Supplementary Data

Supplementary figures

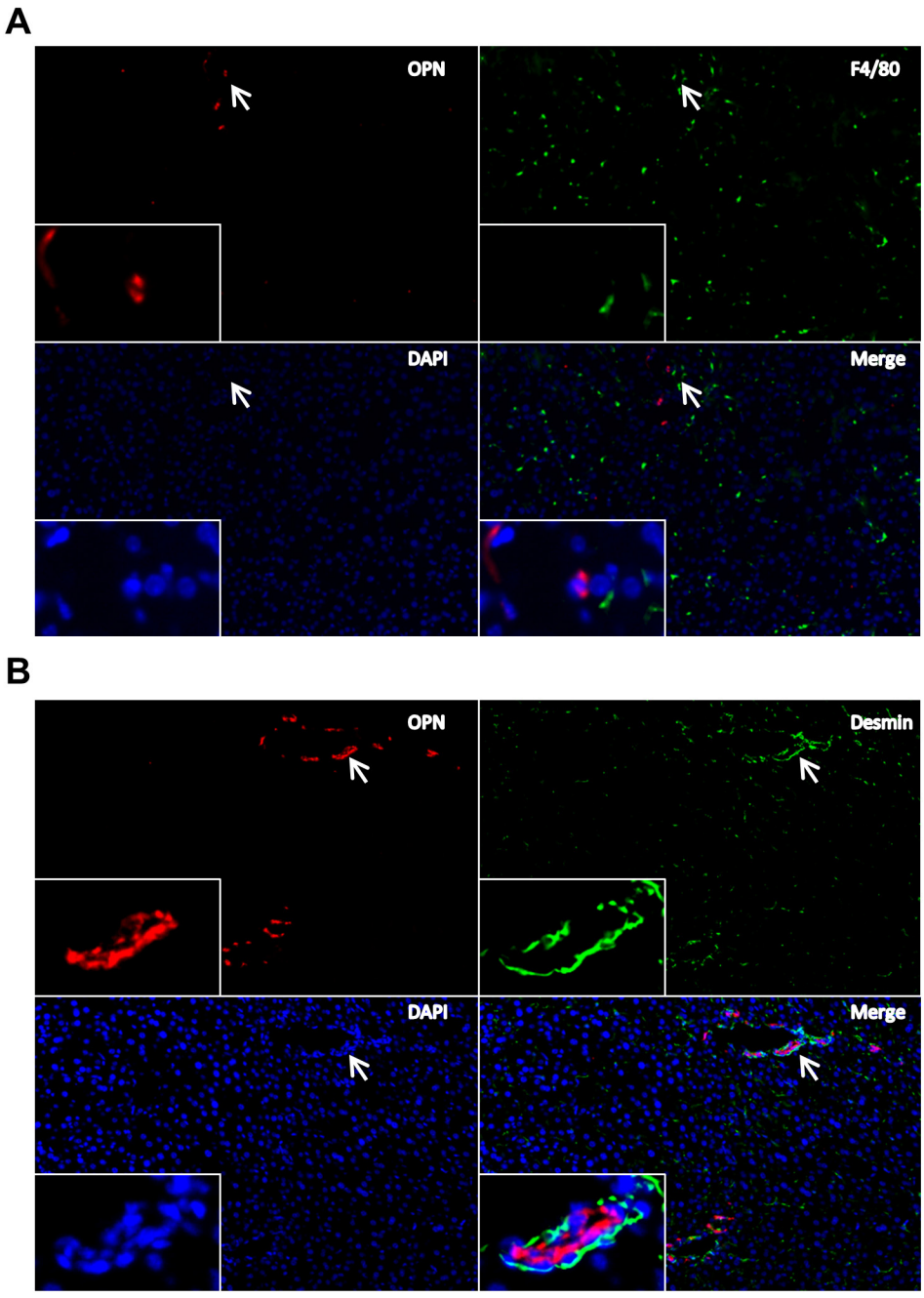

Fig. S1. Kupffer cells and hepatic stellate cells are not responsible for OPN production 3 hours after PHx. Immunofluorescence analysis showed that OPN did not co-label with (A) F4/80 and (B) desmin in the liver 3 hours

after PHx (200× magnification). The small insets show a magnified picture of staining of DAPI, OPN and F4/80 or desmin (white arrows). Three independent experiments were performed with similar results.

