Supplemental Figure Legends

Figure S1. ACAT expression. (A) Immunoblot analyses of adipocytes and the stromal vascular fraction (SVF) isolated from the adipose tissue of WT and *ob/ob* mice. CD11b is a macrophage marker. Three age-matched pairs of WT and *ob/ob* mice were used for adipocytes. Ponceau S staining was used as a loading control. (B) Immunoblot analysis of WT and the stable mutant of ACAT2 (ACAT2-C277A). (C) Quantification of protein levels in (B). Two-tailed Student's *t* test was used: mean ± S.D.; n = 3, **P < 0.01.

Figure S2. ACAT1/2 overexpression decreased LD size. LD morphology in 3T3-L1 cells stably overexpressing ACAT1/2 on (A) day 8 or (B) day 0 and day 2 after differentiation. Immunofluorescence was carried out with anti-Flag primary antibody on cells expressing Flag-tagged ACAT1/2, but not on EV control cells. Bars = 10 μm. Inlay, Bars = 5 μm. (C) LD morphology in HeLa cells upon ACAT1/2 overexpression. Immunofluorescence was carried out with anti-Flag primary antibody on cells expressing Flag-tagged ACAT1/2, but not on EV control cells. Bars = 10 μm. Inlay, Bars = 1 μm. (D) Distribution of LD sizes in HeLa cells. LDs from ~ 10 cells/each cell type were used.

Figure S3. Endogenous mRNA levels of *Acat1* and *Acat2* in 3T3-L1 cells during differentiation. Two-tailed Student's *t* test was used: mean ± S.D.; n = 3, *P < 0.05.**P < 0.01.

Fig S1

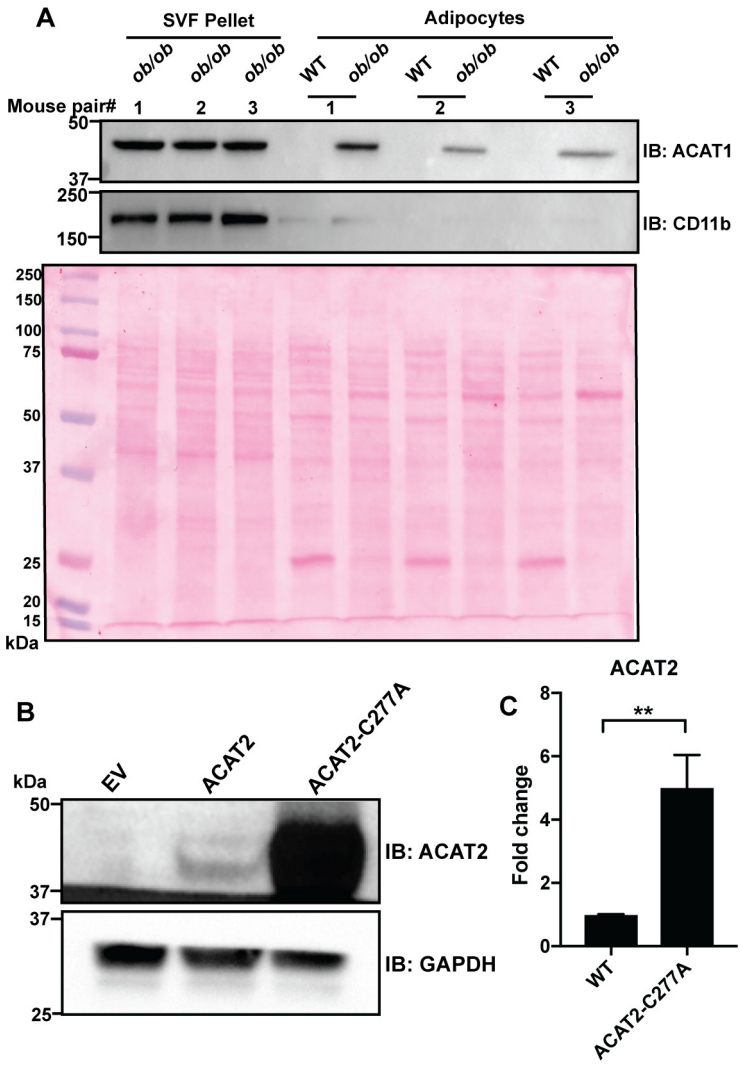
A
SVF Pellet
Adipocytes
ob/ob
ob/ob
ob/ob
WT
ob/ob
WT
ob/ob
WT
ob/ob
Mouse pair#
1
2
3
1
2
3
50
37
250
150
IB: ACAT1
IB: CD11b
250
150
100
75
50
37
25
20
15
kDa
B
kDa
EV
ACAT2
ACAT2-C277A
50
37
37
25
IB: ACAT2
IB: GAPDH
C
ACAT2
**
Fold change
8
6
4
2
0
WT
ACAT2-C277A

