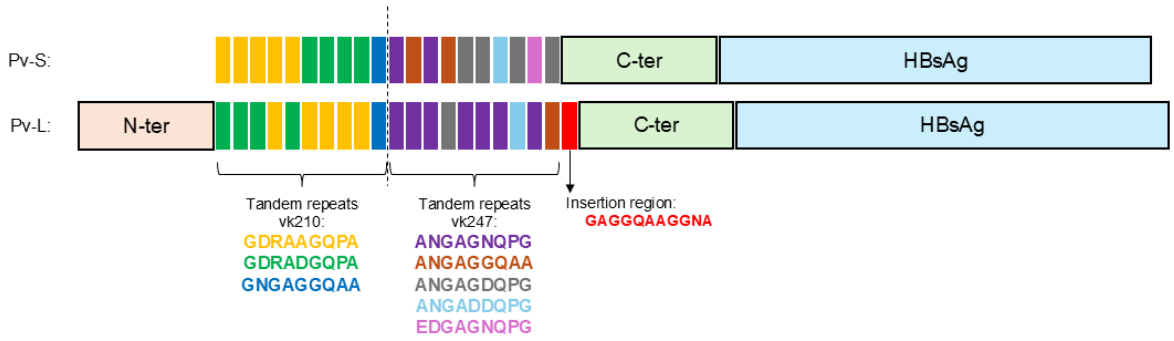
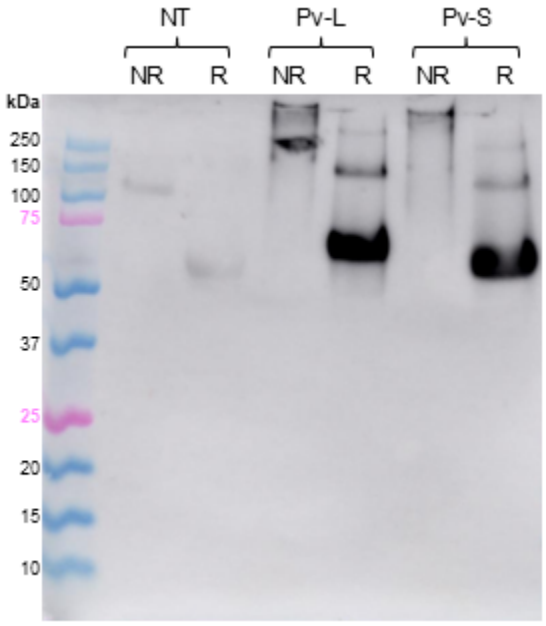
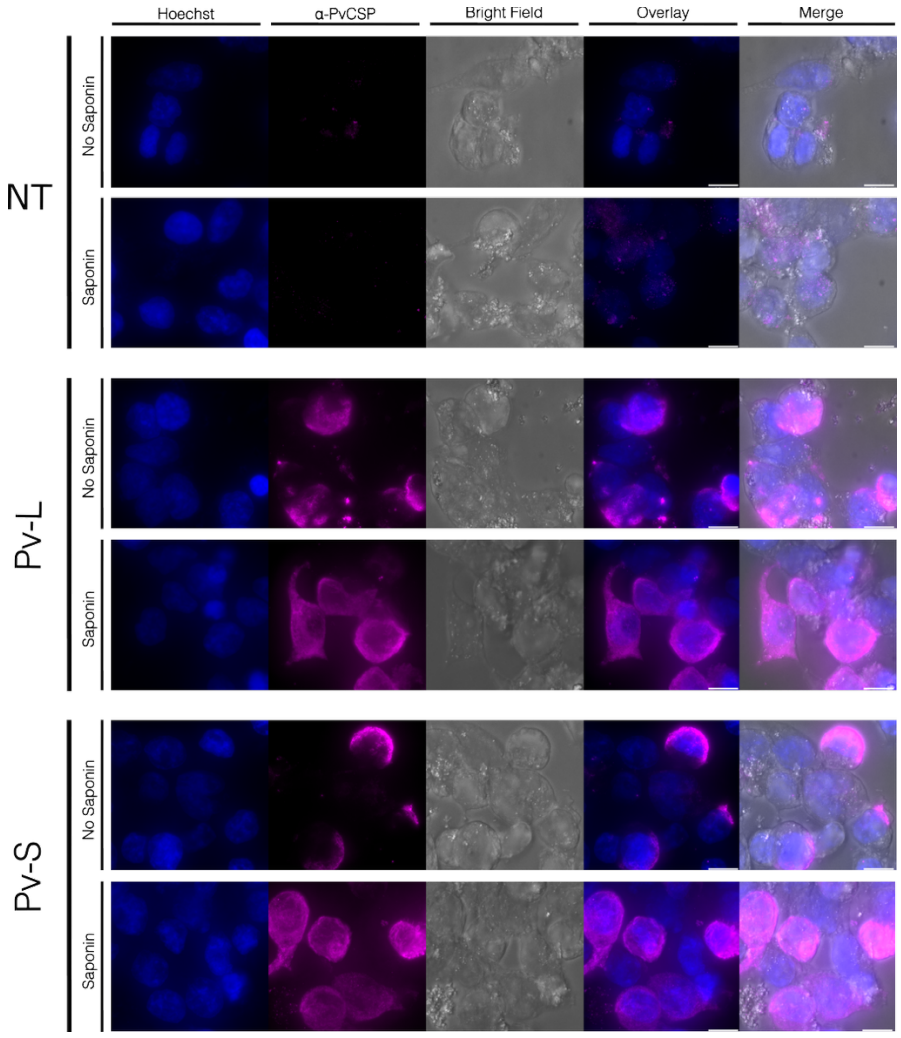