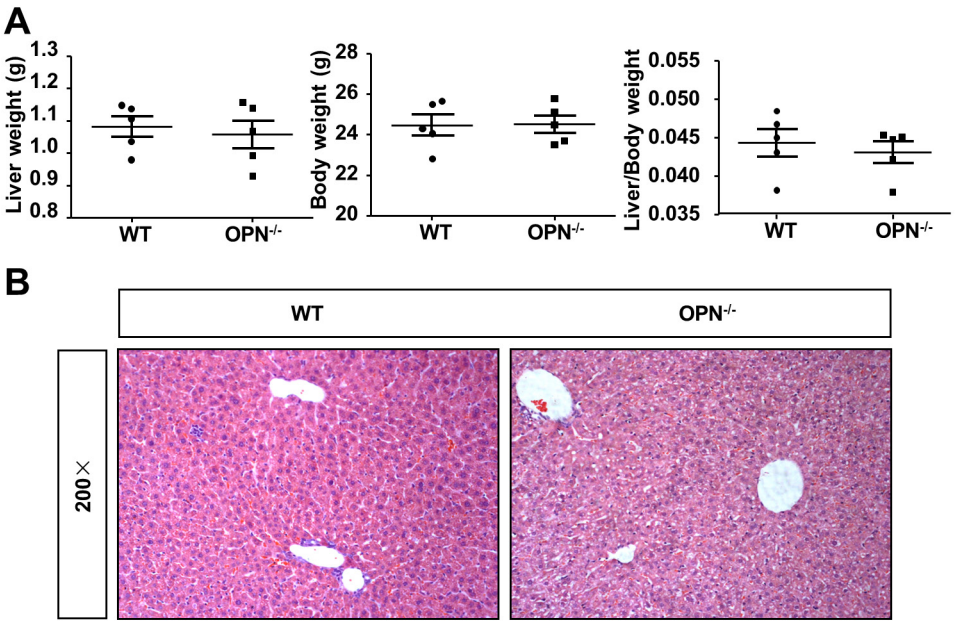

Fig. S2. OPN deficiency does not impact young adult livers. (A) Liver weight, body weight and the liver-to-body weight ratio in WT and OPN$^{-/-}$ mice 8 weeks after birth were determined. The data are shown as the mean ± SEM. (B) H&E staining of WT and OPN$^{-/-}$ livers was performed (200× magnification). Three independent experiments were performed with similar results.

