APR-246 induces early cell death by ferroptosis in acute myeloid leukemia

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Supplemental Figure 1-

(a) Cell death (%) in MOLM-14 cells at 16 h post-APR-246 treatment (60μM) with or without ferrostatin-1 (10μM), necrostatin-1 (20 μM), necrostatin-1s (20 μM) and Necrosulfonamide (2μM) (n=3). Error bars, ± s.e.m. All compounds were added 2 h prior to APR-246 in the medium. Statistics, 2-way ANOVA; *p < 0.05, **p < 0.01, ***p < 0.0001.

(b) Immunoblot showing MLKL and pMLKL protein levels in MOLM-14 and OCI-AML2 cells with or without APR-246 at 16 h. b-actin was used as a loading control (n=2).

Supplemental Figure 2-

(a) Immunoblotting analysis of PARP, caspase 8 and caspase 3 in NB4 cells treated for 16 h with DMSO, APR-246 (60 μM) or puromycin (1 μg/mL). b-actin was used as a loading control (n=2).

(b) Cell death (%) and C11-BODIPY positive cells (%) of the indicated cells at 16 h and 14 h post-APR-246 treatment (60 μM) with or without ferrostatin-1 (10 μM) (n=3). Error bars, ± s.d.

(c-d) Immunoblotting analysis of PARP, caspase 8 and caspase 3 in OCI-AML2 **(c)** or MOLM-14 **(d)** cells treated for 16 h and 24 h with DMSO, APR-246 (30 μM), ferrostatin-1 (10μM) or puromycin (1 μg/mL). b-actin was used as a loading control (n=2).

(d) Immunoblotting analysis of PARP, caspase 8 and caspase 3 in OCI-AML2 cells treated for 16 h with DMSO, APR-246 (30 μM), ferrostatin-1 (10μM) or puromycin (1 μg/mL). b-actin was used as a loading control (n=2).

(e) Cell death (%) for the indicated cells at 16 h and 24 h post-APR-246 treatment (60μM) with or without ferrostatin-1 (10μM) and/or QVD-OPH (25μM) (n=3). Error bars, ± s.e.m. All compounds were added 2 h prior to APR-246 in the medium. Statistics, t-test; *p < 0.05, **p < 0.01, ***p < 0.0001.

Supplemental Figure 3

Electron microscopy analysis of MOLM14 cells treated with vehicle, chloroquine (autophagy inhibitor, 20μM), puromycin (apoptosis inducer, 1 mg/mL), erastin (ferroptosis inducer, 10 μM), RSL3 (ferroptosis inducer, 1 μM) or APR-246 (60 μM) at 16 h post treatment. The cells treated with chloroquine showed an accumulation of autophagolysosomes (*). Puromycin-treated cells showed a nuclear condensation typical of apoptosis induction (white arrow). However, cells treated with the ferroptosis inducers did not show these characteristics.

Supplemental Figure 4

(a) Immunoblotting analysis of SLC7A11 protein levels in MOLM14 and OCI-AML2 cells with or without SLC7A11 overexpression. b-actin was used as a loading control (n=2).

(b) Immunoblotting analysis of SLC7A11 protein levels in MOLM14 and OCI-AML2 cells with or without shRNA targeting SLC7A11 at 72 h post doxycycline induction. b-actin was used as a loading control (n=2).

(c) Cystine uptake in MOLM-14 cells with or without shRNA targeting SLC7A11 at 72 h post doxycycline induction.

(d) Cell death (%) for the indicated cells at 3- and 7-days post doxycycline induction. Error bars, ± s.e.m. All compounds were added 2 h prior to APR-246 in the medium. Statistics, t-test; *p < 0.05, **p < 0.01, ***p < 0.0001.

(e) Cell count for the indicated cells at 3- and 7-days post doxycycline induction. Error bars, ± s.e.m. All compounds were added 2 h prior to APR-246 in the medium. Statistics, t-test; *p < 0.05, **p < 0.01, ***p < 0.0001.

(f) Detection of lipid peroxidation (using C11-BODIPY) by FCM at 14 h post APR-246 (50 μM) treatment in the MOLM14 cell line. Statistics, t-test; *p < 0.05, **p < 0.01, ***p < 0.0001.

(g) Viability curves for the indicated cells at 24 h post-erastin treatment. Error bars, ± s.d.

Supplemental Figure 5-

Synergy score for co-treatment with APR-246 and erastin in the indicated AML cell lines (24 hours). The mean AML cell line viability of three independent experiments was used. One experiment was used for primary AML cells.

Supplemental Figure 6-

(a) Immunoblotting analysis of SLC7A11, GPX4, and TP53 protein level in AML cell lines. b-actin was used as a loading control (n=2).

(b) Correlation analysis between SLC7A11 or GXP4 protein level and APR-246 IC50. Statistics: Pearson index. *p < 0.05, **p < 0.01, ***p < 0.0001.

Supplemental Figure 7-

(a) Immunoblotting analysis of GPX protein levels in MOLM14 and OCI-AML2 cells with or without shRNA targeting GPX4 (Day 3 post-doxycycline induction). b-actin was used as a loading control (n=2).

(b) Cell death (%) for the indicated cell types at day 2-3-4 h post-doxycycline treatment (n=3). Error bars, ± s.d.

Supplemental Figure 8-

Viability curves for the indicated cells and compounds at 24 h post-treatment. Error bars, ± s.d.

Supplemental Figure 9-

Synergy score for co-treatment with APR-246 and RSL3 in the indicated AML cell lines (H24). The mean cell viability of three independent experiments was used.

Supplemental Figure 10-

Synergy score for co-treatment with APR-246 and FINO-2 for the indicated AML cell lines (H24). The mean cell viability of three independent experiments was used.

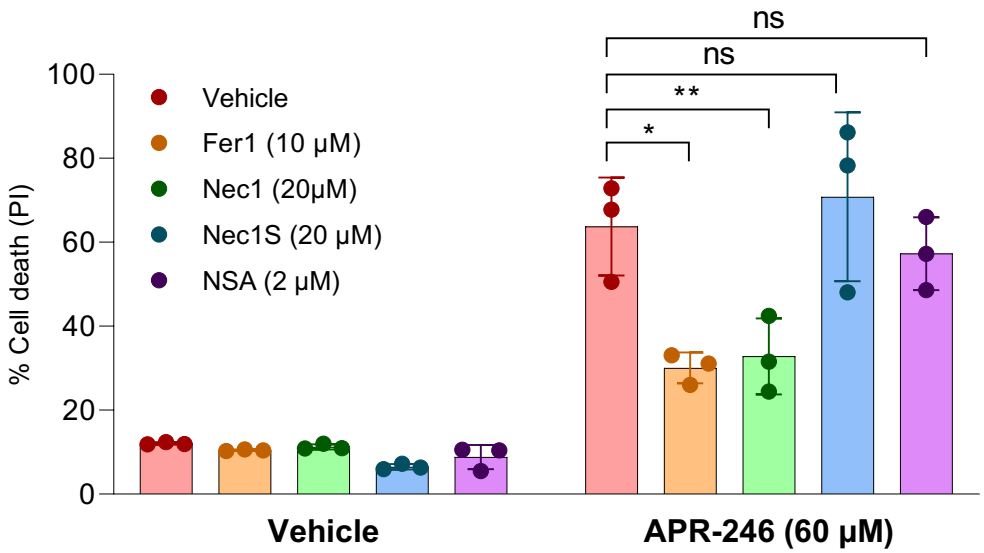
Supplemental Table 1

Primary AML sample characteristics.

