## MATERIALS AND METHODS

### Histopathological analysis

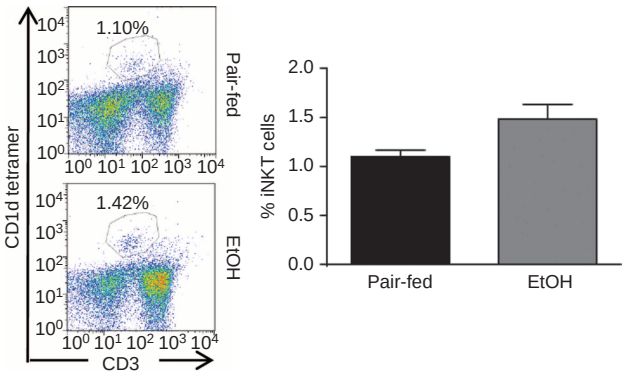
Liver tissue was collected 9 h post-gavage, and the tissue samples were fixed in 10% formalin and paraffin-embedded following a standard procedure. The embedded liver tissue was cut to a thickness of 4 μm and subjected to staining with hematoxylin and eosin (H&E) or subjected to immunohistochemistry using antibodies specific for Cyp2E1 (EMD Millipore Corp., Billerica, MA, USA) or myeloperoxidase (MPO; Biocare Medical, Concord, CA, USA).

### Analysis of blood neutrophils

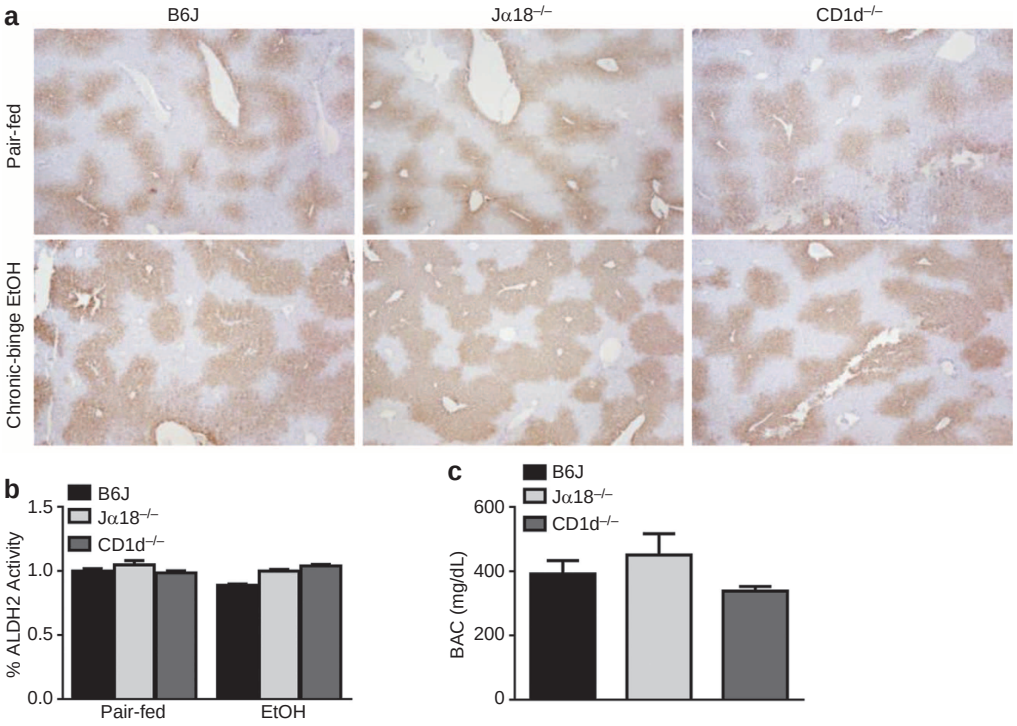
Blood from each mouse was collected in BD Microtainer tubes containing EDTA (BD Biosciences) to prevent coagulation. Following collection, 50 ml of blood was added to 200 ml of normal saline in a fresh BD Microtainer tube containing EDTA and sent to the Department of Laboratory Medicine at the NIH Clinical Center for analysis of neutrophil percentage and number.

