Supplementary Information

Supplementary Materials and methods

Plasmid constructs. pLEX_305-N-dTAG-CDK7 wt and pLEX_305-N-dTAG-CDK7 C312S were gifts from Nathanael S. Gray (Nabet B., 2020). To generate lentiCRISPR v2-Blast-(sgGFP, sgCDK7#3, sgCDK7#5 and sgCDK7#7), sgCDK7 were cloned into lentiCRISPR v2-Blast vector (Addgene, #98293). pCDH-MSCV-T2A-puro, pCDH-MSCV-T2A-puro-Flag-MYC WT and pCDH-MSCV-T2A-puro-Flag-MYC T58A were generously given by Dr. Shideng Bao (Cleveland Clinic, Cleveland, OH).

Immunoblotting. MM cells were lysed with RIPA lysis buffer (Boston Bio Products, #BP-115) supplemented with EDTA-free protease inhibitor cocktail (Thermo Fisher Scientific, #78440). Protein concentrations were determined by the BCA Protein Assay Kit (Thermo Fisher Scientific, #23227) and separated by SDS-PAGE. The following primary antibodies were employed in this study: CDK1 (Bethyl, #A303-663A), phospho-CDK1 (CST (Cell Signaling Technology), #2561), CDK2 (Bethyl, #A301-812A), phospho-CDK2 (CST, #9114), CDK4 (CST, #12790), phospho-CDK4 (ABclonal, AP-0593), CDK7 (Cell Signaling Technology, #2916), cyclin H (CST, #2927), MAT1 (Santa Cruz Biotechnology, sc-13142), Rb (Santa Cruz Biotechnology, sc-73598), phospho-Rb (s780) (CST, #8180), phospho-Rb (s795) (CST, #9301), phospho-Rb (s807/811) (CST, #8516), PARP (CST, #9542), caspase3 (CST, #9662), HA-tag (CST, #3724), RNAPII (Biolegend, #664906), phosphor-RNAPII ser2 (CST, #13499), phosphor-RNAPII ser5 (Abcam, #ab5131), phosphor-RNAPII ser7 (CST, #13780), E2F1 (Santa Cruz Biotechnology, sc-251), MYC (Santa Cruz Biotechnology, sc-40), cyclin D1 (CST, #2978), cyclin B1 (CST, #4138), cyclin A1 (Santa Cruz Biotechnology, sc-271682), LDHA (CST, #3582), cyclin D1 (Cell Signaling Technology, #2978), cyclin B1 (Cell Signaling Technology, #4138), cyclin A1 (Santa Cruz Biotechnology, sc-271682). and HK2 (CST, #2867). GAPDH (CST, #2118), g-Tubulin (CST, #2128), and β-actin (CST, #4970) were used as loading controls.

Transfections. For the inducible CDK7 knockdown experiment, human TRIPZ CDK7 short hairpin RNA (shRNA) vectors were purchased from Dharmacon (Horizon Discovery). Packaged viral particles were used to infect myeloma cells with polybrene media (final concentration 8μg/mL). Infected MM cells were selected by 1 μg/mL puromycin (Gibco, A1113803). shRNA expression was induced by adding 0.5 μg/ml doxycycline. The efficacy of the induction was

confirmed by examining the TurboRFP-positive cells and by western blot analysis after 72h of induction with doxycycline. Functional studies were performed as described above. Sh#1: Clone ID, V2THS_150483; Sh#2: Clone ID, V2THS_150486; Sh#3: Clone ID, V2THS_150487. For the inducible CDK7 knockout experiment, inducible Cas9 and human CDK7 guide RNA plasmids were gifts from Charles Y. Lin. Packaged CDK7 knockout viral particles were used to infect myeloma cells with overexpressing inducible Cas9. Cas9 expression was induced by adding 0.5 μg/mL doxycycline. The efficacy of knockout was confirmed by western blot analysis after 4 days of induction. For the MYC siRNA (CST, #6341) transfection experiment, cells were transformed with the Neon Transfection system, according to the manufacturer's instructions.

Cell proliferation, viability, cell cycle and apoptosis assay. Cell viability was measured by CellTiter-Glo (CTG; Promega, G7572). The cell cycle was evaluated by flow cytometric analysis following propidium iodide (PI, BD Biosciences, 51-6621E) staining. Apoptosis was evaluated by flow cytometric analysis following Annexin-V (BD Biosciences, 550475) and DAPI (BD Biosciences, 564907) staining.

Extracellular flux analysis. The extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured with a Seahorse XFe96 Analyzer (Agilent). MM cells were treated with YKl-5-124 for 24h and then seeded in XFe96 cell culture microplates at 2×10^4 cells/well. A mitochondrial stress assay (Agilent, #103015-100) was performed in XF Base medium containing 10mM glucose, 1mM pyruvate and 2mM Glutamine. The following compounds were injected in sequential order: 1μM oligomycin (OM), 1μM Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP), and 0.5μM rotenone/antimycin (R/A). A glycolysis stress assay (Agilent, #103020-100) was performed in XF Base medium containing 2mM glutamine, and the following compounds were injected in sequential order: 10mM glucose (G), 2mM oligomycin(O), and 50mM 2-DG.

RNA-seq Analysis. Bulk RNA-sequencing experiments were performed on H929 and AMO1 cells. Cells were collected 24 hours after treatment. All RNA-seq analyses were performed with the HG19 ERCC human reference genome build with HG19 ERCC gene annotations. Data were preprocessed with BBDuk for single-end samples, aligned with HISAT2, quantified via Cuffquant 2.2, and normalized with cuffnorm. The top 3 replicates based on correlation were used for each timepoint/treatment. Inactive genes (featuring 0 samples with FPKM > 10) were filtered out for additional analysis. Raw and processed data have been deposited into GEO: GSE172445.

