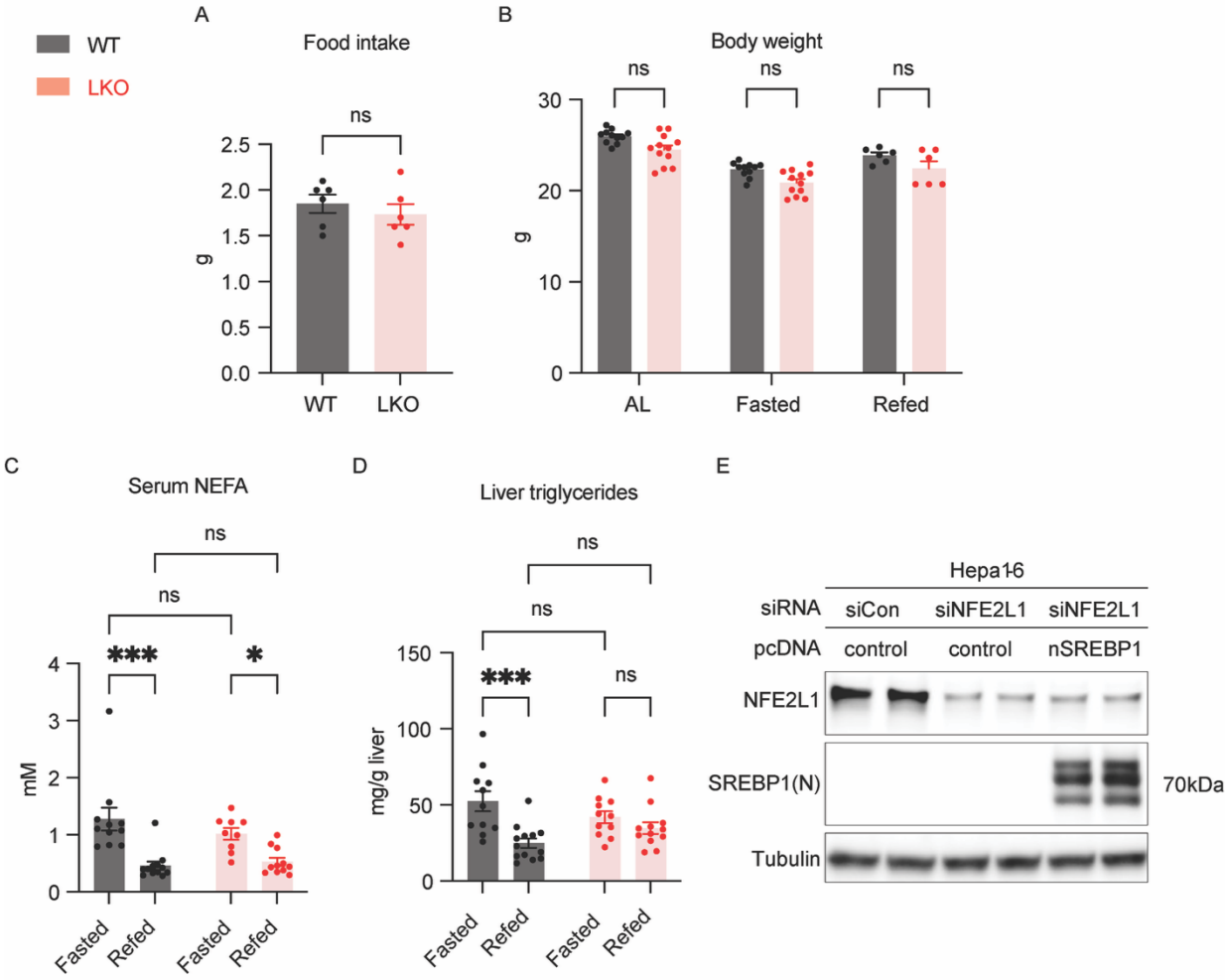


**Figure S1. NFE2L1 deficiency does not alter food intake, body weight, or serum NEFA.**

(A) Food intake during a 6-hour refeeding period after 16-hour fasting in NFE2L1 WT and LKO mice (n=6/group). (B) Body weights of WT and LKO mice under ad libitum (AL), 16-hour fasted (Fasted), and 6-hour refed (Refed) conditions. (C) Serum NEFA levels measured by ELISA (n=11/group). (D) Liver TG levels. (E) Immunoblot analysis and quantification of SREBP1 in Hepa1-6 hepatocytes after transient siRNA-mediated NFE2L1 knockdown (24 hours) and re-expression of SREBP1 (N). Data are mean ± SEM. Statistical significance assessed by two-way ANOVA (C, D) or Student's t-test (A, B) (*P < 0.05, ***P < 0.001).

