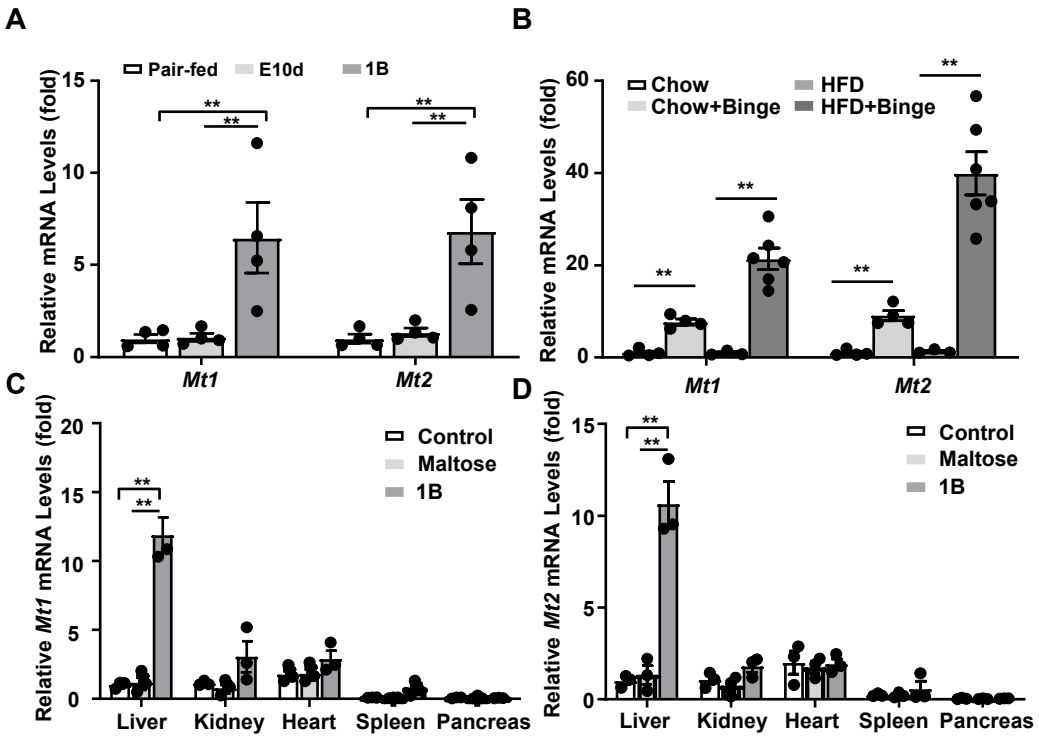
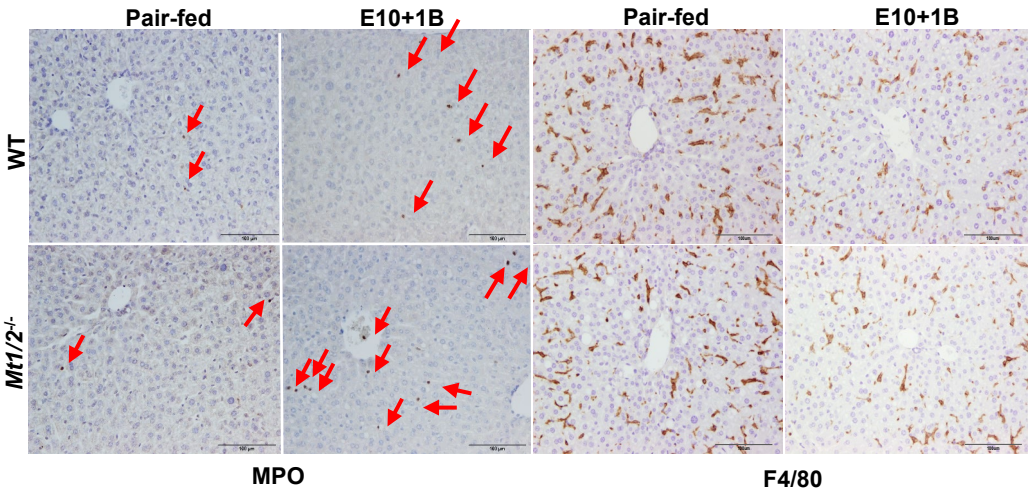
Supporting Table 1

