Supplementary experimental procedures

Antibiotics treatment

NcDase$^{-/-}$ or WT mice were maintained on drinking water containing ampicillin (0.5 g/L), neomycin (1 g/L), vancomycin (0.25 g/L) and metronidazol (1 g/L) for 8-12 weeks while being fed a NCD in order to determine the relative contribution of the microbiota to IgA production phenotype.

Growth of *Desulfovibrio desulfuricans* ATCC 27774 *and Ruminococcaceae sp.* (TSD-27)

The bacterial species *Desulfovibrio desulfuricans* (27774) and *Ruminococcaceae sp.* (TSD-27) were purchased from ATCC. Media for ATCC 27774 was composed of NH_4Cl (1 g/L), Na_2SO_4 (2 g/L), $Na_2S_2O_3 \cdot 5H_2O$ (1 g/L), $MgSO_4 \cdot 7H_2O$ (1 g/L), $CaCl_2 \cdot 2H_2O$ (0.1 g/L), KH_2PO_4 (0.5 g/L), Yeast Extract (1 g/L), Resazurin (0.5 mL/L), cysteine (0.6 g/L), DTT (0.6 g/L) (Sigma), $NaHCO_3$ (1 g/L), pyruvic acid (3 g/L), malic acid (3 g/L), and adjusted to pH of 7.2. Bacteria were grown for 48 hours in an anaerobic chamber and stored in growth media containing 25% glycerol at -70°C.

Transplantation of gut microbiota into germ-free (GF) mice

GF mice were maintained in sterile isolators and verified monthly for GF status by plating and PCR of feces. Feces from 6-months-HFD fed NcDase$^{-/-}$ or WT mice (n=10) were pooled and suspended in 1.5ml PBS per cecum. The suspensions were immediately administered (0.15ml/mouse) to 8-week-old GF C57BL/6 male mice that were maintained in sterile cages with autoclaved normal chow diet food and water for 5 weeks. Some GF

mice were orally given with *D.desulfuricans* (27774), *Ruminococcaceae sp.* (TSD-27) or both at $2X10^9$ CFU and maintained in sterile cages for three weeks.

Reagents, antibodies and flow cytometry

The following assay kits were used in this study: ALT, AST, ALP assay kits, insulin ELISA kit (EMINS), leptin ELISA kit (KMC2281), Infinity Cholesterol (TR13421) and triglyceride (TR22421) from Thermo fisher. Choline kit from Aatbio. Glucose and Insulin were purchased from Sigma. For analysis of surface markers, cells were stained in PBS containing 2% (wt/vol) BSA. Intracellular staining of the transcription factor Foxp3 was performed using the Foxp3 Fix/Perm Buffer Set (eBioscience, Thermo fisher). For detection of intracellular cytokines, cells were first stimulated for 4 h with 50 ng/ml PMA and 1µg/ml ionomycin in the presence of Brefeldin A (5µg/ml; All obtained from Sigma), followed by staining for surface markers. Cells were then fixed and permeabilized using the Foxp3 Fix/Perm Buffer Set and stained for intracellular cytokines. The following antibodies were used at a dilution of 1/200–1/600: PerCP-Cy5.5, PE-, FITC- or APC-labeled anti-IL-17A (TC11–18H10.1), PE- or APC-labeled anti-IL-4 (11B11, eBioscience, Thermo fisher), PE- or APC-labelled anti-IL-10 (JES5–16E3), APC- or PE-Cy7-labeled anti-IFN (XMG1.2), PE-labeled anti-Foxp3 (FJK-16s, eBioscience, Thermo fisher), PE-, FITC- or APC-labeled anti-CD11b (M1/70), PE-, FITC- or APC-labeled anti-CD4 (RM4-5), PE-Cy7-labeled anti-CD3 (145-2C11), PE-anti-Gr-1 (RB6-8C5), PE- or FITC-labeled anti-mouse IgA (MA-6E), APC-or PE-conjugated B220 (RA3-6B2), PE-conjugated anti-IgM (II/41), FITC-, PerCP-Cy5.5 or Pacific Blue-labelled anti-CD45 (30-F11), PE-anti-S1PR1 (FAB7089P,R&D). All antibodies were obtained from ThermoFisher unless

otherwise noted. Flow cytometry data were acquired on a 5-color FACScan (Becton Dickinson) and analyzed using FlowJo software (Treestar). Cell sorting was performed using a FACSAria II.

Plasmids, Cell Culture, and Transfection

Adv-SCD1 was from Applied Biological Material Inc (BC, Canada). pAAV-IRS-hrGFP-mWnt3a was from Addgene (MA, USA). HepG2, HEK293T, L, and L-Wnt3a cells were obtained from ATCC. HepG2, HEK293T, and L-cells were grown in Dulbecco's modified Eagle's medium (DMEM)/10% fetal bovine serum (FBS). L-Wnt3a cells were grown in DMEM/10% FBS and 0.5g/l Geneticin. Primary hepatocytes were infected with AAV-mWnt3a. For quantification of Wnt3a, hepatocyte conditioned medium was concentrated 10-fold by centrifugal filters (Millipore, Ultracel) and analyzed using an ELISA kit (LifeSpan Biosciences, LS-F19433). Hepatocytes and hepatic stellate cells (HSCs) were isolated from 6-week-old WT or NcDase$^{-/-}$ mice.

Preparation of BSA-conjugated fatty acids and treatment of HepG2, primary hepatocytes or HSCs

Oleic acid (O1008), stearic acid (S4751), palmitic acid (P0500), and palmitoleic acid (P9417), and fatty acid free-BSA (A8806) were purchased from Sigma. BSA-conjugated fatty acids were prepared by diluting 50 mM stock fatty acids in DMSO with 100 mg/ml BSA in 1x PBS to produce a 3 mM working solution. Primary hepatocytes were isolated and then treated with control media or media from L-Wnt3a with/without a final concentration of 200 μM BSA-conjugated fatty acid (and a final concentration of 0.1%

BSA) or vehicle control for 12h-48h. Some hepatocytes were also treated with Wnt3a pharmacologic inhibitor ICG-001 (10 μM, Sigma) or DMSO for 12h-24h. Primary HSCs or LXZ, a spontaneously immortalized HSC line, were maintained in low glucose DMEM with 5% FBS and antibiotics. For conditioned medium transfer experiments, primary hepatocytes were cultured in DMEM/F12 containing 0.2% BSA, PA, or POA and incubated for 24h. The media were then transferred to LXZ cells or primary HSCs that had previously been incubated in DMEM, 0.2% BSA for 24 h. After 24 h, the HSCs were assayed for gene expression.

