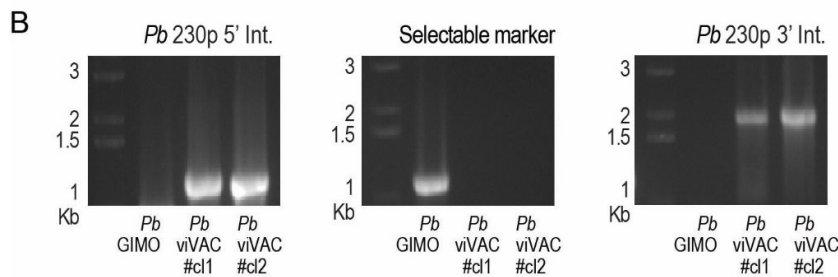
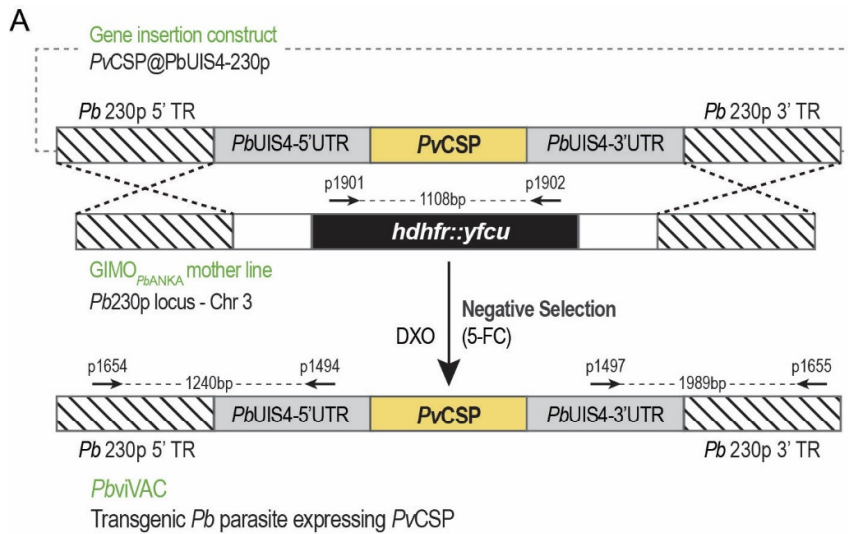
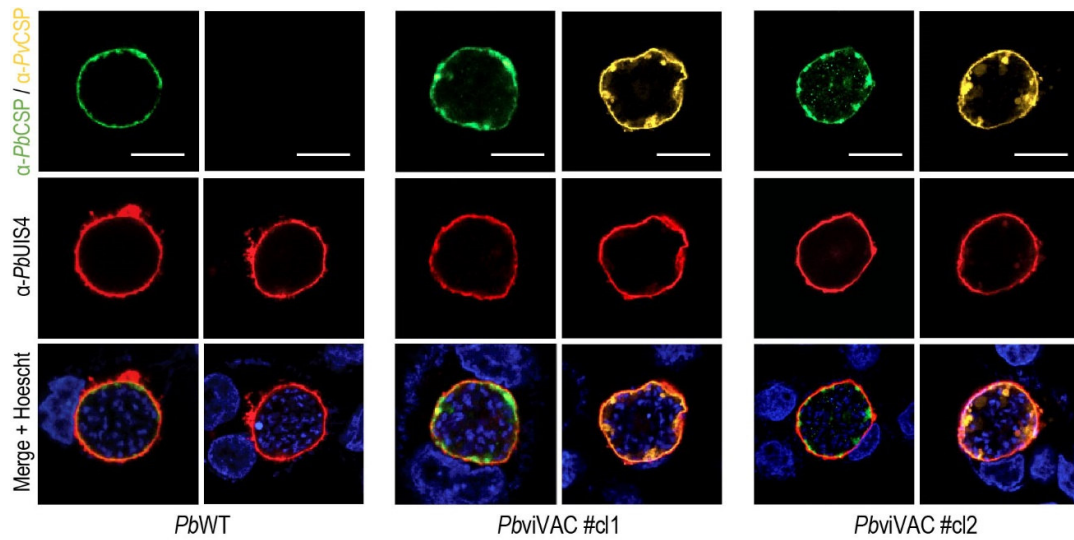


Supplementary Figure 1 - ClustalW alignment of the *PvCSP* gene sequence used to generate *PbviVac* with sequences from reference *Pv* genomes.



