apicoplast to the ER for repeated rounds of carbon chain elongation by ELO A/B/C, KCR, DEH, and ECR.

Figure 4.9. Generating genetic knockouts of regression analysis candidates by CRISPR/Cas9

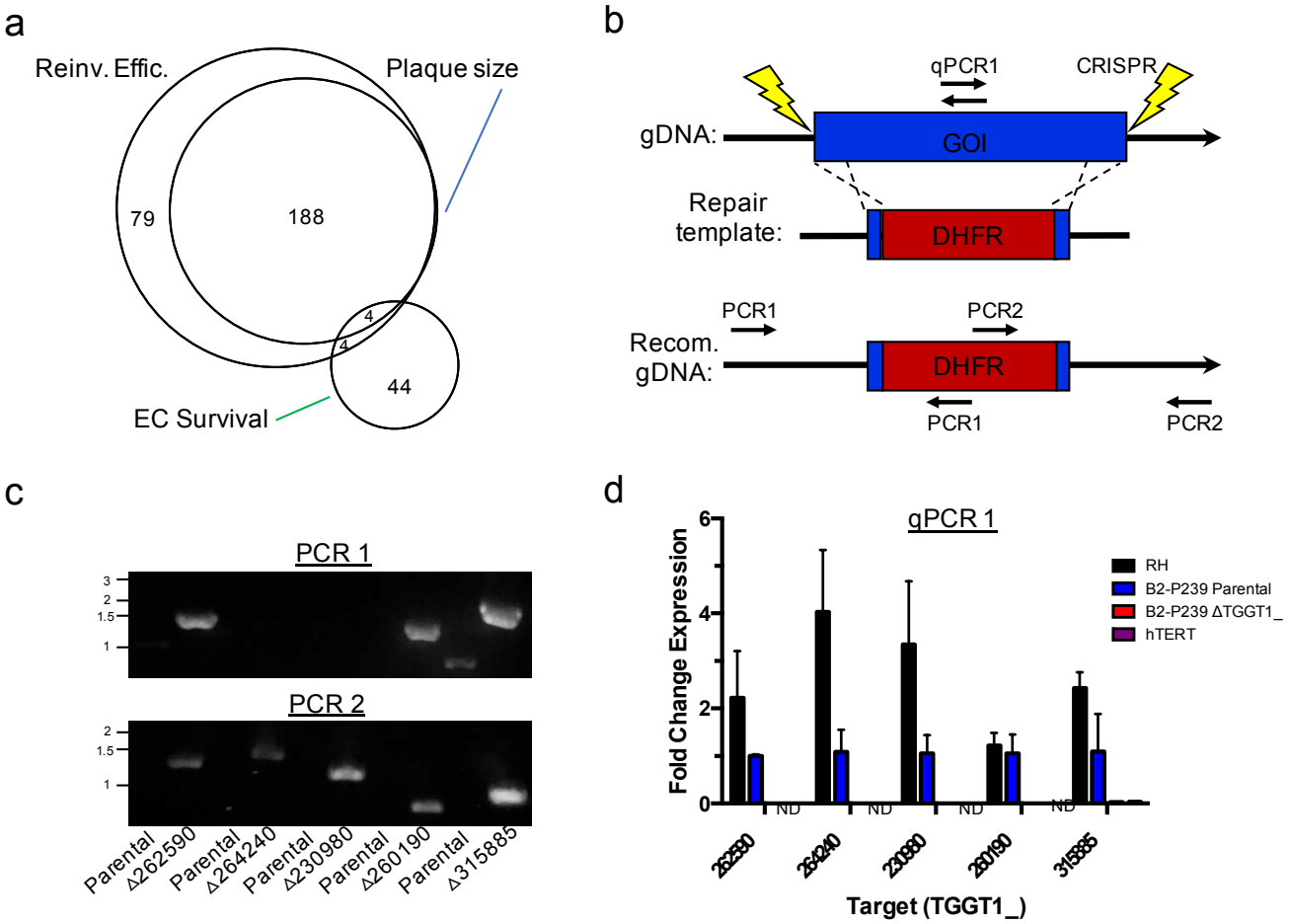


Figure 4.9. Generating genetic knockouts of regression analysis candidates by CRISPR/Cas9

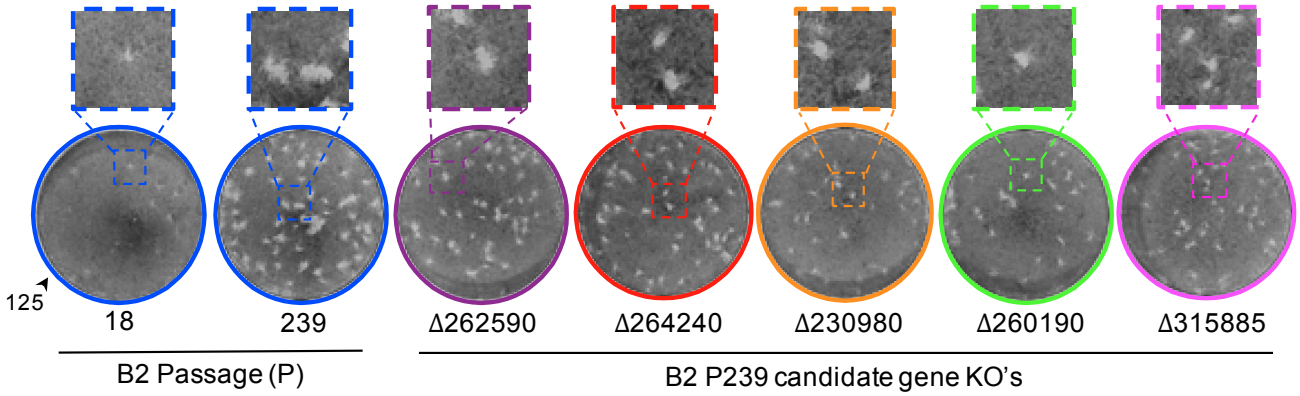
a.) Venn diagram of the 319 differentially expressed genes (DEGS) that strongly correlate ($R_2 \geq 0.70$) with changes in plaque size, reinvasion efficiency, and/or extracellular survival during lab-adaptation. **b.)** Strategy for generating KO of candidate DEGs. Transfection of one or two CRISPR plasmids generates a DSB, allowing for recombination of a co-transfected DHFR selection cassette with short homologous flanks, into the genetic locus and disrupting the gene of interest. Note, sites of recombination are not drawn to scale; relative regions for diagnostic PCR to confirm integration and qPCR to confirm ablation of mRNA expression are shown. **c.)** Diagnostic PCR of clonal KO parasites confirms integration of the DHFR cassette into the gene of interest. **d.)** qPCR of clonal KO parasites confirms ablation of mRNA expression of the gene of interest.

Figure 4.10. Functional analysis of candidate knockouts identified several differentially expressed genes important for optimal *in vitro* virulence, suggesting acquired *in vitro* virulence is a polygenic trait

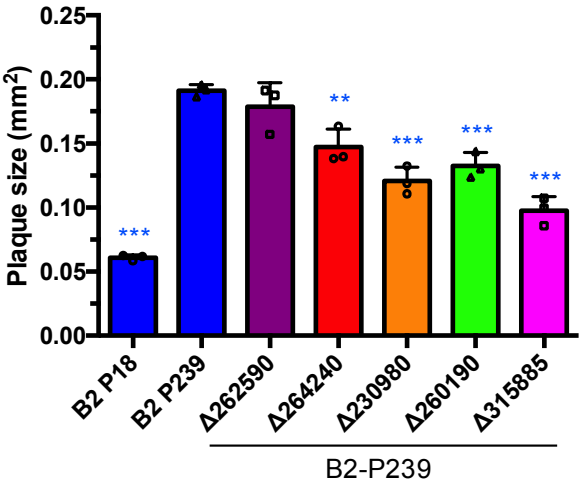
a.

Gene ID	Log_2 FC (Relative to GT1 B2-P11)							Correlation Coefficient (R_2)			Product Description
	RH	P11	P35	P55	P85	P148	P210	Plaque Size	Reinv. Effic.	EC Survival	
TGGT1_262590	2.90		2.06	2.98	2.66	2.58	3.45	0.56	0.56	0.94	hypothetical protein
TGGT1_264240	4.35		0.81	0.36	1.04	3.44	3.22	0.85	1.00	0.05	hypothetical protein
TGGT1_230980	0.97		0.62	0.67	0.69	1.33	1.33	0.85	1.00	0.05	myosin I
TGGT1_260190	0.24		0.06	0.49	0.77	1.30	0.51	0.49	0.74	0.05	microneme protein MIC13
TGGT1_315885	-0.09		0.79	1.23	1.04	1.49	1.94	0.85	1.00	0.05	putative glycosyltransferas

b.



c.



d.

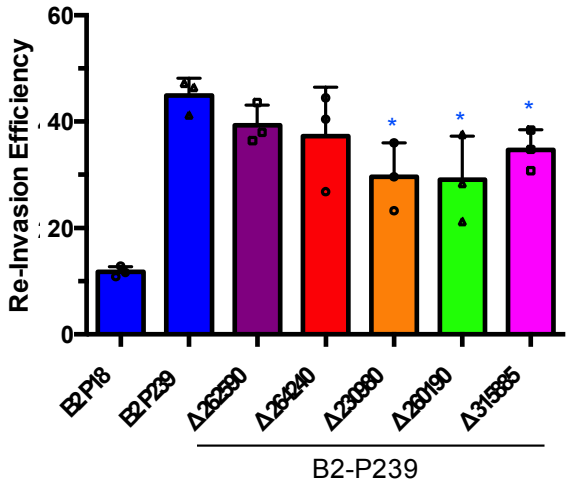


Figure 4.10. Functional analysis of candidate knockouts identified several differentially expressed genes important for optimal *in vitro* virulence, suggesting acquired *in vitro* virulence is a polygenic trait

a.) Expression profiles and phenotype correlation coefficients (R_2) of five candidate DEGs chosen for phenotypic evaluation by CRISPR KO. Upon successful KO, **b.)** 11-day plaque assays were performed and **c.)** plaque size and **d.)** plaque number were quantified**.**

Chapter 5: Conclusions and future directions

5.1. Conclusions

Most E&R experiments utilize lab-adapted organisms in order to identify genomic mutations that bestow adaptation to a single experimental variable, such as limited glucose or presence of a drug. Our E&R experiment differs from more traditional studies as we are utilizing a non-lab-adapted organism with the aim of identifying the genetic basis for augmented phenotypes observed during *in vitro* lab-adaptation. Notably, lab-adaptation does not contain a single variable either; rather it is a combination of variables, such as nutrient availability (glucose, lipids, etc.), oxygen tension, and immune response, which are all altered *in vitro* when compared to natural *in vivo* conditions. Furthermore, with obligate intracellular parasites, such as *Toxoplasma* and *Plasmodium*, *in vitro* serial passaging is cyclically exposing microorganisms to differing nutrient, ionic, pH, oxygen, and axenic environments due to the parasite's lytic cycle. Thus, *in vitro* lab-adaptation, especially for lytic parasites, is actually a complex set of ever-changing variables. While more environmentally dynamic and complex E&R studies are limited, one relatable study in yeast adapting to alternating salt and oxidative stress cycles were found to adapt by increasing their DNA content and gene expression profiles (one mutation was also identified but it increased fitness in control populations as well) [282]. Another study in bacteria found that adaptation to delayed serial passaging, which exposes bacteria to several different environments (lag, log, stationary, and death), is controlled by genetic mutations [264]. Thus, mechanisms of adaptation in microorganisms can range from the accumulation of mutations to mechanisms altering gene expression patterns. In our E&R study, we lab-adapted a strain of *T. gondii* in order to identify the genomic mutations, epistatic interactions, or transcriptomic profiles that are associated with altered

phenotypes during lab-adaptation. In doing so, we could identify polymorphic virulence factors, novel epistatic interactions, or gene expression profiles that contribute to parasite fitness.

A recently published mutation rate of RH closely resembles our calculated mutation rate of GT1, corroborating our analysis, yet both mutations rates are dramatically lower than that of the related apicomplexan species *P. falciparum* [280]. Having such a relatively low mutation rate, *T. gondii*'s population complexity is likely to be impacted upon serial passaging due to genetic drift, resulting in the loss of potentially beneficial mutations [229]. By performing WGS on clonal GT1 populations, we were able to capture some of this genetic diversity within the bulk population and observe that many mutations continue to remain at low frequency or are completely lost following continued lab-adaptation. Maintaining an appropriate population size can reduce the effects of genetic drift, as observed in yeast and bacterial studies [229]. Therefore, our observations agree with many others that it is important to take genetic drift, mutation rate, and population size into account when performing E&R experiments, especially if genomic mutations are of particular interest [229]. Notably, our E&R experiment was designed to identify the basis of phenotypic changes during standard laboratory conditions [8], thus we did not aim to alter the mutation rate (e.g. mutagenesis) or increase the population size of our GT1 cultures (e.g. 1:5 serial transfers as opposed to standard 1:20).

While mutation accumulation or epistatic interactions do not appear to play a significant role in GT1's lab-adaptation, gene expression appeared far more malleable and conducive, suggesting epigenetic mechanisms are at play. While the vast number of E&R studies have revealed genomic mutations as a fundamental basis of evolutionary

adaptations, there is growing evidence for epigenetic control of evolutionary adaptations as well [208-210, 212]. Interestingly, our characterization of GT1's adapting transcriptome illustrates a significant trend toward a more tachyzoite-like transcriptome. Such a transcriptomic trend parallels the behavioral essence of a tachyzoite's tendencies and, therefore, are likely a genetic basis for GT1's evolved virulence *in vitro.* Indeed, our genetic KO's support this hypothesis and, therefore, passage history is an important variable to consider when interpreting plaque assays or cell differentiation. Anecdotally, it has been advised to periodically passage parasites *in vivo* (e.g. mice) in order to maintain the "vigor and biologic characteristics of the strain" [8]. Furthermore, it has been suggested that prolonged cultivation of the RH strain is likely the reason why it does not readily differentiate into bradyzoites anymore [168]. Our data is the first to demonstrate the potential expression changes that likely underlies previous anecdotal observations of pro-tachyzoite tendencies during prolonged serial passaging. Furthermore, in the absence of mutation accumulation, our data indicates that epigenetic control of pro-tachyzoite genes is likely the dominant mechanism of GT1's lab-adaptation.

Aside from passage history, another variable to strongly consider with E&R is the amount of fetal bovine serum (FBS) used in culture media. FBS is a rich source of FAs, lipids, and many other components, and the amount of FBS utilized in *T. gondii* research differs between laboratories, commonly either 1% or 10% FBS. Knowing that RH fitness is unaffected by either concentration, we and many others utilize 1% FBS for economic purposes [8]. However, in the context of other strains, it is becoming clear that the amount of FBS is a significant variable in *T. gondii*, likely due to the availability of exogenous FA and lipids that are scavenged. In a recent study, the avirulent ME49 and Prugniaud strains

(both of type II) were shown to have roughly 2-fold increase in *in vitro* virulence when cultured in 10% FBS, relative to 1% FBS [295]. RH, however, is unaffected by the concentration of FBS, even with 0% FBS, because it is able to rapidly upregulate its *de novo* FASII pathway [295]. Notably, the FASII pathway is not essential for parasite survival due to lipid scavenging capabilities; however, FASII disruption can significantly reduce *in vitro* virulence >50% unless the culture media is supplemented with extra FAs (e.g. C:14-C:18) [289, 295, 308, 309]. Therefore, *in vitro* virulence is likely dependent on *T. gondii's* ability to both scavenge and synthesize lipids. In our study, we culture GT1 in 1% FBS and observed an overall increase in the expression of several FASII-related genes over time. This suggests that non-lab-adapted parasites are starved in 1% FBS conditions and they can adapt to this niche by upregulating genes of the FASII pathway, although future lipidomic work is needed to confirm the *de novo* synthesis of FAs and lipids (see Chapter 5.2.2.). The genomic mutations observed in the P4-flippase gene likely also cater to increase FA access, which will require further validation in the future. In short, FBS concentration is likely an overlooked variable that should be considered when working with non-lab-adapted strains as mechanisms for FA and lipid procurement appears to be naturally selected for during lab-adaptation.

Regulation of the FASII pathway represents only a small fraction of the 988 DEGs identified in this study, suggesting that other adaptive biological processes are at play. First, antioxidant activity was enriched in GOEA, suggesting that the parasite is likely adapting to the 21% pO_2 experienced during the extracellular milieus of lab-adaptation. Secondly, an increase in bradyzoite-associated genes was identified by life-stage analysis and GSEA, likely stemming from an unknown stress signal that is sensed by

extracellular parasites. It is well known that intracellular-stressed parasites will differentiate into bradyzoites and, although less publicized, 12-hour extracellular parasites are >50% more likely to differentiate into bradyzoite upon re-inoculation. Whether or not this quasi-bradyzoite-like state of extracellular parasites confers any adaptive advantage is a topic of future work. Lastly, our functional validation of 4/5 candidate differentially expressed genes identified known and novel factors that can contribute to *in vitro* virulence: MIC13, MyoI, Gnt, and TGGT1_264240. MIC13, which is largely expressed in bradyzoites, was recently shown to be important for optimal growth under stressed-induced conditions [284] and, in our study, was important for GT1's lab-adaptation, further suggesting that non-lab-adapted strains introduced into culture will experience novel stress conditions, for example, prolonged extracellular survival during serial passaging. The discovery of MyoI, Gnt, and a TGGT1_264240 do not point to any obvious mechanisms of *in vitro* virulence, thus novel roles of these proteins are yet to be discovered. For example, MyoI residing at the basal complex could be accommodating enhanced lipid scavenging efficiency.

In conclusion, these results indicate that lab-adaptation of GT1 results in augmented phenotypes largely influenced by the natural selection of extracellular parasites during serial passaging. Furthermore, our work demonstrates the phenotypic and transcriptomic pliability of low passage strains under the most basic laboratory conditions commonly practiced in the *T. gondii* field [8]. These results also indicate that lab-adaptive *in vitro* virulence is a complex and polygenic phenotype, at least in our experiment, likely stemming from epigenetic control. Future directions of this study will aim to understand some of the cis- and trans-regulatory factors that control the expression

of these differentially expressed virulence factors (see Chapter 5.2.3.) to decipher the transcriptional networks potentially leading to new therapeutic targets.

5.2. Future Directions

5.2.1. Functional analysis of P4-flippase mutations

Contrary to our original hypothesis, we only identified one gene, P4-flippase, whose CDS evolved during GT1's lab-adaptation. Interestingly, two different mutations (L270R and A477D) emerged within the same exon and around the same time in two parallel evolving populations. Unfortunately, our attempts to functionally characterize these mutations were unsuccessful, likely due to the parasite's NHEJ repair mechanism that is favored over homologous recombination, with the latter necessary to integrate our allele-containing repair template. Therefore, to pursue functional characterization of L270R and A477D, future allele swapping experiments should be performed in novel GT1ΔKu80 parasites, which will ablate NHEJ [310]. Generating GT1ΔKu80 will be performed with two CRISPR/Cas9-mediated DSBs flanking the Ku80 CDS followed by homologous recombination of a DHFR selection cassette (repair template) into the Ku80 locus. Proper DHFR integration into the genome will confer pyrimethamine-resistant parasites and diagnostic PCR can confirm Ku80's removal from the genome. Our CRISPR KO experiments with GT1 invoked this very same KO approach (Figure 4.9b) which was relatively successful. Upon generation of GT1ΔKu80, allele swapping experiments can be repeated as before, this time with >300-fold greater chances of homologous recombination of the allele-containing repair template [310].

5.2.2. Investigating *de novo* FA biosynthesis as a regulator of GT1's lab-adaptive *in vitro* virulence

Enrichment analysis of our extracellular RNA-seq data identified the upregulation of many genes involved in *de novo* synthesis of FAs within the apicoplast, suggesting that FA synthesis is vital to GT1's lab-adaptive virulence. To confirm the relative output of FA between high and low passage GT1 parasites, metabolic radio labeling experiments will be performed. Briefly, exogenous [$^{13}C_6$]-glucose will supplement the media for 48 hours to allow the incorporation of sugar-derived carbon (^{13}C) into FA. Subsequently, gas chromatography-mass spectrometry (GC-MS) will analyze the relative abundance of isotopologues in high vs low passage GT1, specifically C:14 (myristic acid) and C:16 (palmitic acid) FA's which are primarily produced by the apicoplast (Figure 4.8). Should we confirm a significant difference in ^{13}C incorporation into FAs, functional analysis will be performed by FA supplementation experiments. Briefly, *in vitro* virulence will be assessed in high and low passage GT1 parasites by plaque assays supplemented with or without myristic (C:140 and palmitic acid (C:16). We expect supplementation to result in enhanced *in vitro* virulence in low passage GT1 parasites, which would confirm the FASII pathway as an important factor in GT1's lab-adapted virulence.

5.2.3. Determine chromatin dynamics and identify trans-regulatory factors and cis-regulatory motifs controlling GT1's lab-adaptive *in vitro* virulence

In our study, we identify differential gene expression as a vital process for the evolution of *in vitro* virulence, thus we hypothesize that evolution of GT1 is mediated by trans- and cis-regulatory elements. Trans-regulatory elements, such as ApiAP2 TF's, have been show to control several aspects of *T. gondii* biology including asexual replication and bradyzoite differentiation [147-150, 216, 218-220]. ApiAP2 TF's bind to

regulatory elements, typically within gene promoter regions, in a sequence-specific manner to drive gene expression. In *T. gondii,* for example, AP2XI-5 has been shown to recognize the motif GCTAGC and regulate the expression of over 300 genes, many involved in virulence, such as ROP18 [217]. However, it is well known that TF binding and gene regulation is contingent on chromatin accessibility (e.g. heterochromatin and euchromatin) [311]. In *Plasmodium,* for example, chromatin state has been shown to influence antigenic variation and virulence gene expression [206, 207]. Furthermore, an Assay for Transposase-Accessible Chromatin by sequencing (ATAC-seq) confirms the association between the dynamics in accessible chromatin and temporal gene expression profiles in *Plasmodium* [312]. Such experiments assessing chromatin dynamics, however, are lacking in *T. gondii*.

To better understand the underlying expressionmechanisms of our lab-adaptation study, we will utilize ATAC-seq to identify genome-wide regulatory regions that played an essential role in GT1's evolved *in vitro* virulence. Briefly, using the same GT1 cell lines and timepoints utilized in this study, we will use ATAC-seq to determine the dynamics of chromatin accessibility and co-regulated genes during lab-adaptation; extensive computational analysis will also be employed to define candidate cis-regulatory motifs within these co-regulated genes; and DNA-pulls down and mass spectrometry experiments will be utilized to identify interacting proteins, which can consist of chromatin remodeling complexes, AP2 TF's, and other putative TFs (e.g. Myb-domain containing proteins). With the future work proposed here, we expect to identify cis- and trans-regulatory factors that regulated GT1's lab-adaptive *in vitro* virulence. Not only will this

enhance our understanding of apicomplexan biology, but provide a unique opportunity to identify novel therapeutic targets that are regulators of T. gondii's acute lytic cycle.

Chapter 6: Materials and methods

6.1. Cell culture

The GT1 strain of *Toxoplasma gondii* was obtained through BEI Resources (catalog NR-20728) and propagated into culture using ED1 media supplemented with HEPES buffer: 1% heat-inactivated fetal bovine serum (FBS), 10 mM HEPES pH 7.6, 2 mM L-glutamine, 1% penicillin/streptomycin, Dulbecco's Modified Eagle Medium (DMEM), pH 7.2. After inoculation onto a host monolayer consisting of confluent human telomerase reverse transcriptase (hTERT) cells, parasites are incubated at 37ºC in 5% CO_2. Typically, early passage (P) GT1 parasites require 3-4 days to lyse a T25 (25 cm^2) flask of host cells while later passage GT1 parasites (>P80) require 2-3 days. Passing was performed serially by transferring 500 µl (~5%) of the lysed host cell flask into a new T25 flask of hTERT host cells containing 9 ml of warm ED1+HEPES media. Importantly, 1 ml serological pipettes were used for transferring in order to reduce cross-contamination of separate *T. gondii* populations. Serial passaging of GT1 populations occurred in this fashion for up to >P220. Parasite populations were periodically frozen down for future analysis.

6.2. Plaque assay

Plaque assays were performed with 3-6 week old monolayers of human foreskin fibroblasts (HFF) or goat skin fibroblasts cells (GSF) in 6-well plates. GSF cells are courtesy of Dr. Singh from Fort Valley State University [313]. Upon mechanical lysis (27G needle) of parasite vacuoles, 50 RH parasites or 125 GT1 parasites were inoculated onto host cell monolayers and allowed to form plaques undisturbed for 11 days. Plates were

then fixed in 100% ethanol and stained with crystal violet. Plaque size was quantified using FIJI [314].

6.3. Extracellular survival assay

Infected host cell monolayers containing large vacuoles were washed 3x in warm PBS, cell scraped in ED1+HEPES buffer, passed through a 27G needle, and filtered through a 2 µm filter. The resulting cell suspension was then normalized to a final concentration 10,000 cells/ml in ED1+HEPES buffer. Next, 3 ml of the parasite cell suspension was incubated at 37ºC (5% CO_2) in non-tissue-culture-treated 6-well plates. Plaque assays were performed hourly and quantified as mentioned above. Plaque numbers at each timepoint were then normalized to the 0-hour timepoint to yield percent survival.

6.4. Reinvasion assay

Infected host cell monolayers containing large vacuoles were washed 3x in warm PBS, cell scraped in ED1+HEPES buffer, passed through a 27G needle, and filtered through a 2 µm filter. After counting the number of suspended cells, either 50 RH parasites or 125 GT1 parasites were inoculated onto 6-well host cell monolayers and allowed to form plaques undisturbed for 11 days. Plates were then fixed in 100% ethanol and stained with crystal violet. The number of resulting plaques were quantified using FIJI [314], and normalized to input (50 for RH or 125 for GT1) to yield reinvasion efficiency.

6.5. Replication assay

Mechanically egressed parasites (27G needle) were inoculated onto confluent HFF monolayers grown on coverslips, centrifuged at 1000*g for 5 minutes, allowed to invade at 37ºC (floating in a water bath) for 10 minutes, and subsequently washed 3x with PBS. Intracellular parasites were then allowed to replicate for exactly 24 hours followed by methanol fixation and immunofluorescence assay (IFA) with αTgIMC3 and DAPI. The number of parasites per vacuole was enumerated for 100 vacuoles.

6.6. Egress assay

Mechanically egressed RH and GT1 parasite populations were inoculated onto 6-well HFF plates containing glass coverslips and allowed to replicate for 30-hours. Next fresh media containing either 1mM of A23187, 5% EtoH, or DMSO was added for exactly five minutes before fixation of infected monolayers with 4% PFA and IFA with αTgIMC3 and DAPI. The number of egressed vacuoles was enumerated for a total of 50 vacuoles per conditions.

6.7. DNA-sequencing and analysis

Parasite gDNA was isolated using Qiagen DNAeasy Blood and Tissue kit (catalog 69504) according to manufacturer's protocol. Illumina's Library Prep kit (FC-121-1030) was used to generate ~361bp DNA fragments, on average, which were quantified using Quibit Flex Fluorometer (catalog Q32851) and quality checked using Agilent's TapeStation (catalog 5067-5584, 5067-5585). Next, 150bp paired-end sequencing was performed on Illumina's NextSeq500 platform using their high output flow cell kit (FC-

404-2004) according to manufacturer's protocol. FASTQ reads were then analyzed by RUFUS analysis to call sequence variants between two samples (https://github.com/jandrewrfarrell/RUFUS) [263]. High frequency variants were called if the emerging mutation reached ≥75% allele frequency in at least one evolving population.

6.8. RNA-sequencing and analysis

T. gondii infected (24-36 hours) hTERT monolayers were washed 3x with PBS and immediately lysed and processed on ice for RNA isolation using Qiagen RNAeasy kit (catalog 74104) according to manufacturer's protocol. RNA quality was confirmed by measuring the RNA integrity Number (RIN) using Agilent's TapeStation (kit catalog 5067-5579, 5067-5580). Illumina's Library Prep kit (RS-122-2102) was used to generate ~281bp cDNA fragments, on average, which were quantified using Quibit Flex Fluorometer (catalog Q32852) and quality checked using Agilent's TapeStation (catalog 5067-5584, 5067-5585). Next, 75bp paired-end sequencing was performed on Illumina's NextSeq500 platform using their high-output (150 cycles) flow cell kit (FC-404-2002) according to manufacturer's protocol. FASTQ reads were mapped to the *Toxoplasma gondii* GT1 genome (ToxoDB.org) using HISAT2 (Version 2.0.5). Normalized gene expression expressed as counts per million (CPM) and DEA were calculated using the edgeR R package (Version 3.24.3). Time-course sequencing analysis was performed by TCseq R package (Version 1.6.1). Regression analysis was performed by mixed-effect modelling.

6.9. Plasmids and parasite strain generation

Synthesized and annealed forward and reverse oligos (Table 6.2), serving as sgRNAs, were cloned into BsaI digested pU6-Universal plasmid (Addgene #52694) to generate our final CRISPR/Cas9 plasmids. A DHFR selection cassette was amplified with a 60bp primers (Table 6.3) to yield a 2700bp repair template containing the entire 5'UTR, 3'UTR, and CDS of DHFR, along with 39bp arms complementary to the site of Cas9-mediated DSBs within the gene of interest (GOI). To KO a GOI, 20μg of each CRISPR/Cas9 plasmid were co-transfected with 20μg of the DHFR selection cassette to enabled DSB and homology-directed repair at the locus. Successful homologous recombination of the DHFR selection cassette into the GOI locus was confirmed with diagnostic PCR primer sets (Table 6.4). Successful ablation of mRNA expression was confirmed by qPCR (Tables 6.5).

6.10. Life stage score analysis

Tachyzoite, merozoite, and bradyzoite (tissue cyst) RNA-seq datasets that were used to generate stage-scores were published by Ramakrishnan *et al.* [276]; Sporozoite RNA-seq datasets that were used to generate stage scores were published by Fritz *et al. [315]*; all RNA-seq datasets were obtained from ToxoDB.org and downloaded as TGME49 gene IDs, which were then converted into TGGT1 gene IDs using the "syntenic orthologs" tool on ToxoDB.org. This process excluded 496 TGGT1 gene ID's from the analysis. Four singular timepoints from these available datasets were chosen for DE analysis ("tachyzoites", "tissue cysts", "Mero 3", and "Sporozoite day 4"). For each gene, all three possible differential expression scenarios between these four datasets were

calculated. For each gene, the number of ≥2-fold upregulated scenarios were enumerated and is considered the "stage-score" (score = 0 to 3). To validate the established stage scores, three previously published gene sets were examined for significant enrichment in their indicated life stage [316]. For analysis of our RNA-seq data sets, the DE-score of our upregulated and down-regulated gene sets were calculated. Bootstrap analysis (n = 1000 random sampling) was performed on each individual life stage in order to assign significance to the dataset's sample distribution.

6.11. Enrichment analysis

Previously published gene sets were utilized for GSEA [316] and statistical analysis was run using Fisher's exact test to calculate the significance of enrichment between published datasets and our generated datasets. GOEA and MPEA was performed using the "Analyze results" feature on ToxoDB.org (https://toxodb.org/toxo/analysisTools.jsp). Redundant GO terms were removed from GOEA analysis using the REVIGO analysis feature on ToxoDB (http://revigo.irb.hr/) with 0.4 similarity ("tiny") allowed between GO terms.

6.12. Statistical analysis

A student's two-tailed equal variance *t* test was used to determine the significance (*p*-value) of evolved samples compared to the lowest passage (P) sample. Adjusted *p*-values (*q*-values) were calculated using the false discovery rate (FDR) method.

Table 6.1. Primers used for PCR-sequencing of SNPs

List of 15 primers names and sequences (5' to 3') used to validate SNPs identified by RUFUS sequence analysis. M13 tail sequences (red) were added to the 5' end of PCR primers.

Table 6.1. Primers used for PCR-sequencing of SNPs

Chromosome	Gene ID	Codon Change	Mutation	PCR size	Forward Primer (M13 Forward)	Reverse Primer
TGGT1_chrVIIa	TGGT1_016250	ATT to AAT	Ile to Asn	585	TGTAAAACGACGGCCAGTTGTTGACGAATCCCCCAAAG	CTTTTGCTTTGTGCTGCATG
TGGT1_chrX	TGGT1_081400	GCT to GAT	Ala to Asp	582	TGTAAAACGACGGCCAGTAGAGCAACAAGCCGATACTG	CGACTTTTCTCCCTAGAAGC
TGGT1_chrVIIb	TGGT1_011730	AAT to CAT	Asn to His	576	TGTAAAACGACGGCCAGTTCTTTGCCCTTCTTCTGAAG	GAGCAAGAGGATGTAAATCC
TGGT1_chrII	TGGT1_065060	TGT to TCT	Cys to Ser	557	TGTAAAACGACGGCCAGTCTTTCTAAAGGAACTATCCC	TCTGGCGTTTTCGTTACCTC
TGGT1_chrVIIb	TGGT1_007550	CTC to CGC	Leu to Arg	550	TGTAAAACGACGGCCAGTAAAACTCGTTCTCTGTGTAG	AGAACCAAGGGCGTTTCAAG
TGGT1_chrIV	TGGT1_122460	GTG to GGG	Val to Gly	493	TGTAAAACGACGGCCAGTCACAAGGAACAGACAAATTC	AGTGGCACCTACCGAATATC
TGGT1_chrVIII	TGGT1_116720	GGG to GCG	Gly to Ala	495	TGTAAAACGACGGCCAGTTAGACTGGCGTGAGTTTGAC	ATATACATGGACACCTGTAG
TGGT1_chrXI	TGGT1_098520	GTT to TTT	Val to Phe	490	TGTAAAACGACGGCCAGTGTTCGCACCTGAAACTTATG	TACGGACAATGCGCATTTGG
TGGT1_chrVI	TGGT1_051830	GAG to AAG	Glu to Lys	459	TGTAAAACGACGGCCAGTCATGTGACTTCTGTGTCTTG	ATCTGGCGTTGGAAATCCAC
TGGT1_chrVIII	TGGT1_113990	TCC to TTC	Ser to Phe	526	TGTAAAACGACGGCCAGTTCAGCAAGTGGTTTTTTGGC	TTCTGTGGAGCACATACGTG
TGGT1_chrVIII	TGGT1_060660	TTT to TCT	Phe to Ser	536	TGTAAAACGACGGCCAGTTCCTCTCTCTCGTCACATTC	GCATTTCCAGTTTTCTCTTG
TGGT1_chrIX	TGGT1_041270	CAG to AAG	Gln to Lys	526	TGTAAAACGACGGCCAGTTTCGGAAAAAGACCAAGCAC	GTTCAGAGCATCTCTCAGAG
TGGT1_chrX	TGGT1_071670	GAG to AAG	Glu to Lys	514	TGTAAAACGACGGCCAGTCATTCCTGTTCACATGGTTG	GTTGCATGCGCTAAATCGAC
TGGT1_chrXI	TGGT1_088340	GAC to GCC	Asp to Ala	533	TGTAAAACGACGGCCAGTTTGGACGTTGTTGATCGAAG	ACTTGGAGCTCCAAAGGAAC
TGGT1_chrXII	TGGT1_103920	AGT to ACT	Ser to Thr	512	TGTAAAACGACGGCCAGTCATGCAGGAGGTTCCTTATC	CTTGTTCGCCGTCACTTTTG

Table 6.2. Protospacers for cloning into pU6-Universal plasmid

List of primer sequences (5' to 3') used for cloning into the pU6-Universal plasmid (Addgene plasmid # 52694). sgRNA sequences are red and cloning flanks into the plasmid are in black.

Table 6.2. Protospacers for cloning into pU6-Universal plasmid

Target Gene ID	Direction	Protospacer 1 + cloning flanks	Protospacer 2 + cloning flanks	Product Description
TGGT1_262590	Fwd:	AAGTTGACGCGCGTCTTGTGAGGGCG	AAGTTGCAGACGAACGTACCGACGAG	Hypothetical protein
	Rev:	AAAACGCCCTCACAAGACGCGCGTCA	AAAACTCGTCGGTACGTTCGTCTGCA	
TGGT1_264240	Fwd:	AAGTTGCGGGGTTGTGGTTTCGTGCCG	N/A	Hypothetical protein
	Rev:	AAAACGGCACGAAACCACAACCCCCGCA	N/A	
TGGT1_230980	Fwd:	AAGTTGATGGAGCTGGCGGCGGGCAGG	AAGTTGGGCCAGCCGGAGCTCGAGGG	Myosin I
	Rev:	AAAACCTGCCCGCCGCCAGCTCCATCA	AAAACCCTCGAGCTCCGGCTGGCCCA	
TGGT1_260190	Fwd:	AAGTTGTCTGAAGAAACTGATATCGTG	AAGTTGCAATGCGTGCAGATGCCGAG	MIC13
	Rev:	AAAACACGATATCAGTTTCTTCAGACA	AAAACTCGGCATCTGCACGCATTGCA	
TGGT1_315885	Fwd:	AAGTTGGATGCATGCGGCAGAACGAG	AAGTTGAAGTCACTGCCGAAGAGCACG	Putative glycosyltransferase
	Rev:	AAAACTCGTTCTGCCGCATGCATCCA	AAAACGTGCTCTTCGGCAGTGACTTCA	

Table 6.3. PCR primers used to generate a DHFR cassette

Long PCR primers sequences (5' to 3') used for amplifying the DHFR selection cassette. Sequences that recognize the 5' UTR (Fwd) or 3'UTR (Rev) of the DHFR gene are in red. Primer tails consist of 38-39bp homologous arms (black) which allow for recombination of the DHFR cassette into *T. gondii's* genome.

Table 6.3. PCR primers used to generate a DHFR cassette

Target Gene ID	Direction	DHFR-specific primer + cloning flanks	Product Description
TGGT1_262590	Fwd:	AGACACCAGTGCGACACCTGCAGAAACTGACGCGCGTCCACGAAACCTTGCATTCAAACC	Hypothetical protein
	Rev:	TGTGACTCCGCGCTCCGTCCTTTTTCGTTACGCTTCAGAATCCTGCAAGTGCATAGAAGG	
TGGT1_264240	Fwd:	TGATTCGCTGTGTTCGCAGATGTAGGGGCGGGGTTGTGCACGAAACCTTGCATTCAAACC	Hypothetical protein
	Rev:	CGCACAGCAATAATGACGGAGATGGAGCTGCACGTGGCTATCCTGCAAGTGCATAGAAGG	
TGGT1_230980	Fwd:	CTCGGGCTAGCCGGCGTGTGCTGCAACGATGGAGCTGGCACGAAACCTTGCATTCAAACC	Myosin I
	Rev:	CTTCTTTCTTTTCTGTCTCTGCTTCGCCGCGTTCTCCTCATCCTGCAAGTGCATAGAAGG	
TGGT1_260190	Fwd:	GTCGAGCAGAGCTTTGTATCTGAAGAAACTGATATCACGAAACCTTGCATTCAAACC	MIC13
	Rev:	CCGTTCCTTTGCAGGAATCGGCACATTGCCCTTCGATCCTGCAAGTGCATAGAAGG	
TGGT1_315885	Fwd:	CGTCAGACGGGAACTCGACTGCAAACATGGATGCATGCCACGAAACCTTGCATTCAAACC	Putative glycosyltransferase
	Rev:	TCAAAAGCGAAAGAAGTAATTGAACACCTTCGCGCTGTGATCCTGCAAGTGCATAGAAGG	

Table 6.4. Diagnostic PCR primers for confirming homologous recombination

List of primer sequences (5' to 3') used for confirming the integration of the DHFR selection cassette into the proper genomic locations. Primers in black are specific to the indicated gene of interest while primers in red are specific to DHFR.

Table 6.4. Diagnostic PCR primers for confirming homologous recombination

Target Gene ID	Direction	PCR 1 (DHFR-specific primer)	PCR 2 (DHFR-specific primer)	Product Description
TGGT1_262590	Fwd:	GACCAGGAGGACACAGAGTG	GACGCTCATGGTTGCACACG	Hypothetical protein
	Rev:	AGCGCTGCCTCGTACAGTC	CGTCTCCAGCATATCTCCGAC	
TGGT1_264240	Fwd:	ACGAGCTGATACCAAGACGCG	GACGCTCATGGTTGCACACG	Hypothetical protein
	Rev:	AGCGCTGCCTCGTACAGTC	GGCCGATGTGTTCGTGTCAAC	
TGGT1_230980	Fwd:	GTTTCGTCCGTCCACGTGC	GACGCTCATGGTTGCACACG	Myosin I
	Rev:	AGCGCTGCCTCGTACAGTC	CCGATGAAGAGTCGGCTGTC	
TGGT1_260190	Fwd:	CAGCTGTGCCTCCTAACGTG	GACGCTCATGGTTGCACACG	MIC13
	Rev:	AGCGCTGCCTCGTACAGTC	CTAGCACTCTGTCGAGGCG	
TGGT1_315885	Fwd:	CCATTCAGACCTGAAGTGTTCG	GACGCTCATGGTTGCACACG	Putative glycosyltransferase
	Rev:	AGCGCTGCCTCGTACAGTC	GACACCGTAGAAGACGGTTTG	

Table 6.5. qPCR primers for confirming mRNA ablation

List of primer sequences (5’ to 3’) used for confirming ablation of mRNA expression of the indication gene of interest.

Table 6.5. qPCR primers for confirming mRNA ablation

Target Gene ID	Direction	qPCR	Product Description
TGGT1_262590	Fwd:	ACTCTTTGAATGGGGAGAGCAG	Hypothetical protein
	Rev:	CTGTTCTCCGGCGAAATCTCG	
TGGT1_264240	Fwd:	GGTTGTGGTTTCGTGCCCG	Hypothetical protein
	Rev:	CCCACATGCAGAACTGCTGC	
TGGT1_230980	Fwd:	GAGTCGCTTTGGGAAGTTCACG	Myosin I
	Rev:	GGACAGAAACTGCCTTGCACC	
TGGT1_260190	Fwd:	GCAAGGTGCAGTGTGTCGATG	MIC13
	Rev:	CATTTGGAATGGACTGCAGTGC	
TGGT1_315885	Fwd:	AGCAACCTGAACGAGGAATCC	Putative glycosyltransferase
	Rev:	GAGAAAGCAGAAGTATTCGGG	
TGGT1_245510	Fwd:	GATGGCTCTGTGCCATTCCG	P4-Flippase
	Rev:	CTCCCCGACAATTCGCGAAAC	

Appendix A: Evaluating the differences between intracellular and extracellular tachyzoites

A.1. Introduction

We originally performed RNA-seq on both 30-hour intracellular and 6-hour extracellular parasites across several *in vitro* passages in order to identify DE virulence factors within both milieus of the parasites lytic cycle; however, this data was also analyzed in absence of a temporal context in order to analyze the transcriptomic profiles of intracellular vs extracellular parasites. Previous studies have indicated that the transcriptome of extracellular tachyzoites represent a transcriptomic state similar to that of bradyzoites [316]. Conversely, the transcriptome of extracellular mutant tachyzoites incapable of converting into bradyzoites more closely resemble intracellular parasites, supporting a link between bradyzoite development and the extracellular transcriptome [317]. Moreover, extracellular parasites are also enriched in G1-related transcripts of the cell cycle [316, 318], and G1-blockage of the cell cycle in intracellular parasites inhibits bradyzoite conversion [319]. Taken together, there appears to be an association between the process of bradyzoite conversion and genes found in the extracellular and G1 state.

Being obligate intracellular parasites, *T. gondii* cannot fully convert into bradyzoites while in their extracellular milieu, thus begging the question: why express bradyzoite genes during a period of extracellular survival. Current insights suggest bradyzoites expression is part of a stress response mechanism as the expression of canonical bradyzoite markers are observed in conditions of nutrient deprivation, alkaline pH, and heat shock of intracellular parasites [142, 143]. Interestingly, tachyzoites exposed to stress of 12-hour extracellular conditions can also induce bradyzoite differentiation upon their subsequent reinvasion of host cells [320]. Because bradyzoite differentiation is a critical biological process to ensure *T. gondii's* survival and continued transmission *in*

vivo, these responses to exogenous stress suggest that the parasite is capable of sensing danger in its environment both intracellularly and extracellularly [321]. Such anecdotal suggestions, however, have yet to be confirmed empirically and to understand why extracellular tachyzoites respond this way we must first better understand what the response is at the molecular level. Therefore, we sought to better characterize the molecular response to the extracellular milieu. To do so, we compared our previously generated intracellular and extracellular RNA-seq data sets and applied our RNA-seq analysis pipeline to perform DEA followed by stage scoring, GSEA, GOEA, and MPEA in order to uncover biological insights invoked during extracellular milieus, relative to the intracellular milieu. In summary, our data indicate a unique and mostly metabolically inactive transcriptomic state for extracellular parasites that can be described as anti-tachyzoite, G1a-like, and quasi-bradyzoite. We discuss these profiles in the context of our lab-adaptation experiments (Chapter 4). Furthermore, we also identify many AP2 and Myb-domain containing TF that are DE upon extracellular survival, suggesting these as potential regulators of this unique transcriptional state.

A.2. Results

A.2.1. Extracellular tachyzoites represent a unique transcriptomic state resembling an anti-tachyzoite state halted at an early G1a-like state

In this study, we performed RNA-seq on 30-hour intracellular and 6-hour extracellular RH and GT1 (B2-P11) in order to identify the molecular factors and biological processes involved in extracellular parasites (Figure A.1.a). DEA identified 1597 and 1500 downregulated DEGs, relative to intracellular RH and GT1, respectively, of which 1204 (~75-80%) were in common (Figure A.1.b); also, DEA identified 1584 and 1555 upregulated DEGs in RH and GT1, respectively, of which 1189 (~76%) were in common (Figure A.1.b). Taken together, DEA across these two type I strains reveals a high degree of similarity to the response to extracellular conditions (Figure A.1.b).

Life stage scoring of these DEGs show a significant upregulation on bradyzoite-, merozoite-, and sporozoite-associated genes while there is a significant downregulation of tachyzoite- and bradyzoite-associated genes (Figure A.2). While bradyzoite genes are both upregulated and downregulated, these data strongly indicate an anti-tachyzoite-like transcriptomic state in extracellular parasites. Interestingly, a master regulator of bradyzoite differentiation, BFD1, is significantly upregulated, along with an array of bradyzoite activators and repressors that are both up- and downregulated upon extracellular survival (Table A.1). Such misregulation indicates a complex network of bradyzoite differentiation that is finely balanced to bring about the quasi-bradyzoite state observed in extracellular parasites.

Because extracellular parasites cannot continue their cell cycle, we next investigated the cell cycle state of extracellular parasites with GSEA [130, 316]. As

previously reported [316], both RH and GT1 extracellular tachyzoites are enriched in G1-related genes (Figure A.3). More specifically, we were able to identify a restriction relative to G1a-related genes as there is an abrupt halt before predicted G1b progression [322], indicating that extracellular parasites are being halted at a G1a-to-G1b checkpoint (Figure A.3).

A.2.2. Upon extracellular survival, parasites turn off many metabolic pathways and turn on regulatory genes

Of the DEGs downregulated upon extracellular survival, GSEA identified the downregulation of many genes associated with: metabolism, biosynthesis, DNA replication and repair, protein processing in the ER, bradyzoites, and many more (Figure A.4). GOEA identified the downregulation of many genes associated with: metabolism, Golgi vesicle transport, DNA replication, protein folding, glycosylation, and many more (Figure A.5). MPEA identified the downregulation of genes associated with: tRNA charging, glycolysis, pyruvate metabolism, TCA cycle, and many more (Figure A.6).

Of the DEGs upregulated upon extracellular survival, GSEA identified the upregulation of far fewer genes, which were associated with: micronemes, bradyzoites, and sporogenesis (Figure A.4). GOEA identified the upregulation of genes associated with: protein kinase/phosphorylation activity, microtubule motor activity, TF activity, and a few others (Figure A.5). MPEA identified the upregulation of genes associated with: FA degradation, lipoxygenase, and a few others (Figure A.6). Taken together, these data suggest that, along with the cell cycle, cell metabolism is largely turned off in extracellular parasites when compared to intracellular. Instead, regulatory processes such as protein

phosphorylation and DNA-binding TFs are upregulated. In fact, >50% of *T. gondii*'s AP2 TFs are DE upon extracellular survival (Table A.1). Interestingly, lipid peroxidation appears upregulated due to increase lipoxygenase activity, suggesting lipids may serve a protective role from ROS during extracellular survival conditions.

