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Supporting Information for

Acquired resistance to PRMT5 inhibition induces concomitant collateral sensitivity to paclitaxel

Helen S. Mueller[1,2], Colin E. Fowler[1,2], Simona Dalin[1,2], Enrico Moiso[1], Tee Udomlumleart[1,2], Salil Garg[1,4], Michael T. Hemann[1,2], Jacqueline A. Lees[1,2*]

[1]The David H. Koch Institute for Integrative Cancer Research, [2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. [4]Department of Pathology, Massachusetts General Hospital, Boston, MA 02114, USA.

[*]Corresponding author: Jacqueline A. Lees, jalees@mit.edu

This PDF file includes:

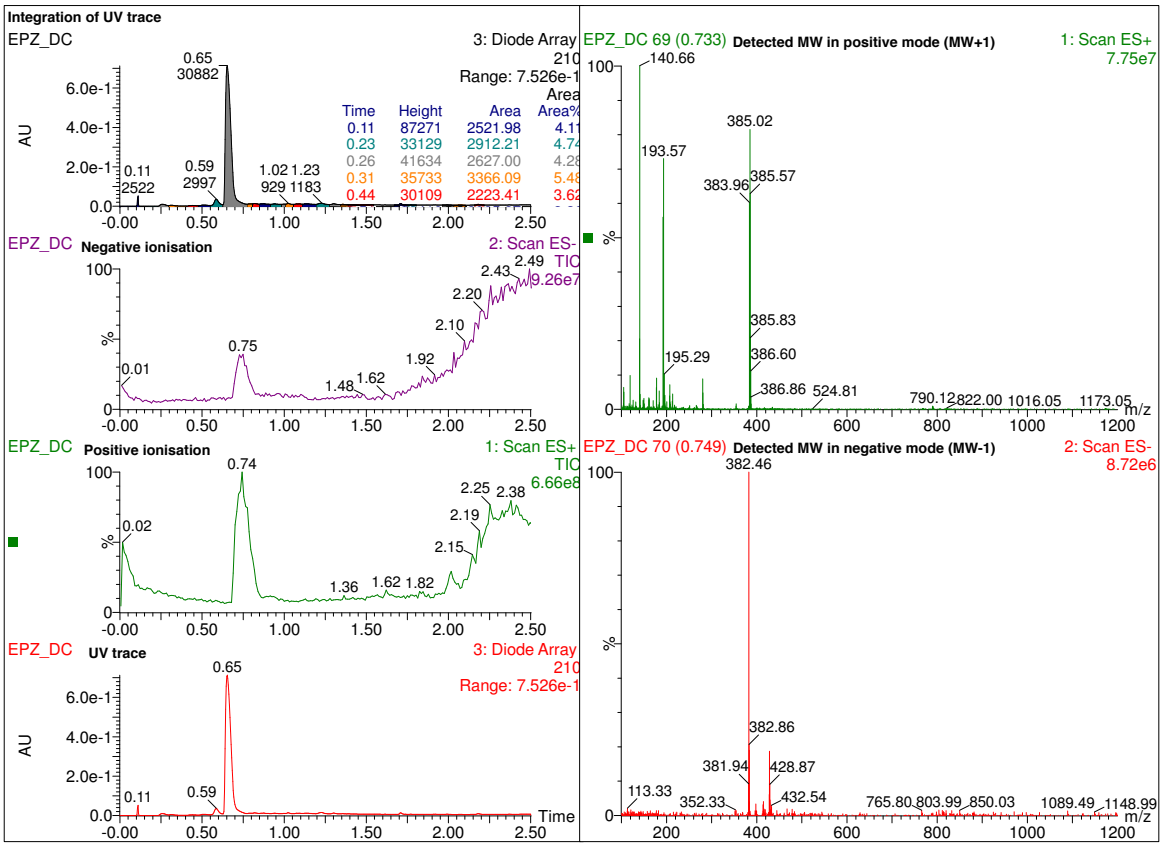
Supplementary Methods
Figures S1 to S5
Table S1
Legends for Datasets S1 and S2

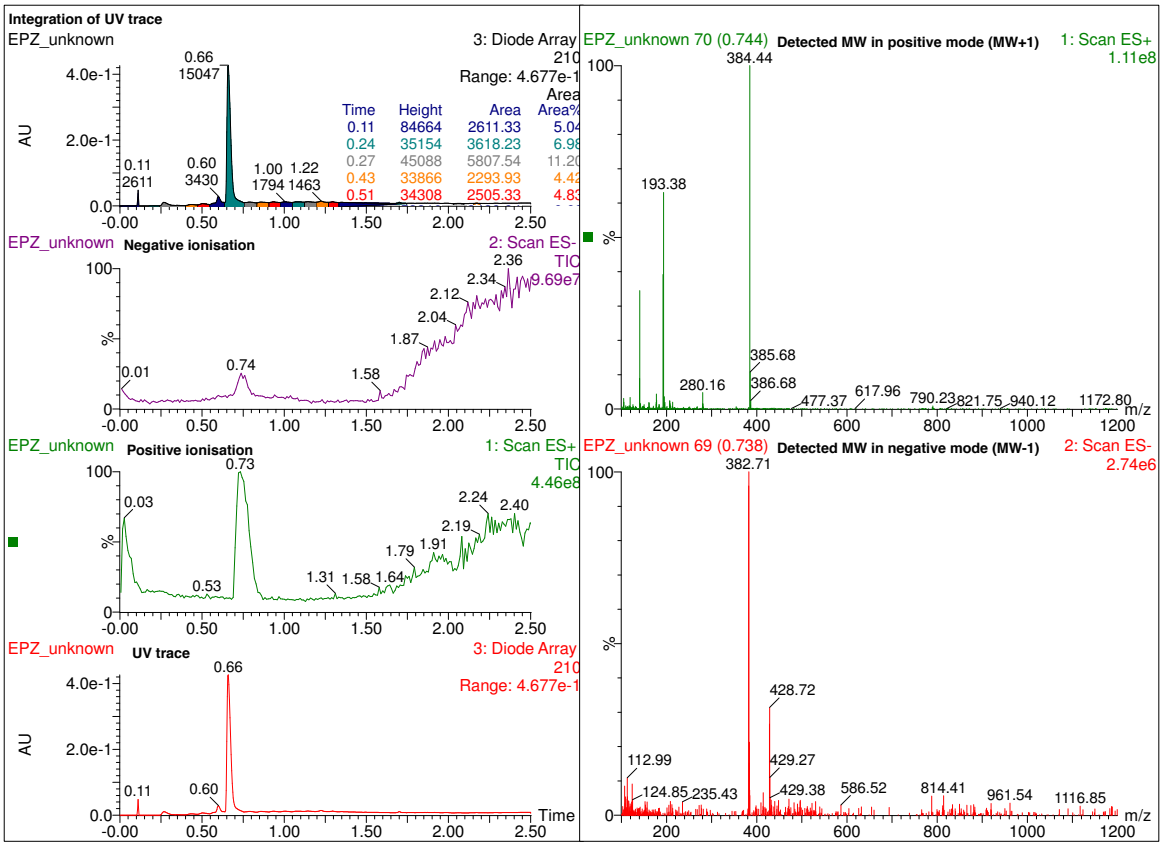
Other supplementary materials for this manuscript include the following:

