Supplemental Information

Molecular mechanisms of snoRNA-IL15 crosstalk in adipocyte lipolysis and NK cell rejuvenation

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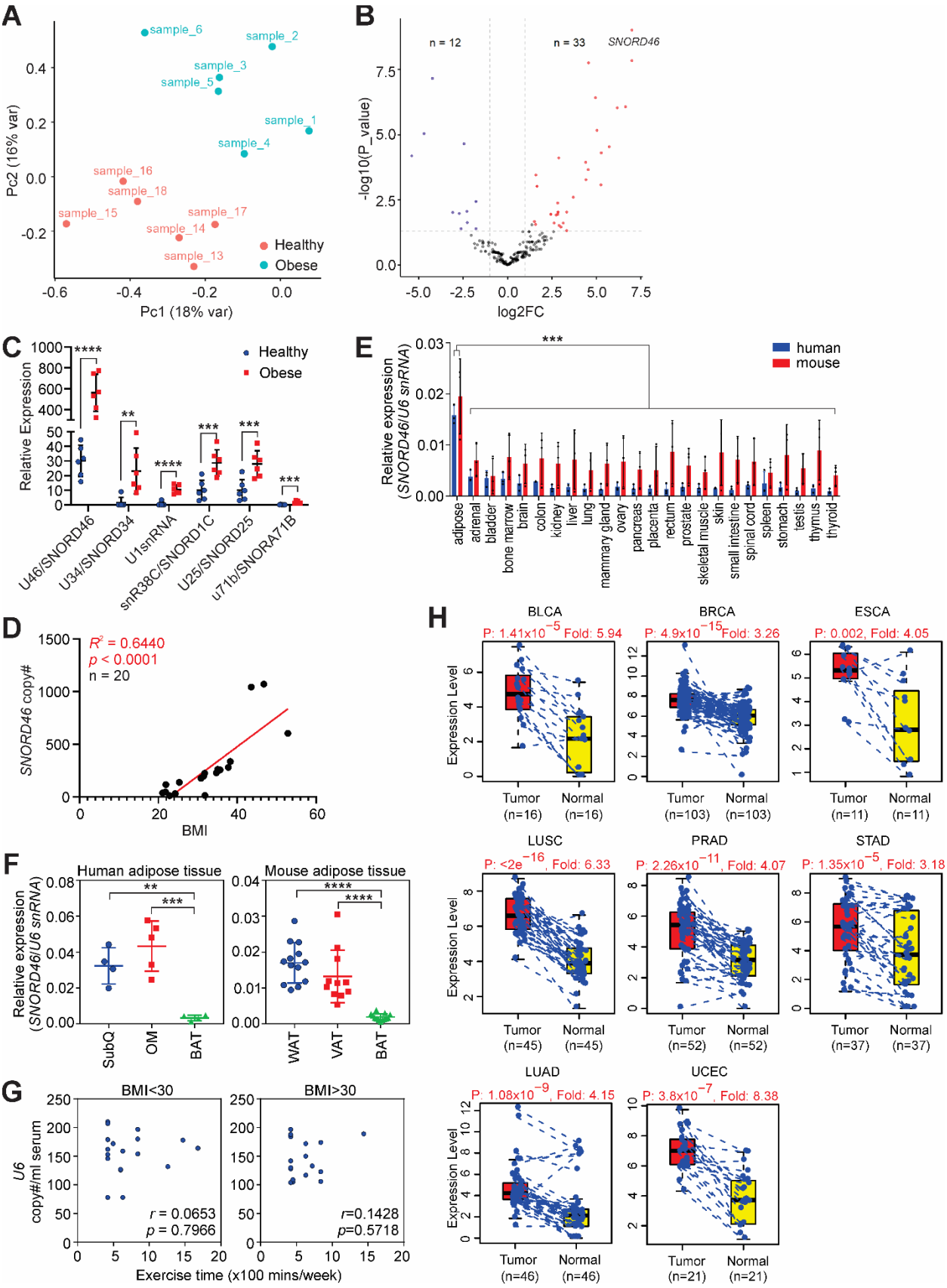
A
sample_6
sample_2
sample_3
sample_5
sample_1
sample_4
sample_16
sample_18
sample_15
sample_17
sample_14
sample_13
Healthy
Obese
Pc2 (16% var)
Pc1 (18% var)
B
n = 12
n = 33
SNORD46
-log10(P_value)
log2FC
C
Healthy
Obese
Relative Expression
U46/SNORD46
U34/SNORD34
U1snRNA
snR38C/SNORD1C
U25/SNORD25
u71b/SNORA71B
D
R² = 0.6440
p < 0.0001
n = 20
SNORD46 copy#
BMI
E
human
mouse
Relative expression
(SNORD46/U6 snRNA)
adipose
adrenal
bladder
bone marrow
brain
colon
kidney
liver
lung
mammary gland
ovary
pancreas
placenta
rectum
prostate
skeletal muscle
skin
small intestine
spinal cord
spleen
stomach
testis
thymus
thyroid
F
Human adipose tissue
Mouse adipose tissue
Relative expression
(SNORD46/U6 snRNA)
SubQ
OM
BAT
WAT
VAT
BAT
G
BMI<30
BMI>30
U6
copy#/ml serum
r = 0.0653
p = 0.7966
r=0.1428
p=0.5718
Exercise time (x100 mins/week)
H
BLCA
P: 1.41x10−5 Fold: 5.94
BRCA
P: 4.9x10−15 Fold: 3.26
ESCA
P: 0.002, Fold: 4.05
LUSC
P: <2e−16, Fold: 6.33
PRAD
P: 2.26x10−11, Fold: 4.07
STAD
P: 1.35x10−5, Fold: 3.18
LUAD
P: 1.08x10−9, Fold: 4.15
UCEC
P: 3.8x10−7, Fold: 8.38
Expression Level
Tumor
Normal
(n=16)
(n=103)
(n=11)
(n=45)
(n=52)
(n=37)
(n=46)
(n=21)

Figure S1. Characterization of *SNORD46* expression, related to Figure 1.
(**A**) PCA blot of the snoRNA arrays of healthy (n = 6) or obese donors (n = 6). (**B**) Volcano blot of the differential expressed genes in the snoRNA arrays of healthy (n = 6) or obese donors (n = 6). (**C**) The abundance of indicated snoRNAs analyzed by Human/Mouse snoRNA PCR Array in the serum of healthy and obese donors (error bars, SD, n = 6 per group, two-way ANOVA). (**D**) Pearson correlation between serum *SNORD46* copy number and the BMI of obese donors (n = 20, Fisher's exact test). (**E**) Relative expression of *SNORD46* in normal human or mouse tissues (error bars, SD, n = 3 independent experiments, one-way ANOVA). (**F**) Relative expression of *SNORD46* in human (left) or mouse (right) adipose tissues (error bars, SD, n = 4, 5, 4 human donors and n = 13, 11, 13 mouse tissues, one-way ANOVA). SubQ: subcutaneous; OM: omental; BAT: Brown adipose tissue; WAT: white adipose tissue; VAT: visceral adipose tissue. (**G**) Pearson correlation between serum *U6* copy number and exercise time of human donors with BMI<30 (left) or BMI>30 (right) (n = 18 per group, Fisher's exact test). (**H**) The expression level of *SNORD46* in paired tumor tissues and adjacent normal tissues of indicated tumor types. Box plots indicate median and interquartile range, paired Student's t-test.

