Supplemental Information

Deadenylase-dependent mRNA decay of GDF15 and FGF21 orchestrates food intake and energy expenditure

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Supplemental Information includes:

Figures S1 to S7

Data S1

Figure S1.

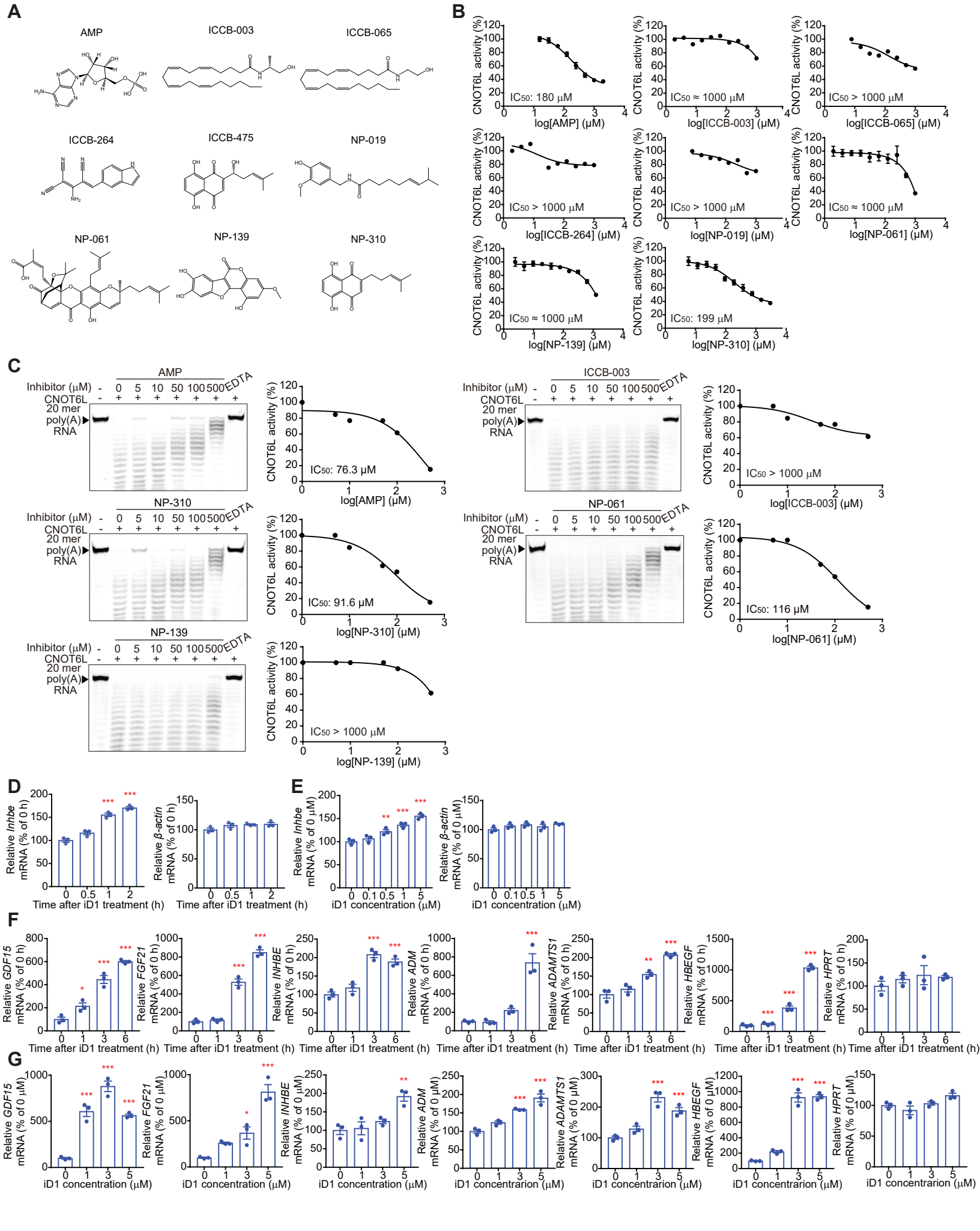
A
AMP
ICCB-003
ICCB-065
ICCB-264
ICCB-475
NP-019
NP-061
NP-139
NP-310
B
CNOT6L activity (%)
IC50: 180 μM
log[AMP] (μM)
IC50 ≈ 1000 μM
log[ICCB-003] (μM)
IC50 > 1000 μM
log[ICCB-065] (μM)
IC50 > 1000 μM
log[ICCB-264] (μM)
IC50 > 1000 μM
log[NP-019] (μM)
IC50 ≈ 1000 μM
log[NP-061] (μM)
IC50 ≈ 1000 μM
log[NP-139] (μM)
IC50: 199 μM
log[NP-310] (μM)
C
AMP
Inhibitor (μM) - 0 5 10 50 100 500 EDTA
CNOT6L - + + + + + + +
20 mer
poly(A)
RNA
IC50: 76.3 μM
log[AMP] (μM)
ICCB-003
IC50 > 1000 μM
log[ICCB-003] (μM)
NP-310
IC50: 91.6 μM
log[NP-310] (μM)
NP-061
IC50: 116 μM
log[NP-061] (μM)
NP-139
IC50 > 1000 μM
log[NP-139] (μM)
D
Relative Inhbe mRNA (% of 0 h)
Time after iD1 treatment (h)
Relative β-actin mRNA (% of 0 h)
E
Relative Inhbe mRNA (% of 0 μM)
iD1 concentration (μM)
Relative β-actin mRNA (% of 0 μM)
F
Relative GDF15 mRNA (% of 0 h)
Relative FGF21 mRNA (% of 0 h)
Relative INHBE mRNA (% of 0 h)
Relative ADM mRNA (% of 0 h)
Relative ADAMTS1 mRNA (% of 0 h)
Relative HBEGF mRNA (% of 0 h)
Relative HPRT mRNA (% of 0 h)
Time after iD1 treatment (h)
G
Relative GDF15 mRNA (% of 0 μM)
Relative FGF21 mRNA (% of 0 μM)
Relative INHBE mRNA (% of 0 μM)
Relative ADM mRNA (% of 0 μM)
Relative ADAMTS1 mRNA (% of 0 μM)
Relative HBEGF mRNA (% of 0 μM)
Relative HPRT mRNA (% of 0 μM)
iD1 concentration (μM)
iD1 concentrarion (μM)

Figure S1. Screening of CNOT6L inhibitors. Related to Figures 1 and 2.

(A) Structures of AMP and the compounds identified in FRET-based HTS.

