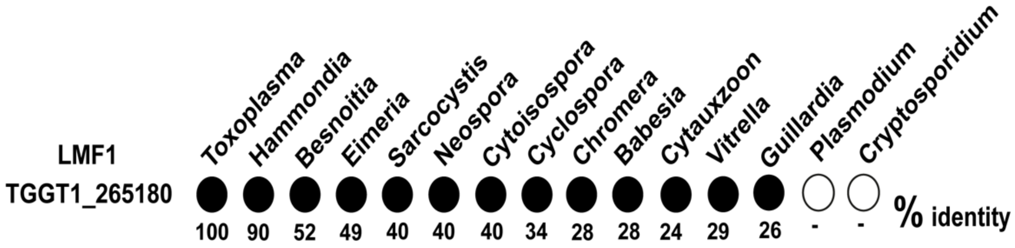


Fig. S1. Domains and phylogenetic analysis of LMF1. List of apicomplexan parasites with LMF1 orthologs. Black filled circle means the presence of an orthologue, and a white-filled circle means the absence of an LMF1 orthologue. Bellow the circles, the percentage of amino acid identity of each homologue found by reciprocal BLASTp.

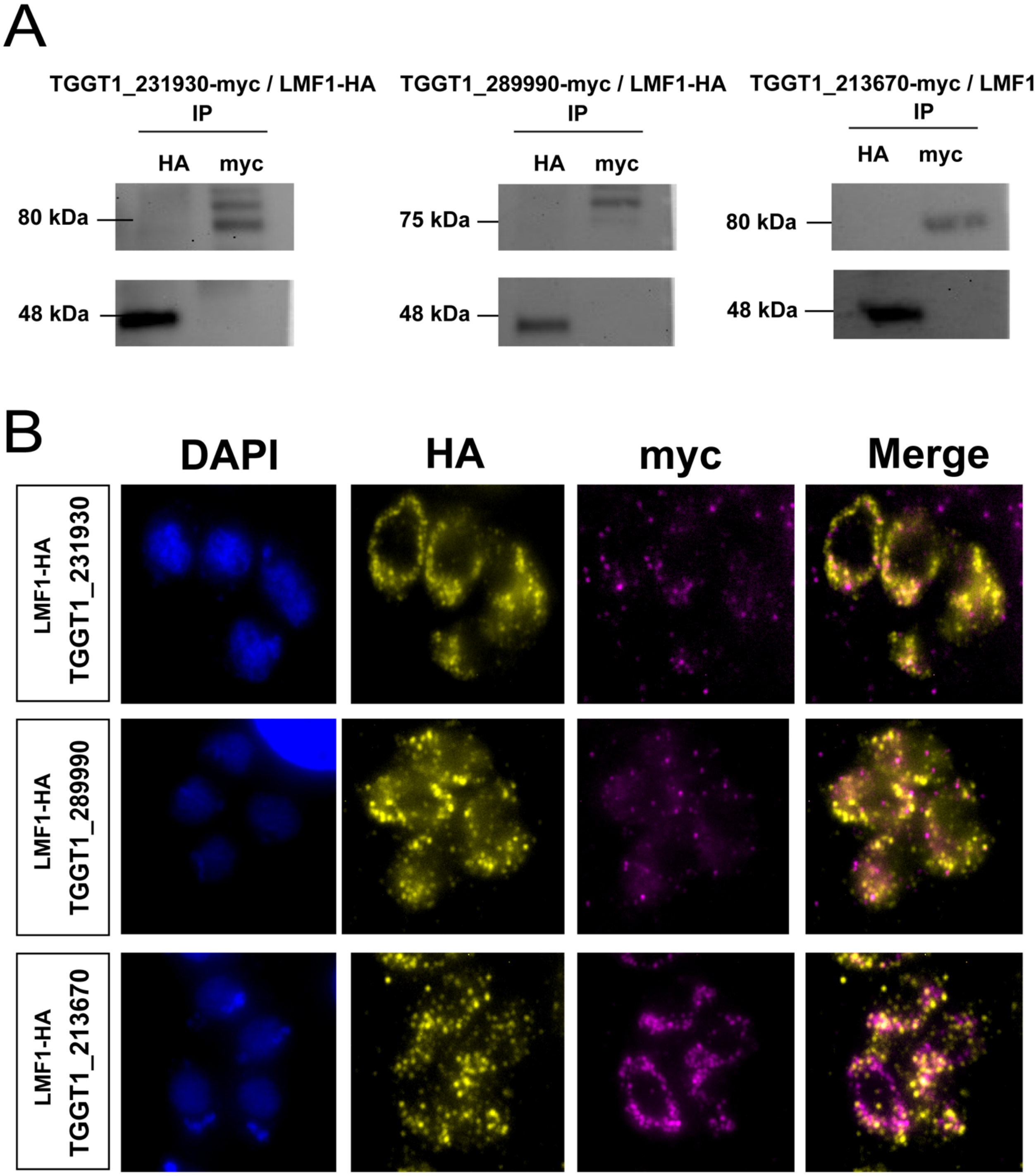


Fig. S2. Characterization of LMF1 interactors. To investigate the localization of LMF1 interactors, we introduced sequences encoding an N-terminal Myc tag to the endogenous locus in the parasite strain expressing an HA-tagged LMF1. A) Reciprocal co-immunoprecipitation of putative LMF1 interactors was performed for the strains expressing LMF1-HA and either TGGT1_231930-Myc, TGGT1_289990-Myc, or

TGGT1_213670-Myc. For each of the three dually tagged parasite strains, proteins were immunoprecipitated with either anti-HA or anti-Myc conjugated beads and probed with either Myc (for the interactor) and for HA (for LMF1). B) Intracellular parasites expressing the Myc tagged versions of TGGT1_231930-myc, TGGT1_289990-myc and TGGT1_213670-myc were stained for HA (yellow) and myc (magenta).

