

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Generation and characterization of NEMO^{Δhepa}/Fas^{lpr} mice. (a) PCR blot of IKKγ/NEMO and Fas shows WT and knockout bands in NEMO^{ff}, NEMO^{Δhepa}, Fas^{lpr} and NEMO^{Δhepa}/Fas^{lpr}. (b) Representative photograph of spleens and peritoneal lymph nodes of Fas^{lpr} and NEMO^{Δhepa}/Fas^{lpr} mice. (c) The spleen size and (d) the number of peritoneal lymph nodes of 8 week-old NEMO^{ff}, NEMO^{Δhepa}, Fas^{lpr} and NEMO^{Δhepa}/Fas^{lpr} is represented. Results are expressed as mean ± SEM ($n=25$, ** $p<0.01$; ** $p<0.01$).

Supplementary Figure 2. Markers of liver injury after Fas^{lpr} deletion. (a) Serum AP and (b) GLDH levels of 8 week-old NEMO^{ff}, NEMO^{Δhepa}, Fas^{lpr} and NEMO^{Δhepa}/Fas^{lpr} mice were determined. Results are expressed as mean ± SEM ($n=25$, ** $p<0.01$; ** $p<0.01$).

Supplementary Figure 3. Cell death, proliferation and inflammation markers after deficient FasL/FasR signaling. mRNA relative expression of (a) TNF and (b) TGFβ was quantified by RT-PCR and graphed.

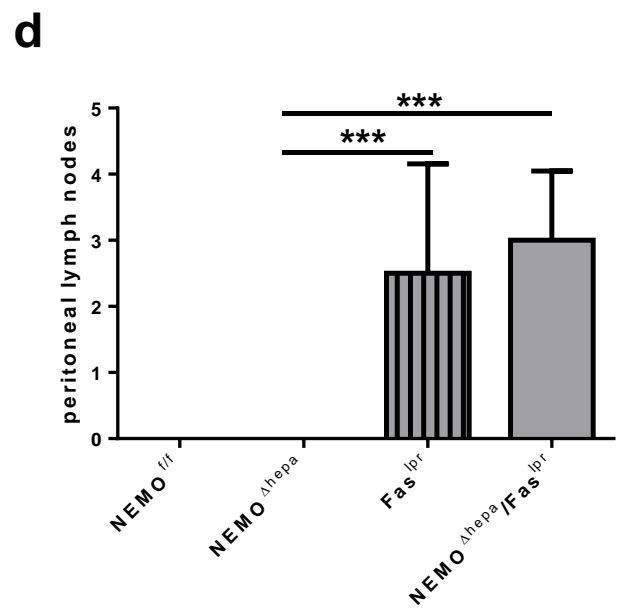
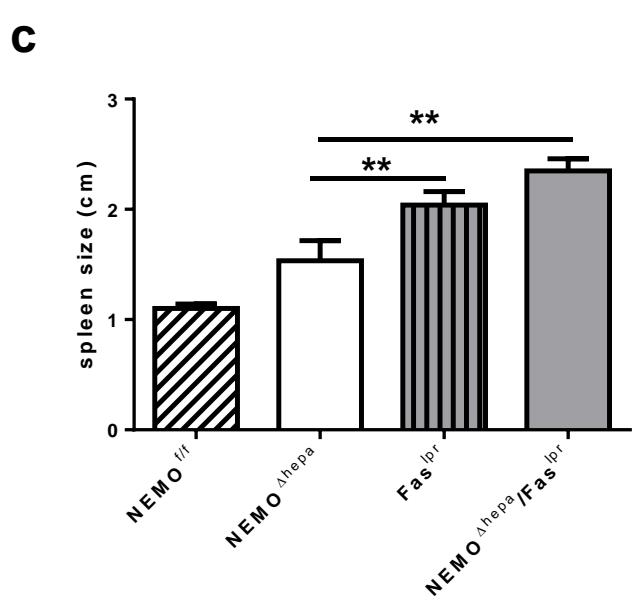
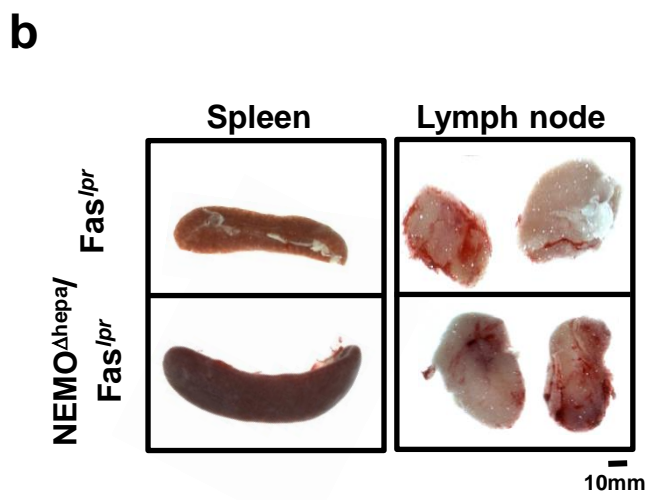
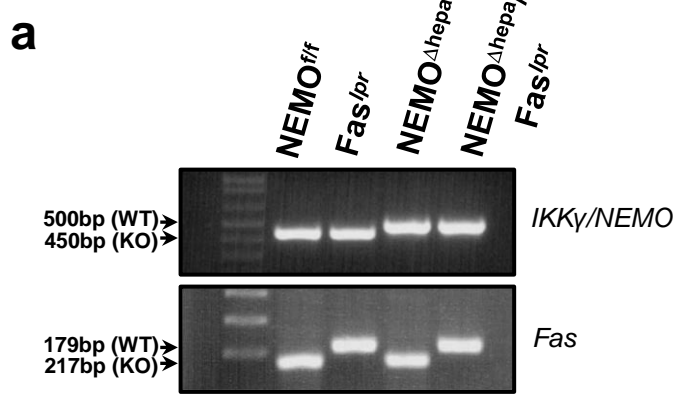
Supplementary Figure 4. Infiltration of inflammatory cells into the liver. (a) FACS blot of proinflammatory macrophages CD11b/F4/80 is shown. (b) Representative CD11b staining of 8 week-old NEMO^{ff}, NEMO^{Δhepa}, Fas^{lpr} and NEMO^{Δhepa}/Fas^{lpr} livers. (c) The positive Area Fraction was quantified in Image J©

