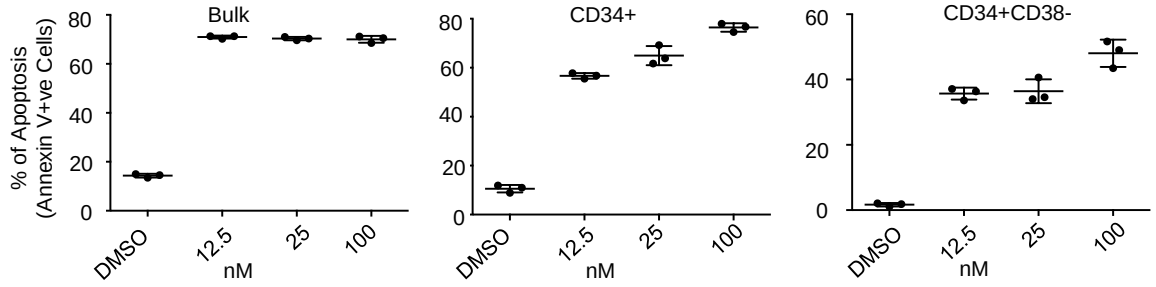
Supplementary Figure 1

F



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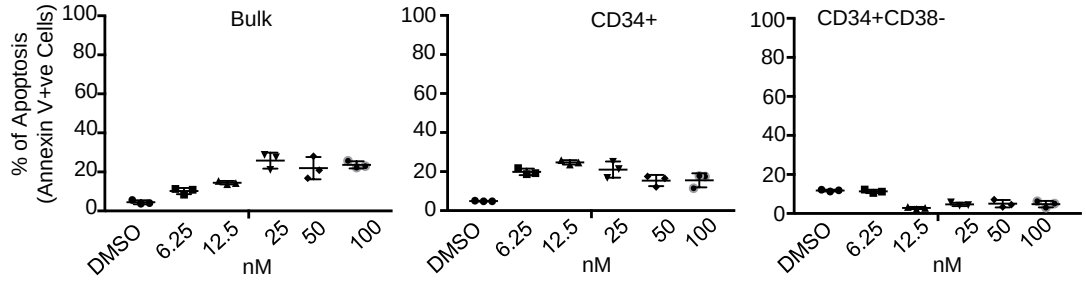
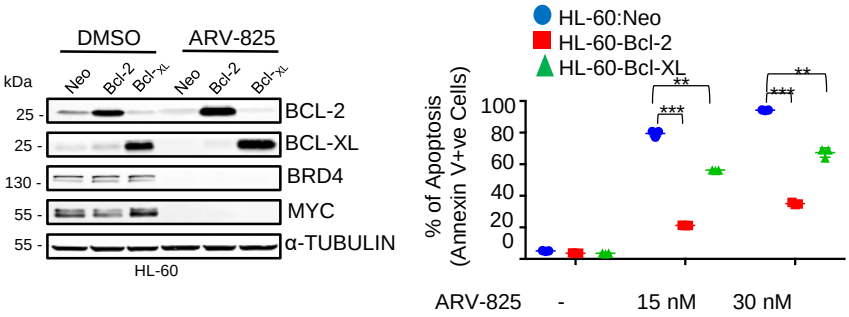
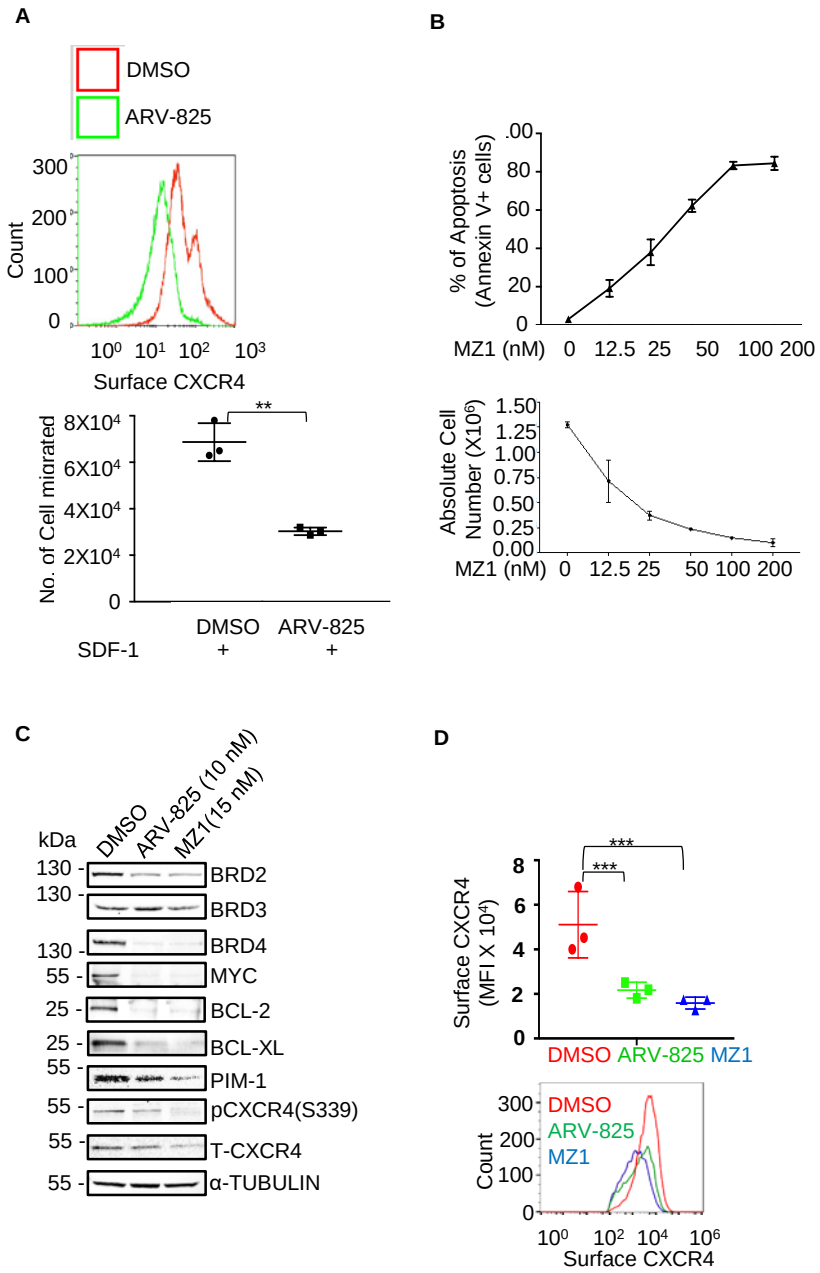