Supplemental Table 2

AML cell lines characteristics.

a



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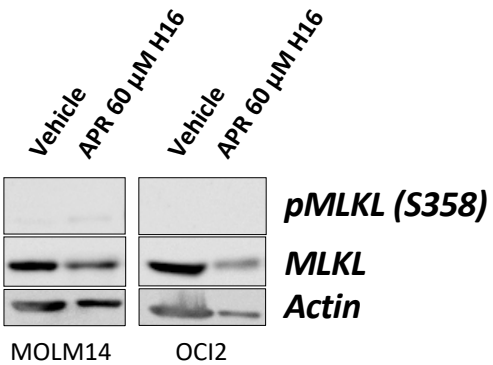
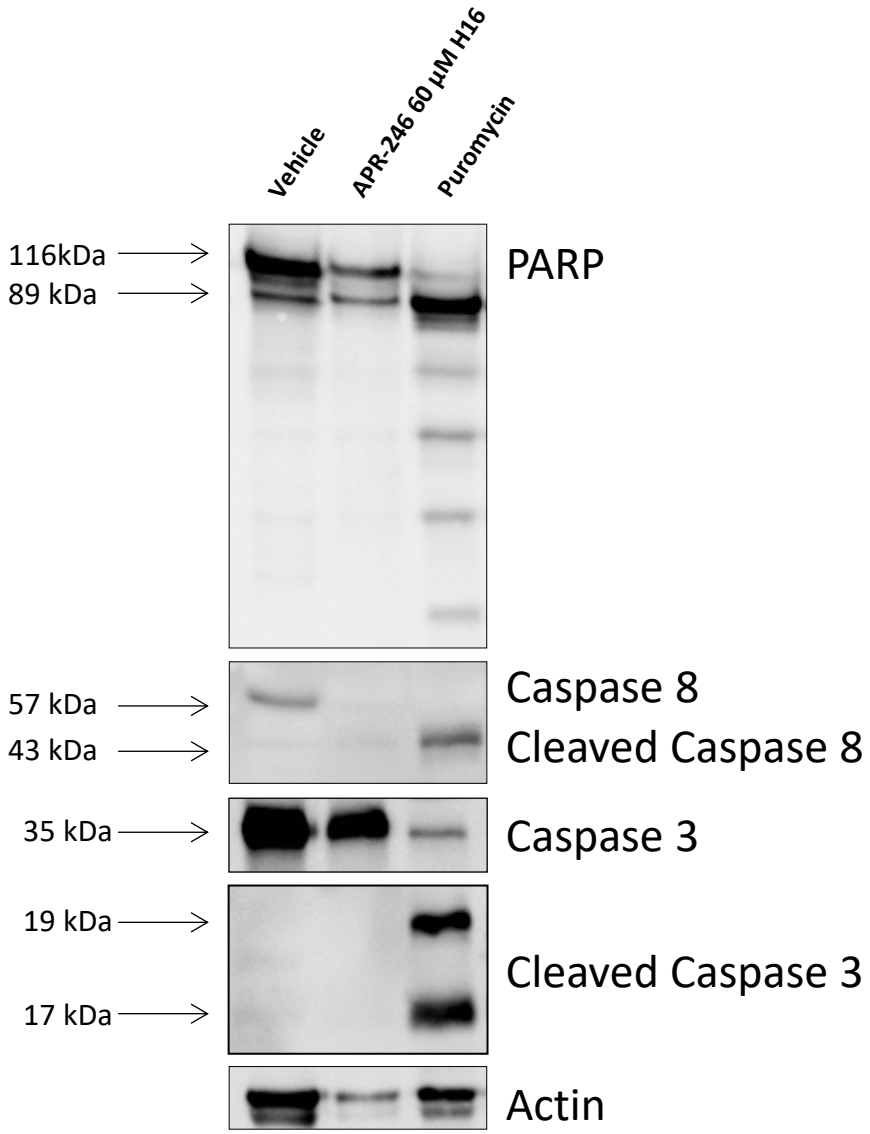