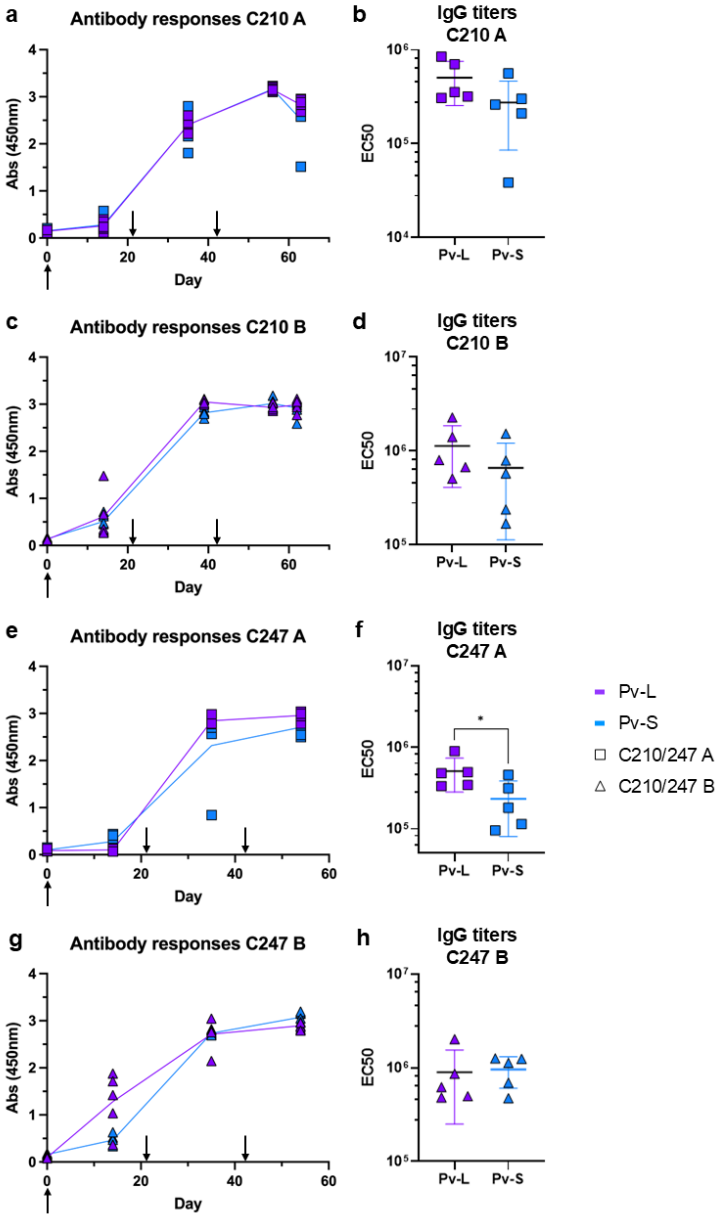
Supplementary Fig. 1. mRNA constructs Pv-S and Pv-L. The Pv-S construct consists in PvCSP central tandem repeats from the vk210 and vk247 alleles, followed by the C-terminal (C-ter) domain of PvCSP, and the hepatitis B surface antigen (HBsAg) sequence. The Pv-L construct includes the N-terminal (N-ter) region of PvCSP, central tandem repeats from the PvCSP allele vk210 and vk247 allles, an insertion region and the C-ter domain of PvCSP fused to the N-terminus of HBsAg.



