Supplementary Figures

The calcium pump ATPase 4 does not influence parasite levels but partially promotes experimental cerebral malaria during murine blood stage malaria

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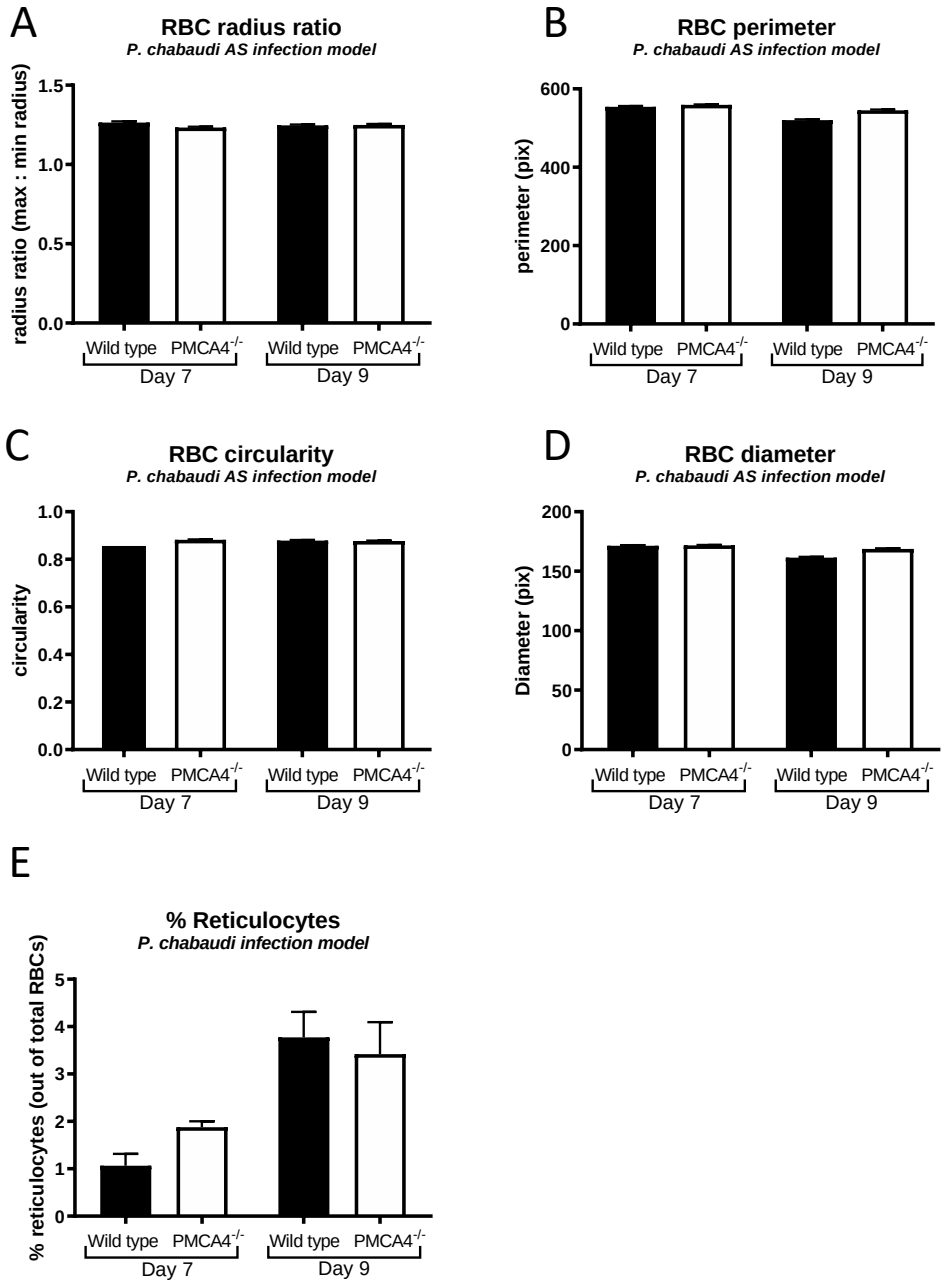
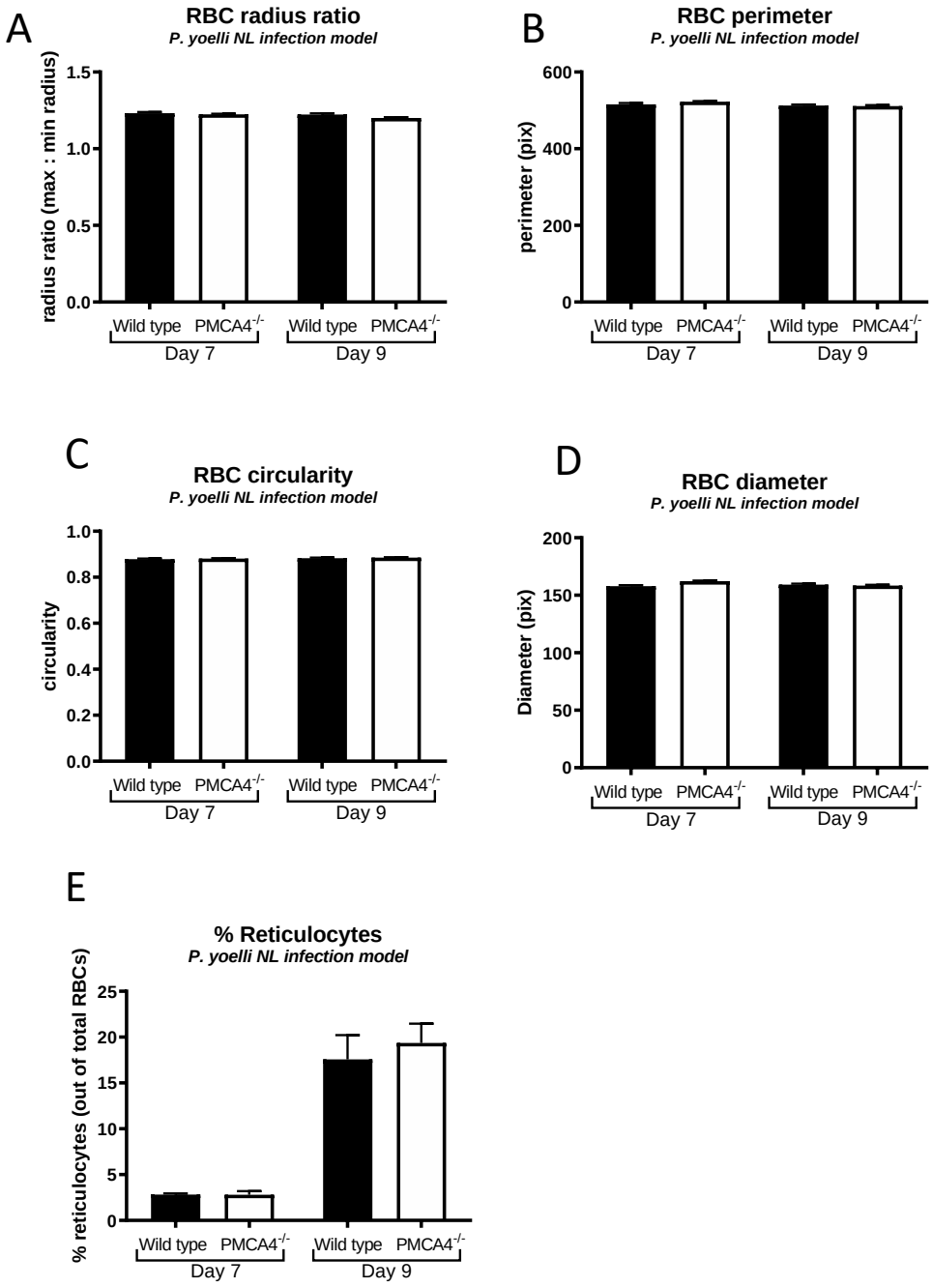
Running title: PMCA4 ablation in malaria infection