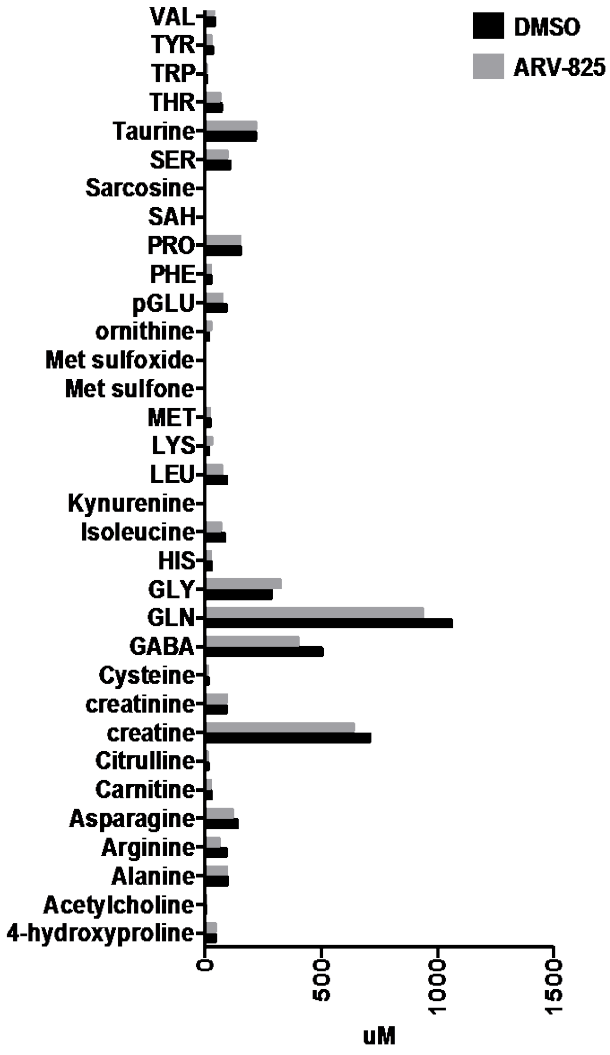
Supplementary Figure 2



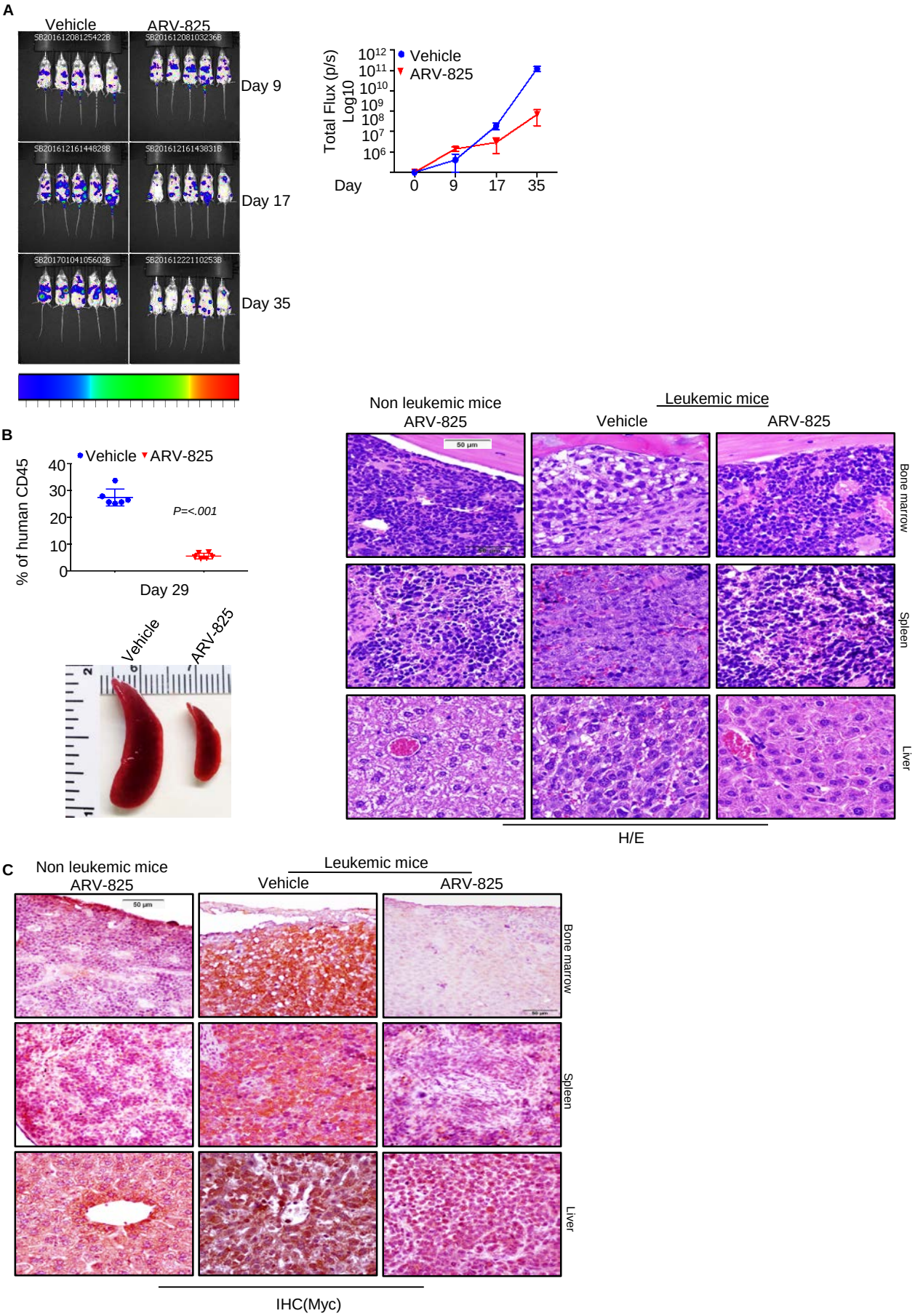
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



