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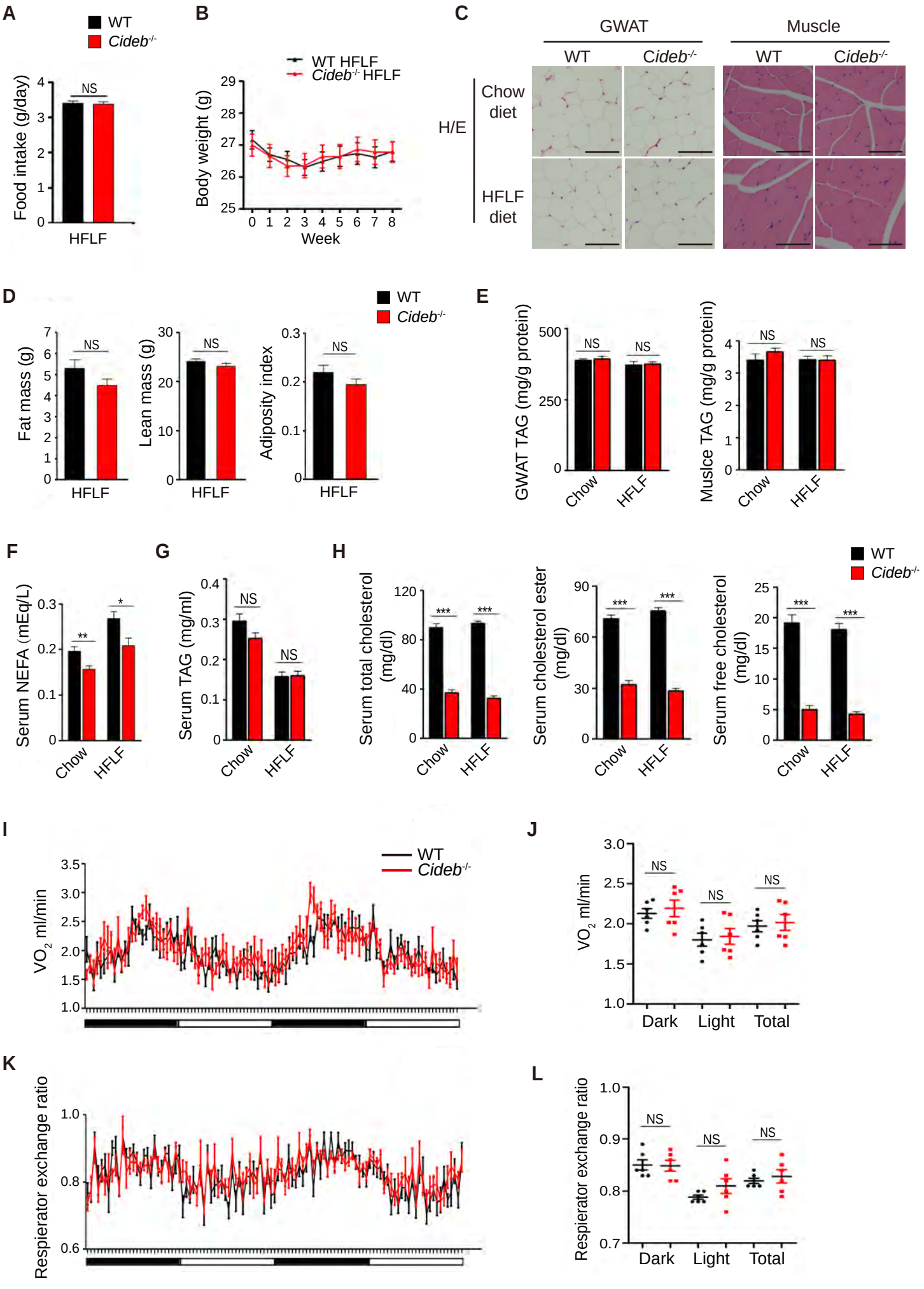
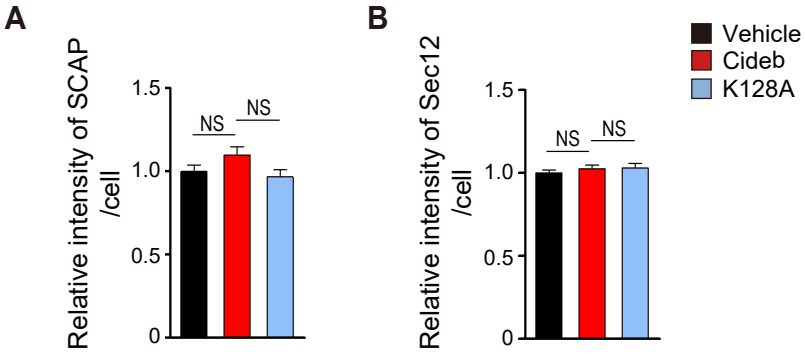
Appendix Figure S1



