Supplemental Figure Legends

Figure S1. Long-term ID inactivates mTORC1
(A) Levels of non-heme iron in response to 150μM DFO for 24 hours in HEK293T cells (n=5 replicates, unpaired t-test, mean ± SE). **(B)** mRNA levels of *TTP* and *TFRC* at various time points after addition of 150μM DFO in HEK293T cells. Internal control: *POLR2A* (n=6 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(C)** Immunoblot of mTORC1 activity 18 hour after treatment with indicated concentrations of DFO ranging from 0 to 150μM in HEK293T cells. **(D)** Immunoblot of mTORC1 activity in response to 3 hours of with 150μM DFO or 50μM BPD in HEK293T cells. **(E)** mRNA levels of *TFRC*, *REDD1*, and *TTP* 3 hours after addition of 150μM DFO or 50μM BPD. Internal control: *POLR2A* (n=4-8 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(F)** Immunoblot of mTORC1 activity in response to 250nM Torin1 at indicated time points.

Figure S2. Iron chelation inhibits mTORC1 activity independent of cell cycle.
(A) Immunoblot of TFRC and mTORC1 activity in freshly isolated primary murine hepatocytes 18 hours after treatment with increasing concentrations of DFO. **(B)** mRNA of *Ttp and Tfrc* in primary murine hepatocytes in response to indicated concentrations of DFO for 18 hours. Internal control: *Polr2a* (n=3 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(C)** Immunoblot of mTORC1 activity in hPS-CM 18 hours after treatment with 150μM DFO. **(D)** Immunoblot of mTORC1 activity in hiPS-neurons 18 hours after treatment with 150μM DFO. **(E)** Immunoblot of HEK293T cells chelated for 18 hours with 150μM DFO followed by the readdition of 300μM ferric ammonium citrate (FAC) for indicated times.

Figure S3. Iron chelation inhibits cell cycle but does not cause cell death within 24 hours.
(A) Fluorescent microscopy of BrdU incorporation in HEK293T cells treated with 150μM DFO for 12 hours. **(B)** Quantification of cellular proliferation by Hoescht staining of nuclei 48 hours after treatment with DFO (n=6 replicates, unpaired t-test, mean ± SE). **(C)** Fluorescent microscopy of cell death using Hoescht and propidium iodide (PI) double staining in HEK293T cells treated with 150μM DFO for 0-24 hours or 250nM Torin-1 for 24 hours. **(D)** Quantification of images in panel C. (n=6 replicates, one-way ANOVA and Tukey's post-hoc test, mean ± SE).

Figure S4. Reducing Transferrin saturation in the culture media and overexpressing the iron exporter Ferroportin inhibits mTORC1 activity.
(A) Immunoblot of mTORC1 activity in HEK293T cells treated with high Tf-sat (66%) or low Tf-sat (6.6%) media for 18 hours. **(B)** Summary of immunoblot in panel A (n=3 replicates, unpaired t-test, mean ± SE). **(C)** Immunoblot of mTORC1 activity in HepG2 cells transfected with the FPN-GFP fusion protein and TET inducible rtTA3 plasmids in the presence and absence of 500ng/ml doxycycline for 48 hours. **(D)** Summary of immunoblot in panel C (n=3 replicates, unpaired t-test, mean ± SE). **(E)** Fluorescent microscopy of cells transfected with FPN-GFP construct and treated with 500ng/ml doxycycline for 24 hours demonstrating appropriate expression and localization of the FPN-GFP fusion protein. **(F)** Immunoblot of puromycin incorporation in rtTA3/FPN-GFP stable HEK293T cells in the presence and absence of 500ng/ml doxycycline for 48 hours. **(G)** mRNA expression of ER stress makers *CHOP* and *BNIP3* in HepG2 cells transfected with rtTA3/FPN-GFP plasmids and treated with 500ng/ml doxycycline for 48 hours. Internal control: *18S* (n=4 replicates, unpaired t-test, mean ± SE).

Figure S5. Analysis of mTORC1 core complex expression and mitogen signaling pathways and mitochondrial function during ID.

(A) Immunoblot of protein levels of the key components of the mTORC1 complex after 24 hours of 150μM DFO in HEK293T cells. **(B)** Summary of results shown in panel A (n=4 replicates, unpaired t-test, mean ± SE). **(C)** Immunoblot of total and phosphorylated TSC2, AKT, ERK, GSK3β, and S6 proteins and total P53 at different time points after treatment with 150μM of DFO in HEK293T cells. **(D)** Immunoblot of mTORC1 activity and mitochondrial function in HepG2 cells treated with DFO for 18 hours at the indicated doses. **(E)** Oxygen Consumption Rate (OCR) measured by the Seahorse Assay in HepG2 cells treated for 24 hours of DFO at the indicated doses (n=8-10 replicates per group).

Figure S6. TSC2 complex and REDD1 are not required for mTORC1 inhibition during ID, and ID activates AMPK signaling.
(A) *Ttp* mRNA in WT and *Tsc2* KO MEFs in the presence and absence of 150μM DFO for 18 hours. Internal control: *Snrk* (n=4 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(B)** Immunoblot of mTORC1 activity in WT and *Tsc2* KO MEFs that were serum starved overnight followed by the addition of serum for 1 hour in the presence or absence of 150μM DFO for 17 hours **(C)** *REDD1* mRNA in HEK293T cells in response to *REDD1* siRNA. Internal control: *POL2RA* (n=6 replicates per condition, unpaired t-test, mean ± SE). **(D)** *TFRC* mRNA in HEK293T cells in response to *REDD1* siRNA and 150μM DFO for 18 hours. Internal control: *POL2RA* (n=6 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(E)** Immunoblot of mTORC1 and AMPK activity in the presence of DFO or serum starvation with AICAR (an AMPK activator)**. (F)** *Ttp* mRNA in the presence and absence of 150μM DFO for 18 hours in WT and *Ampkα1/2* dKO cells. Internal control: *Polr2a* (n=4 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE).

Figure S7. Iron chelation inhibits mTORC1 activity independent of alterations in mitochondrial metabolism.
(A) Targeted metabolomics in HEK293T cells treated with 150μM DFO for 18 hours (n=4 replicates per condition, Mann-Whitney test, median ± quartiles). **(B)** Total cellular ATP pools in HEK293T cells after 18 hours of treatment with 150μM DFO (n=4 replicates per condition, Mann-Whitney test, median ± quartiles). **(C)** Immunoblot of mTORC1 activity in HEK293T cells in the presence and absence of 150μM DFO for 18 hours, supplemented with 1mM dimethyl malate or 500μM NMN. **(D)** Immunoblot of mTORC1 activity in HEK293T cells in the presence and absence of 150μM DFO for 18 hours, supplemented with 100μM nucleoside cocktail (Adenosine, Guanosine, Thymidine and Cytidine), or 500μM dimethyl aspartate.

Figure S8. ID does not impair lysosomal acidification, and Leucine insensitive mTORC1 cell lines are resistant to inhibition by ID but have increased cell death.
(A) Fluorescent confocal microscopy image of acridine orange staining in HEK293T cells treated with 150μM DFO for 24 hours. **(B)** Summary of the results in Panel A (n=6-7 replicates per condition, unpaired t-test, mean ± SE). **(C)** Total leucine levels in HEK293T cells treated with 150μM DFO for 24 hours (n=4-5 replicates per condition, Mann-Whitney test, median ± quartiles). **(D)** SAM levels in HEK293T cells treated with 150μM DFO for 18 hours measured by HPLC-MS (n=4-5 replicates per condition, Mann-Whitney test, median ± quartiles). **(E)** Immunoblot of mTORC1 activity in WT and *NPRL2* KO HEK293T cells deprived of leucine for 16 hours and restimulated with 400μM leucine for 1 hour. **(F)** Fluorescent microscopy of cell death using Hoescht and propidium iodide (PI) double staining in WT and *NPRL2* KO HEK293T cells treated with 150μM DFO for 0, 36 and 60 hours. **(G)** Quantification of images in panel F. (n=6 replicates, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(H)** Immunoblot of mTORC1 activity in *Rraga*$^{+/+}$ (WT) and *Rraga*$^{Q66L/Q66L}$ (KI) MEFs treated with 150μM DFO for 18 hours. **(I)** Densitometry analysis of immunoblot in panel H. (n=3 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE).

Figure S9. ID suppresses leucine uptake and leucine transporter expression and cell-permeable leucine re-localizes mTORC1 to the lysosome during ID
(A) ^{3}H-leucine uptake into MEFs treated with 150μM DFO for 24 hours (n=5-6 replicates per condition, unpaired t-test, mean ± SE). **(B)** ^{3}H-Leucine-leucine uptake into MEF cells treated with 50nM rapamycin for 24 hours (n=5-6 replicates per condition, unpaired t-test, mean ± SE). **(C)** ^{14}C-Leucine uptake into HEK293T cells transfected with rtTA3/FPN-GFP or rtTA3/eGFP control and in the presence and absence of 500ng/ml doxycycline treatment for 48 hours or 150μM of DFO for 18 hours (n=5-6 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(D)** mRNA of various leucine transporters in HepG2 cells transfected with rtTA3/FPN-GFP plasmids in and treated with 500ng/ml doxycycline for 48 hours. Internal control: *18S* (n=5-6 replicates per condition, unpaired t-test, mean ± SE). **(E)** *Slc38a9* mRNA levels in MEFs treated with DFO for 24 hours. Internal control: *Polr2a* (n=8 replicates per condition, unpaired t-test, mean ± SE). **(F)** *TFRC* mRNA levels in HeLa cells treated with 150μM DFO for the indicated time points. Samples matched with Figure 4D. Internal control: *POLR2A* (n=4 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(G)** Immunoblot of mTORC1 activity in cytosolic fraction and LAT3 and PAT1 in the membrane fraction of HeLa cells treated with 150μM DFO for 18 hours. **HA)** Normalized expression (NX) of *SLC43A1* across multiple human tissues (https://www.proteinatlas.org/ENSG00000149150-SLC43A1/tissue). **(I)** Table listing the normalized expression of leucine transporters in the liver. Data for panels A and B was acquired from The Human Protein Atlas version 20.1 and Ensembl version 92.38, available from http://www.proteinatlas.org
"Uhlén M et al., **Tissue-based map of the human proteome.** *Science* (2015) PubMed: 25613900 DOI: 10.1126/science.1260419". **(J)** Recruitment of mTORC1 to the lysosome in HEK293T cells treated with 150μM DFO for 18 hours and supplemented with 400μM LLMOE for 1 hour. **(K)** Summary of the results in panel A (n=6 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE).

Figure S10. ID causes global hyper-methylation of histones
(A-B) Unbiased measure of global changes in histone methylation measured by histone-MS in response to ID. Graphs are presented as rank ordered lists by change in %abundance with 150μM DFO - control (A) and total %abundance in the 150μM DFO group (B). Data matched with Figure 5B.

Figure S11. ID and decreased transferrin saturation increase H3K9 di-methylation independent of cell-cycle, and ID represses mTORC1 activity and increases H3K9 di-methylation independent of ARNT, ATF4, and IRP system.
(A) Immunoblot of H3K9me^{2} in hiPS-CM 18 hours after treatment with 150μM DFO. Data paired with Figure S2C **(B)** Immunoblot of H3K9me^{2} in hiPS-Neurons 18 hours after treatment with 150μM DFO. Data paired with Figure S2D **(C)** Immunoblot of mTORC1 activity and H3K9me^{2} levels in MEFs treated with high Tf-sat (66%), low Tf-sat (6.6%), or high Tf-sat (66%) media supplemented with 150μM DFO for 24 hours. (**D, E**) Summary of immunoblot in panel C (n=3 replicates, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(F)** Immunoblot of H3K9me^{2} in HepG2 cells transfected with rtTA3/GFP-FPN plasmids and treated with 500ng/ml doxycycline for 48 hours. Data paired with Figure S4C **(G)** mRNA of *Ttp* and *Redd1* in WT and *Arnt* KO MEFs treated with 150μM DFO for 12 hours. Internal control: *18S* (n=4 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(H)** Immunoblot of mTORC1 activity and H3K9me^{2} levels in WT and *Atf4* KO MEFs treated with 150μM DFO for 18 hours. **(I)** Summary of immunoblot in panel H (n=3 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(J)** Immunoblot of the IRP targets FPN and FTH1, and

H3K9me^2 levels in WT and *Irp1/2* KD/KO MEFs treated with 150μM DFO for 18 hours. **(K)** Summary of immunoblot in panel J (n=3 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(L)** Immunoblot of the IRP1 levels in *Irp2* KO MEFs treated with control or siRNA targeted against *Irp1*. **(M)** Summary of immunoblot in panel L (n=3 replicates per condition, unpaired t-test, mean ± SE). **(N)** mRNA of *Irp1*, *Tfr1*, *Ttp* and *Lat3* in WT and *Irp1/2* KD/KO MEFs treated with 150μM DFO for 18 hours. Internal control: *Snrk* (n=6 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE).

Figure S12. Increased H3K9 di-methylation during iD is upstream of mTORC1 and cannot be rescued by supra-physiologic levels of α-KG or cell-permeable leucine.
(A) Ratio of 2-HG/succinate levels in HEK293T cells treated 150μM DFO for 18 hours measured by HPLC-MS (n=4-5 replicates per condition, Mann-Whitney test, median ± SE). **(B)** Immunoblot of mTORC1 activity and H3K9me^2 in HEK293T cells treated with 150μM DFO in the presence and absence of 1mM cell permeable dimethyl α-KG (DMKG). **(C)** Immunoblot of mTORC1 activity and H3K9me^2 in HEK293T cells starved of leucine for 16 hours in the presence or absence of 150μM DFO followed by supplementation with 400μM LLOME for 1 hour.

Figure S13. ID alters occupancy of POLR2A at the promoters of multiple genes.
(A) Fold change in POLR2A occupancy within predefined regions of the promoter and gene body to categorize genes defined by increased POLR2A binding, POLR2A loss, and promoter-pausing. **(B)** Gene-ontology (GO) terms of genes enriched in the increased POLR2A group after ID. **(C)** GO terms of genes that had lost POLR2A after ID. **(D)** UCSC genome browser tracks for the LAT3 (top) and PAT1 (bottom) gene loci. POLR2A and H3K9me2 tracks from ChIP-seq analysis were loaded and represented as the difference in normalized reads between the DFO and control groups. Regions of H3K9me^2 enrichment in the DFO group are underlined in red. Direction of transcription is indicated by a black arrow. Encode Histone (LAT3 and PAT1) and Genehancer (PAT1) browser tracks are displayed beneath and represent predicted enhancer regions which align with regions of increased H3K9me^2 signal in response to DFO. Yellow bar, red and grey arrows indicate enhancer regions designated by Encode Histone and Genehancer browser tracks.

Figure S14. ID increases H3K9 di-methylation within the promoter of *RPTOR* and correlates with decreased *RPTOR* mRNA expression.
(A) H3K9me^2 signal and POL2RA occupancy in the promoter for *RPTOR* from ChIP-Seq analysis. **(B)** ChIP-PCR of *RPTOR* in HEK293T cells. Cells treated with 150μM DFO or 250μM IOX1 for 12 hours and vehicle controls were followed by IP of lysates using an antibody against H3K9me^2. IgG was used as a negative control for the IP (n=2 replicates). **(C)** *RPTOR* mRNA levels at indicated time points after the addition of 150μM DFO. Samples matched with Figure 4D. Internal control: *POLR2A* (n=4 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(D)** Immunoblot of RPTOR, mTOR and HIF1-α levels in HEK293T cells treated with 100 μg/ml cycloheximide (CHX) for indicated time points. HIF1-α, which is rapidly turned over under normoxic conditions via the actions of the EGLN and Von-Hippel Lindau (VHL) proteins, was used as a positive control. **(E)** Immunoblot of mTORC1 activity and complex member RAPTOR in A549 cells after treatment with 150μM DFO for 48 hours. **(F)** Immunoblot of lysates from cells transfected with FLAG-RAP2A or FLAG-mTOR and treated with or without 150μM DFO for 48 hours. Immunoprecipitation was performed with anti-FLAG antibody. RAP2A = negative control. **(G)** Summary of IP studies in Panel F (n=3 replicates, unpaired t-test, mean ± SE). **(H)** Immunoblot of mTORC1 activity in WT and *NPRL2* KO HEK293T treated with and without 150μM DFO for 48 hours.