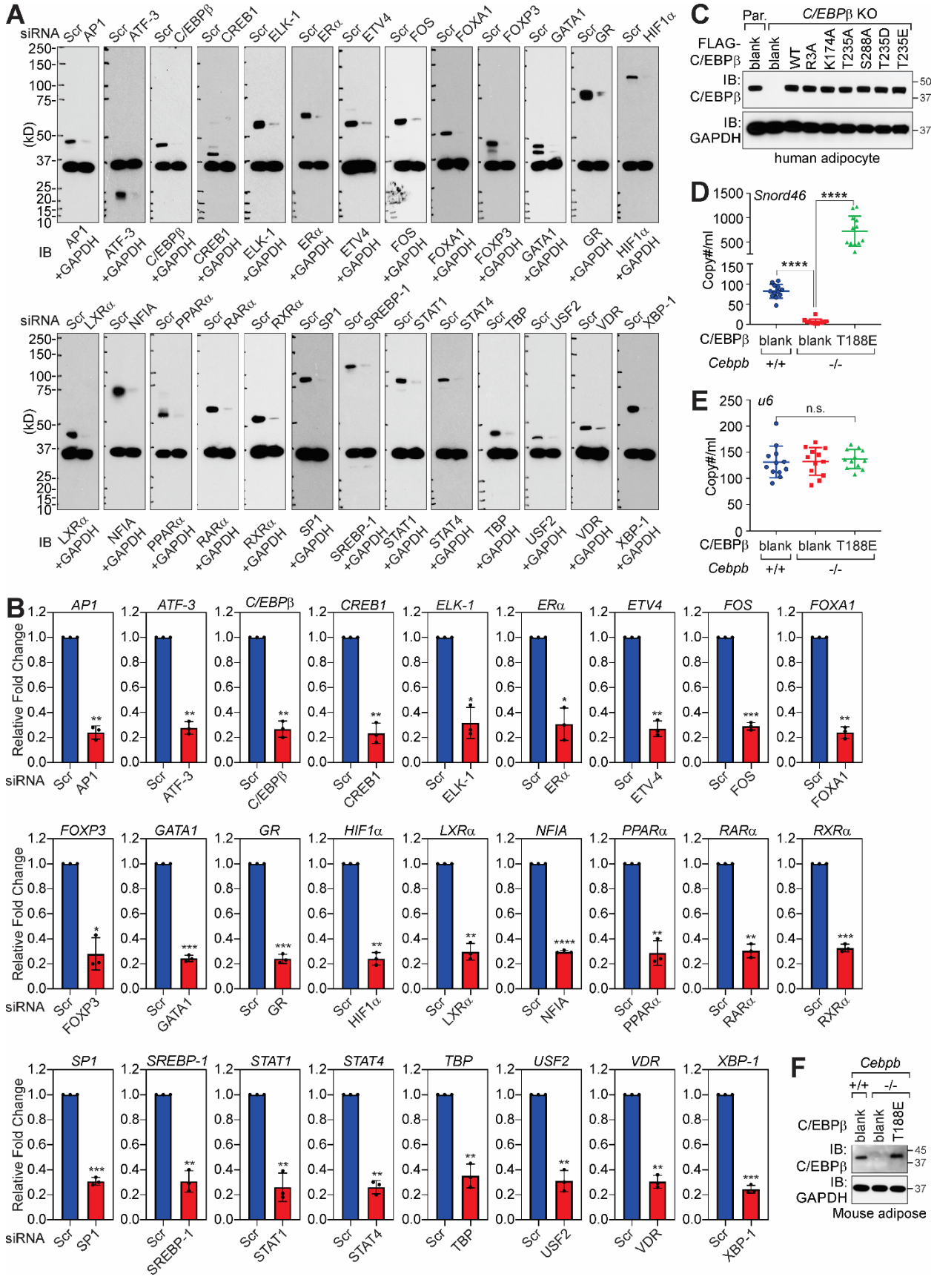
A
siRNA
IB
(kD)
B
Relative Fold Change
siRNA
C
Par.
C/EBPβ KO
FLAG-C/EBPβ
blank
WT
R3A
K174A
T235A
S288A
T235D
T235E
IB: C/EBPβ
IB: GAPDH
human adipocyte
D
Snord46
Copy#/ml
C/EBPβ
Cebpb
+/+
-/-
E
u6
n.s.
F
Mouse adipose

Figure S2. C/EBPβ regulates the expression of *SNORD46*, related to Figure 2.
(**A**) Immunoblotting (IB) analysis of transcription factor knock-down efficiency in human pre-adipocytes. Samples with knock-down of each transcription factor (labeled above) compared to scramble siRNA (Scr) controls. GAPDH was used as the loading control. (**B**) RT-qPCR analysis of transcription factor knock-down efficiency in human pre-adipocytes. *GAPDH* gene was used as an internal control. Data represent the means + S.D. of three independent experiments. *p < 0.05, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, compared with Scr siRNA. (**C**) IB demonstrating endogenous C/EBPβ knockout (KO) and FLAG-C/EBPβ protein expression in differentiated human adipocytes. (**D** and **E**) The copy number of *Snord46* (D) and *U6* (E) in supernatant of wild-type and *Cebpb* knockout mouse adipocytes expressing indicated exogenous C/EBPβ protein (error bars, SD, n = 12 mice per group, one-way ANOVA). (**F**) IB demonstrating exogenous C/EBPβ protein expression in adipocytes derived from wild-type and Cebpb knockout mice, expressing indicated exogenous C/EBPβ protein.

