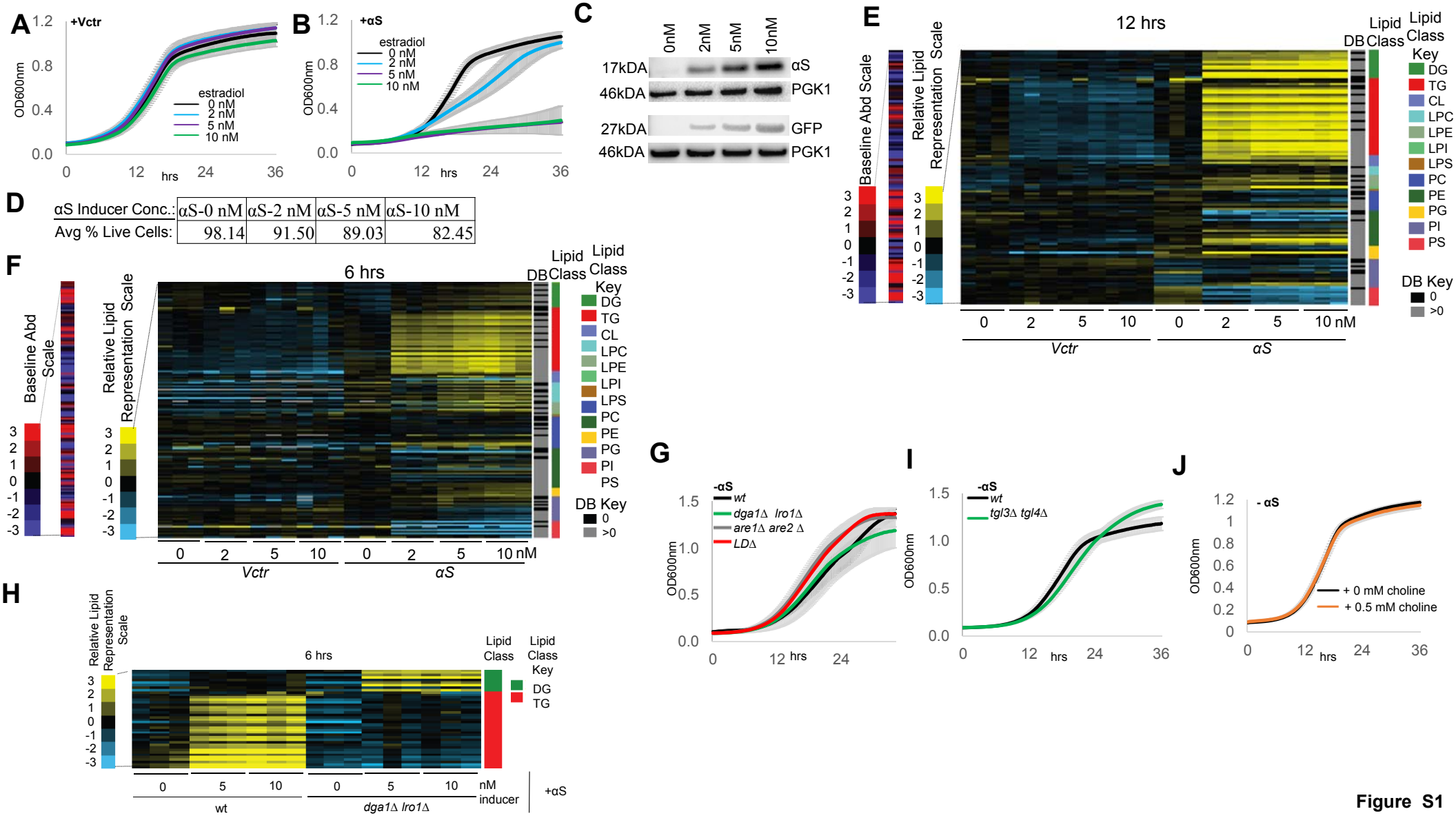
A
+Vctr
OD600nm
estradiol
0 nM
2 nM
5 nM
10 nM
hrs
B
+αS
C
0nM
2nM
5nM
10nM
17kDA
αS
46kDA
PGK1
27kDA
GFP
46kDA
PGK1
D
αS Inducer Conc.: αS-0 nM αS-2 nM αS-5 nM αS-10 nM
Avg % Live Cells: 98.14 91.50 89.03 82.45
E
12 hrs
Baseline Abd Scale
Relative Lipid Representation Scale
Lipid DB
Lipid Class
Lipid Class Key
DG
TG
CL
LPC
LPE
LPI
LPS
PC
PE
PG
PI
PS
DB Key
0
>0
Vctr
αS
F
6 hrs
G
-αS
wt
dga1Δ lro1Δ
are1Δ are2 Δ
LDΔ
I
tgl3Δ tgl4Δ
J
- αS
+ 0 mM choline
+ 0.5 mM choline
H
wt
dga1Δ lro1Δ
nM inducer
+αS

