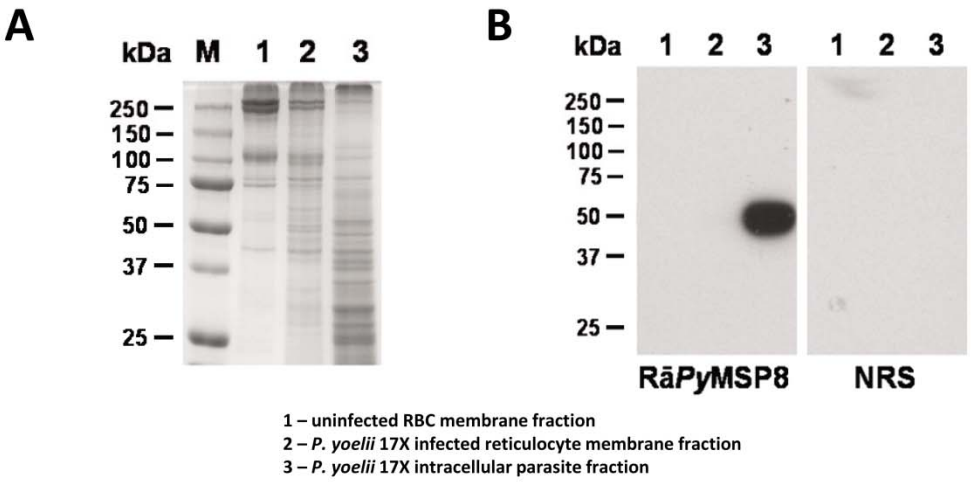
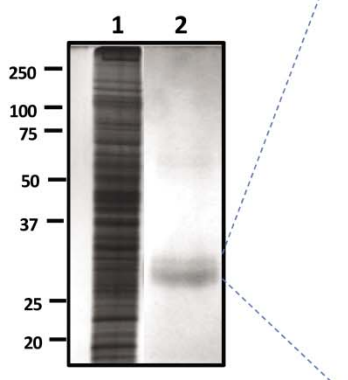


