SUPPLEMENTARY APPENDIX

Concomitant targeting of BCL2 with venetoclax and MAPK signaling with cobimetinib in acute myeloid leukemia models

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Supplemental Methods

Patient samples, AML cell lines, and reagents

The AML cell lines were purchased from the ATCC (Manassas, VA) or Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). Cells were validated by short tandem repeat DNA fingerprinting using the Amp-FISTR Identifier kit according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA). All cells were routinely propagated in RPMI-1640 medium (Cat. 10-040-CV; Mediatech, Inc., Manassas, VA) containing 10% fetal bovine serum (FBS) and 1% each penicillin and streptomycin. Granulocyte-macrophage colony-stimulating factor GM-CSF (2 ng/mL, Cat. NDC 58406-002-01; Immunex Corporation, Seattle, WA) was added to the medium for the TF-1 cell culture. Cobimetinib was provided by Genentech (South San Francisco, CA) and venetoclax by AbbVie (North Chicago, IL).

CellTiter-Glo proliferation assay

For dose-response assays, 11 AML cell lines were seeded into 96-well plates at 2×10^5/mL and left untreated or treated with cobimetinib and/or venetoclax at 0.001, 0.01, 0.1, or 1.0 μM. Cell proliferation was determined by CellTiter-Glo assay (CTG, Cat. G7571; Promega, Madison, WI) 72 hours after adding drug, using standard protocols. Calcusyn software (Biosoft, Cambridge, MA) was used to calculate the median inhibitory concentration (IC_{50}) values and combination index (CI) based on the luminescent intensity that was proportional to the number of viable cells.

Apoptosis of primary AML samples

Primary AML peripheral blood mononuclear cells or AML patient-derived xenograft (PDX) samples were cultured in serum-free Expansion Medium (Cat. 09650) supplemented with BIT 9500 Serum Substitute (Cat. 09500; both from STEMCELL Technologies Inc., Vancouver, BC, Canada) and cytokines including stem cell factor (SCF, 100 ng/mL, Cat. 300-07), Flt3 ligand (50 ng/mL, Cat. 300-19), IL3 (20 ng/mL, Cat. 200-03), and G-CSF (20 ng/mL, Cat. 300-23; all from Peprotech, Rocky Hill, NJ) and StemRegenin 1 (1 μM, Cat. S2858; Selleck Chemicals LLC, Houston, TX) as reported.[1] After treatment with cobimetinib and/or venetoclax for 5 days, cells were stained with a cocktail of antibodies comprising CD45-FITC (Cat. 347463) and Annexin-V–APC (Cat. 550475; both from BD Biosciences, San Jose, CA) for 30 minutes at room temperature in the dark. CD45-PE, CD34-FITC (Cat. 555821), CD38-PE-Cy7 (Cat. 335808) and

CD123-PerCP-Cy5.5 (Cat. 558714) (all from BD Biosciences) were included in 4 AML samples to define LSC fraction.

The cells were then washed and resuspended in phosphate-buffered saline solution (PBS) with 4′6-diamidino-2-phenylindole (DAPI). Viable AML cells were enumerated by using CountBright counting beads (Cat. C36950; Invitrogen, Carlsbad, CA) with concurrent Annexin-V and DAPI detection on a Gallios Flow Cytometer (Beckman Coulter, Indianapolis, IN). Data were analyzed by using Flowjo software (Tree Star, Ashland, OR). Results are presented as percentage of specific apoptosis: 100 × (% apoptosis of treated cells - % apoptosis of control cells) / (100 - % apoptosis of control cells).

Western blot

Cells were treated as indicated for 4 hours and subjected to lysis in lysis buffer (150 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM NaF, 5 mM sodium pyrophosphate, 10 mM β-glycerophosphate, 1% Triton X-100, 10 mM iodoacetamide, 1 mM Na_3VO_4, 0.1% NaN_3, 3 mM phenylmethylsulfonyl fluoride) supplemented with a protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). Lysates were separated on a 10% polyacrylamide gel, transferred to PVDF membranes (Bio-Rad, Hercules, CA), probed with appropriate antibodies, and analyzed on the Odyssey imaging system from LI-COR Biosciences (Lincoln, NE). Antibodies included pERK1/2 (Cat. 4370), pS6 (Ser235/236) (Cat. 4548), pS6 (Ser240/244) (Cat. 2215), PARP (Cat. 9541), c-Myc (cat. 5605), and tubulin (Cat. 15115), all from Cell Signaling Technology (Danvers, MA), and MCL1 (Cat. Sc-819), BCL2 (cat. Sc-7382), and total ERK2 (Cat. Sc-1647), all from Santa Cruz Biotechnology (Dallas, TX).

Electrochemiluminescent ELISA assay

AML cell lines were left untreated or treated with cobimetinib or venetoclax as single agents or in combination at 10 times the IC_{50} value of each compound in each cell line for 4 hours. Cell pellets were harvested post treatment and subjected to the Electrochemiluminescent ELISA (MSD, Gaithersburg, MD) assay as previously described.[2] Briefly, the 384-well MSD assay quantifies expression of BCL-2 protein alone or in complex with MCL1 or BIM. The platform uses SULFO-TAG labels that are conjugated to detection antibodies. These labels generate light when stimulated by electricity in the appropriate chemical environment, which can then be used to measure key proteins and molecules.

Colony-forming cell assay

Four primary AML bone marrow samples were utilized for this assay. Mononuclear cells isolated from 4 patients with AML (100,000) or healthy donors (50,000) were plated in methylcellulose medium (1 mL/well; Cat. 04435; STEMCELL Technologies Inc., Vancouver, BC, Canada) in triplicate per condition. Colonies were scored after 2 weeks of culture.

Reverse-phase protein array

AML cell lines (n=11) were left untreated or treated with cobimetinib or venetoclax as single agents or in combination at 0.5, 1, or 2 times the IC_{50} value of each compound in each cell line for 24 hours. Cell pellets were harvested post treatment and subjected to RPPA analysis as previously reported.[3]

RNA sequencing

AML cells were treated and processed as described for the RPPA assay. RNA was isolated by using the RNeasy kit (QIAGEN, Valencia, CA), at a minimum of 300 ng at a concentration of 20 ng/uL, quantified by RiboGreen in 15 uL nuclease-free water. The RNA samples were sent to Q^2 Solutions | EA Genomics for mRNA sequencing. Briefly, samples were quality-controlled by Agilent Bioanalyzer, and pass/fail criteria were determined by a minimum DV200 of 30 to proceed with the RNA Access kit. Processing occurred in a multiplex of 4 units. Units consisted of samples and controls. A minimum of one control was required for every batch of up to 47 samples. Samples were sequenced on the Illumina HiSeq System.

Antibody conjugation for CyTOF analysis

Antibodies for CyTOF were either purchased from Fluidigm (San Francisco, CA) or conjugated in-house, which was performed as following. Purified, carrier-free antibodies were conjugated with lanthanide isotopes using MaxPar Antibody Labeling Kit (Fluidigm), following manufacturer's instructions. Protein concentrations were determined using NanoDrop 2000 (Thermo Fisher Scientific), and metal contents of the conjugated antibodies were determined by CyTOF in solution mode, using Claritas PPT Grade Multi-Element Solution 1 (SPEX CertiPrep, Metuchen, NJ) at 0.5ppb as a standard.

