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Authors

Tran, Nathan L

Jiang, Jiewei

Ma, Min

et al.

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ZBTB11 Depletion Targets Metabolic Vulnerabilities in K-Ras Inhibitor Resistant PDAC

Nathan L. Tran^{*,1,5}, Jiewei Jiang^{*,1}, Min Ma¹, Gillian E. Gadbois¹, Kevin C. M. Gulay², Alyssa Verano⁶, Cara R. Schiavon⁴, Elena Robollo⁴, Haowen Zhou³, Chun-Teng Huang³, David A. Scott³, Uri Manor⁴, Anne G. Bang³, Herve Tiriach², Andrew M. Lowy², Eric S. Wang^{#,5}, Fleur M. Ferguson^{#,1,7}

1. Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA.

2. Department of Surgery, Division of Surgical Oncology, UCSD Moores Cancer Center, University of California San Diego, La Jolla, CA.

3. Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA.

4. Department of Cell & Developmental Biology, University of California San Diego, La Jolla, CA.

5. Cancer Molecular Therapeutics Program, NCI-Designated Cancer Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA.

6. Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, 02115.

7. Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA.

* These authors contributed equally.

Correspondence: fmferguson@ucsd.edu, ewang@sbgpdiscovery.org

ABSTRACT

Over 95% of pancreatic ductal adenocarcinomas (PDAC) harbor oncogenic mutations in K-Ras. However, upon treatment with K-Ras inhibitors, PDAC cancer cells undergo rapid metabolic reprogramming towards an oxidative phosphorylation-dependent, drug-resistant state. In this work, we demonstrate that this metabolic shift is associated with upregulation of the transcription factor ZBTB11, and both the metabolic state and the K-Ras inhibitor drug resistance can be attenuated by ZBTB11 depletion. We develop molecular glue degraders of ZBTB11 and demonstrate that they reprogram the aberrant transcriptome, proteome, metabolome, and bioenergetics of K-Ras inhibitor resistant PDAC, resensitizing them to K-Ras inhibition. ZBTB11 degradation leverages cell-type and cell-state specific differences in gene regulatory mechanisms controlling OXPHOS-pathway transcripts to selectively target the K-Ras inhibitor resistant state in PDAC while sparing hiPSC-derived neurons. Together, this work establishes ZBTB11 as a druggable vulnerability in K-Ras inhibitor-resistant PDAC and provides a suite of molecular glue degrader tool compounds to investigate its function.

INTRODUCTION

Highly aggressive pancreatic ductal adenocarcinomas (PDAC) make up >90% of all pancreatic cancers and are the third leading cause of cancer deaths in the US.¹ Innovations in surgical management and adjuvant therapy have led to improved outcomes for patients with resectable (Stage 0-IIB) PDAC, who have a 5-year overall survival rate of 21%.² Unfortunately, as early stage PDAC has few symptoms, the majority (>85%) of PDAC patients are diagnosed with unresectable Stage III and Stage IV disease, where the 5-year overall survival remains *below 1%* due to an absence of efficacious anticancer drugs.² Over 95% of PDAC patients harbor tumors driven by activating mutations in K-Ras,³ and inhibitors that target oncogenic K-Ras were anticipated to herald paradigm-shifting improvements in patient outcomes. Unfortunately, the initial results in patients treated with drugs targeting K-Ras^{G12C} suggest these benefits may not be realized, due to the rapid onset of K-Ras inhibitor drug resistance.⁴

All of the oncogenic K-Ras mutants found in PDAC are now targetable by at least one clinical-stage inhibitor, and the development of both reversible and covalent mutant K-Ras inhibitors is now a highly productive field of research.⁵⁻⁹ Currently approved K-Ras inhibitors include sotorasib and adagrasib, which were FDA approved for treatment of K-Ras^{G12C} mutant NSCLC in 2021 and 2022 respectively.^{10,11} Although encouraging responses to sotorasib in PDAC have been reported, resistance swiftly arises.⁴ In a Ph I/II study of patients with K-Ras^{G12C} pancreatic cancer who received at least one previous systemic therapy, a 21% objective overall response rate and an 84% disease control rate were reported, but this led to a meager 4 month progression-free survival benefit and 5.7 month median duration of response.^{4,12}

In both clinical and laboratory studies of K-Ras inhibitor resistance, acquired secondary mutations in K-Ras itself are relatively rare, occurring ~ 3 - 4% of the time.^{13,14} Resistance to K-Ras inhibition is more commonly mediated by the accumulation of mutations and activation events in other pathways, posing a challenge for the development of second-line K-Ras inhibitors, vertical pathway targeting, or combination therapies once resistance has developed.^{15,16} Pre-clinical studies indicate that similar resistance profiles occur in PDAC cancers harboring the more commonly occurring K-Ras^{G12D} and K-Ras^{G12V} mutations in response to K-Ras^{G12D} and pan-RAS inhibitors.¹⁷ These data highlight the urgent need to identify and validate new targets that can combat acquired resistance to K-Ras inhibition in PDAC.

Adaptive K-Ras inhibitor resistance is accompanied by significant metabolic reprogramming of cancer cells, including hyperactivation of the PI3K/AKT pathway^{18,19}, elevated TCA cycle flux²⁰,

Myc amplification²¹, and upregulation of mitochondrial biogenesis²², culminating in heightened reliance on oxidative phosphorylation (OXPHOS).^{22,23} As K-Ras pathway inhibition impairs glycolysis, forcing cancer cells to rely on OXPHOS for their energetic demands, this mechanism of clinical resistance is expected to impact the entire pharmacological class and is already being reported in laboratory studies of other K-Ras mutant targeting drug candidates.^{14,15} For example, K-Ras^{G12D} is the most common K-Ras mutation in PDAC³ and inhibitors that selectively target K-Ras^{G12D} are currently the subject of Ph I clinical trials in PDAC patients (NCT05737706). We recently demonstrated that acquired resistance to the K-Ras^{G12D} inhibitor MRTX1133 in human PDAC cells is associated with feedback activation of ERBB/AKT signaling and enhanced OXPHOS.¹⁴ Resistance to the mechanistically orthogonal pan K-Ras(ON) inhibitor RM-7977 has been recently reported in murine models of PDAC, and is associated with Myc amplification²¹. While metabolic profiling was not performed in this study, Myc overexpression has been shown to result in increased mitochondrial biogenesis²⁴, and over 200 OXPHOS genes are regulated by Myc. These K-Ras inhibitor resistance studies concur with genetic studies in inducible K-Ras^{G12D}/p53^{+/-} PDAC murine models, which identified a subpopulation of highly OXPHOS-dependent cancer cells able to overcome genetic ablation of K-Ras^{G12D} to re-seed tumors.²² Pairing oncogenic K-Ras inhibition with OXPHOS inhibition is therefore an attractive therapeutic strategy in PDAC and other Ras addicted malignancies.

Unfortunately, most existing OXPHOS inhibitors have properties limiting their clinical use, including insufficient potency²⁵ and poor selectivity^{26,27}. Others, such as the mitochondrial complex I inhibitor IACS-010759, display dose-limiting on-target toxicity in the clinic due to their action on actively respiring mitochondria in peripheral neurons.²⁸ We therefore sought to identify, validate, and drug novel OXPHOS-modulating targets hijacked by PDAC, that possess a superior therapeutic index.

RESULTS

Identification of ZBTB11 as a target for PDAC metabolic reprogramming. To validate that K-Ras inhibitor resistance reliably generates a high-OXPHOS state, and to develop in-house PDAC models, we cultured 3 independent K-Ras^{G12C} MIA PaCa-2 lines with escalating sublethal concentrations of sotorasib (AMG-510) to generate sotorasib-resistant cell lines R1, R2 and R3 (Fig. 1A, Fig. S1A), as a complement to our previously reported K-Ras^{G12D} MRTXR SUIT2 lines¹⁴ which are resistant to MRTX1133 (Fig. 1B). We characterized their cellular metabolic states using

Seahorse bioenergetic analysis, where a mitochondrial stress test revealed increased basal and maximal OXPHOS in resistant cells compared to parental cells, as well as more modest increases in extracellular acidification rate (ECAR) resulting in overall higher concentrations of ATP produced per minute per cell in both resistant lines (Fig. 1C-E). We evaluated the growth rates of K-Ras resistant lines relative to parental controls and observed slower cell growth in resistant lines when selective pressure of K-Ras inhibition was maintained (Fig. S1B-C). Next, we characterized changes in cellular and mitochondrial morphology by brightfield imaging (Fig. S1E). In parental MIA PaCa-2 cultures, cells existed as a mixture of flat cells and smaller rounded cells, as previously reported.²⁹ Conversely, resistant cells were predominantly flat and adherent in morphology. To characterize changes in mitochondrial morphology, we performed quantitative fluorescent phase imaging (Fig. S1F), where the flat parental cells and resistant cells had more mitochondrial volume per cell and a greater branch length, as compared to the round parental subpopulation (Fig. S1G). Mitochondrial distribution analysis revealed that Sotorasib resistant cells had reduced peripheral mitochondrial distribution relative to both parental cell populations (Fig. S1H-J). Finally, we performed mass-spectrometry based metabolomics following ¹³C glucose or ¹³C glutamine labeling to identify differences between parental and resistant MIA PaCa-2 cells (Fig. S1K, Dataset 1) and parental and resistant SUIT2 cells (Fig. S1L, Dataset 1). Metabolite set enrichment analysis identified changes in diverse metabolic pathways including glycine, serine and threonine metabolism, glycolysis/gluconeogenesis, and the TCA cycle. These data highlight the breadth of metabolites and pathways altered during the development of resistance, that culminate in the observed increases in OXPHOS, and also establish baseline phenotypes to evaluate the effects of OXPHOS inhibition.

To identify mechanistically orthogonal approaches to combating OXPHOS-mediated adaptive K-Ras inhibitor resistance, we evaluated public datasets to identify targets that met the following four criteria: (1) upregulated at the protein level during the development of K-Ras inhibitor resistance in PDAC cells at a minimum of one time point;²³ (2) essential for cell survival in glycolysis suppression media (low glucose, plus galactose), which forces dependency on OXPHOS;³⁰ (3) not a component of complex I, to identify mechanistically orthogonal targets, with the goal of limiting effects on peripheral neurons and other high-OXPHOS healthy cells;²⁸ and (4) chemically tractable via inhibition or targeted protein degradation (Fig. 1F).^{31,32} This approach identified Zinc Finger and BTB Domain Containing 11 (ZBTB11) as an attractive anti-OXPHOS target for molecular glue degrader development.

ZBTB11 is a zinc finger transcription factor that cooperates with GABPα to regulate the nuclear expression of components of complex I and the mitoribosome, thereby maintaining functional homeostasis in mitochondria, in murine ES and human HEK293 cells.³³⁻³⁵ A recent proteomic dataset showed ZBTB11 upregulation in MIA PaCa-2 cells during the development of resistance to the K-Ras^{G12C} inhibitor Sotorasib in a subset of the sampled timepoints.²³ To validate this data, we used CRISPR/cas9 to knock in a HiBiT tag at the C-terminus of the endogenous ZBTB11 locus in MIA PaCa-2 cells, enabling high-throughput quantification of ZBTB11 protein levels. We then exposed these cells to increasing sublethal concentrations of Sotorasib (up to 10 μM) and monitored ZBTB11 levels over the course of 7 weeks. We observed a sharp upregulation of ZBTB11 coinciding with the transition between the slow-growing adaptive state and robust resistance to Sotorasib (Fig. 1G).

We next examined dependencies on ZBTB11 in low glucose conditions, in cancer cells, and in neurons, using published datasets. Using matched genome-wide CRISPRi screens in K562 leukemia cells grown in normal and glycolysis-suppression media, Arroyo *et al* identified that ZBTB11 depletion impairs cell survival in the glycolysis-suppression media conditions, though it is not a top-ranked hit.³⁰ To evaluate the potential effects of ZBTB11 depletion in cancer cells, we examined the cancer dependency map. Here, ZBTB11 knockdown via RNAi was well tolerated across all cancer cell lines, while complete ablation of ZBTB11 via CRISPR/cas9 knockout was lethal to the majority of evaluated cells (Fig. 1H)³⁶⁻³⁸. We next examined the anticipated effects of ZBTB11 loss on post-mitotic neurons using a dataset from Dräger *et al*, where neurotoxic and neuroprotective genes were mapped via a genome-wide CRISPRi survival screen in human iPSC-derived neurons.³⁹ This study found that ZBTB11 CRISPRi knockdown had minimal effects on neuronal health (Fig. 1I). Taken together, these datasets led us to hypothesize that partial loss of ZBTB11 would impair the fitness of K-Ras inhibitor resistant PDAC due to a synthetic lethal relationship between ZBTB11 and the Kras inhibitor-resistant cell state, with minimal on-target toxicity in post-mitotic neurons and other cell types.⁴⁰

ZBTB11 regulates OXPHOS levels in PDAC cells. To determine the effects of ZBTB11 depletion in PDAC cells and to validate it as a therapeutic target, we developed inducible ZBTB11 CRISPRi systems in both parent and K-Ras inhibitor resistant MIA PaCa-2 cell lines. We observed robust ZBTB11 knockdown following dCas9-KRAB induction at both the mRNA and protein levels (Fig. 1J, Fig. S2A,D). Next, we selected 4 representative mitoribosome genes (MRPL48, MRPL44, MRPL1, MRPL30) and 4 complex I genes (NDUF37, NDUF12, NDUF2, NDUF1) reported to be regulated by murine ZBTB11³⁵, and performed qPCR to quantify their mRNA levels

after 1 and 3 days of hZBTB11 CRISPRi knockdown. in parent and resistant MIA PaCa-2 cells (Fig. 1K, Fig. S2A-F). In both parent and resistant MIA PaCa-2 cells, we observed reduction in mRNA levels of the mitoribosome genes within 24 hrs of dCas9-KRAB induction, and reduction of the complex I genes after 3 days (Fig. 1K, Fig. S2A-F). To map the impact of ZBTB11 knockdown on the global PDAC transcriptome, we performed time-course RNA-seq analysis following dCas9-KRAB induction of ZBTB11 knockdown in the Sotorasib-resistant MiaPaCa-2 cell line (Dataset 2). Gene set enrichment analysis revealed rapid downregulation of genes associated with mitochondrial respiration and biogenesis, followed by downregulation of mitoribosome and ribosome associated genes at later time points, consistent with our qPCR data. Upregulated gene sets included those related to energetic crisis, including the autophagy pathway (Fig. 1L, Fig. S3-10).

To examine the effects of ZBTB11 depletion on cellular bioenergetics, we performed Seahorse bioenergetic analysis and observed the expected reduction in oxygen consumption rate (OCR), with no change to the ECAR 24 hrs following dCas9-KRAB induction (Fig. 1M-N). Finally, we evaluated the impact of ZBTB11 depletion on cell proliferation for 14 days following induction of dCas9-KRAB, alone and in combination with Sotorasib. In parent MiaPaCa-2 cells, ZBTB11 depletion had modest effects on cell proliferation, while all conditions containing Sotorasib resulted in loss of proliferation and cell death (Fig. 1O, Fig. S2G-H). In Sotorasib-resistant MiaPaCa-2 cells, removal of Sotorasib from the culture media allowed cells to proliferate more rapidly, and this was again only modestly impaired by ZBTB11 knockdown. However, the combination of Sotorasib and ZBTB11 knockdown dramatically reduced cell proliferation (Fig. 1P, Fig. S2I,J). Taken together, these data indicate that ZBTB11 loss was well tolerated under normal conditions but was synthetic lethal in K-Ras inhibitor resistant PDAC cells, provided that the selection pressure of K-Ras inhibition was maintained. We conclude that ZBTB11 regulates OXPHOS in K-Ras inhibitor resistant PDAC and is a potential therapeutic target for combination therapy.

Development of a CRBN-recruiting molecular glue degrader of ZBTB11. We next examined the tractability of ZBTB11 as a therapeutic target. ZBTB11 contains several C₂H₂ zinc finger domains with CXXCG sequence motifs in their beta-hairpin loops, making it potentially chemically targetable via molecular glue degrader-mediated recruitment to the CRL4^{Cereblon} (CRBN) E3 ligase complex (Fig. S2K).⁴¹ Examination of publicly available proteomic datasets revealed that ZBTB11 had not previously been identified as a target of the IMiD drugs or their analogues, indicating it may require development of chemically differentiated molecular glues. Moreover, targeted protein

degradation approaches can fail when the protein of interest has a short (< 6 hrs) half-life, as degradation is counteracted by rapid protein resynthesis.⁴² Therefore, we examined the half-life of ZBTB11 protein in publicly available proteomics datasets, and found ZBTB11 has a reported calculated half-life of greater than 8 hrs in all tested cell lines (Fig. S2L). The presence of the canonical CRBN degron motifs and its long cellular half-life identify ZBTB11 as a tractable target for targeted protein degradation approaches.

To screen for ZBTB11 targeting molecular glues, we used our ZBTB11-HiBiT reporter system to profile a proprietary library of ~ 250 CRBN-binding molecular glue candidates (Fig. 2A-B). We identified several multi-targeted hits from the same chemical series that depleted ZBTB11 by greater than 30% (Fig. 2C, Fig. S11A-B). Medicinal chemistry optimization (to be reported elsewhere) culminated in the identification of JWJ-01-306 (Fig. 2C). At the 5 hr time point, JWJ-01-306 degraded ZBTB11 up to a $D_{\max, 10 \mu\text{M}}$ of 60% by HiBiT quantification, which deepened to $D_{\max, 10 \mu\text{M}}$ of 80% and a DC_{50} of 620 nM after 24 hrs treatment (Fig. 2D,F, Fig. S11D). These results were validated by immunoblot, where similar trends were observed (Fig. S11C-D). We confirmed rescue of JWJ-01-306 mediated ZBTB11 degradation by co-treatment with the proteasome inhibitor carfilzomib or the NAE1 inhibitor MLN4924, and by competition with the CRBN binder lenalidomide (Fig. 2E).⁴³

To aid interpretation of phenotypic data and confirm observed effects were on-target, we developed two negative control compounds (Fig. 2C). To rescue all CRBN-dependent pharmacology, we synthesized JWJ-01-334, which contains an N-Methyl glutarimide that prevents binding to CRBN⁴⁴, ZBTB11 ternary complex formation, and ZBTB11 degradation (Fig. 2D, S11E-G). To rescue ZBTB11-dependent pharmacology, we synthesized JWJ-01-368, which lacks the aromatic CF_3 substituent crucial for achieving potent ZBTB11 degradation in our SAR studies. In live cells, JWJ-01-368 efficiently bound CRBN but failed to promote both ZBTB11 ternary complex formation and ZBTB11 degradation (Fig. 2D, S11E-G).

We evaluated the selectivity of JWJ-01-306 and JWJ-01-368 in MIA PaCa-2 cells by global proteomics analysis, following a 5 hr treatment with 10 μM compound (Fig. 2G and Fig. S11M, Dataset 3). In JWJ-01-306 treated cells, we observed potent ZBTB11 downregulation, and off-target degradation of three related C_2H_2 zinc finger transcription factors: ZFP91, ZBTB21 and WIZ. In JWJ-01-368 treated cells, we did not observe ZBTB11 degradation, indicating JWJ-01-368 is a suitable control compound for rescue of ZBTB11-specific pharmacology (Fig. S11M)

To examine potential confounding phenotypic consequences of off-target WIZ and ZFP91 degradation, we performed cell counting experiments in Sotorasib-resistant MiaPaCa-2 and MRTX1133-resistant SUIT2 cells using the ZFP91 degrader mezigdomide⁴⁵ and the selective WIZ degrader dWIZ2.⁴⁶ We observed no effect on cell proliferation over 14 days upon treatment with mezigdomide or dWIZ2 alone, or in combination with K-Ras inhibition (Fig. S11R-S).

Beyond off-targets identified through our proteomics studies, frequently-encountered off-targets from CRBN-recruiting molecular glue degraders such as IKZF1/3, IKZF2, SALL4, and GSPT1 are not expressed in PDAC, so we performed follow-up screening in relevant cell models. JWJ-01-306 exhibited potent IKZF1 and IKZF2 degradation activity (Fig. S11N, Q). To examine the effect on SALL4, we performed western blotting in H1 human ES cells and observed potent degradation (Fig. S11P). Finally, to confirm selectivity against the anti-tumor target GSPT1⁴⁷, we performed follow-up screening in a Jurkat GSPT1-HiBiT cell line, where all 3 compounds were inactive (Fig. S11O). This suite of chemical tools has additional degradation targets in certain lymphoid, myeloid, and stem cell lines and should be used with caution in those contexts.

Molecular glue degraders depend on formation of ternary complexes for their cellular activity. ZBTB11 contains 12 C₂H₂ zinc finger (ZF) domains, 7 of which harbor the CXXCG degron motif for CRBN-based molecular glue degraders (Fig. S11L).⁴¹ To identify which of these ZF domains participates in the interaction with degrader-bound CRBN, we generated NLuc tagged ZF constructs consisting of ZF1-4, ZF5-8 and ZF9-12 (Fig. 2H). Treatment with ZBTB11 degraders resulted in degradation of ZFs 5-8 and 9-12 but not ZFs1-4 (Fig. 2I). We then generated constructs linking individual ZFs 5-9 to NLuc and quantified changes in individual ZF-NLuc fusion protein levels upon treatment with ZBTB11 degrader molecules, elucidating that zinc finger 10 (ZF10) contains the primary degron (Fig. 2J). To measure intracellular ternary complex between our molecules, ZBTB11, and CRBN, we developed a NanoBRET assay and confirmed that JWJ-01-306 promoted formation of the CRBN:molecular glue:ZBTB11 ternary complex (Fig. 2L, Fig. S11F).

To enable structure-based design of improved ZBTB11 degraders and controls, we established ternary complex molecular modeling protocols (Fig. 2K). First, we generated an AlphaFold2⁴⁸ model of ZBTB11 ZF10 and used this structure to perform Rosetta protein-complex docking to JWJ-01-306-bound CRBN.⁴⁹ Manual inspection of the clusters revealed a binding mode consistent with previously reported cryo-EM structures of CRBN-binding molecular glue degraders.⁵⁰ The glutarimide binds in the tri-tryptophan pocket of CRBN where it makes two H-

bonds to H378 and one H-bond to the backbone amide of S379 (Fig. 2K). The aniline ring engages in an edge-to-face π interaction with H353 of CRBN, and 3-substitution orients the benzylic cyclobutane ring towards the interface of CRBN and ZBTB11 ZF10, making hydrophobic contact with CRBN P352. Finally, ZBTB11 residue K866 and CRBN residue E377 form a cation- π -anion stacking interaction with the 4-Cl, 3-CF₃ substituted phenyl ring in JWJ degraders that bridges the ternary complex.⁵¹ Here, the CF₃ group contributes through its electron withdrawing inductive effects on the delocalized pi electron cloud in some structures in the cluster. In these same structure clusters, it participates in halogen-hydrogen bond donor interactions with the protonated lysine. These interactions agree with trends in the ZBTB11 degrader structure activity relationships.

To validate our docking model and to further account for pharmacology that may be mediated by off-target activities of JWJ-01-306, we sought to design a degradation-resistant mutant for use as a genetic control in rescue experiments. Based on our docking model, we predicted that K866 made critical contributions to ternary complex formation between CRBN and ZBTB11 ZF10 (Fig. 2K). In contrast, ZBTB11 ZF3, which has a CXXCG motif but a threonine (T659) in the equivalent position to K866, was not degraded as an NLuc fusion by our molecules (Fig. 2I). We performed site-directed mutagenesis to generate ZBTB11^{K866T}, which ablated both ternary complex formation and degradation of full length ZBTB11 by JWJ-01-306 (Fig. 2M-N). To examine if a lysine in the K866 position alone can promote degradation by JWJ-01-306, we mutated ZF3 T659 to generate ZF3^{T659K}. This construct remained recalcitrant to degradation, indicating that K866 was necessary but not sufficient for degradation by our compounds, highlighting the importance of additional contacts made between CRBN and ZBTB11 ZF10 in the ternary complex that drive the specificity of our degrader proteome-wide (Fig. S11G).

To examine the kinetics of protein resynthesis following degrader washout, we identified conditions for complete JWJ-01-306 washout using our CRBN target engagement assay and ZBTB11 ZF10 CRBN BRET assay. Following washout, we observed complete rescue of CRBN occupancy and ternary complex formation (Fig. S11H-I). We treated parent and resistant MiaPaCa-2 cells with JWJ-01-306 for 24 hrs, followed by washout and measured ZBTB11 levels using the HiBiT assay over a time course of 7 days (Fig. S11J-K); a no-washout condition was included as a control for comparison. Here, we observed minimal rebound of ZBTB11 protein levels up to 7 days following washout, consistent with its reported long half-life (Fig. S2L). We verified these results were not an artifact of the HiBiT assay by immunoblot, which revealed similar trends.

