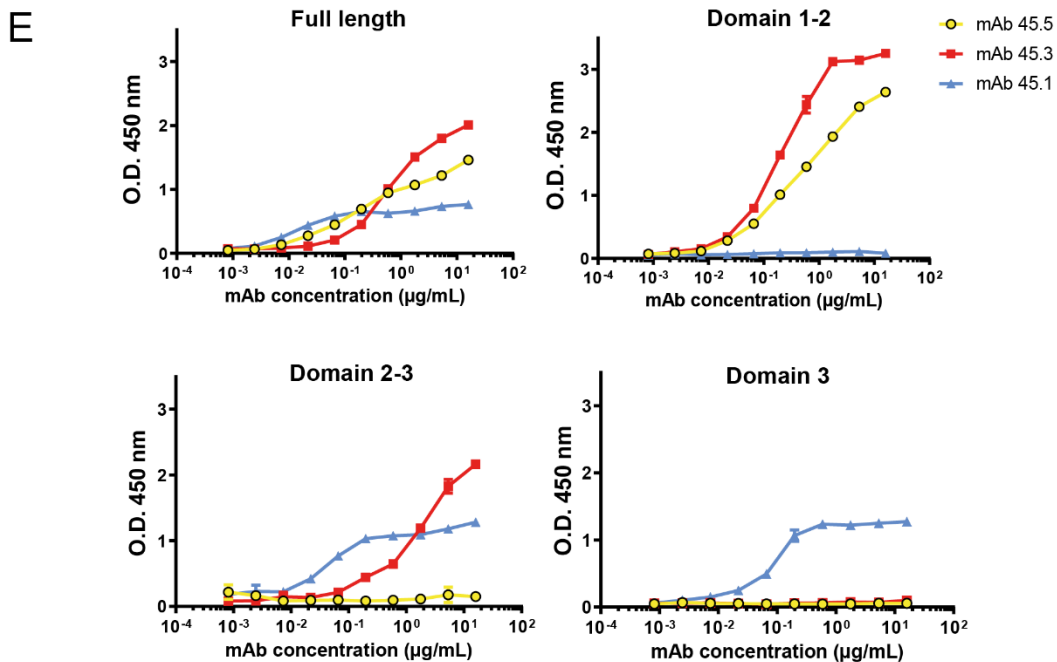
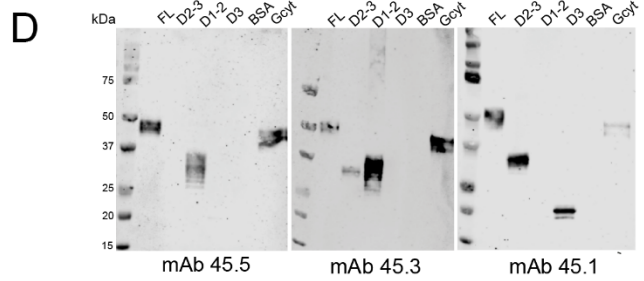
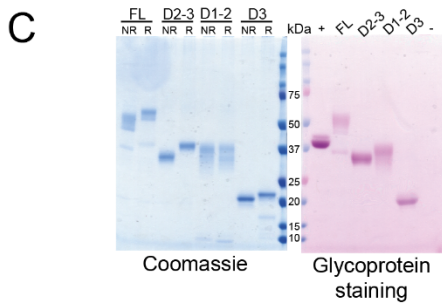
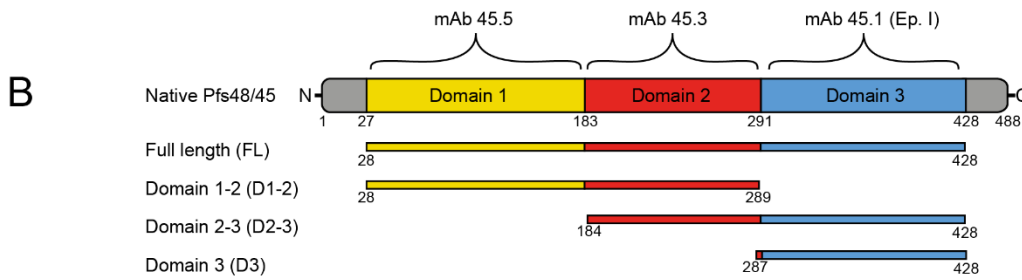
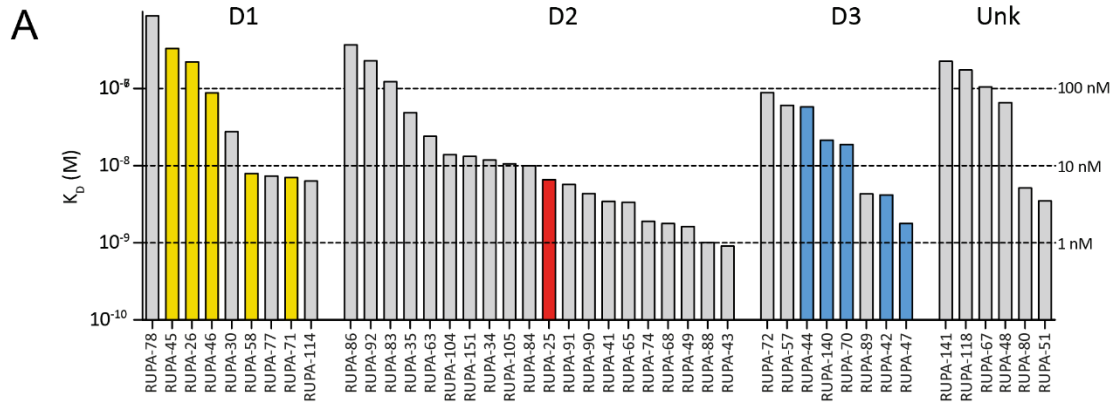


**Supplemental information**

**Highly potent, naturally acquired human  
monoclonal antibodies against Pfs48/45 block**

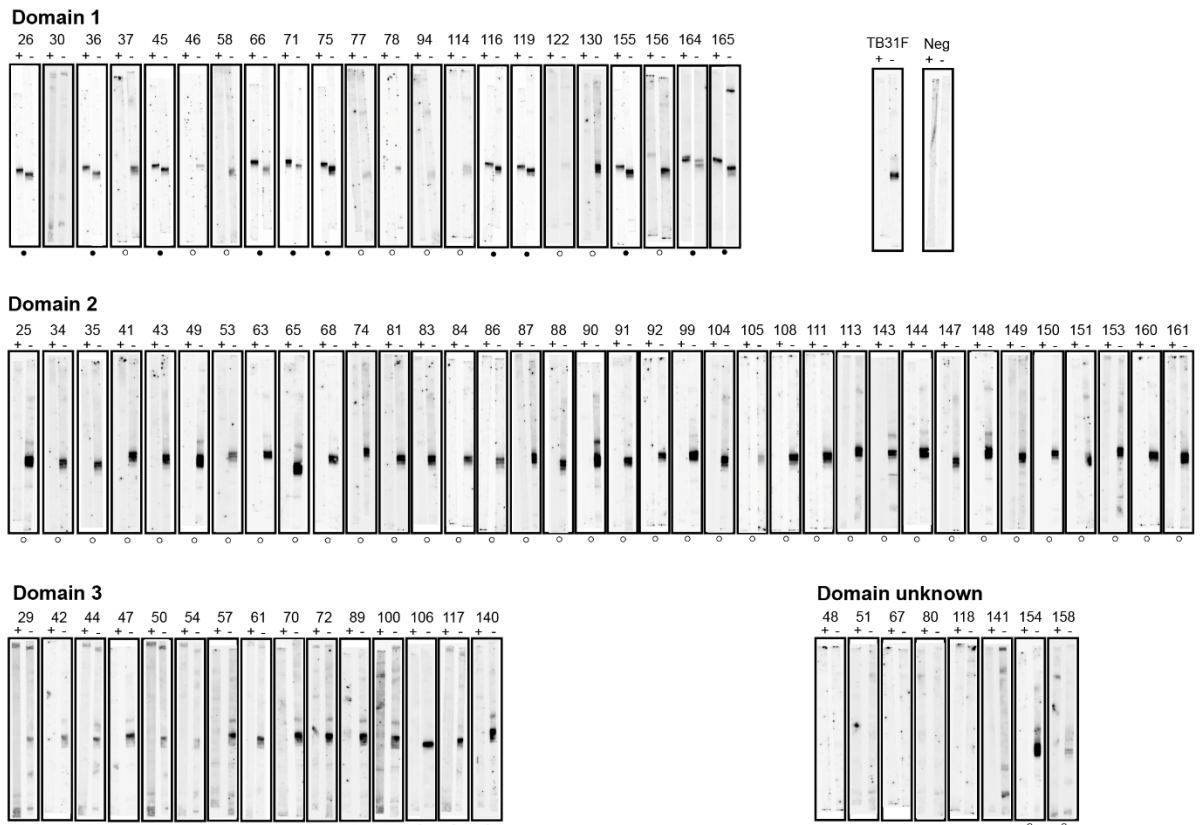
***Plasmodium falciparum* transmission to mosquitoes**

**Amanda Fabra-García, Sophia Hailemariam, Roos M. de Jong, Kirsten Janssen, Karina Teelen, Marga van de Vegte-Bolmer, Geert-Jan van Gemert, Danton Ivanochko, Anthony Semesi, Brandon McLeod, Martijn W. Vos, Marloes H.C. de Bruijni, Judith M. Bolscher, Marta Szabat, Stefanie Vogt, Lucas Kraft, Sherie Duncan, Moses R. Kanya, Margaret E. Feeney, Prasanna Jagannathan, Bryan Greenhouse, Koen J. Dechering, Robert W. Sauerwein, C. Richter King, Randall S. MacGill, Teun Bousema, Jean-Philippe Julien, and Matthijs M. Jore**