[3H]-thymidine incorporation assay. H929, AMO1, JJN3 and MM1S cells (10^4 cells/well) were cultured in 96-well plates at 37°C for 24 hours, in the presence or absence of YKL-5-124. Cells were pulsed with [3H]-thymidine (0.5 microCi/well) for 6 hours, harvested, and radioactivity was counted using the LKB Betaplate scintillation counter (Wallac). In coculture experiments, MM cells (10^4 cells/well) were incubated in BMSCs-coated 96-well plates in the presence or absence of YKL-5-124. All experiments were carried out in triplicate.

LDH and lactate assay. AMO1 cells were treated with YKL-5-124 (50 and 500nM) for 24 hours, harvested and used in an LDH assay kit (Abcam, #ab102526), according to the manufacturer's instructions. H929 and AMO1 cells were treated with YKl-5-124 for 6 and 24 hours, and the medium was collected, diluted 50 times, and used in the Lactate-Glo assay kit (Promega, #J5021), according to the manufacturer's instructions.

Tandem Mass Tag (TMT)-based proteomic analysis. Whole cell lysate from AMO1 cells treated with YKL-5-124 for 24 hours or H929-dTAG-CDK7wt cells treated with $dTAG^v$-1 for 24 hours were subjected to protein quantification with a microBCA assay. 200 ug of lysates were digested with Lys-C and Trypsin, and labeled with TMT tags, then subjected to LC-MS3 proteomic analysis. Peptide-spectrum match level was filtered to 1% false discovery rate (FDR) across the entire combined data set using the target-decoy strategy combined with linear discriminant analysis. P values for the protein differentiation analysis were adjusted for multiple hypothesis testing using the Benjamini-Hochberg method.

Quantitative RT-qPCR analysis. Total RNA extraction was done using the RNeasy Plus Mini Kit (QIAGEN, #74136) according to the manufacturer's instructions. The extracted RNA was reverse transcribed into cDNA using the SuperScript VILO cDNA Synthesis Kit (Thermo Fisher, #11754050) according to the manufacturer's instructions. The obtained cDNA was diluted and mixed with Taqman Fast Advanced Master Mix (Applied Biosystems, #4444557). Relative expression was calculated using the comparative delta delta (Ct) method.

RNA-seq. Libraries for sequencing were prepared with the Lexogen QuantSeq 3' mRNA-Seq Library Prep Kit FWD (SKU: 015.96). The libraries were quantified with the NEBNext Library Quant Kit for Illumina (NEB E7630L), pooled, and sequenced with the Illumina Nextseq 500 with single-end 75 base pair reads. RNA-seq analysis was performed via the HG19 ERCC human reference genome build with HG19 ERCC gene annotations. Data were preprocessed with BBDuk for single-end samples, aligned with HISAT2, quantified via Cuffquant 2.2, and normalized with

cuffnorm. Top 3 replicates based on correlation were used for each timepoint/treatment. Inactive genes (featuring 0 samples with FPKM > 10) were filtered out for additional analysis.

Gene Set Enrichment Analysis. Gene set enrichment analysis (GSEA) was performed via computational platform from the Broad institute. Expression data was formatted to a Gene Cluster Text (.gct) file and phenotype data was formatted as a Categorical Class (.cls) file for each comparison being made. The MM Response Genes, MM SuperEnhancer Genes, and Top E2F Targets gene sets were added to the C2 all curated gene set derived from the GSEA website and was used as the Gene Matrix Transposed (.gmt) file. A normalized enrichment score (NES) scatter plot was generated using the "ggplot2" library in R with indices generated from GSEA, which ranked each gene set by NES and false discovery rate (FDR). Key leading edge enrichment plots were taken from GSEA output.

NES Heatmap Analysis. A normalized enrichment score heatmap was generated using the "ggplot2" library in R. For the cell lines being compared, NES and FDR values for key gene sets derived from a comparison of sensitive cell lines (H929 and MM1S) were compared across all MM cell lines. NES values accompanied by an FDR value > 0.05 were left unfilled on the heatmap.

Log2 Fold Change Analysis. Normalized FPKM values were taken for each MM cell line. Following the filtration of inactive genes, FPKM averages were calculated across treatments/timepoints. Log2 values were calculated using the YKL-5-124/DMSO fold changes and visualized using the "ggplot2" library in R. Statistical analysis was performed using the Wilcoxon Ranked Sum test.

MSigDB Pathway Analysis. MM response genes were input into MSigDB for pathway analysis using the Hallmark gene signature. Results were imported into R and used to generate a horizontal bargraph using "ggplot2" in R.

***In vivo* studies.** All animal experiments were approved by and conducted according to the relevant regulatory standards of the Institutional Animal Care and Use Committee at the Dana-Farber Cancer Institute. For the flank murine model, SCID mice (Charles River) were irradiated (200 cGy) and then inoculated subcutaneously in the right flank with 5×10^6 MM cells. Tumor growth was measured in two dimensions by caliper, and volume was calculated using the following formula: $V=0.5\times length\times width^2$. Body weight was measured every week throughout the study to monitor toxicity. For the orthotopic murine model, NOD/SCID-γ mice (The Jackson Laboratory) were injected i.v. with 1×10^6 H929/Luc cells or 1×10^6 MOLP8/Luc cells. Mice were monitored for

tumor growth with Living Image software version 4.2. For 18F-FDG PET mouse model, 8-week-old female SCID mice (Charles River) were used. Mice were subcutaneously injected with 5×10^6 H929 cells in a 1:1 ratio with Matrigel (Coring). Mice were imaged with PET-CT and started treatment once the tumor reached 200 mm^3. For the Vk*MYC mouse model (Chesi et al., 2008), aged Vk*MYC mice with an M-spike of at least 15 g/L were treated daily with YKL-5-124 via intraperitoneal (i.p.) injection, 5 consecutive days/week for 2 weeks. Serum protein electrophoresis (SPEP) was performed weekly, and drug response was calculated by dividing the gamma/albumin ratio at d0 for each individual M-spike by the gamma/albumin ratio obtained post treatment.

