Abstract
The glideosome of apicomplexan parasites is an actin- and myosin-based machine located at the pellicle, between the plasma membrane (PM) and inner membrane complex (IMC), that powers parasite motility, migration, and host cell invasion and egress. It is composed of myosin A, its light chain MLC1, and two gliding-associated proteins, GAP50 and GAP45. We identify GAP40, a polytopic protein of the IMC, as an additional glideosome component and show that GAP45 is anchored to the PM and IMC via its N- and C-terminal extremities, respectively. While the C-terminal region of GAP45 recruits MLC1-MyoA to the IMC, the N-terminal acylation and coiled-coil domain preserve pellicle integrity during invasion. GAP45 is essential for gliding, invasion, and egress. The orthologous Plasmodium falciparum GAP45 can fulfill this dual function, as shown by transgenera complementation, whereas the coccidian GAP45 homolog (designated here as) GAP70 specifically recruits the glideosome to the apical cap of the parasite.

Reference

DOI : 10.1016/j.chom.2010.09.002
PMID : 20951968
Supplemental Information

Functional Dissection of the Apicomplexan

Glideosome Molecular Architecture

Karine Frénal, Valérie Polonais, Jean-Baptiste Marq, Rolf Stratmann, Julien Limenitakis, and Dominique Soldati-Favre

Inventory of Supplemental Information

**Figure S1:** Mass spectrometry and sequence of TgGAP40, a new component of the glideosome motor complex described in figure 1.

**Figure S2:** Schematic representation of the MLC1 constructs used in figure 2, localization of the corresponding proteins within the pellicle of the parasites and multiple alignment of the conserved N-termini of apicomplexan MLC1.

**Figure S3:** Multiple alignment of apicomplexan GAP45 showing the topology of the protein and the residues discussed in this study. The construct of GAP45 used in figures 3 and 4 are also represented and the mutated residues are highlighted.

**Figure S4:** Scheme recapitulating the steps leading to the conditional knockout (iKO) of GAP45 as well as the name of the strains obtained through this process and characterized in figure 5. The iKO showed no defect in cell division and in its organelles.

**Figure S5:** Schematic representation of the GAP mutants used to complement the iKO and characterized in figures 5 and 6. The localisation and regulation of two of them is presented in absence and presence of ATc. None of the complemented strains showed any defect in cell division.

**Figure S6:** Electron micrographs of GAP45-iKO and complemented strains (described in figure 6 highlighting the role of GAP45 is the cohesion of the pellicle.

**Figure S7:** Detailed localization of GAP70Ty (presented in figure 7) at the apical cap of the tachyzoites. Disruption of GAP70 gene in the ΔKU80 strain shows the non-essential role of GAP70 in the tachyzoites lytic cycle.

**Table S1:** Summary of the phenotypes of GAP45 complementation mutants in presence of ATc

**Table S2:** List of the primers used in this study for cloning.
Figure S2

A

MLC1Ty

NtMLC1GFP Ty

CtMLC1GFP Ty

B

control

\(\alpha\)-SAG1  \(\alpha\)-GAP45  merge

\(\alpha\)-SAG1  \(\alpha\)-MLC1  merge

MLC1 Ty

\(\alpha\)-Ty  \(\alpha\)-SAG1  merge

\(\alpha\)-Ty  \(\alpha\)-GAP45  merge

NtMLC1 Ty

\(\alpha\)-Ty  \(\alpha\)-SAG1  merge

\(\alpha\)-Ty  \(\alpha\)-GAP45  merge

C

TgMLC1  1 -MSKVEKKC-PVCYQKLDNPADVLGPMKDKEKLYFMWWMPGDPWEPWGVE 47
NcMLC1  1 -MSKVEKKC-PVCYQKLDNPADVLGPMKDKEKLYFMWWMPGDPWEPWGVE 47
EtMLC1  1 -MGRAEKCC-PICYHKLQRPGEVLEPKDEELNYMWMIPGDPWEPWGVE 47
PfMTIP  1 -----MKQECNVCYFNLPDPPESTLGPYDEELNYMWMIPGDPWEPWGVE 44
PvMTIP  1 MAAKMEKQC-PVCYFNLDPDEAIAPDEELNYMWMIPGDPWEPWGVE 48
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PbMTIP  1 -----MEQQ-HANCYFELDPDEAKITTIGPYDEELNYMWMIPGDPWEPWGVE 44
FcMTIP  1 -----MEQQ-RTCYFELDPDEAKITTIGPYDEELNYMWMIPGDPWEPWGVE 44
TaMTIP  1 -MKTLVQCLKDYNLQPEFVLSPEDELNYNLYLWMPGFKYQSFQEK 48
TpMTIP  1 -MKTVQCLTDYNLQPEFVLSPEDELNYNLYLWMPGFKYQSFQEK 48
Figure S3

