

SUPPLEMENTARY FIGURES

S1.

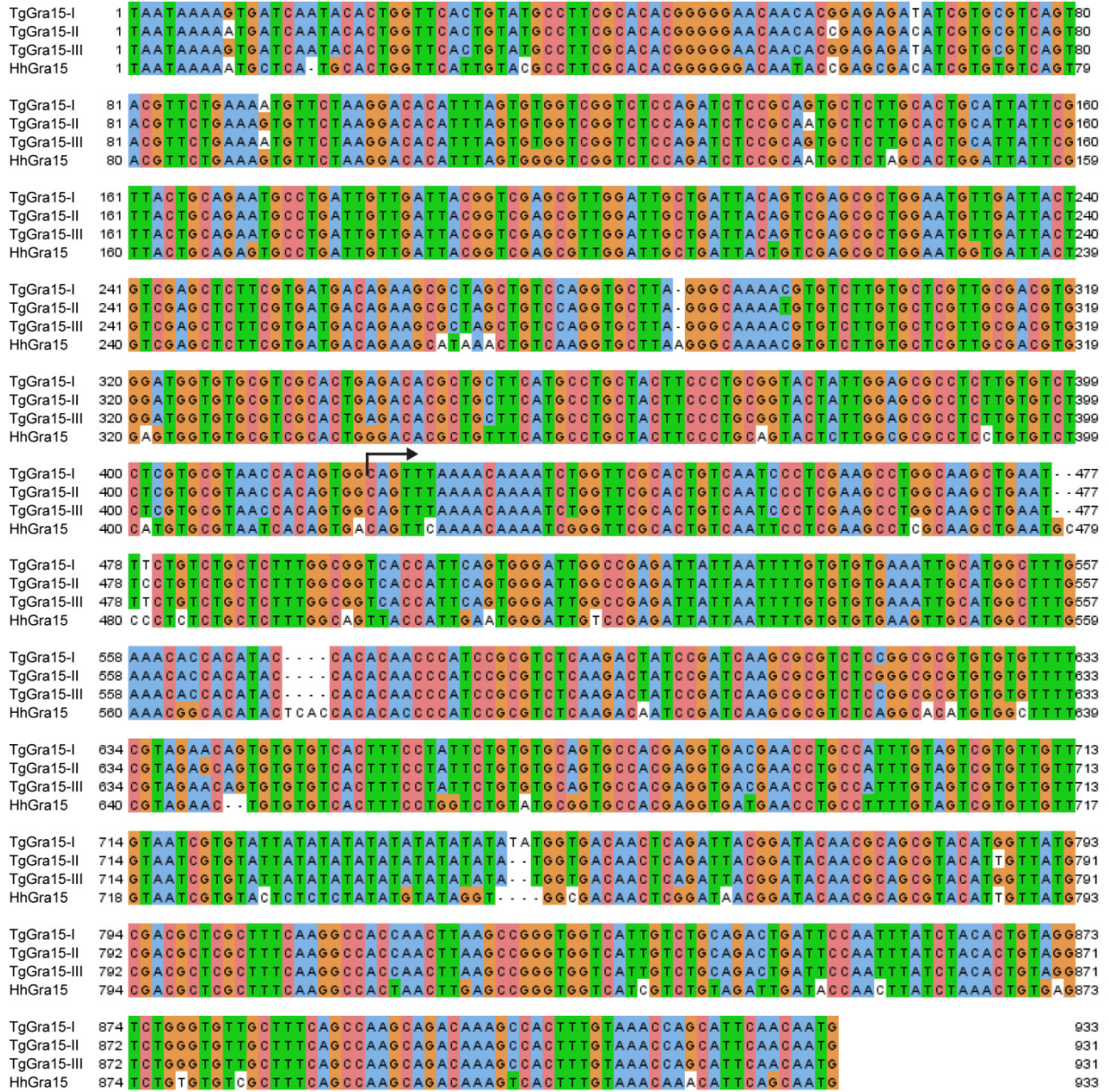


Figure S1: Alignment of the predicted promoters of *GRA15* in *T. gondii*-I,II,III and *H. hammondi*. These are the same upstream regions used in luciferase reporter assays. An arrowhead indicates the putative transcriptional start site.

S2.

