

Supplementary Methods

***In vitro* inhibition of sporozoite invasion assay**

The inhibition of sporozoite invasion (ISI) assay was carried out as previously described¹. Briefly, sporozoites were incubated with serial dilutions of mAb for 30 minutes at RT prior to infection of Hepa 1-6 cells for 90 minutes at 37°C in the presence of fluorescently-labeled dextran. Cells were then washed, fixed and permeabilized prior to staining with fluorescently-labeled anti-CSP antibody. Flow cytometry was performed on an LSRII cytometer (BD Biosciences) and downstream analysis was performed using FlowJo software (TreeStar, Inc.).

ELISAs

Sporozoite lysate was made by double purification of salivary gland sporozoites over an Accudenz gradient². Six million of these sporozoites were then pelleted at 13,000rpm in a table top centrifuge and resuspended in 100uL lysis buffer (50mM Tris-HCl pH7.5; 140nM NaCl; 1% Triton-X; 1mM MgCl; 1mM EDTA) in the presence of protease inhibitor. After 30 minutes of incubation on ice, cell debris was pelleted at 13,000rpm in a table top centrifuge and supernatant collected as lysate. For each ELISA, 50 µL of purified sporozoite lysate or CSP repeat peptide was coated on 96 well high binding polystyrene plates (Corning, inc.) at a concentration of 1µg/mL or 0.1µg/mL, respectively, in carbonate/bicarbonate buffer at 4°C overnight. Plates were washed three times and 50µL of serum samples diluted in dilution buffer (PBS, 0.05% Tween-20, 6% BSA) at 1:10 for sporozoite lysate and 1:200 for CSP peptide were added in duplicate to each well. After 2h incubation at 37°C plates were again

washed three times and 100 μ L 1:2000 dilution (in dilution buffer) of HRP-conjugated secondary anti-mouse IgG antibody(Sigma Aldrich) added to each well and incubate for an additional 2 hours at 37°C. Plate were washed three times, developed with SigmaFast™ OPD (Sigma-Aldrich) and absorbance read at 450nm.

Supplementary Figure Legends

Figure S1. *In vitro* assay shows 2F6 mAb is able to reduce sporozoite invasion and traversal. *Py* salivary gland sporozoites were incubated with the indicated concentrations of mAb specific for the respective CSP repeat region for 15 minutes at RT prior to incubation with 50,000 Hepa1-6 cells/well in a 96-well plate at a 3:1 ratio of hepatoma:sporozoite for 90 minutes at 37°C in the presence of fluorescently-labeled dextran to stain damaged (traversed) cells. Cells were then collected, permeabilized, fixed and stained with a fluorescently labeled anti-CSP antibody to stain intracellularly infected cells. Percentages of infected (**A**) and traversed (**B**) were determined by flow cytometry. Each dilution shows individual points as replicates with horizontal bars representing the mean \pm SD. Asterisks indicate a p value <0.05 as determined by one-way ANOVA and Dunnet's post-test with comparisons made to mock-treated sporozoites.

Figure S2. Challenge by 3,000 *Py* GFP-luc sporozoite iv does not result in significantly different liver stage burden than 15 *Py* GFP-luc-infected mosquito bites. Total flux in BALB/c mice (n=8-15/group) 48 hours after infection with *Py* GFP-luc by either 15 infected mosquito bites or 3,000 sporozoites iv. Comparisons of the two means was conducted by Students t-test and significance cutoff was p<0.05 where NS=non-significant difference.

Figure S3. Anti-sporozoite lysate and CSP antibodies in immunized mice. Antibodies against whole sporozoite lysate or *Py* CSP were measured by ELISA in pooled serum collected from C57BL6 mice immunized with or 2x50,000 sporozoites of indicated GAP 2 weeks after final immunization or from naïve C57BL6 mice. Each column is the average absorbance at 450nm of duplicate samples from each serum source.

Figure S4. FRG-huHep mice reconstituted with different donor hepatocytes show different susceptibilities to *Pf* infection. FRG-huHep mice reconstituted with hepatocytes from two different donors were infected with 1 million *Pf* GFP-luc sporozoites iv and bioluminescence measured 6 days after infection. Images are shown in **A** with quantification of total flux (p/s) in **B**. Dotted line on the y-axis shows background levels of luminescence.

Supplemental References

- 1 Kaushansky, A., Rezakhani, N., Mann, H. & Kappe, S. H. Development of a quantitative flow cytometry-based assay to assess infection by *Plasmodium falciparum* sporozoites. *Molecular and biochemical parasitology* **183**, 100-103, doi:10.1016/j.molbiopara.2012.01.006 (2012).
- 2 Kennedy, M. *et al.* A rapid and scalable density gradient purification method for *Plasmodium* sporozoites. *Malaria journal* **11**, 421, doi:10.1186/1475-2875-11-421 (2012).