Mass cytometry staining

Six primary AML samples were utilized for only CyTOF study and two additional AML samples for both apoptosis and CyTOF assays. Primary AML cells were treated with cobimetinib at 1.0 μM overnight and subjected to viability staining with Cell-ID Cisplatin (Cat. 201064; Fluidigm) at 5 μM for 5 minutes at room temperature. Cells were the subjected to centrifugation in MaxPar Cell Staining Buffer (Cat. 201068; Fluidigm) and then barcoded using the Cell-ID 20-Plex Pd Barcoding Kit (Cat. 201060; Fluidigm). Briefly, cells were resuspended in 1 mL Fix I buffer and incubated for 10 minutes at room temperature, then washed twice with Barcode Perm Buffer. All samples were then barcoded and mixed for 30 minutes at room temperature before processing as one multiplexed sample. Metal-labeled antibodies against surface markers were stained in 50 μL final reaction volumes at room temperature for 30 minutes and then treated with human Fc receptor binding inhibitor (Cat. 14-9161-73, Thermo Fisher Scientific) for 15 minutes at 4°C. Following staining, cells were washed twice with wash buffer and once with PBS and resuspended in 500 μL of 1.6% paraformaldehyde (PFA) for 10 minutes at room temperature. Cells were spun down, washed twice with wash buffer, and permeabilized with 80% cold methanol at –20°C overnight. After washing twice to remove methanol, cells were stained with antibodies against intracellular markers in 50-μL final reaction volumes at room temperature for 30 minutes. Cells were then washed twice with wash buffer and once with PBS and stained in 500 μL of 1:1000 Iridium intercalator (Cat. 201192A; Fluidigm) diluted in PBS with 1.6% PFA for 20 minutes at room temperature. Cells were then washed twice with wash buffer and filtered through blue-capped tubes. Each sample pellet was resuspended in 50 μL deionized water and transferred to a 96-deep well plate containing 50 μL Eu151/153 calibration beads (Cat. 201073, Fluidigm). Samples were analyzed on a CyTOF mass cytometer (Fluidigm) using an AS5 Autosampler (Fluidigm); 0.4 mL deionized water was added to each sample just prior to injection according to published procedures. The data were saved in FCS3.0 format, de-barcorded by the Fluidigm de-barcorder, and analyzed by spanning-tree progression analysis of density-normalized events (SPADE) software.[4]

Spanning-tree progression analysis of density-normalized events analysis

The original FCS files were input into Flowjo software and exported after gating on viable $CD45^{dim}$ blast cells based on DNA (Ir191/193), cell length, cisplatin, and CD45. The exported FCS files were transferred into the SPADE software for analysis. In particular, SPADE was used to analyze six FCS files from each patient. Surface markers (CD34, CD123, CD117, CD135, CD45, CD25, TIM3, CD7, CD15, CD64, CD33, HLA-DR, CD41, and CD38) were chosen to down sample cells, perform clustering, and construct a tree structure. Other surface markers

with low signals were tested but not selected. The SPADE tree representing all cell types was defined by the seven selected surface markers. For each annotated phenotype, the median intensity of the marker expression was computed for the indicated phospho-protein marker for each patient.

In vivo study of cobimetinib in combination with venetoclax in AML xenograft mouse models

As a second xenograft model, MOLM13-Luc-GFP cells (0.8×10^6) were injected into NSGS mice. Leukemia engraftment was confirmed on day 3 post injection by bioluminescence imaging (BLI). Mice were treated for 2 weeks as already described, starting from day 4. Three mice from each group were sacrificed 3 hours after the last dose for CyTOF study. NSG mice were injected with the AML11 PDX sample (1×10^6 per animal). Leukemia engraftment was confirmed on week 4 by human CD45 flow on peripheral blood. Mice were dosed as described above. Overall survival rate was estimated by the Kaplan-Meier method.

Statistical analyses

The RPPA data were generated and normalized in Theranostics Health Inc. Linear mixed models were used to assess the treatment effects, adjusting for cell types. Technical replicates was modeled as random effect. Dose effect was not added to the model because of the limited sample size. The Kenward-Rogers approximation was applied to estimate the degrees of freedom in linear mixed models.[5] Proteins with $P < 0.05$ and fold change $>\pm1.2$ were selected as significantly differentially expressed. The false discovery rate was estimated by using the Benjamini-Hochberg method. For differential gene expression and pathway analysis, R package Limma was used to Voom normalize RNA read counts, and Limma was used for differential gene expression analysis. The per-cell line fold changes following treatment were compared between the synergistic and resistant cell lines using an interaction test. We further filtered our results by including genes with significant treatment-induced differential expression within the "sensitive" cell lines alone. The C2 and Hallmark curated pathway gene sets from MSigDB and the differential gene expression results from Limma were used with hypergeometric over-enrichment tests to determine pathways of interest.

Supplemental Table 1. Antibodies used in CyTOF

Antigen	Conjugate	Clone	Catalogue	Supplier
CD45	89Y	HI30	3089003B	DVS-Fluidigm
CD7	139La	6B7	343102	BioLegend
CD117	143Nd	104D2	3143001B	DVS-Fluidigm
CD123	145Nd	7G3	554527	BD Biosciences
CD64	146Nd	10.1	3146006B	DVS-Fluidigm
CD34	148Nd	581	555820	BD Biosciences
CD25	155Gd	M-A251	555430	BD Biosciences
TIM3	156Gd	F38-2E2	345002	BioLegend
CD33	158Gd	WM53	3158001B	DVS-Fluidigm
CD19	161Dy	HIB19	302202	BioLegend
CD135	162Dy	4G8	558995	BD Biosciences
CD15	164Dy	W6D3	3164001B	DVS-Fluidigm
CD41	166Er	HIP8	303702	BioLegend
CD38	168Er	HIT2	303502	BioLegend
CD3	170Er	UCHT1	300433	BioLegend
CD90	171Yb	5E10	328102	BioLegend
CD11b	173Yb	ICF44	301302	BioLegend
HLA-DR	174Yb	L243	307602	BioLegend
BCL-xL	141Pr	54H6	2764BF	Cell Signaling Technology
BCL2	144Nd	100	658702	BioLegend
MCL1	176Yb	22/MCL1	559027	BD Biosciences
pSTAT3 (Tyr705)	152Sm	M9C6	4113BF	Cell Signaling Technology
pAKT (Ser473)	159Tb	M89-61	560397	BD Biosciences
pERK (Thr202/Tyr204)	167Er	D13.14.4E	3167005A	DVS-Fluidigm
pMEK (Ser217/221)	169Tm	41G9	9154BF	Cell Signaling Technology
pS6 (Ser235/236)	172Yb	N7-548	3172008A	DVS-Fluidigm
pSTAT5 (Tyr694)	175Lu	C71E5	9314BF	Cell Signaling Technology
p21	160Gd	EA10	Ab16767	Abcam

Supplemental Figure legends

Supplemental Figure 1. Specific apoptosis and % reduction in viable cell count compared to the control groups are shown for the indicated AML samples. Cells were left untreated or treated with cobimetinib (Cobi) or venetoclax (Ven), both at 0.1 μM, as single agents or in combination (Combo) and cultured for 5 days.

Supplemental Figure 2. Mononuclear cells collected from AML patients (100,000 cells) or healthy donors (NBM; 50,000 cells) were plated in methylcellulose, left untreated or treated with cobimetininb (Cobi) or ventoclax (Ven), both at 0.1 μM, as single agents or in combination (Combo). Colonies were scored on day 14.

Supplemental Figure 3. AML13 and AML14 samples were treated as described in the legend to Fig 2D. The original FCS files were input into Flowjo software and exported after gating on viable $CD45^{dim}$ blast cells based on DNA (Ir191/193), cell length, cisplatin, and CD45. The exported FCS files were transferred into the SPADE software for analysis. In particular, SPADE was used to analyze six FCS files from each patient. Surface markers (CD34, CD123, CD117, CD135, CD45, CD25, TIM3, CD7, CD15, CD64, CD33, HLA-DR, CD41, and CD38) were chosen to down sample cells, perform clustering, and construct a tree structure. Other surface markers with low signals were tested but not selected. The SPADE tree representing all cell types was defined by the seven selected surface markers. For each annotated phenotype, median intensity of the marker expression was computed for the indicated phosphoprotein marker for each patient and shown as a heatmap.

Supplemental Figure 4. Six AML PB samples were treated were treated with cobi or ven as single agents or combination at 1.0 μM overnight. Cells were fixed, permeabilized, and processed for CyTOF. SPADE trees were generated by using markers shown in Supplemental Fig 3. Frequencies of gated populations in Figure 2D were shown in A. (B) A representative t-SNE plots analyzed by Cytofkit bioconductor from AML 25 shows percentage of leukemia-specific clusters, with normal peripheral blood sample as a reference. (C) Summary of percentage of leukemia-specific clusters in all samples (D) The frequencies of putative LSC fraction defined by $CD34^{+}CD38^{-}CD123^{+}$ were analyzed by Flowjo software,

Supplemental Figure 5. AML cell lines were left untreated or treated with cobimetinib or venetoclax as single agents or in combination at 0.5, 1, or 2 times the IC_{50} value of each compound in each cell line for 24 hours. Cell pellets were harvested post treatment and

subjected to RPPA analysis. The heatmaps showed proteins at baseline differentially expressed between (A) cobimetinib–sensitive and –resistant cell lines (IC_{50} values of cobimetinib below or above 0.3 μM, as shown in Table 1), (B) venetoclax–sensitive and –resistant cell lines (IC_{50} values of venetoclax below or above 0.1 μM, as shown in Table 1). (C) Cells were treated with cobimetinib (Cobi), venetoclax (Ven), or a combination (Combo) as indicated for 4 hours and subjected to lysis; proteins were separated and probed with the antibodies indicated.