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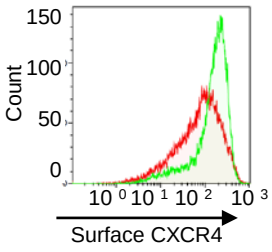
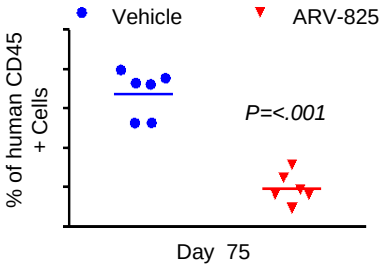


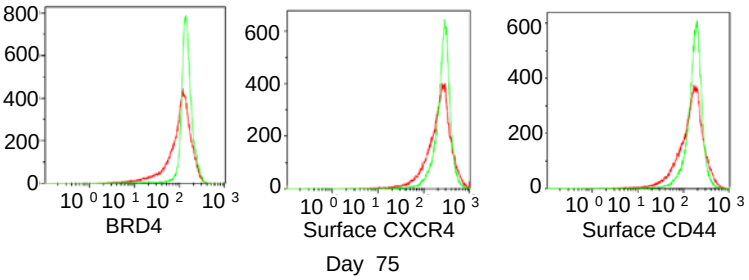
MFI
Vehicle:173.99
ARV-825:85.26

E



F

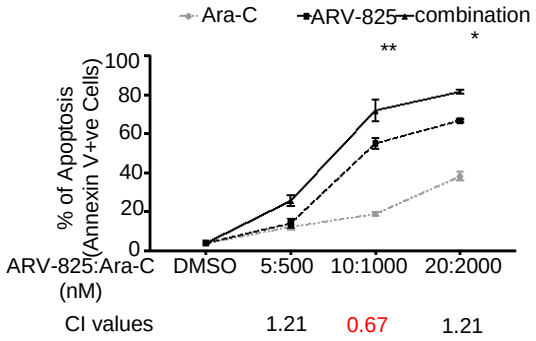
DMSO
ARV-825



Day 75

Supplementary Figure 6

A



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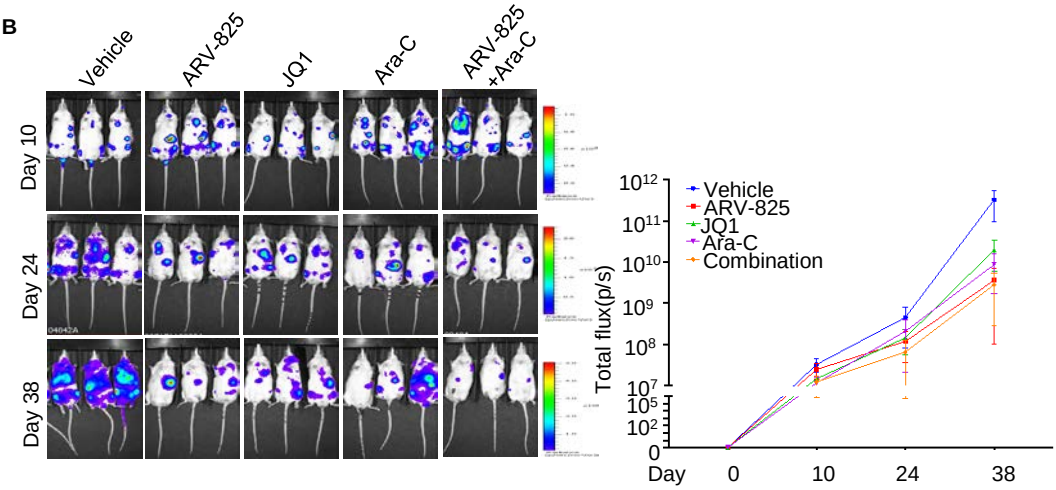