Supplementary Figure Legends

Figure S1. MM cells are selectively sensitive to CDK7 inhibition.
a. Boxplot visualization for CDK7, CCNH or MNAT1 expression by RNA-seq in primary MM cells. b. Whole-cell lysates from a panel of MM cell lines and one primary myeloma sample were subjected to WB analysis and probed with the indicated antibodies, and GAPDH as a loading control. c. Genetic depletion of CDK7 was achieved using 4 different tetracycline-inducible CRISPR/Cas9 vectors containing the target sequence. Transfected H929 cells were plated in growth medium in the absence or presence of 0.5 µg/ml doxycycline for 4 days. Cell proliferation was evaluated over time by CTG. WB analysis was performed to confirm CDK7 expression decrease after treatment with 0.5 µg/ml doxycycline for 4 days. d. Whole-cell lysates from H929 cells in which CDK7 was engineered with a small molecule–coupled degron epitope (dTAG-$CDK7^{WT}$) treated with 50nM $dTAG^V$-1 for 4 and 16 hours were subjected to WB analysis and probed with indicated antibodies, and Tubulin as a loading control.

Figure S2. CDK7 is an upstream regulator of the RB-E2F axis.
a. Whole-cell lysates from H929 cells (dTAG-$CDK7^{WT}$) treated with 50nM $dTAG^V$-1 for 4 and 16 hours were subjected to WB analysis and probed with indicated antibodies, and Tubulin as a loading control. b. H929 and AMO1 dTAG-$CDK7^{WT}$ cells were treated with 50nM or 500nM $dTAG^V$-1 for 24 hours. EMSA assay was performed with E2F1 probe. EMSA reveals changes in the DNA binding ability of E2F1. c. Boxplot visualization for CDK7 expression by RNA-seq in three cohorts of primary MM cells with differential expression of E2F target genes. d-e. E2F scores were calculated from patient samples collected at diagnosis and at the time of relapse (Determination study). After RNA-seq normalization, z scores were calculated for each sample and total score for each patient sample at diagnosis and relapse were compared using paired sample t test analysis (d). Pearson correlation coefficient was calculated between E2F score and CDK7 expression (e). f. AMO1 cells were transduced with control or T121 plasmid and treated with doxycycline (0.5 µg/ml) for 24 hours. T121 fragment was detected by qRT-PCR. The relative mRNA expression level compared to control ($2^{-\Delta\Delta CT}$) is shown in graph. g. Plot showing the distribution of normalized CRISPR viability scores (YKL-5-124 vs control) for genes targeted by sgRNA library. Dots are all targeted genes. RB1 is highlighted.

Figure S3. YKL-5-124 treatment disrupts oncogenic gene expression programs in MM.

a. Boxplot visualizing the log2 fold change for active genes across MM cell lines following a 24-hour treatment with YKL-5-124 at concentrations comparable to IC50 values compared to DMSO, using cell count spike-in normalized RNA-seq. b. GSEA leading edge enrichment score plots for E2F MM gene signature, previously defined in (Fulciniti et al., 2018), in MM cell lines after treatment with YKL-5-124. c. GSEA leading edge enrichment score plots for MYC gene signature in H929 and AMO1 cell lines after treatment with YKL-5-124. NES: normalized enrichment score. FDR: false discovery rate. d. AMO1 cells were treated with YKL-5-124 500nM for 24 hours and submitted to Log2 fold change is shown on the x-axis, while negLog (FDR) is listed on the y-axis. Significantly up-regulated (pink) and down-regulated (blue) proteins were shown under the criteria of fold change >2, and FDR adjusted p value <0.05. e. A scatter plot of the correlation coefficient (r=0.62) between protein and mRNA level changes. The black line represents a hypothetical complete agreement between the two groups. f. Whole-cell lysates from H929 cells treated with several concentrations of YKL-5-124 for 24 hours were subjected to WB analysis and probed with cyclin D and MCL1 antibodies, with GAPDH or Tubulin as a loading control. g. The ratio of MYC/GAPDH protein was analyzed with Image J software and is represented as the fold change from untreated cells, CDK7 degradation or overexpression cell lines. Mean values ± SD in four MM cell lines is displayed. h. MM cells were infected with empty or T121 vector and incubated with YKL-5-124 in the presence of doxycycline for 24 hours. Whole-cell lysate was subjected to western blot analysis and probed with indicated antibodies.

Figure S4. CDK7 regulates MYC levels in MM cells.

a. H929 and AMO1 cells were treated with DMSO or YKL-5-124 in the presence or absence of 20uM cycloheximide (CHX) and the whole-cell lysates were subjected to WB analysis and probed with MYC antibody. Protein levels were measured with densitometric intensity. Half-life of MYC was calculated and shown in right graph. b. H929 and AMO1 cells were treated with DMSO or YKL-5-124 in the presence or absence of 5uM or 10uM MG132 for 3 hours and the whole-cell lysates were subjected to WB analysis and probed with MYC antibody. The ratio of MYC/GAPDH protein was analyzed with Image J software and is represented as the fold change from untreated cells. c. MOLP8, MM1S and RPMI8226 were treated with DMSO or YKL-5-124 in the presence or absence of 5μM MG132 for 6 hours or 10μM MG132 for 3 hours, and the whole cell lysates were subjected to WB analysis and probed with MYC antibody. d. Whole-cell lysates from Raji cells treated with YKL-5-124 for 24 hours were subjected to WB analysis and probed with MYC antibody. e. H929 cells were transfected with MYC WT or mutant (T58A) plasmids, treated with the indicated concentrations of YKL-5-124 for 24h, and whole-cell lysates were subjected to WB analysis.

