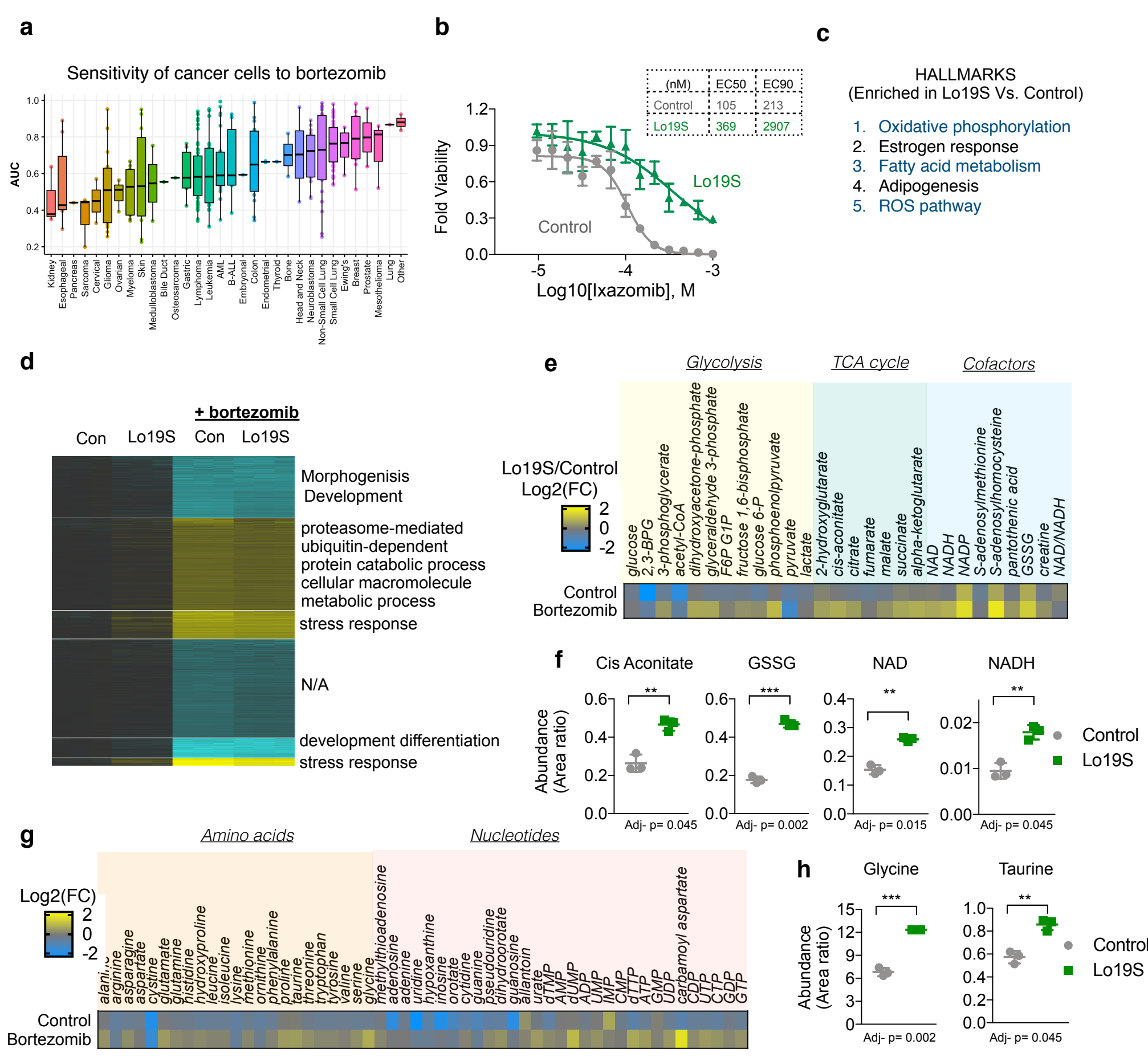
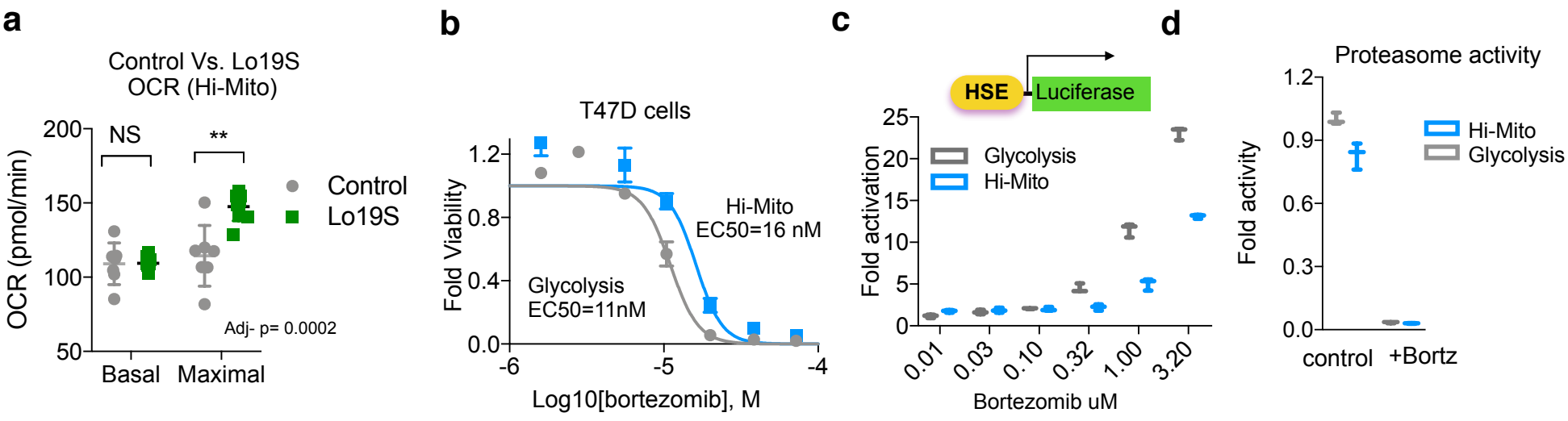


