

Supplementary material for:

An ER-localised, extensive, essential and immunogenic glycoprotein family in trypanosomes

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Figure legends

Figure S1: Bioinformatic filters for identification of predicted type I *trans*-membrane domain surface membrane proteins. Firstly, the TREU927 proteome was downloaded from TriTrypDB (<http://tritrypdb.org/tritrypdb/>). All protein sequences were entered into SignalP HMM (<http://www.cbs.dtu.dk/services/SignalP-2.0/>) and those protein sequences containing predicted signal peptides and signal anchors were retained, whilst all others were discarded. Mitprot (<http://ihg.gsf.de/ihg/mitoprot.html>), Predotar (<http://www.hsls.pitt.edu/obrc/index.php?page=URL1043959648>) and TargetP (<http://www.cbs.dtu.dk/services/TargetP/>) were then used to remove predicted mitochondrial proteins. Using the output from SignalP HMM, signal sequences were removed and thus the mature protein sequence was generated for all retained protein sequences. These sequences were then entered into TMHMM (<http://www.cbs.dtu.dk/services/TMHMM/>). Finally, GPI-SOM (<http://gpi.unibe.ch>) and big-PI (http://mendel.imp.ac.at/gpi/gpi_server.html) were both used to remove all predicted GPI-anchored proteins. Only one GPI-anchored protein was found in this final output; Tb09.v4.0204. The final cohort of 208 protein IDs was parsed through the publicly available web-based mitochondrial proteome database (www.TrypsProteome.org), to check for the absence of mitochondrial-targeted proteins. Accession numbers and details for these sequences are available in Table S2.

Figure S2: Phylogenetic identification of the IGP family. (A) A neighbour joining tree was generated using ClustalW2. Adenylate cyclases are highlighted in pink, ISG and ISG-like proteins are highlighted in blue and light blue respectively. Those proteins which do not form clusters are highlighted in yellow and referred to as singletons. A family of 20 proteins, invariant glycoproteins (IGPs) are highlighted in green. (B) Phylogenetic analysis of IGPs and ISGs, which demonstrates the distinct nature of these two gene families. The IGP family is further divided into three subfamilies, designated IGP34 (two genes), IGP40 (ten genes) and IGP48 (eight genes), based on molecular weight, presence in all Salivarian trypanosomes and *T. cruzi* and also on possession of N-glycosylation sites.

Sequences from the nearest set of genes from the closer analysis in panel A were used as an out group. (C) Phylogenetic analysis of all retrieved IGP orthologs from a range of kinetoplastid genomes (see methods). Leishmania and related species are colour-coded in shades of green, African trypanosomes in shades of red/ochre and south American trypanosomes in shades of blue.

Figure S3: ClustalW alignment of IGP homologues. Panel A: Single representatives of the IGP34, 40 and 48 families are shown to illustrate the homology within the N-terminal region. Panel B: Eighty IGP family sequences, from *T. brucei brucei*, *T. brucei gambiensie*, *T. vivax*, *T. congolense* and *T. cruzi* were aligned using ClustalW. The alignment was used for building the phylogeny shown in Figure S2C.

Figure S4: IGP48 is a tomato lectin-binding protein. 1×10^7 BSF cells over-expressing IGP48 full-length protein, IGP48 ectodomain, dTRIM or wild type cells (negative control) were lysed and HA-tagged proteins immunoprecipitated with anti-HA antibody and Dynabeads® Protein G (Life technologies). This was carried out under both native and denaturing conditions (addition of detergent and heating to 95°C). Samples were separated by SDS-PAGE followed by Western blot with TL-biotin conjugate (1:10,000, Vector Laboratories) and streptavidin-HRP (1:10,000, Sigma Aldrich). Membranes were stripped and re-probed with mouse anti-HA monoclonal antibody. Antibody light (~25kDa) and heavy chains (~50kDa) can be seen for all blots. Bands corresponding to HA-tagged proteins are also seen at the correct molecular weight when probed with mouse anti-HA to verify the immunoprecipitation of HA-tagged proteins. To confirm the specificity of immunoprecipitation, wild type BSF cells were lysed and incubated with mouse anti-HA and then Dynabeads under native and denaturing conditions. Following SDS-PAGE, membrane was probed with rabbit polyclonal antibodies against the highly N-glycosylated VSG221. A protein band corresponding to VSG221 was present in the whole cell sample, and no band was observed in the native or denaturing immunoprecipitated samples, indicating that only HA-tagged constructs were isolated in the immunoprecipitation process. The addition of chitin hydrolysate was used to check the carbohydrate specificity of tomato lectin for proteins containing poly-LacNAc units.

Figure S5: Effects of IGP RNAi on cell cycle progression. Uninduced and cells that have been induced for 24 hrs were fixed and stained with DAPI. Representative images were taken for cells with normal nuclei and kinetoplast content (1K:1N, 2K:1N, 2K:2N), as well as for cells with abnormal DNA content (0K:1N, 1K:0N, 1K:2N, 0K:2N, K:N n>2). The percentage of cells with this type of DNA content out of 200 cells is indicated for induced and uninduced cells beside each image. All images were captured at the same magnification and scale bar represents 2μm.

Figure S6: Induction of IGP48 expression in stumpy and stumpy-like cells. BSF cells ectopically over-expressing HA epitope tagged IGP48 at the C-terminus were incubated at 37°C, 20°C (cold-shock), or in the presence of pCPT-cAMP for 12 hours. A Western blot of

endogenous IGP48 expression levels using rabbit anti-IGP48 antibody (1:100) is shown. Blots were stripped and re-probed with anti-ISG75 whose levels remained unchanged. IGP48 protein levels were determined by densitometry using ImageJ and quantified by normalisation to ISG75. Blots were probed for p67, which is up-regulated in cells treated with pCPT-cAMP and PAD2 (protein associated with differentiation 2), which is under thermoregulated control.

Figure S7: Visualisation of IGP48 by confocal microscopy. Cells were fixed and membranes remained intact (not permeabilised), so that incubation with anti-HA antibody stained only HA epitope-tagged proteins on the surface of the cell, if present. A representative image is shown for each condition of a single optical z-section. Confocal z sections were acquired using an SP2-visible inverted confocal microscope (Leica Microsystems GmbH, Germany). Scale bar is 2 μ m.

Figure S8: Analysis of glycosylation defects on IGP knockdown. (A) Whole cell lysates were prepared from p2T7 IGP48 RNAi cell lines cultured in the presence (induced) or absence (uninduced) of tetracycline for 24 hrs and proteins separated by NuPAGE® Bis-Tris Gel System (Invitrogen) using 4-12% gradient acrylamide gels, allowing high resolution separation of proteins. Analysis of protein abundance and molecular weight was carried out using ImageJ, and a line profile plot of grey value (the sum of the grey values of all the pixels in the selection) against distance from top to bottom of the blot was generated. (B) Samples were separated by SDS-PAGE and lectin blots were carried out with *Erythrina cristigalli* (EC; 1:1000) or *Ricinus communis* (RC; 1:1000) lectins conjugated to biotin and then incubated with streptavidin-HRP. A line profile plot, using ImageJ, was generated for each blot. Protein gels were also stained with Coomassie blue stain, to show equal protein loading. Each experiment was performed in duplicate with a representative experiment shown here.

Figure S9: Analysis of intracellular compartment morphology in IGP48 knockdown cells. (Left) Visualisation of ER structure and morphology of cells in *T. brucei* stained with TbBiP. (Centre and right) Distribution of VSG221 in permeabilised cells (intracellular VSG, centre) and non-permeabilised cells (surface VSG, right). Intracellular staining with BiP and VSG was visualised with confocal microscopy. Numbers on the panels indicate the period after induction when cells were taken for analysis. Scale bar, 2 μ m.

Figure S10: IGP48 knockdown does not induce ATG8-dependant autophagy. BSF IGP48 RNAi cells transfected with YFP-ATG8.2::GL2166. Cells were induced with tetracycline for 48 hours and fixed, permeabilised and stained with anti-GFP. The number of ATG8-positive puncta were counted for induced and uninduced cells ($n = 20$) and plotted as a bar graph. Error bars denote standard error of the mean. Knockdown of IGP48 is verified by Western blot, using anti-IGP48 antibody and anti-tubulin as a loading control. A Western blot probed with anti-GFP to verify molecular weight of YFP-ATG8 as 41kDa.

Table S1: Primer sequences for the verification of RNAi knockdown and localisation studies. The epitope tag is underlined and restriction sites are shown in bold. Primers are shown in 5' to 3' direction. Designation of primers is shown to the left and restriction sites and epitope tags used are given on the right.

