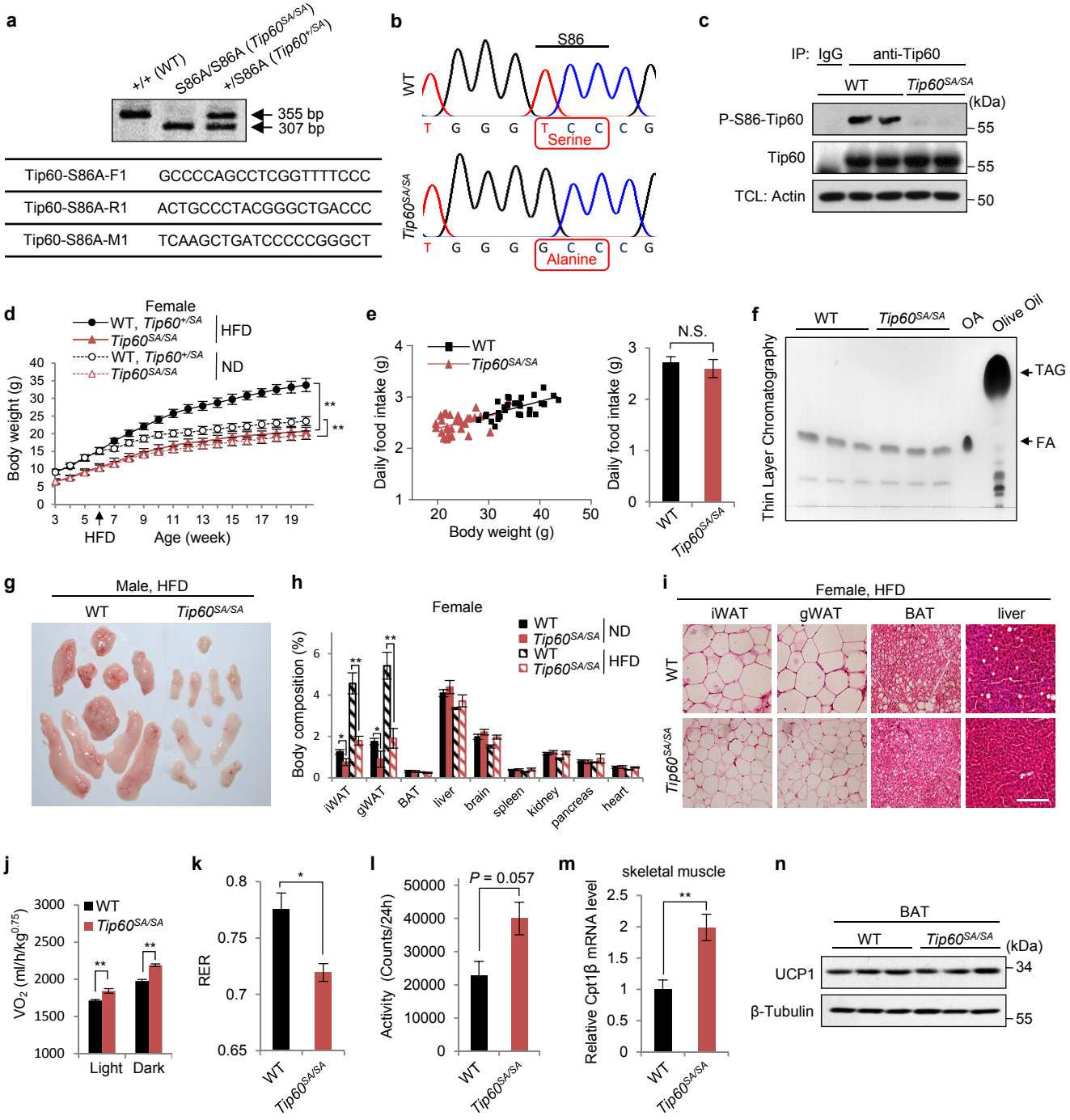
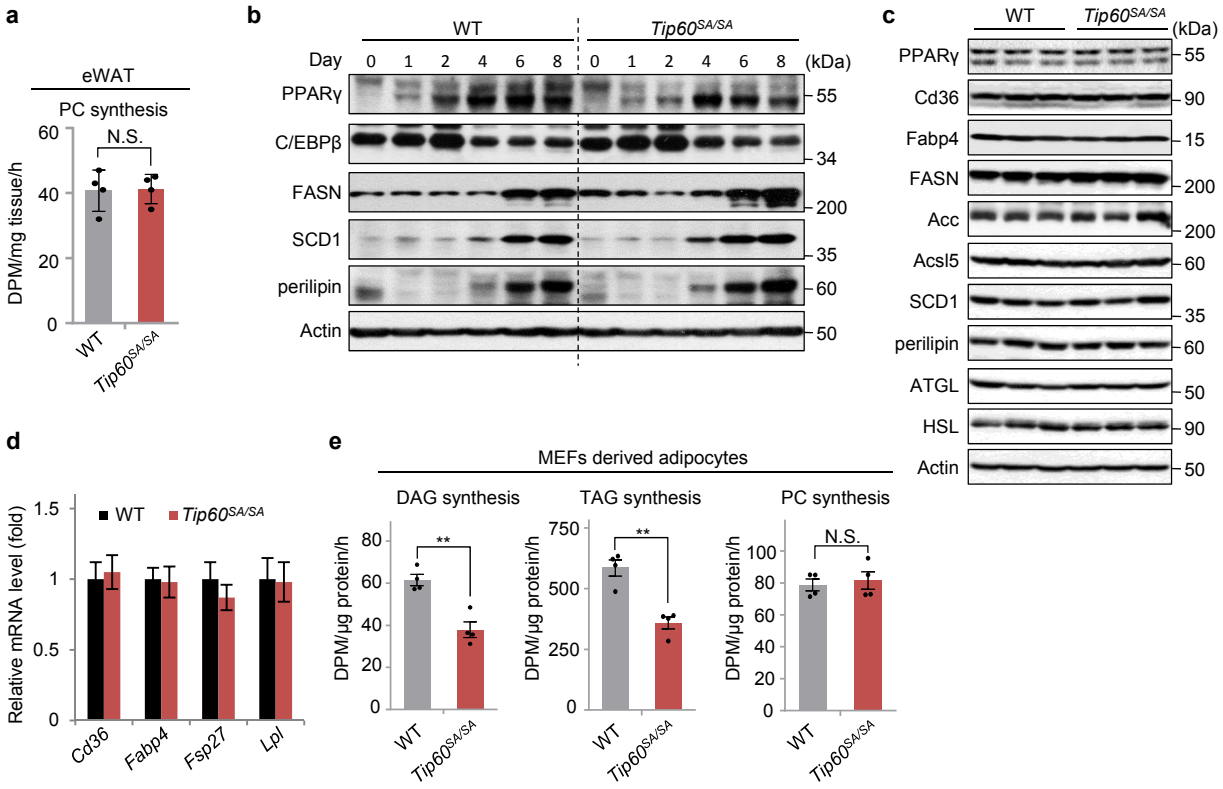
Supplementary Information

Tip60-mediated lipin 1 acetylation and ER translocation determine triacylglycerol synthesis rate

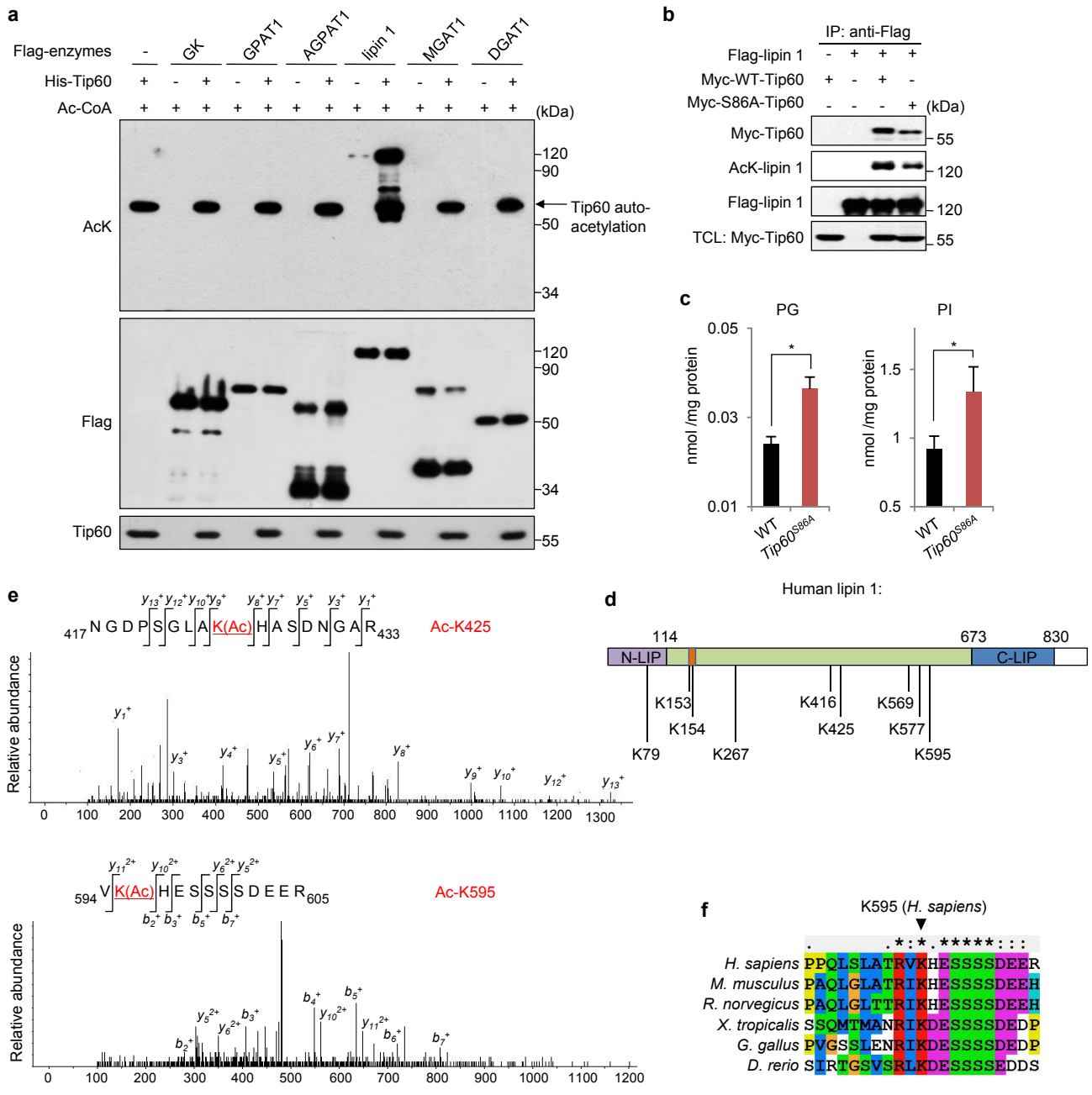
Li *et al*.