Figure S15. ID is an abiotic stress in *Arabidopsis thaliana* and represses *TOR1/KOG1* transcription rate in *Saccharomyces cerevisiae*.
(A) *A. thaliana* root growth on MS medium containing 0 (Control) or 400μM BPD for 4 days. Seedlings were transferred 5 days after stratification on BPD-free MS medium. **(B)** Summary bar graph of root length in panel A. Starting root length (white bar) and new root growth over 4 days (red bar) (n=18-24 replicates per condition, unpaired t-test, mean ± SE). **(C)** Transcriptional rate of *TOR1*, *TOR2* and *KOG1* within 180 minutes of iron chelation in *S. cerevisiae* (n=3 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE, (*****) = $P < 0.05$ for *TOR1,* (†) = $P < 0.05$ for *KOG1*).

Figure S16. The Jumonji-C KDM family inhibitor IOX1 mimics the actions of ID on mTORC1 activity and leucine uptake, and loss of *NPRL2* protects mTORC1 activity from suppression by IOX1.
(A) RT-PCR of AA transporters, *RPTOR* and *TTP* in HEK293T cells treated with various Jmj-C domain inhibitors for 12 hours. Internal control: *POLR2A* (n=3-4 replicates per condition mean ± SE). **(B)** Immunoblot of mTORC1 activity and H3K9me^2 levels in A549 cells treated with 150μM DFO or 250μM IOX1 for 18hrs. **(C)** Immunoblot of indicated proteins in A549 cells treated with 250μM IOX1 for 48 hours. **(D)** Immunoblot of cytosol and membrane fractions from HepG2 cells treated with 250μM IOX1 for 12 hours. **(E)** Densitometry summary of data in Panel D (n=3 replicates per condition, unpaired t-test, mean ± SE). **(F)** ^{14}C-leucine uptake into HEK293T cells treated with 150μM DFO or 100μM IOX1 for 18 hours (n=5-6 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(G)** ^{14}C-leucine uptake into HeLa cells treated with 150μM DFO or 100μM IOX1 for 18 hours (n=5-6 replicates per condition, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(H)** Immunoblot of mTORC1 activity WT and *NPRL2* KO HEK293T cells treated with 250μM IOX1 for 18 hours. **(I)** Quantification of immunoblot in panel H (n=3 replicates per group, one-way ANOVA with Tukey's post-hoc test. mean ± SE).

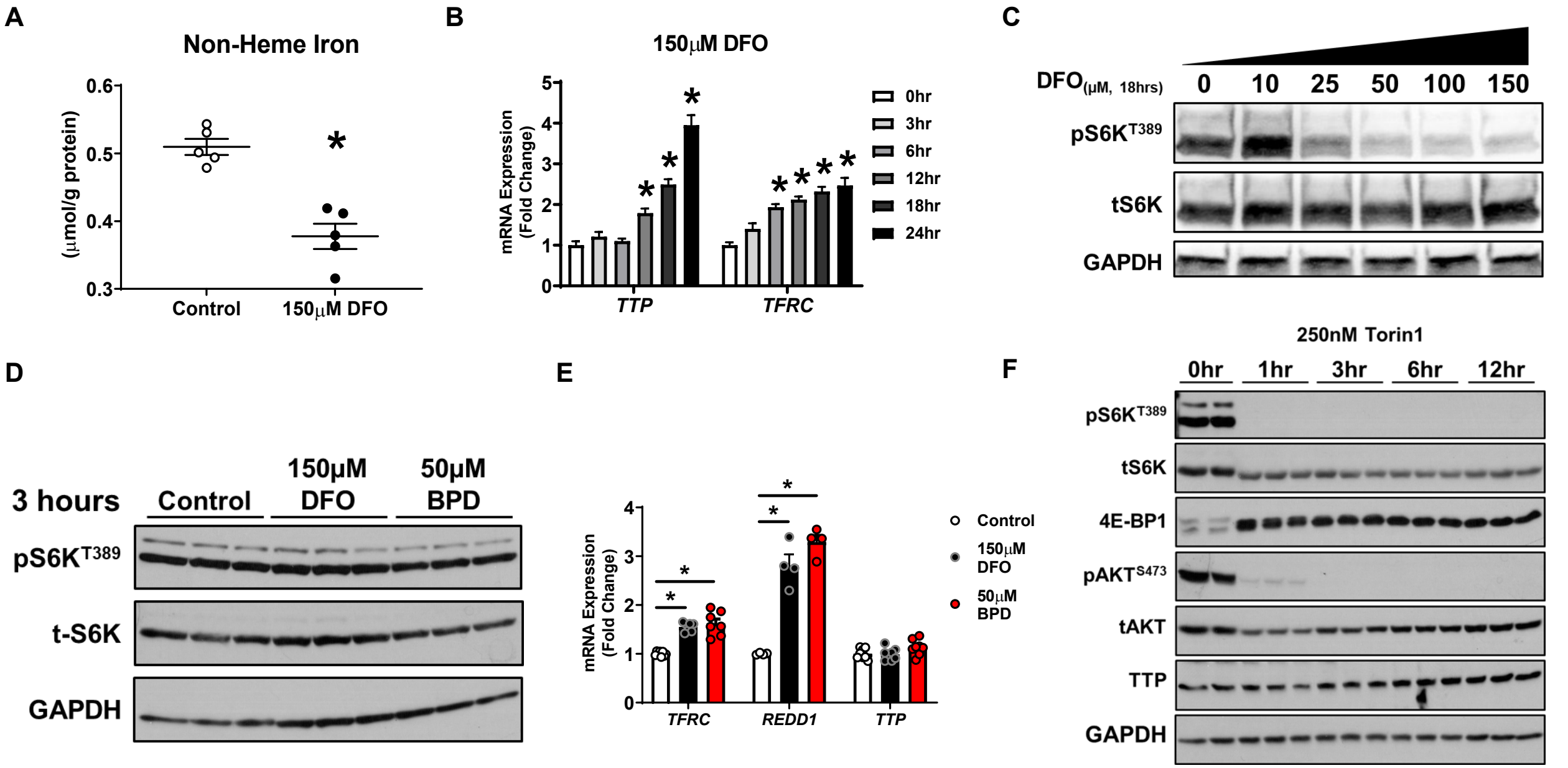
Figure S17. H3K9 di-methyl ChIP-seq signal in iron chelated samples correlate with changes after loss of KDM3A and KDM3B.
(A) Heatmap of the log2FC in H3K9me^2 signal between DFO treatment and control plotted against KDM3A and KDM3B occupancy. K-means cluster =3. **(B)** Browser tracks with KDM3B and H3K9me^2 track data from HCT116 cells treated with control shRNA (shC) or shRNA targeting KDM3A and KDM3B (sh3A3B). Track data for KDM3A was downloaded from the GEO database accession GSE127624. Track data for KDM3B and H3K9me^2 from HCT116 cells were downloaded from the GEO database accession GSE71885. **(C)** Hierarchical clustering and correlation analysis between indicated samples using deepTools plotCorrelation. Values indicate the Pearson correlation between the sample listed in the corresponding row and column.

Figure S18. CRISPR-mediated deletion of KDM3A, 4B, or 4C does not protect mTORC1 activity during ID, and KDM3B KO cells have increased cell death during ID.
(A) Immunoblot showing deletion of KDM4A and 4B, using CRISPR-Cas technology in HEK293T cells. **(B)** Immunoblot of mTORC1 activity and H3K9me^2 in Cas9 (WT) and *KDM3A*, *KDM4B* or *KDM4C* KO HEK293T cells in the presence and absence of 150μM DFO for 18 hours. **(C)** Immunoblot of mTORC1 activity and H3K9me^2 in HEK293T cells with overexpression of HA-tagged KDM4A, KDM4B or combination of the two proteins treated with 150μM DFO for 18 hours. **(D)** Immunoblot of mTORC1 activity and H3K9me^2 in Cas9 (WT) and sg.3B c.7 (*KDM3B* KO) *TSC2* KO HeLa cells in the presence and absence of 150μM DFO for 18 hours.

(E-F) Summary of immunoblot in panel D (n=3 replicates per group, one-way ANOVA with Tukey's post-hoc test. Mean ± SE). **(G)** Fluorescent microscopy of cell death using Hoescht and propidium iodide (PI) double staining in *KDM3B* KO HEK293T cells treated with 150μM DFO for 0, 24, 36 and 60 hours **(H)** Quantification of images in panel G. (n=6 replicates, one-way ANOVA and Tukey's post-hoc test, mean ± SE).

Figure S19. The oxygen-sensor KDM6A has a high affinity for iron, and marker of tumor iron status influences survival and correlates with expression of KDM3B and mTORC1 related genes.
(A) SDS-PAGE gel of purified N-terminal FLAG-tagged WT KDM6A and KDM6A$^{MT/ED}$ mutant expressed using a baculoviral overexpression system. **(B, C)** Enzyme kinetics of WT KDM6A (**B**) and KDM6A$^{MT/ED}$ (**C**) in the presence of increasing concentrations of iron. **(D)** Enzyme kinetics of KDM3B in the presence of increasing concentrations of R-2HG. **(E)** Immunoblot of mTORC1 activity in HEK293T cells after treatment with 150μM DFO or increasing concentrations of octyl-R-2HG. **(F)** Ordered list of tumor types by *TFRC* expression. **(G)** Cox-proportional hazard curves of overall survival in cancer patients stratified by median expression of *TFRC*, *PAT1,* and *RPTOR*. **(H)** Correlation between the expression of *LAT3*, *PAT1,* or *RPTOR* and *KDM3B* in *TFRC* over-expressing tumors.

Figure S20. ID increases cancer cell sensitivity to genotoxic chemotherapy, and represses RAPTOR levels and mTORC1 activity in patient-derived primary tumor cells.
(A-B) Quantification of viable A549 adenocarcinoma (**A**) and HeLa cervical carcinoma (**B**) cells after treatment with the indicated doses of Cisplatin for 48 hours in the presence and absence of 150μM DFO (n=6 replicates, one-way ANOVA and Tukey's post-hoc test, mean ± SE). **(C)** Immunoblot of mTORC1 activity and RAPTOR expression in patient-derived primary tumor cells treated with 150μM DFO for the indicated times. **(D)** Summary of immunoblot in panel D (n=3 replicates per group, one-way ANOVA with Tukey's post-hoc test, mean ± SE).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100
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3 hours
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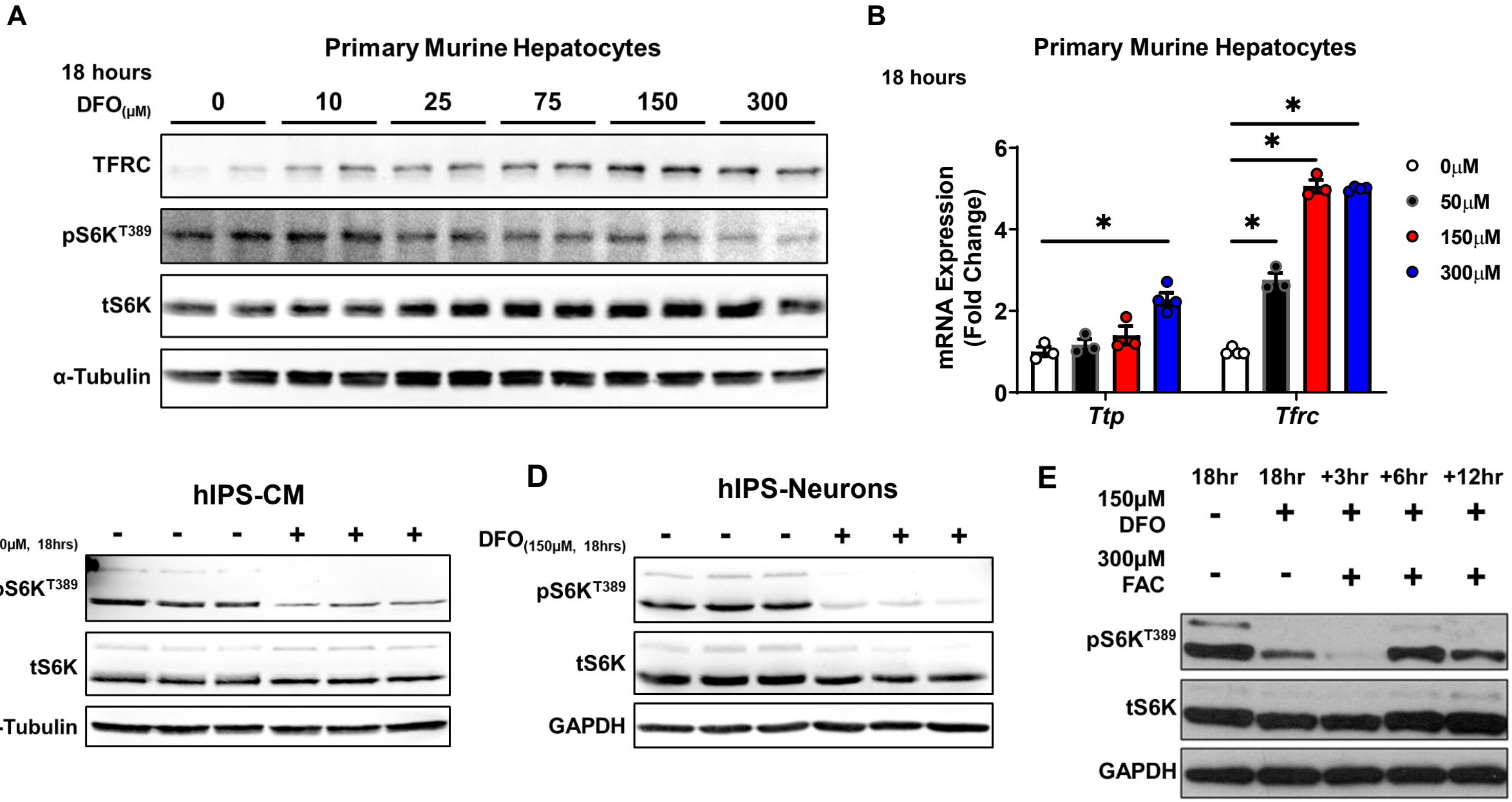
Figure S1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B
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50μM
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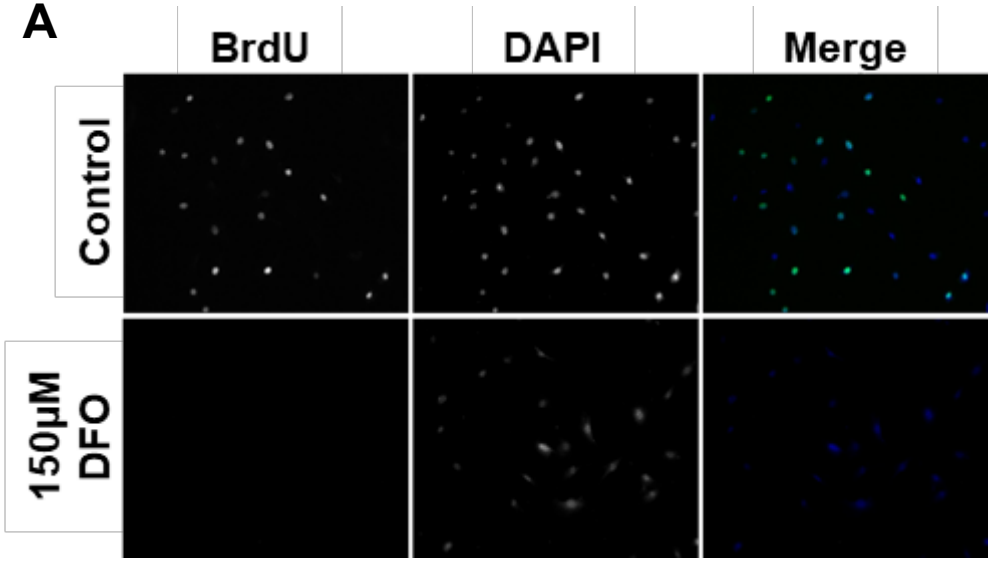
Figure S2