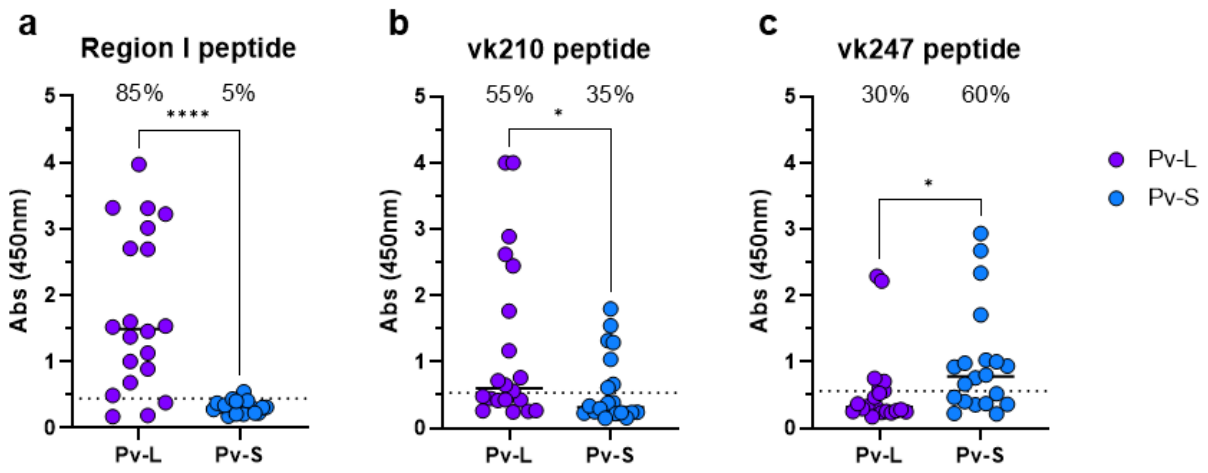
Supplementary Fig. 2. Secretion of Pv-S and Pv-L recombinant proteins after mRNA transfection of HEK cells. Culture supernatants from cells transfected with mRNA constructs Pv-S or Pv-L and non-transfected (NT) cells were analyzed by western blotting under non-reducing (NR) and reducing (R) conditions. Anti-PvCSP mouse sera were used for detection of the proteins.