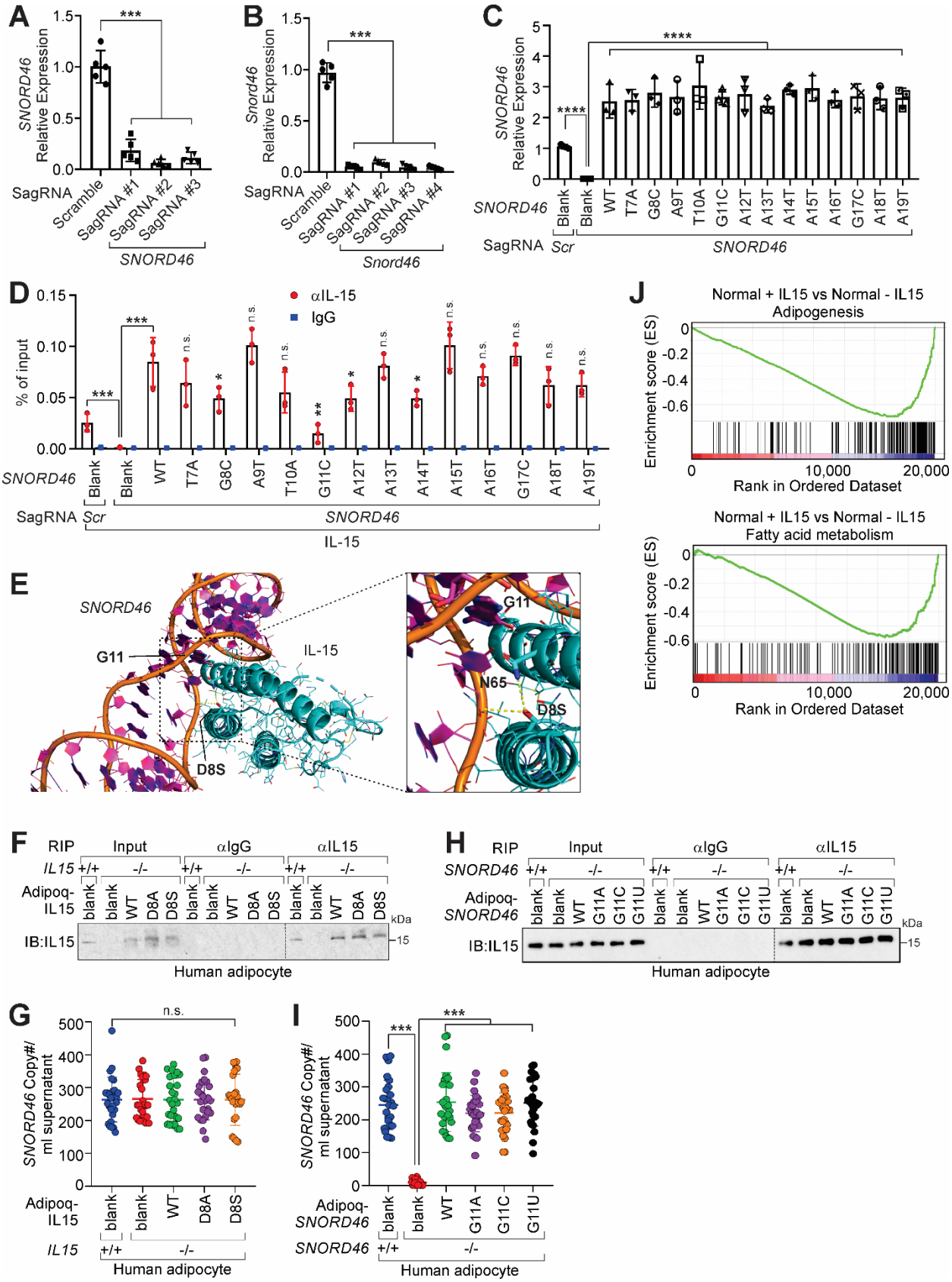

Figure S3. *SNORD46* is associates with IL15, related to Figure 3.

(**A** and **B**) Bar plots showing *SNORD46/Snord46* expression level in differentiated human (A) and mouse (B) adipocytes transduced with CRISPR–Cas9 constructs targeting control (scramble) or *SNORD46/Snord46* (error bars, SD, n = 5 independent experiments, one-way ANOVA). (**C**) Bar plots showing *SNORD46* knockout and exogenous *SNORD46* expression in differentiated human adipocytes (error bars, SD, n = 3 independent experiments, one-way ANOVA). (**D**) RIP-qPCR showing the *SNORD46*-IL15 interaction in *SNORD46*-deficient human differentiated adipocytes expressing wild-type or indicated *SNORD46* mutants (error bars, SD, n = 3 independent experiments, one-way ANOVA). (**E**) Computational modeling demonstrating D8S mutation of IL15 strengthen its interaction with *SNORD46*. Magenta cartoon: SNORD46; Cyan cartoon: IL15; Magenta stick: G11; Cyan stick: S8 and N65. (**F**) IB detection using indicated antibodies in *IL15*-proficient or deficient, differentiated human adipocytes expressing wild-type or indicated IL15 mutants. (**G**) The copy number of *SNORD46* in supernatant of *IL15*-proficient or deficient, differentiated human adipocytes expressing wild-type or indicated IL15 mutants (error bars, SD, n = 26 donors, one-way ANOVA). (**H**) IB detection using indicated antibodies in *SNORD46*-proficient or deficient, differentiated human adipocytes expressing wild-type or indicated *SNORD46* mutants. (**I**) The copy number of *SNORD46* in supernatant of *SNORD46*-proficient or deficient, differentiated human adipocytes expressing wild-type or indicated *SNORD46* mutants (error bars, SD, n = 26 donors, one-way ANOVA). (**J**) Gene set enrichment analysis (GSEA) of RNA-seq data performed on healthy human adipocytes stimulated with or without IL15. Top differentially-regulated pathways Adipogenesis (top) and Fatty acid metabolism (bottom) are shown.