a
+/+ (WT)
S86A/S86A (Tip60SA/SA)
+/S86A (Tip60+/SA)
355 bp
307 bp
Tip60-S86A-F1 GCCCCAGCCTCGGTTTTCCC
Tip60-S86A-R1 ACTGCCCTACGGGCTGACCC
Tip60-S86A-M1 TCAAGCTGATCCCCCGGGCT
b
S86
WT
T G G G T C C C G
Serine
Tip60SA/SA
T G G G G C C C G
Alanine
c
IP: IgG anti-Tip60
WT Tip60SA/SA
(kDa)
P-S86-Tip60
Tip60
TCL: Actin
55
55
50
d
Female
WT, Tip60+/SA
Tip60SA/SA
HFD
WT, Tip60+/SA
Tip60SA/SA
ND
Body weight (g)
HFD
Age (week)
e
WT
Tip60SA/SA
Daily food intake (g)
Body weight (g)
N.S.
Daily food intake (g)
WT
Tip60SA/SA
f
WT
Tip60SA/SA
OA
Olive Oil
Thin Layer Chromatography
TAG
FA
g
Male, HFD
WT
Tip60SA/SA
h
Female
WT
Tip60SA/SA
ND
WT
Tip60SA/SA
HFD
Body composition (%)
iWAT
gWAT
BAT
liver
brain
spleen
kidney
pancreas
heart
i
Female, HFD
iWAT
gWAT
BAT
liver
WT
Tip60SA/SA
j
WT
Tip60SA/SA
VO2 (ml/h/kg0.75)
Light
Dark
k
RER
WT
Tip60SA/SA
l
P = 0.057
Activity (Counts/24h)
WT
Tip60SA/SA
m
skeletal muscle
Relative Cpt1β mRNA level
WT
Tip60SA/SA
n
BAT
WT
Tip60SA/SA
(kDa)
UCP1
β-Tubulin
34
55

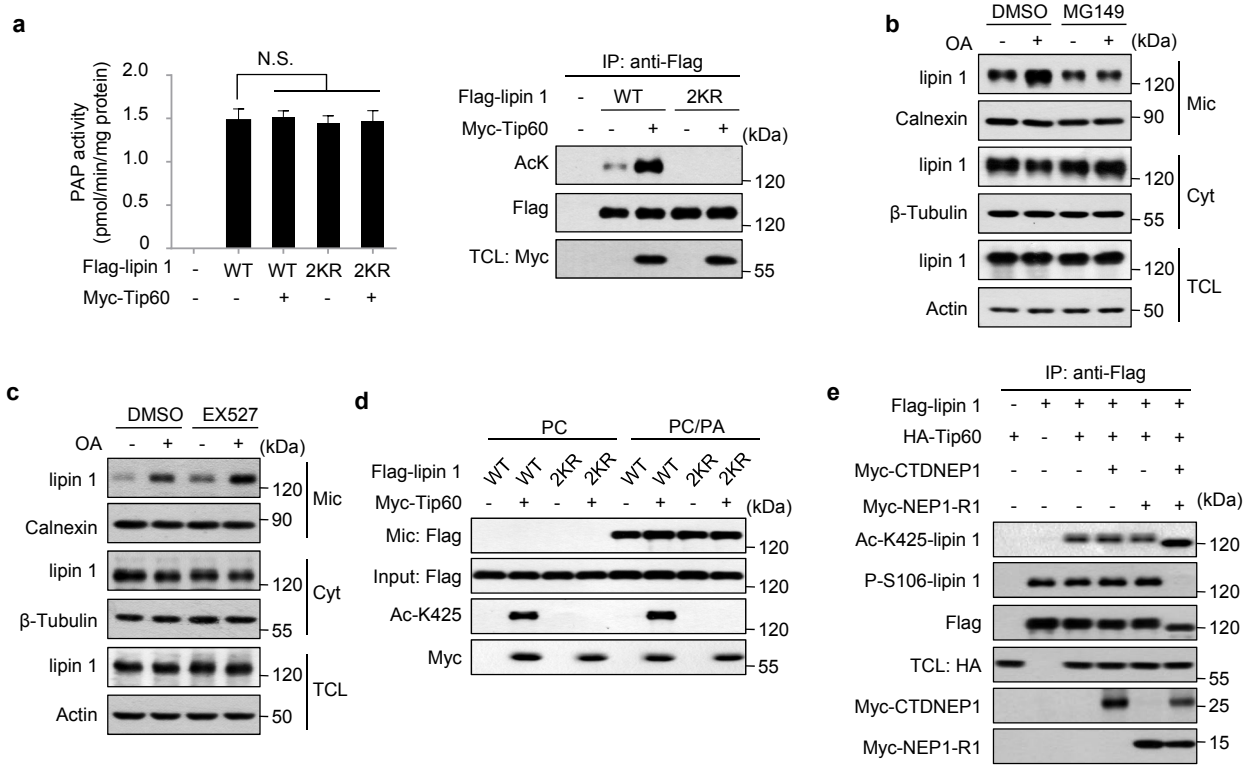
Supplementary Fig. 1 *Tip60*$^{SA/SA}$ mice demonstrate lean phenotypes and are resistant to obesity-associated metabolic disorders. (**a**) PCR analysis of wild-type (WT), heterozygous (*Tip60*$^{+/SA}$) or *Tip60*$^{SA/SA}$ knock-in mice and the primers used for genotyping. (**b**) Nucleotide sequencing results of genomic DNA from WT and *Tip60*$^{SA/SA}$ mice. (**c**) Equal amounts of cell lysates from WT or *Tip60*$^{SA/SA}$ mouse embryonic fibroblasts (MEFs) were immunoprecipitated by antibody to Tip60 and analyzed by immunoblotting as indicated. (**d**) Body weights of female WT, *Tip60*$^{+/SA}$ and *Tip60*$^{SA/SA}$ mice fed a ND *(n* = 16 per group) or HFD starting at 6-week age (*n* = 20 per group). (**e**) Daily food intake of HFD-fed WT and *Tip60*$^{SA/SA}$ mice (male, *n* = 25 per group). Food intake and body weight were collected for 4 consecutive weeks. Squares and triangles correspond to single individuals. Bar graph (right) shows the adjusted means based on a normalized mouse weight of 27.9 g determined by using ANCOVA. (**f**) No evidence of increased fecal fat content in *Tip60*$^{SA/SA}$ mice. Total lipids were extracted from fecal collected from WT and *Tip60*$^{SA/SA}$ mice fed HFD for one week and separated by thin layer chromatography (TLC). OA, oleic acid; TAG, triacylglycerol; FA, fatty acid. (**g**) Representative photographs of different positions of white adipose tissue (WAT) explants isolated from 18-week old WT and *Tip60*$^{SA/SA}$ HFD male mice. (**h**) Percentages of the weights of different tissues from 18-week old WT and *Tip60*$^{SA/SA}$ ND or HFD female mice as compared to their corresponding body weights (*n* = 12 per group). iWAT, inguinal WAT; gWAT, gonadal WAT; BAT, brown adipose tissue. (**i**) Representative sections of iWAT, gWAT, BAT and liver from HFD 18-week old female mice. Scale bar, 50 μm. (**j-m**) O_2 consumption (VO_2) (**j**), respiratory exchange ratio (RER) (**k**), physical activity (**l**), and gene expression level of *Cpt1β* (**m**) of WT and *Tip60*$^{SA/SA}$ mice fed HFD for one week (male, *n* = 6 per group), before the mice diverge too much in body weight. For VO_2, data were collected for 3 consecutive days, expressed as adjusted means based on body weight to the power 0.75. (**n**) Similar expression level of Ucp1 in brown adipose tissue (BAT) of WT and *Tip60*$^{SA/SA}$ mice fed HFD for one week (male, *n* = 6 per group). Error bars denote SEM. Statistical analysis was performed by ANOVA followed by Tukey in (**d**, **h**) and (**j**), or by two-tailed unpaired Student's *t*-test in (**k-m**) (**P* < 0.05; ***P* < 0.01; N.S., not significant). Uncropped blots can be found in Supplementary Fig. 6.



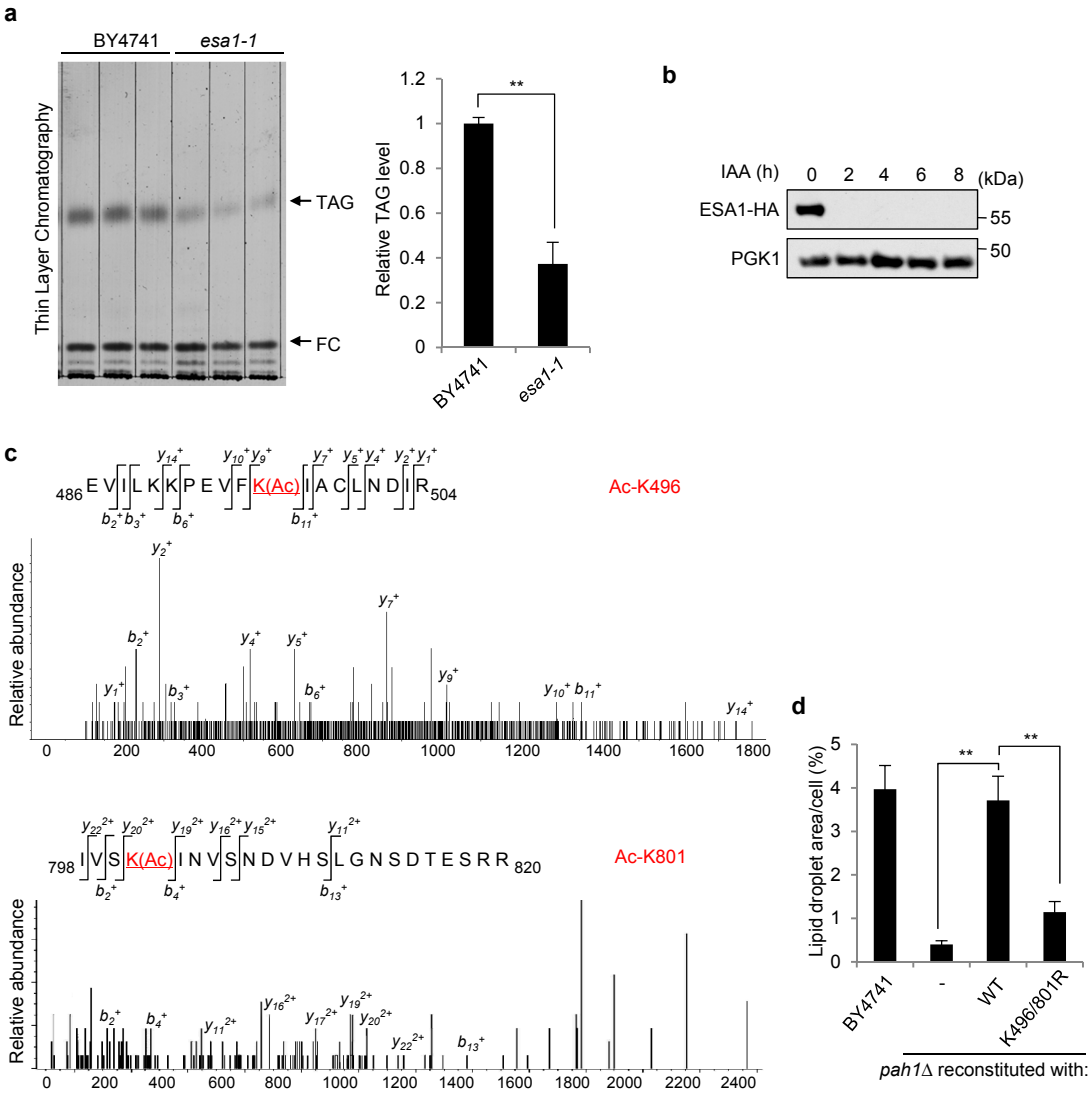
Supplementary Fig. 2 Roles of Tip60 and its Ser[86] phosphorylation in adipogenesis and lipid synthesis. (**a**) Rates of PC synthesis from ^{3}H-OA in eWAT explants isolated from 6-week old WT and *Tip60*$^{SA/SA}$ mice (n = 4 individuals per group). (**b**) Cell lysates of WT and *Tip60*$^{SA/SA}$ MEFs after 0-8 days of adipogenic differentiation induction were analysed by immunoblotting. (**c**) Cell lysates of WT and *Tip60*$^{SA/SA}$ stromal vascular fibroblasts (SVFs) after 8 days of adipogenic differentiation induction were analysed by immunoblotting. (**d**) RT-PCR analysis of WT and *Tip60*$^{SA/SA}$ SVFs after 8 days of differentiation induction (right, n = 8 mice for each group). (**e**) Rates of DAG, TAG and PC synthesis in WT and *Tip60*$^{SA/SA}$ MEFs after 8 days of differentiation (n = 4 experiments). TAG, triacylglycerol, DAG, diacylglycerol, PC, phosphatidylcholine. Error bars denote SEM. Statistical analysis was performed by two-tailed unpaired Student's t-test in (**a**) and (**e**) (**P < 0.01; N.S., not significant). Uncropped blots can be found in Supplementary Fig. 6.


