**Supporting information**
**Bookwalter et. al.**

## S1 figures


**Fig. S1** Sequence comparison of the PfELC (Plasmo DB PF3D7_1017500, GenBank XP_001347455.1) identified here, with essential light chain isoforms from *Toxoplasma gondii*, TgELC1 (Toxo DB TGME49_269442, GenBank XP_002365635.1) and TgELC2 (Toxo DB TGME49_305050, GenBank XP_018636125.1) (1, 2). Identity in all three proteins is shown in salmon, identity in PfELC and one of the TgELC isoforms in yellow. Common charged residues (D/E or R/K) present in all three light chains are indicated in green. PfELC is 20% identical to TgELC1 and 21% identical with TgELC2.

A

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                                                                  52
MASDMEEKFREAFILFSSCSDHIEMYKFFELMNSFGIILTNDEKAALPNDIN
CCCHHHHHHHHHHHHHCCCCHHHHHHHHHHHHHHHHHCCCCCCHHHHCCCCCCCCC
                                                                 104
MDYWLNFAKKHYNYEQPFKHINNVNEQNTNVQIKIDNFLGIMKALDTRLTES
HHHHHHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCCHHHHHHHHHHHHHHCCHH
                                                        134
DLNILLQITNPENKSTLNLKTVSQKLTESI
HHHHHHHHCCCCCCCCCCCHHHHHHHHHHCC
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B

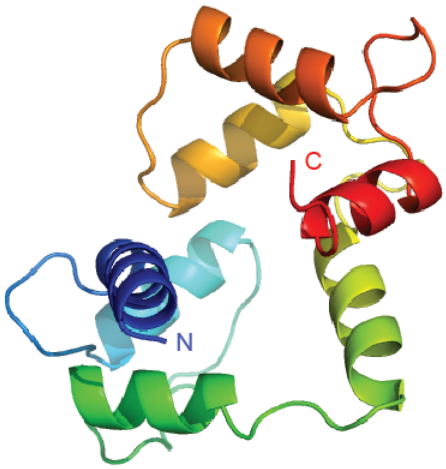


**Fig. S2** Secondary and tertiary **s**tructure prediction of PfELC using the RaptorX web portal (http://raptorx.uchicago.edu/). (A) Secondary structure prediction shows 59% helix (bold red H), and 41% coil (black C) (3-5). (B) Tertiary structure prediction (6) with the N- and C-termini labeled.