A.3. Discussion

In our analysis of the extra- vs intracellular transcriptome, we find that tachyzoite-associated genes are largely downregulated in the extracellular state of RH and GT1 B2-P11 parasites, suggesting that extracellular conditions largely promote an anti-tachyzoite transcriptome. Interestingly, temporal analysis of GT1 during lab-adaptation (Chapter 4) indicates a pro-tachyzoite transcriptome in extracellular parasites- when compared to B2-P11 extracellular GT1 parasites (Figure 4.4). Taken together, DEA across conditions and over time suggest that the extracellular milieu of the lytic cycle induces an anti-tachyzoite response in the cell that can be slowly shifted in a pro-tachyzoite direction with prolonged lab-adaptation. Such a transcriptomic profile is associated with tachyzoite tendencies and *in vitro* virulence (Figure 2.1 & Figure 4.4). Understanding the transcriptional regulators (e.g. AP2 TFs) and biological mechanisms (e.g. lipid peroxidation, lipid biosynthesis) behind this response would aid in our understanding of the understudied extracellular milieu of *T. gondii*'s lytic cycle.

In our analysis of the extra- vs intracellular transcriptome, we find that bradyzoite-associated genes are largely misregulated during the extracellular state of RH and GT1 B2-P11 parasites (Figure A.3 & Figure A.4). Temporal analysis of GT1 during lab-adaptation (Chapter 4) also reveals up- and downregulation of many bradyzoite associated genes- relative to B2-P11 extracellular GT1 parasites (Figure 4.4). We hypothesize this may be due to a stress response pathway of extracellular parasites, perhaps through oxygen sensing mechanisms as extracellular parasites *in vitro* are exposed to near 21% pO_2, as compared to 1-10% pO_2 intracellularly [79]. Interestingly, parasites are more likely to undergo spontaneous differentiation into bradyzoites when

left extracellular for several hours before inoculation [320]; in the context of our analysis, this suggests that the extracellular environment is transcriptionally priming parasites for bradyzoite differentiation in response to stress. Whether or not this response aids in GT1's *in vitro* virulence or extracellular virulence traits still needs to be explored but could provide a new perspective on how we view the extracellular milieu.

Figure A.1. Examining differentially expressed genes (DEGs) between intracellular and extracellular type I parasites

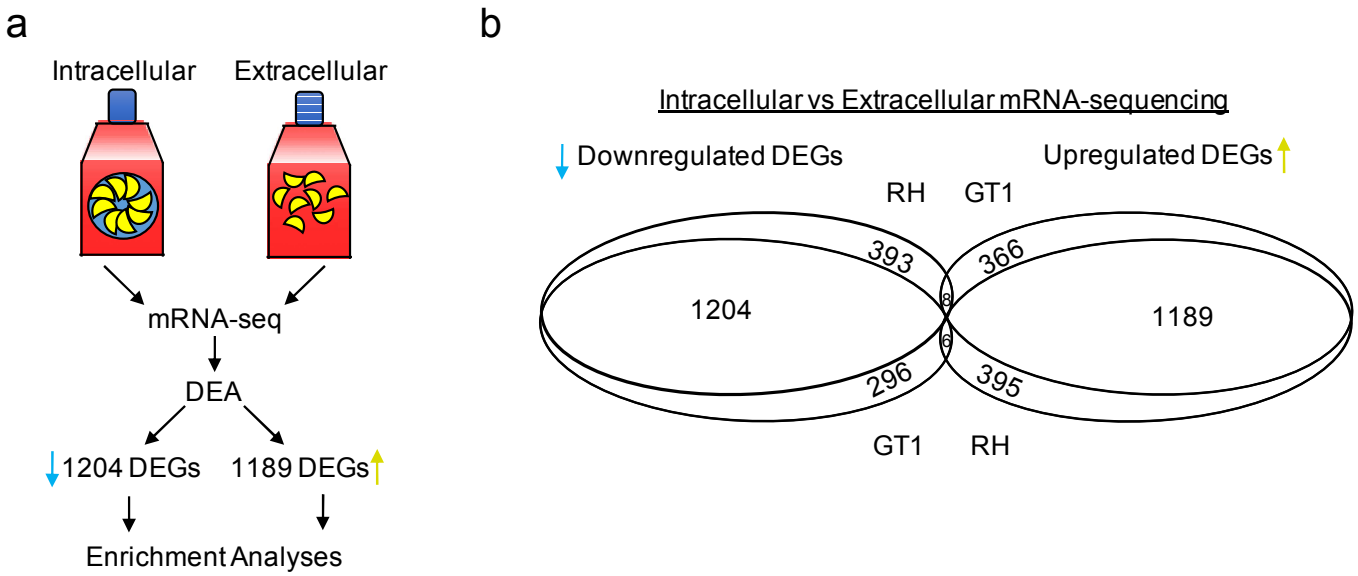


Figure A.1. Examining differentially expressed genes (DEGs) between intracellular and extracellular type I parasites

a.) Roughly 30-hour intracellular and 6-hour extracellular parasites were harvested for mRNA-sequencing (RNA-seq) and data was analyzed by differential expression analysis (DEA), followed by life stage analysis, gene set enrichment analysis (GSEA), gene ontology enrichment analysis (GOEA), and metabolic pathway enrichment analysis (MPEA) in order to identify biological insights into lab-adaptation. **b.)** Venn diagram of DEGs identified across RH and B2-P11 GT1 populations.

Figure A.2. Life stage scoring identified anti-tachyzoite gene expression in extracellular type I parasites

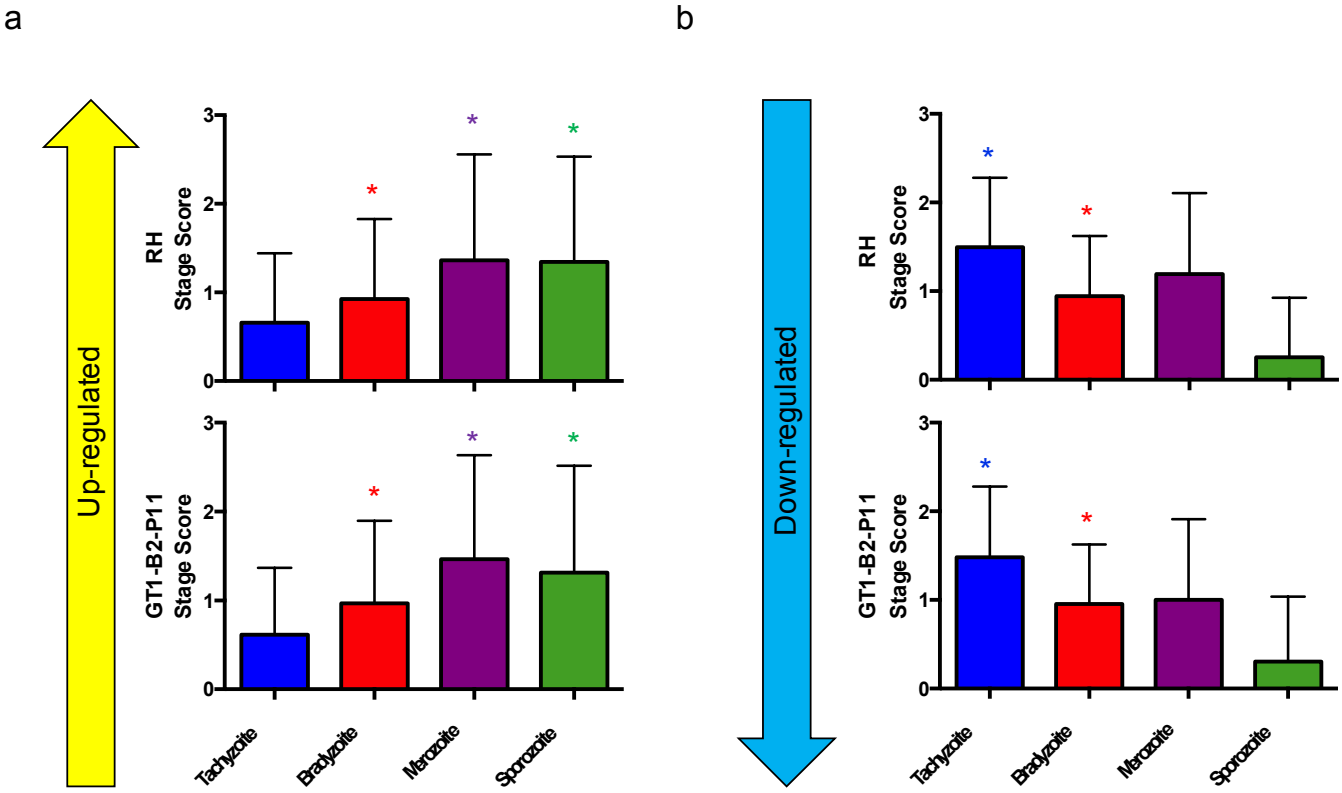


Figure A.2. Life stage scoring identified anti-tachyzoite gene expression in extracellular type I parasites

Life stage scoring was performed on **a.)** upregulated and **b.)** down-regulated DEGs identified by DEA of extracellular parasites, relative to intracellular parasites. Color-coded asterisks (*) indicate *p*-value ≤0.05, as determined by an independent bootstrap analysis (n=1000 random sampling) for each individual life stage.

Figure A.3. The cell cycle of extracellular parasites is halted at a G1a state

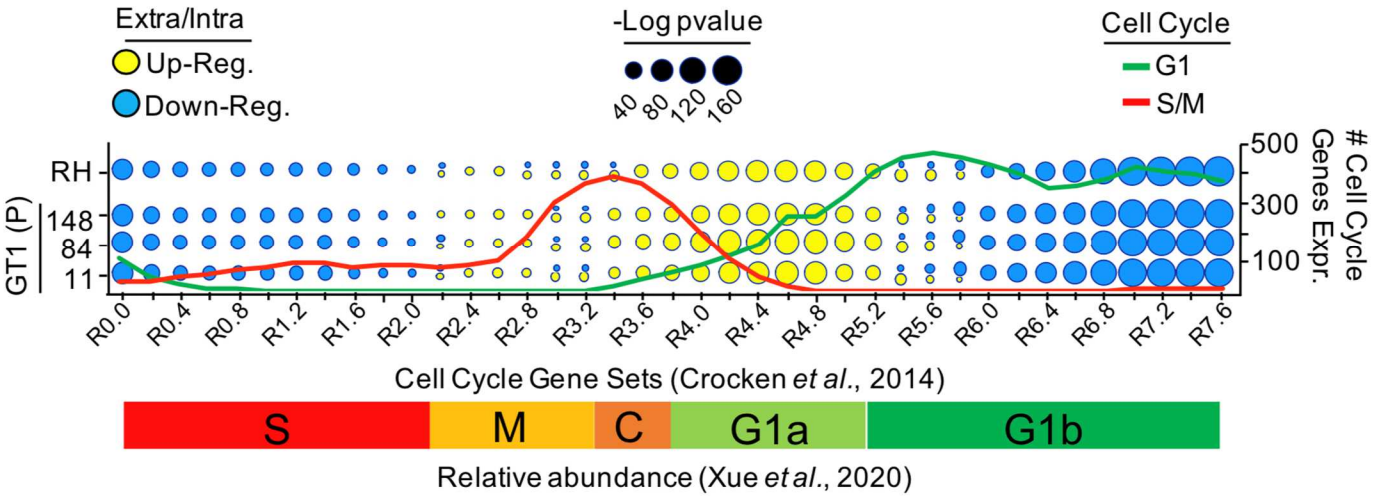


Figure A.3. The cell cycle of extracellular parasites is halted at a G1a state

GSEA analysis of DEGs identified in extracellular parasites, relative to intracellular parasites (left y-axis), in the context of time-coursed cell-cycle data that was compiled and curated by *Crocken et al.* into published GSEA gene lists (x-axis) [316]. The number of S/M or G1 genes expressed at each timepoint is indicated by the right y-axis. Genes we identified as upregulated during extracellular conditions are in yellow, while downregulated genes are in blue. The statistical significance of the overlap between our dataset and published cell cycle gene lists are indicated by the size of the circles. Xue *et al.* recently published, at better resolution, the relative amount of time each step of the cell cycle is [322]; this abundance is aligned to scale with our data set.

Figure A.4. Gene set enrichment analysis (GSEA) of differentially expressed genes (DEGs) identified in extracellular type I parasites

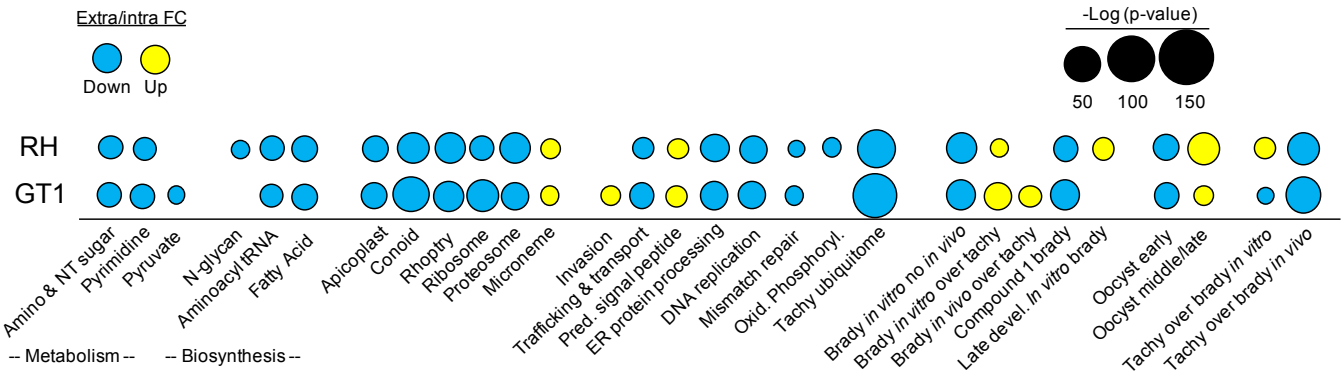


Figure A.4. Gene set enrichment analysis (GSEA) of differentially expressed genes (DEGs) identified in extracellular type I parasites

Up- and down-regulated DEGs identified by DEA of RH and B2-P11 GT1 populations were subject to GSEA, which utilizes previously published gene sets that describe organelle-associated genes, metabolic genes, life stage genes, and genes involved with different biological processes. Genes we identified as upregulated during extracellular conditions are in yellow, while down-regulated genes are in blue. The statistical significance of overlap between our set of genes with these previously published gene sets are indicated by the size of the circle.

Figure A.5. Gene ontology enrichment analysis (GOEA) of differentially expressed genes (DEGs) identified in extracellular type I parasites

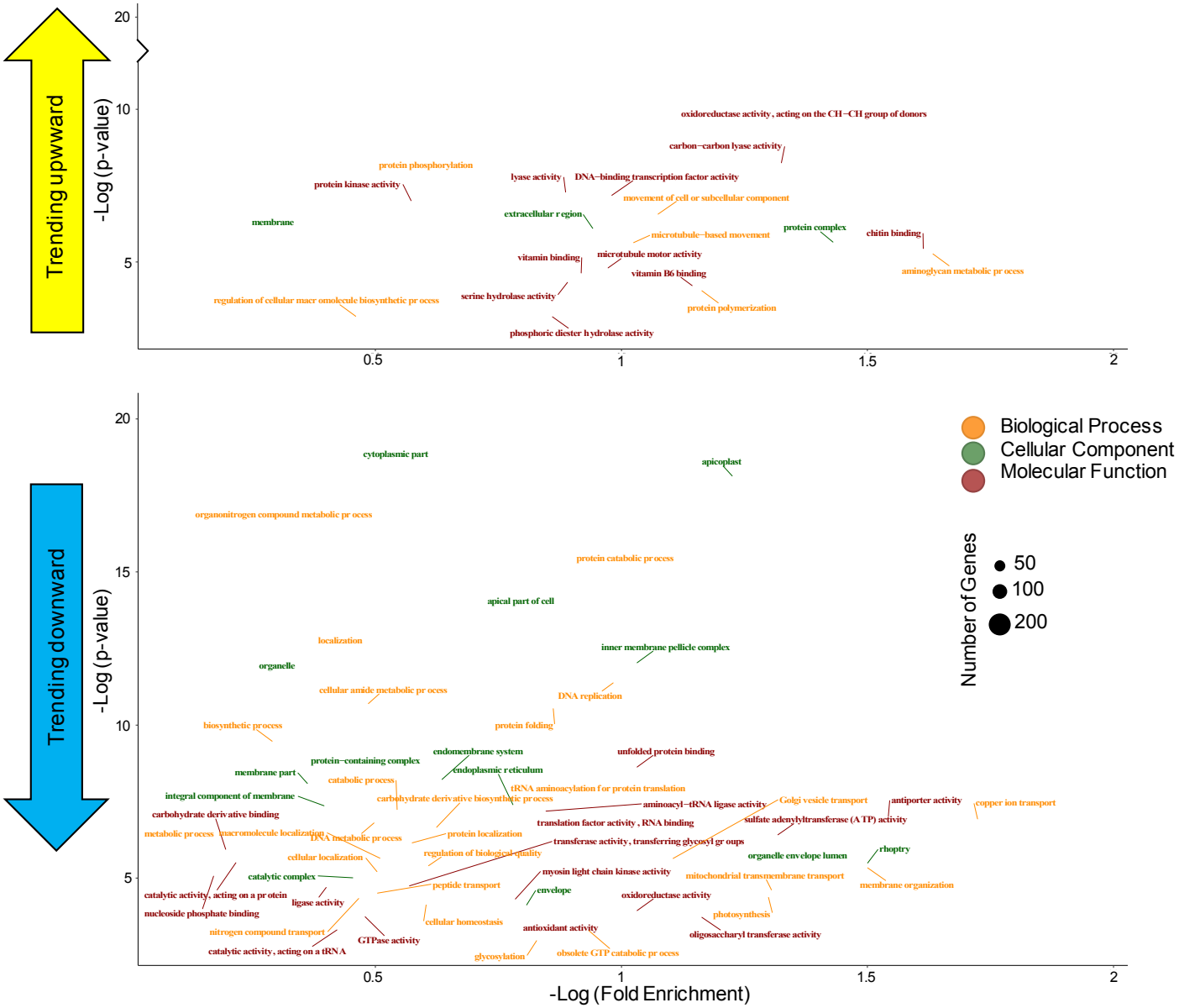


Figure A.5. Gene ontology enrichment analysis (GOEA) of differentially expressed genes (DEGs) identified in extracellular type I parasites

Up- and down-regulated DEGs identified by DEA of B2-P11 GT1 populations were subject to GOEA on ToxoDB.org to identify GO-terms associated with biological processes, molecular functions, and cellular components. Redundant GO terms were removed by REVIGO analysis available on ToxoDB.org (see methods).

Figure A.6. Metabolic pathway enrichment analysis (MPEA) of differentially expressed genes (DEGs) identified in extracellular type I parasites

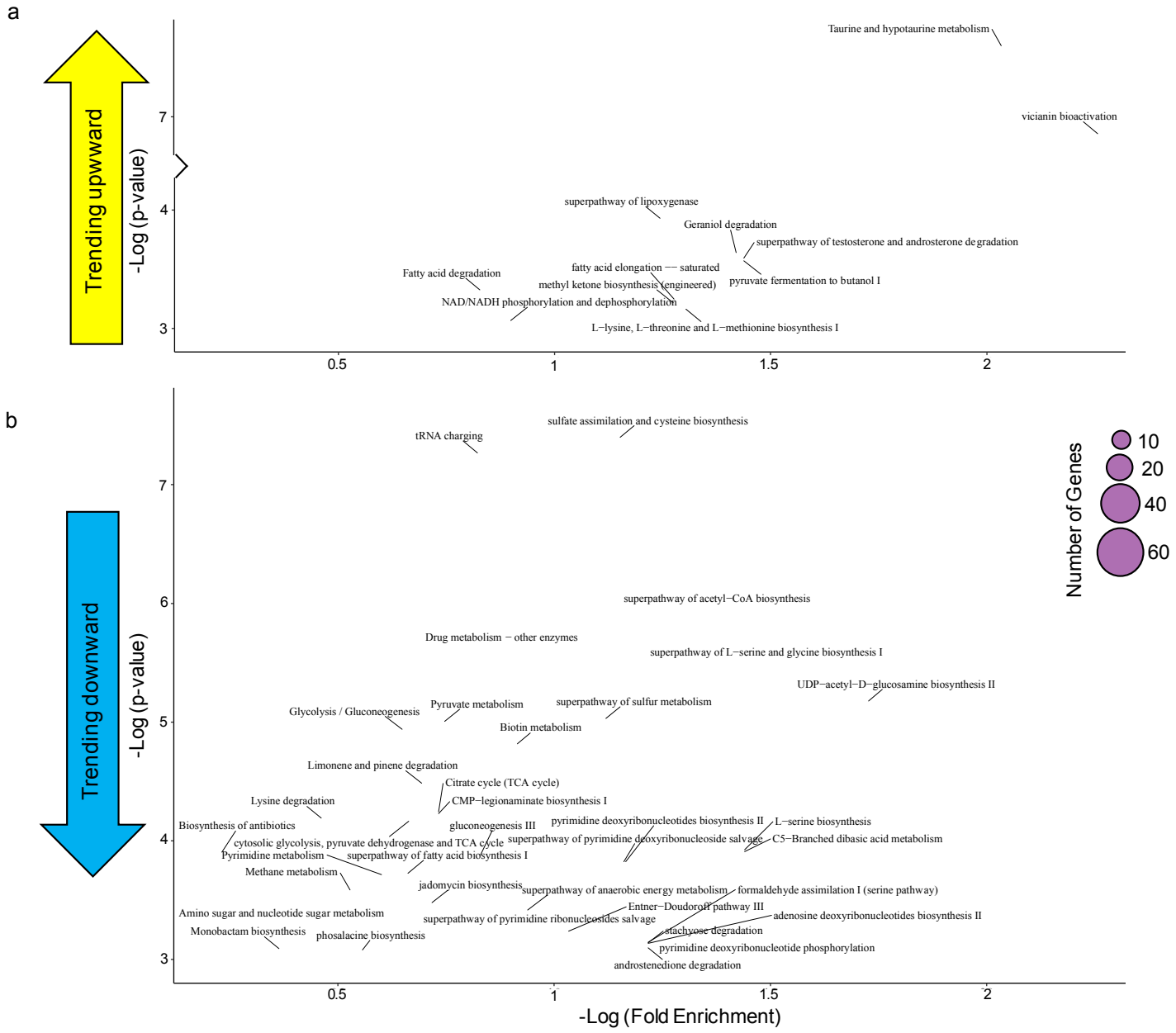


Figure A.6. Metabolic pathway enrichment analysis (MPEA) of differentially expressed genes (DEGs) identified in extracellular type I par8asites

Up- and down-regulated DEGs identified by DEA of B2-P11 GT1 populations were subject to MPEA on ToxoDB.org to identify associated metabolic pathway. Several identified terms have been manually removed due to redundancy (see methods).

Table A.1. DE of many trans-regulatory elements upon prolonged extracellular survival

List of AP2 TFs and Myb-domain containing proteins that are differentially expressed in B2-P11 GT1 populations upon extracellular conditions, relative to intracellular conditions.

Table A.1. DE of many trans-regulator elements upon prolonged extracellular survival

Gene ID	RH	Log2 FC (Relative to intracellular)				Product Description
		P7	P11	P85	P148	
TGGT1_208020	5.70	4.68	4.77	4.54	5.77	AP2 domain transcription factor AP2Ib-1
TGGT1_306620	4.43	3.39	4.37	4.12	4.37	AP2 domain transcription factor AP2IX-9
TGGT1_252370	2.11	1.19	2.62	1.77	3.70	AP2 domain transcription factor AP2III-1
TGGT1_299020	1.01	2.87	3.18	2.20	3.26	AP2 domain transcription factor AP2III-4
TGGT1_215895	2.48	2.20	2.32	2.71	2.84	AP2 domain-containing protein
TGGT1_203050	1.92	2.23	2.33	2.16	2.24	AP2 domain transcription factor AP2VIIa-6
TGGT1_318610	1.77	1.73	2.44	1.16	1.65	AP2 domain transcription factor AP2IV-3
TGGT1_272710	1.76	1.72	1.80	1.52	1.63	AP2 domain transcription factor AP2VIII-4
TGGT1_214960	1.76	1.81	2.02	1.51	1.60	AP2 domain transcription factor AP2X-8
TGGT1_224230	2.42	1.51	1.94	1.51	1.58	AP2 domain transcription factor AP2X-3
TGGT1_264485	4.59	2.37	2.07	1.55	1.49	AP2 domain transcription factor AP2IX-3
TGGT1_280470	0.71	1.38	1.55	1.36	1.42	AP2 domain transcription factor AP2VIIa-1
TGGT1_227900	1.52	0.54	0.75	1.12	1.40	AP2 domain transcription factor AP2X-1
TGGT1_269010	1.04	1.15	1.60	1.12	1.31	AP2 domain transcription factor AP2VIII-7
TGGT1_240900	1.23	1.82	2.02	1.29	1.23	AP2 domain transcription factor AP2VI-2
TGGT1_310900	1.39	1.13	1.17	0.92	1.03	AP2 domain transcription factor AP2XI-2
TGGT1_315760	1.07	0.81	0.88	0.83	0.98	AP2 domain transcription factor AP2XI-4
TGGT1_249190	0.98	1.07	0.80	1.34	0.96	AP2 domain transcription factor AP2XII-6
TGGT1_285895	1.27	0.95	0.99	0.65	0.93	AP2 domain transcription factor AP2V-2
TGGT1_309410	0.47	0.99	1.21	1.05	0.90	AP2 domain transcription factor AP2XI-1
TGGT1_200385	0.83	0.89	0.93	0.83	1.05	Myb family DNA-binding domain-containing protein
TGGT1_321450	1.38	1.03	1.32	0.68	0.91	Myb family DNA-binding domain-containing protein
TGGT1_282210	-0.77	-0.36	-0.47	-0.72	-1.07	AP2 domain transcription factor AP2VIIa-8
TGGT1_215150	-1.19	-1.27	-1.12	-1.51	-1.18	AP2 domain transcription factor AP2X-9
TGGT1_203710	-2.72	-1.12	-0.91	-1.31	-1.25	AP2 domain transcription factor AP2VIIa-4
TGGT1_313450	-1.10	-1.05	-0.86	-0.93	-1.28	AP-2 complex subunit sigma-1, putative
TGGT1_237090	-1.59	-1.94	-1.81	-2.22	-1.60	AP2 domain transcription factor AP2X-5
TGGT1_205650	-2.39	-2.03	-2.08	-1.79	-1.60	AP2 domain transcription factor AP2VIIa-3
TGGT1_251740	-0.70	-1.06	-0.77	-1.72	-1.60	AP2 domain transcription factor AP2XII-9
TGGT1_240460	-1.48	-1.34	-1.43	-1.66	-1.79	AP2 domain transcription factor AP2VI-1
TGGT1_289710	-1.81	-1.21	-1.19	-1.92	-1.90	AP2 domain transcription factor AP2IX-5
TGGT1_318470	-1.06	-0.99	-0.68	-1.90	-2.00	AP2 domain transcription factor AP2IV-4
TGGT1_253380	-1.48	-1.70	-1.63	-2.07	-2.13	AP2 domain transcription factor AP2III-2
TGGT1_244510	-3.14	-3.30	-3.30	-2.48	-2.34	AP2 domain transcription factor AP2VI-3
TGGT1_271200	-3.04	-2.58	-2.22	-2.59	-2.57	AP2 domain transcription factor AP2VIII-5
TGGT1_217700	-2.32	-1.91	-1.70	-2.88	-3.63	AP2 domain transcription factor AP2XII-2
TGGT1_306320	-1.72	-2.33	-2.07	-1.22	-1.16	Myb family DNA-binding domain-containing protein

Appendix B: Tables of mutations and DEGs from Chapter 2 & 3

Table 3.1. Whole genome sequencing (WGS) of RH and low passage GT1

WGS of RH and low passage GT1 populations B0-P4, B2-P15, B4-15, and B6-P10 followed by RUFUS analysis identified 207 SNPs/indels between RH and GT1 and 0 SNPs/indels between low passage GT1 populations. Allele frequencies, the percentage of reads with the indicated allele, are represented by the blue/yellow heat map.

Table 3.1. Whole genome sequencing (WGS) of RH and low passage GT1

Gene ID	Chrom.	Position	Amino Acid	RH	GT1 Clones				Product Description
					B0-P4	B2-P15	B4-P15	B6-P10	
TGGT1_202540	VIIa	3138655	C1462R	0.98	0.00	0.03	0.00	0.00	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_219320	XII	910066	I111N	1.00	0.00	0.04	0.00	0.00	acid phosphatase GAP50
TGGT1_246170	XII	2641576	P1839P	0.99	0.00	0.02	0.00	0.00	ARID/BRIGHT DNA binding domain-containing protein
TGGT1_247390	XII	3244849	G1304S	1.00	0.00	0.05	0.00	0.00	ATPase, AAA family protein
TGGT1_269390	VIII	5844479	A720A	1.00	0.00	0.04	0.00	0.00	CRAL/TRIO domain-containing protein
TGGT1_315770	XI	4916642	G296D	1.00	0.00	0.04	0.00	0.00	cytochrome p450 superfamily protein
TGGT1_313600	XI	3477327	D45E	1.00	0.00	0.03	0.00	0.00	DDHD domain-containing protein
TGGT1_236650	X	5362932	I372N	1.00	0.00	0.00	0.00	0.00	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17
TGGT1_226250	X	1669605	R478C	1.00	0.00	0.06	0.00	0.00	DEAD (Asp-Glu-Ala-Asp) box polypeptide DDX3X
TGGT1_260450	VIIb	2235939	S592L	1.00	0.00	0.00	0.00	0.00	DEAD/DEAH box helicase domain-containing protein
TGGT1_227620	X	780003	G138S	1.00	0.00	0.07	0.00	0.00	dense granule protein GRA2
TGGT1_285540	V	2196073	E1216E	1.00	0.00	0.03	0.00	0.00	DNA-directed DNA polymerase
TGGT1_249560	XII	4577955	F467L	1.00	0.00	0.00	0.00	0.00	DNA-directed RNA polymerase alpha chain rpoA
TGGT1_315920	XI	5020559	C147G	1.00	0.00	0.05	0.00	0.00	DNA-directed RNA polymerase II RPB11A
TGGT1_287980	IX	2089054	S1094L	1.00	0.00	0.00	0.00	0.00	FHA domain-containing protein
TGGT1_287980	IX	2095268	T479M	1.00	0.00	0.01	0.00	0.00	FHA domain-containing protein
TGGT1_265780	IX	1283013	E1022D	1.00	0.00	0.03	0.00	0.00	flagellar/basal body protein
TGGT1_207480	Ib	153771	F4871L	1.00	0.00	0.06	0.00	0.00	GCC2 and GCC3 domain-containing protein
TGGT1_315670	XI	4833635	V193V	1.00	0.00	0.03	0.00	0.00	HEAT repeat-containing protein
TGGT1_285490	V	2242699	H54H	1.00	0.00	0.02	0.00	0.00	helix-hairpin-helix motif domain-containing protein
TGGT1_257750	VIIb	3788597	I218I	1.00	0.00	0.05	0.00	0.00	homocysteine s-methyltransferase domain-containing protein
TGGT1_240330	VI	1158522	A228T	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_213255	V	745145	A3158P	1.00	0.01	0.06	0.04	0.08	hypothetical protein
TGGT1_218370	XII	1496007	C50R	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_299250	III	2074849	D162A	1.00	0.05	0.05	0.00	0.00	hypothetical protein
TGGT1_228120	X	475481	E1397D	1.00	0.00	0.03	0.00	0.00	hypothetical protein
TGGT1_234220	X	4096287	E393K	1.00	0.00	0.03	0.00	0.00	hypothetical protein
TGGT1_258970	VIIb	3033240	F1047I	1.00	0.00	0.02	0.00	0.00	hypothetical protein
TGGT1_259720	VIIb	2669294	F733V	1.00	0.06	0.07	0.00	0.00	hypothetical protein
TGGT1_305790	IX	5372425	F92S	1.00	0.00	0.04	0.00	0.00	hypothetical protein
TGGT1_258450	VIIb	3382360	F983F	1.00	0.00	0.06	0.00	0.00	hypothetical protein
TGGT1_248610	XII	3964466	Frameshift	1.00	0.00	0.07	0.00	0.00	hypothetical protein
TGGT1_218215	XII	1624033	K272R	1.00	0.00	0.04	0.00	0.00	hypothetical protein
TGGT1_462961	VIII	5161927	K4859R	1.00	0.00	0.04	0.00	0.00	hypothetical protein
TGGT1_312840	XI	2910414	L1007H	1.00	0.00	0.06	0.00	0.00	hypothetical protein
TGGT1_289290	IX	2960787	L138F	1.00	0.00	0.04	0.00	0.00	hypothetical protein
TGGT1_312580	XI	2763015	L369V	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_258480	VIIb	3365929	N238H	1.00	0.00	0.12	0.00	0.00	hypothetical protein
TGGT1_245530	XII	2294646	P1680S	1.00	0.00	0.06	0.00	0.00	hypothetical protein
TGGT1_312240	XI	2498041	R1551K	1.00	0.00	0.03	0.00	0.00	hypothetical protein
TGGT1_220920	II	48162	R324S	1.00	0.00	0.04	0.00	0.00	hypothetical protein
TGGT1_308060	XII	490727	R927R	1.00	0.00	0.01	0.00	0.00	hypothetical protein
TGGT1_225860	X	1900464	S297F	1.00	0.00	0.04	0.00	0.00	hypothetical protein
TGGT1_235380	X	4707788	S470R	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_313270	XI	3187357	S472P	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_213010	V	657224	S97C	1.00	0.00	0.05	0.00	0.00	hypothetical protein
TGGT1_285650	V	2177352	T1797R	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_408920	VIIb	3707401	T413A	1.00	0.00	0.07	0.00	0.00	hypothetical protein
TGGT1_301400	IV	2338233	V144X	0.99	0.00	0.05	0.00	0.00	hypothetical protein
TGGT1_280500	VIIa	325741	S932S	1.00	0.00	0.03	0.00	0.00	inorganic anion transporter, sulfate permease (SulP) family protein
TGGT1_311650	XI	2143476	A1093A	1.00	0.00	0.00	0.00	0.00	leucine rich repeat-containing protein
TGGT1_267550	IX	416884	S242R	1.00	0.00	0.00	0.00	0.00	leucine-rich repeat protein LRR1
TGGT1_213890	V	1277457	L528V	1.00	0.00	0.04	0.00	0.00	Myb family DNA-binding domain-containing protein
TGGT1_216440	XI	6065904	F64I	1.00	0.00	0.07	0.00	0.00	OTU family cysteine protease
TGGT1_253750	III	1148147	G1681G	1.00	0.00	0.08	0.00	0.00	PLU-1 family protein
TGGT1_272540	VIII	3908501	A396A	1.00	0.00	0.00	0.00	0.00	protein kinase (incomplete catalytic triad)
TGGT1_246800	XII	2944146	W824*	1.00	0.00	0.04	0.00	0.00	putative acylaminoacyl-peptidase
TGGT1_216790	XI	5800498	P588L	1.00	0.00	0.00	0.00	0.00	putative ATP-binding cassette sub-family E member 1
TGGT1_247560	XII	3348945	A2V	1.00	0.00	0.06	0.00	0.00	putative dynein light chain protein
TGGT1_310010	XI	969734	D166G	1.00	0.00	0.03	0.00	0.00	rhoptry neck protein RON1
TGGT1_207440	Ib	78769	V91A	1.00	0.00	0.06	0.00	0.00	ribosomal protein RPS4
TGGT1_202720	VIIa	2979839	E2279V	1.00	0.00	0.05	0.00	0.00	RNB family domain-containing protein
TGGT1_233460	VIII	2660973	T173T	1.00	0.00	0.04	0.00	0.00	SAG-related sequence SRS29B
TGGT1_231000	VIII	1182181	G351A	1.00	0.00	0.03	0.00	0.00	START domain-containing protein
TGGT1_225960	X	1802390	T3935A	1.00	0.00	0.06	0.00	0.00	STE kinase
TGGT1_213325	V	811115	A2467A	1.00	0.01	0.03	0.00	0.00	TBC domain-containing protein
TGGT1_226940	X	1125867	T47A	1.00	0.00	0.02	0.00	0.00	ubiquitin carboxyl-terminal hydrolase
TGGT1_254480	III	1747687	R300P	0.99	0.00	0.02	0.00	0.00	WD domain, G-beta repeat-containing protein
TGGT1_293300	Ia	227230	N34H	0.99	0.00	0.04	0.00	0.00	Yip1 domain-containing protein
TGGT1_263710	VIIb	351093	Intronic	0.99	0.00	0.02	0.00	0.00	acyl-CoA:cholesterol acyltransferase alpha ACAT1-alpha
TGGT1_265220	IX	1509271	Intronic	0.99	0.00	0.06	0.00	0.00	co-chaperone GrpE protein
TGGT1_219070	XII	1094139	Intronic	1.00	0.00	0.00	0.00	0.00	cyclic nucleotide-binding domain-containing protein
TGGT1_239820	VI	935257	Intronic	1.00	0.00	0.05	0.00	0.00	D-3-phosphoglycerate dehydrogenase
TGGT1_316750	XI	5513225	Intronic	1.00	0.00	0.09	0.00	0.00	DEAD/DEAH box helicase domain-containing protein
TGGT1_297530	II	1852611	Intronic	1.00	0.00	0.00	0.00	0.00	DNA-directed RNA polymerase I RPA2
TGGT1_219630	XII	750964	Intronic	1.00	0.00	0.04	0.00	0.00	flavodoxin domain-containing protein
TGGT1_292020	IX	4287104	Intronic	1.00	0.00	0.04	0.00	0.00	GCC2 and GCC3 domain-containing protein
TGGT1_257770	VIIb	3766328	Intronic	1.00	0.00	0.05	0.00	0.00	histone lysine methyltransferase SET2
TGGT1_202120	VIIa	3505266	Intronic	1.00	0.00	0.11	0.00	0.00	hypothetical protein
TGGT1_205730	VIIa	1108782	Intronic	1.00	0.04	0.13	0.00	0.00	hypothetical protein
TGGT1_205740	VIIa	1099615	Intronic	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_207100	X	7258697	Intronic	1.00	0.00	0.14	0.00	0.00	hypothetical protein
TGGT1_212940	V	584364	Intronic	1.00	0.00	0.04	0.00	0.00	hypothetical protein

TGGT1_214990	X	6562102	Intronic	1.00	0.00	0.03	0.00	0.00	hypothetical protein
TGGT1_219370	XII	902233	Intronic	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_221220	II	138708	Intronic	1.00	0.02	0.09	0.00	0.00	hypothetical protein
TGGT1_225000	X	2512702	Intronic	0.99	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_230220	VIII	694859	Intronic	1.00	0.00	0.05	0.00	0.00	hypothetical protein
TGGT1_240910	VI	1545511	Intronic	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_242260	VI	1840852	Intronic	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_250220	XII	4967308	Intronic	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_250220	XII	4974498	Intronic	1.00	0.00	0.05	0.00	0.00	hypothetical protein
TGGT1_251730	XII	5466327	Intronic	1.00	0.00	0.06	0.00	0.00	hypothetical protein
TGGT1_253615	III	1042206	Intronic	1.00	0.00	0.05	0.00	0.00	hypothetical protein
TGGT1_255635	VIIb	4670127	Intronic	1.00	0.00	0.04	0.00	0.00	hypothetical protein
TGGT1_263810	VIIb	291127	Intronic	1.00	0.00	0.07	0.00	0.00	hypothetical protein
TGGT1_270930	VIII	4856292	Intronic	1.00	0.00	0.07	0.00	0.00	hypothetical protein
TGGT1_272590	VIII	3860171	Intronic	0.99	0.00	0.03	0.00	0.00	hypothetical protein
TGGT1_273885	VIII	3193286	Intronic	1.00	0.00	0.06	0.00	0.00	hypothetical protein
TGGT1_274150	VIII	2980074	Intronic	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_277810	XII	6415386	Intronic	1.00	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_285800	V	2072298	Intronic	1.00	0.00	0.04	0.00	0.00	hypothetical protein
TGGT1_294400	Ia	869056	Intronic	1.00	0.00	0.03	0.00	0.00	hypothetical protein
TGGT1_294940	Ia	1270195	Intronic	0.99	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_313130	XI	3089958	Intronic	0.99	0.00	0.00	0.00	0.00	hypothetical protein
TGGT1_252270	III	438794	Intronic	1.00	0.00	0.00	0.00	0.00	L1P family of ribosomal protein
TGGT1_314900	XI	4342756	Intronic	0.99	0.00	0.00	0.00	0.00	LisH protein
TGGT1_308580	XI	228169	Intronic	1.00	0.00	0.00	0.00	0.00	Lon protease family protein
TGGT1_293180	Ia	126347	Intronic	1.00	0.00	0.00	0.00	0.00	NADP-specific glutamate dehydrogenase
TGGT1_309265	XI	562836	Intronic	1.00	0.00	0.06	0.00	0.00	oxidoreductase, short chain dehydrogenase/reductase family protein
TGGT1_209430	Ib	1227412	Intronic	1.00	0.00	0.00	0.00	0.00	putative 40S ribosomal protein S4
TGGT1_249810	XII	4765687	Intronic	1.00	0.00	0.08	0.00	0.00	putative activating signal cointegrator 1 complex subunit 3
TGGT1_216790	XI	5805722	Intronic	1.00	0.00	0.00	0.00	0.00	putative ATP-binding cassette sub-family E member 1
TGGT1_235020	X	4507941	Intronic	1.00	0.00	0.08	0.00	0.00	putative COPI protein
TGGT1_226700	X	1335364	Intronic	1.00	0.00	0.07	0.00	0.00	putative nuclease
TGGT1_201830	VIIa	3577355	Intronic	1.00	0.00	0.04	0.00	0.00	ribosomal protein L37
TGGT1_259230	VIIb	2814653	Intronic	1.00	0.00	0.00	0.00	0.00	site-specific recombinase, phage integrase family protein
TGGT1_310530	XI	1386907	Intronic	1.00	0.00	0.02	0.00	0.00	SNF2 family N-terminal domain-containing protein
TGGT1_273870	VIII	3202256	Intronic	1.00	0.00	0.08	0.00	0.00	SWI2/SNF2 ISWI-like (AT hook)
TGGT1_291090	IX	3926254	Intronic	1.00	0.00	0.00	0.00	0.00	SWI2/SNF2-containing protein
TGGT1_270070	VIII	5433586	Intronic	1.00	0.00	0.08	0.00	0.00	synaptobrevin family protein
TGGT1_297500	II	1809275	Intronic	1.00	0.02	0.08	0.00	0.00	T-complex protein 1 eta subunit
TGGT1_250920	XII	5249921	Intronic	1.00	0.00	0.03	0.00	0.00	tetratricopeptide repeat-containing protein
TGGT1_234410	X	4255009	Intronic	1.00	0.00	0.08	0.00	0.00	small conductance mechanosensitive ion channel (MscS) family protein
TGGT1_312520	XI	2724670	Intronic	1.00	0.00	0.07	0.00	0.00	tRNA dimethylallyltransferase
Intergenic	Ia	470420	Intergenic	0.99	0.00	0.04	0.00	0.00	N/A
Intergenic	Ib	880169	Intergenic	1.00	0.00	0.07	0.00	0.00	N/A
Intergenic	Ib	1442011	Intergenic	1.00	0.00	0.03	0.00	0.00	N/A
Intergenic	II	537627	Intergenic	1.00	0.00	0.05	0.00	0.00	N/A
Intergenic	III	1536907	Intergenic	1.00	0.00	0.03	0.00	0.00	N/A
Intergenic	III	439992	Intergenic	1.00	0.00	0.00	0.00	0.00	N/A
Intergenic	III	2045363	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	IV	1825960	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	IV	1942258	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	IX	2954953	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	IX	5597650	Intergenic	1.00	0.00	0.05	0.00	0.00	N/A
Intergenic	IX	1057959	Intergenic	1.00	0.00	0.09	0.00	0.00	N/A
Intergenic	IX	2730252	Intergenic	1.00	0.00	0.07	0.00	0.00	N/A
Intergenic	IX	5140738	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	IX	900168	Intergenic	1.00	0.00	0.09	0.00	0.00	N/A
Intergenic	IX	4603155	Intergenic	1.00	0.00	0.03	0.00	0.00	N/A
Intergenic	IX	3685652	Intergenic	0.98	0.00	0.08	0.00	0.00	N/A
Intergenic	IX	591204	Intergenic	0.82	0.00	0.05	0.00	0.00	N/A
Intergenic	IX	5807494	Intergenic	1.00	0.00	0.03	0.00	0.00	N/A
Intergenic	V	1099737	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	V	2987554	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	VI	492205	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	VI	713008	Intergenic	1.00	0.00	0.02	0.00	0.00	N/A
Intergenic	VIIa	358021	Intergenic	1.00	0.00	0.03	0.00	0.00	N/A
Intergenic	VIIa	585218	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	VIIa	1745855	Intergenic	1.00	0.00	0.05	0.00	0.00	N/A
Intergenic	VIIa	2294095	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	VIIa	2305275	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	VIIa	3319699	Intergenic	1.00	0.00	0.00	0.00	0.00	N/A
Intergenic	VIIa	3657742	Intergenic	1.00	0.00	0.00	0.00	0.00	N/A
Intergenic	VIIa	424565	Intergenic	1.00	0.00	0.03	0.00	0.00	N/A
Intergenic	VIIa	3278484	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	VIIb	61563	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	VIIb	2126748	Intergenic	1.00	0.00	0.02	0.00	0.00	N/A
Intergenic	VIIb	2831303	Intergenic	1.00	0.00	0.00	0.00	0.00	N/A
Intergenic	VIIb	4119445	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	VIIb	4783554	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	VIIb	4606259	Intergenic	1.00	0.00	0.05	0.00	0.00	N/A
Intergenic	VIIb	2656622	Intergenic	0.99	0.00	0.03	0.00	0.00	N/A
Intergenic	VIII	98117	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	VIII	607622	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	VIII	898273	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	VIII	903926	Intergenic	1.00	0.00	0.00	0.00	0.00	N/A
Intergenic	VIII	2452573	Intergenic	1.00	0.00	0.09	0.00	0.00	N/A

Intergenic	VIII	3168227	Intergenic	1.00	0.00	0.05	0.00	0.00	N/A
Intergenic	VIII	4792051	Intergenic	1.00	0.00	0.00	0.00	0.00	N/A
Intergenic	VIII	5589730	Intergenic	1.00	0.00	0.02	0.00	0.00	N/A
Intergenic	VIII	2493242	Intergenic	0.98	0.00	0.05	0.00	0.00	N/A
Intergenic	VIII	1618200	Intergenic	0.98	0.00	0.07	0.00	0.00	N/A
Intergenic	VIII	5476330	Intergenic	0.97	0.00	0.00	0.00	0.00	N/A
Intergenic	X	19316	Intergenic	1.00	0.00	0.00	0.00	0.00	N/A
Intergenic	X	50233	Intergenic	1.00	0.00	0.02	0.00	0.00	N/A
Intergenic	X	2928546	Intergenic	1.00	0.00	0.09	0.00	0.00	N/A
Intergenic	X	3762836	Intergenic	1.00	0.00	0.00	0.00	0.00	N/A
Intergenic	X	6663623	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	X	6285025	Intergenic	0.99	0.00	0.00	0.00	0.00	N/A
Intergenic	X	7232649	Intergenic	0.98	0.00	0.03	0.00	0.00	N/A
Intergenic	XI	3281365	Intergenic	1.00	0.00	0.08	0.00	0.00	N/A
Intergenic	XI	4072004	Intergenic	1.00	0.00	0.03	0.00	0.00	N/A
Intergenic	XI	5156138	Intergenic	1.00	0.00	0.02	0.00	0.00	N/A
Intergenic	XI	6172343	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	XI	1420899	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	XI	1688696	Intergenic	1.00	0.00	0.09	0.00	0.00	N/A
Intergenic	XI	5465613	Intergenic	1.00	0.00	0.05	0.00	0.00	N/A
Intergenic	XI	1255287	Intergenic	1.00	0.02	0.06	0.00	0.00	N/A
Intergenic	XI	2245313	Intergenic	1.00	0.00	0.03	0.00	0.00	N/A
Intergenic	XI	5357331	Intergenic	0.99	0.00	0.00	0.00	0.00	N/A
Intergenic	XI	1354223	Intergenic	0.99	0.00	0.06	0.00	0.00	N/A
Intergenic	XII	3338865	Intergenic	1.00	0.00	0.05	0.00	0.00	N/A
Intergenic	XII	3677401	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	XII	4273612	Intergenic	1.00	0.00	0.07	0.00	0.00	N/A
Intergenic	XII	5000288	Intergenic	1.00	0.00	0.06	0.00	0.00	N/A
Intergenic	XII	3585773	Intergenic	1.00	0.00	0.05	0.00	0.00	N/A
Intergenic	XII	2813301	Intergenic	1.00	0.00	0.05	0.00	0.00	N/A
Intergenic	XII	1678844	Intergenic	1.00	0.05	0.00	0.00	0.00	N/A
Intergenic	XII	3583729	Intergenic	1.00	0.04	0.00	0.08	0.00	N/A
Intergenic	XII	1489806	Intergenic	1.00	0.00	0.07	0.00	0.00	N/A
Intergenic	XII	2239394	Intergenic	1.00	0.00	0.04	0.00	0.00	N/A
Intergenic	XII	944795	Intergenic	1.00	0.00	0.05	0.00	0.00	N/A
Intergenic	XII	682963	Intergenic	0.99	0.00	0.00	0.00	0.00	N/A
Intergenic	XII	2697201	Intergenic	0.99	0.00	0.03	0.00	0.00	N/A
Intergenic	XII	6505010	Intergenic	0.99	0.00	0.05	0.00	0.00	N/A

Table 3.2. PCR primers utilized for SNP validation between RH and GT1

Chromosome	Gene ID	Codon Change	Mutation	PCR size	Forward Primer (M13 Forward)	Reverse Primer
TGGT1_chrVIIa	TGGT1_016250	ATT to AAT	Ile to Asn	585	TGTAAAACGACGGCCAGTTGTTGACGAATCCCCCAAAG	CTTTTGCTTTGTGCTGCATG
TGGT1_chrX	TGGT1_081400	GCT to GAT	Ala to Asp	582	TGTAAAACGACGGCCAGTAGAGCAACAAGCCGATACTG	CGACTTTTCTCCCTAGAAGC
TGGT1_chrVIIb	TGGT1_011730	AAT to CAT	Asn to His	576	TGTAAAACGACGGCCAGTTCTTTGCCCTTCTTCTGAAG	GAGCAAGAGGATGTAAATCC
TGGT1_chrII	TGGT1_065060	TGT to TCT	Cys to Ser	557	TGTAAAACGACGGCCAGTCTTTCTAAAGGAACTATCCC	TCTGGCGTTTTCGTTACCTC
TGGT1_chrVIIb	TGGT1_007550	CTC to CGC	Leu to Arg	550	TGTAAAACGACGGCCAGTAAAACTCGTTCTCTGTGTAG	AGAACCAAGGGCGTTTCAAG
TGGT1_chrIV	TGGT1_122460	GTG to GGG	Val to Gly	493	TGTAAAACGACGGCCAGTCACAAGGAACAGACAAATTC	AGTGGCACCTACCGAATATC
TGGT1_chrVIII	TGGT1_116720	GGG to GCG	Gly to Ala	495	TGTAAAACGACGGCCAGTTAGACTGGCGTGAGTTTGAC	ATATACATGGACACCTGTAG
TGGT1_chrXI	TGGT1_098520	GTT to TTT	Val to Phe	490	TGTAAAACGACGGCCAGTGTTCGCACCTGAAACTTATG	TACGGACAATGCGCATTTGG
TGGT1_chrVI	TGGT1_051830	GAG to AAG	Glu to Lys	459	TGTAAAACGACGGCCAGTCATGTGACTTCTGTGTCTTG	ATCTGGCGTTGGAAATCCAC
TGGT1_chrVIII	TGGT1_113990	TCC to TTC	Ser to Phe	526	TGTAAAACGACGGCCAGTTCAGCAAGTGGTTTTTTGGC	TTCTGTGGAGCACATACGTG
TGGT1_chrVIII	TGGT1_060660	TTT to TCT	Phe to Ser	536	TGTAAAACGACGGCCAGTTCCTCTCTCTCGTCACATTC	GCATTTCCAGTTTTCTCTTG
TGGT1_chrIX	TGGT1_041270	CAG to AAG	Gln to Lys	526	TGTAAAACGACGGCCAGTTTCGGAAAAAGACCAAGCAC	GTTCAGAGCATCTCTCAGAG
TGGT1_chrX	TGGT1_071670	GAG to AAG	Glu to Lys	514	TGTAAAACGACGGCCAGTCATTCCTGTTCACATGGTTG	GTTGCATGCGCTAAATCGAC
TGGT1_chrXI	TGGT1_088340	GAC to GCC	Asp to Ala	533	TGTAAAACGACGGCCAGTTTGGACGTTGTTGATCGAAG	ACTTGGAGCTCCAAAGGAAC
TGGT1_chrXII	TGGT1_103920	AGT to ACT	Ser to Thr	512	TGTAAAACGACGGCCAGTCATGCAGGAGGTTCCTTATC	CTTGTTCGCCGTCACTTTTG

Table 4.1. Differential expression analysis identified many differentially expressed gene amongst intracellular RH and non-lab-adapted GT1 populations

Following RNA-seq of 30-hour intracellular parasites, DEA identified 545 DEGs in GT1 population B2-P11 and 764 DEGS in GT1 population B4-P11, both relative to RH. In union, 498 DEGs were found to have similar expression across the two GT1 populations. All genes with Log_2 FC values shown are considered significant (FC $\geq$2; *q*-value $\leq$0.05); N.S. = not significant.