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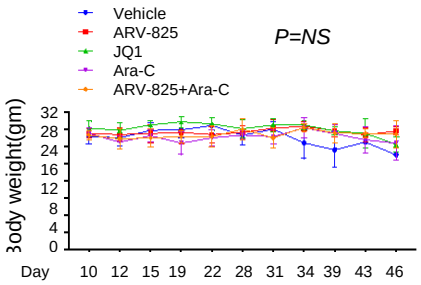
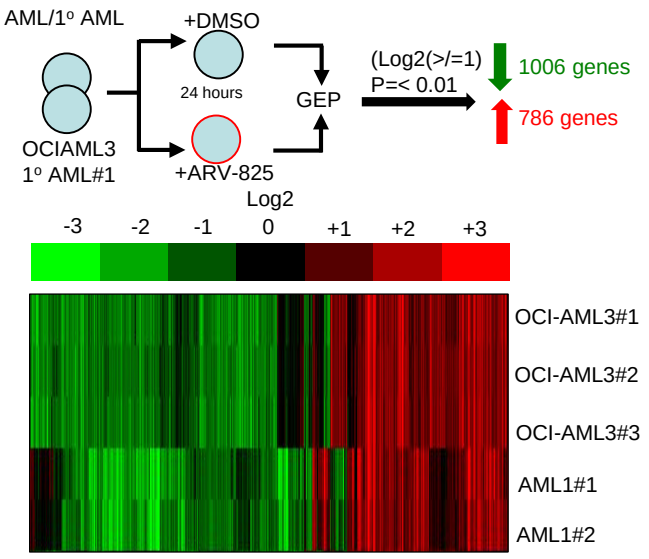
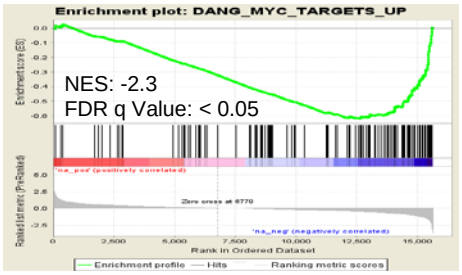
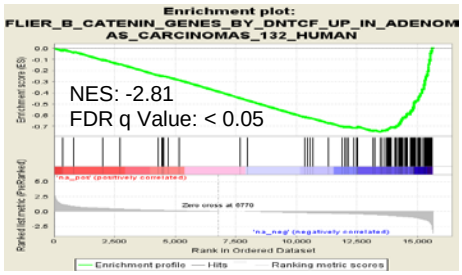


Supplementary Figure 7

A







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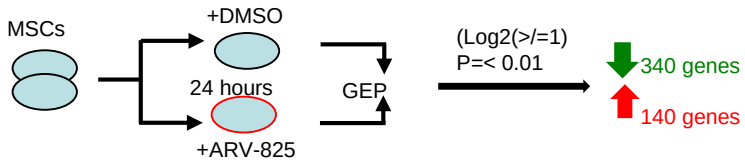

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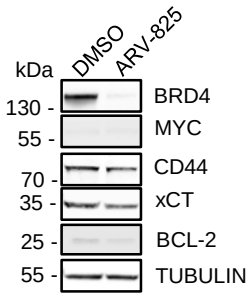
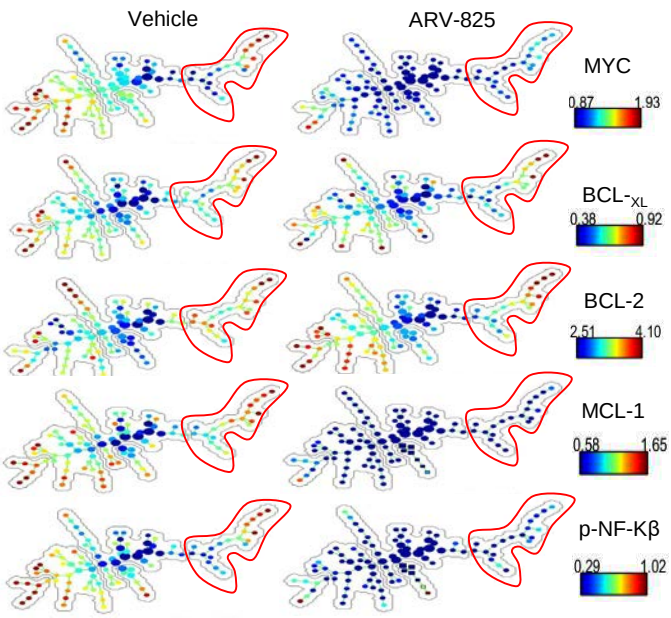


