Table S1: Primers information and sequences

Purpose	Primer name	Sequence
Expression of rPb22	F	5′-CTGGATCCGACGACGACGACAAGTCACATAAAAATATAATTCAAATAAATTAT-3′
	R	5′-CAGCGGCCGCTTATGAATCACCTGTCTGTTGTGTATTTTCA-3′
Expression of rP47	F	5’- CGGGATCCATATTCCCTAATGGATATGTCTG-3’
	R	5’- CGGCGGCCGCTTAAAGCTCGCGTTTTGTTTTGGAG-3’
Expression of rSET	F	5’- CGGGATCCATGAAGAGAGATCGTTCAGA-3’
	R	5’- CGGCGGCCGCTTAATCATCATTATCTTTTCCATC -3’
Generation and identification of Pb22HA-tagging parasite	HA-5UTR-F	5′-CGGGGCCCACGATAAAAGGGTAGGGGAAA -3′
	HA-5UTR-R	5′-TCCCCGCGGTGAATCACCTGTCTGTTGTG -3′
	HA-3UTR-F	5′- CCGCTCGAGATTTGAATGATCCGTAGATG -3′
	HA-3UTR-R	5′-ATTGCGGCCGCGATGAGCCTTTCGCAAACCG -3′
	HA-P1	5′-GAGGCTAGAACTGGCAAATGT-3′
	HA-P2	5′-GGTGGAAAGGGGGTTTTGGT-3′
	HA-P3	5′-CTGGTGCTTTGAGGGGTGAG-3′
	HA-P4	5′-GGTCACAAGCGGCATTCCTA-3′
	HA-P5	5′-TTGTTACTGGTGCCCTCGAC-3′
Generation and identification of KO parasite	KO -5UTR-F	5′-CCCAAGCTTGCCATGGGTTTTGGGCCATGTTATAC-3′
	KO 5UTR-R	5′-GGCTGCAGTTTCCCCTACCCTTTTATCGTTAAT-3′
	KO -3UTR-F	5′-GGGGTACCATTTGAATGATCCGTAGATGTTAAG-3′
	KO -3UTR-R	5′-ATTGCGGCCGCGAGGTGCATTATTCTTTAACTTATG-3′
	KO -P1	5′-GTGAAACTCAAACAAACCACACATAGATTA-3′
	KO -P2	5′-TTATCATAGGTTCGATAGTTTAGCACAT-3′
	KO-P3	5′-CTGGTGCTTTGAGGGGTGAG-3′

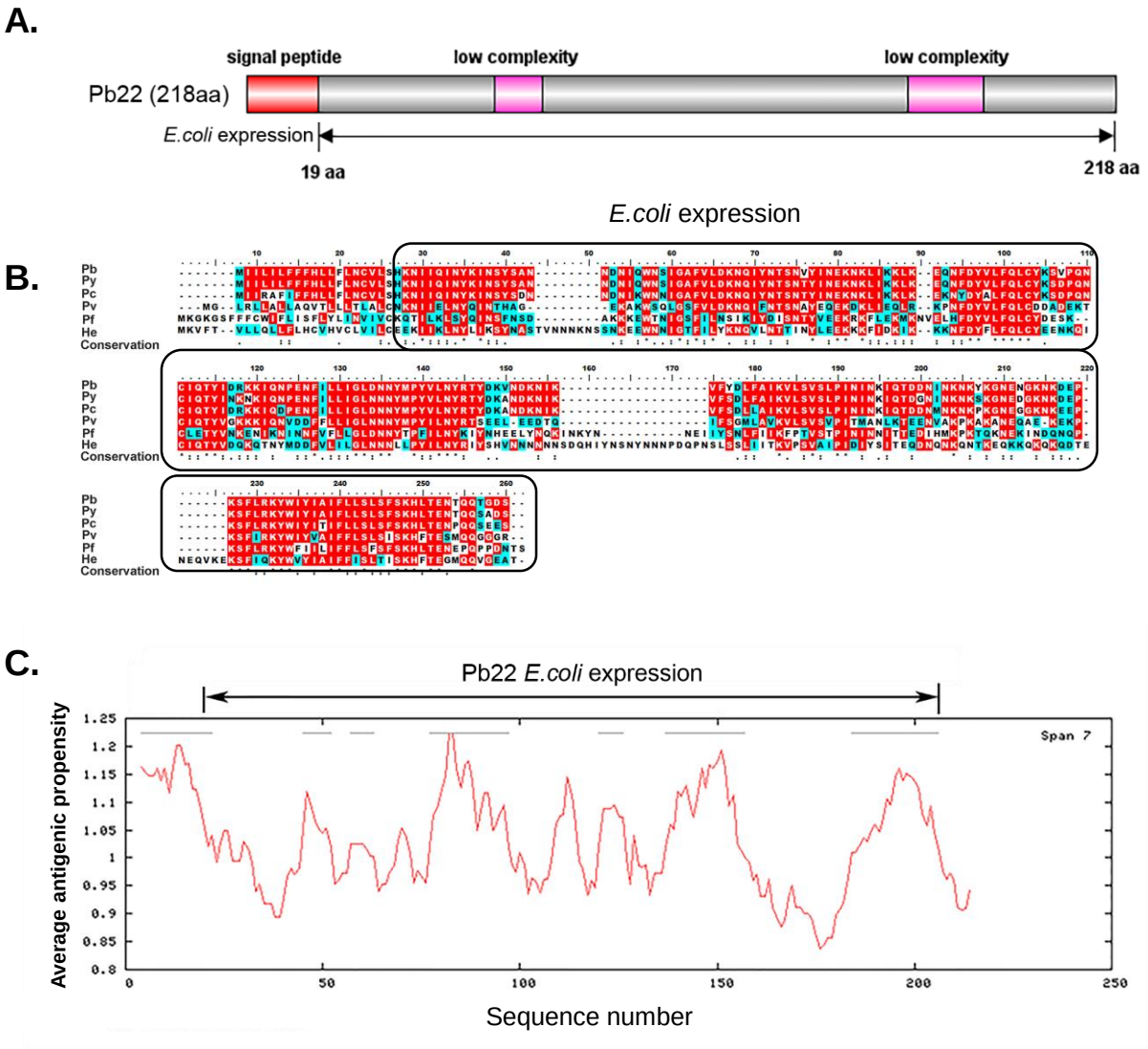
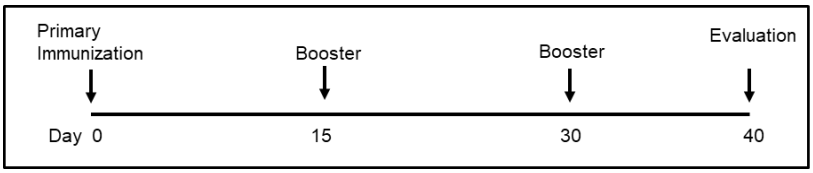