Figure S1

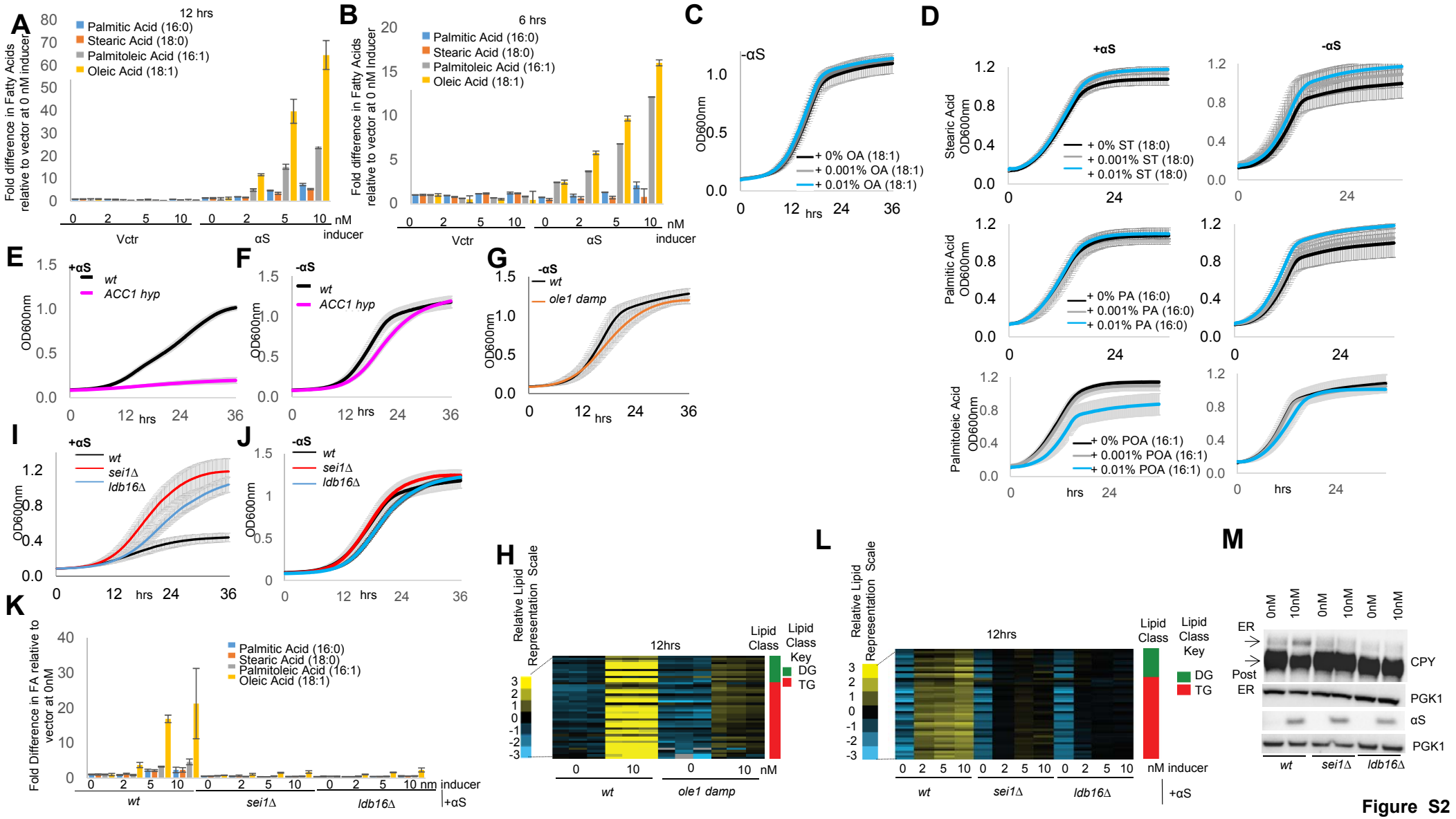
A
12 hrs
Palmitic Acid (16:0)
Stearic Acid (18:0)
Palmitoleic Acid (16:1)
Oleic Acid (18:1)
Fold difference in Fatty Acids relative to vector at 0 nM inducer
Vctr
αS
nM inducer
B
6 hrs
Fold difference in Fatty Acids relative to vector at 0 nM Inducer
C
-αS
OD600nm
+ 0% OA (18:1)
+ 0.001% OA (18:1)
+ 0.01% OA (18:1)
hrs
D
+αS
-αS
Stearic Acid OD600nm
+ 0% ST (18:0)
+ 0.001% ST (18:0)
+ 0.01% ST (18:0)
Palmitic Acid OD600nm
+ 0% PA (16:0)
+ 0.001% PA (16:0)
+ 0.01% PA (16:0)
Palmitoleic Acid OD600nm
+ 0% POA (16:1)
+ 0.001% POA (16:1)
+ 0.01% POA (16:1)
E
+αS
wt
ACC1 hyp
F
-αS
G
ole1 damp
I
sei1Δ
ldb16Δ
J
K
Fold Difference in FA relative to vector at 0nM
wt
sei1Δ
ldb16Δ
+αS
H
Relative Lipid Representation Scale
12hrs
Lipid Class
Lipid Class Key
DG
TG
L
M
ER
Post ER
CPY
PGK1
αS

Figure S2

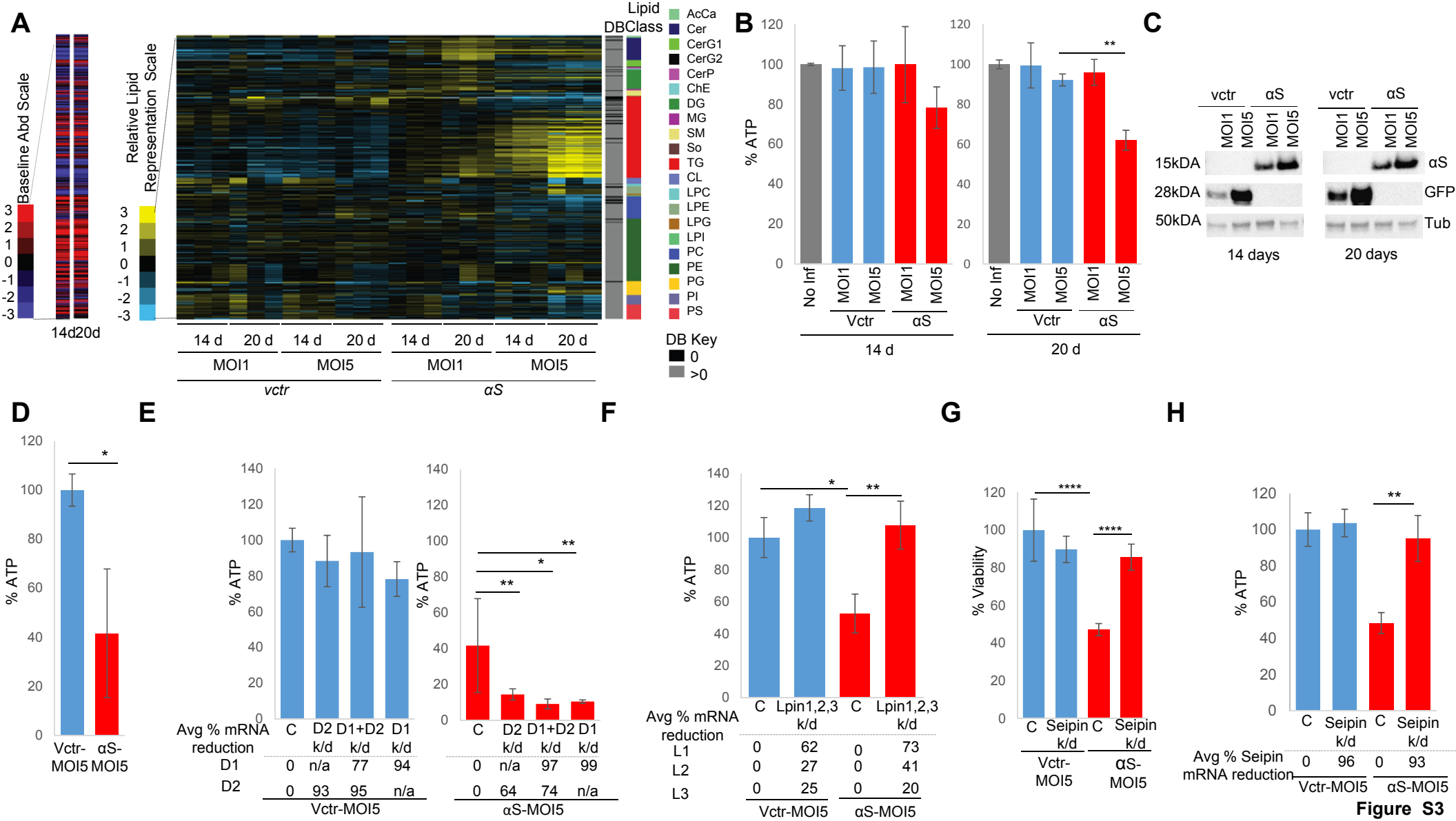
A
Baseline Abd Scale
14d20d
Relative Lipid Representation Scale
Lipid DBClass
AcCa
Cer
CerG1
CerG2
CerP
ChE
DG
MG
SM
So
TG
CL
LPC
LPE
LPG
LPI
PC
PE
PG
PI
PS
DB Key
0
>0
14 d 20 d 14 d 20 d 14 d 20 d 14 d 20 d
MOI1 MOI5 MOI1 MOI5
vctr αS
B
% ATP
No Inf MOI1 MOI5 MOI1 MOI5
Vctr αS
14 d
20 d
C
vctr αS vctr αS
MOI1 MOI5 MOI1 MOI5 MOI1 MOI5 MOI1 MOI5
15kDA
28kDA
50kDA
αS
GFP
Tub
14 days
20 days
D
% ATP
Vctr-MOI5 αS-MOI5
E
% ATP
Avg % mRNA reduction
C D2 k/d D1+D2 k/d D1 k/d
D1 0 n/a 77 94
D2 0 93 95 n/a
Vctr-MOI5
0 n/a 97 99
0 64 74 n/a
αS-MOI5
F
% ATP
C Lpin1,2,3 k/d C Lpin1,2,3 k/d
Avg % mRNA reduction
L1 0 62 0 73
L2 0 27 0 41
L3 0 25 0 20
Vctr-MOI5 αS-MOI5
G
% Viability
C Seipin k/d C Seipin k/d
Vctr-MOI5 αS-MOI5
H
% ATP
C Seipin k/d C Seipin k/d
Avg % Seipin mRNA reduction
0 96 0 93
Vctr-MOI5 αS-MOI5