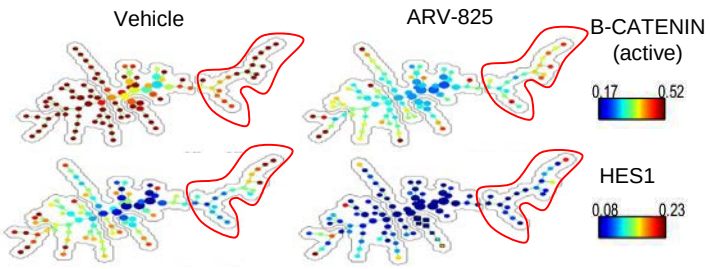
D

MYC Activity



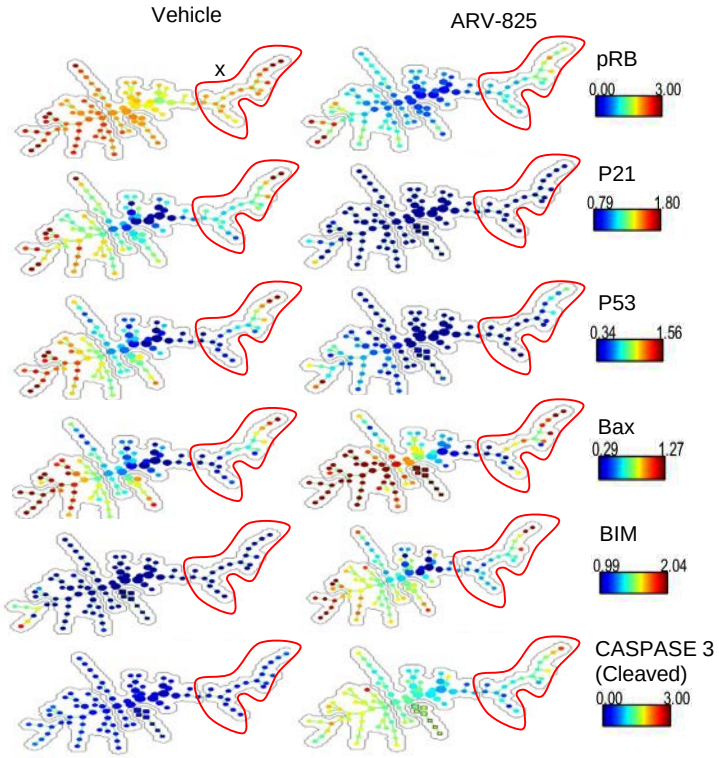
	MYC	BCL-$_{XL}$	BCL-2	MCL-1	p-NF-Kβ
Vehicle	530.8	15.8	33560247.7	262.0	12.2
ARV-825	4.7	9.9	1583032.4	4.2	2.6

E Wnt/b-catenin/Notch pathway



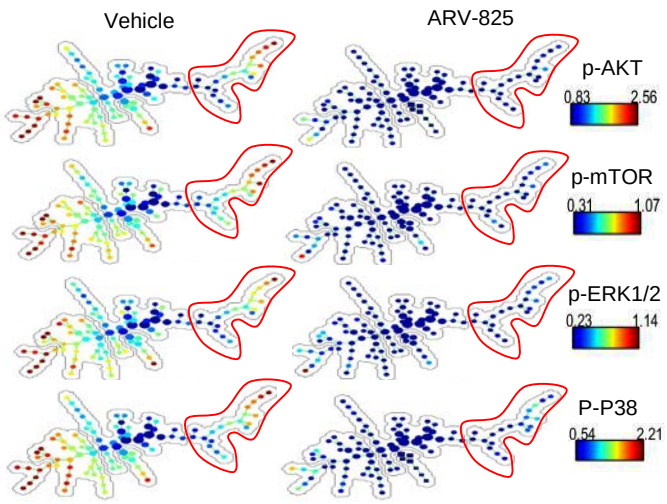
	B-CATENIN(active)	HES1
Vehicle	2.4	0.9
ARV-825	1.0	0.5

F Cell cycle/Apoptosis pathway



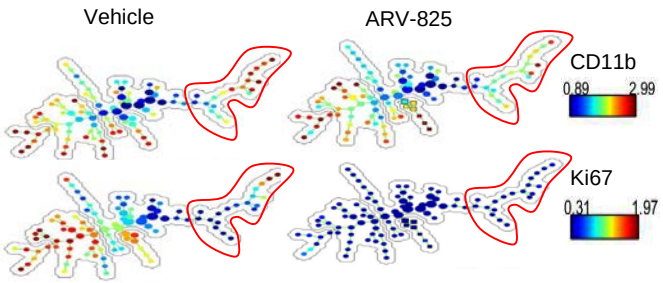
	pRB	P21	P53	Bax	BIM	Caspase 3(cleaved)
Vehicle	44415.9	335.9	22.3	11.3	20.2	2.9
ARV-825	269.2	6.9	3.5	25.9	740.9	205.5

G PI3K/AKT/mTOR Pathway



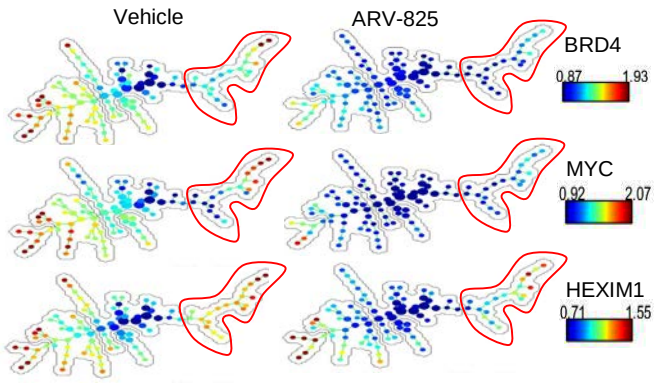
	pAKT	pMTOR	pERK1/2	p-P38
Vehicle	896.8	18.6	8.6	298.5
ARV-825	15.2	2.0	1.4	15.8

