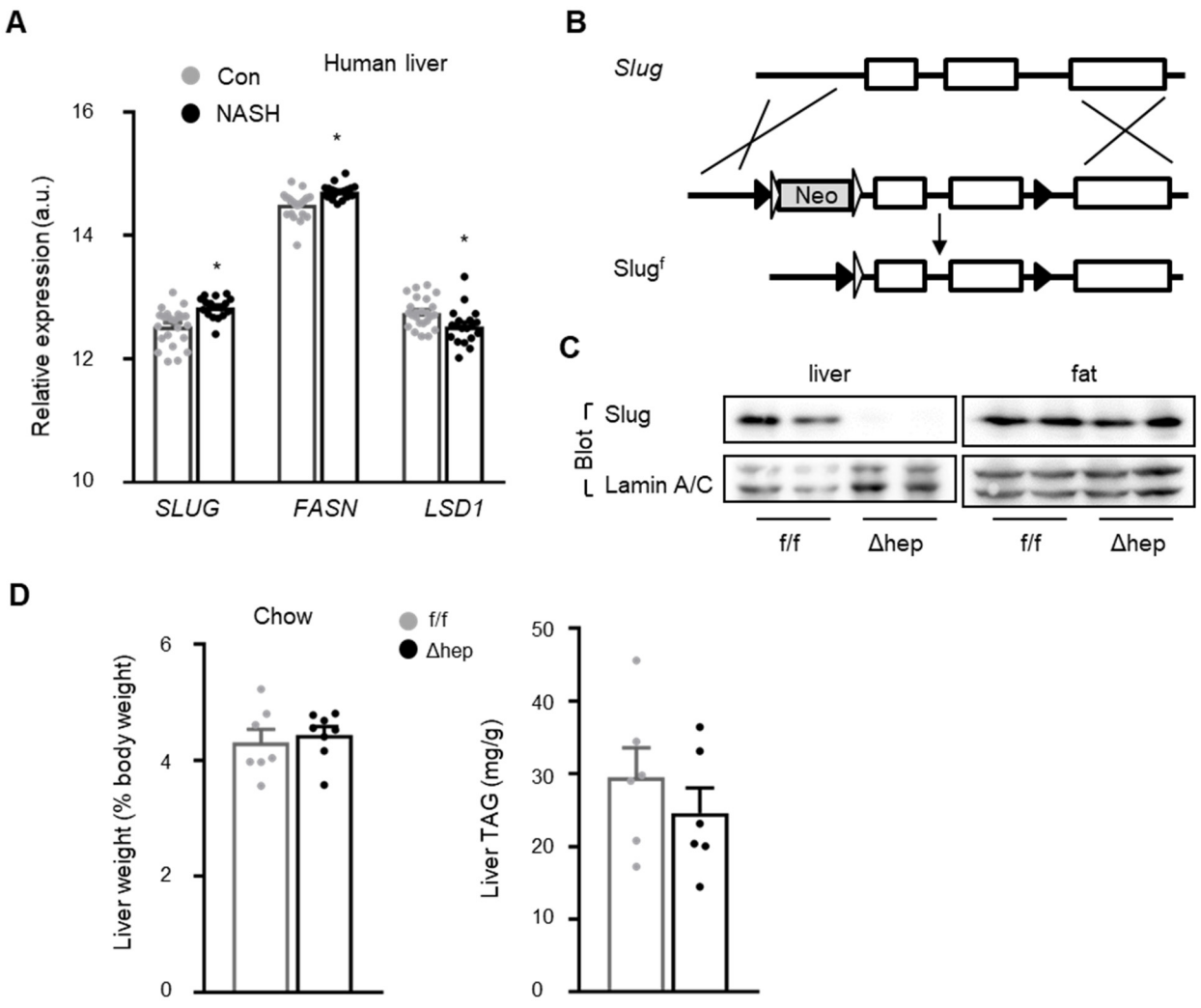
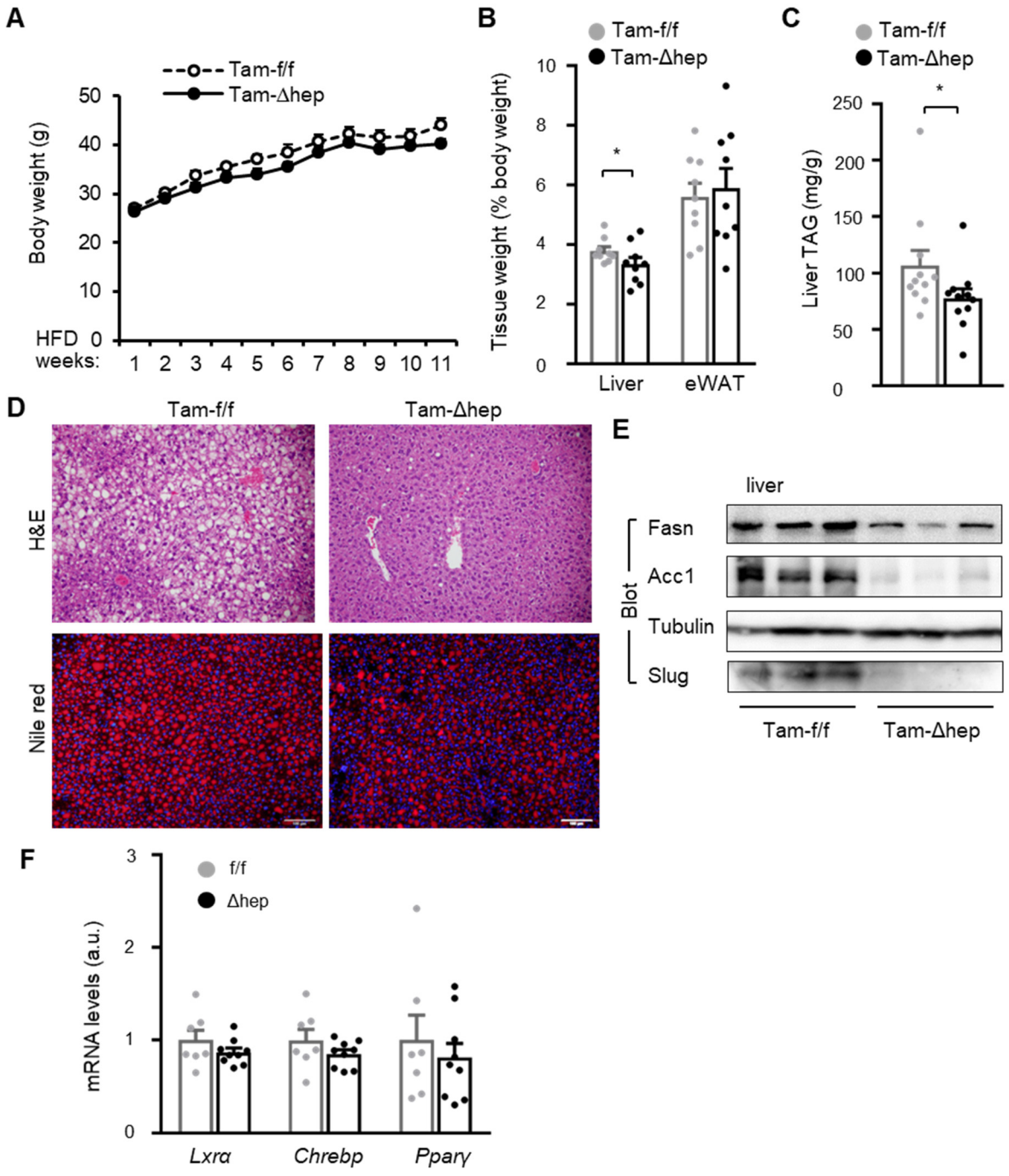
Hepatic Slug epigenetically promotes liver lipogenesis, fatty liver disease, and type 2 diabetes