TgGra15-I	1	MVT TTTPT P P P G A P A V V P I F D V V Y Q L N P H V F R S R F S R R N R A R R V V S S K S R S I I R W L G Y L T	60
TgGra15-II	1 - A	59
TgGra15-III	1 V	60
HhGra15	1 R D M Q H G A K S	60
TgGra15-I	61	V L A A V I L L G A Y A V R R L S R D L S D S V R E T R R G R R I T G S V P P G T T R P R S E S C T G T Q V D G G C G A	120
TgGra15-II	60 V	119
TgGra15-III	61	120
HhGra15	61 I P Q A G P A H S L N A S N Y V	120
TgGra15-I	121	D T S T D G K S E S E Q T E N G E D S R F S T R T P I H V T A S T S P F A T R K A A E E R S S S P R D R K V P E G A Q L	180
TgGra15-II	120	179
TgGra15-III	121	180
HhGra15	121	E A T F R A I T P S Q K H I S R	180
TgGra15-I	181	P T S S T P H A Q R K D S G S D S R N P S T L I P S P G T N T F N M N F Y I I G A G S S A L D F I F P H T P D A Q A T V	240
TgGra15-II	180	239
TgGra15-III	181	240
HhGra15	181	T P T P T D N D S S S V L R T A	240
TgGra15-I	241	V S P P R S A A A A P T V E T V P R V R T Y S T P T T L L P T A P A T A T S N H M H A S A T P S P P E R P Q N F R G G	300
TgGra15-II	240	299
TgGra15-III	241 L V	300
HhGra15	241 T P G H T T R P	300
TgGra15-I	301	L M R Q N G M V E G T S L T T T E A G M P A P L Q S P Q H I E T E A R L T Y S N H L K S P H T P E T P T V H S I D P V V	360
TgGra15-II	300	359
TgGra15-III	301 S T	360
HhGra15	301 E P S D I G P L G F P L Q Q F	360
TgGra15-I	361	G T S G H S V A V G S Q S P A G G P P T D S R T P A A L T P T S S S F S H A D S L E T S E H P Q S G P S L H P L I S G I	420
TgGra15-II	360	419
TgGra15-III	361	420
HhGra15	361 S T L I H I S P V	420
TgGra15-I	421	Q D A V Q S Q L P L S Q Q E T L P V V E N A T F F G P Q Q T P P W M D E T A A A A I P L A P S Q P G S R T Q P I S S P H	480
TgGra15-II	420	479
TgGra15-III	421	480
HhGra15	421 K K L E T V T P H T A N	480
TgGra15-I	481	T L L P L S G G V S A V P G P P R T E N P R Q P Q V P G E N S Y Y S V P T E P Y P A Q D M S P L I R G T H S Q T E T V E	540
TgGra15-II	480 S R - - - - -	517
TgGra15-III	481 P L	540
HhGra15	481 P S R G T A S T P G A	540
TgGra15-I	541	C G V N A S S E G L A A G A P S S K S A E N A Q T G Q G A G K S L L P V F L H P Q E Q S P H S M P T L G A G R F G S G E	600
TgGra15-II		
TgGra15-III	541	600
HhGra15	541 V V T E I I S R F P R R	600
TgGra15-I	601	L Q R T I S D P G P Q R A G A T Q A D G I G A G G P R D T Q S A V T P	635
TgGra15-II	518	- - A	550
TgGra15-III	601	635
HhGra15	601 L E S D G	635

Figure S2. Alignment of the predicted protein sequence region of GRA15 in *T. gondii*-I,II,III and *H. hammondi*. Polymorphisms unique to types II and III are indicated in green and blue, respectively. There are no GRA15 polymorphisms unique to *T. gondii* Type I. Sites in *H. hammondi* GRA15 that are the same as Type II at unique polymorphic sites are also colored green. There were no Type III-specific polymorphisms that were conserved in HhGRA15.

S3.