Appendix Figure S2



Appendix Figure S1. Metabolic parameters of WT and *Cideb*$^{-/-}$ mice under HFLF diets.

A Similar Food intake of WT and *Cideb*$^{-/-}$ mice under HFLF diet (*n* = 6 per group).

B Growth curve reflected by body weight analysis (*n* = 6 per group) of WT and *Cideb*$^{-/-}$ mice under HFLF diet.

C Similar Morphology (H/E staining) of WT and *Cideb*$^{-/-}$ gonadal white adipose tissue (GWAT) and muscle under chow diet and HFLF diet. Scale bar represented 100 μm.

D Adiposity of the WT and *Cideb*$^{-/-}$ mice under HFLF diet. The fat mass and lean mass were calculated with Dual-energy X-ray absorptiometry (*n* = 6 per group).

E GWAT and muscle TAG levels of WT and *Cideb*$^{-/-}$ mice under chow diet and HFLF diet (*n* = 5 per group).

F-H Serum NEFA levels (F), TAG levels (G) and free cholesterol or cholesterol ester levels (H) of WT and *Cideb*$^{-/-}$ mice under chow diet and HFLF diet (*n* = 8 per group).

I-L Oxygen consumption (VO_2, I, J) and respiratory exchange rate (K, L) of WT and *Cideb*$^{-/-}$ mice under HFLF diet monitored for 48 hours (*n* = 6 per group).

Data information: Data represent Mean ± SEM; NS: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, by 2-tailed Student's t test.

Appendix Figure S2. Cideb enhances the targeting of SREBP/SCAP to ER exit sites.

A-B Normalized fluorescent intensity of SCAP (A) or Sec12 (B) per cell under different conditions. Data represent Mean ± SEM; NS: not significant，by 2-tailed Student's t test.

Appendix Table S1. Liver mRNA level of WT and *Cideb*$^{-/-}$ mice under Chow diet and HFLF diet

