

# Supplementary

**a**

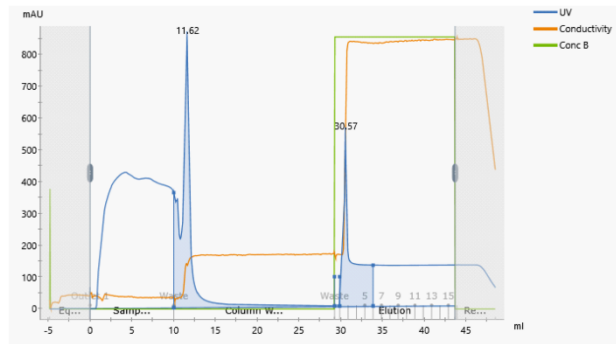
rPfCSP Amino Acid Sequence

PlasmoDB ID: PF3D7\_0304600.1

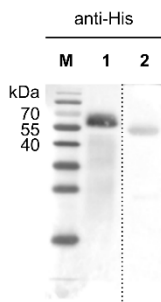
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 HHHHHHHH\*

**b**

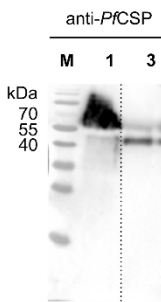
His-TRAP Purification



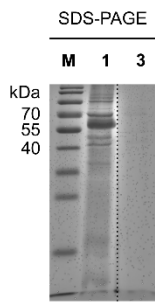
**c**



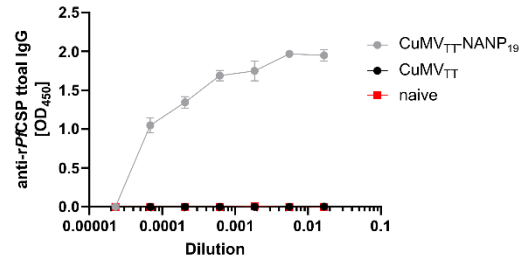
**d**



**e**



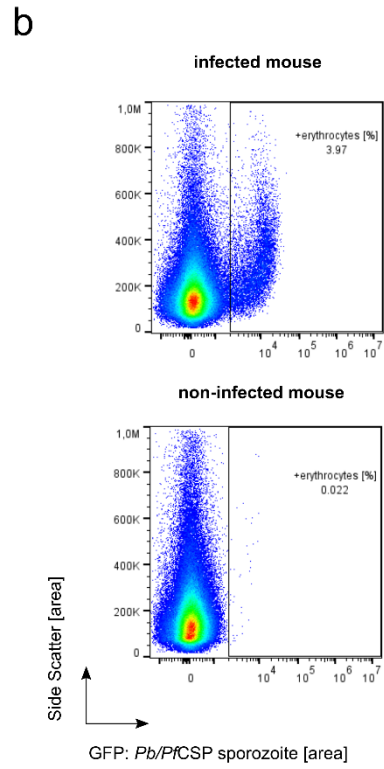
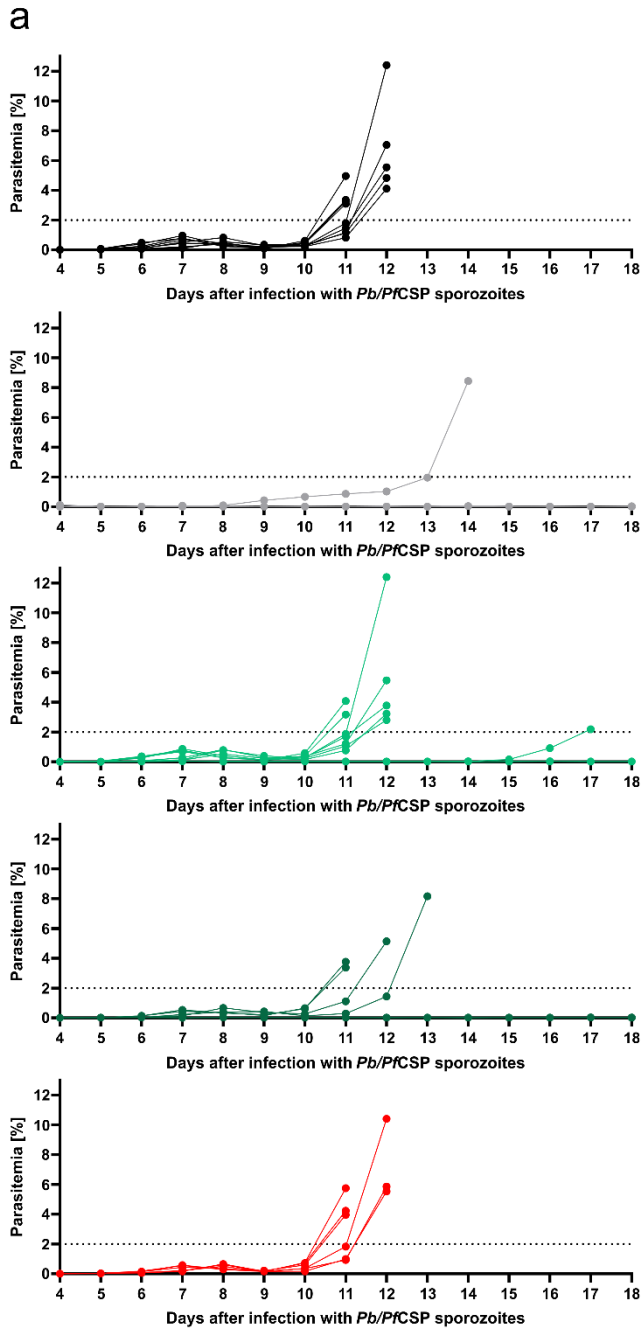
**f**