Supplementary Fig. 1

Mitochondrial energy metabolism is associated with the PI-resistant Lo19S state.

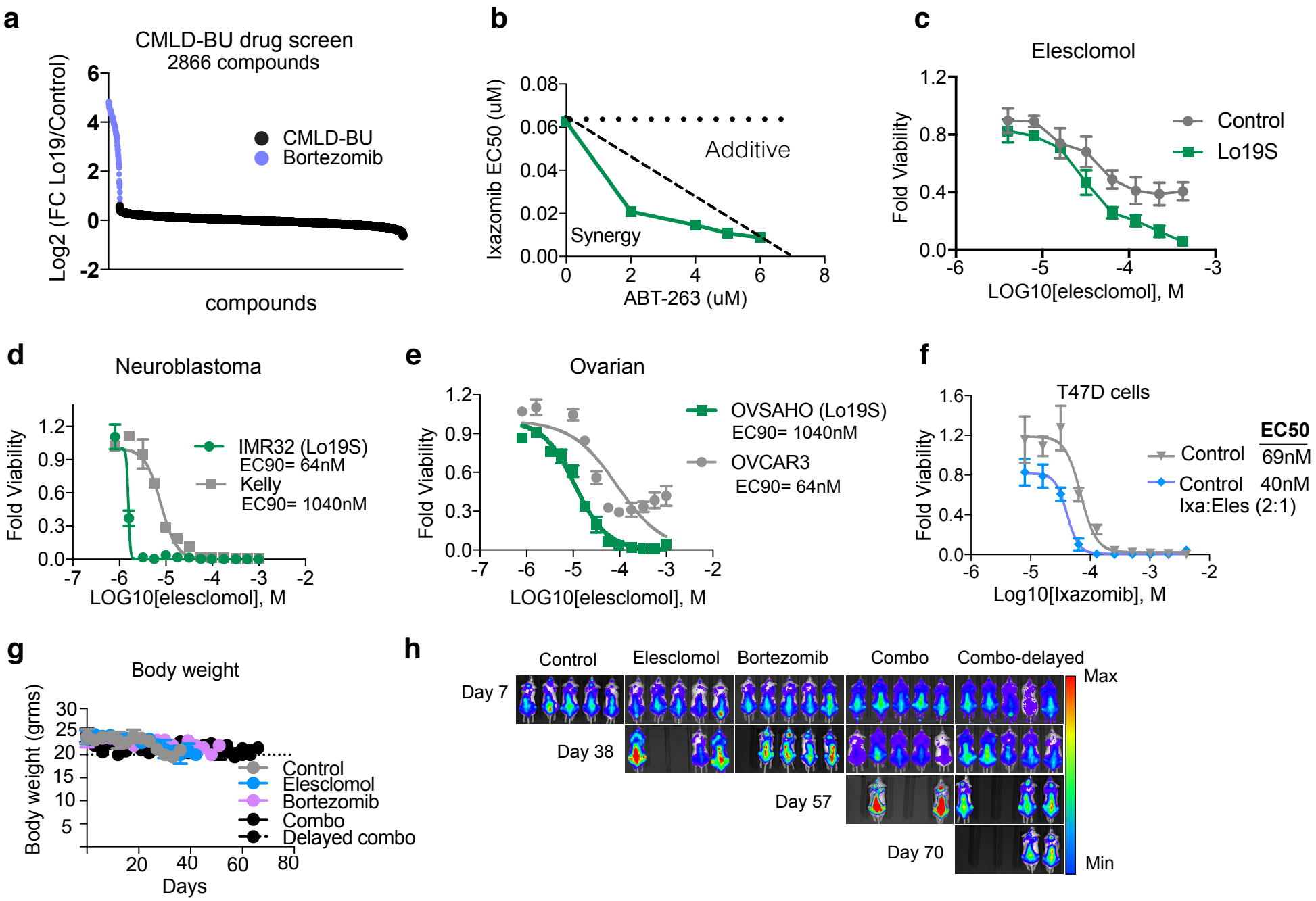
a, The relative sensitivity of different cancer cell line lineages to the proteasome inhibitor bortezomib taken from GDSC (www.cancerrxgene.org) and shown as the calculated area under curve (AUC) (total n= 294 cancer cell lines sub-categorized by lineage) **b**, T47D breast cancer cells harboring a doxycycline-inducible PSMD2 shRNA were grown in the presence or absence of 0.2 µg/ml doxycycline for 72 hours to induce the Lo19S state. Cells were then collected, washed and plated with fresh media without doxycycline. After 24 hours the indicated concentrations of ixazomib was added and the relative viability was measured after 72 hours The calculated EC50s and EC90s are also presented (mean -/+ SD, n= 4 biologically independent samples). **c**, GSEA of genes upregulated in the inducible Lo19S state in T47D cells following 16 hour treatment with 20nM bortezomib. The top 5 Hallmark categories are plotted. Mitochondria-related categories are marked in blue. **d**, Heat map showing fold change in mRNA levels of genes differentially expressed in control and Lo19S cells in the presence or absence of 20nM bortezomib for 16 hours. Triplicate of each condition is presented as fold change in mRNA compared to untreated control. Only genes that have at least Log2 fold change >1 in at least one category are plotted. **e-h**, Metabolite profiling of controls and Lo19S state cells in the presence or absence of 20nM bortezomib treatment for 16 hours. The results are presented as the log2 fold change of the average (triplicates) of each metabolite in the Lo19S state versus control in the untreated (control) and bortezomib treated (bortezomib) conditions. **e-f,** Glycolysis, TCA and co-factor related metabolites are presented. The metabolites that exhibit a statistically significant change in Lo19S compared to control (in the presence of 20nM bortezomib) are presented separately (mean -/+ SD, n= 3 biologically independent samples) (**f**). **g-h,** same as (**e-f**) only for nucleotides and amino acids. For the statistical significance the discovery was determined using the two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 5%.Each row was analyzed individually, without assuming a consistent SD (see Supplementary Dataset 3) .



