Supplemental Figure Legends

Figure S1, related to Figure 1.

(A) Generic sequences of TALEN proteins used for genome editing.

(B) PCR amplicons subjected to electrophoresis on 2.5% agarose gels to discriminate clones with indels. Based on the appearance of the band(s), the number of alleles with indels can be assessed for each clone, allowing for prioritization of clones for subcloning of PCR amplicons and Sanger sequencing.

Figure S2, related to Figure 2.

(A) Sequences at *APOB* TALEN target sites in all clones with mutant alleles, whether biallelic, in which case both alleles are shown, or monoallelic, in which case one allele is shown. Insertions are indicated in bold red.

(B) Representative karyotypes of HuH-7/CD81^{high} cells, a wild-type HUES 9 clone, and a *SORT1*–/– HUES 9 clone (clone D from Fig. 3A). The two HUES 9 clones have identical, normal karyotypes; the HuH-7 cells have severely abnormal karyotypes.

Figure S3, related to Figure 3 and Figure 4.

(A) Differentiation protocol of human pluripotent stem cells (hPSCs) into hepatocyte-like cells (HLCs).

(B) Differentiation with marker staining at various stages, with cultured human HepG2 cells for comparison.

(C) Relative expression of hepatocyte-specific genes in HLCs vs. hPSCs measured by qRT-PCR; expression is indicated as fold change of $2^{-\Delta\Delta Ct}$ with reference to *HPRT*, normalized to levels in hPSCs.

(D) Secreted apoB and apoA-I mass in media from HLCs vs. hPSCs by ELISA and/or Western blot analysis.

Figure S4, related to Figure 3.

(A) Albumin and apoB mass measured by ELISA in media collected from wild-type HLCs differentiated from HUES 1 or HUES 9 cells, normalized to mean levels in HUES 1 HLCs. N = 6.

(B) ApoA-I and apoB mass measured by ELISA in media collected from wild-type and knockout HLCs (two clones each; A and B for HUES 1, D and E for HUES 9 from Figure 3), normalized to mean levels in wild-type HLCs. N = 3 for HUES 1, N = 6 for HUES 9.

(C) ApoA-I and apoB mass in media measured by ELISA and *SORT1* mRNA expression measured by qRT-PCR from wild-type and knockout HUES 9 HLCs (two clones each; clones D and E from Figure 3) infected with *SORT1*- or *GFP*-expressing lentivirus. ELISA measurements are normalized to mean levels in wild-type HLCs. mRNA expression is indicated as fold change of $2^{-\Delta\Delta Ct}$ with reference to 18S rRNA, normalized to mean level in undifferentiated HUES 9 hESCs (not shown). N = 6.

(D) ANGPTL4, ANGPTL6, HGF, and FGF-19 mass in media measured by ELISA from clones in (C), normalized to mean levels in wild-type HLCs. N = 5.

(E) Lipid-related gene expression measured by qRT-PCR from clones in (C); expression is indicated as fold change of $2^{-\Delta\Delta Ct}$ with reference to 18S rRNA, normalized to mean level in undifferentiated HUES 9 hESCs (not shown). N = 6.

Error bars show s.e.m. from experiments with biological replicates. *P* values calculated with unpaired t test.

Figure S5, related to Figure 3.

(A) Ratios of glucose uptake to total protein content in wild-type and knockout HUES 1 adipocytes (two clones each; clones A and B from Figure 3) treated with or without insulin, all normalized to mean ratio in wild-type adipocytes without insulin. N = 6.

(B) *SORT1* and adipose-related gene expression measured by qRT-PCR from wild-type and

knockout HUES 1 adipocytes (one clone each; clone A from Figure 3) infected with *SORT1*-expressing or control lentivirus; expression is indicated as fold change of $2^{-\Delta\Delta Ct}$ with reference to 18S rRNA, normalized to mean level in wild-type adipocytes. N = 6.

(C) Total cell numbers (determined as number of cells marked with DAPI), motor neuron numbers (determined as number of cells doubly positive for TUJ1 and ISL-1), and ratios of motor neuron number to total cell number upon differentiation of wild-type and knockout HUES 9 cells into neurons (two clones each; clones D and E from Figure 3). N = 12.

Error bars show s.e.m. from experiments with biological replicates. *P* values calculated with unpaired t test.

Figure S6, related to Figure 4.

(A) Identification of *AKT2* E17K clones generated by HDR with a 67-bp ssODN. Sanger sequencing identified the two targeted point mutations: the first to introduce the E17K mutation, the second to introduce a new RsaI restriction enzyme cutting site. Screening for the mutation was performed by obtaining PCR amplicons around the TALEN target site and digesting the products with RsaI; two positive clones are shown. Also shown are two other clones with indels introduced by the TALENs via NHEJ.

(B) Gluconeogenesis-related gene expression measured by qRT-PCR from wild-type, knockout, and E17 HUES 9 HLCs (two clones each; A and B for knockout; C and D for E17K from Figure 4) treated with or without dexamethasone and forskolin with or without insulin; expression is indicated as fold change of $2^{-\Delta\Delta Ct}$ with reference to 18S rRNA, all normalized to mean levels in wild-type adipocytes without additives. N = 2.

(C) MCP-1 and PAI-1 mass measured by ELISA in media collected from wild-type, knockout, and E17K HUES 9 adipocytes (two clones each; A and B for knockout; C and D for E17K from Figure 4), normalized to mean levels from wild-type adipocytes. N = 4.

Error bars show s.e.m. from experiments with biological replicates. *P* values calculated with unpaired t test.

Figure S7, related to Figure 5.

(A) Alignment of predicted amino acid sequences of wild-type perilipin, protein expressed from *PLIN1* with the naturally occurring patient-specific Val398fs mutation (Gandotra et al., 2011a), elongated protein expressed from $PLIN1^{558}$ allele (in clone A from Figure 5), and truncated protein expressed from $PLIN1^{415}$ allele (in clone B from Figure 5). Boxes indicate areas of divergence in the sequences.

(B) Brightfield images of wild-type, $PLIN1^{558}$ (clone A from Figure 5), and $PLIN1^{415}$ (clone B from Figure 5) adipocytes.

(C) Western blot for C-terminus of perilipin in wild-type, $PLIN1^{558}$, and $PLIN1^{415}$ adipocytes.

(D) Gene expression measured by qRT-PCR from wild-type, $PLIN1^{558}$, and $PLIN1^{415}$ adipocytes; expression is indicated as fold change of $2^{-\Delta\Delta Ct}$ with reference to 18S rRNA, all normalized to mean levels in wild-type adipocytes without additives. N = 2.

Error bars show s.e.m. from experiments with biological replicates. *P* values calculated with unpaired t test.