and graphed. (d) Representative F4/80 staining of the same livers. (e) The positive Area Fraction was quantified in Image J© and graphed.

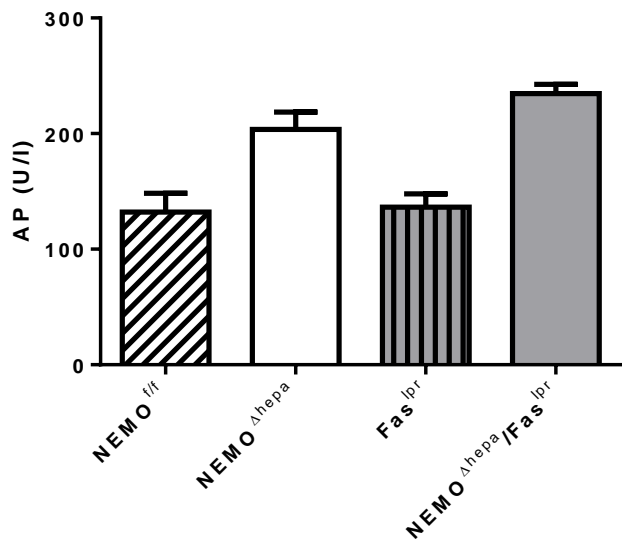
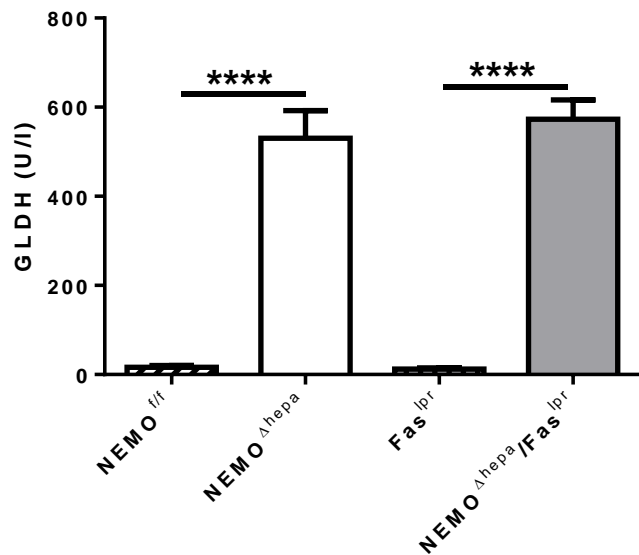
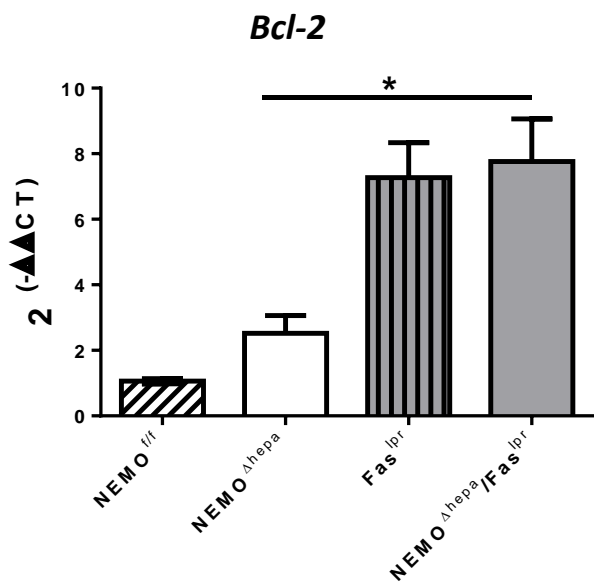
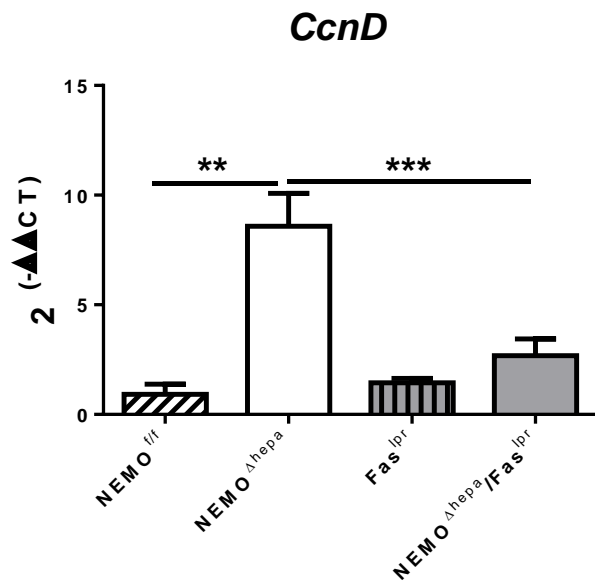
Supplementary Figure 5. Characterization of NEMO^{Δhepa}/Fas^{lpr}/TNFR1^{-/-} mice. (a) Representative photograph of spleens and peritoneal lymph nodes of 8 week-old Fas^{lpr}/TNFR1^{-/-} and NEMO^{Δhepa}/Fas^{lpr}/TNFR1^{-/-} mice. (b) The spleen size and (c) the number of peritoneal lymph nodes of 8 week-old Fas^{lpr}/TNFR1^{-/-} and NEMO^{Δhepa}/Fas^{lpr}/TNFR1^{-/-} compared with NEMO^{f/f}, NEMO^{Δhepa}, Fas^{lpr} and NEMO^{Δhepa}/Fas^{lpr} is represented. Results are expressed as mean ± SEM (n=25, **p<0.01; ***p<0.001).