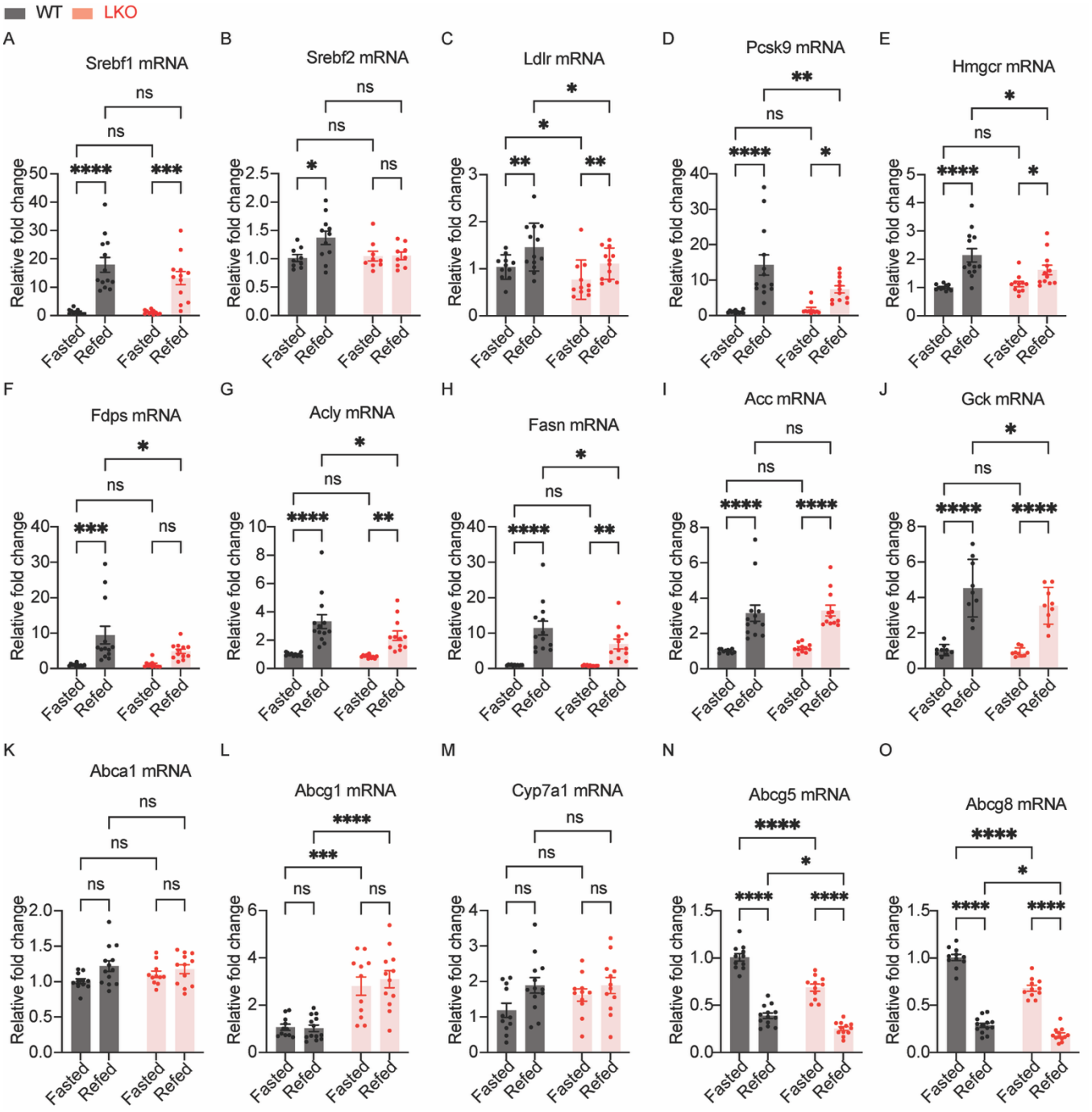


**Figure S2. Hepatic mRNA levels of SREBP and LXR target genes in WT and LKO mice.**

8-week-old NFE2L1 WT or LKO mice were fasted for 16 hours or fasted for 16 hours followed by 6-hour refeeding (n=11/group). Gene expression was normalized to 18S ribosomal RNA. Gray and red bars denote WT and LKO mice, respectively. Statistical significance was assessed by two-way ANOVA (*P < 0.05, **P < 0.0, ***P < 0.001, ****P < 0.0001).

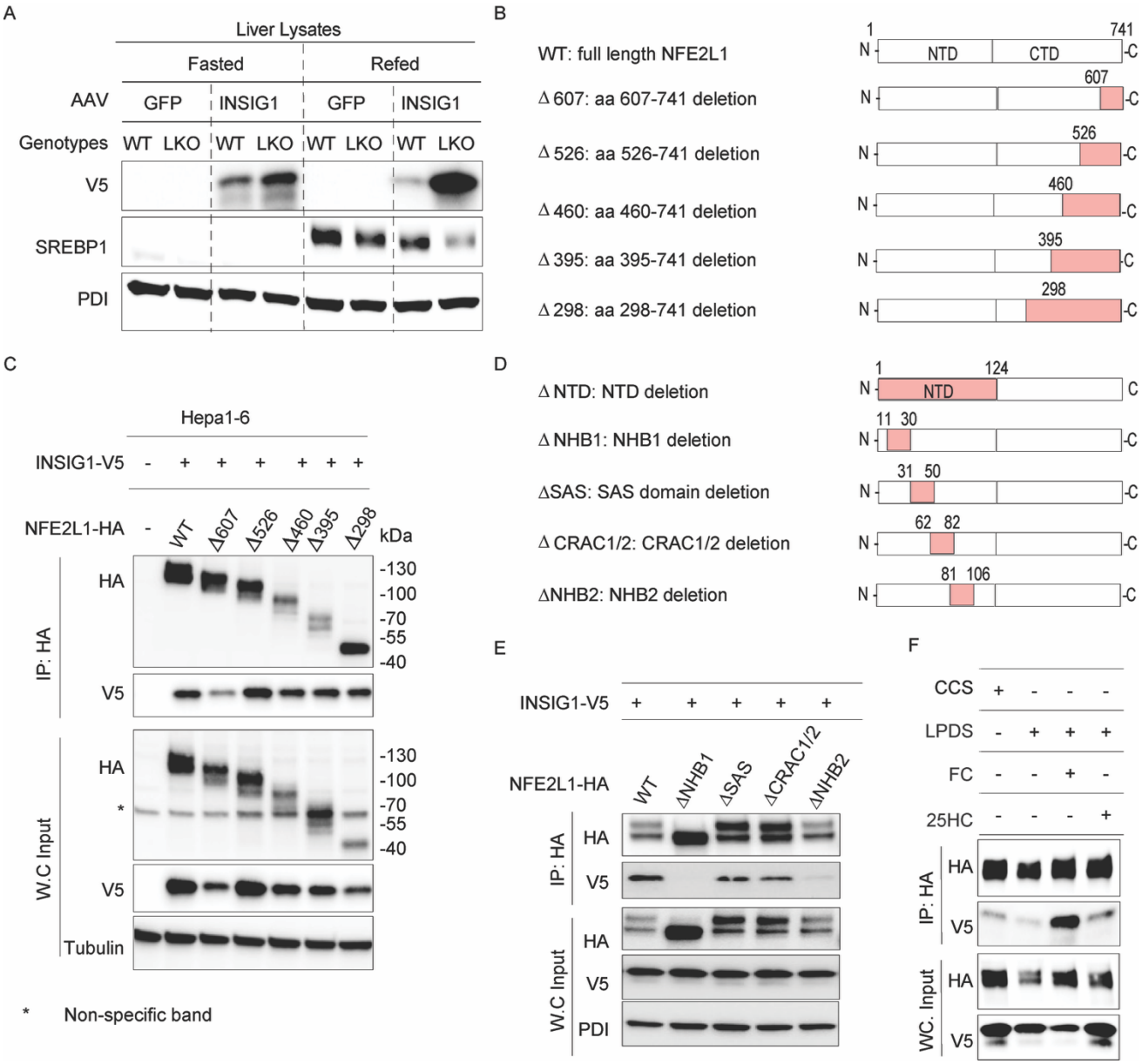


**Figure S3. The N terminal domain (NTD) of NFE2L1 binds to INSIG1 in the ER.**

(A) Western blot analysis of INSIG1-V5 in liver from WT and LKO mice injected with AAV-GFP or INSIG1-V5 under fasting or refed conditions. (B) Schematic representation of NFE2L1 truncation mutants lacking regions in the C-terminal domain (CTD). Red color indicates deleted regions. (C) Co-immunoprecipitation (Co-IP) of INSIG1-V5 with HA-tagged NFE2L1 CTD mutants in Hepa1-6 cells. Lysates were harvested 48 h post-transfection and immunoprecipitated with HA beads. (D) Schematic representation of NFE2L1 truncation mutants lacking regions in the N-terminal domain (NTD). (E) Co-IP of INSIG1-V5 with HA-

tagged NFE2L1 NTD mutants, performed as described in panel (B). (F) Sterol-dependent interaction between NFE2L1 and INSIG1. Cells transfected with NFE2L1-WT and INSIG1-V5 were treated for 3 h with 5% cosmic calf serum (CCS), 5% lipoprotein-deficient serum (LPDS), 50 μM free cholesterol (FC), or 1 μM 25-hydroxycholesterol (25HC). Lysates were subjected to Co-IP with HA beads. Western blots are representative of ≥3 independent experiments.