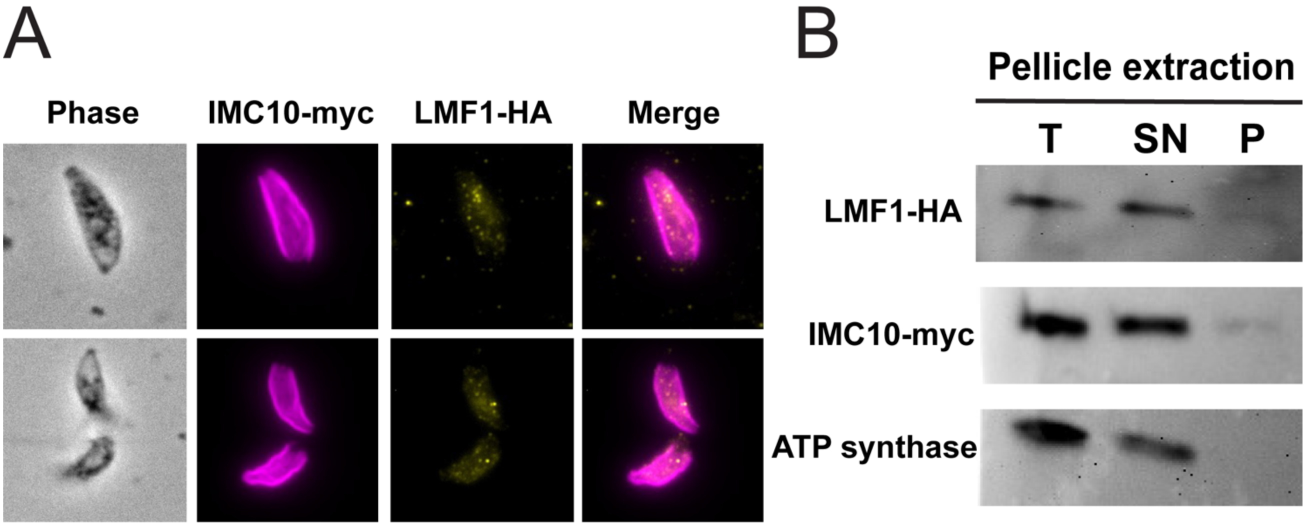


Fig S3. Localization of LMF1 in isolated pellicles. Pellicles were extracted from intracellular parasites with deoxycholate (DOC) and used for IFA (A) and Western Blots (B). A) IFA of DOC extracted pellicles showing IMC10 (magenta) and LMF1 (yellow). B) Representative western blots from total parasite extract (T) and both the supernatant (SN) and pellet (P) from the DOC extracted fraction. Western blots were probed for LMF1 (anti- HA), IMC10 (anti-Myc), and the ATP synthase subunit B. Only IMC10 and LMF1 are detected in the pellet fraction, which contains the parasite pellicle.