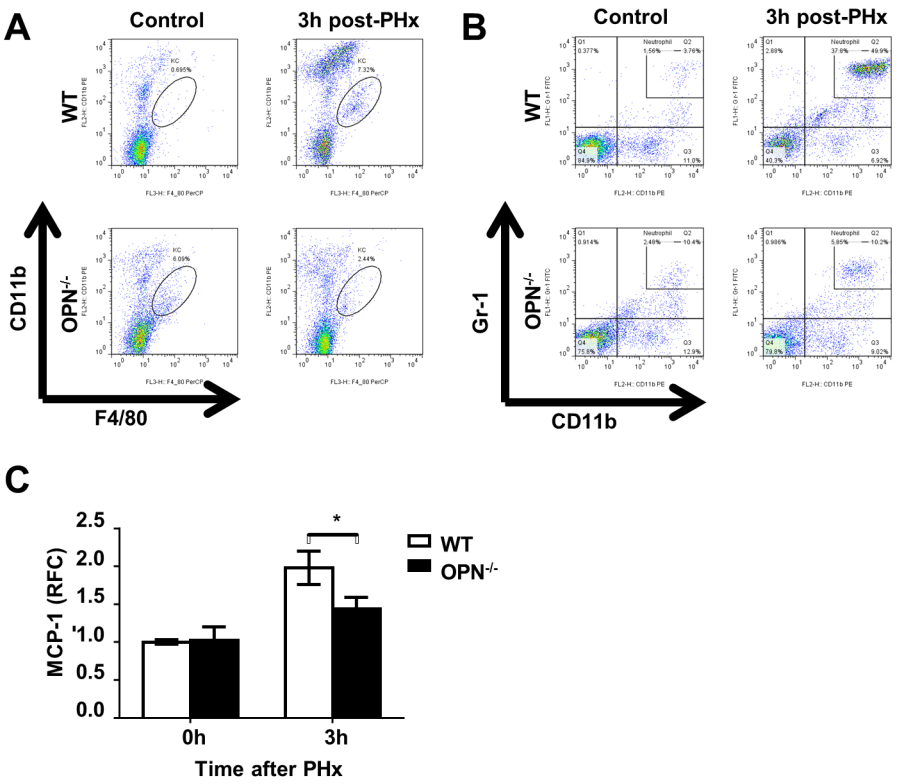

Fig. S3. Loss of OPN impairs inflammatory responses in regenerating livers. (A, B) Liver macrophages ($CD11b^{int}$ $F4/80^{+}$) and neutrophils ($Gr\text{-}1^{high}$ $CD11b^{+}$) were analyzed in WT and $OPN^{-/-}$ mice by FACS analysis after PHx at the indicated time points by staining with FITC-conjugated anti-Gr-1, PE-conjugated anti-CD11b, PerCP-conjugated anti-F4/80, APC-conjugated anti-CD45 antibodies. Representative FACS data are shown. (C) Relative expression of MCP-1 in liver tissues was analyzed after PHx (n = 3-4). The data are shown as the mean ± SEM. *P<0.05. RFC: relative fold change.

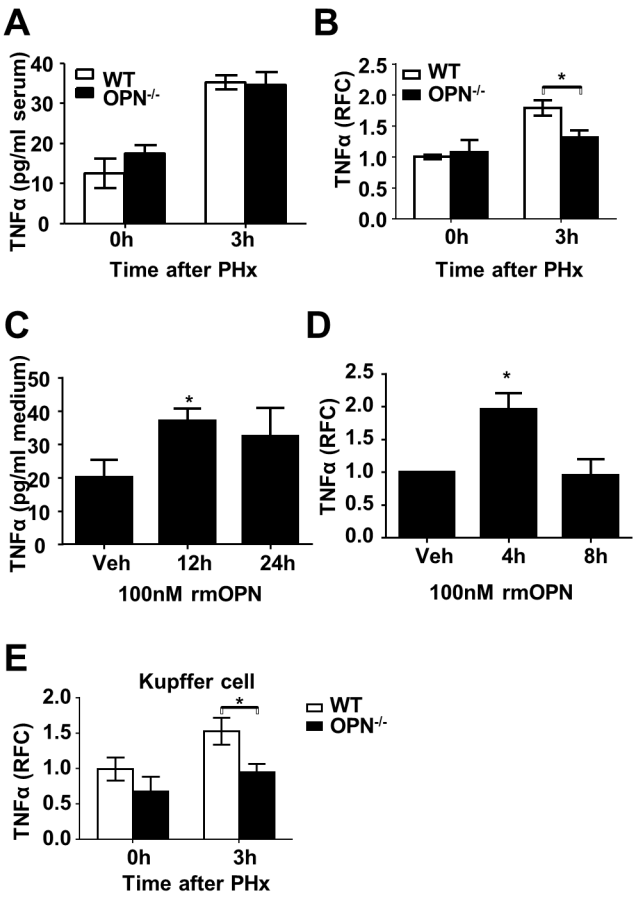

Fig. S4. OPN improves TNFα release in Kupffer cells both *in vivo* and *in vitro.* (A) The serum TNFα level after PHx was determined by ELISA (n = 3-6). The data are shown as the mean ± SEM. (B) TNFα mRNA expression in the liver was analyzed by qPCR (n = 3-6). The data are shown as the mean ± SEM. **P*<0.05. (C) Kupffer cells were isolated from WT mouse liver and treated with 100 nM OPN. Cell culture supernatants were subjected to ELISA for TNFα (n = 3). The data are shown as the mean ± SEM. *P<0.05. (D) Cell lysates were collected for quantitative analysis of TNFα mRNA levels (n = 3). The data are shown as the mean ± SEM. *P<0.05. (E) Kupffer cells were isolated from WT and OPN$^{-/-}$ mice after PHx and collected for quantitative analysis of TNFα mRNA levels (n = 3-6). The data are shown as the mean ± SEM. **P*<0.05. RFC:

relative fold change.