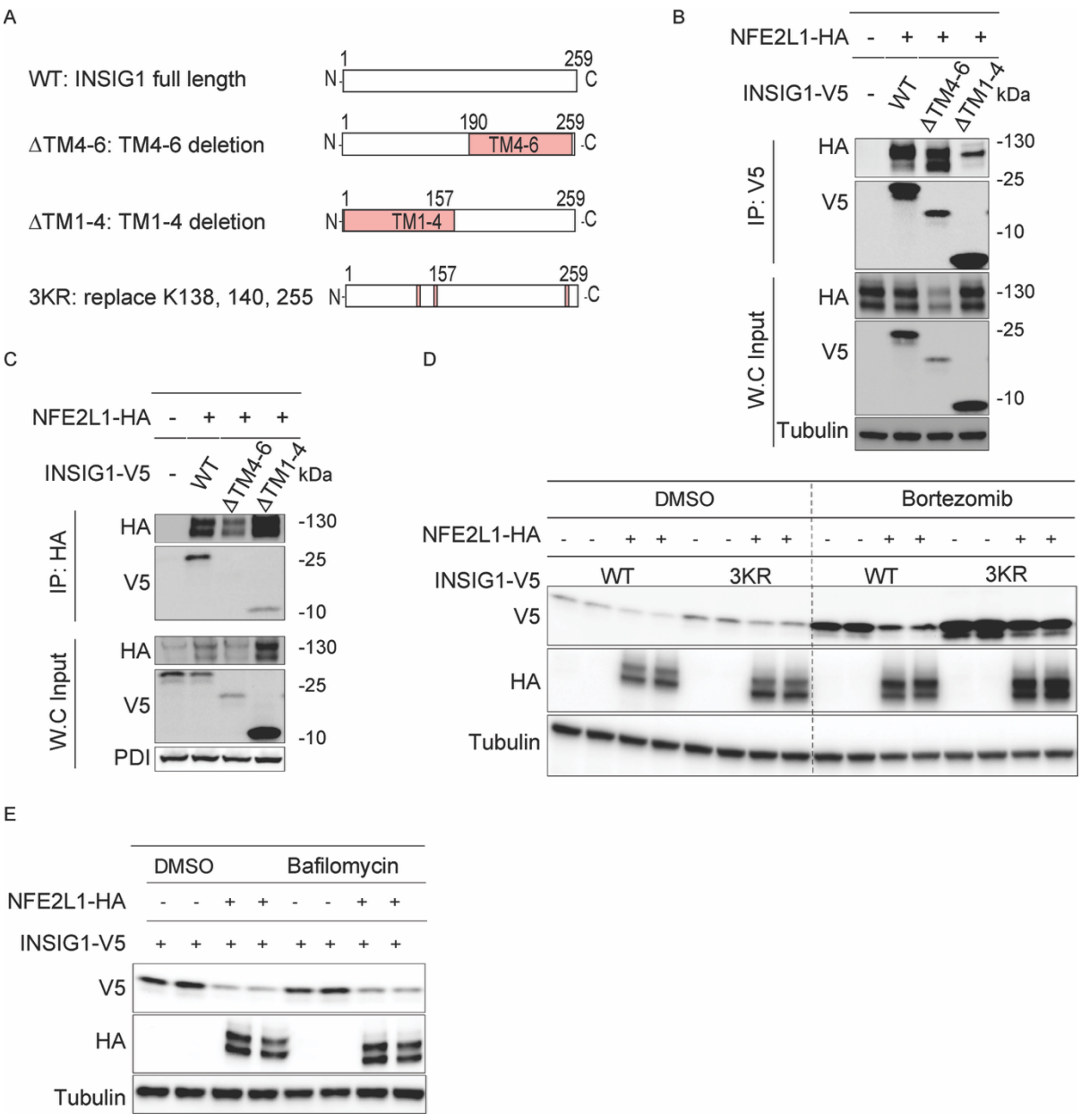


**Figure S4. Mechanisms of INSIG1 degradation.**

(A) Schematic representation of INSIG1 truncation mutants lacking regions in the transmembrane domain (TM). Red indicates deleted regions. (B) Co-immunoprecipitation (Co-IP) of INSIG1-V5 mutants with HA-tagged NFE2L1 in Hepa1-6 cells. Lysates were harvested 48 h post-transfection and immunoprecipitated with HA beads. (C) Co-IP of NFE2L1 with V5-tagged INSIG1 mutants, performed as in (B). (D) Immunoblotting of INSIG1 WT and mutant 3KR after proteasome inhibition. Cells transfected with NFE2L1-WT and INSIG1-V5 were treated for 3 h with proteasome inhibitor Bortezomib. (E) Immunoblotting of INSIG1 WT and

NFE2L1 after lysosome inhibition. Cells transfected with NFE2L1-WT and INSIG1-V5 were treated for 3 h with lysosome inhibitor bafilomycin. Immunoblots are representative of ≥3 independent experiments.

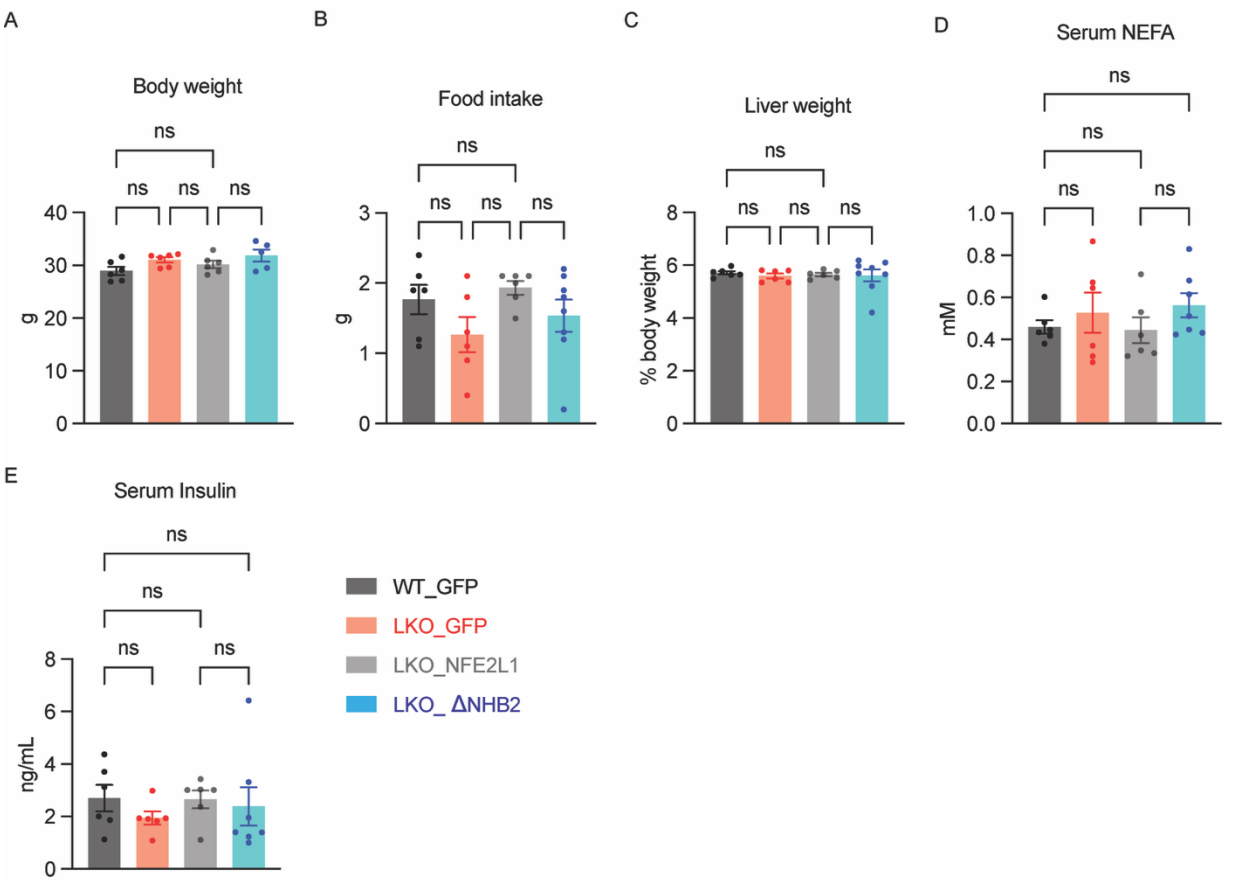


**Figure S5. AAV-mediated NFE2L1 expression does not alter metabolic parameters in mice.**

NFE2L1 wild-type (WT) or liver-specific knockout (LKO) mice (8-week-old) were injected with GFP, NFE2L1-WT, or NFE2L1-ΔNHB2 containing AAV-based expression constructs. Three weeks post-injection, mice were fasted overnight and refed for 6 hours (n=6/group). (A) Body weight. (B) Food intake during refeeding. (C) Liver weights. (D) Serum non-esterified fatty acid (NEFA) levels. (E) Serum insulin levels. Data are mean ± SEM. Statistical significance was assessed by one-way ANOVA.

