SUPPLEMENTAL DATA

Proteolytic Activation of the Essential Parasitophorous Vacuole Cysteine Protease SERA6 accompanies Malaria Parasite Egress from its Host Erythrocyte*

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* Running title: *SERA6 is a cysteine protease in the malarial PV*

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FIGURE S1. ImmunoEM localization of SERA6 to the PV and PVM. Lower magnification (x6000) image of the schizont section view shown in the micrograph in Figure 2D of the main paper. Note association of gold particles with the PMV (small black arrows) as well as the space between merozoites, whilst in contrast the erythrocyte membrane (large black arrowheads) is virtually free of label.

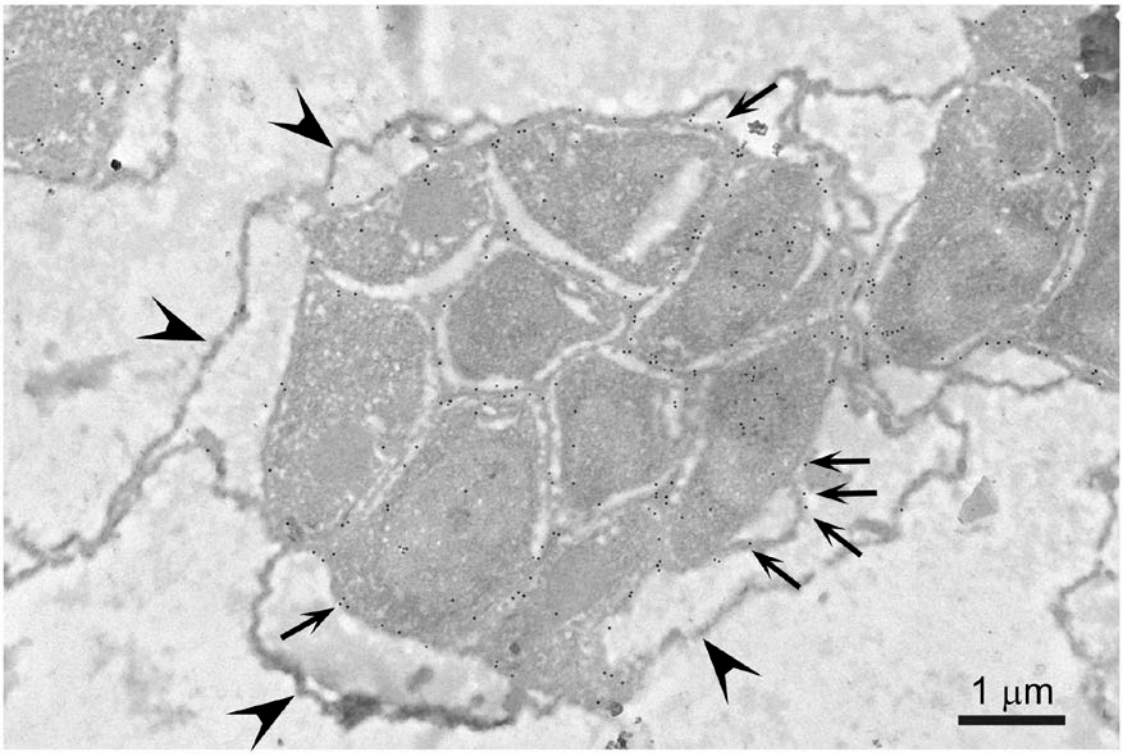

FIGURE S2. Recombinant expression and purification of PbSERA3. A recombinant form of full-length PbSERA3 (rPbSERA3), as well as a mutant in which the putative catalytic Cys641 residue is replaced by an Ala residue (rPbSERA3C641A) were expressed as secreted proteins in baculovirus-infected High Five™ cells in the presence of 0.5 μg/ml tunicamycin to prevent N-glycosylation of the recombinant protein. Culture supernatant (1200 ml) was clarified, adjusted to 50 mM Tris-HCl pH 8.2, applied to a HiPrep Q XL 16/10 ion exchange column (GE Healthcare, UK) and eluted with a 200 ml gradient of 0-400 mM NaCl in the same buffer, collecting 5 ml fractions. Peak fractions were pooled, concentrated, and applied to a Superdex 200 HR 10/30 column (GE Healthcare, UK), equilibrated in 25 mM HEPES pH 7.4, 150 mM NaCl, 12 mM $CaCl_2$, collecting elution fractions of 0.4 ml. Shown are Western blot analyses of the indicated elution fractions, probed in all cases with an antibody reactive with the central papain-like domain of PbSERA3 (anti-PbS3C1). It can be seen that the elution profiles and yields of the two proteins were very similar.