Table S2: Accession numbers of predicted type I proteins in *T. brucei*. The accession numbers of all 208 proteins resulting from the bioinformatics search outlined in Fig. S1 are shown in the table.

Table S3: Serum immunoglobulin responses to IGP48 in *T. b. rhodesiense* patients. Immunoglobulin G and M responses to recombinant IGP48 detected by Western blotting are recorded as either positive (+) or negative (-). For each patient, disease stage, age and thick film parasitaemia (i.e. Number of Giemsa stained parasites observed per 10 fields, at 400x magnification) are recorded.

Trypanosoma brucei predicted proteome (TREU 927)

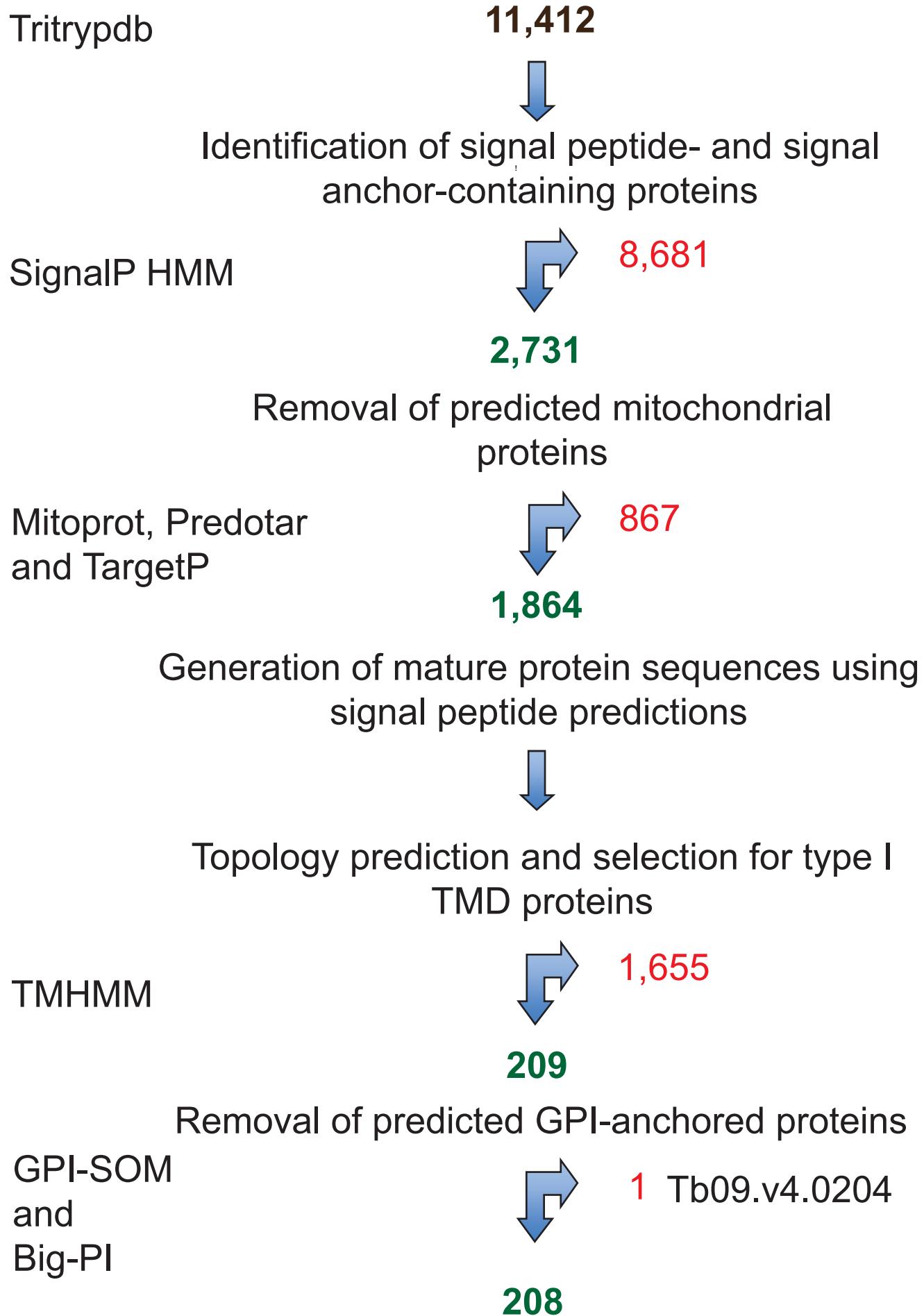


Figure S1

Figure S2A

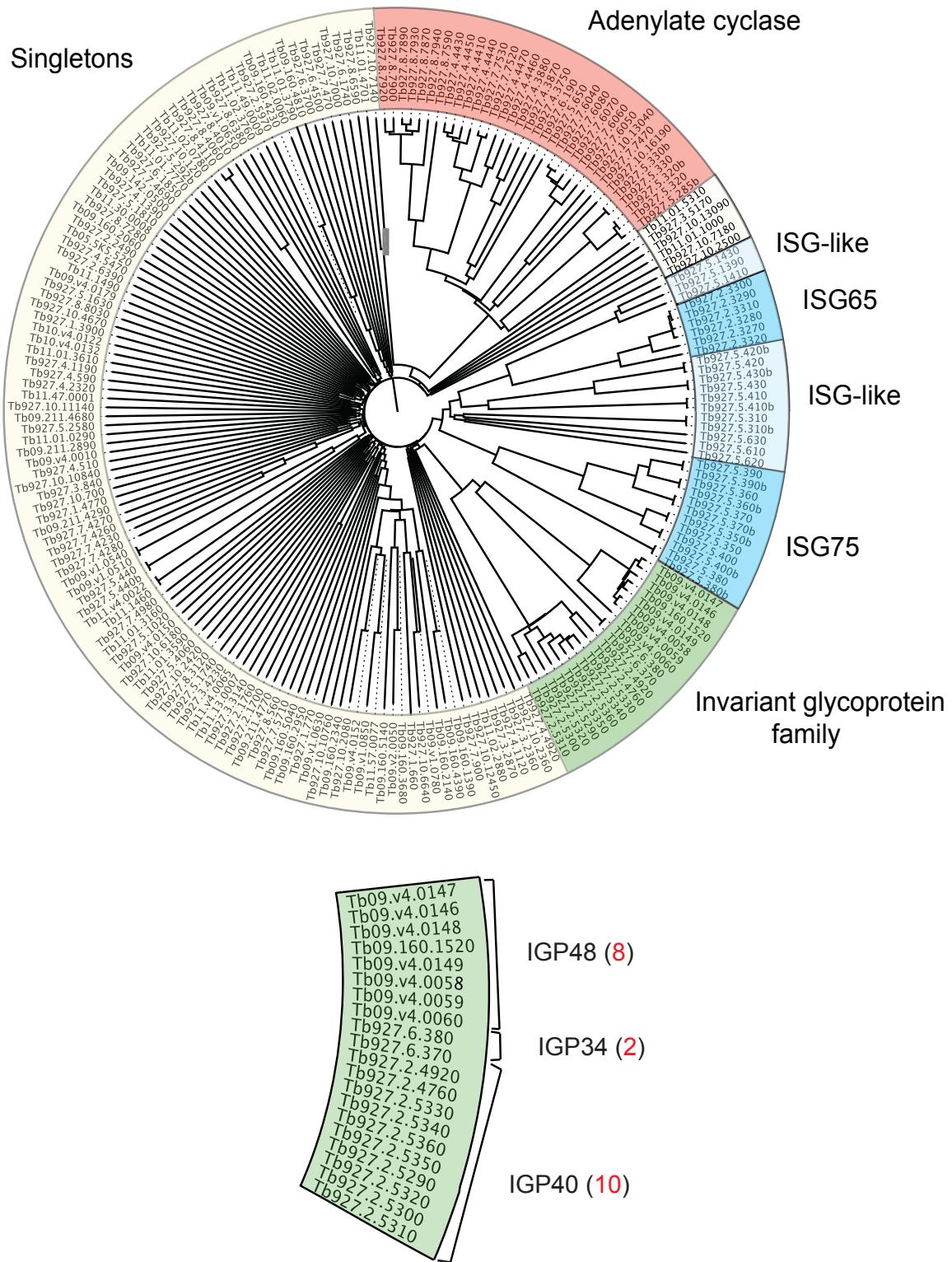
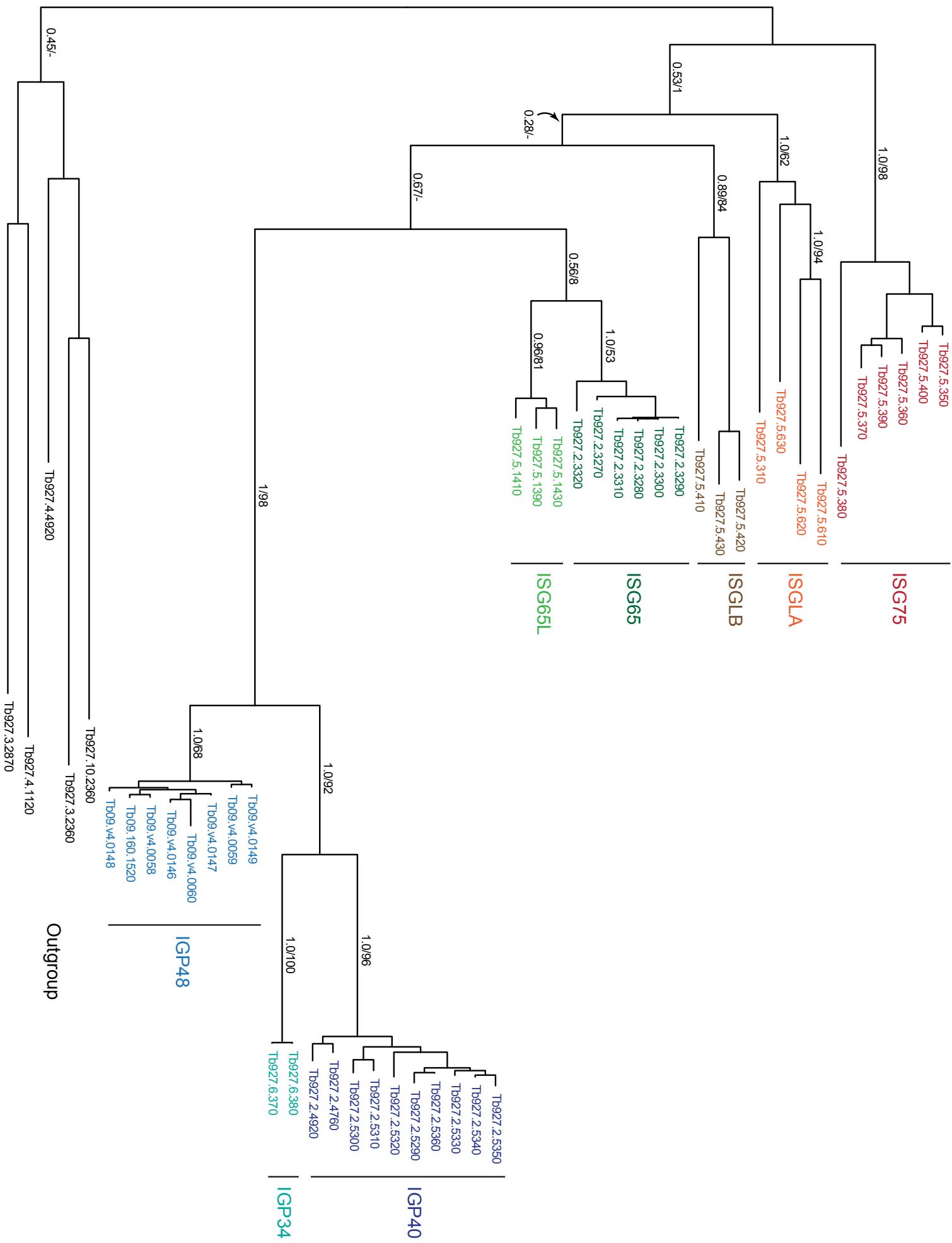