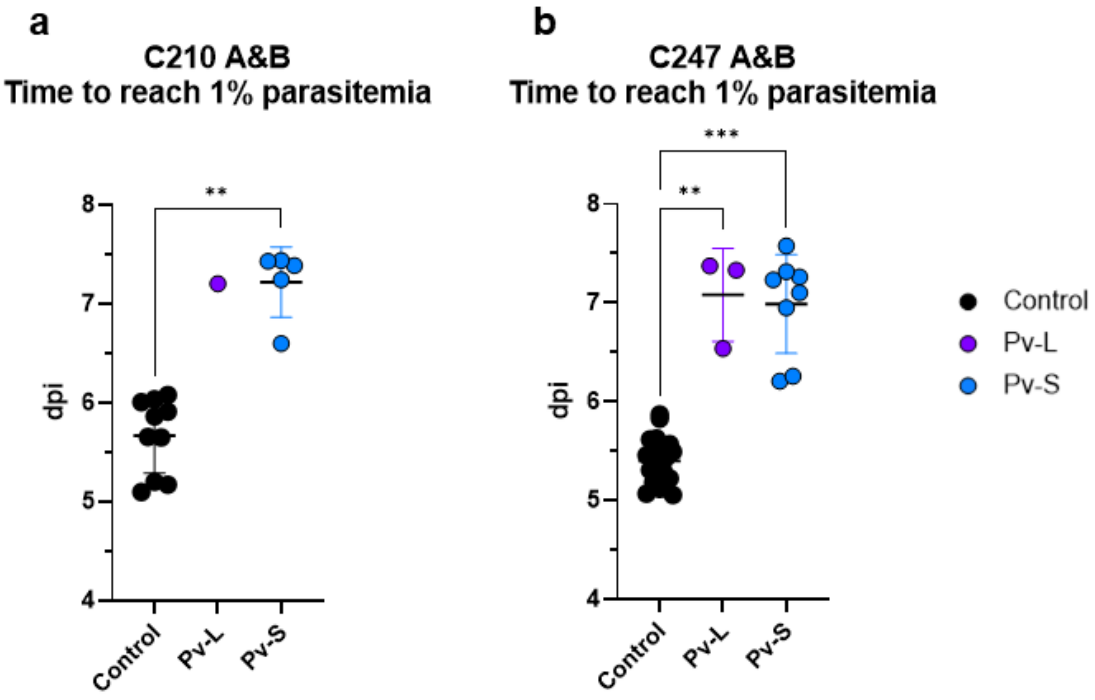
Supplementary Fig. 3. Surface expression of PvCSPCh$_S$-HBsAg and PvCSPCh$_L$-HBsAg detected by immunofluorescence assay (IFA) after transfection of HEK cells with mRNA constructs Pv-S and Pv-L. Non-transfected (NT) cells and cells transfected with mRNA constructs Pv-S or Pv-L were analyzed by immunofluorescence with or without saponin using anti-PvCSP mouse sera. Scale bars represent 10 µm.



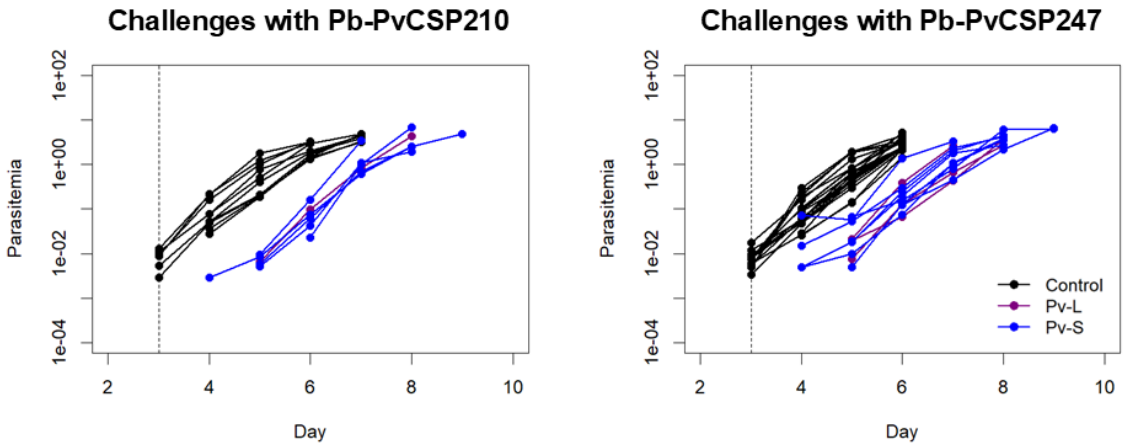
Supplementary Fig. 4. ELISA recognition of recombinant PvCSPCh$_L$ protein in individual challenge experiments. a-c-e-g) Antibody responses were evaluated over the course of the immunizations in all challenges. Individual mice and averages connected by straight lines are reported for each vaccine construct group. Black arrows show the time point of the immunization. **b-d-f-h)** Sera from immunized mice at time point C-2 was used to calculate IgG titers shown as effective serum concentration (EC50) for each vaccine group. Means and standard deviations are shown. Statistical analysis was performed using the Mann-Whitney test (*$p < 0.05$).



