JEM

SUPPLEMENTAL MATERIAL

Beraza et al., http://www.jem.org/cgi/content/full/jem.20082152/DC1

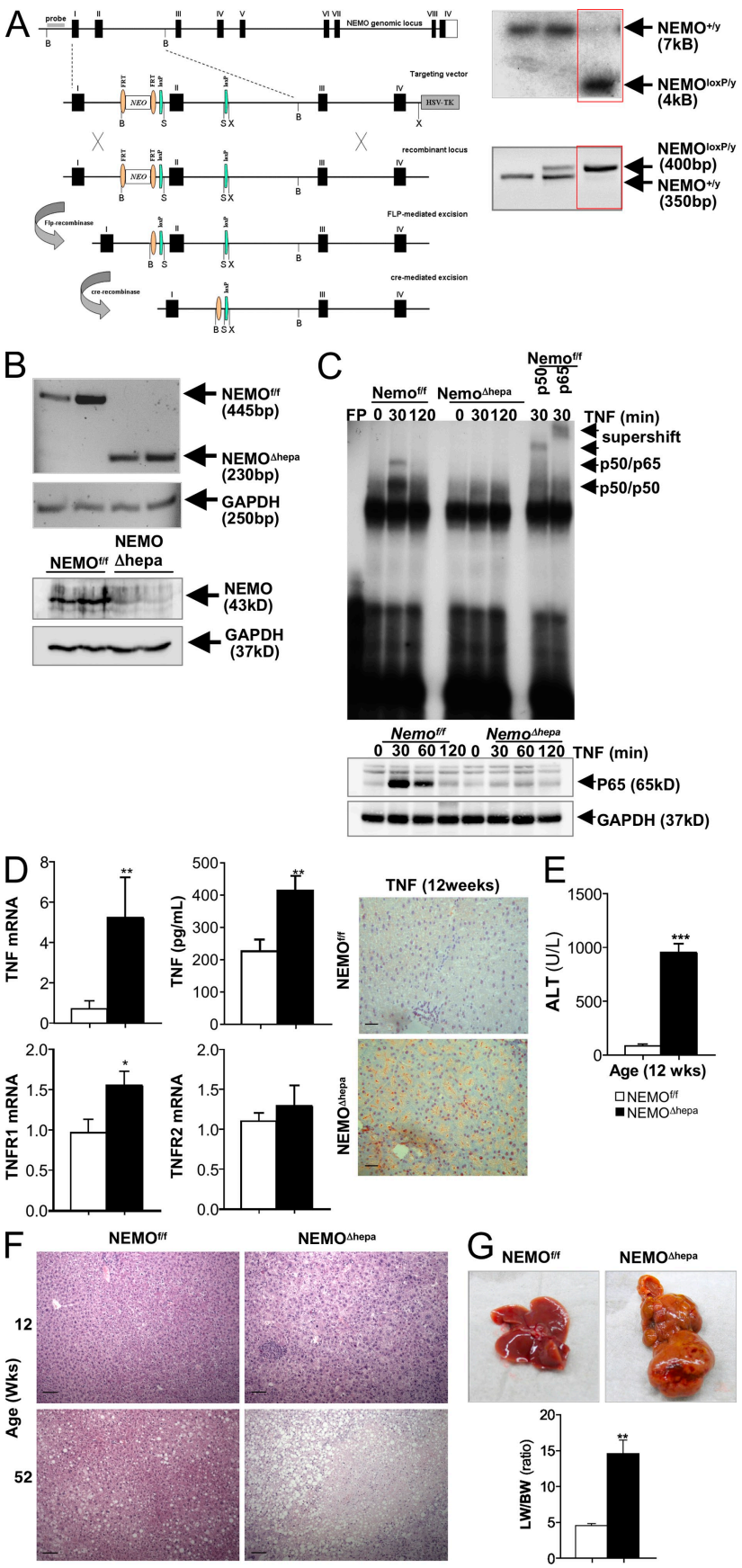
A
probe
NEMO genomic locus
Targeting vector
NEO
HSV-TK
recombinant locus
Flp-recombinase
FLP-mediated excision
cre-recombinase
cre-mediated excision
NEMO+/y (7kB)
NEMOloxP/y (4kB)
NEMOloxP/y (400bp)
NEMO+/y (350bp)
B
NEMOf/f (445bp)
NEMOΔhepa (230bp)
GAPDH (250bp)
NEMO
NEMOf/f Δhepa
NEMO (43kD)
GAPDH (37kD)
C
Nemof/f
p50
p65
Nemof/f NemoΔhepa
FP 0 30 120 0 30 120 30 30 TNF (min)
supershift
p50/p65
p50/p50
Nemof/f NemoΔhepa
0 30 60 120 0 30 60 120 TNF (min)
P65 (65kD)
GAPDH (37kD)
D
TNF mRNA
TNF (pg/mL)
TNF (12weeks)
NEMOf/f
NEMOΔhepa
TNFR1 mRNA
TNFR2 mRNA
E
ALT (U/L)
Age (12 wks)
NEMOf/f
NEMOΔhepa
F
NEMOf/f
NEMOΔhepa
Age (Wks)
12
52
G
NEMOf/f
NEMOΔhepa
LW/BW (ratio)