Figure S1.

A

Generic TALEN amino acid sequences (to recognize 15bp DNA sequences)

```
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPSRVDLRTLGYSQQQQEKIKPKVRSTVAQHH
EALVGHGFTHAHIVALSQHPAALGTVAVTYQHIITALPEATHEDIVGVGKQWSGARALEALLTDAGEL
RGPPLQLDTGQLVKIAKRGGVTAMEAVHASRNALTGAPLNLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGRPALESIVAQLSR
PDPALAALTNDHLVALACLGGRPAMDAVKKGLPHAPELIRRVNRRIGERTSHRVAGSQLVKSELEEKK
SELRHKLKYVPHEYIELIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAIYTVGSPIDY
GVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKA
QLTRLNRKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF
```

```
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPARVDLRTLGYSQQQQEKIKPKVRSTVAQHH
EALVGHGFTHAHIVALSQHPAALGTVAVTYQHIITALPEATHEDIVGVGKQWSGARALEALLTDAGEL
RGPPLQLDTGQLVKIAKRGGVTAMEAVHASRNALTGAPLNLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASXXGGKQALETVQRLLPVLCQAHGLTPEQVVAIASXXGGRPALESIVAQLSR
PDPALAALTNDHLVALACLGGRPAMDAVKKGLPHAPELIRRVNRRIGERTSHRVAGSQLVKSELEEKK
SELRHKLKYVPHEYIELIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAIYTVGSPIDY
GVIVDTKAYSGGYNLPIGQADEMERYVEENQTRDKHLNPNEWWKVYPSSVTEFKFLFVSGHFKGNYKA
QLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF
```

To recognize **C**: **XX = HD**
To recognize **T**: **XX = NG**
To recognize **A**: **XX = NI**
To recognize **G**: **XX = NN** except in last position, where **XX = NK**

Underline indicates N-term and C-term of TAL effector

B

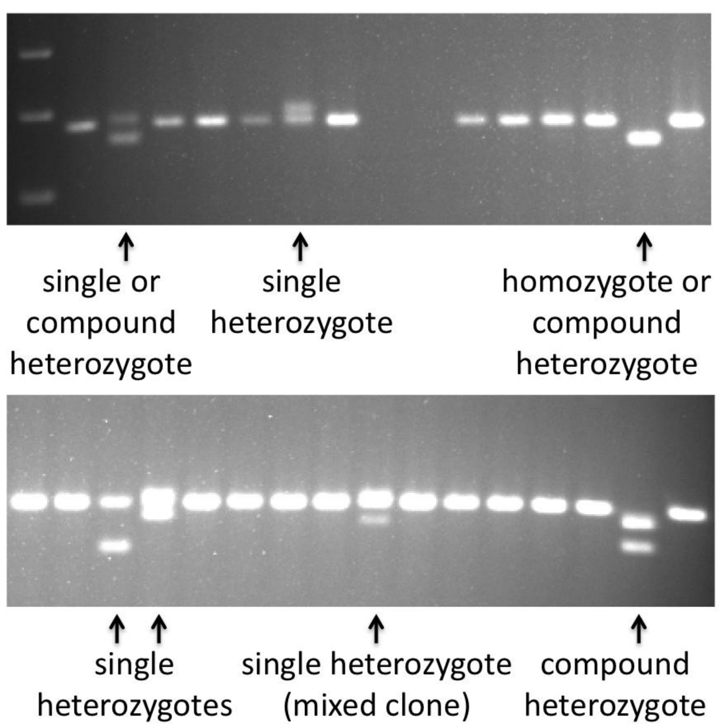


Figure S2.

A

wt: ATGCTTCTCCGGGAGATAAGCGACTGGCTGCCTATCTTATGTTGATGAGGAGTCCTTCACAGGCAGATATTAACA

Biallelic *APOB* knockout in HuH-7 cells = 4/126 clones (3%)

A: ATGCTTCTCCGGGAGATAAGCGACTGGCT----------GTTGATGAGGAGTCCTTCACAGGCAGATATTAACA 11bp del
ATGCTTCTCCGGGAGATAAGCGACTGGCT----------{26bp insert;8bp dup}GTTGATGATGAGCA 11bp del

B: ATGCTTCTCCGGGAGATAAGCGACTGGCTGCCTATC-TATGTTGATGAGGAGTCCTTCACAGGCAGATATTAACA 1bp del
ATGCTTCTCCGGGAGATAAGCGACTGGCTGCCTAT--TATGTTGATGAGGAGTCCTTCACAGGCAGATATTAACA 2bp del

C: ATGCTTCTCCGGGAGATAAGCGACTGGCTGCCTATCTT-GTTGTTGATGAGGAGTCCTTCACAGGCAGATATTAACA 1bp del
ATGCTTCTCCGGGAGATAAGCGACTGGCTGCCTAT-----GTTGATGAGGAGTCCTTCACAGGCAGATATTAACA 5bp del

D: ATGCTTCTCCGGGAGATAAGCGACT--------------TGTTGATGAGGAGTCCTTCACAGGCAGATATTAACA 14bp del
ATGCTTCTCCGGGAGATAAGCGACTGGCTGCCTATCTTA-GTTGATGAGGAGTCCTTCACAGGCAGATATTAACA 1bp del

Monoallelic *APOB* knockout in HuH-7 cells = 4/126 clones (3%)

E: ATGCTTCTCCGGGAGATAAGCGACTGGCT----------GTTGATGAGGAGTCCTTCACAGGCAGATATTAACA 11bp del

F: ATGCTTCTCCGGGAGATAAGCGACTGGCTGCCTA--TTATGTTGATGAGGAGTCCTTCACAGGCAGATATTAACA 2bp del

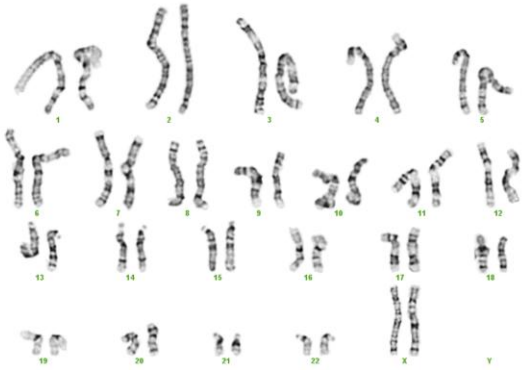
G: ATGCTTCTCCGGGAGATAAG----------TGATGAGGAGTCCTTCACAGGCAGATATTAACA 22bp del

H: ATGCTTCTCCGGGAGATAAGCGACTGGCTGCCTATCTTATATGTTGATGAGGAGTCCTTCACAGGCAGATATTAACA

Other *APOB* targeting in HuH-7 cells = 1/126 clones (1%)

