Supplementary Material

Benoit Poulin et al. doi: 10.1242/bio.20136163

	Toxoplasma-gondii ISP1	1
ISP1	Eimeria-tenella ETH 00012540.1	1 · · · · · · · · · · · · · · · · MGAV - SSCCAV - EDA - · · · DDROVMKEPOPAAAAAAAKEKPHSAKPRKESSSKEOSEOKSKDKERENKEAAKEEKKPKKOOOOEOPAVAVNOPEVEDLRKRL OGGM 101
	Theileria-parva TP01 0108	1 · · · · · · MSVKTSFNNFFGLF - NSCCAHGNSTK0ETFDEDLEE · · · · · · · · · · · · · · · · · ·
	Plasmodium-falciparum_PF3D7_1011000	1 · · · · · · · · · · · · · · · · · · ·
	Plasmodium-berghei PBANKA 120940	1
ISP2	Toxoplasma-gondii_ISP2	1 · · · · · · · · · · · · · · · · · · ·
	Eimeria-tenella_ETH_00020415.1	1
ISP3	Toxoplasma-gondii_ISP3	1
	Eimeria-tenella_ETH_00006440.1	1 EDKANVTL-Q QLALAGLK DVPPRVYAAWLQKYTEGN 46
	Plasmodium-falciparum_PF3D7_1460600	1
ISP4	Plasmodium-berghei_PBANKA_132430	1
	Toxoplasma-gondii_ISP4	1 MP TFGPLL VDRY I HE SYRCFPL Y TC TTI - RQC
	Eimeria-tenella_ETH_00023475.1	
	Toxoplasma-gondii ISP1	77 AVLVLLODG TRLOCILHYNEADSSLSISCED KVRVIPLSDIKAL H TRDOLORVETKANLVDDESCVALHLLESGNCIPLREDGVKDKTCEVDLLKKLKA
ISP1	Eimeria-tenella ETH 00012540.1	102 A I I VLLQDG TKLAC TLHLN PSDKSLSI SCEDKVRVI PLSDVKSLLH TRDQLKRVETKANL VDDENCVALHLIESGNCI PIRFEAVKDKHIFVEMMKQLKEEAEKNRN · · · · · · · · · · · · · · · · · ·
	Theileria-parva_TP01_0108	47 DV I VLLEDG TKLSC TLHVNCETSLVR I ACDKQVRE I DFASVKKI LHTKDELSR I QTTGNSMNYN TTVAFHLLENGNC I PVSF SN TREKRMFLN I LSPF I PT
	Plasmodium-falciparum_PF3D7_1011000	43 Q I VVLLQDG TKLPCNLQAN FQEKTLC I SCHQKVRMINFSD I RSLLYGE EQLKRVE TQANL INDNCCLALHLDDSGNC I P I KFGSVKEKNLF I F I MKDYKKNS
	Plasmodium-berghei_PBANKA_120940	43 Q I V VLL Q DG T KL P C NL Q AN F A E K T L C I S C H Q K V R M I N F S D I R S L Y G E Q L K R V E T Q AN L I S G N C C L A L H L D D S G N C I P I K F E AMKD K N L F I Y I M R D Y K K N · · · · · · · · · · · · · · · · ·
	Toxoplasma-gondii_ISP2	52 T I G L I L Q K S R L D C K V R L T Q G N S A I E L S C E R K S R V V N L S G I R N I L Y T A E Q L K R V D C S A G I S K D D Y C V A L H L T S S G N C I P L F F S N P E D R D C F V L V L Q E R S A S P A V G A S A 160
	Eimeria-tenella_ETH_00020415.1	47 AVGLILODKSRLECVVRLSSSEDSLLLSCEAKSRVVPLQSIKALLHSSSQLLRVDCSAGIRPSDFCAALHLAASGNCIPLFFASLRDK-NLFLITLAHVRTRSSSSSSSSSSSSSGGGGGGGGGGGGGAG 169
ISP3 ISP4	Toxoplasma-gondii_ISP3	50 TMEVL FPDGHRIECNLKIDRPKNFMNLTFNQKVRPIQLDDIAAVLYGSDPRSSECADSKMLRNPCVVGFRLASSGRALAFSFKDITDAQCFVSFLDDEIKKNQESNKSSASNDRN ······164
	Eimeria-tenella_ETH_00006440.1	47 TVEVLFPDGQRIECRLTLDSAKKTLTLSFKEKVRPIPYKDIDSWIYGPSAVDQASADAKLLKDPKVVGFRLSTSGRATAVAFDTTDNAICFVRFLEQILEEAREEEQPNGPKPV160
	Plasmodium-falciparum_PF3D7_1460600	47 SIKVAFPDGNEIQCNFRIFFKEKYFELSLDNKVRVIKFNDINCILHRNSCETLLESEQNLLKSPKVIGIRLISTLKA AFSMDSPGEARIFNDFLQKYCLNA
	Plasmodium-berghei_PBANKA_132430	49 TIRVAFPDGNEIOCYFKIFLNEKCFELSLONKVRIIKFNDIKCVHRNSCESLLESEONLLKSPKVIGIRLISTLKATAFAMDSPGEERM YEFIKKHCLTA 150
	Toxoplasma-gondii_ISP4	50 I C C LMHR DGG S VR I VKLNRAR TAL I LKAGE KGK TVP LEQIHGVL FGDE - LKRVDAFE - ADN TPC VA I YMVSG - SALP I VEPSEQLKQA LNGVGSLR IGPDGHSA TEKKATGE
	Eimeria-tenella_ETH_00023475.1	1 · · · · · · · · · · · · · · · · · · ·

Fig. S1. Phylogenetic analysis of ISP proteins in *Apicomplexa*. Residues are coloured according to degree of conservation. The positions of predicted sites for myristoylation (yellow) and palmitoylation (red) are also shown. ISP4 sequences lack strong predictions for palmitoylation, but potential other sites for S-palmitoylation are indicated (pink dot). For convenience, the C-terminal extension of *Eimeria* ISP2 is not included.



