Supplementary data

Figure 1. PARP$^{+/+}$ and PARP-1$^{-/-}$ mice show similar levels of NTS deposition in kidneys. To determine whether PARP$^{+/+}$ and PARP-1$^{-/-}$ mice show similar NTS deposition, mice were injected with 6ml/kg NTS and kidneys were harvested 30h and 3 days later. Kidneys frozen in OCT were cut, fixed in acetone and stained with Alexa Fluor 488 conjugated donkey anti-sheep IgG. PARP$^{+/+}$ and PARP-1$^{-/-}$ mice injected with NTS showed similar NTS deposition (upper panels). Higher magnification shows a immune deposition with a linear pattern, indicating the specificity of the NTS toward components of the glomerular basement membrane.

Figure 2. PARP-1$^{-/-}$ mice show normal T and B cell development and maturation.

A. We investigated maturation of double negative (DN, $CD4^-CD8^-$) T cells, from early T cell precursors (ETP) ($CD4^-CD8^-CD44^{high}CD25^-$), to their different stages of DN1 ($CD4^-CD8^-CD44^+CD25^-$), DN2 ($CD4^-CD8^-CD44^{high}CD25^+$), DN3 ($CD4^-CD8^-CD44^-CD25^+$), and DN4 ($CD4^-CD8^-CD44^-CD25^-$). We have also investigated their maturation form DN ($CD4^-CD8^-$) to double positive (DP $CD4^+CD8^+$), and finally single positive (SP) T cells ($CD4^-CD8^+$ and $CD4^+CD8^-$). We characterized 3-5 months old male and female mice and found no difference between the PARP-1$^{-/-}$ and PARP-1$^{+/+}$ strain. These results demonstrate that the T cell development in the absence of PARP-1 is normal. B. We performed flow cytometry characterization of precursors and mature B cells by using the following markers- Bone Marrow: PreB cells ($CD19^+B220^{lo}AA4.1^+$) and Immature B cells (Im) ($CD19^+B220^+AA4.1^+$). Transitional B cells in the Spleen: T1 ($CD19^+B220^+AA4.1^+IgM^{hi}CD23^-$), T2 $CD19^+B220^+AA4.1^+IgM^{hi}CD23^+$ and T3 $CD19^+B220^+AA4.1^+IgM^{lo}CD23^+$. Follicular Zone (FO)

($IgD^{+}IgM^{lo}CD23^{+}B220^{hi}AA4.1^{-}$) and Marginal Zone (MZ) B cells ($CD19^{+}B220^{+}IgM^{hi}CD21^{hi}$). We characterized 3-5 months old male and female mice and found no difference between the PARP-$1^{-/-}$ and PARP-$1^{+/+}$ strain. These results demonstrate that the B cell compartment in the absence of PARP-1 is normal.

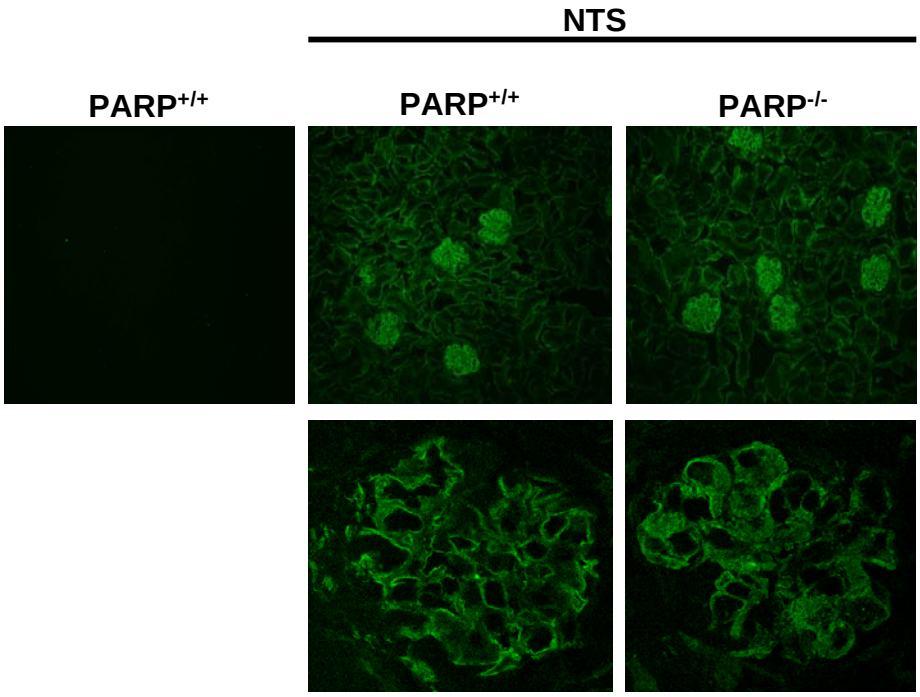
Figure 3. Humoral Immune Response in PARP-$1^{-/-}$ is normal. PARP-$1^{+/+}$ and PARP-$1^{-/-}$ mice were immunized with 100 μg of OVA in PBS emulsified with an equal volume of CFA. Mice were bled at the times indicated and sera were tested for anti-OVA Ab levels by ELISA. PARP-$1^{-/-}$ mice responded to the immunogenic challenge as well as PARP-$1^{+/+}$ mice, as shown by the comparable levels of Abs produced (p= n.s.). Data are represented as mean ± SEM of 5 mice each group.