A

Insertion of tag in Tg constructs

B

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C

GAP45, GC-AAGAP45, MycGAP45, GC-AAMycGAP45, MycGAP45cc-AZ, GC-AAMycGAP45cc-AZ, MycHisGFPCtGAP45, MycHisGFPCtGAP45cc-AZ
Figure S4

A

TATi-1

ΔGAP45e/GAP4Si

TgGAP45

ΔGAP45e/ΔGAP4Si

B

ΔGAP45e/GAP4Si

TATi-1

ΔGAP45e/ΔGAP4Si

ΔGAP45e/ΔGAP4Si

C

α-IMC1  α-ROP2  α-MIC4  α-HSP

D

TATi-1

ΔGAP45e/GAP4Si

-ATc

+ATc

E

% Invasion vs. BM7/YFP

TATi-1  GAP45e/GAP4Si  ΔGAP45e/GAP4Si

F

ΔGAP45e/MycGAP4Si/MycHisMyoA

ΔGAP45e/MycGAP4Si/MycHisMyoA

-ATc  +ATc
Figure S5

A

B

C

D

-ATc

+ATc

-ATc

+ATc

-ATc

+ATc

\( \alpha\)-Myc

\( \alpha\)-MLC1

\( \alpha\)-Ty

\( \alpha\)-MLC1

\( \alpha\)-Myc

\( \alpha\)-MLC1

\( \alpha\)-Ty

\( \alpha\)-MLC1

\( \alpha\)-Myc

\( \alpha\)-MLC1

\( \alpha\)-Ty

\( \alpha\)-MLC1

% vacuoles

parasites/vacuole
Figure S6
SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1. Identification of GAP40, a new component of the glideosome
A. Parasites stably expressing MLC1Ty were used to immunoprecipitate the glideosome complex using anti-Ty antibodies. Elution was loaded on a SDS-PAGE gel and stained with Coomassie blue. The band below 40 kDa was cut out and analyzed by mass spectrometry. The identified peptides are listed in the table. The asterisks indicate the Ig heavy and light chains.
B. Multiple alignment of apicomplexan GAP40 performed with CLUSTAL W (Thompson et al., 1994). Identical residues are in red, strongly similar residues in green and weakly similar residues in blue. Transmembrane domains were predicted using TMHMM2 (Krogh et al., 2001) and are highlighted in yellow. TgGAP40 (Genbank HM751080), NcGAP40 (N. caninum assembled contigs, contig1129), PfGAP40 (Genbank XP_001351714), PbGAP40 (Genbank XP_679446), PyGAP40 (Genbank XP_730242), CpGAP40 (Genbank XP_628246), TpGAP40 (Genbank XP_763908), BbGAP40 (Genbank XP_001609843).

Figure S2, related to Figure 2. The N-terminal extension of MLC1 is the determinant for the IMC localization
A. Schematic representation of the MLC1 constructs used in this study.
B. The distribution of endogenous MLC1 (MLC1e), MLC1Ty and NtMLC1Ty in the pellicle was determined in extracellular tachyzoites treated with A. hydrophila aerolysin using anti-Ty antibody. Anti-GAP45 and anti-SAG1 were used to visualize the IMC (white arrow) and the PM (white arrowhead), respectively. Scale bar 2 µm.
C. Multiple alignment of the conserved part of MLC1/MTIP N-terminal extension sequences from different Apicomplexans performed using CLUSTAL W (Thompson et al., 1994). Identical
residues are in red and strongly similar residues are in green. Accession numbers: TgMLC1 (GenBank AAL08211), NcMLC1 (*N. caninum* assemble contigs, contig 1003), EtMLC1 (GeneDB EIMER_contig_00029432), PfMTIP (GenBank AAN36529), PvMTIP (GenBank EDL44262), PyMTIP (GenBank AAL69979), PbMTIP (GenBank CAH99347), PcMTIP (GenBank CAH79004), TaMTIP (GenBank XP_954256) and TpMTIP (tigrdb, 525.06429).