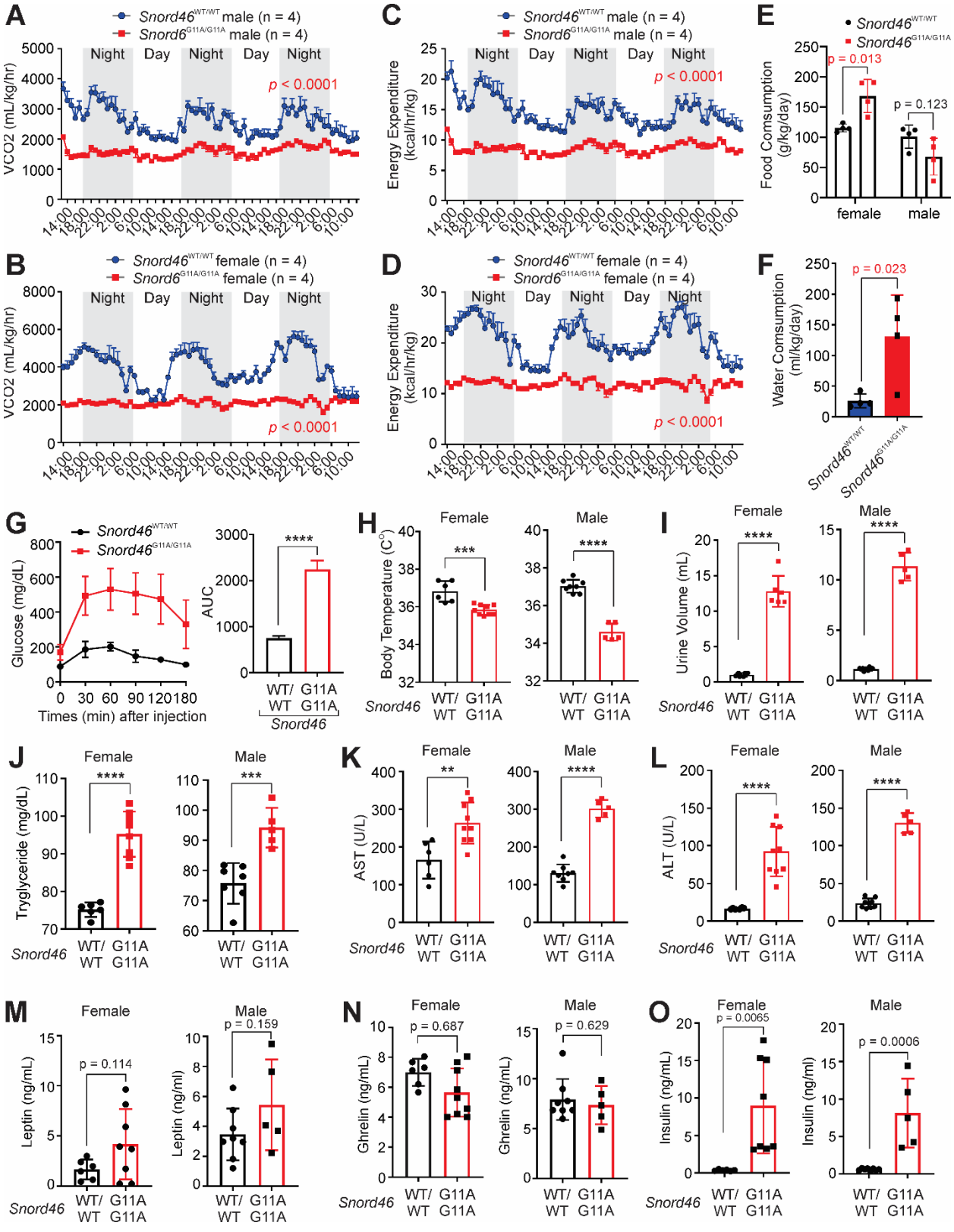

Figure S4. *SNORD46* G11A mice exhibit obesity. Related to Figure 4.

(**A** and **B**) Hourly average of VCO_2 of male (A) or female (B) *Snord46*$^{WT/WT}$ or *Snord46*$^{G11A/G11A}$ mice measured in the indirect calorimetry chambers over 72h (error bars, SD, n = 4 mice per groups, two-way ANOVA). (**C** and **D**) Hourly average of energy expenditure of male (C) or female (D) *Snord46*$^{WT/WT}$ or *Snord46*$^{G11A/G11A}$ mice measured in the indirect calorimetry chambers over 72 hours (error bars, SD, n = 4 mice per group, two-way ANOVA). (**E**) Food intake of female and male *Snord46*$^{WT/WT}$ or *Snord46*$^{G11A/G11A}$ mice (error bars, SD, n = 4 mice per group, two-way ANOVA). (**F**) Water consumption of male *Snord46*$^{WT/WT}$ or *Snord46*$^{G11A/G11A}$ mice (error bars, SD, n = 4 mice per group, Student's t-test). (**G**) Glucose tolerance tests (GTTs) (left) and the corresponding area under the curve (AUC) of female *Snord46*$^{WT/WT}$ (n = 6) or *Snord46*$^{G11A/G11A}$ (n = 8) mice at the indicated time points (error bars, SD, Student's t-test). (**H**) Body temperature of female *Snord46*$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 6 and 9) or male *Snord46*$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 8 and 5) mice. (**I**) Urine volume of female *Snord46*$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 6 and 6) or male *Snord46*$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 6 and 5) mice. (**J**) Serum concentrations of triglyceride in female *Snord46*$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 6 and 8) or male *Snord46*$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 7 and 5) mice. (**K**) Serum concentrations of AST in female *Snord46*$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 6 and 9) or male *Snord46*$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 8 and 5) mice. (**L**) Serum concentrations of ALT in female *Snord46*$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 6 and 9) or male *Snord46*$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 8 and 5) mice. (**M**) Serum concentrations of Leptin in female *Snord*46$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 6 and 8) or male *Snord*46$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 8 and 5) mice. (**N**) Serum concentrations of Ghrelin in female *Snord*46$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 6 and 8) or male *Snord*46$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 8 and 5) mice. (**O**) Serum concentrations of Insulin in female *Snord*46$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 6 and 8) or male *Snord*46$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ (n = 8 and 5) mice. Error bars: SD. Student's t-test (H-O) was used to test significance.

