

1 *Conventional type 1 dendritic cells protect against gut barrier*  
2 *disruption via maintaining A. muciniphila in alcoholic*  
3 *steatohepatitis*

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## 1 ***Supplementary Materials and Methods***

### 2 ***Isolation of liver immune cells***

3 Liver immune cells were isolated, as previously described [1] with some  
4 modifications. Briefly, mice were anesthetized, and the livers were firstly perfusion  
5 with cold PBS via the portal vein and followed by digested for 15 min at 37°C in  
6 digestion buffer: RPMI 1% FCS containing 0.1mg/ml DNase-I (Sigma-Aldrich, St.  
7 Louis, MO), 0.2 mg/ml Collagenase P (Sigma-Aldrich, St. Louis, MO) and 0.8 mg/ml  
8 Dispase II (Sigma-Aldrich, St. Louis, MO). After 30 min digestion, dissociated cells  
9 were collected and filtered through a 100 µm cell strainer (BD Biosciences, San Jose,  
10 CA) followed by centrifuge at 50 g for 3 min at 4°C. Supernatant were collected for  
11 measuring the proportion of neutrophils, monocytes, and macrophage in the liver.

### 12 ***Isolation of immune cells from intestine***

13 The immune cells in SILP, LILP, PP and MLN were isolated as described previously  
14 [2]. Briefly, mice were anesthetized, and the intestinal epithelial cells were dissected  
15 and dissociated by shaking in warm Ca<sup>2+</sup>/Mg<sup>2+</sup>-free HBSS with 5% FBS and 2mM  
16 EDTA buffer for 20 min at 37 °C for twice. Then the tissue were cut into small pieces  
17 and enzymatically digested with Collagenase IV (0.5 mg/ml, Sigma-Aldrich, St. Louis,  
18 MO) and DNase I (12.5 mg/ml, Sigma-Aldrich, St. Louis, MO) for 40 min at 37 °C  
19 while shaking and filtered prior to analysis. For DC purification, intestinal  
20 macrophages were firstly excluded by F4/80 magnetic beads. Then, MojoSort™  
21 Mouse CD11c Nanobeads (Biolegend, Dedham, MA) were used to enrich for DCs  
22 prior to cell sorting by BD FACSMelody™ cell sorter.

### 23 ***Flow cytometry***

1 Cells were counted using a cell count Analyzer.  $2 \times 10^7$  cells were resuspended in 100  
2  $\mu\text{l}$  ice-cold staining buffer containing murine Fc-block (CD16/32) antibody  
3 (BioLegend, Dedham, MA) and incubated for 5 minutes on ice to reduce non-specific  
4 binding. Cell debris and doublets were gated out using FSC-A vs. SSC-A and FSC-A  
5 vs. FSC-H gates, respectively. Dead cells were distinguished using 7-AAD (5  $\mu\text{l}$ /per  
6 test; BioLegend, Dedham, MA) antibody. Single cells were stained in FACS buffer  
7 (BioLegend, Dedham, MA) as described previously [1]. Briefly, cells were  
8 resuspended in 100 $\mu\text{l}$  FACS buffer containing the antibodies (Supplementary table 3)  
9 to various surface markers for further 30 minutes on ice. Following incubation,  
10 samples were analyzed using a BD FACSMelody™ flow cytometer (BD Bioscience,  
11 Heidelberg Germany). For intracellular markers, cells were fixed, permeabilized, and  
12 stained using the Cytofix/Cytoperm kit (BD Biosciences, Heidelberg Germany),  
13 following the manufacturer's guidelines. Data were analyzed with FlowJo software  
14 (TreeStar, Ashland, OR).

### 15 *In vitro cultures*

16 For intestinal dendritic cells isolation and cultures, intestinal macrophages were first  
17 excluded by F4/80<sup>+</sup> magnetic beads (Thermo Fisher Scientific, USA). Next,  
18 nanobeads (Biolegend, Dedham, MA)-enriched CD11c<sup>+</sup> cells were sorted by a cell  
19 sorter and suspended in RPMI 1640 containing with 10% FCS (Sigma-Aldrich, St.  
20 Louis, MO), 1 mM sodium pyruvate (Gibco, USA), 10 mM HEPES, 100 U/ml  
21 Penicillin and 100  $\mu\text{g}/\text{ml}$  Streptomycin, 50  $\mu\text{g}/\text{ml}$  Gentamycin (Gibco, USA) (R10  
22 medium) and plated ( $2.5 \times 10^5$  cells/well) in 96 well plates in the presence/absence of  
23 CpG ODN1585 (7.5  $\mu\text{g}/\text{ml}$ , InvivoGen) for 40h at 37°C. IL-12 levels in culture  
24 supernatants was assessed using the Mouse IL-12 ELISA Kit (R&D System, USA)  
25 according to manufacturers instructions.

### 1 ***Generation of CD103<sup>+</sup> cDC1 in vitro***

2 Bone marrow derived MHCII<sup>+</sup>CD11c<sup>+</sup>CD103<sup>+</sup>CLEC9A<sup>+</sup> cDC1 were generated as  
3 previous described [3]. Briefly, bone marrow was taken from the tibias and femurs of  
4 C57BL/6J mice, single cell suspension was prepared and red cells were lysed using  
5 ACK lysing buffer (Biolegend, MA) according to manufacturer's guidelines. Cells  
6 were cultured in RPMI containing 10% FBS, 1% PS, 0.1% bME and 5 ng/ml GM-  
7 CSF and 200 ng/ml Flt3L for 15 days as described previously [3]. At the day 15, non-  
8 adherent cells were collected and were verified via flow cytometry for MHCII,  
9 CD11c, CLEC9A and CD103 expression before injection.

### 10 ***Animal treatment***

11 Recombinant mouse IFN- $\gamma$  (R&D Systems, CA) or mouse IL-12 (R&D Systems, CA)  
12 was given to AF mice at 300 ng/mouse (IFN- $\gamma$ ) and 5  $\mu$ g/mouse (IL-12), respectively,  
13 twice a week through intraperitoneal injection for the last four weeks. To detect the  
14 role of *A. muciniphila* in ALD. Mouse was orally administered with  $5 \times 10^8$  CFU *A.*  
15 *muciniphila* every other day for the last four weeks. For *Lactobacillus reuteri*  
16 administration,  $1.5 \times 10^9$  CFU *L. reuteri* were orally administrated every other day for  
17 the last four weeks. For the cDC1s adaptive transfer, identified  $1 \times 10^6$  cDC1s were  
18 injected in sterile 150 $\mu$ l PBS intravenously one time/week for the last four weeks.

### 19 ***Cultivation of A. muciniphila and L. reuteri***

20 *A. muciniphila* (ATTC, USA) was were cultured anaerobically in BHI (brain-heart-  
21 infusion) broth (BD Bioscience, San Jose, CA) supplemented with 0.5% porcine  
22 mucin and 0.05% cysteine (Sigma-Aldrich, St. Louis, MO) as previous described [4].  
23 Cultures were washed and concentrated in anaerobic PBS with 25% (vol/vol) glycerol  
24 under anaerobic conditions. Before the administration, *A. muciniphila* was scraped  
25 from the agar plates, diluted in sterile PBS, and kept under anaerobic conditions until

1 administration. *L. Reuteri* (ATTC, USA) was cultured and enriched. Stock cultures  
2 were maintained at  $-80^{\circ}\text{C}$ . The *L. Reuteri* cultures were revived by streak plating on  
3 DeMan, Rogosa Sharpe (MRS) agar (BD Difco™, Franklin Lakes, NJ, USA) and  
4 incubated at  $37^{\circ}\text{C}$  for 24 h in a incubator. A single colony was picked and enriched in  
5 MRS broth for 18 h at  $37^{\circ}\text{C}$ . Then, the cultures were incubated under aerobic  
6 conditions at  $37^{\circ}\text{C}$

### 7 ***Cecal Microbial Community Analysis***

8 Cecal content samples were collected and stored at  $-80^{\circ}\text{C}$  until further processing.  
9 The V4 region of the bacterial 16S rRNA genes were PCR-amplified and sequenced  
10 on Illumina MiSeq platform using the MiSeq Reagent kit V2 (Illumina Inc., San  
11 Diego, CA) after DNA extraction from the mouse cecal samples with DNeasy  
12 PowerLyzer PowerSoil kit (Qiagen, Germantown, MD), as we previously reported.  
13 The acquired 16S rRNA MiSeq data were analyzed using Mothur software package  
14 (v.1.39.5), quality-filtered, aligned against SILVA v132 database, clustered into  
15 operational taxonomic units (OUT) with 97% similarity, and classified against the  
16 Ribosomal Database Project. Distance matrices (beta diversity) between the samples  
17 were explored by Bray-Curtis and J-Class distance matrices. Phylogenetic  
18 investigation of communities by reconstruction of unobserved states (PICRUSt) was  
19 used to predict functional genes of the cecal microbiota based on taxonomy obtained  
20 from the Greengenes reference database 13.5.