Figure S3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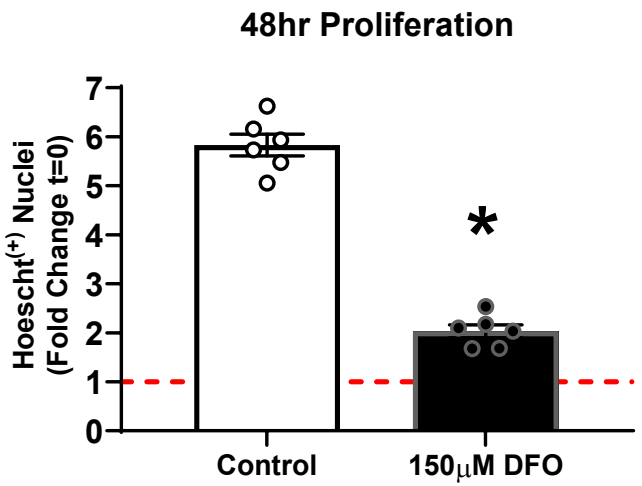
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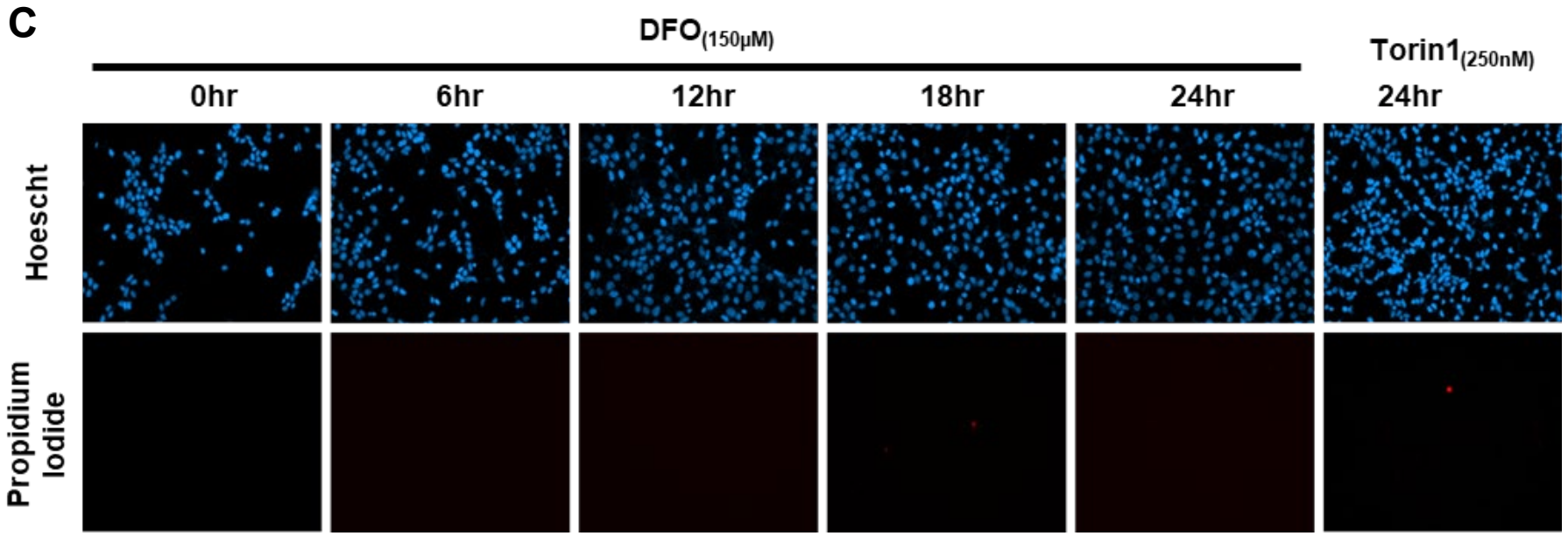
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6hr
12hr
18hr
24hr
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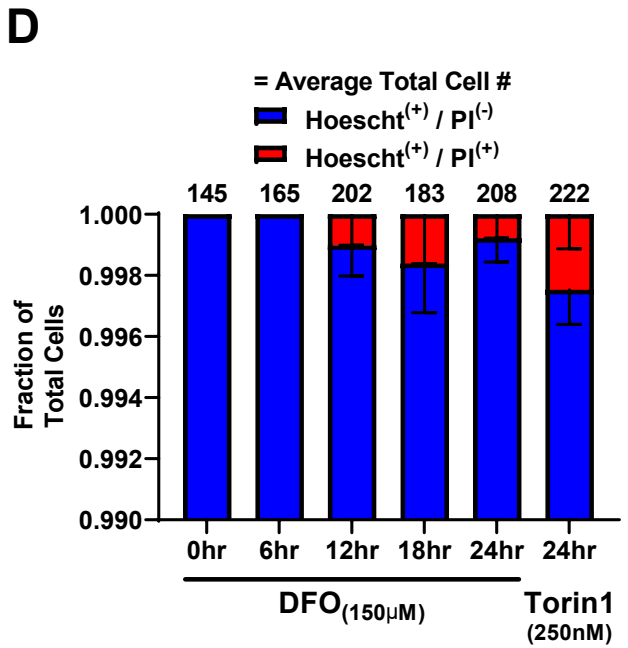
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222
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0.990
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18hr
24hr
24hr
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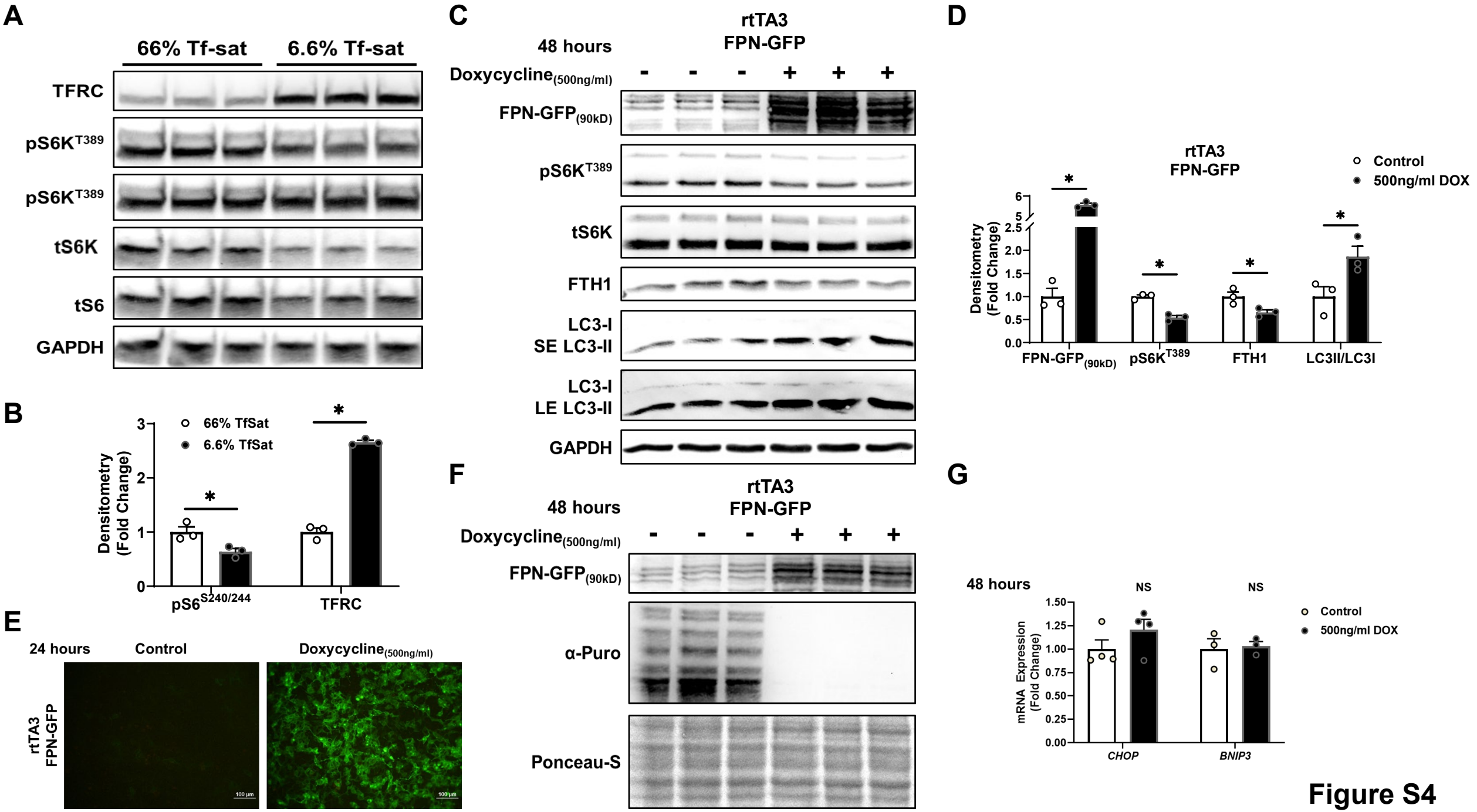
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6.6% TfSat
Densitometry (Fold Change)
pS6 S240/244
TFRC
C
48 hours
rtTA3 FPN-GFP
Doxycycline (500ng/ml)
FPN-GFP (90kD)
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tS6K
FTH1
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GAPDH
D
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LC3II/LC3I
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24 hours
Control
Doxycycline (500ng/ml)
rtTA3 FPN-GFP
F
48 hours
rtTA3 FPN-GFP
Doxycycline (500ng/ml)
FPN-GFP (90kD)
α-Puro
Ponceau-S
G
48 hours
NS
NS
Control
500ng/ml DOX
mRNA Expression (Fold Change)
CHOP
BNIP3

Figure S4

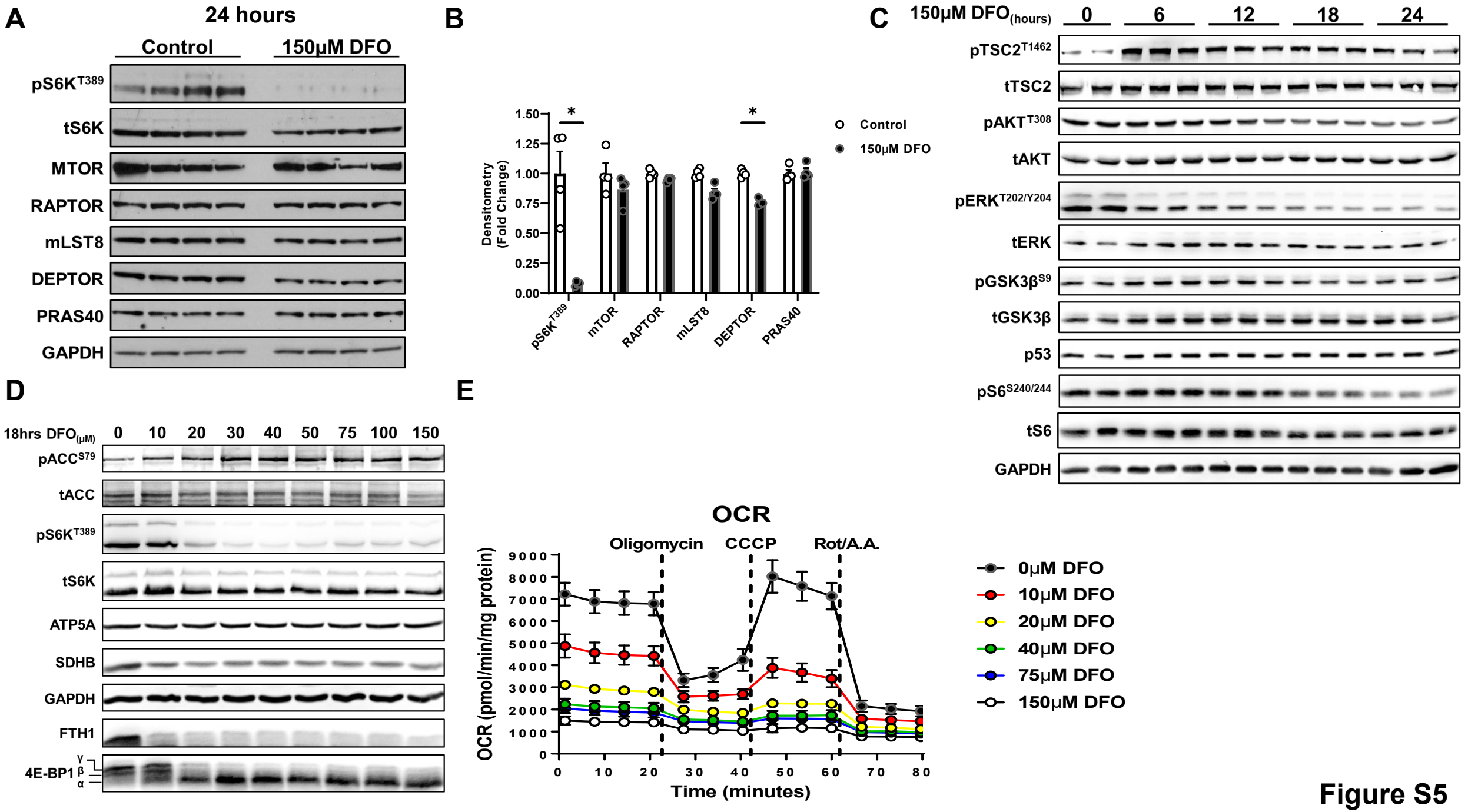

A
24 hours
Control
150μM DFO
pS6K T389
tS6K
MTOR
RAPTOR
mLST8
DEPTOR
PRAS40
GAPDH
B
Densitometry (Fold Change)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
*
*
pS6K T389
mTOR
RAPTOR
mLST8
DEPTOR
PRAS40
Control
150μM DFO
C
150μM DFO (hours)
0
6
12
18
24
pTSC2 T1462
tTSC2
pAKT T308
tAKT
pERK T202/Y204
tERK
pGSK3β S9
tGSK3β
p53
pS6 S240/244
tS6
GAPDH
D
18hrs DFO (μM)
0
10
20
30
40
50
75
100
150
pACC S79
tACC
pS6K T389
tS6K
ATP5A
SDHB
GAPDH
FTH1
4E-BP1
γ
β
α
E
OCR
Oligomycin
CCCP
Rot/A.A.
OCR (pmol/min/mg protein)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
60
70
80
Time (minutes)
0μM DFO
10μM DFO
20μM DFO
40μM DFO
75μM DFO
150μM DFO