Table 4.1. Differential expression analysis identified many differentially expressed gene amongst intracellular RH and non-lab-adapted GT1 populations

Gene ID	Log_2 FC relative to RH		Product Description
	B2-P11	B4-P11	
TGGT1_278365	8.16	8.42	Toxoplasma gondii family A protein
TGGT1_301150	6.83	5.06	SAG-related sequence SRS19B
TGGT1_320190	6.82	7.46	SAG-related sequence SRS16B
TGGT1_242760	6.58	6.35	hypothetical protein
TGGT1_217420	6.07	6.25	hypothetical protein
TGGT1_300980	6.05	6.86	hypothetical protein
TGGT1_285470	5.86	5.65	patched family protein
TGGT1_320105	5.79	7.23	hypothetical protein
TGGT1_265030	5.76	5.95	hypothetical protein
TGGT1_300990	5.72	5.33	Toxoplasma gondii family C protein
TGGT1_410080	5.69	9.87	hypothetical protein
TGGT1_278370	5.67	4.98	Toxoplasma gondii family A protein
TGGT1_301180	5.58	5.03	SAG-related sequence SRS19F
TGGT1_207150	5.51	6.71	SAG-related sequence SRS49C
TGGT1_290970	5.48	6.04	8-amino-7-oxononanoate synthase
TGGT1_201690	5.25	7.04	BT1 family protein
TGGT1_202025	5.18	4.76	hypothetical protein
TGGT1_278400	5.15	4.33	Toxoplasma gondii family A protein
TGGT1_293840	5.07	4.89	hypothetical protein
TGGT1_284310	5.02	5.34	hypothetical protein
TGGT1_278110	4.94	5.19	1,3-beta-glucan synthase component protein
TGGT1_284420	4.87	5.29	hypothetical protein
TGGT1_301370	4.82	4.55	DHHC zinc finger domain-containing protein
TGGT1_305790	4.75	4.94	hypothetical protein
TGGT1_292375	4.72	5.37	KRUF family protein
TGGT1_301170	4.59	4.74	SAG-related sequence SRS19D
TGGT1_278120	4.53	3.03	SCP family extracellular subfamily protein
TGGT1_410090	4.51	5.01	hypothetical protein
TGGT1_306480	4.48	5.09	CAM kinase, CDPK family
TGGT1_411820	4.46	3.45	toxoplasma gondii family C protein
TGGT1_301690	4.36	4.74	hypothetical protein
TGGT1_323310	4.32	4.90	hypothetical protein
TGGT1_225290	4.32	4.10	GDA1/CD39 (nucleoside phosphatase) family protein
TGGT1_462968	4.31	4.40	putative ATPase family protein
TGGT1_292280	4.25	3.74	SAG-related sequence SRS36D
TGGT1_216140	4.23	5.57	tetratricopeptide repeat-containing protein
TGGT1_270655	4.18	4.44	hypothetical protein
TGGT1_255210	4.00	4.30	ATPase, AAA family protein
TGGT1_264660	3.98	3.71	SAG-related sequence SRS44
TGGT1_278380	3.91	3.35	Toxoplasma gondii family A protein
TGGT1_318120	3.91	3.84	hypothetical protein
TGGT1_411360	3.90	3.40	rhoptry kinase family protein
TGGT1_293500	3.89	3.65	hypothetical protein
TGGT1_217400	3.86	3.23	hypothetical protein
TGGT1_278090	3.80	2.74	Toxoplasma gondii family A protein
TGGT1_323320	3.79	4.13	hypothetical protein
TGGT1_255200	3.79	4.09	putative Radial spoke head protein 9

TGGT1_313890	3.77	4.13	hypothetical protein
TGGT1_358470	3.74	3.99	hypothetical protein
TGGT1_356400	3.74	3.94	cAMP-dependent protein kinase
TGGT1_408620	3.70	3.53	hypothetical protein
TGGT1_410720	3.69	6.69	putative protein kinase
TGGT1_200130	3.58	4.09	Toxoplasma gondii family C protein
TGGT1_245530	3.56	4.47	hypothetical protein
TGGT1_307480	3.56	3.26	Toxoplasma gondii family B protein
TGGT1_283450	3.48	3.44	Toxoplasma gondii family B protein
TGGT1_220720	3.47	3.10	hypothetical protein
TGGT1_226450	3.46	2.41	hypothetical protein
TGGT1_255215	3.45	3.93	hypothetical protein
TGGT1_280580	3.44	3.07	SAG-related sequence SRS35B
TGGT1_209985	3.42	3.29	cAMP-dependent protein kinase
TGGT1_293780	3.41	3.48	hypothetical protein
TGGT1_273110	3.40	2.70	SAG-related sequence SRS30D
TGGT1_210450	3.40	3.70	hypothetical protein
TGGT1_363340	3.31	4.07	hypothetical protein
TGGT1_301210	3.30	2.77	putative NAD(P) transhydrogenase subunit beta
TGGT1_239910	3.29	3.65	cyclin-dependent kinase
TGGT1_222080	3.28	5.27	hypothetical protein
TGGT1_244710	3.26	3.68	hypothetical protein
TGGT1_320180	3.22	3.33	SAG-related sequence SRS16C
TGGT1_410610	3.18	4.72	hypothetical protein
TGGT1_306338B	3.18	2.97	putative dynein gamma chain, flagellar outer arm
TGGT1_298820	3.14	4.50	hypothetical protein
TGGT1_410730	3.13	3.90	PT repeat protein
TGGT1_214545	3.10	1.99	hypothetical protein
TGGT1_266105	3.08	3.31	EF hand family protein
TGGT1_318130A	3.07	2.87	Toxoplasma gondii family E protein
TGGT1_307070	3.07	4.19	hypothetical protein
TGGT1_278882	3.06	3.41	GDA1/CD39 (nucleoside phosphatase) family protein
TGGT1_257945	3.03	2.90	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_258230	3.00	3.13	rhoptry kinase family protein ROP20
TGGT1_244700	2.98	3.13	NAD(+)/NADH kinase domain-containing protein
TGGT1_222370	2.98	2.89	SAG-related sequence SRS13
TGGT1_219218	2.98	2.71	hypothetical protein
TGGT1_271015	2.94	3.32	hypothetical protein
TGGT1_411310	2.94	3.09	hypothetical protein
TGGT1_253770	2.91	3.71	hypothetical protein
TGGT1_359770	2.89	3.21	SAG-related sequence protein SRS22E
TGGT1_232770	2.88	2.68	hypothetical protein
TGGT1_311335	2.87	3.52	dual specificity phosphatase, catalytic domain-containing protein
TGGT1_278580	2.87	3.15	hypothetical protein
TGGT1_243360	2.85	1.77	hypothetical protein
TGGT1_206290	2.81	2.25	hypothetical protein
TGGT1_301160	2.80	2.75	SAG-related sequence SRS19C
TGGT1_217724	2.80	2.86	hypothetical protein
TGGT1_207865	2.79	2.42	GCC2 and GCC3 domain-containing protein
TGGT1_315802	2.78	2.52	hypothetical protein
TGGT1_411390	2.77	2.92	hypothetical protein
TGGT1_304920	2.77	4.47	hypothetical protein
TGGT1_215340	2.75	3.72	AP2 domain transcription factor AP2X-10
TGGT1_278390	2.75	2.32	Toxoplasma gondii family A protein
TGGT1_411800	2.74	2.09	hypothetical protein
TGGT1_411270	2.72	3.11	hypothetical protein
TGGT1_210235	2.71	2.26	hypothetical protein
TGGT1_215775	2.71	2.58	rhoptry protein ROP8
TGGT1_293810	2.69	2.96	carboxyvinyl-carboxyphosphonate phosphorylmutase
TGGT1_210245	2.68	2.19	hypothetical protein

TGGT1_409990	2.67	2.81	hypothetical protein
TGGT1_204360	2.64	3.26	subtilisin SUB4
TGGT1_231470	2.61	2.28	hypothetical protein
TGGT1_218400	2.61	2.11	NEK kinase
TGGT1_208730	2.61	3.09	putative microneme protein
TGGT1_215180	2.61	3.23	hypothetical protein
TGGT1_276920	2.60	2.38	protein phosphatase 2C domain-containing protein
TGGT1_247540	2.60	3.48	ATP-binding cassette G family transporter ABCG107
TGGT1_287460	2.58	2.86	hypothetical protein
TGGT1_276980	2.58	3.19	hypothetical protein
TGGT1_356030	2.58	2.03	hypothetical protein
TGGT1_306455	2.54	1.87	hypothetical protein
TGGT1_263750	2.54	2.67	hypothetical protein
TGGT1_409850	2.53	1.66	SAG-related sequence protein SRS47A
TGGT1_411400	2.50	3.04	hypothetical protein
TGGT1_257000	2.49	2.02	hypothetical protein
TGGT1_408770	2.49	1.65	putative transmembrane protein
TGGT1_240470	2.48	2.30	hypothetical protein
TGGT1_313650	2.48	3.43	hypothetical protein
TGGT1_411730	2.48	2.57	hypothetical protein
TGGT1_218390	2.46	2.45	hypothetical protein
TGGT1_306500	2.46	4.03	hypothetical protein
TGGT1_239880	2.45	3.18	hypothetical protein
TGGT1_202020	2.44	3.64	DnAK-TPR
TGGT1_356970	2.44	1.49	hypothetical protein
TGGT1_306450	2.42	3.59	putative short chain dehydrogenase family protein
TGGT1_207140	2.42	2.48	SAG-related sequence SRS49B
TGGT1_225280	2.42	2.97	hypothetical protein
TGGT1_262590	2.40	2.19	hypothetical protein
TGGT1_264240	2.39	2.91	hypothetical protein
TGGT1_409590	2.37	2.15	putative transmembrane protein
TGGT1_254060	2.35	1.82	SAG-related sequence SRS14A
TGGT1_207700	2.35	1.89	rhoptry kinase family protein ROP22 (incomplete catalytic triad)
TGGT1_264260	2.34	2.62	hypothetical protein
TGGT1_219660	2.34	3.63	hypothetical protein
TGGT1_217915	2.33	2.74	hypothetical protein
TGGT1_208760	2.33	2.11	hypothetical protein
TGGT1_253790	2.30	1.70	zinc finger (CCCH type) motif-containing protein
TGGT1_217920	2.28	2.72	hypothetical protein
TGGT1_309405	2.27	2.42	hypothetical protein
TGGT1_202910	2.27	2.09	zinc carboxypeptidase superfamily protein
TGGT1_248140	2.27	2.36	hypothetical protein
TGGT1_249810	2.26	2.36	putative activating signal cointegrator 1 complex subunit 3
TGGT1_210095	2.26	2.96	hypothetical protein
TGGT1_365020	2.25	1.95	cytochrome C family oxidase subunit III subfamily protein
TGGT1_241165	2.24	1.80	hypothetical protein
TGGT1_296231	2.24	2.77	hypothetical protein
TGGT1_243615	2.24	3.00	hypothetical protein
TGGT1_207875	2.24	2.48	GCC2 and GCC3 domain-containing protein
TGGT1_257568	2.23	2.20	hypothetical protein
TGGT1_257572	2.23	2.14	hypothetical protein
TGGT1_266670	2.23	2.02	hypothetical protein
TGGT1_215970	2.21	2.55	hypothetical protein
TGGT1_322110	2.21	2.24	hypothetical protein
TGGT1_321480	2.20	2.68	SAG-related sequence SRS12B
TGGT1_241130	2.20	2.18	hypothetical protein
TGGT1_411000	2.19	2.07	hypothetical protein
TGGT1_215343	2.19	1.97	hypothetical protein
TGGT1_363480	2.18	3.01	hypothetical protein
TGGT1_409830	2.18	2.01	putative cytochrome protein B

TGGT1_220060	2.18	1.92	hypothetical protein
TGGT1_239510	2.17	1.85	hypothetical protein
TGGT1_320230	2.14	2.51	SAG-related sequence SRS15C
TGGT1_410930	2.13	2.28	hypothetical protein
TGGT1_217410	2.13	3.51	hypothetical protein
TGGT1_295720	2.11	2.47	putative sulfite oxidase
TGGT1_266950	2.11	2.31	putative protein kinase
TGGT1_216290A	2.10	2.35	hypothetical protein
TGGT1_283882	2.10	1.61	hypothetical protein
TGGT1_257610	2.09	2.26	hypothetical protein
TGGT1_252070	2.09	2.66	KRUF family protein
TGGT1_259670	2.09	1.98	von Willebrand factor type A domain-containing protein
TGGT1_260480	2.09	2.22	leucine rich repeat-containing protein
TGGT1_269600	2.08	2.12	biotin-requiring enzyme domain-containing protein
TGGT1_315740	2.07	1.85	SAG-related sequence SRS54
TGGT1_285860	2.07	1.61	SAG-related sequence SRS20C
TGGT1_411410	2.06	2.73	putative transmembrane protein
TGGT1_300780	2.04	1.83	hypothetical protein
TGGT1_309930	2.03	2.13	melibiase subfamily protein
TGGT1_219742	2.02	1.64	hypothetical protein
TGGT1_277930	2.02	1.68	hypothetical protein
TGGT1_272680	2.00	2.80	hypothetical protein
TGGT1_286660	2.00	2.63	putative kinesin
TGGT1_254890	1.99	1.57	hypothetical protein
TGGT1_244515	1.98	2.68	hypothetical protein
TGGT1_307030	1.98	2.19	Purine nucleoside phosphorylase
TGGT1_201840	1.98	2.40	aspartyl protease ASP1
TGGT1_214760	1.94	1.63	erythronate-4-phosphate dehydrogenase domain-containing protein
TGGT1_243635	1.93	2.15	hypothetical protein
TGGT1_272440	1.93	2.03	RNA recognition motif-containing protein
TGGT1_248425	1.92	1.73	hypothetical protein
TGGT1_235183	1.90	2.37	PAN domain-containing protein
TGGT1_229405	1.90	2.18	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_314250	1.88	1.79	bradyzoite rhoptry protein BRP1
TGGT1_315260	1.88	1.99	alanine dehydrogenase
TGGT1_215195	1.88	1.75	tetratricopeptide repeat-containing protein
TGGT1_223430	1.87	1.85	hypothetical protein
TGGT1_216290B	1.87	1.85	hypothetical protein
TGGT1_268300	1.84	1.45	hypothetical protein
TGGT1_227430	1.84	2.01	transmembrane amino acid transporter protein
TGGT1_289350	1.84	1.71	ATP-binding cassette G family transporter ABCG84
TGGT1_224810	1.84	1.24	hypothetical protein
TGGT1_269300	1.84	1.93	lipase
TGGT1_271174	1.82	1.60	hypothetical protein
TGGT1_315900	1.82	1.62	hypothetical protein
TGGT1_304930	1.81	1.47	hypothetical protein
TGGT1_223560	1.80	1.58	hypothetical protein
TGGT1_206570	1.79	1.35	putative kinesin
TGGT1_310177	1.78	1.74	hypothetical protein
TGGT1_223060	1.78	1.81	MORN repeat-containing protein
TGGT1_409050	1.77	2.00	hypothetical protein
TGGT1_295960	1.76	1.59	hypothetical protein
TGGT1_216335	1.75	2.05	hypothetical protein
TGGT1_291860	1.74	1.64	HORMA domain-containing protein
TGGT1_265810	1.74	1.73	hypothetical protein
TGGT1_258225	1.73	1.90	hypothetical protein
TGGT1_294225	1.73	1.31	hypothetical protein
TGGT1_313840	1.72	1.06	hypothetical protein
TGGT1_295950	1.72	1.63	KRUF family protein
TGGT1_318675	1.71	2.16	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein

TGGT1_411020	1.71	2.22	hypothetical protein
TGGT1_249290	1.71	2.49	hypothetical protein
TGGT1_411150	1.71	2.39	hypothetical protein
TGGT1_224845	1.70	1.87	hypothetical protein
TGGT1_217722	1.70	3.06	hypothetical protein
TGGT1_235680	1.70	1.68	peptidase M16 inactive domain-containing protein
TGGT1_294595	1.69	2.41	hypothetical protein
TGGT1_273065	1.69	1.24	hypothetical protein
TGGT1_205090	1.67	1.27	Toxoplasma gondii family D protein
TGGT1_328800	1.66	1.77	hypothetical protein
TGGT1_411120	1.66	1.59	putative transmembrane protein
TGGT1_277810	1.65	2.51	hypothetical protein
TGGT1_411520	1.63	2.04	hypothetical protein
TGGT1_288945	1.62	2.05	hypothetical protein
TGGT1_262600	1.61	1.57	hypothetical protein
TGGT1_292260	1.61	1.11	SAG-related sequence SRS36B
TGGT1_244180	1.59	1.53	microneme-like protein
TGGT1_287490	1.59	1.19	hypothetical protein
TGGT1_219240	1.59	1.64	Peptidyl-tRNA hydrolase PTH2 domain-containing protein
TGGT1_205210	1.58	1.65	hypothetical protein
TGGT1_222188	1.57	1.17	hypothetical protein
TGGT1_239020	1.57	1.40	ABC transporter transmembrane region domain-containing protein
TGGT1_217660	1.56	2.22	Tctex-1 family protein
TGGT1_261022	1.56	1.90	dynein heavy chain family protein
TGGT1_252255	1.54	1.11	hypothetical protein
TGGT1_295945	1.54	1.53	hypothetical protein
TGGT1_204500	1.54	2.01	hypothetical protein
TGGT1_243740	1.53	1.85	WD domain, G-beta repeat-containing protein
TGGT1_309860	1.53	1.27	hypothetical protein
TGGT1_271335	1.51	1.49	hypothetical protein
TGGT1_228410	1.51	1.28	Tubulin-tyrosine ligase family protein
TGGT1_234930	1.51	1.28	SAG-related sequence SRS43
TGGT1_316670	1.51	2.01	Toxoplasma gondii family D protein
TGGT1_271310	1.50	1.47	hypothetical protein
TGGT1_321800	1.49	2.50	EGF family domain-containing protein
TGGT1_217020	1.49	1.81	ATPase, AFG1 family protein
TGGT1_207750	1.49	1.52	hypothetical protein
TGGT1_305000	1.49	1.09	hypothetical protein
TGGT1_217728	1.49	1.43	hypothetical protein
TGGT1_411190	1.47	1.97	hypothetical protein
TGGT1_315410	1.47	1.26	SAG-related sequence SRS53F
TGGT1_213820	1.46	1.48	hypothetical protein
TGGT1_244940	1.46	1.64	hypothetical protein
TGGT1_213560	1.45	1.18	hypothetical protein
TGGT1_248430	1.43	1.91	hypothetical protein
TGGT1_263100	1.42	1.76	hypothetical protein
TGGT1_266910	1.41	1.21	putative cell-cycle-associated protein kinase GSK
TGGT1_285865	1.41	1.43	hypothetical protein
TGGT1_236990	1.40	1.32	beta-ketoacyl synthase, N-terminal domain-containing protein
TGGT1_301350	1.40	1.34	SNARE associated protein
TGGT1_308075	1.39	1.49	hypothetical protein
TGGT1_273120	1.39	1.55	SAG-related sequence SRS30C
TGGT1_237270	1.37	1.08	hypothetical protein
TGGT1_242590	1.37	2.08	hypothetical protein
TGGT1_293258	1.34	1.84	hypothetical protein
TGGT1_408760	1.33	1.05	toxoplasma gondii family A protein
TGGT1_268765	1.33	1.43	hypothetical protein
TGGT1_269417	1.32	1.41	hypothetical protein
TGGT1_216680	1.32	1.26	ankyrin repeat-containing protein
TGGT1_213720	1.31	1.37	hypothetical protein

TGGT1_232950	1.30	1.68	hypothetical protein
TGGT1_253930	1.29	1.41	GCC2 and GCC3 domain-containing protein
TGGT1_202255	1.29	1.28	hypothetical protein
TGGT1_222160	1.28	1.18	aldehyde dehydrogenase
TGGT1_238960	1.27	1.24	EF hand domain-containing protein
TGGT1_254340	1.26	1.99	hypothetical protein
TGGT1_301355	1.22	1.00	hypothetical protein
TGGT1_248470	1.22	1.66	subtilisin SUB6
TGGT1_309410	1.17	1.23	AP2 domain transcription factor AP2XI-1
TGGT1_310520	1.17	1.40	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_223725	1.16	1.36	hypothetical protein
TGGT1_316290	1.15	1.72	hypothetical protein
TGGT1_215347	1.14	1.05	hypothetical protein
TGGT1_316510	1.10	1.12	hypothetical protein
TGGT1_254635	1.06	1.30	hypothetical protein
TGGT1_281400	1.06	1.55	phosphofructokinase domain-containing protein
TGGT1_305040	1.06	1.12	HEAT repeat-containing protein
TGGT1_359210	1.06	1.07	hypothetical protein
TGGT1_238140	1.06	1.22	hypothetical protein
TGGT1_280810	1.04	1.24	hypothetical protein
TGGT1_226420	1.02	1.13	peptidase family M3 protein
TGGT1_278060	1.02	1.08	Mre11 DNA-binding domain-containing protein
TGGT1_271025	1.02	1.25	hypothetical protein
TGGT1_306680	1.01	1.30	hypothetical protein
TGGT1_264760	-1.02	-1.05	Oxysterol-binding protein
TGGT1_246210	-1.03	-1.06	hypothetical protein
TGGT1_267560	-1.03	-1.04	folate-binding protein YgfZ protein
TGGT1_267570	-1.05	-1.05	hypothetical protein
TGGT1_267790	-1.05	-1.05	hypothetical protein
TGGT1_240580	-1.05	-1.33	hypothetical protein
TGGT1_288045	-1.06	-1.05	hypothetical protein
TGGT1_255280	-1.07	-1.21	hypothetical protein
TGGT1_267440	-1.08	-1.00	RING zinc finger protein
TGGT1_279400	-1.08	-1.03	putative glutaredoxin
TGGT1_267580	-1.08	-1.10	cyclin2 related protein
TGGT1_313852	-1.09	-1.37	hypothetical protein
TGGT1_267610	-1.09	-1.22	hypothetical protein
TGGT1_264680	-1.10	-1.04	hypothetical protein
TGGT1_216970	-1.10	-1.19	putative coronin
TGGT1_205250	-1.10	-1.25	rhoptry protein ROP18
TGGT1_279360	-1.11	-1.09	hypothetical protein
TGGT1_267840	-1.11	-1.03	tetratricopeptide repeat-containing protein
TGGT1_267650	-1.12	-1.08	hypothetical protein
TGGT1_266410	-1.12	-1.14	hypothetical protein
TGGT1_279370	-1.13	-1.23	SNARE associated Golgi protein
TGGT1_264730	-1.13	-1.04	hypothetical protein
TGGT1_242625	-1.13	-1.01	TPase family associated with various cellular activities (AAA) subfamily protei
TGGT1_264900	-1.13	-1.09	hypothetical protein
TGGT1_288040	-1.14	-1.07	hypothetical protein
TGGT1_297488	-1.14	-1.01	hypothetical protein
TGGT1_264820	-1.14	-1.07	RbAp48
TGGT1_267740	-1.15	-1.06	hypothetical protein
TGGT1_267450	-1.15	-1.15	alpha-tubulin suppressor protein
TGGT1_267855	-1.16	-1.19	hypothetical protein
TGGT1_267620	-1.18	-1.18	multi-pass transmembrane protein
TGGT1_207470	-1.20	-1.06	hypothetical protein
TGGT1_231990	-1.21	-1.39	hypothetical protein
TGGT1_264790	-1.21	-1.18	hypothetical protein
TGGT1_308965	-1.22	-1.24	hypothetical protein
TGGT1_264690	-1.22	-1.22	putative cyclin 4

TGGT1_299010	-1.22	-1.27	hypothetical protein
TGGT1_267430	-1.22	-1.17	DnaJ domain-containing protein
TGGT1_266390	-1.22	-1.25	DNA mismatch repair protein, C-terminal domain-containing protein
TGGT1_264890	-1.23	-1.16	hypothetical protein
TGGT1_295110	-1.23	-1.37	rhoptry protein ROP7
TGGT1_267680	-1.23	-1.42	microneme protein MIC12
TGGT1_264752	-1.24	-1.19	HEAT repeat-containing protein
TGGT1_323020	-1.25	-1.19	KRUF family protein
TGGT1_256650	-1.27	-1.04	hypothetical protein
TGGT1_410770	-1.27	-1.20	histone deacetylase SIR2-like protein
TGGT1_264745	-1.27	-1.04	hypothetical protein
TGGT1_264870	-1.28	-1.07	Sodium:neurotransmitter symporter family protein
TGGT1_288020	-1.29	-1.35	hypothetical protein
TGGT1_267370	-1.29	-1.29	kinesin motor domain-containing protein
TGGT1_229220	-1.30	-1.09	hypothetical protein
TGGT1_264670	-1.30	-1.23	DNA polymerase family B protein
TGGT1_255290	-1.31	-1.39	hypothetical protein
TGGT1_266360	-1.31	-1.08	hypothetical protein
TGGT1_250220	-1.32	-1.04	hypothetical protein
TGGT1_366480	-1.36	-1.37	phosphofructokinase
TGGT1_267630	-1.36	-1.15	hypothetical protein
TGGT1_226100	-1.39	-1.52	haloacid dehalogenase family hydrolase domain-containing protein
TGGT1_294651	-1.39	-1.41	hypothetical protein
TGGT1_409260	-1.40	-1.30	hypothetical protein
TGGT1_244280	-1.40	-1.43	hypothetical protein
TGGT1_267380	-1.40	-1.47	UDP-galactose transporter subfamily protein
TGGT1_224840	-1.41	-1.44	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_267720	-1.44	-1.18	transporter, cation channel family protein
TGGT1_264748	-1.45	-1.21	hypothetical protein
TGGT1_216610	-1.46	-1.54	ribosomal RNA large subunit methyltransferase J protein
TGGT1_226310	-1.46	-1.65	zinc finger (CCCH type) motif-containing protein
TGGT1_270580	-1.46	-1.83	HECT-domain (ubiquitin-transferase) domain-containing protein
TGGT1_279100	-1.47	-1.63	hypothetical protein
TGGT1_221220	-1.47	-1.17	hypothetical protein
TGGT1_287980	-1.48	-1.40	FHA domain-containing protein
TGGT1_306730	-1.49	-1.35	hypothetical protein
TGGT1_230905	-1.51	-1.26	hydrolase, alpha/beta fold family protein
TGGT1_264720	-1.52	-1.46	hypothetical protein
TGGT1_237450	-1.53	-1.27	hypothetical protein
TGGT1_287990	-1.54	-1.22	hypothetical protein
TGGT1_235700	-1.55	-1.44	sedoheptulose-1,7-bisphosphatase
TGGT1_264225	-1.56	-1.86	Toxoplasma gondii family B protein
TGGT1_222420	-1.57	-1.56	hypothetical protein
TGGT1_264940	-1.58	-1.62	kelch repeat-containing protein
TGGT1_310160	-1.59	-1.64	argonaute AGO
TGGT1_202580	-1.62	-1.36	ATPase, AAA family protein
TGGT1_462961	-1.62	-2.13	hypothetical protein
TGGT1_266035	-1.66	-1.37	hypothetical protein
TGGT1_319308	-1.66	-1.59	hypothetical protein
TGGT1_315123	-1.67	-1.84	hypothetical protein
TGGT1_267820	-1.68	-1.62	putative electron transfer flavoprotein subunit beta
TGGT1_410390	-1.68	-1.63	hypothetical protein
TGGT1_217860	-1.69	-1.72	hypothetical protein
TGGT1_272730	-1.69	-1.31	hypothetical protein
TGGT1_233960	-1.69	-1.65	hypothetical protein
TGGT1_254840	-1.74	-1.78	tetratricopeptide repeat-containing protein
TGGT1_297910	-1.75	-1.97	hypothetical protein
TGGT1_266380	-1.80	-1.71	hypothetical protein
TGGT1_263030	-1.81	-1.79	dynein light chain 8 family E protein
TGGT1_315127	-1.84	-1.83	hypothetical protein

TGGT1_322100	-1.84	-1.62	myosin-light-chain kinase
TGGT1_319890	-1.85	-1.94	hypothetical protein
TGGT1_312435	-1.85	-1.58	hypothetical protein
TGGT1_227350	-1.89	-1.81	hypothetical protein
TGGT1_312240	-1.91	-1.38	hypothetical protein
TGGT1_410660	-1.94	-2.16	hypothetical protein
TGGT1_270670	-1.94	-2.05	hypothetical protein
TGGT1_233370	-1.96	-1.99	hypothetical protein
TGGT1_363030	-1.96	-2.00	rhoptry protein ROP8
TGGT1_295125	-1.97	-2.26	rhoptry protein ROP4
TGGT1_264950	-1.98	-2.01	hypothetical protein
TGGT1_274290	-2.01	-2.03	Toxoplasma gondii family B protein
TGGT1_279350	-2.05	-1.64	hypothetical protein
TGGT1_230830	-2.07	-1.93	se family associated with various cellular activities (AAA) domain-containing p
TGGT1_267585	-2.07	-1.71	hypothetical protein
TGGT1_239475	-2.07	-1.53	hypothetical protein
TGGT1_207880	-2.15	-1.57	hypothetical protein
TGGT1_270680	-2.15	-2.21	hypothetical protein
TGGT1_286250	-2.16	-2.03	hypothetical protein
TGGT1_291075	-2.16	-2.42	hypothetical protein
TGGT1_212810	-2.17	-2.11	hypothetical protein
TGGT1_237170	-2.17	-2.31	hypothetical protein
TGGT1_222340	-2.18	-2.21	NOL1/NOP2/sun family protein
TGGT1_411240	-2.18	-2.44	toxoplasma gondii family B protein
TGGT1_254850	-2.24	-2.23	putative myosin heavy chain
TGGT1_279345	-2.27	-1.87	hypothetical protein
TGGT1_283840	-2.29	-2.49	GATA zinc finger domain-containing protein
TGGT1_230900	-2.30	-2.34	hypothetical protein
TGGT1_411560	-2.35	-2.12	hypothetical protein
TGGT1_204010	-2.36	-2.42	hypothetical protein
TGGT1_231125	-2.36	-2.17	hypothetical protein
TGGT1_205265	-2.37	-2.31	transporter, cation channel family protein
TGGT1_277230	-2.38	-1.33	hypothetical protein
TGGT1_238210	-2.39	-2.07	EGF family domain-containing protein
TGGT1_244950	-2.40	-2.37	hypothetical protein
TGGT1_204000	-2.43	-1.91	hypothetical protein
TGGT1_320000	-2.58	-2.49	SCY kinase (incomplete catalytic triad)
TGGT1_233210	-2.60	-2.27	hypothetical protein
TGGT1_202280	-2.62	-2.75	WD domain, G-beta repeat-containing protein
TGGT1_314362	-2.65	-2.48	hypothetical protein
TGGT1_211270	-2.68	-2.44	sushi domain (scr repeat) domain-containing protein
TGGT1_264485	-2.69	-2.69	AP2 domain transcription factor AP2IX-3
TGGT1_222060	-2.70	-2.63	hypothetical protein
TGGT1_259155	-2.74	-2.74	hypothetical protein
TGGT1_268220	-2.74	-2.60	hypothetical protein
TGGT1_267030	-2.75	-2.90	ribonuclease type III Dicer
TGGT1_294650	-2.77	-2.50	hypothetical protein
TGGT1_306700	-2.78	-2.83	hypothetical protein
TGGT1_294400	-2.78	-2.19	hypothetical protein
TGGT1_295440	-2.85	-2.83	hypothetical protein
TGGT1_213255	-2.87	-1.80	hypothetical protein
TGGT1_329600	-2.91	-2.75	hypothetical protein
TGGT1_289218	-2.92	-3.02	hypothetical protein
TGGT1_233770A	-2.95	-2.09	calcium-translocating P-type ATPase, PMCA-type protein
TGGT1_267435	-2.97	-2.37	hypothetical protein
TGGT1_244412	-3.01	-2.63	hypothetical protein
TGGT1_200320	-3.02	-3.18	hypoxanthine-xanthine-guanine phosphoribosyl transferase HXGPRT
TGGT1_289222	-3.02	-3.12	hypothetical protein
TGGT1_289060	-3.09	-2.84	hypothetical protein
TGGT1_295430	-3.13	-3.10	hypothetical protein

TGGT1_245746B	-3.33	-3.20	hypothetical protein
TGGT1_301890	-3.41	-3.55	Toxoplasma gondii family B protein
TGGT1_244408	-3.43	-3.41	hypothetical protein
TGGT1_244406	-3.45	-3.45	cysteine dioxygenase type i protein
TGGT1_289216	-3.47	-3.53	hypothetical protein
TGGT1_215540	-3.61	-3.74	hypothetical protein
TGGT1_312245	-3.61	-3.22	hypothetical protein
TGGT1_233770B	-3.65	-2.67	calcium-translocating P-type ATPase, PMCA-type protein
TGGT1_361030	-3.65	-3.60	hypothetical protein
TGGT1_293420	-3.79	-3.98	transporter, major facilitator family protein
TGGT1_223480	-3.85	-3.87	sushi domain (scr repeat) domain-containing protein
TGGT1_319992	-3.95	-3.87	hypothetical protein
TGGT1_266500	-4.04	-4.24	hypothetical protein
TGGT1_245746A	-4.13	-3.80	hypothetical protein
TGGT1_411650	-4.23	-4.63	hypothetical protein
TGGT1_314340	-4.28	-4.19	Sodium:neurotransmitter symporter family protein
TGGT1_287240	-4.44	-3.99	hypothetical protein
TGGT1_287235	-4.47	-4.26	hypothetical protein
TGGT1_203685	-4.51	-3.78	hypothetical protein
TGGT1_203682	-4.53	-3.71	hypothetical protein
TGGT1_245748	-4.57	-4.22	hypothetical protein
TGGT1_274280	-4.65	-4.93	hypothetical protein
TGGT1_462973	-4.67	-4.16	hypothetical protein
TGGT1_411750	-4.69	-4.81	hypothetical protein
TGGT1_411680	-4.73	-5.05	hypothetical protein
TGGT1_245752	-4.77	-4.48	WD domain, G-beta repeat-containing protein
TGGT1_200595	-4.81	-4.57	hypothetical protein
TGGT1_280500	-4.99	-4.84	inorganic anion transporter, sulfate permease (SulP) family protein
TGGT1_408600	-5.10	-4.74	hypothetical protein
TGGT1_203688	-5.45	-4.81	hypothetical protein
TGGT1_304450	-11.20	-11.41	cation-transporting ATPase
TGGT1_306334	3.61	N.S.	hypothetical protein
TGGT1_311485	2.80	N.S.	hypothetical protein
TGGT1_294320	2.10	N.S.	Oxysterol-binding protein
TGGT1_282000	1.50	N.S.	hypothetical protein
TGGT1_218910	1.43	N.S.	hypothetical protein
TGGT1_246760	1.41	N.S.	hypothetical protein
TGGT1_254603	1.36	N.S.	hypothetical protein
TGGT1_235860	1.26	N.S.	subtilisin SUB11
TGGT1_218540	1.23	N.S.	putative peptidase S15
TGGT1_200700	1.19	N.S.	Toxoplasma gondii family C protein
TGGT1_306070	1.19	N.S.	hypothetical protein
TGGT1_411430	1.19	N.S.	rhoptry protein ROP5
TGGT1_244400	1.17	N.S.	hypothetical protein
TGGT1_200250	1.12	N.S.	microneme protein MIC17A
TGGT1_212970	1.08	N.S.	protein kinase (incomplete catalytic triad)
TGGT1_202010	1.06	N.S.	hypothetical protein
TGGT1_269930	1.05	N.S.	calcium binding egf domain-containing protein
TGGT1_255860	1.03	N.S.	hypothetical protein
TGGT1_258670	1.01	N.S.	hypothetical protein
TGGT1_293650	1.00	N.S.	hypothetical protein
TGGT1_208820	-1.00	N.S.	1-deoxy-D-xylulose-5-phosphate synthase
TGGT1_310650	-1.00	N.S.	hypothetical protein
TGGT1_267725	-1.01	N.S.	hypothetical protein
TGGT1_267530	-1.01	N.S.	hypothetical protein
TGGT1_269050	-1.01	N.S.	hypothetical protein
TGGT1_266366	-1.02	N.S.	BT1 family protein
TGGT1_288030	-1.03	N.S.	hypothetical protein
TGGT1_267700	-1.03	N.S.	hypothetical protein
TGGT1_271770	-1.04	N.S.	hypothetical protein

TGGT1_365840	-1.04	N.S.	hypothetical protein
TGGT1_267775	-1.04	N.S.	hypothetical protein
TGGT1_299000	-1.04	N.S.	hypothetical protein
TGGT1_267800	-1.05	N.S.	dynamin-related protein DRPA
TGGT1_264830	-1.07	N.S.	hypothetical protein
TGGT1_411300	-1.08	N.S.	hypothetical protein
TGGT1_288000	-1.09	N.S.	hypothetical protein
TGGT1_205680	-1.11	N.S.	hypothetical protein
TGGT1_268660	-1.11	N.S.	hypothetical protein
TGGT1_279310	-1.12	N.S.	hypothetical protein
TGGT1_268035	-1.15	N.S.	hypothetical protein
TGGT1_314550	-1.22	N.S.	hypothetical protein
TGGT1_306692	-1.22	N.S.	hypothetical protein
TGGT1_258550	-1.28	N.S.	SAG-related sequence SRS28
TGGT1_221880	-1.29	N.S.	hypothetical protein
TGGT1_410860	-1.38	N.S.	hypothetical protein
TGGT1_294240	-1.77	N.S.	hypothetical protein
TGGT1_306860	-2.27	N.S.	hypothetical protein
TGGT1_207160	N.S.	6.27	SAG-related sequence SRS49D
TGGT1_318130B	N.S.	5.63	Toxoplasma gondii family E protein
TGGT1_252080	N.S.	5.46	hypothetical protein
TGGT1_214550	N.S.	5.16	hypothetical protein
TGGT1_263135	N.S.	4.90	hypothetical protein
TGGT1_278710	N.S.	4.75	hypothetical protein
TGGT1_278080	N.S.	4.29	Toxoplasma gondii family A protein
TGGT1_204870	N.S.	4.06	hypothetical protein
TGGT1_225102	N.S.	3.89	hypothetical protein
TGGT1_245770B	N.S.	3.73	hypothetical protein
TGGT1_218470	N.S.	3.56	putative protein disulfide-isomerase
TGGT1_269315	N.S.	3.54	hypothetical protein
TGGT1_305890	N.S.	3.33	hypothetical protein
TGGT1_205420	N.S.	3.30	aspartate-semialdehyde dehydrogenase
TGGT1_245770A	N.S.	3.28	hypothetical protein
TGGT1_242350	N.S.	3.27	tetratricopeptide repeat-containing protein
TGGT1_220080	N.S.	3.21	Toxoplasma gondii family C protein
TGGT1_215900	N.S.	3.18	hypothetical protein
TGGT1_236900	N.S.	3.18	hypothetical protein
TGGT1_249520	N.S.	3.18	hypothetical protein
TGGT1_311370	N.S.	3.17	putative methylmalonate-semialdehyde dehydrogenase [acylating]
TGGT1_243382	N.S.	3.08	hypothetical protein
TGGT1_356590	N.S.	3.07	hypothetical protein
TGGT1_215185	N.S.	2.94	hypothetical protein
TGGT1_411080	N.S.	2.94	hypothetical protein
TGGT1_293800	N.S.	2.91	putative transmembrane protein 65
TGGT1_300070	N.S.	2.86	hypothetical protein
TGGT1_223045	N.S.	2.78	hypothetical protein
TGGT1_268800	N.S.	2.77	hypothetical protein
TGGT1_270280	N.S.	2.73	hypothetical protein
TGGT1_307045	N.S.	2.69	Toxoplasma gondii family B protein
TGGT1_257010	N.S.	2.68	sporozoite developmental protein
TGGT1_280570	N.S.	2.67	SAG-related sequence SRS35A
TGGT1_221840	N.S.	2.64	hypothetical protein
TGGT1_358440	N.S.	2.59	KRUF family protein
TGGT1_216875	N.S.	2.57	cpw-wpc domain-containing protein
TGGT1_410970	N.S.	2.57	hypothetical protein
TGGT1_224760	N.S.	2.47	SAG-related sequence SRS40E
TGGT1_307050	N.S.	2.47	hypothetical protein
TGGT1_209755B	N.S.	2.47	hypothetical protein
TGGT1_273468	N.S.	2.45	hypothetical protein
TGGT1_313318	N.S.	2.44	hypothetical protein

TGGT1_267980	N.S.	2.43	hypothetical protein
TGGT1_286782	N.S.	2.43	MAM domain-containing protein
TGGT1_259270	N.S.	2.43	SAG-related sequence SRS26E
TGGT1_204560	N.S.	2.42	putative type I fatty acid synthase
TGGT1_305100	N.S.	2.41	hypothetical protein
TGGT1_262160	N.S.	2.40	hypothetical protein
TGGT1_260460	N.S.	2.38	putative oxidoreductase
TGGT1_291040	N.S.	2.38	lactate dehydrogenase LDH2
TGGT1_253760	N.S.	2.37	hypothetical protein
TGGT1_235950	N.S.	2.36	subtilisin SUB8
TGGT1_258200	N.S.	2.33	hypothetical protein
TGGT1_239855	N.S.	2.32	Ras-associated protein Rap1 isoform 1 family protein
TGGT1_238980	N.S.	2.31	hypothetical protein
TGGT1_306688	N.S.	2.31	hypothetical protein
TGGT1_301140	N.S.	2.30	SAG-related sequence SRS19A
TGGT1_299020	N.S.	2.30	AP2 domain transcription factor AP2III-4
TGGT1_273980	N.S.	2.30	hypothetical protein
TGGT1_315520	N.S.	2.29	calcium binding egf domain-containing protein
TGGT1_266785	N.S.	2.28	zinc finger (CCCH type) motif-containing protein
TGGT1_408550	N.S.	2.27	hypothetical protein
TGGT1_225140	N.S.	2.24	BT1 family protein
TGGT1_309840	N.S.	2.24	hypothetical protein
TGGT1_243700	N.S.	2.21	hypothetical protein
TGGT1_240930	N.S.	2.21	MoaC family protein
TGGT1_235010	N.S.	2.19	hypothetical protein
TGGT1_235187A	N.S.	2.18	hypothetical protein
TGGT1_285665	N.S.	2.16	hypothetical protein
TGGT1_271980	N.S.	2.14	SAG-related sequence SRS32
TGGT1_297660	N.S.	2.13	hypothetical protein
TGGT1_268860	N.S.	2.13	enolase 1
TGGT1_252640	N.S.	2.11	P-type ATPase PMA1
TGGT1_411050	N.S.	2.11	KRUF family protein
TGGT1_210950	N.S.	2.08	oocyst wall protein
TGGT1_209755A	N.S.	2.07	hypothetical protein
TGGT1_227090	N.S.	2.06	aspartokinase
TGGT1_225070	N.S.	2.05	hypothetical protein
TGGT1_231900	N.S.	2.05	acyl-CoA dehydrogenase domain-containing protein
TGGT1_227070	N.S.	2.04	hypothetical protein
TGGT1_222935	N.S.	2.03	hypothetical protein
TGGT1_245980	N.S.	2.02	hypothetical protein
TGGT1_320090	N.S.	2.00	hypothetical protein
TGGT1_275380	N.S.	2.00	SAG-related sequence SRS47D
TGGT1_261560	N.S.	2.00	Tat binding protein 1(TBP-1)-interacting protein TBPIP
TGGT1_220585	N.S.	1.99	hypothetical protein
TGGT1_213290	N.S.	1.94	hypothetical protein
TGGT1_269640	N.S.	1.92	hypothetical protein
TGGT1_224080	N.S.	1.91	Kazal-type serine protease inhibitor domain-containing protein
TGGT1_320250	N.S.	1.91	SAG-related sequence SRS15A
TGGT1_286540	N.S.	1.91	hypothetical protein
TGGT1_411490	N.S.	1.90	putative toxoplasma gondii family E protein
TGGT1_291830	N.S.	1.89	putative DNA double-strand break repair rad50 ATPase
TGGT1_237585	N.S.	1.89	hypothetical protein
TGGT1_285825	N.S.	1.88	hypothetical protein
TGGT1_297670	N.S.	1.86	hypothetical protein
TGGT1_299260	N.S.	1.85	hypothetical protein
TGGT1_211050	N.S.	1.83	ATPase, AAA family protein
TGGT1_293250	N.S.	1.82	hypothetical protein
TGGT1_410790	N.S.	1.82	hypothetical protein
TGGT1_225028	N.S.	1.81	putative myosin heavy chain
TGGT1_294410	N.S.	1.81	hypothetical protein