Genotype	WT	WT	*Cideb*$^{-/-}$	*Cideb*$^{-/-}$
Diet	**Chow**	**HFLF**	**Chow**	**HFLF**
SREBP pathway				
Srebp-1c	1.00 ± 0.02	2.74*** ± 0.17	0.60 ± 0.03	0.92 ± 0.13
Srebp-2	1.00 ± 0.07	1.00 ± 0.04	0.67 ± 0.03	0.65 ± 0.03
Other transcription factors				
Chrebp	1.00 ± 0.09	1.10 ± 0.07	0.79 ± 0.05	0.98 ± 0.07
Lxrα	1.00 ± 0.03	1.15 ± 0.08	0.90 ± 0.08	1.10 ± 0.05
Lxrβ	1.00 ± 0.05	1.30** ± 0.08	0.89 ± 0.10	1.08 ± 0.10
Pparα	1.00 ± 0.12	1.65** ± 0.14	0.93 ± 0.08	1.62** ± 0.15
Fatty acids synthesis pathway				
Fasn	1.00 ± 0.03	8.32*** ± 0.98	0.43 ± 0.05	1.33* ± 0.23
Acaca	1.00 ± 0.10	5.60*** ± 0.47	0.43 ± 0.11	1.13** ± 0.11
Scd1	1.00 ± 0.11	8.11*** ± 1.07	0.10 ± 0.12	0.73** ± 0.13
Elovl6	1.00 ± 0.04	7.78** ± 1.24	0.47 ± 0.03	1.81*** ± 0.20
Cholesterol metabolism pathway				
Hmgcr	1.00 ± 0.08	2.18** ± 0.26	0.77 ± 0.02	0.76 ± 0.05
Hmgcs	1.00 ± 0.08	0.86 ± 0.07	0.61 ± 0.07	0.44 ± 0.06
Ldlr	1.00 ± 0.01	1.18** ± 0.04	0.72 ± 0.06	0.65 ± 0.06
Mitochondrial oxidation				
Cox4	1.00 ± 0.08	1.00 ± 0.07	0.95 ± 0.04	0.96 ± 0.06
Cpt1	1.00 ± 0.11	1.76** ± 0.18	1.03 ± 0.11	1.46** ± 0.06
Cpt2	1.00 ± 0.10	1.10 ± 0.04	1.00 ± 0.09	1.15 ± 0.07
Inflammation pathway				
F4/80	1.00 ± 0.04	2.21*** ± 0.16	1.04 ± 0.08	1.32 ± 0.17
Tnfα	1.00 ± 0.10	2.17*** ± 0.13	1.04 ± 0.14	0.99 ± 0.12
Mcp1	1.00 ± 0.06	2.32* ± 0.34	0.89 ± 0.06	0.49** ± 0.10
Ccl3	1.00 ± 0.17	1.77** ± 0.14	1.02 ± 0.07	0.99 ± 0.11
LXR target genes				
Abcg5	1.00 ± 0.08	1.67** ± 0.19	0.88 ± 0.06	1.47** ± 0.15
Abcg8	1.00 ± 0.07	2.28*** ± 0.06	0.80 ± 0.05	1.78*** ± 0.13
Lpcat3	1.00 ± 0.07	1.67** ± 0.19	0.78 ± 0.14	1.54** ± 0.15
Abca1	1.00 ± 0.11	1.37* ± 0.06	0.77 ± 0.09	1.21* ± 0.11

Transcript levels of genes determined by qPCR from WT and *Cideb*$^{-/-}$ liver under

Chow diet and HFLF diet (n = 5 per group). Data represent the Mean ± SEM; *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, by 2-tailed Student's t test.

Appendix Table S2. Sequence of primers for qPCR

	Forward (5'-3')	Reverse (5'-3')
β-*Actin*	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Cidea	TGACATTCATGGGATTGCAGAC	GGCCAGTTGTGATGACTAAGAC
Cideb	TCTGTGATCATAAGCGGACA	GCAGCAGCGAGGAAGTCCAA
Cidec	TCGTGTTAGCACCGCAGAT	GCTCTCTTCTTGCGCTGTT
Srebp-1c	GGAGCCATGGATTGCACATT	GCTTCCAGAGAGGAGGCCAG
Srebp-2	CCCTTGACTTCCTTGCTGCA	GCGTGAGTGTGGGCGAATC
Scap	TGGAGCTTTTGAGACTCAGGA	TCGATTAAGCAGGTGAGGTCG
Insig-1	TCACAGTGACTGAGCTTCAGCA	TCATCTTCATCACACCCAGGAC
Insig-2a	CCCTCAATGAATGTACTGAAGGATT	TGTGAAGTGAAGCAGACCAATGT
Fasn	TCCAAGACTGACTCGGCTACTGAC	GCAGCCAGGTTCGGAATGCTATC
Acaca	AGCTGATCCTGCGAACCT	GCCAAGCGGATGTAAACT
Elovl6	AAGCAGTTCAACGAGAACGAA	CGTACAGCGCAGAAAACAGG
Scd1	AAGCTCTACACCTGCCTCTTC	GTGACTCCCGTCTCCAGTTCT
Acly	ATATTCATCAGCTTCCTCCC	TCCAAGAAGCCAAATCTTATCCTG
Acss2	GCTGCCGACGGGATCAG	TCCAGACACATTGAGCATGTCAT
Hmgcr	TGTGTGTCGCTGGTCCTAGA	TTTCTCTCTTGGTTCGCTCCT
Hmgcs	ACAGTTTGGATGAAGGAATG	GTCTTGGCACTTTCTTAGCA
Ldlr	AGGAGCAGCCACATGGTATGA	CTGTCTTGAGGGGTAGGTAT
Ss	CCAACTCAATGGGTCTGTTCCT	TGGCTTAGCAAAGTCTTCCAACT