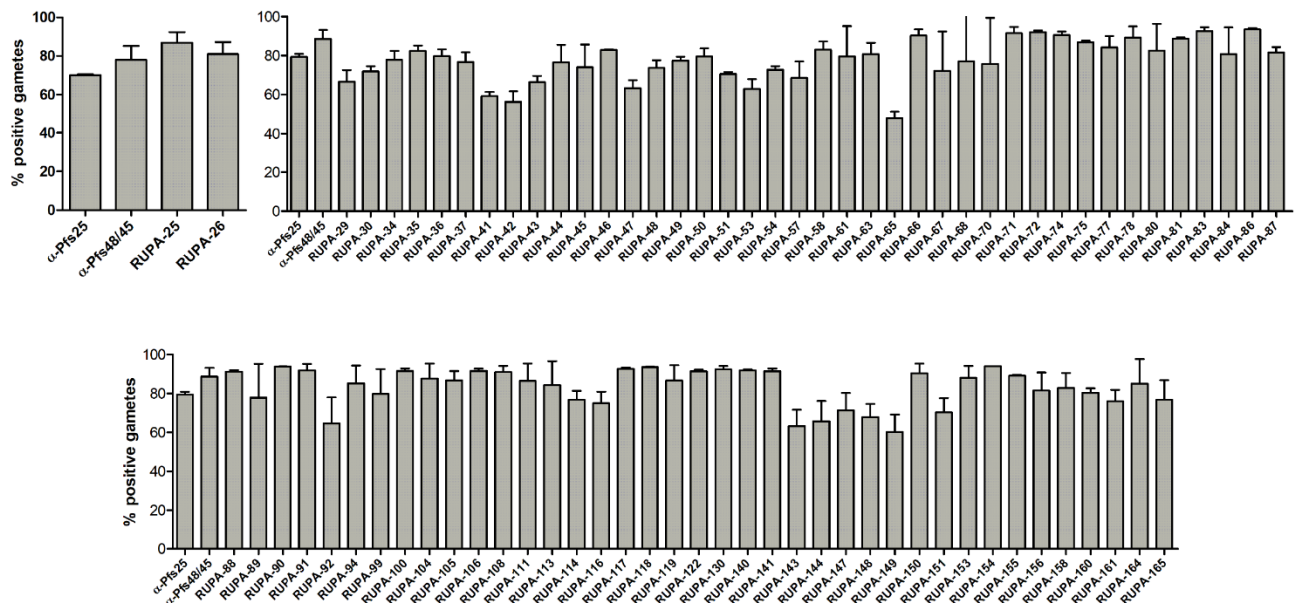


**Figure S1. Binding of mAbs to recombinant Pfs48/45.** (a) Binding affinity ( $K_D$ ) of mAbs for Pfs48/45. Affinity was determined by surface plasmon resonance (SPR) with immobilized antibodies and full-length Pfs48/45 as analyte. mAbs are grouped according to domain specificity. Colored bars represent mAbs with >80% TRA at 100  $\mu\text{g/ml}$ . (b-e) Expression and validation of recombinant Pfs48/45 fragments. (b) Schematic overview of Pfs48/45 fragments produced in *Drosophila melanogaster* S2 cells, including amino acid numbers. Rodent antibodies 85RF45.5, 85RF45.3 and 85RF45.1 recognize D1, D2 and D3, respectively<sup>1</sup>. mAbs 85RF45.3 and 85RF45.1 recognize conformational epitopes, 85RF45.5 recognizes a linear epitope<sup>1</sup>. Ep. I, Epitope I. (c) Purified proteins were separated on a 4-20% Bis-Tris polyacrylamide gel under non-reducing (NR) and reducing (R) conditions and were visualized with coomassie staining (left) or Pierce<sup>®</sup> glycoprotein staining (right). (+) and (-) are positive and negative glycosylation controls, respectively. The presence of (conformational) epitopes in recombinant fragments was confirmed by western blot (d) and ELISA (e). Gcyt, gametocyte extract. BSA, bovine serum albumin. **Related to Figure 1.**

**A**

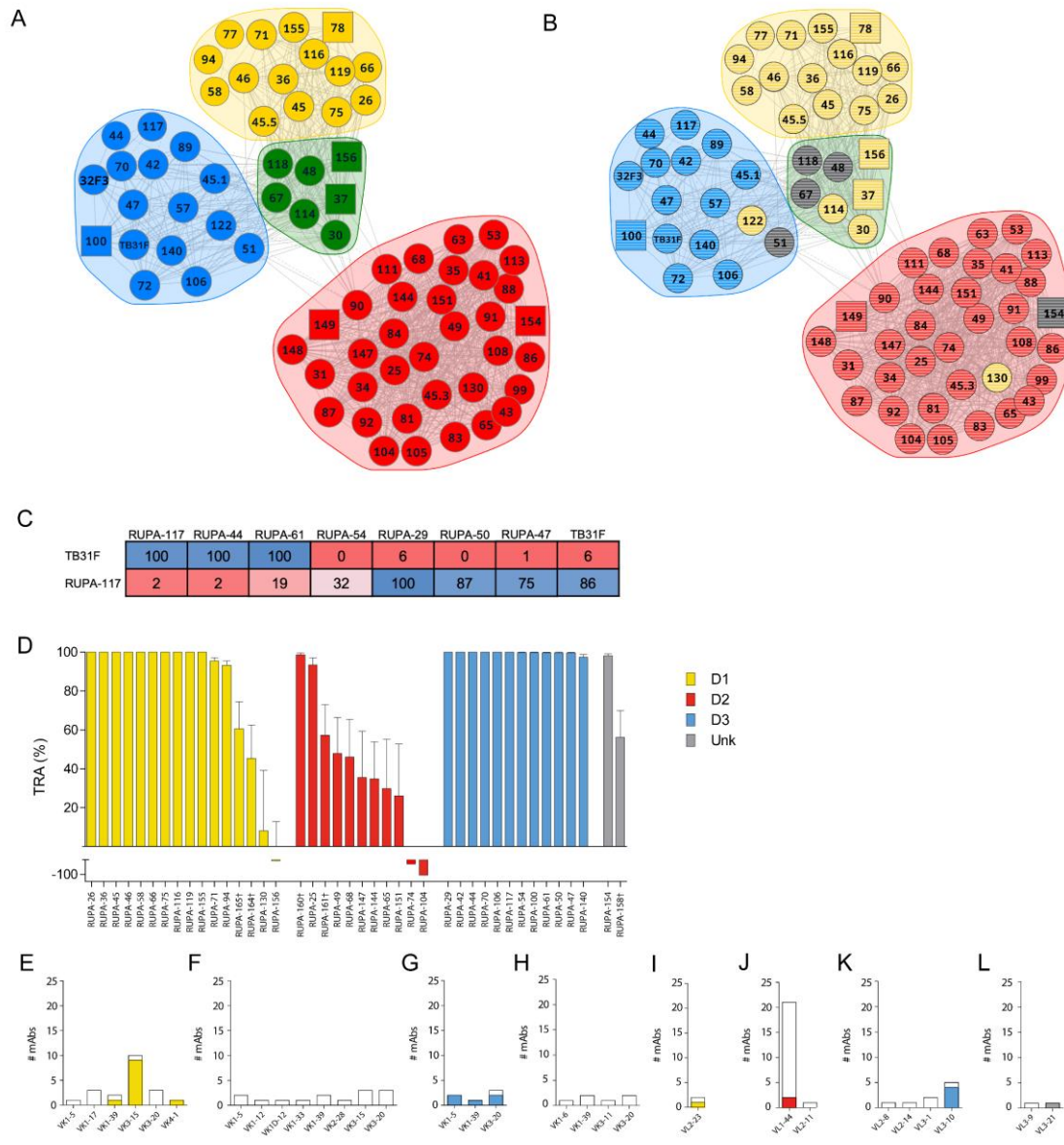


**B**



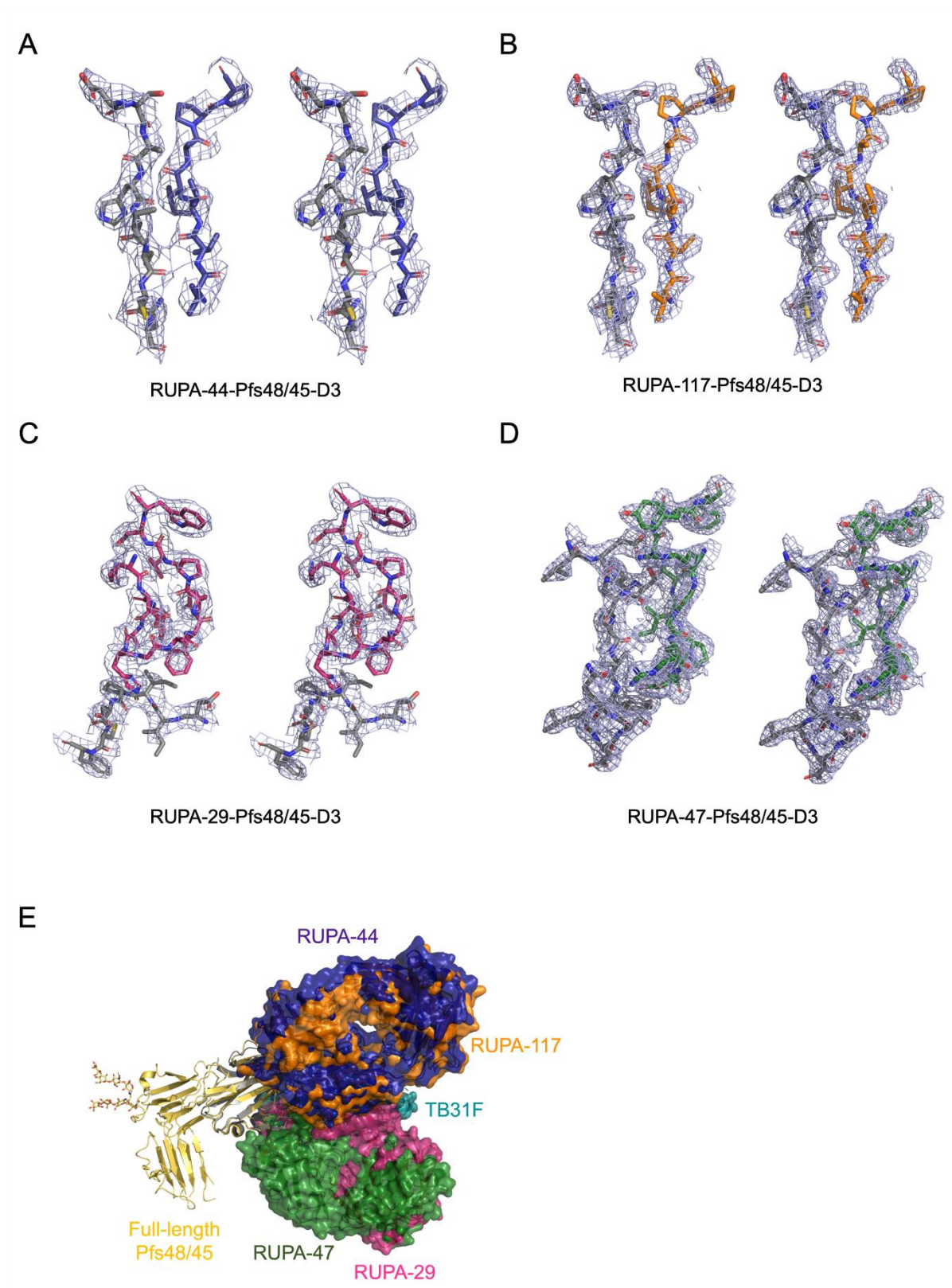
**Figure S2. Recognition of native Pfs48/45 by RUPA antibodies. (a)** Binding of mAbs to gametocyte extract. Gametocyte extract was run on SDS-PAGE gel under reducing (+) and non-reducing (-) conditions. After transfer to nitrocellulose membrane, strips were cut and incubated with individual RUPA antibodies. TB31F was included as a positive control antibody that is conformation-dependent. A non-targeting human IgG was included as negative control (Neg). Closed circles indicate mAbs that