Figure S2B



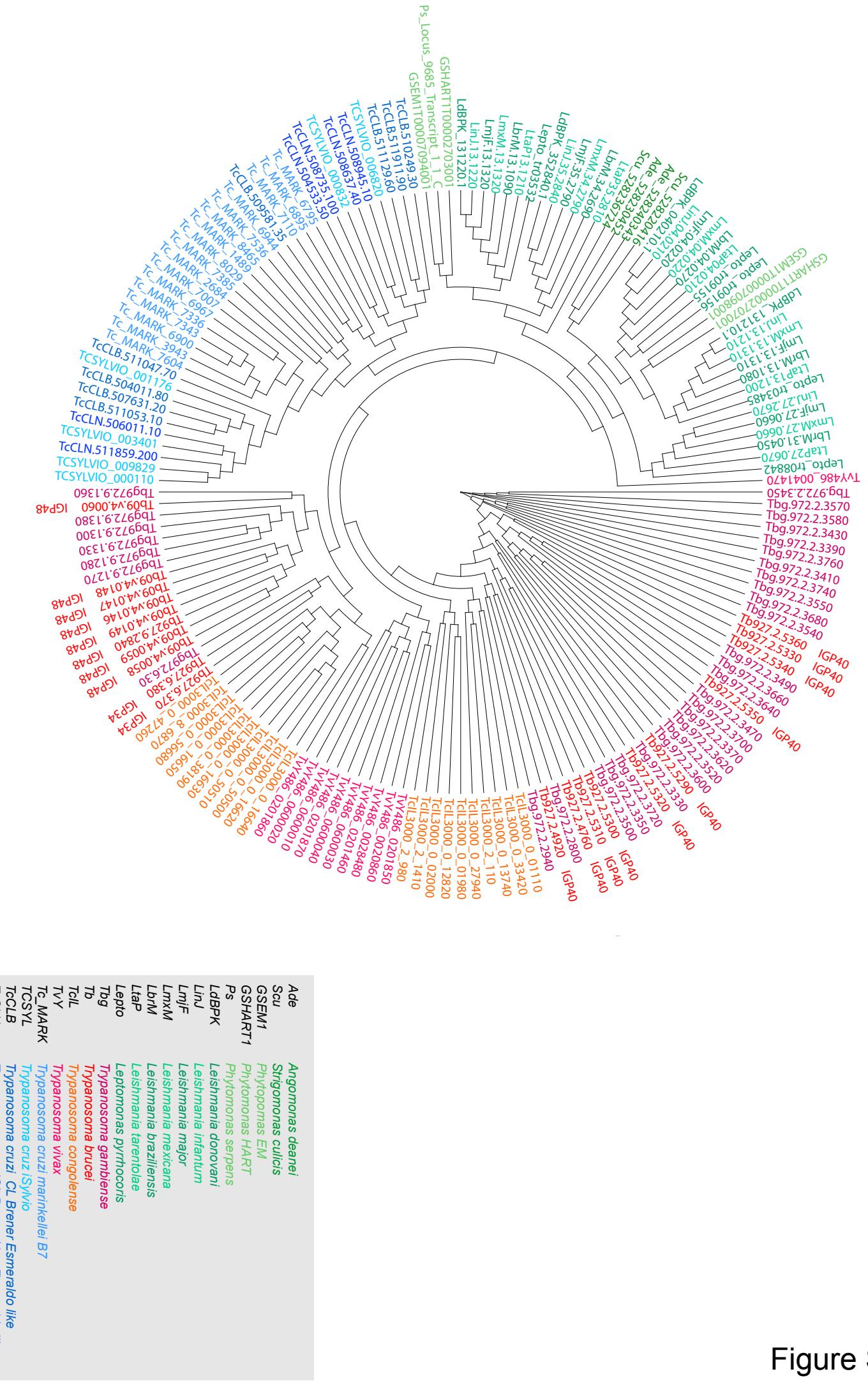


Figure S2C

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6 Tb927.2.5290 MSMDTRMVNFGFTRAALLCG-LLLLTAVCPLHVTADNGRVIVNVKSYSMDNF AFAHR
7 : * . *.*: * * ** . : : . . *. * : * :
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21 Tb927.2.5290 WGSEYPKRGQLYTLSWYHTGDDVTTWYDGKDMMSGRIKWASDNPDTKTENYVILCEVHDSI
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43

Figure S3B

CLUSTAL W (1.8) multiple sequence alignment

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Tb92725310 -----TTTTTT
Tbg97222800 -----TTTTTTTTTT
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TcIL300013740 T-----TTTTTTTTTT
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Tbg972630 VT-----VPQPSNPVPGNGNSP
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	-NETES
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Tb09v40149	-----TTVDGNTNAENTTADEN-----
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Tb091601520-----TTADENATADEN-----
Tb09v40149-----TTADENTTADEN-----
Tb09v40059-----ENTVDENTTADEN-----
Tb09v40058-----TNVDENTTADEN-----
Tb09v40148-----ENTTVDENTTVDEN-----
Tbg97291380-----ENTTADENTNADGNTTVDENTTADGN-----
Tb09v40060-----DESTSDYEESTADEEYSLYNN---GIWKENTTADENTTADENTNVDENNTADEN-----
Tbg97291360-----DESTSDYEESTADEEYSLYNN---GIWKENTTADENTTVDENNTVDENNTVDENTTVDEN-----
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Tc00104705350763120-----VVCETOEGYV-----
Tc00104705350401180-----VVCETOEGYV-----
Tc001047053511859200-----VVCETOEGYV-----

