# Supplementary Information

**Disruption of plasmepsin-4 and merozoites surface protein-7 genes in *Plasmodium berghei* induces combined virulence-attenuated phenotype**

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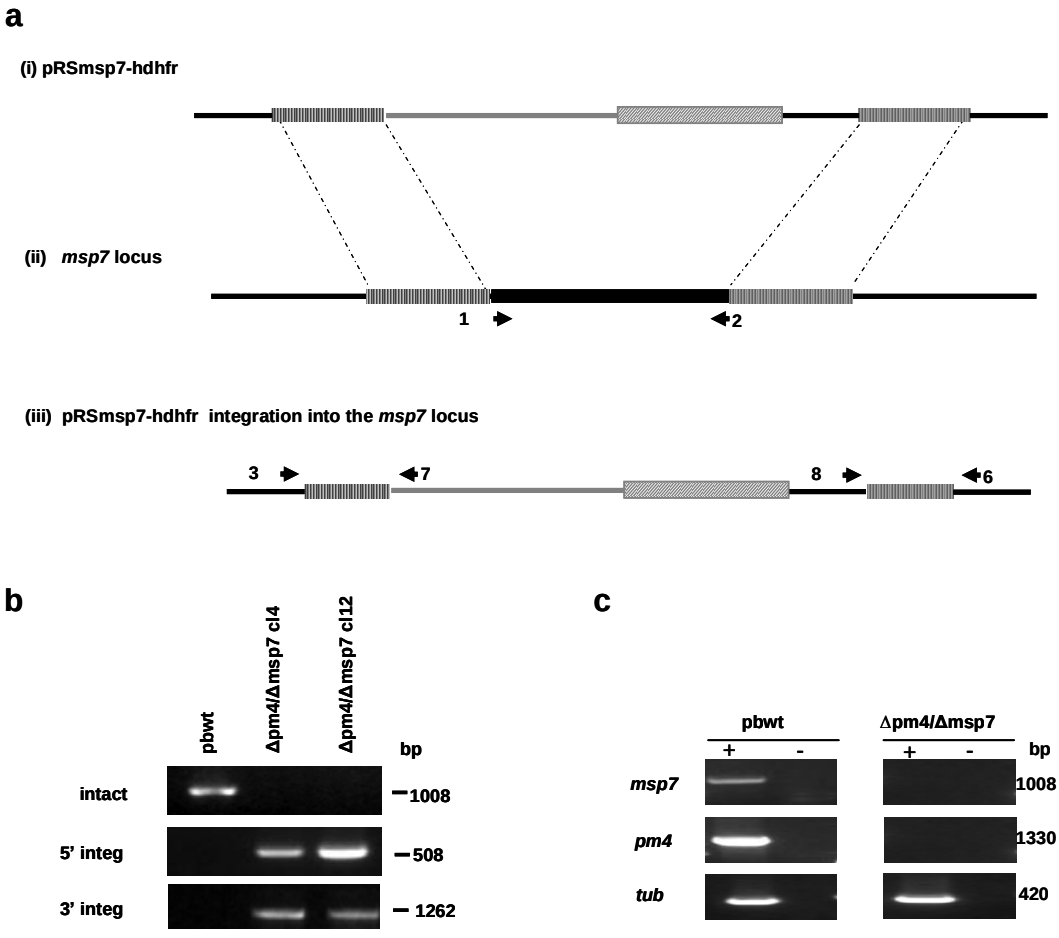
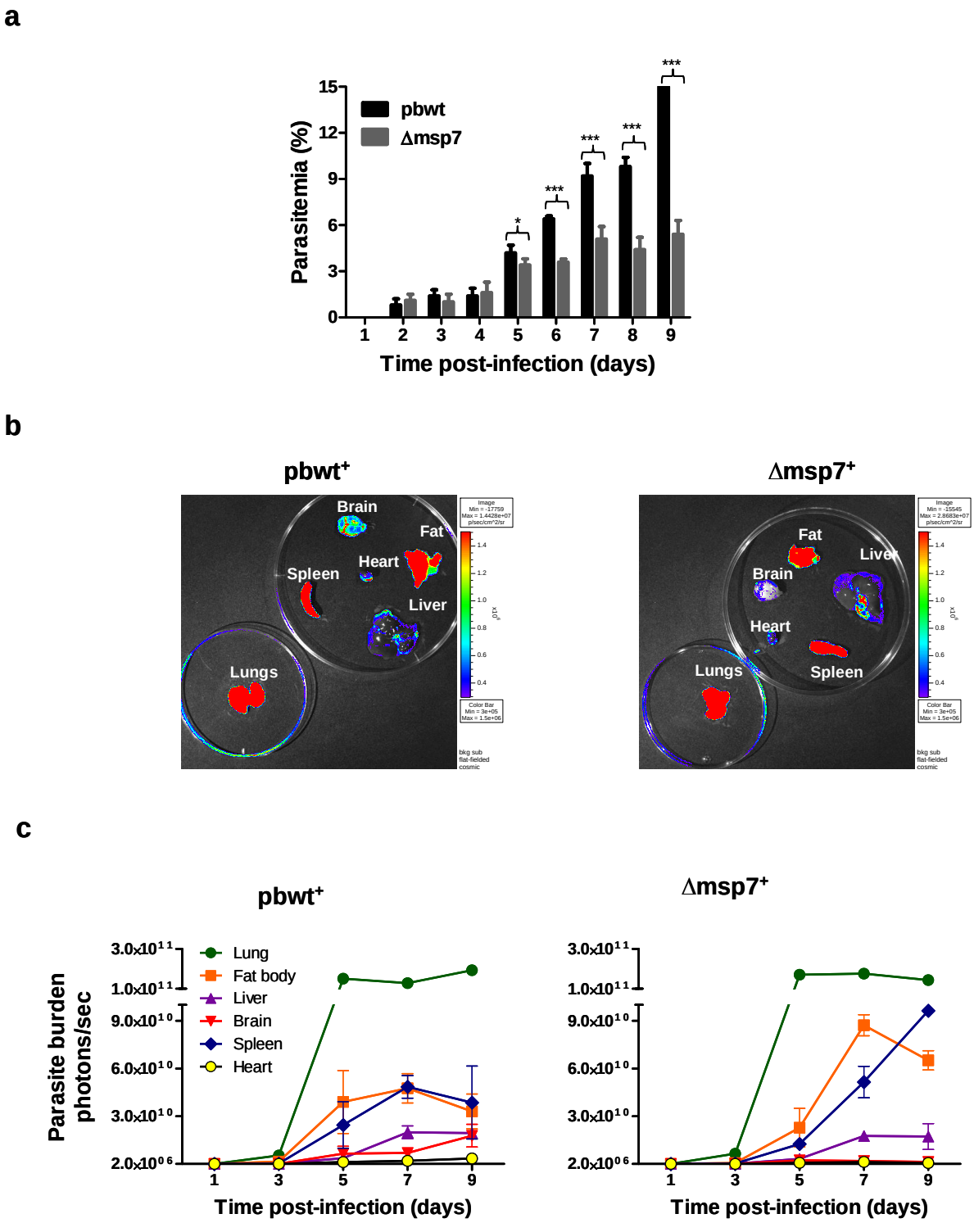
## Supplementary figures