bind to linear epitopes, while open circles indicate mAbs that bind to conformational epitopes. **(b)** Female gamete recognition by mAbs in surface immunofluorescence assay. All antibodies were tested at 5 µg/mL, except for mAbs against Pfs25 (2544, 0.1 µg/mL), Pfs48/45 (TB31F, 0.03 µg/mL). RUPA-25 and RUPA-26 were tested at 5 µg/mL in a separate experiment that included mAbs against Pfs25 (2544, 1 µg/mL) and Pfs48/45 (TB31F, 0.1 µg/mL). Anti-PfCSP mAb 399, which is sporozoite specific and does not recognize female gametes, was used for background correction. Values represent means of two technical replicates after background correction, and error bars show s.d. **Related to Figure 1.**



**Figure S3. Competition experiments identified four groups of monoclonal antibodies, transmission reducing activity (TRA) of potent monoclonal antibodies (mAbs) in standard membrane feeding assay (SMFA) and genetic characteristics of light chains.** (a) Competition between monoclonal antibodies (mAbs) was tested in two orientations; one antibody coupled to a chip and the other one in solution, and vice versa. mAbs with data in two directions are shown in circles and those with data in one direction as squares. (b) As in a, but with individual mAbs colored following domain specificity as determined by construct ELISA (Table S1). Yellow, D1; red, D2; blue, D3 and grey, unknown domain. (c) Epitope binning of anti-Pfs48/45-D3 Fabs using biolayer interferometry. Binning data is shown as a matrix where numbers indicate the percentage of potential binding. High competition is displayed in red (low numbers) and low competition is displayed in blue (high numbers). (d) 36 mAbs that showed the highest potency in barcoded membrane feeding assay (Figure 2a) and five mAbs that were not available at the time of high throughput membrane feeding assay (marked with †) were tested at 100  $\mu\text{g}/\text{ml}$  in SMFA with *Anopheles stephensi* mosquitoes and *Plasmodium falciparum* NF54 gametocytes. TRA was calculated as the percentage reduction in oocyst intensity compared to a non-antibody control and obtained from a single feeding experiment with 20 mosquitoes per condition. Error bars indicate 95% confidence intervals. Raw SMFA data is presented in Supplementary file 1. (e-l) Genetic characteristics of light chains. Bar graphs showing number of monoclonal antibodies (mAbs) per (e-h)

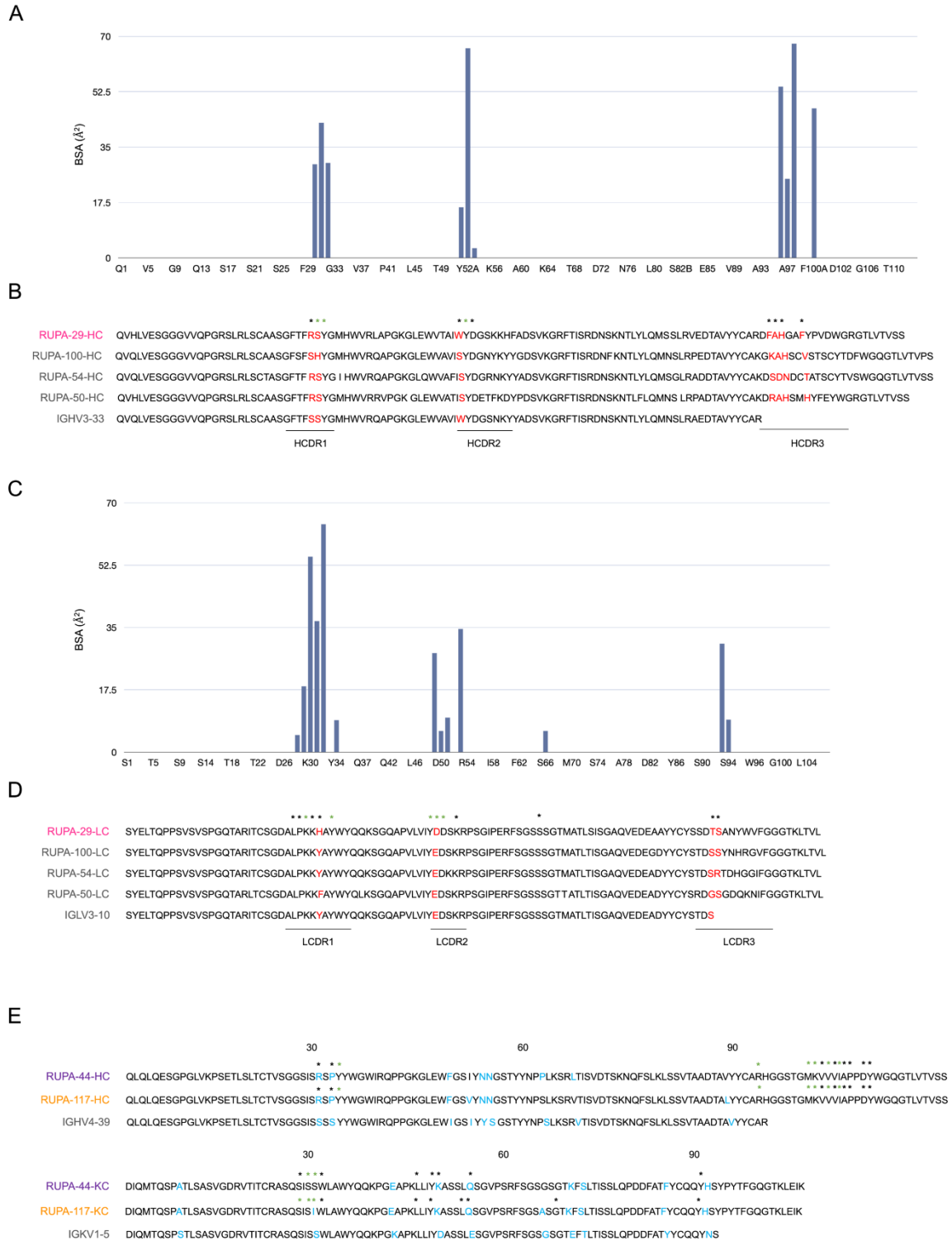
kappa and (i-l) lambda light chain family. mAbs are grouped by (e,i) D1-, (f,j) D2- and (g,k) D3-specific mAbs and (h,l) mAbs with unknown specificity. Colored bars represent mAbs with >80% transmission reducing activity at 100 µg/ml. **Related to Figures 1, 2 and 3.**



**Figure S4. Protective epitopes of potent human antibodies are accessible in full-length Pfs48/45 structure.** (a) Stereo view of the 2mFo-DFc composite omit electron density map contoured at  $1.5 \sigma$ . Structures of interfaces between Pfs48/45-D3 in grey and RUPA-44 (a) in blue, RUPA-117 (b) in orange, RUPA-29 (c) in pink, and RUPA-47 (d) in green. Electron density is shown as a blue mesh. (e) Fabs RUPA-



44 (dark blue), RUPA-117 (orange), TB31F (teal; PDB ID: 6E63), RUPA-29 (pink), and RUPA-47 (dark green) bound to D3 (grey) overlaid onto full-length Pfs48/45 (yellow; PDB:7ZXF). **Related to Figures 4 and 5.**



**Figure S5. Alignment of antibodies from potent antibody lineages targeting Pfs48/45-D3 epitopes Ia and Ib. (a)** Buried surface area of each RUPA-29-HC residue in the Pfs48/45-D3-RUPA-29 complex. **(b)** Alignment of the heavy chain sequences of RUPA-29, RUPA-100, RUPA-54, RUPA-50, and germline precursor, IGHV3-33. **(c)** Buried surface area of each RUPA-29-LC residue in the Pfs48/45-D3-RUPA-29 complex. **(d)** Alignment of the lambda chain sequences of RUPA-29, RUPA-100, RUPA-54, RUPA-50, and germline precursor, IGLV3-10. Green asterisks indicate RUPA-29 residues that form H-bonds/salt-bridges, while black asterisks indicate residues that make van der Waals (VdW) interactions with Pfs48/45-D3. Amino acids in red signify contact residues that are not conserved across all antibodies.