Figure S1. Hepatocyte-specific NEMO deletion impairs NF-κB activation in hepatocytes and leads to spontaneous steatohepatitis and HCC development. For the generation of conditional NEMO KO mice, we designed a gene-targeting vector containing two loxP sites in tandem orientation separated by a unique SalI restriction site and the neomycin resistance gene (neo) under the control of the cmv promoter flanked by FRT recombination sites. (A) Gene-targeting strategy. Exons and introns are represented by vertical bars and horizontal lines, respectively. A neomycin-resistance gene driven by a cmv promoter (NEO) was used as selection marker. NEMO-targeted embryonic stem cell clones were identified by southern blot analysis after BamHI digestion using a 5′ probe (red box, top). Cointegration of the 3′ loxP site was verified by a PCR approach (red box, bottom). An ∼50-bp difference between WT and recombinant clones represents the 34-bp loxP sequence flanked by SalI and XhoI restriction sites. NEMO chimeric mice were generated by injection of embryonic stem cells with homologous integration of the targeting vector in the NEMO locus and with both loxP sites (f) cointegrated into C57/BL6 blastocysts that were retransplanted into pseudo-pregnant foster mice. Heterozygous and neo-deleted NEMO$^{+/f}$ animals were generated by backcrossing chimeras with transgenic mice containing the FLPe recombinase under the control of the ubiquitous human ACTB promoter (Rodriguez, C.I., F. Buchholz, J. Galloway, R. Sequerra, J. Kasper, R. Ayala, A.F. Stewart, and S.M. Dymecki. 2000. *Nat. Genet.* 25:139–140). For the hepatocyte-specific deletion, NEMO$^{f/f}$ mice were crossed with animals expressing a cre transgene under control of the hepatocyte-specific albumin promoter (NEMOΔ^{hepa}; Kellendonk, C., C. Opherk, K. Anlag, G. Schutz, and F. Tronche. 2000. *Genesis.* 26:151–153). The exons are labeled by roman numbers. B, BamHI; S, SalI; X, XhoI. (B) PCR and Western blot analysis demonstrated the efficiency of NEMO deletion. (C) NEMOΔ^{hepa} mice show inability to translocate p65 to the nucleus after TNF stimulation as shown by EMSA and Western blotting. (D) RT-PCR, ELISA, and IHC demonstrated a high presence of TNF in livers from NEMOΔ^{hepa} mice under basal conditions. TNFR1 and TNFR2 mRNA expression were analyzed. (E and F) Serum ALT levels were elevated in NEMOΔ^{hepa} mice (E) and spontaneous liver damage was confirmed by histological analysis on liver sections with H&E staining (F) that showed inflammation and steatohepatitis at 12 wk of age progressing to HCC development in NEMOΔ^{hepa} mice after 52 wk. (G) Macroscopic view of liver of NEMO and NEMOΔ^{hepa} mice at 52 wk of age and liver weight/body weight ratio. Bars, 50 µm. All data are representative of four independent experiments. n = 5–10. *, P < 0.05; **, P < 0.01; ***, P < 0.001 (NEMO$^{f/f}$ NEMOΔ^{hepa}). Error bars represent SD.