Fig. S1. Sequence analysis of P22. (A) Schematic diagram of Pb22 showing the locations of a signal peptide (red) and two low-complexity regions (pink). The *E. coli* expression shows amino acids 19–218. **(B**) Alignment of P22 proteins in *Plasmodium* species: *P. berghei* (Pb), *P. yoelii* (Py), *P. chabaudi* (Pc), *P. vivax* (Pv) and *P. falciparum* (Pf), and *Hepatocystis* sp. (He). Amino acids conserved across four species are marked: identical (red), similar (blue). The *E. coli* expression sequence is boxed. **(C)** Prediction of B-cell epitopes in Pb22. Diagram of Pb22 showing seven epitopes (grey line).

A.



B.

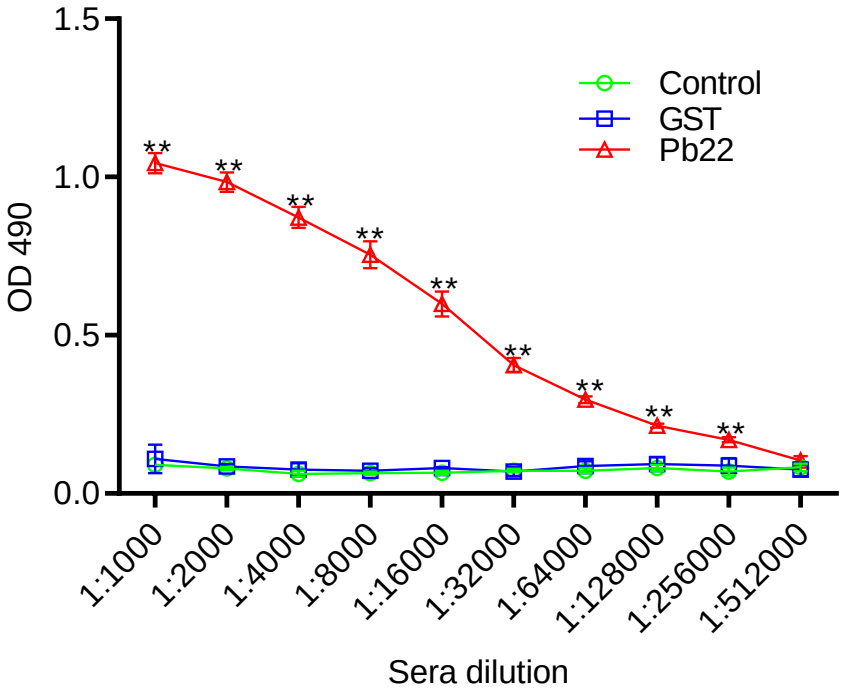


Fig. S2. Antibody response in mice immunized with rPb22. Female BALB/c mice were immunized with the rPb22 protein, a recombinant GST protein as a negative control, and adjuvants in PBS only as control. **(A)** Immunization scheme. **(B)** Total IgG titers after the final immunization were measured by ELISA with the plates coated with rPb22. Error bars indicate mean ± SD (n=5). ** $P < 0.01$ represents the difference between GST and rPb22 groups (Student's *t* test).