(e) Alignment of heavy and light variable chain sequences of RUPA-44 and RUPA-117 with their inferred germline precursor. Mutated residues from the germline are in blue. Asterisks indicate residues that form VdW (black) or electrostatic (green) contacts with Pfs48/45-D3. **Related to Figures 4 and 5.**

**Table S1. Domain specificity of isolated monoclonal antibodies (mAbs) as determined by ELISA.** mAbs that were positive against full-length Pfs48/45, were also tested against fragments of Pfs48/45 (Supplementary figure 3). mAbs were considered Domain 1-specific mAbs if they were positive for Domain 1-2 and negative for Domain 2-3 and Domain 3, Domain 2-specific mAbs if positive for Domain 1-2 and Domain 2-3 and negative for Domain 3, and Domain 3-specific mAbs if positive for Domain 2-3 and Domain 3 and negative for Domain 1-2. Other mAbs have unknown (unk) specificity. Optical density (OD<sub>450</sub>) values are means of three technical replicates and the standard deviation (SD) is shown between brackets. The cutoff was determined by calculating the mean + three standard deviations of seven non-Pfs48/45 mAbs that were included on each ELISA plate. Two mAbs, marked with an asterisk, were negative in full-length Pfs48/45 ELISA but showed high affinity for full-length Pfs48/45 by surface plasmon resonance and were therefore included in further analyses. **Related to Figure 1.**

Name	Full-length			Domain 1-2			Domain 2-3			Domain 3			Domain specificity
	OD <sub>450</sub> (SD)	Cutoff	Pos=1/ Neg=0	OD <sub>450</sub> (SD)	Cutoff	Pos=1/ Neg=0	OD <sub>450</sub> (SD)	Cutoff	Pos=1/ Neg=0	OD <sub>450</sub> (SD)	Cutoff	Pos=1/ Neg=0	
RUPA-25	1.38 (0.51)	0.14	1	2.13 (0.04)	0.30	1	1.62 (0.09)	0.19	1	0.05 (0.01)	0.15	0	2
RUPA-26	1.28 (0.06)	0.14	1	1.54 (0.07)	0.30	1	0.07 (0.02)	0.19	0	0.06 (0.01)	0.15	0	1
RUPA-29	1.60 (0.04)	0.14	1	0.15 (0.00)	0.30	0	1.69 (0.14)	0.19	1	1.84 (0.04)	0.15	1	3
RUPA-30	0.66 (0.72)	0.14	1	0.35 (0.03)	0.30	1	0.15 (0.02)	0.19	0	0.10 (0.03)	0.15	0	1
RUPA-34	1.30 (0.27)	0.14	1	1.85 (0.05)	0.30	1	0.35 (0.02)	0.19	1	0.05 (0.01)	0.15	0	2
RUPA-35	1.33 (0.08)	0.14	1	1.93 (0.03)	0.30	1	1.15 (0.08)	0.19	1	0.13 (0.03)	0.15	0	2
RUPA-36	1.09 (0.04)	0.14	1	1.64 (0.06)	0.30	1	0.05 (0.00)	0.19	0	0.03 (0.00)	0.15	0	1
RUPA-37	0.80 (0.05)	0.14	1	0.79 (0.04)	0.30	1	0.06 (0.00)	0.19	0	0.04 (0.00)	0.15	0	1
RUPA-41	1.58 (0.11)	0.14	1	2.17 (0.09)	0.30	1	1.54 (0.06)	0.19	1	0.05 (0.00)	0.15	0	2
RUPA-42	1.22 (0.05)	0.14	1	0.12 (0.00)	0.30	0	1.36 (0.06)	0.19	1	1.55 (0.11)	0.15	1	3
RUPA-43	1.50 (0.33)	0.14	1	2.20 (0.02)	0.30	1	1.71 (0.07)	0.19	1	0.06 (0.00)	0.15	0	2
RUPA-44	1.81 (0.11)	0.14	1	0.29 (0.01)	0.30	0	1.80 (0.06)	0.19	1	1.81 (0.11)	0.15	1	3
RUPA-45	0.94 (0.20)	0.14	1	1.20 (0.03)	0.30	1	0.11 (0.00)	0.19	0	0.05 (0.01)	0.15	0	1
RUPA-46	0.61 (0.04)	0.14	1	0.56 (0.02)	0.30	1	0.10 (0.01)	0.19	0	0.04 (0.00)	0.15	0	1
RUPA-47	1.67 (0.04)	0.14	1	0.23 (0.01)	0.30	0	1.84 (0.07)	0.19	1	1.81 (0.05)	0.15	1	3
RUPA-48	0.39 (0.02)	0.14	1	0.17 (0.01)	0.30	0	0.07 (0.01)	0.19	0	0.05 (0.01)	0.15	0	Unk
RUPA-49	1.54 (0.07)	0.14	1	2.34 (0.14)	0.30	1	1.69 (0.03)	0.19	1	0.06 (0.01)	0.15	0	2
RUPA-50	1.49 (0.05)	0.14	1	0.14 (0.00)	0.30	0	1.68 (0.03)	0.19	1	1.70 (0.05)	0.15	1	3
RUPA-51	0.46 (0.04)	0.14	1	0.10 (0.01)	0.30	0	0.08 (0.01)	0.19	0	0.08 (0.06)	0.15	0	Unk
RUPA-53	1.86 (0.06)	0.14	1	2.45 (0.15)	0.30	1	1.84 (0.03)	0.19	1	0.06 (0.02)	0.15	0	2
RUPA-54	1.61 (0.10)	0.18	1	0.23 (0.12)	0.26	0	1.87 (0.02)	0.19	1	1.97 (0.04)	0.23	1	3
RUPA-57	1.44 (0.04)	0.18	1	0.08 (0.01)	0.26	0	1.71 (0.02)	0.19	1	1.75 (0.01)	0.23	1	3
RUPA-58	1.29 (0.04)	0.18	1	0.97 (0.04)	0.26	1	0.12 (0.03)	0.19	0	0.19 (0.01)	0.23	0	1
RUPA-59	0.09 (0.01)	0.18	0										
RUPA-61	1.65 (0.06)	0.18	1	0.10 (0.01)	0.26	0	1.78 (0.03)	0.19	1	1.89 (0.15)	0.23	1	3
RUPA-63	1.68 (0.04)	0.18	1	2.14 (0.05)	0.26	1	1.57 (0.00)	0.19	1	0.05 (0.00)	0.23	0	2
RUPA-65	2.05 (0.03)	0.18	1	2.16 (0.15)	0.26	1	1.75 (0.08)	0.19	1	0.19 (0.02)	0.23	0	2
RUPA-66	1.39 (0.02)	0.18	1	1.50 (0.09)	0.26	1	0.10 (0.04)	0.19	0	0.19 (0.01)	0.23	0	1
RUPA-67	0.23 (0.01)	0.18	1	0.06 (0.00)	0.26	0	0.11 (0.04)	0.19	0	0.17 (0.06)	0.23	0	Unk
RUPA-68	1.86 (0.03)	0.18	1	2.02 (0.11)	0.26	1	1.53 (0.05)	0.19	1	0.17 (0.07)	0.23	0	2
RUPA-70	1.76 (0.06)	0.18	1	0.16 (0.00)	0.26	0	1.66 (0.07)	0.19	1	2.23 (0.07)	0.23	1	3
RUPA-71	1.39 (0.02)	0.18	1	1.02 (0.20)	0.26	1	0.13 (0.02)	0.19	0	0.22 (0.01)	0.23	0	1
RUPA-72	1.30 (0.04)	0.18	1	0.06 (0.01)	0.26	0	1.37 (0.03)	0.19	1	1.56 (0.04)	0.23	1	3
RUPA-74	1.96 (0.09)	0.18	1	2.32 (0.11)	0.26	1	1.71 (0.06)	0.19	1	0.09 (0.03)	0.23	0	2
RUPA-75	1.37 (0.06)	0.18	1	1.54 (0.13)	0.26	1	0.10 (0.05)	0.19	0	0.10 (0.07)	0.23	0	1
RUPA-77	1.24 (0.08)	0.18	1	0.97 (0.04)	0.26	1	0.06 (0.00)	0.19	0	0.07 (0.01)	0.23	0	1
RUPA-78	0.89 (0.03)	0.18	1	0.65 (0.08)	0.26	1	0.05 (0.00)	0.19	0	0.06 (0.01)	0.23	0	1
RUPA-80*	0.08 (0.00)	0.18	0										Unk
RUPA-81	1.70 (0.12)	0.18	1	1.93 (0.05)	0.26	1	0.70 (0.04)	0.19	1	0.16 (0.07)	0.23	0	2
RUPA-83	1.79 (0.07)	0.18	1	2.02 (0.11)	0.26	1	1.46 (0.01)	0.19	1	0.15 (0.08)	0.23	0	2
RUPA-84	1.54 (0.07)	0.14	1	2.07 (0.07)	0.27	1	1.63 (0.03)	0.15	1	0.08 (0.01)	0.25	0	2
RUPA-86	1.32 (0.15)	0.14	1	1.84 (0.02)	0.27	1	1.34 (0.05)	0.15	1	0.08 (0.00)	0.25	0	2

