Cell Reports, Volume *17*

Supplemental Information

SEIPIN Regulates Lipid Droplet Expansion and Adipocyte Development by Modulating the Activity of Glycerol-3-phosphate Acyltransferase

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SUPPLEMENTAL FIGURES

Figure S1. Related to Figures 1 and 2.

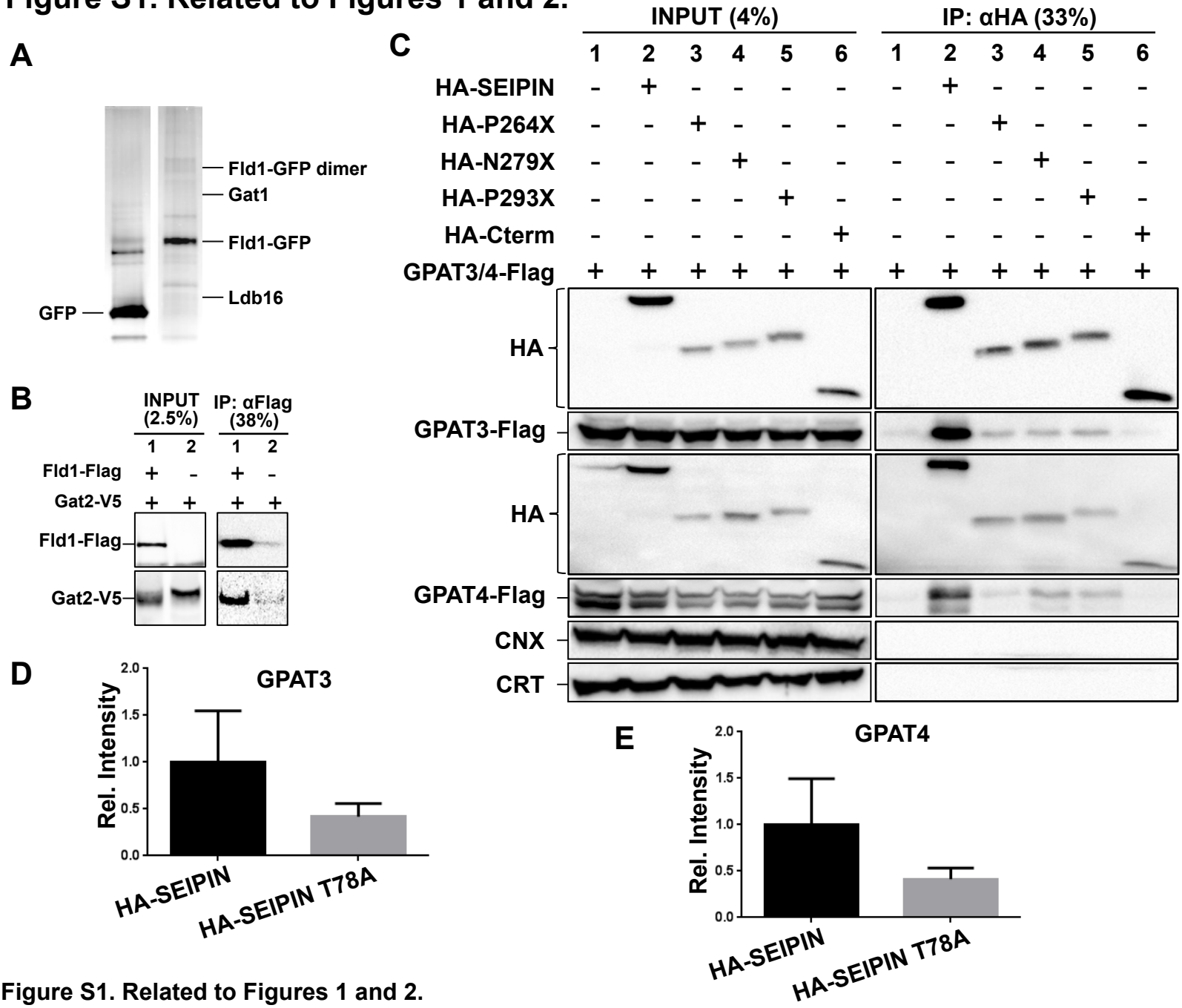


Figure S1. Related to Figures 1 and 2.

A) Coomassie stained SDS-PAGE gel showing yeast proteins that co-eluted with GFP or Fld1-GFP after GFP-Trap affinity purification. See also Table S1. B) Coimmunoprecipitation of endogenous levels of Gat2-V5 and FLAG-Fld1 by Flag antibody from yeast lysates. C) Co-immunoprecipitation analyses of Flag-tagged GPAT3/ GPAT4 with a series of overexpressed HA-tagged C-terminal SEIPIN truncations, and an HA-tagged SEIPIN C-terminus (HA-Cterm). D, E) Western blotting quantification. Relative protein quantification levels for D) Figure 2B (n=3, GPAT3-Flag, co-immunoprecipitated with HA-SEIPIN relative to co-immunoprecipitated with HA-T78A), (E) Figure 2C (n=3, GPAT4-Flag, co-immunoprecipitated with HA-SEIPIN relative to co-immunoprecipitated with HA-T78A). *$p<0.05$, paired t-test, data are shown as mean ± SEM.

Figure S2. Related to Figure 2.

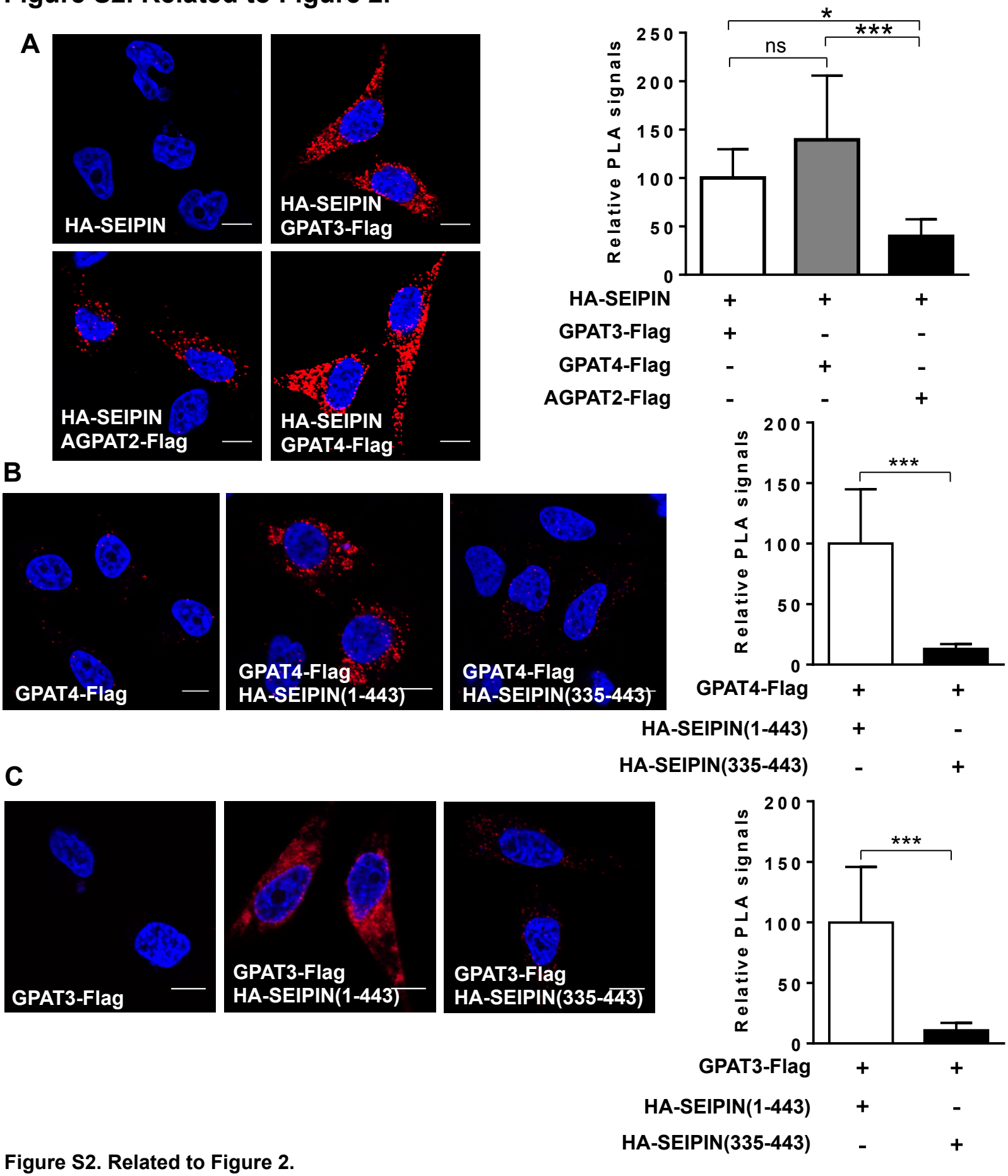
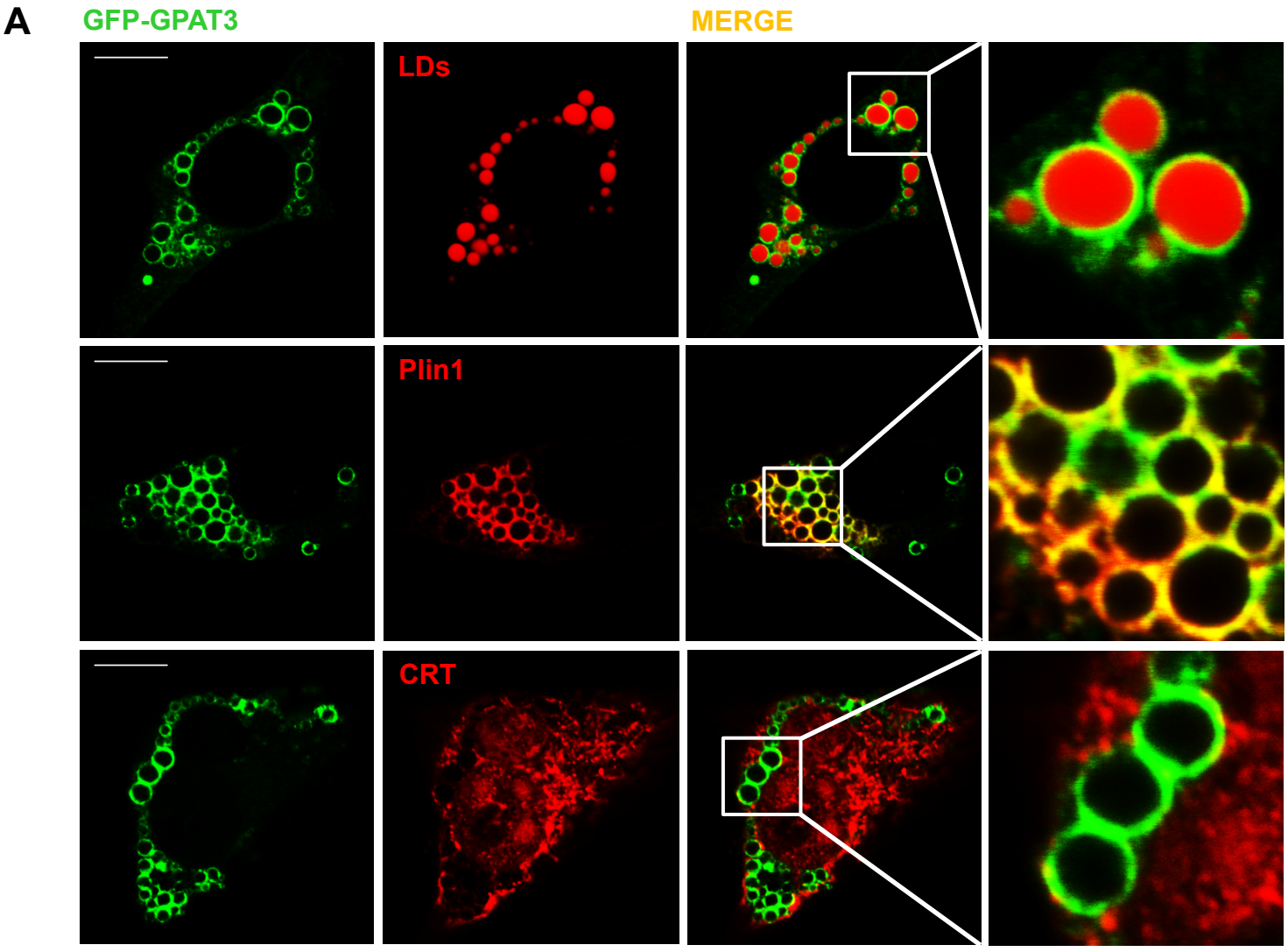