(B) Dose-dependent inhibition curves of CNOT6L activity with the indicated compounds. FRET-based deadenylase assay of CNOT6L with AMP or the selected compound at the indicated concentration was performed. Fluorescence intensity was measured to calculate IC_{50} of the indicated compounds.

(C) Gel-based deadenylase assay of CNOT6L with AMP or the selected compounds. CNOT6L protein at 2.5 μM and a compound at the indicated concentration were incubated with 5'-FITC-poly$(A)_{20}$ RNA. Labeled RNAs were then visualized on a denaturing sequencing gel (left). Dose-dependent inhibition curves of CNOT6L activity with the indicated compounds were calculated from the gel images (right). EDTA was used as a positive control.

(D and E) Levels of the indicated mRNAs in primary hepatocytes treated with iD1 for the indicated time (**D**) or at the indicated concentration (**E**). mRNA levels were determined by qPCR and normalized to *Hprt* mRNA. $n = 3$ per group.

(F and G) Levels of the indicated mRNAs in Huh7 cells treated with iD1 for the indicated time (**F**) or at the indicated concentration (**G**). mRNAs levels were determined by qPCR and normalized to *GAPDH* mRNA. $n = 3$ per group.

Data represent mean ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; p values by one-way ANOVA with Dunnett's multiple comparison test.

Figure S2.

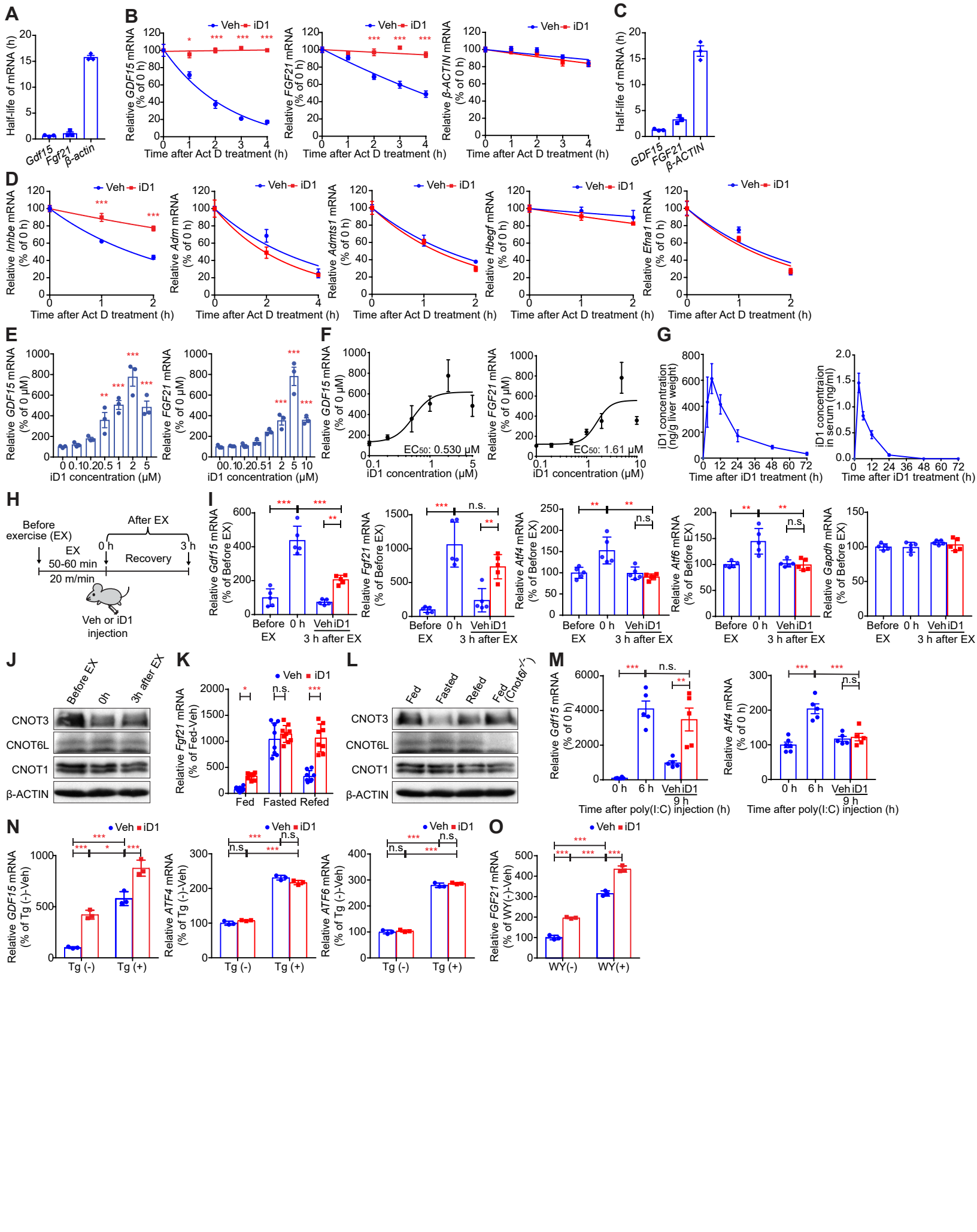
A
Half-life of mRNA (h)
Gdf15
Fgf21
β-actin
B
Veh iD1
Relative GDF15 mRNA (% of 0 h)
Relative FGF21 mRNA (% of 0 h)
Relative β-ACTIN mRNA (% of 0 h)
Time after Act D treatment (h)
C
Half-life of mRNA (h)
GDF15
FGF21
β-ACTIN
D
Relative Inhbe mRNA (% of 0 h)
Relative Adm mRNA (% of 0 h)
Relative Admts1 mRNA (% of 0 h)
Relative Hbegf mRNA (% of 0 h)
Relative Efna1 mRNA (% of 0 h)
Time after Act D treatment (h)
E
Relative GDF15 mRNA (% of 0 μM)
Relative FGF21 mRNA (% of 0 μM)
iD1 concentration (μM)
F
Relative GDF15 mRNA (% of 0 μM)
Relative FGF21 mRNA (% of 0 μM)
EC50: 0.530 μM
EC50: 1.61 μM
iD1 concentration (μM)
G
iD1 concentration (ng/g liver weight)
iD1 concentraion in serum (ng/ml)
Time after iD1 treatment (h)
H
Before exercise (EX)
EX
50-60 min
20 m/min
After EX
0 h
3 h
Recovery
Veh or iD1 injection
I
Relative Gdf15 mRNA (% of Before EX)
Relative Fgf21 mRNA (% of Before EX)
Relative Atf4 mRNA (% of Before EX)
Relative Atf6 mRNA (% of Before EX)
Relative Gapdh mRNA (% of Before EX)
Before EX
0 h
Veh
iD1
3 h after EX
n.s.
J
Before EX
0h
3h after EX
CNOT3
CNOT6L
CNOT1
β-ACTIN
K
Relative Fgf21 mRNA (% of Fed-Veh)
Fed
Fasted
Refed
L
Fed
Fasted
Refed
Fed (Chotoby-/-)
CNOT3
CNOT6L
CNOT1
β-ACTIN
M
Relative Gdf15 mRNA (% of 0 h)
Relative Atf4 mRNA (% of 0 h)
0 h
6 h
Veh
iD1
9 h
Time after poly(I:C) injection (h)
N
Relative GDF15 mRNA (% of Tg (-)-Veh)
Relative ATF4 mRNA (% of Tg (-)-Veh)
Relative ATF6 mRNA (% of Tg (-)-Veh)
Tg (-)
Tg (+)
O
Relative FGF21 mRNA (% of WY(-)-Veh)
WY(-)
WY(+)