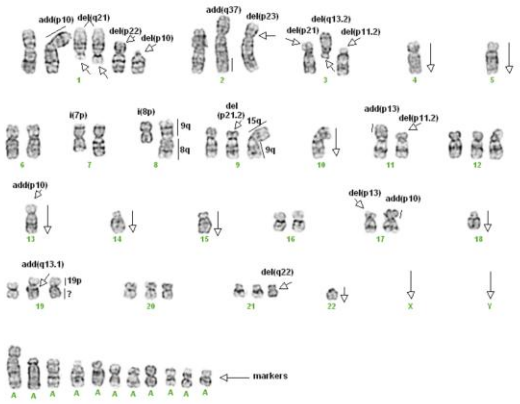
I: ATGCTTCTCCGGGAGATAAGCGACTGGCTGCCTATCTTATTATGTTGATGAGGAGTCCTTCACAGGCAGATATTAACA
ATGCTTCTCCGGGAGATAAGCGACTGGCTGCCTATCTTATTATGTTGATGAGGAGTCCTTCACAGGCAGATATTAACA

HUES 9 wild-type



B

HuH-7



HUES 9 *SORT1*–/–

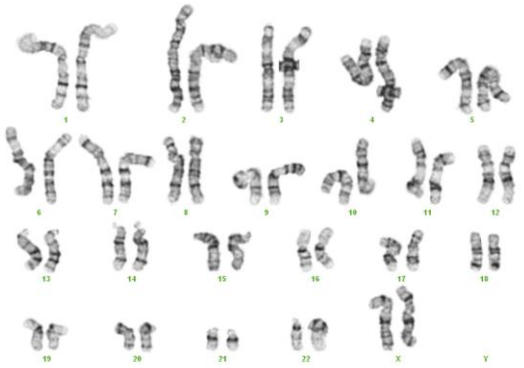


Figure S3.

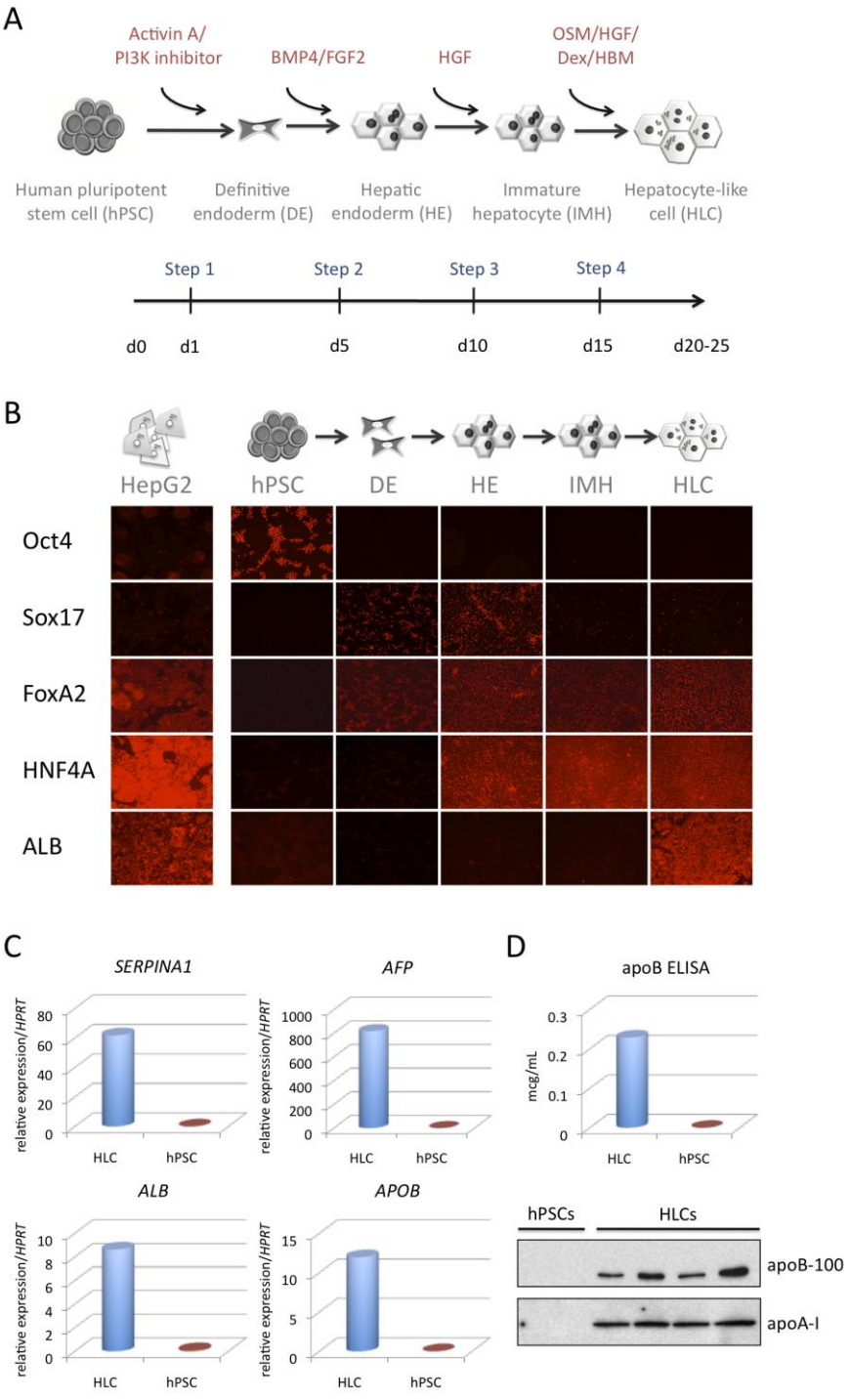

A
Activin A/
PI3K inhibitor
BMP4/FGF2
HGF
OSM/HGF/
Dex/HBM
Human pluripotent stem cell (hPSC)
Definitive endoderm (DE)
Hepatic endoderm (HE)
Immature hepatocyte (IMH)
Hepatocyte-like cell (HLC)
Step 1
Step 2
Step 3
Step 4
d0
d1
d5
d10
d15
d20-25
B
HepG2
hPSC
DE
HE
IMH
HLC
Oct4
Sox17
FoxA2
HNF4A
ALB
C
SERPINA1
AFP
ALB
APOB
relative expression/HPRT
HLC
hPSC
D
apoB ELISA
mcg/mL
hPSCs
HLCs
apoB-100
apoA-I

Figure S4.