Primer sequences for real-time PCR

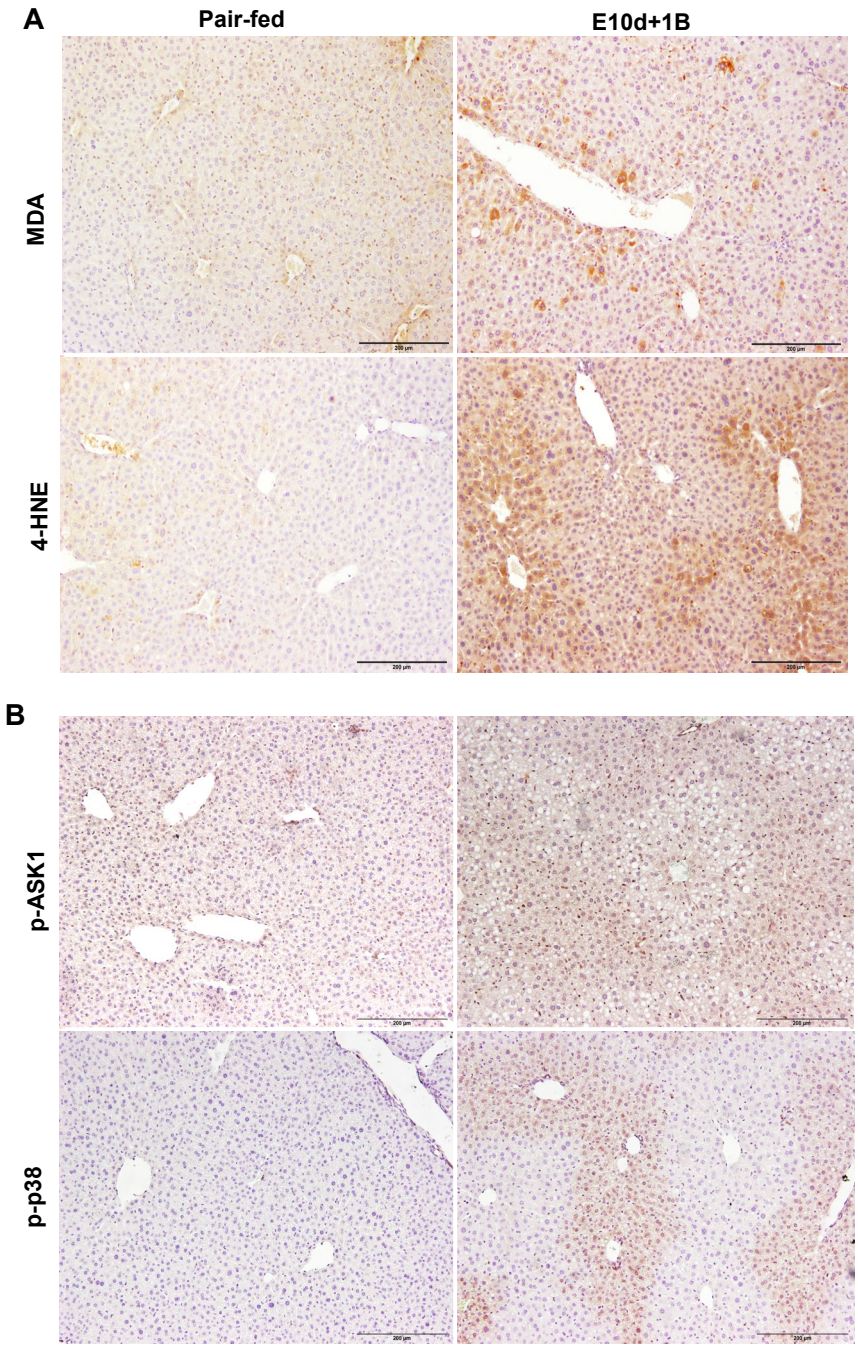
Genes	Forward primer (5'-3')	Reverse primer (5'-3')
18s	ACGGAAGGGCACCACCAGGA	CACCACCACCCACGGAATCG
Ly6g	TGCGTTGCTCTGGAGATAGA	CAGAGTAGTGGGGCAGATGG
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
Bak	AGACCTCCTCTGTGTCCTGG	AAAATGGCATCTGGACAAGG
Bax	GATCAGCTCGGGCACTTTAG	TTGCTGATGGCAACTTCAAC
Bim	GCTCCTGTGCAATCCGTATC	GCCCCTACCTCCCTACAGAC
Chop	GACCAGGTTCTGCTTTCAGG	CAGCGACAGAGCCAGAATAA
Gadd34	GGAGATAGAAGTTGTGGGCG	TTTTGGCAACCAGAACCG
Ero1a	CACAGGTACAGTCGTCCAGGT	CTTGCTCGTTGGACTCCTG
Ero1b	TGACAAAAAGGGGGCCAAGT	TATCGCACCCAACACAGTCC
Pdi	CCCCGTGTGTGGAAAAGAGA	AGCCACAGAGTAATGTGCCC
Il1b	TCGCTCAGGGTCACAAGAAA	CATCAGAGGCAAGGAGGAAAAC
Il6	TCCATCCAGTTGCCTTCTTG	TTCCACGATTTCCCAGAGAAC
Tnfa	AGGCTGCCCCGACTACGT	GACTTTCTCCTGGTATGAGATAGCAAA
Vcam1	TGAACCCAAACAGAGGCAGAGT	GGTATCCCATCACTTGAGCAGG
Icam1	CAATTTCTCATGCCGCACAG	AGCTGGAAGATCGAAAGTCCG
Mcp1	TCTGGACCCATTCCTTCTTGG	TCAGCCAGATGCAGTTAACGC
Mip1a	TGAGAGTCTTGGAGGCAGCGA	TGTGGCTACTTGGCAGCAAACA
Mip1b	AACACCATGAAGCTCTGCGT	AGAAACAGCAGGAAGTGGGA
p47^{phox}	TCCTCTTCAACAGCAGCGTA	CTATCTGGAGCCCCTTGACA
p67^{phox}	TCTATCAGCTGGTTCCCACG	CTATCTGGAGCCCCTTGACA
p40^{phox}	ATCGTCTGGAAGCTGCTCAA	CCCATCCATCTGCTTTTCTG
p22^{phox}	ATGGAGCGATGTGGACAGAAG	TAGATCACACTGGCAATGGCC
gp91^{phox}	GACCATTGCAAGTGAACACCC	AAATGAAGTGGACTCCACGCG
Mt1	AAGAGTGAGTTGGGACACCTT	CGAGACAATACAATGGCCTCC
Mt2	GCCTGCAAATGCAAACAATGC	AGCTGCACTTGTCGGAAGC
Rab11a	TGGGAAAACAATAAAGGCACAGA	ATGTGAGATGCTTAGCAATGTCA
Rab11b	ATTCAAAGTGGTGCTTATTGGGG	TCCGATGGTACTCTTGCTCTC
Rab35	CCACAATCGGAGTGGATTTCA	CGTCGTAAACCACAATGACCC
Rab5b	TGGGTAAAGGAACTACAGCGG	GGCCACCTTACTTCATACTCCA
Rab27a	TCGGATGGAGATTACGATTACCT	TTTTCCCTGAAATCAATGCCCA
Rab27b	CGTCAGGAAAAGCGTTTAAGGT	AGAAGCTCTGTTGACTGGTGA
Vamp7	GACAACTTACGGTTCAAGAGCA	TCTCCACGTTGAGCAACTAAATC
Vamp8	AGTGGGAGTGCCGGAAATG	TGAAGTGTTCAGACGTGGCTT
Hgs	TTCGAGCGTCTCCTAGACAAA	GCTTGTGTGTCCCCCTGAC
Pdcd61p	TAGTGTTTGCACGGAAGACAG	GGGAGGACTGATAGGCTGGA
Tsg101	TCTAACCGTCCGTCAAACTGT	TTGTACCAGTGAGGTTCACCA
Stam1	ACCCCTTCGACCAGGATGTT	CCACAGTTTGATACACATGCTCC
Vta1	AAGAGCATACAGCACCATTTGA	GCTTCATTATCCCCCAACTGTTT
Ykt6	AGTCAACTGATTGTGGAACGC	TCTGGAAGGGTATTCGCTGTC
nSmase2	ACACGACCCCCTTTCCTAATA	GGCGCTTCTCATAGGTGGTG
Bcl2	GTCGCTACCGTCGTGACTTC	CAGACATGCACCTACCCAGC
Bclxl	GACAAGGAGATGCAGGTATTGG	TCCCGTAGAGATCCACAAAAGT