Figure S2. CNOT6L inhibition increases levels of hepatokine mRNAs through their stabilization in cells and mice. Related to Figure 2.

(A) Half-life of the indicated mRNAs in primary hepatocytes calculated from (**Figure 2C**). *n* = 3 per group.

(B and C) Stability of the indicated mRNAs in Huh7 cells incubated with actinomycin D (Act D) and iD1 for the indicated time. mRNA levels were determined by qPCR and normalized to *GAPDH* mRNA (**B**). Half-life of the indicated mRNAs in Huh7 cells (**C**). *n* = 3 per group.

(D) Stability of the indicated mRNAs in primary hepatocytes incubated with Act D and iD1 for the indicated time. mRNA levels were determined by qPCR and normalized to *Hprt*. *n* = 3 per group.

(E and F) EC_{50} of iD1 for *GDF15* and *FGF21* mRNA levels in Huh7 cells. Levels of the indicated mRNAs in Huh7 cells treated with iD1 at the indicated concentration were determined by qPCR and normalized to *GAPDH* mRNA (**E**). EC_{50} for the indicated mRNAs were calculated (**F**). *n* = 3 per group.

(G) Concentrations of iD1 in livers (left) and serum (right) of mice treated with iD1 for the indicated time. iD1 concentrations were determined by LC-MS/MS. *n* = 5 per group.

(H-J) A treadmill exercise and recovery protocol (**H**) and levels of the indicated mRNAs (**I**) and the indicated proteins (**J**) in livers of WT mice before, immediately after (0 h), or 3 h after the high-intensity exercise (EX). Veh or iD1 was immediately injected after the exercise. mRNA levels were quantified by qPCR and normalized to *Hprt* mRNA. *n* = 5 per group. β-ACTIN was used as a loading control.

(K and L) Levels of *Fgf21* mRNA (**K**) and the indicated proteins (**L**) in livers of WT mice fed, fasted for 24 h, or refed for 2 h after 24 h-fasting. mRNA levels were quantified by qPCR and normalized to *Hprt* mRNA. *n* = 5 per group. β-ACTIN was used as a loading control.

(M) Levels of *Gdf15* and *Atf4* mRNAs in livers of WT mice before (0 h), 6 h after, or 9 h after injection of poly(I:C) (10 mg/kg of body weight). Veh or iD1 was injected 6 h after the poly(I:C) injection. mRNA levels were quantified by qPCR and normalized to *Hprt* mRNA. *n* = 5 per group.

(N) Levels of the indicated mRNAs in Huh7 cells treated with an ER stress inducer, thapsigargin (Tg), and/or iD1. mRNA levels were determined by qPCR and normalized to *GAPDH* mRNA. *n* = 3 per group.

(O) Levels of *FGF21* mRNA in Huh7 cells treated with a PPARα agonist, WY-14643 (WY), and/or iD1. mRNA levels were determined by qPCR and normalized to *GAPDH* mRNA. *n* = 3 per group. Data represent mean ± SEM. **p* < 0.05, ***p* < 0.01, ****p* < 0.001; *p* values by two-way ANOVA with Bonferroni's multiple comparison test for (**B, D, I, K, and M-O**) or one-way ANOVA with Dunnett's multiple comparison test for (**E**).

Figure S3.