Fig S2

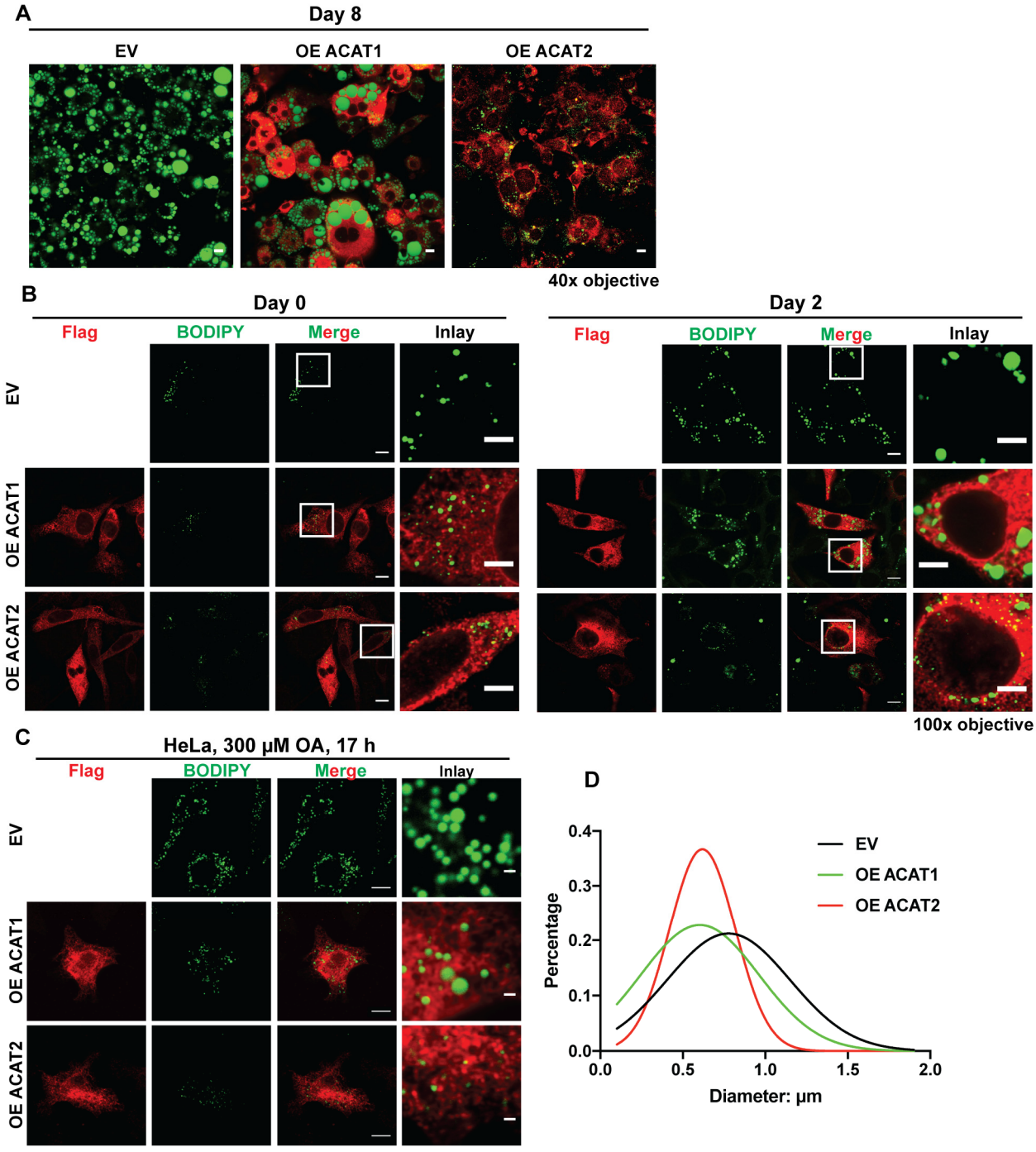
A
Day 8
EV
OE ACAT1
OE ACAT2
40x objective
B
Day 0
Day 2
Flag
BODIPY
Merge
Inlay
EV
OE ACAT1
OE ACAT2
100x objective
C
HeLa, 300 μM OA, 17 h
D
Percentage
Diameter: μm
0.0
0.1
0.2
0.3
0.4
0.5
1.0
1.5
2.0