Serum biochemistry analyses, Insulin- and glucose-tolerance tests

Mouse serum was collected by orbital vein bleeding at the time of euthanasia. Serum alanine aminotransferase (ALT) and AST were analyzed using the Piccolo Xpress system (ABAXIS, Union City, CA) or kits from Thermo Fisher Scientific. Whole blood glucose was measured in overnight (15h) fasted mice. Blood collected from a tail cut was measured using an automatic glucose monitor (Contour; Bayer). Lipids including triglyceride and cholesterol were measured in serum obtained from 5 h fasted mice using Piccolo Xpress system. Serum insulin and leptin levels were measured from blood collected from 6 h fasted mice, by ELISA according to the manufacturer's instructions. For food intake studies, 10 NcDase$^{-/-}$ or WT mice were single caged and acclimated to custom made food racks for at least three days prior to twice daily food intake measurements, by weight, for 4 days. Blood glucose levels in response to insulin challenge were used to examine the insulin sensitivity of NcDase$^{-/-}$ or WT mice on different treatments. Mice were initially fasted for 5 h and subsequently glucose (1.5 g/kg body weight) or insulin (0.25-0.75

units/kg body weight, i.p.) was injected. Blood glucose was determined at 0, 30, 60, 90, and 120 min after injection[1].

Histopathologic Analysis, Immunohistochemistry, and Immunofluorescence

Tissue specimens were fixed in 10% formalin, dehydrated, and then embedded in paraffin. Tissue samples were cut at 5 µm thicknesses and stained with hematoxylin and eosin and evaluated for severity of NAFLD. Inflammatory cells in H&E-stained liver section images were quantified as the number of mononuclear cells per field (10x objective). Liver fibrosis was assessed by Picrosirius (Sirius) red or by Masson's trichrome staining (Sigma, HT15), with aniline blue-positive areas quantified as a measure of collagen content in the trichrome-stained sections. TUNEL staining was conducted using a kit from Roche (#12156792910).

For Oil Red O staining, frozen sections were incubated with 60% isopropanol for 1 minute. Tissues were dried in a 37°C incubator for approximately 10 minutes before incubating with Oil Red O solution. Slides were incubated with Oil Red O solution for 15 minutes. The Oil Red O solution was aspirated from the slides, and 60% isopropanol was added to the slides for several minutes to remove any residual Oil Red O. The slides were washed in PBS and stained for 30 seconds with hematoxylin (GHS132; Sigma,) followed by more washes in dH_2O. Coverslips were mounted on slides with a mounting medium (glycerol in PBS 6:1) followed by microscopy.

For immunofluorescence analysis, tissue sections were subjected to antigen retrieval by boiling the slides in Antigen Unmasking Solution (Vector Laboratories) for 10 minutes

according to instructions. Sections were then blocked for 1 hour at 22°C with 5% BSA in PBS and incubated overnight at 4°C with the primary antibodies BB20, IgA, α-SMA, using a 1:200 dilution. Primary antibodies were detected by Alexa Fluor 488, 594 or 647 conjugated goat anti-mouse, anti-rabbit IgG and anti-rat (1:600, Invitrogen). Tissues were counterstained with DAPI and images were captured on a Zeiss LSM 510 confocal microscope equipped with a digital image analysis system (Pixera). For F/480 staining, frozen sections were fixed in 4% paraformaldehyde for 1 h at room temperature. OCT (Sakura Finetek)-embedded tissue cryosections (9μm-thick) were stained with F4/80 (BM8, ebioscience, ThermoFisher). For most experiments, $F4/80^+$ cells were quantified in immunostained liver section images as the percentage of $F4/80^+$ cells per total DAPI-stained cells. For immunohistochemistry, the deparaffinization, rehydration, and antigen retrieval processes were the same as with immunofluorescence staining. The slides were treated with 3% hydrogen peroxide for 10 min and then blocked with Serum-Free Protein Block (Vector Laboratories) for 30 min. Sections were incubated overnight with antibodies against MPO or α-SMA (1:100), followed by staining with horseradish peroxidase-conjugated anti-IgG second antibodies. Antigens were then visualized with 3,3′-diaminobenzidine substrate (Vector Laboratories) and scanned using an Aperio Imagescope.

Measurement of Liver TGs

Hepatic TG extraction was performed as described previously[2]. Briefly, approximately 20-50 mg of liver tissue was ground into powder and incubated in a chloroform-methanol (2:1) mixture of 1 mL for 1 hour with vigorous shaking. 200 μL of ddH2O was added to

the samples, and the samples were vortexed and centrifuged at 3,000*g* for 5 minutes to separate out the lipid phase. The lower lipid phase was collected and dried, and the remaining pellet was dissolved in a *tert*-butanol, Triton X-114, and methanol (9:4:2) mixture. Triglyceride content was determined using commercially available colorimetric kits (Thermo Fisher, Waltham, MA). Tissue triglyceride liver tissues were homogenized in distilled water and the homogenate was collected for lipid extraction.

Isolation of gut extracellular vesicles

Isolation of Gut EVs was performed as described previously[3]. Briefly, mice were euthanized and the intestine removed. The luminal contents of the intestine were removed by gently flushing the intestine with ice-cold PBS. The intestine was then opened longitudinally and gently washed with ice-cold PBS. The mucous further were scraped off by mild physical separation using a glass slide, and soaked in washing PBS, then agitated on a rotator at 70 r.p.m. min^{-1} for 15 min and centrifuged at 500×*g* for 15 min at 4 °C. The supernatant was then followed by differential centrifugation: 4000 rpm for 30 min, 8000rpm for 60 min, 36000rpm for 90 min. The pellets from 8000 rpm or 36000rpm were used for microparticles and exosomes-like particles, respectively. Protein concentration was determined using the Bio-Rad Protein Quantitation Assay kit with bovine serum albumin as a standard.

Tissue lipid analysis

All the lipid quantification and fatty acid analyses in various lipid fractions were performed as described by the NIH Mouse Metabolic Phenotyping Center (MMPC)/DTRC Lipid laboratory, Vanderbilt University, Nashville, TN. Briefly, lipids were extracted using the

method of Folch-Lees[4]. The extracts were filtered, and lipids recovered in the chloroform phase. Individual lipid classes were separated by thin layer chromatography using Silica Gel 60 A plates developed in petroleum ether, ethyl ether, acetic acid (80:20:1) and visualized by rhodamine 6G. Phospholipids, diglycerides, triglycerides, free fatty acids and cholesteryl esters were scraped from the plates and methylated using BF3 /methanol as described by Morrison and Smith[5]. The methylated fatty acids were extracted and analyzed by gas chromatography. Gas chromatographic analyses were carried out on an Agilent 7890A gas chromatograph equipped with flame ionization detectors, a capillary column (SP2380, 0.25 mm x 30 m, 0.25 µm film, Supelco, Bellefonte, PA). Helium was used as a carrier gas. The oven temperature was programmed from 160°C to 230°C at 4°C/min. Fatty acid methyl esters were identified by comparing the retention times to those of known standards. Inclusion of lipid standards with odd chain fatty acids permitted quantitation of the amount of lipid in the sample. Dipentadecanoyl phosphatidylcholine (C15:0), diheptadecanoin (C17:0), trieicosenoin (C20:1), and cholesteryl eicosenoate (C20:1) were used as standards.