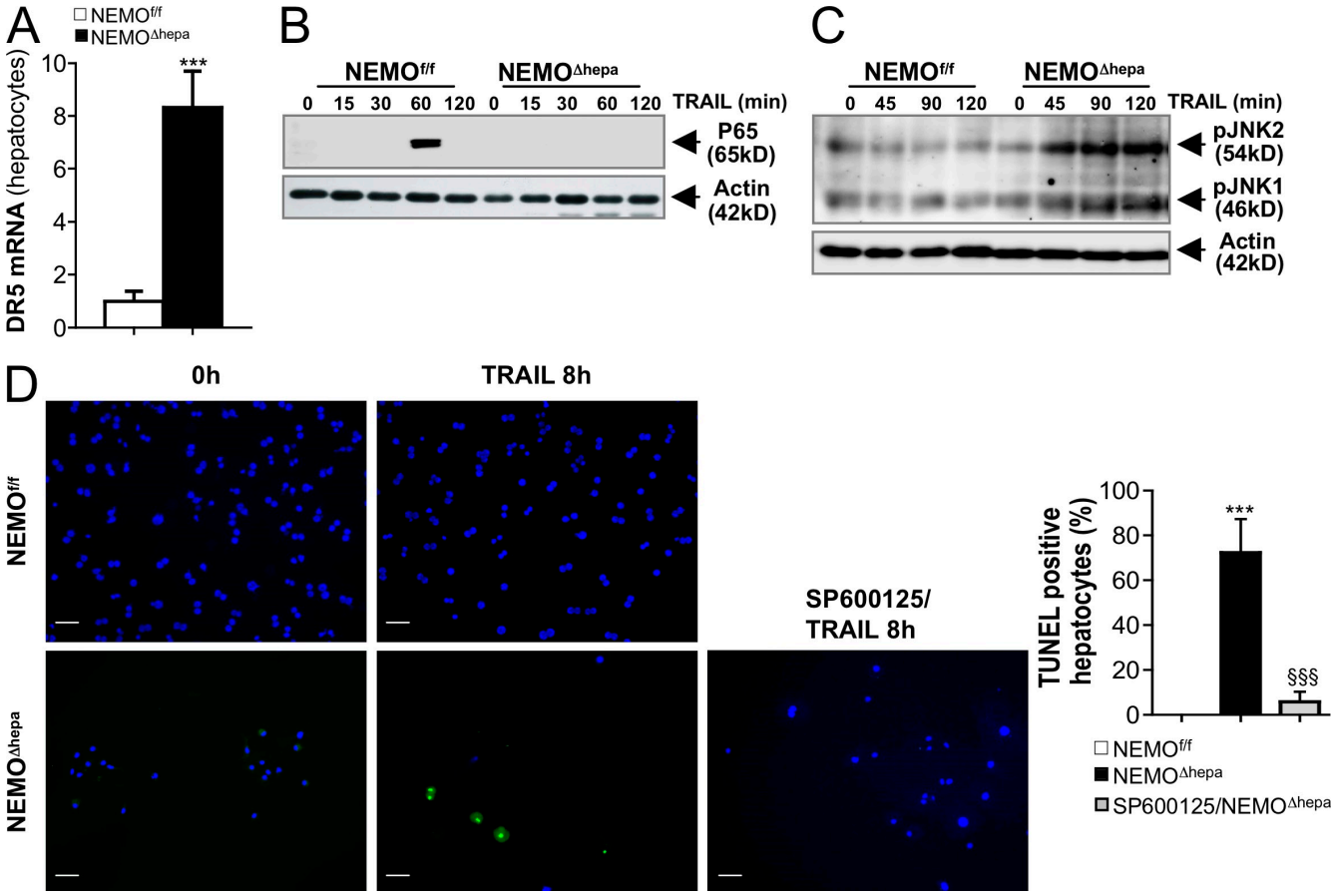

Figure S2. Hepatocytes isolated from NEMOΔhepa mice are hypersensitive to TRAIL–JNK–mediated apoptosis. (A) mRNA analysis of DR5 expression on isolated primary hepatocytes from NEMO$^{f/f}$ and NEMOΔ^{hepa}. (B) Western blotting on nuclear extracts showing transient p65 activation on WT cells that is blunted in NEMOΔ^{hepa} hepatocytes in response to 1 µg/ml TRAIL. (C) Western blot analysis using a p-JNK1/JNK2 antibody showing strong activation in NEMOΔ^{hepa} mice. JNK1 and GAPDH were used as loading controls. (D) TUNEL assay evidencing attenuation of TRAIL apoptosis in the presence of 20 µM of JNK inhibitor AS600125, 1 h before TRAIL treatment. Quantification of TUNEL-positive hepatocytes represented in a percentage related to the number of cells per power field. Bars, 50 µm. All data are representative of three independent experiments. n = 4. ***, P < 0.001 (NEMO$^{f/f}$ vs. NEMOΔ^{hepa}). §§§, P < 0.001 (NEMO$^{f/f}$ vs. SP600125/NEMOΔ^{hepa}). Error bars represent SD.

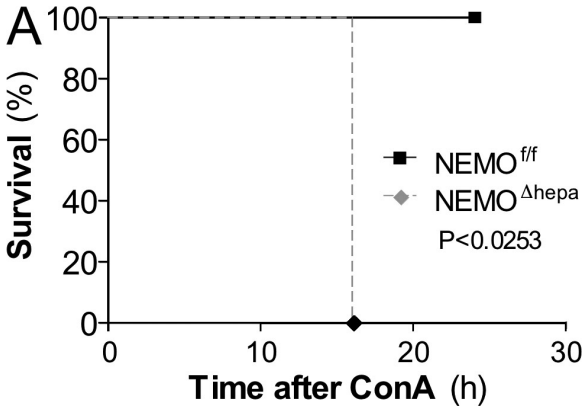

Figure S3. ConA promotes death of NEMOΔhepa mice. 100% of NEMOΔ^{hepa} mice died within 24 h after i.v. administration of 15 mg/kg ConA. All NEMO$^{f/f}$ mice survived the challenge. All data are representative of two independent experiments. *n* = 5–10.