Fig S3

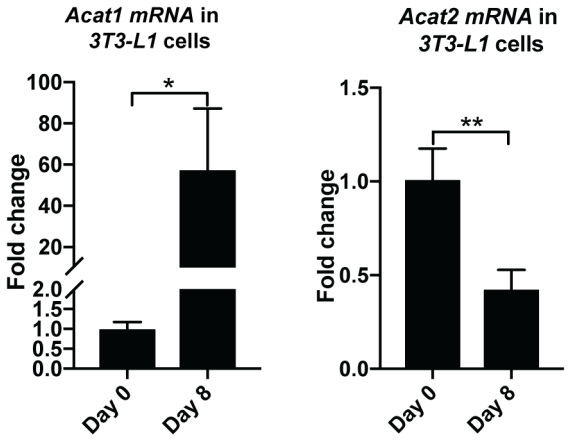
Acat1 mRNA in 3T3-L1 cells
Fold change
100
80
60
40
20
2.0
1.5
1.0
0.5
0.0
*
Day 0
Day 8
Acat2 mRNA in 3T3-L1 cells
Fold change
1.5
1.0
0.5
0.0
**
Day 0
Day 8

Table S1 Primer sequences

Primer	**Sequence**
pBABE-puro-Flag-ACAT1-F	AAAAAGAATTCATGGACTACAAAGATGACGACAAGGTGGGTGAAGAGAAGAT
pBABE-puro-Flag-ACAT1-R	AAAAAGTCGAC CTAAAACACGTAACGACAAGTCC
pBABE-puro-Flag-ACAT2-F	AAAAAGAATTCATGGACTACAAAGACGATGACGACAAGGAGCCAGGCGG
pBABE-puro-Flag-ACAT2-R	AAAAAGTCGAC CTAGGTATGGCAGGACCAAG
RRL-Flag-ACAT1-F	AAAAAGTTAACATGGACTACAAAGACGATGACGACAAGAAGGAAGTTGGCAGTCA
RRL-Flag-ACAT1-R	AAAAATCTAGA CTAAAACACGTAACGACAAGTCC
RRL-Flag-ACAT2-F	AAAAAGTTAACATGGACTACAAAGACGATGACGACAAGGAGCCAGGCGG
RRL-Flag-ACAT2-R	AAAAATCTAGA CTAGGTATGGCAGGACCAAG

Table S2 Mutant construct primers

Primer	**Sequence**
ACAT1 H460A_F	TTTGCTGTATCTGCTGTAGTAGCAGAATATGCCTTGGCTG TTTGC
ACAT1 H460A_R	GCAAACAGCCAAGGCATATTCTGCTACTACAGCAGATAC AGCAAA
ACAT2 H360A_F	CTCATCTTCTTTGCCTTCCTCGCTTGCTGGCTCAACGCCTT TGCC
ACAT2 H360A_R	GGCAAAGGCGTTGAGCCAGCAAGCGAGGAAGGCAAAGA AGATGAG
ACAT2 C277A_F	AGCTACCTCTACTTCCTCTTCGCTCCAACACTCATCTACAG GGAG
ACAT2 C277A_R	CTCCCTGTAGATGAGTGTTGGAGCGAAGAGGAAGTAGAG GTAGCT

Table S3 qRT-PCR primers

Gene	Sequence
36B4	F-5' CCCACTTACTGAAAAGGTCA R-5' TTAGTCGAAGAGACCGAATC
Mus-PPARγ	F-5' ACCACAGTTGATTTCTCCAG R-5' TAGAGCTGGGTCTTTTCAGA
Mus-aP2	F-5' ACATGAAAGAAGTGGGAGTG R-5' GGTTATGATGCTCTTCACCT
Mus-c/EBPα	F-5' CAAGAACAGCAACGAGTACC R-5' TTGACCAAGGAGCTCTCAG
Mus-Pref1	F-5' CCATGAAAGAGCTCAACAAG R-5' TACTGCAACAGGAGGTTCTT
Homo-ACAT1	F-5' GAAGTTGGCAGTCACTTTGATGA R-5' GAGCGCACCCACCATTATCTA
Homo-ACAT2	F-5' ATGGAAACACTGAGACGCACA R-5' GGTAGGATTGTATAGCCTCCCG
Mus-Acat1	F-5' GAAACCGGCTGTCAAAATCTGG R-5' TGTGACCATTTCTGTATGTGTCC
Mus-Acat2	F-5' ACAAGACAGACCTCTTCCCTC R-5' ATGGTTCGGAAATGTTGCACC
Mus-Hmgcr	F-5' ACTCTTGACGCTCTTGTGGA R-5' CGAAGCAGCACATGATCTCC
Mus-Dgat1	F-5' TCCGTCCAGGGTGGTAGTG R-5' TGAACAAAGAATCTTGCAGACGA
Mus-Dgat2	F-5' GCGCTACTTCCGAGACTACTT R-5' GGGCCTTATGCCAGGAAACT

Table S4 Relative ΔCT values of *Acat1/2* in adipocytes comparing to that of *Dgat1/2*

	ΔCT value			
	Dgat1	*Dgat2*	*Acat1*	*Acat2*
Day 0	6.04±0.12	7.37±0.37	10.33±0.22	10.53±0.25
Day 8	1.26±0.15	-0.45±0.09	4.6±0.73	11.78±0.33