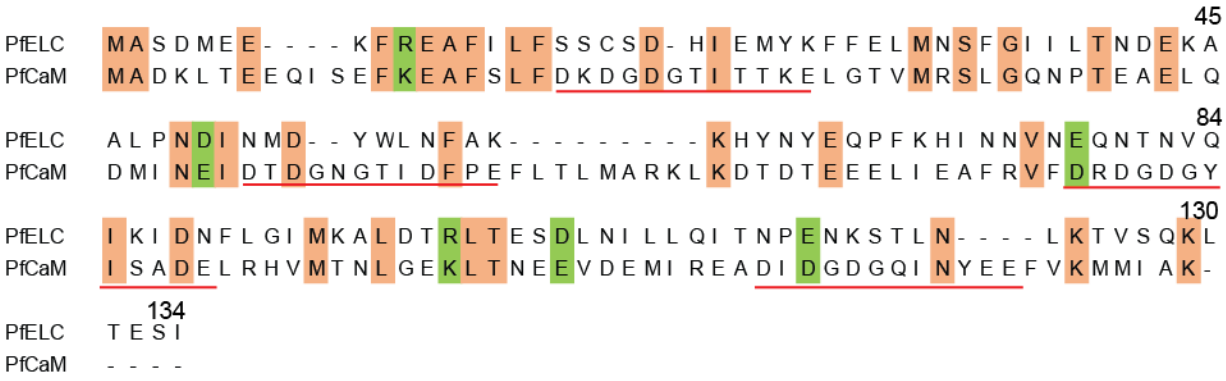


**Fig. S3** Sequence comparison of the PfELC (Plasmo DB PF3D7_1017500, GenBank XP_001347455.1) identified here and Pf calmodulin (PfCaM) (Plasmo DB PfCaM PF3D7_1434200, GenBank XP_001348497). Identity is shown in salmon, common charged residues (D/E or R/K) in green. The calcium-binding sites in PfCaM are indicated by the horizontal red line. Calcium binding requires a D/E at position 12 of the motif, and thus PfELC does not appear to have a functional calcium binding site.

## S1 Materials and Methods

***Plasmodium* Actin 1 Expression and Purification.** Expression and purification of Plasmodium actin 1 was as described in (7) with the following modifications. Infected *Sf*9 cells (2 x$10^9$) were harvested 3 days after infection and lysed by sonication in 50 ml 10 mM HEPES, pH 8.0, 0.25 mM $CaCl_2$, 0.3 M NaCl, 7 mM β-mercaptoethanol, 0.25 mM $Na_2$ATP, clarified, and immediately bound to a nickel affinity column. Non-specifically bound protein was washed off with buffer containing 10 mM imidazole pH 8.0, 10 mM HEPES, pH 8.0, 0.25 mM $CaCl_2$, 0.3 M NaCl, 7 mM β-mercaptoethanol, 0.25 mM $Na_2$ATP, 1 µg/ml leupeptin. Actin was eluted with the wash buffer with 200 mM imidazole, For proteolytic removal of the thymosin and HIS tag, a 1:30 weight ratio of chymotrypsin:actin was used.

**Labeling *Plasmodium* Actin for Visualization in the Motility Assay.** Labeled *Plasmodium* actin monomer was prepared by first adding fresh 1 mM DTT to expressed *Plasmodium* actin in G buffer (5 mM Tris pH 8.26 at 4°C, 0.2 mM $CaCl_2$, 0.2 mM $Na_2$ATP, 0.5 mM DTT). The actin was then dialyzed against 5 mM imidazole, pH 6.8, 0.2 mM $CaCl_2$, 0.2 mM $Na_2$ATP. The pH was raised to 8.0 by adding Tris pH 8.0 to a final concentration of 20 mM. The cysteine reactive dye tetra-methyl-rhodamine maleimide (Sigma) was added in a 2.5-fold molar excess over actin, and incubated for 18 hours at 4°C. The reaction was stopped with a 20-fold molar excess of DTT over the label, and excess label was removed by dialysis against G buffer. The labeled actin was clarified by centrifugation (45 min at 350,000 x *g*), and frozen in liquid nitrogen and stored at -80°C. The degree of labeling was determined using an extinction coefficient of 95,000 $M^{-1}cm^{-1}$ at 560 nm for rhodamine. Actin concentration was quantified with the Bio-Rad Protein Assay. The degree of labeling was 1.7 mole rhodamine/mole actin. For preparing labeled *Plasmodium* actin filaments, 1 mg/ml G-actin (0.75 mg/ml unlabeled actin and 0.25 mg/ml rhodamine-labeled actin) was polymerized by addition of 1/10 volume of a 10x buffer containing 0.5 M KCl, 100 mM imidazole, pH 7.4, 40 mM $MgCl_2$ and 10 mM EGTA. An equal molar ratio of jasplakinolide (Thermo Fisher Scientific) was added during polymerization.