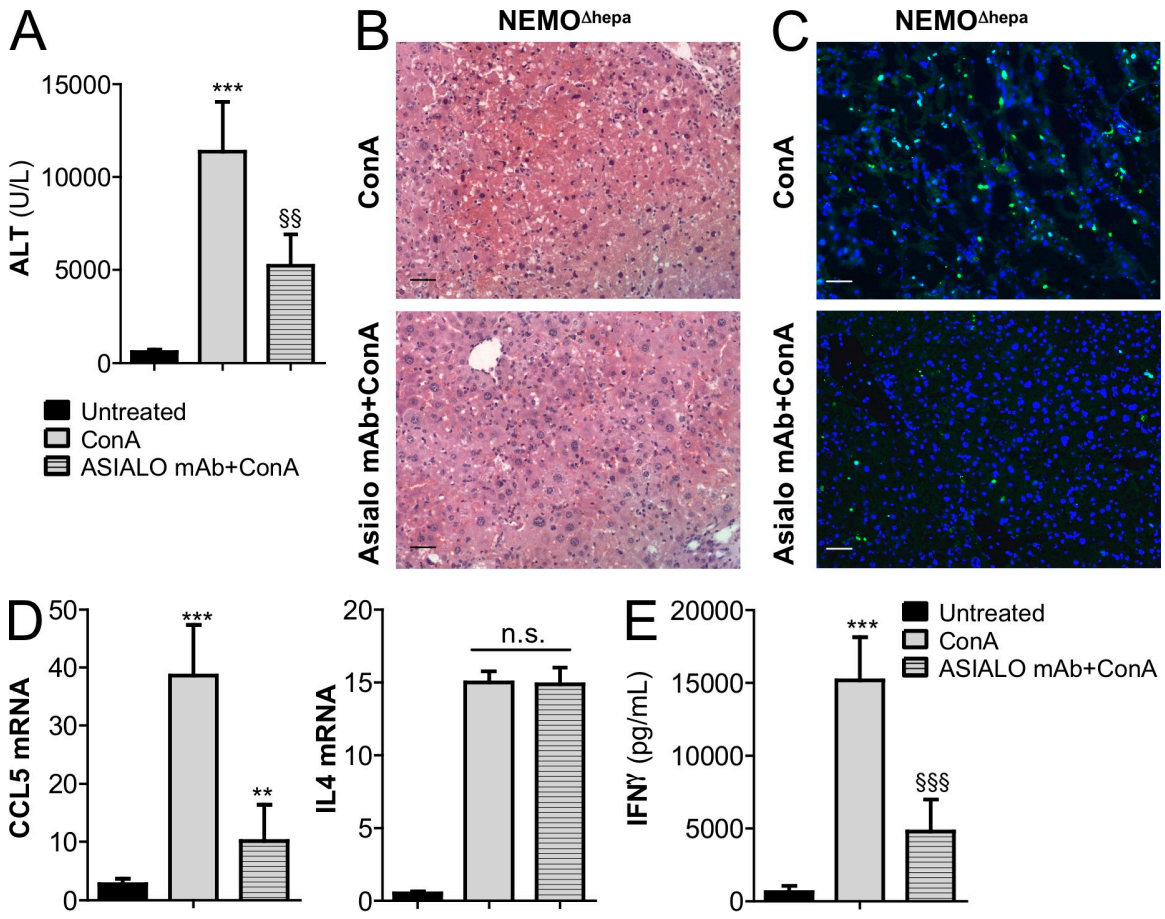

Figure S4. Selective NK cell depletion with asialo-GM1 mAb protects NEMOΔhepa mice against ConA hepatitis. 250 µg/mouse of Asialo-GM1 mAb was administered i.p., 40 h before ConA administration. (A–C) Serum ALT (A), H&E staining (B), and TUNEL assay (C) confirmed protective effect of selective depletion of NK cells against ConA. (D and E) mRNA analysis of CCL5, IL-4 (D), and serum IFN-γ levels (E). Bars, 50 µm. All data are representative of two independent experiments. *n* = 4. ***, P < 0.001 (NEMO$^{f/f}$ vs. NEMOΔ^{hepa}). §§, P < 0.01; §§§, P < 0.001 (NEMO$^{f/f}$ vs. Asialo/NEMOΔ^{hepa}).