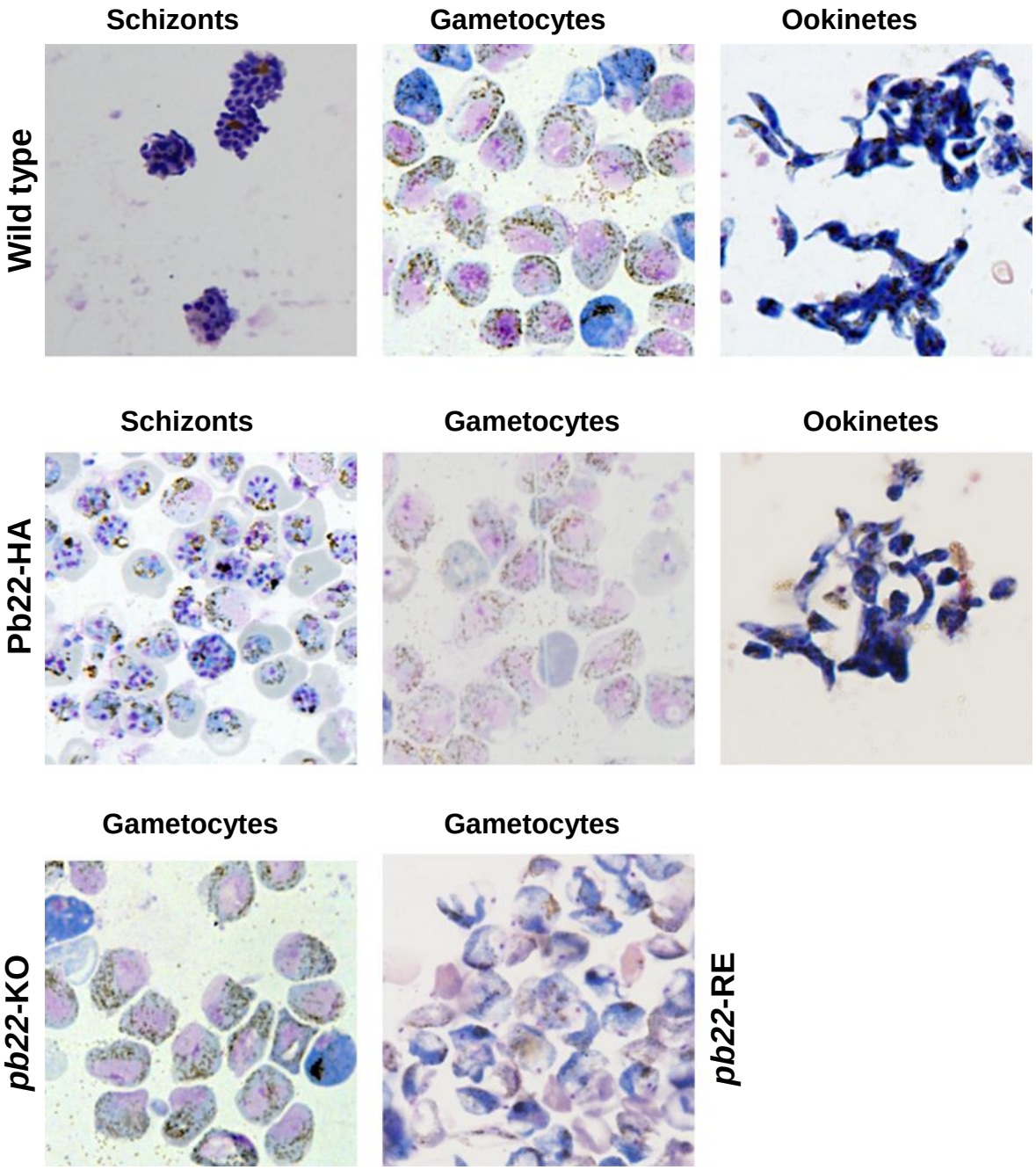


Fig. S3. Giemsa staining of purified parasites. Shown are schizonts, gametocytes, ookinetes of wild-type parasites (top panels), of HA-tagged parasites (middle panels), and gametocytes of *pb22*-KO line and *pb22*-RE line (bottom panels).

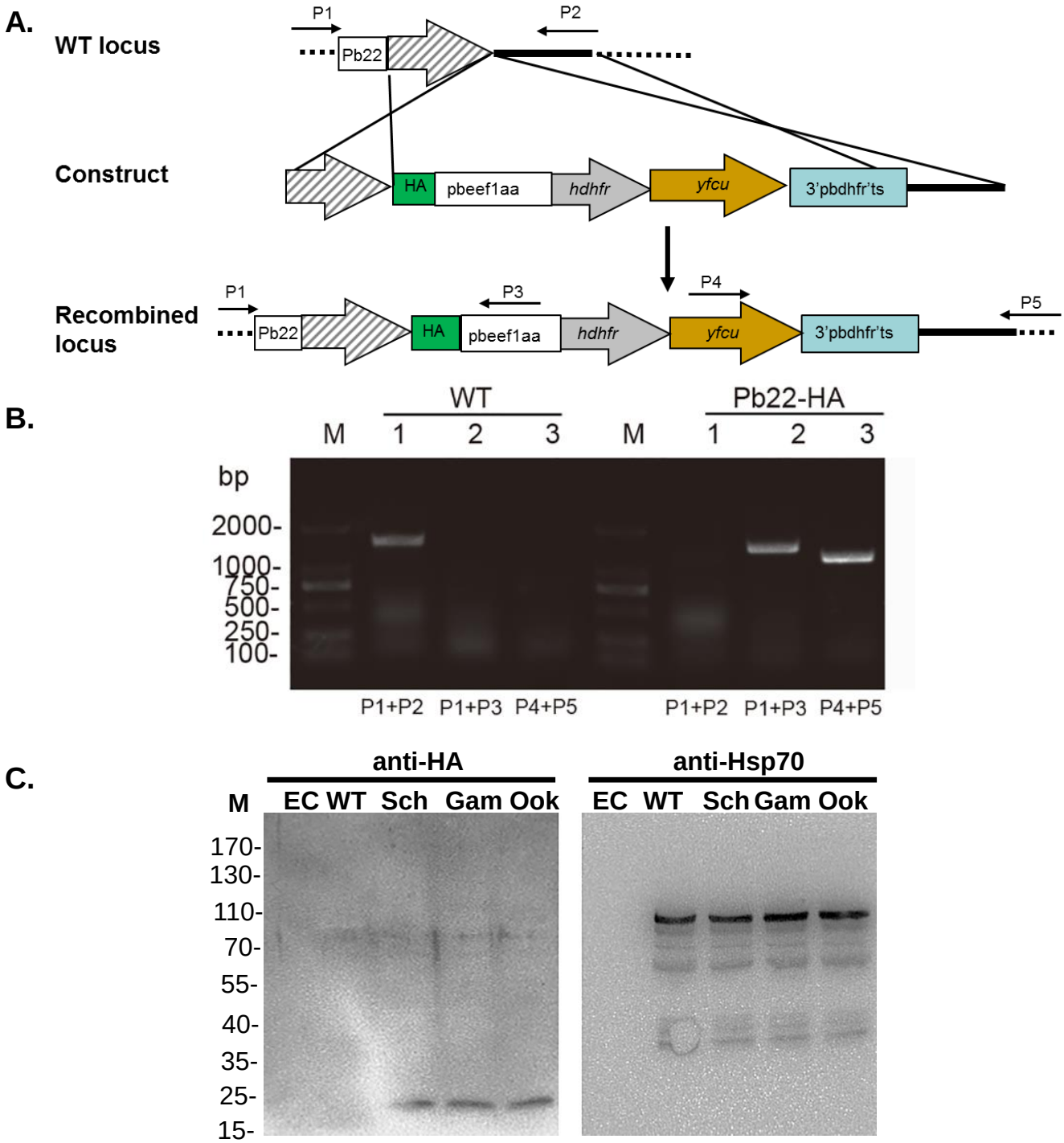


Fig. S4. Tagging of Pb22 with the HA tag. (A) Schematic of the WT *Pb22* locus, the transfection construct and the recombined *Pb22* locus. The h*dhfr* cassette directed by the *pbeef1aa* promoter was for selection of transgenic parasites with pyrimethamine. Primers 1-5 were used for genotyping PCR. **(B)** Diagnostic PCR of wild-type (WT) and Pb22-HA parasites. Lane 1, primers 1 + 2 (1548 bp); Lane 2: primer 1 + 3 (1497 bp); Lane 3: primer 4 + 5 (1229 bp). **(C)** Western blot analysis of proteins extracted from schizonts (Sch), gametocyte (Gam) and ookinete (Ook) of HA-tagged parasites and WT gametocytes (10 µg/lane) with anti-HA antibody and anti-rHsp70 sera (as loading control). Uninfected erythrocyte lysate (EC) was used as a negative control. M, molecular markers in kDa.