Supplemental Figure 1-

a



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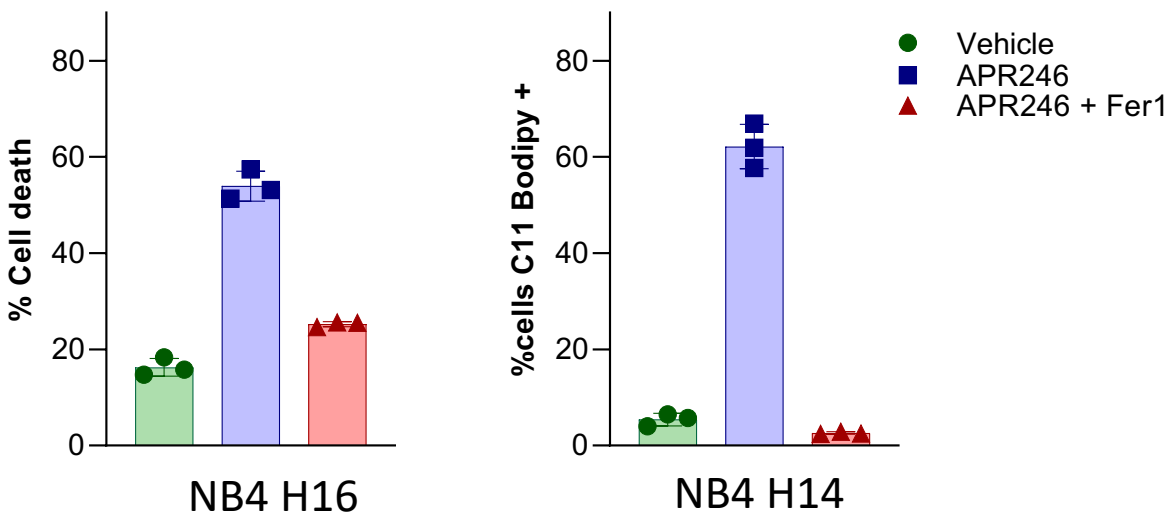
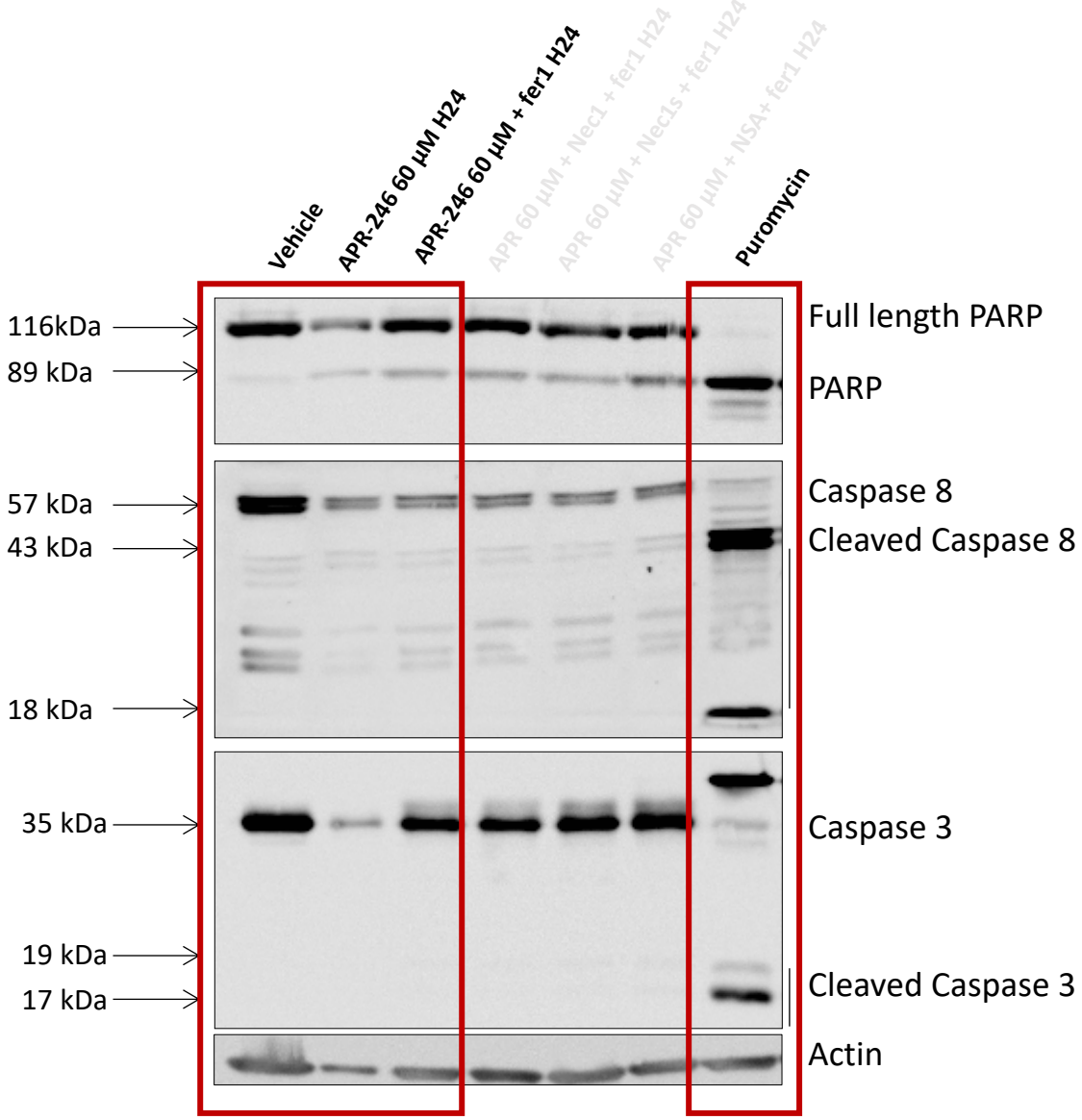
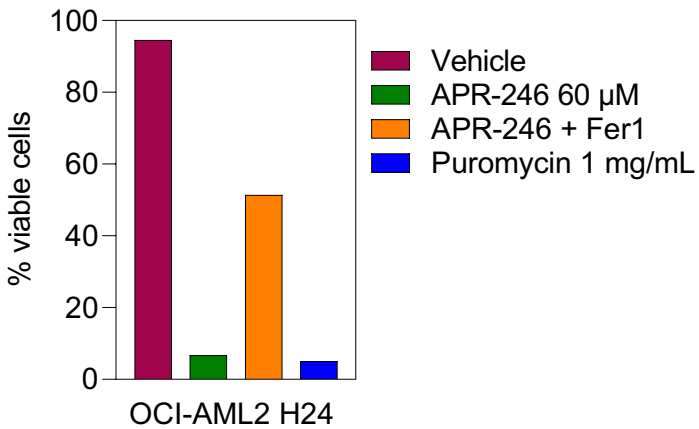