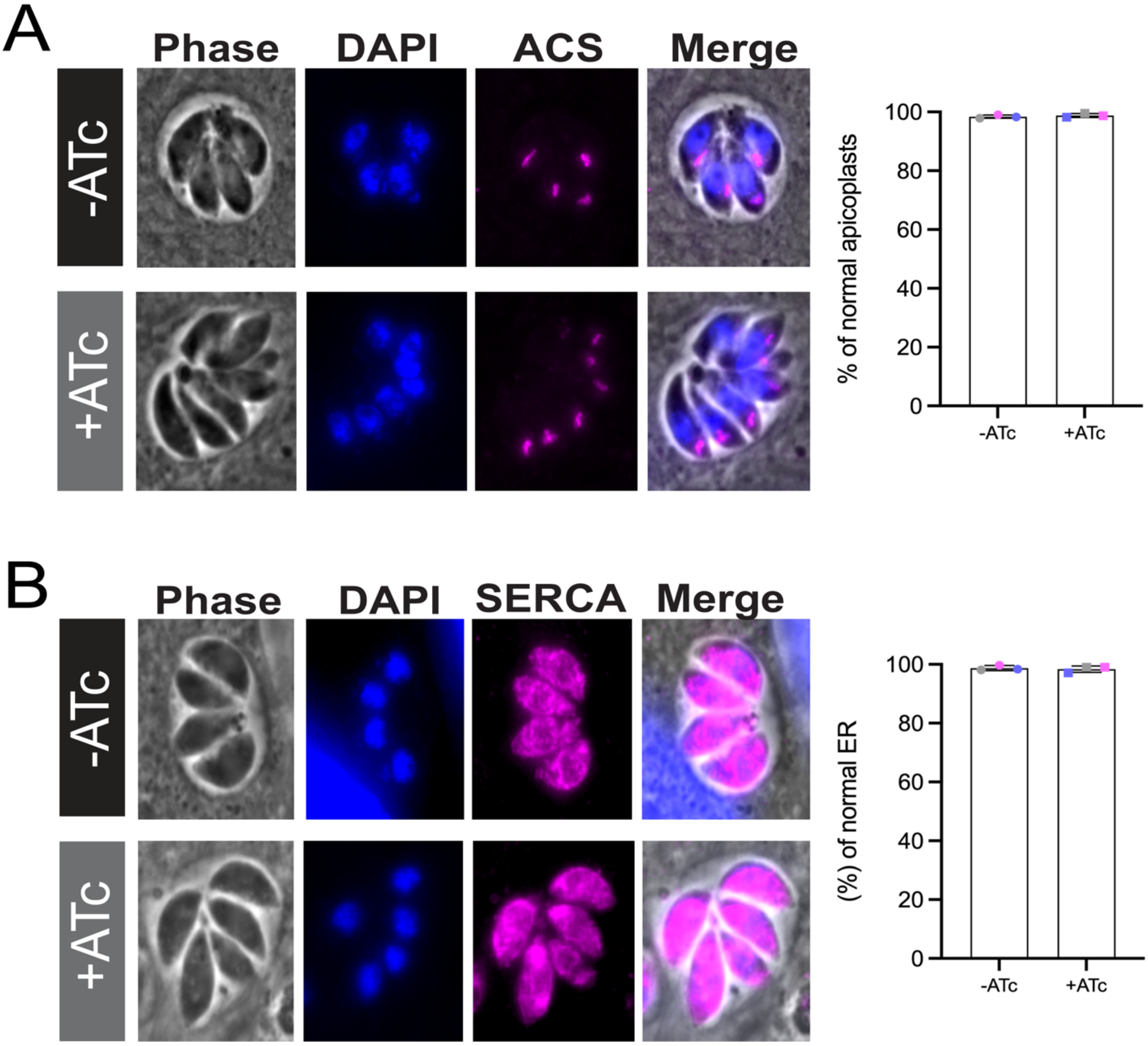


Fig. S4. IMC10 knockdown does not affect apicoplast or endoplasmic reticulum (ER) morphology. IFA of parasites stained for DAPI (blue), apicoplast, and ER (magenta) showing the shape of both structures in parasites maintained with and without ATC for 48h. A) Apicoplast morphology at 48h. B) ER morphology at 48h. All graphs represent the percentage of vacuoles with the related phenotype. At least 150 vacuoles per sample were inspected. For all graphs, n = 3. Error bars represent standard deviation.

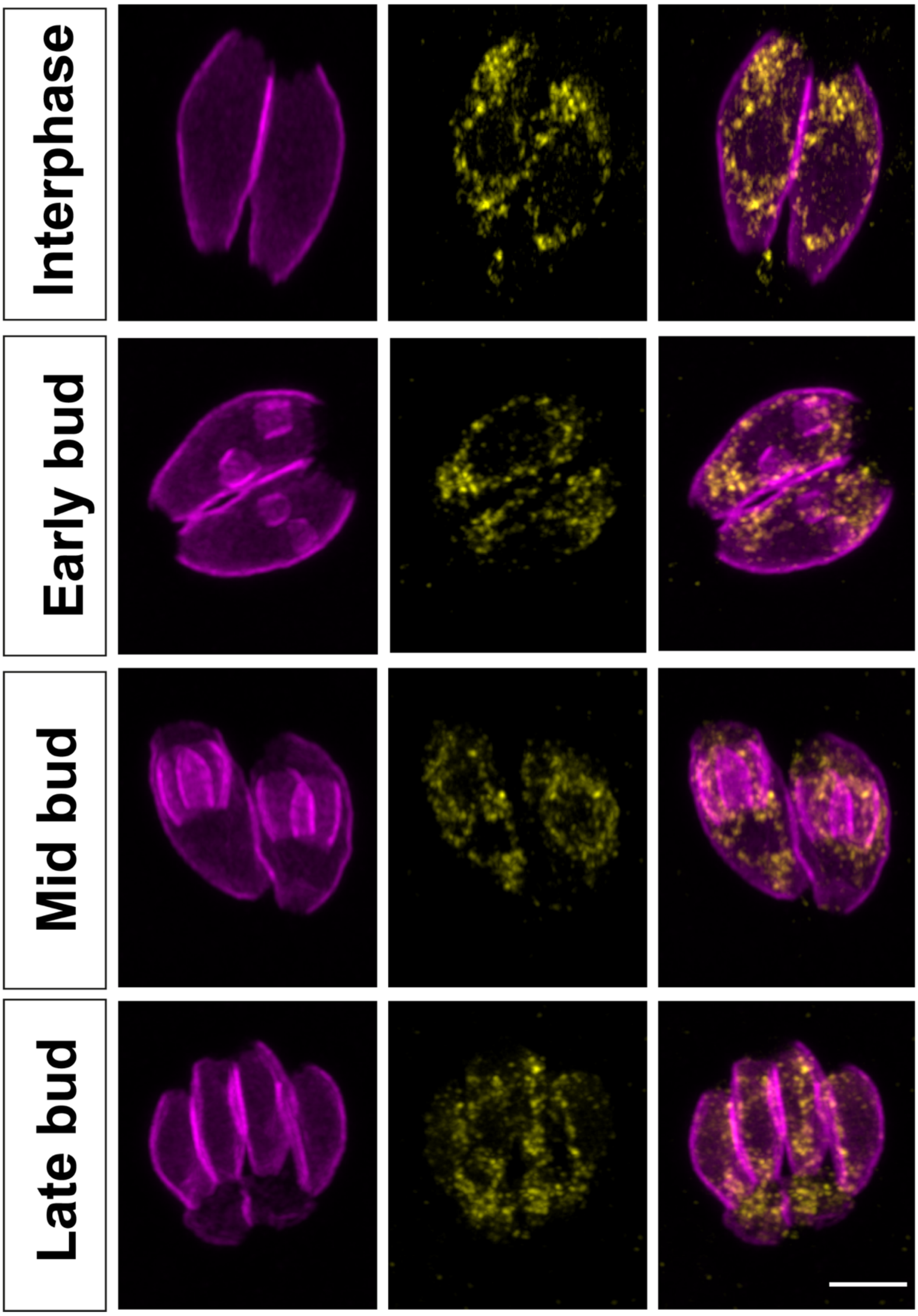
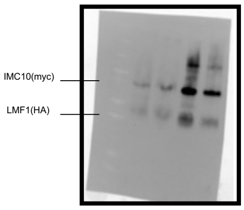


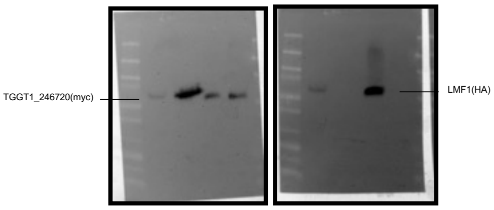
Fig. S5. LMF1 and IMC10 interact during mitochondrial distribution. IFA of intracellular parasites stained for LMF1-HA (yellow) and IMC10-Myc (magenta). Scale bar = 5μm. Images were acquired using a Zeiss LSM 800 microscope with Airyscan processing.