Finally, we performed pharmacokinetic analysis in C57BL/6 mice dosed via intraperitoneal (IP) injection with 10 mg/kg JWJ-01-306.HCl. We observed a half-life of 91 minutes and a C_{max} of 1.67 μ M (Fig. 2O, Dataset 4). Plasma protein binding analysis revealed that JWJ-01-306 was over 99% protein bound (Dataset 4). Taken together, these data indicated that the free drug levels of JWJ-01-306 *in vivo* was unlikely to be sufficient to support ZBTB11 degradation at reasonable dosing regimens, and that further chemistry optimization is needed to improve the stability and physicochemical properties of JWJ-01-306. Therefore, JWJ-01-306 is best suited as a cell-based tool compound for target validation and mechanistic studies.

ZBTB11 degradation reprograms cellular metabolism to reduce OXPHOS. We next sought to mechanistically interrogate the metabolic changes induced by JWJ-01-306 in the context of K-Ras inhibitor-resistant PDAC.

To evaluate the impact of ZBTB11 degradation on OXPHOS levels, we treated parental and sotorasib-resistant MIA PaCa-2 cells with 10 μ M JWJ-01-306, JWJ-01-334 or JWJ-01-368 and performed a mitochondrial stress test after 24 hrs (Fig. 3A-B, Fig. S12A-B). We observed significant reductions in basal and maximal oxidative phosphorylation in cells treated with JWJ-01-306 but not JWJ-01-334 or JWJ-01-368. We also observed changes in the metabolic index (mitoATP/glycoATP ratio) in cells treated with JWJ-01-306 but not JWJ-01-334 or JWJ-01-368, reflecting greater reduction in OXPHOS (Fig. 3A-B, Fig. S12A-B,D-E,G-H). To confirm that these effects were general to K-Ras inhibitor-resistant PDAC, we repeated these experiments in parental and MRTX1133-resistant SUIT2 cells, and again observed JWJ-01-306-dependent reductions in basal, maximal, and mitoATP/glycoATP ratios that were not induced by control compounds (Fig. 3C-D, Fig. S13A-B). To identify how expression of mitochondria and complex I genes downstream of ZBTB11 were altered by ZBTB11 degraders to effect the observed proteomic and metabolic changes, we performed qPCR in parental and sotorasib-resistant MIA PaCa-2 cells treated with 10 μ M JWJ-01-306, JWJ-01-334 or JWJ-01-368 for 24 hrs. We observed downregulation of these transcripts by JWJ-01-306 but not negative controls JWJ-01-334 or JWJ-01-368 (Fig. 3E, Fig. S12C,F,I). We confirmed these results in matched experiments in parental and MRTX1133-resistant SUIT2 cells (Fig. 3F, Fig. S13C). Finally, we examined the effects of ZBTB11 degraders on ZBTB11 and K-Ras transcript levels, where we observed compensatory upregulation of ZBTB11 in the presence of JWJ-01-306 but not negative controls in parental and resistant MIA PaCa-2 cells and SUIT2 cells (Fig. S12C,F,I, J, Fig. S13D). Varied effects on K-Ras transcript levels were observed, suggesting that K-Ras was not transcriptionally regulated by ZBTB11.

To confirm that these effects were specific to degradation of ZBTB11 and not off-targets, we generated sotorasib-resistant MIA PaCa-2 cells that stably expressed full length ZBTB11^{WT} or ZBTB11^{K866T}. We performed a mitochondrial stress test after 24 hrs of treatment with 10 μ M JWJ-01-306 and found that expression of the ZBTB11^{K866T} mutant completely rescued degrader-induced reductions in oxidative phosphorylation and metabolic index relative to WT control (Fig. 3G-H). Consistently, stable expression of ZBTB11^{K866T} in sotorasib-resistant MIA PaCa-2 rescued downregulation of 7/8 representative mitochondrial and Complex I genes (and partially rescued expression of MRPL1) after 24 hrs treatment with 10 μ M JWJ-01-306, while expression of ZBTB11^{WT} did not (Fig. 3I).

To identify the metabolic pathway alterations that mediated the effects of ZBTB11 degradation on cellular respiration, we performed metabolomics on sotorasib-resistant MIA PaCa-2 cells treated with 10 μ M JWJ-01-306 for 24 hrs and labeled with ¹³C-glucose or ¹³C-glutamine. Metabolite set enrichment analysis revealed that JWJ-01-306 treatment induced changes consistent with reduced OXPHOS, including in the TCA cycle and pyruvate metabolism (Fig. S12K, Dataset 5). Manual inspection of metabolite abundances and mass isotopomer distributions aligned with this analysis and indicated significant changes in the TCA cycle pathway, (Fig. 3J-M, Dataset 5). The cellular abundance of citrate, a TCA cycle intermediate, was reduced with JWJ-01-306 treatment (Fig. 3J-K). As tumor cells utilize reductive glutamine metabolism to support various cellular processes when activity of the electron transport chain (ETC) is inhibited or the TCA cycle is perturbed^{52,53}, we sought to determine whether ZBTB11 degradation promoted reductive glutamine metabolism. We found that the labeling pattern of citrate (derived from ¹³C-glutamine) in cells treated with JWJ-01-306 was altered, with an increased proportion of m+3 and m+5 isotopomers, indicative of increased reductive carboxylation of glutamine-derived α -ketoglutarate (Fig. 3K). Consistent with these results, we also observed JWJ-01-306-dependent increases in aspartate, malate and fumarate m+3 isotopomers, indicative of reductive glutamine metabolism (Fig. 3L-M). Furthermore, shifts in TCA substrate abundances and the α -ketoglutarate/citrate ratio were in agreement with previous observations in cells with perturbed mitochondrial respiration^{54,55}.

To evaluate the impact of ZBTB11 degradation on mitochondrial morphology, we performed quantitative fluorescent phase imaging and mitochondrial distribution analysis in sotorasib-resistant MIA PaCa-2 cells treated with 10 μ M JWJ-01-306 for 3 d or 10 nM IACS-010759 for 6 hrs (Fig. 3N-O, Fig. S13E-G). In contrast to IACS-010759, JWJ-01-306 had minimal impact on mitochondrial volume and branch length, and neither treatment altered mitochondrial distribution.

Taken together, these data demonstrated that JWJ-01-306 treatment impaired the ETC and disrupted normal flux through the TCA cycle via a mechanistically distinct pathway than the direct complex I inhibitor IACS-010759.

ZBTB11 degradation impairs proliferation of K-Ras inhibitor resistant PDAC cells. To determine the effectiveness of ZBTB11 degraders for targeting K-Ras inhibitor-resistant PDAC, we performed cell proliferation assays via cell counting over time (Fig. 4A-H, Fig. S14A-H). As expected, proliferation of parental MIA PaCa-2 cells was completely impaired by high concentrations of sotorasib (Fig. S14A-D). In these highly glycolytic cells,⁵⁶ single agent JWJ-01-306 treatment had only mild effects on proliferation over a 2-week time period, comparable to the negative controls JWJ-01-368 and JWJ-01-334 (Fig. S14A-B). K-Ras inhibitor-resistant MIA PaCa-2 cells did not show significantly impaired proliferation in the presence of JWJ-01-306 alone; however, JWJ-01-306 re-sensitized these resistant cells to sotorasib (Fig. 4A), while JWJ-01-368 and JWJ-01-334 did not (Fig. 4B). To confirm that these effects were mediated by ZBTB11, we expressed ZBTB11^{K866T} in parent and sotorasib-resistant MIA PaCa-2 cells, and observed complete rescue of anti-proliferative effects (Fig. 4C). In contrast, expression of ZBTB11^{WT} did not (Fig. S14I,J). Finally, to examine if these effects were general to OXPHOS inhibition, we evaluated the combination of Complex I inhibitor IACS and sotorasib. Here, we observed high single agent-toxicity of IACS-010759 (Fig. 4D, Fig. S14D).

We next examined effects of ZBTB11 degradation in parent and MRTX1133-resistant SUIT2 cells. Proliferation of parent SUIT2 cells was completely impaired by high concentrations (500 nM) of MRTX1133 and also by JWJ-01-306 as a single agent, but not JWJ-01-368 or JWJ-01-334 (10 μ M, Fig. S14E-F). In contrast to MIA PaCa-2 cells, proliferation in MRTX1133-resistant SUIT2 lines restricted by JWJ-01-306 alone, which also further enhanced the effect of MRTX1133 (Fig. 4E). As expected, JWJ-01-368 and JWJ-01-334 did not impact proliferation with or without MRTX1133 (Fig. 4F). As above, these effects could be rescued by expression of ZBTB11^{K866T} (Fig. 4G) but not ZBTB11^{WT} (Fig. S14K,L). Finally, to examine if these effects were general to OXPHOS inhibition, we evaluated the combination of Complex I inhibitor IACS-010759 and MRTX1133. We selected IACS-010759 concentrations based on equivalentce in bioenergetics analysis (Fig. S14m-p) We again observed high single agent-toxicity of IACS, which was increased by combination with MRTX1133 (Fig. 4H). Finally, we performed synergy studies to evaluate combinations of ZBTB11 degradation (JWJ-01-306, vs negative control JWJ-01-368) or complex

I inhibition (IACS-010759) and K-Ras inhibition in MIA PaCa-2, SOTR MIA PaCa-2, SUIT2, and MRTXR SUIT2. We observed no synergy in any of the conditions tested, and conclude that ZBTB11 degradation and K-Ras inhibition combinatorial effects are additive (Fig. S23).

To validate ZBTB11 degrader co-treatment as a potential therapeutic strategy in a more clinically relevant context, we turned to patient-derived organoid models of K-Ras^{G12D} driven PDAC⁵⁷. Personalized tumor drug sensitivity profiling using these models was shown to correlate with patient-responses, and their predictive value is currently being assessed in a clinical trial (NCT04469556). As MRTX1133 is still being evaluated in early phase clinical trials, no MRTX1133-resistant patient-derived organoids were available. We therefore sought to evaluate if JWJ-01-306 could deepen the response to MRTX1133 as a combination therapy in organoids derived from tumor samples from both chemotherapy-naïve (hF44) and highly pretreated multi-drug resistant (hM1E) patients. We first treated organoids with DMSO, JWJ-01-306 (1 μ M), JWJ-01-334 (1 μ M), or JWJ-01-368 (1 μ M) and examined effects on ZBTB11 downstream transcripts, observing reduction only in the presence of JWJ-01-306 (Fig. 4 I,K). We treated organoids with DMSO, JWJ-01-306 (1 μ M), MRTX1133 (100 nM), or JWJ-01-306 (1 μ M) + MRTX1133 (100 nM) combination and monitored cell proliferation by brightfield imaging over 7 d (Fig. 4 J,L). Both organoids exhibited a partial response to MRTX1133, and greater anti-proliferative effects were achieved by combining treatment with JWJ-01-306, indicating that enhanced responses to K-Ras inhibitors can be accomplished by preemptive blockade of metabolic escape pathways.

To further examine the differing single agent effects of ZBTB11 degradation in different cellular contexts, we evaluated the effects of JWJ-01-306 on proliferation in a panel of 6 PDAC cell lines (MIA PaCa-2, SUIT2, CAPAN-1, BxPC-3, AsPC-1, and PANC-1) in the presence of 10 μ M JWJ-01-306 over 14 days (Fig. 4M). We also profiled the bioenergetics and ZBTB11 mRNA expression levels in these cell lines (Fig. 4N, Fig. S14Q-T). While no correlation between ZBTB11 transcript expression and anti-proliferative activity of JWJ-01-306 was observed (Fig. 4P), we observed a significant correlation between single agent JWJ-01-306 activity and the metabolic index of the cells, with cells with higher levels of OXPHOS being more sensitive (Fig. 4O).

Together, these data demonstrated that JWJ-01-306 countered K-Ras inhibitor resistance in multiple types of mutant K-Ras-driven PDAC, and also had single agent antiproliferative activity in some PDAC contexts.

ZBTB11 reprograms the K-Ras inhibitor resistant state. To identify the genes downstream of ZBTB11 that were regulated by JWJ-01-306 treatment, we performed global RNA sequencing

analysis of sotorasib-resistant MIA PaCa-2 cells treated with DMSO, 1 μ M sotorasib (resistant cell media), 10 μ M JWJ-01-306 and 1 μ M sotorasib + 10 μ M JWJ-01-306 for 5hrs, 1, 3 and 5 days (Dataset 2). We then performed gene set enrichment analysis to identify biological pathways that change in response to each treatment group. When comparing DMSO vs JWJ-01-306, and sotorasib vs combo (Fig. 5A, Fig. S15-18), we observed rapid (within 5 hrs) downregulation of pathways associated with mitochondrial biogenesis and respiration, as well as ribosome biogenesis and RNA processing. ER-stress pathways and response to glucose starvation were rapidly upregulated (5 hrs) but subsequently subsided. At later time points, compensatory upregulation of genes associated with fatty acid biogenesis and steroid and neutral lipid biosynthesis were observed (Fig. 5B). Finally, upregulation of gene sets associated with innate immune responses and T cell immunity were observed at 3 and 5 d time points (Fig. 5B, Dataset 2). Comparing these results to the RNA-seq performed in ZBTB11 CRISPRi cells, we observed significant overlap in the modulated pathways, but with more rapid kinetics with JWJ-01-306, reflecting the fast-acting nature of degraders compared to genetic knockdown (Fig. 5A-B, Fig. S3-10, Dataset 2).

To identify how K-Ras inhibitors and ZBTB11 degraders affected the cellular proteome at phenotypically relevant time points, we performed global proteomics analysis of sotorasib-resistant MIA PaCa-2 cells treated with DMSO, 1 μ M sotorasib (resistant cell media), 10 μ M JWJ-01-306 and 1 μ M sotorasib + 10 μ M JWJ-01-306 for 3 and 5 days (Fig. 5C, Fig. S19-20, Dataset 5). We compared the proteomic changes between treatment groups and performed K-nearest neighbor clustering analysis to identify protein groups with the same changes in protein abundance in response to each treatment condition (Fig. 5C, Fig. S19-20, Dataset 5). Next, we performed gene ontology analysis to identify the biological pathways enriched within each protein cluster. Proteins downregulated by JWJ-01-306 treatment correspond to mitochondria, oxidative phosphorylation, mitoribosome, and differentiation pathways (Fig. 5C and Fig. S19 clusters 3, 7 and 9, Fig. S20 cluster 4). Protein sets primarily responsive to sotorasib include upregulation of mitochondrial ATP synthesis, and changes to glycine, serine, and threonine metabolism pathway proteins, consistent with the observed metabolic and metabolomic differences we observed between parental and sotorasib-resistant cell lines (Fig. 5C cluster 8, Fig. S20 cluster 5). Finally, clusters representing proteins that change only in response to the combination treatment are enriched for nucleoside metabolism, ER membrane, and gene expression (upregulated, Fig. S19 cluster 1), and DNA replication (downregulated, Fig. S19 cluster 4). To further elucidate the effects of ZBTB11 depletion on mitochondrial proteins, we evaluated proteins downregulated in JWJ-01-

306 treatment conditions and compared them to the MitoCarta 3.0 database of mitochondrial proteins (Fig. S21A). We observed significant enrichment (554 proteins on day 3, 592 proteins on day 5) in each condition of annotated mitochondrial proteins. We confirmed that ZBTB11 was downregulated in JWJ-01-306 treated samples (Fig. S21C) alongside a subset of ZBTB11 downstream proteins (NDUFS7, NDUFA12, NDUFC2, NDUFAF1, MRPL48, MRPL44, MRPL1, MRPL30, Fig S21D-K). K-Ras levels were also reduced in the 5 d samples in response to JWJ-01-306 but not sotorasib (Fig. S21K), alongside off-targets identified through proteomics selectivity profiling (Fig. S21M-O). Together, these data demonstrated that ZBTB11 degradation reprogramed the pancreatic cancer cell transcriptome and proteome to counter the high-OXPHOS drug resistant state.

ZBTB11 degraders show reduced neurotoxicity compared to complex I inhibitors. Having established the mechanism-of-action of JWJ-01-306, we next sought to compare ZBTB11 degradation to complex I inhibition in human models of neuronal health. The primary safety liability of OXPHOS-targeting drugs are toxic effects on neurons, which rely heavily on mitochondrial metabolism to sustain their bioenergetic needs.²⁸ These effects often go unnoticed in murine studies, due to species differences between the human and murine nervous system, and challenges with accurately measuring neuropathy in mice.⁵⁸ Furthermore, molecular glue degraders often have different target profiles in murine and human systems due to sequence differences in both CRBN and the targets that alter the induced protein-protein interaction interface⁵⁹⁻⁶¹. To overcome these limitations and provide an early readout of the propensity of our ZBTB11 degraders to cause neuropathy, we profiled the neurotoxicity of ZBTB11 degraders using a suite of assays to monitor neuronal viability, morphology, and mitochondrial function in human induced pluripotent stem cell (hiPSC)-derived neurons.⁶²⁻⁶⁵ We found that hiPSC-derived neurons exposed to IACS-010759 for 24 hrs exhibited significantly decreased mitochondrial membrane potential (MMP) and increased reactive oxygen species (ROS), consistent with inhibition of mitochondrial complex I and reported adverse events in clinical trials²⁸ (Fig. 6A-B, Fig. S22A-B). Following treatment with IACS-010759 for 72 hrs, neurite length and neuronal survival were also decreased (Fig. 6C-D, S22C-D). In contrast, JWJ-01-306, and negative control JWJ-01-368, showed minimal effects on all phenotypes assayed (Fig. 6A-D). Next, we examined the bioenergetic effects of 10 μ M JWJ-01-306 on human iPSC-derived neurons, compared to 10 nM IACS-010759 at the 1 and 3 d time point. We found that effects of JWJ-01-306 on OXPHOS and metabolic index in neurons were minimal, in contrast to their effects in PDAC cells. However, IACS-010759 had potent effects, reducing basal and maximal OXPHOS, reducing overall ATP

production, and altering the metabolic index of the neurons (Fig. 6E-F). To better understand the cell-type specific effects of ZBTB11 degradation on bioenergetics, we treated iPSC-derived neurons with JWJ-01-306 and performed qPCR on downstream target genes at the 1 and 3 d time point. We observed minimal changes in transcript levels of components of complex I and upregulation of transcripts of mitoribosome components (Fig. 6G-H). These data suggest that these genes are not exclusively regulated by ZBTB11 in human neurons, and that compensatory mechanisms may exist to compensate for ZBTB11 loss. Together, these data demonstrated that our assay panel accurately identified neurotoxic compounds targeting mitochondrial function, and that ZBTB11 degradation had reduced acute neuronal toxicity and energetic perturbations relative to complex I inhibition in *in vitro* neuronal cultures.

DISCUSSION

In this project, we generated a comprehensive set of chemical and genetic tools to pharmacologically interrogate ZBTB11 function, and used these to identify ZBTB11-governed metabolic networks in human K-Ras inhibitor-resistant PDAC. In contrast to existing OXPHOS inhibitors, most of which are thought to target complex I-V enzymatic activity, ZBTB11 degradation directly targets the transcriptional upregulation of OXPHOS genes associated with cancer drug resistance. Pharmacological degradation of ZBTB11 countered metabolic reprogramming associated with acquired resistance to K-Ras inhibitors in multiple *ex vivo* models of PDAC, validating ZBTB11 as a druggable therapeutic vulnerability in PDAC.

A major limitation for targeting aberrant OXPHOS are the neurotoxic side effects of complex I inhibition, observed as on-target adverse events in clinical trials and in bedside-to-bench murine studies.²⁸ Using human iPSC-derived neuronal models, we demonstrated that ZBTB11 degradation had minimal effects on neuronal viability, bioenergetics, and function at phenotypically relevant concentrations and time points (10 μ M, 3d), in agreement with ZBTB11 CRISPRi, and in contrast to complex I inhibitor IACS-010759 (10 nM). Quantification of ZBTB11-target gene transcripts indicated these differences may be because ZBTB11 has context-specific roles in regulating OXPHOS networks in drug-resistant PDAC vs neurons, or because other factors compensate for ZBTB11 depletion in neurons. Our work marks a critical milestone by identifying a mechanistically orthogonal therapeutic strategy for targeting high OXPHOS cancer states. However, while the data in this manuscript support the hypothesis that ZBTB11 degradation phenotypically mimics non-toxic ZBTB11 knock-down, complete genetic knock-out of ZBTB11 in cancer cells is toxic, and therefore more comprehensive studies in *in vivo*

mammalian models at extended time points will be needed to establish the preclinical therapeutic window. In addition, our first-in-class tool degrader, JWJ-01-306 has off-targets, and further refinement of its selectivity would be desirable prior to advancement into the clinic.

Our patient-derived organoid study demonstrated that concurrent K-Ras and ZBTB11 blockade deepened responses to K-Ras^{G12D} inhibition in PDAC organoids that had not been previously treated with MRTX1133, including in the multi-drug resistant PDAC line hM1E. ZBTB11 degradation may therefore have applications in other cancers that upregulate OXPHOS to drive resistance to therapeutic interventions, including chemotherapies⁶⁶⁻⁶⁸, anti-angiogenic therapies⁶⁹, and targeted therapies such as inhibition of BCL2⁷⁰, tyrosine kinases⁷¹, and BRAF⁷². We anticipate that our suite of chemical and genetic tools will accelerate efforts to map ZBTB11 sensitive cancer cell states and identify additional settings where ZBTB11 depletion can address cancer drug resistance.

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AUTHOR CONTRIBUTIONS

N.L.T. performed Seahorse, RT-qPCR, HiBiT and NanoLuc luminescence assays, NanoBRET ternary complex assays, trypan blue exclusion, computational modeling, generated inducible CRISPRi cell lines, and prepared samples for proteomics and metabolomics studies. N.L.T. and M.M. conducted the proteomics experiments, performed proteomics and metabolomics analysis,

and prepared figures. J.W.J. designed and performed molecule synthesis. C.H. designed and generated CRISPRi constructs. D.A.S. performed metabolomic studies in collaboration with N.L.T. G.E.G performed CRBN target engagement assay. N.L.T and E.S.W. designed and cloned DNA plasmid constructs, generated ZBTB11-HiBiT cell lines, and performed immunoblot experiments. E.S.W supervised the research, performed study design and analysis, prepared figures, and edited the manuscript. F.M.F. supervised the research, performed study design and analysis, funding acquisition, prepared the figures, wrote the manuscript (first draft). K.C.M.G., H.T. and A.M.L. developed MRTX1133-resistant cell lines, performed bright-field proliferation assays and edited the manuscript. C.R.S. and E.R. performed Airyscan confocal microscopy and analysis. U.M. supervised the imaging assays, funding acquisition, and edited the manuscript All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

F.M.F., E.S.W., J.W.J. and N.L.T. are inventors on a patent application relating to this work (US 63/515,472). The Wang lab receives or has received research resources in kind from SK Life Sciences. F.M.F. is a scientific co-founder and equity holder in Proximity Therapeutics, and was previously a scientific advisory board member (SAB) of Triana Biomedicines. F.M.F. is or was recently a consultant or received speaking honoraria from RA Capital, Tocris BioTechne, Eli Lilly and Co., Amgen, Neomorph, and Plexium Inc. The Ferguson lab receives or has received research funding, travel funding, or resources in kind from Ono Pharmaceutical Co. Ltd, Eli Lilly and Co., Promega, and Merck and Co. F.M.F.'s interests have been reviewed and approved by the University of California San Diego in accordance with its conflict-of-interest policies.