Figure S2. Related to Figure 2.

A, B, C) Proximity ligation assay (PLA) of the indicated full-length HA-SEIPIN, HA-SEIPIN truncation (1-443), and HA-tagged C-terminus of SEIPIN (335-443), respectively, and Flag-tagged GPAT4 or GPAT3 or AGPAT2 pairs, respectively, in HeLa cells. Representative images (n=3) are shown. Scale bar: 10 μm. Quantification of PLA assay is described in Experimental procedures. Means ±SD of PLA signal per cell are shown (*p < 0.05; ***p < 0.001), one-way ANOVA and student's T-test, n=3.

Figure S3. Related to Figure 2.



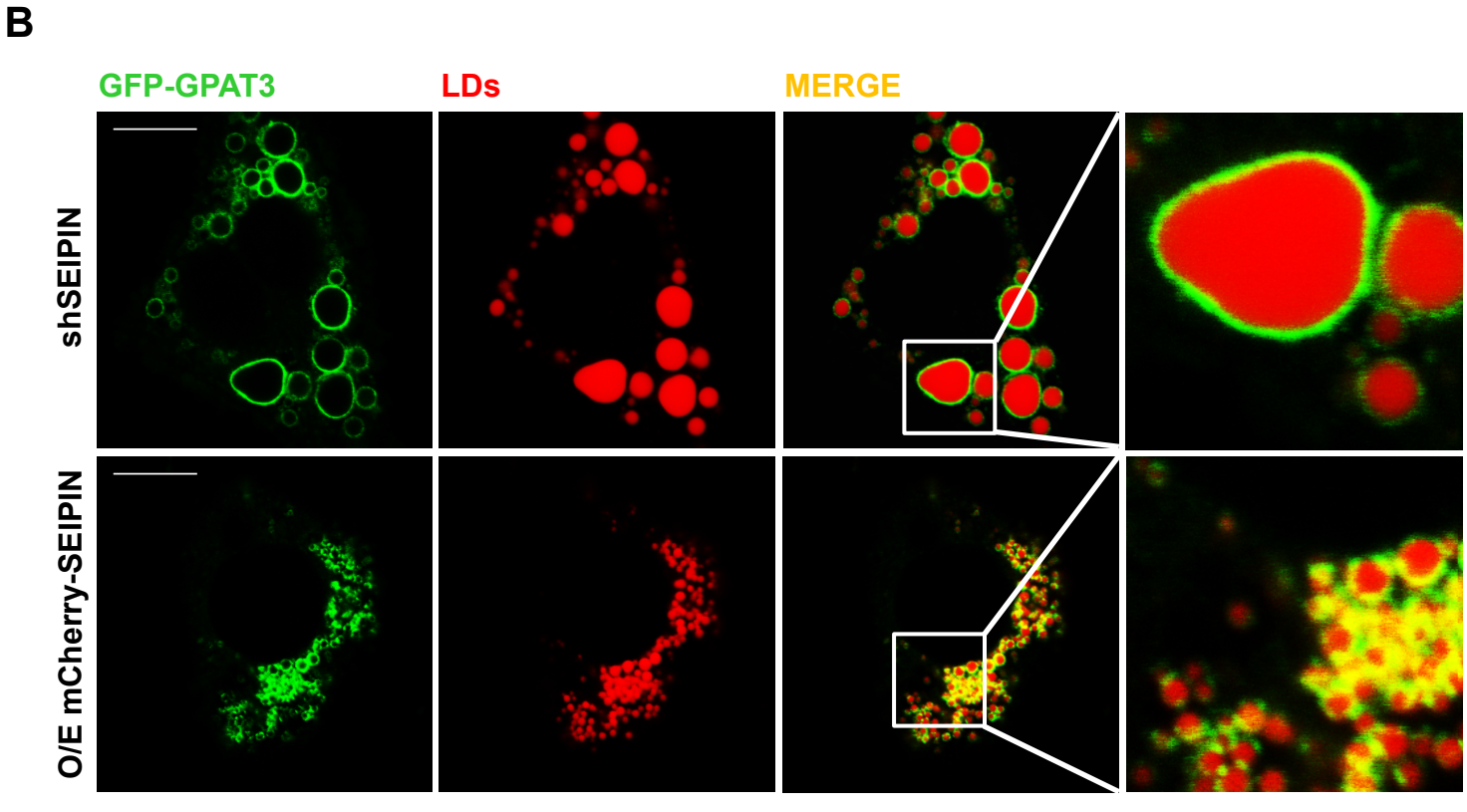
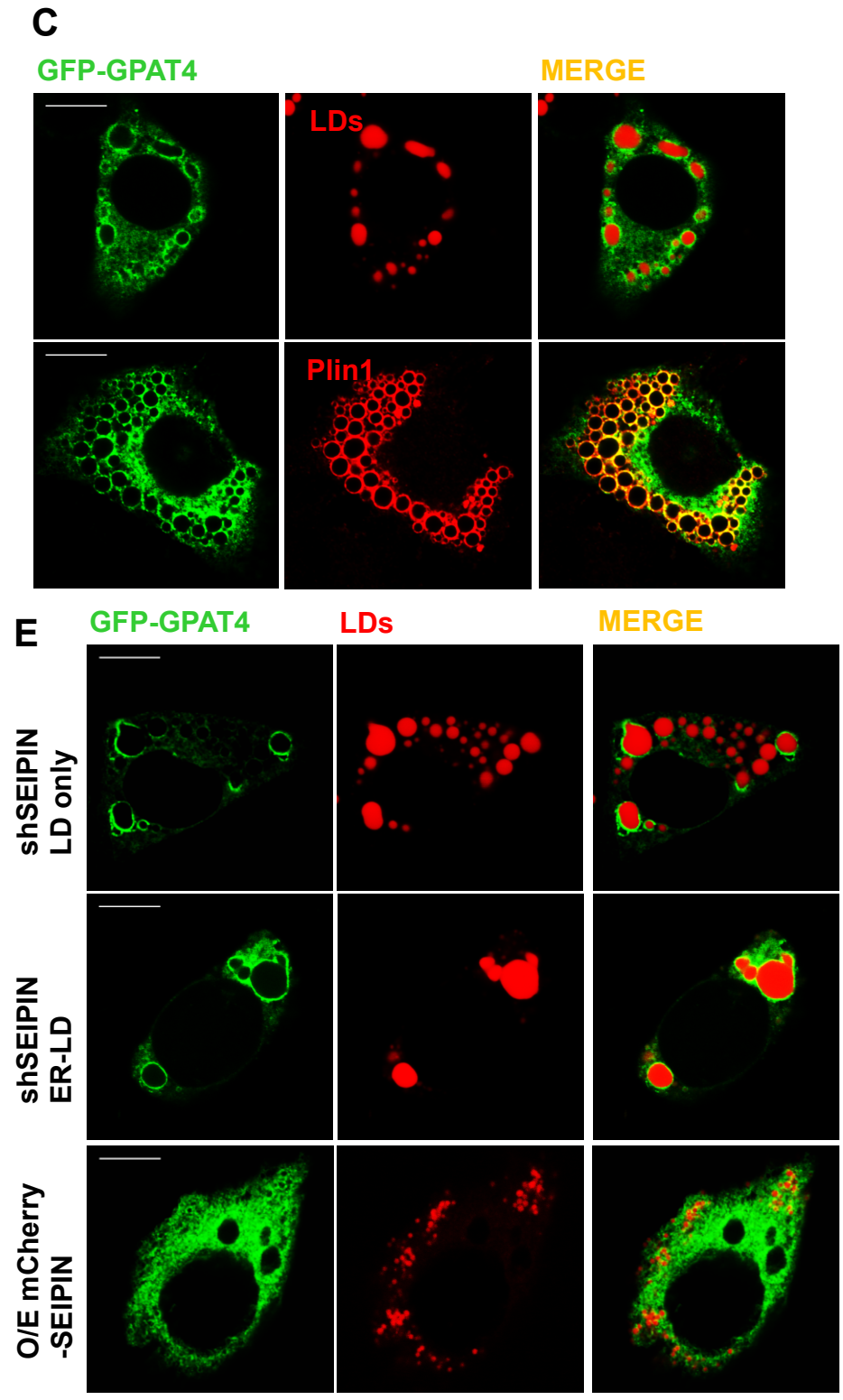
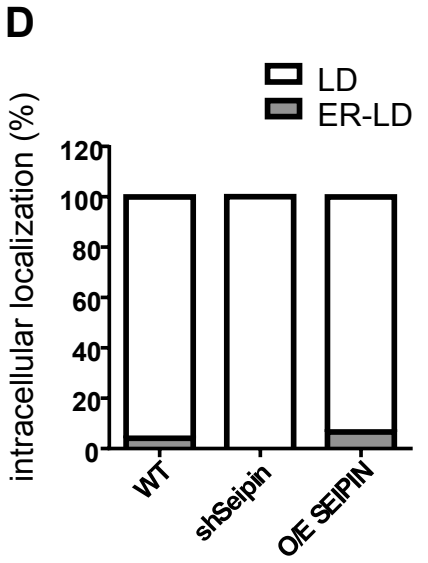