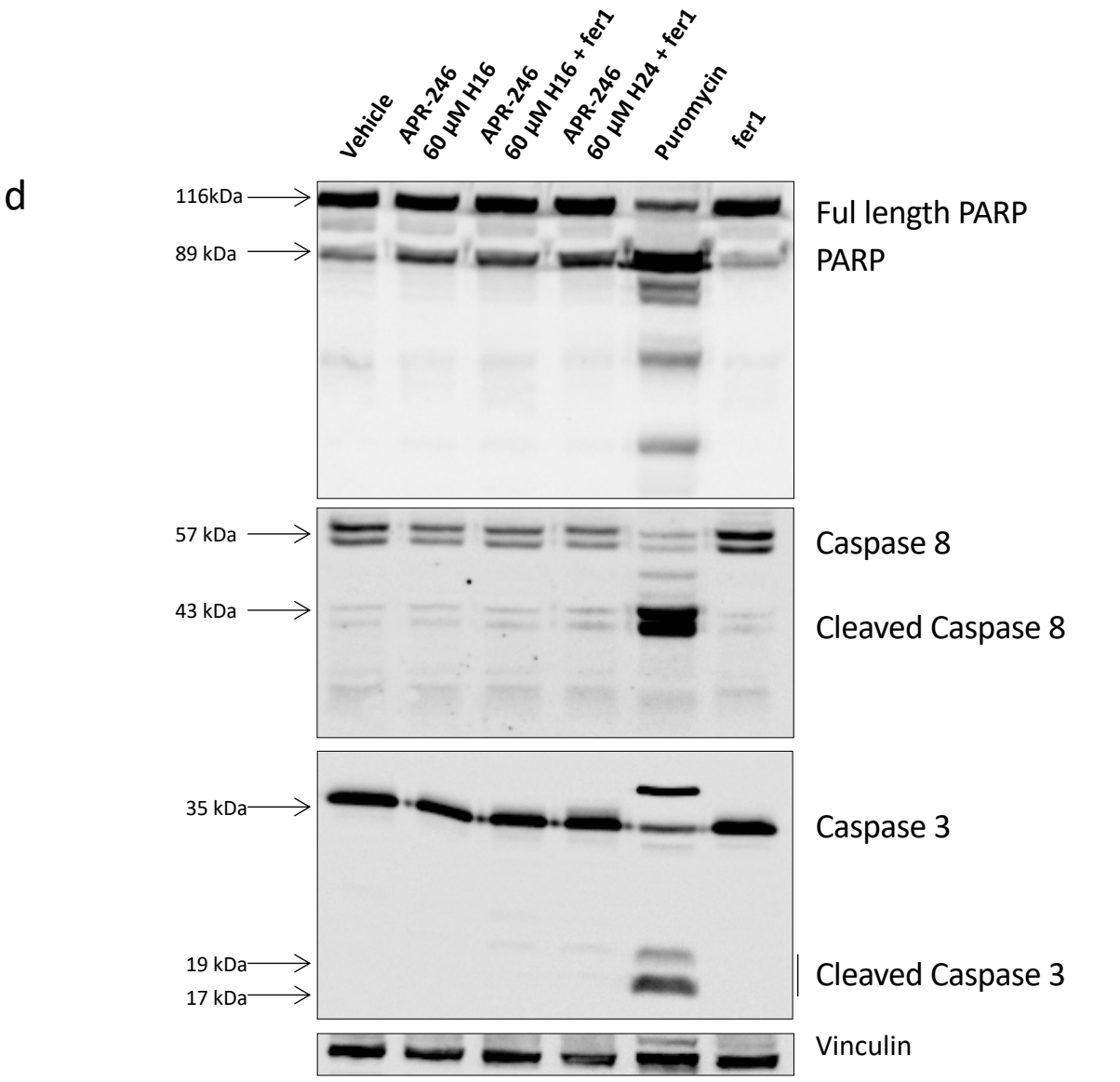
Supplemental Figure 2-

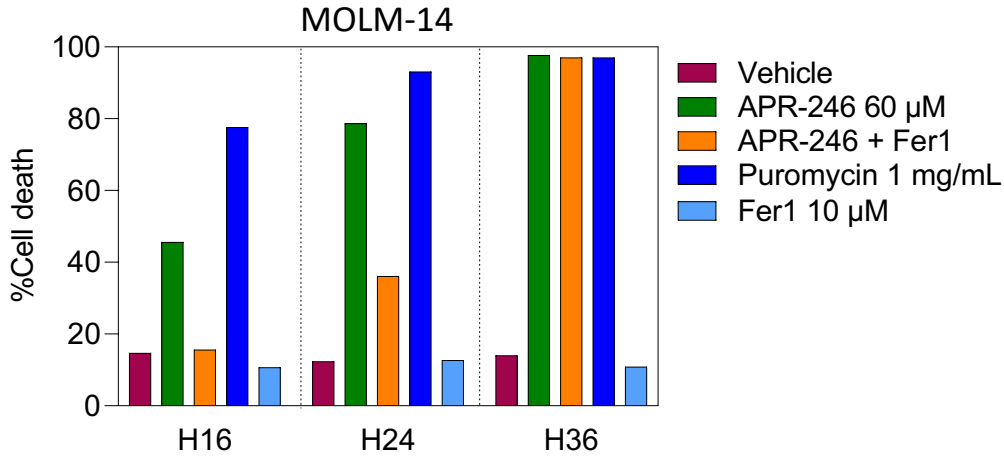
C

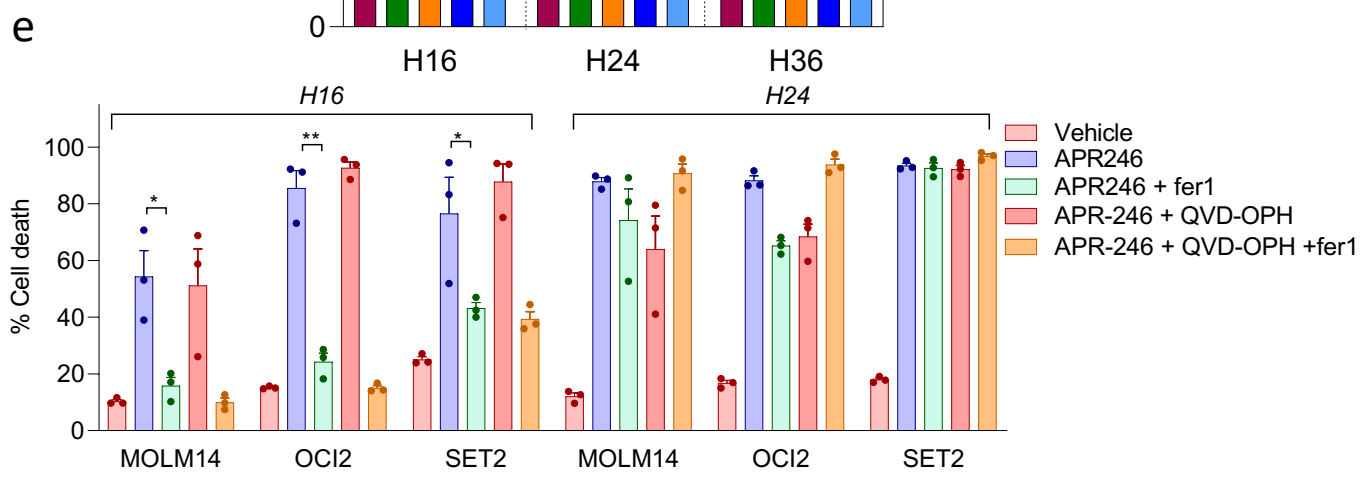




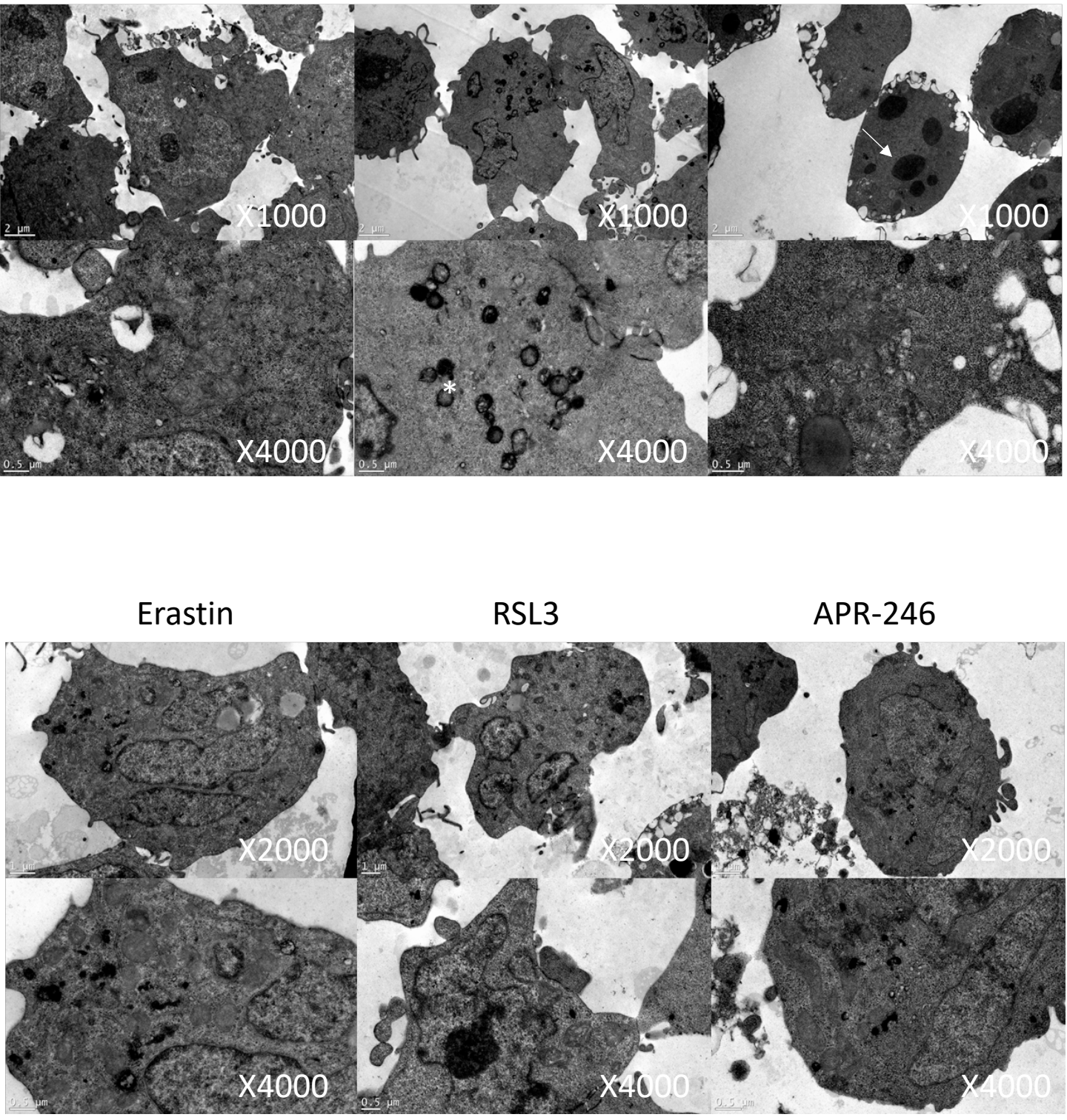
Supplemental Figure 2-



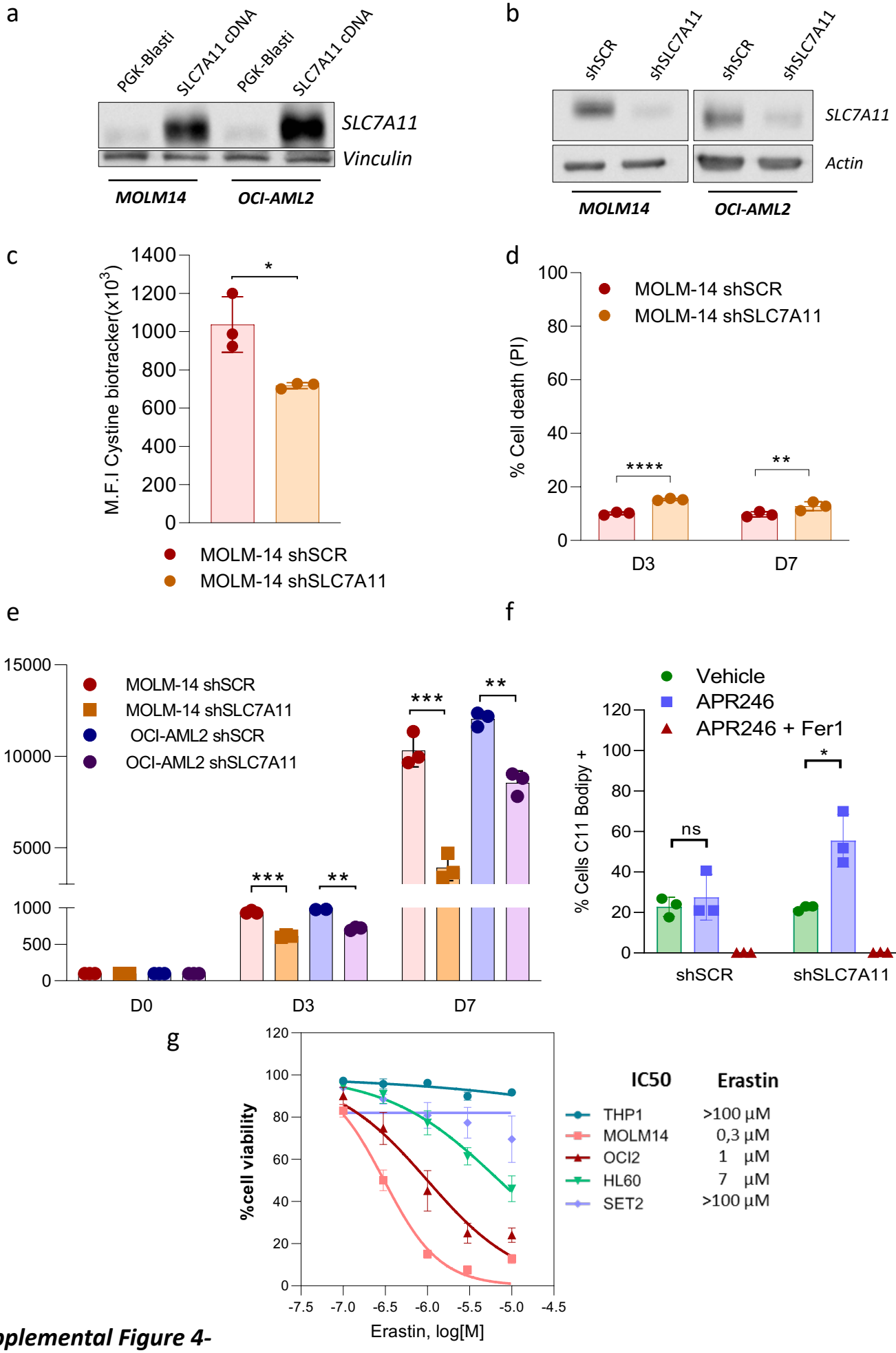




Supplemental Figure 2-