Western blots Figure 1B

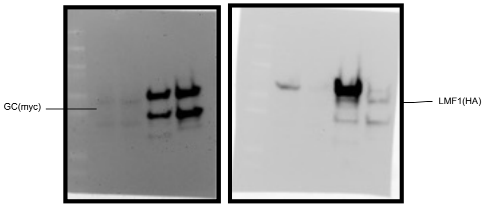
IP
IMC10(myc)/LMF1(HA)
Probed against myc and HA



IP
TGGT1_246720(myc)/LMF1(HA)
Probed against myc and HA

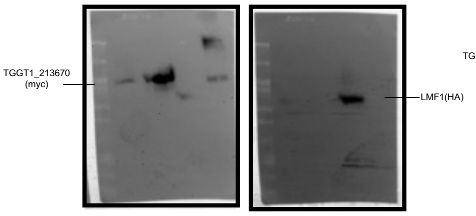


IP
GC(myc)/LMF1(HA)
Probed against myc and HA

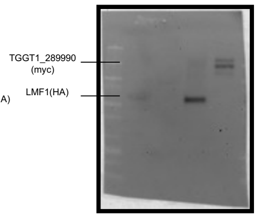


Western blots Figure S2A

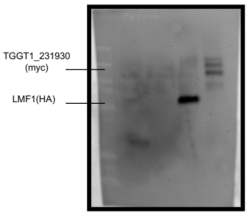
IP
TGGT1_213670(myc)/LMF1(HA)
Probed against myc and HA



IP
TGGT1_289990(myc)/LMF1(HA)
Probed against myc and HA

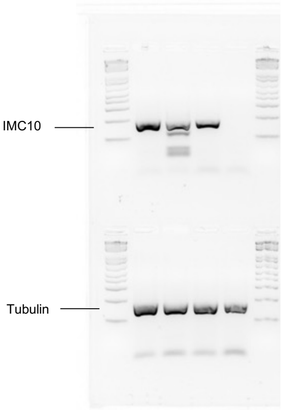


IP
TGGT1_231930(myc)/LMF1(HA)
Probed against myc and HA

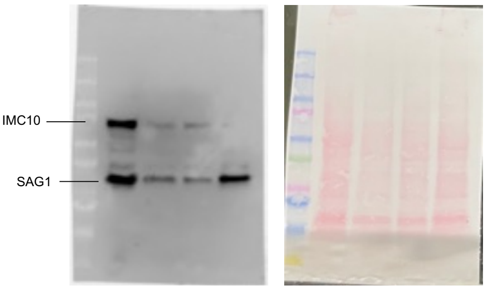


Western blots Figure 3C and D

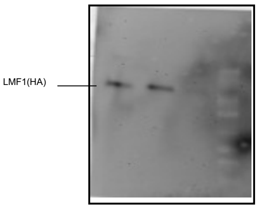
iKD-IMC10 cell line knockdown
PCR using cDNA from intracellular parasites in the presence (or not) of Atc.

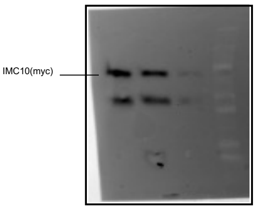


iKD-IMC10 cell line knockdown
Probed against anti-IMC10 and anti-SAG1



Western blots Supplemental Figure S3





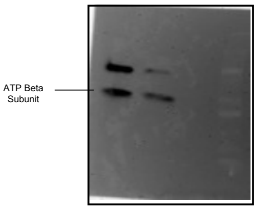


Fig. S6. Original images for the western blots showed in this work with molecular weight.

Table S1. List of candidates LMF1 interactors. Listed are proteins identified by the yeast two-hybrid (Y2H) screen. Included are the Gene ID, the gene annotation, and the global PrBS score for the likelihood of interaction in Y2H.

Gene Name (Best Match)	GenBank ID (NCBI)	ToxoDB ID	Global PBS
ALV12	XM_002367901.1	TGME49_230210	A
SRS20A	XM_002369184.2	TGME49_285870	A
elongation factor 1-gamma, putative	XM_002371696.2	TGME49_300140	A
tRNA ligase class II core domain (G, H, P, S and T) domain-containing protein	XM_002370233.2	TGME49_295050	A
MIC2	XM_018779175.1	TGME49_201780	B
Sel1 repeat-containing protein	XM_018780375.1	TGME49_233810	B
guanylyl cyclase	XM_018781054.1	TGME49_254370	B
hypothetical protein	XM_002366901.2	TGME49_244530	B
myosin A	XM_002368901.2	TGME49_235470	B
phosphoglycerate mutase family protein	XM_018781182.1	TGME49_258540	B
clathrin heavy chain, putative	XM_002368438.2	TGME49_290950	C
hypothetical protein	XM_002365609.1	TGME49_269690	C
hypothetical protein	XM_002366715.2	TGME49_242260	C
hypothetical protein	XM_002364969.2	TGME49_257760	C
hypothetical protein	XM_018779329.1	TGME49_205360	C
hypothetical protein	XM_002371294.2	TGME49_217680	C
hypothetical protein	XM_002370465.2	TGME49_278030	C
hypothetical protein	XM_018782763.1	TGME49_313380	C
ACC2	XM_002370599.2	TGME49_218560	D
CDPK7	XM_018780218.1	TGME49_228750	D
ClpB, putative	XM_018781733.1	TGME49_275690	D
DEAD-family helicase	XM_002371178.2	TGME49_298020	D
DEAD/DEAH box helicase domain-containing protein	XM_002370173.1	TGME49_294350	D
DNA polymerase family B protein	XM_018782969.1	TGME49_319860	D
FRM1	XM_018779357.1	TGME49_206430	D
GRA5	XM_002369230.2	TGME49_286450	D
GRA6	XM_002371898.2	TGME49_275440	D
LisH protein	XM_002364639.2	TGME49_314900	D
PCI domain-containing protein	XM_002366378.2	TGME49_227960	D
RNA pseudouridine synthase superfamily protein	XM_002370387.2	TGME49_306660	D
Sec7 domain-containing protein	XM_018781442.1	TGME49_266830	D
WD domain, G-beta repeat-containing protein	XM_018781644.1	TGME49_272040	D
XPA binding protein 2 family protein	XM_002370276.2	GME49_305240	D
crooked neck family 1 protein isoform 2, putative	XM_018781534.1	TGME49_269200	D
eukaryotic initiation factor-3, subunit 8, putative	XM_018782235.1	TGME49_294620	D
histidine acid phosphatase superfamily protein	XM_018779286.1	TGME49_204080	D
hypothetical protein	XM_002366120.2	TGME49_224870	D
hypothetical protein	XM_002367018.2	TGME49_246720	D
hypothetical protein	XM_002366895.2	TGME49_244470	D