Figure S3

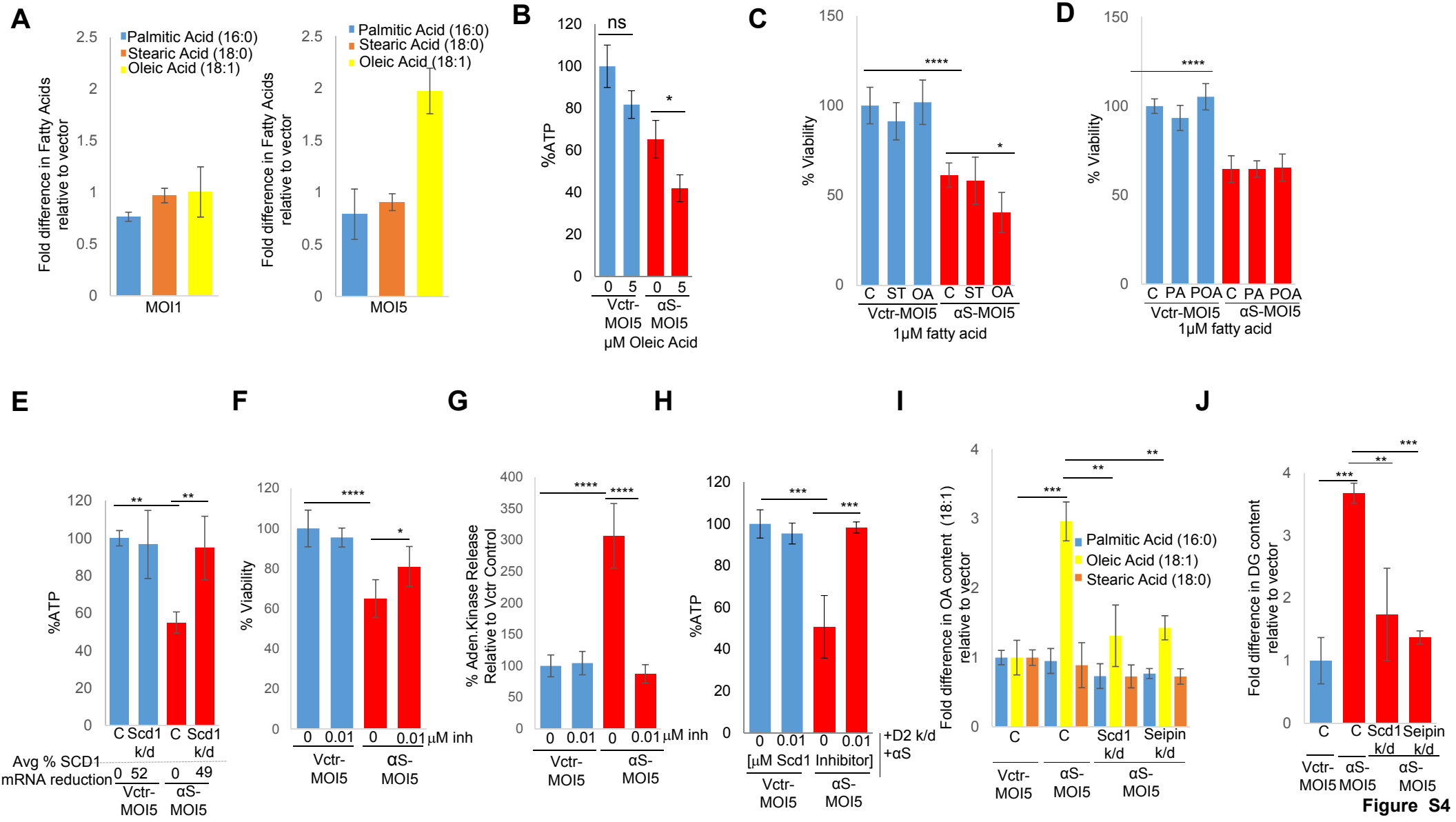
A
Palmitic Acid (16:0)
Stearic Acid (18:0)
Oleic Acid (18:1)
Fold difference in Fatty Acids relative to vector
MOI1
MOI5
B
%ATP
ns
*
0 5 0 5
Vctr-MOI5
αS-MOI5
μM Oleic Acid
C
% Viability
****
*
C ST OA C ST OA
Vctr-MOI5
αS-MOI5
1μM fatty acid
D
% Viability
****
C PA POA C PA POA
Vctr-MOI5
αS-MOI5
1μM fatty acid
E
%ATP
**
**
C Scd1 k/d C Scd1 k/d
Avg % SCD1 mRNA reduction 0 52 0 49
Vctr-MOI5
αS-MOI5
F
% Viability
****
*
0 0.01 0 0.01 μM inh
Vctr-MOI5
αS-MOI5
G
% Aden.Kinase Release Relative to Vctr Control
****
****
0 0.01 0 0.01 μM inh
Vctr-MOI5
αS-MOI5
H
%ATP
***
***
0 0.01 0 0.01
[μM Scd1 Inhibitor]
+D2 k/d
+αS
Vctr-MOI5
αS-MOI5
I
Fold difference in OA content (18:1) relative to vector
***
**
**
Palmitic Acid (16:0)
Oleic Acid (18:1)
Stearic Acid (18:0)
C C Scd1 k/d Seipin k/d
Vctr-MOI5
αS-MOI5
αS-MOI5
J
Fold difference in DG content relative to vector
***
**
***
C C Scd1 k/d Seipin k/d
Vctr-MOI5
αS-MOI5
αS-MOI5