Tc00104705350601110	-----VVCETQEGYV-----
Tc00104705351104770	-----VVCEAQKGYV-----
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Tc001047053508735100	-----VVCEAQKGYV-----
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Tc00104705351191190	-----VVCEAQKGYV-----
Tc00104705351024930	-----VVCEAQKGYV-----
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TvY486_0600050	-----PT-----TTTTT-----TSAPDVSEWV-----
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TvY486_0201850	-----SSGPGGAAGTPSSS-----SG-TASATSPGPV-----
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Tbg97291270	TNTDEISNGSNEASDKTVPSTASDGEKGSGGGAGAAVVIIFILLVVLVLLYFCCFAG--H
Tbg97291300	TNTDEISNGSNEASDKTVPSTASDGEKGSGGGAGAAVVIIFILLVVLVLLYFCCFAG--H
Tb09v40146	TNADEISNGSNEASDKTVPSTASDGEKGSGGGAGAAVVIIFILLAVLLILLYFCCFAG--H
Tb09v40147	TNADEISNGSNEASDKTVPSTASDGEKGSGGGAGAAVVIIFILLAVLLILLYFCCFAG--H
Tb09v1601520	TIADEISNGSNEASDKTVPSTASDGEKGSGGGAGAAVVIIFILLAVLLILLYFCCFAG--H
Tb09v40149	TIADEISNGSNEASDKTVPSTASDGEKGSGGGAGAAVVIIFILLAVLLILLYFCCFAG--H
Tb09v40059	TIADEISNGSNEASDKTVPSTASDGEKGSGGGAGAAVVIIFILLAVLLILLYFCCFAG--H
Tb09v40058	TIADEISNGSNEASDKTVPSTASDGEKGSGGGAGAAVVIIFILLAVLLILLYFCCFAG--H
Tb09v40148	TNADEISNGSNEASDKTVPSTASDGEKGSGGGAGAAVVIIFILLVVLVLLYFCCFAG--H
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Tbg97291360	TNTDEISNGSNEASDKTVPSTASDGEKGSGGGAGAAVVIIFILLVVLVLLYFCCFAG--H
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Tc00104705350763120	VSTTTLPP-----VEIPVWVKRWWYVILIAILVPVIVAVAIIITVYFCRSCGGHDE
Tc00104705350401180	VSTTTLPP-----VEIPVWVKRWWYVILIAILVPVIVAVAIIITACFCRCRGADDE
Tc001047053511859200	VSTTTLPP-----VEISWVRKNWYFVLIAILVPVIVAVALITVCFCRCRGADDE
Tc00104705350601110	VSTTTLPP-----VKISWVKRWWYVILIAILVPVIVAVTLLITVCFCRCRGADDE
Tc00104705351104770	VSTTTLPPA-----VEISWVKRWWYFVLAILVPVIVAVVLITACFCRCRGADDE
Tc00104705350453350	VSTTTLPPA-----IVVPWLQKNWYVVLLIVVLLPVVVAVALITVCFCRGRGVDD
Tc001047053508735100	VSTTTLPPA-----VVVPWLQKNWYVVLLIVVLLPVVVAVALITVCFCRGRGVDD
Tc0010475350894510	VSTTTLPPA-----VVVPWLQKNWYVVLLAVLPVIAAAALITVCFCRGRGV-DE
Tc00104705350863740	VSTTTLPPA-----VVVPWLQKNWYFVLAILLPVIAAAALITVCFCRGRGVYDE
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Tc00104705351024930	VSTTTLPPA-----VVVPWLQKNWYFILAAILLPVIAAAALITVCFCRGRGVDE
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TvY486_0028480	VSTTTLPPA-----GNATSAPPVPGGAGHAGLGAASSTWAERYWYVILLFLSVIVAIVLIVLFLVCRSRSD--D
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Tbg97223580	VTTASLEERS-----NWHIILIALISVSVSLIVLLLFLRWFCDYN--V
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Tbg97223390	VTTASLEERS-----NWHIILIALISVSVSLIVLLLFLRWFCDYN--V
Tbg97223430	VTTASLEERS-----NWHIILIALISVSVSLIVLLLFLRWFCDYN--V
Tbg97223760	VTTASLEERS-----NWHIILIALISVSVSLIVLLLFLRWFCDYN--V
Tbg97223550	VTTASLEERS-----NWHIILIALISVSVSLIVLLLFLRWFCDYN--V
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TcIL3000013740	TTTAQPEASEGK-GSG-----YIILIVLLLALIALLLLAYCCFSAG--G
TcIL3000027940	TTTAQPEASEGK-GSG-----YIILIVLLLALIALLLLAYCCFSAG--G
TcIL3000001110	TTTAQPEASEGSDGSSG-ASTSWGTRNWYIILIVLLLALIALLLLAYCCFSAG--G
TcIL3000033420	TTTAQPEASEGSDGSSG-ASASWGTRNWYIILIVFLLLAALIALLLLAYCCFSAG--G
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Tb9276370	PSATPKPKGE-----AETTLSLSHITICVGHELCLLIVFFSLAIV--C
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TcIL3000016620	VSSTEAPAGK-----VNNGVLAVAILLPIIAIAALLLLLWYFCFRRR--D
TcIL3000016630	VSSTEAPAGK-----VNNGVLAVAILLPIIAIAALLLLLWYFCFRRR--D
TcIL3000047260	VSSTAAPAGK-----VNNGVLAVAILLPIIAIVLLLAWYFCGVWK--N
:	
:	
:	
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Tbg97291280	EKYITVMSLREKVTP---VSNVEAAEVAAP-----SNGEEHLSITSHQEQTP
Tbg97291270	EKYITVMSLREKVTP---VSNVEAAEVAAP-----SNGEEHLSITSHQEQTP
Tbg97291300	EKYITVMSLREKVTP---VSNVEAAEVAAP-----SNGEEHLSITSHQEQTP
Tb09v40146	EKYITVMSLREKVTP---VSNVEAAEVAAP-----SNGEEHLSITSHQEQTP
Tb09v40147	EKYITVMSLREKVTP---VSNVEAAEVAAP-----SNGEEHLSITSHQEQTP
Tb091601520	EKYITVMSLREKVTP---VSNVEAAEVAAP-----SNGEEHLSITSHQEQTP
Tb09v40149	EKYITVMSLREKVTP---VSNVEAAEVAAP-----SNGEEHLSITSHQEQTP
Tb09v40059	EKYITVMSLREKVTP---VSNVEAAEVAAP-----SNGEEHLSITSHQEQTP
Tb09v40058	EKYITVMSLREKVTP---VSNVEAAEVAAP-----SNGEEHLSITSHQEQTP
Tb09v40148	EKYITVMSLREKVTP---VSNVEAAEVAAP-----SNGEEHLSITSHQEQTP
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 Tbg97291360 EKYITVMSLREKVTPSP---VSNVEAAEVAAVP-----SNGEEHLSITSHQOETP
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 Tc00104705350401180 SKWVVHMLREVNGEP---LHPIDGDGGIYND-DSQMMRYSTLGDGVPMTAPSGYENVEV
 Tc001047053511859200 SKWVVHMLREVNGKP---LHPIDGDGGIYND-NSPMMRYSTLGDGVPMNTPFAYENVEV
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 Tc001047053508735100 SKWVAHMLREINDNP---LYAIDRNDGIHNK-DSQMKQDLTLGDGMPTTVPSEHENAEV
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 TcIL3000027940 GKELTPMTLREVTGLP---VRAVEPESYAAPD-----EAYDIPQPLGYHYAQGN
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 TcIL3000033420 GKELTPMTLREVCGLP---LCAAGSESYAAPD-----EAYGIPPPQSYPHNAQGH