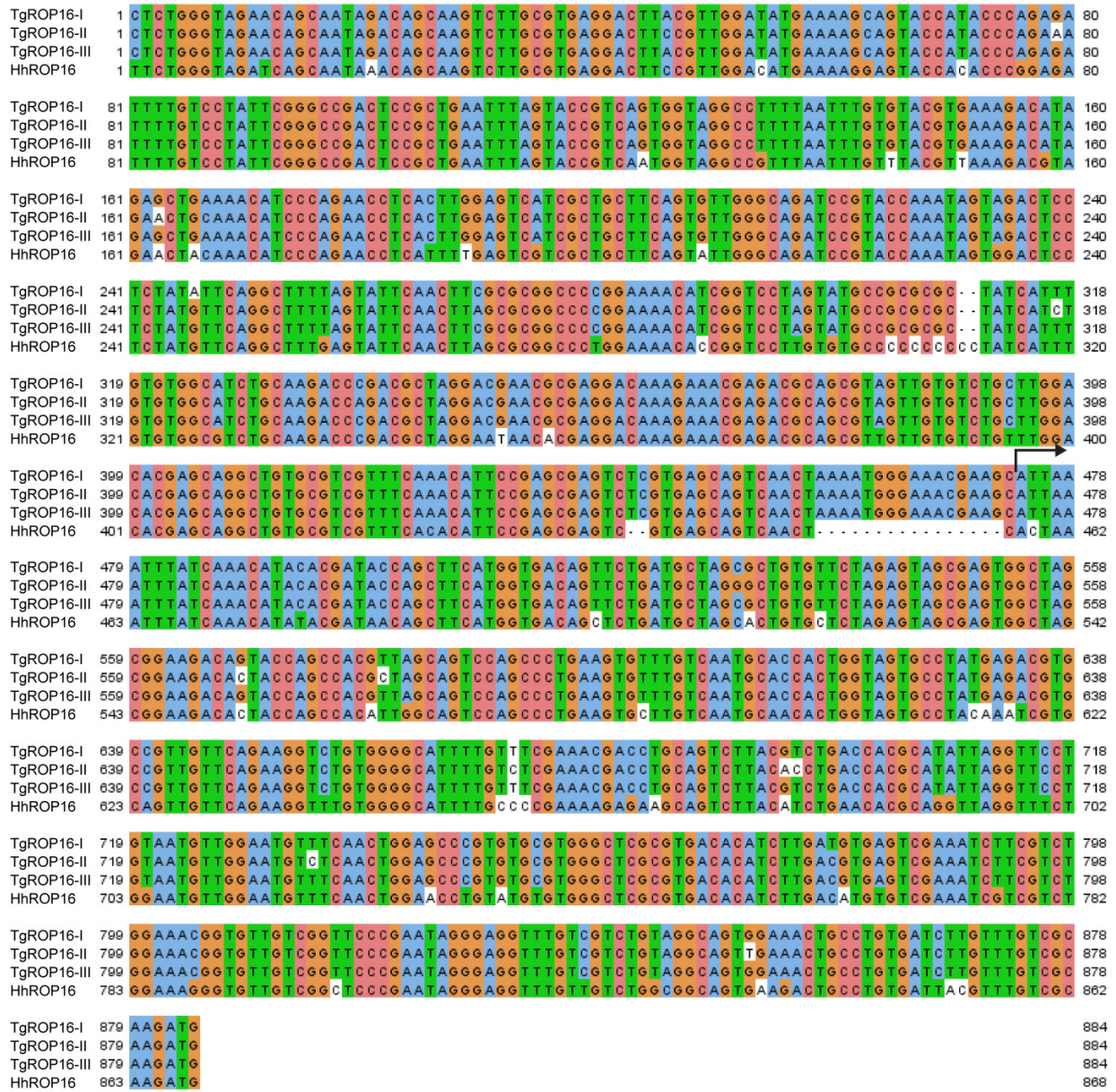


Figure S3. Alignment of the predicted *ROP16* promoters for *T. gondii*-I,II,III and *H. hammondi*. These are the same upstream regions used in luciferase reporter assays. Putative transcriptional start site is indicated by an arrowhead.

S4.