Figure S5. MYC-dependent aerobic glycolysis is impaired in CDK7-inhibited MM cells.

a. Cell lysates from H929-dTAG-CDK7^{wt} treated with 50nM dTAGV-1 for 16 hours were subjected to global quantitative tandem mass tag (TMT)-based proteomic. Log2 fold change is shown on the x-axis, while negLog (adjust p value) is listed on the y-axis. Significantly up-regulated (yellow) and down-regulated (blue) proteins with fold change >2 and adjusted p value <0.05 are colored. b. Whole-cell lysates from a panel of myeloma cells treated with YKL-5-124 for 24 hours were subjected to WB analysis and probed with LDHA antibody, with β-Actin as a loading control. c. Whole-cell lysates from H929 and AMO1 cells treated with YKL-5-124 for 24 hours were subjected to WB analysis and probed with GLUT1 and PDH antibodies, with GAPDH as a loading control. d. CRISPR screen score (CSS) for HK2 and LDHA genes from a CRISPR-

Cas9 screen performed in 17 MM cell lines. e. OPM2, MOLP8 and MM1s cells were treated with YKL-5-124 for 24 hours and analyzed in the context of glycolysis stress assay on a Seahorse XFe96 extracellular flux analyzer. f. H929 dTAG-CDK7^{WT} cells were treated with 50nM dTAG^{V}-1 for 24 hours and analyzed in the context of a glycolysis stress assay on a Seahorse XFe96 extracellular flux analyzer. g. U266 or AMO1 cells infected with empty vector (EV) or MYC-OE virus were subjected to glycolysis stress analysis on a Seahorse XFe-96 device. h-i. AMO1 cells were transfected with a specific siRNA targeting MYC or a non-targeting control and were analyzed in a glycolysis stress assay. The whole-cell lysates were subject to western blot analysis and probed with the indicated antibodies (h). The graphs show glycolysis level (i). j. H929 cells were infected with control or T121 vector and incubated with YKL-5-124 in the presence of doxycycline for 24 hours. Whole-cell lysate was subjected to western blot analysis and probed with HK2 antibody. k. H929 cells were infected with control vector (PCW) or T121 vector and incubated with YKL-5-124 for 24 hours, then analyzed in the context of a glycolysis stress assay. l. H929 and AMO1 cells were treated with YKL-5-124 or 2-DG alone or in combination, and cell survival was assessed by CTG.

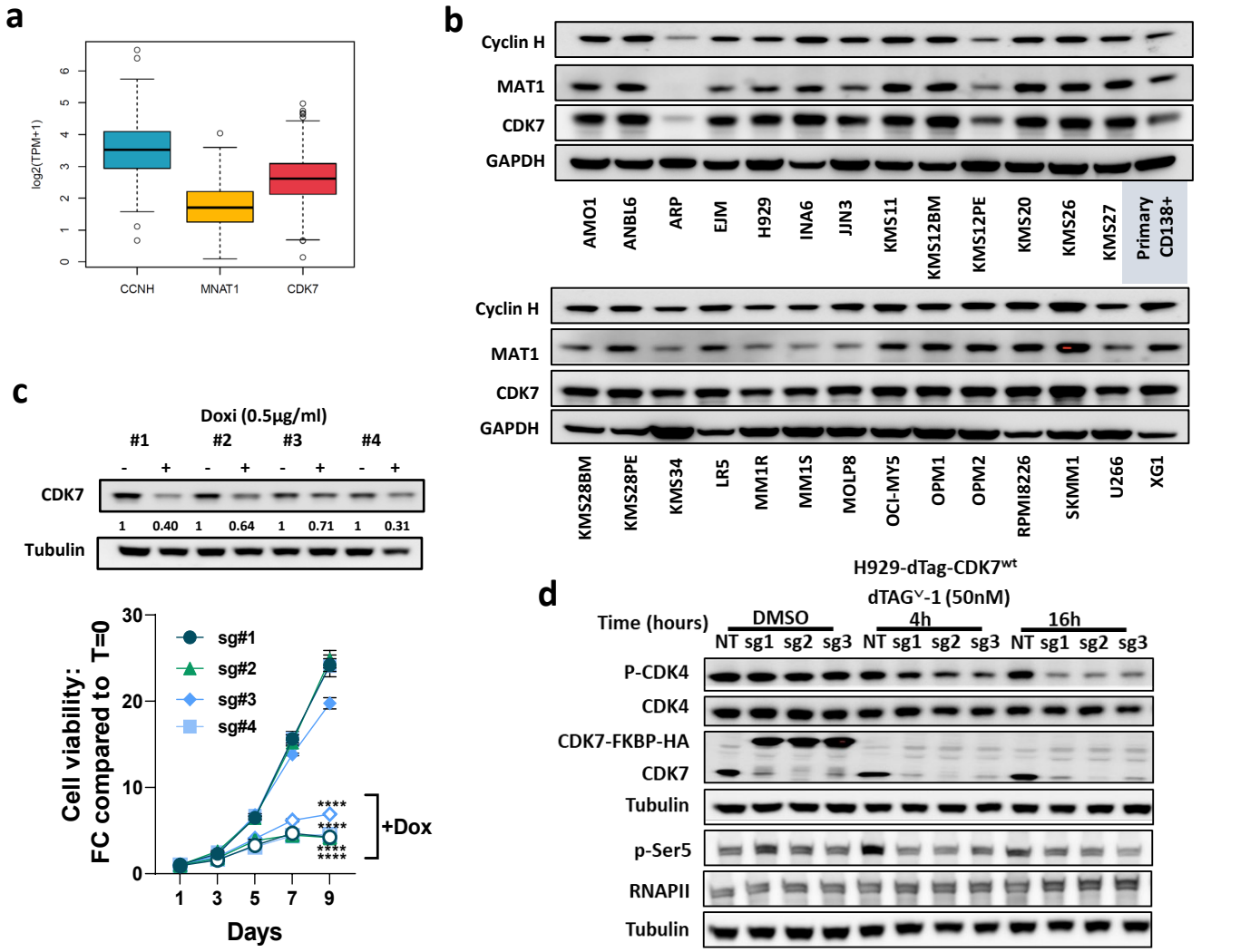
Figure S6. YKL-5-124 synergizes with conventional anti-MM agents.