Supplementary Fig. 2

Increased mitochondrial energy metabolism (Hi-Mito) is sufficient to promote proteotoxic stress tolerance.

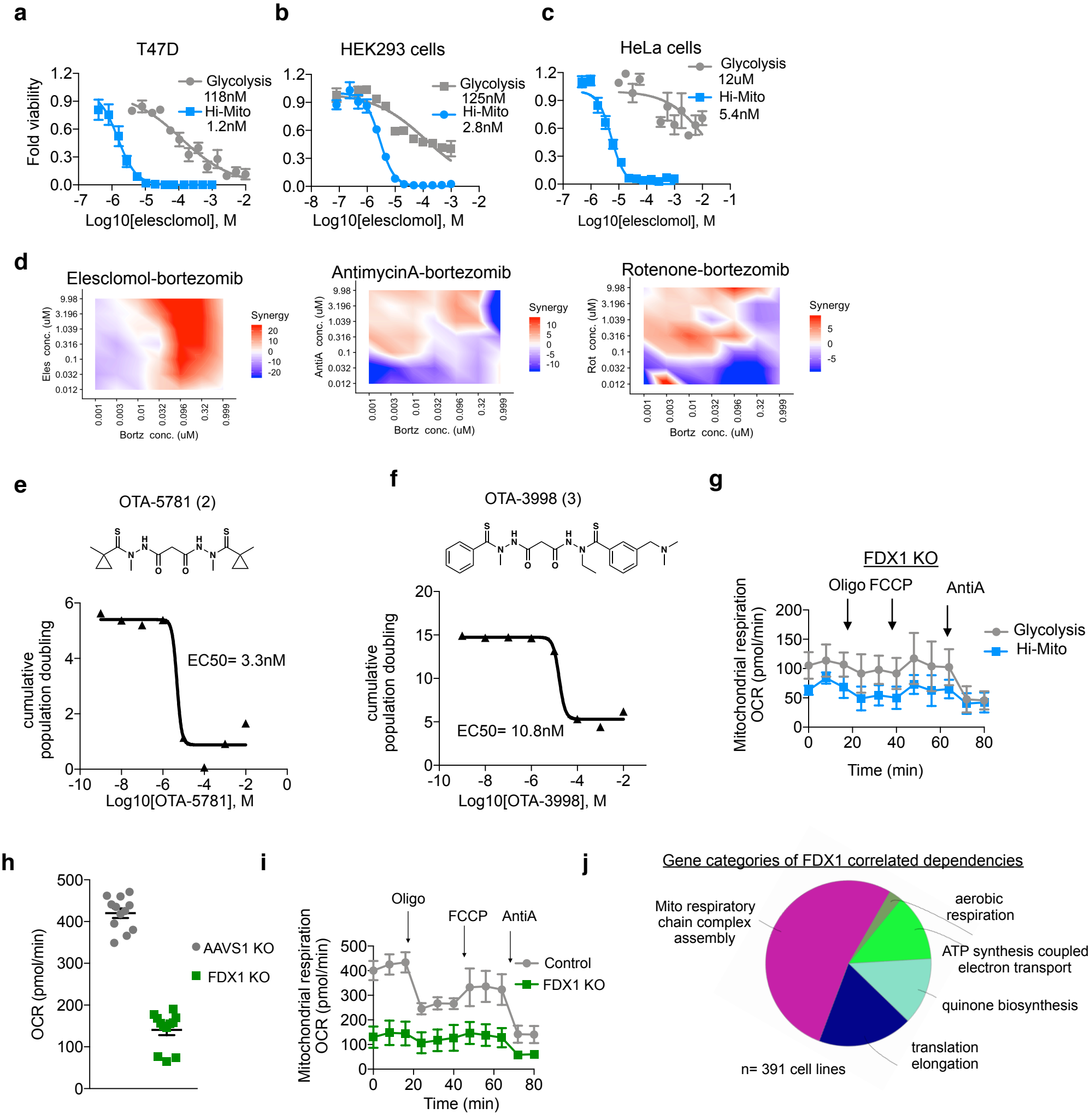
a, Basal and maximal oxygen consumption rate (OCR) was measured in control and inducible Lo19S state cells when grown in media without glucose (with galactose- the Hi-Mito state) (Discovery determined using the Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%. Each row was analyzed individually, without assuming a consistent SD. Plotted mean -/+ SD, n= 7 or 8 biologically independent samples) **b**, Parental T47D breast cancer cells were examined for their viability when grown in the presence of bortezomib and media containing either glucose (control) or galactose (Hi-Mito) (mean -/+ SD, n= 3 biologically independent samples) **c**, HEK293T cells harboring a heat-shock element (HSE) promoter followed by luciferase were examined for the ability of increasing concentrations of bortezomib to induce heat shock when cells were grown in media containing either glucose (control) or galactose (Hi-Mito) (mean -/+ SD, n= 3 biologically independent samples). **d**, The chymotrypsin-like activity of the proteasome was determined in T47D cells grown in the presence of either glucose (control) or galactose (Hi-Mito) with or without pre-treatment for 6 hours with 100nM bortezomib (+Bortz). Plotted as a box and whiskers plot mean -/+ SD, n= 3 biologically independent samples.