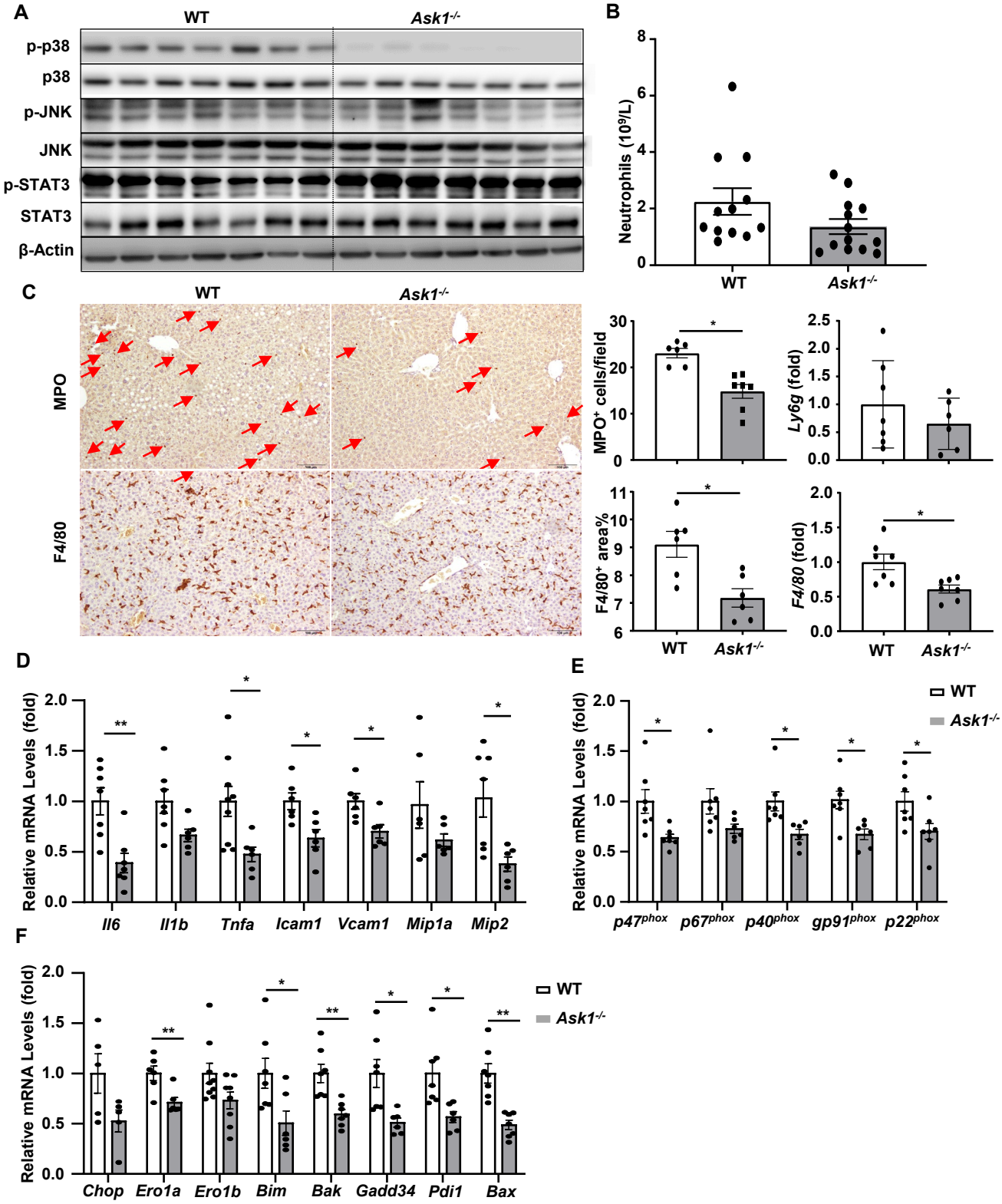
Supporting Fig. S1: *Mt* expression in different alcohol injury models. (A) C57BL6 N mice were pair-fed or fed an ethanol diet for 10 days or one binge (1B). *Mt1* and *Mt2* levels in liver tissue were examined by RT-qPCR. (B) C57BL6 N mice were fed chow diet for 3 months, chow diet for 3 months plus one binge, high fat diet (HFD) for 3 months or HFD for 3 months plus binge. *Mt1* and *Mt2* levels in liver tissue were examined by RT-qPCR. (C) Different tissues from control, maltose or one binge groups were subjected to RT-qPCR for *Mt1* expression. (D) Different tissues from control, maltose or one binge groups were subjected to RT-qPCR for *Mt2* expression. Values represent mean±SEM. Statistical evaluation was performed by Student's t-test or one-way ANOVA with Tukey's post hoc test for multiple comparisons. (**p<0.01)