a. Three MM cell lines (H929, AMO1 and MM1s) were cultured in the presence of different concentrations of YKL-5-124 with or without bortezomib (2.5nM), lenalidomide (5uM), melphalan (2.5uM) and carfilzomib (1nM) and cell survival was assessed by CTG. Data are presented as % of cell death. b. ANBL6 WT and ANBL6 BR cell lines were cultured in the presence of different concentrations of YKL-5-124 with or without bortezomib (2.5nM). Data are presented as combination index. c. H929 or AMO1 cells ectopically overexpressing HK2 were cultured in the presence of YKL-5-24 with or without bortezomib (2nM), lenalidomide (10uM) and melphalan (2.5uM). Data are presented as combination index.

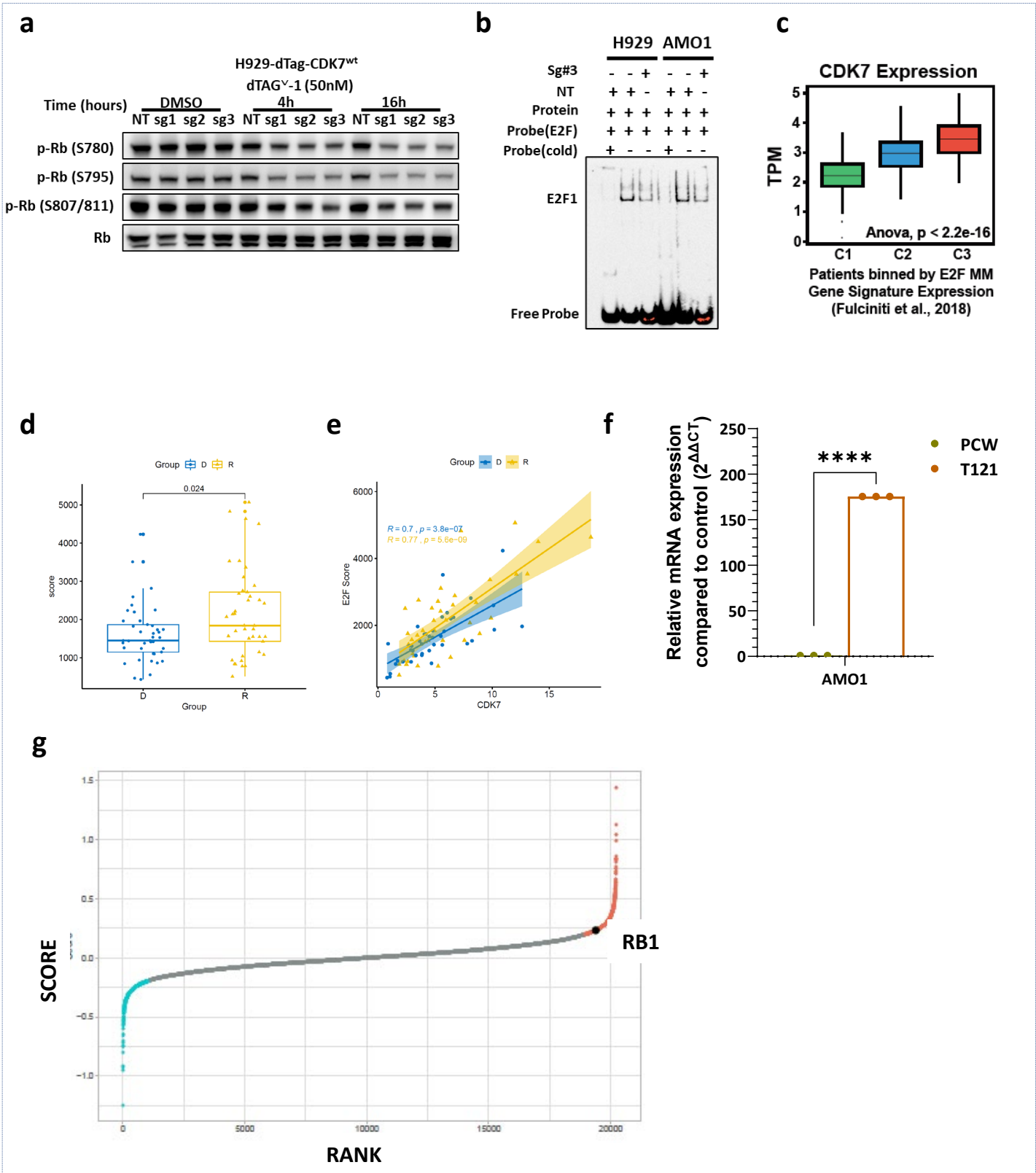
Figure S7. CDK7 inhibition reduces myeloma burden and enhances survival *in vivo* in MM mouse models.