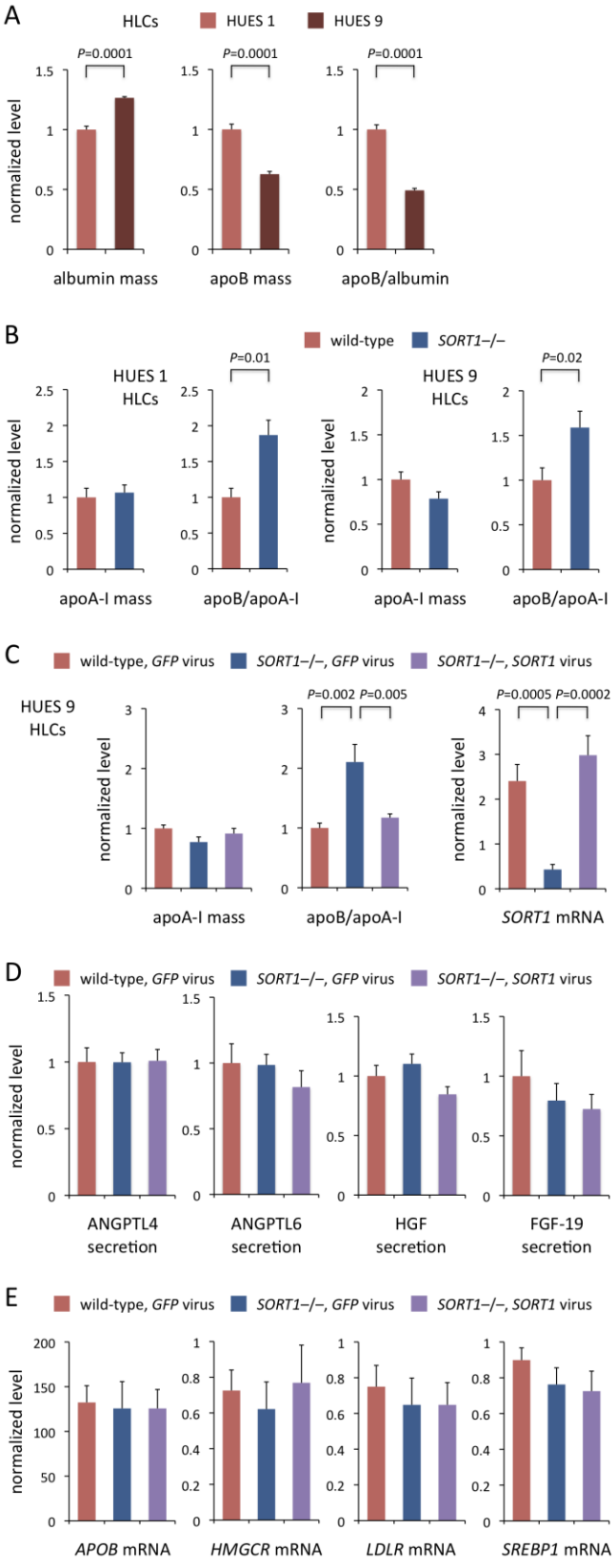

A
HLCs
HUES 1
HUES 9
P=0.0001
P=0.0001
P=0.0001
normalized level
albumin mass
apoB mass
apoB/albumin
B
wild-type
SORT1−/−
HUES 1 HLCs
HUES 9 HLCs
P=0.01
P=0.02
normalized level
apoA-I mass
apoB/apoA-I
apoA-I mass
apoB/apoA-I
C
wild-type, GFP virus
SORT1−/−, GFP virus
SORT1−/−, SORT1 virus
HUES 9 HLCs
P=0.002 P=0.005
P=0.0005 P=0.0002
normalized level
apoA-I mass
apoB/apoA-I
SORT1 mRNA
D
wild-type, GFP virus
SORT1−/−, GFP virus
SORT1−/−, SORT1 virus
normalized level
ANGPTL4 secretion
ANGPTL6 secretion
HGF secretion
FGF-19 secretion
E
wild-type, GFP virus
SORT1−/−, GFP virus
SORT1−/−, SORT1 virus
normalized level
APOB mRNA
HMGCR mRNA
LDLR mRNA
SREBP1 mRNA

Figure S5.

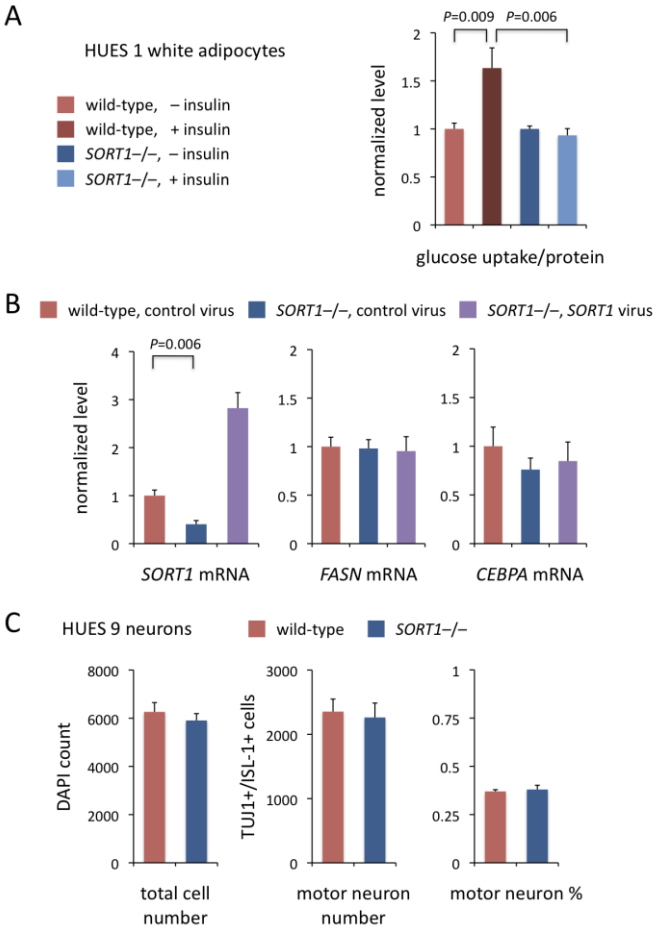

A
HUES 1 white adipocytes
wild-type, – insulin
wild-type, + insulin
SORT1–/–, – insulin
SORT1–/–, + insulin
P=0.009
P=0.006
normalized level
glucose uptake/protein
B
wild-type, control virus
SORT1–/–, control virus
SORT1–/–, SORT1 virus
P=0.006
normalized level
SORT1 mRNA
FASN mRNA
CEBPA mRNA
C
HUES 9 neurons
wild-type
SORT1–/–
DAPI count
TUJ1+/ISL-1+ cells
total cell number
motor neuron number
motor neuron %

Figure S6.