Assay of Ceramidase Activity

The neutral ceramidase activity was measured using C12 NBD Ceramide (d18:1/12:0) (Caymanchem) as the substrates. Small intestine and liver were harvested after mice were fasted for 18 h. Mucosa was obtained by cutting the entire small intestine longitudinally and scraping the mucosa off with a glass slide. Gut Mucosa and liver were homogenized in RIPA buffer with protease inhibitor mixture (Sigma-Aldrich) and centrifuged at 10,000 × g at 4 °C for 15 min. 30-50 µg of homogenate protein in the

supernatant was incubated in 100 µl of reaction buffer (25 mM Tris-HCl, pH 7.5, 1% sodium cholate, and 50 µM NBD C_{12}-ceramide) at 37 °C for 2.5-3 hours. The reaction was terminated by the addition of 300µl of chloroform/methanol (2/1, v/v). This solution was vortexed and centrifuged at 8,000 × g for 1 minute. The lower layer was dried in a SpeedVac concentrator, dissolved in 10µl of chloroform/methanol (1/1, v/v), and applied onto TLC plates (silica gel, Sigma). The TLC was developed with chloroform/methanol/2.5 N ammonia (90/30/0.5, v/v/v). Ceramide and fatty acid were separated by TLC and quantitated using a fluorescent image analyzer.

RNA extraction and PCR

Total RNA was isolated from the tissue or lymphocytes of MLNs, small intestine and colon using the Qiagen RNeasy RNA isolation Kit and was used to synthesize cDNA. RNA (1µg) was reverse-transcribed with Superscript III and random primers (Invitrogen). For quantitation of genes of interest, cDNA samples were amplified in applied biosystems Realtime System using SYBR Green Master Mix (Invitrogen) and specific primers (Supplemental Table 1) according to the manufacturer's instructions. Fold changes in mRNA expression between treatments and controls were determined by the δCT method. Results for each sample were normalized to the concentration of GAPDH mRNA measured in the same samples and expressed as fold increase over baseline levels, which are set at a value of 1. Differences between groups were determined using a two-sided Student's *t*-test and one-way ANOVA. Error bars on plots represent ± SEM, unless otherwise noted. All primers were purchased from Eurofins MWG Operon.

Western blot analysis

Tissue or cells were disrupted in lysis buffer containing 1% Triton X-100, 0.1% SDS, 150 mM NaCl, 50 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 5 mM sodium molybdate and 20 mM phenylphosphate with protease and phosphatase inhibitors (1 mM PMSF, 10 μg ml^{-1} aprotinin, 20 μg ml^{-1} leupeptin, 20 μg ml^{-1} pepstatin A, 50 mM NaF and 1 mM sodium orthovanadate) for 30 min on ice. The samples were centrifuged (16,000*g*, 10 min, 4°C) and the resulting supernatants transferred to fresh tubes. Protein lysates were quantitated using a Bio-Rad protein kit (Bio-Rad) and 50-100 μg of lysates were separated on 10% SDS polyacrylamide gels and transferred to a nitrocellulose membrane. Rabbit anti-phospho-β-catenin, GAPDH (#3683), β-actin (#5125), p-GSK3beta (S9), GSK3beta, p-LRP6 (S1490), SCD1, Non-p-β-catenin (Ser33/37/Thr41, #8814), p-β-catenin (S552), Total β-catenin, p-AKT(S473), Wnt Signaling Antibody Sampler Kit (#2915) and actin were purchased from Cell Signaling Technology (Danvers, MA), α-SMA, Collagen I and Fibronectin were purchased from Abcam, and used at a dilution of 1/1,000. Anti-NcDase was bought from Abcam and Sigma. Membranes were probed with specific antibodies and protein quantity visualized using the ChemiDoc Imaging Systems (Bio-Rad, Hercules, California).

Quantification of fecal immunoglobulins

To quantify luminal IgA, fecal pellets were collected and weighed. Luminal contents were resuspended in 10 μL of sterile 1X HBSS per mg of fecal weight and spun at 100×*g* for 5 min to remove material. Supernatants were centrifuged at 8,000×*g* for 5 min to pellet

bacteria. Supernatants (containing IgA) were then diluted at 1/100 (v/v) for IgA assay using a IgA specific ELISA kit (eBioscience; performed according to the manufacturer's instructions). Absorbance was read at 450 nm and IgA concentrations were calculated using a standard curve. Concentrations were normalized to fecal weight. For detection of bacteria bound IgA, without washing cells, 4 ng/mL of rat anti-mouse IgA (eBioscience clone mA-6E1, PE), rat anti-mouse IgG1 diluted at 1:500 in sterile HBSS containing 10% (v/v) FBS was added and incubated at 4°C for 30 min. The plate was washed twice by spinning at 2,500×*g* for 5 min before removing the supernatant and resuspending cells in sterile HBSS. After a final wash, bacterial wells were resuspended in 250 µL of HBSS containing 5 µL of 1X SYBR green stain (Invitrogen cat #S7563). Wells were incubated for 20 min at 4°C before immediate enumeration on a BD FACSAria II. Flow data were analyzed using FlowJo software (Tree Star).

Isolation and 16S sequencing of fecal and IgA-bound microbial DNA

Mice were sacrificed and fecal and ileal samples were immediately frozen at -70°. DNA was extracted using the QIAamp PowerFecal DNA Kit (Qiagen), according to manufacturer instructions. IgA-bound and -unbound bacteria were isolated from cecal contents of HFD-fed mice and frozen at -70 C before processing. IgA-bound bacteria separation, 16S rDNA amplification, sequencing, and sequence processing was performed as previously described[6].

Isolation of lamina propria lymphocytes (LPLs) and hepatic immune cells

The method used for isolation of LPLs has been previously described[7]. In brief, fat tissues and Peyer's patches (PPs) were removed from small intestine. The intestine was open and cut in pieces 1-cm long and incubated in an HBSS solution containing 5 mM EDTA and 10 mM Hepes for 30 min at 37°C with slow rotation (180 r.p.m.). Pieces were then cut into smaller pieces and incubated in an HBSS solution containing 0.5 mg ml^{-1} DNase I (Roche) and 1 mg ml^{-1} collagenase type IV (Worthington). Finally, the solution containing digested tissue was passed through a 100-µm cell strainer and LPLs were recovered at the interface of the 40 and 72% Percoll (GE Healthcare) solutions. Livers were perfused with saline solution by way of the portal vein which was followed by enzymatic digestion. Liver immune cells were purified by centrifugation using a Percoll gradient as described previously. For flow cytometry analysis, the cells were labelled using standard procedures described above.