Figure S5

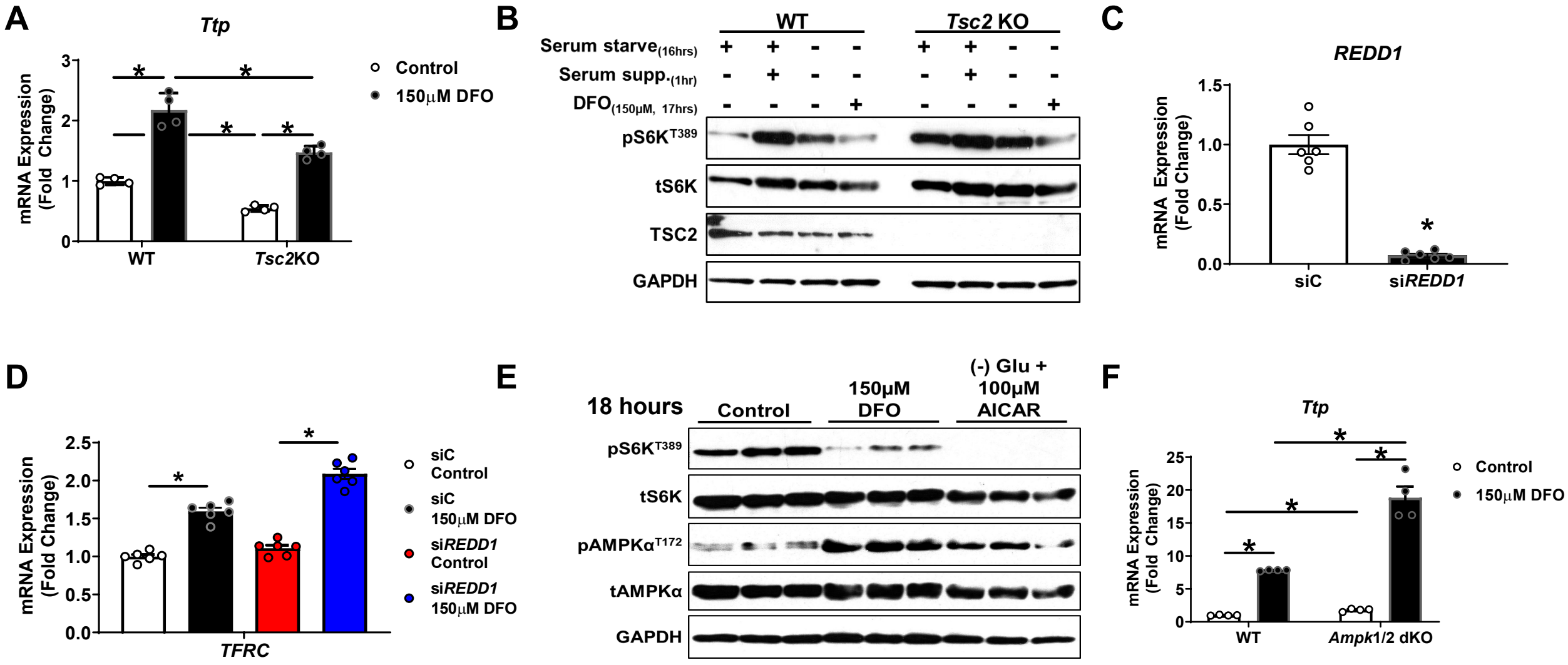

A
Ttp
mRNA Expression (Fold Change)
Control
150μM DFO
WT
Tsc2KO
B
WT
Tsc2 KO
Serum starve(16hrs)
Serum supp.(1hr)
DFO(150μM, 17hrs)
pS6K T389
tS6K
TSC2
GAPDH
C
REDD1
mRNA Expression (Fold Change)
siC
siREDD1
D
mRNA Expression (Fold Change)
TFRC
siC Control
siC 150μM DFO
siREDD1 Control
siREDD1 150μM DFO
E
18 hours
Control
150μM DFO
(-) Glu + 100μM AICAR
pS6K T389
tS6K
pAMPKα T172
tAMPKα
GAPDH
F
Ttp
mRNA Expression (Fold Change)
Control
150μM DFO
WT
Ampk1/2 dKO

Figure S6

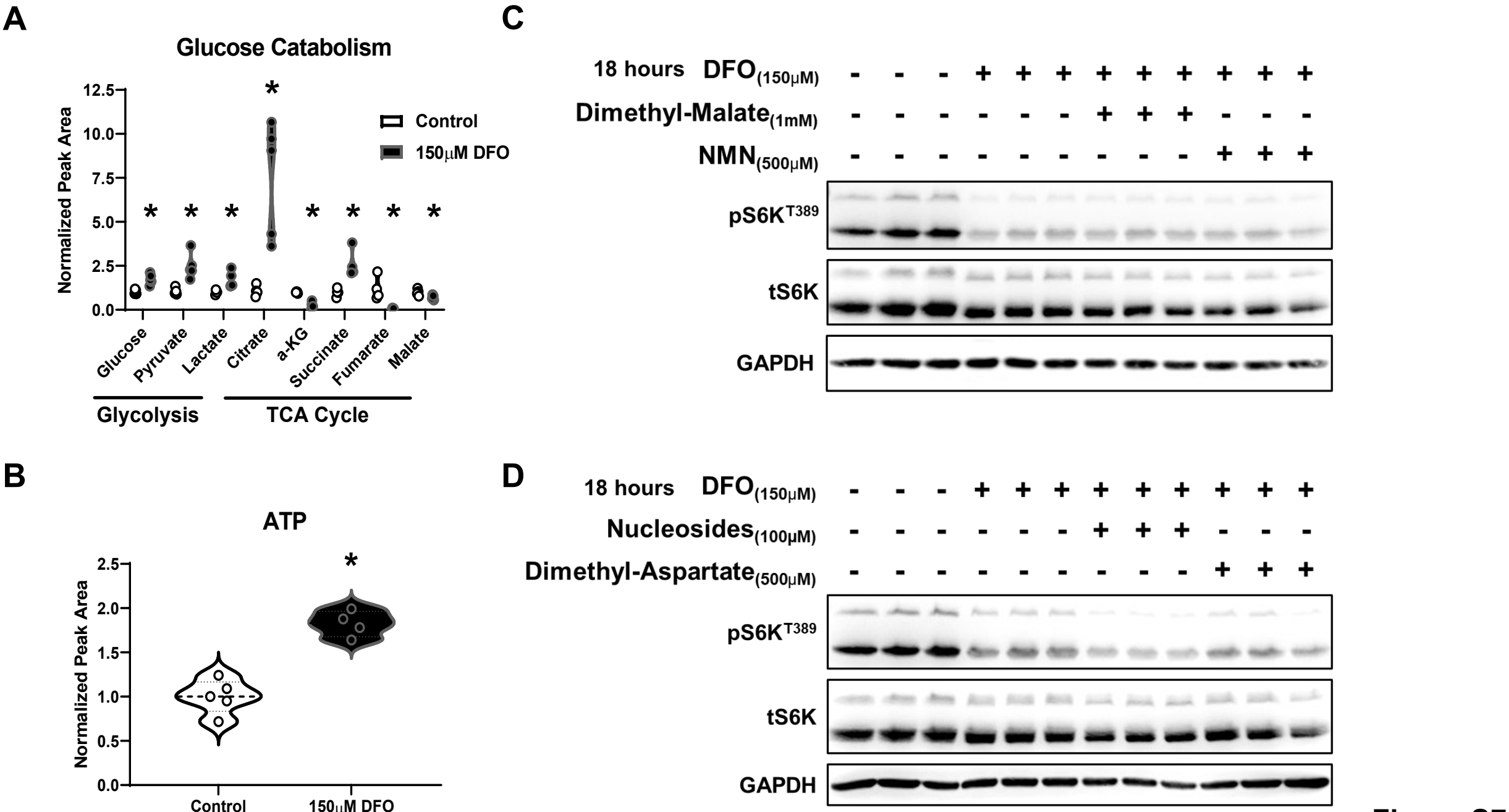
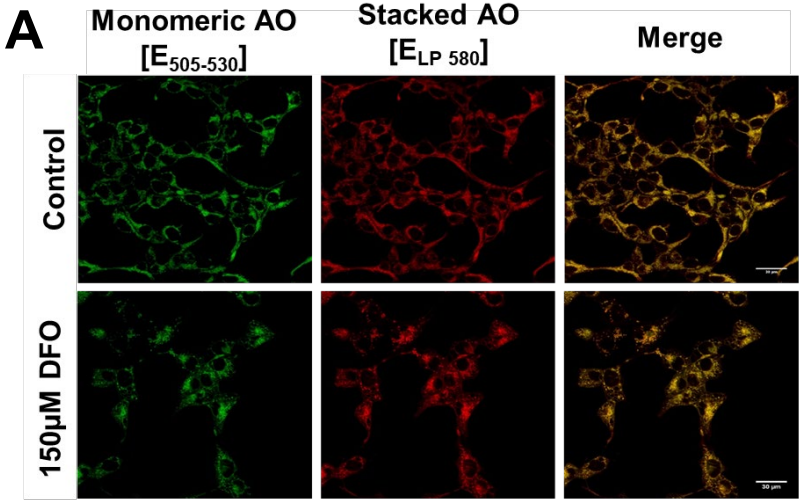
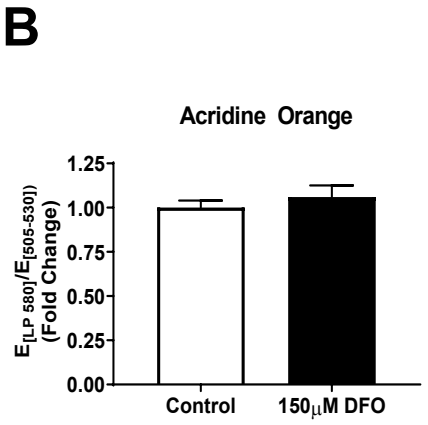
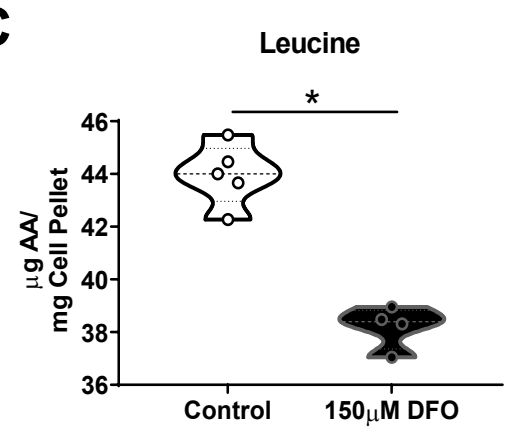
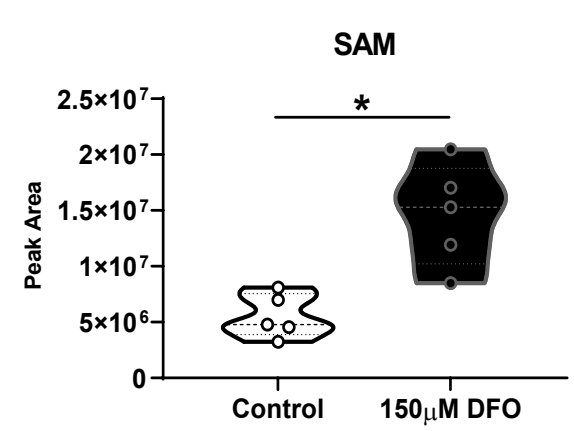

A
Glucose Catabolism
Normalized Peak Area
12.5
10.0
7.5
5.0
2.5
0.0
Control
150μM DFO
Glucose
Pyruvate
Lactate
Citrate
a-KG
Succinate
Fumarate
Malate
Glycolysis
TCA Cycle
B
ATP
Normalized Peak Area
2.5
2.0
1.5
1.0
0.5
0.0
Control
150μM DFO
C
18 hours DFO(150μM)
Dimethyl-Malate(1mM)
NMN(500μM)
pS6KT389
tS6K
GAPDH
D
18 hours DFO(150μM)
Nucleosides(100μM)
Dimethyl-Aspartate(500μM)
pS6KT389
tS6K
GAPDH

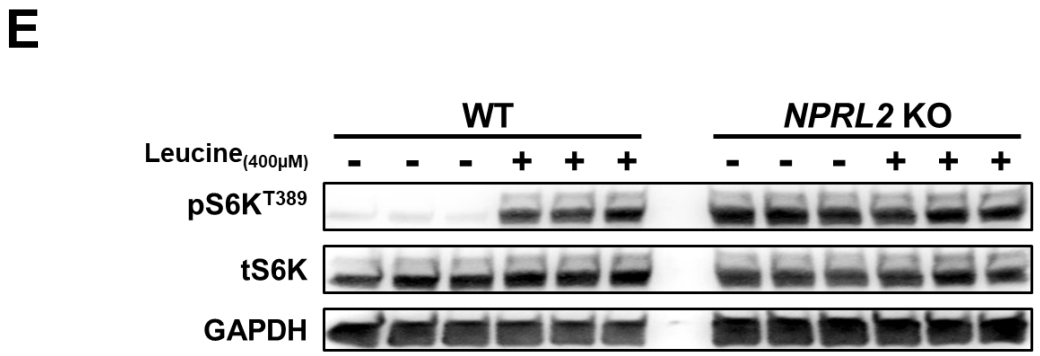
Figure S7

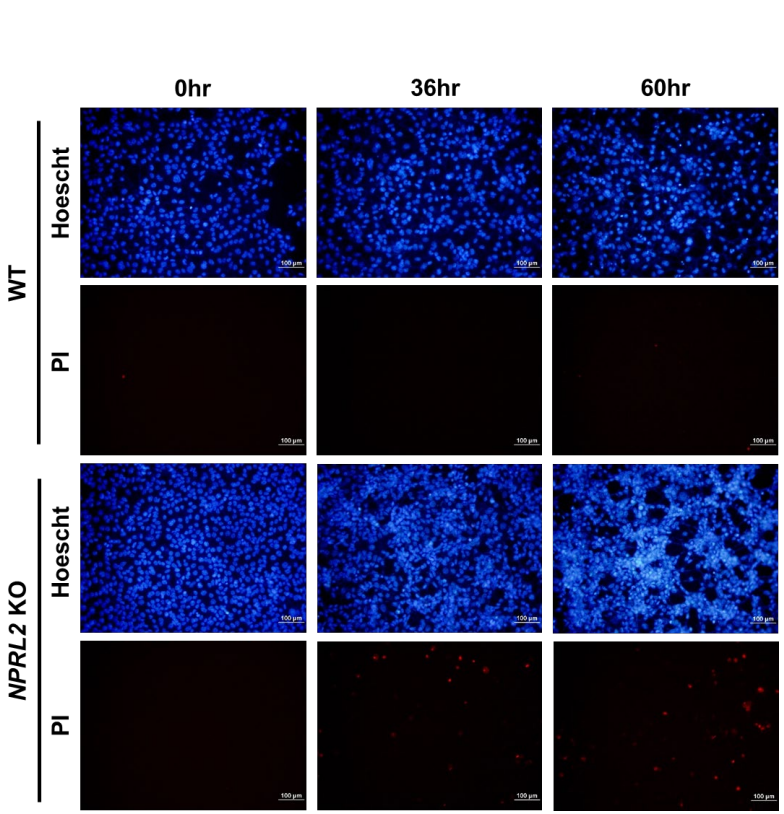

A
Monomeric AO
[E505-530]
Stacked AO
[ELP 580]
Merge
Control
150μM DFO

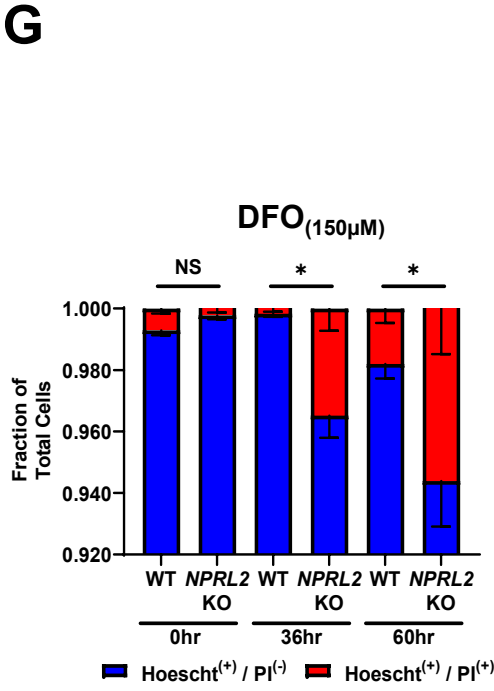

B
Acridine Orange
E[LP 580]/E[505-530]
(Fold Change)
1.25
1.00
0.75
0.50
0.25
0.00
Control
150μM DFO

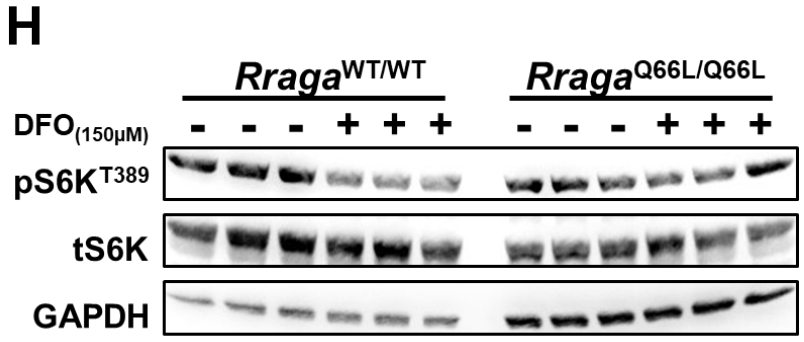

C
Leucine
*
μg AA/
mg Cell Pellet
46
44
42
40
38
36
Control
150μM DFO


D
SAM
*
Peak Area
2.5×10^7
2×10^7
1.5×10^7
1×10^7
5×10^6
0
Control
150μM DFO


E
WT
NPRL2 KO
Leucine(400μM)
- - - + + +
- - - + + +
pS6KT389
tS6K
GAPDH


F
0hr
36hr
60hr
WT
Hoescht
PI
NPRL2 KO
Hoescht
PI


G
DFO(150μM)
NS
*
*
Fraction of
Total Cells
1.000
0.980
0.960
0.940
0.920
WT
NPRL2 KO
WT
NPRL2 KO
WT
NPRL2 KO
0hr
36hr
60hr
Hoescht(+) / PI(-)
Hoescht(+) / PI(+)