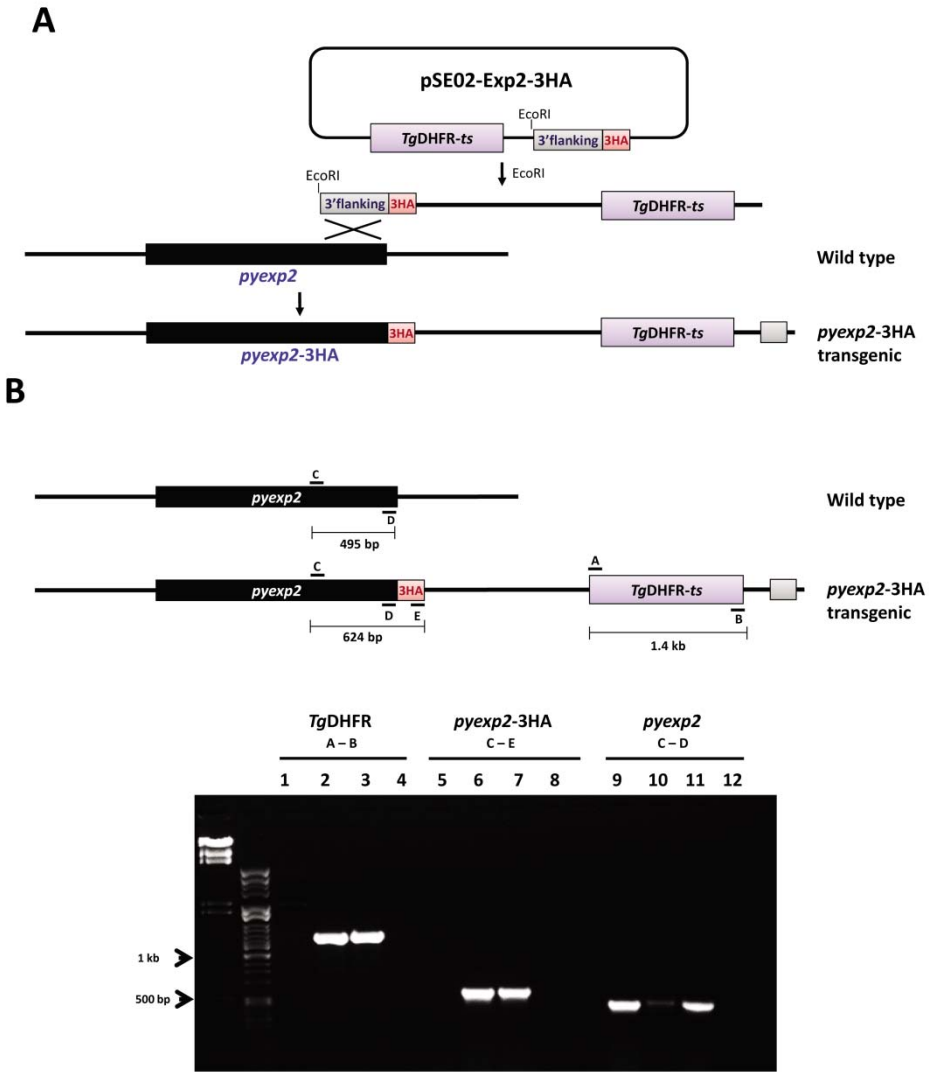

C



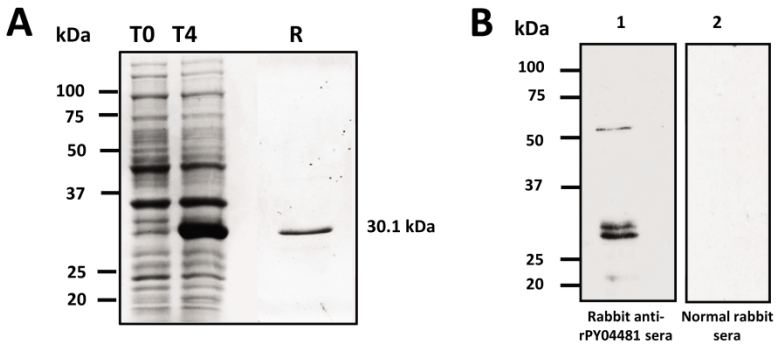

| PlasmodB acc no. | Protein description | Molecular wt (kDa) | Signal peptide | TM domain | Pf PEXEL motif | P value | Number of peptides |
|---|---|---|---|---|---|---|---|
| PY05892 | Exported protein-2 | 31.3 | 1 | 0 | - | 2.92E-06 | 6 |
| PY04481 | hypothetical protein - (*pyst*-a family) | 32.9 | 1 | 0 | - | 5.27E-06 | 3 |
| PY07631 | hypothetical protein - (*pyst*-a family) | 31.5 | 1 | 0 | - | 3.25E-11 | 2 |
| PY03885 | L-lactate dehydrogenase | 34.4 | 1 | 1 | KNLGD | 3.70E-11 | 10 |
| PY03531 | Cof-like hydrolase | 32.7 | 0 | 0 | - | 6.74E-04 | 4 |
| PY05001 | Heat shock protein 70 | 79.1 | 1 | 3 | - | 3.10E-10 | 10 |
| PY00622 | Rhoptry associated protein 1 | 70.8 | 1 | 0 | - | 6.32E-06 | 5 |
| PY01557 | m1-family aminopeptidase | 123.0 | 1 | 1 | - | 3.29E-09 | 3 |
| PY00963 | ATP synthase F1subunit beta | 57.8 | 0 | 0 | - | 8.30E-04 | 1 |

