

Lysine specific demethylase 1 inactivation enhances differentiation and promotes cytotoxic response when combined with all-trans retinoic acid in acute myeloid leukemia across subtypes

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RNA-seq Methods

Cells were seeded in flasks and treated with 0.1% DMSO, 1 μ M GSK2879552, 100 nM ATRA, or the combination of 1 μ M GSK2879552 and 100 nM ATRA for 2 or 4 days. After treatment, a minimum of 10 million cells were harvested, centrifuged, and resuspended in TRIzol (Invitrogen), then snap frozen on dry ice and stored at -80°C. RNA was purified from cells stored in TRIzol using the RNeasy Mini Kit (Qiagen). Libraries were prepared using TruSeq Stranded Total RNA Library Prep Kit (Illumina). Libraries were sequenced on an Illumina HiSeq2500 to ~50 million PE 2 x 101bp reads (Illumina). Trimmomatic¹ adapter reads were aligned to GRCh38 using STAR.² HTSeq-count was used to generate a count matrix of genes using intersection-strict mode for exon feature type (gencode.v24.annotation.gtf).³

Library size normalization and differential gene expression for individual cell line at both timepoints for treatment versus DMSO was performed using DESeq2.⁴ Genes were considered significantly differentially expressed at $|\log_{2}FC| \geq 1$ and adjusted p-value ≤ 0.05 threshold.

The union of significant genes for combination vs DMSO at each timepoint was identified for each cell line. Using the intersect genes from all these unions, enrichment in KEGG pathways (MsigDB) was determined with the hypergeometric test.^{5, 6} These genes were also tested for enrichment of a subset of KEGG pathways using a method based on the central limit theorem (CLT).⁷ A subset of pathways was used to focus the analysis and reduce the number of multiple tests (from n=182 to n=145).

MSigDB_KEGG_pathways_removed_from_CLT_GSEA
KEGG_PORPHYRIN_AND_CHLOROPHYLL_METABOLISM
KEGG_LIMONENE_AND_PINENE_DEGRADATION
KEGG_DORSO_VENTRAL_AXIS_FORMATION
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES
KEGG_CIRCADIAN_RHYTHM_MAMMAL
KEGG_OLFACTORY_TRANSDUCTION
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON
KEGG_MELANOGENESIS
KEGG_TYPE_II_DIABETES_MELLITUS
KEGG_MATURITY_ONSET_DIABETES_OF_THE_YOUNG
KEGG_VASOPRESSIN_REGULATED_WATER_REABSORPTION
KEGG_ALZHEIMERS_DISEASE
KEGG_AMYOTROPHIC_LATERAL_SCLEROSIS_ALS
KEGG_PRION_DISEASES

KEGG_EPITHELIAL_CELL_SIGNALING_IN_HELICOBACTER_PYLORI_INFECTION
KEGG_LEISHMANIA_INFECTION
KEGG_ASTHMA
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS
KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM
KEGG_DILATED_CARDIOMYOPATHY
KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS
KEGG_OOCYTE_MEIOSIS
KEGG_LONG_TERM_DEPRESSION
KEGG_TASTE_TRANSDUCTION
KEGG_PROGESTERONE_MEDIATED_OOCYTE_MATURATION
KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY
KEGG_TYPE_I_DIABETES_MELLITUS
KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION
KEGG_PROXIMAL_TUBULE_BICARBONATE_RECLAMATION
KEGG_PARKINSONS_DISEASE
KEGG_HUNTINGTONS_DISEASE
KEGG_VIBRIO_CHOLERAЕ_INFECTION
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION
KEGG_AUTOIMMUNE_THYROID_DISEASE
KEGG_ALLOGRAFT_REJECTION
KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC
KEGG_VIRAL_MYOCARDITIS

Supplemental References

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Supplemental Figures

Supplemental Table 1: A, Known characteristics of patient-derived samples evaluated in Figure 6.

Supplemental Figure 1: A, Comparison of maximum inhibition values for 10 AML cell lines treated with a titration of GSK2879552 for 6 (x-axis) and 10 (y-axis) days. **B**, Comparison of EC₅₀ values for 10 AML cell lines treated with a titration of GSK2879552 for 6 (x-axis) and 10 (y-axis) days. **C**, Cell cycle phase distribution of MOLM-13 cells treated with a titration of GSK-LSD1 for 6 days. **D**, Representative flow cytometric histograms of CD11b protein expression on THP-1 cells (left) and CD86 protein expression on MOLM-13 cells (right) treated with GSK2879552 for 1 day. **E**, Representative dose response curves of CD11b protein expression on THP-1 cells (top) and CD86 protein expression on MOLM-13 cells (bottom) treated with GSK2879552 for 1 day. **F**, The change (treatment-vehicle) in the percentage of cells positive for CD86 (blue), CD11b (red), and CD71 (green) protein expression (+/- standard error) for 6 AML cell lines treated with 500 nM GSK2879552 (or 1000 nM GSK2879552 for CD86 and CD11b only, indicated by *) for 3 days. Significance ($p < 0.05$) between DMSO and GSK552 treatment is indicated with a †.

Supplemental Figure 2: A, Percent maximum inhibition (blue bars) +/- standard error and IC₅₀ values (red circles) +/- standard error for AML cells treated with a titration of GSK2879552 +/- 1, 10, 100, or 1000 nM ATRA for 4 days. Significance ($p < 0.05$) between DMSO and ATRA treatments is represented as * for maximum inhibition and as † for IC₅₀. Higher concentrations of ATRA for SIG-M5 were not evaluable.

Supplemental Table 2: A-B, Values based on Bliss Independence analysis for 7 AML cell lines treated with indicated concentrations of GSK2879552 and ATRA for 4 days (A) and 6 days (B). Values > 20, representing strong synergy, are colored dark green. Values > 10 - 20, representing weak synergy, are colored light green. Values < -10, representing antagonism, are colored gold.

Supplemental Figure 3: A, Venn diagrams depicting the overlap of significantly differentially expressed genes in the specific treatments (GSK2879552 + ATRA combination relative to DMSO, ATRA relative to DMSO and GSK2879552 relative to DMSO) for MV4-11, HL-60, THP-1, and Kasumi-1 cell lines. The top row shows day 2 samples, and bottom row shows day 4 samples. **B**, Corresponding gene expression heatmaps depicting the union of genes from day 2 and day 4 Venn diagrams (from A) for MV-4-11, THP-1, HL-60 and Kasumi-1 cell lines. Values are row-mean centered, log₂ normalized, gene expression data shown high (yellow) to low (blue). Top row indicates treatment type DMSO, GSK2879552, ATRA or GSK2879552 + ATRA combination in tan, magenta, salmon and green, respectively. Bottom row indicates day 2 in black and day 4 in red. **C**, Gene expression fold change relative to DMSO from RNA-seq study and from qRT-PCR validation. **D**, Colorplot indicating the significantly enriched KEGG pathways (MSigDB) as determined using the central limit theorem

separated by day for combination relative to DMSO. Adjusted p-values are shown for pathways that were significant in at least one condition (day, cell line). Color indicates the level of significance from adjusted p-value 0.1 in white to 0 in red. Significance values for day 2 is shown in the top panel and day 4 in the bottom panel. **E**, Barplots showing the top 10 KEGG pathways found to be significantly enriched from significantly differentially expressed genes for each treatment relative to DMSO for each cell line (union of day 2 and day 4 genes). Bars show the $-\log(\text{FDR p-value})$ on the x-axis and the top ten MSigDB KEGG pathways identified as enriched in at least one treatment on the y-axis.

Supplemental Figure 4: A, Percent CD11b+ expression (blue bars) on AML cell lines treated with the indicated concentrations of GSK2879552 and ATRA for 2 days. The value representing the predicted additive effect at each combination concentration is indicated with a red circle. **B**, Representative histograms of CD11b expression on THP-1 cells treated with DMSO (green), 1000 nM GSK2879552 (orange), 100 nM ATRA (blue), or 1000 nM GSK2879552 plus 100 nM ATRA (red) for 2 days. **C**, Shading represents median fluorescence intensity values of CD11b expression relative to additive threshold on THP-1 cells treated with the indicated concentrations of GSK2879552 and ATRA for 2 days. Dark green shading indicates > 10-fold above additive threshold, light green indicates > 5 – 10 fold above additive threshold, light brown indicates 1 - 5 fold above additive threshold. **D**, May Grunwald/Giemsa staining of MOLM-13 cells treated for 3 days with DMSO, 500 nM GSK-LSD1, 1000 nM ATRA, or the combination of 500 nM GSK-LSD1 plus 1000 nM ATRA.

Supplemental Table 4: A, EC₅₀ values of dose response curves for cell lines treated for two days with a titration of GSK2879552 plus and minus the indicated concentration of ATRA.

Supplemental Figure 5: A, GDI values of AML cell lines treated with 1000 nM GSK2879552, 100 nM ATRA, or 1000 nM GSK2879552 + 100 nM ATRA for 1 to 6 days. Significance ($p < 0.05$) is indicated by † between GSK552 and COMBO; by ‡ between ATRA and COMBO; and by * between both GSK552 and COMBO as well as ATRA and COMBO. Cells must reach a minimum doubling of 0.75 to be evaluated. **B**, Fold caspase 3/7 cleavage values from day of peak induction (blue bars) for AML cell lines treated with the indicated concentrations of GSK2879552 and ATRA. The value representing the additive effect at each combination concentration is indicated with a red circle. **C**, Annexin V staining of MOLM-13 cells treated for 3 days with a titration of GSK-LSD1, a titration of ATRA, or a titration of GSK-LSD1 plus 1000 nM ATRA. **D**, Annexin V and CD11b staining of MOLM-13 cells treated for 1-3 days with the indicated concentrations of GSK-LSD1 or ATRA.

Supplemental Figure 6: A, Maximum percent of inhibition achieved for 9 AML patient samples treated with a titration of GSK2879552 +/- 100 nM ATRA or 100 nM ATRA. **B**, IC₅₀ values of the GSK2879552 dose response curves +/- 100 nM ATRA for 9 AML patient samples.