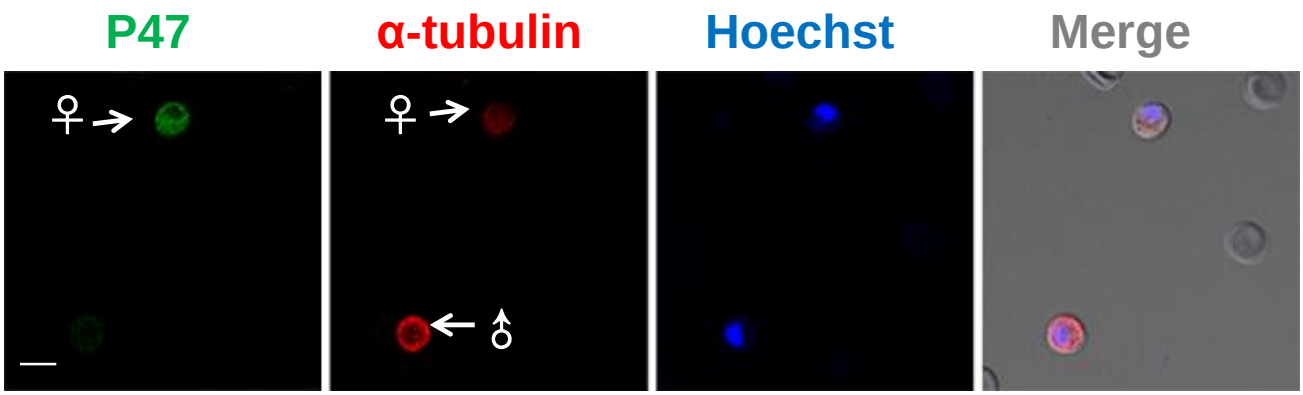


Fig. S5. Differentiation of male and female gametocytes with anti-P47 and anti-α-tubulin II antibodies. Representative images show staining of WT gametocytes. Parasites were permeabilized with Triton X-100 and incubated with mouse anti-P47 antibody (green) and rabbit anti-α-tubulin antibody (red). Nucleus was stained with Hoechst 33258 (blue). Merge includes all staining with the DIC image. Note that anti-α-tubulin antibodies stained both male and female gametocytes, but the fluorescent signal was much stronger in male gametocytes. Scale bar = 20 μm.