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Tbg97291360	AAADE-----
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Tc00104705350453350	PTVIASQESTVYDLGDVF
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Tc0010475350894510	PTVIKNQGSTVYDDGDVA
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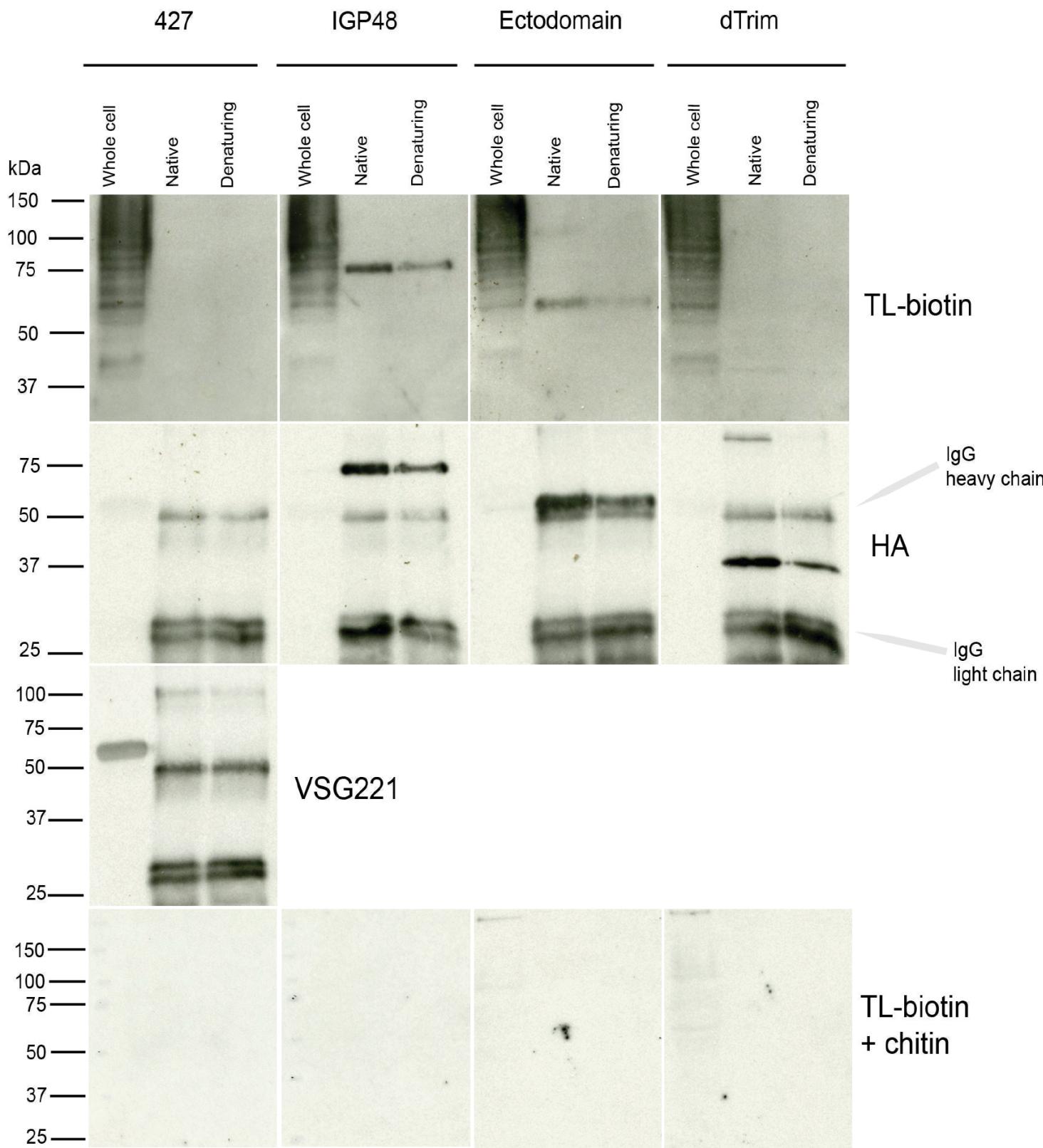