Figure S4

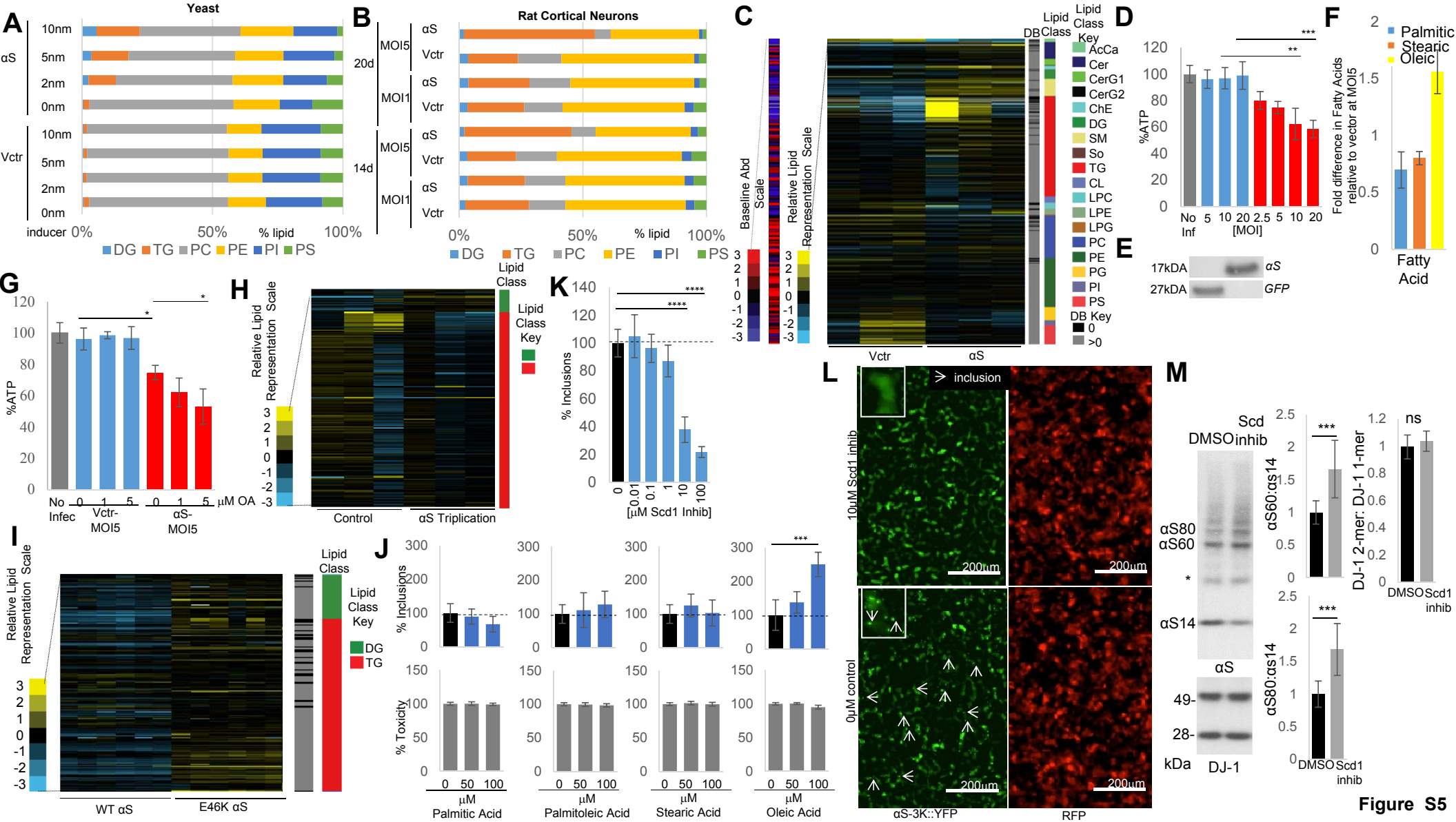
A
Yeast
αS
Vctr
10nm
5nm
2nm
0nm
inducer
0%
50%
% lipid
100%
DG TG PC PE PI PS
B
Rat Cortical Neurons
20d
14d
MOI5
MOI1
αS
Vctr
C
Baseline Abd Scale
Relative Lipid Representation Scale
Lipid Class
Lipid Class Key
DB Class
DB Key
AcCa
Cer
CerG1
CerG2
ChE
DG
SM
So
TG
CL
LPC
LPE
LPG
PC
PE
PG
PI
PS
0
>0
Vctr
αS
D
%ATP
No Inf
5
10
20
2.5
5
10
20
[MOI]
E
17kDA
27kDA
αS
GFP
F
Fold difference in Fatty Acids relative to vector at MOI5
Palmitic
Stearic
Oleic
Fatty Acid
G
%ATP
No Infec
Vctr-MOI5
αS-MOI5
μM OA
H
Relative Lipid Representation Scale
Lipid Class
Lipid Class Key
Control
αS Triplication
K
% Inclusions
[μM Scd1 Inhib]
I
Relative Lipid Representation Scale
Lipid Class
Lipid Class Key
DG
TG
WT αS
E46K αS
J
% Inclusions
% Toxicity
μM
Palmitic Acid
Palmitoleic Acid
Stearic Acid
Oleic Acid
L
inclusion
10μM Scd1 inhib
0μM control
200μm
αS-3K::YFP
RFP
M
Scd
DMSO
inhib
αS80
αS60
αS14
αS
49-
28-
kDa
DJ-1
αS60:αs14
αS80:αs14
DJ-1 2-mer: DJ-1 1-mer
DMSO
Scd1 inhib
ns

Figure S5

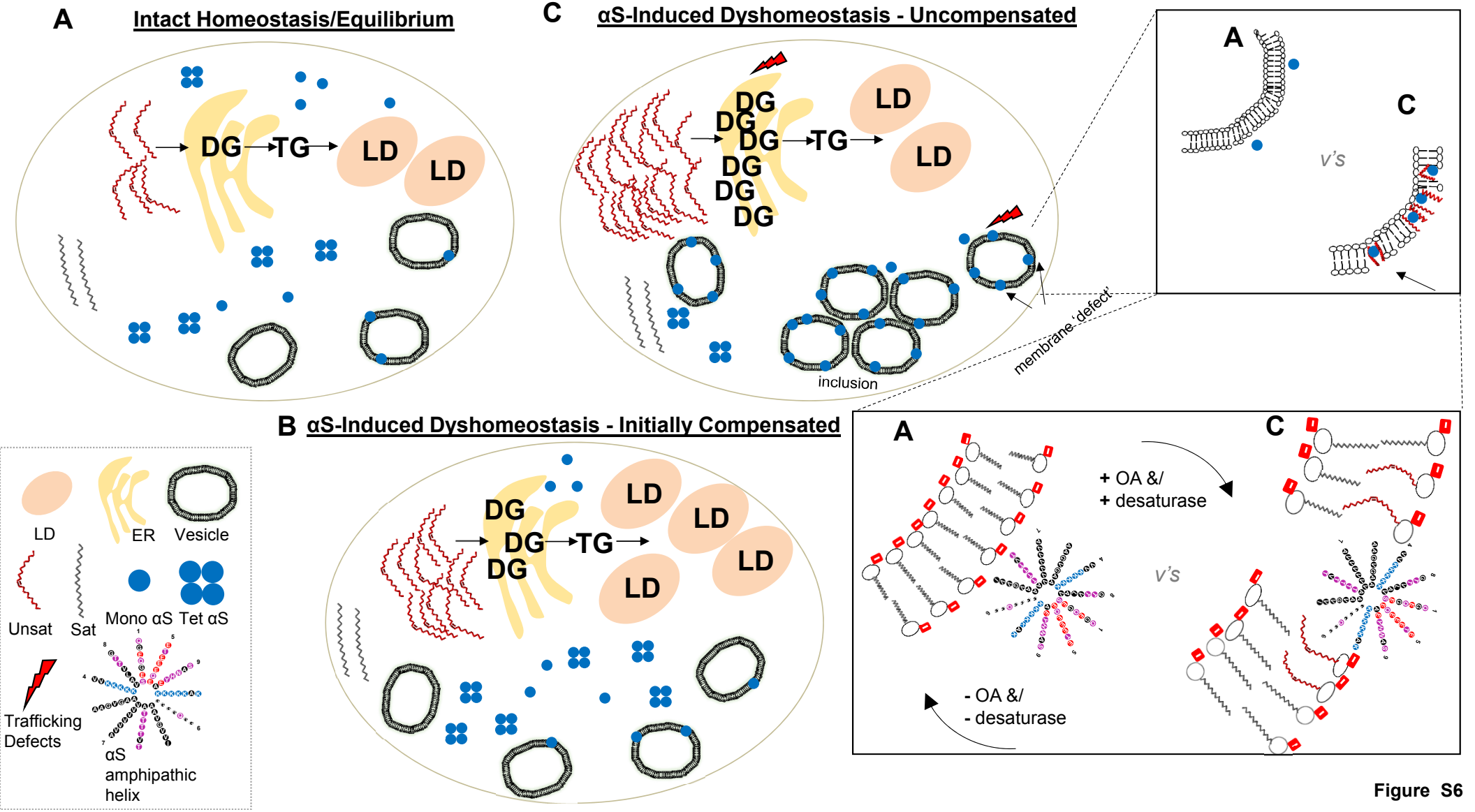
A
Intact Homeostasis/Equilibrium
DG
TG
LD
LD
C
αS-Induced Dyshomeostasis - Uncompensated
DG
DG
DG
DG
DG
DG
TG
LD
LD
inclusion
membrane 'defect'
A
C
v's
B
αS-Induced Dyshomeostasis - Initially Compensated
DG
DG
DG
TG
LD
LD
LD
LD
LD
ER
Vesicle
Unsat
Sat
Mono αS
Tet αS
Trafficking Defects
αS amphipathic helix
A
C
+ OA &/
+ desaturase
v's
- OA &/
- desaturase