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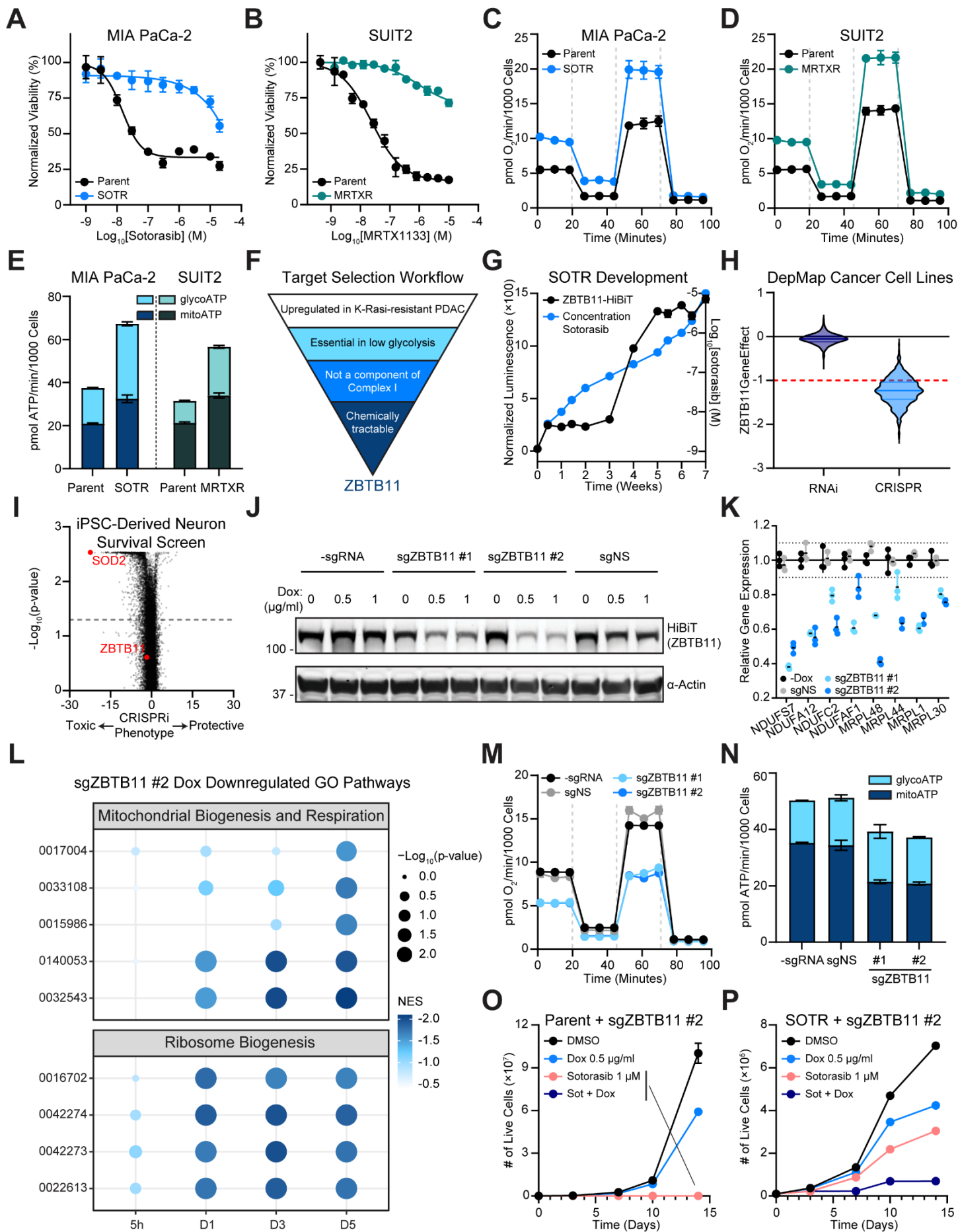


Figure 1 | K-Rasi resistance in PDAC cells is followed by OXPHOS upregulation and is countered by genetic knockdown of ZBTB11. **A-B.** MIA PaCa-2 and SUIT2 cells were treated with the indicated dose of sotorasib or MRTX1133 for 3 days, and viability was measured by CellTiterGlo®. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates and are normalized to DMSO vehicle treatment controls. **C-D.** MIA PaCa-2 and SUIT2 cells were subjected to a mitochondrial stress test protocol using a Seahorse XFe24 analyzer. **E.** Oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) measured during the mitochondrial stress test were used to calculate ATP production by glycolysis (glycoATP) and by mitochondrial respiration (mitoATP). **F.** Sequential workflow to identify suitable target for indirect OXPHOS inhibition. **G.** MIA PaCa-2 parental cells were cultured with increasing sublethal concentrations of sotorasib to generate sotorasib-resistant MIA PaCa-2 cells. ZBTB11 protein abundance was measured throughout the development of sotorasib resistance via CRISPR knock-in of a HiBiT tag to the endogenous locus of ZBTB11 in MIA PaCa-2 parental cells. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates. **H.** Effect of ZBTB11 knockdown (RNAi) or knockout (CRISPR) on the viability of cancer cell lines as determined by DepMap RNAi (DEMETER2) and CRISPR KO (Chronos) screens. Data are depicted as the average (dark, thick line) with the first and third quartiles marked (light, thin lines). **I.** Effect of CRISPRi-mediated gene knockdown on the viability of iPSC-derived neurons as determined by Tian, R. *et al* (2019). **J.** MIA PaCa-2 parental cells were transduced with lentiviruses to introduce the components of a doxycycline-inducible CRISPRi system, followed by transduction with: -sgRNA (no guide RNA), sgZBTB11 #1/#2 (ZBTB11-targeting guide RNAs), or sgNS (non-targeting guide RNA). After FACS and antibiotic selection, these cells were treated with the indicated amount of doxycycline for 3 days, and protein abundance was assessed by Western blotting. **K.** MIA PaCa-2 sgZBTB11 #1/#2 or sgNS cells were treated with 0.5 μ g/ml doxycycline for 3 days, and gene transcript abundance was measured by RT-qPCR. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates and are normalized to H2O vehicle treatment controls. The H2O vehicle treatment control for MIA PaCa-2 sgNS (-Dox) is included as a reference baseline. **L.** MIA PaCa-2 sgZBTB11 #2 cells were treated with 0.5 μ g/ml doxycycline or H2O vehicle for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative top-scoring pathways are shown here as GOBP codes with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates. **M.** MIA PaCa-2 cells were treated with 0.5 μ g/ml doxycycline for 3 days, followed by a mitochondrial stress test protocol using a Seahorse XFe24 analyzer. **N.** OCR and ECAR measured during the mitochondrial stress test were used to calculate glycoATP and mitoATP. **O-P.** MIA PaCa-2 sgZBTB11 #2 cells were treated with the indicated compound(s) over a 14-day period, and cumulative number of live cells was measured by trypan blue exclusion. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates. **C-D, M.** From left to right, gray dotted lines indicate additions of oligomycin, BAM15, and rotenone/antimycin A. Data are depicted as the average $\pm$ S. D. of $n = 2$ biological replicates. **E, N.** Data are depicted as the average $\pm$ S. D. of $n = 2$ biological replicates with $n = 3$ technical replicates each.

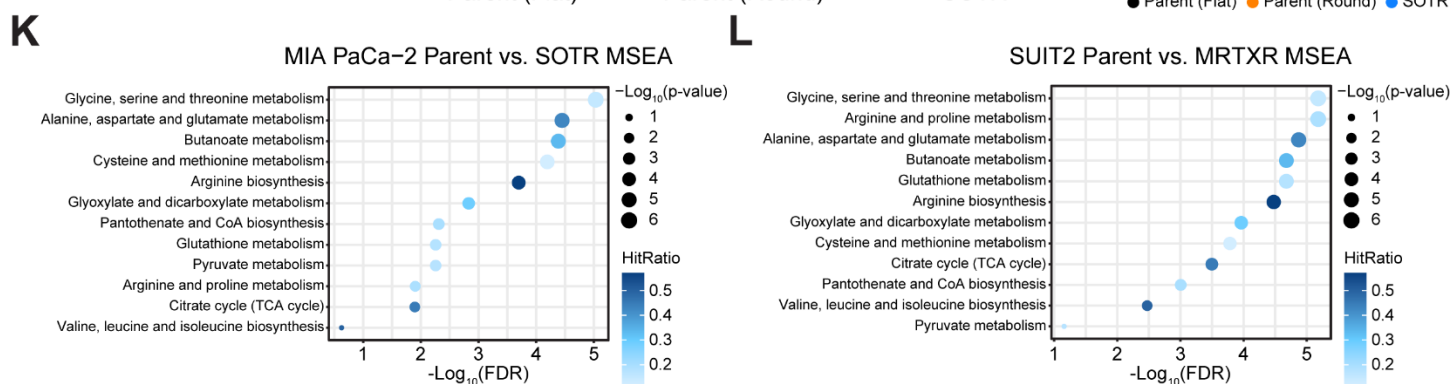
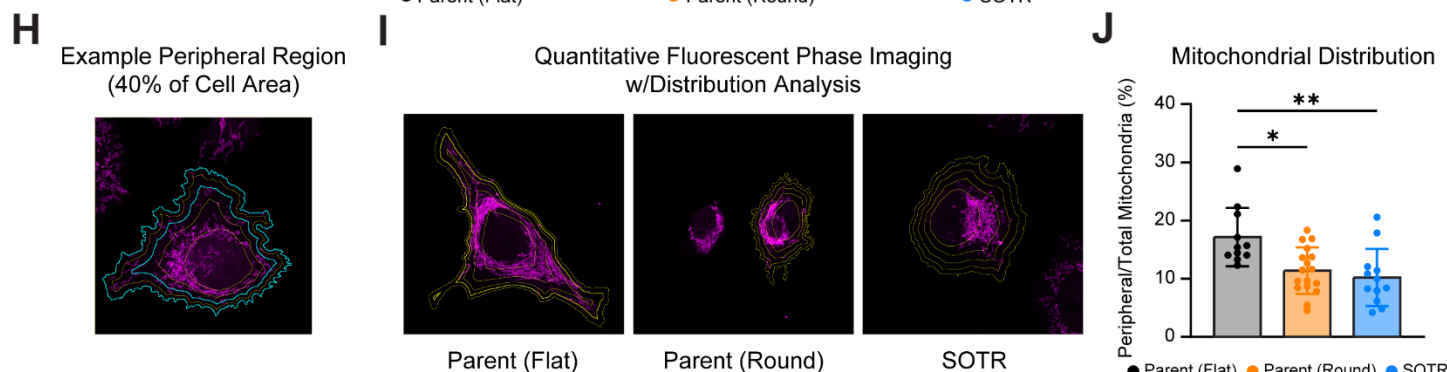
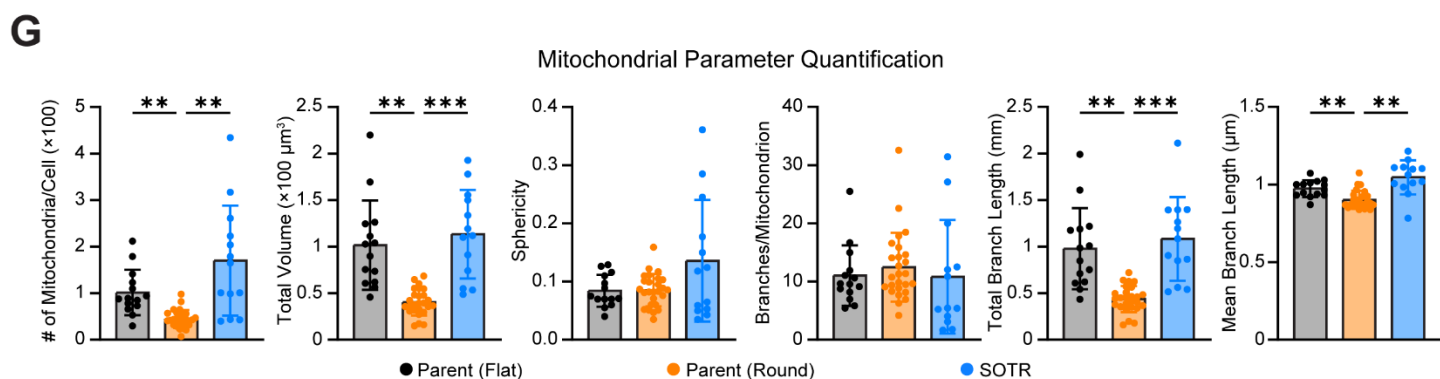
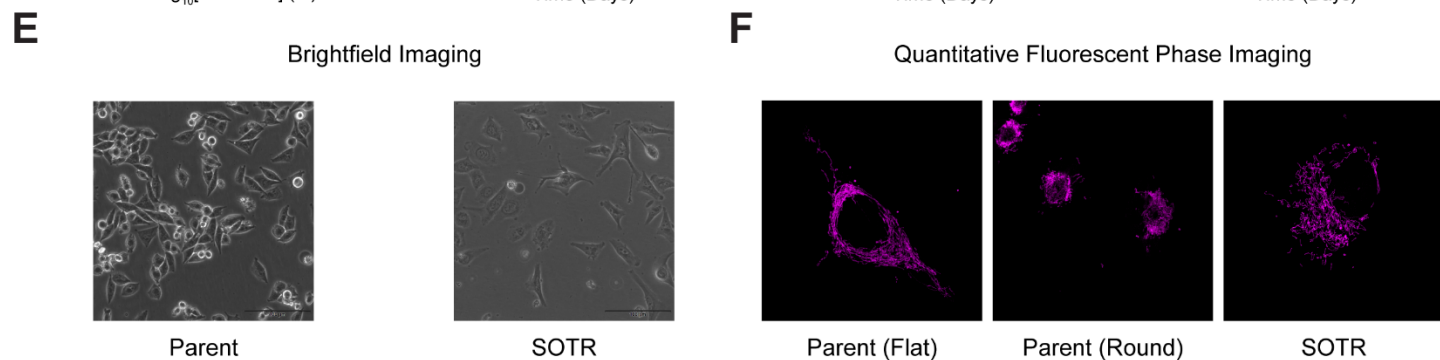
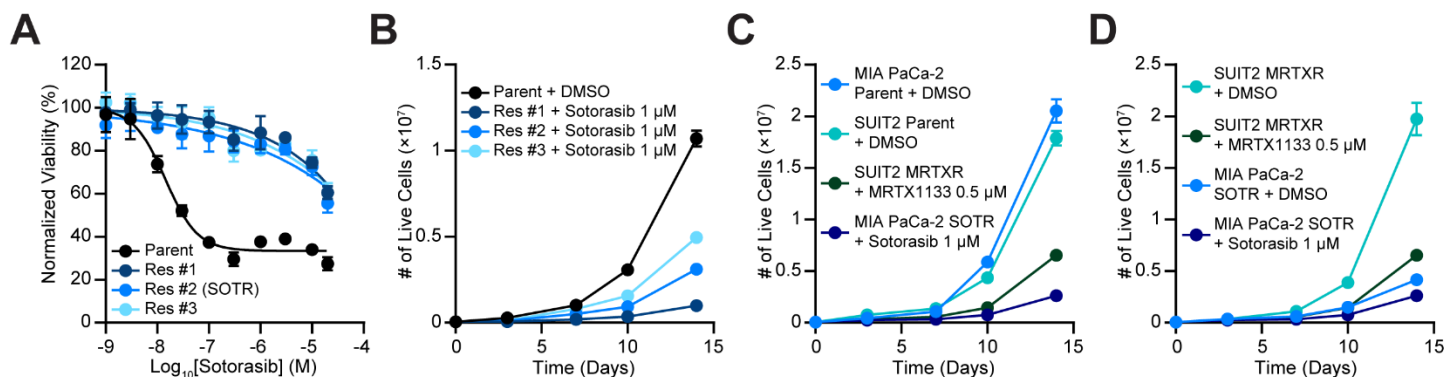


Figure S1 | K-Rasi resistant PDAC cells exhibit significant changes in morphology, mitochondria, and metabolism. **A.** MIA PaCa-2 cells were treated with the indicated dose of sotorasib for 3 days, and viability was measured by CellTiterGlo®. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates and are normalized to DMSO vehicle treatment controls. **B.** MIA PaCa-2 cells were treated with the indicated compound over a 14-day period. **C-D.** MIA PaCa-2 and SUIT2 cells were treated with the indicated compound over a 14-day period. **E.** MIA PaCa-2 cells in culture were imaged using a brightfield microscope with 20X magnification objective lens. **F.** MIA PaCa-2 cells were fixed onto #1.5 coverslips and subjected to an immunofluorescence staining protocol using α -HSP60 1° antibody. The stained cells were then imaged using a Zeiss LSM980 equipped with an Airyscan detector. **G.** 3D mitochondrial morphology in stained MIA PaCa-2 cells was analyzed and quantified using the Mitochondria Analyzer Fiji plugin. Data are depicted as the average $\pm$ S. D. of at least $n = 10$ biological replicates. Significance was determined by performing Brown-Forsythe and Welch ANOVA tests with Dunnett's T3 test for multiple comparisons. **H.** Example image of stained MIA PaCa-2 cells with peripheral region marked (area between the two blue lines). Cell and nucleus outlines defining the regions were extracted from transmission and DAPI images; respectively. **I.** MIA PaCa-2 cells were subjected to same protocol as in Fig. S1E. Acquired images were then processed as described in Fig. S1G. **J.** Distribution of mitochondria in stained MIA PaCa-2 cells was quantified using the ringIntensityDistribution Fiji macro. Data are depicted as the average $\pm$ S. D. of at least $n = 10$ biological replicates. Significance was determined by performing Brown-Forsythe and Welch ANOVA tests with Dunnett's T3 test for multiple comparisons. **K-L.** MIA PaCa-2 and SUIT2 cells were grown with ^{13}C -labeled glucose or glutamine for 24 hours, and targeted metabolomics analysis was performed by GC-MS/MS. Metabolite abundances from these conditions were input to MSEA and clustered by KEGG pathway. Representative top-scoring pathways are shown here with corresponding significance and enrichment (HitRatio) scores of $n = 6$ biological replicates. **B-D.** Cumulative number of live cells was measured by trypan blue exclusion. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates.

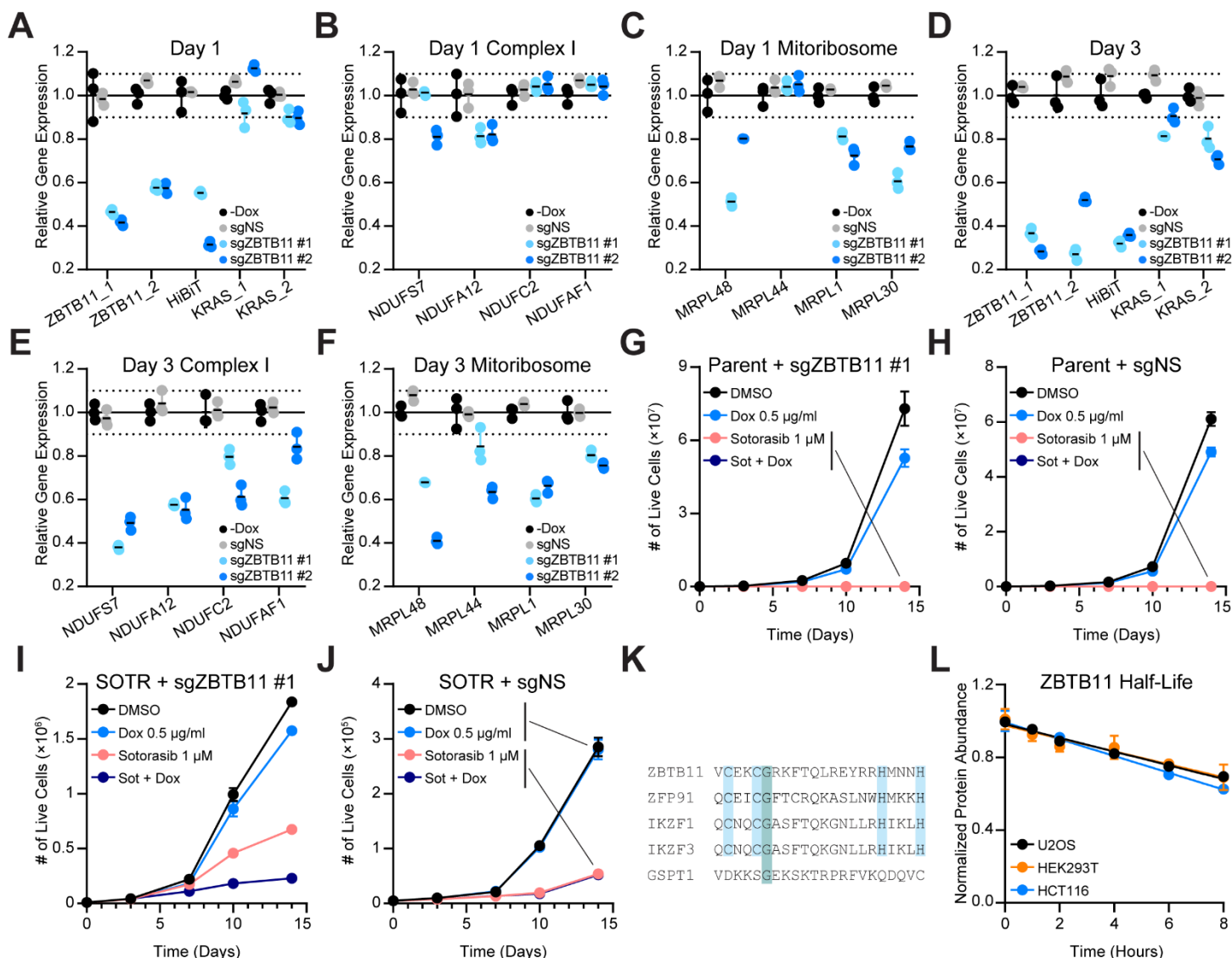


Figure S2 | Genetic knockdown of ZBTB11 induces downregulation of mitochondrial genes and counters K-Rasi resistance in PDAC cells. **A-F.** MIA PaCa-2 sgZBTB11 #1/#2 or sgNS cells were treated with 0.5 μ g/ml doxycycline for the indicated amount of time, and gene transcript abundance was measured by RT-qPCR. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates and are normalized to H₂O vehicle treatment controls. The H₂O vehicle treatment control for MIA PaCa-2 sgNS (-Dox) is included as a reference baseline. **G-J.** MIA PaCa-2 sgZBTB11 #1 or sgNS cells were treated with the indicated compound(s) over a 14-day period, and cumulative number of live cells was measured by trypan blue exclusion. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates. **K.** Sequence alignment of ZBTB11 and other C2H2 zinc finger-containing proteins, as well as GSPT1, with the C2H2 motif and beta hairpin glycine highlighted. Sequences were aligned using blastp algorithm. **L.** Protein half-life of ZBTB11 in the indicated cell line as determined by cycloheximide chase followed by mass spectrometry-based proteomics performed by Li, J. *et al.* (2021).

sgZBTB11 #2 Dox Downregulated GO Pathways
Matched with Compound-Guided RNAseq DMSO GO Terms

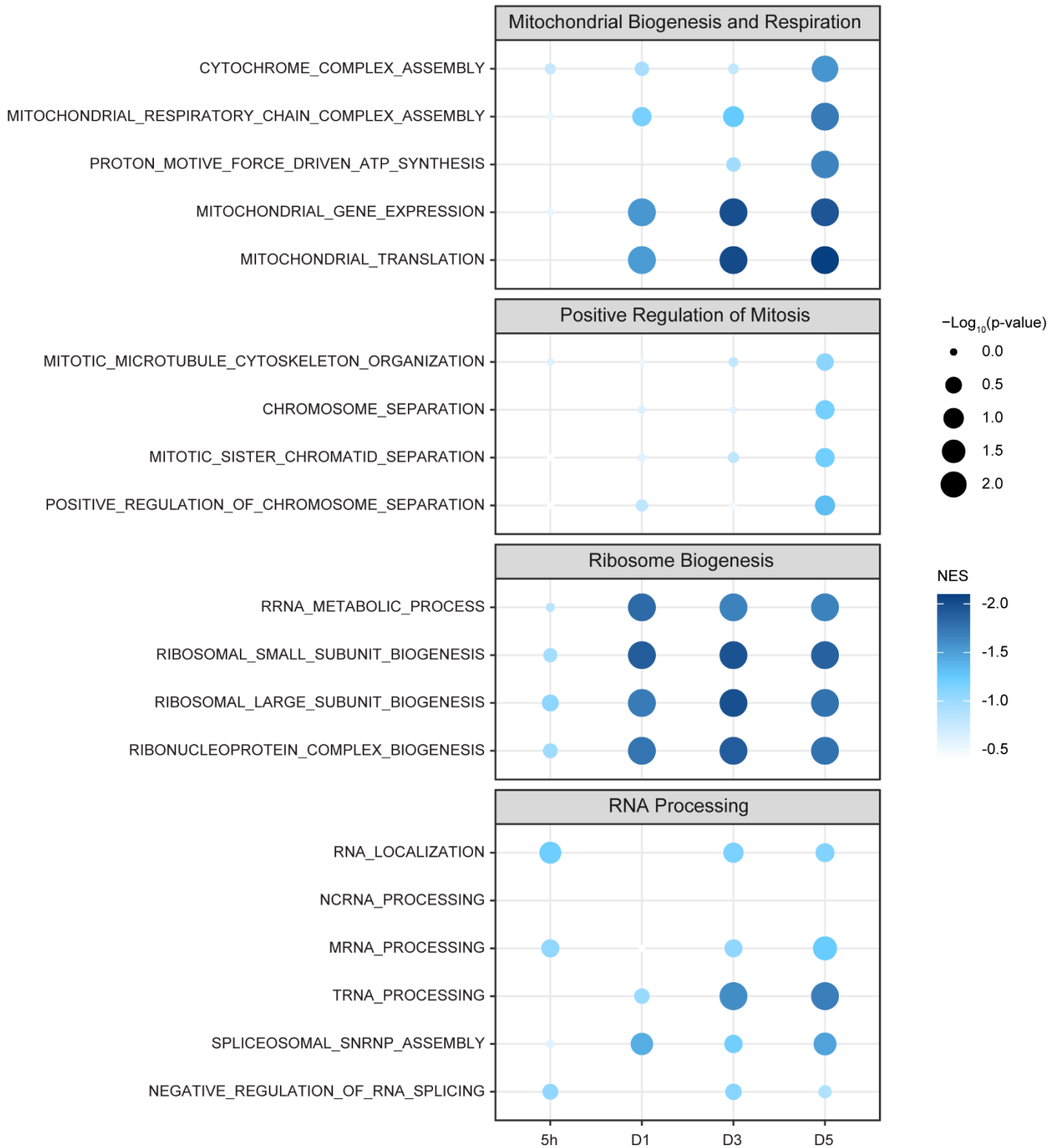


Figure S3 | Genetic knockdown of ZBTB11 induces transcriptome-wide downregulation of mitochondrial and ribosome biogenesis. MIA PaCa-2 sgZBTB11 #2 cells were treated with 0.5 µg/ml doxycycline or H₂O vehicle for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative pathways that coincide with the top-scoring pathways of the DMSO condition in our compound-guided RNAseq analysis are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

sgZBTB11 #2 Dox Downregulated GO Pathways
Matched with Compound-Guided RNAseq Sotorasib GO Terms