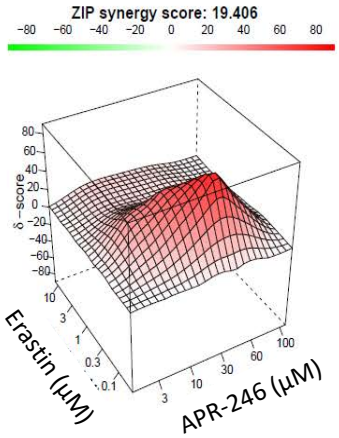


Supplemental Figure 3-



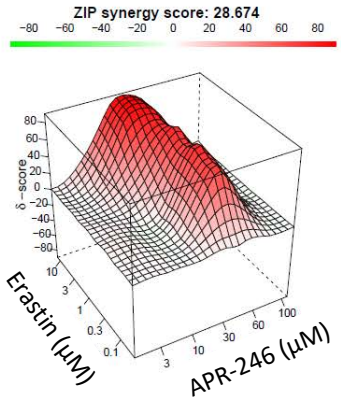
Supplemental Figure 4-

MOLM14



Erastin (μM) \ APR-246 (μM)	0	3	10	30	60	100
0	0	0	0	0	0	0
0,1	0	17	44	81	32	2
0,3	0	37	56	49	20	1
1	0	16	17	14	5	0
3	0	11	12	10	4	0
10	0	12	12	10	4	0