**Mass Spectrometry of *Sf*9 cell expressed PfMyoA.** The phosphorylation status of expressed PfMyoA-MTIP was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). The PfMyoA heavy chain 104kDa) and MTIP (24.6kDa) bands were separated on SDS-polyacrylamide gels, stained with Coomassie, and excised from the gel. The bands were destained with 50% acetonitrile (ACN), dehydrated with 100% ACN and dried in a speed vacuum device. The dried bands were rehydrated with 10 mM dithiothreitol (DTT) in 50 mM ammonium bicarbonate (AB) and incubated (55°C, 45 minutes). The DTT was removed and 55 mM iodoacetamide in 50 mM AB was added. The tubes were incubated in the dark (20°C, 30 minutes). The iodoacetamide was removed, and the gel slices were rinsed for 15 minutes with 50% ACN. Rinsing was repeated three times and the samples were dried in a speed vacuum. A 2-µg aliquot of trypsin (Promega) in 50 mM AB was added to each tube. The tubes were incubated at 4°C for 1 hour and then at 37°C overnight. An aliquot of 7% formic acid in 50 mM AB was added to each tube. The peptides were serially extracted with 3 aliquots of 50 mM AB, with 15 min incubations between extractions. The resultant peptides were dried in a speed vacuum device and then reconstituted in 0.05% trifluoroacetic acid. The peptides were separated on a Acquity UPLC HSS T3 column (100Å, 1.8 µm, 1 mm x 150 mm) (Waters) attached to a Dionex UltiMate 3000 high pressure liquid chromatography system (HPLC) (Dionex). The HPLC effluent was directly injected into a Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometer through an electrospray ionization source (ThermoFisher). Data were collected in data-dependent MS/MS mode with the top 5 most abundant ions being selected for fragmentation. Peptides were identified from the resultant MS/MS spectra using SEQUEST run via Proteome Discoverer 2.1 software (ThermoFisher). These searches were performed against a custom database that reflected the

cloned genes (PfMyoA heavy chain and MTIP) plus tags. Peptide oxidation was accounted for by addition of 15.99 and 31.99 Da to each methionine, carbamidomethylation was accounted for by addition of 57.02 Da to each cysteine, and phosphorylation was accounted for by adding 79.97 Da to each serine, threonine or tyrosine residue. All identification were manually confirmed by inspection of the MS/MS fragmentation spectra.

Quantitation of phosphorylation ion currents, measured for each identified peptide were extracted from the MS spectra using PinPoint software (ThermoFisher). The degree of phosphorylation was estimated in each sample from the loss in abundance of non-phosphorylated peptides using a mass-balance approach. This loss was determined from the ratio of the non-phosphorylated peptides in each sample to those in samples that had been treated with phosphatase (8, 9).