TGGT1_260000	N.S.	1.80	hypothetical protein
TGGT1_273750	N.S.	1.80	hypothetical protein
TGGT1_218510	N.S.	1.77	hypothetical protein
TGGT1_316320	N.S.	1.77	ABC transporter transmembrane region domain-containing protein
TGGT1_293252	N.S.	1.76	hypothetical protein
TGGT1_254335	N.S.	1.75	hypothetical protein
TGGT1_233792	N.S.	1.75	hypothetical protein
TGGT1_270273	N.S.	1.75	hypothetical protein
TGGT1_215895	N.S.	1.73	AP2 domain-containing protein
TGGT1_257720	N.S.	1.73	putative proton ATPase
TGGT1_286570	N.S.	1.73	hypothetical protein
TGGT1_200230	N.S.	1.72	microneme protein MIC17C
TGGT1_212275	N.S.	1.72	hypothetical protein
TGGT1_282030	N.S.	1.70	hypothetical protein
TGGT1_312930	N.S.	1.70	putative cystathione gamma lyase
TGGT1_253755	N.S.	1.69	hypothetical protein
TGGT1_319390	N.S.	1.69	hypothetical protein
TGGT1_356120	N.S.	1.68	putative kelch repeat protein
TGGT1_217961	N.S.	1.66	hypothetical protein
TGGT1_366630	N.S.	1.65	hypothetical protein
TGGT1_201650	N.S.	1.65	hypothetical protein
TGGT1_201180A	N.S.	1.64	hypothetical protein
TGGT1_204325	N.S.	1.64	hypothetical protein
TGGT1_358240	N.S.	1.64	hypothetical protein
TGGT1_255175	N.S.	1.63	hypothetical protein
TGGT1_285300	N.S.	1.62	hypothetical protein
TGGT1_411070	N.S.	1.62	tRNA-Ala
TGGT1_225098	N.S.	1.62	hypothetical protein
TGGT1_224680	N.S.	1.61	hypothetical protein
TGGT1_258520	N.S.	1.60	WD domain, G-beta repeat-containing protein
TGGT1_319360	N.S.	1.59	SAG-related sequence SRS17A
TGGT1_243655	N.S.	1.58	GMC oxidoreductase
TGGT1_265320	N.S.	1.58	hypothetical protein
TGGT1_210320	N.S.	1.57	SAG-related sequence SRS37A
TGGT1_254330	N.S.	1.56	lipase
TGGT1_281660	N.S.	1.54	hypothetical protein
TGGT1_203200	N.S.	1.54	hypothetical protein
TGGT1_293280	N.S.	1.53	cyclin protein
TGGT1_289740	N.S.	1.53	hypothetical protein
TGGT1_269310	N.S.	1.53	hypothetical protein
TGGT1_276200	N.S.	1.52	hypothetical protein
TGGT1_409760	N.S.	1.51	putative transmembrane protein
TGGT1_281675	N.S.	1.50	putative protein kinase
TGGT1_207130	N.S.	1.49	SAG-related sequence SRS49A
TGGT1_357130	N.S.	1.48	WD domain, G-beta repeat protein
TGGT1_212180	N.S.	1.47	hypothetical protein
TGGT1_203230	N.S.	1.46	hypothetical protein
TGGT1_242750	N.S.	1.46	hypothetical protein
TGGT1_203590	N.S.	1.45	hypothetical protein
TGGT1_237510	N.S.	1.44	hypothetical protein
TGGT1_226610	N.S.	1.44	hypothetical protein
TGGT1_408250	N.S.	1.43	hypothetical protein
TGGT1_254790	N.S.	1.42	hypothetical protein
TGGT1_275360	N.S.	1.41	SAG-related sequence SRS47B
TGGT1_247310	N.S.	1.40	cpw-wpc domain-containing protein
TGGT1_316380	N.S.	1.39	hypothetical protein
TGGT1_200350	N.S.	1.37	subtilisin SUB3
TGGT1_209705	N.S.	1.37	hypothetical protein
TGGT1_305150	N.S.	1.37	tetratricopeptide repeat-containing protein
TGGT1_202430	N.S.	1.36	hypothetical protein

TGGT1_408330	N.S.	1.35	toxoplasma gondii family D protein
TGGT1_217940	N.S.	1.35	hypothetical protein
TGGT1_251857	N.S.	1.34	hypothetical protein
TGGT1_237800	N.S.	1.33	dense granule protein GRA11
TGGT1_252370	N.S.	1.32	AP2 domain transcription factor AP2III-1
TGGT1_313220	N.S.	1.32	hypothetical protein
TGGT1_215680	N.S.	1.32	SAG-related sequence SRS18
TGGT1_240200	N.S.	1.32	hypothetical protein
TGGT1_235315	N.S.	1.31	PAN domain-containing protein
TGGT1_223520	N.S.	1.30	EF hand domain-containing protein
TGGT1_280465	N.S.	1.29	hypothetical protein
TGGT1_230640	N.S.	1.28	hypothetical protein
TGGT1_232170	N.S.	1.27	hypothetical protein
TGGT1_212410	N.S.	1.27	dense granule protein GRA11
TGGT1_304730	N.S.	1.27	Mob1/phocein family protein
TGGT1_287430	N.S.	1.27	hypothetical protein
TGGT1_260375	N.S.	1.26	hypothetical protein
TGGT1_274170	N.S.	1.25	protein kinase (incomplete catalytic triad)
TGGT1_311815	N.S.	1.24	hypothetical protein
TGGT1_247570	N.S.	1.23	hypothetical protein
TGGT1_211280	N.S.	1.22	hypothetical protein
TGGT1_212420	N.S.	1.21	hypothetical protein
TGGT1_251855	N.S.	1.21	hypothetical protein
TGGT1_273740	N.S.	1.21	putative acetyl-CoA acyltransferase B
TGGT1_305610	N.S.	1.20	hypothetical protein
TGGT1_253120	N.S.	1.19	putative mandelonitrile lyase
TGGT1_286740	N.S.	1.19	microneme-like protein
TGGT1_240390	N.S.	1.18	calcium-dependent protein kinase
TGGT1_209040	N.S.	1.18	hypothetical protein
TGGT1_233040	N.S.	1.17	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_276810	N.S.	1.16	hypothetical protein
TGGT1_232270	N.S.	1.15	histidine acid phosphatase superfamily protein
TGGT1_318742	N.S.	1.15	hypothetical protein
TGGT1_233153	N.S.	1.14	hypothetical protein
TGGT1_268970	N.S.	1.14	hypothetical protein
TGGT1_311490	N.S.	1.14	hypothetical protein
TGGT1_271010	N.S.	1.14	hypothetical protein
TGGT1_258870B	N.S.	1.13	hypothetical protein
TGGT1_286460	N.S.	1.11	hypothetical protein
TGGT1_201180B	N.S.	1.10	hypothetical protein
TGGT1_220060B	N.S.	1.10	hypothetical protein
TGGT1_293520	N.S.	1.09	hypothetical protein
TGGT1_245428	N.S.	1.08	hypothetical protein
TGGT1_220420	N.S.	1.08	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_200240	N.S.	1.06	microneme protein MIC17B
TGGT1_239600	N.S.	1.06	rhoptry kinase family protein ROP23 (incomplete catalytic triad)
TGGT1_315962	N.S.	1.06	hypothetical protein
TGGT1_310090	N.S.	1.05	hypothetical protein
TGGT1_299790	N.S.	1.05	hypothetical protein
TGGT1_275870	N.S.	1.04	tubulin/FtsZ family, GTPase domain-containing protein
TGGT1_219640	N.S.	1.03	hypothetical protein
TGGT1_248280	N.S.	1.03	hypothetical protein
TGGT1_256020	N.S.	1.02	putative EFHC1
TGGT1_267660	N.S.	-1.01	hypothetical protein
TGGT1_306720	N.S.	-1.01	hypothetical protein
TGGT1_264710	N.S.	-1.02	bucentaur or craniofacial development domain-containing protein
TGGT1_264770	N.S.	-1.02	hypothetical protein
TGGT1_267710	N.S.	-1.03	CPSF A subunit region protein
TGGT1_264472	N.S.	-1.03	hypothetical protein
TGGT1_266400	N.S.	-1.04	hypothetical protein

TGGT1_276155	N.S.	-1.06	hypothetical protein
TGGT1_264875	N.S.	-1.06	hypothetical protein
TGGT1_202970	N.S.	-1.06	hypothetical protein
TGGT1_267500	N.S.	-1.08	hypothetical protein
TGGT1_285445	N.S.	-1.09	hypothetical protein
TGGT1_226985	N.S.	-1.09	hypothetical protein
TGGT1_236670B	N.S.	-1.10	hypothetical protein
TGGT1_279380	N.S.	-1.11	hypothetical protein
TGGT1_205520	N.S.	-1.11	hypothetical protein
TGGT1_266270	N.S.	-1.13	short-chain-acyl-CoA dehydrogenase
TGGT1_237560	N.S.	-1.13	iron-sulfur cluster protein ISCU
TGGT1_226110	N.S.	-1.16	Copper-exporting ATPase
TGGT1_264420	N.S.	-1.23	putative lipoprotein
TGGT1_366510	N.S.	-1.26	hypothetical protein
TGGT1_294660	N.S.	-1.28	hypothetical protein
TGGT1_259150	N.S.	-1.29	hypothetical protein
TGGT1_462971	N.S.	-1.30	hypothetical protein
TGGT1_310830	N.S.	-1.30	enoyl-CoA hydratase/isomerase family protein
TGGT1_411590	N.S.	-1.31	nucleoside-triphosphatase
TGGT1_267642	N.S.	-1.34	hypothetical protein
TGGT1_280680	N.S.	-1.35	hypothetical protein
TGGT1_285400	N.S.	-1.38	hypothetical protein
TGGT1_218750	N.S.	-1.39	hypothetical protein
TGGT1_220280	N.S.	-1.40	mucin family glycoprotein
TGGT1_216710	N.S.	-1.42	transporter, major facilitator family protein
TGGT1_411760	N.S.	-1.48	actin
TGGT1_236290	N.S.	-1.51	hypothetical protein
TGGT1_205050	N.S.	-1.56	hypothetical protein
TGGT1_242100	N.S.	-1.66	hypothetical protein
TGGT1_363220	N.S.	-1.67	hypothetical protein
TGGT1_234400	N.S.	-1.68	hypothetical protein
TGGT1_289280	N.S.	-1.70	hypothetical protein
TGGT1_295662	N.S.	-1.72	hypothetical protein
TGGT1_268360	N.S.	-1.73	hypothetical protein
TGGT1_316550	N.S.	-1.83	hypothetical protein
TGGT1_304480	N.S.	-1.89	3-oxo-5-alpha-steroid 4-dehydrogenase
TGGT1_252890	N.S.	-2.09	hypothetical protein
TGGT1_266740	N.S.	-2.31	RNA recognition motif-containing protein
TGGT1_411210	N.S.	-2.57	hypothetical protein
TGGT1_287250	N.S.	-2.58	hypothetical protein
TGGT1_410520	N.S.	-2.58	putative OTU family cysteine protease
TGGT1_275760	N.S.	-2.99	hypothetical protein
TGGT1_242900	N.S.	-3.10	Toxoplasma gondii family A protein
TGGT1_358120	N.S.	-6.82	hypothetical protein

Table 4.2. Differential expression analysis identified many differentially expressed gene amongst extracellular RH and non-lab-adapted GT1 populations

Following RNA-seq of 6-hour extracellular parasites, DEA identified 927 DEGs in GT1 population B2-P11 and 955 DEGS in GT1 population B4-P11, both relative to RH. In union, 692 DEGs were found to have similar expression across the two GT1 populations. All genes with Log_2 FC values shown are considered significant (absolute FC $\geq$2; *q*-value $\leq$0.05); N.S. = not significant.

Table 4.2. Differential expression analysis identified many differentially expressed gene amongst extracellular RH and non-lab-adapted GT1 populations

Gene ID	Log_2 FC relative to RH		Product Description
	B2-P11	B4-P11	
TGGT1_292375	7.64	8.65	KRUF family protein
TGGT1_300980	7.56	7.38	hypothetical protein
TGGT1_242760	7.02	6.92	hypothetical protein
TGGT1_410080	6.93	6.53	hypothetical protein
TGGT1_298820	6.68	6.49	hypothetical protein
TGGT1_363340	6.62	6.37	hypothetical protein
TGGT1_220720	6.62	5.96	hypothetical protein
TGGT1_285470	6.35	5.89	patched family protein
TGGT1_220080	6.11	5.66	Toxoplasma gondii family C protein
TGGT1_278365	6.02	5.62	Toxoplasma gondii family A protein
TGGT1_300990	5.84	5.55	Toxoplasma gondii family C protein
TGGT1_411310	5.78	5.47	hypothetical protein
TGGT1_301180	5.65	5.33	SAG-related sequence SRS19F
TGGT1_411800	5.49	5.20	hypothetical protein
TGGT1_411520	5.49	5.12	hypothetical protein
TGGT1_293840	5.49	5.06	hypothetical protein
TGGT1_216680	5.19	5.06	ankyrin repeat-containing protein
TGGT1_411000	5.14	5.00	hypothetical protein
TGGT1_307050	5.08	4.97	hypothetical protein
TGGT1_200130	5.01	4.90	Toxoplasma gondii family C protein
TGGT1_265030	4.94	4.83	hypothetical protein
TGGT1_284310	4.91	4.78	hypothetical protein
TGGT1_264260	4.80	4.75	hypothetical protein
TGGT1_300780	4.70	4.65	hypothetical protein
TGGT1_249800	4.70	4.49	hypothetical protein
TGGT1_219218	4.70	4.47	hypothetical protein
TGGT1_278120	4.68	4.36	SCP family extracellular subfamily protein
TGGT1_278110	4.66	4.31	1,3-beta-glucan synthase component protein
TGGT1_411020	4.63	4.31	hypothetical protein
TGGT1_289350	4.60	4.27	ATP-binding cassette G family transporter ABCG84
TGGT1_216290B	4.40	4.27	hypothetical protein
TGGT1_264240	4.35	4.25	hypothetical protein
TGGT1_409050	4.35	4.24	hypothetical protein
TGGT1_216290A	4.17	4.21	hypothetical protein
TGGT1_201690	4.10	4.18	BT1 family protein
TGGT1_255210	4.09	4.09	ATPase, AAA family protein
TGGT1_217724	4.09	3.96	hypothetical protein
TGGT1_293780	4.05	3.91	hypothetical protein
TGGT1_278400	4.03	3.91	Toxoplasma gondii family A protein
TGGT1_210235	4.01	3.88	hypothetical protein
TGGT1_358470	3.99	3.81	hypothetical protein
TGGT1_301170	3.99	3.76	SAG-related sequence SRS19D
TGGT1_210245	3.96	3.75	hypothetical protein
TGGT1_462968	3.96	3.73	putative ATPase family protein
TGGT1_278380	3.94	3.71	Toxoplasma gondii family A protein
TGGT1_311335	3.79	3.70	dual specificity phosphatase, catalytic domain-containing protein

TGGT1_283450	3.79	3.65	Toxoplasma gondii family B protein
TGGT1_202025	3.76	3.63	hypothetical protein
TGGT1_410890	3.70	3.62	hypothetical protein
TGGT1_323320	3.59	3.57	hypothetical protein
TGGT1_411150	3.52	3.51	hypothetical protein
TGGT1_305790	3.50	3.48	hypothetical protein
TGGT1_217020	3.48	3.46	ATPase, AFG1 family protein
TGGT1_323310	3.48	3.42	hypothetical protein
TGGT1_220060	3.48	3.41	hypothetical protein
TGGT1_307480	3.48	3.40	Toxoplasma gondii family B protein
TGGT1_307070	3.45	3.40	hypothetical protein
TGGT1_284420	3.44	3.39	hypothetical protein
TGGT1_278370	3.38	3.26	Toxoplasma gondii family A protein
TGGT1_257945	3.38	3.26	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_216140	3.36	3.24	tetratricopeptide repeat-containing protein
TGGT1_210450	3.36	3.21	hypothetical protein
TGGT1_249810	3.35	3.21	putative activating signal cointegrator 1 complex subunit 3
TGGT1_301210	3.35	3.21	putative NAD(P) transhydrogenase subunit beta
TGGT1_244700	3.33	3.20	NAD(+)/NADH kinase domain-containing protein
TGGT1_410730	3.32	3.20	PT repeat protein
TGGT1_320190	3.32	3.17	SAG-related sequence SRS16B
TGGT1_253770	3.29	3.16	hypothetical protein
TGGT1_410930	3.26	3.07	hypothetical protein
TGGT1_244710	3.25	3.05	hypothetical protein
TGGT1_217728	3.24	3.04	hypothetical protein
TGGT1_287460	3.20	2.99	hypothetical protein
TGGT1_328800	3.20	2.98	hypothetical protein
TGGT1_271015	3.19	2.96	hypothetical protein
TGGT1_306688	3.18	2.95	hypothetical protein
TGGT1_411420	3.17	2.89	hypothetical protein
TGGT1_255215	3.14	2.89	hypothetical protein
TGGT1_276810	3.13	2.89	hypothetical protein
TGGT1_410090	3.06	2.88	hypothetical protein
TGGT1_409590	3.03	2.87	putative transmembrane protein
TGGT1_263730	3.02	2.87	FAD-dependent glycerol-3-phosphate dehydrogenase
TGGT1_411820	2.97	2.87	toxoplasma gondii family C protein
TGGT1_305890	2.96	2.87	hypothetical protein
TGGT1_322110	2.95	2.85	hypothetical protein
TGGT1_281400	2.95	2.83	phosphofructokinase domain-containing protein
TGGT1_410970	2.94	2.83	hypothetical protein
TGGT1_262590	2.90	2.81	hypothetical protein
TGGT1_278410	2.90	2.81	Toxoplasma gondii family A protein
TGGT1_217420	2.90	2.80	hypothetical protein
TGGT1_410790	2.84	2.80	hypothetical protein
TGGT1_216335	2.82	2.79	hypothetical protein
TGGT1_263740	2.79	2.72	ABC transporter transmembrane region domain-containing protein
TGGT1_411390	2.69	2.71	hypothetical protein
TGGT1_262600	2.68	2.69	hypothetical protein
TGGT1_301370	2.66	2.67	DHHC zinc finger domain-containing protein
TGGT1_411730	2.62	2.67	hypothetical protein
TGGT1_245530	2.61	2.65	hypothetical protein
TGGT1_411270	2.60	2.65	hypothetical protein
TGGT1_251857	2.60	2.65	hypothetical protein
TGGT1_252255	2.58	2.65	hypothetical protein
TGGT1_290970	2.58	2.63	8-amino-7-oxononanoate synthase
TGGT1_409990	2.57	2.63	hypothetical protein
TGGT1_260530	2.56	2.62	Sel1 repeat-containing protein
TGGT1_204870	2.56	2.54	hypothetical protein
TGGT1_411400	2.55	2.54	hypothetical protein
TGGT1_251855	2.53	2.49	hypothetical protein

TGGT1_365020	2.52	2.46	cytochrome C family oxidase subunit III subfamily protein
TGGT1_217722	2.52	2.46	hypothetical protein
TGGT1_235680	2.49	2.45	peptidase M16 inactive domain-containing protein
TGGT1_217400	2.48	2.45	hypothetical protein
TGGT1_215180	2.47	2.44	hypothetical protein
TGGT1_220650	2.46	2.43	hypothetical protein
TGGT1_278060	2.45	2.43	Mre11 DNA-binding domain-containing protein
TGGT1_306680	2.44	2.41	hypothetical protein
TGGT1_215343	2.42	2.37	hypothetical protein
TGGT1_231470	2.41	2.37	hypothetical protein
TGGT1_270655	2.39	2.36	hypothetical protein
TGGT1_213635	2.38	2.34	hypothetical protein
TGGT1_207750	2.34	2.32	hypothetical protein
TGGT1_267980	2.34	2.32	hypothetical protein
TGGT1_283855	2.33	2.28	hypothetical protein
TGGT1_304670	2.30	2.25	leucine rich repeat-containing protein
TGGT1_320740	2.30	2.22	hypothetical protein
TGGT1_411120	2.27	2.22	putative transmembrane protein
TGGT1_320105	2.27	2.20	hypothetical protein
TGGT1_306338B	2.25	2.20	putative dynein gamma chain, flagellar outer arm
TGGT1_293500	2.23	2.19	hypothetical protein
TGGT1_231992	2.21	2.18	hypothetical protein
TGGT1_258230	2.20	2.17	rhoptry kinase family protein ROP20
TGGT1_242590	2.19	2.16	hypothetical protein
TGGT1_269600	2.17	2.15	biotin-requiring enzyme domain-containing protein
TGGT1_266105	2.14	2.15	EF hand family protein
TGGT1_217630	2.14	2.13	hypothetical protein
TGGT1_261022	2.13	2.13	dynein heavy chain family protein
TGGT1_244715	2.13	2.10	hypothetical protein
TGGT1_206290	2.11	2.07	hypothetical protein
TGGT1_296231	2.09	2.07	hypothetical protein
TGGT1_287430	2.07	2.07	hypothetical protein
TGGT1_411110	2.07	2.06	hypothetical protein
TGGT1_307640	2.06	2.05	CMGC kinase, CK2 family
TGGT1_216965	2.06	2.02	leucine rich repeat-containing protein
TGGT1_202255	2.04	2.01	hypothetical protein
TGGT1_223060	2.03	2.01	MORN repeat-containing protein
TGGT1_293790	2.02	2.00	hypothetical protein
TGGT1_217660	2.01	1.99	Tctex-1 family protein
TGGT1_285920	2.00	1.96	hypothetical protein
TGGT1_276920	1.99	1.93	protein phosphatase 2C domain-containing protein
TGGT1_301690	1.97	1.93	hypothetical protein
TGGT1_259670	1.96	1.92	von Willebrand factor type A domain-containing protein
TGGT1_217030	1.95	1.91	hypothetical protein
TGGT1_223045	1.89	1.90	hypothetical protein
TGGT1_286770	1.89	1.90	hypothetical protein
TGGT1_359210	1.88	1.89	hypothetical protein
TGGT1_240800	1.88	1.85	MORN repeat-containing protein
TGGT1_408560	1.87	1.84	hypothetical protein
TGGT1_247540	1.87	1.84	ATP-binding cassette G family transporter ABCG107
TGGT1_305030	1.86	1.83	kinase, pfkB family protein
TGGT1_273110	1.86	1.83	SAG-related sequence SRS30D
TGGT1_215185	1.85	1.83	hypothetical protein
TGGT1_239020	1.85	1.83	ABC transporter transmembrane region domain-containing protein
TGGT1_291860	1.81	1.81	HORMA domain-containing protein
TGGT1_243378	1.81	1.80	hypothetical protein
TGGT1_411190	1.81	1.79	hypothetical protein
TGGT1_266785	1.79	1.78	zinc finger (CCCH type) motif-containing protein
TGGT1_222080	1.78	1.78	hypothetical protein
TGGT1_409830	1.78	1.77	putative cytochrome protein B

TGGT1_236990	1.78	1.77	beta-ketoacyl synthase, N-terminal domain-containing protein
TGGT1_258030	1.77	1.77	DNA polymerase
TGGT1_255200	1.77	1.76	putative Radial spoke head protein 9
TGGT1_217040	1.77	1.75	hypothetical protein
TGGT1_200700	1.75	1.74	Toxoplasma gondii family C protein
TGGT1_243635	1.75	1.72	hypothetical protein
TGGT1_316290	1.74	1.72	hypothetical protein
TGGT1_277685	1.74	1.72	hypothetical protein
TGGT1_256020	1.74	1.71	putative EFHC1
TGGT1_313840	1.74	1.68	hypothetical protein
TGGT1_231991	1.73	1.68	hypothetical protein
TGGT1_243600	1.73	1.68	acetyltransferase, GNAT family protein
TGGT1_224130	1.72	1.68	hypothetical protein
TGGT1_253930	1.72	1.68	GCC2 and GCC3 domain-containing protein
TGGT1_263750	1.70	1.67	hypothetical protein
TGGT1_279355	1.68	1.66	hypothetical protein
TGGT1_220060B	1.66	1.66	hypothetical protein
TGGT1_269417	1.66	1.65	hypothetical protein
TGGT1_247310	1.64	1.64	cpw-wpc domain-containing protein
TGGT1_234370	1.64	1.63	SAG-related sequence SRS42
TGGT1_215970	1.64	1.62	hypothetical protein
TGGT1_220420	1.64	1.62	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_243740	1.63	1.61	WD domain, G-beta repeat-containing protein
TGGT1_244590	1.63	1.61	katanin-like family protein
TGGT1_253760	1.63	1.60	hypothetical protein
TGGT1_203070	1.62	1.59	hypothetical protein
TGGT1_248430	1.60	1.59	hypothetical protein
TGGT1_265320	1.59	1.55	hypothetical protein
TGGT1_408550	1.58	1.54	hypothetical protein
TGGT1_411430	1.58	1.52	rhoptry protein ROP5
TGGT1_204360	1.57	1.51	subtilisin SUB4
TGGT1_217726	1.57	1.51	hypothetical protein
TGGT1_222370	1.56	1.51	SAG-related sequence SRS13
TGGT1_235183	1.54	1.51	PAN domain-containing protein
TGGT1_236980	1.54	1.49	AMP-binding enzyme domain-containing protein
TGGT1_320180	1.53	1.49	SAG-related sequence SRS16C
TGGT1_258870B	1.50	1.48	hypothetical protein
TGGT1_202910	1.50	1.48	zinc carboxypeptidase superfamily protein
TGGT1_410160	1.50	1.47	hypothetical protein
TGGT1_306470	1.49	1.47	isoprenylcysteine carboxyl methyltransferase (icmt) family protein
TGGT1_270280	1.49	1.46	hypothetical protein
TGGT1_214850	1.47	1.46	putative 1-deoxy-D-xylulose 5-phosphate reductoisomerase
TGGT1_220060A	1.47	1.45	hypothetical protein
TGGT1_212160	1.46	1.45	hypothetical protein
TGGT1_285410	1.45	1.45	putative calmodulin-binding carboxy-terminal kinesin-like family protein
TGGT1_218910	1.45	1.45	hypothetical protein
TGGT1_217961	1.45	1.44	hypothetical protein
TGGT1_295950	1.44	1.44	KRUF family protein
TGGT1_315900	1.44	1.43	hypothetical protein
TGGT1_203990	1.44	1.43	rhoptry protein ROP12
TGGT1_211860	1.44	1.43	hypothetical protein
TGGT1_307270	1.43	1.43	hypothetical protein
TGGT1_217915	1.43	1.42	hypothetical protein
TGGT1_266950	1.41	1.42	putative protein kinase
TGGT1_257010	1.41	1.42	sporozoite developmental protein
TGGT1_226532	1.41	1.42	hypothetical protein
TGGT1_243700	1.40	1.41	hypothetical protein
TGGT1_297940	1.40	1.41	single-strand binding protein
TGGT1_263100	1.39	1.41	hypothetical protein
TGGT1_235500	1.39	1.41	hypothetical protein

TGGT1_317705	1.39	1.40	enoyl-CoA hydratase/isomerase family protein
TGGT1_286660	1.39	1.40	putative kinesin
TGGT1_298060	1.38	1.40	Toxoplasma gondii family C protein
TGGT1_245770A	1.38	1.39	hypothetical protein
TGGT1_329400	1.38	1.39	Toxoplasma gondii family C protein
TGGT1_257040	1.37	1.39	TB2/DP1, HVA22 family protein
TGGT1_207950	1.37	1.39	hypothetical protein
TGGT1_293200	1.36	1.38	hypothetical protein
TGGT1_252385	1.36	1.38	hypothetical protein
TGGT1_207240	1.36	1.38	hypothetical protein
TGGT1_232770	1.35	1.37	hypothetical protein
TGGT1_277810	1.35	1.37	hypothetical protein
TGGT1_293650	1.35	1.36	hypothetical protein
TGGT1_363480	1.35	1.35	hypothetical protein
TGGT1_238490	1.34	1.35	SAG-related sequence SRS22E
TGGT1_239770	1.34	1.35	alveolin domain containing intermediate filament IMC11
TGGT1_209600	1.33	1.35	hypothetical protein
TGGT1_266910	1.33	1.33	putative cell-cycle-associated protein kinase GSK
TGGT1_257568	1.32	1.33	hypothetical protein
TGGT1_297660	1.32	1.33	hypothetical protein
TGGT1_247350	1.32	1.32	thioredoxin domain-containing protein
TGGT1_276940	1.32	1.32	putative ribosome associated membrane protein RAMP4
TGGT1_313890	1.31	1.30	hypothetical protein
TGGT1_295920	1.30	1.30	hypothetical protein
TGGT1_281470	1.30	1.30	hypothetical protein
TGGT1_215360	1.29	1.29	hypothetical protein
TGGT1_211675	1.29	1.29	hypothetical protein
TGGT1_278580	1.28	1.28	hypothetical protein
TGGT1_297670	1.28	1.27	hypothetical protein
TGGT1_245428	1.27	1.26	hypothetical protein
TGGT1_201840	1.27	1.26	aspartyl protease ASP1
TGGT1_294225	1.27	1.26	hypothetical protein
TGGT1_295960	1.26	1.25	hypothetical protein
TGGT1_239910	1.26	1.24	cyclin-dependent kinase
TGGT1_226420	1.26	1.24	peptidase family M3 protein
TGGT1_223520	1.26	1.24	EF hand domain-containing protein
TGGT1_244180	1.25	1.24	microneme-like protein
TGGT1_271335	1.24	1.23	hypothetical protein
TGGT1_211280	1.24	1.22	hypothetical protein
TGGT1_230610	1.22	1.21	transmembrane protein TMEM222
TGGT1_235515	1.22	1.21	MORN repeat-containing protein
TGGT1_201170	1.22	1.19	hypothetical protein
TGGT1_270040	1.22	1.19	hypothetical protein
TGGT1_228780	1.20	1.18	Toxoplasma gondii family C protein
TGGT1_299570	1.20	1.17	Toxoplasma gondii family B protein
TGGT1_313750	1.19	1.17	hypothetical protein
TGGT1_253755	1.19	1.17	hypothetical protein
TGGT1_292600	1.19	1.16	hypothetical protein
TGGT1_314690	1.19	1.16	hypothetical protein
TGGT1_268610	1.18	1.15	hypothetical protein
TGGT1_215895	1.18	1.14	AP2 domain-containing protein
TGGT1_275860	1.17	1.14	hypothetical protein
TGGT1_287160	1.17	1.13	internal kinesin motor domain protein
TGGT1_279540	1.17	1.13	hypothetical protein
TGGT1_222975	1.17	1.12	hypothetical protein
TGGT1_253180	1.16	1.11	hypothetical protein
TGGT1_258870A	1.16	1.10	hypothetical protein
TGGT1_227900	1.15	1.10	AP2 domain transcription factor AP2X-1
TGGT1_313690	1.14	1.09	Sel1 repeat-containing protein
TGGT1_286595	1.13	1.09	hypothetical protein

TGGT1_232640	1.13	1.09	RNA 2'-phosphotransferase, Tpt1/KptA family protein
TGGT1_219470	1.12	1.08	hypothetical protein
TGGT1_249780	1.12	1.08	hypothetical protein
TGGT1_316600	1.12	1.07	hypothetical protein
TGGT1_411580	1.11	1.07	TPRX1
TGGT1_286130	1.10	1.06	hypothetical protein
TGGT1_307260	1.10	1.06	Toxoplasma gondii family C protein
TGGT1_300052	1.10	1.06	hypothetical protein
TGGT1_256025	1.09	1.06	SPEF1 family protein
TGGT1_208010	1.09	1.05	hypothetical protein
TGGT1_202500	1.08	1.05	GAPM1a
TGGT1_227840	1.08	1.04	Memo family protein
TGGT1_273300	1.07	1.04	hypothetical protein
TGGT1_319690	1.07	1.04	hypothetical protein
TGGT1_299015	1.07	1.04	hypothetical protein
TGGT1_228410	1.07	1.04	Tubulin-tyrosine ligase family protein
TGGT1_275755	1.06	1.03	hypothetical protein
TGGT1_224520	1.04	1.03	alveolin domain containing intermediate filament IMC8
TGGT1_356060	1.04	1.02	hypothetical protein
TGGT1_300055	1.04	1.02	hypothetical protein
TGGT1_245440	1.03	1.02	hypothetical protein
TGGT1_223070	1.02	1.01	hypothetical protein
TGGT1_315750	1.02	1.01	hypothetical protein
TGGT1_223140	1.01	1.00	tRNA binding domain-containing protein
TGGT1_271250	1.01	1.00	hypothetical protein
TGGT1_250940	1.01	1.00	hypothetical protein
TGGT1_211020	1.00	1.00	RNA recognition motif-containing protein
TGGT1_246210	-1.00	-1.00	hypothetical protein
TGGT1_224160	-1.01	-1.00	hypothetical protein
TGGT1_215270	-1.01	-1.01	hypothetical protein
TGGT1_273500	-1.01	-1.01	O-linked N-acetylglucosamine transferase
TGGT1_214800	-1.02	-1.01	hypothetical protein
TGGT1_259200A	-1.02	-1.01	Na+/H+ exchanger NHE1
TGGT1_236970	-1.02	-1.01	SWI2/SNF2-containing PHD finger protein
TGGT1_226700	-1.03	-1.02	putative nuclease
TGGT1_231220	-1.03	-1.02	hypothetical protein
TGGT1_226460	-1.03	-1.03	hypothetical protein
TGGT1_279360	-1.03	-1.03	hypothetical protein
TGGT1_247970	-1.03	-1.03	hypothetical protein
TGGT1_256700	-1.03	-1.04	hypothetical protein
TGGT1_277070	-1.04	-1.05	SWI2/SNF2-containing protein
TGGT1_254625	-1.04	-1.05	hypothetical protein
TGGT1_215960	-1.04	-1.05	hypothetical protein
TGGT1_255380	-1.04	-1.05	hypothetical protein
TGGT1_257580	-1.04	-1.06	hypothetical protein
TGGT1_219660	-1.04	-1.06	hypothetical protein
TGGT1_203800	-1.05	-1.06	hypothetical protein
TGGT1_319320	-1.05	-1.06	hypothetical protein
TGGT1_281675	-1.05	-1.06	putative protein kinase
TGGT1_313630	-1.05	-1.07	hypothetical protein
TGGT1_270670	-1.05	-1.08	hypothetical protein
TGGT1_244450	-1.05	-1.09	protein phosphatase 2C domain-containing protein
TGGT1_295390	-1.05	-1.09	hypothetical protein
TGGT1_304720	-1.05	-1.09	hypothetical protein
TGGT1_271115	-1.05	-1.09	hypothetical protein
TGGT1_214540	-1.06	-1.09	hypothetical protein
TGGT1_244335	-1.06	-1.10	hypothetical protein
TGGT1_217500	-1.06	-1.10	HMG (high mobility group) box domain-containing protein
TGGT1_244910	-1.06	-1.10	MIZ/SP-RING zinc finger domain-containing protein
TGGT1_320690	-1.06	-1.10	putative gamma-soluble NSF attachment protein

TGGT1_236400	-1.07	-1.11	hypothetical protein
TGGT1_209650B	-1.07	-1.11	RNA-directed DNA polymerase
TGGT1_267710	-1.07	-1.11	CPSF A subunit region protein
TGGT1_278660	-1.07	-1.11	putative P-type ATPase4
TGGT1_266035	-1.07	-1.11	hypothetical protein
TGGT1_277940	-1.07	-1.12	hypothetical protein
TGGT1_257490	-1.07	-1.12	prefoldin subunit superfamily protein
TGGT1_267875	-1.08	-1.12	putative NBP2b protein
TGGT1_249170	-1.08	-1.13	Ras family protein
TGGT1_286250	-1.08	-1.14	hypothetical protein
TGGT1_214920	-1.09	-1.14	putative GPI-anchored wall transfer protein GWT1
TGGT1_245570	-1.09	-1.14	origin recognition complex subunit 2 protein
TGGT1_213710	-1.09	-1.14	WD domain, G-beta repeat-containing protein
TGGT1_225430	-1.09	-1.14	Ras-related GTP binding A family protein
TGGT1_409310	-1.09	-1.15	tRNA-Met
TGGT1_229350	-1.09	-1.15	HEAT repeat-containing protein
TGGT1_297920	-1.09	-1.15	hypothetical protein
TGGT1_202010	-1.09	-1.15	hypothetical protein
TGGT1_221895	-1.10	-1.15	hypothetical protein
TGGT1_203350	-1.10	-1.15	hypothetical protein
TGGT1_310730	-1.10	-1.16	hypothetical protein
TGGT1_261730	-1.10	-1.16	hypothetical protein
TGGT1_312075	-1.10	-1.16	hypothetical protein
TGGT1_287970	-1.10	-1.16	hypothetical protein
TGGT1_295970	-1.10	-1.16	hypothetical protein
TGGT1_314370	-1.11	-1.16	hypothetical protein
TGGT1_259250	-1.11	-1.17	ATP-dependent DNA helicase, RecQ family protein
TGGT1_214870	-1.11	-1.17	ribosomal protein L9, N-terminal domain-containing protein
TGGT1_267470	-1.11	-1.17	cold-shock DNA-binding domain-containing protein
TGGT1_267642	-1.11	-1.19	hypothetical protein
TGGT1_288940	-1.11	-1.19	hypothetical protein
TGGT1_261260B	-1.11	-1.19	histone lysine demethylase JMJD6a
TGGT1_206660	-1.12	-1.20	hypothetical protein
TGGT1_264780	-1.12	-1.20	UTP-glucose-1-phosphate uridylyltransferase subfamily protein
TGGT1_226310	-1.12	-1.20	zinc finger (CCCH type) motif-containing protein
TGGT1_264640	-1.12	-1.20	bromodomain-containing protein
TGGT1_255960	-1.13	-1.20	hypothetical protein
TGGT1_271780	-1.13	-1.21	Filamin/ABP280 repeat-containing protein
TGGT1_209650A	-1.13	-1.21	RNA-directed DNA polymerase
TGGT1_312500	-1.13	-1.22	hypothetical protein
TGGT1_262610	-1.13	-1.23	hypothetical protein
TGGT1_206430	-1.13	-1.23	formin FRM1
TGGT1_240730	-1.13	-1.23	hypothetical protein
TGGT1_247485	-1.13	-1.23	zinc finger, C3HC4 type (RING finger) domain-containing protein
TGGT1_231400B	-1.13	-1.23	tubulin/FtsZ family, GTPase domain-containing protein
TGGT1_279330	-1.14	-1.24	DEAD/DEAH box helicase family protein
TGGT1_295980	-1.14	-1.24	hypothetical protein
TGGT1_312480	-1.15	-1.25	putative uracil phosphoribosyltransferase FUR1
TGGT1_220340	-1.15	-1.25	hypothetical protein
TGGT1_202050	-1.15	-1.26	hypothetical protein
TGGT1_215955	-1.15	-1.27	hypothetical protein
TGGT1_231215	-1.16	-1.27	hypothetical protein
TGGT1_281575	-1.17	-1.27	hypothetical protein
TGGT1_290860	-1.17	-1.27	putative amino acid transporter
TGGT1_277550	-1.17	-1.27	UvrD/REP helicase domain-containing protein
TGGT1_267440	-1.17	-1.28	RING zinc finger protein
TGGT1_264760	-1.17	-1.28	Oxysterol-binding protein
TGGT1_295110	-1.17	-1.29	rhoptry protein ROP7
TGGT1_221170	-1.17	-1.29	CAAX metallo endopeptidase
TGGT1_204000	-1.18	-1.29	hypothetical protein

TGGT1_214270	-1.18	-1.29	putative translation initiation factor IF-2
TGGT1_247390	-1.18	-1.30	ATPase, AAA family protein
TGGT1_214510	-1.19	-1.30	hypothetical protein
TGGT1_250010	-1.19	-1.31	Sad1 / UNC family protein
TGGT1_289216	-1.19	-1.31	hypothetical protein
TGGT1_277050	-1.19	-1.31	hypothetical protein
TGGT1_276110	-1.20	-1.32	cytochrome b5 family heme/steroid binding domain-containing protein
TGGT1_318430	-1.20	-1.32	malate dehydrogenase MDH
TGGT1_267370	-1.20	-1.32	kinesin motor domain-containing protein
TGGT1_223025	-1.20	-1.33	hypothetical protein
TGGT1_240325	-1.20	-1.33	Toxoplasma gondii family E protein
TGGT1_277950	-1.20	-1.33	lipase
TGGT1_285990	-1.21	-1.33	Filamin/ABP280 repeat-containing protein
TGGT1_208710	-1.21	-1.33	DNA/RNA non-specific endonuclease
TGGT1_249880	-1.21	-1.34	hypothetical protein
TGGT1_297210	-1.21	-1.34	hypothetical protein
TGGT1_287960	-1.22	-1.34	hypothetical protein
TGGT1_270090	-1.22	-1.34	hypothetical protein
TGGT1_312065	-1.22	-1.34	hypothetical protein
TGGT1_208340	-1.22	-1.34	hypothetical protein
TGGT1_411770	-1.22	-1.35	putative IgA-specific serine endopeptidase
TGGT1_264710	-1.23	-1.35	bucentaur or craniofacial development domain-containing protein
TGGT1_201700	-1.24	-1.35	WD domain, G-beta repeat-containing protein
TGGT1_233870	-1.24	-1.35	hypothetical protein
TGGT1_259230	-1.24	-1.36	site-specific recombinase, phage integrase family protein
TGGT1_270680	-1.24	-1.36	hypothetical protein
TGGT1_239270	-1.24	-1.36	hypothetical protein
TGGT1_267570	-1.24	-1.37	hypothetical protein
TGGT1_214545	-1.25	-1.38	hypothetical protein
TGGT1_266380	-1.25	-1.38	hypothetical protein
TGGT1_246800	-1.25	-1.39	putative acylaminoacyl-peptidase
TGGT1_315345	-1.25	-1.40	SAG-related sequence SRS52F
TGGT1_246070	-1.25	-1.40	SAG-related sequence SRS56
TGGT1_285220	-1.26	-1.40	CAP-Gly domain-containing protein
TGGT1_262800	-1.26	-1.40	hypothetical protein
TGGT1_260680	-1.27	-1.40	putative small subunit DNA primase
TGGT1_202580	-1.27	-1.40	ATPase, AAA family protein
TGGT1_316580	-1.27	-1.40	hypothetical protein
TGGT1_264790	-1.27	-1.41	hypothetical protein
TGGT1_279320	-1.27	-1.41	hypothetical protein
TGGT1_256920	-1.27	-1.41	phosphatidylinositol-4-phosphate 5-Kinase
TGGT1_318770	-1.27	-1.41	aurora kinase(incomplete catalytic triad)
TGGT1_295030	-1.28	-1.41	hypothetical protein
TGGT1_202650	-1.29	-1.41	hypothetical protein
TGGT1_240930	-1.29	-1.41	MoaC family protein
TGGT1_281900	-1.29	-1.42	SET domain containing lysine methyltransferase KMTox
TGGT1_312240	-1.29	-1.42	hypothetical protein
TGGT1_242320	-1.29	-1.43	B-box zinc finger domain-containing protein
TGGT1_311640	-1.29	-1.43	hypothetical protein
TGGT1_264748	-1.29	-1.44	hypothetical protein
TGGT1_289222	-1.29	-1.44	hypothetical protein
TGGT1_248590	-1.29	-1.44	hypothetical protein
TGGT1_266390	-1.30	-1.44	DNA mismatch repair protein, C-terminal domain-containing protein
TGGT1_203135	-1.30	-1.45	dynein heavy chain family protein
TGGT1_282200	-1.30	-1.45	ATPase, AAA family protein
TGGT1_288450	-1.31	-1.46	aldehyde dehydrogenase (NAD) family protein
TGGT1_203330	-1.31	-1.46	hypothetical protein
TGGT1_263120	-1.31	-1.47	hypothetical protein
TGGT1_233180	-1.32	-1.47	hypothetical protein
TGGT1_279370	-1.32	-1.47	SNARE associated Golgi protein

TGGT1_209730	-1.32	-1.48	hypothetical protein
TGGT1_271892	-1.32	-1.49	hypothetical protein
TGGT1_208020	-1.32	-1.49	AP2 domain transcription factor AP2Ib-1
TGGT1_291930	-1.32	-1.50	RNA recognition motif-containing protein
TGGT1_305950	-1.32	-1.51	tetratricopeptide repeat-containing protein
TGGT1_267600	-1.32	-1.51	FHA domain-containing protein
TGGT1_208580	-1.33	-1.52	putative DNA ligase 1
TGGT1_410630	-1.33	-1.52	tRNA-Sec
TGGT1_264610	-1.33	-1.52	RNA recognition motif-containing protein
TGGT1_291900	-1.33	-1.53	Ydr279p family (RNase H2 complex component) protein
TGGT1_279100	-1.33	-1.53	hypothetical protein
TGGT1_263110	-1.34	-1.54	hypothetical protein
TGGT1_211480	-1.34	-1.54	putative GTP-binding protein engA
TGGT1_239810	-1.35	-1.55	hypothetical protein
TGGT1_200430	-1.35	-1.55	ne and deoxycytidylate deaminase zinc-binding region domain-containing pr
TGGT1_263510	-1.36	-1.55	Spc97 / Spc98 family protein
TGGT1_208842	-1.36	-1.56	DEAD/DEAH box helicase domain-containing protein
TGGT1_231200	-1.37	-1.56	hypothetical protein
TGGT1_323020	-1.37	-1.56	KRUF family protein
TGGT1_308945	-1.37	-1.57	hypothetical protein
TGGT1_410770	-1.38	-1.58	histone deacetylase SIR2-like protein
TGGT1_264752	-1.38	-1.58	HEAT repeat-containing protein
TGGT1_288455	-1.38	-1.58	hypothetical protein
TGGT1_236290	-1.38	-1.58	hypothetical protein
TGGT1_254890	-1.38	-1.58	hypothetical protein
TGGT1_214230	-1.39	-1.58	Dopey, N-terminal domain-containing protein
TGGT1_278878	-1.39	-1.58	GDA1/CD39 (nucleoside phosphatase) family protein
TGGT1_232240	-1.39	-1.58	hypothetical protein
TGGT1_218560	-1.40	-1.58	acetyl-coA carboxylase ACC2
TGGT1_267610	-1.40	-1.59	hypothetical protein
TGGT1_300150	-1.40	-1.59	hypothetical protein
TGGT1_267620	-1.40	-1.59	multi-pass transmembrane protein
TGGT1_323200	-1.40	-1.59	OTU family cysteine protease
TGGT1_267480	-1.40	-1.59	putative tRNA (guanine(26)-N(2))-dimethyltransferase
TGGT1_279405	-1.40	-1.59	hypothetical protein
TGGT1_319312	-1.40	-1.60	hypothetical protein
TGGT1_230830	-1.41	-1.60	se family associated with various cellular activities (AAA) domain-containing p
TGGT1_259155	-1.41	-1.60	hypothetical protein
TGGT1_462965	-1.43	-1.60	formin domain-containing protein
TGGT1_287240	-1.43	-1.61	hypothetical protein
TGGT1_224170	-1.43	-1.61	SAG-related sequence SRS60A
TGGT1_203665	-1.43	-1.61	hypothetical protein
TGGT1_215940	-1.43	-1.61	putative Acetyl-coenzyme A transporter
TGGT1_279410	-1.43	-1.61	hypothetical protein
TGGT1_244130	-1.44	-1.62	hypothetical protein
TGGT1_203705	-1.44	-1.62	hypothetical protein
TGGT1_204395	-1.45	-1.62	hypothetical protein
TGGT1_267775	-1.45	-1.62	hypothetical protein
TGGT1_254160	-1.46	-1.64	hypothetical protein
TGGT1_319580	-1.46	-1.64	hypothetical protein
TGGT1_201230	-1.47	-1.64	kinesin motor domain-containing protein
TGGT1_308940	-1.47	-1.66	hypothetical protein
TGGT1_249890	-1.48	-1.66	hypothetical protein
TGGT1_314362	-1.49	-1.66	hypothetical protein
TGGT1_264430	-1.49	-1.68	hypothetical protein
TGGT1_276155	-1.49	-1.68	hypothetical protein
TGGT1_262655	-1.49	-1.69	hypothetical protein
TGGT1_267670	-1.50	-1.69	hypothetical protein
TGGT1_205670	-1.50	-1.69	SF-assemblin/beta giardin protein
TGGT1_264745	-1.51	-1.69	hypothetical protein

