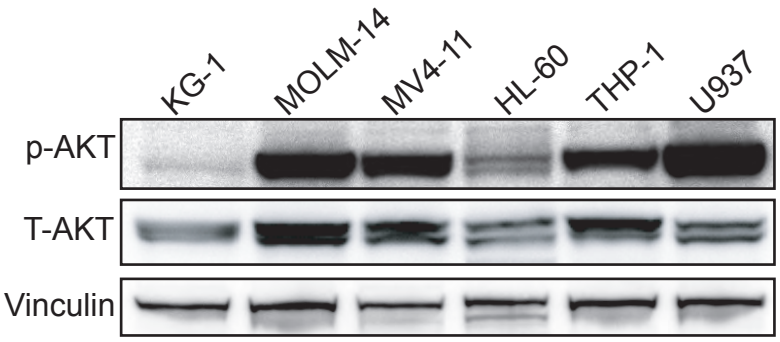
Supplementary Figure S1.



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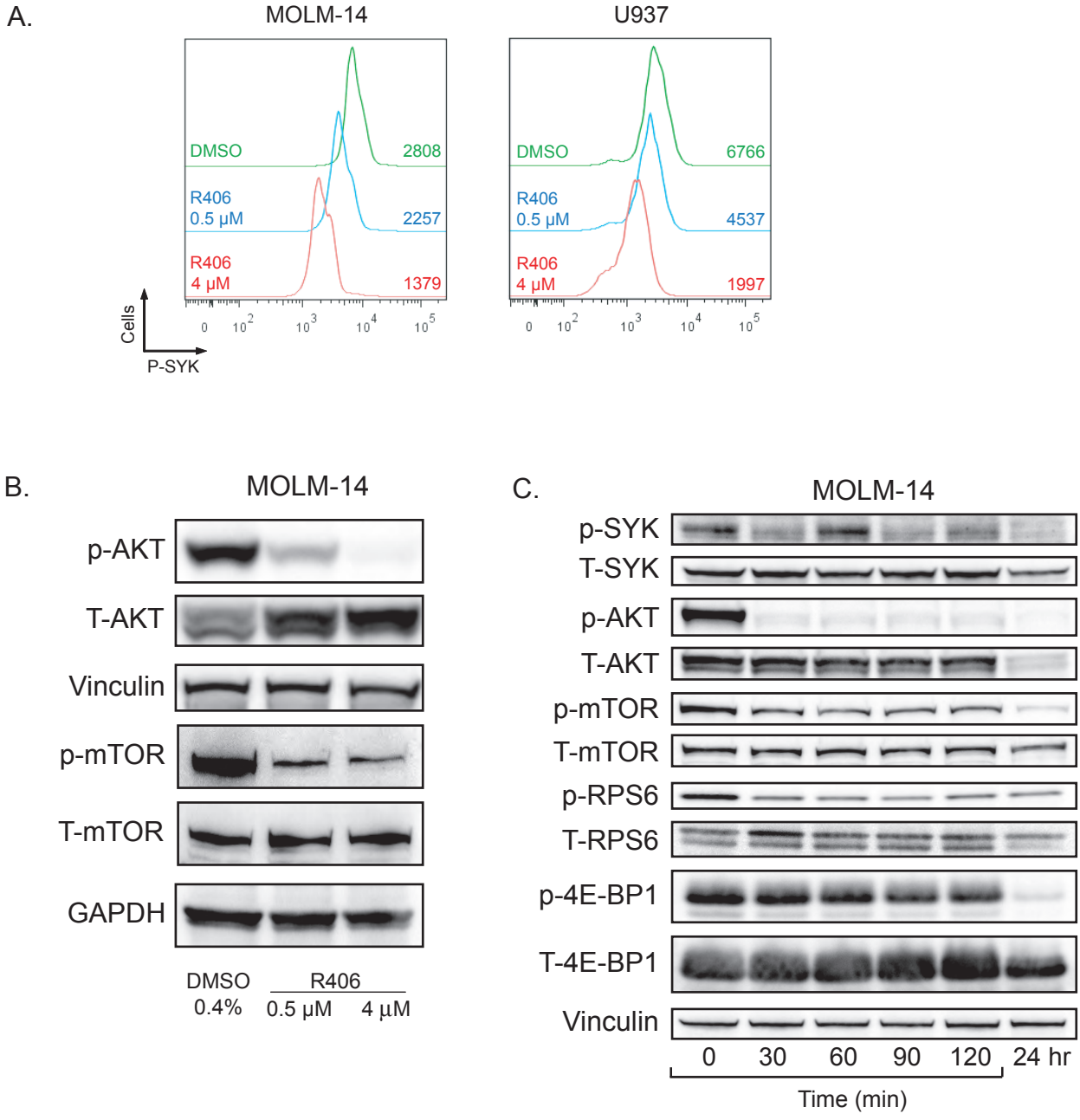