### 21 ***Protein omics analysis of isolated intestinal cDCs.***

#### 22 *Sample preparation*

23 The sorted intestinal dendritic cells (10,000 – 100,000 cells per sample) from eight  
24 mice in the control group and seven excessed ethanol feeding mice group extracted  
25 the proteins by adding 20  $\mu\text{L}$  of 0.1% DDM (n-Dodecyl- $\beta$ -D-Maltoside, Sigma

1 Aldrich, Cat# D4641) with 5 mM DTT (Dithiothreitol, Sigma Aldrich, Cat#  
2 11583786001) in 50 mM TEAB followed by water-based sonication for 10 min and  
3 heating at 70°C for 40 min. The lysate was alkylated by adding 5 mM IAA  
4 (Iodoacetamide, Sigma Aldrich, Cat# I6125) and incubated at room temperature for  
5 60 min in dark. Enzymatic digestion was performed by adding 1:5 enzyme to protein  
6 ratio of Trypsin/Lys-C (Protease Mix, Thermo science, Cat# A40009) and incubation  
7 overnight at 37°C, followed by acidifying the peptides in 1% formic acid.

#### 8 *Liquid chromatography and mass spectrometry*

9 The concentration of peptides in each sample was measured by a fluorescence peptide  
10 assay kit (Thermo Fisher Scientific, Cat# 23290), and 200 ng peptides per sample  
11 were loaded into EvoTips, which are disposable trap columns designed for the Evosep  
12 One TM LC system (EV-1000, Evosep, Denmark) and separated by an analytical  
13 column (3µm beads, 100µm ID, 8cm long) in 21 min (60 SPD method provided by  
14 Evosep). With the Orbitrap Exploris TM 240 MS (OE240, Thermo Fisher, Germany),  
15 MS spectra were scanned from 375 to 1500 m/z at a resolution of 60 k at 200 m/z  
16 followed by data-dependent HCD (30%) MS/MS at a resolution of 15 k at 200 m/z for  
17 1.8 sec cycle time. Other settings of the OE240 used for this study include a full MS  
18 AGC target of 3e6, MS/MS AGC target of 1e5, dynamic exclusion of 20 sec, and  
19 mass isolation window of 1.4 m/z, and a minimum intensity threshold of 1e5.

#### 20 *Data analysis and normalization*

21 Maxquant (Ver. 1.6.17.0, <https://www.maxquant.org/>) proteomics database search  
22 tool with an Android search engine was utilized to search the database (UniprotKB,  
23 08/06/2021, Mus Musculus). Default settings were used with the slight modification  
24 of some parameters. Briefly, identifications of peptides or proteins were cut off with a  
25 global FDR of less than 1%, and the minimum and maximum peptide length was set

1 to 6 and 25, respectively. Variable modifications were set with methionine oxidation  
2 and protein N-term acetylation, while fixed modification was set with cysteine  
3 carbamidomethyl. The match between runs feature was toggled on with 0.7 min of  
4 matching time window and 20 min of an alignment time window. Label-free  
5 quantification among the samples was quantified when there were at least two  
6 quantifiable peptides for a protein.

### 7 *Statistics and bioinformatics*

8 For the statistic analysis, the LFQ intensities from the Maxquant result were  
9 processed in Perseus (Ver. 1.6.14.0, <https://maxquant.net/perseus/>). Potential  
10 contaminants and reverse proteins were removed and all intensities were transformed  
11 to log2. If the valid values per protein existed more than 70% in at least one group, it  
12 was considered a quantifiable protein. The missing values were replaced by  
13 imputations based on the normal distribution of protein abundances. We also removed  
14 the batch effects from mice batches using Limma algorithm in the Perseus-R package.  
15 Differentially expressed proteins (DEPs) between two groups with the normalized  
16 abundances were defined by a two-sample t-test (permutation-based FDR<0.05, and  
17  $S_0=0.05$ ). The list of up- or down-regulated DEPs in the AF group was analyzed in  
18 Cytoscape (Ver 3.9.1) with the plug-in ClueGO app (Ver 2.5.8) to enrich biological  
19 functions based on the KEGG pathway database.

### 20 ***Immunofluorescence***

21 Immunofluorescence was applied to determine the levels of ileal tight junction protein.  
22 Cryostat sections of mouse ileum were incubated with anti-ZO-1 (Millipore,  
23 Burlington, MA) or anti-Occludin (Thermo Fisher Scientific, USA) followed by  
24 Alexa Fluor 594-conjugated donkey anti-rat IgG (Jackson ImmunoResearch  
25 Laboratories, West Grove, PA, United States) or Alexa Fluor 594-conjugated donkey

1 anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA,  
2 United States), respectively. For liver LCN2 staining, cryostat sections of mouse liver  
3 were incubated with anti-LCN2 (R&D System, USA) followed by Alexa Fluor 488-  
4 conjugated donkey anti-goat IgG. The nuclei were counterstained by 4',6-diamidino-  
5 2-phenylindole (DAPI; Thermo Fisher Scientific).

### 6 ***Biochemical analysis***

7 The levels of triglycerides and free fatty acids in liver were measured with  
8 Triglyceride Assay Kit and Free Fatty Acid Assay Kit (Biovision, CA), respectively,  
9 according to manufacturer's instructions as described previously [5]. Serum ALT and  
10 AST activity was calorimetrically measured using Infinity kits (Thermo Scientific,  
11 MA, USA) according to the manufacturer's instructions as previously described [6].  
12 Intestinal IFN- $\gamma$  and IL-12 protein levels were measured by commercial kits (R&D  
13 System, USA), respectively, according to manufacturer's instructions. Endotoxin  
14 levels in mouse blood and livers were determined using a chromogenic kinetic  
15 limulus amoebocyte lysate assay kit (QCL-1000, Lonza, Walkersville, MD). The  
16 concentrations of endotoxin were expressed in endotoxin units (EU) per milliliter for  
17 plasma and EU per milligram liver tissue.

### 18 ***Quantitative Real Time-PCR***

19 Total RNA was isolated from the intestine or liver and reverse-transcribed with  
20 TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA). The  
21 gene expression of related mRNAs was measured in triplicate by the comparative  
22 cycle threshold method using a 7500 real-time PCR system (Applied Biosystems,  
23 Foster City, CA). The primer sequences (Integrated DNA Technologies, IL, USA)  
24 were shown in Supplementary Table 1. The data were normalized to 18s rRNA



1 mRNA levels and presented as fold changes, setting the value of controls as 1. For the  
2 *A. muciniphila* detection. DNAs were isolated from approximately 250 mg of cecal  
3 contents using the QIAamp DNA Mini Kit (Qiagen, Germantown, MD) according to  
4 the manufacturer's instructions. Quantitative PCR (qPCR) was performed on a 7500  
5 Real-Time PCR System (Applied Biosystems, Carlsbad, CA) using *A. muciniphila*  
6 primers (forward 5'-CAGCACGTGAAGGTGGGGAC-3', reverse 5'-  
7 CCTTGCGGTTGGCTTCAGAT-3').

### 8 ***Western blot***

9 Whole protein lysates of livers were extracted using 10% Nonidet P-40 lysis buffer  
10 supplemented with protease inhibitor and phosphatase inhibitor (Sigma-Aldrich,  
11 USA). Aliquots containing 50 µg of proteins were loaded onto 8–12% SDS-PAGE,  
12 trans-blotted onto PVDF membrane, blocked with 4% nonfat milk for 1 h at room  
13 temperature, and incubated with antibodies (Supplementary Table 2) respectively.  
14 Membranes were washed and incubated with horseradish peroxidase–conjugated  
15 secondary antibodies (Thermo Scientific, Rockford, IL). Bound complexes were  
16 detected via enhanced chemiluminescence (GE Healthcare, Piscataway, NJ). Bands  
17 were quantified, and the ratio to β-actin was calculated and given as fold changes,  
18 setting the values of pair-fed at 1.