THP1



Erastin (μM) \ APR-246 (μM)	0	3	10	30	60	100
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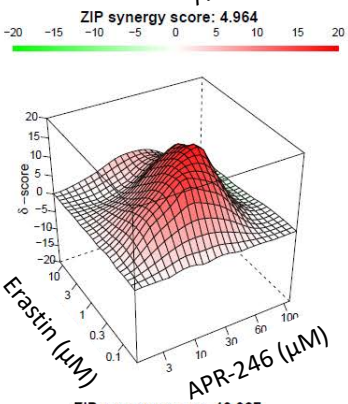
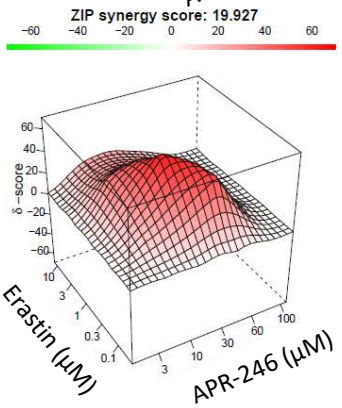
0,1	0	1	4	72	9	2
0,3	0	1	7	79	10	2
1	0	2	35	80	10	2
3	0	10	79	75	9	2
10	0	27	86	75	10	3

SET2



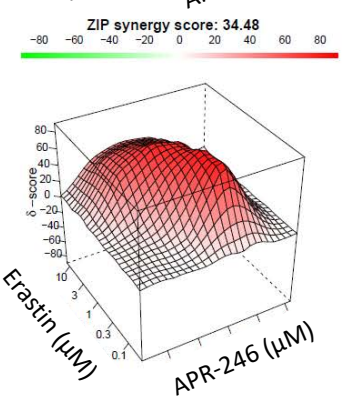
Erastin (μM) \ APR-246 (μM)	0	3	10	30	60	100
0	0	0	0	0	0	0
0,1	0	8	15	11	1	0
0,3	0	7	14	18	1	0
1	0	3	9	14	1	-1
3	0	-1	2	6	0	-2
10	0	2	6	5	-1	-2

OCI2



Erastin (μM) \ APR-246 (μM)	0	3	10	30	60	100
0	0	0	0	0	0	0
0,1	0	15	32	51	7	1
0,3	0	29	64	45	6	1
1	0	36	44	27	3	0
3	0	21	25	14	1	-1
10	0	20	23	14	1	0

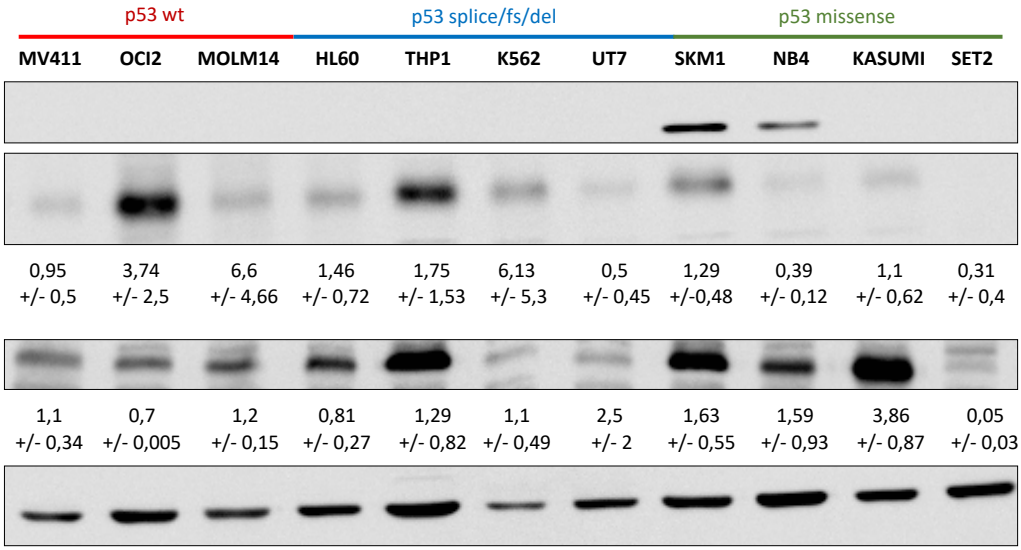
HL60



Erastin (μM) \ APR-246 (μM)	0	3	10	30	60	100
0	0	0	0	0	0	0
0,1	0	1	4	72	9	2
0,3	0	1	7	79	10	2
1	0	2	35	80	10	2
3	0	10	79	75	9	2
10	0	27	86	75	10	3

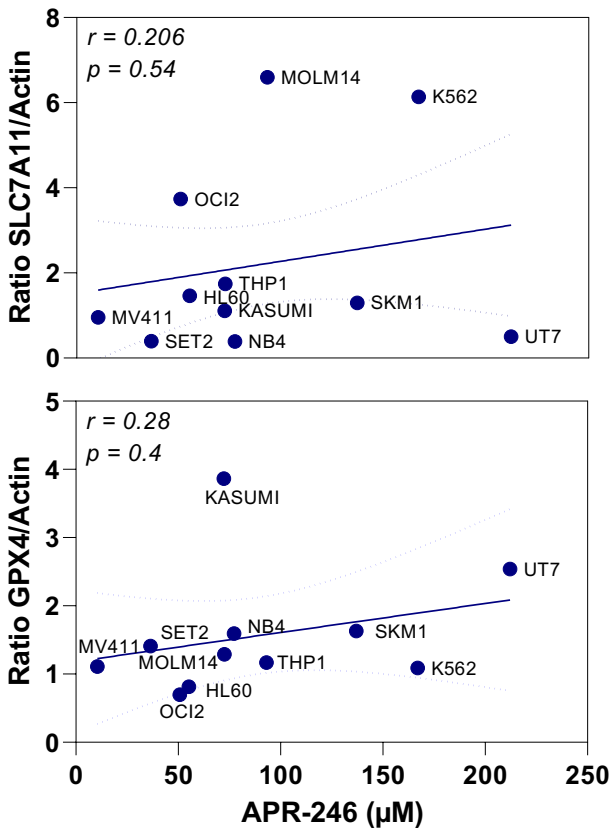
Supplemental Figure 5-

a



	MV411	OCI2	MOLM14	HL60	THP1	K562	UT7	SKM1	NB4	KASUMI	SET2
Ratio SLC7A11/actin (mean +/- SD; n=4)	0,95 +/- 0,5	3,74 +/- 2,5	6,6 +/- 4,66	1,46 +/- 0,72	1,75 +/- 1,53	6,13 +/- 5,3	0,5 +/- 0,45	1,29 +/-0,48	0,39 +/- 0,12	1,1 +/- 0,62	0,31 +/- 0,4
Ratio GPX4/actin (mean +/- SD; n=2)	1,1 +/- 0,34	0,7 +/- 0,005	1,2 +/- 0,15	0,81 +/- 0,27	1,29 +/- 0,82	1,1 +/- 0,49	2,5 +/- 2	1,63 +/- 0,55	1,59 +/- 0,93	3,86 +/- 0,87	0,05 +/- 0,03

b



Supplemental Figure 6-

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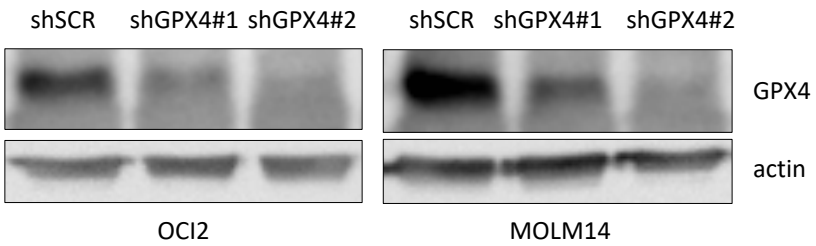