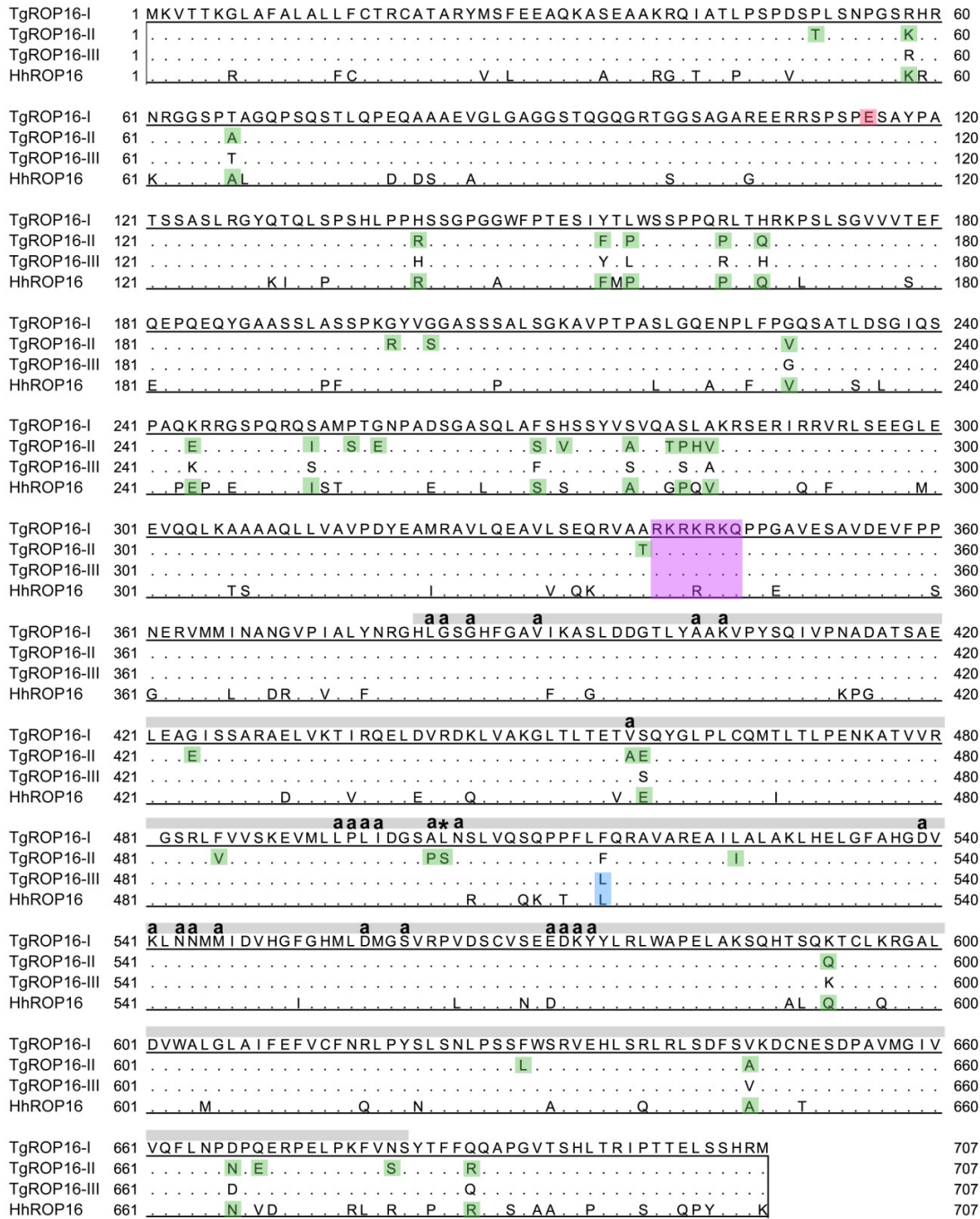


Figure S4. Alignment of the predicted protein sequence region of ROP16 in *T. gondii*-I,II,III and *H. hammondi*. Polymorphisms unique to types I, II and III are indicated in red, green and blue, respectively. At sites with unique polymorphisms in any of the 3 lineages, the *H. hammondi* sequence is also colored red, green or blue if it has the same amino acid at that site. The predicted class 4 NLS is highlighted in magenta. The kinase domain is indicated by the grey bar above the alignment, with active or ATP binding sites indicated by "a". The polymorphic leucine residue required for ROP16-driven STAT3 phosphorylation in TgROP16_{I,III} is indicated by an asterisk.

S5.

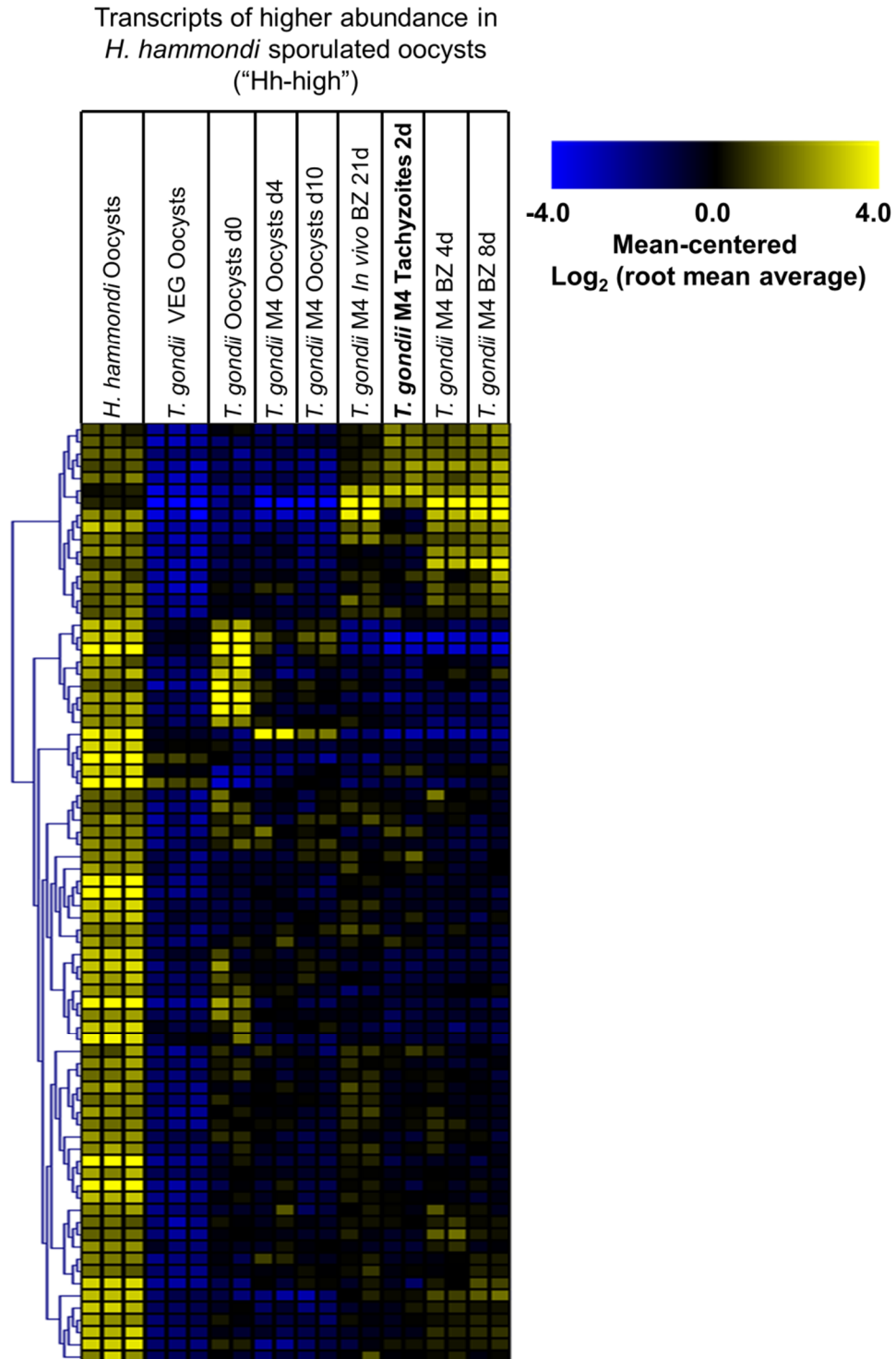


Figure S5. Heatmap of mean-centered, normalized data for *H. hammondi* HhGer041 and *T. gondii* VEG sporozoites showing genes with transcripts that were of higher abundance (FDR $P < 0.01$) in *H. hammondi* compared to *T. gondii* VEG ("Hh-high"). Data from multiple life cycle stages of *T. gondii* strain M4 are shown for comparison. A subset of these genes (top cluster) are developmentally regulated during the tachyzoite to bradyzoite transition in *T. gondii* strain M4.

S6.

