Electronic supplementary material

Materials and methods

Constructs

*Pb*YOP1 was PCR-amplified from the genome of *P. berghei* ANKA. The intron was removed using overlapping PCR, and the resulting coding region with a C-terminal HA-tag was ligated into pET28a plasmid via the NheI/Xhol1 sites. Mutation of *Pb*YOP1 was performed using the QuikChange Lightning Site-Directed Mutagenesis Kit (Stratagene). For yeast expression, *Pb*YOP1 was amplified and inserted into pESC-URA. All constructs were verified by sequencing.

Protein purification

Plasmids expressing wild-type or mutant His-*Pb*YOP1-HA were transformed into DE3 strains and cultured in 1L Luria Broth medium. IPTG (300 μM) was added when the cultures reached an OD600 of 0.8 and grown overnight at 16˚C. Cells were harvested, resuspended in TSG (50 mM Tris pH 8.0, 150 mM NaCl, 20 mM imidazole, 10% glycerol), and lysed by sonication. The lysates were then mixed with 1% Foscholine-12 (Anatrace) for 1 h and cleared by centrifugation for 1 h at 4˚C in a rotor (45 Ti; Beckman Coulter) at 30,000 rpm. The supernatant was then incubated with 1 mL Ni-NTA sepharose (GE healthcare), washed with TSG containing 0.1% Foscholine-12, and eluted with TSG containing 300 mM imidazole and 0.1%

Foscholine-12. The elution was collected and concentrated to 500 µL and 30U thrombin added to remove the His-tag overnight. The protein was then further purified by gel filtration (Superdex 200, GE healthcare) in TSG containing 0.1% Foscholine-12. Selected fractions were concentrated to 2 mg/mL for reconstitution.

Reconstitution

For SUV production, POPC and DOPS (Avanti Polar Lipid) were mixed at a molar ratio of 85:15 (10 mM of total lipid). The mixture was then dried under a stream of N_2 and rehydrated in TSG1 buffer (50 mM Tris pH 8.0, 150 mM NaCl, 10% glycerol). After 10 freeze-thaw cycles, the vesicles were then extruded 11 times through the extruder (Avanti Polar Lipid) with 100 nm or 400 nm filter membranes. Purified *Pb*YOP1 (final concentration 0.4 mg/mL) in Foscholine-12 was then mixed with the pre-formed SUV (final concentration 0.4 mM) for 30 min at room temperature. The detergent was removed by addition of SM-2 Bio-beads four times at room temperature. Finally, the proteoliposomes were cleared by centrifugation at 14,000 rpm for 10 min.

Electron microscopy

Negative staining was performed with 2% uranyl acetate. First, 5 µL of the 5X diluted proteoliposome sample was placed onto a carbon-coated copper grid for 1 min, the excessive sample dried by filter paper, and the grid washed with deionized water. A total of 5 µL of filtered 2% uranyl acetate was placed on the grid and

excessive stain dried by filter paper. Images were collected at room temperature using a Hitachi TEM system operated at an acceleration voltage of 100 kV. Images were recorded at a magnification of 30,000 and a defocus value of 1.5 μm. All images were recorded on a 2k x 2k CCD camera.

Sucrose gradient centrifugation

A 5-layer sucrose gradient (5%, 10%, 15%, 20%, 25%, 50 μL each) in TS buffer (50 mM Tris pH 8.0 and 150 mM NaCl) was prepared in centrifugation tubes. Proteoliposomes treated with 20 μL 1% digitonin, Triton X-100, or SDS were loaded onto the top layer of the gradient. The samples were then centrifuged at 174,000 rpm in a TLS-55 rotor (Beckman Coulter) for 2 h at 4˚C. Each fraction sample was analyzed by Western blot with HA-tag antibody.

Circular dichroism

Synthesized peptide (50 μM) in 10 mM potassium phosphate (pH 7.5) was mixed with TSG buffer and 100 nm SUV (1 mM lipids) or 400 nm SUV (1 mM lipids) separately. Circular dichroism was performed on a Biologic MOS-450 instrument at room temperature. Spectra were collected from 190-260 nm at a bandwidth of 1 nm.

Mammalian cell culture and transfection

COS-7 cells (American Type Culture Collection) were maintained in complete Dulbecco's minimum essential medium supplemented with 10% fetal bovine serum

in 5% CO_2 at 37°C. Transfections were performed using Turbo (Thermo) according to the manufacturer's instructions. To generate ATL-deleted cells by CRISPR/Cas9 genome editing, guide RNA (gRNA) sequences were designed using the CRISPR design tool as following, with protospacer adjacent motifs (PAMs) underlined:

ATL2: 5′-GACGAGATCTTAACATAGTAGTGG-3′.