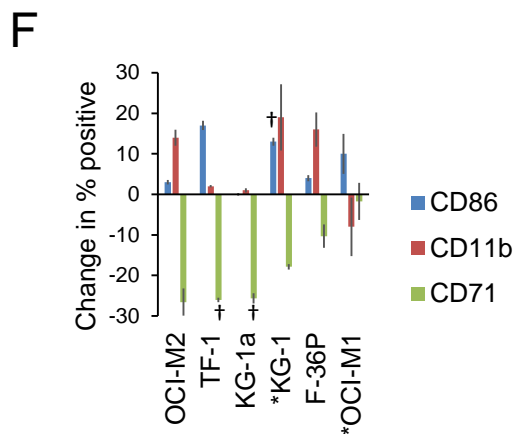
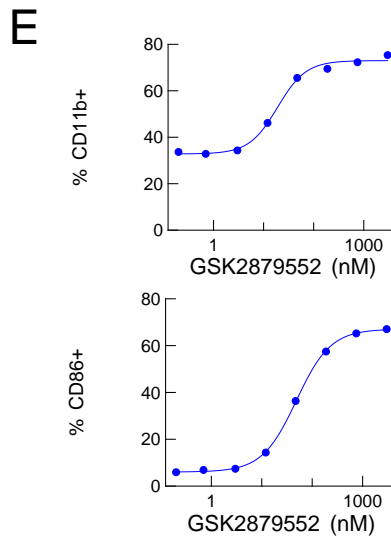
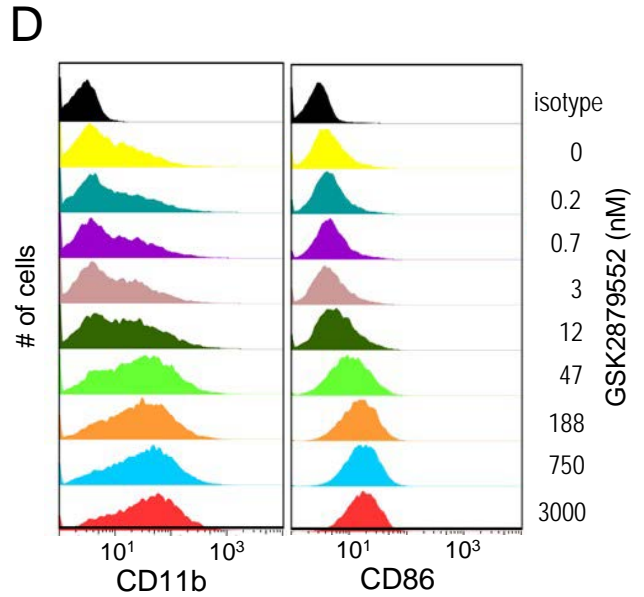
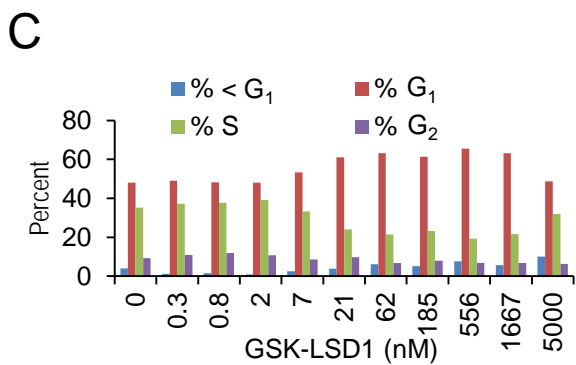
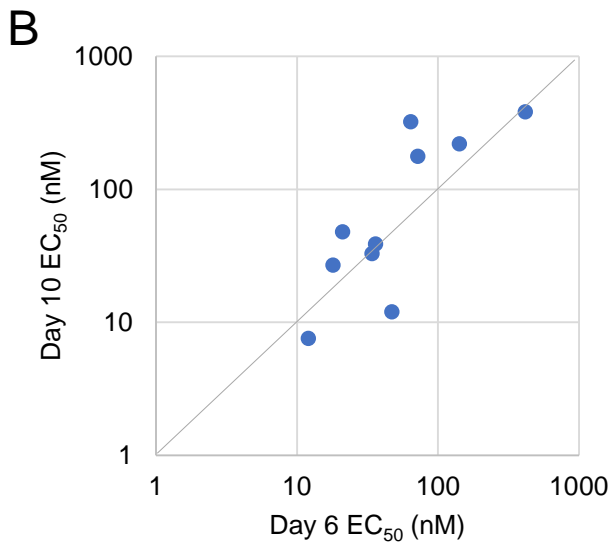
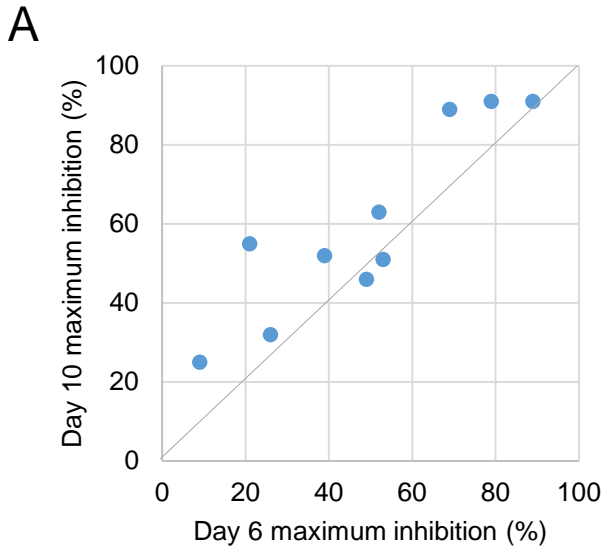
Supplemental Table 1

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Donor ID	Abbreviation	Disease Status	Gender	Age
VM-BM0007	AML0007	Newly diagnosed	Male	76
EBO-BM0169	AML0169	Newly diagnosed	Female	Not available
BBM1000-E1110032867062013SH	AML620	Newly diagnosed	Male	Not available
BBM1000-E1110032840070113SH	AML701	Newly diagnosed	Male	58
BBM1000-H1120108453061213SH	AML612	Newly diagnosed	Male	29

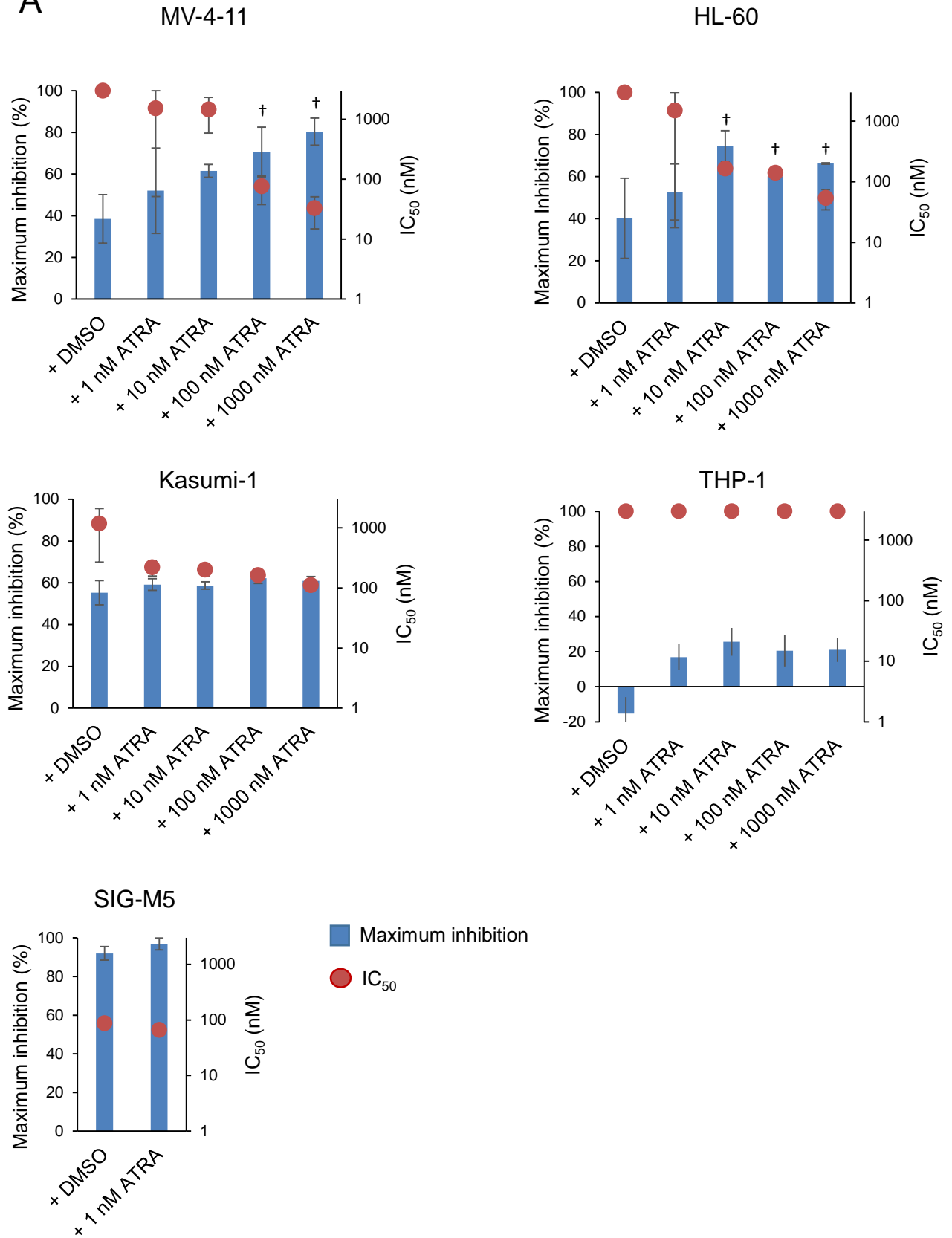
Donor ID	Abbreviation	Primary Diagnosis	Gender	Age	Race	Clinical Stage
BBM1000-E2110029229111912SH	11912SH	Acute Myeloid Leukemia	Female	76	White	Not Available
BBM1000-A1120078464031113SH	4031113SH	Acute Myeloid Leukemia	Male	69	White	Not Available
BBM1000-E7120166684103113SH	4103113SH	Acute Myeloid Leukemia	Male	45	White	M5
BBM1000-T6110001096071514HS	71514SH	Acute Myeloid Leukemia	Female	83	Non-hispanic	Not Available