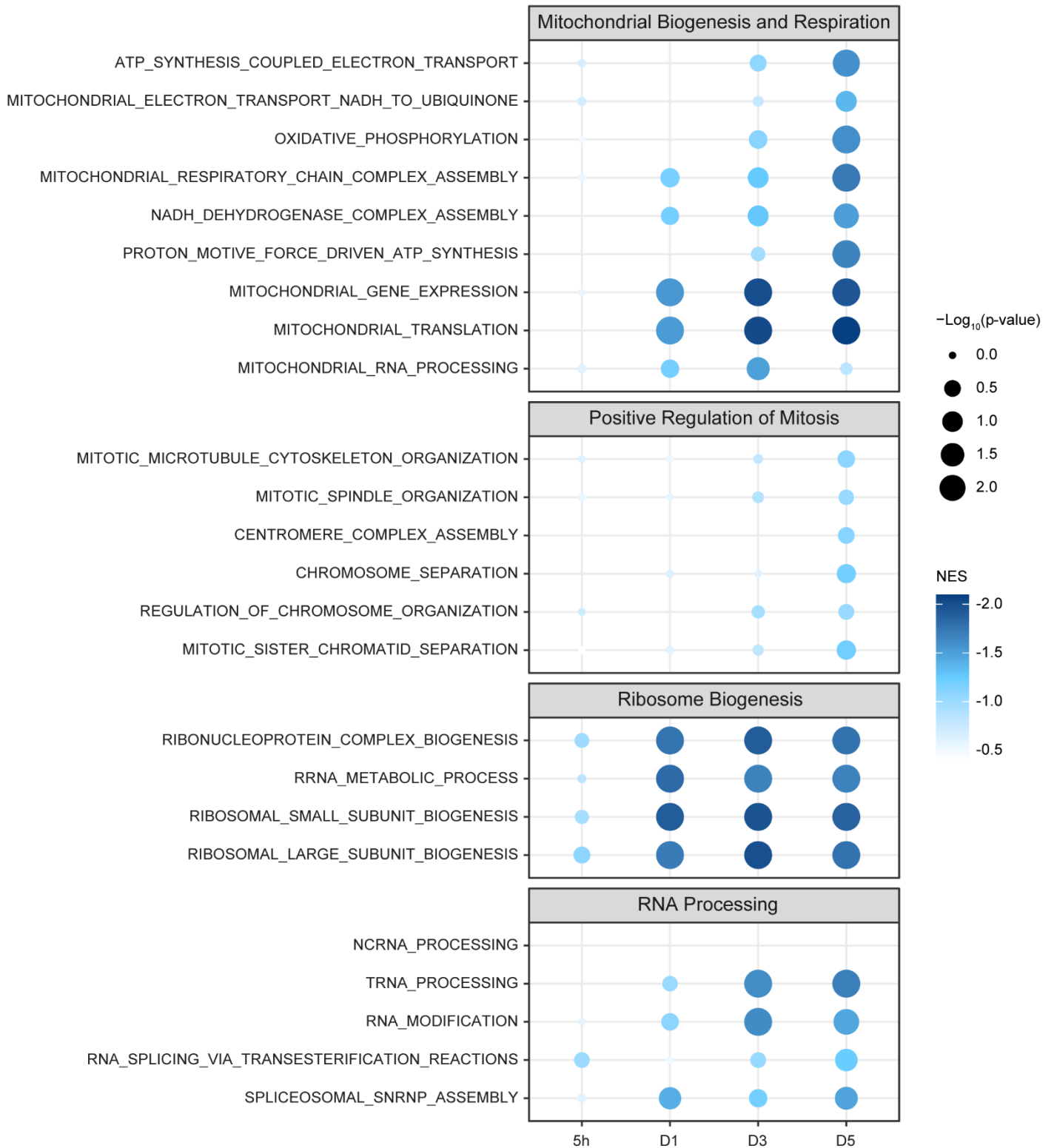


Figure S4 | Genetic knockdown of ZBTB11 induces transcriptome-wide downregulation of mitochondrial and ribosome biogenesis. MIA PaCa-2 sgZBTB11 #2 cells were treated with 0.5 µg/ml doxycycline or H₂O vehicle for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative pathways that coincide with the top-scoring pathways of the sotorasib condition in our compound-guided RNAseq analysis are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

sgZBTB11 #2 Dox Upregulated GO Pathways
Matched with Compound-Guided RNAseq JWJ-01-306 GO Terms

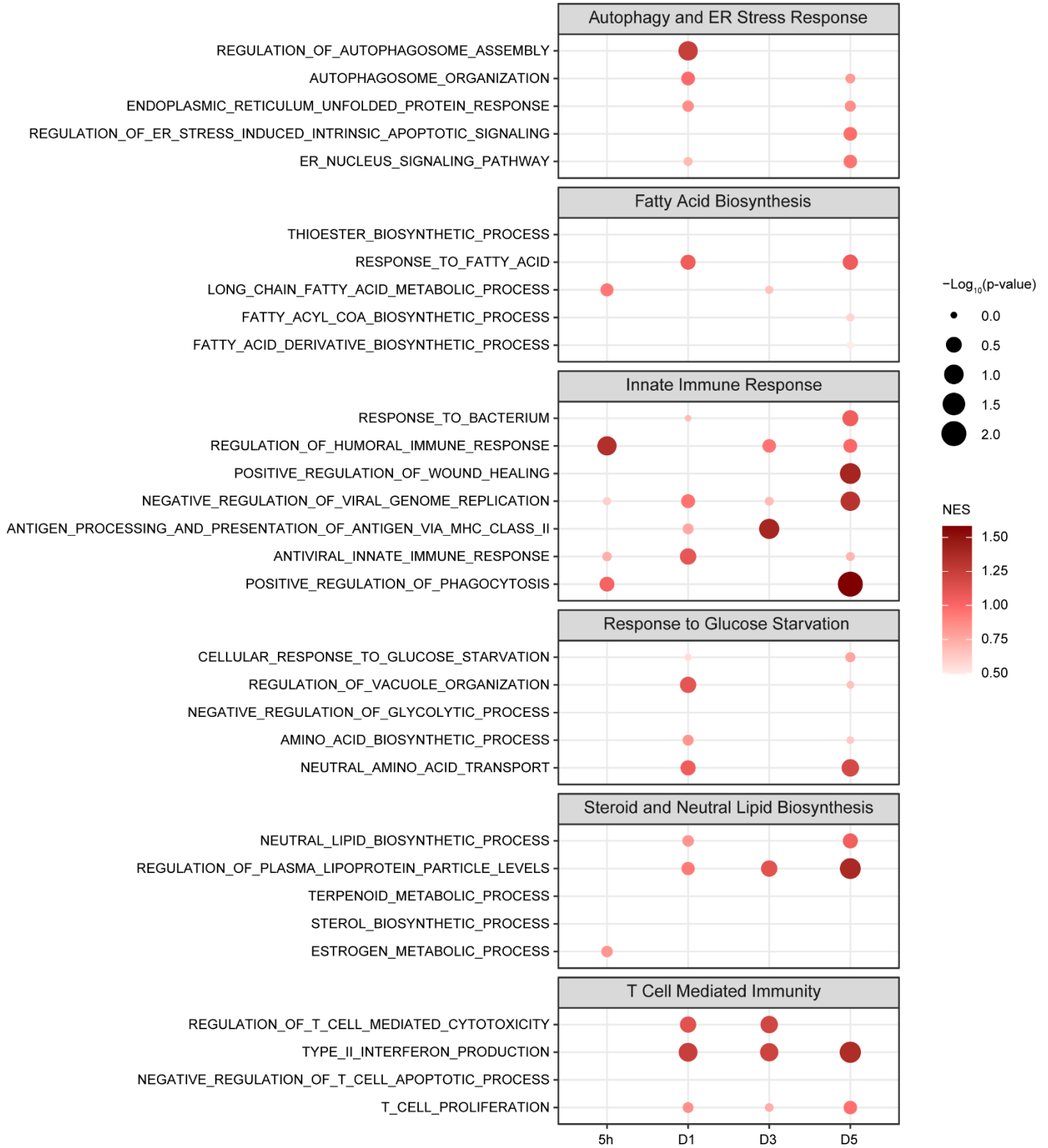


Figure S5 | Few pathways are upregulated on a transcriptome-wide level by genetic ZBTB11 knockdown. MIA PaCa-2 sgZBTB11 #2 cells were treated with 0.5 µg/ml doxycycline or H₂O vehicle for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative pathways that coincide with the top-scoring pathways of the JWJ-01-306 condition in our compound-guided RNAseq analysis are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

sgZBTB11 #2 Dox Upregulated GO Pathways
Matched with Compound-Guided RNAseq Combo GO Terms

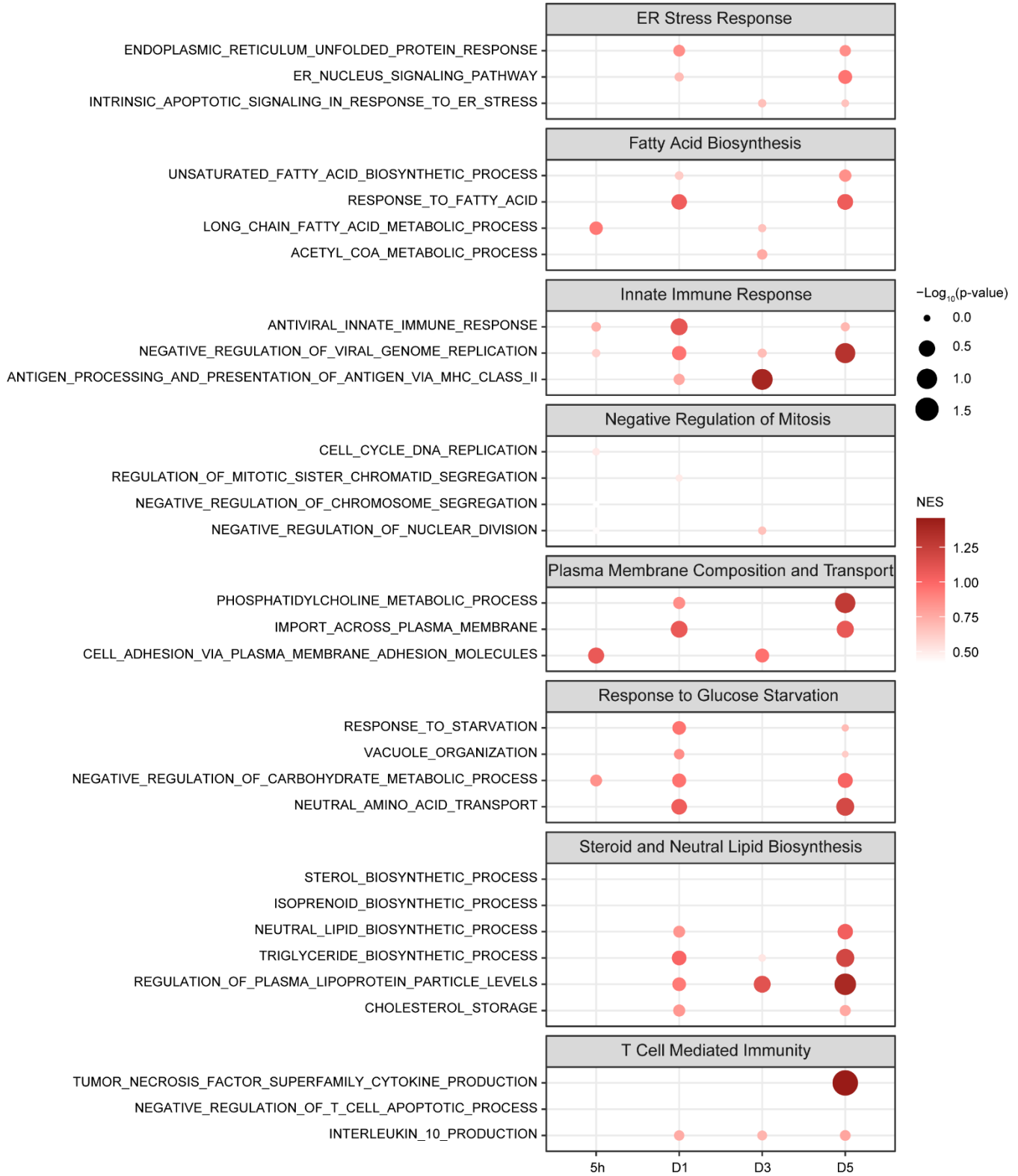


Figure S6 | Few pathways are upregulated on a transcriptome-wide level by genetic ZBTB11 knockdown. MIA PaCa-2 sgZBTB11 #2 cells were treated with 0.5 µg/ml doxycycline or H₂O vehicle for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative pathways that coincide with the top-scoring pathways of the combo (sotorasib + JWJ-01-306) condition in our compound-guided RNAseq analysis are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

sgNS Dox Downregulated GO Pathways
Matched with Compound-Guided RNAseq DMSO GO Terms

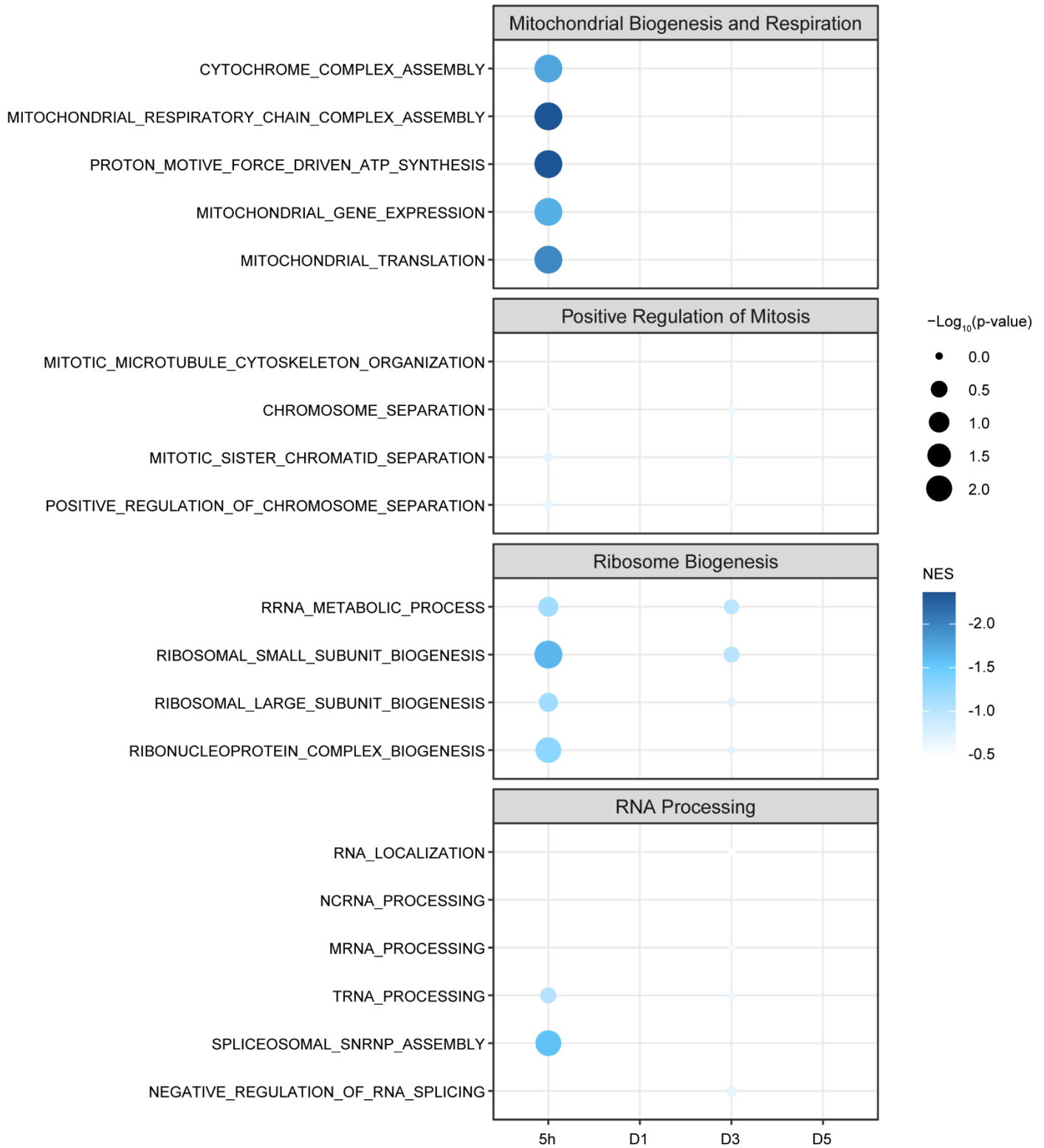


Figure S7 | Non-targeting sgRNA control does not induce any sustained changes in the transcriptome. MIA PaCa-2 sgNS cells were treated with 0.5 µg/ml doxycycline or H₂O vehicle for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative pathways that coincide with the top-scoring pathways of the DMSO condition in our compound-guided RNAseq analysis are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

sgNS Dox Downregulated GO Pathways
Matched with Compound-Guided RNAseq Sotorasib GO Terms



Figure S8 | Non-targeting sgRNA control does not induce any sustained changes in the transcriptome. MIA PaCa-2 sgNS cells were treated with 0.5 µg/ml doxycycline or H₂O vehicle for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative pathways that coincide with the top-scoring pathways of the sotorasib condition in our compound-guided RNAseq analysis are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

sgNS Dox Upregulated GO Pathways
Matched with Compound-Guided RNAseq JWJ-01-306 GO Terms

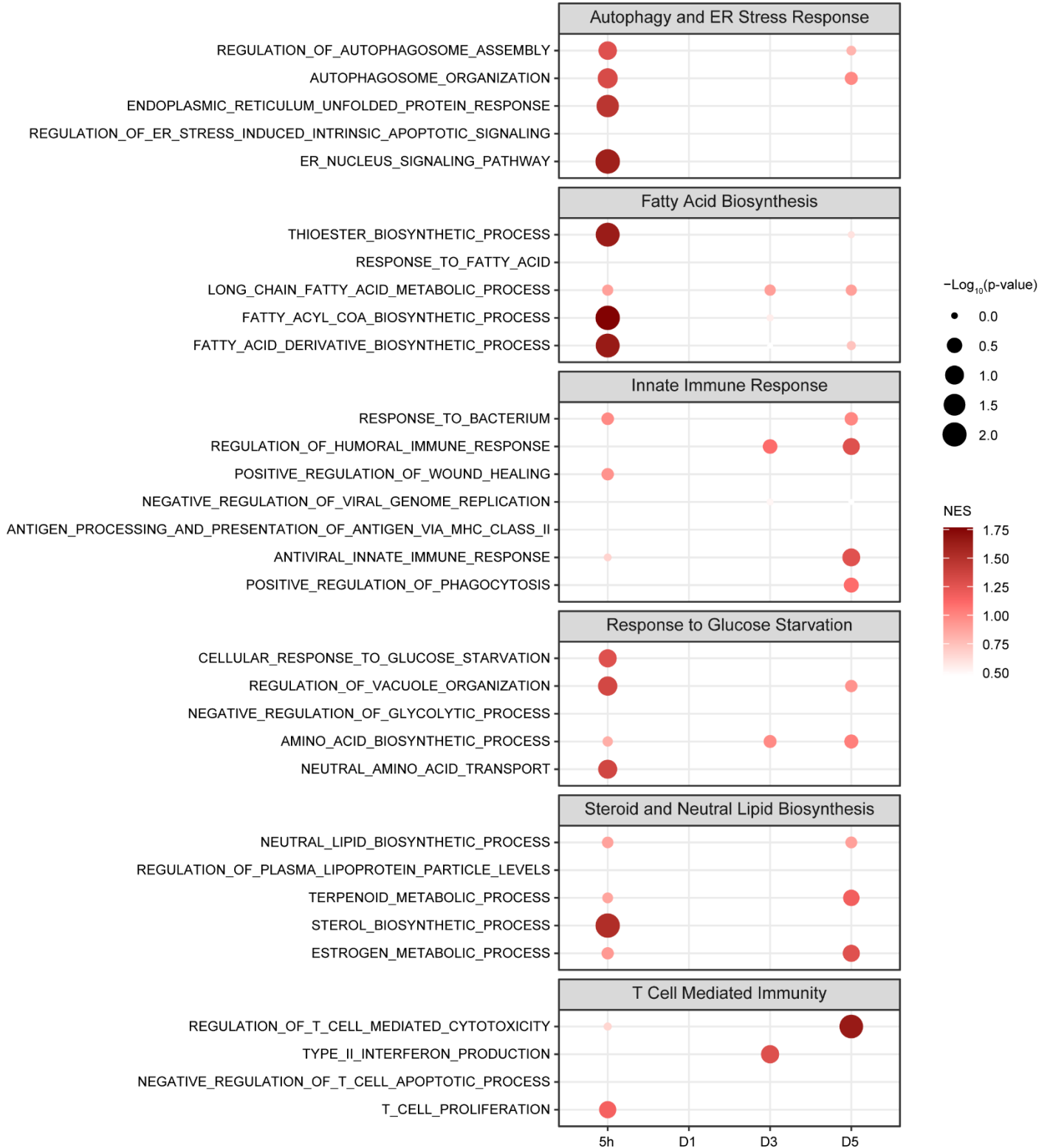


Figure S9 | Non-targeting sgRNA control does not induce any sustained changes in the transcriptome. MIA PaCa-2 sgNS cells were treated with 0.5 µg/ml doxycycline or H₂O vehicle for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative pathways that coincide with the top-scoring pathways of the JWJ-01-306 condition in our compound-guided RNAseq analysis are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

sgNS Dox Upregulated GO Pathways
Matched with Compound-Guided RNAseq Combo GO Terms

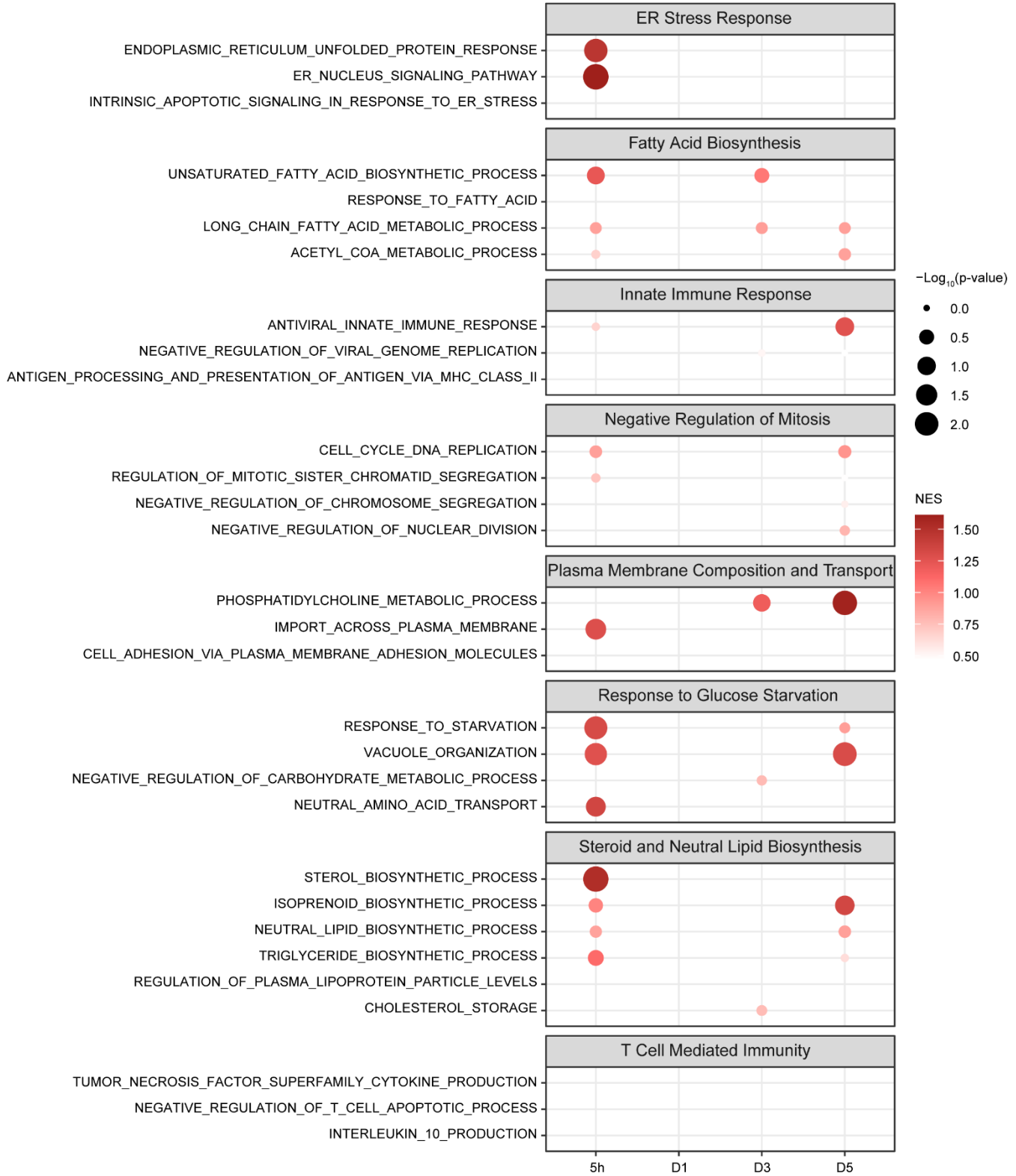


Figure S10 | Non-targeting sgRNA control does not induce any sustained changes in the transcriptome.