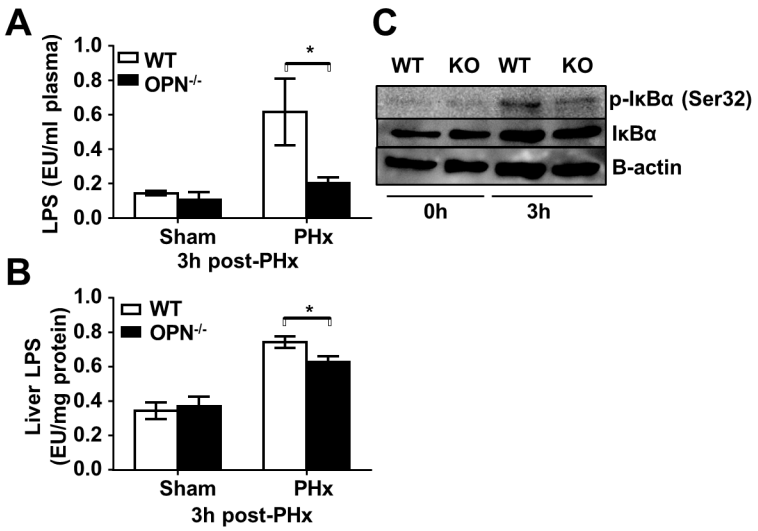

Fig. S5. Plasma and hepatic LPS and phosphorylation of IκBα in Kupffer cells are higher in WT mice than in OPN$^{-/-}$ mice 3 hours after PHx. (A) Plasma from blood in the portal vein and (B) liver homogenates in WT and OPN$^{-/-}$ mice at 3 hours after PHx were subjected to endotoxin assays (n = 4-6). The data are shown as the mean ± SEM. *P<0.05. (C) Phosphorylation of IκBα in Kupffer cells isolated from both genotypes of mice 3 hours post-PHx was analysed by western blot. Three independent experiments were performed with similar results.

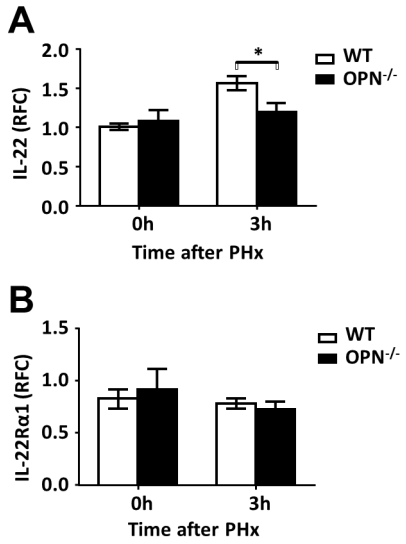

Fig. S6. IL-22 is increased in WT livers but not in OPN deficient livers 3 hours after PHx, while IL-22Rα1 is not altered. (A) IL-22 and (B) IL-22Rα1 mRNA expression was analyzed by qPCR 3 hours after PHx (n = 3-6). The data are shown as the mean ± SEM. *P<0.05. RFC: relative fold change.

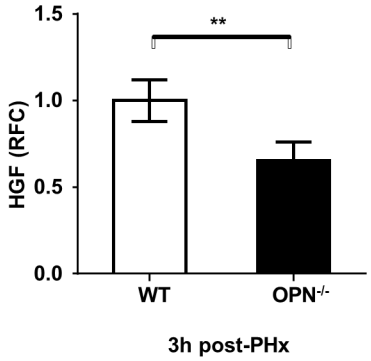

Fig. S7. HGF expression is higher in WT mice than in OPN$^{-/-}$ mice 3 hours after PHx. HGF mRNA expression was analyzed in livers at 3 hours post-PHx (n = 3-5). The data are shown as the mean ± SEM. ***P*<0.01. RFC: relative fold change.