ATL3: 5′-GTTTTCACTGTGGAGAAGCCAGG-3′.

gRNA containing oligonucleotides were introduced into the pX330 vector. CRISPR/Cas9 plasmids were transiently transfected into COS-7 cells along with pLKO.1-puro at 1:1:1 ratio using TurboFect transfection reagent (Thermo). 24 hours later, transfected cells were selected with 1 μg/ml puromycin for 1 week. The cells were then sorted for single cell into a 96 well plate by a BD FACS Aria-II sorter. Different single clones were verified by immunoblotting and sequencing.

Immunofluorescence and confocal microscopy

Indirect immunofluorescence of paraformaldehyde-fixed cells was described previously (Hu et al., 2009). Transfected cells were grown on coverslips and immunostained with anti-calreticulin (abcam; 1:500) and anti-HA antibodies (Sigma; 1:500) as primary antibodies and various Alexa Fluor-conjugated secondary antibodies (AlexaFluor 488 anti-mouse or AlexaFluor 568 anti-rabbit, 1:1000, Invitrogen). Yeast cells were imaged live as described previously (Hu et al., 2009). All images were captured on an OLYMPUS FV1200 confocal microscope with a

60×/1.40 NA Plan Apochromat oil immersion objective lens using the Olympus Fluoview Version 2.0b Viewer. Brightness and contrast were adjusted across the entire image using Adobe Photoshop.

Figure S1. Sequence alignment of *Pb*YOP1, *Pb*YOP1L and HVA22. Labeling is as in Fig. 1A.

