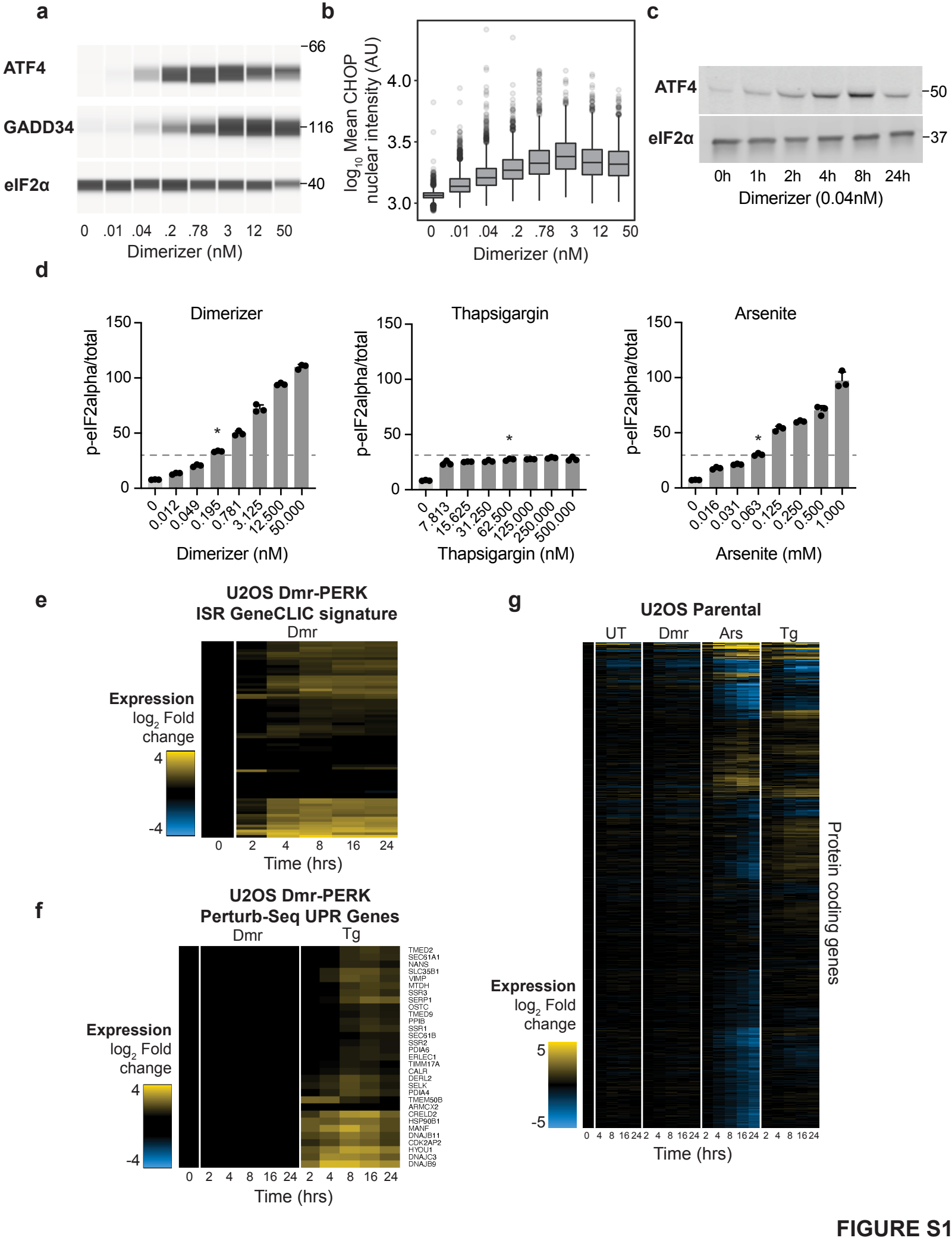
a
ATF4
GADD34
eIF2α
66
116
40
0 .01 .04 .2 .78 3 12 50
Dimerizer (nM)
b
log10 Mean CHOP nuclear intensity (AU)
4.0
3.5
3.0
0 .01 .04 .2 .78 3 12 50
Dimerizer (nM)
c
ATF4
eIF2α
50
37
0h 1h 2h 4h 8h 24h
Dimerizer (0.04nM)
d
Dimerizer
Thapsigargin
Arsenite
p-eIF2alpha/total
150
100
50
0
0 0.012 0.049 0.195 0.781 3.125 12.500 50.000
Dimerizer (nM)
0 7.813 15.625 31.250 62.500 125.000 250.000 500.000
Thapsigargin (nM)
0 0.016 0.031 0.063 0.125 0.250 0.500 1.000
Arsenite (mM)
e
U2OS Dmr-PERK
ISR GeneCLIC signature
Dmr
Expression
log2 Fold change
4
-4
0 2 4 8 16 24
Time (hrs)
f
U2OS Dmr-PERK
Perturb-Seq UPR Genes
Dmr
Tg
TMED2
SEC61A1
NANS
SLC35B1
VIMP
MTDH
SSR3
SERP1
OSTC
TMED9
PPIB
SSR1
SEC61B
SSR2
PDIA6
ERLEC1
TIMM17A
CALR
DERL2
SELK
PDIA4
TMEM50B
ARMCX2
CRELD2
HSP90B1
MANF
DNAJB11
CDK2AP2
HYOU1
DNAJC3
DNAJB9
Expression
log2 Fold change
4
-4
0 2 4 8 16 24 2 4 8 16 24
Time (hrs)
g
U2OS Parental
UT
Dmr
Ars
Tg
Expression
log2 Fold change
5
-5
0 4 8 16 24 2 4 8 16 24 2 4 8 16 24 2 4 8 16 24
Time (hrs)
Protein coding genes

FIGURE S1

Supplementary Figure 1. Dmr-PERK selectively activates the ISR. a Wes analysis of ATF4, GADD34 and eIF2α protein in Dmr-PERK cells treated with indicated dose of dimerizer for 4 hours. **b** Immunofluorescence measurement of CHOP protein in Dmr-PERK cells treated with indicated doses of dimerizer for 24 hours. Nuclear fluorescence was quantified from 13 fields of view for each condition. AU = arbitrary units. **c** Western blot for ATF4 and eIF2 in lysates from Dmr-PERK cells treated with dimerizer (Dmr, 0.04 nM) for the indicated amount of time. **d** Bar plots representing AlphaLISA measurement of p-eIF2/eIF2 ratio in Dmr-PERK cells treated with the indicated concentrations of dimerizer, thapsigargin, or arsenite for 2 hours. Asterisks indicate the concentrations chosen for RNA-seq analysis that elicit comparable levels of p-eIF2 (dotted line). **e** Heatmap of Gene-CLIC ISR gene signature from Wong et al. in Dmr-PERK cells treated with dimerizer (Dmr, 0.2nM) for the indicated time points. Each gene was normalized to its mean expression at t=0 on a $\log_2$ scale. Each column shows the mean expression of three technical replicates for each timepoint. **f** Heatmap of Perturb-seq UPR gene signature from Adamson et al. in Dmr-PERK cells treated with dimerizer (0.2nM) or thapsigargin (Tg, 100nM) for the indicated time points. Each gene was normalized to its mean expression at t=0 on a $\log_2$ scale. Each column shows the mean expression of three technical replicates for each timepoint. **g** Heatmap of protein coding gene expression in parental U2OS cells that were untreated (UT) or treated with dimerizer (Dmr, 0.2nM), thapsigargin (Tg, 100 nM), or arsenite (Ars, 0.05 mM) for the indicated amount of time. Each gene is normalized to its mean expression at t=0 on a $\log_2$ scale. Columns show the mean expression of three technical replicates for each timepoint. Source data are provided as a Source Data file.