Supplementary Fig. 3

The PI-resistant Lo19S state exhibits increased sensitivity to elesclomol.

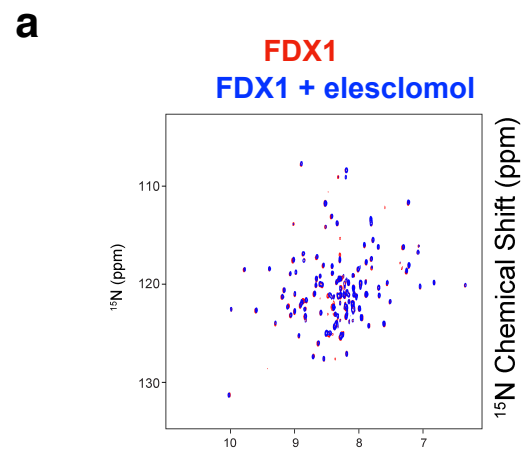
a, T47D inducible Lo19S state cells and control cells (as described above) were subjected to 2866 compounds from the Boston University's Chemical Methodology and Library Development (CMLD-BU) in one dose (10uM) and bortezomib as controls. The relative viability of the Lo19S cells versus control cells was calculated for replica experiments and the log2 of the ratio is plotted. Black dots: drugs in the library; purple dots: bortezomib controls. **b**, The effect of sub lethal concentrations of ABT-263 on the sensitivity of control (Control) cells or Lo19S cells with the proteasome inhibitor ixazomib. The dashed line connects the EC50 of ixazomib alone with that of ABT-263. Synergistic effect should result in EC50 changes under the dashed line. **c**, The relative viability of control and Lo19S cells in the presence of indicated concentrations of elesclomol (mean -/+ SD, n= 4 biologically independent samples). **d-e**, Two pairs of neuroblastoma (**d**) and ovarian (**e**) cancer cell lines with a natural occurring Lo19S state (green) and the paired controls (gray) were examined for the relative cell growth following treatment with eleslcomol for 72 hours hours. The calculated EC90 is presented (mean -/+ SD, n= 3 biologically independent samples). **f**, The effect on relative cell growth of elesclomol added with the proteasome inhibitor ixazomib at a 1:2 (elesclomol:ixazomib) ratio (the ratio of the EC50s) to control cells compared to the effect of ixazomib. Plotted are the mean -/+ SD n=3 biologically independent samples. **g,** Body weight of mice in the experimental set up described in figure 3. **h,** Representative *in vivo* images of MM1S LUC/GFP tumor-bearing SCID mice over the course of the indicated treatments.