**Figure S3, related to Figures 3 and 4. GAP45 organization and generated constructs**

A. Multiple alignment of GAP45 sequences from different apicomplexans performed using CLUSTAL W (Thompson *et al*., 1994). Identical residues are in red, strongly similar residues in green and weakly similar residues in blue. The myristoylated glycine 2 and palmitoylated cysteine 5 were predicted using myristoylator (Bologna *et al*., 2004) and CSS-Palm 2.0 (Ren *et al*., 2008), respectively and are indicated by a red arrow. The serines 163 and 167 are indicated by blue arrows and the two conserved C-terminal cysteines are indicated by black arrows. The coiled-coil domain prediction was performed using Coils (Lupas *et al*., 1991) and is indicated by a blue spring. The position of the tag introduced in TgGAP45 and in PfGAP45 constructs is specified. Accession numbers: TgGAP45 (GenBank AF453384), NcGAP45 (*N. caninum* assemble contigs, contig 1114), EtGAP45 (GenedDB EIMER_contig_00031646), PfGAP45 (GenBank XP_001350624), PbGAP45 (GenBank XP_674811), TaGAP45 (GenBank CAI74487).

B. The only prediction palmitoylated cysteines of TgGAP45 are presented in the table according to CSS-Palm 2.0 (Ren *et al*., 2008).

C. Schematic representation of TgGAP45 constructs used in this study to determine the topology of the protein in the pellicle. The mutations introduced on G2, C5, C230 or C233 are specified on the right for each construct.
**Figure S4, related to Figure 5. Generation of the ΔGAP45e/GAP45i strain**

A. Schematic representation of the steps leading to the GAP45 conditional KO. 5 kb of the 5’ and 3’ flanking regions were amplified and cloned upstream and downstream the DHFR resistant cassette to generate the plasmid creating the genomic KO of GAP45.

B. Intracellular growth assays of TATi-1, GAP45e/GAP45i and ΔGAP45e/GAP45i strains were performed by determining the number of parasites per vacuole after 48 hr ± ATc. Data are represented as mean +/- SEM.

C. The organelles of the ΔGAP45e/GAP45i strain were stained in IFAs after treatment of 48 hr ± ATc. Scale bar 2 µm.

D. Motility was induced on freshly released parasites by treatment with ionomycin. Trails formation was visualized by IFA using anti-SAG1 antibodies.

E. Invasion efficiency of TATi-1, GAP45e/GAP45i and ΔGAP45e/GAP45i was assessed by invasion assay after 36 hr ± ATc using RH-2YFP parasites as an internal standard. The results are expressed as a percentage of invasion compared to RH-2YFP. Data are represented as mean +/- SEM.

F. ΔGAP45e/GAP45i parasites were treated for 24 hr ± ATc, transiently transfected with MycHisMyoA (Hettmann et al., 2000) and then fixed after 24 hr ± ATc. The localization of MycHisMyoA was determined using anti-Myc (in the more intense parasites expressing at the same time MycHisMyoA and MycGAP45 in absence of ATc) and anti-His antibodies. Scale bar 2 µm.
Figure S5, related to Figure 5 and 6. Complementation of the ΔGAP45e/GAP45i strain with several mutants of GAP45

A. Schematic representation of the constructs used to complement GAP45-iKO. The mutations introduced on G2, C5, C230 or C233 are specified on the right for each construct. The localization of TyGAP45Δcoil (B) and TyGAP45-2coils (C) in ΔGAP45e/GAP45i was assessed by IFA in intracellular tachyzoites cultured for 48 hr ± ATc using anti-Ty, anti Myc and anti-MLC1 antibodies. Scale bar 2 μm.

D. Intracellular growth assays of ΔGAP45e/GAP45i and complementation mutants were performed by determining the number of parasites per vacuole after 48 hr ± ATc. Data are represented as mean +/- SEM.

Figure S6, related to Figure 6. GAP45 plays a role in pellicle integrity

Electron micrographs of (A) GAP45-iKO parasites without ATc, (B) GAP45-iKO complemented with MycGAP45 treated with ATc, (C) complemented with TyGAP45CC-AA treated with ATc, (D) complemented with GC-AAMycGAP45 without ATc, (E) GAP45-iKO parasites treated with ATc for 48 hr, (F) GAP45-iKO complemented with TyGAP45Δcoil and treated with ATc for 48 hr, (G-J) complemented with GC-AAMycGAP45 and treated with ATc for 48 hr. DG: dense granule, M: mitochondrion, N: nucleus, R: rhoptries. The arrows point to the defect observed in the pellicle. Scale bars 0.5 μm for A, B, D, E, F, G and 1 μm for C, H, I, J.

