Supplementary Information

Structure of the malaria vaccine candidate Pfs48/45 and its recognition by transmission blocking antibodies

Supplementary Data Figures and Tables

**Supplementary Figure 1: surface plasmon resonance analysis**

The top panel shows surface plasmon resonance traces for binding to immobilised Pfs48/45 for antibodies 85RF45.1 (a two-fold dilution series from a top concentration of 7.8nM), 10D8 and 32F3 (each two-fold concentrations series from top concentrations of 125nM). The red dotted lines show the fitting to a one-to-one binding model. The lower panel shows the binding kinetics for these three sets of data based on the one-to-one fitting.

|       | kₐ (1/Ms)   | kₐ (1/s)   | Kᵢₐ (M)   | χ²         |
|-------|-------------|-------------|-----------|------------|
| 10D8  | 7.55 x 10⁴  | 0.002702    | 3.58 x 10⁻⁸| 5.23       |
| 32F3  | 2.94 x 10⁶  | 1.21 x 10⁻⁴| 4.10 x 10⁻⁹| 8.52       |
| 85RF45.1 | 1.38 x 10⁶  | 0.001862    | 1.34 x 10⁻⁹| 6.58       |
Supplementary Figure 2: the domains of Pfs48/45 determined by x-ray crystallography compared with AlphaFold2 models

The upper panels show the N-terminal, central and C-terminal domains of Pfs48/45. Disulphide bonds are shown as sticks, with sulphur residues coloured yellow. These are taken from the structure of full-length Pfs48/45 in complex with 10D8, and the equivalent map is shown at a contour level of 1.0 in blue mesh. The lower panel shows the same three domains aligned with the equivalent AlphaFold2 model. AlphaFold models are shown in the colour scheme indicated at the bottom of the figure, with green used for regions of highest confidence and red for lowest confidence.
Supplementary Figure 3: molecular dynamics simulations

A. Graphs showing the observed interdomain angles in full-length Pfs48/45 during atomistic molecular dynamics simulations. Three independent sets of simulations were run, using full-length Pfs48/45 taken from the crystal structure of the complex indicated above each graph. The interdomain angle is that between the line linking the centres of mass of the N-terminal and central domains and that linking the centres of mass of the central and C-terminal domains. The five lines on each graph show five independent simulations and were smoothened using a Savistky-Golay filter. B. A representation of the degree of flexibility of the epitopes for 32F3 (left), 85RF45.1 (centre) and 10D8 (right). In each case, the structure of Pfs48/45 is shown in blue with the residues which contact the antibody in orange, red or yellow. Five structures are shown, from the simulations shown in A, to indicate the degree of epitope flexibility.
Supplementary Figure 4: small angle x-ray scattering

A. Parameters derived from solution scattering data. \( R_g^{\text{exp}} \) is the radius of gyration, \( D_{\text{max}} \) the maximum particle diameter, \( V_{\text{Porod}} \) is the Porod volume. \( M_r^{\text{app}} \) is the molecular mass calculated from the excluded volume in the final volumetric representation, and \( M_r^{\exp} \) is the expected molecular mass.

B. Fit between the SEC-SAXS data and the three structures of Pfs48/45 constructed based on the crystal structures of Pfs48/45 bound to different antibody combinations and the structure from MD simulations conducted on Pfs48/45 that shows the lowest \( \chi^2 \) when fitted to the SEC-SAXS data (Pfs48/45FL-MD). The red line shows the scattering curve calculated from the model and the grey line shows scattering data. Shown below are the fits of these structures to the envelope calculated from the SEC-SAXS data, docked using Chimera.
**Supplementary Figure 5: negative stain electron microscopy**