### Real-time quantitative polymerase chain reaction (real-time PCR)

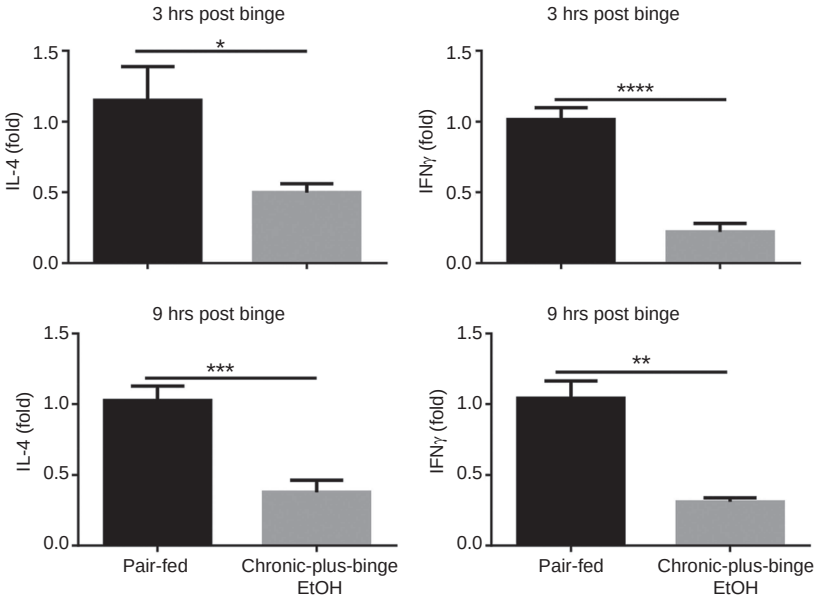
Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed in duplicate for each sample using the ABI PRISM 7500 Real-Time PCR System (Applied Biosystems). The reaction mixture contained 10 ml of SYBR Green Master Mix (Applied Biosystems), 0.5 mmol of forward and reverse primers (Invitrogen) and 2 ml of cDNA in a total volume of 20 μl. The PCR conditions were: 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Amplification of specific transcripts was confirmed *via* melting curve profiles generated at the end of the PCR program. Expression levels of the target genes were normalized to the expression of the 18 s ribosomal subunit and calculated based on the comparative cycle threshold $C_t$ method ($2^{-\Delta\Delta Ct}$). Primer sequences are shown in Supplementary Table 1.



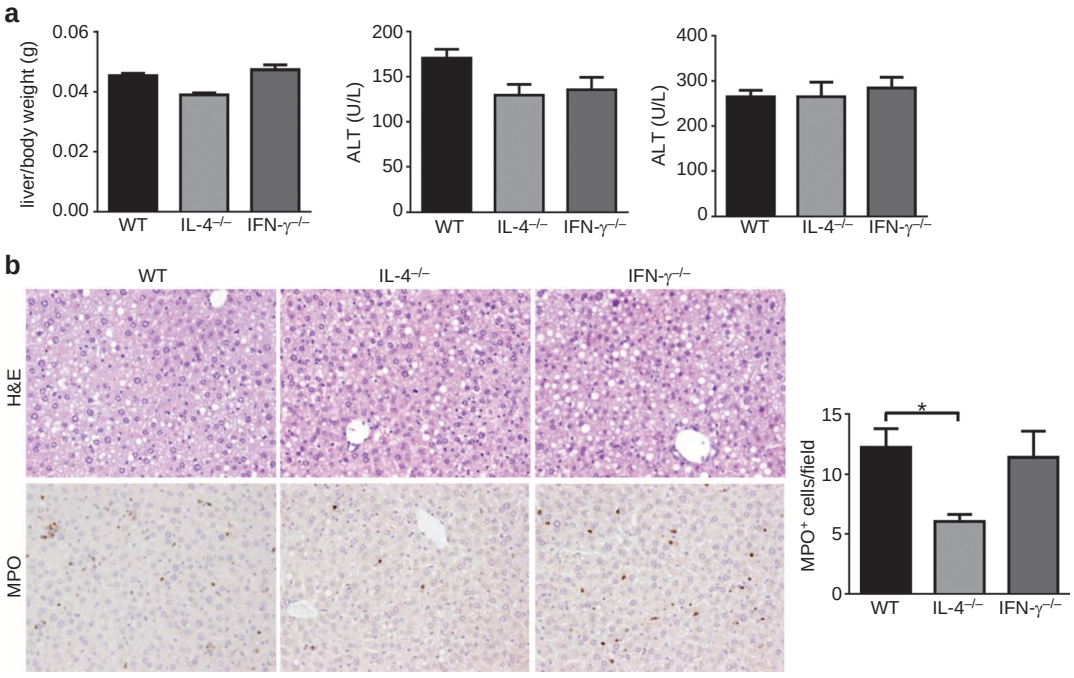

**Supplemental Figure 1** Chronic-plus-binge ethanol feeding does not increase splenic iNKT cell numbers in C57BL/6J mice. Lymphocytes were isolated from the spleens of chronic-plus-binge ethanol- or pair-fed WT mice and subjected to FACS analysis. A representative flow cytometry plot is shown on the left. The percentage of iNKT cells is shown on the right.