MIA PaCa-2 sgNS cells were treated with 0.5 µg/ml doxycycline or H₂O vehicle for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative pathways that coincide with the top-scoring pathways of the combo (sotorasib + JWJ-01-306) condition in our compound-guided RNAseq analysis are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

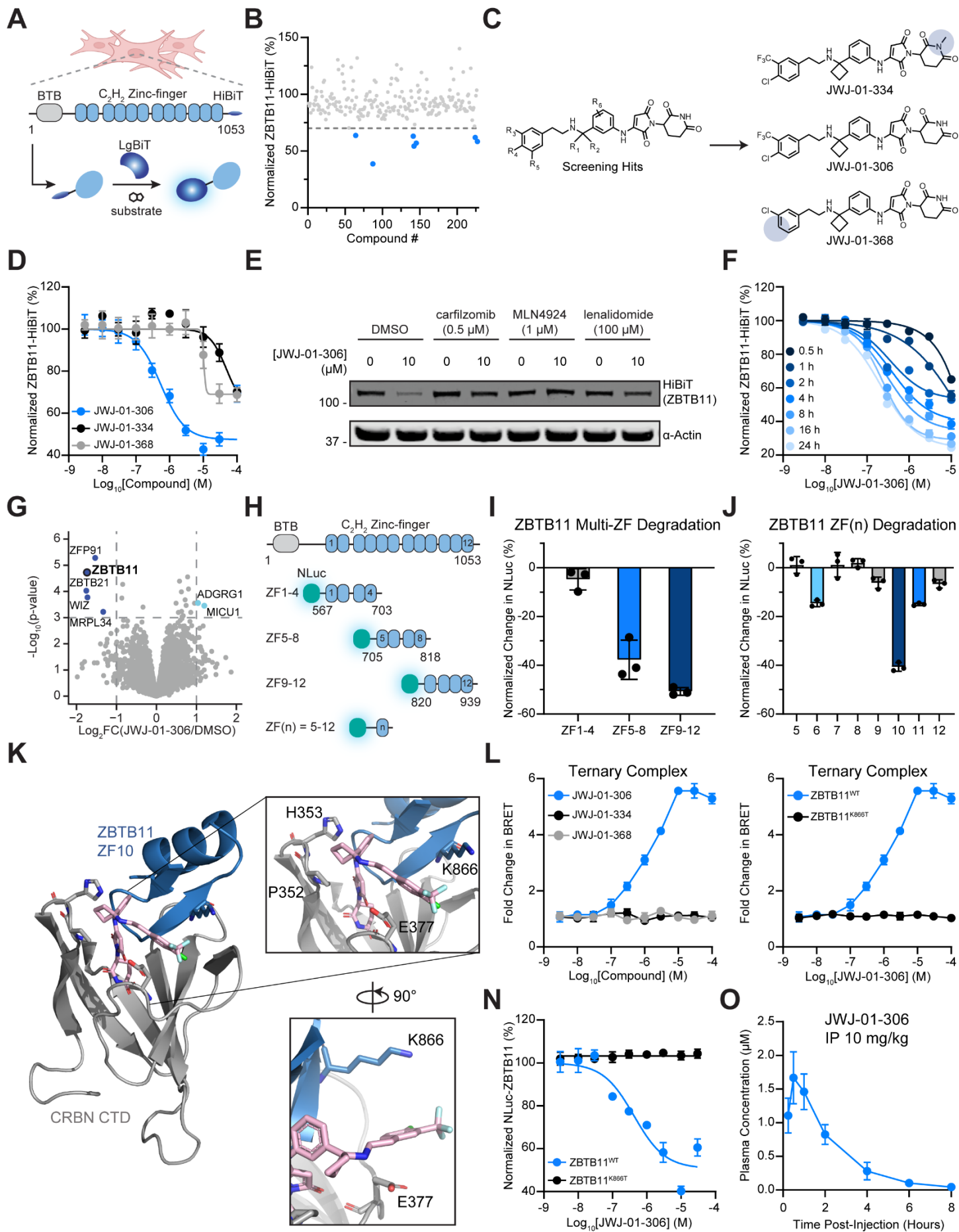


Figure 2 | High-throughput screening and chemical optimization enable development of a potent and selective molecular glue degrader of ZBTB11. **A.** Schematic of endogenous ZBTB11-HiBiT gene structure and HiBiT luminescence assay. **B.** MOLT-4 cells were treated with 20 μ M of the indicated compound for 8 hours. Data are depicted as $n = 1$ biological replicate from compound library screening and are normalized to DMSO vehicle treatment controls. Screening hits were defined as compounds inducing $\geq 30\%$ degradation of ZBTB11-HiBiT ($\leq 70\%$ normalized ZBTB11-HiBiT signal) and are highlighted in blue. **C.** Chemical structures of generalized validated screening hits and optimized final compound (JWJ-01-306), as well as negative control compounds (JWJ-01-334 and JWJ-01-368). **D.** MIA PaCa-2 cells were treated with the indicated concentration of compound for 5 hours. **E.** MIA PaCa-2 cells were pre-treated with the indicated mechanistic inhibitor or competitor for 1 hour prior to treatment with JWJ-01-306 or DMSO vehicle for 5 hours, and protein abundance was assessed by Western blotting. **F.** MIA PaCa-2 cells were treated with the indicated concentration of JWJ-01-306 for varying amounts of time. **G.** MIA PaCa-2 cells were treated with 10 μ M JWJ-01-306 or DMSO vehicle for 5 hours, and global proteomics analysis was performed by LC-MS/MS. Protein abundances from these two conditions were processed using R packages and are shown here with corresponding significance and fold change values of $n = 3$ biological replicates. Significant hits were defined as proteins with ≥ 2 -fold change and p -value ≥ 0.05 (two-sample moderated t-test with two-tailed distribution) and are highlighted in blue with identity labels. **H.** Schematic of endogenous ZBTB11 gene structure and NanoLuc tagging strategy. **I-J.** MOLT-4 cells transfected with multi-ZF or single-ZF NLuc-ZBTB11 constructs were treated with 10 μ M ALV-05-184 for 5 hours. **K.** Rosetta-generated model of the ternary complex between CRBN, JWJ-01-306, and ZBTB11 ZF10. Protein residues predicted to make contacts at the ternary complex interface are shown in stick representation and labeled with residue identity and position. **L.** MOLT-4 cells transfected with NLuc-ZBTB11 and CRBN-HT were pre-treated with 100 nM proteasomal inhibitor carfilzomib and 100 nM HaloTag® NanoBRET™ 618 Ligand for 1 hour prior to treatment with the indicated concentration of compound for 5 hours. **M.** MOLT-4 cells transfected with CRBN-HT and either NLuc-ZBTB11^{WT} or NLuc-ZBTB11^{K866T} were pre-treated with 100 nM proteasomal inhibitor carfilzomib and 100 nM HaloTag® NanoBRET™ 618 Ligand for 1 hour prior to treatment with the indicated concentration of compound for 5 hours. **N.** MIA PaCa-2 cells transfected with either NLuc-ZBTB11^{WT} or NLuc-ZBTB11^{K866T} were treated with the indicated concentration of JWJ-01-306 for 5 hours. **O.** C57BL/6 mice were dosed intraperitoneally with 10 mg/kg JWJ-01-306-HCl, and plasma concentration of JWJ-01-306 was measured by LC-MS. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates. **B, D, F.** ZBTB11 protein abundance was measured by HiBiT luminescence assay. **I-J, N.** ZBTB11 protein abundance was measured by NLuc luminescence assay. **L-M.** Ternary complex formation was measured by NanoBRET™ assay. **D, F, I-J, L-N.** Data are depicted as the average $\pm$ S.D. of $n = 3$ biological replicates and are normalized to DMSO vehicle treatment controls.

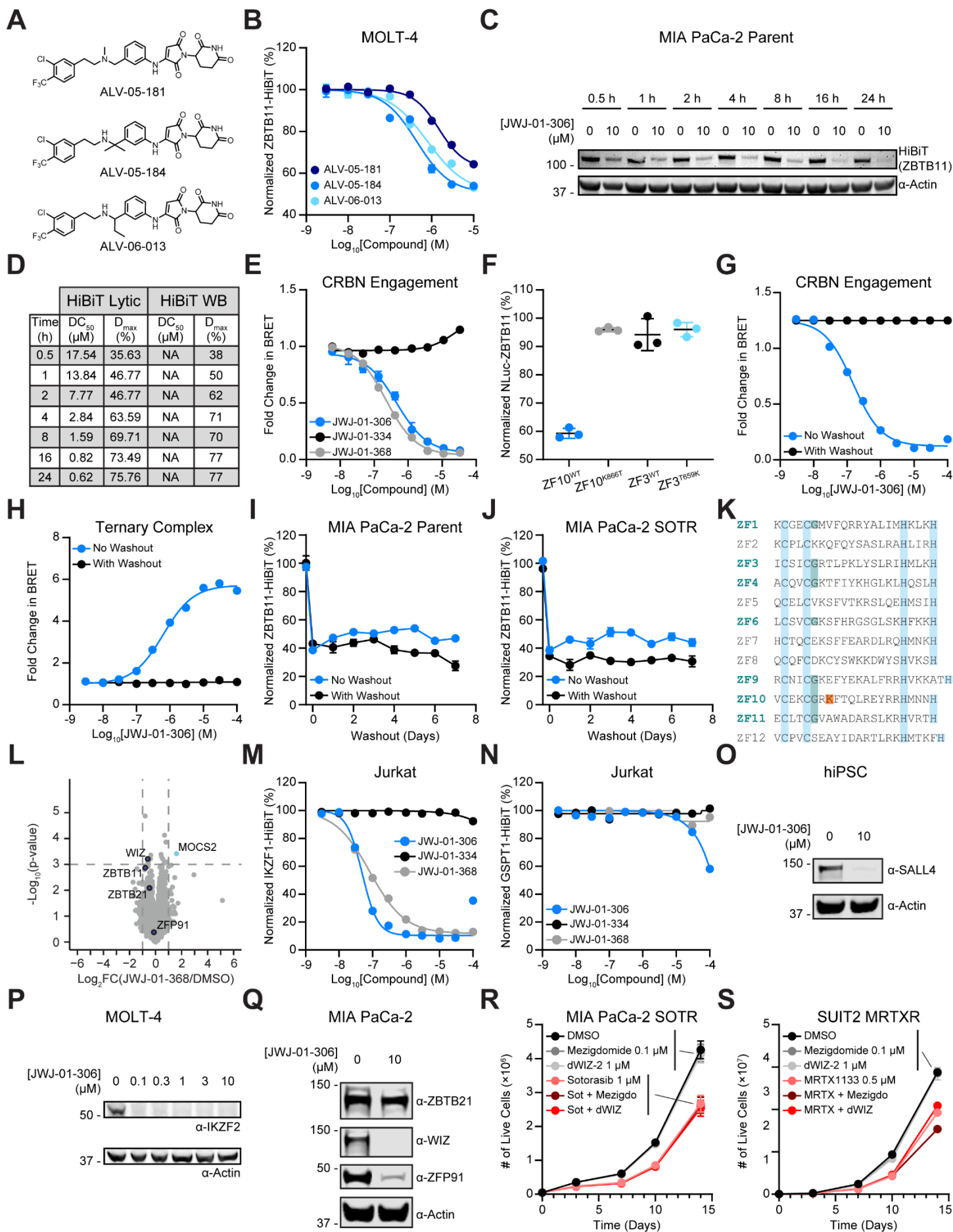


Figure S11 | A suite of cell-based biochemical assays facilitates development and characterization of a potent and selective ZBTB11 degrader. **A.** Chemical structures of validated screening hits. **B.** MIA PaCa-2 cells were treated with the indicated concentration of compound for 5 hours. **C.** MIA PaCa-2 cells were treated with 10 μ M JWJ-01-306 or DMSO vehicle for the indicated amount of time. **D.** Data table of DC_{50} and D_{max} values of JWJ-01-306 against ZBTB11 calculated from nonlinear regression of HiBiT lytic assay (Fig. 2F) and from quantification of Western blot (Fig. S11C). **E.** HEK293 cells transfected with NLuc-CRBN and DDB1 were treated with 0.5 μ M NanoBRET™ Tracer Reagent and the indicated concentration of compounds for 2 hours. **F.** MOLT-4 cells transfected with the indicated single-ZF NLuc-ZBTB11 constructs were treated with 10 μ M ALV-05-184 for 5 hours, and protein abundance was measured by NLuc luminescence assay. **G.** HEK293 cells transfected with NLuc-CRBN and DDB1 were treated with the indicated concentration of JWJ-01-306 for 2 hours prior to washout and subsequent addition of 0.5 μ M NanoBRET™ Tracer Reagent. **H.** HEK293T cells transfected with NLuc-ZBTB11 and CRBN-HT were pre-treated with 100 nM proteasomal inhibitor carfilzomib and 100 nM HaloTag® NanoBRET™ 618 Ligand for 1 hour prior to treatment with the indicated concentration of JWJ-01-306 for 5 hours followed by washout. Ternary complex formation was measured by NanoBRET™ assay. **I-J.** MIA PaCa-2 cells were treated with 10 μ M of JWJ-01-306 for 5 hours prior to washout and subsequent incubation for the indicated amount of time. **K.** Sequence alignment of the 12 C2H2 zinc fingers of ZBTB11 with the C2H2 motif, beta hairpin glycine, and ZF10-unique lysine highlighted. Sequences were aligned using blastp algorithm. **L.** MIA PaCa-2 cells were treated with 10 μ M JWJ-01-368 or DMSO vehicle for 5 hours, and global proteomics analysis was performed by LC-MS/MS. Protein abundances from these two conditions were processed using R packages and are shown here with corresponding significance and fold change values of $n = 3$ biological replicates. Significant hits were defined as proteins with ≥ 2 -fold change and p -value ≥ 0.05 (two-sample moderated t-test with two-tailed distribution) and are highlighted in blue with identity labels. **M-N.** Jurkat cells with HiBiT tag knocked into endogenous locus of IKZF1 or GSPT1 were treated with the indicated concentration of compound for 8 hours. **O.** Human induced-pluripotent stem cells (hiPSCs) were treated with 10 μ M of JWJ-01-306 or DMSO vehicle for 5 hours. **P.** MOLT-4 cells were treated with the indicated concentration of JWJ-01-306 for 5 hours. **Q.** MIA PaCa-2 cells were treated with 10 μ M JWJ-01-306 or DMSO vehicle for 5 hours. **R-S.** MIA PaCa-2 or SUIT2 cells were treated with the indicated compound(s) over a 14-day period, and cumulative number of live cells was measured by trypan blue exclusion. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates. **B, I-J, M-N.** Protein abundance was measured by HiBiT luminescence assay. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates and are normalized to DMSO vehicle treatment controls. **E, G.** CRBN engagement was measured by NanoBRET™ assay. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates and are normalized to DMSO vehicle treatment controls. **C, O-Q.** Protein abundance was assessed by Western blotting.

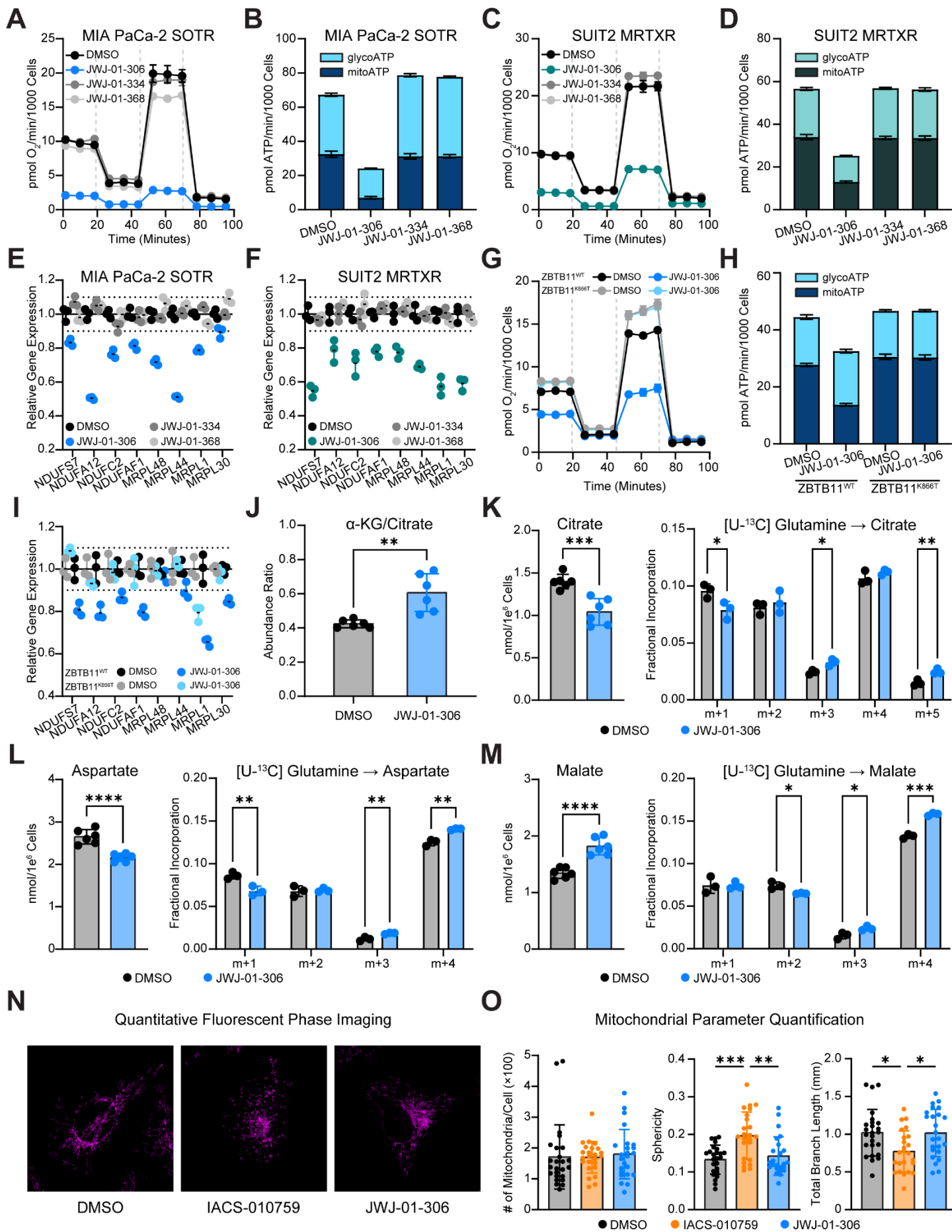


Figure 3 | Chemical depletion of ZBTB11 induces OXPHOS downregulation and reprogramming of mitochondrial metabolism in PDAC cells. **A.** MIA PaCa-2 cells were treated with 10 μ M of the indicated compound for 24 hours. **B.** OCR and ECAR measured during the mitochondrial stress test were used to calculate glycoATP and mitoATP. **C.** SUIT2 cells were treated with 10 μ M of the indicated compound for 24 hours. **D.** OCR and ECAR measured during the mitochondrial stress test were used to calculate glycoATP and mitoATP. **E-F.** MIA PaCa-2 and SUIT2 cells were treated with 10 μ M of the indicated compound for 24 hours. **G.** MIA PaCa-2 cells transfected with either NLuc-ZBTB11^{WT} or NLuc-ZBTB11^{K866T} were treated with 10 μ M JWJ-01-306 or DMSO vehicle for 24 hours. **H.** OCR and ECAR measured during the mitochondrial stress test were used to calculate glycoATP and mitoATP. **I.** MIA PaCa-2 cells transfected with either NLuc-ZBTB11^{WT} or NLuc-ZBTB11^{K866T} were treated with 10 μ M JWJ-01-306 or DMSO vehicle for 24 hours. **J-M.** MIA PaCa-2 cells were treated with 10 μ M JWJ-01-306 or DMSO vehicle for 24 hours prior to incubation with media containing 10 μ M JWJ-01-306 or DMSO vehicle and ¹³C-labeled glucose or glutamine for 24 hours, and targeted metabolomics analysis was performed by GC-MS/MS. Metabolite abundances and abundance ratios are adjusted for metabolite quantity in parallel-run standards and recovery of the internal norvaline standard in each condition. Abundance data are depicted as the average $\pm$ S. D. of $n = 6$ biological replicates. Mass isotopomer distribution (MID) data, quantified by fractional incorporation, are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates. Significance was determined by performing unpaired t-tests between the DMSO and JWJ-01-306 conditions. **N.** MIA PaCa-2 cells were treated with 100 nM IACS-010759 for 24 hours, 10 μ M JWJ-01-306 for 3 days, or DMSO vehicle for 3 days. These cells were subsequently fixed onto #1.5 coverslips and subjected to a fluorescent dye-based staining protocol using MitoTracker® Deep Red FM. The stained cells were then imaged using a Zeiss LSM980 equipped with an Airyscan detector. **O.** 3D mitochondrial morphology in stained MIA PaCa-2 cells was analyzed and quantified using the Mitochondria Analyzer Fiji plugin. Data are depicted as the average $\pm$ S. D. of at least $n = 20$ biological replicates. Significance was determined by performing Brown-Forsythe and Welch ANOVA tests with Dunnett's T3 test for multiple comparisons. **A, C, G.** OCR and ECAR were measured by a mitochondrial stress test protocol using a Seahorse XFe24 analyzer. From left to right, gray dotted lines indicate additions of oligomycin, BAM15, and rotenone/antimycin A. Data are depicted as the average $\pm$ S. D. of $n = 2$ biological replicates. **B, D, H.** Data are depicted as the average $\pm$ S. D. of $n = 2$ biological replicates with $n = 3$ technical replicates each. **E-F, I.** Gene transcript abundance was measured by RT-qPCR. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates and are normalized to DMSO vehicle treatment controls, which are included as reference baselines.