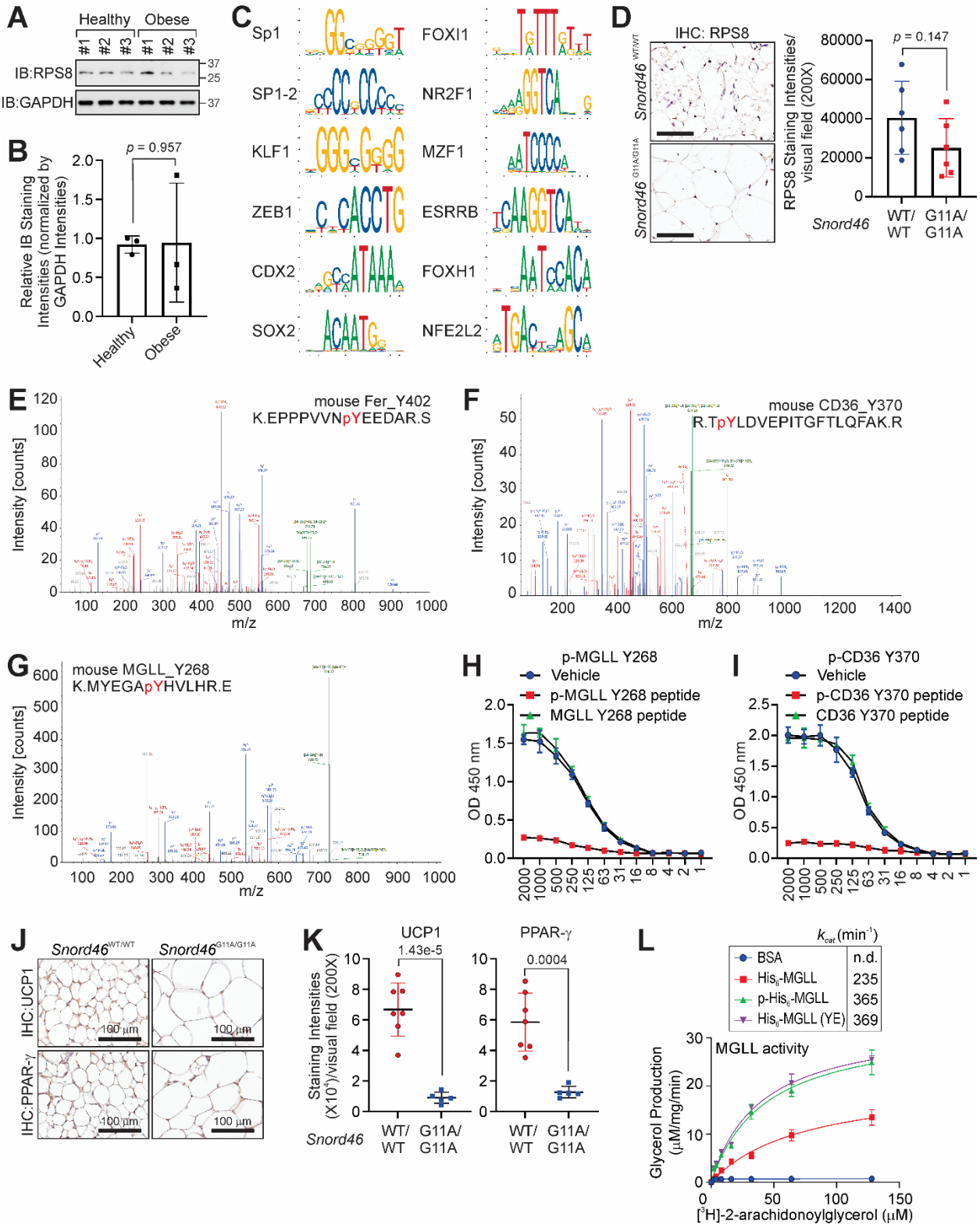

Figure S5. Characterization of CD36 and MGLL phosphorylation, related to Figure 5. (**A** and **B**) IB analysis (A) and densitometry-based quantification (B) of RPS8 in healthy and obese pro-adipocytes. Error bars (B), SD, n = 3 independent experiments, Student's t-test. (**C**) Prediction

of transcription factor (TF) binding motifs extracted by JASPAR. The consensus sequence of TF targeting RSP8 with the best-scoring are shown. (**D**) Representative IHC images (left panel) and staining intensities analysis (right panel) of RPS8 in WAT of *Snord46*$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ mice. Scale bars, 100 μm. Error bars, SD, n = 6, 6 animals, Student's t-test. (**E-G**) Annotated MS/MS spectrum assigned to the mouse FER peptide EPPPVVNpYEEDAR (E), CD36 peptide TpYLDVEPITGFTLQFAK (F), and MGLL peptide MYEGApYHVLHR (G) acquired from analysis of tryptic digest by high-sensitivity LC-MS/MS on an Orbitrap Elite high-resolution mass spectrometer. (**H** and **I**) Peptide blocking assay using anti-p-MGLL (Y268) (H) or p-CD36 (Y370) (I) at indicated concentration in the presence of indicated synthetic peptides. OD450 were measured using HRP conjugated mAb (error bars: SD, n = 3 independent replicates). (**J** and **K**) IHC staining (J) and staining intensities analysis (K) for UCP1 and PPARγ in adipose tissues of *Snord*46$^{WT/WT}$ and *Snord46*$^{G11A/G11A}$ mice (n = 7 and 5, error bars: SD, Student's t-test). Scale bars, 100 μm. (**L**) Measurement of glycerol production catalyzed by wild-type or MGLL mutant protein (error bars: SD, n = 3 independent experiments).