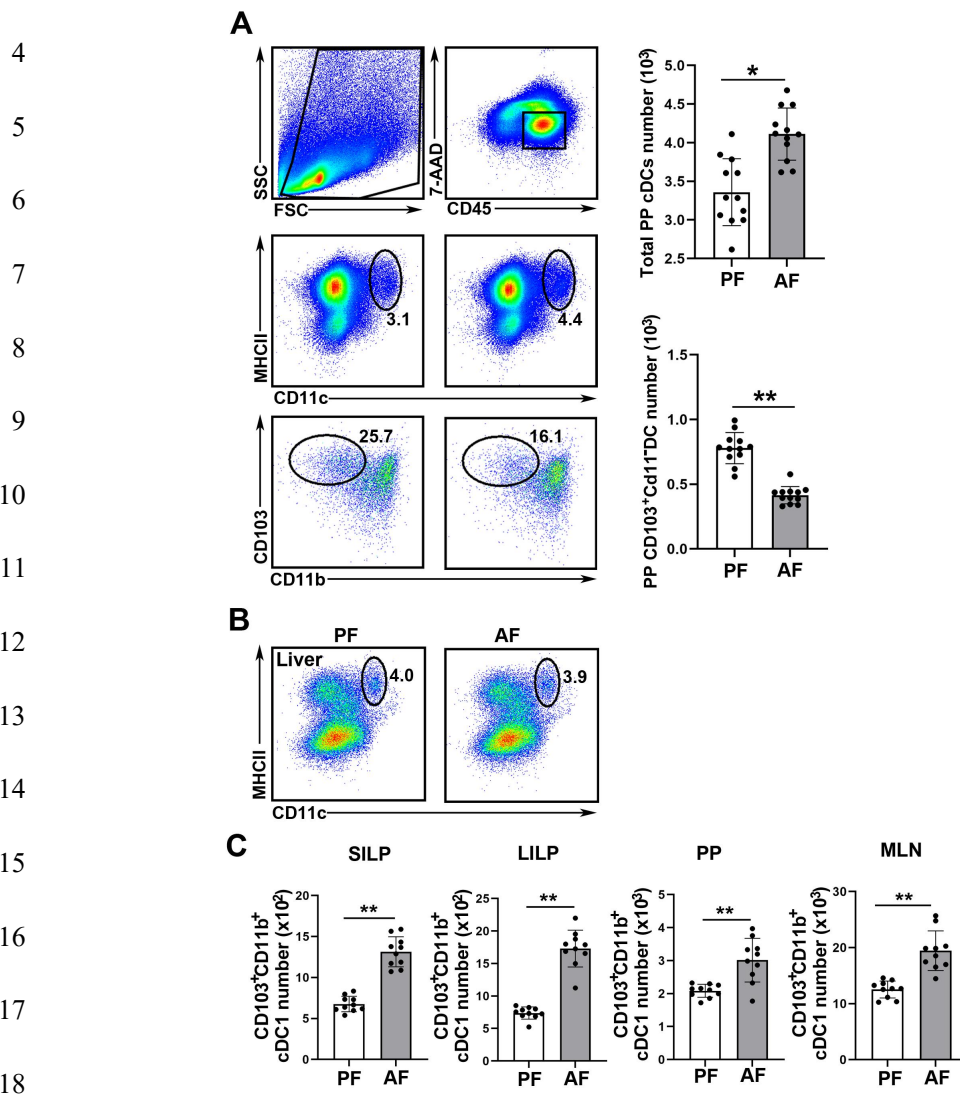
### 19 ***Statistical Analysis***

20 The analyses were performed using SPSS 21.0 software (SPSS, IL, USA). Data are  
21 expressed as the mean ± standard deviation (SD). Results were analyzed using the  
22 two-way analysis of variance (ANOVA) or Student's t-test where it was appropriate.  
23 In all tests, P values less than 0.05 were considered statistically significant.

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1 **Supplementary Figure**2 **Supplementary Fig. 1**

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19 **Supplementary Fig. 1. The effects of chronic alcohol consumption on cDCs**20 **number in PP and liver.** C57BL/6J WT mice were fed Lieber-DeCarli liquid diets

21 containing alcohol (alcohol-fed, AF) or isocaloric dextran (pair-fed, PF) for 8 weeks

22 plus a single binge (4 g/kg). (A) Gating strategy and representative dot plot for cDCs

23 and cDC1s in the PP (n=12). (B) Gating strategy and representative dot plot for

24 CD11c<sup>+</sup>MHCII<sup>+</sup> cDCs in the liver. (C) The role of chronic alcohol consumption on25 intestinal cDC2 numbers (n=10). Data are presented as means  $\pm$  SD. \* $P$ <0.05,26 \*\* $P$ <0.01 vs. PF mice.

1 **Supplementary Fig. 2**

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16 **Supplementary Fig. 2. Chronic alcohol consumption changes ileal cDCs**

17 **functions.** C57BL/6J WT mice were fed Lieber-DeCarli liquid diets containing

18 alcohol (alcohol-fed, AF) or isocaloric dextran (pair-fed, PF) for 8 weeks plus a single

19 binge (4 g/kg). (A) Principal components analysis plot. (B) Volcano plot. (C) Net

20 work of functional enrichment analysis.