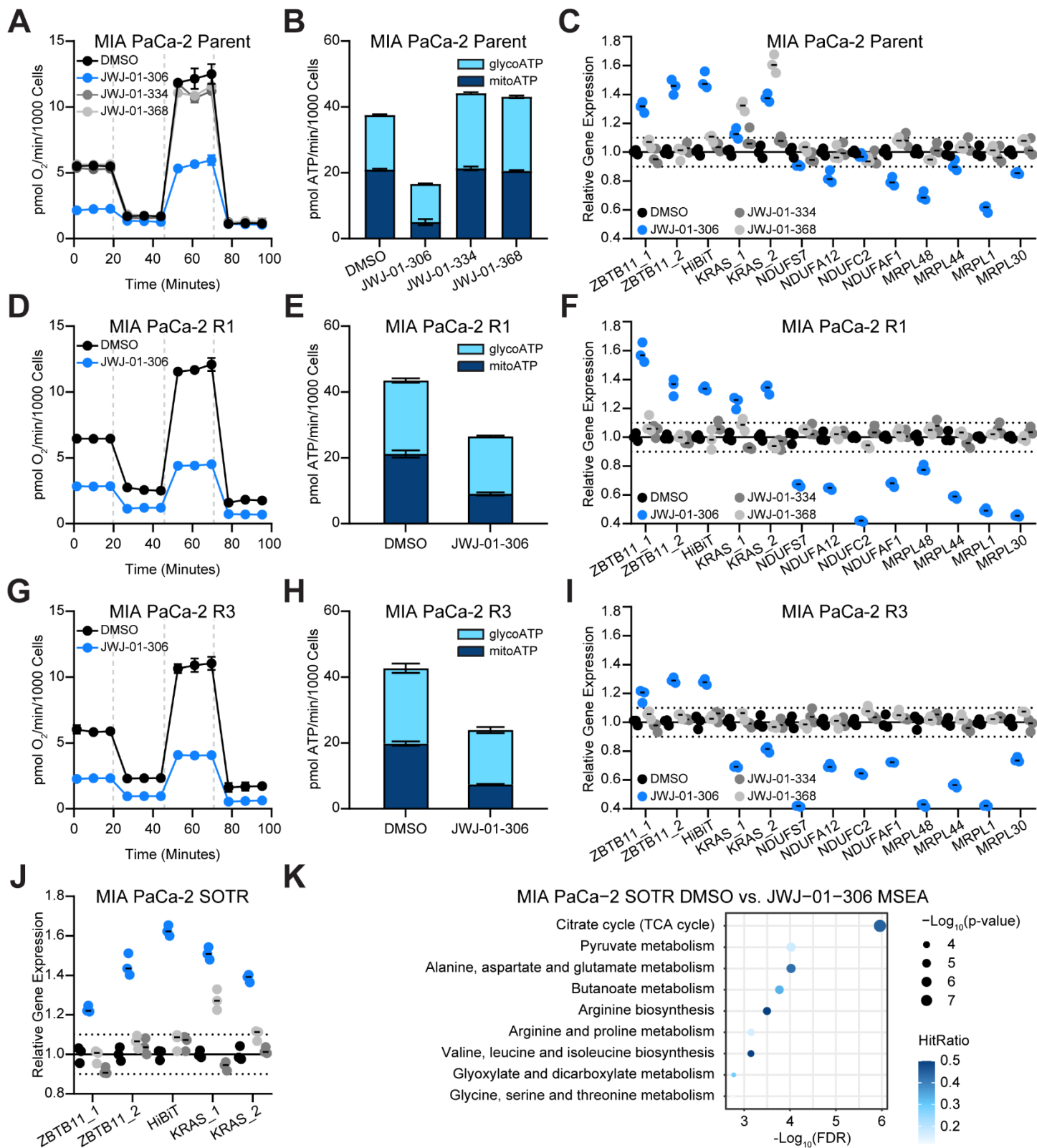


Figure S12 | Chemical depletion of ZBTB11 induces OXPHOS downregulation and reprogramming of mitochondrial metabolism in PDAC cells. **A, D, G.** MIA PaCa-2 cells were treated with 10 μ M of the indicated compound for 24 hours. OCR and ECAR were measured by a mitochondrial stress test protocol using a Seahorse XFe24 analyzer. From left to right, gray dotted lines indicate additions of oligomycin, BAM15, and rotenone/antimycin A. Data are depicted as the average $\pm$ S. D. of $n = 2$ biological replicates. **B, E, H.** OCR and ECAR measured during the mitochondrial stress test were used to calculate glycoATP and mitoATP. Data are depicted as the average $\pm$ S. D. of $n = 2$ biological replicates with $n = 3$ technical replicates each. **C, F, I-J.** MIA PaCa-2 cells were treated with 10 μ M of the indicated compound for 24 hours. Gene transcript abundance was measured by RT-qPCR. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates and are normalized to DMSO vehicle treatment controls, which are included as reference baselines. **K.** MIA PaCa-2 cells were treated with 10 μ M JWJ-01-306 or DMSO vehicle for 24 hours prior to incubation with media containing 10 μ M JWJ-01-306 or DMSO vehicle and ^{13} C-labeled glucose or glutamine for 24 hours, and targeted metabolomics analysis was performed by GC-MS/MS. Metabolite abundances from these conditions were input to MSEA and clustered by GOBP/KEGG pathway. Representative top-scoring pathways are shown here with corresponding significance and enrichment (HitRatio) scores of $n = 6$ biological replicates.

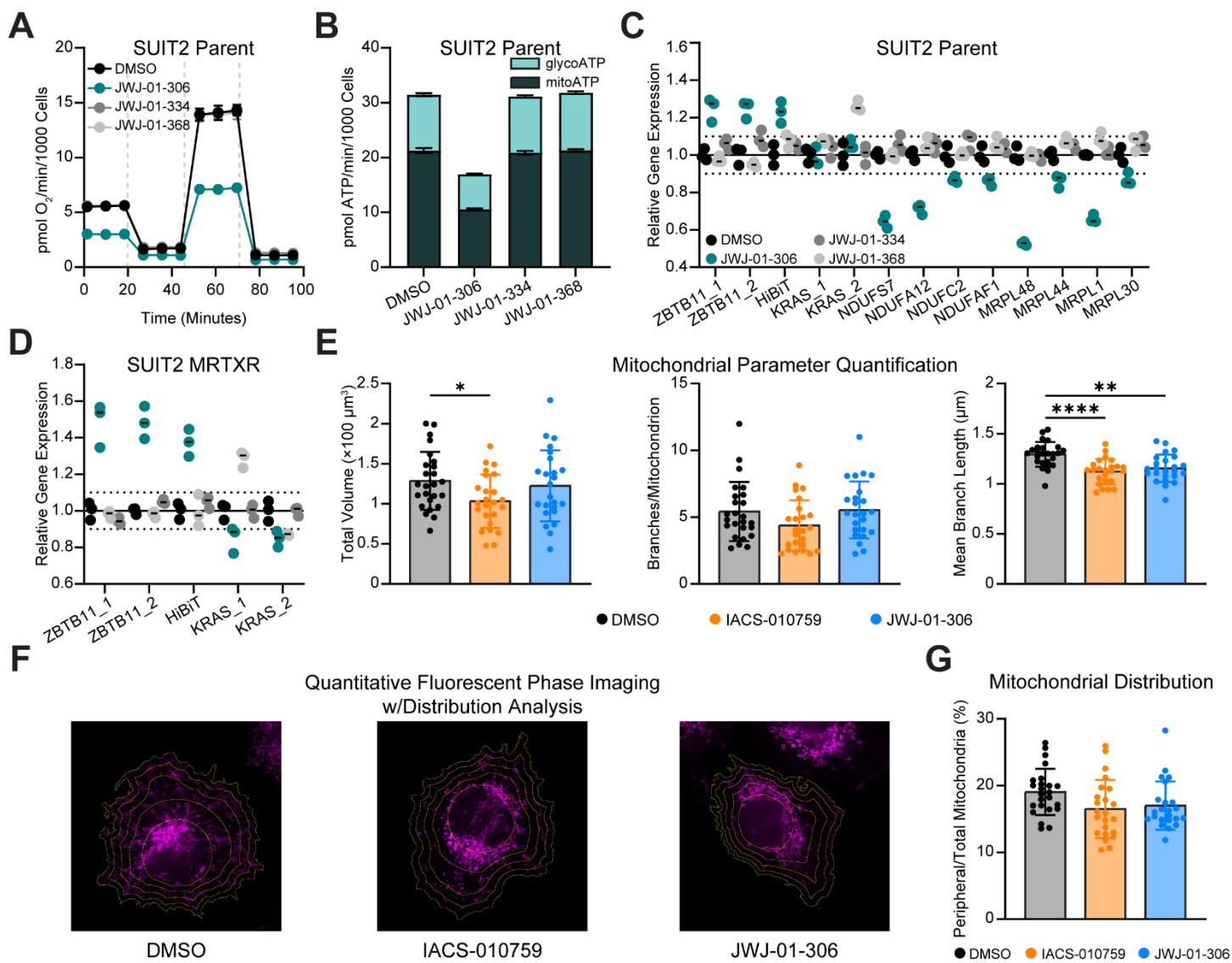


Figure S13 | Chemical depletion of ZBTB11 induces OXPHOS downregulation and reprogramming of mitochondrial metabolism in PDAC cells. **A.** SUIT2 cells were treated with 10 μ M of the indicated compound for 24 hours. OCR and ECAR were measured by a mitochondrial stress test protocol using a Seahorse XFe24 analyzer. From left to right, gray dotted lines indicate additions of oligomycin, BAM15, and rotenone/antimycin A. Data are depicted as the average $\pm$ S. D. of $n = 2$ biological replicates. **B.** OCR and ECAR measured during the mitochondrial stress test were used to calculate glycoATP and mitoATP. Data are depicted as the average $\pm$ S. D. of $n = 2$ biological replicates with $n = 3$ technical replicates each. **C-D.** SUIT2 cells were treated with 10 μ M of the indicated compound for 24 hours. Gene transcript abundance was measured by RT-qPCR. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates and are normalized to DMSO vehicle treatment controls, which are included as reference baselines. **E.** 3D mitochondrial morphology in stained MIA PaCa-2 cells was analyzed and quantified using the Mitochondria Analyzer Fiji plugin. Data are depicted as the average $\pm$ S. D. of at least $n = 20$ biological replicates. Significance was determined by performing Brown-Forsythe and Welch ANOVA tests with Dunnett's T3 test for multiple comparisons. **F.** MIA PaCa-2 cells were treated with 100 nM IACS-010759 for 24 hours, 10 μ M JWJ-01-306 for 3 days, or DMSO vehicle for 3 days. These cells were subsequently fixed onto #1.5 coverslips and subjected to a fluorescent dye-based staining protocol using MitoTracker® Deep Red FM. The stained cells were then imaged using a Zeiss LSM980 equipped with an Airyscan detector. Cell and nucleus outlines defining the regions were extracted from transmission and DAPI images; respectively. **G.** Distribution of mitochondria in stained MIA PaCa-2 cells was quantified using the ringIntensityDistribution Fiji macro. Data are depicted as the average $\pm$ S. D. of at least $n = 20$ biological replicates. Significance was determined by performing Brown-Forsythe and Welch ANOVA tests with Dunnett's T3 test for multiple comparisons.

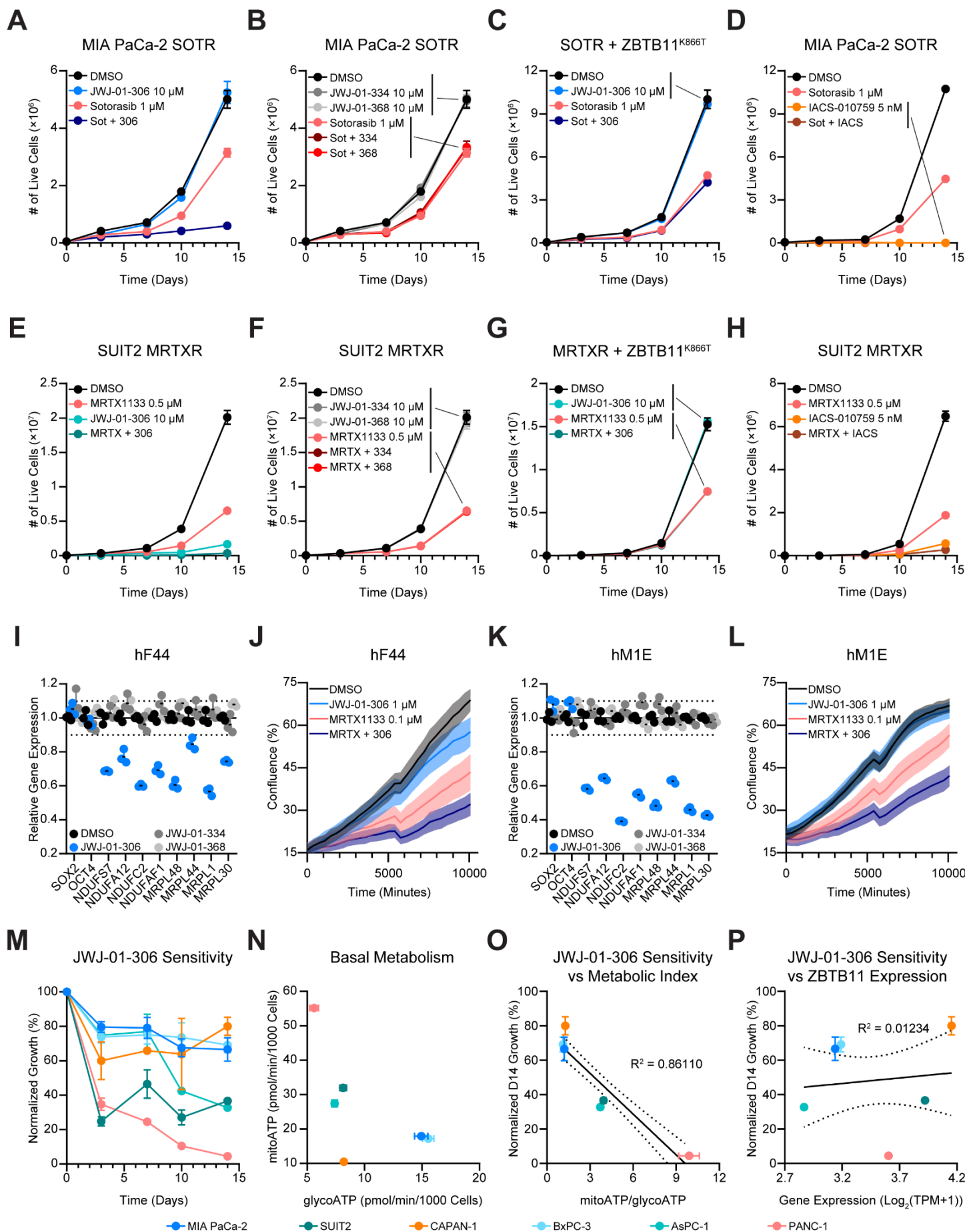


Figure 4 | ZBTB11 degradation synergizes with K-Rasi in PDAC cells and patient-derived organoids. A-D. MIA PaCa-2 cells were treated with the indicated compound(s) over a 14-day period. **E-H.** SUI2 cells were treated with the indicated compound(s) over a 14-day period. **I.** hF44 organoids derived from a drug-naïve PDAC patient were treated with 10 μ M of the indicated compound for 24 hours. **J.** hF44 organoids were treated with the indicated compound(s) over a 14-day period. **K.** hM1E organoids derived from a multiple drug-resistant PDAC patient (gemcitabine/Nab/paclitaxel/FOLFIRINOX resistant) were treated with 10 μ M of the indicated compound for 24 hours. **L.** hM1E organoids were treated with the indicated compound(s) over a 14-day period. **M.** A panel of six PDAC cell lines were treated with 10 μ M JWW-01-306 or DMSO vehicle over a 14-day period, and cumulative number of live cells was measured by trypan blue exclusion. Data are depicted as the number of live cells treated with JWW-01-306 divided by the number of live cells treated with DMSO expressed as a percentage and represent the average $\pm$ S. D. of $n = 3$ biological replicates. **N.** A panel of six PDAC cell lines were subjected to a mitochondrial stress test protocol using a Seahorse XFe24 analyzer. OCR and ECAR measured during the mitochondrial stress test were used to calculate glycoATP and mitoATP. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates. **O.** Sensitivity to JWW-01-306, shown as the number of live cells treated with JWW-01-306 divided by the number of live cells treated with DMSO at the end of a 14-day period expressed as a percentage, plotted against the metabolic index, calculated by dividing mitoATP by glycoATP, for the panel of six PDAC cell lines. **P.** Sensitivity to JWW-01-306, shown as the number of live cells treated with JWW-01-306 divided by the number of live cells treated with DMSO at the end of a 14-day period expressed as a percentage, plotted against ZBTB11 transcript abundance determined by the latest DepMap gene expression dataset (Expression Public 24Q4). **A-H.** Cumulative number of live cells was measured by trypan blue exclusion. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates. **I, K.** Gene transcript abundance was measured by RT-qPCR. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates and are normalized to DMSO vehicle treatment controls, which are included as reference baselines. **J, L.** Confluence was measured by real-time brightfield imaging using a Cellcyte instrument. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates with $n = 4$ technical replicates each. **O-P.** The line of best fit, 95% confidence intervals, and coefficient of determination (R^2) are shown from the calculated linear regression model.

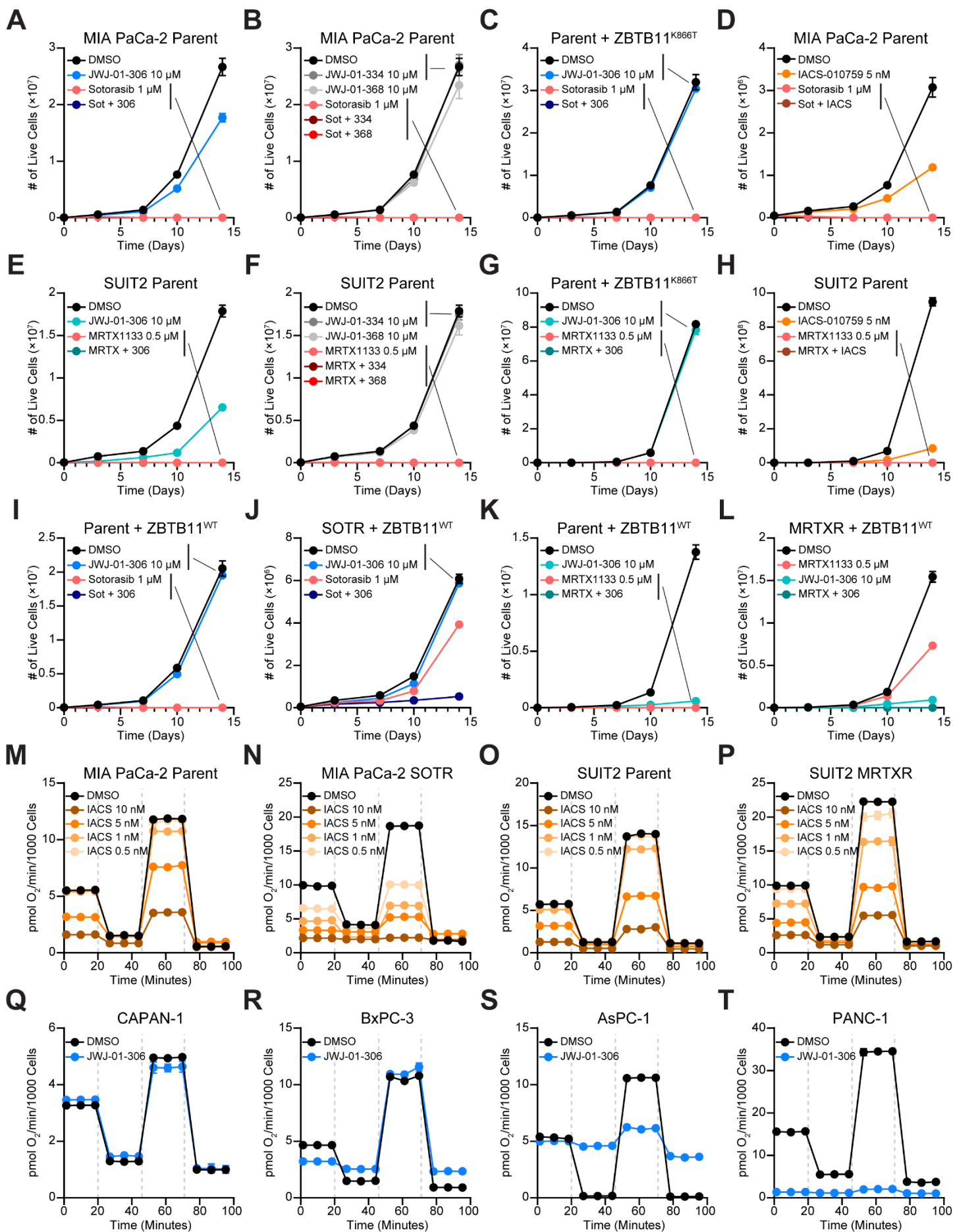
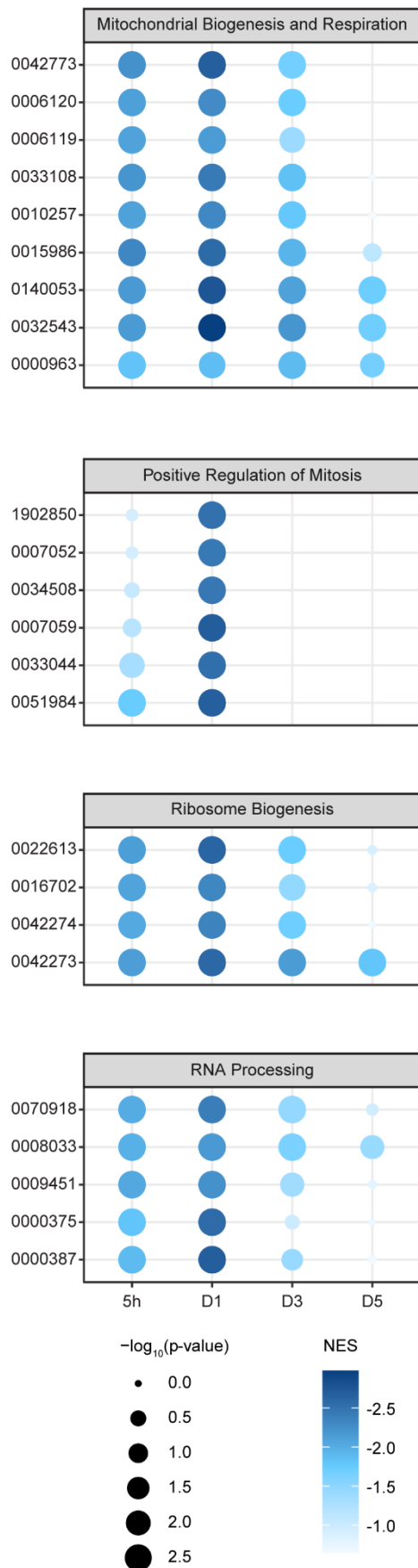


Figure S14 | Parental PDAC cells are highly sensitive to K-Rasi and direct OXPHOS inhibition. A-D. MIA PaCa-2 cells were treated with the indicated compound(s) over a 14-day period. **E-H.** SUI2 cells were treated with the indicated compound(s) over a 14-day period. **I-J.** MIA PaCa-2 cells transfected with NLuc-ZBTB11^{WT} were treated with the indicated compound(s) over a 14-day period. **K-L.** SUI2 cells transfected with NLuc-ZBTB11^{WT} were treated with the indicated compound(s) over a 14-day period. **M-P.** MIA PaCa-2 and SUI2 cells were treated with the indicated concentration of IACS-010759 for 24 hours. **Q-T.** CAPAN-1, BxPC-3, AsPC-1, and PANC-1 PDAC cell lines were treated with 10 μ M JWJ-01-306 or DMSO vehicle for 24 hours. **A-L.** Cumulative number of live cells was measured by trypan blue exclusion. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates. **M-T.** OCR and ECAR were measured by a mitochondrial stress test protocol using a Seahorse XFe24 analyzer. From left to right, gray dotted lines indicate additions of oligomycin, BAM15, and rotenone/antimycin A. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates.