Figure S6

Supplementary Table 1: Abbreviations (See STAR Methods)

αS	α-synuclein
AcCa	Acylcarnitine
Cer	Ceramide
Cer G1/G2	Glucosylceramide
Cer P	Ceramide phosphate
ChE	Cholesterol
CL	Cardiolipin
CPY	Carboxypeptidase Y
DAergic	Dopaminergic
DG	Diglyceride
FA	Fatty acid
fPD	Familial Parkinson's disease
GWAS	Genome-wide association studies
iPSC	Induced pluripotent stem cells
LCMS	Liquid chromatography/mass spectrometry
LD	Lipid droplet
LPC	Lysophosphatidylcholine
LPE	Lysophosphotidyethanolamine
LPG	Lysophosphatidylglycerol
LPI	Lysophosphatidylinositol
LPS	Lysophosphatidylserine
MG	Monoglyceride
OA	Oleic acid
PA	Palmitic acid
PC	Phosphatidylcholine
PD	Parkinson's disease
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
POA	Palmitoleic acid
PS	Phosphatidylserine
SCD	Stearoyl-coA desaturase
Sm	Sphingomyelin
So	Sphigosine
SE	Sterol ester
ST	Stearic acid
TG	Triglyceride
UFA	Unsaturated fatty acid

Table S2: Statistical Analysis of Yeast Curves (Fig 1, 2, 3, S1, S2)

		T-TEST with Bonferroni (p value)	
Figure Reference	**Comparison Strains & (Condition)**	**Max Growth**	**Area Under the Curve**
S1G	WT vs DGA1Δ/LRO1Δ (-αS)	0.182250756272921	0.79786106193493
1D	WT vs DGA1Δ/LRO1Δ (+αS)	0.0172061250774888	0.0165862574014929
S1G	WT vs ARE1Δ/ARE2Δ (-αS)	0.403801909434348	0.133663413791191
1D	WT vs ARE1Δ/ARE2Δ (+αS)	0.386848035791176	0.135108422221598
S1G	WT vs ΔLD (-αS)	0.563809630586069	0.0948601347107356
1D	WT vs ΔLD (+αS)	0.00519915343722351	0.0287169654024982
S2G	WT vs OLE1 damp (-αS)	0.301500600676801	0.586087132035938
3E	WT vs OLE1 damp (+αS)	2.36280800792935e-05	0.00536958614707013
S1I	WT vs TGL3Δ/TGL4Δ (-αS)	0.0207775806052023	0.823429457242472
2C	WT vs TGL3Δ/TGL4Δ (+αS)	0.00163792253496253	0.012519305844174
S2F	WT vs Acc1 hyp (-αS)	0.804850663364776	0.0739183879855935
S2E	WT vs Acc1 hyp (+αS)	9.05423467869632e-06	1.57804867746754e-05
S2C	WT VS WT+0.001% OA (-αS) (18:1)	0.647017250389983	0.662965586287573
3D	WT VS WT+0.001% OA (+αS) (18:1)	0.0506284477546262	0.0712261344554862
S2C	WT VS WT+0.01% OA (-αS) (18:1)	0.481618163175302	0.368538565614384
3D	WT VS WT+0.01% OA (+αS) (18:1)	0.00372373215149859	4.61887825054233e-06
S2D	WT VS WT+0.001% ST (-αS) (18:0)	0.937349948778449	0.633861510654645
S2D	WT VS WT+0.001% ST (+αS) (18:0)	0.35178695637417	0.748302525319572
S2D	WT VS WT+0.01% ST (-αS) (18:0)	0.304819828711373	0.377256773721823
S2D	WT VS WT+0.01% ST (+αS) (18:0)	0.00496341004388248	0.765970441122609
S2D	WT VS WT+0.001% PA (-αS) (16:0)	0.677902161134442	0.743927345245882
S2D	WT VS WT+0.001% PA (+αS) (16:0)	0.0149645665179657	0.0471876452323082
S2D	WT VS WT+0.01% PA (-αS) (16:0)	0.250593753363389	0.177705977447314
S2D	WT VS WT+0.01% PA (+αS) (16:0)	0.332658988866257	0.277160328702035
S2D	WT VS WT+0.001% POA (-αS) (16:1)	0.677902161134442	0.743927345245882
S2D	WT VS WT+0.001% POA (+αS) (16:1)	0.332658988866257	0.277160328702035
S2D	WT VS WT+0.01% POA	0.377969868162975	0.44058044646484

	(-αS) (16:1)		
S2D	WT VS WT+0.01% POA (+αS) (16:1)	0.0688411083808073	0.0487465831390694
S1A	Vctr 0nm vs Vctr 2nm	0.428486307790048	0.359443484136673
S1A	Vctr 0nm vs Vctr 5nm	0.417541591955535	0.526318640832264
S1A	Vctr 0nm vs Vctr 10nm	0.303235805224668	0.269959826856608
S1B	WT 0nm vs αS 2nm	0.233871972757606	0.0628619227761856
S1B	WT 0nm vs αS 5nm	0.00101397571107858	0.00190964232203463
S1B	WT 0nm vs αS 10nm	0.00532226268016584	0.000397058609490757
S1J	WT (-αS) vs WT (-αS) +choline	0.321068877475049	0.585482632878353
2F	WT (+αS) vs WT (+αS) +choline	0.00971375157070605	0.0224402622338679
S2J	WT vs FLD1Δ (-αS)	0.356165004376557	0.488579631656867
S2I	WT vs FLD1Δ (+αS)	0.00721956351927037	0.0102584087608092
S2J	WT vs LBD16Δ (-αS)	0.491756144749409	0.243520413749671
S2I	WT vs LBD16Δ (+αS)	0.00125959263791623	0.00421924045444203