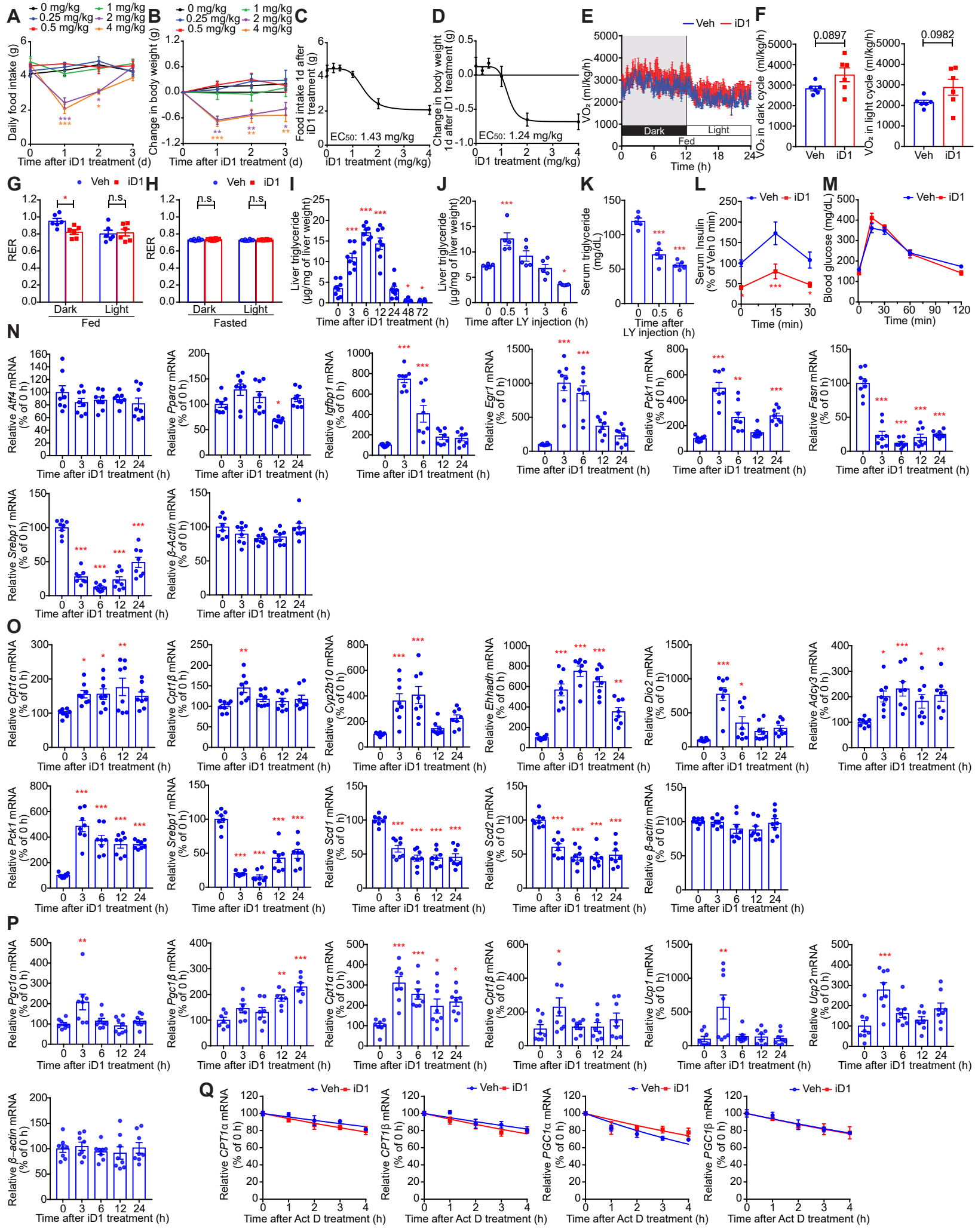
A
0 mg/kg
0.25 mg/kg
0.5 mg/kg
1 mg/kg
2 mg/kg
4 mg/kg
Daily food intake (g)
Time after iD1 treatment (d)
B
Change in body weight (g)
Time after iD1 treatment (d)
C
Food intake 1d after iD1 treatment (g)
EC50: 1.43 mg/kg
iD1 treatment (mg/kg)
D
Change in body weight 1d after iD1 treatment (g)
EC50: 1.24 mg/kg
iD1 treatment (mg/kg)
E
Veh
iD1
VO2 (ml/kg/h)
Dark
Light
Fed
Time (h)
F
0.0897
0.0982
VO2 in dark cycle (ml/kg/h)
VO2 in light cycle (ml/kg/h)
Veh
iD1
G
Veh
iD1
RER
n.s
Dark
Light
Fed
H
Veh
iD1
RER
n.s
n.s
Dark
Light
Fasted
I
Liver triglyceride (μg/mg of liver weight)
Time after iD1 treatment (h)
J
Liver triglyceride (μg/mg of liver weight)
Time after LY injection (h)
K
Serum triglyceride (mg/dL)
Time after LY injection (h)
L
Veh
iD1
Serum Insulin (% of Veh 0 min)
Time (min)
M
Veh
iD1
Blood glucose (mg/dL)
Time (min)
N
Relative Atf4 mRNA (% of 0 h)
Relative Ppara mRNA (% of 0 h)
Relative Igfbp1 mRNA (% of 0 h)
Relative Egr1 mRNA (% of 0 h)
Relative Pck1 mRNA (% of 0 h)
Relative Fasn mRNA (% of 0 h)
Relative Srebp1 mRNA (% of 0 h)
Relative β-Actin mRNA (% of 0 h)
Time after iD1 treatment (h)
O
Relative Cpt1α mRNA (% of 0 h)
Relative Cpt1β mRNA (% of 0 h)
Relative Cyp2b10 mRNA (% of 0 h)
Relative Ehhadh mRNA (% of 0 h)
Relative Dio2 mRNA (% of 0 h)
Relative Adcy3 mRNA (% of 0 h)
Relative Pck1 mRNA (% of 0 h)
Relative Srebp1 mRNA (% of 0 h)
Relative Scd1 mRNA (% of 0 h)
Relative Scd2 mRNA (% of 0 h)
Relative β-actin mRNA (% of 0 h)
Time after iD1 treatment (h)
P
Relative Pgc1α mRNA (% of 0 h)
Relative Pgc1β mRNA (% of 0 h)
Relative Cpt1α mRNA (% of 0 h)
Relative Cpt1β mRNA (% of 0 h)
Relative Ucp1 mRNA (% of 0 h)
Relative Ucp2 mRNA (% of 0 h)
Relative β-actin mRNA (% of 0 h)
Time after iD1 treatment (h)
Q
Veh
iD1
Relative CPT1α mRNA (% of 0 h)
Relative CPT1β mRNA (% of 0 h)
Relative PGC1α mRNA (% of 0 h)
Relative PGC1β mRNA (% of 0 h)
Time after Act D treatment (h)

Figure S3. CNOT6L inhibition decreases food intake and body weight and increases energy expenditure and fat utilization through up-regulation of hepatokine mRNAs. Related to Figure 3.

(A-D) Daily food intake (**A**) and change in body weight (**B**) after a single administration of iD1 at the indicated concentration. EC_{50} for daily food intake (**C**) and change in body weight (**D**) was calculated from (**A** and **B**). *n* = 5 per group.

(E-G) Oxygen consumption (VO_2) for 24 h (**E**), average VO_2 of dark- or light-cycle (**F**), and average RER of dark- or light-cycle (**G**) after a single administration of Veh or iD1 into WT mice under a fed condition. VO_2 was normalized to body weight. *n* = 6 per group.

(H) Average RER of dark- or light-cycle after a single administration of Veh or iD1 into WT mice under a fasted condition. *n* = 7 per group.

(I) Levels of liver triglycerides in WT mice treated with iD1 for the indicated time. Liver triglyceride levels were normalized to liver weight. *n* = 8 per group.

(J and K) Levels of liver triglycerides (**J**) and serum triglycerides (**K**) in WT mice treated with an FGF21 analog, LY2405319 (LY), for the indicated time. Liver triglyceride levels were normalized to liver weight. *n* = 5 per group.

(L and M) Glucose tolerance tests (GTTs) in WT mice treated with Veh or iD1 for 3 h. Levels of serum insulin (**L**) and blood glucose (**M**) in glucose-injected WT mice for the indicated time under a fed condition. *n* = 7-8 per group.

(N-P) Levels of the indicated mRNAs in livers (**N**), BAT (**O**), and ingWAT (**P**) of WT mice administrated with iD1 for the indicated time. mRNA levels were quantified by qPCR and normalized to *Hprt* mRNA. *n* = 8 per group.

(Q) Stability of the indicated mRNAs in Huh7 cells incubated with Act D and iD1 for the indicated time. mRNA levels were determined by qPCR and normalized to *GAPDH* mRNA. $n = 3$ per group. Data represent mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; p values by two-way ANOVA with Bonferroni's multiple comparison test for (**A, B, G, H, L, M, and Q**), one-way ANOVA with Dunnett's multiple comparison test for (**I-K and N-P**), or student's t-test for (**F**).

Figure S4.