RUPA-87	1.99 (0.15)	0.14	1	2.15 (0.23)	0.27	1	1.81 (0.09)	0.15	1	0.10 (0.01)	0.25	0	2
RUPA-88	1.88 (0.09)	0.14	1	2.07 (0.14)	0.27	1	1.71 (0.10)	0.15	1	0.09 (0.00)	0.25	0	2
RUPA-89	1.53 (0.05)	0.14	1	0.13 (0.01)	0.27	0	1.54 (0.01)	0.15	1	1.76 (0.01)	0.25	1	3
RUPA-90	1.64 (0.15)	0.14	1	2.14 (0.06)	0.27	1	1.75 (0.13)	0.15	1	0.08 (0.01)	0.25	0	2
RUPA-91	1.45 (0.06)	0.14	1	2.05 (0.14)	0.27	1	1.48 (0.06)	0.15	1	0.06 (0.00)	0.25	0	2
RUPA-92	0.95 (0.03)	0.14	1	1.57 (0.07)	0.27	1	0.79 (0.09)	0.15	1	0.07 (0.01)	0.25	0	2
RUPA-94	0.82 (0.06)	0.14	1	0.57 (0.07)	0.27	1	0.06 (0.00)	0.15	0	0.07 (0.01)	0.25	0	1
RUPA-99	1.70 (0.08)	0.14	1	2.06 (0.19)	0.27	1	1.75 (0.03)	0.15	1	0.08 (0.00)	0.25	0	2
RUPA-100	1.54 (0.08)	0.14	1	0.14 (0.01)	0.27	0	1.77 (0.05)	0.15	1	1.92 (0.05)	0.25	1	3
RUPA-102	0.05 (0.00)	0.14	0										
RUPA-104	1.96 (0.08)	0.14	1	2.21 (0.16)	0.27	1	1.81 (0.06)	0.15	1	0.08 (0.01)	0.25	0	2
RUPA-105	1.55 (0.08)	0.14	1	1.90 (0.14)	0.27	1	1.51 (0.04)	0.15	1	0.07 (0.00)	0.25	0	2
RUPA-106	0.97 (0.09)	0.14	1	0.06 (0.02)	0.27	0	1.14 (0.06)	0.15	1	1.25 (0.08)	0.25	1	3
RUPA-107	0.06 (0.00)	0.14	0										
RUPA-108	1.25 (0.10)	0.14	1	1.73 (0.10)	0.27	1	0.35 (0.05)	0.15	1	0.07 (0.01)	0.25	0	2
RUPA-110	0.05 (0.01)	0.14	0										
RUPA-111	1.86 (0.17)	0.14	1	2.23 (0.21)	0.27	1	1.94 (0.08)	0.15	1	0.09 (0.03)	0.25	0	2
RUPA-113	1.59 (0.05)	0.14	1	2.01 (0.08)	0.27	1	1.62 (0.05)	0.15	1	0.07 (0.00)	0.25	0	2
RUPA-114	0.67 (0.05)	0.21	1	0.45 (0.05)	0.30	1	0.14 (0.01)	0.22	0	0.19 (0.01)	0.31	0	1
RUPA-116	1.16 (0.07)	0.21	1	1.43 (0.06)	0.30	1	0.03 (0.00)	0.12	0	0.17 (0.01)	0.31	0	1
RUPA-117	1.65 (0.06)	0.21	1	0.24 (0.02)	0.30	0	2.07 (0.09)	0.22	1	2.07 (0.11)	0.31	1	3
RUPA-118*	0.12 (0.01)	0.21	0										Unk
RUPA-119	1.23 (0.09)	0.21	1	1.13 (0.04)	0.30	1	0.05 (0.00)	0.22	0	0.19 (0.04)	0.31	0	1
RUPA-120	0.06 (0.00)	0.21	0										
RUPA-121	0.11 (0.11)	0.21	0										
RUPA-122	0.82 (0.03)	0.21	1	0.35 (0.03)	0.30	1	0.11 (0.03)	0.22	0	0.18 (0.01)	0.31	0	1
RUPA-124	0.05 (0.00)	0.21	0										
RUPA-125	0.05 (0.01)	0.21	0										
RUPA-127	0.06 (0.01)	0.21	0										
RUPA-130	1.06 (0.03)	0.21	1	1.40 (0.10)	0.30	1	0.11 (0.00)	0.22	0	0.07 (0.05)	0.31	0	1
RUPA-132	0.06 (0.01)	0.21	0										
RUPA-140	1.54 (0.02)	0.21	1	0.11 (0.01)	0.30	0	1.78 (0.09)	0.22	1	1.65 (0.10)	0.31	1	3
RUPA-141	0.26 (0.07)	0.21	1	0.08 (0.01)	0.30	0	0.15 (0.01)	0.22	0	0.07 (0.01)	0.31	0	Unk
RUPA-142	0.06 (0.00)	0.21	0										
RUPA-143	0.37 (0.06)	0.21	1	0.42 (0.04)	0.30	1	0.32 (0.03)	0.22	1	0.06 (0.03)	0.31	0	2
RUPA-144	1.54 (0.04)	0.21	1	1.99 (0.07)	0.30	1	1.58 (0.09)	0.22	1	0.14 (0.08)	0.31	0	2
RUPA-145	0.15 (0.09)	0.21	0										
RUPA-146	0.06 (0.00)	0.25	0										
RUPA-147	1.82 (0.04)	0.25	1	2.08 (0.06)	0.22	1	1.62 (0.03)	0.10	1	0.05 (0.00)	0.13	0	2
RUPA-148	1.78 (0.05)	0.25	1	2.14 (0.04)	0.22	1	1.66 (0.02)	0.10	1	0.05 (0.00)	0.13	0	2
RUPA-149	1.01 (0.04)	0.25	1	1.21 (0.22)	0.22	1	0.75 (0.15)	0.10	1	0.05 (0.00)	0.13	0	2
RUPA-150	0.71 (0.03)	0.25	1	0.78 (0.17)	0.22	1	0.45 (0.08)	0.10	1	0.04 (0.00)	0.13	0	2
RUPA-151	1.73 (0.05)	0.25	1	2.15 (0.06)	0.22	1	1.53 (0.03)	0.10	1	0.05 (0.01)	0.13	0	2
RUPA-152	0.22 (0.01)	0.25	0										
RUPA-153	0.51 (0.03)	0.25	1	0.85 (0.08)	0.22	1	0.34 (0.04)	0.10	1	0.09 (0.00)	0.13	0	2
RUPA-154	1.67 (0.10)	0.25	1	1.99 (0.05)	0.22	1	1.69 (0.01)	0.10	1	0.22 (0.03)	0.13	1	Unk
RUPA-155	1.24 (0.14)	0.25	1	1.48 (0.20)	0.22	1	0.07 (0.00)	0.10	0	0.05 (0.00)	0.13	0	1
RUPA-156	0.50 (0.09)	0.25	1	0.49 (0.02)	0.22	1	0.08 (0.01)	0.10	0	0.05 (0.00)	0.13	0	1
RUPA-157	0.06 (0.00)	0.25	0										
RUPA-158	0.38 (0.05)	0.25	1	0.15 (0.01)	0.22	0	0.07 (0.00)	0.10	0	0.04 (0.00)	0.13	0	Unk
RUPA-159	0.09 (0.01)	0.25	0										
RUPA-160	1.33 (0.09)	0.25	1	1.63 (0.15)	0.22	1	1.01 (0.03)	0.10	1	0.04 (0.00)	0.13	0	2
RUPA-161	1.76 (0.02)	0.25	1	2.10 (0.06)	0.22	1	1.72 (0.10)	0.10	1	0.05 (0.00)	0.13	0	2
RUPA-162	0.07 (0.01)	0.25	0										
RUPA-163	0.12 (0.03)	0.25	0										
RUPA-164	0.32 (0.04)	0.25	1	0.58 (0.06)	0.22	1	0.05 (0.00)	0.10	0	0.04 (0.00)	0.13	0	1
RUPA-165	0.85 (0.10)	0.25	1	1.12 (0.09)	0.22	1	0.05 (0.00)	0.10	0	0.04 (0.00)	0.13	0	1

**Table S2. Gene characteristics of Pfs48/45-specific monoclonal antibodies (mAbs).** mAbs are grouped by donor (D=Dutch expatriate, U=Ugandan donor) and domain specificity. **Related to Figure 3.**