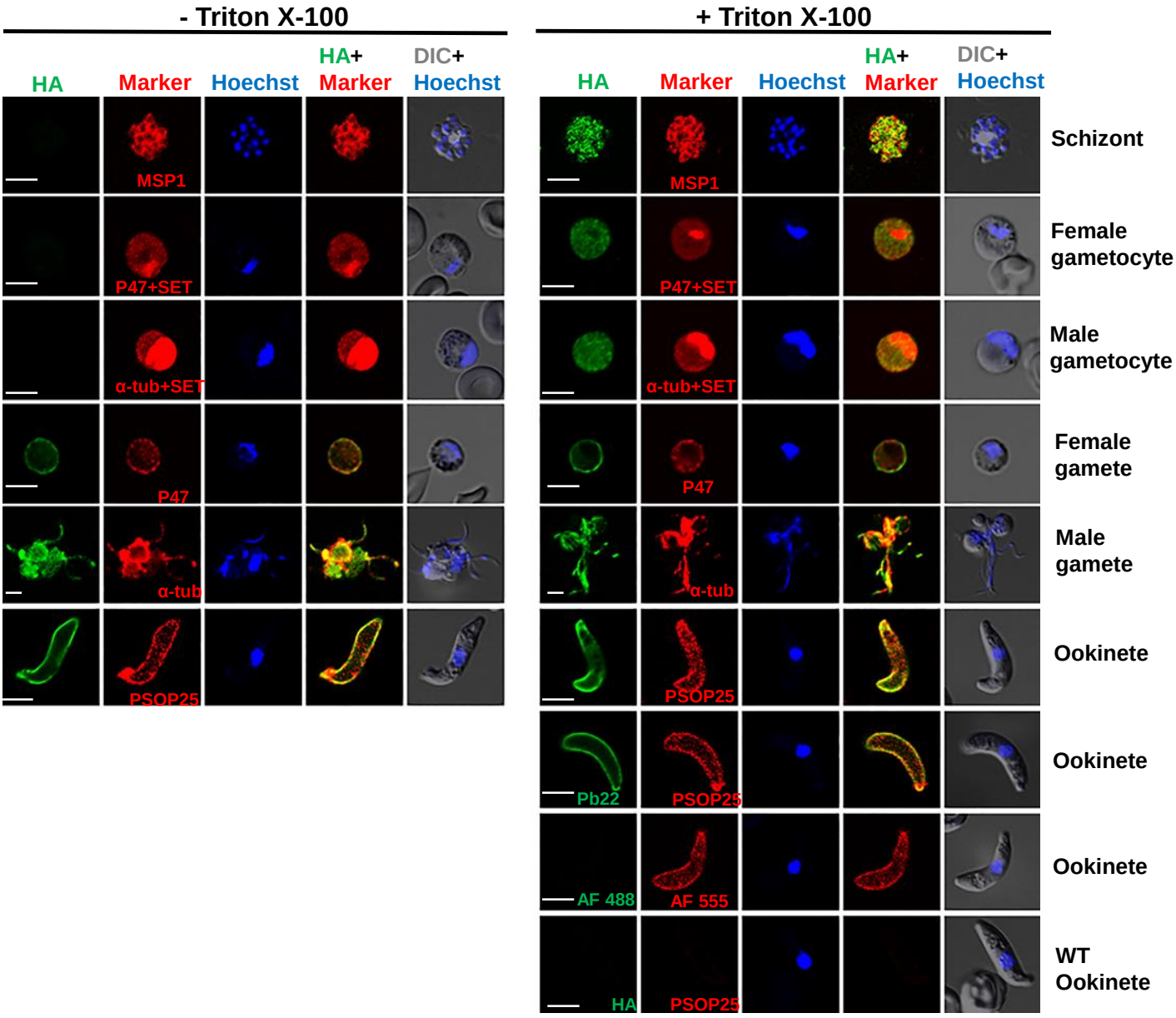


Fig. S6. Localization of HA-tagged Pb22 by IFA. Representative images show staining of Pb22-HA parasites at different developmental stages. Parasites were incubated with anti-HA monoclonal antibody (green) without (left) or with (right) permeabilization. After three washes, the left panel parasites were permeabilized with Triton X-100. All the parasites were labeled with antibodies against the marker proteins (red) for different stages (PbMSP1 for schizonts, Pbs21 for female gametocytes and macrogametes, α-tubulin for male gametocytes and microgametes, SET for nuclei of female and male gametocytes, and PSOP25 for ookinetes). Nucleus was stained with Hoechst 33258 (blue). DIC, differential interference contrast microscopy. HA-tagged ookinetes (+TX) labeled with anti-rPb22 sera were used as a positive control (right panel, third row from bottom). Two negative controls are HA-tagged ookinetes (+TX) labeled with only the secondary antibodies (AF488 – Alexa Fluor 488, and AF555 – Alexa Fluor 555) and WT ookinetes labeled with anti-HA monoclonal antibody (right panel, two bottom rows). Scale bar = 5 μm.

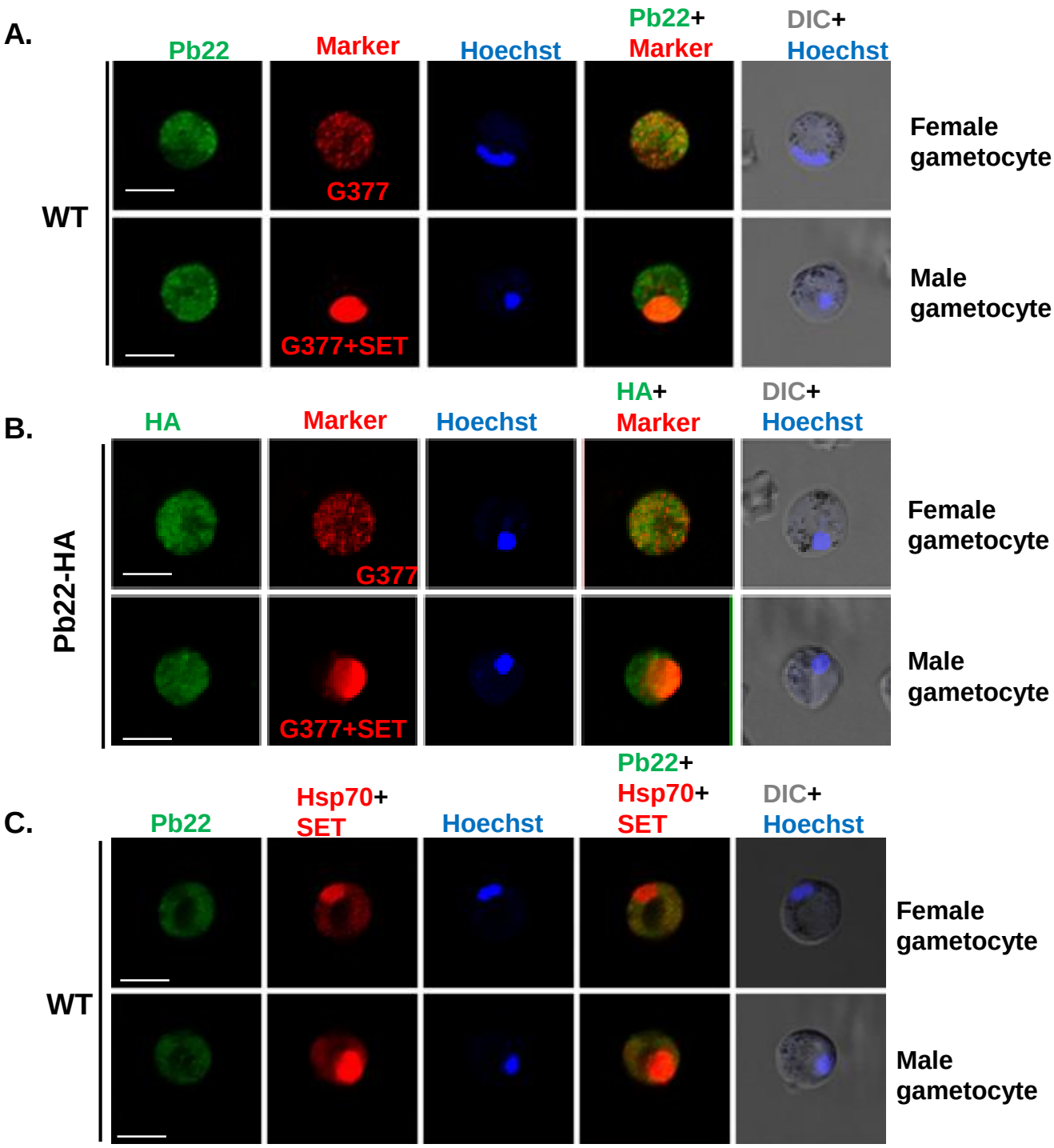


Fig. S7. Pb22 localization in gametocytes. (A) WT gametocytes were incubated with anti-Pb22 sera (green). Females were stained with anti-PbG377 (red), while males were incubated with anti-Pb22 sera (green) and anti-PbG377 and anti-SET antibodies (G377+SET) (red). **(B)** Same staining procedure was performed for Pb22-HA parasite line with Pb22 detected by HA staining (green). **(C)** WT gametocytes were incubated with anti-Pb22 sera (green) and anti-Hsp70 and anti-SET sera (red). All parasites were permeabilized with Triton X-100. Antibodies for G377, SET, and Hsp70 are for staining female gametocytes, nuclei, and cytoplasm. Nucleus was counter-stained with Hoechst 33258 (blue). DIC, differential interference contrast microscopy. Scale bar = 5 μm.