Figure S3 (continued). Related to Figure 2.





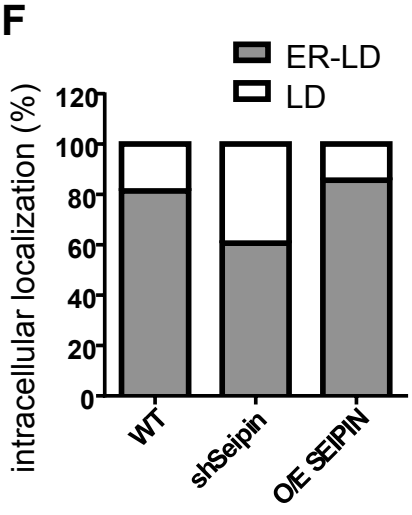


Figure S3. Related to Figure 2.

Variations of SEIPIN expression alter subcellular localization of GPAT4, but not GPAT3, in preadipocytes. Co-localization of GPAT3 (A) with ER marker CRT (calreticulin) and LD surface protein Plin1 (Perilipin 1) and of GPAT4 (C) with Plin1. 3T3-L1 preadipocytes were co-transfected with FLAG-Plin1 and GFP-GPAT3/GFP-GPAT4 for 48h, followed by incubation with 400 μM oleate for 16h. FLAG-Plin1 and CRT were labelled by immunofluorescence staining using FLAG and CRT antisera, respectively. LDs were stained using LipidTox. B, E) Localization of GPAT3 (B) and GPAT4 (E) was also examined in shSEIPIN (SEIPIN knockdown) or O/E mCherry-SEIPIN (overexpressed mCherry-tagged SEIPIN) 3T3-L1 preadipocytes in the presence of LipidTox stained LDs. 3T3-L1 preadipocytes with lentiviral-based stable knockdown of SEIPIN or transiently transfected with mCherry-SEIPIN were co-transfected with either GFP-GPAT3 or GFP-GPAT4, followed by incubation with 400 μM oleate for 16h. Images were taken using confocal microscope. Bar = 10 μm. D, F) The percentage of cells with different intracellular localization patterns, either LD only or both ER and LD, was quantified from more than 25 images in each group setting.

Figure S4. Related to Figure 3.

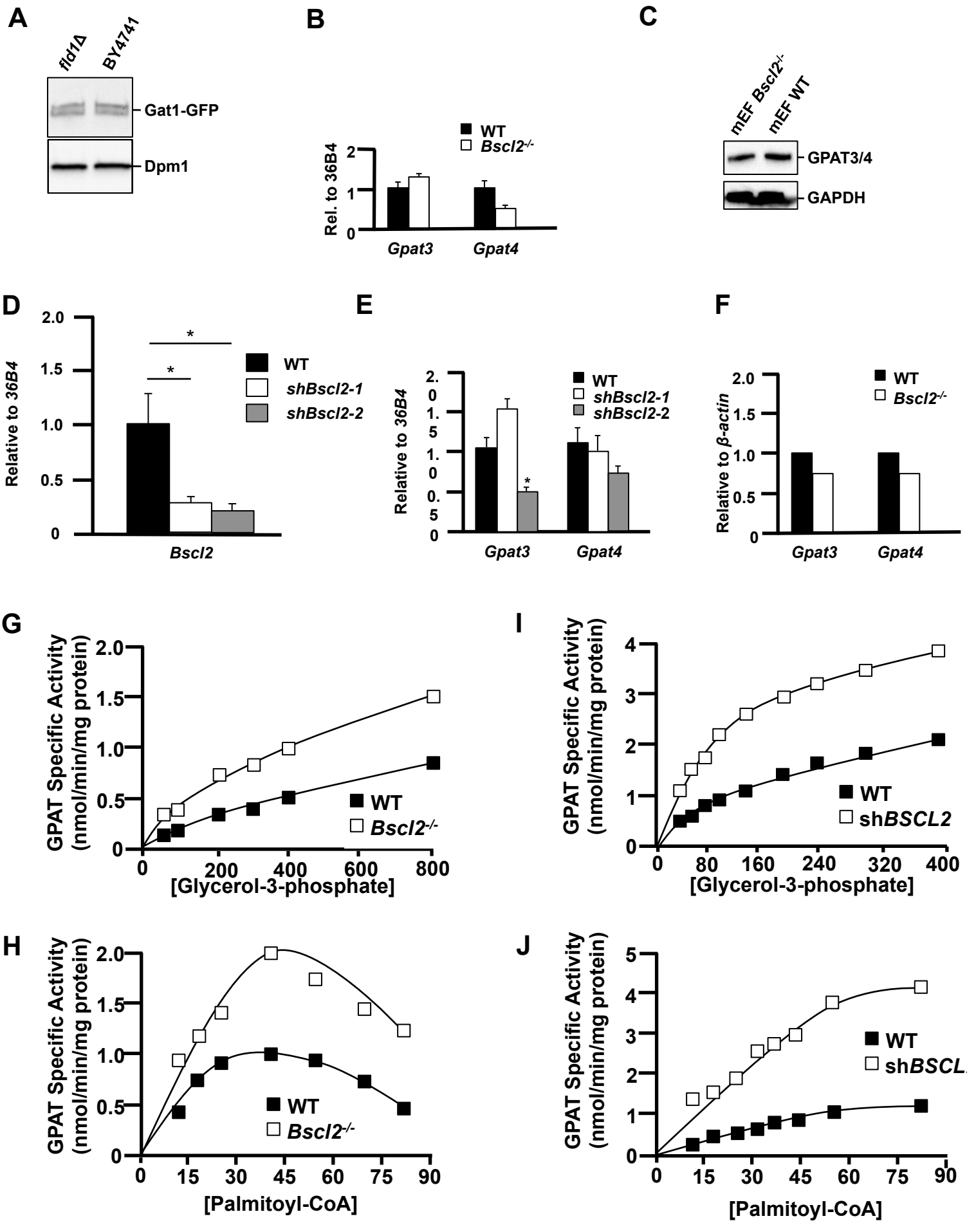


Figure S4 (continued). Related to Figure 3.