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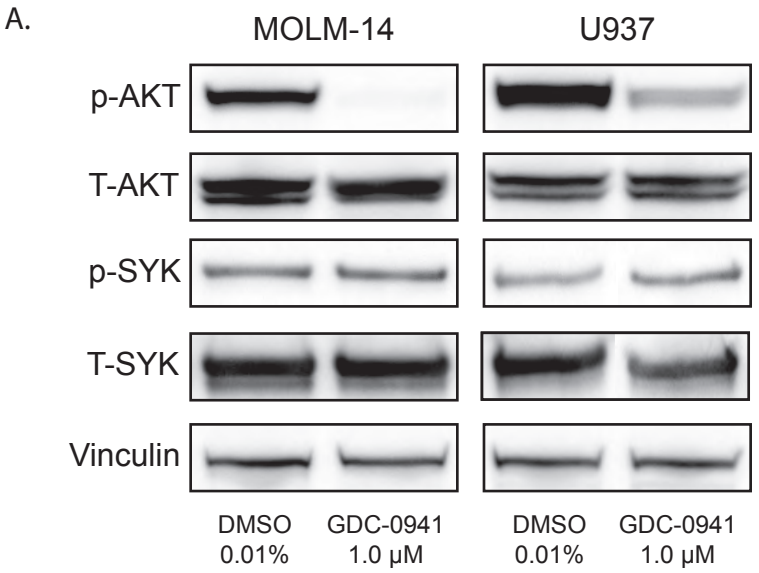
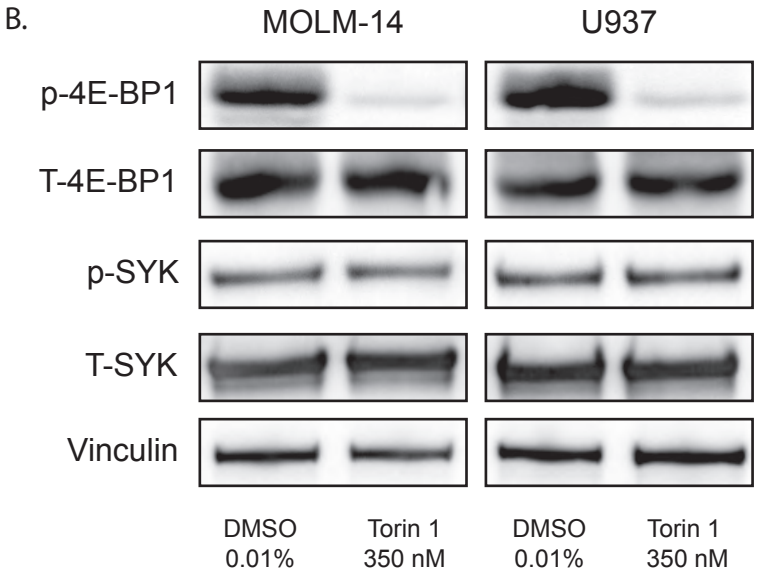
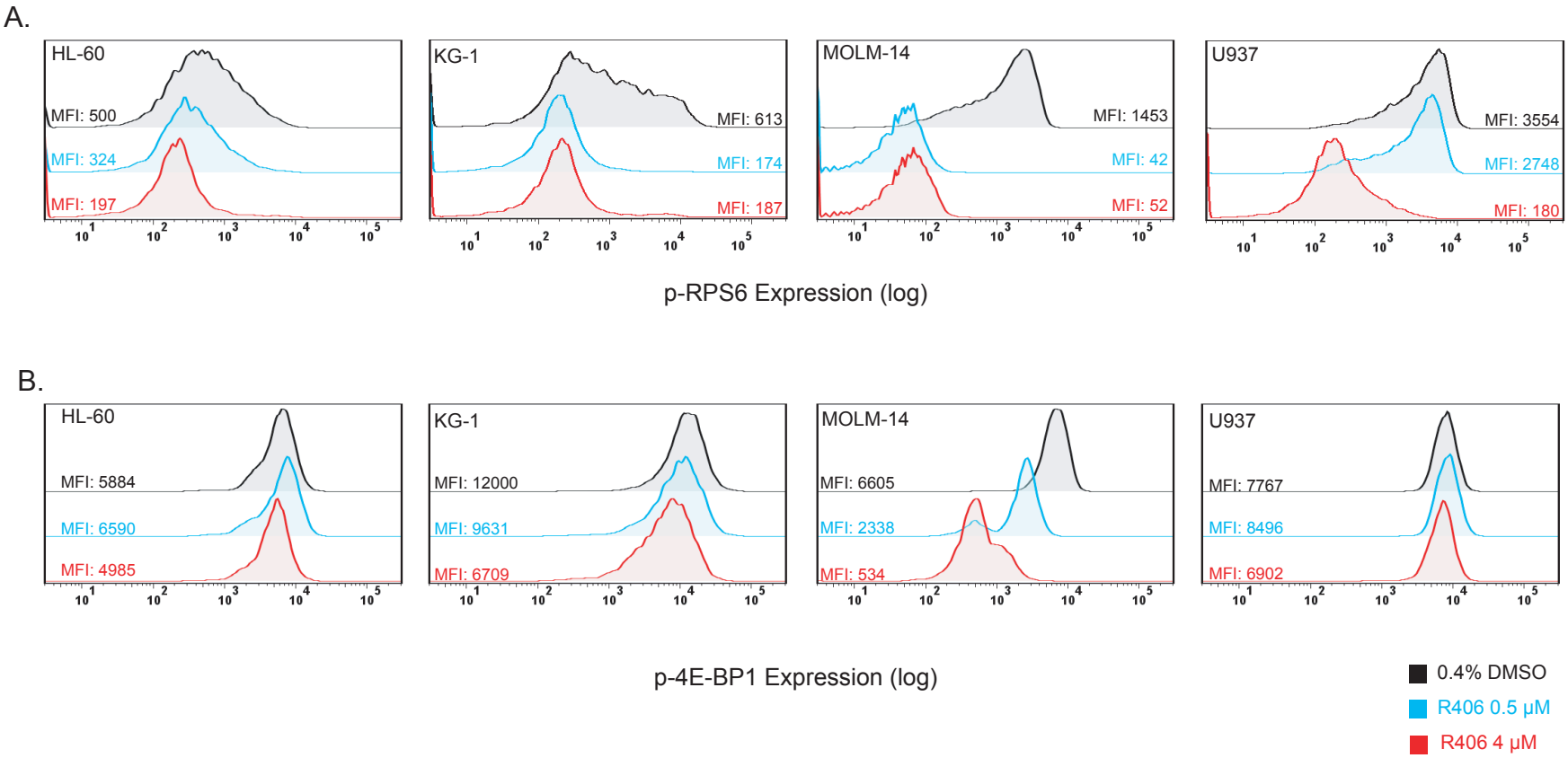
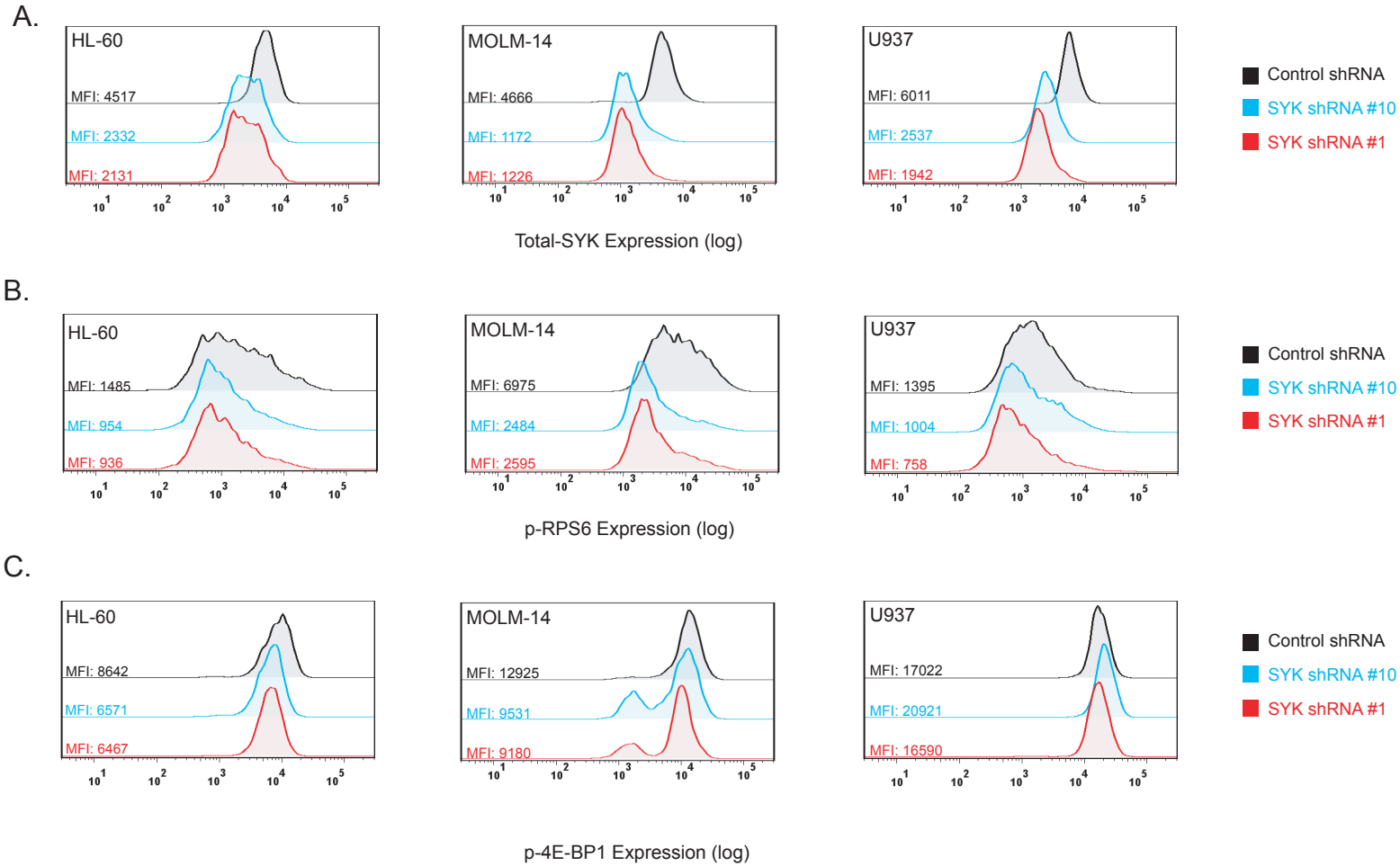