Datasets S1 to S2

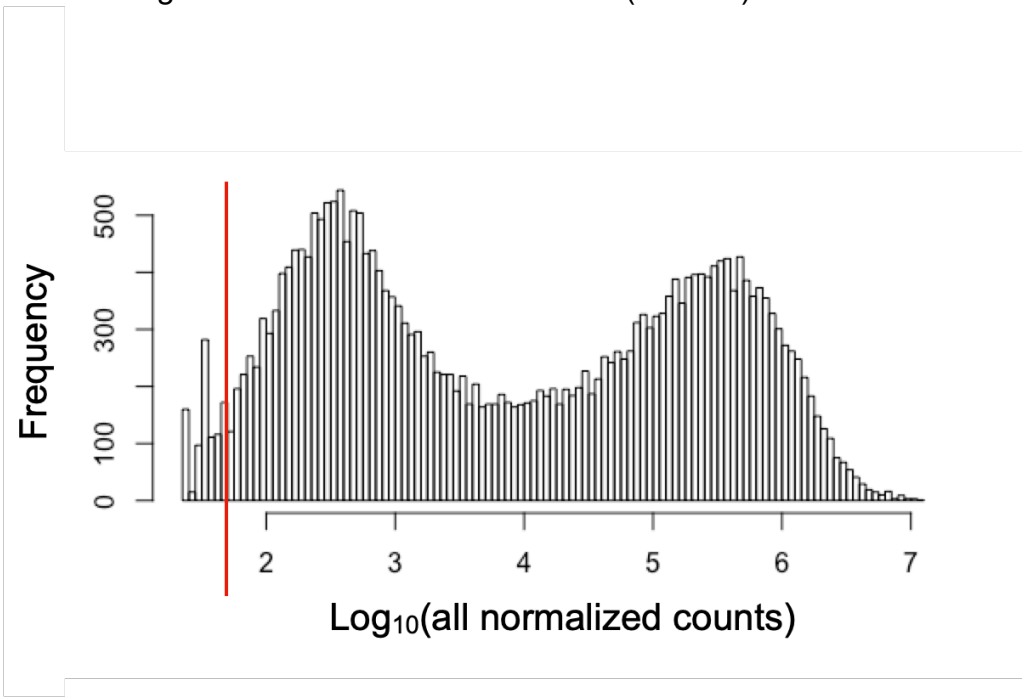
Supplementary Methods

1). HPLC traces show that EPZ015666 (MW: 383.44) purchased from DC Chemicals (EPZ_DC) and the EPZ015666 used in the experiments in this study (received as a gift; EPZ_unknown) have similar traces and are both pure.




Integration of UV trace
EPZ_unknown
3: Diode Array
210
Range: 4.677e-1
Time Height Area Area%
0.11 84664 2611.33 5.04
0.24 35154 3618.23 6.98
0.27 45088 5807.54 11.20
0.43 33866 2293.93 4.42
0.51 34308 2505.33 4.83
AU
Negative ionisation
2: Scan ES-
TIC
9.69e7
Positive ionisation
1: Scan ES+
TIC
4.46e8
UV trace
Time
EPZ_unknown 70 (0.744)
Detected MW in positive mode (MW+1)
1: Scan ES+
1.11e8
EPZ_unknown 69 (0.738)
Detected MW in negative mode (MW-1)
2: Scan ES-
2.74e6
m/z

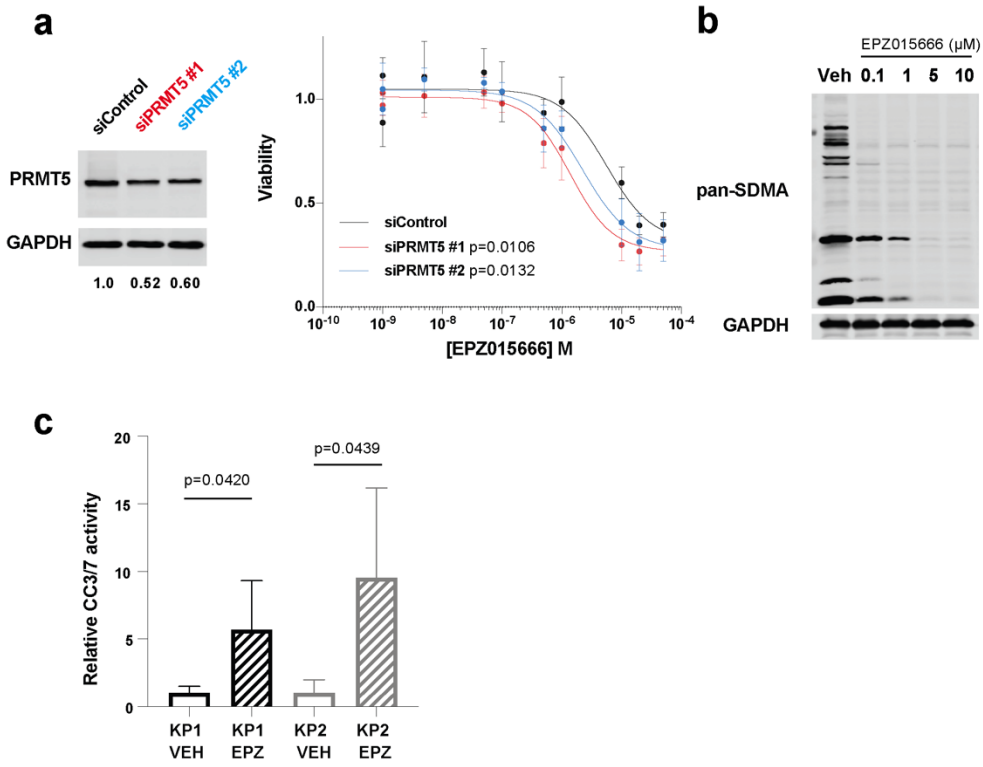
2). Histogram of the distribution of normalized counts for the barcodes. Threshold for including barcodes was set at 50 counts (red line).



Figures S1-S5:

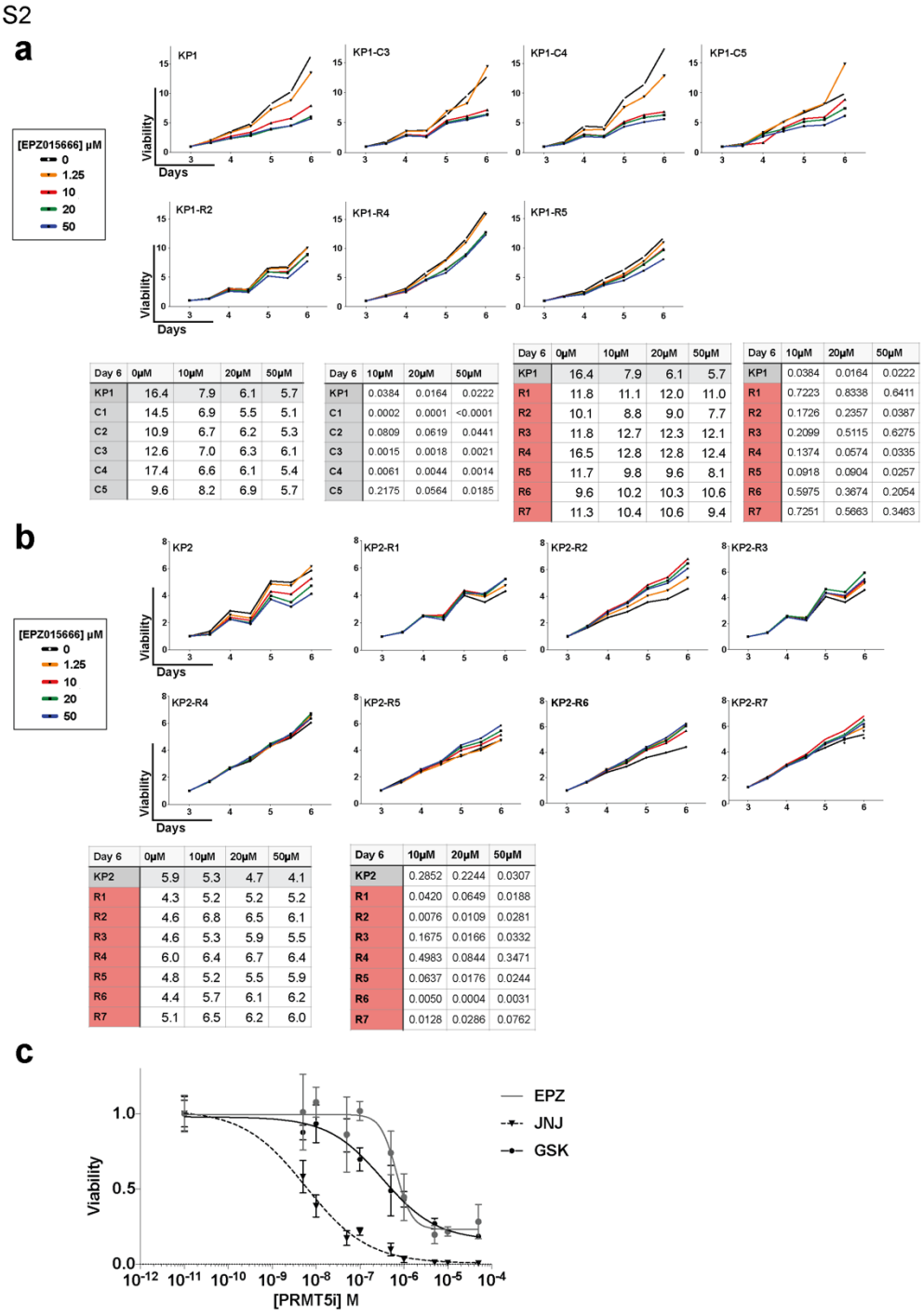
S1:

S1



(a) Left panel: western blot of KP1 cells transfected with siControl or two different siPRMT5s (#1, #2). Numbers below show quantification of PRMT5 levels relative to siControl condition and normalized to GAPDH levels. Right panel: nine-point dose response curves of siRNA transfected cells treated with EPZ015666 for 5 days. Significance determined using Student's t-test. **(b)** Western blot for pan-SDMA in KP1 cells treated for 5 days with vehicle (DMSO), 0.1, 1, 5 or 10 μM EPZ015666. **(c)** Relative cleaved caspase 3 and 7 activity after 6 day treatment with 10 μM EPZ015666 in KP1+ vehicle, KP1 + EPZ, KP2 + vehicle and KP2+ EPZ cells, relative to KP1 or KP2 vehicle control. Significance determined using Welch's t-test.

S2:



Day 6	0µM	10µM	20µM	50µM
KP1	16.4	7.9	6.1	5.7
C1	14.5	6.9	5.5	5.1
C2	10.9	6.7	6.2	5.3
C3	12.6	7.0	6.3	6.1
C4	17.4	6.6	6.1	5.4
C5	9.6	8.2	6.9	5.7

Day 6	10µM	20µM	50µM
KP1	0.0384	0.0164	0.0222
C1	0.0002	0.0001	<0.0001
C2	0.0809	0.0619	0.0441
C3	0.0015	0.0018	0.0021
C4	0.0061	0.0044	0.0014
C5	0.2175	0.0564	0.0185

Day 6	0µM	10µM	20µM	50µM
KP1	16.4	7.9	6.1	5.7
R1	11.8	11.1	12.0	11.0
R2	10.1	8.8	9.0	7.7
R3	11.8	12.7	12.3	12.1
R4	16.5	12.8	12.8	12.4
R5	11.7	9.8	9.6	8.1
R6	9.6	10.2	10.3	10.6
R7	11.3	10.4	10.6	9.4

Day 6	10µM	20µM	50µM
KP1	0.0384	0.0164	0.0222
R1	0.7223	0.8338	0.6411
R2	0.1726	0.2357	0.0387
R3	0.2099	0.5115	0.6275
R4	0.1374	0.0574	0.0335
R5	0.0918	0.0904	0.0257
R6	0.5975	0.3674	0.2054
R7	0.7251	0.5663	0.3463

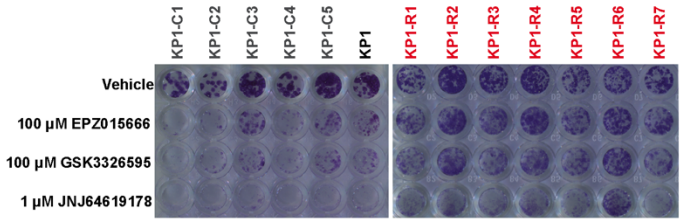
Day 6	0µM	10µM	20µM	50µM
KP2	5.9	5.3	4.7	4.1
R1	4.3	5.2	5.2	5.2
R2	4.6	6.8	6.5	6.1
R3	4.6	5.3	5.9	5.5
R4	6.0	6.4	6.7	6.4
R5	4.8	5.2	5.5	5.9
R6	4.4	5.7	6.1	6.2
R7	5.1	6.5	6.2	6.0

Day 6	10µM	20µM	50µM
KP2	0.2852	0.2244	0.0307
R1	0.0420	0.0649	0.0188
R2	0.0076	0.0109	0.0281
R3	0.1675	0.0166	0.0332
R4	0.4983	0.0844	0.3471
R5	0.0637	0.0176	0.0244
R6	0.0050	0.0004	0.0031
R7	0.0128	0.0286	0.0762

(a) Relative growth of parental (KP1) cell line compared to other control (KP1-C3, C4, C5) and resistant (KP1-R2, R4, R5) cell lines. Growth is normalized to the day 3 time point (n=3 replicates/condition). Tables of mean normalized viability values and significance (Student's t-test) calculated at the day 6 time point of PRMT5i treatment, relative to the same cell line in 0µM EPZ015666, for all of the KP1-C and -R samples (shown in Figure 2b or in Supplementary Figure 2a above). **(b)** Relative growth of parental (KP2) cell line compared to resistant (KP2-R1 to R7) cell lines. Growth is normalized to the day 3 time point (n=3 replicates/condition). Tables of mean normalized viability values as well as tables with significance (Student's t-test), were calculated at the day 6 time point relative to the same cell line in 0 µM EPZ015666 for KP2 and all KP2-R samples. **(c)** Graph shows the response of KP1 to the three PRMT5 inhibitors: EPZ015666 (gray), its derivative GSK3326595 (GSK; black) and the more potent inhibitor JNJ64619178 (JNJ; black-dashed). Response after a five-day treatment, viability is relative to DMSO control.