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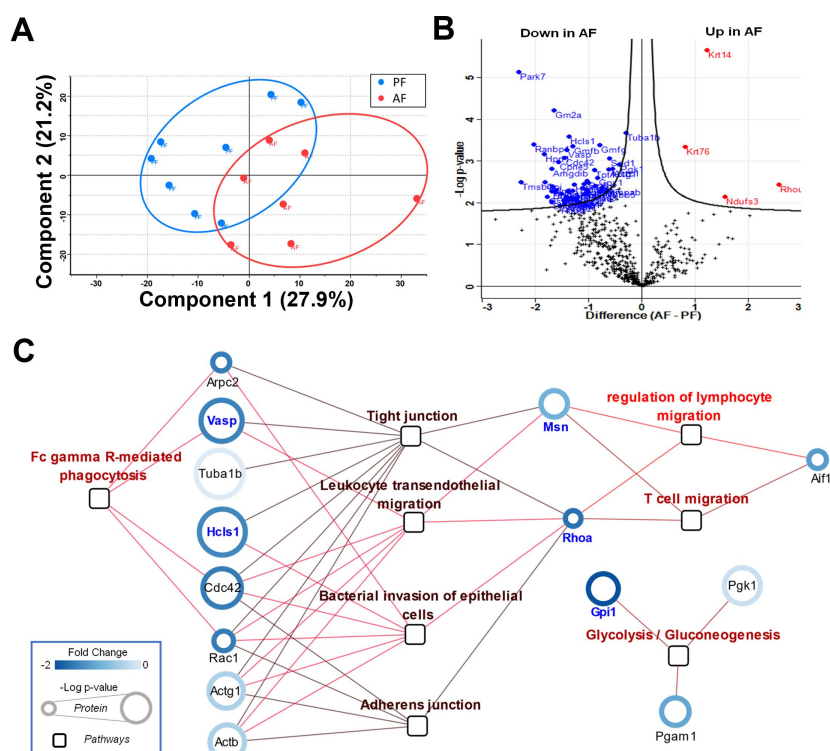
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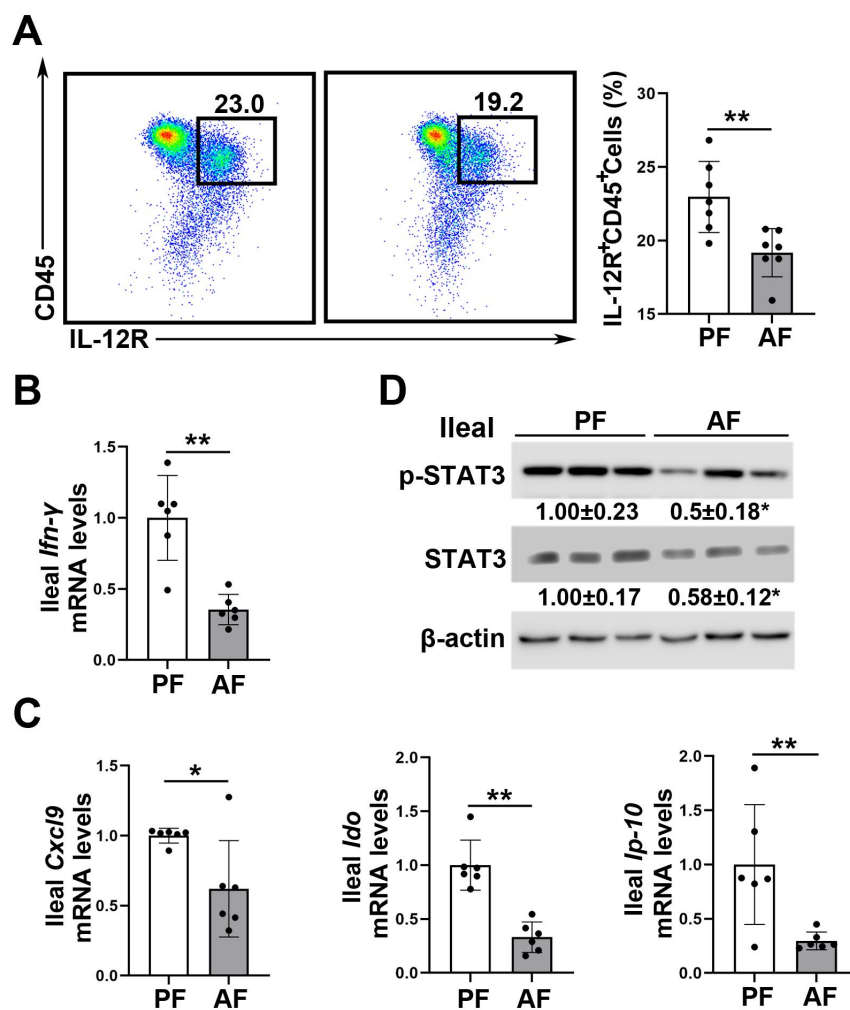
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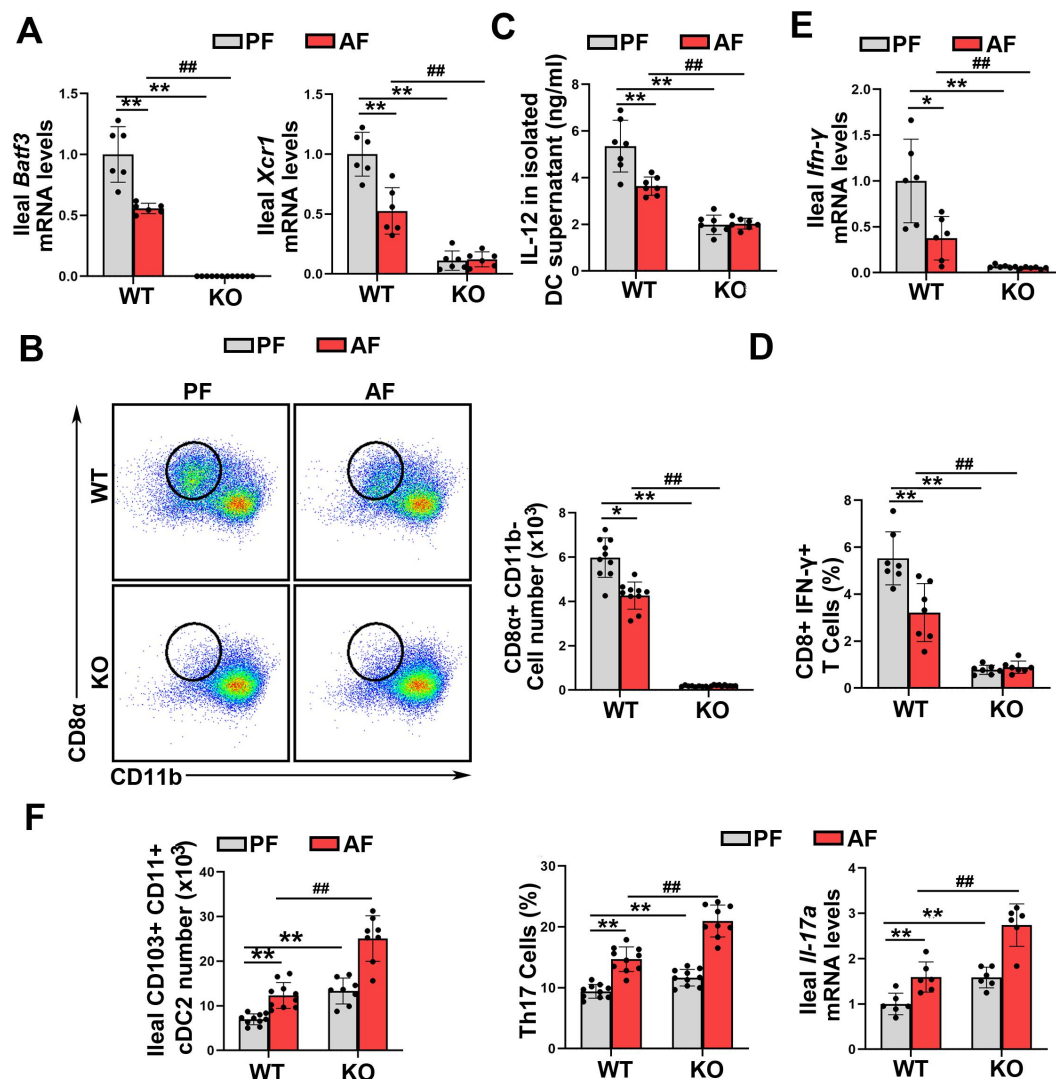


## Supplementary Fig. 3



**Supplementary Fig. 3. The effects of chronic alcohol consumption on ileal IL12-IFN- $\gamma$  signaling pathway in mice.** C57BL/6J WT mice were fed Lieber-DeCarli liquid diets containing alcohol (alcohol-fed, AF) or isocaloric dextran (pair-fed, PF) for 8 weeks plus a single binge (4 g/kg). (A) Gating strategy and representative dot plot for ileal CD45<sup>+</sup>IL-12R<sup>+</sup> cells. (B) Relative mRNA levels of *Ifn- $\gamma$*  in the ileum. (C) Relative mRNA levels of *Cxcl9*, *Ido*, and *Ip-10* in the ileum. (D) IProtein levels of ileal p-STAT3 and STAT3 Data are presented as means  $\pm$  SD. \* $P$ <0.05, \*\* $P$ <0.01 vs. PF mice.

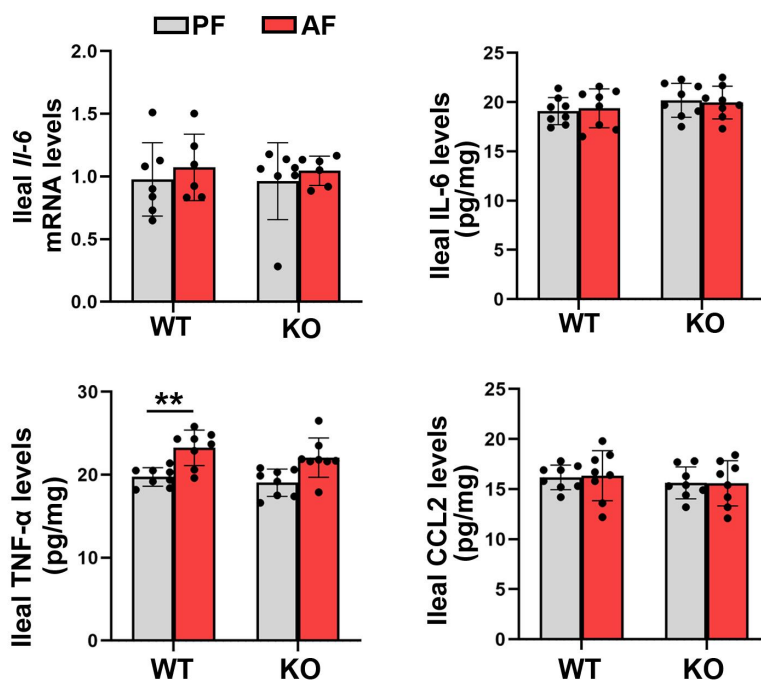
1 Supplementary Fig. 4  
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5 **Supplementary Fig. 4. cDC1s deficiency exacerbates alcohol-decreased the**  
6 **mRNA levels of ileal *Ifn-gamma* but increased the mRNA levels of ileal *Il-17a*. (A)**  
7 **Relative mRNA levels of ileal *Batf3* and *Xcr1*. (B) Representative dot plot of**  
8 **CD8 $\alpha$ +CD11b- DC1s in MLN (n=8-10). (C) IL-12 levels in isolated cDCs treated with**  
9 **CpG ODN. (D) The frequency of ileal CD8+IFN- $\gamma$ + T cells. (E) Relative mRNA levels**  
10 **of *Ifn-gamma* in the ileum of mouse. (F) The number of ileal cDC2s (n=8-10), the frequency**  
11 **of ileal Th17 cells (n=10), and the relative mRNA levels of ileal *Il-17a* (n=6). Data**

1 are presented as means  $\pm$  SD. \* $P$ <0.05, \*\* $P$ <0.01 vs. WT/PF mice; # $P$ <0.05,  
 2 ### $P$ <0.01 vs. WT/AF mice. PF, pair-fed; AF, alcohol-fed.