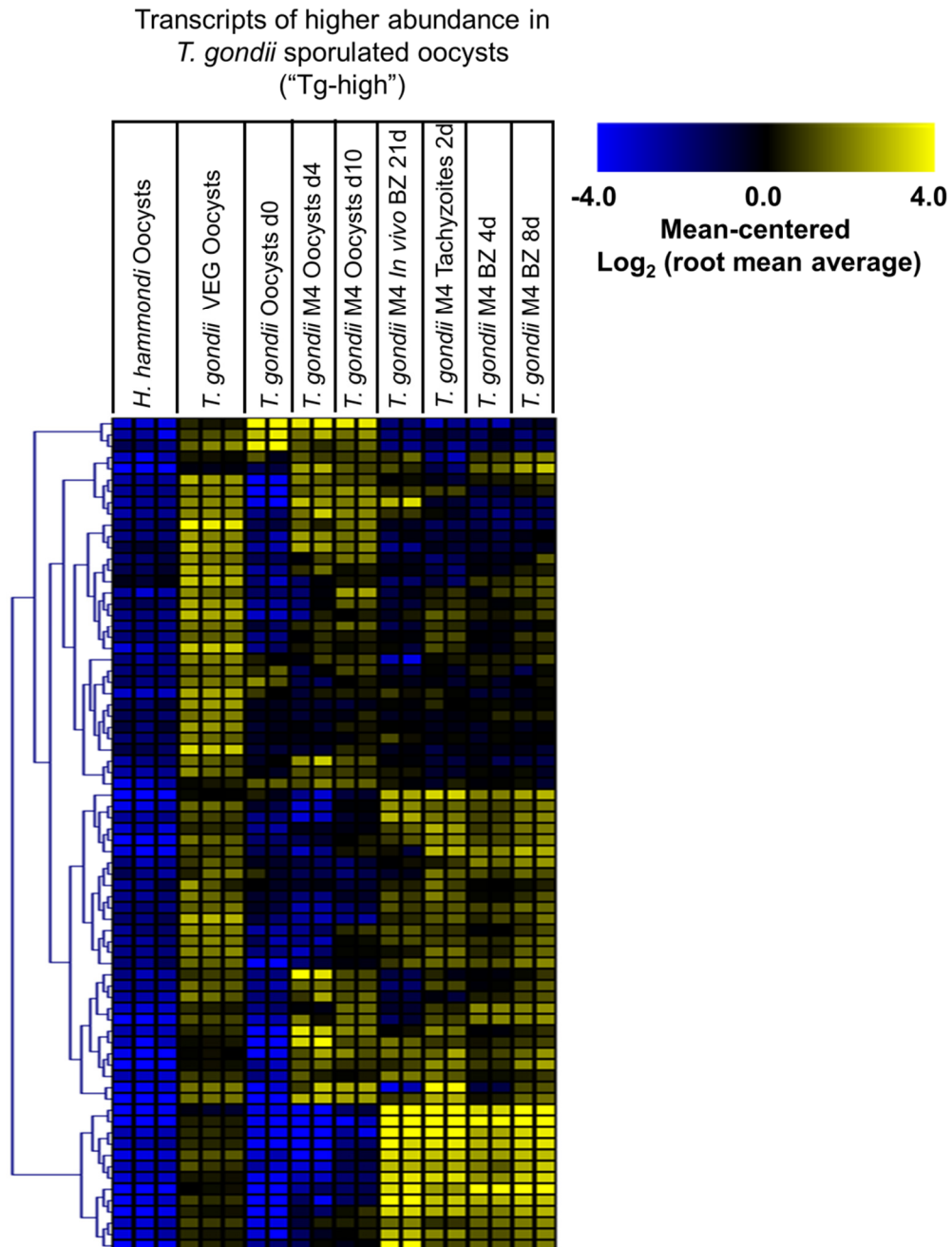


Figure S6. Heatmap of mean-centered, normalized data for *H. hammondi* HhCatGer041 and *T. gondii* VEG sporozoites showing genes with transcripts that were of higher abundance (FDR $P < 0.01$) in *T. gondii* VEG compared to *H. hammondi* ("Tg-high"). Data from multiple life cycle stages of *T. gondii* strain M4 are shown for comparison.

S7.