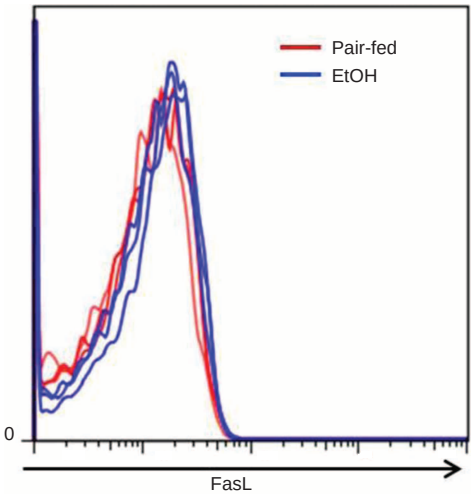

**Supplemental Figure 2** Hepatic CYP2E1 expression, hepatic ALDH2 activity, and blood alcohol concentration (BAC) are comparable between WT and iNKT-deficient mice following chronic-plus-binge ethanol feeding. (**a**) Liver tissue from ethanol-fed WT and iNKT cell-deficient mice was subjected to immunohistochemistry to assess the expression of CYP2E1. Representative immunostaining is shown. (**b**) Mitochondrial ALDH2 activity was measured in hepatic tissues via commercially available assay. (**c**) Blood alcohol content was measured 1 hour post-binge of a single gavage of ethanol (5g/kg body weight). No statistical differences were observed between WT and KO mice.




**Supplemental Figure 3** Hepatic IL-4 and IFN-$\gamma$ mRNA are downregulated in response to chronic-plus-binge ethanol feeding in C57BL/6J mice. RNA was isolated from the livers of chronic-plus-binge or pair-fed mice and analyzed by real-time PCR for the expression of IL-4 and IFN-$\gamma$ mRNA at 3 h or 9 h post-binge.




**Supplemental Figure 4** IL-4 contributes to chronic-plus-binge ethanol-induced neutrophil infiltration. WT, IL-4$^{-/-}$, and IFN-$\gamma^{-/-}$ mice were subjected to chronic-plus-binge ethanol feeding. Mice were euthanized 9 hours post-binge. (**a**) Liver/body weight ratios and serum ALT and AST levels were measured. (**b**) Liver tissues were subjected to H&E staining and MPO staining. Representative images are shown. The numbers of MPO$^{+}$ cells per field were calculated and are shown on the right. *$p<0.05$ compared to WT.




**Supplemental Figure 5** Chronic-plus-binge ethanol feeding does not induce FasL expression on NKT cells in C57BL/6J mice. Hepatic lymphocytes were isolated from chronic-plus-binge ethanol-fed WT mice, and iNKT cells were analyzed for Fasl expression.

**Supplemental Table 1 Primer sequences for real-time PCR**

| *Gene* | *Forward (5′→3′)* | *Reverse (5′→3′)* |
|---|---|---|
| *18 s* | CTCAACACGGGAAACCTCAC | CGCTCCACCAACTAAGAACG |
| *TNF-alpha* | GAAGTTCCCAAATGGCCTCC | GTGAGGGTCTGGGCCATAGA |
| *IL-1beta* | AAAAAAGCCTCGTGCTGTCG | GTCGTTGCTTGGTTCTCCTTG |
| *IL-4* | GGTCTCAACCCCCAGCTAGT | GCCGATGATCTCTCTCAAGTGAT |
| *IL-6* | TCCATCCAGTTGCCTTCTTG | TTCCACGATTTCCCAGAGAAC |
| *IFN-gamma* | ACTGGCAAAAGGATGGTGAC | TGAGCTCATTGAATGCTTGG |
| *ICAM-1* | CAATTTCTCATGCCGCACAG | AGCTGGAAGATCGAAAGTCCG |
| *Ly6G* | TGCGTTGCTCTGGAGATAGA | CAGAGTAGTGGGGCAGATGG |
| *MCP-1* | TCAGCCAGATGCAGTTAACGC | TCTGGACCCATTCCTTCTTGG |
| *MIP-1alpha* | TGAGAGTCTTGGAGGCAGCGA | TGTGGCTACTTGGCAGCAAACA |
| *MIP-2* | AAAATCATCCAAAAGATACTGAACAA | CTTTGGTTCTTCCGTTGAGG |
| *OPN* | GATGATGATGACGATGGAGACC | CGACTGTAGGGACGATTGGAG |
| *SELE* | AGCAGAGTTTCACGTTGCAGG | TGGCGCAGATAAGGCTTCA |