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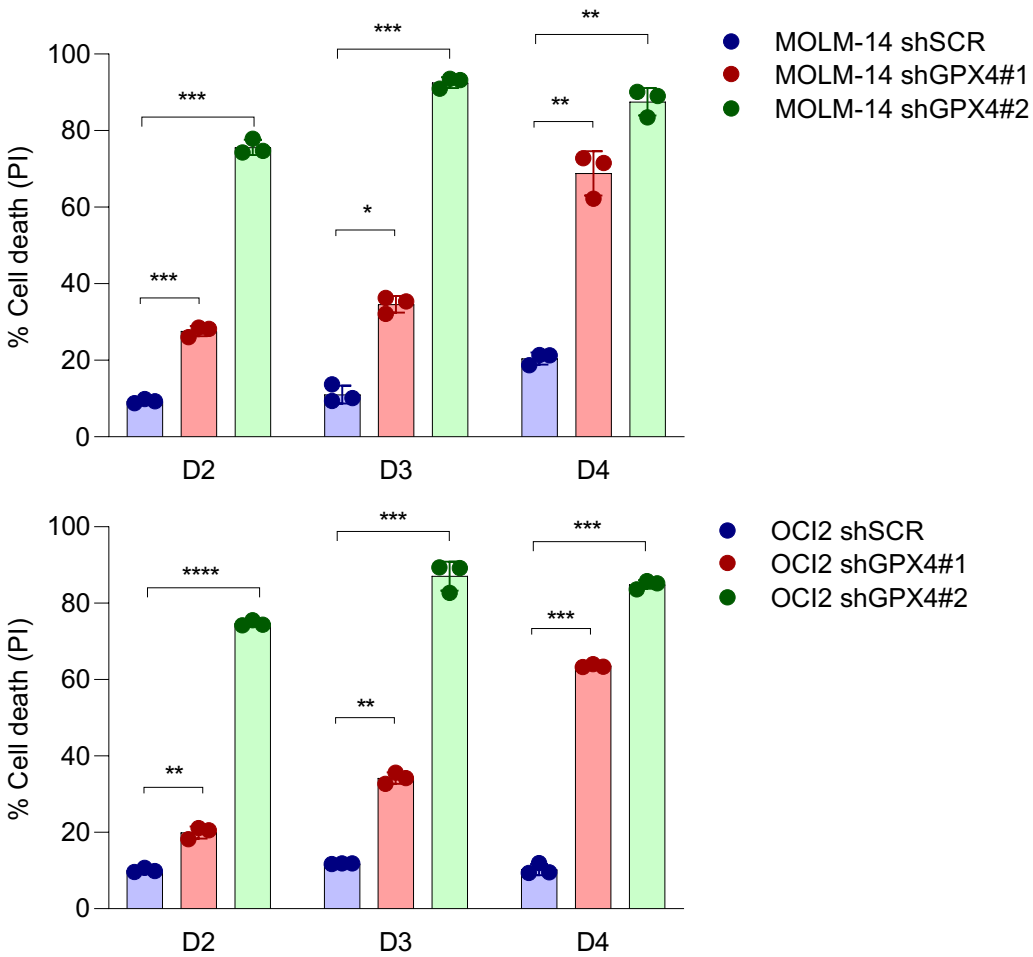
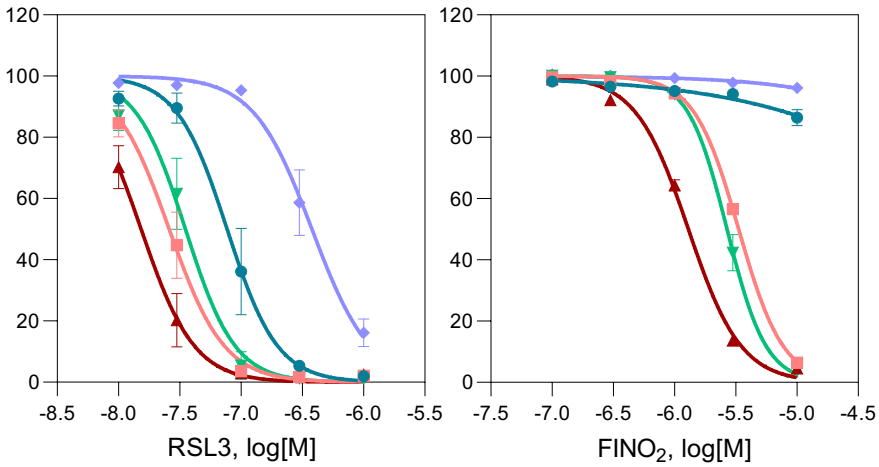


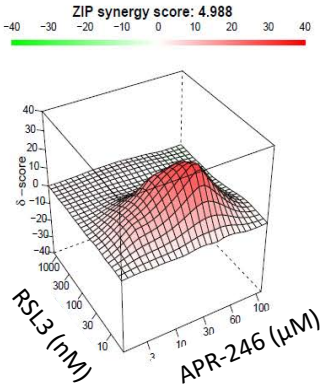
Supplemental Figure 7-



IC50	RSL3	$FINO_2$
THP1	77 nM	>100 µM
MOLM14	26 nM	3,3 µM
OCI2	15 nM	1,3 µM
HL60	35 nM	2,7 µM
SET2	378 nM	>100 µM

Supplemental Figure 8-

MOLM14



RSL3 (nM) \ APR-246 (μM)	0	3	10	30	60	100
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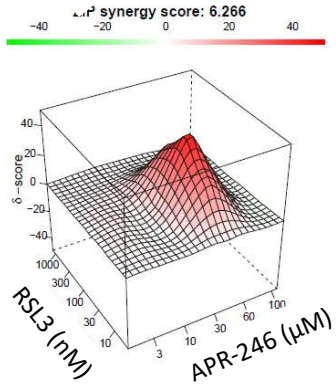
30	0	8	21	23	4	-1
100	0	2	2	1	-2	-3
300	0	0	1	0	-1	-2
1000	0	0	0	0	-2	-2