S2

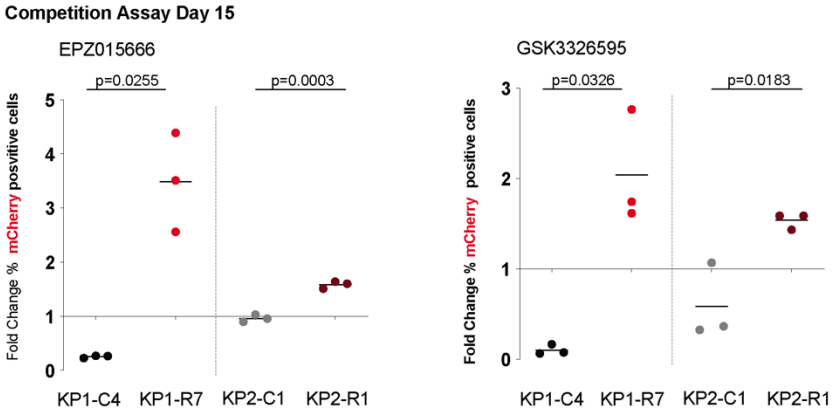
d



e

IC50 (M)	EPZ	GSK	JNJ
KP1	1.582E-07	5.565E-08	1.362E-08
KP1-R6	>5E-05	7.137E-05	6.161E-07
Fold Change	> 316	1282	45

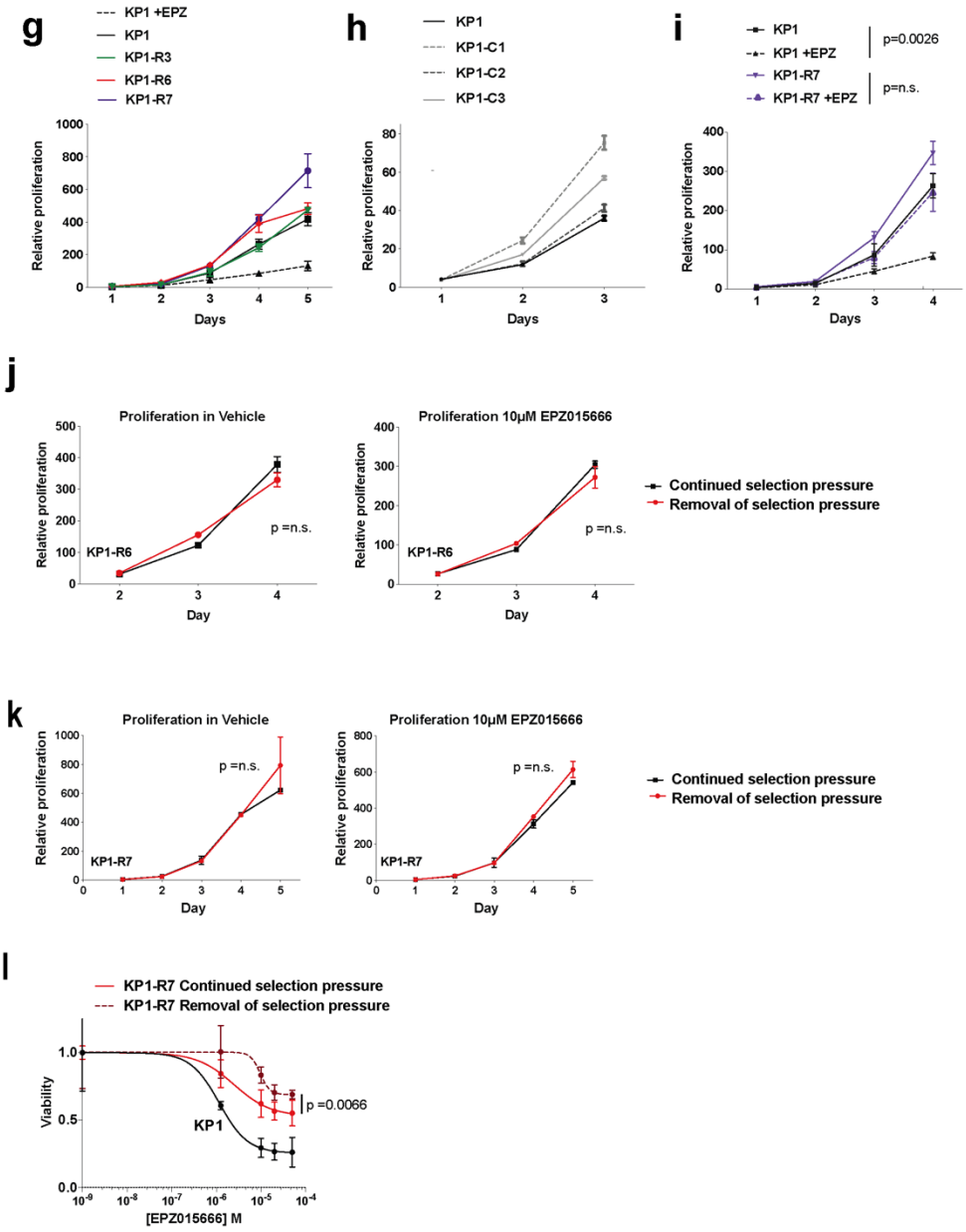
f



Range of % mCherry positive cells	Vehicle (DMSO)	EPZ015666 (10µM)	GSK3326595 (10µM)
KP1 C4	29.1-47.6	9.4-11.3	2.7-6.9
KP1 C5	38.0-45.3	6.5-10.2	10.5-19.0
KP1 R6	28.4-32.1	71.9-85.5	39.6-76.5
KP1 R7	16.2-24.0	52.6-90.1	33.2-56.8
KP2 C1	50.4-54.1	47.4-54.3	17.2-56.4
KP2 R1	54.5-58.2	85.2-92.4	80.8-89.6

(d) KP1, KP1-C1 to KP1-C5 (gray), KP1-R1 to KP1-R7 (red) were grown in vehicle, 100 µM EPZ015666, 100 µM GSK3326595 or 1 µM of JNJ64619178 for six days. Cells were stained with crystal violet. Viability is quantified in Figure 2c. **(e)** IC50s of the dose response curves in Fig. 2d, estimated from the nonlinear fit. Fold change is the amount KP1-R6 is more resistant than KP1 for EPZ015666, GSK3326595 and JNJ64619178. **(f)** Graphs show the results of the competition assay comparisons between additional C and R lines for KP1 (C4 and R7) and KP2 (C1 and R1) using the same conditions and quantification as shown in Fig. 2e. Significance was determined using Student's t-test. Also shown, in the table, is the range of the percent mCherry-positive cells for each cell line when grown in vehicle control or the two PRMT5i.

