Fig.5. Known stages of parasite egress program, timing of observable events, molecular agents, and inhibitors affecting egress. A mature schizont initiates a stereotypical, Ca^{2+} -dependent egress program approximately 9 minutes prior to parasite egress. The first mechanically observable step is a rounding of the parasite vacuole lasting only a few minutes. Vacuole rounding is prevented by intracellular (but not extracellular) Ca^{2+} chelators. The rounding stage is followed by macroscopic vacuolar membrane rupture and sudden release of vacuolar contents into the erythrocyte compartment. Compounds CWHM-117 and C2 block vacuolar rupture, leaving the parasite in the vacuole rounding stage; exit from this stage apparently requires PMX-dependent SUB1 maturation and PfPKG-dependent SUB1 release from parasite exonemes. For the following ~ 7 min, the vacuolar membrane gradually degrades and the erythrocyte membrane gradually collapses on the schizont body. Two cysteine proteases, SERA 6 and host calpain-1, appear to degrade the erythrocyte cytoskeleton explaining erythrocyte shape change. The last minutes of the erythrocyte cycle is characterized by a mechanical separation of mature merozoites and perforation of erythrocyte membrane. Final parasite release completes the egress program, blocked by cysteine protease inhibitor E64 or a conditional knockout of SERA6 (SERA6-KO, Thomas et al., 2018).

SUPPLEMENTARY FIGURES

Supplementary Fig.1. Monitoring of vacuolar membrane fluorescence prior to and during the rounded vacuolar stage, after PVM and EPM rupture.

Supplementary Fig.2. Evaluation of parasite egress kinetics using single-tagged parasites and a single laser illumination. 22 recordings of egress from NF54-Exp2-mNeonGreen-infected erythrocytes (illumination with 488 nm laser) and 18 recordings of egress from NF54-mRuby3-infected erythrocytes (illumination with 561 nm laser). T-test shows no statistically significant differences between datasets as well as between single- and double-labeled parasites.

Movie 1. Experimental swelling of NF54-Exp2-mNeonGreen/PV-mRuby3 infected erythrocyte by laser wounding of the cell using 800 nm two-photon illumination of the area outside the schizont location marked by arrow in the beginning of the movie. Note the relocation of Exp2-mNeonGreen signal to the periphery of schizont upon vacuolar membrane stretching. Scale bar 5 μm.

Movie 2. Transformation of vacuolar shape from irregular to rounded prior to PVM rupture. NF54-Exp2-mNeonGreen-infected erythrocyte. Note that intervals between frames was different to minimize photodamage.

Movie 3. Visualizing the sequence of events preceding parasite egress from an erythrocyte infected with NF54-Exp2-mNeonGreen/PV-mRuby3 to follow vacuolar membrane and vacuolar lumen. See the description of Fig.3A in the figure legend. Green color - Exp2-mNeonGreen, PVM-associated protein; red color - mRuby3, a soluble protein in the vacuolar lumen.

Movie 4. A piecemeal pattern of PVM degradation: a part of parasite egress recording. Green color – Exp2-mNeonGreen, a PVM-associated protein.

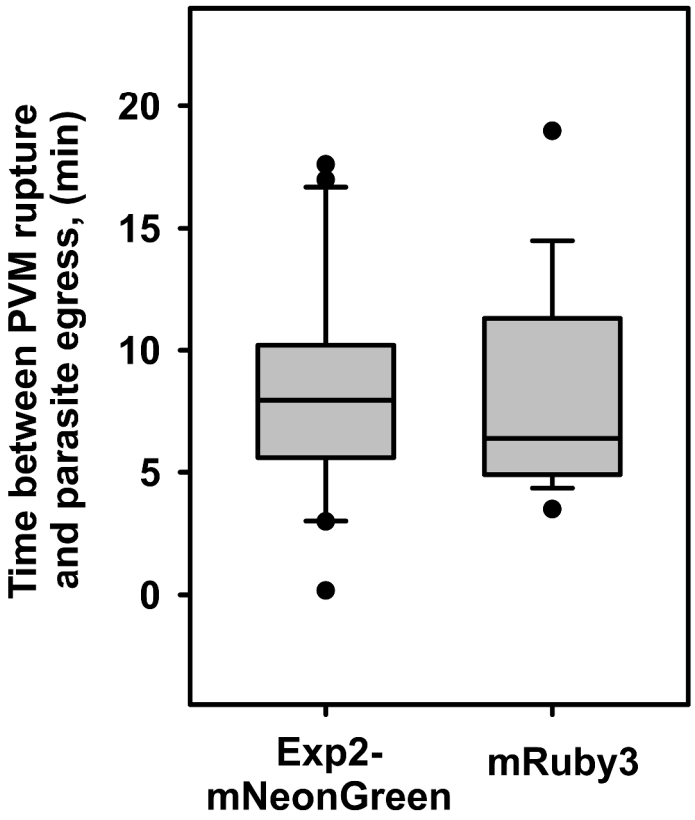
Movie 5. Parasite egress from the double-infected erythrocyte. Note that rupture of one vacuole does not affect the integrity of the second one but rupture of erythrocyte lead to the permeation of the second vacuole.

Movie 6. Dissociation of merozoite prior to parasite egress from the infected erythrocyte. See text for the Fig.3F description.

ACKNOWLEDGEMENTS

We thank Robyn Roth for assistance with sample preparation for electron microscopy, Alan Hoofring for artwork, and Kamran Melikov for fruitful discussion. This study was funded by the Intramural Research Programs of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. Daniel Goldberg and Josh R. Beck acknowledge funding from the National Institutes of Health including K99/R00 HL133453 to Josh R. Beck.

Supplementary Fig. 2



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