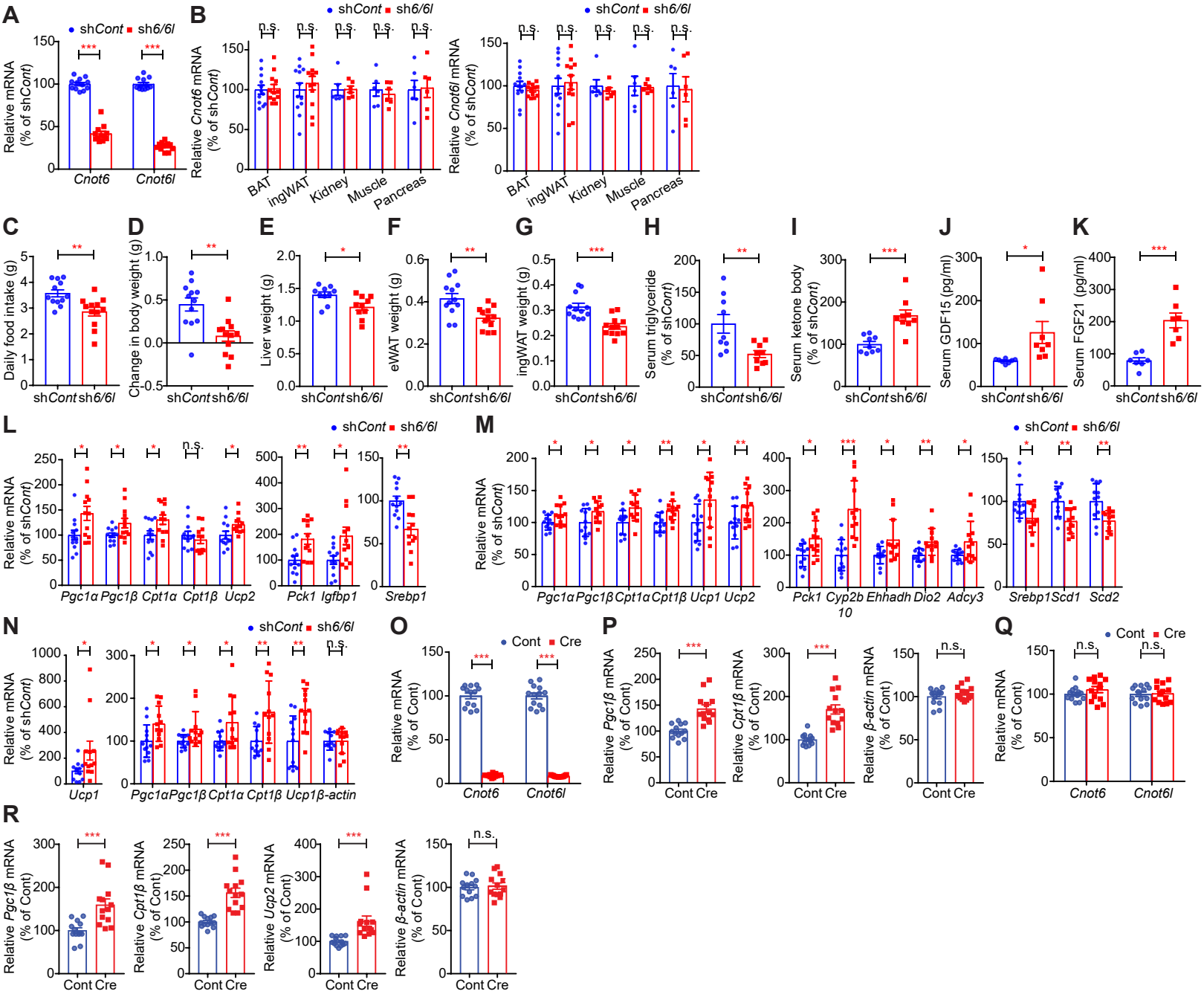
A
shCont sh6/6l
Relative mRNA (% of shCont)
Cnot6
Cnot6l
B
Relative Cnot6 mRNA (% of shCont)
Relative Cnot6l mRNA (% of shCont)
n.s.
BAT
ingWAT
Kidney
Muscle
Pancreas
C
Daily food intake (g)
D
Change in body weight (g)
E
Liver weight (g)
F
eWAT weight (g)
G
ingWAT weight (g)
H
Serum triglyceride (% of shCont)
I
Serum ketone body (% of shCont)
J
Serum GDF15 (pg/ml)
K
Serum FGF21 (pg/ml)
L
Pgc1α Pgc1β Cpt1α Cpt1β Ucp2
Pck1 Igfbp1
Srebp1
M
Pgc1α Pgc1β Cpt1α Cpt1β Ucp1 Ucp2
Pck1 Cyp2b 10 Ehhadh Dio2 Adcy3
Srebp1 Scd1 Scd2
N
Ucp1
Pgc1α Pgc1β Cpt1α Cpt1β Ucp1 β-actin
O
Cont Cre
Cnot6
Cnot6l
P
Relative Pgc1β mRNA (% of Cont)
Relative Cpt1β mRNA (% of Cont)
Relative β-actin mRNA (% of Cont)
Q
R
Relative Pgc1β mRNA (% of Cont)
Relative Cpt1β mRNA (% of Cont)
Relative Ucp2 mRNA (% of Cont)
Relative β-actin mRNA (% of Cont)

Figure S4. Hepatic *Cnot6* and *Cnot6l* double knockdown/knockout induces loss of food intake and body weight and stimulates fat utilization through an increase in hepatokines. Related to Figure 4.

(A and B) Levels of *Cnot6* (left) and *Cnot6l* (right) mRNAs in livers (**A**) and the indicated tissues (**B**) of WT mice maintained with a standard diet (SD) and injected with adenovirus expressing shRNA against *Gfp* (sh*Cont*) or *Cnot6* and *Cnot6l* (sh*6/6l*). mRNA levels were normalized to *Hprt* mRNA. *n* = 12 per group

(C-G) Daily food intake (**C**), change in body weight (**D**), and tissue weights of livers (**E**), eWAT (**F**), and ingWAT (**G**) of sh*Cont* and sh*6/6l* mice fed with SD. *n* = 10-12 per group.

(H-K) Levels of serum triglycerides (**H**), serum ketone bodies (**I**), serum GDF15 (**J**), and serum FGF21 (**K**) of sh*Cont* and sh*6/6l* mice fed with SD. *n* = 7-9 per group.

(L-N) Levels of the indicated mRNAs in livers (**L**), BAT (**M**), and ingWAT (**N**) of sh*Cont* and sh*6/6l* mice. mRNA levels were normalized to *Hprt* mRNA. *n* = 12 per group.