Supplemental Figure 6. The complete data sets in heatmaps to supplement Figure 3A and 3B including data from treatment with Cobi (Fig S6A) and Ven (Fig S6B) as single agents and in combination (Fig S6C)

Supplemental Figure 7. MV4-11 cells were exposed to 10 nM venetoclax (Ven) or 200 nM cobimetinib (Cobi) alone or together (Combo) for the designated intervals, after which cells were subjected to lysis and Western blot analysis with the indicated antibodies. C, control.

Supplemental Figure 8. NSGS mice were injected with MOLM13-Luc-GFP cells (1×10^6 per animal). Leukemia engraftment was confirmed on day 3 using BLI. Mice were dosed daily with an orally active form of cobimetinib (Cobi; 10 mg/kg) or venetoclax (Ven; 100 mg/kg) or the combination (Combo) for 14 days. (A) Human CD45 engraftment in bone marrow (BM) and spleen was determined by CyTOF. (B) Viable cells were counted by using Vi-Cell.

Supplemental Figure 9. NSG mice were injected with the AML11 PDX sample (1×10^6 per animal). Leukemia engraftment (2%) was confirmed on week 4 by human CD45 flow on peripheral blood. Mice were dosed as described in Supplemental Figure 8. Overall survival rate was estimated by the Kaplan-Meier method.

Supplemental Figure 10. Summary of proposed mechanisms of action.

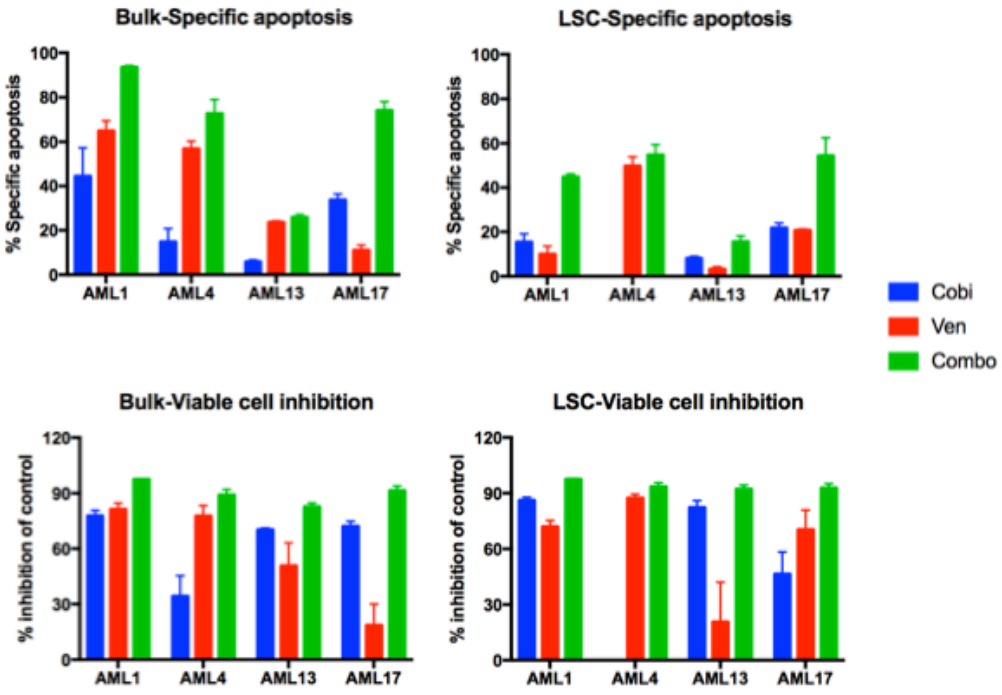
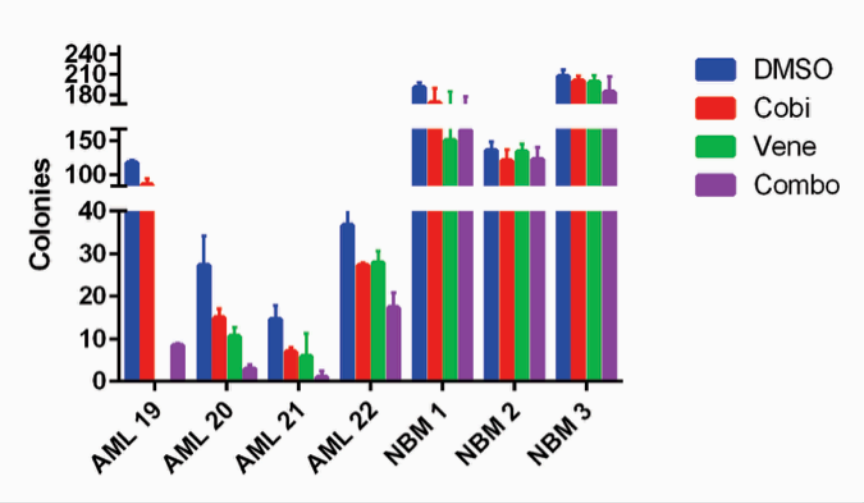
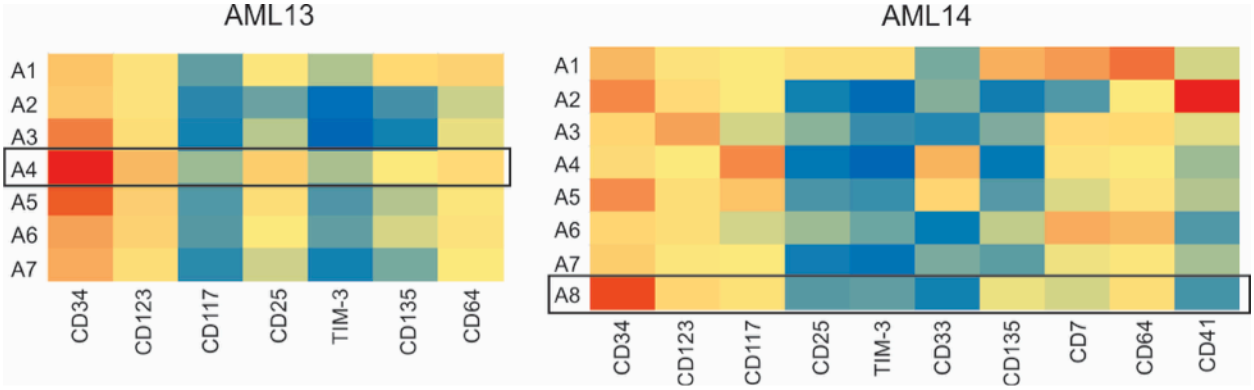


Fig S1

Supplemental Figure 1.



Supplemental Figure 2.