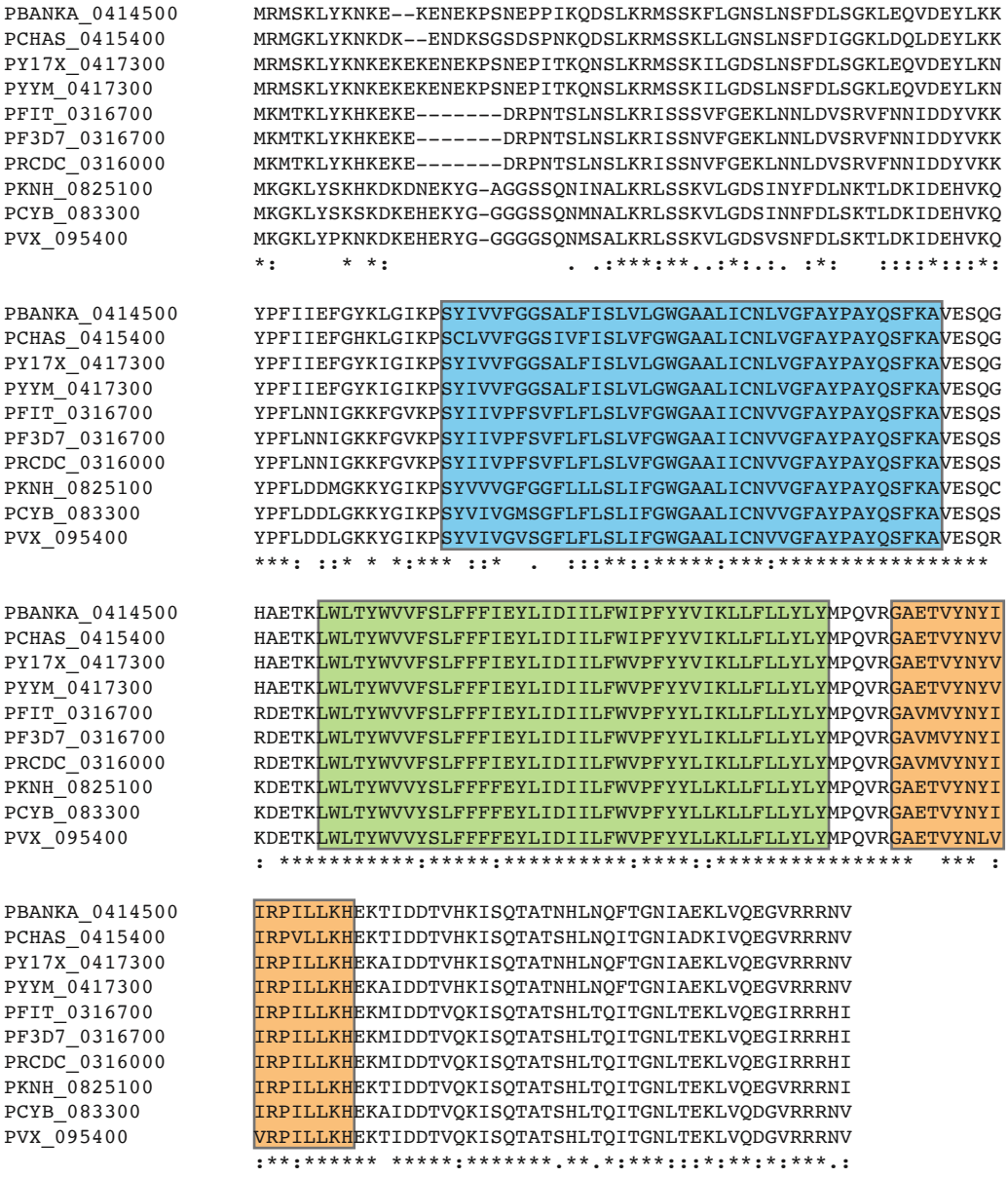

Figure S2. Sequence alignment of *Plasmodium* homologs of *Pb*YOP1. Labeling is as in Fig. 1A.

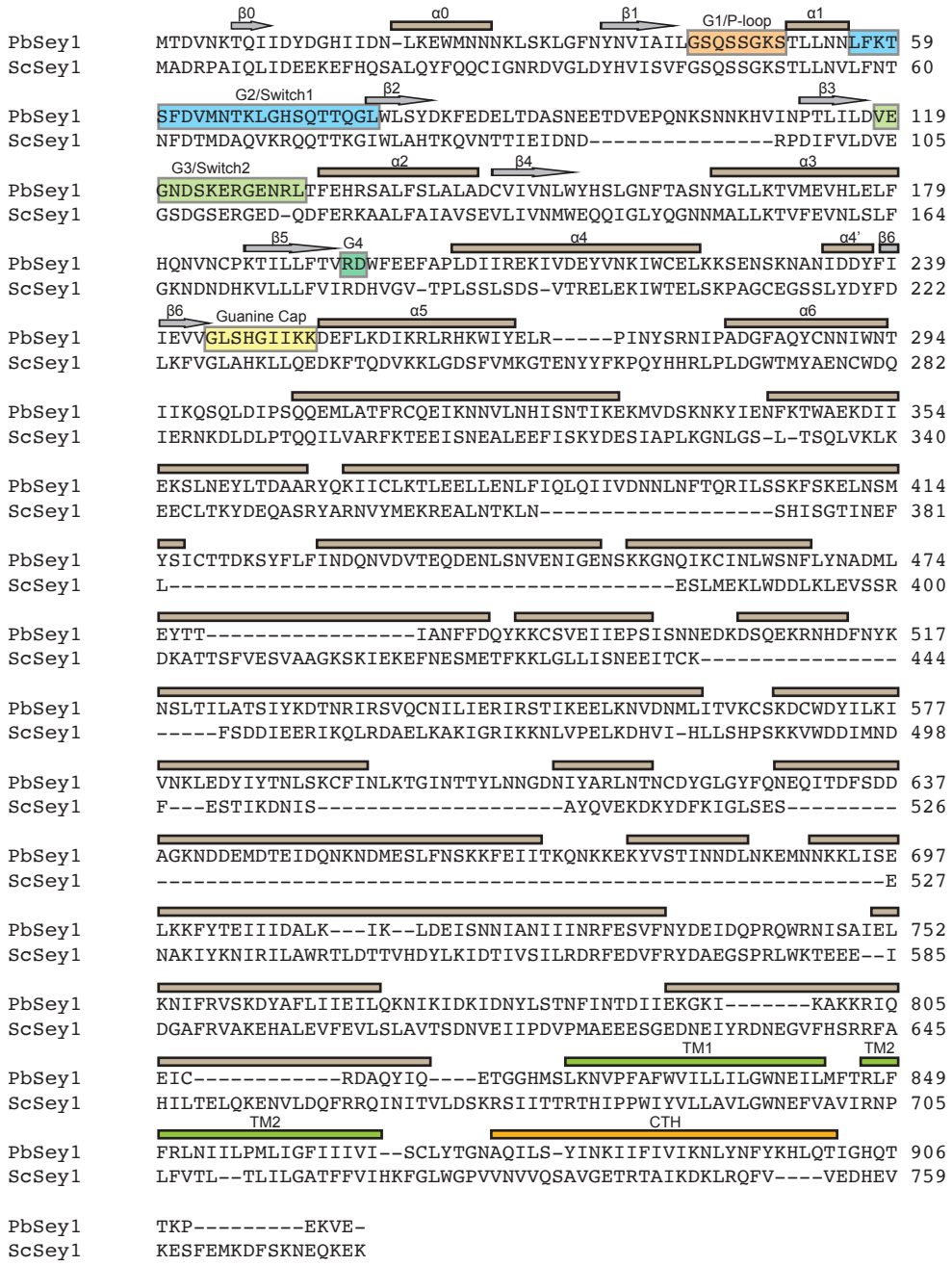

Figure S3. Sequence alignment of *Pb*SEY1 and *Sc*Sey1p. Predicted secondary structures are labeled and conserved elements are highlighted.