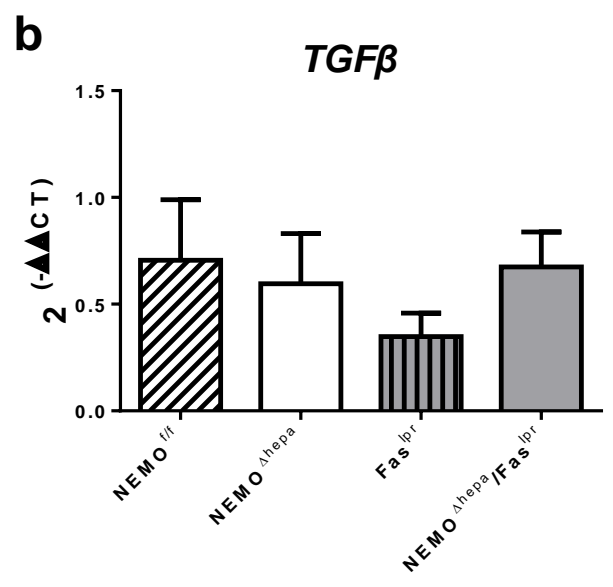
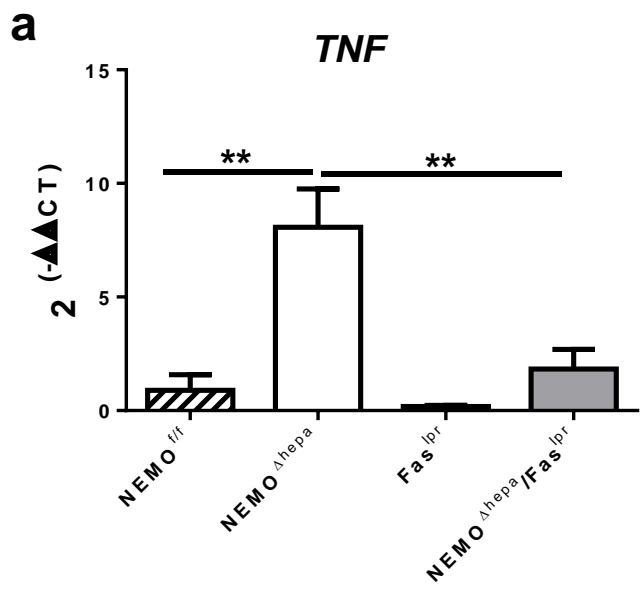
Supplementary Figure 6. Deletion of TNFR1 further protects against the NEMO-dependent hepatitis. Fas^{lpr}/TNFR1^{-/-} and NEMO^{Δhepa}/Fas^{lpr}/TNFR1^{-/-} mice at the age of 8 weeks were sacrificed and livers extracted and analyzed. (a) Macroscopic appearance of livers. (b) Representative H&E staining of liver sections. (c) Serum ALT, (d) AP and (e) GLDH were determined in the same mice and compared to 8 week-old NEMO^{f/f}, NEMO^{Δhepa}, Fas^{lpr} and NEMO^{Δhepa}/Fas^{lpr} mice. Results are expressed as mean ± SEM (n=25, **p<0.05; ***p<0.001).