H Differentiation/Proliferation



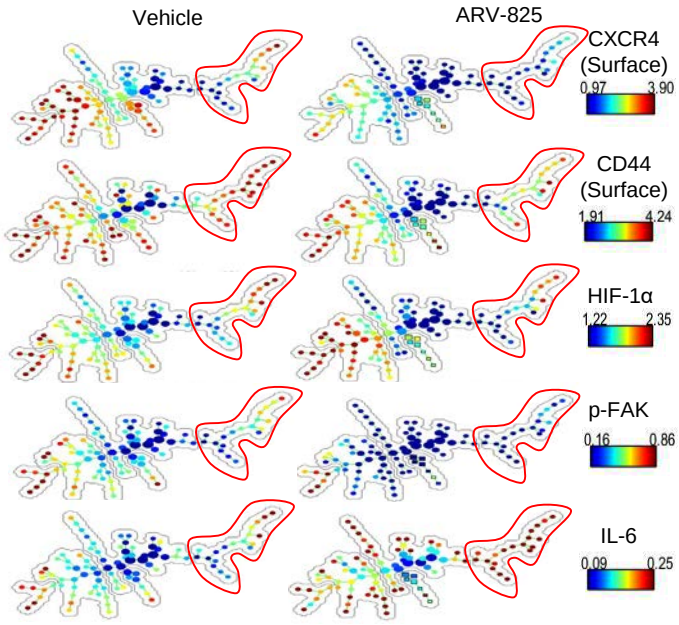
	CD11b	Ki67
Vehicle	28994.3	18.5
ARV-825	5197.1	0.8

I Target inhibition



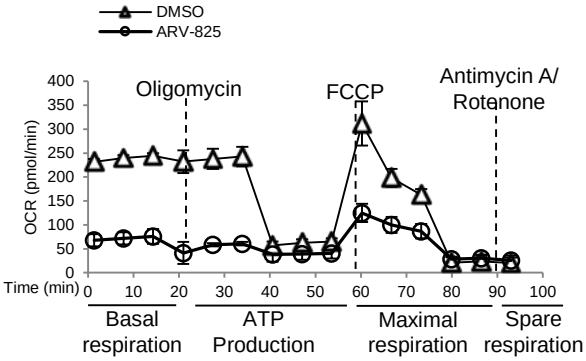
	BRD4	MYC	HEXIM1
Vehicle	500.4	530.8	369.8
ARV-825	7.7	4.7	13.1

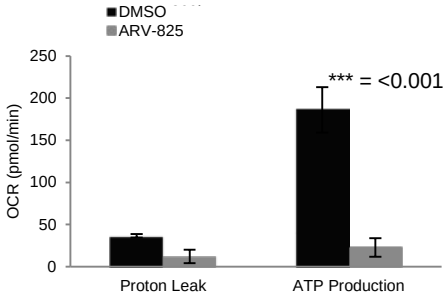
J Tumor microenvironment



	CXCR4	CD44	HIF1a	p-FAK	IL-6
Vehicle	12699.9	115855330.5	4347.5	3.5	0.9
ARV-825	203.7	2772757.4	575.3	1.0	2.7

Supplementary Figure 8





Supplementary Figure 1: (A) AML cell lines were treated with ARV-825 or JQ1 in wide range of concentration for 72 hours. Absolute cell numbers were enumerated using trypan blue staining based Beckmen Vi-CELL counter.

(B/C/) Ficolled AML mononuclear cells were cultured with increasing concentration of either ARV-825 or JQ1 for 72 hours and stained with Annexin V, CD45, CD34 and CD38 to quantify the percentage of apoptosis by flow cytometry.

(D/E/F) Ficolled AML mononuclear cells were cultured with increasing concentration of ARV-825 for 72 hours and stained with Annexin V, CD45, CD34 and CD38 to quantify the percentage of apoptosis by flow cytometry.

(G) normal bone marrow samples were cultured with increasing concentration of ARV-825 as indicated. After 72 hours, cell pellets were stained with Annexin V, CD45, CD34 and CD38 and cell survival in different subpopulations were determined by flow cytometry.

Data are representative of mean ± SD from 3 independent seeding are plotted. The statistical significance between two groups was calculated by a standard Student's *t*-test (*** = <0.001, ** = <0.01, * = <0.05).

Supplementary Figure 2: Stably BCL-2 or BCl-$_{XL}$ overexpressed HL-60 cells were treated with ARV-825 for indicated different concentration for 24 hours or 72hours and then determined the overexpression of protein (Left) and apoptosis (Right) by immunoblotting and flow cytometry respectively. Data represent the mean +/- SD *** = <0.001, ** = <0.01 by Student's t test.

Supplementary Figure 3: Ficolled AML mononuclear cells were treated with ARV-825 (25 nM) or DMSO for 24 hours and surface expression of CXCR4 was determined by flow cytometry. Duplicate samples were subjected to 4 hours post incubation in SDF-1 (100 ng) containing media, the total number of migrated cells were measured (n=3)**.** Data represent the mean +/- SD *** = <0.001, ** = <0.01 by Student's t test.