Figure S4

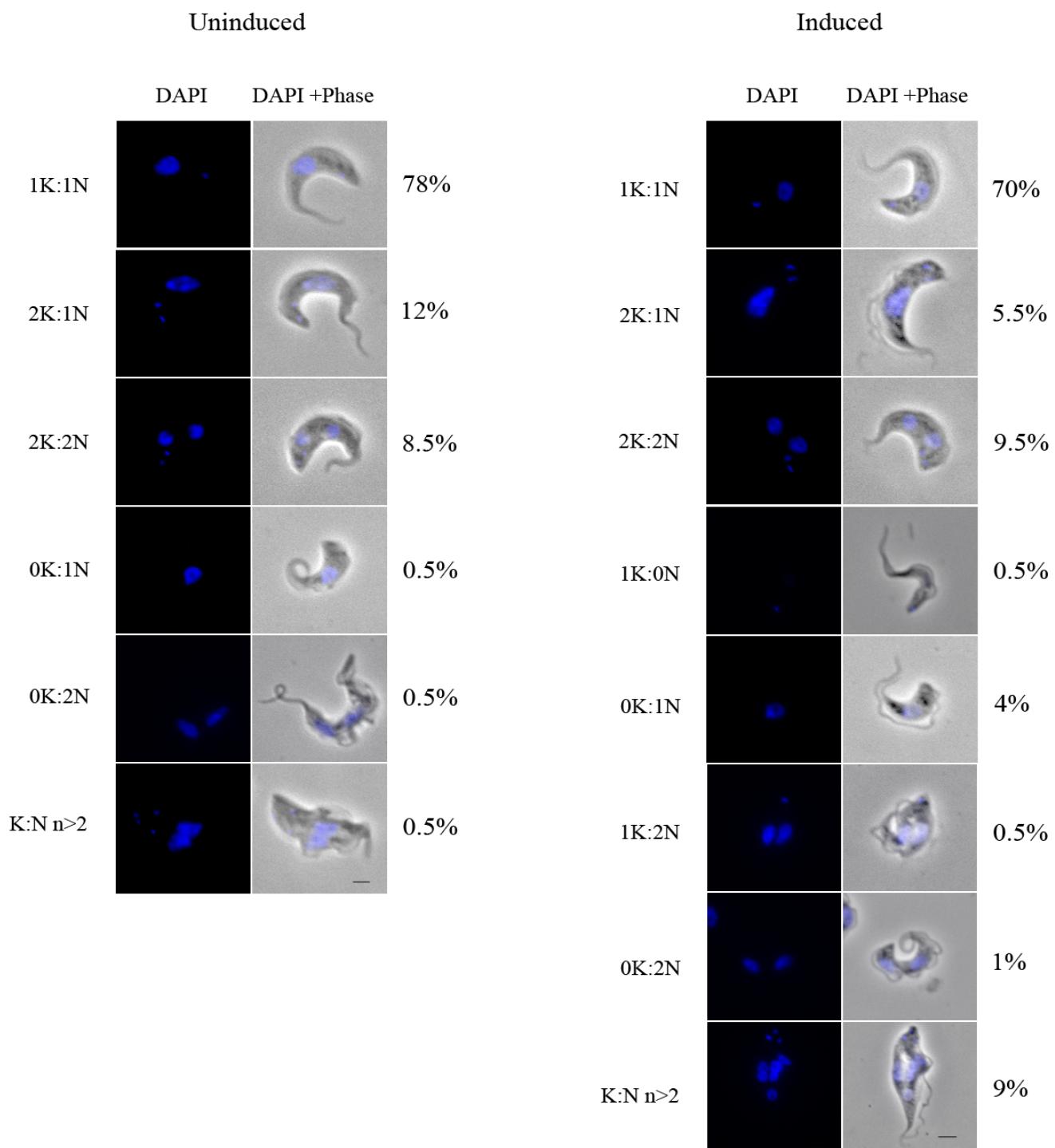


Figure S5

Figure S6

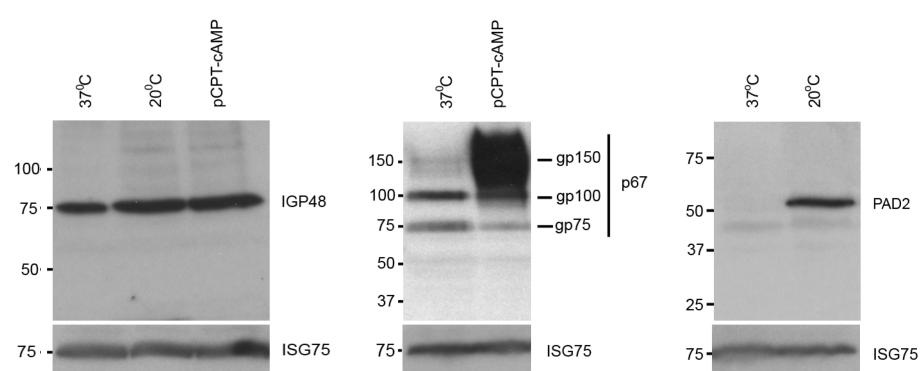


Figure S7

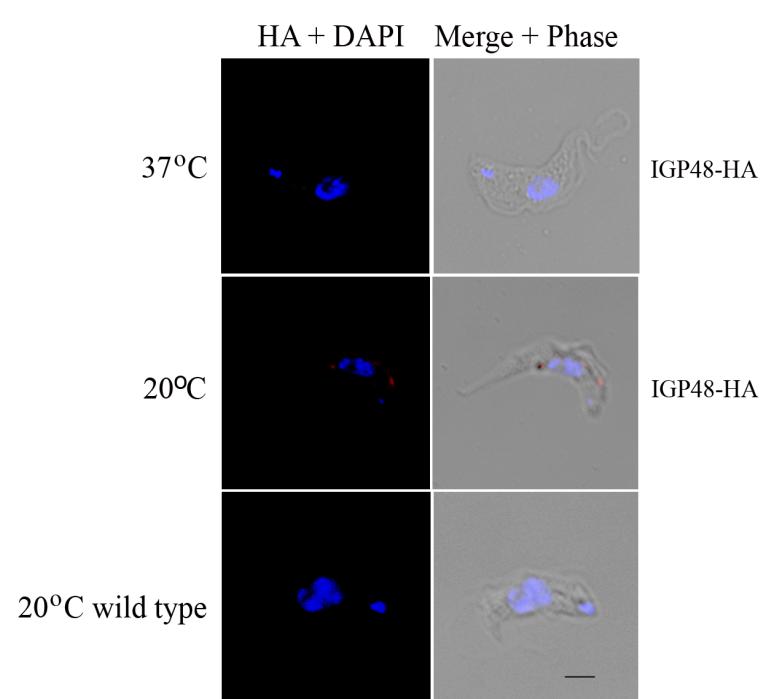
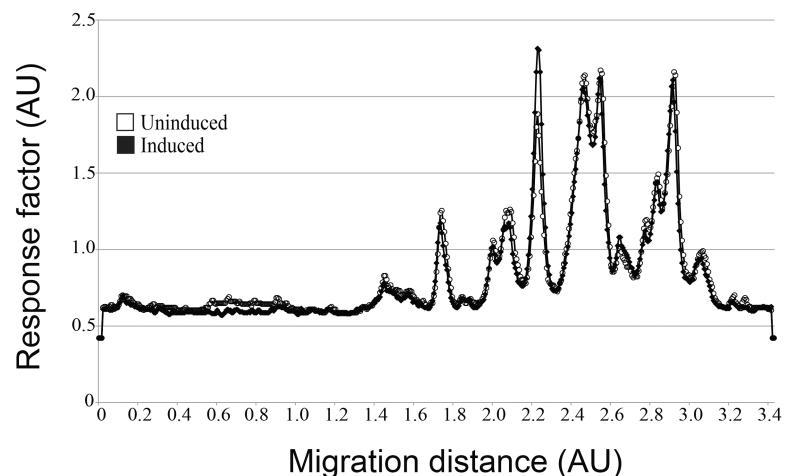
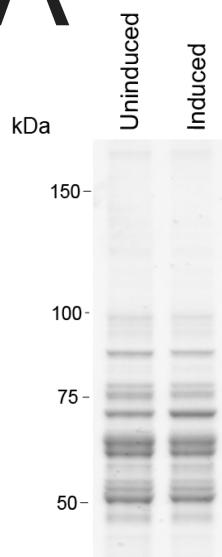


Figure S8

A



B

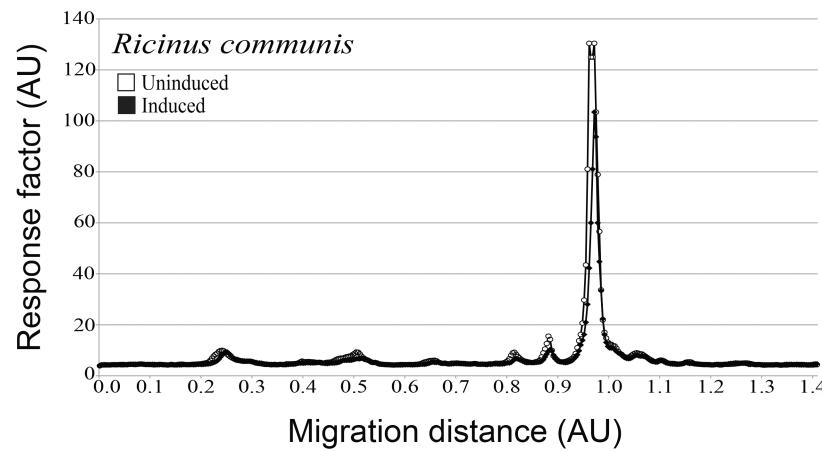
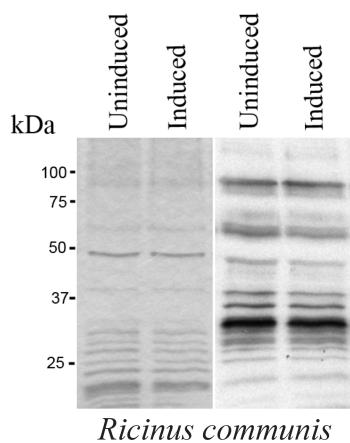
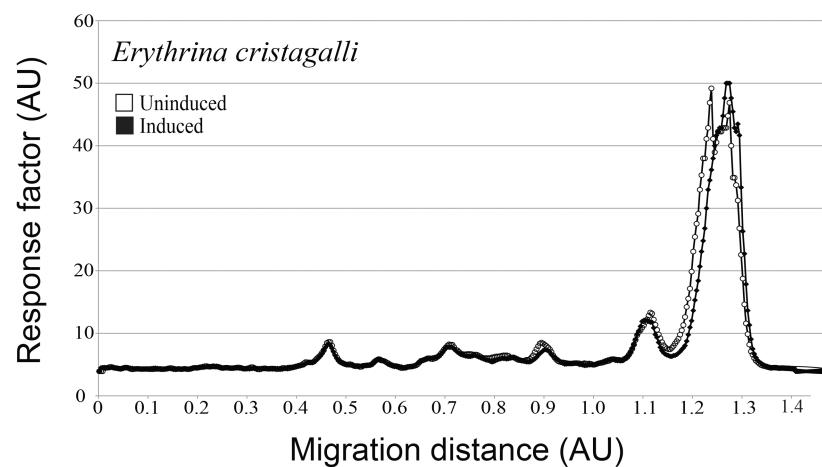
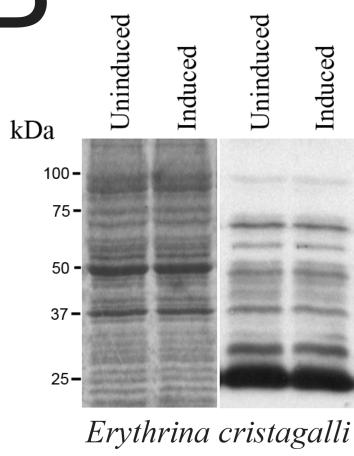