S2



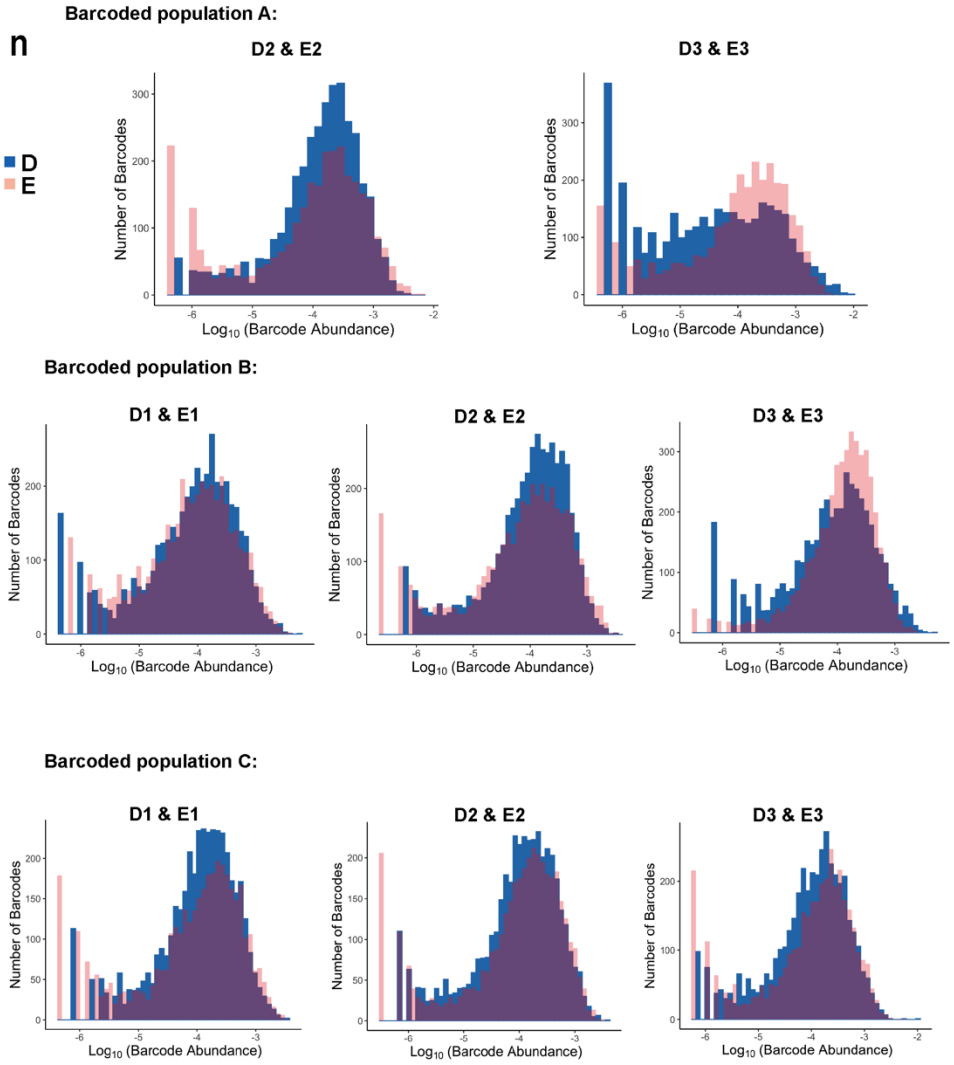
(g-i) Relative proliferation of: **(g)** KP1 (black), KP1 in the presence of 10μM EPZ015666 (KP1+EPZ; black-dashed), KP1-R3, R6, R7 (colored), and **(h)** KP1 (black) and KP1-C1 to C3 (gray) and **(i)** KP1 (black), KP1 +EPZ (black-dashed), KP1-R7 (purple), KP1-R7 +EPZ (purple-dashed). Significance determined by Welch's t-test with the Holm-Sidak correction. **(j-l)** KP1-R6 and KP1-R7 variants were grown for ten passages in the presence (black; continued selection pressure) or absence (red; removal of selection pressure) of 10 μM EPZ015666 were assessed for: **(j,k)** relative proliferation of **(j)** KP1-R6 or **(k)** KP1-R7 in the presence of vehicle control (left) or 10 μM EPZ015666 (right), with significance determined by Welch's t-test with the Holm-Sidak correction; and **(l)** response of KP1-R7 (here continued selection pressure: red solid, removal of selection pressure: red dashed) to a range of EPZ015666 concentrations, relative to the parental KP1 control (black), after a five-day treatment. Viability is relative to DMSO control. Significance determined by Student's t-test.

S2

m

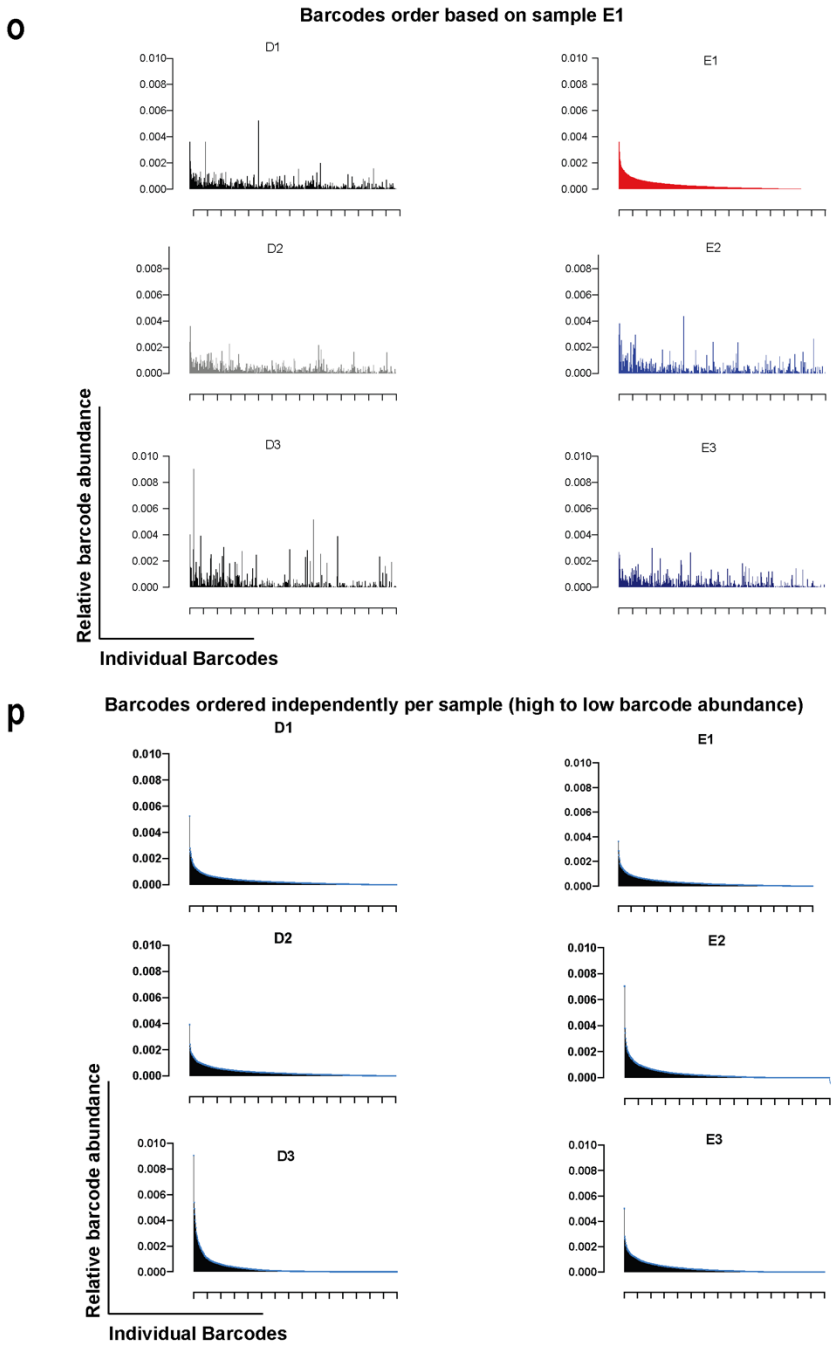
	A	B	C
Total shared	3514	4603	4277
E1	3406 (97%)	4336 (94%)	3701 (87%)
E2	2817 (80%)	4107 (89%)	3762 (88%)
E3	3005 (86%)	4520 (98%)	3779 (88%)

n



(m) For each barcoded population (A, B, & C), the table shows the total number of barcodes that were present in all of the three initial and three vehicle treated samples (total shared), and the number (and fraction of the total shared) that were present in each of the three EPZ015666 treated samples (E1, E2, E3). **(n)** For each barcoded population (A, B, C), the graphs show the distribution of barcode abundances for one vehicle treated sample (D1, D2 or D3; blue) compared to one EPZ treated sample (E1, E2, E3; pink), with the overlap in purple.