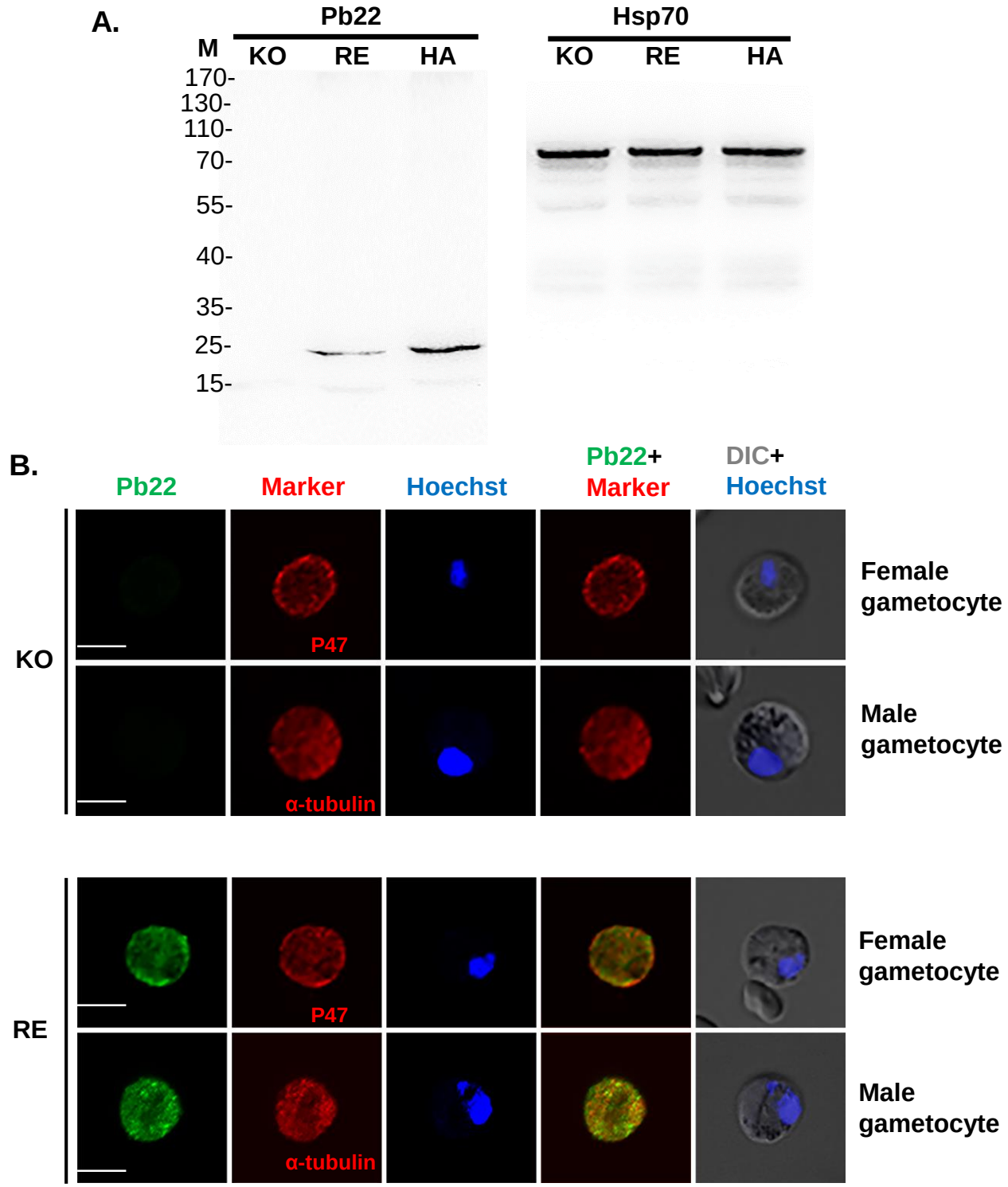


Fig. S8. Detection of Pb22 expression in *pb22*-KO and *pb22*-RE gametocytes. (A) Western blot. Lysates extracted from *pb22*-KO, -RE and -HA gametocytes were incubated with anti-rPb22 and Hsp70 antisera. M, molecular markers in kDa. **(B) IFA.** Gametocytes were incubated with anti-rPb22 sera (green) as primary antibody after permeabilization with Triton X-100. The parasites were also labeled with antibodies against the marker proteins (red) for females (P47) or males (α-tubulin). Nucleus was stained with Hoechst 33258 (blue). DIC, differential interference contrast. Scale bar = 5 μm.