Pcsk9	ACCCTCATAGGCCTGGAGTT	CTGTGATGACCTCTGGAGCA
Pparα	ACGGCAATGGCTTTATCA	CGCTGCGTCGGACTCGGT
Lxrα	ATGTCCACGAGTGACTGTT	TTGACTCTCCCTTAATGCTAC
Lxrβ	TATGCCTTGCTTATCGCCATC	CTTGTCCTGGAGTCGCAATG
Cpt1	ACCACTGGCCGCATGT	CTCCATGGCGTAGTAGTTGCT
Cpt2	CCACCGACTCCTCCGTGTC	GCTGCTGCCAGATACCGTAGA
Cox4	CGGCGTGACTACCCCTTG	TGAGGGATGGGGCCATACA
Chrebp	GCTTACAGTGGCAAGCTGGT	GTCACGAAACCACACACTGG
Mcp1	AGGTCCCTGTCATGCTTCTG	GCTGCTGGTGATCCTCTTGT
Tnfα	CCAGACCCTCACACTCAGATC	CACTTGGTGGTTTGCTACGAC
F4/80	TGTCTGACAATTGGGATCTGCCCT	ATACGTTCCGAGAGTGTTGTGGCA
Ccl3	TTTGAAACCAGCAGCCTTTGCTCC	TCAGGCATTCAGTTCCAGGTCAGT
Apoe	GCTGGGTGCAGACGCTTT	TGCCGTCAGTTCTTGTGTGACT
Pepck	CCACAGCTGCTGCAGAACA	GAAGGGTCGCATGGCAAA
Abcg5	TGGATCCAACACCTCTATGCTAAA	GGCAGGTTTTCTCGATGAACTG
Abcg8	ATGAGCTGGAAGACGGGCTG	GCCAGTGAGAGCAAGGCTGA
Lpcat3	TTTCTGGTTCCGCTGCATGT	CCGACAGAATGCACACTCCTTC
Abca1	TCTCTGCTATCTCCAACCTCATC	ACGTCTTCACCAGGTAATCTGAA