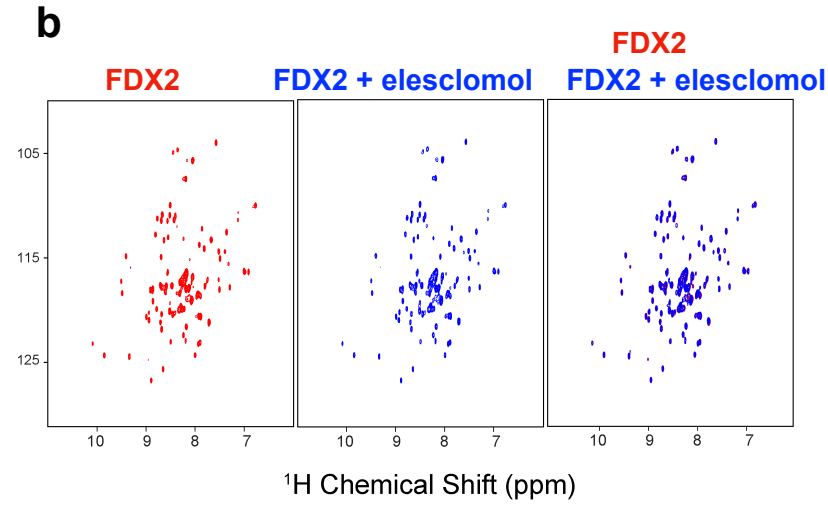


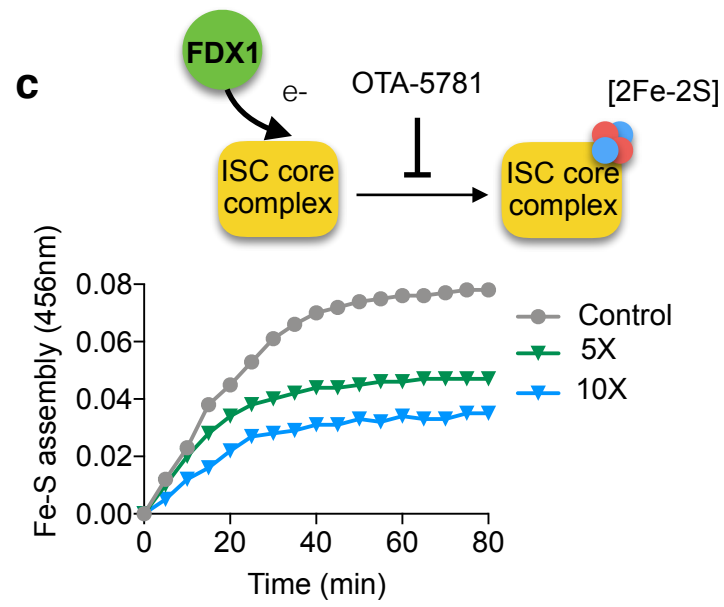
Supplementary Fig. 4

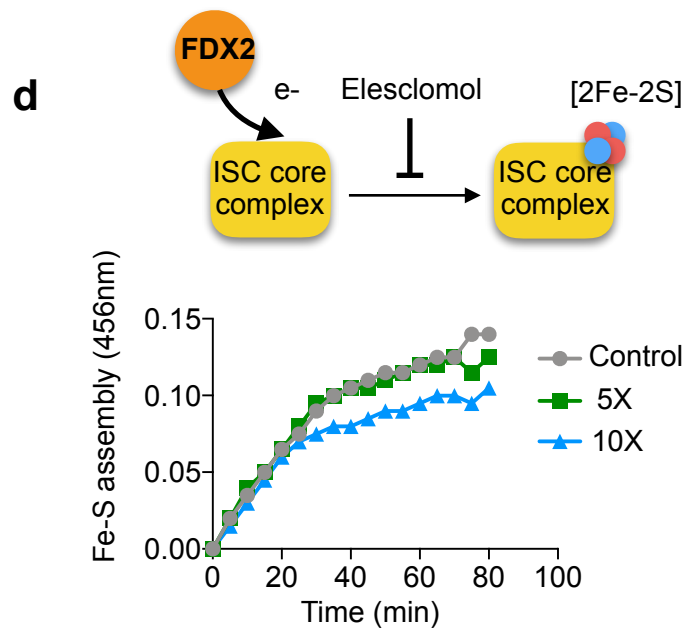
FDX1 is the primary mediator fo elesclomol induced toxicity.

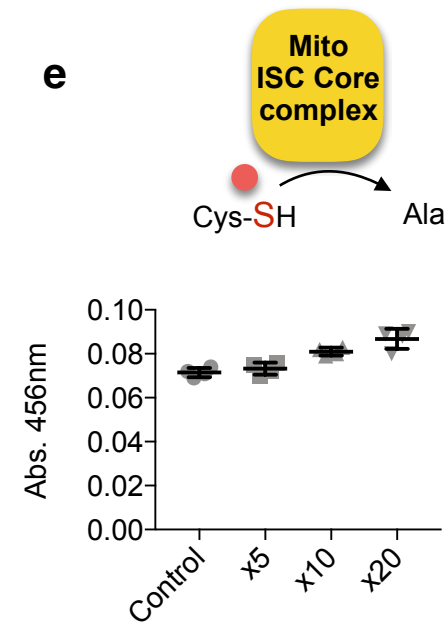
a-d, T47D (**a**), HEK293 (**b**) and HeLa (**c**) cells were grown in the presence of either glucose (control, gray) or galactose (Hi-Mito, blue) as the carbon source and the relative viability was analyzed 72 hours after addition of the indicated concentrations of elesclomol. Plotted are the mean -/+ SD n=3 biologically independent samples. **d,** T47D inducible Lo19S state cells were treated with indicated concentrations of bortezomib and either elesclomol, antimycin A or rotenone. Synergy plots were created using Synergyfinder and the HSA synergy score is presented as calculated from the relative viability after 48 hours of treatment. **e-f**, The effect of the indicated concentrations OTA-5781 (**e**) or OTA-3998 (**f**) on accumulative cell replications over 8 days. **g**, The oxygen consumption rate (OCR) was detected in control AAVS1 KO and FDX1 KO cells grown in the presence of either glucose (gray) or galactose (blue) under basal conditions or following the addition of oligomycin (1 µM), the uncoupler FCCP (2.5 µM) or the electron transport inhibitor antimycin A (1 µM) (mean -/+ SD, n= 4 biologically independent samples) **h-i**, The oxygen consumption rate (OCR) was detected in control AAVS1 KO and FDX1 KO cells under basal conditions or following the addition of oligomycin (1 µM), the uncoupler FCCP (2.5 µM) or the electron transport inhibitor antimycin A (1 µM). Basal OCR was decreased in FDX1 KO cells (**h**) (mean -/+ SD, n= 12 biologically independent samples) and both basal as well as maximal respiration was reduced in FDX1 KO cells (**i**) (mean -/+ SD, n= 4 biologically independent samples). **j**, Categories of genes that are enriched whose essentiality pattern phenocopies that of FDX1 deletion. Taken from the CCLE data sets for 391 cell lines. Go categories showing enrichment are visualized using ClueGo in Cytoscape.