(O-R) Levels of the indicated mRNAs in livers (**O** and **P**) and BAT (**Q** and **R**) of *Cnot6*/*Cnot6l* conditional KO mice injected with AAV8 harboring mock (Cont) or Cre recombinase (Cre) under the liver-specific TBG promoter. *n* = 12 per group.

Data represent mean ± SEM. **p* < 0.05, ***p* < 0.01, ****p* < 0.001; *p* value by Student's *t*-tests.

Figure S5.

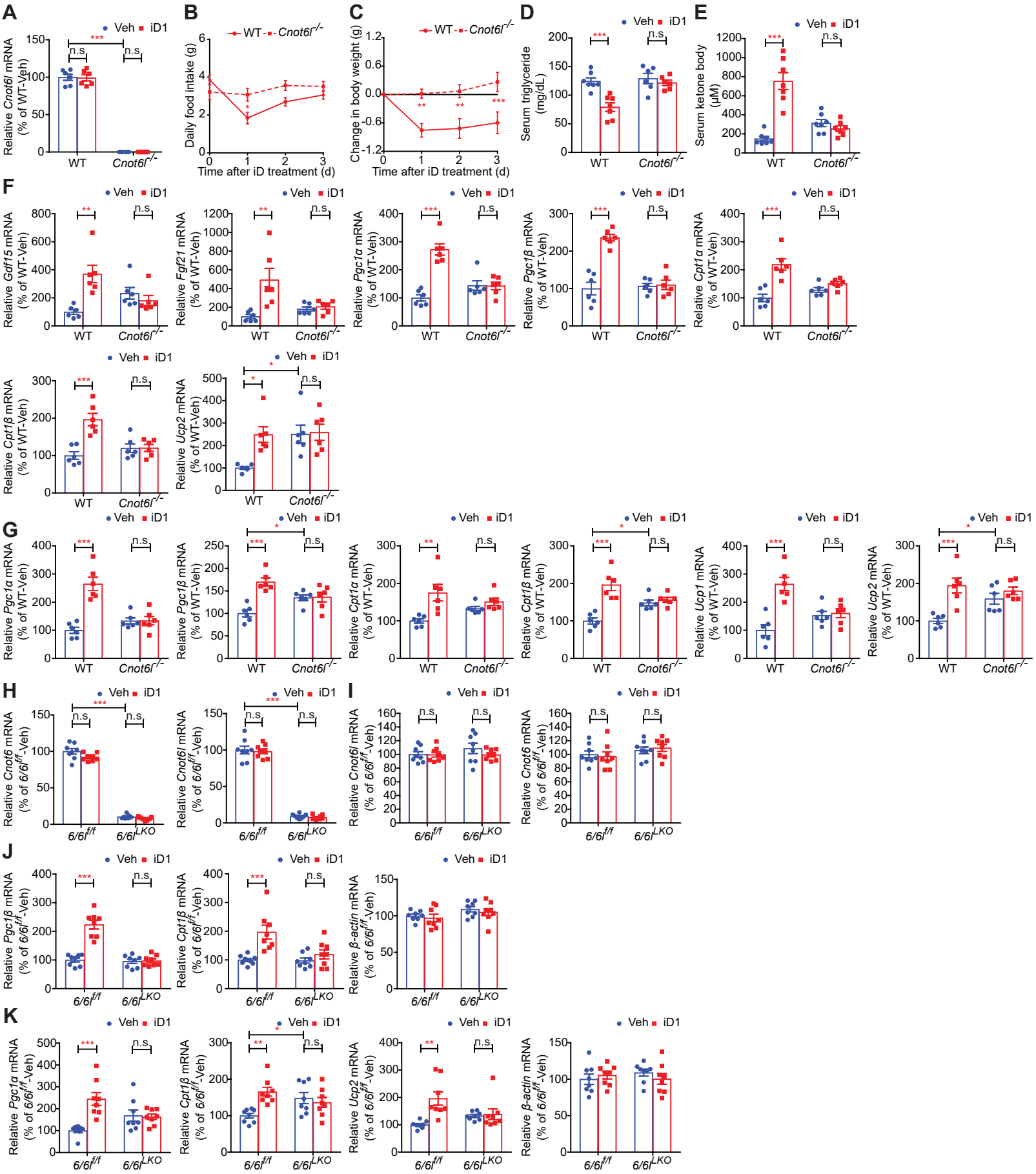
A
B
C
D
E
F
G
H
I
J
K
Veh
iD1
WT
Cnot6l-/-
n.s
Relative Cnot6l mRNA (% of WT-Veh)
Daily food intake (g)
Time after iD treatment (d)
Change in body weight (g)
Serum triglyceride (mg/dL)
Serum ketone body (μM)
Relative Gdf15 mRNA (% of WT-Veh)
Relative Fgf21 mRNA (% of WT-Veh)
Relative Pgc1α mRNA (% of WT-Veh)
Relative Pgc1β mRNA (% of WT-Veh)
Relative Cpt1α mRNA (% of WT-Veh)
Relative Cpt1β mRNA (% of WT-Veh)
Relative Ucp2 mRNA (% of WT-Veh)
Relative Ucp1 mRNA (% of WT-Veh)
Relative Cnot6 mRNA (% of 6/6l f/f-Veh)
Relative β-actin mRNA (% of 6/6l f/f-Veh)
6/6l f/f
6/6l LKO

Figure S5. Hepatic CNOT6 and CNOT6L are required for the iD1 actions on food intake, body weight, fat utilization, and hepatokine mRNAs. Related to Figure 4.

(A-G) Levels of *Cnot6l* mRNA (**A**), daily food intake (**B**), change in body weight (**C**), and levels of serum triglycerides (**D**), serum ketone bodies (**E**), and the indicated mRNAs in livers (**F**) and BAT (**G**) of WT and *Cnot6l* knockout (*Cnot6l*$^{-/-}$) mice after a single administration of Veh or iD1. mRNA levels were normalized to *Hprt* mRNA. *n* = 6-9 per group.

(H-K) Levels of *Cnot6* (left) and *Cnot6l* (right) mRNAs in livers (**H**) and BAT (**I**) and the indicated mRNAs in livers (**J**) and BAT (**K**) of control (*6/6l*$^{f/f}$) and *Cnot6*/*Cnot6l* liver-specific double KO (*6/6l*^{LKO}) mice after a single administration of Veh or iD1. mRNA levels were normalized to *Hprt* mRNA. *n* = 8 per group.

Data represent mean ± SEM. **p* < 0.05, ***p* < 0.01, ****p* < 0.001; *p* value by two-way ANOVA with Bonferroni's multiple comparison test.

Figure S6.