Statistical Analysis

Values are shown as Mean $\pm$ SEM except where otherwise indicated. Comparison of multiple experimental groups was performed by two-way ANOVA with appropriate multiple comparison tests. A t test was used to compare the means of two groups. The p values < 0.05 were considered to be statistically significant. Sample sizes are calculated to allow significance to be reached.

Supplemental table 1. Primers used for Real-time PCR

Gene name	Forward primer	Reverse primer
GAPDH	AGGTCATCCCAGAGCTGAACG	ACCCTGTTGCTGTAGCCGTAT
β-Actin	ACGGCCAGGTCATCACTATTC	AGGAAGGCTGGAAAAGAGCC
TNF-α	TCTATGGCCCAGACCCTCAC	GACGGCAGAGAGGAGGTTGA
MMP9	AATCTCTTCTAG AGACTG GGAAGGAG	AGC TGA TTG ACT AAA GTA GCT GGA
SK2	CCACCTGAGCTCCGAGCTGTT	GGCACATGAACCAGGTATGGA
SK1	GAGCTCCGAGCTGTTTGCA	TGACACCCCCGCACGTA
Tgfb1	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG
ASAH2	GTGACATATGGGCTATGCG	CTCCCGAGATTTGATGAAGCA
IL-17A	TTTAACTCCCTTGGCGCAAAA	CTTTCCCTCCGCATTGACAC
IFN-γ	TCAGCAACAGCAAGGCGAAAAAGG	CCACCCCGAATCAGCAGCGA
Fzd6	ATGGAAAGGTCCCCGTTTCTG	GGGAAGAACGTCATGTTGTAAGT
IL-6	GAGAGGAGACTTCACAGAGGATAC	GTACTCCAGAAGACCAGAGG
CXCL1	GCACCCAAACCGAAGTCA	AAGCCAGCGTTCACCAGA
CD36	TGAGACTGGGACCATTGGTGAT	CCCAAGTAAGGCCATCTCTACCAT
SCD1	AGAGGAGGTACTACAAGCC	GAGTGTATCGCAAGAAGG
S100A8	ACAATGCCGTCTGAACTGG	CTCTGCTACTCCTTGTGGCTGTCT
S100A9	GGAGCGCAGCATAACCAC	GCCATTGAGTAAGCCATTCC
MUC5b	GTGGCCTTGCTCATGGTGT	GGACGAAGGTGACATGCCT
ACC1	ATGGGCGGAATGGTCTCTTTC	TGGGGACCTTGTCTTCATCAT
Elovl6	GAAAAGCAGTTCAACGAGAACG	AGATGCCGACCACCAAAGATA
Fasn	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
AOX1	GAGGAAGAATCTCCGACTCACA	TGGTGACTGCTGTACCATGTAG
Hmgcr	AGCTTGCCCGAATTGTATGTG	TCTGTTGTGAACCATGTGACTTC
Hmgcs1	AACTGGTGCAGAAATCTCTAGC	GGTTGAATAGCTCAGAACTAGCC

Dgat1	GTGCACAAGTGGTGCATCAG	CAGTGGGATCTGAGCCATC
Dgat2	AGTGGCAATGCTATCATCATCGT	TCTTCTGGACCCATCGGCCCCAGGA
Pparg	AAGAGCTGACCCAATGGT	ATGGTTCTTCGGAAAAAA
Wnt7a	CCTTGTTGCGCTTGTTCTCC	GGCGGGGCAATCCACATAG
Wnt5a	CAACTGGCAGGACTTTCTCAA	CATCTCCGATGCCGGAACT
Wnt3a	CTCCTCTCGGATACCTCTTAGTG	GCATGATCTCCACGTAGTTCCTG
Elovl5	GAACATTTCGATGCGTCACTCA	GGAGGAACCATCCTTTGACTCTT
Ccnd1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
Srebf1	GCAGCCACCATCTAGCCTG	CAGCAGTGAGTCTGCCTTGAT
Srebf2	GCAGCAACGGGACCATTCT	CCCCATGACTAAGTCCTTCAACT
ChREBP	AGATGGAGAACCGACGTATCA	ACTGAGCGTGCTGACAAGTC
Col1a1	CCCGCCGATGTCGCTAT	GCTACGCTGTTCTTGCAGTGAT
α-SMA	GTGAAGAGGAAGACAGCACAG	GCCCATTCCAACCATTACTCC
TIMP 1	CACAAGCCTGGATTCCGTGG	TCCCTTGCAAACTGGAGAGTGAC
Fibronectin	GGTCTGCAGAGGTTGACAGTG	GGAGAAGTTTGTGCATGGTGTCC
Cpt1a	CTCCGCCTGAGCCATGAAG	CACCAGTGATGATGCCATTCT
axin-2	TGACTCTCCTTCCAGATCCCA	TGCCCACACTAGGCTGACA
c-myc	ATGCCCCTCAACGTGAACTTC	CGCAACATAGGATGGAGAGCA
Jag1	CCTCGGGTCAGTTTGAGCTG	CCTTGAGGCACACTTTGAAGTA
Opn	CTGACCCATCTCAGAAGCAGAATCT	TCCATGTGGTCATGGCTTTCATTGG
Fabp4	CCCGCATGGAGGGTGTATG	TGGAGGGATCACGAGCTTGAA
Fabp5	TGAAAGAGCTAGGAGTAGGACTG	CTCTCGGTTTTGACCGTGATG
Fabp1	ATGAACTTCTCCGGCAAGTACC	CTGACACCCCCTTGATGTCC
Acat2	ATGTTCTACCGGGACTGGTG	CCCGAAAACAAGGAATAGCA
Ppara	AGAGCCCCATCTGTCCTCTC	ACTGGTAGTCTGCAAAACCAAA
SCD1	ACGCCGACCCTCACAATTC	CAGTTTTCCGCCCTTCTCTTT
SCD2	GCTCTCGGGAGAACATCTTG	CAGCCCTGGACACTCTCTTC

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Supplementary Figures:

Figure S1

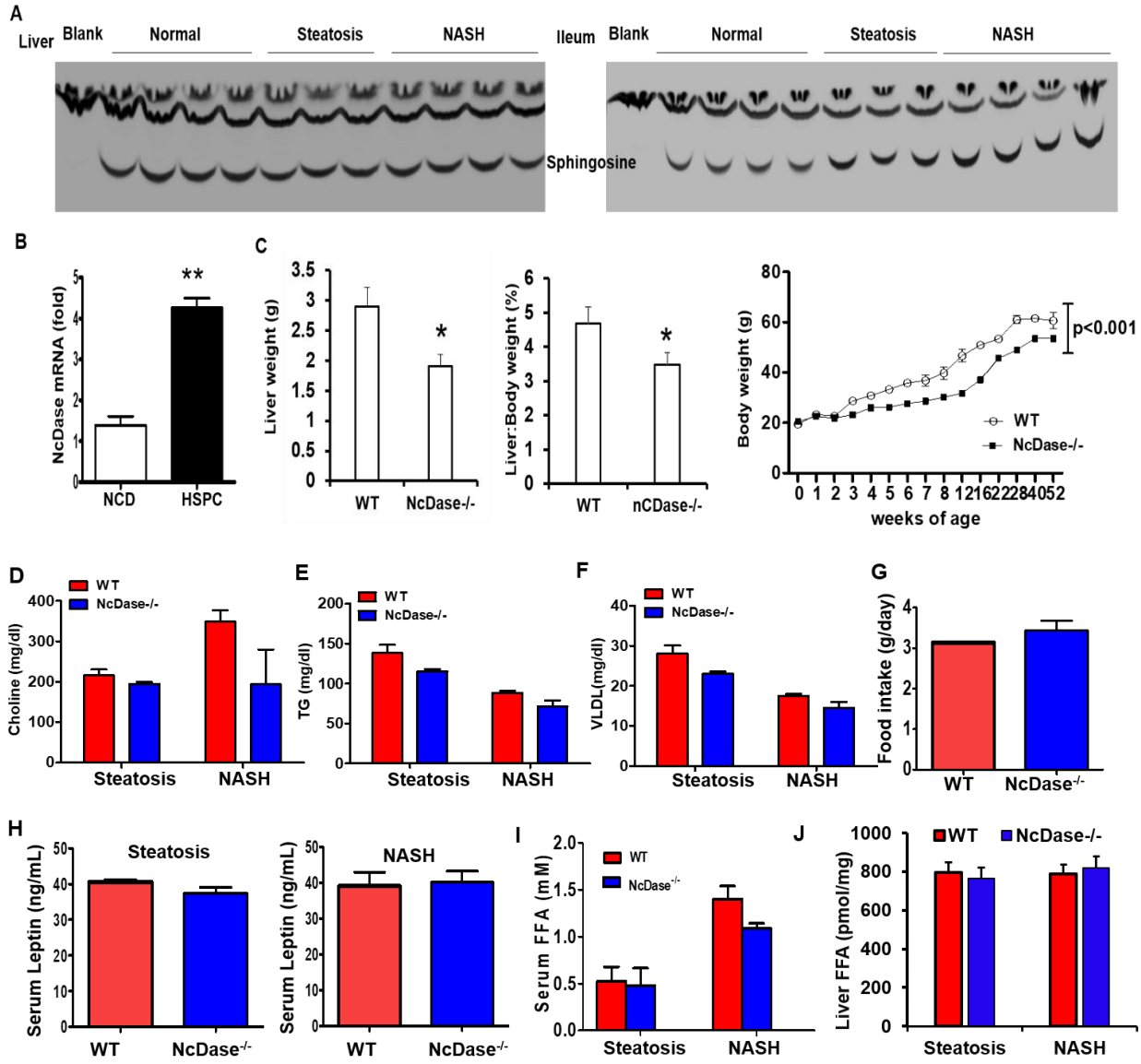

Figure S1. NcDase deficiency reduces liver inflammation, fibrosis, and cell death in HFD-fed mice

(**A, C-H**) Male WT mice or NcDase-/- mice were fed the HFD for 7 months (Steatosis) or 14 months (NASH).

(A) Liver or ileum mucosa from WT mice with/without steatosis or NASH was incubated with NBD C12-ceramide) at 37 °C for 3 h, and lipids were separated by TLC plate and detected using a luminescent image analyzer. Released Sphingosine indicates the activity of NcDase, which is shown as graph in Figure 1C.

(B) Quantification of NcDase mRNA in guts in mice fed the NCD or HSPC diet.

(C) Body weights of mice over time and the ratio of liver to body weight at 14 months after HFD feeding.

(D) Plasma choline levels in mice with steatosis or NASH.

(E-F) Plasma TG (E) and VLDL (F) in mice with steatosis or NASH.

(G) Food intake.

(H) Serum leptin levels in mice with steatosis or NASH.

(I-J) Free fatty acid (FFA) levels in the serum (I) and liver (J) mice with steatosis or NASH.

Mean ± SEM; n = 10, *$p < 0.05$, **$p < 0.01$.