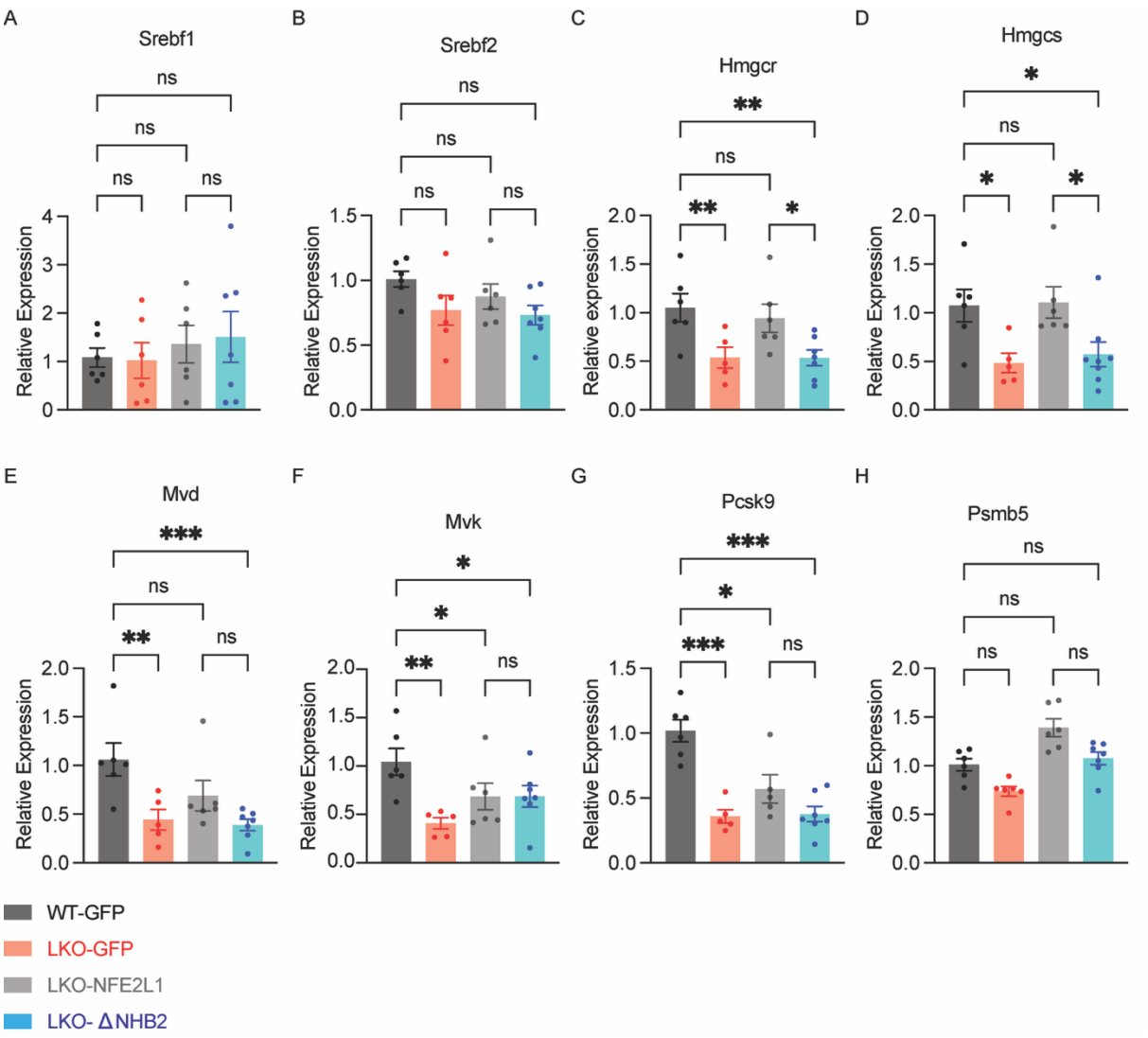


**Figure S6. AAV-mediated restoration of NFE2L1 rescues SREBP target gene expression in livers of NFE2L1-LKO mice.** NFE2L1 wild-type (WT) or liver-specific knockout (LKO) mice (8-week-old) were injected with GFP, NFE2L1-WT, or NFE2L1-ΔNHB2 containing AAV-based expression constructs. Three weeks after injection, mice were fasted overnight then refed for 6 hours (n=6/group). (A-H) Relative mRNA levels of SREBP target genes in refed livers. Expression of the genes were normalized to 18S. Data are the means ± SEM. Statistical significance was assessed by one-way ANOVA.