H
RragaWT/WT
RragaQ66L/Q66L
DFO(150μM)
- - - + + +
- - - + + +
pS6KT389
tS6K
GAPDH

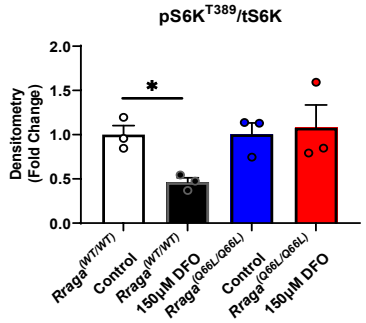

I
pS6KT389/tS6K
Densitometry
(Fold Change)
2.0
1.5
1.0
0.5
0.0
*
RragaWT/WT
Control
RragaWT/WT
150μM DFO
RragaQ66L/Q66L
Control
RragaQ66L/Q66L
150μM DFO

Figure S8

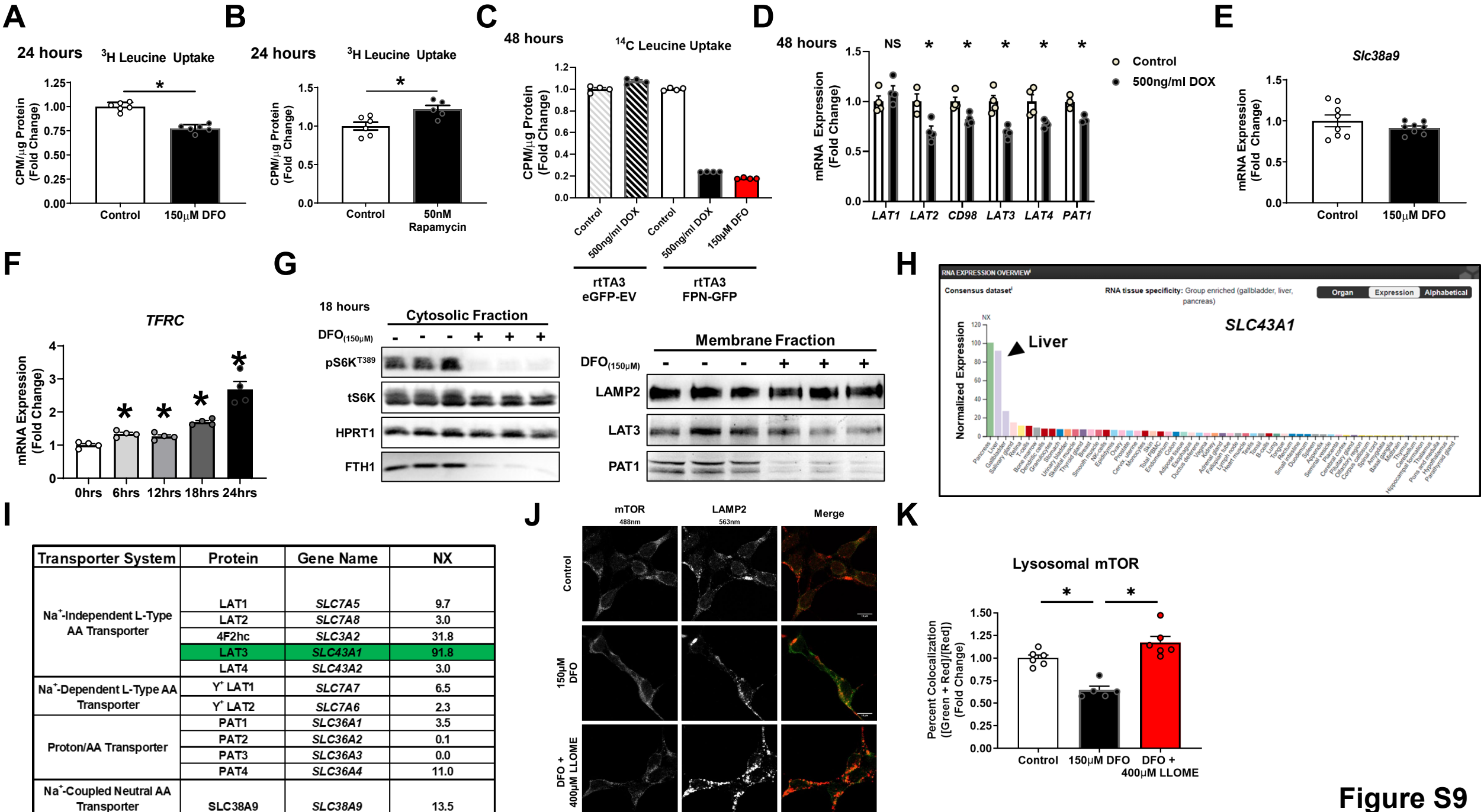

A
24 hours
^{3}H Leucine Uptake
CPM/μg Protein (Fold Change)
Control
150μM DFO
B
24 hours
^{3}H Leucine Uptake
CPM/μg Protein (Fold Change)
Control
50nM Rapamycin
C
48 hours
^{14}C Leucine Uptake
CPM/μg Protein (Fold Change)
Control
500ng/ml DOX
Control
500ng/ml DOX
150μM DFO
rtTA3 eGFP-EV
rtTA3 FPN-GFP
D
48 hours
NS
mRNA Expression (Fold Change)
Control
500ng/ml DOX
LAT1
LAT2
CD98
LAT3
LAT4
PAT1
E
Slc38a9
mRNA Expression (Fold Change)
Control
150μM DFO
F
TFRC
mRNA Expression (Fold Change)
0hrs
6hrs
12hrs
18hrs
24hrs
G
18 hours
Cytosolic Fraction
DFO(150μM)
pS6K^{T389}
tS6K
HPRT1
FTH1
Membrane Fraction
DFO(150μM)
LAMP2
LAT3
PAT1
H
RNA EXPRESSION OVERVIEW
Consensus dataset
RNA tissue specificity: Group enriched (gallbladder, liver, pancreas)
Organ
Expression
Alphabetical
SLC43A1
Normalized Expression
Liver
I
Transporter System | Protein | Gene Name | NX
Na+-Independent L-Type AA Transporter | LAT1 | SLC7A5 | 9.7
Na+-Independent L-Type AA Transporter | LAT2 | SLC7A8 | 3.0
Na+-Independent L-Type AA Transporter | 4F2hc | SLC3A2 | 31.8
Na+-Independent L-Type AA Transporter | LAT3 | SLC43A1 | 91.8
Na+-Independent L-Type AA Transporter | LAT4 | SLC43A2 | 3.0
Na+-Dependent L-Type AA Transporter | Y+ LAT1 | SLC7A7 | 6.5
Na+-Dependent L-Type AA Transporter | Y+ LAT2 | SLC7A6 | 2.3
Proton/AA Transporter | PAT1 | SLC36A1 | 3.5
Proton/AA Transporter | PAT2 | SLC36A2 | 0.1
Proton/AA Transporter | PAT3 | SLC36A3 | 0.0
Proton/AA Transporter | PAT4 | SLC36A4 | 11.0
Na+-Coupled Neutral AA Transporter | SLC38A9 | SLC38A9 | 13.5
J
mTOR
488nm
LAMP2
563nm
Merge
Control
150μM DFO
DFO + 400μM LLOME
K
Lysosomal mTOR
Percent Colocalization ([Green + Red]/[Red]) (Fold Change)
Control
150μM DFO
DFO + 400μM LLOME

Figure S9

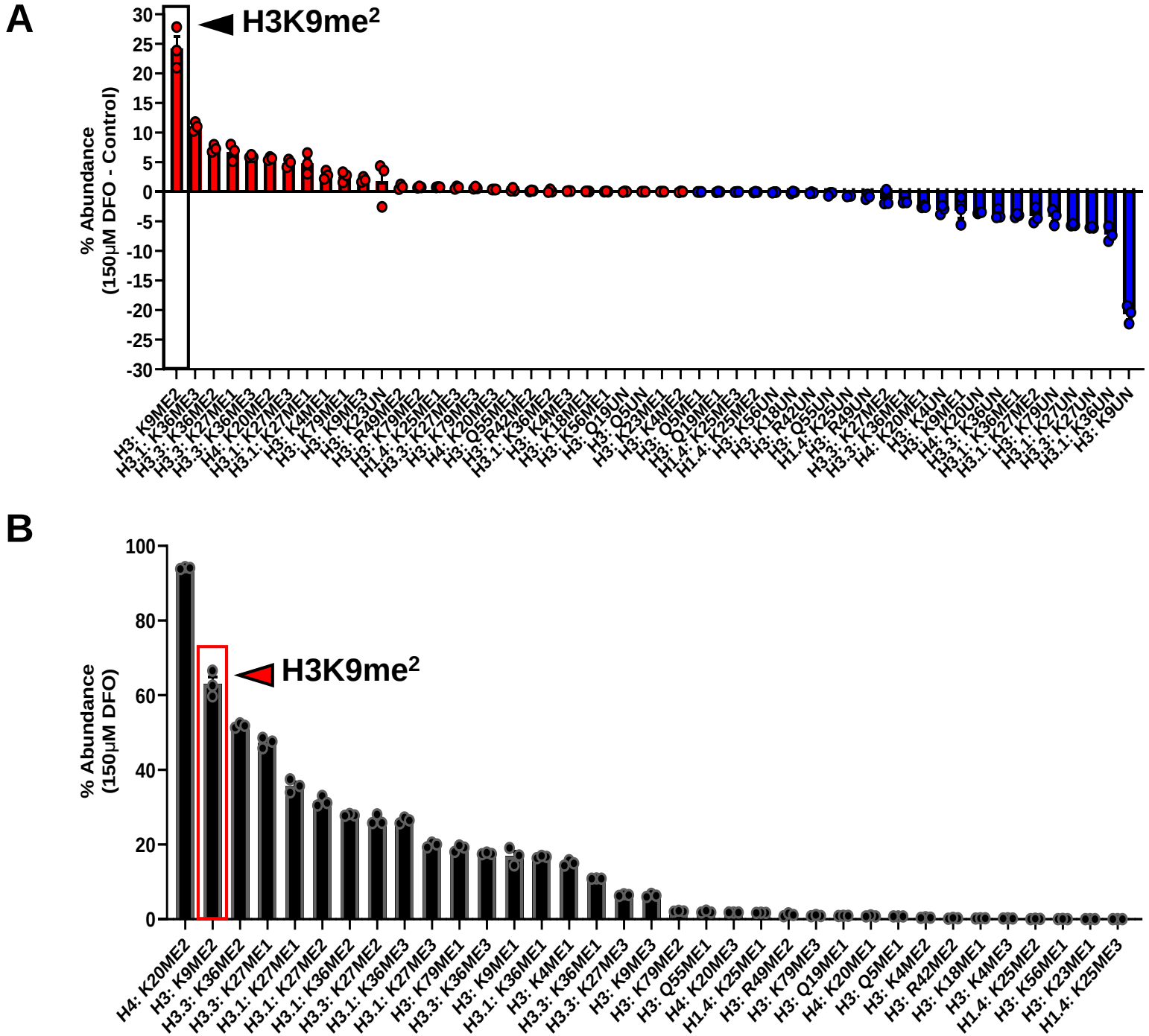

A
H3K9me2
% Abundance
(150μM DFO - Control)
30
25
20
15
10
5
0
-5
-10
-15
-20
-25
-30
H3: K9ME2
H3.1: K36ME3
H3.3: K36ME2
H3.3: K27ME1
H4: K20ME2
H3.1: K27ME3
H3.1: K27ME1
H3: K4ME1
H3: K79ME1
H3: K9ME3
H3: K23UN
H3: R49ME2
H1.4: K79ME2
H3.3: K25ME1
H4: K27ME3
H3: K79ME3
H3: K20ME3
H3: Q55ME1
H3: R42ME2
H3.1: K36ME2
H3: K4ME3
H3: K18ME1
H3: K56ME1
H3: Q19UN
H3: Q5UN
H3: K23ME1
H3: K4ME2
H3: Q5ME1
H3: Q19ME1
H1.4: K25ME3
H1.4: K25ME2
H3: K56UN
H3: K18UN
H3: R42UN
H3: Q55UN
H1.4: K25UN
H3: R49UN
H3.3: K27ME2
H3.3: K36ME1
H4: K20ME1
H3: K4UN
H3: K9ME1
H4: K20UN
H3.3: K36UN
H3.1: K36ME1
H3.1: K27ME2
H3: K79UN
H3.1: K27UN
H3.3: K27UN
H3.1: K36UN
H3: K9UN
B
H3K9me2
% Abundance
(150μM DFO)
100
80
60
40
20
0
H4: K20ME2
H3: K9ME2
H3.3: K36ME2
H3.3: K27ME1
H3.1: K27ME1
H3.1: K27ME2
H3.1: K36ME2
H3.3: K27ME2
H3.1: K36ME3
H3.1: K27ME3
H3: K79ME1
H3.3: K36ME3
H3: K9ME1
H3.1: K36ME1
H3: K4ME1
H3.3: K36ME1
H3.3: K27ME3
H3: K9ME3
H3: K79ME2
H3: Q55ME1
H4: K20ME3
H1.4: K25ME1
H3: R49ME2
H3: K79ME3
H3: Q19ME1
H4: K20ME1
H3: Q5ME1
H3: K4ME2
H3: R42ME2
H3: K18ME1
H3: K4ME3
H1.4: K25ME2
H3: K56ME1
H3: K23ME1
H1.4: K25ME3