Figure S2

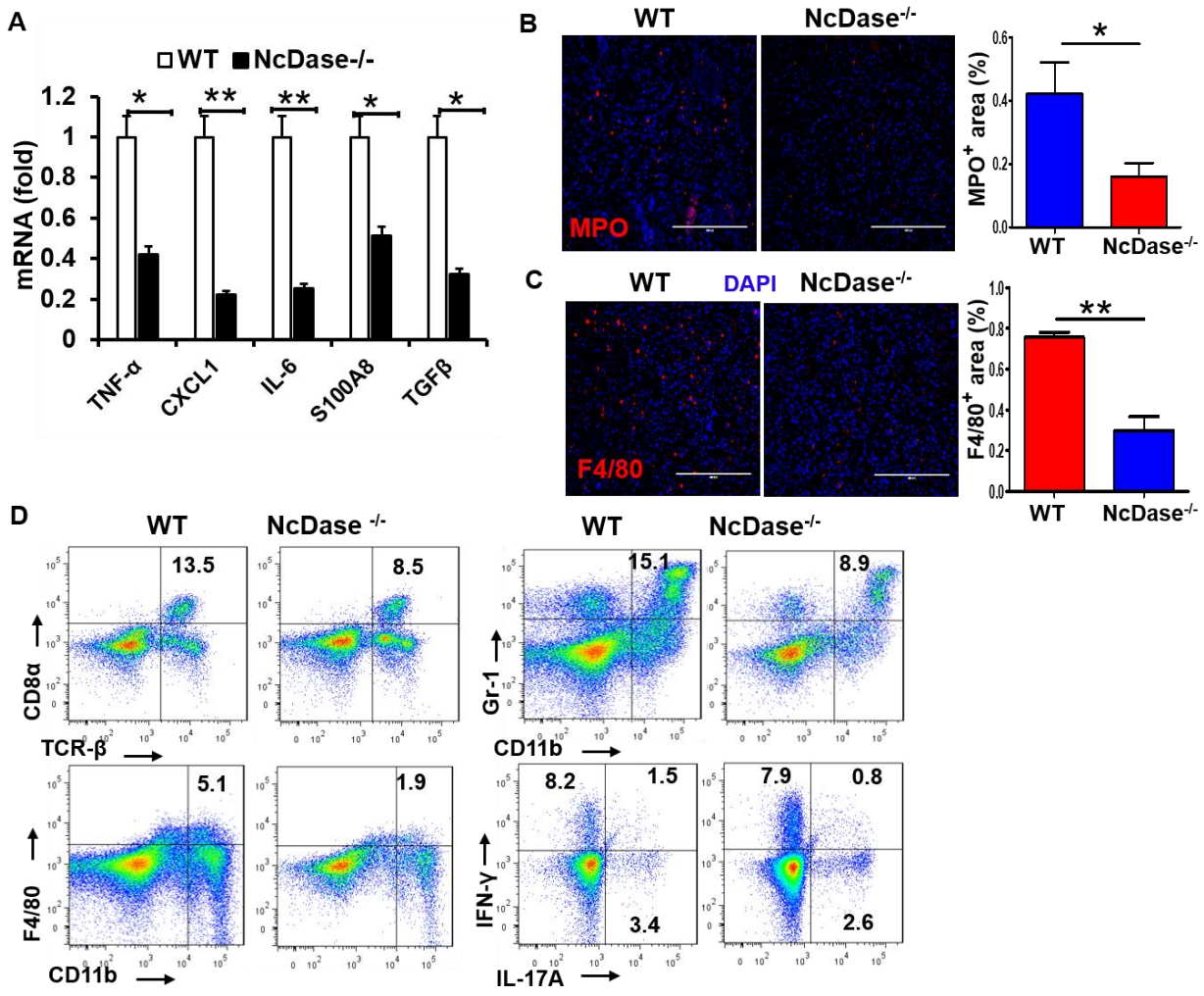

Figure S2 NcDase deficiency reduces liver inflammation in mice during HFD feeding.

(A) mRNA levels of TNF-α, MCP-1, IL-6, TGF-β and S100A8 in the liver.

(B) DAPI counterstain (blue) and MPO immunofluorescence (red) of liver and quantification.

(C) DAPI counterstain (blue) and F4/80 immunofluorescence (red) of liver and quantification.

(D) FACS analysis of $CD8^+$ T cells, $CD11b^+F4/80^+$ macrophages, $CD11b^+Gr\text{-}1^+$ immature myeloid cells/MDSCs, $CD4^+$ $IL\text{-}17A^+$ and $CD4^+$ $IFN\text{-}\gamma^+$ T cells in the liver.

Mean ± SEM; n = 10, *p < 0.05, **p < 0.01.

Figure S3

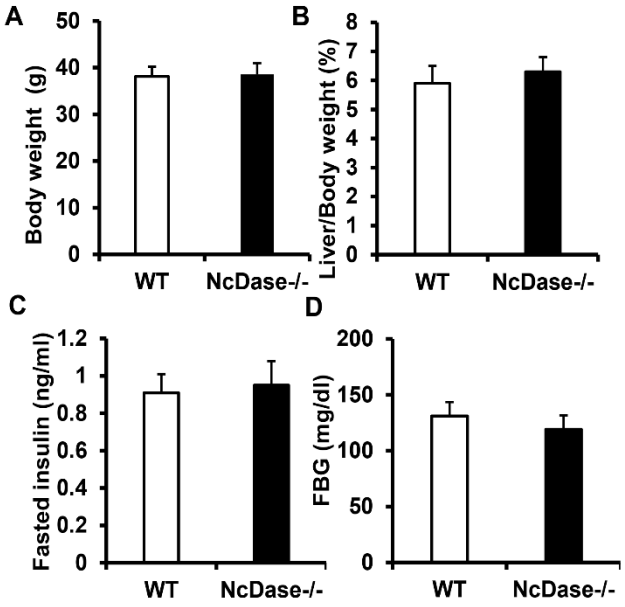

Figure S3 NcDase deficiency reduces liver inflammation, fibrosis, and cell death in HSPC-fed mice

(A) Body weights of mice.

(B) The ratio of liver to body weight

(C) Fasted serum insulin levels.

(D) Fasted blood glucose.

Mean ± SEM; n = 10, *p < 0.05, **p < 0.01.

Figure S4

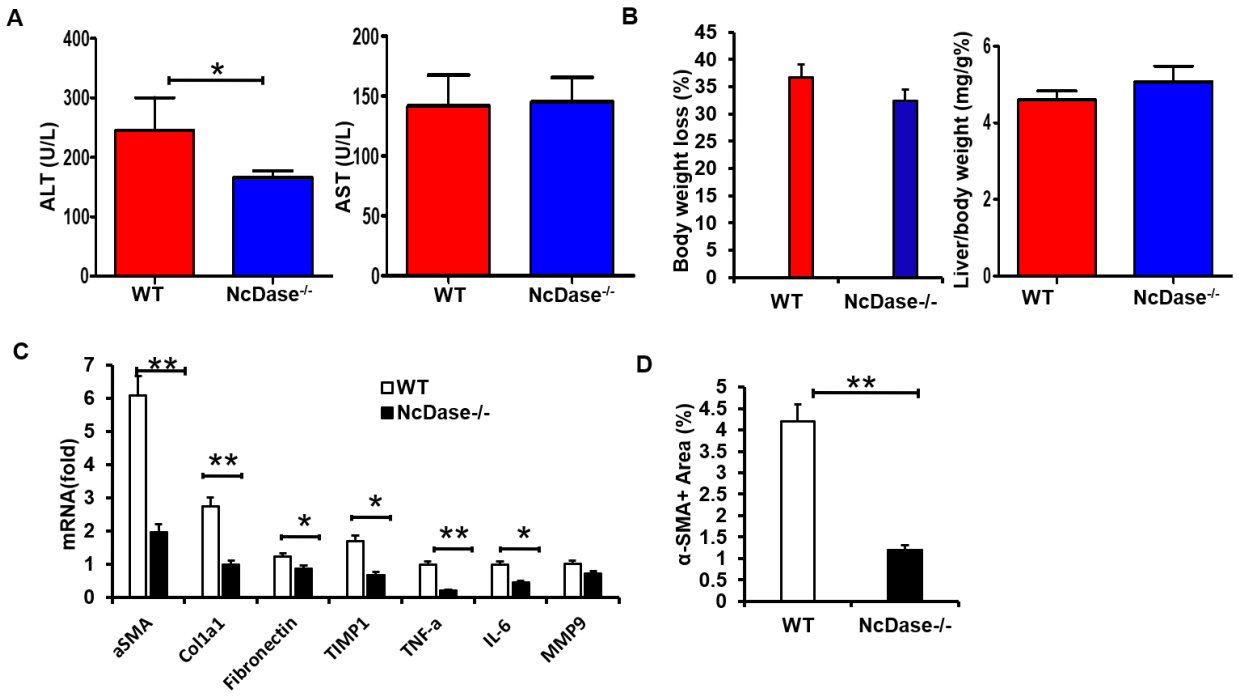

Figure S4 NcDase deficiency reduces liver inflammation and fibrosis in MCD-fed mice

(A) Levels of serum ALT and AST.

(B) Body weights of mice and the ratio of liver to body weight.

(C) mRNA levels of the indicated genes related to inflammation and fibrosis in liver.

(D) αSMA^{+} cells.