Supplementary Figure 7. Liver fibrogenesis is further decreased in 1 year-old NEMO^{Δhepa}/Fas^{lpr}/TNFR1^{-/-} livers. Sirius red staining of paraffin-embedded liver tissue was performed and photomicrographs in polarized and normal light were taken in NEMO^{f/f}, NEMO^{Δhepa}, Fas^{lpr} and NEMO^{Δhepa}/Fas^{lpr}, Fas^{lpr}/TNFR1^{-/-} and NEMO^{Δhepa}/Fas^{lpr}/TNFR1^{-/-} mice at the age of 1 year.

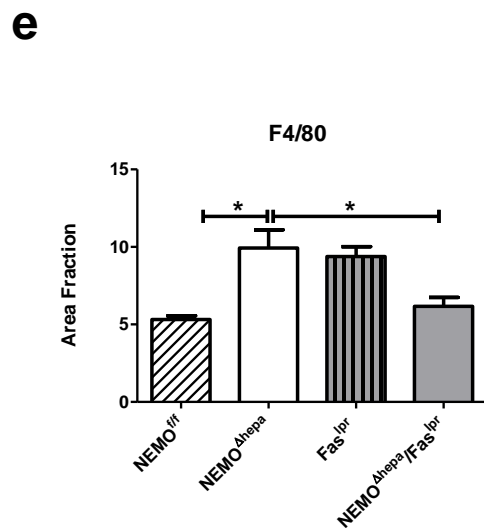
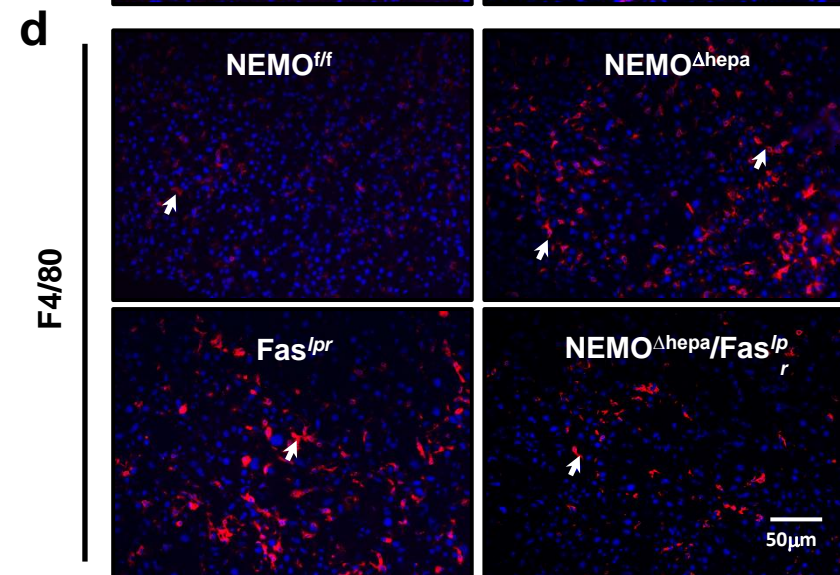
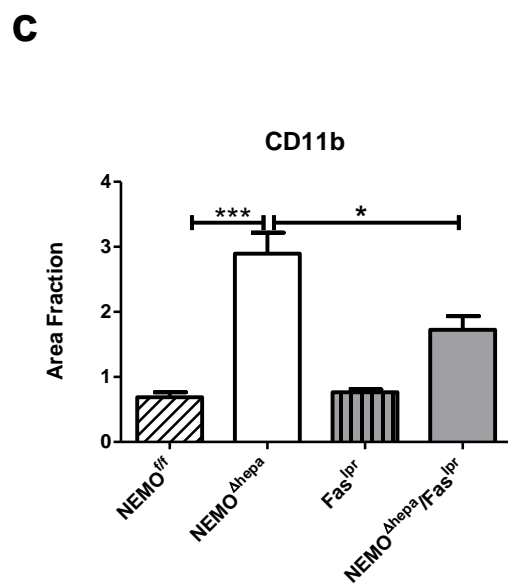
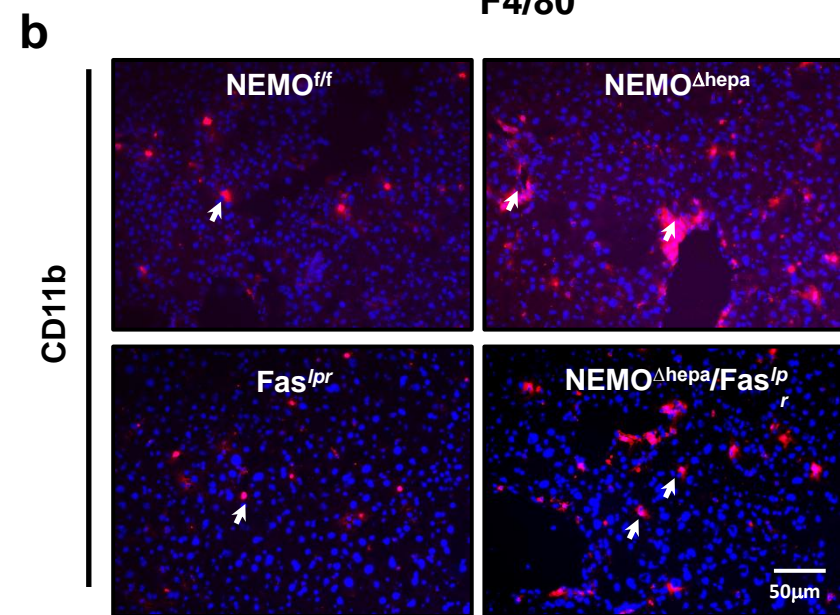
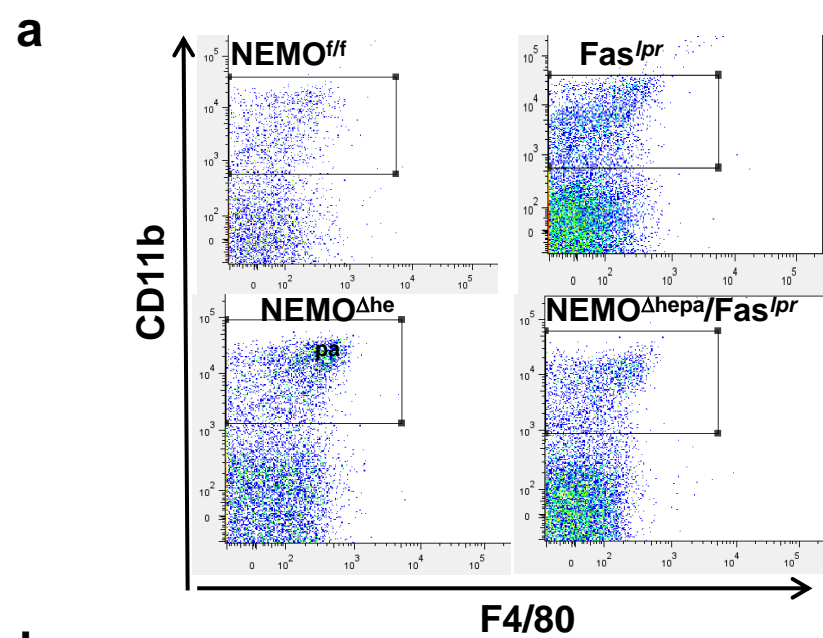