3 **Supplementary Fig. 5**



16 **Supplementary Fig. 5. The role of cDC1 deficiency on ileal IL-6, TNF- $\alpha$ , and**  
 17 **CCL2.** C57BL/6J WT mice and *Batf3*<sup>-/-</sup> mice were fed Lieber-DeCarli liquid diets  
 18 containing alcohol or isocaloric dextran for 8 weeks plus a single binge (4 g/kg)  
 19 before 4 hours of tissue collection. Data are presented as means  $\pm$  SD. \*\* $P$ <0.01 vs.  
 20 WT/PF mice; PF, pair-fed; AF, alcohol-fed.

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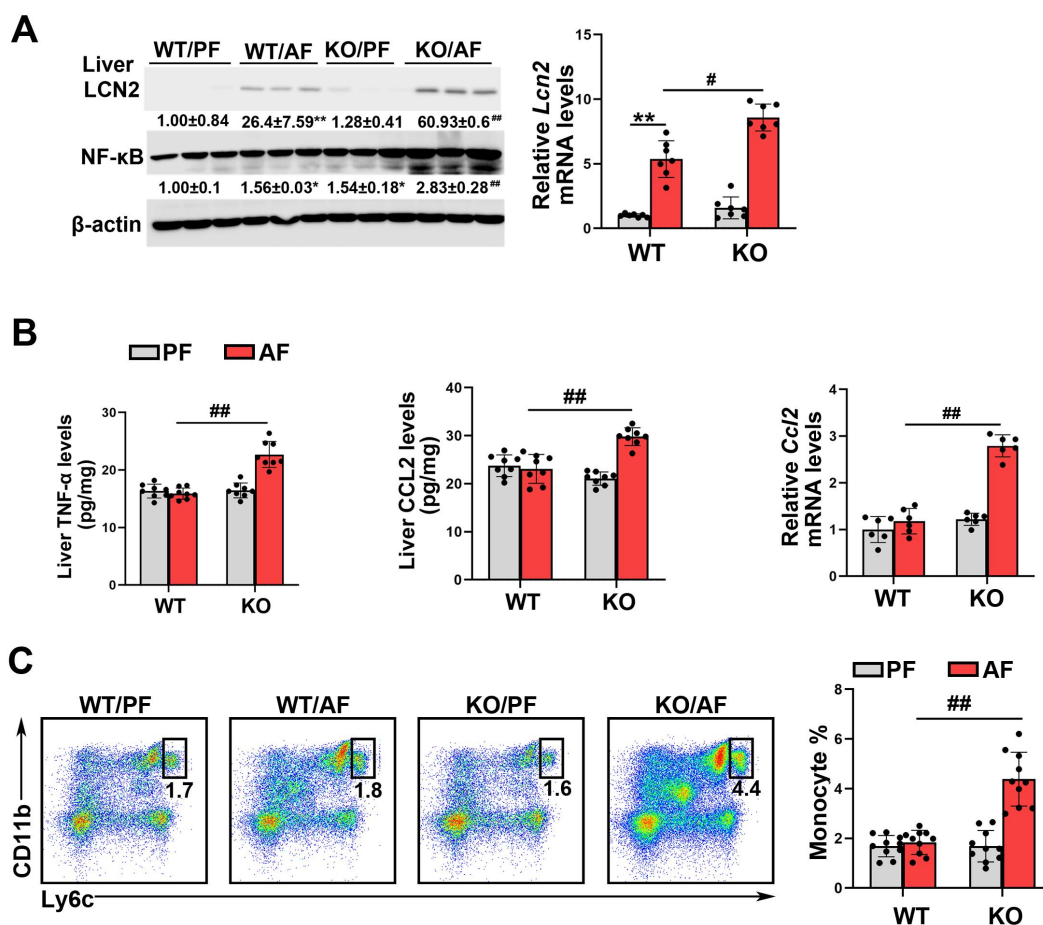
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1 **Supplementary Fig. 6**

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3 **Supplementary Fig. 6. Mice lack of cDC1s exacerbated alcohol-induced liver**4 **inflammation.** C57BL/6J WT mice and *Batf3*<sup>-/-</sup> mice were fed Lieber-DeCarli liquid

5 diets containing alcohol or isocaloric dextran for 8 weeks plus a single binge (4 g/kg)

6 before 4 hours of tissue collection. (A) The protein levels of hepatic LCN2 and NF-

7 κB. (B) Relative mRNA levels of hepatic *Il-1β* and the protein levels of hepatic TNF-

8 α and CCL2. (C) The frequency of monocytes in the liver. Data are presented as

9 means ± SD. \**P*<0.05, \*\**P*<0.01 vs. WT/PF mice; #*P*<0.05, ##*P*<0.01 vs. WT/AF

10 mice. PF, pair-fed; AF, alcohol-fed.

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1 **Supplementary Fig. 7.**

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10 **Supplementary Fig. 7. *Batf3*-dependent cDC1s deficiency has no effects on**  
 11 **alcohol metabolism in the liver.** C57BL/6J WT mice and *Batf3*<sup>-/-</sup> mice were fed  
 12 Lieber-DeCarli liquid diets containing alcohol or isocaloric dextran for 8 weeks plus a  
 13 single binge (4 g/kg) before 4 hours of tissue collection. (A) The protein levels of  
 14 hepatic ADH and CYP2E1 (n=3). (B) The serum ethanol levels (n=7). Data are  
 15 presented as means ± SD. \*\**P*<0.01 vs. WT/PF mice. PF, pair-fed; AF, alcohol-fed.

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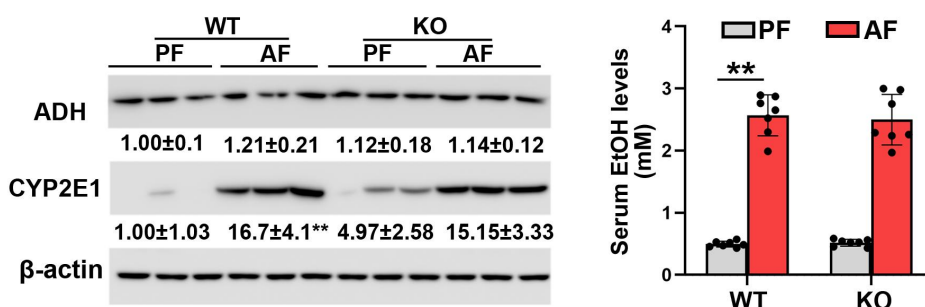
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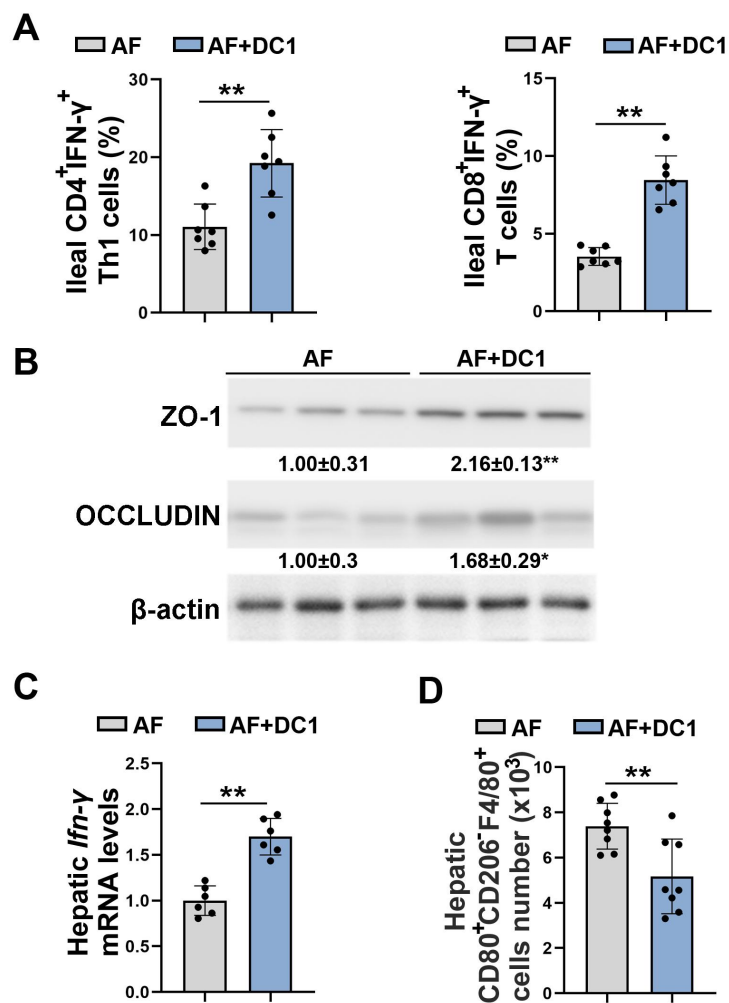
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1 **Supplementary Fig. 8.**18 **Supplementary Fig. 8. The effects of cDC1s transfer on ileal Th1 cells, Tc cells,**19 **tight junction disruption, and liver inflammation.** Alcohol-fed C57BL/6J WT mice