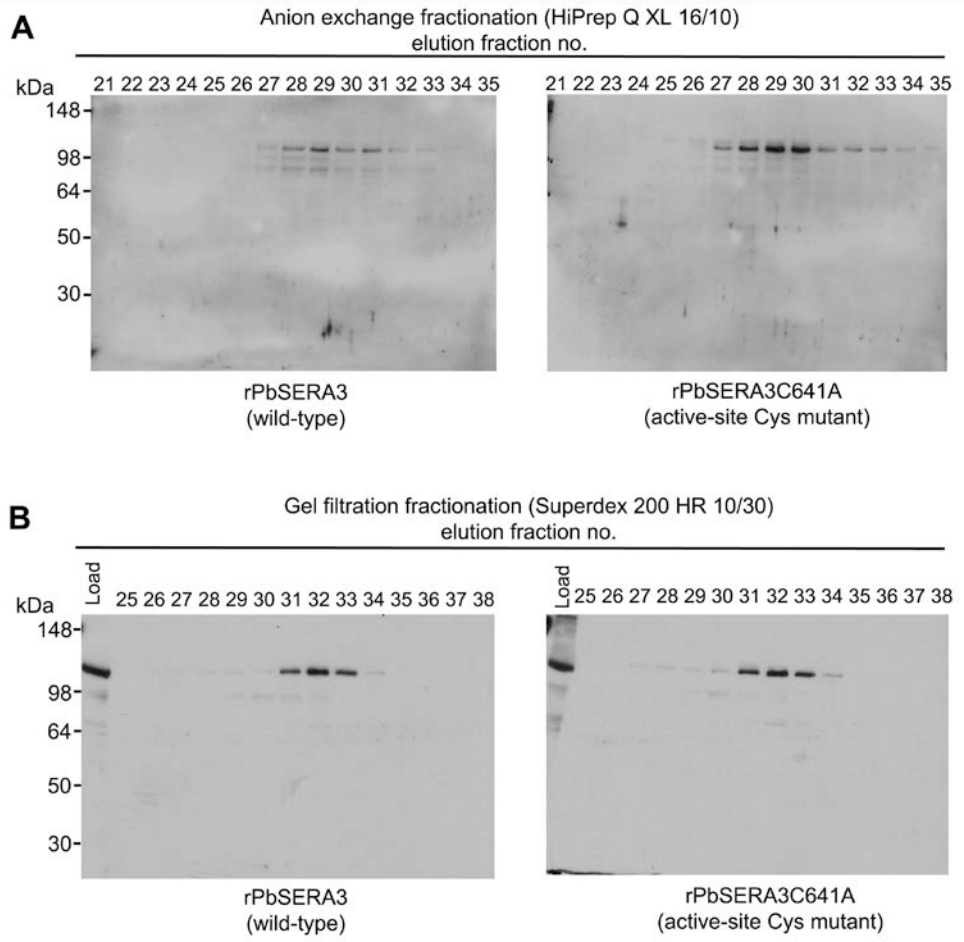

FIGURE S3. PfSUB1 correctly cleaves synthetic peptides based on the two predicted PbSUB1 cleavage sites in PbSERA3. A. Alignment of the predicted *P. falciparum* FCBR SERA6 sequence (GenBank M55428) with the PbSERA3 sequence (PlasmodDB ID PBANKA_030490). Sequences were pairwise aligned using a fast/approximate CLUSTALW output tool from the Kyoto University Bioinformatics Center. The alignment was then analysed and modified using Jalview 2.7 (1,2) . Positions of identity are shaded in light blue. Gaps inserted to improve the alignment are shown as hyphens. The eight residues (P4-P4') flanking PfSUB1 cleavage sites in SERA6 (identified in this study) are shown in red, as are predicted site 1 and 2 cleavage sites in PbSERA3. The papain-like domain of both sequences (Ser606-Lys880 in SERA6) is indicated in dark blue, with predicted catalytic triad Cys, His and Asn residues in green. B. Progress curve showing cleavage of fluorogenic peptide substrate PbSERA3st1F-6R (0.1 μM) by recombinant PfSUB1 (1.7 U/ml), as detected by a time-dependent increase in fluorescence. No cleavage occurred in the absence of PfSUB1 or with the additional presence of recombinant PD (85 nM). C. RP-HPLC fractionation of partially digested PbSERA3st1F-6R. Only two major new fluorescent peaks were produced. Their identities, as determined by electrospray mass spectrometry, are indicated. Cysteine side chains modified by 6-iodoacetamido tetramethylrhodamine (3) are shown (-R). D. RP-HPLC elution profiles of undigested and partially-digested peptide PbSERA3st2 (Ac-DVSGQSENHQ). Identities of major peaks determined by mass spectrometry (see Supplemental Table S2) are indicated. Note that the highly polar C-predicted terminal cleavage product (NH_2-SENHQ-OH) was not observed, probably due to it not being retained by the column as found previously for other digestion products of PfSUB1 peptide substrates (4-6).