Supplemental Figure 3

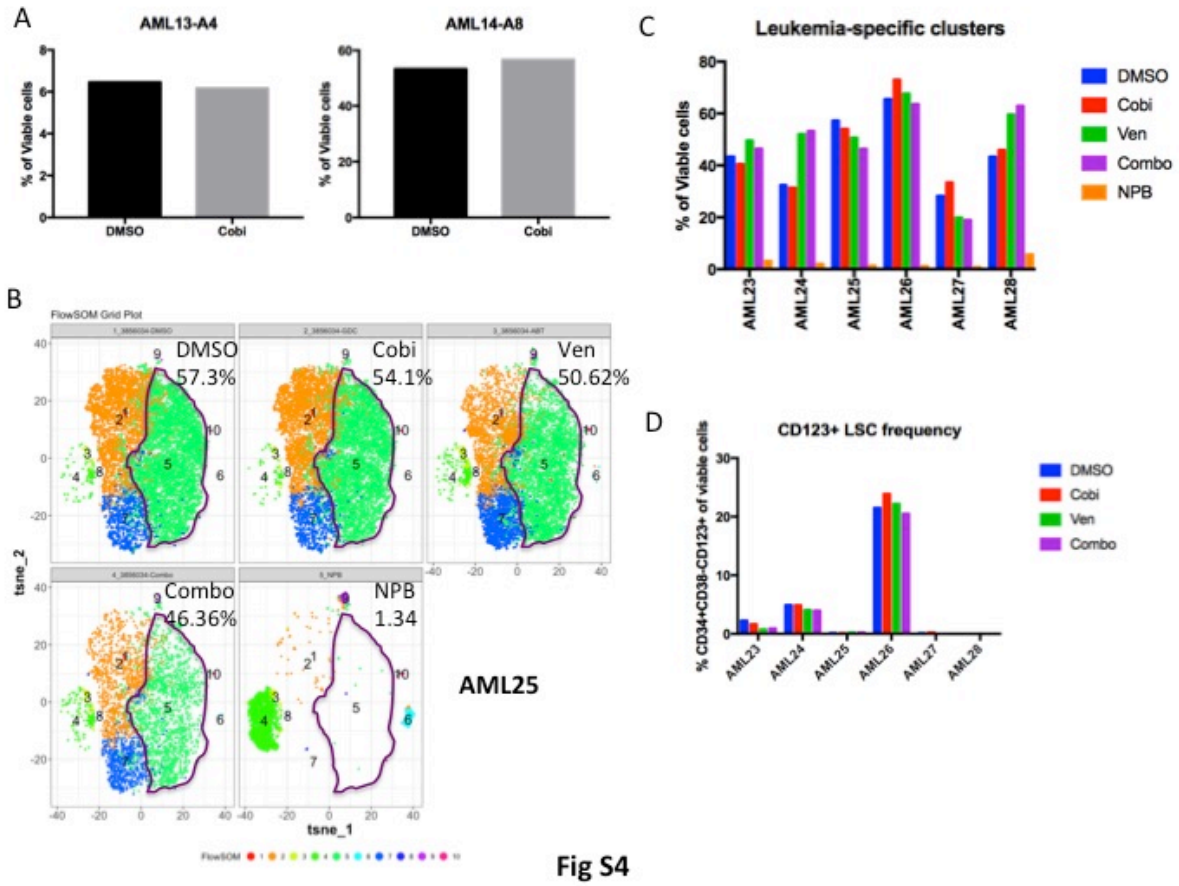
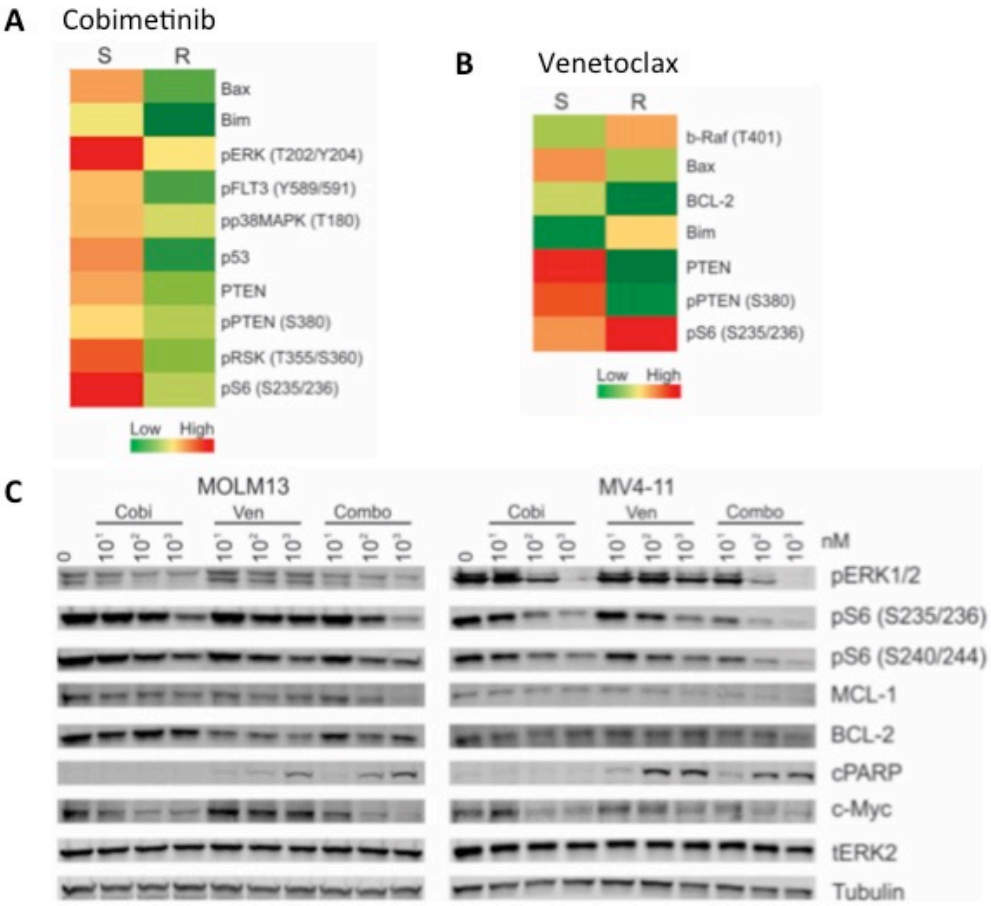


Fig S4

Supplemental Figure 4.