M: Protein marker  
 1: rPfCSP  
 2: His-tag control  
 3: *Pb/PfCSP* sporozoites

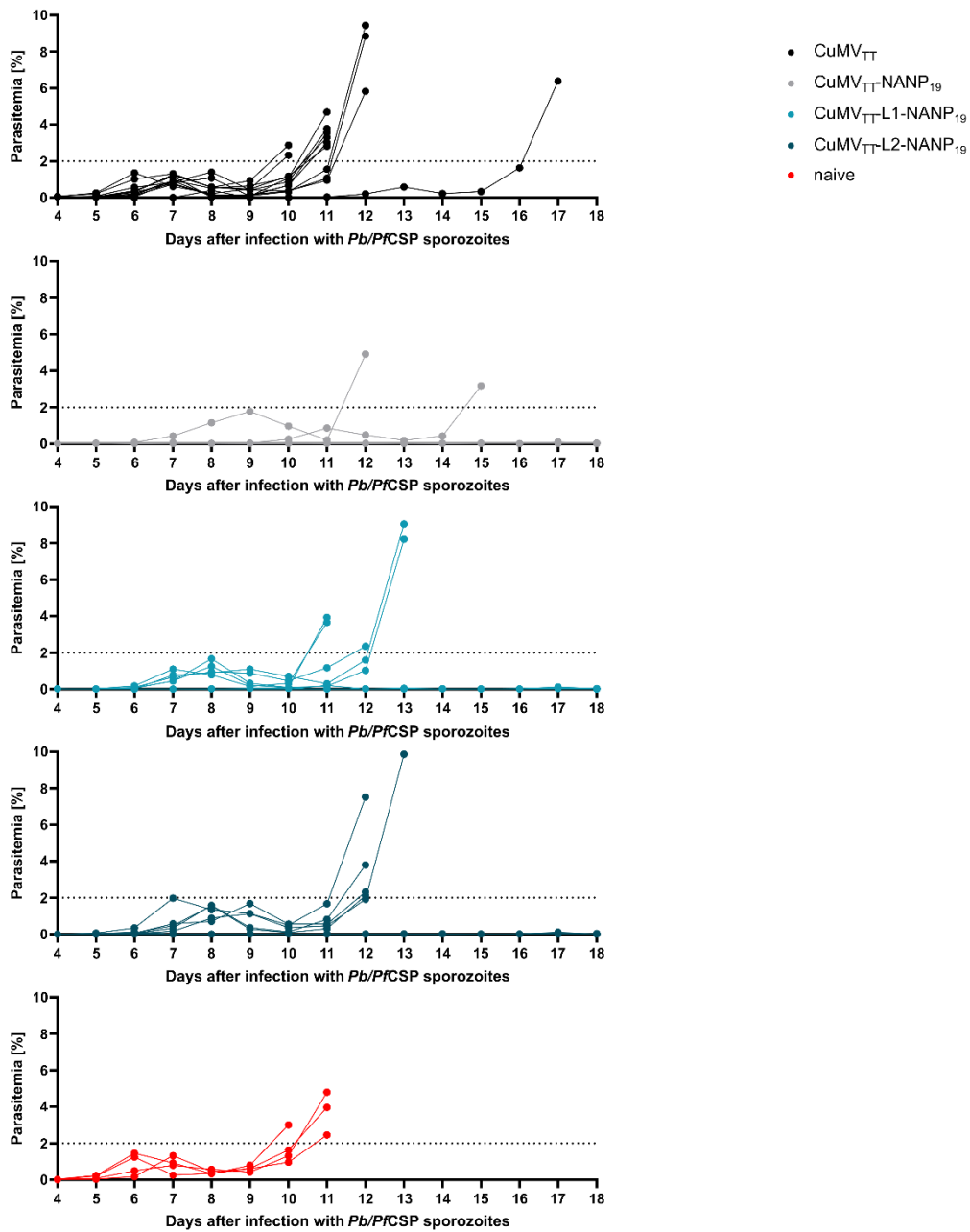
Supplementary Figure 1

**Supplementary Figure 1: Characterization of recombinantly expressed rPfCSP.** rPfCSP was expressed as previously described by *Kisalu et al.(41)*. a) Amino acid sequence of rPfCSP (Pf3D7 strain; PlasmoDB ID: PF3D7\_0304600.1). The first 20 leader amino acids were changed to a mammalian secretory signal peptide derived from the modified bovine lactin (blue), and the GPI-anchor residues were replaced with a GSG-linker (green) followed by a histidine-tag (red). b) Affinity chromatography (His-Trap HP column) of rPfCSP after expression in Expi293™ HEK cells. c) Western blot specific for histidine. M. protein marker, 1. rPfCSP, 2. control protein with histidine-tag. d) Western blot specific for PfCSP. M. protein marker, 1. rPfCSP, 3. Pb/PfCSP sporozoites. e) 12 % SDS-PAGE of M. protein marker, 1. rPfCSP, 3. Pb/PfCSP sporozoites. Binding capacity of CuMV<sub>TT</sub>-NANP<sub>19</sub>-induced IgG antibodies to rPfCSP tested by ELISA. Serum samples from CuMV<sub>TT</sub>-immunized or naïve mice were used as controls. OD<sub>450</sub> absorbance of the titration curve is shown. N = 2.