Supplementary Figure 1

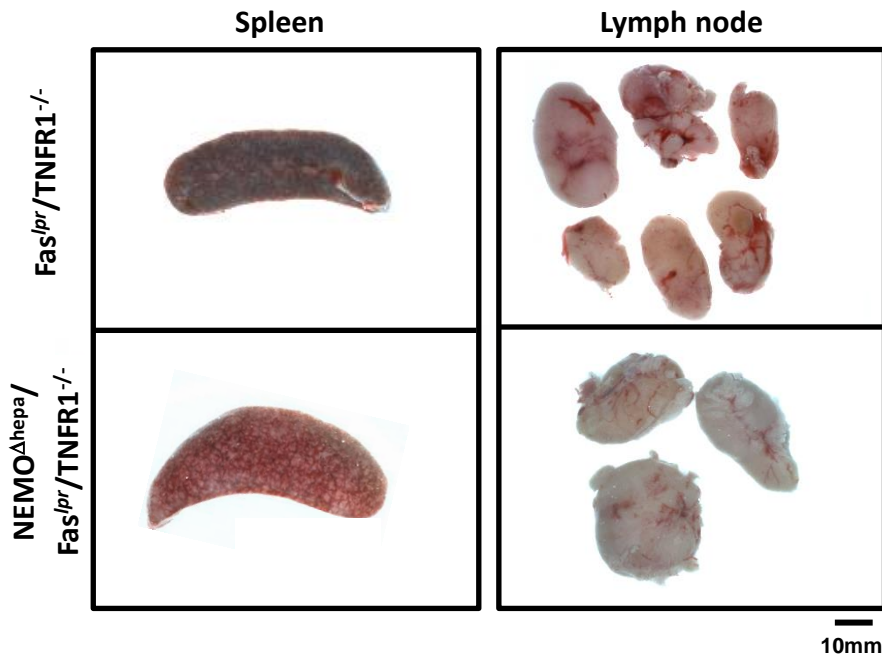
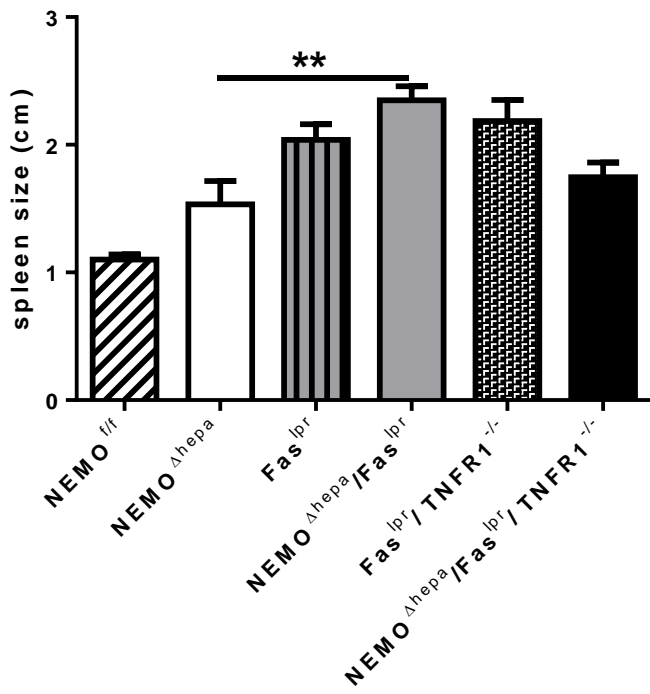
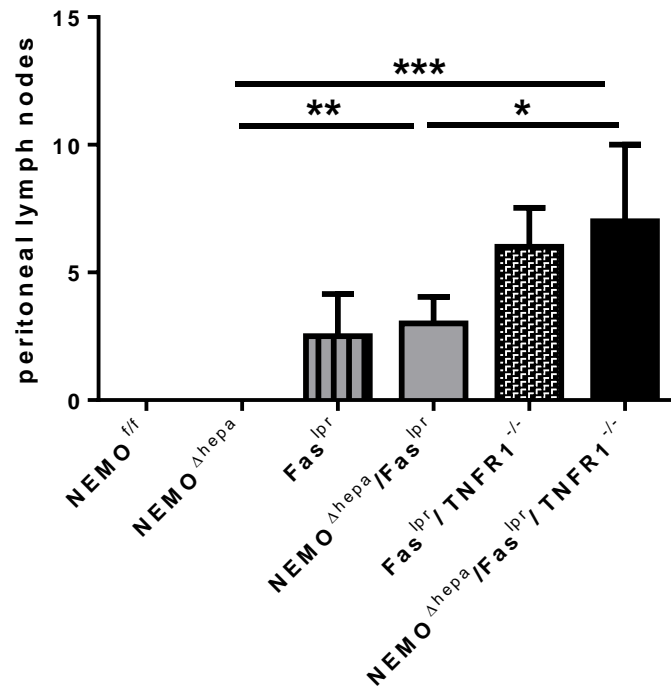
a**b****c****d**