Supporting Fig. S2: Infiltration of neutrophils and macrophages in the liver of *Mt1/2*$^{-/-}$ mice subjected to E10d+1B treatment. Mice were subjected to chronic-plus-binge ethanol feeding or pair-feeding. Mice were euthanatized 9 hours post gavage. Liver tissues from WT and *Mt1/2*$^{-/-}$ were subjected to immunostaining with an anti-MPO or anti-F4/80 antibody. Representative photographs are shown (scale bar:100μm).



Supporting Fig. S3: Representative images of oxidative stress and stress kinase activation staining in the livers of E10+1B model (scale bar:200μm). C57BL6N mice were pair-fed or ethanol diet for 10 day (E10d) or 10 day plus one binge (E10d+1B) and were euthanized 9 hours after gavage. (A) Liver tissues were subjected to immunostaining with anti-MDA and anti-4-HNE antibodies. (B) Liver tissues were subjected to immunostaining with anti-p-ASK1 and p-p38 antibodies.


A
WT
Ask1-/-
p-p38
p38
p-JNK
JNK
p-STAT3
STAT3
β-Actin
B
Neutrophils (10⁹/L)
WT
Ask1-/-
C
WT
Ask1-/-
MPO
F4/80
MPO+ cells/field
Ly6g (fold)
F4/80+ area%
F4/80 (fold)
D
Relative mRNA Levels (fold)
Il6
Il1b
Tnfa
Icam1
Vcam1
Mip1a
Mip2
E
WT
Ask1-/-
Relative mRNA Levels (fold)
p47phox
p67phox
p40phox
gp91phox
p22phox
F
WT
Ask1-/-
Relative mRNA Levels (fold)
Chop
Ero1a
Ero1b
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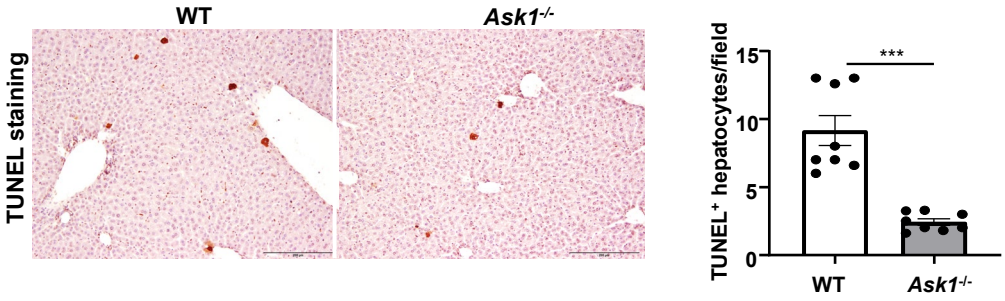
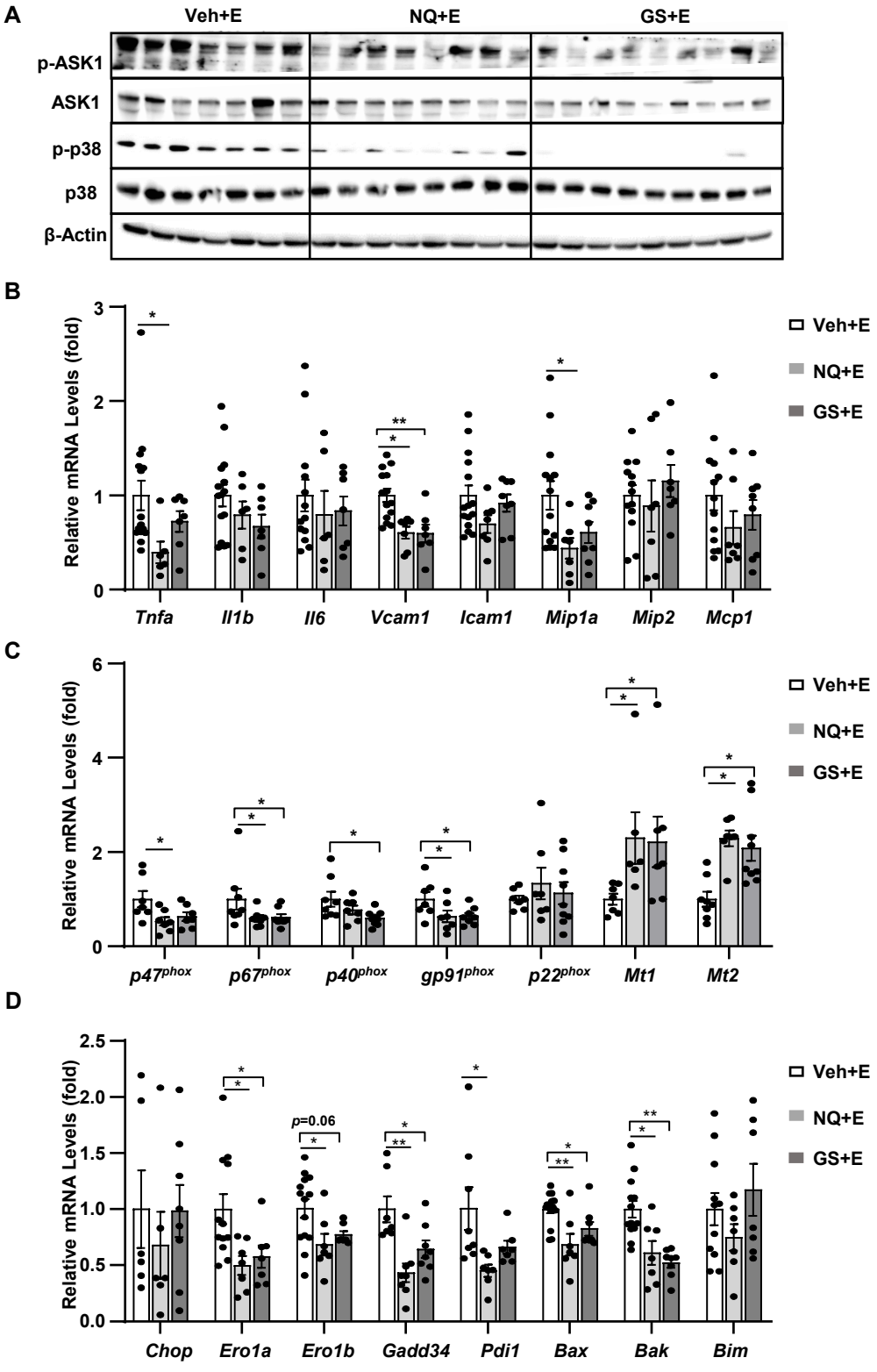
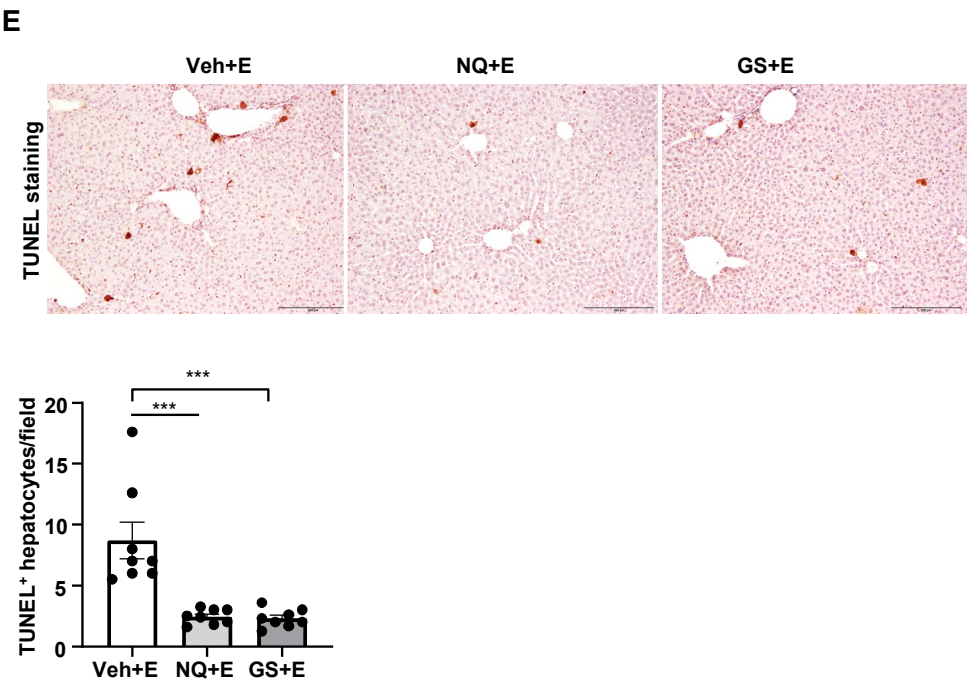
Bak
Gadd34
Pdi1
Bax