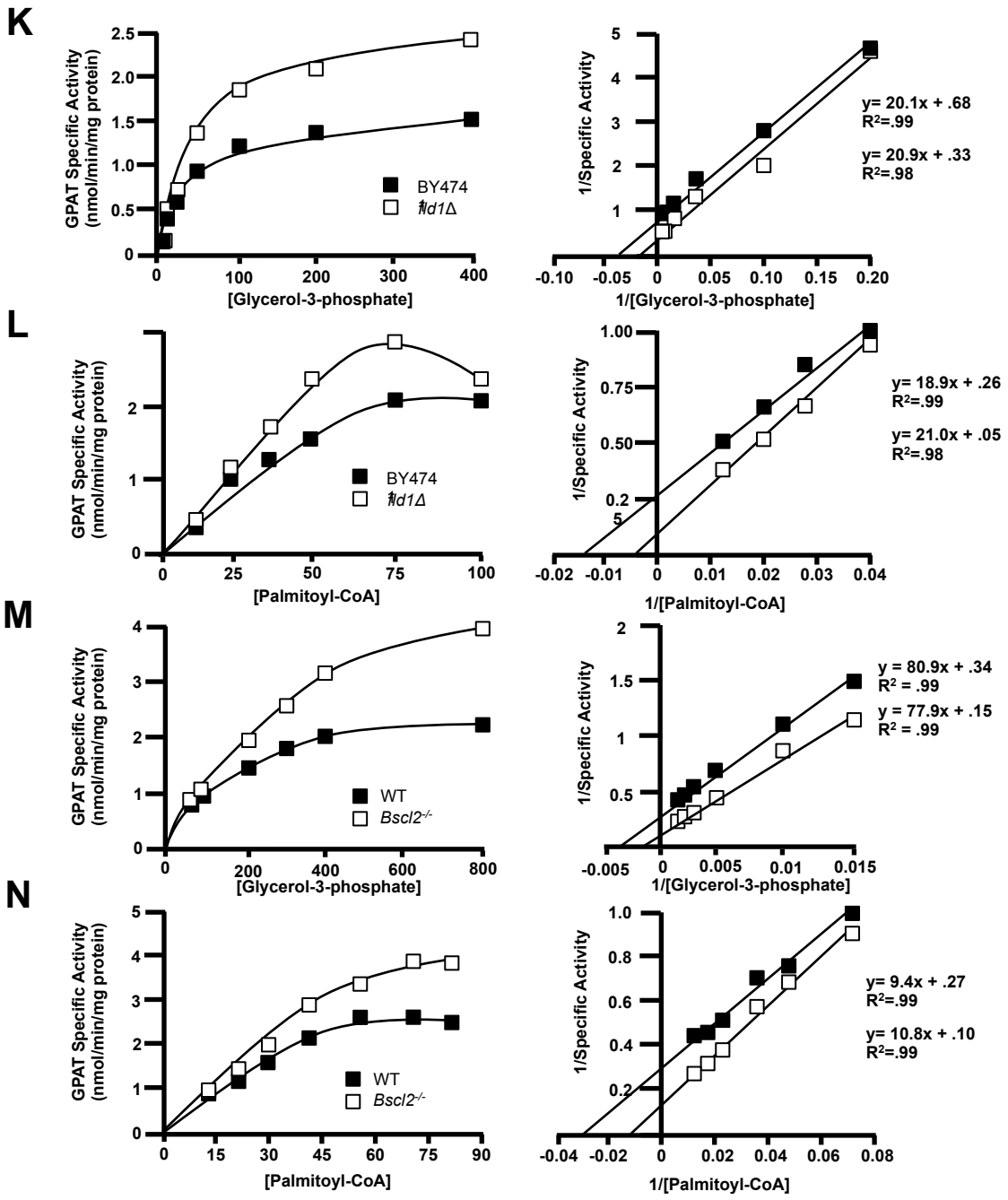


Figure S4. Related to Figure 3.

A) Immunoblot for Gat1-GFP in BY4741 and *fld1*Δ yeast. B) mRNA and C) protein levels of GPAT3/ GPAT4 in control and *Bscl2*$^{-/-}$ MEFs. D) *Bscl2* mRNA and E) *Gpat3* and *Gpat4* mRNA expression in control and *shBscl2*-infected 3T3-L1 preadipocytes. F) mRNA of *Gpat3* and *Gpat4* in control and *Bscl2*$^{-/-}$ testis. mRNA expression normalized to *36B4* or β-actin as indicated. Dpm1 and GAPDH used as protein loading controls as indicated. Data are presented as mean ± S.E.M. GPAT dependence on glycerol-3-phosphate or Palmitoyl-CoA in G, H) control and *Bscl2*$^{-/-}$ testis, I, J) control and *shBSCL2* 3T3-L1 preadipocytes. Glycerol-3-phosphate and Palmitoyl-CoA concentrations were varied as indicated. All experiments were performed in triplicate. GPAT dependence on glycerol-3-phosphate or Palmitoyl-CoA and double-reciprocal plots in K, L) BY4741 and *fld1*Δ yeast, M, N) control and *Bscl2*$^{-/-}$ MEFs. All experiments were performed in triplicate. Data are presented as mean ± S.E.M.

Figure S5. Related to Figure 4.

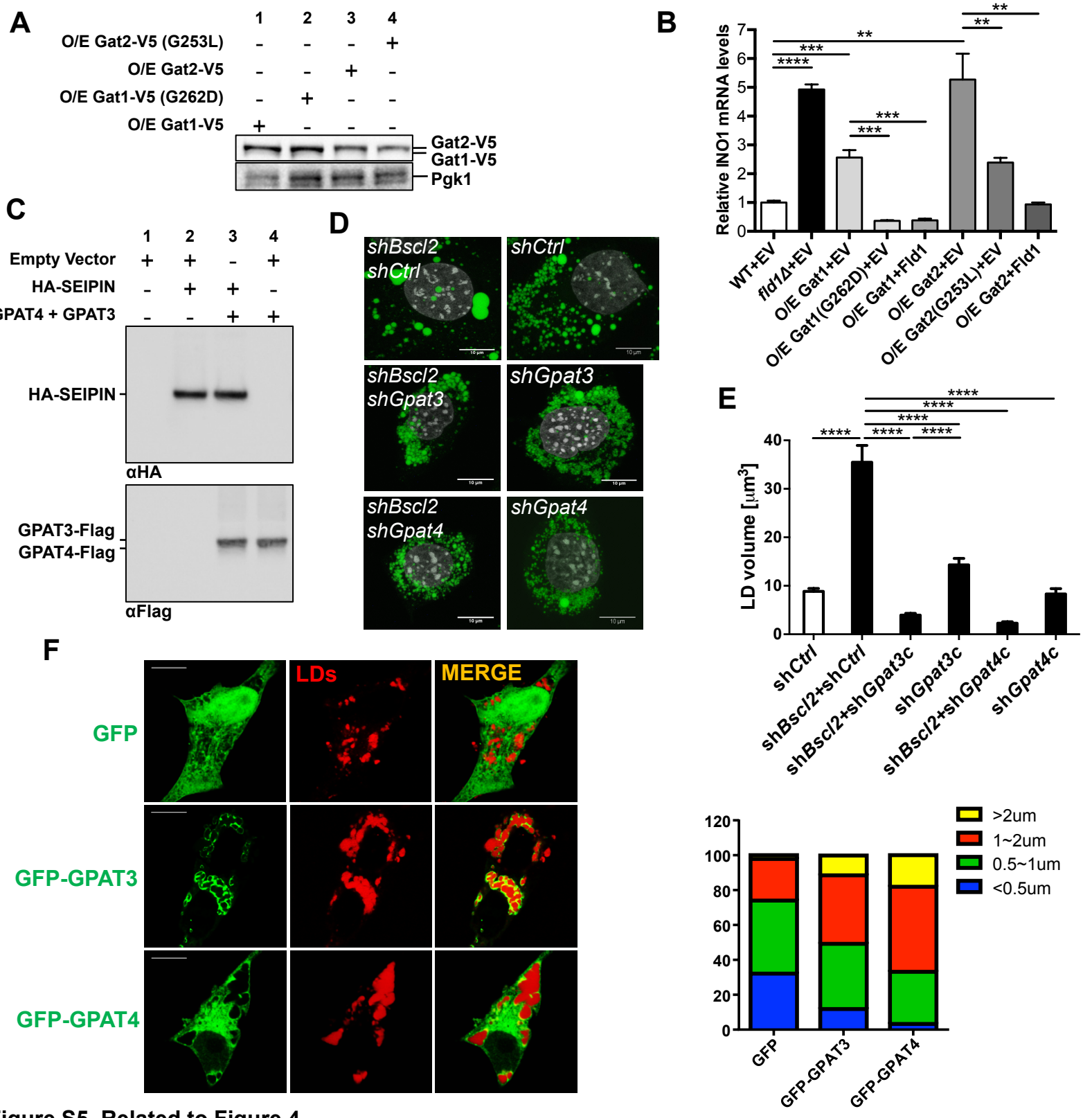
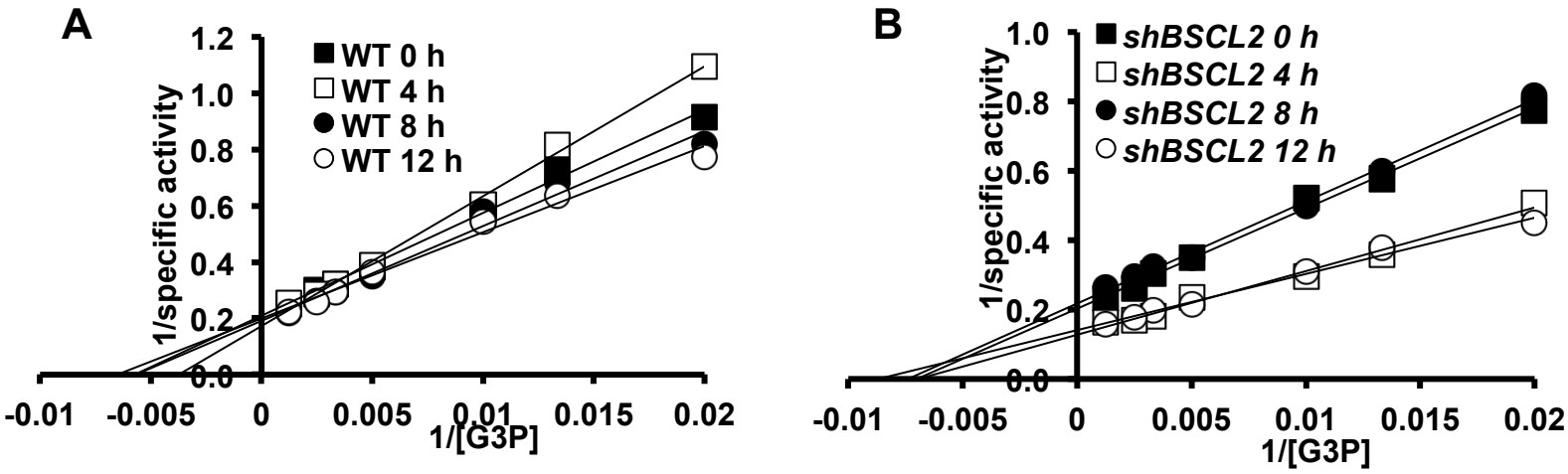


Figure S5. Related to Figure 4.