Supplemental Figure 1



Supplemental Figure 2

A



Supplemental Table 2

A

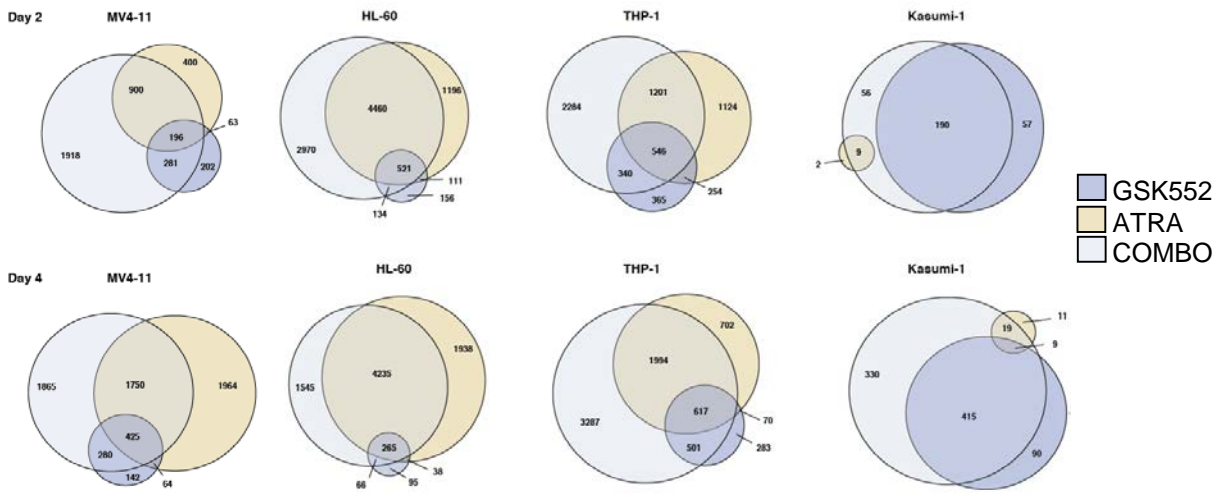
		Bliss Independence Day 4										
		GSK552 (nM)										
		3000	1000	333	111	37	12	4.1	1.4	0.5	0.2	
ATRA (nM)	1000	30.2	30.5	35.9	39.4	41.3	32.9	28.1	23.4	29.0	27.3	THP-1
	100	34.4	37.9	47.3	39.9	48.3	41.4	36.4	37.1	32.0	33.9	
	10	40.8	44.7	45.2	48.7	46.8	38.2	35.3	25.4	35.9	30.2	
	1	36.8	39.6	45.3	45.4	41.2	33.7	33.4	27.7	21.5	30.4	
ATRA (nM)	1000	7.6	10.1	9.9	9.1	5.5	6.6	4.7	5.4	2.3	5.9	OCI-AML3
	100	12.5	14.1	15.4	13.0	5.6	6.1	8.1	7.0	3.9	8.7	
	10	24.8	24.7	19.9	20.6	15.7	16.8	18.2	18.3	16.6	27.6	
	1	17.6	25.3	18.9	23.6	12.5	31.3	31.7	31.1	18.7	41.2	
ATRA (nM)	1000	1.6	1.0	1.6	1.5	1.2	0.9	0.5	0.6	-1.0	0.7	MV-4-11
	100	41.1	30.6	37.7	41.4	44.7	51.5	50.7	46.8	39.0	49.6	
	10	2.4	-7.5	0.1	0.2	-0.3	-2.1	-0.4	0.8	-1.1	2.4	
	1	12.6	10.4	21.6	14.2	18.7	8.2	8.5	14.2	-12.0	20.8	
ATRA (nM)	1000	4.1	4.9	6.9	7.3	5.2	7.3	6.7	5.2	8.0	5.9	HL-60
	100	2.8	6.0	6.9	5.5	4.2	5.3	6.6	4.2	7.9	8.5	
	10	7.4	10.3	10.7	8.6	7.5	12.8	11.0	10.5	16.3	9.7	
	1	11.6	16.1	20.0	12.1	27.0	8.8	10.8	-5.6	21.8	25.9	
ATRA (nM)	1000	0.7	0.8	0.8	0.8	0.5	-0.8	-1.6	-0.7	-0.9	-0.9	MOLM-13
	100	20.1	25.1	29.4	37.7	38.3	36.4	31.6	34.6	1.7	-1.8	
	10	21.2	25.0	22.5	17.5	9.6	-3.3	-3.4	-1.1	-2.6	12.6	
	1	1.6	7.1	0.2	1.7	-5.2	-19.7	-12.1	-7.4	1.9	8.1	
ATRA (nM)	1000	7.5	6.3	7.3	11.6	9.3	5.5	2.1	2.9	-1.1	0.9	Kasumi-1
	100	9.0	6.4	6.8	7.3	7.2	6.0	4.2	-0.2	0.2	2.2	
	10	5.1	3.7	5.9	-23.2	4.2	0.7	1.0	-3.7	-4.5	-3.7	
	1	6.3	5.2	4.1	5.4	4.5	1.1	2.0	3.4	7.4	1.5	
ATRA (nM)	1000	-1.6	-0.6	-0.3	0.3	1.1	1.4	1.7	1.6	1.4	1.3	SIG-M5
	100	-1.6	-0.7	-0.3	0.2	1.1	1.4	1.6	1.6	1.5	1.2	
	10	-1.7	-0.9	-0.6	0.1	1.6	1.4	-1.1	1.6	2.0	0.2	
	1	1.2	3.3	0.6	3.2	15.5	10.4	15.4	14.9	6.9	12.7	

B

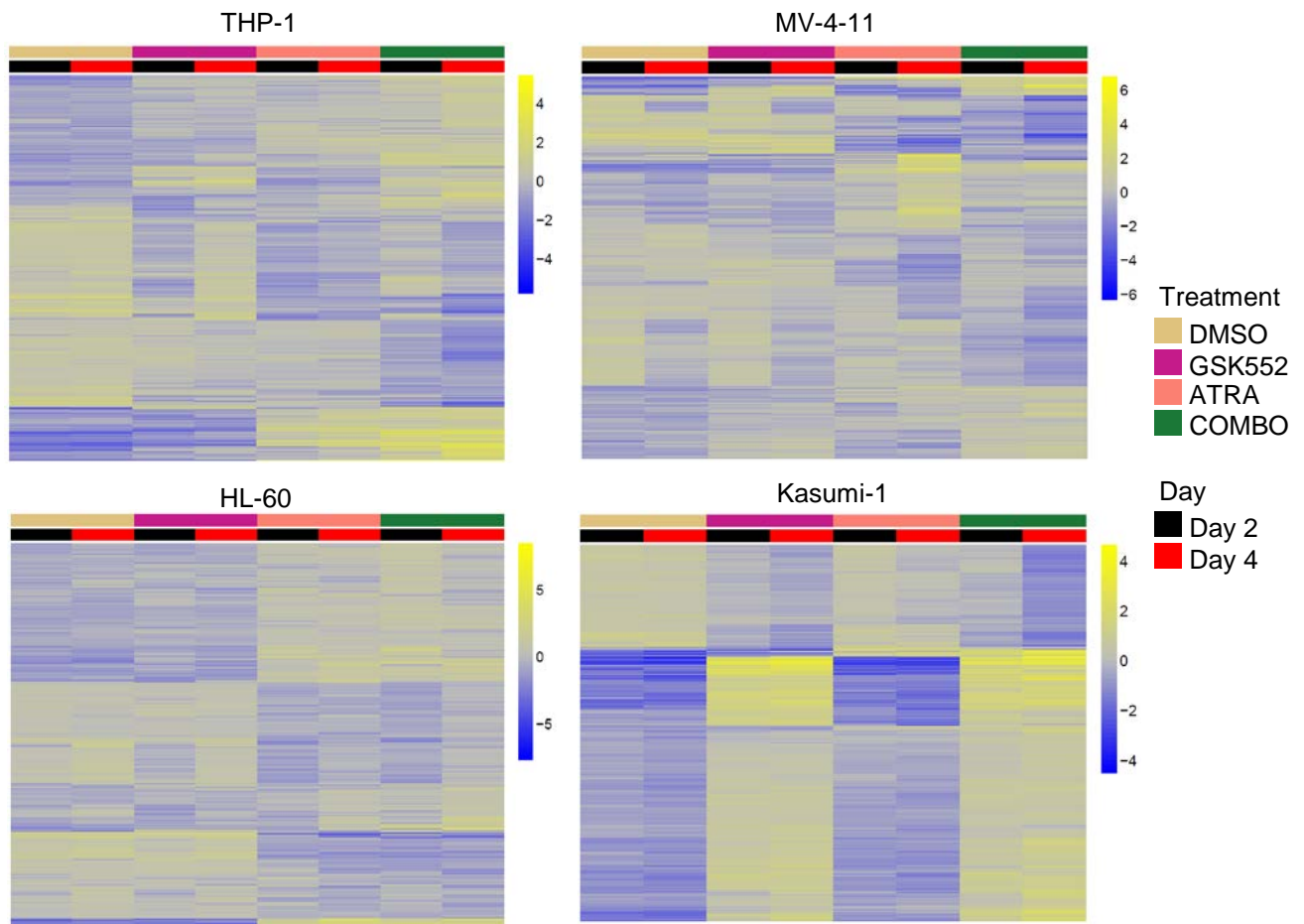
		Bliss Independence Day 6										
		GSK552 (nM)										
		3000	1000	333	111	37	12	4.1	1.4	0.5	0.2	
ATRA (nM)	1000	10.9	10.8	14.4	20.0	22.9	18.3	17.2	15.2	15.2	16.4	THP-1
	100	21.1	23.2	28.6	35.0	39.1	34.3	26.3	28.6	28.1	29.2	
	10	24.6	27.8	32.8	41.2	45.3	36.9	35.0	26.2	31.1	26.8	
	1	39.0	43.2	48.8	57.0	52.4	37.2	32.9	29.9	30.9	35.6	
ATRA (nM)	1000	0.1	0.1	0.0	0.0	0.0	-0.1	-0.2	-0.1	-0.1	-0.1	MOLM-13
	100	19.9	28.5	36.2	54.2	45.2	50.3	43.2	43.7	6.6	1.5	
	10	28.0	33.6	35.3	33.4	22.9	6.5	8.0	6.8	8.8	21.3	
	1	-15.3	-7.2	-10.2	-2.7	0.5	-4.5	-0.2	0.8	10.9	16.6	
ATRA (nM)	1000	10.8	10.6	13.1	19.3	23.5	17.3	13.2	11.3	17.1	9.7	Kasumi-1
	100	8.7	7.4	11.7	13.2	16.3	12.7	12.4	5.0	0.3	11.6	
	10	9.9	6.8	9.5	10.7	19.0	9.1	16.3	10.8	4.3	15.0	
	1	6.6	2.9	2.5	5.8	10.3	13.3	23.2	3.5	4.9	18.3	
ATRA (nM)	1000	1.0	1.7	1.9	2.1	1.5	0.8	0.7	0.5	0.6	0.4	OCI-AML3
	100	2.2	3.4	3.8	4.3	3.2	1.9	1.6	2.0	2.2	0.7	
	10	5.4	5.6	3.7	3.8	-2.0	-3.1	1.1	5.6	6.2	5.2	
	1	9.0	34.7	33.1	41.9	25.0	25.0	20.7	25.0	36.3	17.4	
ATRA (nM)	1000	0.9	1.2	1.4	1.7	2.1	1.3	1.5	1.8	1.4	0.9	HL-60
	100	1.5	1.9	2.5	2.8	3.6	2.3	1.8	2.2	2.0	2.0	
	10	2.3	3.0	3.7	4.8	5.6	4.6	2.3	3.7	3.9	1.7	
	1	5.9	8.0	8.7	9.9	21.3	6.1	4.7	1.2	9.9	19.0	
ATRA (nM)	1000	0.2	0.2	0.3	0.4	0.3	0.1	0.1	0.0	0.0	0.0	MV-4-11
	100	0.8	0.9	1.2	1.3	0.7	0.0	0.2	-0.2	-0.5	-0.1	
	10	0.9	0.9	1.1	0.6	0.3	-1.8	0.1	0.0	-0.4	0.1	
	1	15.5	20.4	24.5	23.2	19.8	-1.7	10.7	10.0	10.0	12.2	
ATRA (nM)	1000	-1.0	-0.4	-0.2	0.2	0.8	0.8	0.8	0.8	0.8	0.6	SIG-M5
	100	-1.1	-0.4	-0.2	0.2	0.7	0.8	0.7	0.8	0.7	0.6	
	10	-1.1	-0.5	-0.3	0.1	0.8	0.9	0.7	0.8	0.9	0.5	
	1	0.9	2.6	5.2	22.7	29.9	13.7	4.3	8.8	7.2	9.4	