Figure S10

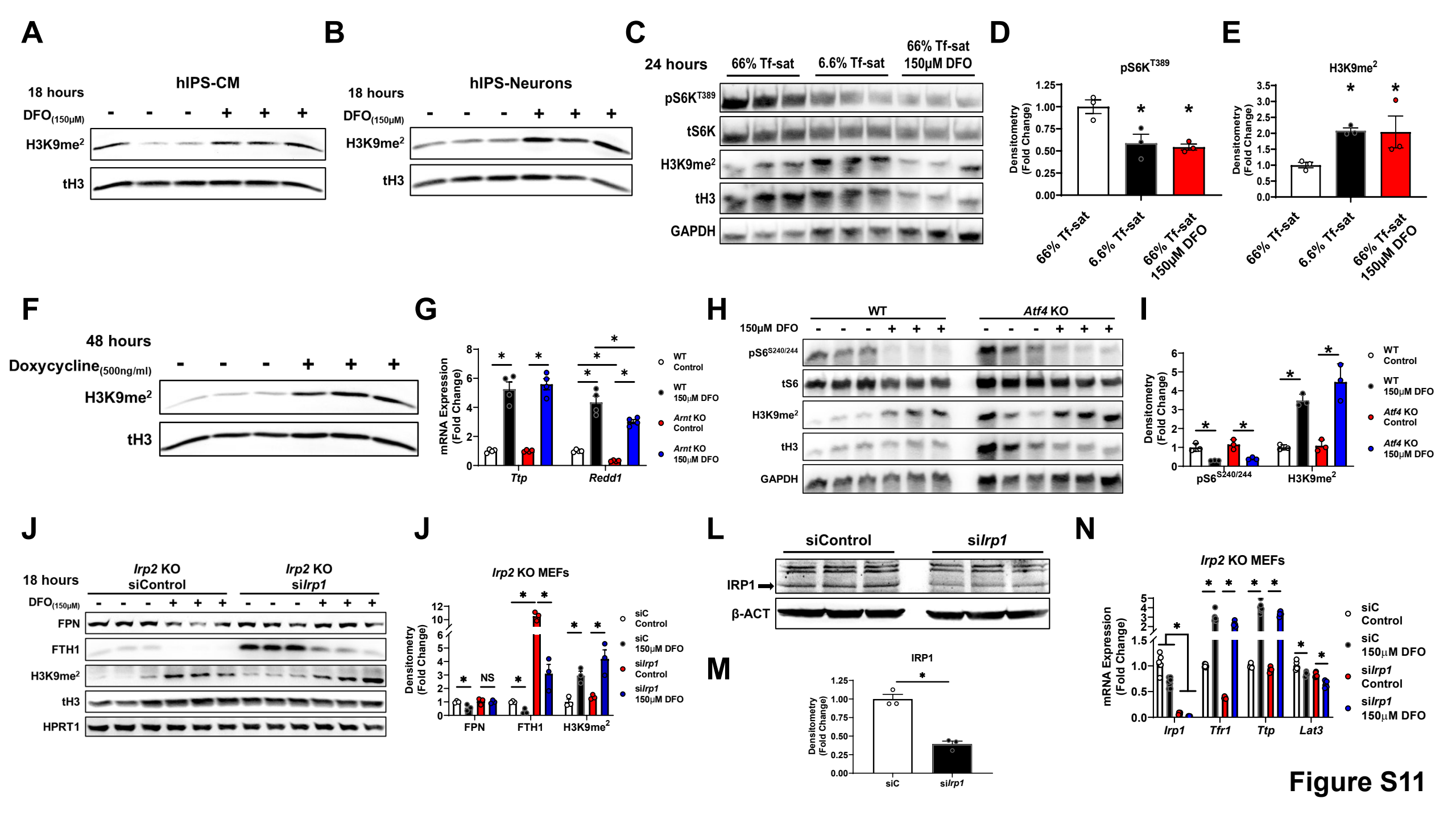

A
hIPS-CM
18 hours
DFO(150μM)
H3K9me2
tH3
B
hIPS-Neurons
18 hours
DFO(150μM)
H3K9me2
tH3
C
24 hours
66% Tf-sat
6.6% Tf-sat
66% Tf-sat 150μM DFO
pS6KT389
tS6K
H3K9me2
tH3
GAPDH
D
pS6KT389
Densitometry (Fold Change)
66% Tf-sat
6.6% Tf-sat
66% Tf-sat 150μM DFO
E
H3K9me2
Densitometry (Fold Change)
66% Tf-sat
6.6% Tf-sat
66% Tf-sat 150μM DFO
F
48 hours
Doxycycline(500ng/ml)
H3K9me2
tH3
G
mRNA Expression (Fold Change)
Ttp
Redd1
WT Control
WT 150μM DFO
Arnt KO Control
Arnt KO 150μM DFO
H
150μM DFO
WT
Atf4 KO
pS6S240/244
tS6
H3K9me2
tH3
GAPDH
I
Densitometry (Fold Change)
pS6S240/244
H3K9me2
WT Control
WT 150μM DFO
Atf4 KO Control
Atf4 KO 150μM DFO
J
18 hours
Irp2 KO siControl
Irp2 KO siIrp1
DFO(150μM)
FPN
FTH1
H3K9me2
tH3
HPRT1
J
Irp2 KO MEFs
Densitometry (Fold Change)
FPN
NS
FTH1
H3K9me2
siC Control
siC 150μM DFO
siIrp1 Control
siIrp1 150μM DFO
L
siControl
siIrp1
IRP1
β-ACT
M
IRP1
Densitometry (Fold Change)
siC
siIrp1
N
Irp2 KO MEFs
mRNA Expression (Fold Change)
Irp1
Tfr1
Ttp
Lat3
siC Control
siC 150μM DFO
siIrp1 Control
siIrp1 150μM DFO

Figure S11

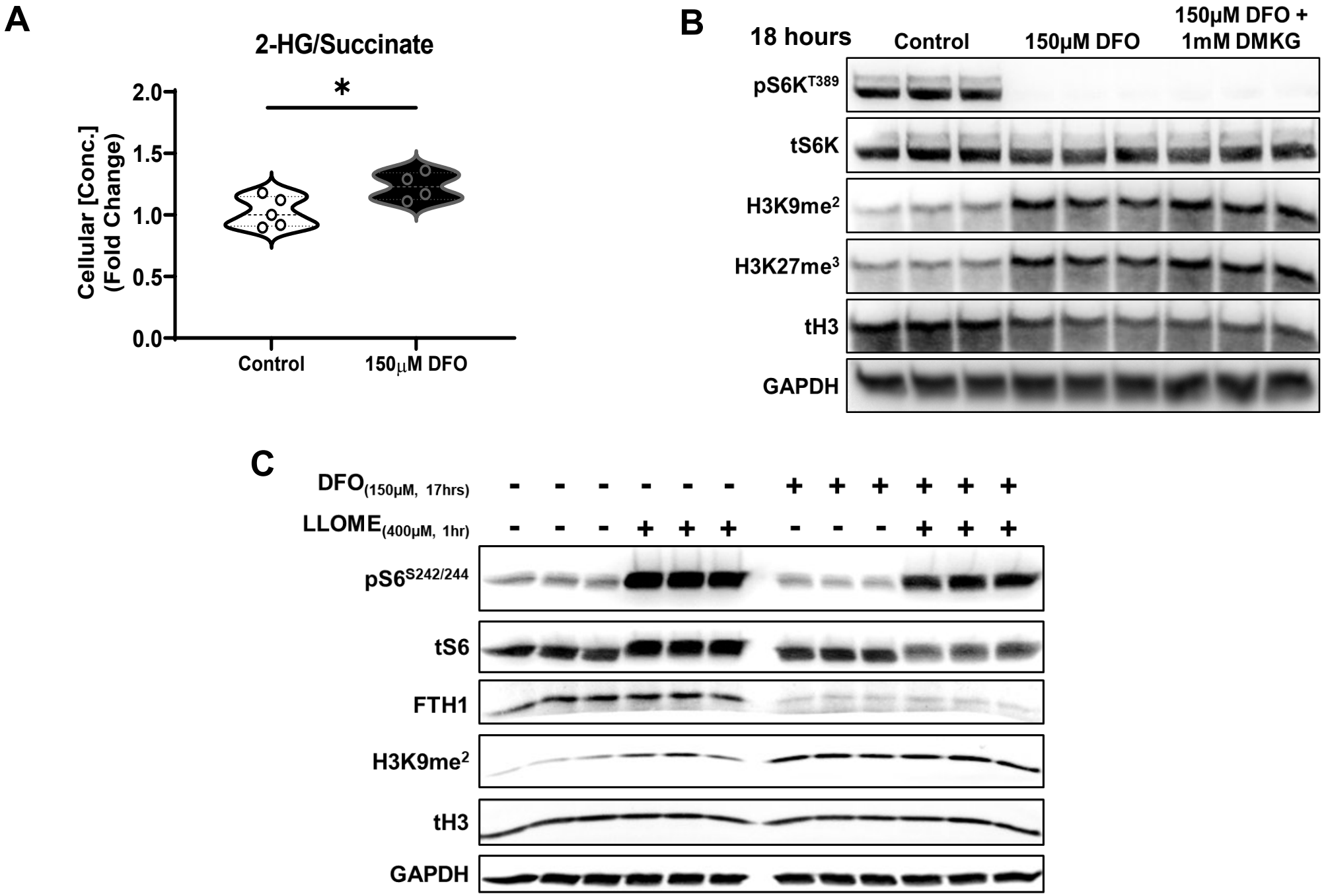
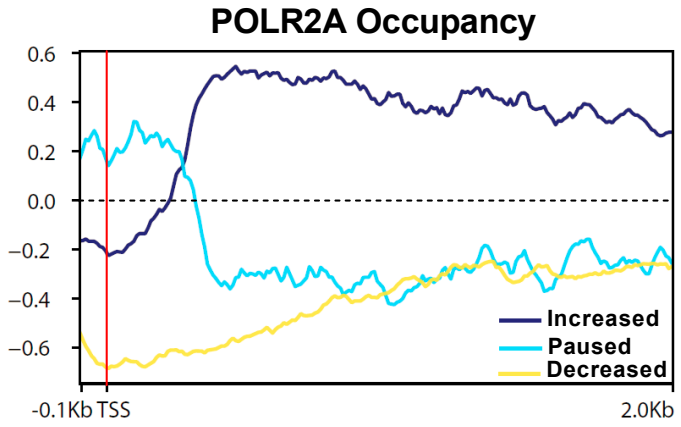

A
2-HG/Succinate
*
Cellular [Conc.] (Fold Change)
2.0
1.5
1.0
0.5
0.0
Control
150μM DFO
B
18 hours
Control
150μM DFO
150μM DFO + 1mM DMKG
pS6K T389
tS6K
H3K9me2
H3K27me3
tH3
GAPDH
C
DFO (150μM, 17hrs)
LLOME (400μM, 1hr)
pS6 S242/244
tS6
FTH1
H3K9me2
tH3
GAPDH

Figure S12

A



B

Increased

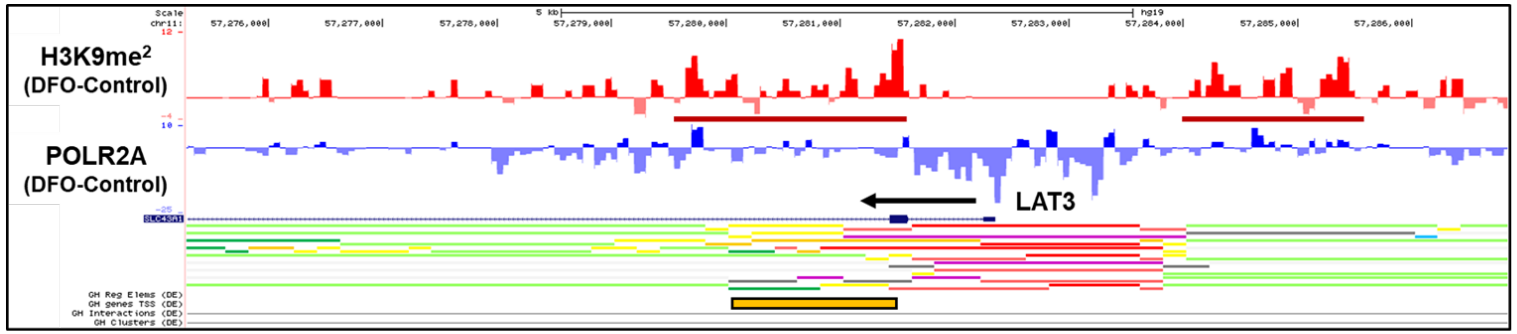
PANTHER GO - Biological Process	Fold Enrichment	FDR
carbohydrate transport (GO:0008643)	4.15	2.70E-02
activation of MAPK activity (GO:0000187)	2.9	4.19E-02
positive regulation of I-kappaB kinase/NF-kappaB signaling (GO:0043123)	2.84	2.35E-02
cellular response to oxygen levels (GO:0071453)	2.7	2.30E-02
cellular response to decreased oxygen levels (GO:0036294)	2.66	3.87E-02
cellular response to hypoxia (GO:0071456)	2.66	4.97E-02
axon guidance (GO:0007411)	2.5	2.29E-02
neuron projection guidance (GO:0097485)	2.5	2.31E-02
response to oxygen levels (GO:0070482)	2.45	3.07E-03
response to hypoxia (GO:0001666)	2.33	2.38E-02
response to decreased oxygen levels (GO:0036293)	2.32	2.21E-02
axonogenesis (GO:0007409)	2.13	3.92E-02
cell morphogenesis involved in neuron differentiation (GO:0048667)	2.07	3.27E-02
chemotaxis (GO:0006935)	2.01	2.27E-02
taxis (GO:0042330)	2.01	2.30E-02

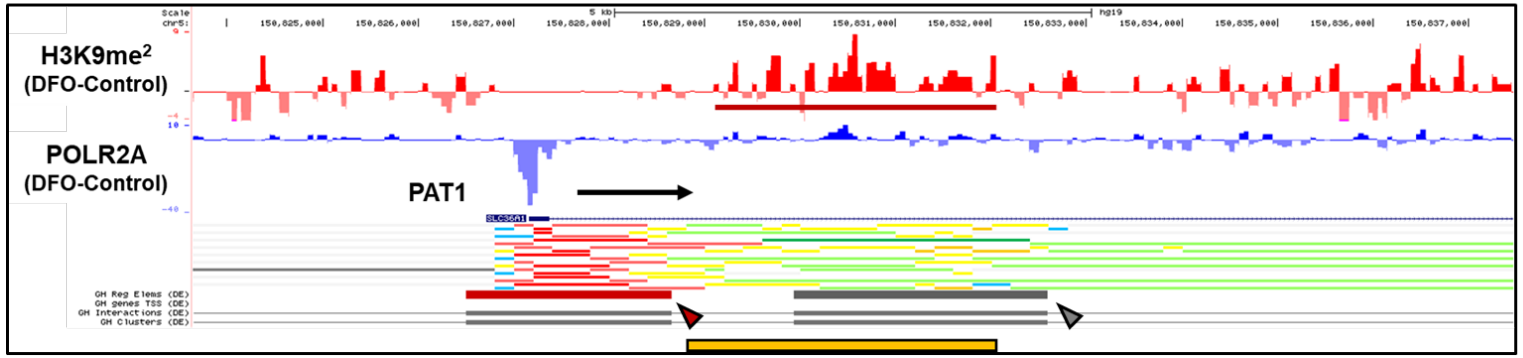
C

Decreased

PANTHER GO - Biological Process	Fold Enrichment	FDR
cytoplasmic translation (GO:0002181)	3.61	0.00239
cell-substrate adhesion (GO:0031589)	3.32	0.0145
peptide biosynthetic process (GO:0043043)	1.9	0.0186
translational elongation (GO:0006414)	1.88	0.0227
translation (GO:0006412)	1.88	0.0225
amide biosynthetic process	1.86	0.0132
peptide metabolic process (GO:0006518)	1.77	0.0216
nervous system development (GO:0007399)	1.76	0.00245
organonitrogen compound biosynthetic process (GO:1901566)	1.66	0.00135
cell development (GO:0048468)	1.63	0.0378
cellular amide metabolic process (GO:0043603)	1.62	0.0354
multicellular organism development (GO:0007275)	1.55	0.00216
system development (GO:0048731)	1.53	0.0056
cell differentiation (GO:0030154)	1.51	0.00709
developmental process (GO:0032502)	1.51	0.0003
anatomical structure development (GO:0048856)	1.5	0.00118

D





Encode Histone Color Code
Green: Transcription
Red: Active Promoter
Yellow/Orange: Enhancer
Purple: Poised Promoter
Grey: Heterochromatin