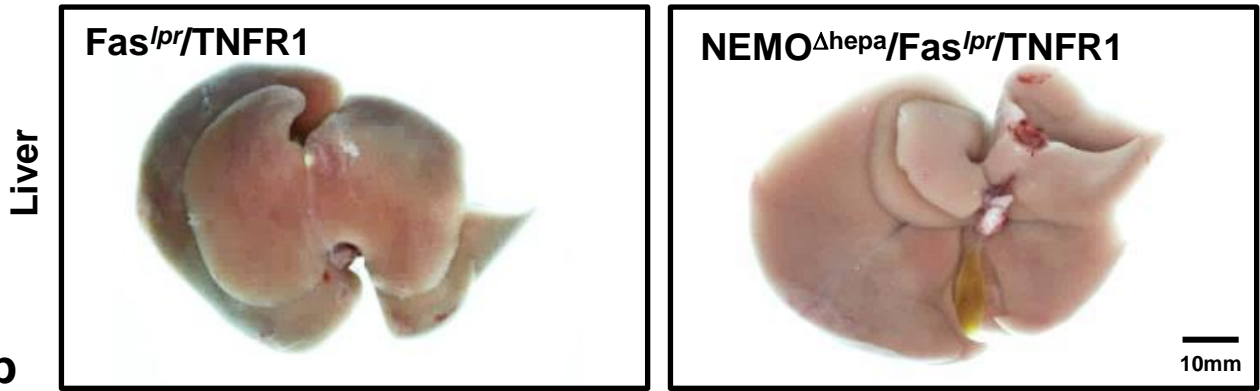
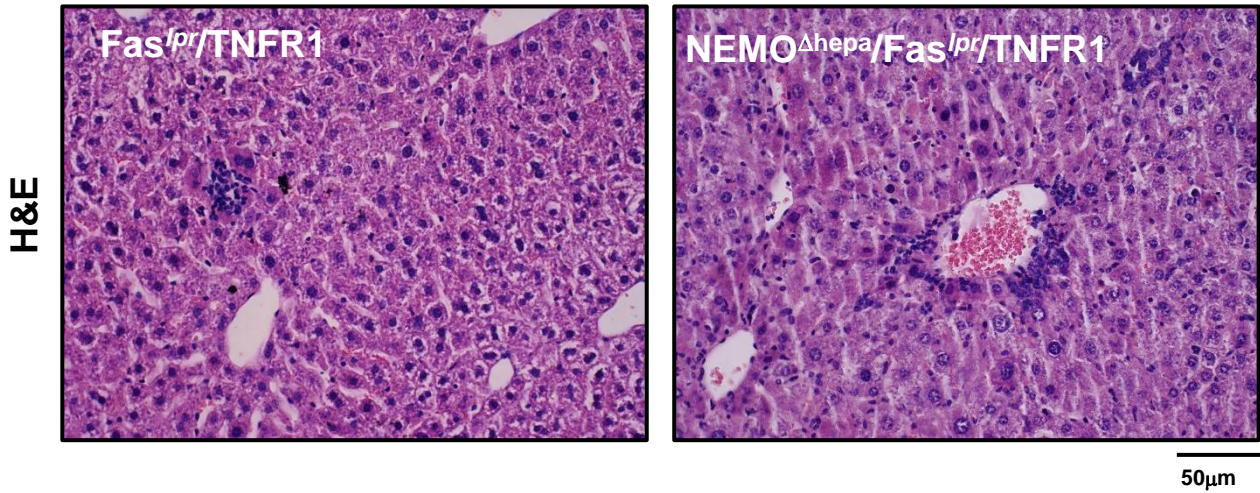
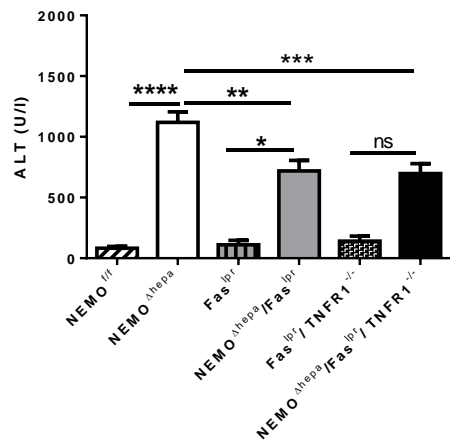
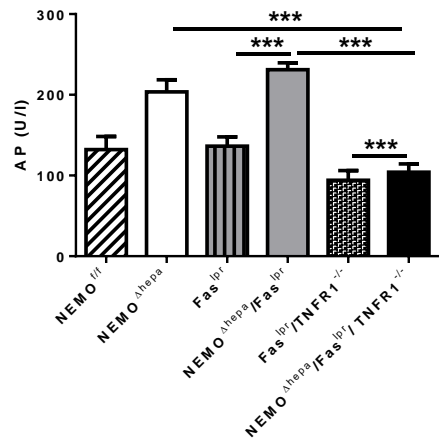
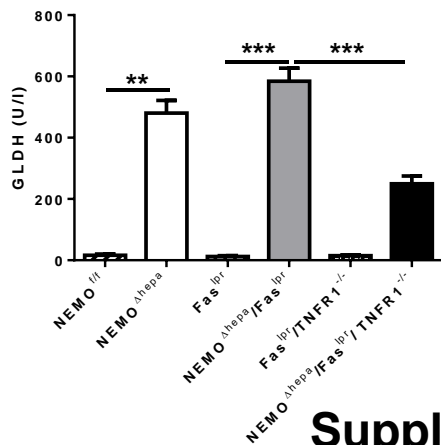