Supplemental Figure 3

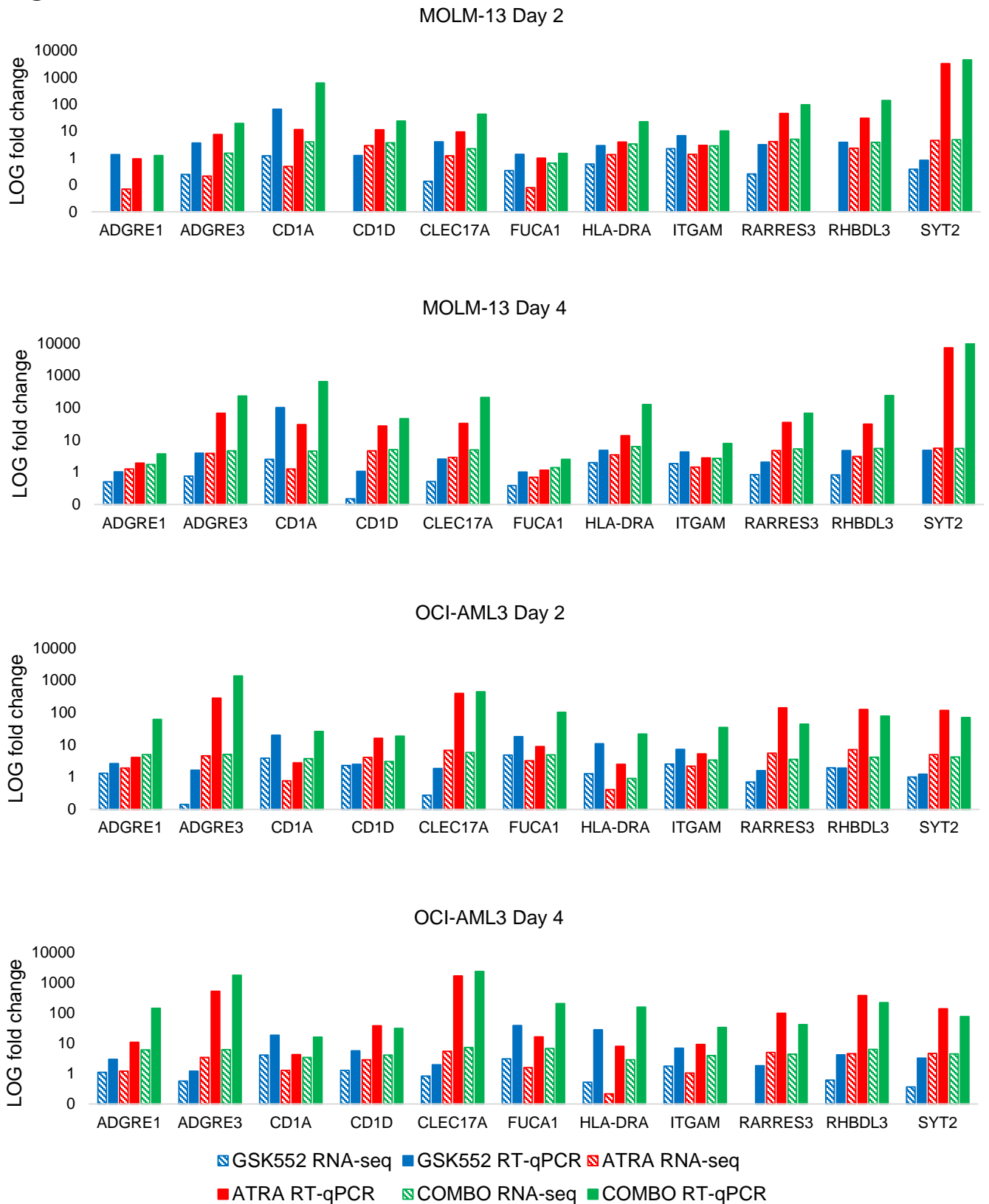
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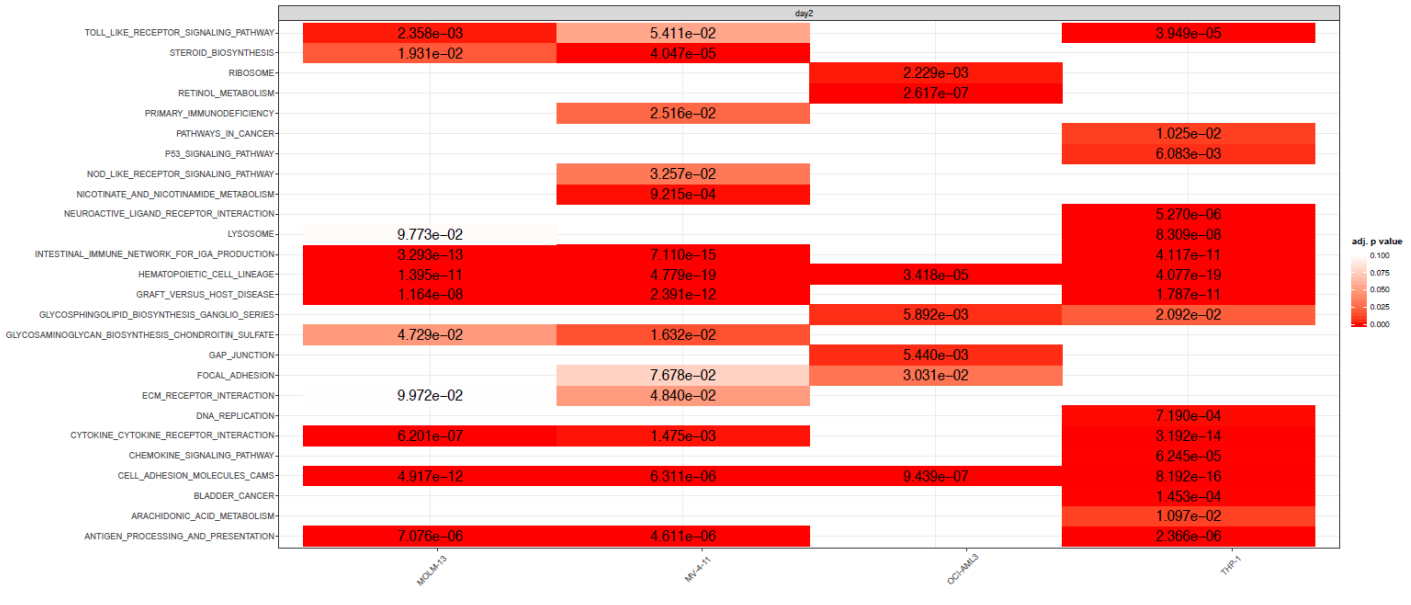
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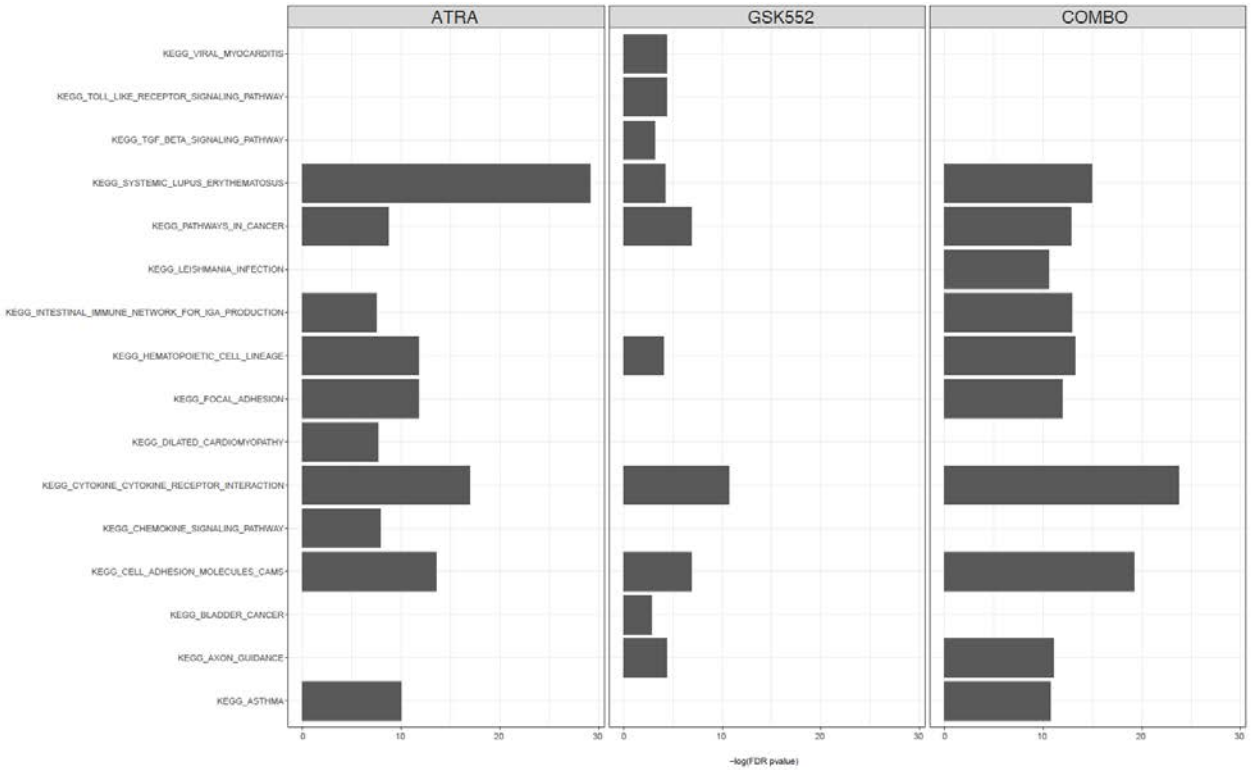