G

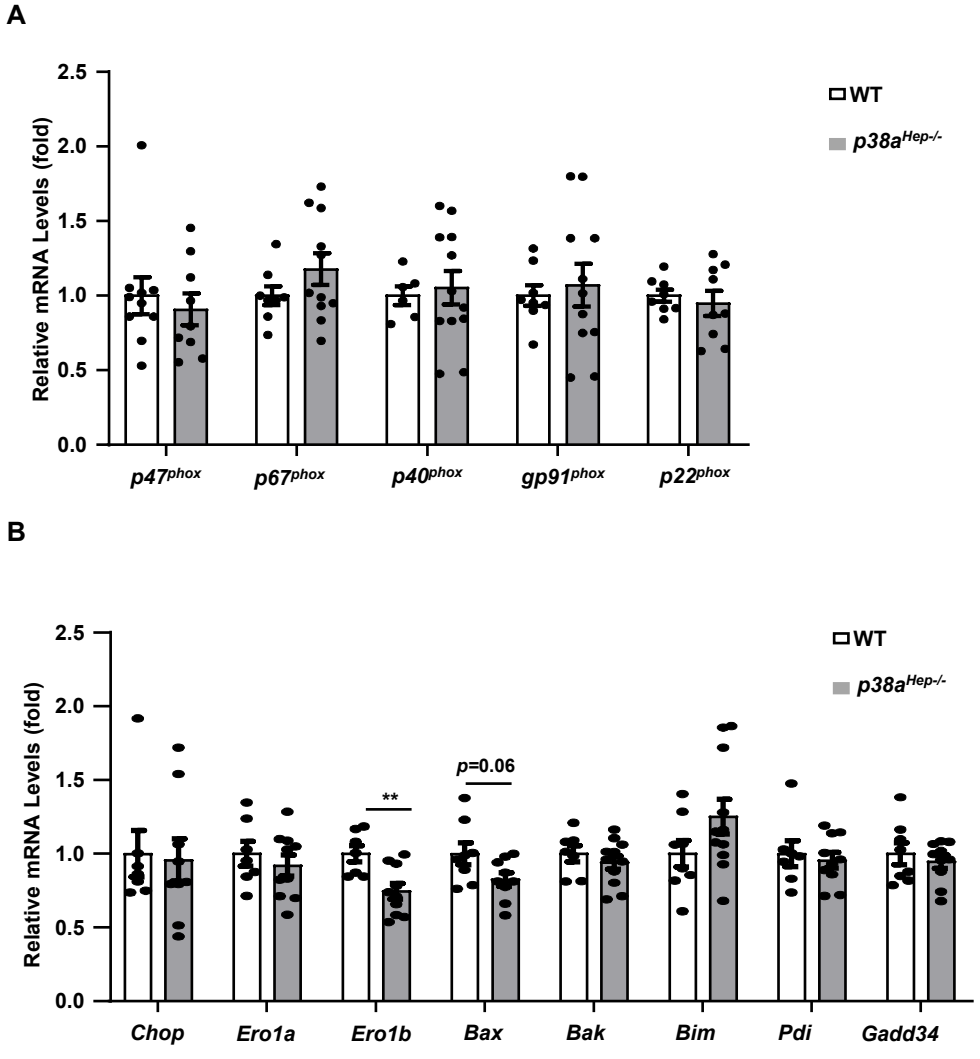


Supporting Fig. S4: Deletion of *Ask1* ameliorates chronic-plus-binge ethanol-induced liver injury. WT and *Ask1*$^{-/-}$ mice were subjected to E10d+1B feeding and were euthanized 9 hours after gavage. (A) Liver tissues were subjected to western blot analysis of p-p38, p38, p-JNK, JNK, p-STAT3 and STAT3 and β-actin; (B) Circulating neutrophil numbers were counted. (C) Liver tissues were subjected to immunostaining of MPO and F4/80. Representative photographs are shown on the left (scale bar:100μm). The number of MPO^{+} cells and the percentage of F4/80^{+} area were quantified on the right. Hepatic *Ly6g* and *F4/80* were detected by real-time PCR and were shown on the right; (D, E, F) Liver tissues were subjected to RT-qPCR analyses of inflammatory cytokine genes (panel D), ROS-associated genes (panel E), ER-stress-associated genes (panel F). (G) Liver tissues were subjected to TUNEL staining. Representative images are shown. Values represent mean ± SEM. Statistical evaluation was performed by Student's t-test. (*p<0.05; **p<0.01; ***p<0.001)