a
Flag-enzymes
-
GK
GPAT1
AGPAT1
lipin 1
MGAT1
DGAT1
His-Tip60
Ac-CoA
(kDa)
AcK
Flag
Tip60
120
90
50
34
55
Tip60 auto-acetylation
b
IP: anti-Flag
Flag-lipin 1
Myc-WT-Tip60
Myc-S86A-Tip60
Myc-Tip60
AcK-lipin 1
Flag-lipin 1
TCL: Myc-Tip60
c
PG
PI
nmol /mg protein
0.05
0.03
0.01
1.5
1
0.5
WT
Tip60S86A
d
Human lipin 1:
114
673
830
N-LIP
C-LIP
K153
K154
K79
K267
K416
K425
K569
K577
K595
e
417 N G D P S G L A K(Ac) H A S D N G A R 433
Ac-K425
Relative abundance
594 V K(Ac) H E S S S S D E E R 605
Ac-K595
f
K595 (H. sapiens)
H. sapiens PPQLSLATRVKHESSSSDEER
M. musculus PAQLGLATRIKHESSSSDEEH
R. norvegicus PAQLGLTTRIKHESSSSDEEH
X. tropicalis SSQMTMANRIKDESSSSDEDP
G. gallus PVGSSLENRIKDESSSSDEDP
D. rerio SIRTGSVSRLKDESSSSEDDS

Supplementary Fig. 3 Identification of Lys^{425} and Lys^{595} as amino acid sites on lipin 1 acetylated by Tip60. (**a**) Acetylation of lipin 1 by Tip60. Tip60 specifically acetylates lipin 1 but not other enzymes in the TAG synthesis pathway. Immunoprecipitated Flag-tagged enzymes in the TAG biosynthesis pathway were incubated with or without bacterially expressed His-Tip60 in the presence of Ac-CoA and immunoblotted as indicated. (**b**) S86A-Tip60 mutant demonstrates attenuated binding affinity with lipin 1. HEK293T cells were transfected with Flag tagged lipin 1 and Myc tagged WT or S86A mutant of Tip60, immunoprecipitated with antibody to Flag and immunoblotted as indicated. (**c**) Total levels of phosphatidylglycerol (PG) and phosphatidylinositol (PI) normalized to cellular protein in eWAT from 6-week old WT and $Tip60^{SA/SA}$ mice (n = 3 individuals for each group). (**d**) Mass spectrometric analysis for acetylation sites on lipin 1. N-LIP, N-terminus lipin homology domain; C-NLP, C-terminus lipin homology domain. (**e**) Identification of K425 and K595 acetylation on lipin 1 by liquid chromatography-mass spectrometry (LC-MS/MS) analysis. The MS/MS spectrum of the acetylated peptide NGDPSGLAK(Ac)HASDNGAR (top) and VK(Ac)HESSSSDEER (bottom) are shown. The labeled peaks show the masses of y or b ions of the acetylated peptides. (**f**) Sequence alignment of the residues flanking Lys^{595} across different species. Arrow heads point to Lys^{595} residues corresponding to human lipin 1. Error bars denote SEM. Statistical analysis was performed by two-tailed unpaired Student's t-test in (**b**) ($^*P < 0.05$). Uncropped blots can be found in Supplementary Fig. 6.



Supplementary Fig. 4 Regulation of PAP activity and ER localization of lipin 1 by Tip60 or Sirt1 inhibitors. (**a**) Tip60-mediated acetylation of lipin 1 doesn't affect its PAP activity. Flag tagged WT or 2KR-lipin 1 were transfected with or without Tip60 in HEK293 cells, immunopurified with Flag antibody and measured for the Mg^{2+}-dependent PAP activity (left) (n = 4 experiments). Representative western blots detecting the overexpressed proteins are shown (right). (**b**) Tip60 inhibitor MG149 blocks the ER localization of lipin 1. 3T3-L1 adipocytes were pretreated with DMSO or MG149 for 3 h and co-treated with BSA or OA for 3 h and analyzed by fractionation, followed by western blotting. (**c**) Sirt1 inhibitor EX527 promotes lipin 1 ER localization. 3T3-L1 adipocytes (differentiation day 8) were pretreated with DMSO or EX527 for 3 h and co-treated with or without OA for 3 h, analyzed by fractionation, followed by western blotting. (**d**) Binding of lipin 1 with artificial liposomes. Immuno-purified Flag-tagged WT-lipin 1 or 2KR mutant co-expressed with or without Tip60 in HEK293T cells were incubated with liposomes consist of phosphatidylcholine (PC) or PC with 20 mol% of phosphatidic acid (PA) (PC/PA) for 20 min. The lipin 1 protein binds with liposomes (mic) were purified by ultracentrifuge and analysed by western blotting. (**e**) Co-expression of CTDNEP1 and NEP1-R1 with lipin 1 leads to lipin 1 dephosphorylation and promotes its acetylation by Tip60. HEK293T cells were transfected with lipin 1, Tip60, CTDNEP1 and/or NEP1-R1, immunoprecipitated with antibody to Flag and immunoblotted as indicated. Error bars denote SEM. Statistical analysis was performed by ANOVA followed by Tukey in (**a**) (N.S., not significant). Uncropped blots can be found in Supplementary Fig. 6.



Supplementary Fig. 5 Role of ESA1 (Yeast Tip60) in TAG accumulation in *S. cerevisiae* and PAH1 (Yeast Lipin 1) acetylation by ESA1. (**a**) Impaired accumulation of TAG in *ESA1* loss of function mutant (*esa1-1*, an auxin-sensitive mutant strain of ESA1). TAG were extracted from cells grown in synthetic complete (SC) medium, separated by thin TLC, stained by iodine vapor and quantified. FC, free cholesterol (n = 3 experiments). (**b**) Rapid depletion of ESA1 by auxin indole-3-acetic acid (IAA) treatment in the auxin-sensitive mutant strain of ESA1. Cells grown in SC medium were collected for analysis at indicated time points after addition of 500 μM IAA. (**c**) Identification of K496 and K801 acetylation on PAH1 by liquid chromatography-mass spectrometry (LC-MS/MS) analysis. The MS/MS spectrum of the acetylated peptide EVILKKPEVFK(Ac)IACLNDIR (top) and IVSK(Ac)INVSNDVHSLGNSDTESRR (bottom) are shown. The labeled peaks show the masses of *y* or *b* ions of the acetylated peptides. (**d**) Percentage of lipid droplet area in WT (BY4741) or PAH1 knockout (*pah1Δ*) cells reconstituted with vector, WT-PAH1 or K496/801R-PAH1 (n = 100 cells quantified for each group). Error bars denote SEM. Statistical analysis was performed by two-tailed unpaired Student's *t*-test in (**a**) or by ANOVA followed by Tukey in (**d**), (**$P < 0.01$). Uncropped blots can be found in Supplementary Fig. 6.

Supplementary Figure 6 Uncropped blots with size marker indications.

Figure 2e

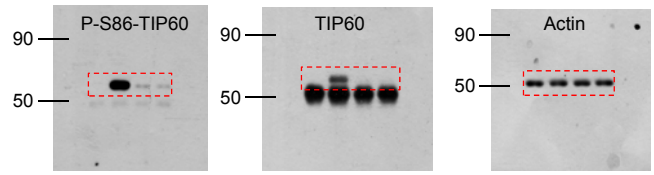


Figure 3b

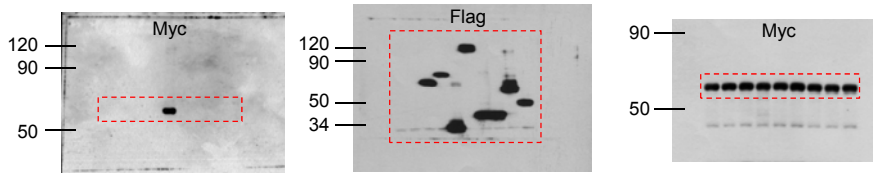
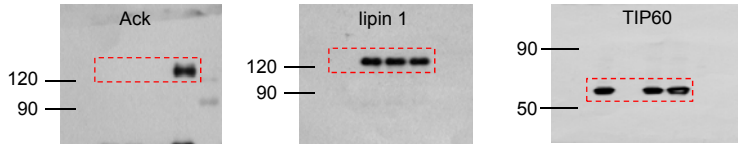


Figure 3c



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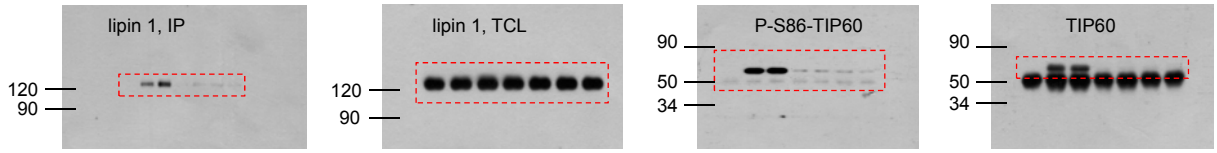

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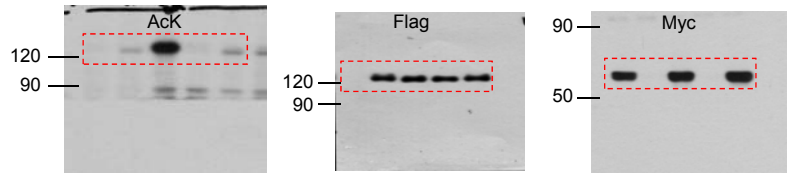


Figure 3f

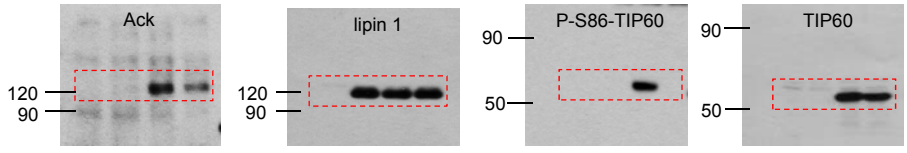
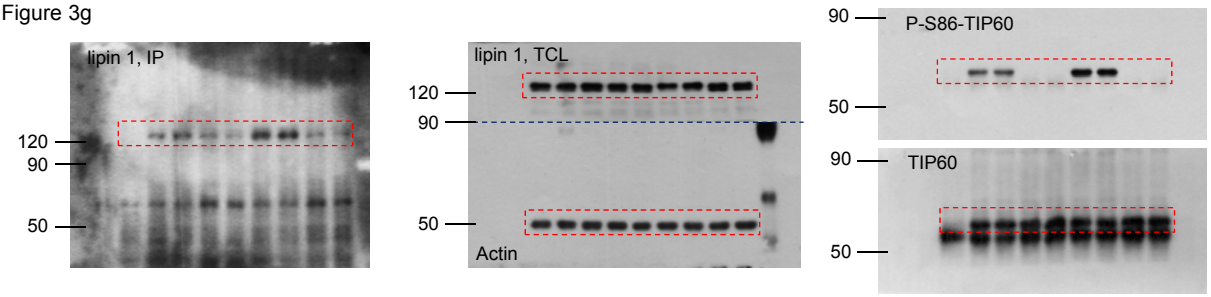


Figure 3g



Supplementary Figure 6 Uncropped blots with size marker indications (continued).