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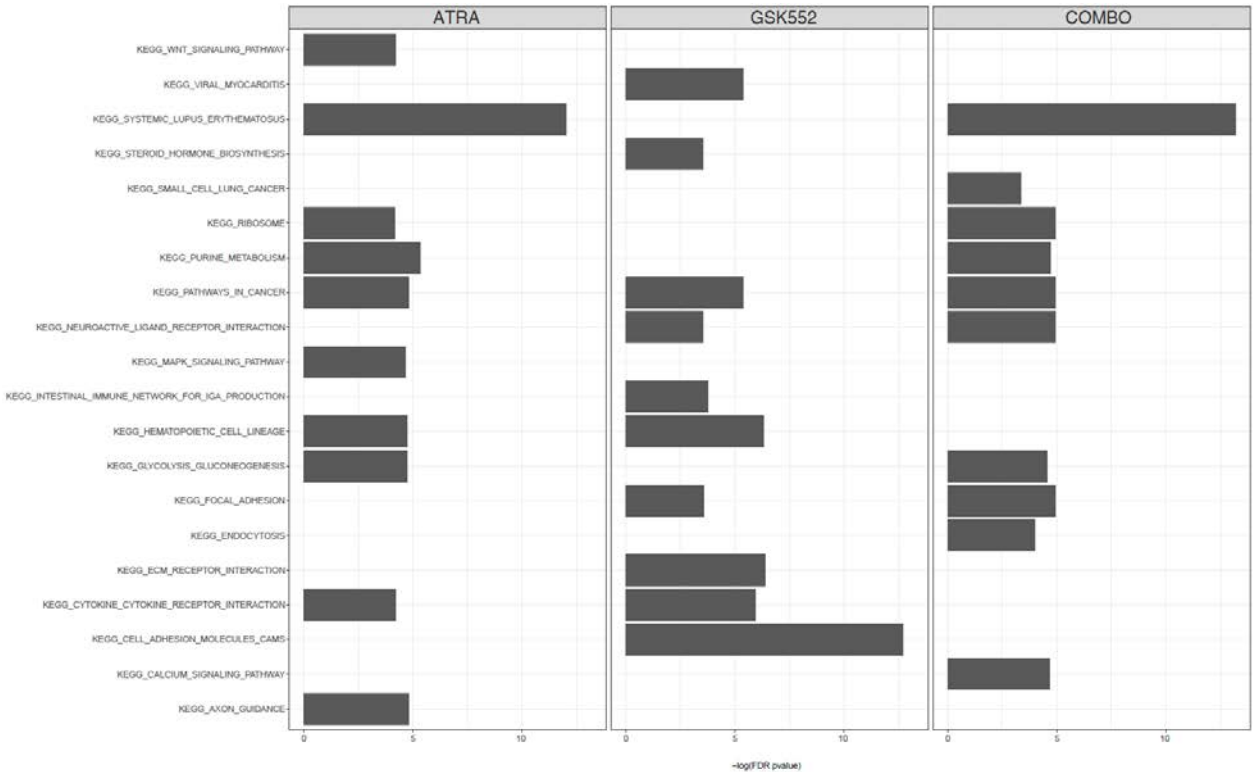


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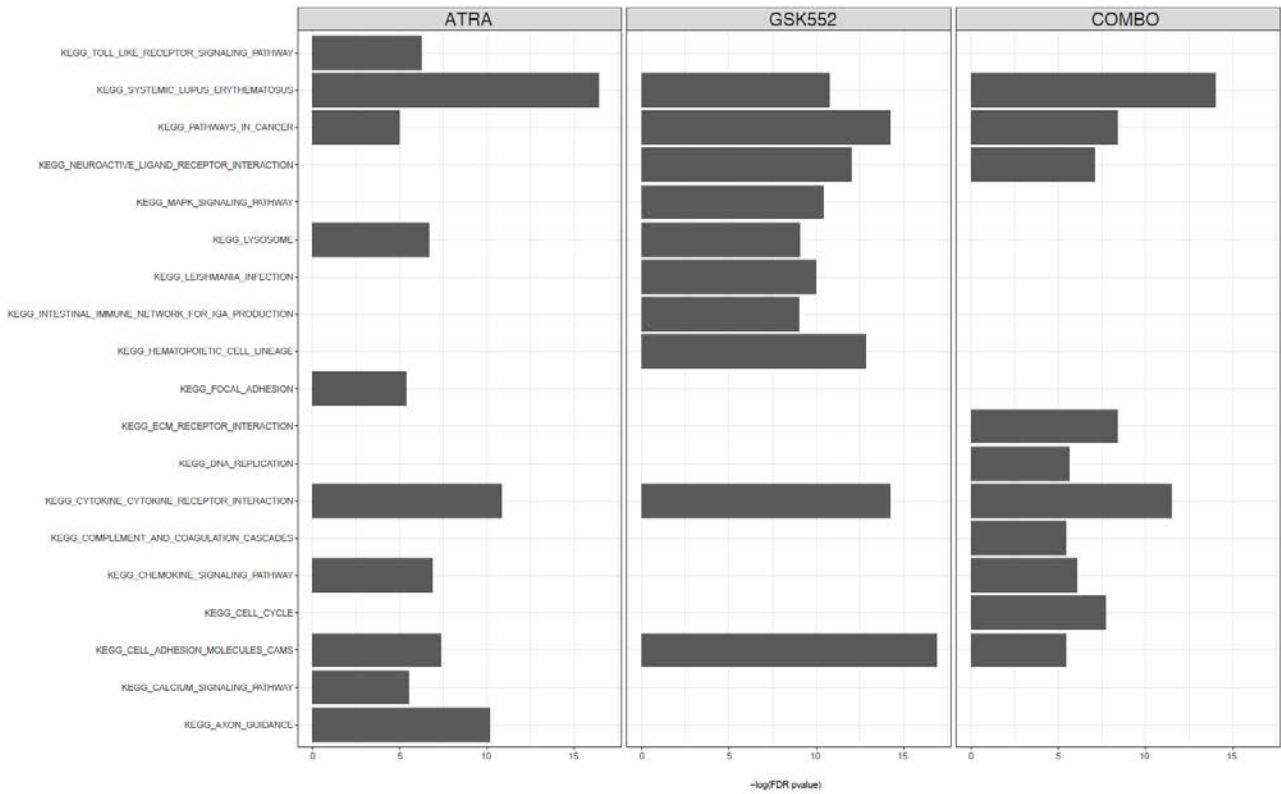
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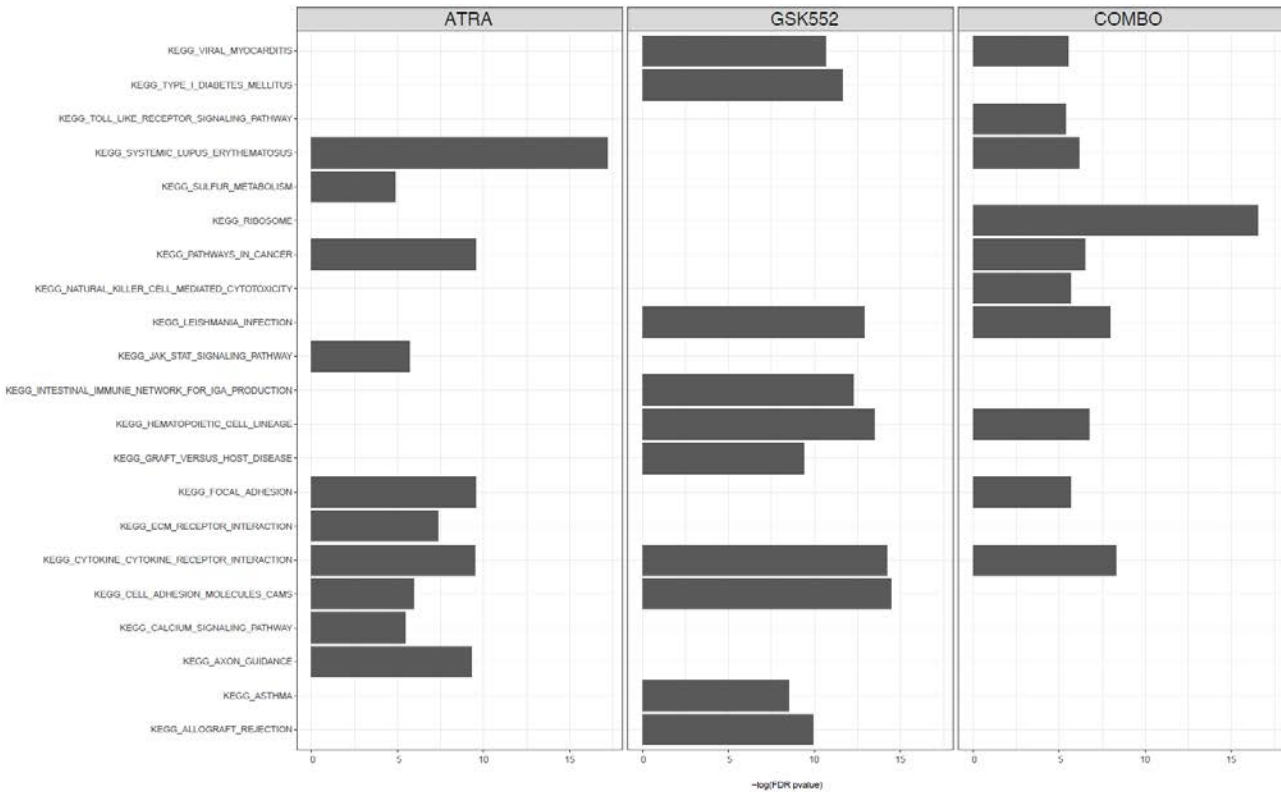
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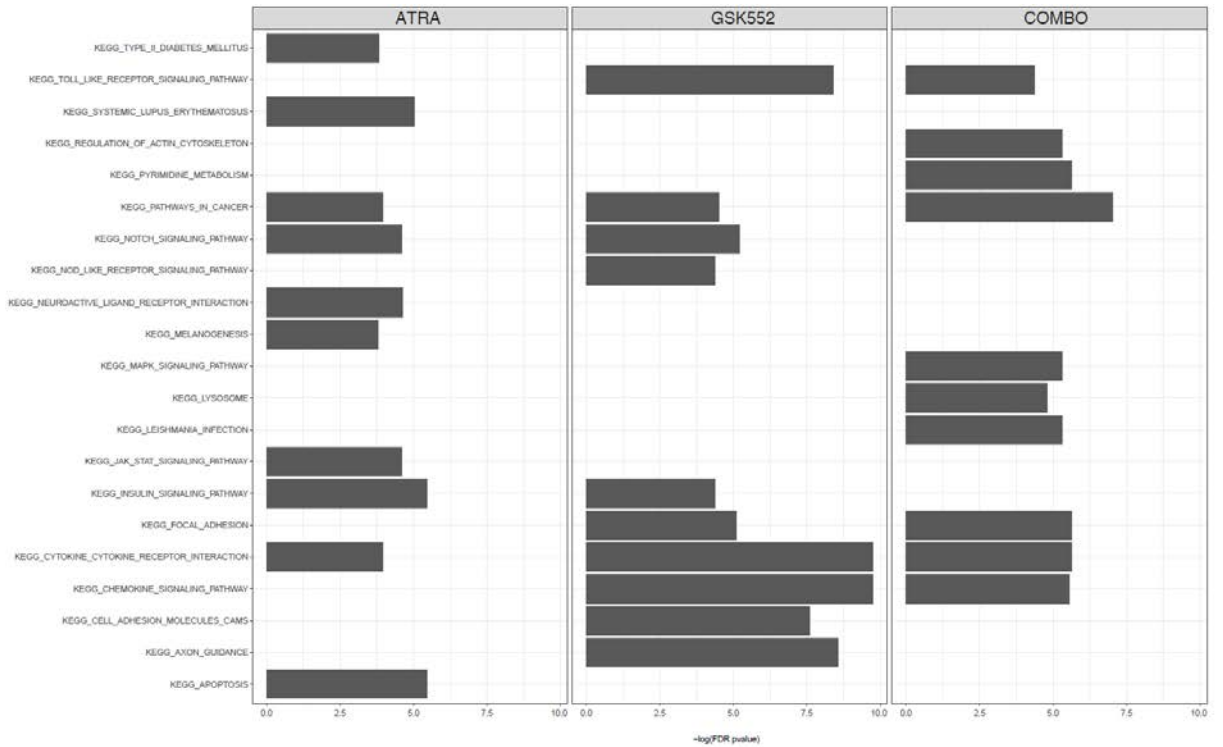
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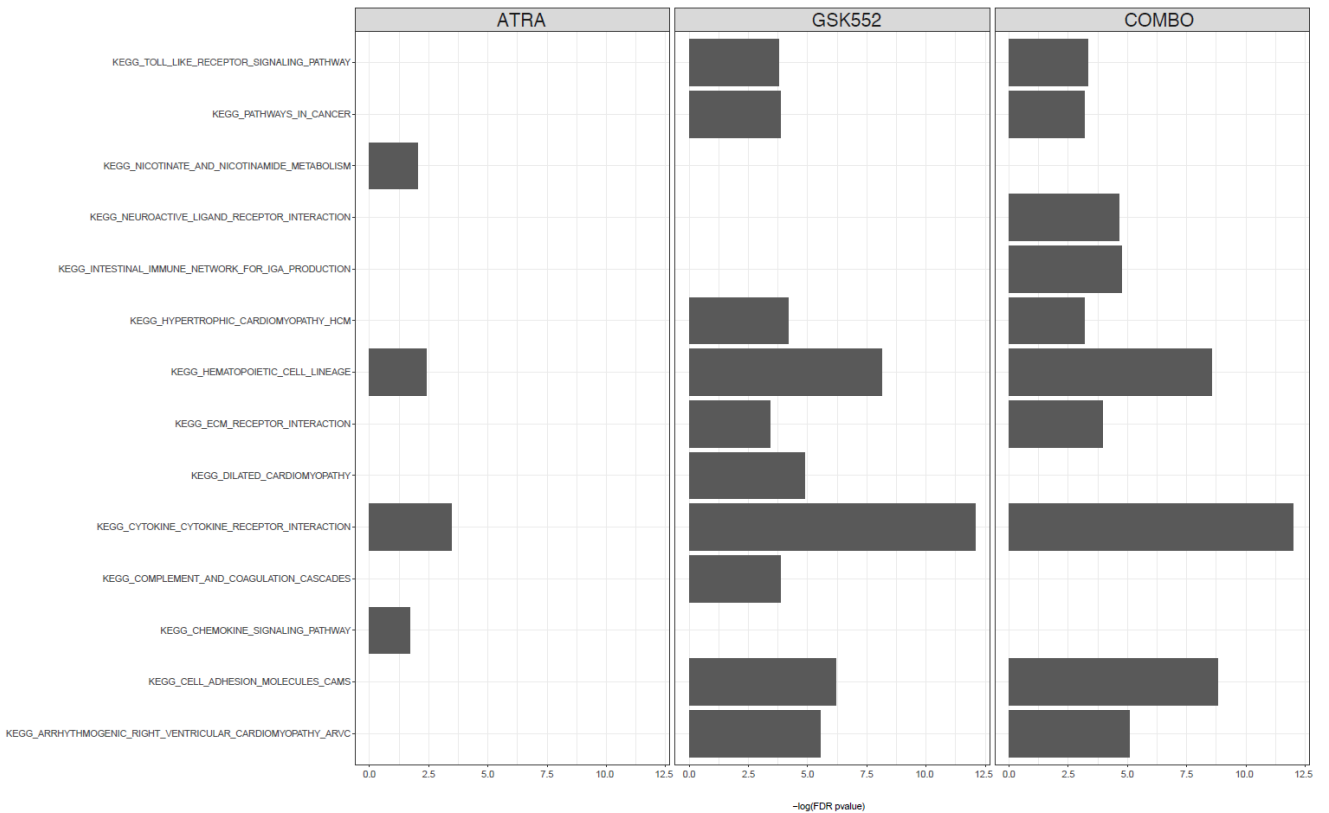
MV-4-11