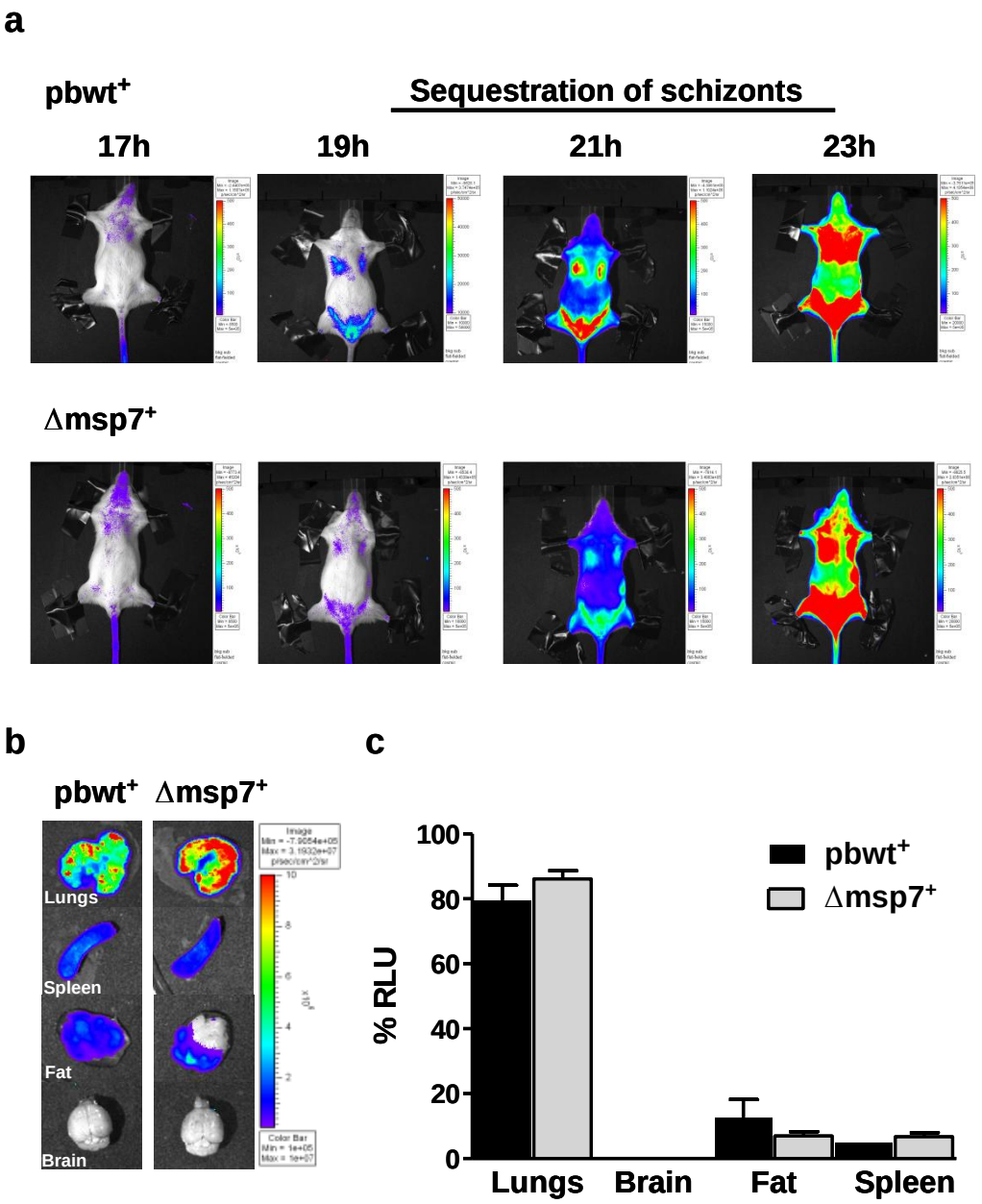



**Supplemental Figure 1. Targeted disruption of the *msp7* locus in the Δpm4 cl6 parasite clone.** (A) Schematic representation of the replacement vector pRSmsp7-hdhfr/ts (i) and the target *msp7* locus (ii). The wild-type *msp7* locus is targeted with a linear replacement fragment containing the 5' and 3' UTRs (striped bars) of the *msp7* coding sequence (solid black bars) and the positive selectable marker *hdhfr* (gray striped bars). Integration of the construct by double crossover recombination results in the replacement of the *msp7* gene with the *hdhfr* drug selectable marker (iii). The position and orientation of primers used for sequence amplification and diagnostic analysis by PCR are indicated with arrow heads (B). Diagnostic PCR experiments demonstrated the correct integration of the construct. The primer pairs 1+2 amplified a fragment of 1008 bp showing the presence of the intact *msp7* locus in pbwt parasites, whereas no product was obtained from Δpm4/Δmsp7 parasites clones. The primer pairs 3+7 and 8+6 amplified two fragments of 508 bp and 1262 bp, respectively, only in the genomic DNA from Δpm4/Δmsp7 parasites demonstrating the correct integration of the construct. (C) RT-PCR analysis failed to amplify both *msp7* and *pm4* genes transcripts in RNA isolated from Δpm4/Δmsp7 blood stage parasites. *P. berghei tubulin* primers were used as a positive control to amplify the *tub* transcript.




**Supplemental Figure 2. Time course of Δmsp7 infections and parasites burden**. (A) The graph shows the level of parasitemia in C57BL/6 mice injected with the same parasite dose (i.p. $10^5$) of either pbwt (n=10), or Δmsp7 (n=10). Mice infected with Δmsp7 did not show signs of ECM and develop significant lower

levels of parasitemia compared to pbwt. These data are representative of 2 two independent experiments. Mann-Whitney: * $p<0.05$; *** $p<0.001$. (B) Representative bioluminescent images showing the schizonts generated bioluminescence signal in isolated organs from CD1 mice infected with $pbwt^{+}$ and $\Delta msp7^{+}$ at day 7 post-infection. Rainbow images show the relative levels of luminescence ranging from low (blue), to medium (green), to high (yellow/red). (C) Quantitative bioluminescent analysis (photons/sec/$cm^2$) is utilized to compare the parasites burden in the isolated organs at different time points during the infection.




**Supplemental Figure 3. Distribution of Δmsp7$^+$ and pbwt$^+$ parasites in live infected mice.** (A) Time course (hours) bioluminescence analysis of CD1 mice infected with synchronous pbwt$^+$ and Δmsp7$^+$. At 19 and 23h both pbwt$^+$ and Δmsp7$^+$ showed an identical bioluminescence signal distribution in the adipose tissue, lungs and spleen. (B) Bioluminescence imaging of dissected organs isolated at 21h after synchronous infection. (C) Quantification of luciferase signals in organs of mice infected with Δmsp7$^+$ and pbwt$^+$ showed no significant differences in parasite distribution and intensity of infection. Bars represent the mean ± SD from 6 mice.