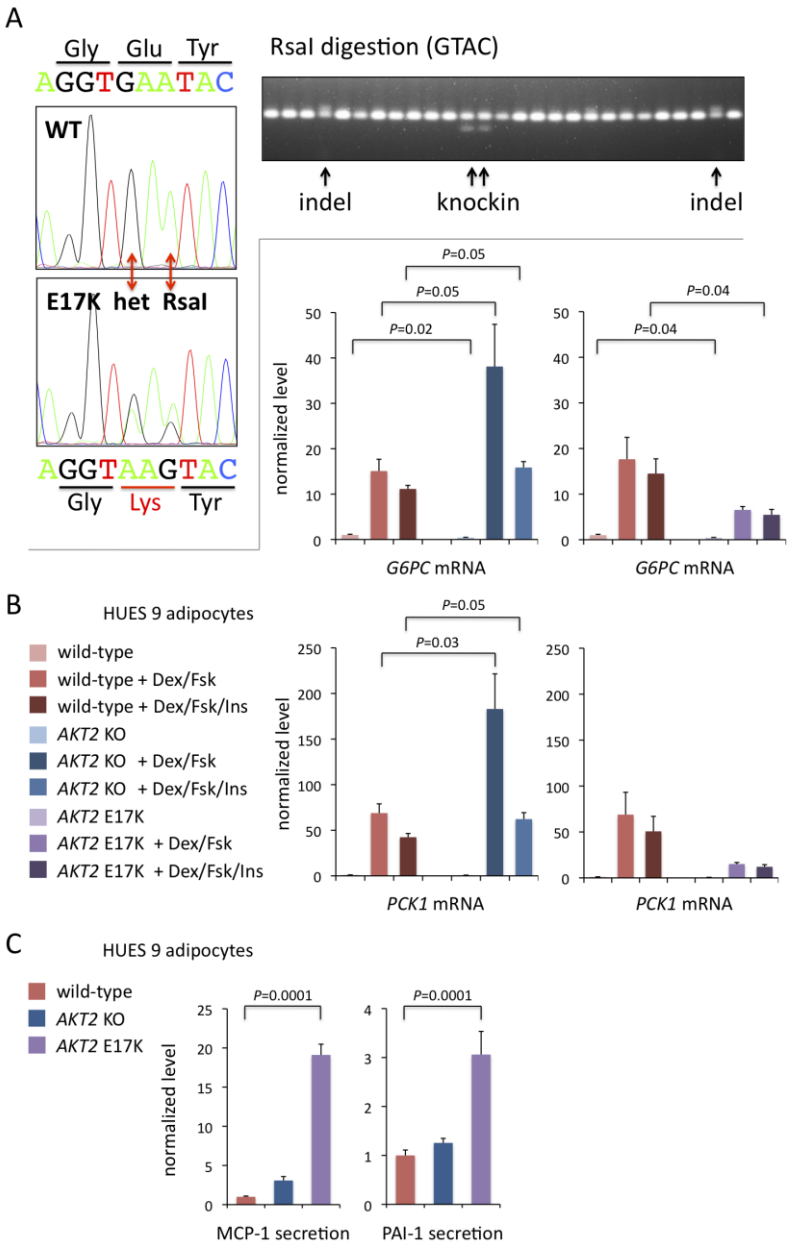
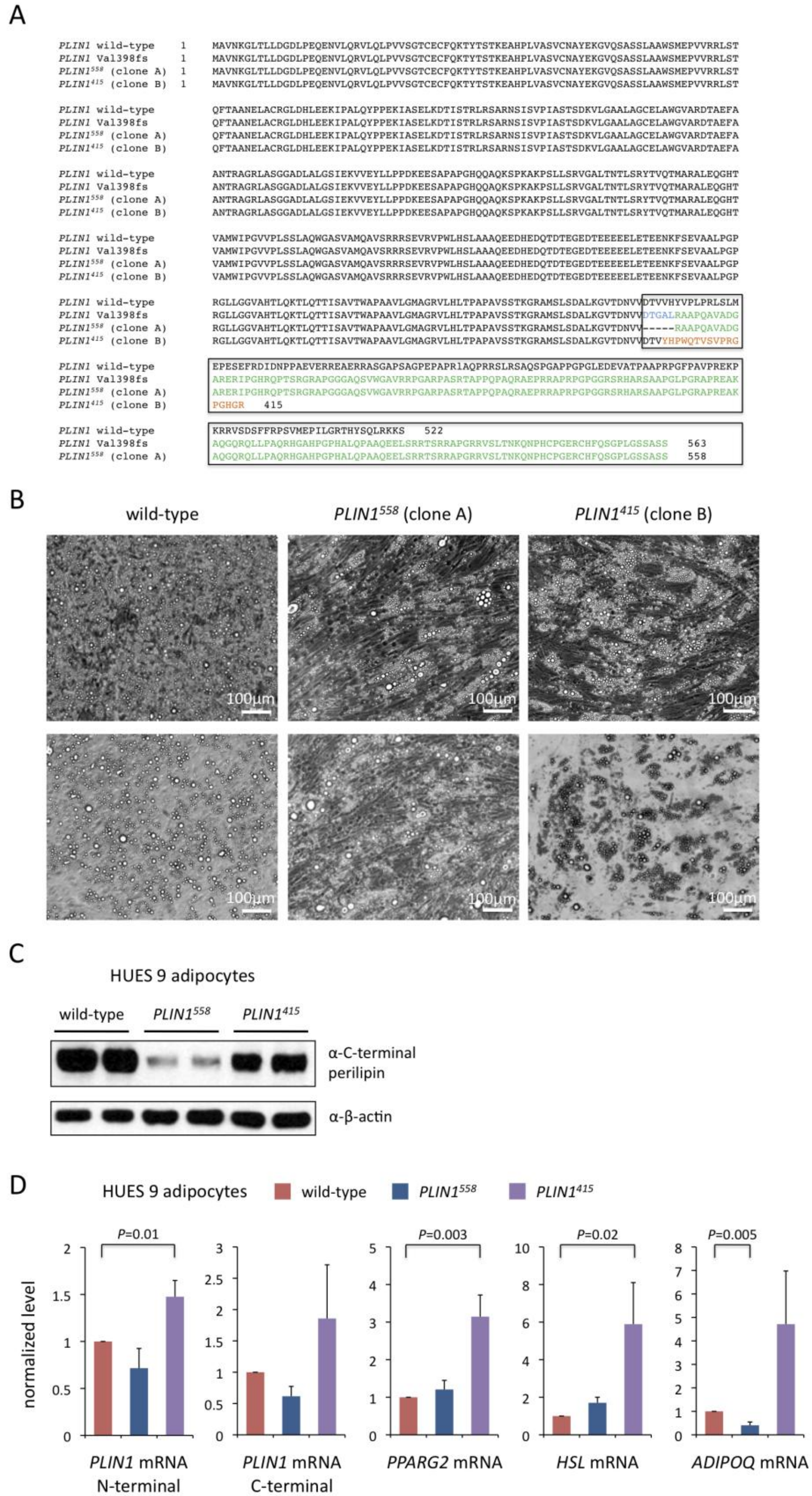