20 were administrated with or without cDC1s adoptive transfer. (A) The frequency of

21 ileal Th1 and CD8+ Tc cells (n=7). (B) Ileal ZO-1 and OCCLUDIN protein levels

22 (n=3). (C) Hepatic *Ifn-γ* mRNA levels (n=6) (D) The number of hepatic23 CD80<sup>+</sup>CD206<sup>+</sup>F4/80<sup>+</sup> M1 macrophages (n=8). Data are presented as means ± SD.24 \*\**P*<0.01 vs. AF mice.

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1 **Supplementary Fig. 9.**

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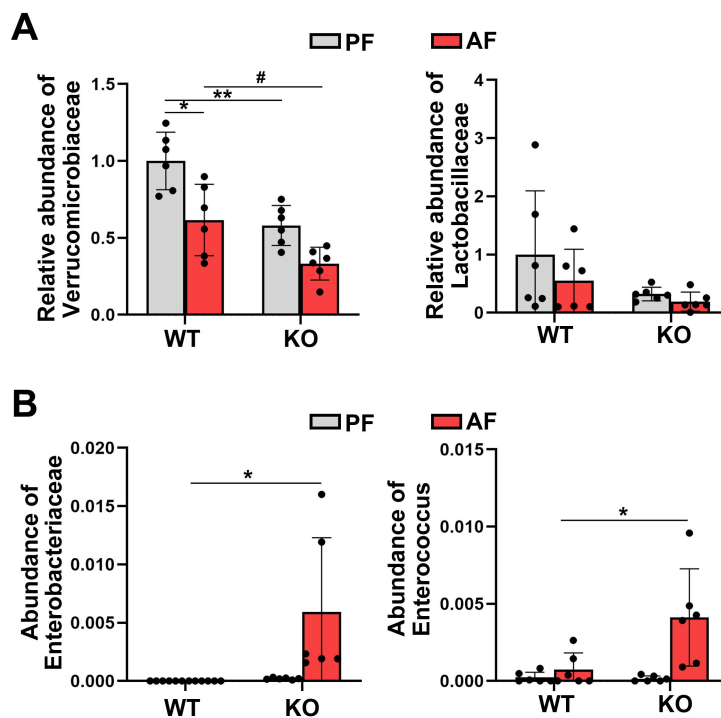
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14 **Supplementary Fig. 9. The effects of cDC1s deficiency on fecal microbiota in**15 **mice.** C57BL/6J WT mice and *Batf3*<sup>-/-</sup> mice were fed Lieber-DeCarli liquid diets

16 containing alcohol or isocaloric dextran for 8 weeks plus a single binge (4 g/kg)

17 before 4 hours of tissue collection. (A) Relative abundance of fecal

18 Verrucomicrobiaceae and Lactobacillaceae. (B) The abundance of fecal

19 Enterobacteriaceae and Enterococcus. Data are presented as means ± SD. (A)

20 \**P*<0.05 vs. PF mice, \*\**P*<0.01 vs. PF mice; #*P*<0.05 vs. AF mice. (B) \**P*<0.05 vs.

21 AF mice.

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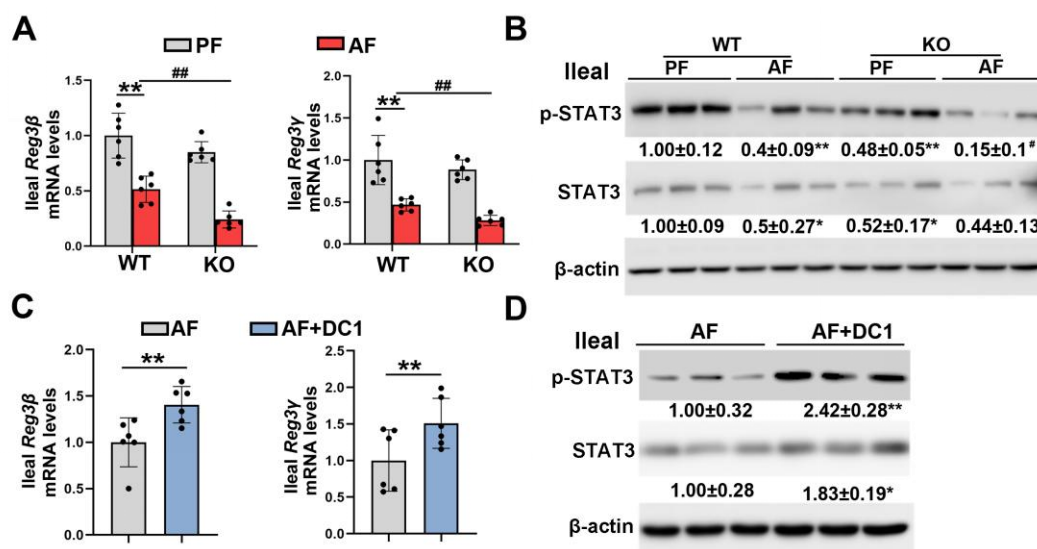
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2 **Supplementary Fig. 10.**

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6 **Supplementary Fig. 10. Lack of cDC1 exacerbates alcohol-perturbed ileal p-**7 **STAT3-AMPs signaling pathway. (A-B) C57BL/6J WT mice and *Batf3*<sup>-/-</sup> mice**

8 were fed Lieber-DeCarli liquid diets containing alcohol or isocaloric dextran for 8

9 weeks plus a single binge (4 g/kg) before 4 hours of tissue collection. (A) Relative

10 mRNA levels of ileal *Reg3β* and *Reg3γ* (n=6). (B) The protein levels of ileal p-