Genehancer Color Code
Red: Promoter
Grey: Enhancer Element

Figure S13

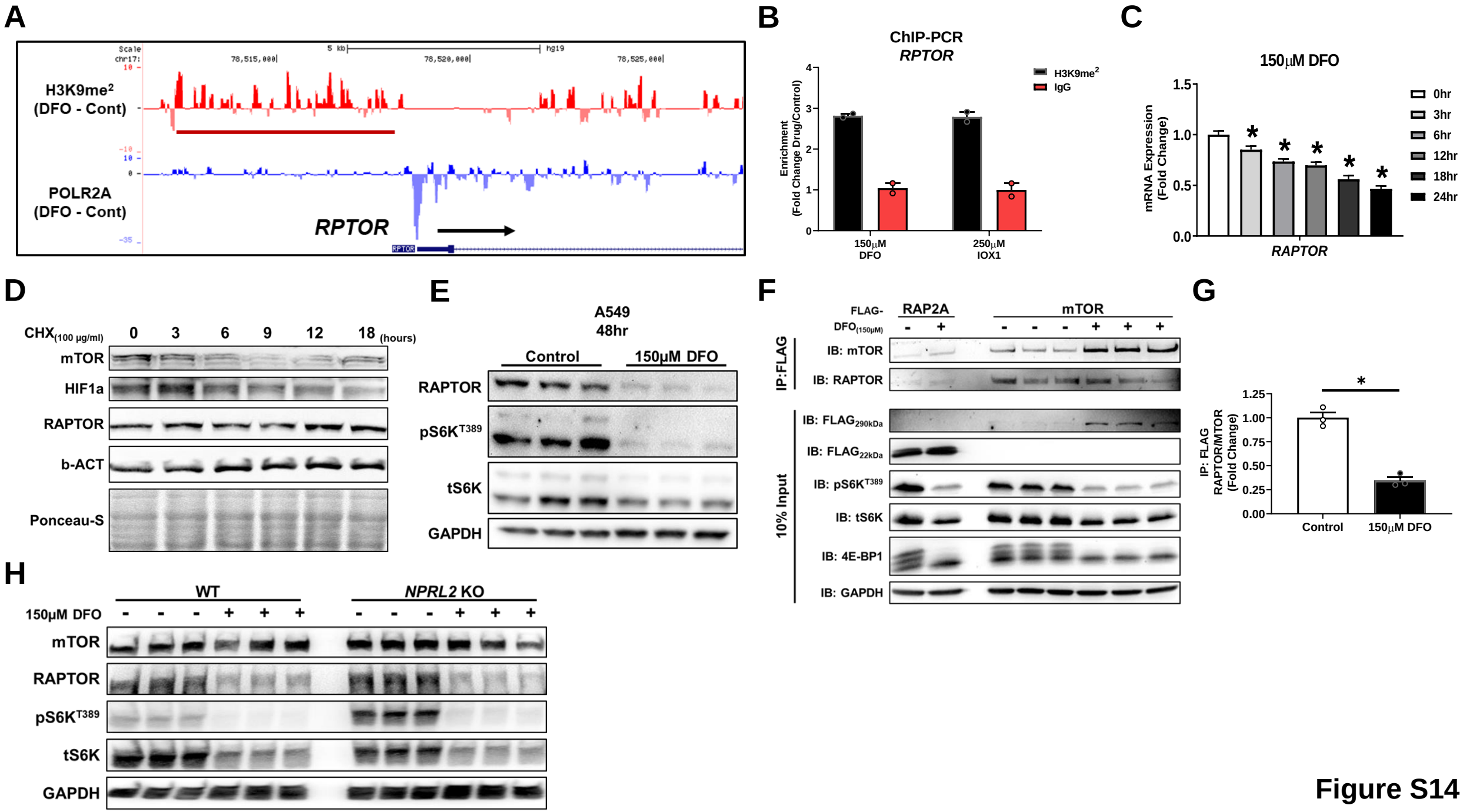
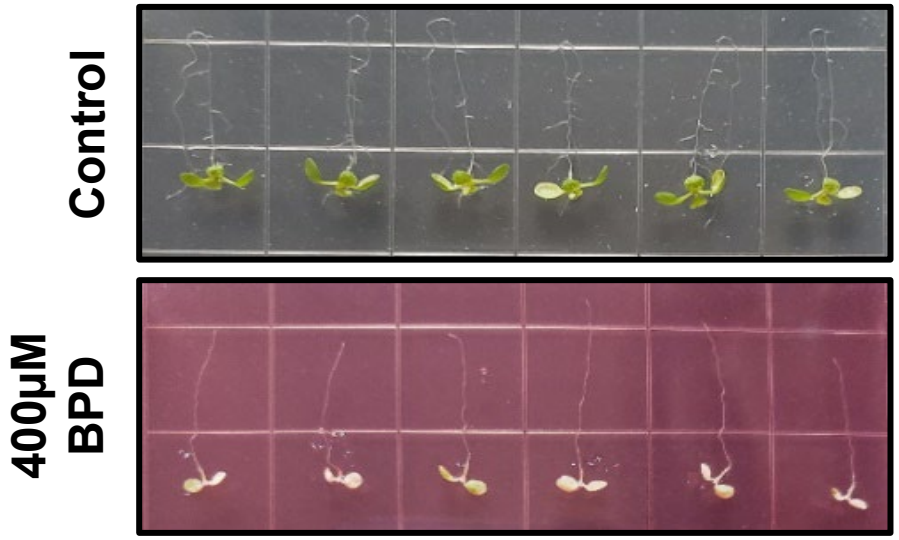
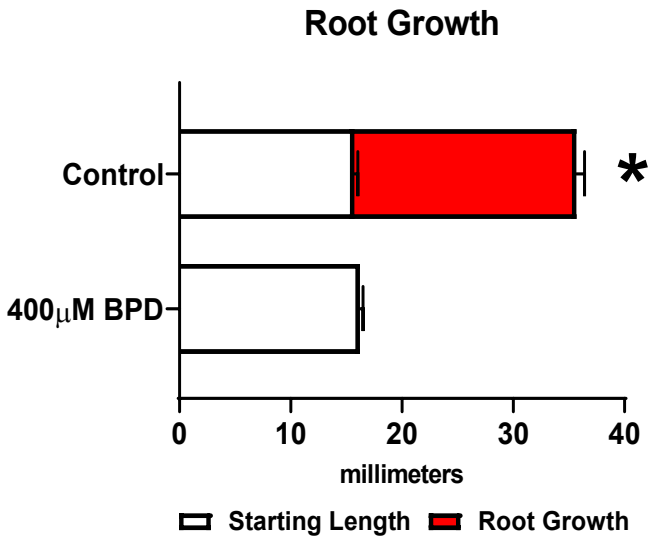

A
H3K9me2 (DFO - Cont)
POLR2A (DFO - Cont)
RPTOR
B
ChIP-PCR
RPTOR
Enrichment (Fold Change Drug/Control)
H3K9me2
IgG
150μM DFO
250μM IOX1
C
150μM DFO
mRNA Expression (Fold Change)
0hr
3hr
6hr
12hr
18hr
24hr
RAPTOR
D
CHX(100 μg/ml)
0 3 6 9 12 18 (hours)
mTOR
HIF1a
RAPTOR
b-ACT
Ponceau-S
E
A549
48hr
Control
150μM DFO
RAPTOR
pS6KT389
tS6K
GAPDH
F
FLAG-
RAP2A
mTOR
DFO(150μM)
IP:FLAG
IB: mTOR
IB: RAPTOR
10% Input
IB: FLAG290kDa
IB: FLAG22kDa
IB: pS6KT389
IB: tS6K
IB: 4E-BP1
IB: GAPDH
G
IP: FLAG RAPTOR/MTOR (Fold Change)
Control
150μM DFO
H
WT
NPRL2 KO
150μM DFO
mTOR
RAPTOR
pS6KT389
tS6K
GAPDH

Figure S14

A



B



C

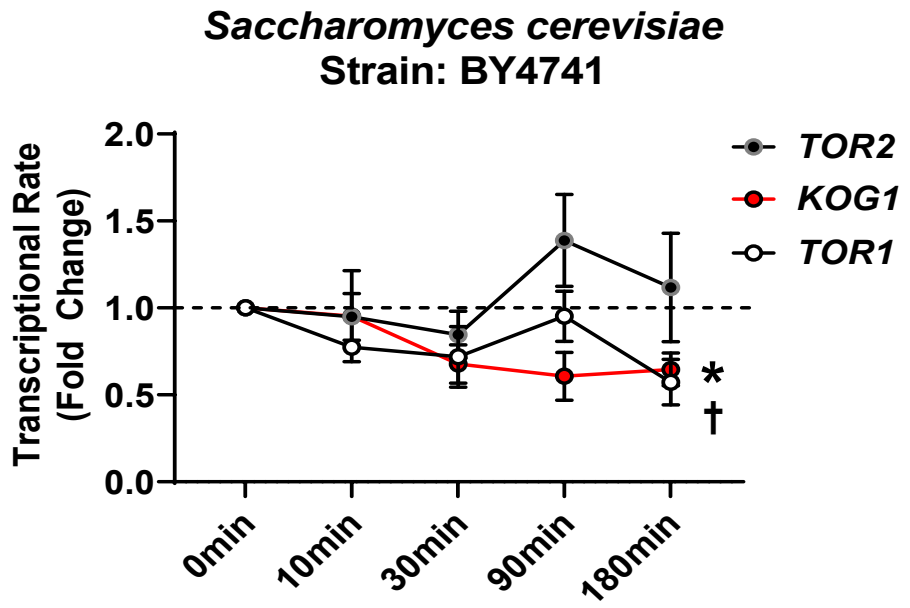


Figure S15

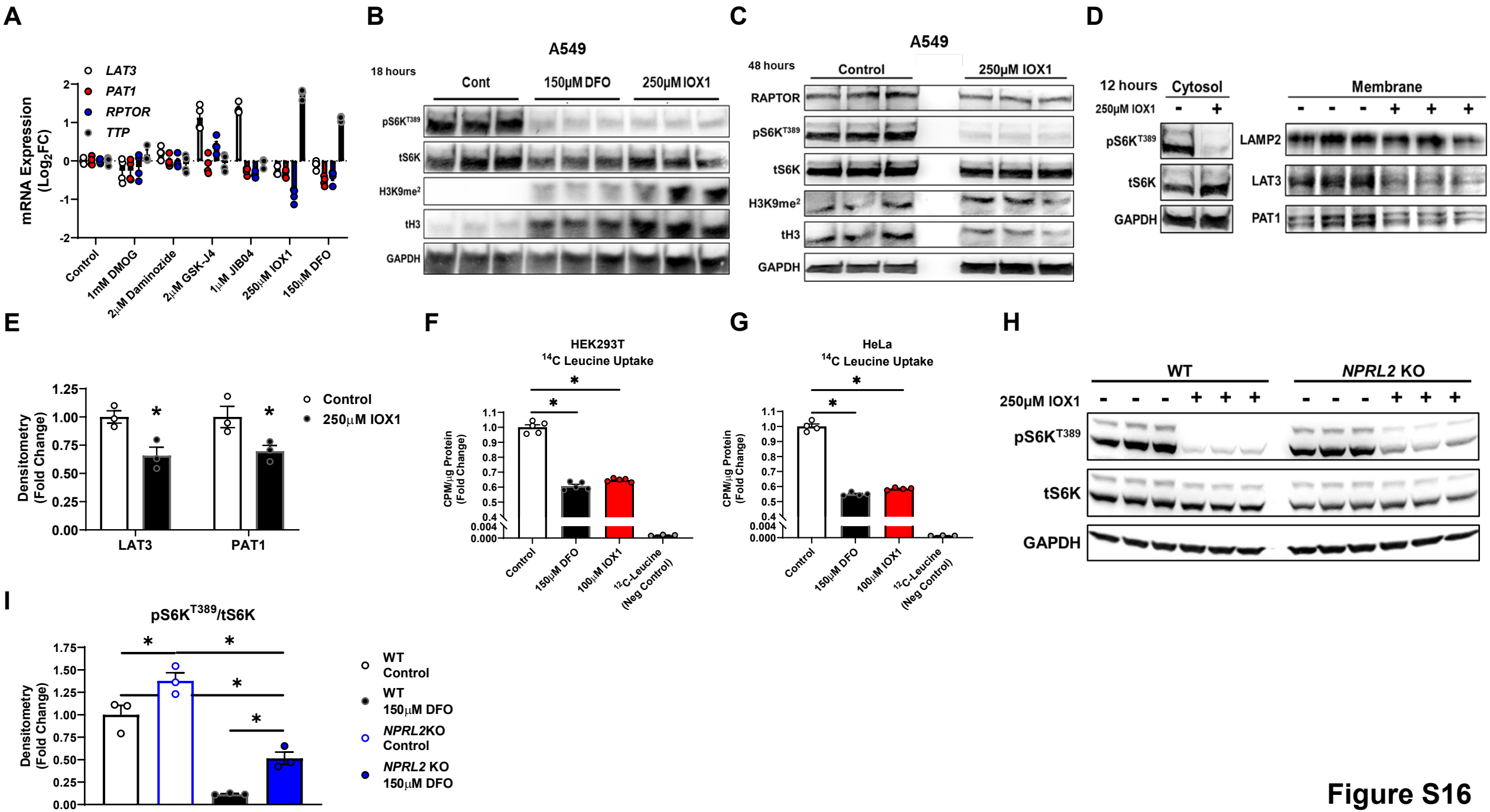

A
LAT3
PAT1
RPTOR
TTP
mRNA Expression (Log2FC)
Control
1mM DMOG
2μM Daminozide
2μM GSK-J4
1μM JIB04
250μM IOX1
150μM DFO
B
A549
18 hours
Cont
150μM DFO
250μM IOX1
pS6K T389
tS6K
H3K9me2
tH3
GAPDH
C
A549
48 hours
Control
250μM IOX1
RAPTOR
pS6K T389
tS6K
H3K9me2
tH3
GAPDH
D
12 hours
Cytosol
Membrane
250μM IOX1
pS6K T389
tS6K
GAPDH
LAMP2
LAT3
PAT1
E
Densitometry (Fold Change)
Control
250μM IOX1
LAT3
PAT1
F
HEK293T
14C Leucine Uptake
CPM/μg Protein (Fold Change)
Control
150μM DFO
100μM IOX1
12C-Leucine (Neg Control)
G
HeLa
14C Leucine Uptake
CPM/μg Protein (Fold Change)
Control
150μM DFO
100μM IOX1
12C-Leucine (Neg Control)
H
WT
NPRL2 KO
250μM IOX1
pS6K T389
tS6K
GAPDH
I
pS6K T389/tS6K
Densitometry (Fold Change)
WT Control
WT 150μM DFO
NPRL2KO Control
NPRL2 KO 150μM DFO

Figure S16

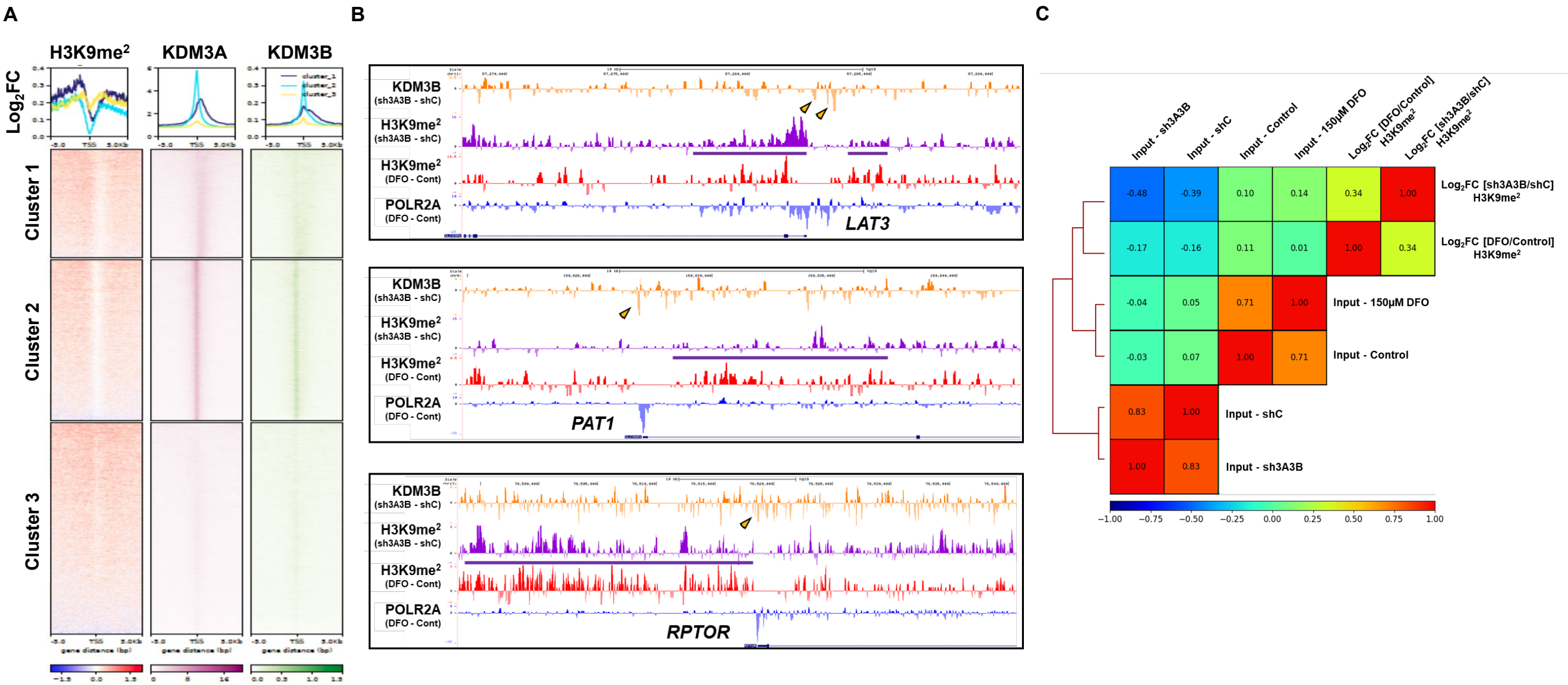

A
H3K9me2
KDM3A
KDM3B
Log2FC
Cluster 1
Cluster 2
Cluster 3
TSS
gene distance (bp)
B
KDM3B (sh3A3B - shC)
H3K9me2 (sh3A3B - shC)
H3K9me2 (DFO - Cont)
POLR2A (DFO - Cont)
LAT3
PAT1
RPTOR
C
Input - sh3A3B
Input - shC
Input - Control
Input - 150μM DFO
Log2FC [DFO/Control] H3K9me2
Log2FC [sh3A3B/shC] H3K9me2
-0.48 -0.39 0.10 0.14 0.34 1.00
-0.17 -0.16 0.11 0.01 1.00 0.34
-0.04 0.05 0.71 1.00
-0.03 0.07 1.00 0.71
0.83 1.00
1.00 0.83
−1.00 −0.75 −0.50 −0.25 0.00 0.25 0.50 0.75 1.00

