

Sup Table 1 List of primers and vectors used in this study

primer	sequence	direction	site	use	plasmid	linearisation	strain
<i>T. gondii</i>							
ASH1ann-s	GGAAATCCTCTCCCCCGAG	sense	-	annotation of TgASH1	-	-	-
ASH1ann-a	CCTCGCTCTCTCTCGCTCGCTCGG	antisense	-				
ASH1-s	GAATTCCTGGAGGAAATTCACACATGCCGC	sense	EcoRI	overexpression of Tub8 promoter controlled second copy with C-term Ty tag	pT8-ASH1Ty-HX	BamHI	ASH1-Ty
ASH1-a	ATGCATCGTTTGTGTGAGGACGTTTCGATGAATCGTCG	antisense	Nsil				
ASH1-KI-s	CCGGGTACCGCGGGTGGGCCACCTCG	sense	KpnI	C-term epitope tagging of endogenous gene	ASH1-3Ty-KI-HX	AvrII	ASH1-3Ty
ASH1-KI-a	CCATGCATGTTTGTGTGAGGACGTTTCGATG	antisense	Nsil				
ASH1-KO-s	CCGTTAATTAATCCATATTATGTCTAGATGGGAATCTGG	sense	Pacl	Knock-out of endogenous gene -5'UTR fragment cloned into ASH1-3Ty-KI-HX	ASH1-KO-HX	Pacl	ash1-ko
ASH1KO-a	CCGGTTAACGTGCGGTGCACAGCAGCCAGC	antisense	Hpal				
ASH1KO-c (P1)	CATATGCCGCCCTTGCTCTTGAAGTTTC	sense	NdeI	confirmation of ASH1KO - WT locus	-	-	-
ASH1KO-c (P2)	AAGCTTTGCATCGTTTGTGTGAGGACGTTTTTC	antisense	HindIII				
ASH1KO-c (P3)	TAATACGACTCACTATAGGG	sense	-	confirmation of ASH1KO - 3' integration	-	-	-
ASH1KO-c (P4)	CCTCGCTCTCTCTCGCTCGCTCGG	antisense	-				
ASH1KO-c (P5)	GGAGCGGCCGCGGGGTGGGCCACCTCG	sense	NotI	control	-	-	-
ASH2-KI-s	TACTTCCAATCCAATTTAATGCCAATCTTTTGTCTGAGC	sense	KpnI	C-term epitope tagging of endogenous gene	ASH2-3Ty-KI-HX	SnaBI	ASH2-3Ty
ASH2-KI-a	TCCTCCACTTCCAATTTTAGCAAATGCATTTTCAGACCTTCTTCGCAAGTCG	antisense	Nsil				
ASH2-KO-s	TACTTCCAATCCAATTTAATGCCAATCTTTTGTCTGAGC	sense	KpnI	Endogenous gene truncation	ASH2-3Ty-KO-HX	EcoRI	ash2-ko
ASH2-KO-a	TCCTCCACTTCCAATTTTAGCAAATGCATTTCTGGTGAGGTCTCTGCACCG	antisense	Nsil				
ASH2KO-c (P1)	TACTTCCAATCCAATTTAATGCCAATCTTTTGTCTGAGC	sense	KpnI	control	-	-	-
ASH2KO-c (P2)	TCCTCCACTTCCAATTTTAGCAAATGCATTTCTGGTGAGGTCTCTGCACCG	antisense	Nsil				
ASH2KO-c (P3)	GTGACACCTGCAAGCCACAGCGG	antisense	-	Integration 5'	-	-	-
ASH2KO-c (P4)	CCCAACGTTTCAGAGCTATGCAGCTCTAGG	sense	-	WT locus/recombinant locus	-	-	-