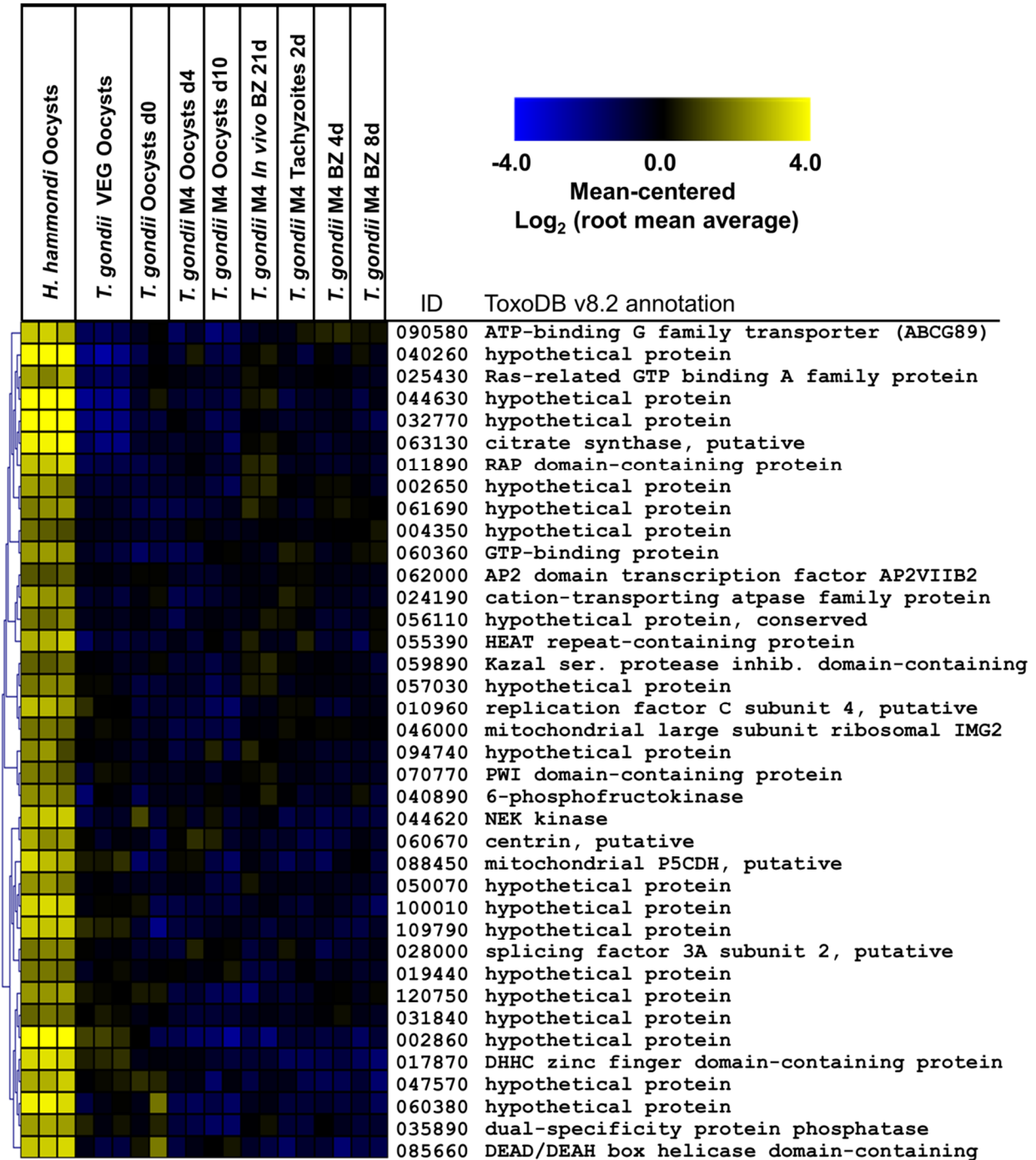


Figure S7. Heatmap of mean-centered, normalized data for *H. hammondi* HhGer041 and *T. gondii* VEG sporozoites showing genes with transcripts that were found to significantly (FDR $P < 1 \times 10^{-5}$) match the template (using Pavlidis Template Matching; see Materials and Methods) of high transcript level in *H. hammondi* sporozoites but lower in *T. gondii* VEG sporozoites and all life stages for *T. gondii* strain M4.

S8.