Figure 3k

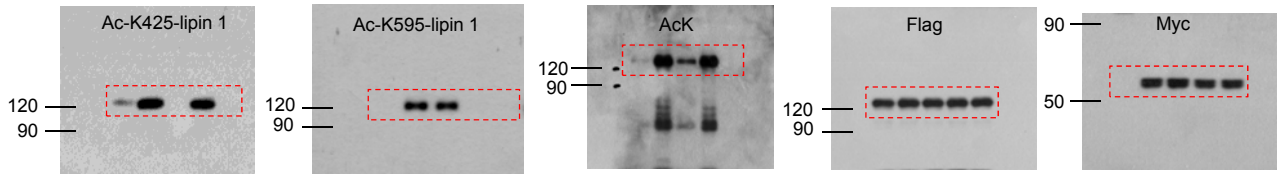
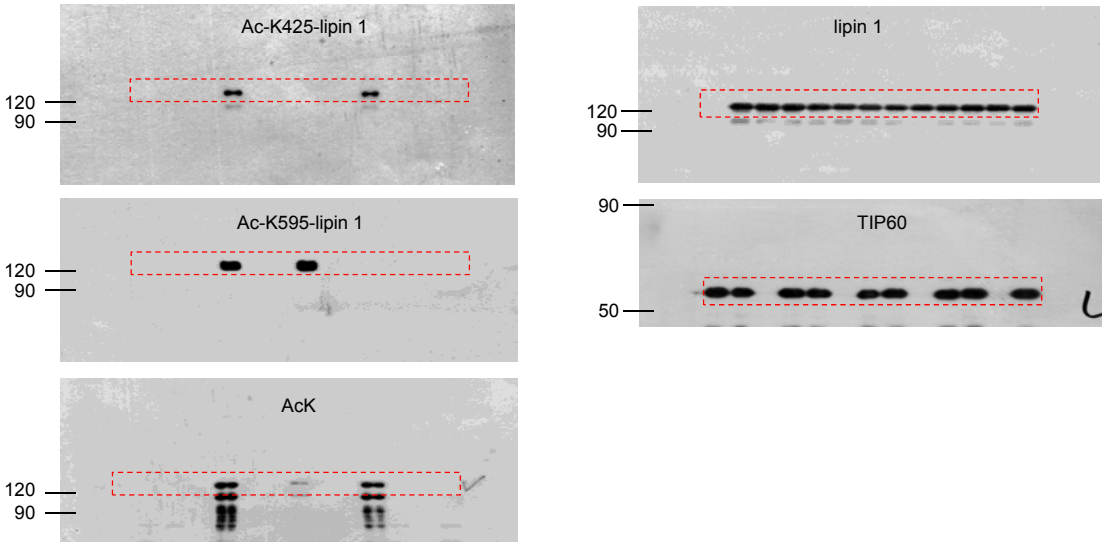


Figure 3l



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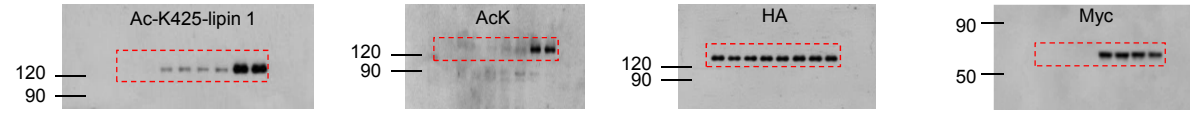

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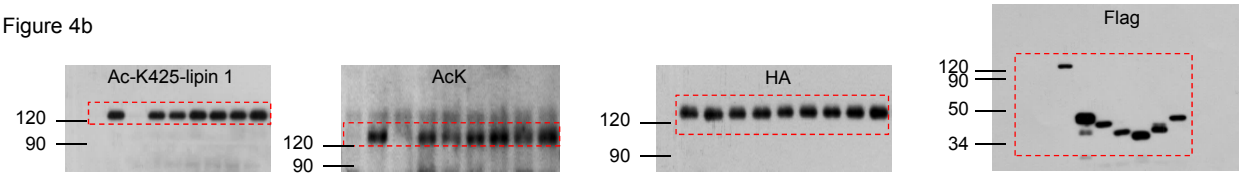

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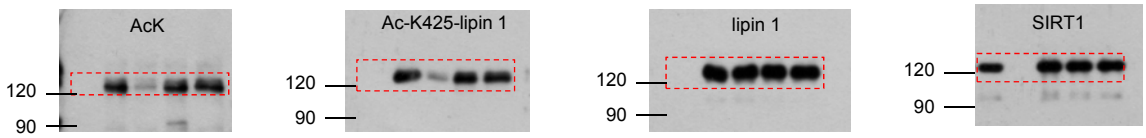

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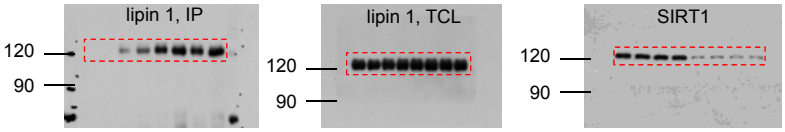

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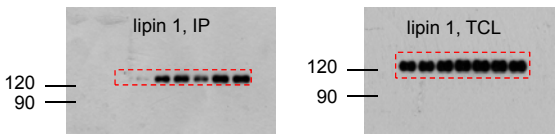
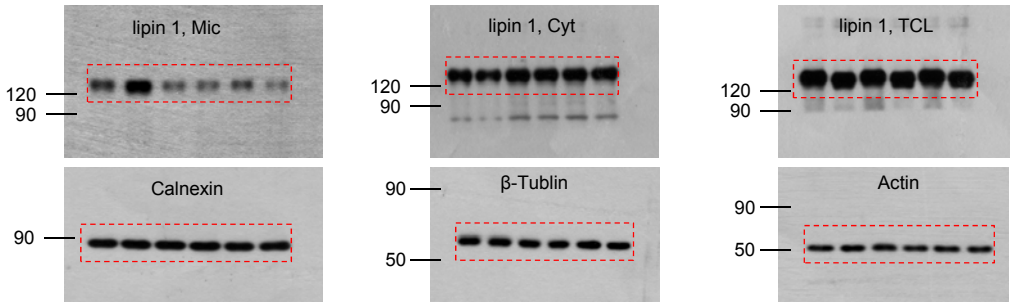


Supplementary Figure 6 Uncropped blots with size marker indications (continued).

Figure 5b



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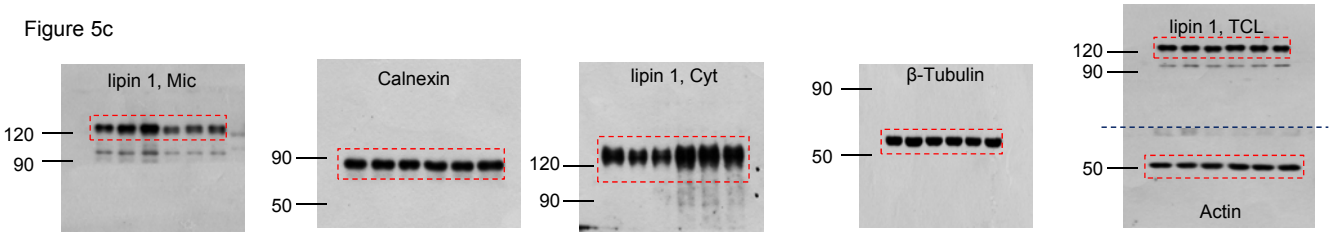


Figure 5d

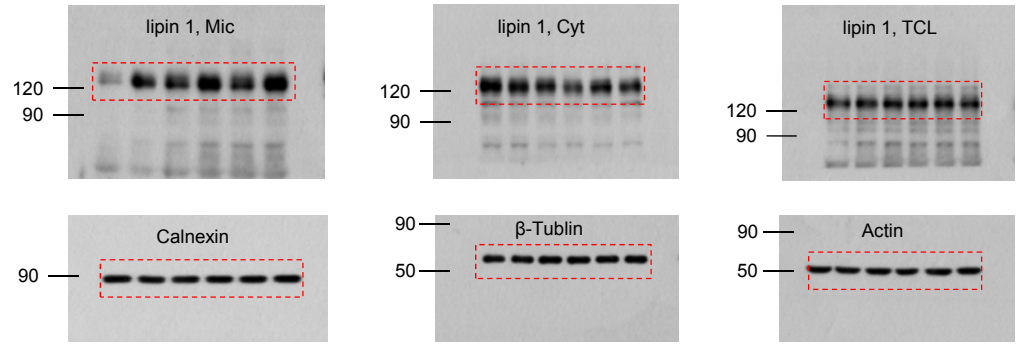
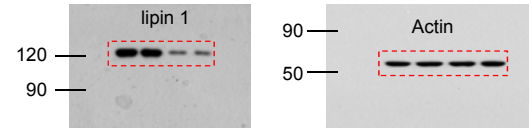


Figure 5e



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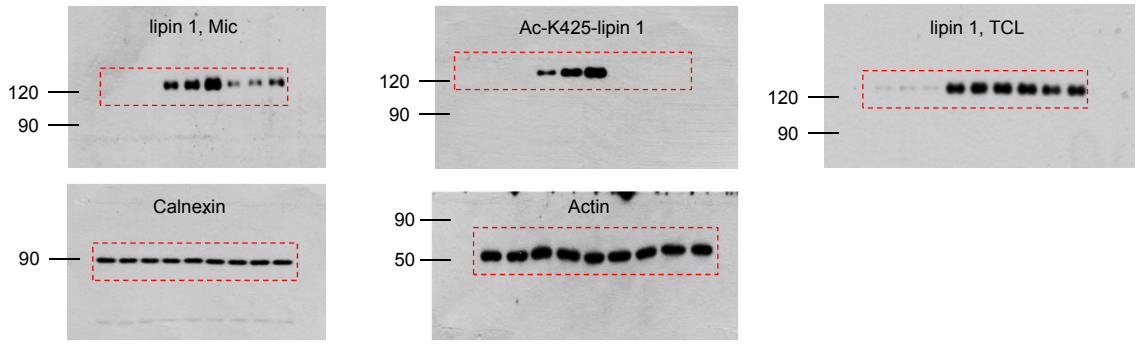
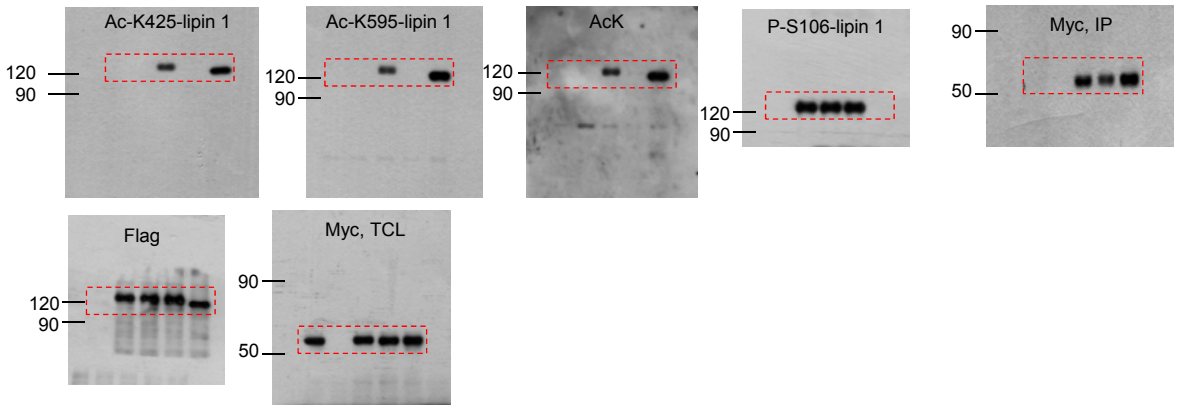


Supplementary Figure 6 Uncropped blots with size marker indications (continued).