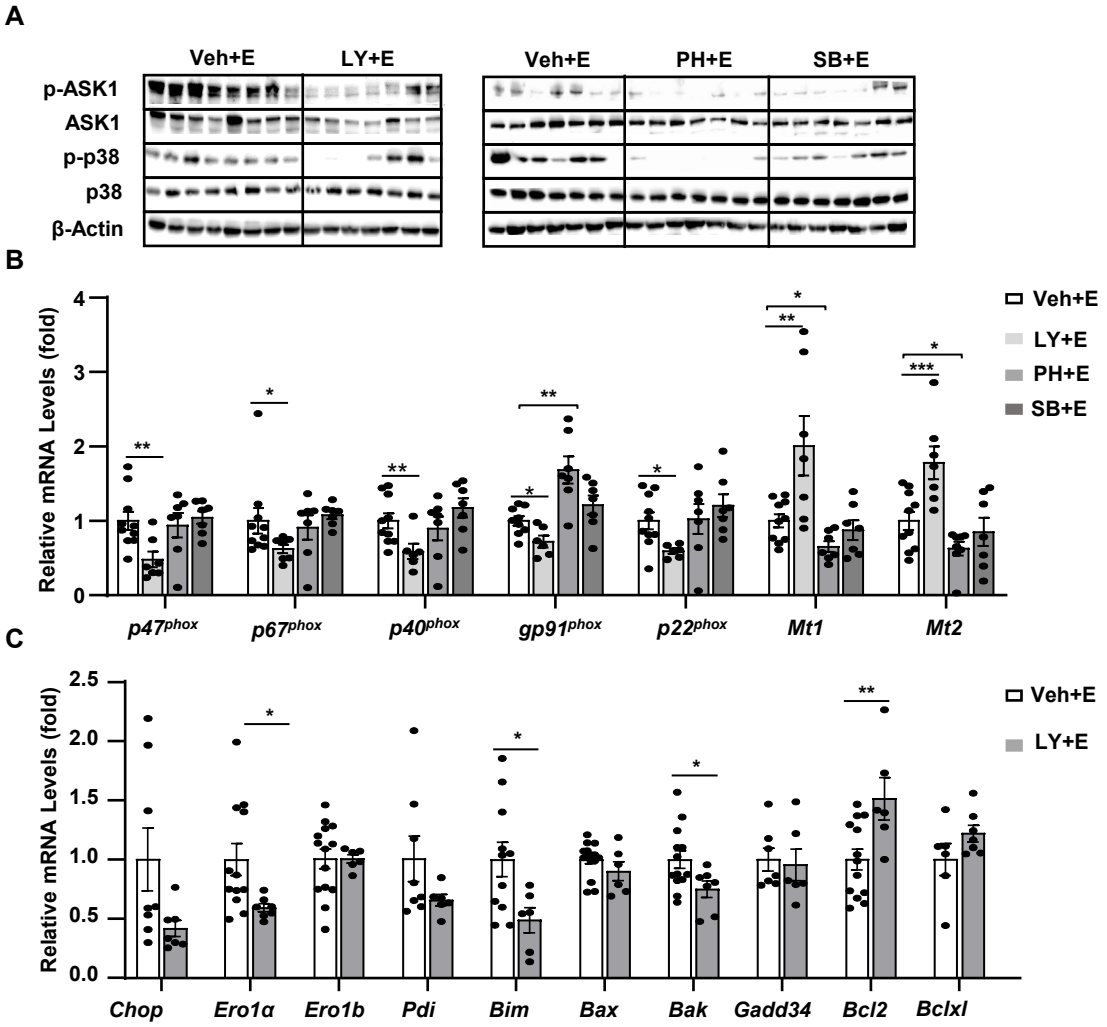

A
Veh+E
NQ+E
GS+E
p-ASK1
ASK1
p-p38
p38
β-Actin
B
Relative mRNA Levels (fold)
Veh+E
NQ+E
GS+E
Tnfa
Il1b
Il6
Vcam1
Icam1
Mip1a
Mip2
Mcp1
C
Relative mRNA Levels (fold)
Veh+E
NQ+E
GS+E
p47phox
p67phox
p40phox
gp91phox
p22phox
Mt1
Mt2
D
Relative mRNA Levels (fold)
p=0.06
Veh+E
NQ+E
GS+E
Chop
Ero1a
Ero1b
Gadd34
Pdi1
Bax
Bak
Bim



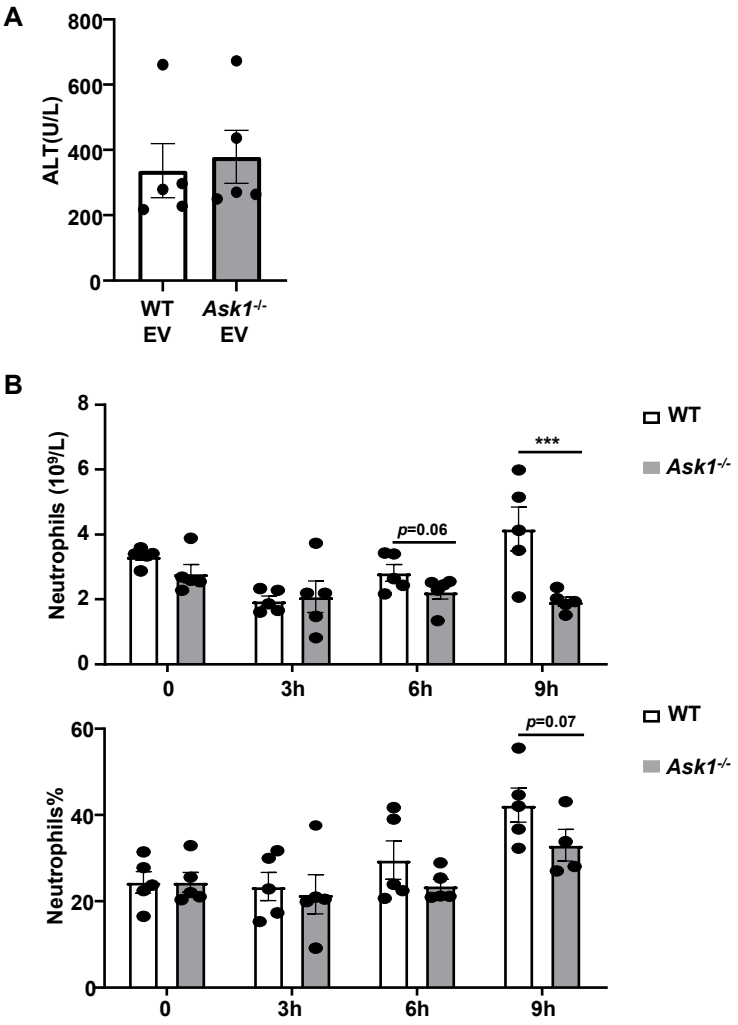
Supporting Fig. S5: Inhibition of ASK1 ameliorates chronic-plus-binge ethanol-induced liver injury. C57BL/6J mice were subjected to E10d+1B feeding and received i.p. injection of ASK1 inhibitors (GS-4997 at 3mg/kg; NQDI-1 at 4mg/kg) 30min before gavage. Mice were euthanized 9 hours after gavage. (A) Western blot analysis of p-ASK1, ASK1, p-p38, p38 and β-Actin. (B, C, D) Liver tissues were subjected to RT-qPCR analyses of inflammatory genes (panel B), reactive oxygen speices (ROS)-associated genes (panel C), and ER-stress-associated genes (panel D). (E) Liver tissues were subjected to TUNEL staining. Representative images are shown. Values represent mean ± SEM. Statistical evaluation was performed by Student's t-test or one-way ANOVA with Tukey's post hoc test for multiple comparisons. (*p<0.05; **p<0.01; ***p<0.001).