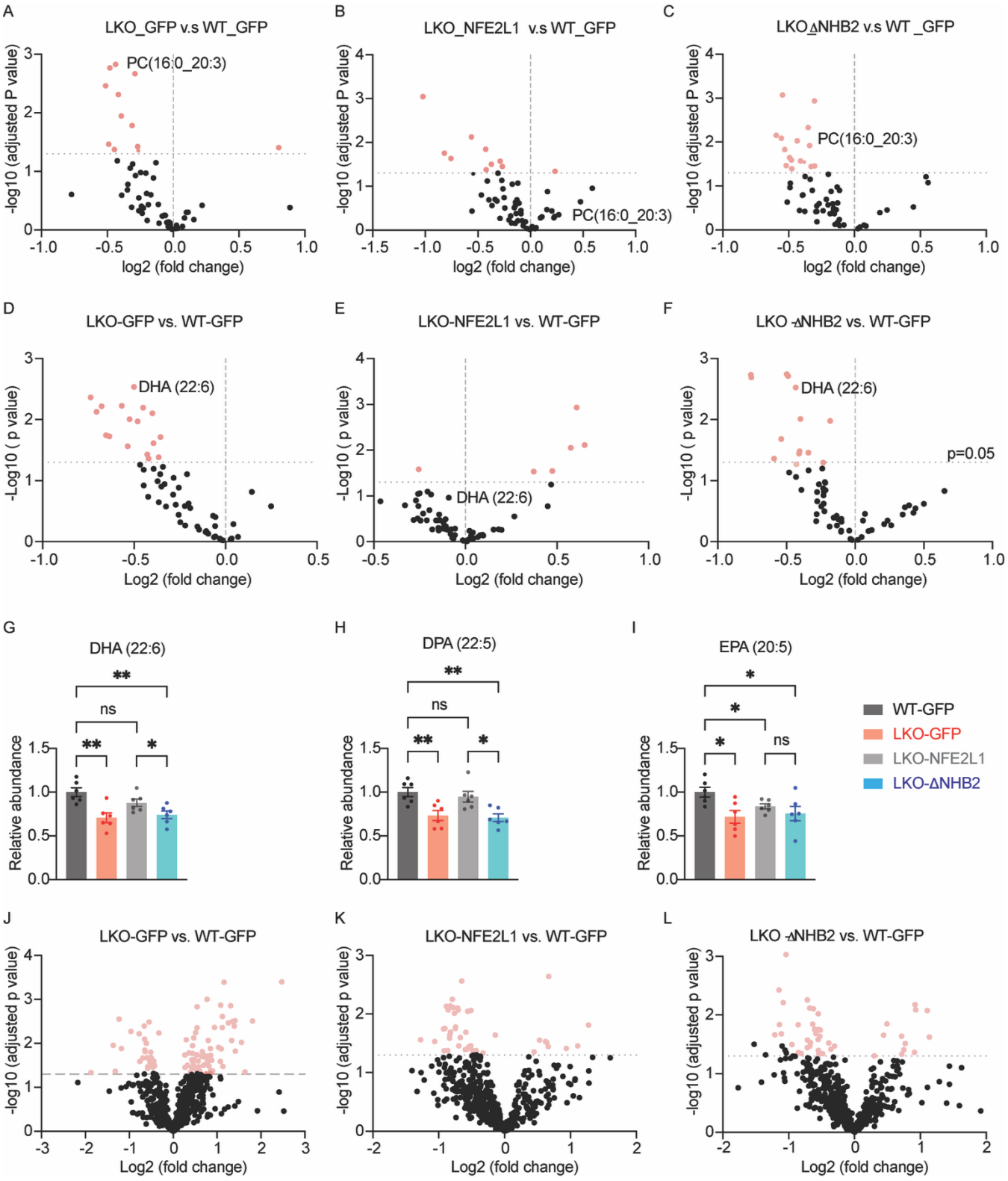


**Figure S7. Effect of NFE2L1 -INSIG1 axis on serum phosphatidylcholine (PC) and PUFAs.**

(A-C) Serum lipidomic analysis for phospholipid species with different fatty acid compositions (n=6 per group). (D-F) Fatty acid composition analysis within serum TG pool (n=6/group). (G-I)

Serum DHA, DPA and EPA within TG pool. (J-L) Liver lipidomic analysis for TG species with different fatty acid compositions (n=6 per group). Red indicates $p<0.05$. Statistical significance was assessed by one-way ANOVA (G, H, I), *$p<0.05$.

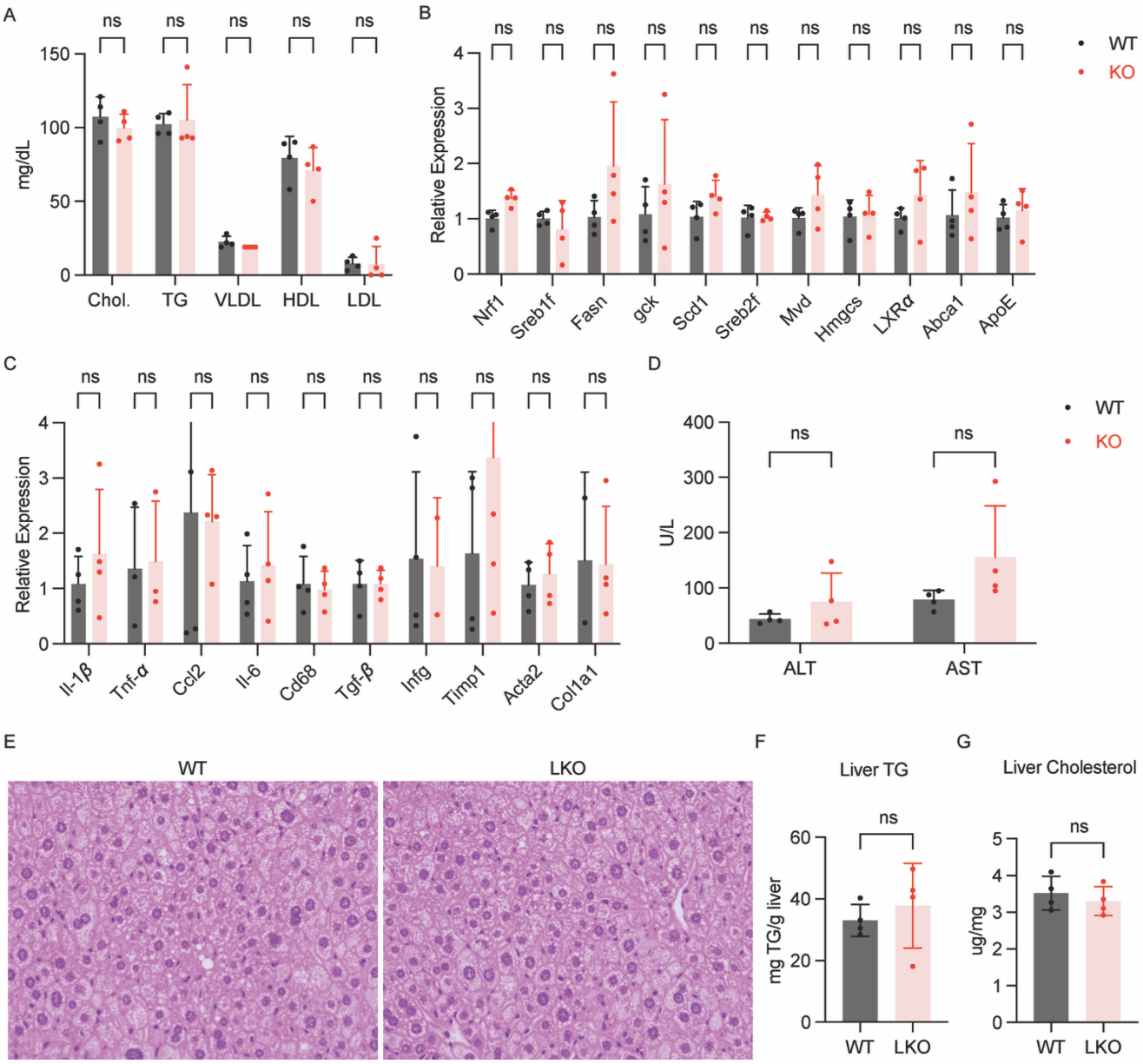


**Figure S8. Role of NFE2L1 in high fat diet induced fatty liver.**

Liver-specific knockout mice and their littermates were fed with fed with high fat diet for 2 months. Mice were fed ad libitum before samples collection (n=4-5/group). (A) Serum lipids profiles measured via Piccolo Lipid Panel Plus. (B) Liver mRNA levels of SREBP1/2 and LXR target genes normalized to 18S ribosomal RNA (18S). (C) mRNA levels of liver inflammation and fibrosis markers. (D) Serum ALT and AST levels (Piccolo Lipid Panel Plus). (E) Representative histology images of liver section staining H&E. (F) Quantification of liver TG and cholesterol. Data: Mean ± SEM. Statistics: t-test (*P < 0.05).