Donor	Domain	mAb	V <sub>H</sub>	V <sub>H</sub> Identity to germline (%)	D <sub>H</sub>	J <sub>H</sub>	HCDR3 (AA)	V <sub>L</sub>	V <sub>L</sub> Identity to germline (%)	J <sub>L</sub>	LCDR3 (AA)		
U	1	RUPA-26	VH4-34	90.4	DH6-19	JH5	ARRSSGYRGSRYTGWFDP	VK3-15	96.1	JK4	QQYNNWPGLT		
		RUPA-30	VH1-46	92.2	DH2-15	JH3	ARAGEYCRGIRCYIDGLDI	VK1-17	97.9	JK1	LQHNDSPWT		
		RUPA-36	VH4-34	89.0	DH6-19	JH5	ARRASGWYRQSRYTGWFDP	VK3-15	98.2	JK4	QQYNNWPGLS		
		RUPA-37	VH1-46	88.5	DH2-15	JH3	ARAGEYCRGIRCYVDGIDM	VK1-17	97.5	JK1	LQHNSPWT		
		RUPA-45	VH4-34	89.0	DH6-19	JH5	ARRSSGYRGSRYTGWFDP	VK3-15	96.1	JK4	QQYNNWPGLT		
		RUPA-46	VH3-11	92.6	DH2 or 15-2a	JH3	ARDRKRNSSETYYGALDV	VK4-1	95.4	JK1	QQYTTPTWT		
		RUPA-58	VH1-2	94.6	DH2-2	JH4	ARDYCTGSSCYRTDYDY	VK1-39	94.0	JK5	QQSYRDPIT		
		RUPA-66	VH4-34	96.2	DH6-19	JH5	ARRSSGWYRRSRYTGWFDP	VK3-15	96.5	JK4	QQYNNWPGLS		
		RUPA-71	VH3-49	90.3	DH3-22	JH3	TRNGLRWYDSSGPRGWAFDI	VK3-15	97.6	JK1	QQYNNWPPWT		
		RUPA-75	VH4-34	91.8	DH3-22	JH5	ARRSSGYRGSRYTGWLDP	VK3-15	96.1	JK4	QQYNNWPGLT		
		RUPA-77	VH2-5	92.3	DH6-6	JH5	AHRILAAANYFDY	VK1-39	93.3	JK4	QQSYTSPLT		
		RUPA-78	VH3-23	90.1	DH6-6	JH3	AKFRTPRFSSSGDALDL	VK3-20	96.5	JK2	QQYGSVPGT		
		RUPA-94	VH4-39	92.3	DH3-16	JH4	ARRPNPGFSGGGYFDY	VL2-23	98.0	JL1	CSYADSSTRNVV		
		RUPA-114	VH1-18	92.4	DH5-12	JH6	ARGGDRGDGLRFYFALDV	VK3-20	96.5	JK1	QLYGRSSWT		
		RUPA-116	VH4-34	94.8	DH6-19	JH5	ARRSSGYRQSRYTGWFDP	VK3-15	96.8	JK4	QQYNNWPGLT		
		RUPA-119	VH4-34	89.0	DH6-19	JH5	ARRSSGWYRQSRYTGWFDP	VK3-15	98.2	JK4	QQYNNWPGLT		
		RUPA-122	VH2-5	91.3	DH3-10	JH5	ARTMKKITMIRGVPEYEGGVQGFDP	VK1-17	94.7	JK4	QQYSGYFT		
		RUPA-155	VH4-34	90.2	DH6-19	JH5	ARRASGWYRQSRYTGWFDPPW	VK3-15	98.2	JK4	QQYNNWPGLSF		
		RUPA-156	VH3-48	91.3	DH1-26	JH4	ARDGRKDSGSFLDHW	VK1-5	93.9	JK1	QQFDTYWTF		
		RUPA-164	VH4-59	95.1	DH2-2	JH6	ARDRVVGSYYYGMDVW	VK3-20	95.0	JK1	QHYGGSPPRETF		
		RUPA-165	VH4-34	95.1	DH6-19	JH5	ARRSSGYRGSRYTGWLDPPW	VK3-15	94.6	JK4	QQYNNWPGLTF		
		D	2	RUPA-25	VH1-8	91.6	DH3-10	JH5	VRGVGSYHSGGWFDP	VL1-44	93.8	JL3	ATWDASLKRRV
				RUPA-34	VH3-53	92.7	DH3-22	JH4	VRDSRGWGYFDD	VK3-20	95.5	JK1	QQYQNSPWT
				RUPA-35	VH1-8	94.6	DH3-10	JH5	SRGVGYTHGSGGWFDP	VL1-44	95.5	JL3	ATWDDSLKRGV
				RUPA-41	VH1-8	93.9	DH3-10	JH5	ARGVGYTHGAGGWFDP	VL1-44	94.8	JL3	ATWDDSLKRGV
				RUPA-43	VH1-8	93.6	DH3-10	JH5	ARGVGSYHSGGWLDP	VL1-44	94.5	JL3	GTWDDSLKRRV
				RUPA-49	VH1-8	90.5	DH3-10	JH5	ARGVGYTHGSGGWFDP	VL1-44	95.5	JL3	ATWDDSLKRGV
				RUPA-53	VH1-8	93.6	DH3-10	JH5	ARGVGSYHSGGWLDP	VK1-33	98.9	JK4	QQYDNLPLT
RUPA-63	VH1-8			92.2	DH3-10	JH5	ARGVGYTHGSEGWLDP	VL1-44	95.2	JL3	AAWDDSLKRGV		
RUPA-65	VH1-8			93.2	DH3-10	JH5	ARGVGYTHGSGGWFDP	VL1-44	97.9	JL3	ATWDDSLKRGV		
RUPA-68	VH1-8			93.9	DH3-10	JH5	ARGVGYTHGAGGWFDP	VL1-44	95.5	JL3	ATWDDSLKRGV		
RUPA-74	VH1-8			90.2	DH3-10	JH5	ARGVGSYHSGGWIDP	VL1-44	94.5	JL3	ETWDDSLKRRV		
RUPA-81	VH3-53			88.7	DH3-10	JH4	ARDVRGWGYLDF	VK3-20	95.5	JK1	QQYGYSPWT		
RUPA-83	VH1-18			91.9	DH4-11	JH5	ARDSRSSQLLQKFDL	VK1-39	92.3	JK1	QQSFSSPT		
RUPA-84	VH1-8			93.2	DH3-10	JH5	ARGVGYTHGSEGWLDP	VL1-44	95.5	JL3	ATWDDSLKRGV		
RUPA-86	VH1-2			95.2	DH3-16	JH6	ARLVAYDQGLDV	VK3-15	98.6	JK2	QQYNNWPPPLYT		
RUPA-87	VH1-2			93.2	DH6-19	JH4	ARVVAEAGYFDY	VK1-12	97.9	JK4	QQANSFPLT		
RUPA-88	VH1-8			91.2	DH3-10	JH5	ARGVGSYHSGGWLDP	VL1-44	94.8	JL3	ATWDDSLKRRV		
RUPA-90	VH3-7			94.9	DH2-2	JH6	AREGDVFAVAPSARVKGKGGVYGMV	VK2-28	99.3	JK3	MQUALQTPYRVT		
RUPA-91	VH1-8			93.2	DH3-10	JH5	ARGVGYTHGSEGWLDP	VL1-44	95.2	JL3	ATWDDSLKRGV		
RUPA-92	VH1-8			95.6	DH3-10	JH5	ARGVGYRSGGWFDP	VL2-11	96.2	JL2	CSYAGNYIEI		
RUPA-99	VH1-8			90.5	DH3-10	JH5	ARGVGSYHSGGWLDP	VL1-44	94.1	JL3	ATWDDSLKRRV		
RUPA-104	VH1-8			95.3	DH4-17	JH4	ARGVTPDPYFDS	VK1D-12	95.1	JK5	LQANSLPVT		
RUPA-105	VH1-8			89.9	DH3-10	JH5	VRGVGSYHSGGWLDP	VL1-44	93.4	JL3	ATWDASLKRRV		
RUPA-108	VH3-53			90.7	DH3-22	JH4	VRDSRGWGYFDD	VK3-20	94.8	JK1	QQYQNSPWT		
RUPA-111	VH3-48			91.6	DH1-26	JH4	ARDGRKDSGSFLDH	VK1-5	95.8	JK1	QQFDTYWTF		
RUPA-113	VH1-8			91.6	DH3-10	JH5	TRGVGYTHGAGGWFDP	VL1-44	96.9	JL3	ATWDDSLKRRV		
RUPA-143	VH1-18			66.7	DH3-16	JH6	ARANWYDNNWQEFYFGMDVW	VK1-5	90.3	JK1	QHYDAYPWTFF		
RUPA-144	VH1-8			92.7	DH3-10	JH5	ARGVGYTHGSEGWLDPW	VL1-44	95.4	JL3	ATWDDSLKRGV		
RUPA-147	VH1-8			91.7	DH3-10	JH5	VRGVGSYHSGGWFDPPW	VL1-44	93.7	JL3	ATWDASLKRRV		
RUPA-148	VH1-8			91.7	DH3-10	JH5	VRGVGSYHSGGWFDPPW	VL1-44	93.0	JL3	ATWDASLKRRV		

		RUPA-149	VH3-30	91.3	DH3-3	JH6	AKDKVFGVVANHHQYGLDVW	VK1-39	88.5	JK2	QQSYTTPHFTF	
		RUPA-150	VH4-34	90.5	DH6-19	JH5	ARRSSGYRGSRYTGWFDPW	VK3-15	97.9	JK1	QQYNNWPPPWF	
		RUPA-151	VH1-8	91.0	DH3-10	JH5	ARGVGTYHGSEGWLDPW	VL1-44	94.0	JL3	CAAWDDSLKRGVF	
		RUPA-153	VH4-31	69.4	DH3-10	JH4	ARAVGVGRYFYFPSW	VK3-15	97.5	JK1	QQYNNWPPPWF	
		RUPA-160	VH1-8	91.3	DH3-10	JH5	VRAVGSYYGGGWFDPW	VL1-44	94.7	JL3	ATWDDSMKREVF	
		RUPA-161	VH1-8	91.3	DH3-10	JH5	ARGVGTYHGSGGWFDPW	VL1-44	96.1	JL3	ATWDDSLKRGVF	
	3	RUPA-29	VH3-33	93.9	DH4 or 15-4a	JH4	ARDFAHGAFYVD	VL3-10	96.8	JL3	YSSDTSANYVW	
		RUPA-42	VH4-31	95.0	DH5-12	JH4	ARAGLVAAIRGSRGQQGDYFDH	VL3-1	94.0	JL2	QAWDSSTGV	
		RUPA-44	VH4-39	96.3	DH3-22	JH4	ARHGGSTGMKVVIAPPDY	VK1-5	95.4	JK2	QQYHSYPYT	
		RUPA-47	VH1-18	89.2	DH3-16	JH4	VRGGRDSPILGGY	VK3-20	95.8	JK4	HQYGSPLT	
		RUPA-50	VH3-30	90.2	DH1-26	JH4	AKDRAHSMHYFEY	VL3-10	95.1	JL2	YSRDGSGDQKNI	
		RUPA-54	VH3-30	94.6	DH2-2	JH5	AKSDNDCTATSCYTVS	VL3-10	96.2	JL2	YSTDRSDHGGI	
		RUPA-57	VH3-30	93.9	DH3-22	JH4	AKSNDHYDNSGYFVY	VL3-10	96.2	JL3	YSRDSNTQRV	
		RUPA-61	VH1-46	93.5	DH5-24	JH6	ARVRKRDAYNRRNWGSVFGGMDV	VL2-8	97.0	JL3	SSFSGSNNLV	
		RUPA-70	VH4-31	96.6	DH5-12	JH4	ARTGLVAAIRGSRGQQGDYFDY	VL3-1	97.2	JL2	QAWDSNTGV	
		RUPA-72	VH3-74	93.8	DH2-8	JH6	ARAGCSNGVCPPRYYHGMVDV	VK3-20	96.9	JK1	QHYGNSRVT	
		RUPA-89	VH3-23	92.2	DH3-9	JH5	AKEGILRPFDP	VL2-14	96.2	JL1	SSYTSITYHHV	
		RUPA-100	VH3-30	96.6	DH2-2	JH4	AKGKAHSCVSTSCYTDF	VL3-10	97.9	JL2	YSTDSSYNHRGV	
		RUPA-106	VH3-23	95.9	DH2-8	JH4	AKDTHSNILYLFDY	VK3-20	97.2	JK1	HQYGISPAT	
		RUPA-117	VH4-39	96.3	DH3-22	JH4	ARHGGSTGMKVVIAPPDY	VK1-5	94.7	JK2	QQYHSYPYT	
		?	RUPA-48	VH3-9	96.6	DH2-2	JH6	AKDGGYCSGIRCYHLRGMDV	VK3-11	98.3	JK2	QQRSSWPPYT
			RUPA-51	VH1-18	92.8	DH5-12	JH6	ARGGDRGDGPRFYGLDV	VK3-20	94.7	JK1	SLYGRSSWT
			RUPA-67	VH4-4	92.5	DH2-2	JH6	ARDVSPEECVSTKCYRRPGYMDV	VK1-39	95.1	JK1	QQSYTTPRT
	RUPA-80*		VH1-8	99.7	DH2-2	JH5	ARGLPKIYIRGIWFDP	VL3-9	99.3	JL2	QVWDTSTRGV	
	RUPA-118*		VH4-4	94.5	DH2-2	JH6	ARDLSPEECVSTKCYRRPGYMDV	VK1-39	95.5	JK1	QQSYTTPRT	
	RUPA-154		VH1-2	92.4	DH6-25	JH6	ARLLYSRAVYYYGMDVW	VL3-21	96.8	JL1	QVWDSSTDHYVF	
	RUPA-158	VH1-18	93.1	DH5-12	JH6	ARGGDRGDGPRYYYGLDVW	VK3-20	93.6	JK1	SLYGRSSWTF		
U	1	RUPA-130	VH3-15	93.7	DH1-14	JH4	TTDSWAGEVGNYYFDN	VL2-23	95.9	JL1	CSYSKSSTYV	
	3	RUPA-140	VH3-23	93.6	DH3-3	JH4	AKVGGRSIFGEATDY	VK1-39	94.4	JK4	QQTYTLPLI	
	?	RUPA-141	VH3-7	94.9	DH6-19	JH3	ARDNGARYSGVWYDGWYDAFDI	VK1-6	97.2	JK1	LQDYNYPRT	