Figure S9

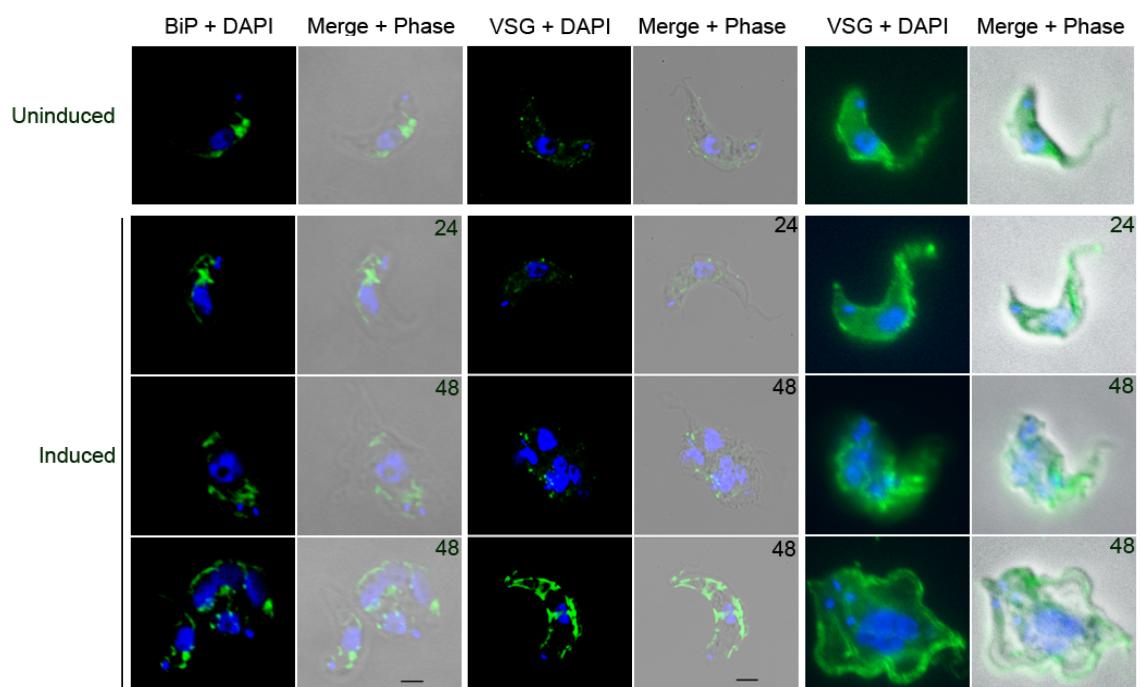


Figure S10

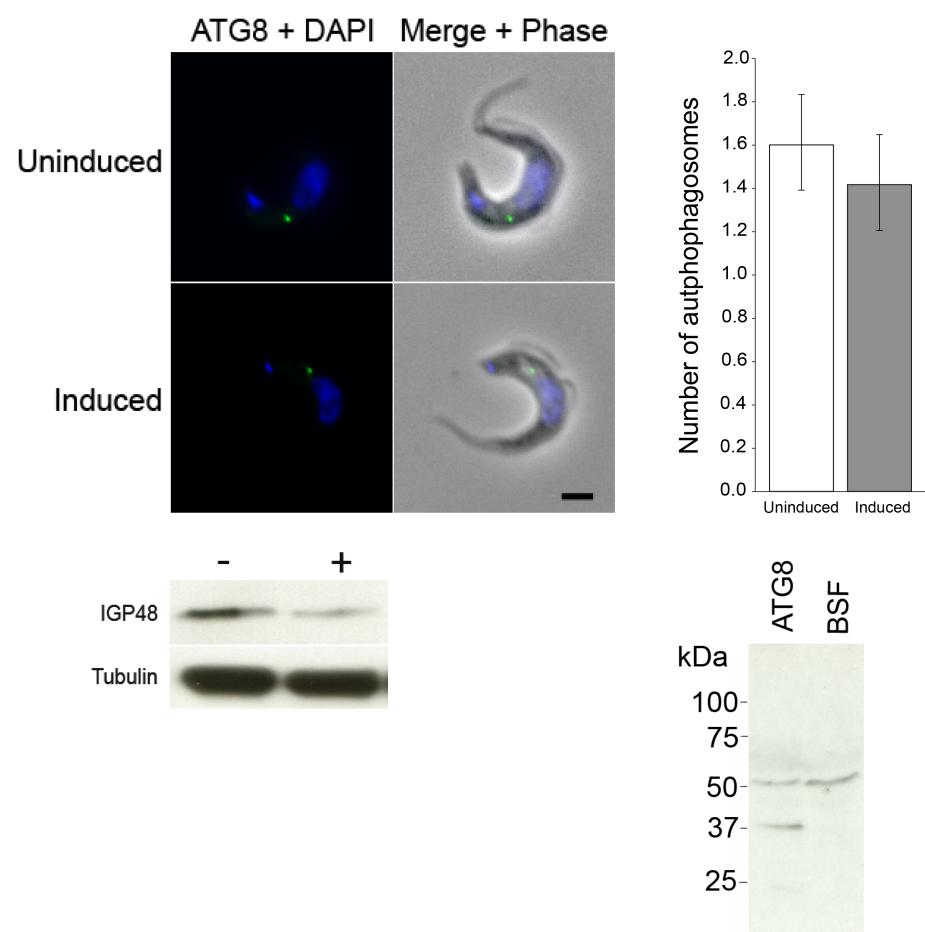


Table S1

Oligomers	Sequence	Restriction site	Epitope tag
<i>RNAi knock-down confirmation</i>			
IGP48:FP	GCCGGTGCTGCTGTTGTTA	-	-
IGP48:RP	ATACGGGTGAGGTAAACCTTT	-	-
IGP40:FP	TTGTGTGTATCGTTGGAATAAG	-	-
IGP40:RP	AGGATGGGTAAACCATTCAATTG	-	-
IGP34:FP	CTCAAGATCAACCTTCGGCAA	-	-
IGP34:RP	GCACACAATTGCGAGGGAAA	-	-
		-	
<i>Localisation</i>			
IGP48-CO ₂ H:FP	CTCGAA <u>AAGCTT</u> ATGGAAATCCGCATGGTGCCA	HindIII	-
IGP48-CO ₂ H:RP (HA)	TGCTAT <u>CCCCGG</u> TCA <u>AGCGTA</u> ATCTGGAACATCGTATGGGTACTCATCAGCTGCTGGAGT	SmaI	HA
IGP48-CO ₂ H:RP (FLAG)	AGCCAT <u>GGATC</u> CTCA <u>TTGTC</u> GT <u>ATCGT</u> CTTGTAGCCTCATCAGCTGCTGGAGTTTC	BamHI	FLAG
IGP48-65:RP	ACTATCAAAGTCGGCACCAACGCCACTCCCTC	-	-
48-ISG65:FP	GGCGGTGGTGCCGACTTGATAGTCTCCTAGAT	-	-
ISG65:RP	GCCAC <u>CCCCGG</u> T <u>CAAGCGTA</u> ATCTGGAACATCGTATGGTACATTACTACTTTACGCTAGA	SmaI	HA
BiP-IGP48:FP	GC <u>GGCCG</u> T <u>AGC</u> AGTAATGAAGCATCAGATAAG	NheI	-
BiP-IGP48:RP	TGC <u>CTTGA</u> ATT <u>CTCA</u> CTCATCAGCTGCTGG	EcoRI	-
IGP40-CO ₂ H:FP	GCTAT <u>GAGCTT</u> ATGAGCATGGACACCCGT	HindIII	-
IGP40-CO ₂ H:RP	ACTGC <u>ACCCGG</u> T <u>TAAGCGTA</u> ATCTGGAACATCGTATGGTATTCA <u>CTGGT</u> TCATTG	SmaI	HA
IGP40-65: RP	ACTATCAAAGTCGTGCCAATTACTTCTCTCTTC	-	-
40-ISG65:FP	AGTAATTGGCAC <u>GACTT</u> GATAGTCTCCTAGAT	-	-
BiP-IGP40:FP	GCAGAT <u>GCTAGC</u> GAAGTACCGGAACCAACTGTG	NheI	-
BiP-IGP40:RP	GAC <u>CTAGA</u> ATT <u>CTTATT</u> CACTGGTCTCATTG	EcoRI	-
dCLECT:FP	TCTATT <u>GCTAGC</u> CCAA <u>CTCCG</u> CTCCACCTGCT	NheI	-
dCLECT:RP	TGC <u>CTTGA</u> ATT <u>CTCA</u> CTCATCAGCTGCTGG	EcoRI	-
dTRIM:FP	ATCGTC <u>GAATT</u> C <u>GTGCC</u> ATCTACAGCTCCGAT	EcoRI	-
dTRIM:RP	GTACAT <u>GAATT</u> CAGCAGGTGGAA <u>GC</u> GGAGTTGG	EcoRI	-