Electron microscopy imaging of complexes containing Pfs48/45 (white), the Fab of 85RF45.1 (red) and one additional antibody Fab, as indicated to the left-hand side of each row. Each row shows a representative micrograph (n>100 as described in Supplementary Table 4, left), two-dimensional class averages (centre) and a low-resolution reconstruction (right).
**Supplementary Table 1: crystallographic statistics for structures with incomplete Pfs48/45 constructs**

| Data collection | Pfs48/45-6C:32F3 | Pfs48/45-D2+3:10D8:32F3 |
|-----------------|------------------|-------------------------|
| **Space group** | P12,1            | P2₁,2,2₁                |
| **Cell dimensions:** |               |                         |
| a, b, c         | 78.66, 78.66, 110.62 | 108.58, 158.45, 186.16 |
| α, β, γ         | 90°, 99.5°, 90°    | 90°, 90°, 90°           |
| **Resolution**  | 77.57 – 1.90 Å (1.93 – 1.90 Å) | 108.58 – 3.69 Å (3.75 – 3.69 Å) |
| **Total observations** | 781771 (36476) | 230227 (11072) |
| **Total unique** | 116014 (5704) | 35355 (1713) |
| R<sub>pim</sub> | 0.037 (0.282) | 0.065 (0.338) |
| CC<sub>1/2</sub> | 0.999 (0.780) | 0.971 (0.438) |
| I/σ(I)          | 11.6 (2.2) | 7.2 (1.8) |
| **Completeness (%)** | 99.9 (97.8) | 100.0 (98.0) |
| **Multiplicity** | 6.7 (6.4) | 6.5 (6.5) |
| **Wilson B factor (Å<sup>2</sup>)** | 27 | 119 |
| **Refinement** |               |                         |
| **Number of reflections** | 115991 | 35298 |
| R<sub>work</sub> / R<sub>free</sub> | 0.189 / 0.217 | 25.5 / 28.0 |
| **B factors (Å<sup>2</sup>)** |               |                         |
| Average        | 42              | 161                     |
| Pfs48/45-D2    | N/A             | (A) 169 (F) 157         |
| Pfs48/45-D3    | (A) 34 (B) 32   | (A) 138 (F) 145         |
| 32F3 heavy chain | (B) 41 (E) 44 | (B) 144 (G) 145         |
| 32F3 light chain | (C) 48 (F) 48 | (C) 153 (H) 136         |
| 10D8 heavy chain | N/A             | (D) 157 (I) 189         |
| 10D8 light chain | N/A             | (E) 159 (J) 220         |
| **Number of residues:** |               |                         |
| Amino acid residues | 1123 | 2179 |
| Sugars         | 3               | 10                      |
| Waters         | 963             | 0                       |
| **RMSZ deviations** |               |                         |
| Bond lengths   | 0.009           | 0.004                   |
| Bond angles    | 1.02            | 0.67                    |
| **Ramachandran plot** |               |                         |
| Favoured       | 98.5 %          | 94.8 %                  |
| Allowed        | 1.5 %           | 5.2 %                   |
| Outliers       | 0 %             | 0 %                     |
### Supplementary Table 2: crystallographic statistics for structures with complete Pfs48/45 constructs

| Data collection | Pfs48/45-FL:10D8 | Pfs48/45-FL:10D8:85RF45.1 | Pfs48/45-FL:32F3 scFv |
|----------------|------------------|---------------------------|----------------------|
| Space group    | P2₁2₁₂           | P4₁2₂                  | C222₁                |
| Cell dimensions: |                  |                          |                      |
| a, b, c        | 81.77, 125.80, 216.60 | 156.88, 156.80, 148.76 | 81.83, 126.12, 146.79 |
| α, β, γ        | 90°, 90°, 90°    | 90°, 90°, 90°           | 90°, 90°, 90°        |
| Resolution     | 76.27 – 4.20 Å  | 108.00 – 3.72 Å         | 73.39 – 2.13 Å       |
|                | (4.70 – 4.20Å)  | (4.06 – 3.72Å)          | (2.17 – 2.13Å)       |
| Total observations | 109594 (31747) | 456518 (56247)          | 586552 (28745)       |
| Total unique   | 16968 (4732)    | 17015 (1893)            | 42648 (2277)         |
| R_{sym}        | 0.088 (0.668)   | 0.027 (0.875)           | 0.037 (2.26)         |
| CC_{1/2}       | 0.998 (0.543)   | 0.999 (0.377)           | 0.999 (0.384)        |
| I/σ(I)         | 7.5 (1.6)       | 15.7 (1.2)              | 11.2 (0.4)           |
| Completeness (%) | 99.9 (100.0) | 94.9 (74.3)             | 99.8 (99.4)          |
| Multiplicity   | 6.5 (6.7)       | 26.8 (29.7)             | 13.8 (14.2)          |
| Wilson B factor (Å²) | 166        | 191                     | 59                   |