A
Gly Glu Tyr
AGGTGAATAC
WT
E17K het RsaI
AGGTAAGTAC
Gly Lys Tyr
RsaI digestion (GTAC)
indel
knockin
indel
P=0.05
P=0.05
P=0.02
P=0.04
P=0.04
normalized level
G6PC mRNA
G6PC mRNA
B
HUES 9 adipocytes
wild-type
wild-type + Dex/Fsk
wild-type + Dex/Fsk/Ins
AKT2 KO
AKT2 KO + Dex/Fsk
AKT2 KO + Dex/Fsk/Ins
AKT2 E17K
AKT2 E17K + Dex/Fsk
AKT2 E17K + Dex/Fsk/Ins
P=0.05
P=0.03
normalized level
PCK1 mRNA
PCK1 mRNA
C
HUES 9 adipocytes
wild-type
AKT2 KO
AKT2 E17K
P=0.0001
P=0.0001
normalized level
MCP-1 secretion
PAI-1 secretion

Figure S7.



A
PLIN1 wild-type
PLIN1 Val398fs
PLIN1558 (clone A)
PLIN1415 (clone B)
B
wild-type
PLIN1558 (clone A)
PLIN1415 (clone B)
100µm
C
HUES 9 adipocytes
wild-type
PLIN1558
PLIN1415
α-C-terminal perilipin
α-β-actin
D
HUES 9 adipocytes
wild-type
PLIN1558
PLIN1415
normalized level
P=0.01
P=0.003
P=0.02
P=0.005
PLIN1 mRNA N-terminal
PLIN1 mRNA C-terminal
PPARG2 mRNA
HSL mRNA
ADIPOQ mRNA

Supplemental Experimental Procedures

Differentiation of hPSCs into HLCs. Following the protocol of Si-Tayeb et al. (2010), we used feeder cell-free, virus-free differentiation conditions entailing the addition of a variety of growth factors and chemicals to the growth media (Figure S3). We incubated cells in (1) RPMI-B27 (RPMI-1640 from Sigma; B27 supplement from Invitrogen) medium supplemented with recombinant activin A (100 ng/mL; PeproTech) and LY-294002 (5 μM; Promega), a phosphatidylinositol 3-kinase (PI3K) chemical inhibitor, for 4 days to obtain definitive endoderm; (2) RPMI-B27 supplemented with the growth factors BMP4 (20 ng/mL; PeproTech) and FGF2 (5 ng/mL; Millipore) and 0.5% DMSO for 5 days, yielding hepatoblasts; (3) RPMI-B27 supplemented with the growth factor HGF (20 ng/mL; PeproTech) and 0.5% DMSO for 5 days, yielding immature hepatocytes; and (4) HCM Hepatocyte Culture Medium (Lonza) supplemented with HGF (20 ng/mL), Oncostatin M (20 ng/mL; PeproTech), dexamethasone (100 nM; Sigma), and 0.5% DMSO for 10 days, yielding mature HLCs. Infection with lentiviruses expressing *SORT1* or GFP was performed by standard methods at the end of the 3rd stage (day 15) for 2 hours, followed by the 4th stage of differentiation.

Differentiation of hPSCs into white adipocytes. Differentiation of white adipocytes from HUES 1 and HUES 9 was performed as previously described (Ahfeldt et al., 2012). In brief, cells were grown as embryoid bodies, replated, serially passaged to obtain mesenchymal progenitor cells (MPCs), and programmed with inducible *PPARG2* expression to obtain white adipocytes. For *SORT1* reconstitution experiments, *SORT1*–/– MPCs were either co-infected with lentiviruses expressing *PPARG2*, rtTA, and *SORT1* or, for control conditions, with lentiviruses expressing *PPARG2* and rtTA only, followed by adipocyte differentiation.

Differentiation of hPSCs into motor neurons. Differentiation of motor neurons from HUES 9 was performed as previously described (Di Giorgio et al., 2008; Chambers et al., 2009). In brief, cells were grown as embryoid bodies in DMEM/F12 containing 2% B27 and 1% N2 (Life

Technologies). The embryoid bodies were treated with Dorsomorphin and SB 431542 (Stemgent) to induce a neural lineage and, additionally, with retinoic acid (Sigma) and a smoothened agonist (Millipore). After 10 days of embyroid body formation, colonies were plated on poly-ornithine and laminin (PO/LAM)-coated plates to differentiate neural progenitors to motor neurons. After 30 days of differentiation, the cultures were dissociated to single cells with a papain solution (Worthington) and plated on PO/LAM-coated 384-well plates in Neurobasal-containing 2% B27 and 1% N2 (Life Technologies). After 5 additional days of culture, cells were treated with 10 ng/mL of GDNF and BDNF (R&D Systems) or proBDNF (ProSpecBio). After 3 days of treatment, cells were fixed with 4% PFA (Electron Microscopy Sciences) and subjected to immunocytochemistry.

Enzyme-linked immunosorbent assays (ELISAs). For HuH-7 clones, cells were seeded at 50,000 cells/well in 24-well cell culture plates and grown to 80% confluency. Growth medium was added fresh at time = 0 hr and collected at time = 8 hr. The secreted apoB mass was quantitated using an apoB ELISA kit (Mabtech) according to manufacturer instructions. The measurements were expressed as absorbance at 450 nm, adjusted for the background absorbance level.

For HLCs, after 25 days of differentiation, medium was added fresh at time = 0 hr and collected at time = 16 hr, and ELISAs were performed according to manufacturer instructions for apoB and apoA-I (Mabtech); albumin (Bethyl Laboratories); and ANGPTL4, ANGPTL6, HGF, and FGF-19 (Millipore). For adipocytes, after 21 days of differentiation, medium was added fresh at time = 0 hr and collected at time = 12 hr, and ELISAs were performed according to manufacturer instructions for adiponectin, MCP-1, IL-8, and PAI-1 (Millipore).