Supplementary Figure Legends

Suppl. Fig 1 (Relates to Fig 1 and Fig 2) - (A) Growth curves for vector strain with increasing concentrations of inducer (estradiol) treatment. Expression of vector in wt strain background. (B) Growth curves for αS strain with increasing concentrations of inducer (estradiol) treatment. Expression of αS in wt strain background. See Table S2 for statistical analysis of yeast growth curves. (C) Protein expression in strains used for lipid profile. Western blot showing protein expression of vector control and αS in lipid-profiled strains with increasing concentrations of inducer (estradiol). Quantification of this blot at αS 2 nm:5 nm and 5 nm:10 nm yields ratios of 1:1.9 αS and 1:1.8 αS, respectively. Quantification of this blot at gfp 2 nm:5 nm and 5 nm:10 nm yields ratios of 1:1.7 gfp and 1:2.0 gfp, respectively (post normalization to PGK1 loading control). (D) Cell viability of αS–expressing cells at 12h post induction. Data on percent cell viability of yeast cells expressing αS with increasing concentrations of inducer (estradiol). Numbers presented are average of duplicates. (E) Lipid profiles of human αS expression in yeast at 12h post induction. These profiles show triplicate samples for averages presented in Fig 1A. Baseline abundance (Abd) of each lipid species is indicated by a red/blue bar on the left of the heat map (relative scale from -3 to 3, see key). Baseline abundance was calculated on the basis of the vector strain with 0 nanomolar (nM) inducer (estradiol). Yellow/Blue heatmap coloring is a representation of a given lipid species relative to the median of the logs across all samples for that lipid species; data for 0, 2, 5 and 10 nM of inducer are shown. (relative log scale from -3 to 3, see key). Status of saturation (presence of double bonds [DB]) of each lipid species is indicated by grey (>1 DB) or black (0 DB) bar on the right of the heat map. Lipid species are indicated by color and in the order of the key on the right of the map. DG; TG; CL; LPC; LPE; LPI; LPS; PC; PE; PG; PI; PS. (F) Lipid profiles of human αS expression in yeast at 6h post induction. Baseline abundance (Abd) of each

lipid species is indicated by a red/blue bar on the left of the heat map (relative scale from -3 to 3, see key). Baseline abundance was calculated on the basis of the vector strain with 0 nanomolar (nM) inducer (estradiol). Yellow/Blue heatmap coloring is a representation of a given lipid species relative to the median of the logs across all samples for that lipid species; data for 0, 2, 5 and 10 nM of inducer are shown. (relative log scale from -3 to 3, see key). Status of saturation (presence of double bonds [DB]) of each lipid species is indicated by grey (>1 DB) or black (0 DB) bar on the right of the heat map. Lipid species are indicated by color and in the order of the key on the right of the map. DG; TG; CL; LPC; LPE; LPI; LPS; PC; PE; PG; PI; PS. (G) Growth curves for uninduced wt, *dga1*Δ *lro1*Δ, *are1*Δ *are2*Δ and (LDΔ) *dga1*Δ *lro1*Δ+*are1*Δ *are2*Δ. Growth curves of yeast strains were compared without αS expression (uninduced) in wt, *dga1*Δ *lro1*Δ, *are1*Δ *are2*Δ and (LDΔ) *dga1*Δ *lro1*Δ+*are1*Δ *are2*Δ strain backgrounds. X-axis: hrs; Y-axis: OD600nm. See Table S2 for statistical analysis of yeast growth curves. (H) Neutral lipid profiles of human αS expression in yeast in wt and *dga1*Δ *lro1*Δ strain backgrounds. These profiles show triplicate samples for averages presented in Fig 2A. Yellow/Blue heatmap coloring is a representation of a given lipid species relative to the median of the logs across all samples for that lipid species; data for 0, 2, 5 and 10 nM of inducer are shown. (relative log scale from -3 to 3, see key). The time point of 6 h post induction was chosen because the *dga1*Δ *lro1*Δ strain has enhanced toxicity over wt and we wanted to ensure that profiling was performed before significant toxicity differences were evident. (I) Growth curves for wt, *tgl3*Δ*/tgl4*Δ strains without αS expression. Growth curves of yeast strains were compared without αS expression in wt and *tgl3*Δ*/tgl4*Δ strain backgrounds. X-axis: hr; Y-axis: OD 600 nm. See Table S2 for statistical analysis of yeast growth curves. (J) Growth curves for yeast untreated and treated with choline in the absence of αS. X-axis: hrs; Y-axis: OD600nm. See Table S2 for statistical analysis of yeast growth curves.

Suppl. Fig 2 – (Relates to Fig 3) (A) FA profiles for αS expressing yeast strains at 12h post induction. Intracellular FA analysis of αS expressing and vector control wt yeast at 12h post induction. Results are the average of triplicates and error bars represent std deviation. 0, 2, 5, 10 refer to nM concentrations of estradiol inducer. (B) FA profiles for αS expressing yeast strains at 6h post induction. Intracellular FA analysis of αS expressing and vector control wt yeast at 6h post induction. Results are the average of triplicates and error bars represent std deviation. 0, 2, 5, 10 refer to nM concentrations of estradiol inducer. (C) Growth curves for wt yeast strain treated with OA without αS expression. X-axis: hrs; Y-axis: OD600 nm. See Table S2 for statistical analysis of yeast growth curves. (D) Growth curves for wt yeast strain treated with palmitic acid (PA) (16:0), palmitoleic acid (POA) (16:1) and stearic acid (ST) (18:0) in the presence (2nm inducer) and absence (0nm inducer) of αS. 2nm estradiol was used to induce to best visualize the impact of fatty acids on αS toxicity. X-axis: hrs; Y-axis: OD600nm. See Table S2 for statistical analysis of yeast growth curves. (E) Growth curves for αS expressing in wt and *ACC1* hyp strain backgrounds. Growth curves of wt and *ACC1* hyp yeast strains expressing αS. 2nm estradiol was used to induce to best visualize the impact of *ACC1* hyp on αS toxicity. X-axis: hrs; Y-axis: OD600 nm. See Table S2 for statistical analysis of yeast growth curves. (F) Growth curves for wt and *ACC1* hyp strains without αS expression. Growth curves of wt and ACC1 hyp strain backgrounds without αS expression. X-axis: hrs; Y-axis: OD600nm. See Table S2 for statistical analysis of yeast growth curves. (G) Growth curves for wt and ole1 damp strains without αS expression (0nm inducer). X-axis: hrs; Y-axis: OD600nm. See Table S2 for statistical analysis of yeast growth curves. (H) Neutral lipid profiles of human αS expression in yeast at 12 h post induction in wt and ole1 damp strain backgrounds. These profiles show triplicate samples for averages presented in Fig 3F. Yellow/Blue heatmap coloring is a representation of a given lipid species relative to the median of the logs across all samples for that lipid species. The relative log scale (-3 to 3) for yellow/blue