HL-60

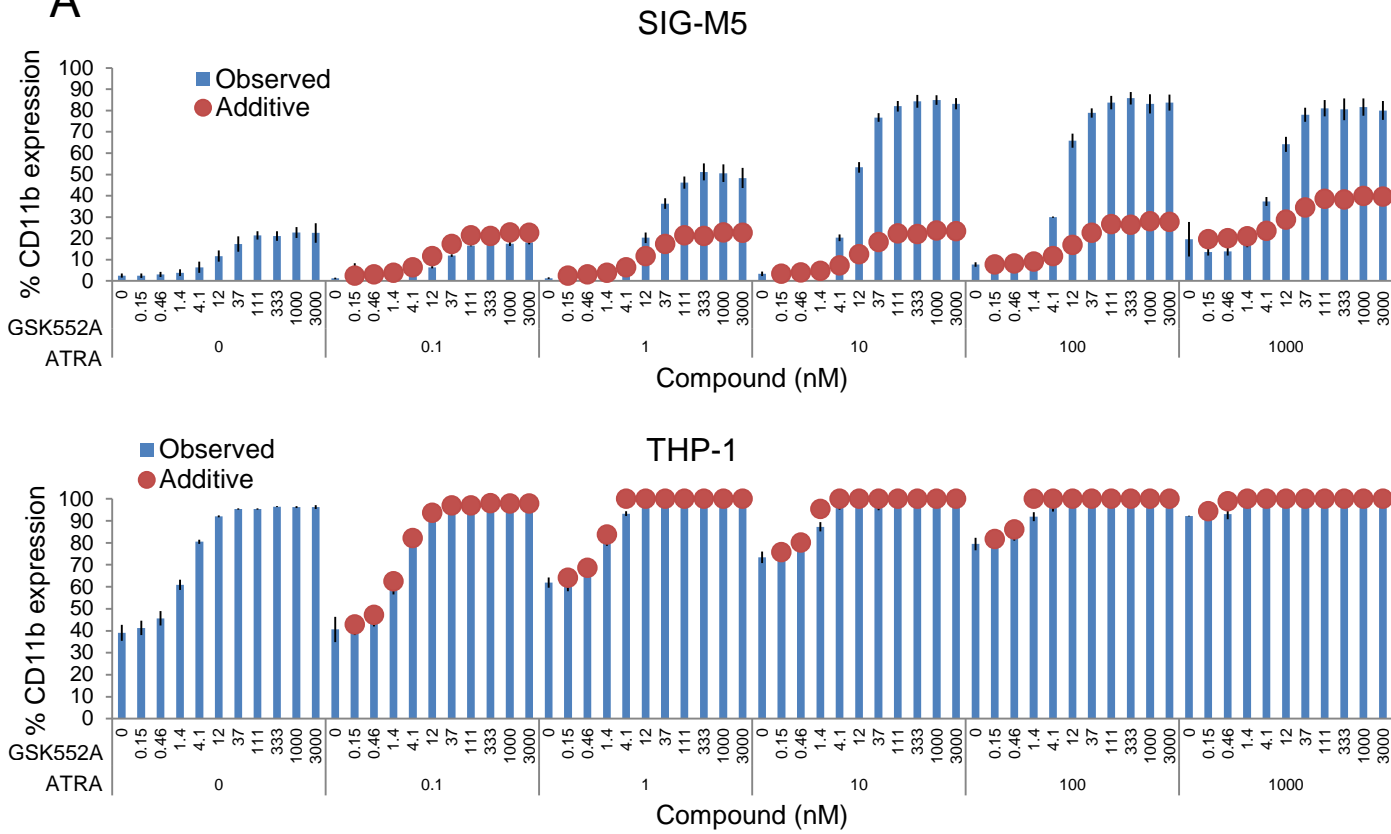


Kasumi-1

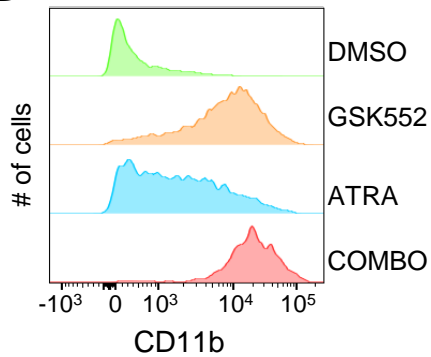


Supplemental Figure 4

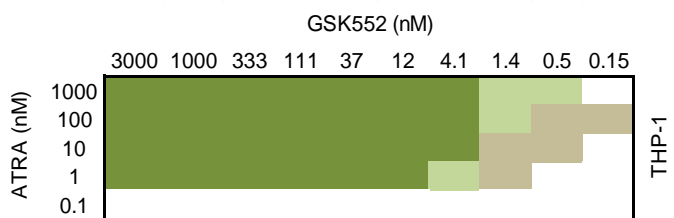
A



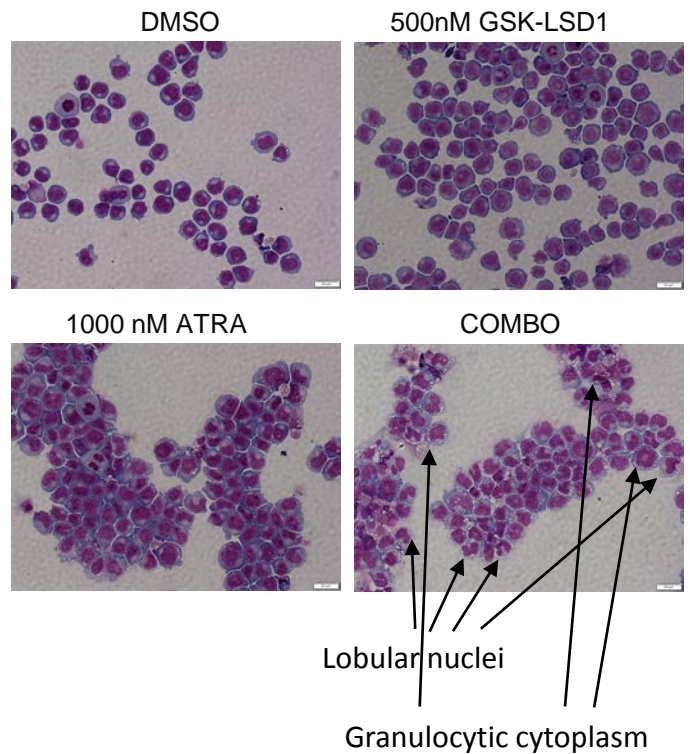
B



C



D

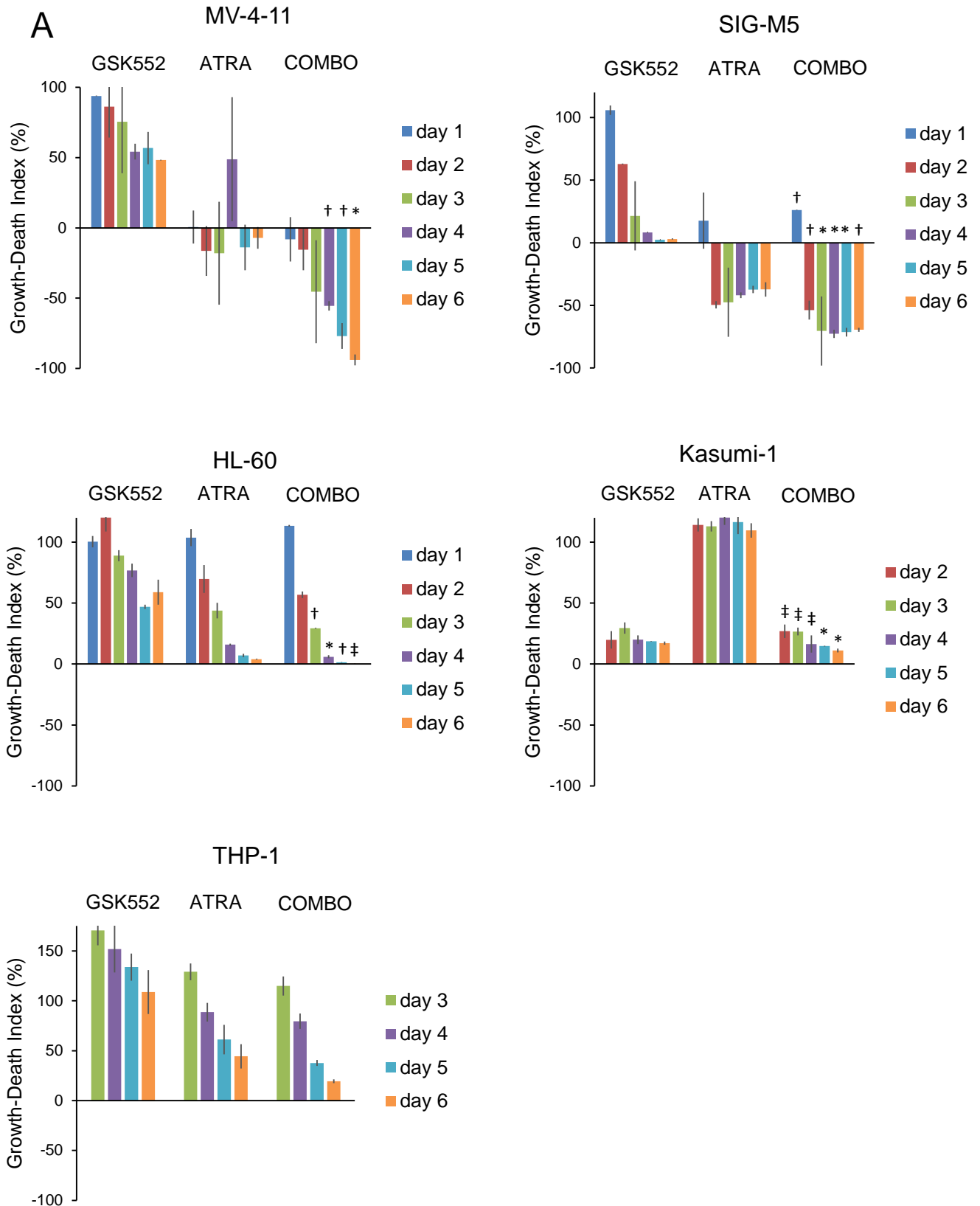


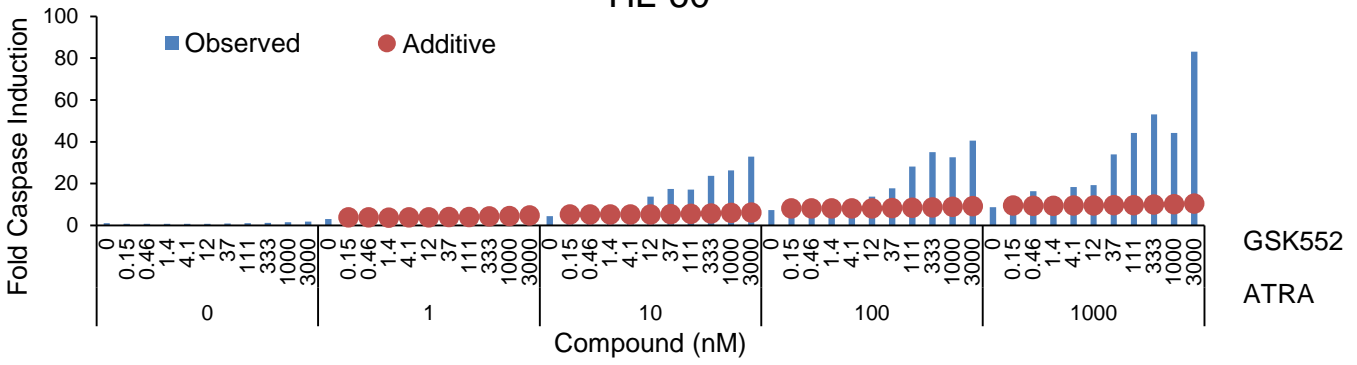
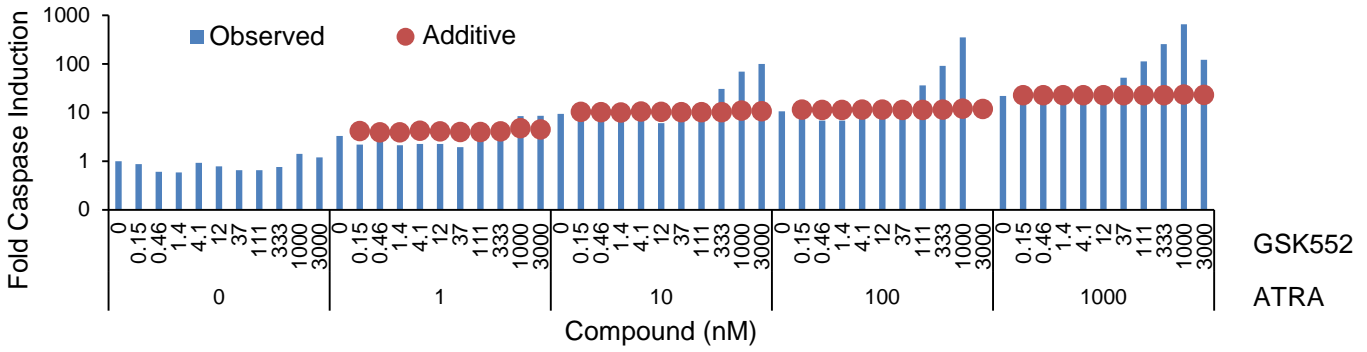
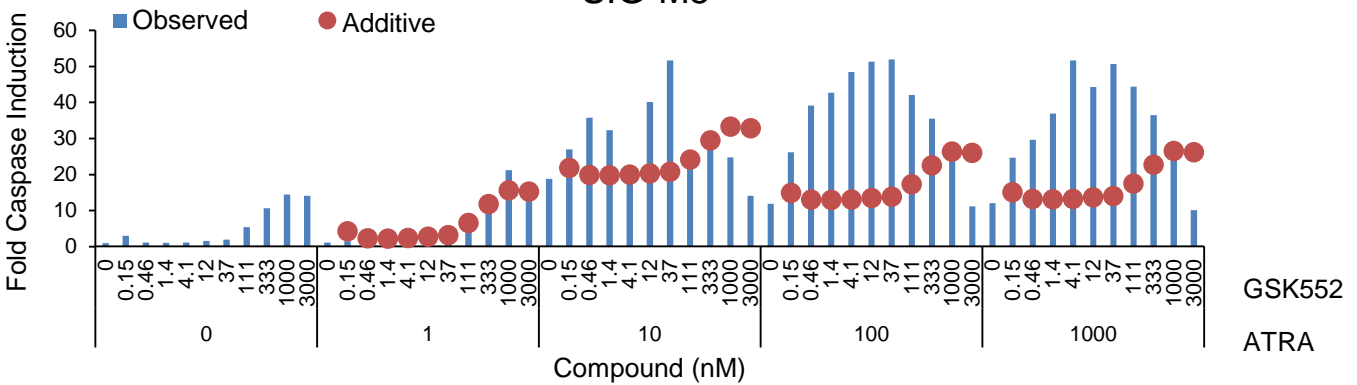