Supplementary Figure 3



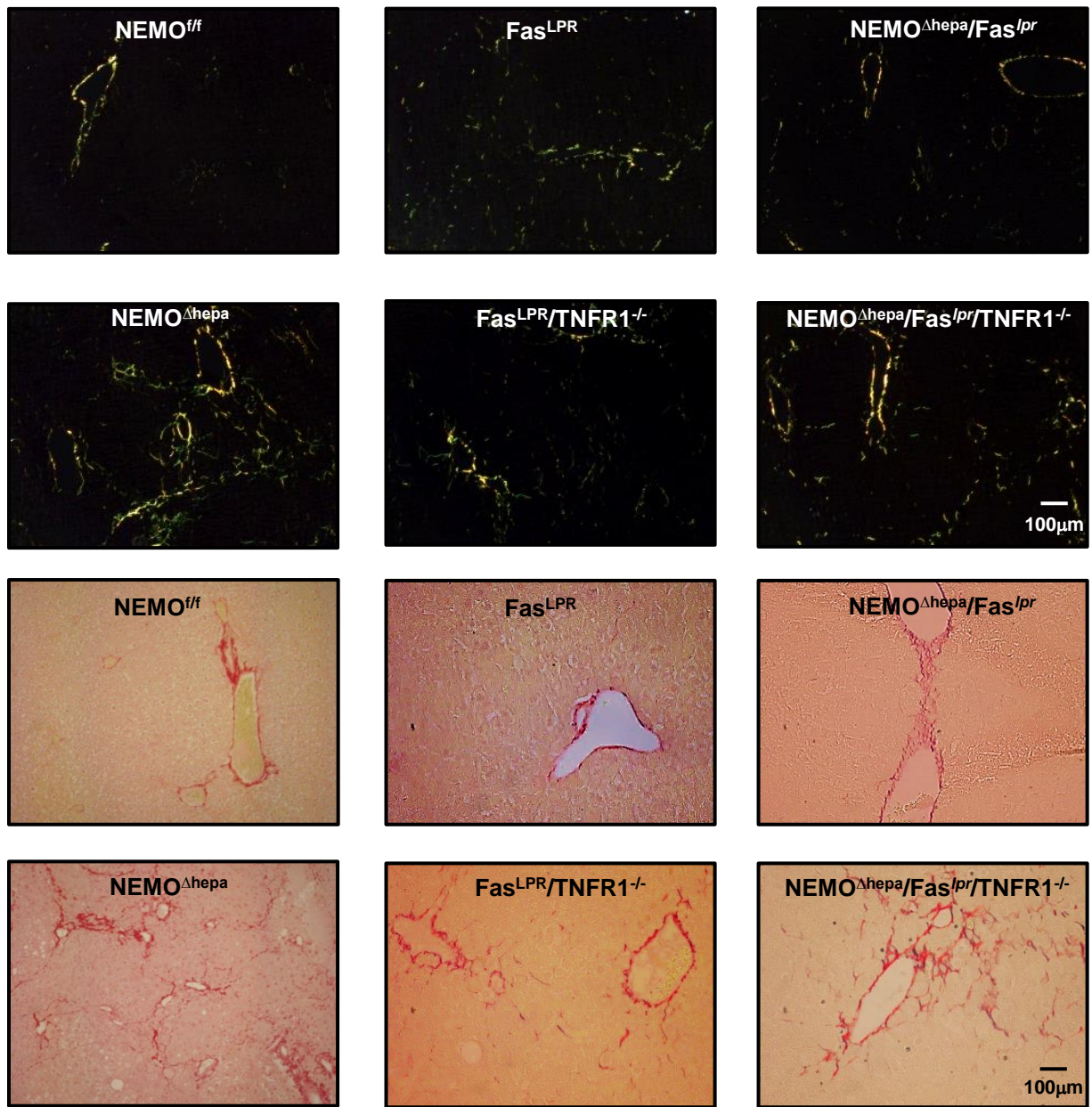
Supplementary Figure 4

a**b****c**

a**b****c****d****e**

Supplementary Figure 6

Sirius red



Supplementary Figure 7