hypothetical protein	XM_018780307.1	TGME49_231930	D
hypothetical protein	XM_018782084.1	TGME49_289520	D
hypothetical protein	XM_002368014.1	TGME49_231840	D
hypothetical protein	XM_018779183.1	TGME49_202040	D
hypothetical protein	XM_018780814.1	TGME49_247290	D
hypothetical protein	XM_018781060.1	TGME49_254470	D
hypothetical protein	XM_018781216.1	TGME49_259720	D
hypothetical protein	XM_018780111.1	TGME49_225745	D
hypothetical protein	XM_018779615.1	TGME49_213670	D
hypothetical protein	XM_018781450.1	TGME49_267020	D
hypothetical protein	XM_018781196.1	TGME49_258870	D
hypothetical protein	XM_018780237.1	TGME49_229790	D
hypothetical protein	XM_018780016.1	TGME49_223760	D
hypothetical protein	XM_002370104.2	TGME49_293430	D
hypothetical protein	XM_018782540.1	TGME49_308070	D
hypothetical protein	XM_018781162.1	TGME49_258070	D
hypothetical protein	XM_002367593.2	TGME49_203520	D
hypothetical protein	XM_002369737.1	TGME49_318390	D
hypothetical protein	XM_018781098.1	TGME49_255300	D
hypothetical protein	XM_002367173.2	TGME49_248680	D
hypothetical protein	XM_002369325.2	TGME49_252880	D
hypothetical protein	XM_018781674.1	TGME49_272695	D
hypothetical protein	XM_002368383.2	TGME49_289990	D
kinesin heavy chain, putative	XM_002365916.1	TGME49_273560	D
myosin H	XM_002366784.2	TGME49_243250	D
root hair defective 3 gtp-binding protein (rhd3) protein	XM_018782846.1	TGME49_314970	D
tetratricopeptide repeat domain containing protein	XM_002369289.2	TGME49_252220	D
tetratricopeptide repeat-containing protein	XM_002371471.2	TGME49_295730	D
ubiquitin family protein	XM_002371852.2	TGME49_304680	D
zinc finger (CCCH type) motif-containing protein	XM_018780934.1	TGME49_250690	D
hypothetical protein	XM_002370830.2	TGME49_215520	E

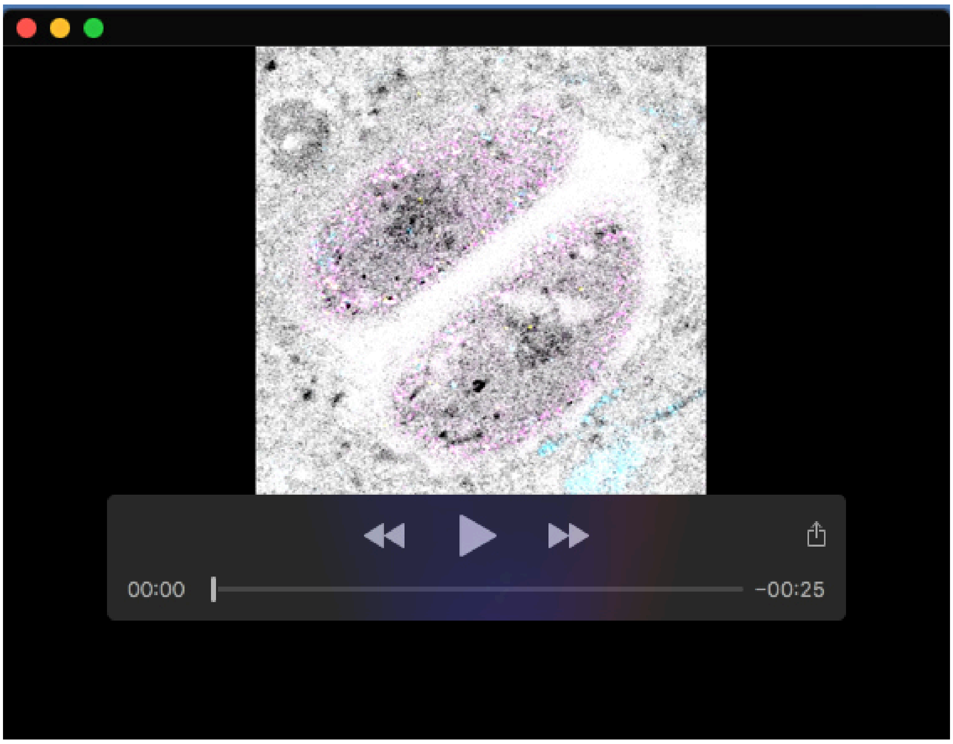
Table S2. LMF1 interactors identified by immunoprecipitation. Listed are proteins that had at least four peptides in the LMF1 IP and zero in control (parental parasites). LMF1 is in yellow and IMC10 in pink. Proteins that are known to localize to the pellicle are highlighted in grey. Included are the Gene ID, the gene annotation, and the number of peptides detected by mass spectrometry.