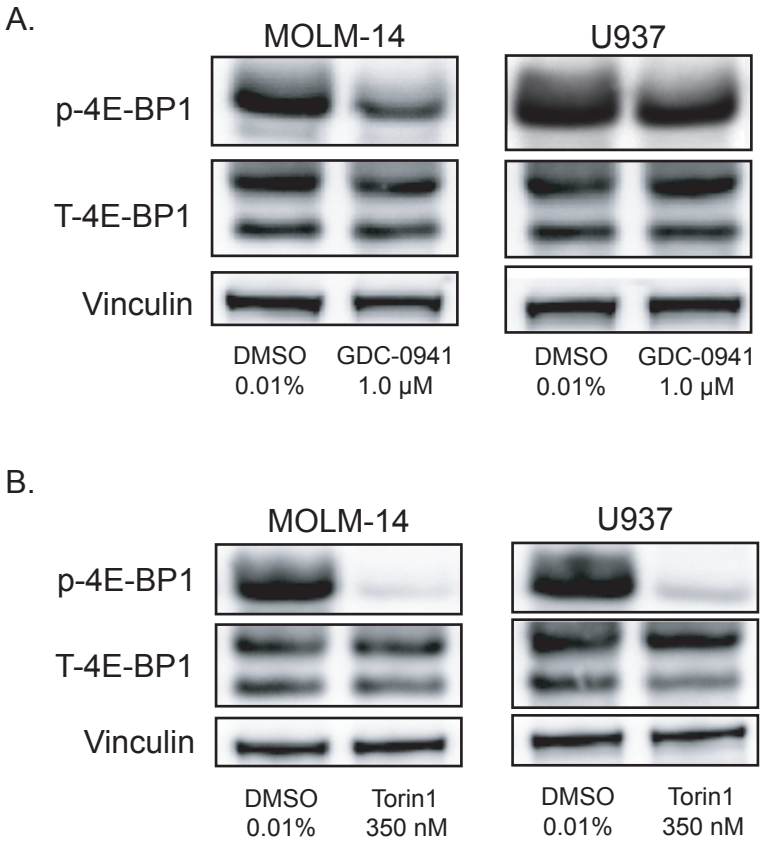
Supplementary Figure S4.