ASH2KO-c (P5)	ATGCATCTTCAGACCTTCTTCGCAAGTCGCATC	antisense	Nsil	WT locus	-	-	-
ASH3-KI-s	TACTTCCAATCCAATTTAATGGTACCAAGAAAGCGGTGCAGGTGGG	sense	KpnI	C-term epitope tagging of endogenous gene	ASH3-3Ty-KI-HX	StuI	ASH3-3Ty
ASH3-KI-a	TCCTCCACTTCCAATTTTAGCAAATGCATGACCCCATGGCAATTCGACG	antisense	Nsil				
ASH3-KO-s	TACTTCCAATCCAATTTAATGGTACCAAGAAAGCGGTGCAGGTGGG	sense	KpnI	Endogenous gene truncation	ASH3-3Ty-KO-HX	StuI	ash3-ko
ASH3-KO-a	TCCTCCACTTCCAATTTTAGCAAATGCATGGGCATTTCAGTGCCTCTCTCTC	antisense	Nsil				
ASH3KO-c (P1)	TACTTCCAATCCAATTTAATGGTACCAAGAAAGCGGTGCAGGTGGG	sense	KpnI	control	-	-	-
ASH3KO-c (P2)	TCCTCCACTTCCAATTTTAGCAAATGCATGGGCATTTCAGTGCCTCTCTCTC	antisense	Nsil				
ASH3KO-c (P3)	GTGACACCTGCAAGCCACAGCGG	antisense	-	Integration 5'	-	-	-
ASH3KO-c (P4)	CGTACGGGGTTGCCGAGGCTTG	sense	-	WT locus/recombinant locus	-	-	-
ASH3KO-c (P5)	CAATCGTATCTGTGCTTCTCACC	antisense	-	WT locus	-	-	-
ASH4-KI-s	TACTTCCAATCCAATTTAATGCGGGAAGCGTGCAGAGCAGGGAGTGATG	sense	KpnI	C-term epitope tagging of endogenous gene	ASH4-3Ty-KI-HX	NcoI	ASH4-3Ty
ASH4-KI-a	TCCTCCACTTCCAATTTTAGCAAATGCAGTTCTGACGAGACGCGCGTGGAACTGAGG	antisense	PstI				
ASH4-KO-s	TACTTCCAATCCAATTTAATGCTCACCATCATCTTCAGTCACGGAAATG	sense	KpnI	Endogenous gene truncation	ASH4-3Ty-KO-HX	SnaBI	-
ASH4-KO-a	TCCTCCACTTCCAATTTTAGCAAATGCATTTGCCCATATCTGGAAAGATCAAACG	antisense	Nsil				
ASH4KO-c (P1)	TACTTCCAATCCAATTTAATGCTCACCATCATCTTCAGTCACGGAAATG	sense	KpnI	control	-	-	-
ASH4KO-c (P2)	TCCTCCACTTCCAATTTTAGCAAATGCATTTGCCCATATCTGGAAAGATCAAACG	antisense	Nsil				
ASH4KO-c (P3)	GTGACACCTGCAAGCCACAGCGG	antisense	-	Integration 5'	-	-	-
ASH4KO-c (P4)	GGATCCATGGGAAACGCTCTGAAAGCGGATG	sense	BamHI	WT locus/recombinant locus	-	-	-
ASH4KO-c (P5)	GCGGCCGCTCATCTGACGAGACGCGCCG	antisense	NotI	WT locus	-	-	-
<i>E. coli</i>							
rASH1-His-s	GGATCCATGGCGTCTCTCCAGCCAGGCGACGGCTA	sense	BamHI	producing recombinant ASH1 with 8-His N-term tag	His-rASH1	-	-
rASH1-His-a	CTCGAGTCAGTTTGTGTGAGGACGTTTCGATGAATC	antisense	XhoI				