**Supplemental Figure 1. A. Analysis of *P. yoelii* 17X reticulocyte host cell membrane and parasite associated protein fractions.** Proteins associated with the membrane of uninfected (lane 1) or *P. yoelii* 17X infected reticulocytes (lane 2) and intracellular *P. yoelii* 17X parasites (lane 3) were separated by SDS-PAGE and analyzed by immunoblot. **A.** Coomassie Blue stained gel of each antigen preparation. **B.** Immunoblot analysis of each preparation using rabbit antisera against r*Py*MSP-8 or normal rabbit sera. **C.** Silver stained SDS-PAGE gel showing separation of *P. yoelii* 17X reticulocyte membrane fraction (lane 1) and a purified 29-32 kDa protein band (lane 2) from the membrane fraction by a gel electro-elution method. The purified band was subjected to in-gel trypsin digestion followed by mass spectrometric analysis (LC-MS/MS). The experiment was repeated three times and the table on the right shows nine *P. yoelii* proteins obtained at least two out of three times from the analysis. A *p* value <0.001 was set as the threshold for significance (probability of a match in an MS/MS search with equal or better scores than are expected to occur by chance alone). Signal peptides, TM domains and PEXEL motifs were based on bioinformatic data available in PlasmoDB (www.plasmodb.org). A putative PEXEL motif in *Pf*LDH was reported (1).

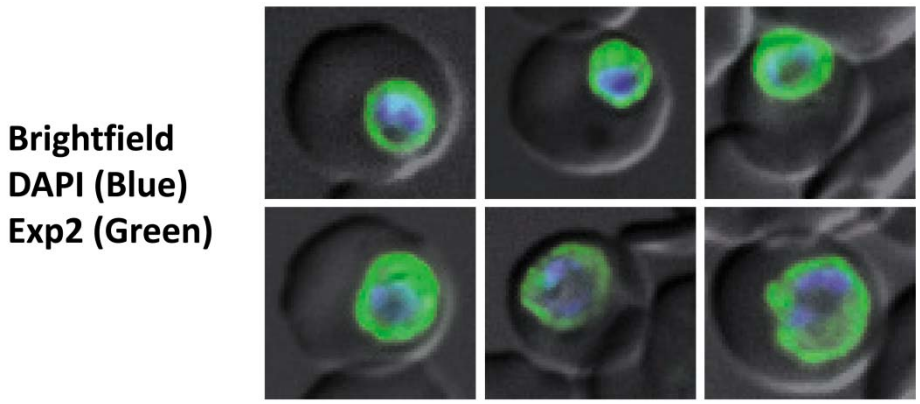
1. **Lanzer M, Wickert H, Krohne G, Vincensini L, Braun Breton C**. 2006. Maurer's clefts: a novel multi-functional organelle in the cytoplasm of *Plasmodium falciparum*-infected erythrocytes. Int J Parasitol 36:23-36.