11 STAT3 and STAT3 (n=3). (C-D) Alcohol-fed C57BL/6J WT mice were

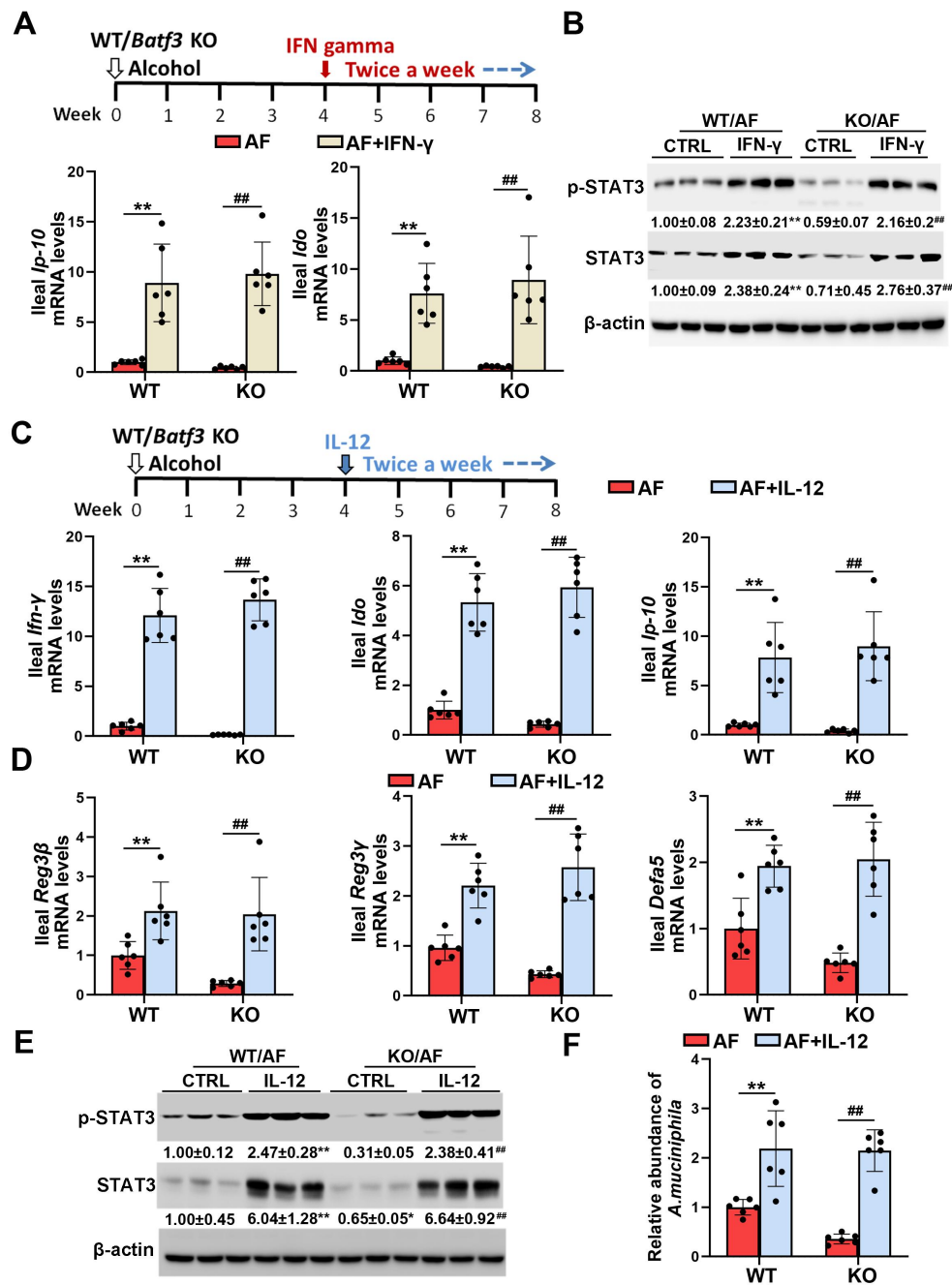
12 administrated with or without cDC1s adoptive transfer. (C) Relative mRNA levels of

13 ileal *Reg3β* and *Reg3γ* (n=6). (D) The protein levels of ileal p-STAT3 and STAT3.14 (A-B) Data are presented as means ± SD. \**P*<0.05, \*\**P*<0.01 vs. WT/PF mice;15 ###*P*<0.01 vs. WT/AF mice. PF, pair-fed; AF, alcohol-fed. (C-D) Data are presented16 as means ± SD. \**P*<0.05, \*\**P*<0.01 vs. AF mice.

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## Supplementary Fig. 11.



## Supplementary Fig. 11. cDC1s control ileal AMPs levels via regulating IL-12-

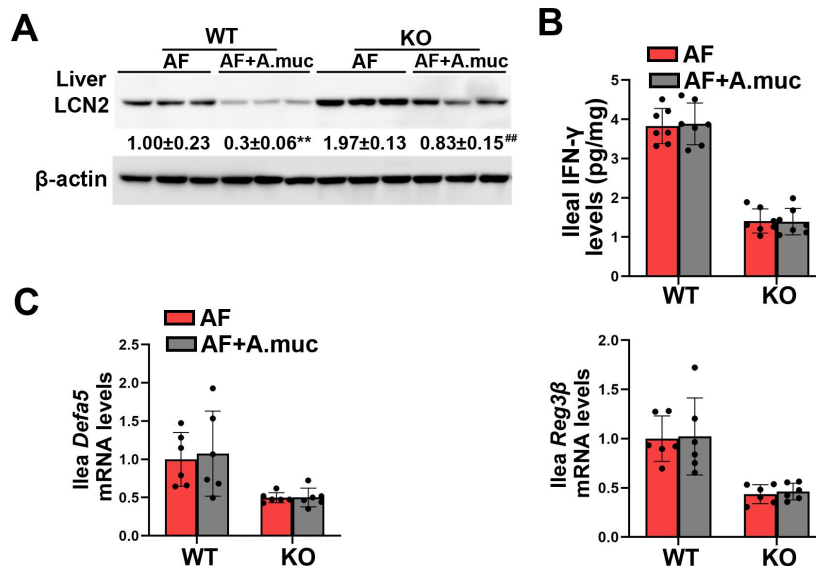
IFN- $\gamma$  signaling pathway in ALD. (A-B) Alcohol-fed C57BL/6J WT mice and*Batf3*<sup>-/-</sup> mice were administrated with or without IFN- $\gamma$ , respectively. (A) RelativemRNA levels of ileal *Ip-10* and *Ido* (n=6). (B) The protein levels of ileal p-STAT3

1 and STAT3 (n=3). (C-F) Alcohol-fed C57BL/6J WT mice and *Batf3* KO mice were  
 2 administrated with or without IL-12, respectively. (C) Relative mRNA levels of ileal  
 3 *IFN- $\gamma$* , *Ip-10*, and *Ido* (n=6). (D) Relative mRNA levels of ileal *Defa5*, *Reg3 $\beta$*  and  
 4 *Reg3 $\gamma$*  (n=6). (E) The protein levels of ileal p-STAT3 and STAT3 (n=3). (F) Relative  
 5 *A. muciniphila* abundance in the gut (n=6). Data are presented as means  $\pm$  SD. PF,  
 6 pair-fed; AF, alcohol-fed. (A-B) \*\*  $P < 0.01$  vs WT/AF mice. ##  $P < 0.01$  vs KOAF  
 7 mice. (C-F) \*\*  $P < 0.01$  vs WT/AF mice. ##  $P < 0.01$  vs KOAF mice.

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9 **Supplementary Fig. 12.**