A) Protein level comparison of Gat1-V5 vs. Gat1-V5 (G262D), and Gat2-V5 vs. Gat2-V5 (G253L) by anti-V5 Western blot analysis. B) qRT-PCR analysis of *INO1* mRNA levels in yeast strains (shown in Figure 4A) grown to exponential growth phase. *INO1* mRNA levels were normalized with *TCM1* mRNA levels. Data represent mean ± SD, significance was determined by one-way ANOVA multiple-comparison analysis (**p < 0.001, ***p < 0.0005, ****p < 0.0001). C) Immunoblots for HA-SEIPIN, Flag-tagged GPAT3 and GPAT4 in 3T3-L1 preadipocytes. D) BODIPY-stained LDs in 3T3-L1 preadipocytes, transduced with shRNA-mediated knock-down vectors and incubated with 800 μM oleate for 16 h. Representative confocal images are shown. E) Diameters of top 3 largest lipid droplets in 100 of each cell line were measured to determine the droplet volumes. Data represent mean ± SD (****p < 0.0001). F) Confocal microscopy images of Huh7 cells overexpressing GFP-GPAT3, GFP-GPAT4, or GFP alone. LD diameter quantifications from GFP, GFP-GPAT3, and GFP-GPAT4 groups, respectively.

Figure S6. Related to Figure 6.



C

	0 h	4 h	8 h	12 h
WT	217 ± 46 μM	302 ± 68 μM	179 ± 18 μM	177 ± 36 μM
sh*Bscl2*	118 ± 9 μM	195 ± 37 μM	164 ± 19 μM	140 ± 22 μM

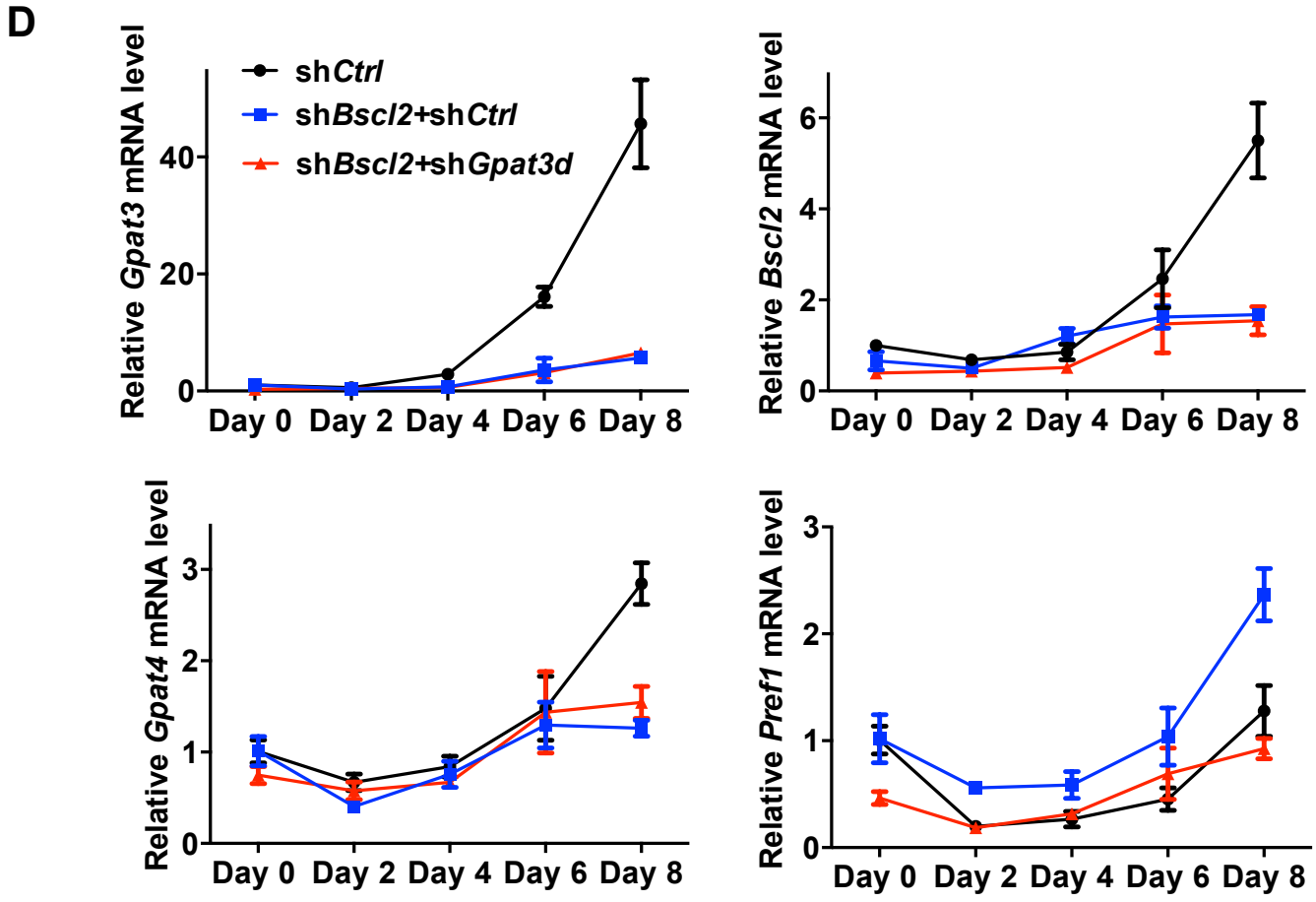


FIGURE S5. Related to Figure 6.
A, B) Control and *shBSCL2* 3T3L1 preadipocytes were differentiated for 0, 4, 8, or 12 h, and GPAT dependence on glycerol-3-phosphate was determined. Data are presented as double-reciprocal plots. n=3 from independent cell passages and differentiation time courses. C) Summary table of apparent Km values for glycerol-3-P. D) *Gpat3, Gpat4, Bscl2,* and *Pref1* mRNA levels were measured by qRT-PCR in 3T3-L1 cells transduced with the indicated lentiviral knock-down vectors at the indicated time-points post-differentiation induction (see Figure 6D).

Figure S7. Related to Figure 6.