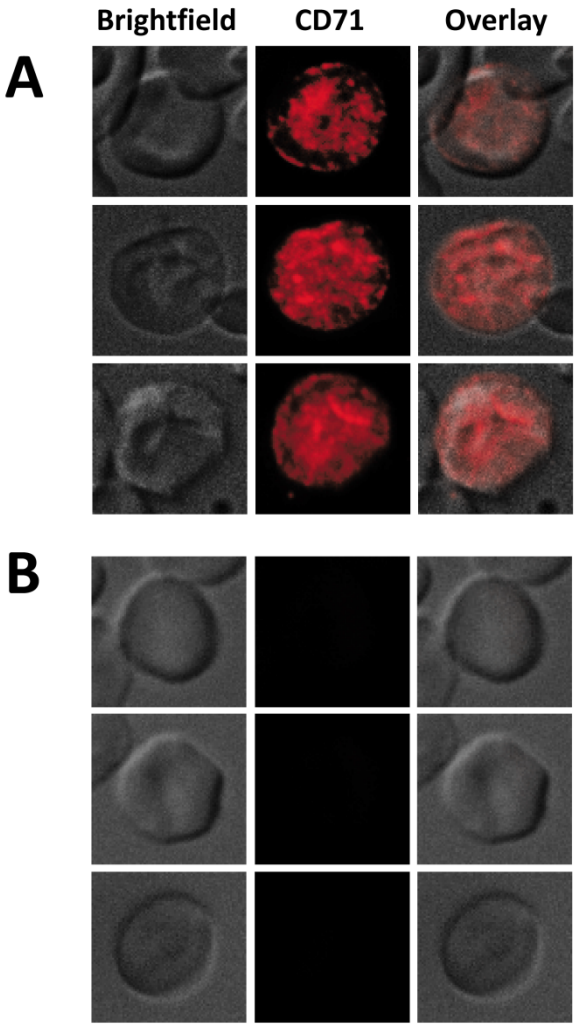

**Supplemental Figure 2. The 3' end of the *pyexp2* genomic locus is accessible for manipulation. A.** Schematic of pSE02-Exp2-3HA construct and 3' triple HA integration event in the *pyexp2* genomic locus by a single cross-over recombination. **B.** Diagnostic PCR showing integration of triple HA at the 3' end of *pyexp2* gene in two parasite lines (#3, #4) generated by two independent transfections. Parasite genomic DNA isolated from wild type *P. yoelii* 17X (lane 1, 5, 9), *P. yoelii* 17X-*Py*Exp2-3HA transgenic lines #3 (lane 2, 6, 10) and #4 (lane 3, 7, 11) was used for diagnostic PCR using *Tg*DHFR-ts specific primers (A-B), *pyexp2* gene specific primers (C-D), and *pyexp2*-3HA specific primers (C-E). Lanes 4, 8 and 12 represent negative controls for PCR.