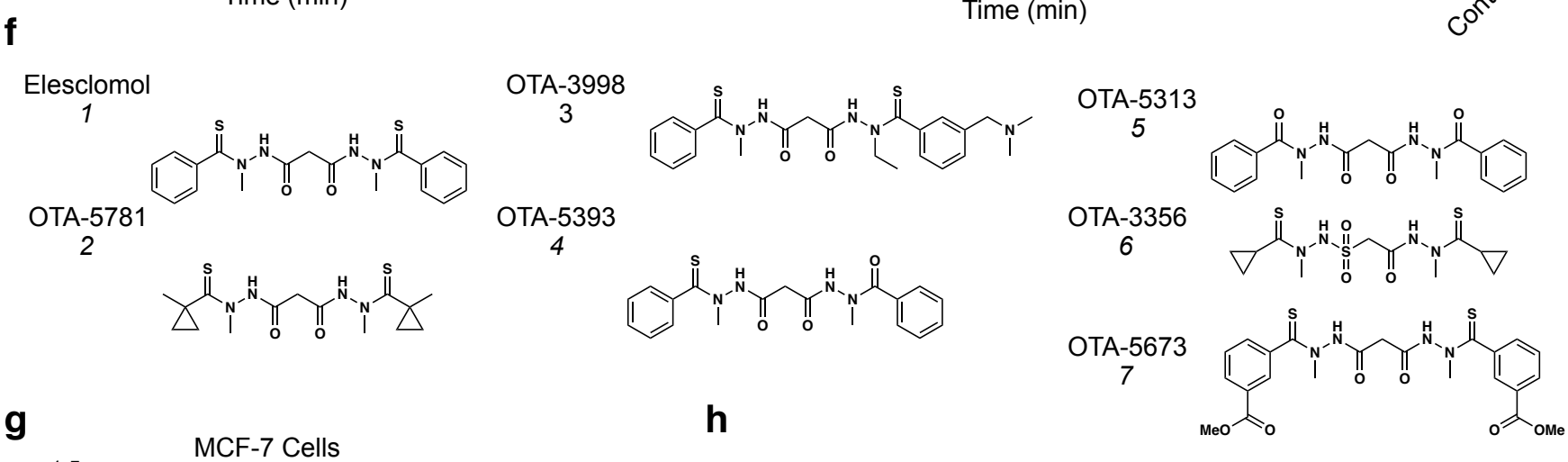


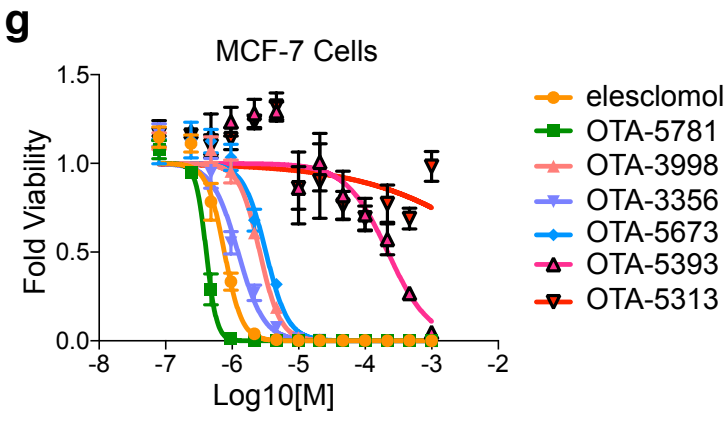


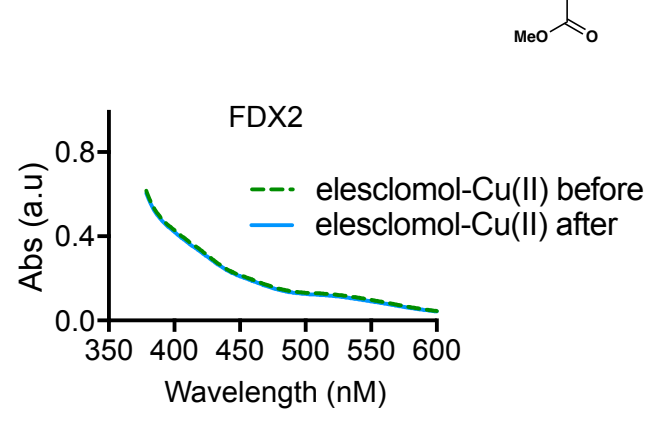






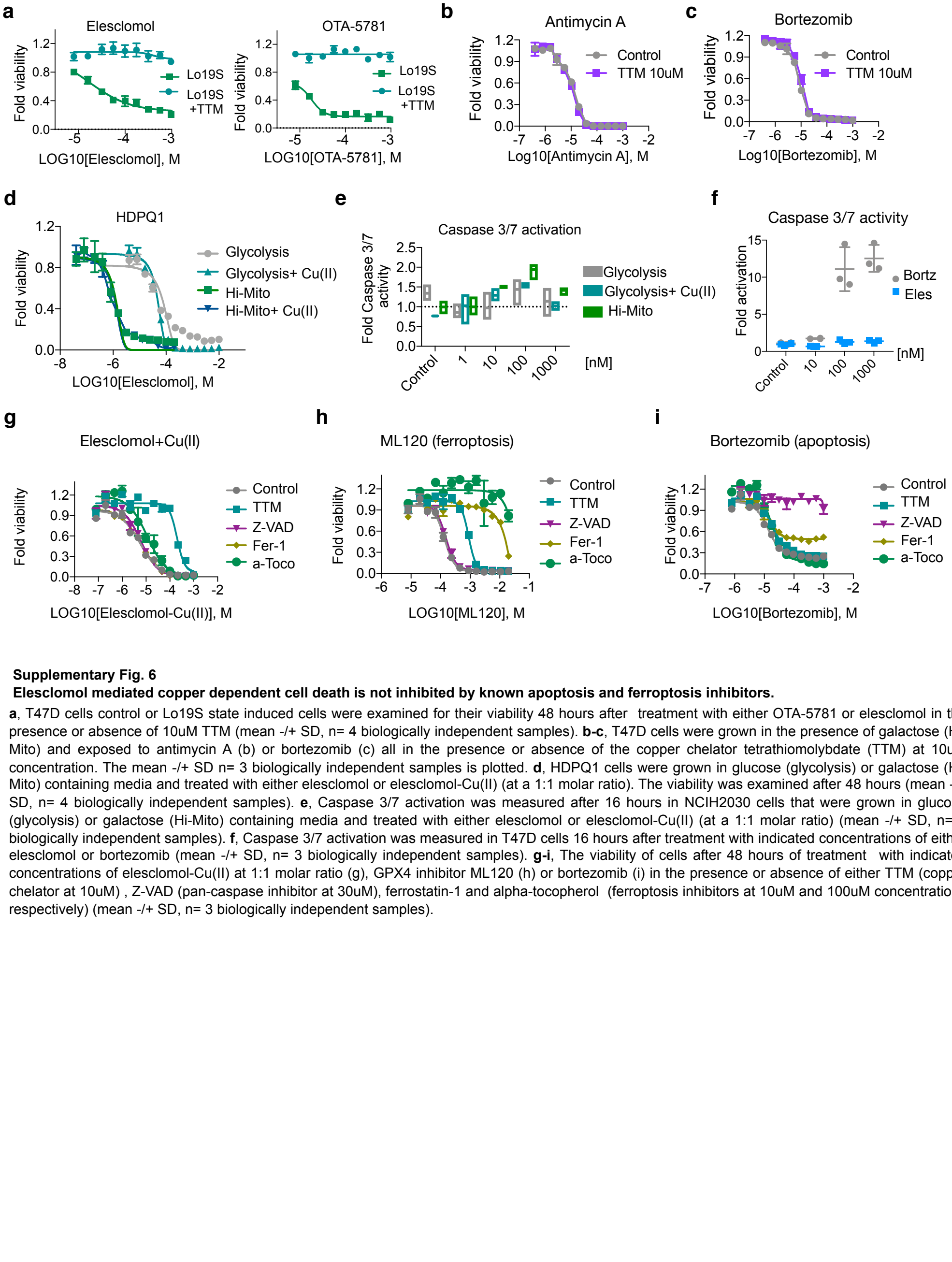






Supplementary Fig. 5
Elesclomol inhibits the natural function of FDX1 in the Fe-S cluster biosynthesis, serving as a neo-substrate when bound to copper.

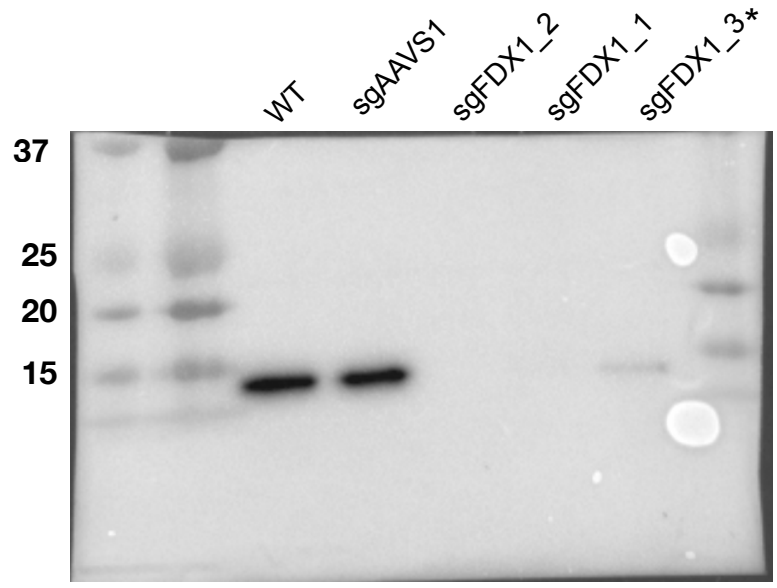
a, $^{1}H,^{15}N$ TROSY-HSQC NMR spectra of [U-^{15}N]-FDX1 before (red) and after (blue) titration of 5X unlabeled elesclomol. **b,** ^{1}H-^{15}N TROSY-HSQC NMR spectrum of [U-^{15}N]-FDX2 (left), ^{1}H-^{15}N TROSY-HSQC NMR spectrum of [U-^{15}N]-FDX2 mixed with 5 molar equivalent of elesclomol (middle) and overlay view of the NMR spectra (right). No significant chemical shift perturbations were observed, indicating no interaction between FDX2 and elesclomol. **c,** The *in vitro* Fe-S cluster assembly with reduced FDX1 as the reducing agent was measured by the increase of absorbance at 456 nm in the presence or absence of either of 5X (green) or 10X (blue, both relative to FDX1) OTA-5781. **d,** *In vitro* Fe-S cluster assembly was carried out with reduced FDX2 (instead of FDX1) as the reducing agent in the presence or absence of 5X (green, relative to FDX1) and 10X (yellow, relative to FDX1) elesclomol. **e,** Cysteine desulfurase activity was measured in the presence or absence of indicated molar ratio of elesclomol (mean -/+ SD, n= 3 biologically independent samples). Data in c-e is representative of two independent experiments **f,** The structures of the different elesclomol analogs used. See also supplementary data. **g**, MCF-7 cells were grown in the presence of galactose (Hi-Mito) and exposed to different elesclomol analogs and relative cell viability (color coded) was analyzed 72 hours post compound addition mean -/+ SD, n= 3 biologically independent samples). **h**, The UV/vis spectra of reduced FDX2 before and after incubation with elesclomol-Cu(II) or Cu(II) alone.