TGGT1_322100	-1.51	-1.70	myosin-light-chain kinase
TGGT1_221310	-1.51	-1.71	aminopeptidase N protein
TGGT1_278518	-1.51	-1.72	N-acetylgalactosaminyl transferase
TGGT1_275430	-1.52	-1.72	hypothetical protein
TGGT1_313940	-1.52	-1.72	hypothetical protein
TGGT1_222340	-1.52	-1.72	NOL1/NOP2/sun family protein
TGGT1_267585	-1.53	-1.72	hypothetical protein
TGGT1_203740	-1.53	-1.73	hypothetical protein
TGGT1_237220	-1.53	-1.74	putative DNA replication licensing factor Mcm7
TGGT1_246010	-1.54	-1.75	hypothetical protein
TGGT1_244550	-1.54	-1.75	hypothetical protein
TGGT1_214190	-1.54	-1.75	SAG-related sequence SRS46
TGGT1_410390	-1.54	-1.75	hypothetical protein
TGGT1_227370	-1.54	-1.77	hydrolase CocE/NonD family protein
TGGT1_269630	-1.54	-1.77	hypothetical protein
TGGT1_233540	-1.55	-1.77	transporter, major facilitator family protein
TGGT1_233210	-1.55	-1.78	hypothetical protein
TGGT1_267490	-1.55	-1.78	putative preprocathepsin c precursor
TGGT1_233360	-1.55	-1.78	hypothetical protein
TGGT1_271860	-1.55	-1.79	tRNA (Uracil-5-)-methyltransferase
TGGT1_249380	-1.56	-1.79	DHHC zinc finger domain-containing protein
TGGT1_311710	-1.56	-1.80	hypothetical protein
TGGT1_213700	-1.56	-1.80	hypothetical protein
TGGT1_267810	-1.56	-1.80	Rab 5
TGGT1_269380	-1.56	-1.81	hypothetical protein
TGGT1_256650	-1.56	-1.81	hypothetical protein
TGGT1_312175	-1.56	-1.81	hypothetical protein
TGGT1_362090	-1.57	-1.82	hypothetical protein
TGGT1_231780	-1.58	-1.83	hypothetical protein
TGGT1_267800	-1.58	-1.84	dynamin-related protein DRPA
TGGT1_224570	-1.58	-1.85	hypothetical protein
TGGT1_267380	-1.59	-1.86	UDP-galactose transporter subfamily protein
TGGT1_238940	-1.59	-1.86	putative GDP mannose 4,6-dehydratase
TGGT1_304480	-1.59	-1.86	3-oxo-5-alpha-steroid 4-dehydrogenase
TGGT1_295440	-1.59	-1.87	hypothetical protein
TGGT1_264805	-1.61	-1.87	hypothetical protein
TGGT1_229230	-1.61	-1.89	hypothetical protein
TGGT1_264420	-1.62	-1.89	putative lipoprotein
TGGT1_363030	-1.62	-1.90	rhoptry protein ROP8
TGGT1_221675B	-1.63	-1.91	hypothetical protein
TGGT1_229220	-1.63	-1.93	hypothetical protein
TGGT1_287235	-1.64	-1.93	hypothetical protein
TGGT1_243920	-1.64	-1.93	putative DNA replication licensing factor MCM5
TGGT1_256950	-1.66	-1.94	hypothetical protein
TGGT1_228160	-1.68	-1.94	acid phosphatase
TGGT1_267540	-1.69	-1.95	AGC kinase
TGGT1_265770	-1.69	-1.95	hypothetical protein
TGGT1_255280	-1.69	-1.96	hypothetical protein
TGGT1_267790	-1.70	-1.97	hypothetical protein
TGGT1_248170	-1.70	-1.97	hypothetical protein
TGGT1_266366	-1.70	-1.97	BT1 family protein
TGGT1_278910	-1.72	-1.98	putative O-acetylserine (thiol) lyase 2
TGGT1_219370	-1.72	-1.98	hypothetical protein
TGGT1_282130	-1.73	-2.00	hypothetical protein
TGGT1_294240	-1.74	-2.00	hypothetical protein
TGGT1_244280	-1.76	-2.00	hypothetical protein
TGGT1_237450	-1.76	-2.00	hypothetical protein
TGGT1_274160	-1.77	-2.01	hypothetical protein
TGGT1_410900	-1.78	-2.02	toxoplasma gondii family A protein
TGGT1_208820	-1.81	-2.03	1-deoxy-D-xylulose-5-phosphate synthase

TGGT1_280690	-1.83	-2.03	DNA polymerase epsilon subunit B protein
TGGT1_233370	-1.83	-2.03	hypothetical protein
TGGT1_273940	-1.84	-2.05	hypothetical protein
TGGT1_218810	-1.84	-2.06	histidyl-tRNA synthetase
TGGT1_203710	-1.86	-2.06	AP2 domain transcription factor AP2VIIa-4
TGGT1_255290	-1.86	-2.08	hypothetical protein
TGGT1_254840	-1.87	-2.08	tetratricopeptide repeat-containing protein
TGGT1_200320	-1.89	-2.10	hypoxanthine-xanthine-guanine phosphoribosyl transferase HXGPRT
TGGT1_261610	-1.89	-2.11	hypothetical protein
TGGT1_221220	-1.90	-2.11	hypothetical protein
TGGT1_232320	-1.90	-2.11	hypothetical protein
TGGT1_208850	-1.92	-2.11	SAG-related sequence SRS11
TGGT1_311700	-1.92	-2.12	hypothetical protein
TGGT1_289218	-1.92	-2.13	hypothetical protein
TGGT1_264840	-1.93	-2.14	ATP-dependent DNA helicase, RecQ family protein
TGGT1_295130	-1.93	-2.14	hypothetical protein
TGGT1_267435	-1.94	-2.15	hypothetical protein
TGGT1_221675A	-1.94	-2.18	hypothetical protein
TGGT1_319370	-1.96	-2.18	hypothetical protein
TGGT1_202445	-2.00	-2.20	hypothetical protein
TGGT1_267820	-2.00	-2.23	putative electron transfer flavoprotein subunit beta
TGGT1_409410	-2.00	-2.23	tRNA-Leu
TGGT1_221320	-2.01	-2.24	acetyl-CoA carboxylase ACC1
TGGT1_237045	-2.02	-2.27	hypothetical protein
TGGT1_411550	-2.03	-2.27	toxoplasma gondii family A protein
TGGT1_204010	-2.04	-2.29	hypothetical protein
TGGT1_244950	-2.05	-2.31	hypothetical protein
TGGT1_267410	-2.05	-2.31	scavenger receptor protein SR1 precursor
TGGT1_321470	-2.05	-2.32	SAG-related sequence SRS12D
TGGT1_267855	-2.06	-2.34	hypothetical protein
TGGT1_245746B	-2.06	-2.34	hypothetical protein
TGGT1_410520	-2.07	-2.35	putative OTU family cysteine protease
TGGT1_211730	-2.07	-2.36	histone lysine methyltransferase SET8
TGGT1_295125	-2.09	-2.36	rhoptry protein ROP4
TGGT1_203770	-2.09	-2.37	hypothetical protein
TGGT1_222420	-2.10	-2.38	hypothetical protein
TGGT1_213340	-2.10	-2.38	GMC oxidoreductase
TGGT1_205050	-2.10	-2.39	hypothetical protein
TGGT1_315127	-2.11	-2.40	hypothetical protein
TGGT1_205520	-2.11	-2.44	hypothetical protein
TGGT1_205658	-2.11	-2.44	F5/8 type C domain-containing protein
TGGT1_269075	-2.13	-2.49	hypothetical protein
TGGT1_233770A	-2.13	-2.50	calcium-translocating P-type ATPase, PMCA-type protein
TGGT1_221890	-2.15	-2.50	WD domain, G-beta repeat-containing protein
TGGT1_312245	-2.15	-2.50	hypothetical protein
TGGT1_238210	-2.18	-2.50	EGF family domain-containing protein
TGGT1_236670B	-2.18	-2.52	hypothetical protein
TGGT1_363220	-2.18	-2.54	hypothetical protein
TGGT1_308965	-2.18	-2.57	hypothetical protein
TGGT1_280500	-2.19	-2.58	inorganic anion transporter, sulfate permease (SulP) family protein
TGGT1_267030	-2.19	-2.58	ribonuclease type III Dicer
TGGT1_239290	-2.20	-2.58	hypothetical protein
TGGT1_411750	-2.21	-2.61	hypothetical protein
TGGT1_245752	-2.24	-2.61	WD domain, G-beta repeat-containing protein
TGGT1_237170	-2.25	-2.62	hypothetical protein
TGGT1_203688	-2.30	-2.65	hypothetical protein
TGGT1_252890	-2.31	-2.66	hypothetical protein
TGGT1_256960	-2.32	-2.68	FYVE zinc finger domain-containing protein
TGGT1_272730	-2.33	-2.68	hypothetical protein
TGGT1_255390	-2.33	-2.69	HEAT repeat-containing protein

TGGT1_244406	-2.34	-2.69	cysteine dioxygenase type i protein
TGGT1_223480	-2.35	-2.70	sushi domain (scr repeat) domain-containing protein
TGGT1_221880	-2.39	-2.70	hypothetical protein
TGGT1_203685	-2.40	-2.71	hypothetical protein
TGGT1_221830	-2.40	-2.74	subtilisin SUB12
TGGT1_265255	-2.43	-2.74	hypothetical protein
TGGT1_205662	-2.48	-2.75	LCCL domain-containing protein
TGGT1_410860	-2.51	-2.78	hypothetical protein
TGGT1_203682	-2.51	-2.78	hypothetical protein
TGGT1_305630	-2.52	-2.80	hypothetical protein
TGGT1_269085	-2.53	-2.81	hypothetical protein
TGGT1_319992	-2.54	-2.82	hypothetical protein
TGGT1_274280	-2.54	-2.83	hypothetical protein
TGGT1_296020	-2.55	-2.84	hypothetical protein
TGGT1_211270	-2.56	-2.84	sushi domain (scr repeat) domain-containing protein
TGGT1_289060	-2.56	-2.89	hypothetical protein
TGGT1_411680	-2.60	-2.90	hypothetical protein
TGGT1_319860	-2.69	-2.90	DNA polymerase family B protein
TGGT1_218192	-2.72	-2.96	hypothetical protein
TGGT1_319308	-2.73	-2.99	hypothetical protein
TGGT1_409630	-2.74	-3.01	toxoplasma gondii family E protein
TGGT1_279460	-2.75	-3.05	Toxoplasma gondii family B protein
TGGT1_222060	-2.75	-3.07	hypothetical protein
TGGT1_227100	-2.77	-3.12	hypothetical protein
TGGT1_295430	-2.78	-3.14	hypothetical protein
TGGT1_293420	-2.80	-3.17	transporter, major facilitator family protein
TGGT1_291910	-2.80	-3.17	hypothetical protein
TGGT1_410480	-2.80	-3.17	hypothetical protein
TGGT1_320000	-2.83	-3.18	SCY kinase (incomplete catalytic triad)
TGGT1_240370	-2.83	-3.24	Toxoplasma gondii family E protein
TGGT1_322210	-2.83	-3.42	putative apocytochrome b
TGGT1_240365	-2.83	-3.42	hypothetical protein
TGGT1_264950	-2.88	-3.48	hypothetical protein
TGGT1_291920	-2.90	-3.50	hypothetical protein
TGGT1_233770B	-2.93	-3.54	calcium-translocating P-type ATPase, PMCA-type protein
TGGT1_244408	-3.04	-3.56	hypothetical protein
TGGT1_200595	-3.05	-3.61	hypothetical protein
TGGT1_207880	-3.14	-3.61	hypothetical protein
TGGT1_212810	-3.23	-3.65	hypothetical protein
TGGT1_215540	-3.31	-3.74	hypothetical protein
TGGT1_239475	-3.49	-3.80	hypothetical protein
TGGT1_314340	-3.62	-3.82	Sodium:neurotransmitter symporter family protein
TGGT1_244412	-3.72	-3.91	hypothetical protein
TGGT1_227350	-4.06	-3.98	hypothetical protein
TGGT1_266500	-4.85	-5.04	hypothetical protein
TGGT1_304450	-5.42	-5.97	cation-transporting ATPase
TGGT1_361230	5.12	N.S.	toxoplasma gondii family B protein
TGGT1_207150	3.72	N.S.	SAG-related sequence SRS49C
TGGT1_277930	3.40	N.S.	hypothetical protein
TGGT1_202020	3.24	N.S.	DnAK-TPR
TGGT1_301150	3.05	N.S.	SAG-related sequence SRS19B
TGGT1_305040	3.05	N.S.	HEAT repeat-containing protein
TGGT1_292280	3.04	N.S.	SAG-related sequence SRS36D
TGGT1_207865	2.61	N.S.	GCC2 and GCC3 domain-containing protein
TGGT1_207140	2.61	N.S.	SAG-related sequence SRS49B
TGGT1_356400	2.60	N.S.	cAMP-dependent protein kinase
TGGT1_209985	2.40	N.S.	cAMP-dependent protein kinase
TGGT1_293254	2.11	N.S.	hypothetical protein
TGGT1_244515	2.11	N.S.	hypothetical protein
TGGT1_293252	2.06	N.S.	hypothetical protein

TGGT1_315740	2.06	N.S.	SAG-related sequence SRS54
TGGT1_304920	2.03	N.S.	hypothetical protein
TGGT1_211370	2.01	N.S.	tetratricopeptide repeat-containing protein
TGGT1_213480	1.99	N.S.	hypothetical protein
TGGT1_225950	1.96	N.S.	hypothetical protein
TGGT1_285860	1.93	N.S.	SAG-related sequence SRS20C
TGGT1_239880	1.93	N.S.	hypothetical protein
TGGT1_225290	1.89	N.S.	GDA1/CD39 (nucleoside phosphatase) family protein
TGGT1_293800	1.89	N.S.	putative transmembrane protein 65
TGGT1_283882	1.87	N.S.	hypothetical protein
TGGT1_408760	1.87	N.S.	toxoplasma gondii family A protein
TGGT1_205420	1.82	N.S.	aspartate-semialdehyde dehydrogenase
TGGT1_205410	1.80	N.S.	RNA pseudouridine synthase superfamily protein
TGGT1_285790	1.71	N.S.	hypothetical protein
TGGT1_230663	1.70	N.S.	hypothetical protein
TGGT1_235187A	1.70	N.S.	hypothetical protein
TGGT1_207665	1.67	N.S.	kinesin motor domain-containing protein
TGGT1_356030	1.66	N.S.	hypothetical protein
TGGT1_293256	1.65	N.S.	hypothetical protein
TGGT1_253500	1.65	N.S.	hypothetical protein
TGGT1_269300	1.64	N.S.	lipase
TGGT1_212275	1.64	N.S.	hypothetical protein
TGGT1_268300	1.60	N.S.	hypothetical protein
TGGT1_310173	1.58	N.S.	hypothetical protein
TGGT1_210478	1.57	N.S.	hypothetical protein
TGGT1_286782	1.55	N.S.	MAM domain-containing protein
TGGT1_230598	1.55	N.S.	hypothetical protein
TGGT1_307090	1.54	N.S.	hypothetical protein
TGGT1_211030	1.52	N.S.	hypothetical protein
TGGT1_207740	1.52	N.S.	hypothetical protein
TGGT1_288945	1.51	N.S.	hypothetical protein
TGGT1_280580	1.49	N.S.	SAG-related sequence SRS35B
TGGT1_241130	1.49	N.S.	hypothetical protein
TGGT1_301160	1.49	N.S.	SAG-related sequence SRS19C
TGGT1_242360	1.49	N.S.	tetratricopeptide repeat-containing protein
TGGT1_359770	1.48	N.S.	SAG-related sequence protein SRS22E
TGGT1_315962	1.48	N.S.	hypothetical protein
TGGT1_248445	1.48	N.S.	hypothetical protein
TGGT1_253020	1.48	N.S.	hypothetical protein
TGGT1_309990	1.47	N.S.	hypothetical protein
TGGT1_298050	1.46	N.S.	hypothetical protein
TGGT1_217920	1.45	N.S.	hypothetical protein
TGGT1_241165	1.45	N.S.	hypothetical protein
TGGT1_314310	1.45	N.S.	hypothetical protein
TGGT1_308090	1.45	N.S.	rhoptry protein ROP5
TGGT1_203720	1.42	N.S.	vitamin k epoxide reductase family protein
TGGT1_260480	1.42	N.S.	leucine rich repeat-containing protein
TGGT1_307045	1.42	N.S.	Toxoplasma gondii family B protein
TGGT1_221840	1.40	N.S.	hypothetical protein
TGGT1_286040	1.39	N.S.	hypothetical protein
TGGT1_282000	1.37	N.S.	hypothetical protein
TGGT1_272490	1.37	N.S.	protoporphyrinogen oxidase
TGGT1_214350	1.36	N.S.	putative GTP-binding protein
TGGT1_222160	1.33	N.S.	aldehyde dehydrogenase
TGGT1_228370	1.33	N.S.	hypothetical protein
TGGT1_308075	1.33	N.S.	hypothetical protein
TGGT1_278090	1.32	N.S.	Toxoplasma gondii family A protein
TGGT1_295035	1.31	N.S.	hypothetical protein
TGGT1_319560	1.31	N.S.	microneme protein MIC3
TGGT1_275710	1.29	N.S.	hypothetical protein

TGGT1_214760	1.29	N.S.	erythronate-4-phosphate dehydrogenase domain-containing protein
TGGT1_230200	1.28	N.S.	hypothetical protein
TGGT1_217940	1.28	N.S.	hypothetical protein
TGGT1_249290	1.26	N.S.	hypothetical protein
TGGT1_320730	1.26	N.S.	putative homoserine O-acetyltransferase
TGGT1_233460	1.26	N.S.	SAG-related sequence SRS29B
TGGT1_233040	1.25	N.S.	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_217890	1.25	N.S.	putative alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allerger
TGGT1_306310	1.25	N.S.	RecF/RecN/SMC N terminal domain-containing protein
TGGT1_254855	1.24	N.S.	hypothetical protein
TGGT1_285700	1.23	N.S.	ubiquitin fusion degradation protein UFD1AP
TGGT1_278390	1.22	N.S.	Toxoplasma gondii family A protein
TGGT1_271310	1.22	N.S.	hypothetical protein
TGGT1_257000	1.22	N.S.	hypothetical protein
TGGT1_409850	1.21	N.S.	SAG-related sequence protein SRS47A
TGGT1_257330	1.21	N.S.	hypothetical protein
TGGT1_310940	1.20	N.S.	hypothetical protein
TGGT1_230602	1.20	N.S.	hypothetical protein
TGGT1_217800	1.20	N.S.	hypothetical protein
TGGT1_224760	1.19	N.S.	SAG-related sequence SRS40E
TGGT1_264660	1.18	N.S.	SAG-related sequence SRS44
TGGT1_278882	1.18	N.S.	GDA1/CD39 (nucleoside phosphatase) family protein
TGGT1_230970	1.17	N.S.	hypothetical protein
TGGT1_244940	1.16	N.S.	hypothetical protein
TGGT1_322400	1.15	N.S.	hypothetical protein
TGGT1_240290	1.15	N.S.	hypothetical protein
TGGT1_270840	1.15	N.S.	poly(ADP-ribose) polymerase catalytic domain-containing protein
TGGT1_293680	1.14	N.S.	hypothetical protein
TGGT1_215195	1.13	N.S.	tetratricopeptide repeat-containing protein
TGGT1_214080	1.13	N.S.	toxofilin
TGGT1_252070	1.13	N.S.	KRUF family protein
TGGT1_202550	1.13	N.S.	NLI interacting factor family phosphatase
TGGT1_238110	1.13	N.S.	replication factor a protein 3 protein
TGGT1_360000	1.12	N.S.	hypothetical protein
TGGT1_203090	1.12	N.S.	hypothetical protein
TGGT1_227280	1.12	N.S.	dense granule protein GRA3
TGGT1_286150	1.12	N.S.	PAN/Apple domain-containing protein
TGGT1_307570	1.11	N.S.	putative glycerol-3-phosphate dehydrogenase (gpdh)
TGGT1_217930	1.11	N.S.	hypothetical protein
TGGT1_320030	1.11	N.S.	hypothetical protein
TGGT1_307780	1.11	N.S.	hypothetical protein
TGGT1_408250	1.11	N.S.	hypothetical protein
TGGT1_215900	1.10	N.S.	hypothetical protein
TGGT1_222410	1.10	N.S.	hypothetical protein
TGGT1_289900	1.10	N.S.	n-acetyltransferase family protein
TGGT1_213780	1.09	N.S.	radical SAM domain-containing protein
TGGT1_293810	1.09	N.S.	carboxyvinyl-carboxyphosphonate phosphorylmutase
TGGT1_315520	1.09	N.S.	calcium binding egf domain-containing protein
TGGT1_312380	1.09	N.S.	tetratricopeptide repeat-containing protein
TGGT1_315110	1.09	N.S.	prefoldin, alpha subunit protein
TGGT1_230205	1.09	N.S.	hypothetical protein
TGGT1_311740	1.08	N.S.	hypothetical protein
TGGT1_286720	1.08	N.S.	heat shock protein HSP28
TGGT1_306570	1.08	N.S.	hypothetical protein
TGGT1_269170	1.08	N.S.	hypothetical protein
TGGT1_290300	1.07	N.S.	hypothetical protein
TGGT1_209180	1.07	N.S.	PAN domain-containing protein
TGGT1_212930	1.07	N.S.	NifU family domain-containing protein
TGGT1_357130	1.07	N.S.	WD domain, G-beta repeat protein
TGGT1_215340	1.07	N.S.	AP2 domain transcription factor AP2X-10

TGGT1_300030	1.06	N.S.	hypothetical protein
TGGT1_227430	1.05	N.S.	transmembrane amino acid transporter protein
TGGT1_211330	1.05	N.S.	methionine aminopeptidase
TGGT1_227030	1.05	N.S.	hypothetical protein
TGGT1_219140	1.05	N.S.	EF-1 guanine nucleotide exchange domain-containing protein
TGGT1_275310	1.05	N.S.	hypothetical protein
TGGT1_253300	1.04	N.S.	hypothetical protein
TGGT1_310270	1.04	N.S.	hypothetical protein
TGGT1_301420	1.03	N.S.	hypothetical protein
TGGT1_254820	1.03	N.S.	hypothetical protein
TGGT1_266300	1.03	N.S.	hypothetical protein
TGGT1_224810	1.03	N.S.	hypothetical protein
TGGT1_219170	1.03	N.S.	hypothetical protein
TGGT1_315330	1.03	N.S.	SAG-related sequence SRS52D
TGGT1_286750	1.03	N.S.	MA3 domain-containing protein
TGGT1_313380	1.03	N.S.	hypothetical protein
TGGT1_240840	1.03	N.S.	histone lysine demethylase JmjC NO66
TGGT1_362290	1.02	N.S.	hypothetical protein
TGGT1_321170	1.02	N.S.	Toxoplasma gondii family C protein
TGGT1_219175	1.02	N.S.	hypothetical protein
TGGT1_224210	1.02	N.S.	hypothetical protein
TGGT1_211390	1.01	N.S.	hypothetical protein
TGGT1_239795	1.01	N.S.	hypothetical protein
TGGT1_203120	1.01	N.S.	hypothetical protein
TGGT1_235370	1.01	N.S.	hypothetical protein
TGGT1_271080	1.01	N.S.	hypothetical protein
TGGT1_230070	1.00	N.S.	BolA family protein
TGGT1_293510	1.00	N.S.	DP-ribose) polymerase and DNA-Ligase Zn-finger region domain-containing
TGGT1_247260	1.00	N.S.	hypothetical protein
TGGT1_215160	-1.00	N.S.	hypothetical protein
TGGT1_214910	-1.00	N.S.	hypothetical protein
TGGT1_203780	-1.00	N.S.	hypothetical protein
TGGT1_408850	-1.01	N.S.	hypothetical protein
TGGT1_310055	-1.01	N.S.	hypothetical protein
TGGT1_266290	-1.01	N.S.	hypothetical protein
TGGT1_255510	-1.01	N.S.	ankyrin repeat-containing protein
TGGT1_211470	-1.02	N.S.	Fcf2 pre-rRNA processing protein
TGGT1_286470	-1.02	N.S.	AGC kinase
TGGT1_287990	-1.02	N.S.	hypothetical protein
TGGT1_268380	-1.03	N.S.	RNA recognition motif-containing protein
TGGT1_305080	-1.03	N.S.	hypothetical protein
TGGT1_264940	-1.03	N.S.	kelch repeat-containing protein
TGGT1_223020	-1.04	N.S.	coproporphyrinogen III oxidase
TGGT1_239365	-1.04	N.S.	hypothetical protein
TGGT1_318130A	-1.04	N.S.	Toxoplasma gondii family E protein
TGGT1_269620	-1.05	N.S.	hypothetical protein
TGGT1_235390	-1.06	N.S.	PAN domain-containing protein
TGGT1_313900	-1.06	N.S.	non-specific serine/threonine protein kinase
TGGT1_205160	-1.07	N.S.	hypothetical protein
TGGT1_309060	-1.07	N.S.	ubiquitin carboxyl-terminal hydrolase
TGGT1_245560	-1.08	N.S.	hypothetical protein
TGGT1_261260A	-1.08	N.S.	histone lysine demethylase JMJD6a
TGGT1_321340	-1.08	N.S.	putative membrane protein
TGGT1_264720	-1.08	N.S.	hypothetical protein
TGGT1_209510	-1.09	N.S.	hypothetical protein
TGGT1_306700	-1.09	N.S.	hypothetical protein
TGGT1_258910	-1.12	N.S.	hypothetical protein
TGGT1_268220	-1.13	N.S.	hypothetical protein
TGGT1_214420	-1.13	N.S.	hypothetical protein
TGGT1_264700	-1.14	N.S.	hypothetical protein

TGGT1_329800	-1.14	N.S.	hypothetical protein
TGGT1_237100	-1.15	N.S.	RAP domain-containing protein
TGGT1_267560	-1.15	N.S.	folate-binding protein YgfZ protein
TGGT1_266065	-1.16	N.S.	hypothetical protein
TGGT1_287980	-1.17	N.S.	FHA domain-containing protein
TGGT1_277055	-1.17	N.S.	hypothetical protein
TGGT1_294400	-1.17	N.S.	hypothetical protein
TGGT1_264740	-1.17	N.S.	hypothetical protein
TGGT1_267720	-1.19	N.S.	transporter, cation channel family protein
TGGT1_267725	-1.20	N.S.	hypothetical protein
TGGT1_224840	-1.21	N.S.	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_264890	-1.22	N.S.	hypothetical protein
TGGT1_268360	-1.22	N.S.	hypothetical protein
TGGT1_262620	-1.23	N.S.	RNA recognition motif-containing protein
TGGT1_408750	-1.25	N.S.	hypothetical protein
TGGT1_268340	-1.25	N.S.	glycosyltransferase family 28 C-terminal domain-containing protein
TGGT1_264860	-1.25	N.S.	zinc finger, C3HC4 type (RING finger) domain-containing protein
TGGT1_214430	-1.25	N.S.	hypothetical protein
TGGT1_228500	-1.28	N.S.	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_279310	-1.30	N.S.	hypothetical protein
TGGT1_242625	-1.30	N.S.	TPase family associated with various cellular activities (AAA) subfamily protei
TGGT1_213880	-1.32	N.S.	hypothetical protein
TGGT1_205140	-1.32	N.S.	hypothetical protein
TGGT1_366510	-1.33	N.S.	hypothetical protein
TGGT1_261240	-1.33	N.S.	histone H3
TGGT1_316690	-1.35	N.S.	hypothetical protein
TGGT1_279315	-1.35	N.S.	hypothetical protein
TGGT1_280680	-1.35	N.S.	hypothetical protein
TGGT1_268350	-1.38	N.S.	hypothetical protein
TGGT1_270970	-1.40	N.S.	hypothetical protein
TGGT1_239430	-1.42	N.S.	EF hand domain-containing protein
TGGT1_410820	-1.43	N.S.	tRNA-Ser
TGGT1_410870	-1.49	N.S.	tRNA-Pro
TGGT1_204290	-1.58	N.S.	hypothetical protein
TGGT1_237894	-1.63	N.S.	OTU family cysteine protease
TGGT1_366530	-1.65	N.S.	KRUF family protein
TGGT1_361030	-1.74	N.S.	hypothetical protein
TGGT1_226880	-1.77	N.S.	hypothetical protein
TGGT1_264620	-1.81	N.S.	hypothetical protein
TGGT1_236670A	-1.83	N.S.	hypothetical protein
TGGT1_408600	-2.23	N.S.	hypothetical protein
TGGT1_364790	N.S.	2.27	putative transmembrane protein
TGGT1_258520	N.S.	1.89	WD domain, G-beta repeat-containing protein
TGGT1_408770	N.S.	1.83	putative transmembrane protein
TGGT1_409300	N.S.	1.60	tRNA-Gly
TGGT1_203375	N.S.	1.55	cpw-wpc domain-containing protein
TGGT1_320500	N.S.	1.55	patched family protein
TGGT1_208730	N.S.	1.43	putative microneme protein
TGGT1_261530	N.S.	1.40	eukaryotic aspartyl protease superfamily protein
TGGT1_225110	N.S.	1.40	AP2 domain transcription factor AP2X-2
TGGT1_305530	N.S.	1.35	hypothetical protein
TGGT1_315130	N.S.	1.34	L-isoaspartyl protein carboxyl methyltransferase family protein
TGGT1_200340	N.S.	1.33	hypothetical protein
TGGT1_216830	N.S.	1.32	DTW domain-containing protein
TGGT1_217720	N.S.	1.28	hypothetical protein
TGGT1_309930	N.S.	1.25	melibiase subfamily protein
TGGT1_409780	N.S.	1.24	tRNA-Ile
TGGT1_310020	N.S.	1.24	hypothetical protein
TGGT1_270320	N.S.	1.24	protein phosphatase 2C domain-containing protein
TGGT1_239855	N.S.	1.23	Ras-associated protein Rap1 isoform 1 family protein

TGGT1_289590	N.S.	1.23	hypothetical protein
TGGT1_294990	N.S.	1.20	hypothetical protein
TGGT1_314680	N.S.	1.19	hypothetical protein
TGGT1_297647	N.S.	1.17	hypothetical protein
TGGT1_256100	N.S.	1.15	tetratricopeptide repeat-containing protein
TGGT1_233490	N.S.	1.15	hypothetical protein
TGGT1_411080	N.S.	1.15	hypothetical protein
TGGT1_217000	N.S.	1.14	hypothetical protein
TGGT1_316660	N.S.	1.14	cullin family protein
TGGT1_316340	N.S.	1.14	hypothetical protein
TGGT1_248130	N.S.	1.13	hypothetical protein
TGGT1_237270	N.S.	1.12	hypothetical protein
TGGT1_271790	N.S.	1.11	hypothetical protein
TGGT1_219460	N.S.	1.11	hypothetical protein
TGGT1_297410	N.S.	1.11	hypothetical protein
TGGT1_305450	N.S.	1.10	acetyltransferase, GNAT family protein
TGGT1_253100	N.S.	1.10	hypothetical protein
TGGT1_257470	N.S.	1.09	myosin J
TGGT1_310290	N.S.	1.09	regulator of chromosome condensation (RCC1) repeat-containing protein
TGGT1_219240	N.S.	1.08	Peptidyl-tRNA hydrolase PTH2 domain-containing protein
TGGT1_304730	N.S.	1.08	Mob1/phocein family protein
TGGT1_261970	N.S.	1.08	hypothetical protein
TGGT1_307030	N.S.	1.08	Purine nucleoside phosphorylase
TGGT1_314900	N.S.	1.08	LisH protein
TGGT1_230340	N.S.	1.07	hypothetical protein
TGGT1_257300	N.S.	1.07	hypothetical protein
TGGT1_200350	N.S.	1.06	subtilisin SUB3
TGGT1_215910	N.S.	1.06	hypothetical protein
TGGT1_253570	N.S.	1.06	hypothetical protein
TGGT1_306720	N.S.	1.05	hypothetical protein
TGGT1_258050	N.S.	1.05	actin like protein ALP2a
TGGT1_250000	N.S.	1.05	hypothetical protein
TGGT1_251470	N.S.	1.05	hypothetical protein
TGGT1_306300	N.S.	1.05	hypothetical protein
TGGT1_311760	N.S.	1.05	hypothetical protein
TGGT1_222240	N.S.	1.05	hypothetical protein
TGGT1_216090	N.S.	1.03	hypothetical protein
TGGT1_258105	N.S.	1.02	hypothetical protein
TGGT1_289620	N.S.	1.01	cathepsin CPC1
TGGT1_254700	N.S.	1.01	hypothetical protein
TGGT1_218510	N.S.	1.01	hypothetical protein
TGGT1_309960B	N.S.	1.00	hypothetical protein
TGGT1_247220	N.S.	1.00	nudix -type motif 9 isoform a family protein
TGGT1_311110	N.S.	1.00	putative Ubiquitin-fold modifier 1 precursor family protein
TGGT1_294340	N.S.	1.00	hypothetical protein
TGGT1_217050	N.S.	1.00	ADA2-A transcriptional co-activator SAGA component
TGGT1_225680	N.S.	1.00	hypothetical protein
TGGT1_215650	N.S.	1.00	hypothetical protein
TGGT1_206440	N.S.	-1.00	cpw-wpc domain-containing protein
TGGT1_234970	N.S.	-1.01	Tyrosine kinase-like (TKL) protein
TGGT1_225270	N.S.	-1.01	hypothetical protein
TGGT1_226220	N.S.	-1.01	alveolin domain containing intermediate filament IMC9
TGGT1_225410	N.S.	-1.01	histone H3 centromeric CENH3
TGGT1_240450	N.S.	-1.02	Maf family protein
TGGT1_239340	N.S.	-1.02	hypothetical protein
TGGT1_218840	N.S.	-1.02	mutS domain protein
TGGT1_237160	N.S.	-1.03	hypothetical protein
TGGT1_258480	N.S.	-1.03	hypothetical protein
TGGT1_221280	N.S.	-1.03	hypothetical protein
TGGT1_295610	N.S.	-1.04	putative histone lysine methyltransferase, SET

TGGT1_292170	N.S.	-1.04	putative histone lysine methyltransferase, SET
TGGT1_239320	N.S.	-1.04	BolA family protein
TGGT1_281560	N.S.	-1.04	hypothetical protein
TGGT1_320110	N.S.	-1.04	proliferating cell nuclear antigen PCNA2
TGGT1_217620	N.S.	-1.04	hypothetical protein
TGGT1_255890	N.S.	-1.05	pyridine nucleotide-disulfide oxidoreductase domain-containing protein
TGGT1_233920	N.S.	-1.05	zinc finger, C3HC4 type (RING finger) domain-containing protein
TGGT1_202290	N.S.	-1.05	putative membrane protein
TGGT1_321660	N.S.	-1.05	putative mannosyltransferase
TGGT1_211220	N.S.	-1.05	hypothetical protein
TGGT1_280540	N.S.	-1.05	HEAT repeat-containing protein
TGGT1_310350	N.S.	-1.05	PGAP1 family protein
TGGT1_257595	N.S.	-1.05	hypothetical protein
TGGT1_306080	N.S.	-1.06	ATP-dependent DNA helicase, RecQ family protein
TGGT1_209930	N.S.	-1.06	hypothetical protein
TGGT1_251550	N.S.	-1.06	acyl-coa-binding protein
TGGT1_240950	N.S.	-1.07	hypothetical protein
TGGT1_264770	N.S.	-1.07	hypothetical protein
TGGT1_279390	N.S.	-1.07	putative proliferation-associated protein 2G4
TGGT1_286140	N.S.	-1.07	hypothetical protein
TGGT1_309265	N.S.	-1.07	oxidoreductase, short chain dehydrogenase/reductase family protein
TGGT1_253370	N.S.	-1.07	hypothetical protein
TGGT1_284645	N.S.	-1.07	hypothetical protein
TGGT1_236950	N.S.	-1.08	hypothetical protein
TGGT1_301480	N.S.	-1.08	hypothetical protein
TGGT1_227650	N.S.	-1.08	putative microtubule-associated protein RP/EB family
TGGT1_279430	N.S.	-1.09	cwf18 pre-mRNA splicing factor protein
TGGT1_293380	N.S.	-1.09	histone lysine acetyltransferase HAT1
TGGT1_226985	N.S.	-1.09	hypothetical protein
TGGT1_207100	N.S.	-1.10	hypothetical protein
TGGT1_279440	N.S.	-1.10	PA14 domain-containing protein
TGGT1_307850	N.S.	-1.10	6-phosphogluconate dehydrogenase
TGGT1_223000	N.S.	-1.11	dynein light chain DLC
TGGT1_289710	N.S.	-1.11	AP2 domain transcription factor AP2IX-5
TGGT1_312520	N.S.	-1.11	tRNA dimethylallyltransferase
TGGT1_289720	N.S.	-1.11	hypothetical protein
TGGT1_258710	N.S.	-1.11	T-complex protein 10 domain-containing protein
TGGT1_249840	N.S.	-1.11	putative dynein heavy chain 2
TGGT1_320750	N.S.	-1.11	hypothetical protein
TGGT1_238040B	N.S.	-1.12	protein disulfide-isomerase domain-containing protein
TGGT1_225090	N.S.	-1.12	hypothetical protein
TGGT1_270910	N.S.	-1.13	acyltransferase domain-containing protein
TGGT1_365080	N.S.	-1.13	rhoptry protein ROP7
TGGT1_247370	N.S.	-1.13	hypothetical protein
TGGT1_228180	N.S.	-1.13	putative cytochrome C oxidase assembly factor COX15
TGGT1_267390	N.S.	-1.13	DNA-directed RNA polymerase I RPAC1
TGGT1_258380	N.S.	-1.14	elongation factor p (ef-p) kow family domain-containing protein
TGGT1_243940	N.S.	-1.14	hypothetical protein
TGGT1_250360	N.S.	-1.14	alpha/beta hydrolase fold domain-containing protein
TGGT1_240580	N.S.	-1.15	hypothetical protein
TGGT1_273330	N.S.	-1.15	hypothetical protein
TGGT1_206650	N.S.	-1.15	zinc finger, c2h2 type domain-containing protein
TGGT1_273530	N.S.	-1.15	flagellar associated protein
TGGT1_288700	N.S.	-1.16	RecF/RecN/SMC N terminal domain-containing protein
TGGT1_203830	N.S.	-1.16	FHA domain-containing protein
TGGT1_274170	N.S.	-1.16	protein kinase (incomplete catalytic triad)
TGGT1_237425	N.S.	-1.16	AP2 domain transcription factor AP2X-6
TGGT1_312230	N.S.	-1.17	putative DNA topoisomerase 2
TGGT1_224910	N.S.	-1.18	divalent cation tolerance protein, CutA1 family protein
TGGT1_249935	N.S.	-1.18	hypothetical protein

TGGT1_222030	N.S.	-1.18	hypothetical protein
TGGT1_215010	N.S.	-1.18	hypothetical protein
TGGT1_202040	N.S.	-1.18	hypothetical protein
TGGT1_206700	N.S.	-1.18	hypothetical protein
TGGT1_310130	N.S.	-1.19	Spc97 / Spc98 family protein
TGGT1_359590	N.S.	-1.19	hypothetical protein
TGGT1_294640	N.S.	-1.19	ribonucleoside-diphosphate reductase large chain
TGGT1_264080	N.S.	-1.19	acyl carrier protein ACP
TGGT1_203340	N.S.	-1.20	metallo-beta-lactamase domain-containing protein
TGGT1_311625	N.S.	-1.20	WD domain, G-beta repeat-containing protein
TGGT1_276220	N.S.	-1.20	hypothetical protein
TGGT1_232550	N.S.	-1.21	hypothetical protein
TGGT1_281930	N.S.	-1.21	SAG-related sequence SRS39
TGGT1_280810	N.S.	-1.22	hypothetical protein
TGGT1_232450	N.S.	-1.22	SWI2/SNF2-containing protein RAD54
TGGT1_273540	N.S.	-1.22	putative phosphatidylserine synthase
TGGT1_203700	N.S.	-1.23	SFT2 family protein
TGGT1_227640	N.S.	-1.23	hypothetical protein
TGGT1_254110	N.S.	-1.23	tryptophanyl-tRNA synthetase (TrpRS1)
TGGT1_268990	N.S.	-1.23	hypothetical protein
TGGT1_267430	N.S.	-1.23	DnaJ domain-containing protein
TGGT1_246200	N.S.	-1.23	zinc finger (CCCH type) motif-containing protein
TGGT1_220560	N.S.	-1.24	hypothetical protein
TGGT1_247680	N.S.	-1.24	hypothetical protein
TGGT1_246190	N.S.	-1.25	hypothetical protein
TGGT1_222270	N.S.	-1.26	hypothetical protein
TGGT1_300270	N.S.	-1.26	hypothetical protein
TGGT1_205640	N.S.	-1.26	hypothetical protein
TGGT1_254580	N.S.	-1.26	UDP-galactose transporter family protein
TGGT1_210280	N.S.	-1.27	PEK kinase
TGGT1_216200	N.S.	-1.27	hypothetical protein
TGGT1_218362	N.S.	-1.27	zinc finger protein ZFP1
TGGT1_266150	N.S.	-1.27	hypothetical protein
TGGT1_267460	N.S.	-1.27	AP2 domain transcription factor AP2IX-1
TGGT1_319590	N.S.	-1.29	hypothetical protein
TGGT1_202510	N.S.	-1.29	multi-pass transmembrane protein
TGGT1_218530	N.S.	-1.29	proteasome-interacting thioredoxin domain-containing protein
TGGT1_253780	N.S.	-1.29	putative GTP cyclohydrolase I
TGGT1_202120	N.S.	-1.31	hypothetical protein
TGGT1_279420	N.S.	-1.31	hypothetical protein
TGGT1_267400	N.S.	-1.32	ribosomal protein RPL32
TGGT1_229370	N.S.	-1.32	AP2 domain transcription factor AP2VIII-1
TGGT1_203170	N.S.	-1.33	OB-fold nucleic acid binding domain-containing protein
TGGT1_262470	N.S.	-1.34	putative C protein immunoglobulin-A-binding beta antigen
TGGT1_202065	N.S.	-1.35	hypothetical protein
TGGT1_231360	N.S.	-1.35	hypothetical protein
TGGT1_208560	N.S.	-1.37	carrier superfamily protein
TGGT1_212940	N.S.	-1.37	hypothetical protein
TGGT1_268010	N.S.	-1.38	hypothetical protein
TGGT1_257770	N.S.	-1.38	histone lysine methyltransferase SET2
TGGT1_311485	N.S.	-1.39	hypothetical protein
TGGT1_257540	N.S.	-1.39	hypothetical protein
TGGT1_289070	N.S.	-1.41	E1-E2 ATPase subfamily protein
TGGT1_217900	N.S.	-1.42	hypothetical protein
TGGT1_209060	N.S.	-1.43	thrombospondin type 1 domain-containing protein
TGGT1_249160	N.S.	-1.44	UAA transporter family protein
TGGT1_240380	N.S.	-1.44	hypothetical protein
TGGT1_301460	N.S.	-1.45	hypothetical protein
TGGT1_247580	N.S.	-1.45	glutaredoxin domain-containing protein
TGGT1_254365	N.S.	-1.45	phosphatidate cytidylyltransferase

TGGT1_251410	N.S.	-1.46	tetratricopeptide repeat-containing protein
TGGT1_294651	N.S.	-1.46	hypothetical protein
TGGT1_253870	N.S.	-1.47	hypothetical protein
TGGT1_266270	N.S.	-1.47	short-chain-acyl-CoA dehydrogenase
TGGT1_267070	N.S.	-1.47	aquaporin 2
TGGT1_249020	N.S.	-1.47	kinesin motor domain-containing protein
TGGT1_271200	N.S.	-1.47	AP2 domain transcription factor AP2VIII-5
TGGT1_289890	N.S.	-1.48	hypothetical protein
TGGT1_260490	N.S.	-1.48	hypothetical protein
TGGT1_205265	N.S.	-1.48	transporter, cation channel family protein
TGGT1_320620	N.S.	-1.49	queuine tRNA ribosyl transferase
TGGT1_231890	N.S.	-1.49	putative beta-ketoacyl-acyl carrier protein synthase III
TGGT1_278740	N.S.	-1.49	diaminopimelate decarboxylase
TGGT1_275370	N.S.	-1.49	SAG-related sequence SRS47C
TGGT1_232500	N.S.	-1.50	hypothetical protein
TGGT1_311490	N.S.	-1.52	hypothetical protein
TGGT1_237140	N.S.	-1.55	putative ethylene inducible protein
TGGT1_288730	N.S.	-1.57	hypothetical protein
TGGT1_272383	N.S.	-1.57	hypothetical protein
TGGT1_262660	N.S.	-1.60	hypothetical protein
TGGT1_295662	N.S.	-1.60	hypothetical protein
TGGT1_223258	N.S.	-1.61	hypothetical protein
TGGT1_294650	N.S.	-1.65	hypothetical protein
TGGT1_272285	N.S.	-1.66	tetratricopeptide repeat-containing protein
TGGT1_237880	N.S.	-1.68	hypothetical protein
TGGT1_260270	N.S.	-1.68	HEAT repeat-containing protein
TGGT1_233960	N.S.	-1.71	hypothetical protein
TGGT1_219100	N.S.	-1.72	cyclin-dependent kinase regulatory subunit protein
TGGT1_269015	N.S.	-1.72	hypothetical protein
TGGT1_286050	N.S.	-1.73	hypothetical protein
TGGT1_225370	N.S.	-1.73	hypothetical protein
TGGT1_231815	N.S.	-1.74	hypothetical protein
TGGT1_264650	N.S.	-1.74	phosphoacetylglucosamine mutase
TGGT1_216700	N.S.	-1.75	hypothetical protein
TGGT1_269020	N.S.	-1.78	hypothetical protein
TGGT1_204505	N.S.	-1.80	hypothetical protein
TGGT1_410660	N.S.	-1.80	hypothetical protein
TGGT1_249660	N.S.	-1.81	hypothetical protein
TGGT1_315560	N.S.	-1.82	ATP-binding cassette G family transporter ABCG77
TGGT1_260410	N.S.	-1.83	hypothetical protein
TGGT1_284010	N.S.	-1.84	5'-3' exonuclease, N-terminal resolvase family domain-containing protein
TGGT1_238060	N.S.	-1.92	EF hand domain-containing protein
TGGT1_273130	N.S.	-1.96	SAG-related sequence SRS30A
TGGT1_266335	N.S.	-2.09	Toxoplasma gondii family A protein
TGGT1_217855	N.S.	-2.09	hypothetical protein
TGGT1_411350	N.S.	-2.13	rhoptry kinase family (incomplete catalytic triad) protein
TGGT1_231170	N.S.	-2.17	RecF/RecN/SMC N terminal domain-containing protein
TGGT1_206415	N.S.	-2.19	myosin K
TGGT1_236595	N.S.	-2.20	ankyrin repeat-containing protein
TGGT1_308093	N.S.	-2.32	rhoptry kinase family protein (incomplete catalytic triad)
TGGT1_358120	N.S.	-2.50	hypothetical protein
TGGT1_218340	N.S.	-2.51	hypothetical protein
TGGT1_258085	N.S.	-2.61	hypothetical protein
TGGT1_266330	N.S.	-2.61	Toxoplasma gondii family A protein
TGGT1_312690	N.S.	-2.71	hypothetical protein
TGGT1_367020	N.S.	-2.90	hypothetical protein
TGGT1_207650	N.S.	-3.08	OTU family cysteine protease
TGGT1_237070	N.S.	-3.26	hypothetical protein
TGGT1_275660	N.S.	-3.27	hypothetical protein
TGGT1_225413	N.S.	-3.30	hypothetical protein

TGGT1_254060	N.S.	-3.31	SAG-related sequence SRS14A
TGGT1_243302	N.S.	-3.49	hypothetical protein
TGGT1_266340	N.S.	-3.92	Toxoplasma gondii family A protein
TGGT1_240360	N.S.	-5.66	Toxoplasma gondii family E protein

Table 4.3. Differential expression analysis identified few intracellular differentially expressed gene during GT1's lab-adaptation

Following RNA-seq of 30-hour intracellular parasites, DEA identified 12 significantly DEGs amongst the lab-adapting B2 GT1 population, relative to the starting B2-P11 clonal population. DEGs were considered significant if the absolute FC ≥2 and *q*-value ≤0.05 (red text) in at least one time point (i.e. passage).