Fig. S2. Generation of transgenic isp lines. A) Schematic representation of the gene targeting strategy employed for tagging the endogenous isp1 and isp3 loci with gfp via single homologous recombination. The C-fusion tag construct contains an insert (white box) homologous to the 3' end of the isp1 or isp3 ORF fused to gfp. A human dihydrofolate reductase selectable marker (hdhfr) allows for selection of transgenic parasites. Arrows 1 and 2 indicate primers used for diagnostic PCR. B) Diagnostic integration PCR for isp3-gfp parasites showing the expected 1.1 kb integration band (primers 1 and 2), thus confirming successful integration of the tagging construct. A second set of primers was used as positive control (+ve cont.) to amplify an unrelated locus to confirm presence of DNA in the PCR mix. Wild type (WT) gDNA was used as control. C) Diagnostic integration PCR for isp1-gfp parasites showing the expected 0.9 kb integration band (primers 1 and 2), thus confirming successful integration of the tagging construct. A second set of primers was used as positive control (+ve cont.) to amplify an unrelated 1 kb locus to confirm presence of DNA. Wild type (WT) gDNA was used as control. D) Western blot analysis using an anti-GFP (Invitrogen) antibody against protein extracted from blood infected with WT P. berghei ANKA 507 clone 1 constitutively expressing GFP (WT-GFP) and transgenic (ISP1-GFP and ISP3-GFP) parasites showing bands of expected size of 29 kDa for wild-type-GFP, 46.5 for ISP3-GFP and 45.4 kDa for ISP1-GFP. E) Schematic representation of the endogenous isp3 locus, the knockout construct and the recombined isp3 locus following double cross-over recombination. The knockout construct contains a T. gondii dihydrofolate reductase/thymidylate synthase (tg dhfr/ts) cassette with a Pbdhfr 3' UTR for selection of transgenic parasites with pyrimethamine. Arrows 3, 4, 5 and 6 indicate binding sites for primers used in integration PCR and knockout PCR. XbaI and SpeI restriction sites and probe binding sites used for Southern blot analysis are shown. F) Genotypic analysis of *Aisp3* parasites by integration PCR and knockout PCR. Presence of a 0.9 kb band using integration specific primers 3 and 4 (Int isp3-KO) on gDNA of mutants (cl2 and cl6) confirms correct integration of the targeting construct. Absence of the 0.3 bp wild type specific band amplified by primers 5 and 6 (wt isp3) in cl2 and cl6 shows loss of the endogenous isp3 gene. Wild type (WT) gDNA was used as control. G) Genotypic analysis of *Aisp3* parasites by Southern blot. gDNA of wild type (WT) parasites, *Aisp3* mutants cl2 and cl6 was probed following Xbal and Spel digestion. The probe homologous to the isp3 5' UTR recognizes a 2.9 kb fragment for the endogenous locus and a 7.2 kb fragment for the recombined locus. H) Pulse-field gel electrophoresis blot (PFGE) hybridized with a Pbdhfr 3'UTR probe. The probe hybridizes to the endogenous dhfr locus on chromosome 7 and the gfp cassette integrated in the 230p locus of the parental line PbANKA 507 clone 1 as well as the disrupted isp3 locus on chromosome 13 I) Diagnostic PCR for isp1-gfp tagged parasites in *Aisp3* genetic background. Successful integration of the isp1 tagging construct is confirmed by a 0.9 kb band amplified with primers 1 and 2 (Int isp1-gfp). Integration of the isp3 knockout construct is confirmed by a 0.9 kb band amplified with primers 3 and 4 (Int isp3-KO), whereas the loss of the endogenous isp3 is confirmed by the absence of a 0.3 kb band amplified by primers 5 and 6 (wt isp3). A fourth set of primers was used as positive control (+ve cont.) to amplify an unrelated locus to confirm presence of DNA. Wild type (WT) gDNA was used as control. J) PFGE for isp1-gfp tagged parasites in *Aisp3* genetic background hybridized with the same Pbdhfr 3'UTR probe as in H). The probe hybridizes to the disrupted isp3 locus on chromosome 13 and to the isp1-gfp on chromosome 12.



Fig. S3. Liver stages and indirect immunofluorescence with GAP45 antibodies. A) Expression of ISP1-GFP and ISP3-GFP in hepatocytes *in vitro* at 24 h and 68 h (Scale bar = 5 µm). B) Indirect immunofluorescence of ISP1-GFP with respect to the IMC marker GAP45, at gametocyte and ookinete stages (Scale bar = 5 µm). Co-localisation with GAP45 is observed at the apical end of the parasite. C) Co-staining of ISP3-GFP with GAP45, at schizont and ookinete stage parasites (Scale bar = 5 µm). In schizonts, the GAP45 staining overlaps with the ISP3-GFP at the membrane of the individual merozoites. At ookinete stage, ISP3-GFP co-localises with the IMC marker at the apical end.