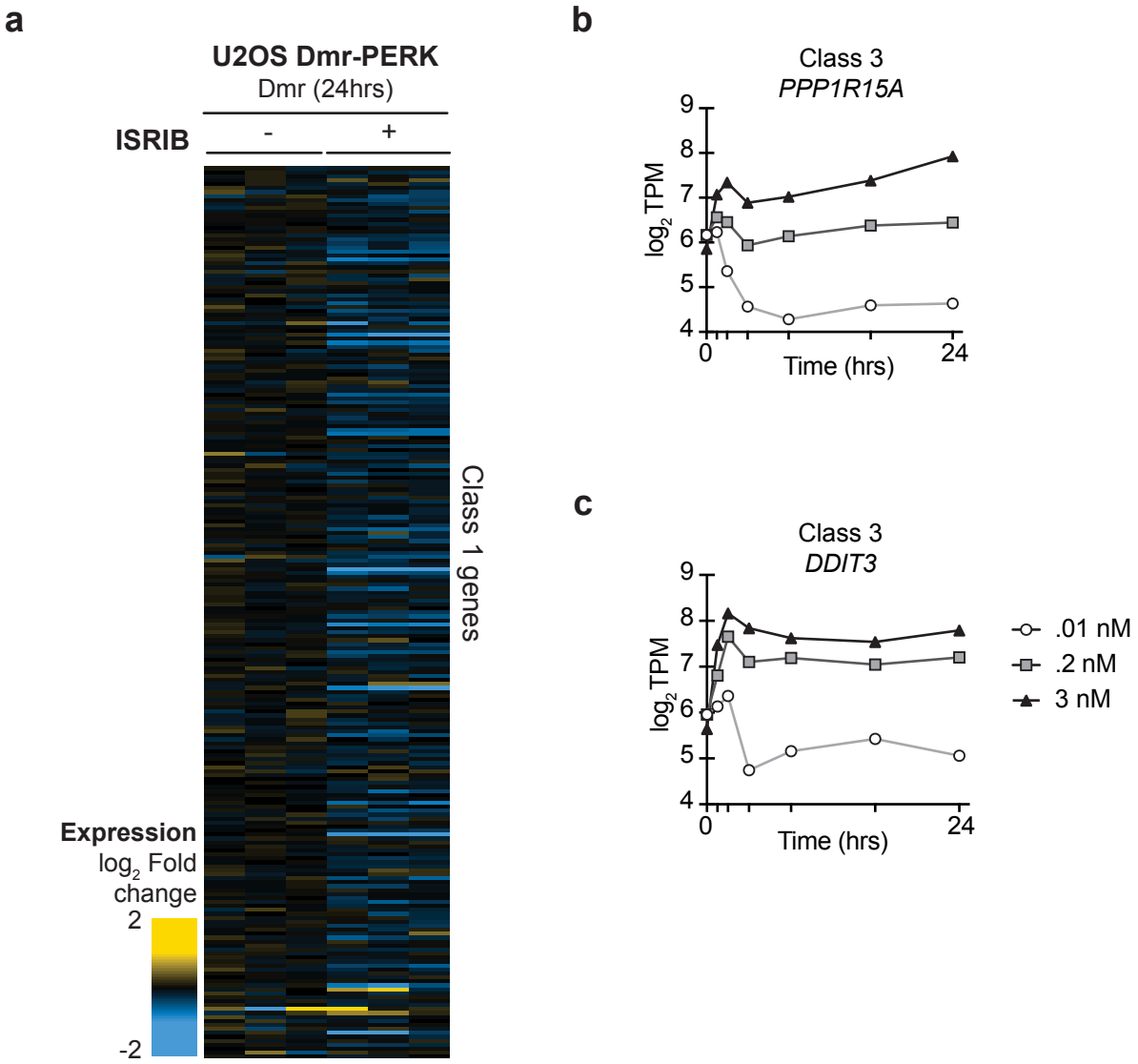
a
U2OS Dmr-PERK
Dmr (24hrs)
ISRIB
-
+
Class 1 genes
Expression
log2 Fold change
2
-2
b
Class 3
PPP1R15A
log2 TPM
9
8
7
6
5
4
0
24
Time (hrs)
c
Class 3
DDIT3
log2 TPM
9
8
7
6
5
4
0
24
Time (hrs)
.01 nM
.2 nM
3 nM

FIGURE S2

Supplementary Figure 2. Dose response behaviors of Dmr-PERK responsive genes. a Heatmap of Class 1 genes in Dmr-PERK cells treated with dimerizer (0.01nM) +/- ISRIB (500nM) for 4h. In cells treated without ISRIB, each gene was normalized to its mean expression at t=0 on a $\log_2$ scale. For cells treated with ISRIB, each gene was normalized to its mean expression at 4h dimerizer treatment without ISRIB, on a $\log_2$ scale. Each column shows the mean expression of three technical replicates for each timepoint. **b,c** Plot of $\log_2$ transcripts per million (TPM) over time at the indicated doses of dimerizer for Class 3 genes *PPP1R15A* (a) and *DDIT3* (b), as described in Fig. 2a.

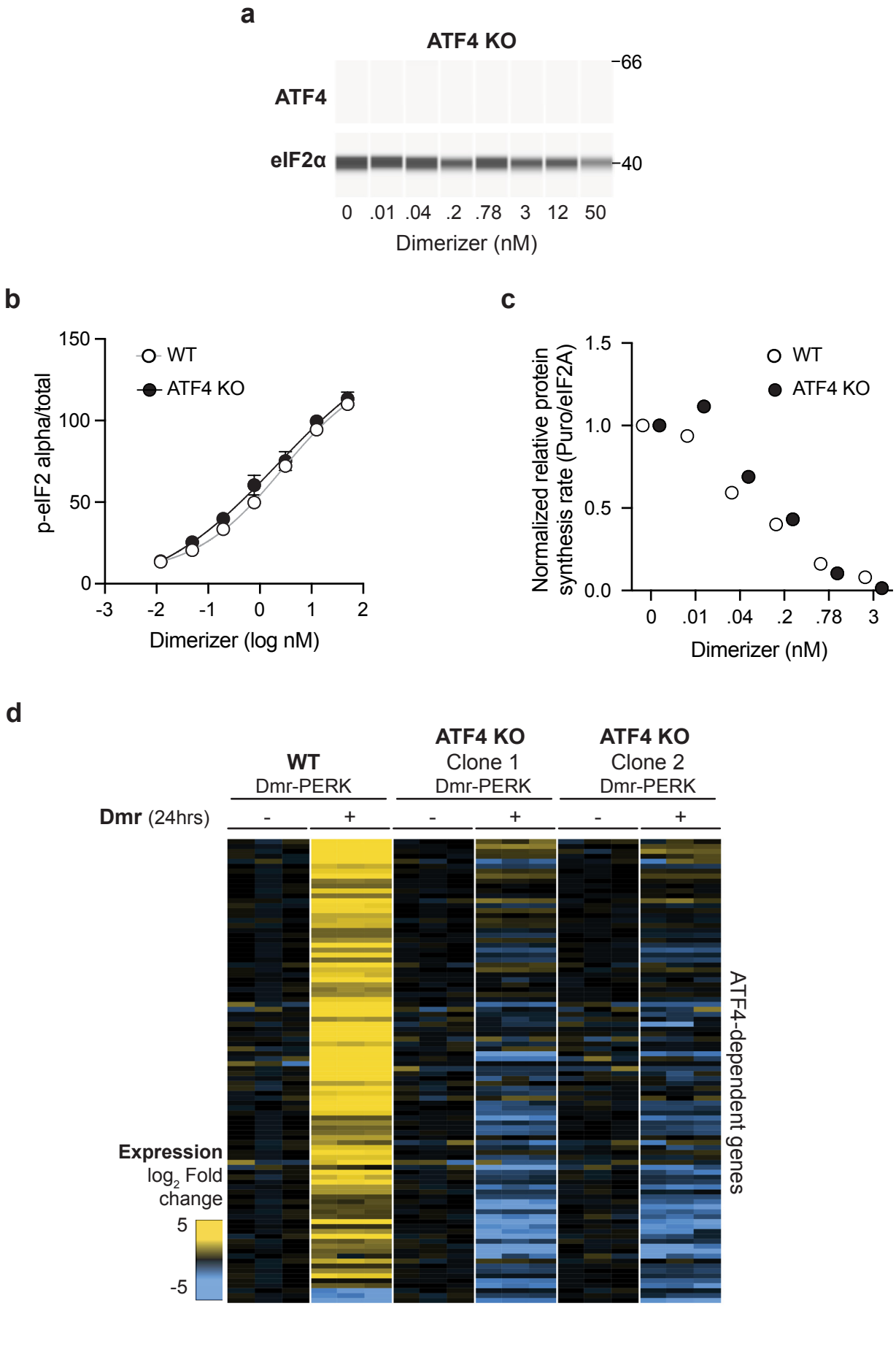
a
ATF4 KO
66
ATF4
eIF2α
40
0 .01 .04 .2 .78 3 12 50
Dimerizer (nM)
b
150
100
50
0
WT
ATF4 KO
p-eIF2 alpha/total
-3 -2 -1 0 1 2
Dimerizer (log nM)
c
1.5
1.0
0.5
0.0
WT
ATF4 KO
Normalized relative protein synthesis rate (Puro/eIF2A)
0 .01 .04 .2 .78 3
Dimerizer (nM)
d
WT
Dmr-PERK
ATF4 KO
Clone 1
Dmr-PERK
ATF4 KO
Clone 2
Dmr-PERK
Dmr (24hrs)
- + - + - +
Expression
log2 Fold change
5
-5
ATF4-dependent genes

FIGURE S3

Supplementary Figure 3. Evaluation of transcriptome in additional Dmr-PERK ATF4KO clones. a Wes analysis of ATF4 and eIF2α protein in ATF4 KO Dmr-PERK cells treated with indicated dose of dimerizer for 4 hours. **b** AlphaLISA measurement of the ratio of p-eIF2/eIF2 in WT and ATF4 KO Dmr-PERK cells following 2 hrs of dimerizer treatment at the indicated concentrations. Error bars show mean +/- SD of three technical replicates. **c** Quantification of OP-puromycin incorporation after treatment of WT and ATF4 KO Dmr-PERK cells with the indicated concentration of dimerizer in. Ratio of puromycin to eIF2α protein level was measured by Western blot and normalized to levels in untreated cells. **d** Heatmap showing the change in expression of ATF4-dependent target genes after 24 hours of treatment with dimerizer (0.2 nM). Target genes were identified by comparison of WT and ATF4KO Dmr-PERK in WT cells and are shown for two independent ATF4 KO clones. Each column shows the expression of a single replicate, with three technical replicates for each condition, normalized to mean expression in untreated cells on a $\log_2$ scale. Source data are provided as a Source Data file.