Figure S7, related to Figure 7. GAP70 is located at the apical cap and is dispensable in tachyzoites

A. Multiple alignment of GAP70 and GAP45 sequences from different apicomplexans performed
using CLUSTAL W (Thompson et al., 1994). Identical residues are in red, strongly similar residues in green and weakly similar residues in blue. The myristoylated glycine 2 and palmitoylated cysteine 5 were predicted using myristoylator (Bologna et al., 2004) and CSS-Palm 2.0 (Ren et al., 2008), respectively and are indicated by a red arrow. The coiled-coil domain prediction was performed using Coils (Lupas et al., 1991) and is indicated by a blue spring.

TgGAP70 (GenBank HM117968), NcGAP70 (N. caninum assembled contigs, contig 835), TgGAP45 (AAP41369), NcGAP45 (N. caninum assemble contigs, contig 1114), PfGAP45 (GenBank XP_001350624) and PbGAP45 (GenBank XP_674811).

B. The localization of GAP70Ty expressed in RH was assessed by IFA in intracellular tachyzoites using anti-Ty and anti-MLC1 antibodies. Scale bar 2 µm.

C. The distribution of GAP70Ty in the pellicle was determined on extracellular tachyzoites treated with A. hydrophila aerolysin using anti-Ty antibody. Anti-MLC1 and anti-SAG1 were used to visualize the IMC (white arrow) and the PM (white arrowhead), respectively. Scale bar 2 µm.

D. The localization of TyGAP70 in ΔGAP45e/GAP45i and the regulation of MycGAP45i were assessed by IFA on intracellular tachyzoites cultured for 48 hr ± ATc using anti-Ty, anti-Myc and anti-MLC1 antibodies. White arrows point to depletion of MycGAP45 at the apical cap upon the expression of TyGAP70. Scale bar 2 µm.

E. Scheme of the double homologous recombination leading to the ΔGAP70 in the ΔKU80 strain. Position of the primers used to characterize the endogenous and modified loci are shown by black arrows and arrowheads, respectively.

F. PCR performed on genomic DNA extracted from ΔKU80 and ΔKU80ΔGAP70 strains with the primers described in the previous scheme.
G. RT-PCR performed on RNA extracted from \( \Delta KU80 \) and \( \Delta KU80 \Delta GAP70 \) strains with the primers specific for the coding sequence of \( GAP70 \) (A and B).

H. Plaque assay performed by incubating host cells with \( \Delta KU80 \) and \( \Delta KU80 \Delta GAP70 \) strains for 7 days.
SUPPLEMENTAL TABLES

Table S1: Phenotypes of GAP45 complementation mutants in presence of ATc

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<th>ΔTgGAP45e/TgGAP45i complemented with:</th>
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<th>Interaction with:</th>
<th>Plaque formation</th>
<th>Induced egress $^a$</th>
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d: not done

$^a$ the induced egress in presence of ATc is expressed as a percentage of the induced egress in absence of ATc for each strain.

Table S2: Oligonucleotide primers used in this study for cloning

The EcoRI, NsiI, PacI, PstI, NarI, Shfl and SacII restriction sites are underlined, the mutated codons are in bold and the non-coding sequences are shown in lowercase.
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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Preparation of *T. gondii* genomic DNA and RNA

Genomic DNAs have been prepared from tachyzoites (RH strain) using the Wizard SV genomic DNA purification system (Promega). RNA was isolated from tachyzoites using Trizol (Invitrogen). cDNA was generated by RT-PCR performed with the Superscript II reverse transcriptase (Invitrogen) according to the manufacturer’s instructions.