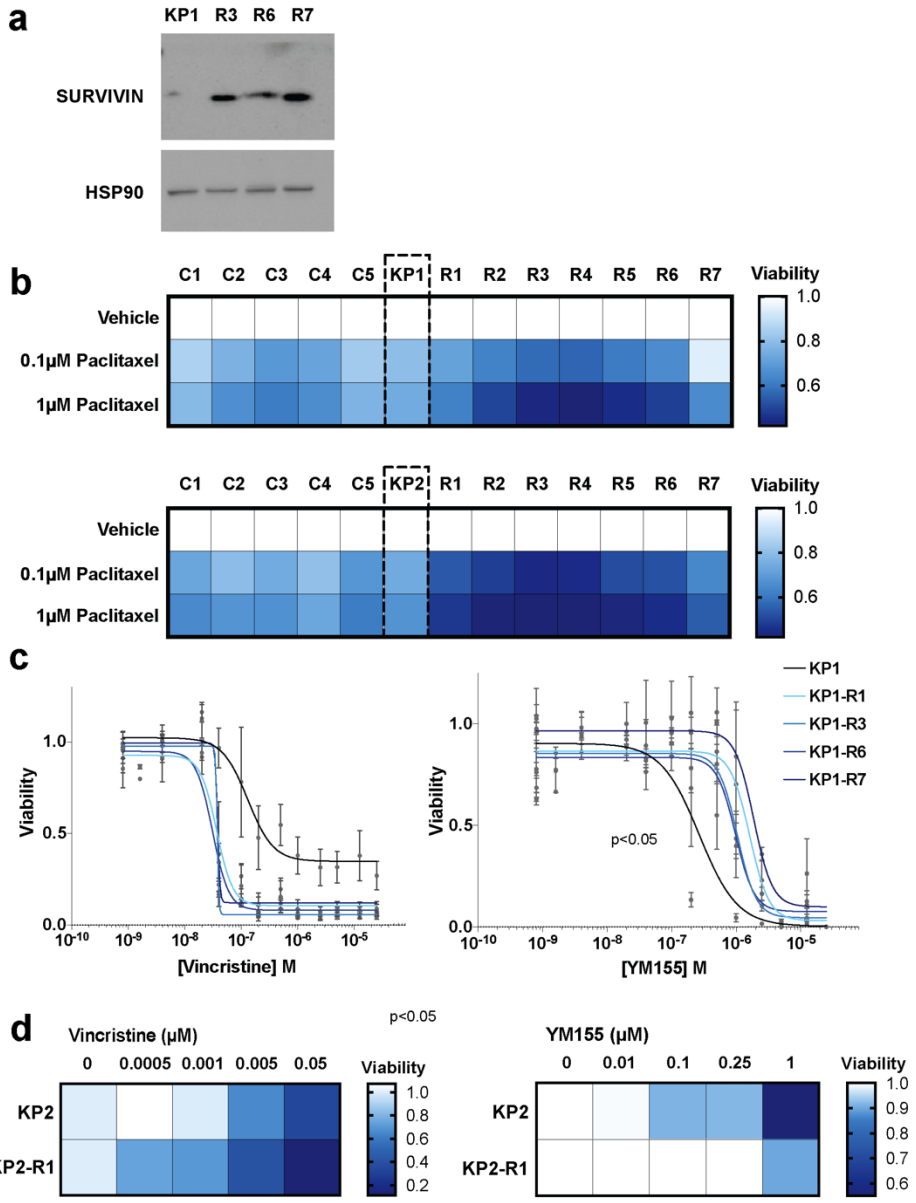
S2



(o) Relative barcode abundances for each of the individual vehicle treated (D1, D2 and D3) and EPZ treated (E1, E2 and E3) population A samples, with the barcodes ordered based on their abundance in E1 (from most to least). **(p)** Also shown are relative barcode abundances of each sample, where the individual barcodes are ordered from most to least for that sample. There are no barcodes in the E1-E3 samples that are higher than the range of the three D1-D3 relative barcode abundances.

S3:

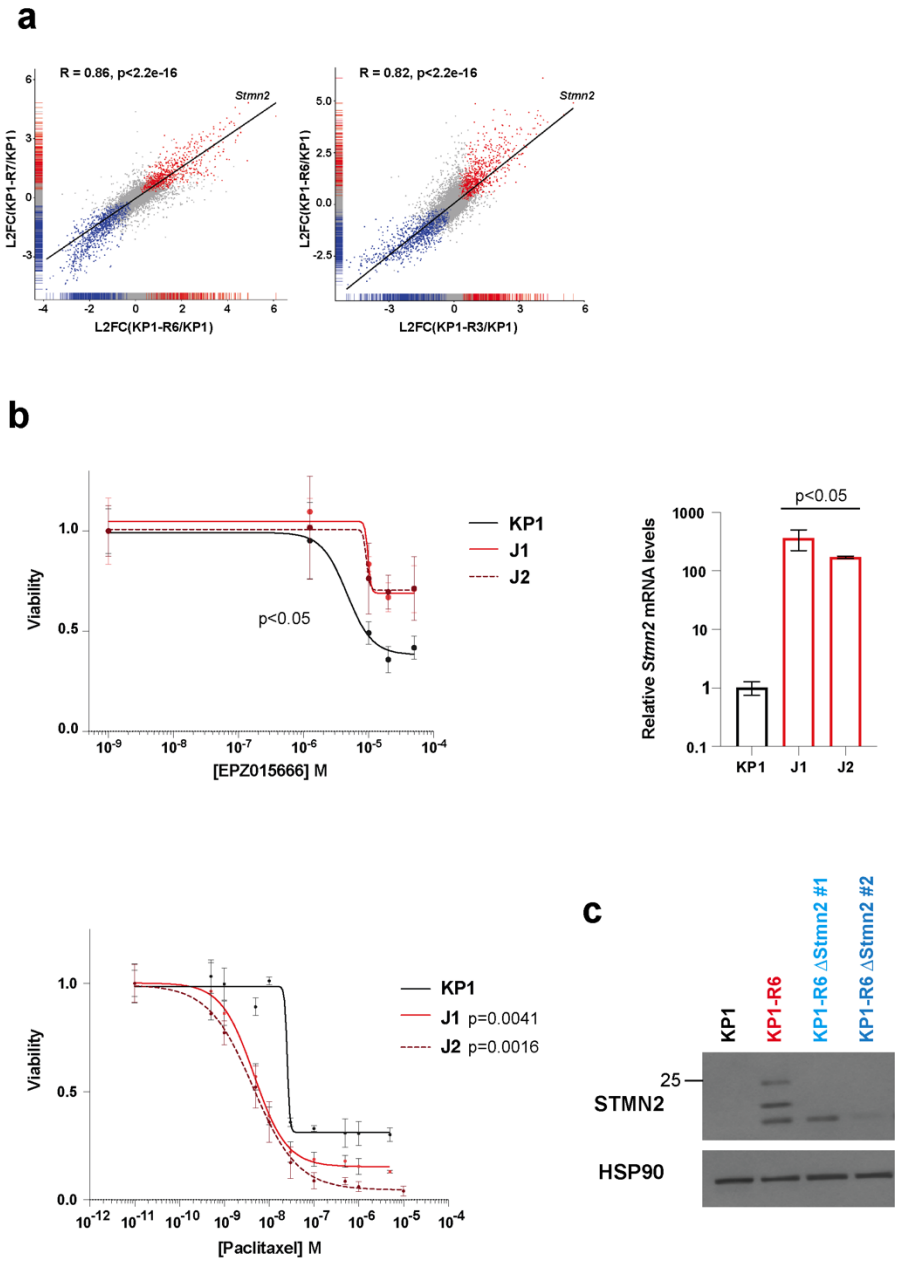
S3



(a) Western blot for SURVIVIN/BIRC5 in KP1, KP1-R3, -R6 and -R7 cells. **(b)** Viability measured with resazurin of KP1 and KP2 C and R cell lines after a two-day treatment with 0.1 and 1 μM of paclitaxel. **(c)** Graphs show response of KP1 (black), KP1-R1, R3, R6, and R7 (blue) to vincristine and YM155 after a five-day treatment. Viability is relative to DMSO control. Significance was determined for vincristine using Welch's t-test, KP1-R1: p=0.0390, KP1-R3: p=0.0406, KP1-R6: p=0.0418, KP1-R7: p=0.0474. Significance was determined for YM155 using Student's t-test, KP1-R1: p=0.0302, KP1-R3: p=0.0001, KP1-R6: p=0.0047, KP1-R7: p=0.0152. **(d)** Viability after KP2 and KP2-R1 were treated with five doses of Vincristine (left) or YM155 (right) for 4 days, indicating that the increased sensitivity to vincristine and resistance to YM155 is also seen for an R line derived from KP2.

S4:

S4



(a) Additional plots showing correlation of the differentially expressed genes between KP1-R6 and KP1-R3 or KP1-R7. Significance determined by the Pearson correlation. **(b)** (i) Graph shows response of KP1 and two KP1 JNJ-R lines (J1, J2) to a five-point dose curve of EPZ015666 after five days of treatment. (ii) Graph of *Stmn2* mRNA levels, relative to *Gapdh,* in KP1 and KP1-J1, -J2. Significance was determined using Welch's t-test. (iii) Graph shows the response of KP1 and KP1-J1,-J2 over a ten-point paclitaxel curve. Significance was determined using Student's t-test unless noted. **(c)** Western blot for STMN2 in the KP1-R6Δ*Stmn2* CRISPR knock-out clones.

S4

d

Early response to PRMT5 inhibition

Differentially expressed genes in **Parental EPZ015666:vehicle**

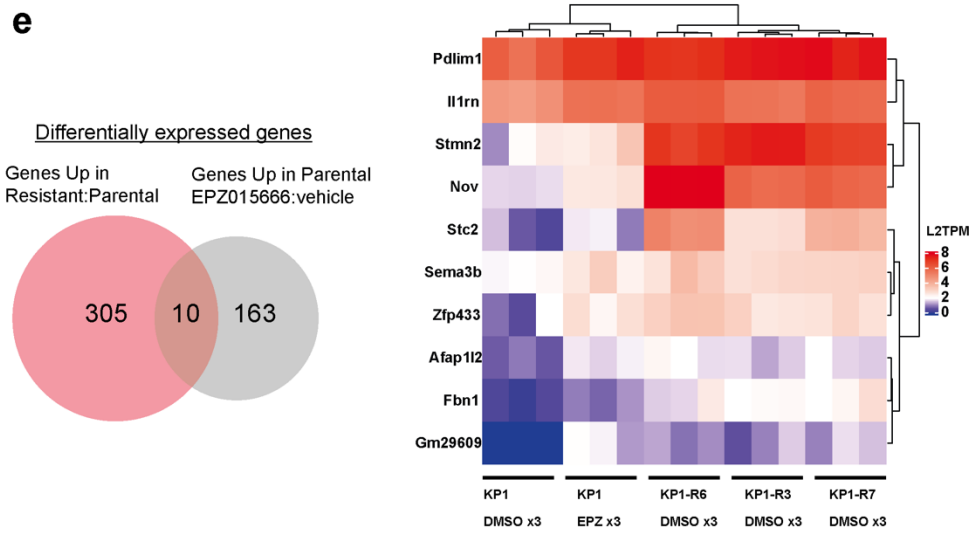
	L2FC>1	L2FC>2
Up	**173**	3
Down	**168**	4
Total	**341**	7

Resistant state

Differentially expressed genes in all **Resistant:Parental**

	L2FC>1	L2FC>2
Up	**315**	81
Down	**636**	177
Total	**951**	258

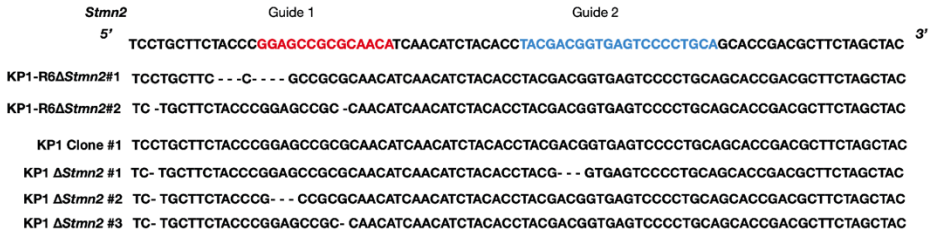
e



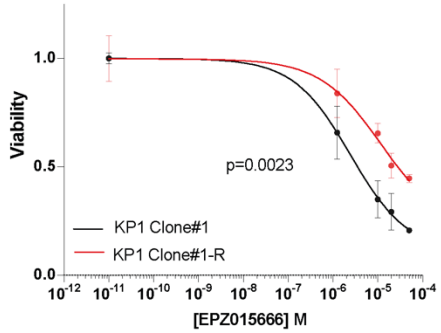
(d) Number of genes that were differentially expressed in the parental cell line after 72 hours of treatment with EPZ015666 (EPZ) (L2FC>1 and L2FC>2, $p<0.05$). Since there were so few genes that had L2FC>2, we chose to move forward with the L2FC>1 list. Also shown, the number of genes differentially expressed in common between all resistant lines and the KP1 parental (L2FC>1, $p<0.05$). **(g)** Analysis of genes that were L2FC>1 in resistant lines and also in parental lines after 72 hours of EPZ treatment showed that there were only ten genes, including *Stmn2*, that were differentially expressed in the resistant lines and also upregulated after 72 hours of EPZ treatment (Venn diagram). Heatmap shows the expression level (log_2TPM) of each of these ten genes across KP1, KP1+EPZ, KP1-R6, R3 and R7 (triplicates shown).

S4

f



g



(f) DNA sequencing of *Stmn2* showing deletions in KP1-R6 and KP1 CRISPR *Stmn2* knock-out and wild-type clones. **(g)** Graph shows response of KP1 Clone #1 and a resistant line generated from it (KP1 Clone #1-R) to EPZ015666 treatment for five days. Significance was determined using Student's t-test.