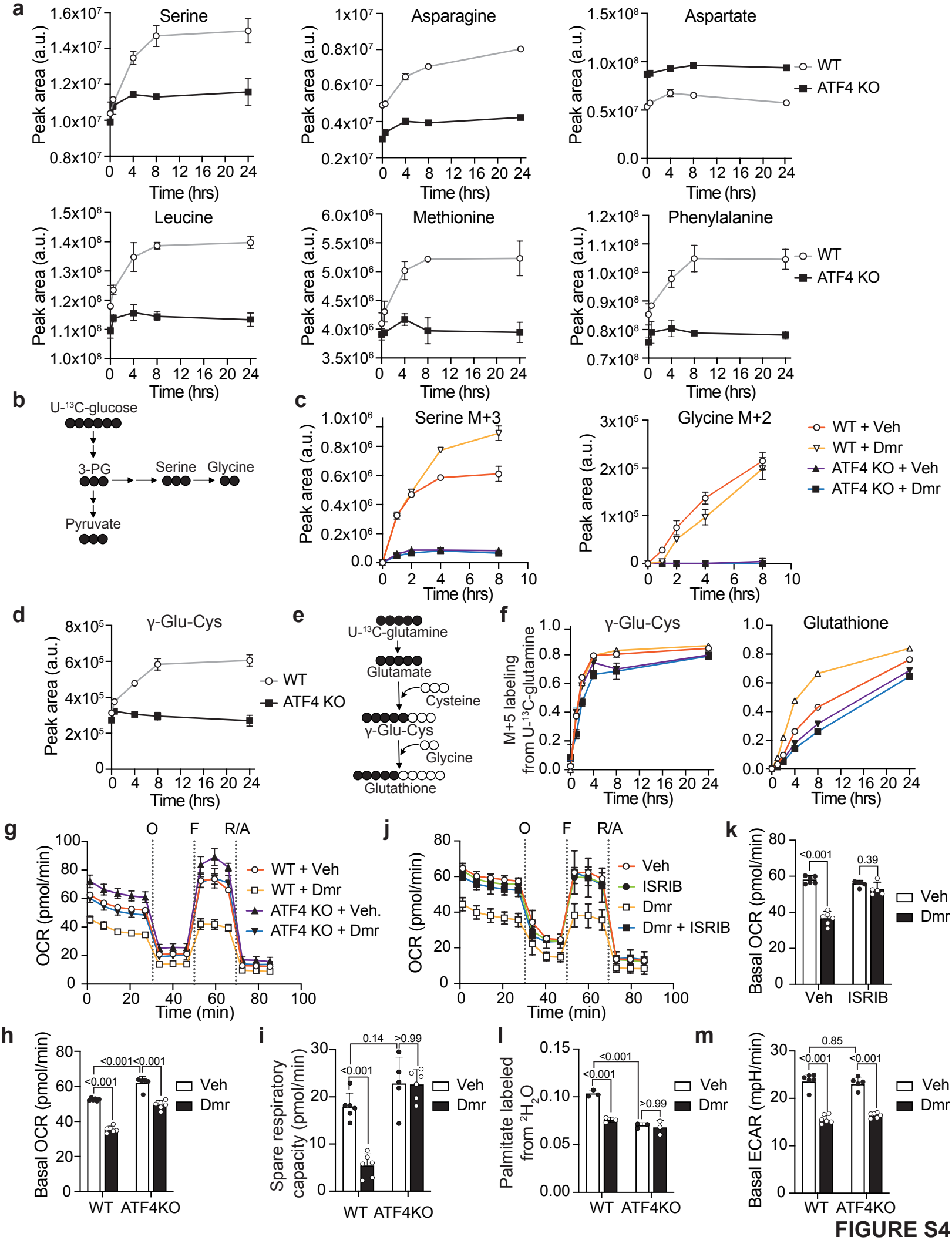
a
Serine
Asparagine
Aspartate
Leucine
Methionine
Phenylalanine
Peak area (a.u.)
Time (hrs)
WT
ATF4 KO
b
U-13C-glucose
3-PG
Serine
Glycine
Pyruvate
c
Serine M+3
Glycine M+2
WT + Veh
WT + Dmr
ATF4 KO + Veh
ATF4 KO + Dmr
d
γ-Glu-Cys
e
U-13C-glutamine
Glutamate
Cysteine
γ-Glu-Cys
Glycine
Glutathione
f
γ-Glu-Cys
Glutathione
M+5 labeling from U-13C-glutamine
g
O
F
R/A
OCR (pmol/min)
Time (min)
WT + Veh
WT + Dmr
ATF4 KO + Veh.
ATF4 KO + Dmr
j
Veh
ISRIB
Dmr
Dmr + ISRIB
k
Basal OCR (pmol/min)
<0.001
0.39
Veh
ISRIB
Dmr
h
Basal OCR (pmol/min)
<0.001
<0.001
<0.001
WT
ATF4KO
i
Spare respiratory capacity (pmol/min)
0.14
>0.99
<0.001
l
Palmitate labeled from 2H2O
<0.001
<0.001
>0.99
m
Basal ECAR (mpH/min)
0.85
<0.001
<0.001

FIGURE S4

Supplementary Figure 4. Changes in cellular metabolites upon ISR activation in Dmr-PERK cells. **a** Levels of amino acids in WT or ATF4 KO Dmr-PERK cells following addition of dimerizer (0.05nM) as described in Figure 4a. Error bars represent mean +/- SD of three technical replicates. **b** Schematic of incorporation of ^{13}C-label from U-^{13}C6-glucose into serine and glycine. **c** Levels of M+3 serine and M+2 glycine labeled from U-^{13}C6-glucose in WT or ATF4KO U2OS treated with vehicle (Veh.) or dimerizer (Dmr, 0.05nM) for the indicated amount of time. **d** Levels of gamma-glutamylcysteine (γ-Glu-Cys) in WT or ATF4 KO U2OS cells following addition of dimerizer (0.05nM) as described in Figure 4a. **e** Schematic of incorporation of 13C-label from U-^{13}C5-glutamine into synthesis of glutathione. **f** Fractional labeling of γ-Glu-Cys (top) and glutathione (bottom) from U-^{13}C5-glutamine in WT or ATF4KO at the indicated time points following treatment with Vehicle (Veh) or dimerizer (Dmr, 0.05nM). **g** Traces of oxygen consumption rate (OCR) in WT or ATF4 KO cells following treatment with Vehicle (Veh) or dimerizer (Dmr, 0.05nM) for 16 hours then subjected to a Mito Stress Test (O: oligomycin, F: FCCP, R/A: rotenone/antimycin A). Error bars depict mean +/- SD of 6 technical replicates. **h,i** Bar plots representing the basal OCR (h) and spare respiratory capacity (i) for WT or ATF4 KO cells calculated from measurements in g. Error bars represent mean +/- SD of 6 technical replicates. **j** Traces of oxygen consumption rate (OCR) in WT Dmr-PERK cells following treatment with Vehicle (Veh), dimerizer (Dmr, 0.05nM) or ISRIB (500nM) for 16 hours then subjected to a Mito Stress Test (O: oligomycin, F: FCCP, R/A: rotenone/antimycin A). Error bars depict mean +/- SD of six technical replicates. **k** Bar plots representing the basal OCR for WT Dmr-PERK cells following treatment with Vehicle (Veh), dimerizer (Dmr, 0.05nM) or ISRIB (500nM) for 16 hours, calculated from measurements in j. Error bars represent mean +/- SD of six technical replicates. **l** Bar plot representing fraction of palmitate hydrogens labeled after 24h from heavy water ($^{2}H_2O$) in lipids from WT or ATF4 KO cells treated with vehicle (Veh) or dimerizer (Dmr, 0.05nM). Error bars represent mean +/- SD of three technical replicates. **m** Bar plot representing the basal extracellular acidification rate (ECAR) for WT or ATF4 KO cells. Error bars represent mean +/- SD of three technical replicates. Statistical significance was evaluated by two-way ANOVA followed by Tukey's HSD test. Source data are provided as a Source Data file.

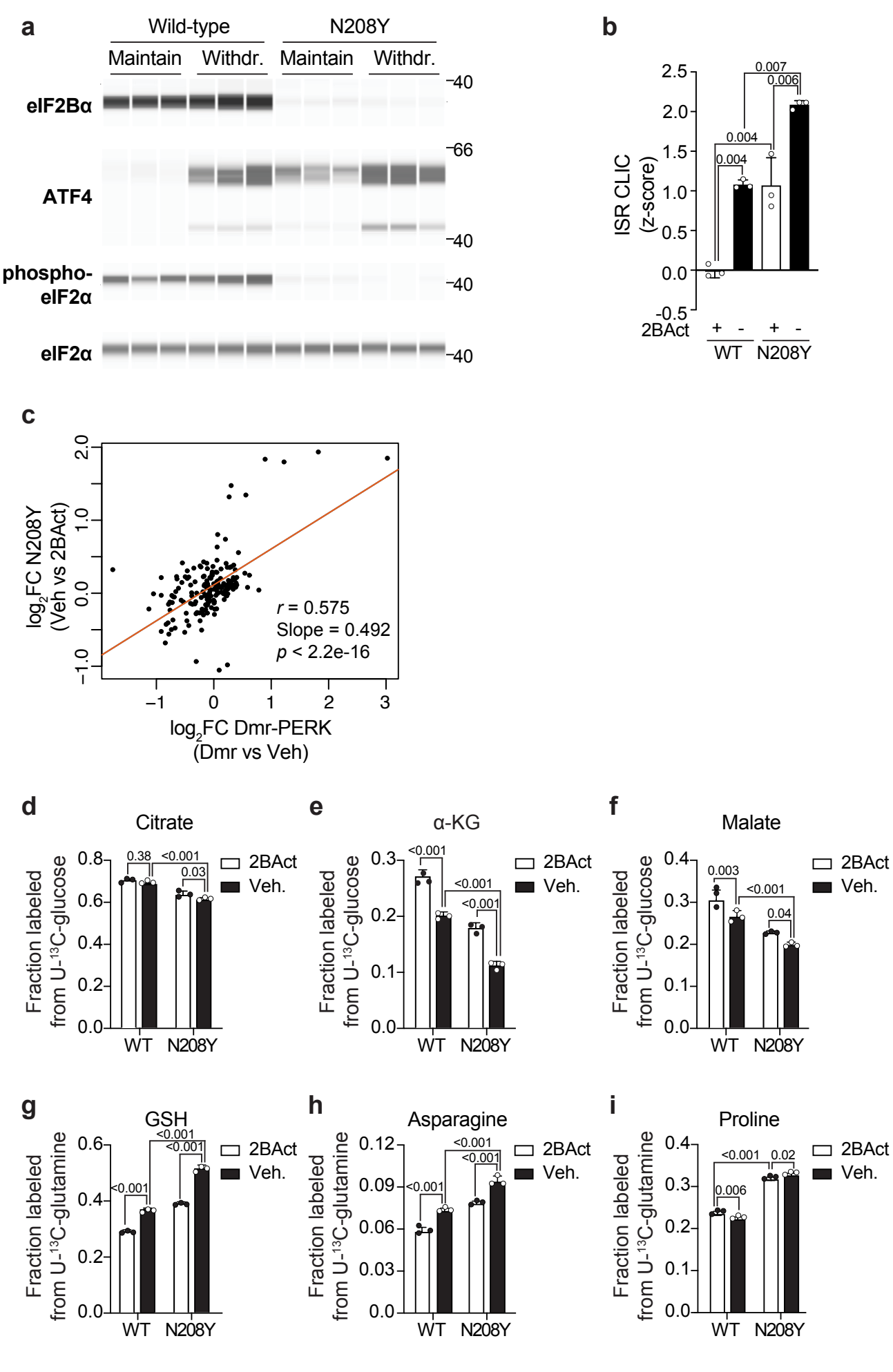
a
Wild-type
N208Y
Maintain
Withdr.
Maintain
Withdr.
eIF2Bα
ATF4
phospho-eIF2α
eIF2α
40
66
40
40
40
b
ISR CLIC (z-score)
0.007
0.006
0.004
0.004
2BAct
WT
N208Y
c
log2FC N208Y (Veh vs 2BAct)
log2FC Dmr-PERK (Dmr vs Veh)
r = 0.575
Slope = 0.492
p < 2.2e-16
d
Citrate
e
α-KG
f
Malate
g
GSH
h
Asparagine
i
Proline
Fraction labeled from U-13C-glucose
Fraction labeled from U-13C-glutamine
2BAct
Veh.
WT
N208Y