Cloning of DNA constructs

*pTUB8MLC1Ty, pTUB8NtMLC1GFPTy, pTUB8CtMLC1GFPTy and pTUB8MLC1Ty* N-terminal mutants

MLC1 ORF was amplified from cDNA using the primers pair 757/758 and cloned into pTUB8MycGFPPfMyoAtailTy-HX (Herm-Gotz et al., 2002) between the *EcoRI* and *NsiI* sites. The GFP sequence was amplified by PCR with primers 783/784, digested by *NsiI* and *PstI*, and cloned into the *NsiI* site of pTUB8MLC1Ty to create pTUB8MLC1GFPTy. Fragments corresponding to the N-terminal extension (aa 1 to 70) and to the 4 EF-hands of MLC1 (aa 71 to 250) were amplified by PCR using pTUB8MLC1Ty as template and the primers pairs 757/786 and 785/758, respectively. The two fragments were then cloned into the pTUB8MLC1GFPTy, between the *EcoRI* and *NsiI* sites. Mutations in the N-terminal extension of MLC1 were introduced by site-directed mutagenesis reaction with pTUB8MLC1Ty as template using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene) and the primer pairs 2439/2440, 2441/2442 and 2534/2535 for D26A-E28A, P36A-G37I-F38A and C8A-C11A mutations, respectively.

*pTUB8GAP40Ty*
The coding sequence of GAP40 was amplified with the primers 3092/3095 and cloned into the pTUB8MycGFPPfMycAtailTy-HX (Herm-Gotz et al., 2002) between MfeI and NsiI sites to generate pTUB8GAP40Ty.

**pTUB8GAP45Ty and pTUB8GC-AAGAP45Ty**

GAP45 and GC-AAGAP45 (G2A-C5A) were amplified from total cDNA using the following primers pairs 2258/2259 and 2260/2259, respectively and cloned into the pTUB8MycGFPPfMycAtailTy-HX (Herm-Gotz et al., 2002) between the EcoRI and NsiI sites.

**pTUB8GAP70Ty and pTUB8MycGAP70**

GAP70 was annotated after amplification from cDNA using primers 2025/2026 (GenBank HM117968). The coding sequence was then subcloned into the pTUB8MycGFPPfMycAtailTy-HX (Herm-Gotz et al., 2002) between the MfeI and NsiI restriction sites to generate pTUB8GAP70Ty or amplified with primers 2380/2381 and cloned in the same vector between NsiI and PacI restriction sites pTUB8MycGAP70.

**pTUB8MycHisGFPCtGAP45, pTUB8MycHisGFPCtGAP45CC-AA and pTUB8MycHisGFPGAP70**

The C-terminal parts of GAP45 (aa 197 to 245) and GAP70 (aa 203 to 313) were amplified using the primers pairs 2386/2385 and 2443/2444, respectively, and cloned into pTUB8MycHisGFP-HX vector (derived from (Hettmann et al., 2000)) downstream the GFP between the PstI and PacI restriction sites. C230A-C233A mutations were introduced by site-directed mutagenesis reaction with pTUB8MycHisGFPCtGAP45 as template using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene) and the primers 2536/2537.

**pTetO7Sac4MycGAP45, pTetO7Sac4MycGFPGAP45, pTetO7Sac1GC-AAMycGAP45, pTetO7Sac4MycGAP45CC-AA, pTetO7Sac1GC-AMycGAP45CC-AA, pTUB8MycGAP45, pTUB8GC-AAMycGAP45, pTUB8MycGFPGAP45, and pTUB8MycGAP45CC-AA**
GAP45 was cloned in two steps into the pTetO7Sag4MycGFP vector (Meissner et al., 2002). The N-terminal part (aa 1 to 40) was amplified with a Myc-tag sequence by PCR with primers 2258/2492 for the WT sequence or with primers 2260/2492 for the G2A-C5A mutated sequence and cloned between EcoRI and NsiI restriction sites. The rest of GAP45 sequence (aa 41 to 245) has been amplified with primers 2493/2383 and cloned between the NsiI and PacI sites to obtain pTetO7Sag4MycGAP45 and pTetO7Sag1GC-AAMycGAP45 or between the PstI and PacI sites to obtain pTetO7Sag4MycGFPGAP45. C230A-C233A mutations were introduced in pTetO7Sag4MycGAP45 and pTetO7Sag1GC-AAMycGAP45 by site-directed mutagenesis reaction using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene) and the primer pair 2536/2537. pTUB8MycGAP45, pTUB8GC-AAMycGAP45, pTUB8MycGFPGAP45, and pTUB8MycGAP45CC-AA were then generated by cutting MycGAP45, GC-AAMycGAP45, MycGFPGAP45, and MycGAP45CC-AA of the pTetO7Sag4 using EcoRI and PacI restriction sites and subcloning into the same sites of pTUB8MycGFPPfMyoAtailTy-HX vector (Herm-Gotz et al., 2002).