Supplemental Figure 5

A

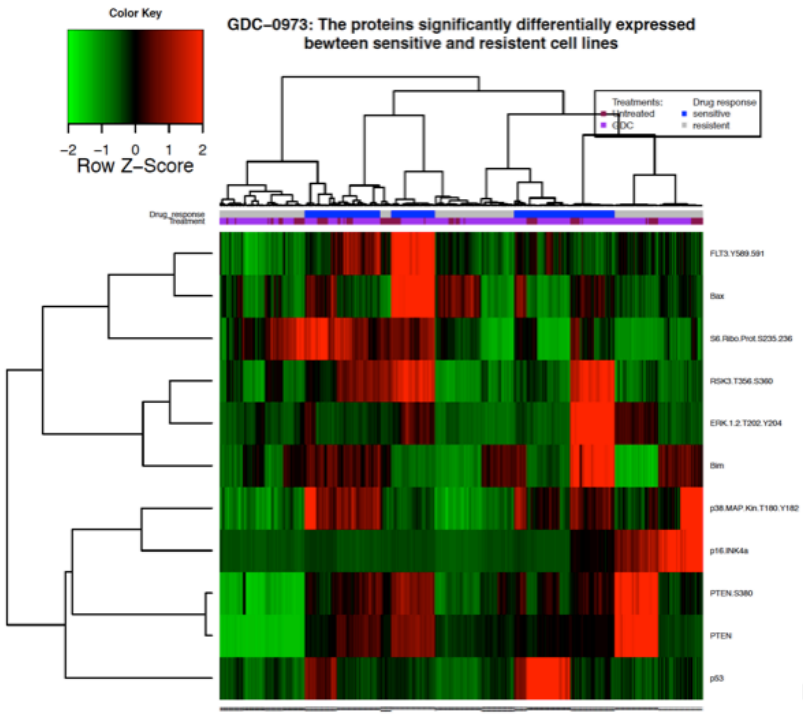


Fig S6

B

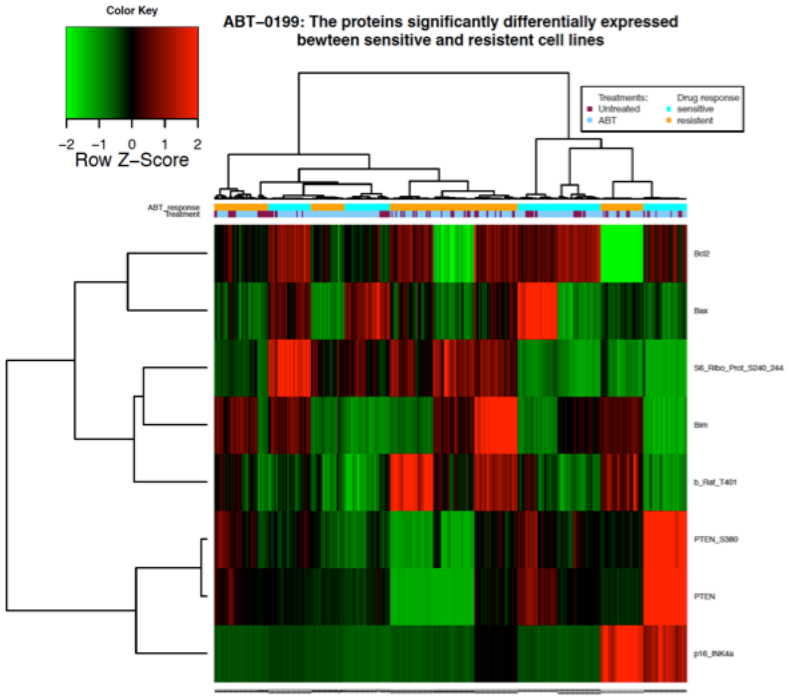


Fig S6

C

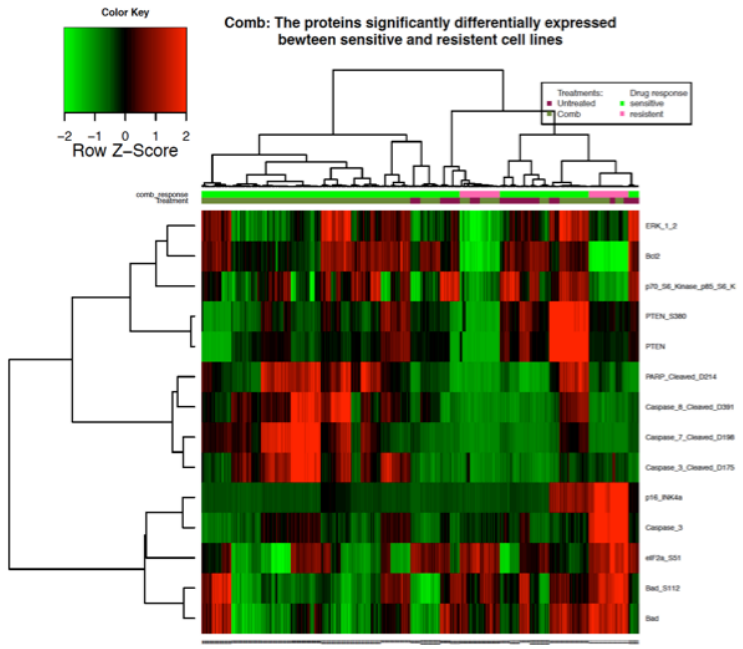
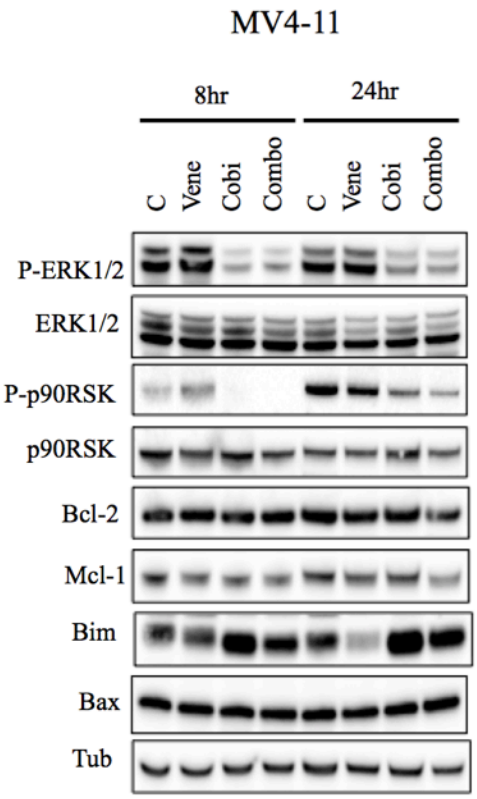
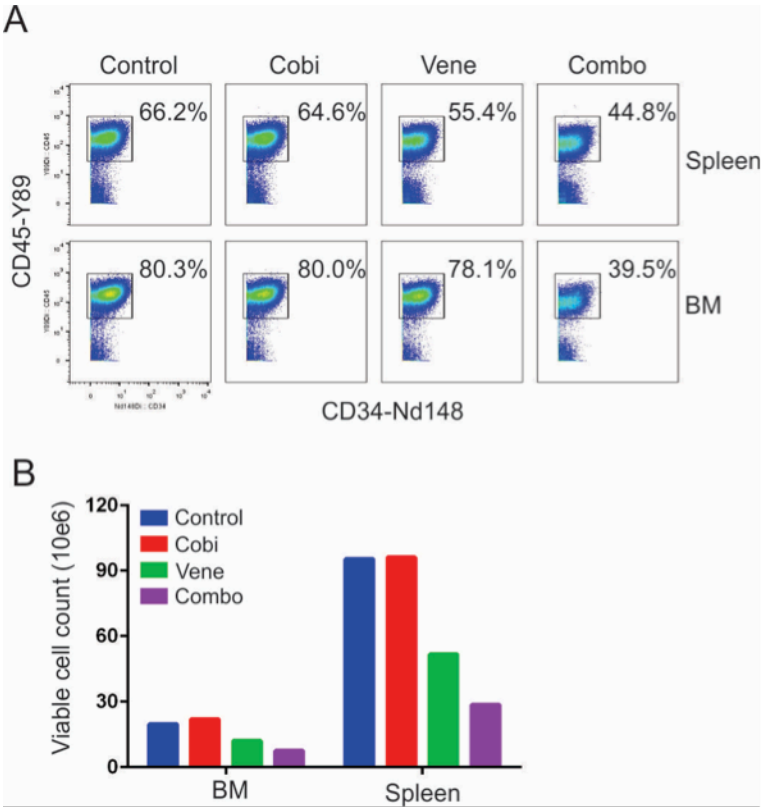