representation is indicated by a smaller yellow/blue bar on the left of the heatmap. 0, 0 refer to nanomolar (nM) of estradiol (inducer). (I) Growth curves for αS expressing wt, *sei1*Δ, *ldb16*Δ strain backgrounds. 10nm estradiol inducer was used to best visualize the suppression of αS toxicity in these mutants relative to wt. X-axis: hrs; Y-axis: OD600nm. See Table S2 for statistical analysis of yeast growth curves. (J) Growth curves for wt, *sei1*Δ, *ldb16*Δ strain backgrounds without αS expression (0nm inducer). X-axis: hrs; Y-axis: OD600nm. See Table S2 for statistical analysis of yeast growth curves. (K) FA profiles of αS-expressing wt, *sei1*Δ, *ldb16*Δ strain backgrounds. Intracellular FA analysis of αS expressing wt, *sei1*Δ, *ldb16*Δ strains at 12 h post induction. Results are the average of triplicates and error bars represent standard deviation. 0, 2, 5, 10 refer to nM concentrations of estradiol inducer. (L) Neutral lipid profiles of human αS expression in wt, *sei1*Δ, *ldb16*Δ strain backgrounds at 12h post induction. Yellow/Blue heatmap coloring is a representation of a given lipid species relative to the median of the logs across all samples for that lipid species. The relative log scale (-3 to 3) for yellow/blue representation is indicated by a smaller yellow/blue bar on the left of the heatmap. 0, 5, 10 refer to nanomolar (nM) of estradiol (inducer). The time point of 12 h post induction was chosen because the *sei1*Δ and *ldb16*Δ strains suppress toxicity so profiling was possible without significant toxicity at a 12hr time point. (M) αS-induced ER accumulation of CPY was ameliorated in *sei1*Δ, *ldb16*Δ strain backgrounds relative to wt at 12h post induction. Immunoblot of CPY in wt, *sei1*Δ and *ldb16*Δ strain backgrounds. CPY was not retained in the ER in the *sei1*Δ and *ldb16*Δ strains expressing αS relative to wt expressing αS. ImageJ was used to quantify retention of CPY in the ER. T-test analysis generated $p=0.04$ for wt compared with *sei1*Δ and $p=0.01$ for wt vs *ldb16*Δ at $n=3$ for each strain type.

Suppl. Fig 3 – (Relates to Fig 4) (A) Lipid profiles of human αS expression in rat cortical neurons. These profiles show triplicate samples for averages presented in Fig 4A. Baseline abundance (Abd) of each lipid species is indicated by a red/blue bar on the left of the heat map (relative scale from -3 to 3, see key). Baseline abundance was calculated on the basis of MOI1 at day 14 and day 20. Yellow/Blue heatmap coloring is a representation of a given lipid species relative to the median of the logs across all samples for that lipid species. The relative log scale (-3 to 3) for yellow/blue representation is indicated by a smaller yellow/blue bar on the left of the heatmap. Status of saturation (presence of double bonds [DB]) of each lipid species is indicated by grey (>1 DB) or black (0 DB) bar on the right of the heat map. Lipid species are indicated by color and in the order of the key on the right of the map. AcCa; Cer; CerG1/G2; CerP; ChE; MG; DG; SM; SO; TG; CL; LPC; LPE; LPG; LPI; LPS; PC; PE; PG; PI; PS. Two MOIs were analyzed: MOI1 and MOI5. (B) Toxicity (% ATP) measurements for rat cortical neurons expressing human αS. No Inf: no infection at 14d and 20d. **p<0.005by one way Anova. (C) Protein expression in lipid profiled lines. Western blot showing protein expression of αS and vector in lipid-profiled neuronal samples. Quantification of the day 14 blot at αS MOI1:MOI5 and gfp MOI1:MOI5 yields ratios of ~ 1:4.2 αS and 1:3.6 gfp, respectively. Quantification of the day 21 blot at αS MOI1:MOI5 and gfp MOI1:MOI5 yields ratios of ~ 1:3.2 αS and 1:3.1 gfp, respectively (post normalizing to tubulin loading control). (D) Toxicity (%ATP) data for vector vs αS expression in rat cortical neurons, 3 weeks post infection. $p<0.05$ by unpaired t-test. (E) Toxicity (%ATP) data in rat cortical neurons, 3 weeks post infection, annotated with RT-PCR expression analysis of knockdown of *DGAT1* and *DGAT2* in vector and αS expressing rat cortical neurons. C: control; D1 k/d: knockdown of *DGAT1*; D2 k/d: knockdown of *DGAT2*; D1+D2 k/d: knockdown of *DGAT1* and *DGAT2*. Significance was tested by unpaired t-test, *$p<0.05$; **$p<0.01$. (F) Toxicity data annotated with RT-PCR expression analysis of knockdown of *LPIN1*, 2,3 in vector and αS expressing rat cortical

neurons. C: control; L1 k/d: knockdown of *LPIN1*; L2 k/d: knockdown of *LPIN2*; L3 knockdown of *LPIN3*. *p<0.01, ** p<0.005 by one way Anova. (G) Inhibition of Seipin rescues αS toxicity. Survival of neurons was measured following knockdown of Seipin in control rat cortical neurons and rat cortical neuron expressing human αS. All graphed data are relative to vector control set at 100%. Y-axis: % Viability on the basis of conversion of Resazurin to Resorufin (Cell Titer Blue, Promega) **** p<0.0001 by one way Anova. (H) Toxicity (% ATP) data annotated with RT-PCR expression analysis of knockdown of seipin in vector and αs expressing rat cortical neurons. C: control. ** p<0.001 by one way Anova.

Suppl. Fig 4 - (Relates to Fig 4) (A) Intracellular FA analysis of αS expression in rat cortical neurons. Bars represent the average of triplicates and error bars represent standard deviation of triplicates. Time point is 14 d. (B) Treatment with exogenous OA enhances αS toxicity in rat cortical neurons. Treatment of αS-expressing rat cortical neurons with two concentrations of OA. ATP readings are 14 days after addition of OA. * p<0.05 by one way Anova. (C & D) Treatment with exogenous fatty acids: C=control, ST=stearic acids, OA=oleic acid, PA=palmitic acid, POA=palmitoleic acid. αS toxicity in rat cortical neurons. Cell Titer Blue (Promega) readings were taken 21 days after fatty acid addition. ****p<0.0001, * p<0.05 by one way Anova. (E) Toxicity data annotated with RTPCR expression analysis of *SCD1* knockdown in vector and αS expressing rat cortical neurons. C=control. ** p<0.005 by one way Anova. (F) Inhibition of Scd1 rescues αS toxicity. Survival of neurons was measured following treatment with Scd1 inhibitor (ab142089) in control rat cortical neurons and rat cortical neuron expressing human αS. All graphed data are relative to vector control set at 100%. Data were generated 14 days after inhibitor was applied to cells. Y-axis: % Viability on the basis of conversion of Resazurin to Resorufin (Cell Titer Blue, Promega) X-axis: concentration of inhibitor. **** p<0.0001; * p<0.05 by one way

Anova. (G) Inhibition of Scd1 rescues AK release associated with αS expression. Survival of neurons was measured following treatment with Scd1 inhibitor (CAY10566) in control rat cortical neurons and rat cortical neuron expressing human αS. All graphed data are relative to vector control set at 100%. Data were generated 14 days after inhibitor was applied to cells. Y-axis: % Adenylate kinase released relative to the vector control. **** $p<0.0001$ by one way Anova. (H) Inhibition of Scd1 rescues *DGAT*-associated toxicity in rat cortical neurons. *DGAT* expression was knocked down in control rat cortical neurons and in rat cortical neurons expressing human αS. Neurons were treated with DMSO or Scd inhibitor and neuronal survival was measured. All graphed data is relative to vector control set at 100%. Data were generated 14 days after inhibitor was applied to cells. Y-axis: % Viability calculated on the basis of n=3 for all samples. *** $p\leq0.0007$ by one way Anova. (I) Intracellular FA analysis of αS expression in rat cortical neurons relative to a vector control in control (C) neurons, neurons with Scd1 knockdown and neurons with Seipin knockdown. Bars represent the average of triplicates and error bars represent standard deviation of triplicates. Time point is 14 d. ** $p<0.001$, *** $P<0.005$ by one way Anova. (J) Analysis of DG content in rat cortical neurons relative to a vector control in control (C) neurons, neurons with Scd1 knockdown and neurons with Seipin knockdown. Bars represent the average of triplicates and error bars represent standard deviation of triplicates. ** $p<0.001$, *** $P<0.005$ by one way Anova.