**p2854-DHRF-5’3’GAP45**

Around 5 kb of the 5’ and 3’ flanking regions of GAP45 were amplified by PCR with primers 2528/2529 and 2530/2531, respectively. The 5’ flanking region was then cloned into the HindIII restriction site of the p2854-DHRF (Roos et al., 1994) and the 3’ flanking region into the NotI site. A SbfI site was introduced in primers 2528 and 2531 to linearize the plasmid.

**pTUB8MycGAP45-2coils, pTUB8TyGAP45CC-AA and pTUB8TyGAP45-2coils**

The coiled-coil domain of GAP45 was amplified by PCR using the primers pair 2627/2628, digested with NsiI and PstI restriction enzymes and cloned into pTUB8MycGAP45 in the PstI site downstream the Myc-tag. The Ty-tag was introduced by PCR by amplifying the tubulin promoter and the N-terminal part of GAP45 with primers T7/2783 and by sub-cloning this insert.
between the *Kpn*I and *Nsi*I sites of pTUB8MycGAP45CC-AA and pTUB8MycGAP45-2coils. The bleomycin cassette was then amplified with primers 2787/2642 and cloned in the *Sac*II site of these two vectors.

\[ pTUB8TyPfGAP45 \]

PfGAP45 synthetic gene was designed according to the amino acid codon usage of *T. gondii* with an internal Ty-tag. The sequence of the gene is the following one, with the Ty tag in lower case:

ATGGGCAACAAAGTGCTCTCGCTCGAAAAGTCAAAAGAGCCGAAAGCCGAAGGACATCGACGAAGCTCGCGGAGCGCGAGAACCTCAAGAAGCAGTCGGAGGAAATCATCGAGGAAAAGCCGGAActcgaggtccacacgagacccgctcgaCGTCGAACAGGTCGAGGAAACGCACGAAGGAACCGCTCGAGCAGGAACAGGAACTCGACGAGCAGAAGATCGAGGAAGAGGAAAGAACCTGAGCAGGTCCCGAAAGAGGAAATCGACTACGCGACGCAGGAAAACGTCGTTCGAAGAGAAGCACCTCGAGGACCTGGAGCGCTCTAACTCTGACATCTACTCGAGTGTCAGAAGCAACGACAGCGTGTCGAAACACTCAAGCTCGAAACGGGCACGCAGCTACGCTGTCTACGGAAGCGACAGGCCGCGGTCCAGCAGATCACGAAGCTCTCGGAGCCGGCGCACGAAGAGTCTATCTACTTCACGTACCGCTCTGTCACGCCGTGCAGACATGAAAGACACGACGAGACACGCCAGTGGTGTTCTCTAGACGCTGAGGCGACCTCGGCGAGAGACACGACGAGAACGCGTGCAAGATCTGCCGCAAGATCGACCTCTCTGACACGCCTCCTCTCGTGA. The synthetic gene was generated by Geneart AG and cloned into pMA vector. TyPfGAP45 sequence was cut out of pMA vector using *Eco*RI and *Pac*I restriction sites and subcloned into the same restriction sites of pTUB8MycGFPPfMyoAtailTy-HX vector (Herm-Gotz *et al.*, 2002).

\[ pTUB8TyGAP70 \text{ and } pTUB8TyGAP70Ct45 \]
To create pTUB8TyGAP70 and pTUB8TyGAP70-Ct45, the N-terminal part of GAP70 with the Ty-tag was amplified with primers 2025/2613 and cloned between the EcoRI and PstI restriction sites of pTUB8MycHisGFPCtGAP70 and pTUB8MycHisGFPCtGAP45, respectively.

*pTUB8TyGAP45Δcoil*

The N-terminal part of GAP45 (aa 1 to 40) was amplified from GAP45Ty with primers T7/2605 and cloned into pTUB8GAP70TyCtGAP45 between the KpnI and NarI restriction sites. The bleomycin cassette was then amplified with primers 2787/2642 and cloned in the SacII site.

*p2855-HXGPRT-5'3'GAP70*

Around 3 kb of the 5’ and 3’ flanking regions of GAP70 were amplified by PCR with primers 2665/2666 and 2667/2668, respectively. The 5’ flanking region was then cloned between the KpnI and ApaI restriction sites of the p2855-HXGPRT (Donald and Roos, 1998) and the 3’ flanking region into the BamHI site. A SbfI site was introduced in primers 2665 and 2668 to linearize the plasmid.