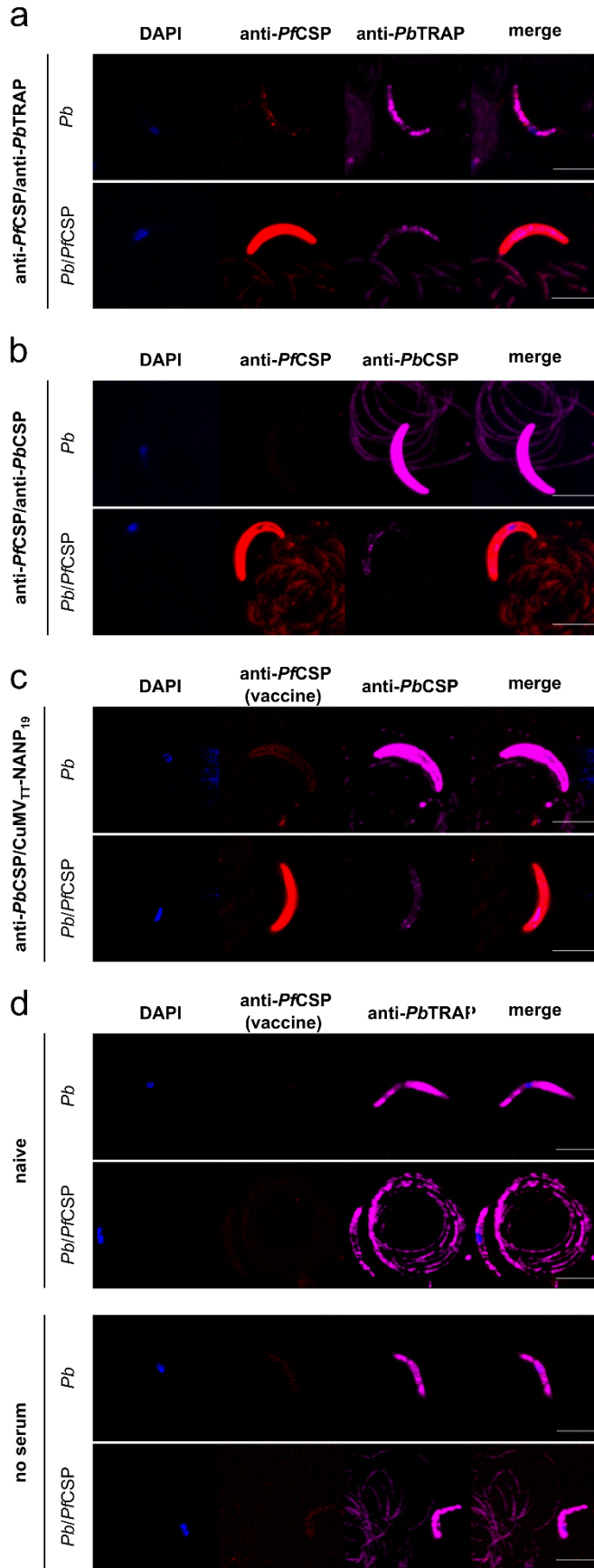
Supplementary Figure 2

**Supplementary Figure 2: Parasitemia in mice infected with *Pb/PfCSP* sporozoites following immunization with CuMV<sub>TT</sub>-based malaria vaccine candidates (data corresponding to Figure 2f).** a) Parasitemia of CuMV<sub>TT</sub>, CuMV<sub>TT</sub>-NANP<sub>19</sub>, CuMV<sub>TT</sub>-J1-NANP<sub>1</sub>, CuMV<sub>TT</sub>-J2-NANP<sub>6</sub> immunized, and naïve mice after challenge with 5000 *Pb/PfCSP* sporozoites injected i.d. b) Parasitemia was measured by flow cytometric detection of GFP-positive (*Pb*-infected) erythrocytes. Representative flow cytometry plots of an infected and a non-infected mouse are shown. N = 9.



Supplementary Figure 3

**Supplementary Figure 3: Parasitemia in mice infected with *Pb/Pf*CSP sporozoites following immunization with optimized CuMV<sub>TT</sub>-based malaria vaccine candidates (data corresponding to Figure 4g).** Parasitemia of CuMV<sub>TT</sub>, CuMV<sub>TT</sub>-NANP<sub>19</sub>, CuMV<sub>TT</sub>-L1-NANP<sub>19</sub>, CuMV<sub>TT</sub>-L2-NANP<sub>19</sub> immunized, and naïve mice after challenge with 5000 *Pb/Pf*CSP sporozoites injected i.d. Data of two experiments was combined. N = 12.



Supplementary Figure 4

**Supplementary Figure 4: Binding capacity of control antibodies to *Pb/PfCSP* sporozoites.** Immunofluorescence assay testing the binding capacity to *Pb/PfCSP* sporozoites of anti-*PfCSP* (red) and anti-*PbTRAP* (purple) in a), anti-*PfCSP* (red) and anti-*PbCSP* (purple) in b), anti-*PfCSP* (red) and CuMV<sub>TT</sub>-NANP<sub>19</sub>-induced IgG (purple) in c), naive serum IgG or no serum IgG (red) and anti-*PbTRAP* (purple) in d). DAPI nucleic acid stain is depicted in blue. As control *Pb* sporozoites were included in the analysis. Scale bar 5  $\mu$ m. One representative of two similar experiments is shown.