FIGURE S5

Supplementary Figure 5. Metabolic changes upon ISR activation in N208Y MEFs are similar to Dmr-PERK cells. a Wes analysis of eIF2Bα, ATF4 and eIF2α protein in lysates from WT or N208Y MEFs maintained or withdrawn from 2BAct for 24 h. **b** Average z-core of ISR CLIC genes calculated from nCounter gene expression profiling of WT or N208Y MEFs maintained or withdrawn from 2BAct for 24 h. Error bars show mean +/- SD of 3 technical replicates. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. **c** Scatter plot of $\log_2$ fold change in abundance of metabolites in U2OS Dmr-PERK and N208Y MEFs 24h after addition of 0.05 nM dimerizer or withdrawal of 2BAct, respectively. *r* = Pearson correlation coefficient. **d-f** Bar plots representing fraction labeling of TCA cycle metabolites from U-^{13}C6-glucose in WT and N208Y MEFs maintained on 2BAct or withdrawn (Veh) for 24 hrs. Error bars depict mean +/- SD of three technical replicates. Statistical significance was evaluated by two-way ANOVA followed by Tukey's HSD test. **g-i** Bar plots representing fraction labeling of glutathione (GSH), asparagine and proline from U-^{13}C5-glutamine in WT and N208Y MEFs maintained on 2BAct or withdrawn (Veh) for 24 hrs. Error bars depict mean +/- SD of three technical replicates. Statistical significance was evaluated by two-way ANOVA followed by Tukey's HSD test. Source data are provided as a Source Data file. Source data are provided as a Source Data file.

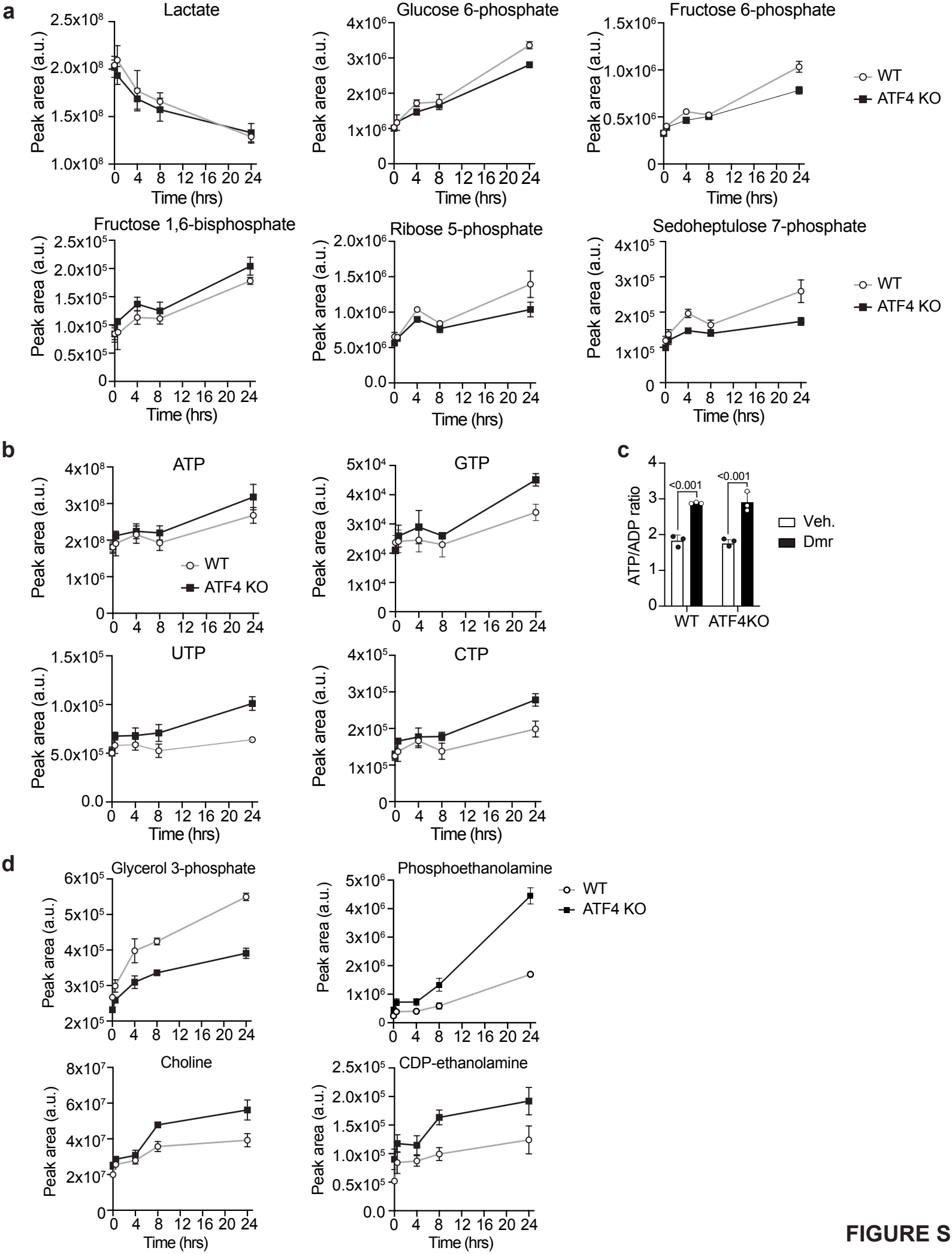
a
Lactate
Glucose 6-phosphate
Fructose 6-phosphate
Fructose 1,6-bisphosphate
Ribose 5-phosphate
Sedoheptulose 7-phosphate
Peak area (a.u.)
Time (hrs)
WT
ATF4 KO
b
ATP
GTP
UTP
CTP
c
ATP/ADP ratio
<0.001
<0.001
Veh.
Dmr
WT
ATF4KO
d
Glycerol 3-phosphate
Phosphoethanolamine
Choline
CDP-ethanolamine

FIGURE S6

Supplementary Figure 6. ATF4-independent changes in cellular metabolites upon ISR activation. a Levels of glycolytic and pentose phosphate pathway metabolites in WT or ATF4 KO Dmr-PERK cells following addition of dimerizer (0.05nM) as described in Figure 4a. Error bars depict mean +/- SD of three technical replicates. **b** Levels of nucleotide triphosphates in WT or ATF4 KO Dmr-PERK cells following addition of dimerizer (0.05nM) as described in Figure 4a. Error bars depict mean +/- SD of three technical replicates. **c** Ratio of ATP/ADP calculated from data shown in b. Error bars depict mean +/- SD of three technical replicates. Statistical significance was evaluated by two-way ANOVA followed by Tukey's HSD test. **d** Levels of phospholipid synthesis intermediates in WT or ATF4 KO Dmr-PERK cells following addition of dimerizer (0.05nM) as described in Figure 4a. Error bars depict mean +/- SD of three technical replicates. Source data are provided as a Source Data file.