### Refinement

| Number of reflections | 16933 | 16936 | 40794 |
| R_{work} / R_{free}  | 25.9 / 27.6 | 28.1 / 30.2 | 27.6 / 28.6 |
| B factor (Å²)        | 212   | 215   | 98    |
| Pfs48/45-D1          | (A) 265 (D) 270 | 249 | 178   |
| Pfs48/45-D2          | (A) 199 (D) 195 | 212 | 130   |
| Pfs48/45-D3          | (A) 213 (D) 191 | 190 | 86    |
| 32F3scFv             | N/A   | N/A   | 83    |
| 85RF45.1 heavy       | N/A   | 202   | N/A   |
| 85RF45.1 light       | N/A   | 22    | N/A   |
| 10D8 heavy chain     | (B) 180 (E) 204 | 210 | N/A   |
| 10D8 light chain     | (C) 194 (F) 231 | 230 | N/A   |
| Number of residues: | 1574  | 1190  | 497   |
| Amino acid residues  | 15    | 8     | 2     |
| Sugars               | 0     | 0     | 94    |
| Waters               | 0     | 0     |       |
| RMSZ deviations      | 0.003 | 0.003 | 0.008 |
| Bond lengths         | 0.661 | 0.593 | 0.99  |
| Bond angles          |       |       |       |
| Ramachandran plot    | 91.9 %| 92.8 %| 92.8 %|
| Favoured             | 8.1%  | 7.2%  | 7.2%  |
| Allowed              | 0 %   | 0 %   | 0 %   |
| Outliers             |       |       |       |
## Supplementary Table 3: Interactions between Pfs48/45 and antibodies 32F3 and 10D8

### Pfs48/45-6C

| Residue | Group       | Chain/Residue | Group       | Type of interaction   |
|---------|-------------|---------------|-------------|-----------------------|
| Ser322  | Mainchain (O) | Heavy/ Arg98  | Sidechain    | Hydrogen bond         |
| Ser322  | Mainchain (O) | Heavy/ Tyr32  | Sidechain    | Hydrogen bond         |
| Ser322  | Sidechain    | Heavy/ Tyr109 | Sidechain    | Hydrogen bond         |
| His324  | Sidechain    | Heavy/ Tyr32  | Sidechain    | Hydrogen bond         |
| Tyr357  | Sidechain    | Heavy/ Tyr101 | Mainchain (O) | Hydrogen bond       |
| Glu360  | Sidechain    | Heavy/ Tyr33  | Sidechain    | Hydrogen bond         |
| Glu363  | Sidechain    | Heavy/ Ser57  | Sidechain    | Hydrogen bond         |
| Thr409  | Sidechain    | Heavy/ Tyr101 | Sidechain    | Hydrogen bond         |
| Asp347  | Sidechain    | Light/ Ser52  | Sidechain    | Hydrogen bond         |
| Leu364  | Mainchain (O) | Light/ Asn93  | Sidechain    | Hydrogen bond         |
| Glu365  | Sidechain    | Light/ Asn93  | Sidechain    | Hydrogen bond         |
| Asn368  | Sidechain    | Light/ Ser91  | Sidechain, mainchain | Hydrogen bond       |
| Ile369  | Mainchain (N) | Light/ Thr30  | Sidechain    | Hydrogen bond         |
| Lys413  | Sidechain    | Light/ Gln48  | Sidechain    | Hydrogen bond         |
| Lys413  | Sidechain    | Light/ Ser52  | Sidechain    | Hydrogen bond         |
| Lys413  | Sidechain    | Light/ Leu53  | Sidechain    | Hydrogen bond         |
| Asp415  | Mainchain (O) | Light/ Ser55  | Sidechain    | Hydrogen bond         |