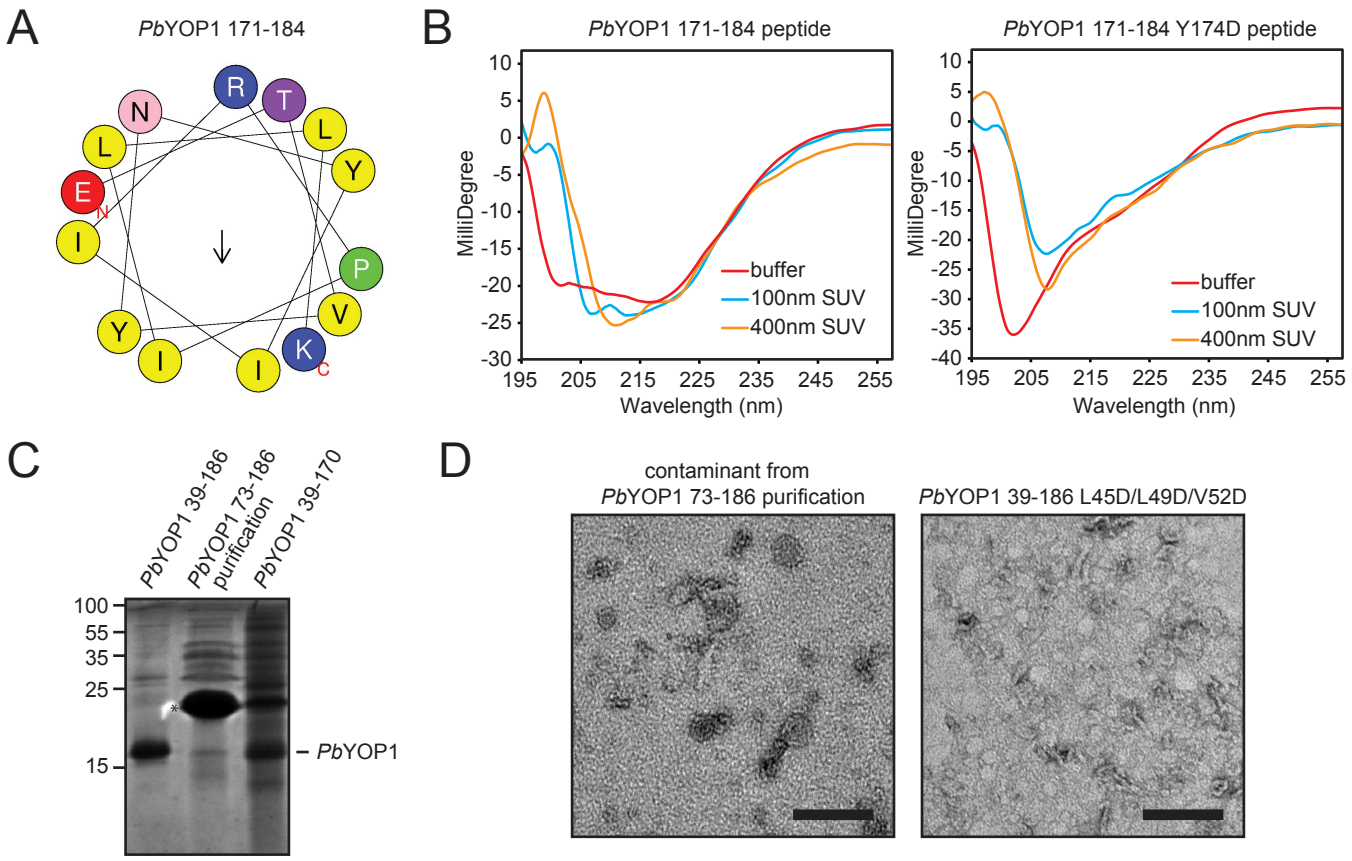

Figure S4. Amphipathic helices flanking the RHD domain of *Pb*YOP1. (A) Helical wheel analysis of the C-terminal helix. Hydrophobic residues are labeled in yellow. (B) CD measurements of wild type or Y174D mutant peptide in the absence or presence of liposomes as indicated. (C) Purification of *Pb*YOP1 mutants lacking the amphipahtic helices. Asterisk indicates a contaminant. Molecular marker is shown in kDa. (D) Reconsitututed proteoliposomes were analyzed by negative stain EM. Scale bar = 200 nm.