**Table S3. Data collection and refinement statistics for the Pfs48/45-D3-RUPA-29 Fab-RUPA-44 Fab co-crystal structure. Related to Figure 4.**

	<i>Pfs48/45-D3-RUPA-29 Fab-RUPA-44 Fab</i>
<b>Wavelength (Å)</b>	1.033190
<b>Space group</b>	I23
<b>Cell dimensions</b>	
<b>a, b, c (Å)</b>	223.1 223.1 223.1
<b>α, β, γ (°)</b>	90 90 90
<b>Resolution (Å)</b>	29.81 - 2.86 (2.91 - 2.86)*
<b>No. molecules in ASU</b>	1
<b>No. unique observations</b>	42,583 (2126)
<b>Multiplicity</b>	40.8 (41.0)
<b>R<sub>meas</sub><sup>1</sup> (%)</b>	18.3 (312.1)
<b>R<sub>pim</sub><sup>2</sup> (%)</b>	2.5 (14.6)
<b>&lt;I/sigma I&gt;</b>	23.2 (1.5)
<b>CC<sub>1/2</sub> (%)</b>	99.9 (61.2)
<b>Completeness (%)</b>	99.9 (100)
<b>Refinement Statistics</b>	
<b>Non-hydrogen atoms</b>	9,216
<b>Macromolecule</b>	9,202
<b>Water</b>	0
<b>Hetero atom</b>	14
<b>R<sub>factor</sub><sup>3</sup>/ R<sub>free</sub><sup>4</sup> (%)</b>	18.5/ 22.3
<b>Rms deviations from ideality</b>	
<b>Bond lengths (Å)</b>	0.002
<b>Bond angle (°)</b>	0.54
<b>Ramachandran plot</b>	
<b>Favoured regions (%)</b>	96.0
<b>Allowed regions (%)</b>	4.0
<b>B-factors (Å<sup>2</sup>)</b>	
<b>Wilson B-factor</b>	85.7
<b>Average B-factors</b>	95.2
<b>Average macromolecule</b>	95.1
<b>Average hetero atom</b>	156.4
<b>Average water</b>	-

\*Values in parentheses are for the highest-resolution shell.

$$^1R_{\text{meas}} = \sum_{\text{hkl}} [N/(N-1)]^{1/2} \sum_i |I_{\text{hkl},i} - \langle I_{\text{hkl}} \rangle| / \sum_{\text{hkl}} \langle I_{\text{hkl}} \rangle$$

$$^2R_{\text{pim}} = \sum_{\text{hkl}} [1/(N-1)]^{1/2} \sum_i |I_{\text{hkl},i} - \langle I_{\text{hkl}} \rangle| / \sum_{\text{hkl}} \langle I_{\text{hkl}} \rangle$$

$$^3R_{\text{work}} = (\sum | |F_o| - |F_c| |) / (\sum |F_o|).$$

<sup>4</sup>R<sub>free</sub> is calculated for 5% of the data that was not included in refinement.



**Table S4. Intermolecular contacts for the Pfs48/45-D3-RUPA-29 Fab and Pfs48/45-D3-RUPA-47 Fab co-complexes.** VDW=van Der Waals, HB=Hydrogen Bond, SB=Salt Bridge. **Related to Figure 4.**

Pfs48/45-D3-RUPA-29 Fab co-complex			Pfs48/45-D3-RUPA-47 Fab co-complex		
RUPA-29 Residue (BSA Å <sup>2</sup> )	Interaction Type	Pfs48/45-D3 Residue	RUPA-47 Residue (BSA Å <sup>2</sup> )	Interaction Type	Pfs48/45-D3 Residue
<b>H-Arg30 (29.6)</b>			<b>H-Gln 1 (16.2)</b>		
Arg	VDW	Gln355, Tyr371	Gln	VDW	Glu362
<b>H-Ser31 (42.7)</b>			<b>H-Val 2 (18.3)</b>		
Ser	VDW	Gln355, Ile369, Tyr371	Val	VDW	Glu362, Glu360
Ser <sup>O</sup>	HB	Gln355 <sup>NE2</sup>	<b>H-Gly 26 (5.5)</b>		
Ser <sup>OG</sup>	HB	Tyr371 <sup>OH</sup>	Gly	VDW	Glu362
<b>H-Tyr32 (30.0)</b>			<b>H-Tyr 27 (7.7)</b>		
Tyr	VDW	Ser367, Ile369	Tyr	VDW	Glu360
Tyr <sup>OH</sup>	HB	Ser367 <sup>O</sup>	<b>H-Asn 31 (13.5)</b>		
<b>H-Trp52 (16.0)</b>			Asn	VDW	Gln 358
Trp	VDW	Asp351	Asn <sup>O</sup>	HB	Gln358 <sup>NE2</sup>
<b>H-Tyr52A (66.2)</b>			<b>H-Phe 32 (53.6)</b>		
Tyr	VDW	Asp351, Phe354, Gln355, Tyr371	Phe	VDW	Gln358, Ser367, Glu360, Asn368
Tyr <sup>OH</sup>	HB	Asp351 <sup>OD2</sup>	<b>H-Tyr 53 (25.7)</b>		
<b>H-Asp53 (3.1)</b>			Tyr	VDW	Ser374
Asp	VDW	Asp351	<b>H-Arg94 (46.4)</b>		
<b>H-Phe96 (54.1)</b>			Arg	VDW	Ser367, Glu365, Glu360, Asn368
Phe	VDW	Ile349, Tyr357, Glu365	Arg <sup>NH1</sup>	SB	Glu360 <sup>OE2</sup>
<b>H-Ala97 (25.0)</b>			Arg <sup>NE</sup>	HB	Ser367 <sup>OG</sup>
Ala	VDW	Ile349, Tyr357, Ile369	Arg <sup>NH2</sup>	HB	Asn368 <sup>OD1</sup>
<b>H-His98 (67.7)</b>			Arg <sup>NH2</sup>	HB	Ser367 <sup>OG</sup>
His	VDW	Ile348, Ile349, Pro350, Asp351, Gln 355, Ile369	Arg <sup>NH2</sup>	HB	Glu360 <sup>OE1</sup>
<b>H-Phe100A (47.2)</b>			<b>H-Gly95 (4.7)</b>		
Phe	VDW	Asp347, Ile348, Ile349	Gly	VDW	Ser367
<b>L-Leu28 (4.8)</b>			<b>H-Gly96 (17.6)</b>		
Leu	VDW	Lys416	Gly	VDW	Ser367, Ile369
<b>L-Pro29 (18.5)</b>			<b>H-Arg97 (89.5)</b>		
Pro	VDW	Asp321, Lys416	Arg	VDW	Gln358, Val370, Ile369, Ser367, Ser374, Gln375
<b>L-Lys30 (54.9)</b>			Arg <sup>NH1</sup>	HB	Gln375 <sup>OE1</sup>
Lys	VDW	Asp415, Lys416	Arg <sup>NH2</sup>	HB	Gln375 <sup>OE1</sup>
Lys <sup>O</sup>	HB	Lys416 <sup>N</sup>	<b>H-Asp98 (67.8)</b>		
<b>L-Lys31 (36.8)</b>			Asp	VDW	Ile369, Tyr371, Val370, Ser374, Gln375
Lys	VDW	Asp347, Asp415, Lys416	Asp <sup>OD2</sup>	HB	Tyr371 <sup>N</sup>
<b>L-His32 (64.0)</b>			<b>H-Ser99 (32.6)</b>		
His	VDW	Lys413, Asp415, Lys416	Ser	VDW	Tyr371, Gln355
<b>L-Tyr34 (9.0)</b>			<b>H-Ile100A (60.3)</b>		
Tyr	VDW	Lys413	Ile	VDW	Pro366, Ile369, Ser367, Gln 355
Tyr <sup>OH</sup>	HB	Lys413 <sup>NZ</sup>	<b>H-Leu100B (11.5)</b>		
<b>L-Tyr49 (27.8)</b>			Leu	VDW	Pro366, Ser367

Tyr	VDW	Leu364, Glu365	<b>H-Gly100C (23.6)</b>		
Tyr <sup>OH</sup>	HB	Glu365 <sup>OE2</sup>	Gly	VDW	Ser367, Glu365, Pro366
<b>L-Asp50 (5.9)</b>			<b>H-Gly101 (10.7)</b>		
Asp	VDW	Lys413	Gly	VDW	Glu365
Asp <sup>OD1</sup>	SB	Lys413 <sup>NZ</sup>	Gly <sup>N</sup>	HB	Glu365 <sup>OE2</sup>
<b>L-Asp51 (9.7)</b>			<b>H-Tyr102 (51.5)</b>		
Asp	VDW	Lys416	Tyr	VDW	Glu365, Ser367, Glu362, Glu363
Asp <sup>OD2</sup>	SB	Lys416 <sup>NZ</sup>	Tyr <sup>N</sup>	HB	Glu365 <sup>OE2</sup>
<b>L-Lys53 (34.6)</b>			<b>K-Arg29 (54.1)</b>		
Lys	VDW	Leu364, Glu365	Arg	VDW	Asp351, Phe354, Gln355,
<b>L-Ser66 (5.9)</b>			Arg <sup>NH1</sup>	SB	Asp351 <sup>OD2</sup>
Ser	VDW	Lys416	Arg <sup>NH1</sup>	HB	Gln355 <sup>OE1</sup>
Ser <sup>OG</sup>	HB	Lys416 <sup>NZ</sup>	Arg <sup>NH2</sup>	HB	Gln355 <sup>OE1</sup>
<b>L-Thr93 (30.5)</b>			<b>K-Ser30 (37.2)</b>		
Thr	VDW	Asp347	Ser	VDW	Pro350, Asp351, Ile348
<b>L-Ser94 (9.1)</b>			<b>K-Met31 (36.9)</b>		
Ser	VDW	Asp390	Met	VDW	Asp347
			<b>K-Tyr32 (68.5)</b>		
			Tyr	VDW	Pro350, Asp351, Gln355, Ile369
			Tyr <sup>OH</sup>	HB	Asp351 <sup>N</sup>
			Tyr <sup>OH</sup>	HB	Gln355 <sup>OE1</sup>
			<b>K-Leu46 (25.4)</b>		
			Leu	VDW	Glu365, Pro366
			<b>K-His49 (55.4)</b>		
			His	VDW	Tyr357, Leu364, Glu365, Pro366
			<b>K-Ser52 (37.4)</b>		
			Ser	VDW	Lys413, Asp347
			Ser <sup>OG</sup>	HB	Lys413 <sup>NZ</sup>
			<b>K-Ile53 (65.6)</b>		
			Ile	VDW	Ile349, Tyr357, Lys413
			<b>K-Thr56 (35.9)</b>		
			Thr	VDW	Glu363
			<b>K-Tyr91 (31.9)</b>		
			Tyr	VDW	Ile369, Pro366