Mean ± SEM; n = 10, *p < 0.05, **p < 0.01.

Figure S5

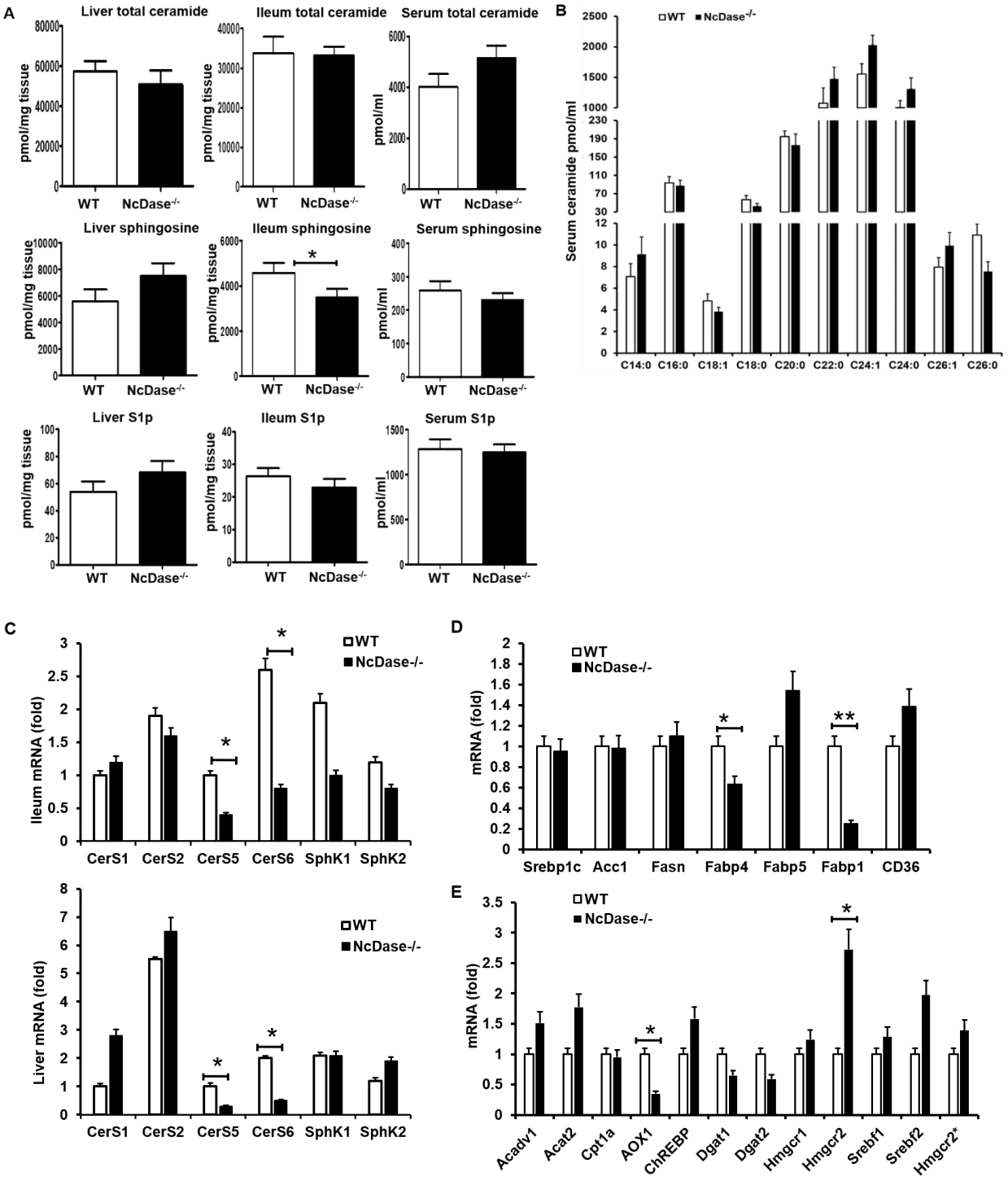

Figure S5 Decreased expression of hepatic lipogenic enzymes in NcDase$^{-/-}$ mice

WT mice or NcDase$^{-/-}$ mice were fed the HFD for 14 months.

(A) Levels of total ceramides, sphingosine and S1p in the liver, small intestine and serum.

(B Levels of acyl-chain ceramides in the serum.

(C) Real-time PCR analysis of ceramide synthases (CerS1, CerS2, CerS5, and CerS6) mRNA levels and sphingosine kinase (SphK1 and SphK2) mRNA levels in the ileum and liver.

(D-E) Real-time PCR analysis of mRNA levels of the indicated genes related to lipid metabolism in the liver.

Mean ± SEM; n = 10, *p < 0.05, **p < 0.01.