Gene ID	**Product Description**	Total Peptides
TGGT1_265180	**LMF1**	**53**
TGGT1_286580	IMC17	40
TGGT1_219320	GAP50	38
TGGT1_230210	**IMC10**	**30**
TGGT1_324600	HSP20	26
TGGT1_222220	IMC7	23
TGGT1_308860	AC3	21
TGGT1_232410	TrxL1	20
TGGT1_258410	PhIL1	16
TGGT1_248700	IMC12	13
TGGT1_241170	hypothetical	12
TGGT1_252360	ROP24	10
TGGT1_219270	GAPM2a	9
TGGT1_219310	DnaK family protein (HSP70)	9
TGGT1_249970	Acylated Pleckstrin-Homology (APH)	7
TGGT1_262050	ROP39	6
TGGT1_287500	T complex chaperonin, putative	4
TGGT1_316540	ISP3	4

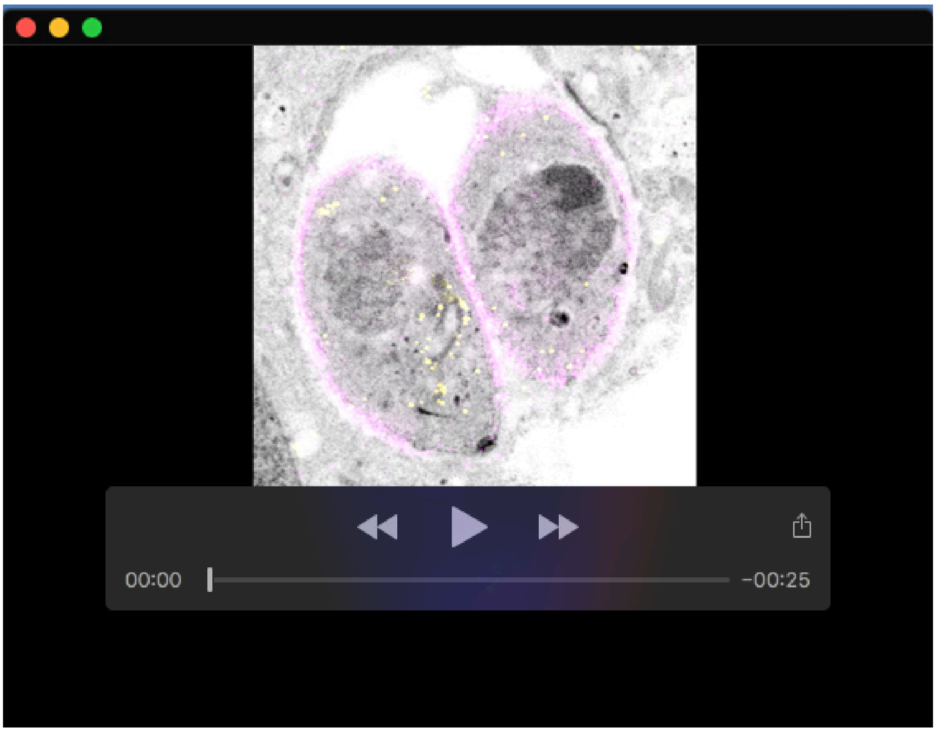
Table S3. Sequences of primers were used in this study. Small caps indicate overhangs with homology to the targeted region for recombination.

Use	#	Primers (5’-3’)
IMC10 guide RNA targeting	P001	acaacacacaGTTTTAGAGCTAGAAATAGC
	P002	gggacgtctcAACTTGACATCCCCATTTAC
IMC10 C-terminal homology regions for myc tagging	P003	GCCAGCGGAGTAGGATTGGGCGAAGAGGCACAGATCAGCGCCttaattaaaattggaagtggagg
	P004	CATGCCCTGTCCCTAAAAATTAGTTCCCTTTCTCAGTTGTAGGTTTTCCCAGTCACGACG
TGGT1_246720 guide RNA	P005	tggatgtcagGTTTTAGAGCTAGAAATAGC
	P006	aacctctttcAACTTGACATCCCCATTTAC
TGGT1_246720 homology regions for myc tagging	P007	TACCACGGCCACGGAAATATCATCACATGGGGAAAATCTTGTttaattaaaattggaagtggagg
	P008	ATCACGCGTCCGGCTGGGTTTCTTTTAACGGAGACATACGAAGTTTTCCCAGTCACGACG
ATPase-GC guide RNA	P009	aacgcagaacAACTTGACATCCCCATTTAC
	P010	gctccagaggGTTTTAGAGCTAGAAATAGC
ATPase-GC homology regions for Myc tagging	P011	CCTTCGGATATAGGGTCGACACCTGGCTCTGCACTCGGGTCGttaattaaaattggaagtggagg
	P012	GCCTCTGTCTGTCTCGACTGCCCGAAGCGGCAGGACACAGACGTTTTCCCAGTCACGACG
TGGT1_213670 guide RNA	P013	ggtgtgttgcGTTTTAGAGCTAGAAATAGC
	P014	cacccccgtcAACTTGACATCCCCATTTAC
TGGT1_213670 homology regions for myc tagging	P015	GCATGCATCAATGCGGATCTGCTTGCCGGTGCTAAATCCCCAttaattaaaattggaagtggagg
	P016	TCTTCAAGTCTAGGTCGGCGAATAGTTCGTACCACCTAGCATGTTTTCCCAGTCACGACG
Guide RNA change using Q5	P017	gcgagggacgGTTTTAGAGCTAGAAATAGC
	P018	actctgttgcAACTTGACATCCCCATTTAC
TGGT1_246720 homology regions for Myc tagging	P019	CGAGACGAGGAAGACGACGTTTCCTCGCTTTCCGAAGACGAGttaattaaaattggaagtggagg
	P020	GAACAGAAGACTCCCGTGCACAGCGTCTGCTTCCATCGTGTGTGTTTTCCCAGTCACGACG
Guide RNA change using Q5	P021	gagacgaattGTTTTAGAGCTAGAAATAGC
	P022	aggtggagtcAACTTGACATCCCCATTTAC
TGGT1_246720 homology regions for Myc tagging	P023	CTCCGACAGCGGCCTGCAGCAGGATTCTCTCTGCAGAACGAGttaattaaaattggaagtggagg
	P024	GGAGACAAGGAGCCCTTGGTACTTTAGTGCCACGCACGCTCAGTTTTCCCAGTCACGACG
Guide RNA change using Q5	P025	taataggagtGTTTTAGAGCTAGAAATAGC
	P026	tcagaggtccAACTTGACATCCCCATTTAC
	P027	TTCAGGAATCGCCAAACAAACATTCCAGTGGCTTTTCCTACTcatgtttgcggatccgggg
	P028	TGTGTGTGTGCGAAAAGGGAATCGAGGATAGCGTTAGGACGACAGGTCCTCCTCGGAGATGA
qPCR IMC10	P029	AGGAGGTCGAGGTTGTTCAG
	P030	TCAAAGGAGAGCAGTAGGGC
qPCR Tubulin	P031	TGTCGCTGAGATCACCAACT