**Parasite transfection and selection of stable transformants**

Parasite transfections were performed by electroporation as previously described (Soldati and Boothroyd, 1993). The hypoxanthine-xanthine-guanine phosphoribosyl transferase (hxgprt) gene was used as a positive selectable marker in the presence of mycophenolic acid (25 mg/mL) and xanthine (50 mg/mL), as described before (Donald et al., 1996). RHΔku80 strain (Fox et al., 2009; Huynh and Carruthers, 2009) have been transfected with 20 µg of p2855-HXGPRT-5’3’GAP70 vector (linearized with SbfI). RHΔku80Δgap70 clones were tested by PCR using the primer pairs A to F indicated in figure S8 and table S2.

**Antibodies**
The antibodies used to check the integrity of the organelles in ΔGAP45e/GAP45i were described before as follow: polyclonal rabbit α-HSP {Pino, #53}, α-MIC4 {Brecht, 2001 #61}, monoclonal mouse α-IMC1 (Mann, 2001 #44), kindly provided by Dr. G Ward, α-ROP2 (Herion, 1993 #60).

**Induced gliding assay**

Parasites were grown for 48 hr ± ATc. Freshly released parasites were resuspended in calcium-saline solution containing 1 mM of ionomycin, incubated for 15 min on poly-L-lysine coated coverslips and fixed with PFA/GA. Anti-SAG1 antibody was used to visualize the trails.

**Invasion assay**

Invasion assays were performed using the RH-2YFP strain as internal standard mainly as described in Sawmynaden et al. (2008). Briefly, confluent HFFs have been heavily infected with a mixture of the strain of interest and RH-2YFP parasites and washed out after some hr. Parasites were incubated ± ATc after 12 hr and for 36 hr. Extracellular parasites were then collected and the ratio of non-YFP to YFP parasites was determined. At the same time these parasites were transferred on new host cells and allowed to invade for 1 hr at 37°C ± ATc before washing. Then, incubation continued for 24 hr ± ATc and cells were fixed. Parasites were stained with α-SAG1 and the ratio between non-YFP and YFP parasite vacuoles was calculated. The efficiency of invasion was determined by counting vacuoles in 20 fields (e.g. around 400 vacuoles) for each condition and for three independent experiments.

**Intracellular growth assay**

Parasites were grown for 24 hr ± ATc prior egress. New HFF were then inoculated with parasites and allowed to grow for 24 hr ± ATc before fixation with PFA/GA. Double IFA were performed using α-MLC1 or α-PRF and α-SAG1 antibodies. The number of parasites per vacuole was
determined by counting the parasites in 100 vacuoles in duplicate and for three independent experiments.

**MALDI-TOF-TOF Analysis**

Samples obtained after co-immunoprecipitation assays were separated on a 12% SDS-PAGE gel and stained with Coomassie blue. Band of interest were cut out from the gel and sent to the Proteomics Core Facility (Faculty of Medicine, University of Geneva, Switzerland) for analysis. Gel fragments were first destained, dried and subjected to protein digestion by trypsin according to standard protocols. Control extractions (blanks) were performed using pieces of gels devoid of proteins. MALDI-TOF/TOF analysis was performed with a 4800 Proteomics Analyzer mass spectrometer (Applied Biosystems, Framingham, MA, USA) according to the tuning procedures suggested by the manufacturer. Peak lists were generated with the Launch peak to MASCOT tools with the following settings: mass range 60–2000; peak density of maximum 50 peaks per 200 Da, minimal S/N ratio of 5, minimal area of 20, and maximum number of peak set at 200. The resulting peaklist were searched against the SwissProt/trEMBL database (Release 15.10 of 03-Nov-2009) restricted to *Alveolata species* (200868 entries) using Mascot (version 2.2.03, Matrix Science, London, UK). Search settings allowed one missed cleavage with the trypsin enzyme selected, one fixed modification (carboxymethylated cysteine) and a variable modification (oxidation of methionine). Scaffold (version Scaffold_3_00_02, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 95.0% probability as specified by the Peptide Prophet algorithm (Keller *et al.*, 2002). Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii *et al.*, 2003). Proteins that contained similar peptides and could
not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.
SUPPLEMENTAL REFERENCES