### Pfs48/45-FL

| Residue | Group       | Chain/Residue | Group       | Type of interaction   |
|---------|-------------|---------------|-------------|-----------------------|
| Ser206  | Mainchain (O) | Light/ Asn34  | Sidechain    | Hydrogen bond         |
| Asn207  | Sidechain    | Heavy/ Asp104 | Mainchain (O) | Hydrogen bond       |
| Phe208  | Mainchain (O) | Heavy/ Arg105 | Sidechain    | Hydrogen bond         |
| Glu214  | Sidechain    | Heavy/ His59  | Sidechain    | Hydrogen bond         |
| Glu216  | Sidechain    | Heavy/ Ser52  | Sidechain    | Hydrogen bond         |
| Glu216  | Sidechain    | Heavy/ Ser54  | Mainchain (N) | Hydrogen bond       |
| Glu216  | Sidechain    | Heavy/ Ser56  | Sidechain, mainchain | Hydrogen bond       |
| Lys287  | Sidechain    | Heavy/ Ser56  | Mainchain (O) | Hydrogen bond       |
| Glu205  | Sidechain    | Light/ Ser33  | sidechain    | Hydrogen bond         |
| Phe208  | Mainchain (O) | Light/ Tyr31  | Sidechain    | Hydrogen bond         |
| Phe208  | Mainchain (O) | Heavy/ Arg105 | Sidechain    | Hydrogen bond         |
| Val209  | Sidechain    | Heavy/ Leu107 | Sidechain    | Hydrogen bond         |

### 10D8Fab

| Residue | Group       | Chain/Residue | Group       | Type of interaction   |
|---------|-------------|---------------|-------------|-----------------------|
| Ser206  | Mainchain (O) | Light/ Asn34  | Sidechain    | Hydrogen bond         |
| Asn207  | Sidechain    | Heavy/ Asp104 | Mainchain (O) | Hydrogen bond       |
| Phe208  | Mainchain (O) | Heavy/ Arg105 | Sidechain    | Hydrogen bond         |
| Glu214  | Sidechain    | Heavy/ His59  | Sidechain    | Hydrogen bond         |
| Glu216  | Sidechain    | Heavy/ Ser52  | Sidechain    | Hydrogen bond         |
| Glu216  | Sidechain    | Heavy/ Ser54  | Mainchain (N) | Hydrogen bond       |
| Glu216  | Sidechain    | Heavy/ Ser56  | Sidechain, mainchain | Hydrogen bond       |
| Asn286  | Sidechain    | Heavy/ Ser56  | Mainchain (O) | Hydrogen bond       |
| Glu215  | Sidechain    | Light/ Ser33  | sidechain    | Hydrogen bond         |
| Phe208  | Mainchain (O) | Light/ Tyr31  | Sidechain    | Hydrogen bond         |
| Phe208  | Mainchain (O) | Heavy/ Arg105 | Sidechain    | Hydrogen bond         |
| Val209  | Sidechain    | Heavy/ Leu107 | Sidechain    | Hydrogen bond         |

| Residue | Group       | Chain/Residue | Group       | Type of interaction   |
|---------|-------------|---------------|-------------|-----------------------|
| Ser206  | Mainchain (O) | Light/ Asn34  | Sidechain    | Hydrogen bond         |
| Asn207  | Sidechain    | Heavy/ Asp104 | Mainchain (O) | Hydrogen bond       |
| Phe208  | Mainchain (O) | Heavy/ Arg105 | Sidechain    | Hydrogen bond         |
| Glu214  | Sidechain    | Heavy/ His59  | Sidechain    | Hydrogen bond         |
| Glu216  | Sidechain    | Heavy/ Ser52  | Sidechain    | Hydrogen bond         |
| Glu216  | Sidechain    | Heavy/ Ser54  | Mainchain (N) | Hydrogen bond       |
| Glu216  | Sidechain    | Heavy/ Ser56  | Sidechain, mainchain | Hydrogen bond       |
| Lys287  | Sidechain    | Heavy/ Ser56  | Mainchain (O) | Hydrogen bond       |
| Glu215  | Sidechain    | Light/ Ser33  | sidechain    | Hydrogen bond         |
| Phe208  | Mainchain (O) | Light/ Tyr31  | Sidechain    | Hydrogen bond         |
| Phe208  | Mainchain (O) | Heavy/ Arg105 | Sidechain    | Hydrogen bond         |
| Val209  | Sidechain    | Heavy/ Leu107 | Sidechain    | Hydrogen bond         |