THP1



RSL3 (nM) \ APR-246 (μM)	0	3	10	30	60	100
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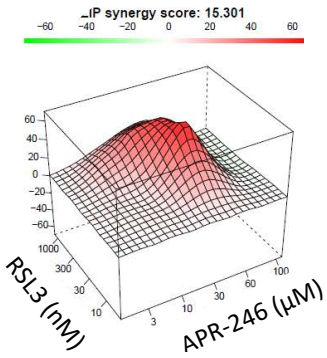
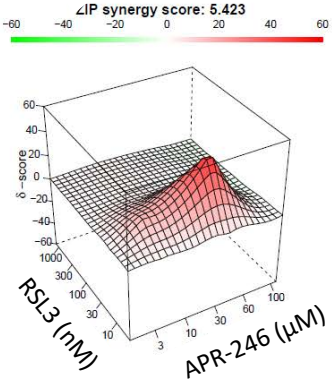
10	0	2	3	27	2	0
30	0	3	7	48	5	1
100	0	3	12	30	2	0
300	0	0	2	2	-1	-1
1000	0	1	2	2	-1	-2

SET2



RSL3 (nM) \ APR-246 (μM)	0	3	10	30	60	100
0	0	0	0	0	0	0
30	0	4	7	22	1	1
100	0	6	19	64	2	0
300	0	17	45	48	1	-1
1000	0	7	20	28	0	-1

OCI2



RSL3 (nM) \ APR-246 (μM)	0	3	10	30	60	100
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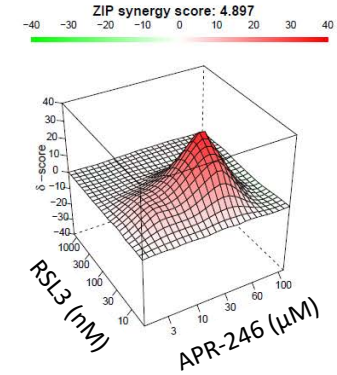
10	0	17	24	52	7	0
30	0	8	13	15	0	-2
100	0	1	1	0	-2	-3
300	0	1	1	0	-2	-3
1000	0	1	1	0	-2	-3

HL60



RSL3 (nM) \ APR-246 (μM)	0	3	10	30	60	100
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10	0	10	12	23	2	0
30	0	9	16	36	2	0
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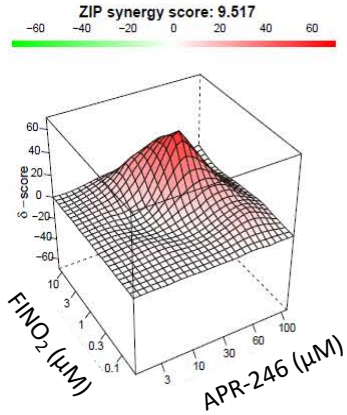
1000	0	-1	0	0	0	0

Supplemental Figure 9-

MOLM14



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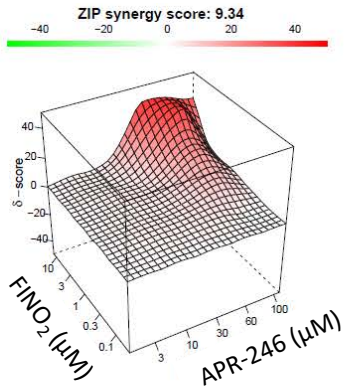
THP1



FINO$_2$ (μM) \ APR-246 (μM)	0	3	10	30	60	100
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0,3	0	2	2	6	11	2
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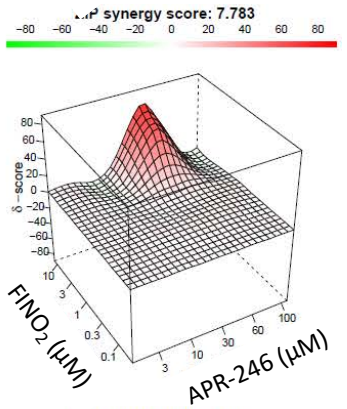
3	0	2	4	21	38	4
10	0	3	6	43	36	3

SET2



FINO$_2$ (μM) \ APR-246 (μM)	0	3	10	30	60	100
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0,3	0	0	1	2	3	0
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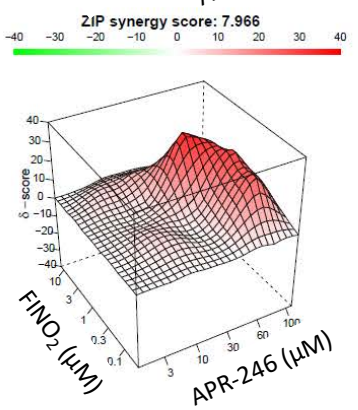
3	0	1	5	59	5	0
10	0	1	8	80	5	-1

OCI2



FINO$_2$ (μM) \ APR-246 (μM)	0	3	10	30	60	100
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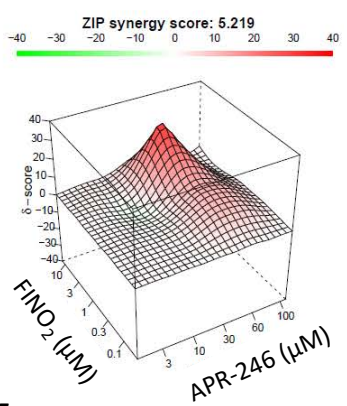
0,1	0	3	4	4	27	2
0,3	0	3	5	8	31	2
1	0	2	4	36	25	1
3	0	6	10	11	5	-1
10	0	0	2	2	0	-1

HL60



FINO$_2$ (μM) \ APR-246 (μM)	0	3	10	30	60	100
0	0	0	0	0	0	0
0,1	0	1	1	8	7	1
0,3	0	0	1	11	9	1
1	0	0	0	18	10	1
3	0	2	6	33	6	0
10	0	2	4	4	0	-1

Supplemental Figure 10-

Patient	Age	BM vs PB	WBC (G/L)	Blast (%)	Cytogenetics	Molecular status
AML#1	35	BM	50	97%	Normal	FLT3-ITD+, STAG2 +, TP53 -
AML#2	58	BM	9,4	47%	Normal	NPM1 +, TP53-
AML#3	31	BM	200	42%	Normal	NPM1+, FLT3-ITD+, TP53-
AML#4	44	BM	100	97%	t(10,11)	FLT3-, TP53-
AML#5	70	BM	125	90	ND	ND

Supplemental Table 1

Name	Cytogenetic	Molecular
MOLM14	MLL-AF9	FLT3-ITD (heterozygous)
THP1	MLL-AF9	Nras, TP53 (mut)
MV4-11	Complex	FLT3-ITD (homozygous)
HL-60	MYC amplification	Nras, TP53 (homozygous deletion)
KASUMI	t(8;21)	c-KIT, TP53 (mut)
OCI-AML2	complex	DNMT3A
OCI-AML3	complex	DNMT3A, NPM1
K562	complex	Bcr-Abl, TP53 (mut)
SKM1	complex	KRAS, TP53 (mut)
UT7-EPO	complex	TP53 (deletion)
NB4	t(15;17)	KRAS, TP53 (homozygous mutation)
SET2	Complex	JAK2 V617F, DNMT3A, TP53 (mut)

Supplemental Table 2