Table 4.3. Differential expression analysis identified few intracellular differentially expressed gene during GT1's lab-adaptation

Gene ID	Log_2 FC (relative to GT1 B2-P11)				Product Description
	RH	P11	P85	P148	
TGGT1_411800	2.74		0.88	2.55	hypothetical protein
TGGT1_262590	2.40		2.75	2.82	hypothetical protein
TGGT1_264240	2.39		0.29	2.43	hypothetical protein
TGGT1_264260	2.34		0.31	2.35	hypothetical protein
TGGT1_216290B	1.87		2.18	0.42	hypothetical protein
TGGT1_409050	1.77		0.14	2.27	hypothetical protein
TGGT1_262600	1.61		1.56	1.62	hypothetical protein
TGGT1_213620	0.39		1.60	1.55	ABC1 family protein
TGGT1_213635	0.23		3.09	3.34	hypothetical protein
TGGT1_280400	0.14		1.01	1.50	hypothetical protein
TGGT1_254840	-1.74		1.95	1.90	tetratricopeptide repeat-containing protein
TGGT1_280500	-4.99		1.71	1.04	inorganic anion transporter, sulfate permease (SulP) family protein

Table 4.4. Differential expression analysis followed by time-course sequencing analysis identified many extracellular differentially expressed genes that trend during GT1's lab-adaptation

Following RNA-seq and DEA of 6-hour extracellular GT1 parasites, TC-seq identified 988 significant DEGs that generally trend u*p* (yellow) or down (blue) over time (i.e. passages). DEGs were considered significant if the absolute FC ≥2 and *q*-value ≤0.05 (red text) in at least one time point (i.e. passage). All DEGs shown here fit one of the expression patterns shown in Figure 4.2.

Table 4.4. Differential expression analysis followed by time-course sequencing analysis identified many extracellular differentially expressed genes that trend during GT1's lab-adaptation

Gene ID	Log_2 FC (relative to GT1 B2-P11)							Product Description
	RH	P11	P35	P55	P85	P148	P210	
TGGT1_411800	5.49		1.90	1.89	1.22	3.95	5.06	hypothetical protein
TGGT1_409050	4.35		1.87	1.82	1.05	3.68	4.74	hypothetical protein
TGGT1_264260	4.80		1.21	1.19	1.01	3.54	4.07	hypothetical protein
TGGT1_249800	4.70		-0.30	0.17	0.60	3.17	3.98	hypothetical protein
TGGT1_213635	2.38		1.94	2.67	3.41	3.71	3.73	hypothetical protein
TGGT1_361230	5.12		1.22	1.48	2.96	4.20	3.49	toxoplasma gondii family B protein
TGGT1_262590	2.90		2.06	2.98	2.66	2.58	3.45	hypothetical protein
TGGT1_264240	4.35		-0.07	0.36	1.04	3.44	3.22	hypothetical protein
TGGT1_280400	0.90		3.08	3.23	2.03	2.91	3.11	hypothetical protein
TGGT1_253330	0.10		0.91	0.62	1.84	3.04	2.73	Rhoptry kinase family protein
TGGT1_216290B	4.40		1.04	1.98	3.06	1.15	2.63	hypothetical protein
TGGT1_249810	3.35		0.14	0.24	0.10	1.68	2.60	putative activating signal cointegrator 1 complex subunit 3
TGGT1_301180	5.65		1.05	1.76	2.39	4.41	2.51	SAG-related sequence SRS19F
TGGT1_301890	-1.38		0.95	1.17	1.87	2.85	2.49	Toxoplasma gondii family B protein
TGGT1_262600	2.68		1.36	2.10	2.10	2.18	2.48	hypothetical protein
TGGT1_306688	3.18		1.01	1.32	0.87	1.63	2.47	hypothetical protein
TGGT1_266760	0.94		0.67	1.50	2.53	2.46	2.31	isocitrate dehydrogenase
TGGT1_216290A	4.17		1.22	1.80	2.69	0.99	2.30	hypothetical protein
TGGT1_276810	3.13		1.58	1.70	2.55	2.70	2.29	hypothetical protein
TGGT1_309930	0.88		1.18	1.01	1.79	2.37	2.21	melibiase subfamily protein
TGGT1_216680	5.19		0.29	0.78	1.22	1.64	2.17	ankyrin repeat-containing protein
TGGT1_358470	3.99		1.81	0.71	0.88	1.14	2.06	hypothetical protein
TGGT1_306692	0.20		1.14	0.92	1.33	1.71	2.02	hypothetical protein
TGGT1_304670	2.30		1.64	1.74	1.16	1.37	2.01	leucine rich repeat-containing protein
TGGT1_301210	3.35		0.34	0.87	0.93	2.93	2.01	putative NAD(P) transhydrogenase subunit beta
TGGT1_222020	0.92		-0.28	0.60	3.19	2.46	1.99	phosphoglycerate kinase PGKII
TGGT1_323310	3.48		0.36	0.79	0.56	1.51	1.97	hypothetical protein
TGGT1_216335	2.82		0.78	1.44	1.85	0.58	1.95	hypothetical protein
TGGT1_207140	2.61		0.07	0.46	3.46	2.88	1.95	SAG-related sequence SRS49B
TGGT1_240820	0.97		1.35	1.62	1.61	1.65	1.95	hypothetical protein
TGGT1_315885	0.63		0.79	1.23	1.04	1.49	1.94	putative glycosyltransferase
TGGT1_410970	2.94		1.32	0.80	0.17	1.34	1.93	hypothetical protein
TGGT1_411240	-0.78		0.65	1.00	1.25	1.98	1.92	toxoplasma gondii family B protein
TGGT1_236670B	-2.18		1.04	1.25	-0.35	0.14	1.91	hypothetical protein
TGGT1_286420A	0.44		1.09	1.41	1.06	1.33	1.89	putative elongation factor 1-alpha (EF-1-ALPHA)
TGGT1_313640	0.89		0.42	0.92	1.61	1.87	1.87	hypothetical protein
TGGT1_277685	1.74		1.43	1.60	1.36	1.79	1.82	hypothetical protein
TGGT1_231992	2.21		1.32	1.35	1.02	1.00	1.80	hypothetical protein
TGGT1_289170	0.92		1.10	1.38	1.04	1.31	1.80	adenylate and guanylate cyclase catalytic domain-containing protein
TGGT1_203990	1.44		1.45	1.55	1.00	0.92	1.77	rhoptry protein ROP12
TGGT1_220060B	1.66		0.75	0.89	0.33	0.99	1.77	hypothetical protein
TGGT1_243520	0.33		0.82	1.34	1.35	1.18	1.76	hypothetical protein
TGGT1_230705	0.31		1.11	1.42	0.87	1.45	1.75	hypothetical protein
TGGT1_263520	0.68		1.72	1.72	0.56	0.51	1.74	microtubule associated protein SPM1
TGGT1_285700	1.23		0.87	1.24	2.08	2.02	1.73	ubiquitin fusion degradation protein UFD1AP
TGGT1_316700			0.80	0.88	1.19	2.00	1.72	uridine kinase
TGGT1_306860	-1.68		0.75	0.39	-0.03	0.74	1.71	hypothetical protein
TGGT1_410930	3.26		0.47	0.28	0.66	1.92	1.70	hypothetical protein
TGGT1_285865	1.14		1.04	1.36	1.46	1.86	1.70	hypothetical protein
TGGT1_220420	1.64		1.03	1.09	0.99	1.74	1.68	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_260480	1.42		1.01	1.09	1.96	2.49	1.68	leucine rich repeat-containing protein
TGGT1_254840	-1.87		0.38	0.95	1.67	1.48	1.68	tetratricopeptide repeat-containing protein
TGGT1_206320	0.60		0.90	1.23	1.47	1.39	1.67	hypothetical protein
TGGT1_224130	1.72		1.15	1.20	1.40	1.88	1.67	hypothetical protein
TGGT1_262655	-1.49		1.28	1.43	0.47	0.52	1.67	hypothetical protein
TGGT1_287270	0.52		0.63	0.52	1.55	1.60	1.66	hypothetical protein
TGGT1_270840	1.15		1.13	1.34	1.80	2.42	1.66	poly(ADP-ribose) polymerase catalytic domain-containing protein
TGGT1_305980	0.19		0.31	0.50	1.88	1.47	1.65	pyruvate dehydrogenase complex subunit PDH-E3I
TGGT1_202970	-0.70		0.35	0.92	0.40	0.50	1.65	hypothetical protein
TGGT1_363250	-0.68		0.68	0.91	0.69	0.98	1.65	hypothetical protein
TGGT1_226330	-0.30		1.03	1.39	0.80	1.04	1.64	hypothetical protein
TGGT1_255330	0.78		0.66	1.37	1.33	1.72	1.64	hypothetical protein
TGGT1_258410	0.64		1.56	1.64	0.66	0.82	1.63	photosensitized INA-labeled protein PHIL1
TGGT1_207665	1.67		0.78	0.82	1.33	1.65	1.63	kinesin motor domain-containing protein
TGGT1_280500	-2.19		0.64	1.37	1.86	1.14	1.61	inorganic anion transporter, sulfate permease (SulP) family protein
TGGT1_360830	-0.13		1.00	1.06	0.50	0.81	1.60	nucleoside-triphosphatase
TGGT1_247350	1.32		0.94	0.82	1.39	1.37	1.60	thioredoxin domain-containing protein
TGGT1_296020	-2.55		1.64	1.12	1.24	1.54	1.60	hypothetical protein
TGGT1_269775	0.99		1.04	1.36	0.99	1.27	1.60	hypothetical protein

TGGT1_254820	1.03		0.50	0.83	1.32	1.60	1.59	hypothetical protein
TGGT1_408600	-2.23		1.40	1.48	0.65	0.94	1.59	hypothetical protein
TGGT1_320740	2.30		1.07	1.21	1.71	1.80	1.58	hypothetical protein
TGGT1_252255	2.58		1.19	1.51	1.39	1.67	1.58	hypothetical protein
TGGT1_323320	3.59		0.13	0.26	0.80	1.79	1.57	hypothetical protein
TGGT1_222270	-0.24		0.80	1.15	1.48	1.61	1.57	hypothetical protein
TGGT1_318430	-1.30		0.56	0.76	1.16	1.23	1.56	malate dehydrogenase MDH
TGGT1_290300	1.07		1.30	1.14	0.59	0.97	1.55	hypothetical protein
TGGT1_240610	0.92		0.43	0.87	1.34	1.45	1.55	hypothetical protein
TGGT1_409590	3.03		0.09	0.58	0.72	1.19	1.55	putative transmembrane protein
TGGT1_220060	3.48		0.77	0.91	1.12	1.55	1.55	hypothetical protein
TGGT1_240410	-0.36		0.73	0.78	0.75	0.96	1.55	protein kinase (incomplete catalytic triad)
TGGT1_285170	-3.00		0.16	0.97	1.30	1.60	1.54	putative ribosomal RNA large subunit methyltransferase I
TGGT1_235515	1.22		0.69	1.19	1.47	1.73	1.54	MORN repeat-containing protein
TGGT1_289210	0.54		1.03	1.33	1.12	1.06	1.52	prefoldin subunit protein
TGGT1_297245	0.41		0.21	0.71	1.54	1.26	1.52	transporter, major facilitator family protein
TGGT1_257000	1.22		0.74	0.99	1.51	2.00	1.51	hypothetical protein
TGGT1_200700	1.75		0.73	0.86	0.96	1.50	1.51	Toxoplasma gondii family C protein
TGGT1_217020	3.48		0.46	0.88	1.36	0.46	1.51	ATPase, AFG1 family protein
TGGT1_265005	0.15		1.12	1.13	0.78	1.24	1.50	hypothetical protein
TGGT1_245670	-0.11		-0.49	-0.03	2.33	1.55	1.50	pyruvate dehydrogenase complex subunit PDH-E1Alpha
TGGT1_317720	0.24		0.71	0.98	0.73	0.92	1.49	putative eukaryotic translation initiation factor 3 subunit 7
TGGT1_220640			1.21	1.27	0.16	0.33	1.48	hypothetical protein
TGGT1_214540	-1.06		0.29	0.70	1.23	1.25	1.47	hypothetical protein
TGGT1_286580	0.76		1.15	1.20	0.74	0.81	1.47	hypothetical protein
TGGT1_213460	0.52		0.29	0.56	1.06	3.04	1.47	hypothetical protein
TGGT1_290580	0.55		0.27	0.84	1.67	1.93	1.46	ATP-binding cassette G family transporter ABCG89
TGGT1_265370	0.07		1.19	1.33	0.58	0.57	1.45	hypothetical protein
TGGT1_253540	-0.21		-1.02	-0.06	1.15	1.61	1.44	hypothetical protein
TGGT1_231990	-0.49		1.17	1.42	1.10	1.12	1.42	hypothetical protein
TGGT1_284310	4.91		0.70	1.07	1.01	1.22	1.42	hypothetical protein
TGGT1_212735	0.58		0.87	0.76	1.17	1.32	1.41	hypothetical protein
TGGT1_211030	1.52		0.83	0.95	1.76	1.74	1.41	hypothetical protein
TGGT1_223045	1.89		0.92	1.02	0.50	1.21	1.40	hypothetical protein
TGGT1_211060	0.21		1.01	1.36	0.66	0.53	1.40	hypothetical protein
TGGT1_309860	0.87		1.00	1.24	0.77	1.39	1.39	hypothetical protein
TGGT1_222410	1.10		1.02	1.28	1.14	1.07	1.39	hypothetical protein
TGGT1_298050	1.46		0.81	1.06	1.73	1.87	1.39	hypothetical protein
TGGT1_244700	3.33		0.27	0.43	0.20	0.50	1.39	NAD(+)/NADH kinase domain-containing protein
TGGT1_260860	0.24		0.60	0.97	0.45	0.59	1.39	hypothetical protein
TGGT1_306470	1.49		0.77	0.88	1.00	1.07	1.39	isoprenylcysteine carboxyl methyltransferase (icmt) family protein
TGGT1_253100	0.92		1.22	1.22	0.24	0.36	1.38	hypothetical protein
TGGT1_264990	1.00		1.37	1.43	0.55	0.49	1.37	hypothetical protein
TGGT1_268170	0.61		1.07	1.28		3.03	1.37	hypothetical protein
TGGT1_240830	1.00		0.84	1.16	1.28	1.50	1.36	hydrolase, alpha/beta fold family protein
TGGT1_252210	0.07		0.15	0.21	0.32	1.35	1.36	pentatricopeptide repeat domain-containing protein
TGGT1_322110	2.95			0.33	0.45	1.16	1.35	hypothetical protein
TGGT1_315900	1.44		0.78	0.79	0.46	0.90	1.35	hypothetical protein
TGGT1_315690	0.50		0.73	0.77	0.88	1.05	1.35	DnaJ domain-containing protein
TGGT1_410360			1.12	1.22	0.24	0.46	1.35	putative transmembrane protein
TGGT1_228990	-0.13		1.11	0.83	0.64	0.99	1.34	zinc finger (CCCH type) motif-containing protein
TGGT1_228370	1.33		1.16	1.13	1.17	1.20	1.34	hypothetical protein
TGGT1_264660	1.18		1.29	0.81	0.54	1.49	1.34	SAG-related sequence SRS44
TGGT1_278630	0.55		0.81	0.94	1.08	1.31	1.33	tetratricopeptide repeat-containing protein
TGGT1_217810	-0.70		0.59	0.94	0.15	0.14	1.33	hypothetical protein
TGGT1_230980	0.97		0.62	0.67	0.69	1.33	1.33	myosin I
TGGT1_229280	0.68		0.85	0.90	0.18	0.74	1.33	hypothetical protein
TGGT1_230520	0.35		0.22	0.53	1.62	1.89	1.32	putative cyclophilin 1
TGGT1_281500	0.62		0.66	0.81	0.81	0.82	1.32	hypothetical protein
TGGT1_248900	-0.51		0.90	0.87	0.53	1.16	1.32	hypothetical protein
TGGT1_247510	0.27		0.81	0.92	0.66	1.07	1.32	fructose-bisphospatase II
TGGT1_313290	0.63		0.99	1.05	0.45	0.62	1.31	MORN repeat-containing protein
TGGT1_286810	0.54		1.18	1.17	0.35	0.38	1.31	hypothetical protein
TGGT1_294990	0.88		0.83	1.02	0.60	0.90	1.31	hypothetical protein
TGGT1_361030	-1.74		0.91	0.98	0.91	1.16	1.30	hypothetical protein
TGGT1_252280	0.76		0.31	0.73	0.81	1.20	1.29	hypothetical protein
TGGT1_282150	0.27		0.99	0.84	1.30	1.53	1.29	hypothetical protein
TGGT1_240600	0.73		0.16	0.69	1.46	1.57	1.29	putative chaperonin cpn60
TGGT1_203450	0.64		0.62	0.96	0.97	0.87	1.29	DUF3228 domain-containing protein
TGGT1_217728	3.24		1.21	0.91	0.98	0.95	1.29	hypothetical protein
TGGT1_273800	-0.72		0.73	0.84	0.51	1.11	1.29	WD domain, G-beta repeat-containing protein
TGGT1_249935	-0.14		0.60	0.93	1.47	1.64	1.29	hypothetical protein
TGGT1_247520	0.24		1.10	1.17	0.67	0.66	1.28	hypothetical protein
TGGT1_292920	0.28		0.25	0.50	0.91	1.20	1.27	putative heat shock protein 75
TGGT1_219170	1.03		1.26	1.25	0.71	0.82	1.27	hypothetical protein
TGGT1_243545	0.48		1.17	1.22	0.60	0.65	1.27	hypothetical protein
TGGT1_411390	2.69		0.83	0.22	0.46	1.18	1.26	hypothetical protein
TGGT1_242820	0.29		1.08	1.18	0.72	0.81	1.26	hypothetical protein
TGGT1_241155	0.41		0.88	0.85	0.54	0.70	1.26	hypothetical protein
TGGT1_286720	1.08		0.72	1.07	1.09	1.22	1.26	heat shock protein HSP28
TGGT1_305050	0.84		1.06	1.17	1.06	0.93	1.25	putative calmodulin
TGGT1_231140	0.36		0.79	0.90	0.95	1.04	1.25	ribosomal protein RPS25
TGGT1_357130	1.07		2.22	1.33	0.10	0.25	1.25	WD domain, G-beta repeat protein
TGGT1_235635	0.30		1.36	1.03	0.10	0.25	1.24	hypothetical protein
TGGT1_329600			0.16	0.14	1.32	1.70	1.24	hypothetical protein
TGGT1_292120	0.63		0.96	0.91	0.45	0.59	1.24	membrane occupation and recognition nexus protein MORN2
TGGT1_409260	-0.24		0.19	0.44	1.16	1.50	1.24	hypothetical protein

TGGT1_316340	0.89		0.94	0.95	0.62	0.92	1.24	hypothetical protein
TGGT1_267500	-0.28		1.05	1.21	0.64	0.55	1.23	hypothetical protein
TGGT1_230830	-1.41		0.51	0.76	0.79	1.13	1.23	Pase family associated with various cellular activities (AAA) domain-containing prot
TGGT1_320030	1.11		1.30	1.20	0.38	0.25	1.23	hypothetical protein
TGGT1_275470	0.78		0.93	1.03	0.23	0.48	1.23	dense granule protein GRA15
TGGT1_268010	-0.54			0.03	0.46	0.67	1.23	hypothetical protein
TGGT1_221470	0.95		0.68	0.71	1.04	1.32	1.22	hypothetical protein
TGGT1_312380	1.09		1.03	0.93	1.31	1.37	1.22	tetratricopeptide repeat-containing protein
TGGT1_254720	0.15		0.81	0.97		0.23	1.22	dense granule protein GRA8
TGGT1_243600	1.73		0.89	1.11	0.78	1.01	1.22	acetyltransferase, GNAT family protein
TGGT1_410790	2.84		0.41		0.45	1.63	1.22	hypothetical protein
TGGT1_411350	-1.43		0.24	1.01	0.98	2.69	1.21	rhoptry kinase family (incomplete catalytic triad) protein
TGGT1_215270	-1.01		0.63	0.91		0.43	1.21	hypothetical protein
TGGT1_219700	0.99			0.40	1.10	1.16	1.21	putative DNA replication licensing factor MCM4
TGGT1_260850	0.47		0.40	0.83	0.64	0.97	1.21	putative mitochondrial import inner membrane translocase subunit TIM10
TGGT1_264080	-0.48			0.40	1.50	1.30	1.21	acyl carrier protein ACP
TGGT1_265010	0.19		0.73	0.89	0.84	1.02	1.21	glutamate 5-kinase domain-containing protein
TGGT1_220060A	1.47		0.45	0.65	1.00	1.24	1.20	hypothetical protein
TGGT1_275770	0.26		0.99	1.16	0.68	0.66	1.20	hypothetical protein
TGGT1_283702	0.49		-0.50	-0.26	1.61	1.24	1.18	FATC domain-containing protein
TGGT1_293780	4.05		1.13	0.58	1.53	2.82	1.17	hypothetical protein
TGGT1_248445	1.48		1.44	1.21	0.94	0.98	1.17	hypothetical protein
TGGT1_262910	0.68		0.78	1.01	0.82	0.99	1.16	putative NADH-cytochrome b5 reductase 1
TGGT1_312075	-1.10		1.11	1.07	0.28	0.25	1.16	hypothetical protein
TGGT1_409300	0.89		0.96	1.15	0.41	0.65	1.16	tRNA-Gly
TGGT1_291010	0.21		0.91	0.97	0.85	0.94	1.16	hypothetical protein
TGGT1_213350	0.34		0.58	0.68	0.90	1.21	1.16	ribosomal protein RPS15
TGGT1_319695	0.41		0.60	1.07	0.88	1.03	1.16	hypothetical protein
TGGT1_231890	-0.06		0.18	0.66	1.34	1.41	1.16	putative beta-ketoacyl-acyl carrier protein synthase III
TGGT1_294980	0.72		0.60	0.78	0.42	0.73	1.15	hypothetical protein
TGGT1_292290	-0.03		1.01	0.92	0.68	1.13	1.15	hypothetical protein
TGGT1_233030	0.33		1.19	1.15	0.42	0.39	1.15	gliding-associated protein GAP70
TGGT1_226420	1.26		0.69	0.65	0.91	1.22	1.14	peptidase family M3 protein
TGGT1_245746A	-1.84		0.80	0.84	0.49	0.68	1.14	hypothetical protein
TGGT1_216280	0.54		0.82	1.24	1.57	2.42	1.14	adenylate and guanylate cyclase catalytic domain-containing protein
TGGT1_258470	-0.16		0.69	0.79	0.61	1.17	1.13	hypothetical protein
TGGT1_293510	1.00		0.88	0.92	1.07	1.42	1.13	y(ADP-ribose) polymerase and DNA-Ligase Zn-finger region domain-containing pro
TGGT1_255370	0.79		0.32	0.74	1.08	1.31	1.13	TIC20 protein
TGGT1_294340	0.75		0.87	0.88	0.55	0.63	1.12	hypothetical protein
TGGT1_263850	0.53		0.82	0.92	0.73	0.69	1.12	hypothetical protein
TGGT1_270580	0.25		0.83	0.89	0.60	0.68	1.12	HECT-domain (ubiquitin-transferase) domain-containing protein
TGGT1_306310	1.25		0.53	0.79	1.28	1.43	1.12	RecF/RecN/SMC N terminal domain-containing protein
TGGT1_227840	1.08		0.34	0.52	0.73	0.61	1.12	Memo family protein
TGGT1_297940	1.40		0.33	0.38	0.75	1.15	1.12	single-strand binding protein
TGGT1_284010	-0.36		-0.32	-0.23	1.46	1.28	1.12	5'-3' exonuclease, N-terminal resolvase family domain-containing protein
TGGT1_409990	2.57		0.60	0.88	0.43	1.14	1.12	hypothetical protein
TGGT1_293460	0.67		0.62	0.99	0.74	1.06	1.11	ATP-dependent DNA ligase domain-containing protein
TGGT1_270250	0.74		1.03	1.04	0.64	0.85	1.11	dense granule protein GRA1
TGGT1_293590			0.49	0.77	1.21	0.99	1.11	putative 3-oxoacyl-acyl-carrier protein synthase I/II
TGGT1_224235	0.87		0.60	0.92	1.21	1.40	1.11	translation initiation factor IF-3 protein
TGGT1_265020	-0.13		0.98	1.10	0.10	0.12	1.11	putative arabinogalactan protein
TGGT1_286630	0.74		0.42	0.59	1.49	1.57	1.11	redoxin domain-containing protein
TGGT1_305030	1.86		0.71	0.68	1.16	1.45	1.10	kinase, pfkB family protein
TGGT1_266372	-0.22		0.72	0.89	0.50	0.52	1.10	hypothetical protein
TGGT1_263730	3.02		0.38	0.70	0.71	0.79	1.10	FAD-dependent glycerol-3-phosphate dehydrogenase
TGGT1_207760	0.40		0.24	0.50	0.61	0.71	1.10	DnaJ C terminal region domain-containing protein
TGGT1_261075			0.40	0.46	1.11	0.99	1.10	hypothetical protein
TGGT1_291350	0.78		0.53	0.83	0.38	0.55	1.09	hypothetical protein
TGGT1_261690	0.84		0.67	0.81	1.00	1.02	1.09	hypothetical protein
TGGT1_237170	-2.25		0.14	0.48	0.99	1.02	1.09	hypothetical protein
TGGT1_310770	0.76		0.98	0.80	0.56	0.60	1.09	hypothetical protein
TGGT1_306320	0.76		0.35	0.89	1.01	1.13	1.08	Myb family DNA-binding domain-containing protein
TGGT1_411560	-0.66		0.68	0.82	0.43	0.59	1.08	hypothetical protein
TGGT1_200595	-3.05		0.48	0.42	0.84	1.24	1.08	hypothetical protein
TGGT1_261950	-0.08		0.41	0.70	0.72	0.76	1.08	ATP synthase beta subunit ATP-B
TGGT1_256990	0.61		0.47	0.64	0.75	0.91	1.08	glycyl-tRNA synthetase
TGGT1_202300	0.88		0.70	0.87	0.91	0.92	1.08	putative inosine triphosphate pyrophosphatase
TGGT1_226570	0.12		0.47	0.74	0.44	0.52	1.08	hypothetical protein
TGGT1_264040	0.72		0.68	0.89	1.08	0.96	1.08	hypothetical protein
TGGT1_228360	0.38		0.66	0.83	0.59	0.55	1.07	putative peptidyl-prolyl isomerase FKBP12
TGGT1_262935	-0.18		0.67	0.77	0.57	0.80	1.07	hypothetical protein
TGGT1_411400	2.55		0.57	0.97	0.34	1.09	1.07	hypothetical protein
TGGT1_204050	-0.37		0.97	1.04		0.09	1.07	subtilisin SUB1
TGGT1_286050				0.60	1.56	1.60	1.07	hypothetical protein
TGGT1_234340	0.75		0.95	0.86	0.56	0.61	1.07	hypothetical protein
TGGT1_285470	6.35		0.63	0.69	0.92	2.20	1.07	patched family protein
TGGT1_272460	0.15		0.54	0.58	0.51	0.83	1.07	hypothetical protein
TGGT1_202620	0.95		0.65	0.77	0.31	0.57	1.07	hypothetical protein
TGGT1_309590	0.96		0.99	1.00	0.38	0.46	1.07	rhoptry protein ROP1
TGGT1_314885	-0.10		0.86	0.85	-0.31	-0.22	1.07	hypothetical protein
TGGT1_230210	0.82		1.09	1.11	0.36	0.35	1.06	alveolin domain containing intermediate filament IMC10
TGGT1_225690	0.54		1.31	1.18			1.06	hypothetical protein
TGGT1_282180	0.30		0.78	0.89	0.47	0.41	1.06	hypothetical protein
TGGT1_228110	0.67		0.93	1.13	0.60	0.50	1.06	hypothetical protein
TGGT1_234450	0.80		0.56	0.58	1.09	1.31	1.06	ribosomal protein RPS15A
TGGT1_225050	-0.35			0.38	1.06	1.40	1.06	putative adenosylhomocysteinase
TGGT1_411160	-0.28		1.17	1.32	0.44	0.23	1.06	putative toxoplasma gondii family C protein

TGGT1_259090	0.12		0.77	0.89	0.22	0.24	1.05	ubiquitin-conjugating enzyme subfamily protein
TGGT1_209710	0.55		0.35	0.65	1.16	1.13	1.05	ribosomal protein RPL28
TGGT1_307605	0.64		0.37	0.69	1.15	1.25	1.05	hypothetical protein
TGGT1_278160	0.41		0.55	0.83	1.20	1.46	1.05	vesicle transport v-snare protein
TGGT1_313710	0.39		0.38	0.58	1.08	1.55	1.05	hypothetical protein
TGGT1_293650	1.35		0.35	1.00	1.42	1.25	1.05	hypothetical protein
TGGT1_313690	1.14		0.62	0.73	0.92	1.07	1.05	Sel1 repeat-containing protein
TGGT1_410470	0.35		1.06	1.16	0.46	0.74	1.05	tRNA-Tyr
TGGT1_314415	0.12		0.91	1.13	0.42	0.43	1.05	hypothetical protein
TGGT1_245752	-2.24		0.98	0.93	0.44	0.66	1.05	WD domain, G-beta repeat-containing protein
TGGT1_306330	0.60		0.75	0.90	0.42	0.61	1.05	phospholipase
TGGT1_224210	1.02		0.61	0.90	0.72	0.81	1.04	hypothetical protein
TGGT1_285840	-0.55		0.18	0.66	0.29	0.42	1.04	RAP domain-containing protein
TGGT1_244670	0.78		0.10	0.52	0.64	0.68	1.03	hypothetical protein
TGGT1_260180	0.68		0.61	0.75	0.74	0.80	1.03	hypothetical protein
TGGT1_209820	-0.21		1.06	0.93	0.74	0.54	1.03	syntaxin protein
TGGT1_286450	0.66		0.71	0.79	0.74	1.02	1.03	dense granule protein GRA5
TGGT1_321590	-0.13		0.32	0.63	1.14	1.25	1.03	hypothetical protein
TGGT1_269438	0.16		1.13	1.10	0.22	0.17	1.03	hypothetical protein
TGGT1_206430	-1.13		0.73	0.75	-0.82	-0.38	1.02	formin FRM1
TGGT1_291940	0.63		0.66	0.64	1.08	1.29	1.02	hypothetical protein
TGGT1_275440	0.33		0.72	0.79	0.58	0.88	1.02	dense granule protein GRA6
TGGT1_227030	1.05		0.69	0.75	1.17	1.11	1.02	hypothetical protein
TGGT1_236990	1.78		0.69	0.64	0.55	1.06	1.02	beta-ketoacyl synthase, N-terminal domain-containing protein
TGGT1_311460	0.07		0.33	0.52	0.73	0.98	1.02	hypothetical protein
TGGT1_233470	0.52		0.95	0.23	0.46	0.68	1.02	hypothetical protein
TGGT1_257380	0.66		0.61	0.70	0.55	0.62	1.02	hypothetical protein
TGGT1_243570	0.29		0.68	0.78	0.72	0.87	1.02	ribosomal protein RPS26
TGGT1_263720	0.61		0.98	0.82	0.96	1.20	1.02	HMG (high mobility group) box domain-containing protein
TGGT1_216435	0.54		0.80	0.93	0.63	0.69	1.01	hypothetical protein
TGGT1_297060	0.46		0.50	0.73	1.04	1.07	1.01	phosphoglycerate mutase PGMII
TGGT1_211680	0.95		0.38	0.58	1.37	1.27	1.01	protein disulfide isomerase
TGGT1_214220	0.06		0.68	0.46	0.61	1.24	1.01	hypothetical protein
TGGT1_263633	-0.31		0.97	0.97	-0.55	-0.29	1.01	hypothetical protein
TGGT1_242570	0.97		0.81	0.83	0.54	0.74	1.01	hypothetical protein
TGGT1_203790			0.69	0.84	0.27	0.69	1.00	hypothetical protein
TGGT1_230340	0.88		1.16	1.13	0.26	0.29	1.00	hypothetical protein
TGGT1_298040	0.59		0.65	0.68	0.90	1.02	1.00	hypothetical protein
TGGT1_240840	1.03		0.57	0.56	1.00	1.01	0.99	histone lysine demethylase JmjC NO66
TGGT1_263630	0.13		1.04	1.16	0.36	0.29	0.99	hypothetical protein
TGGT1_284040	0.23		0.32	0.48	0.79	1.03	0.98	hypothetical protein
TGGT1_231630	0.88		1.01	0.97	0.40	0.20	0.97	alveolin domain containing intermediate filament IMC4
TGGT1_201840	1.27		0.56	0.46	1.16	1.70	0.97	aspartyl protease ASP1
TGGT1_275330	0.10		1.04	0.97	0.34	0.42	0.97	ribosomal protein RPL29
TGGT1_239600	0.76		0.13	0.28	0.94	1.65	0.97	rhoptry kinase family protein ROP23 (incomplete catalytic triad)
TGGT1_313380	1.03		1.12	1.05	0.57	0.44	0.96	hypothetical protein
TGGT1_293680	1.14		0.64	0.78	1.10	1.22	0.96	hypothetical protein
TGGT1_233870	-1.24		0.36	0.27	0.72	1.07	0.96	hypothetical protein
TGGT1_200340	0.98		0.96	1.09	0.55	0.36	0.96	hypothetical protein
TGGT1_216000	0.58		1.14	1.11	0.15	0.06	0.95	alveolin domain containing intermediate filament IMC3
TGGT1_299990	0.52		1.19	0.85	0.15	0.20	0.95	archease family protein
TGGT1_229140	0.31		0.88	1.04	0.41	0.26	0.95	MaoC family domain-containing protein
TGGT1_297880	-0.34		0.26	0.10	0.87	1.19	0.95	dense granule protein DG32
TGGT1_251930	0.36		-0.23	-0.16	2.05	1.52	0.95	enoyl-acyl carrier reductase ENR
TGGT1_275690	0.26		0.25	0.40	1.03	1.36	0.94	putative ClpB
TGGT1_225250	-0.40		0.83	1.02	0.29	0.22	0.94	putative LSU ribosomal protein L14P
TGGT1_230510	0.14		0.17	0.33	1.13	1.10	0.94	hypothetical protein
TGGT1_237880	-0.48		0.05	0.09	1.12	1.26	0.93	hypothetical protein
TGGT1_314730	0.64		0.21	0.45	0.96	1.11	0.93	ALG6, ALG8 glycosyltransferase family protein
TGGT1_312500	-1.13		0.09	0.30	0.71	1.06	0.93	hypothetical protein
TGGT1_254620	0.69		0.62	0.59	1.07	1.35	0.93	ribosomal protein RPL39
TGGT1_220270	0.76		1.02	1.02	0.47	0.37	0.92	alveolin domain containing intermediate filament IMC6
TGGT1_266620	0.71		0.30	0.56	1.08	1.14	0.92	thioredoxin domain-containing protein
TGGT1_264220	0.56		0.35	0.38	1.03	1.14	0.92	hypothetical protein
TGGT1_213069	-0.49		1.03	1.12	0.18	0.11	0.92	hypothetical protein
TGGT1_266300	1.03		1.14	0.89	0.99	0.31	0.91	hypothetical protein
TGGT1_411270	2.60		0.47	0.12	0.48	1.18	0.91	hypothetical protein
TGGT1_318750	0.24		0.57	0.67	1.07	1.39	0.91	deoxyribose-phosphate aldolase
TGGT1_248530	-0.12		0.36	0.50	0.83	1.19	0.90	FATC domain-containing protein
TGGT1_315130	0.94		0.65	0.80	0.84	1.01	0.89	L-isoaspartyl protein carboxyl methyltransferase family protein
TGGT1_263300	0.95		0.42	0.59	1.16	1.16	0.88	eukaryotic porin protein
TGGT1_222340	-1.52		0.47	1.16	0.62	0.94	0.87	NOL1/NOP2/sun family protein
TGGT1_214350	1.36		0.07	0.34	1.47	1.40	0.87	putative GTP-binding protein
TGGT1_209060	-0.39		0.39	0.51	0.81	1.00	0.87	thrombospondin type 1 domain-containing protein
TGGT1_218780	0.97		0.26	0.54	1.00	0.90	0.87	putative phosphoserine aminotransferase
TGGT1_244300			0.14	0.11	1.01	1.06	0.87	hypothetical protein
TGGT1_314310	1.45		0.45	0.71	1.04	1.16	0.87	hypothetical protein
TGGT1_240580	-0.84		0.27	0.46	1.15	1.16	0.87	hypothetical protein
TGGT1_204460			1.58	0.68	0.12	1.76	0.86	putative hydroxymethylglutaryl-CoA lyase
TGGT1_246995	0.23		0.32	0.68	0.95	1.65	0.86	hypothetical protein
TGGT1_285425	0.37		1.23	0.93	0.32	0.31	0.86	hypothetical protein
TGGT1_243510	0.79		0.35	0.53	1.09	1.02	0.85	OTU family cysteine protease
TGGT1_246100	0.47		0.56	0.69	1.01	1.03	0.85	putative phosducin
TGGT1_297270	0.28		0.11	0.36	1.12	1.16	0.84	hypothetical protein
TGGT1_207865	2.61		0.62	1.12	1.57	3.17	0.84	GCC2 and GCC3 domain-containing protein
TGGT1_320620	-0.92		0.41	0.48	1.01	1.17	0.83	queuine tRNA ribosyl transferase
TGGT1_215540	-3.31		0.31	0.45	1.23	1.19	0.83	hypothetical protein

TGGT1_306895	0.52		1.05	0.97	0.19		0.83	hypothetical protein
TGGT1_208050	-0.27		-0.31	0.36	0.56	1.03	0.82	putative ABC transporter
TGGT1_409790	-0.11		0.84	1.02	-0.10	-0.16	0.81	putative transmembrane protein
TGGT1_297910	0.95		1.05	0.56	0.35	0.99	0.81	hypothetical protein
TGGT1_220720	6.62		1.00	1.24	0.94	0.70	0.80	hypothetical protein
TGGT1_249590	0.78		0.36	0.50	1.05	0.94	0.80	putative proteasome subunit alpha type 5-2
TGGT1_261580	0.30		0.98	0.66	1.10	1.87	0.79	histone H2AX
TGGT1_202440	1.03		-0.11	0.69	1.40	1.61	0.78	hypothetical protein
TGGT1_259550	-0.05		-0.18	0.19	0.98	1.17	0.78	dihydropteroate synthase
TGGT1_409020			1.01	0.86	-0.10	-0.14	0.76	hypothetical protein
TGGT1_309210	0.67		0.24	0.31	1.14	1.18	0.75	putative peroxiredoxin 6
TGGT1_261070			-0.45	-0.12	1.28	1.23	0.75	apicoplast triosephosphate translocator APT1
TGGT1_321570	0.55		0.46	0.66	0.94	1.10	0.73	beta-hydroxyacyl-acyl carrier protein dehydratase (FABZ)
TGGT1_226515	0.62		1.18	0.96	-0.03	-0.03	0.73	hypothetical protein
TGGT1_301420	1.03		1.00	0.91	0.54	0.27	0.72	hypothetical protein
TGGT1_232770	1.35		0.99	1.14	-0.30	-0.63	0.72	hypothetical protein
TGGT1_281470	1.30		1.73	1.32	-0.57	0.56	0.71	hypothetical protein
TGGT1_289740	0.97		0.05	0.15	0.89	1.38	0.70	hypothetical protein
TGGT1_304450	-5.42		0.31	0.33	0.89	1.22	0.70	cation-transporting ATPase
TGGT1_356950	1.21		2.10	0.78	-0.93	-0.11	0.69	hypothetical protein
TGGT1_300120	-0.32		-0.43	0.15	0.80	1.37	0.68	aminotransferase, class V superfamily protein
TGGT1_295730	0.62		-0.37	0.23	1.03	1.11	0.66	tetratricopeptide repeat-containing protein
TGGT1_205558	0.54		0.22	0.24	0.98	1.04	0.65	NAC domain-containing protein
TGGT1_226410	0.98		-0.14	0.16	1.11	1.01	0.65	EF-1 guanine nucleotide exchange domain-containing protein
TGGT1_319890	-0.05		0.24	1.01	1.43	3.87	0.64	hypothetical protein
TGGT1_249000	0.30		1.21	0.69	-0.13		0.64	regulator of chromosome condensation (RCC1) repeat-containing protein
TGGT1_222160	1.33		-0.25		1.15	1.33	0.64	aldehyde dehydrogenase
TGGT1_245440	1.03		1.19	0.70	-0.09	-0.21	0.63	hypothetical protein
TGGT1_271320			0.66	0.63	0.79	1.11	0.63	hypothetical protein
TGGT1_239770	1.34		0.36	0.51	0.99	1.02	0.62	alveolin domain containing intermediate filament IMC11
TGGT1_239100	0.29		0.23	0.29	0.88	1.02	0.61	ribosomal protein RPS7
TGGT1_261790	0.16		1.03	0.60	-0.07	-0.06	0.61	hypothetical protein
TGGT1_255210	4.09		0.25	0.18	1.50	1.39	0.60	ATPase, AAA family protein
TGGT1_233153	0.45		0.35	0.45	0.91	1.33	0.60	hypothetical protein
TGGT1_218910	1.45		0.43	1.08	0.75	0.40	0.60	hypothetical protein
TGGT1_223258	-0.82		-0.04	0.37	0.67	1.16	0.60	hypothetical protein
TGGT1_269780	0.84			0.24	0.82	1.05	0.60	putative ADP-ribosylation factor
TGGT1_310380	0.65		1.35	0.72	-0.06	-0.29	0.58	brix domain containing protein
TGGT1_212290	0.11			0.14	0.74	1.01	0.58	ribosomal protein RPS19
TGGT1_208560	-0.69		-0.26	-0.03	0.67	1.00	0.57	carrier superfamily protein
TGGT1_299570	1.20		1.18	0.68	-0.16	0.27	0.56	Toxoplasma gondii family B protein
TGGT1_209700	0.61		0.17	0.13	0.90	1.20	0.56	hypothetical protein
TGGT1_222220	0.38			0.19	0.82	1.04	0.56	alveolin domain containing intermediate filament IMC7
TGGT1_297960A	0.29		1.09	0.90	-0.24	-0.45	0.56	rhoptry neck protein RON6
TGGT1_221295	0.74		0.46	0.51	0.84	1.06	0.55	hypothetical protein
TGGT1_269588	0.16		1.08	0.68	-0.31	-0.47	0.54	hypothetical protein
TGGT1_317705	1.39		-0.25	0.07	1.05	1.13	0.54	enoyl-CoA hydratase/isomerase family protein
TGGT1_224540	-0.51		1.01	0.88	-0.61	-0.49	0.54	hypothetical protein
TGGT1_232300	0.36		0.12	0.17	0.82	1.05	0.52	ribosomal protein RPS3
TGGT1_295350	0.24		0.05	0.10	0.93	1.15	0.51	putative nucleoside diphosphate kinase
TGGT1_260190	0.24		0.06	0.49	0.77	1.30	0.51	microneme protein MIC13
TGGT1_236800	0.44		0.15	0.40	1.09	0.95	0.51	hypothetical protein
TGGT1_287460	3.20		-0.18	-0.74	0.32	1.20	0.50	hypothetical protein
TGGT1_411170	-0.73		1.03	0.86	-0.76	-0.77	0.48	hypothetical protein
TGGT1_305520	0.49		0.21	0.08	0.68	1.09	0.48	ribosomal protein RPS2
TGGT1_260530	2.56		1.86	0.90	0.10	0.48	0.47	Sel1 repeat-containing protein
TGGT1_218850	-0.50		-0.19	0.02	0.75	1.07	0.47	ribosomal protein RPS9
TGGT1_240310B	0.26		1.14	0.56	-0.39	0.58	0.46	Toxoplasma gondii family E protein
TGGT1_313780	0.18		1.08	0.79	-0.72	-0.92	0.45	hypothetical protein
TGGT1_285870	0.33		0.33	0.33	0.89	1.04	0.42	SAG-related sequence SRS20A
TGGT1_269582	0.33		1.09	0.61	-0.31	-0.42	0.42	hypothetical protein
TGGT1_208850	-1.92		-0.27	-0.03	1.11	1.62	0.41	SAG-related sequence SRS11
TGGT1_286420B	0.72		-0.56	-0.20	1.16	1.43	0.41	putative elongation factor 1-alpha (EF-1-ALPHA)
TGGT1_289350	4.60		0.03	0.13	0.54	2.04	0.41	ATP-binding cassette G family transporter ABCG84
TGGT1_234550	0.37		0.21	0.16	0.78	1.04	0.38	ribosomal protein RPL29
TGGT1_311335	3.79		0.27	1.14	1.86	0.10	0.36	dual specificity phosphatase, catalytic domain-containing protein
TGGT1_224310	0.53		-0.20		0.51	1.07	0.35	DHHC zinc finger domain-containing protein
TGGT1_216285	0.45			0.67	1.11	0.74	0.35	hypothetical protein
TGGT1_204325	0.61		1.19	0.97	-0.19	-0.62	0.34	hypothetical protein
TGGT1_312090	0.13		0.05		0.89	1.02	0.33	ribosomal protein RPL23
TGGT1_307270	1.43		-0.34		1.00	1.30	0.32	hypothetical protein
TGGT1_286770	1.89		-0.09	0.39	1.53	1.75	0.32	hypothetical protein
TGGT1_291850	0.49				0.90	1.10	0.30	ribosomal protein RPS30
TGGT1_285400	-0.26		-0.11	0.41	1.19	1.27	0.30	hypothetical protein
TGGT1_308960	0.53		1.23	0.29	-0.83	-0.48	0.30	ATPase, AAA family protein
TGGT1_263530			-0.33	-0.11	0.85	1.02	0.29	putative chaperonin
TGGT1_248390	0.28		-0.05		0.92	1.08	0.28	ribosomal protein RPL26
TGGT1_307260	1.10		-0.10	0.08	0.81	1.01	0.27	Toxoplasma gondii family C protein
TGGT1_288860	0.74		-0.17		1.63	0.70	0.27	RuvB family 2 protein
TGGT1_307030	0.92		0.39	-0.46	0.39	1.29	0.24	Purine nucleoside phosphorylase
TGGT1_305040	3.05		-0.38	0.15	0.89	2.37	0.24	HEAT repeat-containing protein
TGGT1_408550	1.58		1.20	0.46	-0.12	0.53	0.23	hypothetical protein
TGGT1_215343	2.42		1.51	0.62	0.19	0.78	0.20	hypothetical protein
TGGT1_410250	-0.56		1.35	0.65	-0.72	-0.36	0.16	hypothetical protein
TGGT1_252385	1.36		1.05	0.18	-0.11	-0.24	0.15	hypothetical protein
TGGT1_259060	0.93		1.09	0.38	0.21	-0.77	0.15	hypothetical protein
TGGT1_410160	1.50				1.19	0.78	0.14	hypothetical protein