Figure 4. PARP-$1^{-/-}$ splenocytes are resistant to necrotic cell death.

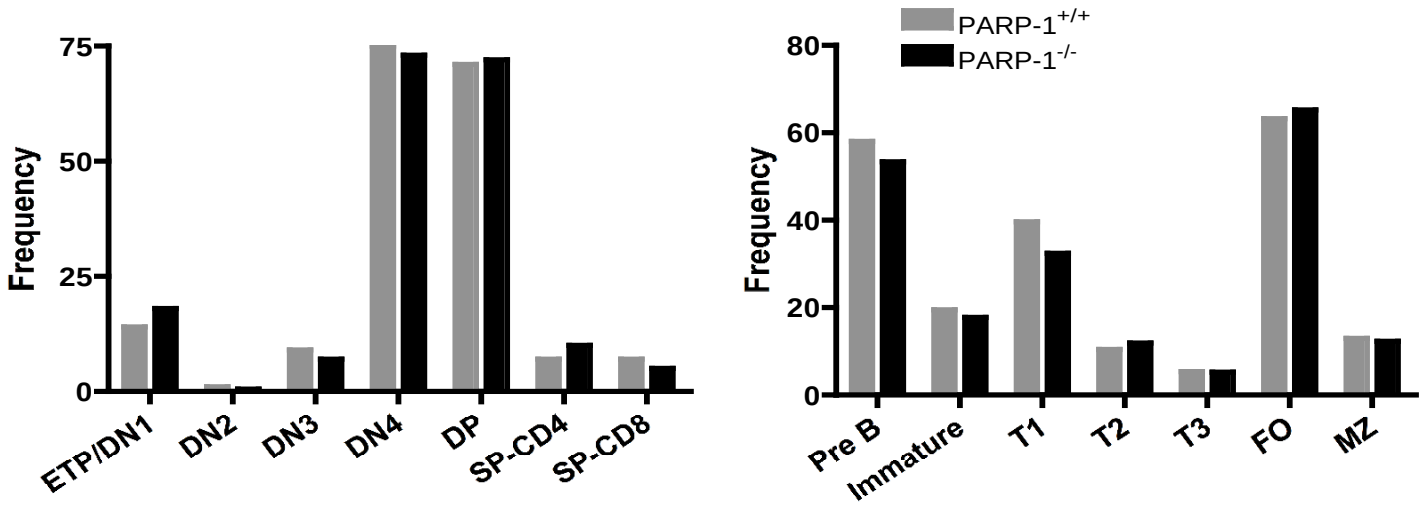
A, Splenocytes ($1X10^{6}$/ml in complete medium and 10% FCS) from male aged-matched PARP-$1^{+/+}$ or PARP-$1^{-/-}$ mice were incubated for 4 hrs w/ or w/o H_2O_2. Cells were harvested and stained for 5 minutes with 1mg/ml of 7AAD. And analyzed by a FACSCalibur (BD) for 7AAD permeability. In contrast to PARP-$1^{-/-}$ cells, PARP-$1^{+/+}$ cells showed early complete positivity, suggesting early necrotic cell death. B. 12 hrs after Starvation or H_2O_2 treatment, cells were harvested and the side scatter analyzed by flow cytometry. PARP-$1^{-/-}$ cells starved or H_2O_2 treated, and PARP-$1^{+/+}$ cells starved, show very similar scatters. In contrast PARP-$1^{+/+}$ cells treated with H_2O_2 show homogenous size reduction, indicating necrotic synchronization. C. Caspase-3 activation was tested with a FITC-conjugated rabbit polyclonal Ab. The caspase was

activated during starvation in both groups. In contrast caspase-3 was activated only in the absence of PARP-1, demonstrating that only PARP-$1^{-/-}$ cells under went apoptotic cell death.

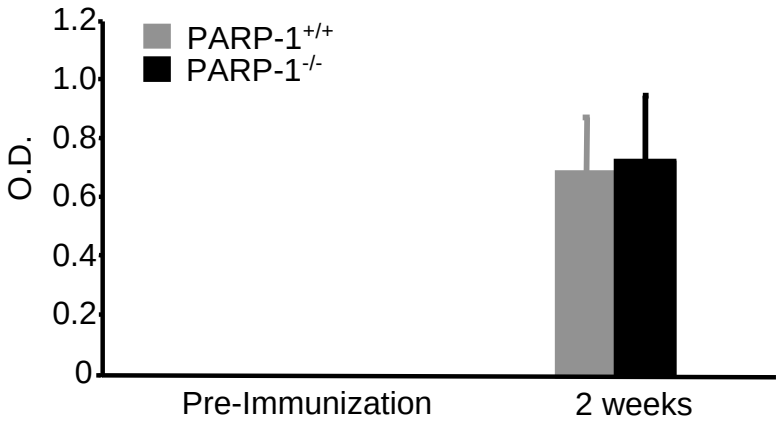
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4