Supplementary figure 1



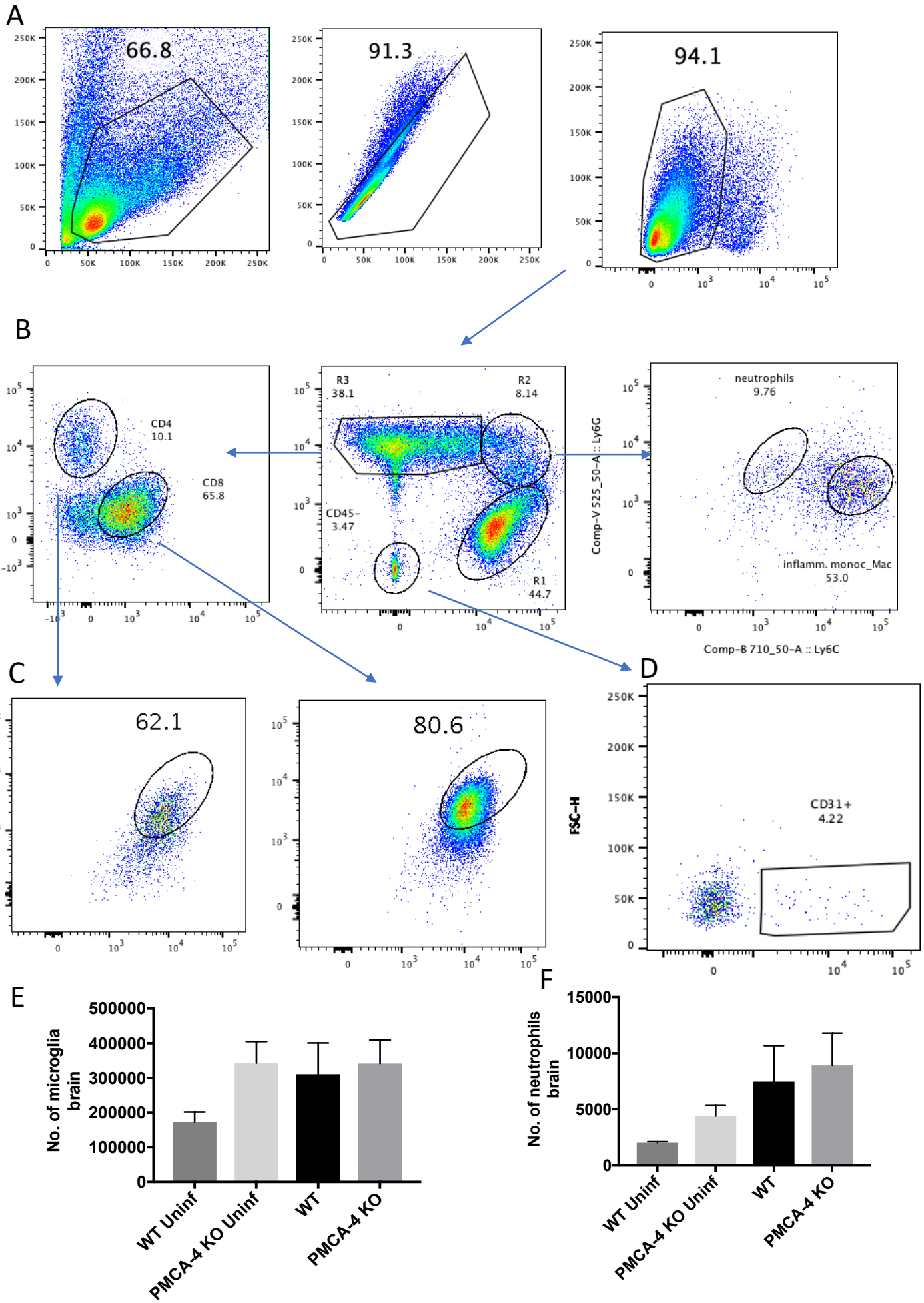
Supplementary Figure 1: Analysis of RBCs during *P. chabaudi* AS infection. RBCs of the Giemsa-stained thin blood smears from WT and PMCA4$^{-/-}$ mice on days 7 and 9 of *P. chabaudi* AS infection were analysed using Image Pro Premier image analysis software. There was no marked difference on **A)** RBC radius ratio, **B)** RBC perimeter, **C)** RBC circularity and **D)** RBC diameter between WT and PMCA4$^{-/-}$ mice at days 7 and 9 of *P. chabaudi* AS infection. **E)** Relative frequency of reticulocytes was analysed on the Giemsa-stained blood smears. No difference in reticulocytes proportion between WT and PMCA4$^{-/-}$ mice at days 7 and 9 following *P. chabaudi* AS infection. A total of 38 fields of view from 3 mice per group per time point was analysed.

Supplementary figure 2



Supplementary Figure 2: Analysis of RBCs during *P. yoelii* NL infection.
Computational analysis of RBCs showed that there was no marked difference on **A)** RBC radius ratio, **B)** RBC perimeter, **C)** RBC circularity and **D)** RBC diameter between WT and PMCA4$^{-/-}$ mice at days 7 and 9 of *P. yoelii* NL infection. **E)** Consistent with the data from *P.chabaudi* AS model there was no difference in reticulocytes proportion between WT and PMCA4$^{-/-}$ mice at days 7 and 9 following *P. yoelii* NL infection. A total of 38 fields of view from 3 mice per group per time point was analysed.

Supplementary figure 3



Supplementary Figure 3. The gating strategy for cells within the brain.
Representative flow cytometry plots showing in order: (A) gating of single cells, singlets and live cells. (B) Gating of $CD45^{+}CD11b^{-}$ (R3) populations, $CD45^{+}CD11b^{+}$ (R2) populations, $CD45^{low}$, $CD11b^{+}$ (R1) microglial cells and $CD45^{-}CD11b^{-}$ cells. R2 cells were gated into $Ly6G^{+}Ly6C^{low}$ Neutrophils and $Ly6C^{+}Ly6G^{low}$ inflammatory monocytes. R3 cells were gated into $CD4^{+}$ and $CD8^{+}$ cells. (C) Granzyme B expression by $CD4^{+}$ and $CD8^{+}$ cells. (D) Identification of $CD31^{+}$ endothelial cells within $CD45^{-}CD11b^{-}$ population. (E, F) The calculated numbers of microglia and neutrophils within the brain in uninfected WT and PMCA-4 KO mice and mice infected with 10^{4} *P. berghei* ANKA pRBCs (analysis on day 6 of infection).