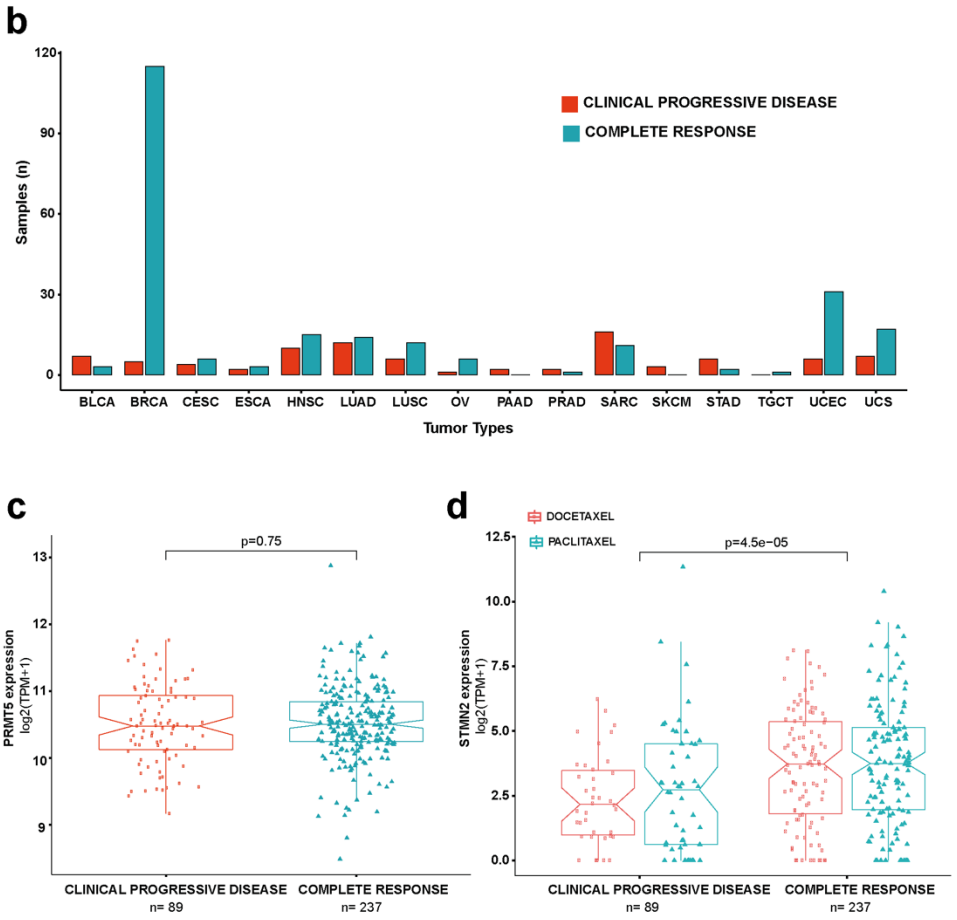
S5:

S5

a

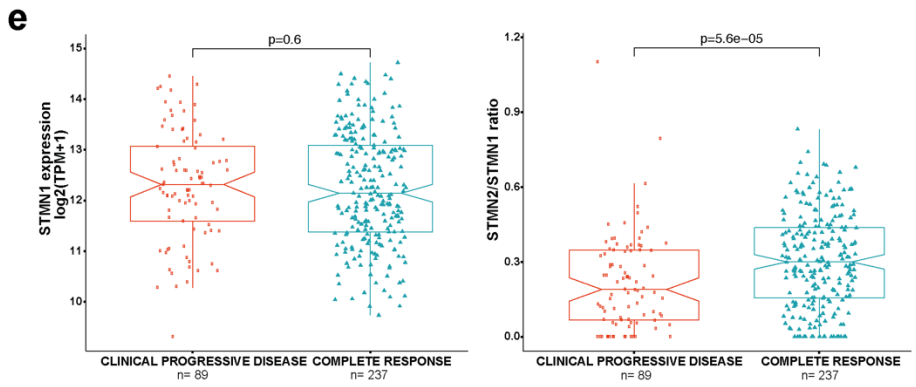
p-values from Fig. 5e

	0.78 nM	1.56 nM	3.12 nM	6.25 nM
A549	0.377	0.1671	0.3605	0.0026
H23	0.6458	0.0057	0.002	0.0034
MDA-MB-468	0.0064	0.1996	0.0014	0.0039
MDA-MB-231	0.0333	0.0066	0.5718	0.2318
CAL51	0.0023	0.0001	0.0001	0.0002
HCT15	0.0025	0.1434	0.5779	0.9279
HepG2	0.0037	0.0016	0.1292	0.0541



(a) Significance of data in Figure 5e, determined by the one sample t-test, compared to a mean of zero. p-values shown, with significant values highlighted. (**b)** Graph showing the representation of the different tumor types that were included in the TCGA analysis, split by patients who showed complete response (n=237) versus clinical progressive disease (n=89) after taxane treatment. **(c)** TCGA analysis relating PRMT5 levels (log_2TPM+1) to clinical response to taxane treatment. **(d)** TCGA analysis relating STMN2 levels (log_2TPM+1) to clinical response to taxane treatment, as shown in Figure 4f, but separating patients treated with paclitaxel versus docetaxel. For **(c)-(d),** significance was determined using the Wilcoxon Rank Sum test.

S5



(e) TCGA analysis relating Stmn1 levels (log_2TPM+1) and the ratio of Stmn2:Stmn1 levels to clinical response to taxane treatment. Significance was determined using the Wilcoxon Rank Sum test.

Table S1. Individual drug concentrations used for drug screen

Supplementary Table 1

Doses (μM) used for the dose response curves of each respective drug in the drug screen (Fig 3a)

	Panobinostat	Fluvistatin	Methotrexate	YM155	Simvastatin	Sorafanib	Paclitaxel	Cycloheximide	Vincristine	Topotecan	Cisplatin	Pemetrexed	Palbociclib	Doxorubicin
1	10	250	25	25	250	25	25	25	25	10	200	625	1010	10
2	5	125	12.5	12.5	125	12.5	12.5	12.5	12.5	5	100	312.5	671	5
3	2	50	5	5	50	5	5	5	5	2	40	125	250	2
4	1	25	2.5	2.5	25	2.5	2.5	2.5	2.5	1	20	62.5	125	1
5	0.4	10	1	1	10	1	1	1	1	0.4	8	25	40	0.4
6	0.2	5	0.5	0.5	5	0.5	0.5	0.5	0.5	0.2	4	12.5	27	0.2
7	0.08	2	0.2	0.2	2	0.2	0.2	0.2	0.2	0.08	1.6	5	10	0.08
8	0.04	1	0.1	0.1	1	0.1	0.1	0.1	0.1	0.04	0.8	2.5	5	0.04
9	0.016	0.4	0.04	0.04	0.4	0.04	0.04	0.04	0.04	0.016	0.32	1	1.62	0.016
10	0.008	0.2	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.008	0.16	0.5	1	0.008
11	0.0016	0.04	0.004	0.004	0.04	0.004	0.004	0.004	0.004	0.0016	0.032	0.1	0.20	0.0016
12	0.00064	0.016	0.0016	0.0016	0.016	0.0016	0.0016	0.0016	0.0016	0.00064	0.0128	0.04	0.06	0.00064
13	0.00032	0.008	0.0008	0.0008	0.008	0.0008	0.0008	0.0008	0.0008	0.00032	0.0064	0.02	0.04	0.00032

Dataset S1 (separate file). This file contains the results of the DESeq2 analysis comparing KP1 and KP1+PRMT5i, KP1 and KP1-R3, KP1 and KP1-R6 and KP1 and KP1-R7. This file includes a results sheet and a key sheet.

Dataset S2 (separate file). This file contains all of the statistical tests used and the results of the statistical tests. The label of each sheet denotes the relevant figure.