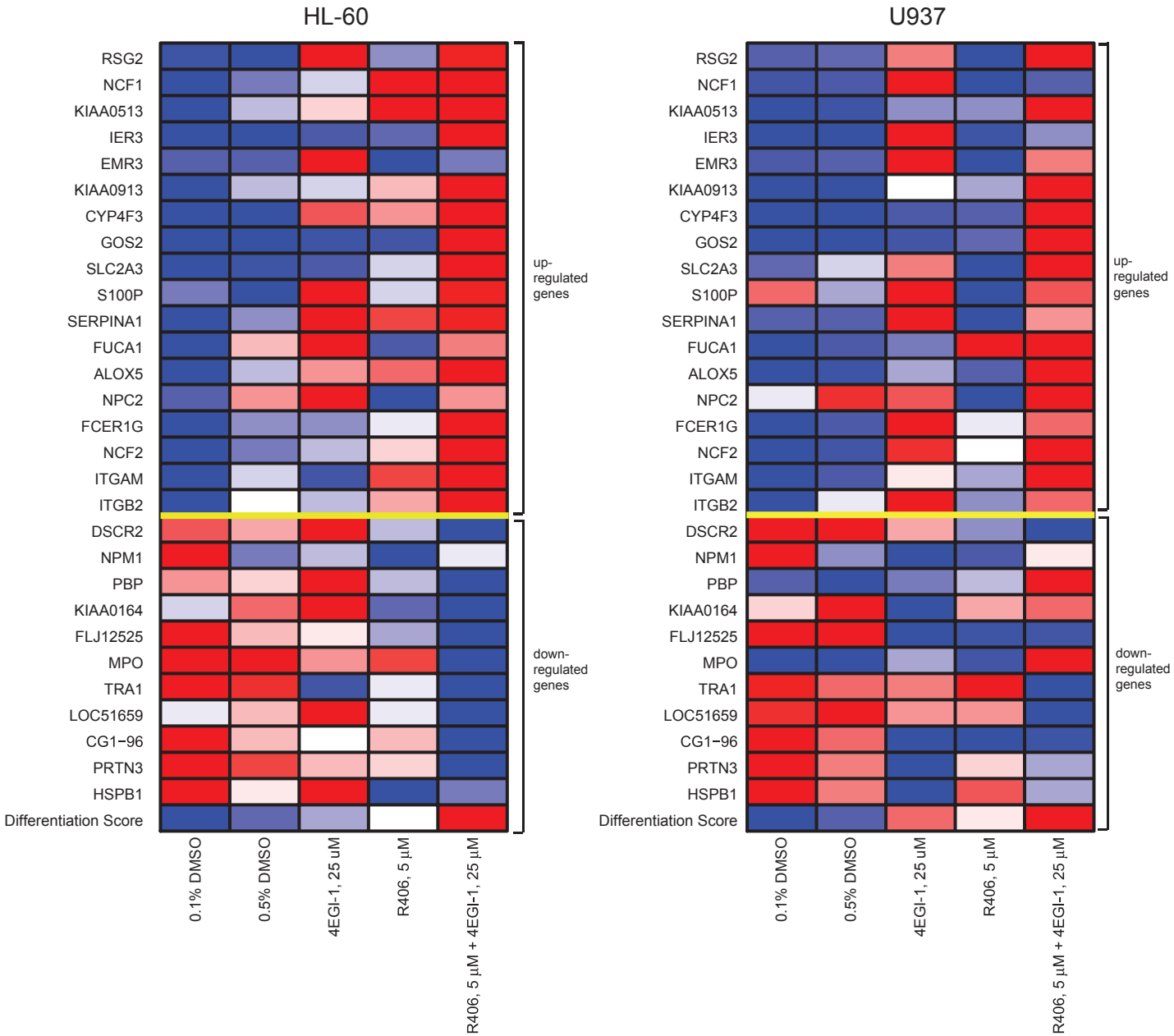
Supplementary Figure S5.



Supplementary Figure S6.

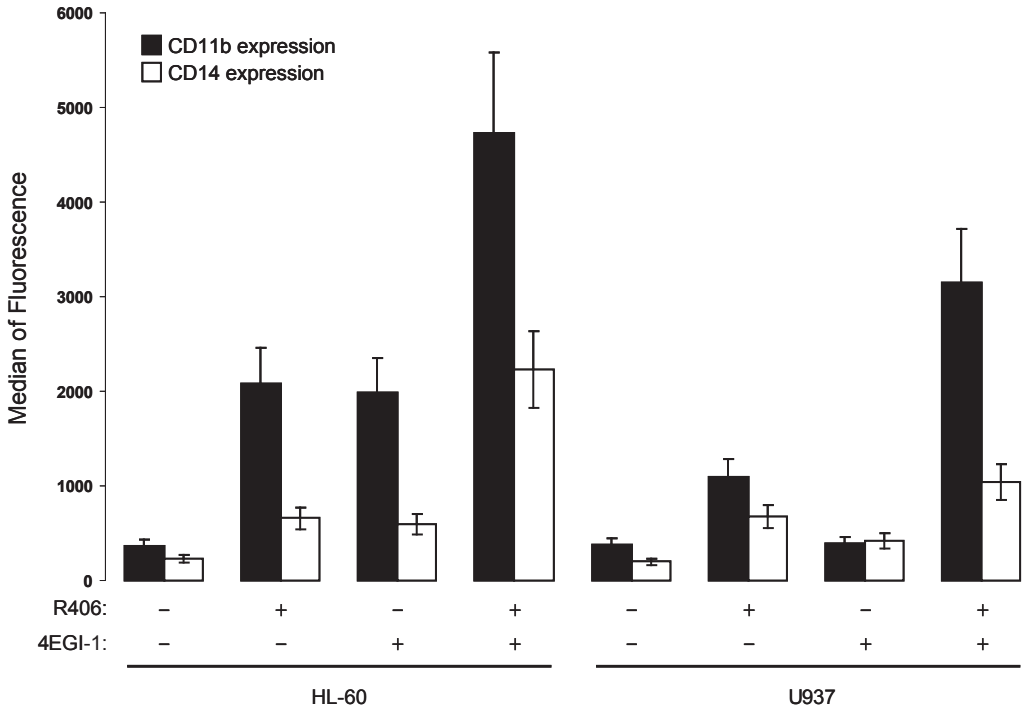


Supplementary Figure S7.



Supplementary Figure S8.

A.



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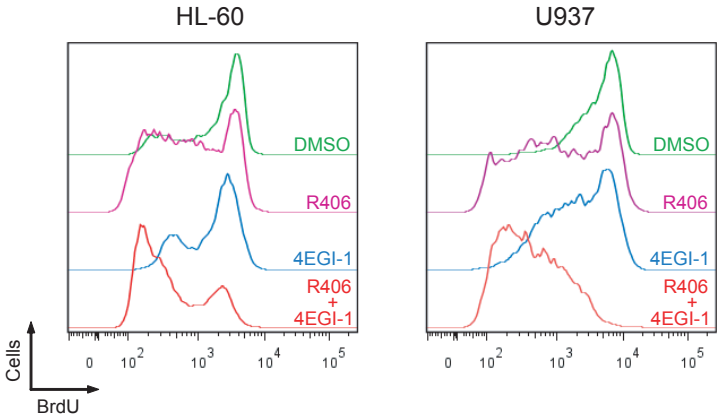