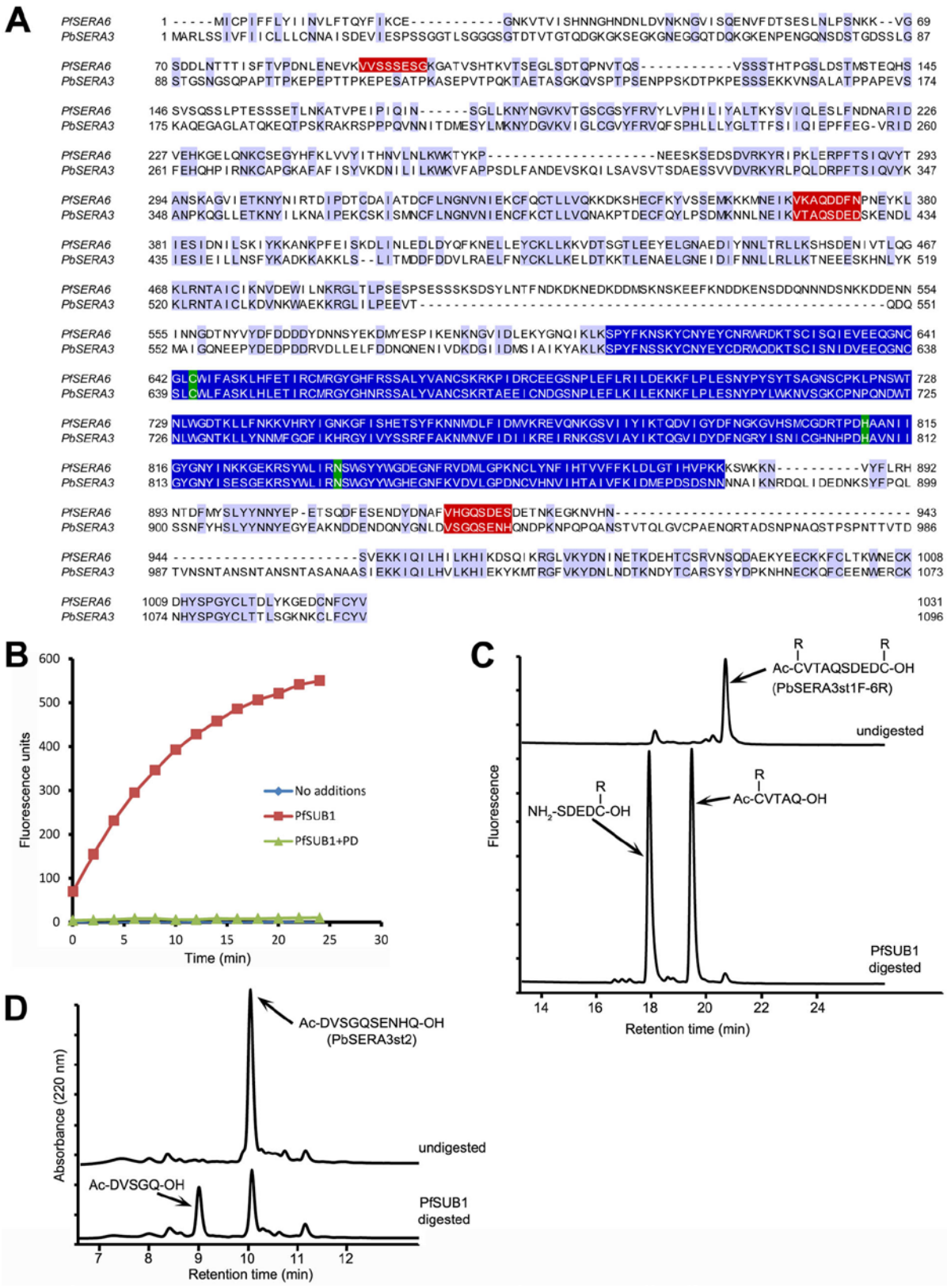
A
PfSERA6
PbSERA3
B
Fluorescence units
Time (min)
No additions
PfSUB1
PfSUB1+PD
C
Ac-CVTAQSDEDC-OH
(PbSERA3st1F-6R)
undigested
NH2-SDEDC-OH
Ac-CVTAQ-OH
PfSUB1
digested
Fluorescence
Retention time (min)
D
Ac-DVSGQSENHQ-OH
(PbSERA3st2)
undigested
Ac-DVSGQ-OH
PfSUB1
digested
Absorbance (220 nm)
Retention time (min)