A
B
C
D
E
F
G
H
I
J
K
L
M
Veh
iD1
shCont
shGdf15
shFgf21
Relative Gdf15 mRNA (% of shCont-Veh)
Relative Gdf15 mRNA in kidney (% of shCont)
Serum GDF15 (pg/ml)
Relative Fgf21 mRNA (% of shCont-Veh)
Relative β-actin mRNA (% of shCont-Veh)
Relative Pgc1α mRNA (% of shCont-Veh)
Relative Pgc1β mRNA (% of shCont-Veh)
Relative Cpt1α mRNA (% of shCont-Veh)
Relative Cpt1β mRNA (% of shCont-Veh)
Relative Ucp1 mRN (% of shCont-Veh)
Relative Ucp2 mRNA (% of shCont-Veh)
Relative Fgf21 mRNA (% of shCont)
BAT
ingWAT
Kidney
Muscle
Pancreas
Serum FGF21 (pg/ml)
Relative Gdf15 mRNA (% of shCont-Veh)
Relative Pgc1β mRNA (% of shCont-Veh)
Relative Cpt1β mRNA (% of shCont-Veh)
Relative Ucp1 mRNA (% of shCont-Veh)
Relative Ucp2 mRNA (% of shCont-Veh)
Relative β-actin mRNA (% of shCont-Veh)
Relative Gdf15 mRNA (% of Fgf21f/f-Veh)
Fgf21f/f
Fgf21LKO
n.s.

Figure S6. GDF15 and FGF21 mainly mediate the effects of CNOT6L inhibition on food intake and energy and lipid consumption, respectively. Related to Figures 5 and 6.

(A-C) Levels of *Gdf15* mRNA in livers (**A**) and BAT (**B**) and serum GDF15 (**C**) of WT mice injected with adenovirus expressing shRNA against *Gfp* (sh*Cont*) or *Gdf15* mRNA (sh*Gdf15*) after a single administration of Veh or iD1. mRNA levels were normalized to *Hprt* mRNA. *n* = 6-7 per group.

(D-F) Levels of the indicated mRNAs in livers (**D**), BAT (**E**), and ingWAT (**F**) of sh*Cont* and sh*Gdf15* mice after a single administration of Veh or iD1. mRNA levels were normalized to *Hprt* mRNA. *n* = 7 per group.

(G-I) Levels of *Fgf21* mRNA in livers (**G**) and the indicated tissues (**H**) and serum FGF21 (**I**) of WT mice injected with adenovirus expressing shRNA against *Gfp* (sh*Cont*) or *Fgf21* mRNA (sh*Fgf21*) mice after a single administration Veh or iD1. *n* = 6-7 per group.

(J-L) Levels of the indicated mRNAs in livers (**J**), BAT (**K**), and ingWAT (**L**) of sh*Cont* and sh*Fgf21* mice after a single administration of Veh or iD1. mRNA levels were normalized to *Hprt* mRNA. *n* = 7-8 per group.

(M) Levels of *Gdf15* mRNA in livers of control (*Fgf21l*$^{f/f}$) and *Fgf21* liver-specific KO (*Fgf21l*^{LKO}) mice after a single administration of Veh or iD1. mRNA levels were normalized to *Hprt* mRNA. *n* = 7 per group

Data represent mean ± SEM. **p* < 0.05, ***p* < 0.01, ****p* < 0.001; *p* value by two-way ANOVA with Bonferroni's multiple comparison test for (**A, C-G, and I-M**) or Student's *t*-tests for (**B and H**).

Figure S7.

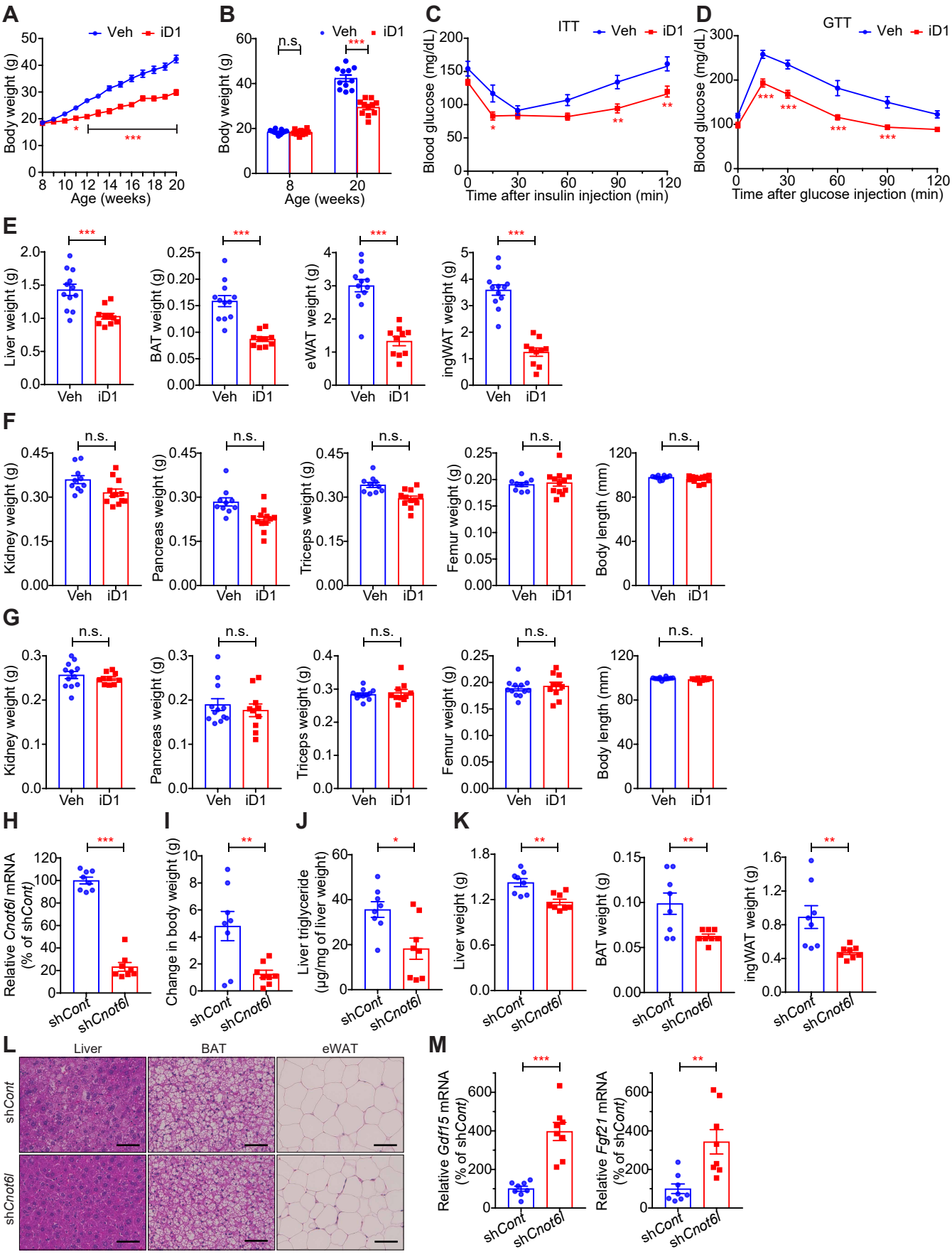
A
Veh
iD1
Body weight (g)
Age (weeks)
B
n.s.
C
ITT
Blood glucose (mg/dL)
Time after insulin injection (min)
D
GTT
Time after glucose injection (min)
E
Liver weight (g)
BAT weight (g)
eWAT weight (g)
ingWAT weight (g)
F
Kidney weight (g)
Pancreas weight (g)
Triceps weight (g)
Femur weight (g)
Body length (mm)
G
H
Relative Cnot6l mRNA (% of shCont)
shCont
shCnot6l
I
Change in body weight (g)
J
Liver triglyceride (μg/mg of liver weight)
K
L
Liver
BAT
eWAT
M
Relative Gdf15 mRNA (% of shCont)
Relative Fgf21 mRNA (% of shCont)