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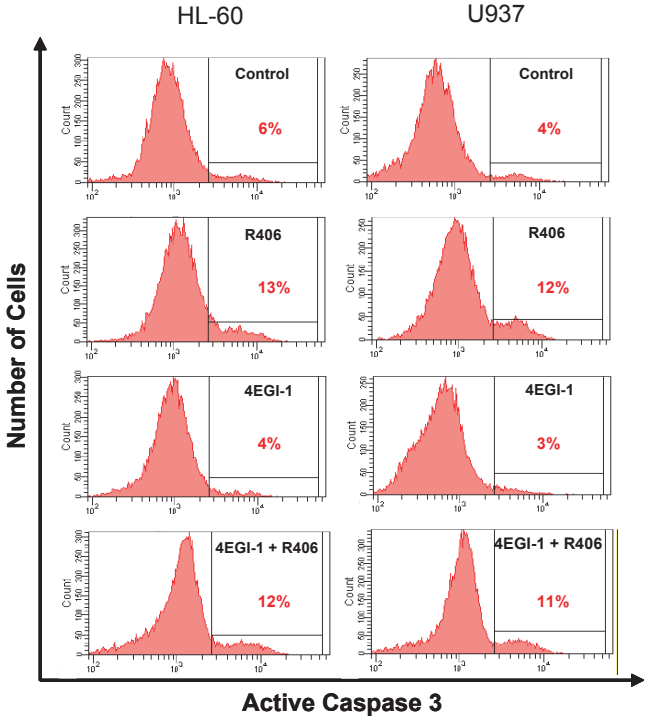
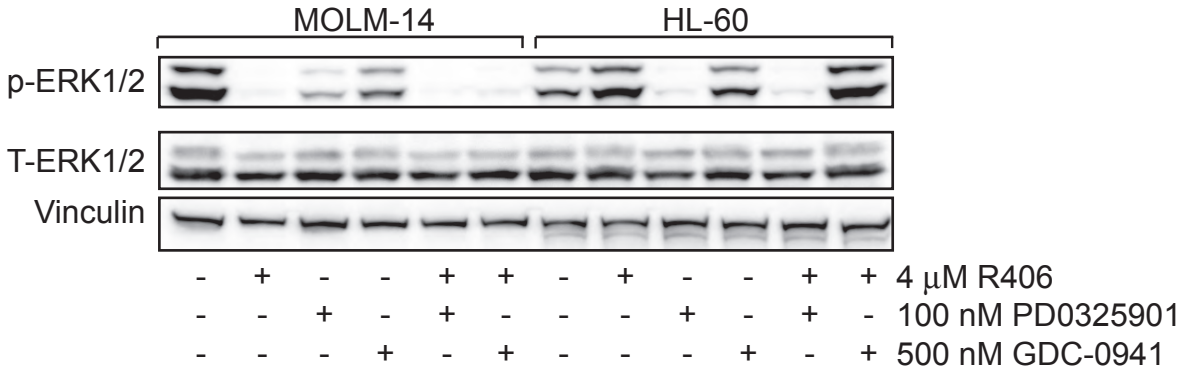
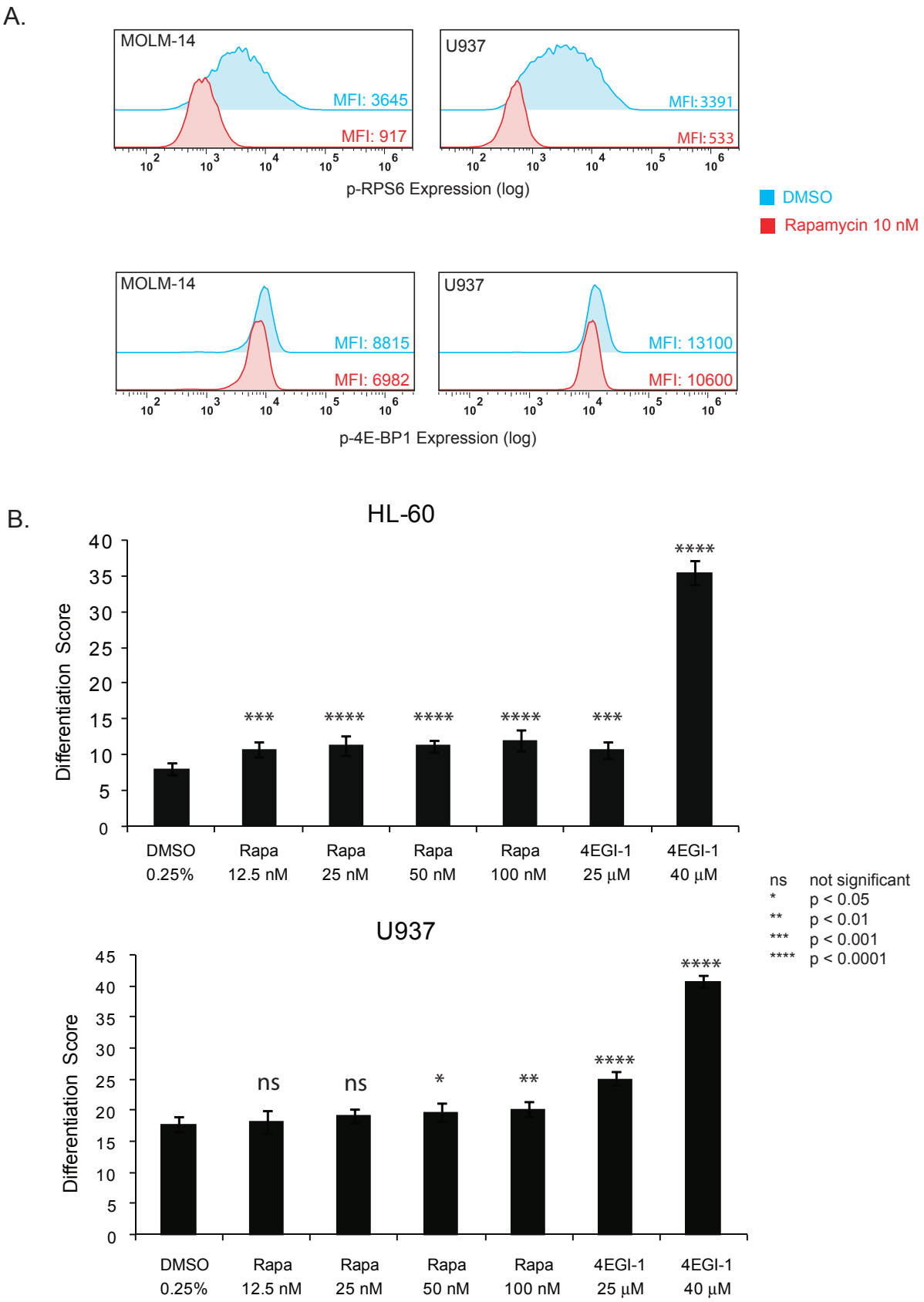


Supplementary Figure S9.