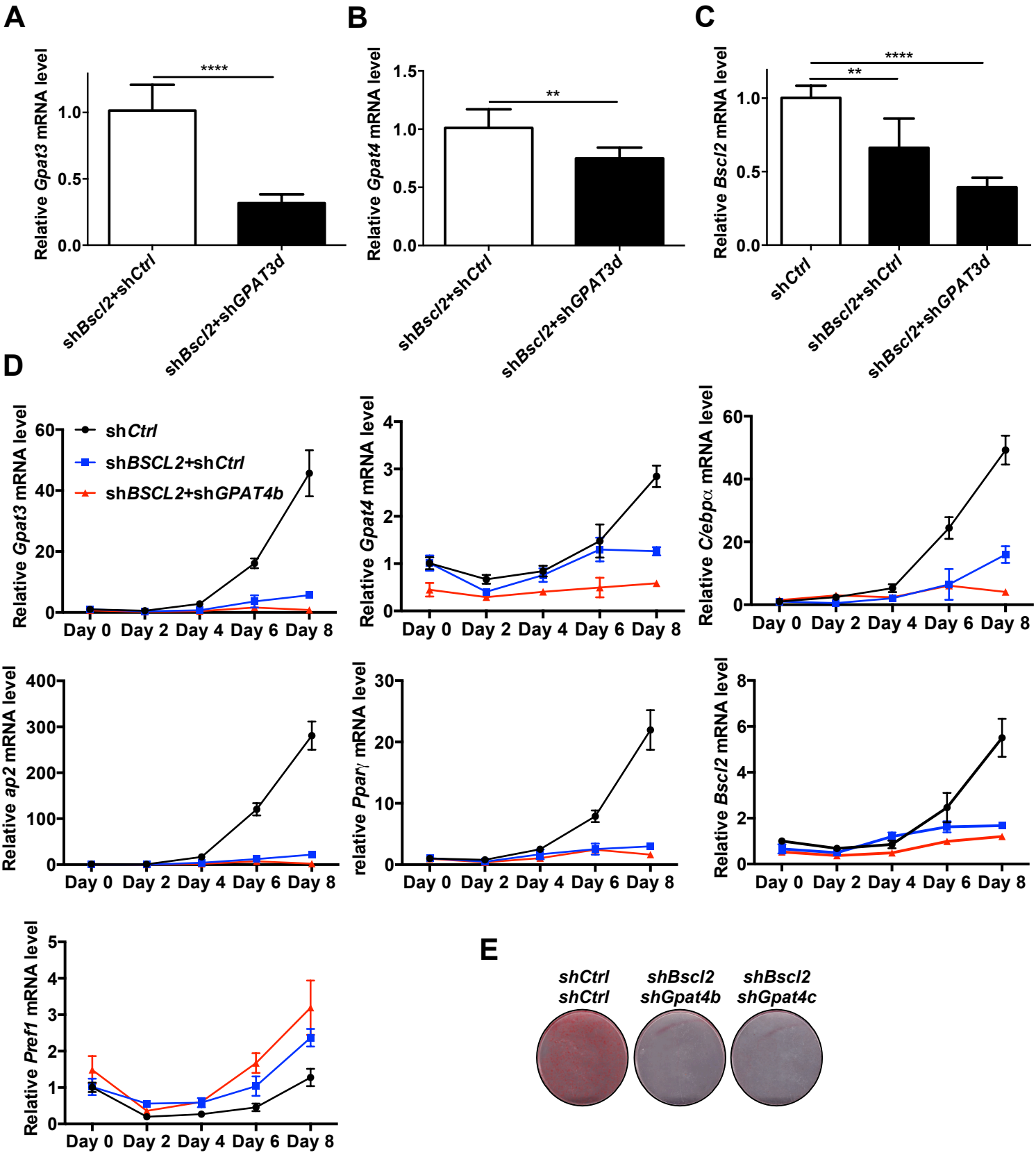


Figure S7 (continued). Related to Figure 6.

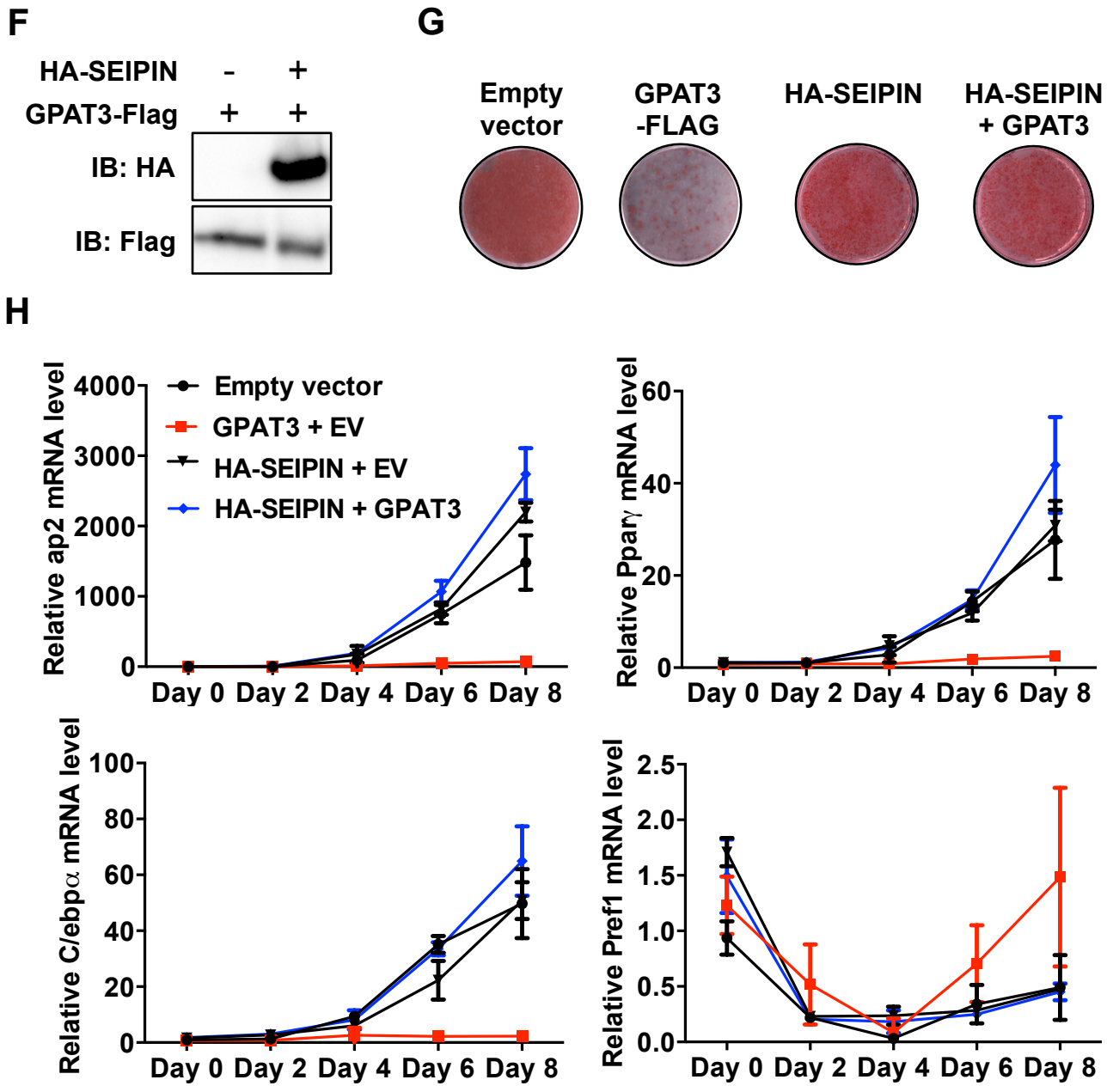


FIGURE S7. Related to Figure 6.

A-C) mRNA levels of *Gpat3*, *Gpat4* and *Bscl2* in 3T3-L1 cells used for the differentiation experiments described in Figure 6. mRNA expression normalized to *36B4*. Data are presented as mean ± SD (**p < 0.001, ****p < 0.0001), one-way ANOVA test.

D) Indicated mRNA levels were measured by qRT-PCR in 3T3L1 cells transduced with the indicated double *shRNA* vectors at the indicated time-points after induction of differentiation. E) Oil red O stains of differentiated/undifferentiated 3T3-L1 cells stably transfected with the indicated lentiviral knock-down vectors. F) Immunoblots for HA-SEIPIN and Flag-tagged GPAT3 in 3T3-L1 preadipocytes used for differentiation experiments. G) Oil red O staining. 3T3-L1 preadipocytes were stably transfected with pBABE-puro empty vector, GPAT3-Flag and/or HA-SEIPIN, then differentiated for 8 days. H) Same as in Figure S7D), except that 3T3L1 cells were transduced with with the indicated lentiviral expression vectors. EV: Empty Vector control.

Figure S8: Model of SEIPIN Function

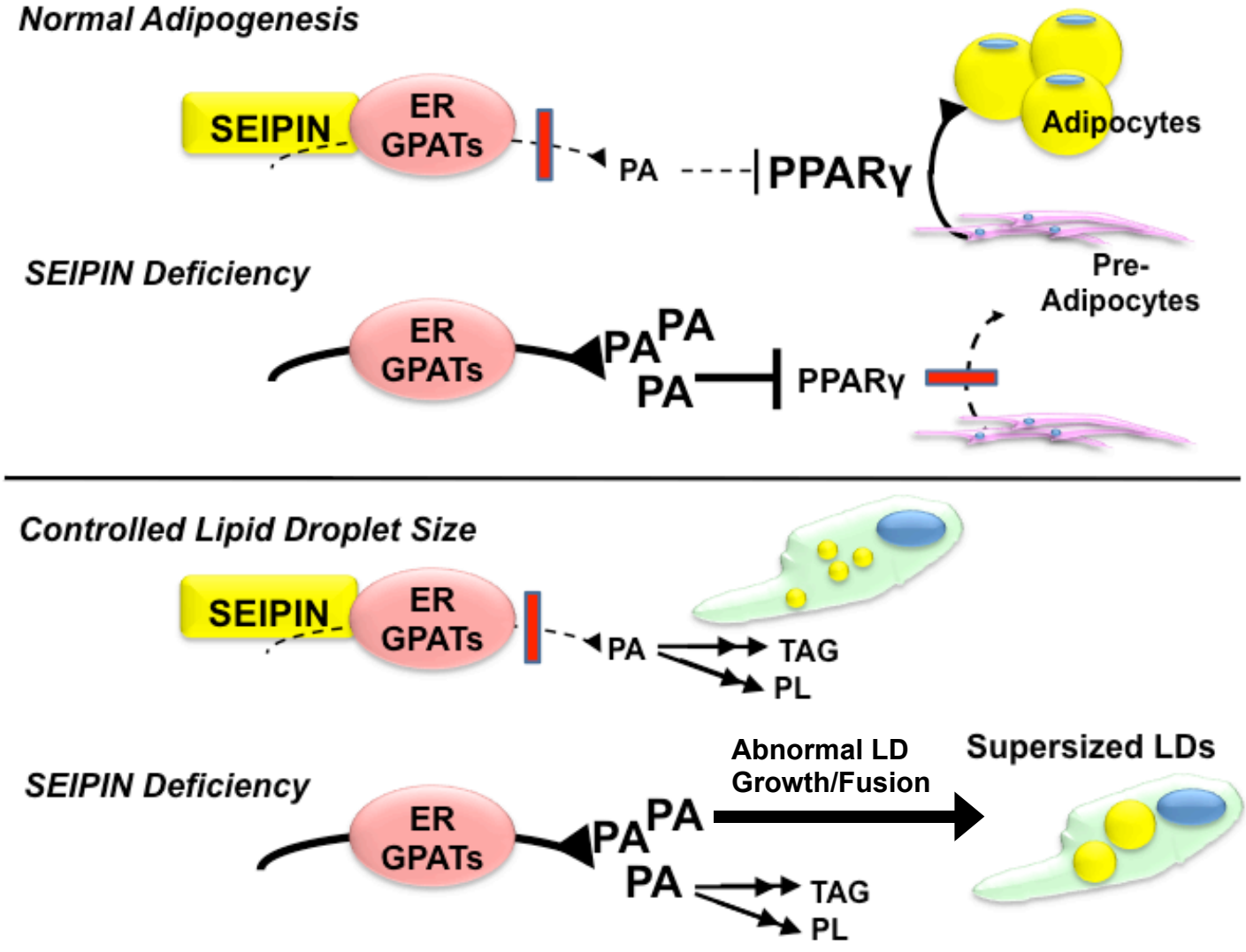


FIGURE S8. MODEL OF SEIPIN FUNCTION.
In preadipocytes, SEIPIN interacts with the ER GPAT isoforms to decrease their specific activity. This inhibition diminishes the over-production of toxic lipid intermediates, e.g. phosphatidic acid (PA), which would otherwise block PPARγ-mediated adipogenesis. In non-preadipocytes, the SEIPIN-GPAT interaction limits the size of LDs, at least in part, by reducing the production of the fusogenic lipid, PA.