| Residue | Group       | Chain/Residue | Group       | Type of interaction   |
|---------|-------------|---------------|-------------|-----------------------|
| Ser206  | Mainchain (O) | Light/ Asn34  | Sidechain    | Hydrogen bond         |
| Asn207  | Sidechain    | Heavy/ Asp104 | Mainchain (O) | Hydrogen bond       |
| Phe208  | Mainchain (O) | Heavy/ Arg105 | Sidechain    | Hydrogen bond         |
| Glu214  | Sidechain    | Heavy/ His59  | Sidechain    | Hydrogen bond         |
| Glu216  | Sidechain    | Heavy/ Ser52  | Sidechain    | Hydrogen bond         |
| Glu216  | Sidechain    | Heavy/ Ser54  | Mainchain (N) | Hydrogen bond       |
| Glu216  | Sidechain    | Heavy/ Ser56  | Sidechain, mainchain | Hydrogen bond       |
| Lys287  | Sidechain    | Heavy/ Ser56  | Mainchain (O) | Hydrogen bond       |
| Glu215  | Sidechain    | Light/ Ser33  | sidechain    | Hydrogen bond         |
| Phe208  | Mainchain (O) | Light/ Tyr31  | Sidechain    | Hydrogen bond         |
| Phe208  | Mainchain (O) | Heavy/ Arg105 | Sidechain    | Hydrogen bond         |
| Val209  | Sidechain    | Heavy/ Leu107 | Sidechain    | Hydrogen bond         |

### Pfs48/45-FL

| Residue | Group       | Chain/Residue | Group       | Type of interaction   |
|---------|-------------|---------------|-------------|-----------------------|
| Ser206  | Mainchain (O) | Light/ Asn34  | Sidechain    | Hydrogen bond         |
| Asn207  | Sidechain    | Heavy/ Asp104 | Mainchain (O) | Hydrogen bond       |
| Phe208  | Mainchain (O) | Heavy/ Arg105 | Sidechain    | Hydrogen bond         |
| Glu214  | Sidechain    | Heavy/ His59  | Sidechain    | Hydrogen bond         |
| Glu216  | Sidechain    | Heavy/ Ser52  | Sidechain    | Hydrogen bond         |
| Glu216  | Sidechain    | Heavy/ Ser54  | Mainchain (N) | Hydrogen bond       |
| Glu216  | Sidechain    | Heavy/ Ser56  | Sidechain, mainchain | Hydrogen bond       |
| Lys287  | Sidechain    | Heavy/ Ser56  | Mainchain (O) | Hydrogen bond       |
| Glu215  | Sidechain    | Light/ Ser33  | sidechain    | Hydrogen bond         |
| Phe208  | Mainchain (O) | Light/ Tyr31  | Sidechain    | Hydrogen bond         |
| Phe208  | Mainchain (O) | Heavy/ Arg105 | Sidechain    | Hydrogen bond         |
| Val209  | Sidechain    | Heavy/ Leu107 | Sidechain    | Hydrogen bond         |
|                      | 1F10: Pfs48/45: 85RF45.1 | 3H6: Pfs48/45: 85RF45.1 | 6A10: Pfs48/45: 85RF45.1 | 9A6: Pfs48/45: 85RF45.1 | 10D8: Pfs48/45: 85RF45.1 |
|----------------------|--------------------------|-------------------------|--------------------------|--------------------------|--------------------------|
| **Microscope**       | Talos F200c              | Talos F200c              | Talos F200c              | Talos F200c              | Talos F200c              |
| **Voltage (kV)**     | 200                      | 200                     | 200                      | 200                      | 200                      |
| **Magnification**    | 45,000                   | 57,000                  | 57,000                   | 57,000                   | 57,000                   |
| **Pixel size (Å)**   | 2.3                      | 1.8                     | 1.8                      | 1.8                      | 1.8                      |
| **Defocus (µm)**     | -5                       | -1.5                    | -1.5                     | -1.5                     | -1.5                     |
| **Number of micrographs** | 101                     | 116                     | 120                      | 111                      | 101                      |
| **Number of picked particles** | 95,717                  | 51,993                  | 94,752                   | 67,240                   | 57,853                   |
| **Particles after 2D classification** | 27,759                  | 7,586                   | 4,893                    | 13,780                   | 7,988                    |
| **Particles in the final map** | 10,137                  | 1,815                   | 3,319                    | 2,925                    | 4,664                    |