Figure 6a



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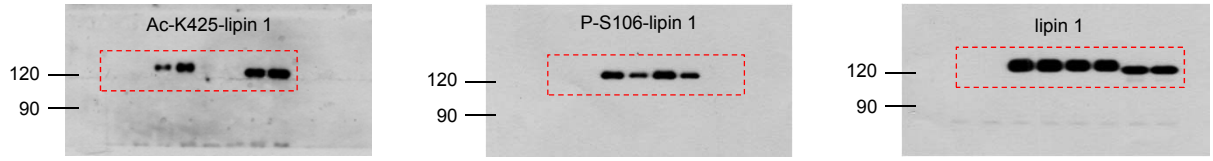

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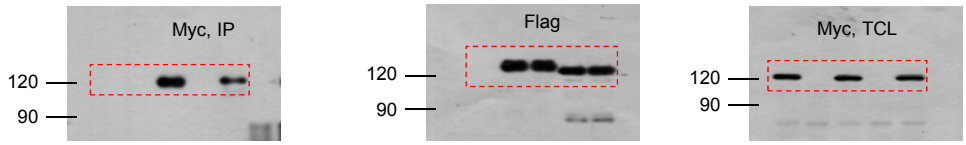

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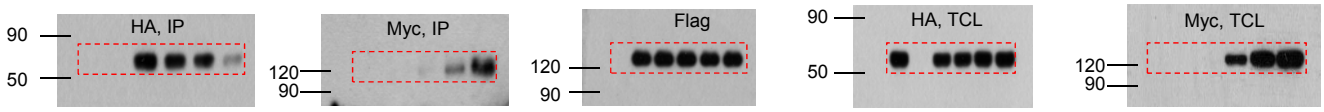

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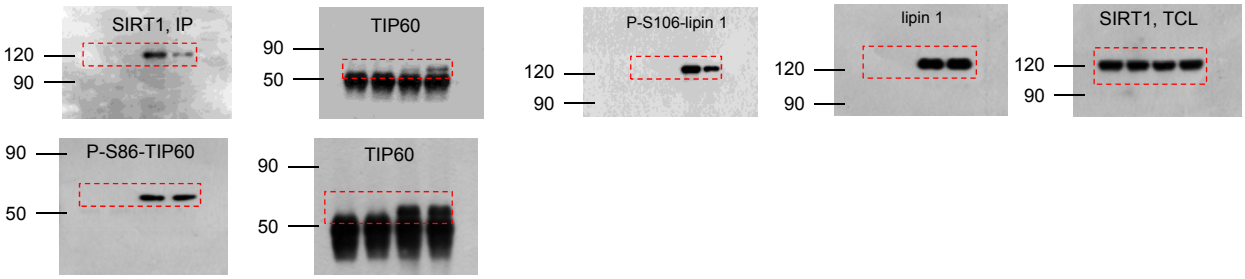

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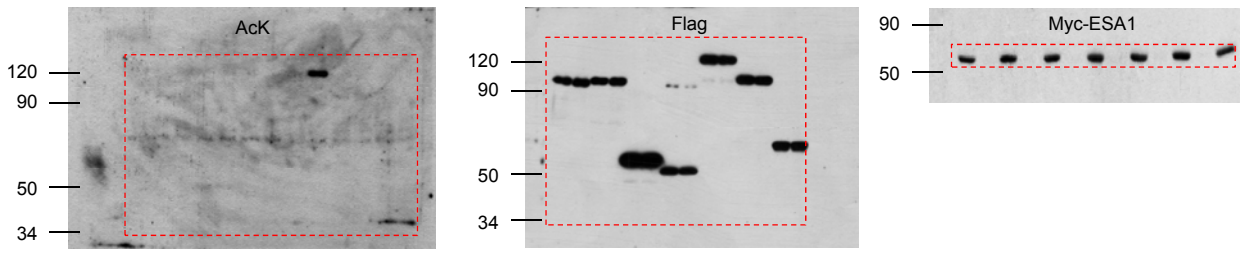


Supplementary Figure 6 Uncropped blots with size marker indications (continued).

Figure 7f

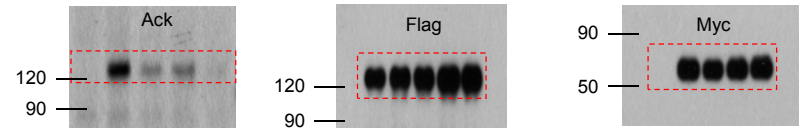
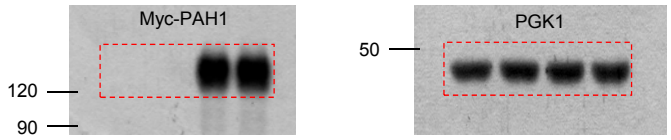
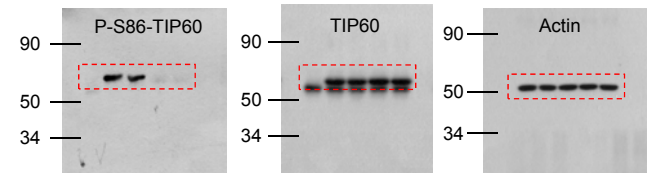


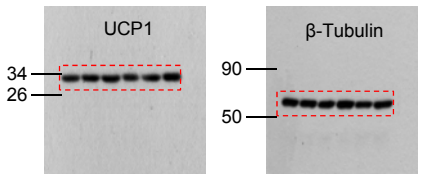
Figure 7g



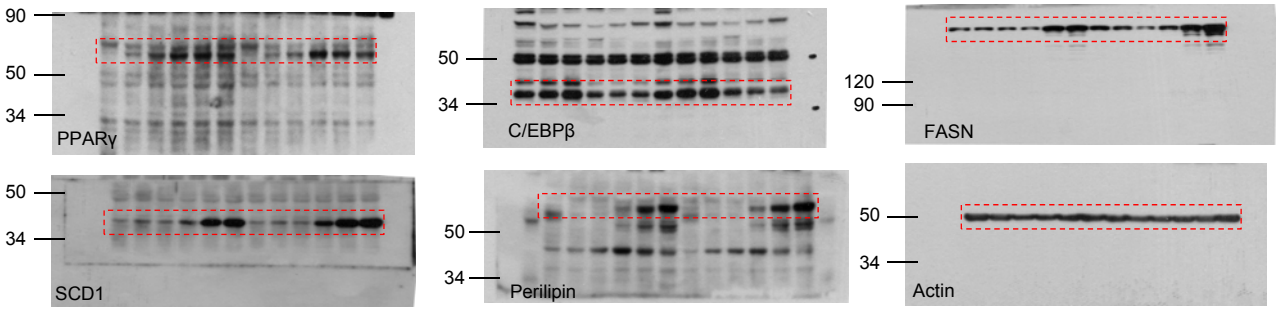
Supplementary Figure 1c



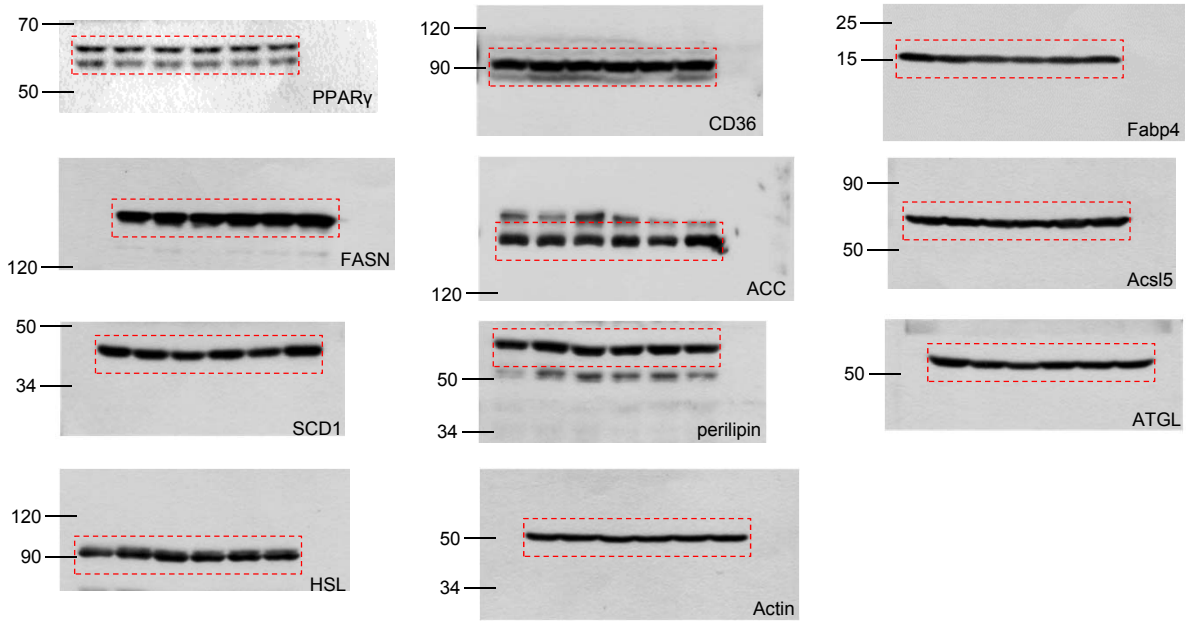
Supplementary Figure 1n



Supplementary Figure 2b

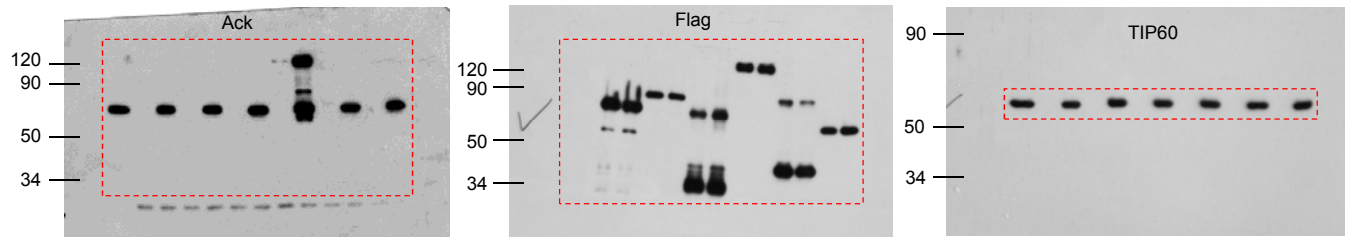


Supplementary Figure 2c

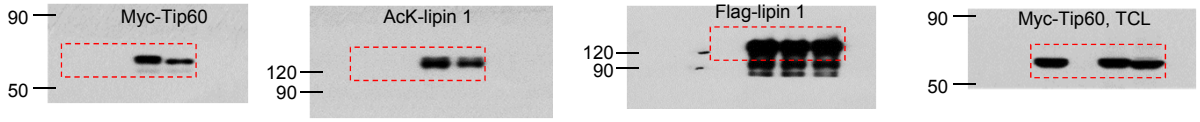


Supplementary Figure 6 Uncropped blots with size marker indications (continued).