Fig S6

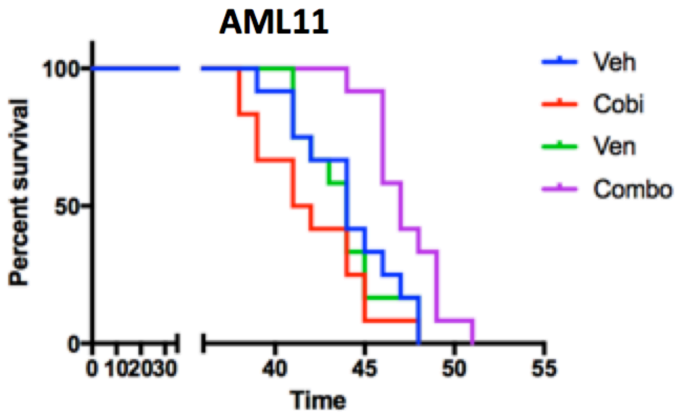
Supplemental Figure 6



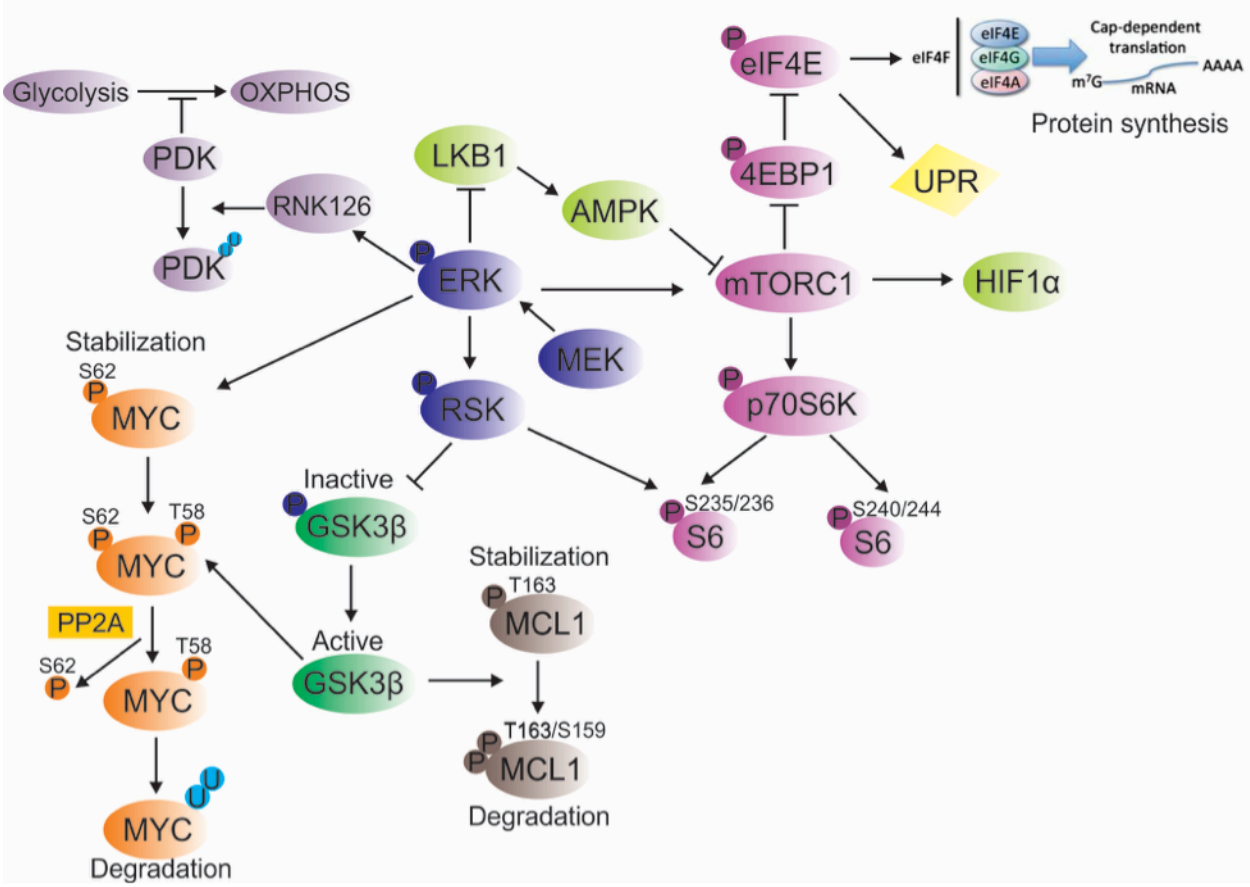
Supplemental Figure 7



Supplemental Figure 8



Supplemental Figure 9



Supplemental Figure 10

References

1. Pabst C, Krosl J, Fares I, et al. Identification of small molecules that support human leukemia stem cell activity ex vivo. *Nat Methods*. 2014;11(4):436-442.
2. Jin S, Tapang P, Osterling DJ, et al. Abstract 6: Quantitative assessment of BCL-2:BIM complexes as a pharmacodynamic marker for venetoclax (ABT-199). *Cancer Research*. 2015;75(15 Supplement):6-6.
3. Konopleva MY, Walter RB, Faderl SH, et al. Preclinical and Early Clinical Evaluation of the Oral AKT Inhibitor, MK-2206, for the Treatment of Acute Myelogenous Leukemia. *Clinical Cancer Research*. 2014;20(8):2226-2235.
4. Qiu P, Simonds EF, Bendall SC, et al. Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE. *Nat Biotechnol*. 2011;29(10):886-891.
5. Barr DJ, Levy R, Scheepers C, Tily HJ. Random effects structure for confirmatory hypothesis testing: Keep it maximal. *J Mem Lang*. 2013;68(3).
6. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550.
7. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. 2005;102(43):15545-15550.
8. Mootha VK, Lindgren CM, Eriksson KF, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet*. 2003;34(3):267-273.

Table S2. HALLMARK_E2F_TARGETS

logFC	AveExpr	adj.P.Val	Symbol
-0.29	8.2	0.04	CDK4
-1	2.09	0.07	CDKN1A
-0.15	8.61	0.13	SYNCRIP
-0.14	8.77	0.12	IPO7
-0.25	8.79	0.13	NOP56
-0.52	7.53	0.01	PAICS
-0.26	4.39	0.2	RAD51AP1
-0.19	7.03	0.02	NUDT21
-0.26	6.23	0.12	CHEK1
-0.27	6.04	0.02	EXOSC8
-0.28	8.84	0.06	CSE1L
-0.22	4.15	0.17	DCK
-0.34	6.76	0.02	EIF2S1
-0.2	8.48	0.06	NUP205
-0.28	6.07	0.07	ORC6
-0.2	6.66	0.11	MMS22L
-0.23	8.57	0.05	ATAD2
-0.15	5.49	0.12	DONSON
-0.25	6.98	0.1	H2AFZ
-0.29	8.51	0.02	HMGA1
-0.4	5.16	0.02	MAD2L1
-0.25	7.77	0.1	MCM2
-0.22	8.31	0.16	MCM6
-0.26	8.79	0.08	MCM7
-0.27	7.69	0.05	MSH2
-0.29	7.81	0.14	MYBL2
-0.28	7.45	0.11	PCNA
0.2	7.54	0.05	LUC7L3
-0.21	6.56	0.17	PLK1
-0.26	6.5	0.06	POLD2
-0.38	5.13	0.02	TIPIN
-0.3	7.07	0.03	PRPS1
-0.19	7.41	0.11	NUP107
-0.28	4.76	0.02	RAD1
-0.1	9.09	0.18	RAD21
-0.18	6.2	0.12	RAD51C
-0.27	8.01	0.05	RAN
-0.16	7.4	0.07	RFC1
-0.21	6.04	0.12	RFC2
-0.26	6.13	0.14	RFC3
-0.14	6.13	0.18	RPA2
-0.43	7.13	0.02	RRM2
-0.17	7.83	0.08	TRA2B
-0.24	6.34	0.05	SNRPB
-0.45	9.76	0.01	TFRC
-0.28	6.79	0.09	TUBG1
-0.18	6.57	0.2	USP1
-0.37	5.29	0.05	DSCC1
0.08	6.71	0.17	SMC6
-0.41	5.28	0.02	E2F8
-0.28	7.72	0.03	ANP32E

-0.22	3.42	0.08	PHF5A
-0.27	4.22	0.13	CCNE1
-0.29	5.31	0.01	TBRG4
-0.2	5.83	0.06	CCP110
-0.29	6.4	0.08	CDK1
-0.51	5.54	0.02	CDC25A
-0.19	8.55	0.05	NUP153

Table S2. HALLMARK_GLYCOLYSIS

logFC	AveExpr	adj.P.Val	Symbol
-0.32	3.4	0.06	GNPDA1
-0.14	5.7	0.07	GNE
-0.22	6.91	0.02	HAX1
-0.08	6.25	0.15	ECD
-0.81	-1.14	0.14	COL5A1
-0.26	1.99	0.18	NANP
0.2	3.85	0.09	CTH
0.16	6.87	0.03	AGL
-1.57	1.22	0	ENO2
-0.45	10.7	0	ALDOA
-0.12	5.93	0.15	PAXIP1
-0.21	8.19	0.08	ZNF292
-0.29	4.71	0.04	SLC37A4
0.15	5.29	0.12	PGLS
-0.23	4.56	0.09	GALE
-0.27	4.8	0.06	GALK1
0.1	5.62	0.09	GALK2
-0.14	3.91	0.13	B3GAT3
-0.52	4.88	0.01	FAM162A
0.46	4.58	0.01	GLRX
0.09	7.63	0.08	GUSB
-0.24	6.69	0.05	GYS1
-0.27	8.84	0.02	HSPA5
-0.1	6.76	0.19	IDH1
0.5	2.97	0.04	IDUA
-0.12	7	0.18	KIF2A
-0.34	1.2	0.14	LCT
-0.1	7.66	0.19	MDH1
-0.2	5.95	0.04	MPI
-0.51	4.52	0	MXI1
-0.89	6.92	0	P4HA1
-0.35	4.99	0.01	PAM
-0.29	3.49	0.11	POLR3K
-0.3	7.8	0.02	PLOD1
-0.2	4.86	0.12	ANKZF1
-0.15	5.35	0.07	PPP2CB
-0.17	6.89	0.01	PGM2
-0.3	7.07	0.03	PRPS1
-0.11	6.39	0.19	QSOX1
0.17	5.87	0.03	PYGB
-0.35	8.36	0	PYGL
-0.3	3.11	0.11	RRAGD
-0.98	-4.23	0.01	SDC1
-0.27	1.04	0.2	SPAG4
-1.57	-3.49	0.03	STC1
-0.26	8.65	0.02	TPI1
-0.19	8.01	0.06	TXN
0.43	6.46	0.03	CXCR4
-0.59	1.05	0.01	CHPF
0.15	4.88	0.05	SRD5A3
0.38	2.92	0.02	CASP6