**Large scale FLAG-tagged immunoprecipitation of PfMyoA-2cMyc-2FLAG culture.** 3D7 parasites containing FLAG-tagged PfMyoA were maintained in standard cultures and synchronised at ring stage to 8% parasitemia by sorbitol treatment (10). This was further scaled up to 3L culture and harvested at mature schizont stage by lysing cultures with 0.1% saponin/PBS followed by washes with cold PBS to separate lysed erythrocytes from parasites. The parasite pellet was then immediately processed to purify the native PfMyoA complex. Fresh parasite pellet was resuspended in lysis buffer (10mM Imidazole, 300 mM NaCL, 1mM EGTA, 5mM $MgCl_2$, 1% v/v triton X100, 2mM ATP, 2 mM TCEP) and lysed using a syringe homogeniser on ice. Lysate was centrifuged at 70,000 rpm for 45 mins at 4 degrees. Anti-FLAG M2 resin (A4596, Sigma Aldrich) was prepared based on product information and was further washed with lysis buffer. Supernatant of parasite lysate was then mixed with prepared Anti-FLAG resin and incubated at 4 degrees for 1-2 hrs. The resin was further washed with wash buffer (10mM Imidazole, 300 mM NaCL, 1mM EGTA, 5mM $MgCl_2$, 2 mM TCEP) to remove Triton X100 and protein was eluted using elution buffer (10 mM Imidazole, 300mM NaCl, 1mM EGTA, 5% glycerol, 2mM TCEP, 3X FLAG peptide, F4799 Sigma-Aldrich). Samples were eluted from beads by boiling in Laemmli sample buffer and the eluates separated by SDS-PAGE until dye front had moved 1cm into separating gel. The gel lane was excised and subjected to in-gel tryptic digestion using a DigestPro automated digestion unit (Intavis Ltd.). Resulting peptides were fractionated using an Ultimate 3000 nanoHPLC system in line with an LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific). In brief, peptides in 1% (vol/vol) formic acid were injected onto an Acclaim PepMap C18 nano-trap column (Thermo Fisher Scientific). After washing with 0.5% (vol/vol) acetonitrile 0.1% (vol/vol) formic acid peptides were resolved on a 250 mm × 75 μm Acclaim PepMap C18 reverse phase analytical column (Thermo Fisher Scientific) over a 150 min organic gradient, using 7 gradient segments (1-6% solvent B over 1 min, 6-15% B over 58 min, 15-32% B over 58 min, 32-40% B over 5min., 40-90% B over 1min, held at 90% B for 6 min and then reduced to 1% B over 1 min) with a flow rate of 300 nl min−1. Solvent A was 0.1% formic acid and Solvent B was aqueous 80% acetonitrile in 0.1% formic acid. Peptides were ionized by nano-electrospray ionization at 2.1 kV using a stainless-steel emitter with an internal diameter of 30 μm (Thermo Fisher Scientific) and a capillary temperature of 250°C. Tandem mass spectra were acquired using an LTQ-Orbitrap Velos mass spectrometer controlled by Xcalibur 2.1 software (Thermo Fisher Scientific) and operated in data-dependent acquisition mode. The Orbitrap was set to analyze the survey scans at 60,000 resolution (at m/z 400) in the mass range m/z 300 to 2000 and the top ten multiply charged ions in each duty cycle selected for MS/MS in the LTQ linear ion trap. Charge state filtering, where unassigned precursor ions were not selected for fragmentation, and dynamic exclusion (repeat count, 1; repeat duration, 30s; exclusion list size, 500) were used. Fragmentation conditions in the LTQ were as follows: normalized collision energy, 40%; activation q, 0.25; activation time 10ms; and minimum ion selection intensity, 500 counts.

The raw data files were processed and quantified using Proteome Discoverer software v1.4 (Thermo Fisher Scientific) and searched against the UniProt *Plasmodium falciparum* isolate 3D7 database (5365 entries) using the SEQUEST (Ver. 28 Rev. 13) algorithm. Peptide precursor mass tolerance was set at 10 ppm, and MS/MS tolerance was set at 0.8Da. Search criteria included carbamidomethylation of cysteine (+57.0214) as a fixed modification and oxidation of methionine (+15.9949) as a variable modification. Searches were performed with full tryptic digestion and a maximum of 1 missed cleavage was allowed. The reverse database search option was enabled and all peptide data was filtered to satisfy false discovery rate (FDR) of 5%. Comparable eluates from a mirrored Anti-FLAG resin using untagged, parental 3D7 parasites were assayed as controls (see Dataset S1).

**Other supporting information**

**Dataset S1.** Supplementary excel spreadsheet for proteins identified by mass spectrometry from immunoprecipitation of PfMyoA-2cMyc-2FLAG lysate. Protein hits are listed from control 3D7 cultures (A) versus transgenic cultures in which PfMyoA is tagged with a 2cMyc-2FLAG C terminal tag. **Area** refers to the average area of the three unique peptides with the largest peak area. **Score** refers to the protein score, which is the sum of the scores of the individual peptides. **PSM** refers to the total number of identified peptide sequences (peptide spectrum matches) for the protein, including those redundantly identified. **AA**, amino acids of identified target. **MW**, predicted molecular weight. **Calculated pI** refers to the theoretically calculated isoelectric point. PfELC is highlighted in yellow.

Excel file to download
**Dataset S1** (XLSX)

## REFERENCES


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