a. H929, AMO1, MM1s and JJN3 MM cell lines were incubated with YKL-5-124 for 24 hours in the absence or presence of BMSCs. Cell proliferation was assessed by (3H)-thymidine uptake and presented as % of DNA synthesis compared with control group. b. In the late treatment model, mice started 1 cycle of treatment when the tumor volume was approximately 500 mm^3. c. Sub-lethally irradiated SCID mice were injected subcutaneously with MM1s MM cells. Mice were randomized and treated with either YKL-5-124 or vehicle at first detection of tumor (tumor volume approximately 100 mm^3). Mice received 1 mg/kg YKL-5-124 for 5 consecutive days/week for 2 weeks. Tumor volume was measured in 2 perpendicular dimensions by caliper once every week. d. H929 and AMO1 cells were engineered to express the dTAG-CDK7^{WT} or dTAG- CDK7^{C312S} plasmid with a silent mutation to confer resistance to CDK7 sgRNAs, allowing for simultaneous expression/KO. Cell viability was measured by CTG after treatment with YKL-5-124. e-f. NOD/SCID-γ (NSG) mice were orthotopically xenografted with intravenous injection of H929-luciferase cells. Upon detection of MM lesions (approximately 2 weeks after tumor cell injection), mice were randomly assigned to receive YKL-5-124 (5 mg/kg, i.p daily) or vehicle control. Whole-body bioluminescence images and fold change increase compared to start of treatment (T0) of BLI measurements (mean +/- SD) (ft) are shown (f).

Supplemental Figure 1



Supplemental Figure 2



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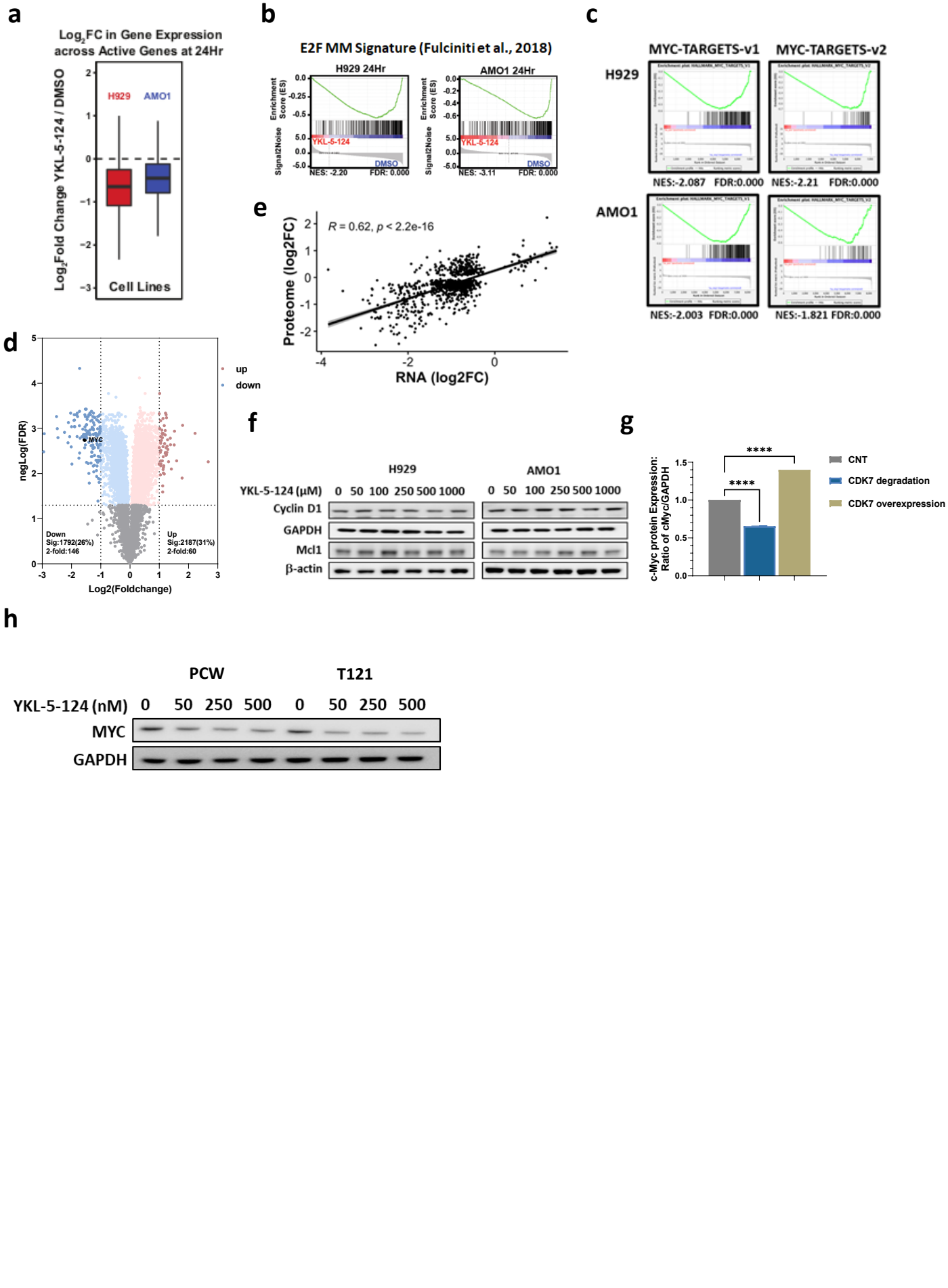