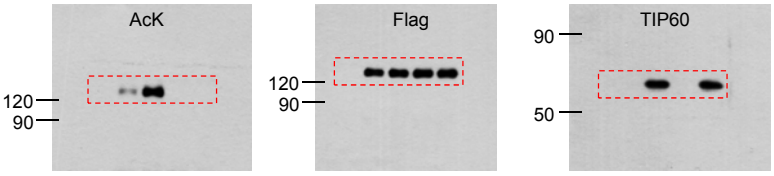
Supplementary Figure 3a



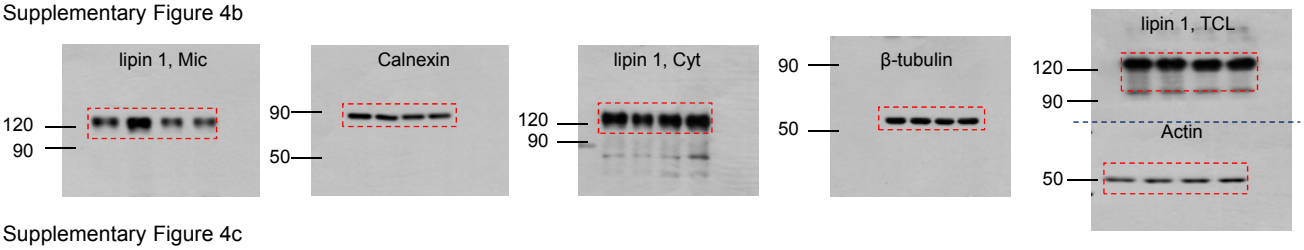
Supplementary Figure 3b



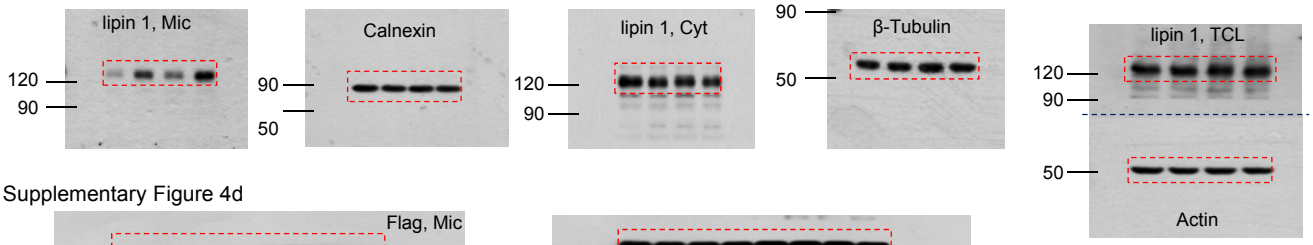
Supplementary Figure 4a



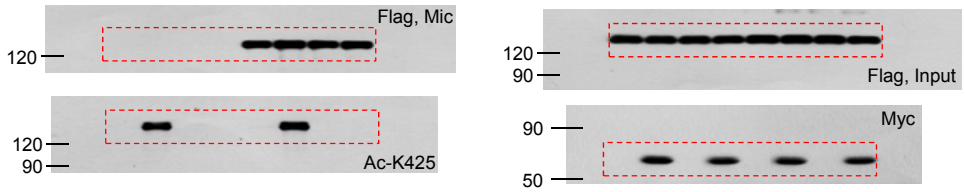
Supplementary Figure 4b



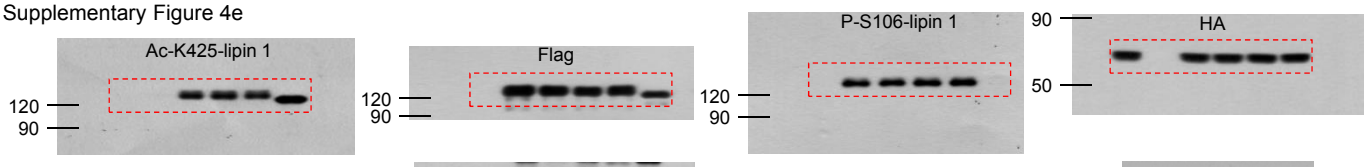
Supplementary Figure 4c

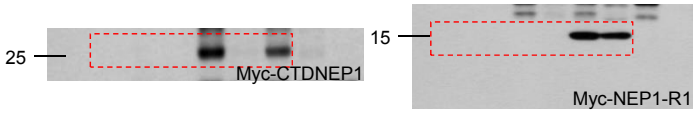


Supplementary Figure 4d

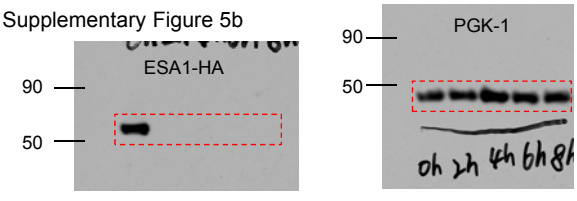


Supplementary Figure 4e





Supplementary Figure 5b



Supplementary Table S1 Lipidomic Profiling of WT and *Tip60*$^{SA/SA}$ Mice eWAT by LC-MS.

	WT-1	WT-2	WT-3	S86A-1	S86A-2	S86A-3	WT-mean	S86A-mean	fold change (S86A/WT)	*P*-values
DAG34:3(18:2/16:1)	2.69600157	2.98579008	2.94658427	1.86371023	2.46053859	2.43990354	2.87612531	2.25471745	0.7839427	0.0495
DAG36:4(18:2/18:2)	10.1260838	10.1764994	11.6386407	8.1664648	9.59002327	8.30241527	10.6470746	8.68630111	0.81583922	0.0495
DAG36:3(18:2/18:1)	12.9123903	12.0256068	14.44311	10.3365188	12.38991	10.0793701	13.1270357	10.9352663	0.83303394	0.1266
DAG34:3(16:2/18:1)	0.14683384	0.12716175	0.14717302	0.12758368	0.1209062	0.11305434	0.14038954	0.12051474	0.85843105	0.1266
DAG32:2(16:2/16:0)	0.05039456	0.05955278	0.05785828	0.05134467	0.05503613	0.04083276	0.0559352	0.04907119	0.87728626	0.2752
DAG36:2(18:1/18:1)	10.2371479	9.34761668	11.3764287	8.94279858	8.96579359	8.40643043	10.3203978	8.7716742	0.84993567	0.1266
DAG34:2(16:1/18:1)	2.25326634	2.2382233	2.533602	1.70432722	2.17728282	2.33573726	2.34169721	2.0724491	0.8850201	0.2752
GM3 18:0/18:0	0.00033346	0.00032316	0.00011842	0.00029621	0.00014103	0.00027589	0.00025835	0.00023771	0.92012072	0.5127
PE38:4p	0.06112815	0.04810464	0.06646267	0.05571995	0.05401127	0.05378806	0.05856515	0.05450642	0.93069722	0.5127
DAG32:2(16:1/16:1)	0.53459385	0.60819964	0.51648485	0.45904273	0.41021118	0.69588798	0.55309278	0.52171396	0.94326664	0.5127
DAG38:4(18:1/20:3)	0.17188626	0.16963364	0.17735656	0.19837691	0.13232093	0.16241942	0.17295882	0.16437242	0.95035581	0.5127
DAG	65.9151289	64.8768009	66.9346427	60.6302128	63.5302239	63.5996522	65.9088575	62.5866963	0.94959462	0.0495
DAG32:1(14:0/18:1)	0.34410147	0.37154682	0.35059432	0.33027347	0.32441336	0.3635994	0.35541421	0.33942874	0.955023	0.2752
PI 34:1	0.02141972	0.01464336	0.02241084	0.02305576	0.01516732	0.018123	0.01949131	0.01878202	0.96361016	0.8273
PS 34:1	0.0103786	0.00902592	0.00868296	0.00931238	0.00885628	0.00892045	0.00936249	0.0090297	0.9644552	0.8273
PE34:1p	0.0232403	0.02413007	0.02163804	0.02709045	0.01756018	0.02307818	0.0230028	0.02257627	0.98145749	0.8273
DAG32:2(18:2/14:0)	0.64613084	0.78782064	0.69426741	0.59449606	0.61734372	0.87777549	0.7094063	0.69653842	0.98186106	0.5127
PE36:3p	0.02339785	0.02474244	0.01852521	0.02135983	0.02080886	0.02345857	0.02222183	0.02187575	0.98442597	0.8273
PC36:2p	0.00397417	0.00529339	0.00534223	0.00539701	0.0051523	0.00387035	0.00486993	0.00480656	0.98698625	0.8273
DAG30:1(14:0/16:1)	0.08989291	0.11325375	0.08343669	0.07118227	0.07582105	0.13621815	0.09552778	0.09440716	0.9882691	0.5127
PE36:4p	0.09168151	0.0921776	0.0950782	0.09403041	0.08511005	0.09744999	0.0929791	0.09219682	0.99158642	0.8273
PI 40:6	0.00650364	0.00507054	0.01269713	0.00988526	0.00745521	0.00682107	0.00809043	0.00805385	0.99547758	0.5127
PC32:2	0.01042903	0.00889433	0.02011728	0.01357604	0.01138376	0.01495779	0.01314688	0.01330586	1.01209287	0.5127
DAG40:6(18:1/22:5)	0.18904977	0.1608413	0.12494203	0.18126212	0.1381758	0.16333117	0.1582777	0.16092303	1.01671322	0.8273
GM3 18:1/18:1	0.00013349	0.00023514	6.7256E-05	4.9519E-05	0.00024567	0.00015351	0.0001453	0.00014956	1.02936812	0.8273
LPA18:1	0.04904401	0.04654232	0.06346141	0.06986236	0.04946468	0.04514659	0.05301592	0.05482454	1.03411479	0.8273
PE36:4	0.10999395	0.07825909	0.06606623	0.10371774	0.07042086	0.08905456	0.08477309	0.08773105	1.03489271	0.8273
GM3 18:1/22:0	0.00106696	0.00196658	0.00062403	0.0011338	0.00098266	0.00171167	0.00121919	0.00127604	1.04663257	0.8273
DAG34:1(16:1/18:0)	0.22991668	0.20087242	0.1937095	0.18904151	0.19701993	0.27029593	0.2081662	0.21878579	1.05101495	0.8273
PC40:5	0.01189307	0.01274124	0.02202173	0.02333288	0.01058309	0.0152403	0.01555201	0.01638543	1.05358867	0.8273
PI 36:1	0.01109958	0.00867773	0.01542865	0.01712751	0.00967335	0.01070001	0.01173532	0.01250029	1.06518563	0.8273
GM3 18:0/22:0	0.00036752	0.00055741	0.0002699	0.00068918	0.00035091	0.00024477	0.00039828	0.00042829	1.07535122	0.8273
DAG34:1(16:0/18:1)	6.23308958	5.92479521	5.35644496	6.36121595	6.00625367	6.52835428	5.83810992	6.29860797	1.07887793	0.1266
PE40:4p	0.07799852	0.08290325	0.06282909	0.08280374	0.08473791	0.07552452	0.07457695	0.08102206	1.0864222	0.5127
DAG32:1(16:1/16:0)	0.86588153	1.08622292	0.83282202	0.92215878	0.87338113	1.2474694	0.92830882	1.01433644	1.09267133	0.2752
PE38:6p	0.14118468	0.15867086	0.11567863	0.17990586	0.13395779	0.14645243	0.13851139	0.15343869	1.1077695	0.5127
PS 40:7	0.03865234	0.03445199	0.02999675	0.05389696	0.03300327	0.02755974	0.03436703	0.03815333	1.11017242	0.8273
CE:18:1	0.27916352	0.38990964	0.30718565	0.39488828	0.32498712	0.3640371	0.3254196	0.36130417	1.11027167	0.2752
PI 34:2	0.02170286	0.01394911	0.01492335	0.02207264	0.01677198	0.01760262	0.01685844	0.01881574	1.11610228	0.2752
DAG34:2(16:0/18:2)	12.1858046	12.1649974	10.7147984	12.9008248	12.9505898	13.6791171	11.6885335	13.1768439	1.12733081	0.0495
DAG36:1(18:1/18:0)	0.92971966	1.00450286	0.78551744	0.99171675	0.92327902	1.17234921	0.90657999	1.02911499	1.13516182	0.5127
DAG36:2(18:2/18:0)	1.7348532	1.86183806	1.52727893	1.95741566	1.7289516	2.15409606	1.70799006	1.94682111	1.13983164	0.2752
PA34:1	0.02051844	0.018021	0.01596654	0.02631283	0.01788765	0.0179992	0.01816866	0.02073323	1.14115338	0.8273
PC34:1	0.29730082	0.33002332	0.32446567	0.40637769	0.31253223	0.36801758	0.31726327	0.36230917	1.14198272	0.2752
PE38:5p	0.06016842	0.06171003	0.06037305	0.07640937	0.06716071	0.06559881	0.0607505	0.06972296	1.14769362	0.0495
LPE18:1	0.03597982	0.02739321	0.03838407	0.0480991	0.0308177	0.03788274	0.03391903	0.03893318	1.14782705	0.5127
PE42:1p	0.0109874	0.0108332	0.00959291	0.01322153	0.00820899	0.01488301	0.01047117	0.01210451	1.15598462	0.5127
PC36:4	0.15754733	0.1514319	0.13071968	0.20381965	0.13664875	0.16919402	0.1465663	0.16988747	1.15911687	0.2752
PC32:1	0.08280728	0.09855956	0.11638809	0.11932923	0.1088406	0.12039846	0.09925164	0.11618943	1.17065502	0.1266
PC38:4	0.09778313	0.12217969	0.11560635	0.16452925	0.1091851	0.11995048	0.11185639	0.13122161	1.17312573	0.5127
LPE16:0	0.01733326	0.01874128	0.00875532	0.01585485	0.01566655	0.02172351	0.01494329	0.0177483	1.18771074	0.8273
PC38:5	0.04803209	0.04860675	0.0662931	0.08451011	0.05146331	0.05754461	0.05431065	0.06450601	1.18772315	0.2752
PC38:3	0.06046441	0.07295956	0.05829457	0.09680016	0.06305905	0.06996376	0.06390618	0.07660766	1.19875197	0.2752
CE:18:0	0.11753994	0.10072028	0.05217228	0.10740095	0.08978682	0.12950327	0.09014417	0.10889701	1.20803173	0.5127
DAG34:0(16:0/18:0)	0.64157852	0.63076337	0.48325407	0.73017336	0.5670922	0.83048755	0.58519865	0.70925103	1.21198337	0.2752
DAG38:4(18:0/20:4)	0.25691075	0.25118961	0.19121234	0.32366406	0.25648469	0.26788981	0.23310423	0.28267952	1.21267434	0.1266
GM3 18:1/18:0	0.00060045	0.00079333	0.00040473	0.00093663	0.00045604	0.00079472	0.0005995	0.00072913	1.21621913	0.2752
PE34:1	0.07910394	0.07701804	0.0493598	0.08962424	0.07117649	0.08924905	0.06849393	0.08334992	1.21689512	0.2752
PC40:6	0.03173789	0.04146118	0.04230668	0.06241112	0.03610499	0.04306326	0.03850191	0.04719312	1.22573451	0.2752
LPI16:1	0.00267747	0.00237151	0.00133006	0.00244902	0.00240483	0.00300775	0.00212634	0.00262053	1.23241205	0.2752
PE42:2p	0.01018764	0.01407858	0.00946278	0.01211499	0.01392789	0.01563815	0.011243	0.01389368	1.23576248	0.2752
GM3	0.00777145	0.00960109	0.0050603	0.01093819	0.00768582	0.00920113	0.00747761	0.00927505	1.24037563	0.5127
PC36:3	0.23305945	0.26746142	0.19901587	0.36179311	0.24210051	0.26425085	0.23317891	0.28938149	1.24102769	0.2752
PC38:6	0.04570061	0.04352616	0.0454238	0.06889029	0.04718164	0.05145461	0.04488353	0.05584218	1.24415754	0.0495
PC34:3	0.02480465	0.02997852	0.02743743	0.04152165	0.02593833	0.03552366	0.02740687	0.03432788	1.25252846	0.2752
PC36:1	0.19936475	0.27758406	0.28597147	0.37572139	0.26632881	0.31601599	0.25430676	0.3193554	1.25578808	0.2752
PC32:2e	0.00681293	0.00723226	0.00710754	0.00946563	0.00807659	0.00907129	0.00705091	0.00887117	1.25815923	0.0495
PE34:2p	0.0178301	0.02041843	0.0145069	0.02664448	0.02385715	0.01601854	0.01758514	0.02217339	1.26091595	0.2752
GM3 18:1/16:0	0.00323331	0.00355201	0.00227666	0.00458123	0.00326139	0.00360587	0.00302066	0.00381616	1.26335283	0.1266
CE	0.87459623	1.04764967	0.90688781	1.304008	1.01151165	1.26852945	0.94304457	1.19468304	1.26683624	0.1266
CE:18:2	0.23817311	0.29529966	0.31304777	0.40791522	0.32658264	0.33854421	0.28217351	0.35768069	1.26759132	0.0495
PC32:0	0.15868396	0.19515276	0.16287755	0.24368702	0.19529817	0.2193724	0.17223809	0.21945253	1.27412313	0.0495
LPS16:0	0.00167664	0.00158812	0.00118861	0.00186235	0.00148596	0.00233148	0.00148446	0.00189326	1.27539006	0.2752