Immunocytochemistry. HuH-7 clones were plated on glass coverslips in a 24-well plate and allowed to adhere for 48 hr in standard growth medium. The cells were then fixed for 10 min in 4% formaldehyde, washed extensively with PBS, permeabilized with 0.1% Triton X-100 for 10

min, and blocked for 1 hr in 3% BSA in PBS. The cells were then incubated with a 1:1000 dilution of mouse monoclonal anti-apoB (C1.4) antibody (sc-13538; Santa Cruz) at 4°C overnight, washed with PBS, incubated for 1 hr at room temperature with Alexa 488-conjugated anti-mouse secondary antibody (1:500 dilution) (Invitrogen). Hoechst (1:5000 dilution) (Invitrogen) was used for nuclear staining. Cells were then mounted with an anti-fade mounting medium (Vector Laboratories), and immunofluorescence was visualized by confocal microscopy (LSM 710; Zeiss).

For studies of FoxO1 nuclear localization, HLCs after 25 days of differentiation in a 24-well plate were serum starved overnight. The cells were then incubated in DMEM with or without insulin (100 nM) stimulation for 15 min. The cells were then treated as described above, with use of a 1:1000 dilution of rabbit monoclonal anti-FoxO1 antibody (C29H4; Cell Signaling Technology), Alexa 488-conjugated anti-rabbit secondary antibody (1:500 dilution) (Invitrogen), and Hoescht staining. Immunofluorescence was visualized by inverted microscopy (Eclipse T*i*; Nikon), and the NIS-Elements software package (Nikon) was used for image analysis.

For *PLIN1* adipocytes, immunostaining was performed using a 1:200 dilution of anti-perilipin antibody (Novus Biologicals), Alexa 594-conjugated anti-goat secondary antibody (1:1000 dilution) (Invitrogen), BODIPY neutral lipid dye (1:20000 dilution) (Invitrogen), and Hoechst staining. Immunofluorescence was visualized by inverted microscopy (Eclipse T*i*; Nikon), and the NIS-Elements software package (Nikon) was used for image analysis.

For studies of motor neurons, immunostaining was performed with anti-TUJ1 (Covance) and anti-ISL-1 (DSHB) antibodies. Alexa 488- and Alexa 555-conjugated secondary antibodies (Invitrogen) were used to visualize motor neurons, and DAPI was used to mark the nuclei of all cells. Immunostained wells (N = 12 for each condition) were imaged with an Operetta high content imager and analyzed with a Columbus Image Data Storage and Analysis System (PerkinElmer).

Western blot analysis. Western blot analyses were performed using standard methods with antibodies against sortilin (AF3154; R&D Systems), albumin (A80-129A; Bethyl Fisher), beta-actin (A5316; Sigma), AKT2 (L79B2; Cell Signaling Technology), AKT1 (C73H10; Cell Signaling Technology), apoB (sc-13538 from Santa Cruz; ab20737 from Abcam), and perilipin (for N-terminus recognition, GP29 from Progen; for C-terminus recognition, 3470 from Cell Signaling Technology), as well as anti-HCV core 6G7 monoclonal antibody (a kind gift of Drs. Harry Greenberg and Xiaosong He, Stanford University).

Glucose production. Glucose production was measured using a protocol adapted from Hagiwara et al. (2012). Briefly, after 25 days of differentiation, HLCs were serum starved overnight, followed by culturing in low-glucose DMEM containing dexamethasone (100 nM; Sigma) in the presence or absence of insulin (100 nM; Sigma) for 16 hrs. Cells were then incubated with glucose production solution containing sodium pyruvate (2 mM; Sigma), lactate (20 mM; Sigma), dexamethasone (100 nM), and forskolin (10 μM; Sigma) with or without insulin (100 nM). After 12 hr of incubation, the glucose concentration in the media was measured using the Amplex Red Glucose/Glucose Oxidase Assay Kit (Sigma) according to manufacturer instructions. Albumin mass in the media was measured by ELISA (Bethyl Fisher).

Glucose uptake. Glucose uptake was measured as previously described (Ahfeldt et al., 2012). Briefly, white adipocytes after 21 days of differentiation were starved overnight in DMEM supplemented with 0.2% BSA and then incubated in KRH buffer (121 mM NaCl, 4.9 mM KCl, 1.2 mM $MgSO_4$, 0.33 mM $CaCl_2$ and 12 mM HEPES, at pH 7.4) for another 5 hours. Glucose uptake was measured by incubating cells with 0.5 μCi mL^{-1} 2-deoxy-D-[^{3}H]glucose (PerkinElmer) for 5 min after insulin (100 nM) stimulation for 10 min at 37 °C. After three washes with cold PBS, cells were lysed with 0.1% Triton X-100 solution and subjected to scintillation counting. CPM values were normalized to total protein content measured by the Bradford assay (Bio-Rad).

Triglyceride content. Cell triglyceride content was measured using Infinity Triglycerides solution (VWR International). White adipocytes after 16 days of differentiation were lysed using 0.1% SDS solution; 30 μL of cell lysate was mixed with 150 μL Infinity Triglycerides solution, followed by incubation at 37°C for 5 min. Absorbance at 525 nm was measured and normalized to total protein content measured by the Bradford assay (Bio-Rad).

Lipolysis. To measure lipolysis activity, white adipocytes after 24 days of differentiation were starved in DMEM containing 1% FBS for 1 hour, followed by incubation in Hank's Balanced Salt Solution (HBSS) with 2% fatty acid-free BSA alone or with 10 μM forskolin for 1 hour. The culture media was collected for glycerol measurement using the Free Glycerol reagent (Sigma). Absorbance at 540 nm was measured and normalized to total protein content measured by the Bradford assay (Bio-Rad) and total triglyceride content.

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). mRNA was extracted from cells using TRIZOL (Invitrogen) and purified using the RNeasy Mini Kit (QIAGEN). cDNA was generated using the Superscript III RT Kit (Invitrogen) and reverse priming with a 1:1 mixture of random hexamers and oligo-dT. Gene expression was measured in isolates using SYBR Green (Applied Biosystems) and oligonucleotides with the following sequences: *APOB*, 5'-ACCAGCACAGACCATTTCAG-3' and 5'-GCGTAGAGACCCATCACATG-3'; *ADIPOQ*, 5'-GATGAAGTCCTGTCTTGGAAGG-3' and 5'-CAGCACTTAGAGATGGAGTTGG-3'; *G6PC*, 5'-TTCTACGTCTTGTCCTTCTGC-3' and 5'-AACACCGAAGACTCCACATC-3'; *HMGCR*, 5'-CTTTGCATGCTCCTTGAACAC-3' and 5'-CTTTAATGGAAGCAAGTGGTCC-3'; *HSL*, 5'-CTCAGTGTGCTCTCCAAGTG-3' and 5'-CACCCAGGCGGAAGTCTC-3'; *LDLR*, 5'-CACATTTGCCACAACCAGG-3' and 5'-TCTTTGAATAAAACAAGGCCGG-3'; *PCK1*, 5'-AGTGGAGCTCAGAGGATGG-3' and 5'-GCCTCCAAAGATAATGCCTTC-3'; *PLIN1* (N-terminus), 5'-CCCCCTGAAAAGATTGCTTCT-3' and 5'-GGAACGCTGATGCTGTTTCTG-3'; *PLIN1* (C-terminus), 5'-GCCATGTCCCTATCAGATGC-3' and 5' –GTTGTCGATGTCCCGGAATT-3';