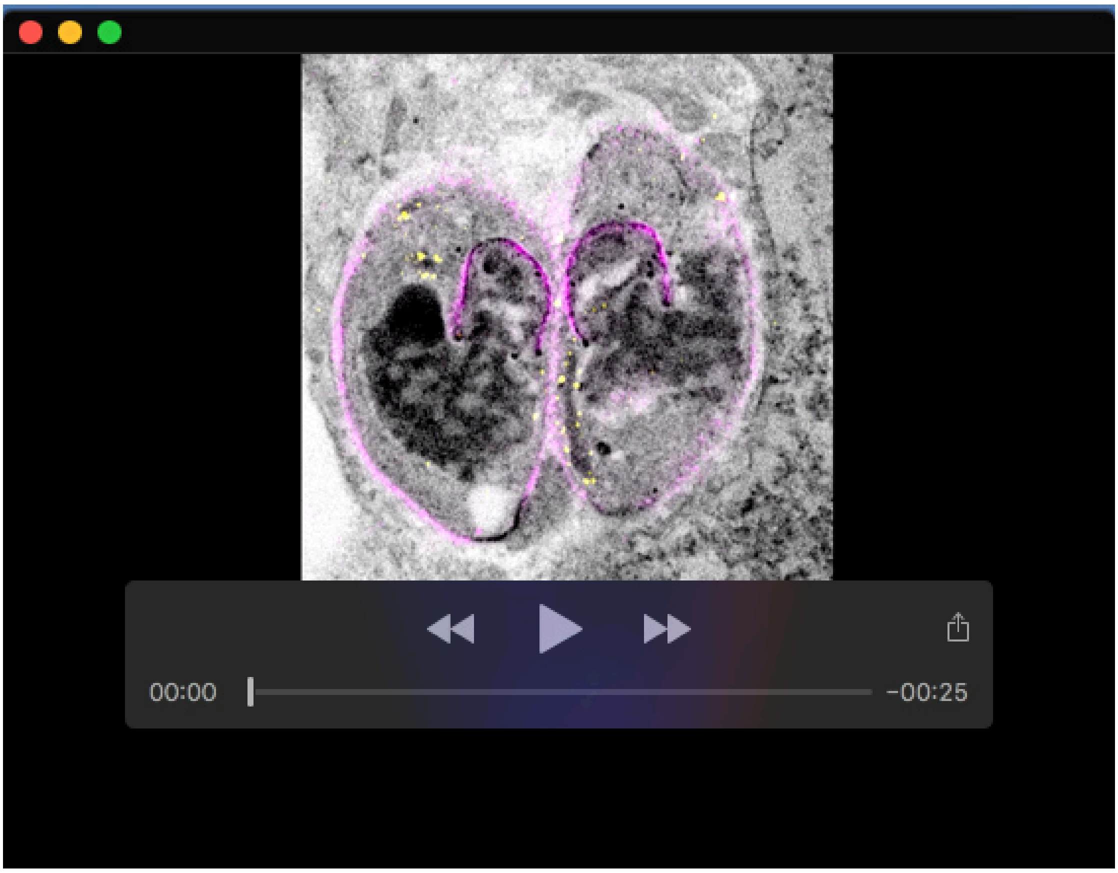
	P032	AGTCGACGAACTGGATGGTT
Promoter replacement confirmation	P033	gcctacgtgacttgctgatg
	P034	Gagtggaggattcgcagtct
Generation of pET-LIC-TEV IMC10 expressing construct	P035	tacttccaatccaatgcaGGAACTGCCACACACAAGGT
	P036	ttatccacttccaatgttattaGGCGCTGATCTGTGCCTCTT



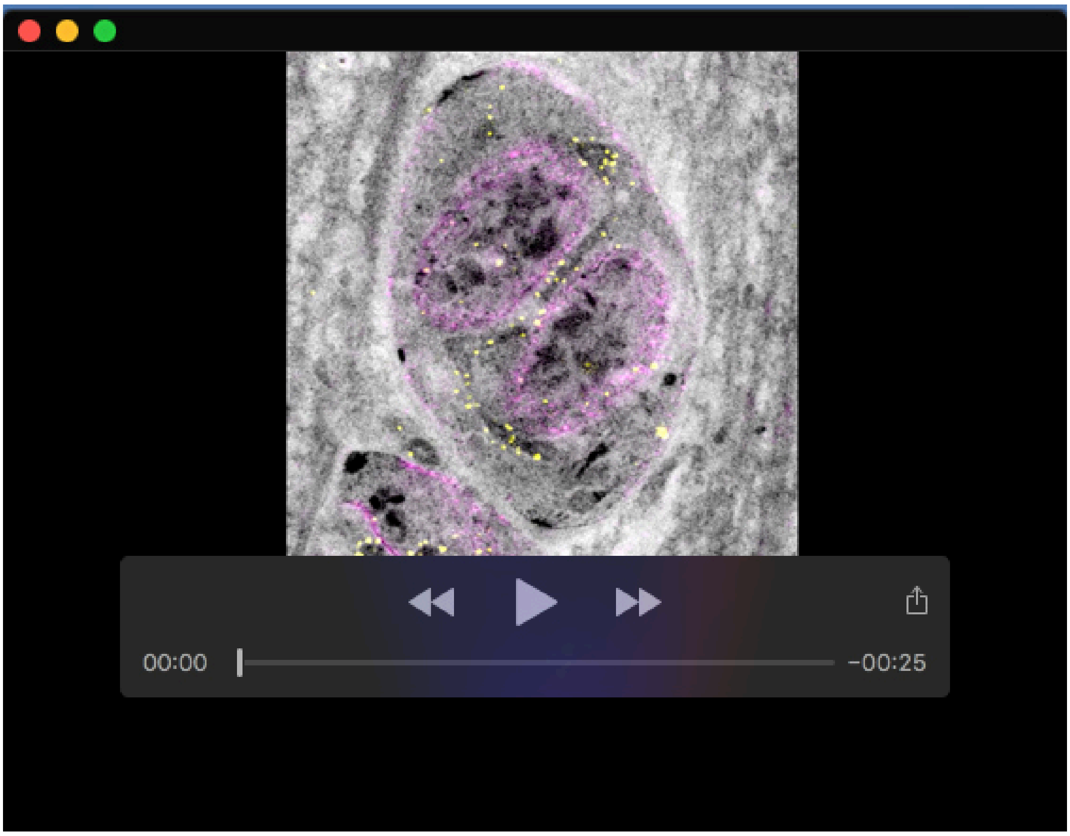
Movie 1. Full Z-stacking of expanded parasites showed in Fig 1B. Images were acquired using a Zeiss LSM 900 with Airyscan processing. The movie was edited and processed in FIJI. The IMC is represented in magenta, LMF1 in yellow and Mitochondrion in cyan.