(B) OCIAML3 cells were treated with different concentration of MZ1 for 72 hours and apoptosis and cell proliferation were determined by flow cytometry in three independent samples. (C) Immunoblot analysis of different proteins (left) and flow cytometry to surface expression of CXCR4 (Right) in OCIAML3 treated with ARV-825 (10 nM), MZ1(15 nM) for 24 hours. Data represent the mean +/- SD *** = <0.001, by Student's t test.

Supplementary Figure 4: OCI-AML3 cells treated with ARV-825 (10 nM) for 24 hours and mass

spectrometry-based analysis was performed to assess changes in intracellular amino acids.

Supplementary Figure 5: NOD/SCID/IL-2rγnull (NSG) mice (6-wk-old) were injected with luciferase-labeled OCI-AML3 cells ($1X10^6$ cells) or AML-PDX ($1X10^6$ cells) through tail vein and ARV-825 (10 mg/kg) administered twice a week intra-peritoneally. (A) Mice were imaged and total flux counts compared to document engraftment and reduction of leukemia burden as indicated. (B) At d 29, 50 μL of peripheral blood was collected by intra-orbital bleeding and tumor burden measured by detection of human CD45 cells (left top panel). At day 34 one mice from each group were sacrificed assess spleen size (left bottom panel) and Infiltration of Leukemia in other organs as indicated by hematoxylin and eosin staining (Right panel) (C) Bone marrow, spleen and Liver from each group were subjected to immunohistochemistry to detect the target inhibition, as Myc. The images were under the 60X magnification and 50 μM scale. (D) Surface expression of CXCR4 in BM flushed by flow cytometry.

AML-PDX: (E) At d 75, 50 μL of peripheral blood was collected by intra-orbital bleeding and tumor burden measured by detection of human CD45 cells. *** = <0.001, ** = <0.01 by Student's t test.

(F) Target inhibition as BRD4 and surface expression of CXCR4 and CD44 was confirmed in PB.

Supplementary Figure 6: (A) OCIAML3 cells were treated with different concentration of ARV-825 and Ara-C in single or combination for 72 hours and apoptosis was measured with flow cytometry in 3 independent samples. Cumulative index (CI) at Ara-C (1000 nM) and ARV-825 (10nM) in highlighted as red showing the significant synergistic effect. Data represent the mean +/- SD ** = <0.01, * = <0.05 by Student's t test.

NOD/SCID/IL-2rγnull (NSG) mice (6-wk-old) were injected with luciferase-labeled OCI-AML3 cells ($1X10^6$ cells) through tail vein and ARV-825 (10 mg/kg), JQ1 (50 mg/kg), Ara-C (50 mg/kg) and combination of ARV-825 and Ara-C were administered twice a week intra-peritoneally. (B) Mice were imaged and total flux counts compared to document engraftment and reduction of leukemia burden as indicated. (C) Body weight of all group mice were measured from day 10 to 46 (NS=non-significant).

Supplementary Figure 7: Gene set enrichment analysis (GSEA) was performed on Illumina GEP data on OCIAML3/Primary AML or MSCs cells treated with ARV-825 for 24 hours. (n=3) (A) AML: Gene expression profiling analysis (Top panel) and the heatmap (Bottom panel) generated shows mRNAs with a change in Log2 value ≥ 1) and $P \leq 0.01$. Gene set enrichment analysis (GSEA) for Myc target and Wnt/B-catenin signaling in ARV-825 treated case (right panel).
(B) MSC: Gene expression profiling analysis shows mRNAs with a change in Log2 value ≥ 1) and $P \leq 0.01$ (C) Validation of inhibition of target in MSCs with ARV-825 treatment MSCs were treated with DMSO or ARV-825 for 24 hours. The whole cell lysates were subjected to immunoblotting with specific antibodies. Tubulin was used as loading control
BM cells were collected from Vehicle/ARV-825 treated mice with AML-PDX and SPADE tree analysis of mouse BM cell populations determined by CyTOF. COLORS SPADE tree according to its colored versions based on (intensities) of different proteins were illustrated while ArcSinh-transformed counts for each proteins were illustrated in Boxes in CD34+CD38-CD90-CD45RA+ LSC (dotted red box) for (D) Myc activity PI3K/AKT/mTOR, (E) WNT/B-CATENIN (F) Cell cycle/Apoptosis, (G) PI3K/AKT/mTOR (H)differentiation/proliferation (I) target inhibition and (J)tumor microenvironment related protein expression.

Supplementary Figure 8: Oxygen consumption rate (OCR) measurements were obtained over time (in min) using an extracellular flux analyzer (Seahorse Bioscience). The mitochondrial metabolic activity test was used to obtain bioenergetics parameters, by adding the ATP synthase inhibitor Oligomycin A (2 μM), to derive ATP-linked OCR, FCCP (1.6 μM) to uncouple the mitochondria for maximal OCR, and antimycin A (0.5 μM).

Supplementary table

Table 1: Comparison of IC50 between ARV-825 and JQ1

Cell line	Cell types	Characteristics	IC_{50} to ARV-825	IC_{50} to JQ1
MV4;11	AML-M5	ITD(m/m), t(4;11)	2.02 pM	60 nM
OCI-AML3	AML-M4	NPMc+, DNMT3Am (R822C), NRASm(Q61L)	8.3 nM	102 nM
HL60	AML-M2	NRASm (Q61L)	13.7 nM	349.4 nM
MOLM14	AML-M5a	ITD, t(9;11)	18.7 nM	98.3 μM
KBM5	CML-BC	BCRABL	26.7 nM	2.5 μM
OCI-AML2	AML-M4	NPMwt, DNMT3Am(R635W)	35 nM	525 nM

U937	AML-M5	FLT3WT/CALM-AF10	44.1 nM	151.7 nM
MOLM13	AML-M5a	ITD (m/w), t(9;11)	54.7 nM	2321 nM
THP1	AML-M5	NRASm (G12D)	64.2 nM	528.5 nM

AML cell lines were treated with ARV-825 or JQ1 in wide range of concentration for 72 hours. Based on inhibition of cell proliferation IC50 values were calculated using Calcusyn software.