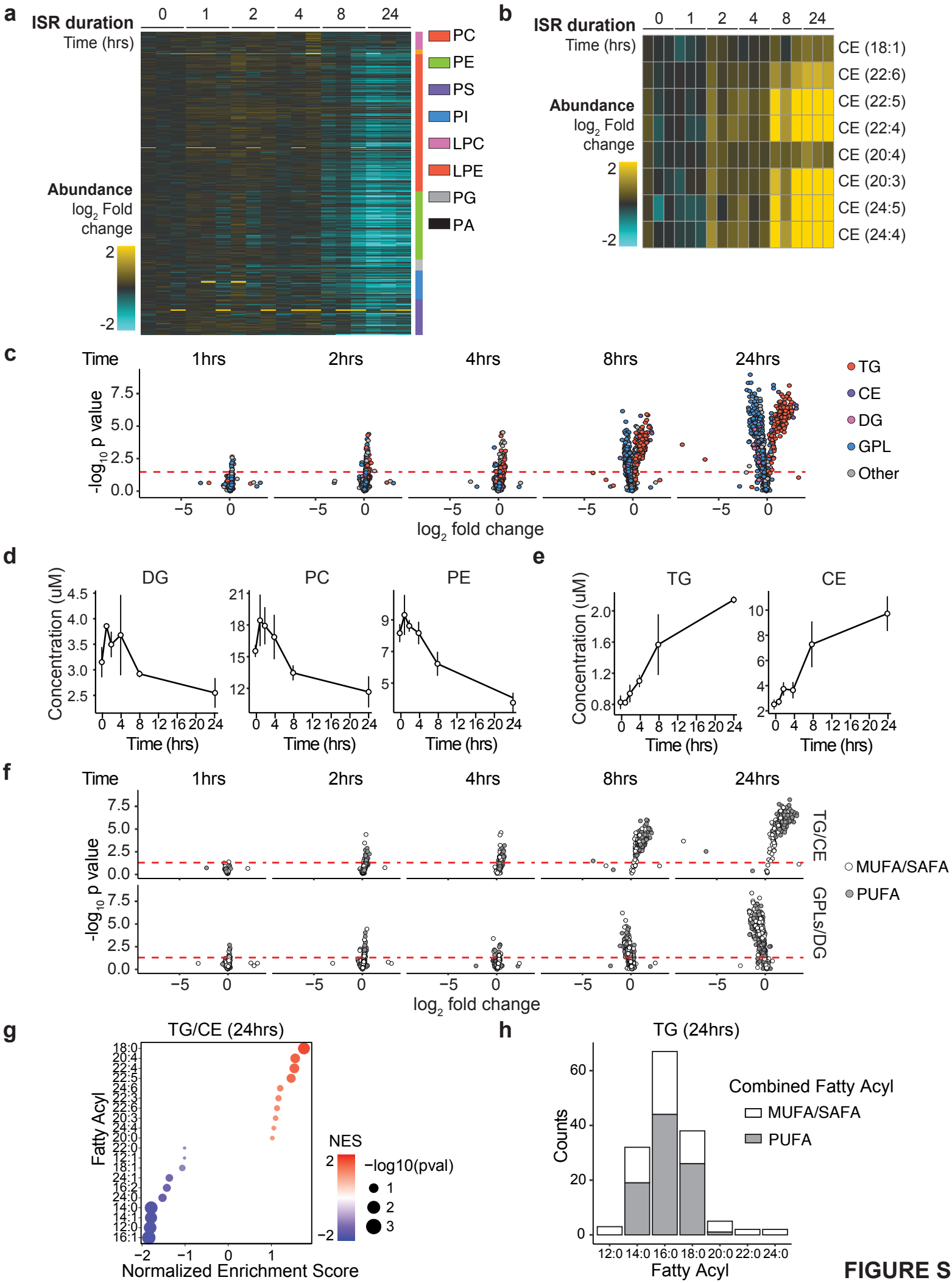
a
ISR duration
Time (hrs)
0 1 2 4 8 24
PC
PE
PS
PI
LPC
LPE
PG
PA
Abundance
log2 Fold change
2
-2
b
ISR duration
Time (hrs)
0 1 2 4 8 24
CE (18:1)
CE (22:6)
CE (22:5)
CE (22:4)
CE (20:4)
CE (20:3)
CE (24:5)
CE (24:4)
Abundance
log2 Fold change
2
-2
c
Time
1hrs
2hrs
4hrs
8hrs
24hrs
-log10 p value
log2 fold change
TG
CE
DG
GPL
Other
d
DG
PC
PE
Concentration (uM)
Time (hrs)
e
TG
CE
Concentration (uM)
Time (hrs)
f
Time
1hrs
2hrs
4hrs
8hrs
24hrs
-log10 p value
log2 fold change
TG/CE
GPLs/DG
MUFA/SAFA
PUFA
g
TG/CE (24hrs)
Fatty Acyl
Normalized Enrichment Score
NES
-log10(pval)
h
TG (24hrs)
Counts
Fatty Acyl
12:0 14:0 16:0 18:0 20:0 22:0 24:0
Combined Fatty Acyl
MUFA/SAFA
PUFA

FIGURE S7

Supplementary Figure 7. Quantification of lipids during ISR activation. a Heatmap of phospholipid species shown in Figure 5a. Color bars indicate phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidic acid (PA), lyso-phosphatidylcholine (LPC) and lyso-phosphatidylethanolamine (LPE). **b** Heatmap of cholesterol ester (CE) species in Figure 5a. **c** Volcano plot of lipid fold-changes relative to time 0 as measured by LC-MS at the indicated time points following addition of 0.05 nM dimerizer, the dotted line represents a p value = 0.05. Lipids were labeled as: orange for lipids belonging to triacylglycerides (TG), purple for cholesterol esters (CE), pink for diacylglycerols (DG), blue for glycerophospholipids (GPL), gray labels for other lipid classes. **d** Absolute concentration of diacylglycerol (DG), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) as measured in a. Error bars represent mean of all species within the class +/- SD of three technical replicates of $5x10^5$ cells. **e** Absolute concentration of triglycerides (TG) and cholesterol esters (CE) as measured in a. Error bars represent mean of all species within the class +/- SD of three technical replicates of $5x10^5$ cells. **f** Volcano plot of acyl chains fold-changes relative to time 0 in triacylglycerides (TG) or cholesterol esters (CE) (top panel) or to glycerophospholipids (GLP) or diacylglycerol (DG) (bottom panel), as measured by LC-MS at the indicated time points following addition of 0.05 nM dimerizer, the dotted line represents a p value = 0.05. Acyl chains were labeled as polyunsaturated fatty acids (PUFA) in gray or monounsaturated (MUFA) or saturated (SAFA) fatty acids in white. **g** Dot plot of TG and CE fatty acyl enrichment relative to time 0 following addition of 0.05 nM dimerizer for 24 hours. The y axis lists the significantly enriched fatty acyls, the x axis enrichment score reflects the degree to which a fatty acyl chain is overrepresented at the top or bottom of a ranked list of TG/CE acyl chain compositions, dot size is the log10 transformation of p value, as calculated using R package “fgsea”. **h** Bar graph depicting the frequency (y axis) of the indicated acyl chain (x axis) being combined with either a polyunsaturated fatty acids (PUFA) (in gray or monounsaturated (MUFA) or saturated (SAFA) fatty acids (in white). Source data are provided as a Source Data file.

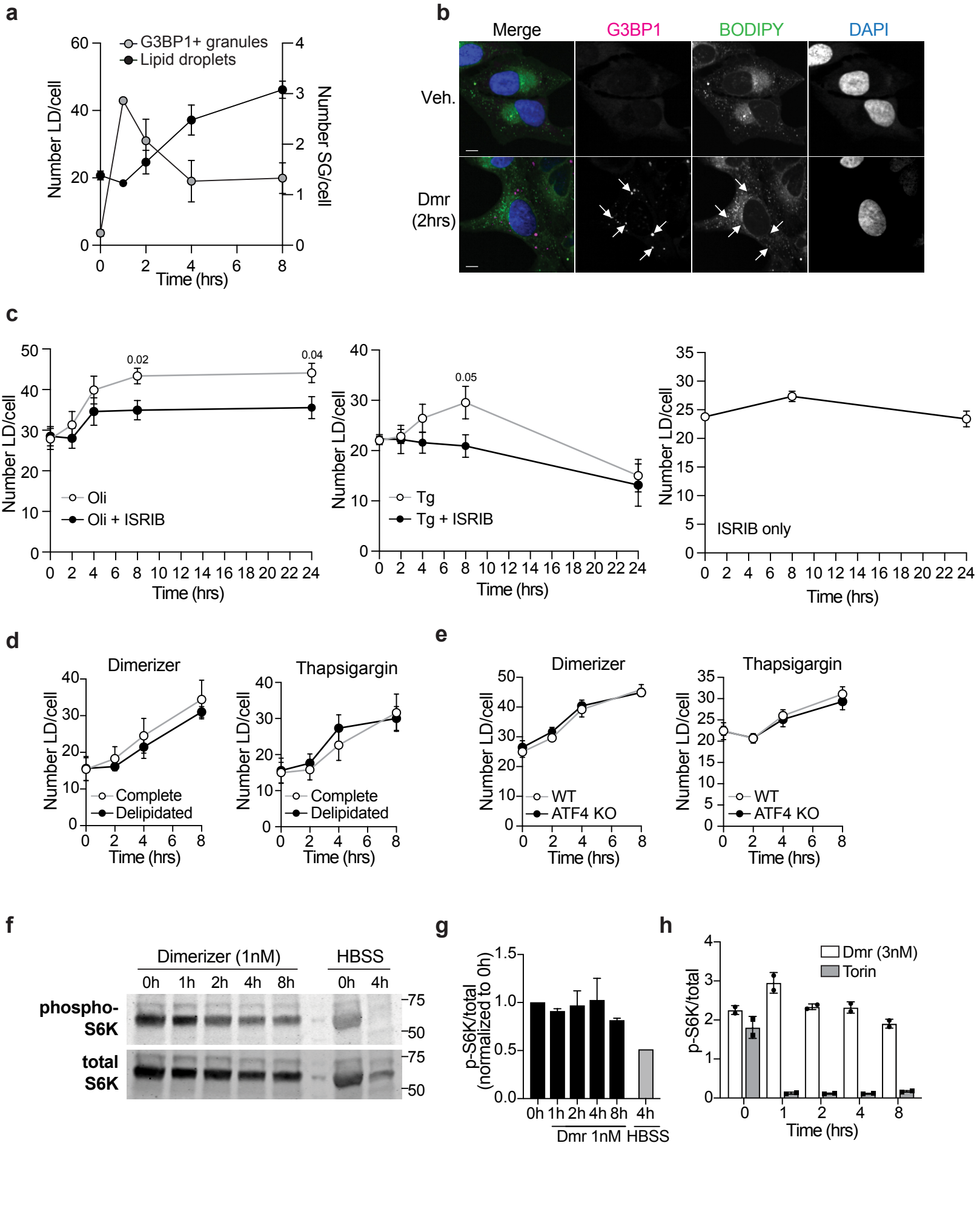
a
G3BP1+ granules
Lipid droplets
Number LD/cell
Number SG/cell
Time (hrs)
b
Merge
G3BP1
BODIPY
DAPI
Veh.
Dmr (2hrs)
c
Oli
Oli + ISRIB
Tg
Tg + ISRIB
ISRIB only
0.02
0.04
0.05
Number LD/cell
Time (hrs)
d
Dimerizer
Thapsigargin
Complete
Delipidated
e
WT
ATF4 KO
f
Dimerizer (1nM)
HBSS
0h 1h 2h 4h 8h 0h 4h
phospho-S6K
total S6K
g
p-S6K/total (normalized to 0h)
Dmr 1nM
HBSS
h
Dmr (3nM)
Torin
p-S6K/total