Table S1. Oligonucleotide primers used in this study

SERA6_C/A_fwd	Forward amplification to mutate C644A in *sera6*$_{synth}$	5′- GCAGGGAAACTGTGGTTTGGCTTGGATCTTCGCTTCCAAG -3′
SERA6_C/A_rev	Reverse amplification to mutate C644A in *sera6*$_{synth}$	5′- CTTGGAAGCGAAGATCCAAGCCAAACCACAGTTTCCCTGC -3′
SERA6st1_mut_fwd	Forward amplification to mutate SERA6st1 from AQ to LL in *sera6*$_{synth}$	5′- GAAGAAAAAGATGAACGAGATCAAGGTTAAGTTATT GGATGACTTCAACCCAAACGAGTACAAGTTG -3′
SERA6st1_mut_rev	Reverse amplification to mutate SERA6st1 in *sera6*$_{synth}$	5′- CTTCTTTTTCTACTTGCTCTAGTTCCAATTCAATAA CCTACTGAAGTTGGGTTTGCTCATGTTCAAC-3′
SERA6st2_mut_fwd	Forward amplification to mutate SERA6st2 from GQ to LL in *sera6*$_{synth}$	5′- GAACGATTACGACAACGCTTTCGTTCACTTATT ATCTGATGAATCCGACGAGACTAACAAA -3′
SERA6st2_mut_rev	Reverse amplification to mutate SERA6st2 in *sera6*$_{synth}$	5′- CTTGCTAATGCTGTTGCGAAAGCAAGTGAATAA TAGACTACTTAGGCTGCTCTGATTGTTT -3′
PbSERA3_C/A_fwd	Forward amplification to Mutate C641A in *pbsera3*$_{synth}$	5′- GGCAACTGCTCCCTGGCCTGGCTGTTCGCTTC -3′
PbSERA3_C/A_rev	Reverse amplification to mutate C641A in *pbsera3*$_{synth}$	5′- GAAGCGAACAGCCAGGCCAGGGAGCAGTTGCC -3′
SERA6prom_fwd	Forward amplification of *SERA6* promoter for pHH-SERA6$_{synth}$	5′- GAGATCTGTATCCTTTAATTTTTAAATAGGGCATAAC -3′
SERA6prom_rev	Forward amplification of *SERA6* promoter for pHH-SERA6$_{synth}$	5′- GATATCACATAATAATGGACATAACGATAATCTCGAGC -3′
SERA6synth_fwd	Forward amplification of *sera6*$_{synth}$ for pHH-SERA6chim	5′- GCTCGAGGTTAACAAGAACGGTGTTATCTCC -3′
SERA6synth_rev	Reverse amplification of *sera6*$_{synth}$ for pHH-SERA6chim	5′- GGTGAAGATTGTAACTTCTGTTATGTTCTCGAGC -3′
SERA6gen_homology_fwd	Forward amplification of genomic *SERA6* (region of homology) for pHH-SERA6chim	5′- CAGTATACATGATATGTCCTATTTTTTAGCTTTTTTAGCTTT -3′