-1.62	-2.55	0	STC2
-0.46	-4.45	0.12	HS6ST2
-0.68	5.78	0	SLC16A3
1.03	-2.16	0.08	NDST3
-0.36	4.07	0.01	HOMER1
-0.43	7.85	0.11	CD44
-0.12	4.99	0.15	HS2ST1
1.03	-2.81	0.05	SDC3
-0.29	6.4	0.08	CDK1

Table S2. HALLMARK_HYPOXIA

logFC	AveExpr	adj.P.Val	Symbol
-1	2.09	0.07	CDKN1A
-1.09	6.56	0.01	NDRG1
0.37	5.31	0.1	PNRC1
-0.81	-1.14	0.14	COL5A1
0.68	-3.65	0.12	CTGF
-1.57	1.22	0	ENO2
-0.45	10.7	0	ALDOA
-1.35	3.42	0.01	ALDOC
-0.21	8.19	0.08	ZNF292
-0.2	4.2	0.13	JMJD6
-0.99	-3.53	0.13	MAFF
-0.29	4.71	0.04	SLC37A4
-0.15	4.84	0.12	CCRN4L
-0.27	4.8	0.06	GALK1
-0.23	10.81	0	GAPDH
0.3	4.73	0.03	TIPARP
-0.73	5.57	0	GBE1
-0.52	4.88	0.01	FAM162A
0.46	4.58	0.01	GLRX
-0.49	10.44	0	GPI
-0.24	6.69	0.05	GYS1
0.42	5.16	0.04	ANXA2
-0.67	7.4	0.02	HK1
-0.84	-4.01	0.04	HOXB9
-0.27	8.84	0.02	HSPA5
0.22	5.42	0.06	IDS
-0.64	-0.81	0.16	CYR61
-0.16	7.06	0.04	RBPJ
-0.26	4.62	0.17	MT2A
-0.51	4.52	0	MXI1
0.12	11.12	0.04	MYH9
-0.41	1.33	0.07	ATF3
-0.89	6.92	0	P4HA1
-0.35	4.99	0.01	PAM
-0.47	5.86	0.02	PDK1
-0.41	4.91	0.01	PFKFB3
-0.23	5.68	0.05	PGM1
0.24	4.72	0.17	ATP7A
-0.6	1.05	0.03	ERRFI1
0.47	4.3	0.08	KLHL24
0.67	-3.58	0.15	PPP1R3C
-1.14	0.98	0	TMEM45A
-0.2	4.86	0.12	ANKZF1
-0.17	6.89	0.01	PGM2
-0.34	6.7	0.04	KDM3A
-0.3	3.11	0.11	RRAGD
0.62	0.36	0.03	RORA
-0.79	1.48	0.01	SDC4
-0.39	2.5	0.01	SIAH2
-0.87	5.95	0	SLC2A1
-1.57	-3.49	0.03	STC1

-0.8	3.4	0.01	TNFAIP3
-0.26	8.65	0.02	TPI1
0.11	5.99	0.06	VHL
0.43	6.46	0.03	CXCR4
0.38	2.92	0.02	CASP6
0.22	5.83	0.04	NDST2
0.28	2.49	0.16	KLF7
-1.62	-2.55	0	STC2
-0.39	0.81	0.14	STBD1
0.78	-3.48	0.11	SELENBP1
0.63	-0.24	0.09	AKAP12
1.03	-2.81	0.05	SDC3

Table S2. HALLMARK_MTORC1_SIGNALING

logFC	AveExpr	adj.P.Val	Symbol
-0.33	5.57	0.06	NAMPT
-0.29	7.16	0.02	PSMD14
-1	2.09	0.07	CDKN1A
-0.11	8.45	0.15	CTSC
0.6	1.7	0.18	FGL2
-0.08	6.93	0.19	COPS5
-0.15	7.54	0.05	IMMT
-0.53	2.77	0.01	STARD4
0.2	3.85	0.09	CTH
-0.22	9.26	0.02	EPRS
-0.3	6.1	0.05	ETF1
-0.45	10.7	0	ALDOA
0.25	7.66	0.01	GGA2
-0.2	8.48	0.06	NUP205
-0.15	6.88	0.07	PITPNB
-0.29	4.71	0.04	SLC37A4
-0.23	10.81	0	GAPDH
-0.73	5.57	0	GBE1
-0.22	3.95	0.1	NUFIP1
-0.34	5.6	0.02	CACYBP
-0.09	5.74	0.17	SERP1
0.46	4.58	0.01	GLRX
-0.49	10.44	0	GPI
-0.19	7.92	0.11	GSR
-0.27	8.84	0.02	HSPA5
-0.44	10.35	0.02	HSPD1
-0.47	6.7	0.09	HSPE1
-0.1	6.76	0.19	IDH1
-0.54	6	0	IDI1
-0.18	6.5	0.08	IFRD1
-0.11	7.43	0.06	M6PR
-0.25	7.77	0.1	MCM2
-0.89	6.92	0	P4HA1
-0.47	5.86	0.02	PDK1
-0.23	5.68	0.05	PGM1
-0.21	6.56	0.17	PLK1
-0.27	7.81	0.01	PPA1
-0.14	6.79	0.2	PSMA4
-0.38	4.23	0.04	PNO1
-0.26	5.19	0.02	PSMB5
-0.11	7.09	0.15	PSMC2
-0.16	5.41	0.05	PSMC6
-0.26	6.26	0.02	PSMD12
-0.21	7.29	0.03	PSMD13
-0.24	4.23	0.13	PSPH
-0.19	5.36	0.05	QDPR
-0.19	6.42	0.01	ELOVL5
-0.43	7.13	0.02	RRM2
-0.87	5.95	0	SLC2A1
-0.32	5.42	0.02	SORD
-1.57	-3.49	0.03	STC1

-0.45	9.76	0.01	TFRC
-0.26	8.65	0.02	TPI1
-0.2	10.36	0.05	HSP90B1
-0.28	6.79	0.09	TUBG1
0.43	6.46	0.03	CXCR4
-0.48	0.49	0.14	ELOVL6
-0.18	4.39	0.09	ADIPOR2
-0.16	10.63	0.03	CANX
0.55	3.73	0	PIK3R3
-0.33	5.85	0.04	PSMG1
-0.16	7.46	0.06	GMPS
0.17	6.8	0.15	SQSTM1
-0.26	6.05	0.01	EIF2S2
-0.3	8.45	0.04	CCT6A
-0.56	4.85	0.01	EEF1E1
-0.19	6.29	0.03	EDEM1
-0.51	5.54	0.02	CDC25A

Table S2. HALLMARK_MYC_TARGETS_V1

logFC	AveExpr	adj.P.Val	Symbol
-0.3	8.05	0.01	UBA2
-0.15	8.75	0.1	TRIM28
-0.2	5.16	0.16	CDK2
-0.29	8.2	0.04	CDK4
-0.29	7.16	0.02	PSMD14
-0.15	8.61	0.13	SYNCRIP
-0.25	8.79	0.13	NOP56
-0.39	8.35	0	CCT4
-0.38	8.55	0.01	CCT2
-0.2	7.12	0.03	PTGES3
-0.16	5.3	0.15	TXNL4A
-0.18	5.09	0.06	RNPS1
-0.12	7.79	0.12	PRDX3
-0.08	6.93	0.19	COPS5
-0.12	6.86	0.19	PWP1
-0.26	8.35	0.02	XPOT
-0.27	8.05	0.01	PHB2
-0.24	7.1	0.02	CBX3
-0.1	5.19	0.16	AP3S1
-0.16	8.97	0.05	DHX15
-0.21	3.01	0.13	EIF1AX
-0.34	6.76	0.02	EIF2S1
-0.11	8.72	0.12	EIF4A1
-0.14	10.68	0.06	EIF4G2
-0.22	9.26	0.02	EPRS
-0.3	6.1	0.05	ETF1
-0.17	8.96	0.07	SF3B3
-0.29	6.84	0.01	SERBP1
-0.26	8.31	0.01	RSL1D1
-0.26	7.88	0.06	GNL3
-0.14	5.73	0.05	MRPS18B
-0.25	6.98	0.1	H2AFZ
-0.22	7.69	0.01	HDAC2
-0.12	7.69	0.19	HDGF
-0.1	9.36	0.2	HNRNPC
-0.33	11.81	0.01	HSP90AB1
-0.44	10.35	0.02	HSPD1
-0.47	6.7	0.09	HSPE1
-0.38	9.37	0.01	IARS
-0.18	6.5	0.08	IFRD1
-0.16	8.69	0.12	ILF2
-0.17	9.4	0.1	IMPDH2
-0.2	9.12	0.04	KARS
-0.23	9.78	0.04	KPNB1
-0.4	5.16	0.02	MAD2L1
-0.25	7.77	0.1	MCM2
-0.22	8.31	0.16	MCM6
-0.26	8.79	0.08	MCM7
-0.36	8.92	0	NPM1
-0.3	7.77	0.08	ODC1
-0.12	6.04	0.14	HDDC2