FIGURE S8

Supplementary Figure 8. ISR induction of lipid droplets is independent of lipid uptake and mTorc1. a Quantification of LD (left axis) and G3BP1-labeled SG (right axis) in Dmr-PERK cells treated with dimerizer (1nM) for the indicated amount of time. Error bars show mean +/- SD of three independent experiments. **b** Representative image of Dmr-PERK cells co-labeled with BODIPY (green) and immunofluorescence for endogenous G3BP1 (magenta) following 2 hrs treatment with vehicle (Veh.) or dimerizer (Dmr, 1nM). Arrows indicate G3BP1+ SGs. Scale bar = 5µm. **c** Quantification of the number of LD in Dmr-PERK cells treated with oligomycin (Oli, 10nM), thapsigargin (Tg) or ISRIB (500nM), as indicated, for the indicated time points. Error bars show mean +/- SD of three independent experiments. Statistical significance was evaluated by two-sided unpaired t-test. **d** Quantification of the number of LD in Dmr-PERK cells treated with dimerizer (left panel, 1nM) or thapsigargin (right panel, 100nM) in complete or delipidated media for the indicated amount of time. Error bars show mean +/- SD of three independent experiments. **e** Quantification of the number of LD in WT or ATF4 KO Dmr-PERK cells treated with dimerizer (left panel, 1nM) or thapsigargin (right panel, 100nM) for the indicated amount of time. Error bars show mean +/- SD of three independent experiments. **f** Western blot for phospho- and total levels of p70 S6K in lysates from Dmr-PERK cells treated with dimerizer (Dmr, 1nM) or starved in HBSS for the specified amount of time. **g** Quantification of the relative density of phospho/total shown in f, normalized to 0h. Error bars show mean +/- SD of two independent experiments. **h** AlphaLISA measurement of the ratio of p-S6K/total in Dmr-PERK U2OS cells following treatment with dimerizer (3nM) or Torin (100nM) for the indicated amount of time. Error bars show mean +/- SD of three technical replicates. Source data are provided as a Source Data file.

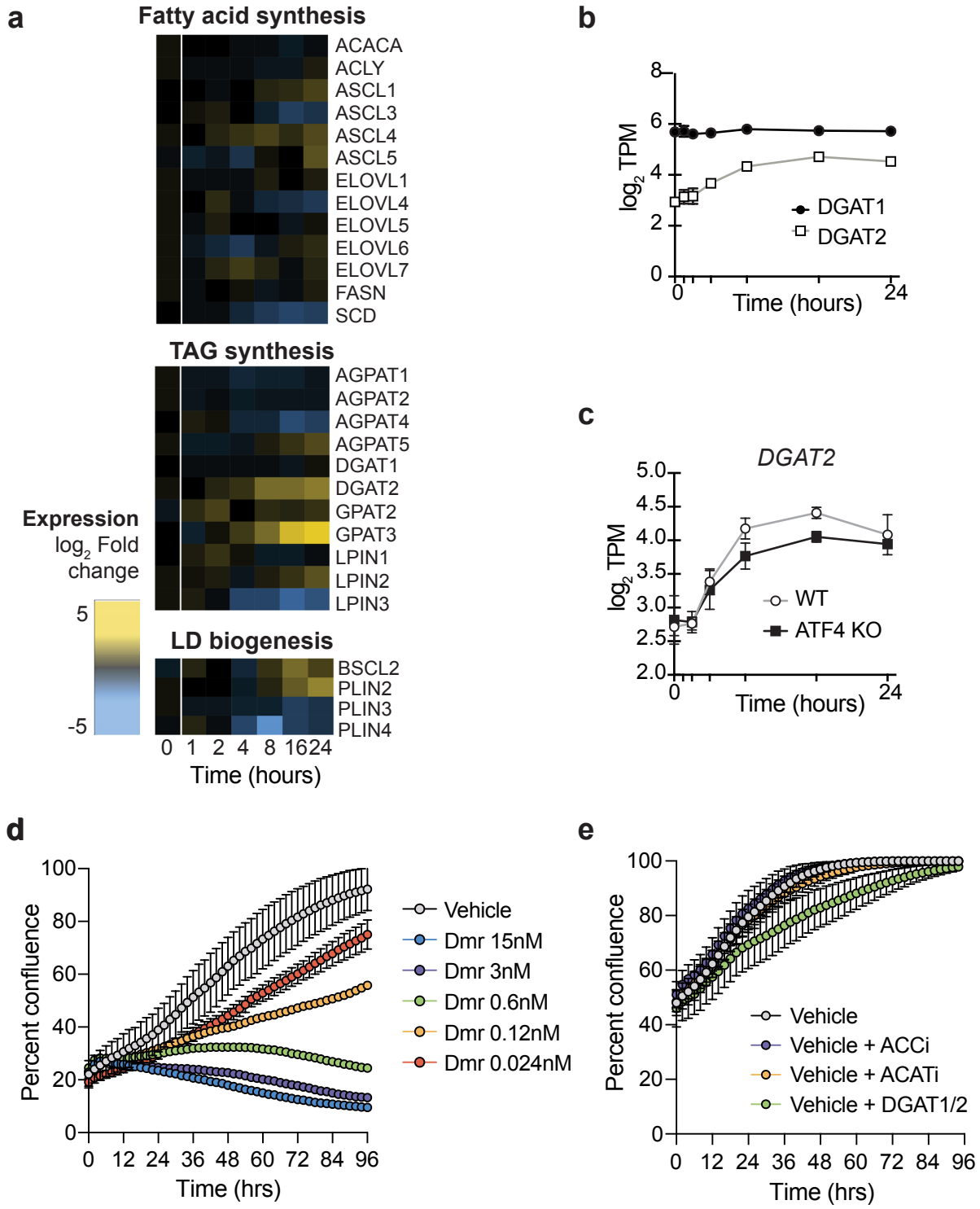
a
Fatty acid synthesis
ACACA
ACLY
ASCL1
ASCL3
ASCL4
ASCL5
ELOVL1
ELOVL4
ELOVL5
ELOVL6
ELOVL7
FASN
SCD
TAG synthesis
AGPAT1
AGPAT2
AGPAT4
AGPAT5
DGAT1
DGAT2
GPAT2
GPAT3
LPIN1
LPIN2
LPIN3
LD biogenesis
BSCL2
PLIN2
PLIN3
PLIN4
Expression
log2 Fold change
5
-5
0 1 2 4 8 1624
Time (hours)
b
log2 TPM
DGAT1
DGAT2
Time (hours)
c
DGAT2
log2 TPM
WT
ATF4 KO
Time (hours)
d
Percent confluence
Vehicle
Dmr 15nM
Dmr 3nM
Dmr 0.6nM
Dmr 0.12nM
Dmr 0.024nM
Time (hrs)
e
Percent confluence
Vehicle
Vehicle + ACCi
Vehicle + ACATi
Vehicle + DGAT1/2
Time (hrs)

FIGURE S9

Supplementary Figure 9. TG synthesis genes are upregulated by the ISR. a Heatmap of transcript levels in Dmr-PERK cells treated with dimerizer (3 nM) for the indicated amount of time. Columns show the mean expression of three technical replicates relative to the mean expression at t=0 on a $\log_2$ scale. **b** Plot of $\log_2$ transcripts per million (TPM) over time of *DGATa* and *DGAT2* transcripts in Dmr-PERK cells following treatment with dimerizer (0.2nM) as described in Figure 3a. **c** Plot of $\log_2$ transcripts per million (TPM) over time of *DGAT2* transcripts in WT and ATF4 KO cells following treatment with dimerizer (0.2nM) as described in Figure 3a. **d** Quantification of the percent confluence of Dmr-PERK cells treated with vehicle or the indicated dose of dimerizer (Dmr). Error bars show mean +/- SD of two independent experiments. **e**. Quantification of the percent confluence of Dmr-PERK cells treated with vehicle, ACATi (10μM), DGAT1i (20μM) or ACCi (2μg/mL), as indicated. Error bars show mean +/- SD of two independent experiments. Source data are provided as a Source Data file.