Yan Liu, Haiyan Lin, Lin Jiang, Qingsen Shang, Yin Lei, Jiandie Lin, Wen-Shu Wu, Liangyou Rui

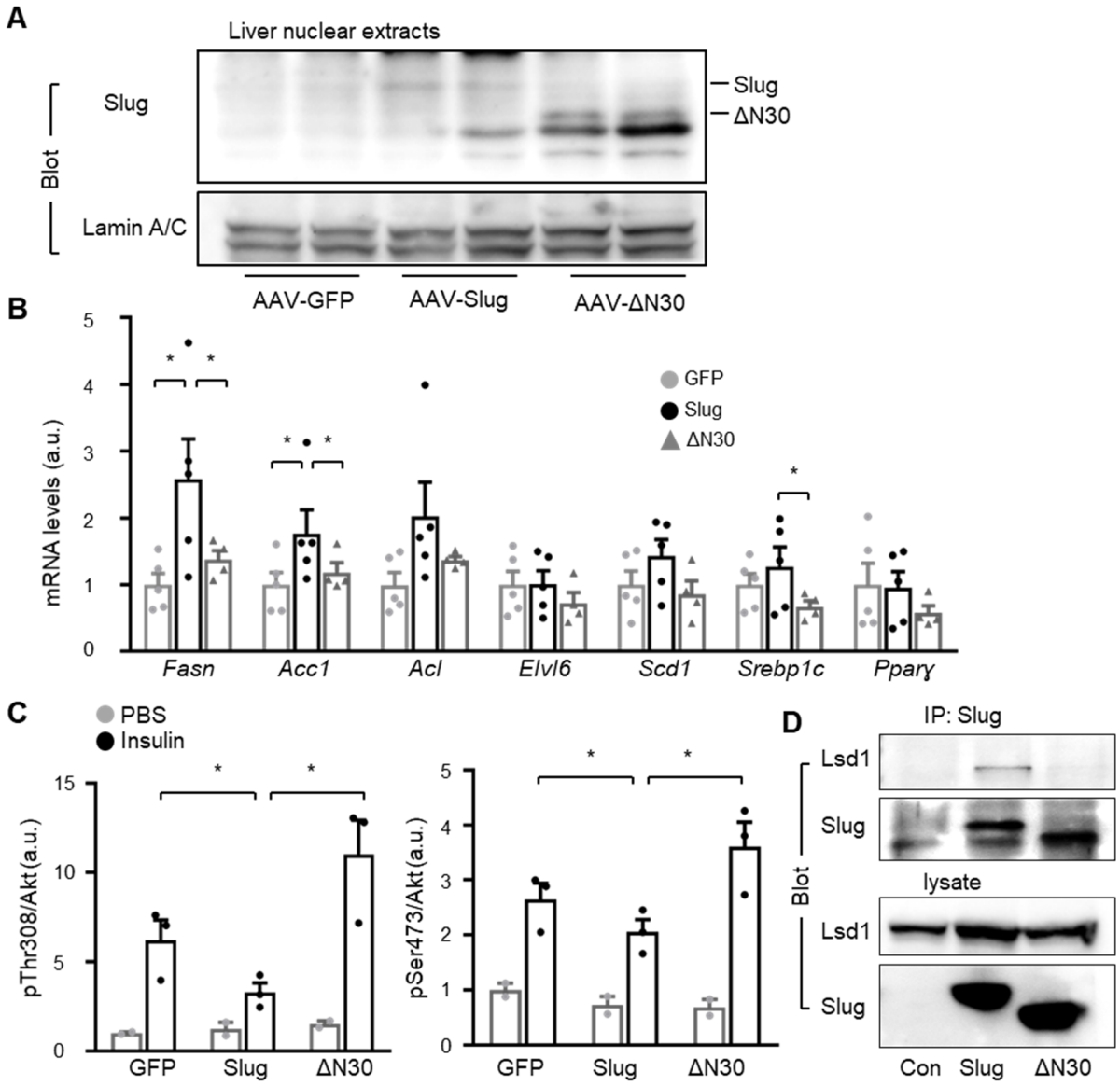
Supplementary information



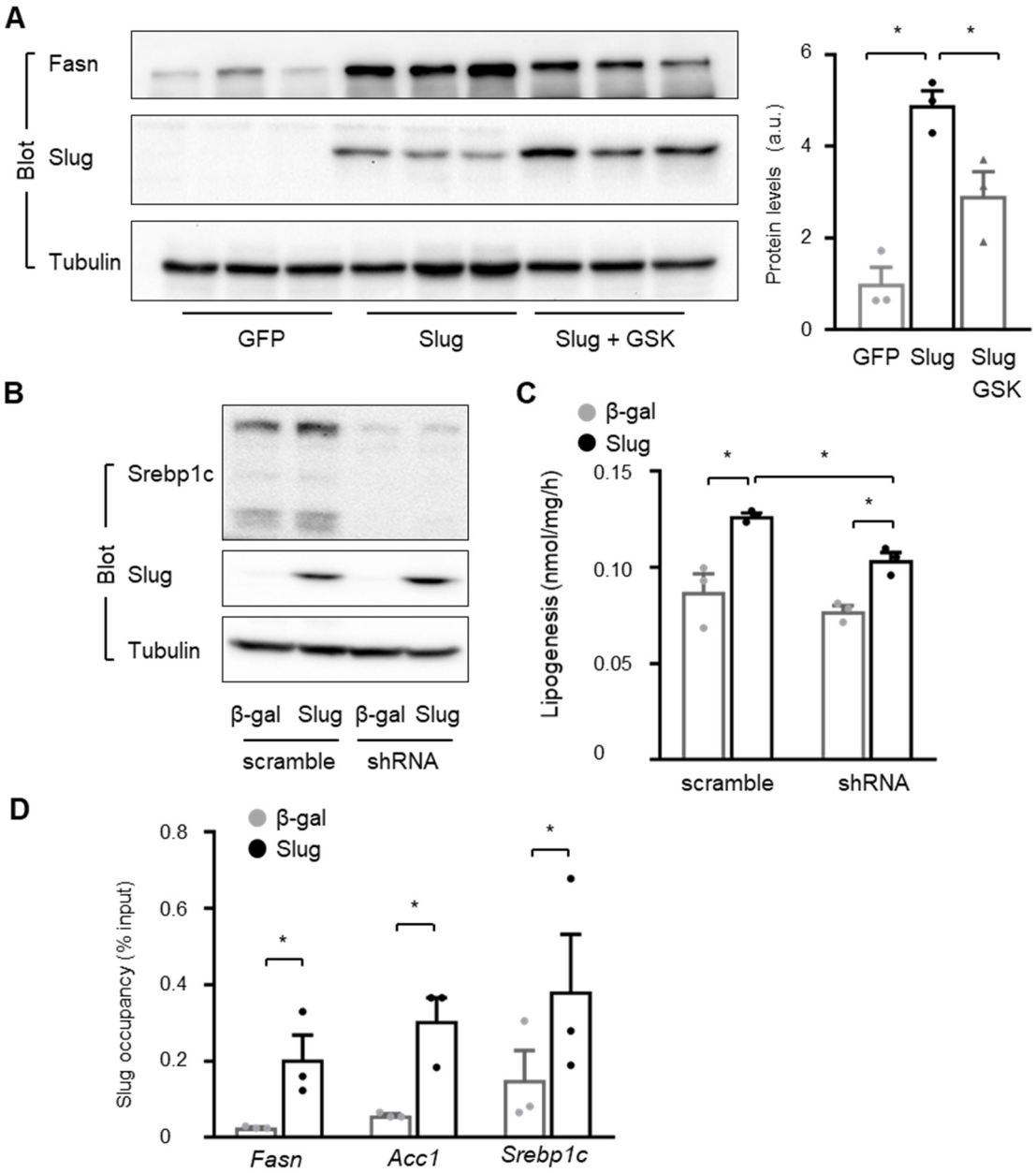
Supplemental Figure 1. Hepatocyte-specific deletion of hepatic *Snai2* in mice. (A) SLUG, FASN and LSD1 expression profile information was retrieved from RNA-seq GSE89632 datasets (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE89632) using GEO2R tools and illumina ID ILMN_1655740 (SLUG), ILMN_1784871(FASN), and ILMN_1813840 (LSD1). Retrieved SLUG, FASN and LSD1 expression values were statistically analyzed. Con: n=24, NASG: n=19. **(B)** Schematic representation of $Slug^{f/f}$ allele. **(C)** Nuclear extracts were prepared from fat and liver and immunoblotted with antibodies against Slug and lamin A/C. **(D)** $Slug^{\Delta hep}$ (n=7) and $Slug^{f/f}$ (n=8) male mice were fed a standard chow diet for 18 weeks. Liver/body weight ratios, liver TAG and levels (normalized to liver weight were measured. Data are presented as mean ± SEM. Data are presented as mean ± SEM. *p<0.05, 2-tailed unpaired Student's *t* test.