SERA6gen_homolgy_rev	Reverse amplification of genomic *SERA6* (region of homology) for pHH-SERA6chim	5′- CAAGGATTGATGTTGAGCACAAGGGTGAG -3′
a	Forward primer for diagnostic PCR, designed to hybridize to the 5′ UTR of the *SERA6* gene	5′- AAAAGTAAAAGACCAAATGATA -3′
b	Reverse primer for diagnostic PCR, designed to hybridize to the	5′- AAGTAGGAGTCGGACTTAGAA -3′

	sera6$_{synth}$ sequence	
c	Reverse primer for diagnostic PCR, designed to hybridize to the endogenous *SERA6* coding sequence	5′- GATTCACTAGGTAATGTTAGACCTC -3′
SERA6int_probe_fwd	Forward amplification of genomic *SERA6* for Southern blot probe	5′- CTGTGATATCACATAATAATGGAC -3′
SERA6int_probe_rev	Reverse amplification of genomic *SERA6* for Southern blot probe	5′- CAGATGATGATTCAGTTGGAAG -3′

Table S2. Correct cleavage by rPfSUB1 of peptide substrates based on predicted PbSUB1 processing sites within PbSERA3.

Peptide name	Peptide sequence[a]	Digestion product identified[b]	Predicted *m/z*[c]	Measured *m/z*[c]
PbSERA3st2	Ac-DVSGQ↓SENHQ	Ac-DVSGQ	547.2	547.2
PbSERA3st1F-6R	Ac-CVTAQ↓SDEDC[d] I I R R	Ac-CVTAQ[d] I R	1004.5	1004.6
		NH_2-SDEDC[d] I R	1009.4	1009.5

[a] See Fig. S3 and main paper for the details of predicted PbSERA3 processing sites and relevant references. Expected scissile bond is indicated by a downward-pointing arrow.

[b] Digestion products were fractionated by RP-HPLC (Fig. S3) and identified by electrospray mass spectrometry.

[C] Monoisotopic *m/z* values.

[d] PbSERA3st1F-6R is the peptide Ac-CVTAQSDEDC labelled on both cysteine side chains with 6-iodoacetamido tetramethylrhodamine, as described previously (3) (see also Fig. S3). Cysteine side chains modified by 6-iodoacetamido tetramethylrhodamine are shown (-R). The modification results in an increase in mass of each Cys residue of 441.2 Da (3).

References

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