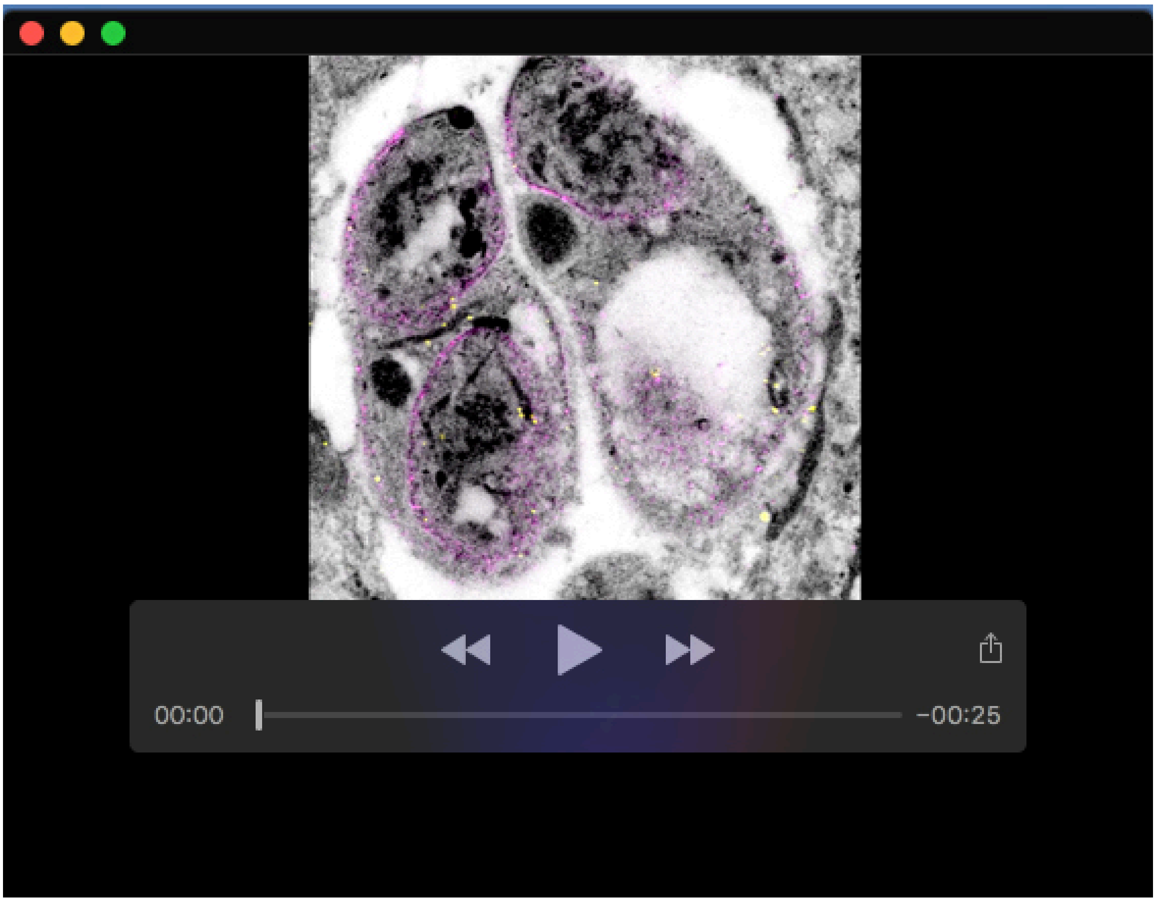
Movie 2. Full Z-stacking of expanded parasites during interphase as shown in Fig 7A. Images were acquired using a Zeiss LSM 900 with Airyscan processing. The movie was edited and processed in FIJI. The IMC is represented in magenta, LMF1 in yellow.



Movie 3. Full Z-stacking of expanded parasites undergoing division during an early stage as shown in Fig 7A. Images were acquired using a Zeiss LSM 900 with Airyscan processing. The movie was edited and processed in FIJI. The IMC is represented in magenta, LMF1 in yellow.



Movie 4. Full Z-stacking of expanded parasites undergoing division during a mid-bud stage as shown in Fig 7A. Images were acquired using a Zeiss LSM 900 with Airyscan processing. The movie was edited and processed in FIJI. The IMC is represented in magenta, LMF1 in yellow.



Movie 5. Full Z-stacking of expanded parasites undergoing division during a late stage as 96 shown in Fig 7A. Images were acquired using a Zeiss LSM 900 with Airyscan processing. The movie was edited and processed in FIJI. The IMC is represented in magenta, LMF1 in yellow.