SUPPLEMENTAL TABLES

Table S1. Related to Figure 1

Gene	Protein function	Cellular localization
GAT1/ GPT2	Glycerol-3 phosphate/dihydroxyacetone phosphate *sn-1* acyltransferase	Lipid droplets and ER
LDB16	Putative yeast SEIPIN homologue	ER
SCJ1	Homologue of bacterial chaperone DnaJ	ER lumen
DFM1	Der1-like Family Member involved in ERAD	ER
NSG2	Protein involved in regulation of sterol biosynthesis	ER
ERG3	C-5 sterol desaturase; glycoprotein that catalyzes the introduction of a double bond into episterol	ER lumen
SVP26	Involved in COP II vesicle transport	ER and Golgi
PSE1	Karyopherin/importin that interacts with the nuclear pore complex	Nuclear pore
SXM1	Nuclear transport factor (karyopherin)	Nuclear pore
KAP123/114/95	Karyopherin	Nuclear pore
PEX30	Negative regulator of peroxisome number	Peroxisome
ANT1	Peroxisomal adenine nucleotide transporter	Peroxisome
ERP1	Member of the p24 family involved in ER to Golgi transport	COPII vesicles
SEC7	Guanine nucleotide exchange factor (GEF) for ADP ribosylation factors	Cytoplasm and Golgi
GET4	Protein involved in inserting tail-anchored proteins into ER membranes	Cytoplasm
FKS1	Catalytic subunit of 1,3-beta-D-glucan synthase	Sites of cell wall remodeling
YDR476C	Dubious open reading frame	unknown
YMR147W	unknown	unknown
YLR312C	unknown	unknown

Table S1. Related to Figure 1.
Table listing nineteen proteins that were identified by Tandem mass spectrometry analysis to coimmunoprecipitate with Fld1-GFP.

Table S2. Related to Figure 3

Tissue/Cells		Substrate			
		G-3-P		16:0-CoA	
		Km[1]	Vmax[2]	Km[1]	Vmax[2]
yeast	Wildtype	29.4 ± 01.0	1.5 ± 0.1	72.3 ± 21.5	3.8 ± 1.4
	*fld1*Δ	62.6 ± 11.1*	3.0 ± 0.4	384.9 ± 74.6*	18.3 ± 3.8*
mouse embryonic fibroblasts	Wildtype	241.3 ± 45.1	3.0 ± 0.5	34.8 ± 16.6	3.7 ± 1.7
	Bscl2$^{-/-}$	517.9 ± 26.8*	6.8 ± 0.6*	107.8 ± 31.4*	10.0 ± 3.0*
3T3L1 preadipocytes	Control	216.6 ± 46.2	3.1 ± 1.6	205.0 ± 43.2	3.1 ± 0.7
	sh*BSCL2*	117.6 ± 08.9*	5.4 ± 1.8*	107.8 ± 11.4*	8.3 ± 0.7*
testis	Wildtype	578.1 ± 34.0	1.2 ± 0.2	111.5 ± 24.9	3.9 ± 0.8
	Bscl2$^{-/-}$	454.2 ± 25.3*	2.2 ± 0.3*	58.4 ± 09.2	4.2 ± 0.9

[1]μM, [2]nmol/min/mg protein

Table S2. Related to Figure 3.
Summary of apparent Km and Vmax values for each of GPATs substrates, glycerol-3-phosphate and C16:0-CoA. All experiments were performed in triplicate. Data are presented as mean ± S.E.M. *p<.05, student's T-test.

Table S3. Primer sets used for plasmid constructions and for site-directed mutagenesis. Related to Experimental Procedures and Figures 2 and 4.

Primer ID	5'-3' sequence	Cut site	Purpose	Origin
F-GAT1-V5	AAAAAAGCTTAGCGTGTACAATAGTCTCTGCTTAAG	HindIII	Cloning of GAT1-V5 into YCplac111	This study
R-GAT1-V5	AAAAGGATCCTCAACCGGTACGCGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCACCTTCTTTCTTTTCGTGTTCTCTTTTCTGTC	BamHI	"	This study
F-GAT2-V5	AAAAAAGCTTATTCTGCCGCCGAACTACTTAAC	HindIII	Cloning of GAT2-V5 into YCplac111	This study
R-GAT2-V5	AAAAGGATCCTCAACCGGTACGCGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCACCCGCATCTCCTTCTTTCCCTTCTTCTTC	BamHI	"	This study
F-FLD1-YCp	AAAAAAGCTTTTACCATGCACGTTGTCG	HindIII	Cloning of FLD1-Flag into YCplac111 and YCplac33	This study
R-FLD1-YCp	AAAAAGGATCCTCAGATCTTATCGTCGTCATCCTTGTAATCCATCGAGCTATGTTTCTTGGATTTTTCC	BamHI	"	This study
F-GAT2	CAGCTAAGCTTATAATGCCTGCACCAAAACTCACGG	HindIII	Cloning of GAT2-V5 into pYES2NTB	This study
R-GAT2	CAGCTGCGGCCGCTCGAGCGCATCTCCTTCTTTCCCTTC	NotI	"	This study
F-GPAT4	AGAATGGATCCATGTTCCTGTTGCTACCTT	BamHI	Cloning of GPAT4-Myc-Flag into pBABE-puro	This study
R-GPAT4	AATATGTCGACTTAAACCTTATCGTCGTCA	SalI	"	This study
F-GPAT3	AGAATGGATCCATGGAGGGCGCAGACCTGG	BamHI	Cloning of GPAT3-Myc-Flag into pBABE-puro	This study
R-GPAT3	AATATGTCGACTTAAACCTTATCGTCGTCA	SalI	"	This study

Table S3 (continued). Primer sets used for plasmid constructions and for site-directed mutagenesis.

Related to Experimental Procedures and Figures 2 and 4.

Primer ID	5'-3' sequence	Purpose	Origin
F-GAT1-G262D	TATTTTCCCCGAGGGTGATTCTCATGACCGTCCTTC	Active site mutation	This study
R-GAT1-G262D	GAAGGACGGTCATGAGAATCACCCTCGGGGAAAATA	"	This study
F-GAT2-G253L	GGGATCTTTCCTGAACTTGGGTCCCACGACAGAAC	"	This study
R-GAT2-G253L	GTTCTGTCGTGGGACCCAAGTTCAGGAAAGATCCC	"	This study
F-T78A	GGACAGACTGTGATTCCTCCGCCGCCTCTCTCTGCTCTTTCCC	Mutation in HA-SEIPIN	This study
R-T78A	GGGAAAGAGCAGAGAGAGGCGGCGGAGGAATCACAGTCTGTCC	"	This study
F-A212P	AAGCGCATCCAGATGTATGGACCCTACCTCCGGATCCATGCCCAC	"	This study
R-A212P	GTGGGCATGGATCCGGAGGTAGGGTCCATACATCTGGATGCGCTT	"	This study
F-SEIPIN-P264X	TGGGGTGCTGTCTGGTGACGCCACCGCTTCTC	C-terminal truncation of SEIPIN	This study
R-SEIPIN-P264X	GAGAAGCGGTGGCGTCACCAGACAGCACCCCA	"	This study
F-SEIPIN-N279X	ACAAAGGGATTGATCACACCACGG	"	This study
R-SEIPIN-N279X	CCGTGGTGTGATCAATCCCTTTGT	"	This study
F-SEIPIN-P293X	TCTCGCGCCATCAGTGAGGTCAGGAATCTAC	"	This study
R-SEIPIN-P293X	GTAGATTCCTGACCTCACTGATGGCGCGAGA	"	This study
F-36B4	CCCACTTACTGAAAAGGTCA	Mammalian qRT-PCR	Designed by Dr. Fei
R-36B4	TTAGTCGAAGAGACCGAATC	"	"
F-ap2	ACATGAAAGAAGTGGGAGTG	"	"
R-ap2	GGTTATGATGCTCTTCACCT	"	"
F-Bscl2	GTCTCACTGGCTAAGAGTGG	"	"
R-Bscl2	ATCACTGAGCGTGAAGAAGT	"	"
F-C/ebpα	CAAGAACAGCAACGAGTACC	"	"
R-C/ebpα	TTGACCAAGGAGCTCTCAG	"	"
F- Pparγ	ACCACAGTTGATTTCTCCAG	"	"
R- Pparγ	TAGAGCTGGGTCTTTTCAGA	"	"
F-Pref-1	CCATGAAAGAGCTCAACAAG	"	"
R-Pref-1	TACTGCAACAGGAGGTTCTT	"	"
F-Gpat3	GGAGGATGAAGTGACCCAGA	"	"
R-Gpat3	CCAGTTTTTGAGGCTGCTGT	"	"
F-Gpat4	TGTCTGGTTTGAGCGTTCTG	"	"
R-Gpat4	TTCTGGGAAGATGAGGATGG	"	"
F-TCM1-ORF	CCAGAGCTGGTCAAAGAGGT	Yeast qRT-PCR	"
R-TCM1-ORF	ACCGTAGTGGACGAAACCAC	"	"
F-INO1-ORF2	GTATTAAACCGGTCTCCATTGC	"	"
R-INO1-ORF2	CCGACGGGCTTCATATATTTG	"	"

Table S4. Primer sets used for qRT-PCR.