Supplementary Figure S10.



Supplementary Table S1.
Differentiation Signature (32-Gene Signature)

GENE NAME	RefSeq	PROBE SEQUENCE
RGS2	NM_002923	5'-TAATACGACTCACTATAGGGCTTTAATCTCAATCAATACAAATCAGGGAATAGGTGGTCTGAAC-3'
		5'-Phos-GTGGTGTCTCACTCTGAAAATCCCTTTAGTGAGGGTTAAT-3'
NCF1	NM_000265	5'-TAATACGACTCACTATAGGGCTTTATCAATACATACTACAATCATGGACGCCGAGGGCAGCCCC-3'
		5'-Phos-GACCCCTGTCCAGCGCGGCTTCCCTTTAGTGAGGGTTAAT-3'
KIAA0513	NM_014732	5'-TAATACGACTCACTATAGGGTACACTTTATCAAATCTTACAATCCACCAGTATCTTCTCTGTTG-3'
		5'-Phos/CATTTTTGCAATCTTGTGTCTCCCTTTAGTGAGGGTTAAT-3'
IER3	NM_003897	5'-TAATACGACTCACTATAGGGTACATTACCAATAATCTTCAAATCGCTGTCACGGAGCGACTGTC-3'
		5'-Phos-GAGATCGCCTAGTATGTTCTTCCCTTTAGTGAGGGTTAAT-3'
EMR3	NM_032571	5'-TAATACGACTCACTATAGGGCAATTCAAATCACAATAATCAATCGATGGGTCCTGACTCAAAAC-3'
		5'-Phos-CCAGTGAGGGGGATGTTTTTTCCCTTTAGTGAGGGTTAAT-3'
KIAA0913	NM_015037	5'-TAATACGACTCACTATAGGGTAATCTTCTATATCAACATCTTACTGGGAGGGGGCGTTGGGTGG-3'
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CYP4F3	NM_000896	5'-TAATACGACTCACTATAGGGATACTTCATTCATTCATCAATTCATCTGGATTTTCTATCTATTC-3'
		5'-Phos-CATGTTGGACCAATACCACATCCCTTTAGTGAGGGTTAAT-3'
PSMG1	NM_003720	5'-TAATACGACTCACTATAGGGTCAATTACCTTTTCAATACAATACAACAACCGAATATAGTACAC-3'
		5'-Phos-GACCTTCCTGCAGCAGTTCTTCCCTTTAGTGAGGGTTAAT-3'
NPM1	NM_002520	5'-TAATACGACTCACTATAGGGCTTTTCAATTACTTCAAATCTTCATGATAGGACATAGTAGTAGC-3'
		5'-Phos-GGTGGTCAGACATGGAAATGTCCCTTTAGTGAGGGTTAAT-3'
PEBP1	NM_002567	5'-TAATACGACTCACTATAGGGTTACTCAAAATCTACACTTTTTCAGAAAAGCTGGTCTGGAGTTG-3'
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ANP32E	NM_030920	5'-TAATACGACTCACTATAGGGAAAGTTGAGTATTGATTTGAAAAGATATTTTGTAGAAGTTTTCG-3'
		5'-Phos-GTCCTATTTTAATGCTTTTGTCCCTTTAGTGAGGGTTAAT-3'
BCLAF1	NM_001077440	5'-TAATACGACTCACTATAGGGTTCACTTTTCAATCAACTTTAATCAAATAACTCACTGATACCTG-3'
		5'-Phos-CGTTAACATACTTTGTTTTGTCCCTTTAGTGAGGGTTAAT-3'
LAS1L	NM_031206	5'-TAATACGACTCACTATAGGGCAATTTCATCATTCATTCATTTCATGGGAGACAGCCTGGATCAG-3'
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MPO	NM_000250	5'-TAATACGACTCACTATAGGGCTTTCTACATTATTCACAACATTATTCCTCACCCTGATTTCTTG-3'
		5'-Phos-CTTATTCACTGAAGTTCTCCTCCCTTTAGTGAGGGTTAAT-3'
HSP90B1	NM_003299	5'-TAATACGACTCACTATAGGGCTATCTTCATATTTCACTATAAACGGAGAGACTTGTTTTGGATG-3'
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GINS2	NM_016095	5'-TAATACGACTCACTATAGGGCTTTCAATTACAATACTCATTACAGCCAACAATGCTGACCGGTG-3'
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RRP7A	NM_015703	5'-TAATACGACTCACTATAGGGTCATTTCACAATTCAATTACTCAACCTCAATGCAAAAGCCCTTG-3'
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PRTN3	NM_002777	5'-TAATACGACTCACTATAGGGTCATTTCAATCAATCATCAACAATCTTCGTGATCTGGGGATGTG-3'
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HSPB1	NM_001537	5'-TAATACGACTCACTATAGGGTACACTTTAAACTTACTACACTAAAAATCCGATGAGACTGCCGC-3'
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G0S2	NM_015714	5'-TAATACGACTCACTATAGGGCTATTACACTTTAAACATCAATACTAGAACTGACCTACCACAAG-3'
		5'-Phos-CATCCACCAAAGGAGTTTGGTCCCTTTAGTGAGGGTTAAT-3'
SLC2A3	NM_006931	5'-TAATACGACTCACTATAGGGCTTTCTATTCATCTAAATACAAACACTTCATGTCAACTTTCTGG-3'
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S100P	NM_005980	5'-TAATACGACTCACTATAGGGCTTTCTATCTTTCTACTCAATAATTCACAGATTCCTGGCAGAGC-3'
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SERPINA1	NM_000295	5'-TAATACGACTCACTATAGGGAATCATACTCAACTAATCATTCAATTACATTTACCCAAACTGTC-3'
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FUCA1	NM_000147	5'-TAATACGACTCACTATAGGGAATCTACACTAACAATTTCATAACGGAAAAGGCTTACCAGGCTG-3'
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ALOX5	NM_000698	5'-TAATACGACTCACTATAGGGCTACTATACATCTTACTATACTTTCTCAGCATTTCCACACCAAG-3'
		5'-Phos-CAGCAACAGCAAATCACGACTCCCTTTAGTGAGGGTTAAT-3'
NPC2	NM_006432	5'-TAATACGACTCACTATAGGGATACTAACTCAACTAACTTTAAACCAGAAACTGAGCTCCGGGTG-3'
		5'-Phos-GCTGGTTCTCAGTGGTTGTCTCCCTTTAGTGAGGGTTAAT-3'
FCER1G	NM_004106	5'-TAATACGACTCACTATAGGGTCATTTACCAATCTTTCTTTATACCCAGGAACCAGGAGACTTAC-3'
		5'-Phos-GAGACTCTGAAGCATGAGAATCCCTTTAGTGAGGGTTAAT-3'
NCF2	NM_000433	5'-TAATACGACTCACTATAGGGCTACAAACAAACAAACATTATCAAAAGGGCACGAGAGAGTCTTC-3'
		5'-Phos-CAGGTACTGATCCTGTTTCTTCCCTTTAGTGAGGGTTAAT-3'
ITGAM	NM_000632	5'-TAATACGACTCACTATAGGGTACACTTTCTTTCTTTCTTTCTTTGGTTTCCTTCAGACAGATTC-3'
		5'-Phos-CAGGCGATGTGCAAGTGTATTCCCTTTAGTGAGGGTTAAT-3'
ITGB2	NM_000211	5'-TAATACGACTCACTATAGGGTACACAATCTTTTCATTACATCATAGAAATCCAGTTATTTTCCG-3'
		5'-Phos-CCCTCAAAATGACAGCCATGTCCCTTTAGTGAGGGTTAAT-3'
GAPDH	NM_002046	5'-TAATACGACTCACTATAGGGTCATTCATATACATACCAATTCATATCTCCCCTCCTCACAGTTG-3'
		5'-Phos-CCATGTAGACCCCTTGAAGATCCCTTTAGTGAGGGTTAAT-3'
HNRNPAB	NM_031266	5'-TAATACGACTCACTATAGGGTCAATCATCTTTATACTTCACAATGCCTGGACCTGTGGACCCTG-3'
		5'-Phos-GTTGTAAAGAGTAAATTGTATCCCTTTAGTGAGGGTTAAT-3'