PPARG2, 5'-GCAGGAGATCTACAAGGACTTG-3' and 5'-CCCTCAGAATAGTGCAACTGG-3'; *SORT1*, 5'-CAGGAGTGCTCATTGTGAAGA-3' and 5'-TTTATTAGTGTGGGAGGCTGTG-3'; *SREBP1*, 5'-TGCTTCTCTTTGTCTACGGTG-3' and 5'- GCCTGGGCAAAGTCTCC-3'; 18S rRNA, 5'-CGGCTACCACATCCAAGGAA-3' and 5'- GCTGGAATTACCGCGGCT-3'. The last was used as a reference control. Biological replicates were performed, and reactions were carried out using a ViiA 7 System (Applied Biosystems) using standard real-time PCR conditions. Relative quantitation of mRNA levels was performed using the $2^{-\Delta\Delta Ct}$ method.

For measurement of the expression of genes involved in gluconeogenesis (*G6PC*, *PCK1*) in *AKT2* clones, HLCs after 25 days of differentiation were serum starved overnight and then incubated in DMEM with dexamethasone (100 nM) and forskolin (10 μM) with or without insulin (100 nM) for 16 hrs before RNA extraction.

HCV infection of HuH-7 cells. HuH-7 cells were grown in DMEM supplemented with 10% FBS and penicillin/streptomycin. Cells were seeded at 75,000 cells/well in 12-well cell culture plates with 1 mL of media in each well and allowed to attach overnight. The growth media was then replaced with 250 μL of media containing the full-length, genotype 2b, tissue culture-infectious JFH-1 hepatitis C virus for 6 hrs; mock-infected wells were replaced with 250 μL of virus-free media. After 6 hours, the virus media was removed and replaced with fresh DMEM supplemented with 10% lipid-depleted FBS (Gemini Bio-Products) and penicillin-streptomycin. To restore intracellular apoB-100, LDL (Sigma) was added to the media to give a final LDL concentration of 9.68 mg/mL.

Protein and RNA were isolated from cell lysates 72 hrs following infection. RNA was isolated using the RNeasy Mini Kit (QIAGEN) and reverse transcribed into cDNA using the GeneAmp RNA PCR Kit (Applied Biosystems) according to manufacturer instructions. HCV RNA levels were quantitated using the DyNAmo HS SYBR Green qPCR kit (Finnzyme). The

following primers were used: JFH-1 RNA, 5'-CTGTCTTCACGCAGAAAGCG-3' and 5'-TCGCAACCCAACGCTACTCG-3'; and *GAPDH*, 5'-ACCTTCCCCATGGTGTCTGA-3' and 5'-GCTCCTCCTGTTCGACAGTCA-3'. Relative quantitation of viral RNA levels was performed using the $2^{-\Delta\Delta Ct}$ method with *GAPDH* as the reference control.

Exome and whole-genome sequence analyses. Genomic DNA from six hESC clones was extracted using the DNeasy Tissue Kit (QIAGEN) and subjected to quality assessment. For exome sequencing, we followed previously described procedures (Gnirke et al., 2009; Stransky et al., 2011) to generate exome-enriched libraries, which were sequenced to ~100× haploid coverage on Illumina GA-II or HiSeq sequencers as paired-end 76-nucleotide reads as previously described (Stransky et al., 2011). For whole-genome sequencing, genomic DNA was sequenced to between 6× and 12× haploid coverage on Illumina GA-II or HiSeq sequencers as paired-end 101-nucleotide reads as previously described (Stransky et al., 2011). Pre-processing, alignment to the human reference genome (assembly 19), and post-filtering of Illumina sequence data was performed using the Broad Institute Sequencing Platform's "Picard" pipeline and Genome Analysis Toolkit (McKenna et al., 2010), including table recalibration (TableRecalibration), BWA alignment, and local realignment near indels (IndelRealigner).

For the exome sequences, all putative novel indels and SNVs observed in any of the clones exposed to TALENs but not observed in the parental HUES 1 cell line (clone X) were considered. Additionally, for the whole-genome sequences, we identified probable sites of off-target TALEN activity using the TAL monomer–nucleotide association frequency-based matrix presented by Doyle et al. (2012). The scoring function of Doyle et al. summates the negative logarithms of the association probabilities between each of the TAL monomers (for our system, monomers containing HD, NG, NI, NN, or NK) and individual nucleotides [$S = -\Sigma \log(P_{\text{monomer–nucleotide}})$; refer to Supplementary Table 1 of Doyle et al. (2012) for monomer–nucleotide association probabilities] for a given TALEN. A sequence was considered a possible off-target TALEN binding site if its score was within a fixed constant of the on-target sequence's score

($S_{\text{off-target}} < S_{\text{on-target}} + k$). To capture any sequence whose TALEN association probability was greater than 0.5% that of the on-target binding site (i.e., $P_{\text{off-target}}/P_{\text{on-target}} > 0.005$), the score cutoff k was set to log(1/0.005). This yielded a set of 10,056 sequences, which was complemented with 987 additional sequences within 2 substitutions of the on-target binding site but not meeting the association probability cutoff (i.e, $P_{\text{off-target}}/P_{\text{on-target}} < 0.005$). We identified sites matching these sequences (98,905 in total) in the human reference genome using a Python script, and we recorded every potential indel (in any mapped read) within 30 bp downstream of the site. Indels were further considered if they appeared in regions of good coverage (≥4×) in at least one of the clones that was exposed to the *SORT1* exon 2 TALENs (A, B, C, and W), appeared in >2 of the total available sequence reads in the clone, and did not appear in either of the two control clones (clones X and Y). Indels meeting these criteria were manually inspected using the SAMtools Text Alignment Viewer, and any potential indel appearing at a site of highly repetitive DNA was eliminated as being likely to have resulted from sequencing artifact.

Candidate indels and SNVs were confirmed or ruled out with Sanger sequencing of PCR products of 200- to 300-bp length surrounding the putative variants. The primer sets for amplification were designed using the Primer3 program (http://frodo.wi.mit.edu/primer3); internal primers were used for Sanger sequencing.

Supplemental References

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