**Table S5. Binding affinities of RUPA-29, RUPA-47, RUPA-44, and RUPA-117 Fabs to Pfs48/45-D3 single point mutants. Related to Figures 4 and 5.**

Fab	Pfs48/45-D3 constructs	$K_D$ (M) – binding affinity	$K_{on}$ (1/Ms) – on rate	$K_{off}$ (1/s) – off rate	$R^2$ – the coefficient of determination
<b>RUPA-29</b>	Stabilized WT	$3.7E-10 \pm 4E-11$	$5.1E+5$	$2.1E-4$	0.982
	K416N	$1.1E-7 \pm 4E-9$	$4.2E+5$	$4.5E-2$	0.991
<b>RUPA-47</b>	Stabilized WT	$3.3E-10 \pm 1E-11$	$3.9E+5$	$1.2E-4$	0.999
	K416N	$5.3E-10 \pm 2E-11$	$4.5E+5$	$2.2E-4$	0.997
<b>RUPA-44</b>	Stabilized WT	$3.9E-9 \pm 1E-11$	$2.2E+5$	$8.5E-4$	0.984
	L314I	$3.2E-9 \pm 8E-11$	$2.8E+5$	$8.9E-4$	0.986
	D320H	$1.6E-8 \pm 3E-10$	$2.0E+5$	$3.3E-3$	0.986
	S322N	$6.8E-9 \pm 2E-10$	$2.1E+5$	$1.4E-3$	0.985
	K416N	$3.1E-9 \pm 1E-10$	$3.0E+5$	$9.2E-3$	0.976
<b>RUPA-117</b>	Stabilized WT	$1.8E-9 \pm 5E-11$	$4.6E+5$	$7.6E-4$	0.984
	L314I	$1.9E-9 \pm 6E-11$	$4.2E+5$	$7.2E-4$	0.985
	D320H	$7.7E-9 \pm 9E-11$	$4.3E+5$	$3.3E-3$	0.988
	S322N	$2.8E-9 \pm 5E-11$	$4.4E+5$	$1.2E-3$	0.981
	K416N	$2.4E-9 \pm 6E-11$	$4.7E+5$	$1.1E-3$	0.983

**Table S6. Intermolecular contacts for the Pfs48/45-D3-RUPA-117 Fab and Pfs48/45-D3-RUPA-44 Fab co-complexes.** VDW=van Der Waals, HB=Hydrogen Bond, SB=Salt Bridge. **Related to Figure 5.**

Pfs48/45-D3-RUPA-117 Fab co-complex			Pfs48/45-D3-RUPA-44 Fab co-complex		
RUPA-117 Residue (BSA Å <sup>2</sup> )	Interaction Type	Pfs48/45-D3 Residue	RUPA-44 Residue (BSA Å <sup>2</sup> )	Interaction Type	Pfs48/45-D3 Residue
<b>H-Arg31 (14.2)</b>			<b>H-Arg31 (20.4)</b>		
Arg	VDW	Leu318	Arg	VDW	Leu318
<b>H-Pro33 (59.1)</b>			<b>H-Pro33 (59.4)</b>		
Pro	VDW	Leu314, Val319, Leu318,	Pro	VDW	Leu314, Leu318, Val319
<b>H-Tyr34 (46.5)</b>			<b>H-Tyr34 (50.1)</b>		
Tyr	VDW	Leu318, Ala323, Val319, Asp320	Tyr	VDW	Leu318, Val319, Asp320, Ala323
Tyr <sup>OH</sup>	HB	Asp320 <sup>N</sup>	Tyr <sup>OH</sup>	HB	Asp320 <sup>N</sup>
<b>H-Arg94 (19.1)</b>			<b>H-Arg94 (17.6)</b>		
Arg	VDW	Asp320	Arg	VDW	Asp320
Arg <sup>NH2</sup>	SB	Asp320 <sup>OD2</sup>	Arg <sup>NH2</sup>	SB	Asp320 <sup>OD2</sup>
<b>H-Met 100A (10.4)</b>			<b>H-Met 100A (9.8)</b>		
Met	VDW	Asn328, Ser326	Met	VDW	Asn328
Met <sup>O</sup>	HB	Asn328 <sup>ND2</sup>	Met <sup>O</sup>	HB	Asn328 <sup>ND2</sup>
<b>H-Lys100B (81.5)</b>			<b>H-Lys100B (89.3)</b>		
Lys	VDW	Asn328, Ser326, Cys327, Asp312, Val329, His330	Lys	VDW	Asp312, Ser326, Cys327, Asn328, Val329, His330
Lys <sup>O</sup>	HB	Asn328 <sup>N</sup>	Lys <sup>O</sup>	HB	Asn328 <sup>N</sup>
Lys <sup>O</sup>	HB	Asn328 <sup>ND2</sup>	Lys <sup>O</sup>	HB	Asn328 <sup>ND2</sup>
Lys <sup>NZ</sup>	HB	Asn328 <sup>O</sup>	Lys <sup>NZ</sup>	HB	Asn328 <sup>O</sup>
Lys <sup>NZ</sup>	SB	Asp312 <sup>OD2</sup>	Lys <sup>NZ</sup>	SB	Asp312 <sup>OD2</sup>
<b>H-Val100C (89.9)</b>			<b>H-Val100C (91.7)</b>		
Val	VDW	Ser326, Asn328, Cys327, Leu314, Asp312, Ser313	Val	VDW	Asp312, Ser313, Leu314, Ser326, Cys327, Asn328
<b>H-Val100D (39.7)</b>			<b>H-Val100D (40.1)</b>		
Val	VDW	Ser326, His324, Ile325	Val	VDW	Leu314, His324, Ile325, Ser326
Val <sup>N</sup>	HB	Ser326 <sup>O</sup>	Val <sup>N</sup>	HB	Ser326 <sup>O</sup>
Val <sup>O</sup>	HB	Ser326 <sup>N</sup>	Val <sup>O</sup>	HB	Ser326 <sup>N</sup>
<b>H-Val100E (45.0)</b>			<b>H-Val100E (48.2)</b>		
Val	VDW	His324, Leu314, Ile325, Val319, Ala323	Val	VDW	Leu314, Val319, Ala323, His324, Ile325
<b>H-Ile100F (53.3)</b>			<b>H-Ile100F (48.5)</b>		
Ile	VDW	Ile325, Ala323, His324, Ser322, Ser326	Ile	VDW	Ser322, Ala323, His324, Ile325, Ser326
Ile <sup>O</sup>	HB	His324 <sup>N</sup>	Ile <sup>O</sup>	HB	His324 <sup>N</sup>
Ile <sup>N</sup>	HB	His324 <sup>O</sup>	Ile <sup>N</sup>	HB	His324 <sup>O</sup>
<b>H-Ala100G (12.6)</b>			<b>H-Ala100G (15.7)</b>		
Ala	VDW	Ser322, Ala323, His324	Ala	VDW	Ser322, Ala323, His324
<b>H-Pro100H (24.6)</b>			<b>H-Pro100H (21.7)</b>		
Pro	VDW	Ser322	Pro	VDW	Ser322
<b>H-Asp101 (19.5)</b>			<b>H-Asp101 (21.8)</b>		

Asp	VDW	Ser322, Asp320	Asp	VDW	Asp320, Ser322
<b>H-Tyr102 (6.7)</b>			<b>H-Tyr102 (9.9)</b>		
Tyr	VDW	Asp320	Tyr	VDW	Asp320
<b>K-Ser28 (26.3)</b>			<b>K-Ser28 (27.8)</b>		
Ser	VDW	Ser361	Ser	VDW	Glu362
Ser <sup>OG</sup>	HB	Ser361 <sup>O</sup>			
<b>K-Ser30 (24.3)</b>			<b>K-Ser30 (38.4)</b>		
Ser	VDW	Glu363	Ser	VDW	Glu362, Glu363, Leu364
Ser <sup>OG</sup>	HB	Glu363 <sup>OE2</sup>	Ser <sup>OG</sup>	HB	Glu363 <sup>O</sup>
<b>K-Ile31 (30.5)</b>			<b>K-Ser31 (34.7)</b>		
Ile	VDW	Glu363	Ser	VDW	Glu363, Leu364
Ile <sup>N</sup>	HB	Glu363 <sup>OE2</sup>	Ser <sup>N</sup>	HB	Glu363 <sup>O</sup>
<b>K-Trp32 (67.0)</b>			<b>K-Trp32 (54.9)</b>		
Trp	VDW	His324, Tyr420	Trp	VDW	His324
<b>K-Leu46 (11.1)</b>			<b>K-Leu46 (10.7)</b>		
Leu	VDW	Ser322	Leu	VDW	Ser322
<b>K-Tyr49 (76.0)</b>			<b>K-Tyr49 (52.7)</b>		
Tyr	VDW	Ser322, Asp321, Lys416	Tyr	VDW	Asp321, Ser322
<b>K-Leu54 (6.6)</b>			<b>K-Lys50 (61.6)</b>		
Leu	VDW	Lys416	Lys	VDW	Asp321, Ser322, Ala323, His324, Leu364
<b>K-Gln55 (7.2)</b>			<b>K-Gln55 (7.6)</b>		
Gln	VDW	Ser322	Gln	VDW	Ser322
<b>K-Gly68 (10.0)</b>			<b>K-Tyr91 (8.5)</b>		
Gly	VDW	Glu362	Tyr	VDW	Ser322
<b>K-Tyr91 (13.5)</b>					
Tyr	VDW	Ser322			

## References

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