Suppl. Fig 5 – (Relates to Fig 1, Fig 4, Fig 5, Fig 7) (A & B) Summary (based on triplicates) of % changes of DG, TG, PC, PE, PI, PS (presented in Fig 1A, 4A) at 12 h post induction (yeast) and in rat cortical neurons at 14d, 20d at MOI1 and MOI5 for expression of human αS and a vector control. Stacked bar charts were constructed in Microsoft Excel. In summary: Yeast: DG increases upon αS expression (light blue bars). Rat cortical neurons: DG increases at MOI1 (light blue bars),

particularly at day 14. Yeast: TG increase (orange bars) upon αS expression. Rat cortical neurons: TG (orange bars) increase is at both time points at MOI5. Yeast: PC decrease upon αS expression (gray bars). Rat cortical neurons: PC decrease (gray bars) at MOI5 at both time points. PE is more abundant as total % of lipid in the rat cortical neurons than yeast. Yeast: PE shows no major change. Rat cortical neurons: PE is decreased at MOI5 at both time points. Yeast: PI (dark blue bars) decreases slightly upon αS expression relative to vector. Rat cortical neurons: PI decreases (dark blue bars) upon αS expression. Yeast: PS decreases (green bars) upon αS expression. Rat cortical neurons: PS decreases slightly at MOI5 at both time points and at MOI1 at day 20. (C) Lipid profiles of αs overexpression in human iPSC-derived neurons. These profiles show triplicate samples for averages presented in Fig 5A. Baseline abundance (Abd) of each lipid species is indicated by a red/blue bar on the left of the heat map (relative scale from -3 to 3, see key). Baseline abundance was calculated on the basis of control cells. Degree of baseline abundance (Baseline Abd Scale) is indicated by a smaller red/blue bar on the left of the heatmap. Yellow/Blue heatmap coloring is a representation of a given lipid species relative to the median of the logs across all samples for that lipid species. The relative log scale (-3 to 3) for yellow/blue representation is indicated by a smaller yellow/blue bar on the left of the heatmap. Status of saturation (presence of double bonds [DB]) of each lipid species is indicated by grey (>1 DB) or black (0 DB) bar on the right of the heat map. Lipid species are indicated by color and in the order of the key on the right of the map. AcCa; Cer; CerG1/G2; CerP; ChE; MG; DG; SM; SO; TG; CL; LPC; LPE; LPG; LPI; LPS; PC; PE; PG; PI; PS. Lipid profiling was performed following αS overexpression (MOI-5 and day 14 post-transduction) and compared with control neurons (transduced with control Lentivirus). (D) Toxicity measurements of overexpressing αS in human iPSCs-derived neurons. No Inf: no infection. Y axis: % ATP. ** $p<0.005$, *** $p<0.005$ by one way Anova. (E) Protein expression in lipid profiled neurons. Western blot showing protein expression of vector and αS in

lipid-profiled lines. (F) Intracellular FA analysis of αS overexpression in human iPS-derived neurons. Bars represent the average of triplicates and error bars represent standard deviation of triplicates. (G) Treatment with exogenous OA enhances αS toxicity in human iPS neurons. Viability readings are 14 days after addition of OA. Y axis: %ATP. n=3 for all samples. * $p<0.05$ by one way Anova. (H) Neutral lipid profiles of human patient αS triplication neurons and isogenic corrected control. Yellow/Blue heatmap coloring is a representation of a given lipid species relative to the median of the logs across all samples for that lipid species. The relative log scale (-3 to 3) for yellow/blue representation is indicated by a smaller yellow/blue bar on the left of the heatmap. Lipid species are indicated by color and in the order of the key on the right of the map. DG; TG. Lipid profile was performed following 24days after differentiation to neurons. (I) Neutral lipid profiles of human neurons expressing wt αS and the E46K familial αS PD mutation (Dettmer et al., 2017; Soldner et al., 2011b; Soldner et al., 2016). Yellow/Blue heatmap coloring is a representation of a given lipid species relative to the median of the logs across all samples for that lipid species. The relative log scale (-3 to 3) for yellow/blue representation is indicated by a smaller yellow/blue bar on the left of the heatmap. Lipid species are indicated by color and in the order of the key on the right of the map. DG; TG. Lipid profile was performed following 36 days after differentiation to neurons. (J) Exogenous palmitic acid (PA), palmitoleic acid (POA), stearic acid (ST) and oleic acid (OA) were assayed for impact on αS inclusion formation in M17D cells. After incubating M17D/αS-3K:YFP (YFP tag αS-3K under doxycycline inducible promoter) cells under serum-starved condition with two concentrations of each fatty acid for 6h, cells were induced to express the αS-3K::YFP mutant and formation of inclusions was followed over 24h. After 24h, the number of inclusions (normalized by the signal of constitutively expressed mCherry) was measured and expressed in % compared to the 0 μM OA control (n=12). Dotted line represents 100%. Y-axis: % inclusions. X-axis: concentration of fatty acid. *** $p<0.0005$ by one way Anova.

(K) *SCD1* Inhibition Decreases αS Inclusions. M17D/αS-3K:YFP cells were treated with Scd1 inhibitor (HY15700) and induced with Dox for 24h. The number of inclusions of αS (normalized by the signal of mCherry constitutively expressed) was measured and expressed in % compared to the 0μM control (n=12). X-axis: μM concentration of Scd1 inhibitor (HY15700). Y-axis: % inclusions. **** $p<0.0001$ by one way Anova. (L) *SCD1* inhibition decreases αS inclusions. Microscopy images showing decreased inclusions of αS (green) upon treatment with 10μM *SCD1* inhibitor HY19762 but similar signal of mCherry (red channel). (M) Scd1 inhibition (CAY10566) increases 60kDa αS:14kDa αS and 80kDa αS: 14kDa αS. M17D/αS-E46K (untagged E46K αS expressed constitutively) were incubated for 48h with 10μM of Scd1 inhibitor (or DMSO control) then submitted to DSG crosslinking. Cell lysates were immunoblotted to detect and quantify αS14, αS60 and αS80. N=2, n=6. Bar charts quantify differences between DMSO and Scd1 inhibitor treatments.

Suppl. Fig 6 – (Relates to Discussion Section, Fig 1-7, S1-5) Model of cellular αS biology in health and disease based on findings in this study. (A) Intact homeostasis/equilibrium: αS is in equilibrium between unfolded monomers and physiological, α-helical tetramers. The tetramers are principally soluble (cytosolic) while the monomers occur both in membrane-associated and cytosolic pools (Dettmer et al., 2017); (B) αS-induced dyshomeostasis (initially compensated): mild accumulation of αS in the absence of a genetic abnormality in lipid metabolism triggers increased OA (and other unsaturated FA) but the dyshomeostasis is tolerated and excess OA and DG are shuttled into TG and LD. (C) αS-Induced dyshomeostasis (uncompensated): Substantial αS accumulation +/- a genetic abnormality in lipid metabolism precludes compensation, so DG accumulate in the ER and aggravate trafficking defects. Excess OA or desaturase activity result in membrane defects. Clusters of vesicles with excess αS monomers may form cytoplasmic

inclusions as reported in (Dettmer et al., 2017; Soper et al., 2008). Arrows represent genetic components of lipid metabolism pathways.