Supplementary Table S1

Table S2

Accession Number	Description	Accession Number
Tb927.5.390	ISG75	Tb09.v4.0147
Tb927.5.390b	ISG75	Tb09.v4.0146
Tb927.5.360	ISG75	Tb09.v4.0148
Tb927.5.360b	ISG75	Tb09.160.1520
Tb927.5.370	ISG75	Tb09.v4.0149
Tb927.5.370b	ISG75	Tb09.v4.0058
Tb927.5.350b	ISG75	Tb09.v4.0059
Tb927.5.350	ISG75	Tb09.v4.0060
Tb927.5.400	ISG75	
Tb927.5.400b	ISG75	Tb927.2.4920
Tb927.5.380	ISG75	Tb927.2.4760
Tb927.5.380b	ISG75	Tb927.2.5330
		Tb927.2.5340
		Tb927.2.5360
Accession Number	Description	Tb927.2.5350
Tb927.6.1850	Singleton	Tb927.2.5290
Tb09.160.4230	Singleton	Tb927.2.5300
Tb11.01.4701	Singleton	Tb927.2.5310
Tb927.10.7140	Singleton	Tb927.2.5320
Tb11.01.5310	Singleton	
Tb09.160.1390	Singleton	Tb927.6.380
Tb09.v1.0780	Singleton	Tb927.6.370
Tb927.8.4110	Singleton	
Tb927.8.4060	Singleton	
Tb927.8.4010	Singleton	Accession Number
Tb11.01.3590	Singleton	Tb927.5.1430
Tb09.160.3680	Singleton	Tb927.5.1390
Tb927.2.1700	Singleton	Tb927.5.1410
Tb927.2.1760	Singleton	Tb927.5.420b
Tb11.57.0077	Singleton	Tb927.5.420
Tb09.v4.0152	Singleton	Tb927.5.430b
Tb11.1460	Singleton	Tb927.5.430
Tb11.v4.0022	Singleton	Tb927.5.410
Tb927.7.4280	Singleton	Tb927.5.410b
Tb927.7.4230	Singleton	Tb927.5.310

Tb927.7.4260	Singleton	Tb927.5.310b
Tb927.5.440b	Singleton	
Tb927.5.440	Singleton	
Tb09.v1.0510	Singleton	
Tb09.v1.0540	Singleton	
Tb927.7.4270	Singleton	
Tb927.10.6180	Singleton	
Tb11.01.3610	Singleton	
Tb927.5.610	ISG-like	
Tb927.5.620	ISG-like	
Tb927.5.630	ISG-like	
Tb927.10.2360	Singleton	
Tb11.1490	Singleton	
Tb927.10.120	Singleton	
Tb927.3.2870	Singleton	
Tb927.10.11140	Singleton	
Tb09.160.2060	Singleton	
Tb927.1.3900	Singleton	
Tb11.47.0001	Singleton	
Tb927.1.660	Singleton	
Tb927.3.5170	Singleton	
Tb927.1.770	Singleton	
Tb09.v4.0010	Singleton	
Tb927.8.8030	Singleton	
Tb927.8.7280	Singleton	
Tb09.160.4390	Singleton	
Tb927.6.1740	Singleton	
Tb927.1.4770	Singleton	
Tb927.3.4230	Singleton	
Tb09.160.4810	Singleton	
Tb09.v1.0630	Singleton	
Tb927.4.590	Singleton	
Tb927.10.2080	Singleton	
Tb927.8.3120	Singleton	
Tb927.5.1810	Singleton	
Tb11.01.0290	Singleton	

Tb927.8.6380	Singleton	
Tb927.5.4060	Singleton	
Tb927.4.2320	Singleton	
Tb09.160.5140	Singleton	
Tb927.5.2580	Singleton	
Tb11.13.0007	Singleton	
Tb927.10.2420	Singleton	
Tb09.160.2950	Singleton	
Tb09.v1.0650	Singleton	
Tb09.160.2340	Singleton	
Tb927.10.10840	Singleton	
Tb09.211.4290	Singleton	
Tb09.160.2140	Singleton	
Tb927.4.1120	Singleton	
Tb927.10.12450	Singleton	
Tb927.3.840	Singleton	
Tb927.7.5710	Singleton	
Tb11.02.2880	Singleton	
Tb09.160.5040	Singleton	
Tb927.4.1390	Singleton	
Tb11.02.3760	Singleton	
Tb09.211.2890	Singleton	
Tb10.v4.0132	Singleton	
Tb927.10.10760	Singleton	
Tb927.3.2360	Singleton	
Tb927.7.900	Singleton	
Tb09.v4.0150	Singleton	
Tb09.v2.0050	Singleton	
Tb927.5.1020	Singleton	
Tb927.3.3140	Singleton	
Tb927.8.560	Singleton	
Tb927.2.6390	Singleton	
Tb927.4.5470	Singleton	
Tb927.10.13090	Singleton	
Tb927.2.5970	Singleton	
Tb927.5.1740	Singleton	

Tb927.7.470	Singleton	
Tb11.01.1000	Singleton	
Tb09.v1.0850	Singleton	
Tb09.211.4680	Singleton	
Tb927.10.700	Singleton	
Tb11.02.0780	Singleton	
Tb09.211.4770	Singleton	
Tb927.6.4500	Singleton	
Tb11.01.7770	Singleton	
Tb927.4.4920	Singleton	
Tb11.01.3160	Singleton	
Tb927.8.6590	Singleton	
Tb927.4.510	Singleton	
Tb11.01.3790	Singleton	
Tb11.49.0009	Singleton	
Tb11.30.0008	Singleton	
Tb09.v4.0179	Singleton	
Tb10.v4.0122	Singleton	
Tb09.142.0500	Singleton	
Tb11.02.0060	Singleton	
Tb927.10.2500	Singleton	
Tb05.5K5.520	Singleton	
Tb927.10.4670	Singleton	
Tb927.5.292b	Singleton	
Tb927.2.2490	Singleton	
Tb927.7.4690	Singleton	
Tb11.v4.0065	Singleton	
Tb927.4.1190	Singleton	
Tb927.6.3700	Singleton	
Tb927.7.4980	Singleton	
Tb927.10.6640	Singleton	
Tb927.5.1630	Singleton	
Tb927.10.7000	Singleton	
Tb927.10.7180	Singleton	

Description	Accession Number	Description
IGP48	Tb927.2.3300	ISG65
IGP48	Tb927.2.3290	ISG65
IGP48	Tb927.2.3310	ISG65
IGP48	Tb927.2.3280	ISG65
IGP48	Tb927.2.3270	ISG65
IGP48	Tb927.2.3320	ISG65
IGP48		
IGP48		
Accession Number		Description
IGP40	Tb927.8.7920	Adenylate Cyclase
IGP40	Tb927.8.7900	Adenylate Cyclase
IGP40	Tb927.8.7890	Adenylate Cyclase
IGP40	Tb927.8.7930	Adenylate Cyclase
IGP40	Tb927.8.7870	Adenylate Cyclase
IGP40	Tb927.8.7940	Adenylate Cyclase
IGP40	Tb927.8.7590	Adenylate Cyclase
IGP40	Tb927.4.4430	Adenylate Cyclase
IGP40	Tb927.4.4450	Adenylate Cyclase
IGP40	Tb927.4.4410	Adenylate Cyclase
	Tb927.4.4440	Adenylate Cyclase
IGP34	Tb927.7.7530	Adenylate Cyclase
IGP34	Tb927.7.7520	Adenylate Cyclase
	Tb927.4.4470	Adenylate Cyclase
	Tb927.4.4460	Adenylate Cyclase
Description	Tb927.4.3880	Adenylate Cyclase
ISG-like	Tb927.4.3870	Adenylate Cyclase
ISG-like	Tb927.4.3750	Adenylate Cyclase
ISG-like	Tb927.6.190	Adenylate Cyclase
ISG-like	Tb927.5.650	Adenylate Cyclase
ISG-like	Tb927.7.6040	Adenylate Cyclase
ISG-like	Tb927.7.6080	Adenylate Cyclase
ISG-like	Tb927.7.6070	Adenylate Cyclase
ISG-like	Tb927.7.6060	Adenylate Cyclase
ISG-like	Tb927.7.6050	Adenylate Cyclase
ISG-like	Tb927.10.13040	Adenylate Cyclase

Table S3: Serum immunoglobulin responses to IGP48 in *T. b. rhodesiense* patients.

	Patient ID	IgG response to rIGP48 (+/-)	IgM response to rIGP48 (+/-)	Disease stage	Age (years)	Parasitaemia	Serum IgG (g/l)	Serum IgM (g/l)
INFECTED								
	L2	-	-	Late	12	0.25	20.5	9.4
	L3	+	-	Late	15	1	30.1	10.2
	L16	+	+	Early	40	20	25.2	21.7
	L30	+	-	Late	18	3	45.3	7.6
	S3	-	-	Late	12	0	17.3	5.7
	S13	+	-	Early	16	50	16.6	1.3
	S48	+	+	Late	40	100	3.1	18.8
	S69	+	+	Early	15	20	51.3	2.7
	S79	+	-	Late	50	2	17.8	51.6
UNINFECTED								
	LC1	-	-	N/A				
	LC15	-	-					
	EUM1	-	-					
	EUF1	-	-					

a: Disease stage: Later stage (meningoencephalitic) criteria either CSF white cells $>5/\text{mm}^3$ or parasites observed in the CSF.

b: Parasitaemia: Number of Giemsa stained parasites observed per 10 fields, at 400x magnification.