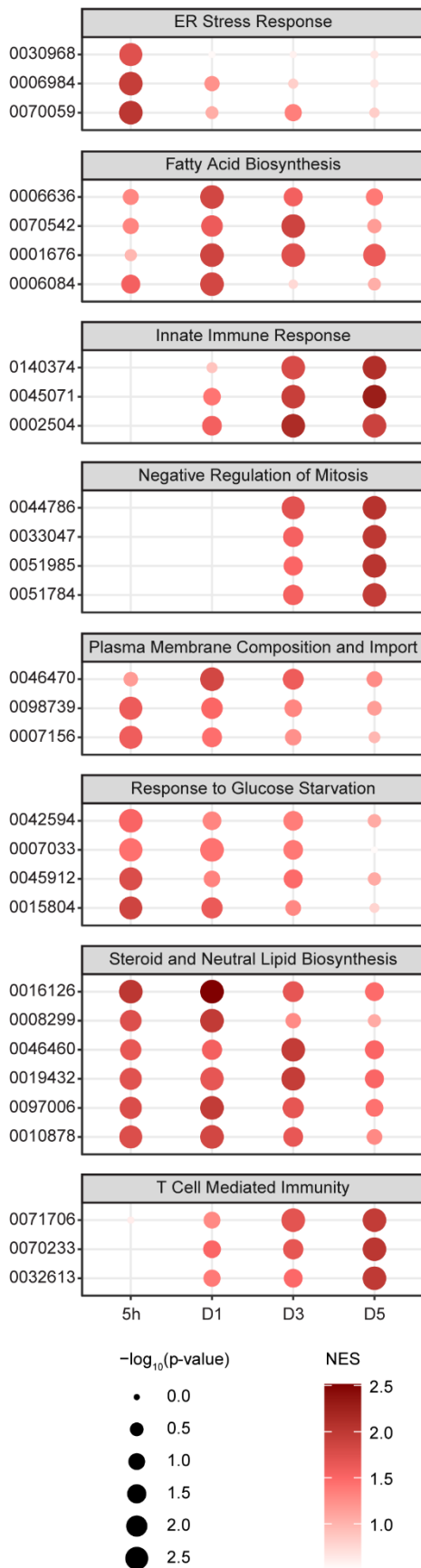
A

Sotorasib + JWJ-01-306
Downregulated GO Pathways



B

Sotorasib + JWJ-01-306
Upregulated GO Pathways



C

D3 Global Proteomics

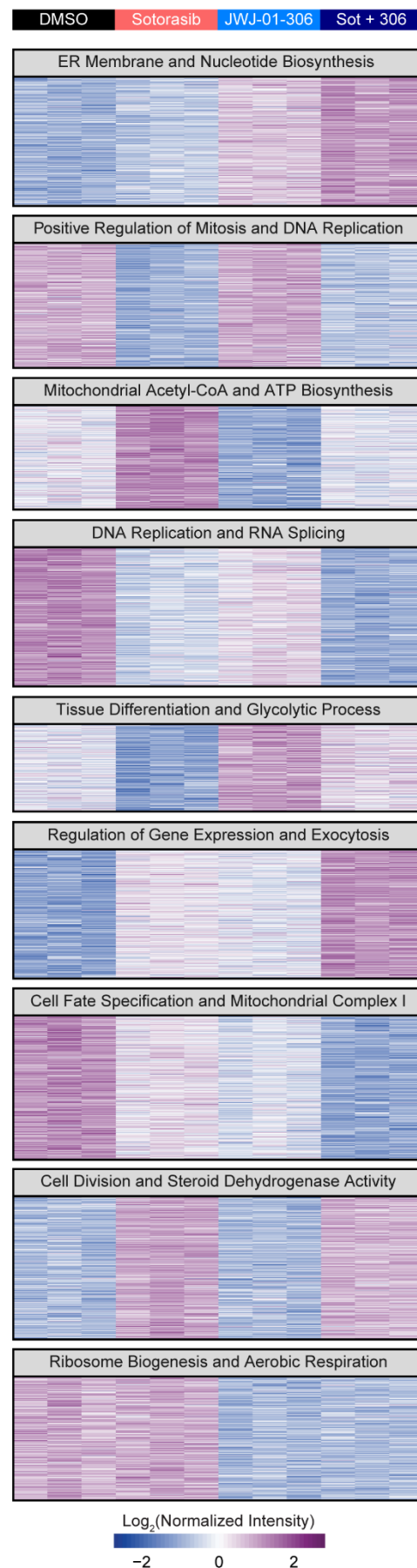


Figure 5 | ZBTB11 degradation induces transcriptome- and proteome-wide changes in mitochondria, metabolism, and cell cycle. A-B. MIA PaCa-2 SOTR cells were treated with 1 μ M sotorasib + 10 μ M JWJ-01-306 or 1 μ M sotorasib for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative top-scoring pathways are shown here as GOBP codes with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates. **C.** MIA PaCa-2 SOTR cells were treated with 1 μ M sotorasib, 10 μ M JWJ-01-306, 1 μ M sotorasib + 10 μ M JWJ-01-306, or DMSO vehicle for 3 days, and global proteomics analysis was performed by LC-MS/MS. Protein abundances were filtered by one-way ANOVA (FDR < 0.05) and clustered using K-nearest neighbors clustering. Each protein cluster was then input to GSEA for pathway enrichment analysis using GO and KEGG pathways. Protein abundances and associated clusters are shown here with corresponding fold change values of $n = 3$ biological replicates.

Sotorasib + JWJ-01-306
Downregulated GO Pathways

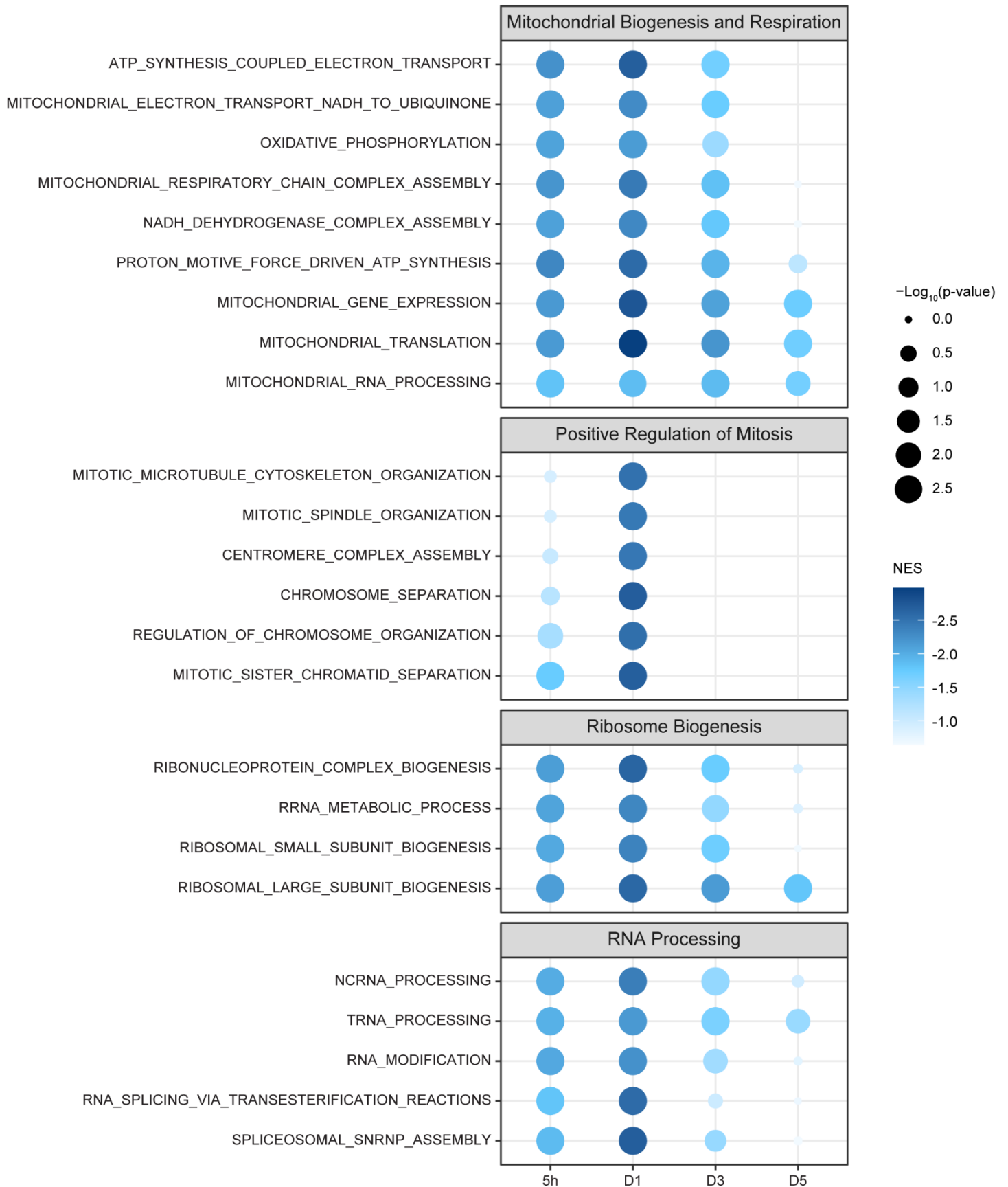


Figure S15 | Chemical depletion of ZBTB11 induces transcriptome-wide downregulation of mitotic processes, RNA processing, and mitochondrial and ribosome biogenesis. MIA PaCa-2 SOTR cells were treated with 1 μ M sotorasib + 10 μ M JWJ-01-306 or 1 μ M sotorasib for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative top-scoring pathways are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

Sotorasib + JWJ-01-306
Upregulated GO Pathways

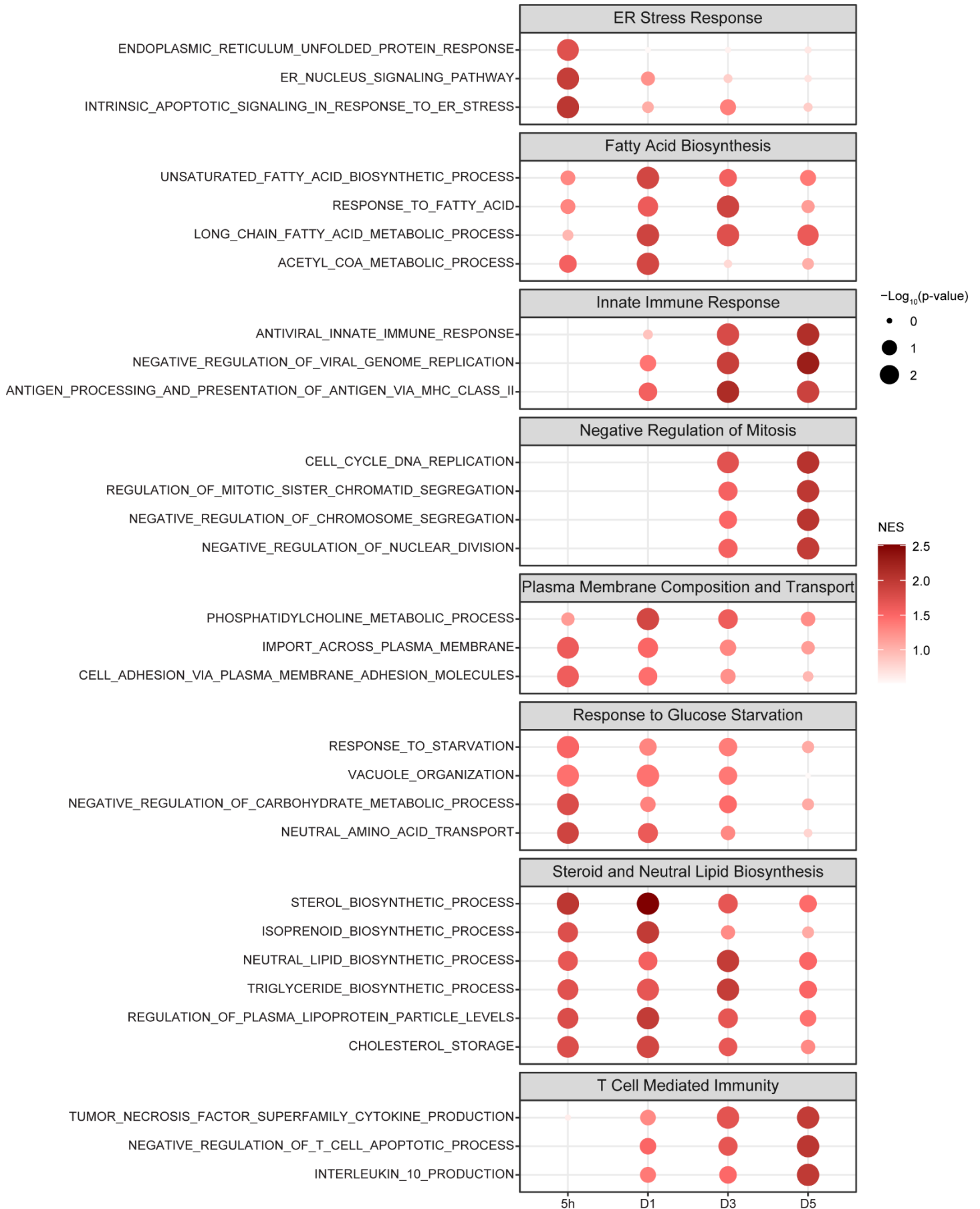


Figure S16 | Chemical depletion of ZBTB11 induces transcriptome-wide upregulation of mitotic blockage, cellular stress and immune responses, and fatty acid and lipid biosynthesis. MIA PaCa-2 SOTR cells were treated with 1 μ M sotorasib + 10 μ M JWJ-01-306 or 1 μ M sotorasib for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative top-scoring pathways are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

JWJ-01-306
Downregulated GO Pathways

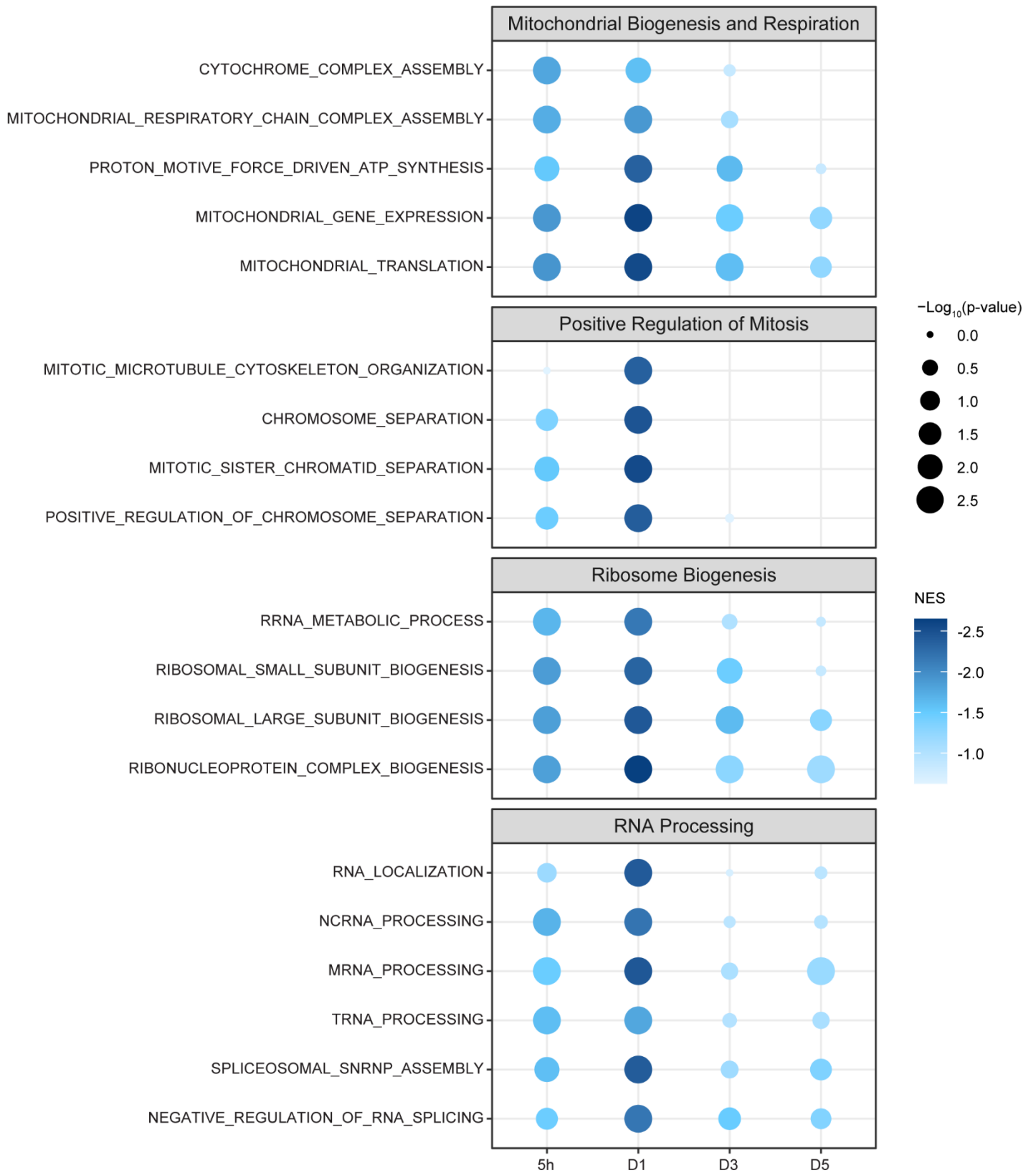


Figure S17 | Chemical depletion of ZBTB11 induces transcriptome-wide downregulation of mitotic processes, RNA processing, and mitochondrial and ribosome biogenesis. MIA PaCa-2 SOTR cells were treated with 10 μ M JJJ-01-306 or DMSO vehicle for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative top-scoring pathways are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

JWJ-01-306
Upregulated GO Pathways

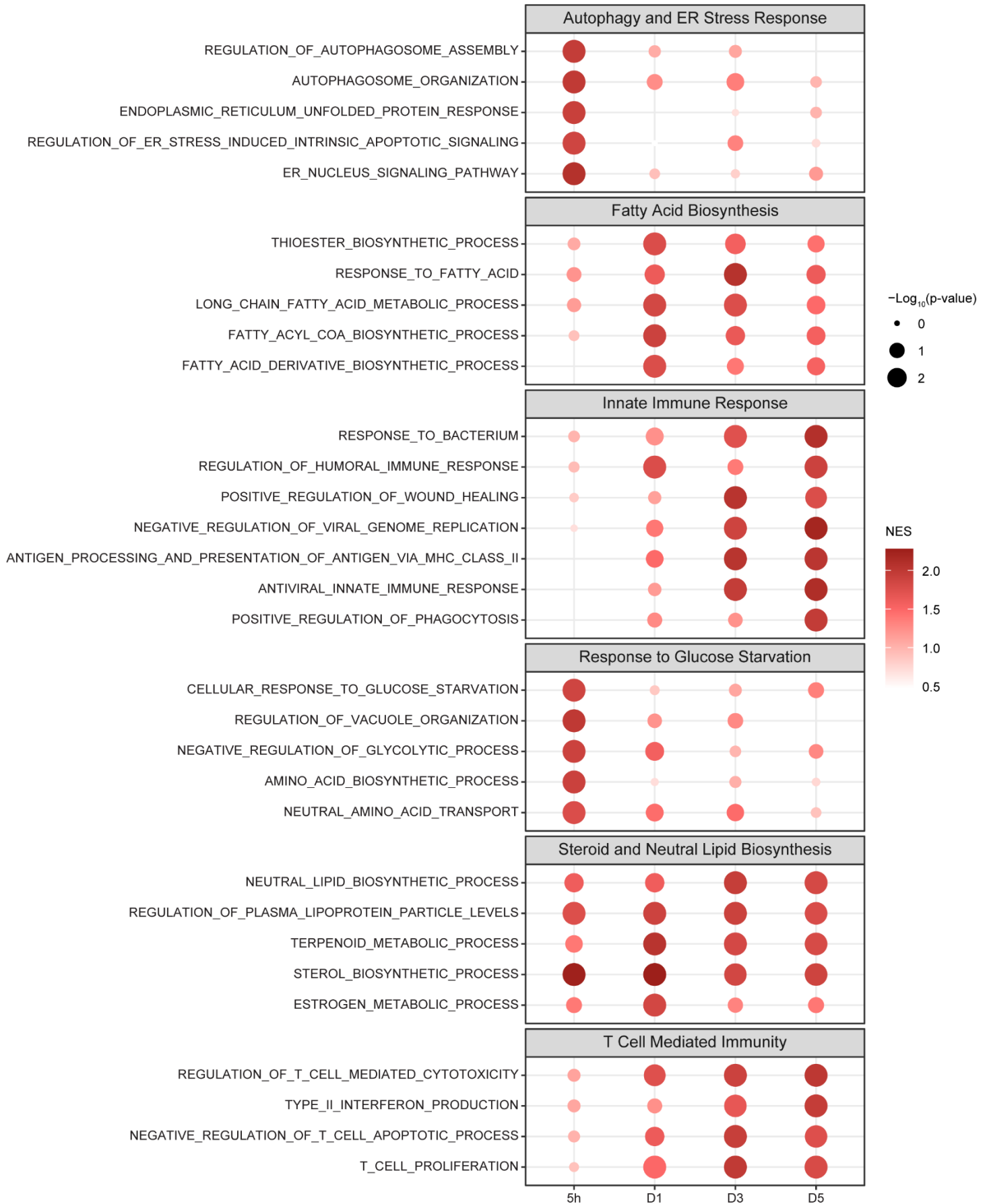


Figure S18 | Chemical depletion of ZBTB11 induces transcriptome-wide upregulation of mitotic blockage, cellular stress and immune responses, and fatty acid and lipid biosynthesis. MIA PaCa-2 SOTR cells were treated with 10 μ M JWJ-01-306 or DMSO vehicle for the indicated amount of time, and global transcriptomics analysis was performed by RNA-seq. Transcript abundances from these two conditions were input to GSEA and clustered by Gene Ontology Biological Process (GOBP) pathway. Representative top-scoring pathways are shown here with corresponding significance and nominal enrichment scores (NES) of $n = 3$ biological replicates.

D3 Global Proteomics

DMSO Sotorasib JWJ-01-306 Sot + 306

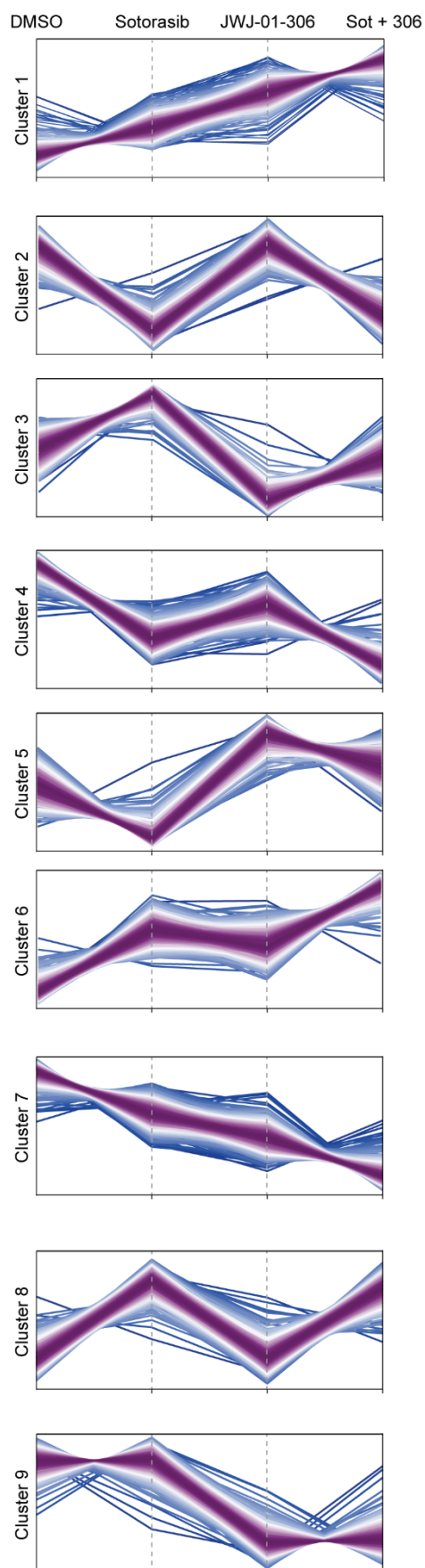
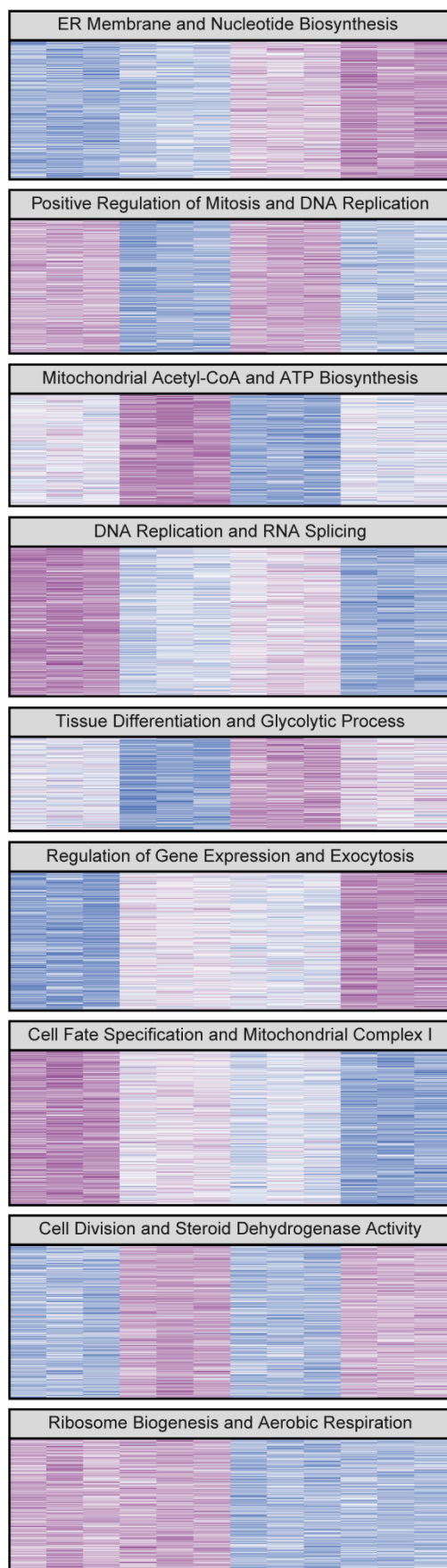


Figure S19 | Sotorasib and JWJ-01-306 as single agents and in combination alter the cellular proteome to effect observed phenotypes. MIA PaCa-2 SOTR cells were treated with 1 μ M sotorasib, 10 μ M JWJ-01-306, 1 μ M sotorasib + 10 μ M JWJ-01-306, or DMSO vehicle for 3 days, and global proteomics analysis was performed by LC-MS/MS. Protein abundances were filtered by one-way ANOVA (FDR < 0.05) and clustered using K-nearest neighbors clustering. Each protein cluster was then input to GSEA for pathway enrichment analysis using GO and KEGG pathways. The abundance of each protein in a given cluster is visualized as a line plot and is color-coded according to its distance from the trend of the cluster center, ranging from purple (close) to blue (far). Protein abundances and associated clusters are shown here with corresponding fold change values of $n = 3$ biological replicates.