Supplemental Table 4

A

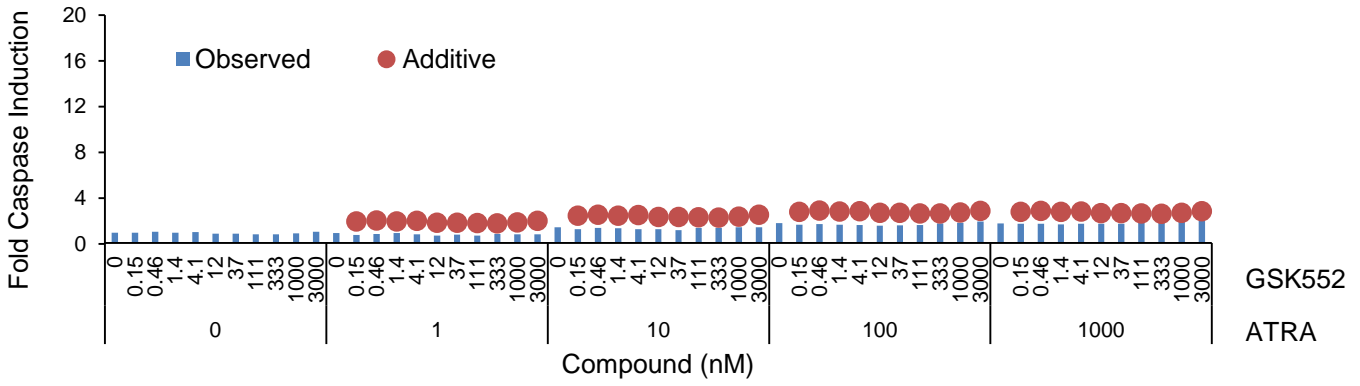
	EC ₅₀ (nM)							
	MOLM-13		OCI-AML3		SIG-M5		THP-1	
GSK2879552	AVG	SEM	AVG	SEM	AVG	SEM	AVG	SEM
+ DMSO	16	3	4	2	15	3	2	0.05
+ 0.1 nM ATRA	17	7	4	1	27	2	2	0.04
+ 1 nM ATRA	25	12	5	2	18	0.4	1	0.02
+ 10 nM ATRA	11	3	5	2	9	1	1	0.1
+ 100 nM ATRA	13	5	6	1	7	0.1	1	0.1
+ 1000 nM ATRA	5	1	5	0.3	6	1	1	0.03