Supplementary Table S2.
Primary Patient Sample Characteristics

Patient	Diagnosis	Cytogenetics
1	New diagnosis M5-AML	46 XY
2	Progressive MDS (RAEB-2)	46 XY
3	New diagnosis AML with monocytic differentiation	46,XY,t(11;17)(q23;q12)
4	New diagnosis mixed phenotype acute leukemia (T/myeloid)	49,XX,+4,+10,+19[8]/46,XX [cp12]
5	Relapsed AML	47,XY,+11[15]/47,XY,+del(11)(q11q21)[3]/46,XY[2]

Supplementary Table S3.
Significance values for viability comparisons in Figure 4A.

	One-way analysis of variance			p VALUE	
	Bonferroni's Multiple Comparison Test			Day 3	Day 6
HL-60	Control shRNA + DMSO	x	Control shRNA + GDC-0941, 1000nM	***	***
HL-60	Control shRNA + DMSO	x	Control shRNA + Torin 1, 250nM	***	***
HL-60	Control shRNA + DMSO	x	Control shRNA + 4EGI-1, 25μM	***	***
HL-60	Control shRNA + DMSO	x	SYK shRNA#1 + DMSO	***	***
HL-60	Control shRNA + DMSO	x	SYK shRNA#1 + GDC-0941, 1000nM	***	***
HL-60	Control shRNA + DMSO	x	SYK shRNA#1 + Torin 1, 250nM	***	***
HL-60	Control shRNA + DMSO	x	SYK shRNA#1 + 4EGI-1, 25μM	***	***
HL-60	Control shRNA + GDC-0941, 1000nM	x	SYK shRNA#1 + GDC-0941, 1000nM	***	***
HL-60	Control shRNA + Torin 1, 250nM	x	SYK shRNA#1 + Torin 1, 250nM	***	***
HL-60	Control shRNA + 4EGI-1, 25μM	x	SYK shRNA#1 + 4EGI-1, 25μM	***	***
HL-60	SYK shRNA#1 + DMSO	x	SYK shRNA#1 + GDC-0941, 1000nM	***	***
HL-60	SYK shRNA#1 + DMSO	x	SYK shRNA#1 + Torin 1, 250nM	**	***
HL-60	SYK shRNA#1 + DMSO	x	SYK shRNA#1 + 4EGI-1, 25μM	***	***
				Day 3	Day 6
U937	Control shRNA + DMSO	x	Control shRNA + GDC-0941, 1000nM	***	***
U937	Control shRNA + DMSO	x	Control shRNA + 4EGI-1, 25μM	***	***
U937	Control shRNA + DMSO	x	SYK shRNA#1 + DMSO	***	***
U937	Control shRNA + DMSO	x	SYK shRNA#1 + GDC-0941, 1000nM	***	***
U937	Control shRNA + DMSO	x	SYK shRNA#1 + 4EGI-1, 25μM	***	***
U937	Control shRNA + GDC-0941, 1000nM	x	SYK shRNA#1 + DMSO	***	***
U937	Control shRNA + GDC-0941, 1000nM	x	SYK shRNA#1 + GDC-0941, 1000nM	***	***
U937	Control shRNA + 4EGI-1, 25μM	x	SYK shRNA#1 + DMSO	***	***
U937	Control shRNA + 4EGI-1, 25μM	x	SYK shRNA#1 + 4EGI-1, 25μM	***	***
U937	SYK shRNA#1 + DMSO	x	SYK shRNA#1 + GDC-0941, 1000nM	ns	***
U937	SYK shRNA#1 + DMSO	x	SYK shRNA#1 + 4EGI-1, 25μM	ns	***
U937	Control shRNA + DMSO	x	Control shRNA + Torin 1, 50nM	***	***
U937	Control shRNA + DMSO	x	SYK shRNA#1 + DMSO	***	***
U937	Control shRNA + DMSO	x	SYK shRNA#1 + Torin 1, 50nM	***	***
U937	Control shRNA + Torin 1, 50nM	x	SYK shRNA#1 + Torin 1, 50nM	***	***
U937	SYK shRNA#1 + DMSO	x	SYK shRNA#1 + Torin 1, 50nM	***	***
	ns not significant				
	** p<0.01				
	*** p<0.001				

Supplementary Figure Legends

Supplementary Figure S1.