TGGT1_362290	1.02		0.25	0.70	1.13	0.38	0.12	hypothetical protein
TGGT1_233350	0.42		-0.39	0.12	1.21	0.79	0.11	putative nuclear transport factor 2
TGGT1_307640	2.06		1.80	1.05	0.30	-0.63	-0.10	CMGC kinase, CK2 family
TGGT1_233490	0.88		1.06	0.03	-0.56	-0.34	0.09	hypothetical protein
TGGT1_205430	-0.33		1.58	0.71	-0.57	1.21	0.09	isovaleryl-CoA dehydrogenase
TGGT1_207610	0.55		1.13		-0.94	-0.67	0.09	rhoptry kinase family protein ROP36 (incomplete catalytic triad)
TGGT1_297680	0.56		1.49	0.59	-0.41	-0.80	-0.08	putative calmodulin
TGGT1_269680	0.35		1.04	0.14	-0.65		-0.08	putative acyl-CoA carboxyltransferase beta chain
TGGT1_221818	-0.23		1.17	0.27	-0.40	-0.51	-0.08	hypothetical protein
TGGT1_260260	0.78		-0.44	-0.44	0.99	1.17	-0.08	ribosomal protein RPP1
TGGT1_239730	0.69		1.41	0.39	-0.98	-1.16	-0.09	MORN repeat-containing protein
TGGT1_310480	-0.26		1.15	0.09	-1.14	-0.98	-0.10	flagellar/basal body protein, PACRG family protein
TGGT1_314460	0.49		1.62	0.37	0.35	-0.20	-0.15	hypothetical protein
TGGT1_266070	0.35		-0.53	-0.50	0.98	1.04	-0.17	ribosomal protein RPL31
TGGT1_255215	3.14		-0.57	-0.37	1.57	1.34	-0.18	hypothetical protein
TGGT1_210400			1.54	0.36	-1.02		-0.26	hypothetical protein
TGGT1_278110	4.66		-0.62	-0.16	1.19	1.46	-0.27	1,3-beta-glucan synthase component protein
TGGT1_410820	-1.43		1.05	0.03	-1.41	-1.78	-0.41	tRNA-Ser
TGGT1_208810			1.13	0.46	-0.39	0.52	-0.42	hypothetical protein
TGGT1_314670	0.65		1.36		-1.20	-0.67	-0.55	hypothetical protein
TGGT1_244500			0.51	0.13	-0.56	-1.00	-0.09	Tubulin-tyrosine ligase family protein
TGGT1_244050	-0.36		0.56	0.08	-0.86	-1.13	-0.29	hypothetical protein
TGGT1_251740	0.37		0.26	0.12	-0.95	-1.10	-0.35	AP2 domain transcription factor AP2XII-9
TGGT1_246190	-0.91		0.35	0.15	-1.02	-0.72	-0.35	hypothetical protein
TGGT1_248240			0.97	0.20	-1.15	-0.48	-0.38	leucine rich repeat-containing protein
TGGT1_271070	0.67		-1.50	-0.30	0.63	0.88	-0.38	hypothetical protein
TGGT1_312430	-0.95		-0.17		-0.67	-1.01	-0.40	hypothetical protein
TGGT1_291830	0.25		0.32	-0.50	-1.24	-0.39	-0.40	putative DNA double-strand break repair rad50 ATPase
TGGT1_276230	-0.17		-1.07	-0.53	0.43	0.12	-0.43	Toxoplasma gondii family B protein
TGGT1_261300					-0.92	-1.15	-0.53	hypothetical protein
TGGT1_295620	-0.44			-0.32	-0.96	-1.00	-0.55	hypothetical protein
TGGT1_228680	-0.40		0.21	-0.36	-1.02	-1.02	-0.55	mitochondrial carrier superfamily protein
TGGT1_252370	-0.31		0.77	0.16	-1.32	0.22	-0.60	AP2 domain transcription factor AP2III-1
TGGT1_306020	-0.28			-0.20	-1.01	-0.82	-0.61	hypothetical protein
TGGT1_245520	0.35		-1.10	-0.95	0.34	0.33	-0.68	hypothetical protein
TGGT1_271000	-0.19		0.22	-0.16	-0.81	-1.14	-0.68	hypothetical protein
TGGT1_205370	0.10		0.13	-0.16	-0.94	-1.15	-0.69	hypothetical protein
TGGT1_277050	-1.19			-0.50	-0.97	-1.05	-0.71	hypothetical protein
TGGT1_304730	0.45		0.51	-0.30	-1.05	-1.29	-0.71	Mob1/phocein family protein
TGGT1_220930	-0.67			-0.56	-0.80	-1.16	-0.72	hypothetical protein
TGGT1_280770	-0.62		-0.29	-0.67	-0.48	-1.15	-0.73	regulator of chromosome condensation (RCC1) repeat-containing protein
TGGT1_257360	-0.33		0.61	0.14	-1.12	-1.21	-0.73	hypothetical protein
TGGT1_239810	-1.35		-0.55	-0.54	-0.82	-1.00	-0.73	hypothetical protein
TGGT1_308010	-0.93			-0.26	-0.94	-1.18	-0.75	hypothetical protein
TGGT1_319600	-0.32		0.11	-0.25	-0.91	-1.25	-0.75	putative alpha-tubulin N-acetyltransferase
TGGT1_213570	-0.57		-1.10	-1.00			-0.76	hypothetical protein
TGGT1_318470	-0.05		0.68	-0.32	-1.28	-1.41	-0.76	AP2 domain transcription factor AP2IV-4
TGGT1_212780	-0.28		-0.16	-0.58	-0.83	-1.15	-0.76	hypothetical protein
TGGT1_309810	0.55		-0.99	-1.02	0.61	0.73	-0.76	ribosomal protein RPP2
TGGT1_318632	-0.14		-1.14	-1.07	0.14	0.15	-0.76	hypothetical protein
TGGT1_310740			0.08	-0.26	-0.73	-1.03	-0.77	hypothetical protein
TGGT1_205360	-0.46		0.10	-0.63	-1.06	-0.94	-0.79	hypothetical protein
TGGT1_226650	0.26		-1.01	-0.71	0.16	0.08	-0.79	hypothetical protein
TGGT1_279345	-0.19		-1.10	-0.96	0.47	0.39	-0.79	hypothetical protein
TGGT1_270760	-0.51		-0.17	-0.33	-0.65	-1.09	-0.79	asparagine synthase
TGGT1_221250			-0.12	-0.54	-0.69	-1.08	-0.79	hypothetical protein
TGGT1_252450	-0.80		0.57	0.31	-0.96	-1.52	-0.80	hypothetical protein
TGGT1_238410	-0.94		0.32	-0.47	-1.16	-1.23	-0.81	hypothetical protein
TGGT1_278390	1.22		-0.74	-1.02	-0.26	0.39	-0.81	Toxoplasma gondii family A protein
TGGT1_293050	0.35		-1.21	-1.05			-0.82	sybindin family protein
TGGT1_295460	0.10		-1.02	-0.76	0.03	-0.34	-0.83	Got1 family protein
TGGT1_221850	0.15		-0.92	-1.04	0.10	0.12	-0.84	putative prohibitin family protein
TGGT1_200230	0.17		0.07	-1.28	-0.63	1.39	-0.84	microneme protein MIC17C
TGGT1_360740	-0.94		0.03	-0.32	-0.80	-1.24	-0.84	hypothetical protein
TGGT1_315580	-0.22			-0.31	-0.76	-1.25	-0.85	hypothetical protein
TGGT1_209000	-0.82		0.00	-0.33	-1.39	-1.07	-0.85	HECT-domain (ubiquitin-transferase) domain-containing protein
TGGT1_306620	0.23			-0.46	-0.86	-1.24	-0.85	AP2 domain transcription factor AP2IX-9
TGGT1_250760	-0.83		-0.35	-0.47	-0.85	-1.10	-0.85	pentatricopeptide repeat domain-containing protein
TGGT1_210345	-0.40		0.06	-0.45	-1.14	-1.00	-0.86	hypothetical protein
TGGT1_222330	0.05		0.21	-0.26	-1.14	-1.41	-0.87	hypothetical protein
TGGT1_205340	0.05		-1.00	-1.07	0.42	0.46	-0.87	ribosomal protein RPS12
TGGT1_215390	-0.29		-1.04	-0.92	0.74	0.58	-0.88	putative TIM10 family protein
TGGT1_291170	-0.38		0.09	-0.45	-1.15	-1.05	-0.91	hypothetical protein
TGGT1_269460	-0.41		-0.22	-0.36	-0.79	-1.13	-0.91	Ser/Thr phosphatase family protein
TGGT1_243910	-0.98		-1.08	-0.85	-0.27	-0.39	-0.92	Cof family hydrolase subfamily protein
TGGT1_263120	-1.31		-0.19	-0.28	-0.99	-1.30	-0.92	hypothetical protein
TGGT1_244130	-1.44		-0.43	-0.58	-1.01	-1.13	-0.92	hypothetical protein
TGGT1_409310	-1.09		-1.05	-1.16	-0.38	0.10	-0.92	tRNA-Met
TGGT1_213560	-0.49			-0.87	-1.62	-0.75	-0.92	hypothetical protein
TGGT1_273530	-0.59		-1.11	-1.03	-0.32	-0.17	-0.93	flagellar associated protein
TGGT1_309080	-0.65			-0.44	-0.97	-1.08	-0.93	hypothetical protein
TGGT1_261220	-0.36		-0.99	-1.05	-0.17	-0.20	-0.96	transcription elongation factor SPT4
TGGT1_233720	0.33		-1.39	-1.20	0.18	0.09	-0.96	DNA-directed RNA polymerase II RPBABC8
TGGT1_322100	-1.51		-0.82	-1.04	0.07	0.19	-0.98	myosin-light-chain kinase
TGGT1_408250	1.11		0.15	0.15	-0.45	-1.10	-0.99	hypothetical protein
TGGT1_232320	-1.90		-0.84	-0.63	-1.01	-1.19	-1.00	hypothetical protein
TGGT1_267360	-0.60		-0.19	-0.84	-0.84	-0.78	-1.00	histone deacetylase

TGGT1_273620			-1.10	-0.98	0.15	0.12	-1.00	hypothetical protein
TGGT1_207875	-0.64		-0.46	-0.58		0.20	-1.00	GCC2 and GCC3 domain-containing protein
TGGT1_294690	-0.28		-0.66	-0.80	-0.55	-0.73	-1.01	rhomboid protease ROM5
TGGT1_251490	-0.87		-0.75	-0.72	-0.54	-0.51	-1.01	ACR, COG2135 domain-containing protein
TGGT1_216610	-0.63		-0.87	-0.64		0.66	-1.01	ribosomal RNA large subunit methyltransferase J protein
TGGT1_228175	-0.22		0.63	-0.41	-1.48	-1.27	-1.01	hypothetical protein
TGGT1_245630	-0.17		-0.64	-0.75	-0.15	-0.32	-1.01	hypothetical protein
TGGT1_243770			-1.06	-0.84		-0.10	-1.02	hypothetical protein
TGGT1_409250	-0.44		0.29	-0.17	-1.24	-1.00	-1.02	hypothetical protein
TGGT1_315260	0.60		-0.35	-0.59	-0.48	-0.37	-1.02	alanine dehydrogenase
TGGT1_286190	-0.54		-0.91	-0.91	-0.72	-0.70	-1.02	hypothetical protein
TGGT1_221410	-0.60		-0.77	-0.97	-0.33	-0.30	-1.02	actin-like protein ALP4
TGGT1_297643	0.64		-0.19	-0.32	-0.11	-0.35	-1.02	hypothetical protein
TGGT1_266980	0.23		-0.81	-0.61	0.20	-0.39	-1.02	hypothetical protein
TGGT1_357490	0.37		-0.78	-0.97		-0.19	-1.02	hypothetical protein
TGGT1_269630	-1.54		-0.71	-0.72	-0.87	-0.90	-1.03	hypothetical protein
TGGT1_315798	-0.51		-0.62	-0.19		1.04	-1.03	hypothetical protein
TGGT1_249380	-1.56		-0.66	-0.60	-0.96	-1.15	-1.03	DHHC zinc finger domain-containing protein
TGGT1_229910	0.41		-1.16	-1.01	-0.19	-0.33	-1.03	EF hand domain-containing protein
TGGT1_271060	-0.82		-0.71	-0.83	-0.45	-0.68	-1.03	Sec1 family protein
TGGT1_280540	-0.98		0.19	-0.40	-0.89	-1.12	-1.03	HEAT repeat-containing protein
TGGT1_233960	-0.85		-0.47	-0.15	-0.42	1.33	-1.03	hypothetical protein
TGGT1_208722	-0.38		-1.05	-1.04	-0.44	-0.58	-1.03	hypothetical protein
TGGT1_280510	-0.59			-0.65	-0.45	-0.56	-1.04	Dpy-30 motif protein
TGGT1_272630	0.38		-1.24	-1.01	0.32	0.15	-1.04	putative U6 snRNA-associated Sm family protein LSm8
TGGT1_293290	0.58		0.19	-0.80	-0.79	-0.65	-1.04	hypothetical protein
TGGT1_308050	-0.22		-0.84	-0.96		-0.30	-1.04	thioredoxin domain-containing protein
TGGT1_229260	-0.34		0.10	-0.64	-1.35	-1.63	-1.04	hypothetical protein
TGGT1_274040	1.69		0.37	-0.39		1.96	-1.04	hypothetical protein
TGGT1_356400	2.60		0.78	-0.75	-1.94	0.49	-1.05	cAMP-dependent protein kinase
TGGT1_231230	-0.97		-0.81	-0.87	-0.79	-0.99	-1.05	hypothetical protein
TGGT1_242100	-0.34		-0.21	-0.68	0.12	0.42	-1.05	hypothetical protein
TGGT1_321360	-0.42		-0.88	-0.74	-0.35	-0.60	-1.05	clustered-asparagine-rich protein
TGGT1_248350				-0.50	-0.66	-0.65	-1.05	hypothetical protein
TGGT1_268860	-0.72		1.18	-0.19	-1.49	-1.80	-1.05	enolase 1
TGGT1_261260A	-1.08		-0.80	-0.86	-0.68	-0.80	-1.05	histone lysine demethylase JMJD6a
TGGT1_218955	-0.55		-0.92	-1.04	-0.53	-0.50	-1.06	hypothetical protein
TGGT1_267435	-1.94		-0.24	-0.29	-0.88	-0.32	-1.06	hypothetical protein
TGGT1_251750	-0.13		0.79	-0.46	-1.55	-0.13	-1.06	MORN repeat-containing protein
TGGT1_268350	-1.59		-0.87	-0.72	-0.61	-0.82	-1.06	hypothetical protein
TGGT1_232140	0.73		-0.27	-0.29	-0.62	-0.23	-1.06	hypothetical protein
TGGT1_321390	-0.20		0.17	-0.47	-0.66	-0.33	-1.07	hypothetical protein
TGGT1_247340	0.64		0.16	-0.56	-0.85	-0.26	-1.07	hypothetical protein
TGGT1_285825	0.42		-0.79	-0.58	-0.22	-0.64	-1.08	hypothetical protein
TGGT1_203175	-0.15		-0.48	-0.81	-0.67	-0.55	-1.08	hypothetical protein
TGGT1_299800	0.64		0.56	-0.19	-1.19	-1.24	-1.08	hypothetical protein
TGGT1_222188	0.74		-0.46	-0.78	-0.91		-1.09	hypothetical protein
TGGT1_202850	-0.75		-0.39	-0.79	-0.85	-1.08	-1.09	ATP-binding domain-containing protein
TGGT1_246910	-0.40		-0.95	-0.77	-0.29	-0.26	-1.09	putative histone lysine methyltransferase, SET
TGGT1_318420	0.44		-1.29	-1.01			-1.09	putative 30S ribosomal protein S16
TGGT1_269310	0.57		-0.42	-0.76	-0.77	-0.22	-1.09	hypothetical protein
TGGT1_227630			0.32	-0.69	-1.35	-1.01	-1.09	hypothetical protein
TGGT1_237210	-0.79		-0.28	-0.65	-0.93	-1.18	-1.09	Tyrosine kinase-like (TKL) protein
TGGT1_205170	-0.79		-0.98	-0.96	-0.30	-0.35	-1.09	hypothetical protein
TGGT1_219370	-1.72		-0.26	-0.45		0.27	-1.09	hypothetical protein
TGGT1_273750	-0.25		-0.78	-1.29	-0.22	0.66	-1.09	hypothetical protein
TGGT1_289490	-0.36		-0.77	-0.97	-0.89	-1.05	-1.10	serine/threonine specific protein phosphatase
TGGT1_294820	0.70		-0.76	-0.50	-0.56	1.40	-1.10	putative type I fatty acid synthase
TGGT1_206520	0.13		-0.75	-0.75	-0.37	-0.37	-1.11	hypothetical protein
TGGT1_250030	-0.91		-0.65	-0.79	-0.34	-0.59	-1.11	hypothetical protein
TGGT1_213580	0.51		-1.62	-1.48	1.03	0.94	-1.11	hypothetical protein
TGGT1_209985	2.40		0.78	-0.67	-1.98	0.30	-1.12	cAMP-dependent protein kinase
TGGT1_208760	0.47		-0.36	-0.74	-0.54	-0.64	-1.12	hypothetical protein
TGGT1_462964	0.10		-0.30	-0.62	-0.94	-1.45	-1.12	hypothetical protein
TGGT1_312680			-1.16	-1.07		-0.15	-1.12	putative 60S ribosomal protein L27
TGGT1_261955B			-0.35	-0.84	-0.73	-0.91	-1.12	hypothetical protein
TGGT1_227980	0.32		-0.94	-0.93	-0.35	-0.39	-1.12	hypothetical protein
TGGT1_263110	-1.34		-0.27	-0.37	-0.79	-1.22	-1.13	hypothetical protein
TGGT1_267030	-2.19		-0.64	-0.38	-0.43	0.17	-1.13	ribonuclease type III Dicer
TGGT1_220580	-0.43		-1.16	-1.15	-0.23	-0.35	-1.13	hypothetical protein
TGGT1_261610	-1.89		-0.81	-0.70	-0.40	-0.38	-1.13	hypothetical protein
TGGT1_232240	-1.38		-0.72	-0.57	-0.89	-0.75	-1.13	hypothetical protein
TGGT1_236400	-1.07		-0.79	-0.79	-0.27	-0.16	-1.13	hypothetical protein
TGGT1_316670			-0.23	-0.51	-0.91	-0.73	-1.13	Toxoplasma gondii family D protein
TGGT1_238970	-0.70		-0.50	-0.35	-0.38	1.98	-1.13	hypothetical protein
TGGT1_239290	-2.20		-0.66	-0.36	-0.17	-0.59	-1.14	hypothetical protein
TGGT1_205230			-0.63	-0.60	-0.99	-0.51	-1.14	hypothetical protein
TGGT1_243382	0.24		-0.57	-0.20	-0.38	0.68	-1.14	hypothetical protein
TGGT1_277070	-1.04		-0.59	-0.63	-0.99	-1.25	-1.14	SWI2/SNF2-containing protein
TGGT1_272680	-0.19		-0.30	-0.54	-0.60	-0.87	-1.14	hypothetical protein
TGGT1_306050	0.31		-0.48	-0.60	-0.72	-0.80	-1.15	hypothetical protein
TGGT1_209040	-0.17			-0.71	-1.00	-0.83	-1.15	hypothetical protein
TGGT1_264485	-0.16			-0.46	-1.25	-1.22	-1.15	AP2 domain transcription factor AP2IX-3
TGGT1_248330	-0.29		-0.41	-0.45	-0.54	-1.17	-1.15	zinc finger, C3HC4 type (RING finder) domain-containing protein
TGGT1_312930	0.86			-0.71	-0.82	-0.65	-1.15	putative cystathione gamma lyase
TGGT1_212910	0.23		-0.32			-0.51	-1.16	rhomboid protease ROM3
TGGT1_262825	-0.73		-0.21	-0.50	-1.29	-1.30	-1.16	peptidase family c50 protein

TGGT1_206650	-0.96		-0.64	-0.74	-0.78	-0.61	-1.16	zinc finger, c2h2 type domain-containing protein
TGGT1_271090	0.63		-0.40	-1.14	-0.76	-1.04	-1.17	hypothetical protein
TGGT1_250670	-0.28		-0.49	-0.68	-0.87	-0.98	-1.17	hypothetical protein
TGGT1_223025	-1.20		-0.77	-0.37	-0.32	-0.59	-1.17	hypothetical protein
TGGT1_236960	0.07		-0.71	-0.95	-0.36	-0.19	-1.17	transporter, major facilitator family protein
TGGT1_285690	-0.23		0.34	0.09	-0.93	-0.88	-1.17	notch (dsl) domain-containing protein
TGGT1_221500	-0.63		-0.78	-0.74	-0.65	-0.80	-1.17	dual specificity phosphatase, catalytic domain-containing protein
TGGT1_232420	0.30		-0.21	-0.50	-0.91	-1.00	-1.17	hypothetical protein
TGGT1_280530	-0.41		-0.49	-0.45	-0.71	-1.07	-1.18	hypothetical protein
TGGT1_203770	-2.09		-0.11	-0.25	-0.38	-0.09	-1.18	hypothetical protein
TGGT1_242720	-0.94		-0.81	-0.90	-0.74	-0.88	-1.19	aspartyl protease ASP5
TGGT1_246010	-1.54		-0.80	-0.77	-0.74	-0.94	-1.20	hypothetical protein
TGGT1_213290	0.37		-0.23	-0.77	-0.66	-0.91	-1.20	hypothetical protein
TGGT1_285940	0.09		-0.14	-0.60	-1.16	-1.34	-1.20	hypothetical protein
TGGT1_201180B	-0.23			-0.48	-1.23	-1.12	-1.21	hypothetical protein
TGGT1_279440	-0.97		-0.61	-0.93		-0.25	-1.21	PA14 domain-containing protein
TGGT1_251830	0.13		-0.51	-0.35	-0.37	-0.90	-1.21	hypothetical protein
TGGT1_265340	-0.50		-0.76	-0.90	-0.29	-0.63	-1.21	hypothetical protein
TGGT1_219110	0.14			-0.90	-1.33	-1.43	-1.21	hypothetical protein
TGGT1_258810	-0.20		-0.75	-0.16	-0.54	-0.34	-1.22	SAG-related sequence SRS27B
TGGT1_316560	0.30		0.34	-0.74	-1.49	-1.54	-1.22	Toxoplasma gondii family D protein
TGGT1_237490	-0.54		-0.61	-0.64	-0.60	0.58	-1.22	centrin family protein
TGGT1_204055			-0.99	-1.10	-0.40	-0.56	-1.23	hypothetical protein
TGGT1_213885			-1.11	-1.06		-0.12	-1.23	hypothetical protein
TGGT1_223725	0.20		-0.13	-0.40	-1.67	-0.73	-1.23	hypothetical protein
TGGT1_319510	0.14		-1.19	-1.13		-0.16	-1.23	hypothetical protein
TGGT1_277705	0.07		-0.65	-0.37	-0.10	0.06	-1.23	hypothetical protein
TGGT1_321480	0.10		-0.33	-0.33	0.00	-0.07	-1.23	SAG-related sequence SRS12B
TGGT1_268800	-0.63		-0.25	-0.83	0.26	0.09	-1.23	hypothetical protein
TGGT1_294400	-1.17		0.06		-0.47	-0.38	-1.23	hypothetical protein
TGGT1_277950	-1.20		-0.46	-0.72	-0.73	-0.89	-1.24	lipase
TGGT1_290645	-0.28		-0.57	-0.70	-0.52	-1.05	-1.24	hypothetical protein
TGGT1_216415	0.32		-1.05	-1.14	-0.54	-0.25	-1.24	hypothetical protein
TGGT1_301470	-0.50		-0.74	-0.60	-0.54	-0.59	-1.24	hypothetical protein
TGGT1_273280	0.43		-0.14	-0.21	-0.95	-1.47	-1.24	hypothetical protein
TGGT1_309330	0.18		0.19	-0.85	-1.21		-1.25	SAG-related sequence SRS55F
TGGT1_275790	0.15			-0.52	-1.06	0.14	-1.25	EGF family domain-containing protein
TGGT1_316580	-1.27		-0.68	-0.37	-0.44	-0.25	-1.26	hypothetical protein
TGGT1_320250	0.81		-0.14	-0.60	-0.79	0.65	-1.26	SAG-related sequence SRS15A
TGGT1_318610	-0.39			-0.76	-0.73	-0.52	-1.26	AP2 domain transcription factor AP2IV-3
TGGT1_316190	-0.66		0.41	0.17	-0.67	-0.45	-1.26	superoxide dismutase SOD3
TGGT1_246160			-0.53	-0.90	-0.88	-1.20	-1.26	hypothetical protein
TGGT1_272645	0.13		-0.75	-0.89	-0.48	-0.56	-1.26	hypothetical protein
TGGT1_290585	-0.84		-0.10	-0.66	-0.82	2.41	-1.26	hypothetical protein
TGGT1_289610	-0.54		-0.86	-0.87	0.22	-0.47	-1.27	hypothetical protein
TGGT1_206440	-0.96		-0.70	-0.62	-0.78	-1.45	-1.27	cpw-wpc domain-containing protein
TGGT1_365080	-0.51		-0.60	-0.88		-0.19	-1.27	rhoptry protein ROP7
TGGT1_245600			-0.50	-0.65	-1.04	-1.02	-1.27	hypothetical protein
TGGT1_301000	0.13		-0.37	-0.55	-0.41	-0.33	-1.27	methyltransferase
TGGT1_238210	-2.18		-0.61	-1.15	0.13	0.22	-1.28	EGF family domain-containing protein
TGGT1_239083	-0.79		-0.72	-1.03	-0.81	-0.82	-1.28	hypothetical protein
TGGT1_269400	0.40		0.73	-0.51	-1.00	-0.61	-1.28	oxidoreductase, short chain dehydrogenase/reductase family protein
TGGT1_218290	0.22		-0.60	-0.67	-0.66	-0.24	-1.28	WD domain, G-beta repeat-containing protein
TGGT1_217700	-0.37		-0.64	-1.16	-1.20	-1.79	-1.28	AP2 domain transcription factor AP2XII-2
TGGT1_282160			-1.45	-1.43	-0.47	-0.57	-1.28	hypothetical protein
TGGT1_222080	1.78		-0.24	-0.15			-1.29	hypothetical protein
TGGT1_217490	-0.75		-0.27	-0.94	-0.83	-0.75	-1.29	hypothetical protein
TGGT1_305610	-0.13		-0.42	-0.34	-0.42	-0.67	-1.29	hypothetical protein
TGGT1_243330	-0.27		-0.80	-0.52	-0.59	0.29	-1.29	hypothetical protein
TGGT1_270273	0.17			-0.08	-0.83	-0.66	-1.30	hypothetical protein
TGGT1_293890	0.20			-0.61	-0.96	-1.03	-1.30	intraflagellar transport 80 family protein
TGGT1_214430	-1.75		0.14	-0.55	-1.37	-1.18	-1.30	hypothetical protein
TGGT1_218510	0.41		-0.21	-0.31	-0.51	-1.13	-1.30	hypothetical protein
TGGT1_209670			-1.40	-1.38	-0.18	-0.27	-1.30	hypothetical protein
TGGT1_268190	0.11		0.29	-0.51	-1.61	-1.02	-1.30	hypothetical protein
TGGT1_208380	0.17		0.23	-0.81	-1.16	-0.80	-1.30	hypothetical protein
TGGT1_305790	3.50			-0.22	-0.46	-0.33	-1.30	hypothetical protein
TGGT1_244726	-0.45		0.27	-0.31	-1.31	-0.56	-1.31	hypothetical protein
TGGT1_269270	-0.42			-0.76	-0.43	-0.14	-1.31	leucine rich repeat-containing protein
TGGT1_255200	1.77		-0.52	-0.84	-1.07	-0.91	-1.31	putative Radial spoke head protein 9
TGGT1_411770	-1.22		-0.38	-0.80	-0.74	-0.70	-1.32	putative IgA-specific serine endopeptidase
TGGT1_266650			0.21	-0.30	-0.60	-1.44	-1.32	hypothetical protein
TGGT1_306070	0.68		-0.60	-0.69	-0.59	-0.99	-1.32	hypothetical protein
TGGT1_293580	-0.41		-1.35	-1.29		-0.31	-1.32	prefoldin subunit protein
TGGT1_306510	0.58		-0.59	-0.82	-0.76	-0.93	-1.32	hypothetical protein
TGGT1_294240	-1.74		-0.83	-0.36	-0.14	0.80	-1.33	hypothetical protein
TGGT1_243720	0.50		-0.57	-0.83	0.20	1.03	-1.34	peroxisomal biogenesis factor PEX11
TGGT1_240390				-1.05	-1.37	-0.84	-1.34	calcium-dependent protein kinase
TGGT1_208770	0.36		-0.19	-0.48	-0.66	-1.00	-1.34	hypothetical protein
TGGT1_293520	-0.33			-0.68	-1.64	-1.12	-1.34	hypothetical protein
TGGT1_235010	-0.42		-0.13	-0.69	-0.63	0.28	-1.34	hypothetical protein
TGGT1_229405	0.41		-0.70	-1.05	-0.42	-0.49	-1.35	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_201690	4.10		-0.16	-0.45	-0.61	0.79	-1.35	BT1 family protein
TGGT1_262110	-0.26		-0.31	-0.90	-0.46		-1.35	hypothetical protein
TGGT1_267490	-1.55		0.19	-0.61	-1.01	-1.44	-1.35	putative preprocathepsin c precursor
TGGT1_227875	-0.44		-0.73	-0.74	-0.76	-1.07	-1.35	hypothetical protein
TGGT1_203300			-0.29	-0.85	-1.02	-0.85	-1.36	hypothetical protein

TGGT1_225105	-0.99		-0.87	-0.87	-1.05	-1.11	-1.38	hypothetical protein
TGGT1_255730	0.25		-0.31	-0.83	-0.67	-0.56	-1.38	hypothetical protein
TGGT1_314370	-1.11		-0.52	-0.72	-0.76	-1.11	-1.38	hypothetical protein
TGGT1_217860	-1.22		-0.87	-0.81	-0.60	0.39	-1.38	hypothetical protein
TGGT1_226220	-0.58		-0.51	-0.84	-0.99	-1.35	-1.38	alveolin domain containing intermediate filament IMC9
TGGT1_315495	0.10		-0.95	-1.03	-0.30	0.22	-1.37	hypothetical protein
TGGT1_216200	-0.65			-0.46	-0.76	-0.74	-1.37	hypothetical protein
TGGT1_220340	-1.15		-0.77	-0.98	-1.54	0.60	-1.37	hypothetical protein
TGGT1_225280	0.12		-0.18	-0.98	-1.06	-0.45	-1.37	hypothetical protein
TGGT1_259020	-0.33		0.35	-0.80	-0.83	-1.65	-1.37	bradyzoite antigen BAG1
TGGT1_410860	-2.51		-1.70	-1.68	-0.12	-0.47	-1.37	hypothetical protein
TGGT1_270670	-1.05		-0.81	-0.86	-0.76	-0.96	-1.37	hypothetical protein
TGGT1_237070	-1.14		-0.78	-0.64	0.41	3.29	-1.38	hypothetical protein
TGGT1_320540	0.10			-1.00	-1.46	-1.17	-1.38	hypothetical protein
TGGT1_318240	-0.13		-0.71	-0.45	-0.33	1.53	-1.38	Tubulin-tyrosine ligase family protein
TGGT1_264700	-1.14		-0.15	-0.58	-0.70	-0.76	-1.39	hypothetical protein
TGGT1_231900	0.60		-0.55	-0.92	-0.81	-1.09	-1.39	acyl-CoA dehydrogenase domain-containing protein
TGGT1_269020	-0.90		-0.66	-0.87	-0.72	-0.28	-1.39	hypothetical protein
TGGT1_318500	0.74		-0.98	-0.76	0.13	-0.49	-1.41	cpw-wpc domain-containing protein
TGGT1_209410			-0.13	-0.74	-1.17	-1.08	-1.41	hypothetical protein
TGGT1_203340	-0.60		-0.96	-0.77	0.17		-1.41	metallo-beta-lactamase domain-containing protein
TGGT1_272265	-0.20		-0.62	-0.33	-0.39	-1.09	-1.41	hypothetical protein
TGGT1_228050	0.27			-0.36	-0.39	-0.28	-1.41	hypothetical protein
TGGT1_310580	0.37		0.24	-0.56	-0.99	0.29	-1.42	1,3(4)-beta-glucanase
TGGT1_319360	0.89		-0.66	-0.74	-0.56	-0.98	-1.42	SAG-related sequence SRS17A
TGGT1_273740	-0.65			-0.63	-0.45	0.40	-1.42	putative acetyl-CoA acyltransferase B
TGGT1_205590	-0.32		-1.10	-0.72	-0.24	0.90	-1.42	hypothetical protein
TGGT1_264178B	0.10			-0.74	-1.46	-0.39	-1.43	flagellar associated protein
TGGT1_222700	-0.15		-0.13	-0.17	-0.83	0.35	-1.43	hypothetical protein
TGGT1_253480	0.25		0.10	-1.27	-1.42	-1.12	-1.42	putative topoisomerase VIA
TGGT1_240325	-1.20		0.31	-0.20	-1.32	-0.30	-1.43	Toxoplasma gondii family E protein
TGGT1_205450	-0.37		-1.48	-1.69	0.07	0.08	-1.43	hypothetical protein
TGGT1_297920	-1.09		-0.33	-0.84	-1.30	0.15	-1.43	hypothetical protein
TGGT1_277055	-1.37		-0.73	-1.06	-1.05	-1.20	-1.43	hypothetical protein
TGGT1_236880	0.08		0.10	-1.01	-1.63	-1.05	-1.43	hypothetical protein
TGGT1_408340			-0.42	-0.67	-0.69	-0.85	-1.44	toxoplasma gondii family D protein
TGGT1_263135	-0.66		-0.20	0.08	-0.40	-1.75	-1.44	hypothetical protein
TGGT1_247540	1.87		-0.41	-0.66	-0.61	0.32	-1.44	ATP-binding cassette G family transporter ABCG107
TGGT1_410900	-1.78		0.17	-0.24	-0.30	1.97	-1.45	toxoplasma gondii family A protein
TGGT1_295740	-0.41		-0.65	-0.50	-1.18	1.08	-1.45	hypothetical protein
TGGT1_239365	-1.04		0.20	-0.62	-1.36	-0.25	-1.45	hypothetical protein
TGGT1_203230	-0.42		-0.64	-0.56	0.10	-0.13	-1.45	hypothetical protein
TGGT1_290590	-0.21		-0.36	-0.46	-0.93	1.86	-1.45	hypothetical protein
TGGT1_273915	-0.33		-1.47	-1.07	0.07	-0.23	-1.46	hypothetical protein
TGGT1_201190	0.10		-0.99	-0.81	0.18	0.14	-1.46	hypothetical protein
TGGT1_262440	-0.33		-0.40	-0.72	-1.35	-0.67	-1.46	hypothetical protein
TGGT1_408330	-0.10		-0.67	-0.95	-0.81	-0.56	-1.46	toxoplasma gondii family D protein
TGGT1_269350	0.69		0.14	-0.80	-0.52	0.42	-1.47	hypothetical protein
TGGT1_223870	-0.58		-0.80	-0.84	-1.06	-0.79	-1.47	hypothetical protein
TGGT1_294595	0.39		0.12	-1.25	-0.84	-1.09	-1.48	hypothetical protein
TGGT1_323200	-1.40		-0.49	-1.03	-0.96	0.95	-1.48	OTU family cysteine protease
TGGT1_220460	0.05		0.19	-0.78	-1.23	-1.04	-1.48	SNF7 family protein
TGGT1_222935	-0.38		-0.53	-0.69	0.07	0.14	-1.49	hypothetical protein
TGGT1_273980	0.10		-0.48	-1.09	-1.45	-1.70	-1.49	hypothetical protein
TGGT1_272540	0.34		0.22	-0.61	-1.23		-1.50	protein kinase (incomplete catalytic triad)
TGGT1_214420	-1.13		0.12	-0.84	-1.47	-1.52	-1.50	hypothetical protein
TGGT1_310680	0.46		0.16	-0.91	-0.96	-0.36	-1.50	aminotransferase, class I/II superfamily protein
TGGT1_289040	0.14		0.41	-0.85	-1.30	-1.70	-1.51	Armadillo/beta-catenin family repeat-containing protein
TGGT1_213340	-2.10		-0.31	-1.12	-1.49	-1.19	-1.51	GMC oxidoreductase
TGGT1_318130A	-1.04		-0.56	-1.40	-2.51	-0.76	-1.52	Toxoplasma gondii family E protein
TGGT1_411040	-0.57		-0.63	-0.99	-0.86	-0.93	-1.52	putative IgA-specific serine endopeptidase
TGGT1_231780	-1.59			-1.08	-1.13	-0.35	-1.52	hypothetical protein
TGGT1_410870	-1.49		-1.35	-1.20	-0.81	-0.86	-1.52	tRNA-Pro
TGGT1_319090	-0.81		-0.76	-1.04	-0.68	-0.72	-1.53	IgA-specific serine endopeptidase
TGGT1_218900	1.21		-1.42	-0.80	-0.23	1.81	-1.53	hypothetical protein
TGGT1_293258	0.42		-0.33	-0.62	-0.79	-1.11	-1.53	hypothetical protein
TGGT1_201180A	0.27		-0.36	-0.72	-1.04	-1.02	-1.53	hypothetical protein
TGGT1_224710	0.18		0.12	-0.79	-1.09	-1.16	-1.54	hypothetical protein
TGGT1_238490	1.34		-1.06	-1.51	-1.02	-0.80	-1.54	SAG-related sequence SRS22E
TGGT1_243420	-0.36		-0.33	-0.97	-0.95	-1.17	-1.54	hypothetical protein
TGGT1_278878	-1.39		-1.15	-1.05	-0.59	-0.76	-1.55	GDA1/CD39 (nucleoside phosphatase) family protein
TGGT1_357300	0.15		-0.52	-0.44	-0.63	-0.79	-1.55	hypothetical protein
TGGT1_221265	-0.29		0.11	-1.10	-1.53	-1.14	-1.55	hypothetical protein
TGGT1_273940	-1.84		-0.73	-0.34	-0.49	1.94	-1.55	hypothetical protein
TGGT1_219450			-0.53	-0.59	-0.74	2.88	-1.56	WD domain, G-beta repeat-containing protein
TGGT1_206660	-1.12		0.10	-0.81	-1.37	-1.42	-1.56	hypothetical protein
TGGT1_257910B	-0.77		-0.30	-0.48	-1.07	2.20	-1.56	hypothetical protein
TGGT1_219660	-1.04		-0.90	-0.63	-0.83	-0.30	-1.56	hypothetical protein
TGGT1_301240			-0.76	-0.72	-0.15	-0.24	-1.56	hypothetical protein
TGGT1_289060	-2.56		-0.86	-0.86	-0.38	-0.33	-1.57	hypothetical protein
TGGT1_285665			-0.24	-0.55	-0.61	-1.08	-1.57	hypothetical protein
TGGT1_257970	0.80		-0.49	-0.87	-0.91	-0.29	-1.57	hypothetical protein
TGGT1_295640	-0.74		0.11	-0.82	-1.09	-0.37	-1.57	peptidase family M13 protein
TGGT1_267410	-2.05		-0.27	-1.38	-1.32	-1.47	-1.58	scavenger receptor protein SR1 precursor
TGGT1_289240	-0.29		-0.58	-0.59	-0.36	1.90	-1.58	DnaJ domain-containing protein
TGGT1_229350	-1.09		-0.87	-1.14	-0.78	-0.96	-1.58	HEAT repeat-containing protein
TGGT1_267670	-1.50		-0.63	-0.98	-0.65	-0.76	-1.58	hypothetical protein

TGGT1_259960	0.95		-0.87	0.16		3.18	-1.58	Nucleoside-diphosphatase
TGGT1_221895	-1.10		-0.28	-0.58	-0.50	0.28	-1.59	hypothetical protein
TGGT1_286460	0.22		-0.90	-0.93	-0.68	-0.89	-1.59	hypothetical protein
TGGT1_227350	-4.06		-1.11	-1.05	-1.17	-2.19	-1.59	hypothetical protein
TGGT1_203330	-1.31		-1.65	-1.37	-0.91	-0.59	-1.60	hypothetical protein
TGGT1_293030	-0.19		-0.03	-0.72	-0.99	0.53	-1.60	hypothetical protein
TGGT1_409850	1.21			-0.53	-1.67	-0.20	-1.60	SAG-related sequence protein SRS47A
TGGT1_204045	0.11		-0.71	-1.08	-0.58	-0.58	-1.60	hypothetical protein
TGGT1_270680	-1.24		-1.14	-1.06	-0.97	-1.12	-1.61	hypothetical protein
TGGT1_236290	-1.36		-0.76	-1.52	-0.49	0.16	-1.62	hypothetical protein
TGGT1_247970	-1.03		-0.29	-1.01	-1.69	-1.54	-1.62	hypothetical protein
TGGT1_252860	0.33		-0.35	-0.86	-1.52	-1.54	-1.62	hypothetical protein
TGGT1_300070	0.51		0.77	-1.30	-1.78	-1.62	-1.62	hypothetical protein
TGGT1_253120	0.41		-0.14	-0.76	-1.31	-0.77	-1.62	putative mandelonitrile lyase
TGGT1_233210	-1.55		-0.18	-0.70	-1.24	-0.98	-1.62	hypothetical protein
TGGT1_205020	-0.30		-1.10	-1.31	-0.53	-0.64	-1.63	hypothetical protein
TGGT1_250360	-0.65		-0.79	-1.09	-1.02	-0.68	-1.63	alpha/beta hydrolase fold domain-containing protein
TGGT1_301260	-0.37		-1.22	-1.26	-0.92	-1.17	-1.63	hypothetical protein
TGGT1_293250	0.42			-0.50	-0.70	2.89	-1.64	hypothetical protein
TGGT1_316690	-1.55		-0.33	-1.18	-1.41	-0.19	-1.64	hypothetical protein
TGGT1_225400	0.14		-0.51	-0.74		0.58	-1.64	hypothetical protein
TGGT1_310260	0.13		-0.56	-0.62	0.26	1.09	-1.64	hypothetical protein
TGGT1_267160	-0.73		-0.90	-1.75	-1.60	-1.60	-1.64	SAG-related sequence SRS38D
TGGT1_210095	1.02		0.46	-0.88	-1.77	-0.65	-1.64	hypothetical protein
TGGT1_281600	0.74		-0.41	-1.08	-0.23		-1.65	hypothetical protein
TGGT1_293500	2.23		-0.58	-1.16	-0.93	0.59	-1.66	hypothetical protein
TGGT1_240340A	-0.50		-0.65	-0.68	-0.99	-0.23	-1.66	Toxoplasma gondii family E protein
TGGT1_238980	-0.98		-0.98	-1.09	-1.50	-1.80	-1.68	hypothetical protein
TGGT1_272360	0.45		-0.41	-1.33	-1.20	0.56	-1.68	EF hand family protein
TGGT1_225190	-0.73		-0.66	-1.03	-1.31	-1.46	-1.69	hypothetical protein
TGGT1_209300	-0.22		-0.48	-1.05	-1.38	-1.19	-1.69	WD domain, G-beta repeat-containing protein
TGGT1_318120	-0.92		-0.93	-1.07	-1.96	-1.15	-1.70	hypothetical protein
TGGT1_253150	-0.34		-0.17	-0.58	-1.16	2.85	-1.70	hypothetical protein
TGGT1_271892	-1.32		-0.45	-1.21	-1.11	-0.51	-1.70	hypothetical protein
TGGT1_205658	-2.11		-0.24	-0.92	-1.63	-0.11	-1.71	F5/8 type C domain-containing protein
TGGT1_292335	-0.79		-0.55	-1.11	-1.30	-1.97	-1.72	hypothetical protein
TGGT1_411550	-2.03			-0.56	-0.36	1.85	-1.72	toxoplasma gondii family A protein
TGGT1_309030	-0.45			-0.70	-0.63	0.44	-1.73	hypothetical protein
TGGT1_227880	-0.24		-0.99	-1.15	-0.48	-0.62	-1.73	hypothetical protein
TGGT1_210235	4.01		-1.18	-0.70	0.53	0.38	-1.73	hypothetical protein
TGGT1_209510	-1.09		0.73	-0.77	-2.12	-1.48	-1.73	hypothetical protein
TGGT1_315480	0.08			-0.62	-1.64	-1.09	-1.73	acyl-CoA dehydrogenase, middle domain-containing protein
TGGT1_211050	-0.14		-0.43	-1.30	-0.64	1.13	-1.74	ATPase, AAA family protein
TGGT1_248760	-0.09		-0.49	-0.60	-0.93	0.74	-1.74	subtilisin SUB7
TGGT1_218210	-0.17		-0.28	-0.86	-0.89	-0.61	-1.74	putative Chromosome-associated kinesin KLP1
TGGT1_269315	0.83			-0.84	-0.35	1.66	-1.75	hypothetical protein
TGGT1_255500	0.85		-0.64	-1.14	-1.58	-0.35	-1.75	hypothetical protein
TGGT1_218340	-0.83		-0.83	-1.09	-0.97	0.97	-1.76	hypothetical protein
TGGT1_240370	-2.83		-0.31	-0.47	-1.14	-0.27	-1.76	Toxoplasma gondii family E protein
TGGT1_272020	0.24		-0.22	-0.64	-1.40	-1.26	-1.76	hypothetical protein
TGGT1_306480	1.95		-0.42	-1.28	-1.77	-1.15	-1.76	CAM kinase, CDPK family
TGGT1_281555	0.80		0.24	-1.24	-0.98	-0.12	-1.76	hypothetical protein
TGGT1_221665	-0.78			-0.67	-0.90	0.72	-1.77	hypothetical protein
TGGT1_409760	0.36		0.20	-0.80	-1.29	-1.04	-1.77	putative transmembrane protein
TGGT1_243940	-0.70		-0.16	-0.78	-1.10	-0.95	-1.77	hypothetical protein
TGGT1_215670	-0.33		-0.20	-0.93	-1.23	1.44	-1.78	cAMP-dependent protein kinase
TGGT1_316130	0.27		-0.84	-1.11	-1.04		-1.78	hypothetical protein
TGGT1_270970	-1.40			-0.94	-0.36	0.42	-1.78	hypothetical protein
TGGT1_205210	-0.60		-0.68	-1.13	-1.06	-0.58	-1.78	hypothetical protein
TGGT1_318675	0.78			-1.08	-1.09	-0.54	-1.79	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_208020	-1.32		-0.41	-0.87	-1.17	-1.38	-1.79	AP2 domain transcription factor AP2Ib-1
TGGT1_312970	0.14		-0.27	-1.00	-1.04	0.93	-1.79	hypothetical protein
TGGT1_240730	-1.13		-1.01	-1.09	-0.71	-0.84	-1.79	hypothetical protein
TGGT1_289850	-0.50		-0.59	-0.85	-0.82	2.55	-1.80	hypothetical protein
TGGT1_265120	0.66		-0.42	-0.99	-1.76	-0.31	-1.80	putative rhoptry neck protein
TGGT1_220840	-0.49		-0.90	-0.72	-0.68	-1.29	-1.80	threonine synthase
TGGT1_258225	0.23		-0.80	-0.76	-0.77	-0.81	-1.80	hypothetical protein
TGGT1_234260	-0.72		-0.77	-0.97	-0.92	-0.98	-1.80	hypothetical protein
TGGT1_320090	-1.00		-0.65	-1.48	-1.53	-1.45	-1.81	hypothetical protein
TGGT1_224080	0.12		0.15	-0.15	-1.47	1.02	-1.82	Kazal-type serine protease inhibitor domain-containing protein
TGGT1_269640	-0.42		-0.90	-1.30	-1.30	-1.14	-1.82	hypothetical protein
TGGT1_228500	-1.38			-1.07	-1.41	-0.95	-1.82	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_202065	-0.70		-0.49	-0.61	-1.36	-0.71	-1.82	hypothetical protein
TGGT1_316550	0.79		-0.61	-0.92	-1.46	-1.00	-1.83	hypothetical protein
TGGT1_271980	-0.50		0.25	-0.89	-1.71	-1.62	-1.84	SAG-related sequence SRS32
TGGT1_221560	0.97		0.28	-0.68	-1.22	1.32	-1.84	hypothetical protein
TGGT1_221550	0.85		-1.48	-0.78	-0.49	0.79	-1.86	CMGC kinase, MAPK family, MEK kinase-related (incomplete catalytic triad)
TGGT1_281675	-1.05		-0.45	-0.75	-1.32	-1.64	-1.86	putative protein kinase
TGGT1_203682	-2.51		-1.00	-1.64	-0.80	-0.35	-1.86	hypothetical protein
TGGT1_295662	-0.68		-0.64	-1.35	-1.33	-1.11	-1.87	hypothetical protein
TGGT1_279310	-1.30		-1.64	-1.45	0.28	-0.54	-1.87	hypothetical protein
TGGT1_231060	-0.50		0.10	-0.55	-1.30	-0.17	-1.87	subtilisin SUB9
TGGT1_294210	-0.77		0.21	-0.94	-1.56	-0.65	-1.88	hypothetical protein
TGGT1_289840	-0.36		-0.39	-1.35	-1.27	1.60	-1.88	hypothetical protein
TGGT1_229660	-0.21		-1.10	-1.19	-0.30	-0.86	-1.89	hypothetical protein
TGGT1_276980	-0.12		-1.06	-1.20	-0.65	-0.50	-1.90	hypothetical protein
TGGT1_360460	0.50		-0.69	-0.64	-0.49	1.57	-1.91	SAG-related sequence protein SRS40D