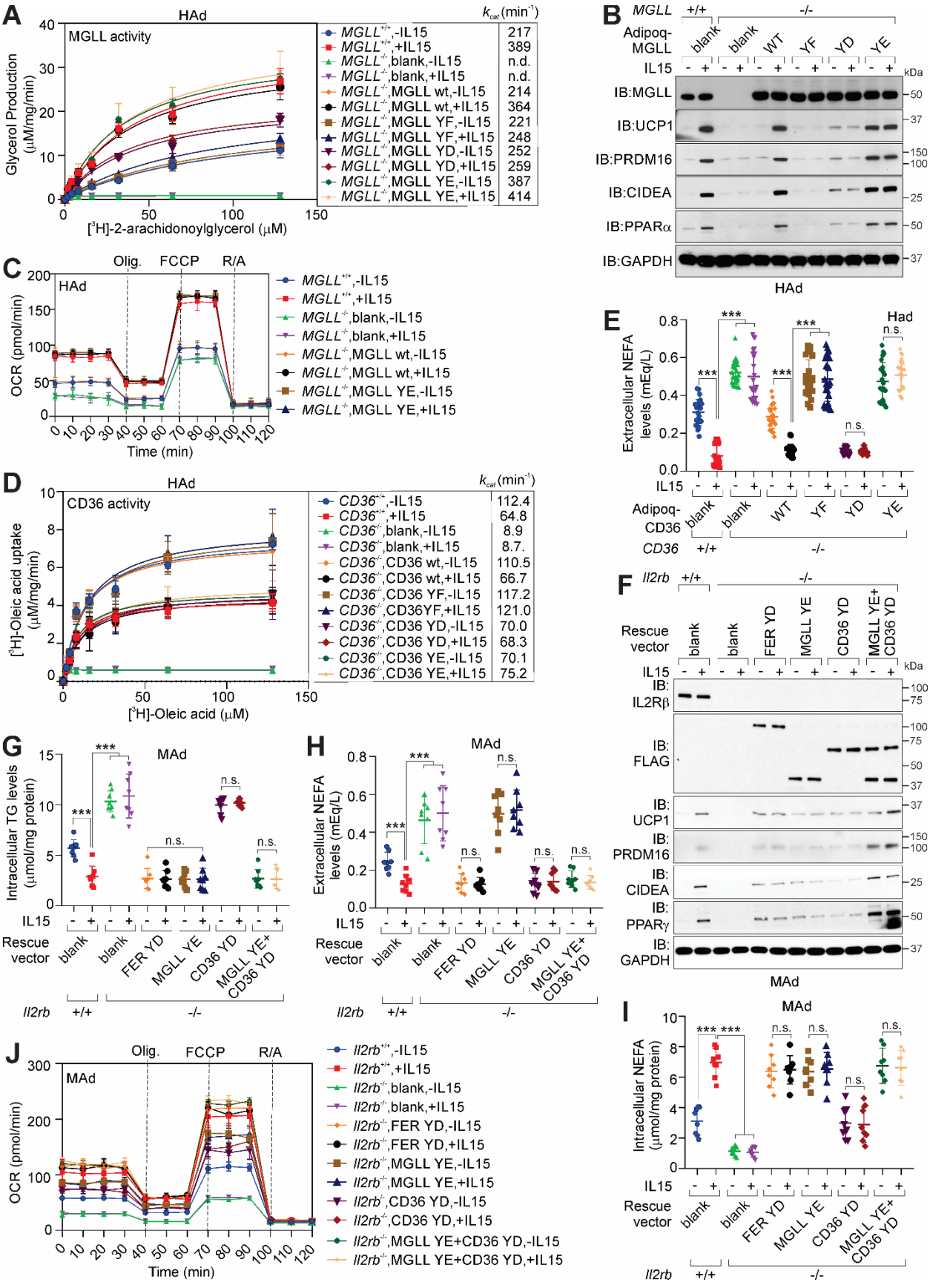
A
HAd
MGLL activity
Glycerol Production (μM/mg/min)
[3H]-2-arachidonoylglycerol (μM)
kcat (min-1)
MGL+/+,-IL15 217
MGL+/+,+IL15 389
MGL-/-,blank,-IL15 n.d.
MGL-/-,blank,+IL15 n.d.
MGL-/-,MGLL wt,-IL15 214
MGL-/-,MGLL wt,+IL15 364
MGL-/-,MGLL YF,-IL15 221
MGL-/-,MGLL YF,+IL15 248
MGL-/-,MGLL YD,-IL15 252
MGL-/-,MGLL YD,+IL15 259
MGL-/-,MGLL YE,-IL15 387
MGL-/-,MGLL YE,+IL15 414
B
MGLL +/+ -/-
Adipoq-MGLL
blank blank WT YF YD YE
IL15
kDa
IB:MGLL
IB:UCP1
IB:PRDM16
IB:CIDEA
IB:PPARα
IB:GAPDH
HAd
C
Olig. FCCP R/A
HAd
OCR (pmol/min)
Time (min)
MGL+/+,-IL15
MGL+/+,+IL15
MGL-/-,blank,-IL15
MGL-/-,blank,+IL15
MGL-/-,MGLL wt,-IL15
MGL-/-,MGLL wt,+IL15
MGL-/-,MGLL YE,-IL15
MGL-/-,MGLL YE,+IL15
D
HAd
CD36 activity
[3H]-Oleic acid uptake (μM/mg/min)
[3H]-Oleic acid (μM)
kcat (min-1)
CD36+/+,-IL15 112.4
CD36+/+,+IL15 64.8
CD36-/-,blank,-IL15 8.9
CD36-/-,blank,+IL15 8.7
CD36-/-,CD36 wt,-IL15 110.5
CD36-/-,CD36 wt,+IL15 66.7
CD36-/-,CD36 YF,-IL15 117.2
CD36-/-,CD36YF,+IL15 121.0
CD36-/-,CD36 YD,-IL15 70.0
CD36-/-,CD36 YD,+IL15 68.3
CD36-/-,CD36 YE,-IL15 70.1
CD36-/-,CD36 YE,+IL15 75.2
E
Had
Extracellular NEFA levels (mEq/L)
n.s.
IL15
Adipoq-CD36
blank blank WT YF YD YE
CD36 +/+ -/-
F
Il2rb +/+ -/-
Rescue vector
blank blank FER YD MGLL YE CD36 YD MGLL YE+ CD36 YD
IL15
kDa
IB: IL2Rβ
IB: FLAG
IB: UCP1
IB: PRDM16
IB: CIDEA
IB: PPARγ
IB: GAPDH
MAd
G
MAd
Intracellular TG levels (μmol/mg protein)
n.s.
IL15
Rescue vector
blank blank FER YD MGLL YE CD36 YD MGLL YE+ CD36 YD
Il2rb +/+ -/-
H
MAd
Extracellular NEFA levels (mEq/L)
n.s.
IL15
Rescue vector
blank blank FER YD MGLL YE CD36 YD MGLL YE+ CD36 YD
Il2rb +/+ -/-
I
MAd
Intracellular NEFA levels (μmol/mg protein)
n.s.
IL15
Rescue vector
blank blank FER YD MGLL YE CD36 YD MGLL YE+ CD36 YD
Il2rb +/+ -/-
J
Olig. FCCP R/A
MAd
OCR (pmol/min)
Time (min)
Il2rb+/+,-IL15
Il2rb+/+,+IL15
Il2rb-/-,blank,-IL15
Il2rb-/-,blank,+IL15
Il2rb-/-,FER YD,-IL15
Il2rb-/-,FER YD,+IL15
Il2rb-/-,MGLL YE,-IL15
Il2rb-/-,MGLL YE,+IL15
Il2rb-/-,CD36 YD,-IL15
Il2rb-/-,CD36 YD,+IL15
Il2rb-/-,MGLL YE+CD36 YD,-IL15
Il2rb-/-,MGLL YE+CD36 YD,+IL15

Figure S6. Molecular linkage of FER/MGLL/CD36 pathway in adipocytes, related to Figure 5.

(**A**) Glycerol production in MGLL-proficient or -deficient differentiated human adipocytes expressing the indicated MGLL protein, with or without IL15 stimulation. Error bars, SD, n = 3 independent experiments. (**B**) IB detection of indicated proteins in MGLL-proficient or -deficient differentiated human adipocytes expressing the indicated MGLL protein, with or without IL15 stimulation. (**C**) OCR across time for MGLL-proficient or -deficient differentiated human adipocytes expressing the indicated MGLL protein, with or without IL15 stimulation. Dot lines indicate the addition of mitochondrial inhibitors (oligomycin; FCCP; antimycin A/rotenone) (n = 8 wells of adipocytes, error bars: SD). (**D**) Measurement of [H^3]-Oleic acid uptake in CD36-proficient or -deficient differentiated human adipocytes expressing the indicated CD36 protein, with or without IL15 stimulation. Error bars, SD, n = 3 independent experiments. (**E**) Measurement of extracellular NEFA concentration in CD36-proficient or -deficient differentiated human adipocytes expressing the indicated CD36 protein, with or without IL15 stimulation. Error bars, SD, n = 24 donors, two-way ANOVA. (**F**) IB demonstrating the expression of indicated FLAG-tagged protein and brown adipocyte markers in mouse wild-type or *Il2rb* knockout adipocytes (MAd), expressing the indicated FER, MGLL or CD36 proteins, with or without IL15 stimulation. (**G-I**) Measurement of intracellular TG (G), extracellular NEFA (H), and intracellular NEFA (I) concentration in mouse wild-type or *Il2rb* knockout adipocytes expressing the indicated FER, MGLL or CD36 proteins, with or without IL15 stimulation (n = 8 mice per group, error bars: SD, one-way ANOVA). (**J**) Oxygen consumption rate (OCR) across time for mouse wild-type or *Il2rb* knockout adipocytes (MAd), expressing the indicated FER, MGLL or CD36 proteins, with or without IL15 stimulation. Arrows indicate the addition of mitochondrial inhibitors (oligomycin; FCCP; antimycin A/rotenone) (n = 8 wells of adipocytes, error bars: SD).