AKT Activation in AML cell lines. Basal levels of phosphorylated AKT in six untreated AML cell lines. AKT activation was assessed by immunoblotting to p-AKT (Ser473).

Supplementary Figure S2.

R406 inhibits SYK and modulates AKT and mTOR activation. (a) Cells from two AML cell lines were treated for 24 hours with R406 versus DMSO and SYK phosphorylation at Tyr525/526 evaluated by intracellular flow cytometry. (b) Western blot of p-AKT (Ser473) and p-mTOR (Ser2448) in MOLM-14 treated with R406 for 6 hours. (c) Cells were treated with 4 μM R406 for various time points, as indicated, and analyzed for p-SYK (Tyr525/526), p-AKT (Ser473), p-mTOR (Ser2448), p-RPS6 (Ser240/244) and p-4E-BP1 (Thr37/46). The x-axis denotes expression (log scale) for each protein of interest, and each is labeled with the median fluorescent intensity (MFI) per condition.

Supplementary Figure S3.

Feedback on SYK is not observed with inhibitors of PI3K or mTORC1/2. (a) MOLM-14 and U937 cells were treated with GDC-0941 or vehicle for 24 hours prior to western blot analysis with p-AKT Ser (473) or p-SYK (Tyr525/526). (b) MOLM-14 and U937 cells were treated with Torin 1 or vehicle for 24 hours prior to western blot analysis with p-4E-BP1 (Thr37/46) or p-SYK (Tyr525/526). For p-SYK analysis, cell extracts were first immunoprecipitated with antibody to SYK before western blotting with p-SYK.

Supplementary Figure S4.

Chemical inhibition of SYK leads to more consistent inhibition of RPS6 than 4E-BP1 when assessed by intracellular phospho-flow cytometry. Four AML cell lines were treated with either vehicle (DMSO) or R406 (0.5 μM and 4 μM) for 24 hours and levels of (a) RPS6 and (b) 4E-BP1 phosphorylation were assessed by intracellular phospho-flow cytometry. Phosphorylation of RPS6 at the Ser240/244 site was inhibited across all cell lines with R406 treatment, but the effects on 4E-BP1 T37/46 phosphorylation were more variable. The histograms for each condition are representative of at least two biological replicates across up to 3 repeated experiments. The x-axis denotes expression (log scale) for each protein of interest, and each is labeled with the median fluorescent intensity (MFI) per condition.

Supplementary Figure S5.

Genetic inhibition of SYK leads to more consistent alterations of RPS6 than 4E-BP1 when assessed by phospho-flow cytometry. RPS6 and 4E-BP1 phosphorylation were assessed in three AML cell lines 72 hours after lentiviral infection with two *SYK*-directed shRNAs or a control shRNA. (a) Immunostaining for total-SYK shows that expression was decreased with both hairpins in each cell line. (b) RPS6 phosphorylation at the Ser240/244 site was consistently inhibited in all cell lines tested with *SYK*-directed shRNAs. (c) The effects of *SYK*-directed shRNA on 4E-BP1 phosphorylation were subtle or absent. The histograms for each condition are representative of at least two biological replicates across two *SYK*-directed shRNAs. The x-axis denotes expression (log scale) for each protein of interest and each is labeled with the median fluorescent intensity (MFI) per condition.

Supplementary Figure S6.

Effects of GDC-0941 and Torin 1 treatment on 4E-BP1 phosphorylation in AML. Two AML cell lines treated for 24 hours with vehicle versus (a) GDC-0941 or (b) Torin 1. Activated 4E-BP1 was assessed by immunoblotting to p-4E-BP1 (Thr37/46).

Supplementary Figure S7.

Inhibition by R406 and 4EGI-1 shows variable effects on individual gene expression. HL-60 and U937 were grown in the presence of R406, 4EGI-1 or R406 + 4EGI-1 for 3 days at which time, GE-HTS analysis was performed. The heat maps are derived from the resulting row-wise comparisons of the performance of individual genes in the signature. Red color indicates a relative increase in gene expression, whereas, blue indicates a relative decrease.

Supplementary Figure S8.

Combined treatment with 4EGI-1 and R406 induces differentiation and inhibits proliferation. HL-60 and U937 cell lines were treated with 4 μM R406, 25 μM 4EGI-1 or a combination of these two molecules for 5 days before flow analysis of (a) myeloid differentiation markers (CD11b and CD14), (b) BrdU incorporation, and (c) apoptosis induction marker (active caspase 3).

Supplementary Figure S9.

MEK inhibition blocks MAPK activation in RAS-mutated AML cell lines. AML cells were treated with vehicle or 4 μM R406 alone, or in combination with the MEK inhibitor PD0325901

(100 nM) or the PI3K inhibitor GDC-0941 (500 nM) for 24 hours before western blot analysis with p-ERK1/2 (Thr202/Tyr204).

Supplementary Figure S10.

Effects of rapamycin on RPS6 and 4E-BP1 activation and differentiation in AML. (a) Two AML cell lines were treated with either vehicle (DMSO) or 10 nM rapamycin for 24 hours and levels of RPS6 and 4E-BP1 phosphorylation were assessed by intracellular phospho-flow cytometry. The histograms for each condition are representative of at least two biological replicates across up to 3 repeated experiments. The x-axis denotes expression (log scale) for each protein of interest, and each is labeled with the median fluorescent intensity (MFI) per condition. (b) Cells were treated with rapamycin or 4EGI-1 for 3 days and the Differentiation Score was determined. Data was analyzed and P-values determined as described in Figure 5. Statistical differences between all chemically-treated samples and DMSO-treated samples are displayed as P-value symbols.

Supplementary Table Legends

Supplementary Table S1.

Differentiation signature (32-gene signature). Listed are the gene names, Ref Seq numbers, Luminex LUA tags, and the probe sequence for the 32 myeloid differentiation signature genes.

Supplementary Table S2.

Primary patient AML/MDS sample information including clinical diagnosis and cytogenetics.

Supplementary Table S3.

Significance values for viability comparisons in Figure 4A. P value symbols are indicated for the various pairwise comparison tests. All comparisons for HL-60 are from the same experiment. The comparisons for U937 derive from two separate experiments. The first 9 rows show comparisons for U937 treated with GDC-0941 and 4EGI-1, and the last 5 rows show comparisons from Torin 1 treatment.