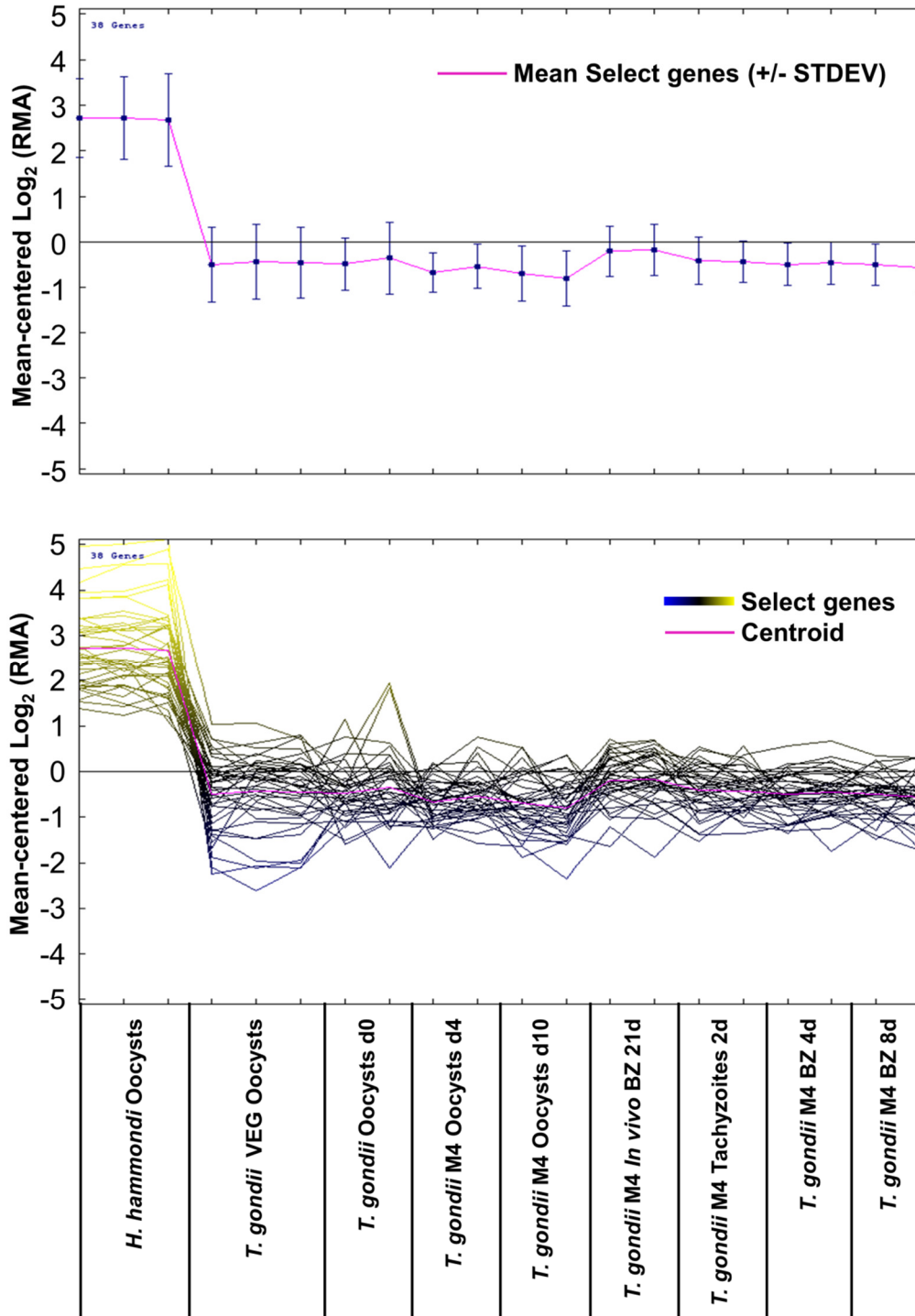


Figure S8. Mean +/- standard deviation (upper panel) or mean-centered hybridization intensity (lower panel) for all 38 genes shown in Fig. S7 in the *H. hammondi* and *T. gondii* sporozoite data obtained in the present study as well as that from the previously published dataset from *T. gondii* strain M4.