Figure S6

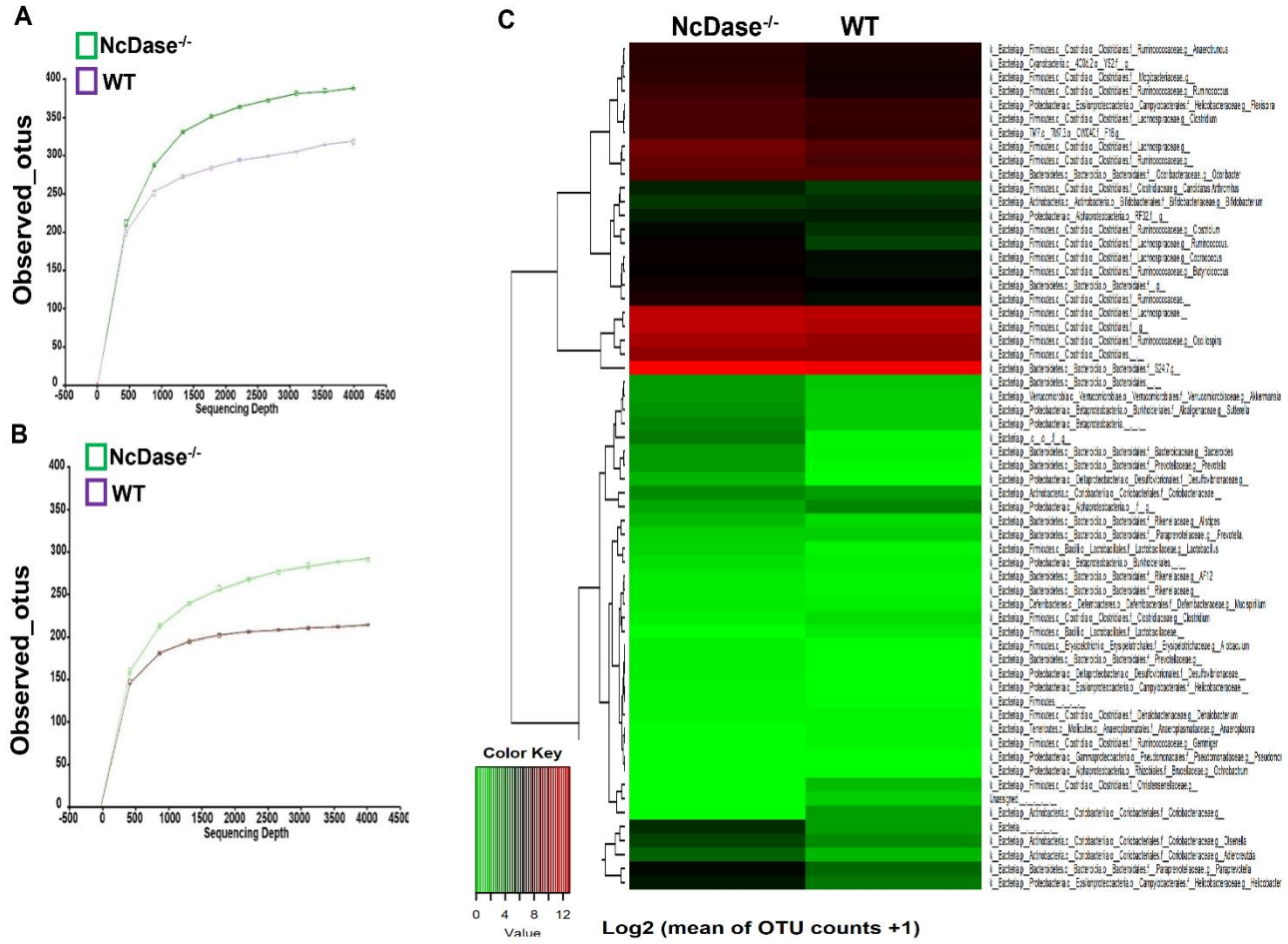

Figure S6 Decreased hepatic MUFAs levels are associated with gut microbiota in NcDase$^{-/-}$ mice.

(A and B) Rarefaction curves of species richness and diversity between HFD-fed WT and HFD-fed NcDase$^{-/-}$ mice. Samples based on Faith's diversity measure (PD) (A) and observed OTUs (B).

(C) Heatmap of differentially represented bacterial species in feces between NCD-fed WT and NCD-fed NcDase$^{-/-}$ mice.

Figure S7

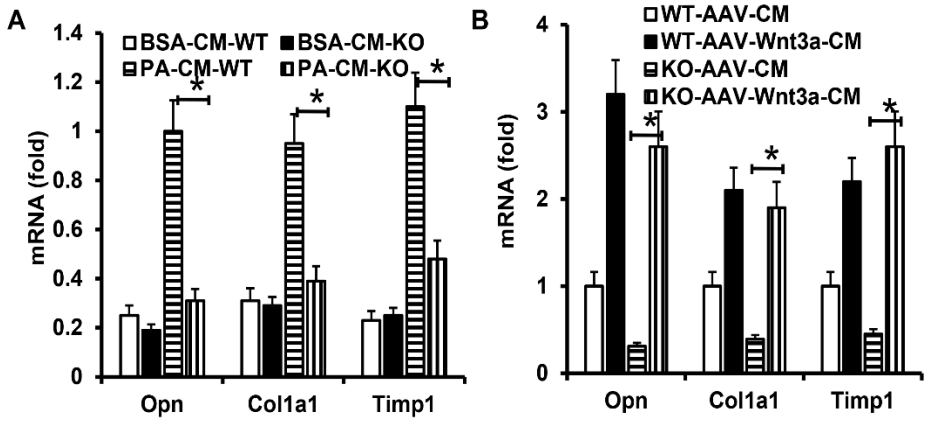

Figure S7 NcDase-induced hepatocyte Wnt3a increases the expression of fibrosis-related genes in hepatic stellate cells (HSCs)

(A) HSCs were incubated for 72 hr with conditioned media (CM) or non-CM obtained from WT or NcDase-null hepatocytes with/without PA treatment and then assayed for Opn, Timp1, and Col1a1 mRNA.

(B) WT and NcDase$^{-/-}$ hepatocytes were infected with AAV-Wnt3a or control AAV. Conditioned media were collected for treatment of HSC. HSCs were incubated for 72 hr with AAV-Wnt3a-CM or AAV-CM and then assayed for Opn, Timp1, and Col1a1 mRNA.

Figure S8

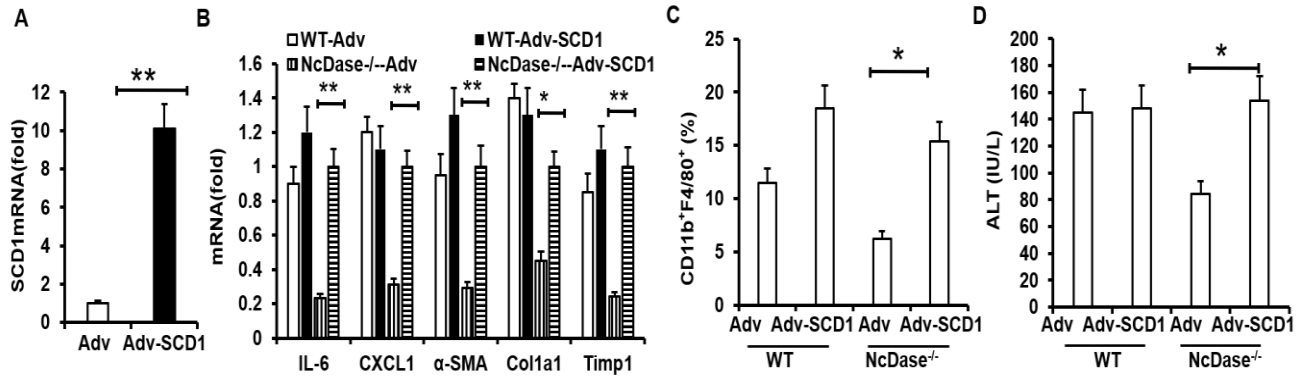

Figure S8 SCD1 overexpression promotes the development of NASH by in NcDase$^{-/-}$ mice

Four groups of mice, including WT mice injected with control adenovirus (WT-Adv), WT mice injected with SCD1 adenovirus (WT-Adv-SCD1), NcDase$^{-/-}$ mice injected with control adenovirus (NcDase$^{-/-}$-Adv), NcDase$^{-/-}$ mice injected with SCD1 adenovirus (NcDase$^{-/-}$-Adv-SCD1), were fed with HSPC for 16 weeks.

(A) mRNA levels of SCD1 after SCD1 adenovirus infection in WT mice.

(B) mRNA levels of the indicated genes related to inflammation and fibrosis in liver.

(C) FACS analysis of CD11b^{+}F4/80^{+} macrophages in the liver.

(D) Levels of serum ALT.

Mean ± SEM; n = 5, *p < 0.05, **p < 0.01.