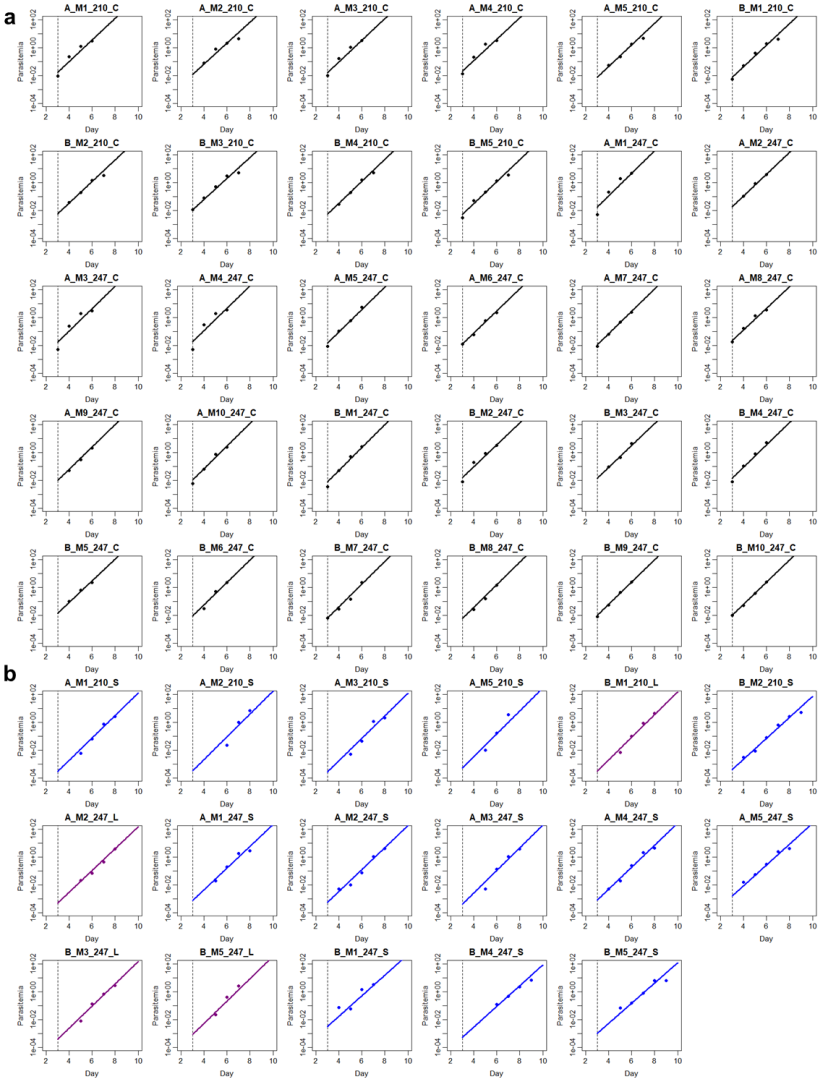
Supplementary Fig. 5. Recognition of peptides by immunogenic mouse sera. Sera from immunized mice at time point C-2 was used for recognition of region I peptide **(a)**, tandem repeat peptide vk210 **(b)**, and tandem repeat peptide vk247 **(c)**. Mice from all challenges were tested and considered above the cut-off (dotted line) when signal was superior to the signal of pooled control sera plus three times the standard deviation. Percentages of mice immunized with mRNA constructs Pv-S and Pv-L having signals above the cut-off are reported. Means are shown as horizontal lines. Statistical analysis was performed using the Mann-Whitney test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).