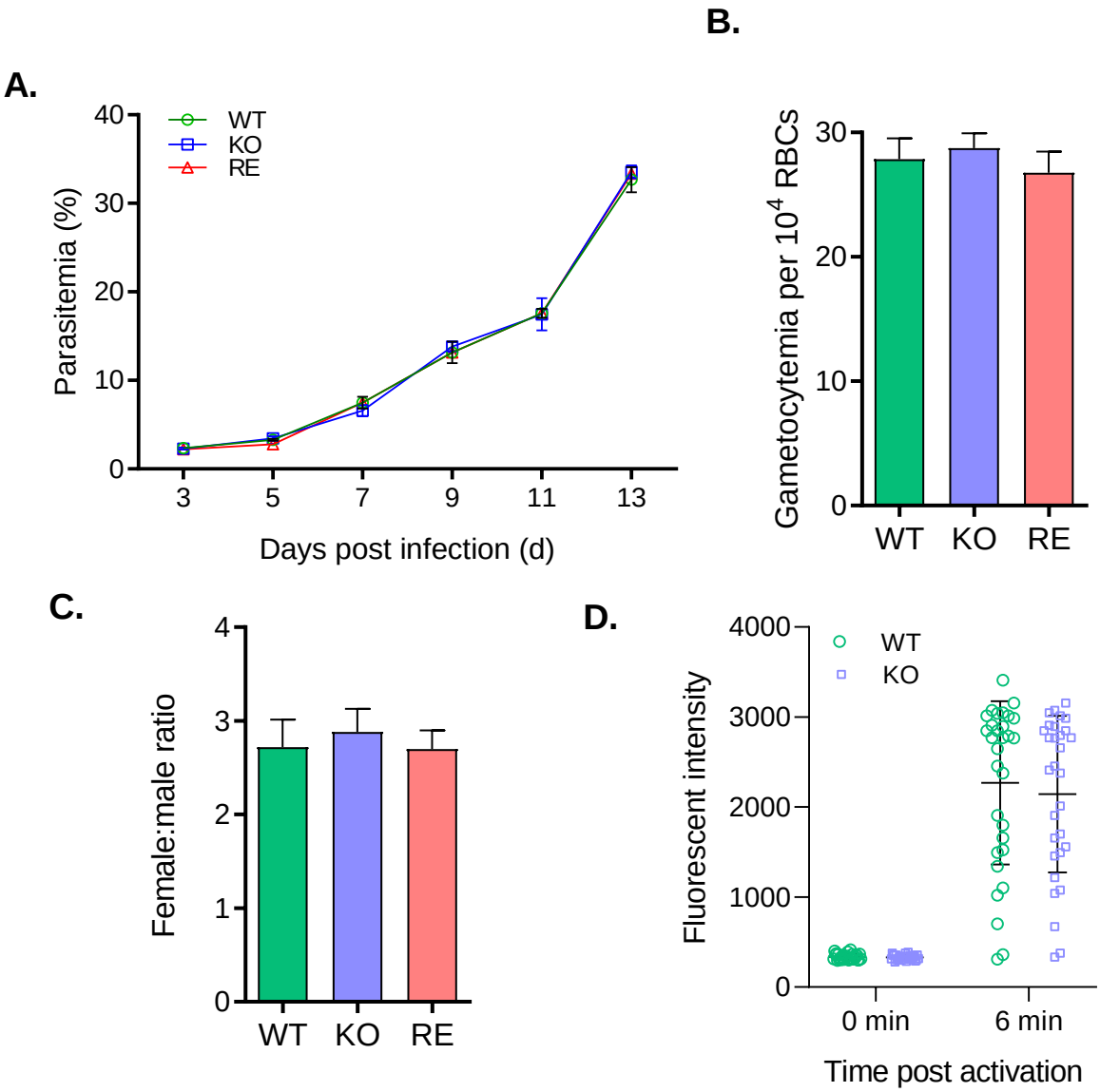


Fig. S9. Parasitemia, gametocytemia, gametocyte sex ratio and DNA content of male gametes in WT, KO, and RE parasites. BALB/c mice were infected by intraperitoneal injection with 1×10^6 parasite-infected red blood cells**.** Error bars indicate mean ± SD (n = 3). **(A)** Parasitemia, **(B)** gametocytemia (gametocytes per 10^4 RBCs) and **(C)** gametocyte sex ratio (mature female gametocyte/mature male gametocyte). Parasitemia, gametocytemia and sex ratios were not significantly different among the three groups ($P > 0.05$). **(D)** DNA contents of male gametocytes between WT and KO groups were quantified by using the Nikon software.