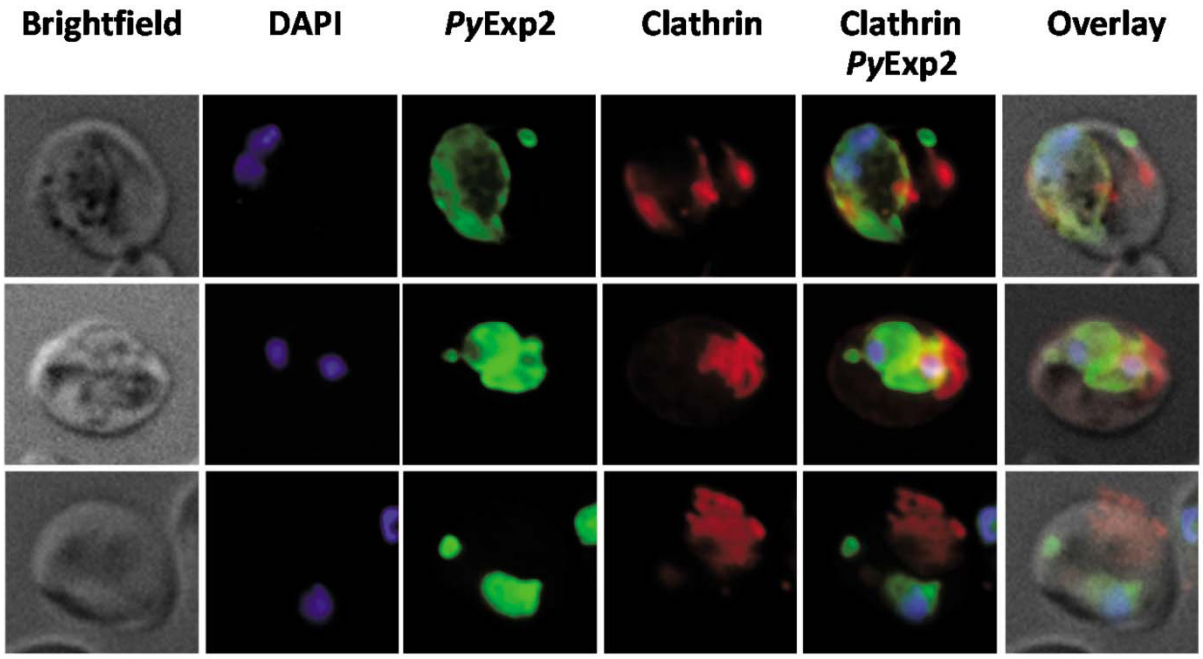

**Supplemental Figure 3. Expression and purification of rPY04481, a member of the *pyst-a* multigene family and production of rabbit antisera. A.** Coomassie-blue stained SDS-PAGE gel showing *E. coli* lysate at the time of IPTG induction (T0), 4 hrs post induction (T4) and rPY04481 (R) (3 µg) purified by nickel-chelate affinity chromatography. **B**. Immunoblot analysis of *P. yoelii* 17X parasite antigen lysate (10 µg) probed with rabbit anti-rPY04481 sera (1) or normal rabbit sera (2). Molecular weight markers in kilodaltons (kDa) are indicated.



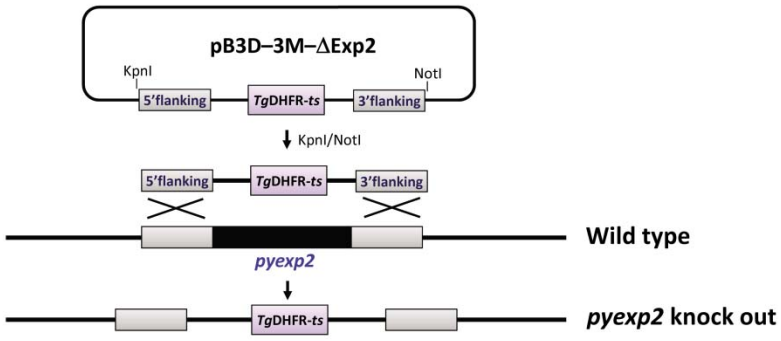

**Supplemental Figure 4. *Py*Exp2 is localized exclusively to the PVM in lethal *P. yoelii* 17XL infected RBCs.** Immunofluorescence image of a representative field showing an overlay of brightfield, *Py*Exp2 (green) and DAPI (blue).




**Supplemental Figure 5. CD71 is expressed on the surface of reticulocytes but absent from mature RBCs.** Immunofluorescence images showing the labeling of mouse **A)** reticulocytes or **B)** mature erythrocytes with anti – mouse CD71 antibodies (red).




**Supplemental Figure 6. *Py*Exp2 vesicles do not co-localize with mouse clathrin in *P. yoelii* 17X infected reticulocytes.** Immunofluorescence images showing co-localization of *Py*Exp2 (green) and mouse clathrin (red) using rabbit anti-r*Py*Exp2 sera and mouse anti-clathrin heavy chain antibodies respectively. DAPI was used to stain the parasite nuclei (blue).




**Supplemental Figure 7.** Schematic structure of *P. yoelii exp2* knockout targeting construct and the predicted double crossover homologous recombination in the *pyexp2* genomic locus.

## Supplemental Movie Legends

**Supplemental movie S1**

Rotation of a reconstructed 3D-SIM image showing a *P. yoelii* 17X infected reticulocyte labeled for *Py*Exp2 (green) and parasite nuclei (blue).

**Supplemental movie S2**

A 3D volume view of de-convoluted z-stacks showing co-localization of *Py*Exp2 (green), BODIPY TR Ceramide (red) and parasite nuclei (blue) in a *P. yoelii* 17X infected reticulocyte.

**Supplemental movie S3**

Rotation of a reconstructed 3D-SIM image showing the co-localization of *Py*Exp2 (green), BODIPY TR Ceramide (red) and parasite nuclei (blue) in a *P. yoelii* 17X infected reticulocyte.

**Supplemental movie S4**

Rotation of a reconstructed 3D-SIM image showing the absence of *Py*Exp2 (green) positive vesicles in CD71 (red) negative mature RBC infected with *P. yoelii* 17XL parasite.

**Supplemental movie S5**

Rotation of a reconstructed 3D-SIM image showing the presence of *Py*Exp2 (green) positive vesicle in a CD71 (red) positive reticulocyte infected with *P. yoelii* 17XL parasite.