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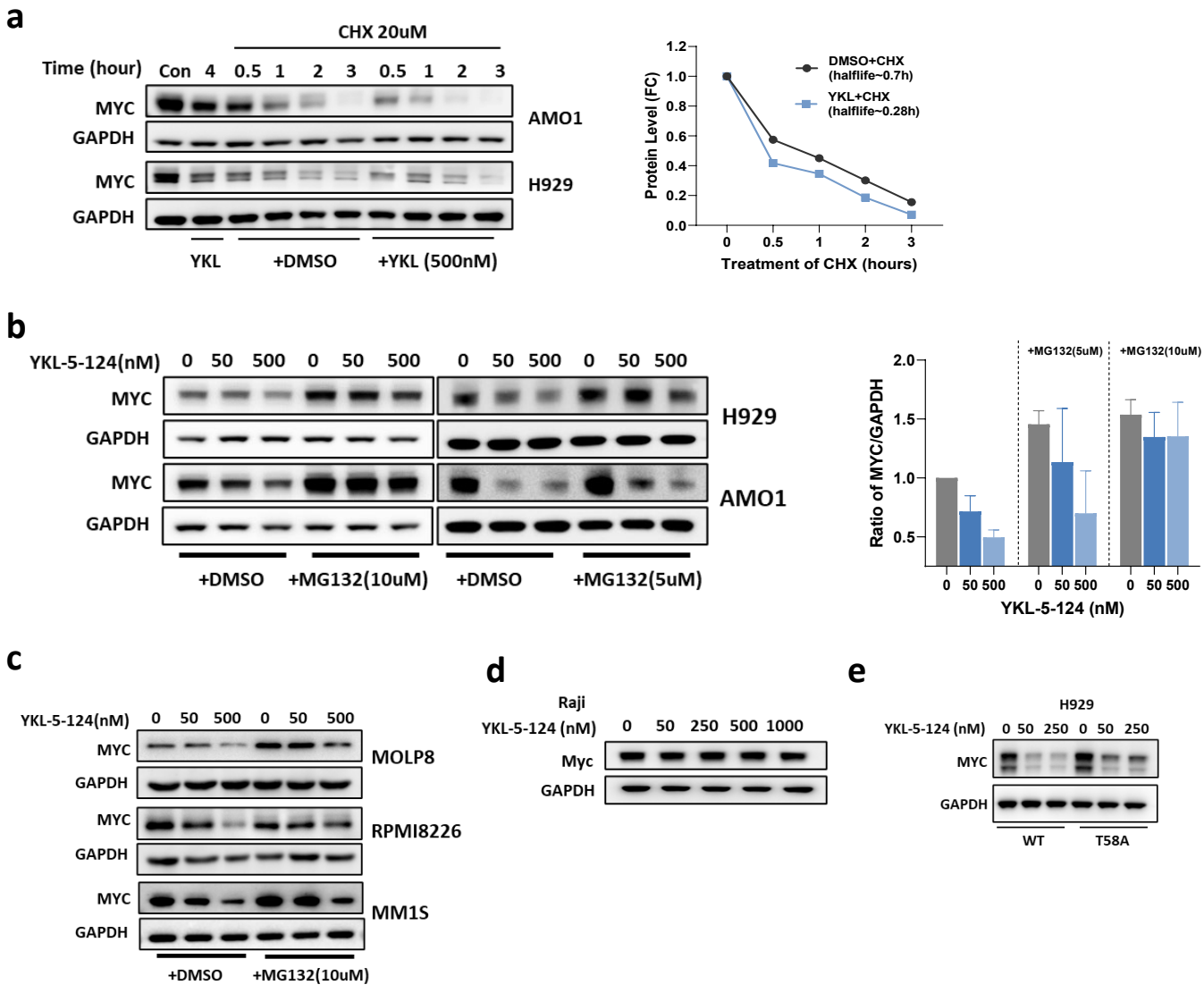
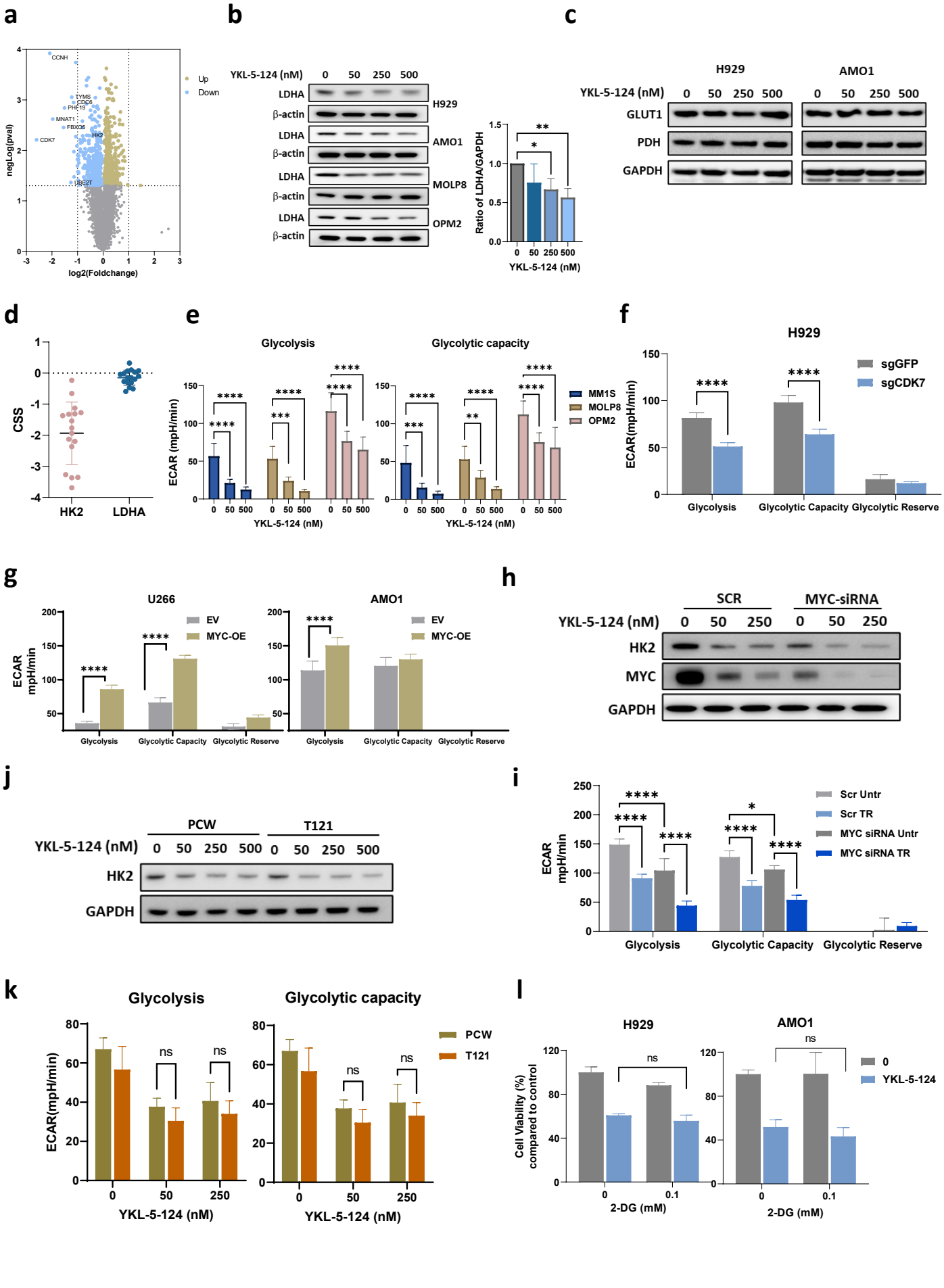