Supplemental Figure 2. Deletion of hepatic Slug protects against liver steatosis in obesity. Tam-f/f (n=10) and Tam-Δhep (n=10) male mice (7 weeks) were fed a HFD for 11 weeks and euthanized under randomly fed conditions. **(A)** Growth curves. **(B)** Liver/body weight and epididymal WAT/body weight ratios. **(C)** Liver TAG levels (normalized to liver weight). **(D)** Reprehensive liver sections (4 pairs). **(E)** Liver extracts were immunoblotted with the indicated antibodies. Slug and other proteins were resolved in parallel gels. **(F)** $Slug^{\Delta hep}$ (n=9) and $Slug^{f/f}$ (n=7) males were fed a HFD for 11 weeks. Liver mRNA abundance was measured by qPCR (normalized to 34B4 levels). Data are presented as mean ± SEM. *p<0.05, 2-tailed unpaired Student's *t* test.



Supplemental Figure 3. Liver-specific overexpression of Slug but not epigenetic defective ΔN30 promotes liver steatosis. (A) C57BL/6J mice were transduced with the indicated AAV vectors for 3 weeks. Liver nuclear extracts were immunoblotted with the indicated antibodies. **(B-C)** C57BL/6 male mice were transduced with AAV-GFP, -Slug, or -ΔN30 vectors via tail vein injection, and then fed a HFD for 11 weeks. **(B)** Mice were fasted overnight and stimulated with insulin (1 unit/kg body weight, iv) for 5 min. Livers extracts were immunoblotted with phospho-Akt (pThr308 and pSer473). Akt phosphorylation was quantified and normalized to total Akt levels. GFP: n=3, Slug: n=3, ΔN3: n=3. **(C)** Liver gene expression (normalized to 36B4 expression). GFP: n=5, Slug: n=5, ΔN3: n=4. **(D)** Lsd1 was coexpressed with Slug or ΔN30 in HEK293 cells. Cell extracts were immunoprecipitated with anti-Slug antibody and immunoblotted with anti-Lsd1 or Slug antibodies. Data are presented as mean ± SEM. *p<0.05, 2-tailed unpaired Student's *t* test.



Supplemental Figure 4. Lsd1 mediates Slug lipogenic function in the liver. (A) C57BL/6J male mice were transduced with GFP or Slug adenoviral vectors and treated with GSK or empty vehicles (control). Liver extracts were prepared 5 days after GSK treatment and immumoblotted with the indicated antibodies. Fasn levels were normalized to α-tubulin levels. **(B-C)** Mouse primary hepatocytes were transduced with the indicated adenoviral vectors for 3 days. **(B)** Cell extracts were immunoblotted with the indicated antibodies. **(C)** *De novo* lipogenesis assays were performed on these cells (n=3 per group). **(D)** Primary hepatocytes were transduced with Slug or β-gal adenoviral vectors. Slug occupancy on the *Fasn*, *Acc1*, and *Srebp1c* promoters was assessed by ChIP-qPCR and normalized to inputs (n=3 per group). Data are presented as mean ± SEM. *p<0.05, 2-tailed unpaired Student's *t* test.