PS 36:1	0.26575004	0.28069763	0.26121344	0.37566097	0.3248546	0.33347693	0.26922037	0.34466417	1.28023064	0.0495
PA38:4	0.01742665	0.0301587	0.01907011	0.03198939	0.02766613	0.02581511	0.02221849	0.02849021	1.28227495	0.2752
PE36:1p	0.02133501	0.01763015	0.01632569	0.02686254	0.02099492	0.02307818	0.01843028	0.02364521	1.28295469	0.1266
PA36:1	0.01018401	0.01462002	0.01431876	0.01870623	0.01583423	0.01579574	0.01304093	0.01677873	1.28662075	0.0495
PC34:0p	0.01479149	0.01880462	0.01721205	0.02146402	0.02572771	0.01818271	0.01693605	0.02179148	1.28669187	0.1266
PS 38:4	0.16050706	0.15008294	0.12305863	0.23007736	0.17472524	0.15363543	0.14454954	0.18614601	1.28776617	0.1266
PA32:0	0.00294368	0.00309704	0.00228447	0.00350725	0.00307877	0.00415413	0.00277506	0.00358005	1.29007773	0.1266
PS 40:4	0.03134114	0.03187226	0.02831646	0.04254354	0.0371876	0.03850973	0.03050996	0.03941362	1.29182817	0.0495
PC36:1p	0.02525194	0.03456481	0.03073434	0.04276585	0.0362443	0.03802245	0.0301837	0.03901086	1.29244824	0.0495
PE	2.26432032	2.15208506	1.62000986	3.02964008	2.26552494	2.50953958	2.01213842	2.6015682	1.292937	0.0495
PE38:5	0.09979917	0.07609469	0.06179738	0.12860178	0.09274664	0.08600808	0.07923041	0.10245217	1.29309142	0.2752
PC36:3p	0.0057074	0.00517029	0.00531904	0.00560463	0.01023489	0.00512024	0.00539891	0.00698659	1.29407329	0.8273
PS 38:5	0.02078148	0.02014173	0.01828177	0.03354827	0.02348908	0.01968553	0.01973499	0.02557429	1.29588571	0.2752
PC36:0p	0.01341699	0.01686597	0.01347232	0.01847453	0.0195999	0.01870717	0.01458509	0.0189272	1.29770838	0.0495
LPA	0.31674626	0.33266593	0.30328472	0.52821212	0.37551219	0.33657932	0.31756564	0.41343454	1.30188691	0.0495
LPS18:1	0.01142842	0.01208017	0.01154875	0.01524592	0.01495176	0.01550042	0.01168578	0.0152327	1.30352432	0.0495
PS 38:3	0.07720206	0.07501516	0.0652268	0.11985092	0.08662007	0.07877226	0.07248134	0.09508108	1.31180082	0.0495
PI 36:4	0.10674988	0.12364191	0.08766514	0.18531211	0.09855191	0.13365965	0.10601898	0.13917456	1.31273254	0.2752
PE40:4	0.02610773	0.03866094	0.02591278	0.03942351	0.03511172	0.04463171	0.03022715	0.03972231	1.31412697	0.1266
PA38:5	0.00437367	0.00572669	0.00348464	0.00579968	0.00549828	0.00659827	0.00452833	0.00596541	1.31735203	0.1266
GM3 18:1/20:0	0.00076745	0.00055786	0.00048896	0.00078822	0.00063224	0.00097794	0.00060476	0.00079947	1.32196228	0.1266
PE34:2	0.21632291	0.19022409	0.12772957	0.26093265	0.19826195	0.24829596	0.17809219	0.23583019	1.32420285	0.1266
PE34:0	0.01273754	0.01082158	0.01010594	0.01696032	0.01069069	0.01697395	0.01122169	0.01487499	1.3255569	0.2752
PC	2.96161219	3.67384598	2.99950282	5.13360384	3.57529742	4.08410988	3.21165366	4.26433705	1.3277699	0.1266
DAG32:0(16:0/16:0)	2.14730821	2.31798293	1.50567851	2.82752029	2.21253409	2.88954185	1.99032321	2.64319874	1.32802488	0.1266
PS	1.46951122	1.51202813	1.22851638	2.25659442	1.74320487	1.5965504	1.40335191	1.8654499	1.32928162	0.0495
PA32:2	0.0030195	0.00433362	0.00272443	0.00459546	0.00382058	0.00505193	0.00335918	0.00448933	1.33643503	0.1266
PE38:6	0.12112934	0.11660916	0.06335176	0.17044962	0.11869324	0.11385469	0.10036342	0.13433252	1.33846095	0.5127
PC32:1e	0.01243067	0.01501847	0.0138902	0.02096549	0.01667536	0.01769923	0.01377978	0.0184467	1.33867831	0.0495
PS 40:6	0.53507993	0.5615751	0.41339473	0.87442963	0.5999648	0.55647013	0.50334992	0.67695485	1.3448991	0.1266
PG36:2	0.00326429	0.00295394	0.00284643	0.0037927	0.00342389	0.004998	0.00302155	0.00407153	1.34749525	0.0495
PI 36:3	0.04468254	0.04454816	0.02903636	0.07224171	0.04187065	0.04529827	0.03942235	0.05313688	1.34788702	0.2752
LPE	0.13699527	0.12468815	0.10337245	0.19073259	0.13744424	0.16451627	0.12168529	0.16423103	1.34963755	0.0495
PE32:0	0.00748249	0.00479496	0.00505392	0.00793022	0.00649168	0.00897045	0.00577712	0.00779745	1.34971157	0.1266
PE38:4	0.18751255	0.17537765	0.13122904	0.28559529	0.1862404	0.19528037	0.16470641	0.22237202	1.35011149	0.1266
PE36:3	0.18591282	0.15945034	0.1154202	0.25256963	0.17440764	0.19527178	0.15359445	0.20741635	1.35041559	0.1266
LPI20:4	0.00573443	0.00835278	0.00601113	0.01086641	0.00832719	0.00803223	0.00669944	0.00907528	1.35463102	0.2752
LPA16:0	0.05200857	0.0510788	0.05309402	0.08496336	0.06893552	0.05781741	0.05206046	0.07057209	1.35557946	0.0495
PC34:1p	0.00893477	0.00994045	0.00884951	0.01245466	0.01232372	0.01290136	0.00924158	0.01255992	1.35906633	0.0495
PE38:7	0.02786019	0.02119556	0.0136054	0.03347164	0.02252333	0.0293769	0.02088705	0.02845729	1.36243705	0.1266
GM3 18:1/20:1	0.00053442	0.00055864	0.0003205	0.00103491	0.00052711	0.00036659	0.00047119	0.00064287	1.36436549	0.8273
PC40:7	0.01018951	0.01705006	0.01646896	0.02275092	0.01580523	0.02116624	0.01456951	0.01990746	1.36637837	0.2752
PE40:5	0.04076001	0.04051905	0.02824634	0.05835235	0.04064727	0.05091902	0.03650847	0.04997288	1.36880243	0.1266
PS 34:2	0.01035234	0.00830408	0.00654447	0.0129644	0.01141584	0.01024231	0.0084003	0.01154085	1.37386182	0.1266
PG36:3	0.0013367	0.00234436	0.00104151	0.00222171	0.0018159	0.00245344	0.00157419	0.00216368	1.37447172	0.2752
PS 40:5	0.13230814	0.13308907	0.09947614	0.21377487	0.15109808	0.13957855	0.12162445	0.1681505	1.38253862	0.0495
LPE16:1	0.0091087	0.00864656	0.00707902	0.01132238	0.01163436	0.0115512	0.00827809	0.01150265	1.38952835	0.0495
PI 36:2	0.04446829	0.03386174	0.03126179	0.06751556	0.03885555	0.04599341	0.03653061	0.05078818	1.39029111	0.1266
PE32:2	0.01226004	0.01268438	0.00816476	0.01651783	0.0164123	0.01316387	0.01103639	0.01536467	1.3921819	0.0495
PE40:6p	0.05873822	0.05892622	0.04754301	0.08456417	0.06660534	0.07895999	0.05506915	0.07670983	1.39297294	0.0495
PA	0.1231879	0.17595874	0.12748246	0.22769695	0.18911846	0.18877378	0.1422097	0.20186306	1.41947461	0.0495
PC34:2	0.59032488	0.75423806	0.53848179	1.08717398	0.71611749	0.84385478	0.62768158	0.88238208	1.4057798	0.1266
PE40:6	0.1279793	0.12821491	0.08174684	0.20610769	0.12250876	0.14837465	0.11264702	0.15899703	1.41146241	0.2752
PA34:2	0.00724154	0.00657923	0.0064628	0.0105019	0.0070811	0.01124221	0.00676119	0.00960841	1.42111205	0.1266
PS 36:2	0.18715821	0.20777202	0.1743243	0.29053497	0.29198989	0.22969906	0.18975151	0.27074131	1.42682029	0.0495
GM3 18:1/22:1	0.00016701	0.00029323	0.0001181	0.00029621	0.00031661	0.00021449	0.00019278	0.00027577	1.43051629	0.1266
PE36:2p	0.01798535	0.01623829	0.01010785	0.02752977	0.01603733	0.02002606	0.01477716	0.02119772	1.43449161	0.2752
PE36:1	0.06383319	0.067739	0.05647913	0.09733988	0.08071078	0.0923068	0.06268378	0.09011915	1.43767908	0.0495
PG32:1	0.00223501	0.00173211	0.00142369	0.00280277	0.00263717	0.00232711	0.00179694	0.00258902	1.44079427	0.0495
LPI16:0	0.00231698	0.00242256	0.0024507	0.00321376	0.00304743	0.00411554	0.00239674	0.00345891	1.44317072	0.0495
LPI	0.02067908	0.02736215	0.02021147	0.03545484	0.03034883	0.03278349	0.0227509	0.03286239	1.44444331	0.0495
PI	0.93857269	1.07317851	0.74951777	1.70011307	1.14636536	1.16653551	0.92042299	1.33767131	1.45332236	0.0495
PE42:3p	0.01003481	0.01299761	0.01166621	0.01873742	0.01431434	0.01773768	0.01156621	0.01692981	1.46373048	0.0495
LPS	0.0324314	0.03966596	0.0326238	0.04836003	0.04453657	0.06045407	0.03490705	0.05111689	1.4643714	0.0495
GM3 18:0/16:0	0.00033346	0.00055819	0.00020288	0.00068918	0.00049133	0.00042757	0.00036485	0.00053603	1.46918733	0.2752
PI 38:5	0.09798749	0.11614636	0.07304452	0.18290292	0.11035285	0.12926033	0.09572612	0.1408387	1.47126713	0.1266
LPE18:0	0.04379478	0.03640181	0.02862398	0.06452244	0.04244441	0.05359835	0.03627352	0.05352173	1.47550419	0.1266
CE:16:0	0.21420106	0.21822721	0.21220634	0.33956055	0.22963181	0.38443947	0.21487821	0.31787728	1.47933698	0.0495
DAG40:6(18:0/22:6)	0.29229264	0.26188966	0.25344776	0.39910016	0.35686126	0.44307581	0.26921002	0.39967908	1.48463671	0.0495
PA36:2	0.01870611	0.02884337	0.01780627	0.03477414	0.02998644	0.0332257	0.02178525	0.03266209	1.49927558	0.0495
PC36:2	0.79328419	1.07294896	0.71209624	1.58707205	1.06891251	1.21310873	0.85944313	1.28969776	1.50062025	0.1266
LPE18:2	0.0307786	0.03350529	0.02053006	0.05093383	0.03688122	0.03976046	0.02827132	0.04252517	1.50418074	0.0495
PI 40:5	0.0080636	0.00798147	0.00703073	0.01603415	0.00984758	0.0088906	0.00769193	0.01159077	1.50687422	0.0495
PG	0.02458798	0.02661361	0.021098	0.03863817	0.03099168	0.03945091	0.02409986	0.03636025	1.50873266	0.0495
PG34:1	0.0090174	0.00954836	0.00805287	0.01584955	0.01112773	0.01320091	0.00887288	0.01339273	1.50940057	0.0495
LPA18:0	0.04341539	0.09073492	0.08233079	0.10496996	0.10157294	0.12030439	0.07216037	0.1089491	1.50981901	0.0495
PI 38:4	0.48553962	0.59461558	0.38863741	0.92205035	0.68001484	0.62929279	0.48959754	0.74378599	1.51917837	0.0495
LPI18:0	0.00995021	0.01421531	0.01041967	0.01892581	0.01656937	0.01762811	0.01152839	0.01770776	1.53601315	0.0495