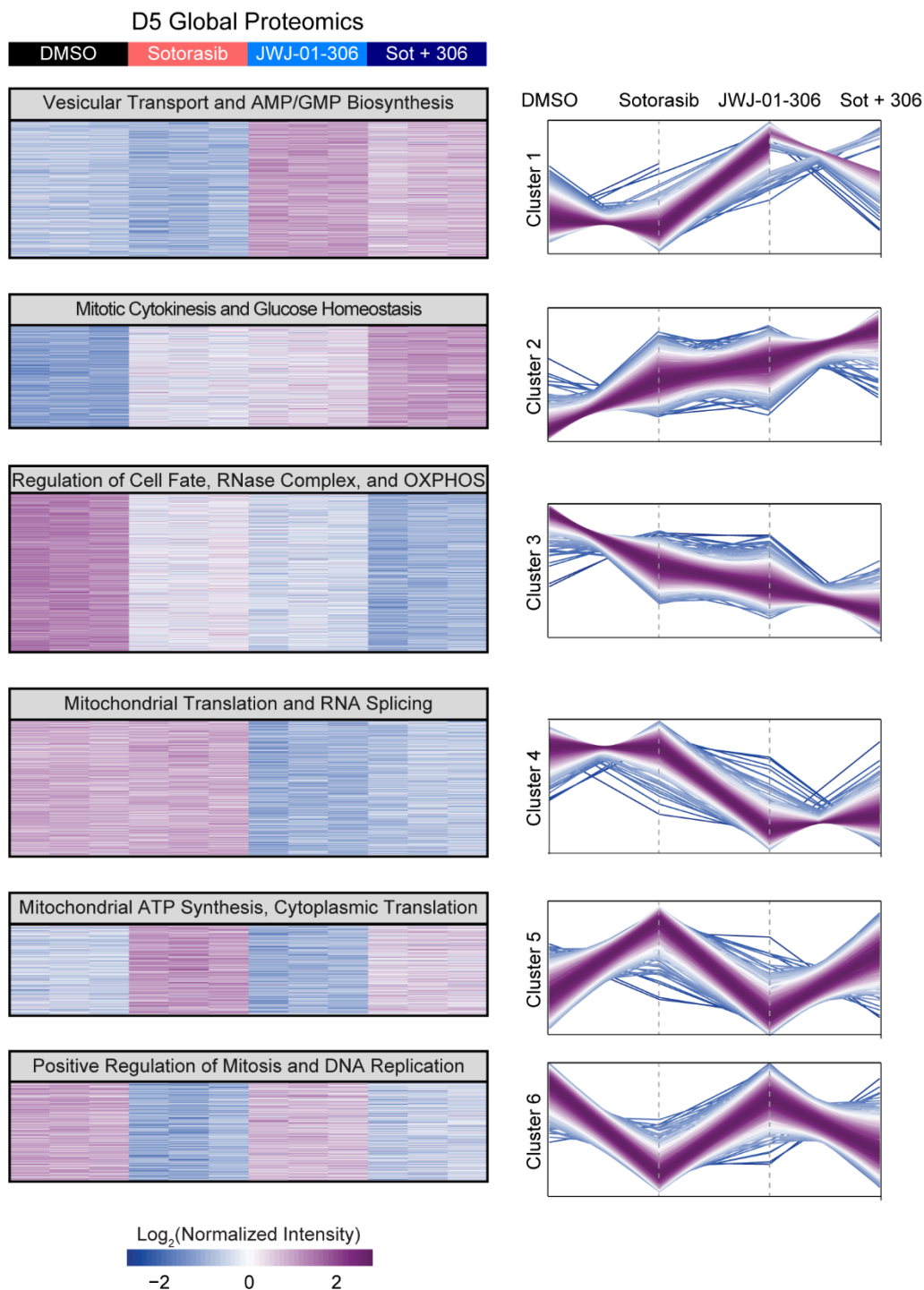


Figure S20 | Sotorasib and JWJ-01-306 as single agents and in combination alter the cellular proteome to effect observed phenotypes. MIA PaCa-2 SOTR cells were treated with 1 μ M sotorasib, 10 μ M JWJ-01-306, 1 μ M sotorasib + 10 μ M JWJ-01-306, or DMSO vehicle for 5 days, and global proteomics analysis was performed by LC-MS/MS. Protein abundances were filtered by one-way ANOVA (FDR < 0.05) and clustered using K-nearest neighbors clustering. Each protein cluster was then input to GSEA for pathway enrichment analysis using GO and KEGG pathways. The abundance of each protein in a given cluster is visualized as a line plot and is color-coded according to its distance from the trend of the cluster center, ranging from purple (close) to blue (far). Protein abundances and associated clusters are shown here with corresponding fold change values of $n = 3$ biological replicates.

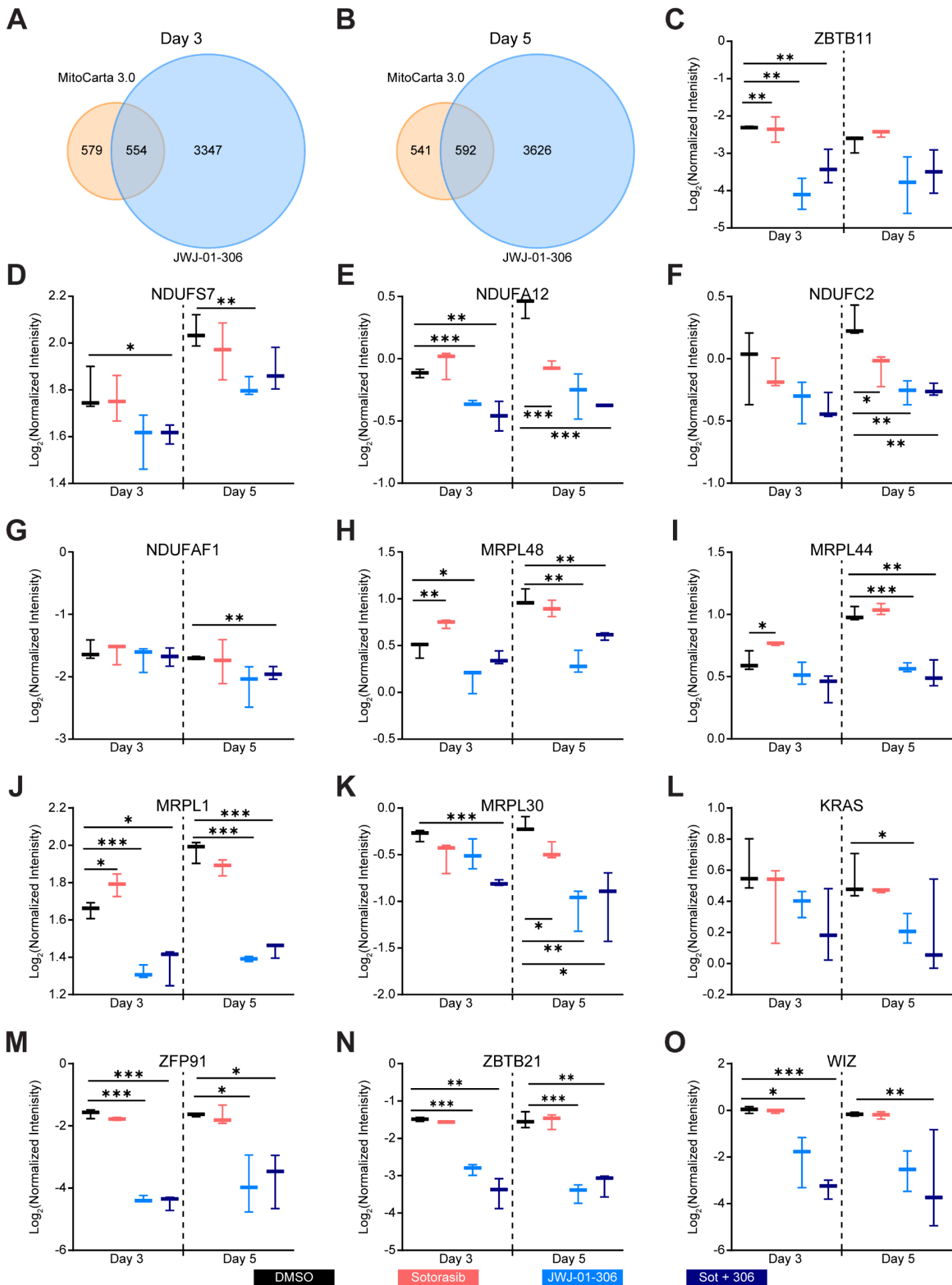


Figure S21 | Proteomic changes upon JWJ-01-306 treatment are enriched in mitochondrial proteins. A-B. Significant protein hits from the JWJ-01-306 treatment condition of the 3-day global proteomics (Fig. S19) and 5-day global proteomics (Fig. S20) were compared with protein entries in the MitoCarta 3.0 database. **C-O.** Protein abundances of ZBTB11, genes regulated by ZBTB11, and select other gene products in each treatment condition for both 3- and 5-day global proteomics experiments. Data are depicted as the median value, with the bounds indicating the 25th and 75th percentiles. Significance was determined by performing two-sample moderated t-tests with two-tailed distribution.

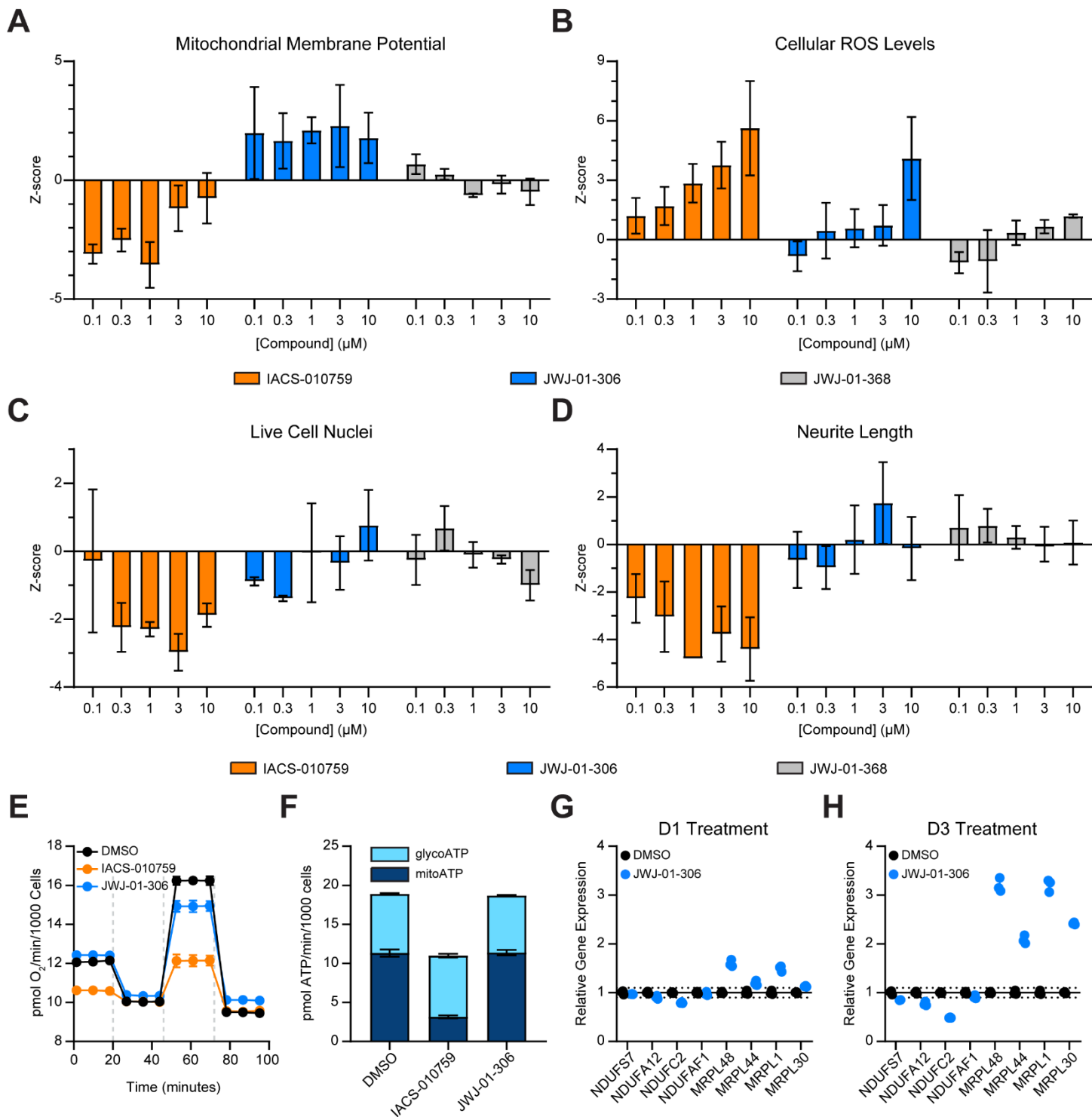


Figure 6 | ZBTB11 degradation is better tolerated than direct OXPHOS inhibition in iPSC-derived neurons. **A-B.** iPSC-derived neurons were treated with the indicated concentration of compound for 24 hours, and mitochondrial membrane potential or cellular reactive oxygen species (ROS) levels were quantified by multiplexing TMRE, CellRox™ Deep Red, MitoTracker® Green, and Hoechst dyes in a high-throughput live-cell imaging assay. Data are depicted as the average $\pm$ S. D. of $n = 3000$ cells split amongst $n = 3$ separate biological replicates. **C-D.** iPSC-derived neurons were treated with the indicated concentration of compound for 3 days, and live-cell nuclei or neurite branch length were quantified by multiplexing TuJ1 and Hoechst dyes in a high-throughput live-cell imaging assay. Data are depicted as the average $\pm$ S. D. of $n = 3000$ cells split amongst $n = 3$ separate biological replicates. **E.** iPSC-derived neurons were treated with 100 nM IACS-010759, 10 μ M JWW-01-306, or DMSO vehicle for 24 hours and were subjected to a mitochondrial stress test protocol using a Seahorse XFe24 analyzer. From left to right, gray dotted lines indicate additions of oligomycin, BAM15, and rotenone/antimycin A. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates. **F.** OCR and ECAR measured during the mitochondrial stress test were used to calculate glycoATP and mitoATP. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates with $n = 3$ technical replicates each. **G-H.** iPSC-derived neurons were treated with 10 μ M JWW-01-306 or DMSO vehicle for 1 or 3 days, and gene transcript abundance was measured by RT-qPCR. Data are depicted as the average $\pm$ S. D. of $n = 3$ biological replicates and are normalized to DMSO vehicle treatment controls, which are included as reference baselines.

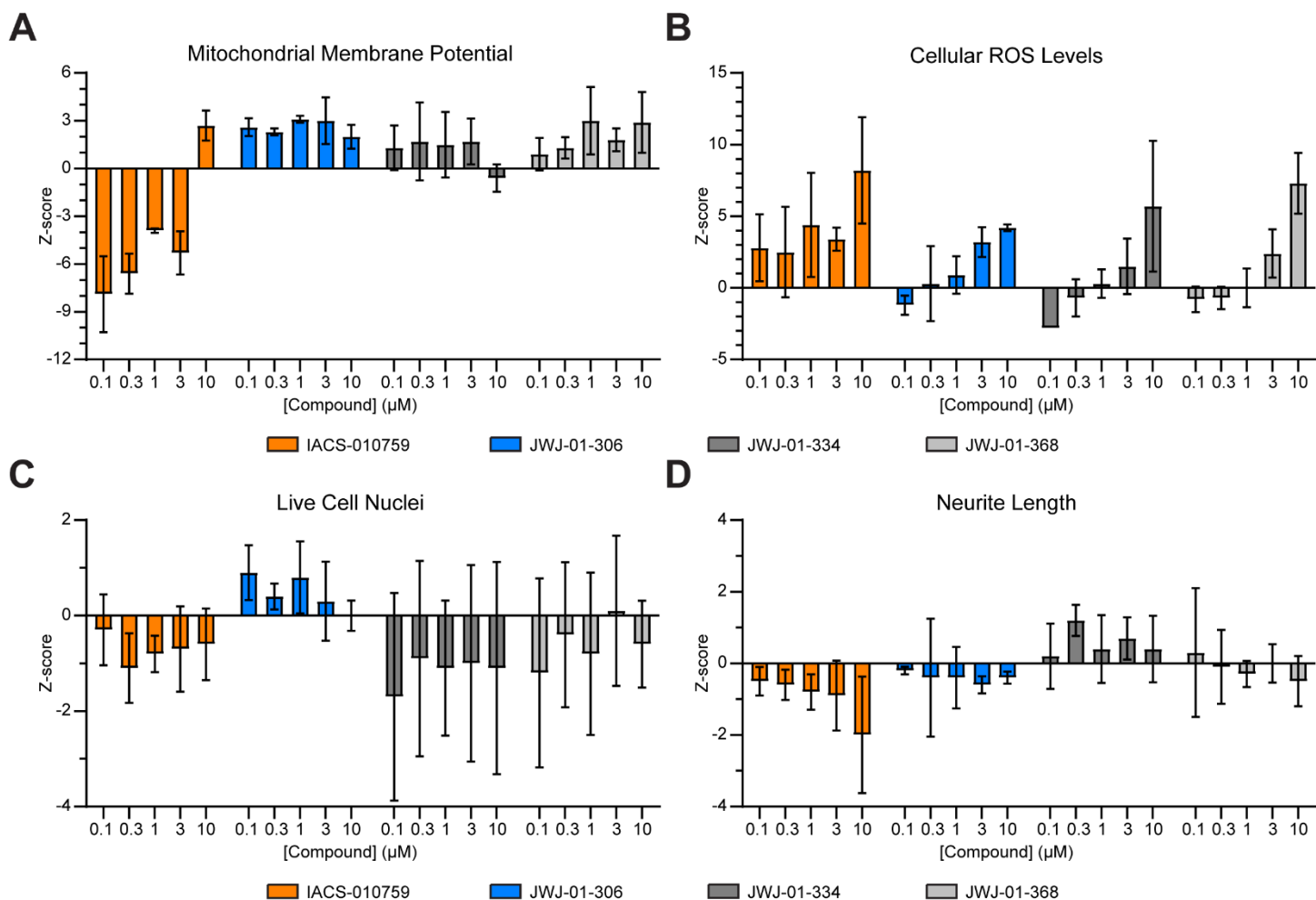


Figure S22 | ZBTB11 degradation is better tolerated than direct OXPHOS inhibition in iPSC-derived neurons. **A-B.** iPSC-derived neurons were treated with the indicated concentration of compound for 24 hours, and mitochondrial membrane potential or cellular reactive oxygen species (ROS) levels were quantified by multiplexing TMRE, CellRox™ Deep Red, MitoTracker® Green, and Hoechst dyes in a high-throughput live-cell imaging assay. Data are depicted as the average $\pm$ S. D. of $n = 3000$ cells split amongst $n = 3$ separate biological replicates. **C-D.** iPSC-derived neurons were treated with the indicated concentration of compound for 3 days, and live-cell nuclei or neurite branch length were quantified by multiplexing TuJ1 and Hoechst dyes in a high-throughput live-cell imaging assay. Data are depicted as the average $\pm$ S. D. of $n = 3000$ cells split amongst $n = 3$ separate biological replicates.

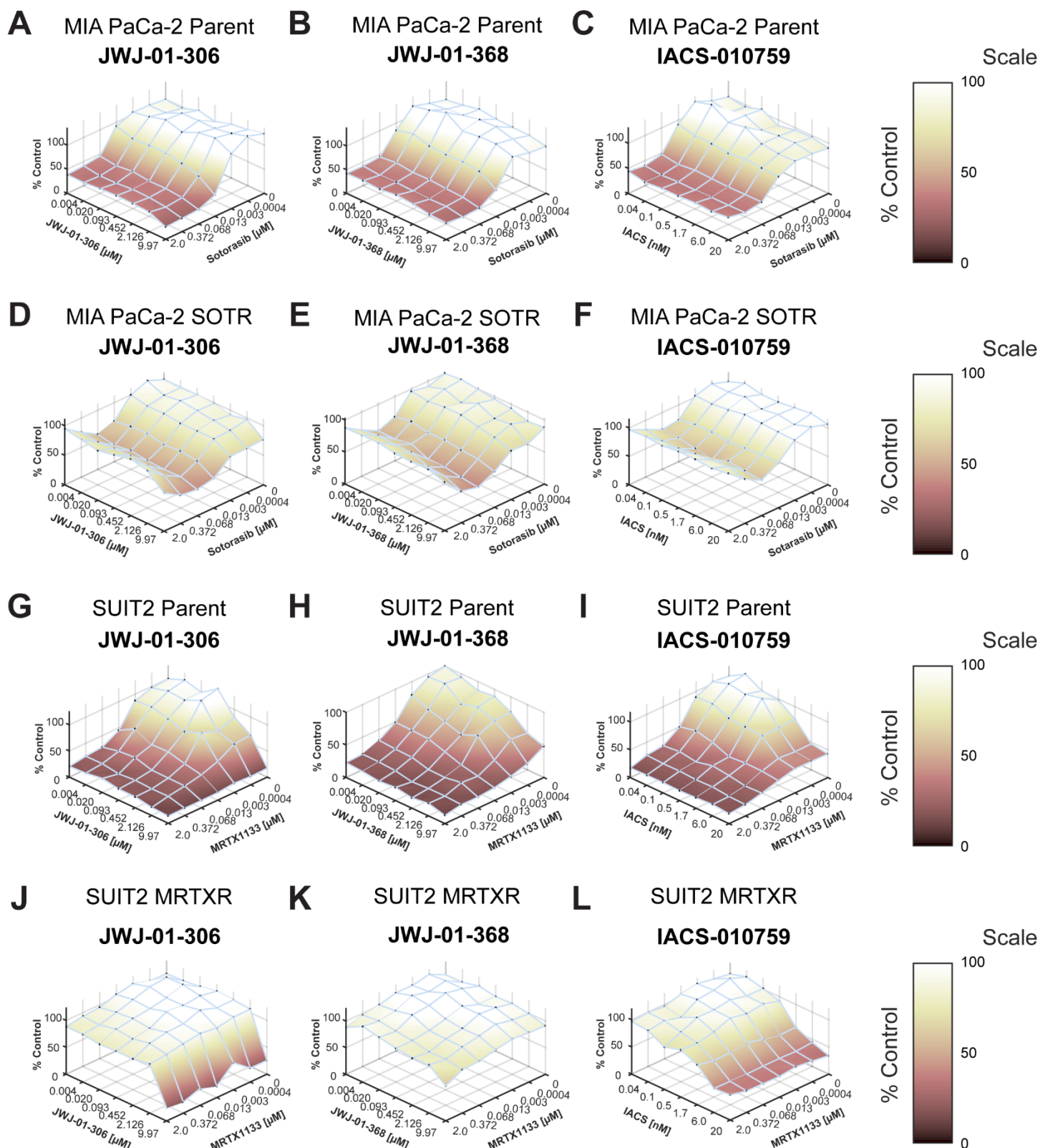


Figure S23 | ZBTB11 degradation and direct OXPHOS inhibition exhibit similar levels of growth inhibition on PDAC cells. A-F. MIA PaCa-2 cells were treated with the indicated concentrations of sotorasib and JWJ-01-306, JWJ-01-368, or IACS-01-0759 for 7 days, and cell viability was measured by CellTiterFluor™. Data are depicted as the average of $n = 2$ biological replicates. **G-L.** SUIT2 cells were treated with the indicated concentrations of MRTX1133 and JWJ-01-306, JWJ-01-368, or IACS-01-0759 for 7 days, and cell viability was measured by CellTiterFluor™. Data are depicted as the average of $n = 2$ biological replicates.