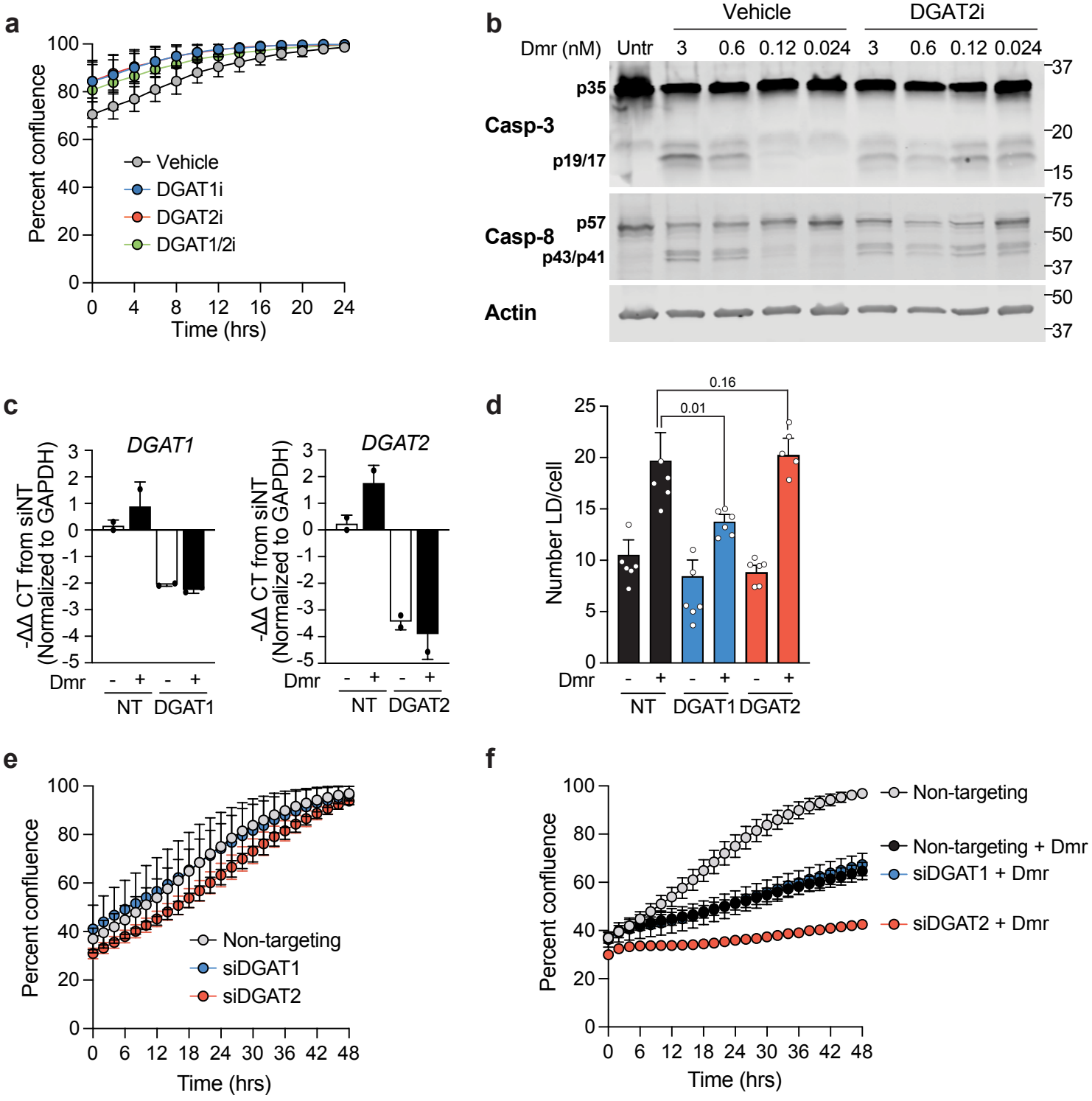
a
Percent confluence
Time (hrs)
Vehicle
DGAT1i
DGAT2i
DGAT1/2i
b
Vehicle
DGAT2i
Dmr (nM)
Untr
3
0.6
0.12
0.024
Casp-3
p35
p19/17
Casp-8
p57
p43/p41
Actin
c
DGAT1
DGAT2
-ΔΔ CT from siNT (Normalized to GAPDH)
Dmr
NT
DGAT1
DGAT2
d
Number LD/cell
0.16
0.01
e
Non-targeting
siDGAT1
siDGAT2
f
Non-targeting
Non-targeting + Dmr
siDGAT1 + Dmr
siDGAT2 + Dmr

FIGURE S10

Supplementary Figure 10. DGAT1 and DGAT2 play distinct roles downstream of ISR activation. **a** Quantification of the percent confluence of Dmr-PERK cells treated with vehicle, DGAT1i (20μM) or DGAT2i (20μM), as indicated. Error bars show mean +/- SD of two replicates and is representative of >3 independent experiments. **b** Western blot for cleaved caspase-3 and -8 in lysates from Dmr-PERK cells treated with dimerizer (Dmr), vehicle or DGAT2i (20μM), as indicated. **c** Quantitative real-time PCR analysis of DGAT1 (left) and DGAT2 (right) transcripts in Dmr-PERK cells transfected with non-targeting siRNA (NT), or siRNA targeting DGAT1 or DGAT2 and treated with dimerizer (Dmr, 0.05nM) for 8 h. **d** Bar plots representing the mean number of LD per cell per well in Dmr-PERK cells treated for 8h with dimerizer (Dmr, 1nM), in the presence of non-targeting siRNA (NT), or siRNA targeting DGAT1 or DGAT2, as indicated. Error bars show mean +/- SD across two independent experiments. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. **e- f** Quantification of the percent confluence of Dmr-PERK cells treated with or without dimerizer (Dmr, 0.12nM), in the presence of non-targeting siRNA (NT), or siRNA targeting DGAT1 or DGAT2, as indicated. Error bars show mean +/- SD of two technical replicates and is representative of 2 independent experiments. Source data are provided as a Source Data file.

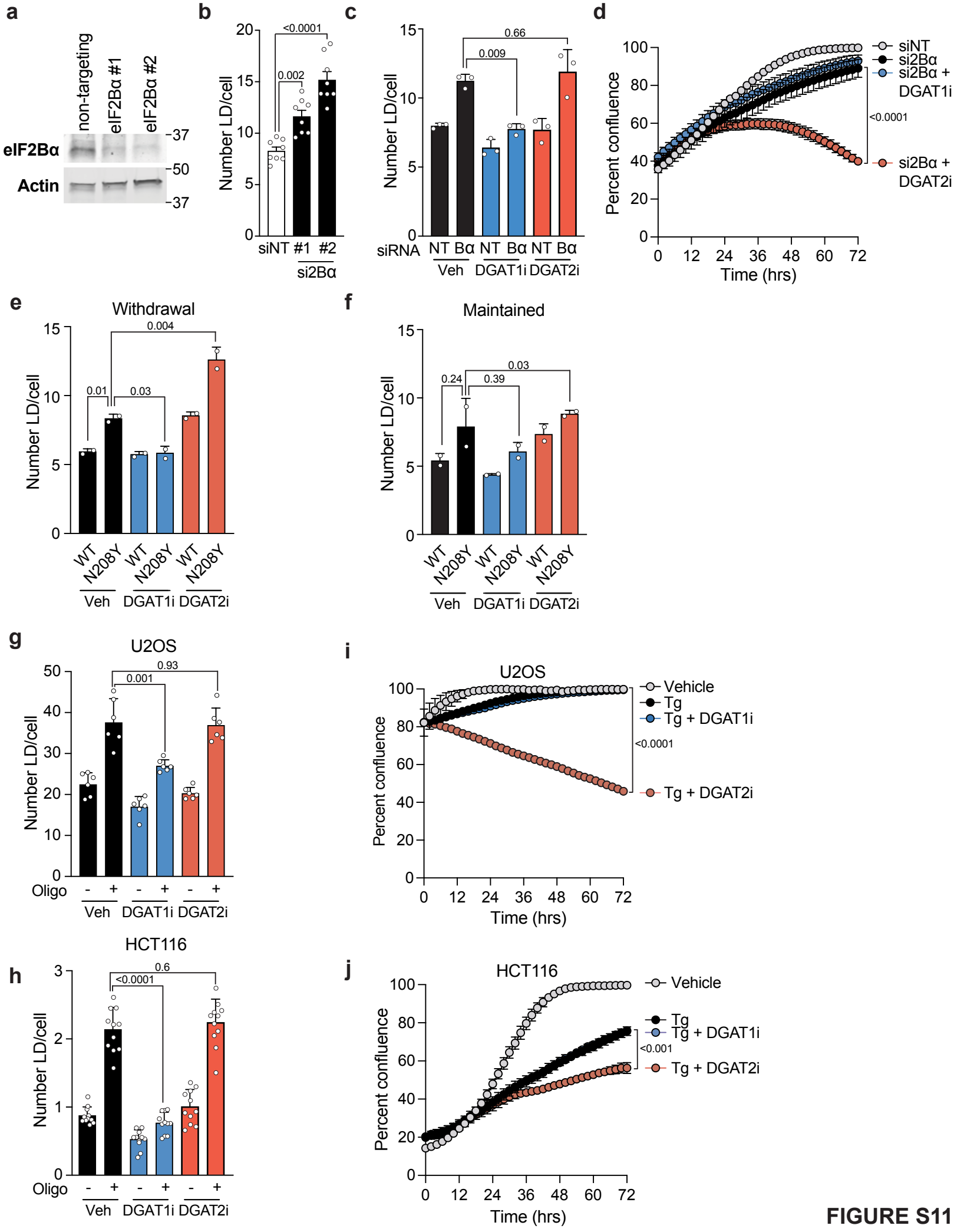
a
non-targeting
eIF2Bα #1
eIF2Bα #2
eIF2Bα
Actin
37
50
37
b
Number LD/cell
<0.0001
0.002
siNT #1 #2
si2Bα
c
Number LD/cell
0.66
0.009
siRNA NT Bα NT Bα NT Bα
Veh DGAT1i DGAT2i
d
Percent confluence
siNT
si2Bα
si2Bα + DGAT1i
<0.0001
si2Bα + DGAT2i
Time (hrs)
e
Withdrawal
Number LD/cell
0.004
0.01
0.03
WT N208Y WT N208Y WT N208Y
Veh DGAT1i DGAT2i
f
Maintained
Number LD/cell
0.03
0.24
0.39
WT N208Y WT N208Y WT N208Y
Veh DGAT1i DGAT2i
g
U2OS
Number LD/cell
0.93
0.001
Oligo - + - + - +
Veh DGAT1i DGAT2i
i
U2OS
Percent confluence
Vehicle
Tg
Tg + DGAT1i
<0.0001
Tg + DGAT2i
Time (hrs)
h
HCT116
Number LD/cell
0.6
<0.0001
Oligo - + - + - +
Veh DGAT1i DGAT2i
j
HCT116
Percent confluence
Vehicle
Tg
Tg + DGAT1i
<0.001
Tg + DGAT2i
Time (hrs)