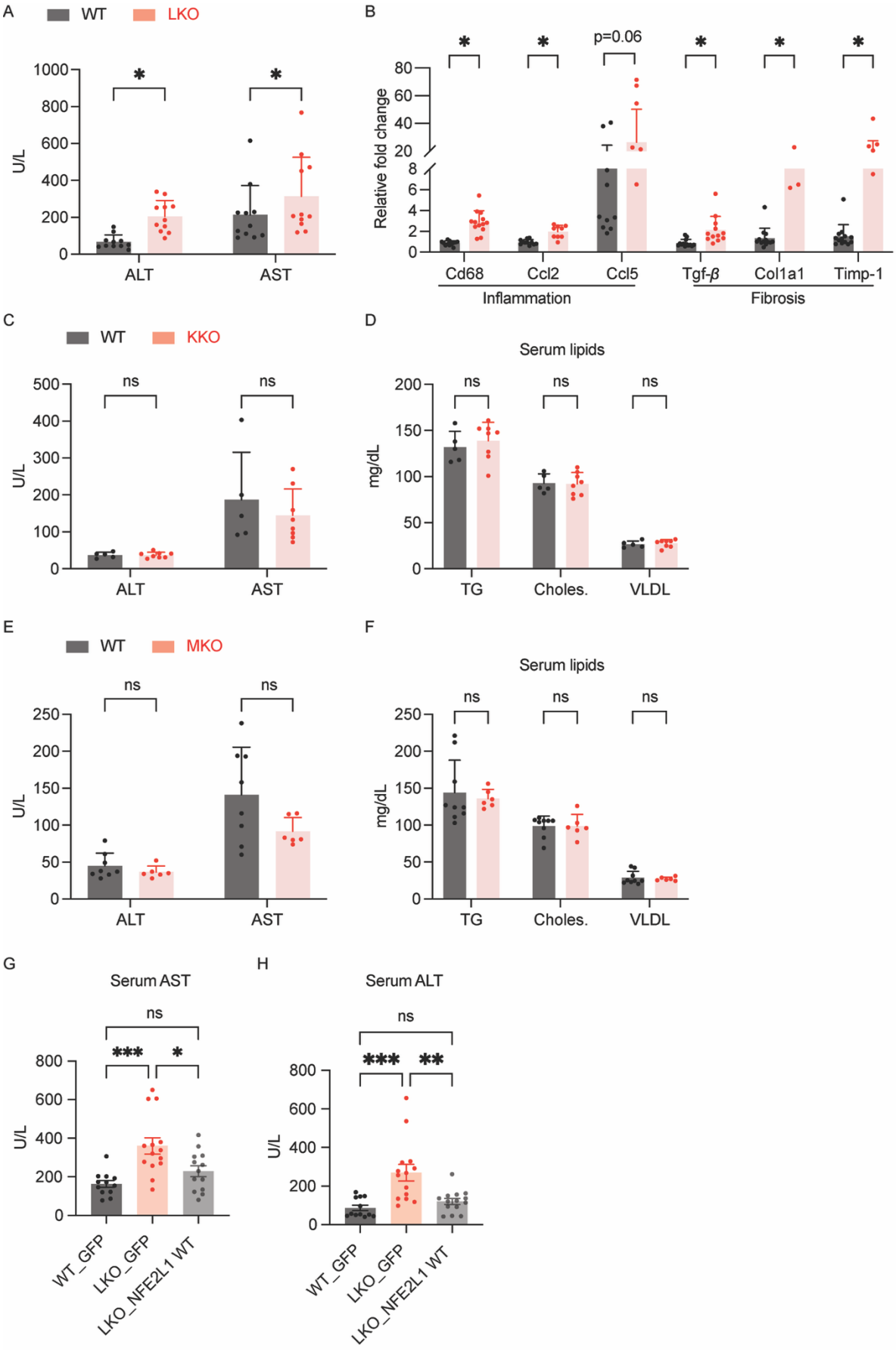
A
WT
LKO
U/L
ALT
AST
B
Relative fold change
p=0.06
Cd68
Ccl2
Ccl5
Tgf-β
Col1a1
Timp-1
Inflammation
Fibrosis
C
WT
KKO
ns
D
Serum lipids
mg/dL
TG
Choles.
VLDL
E
WT
MKO
F
Serum lipids
G
Serum AST
H
Serum ALT
WT_GFP
LKO_GFP
LKO_NFE2L1 WT

**Figure S9. NFE2L1 deficiency exacerbates liver injury, inflammation, and fibrosis.**

NFE2L1-WT or -LKO mice (8-week-old) mice were either fasted for 16 hours or fasted for 16 hours then refed for 6 hours (n=11/group). (A) Serum ALT and AST. (B) Relative mRNA levels of genes involved in inflammation and fibrogenesis. Total RNA was extracted from livers and subjected to quantitative real-time PCR analysis. Expression of the genes was normalized to 18S. (C-D) 8-week-old NFE2L1-WT or Kupffer cell-specific deletion (Nfe2l1-Clec4f-Cre, KKO) mice were fasted overnight (n=6-8/group). Serum ALT and AST (C) and lipid profiles (D) were measured. (E-F) 8-week-old NFE2L1-WT or myeloid-specific NFE2L1 deletion (Nfe2l1-LysM-Cre, MKO) mice were fasted overnight (n=6-8/group). Serum ALT and AST (E) and lipid profiles (F) were measured. (G-H) 8-week-old NFE2L1-WT or -LKO mice were injected with AAV-GFP, AAV-NFE2L1-WT. Three weeks after injection, mice were fasted overnight then refed for 6 hours (n=6/group). Serum ALT (G) and AST (H) were measured. Data are the means ± SEM. Statistical significance was assessed by t-test (*$P < 0.05$).

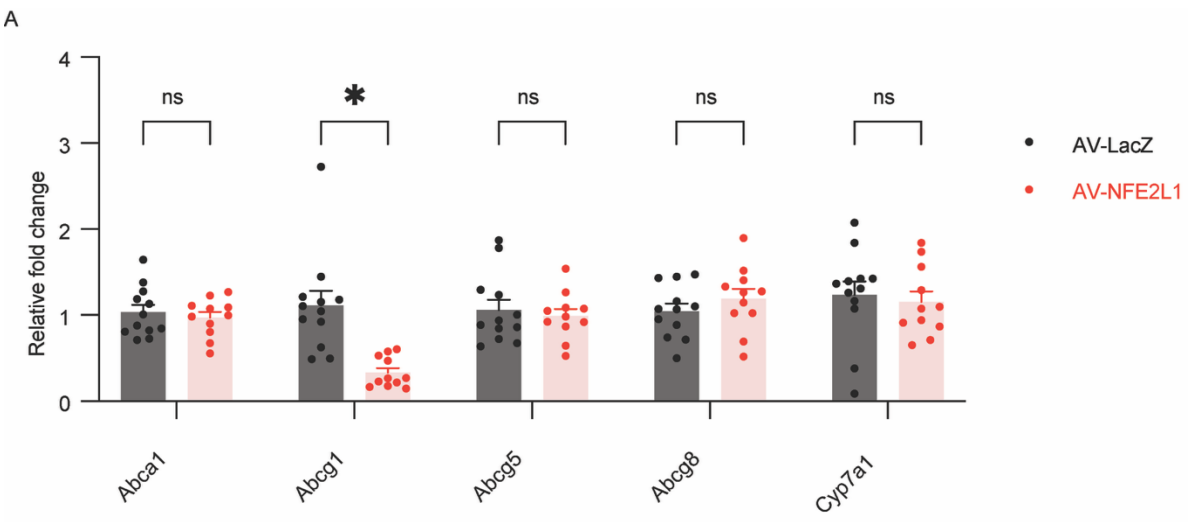


**Figure S10. The role of NFE2L1 in LXR activity.**

8-week-old db/db mice were injected with either AV-LacZ or AV-NFE2L1 and fed with MCD diet for 2 weeks. Mice were fed ad libitum before sample collection (n=12/group). (A) Liver mRNA levels of LXR target genes normalized to 18S ribosomal RNA (18S). Data: Mean ± SEM. Statistics: t-test (*P < 0.05).