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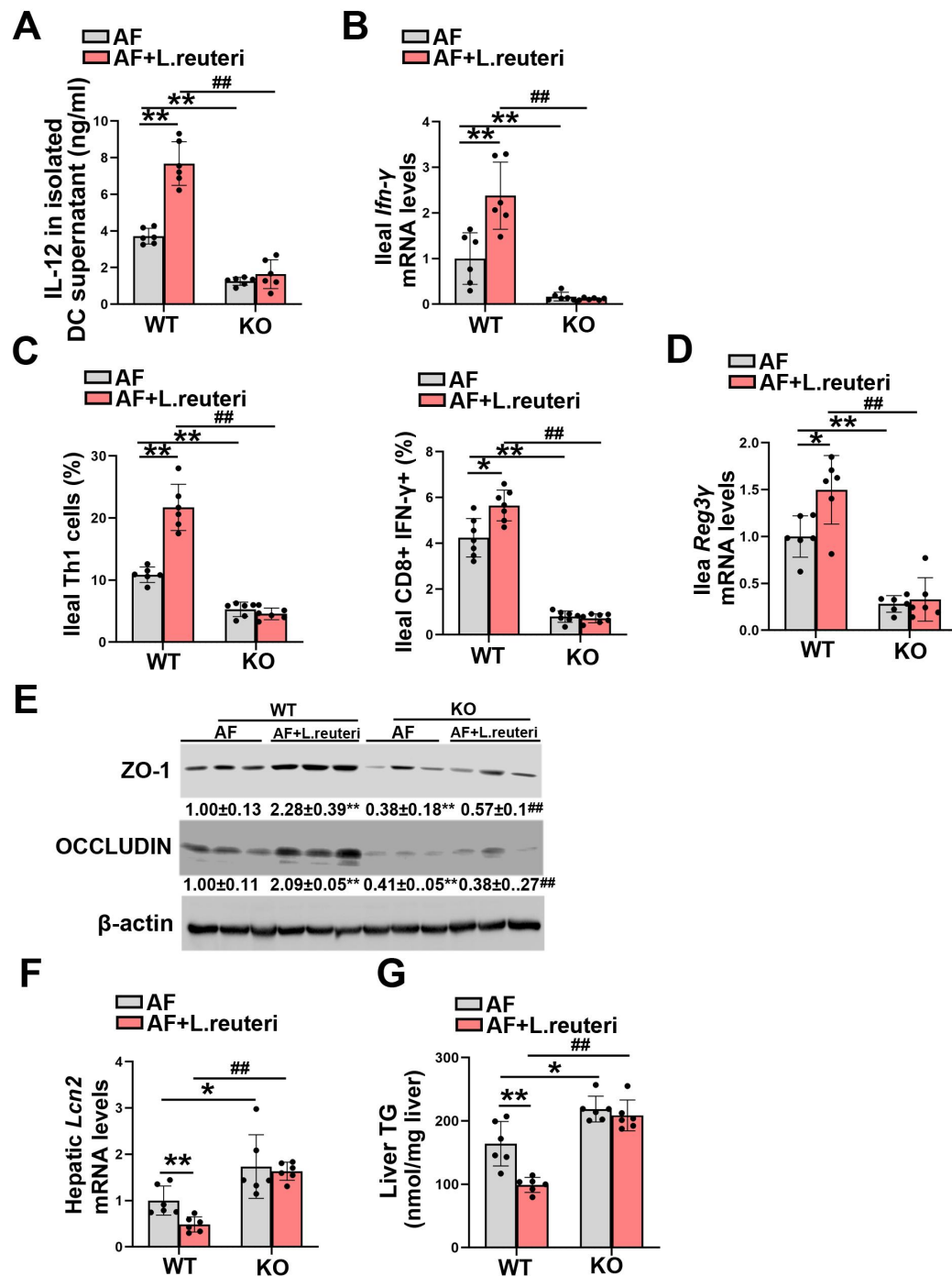


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19 **Supplementary Fig. 12. *A. muciniphila* supplementation did not affect intestinal**20 **IFN- $\gamma$  and AMPs expression levels.** Alcohol-fed C57BL/6J WT mice and *Batf3*<sup>-/-</sup>21 mice were administrated with or without *A. muciniphila*, respectively. (A) Hepatic22 LCN2 protein levels (n=3). (B) The ileal IFN- $\gamma$  protein levels (n=7). (C) Relative23 mRNA levels of ileal *Defa5*, *Reg3 $\beta$*  and *Reg3 $\gamma$*  (n=6). Data are presented as means  $\pm$ 24 SD. \*\*  $P < 0.01$  vs WT/AF mice. ##  $P < 0.01$  vs KO/AF mice.

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## 1 Supplementary Fig. 13.



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3 **Supplementary Fig. 13. Intestinal *cDC1s* are required for the protective role of *L.***  
 4 ***reuteri* in alcohol-induced steatohepatitis.** Alcohol-fed C57BL/6J WT mice and  
 5 *Batf3*<sup>-/-</sup> mice were administrated with or without *L. reuteri*, respectively. (A) The IL-  
 6 12 levels from isolated cDCs in small intestine (n=6). (B) Relative mRNA levels of

1 ileal *Ifn-γ* (n=6). (C) Ileal Th1 cells and CD8<sup>+</sup>IFN-γ<sup>+</sup> cells frequency (n=7). (D)  
2 Relative mRNA levels of ileal *Reg3γ* (n=6). (E) Relative expression levels of ileal  
3 *ZO-1* and *OCCLUDIN* (n=3). (F) Relative mRNA levels of hepatic *Lcn2* (n=6). (G)  
4 Hepatic TG contents (n=6). Data are presented as means ± SD. \**P*<0.05, \*\**P*<0.01 vs.  
5 WT/AF mice; ###*P*<0.01 vs. WT/AF+*L. reuteri*. PF, pair-fed; AF, alcohol-fed.

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1 **Supplementary tables**2  
3 Supplementary Table 1. Primers design for Real-Time qPCR.  
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Gene name	Forward	Reverse
<i>Rn 18s</i>	GTAACCCGTTGAACCCCAT	CCATCCAATCGGTAGTAGCG
<i>Cxcl1</i>	ACTGCACCCAAACCGAAGTC	TGGGGACACCTTTTAGCATCTT
<i>Ifn-γ</i>	CTCTTCCTCATGGCTGTTTCT	TTCTTCCACATCTATGCCACTT
<i>Batf3</i>	GAGCCCAAGGACGATGAC	CTCCTCGTGGAGCTTGTCAG
<i>Clec9a</i>	TGGCAGAGGAAATACACGCT	CAGTCACTACCTGAATGGAGAG
<i>Xcr1</i>	CATGGGTTCTTGGCCTCAGT	ATGCTACCACGACGGTGAAG
<i>Zo-1</i>	GCCGCTAAGAGCACAGCAA	GCCCTCCTTTTAACACATCAGA
<i>Occludin</i>	CTCCCATCCGAGTTTCAGGT	GCTGTCGCCTAAGGAAAGAG
<i>Claudin1</i>	GTTTGCAGAGACCCCATCAC	AGAAGCCAGGATGAAACCCA
<i>Cxcl9</i>	CGAGGCACGATCCACTACAA	AGGCAGGTTTGATCTCCGTT
<i>Ido</i>	ATGTGGGCTTTGCTCTACCA	AAGCTGCCCGTTCTCAATCA
<i>Ip-10</i>	CCAAGTGCTGCCGTCATTTTC	TCCCTATGGCCCTCATTCTCA
<i>Ccl2</i>	TGCTGTCTCAGCCAGATGCAG TTA	TACAGCTTCTTTGGGACACCTGCT
<i>Tnf-α</i>	CCTGTAGCCCACGTCGTAG	GGGAGTAGACAAGGTACAACCC
<i>Lcn2</i>	TCCTCAGGTACAGAGCTACAA	GCTCCTTGGTTCTTCCATACA
<i>Defa5</i>	CAGGCTGATCCTATCCACAAA	CTTGGCCTCCAAAGGAGATAG
<i>Reg3β</i>	AATGGAGGTGGATGGGAATG	CCACAGAAAGCACGGTCTAA
<i>Reg3γ</i>	TTCTCAGGTGCAAGGTGAAG	GGCATAGCAATAGGAGCCATAG
<i>Il-1β</i>	GCCACCTTTTGACAGTGATG	CGTCACACACCAGCAGGTTA
<i>Il-17A</i>	TACCTCAACCGTTCCACGTC	TTCCCTCCGCATTGACACAG
<i>Il-6</i>	CACTTCACAAGTCGGAGGCT	GCCACTCCTTCTGTGACTCC

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## 1 Supplementary Table 2. Antibodies for WB, IF, and IHC.

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Antigen	Origin	Vendor	Catalog No.
CYP2E1	Rabbit	Abcam	ab28146
CHOP	Rabbit	Cell signaling technology	5554S
ATF4	Rabbit	Cell signaling technology	11815S
ADH	Rabbit	Novus	NBP1-45335
$\beta$ -actin	Mouse	Sigma Aldrich	A5316
STAT3	Mouse	Cell signaling technology	9139S
P-STAT3	Rabbit	Cell signaling technology	9145S
LCN2	Mouse	R&D Systems	AF1857
ZO-1	Rat	Millipore	MABT11
OCCLUDIN	Rabbit	Thermo Scientific	40-4700
NF- $\kappa$ B	Rabbit	Cell signaling technology	4764S

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## 1 Supplementary Table 3. Antibodies for flow cytometry.

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Antigen	Vendor	Catalog No.
CD45	Biolegend	103108
MHCII	Biolegend	116417
CD11c	Biolegend	117318
CD103	Biolegend	121432
CD11b	Biolegend	101208
IFN- $\gamma$	Biolegend	505810
IL-17A	Biolegend	506940
CD3	Biolegend	100320
CD4	Biolegend	100406
CD8	Biolegend	100714
Ly6g	Biolegend	127618
Ly6c	Biolegend	128026
F4/80	Biolegend	123116
Clec9A	Biolegend	143504
IL-12R	R&D Systems	FAB1959G
CD64	Biolegend	139314
7-AAD	Biolegend	420404

1 ***Supplementary Reference***

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