Supplementary Fig. 6

Elesclomol mediated copper dependent cell death is not inhibited by known apoptosis and ferroptosis inhibitors.

a, T47D cells control or Lo19S state induced cells were examined for their viability 48 hours after treatment with either OTA-5781 or elesclomol in the presence or absence of 10uM TTM (mean -/+ SD, n= 4 biologically independent samples). **b-c**, T47D cells were grown in the presence of galactose (Hi-Mito) and exposed to antimycin A (b) or bortezomib (c) all in the presence or absence of the copper chelator tetrathiomolybdate (TTM) at 10uM concentration. The mean -/+ SD n= 3 biologically independent samples is plotted. **d**, HDPQ1 cells were grown in glucose (glycolysis) or galactose (Hi-Mito) containing media and treated with either elesclomol or elesclomol-Cu(II) (at a 1:1 molar ratio). The viability was examined after 48 hours (mean -/+ SD, n= 4 biologically independent samples). **e**, Caspase 3/7 activation was measured after 16 hours in NCIH2030 cells that were grown in glucose (glycolysis) or galactose (Hi-Mito) containing media and treated with either elesclomol or elesclomol-Cu(II) (at a 1:1 molar ratio) (mean -/+ SD, n= 3 biologically independent samples). **f**, Caspase 3/7 activation was measured in T47D cells 16 hours after treatment with indicated concentrations of either elesclomol or bortezomib (mean -/+ SD, n= 3 biologically independent samples). **g-i**, The viability of cells after 48 hours of treatment with indicated concentrations of elesclomol-Cu(II) at 1:1 molar ratio (g), GPX4 inhibitor ML120 (h) or bortezomib (i) in the presence or absence of either TTM (copper chelator at 10uM) , Z-VAD (pan-caspase inhibitor at 30uM), ferrostatin-1 and alpha-tocopherol (ferroptosis inhibitors at 10uM and 100uM concentrations respectively) (mean -/+ SD, n= 3 biologically independent samples).



Supplementary Fig. 7
Uncropped western blots shown in Fig. 4g. The lane indicated with an * was not used or shown the Fig. 4g.

Captions for datasets.

Supplementary Data 1.
The drug sensitivity of cancer cell lines to bortezomib (n= 294) as downloaded from GDSC (www.cancerrxgene.org).

Supplementary Data 2.
Gene expression of the inducible Lo19S state in the presence or absence of bortezomib (n= 3 biologically independent samples). Gene expression data, GSEA using HALLMARK categories, genes selected for cluster analysis and GO enrichment of each cluster are presented in this table.

Supplementary Data 3.
Metabolite profiling of controls and Lo19S state cells in the presence or absence of 20nM bortezomib treatment for 16 hours. n= 3 biologically independent samples. Discovery determined using the Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 5%. Each row was analyzed individually, without assuming a consistent SD.

Supplementary Data 4.
Gene expression of MM.1S orthotopic tumors grown out from control and bortezomib-treated mice. Gene expression and GSEA using HALLMARK categories. n= 3 biologically independent samples.

Supplementary Data 5.
Gene set enrichment analysis (GSEA) of genes upregulated in Lo19S but not control tumors was conducted for breast, prostate, thyroid, skin and kidney cancers from the TCGA. Tumor samples as previously described 11.

Supplementary Data 6.
Viability results from the PRISM experiment where cells were grown in either glucose or galactose containing media with and without bortezomib.

Supplementary Data 7.
Drug libraries used in the chemical screens.

Supplementary Data 8.
The cell viability measurements from the drug screens conducted comparing the control versus Lo19S states and the glucose versus galactose media states.

Supplementary Data 9.
The viability measurements from the PRISM experiment with elesclomol and the overall gene expression and gene deletion associations used in the study.