## TABLES

### Table S1. Lipidomic Profiling of Serum Triglycerides Species

| Compared to WT-GFP mice | | | | | |
|---|---|---|---|---|---|
| **LKO-GFP** | | **LKO-NFE2L1** | | **LKO-NHB2** | |
| **TG species** | **-Log10** | **TG species** | **-Log10** | **TG species** | **-Log10** |
| TG(20:0_18:1_22:6) | 3.804 | TG(O-18:0_16:0_18:1) | 3.089 | TG(20:0_18:1_22:6) | 4.452 |
| TG(18:1_18:2_22:6) | 3.766 | TG(16:0_16:0_18:0) | 2.480 | TG(19:1_18:2_22:6) | 3.790 |
| TG(16:0_18:2_20:5) | 3.584 | TG(16:0_18:2_18:3) | 2.316 | TG(18:1_20:1_22:6) | 3.759 |
| TG(19:1_18:2_22:6) | 3.536 | TG(O-20:0_16:0_18:1) | 2.110 | TG(14:0_16:0_18:0) | 3.523 |
| TG(18:1_19:1_22:6) | 3.460 | TG(O-24:2_16:0_18:1) | 2.055 | TG(18:1_20:4_24:5) | 3.449 |
| d5-TG(40:9_18:0) | 3.177 | TG(10:0_14:0_16:0) | 2.041 | TG(16:0_16:0_18:0) | 3.275 |
| TG(18:1_20:1_22:6) | 3.013 | TG(14:0_16:0_18:0) | 1.929 | TG(15:0_18:1_22:6) | 3.166 |
| TG(16:1_18:1_22:1) | 2.986 | TG(16:0_18:2_20:5) | 1.912 | TG(17:0_18:1_22:6) | 3.026 |
| TG(14:0_15:0_22:6) | 2.890 | TG(15:0_18:1_22:6) | 1.872 | TG(16:1_18:3_18:3) | 2.897 |
| TG(17:0_18:1_22:6) | 2.817 | TG(16:0_18:2_22:6) | 1.769 | d5-TG(40:9_18:0) | 2.668 |
| TG(19:0_18:1_18:2) | 2.803 | TG(O-24:2_16:0_18:0) | 1.718 | TG(16:0_22:0_22:6) | 2.448 |
| TG(16:0_22:0_22:6) | 2.750 | TG(O-16:0_16:0_18:1) | 1.679 | TG(17:0_18:1_24:6) | 2.431 |
| TG(18:1_19:1_18:2) | 2.739 | TG(28:1_9:0)+OO:(s) | 1.560 | TG(16:0_20:0_22:6) | 2.385 |
| TG(18:1_22:1_18:2) | 2.570 | TG(O-20:1_16:0_18:1) | 1.545 | TG(16:0_24:5_22:6) | 2.382 |
| TG(22:0_18:1_18:2) | 2.564 | TG(15:0_18:2_22:6) | 1.534 | TG(15:0_16:1_22:6) | 2.361 |
| TG(21:0_18:1_18:2) | 2.547 | TG(O-16:0_16:0_18:2) | 1.495 | TG(18:1_20:3_22:6) | 2.349 |
| TG(17:1_18:2_22:6) | 2.527 | TG(18:2_22:6_22:6) | 1.481 | TG(16:0_18:2_22:6) | 2.277 |
| TG(16:0_24:1_22:6) | 2.518 | TG(18:2_22:6_24:6) | 1.344 | TG(14:0_15:0_22:6) | 2.269 |
| TG(18:1_22:1_22:6) | 2.514 | TG(18:2_18:2_22:6) | 1.338 | TG(14:0_18:1_18:3) | 2.219 |
| TG(15:0_16:1_22:6) | 2.448 | TG(16:0_17:0_18:0) | 1.335 | TG(17:1_18:1_22:6) | 2.213 |
| TG(18:1_18:1_18:2) | 2.439 | TG(16:1_18:3_18:3) | 1.319 | TG(14:0_18:1_18:2) | 2.156 |

| | | | | | |
|---|---|---|---|---|---|
| TG(15:0_18:1_22:6) | 2.420 | TG(14:0_18:1_18:3) | 1.302 | TG(16:1_18:1_22:1) | 2.110 |
| TG(17:0_18:1_24:6) | 2.397 | | | TG(19:0_18:1_18:2) | 2.099 |
| TG(16:0_18:1_23:1) | 2.364 | | | TG(16:0_24:1_22:6) | 2.079 |
| TG(17:1_18:1_22:6) | 2.313 | | | TG(16:0_18:1_22:5) | 2.071 |
| TG(16:0_21:0_22:6) | 2.312 | | | TG(16:0_21:0_22:6) | 2.069 |
| d5-TG(36:4_18:2) | 2.281 | | | TG(16:0_18:1_22:6) | 2.050 |
| TG(15:0_18:2_22:6) | 2.229 | | | TG(16:0_18:2_20:5) | 2.015 |
| TG(16:0_24:5_22:6) | 2.174 | | | TG(15:0_18:2_22:6) | 2.005 |
| TG(16:0_24:0_22:6) | 2.170 | | | TG(18:0_18:1_22:6) | 1.972 |
| TG(16:0_20:5_22:6) | 2.166 | | | TG(12:0_18:1_18:3) | 1.922 |
| TG(16:0_18:1_22:6) | 2.165 | | | TG(18:1_22:6_24:6) | 1.908 |
| TG(16:0_18:2_22:6) | 2.157 | | | TG(16:0_18:2_18:3) | 1.898 |
| TG(16:1_22:6_22:6) | 2.151 | | | TG(17:1_18:2_22:6) | 1.862 |
| TG(18:1_20:1_20:3) | 2.034 | | | TG(16:0_20:5_22:6) | 1.853 |
| TG(18:1_22:6_22:6) | 1.896 | | | TG(18:2_22:6_24:6) | 1.825 |
| TG(17:1_18:1_18:2) | 1.868 | | | TG(18:1_22:5_22:6) | 1.810 |
| TG(22:1_18:2_22:6) | 1.849 | | | TG(18:1_22:6_22:6) | 1.750 |
| TG(18:0_18:1_22:6) | 1.849 | | | TG(18:2_22:5_22:6) | 1.735 |
| TG(18:0_18:1_18:2) | 1.809 | | | TG(16:0_24:0_22:6) | 1.627 |
| TG(16:0_18:1_24:1) | 1.806 | | | TG(17:0_18:1_22:5) | 1.616 |
| TG(18:2_18:2_22:6) | 1.804 | | | TG(18:1_24:5_22:6) | 1.601 |
| TG(18:2_18:2_20:5) | 1.797 | | | TG(17:0_18:1_20:4) | 1.559 |
| TG(16:1_18:3_18:3) | 1.754 | | | TG(18:1_18:2_22:6) | 1.543 |
| TG(14:0_16:0_18:0) | 1.750 | | | TG(18:1_20:1_20:3) | 1.541 |
| TG(16:0_18:1_22:5) | 1.742 | | | TG(14:0_18:2_18:3) | 1.491 |
| TG(16:0_18:2_18:2) | 1.735 | | | TG(14:0_16:0_18:3) | 1.473 |