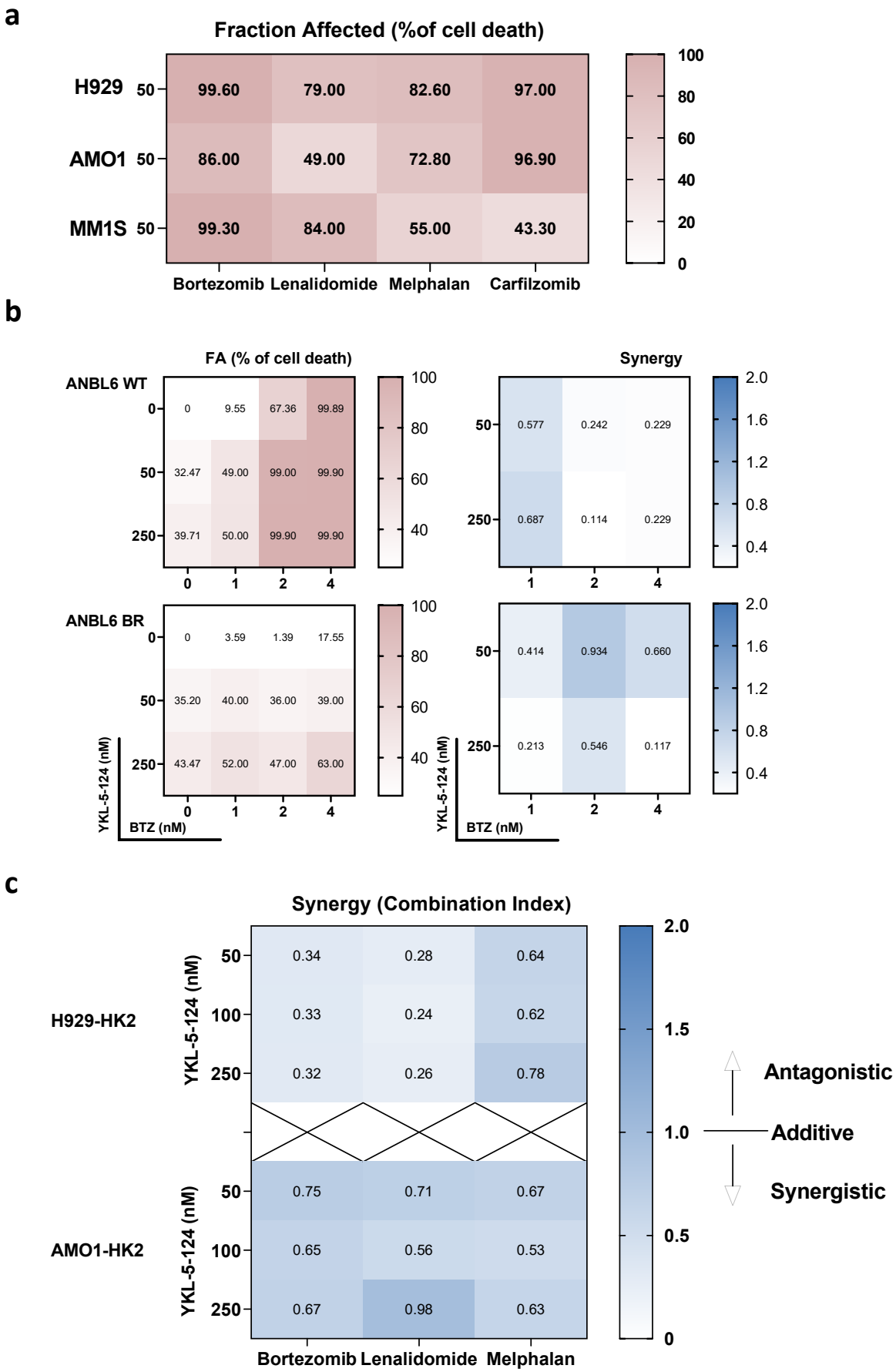


Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7