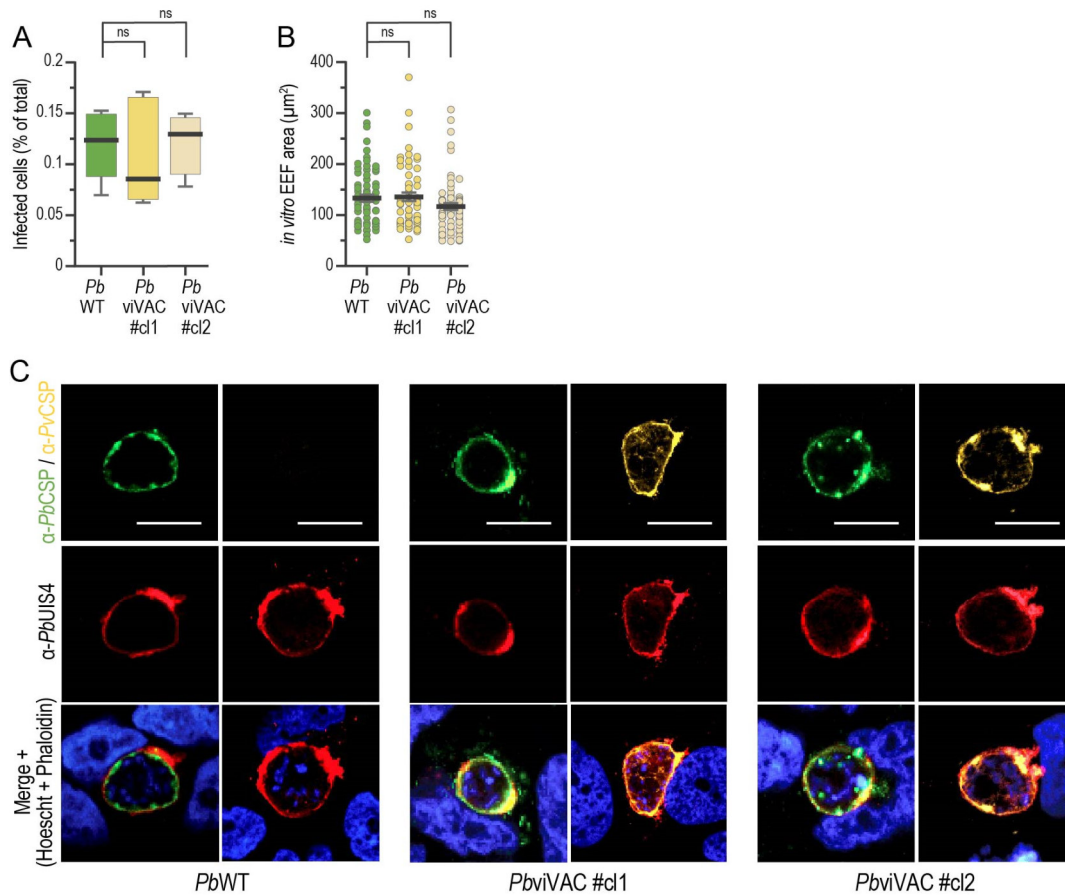
Supplementary Figure 2 - Generation and genotyping of the transgenic *PbvVac* parasite line.

(A) Schematic representation of the strategy used to generate the transgenic *PbvVac* line. The selectable marker (SM; *hdhfr::yfcu*) in the GIMO_{PbANKA} mother line was replaced by the PvCSP coding sequence placed under the control of the *PbUIS4* gene promoter (5'-UTR) and *Pbuis4* transcriptional terminator sequence (3'-UTR) by double cross-over homologous recombination (DXO) using 5' and 3' targeting sequences (TR) for the neutral 230p locus in chromosome 3. Subsequent administration of 5-fluorocytosine (5-FC) allows negative selection of SM-free parasites. Black arrows indicate the location and identifier of primers used for diagnostic PCR, as well as the expected PCR product sizes; **(B)** Genotype analysis by diagnostic PCR analysis of cloned parasite *PbvVac* lines confirms correct integration of the PvCSP expression cassette in the neutral 230p locus, including correct integration at the 5' and 3' regions (5'int. and 3'int.), as well as the absence of selectable marker.



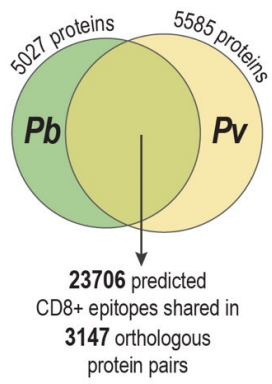
Supplementary Figure 3 – Expression of PvCSP by *PbviVac* parasites developing *in vitro* in HepG2 cells.

Representative immunofluorescence microscopy images of *PbWT* and *PbviVac* parasites developing in HepG2 hepatoma cells 48 hpi. Immunofluorescence staining with the anti-*PbCSP* (green) and anti-*PvCSP* VK210 (yellow), as well as with the anti-*PbUIS4* antibodies, confirms the expression of both proteins by *PbviVac* and their localization to the parasite membrane. Scale bar: 20 μ m.



Supplementary Figure 4 - *PbvVac* in vitro pre-erythrocytic development in Huh 7 human hepatoma cells and expression of *PvCSP*.

(A,B) Compared *in vitro* infectivity **(A)** and parasite development **(B)** of *PbWT* and *PbvVac* parasites in Huh7 human hepatoma cells; **(C)** Representative immunofluorescence microscopy images of *PbWT* and *PbvVac* parasites developing in Huh7 cells at 48 hpi. Immunofluorescence staining with the anti-*PbCSP* (green) and anti-*PvCSP* VK210 (yellow), as well as anti-*PbUIS4* antibodies confirms the expression of both proteins by *PbvVac* and their localization to the parasite membrane. Scale bar: 20 µm. The boxes correspond to the 25th and 75th percentiles in **(A)** and the black and grey lines correspond to mean and standard error of the mean, respectively (ns: not significant, Mann-Whitney *U* test).



Supplementary Figure 5 - *In silico* analyses of *Pb* and *Pv* predicted CD8⁺ T cell epitopes.

Distribution of orthologous protein pairs and strong binding epitopes shared between the *Pb* and *Pv* proteomes.