TGGT1_202050	-1.15		-0.53	-0.76	-1.35	-0.68	-1.91	hypothetical protein
TGGT1_205140	-1.32		-1.03	-1.25	-1.87	0.51	-1.92	hypothetical protein
TGGT1_238915	0.10		0.15	-0.71	-1.46	-0.22	-1.92	hypothetical protein
TGGT1_218890	-0.37		-0.50	-1.42		2.95	-1.92	hypothetical protein
TGGT1_254890	-1.38		-0.86	-1.35	-1.42	-1.12	-1.93	hypothetical protein
TGGT1_315560	-1.11		-1.02	-1.35	-1.84	1.20	-1.93	ATP-binding cassette G family transporter ABCG77
TGGT1_268360	-1.22		-1.96	-1.37	-0.94	0.71	-1.93	hypothetical protein
TGGT1_305580	0.29		-0.64	-0.79	-1.56	-0.56	-1.93	ankyrin repeat-containing protein
TGGT1_269035	-0.18		-0.32	-0.68	-0.79	0.22	-1.93	nucleoside diphosphate kinase
TGGT1_321470	-2.05		-0.89	-0.73	-0.09	0.48	-1.96	SAG-related sequence SRS12D
TGGT1_316380	0.70		-0.16	-0.14	-0.33	1.19	-1.96	hypothetical protein
TGGT1_260000	-0.21		-0.53	-1.22	-1.06	0.79	-1.96	hypothetical protein
TGGT1_203688	-2.30		-1.39	-1.93	-1.04	-0.47	-1.96	hypothetical protein
TGGT1_254750	-0.34			-0.71	-1.25	1.04	-1.97	c3orf15 protein
TGGT1_201230	-1.47		-0.68	-1.22	-1.54	-1.49	-1.97	kinesin motor domain-containing protein
TGGT1_411250	0.16		-0.21	0.28	-0.50	1.07	-1.97	SAG-related sequence protein SRS22I
TGGT1_209485	-1.03		-0.62	-0.63	-0.59	2.14	-1.98	hypothetical protein
TGGT1_254160	-1.46		-1.11	-0.82	-0.61	0.60	-1.98	hypothetical protein
TGGT1_258575	1.08		-0.49	-2.31	-2.03	-2.70	-1.98	Dpy-30 motif protein
TGGT1_313250	-0.42		-1.54	-0.95	-0.22	-1.14	-1.98	hypothetical protein
TGGT1_246070	-1.26		-1.07	-1.39	-0.95	0.21	-1.99	SAG-related sequence SRS56
TGGT1_203250	-0.33		-0.21	-1.12	-0.90	1.50	-2.00	hypothetical protein
TGGT1_275590	0.10		-0.65	-1.02	-1.63		-2.00	mono- or diacylglycerol acyltransferase
TGGT1_274170	-0.65		-0.97	-1.39	-0.79	-0.31	-2.00	protein kinase (incomplete catalytic triad)
TGGT1_409630	-2.74		-0.60	-0.66	-1.05	-0.32	-2.01	toxoplasma gondii family E protein
TGGT1_269380	-1.56		-1.11	-0.94	-1.34	-1.00	-2.01	hypothetical protein
TGGT1_202400	-0.30			-0.33	-0.86	1.19	-2.01	calcium binding egf domain-containing protein
TGGT1_249040	0.27		0.45	-0.39	-1.42	-0.95	-2.01	hypothetical protein
TGGT1_411540	-0.25		-1.52	-1.83	-0.39	-0.48	-2.01	hypothetical protein
TGGT1_233670	-0.46		0.28	-0.61	-0.66		-2.02	hypothetical protein
TGGT1_315345	-1.25		-0.59	-0.76	-1.58	-2.27	-2.02	SAG-related sequence SRS52F
TGGT1_286020	-0.86		-0.96	-1.29	-1.97	-1.27	-2.03	hypothetical protein
TGGT1_275798	0.21		-0.98	-1.34	-0.89	-1.41	-2.03	putative microneme protein
TGGT1_237894	-1.63		-0.75	-1.60	-1.58	-0.78	-2.04	OTU family cysteine protease
TGGT1_266330	-0.66		-0.14	-0.11	-0.39	1.74	-2.04	Toxoplasma gondii family A protein
TGGT1_275360			-0.74	-0.87	-0.13	1.08	-2.04	SAG-related sequence SRS47B
TGGT1_231400A	-0.88		-1.02	-0.87	-0.60	0.19	-2.04	tubulin/FtsZ family, GTPase domain-containing protein
TGGT1_267150	-0.30		-0.88	-1.13	-0.73	-0.39	-2.05	SAG-related sequence SRS38C
TGGT1_202445	-2.00		-1.09	-1.63	-1.48	-1.35	-2.05	hypothetical protein
TGGT1_204560	0.74		-0.53	-0.44	-1.17	1.43	-2.06	putative type I fatty acid synthase
TGGT1_319390	0.69		0.19	-1.26	-1.15	0.80	-2.06	hypothetical protein
TGGT1_272430	-0.34		0.14	-1.40	-1.85	0.47	-2.08	MAC/Perforin domain-containing protein
TGGT1_249150	0.43		-0.37	-0.96	-1.17	-0.58	-2.09	PAN domain-containing protein
TGGT1_243190	-0.74		-0.86	-1.27	-0.94	-0.72	-2.09	Toxoplasma gondii family A protein
TGGT1_411360	0.39		-0.65	-0.59	-0.92	-0.93	-2.10	rhoptry kinase family protein
TGGT1_233180	-1.02		-1.00	-1.22	-1.00	-0.84	-2.10	hypothetical protein
TGGT1_258840	-0.33		-0.90	-0.96	-0.86	-0.74	-2.11	hypothetical protein
TGGT1_239430	-1.42		-0.94	-1.60	-1.78	-1.59	-2.11	EF hand domain-containing protein
TGGT1_204300	-0.84		-0.46	-1.62	-1.23	-1.10	-2.12	hypothetical protein
TGGT1_281660	-1.27		-0.86	-0.91	-1.60	-2.01	-2.14	hypothetical protein
TGGT1_311710	-1.56		-0.19	-1.57	-1.83	-2.14	-2.15	hypothetical protein
TGGT1_293020	0.68		-0.40	-1.11	-1.70	0.08	-2.15	hypothetical protein
TGGT1_218470	0.30		-1.73	-0.88	0.23	2.51	-2.16	putative protein disulfide-isomerase
TGGT1_240930	-1.39		-0.87	-1.06	-1.10	-1.21	-2.16	MoaC family protein
TGGT1_231470	2.41		-0.71	-1.30		2.08	-2.17	hypothetical protein
TGGT1_242740	-0.66		-0.95	-1.16	-2.16	-2.22	-2.18	hypothetical protein
TGGT1_233405	-0.62		-0.42	-1.35	-1.24	-1.49	-2.19	hypothetical protein
TGGT1_231400B	-1.13		-0.84	-0.81	-0.51		-2.19	tubulin/FtsZ family, GTPase domain-containing protein
TGGT1_408360	-0.78		-0.45	-1.13	-1.09	-1.07	-2.19	tRNA-Glu
TGGT1_227370	-1.54		-0.24	-1.23	-1.95	-0.50	-2.22	hydrolase CocE/NonD family protein
TGGT1_297090	-0.58		-0.38	-1.75	-1.37	0.90	-2.22	hypothetical protein
TGGT1_217420	2.90		-0.45	-1.04	-1.24		-2.23	hypothetical protein
TGGT1_364540	-0.30		-1.50	-0.27	-0.23	2.19	-2.24	putative type I fatty acid synthase
TGGT1_286465	-0.37		-1.44	-1.97	-1.08	-1.00	-2.24	hypothetical protein
TGGT1_219742	-0.66		-0.38	-1.08	-1.49	-0.21	-2.27	hypothetical protein
TGGT1_411630	-0.41		-2.61	-1.93	0.12	0.30	-2.28	disulfide-isomerase domain protein
TGGT1_278400	4.03		-1.29	-1.17	-0.63	0.86	-2.33	Toxoplasma gondii family A protein
TGGT1_228160	-1.68		-0.53	-1.25	-1.51	-1.74	-2.34	acid phosphatase
TGGT1_258910	-1.12		0.14	-0.95	-1.96	-1.89	-2.34	hypothetical protein
TGGT1_264805	-1.81		-1.18	-1.16	-1.56	-1.69	-2.35	hypothetical protein
TGGT1_295995	1.04		-0.22	-0.86	-1.05	-0.44	-2.35	hypothetical protein
TGGT1_227100	-2.77		-1.12	-1.44	-0.51	0.30	-2.35	hypothetical protein
TGGT1_299020	-0.79		-0.51	-1.24	-1.83	-1.87	-2.36	AP2 domain transcription factor AP2III-4
TGGT1_294410	0.29		-0.70	-1.28	-1.45	0.31	-2.37	hypothetical protein
TGGT1_239755	-0.44		-1.27	-0.90	0.31	1.61	-2.37	hypothetical protein
TGGT1_271040	-0.73		-0.34	-1.50	-1.89	-1.22	-2.37	hypothetical protein
TGGT1_204290	-1.98		-0.66	-1.71	-1.86	-1.70	-2.38	hypothetical protein
TGGT1_258570	0.55		-0.11	-1.66	-1.44	-0.13	-2.41	hypothetical protein
TGGT1_226450	-0.65		-1.15	-1.32	-1.18	0.51	-2.42	hypothetical protein
TGGT1_257670	-0.46		-0.49	-1.08	-1.66	-2.00	-2.43	hypothetical protein
TGGT1_231880	-0.69		-0.85	-1.32	-1.27	-1.08	-2.44	hypothetical protein
TGGT1_264620	-1.81		-1.03	-1.71	-1.30	0.97	-2.45	hypothetical protein
TGGT1_274140	-0.42		-1.22	-1.01	-0.29	1.29	-2.46	hypothetical protein
TGGT1_256800	-0.10		-0.58	-1.30	-1.93	0.25	-2.46	hypothetical protein
TGGT1_215775	0.89		-0.39	-1.23	-1.78	-1.54	-2.48	rhoptry protein ROP8
TGGT1_312690	-1.02		-1.15	-1.76	-1.20	1.04	-2.49	hypothetical protein
TGGT1_294570	0.10		-0.58	-0.83	-0.77	2.20	-2.50	rhodanese family domain-containing protein

TGGT1_236595	-0.97		-0.92	-1.09	-1.01	-0.88	-2.53	ankyrin repeat-containing protein
TGGT1_320240	0.35		0.29	-1.48	-2.09	0.35	-2.55	SAG-related sequence SRS15B
TGGT1_266270	-0.83		-1.10	-1.38	-0.90	-0.32	-2.56	short-chain-acyl-CoA dehydrogenase
TGGT1_235390	-1.06		-0.74	-1.39	-1.54	0.56	-2.57	PAN domain-containing protein
TGGT1_224010	-0.17			-0.50	-0.96	2.19	-2.63	hypothetical protein
TGGT1_305220	0.39		-1.13	-0.97		-0.15	-2.64	C-Myc-binding family protein
TGGT1_313940	-1.52			-1.23	-2.12	-2.27	-2.65	hypothetical protein
TGGT1_410600	1.54		0.33	-0.88	-2.04	-1.07	-2.66	putative cAMP-dependent protein kinase
TGGT1_277060	0.52		-0.68	-1.50	-1.57	-1.59	-2.67	hypothetical protein
TGGT1_411410	1.00		-0.19	-1.28	-2.10	-1.24	-2.72	putative transmembrane protein
TGGT1_252640	0.84		-1.58	-0.95	-0.73	0.98	-2.73	P-type ATPase PMA1
TGGT1_243655	0.37		-0.82	-1.55	-1.37	-0.22	-2.73	GMC oxidoreductase
TGGT1_217710	0.35		-0.36	-0.89	-0.99	1.89	-2.75	DnaJ domain-containing protein
TGGT1_410610	1.80		-0.57	-1.85	-1.89	-1.16	-2.76	hypothetical protein
TGGT1_314250	1.86		-0.32	-1.53	-2.04	-1.55	-2.77	bradyzoite rhoptry protein BRP1
TGGT1_301370	2.66		-1.14	-2.12	-0.82	-0.83	-2.78	DHHC zinc finger domain-containing protein
TGGT1_201650	-0.15		-1.51	-1.73	-2.05	-0.42	-2.79	hypothetical protein
TGGT1_411220	0.40		-0.80	-1.48	-2.40	-1.73	-2.82	hypothetical protein
TGGT1_297820	0.25		-0.55	-1.42	-0.67	0.97	-2.82	putative sperm associated antigen 6
TGGT1_203900	-0.65		-0.33	-1.48	-1.67	-1.66	-2.84	hypothetical protein
TGGT1_218192	-2.72		-1.71	-1.90	-1.30	-0.76	-2.85	hypothetical protein
TGGT1_237045	-2.02		-1.79	-1.92	-0.35	1.03	-2.85	hypothetical protein
TGGT1_201640	-0.47		-1.23	-1.41	-1.91	0.27	-2.86	hypothetical protein
TGGT1_410720	2.05		-1.69	-2.20	-1.74	-0.95	-2.86	putative protein kinase
TGGT1_306500	2.02		-0.69	-1.76	-1.44		-2.89	hypothetical protein
TGGT1_240365	-2.83		-0.56	-1.16	-1.49	-1.44	-2.90	hypothetical protein
TGGT1_320105	2.27		-1.03	-1.80	-1.38	-2.02	-2.90	hypothetical protein
TGGT1_217370	-0.97		-0.63	-0.80	-0.59	1.39	-2.95	hypothetical protein
TGGT1_203800	-1.05		-1.49	-1.75	-1.83	-0.89	-2.95	hypothetical protein
TGGT1_289930	-0.70		-0.70	-1.44	-1.68	-0.87	-2.98	putative phosphoenolpyruvate carboxykinase
TGGT1_306455	1.40		-0.56	-2.39	-2.30	-1.05	-2.99	hypothetical protein
TGGT1_410590	1.69		0.34	-0.90	-1.86	-0.80	-2.99	cAMP-dependent protein kinase
TGGT1_244620	0.06		-0.72	-1.83	-2.34	-1.51	-3.02	NEK kinase
TGGT1_410630	-1.33		-2.30	-2.69	-0.53	-1.80	-3.04	tRNA-Sec
TGGT1_409410	-2.00		-0.63	-1.90	-2.45	-2.11	-3.10	tRNA-Leu
TGGT1_278410	2.90		-0.91	-0.56	-1.28	0.40	-3.28	Toxoplasma gondii family A protein
TGGT1_205662	-2.48			-1.83	-2.02	-2.24	-3.35	LCCL domain-containing protein
TGGT1_282200	-1.39		-1.25	-1.97	-1.73	-1.51	-3.44	ATPase, AAA family protein
TGGT1_247660	0.07		-2.16	-0.81	-0.43	0.27	-3.44	thioredoxin domain-containing protein
TGGT1_237893	-0.42		-1.38	-2.24	-0.77		-3.48	hypothetical protein
TGGT1_305100	0.40			-0.91	-0.85	2.14	-3.53	hypothetical protein
TGGT1_237585	0.09		-0.96	-2.15	-2.28	-1.71	-3.59	hypothetical protein
TGGT1_269120	0.11		0.28	-0.41		3.45	-3.61	putative oxidoreductase
TGGT1_264630	-1.43		-1.34	-1.85	-1.22	-0.49	-3.87	hypothetical protein
TGGT1_291910	-2.80		-1.62	-1.95	-1.43	-1.12	-3.98	hypothetical protein
TGGT1_306450	1.82		-1.51	-2.73	-2.20	-1.08	-3.99	putative short chain dehydrogenase family protein
TGGT1_311700	-1.92		-1.43	-2.57	-2.23	-2.25	-4.02	hypothetical protein
TGGT1_207050	-0.99		-1.00	-2.24	-1.81	-2.44	-4.24	hypothetical protein
TGGT1_291920	-2.90		-1.76	-2.18	-1.82	-1.17	-4.24	hypothetical protein

Table 4.5. Regression analysis identified 319 differentially expressed genes whose extracellular expression over time strongly correlated with GT1's lab-adapting phenotypes

The relationship between the 988 DEGs identified by TC-seq and our lab-adaptive phenotypes were examined by regression analysis (RA) in order to identify correlating DEGs. A total of 319 DEGs were identified as strongly correlating (R_2 = ≥0.70; indicated by green text) with at least one lab-adaptive phenotype (extracellular survival, reinvasion efficiency, plaque size).

Table 4.5. Regression analysis identified 319 differentially expressed genes whose extracellular expression over time strongly correlated with GT1's lab-adapting phenotypes

Gene ID	Log$_2$ FC (relative to GT1 B2-P11)							Correlation Coefficient (R_2)			Product Description
	RH	P11	P35	P55	P85	P148	P210	Plaque Size	Reinv. Effic	EC Surv.	
TGGT1_409050	4.35		1.87	1.82	1.05	3.68	4.74	0.70	0.88	-0.08	hypothetical protein
TGGT1_264260	4.80		1.21	1.19	1.01	3.54	4.07	0.80	0.96	-0.10	hypothetical protein
TGGT1_249800	4.70		-0.50	0.17	0.60	3.17	3.98	0.84	0.97	0.04	hypothetical protein
TGGT1_262590	2.90		2.06	2.98	2.66	2.58	3.45	0.56	0.56	0.92	hypothetical protein
TGGT1_264240	4.35			0.36	1.04	3.44	3.22	0.85	0.99	0.05	hypothetical protein
TGGT1_253330	0.10		0.91	0.62	1.84	3.04	2.73	0.85	0.99	0.05	Rhoptry kinase family protein
TGGT1_249810	3.35		0.14	0.24	-0.50	1.68	2.60	0.77	0.90	0.00	putative activating signal cointegrator 1 complex subunit 3
TGGT1_262600	2.68		1.36	2.10	2.10	2.18	2.48	0.82	0.83	0.65	hypothetical protein
TGGT1_309930	0.98		1.18	1.01	1.79	2.37	2.21	0.82	0.97	0.05	melibiase subfamily protein
TGGT1_216680	5.19		0.29	0.76	1.22	1.64	2.17	0.85	0.99	0.05	ankyrin repeat-containing protein
TGGT1_304670	2.30		1.64	1.74	1.16	1.37	2.01	0.47	0.39	0.75	leucine rich repeat-containing protein
TGGT1_301210	3.35		0.34	0.87	0.93	2.93	2.01	0.68	0.87	0.05	putative NAD(P) transhydrogenase subunit beta
TGGT1_216335	2.82		0.78	1.44	1.85	0.58	1.95	0.06	0.00	0.78	hypothetical protein
TGGT1_315885	-0.03		0.79	1.23	1.04	1.49	1.94	0.85	0.99	0.05	putative glycosyltransferase
TGGT1_286420A	0.44		1.09	1.41	1.06	1.33	1.89	0.67	0.78	0.78	putative elongation factor 1-alpha (EF-1-ALPHA)
TGGT1_313640	0.89		0.42	0.92	1.61	1.87	1.87	0.77	0.93	0.05	hypothetical protein
TGGT1_277685	1.74		1.43	1.60	1.36	1.79	1.82	0.61	0.77	0.27	hypothetical protein
TGGT1_231992	2.21		1.32	1.35	1.02	1.00	1.80	0.41	0.26	0.75	hypothetical protein
TGGT1_289170	0.82		1.10	1.38	1.04	1.31	1.80	0.62	0.69	0.70	adenylate and guanylate cyclase catalytic domain-containing protein
TGGT1_230705	0.31		1.11	1.42	0.87	1.45	1.75	0.67	0.77	0.47	hypothetical protein
TGGT1_316700	-0.03		0.80	0.80	1.19	2.00	1.72	0.85	0.99	0.05	uridine kinase
TGGT1_220420	1.64		1.03	1.09	0.99	1.74	1.68	0.70	0.91	0.06	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_224130	1.72		1.15	1.20	1.40	1.88	1.67	0.66	0.86	0.05	hypothetical protein
TGGT1_270840	1.15		1.13	1.34	1.80	2.42	1.66	0.46	0.71	0.05	poly(ADP-ribose) polymerase catalytic domain-containing protein
TGGT1_305980	0.19		0.31	0.90	1.88	1.47	1.65	0.85	0.99	0.05	pyruvate dehydrogenase complex subunit PDH-E3I
TGGT1_202970	-0.70		0.35	0.92	0.40	0.60	1.65	0.62	0.56	0.84	hypothetical protein
TGGT1_360830	-0.12		1.00	1.06	0.50	0.81	1.60	0.85	0.99	0.05	nucleoside-triphosphatase
TGGT1_247350	1.32		0.94	0.82	1.39	1.37	1.60	0.85	0.99	0.05	thioredoxin domain-containing protein
TGGT1_296020	-2.55		1.64	1.12	1.24	1.54	1.60	0.85	0.99	0.05	hypothetical protein
TGGT1_254820	1.03		0.50	0.83	1.32	1.60	1.59	0.85	0.99	0.05	hypothetical protein
TGGT1_408600	-2.23		1.40	1.48	0.65	0.94	1.59	0.85	0.99	0.05	hypothetical protein
TGGT1_222270	-0.24		0.80	1.15	1.48	1.61	1.57	0.81	0.96	0.05	hypothetical protein
TGGT1_318430	-1.20		0.38	0.78	1.16	1.23	1.56	0.85	0.99	0.05	malate dehydrogenase MDH
TGGT1_240610	0.92		0.43	0.87	1.34	1.45	1.55	0.47	0.76	0.05	hypothetical protein
TGGT1_240410	-0.36		0.75	0.78	0.75	0.96	1.55	0.85	0.99	0.05	protein kinase (incomplete catalytic triad)
TGGT1_285170	-0.03		0.16	0.97	1.30	1.60	1.54	0.67	0.86	0.05	putative ribosomal RNA large subunit methyltransferase I
TGGT1_289210	0.04		1.03	1.33	1.12	1.06	1.52	0.57	0.56	0.92	prefoldin subunit protein
TGGT1_297245	0.41		0.21	0.71	1.54	1.26	1.52	0.85	0.99	0.05	transporter, major facilitator family protein
TGGT1_200700	1.75		0.73	0.86	0.96	1.50	1.51	0.85	0.99	0.05	Toxoplasma gondii family C protein
TGGT1_217020	3.48		0.09	0.88	1.36	0.46	1.51	0.33	0.32	0.77	ATPase, AFG1 family protein
TGGT1_245670	-0.11		-0.49	-0.03	2.33	1.55	1.50	0.81	0.96	0.05	pyruvate dehydrogenase complex subunit PDH-E1Alpha
TGGT1_317720	0.24		0.71	0.98	0.73	0.92	1.49	0.71	0.82	0.79	putative eukaryotic translation initiation factor 3 subunit 7
TGGT1_214540	-1.99		0.29	0.76	1.23	1.25	1.47	0.85	0.99	0.05	hypothetical protein
TGGT1_213460	0.82		0.78	0.56	1.06	3.04	1.47	0.61	0.83	0.05	hypothetical protein
TGGT1_290580	0.55		0.27	0.84	1.67	1.93	1.46	0.44	0.70	0.05	ATP-binding cassette G family transporter ABCG89
TGGT1_231990	-0.49		1.17	1.42	1.10	1.12	1.42	0.11	-0.06	0.78	hypothetical protein
TGGT1_212735	0.05		0.87	0.76	1.17	1.32	1.41	0.85	0.99	0.05	hypothetical protein
TGGT1_211030	1.52		0.83	0.95	1.76	1.74	1.41	0.46	0.73	0.05	hypothetical protein
TGGT1_211060	0.21		1.01	1.36	0.66	0.53	1.40	0.18	-0.02	0.70	hypothetical protein
TGGT1_309860	0.87		1.00	1.24	0.77	1.39	1.39	0.62	0.76	0.26	hypothetical protein
TGGT1_222410	1.10		1.02	1.28	1.14	1.07	1.39	0.38	0.38	0.95	hypothetical protein
TGGT1_244700	3.33		0.27	0.43	0.20	0.50	1.39	0.74	0.96	0.21	NAD(+)/NADH kinase domain-containing protein
TGGT1_306470	1.49		0.77	0.88	1.00	1.07	1.39	0.85	0.99	0.05	isoprenylcysteine carboxyl methyltransferase (icmt) family protein
TGGT1_252210	-0.03		0.15	0.21	0.32	1.35	1.36	0.85	0.99	0.05	pentatricopeptide repeat domain-containing protein
TGGT1_322110	2.95			0.33	0.45	1.16	1.35	0.85	0.99	0.05	hypothetical protein
TGGT1_315900	1.44		0.76	0.78	0.46	0.90	1.35	0.67	0.73	0.21	hypothetical protein
TGGT1_278630	0.85		0.81	0.94	1.08	1.31	1.33	0.85	0.99	0.05	tetratricopeptide repeat-containing protein
TGGT1_230980	0.97		0.62	0.67	0.69	1.33	1.33	0.85	0.99	0.05	myosin I
TGGT1_248900	-0.51		0.90	0.87	0.53	1.16	1.32	0.85	0.99	0.05	hypothetical protein
TGGT1_247510	0.27		0.81	0.92	0.86	1.07	1.32	0.72	0.94	0.32	fructose-bisphospatase II
TGGT1_294990	0.88		0.83	1.02	0.60	0.90	1.31	0.85	0.99	0.05	hypothetical protein
TGGT1_361030	-1.74		0.91	0.98	0.91	1.16	1.30	0.85	0.99	0.06	hypothetical protein
TGGT1_252280	0.76		0.31	0.73	0.81	1.20	1.29	0.85	0.99	0.05	hypothetical protein
TGGT1_282150	0.27		0.99	0.84	1.30	1.53	1.29	0.51	0.76	0.05	hypothetical protein
TGGT1_240600	0.73		0.16	0.84	1.46	1.57	1.29	0.55	0.79	0.05	putative chaperonin cpn60
TGGT1_203450	0.84		0.62	0.96	0.97	0.87	1.29	0.86	0.84	0.40	DUF3228 domain-containing protein
TGGT1_273800	-0.72		0.73	0.84	0.51	1.11	1.29	0.85	0.99	0.05	WD domain, G-beta repeat-containing protein
TGGT1_292920	0.28		0.25	0.50	0.91	1.20	1.27	0.54	0.79	0.05	putative heat shock protein 75
TGGT1_286720	1.08		0.72	1.07	1.09	1.22	1.26	0.85	0.99	0.05	heat shock protein HSP28
TGGT1_305050	0.84		1.06	1.17	1.06	0.93	1.25	0.18	0.07	0.90	putative calmodulin
TGGT1_231140	0.36		0.75	0.90	0.95	1.04	1.25	0.85	0.99	0.05	ribosomal protein RPS25
TGGT1_409260	-0.24		0.19	0.44	1.16	1.50	1.24	0.75	0.91	0.05	hypothetical protein
TGGT1_267500	-0.28		1.05	1.21	0.64	0.55	1.23	0.31	0.14	0.74	hypothetical protein
TGGT1_230830	-1.41		0.31	0.78	0.79	1.13	1.23	0.82	0.97	0.05	ATPase family associated with various cellular activities (AAA) domain-containi
TGGT1_268010	-0.54			0.16	0.46	0.67	1.23	0.85	0.99	0.05	hypothetical protein
TGGT1_221470	0.95		0.68	0.71	1.04	1.32	1.22	0.85	0.99	0.05	hypothetical protein
TGGT1_219700	0.99			0.40	1.10	1.16	1.21	0.84	0.99	0.05	putative DNA replication licensing factor MCM4
TGGT1_264080	-0.48		0.08	0.40	1.50	1.30	1.21	0.62	0.84	0.05	acyl carrier protein ACP
TGGT1_265010	0.19		0.73	0.89	0.93	1.02	1.21	0.68	0.84	0.48	glutamate 5-kinase domain-containing protein
TGGT1_275770	0.26		0.99	1.16	0.68	0.88	1.20	0.42	0.30	0.80	hypothetical protein
TGGT1_262910	0.08		0.78	1.01	0.82	0.99	1.16	0.71	0.86	0.72	putative NADH-cytochrome b5 reductase 1
TGGT1_213350	0.34		0.50	0.83	0.90	1.21	1.16	0.79	0.94	0.05	ribosomal protein RPS15
TGGT1_231890	-0.03		0.18	0.68	1.34	1.41	1.16	0.49	0.75	0.05	putative beta-ketoacyl-acyl carrier protein synthase III
TGGT1_294980	0.72		0.60	0.78	0.42	0.73	1.15	0.85	0.99	0.05	hypothetical protein
TGGT1_292290			1.01	0.92	0.68	1.13	1.15	0.68	0.80	0.40	hypothetical protein
TGGT1_245746A	-1.84		0.80	0.84	0.49	0.68	1.14	0.50	0.41	0.77	hypothetical protein
TGGT1_258470	-0.12		0.83	0.79	0.61	1.17	1.13	0.65	0.82	-0.06	hypothetical protein

TGGT1_202850	-0.75		-0.39	-0.79	-0.86	-1.82	-1.68	0.62	0.83	0.05	ATP-binding domain-containing protein
TGGT1_227630			0.32	-0.69	-1.35	-1.01	-1.68	0.76	0.92	0.05	hypothetical protein
TGGT1_237210	-0.79		-0.28	-0.65	-0.93	-1.19	-1.68	0.83	0.93	0.05	Tyrosine kinase-like (TKL) protein
TGGT1_208760	0.47		-0.36	-0.74	-0.54	-0.64	-1.12	0.85	0.95	0.05	hypothetical protein
TGGT1_263110	-1.34		-0.27	-0.37	-0.79	-1.22	-1.13	0.76	0.92	0.05	hypothetical protein
TGGT1_232240	-1.39		-0.72	-0.57	-0.88	-0.75	-1.13	0.85	0.95	0.05	hypothetical protein
TGGT1_239290	-2.20		-0.66	-0.36	-0.17	-0.59	-1.14	0.85	0.95	0.05	hypothetical protein
TGGT1_243382	0.24		-0.57	-0.20	-0.38	0.68	-1.14	0.92	0.97	0.23	hypothetical protein
TGGT1_277070	-1.64		-0.59	-0.63	-0.98	-1.25	-1.14	0.81	0.96	0.05	SWI2/SNF2-containing protein
TGGT1_272680	-0.19		-0.30	-0.54	-0.60	-0.87	-1.14	0.85	0.95	0.05	hypothetical protein
TGGT1_209040	-0.17			-0.71	-1.00	-0.83	-1.15	0.68	0.70	0.33	hypothetical protein
TGGT1_248330	-0.29		-0.41	-0.45	-0.54	-1.17	-1.15	0.85	0.95	0.05	zinc finger, C3HC4 type (RING finger) domain-containing protein
TGGT1_312930	0.86			-0.71	-0.82	-0.65	-1.15	0.85	0.95	0.05	putative cystathione gamma lyase
TGGT1_262825	-0.73		-0.21	-0.50	-1.28	-1.30	-1.15	0.44	0.71	0.05	peptidase family c50 protein
TGGT1_206650	-0.98		-0.64	-0.74	-0.78	-0.61	-1.16	0.85	0.95	0.05	zinc finger, c2h2 type domain-containing protein
TGGT1_271090	-0.09		-0.40	-1.14	-0.76	-1.04	-1.17	0.30	0.40	0.84	hypothetical protein
TGGT1_236960	-0.67		-0.71	-0.85	-0.36	-0.19	-1.17	0.37	0.25	0.94	transporter, major facilitator family protein
TGGT1_221500	-0.83		-0.78	-0.74	-0.65	-0.89	-1.17	0.65	0.71	0.64	dual specificity phosphatase, catalytic domain-containing protein
TGGT1_232420	0.30		-0.21	-0.50	-0.91	-1.89	-1.17	0.85	0.95	0.05	hypothetical protein
TGGT1_280530	-0.41		-0.49	-0.45	-0.71	-1.97	-1.18	0.85	0.95	0.05	hypothetical protein
TGGT1_242720	-0.94		-0.81	-0.89	-0.74	-0.88	-1.20	0.68	0.84	0.60	aspartyl protease ASP5
TGGT1_246010	-1.54		-0.80	-0.77	-0.74	-0.94	-1.20	0.75	0.96	0.25	hypothetical protein
TGGT1_321480	0.16		-0.33	-0.33	-0.93	-0.97	-1.23	0.85	0.95	0.05	SAG-related sequence SRS12B
TGGT1_294400	-1.17		0.05		-0.47	-0.38	-1.23	0.85	0.95	0.05	hypothetical protein
TGGT1_277950	-1.20		-0.46	-0.72	-0.73	-0.89	-1.24	0.85	0.95	0.05	lipase
TGGT1_301470	-0.50		-0.74	-0.60	-0.54	-0.59	-1.24	0.78	0.96	0.12	hypothetical protein
TGGT1_273280	0.43		-0.11	-0.21	-0.98	-1.47	-1.24	0.68	0.87	0.05	hypothetical protein
TGGT1_318610	-0.39			-0.76	-0.73	-0.52	-1.26	0.85	0.95	0.05	AP2 domain transcription factor AP2IV-3
TGGT1_246160			-0.53	-0.80	-0.80	-1.20	-1.26	0.79	0.95	0.05	hypothetical protein
TGGT1_206440	-0.96		-0.70	-0.62	-0.78	-1.45	-1.27	0.79	0.92	0.03	cpw-wpc domain-containing protein
TGGT1_245600			-0.50	-0.65	-1.04	-1.02	-1.27	0.85	0.95	0.05	hypothetical protein
TGGT1_301000	-0.11		-0.37	-0.55	-0.41	-0.33	-1.27	0.68	0.82	0.44	methyltransferase
TGGT1_238210	-2.18		-0.61	-1.15	-0.12	0.22	-1.28	0.43	0.29	0.77	EGF family domain-containing protein
TGGT1_218290	0.22		-0.60	-0.67	-0.66	-0.24	-1.28	0.33	0.15	0.75	WD domain, G-beta repeat-containing protein
TGGT1_305610	-0.14		-0.42	-0.34	-0.42	-0.67	-1.29	0.85	0.95	0.05	hypothetical protein
TGGT1_243330	-0.27		-0.80	-0.52	-0.59	0.29	-1.29	0.87	0.96	0.14	hypothetical protein
TGGT1_214430	-1.28		0.14	-0.55	-1.37	-1.18	-1.30	0.72	0.89	0.05	hypothetical protein
TGGT1_218510	0.41		-0.21	-0.31	-0.51	-1.13	-1.30	0.85	0.95	0.05	hypothetical protein
TGGT1_208380	0.17		0.23	-0.81	-1.16	-0.80	-1.30	0.85	0.95	0.05	hypothetical protein
TGGT1_306510	-0.99		-0.59	-0.82	-0.76	-0.93	-1.32	0.75	0.97	0.23	hypothetical protein
TGGT1_294240	-1.74		-0.82	-0.36	-0.14	0.80	-1.33	0.85	0.95	0.05	hypothetical protein
TGGT1_262110	-0.26		-0.31	-0.86	-0.46		-1.35	0.26	0.11	0.91	hypothetical protein
TGGT1_267490	-1.55		0.19	-0.61	-1.01	-1.44	-1.35	0.81	0.96	0.05	putative preprocathepsin c precursor
TGGT1_227875	-0.44		-0.79	-0.74	-0.76	-1.97	-1.35	0.85	0.95	0.05	hypothetical protein
TGGT1_203300			-0.29	-0.85	-1.02	-0.85	-1.36	0.85	0.95	0.05	hypothetical protein
TGGT1_225105	-0.98			-0.87	-1.05	-1.11	-1.36	0.84	0.96	0.05	hypothetical protein
TGGT1_314370	-1.11		-0.52	-0.72	-0.76	-1.11	-1.36	0.85	0.95	0.05	hypothetical protein
TGGT1_217860	-1.22		-0.87	-0.81	-0.60	0.39	-1.36	0.85	0.95	0.05	hypothetical protein
TGGT1_226220	-0.58		-0.51	-0.84	-0.99	-1.35	-1.36	0.83	0.96	0.05	alveolin domain containing intermediate filament IMC9
TGGT1_216200	-0.65			-0.46	-0.76	-0.74	-1.37	0.85	0.95	0.05	hypothetical protein
TGGT1_225280	0.12		-0.18	-0.89	-1.06	-0.45	-1.37	0.32	0.33	0.94	hypothetical protein
TGGT1_270670	-1.68		-0.81	-0.86	-0.76	-0.96	-1.37	0.69	0.91	0.34	hypothetical protein
TGGT1_318240	-0.13		-0.71	-0.45	-0.33	1.53	-1.39	0.85	0.95	0.05	Tubulin-tyrosine ligase family protein
TGGT1_269020	-0.90		-0.66	-0.87	-0.72	-0.28	-1.39	0.50	0.43	0.91	hypothetical protein
TGGT1_209410			-0.13	-0.74	-1.17	-1.08	-1.41	0.85	0.95	0.05	hypothetical protein
TGGT1_319360	0.80		-0.66	-0.74	-0.56	-0.98	-1.42	0.85	0.95	0.05	SAG-related sequence SRS17A
TGGT1_277055	-1.17		-0.73	-1.00	-1.05	-1.20	-1.43	0.85	0.95	0.05	hypothetical protein
TGGT1_236880	-0.89		-0.10	-1.01	-1.03	-1.05	-1.43	0.85	0.95	0.05	hypothetical protein
TGGT1_247540	1.87		-0.41	-0.66	-0.61	-0.32	-1.44	0.85	0.95	0.05	ATP-binding cassette G family transporter ABCG107
TGGT1_223870	-0.58		-0.80	-0.84	-1.06	-0.79	-1.47	0.85	0.95	0.05	hypothetical protein
TGGT1_323200	-1.40		-0.49	-1.03	-0.96	0.95	-1.48	0.75	0.73	0.52	OTU family cysteine protease
TGGT1_222935	-0.38		-0.53	-0.69		0.14	-1.49	0.85	0.95	0.05	hypothetical protein
TGGT1_273980			-0.48	-1.09	-1.45	-1.70	-1.49	0.45	0.72	0.05	hypothetical protein
TGGT1_213340	-2.10		-0.31	-1.12	-1.49	-1.19	-1.51	0.42	0.71	0.05	GMC oxidoreductase
TGGT1_411040	-0.57		-0.63	-0.99	-0.86	-0.93	-1.52	0.85	0.95	0.05	putative IgA-specific serine endopeptidase
TGGT1_319090	-0.91		-0.76	-1.04	-0.68	-0.72	-1.53	0.85	0.95	0.05	IgA-specific serine endopeptidase
TGGT1_293258	0.42		-0.33	-0.62	-0.79	-1.11	-1.53	0.85	0.95	0.05	hypothetical protein
TGGT1_201180A	0.27		-0.36	-0.72	-1.04	-1.02	-1.53	0.75	0.94	0.10	hypothetical protein
TGGT1_224710	0.18		-0.12	-0.79	-1.09	-1.16	-1.54	0.85	0.95	0.05	hypothetical protein
TGGT1_357300	0.15		-0.52	-0.44	-0.63	-0.79	-1.55	0.85	0.95	0.05	hypothetical protein
TGGT1_206660	-1.12			-0.81	-1.37	-1.42	-1.56	0.73	0.90	0.05	hypothetical protein
TGGT1_219660	-1.94		-0.90	-0.63	-0.83	-0.30	-1.56	0.85	0.95	0.05	hypothetical protein
TGGT1_301240			-0.76	-0.72	-0.15	-0.24	-1.56	0.85	0.95	0.05	hypothetical protein
TGGT1_267410	-2.05		-0.27	-1.36	-1.32	-1.47	-1.58	0.52	0.78	0.05	scavenger receptor protein SR1 precursor
TGGT1_229350	-1.99		-0.87	-1.14	-0.79	-0.96	-1.58	0.63	0.70	0.74	HEAT repeat-containing protein
TGGT1_267670	-1.50		-0.63	-0.88	-0.65	-0.76	-1.58	0.85	0.95	0.05	hypothetical protein
TGGT1_286460	0.22		-0.80	-0.87	-0.68	-0.89	-1.59	0.78	0.92	0.54	hypothetical protein
TGGT1_227350	-4.06		-1.11	-1.05	-1.17	-2.19	-1.59	0.66	0.84	0.03	hypothetical protein
TGGT1_204045	0.11		-0.71	-1.03	-0.58	-0.58	-1.60	0.69	0.70	0.92	hypothetical protein
TGGT1_270680	-1.24		-1.14	-1.06	-0.97	-1.12	-1.61	0.85	0.95	0.06	hypothetical protein
TGGT1_233210	-1.55		-0.19	-0.70	-1.24	-0.98	-1.62	0.85	0.95	0.05	hypothetical protein
TGGT1_250360	-0.59		-0.79	-1.09	-1.09	-0.68	-1.63	0.92	0.96	0.25	alpha/beta hydrolase fold domain-containing protein
TGGT1_293500	2.23		-0.58	-1.16	-0.93	0.59	-1.66	0.85	0.95	0.05	hypothetical protein
TGGT1_225190	-0.73		-0.66	-1.03	-1.31	-1.46	-1.69	0.85	0.95	0.05	hypothetical protein
TGGT1_209300	-0.22		-0.48	-1.05	-1.38	-1.19	-1.69	0.85	0.95	0.05	WD domain, G-beta repeat-containing protein
TGGT1_271892	-1.32		-0.45	-1.21	-1.11	-0.51	-1.70	0.85	0.95	0.05	hypothetical protein
TGGT1_205658	-2.11		-0.24	-0.87	-1.01	-0.14	-1.71	0.85	0.95	0.05	F5/8 type C domain-containing protein
TGGT1_218210	-0.17		-0.28	-0.86	-0.89	-0.61	-1.74	0.71	0.81	0.81	putative Chromosome-associated kinesin KLP1
TGGT1_218340	-0.83		-0.83	-1.09	-0.97	0.97	-1.76	0.85	0.95	0.05	hypothetical protein
TGGT1_240370	-2.83		-0.31	-0.47	-1.14	-0.27	-1.76	0.85	0.95	0.05	Toxoplasma gondii family E protein
TGGT1_243940	-0.70		-0.15	-0.70	-1.10	-0.95	-1.77	0.85	0.95	0.05	hypothetical protein
TGGT1_316130	0.27		-0.84	-1.11	-1.04		-1.78	0.85	0.95	0.05	hypothetical protein
TGGT1_318675	0.78		-0.12	-1.06	-1.09	-0.54	-1.79	0.85	0.95	0.05	3'5'-cyclic nucleotide phosphodiesterase domain-containing protein
TGGT1_208020	-1.32		-0.41	-0.87	-1.17	-1.38	-1.79	0.85	0.95	0.05	AP2 domain transcription factor AP2Ib-1
TGGT1_240730	-1.13		-1.01	-1.09	-0.71	-0.84	-1.79	0.69	0.90	0.41	hypothetical protein
TGGT1_269640	-0.42		-0.90	-1.39	-1.30	-1.14	-1.82	0.78	0.97	0.27	hypothetical protein
TGGT1_281675	-1.85		-0.45	-0.72	-1.27	-1.84	-1.86	0.85	0.95	0.05	putative protein kinase
TGGT1_203682	-2.51		-1.06	-1.64	-0.80	-0.35	-1.86	0.22	0.03	0.78	hypothetical protein
TGGT1_276980	0.12		-1.08	-1.20	-0.65	-0.50	-1.90	0.58	0.50	0.83	hypothetical protein
TGGT1_202050	-1.15		-0.53	-0.76	-1.25	-0.68	-1.91	0.85	0.95	0.05	hypothetical protein

TGGT1_254890	-1.38		-0.66	-1.35	-1.42	-1.12	-1.93	0.85	0.00	0.05	hypothetical protein
TGGT1_201230	-1.47		-0.68	-1.22	-1.54	-1.49	-1.97	0.85	0.00	0.05	kinesin motor domain-containing protein
TGGT1_246070	-1.25		-1.87	-1.38	-0.95	0.21	-1.99	0.85	0.00	0.05	SAG-related sequence SRS56
TGGT1_274170	-0.65		-3.87	-1.39	-0.79	-0.31	-2.00	0.52	0.43	0.01	protein kinase (incomplete catalytic triad)
TGGT1_409630	-2.74		-0.60	-0.66	-1.05	-0.32	-2.01	0.85	0.00	0.05	toxoplasma gondii family E protein
TGGT1_315345	-1.25		-0.59	-0.76	-1.58	-2.27	-2.02	0.63	0.84	0.05	SAG-related sequence SRS52F
TGGT1_286020	-0.67		-4.98	-1.29	-1.27	-1.27	-2.03	0.85	0.00	0.05	hypothetical protein
TGGT1_249150	0.43		-0.37	-0.95	-1.17	-0.58	-2.09	0.85	0.00	0.05	PAN domain-containing protein
TGGT1_233180	-1.32		-1.20	-1.22	-1.00	-0.84	-2.10	0.85	0.00	0.05	hypothetical protein
TGGT1_311710	-1.56		-0.19	-1.37	-1.83	-2.14	-2.15	0.73	0.90	0.05	hypothetical protein
TGGT1_240930	-1.29		-0.97	-1.05	-1.10	-1.21	-2.16	0.85	0.00	0.05	MoaC family protein
TGGT1_227370	-1.54		-0.24	-1.23	-1.55	-0.50	-2.22	0.85	0.00	0.05	hydrolase CocE/NonD family protein
TGGT1_228160	-1.68		-0.53	-1.25	-1.51	-1.74	-2.34	0.85	0.00	0.05	acid phosphatase
TGGT1_294410	0.29		-0.70	-1.26	-1.45	0.31	-2.37	0.85	0.00	0.05	hypothetical protein
TGGT1_257670	-0.48		-0.49	-1.00	-1.66	-2.00	-2.43	0.85	0.00	0.05	hypothetical protein
TGGT1_215775	0.69		-0.39	-1.23	-1.78	-1.54	-2.48	0.85	0.00	0.05	rhoptry protein ROP8
TGGT1_312690	-1.02		-1.15	-1.76	-1.20	1.04	-2.49	0.41	0.34	0.00	hypothetical protein
TGGT1_236595	-0.97		-0.92	-1.09	-1.01	-0.88	-2.53	0.75	0.87	0.15	ankyrin repeat-containing protein
TGGT1_266270	-0.83		-4.19	-1.38	-0.90	-0.32	-2.56	0.85	0.00	0.05	short-chain-acyl-CoA dehydrogenase
TGGT1_235390	-1.06		-0.74	-1.39	-1.54	-0.96	-2.57	0.79	0.89	0.19	PAN domain-containing protein
TGGT1_313940	-1.52		-0.91	-1.23	-2.12	-2.27	-2.65	0.84	0.00	0.05	hypothetical protein
TGGT1_277060	0.52		-0.68	-1.50	-1.57	-1.59	-2.67	0.85	0.00	0.05	hypothetical protein
TGGT1_410610	1.80		-0.57	-1.85	-1.89	-1.16	-2.76	0.82	0.92	0.08	hypothetical protein
TGGT1_314250	1.86		-0.32	-1.53	-2.04	-1.55	-2.77	0.85	0.00	0.05	bradyzoite rhoptry protein BRP1
TGGT1_411220	0.40		-0.80	-1.48	-2.40	-1.73	-2.82	0.82	0.92	0.05	hypothetical protein
TGGT1_218192	-2.72		-1.71	-1.90	-1.30	-0.76	-2.85	0.64	0.69	0.73	hypothetical protein
TGGT1_203800	-1.28		-1.49	-1.75	-1.83	-0.89	-2.95	0.88	0.84	0.54	hypothetical protein
TGGT1_289930	-0.70		-0.70	-1.34	-1.68	-0.87	-2.98	0.85	0.00	0.05	putative phosphoenolpyruvate carboxykinase
TGGT1_306455	1.40		-0.56	-2.39	-2.30	-1.05	-2.99	0.23	0.23	0.00	hypothetical protein
TGGT1_282200	-1.30		-1.25	-1.97	-1.73	-1.51	-3.44	0.85	0.00	0.05	ATPase, AAA family protein
TGGT1_237585	-0.68		-0.96	-2.15	-2.28	-1.71	-3.59	0.85	0.00	0.05	hypothetical protein
TGGT1_291910	-2.80		-1.62	-1.95	-1.43	-1.12	-3.98	0.69	0.83	0.66	hypothetical protein
TGGT1_306450	1.82		-1.51	-2.73	-2.20	-1.08	-3.99	0.30	0.18	0.02	putative short chain dehydrogenase family protein
TGGT1_311700	-1.92		-1.43	-2.57	-2.23	-2.25	-4.02	0.90	0.96	0.22	hypothetical protein
TGGT1_291920	-2.90		-1.78	-2.18	-1.62	-1.17	-4.24	0.69	0.81	0.76	hypothetical protein

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