Figure S17

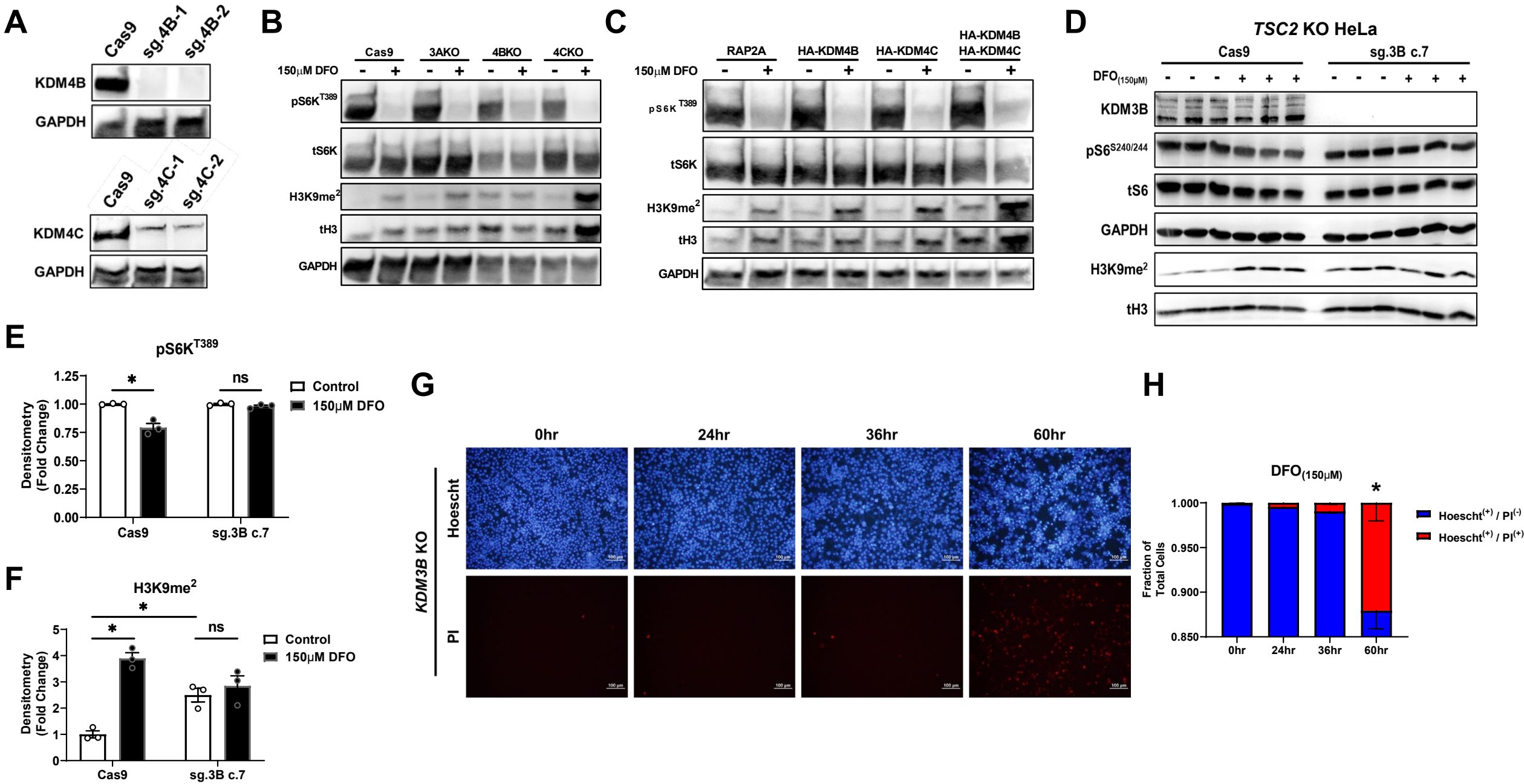

A
Cas9
sg.4B-1
sg.4B-2
KDM4B
GAPDH
Cas9
sg.4C-1
sg.4C-2
KDM4C
GAPDH
B
Cas9
3AKO
4BKO
4CKO
150μM DFO
pS6K T389
tS6K
H3K9me2
tH3
GAPDH
C
RAP2A
HA-KDM4B
HA-KDM4C
HA-KDM4B HA-KDM4C
150μM DFO
pS6K T389
tS6K
H3K9me2
tH3
GAPDH
D
TSC2 KO HeLa
Cas9
sg.3B c.7
DFO(150μM)
KDM3B
pS6S240/244
tS6
GAPDH
H3K9me2
tH3
E
pS6K T389
Densitometry (Fold Change)
1.25
1.00
0.75
0.50
0.25
0.00
*
ns
Control
150μM DFO
Cas9
sg.3B c.7
F
H3K9me2
Densitometry (Fold Change)
5
4
3
2
1
0
*
*
ns
Control
150μM DFO
Cas9
sg.3B c.7
G
0hr
24hr
36hr
60hr
KDM3B KO
Hoescht
PI
H
DFO(150μM)
Fraction of Total Cells
1.000
0.950
0.900
0.850
*
0hr
24hr
36hr
60hr
Hoescht(+) / PI(-)
Hoescht(+) / PI(+)

Figure S18

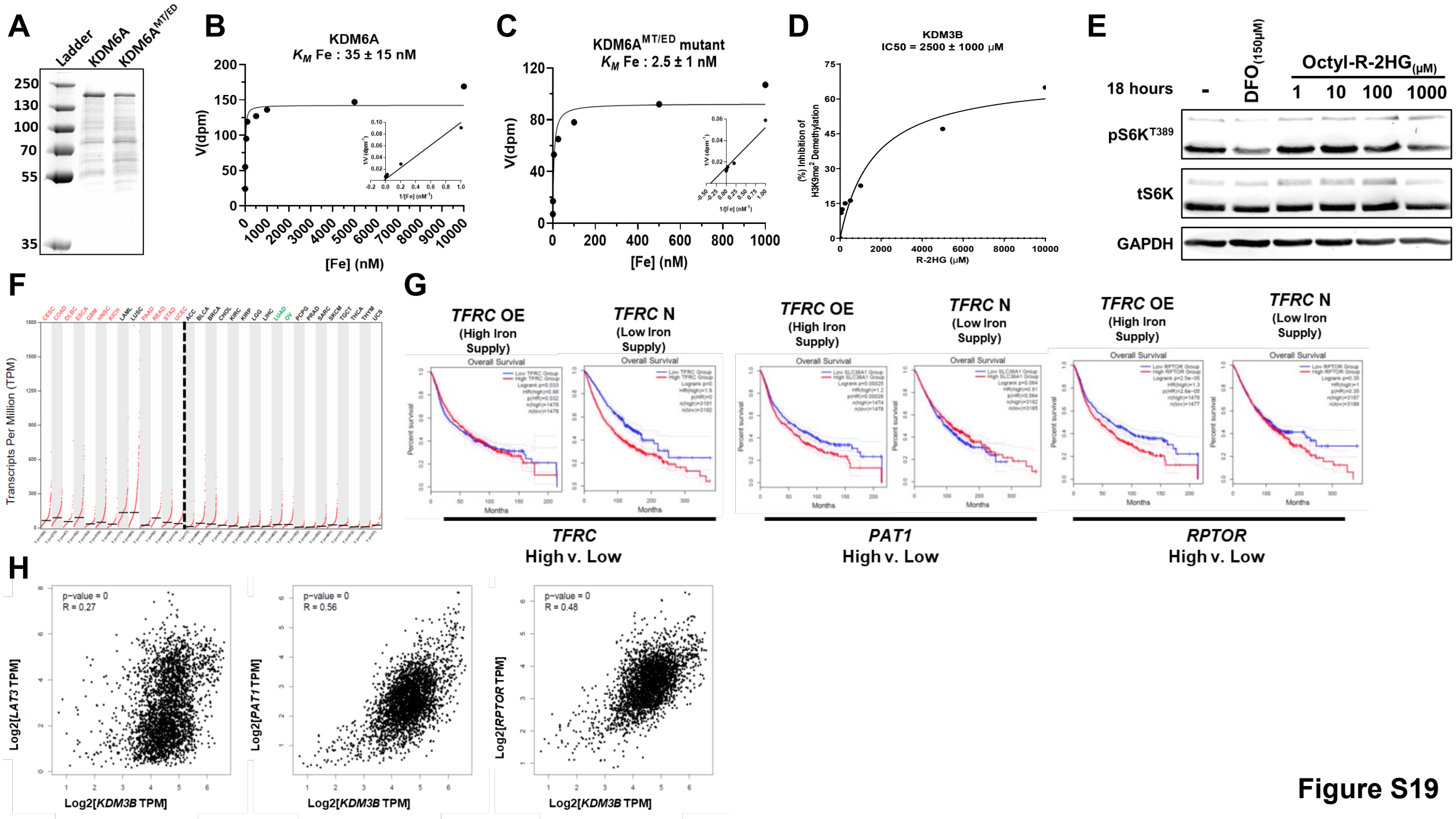
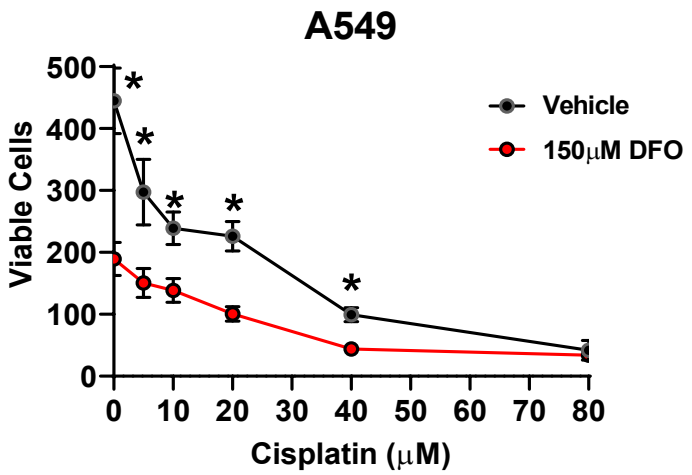
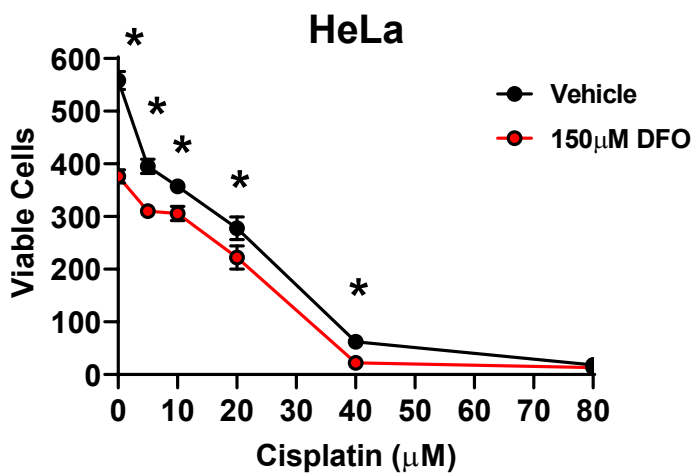

A
Ladder
KDM6A
KDM6A^MT/ED
250
130
100
70
55
35
B
KDM6A
K_M Fe : 35 ± 15 nM
V(dpm)
[Fe] (nM)
C
KDM6A^MT/ED mutant
K_M Fe : 2.5 ± 1 nM
V(dpm)
[Fe] (nM)
D
KDM3B
IC50 = 2500 ± 1000 µM
(%) Inhibition of H3K9me2 Demethylation
R-2HG (µM)
E
18 hours
-
DFO(150µM)
Octyl-R-2HG(µM)
1
10
100
1000
pS6K^T389
tS6K
GAPDH
F
Transcripts Per Million (TPM)
CESC COAD DLBC ESCA GBM HNSC KICH LAML LUSC PAAD READ STAD UCEC
ACC BLCA BRCA CHOL KIRC KIRP LGG LIHC LUAD OV PCPG PRAD SARC SKCM TGCT THCA THYM UCS
G
TFRC OE (High Iron Supply)
TFRC N (Low Iron Supply)
Overall Survival
Percent survival
Months
TFRC
High v. Low
PAT1
High v. Low
RPTOR
High v. Low
H
p-value = 0
R = 0.27
Log2[LAT3 TPM]
Log2[KDM3B TPM]
p-value = 0
R = 0.56
Log2[PAT1 TPM]
Log2[KDM3B TPM]
p-value = 0
R = 0.48
Log2[RPTOR TPM]
Log2[KDM3B TPM]

Figure S19

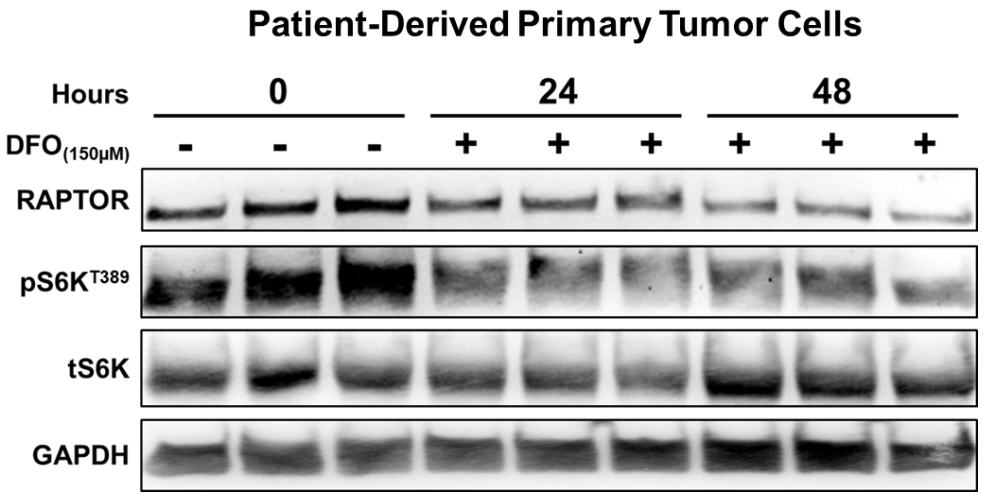
A



B



C



D

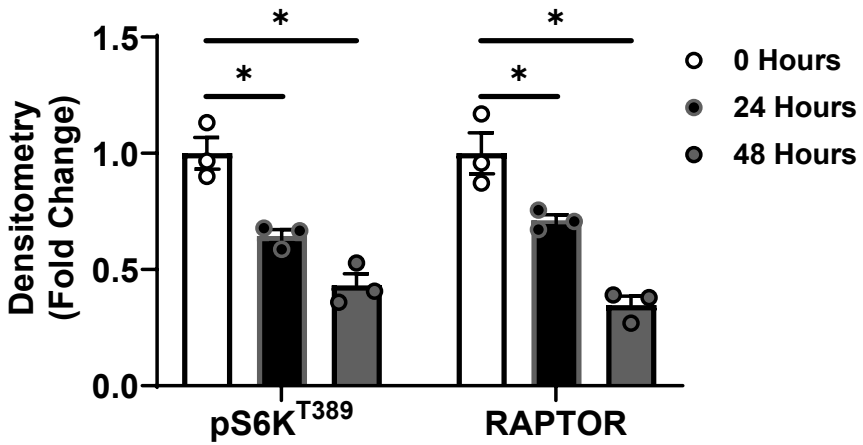


Figure S20