ANTIBODY	SOURCE	IDENTIFIER	DILUTION
Slug	ABclonal Technology	A1057	1:600
Slug	Cell Signaling Technology	#9585	1:1000
Lamin A/C	Cell Signaling Technology	#4777	1:2000
p-Akt (pThr308)	Cell Signaling Technology	#13038	1:5000
p-Akt (pSer473)	Cell Signaling Technology	#4060	1:5000
Akt	Cell Signaling Technology	#4685	1:2000
α-tubulin	Santa Cruz	sc-5286	1:10000
Fasn	Cell Signaling Technology	#3180	1:5000
Acc1	Cell Signaling Technology	#3676	1:5000
Acl	Cell Signaling Technology	#13390	1:5000
H3K9me1	Cell Signaling Technology	#14186	1:200
H3K9me2	Cell Signaling Technology	#4658	1:200
H3K4me2	Cell Signaling Technology	#9725	1:200
Lsd1	Cell Signaling Technology	#2184	1:3000
Ubiquitin	Santa Cruz	sc-8017	1:1000
Srebp-1c	Santa Cruz	Sc-13551	1:500

Supplemental Table 1. Antibody list

Genes	Forward	Reverse
Slug (mouse)	ATTGCCTTGTGTCTGCAAGAT	TTTTGGAGCAGTTTTTGCACT
Slug (human)	ACTGGACACACATACAGTGATT	GGAGAGAGGCCATTGGGTAG
Fasn	TTGACGGCTCACACACCTAC	CGATCTTCCAGGCTCTTCAG
Acc1	CAGGGACTATGTCCTGAAGCA	GGAATCCATTGTGGAGAGGA
Srebp-1c	AACGTCACTTCCAGCTAGAC	CCACTAAGGTGCCTACAGAGC
Mttp	CTCCACAGTGCAGTTCTCACA	AGAGACATATCCCCTGCCTGT
Cpt1α	CTGATGACGGCTATGGTGTTT	GTGAGGCCAAACAAGGTGATA
Gck	GAAAAGATCATTGGCGGAAA	CCCAGAGTGCTCAGGATCTT
LPK	GAGGCTTCCTTCAAGTGCTG	GAGGCTCACGGTAGAGCAAG
Cidea	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
Cideb	GACCCTTCCGTGTCTGTGAT	GTAGCAGCAAGGTCTCCAGG
Cidec	CCTATGACCTGCACTGCTACAAG	CATGTAGCTGGAGGTGCCAAG
CD36	GGAGTGGTGATGTTTGTTGCT	GCACACACCACCATTTCTTCT
Chrebp	CTGGGGACCTAAACAGGAGC	GAAGCCACCCTATAGCTCCC
Elvl6	AAAGCACCCGAACTAGGTGACA	ACCAGTGCAGGAAGATCAGTTTC
Scd1	AGGTGCCTCTTAGCCACTGA	CCAGGAGTTTCTTGGGTTGA
Dgat1	CGTGGTATCCTGAATTGGTG	GGCGCTTCTCAATCTGAAAT
Dgat2	ATCTTCTCTGTCACCTGGCT	ACCTTTCTTGGGCGTGTTCC
Acl	CCTCAAGGACTTCGTCAAACA	GCCCATACTCCTTCCTAGCAC
34B4	AAGCGCGTCCTGGCATTGTCT	CCGCAGGGGCAGCAGTGGT
GAPDH (human)	CGACCACTTTGTCAAGCTCA	AGGGGTCTACATGGCAACTG
Fasn (ChIP)	TGCAACCGTAGTCCAACGAG	GCCTCAGCGGAAGTCATCAG
NCH (ChIP)	GGATGGCTCCAAGATAAGGCA	ACAACATCCACACGTCCAGT
Actb (ChIP)	AATAGCCTCCGCCCTTGTG	CGTGACATCCACACCCAGA
Acc1 (ChIP)	CCTGGCTGTCCTGGAAATCA	TTAAATGTTGTCCTTGCCGGG

Srebp1c (ChIP)	ACACCTCATCTTCTCGCCATC	ATCCCCATCCCCATCTTCTCA

Supplemental Table 2. Primer list.