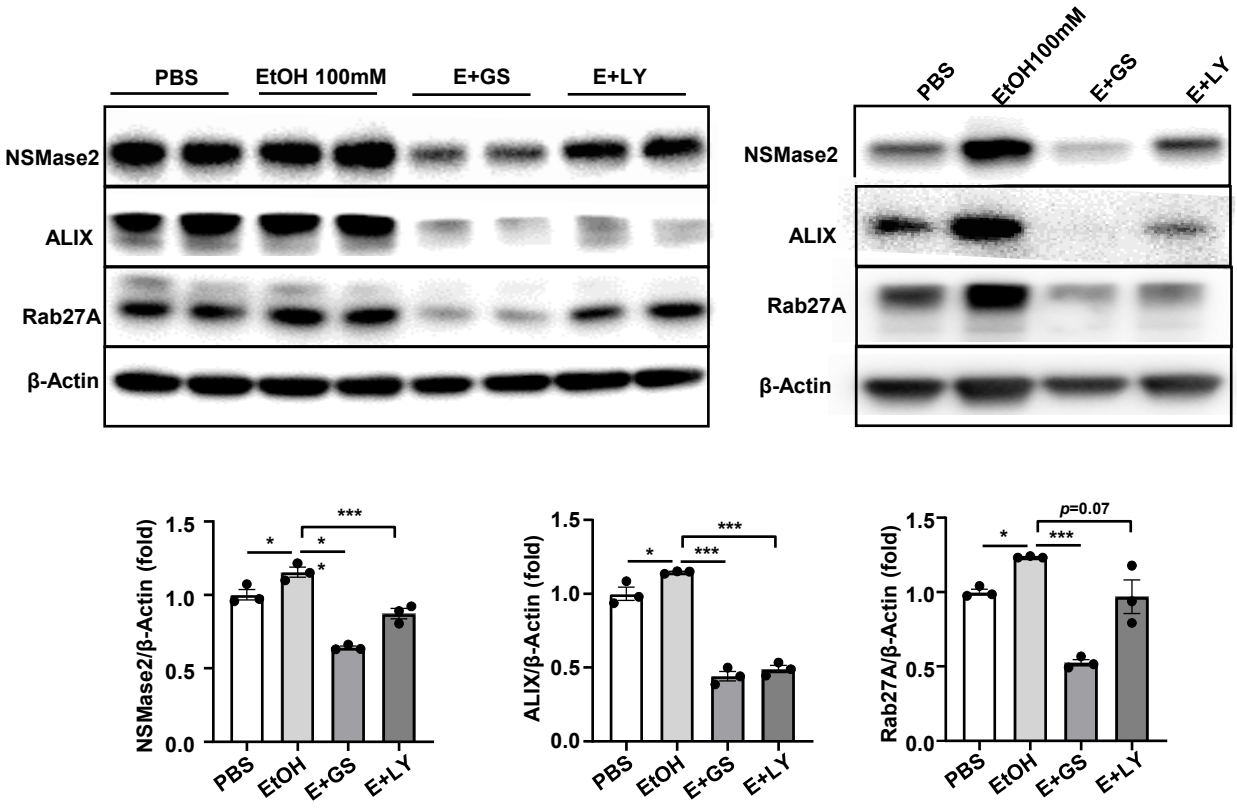
Supporting Fig. S6: Hepatocyte-specific deletion of *p38a* gene ameliorates chronic-plus-binge ethanol-induced liver injury. WT and *p38a*$^{Hep-/-}$ mice were subjected to E10d+1B feeding and were euthanized 9 hours after gavage. Liver tissues were subjected to RT-qPCR analyses of ROS-associated genes (panel A) and ER-stress-associated genes (panel B). Values represent mean ± SEM. Statistical evaluation was performed by Student's t-test. (**p<0.01).



Supporting Fig. S7: Inhibition of p38 ameliorates chronic-plus-binge ethanol-induced liver injury. C57BL/6J mice were subjected to E10d+1B feeding and received i.p. injection of p38 inhibitors (LY2228820 at 3mg/kg, PH797804 at 12mg/kg and SB239063 at 10mg/kg) 30min before gavage. Mice were euthanized 9 hours after gavage. (A) Liver tissues were subjected to western blot analysis of p-ASK1, ASK1, p-p38, p38 and β-actin; (B, C) Liver tissues were subjected to RT-PCR analyses of ROS-associated genes (panel B) and ER-stress-associated genes (panel C). Values represent mean ± SEM. Statistical evaluation was performed by Student's t-test or one-way ANOVA with Tukey's post hoc test for multiple comparisons. (*p<0.05; **p<0.01; *** p<0.001).



Supporting Fig. S8: EVs derived from E10d+1B treated WT but not *Ask1*$^{-/-}$ mice induce neutrophilia in E10d-treated mice. E10d-fed B6J mice were i.v. injected with EVs isolated from either E10d + 1B treated WT or *Ask1*$^{-/-}$ mice. Mice were euthanized 9 hours after injection. (A) Serum ALT levels and (B) the numbers and percentage of peripheral neutrophils were measured (n = 5 per group). Student's t test was performed. ***p< 0.001.



Supporting Fig. S9: Inhibition of ASK1 and p38 kinases attenuates the expression of EV biogenesis induced by ethanol in AML12 cells. AML12 cells were pretreated with GS-4997 (30μM, 1 hr) or LY2228820 (2 μM, 1 hr) and then exposed to ethanol (100mM) for 24h. AML12 cells were collected for western blot analysis of nSMase2, ALIX, Rab27A and β-Actin. Statistical evaluation was performed by Student's t-test. (*p<0.05; ***p<0.001). PBS: phosphate-buffered solution.