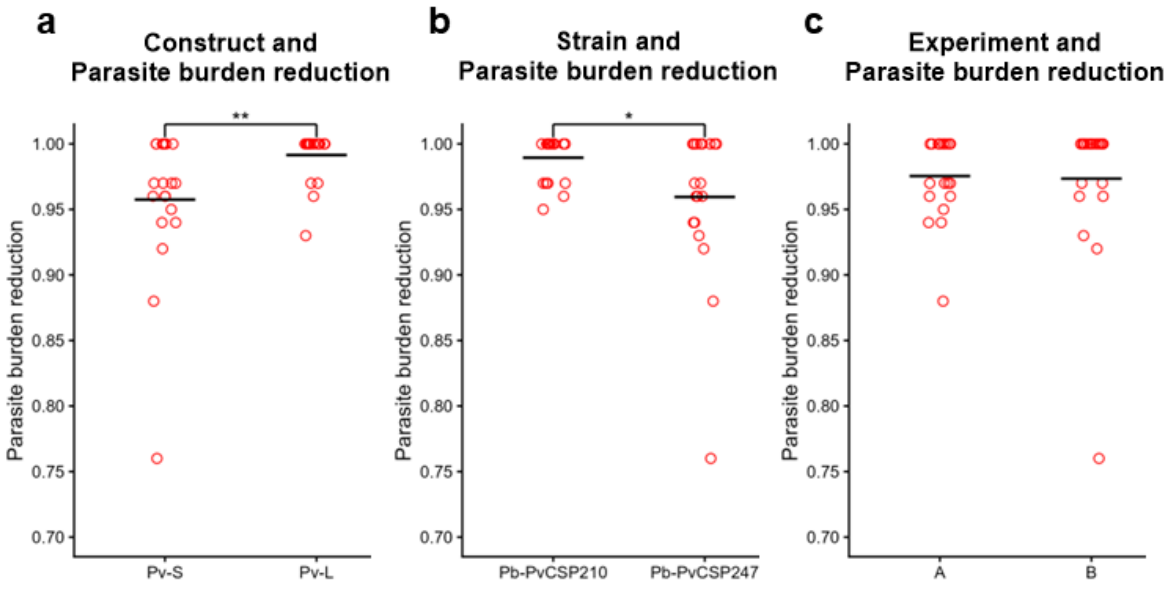
Supplementary Fig. 6. Delays on time to reach 1% parasitemia in C57BL/6 mice immunized with mRNA constructs Pv-S and Pv-L. The log10-transformed parasitemia from infected mice was fitted with a line and used to find the day post-infection (dpi) at which 1% parasitemia was reached. Mice immunized with mRNA constructs Pv-S or Pv-L in challenges C210 A and C210 B **(a)** or C247 A and C247 B **(b)** were compared with unvaccinated controls in their respective challenges. Means and standard deviations are shown. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's correction for multiple comparisons (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).