PA40:6	0.03771656	0.06295175	0.04428388	0.0892087	0.06667962	0.06677138	0.0483174	0.0742199	1.53609061	0.0495
PI 38:3	0.08533323	0.10164676	0.06115701	0.16467054	0.10831176	0.1097576	0.08271234	0.12757997	1.54245394	0.0495
PE32:1	0.01178012	0.01422704	0.00726126	0.02003871	0.01316942	0.01831256	0.01108947	0.01717356	1.54863641	0.1266
PE36:2	0.28220949	0.2712622	0.18679496	0.44700164	0.34290759	0.36404147	0.24675555	0.38465023	1.55883113	0.0495
LPS18:0	0.01932633	0.02599767	0.01988643	0.03125175	0.02809885	0.04262203	0.02173681	0.03399088	1.56374714	0.0495
PG34:2	0.00560016	0.00611141	0.00478036	0.00840407	0.00760118	0.00990163	0.00549731	0.00863563	1.57088212	0.0495
PA32:1	0.00105774	0.00162744	0.00108048	0.00230137	0.00158553	0.00212037	0.00125522	0.00200242	1.59527964	0.1266
PE40:5p	0.02563737	0.02537111	0.02189447	0.04801097	0.03511172	0.03376199	0.02430098	0.03896156	1.60329145	0.0495
PC36:5	0.01688487	0.01615809	0.01358923	0.03371576	0.02377929	0.0174577	0.01554406	0.02498425	1.60731791	0.0495
CE:18:3	0.02551849	0.04349288	0.02227577	0.05424284	0.04052326	0.0520054	0.03042905	0.04892383	1.60780051	0.1266
PG36:1	0.00190133	0.00173322	0.00161494	0.00283654	0.00222727	0.00339547	0.00174983	0.00281976	1.61144569	0.0495
PG36:4	0.00123309	0.00219043	0.00133827	0.00273099	0.00215855	0.00317436	0.00158726	0.00268796	1.69345761	0.1266
GM3 18:0/20:0	0.00023403	0.00020565	0.0001687	0.00044325	0.00028095	0.00042799	0.00020279	0.00038406	1.89389051	0.0495
PI 40:4	0.00502224	0.00839567	0.00622485	0.01724442	0.00949235	0.01113631	0.00654759	0.01262436	1.92809299	0.0495

lipid name abbreviations

CE	**cholesteryl ester**
TAG	**triacylglycerol**
DAG	**diacylglycerol**
PC	**phosphatidylcholine**
PE	**phosphatidylethanolamine**
PA	**phosphatidic acid**
PS	**phosphatidylserine**
PI	**phosphatidylinositol**
PG	**phosphatidylglycerol**
LBPA	**lyso-bisphosphatidic acid**
LPA	**lyso-PA**
LPI	**lyso-PI**
LPS	**lyso-PI**
LPE	**lyso-PE**
GM3	**monosialo-dihexosylganglioside**

P values were caculated by two-tailed unpaired Student's *t*-test comparing lipid species betweeen WT and SA/SA groups.
P < 0.05 was considered as significant. The significant *P* values are highlighted in pink.

Supplementary Table 2 Yeast strains used in this study.

Name	Parent	Genotype	Reference
wild-type	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ gcn5Δ::kanMX6*	(30)
gcn5Δ	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ elp3Δ::kanMX6*	(30)
elp3Δ	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ hpa2Δ::kanMX6*	(30)
sas2Δ	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ sas2Δ::kanMX6*	(30)
sas3Δ	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ sas3Δ::kanMX6*	(30)
rtt109Δ	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ rtt109Δ::kanMX6*	(30)
esa1-HA-aid	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ ADH1-9XMYC-AtTIR1::HisMX6, esa1-HA-aid::URA3*	This study
Wild-type-GFP-Faa4	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ ADH1-9XMYC-AtTIR1::HisMX6, Faa4-GFP::KanMX6*	This study
esa1-HA-aid-GFP-Faa4	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ ADH1-9XMYC-AtTIR1::HisMX6, esa1-HA-aid::URA3, Faa4-GFP::KanMX6*	This study
pah1Δ	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ pah1Δ::URA3*	This study
pah1Δ-vector	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ pah1Δ::URA3 pRS316 [vector-HIS3]*	This study
pah1Δ-WT-PAH1	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3Δ pah1Δ::URA3 pRS316 [Myc-WT-PAH1 HIS3]*	This study
pah1Δ-K496/K801R-PAH1	BY4741	*MATa his3Δ1 leu2Δ met15Δ ura3 Δpah1Δ::URA3 pRS316 [Myc-K496/K801R-PAH1 HIS3]*	This study