Appendix Supplementary Methods

In vitro reconstitution using semi-intact cells

The *in vitro* reconstitution of COPII-mediated cargo packaging with permeabilized semi-intact cells were performed as previously described (Nie, Wang et al., 2018) with slight modifications. Two 10-cm plates of primary hepatocytes at ~70 to 80% confluency were washed with PBS, digested by trypsin and collected by centrifugation at 2200 rpm for 3 minutes at 4 °C. Cells were resuspended in 6 mL cold B88-0 buffer (20 mM HEPES, pH 7.2, 250 mM Sorbitol, and 150 mM KOAc) and permeabilized with 40 μg/mL digitonin on ice for 5 min. Permeabilization was stopped by adding 8 mL ice-cold B88-0 buffer, and cells were collected by centrifugation at 2200 rpm for 3 minutes at 4 °C. After washing twice with 6 mL B88-0 buffer at 4 °C, permeabilized cells were resuspended in 1 mL B88-0 buffer and the Permeabilization was checked by using trypan blue stain. Cells were then centrifuged at 10,000 × g for 30 sec at 4 °C and finally resuspended in 300 to 400 μL B88-0 buffer. Budding reactions (100 μL) were set up in nonstick Eppendorf tubes on ice with 20 μL (OD600 0.1 to 0.2) semi-intact cells as donor membranes, ATP regeneration system (1 mM ATP, 40 mM creatine phosphate, 200 μg/mL creatine phosphokinase, and 500 μM MgOAc in B88-0 buffer), 1.5 μL 10 mM GTP, and 400 μg liver cytosol. Reactions were performed at 30 °C for 60 min and stopped by centrifugation at 12,000 × g for 10 min at 4 °C. Seventy microliters of supernatant was collected by centrifugation in a TLA100 rotor at 135,000 × g for 20 min at 4 °C.

The supernatants were discarded by pipetting with gel-loading tips, and the high-speed pellet fractions (including COPII vesicles) were thoroughly resuspended in 20 μL of 1× SDS sample buffer and heated at 55 °C for 20 min before SDS/PAGE.

SiRNA experiment

Small interfering RNAs (siRNAs) were synthesized at GenePharma (China). SiRNA depleted Cideb mRNA by at least 80% were selected for further experiments. The sequences of siRNAs was listed below: siCideb: 5'-CCTCTGCATGGAGTACCTT-3'. The siRNA was transfected into primary hepatocytes by Lipofectamin 2000 (Invitrogen) for 48 hours before other experiments.

***In vivo* VLDL secretion assay**

The *in vivo* VLDL secretion assay was performed as described before (Ye, Li et al., 2009) with slight modifications. After fasting overnight (around 16 hours), mice were injected with TritonWR-1339 (500 mg/kg in PBS) through the tail veins. Fifty microliters of blood was collected through the tail at 0, 1, 2, 4 hours after injection, and serum TAG concentrations were measured by TAG reagent (Sigma). Levels of serum ApoB-48, ApoB-100, and liver Cideb were detected by immune blotting.

CRISPR/Cas9 system mediated knockin

The knockin experiment was conducted by using CRISPR/Cas9 system as described previously (Ran, Hsu et al., 2013) with slight modifications. Three sets of sgRNA

targeting the C-terminus of Cideb genome were designed at http://tools.genome-engineering.org and constructed into PX459 backbone (Ran et al., 2013). GFP tag flanked with the C-terminus of Cideb genome and its 3' downstream sequences were constructed into another plasmid for homogenous recombination. Both plasmids were then co-transfected into AML12 cells by Lipofectamin 2000 for 48 hours, and the positive colonies were selected by puromycin (1 μg/ml). After selection, the insertion of GFP at the C-terminus of Cideb genome was confirmed by genomic PCR and sequencing. The sequence of sgRNA were listed below,

sg*Cideb*#1: 5'-CCCCCTCCACCACATGGCGA-3',

sg*Cideb*#2: 5'-CTCCACCACATGGCGAAGGG-3',

sg*Cideb*#3: 5'-CCCCTCCACCACATGGCGAA-3'.

References

Nie C, Wang H, Wang R, Ginsburg D, Chen XW (2018) Dimeric sorting code for concentrative cargo selection by the COPII coat. Proc Natl Acad Sci U S A 115: E3155-E3162

Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F (2013) Genome engineering using the CRISPR-Cas9 system. Nat Protoc 8: 2281-2308

Ye J, Li JZ, Liu Y, Li X, Yang T, Ma X, Li Q, Yao Z, Li P (2009) Cideb, an ER- and lipid droplet-associated protein, mediates VLDL lipidation and maturation by interacting with apolipoprotein B. Cell Metab 9: 177-90