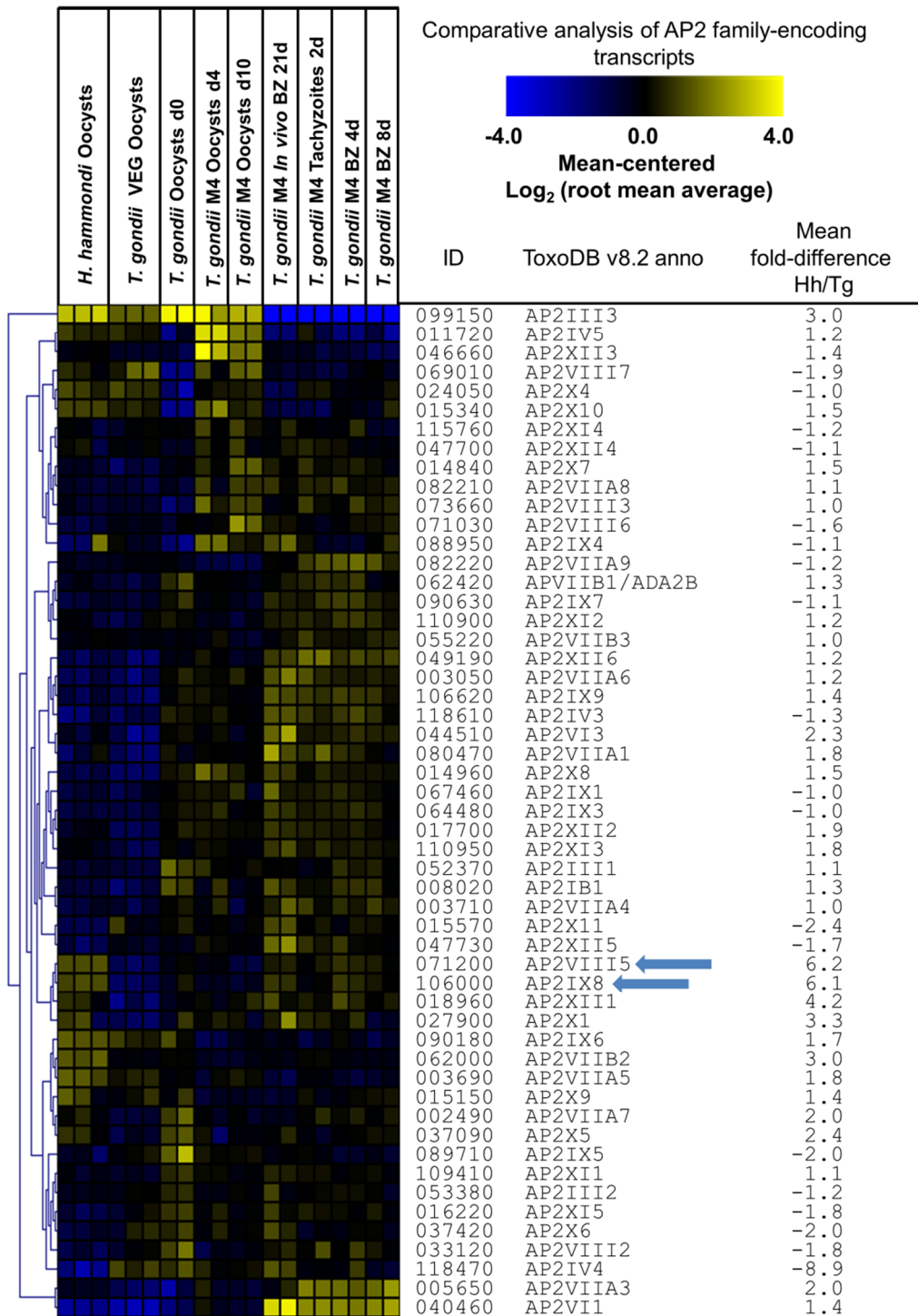


Figure S9. Heatmap of mean-centered, normalized data for *H. Hammondii* HhCatGer041 and *T. gondii* VEG sporozoites showing hybridization data for 53 genes annotated as AP2 transcription factors. Data are shown from multiple life cycle stages of *T. gondii* strain M4 for comparison. Blue arrowheads indicate two genes for which transcript abundance was > 6-fold higher in *H. Hammondii* compared to *T. gondii* VEG (discussed in the manuscript text).

Table S1: Raw luminescence values for *GRA15* and *ROP16* promoter assays

GRA15 Promoter Assays:												
	Rep 1			Rep 2			Rep 3			Rep 4		
Firefly Construct	Firefly	<i>Renilla</i>	F/R*	Firefly	<i>Renilla</i>	F/R	Firefly	<i>Renilla</i>	F/R	Firefly	<i>Renilla</i>	F/R
None[†]	ND	ND	ND	ND	ND	ND	484	1887830	0.0003	722	1093746	0.0007
<i>T. gondii</i> Type II	1547683	160438	9.7	1857258	161149	11.6	556312	44908	12.4	4206383	292684	10.23
<i>H. hammondi</i>	432109	129232	3.3	1402270	571176	2.5	1358314	576972	2.4	2071420	999741	1.9
Fold <i>Tg:Hh</i>			2.9			4.7			5.3			5.4
Fold <i>Hh:None</i>			ND			ND			9183.0			2697.0

ROP16 Promoter Assays:						
	Rep 1			Rep 2		
Firefly Construct	Firefly	<i>Renilla</i>	F/R	Firefly	<i>Renilla</i>	F/R
None[†]	722	1025570	0.0007	564	2671755	0.0002
<i>T. gondii</i> Type II	2394044	1669062	1.4	2540126	1340490	1.9
<i>H. hammondi</i>	68987	1229397	0.056	92930	1289598	0.072
Fold <i>Tg:Hh</i>			25.6			26.3
Fold <i>Hh:None</i>			79.7			341.6

ROP16 Promoter deletion assays:									
	Rep 1			Rep 2			Rep 3		
Firefly Construct	Firefly	<i>Renilla</i>	F/R	Firefly	<i>Renilla</i>	F/R	Firefly	<i>Renilla</i>	F/R
None	ND	ND	ND	203	45993.5	0.004	ND	ND	ND
<i>T. gondii</i> Type II	7350.5	70438	0.099	5416	24946.5	0.217	33167.5	145277	0.228
<i>T. gondii</i> Type IIΔ16 bp	488	20520	0.023	454	16493.5	0.028	6781	137115	0.049
<i>H. hammondi</i>	586.5	57163	0.011	323	12740	0.025	2457	90171.5	0.027
<i>H. hammondi</i> + 16 bp	1438.5	30591	0.047	3160.5	40279.5	0.078	9066.5	78437.5	0.116

*Firefly/*Renilla* signal ratio; [†]Parasites transfected with only the *Renilla* construct.

Table S2. Ten transcripts with the Gene Ontology term GO:0003735, "Structural Constituent of Ribosome" that are of greater abundance in *T. gondii* Sporulated Oocysts compared to *H. Hammondii* Sporulated Oocysts

v8* ID	v7† ID	Genomic Location	Product Description	Mean RMA‡ <i>H. hammondi</i>	Mean RMA <i>T. gondii</i>	Fold-difference <i>Hh/Tg</i>
238010	038010	ME49 chrVI	ribosomal protein RPL23A	6.02	9.79	-13.66
242330	042330	ME49 chrVI	ribosomal protein RPS5	5.20	10.62	-42.95
239760	039760	ME49 chrVI	ribosomal protein RPL22	8.73	13.04	-19.77
238250	038250	ME49 chrVI	ribosomal protein RPL36	5.93	9.49	-11.84
203630	003630	ME49 chrVIIa	ribosomal protein RPL44	7.44	10.87	-10.72
262690	062690	ME49 chrVIIb	ribosomal protein RPL27	6.94	11.85	-29.98
232300	032300	ME49 chrVIII	ribosomal protein RPS3	8.64	13.44	-27.84
266060	066060	ME49 chrIX	ribosomal protein RPSA	9.30	13.41	-17.32
267060	067060	ME49 chrIX	ribosomal protein RPL14	8.24	12.50	-19.08
309120	109120	ME49 chrXI	ribosomal protein RPL4	6.76	11.52	-27.02

*Current gene name based on version 8.2 of the *T. gondii* ME49 genome annotation; †Gene name based on version 7.3 of the *T. gondii* ME49 genome annotation; ‡Root mean average, determined as described in Materials and Methods. N=3 microarrays per species.