FIGURE S11

Supplementary Figure 11. The distinct roles of DGAT1 and DGAT2 do not depend on the mode of ISR activation. a Western blot for eIF2Bα in lysates from Dmr-PERK cells treated with non-targeting siRNA (NT), or siRNA targeting eIF2Bα (si2Bα). **b** Bar plots representing the mean number of LD per cell per well in Dmr-PERK cells treated with non-targeting siRNA (NT), or siRNA targeting eIF2Bα (si2Bα). Error bars show mean +/- SD of three technical replicates. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. **c** Bar plots representing the mean number of LD per cell per well in Dmr-PERK cells treated with non-targeting siRNA (NT), or siRNA targeting eIF2Bα (si2Bα), with or without DGAT1i (20μM) or DGAT2i (20μM) for 8h. Error bars show mean +/- SD of three technical replicates. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. **d** Quantification of the percent confluence of Dmr-PERK cells treated with non-targeting siRNA (NT), or siRNA targeting eIF2Bα (si2Bα), with or without DGAT1i (20μM) or DGAT2i (20μM). Error bars show mean +/- SD of three technical replicates and is representative of 2 independent experiments. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. **e-f** Bar plots representing the mean number of LD per cell per well in WT and N208Y MEFs withdrawn (e) or maintained (f) in 2BAct (500nM) for 8 h with or without DGAT1i (20μM) or DGAT2i (20μM). Error bars show mean +/- SD of two technical replicates and is representative of 2 independent expriments. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. **g-h** Bar plots representing the mean number of LD per well per well in U2OS (g) or HCT116 (h) cells treated with oligomycin (100nM) and either vehicle (Veh), DGAT1i (20μM) or DGAT2i (20μM) for 8h. Error bars show mean +/- SD across two independent experiments. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. **i-j** Quantification of the percent confluence of U2OS (i) or HCT116 (j) cells treated with thapsigargin (Tg, 3nM) and either vehicle, DGAT1i (20μM) or DGAT2i (20μM). Error bars show mean +/- SD of two replicates and is representative of 2 independent experiments.

Statistical significance was evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. Source data are provided as a Source Data file.

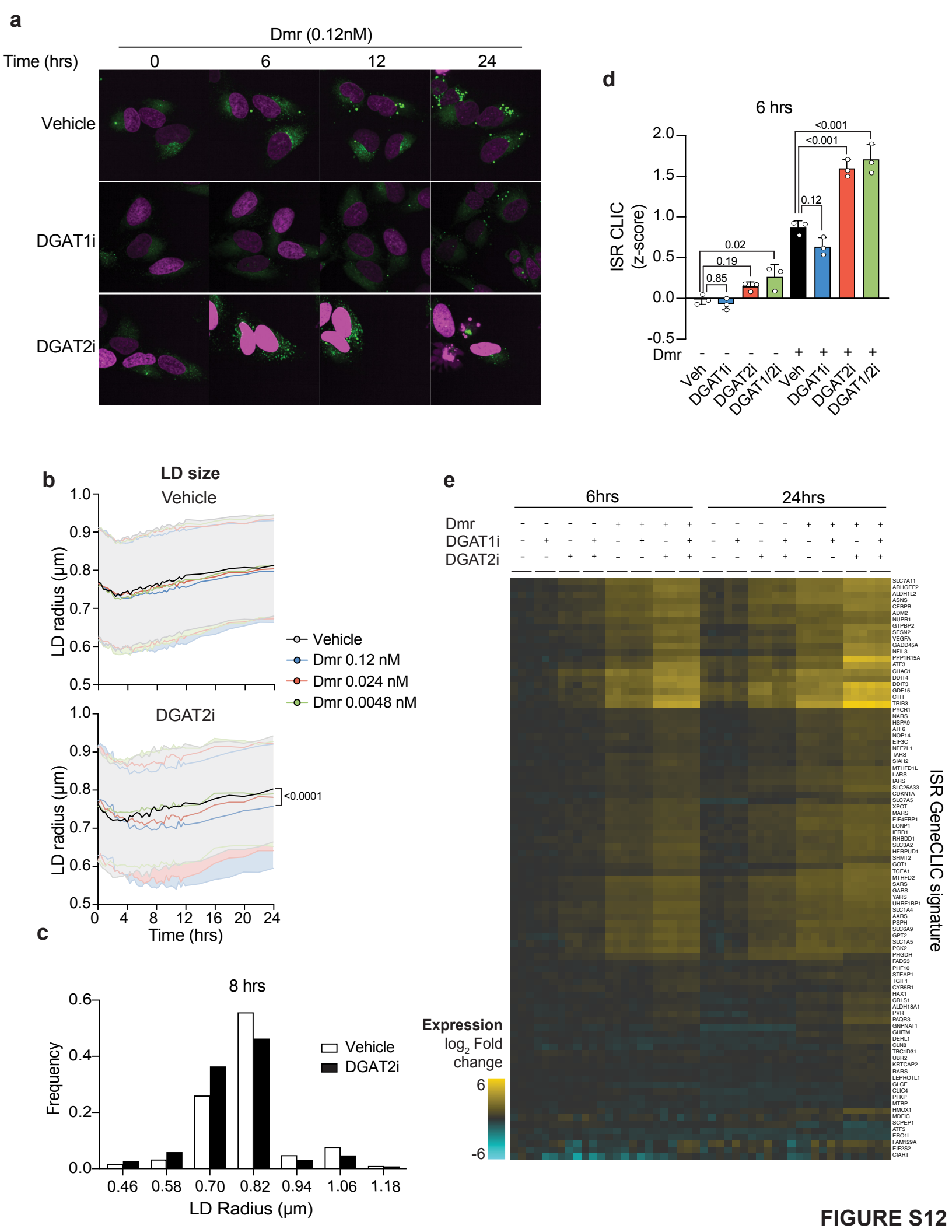
a
Dmr (0.12nM)
Time (hrs)
0
6
12
24
Vehicle
DGAT1i
DGAT2i
d
6 hrs
ISR CLIC (z-score)
2.0
1.5
1.0
0.5
0.0
-0.5
<0.001
<0.001
0.12
0.02
0.19
0.85
Dmr
Veh
DGAT1i
DGAT2i
DGAT1/2i
b
LD size
Vehicle
LD radius (µm)
1.0
0.9
0.8
0.7
0.6
0.5
Vehicle
Dmr 0.12 nM
Dmr 0.024 nM
Dmr 0.0048 nM
DGAT2i
<0.0001
0
4
8
12
16
20
24
Time (hrs)
c
8 hrs
Frequency
0.6
0.4
0.2
0.0
Vehicle
DGAT2i
0.46
0.58
0.70
0.82
0.94
1.06
1.18
LD Radius (µm)
e
6hrs
24hrs
Dmr
DGAT1i
DGAT2i
Expression
log2 Fold change
6
-6
ISR GeneCLIC signature

FIGURE S12

Supplementary Figure 12. Loss of DGAT2i induces an ISR a Representative images of BODIPY-stained LDs (green) and nuclei (magenta) in Dmr-PERK cells treated for the indicated amount of time with dimerizer (Dmr, 0.12nM) and either vehicle, DGAT1i (20μM) or DGAT2i (20μM). **b** Quantification of LD size in Dmr-PERK cells from Figure 6e, f, treated with the indicated doses of dimerizer (Dmr) over 24h in the presence of vehicle or DGAT2i (20μM). Error bars show mean +/- SD of the total distribution of LD size within a condition across 3 independent experiments. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. **c** Bar plot depicting the frequency of LDs of the indicated radius (0.14μm bins) after 8h of dimerizer (0.12nM) treatment in the presence of vehicle or DGAT2i (20μM). **d** Average z-core of ISR CLIC genes calculated from nCounter gene expression profiling of Dmr-PERK cells treated for 6h with dimerizer (Dmr, 0.05nM), DGAT1i (20μM) or DGAT2i (20μM), as indicated. Error bars show mean +/- SD of 3 replicates. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. **e** Heatmap of ISR CLIC genes calculated from nCounter gene expression profiling of Dmr-PERK cells treated for 6h or 24h with dimerizer (Dmr, 0.05nM), DGAT1i (20μM) or DGAT2i (20μM), as indicated. Each gene is normalized to its mean expression at t=0 on a $\log_2$ scale. Columns show the mean expression of three technical replicates for each timepoint. Source data are provided as a Source Data file.