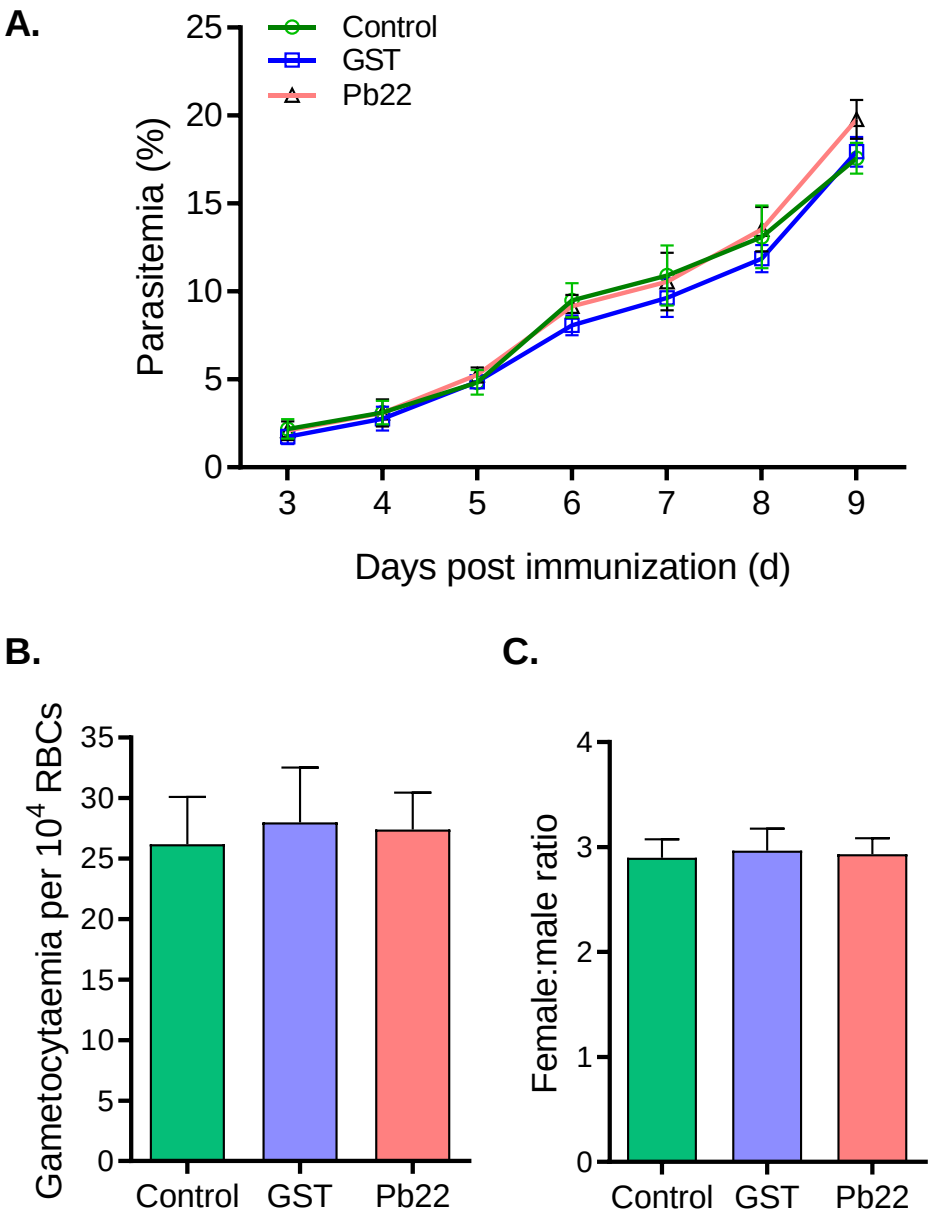


Fig. S10. Parasitemia (A), gametocytemia (B) and gametocyte sex ratio (C) in mice immunized with rPb22, GST and adjuvant control. Mice were infected with WT *P. berghei* after the third immunization. Parasitemia, gametocytemia (gametocyte per 10^4 RBCs) and gametocyte sex ratio (mature female gametocyte/mature male gametocyte) on day 3 were not significantly different among the three groups ($P > 0.05$). Error bars indicate mean ± SD (n = 3).

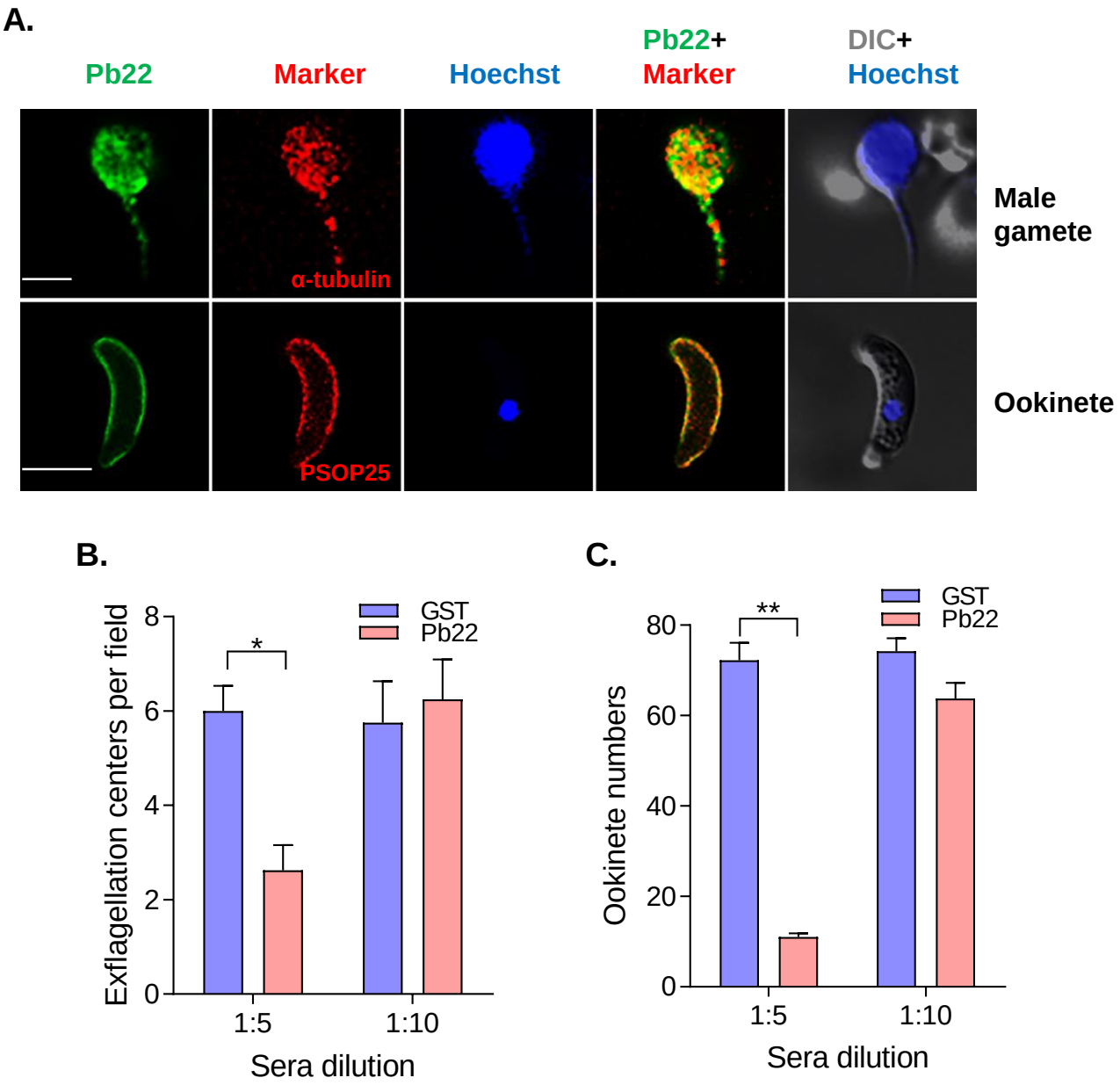


Fig. S11. *In vitro* transmission-blocking activity (TBA) of anti-rPb22 antibodies (A) Antisera after immunization with anti-rPb22 recognized male gametes and ookinetes. Parasites were incubated with anti-rPb22 antisera (green) without permeabilization. Then parasites were permeabilized and labeled with α-tubulin and PSOP25 which were used as male gamete and ookinete markers, respectively. Scale bar = 5 μm. **(B–C) *In vitro* TBA of rPb22 antisera and control anti-GST sera at two dilutions (1:5 and 1:10).** WT *P. berghei* infected blood collected at 3 days post infection was incubated with the respective sera. **(B)** Exflagellation centers per field were counted at 15 min. **(C)** Ookinete numbers in 0.01 μl of blood were counted 24 h later**.** Error bars indicate mean ± SD. *, $P < 0.05$. **, $P < 0.01$. Data are representative of three independent experiments.