Table 2: Sustained effect of ARV-825

Day 3 Absolute Cell number($X10^6$)			Day 3 % of Apoptosis(Annexin V+ cells)		
DMSO	ARV-825	JQ1	DMSO	ARV-825	JQ1
0.75 ± 0.06	0.17 ± 0.02	0.6 ± 0.05	2.63 ± 0.02	66.9 ± 6.6	4.4 ± 0.03

OCI-AML3 cells were treated with ARV-825 (10 nM), JQ1 (100 nM) or DMSO for 24 h. Cells were washed with PBS to remove the respective drugs and re-plated in equal numbers in complete media without any drug for an additional 48 h. After 48 h, cell numbers and percentage apoptosis were assessed.

Table 3: Antibodies used in CyTOF

Antigen	Conjugate	Supplier	Catalog number
ARC	170Er	SantaCruz	sc-374177
Bax	173Yb	CST	2774BF
b-catenin, active	150Nd	EMD	05-665
Bcl-2	158Gd	BioLegend	658702
Bcl-xL	141Pr	CST	2764BF
Bcl-xL	141Pr	CST	2764BF
BIM (EL, L, S)	146Nd	CST	2819BF
BRD4	153Eu	BETHYL	A301-985A50
Caspase 3, Cleaved	142Nd	DVS-Sunnyvale	3142004A
CD11b	144Nd	DVS-Fluidigm	3144001B
CD123	145Nd	BD	554527
CD134	148Nd	BioLegend	350002
CD34	148Nd	BD	555820
CD38	168Er	BioLegend	303502
CD44	171Yb	BioLegend	103002
CD44	171Yb	DVS-Sunnyvale	3171003B
CD45	139La	BioLegend	304002
CD45RA	170Er	BioLegend	304102
CD86	150Nd	DVS-Fluidigm	3150020B
CD90	143Nd	BioLegend	328102
c-Myc	163Dy	CST	5605BF
CXCR4	172Yb	BioLegend	306502
Hes1	151Eu	Abcam	ab55265

HEXIM1	161Dy	Proteintech	15676-1-AP
HIF-1a	165Ho	Novus	NB100-479
IL-6	147Sm	DVS-Fluidigm	3147002B
Ki67	176Yb	BioLegend	350502
LKB1	154Sm	CST	3047BF
Mcl-1	160Gd	CST	5453BF
Mcl-1	176Yb	BD	559027
p21	160Gd	Abcam	ab16767
p21, WAF1/Cip1	154Sm	Sigma	P1484
p53	169Tm	BioLegend	645702
p-AKT	144Nd	BD	560397
p-AKT 152Sm	152Sm	DVS-Fluidigm	3152005A
p-AMPKa(T172)	164Dy	CST	2535BF
p-ERK1/2	167Er	DVS-Sunnyvale	3167005A
p-FAK(Y397)	175Lu	CST	8556BF
P-mTOR	164Dy	CST	5536BF
p-NFkB	149Sm	CST	3033
P-p38(180/182)	156Gd	DVS-Sunnyvale	3156002A
p-Rb(S807/S811)	162Dy	BD	558389
p-STAT5(Y694)	175Lu	CST	9314BF
p-SYK	149Sm	BD	558167
p-JNK	153Eu	CST	9255BF

Table 4: Primer Sequences

No.	Primer	Forward (5'-3')	Reverse (5'-3')
1.	CD44	GCAGTCAACAGTCGAAGAAGG	TGTCCTCCACAGCTCCATT
2.	CD44v6	TCC AGG CAA CTC CTA	CAG CTG TCC CTG TTG
3.	CD44v8-10	TCCCAGACGAAGACAGTCCCTGGAT	CACTGGGGTGGAATGTGTCTTGGTC
4.	AXIN2	CCACACCCTTCTCCAATCC	TGCCAGTTTCTTTGGCTCTT
5.	Fra-1	AGTCAGGAGCTGCAGTGGATGGT	TCAGTTCCTTCCTCCGGTTCCTGC
6.	18S rRNA	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG

Table 5: Details of primary human AML sample used in this study

Patient	Sex	Age(yrs)	Mutation	Blast	Experimental figure
PT1	**M**	**58**	**FLT3(D835)**	**91%**	**1D**
PT2	**M**	**86**	**ASXL, TP53, MPL**	**39%**	**2C/Suppl 1B**
PT3	**F**	**64**	**JAK2, MPL, WT1, CEBPA**	**98%**	**Suppl 1C**
PT4	**M**	**33**	**KIT, IDH2**	**76%**	**Supp. 1D**
PT5	**F**	**62**	**RUNX1, IDH2, FLT-ITD**	**70%**	**Suppl 1E**

	PT6	F	82	NPM1,IDH2	77%	Suppl 1F/ suppl 3A
	PT7	M	33	FLT3(D835),NRAS,KRAS	58%	7A/D / supple 7A

104
105

106