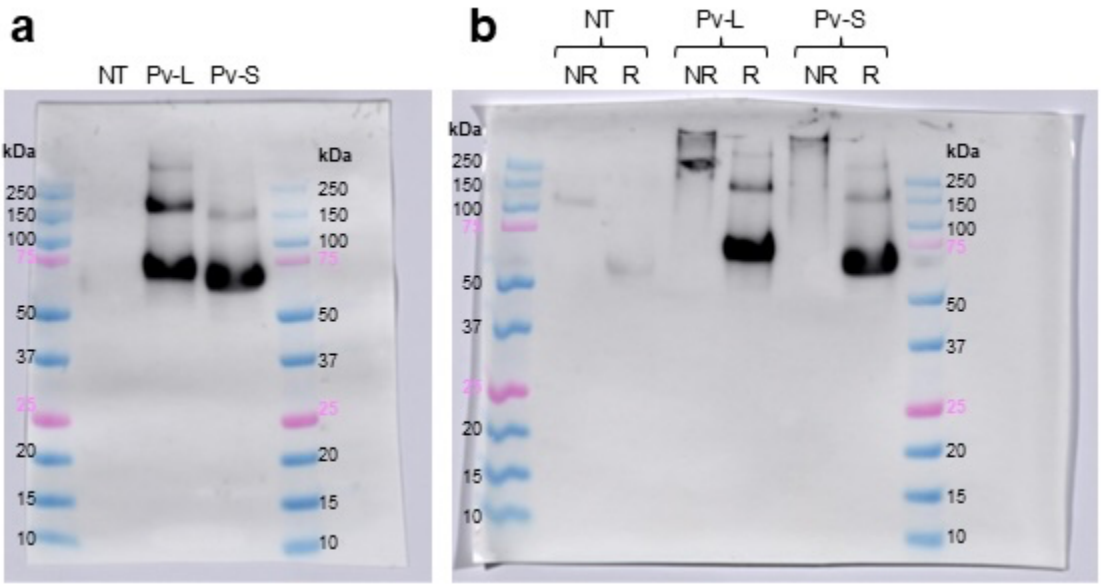
Supplementary Fig. 7. Parasitemia progression in infected mice. Log10-transformed parasitemia values from all infected mice, including controls and mice immunized with Pv-S and Pv-L constructs, are shown over time for challenges with Pb-PvCSP210 and Pb-PvCSP247 strains. Individual data points are connected by lines for each mouse. Dashed vertical line indicates day 3 post challenge.



Supplementary Fig. 8. Parasitemia trajectories fitted with the linear mixed-effects model for each infected mouse for both Pb-PvCSP strains. The log10-transformed parasitemia was fitted using the linear mixed-effects model: log(parasitemia) ~ experiment + strain + group + day + (1 + day | mouse). Plots show parasitemia points over the course of the challenges, the linear mixed-effects model as a straight line, and a dashed vertical line indicating day 3 post-infection. Titles in each plot are titled after the mouse number (M1 to M10), experiment (A or B), Pb-PvCSP strain (210 or 247), and construct (Control, C; Pv-S, S; or Pv-L, L). Plots from unvaccinated controls **(a)** and vaccinated infected mice **(b)** are shown.



Supplementary Fig. 9. Parasite burden reduction comparisons across mouse strains, vaccine constructs, and experiments. Parasite burden reduction was calculated for each mouse in all challenges and compared among the mRNA vaccine constructs **(a)**, PvCSP-expressing transgenic *P. berghei* sporozoites **(b)**, and experiments **(c)**. Means are shown in horizontal bars. Statistical analysis was performed using the Mann-Whitney test (*$p < 0.05$; **$p < 0.01$).



Supplementary Fig. 10. Uncropped and unprocessed western blots showing secretion of Pv-S and Pv-L recombinant proteins following transfection of HEK293 cells with mRNA constructs. a) Uncropped and unprocessed western blots corresponding to Fig. 1a, showing secretion of Pv-S or Pv-L proteins in culture supernatants of HEK293 cells transfected with corresponding mRNA constructs as detected with anti-PvCSP mouse sera. Culture supernatants of non-tranfected HEK293 cells (NT) were also probed by western blotting with anti-PvCSP mouse sera as control. **b)** Uncropped and unprocessed blot corresponding to Supplementary Fig. 2, including NT, Pv-S and Pv-L culture supernatants under non-reducing (NR) and reducing (R) conditions. Anti-PvCSP mouse sera were used for detection of secreted Pv-S and Pv-L under NR and R conditions.

Supplementary Table 1. Proportions immunized mice protected or non-protected across mouse strains, vaccine constructs, and experiments.

	Number of protected mice	Number of non-protected mice
Construct (**)		
Pv-S	7	13
Pv-L	16	4
Strain (ns)		
Pb-PvCSP210	14	6
Pb-PvCSP247	9	11
Experiment (ns)		
A	10	10
B	13	7

Statistical analysis was performed using the Fisher's exact test adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) method (**$p < 0.01$).