| | | | | | |
|---|---|---|---|---|---|
| TG(O-15:2_3:0_18:2) | 1.732 | | | TG(27:1_9:0)+OO:(s) | 1.460 |
| TG(16:0_16:0_18:0) | 1.732 | | | TG(21:0_18:1_18:3) | 1.431 |
| TG(22:7_16:0_20:5) | 1.714 | | | TG(18:1_22:1_18:2) | 1.413 |
| TG(O-18:0_18:1_18:2) | 1.701 | | | TG(18:1_20:4_22:6) | 1.396 |
| TG(18:2_22:5_22:6) | 1.689 | | | TG(28:0_20:5) | 1.362 |
| TG(15:0_18:1_18:2) | 1.685 | | | d5-TG(36:6_20:2) | 1.362 |
| TG(17:0_18:1_20:4) | 1.671 | | | TG(10:0_14:0_18:1) | 1.352 |
| TG(17:0_18:1_18:2) | 1.654 | | | TG(15:0_16:0_18:0) | 1.342 |
| TG(16:0_20:0_22:6) | 1.647 | | | TG(16:0_18:1_18:2) | 1.335 |
| TG(18:1_20:4_22:6) | 1.611 | | | TG(15:0_18:1_18:2) | 1.310 |
| TG(18:1_16:0_18:3) | 1.513 | | | | |
| TG(14:0_18:1_18:2) | 1.491 | | | | |
| TG(16:0_18:1_22:1) | 1.468 | | | | |
| TG(12:0_18:1_18:3) | 1.468 | | | | |
| TG(12:0_18:1_22:6) | 1.448 | | | | |
| TG(18:1_20:3_22:6) | 1.436 | | | | |
| TG(18:1_20:1_18:2) | 1.422 | | | | |
| TG(17:0_18:1_20:1) | 1.414 | | | | |
| TG(14:0_18:2_22:6) | 1.413 | | | | |
| TG(14:0_18:1_18:3) | 1.412 | | | | |
| TG(18:2_18:3_20:5) | 1.395 | | | | |
| TG(16:0_18:2_18:3) | 1.363 | | | | |
| TG(O-15:2_3:0_22:6) | 1.361 | | | | |
| TG(19:0_18:1_20:4) | 1.361 | | | | |

Note: The table includes only TG species meeting statistical significance threshold [(-Log10(adjusted p value)>1.3, equivalent to p<0.05]

## Table S2. Lipidomic Profiling of FA species within Serum Triglycerides Pool

| Compared to WT-GFP mice | | | | | |
|---|---|---|---|---|---|
| **LKO-GFP** | | **LKO-NFE2L1** | | **LKO-NHB2** | |
| **FA species** | **-Log10** | **FA species** | **-Log10** | **FA species** | **-Log10** |
| (22:6) | 2.537 | (O-18:0) | 2.930 | (22:5) | 2.744 |
| (23:1) | 2.364 | (O-20:0) | 2.110 | (24:5) | 2.735 |
| (18:2) | 2.226 | (O-24:2) | 2.051 | (20:3) | 2.714 |
| (22:1) | 2.218 | (20:5) | 1.580 | (24:6) | 2.692 |
| (22:5) | 2.196 | (O-20:1) | 1.545 | (22:6) | 2.527 |
| (22:0) | 2.129 | (O-16:0) | 1.531 | (18:3) | 2.012 |
| (20:3) | 2.104 | | | (9:0+OO:(s)) | 1.978 |
| (24:5) | 2.007 | | | (22:0) | 1.680 |
| (20:5) | 1.971 | | | (20:5) | 1.481 |
| (24:1) | 1.743 | | | (27:1) | 1.460 |
| (3:0) | 1.726 | | | (18:2) | 1.444 |
| (O-15:2) | 1.726 | | | (28:0) | 1.362 |
| (22:7) | 1.714 | | | (18:1) | 1.295 |
| (19:0) | 1.614 | | | | |
| (24:6) | 1.565 | | | | |
| (21:0) | 1.429 | | | | |
| (18:1) | 1.384 | | | | |
| (19:1) | 1.360 | | | | |

Note: The table includes only FA species meeting statistical significance threshold [(-Log10(adjusted p value)>1.3, equivalent to $p<0.05$]

**Table S3. Key resources**

| Antibodies | Source | Identifier |
|---|---|---|
| Anti-Histone H3 | Cell Signaling Technology | Cat# 9715 |
| Anti-SCAP | Abcam | Cat# ab153933 |
| Anti-NFE2L1 | Cell Signaling Technology | Cat# 8052 |
| Anti-PDI | Cell Signaling Technology | Cat# 3501 |
| Anti-V5 | Thermo scientific | Cat# R961-25 |
| Anti-HA | Cell Signaling Technology | Cat# 3724 |
| Anti-AKT | Cell Signaling Technology | Cat# 4691 |
| Anti-pAKT | Cell Signaling Technology | Cat# 9271 |
| Anti-INSIG1 | Abcam | Cat# ab70784 |
| Anti-SREBP1 | Abcam | Cat# ab28481 |
| Anti-CD68 | Proteintech | Cat# 25747-1-AP |
| Anti-LDLR | Abcam | Cat# ab52818 |
| Anti-beta Tubulin HRP | Cell Signaling Technology | Cat# ab21058 |
| Chemicals and Reagents | | |
| Bortezomib (PS-341) | Selleckchem | Cat# S1013 |
| Epoxomycin | Millipore | Cat# 324800 |
| Methyl-b-cyclodextrin | Sigma Aldrich | Cat# C4555 |
| TRIzol | ThermoFisher Scientific | Cat# 15596018 |
| Cholesterol | Sigma Aldrich | Cat# C8667 |
| DMEM | GIBCO | Cat# 11965 |
| Cosmic Calf Serum | Hyclone | Cat# SH30087.03 |
| Sandoz 58-035 | Sigma-Aldrich | Cat# S9318-5MG |
| Fos-Choline-13 | Anagrade | Cat# 85775-42-4 |
| Methyl-b-cyclodextrin | Sigma Aldrich | Cat# C4555 |

| | | |
|---|---|---|
| Adenovirus expressing b-galactosidase | Vector Biolabs | Cat# 1080 |
| Adenovirus expressing wild type mouse Nfe2l1 | Vector Biolabs | Cat# 1080 |
| AAV-GFP | Penn Vector Core | V7400S |
| AAV-NFE2L1-HA-WT | Penn Vector Core | V7401S |
| AAV-dNHB2-HA | Penn Vector Core | V7397S |

Critical Commercial Assays

| | | |
|---|---|---|
| Piccolo Lipid Panel | Abaxis | Cat# 07-P02-12A |
| Cholesterol Assay Kits | Cell Biolabs Inc. | Cat# STA-384 |
| NE-PER™ Nuclear Extraction Reagents | Thermo Scientific | Cat# 78833 |

Cell Lines and Mice

| | | |
|---|---|---|
| Hepa1-6 cells | ATCC | CRL-1830 |
| *db/db* mice | The Jackson Laboratory | Jax: 000664 |
| Nfe2l1-flox mouse | International Mouse Phenotyping Consortium (IMPC) | Generated previously from Ref (13) |
| Albumin-Cre mice | The Jackson Laboratory | Jax: 003574 |
| Nfe2l1-Clec4f-Cre mouse | The Jackson Laboratory | Jax: 004781 |
| Nfe2l1-LysM-Cre mouse | The Jackson Laboratory | Jax: 033296 |
| Regular chow diet | Picolab Rodent diet 20 | |
| High fat diet | Research Diets | Cat#D12492 |
| MCD diet | Research Diets | Cat#A02082002BR |