-0.28	7.45	0.11	PCNA
-0.13	5.37	0.11	ACP1
-0.1	9.37	0.09	SLC25A3
-0.26	6.5	0.06	POLD2
-0.19	6.61	0.01	PSMA1
-0.28	6.97	0.03	PSMA2
-0.14	6.79	0.2	PSMA4
-0.26	6.94	0.09	PSMA7
-0.16	5.41	0.05	PSMC6
-0.16	7.91	0.17	PSMD1
-0.27	6.99	0.01	PSMD7
-0.27	8.01	0.05	RAN
-0.2	6.48	0.13	RFC4
-0.29	6.88	0.02	ABCE1
-0.12	9.81	0.07	RPS5
-0.29	7.89	0.04	RRM1
-0.2	7.98	0.11	SET
-0.11	8.22	0.19	SRSF3
-0.17	7.83	0.08	TRA2B
-0.14	5.87	0.06	MRPL9
-0.27	5.24	0.01	SNRPA1
-0.18	5.84	0.02	SNRPB2
-0.17	6.96	0.11	SNRPD2
-0.59	5.73	0.01	SRM
-0.34	7.48	0.01	SRPK1
-0.31	7.28	0.03	SSB
-0.39	8.75	0	TCP1
-0.33	6.96	0.01	TFDP1
-0.44	7.32	0.02	C1QBP
-0.3	8.73	0.03	CCT3
-0.25	7.44	0.14	TYMS
-0.18	6.57	0.2	USP1
-0.16	4.98	0.05	VBP1
-0.16	7.78	0.12	VDAC3
-0.2	4.72	0.13	EIF4H
-0.23	7.73	0.01	CNBP
-0.16	10.63	0.03	CANX
-0.13	6.5	0.17	CUL1
-0.15	8.53	0.02	EIF3D
-0.2	5.61	0.07	EIF3J
-0.15	8.18	0.05	PABPC4
-0.26	6.05	0.01	EIF2S2
-0.33	7.03	0.04	CCNA2
-0.21	6.28	0.06	COX5A
-0.22	6.52	0.01	TOMM70A

Table S2. HALLMARK_ MYC_TARGETS_V2

logFC	AveExpr	adj.P.Val	Symbol
-0.2	5.22	0.18	RCL1
-0.29	8.2	0.04	CDK4
-0.18	5.7	0.12	PRMT3
-0.23	4.43	0.04	RABEPK
-0.25	8.79	0.13	NOP56
-0.24	7.1	0.02	CBX3
-0.29	7.89	0.01	FARSA
-0.35	7.59	0.02	WDR43
-0.26	7.88	0.06	GNL3
-0.44	10.35	0.02	HSPD1
-0.47	6.7	0.09	HSPE1
-0.36	8.92	0	NPM1
-0.21	6.56	0.17	PLK1
-0.31	5.72	0.02	PPAN
-0.35	4.22	0.03	EXOSC5
-0.31	5.59	0.02	TFB2M
-0.45	4.31	0.01	SLC19A1
-0.32	5.42	0.02	SORD
-0.59	5.73	0.01	SRM
-0.39	4.43	0.01	BYSL
-0.3	7.16	0.03	LAS1L
-0.29	5.31	0.01	TBRG4

Table S2. HALLMARK_P53_PATHWAYS

logFC	AveExpr	adj.P.Val	Symbol
0.2	6.66	0.15	ADA
0.49	3.05	0.01	TOB1
-1	2.09	0.07	CDKN1A
-1.09	6.56	0.01	NDRG1
-0.31	2.27	0.13	PROCR
0.57	4.61	0	HEXIM1
0.24	1.88	0.14	BLCAP
0.13	5.26	0.14	TRAFD1
0.14	5.82	0.08	WWP1
-0.51	1.93	0.02	GADD45A
0.89	2.85	0	ABAT
0.12	7.93	0.17	ERCC5
-0.37	1.2	0.17	F2R
0.71	-4.41	0.15	ZNF365
0.21	5.72	0.01	KIF13B
-0.14	3.57	0.13	RRP8
0.46	5.01	0.03	FUCA1
-0.24	3.56	0.01	RCHY1
-0.52	4.88	0.01	FAM162A
-0.45	5.18	0.01	CYFIP2
-0.21	6.52	0.05	DNTTIP2
-0.22	3.88	0.1	HRAS
-0.2	7.07	0.08	IRAK1
-0.29	10.1	0.01	LDHB
-1.18	-2.12	0.02	LIF
0.21	5.94	0.11	LRMP
-0.41	1.33	0.07	ATF3
-0.2	4.2	0.02	TPRKB
-0.28	7.45	0.11	PCNA
0.62	2.97	0.03	IER5
-0.27	2.57	0.09	TRIAP1
-0.92	-0.37	0.02	PDGFA
0.11	4.28	0.17	RETSAT
-0.09	7.17	0.18	NOL8
-0.23	4.5	0.07	STEAP3
-0.35	1.93	0.04	DRAM1
0.17	3.58	0.11	PRKAB1
-0.3	3.94	0.01	BAX
-0.18	6.2	0.12	RAD51C
-0.31	4.19	0.03	RXRA
0.38	4.12	0.14	SAT1
-0.98	-4.23	0.01	SDC1
-0.75	-2.25	0.13	PERP
-0.38	6.67	0	SLC3A2
-0.4	6.48	0.01	TGFB1
0.89	1.73	0.03	TCHH
-0.23	3.19	0.17	RHBDF2
1.62	-1.98	0.02	SOCS1
0.17	6.6	0.09	VAMP8
0.27	3.97	0.03	TSC22D1
1.24	0.81	0.01	KLF4

-0.15	7.16	0.16	EI24
-0.42	3.47	0	TRAF4
-0.2	5.83	0.06	CCP110

Table S2. HALLMARK_E2F_UNFOLDED_PROTEIN_RESPO

logFC	AveExpr	adj.P.Val	Symbol
-0.19	8.37	0.04	PDIA6
-0.27	7.75	0.03	HYOU1
-0.25	8.79	0.13	NOP56
-0.16	5.27	0.14	YIF1A
-0.16	5.81	0.11	PDIA5
-0.26	8.35	0.02	XPOT
0.12	8.22	0.05	DCTN1
-0.23	6.31	0.09	DDX10
-0.28	8.59	0.08	DKC1
-0.12	10.76	0.17	EEF2
-0.34	6.76	0.02	EIF2S1
-0.11	8.72	0.12	EIF4A1
0.09	6.79	0.11	ATF6
-0.1	7.81	0.19	SKIV2L2
-0.09	5.74	0.17	SERP1
-0.27	8.84	0.02	HSPA5
-0.38	9.37	0.01	IARS
-0.63	-0.11	0.18	IFIT1
-0.1	8.33	0.11	KIF5B
-0.41	1.33	0.07	ATF3
-0.27	6.49	0.01	ATF4
-0.36	8.92	0	NPM1
-0.36	4.71	0.05	GEMIN4
0.13	6.59	0.04	PARN
-0.18	6.5	0.1	EXOSC9
0.17	5.47	0.03	EXOC2
-0.35	4.22	0.03	EXOSC5
-0.27	8.36	0.02	ALDH18A1
-0.22	4.45	0.07	SPCS3
-0.1	9.2	0.17	RPS14
-0.13	7.32	0.04	SRPR
-0.2	10.36	0.05	HSP90B1
-0.24	7.5	0.06	NOP14
-1.62	-2.55	0	STC2
0.17	5.75	0.1	ATP6V0D1
-0.18	4.57	0.06	BAG3
0.1	6.22	0.16	GOSR2
-0.19	6.29	0.03	EDEM1
-0.17	7.27	0.06	EIF4A3