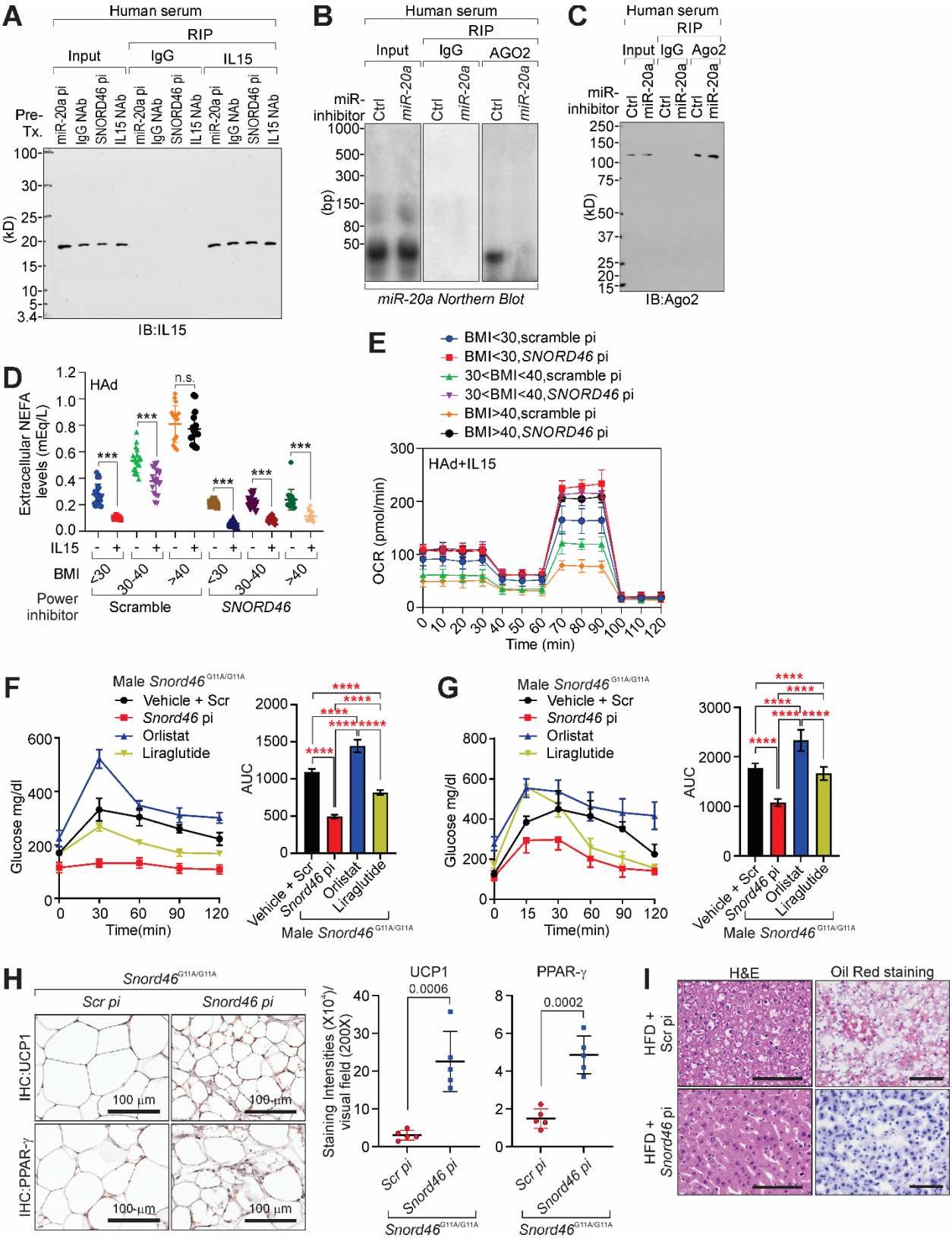

Figure S7. *SNORD46* power inhibitors antagonize obesity, related to Figure 6.

(**A**) RIP assay followed with IB detection using indicated antibodies of human serum pre-treated with miRNA/snoRNA power inhibitors or IL15 neutralizing antibodies as indicated. (**B**) Northern blot analysis of *miR-20a-5p* enriched by Ago2 RIP in human serum pre-treated with indicated miRNA power inhibitors. (**C**) RIP assay followed with IB detection using indicated antibodies in human serum pre-treated with miRNA power inhibitors. (**D**) Measurement of extracellular NEFA concentration of differentiated human adipocytes with indicated BMI, treated with indicated power inhibitors, in the presence or absence of IL15 (n = 24, 19, 17 donors per BMI group, error bars: SD, one-way ANOVA). (**E**) OCR across time for differentiated human adipocytes collected from donors with indicated BMI and treated with indicated power inhibitors, in the presence or absence of IL15 (n = 8 wells of adipocytes, error bars: SD). (**F**) Glucose tolerance tests (GTTs) (left) and the corresponding area under the curve (AUC) of male *Snord46*$^{G11A/G11A}$ mice with the indicated treatment (n = 5, 6, 5, 4 mice, error bars: SD, one-way ANOVA). (**G**) Insulin tolerance tests (ITTs) and the corresponding area under the curve (AUC) of male *Snord46*$^{G11A/G11A}$ mice with the indicated treatment (n = 5, 6, 5, 4 mice, error bars: SD, one-way ANOVA). (**H**) IHC staining (left) and staining intensities analysis (right) for the UCP1 and PPARγ in adipose tissues of *Snord46*$^{G11A/G11A}$ mice treated with scramble or *Snord46* power inhibitor (n = 5 mice per treatment group, error bars: SD, Student's t-test). Scale bars, 100 μm. (**I**) Representative H&E and Oil Red O staining of liver tissue from HFD-fed mice treated with scramble or *Snord46* power inhibitor. Scale bars, 100 μm.