Figure S7. CNOT6L inhibition ameliorates diet-induced metabolic disorders in male and female mice. Related to Figure 7.

(A and B) Body weight curves for 12 weeks (**A**) and starting (8-week-old) and ending (20-week-old) body weight (**B**) of WT female mice fed with a high-fat diet (HFD) and treated with Veh or iD1 for 12 weeks. HFD feeding and iD1 treatment started at 8 weeks of age. *n* = 10-11 per group.

(C-E) ITTs (**C**), GTTs (**D**), and weights of the indicated tissues (**E**) of 20-week-old WT female mice fed with HFD and treated with Veh or iD1 for 12 weeks. *n* = 9-12 per group.

(F and G) Weights of the indicated tissues and body length of 20-week-old WT male (**F**) and female (**G**) mice fed with HFD and treated with Veh or iD1 for 12 weeks. *n* = 10-12 per group.

(H-M) Levels of *Cnot6l* mRNA in livers (**H**), change in body weight (**I**), and liver triglycerides (**J**), weights of the indicated tissues (**K**), representative H&E staining images of the indicated tissues (**L**), and levels of the indicated hepatokine mRNAs (**M**) of WT mice injected with adenovirus expressing shRNA against *Gfp* (sh*Cont*) or *Cnot6l* mRNA (sh*Cnot6l*) and maintained with HFD for 2 weeks. mRNA levels were normalized to *Hprt* mRNA. *n* = 8 per group. Scale bars represent 50 μm.

Data represent mean ± SEM. **p* < 0.05, ***p* < 0.01, ****p* < 0.001; *p* value by two-way ANOVA with Bonferroni's multiple comparison test for (**A-D**) or Student's *t*-tests for (**E-M**).

Data S1. Unprocessed data underlying the display items in the manuscript. Related to Figures 2 and S2.

Figure 2D

Figure 2L

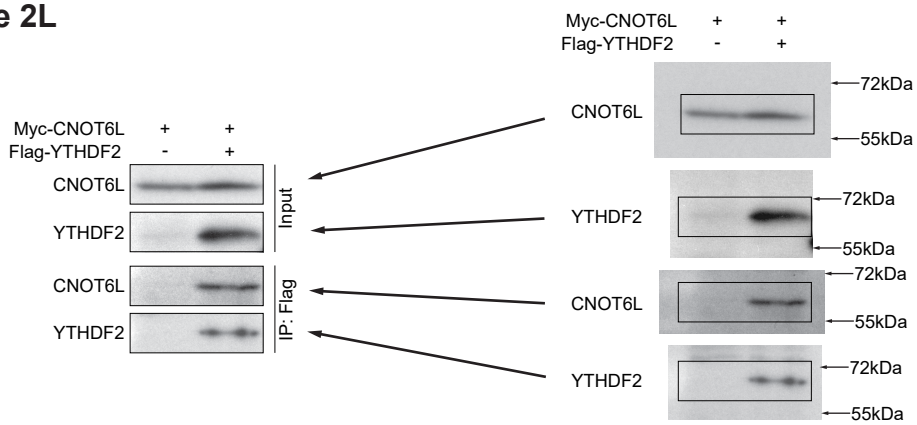

Figure 2R

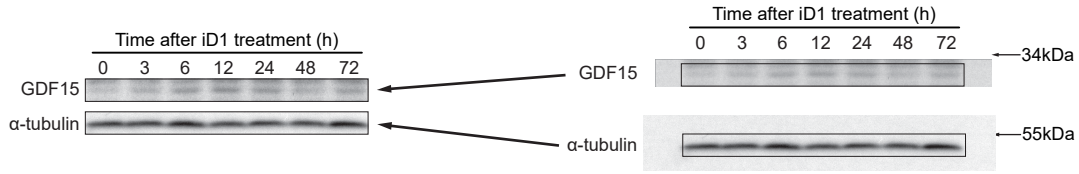

Figure S2J

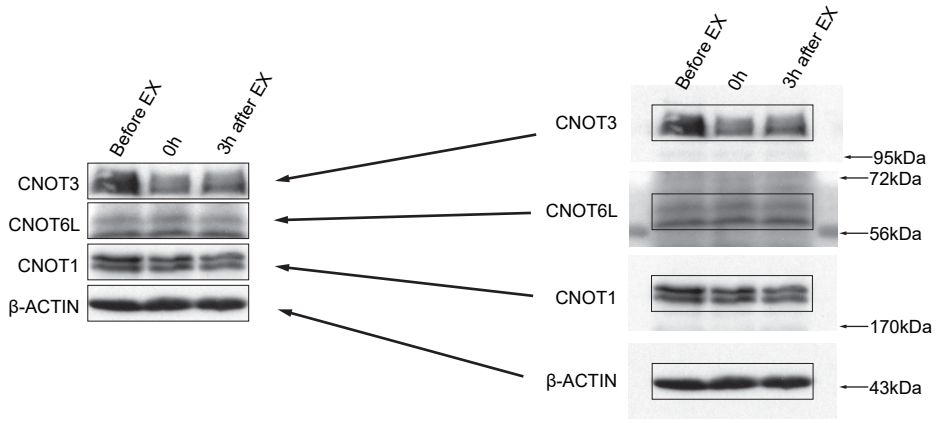

Figure S2L