Related to Experimental Procedures and Figures 3 - 6.

Primer ID	5'-3' sequence	Purpose	Origin
F-36B4	CCCACTTACTGAAAAGGTCA	Mammalian qRT-PCR	Designed by Dr. S. Fei
R-36B4	TTAGTCGAAGAGACCGAATC	"	"
F-ap2	ACATGAAAGAAGTGGGAGTG	"	"
R-ap2	GGTTATGATGCTCTTCACCT	"	"
F-Bscl2	GTCTCACTGGCTAAGAGTGG	"	"
R-Bscl2	ATCACTGAGCGTGAAGAAGT	"	"
F-C/ebpα	CAAGAACAGCAACGAGTACC	"	"
R-C/ebpα	TTGACCAAGGAGCTCTCAG	"	"
F- Pparγ	ACCACAGTTGATTTCTCCAG	"	"
R- Pparγ	TAGAGCTGGGTCTTTTCAGA	"	"
F-Pref-1	CCATGAAAGAGCTCAACAAG	"	"
R-Pref-1	TACTGCAACAGGAGGTTCTT	"	"
F-Gpat3	GGAGGATGAAGTGACCCAGA	"	(Lu et al., 2010)
R-Gpat3	CCAGTTTTTGAGGCTGCTGT	"	"
F-Gpat4	TGTCTGGTTTGAGCGTTCTG	"	"
R-Gpat4	TTCTGGGAAGATGAGGATGG	"	"
F-TCM1-ORF	CCAGAGCTGGTCAAAGAGGT	Yeast qRT-PCR	(Shetty and Lopes, 2010)
R-TCM1-ORF	ACCGTAGTGGACGAAACCAC	"	"
F-INO1-ORF2	GTATTAAACCGGTCTCCATTGC	"	"
R-INO1-ORF2	CCGACGGGCTTCATATATTTG	"	"

Table S5. Yeast and mammalian expression plasmids.

Related to Experimental Procedures and Figures 1, 2, 4, 6.

Plasmid ID	Backbone	Insert	Purpose	Origin
GAT1-V5-pYESNTB	pYESNTB	GAT1-V5	Co-IP, LD study	(Pagac et al., 2012)
GAT2-V5-pYESNTB	pYESNTB	GAT2-V5	Co-IP, LD study	This study
FLD1-Flag-pESC-HIS	pESC-HIS	FLD1-Flag	Co-IP, LD study	This study
GAT1-V5-YCplac111	YCplac111	GAT1-V5	Co-IP experiments	This study
SLC4-V5-pYES2NTB	pYESNTB	SLC4-V5	Co-IP experiments	(Benghezal et al., 2007)
FLD1-Flag-YCplac111	YCplac111	FLD1-Flag	Co-IP experiments	This study
FLD1-Flag-YCplac33	YCplac33	FLD1-Flag	Co-IP experiments	This study
GPAT4-Myc-Flag-pBABE-puro	pBABE-puro	mouse GPAT4-Myc-Flag	Co-IP, LD study, differentiation experiments	This study
GPAT3-Myc-Flag-pBABE-puro	pBABE-puro	mouse GPAT3-Myc-Flag	LD study, differentiation experiments	This study
GPAT4-Myc-Flag-pCMV6-Entry	pCMV6-Entry	mouse GPAT4-Myc-Flag	Co-IP, PLA assay	Origene
GPAT3-Myc-Flag-pCMV6-Entry	pCMV6-Entry	mouse GPAT3-Myc-Flag	Co-IP, PLA assay	Origene
HA-SEIPIN-P264X-pBABE	pBABE-puro	HA-SEIPIN-P264X	Co-IP	This study
HA-SEIPIN-N279X-pBABE	pBABE-puro	HA-SEIPIN-N279X	Co-IP	This study
HA-SEIPIN-P293X-pBABE	pBABE-puro	HA-SEIPIN-P293X	Co-IP	This study
HA-SEIPIN-pBABE	pBABE-puro	HA-seipin	Co-IP, differentiation, LD study	This study
Flag-SEIPIN-pBABE	pBABE-puro	Flag-seipin	Co-IP, BirA	This study
HA-SEIPIN C-term (335-443)	pBABE-puro	HA seipin (335-443)	Co-IP, PLA	This study
HA-SEIPIN-T78A	pBABE-puro	HA-seipin T78A	Co-IP	This study
HA-SEIPIN-A212P	pBABE-puro	HA-seipin A212P	Co-IP	This study
HA-SEIPIN-P264X	pBABE-puro	HA-seipin P264X	Co-IP	This study
HA-SEIPIN-N279X	pBABE-puro	HA-seipin N279X	Co-IP	This study
HA-SEIPIN-P293X	pBABE-puro	HA-seipin P293X	Co-IP	This study
Flag-AGPAT2-pBABE	pBABE-puro	Flag-AGPAT2	PLA	This study
BirA-Flag	pBABE-puro	BirA-Flag	BirA	This study
SEIPIN-BirA-Flag	pBABE-puro	Seipin-BirA-Flag	BirA	This study

Table S6. Antibodies used for Western blotting and immunoprecipitations. Related to Experimental Procedures and Figures 1, 2, 3, 4, 6.

Antibody ID	Antigen	Host	Purpose	Origin	Catalog No
Rabbit Polyclonal AGPAT6 Antibody	AGPAT6/GPAT 4 (human)	Rabbit	WB	Origene	TA309568
Anti-DDK (Flag)	Flag	mouse	Co-IP, WB	Origene	TA50011
Anti-HA Antibody	HA	Rabbit	Co-IP, WB	Cell Signaling	3724
Anti-HA Antibody	HA	Mouse	Co-IP, WB	Covance	MMS-101P
Anti-Flag Antibody	Flag	Mouse	Co-IP, WB	Clontech	635691
Anti-V5 Antibody	V5	Mouse	Co-IP, WB	Santa Cruz Biotechnology	Sc-58052
Anti-V5 Antibody	V5	Rabbit	Co-IP, WB	Santa Cruz Biotechnology	Sc-83849
Anti-Calnexin Antibody	Calnexin	Rabbit	WB	Cell Signaling	2433
Anti-Calreticulin Antibody	Calreticulin	Rabbit	WB	Abcam	ab4
Anti-Actin Antibody	Actin	Rabbit	WB	Cell Signaling	9272
Anti-GAPDH	GAPDH	Rabbit	WB	Cell Signaling	5174
Anti-Dpm1 Antibody	Dpm1 (Yeast)	Mouse	WB	Life Technologies	A-6429
Anti-Ldb16 Antibody	Ldb16 (Yeast)	Rabbit	WB	Wang CW et al., 2014	
Anti-Erg1 Antibody	Erg1 (Yeast)	Rabbit	WB	Wang CW et al., 2014	
Anti-Pgk1 Antibody	Pgk1 (Yeast)	Mouse	WB	Life Technologies	459250

Table S7. shRNA plasmids.

Related to Experimental Procedures and Figures 3, 4, 6.

shRNA ID	5'-3' sequence	Target	Purpose	Origin
pLKO.1-CMV-neo-*Bscl2*	CCGGAGTAGAACTCTACTCTGACTACTCGAGTAGTCAGAGTAGAGTTCTACTTTTTTG	Mouse *Bscl2*	Lentiviral KD of *Bscl2* for differentiation experiments	Sigma-Aldrich
pLKO.1-*Gpat3* shRNA(c)	CCGGCCTCTCCCATTGATGTCCTAACTCGAGTTAGGACATCAATGGGAGAGGTTTTTG	Mouse *Gpat3*	Lentiviral KD of *Gpat3* for differentiation experiments	Sigma-Aldrich
pLKO.1-*Gpat3* shRNA(d)	CCGGGAAGAATTACAGCAAGATGATCTCGAGATCATCTTGCTGTAATTCTTCTTTTTG	Mouse *Gpat3*	Lentiviral KD of *Gpat3* for differentiation experiments	Sigma-Aldrich
pLKO.1-*Gpat4* shRNA(b)	CCGGCCTGCCAAATGGGAGATTTAACTCGAGTTAAATCTCCCATTTGGCAGGTTTTTG	Mouse *Gpat4*	Lentiviral KD of *Gpat4* for differentiation experiments	Sigma-Aldrich
pLKO.1-*Gpat4* shRNA(c)	CCGGGCAGAAGCTATATAGCAAGATCTCGAGATCTTGCTATATAGCTTCTGCTTTTTG	Mouse *Gpat4*	Lentiviral KD of *Gpat4* for differentiation experiments	Sigma-Aldrich
pLKO.1-Scrambled shRNA	CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG	None	Control shRNA	Addgene