Supplemental Figure 5

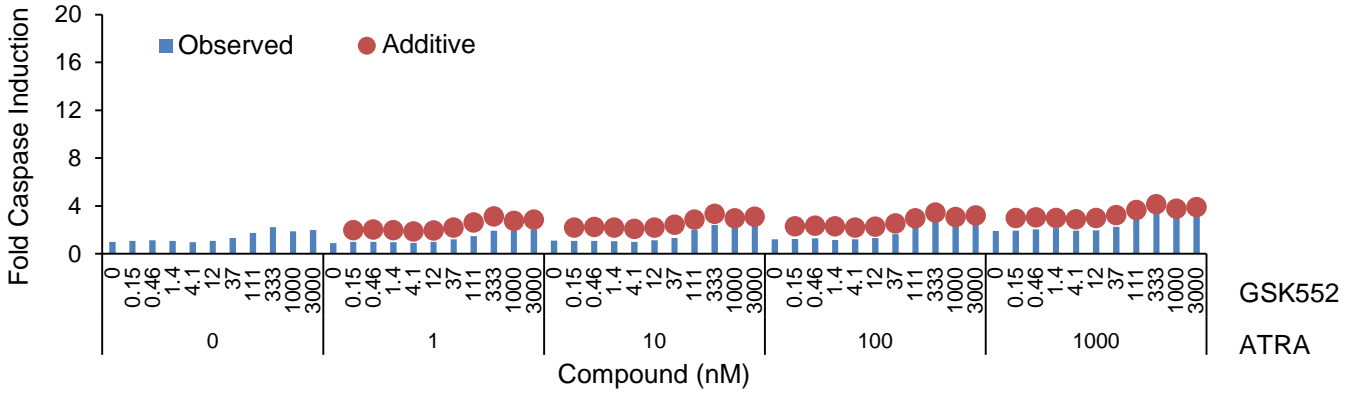


B**HL-60****MV-4-11****SIG-M5**

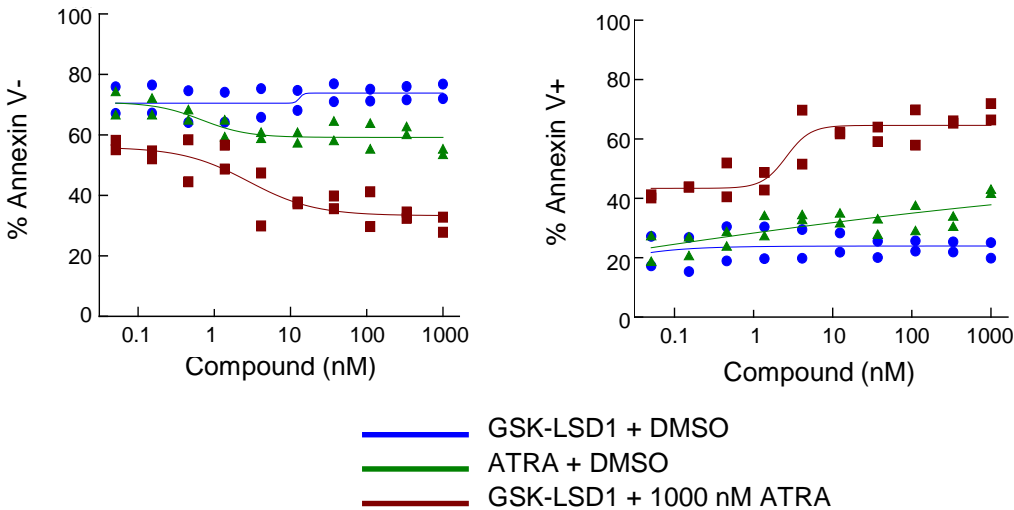
THP-1

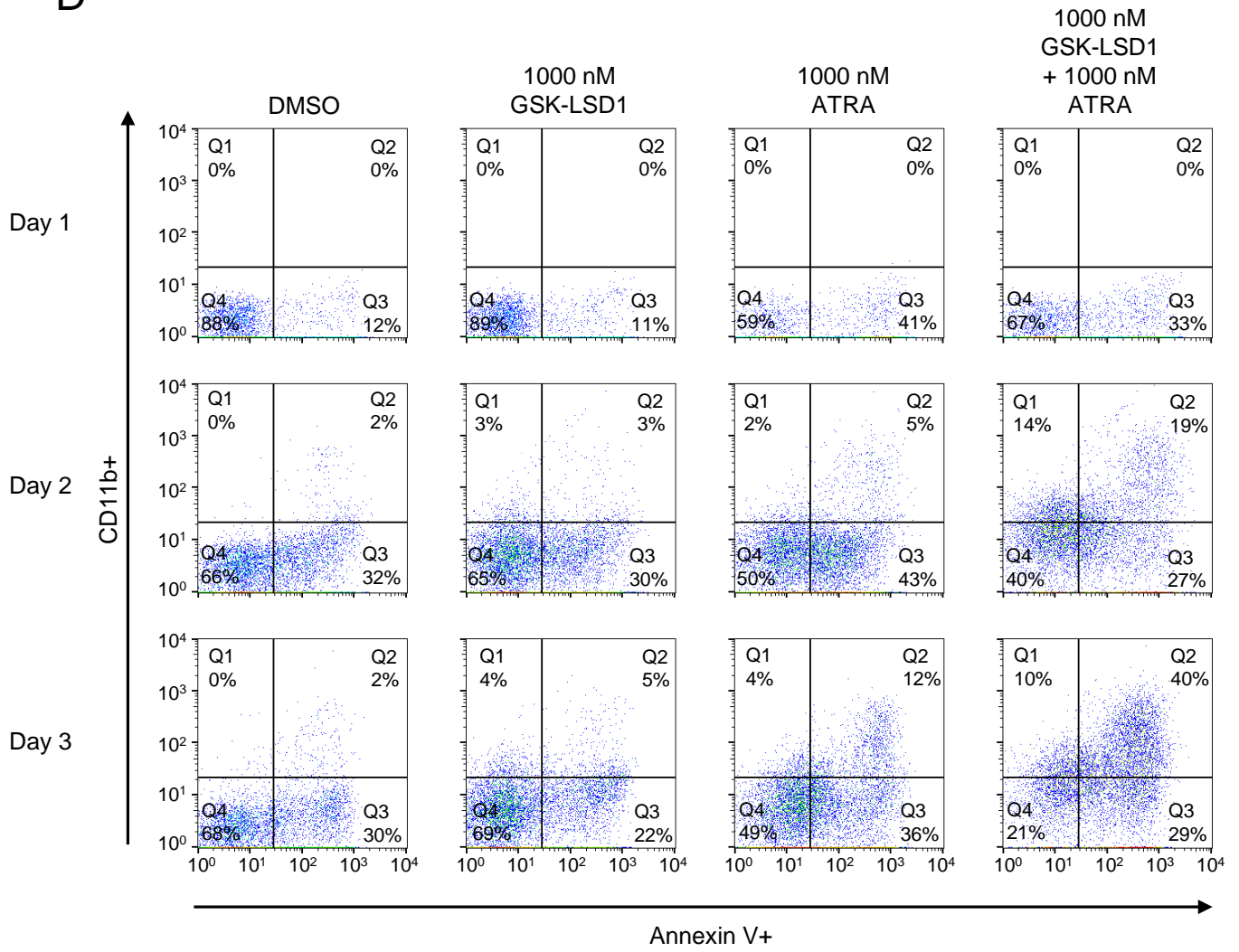


Kasumi-1



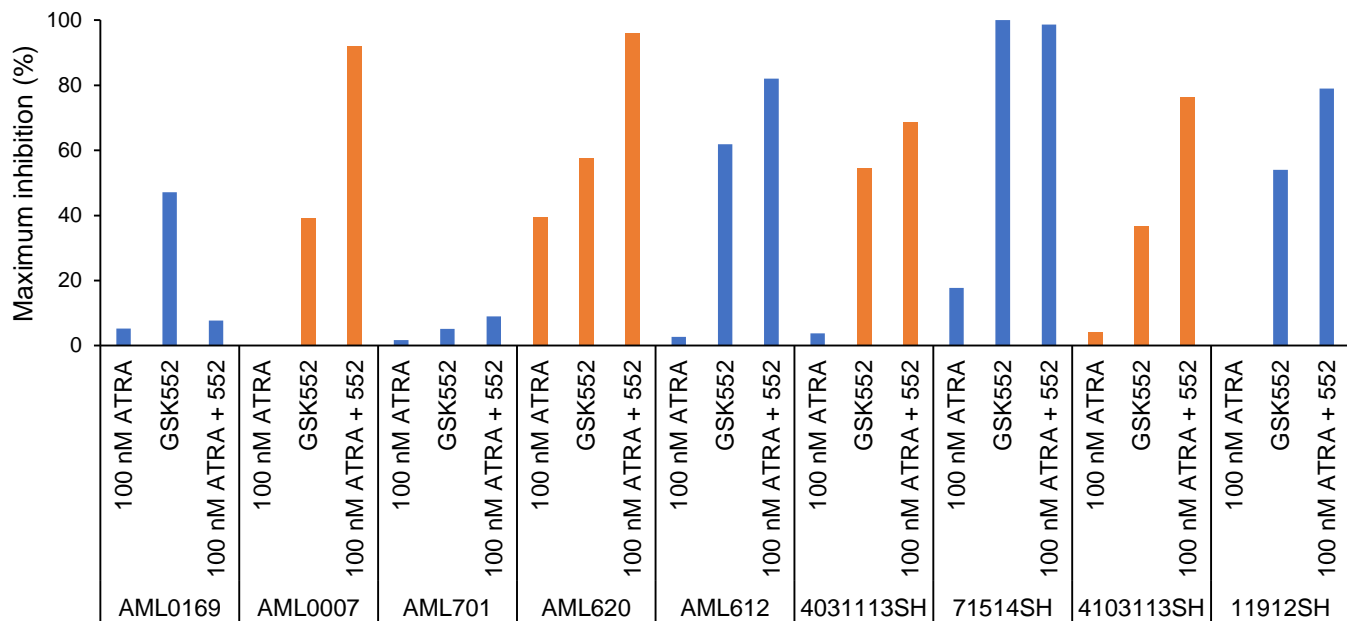
C



D

Supplemental Figure 6

A



B