FIGURE S1 - A - Mass spectrometry spectra of identified inhibitor targets. Parasite lysates treated first with the inhibitors, then with FP-Rh probe were run on a gel next to active serine hydrolases purified using biotinylated FP (FP-biotin). Bands were cut at appropriate positions for inhibitor targets (as identified by the disappearance of a band in the lysate lanes compared to the RH control) and analysed by mass spectrometry

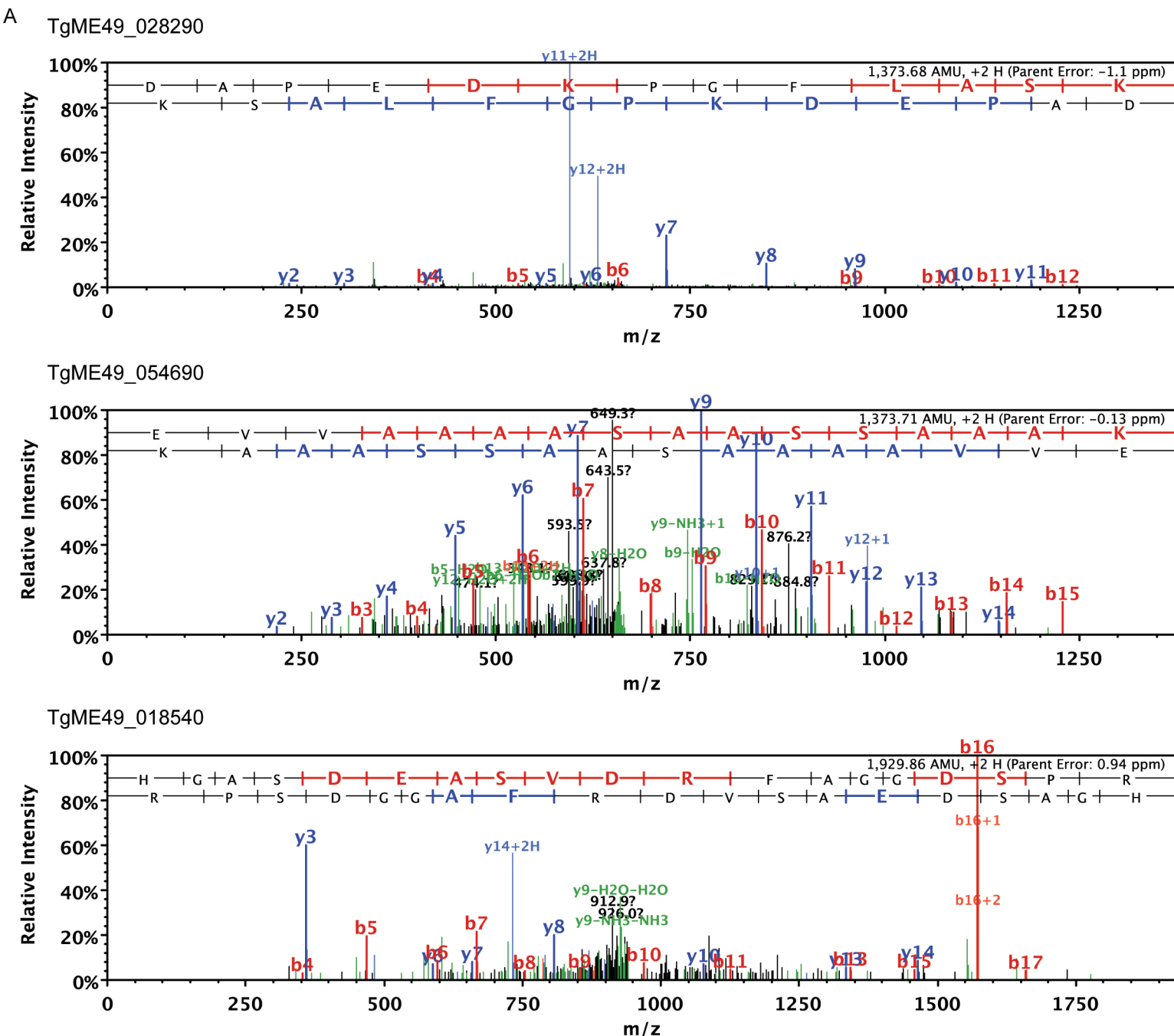


Figure S2- A- Alignment of APT1 homologues and alternative candidates across multiple species. **B-** Alignment of human APT1 with related coccidian proteins using ClustalOmega (EMBL-EBI). For the identity of the genes and organisms, see legend for Fig. 4.