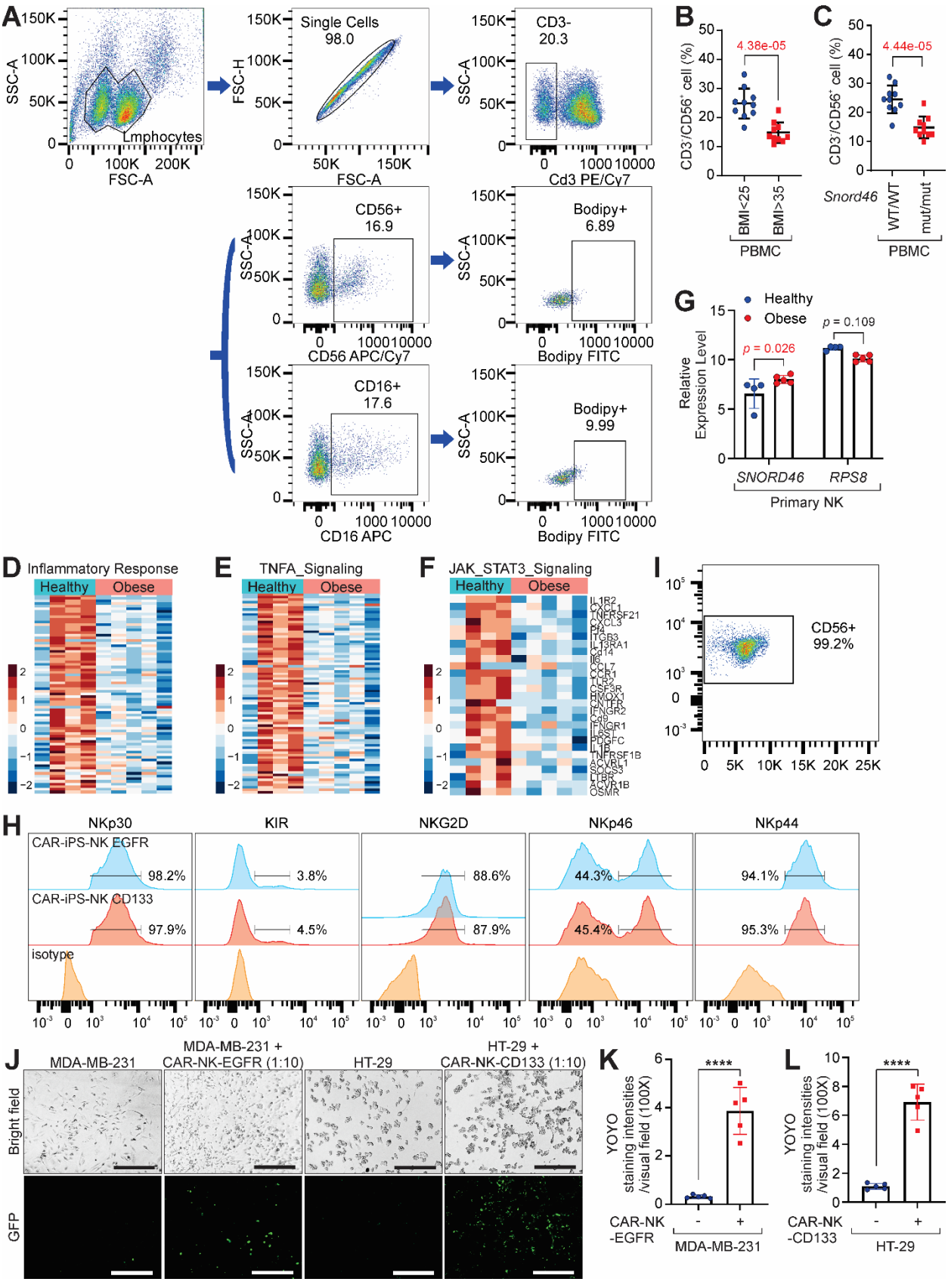
A
SSC-A
FSC-A
Lmphocytes
FSC-H
Single Cells
98.0
CD3-
20.3
Cd3 PE/Cy7
CD56+
16.9
CD56 APC/Cy7
Bodipy+
6.89
Bodipy FITC
CD16+
17.6
CD16 APC
Bodipy+
9.99
B
CD3+/CD56+ cell (%)
4.38e-05
BMI<25
BMI>35
PBMC
C
4.44e-05
Snord46
WT/WT
mut/mut
PBMC
G
Healthy
Obese
p = 0.026
p = 0.109
Relative Expression Level
SNORD46
RPS8
Primary NK
D
Inflammatory Response
E
TNFA_Signaling
F
JAK_STAT3_Signaling
I
CD56+
99.2%
H
NKp30
KIR
NKG2D
NKp46
NKp44
CAR-iPS-NK EGFR
98.2%
3.8%
88.6%
44.3%
94.1%
CAR-iPS-NK CD133
97.9%
4.5%
87.9%
45.4%
95.3%
isotype
J
MDA-MB-231
MDA-MB-231 + CAR-NK-EGFR (1:10)
HT-29
HT-29 + CAR-NK-CD133 (1:10)
Bright field
GFP
K
YOYO staining intensities /visual field (100X)
****
CAR-NK -EGFR
MDA-MB-231
L
YOYO staining intensities /visual field (100X)
****
CAR-NK -CD133
HT-29

Figure S8. *SNORD46* power inhibitor restores autophagy of obese NK cells, related to Figure 7.

(**A**) Flow cytometry gating method used in the analysis of $CD3^-/CD56^+$ NK cells in human PBMC. (**B**) Percentage of $CD3^-/CD56^+$ NK cells of healthy or obese PBMC (n = 10 per BMI group, error bars: SD, Student's t-test). (**C**) Percentage of $CD3^-/CD56^+$ NK cells in *Snord46*$^{WT/WT}$ or *Snord46*$^{G11A/G11A}$ mice (n = 10 mice per group, error bars: SD, Student's t-test). (**D-F**) Heatmap presentation of gene expression related to Inflammatory Response (D), TNFA Signaling (E), or JAK_STA3 signaling (F) in NK cells isolated from obese (n = 5) or healthy (n = 4) donors. (**G**) Relative expression of *SNORD46* or *RPS8* of the bulk RNA-seq using NK cells isolated from donors with normal body weight (healthy) or BMI> 35 (obese). Error bars, SD, n = 4, 5 donors respectively, two-way ANOVA. (**H**) Flowcytometry determination of indicated markers of CAR-iPSC-NK cells. (**I**) Flowcytometry determination of $CD56^+$ expression of CAR-iPSC-NK cells. (**J**) Bright field (top) and GFP immunofluorescence imaging (bottom) of MDA-MB-231 or HT-29 cells co-cultured with or without CAR-NK cells. Scale bars, 100 μm. (**K** and **L**) YOYO staining intensities of MDA-MB-231 (J) or HT-29 (K) co-cultured with or without indicated CAR-NK cells (n = 5 independent experiments, error bars: SD, Student's t-test).