

Activation of the p53-MDM4 regulatory axis defines the anti-tumour response to PRMT5 inhibition through its role in regulating cellular splicing.

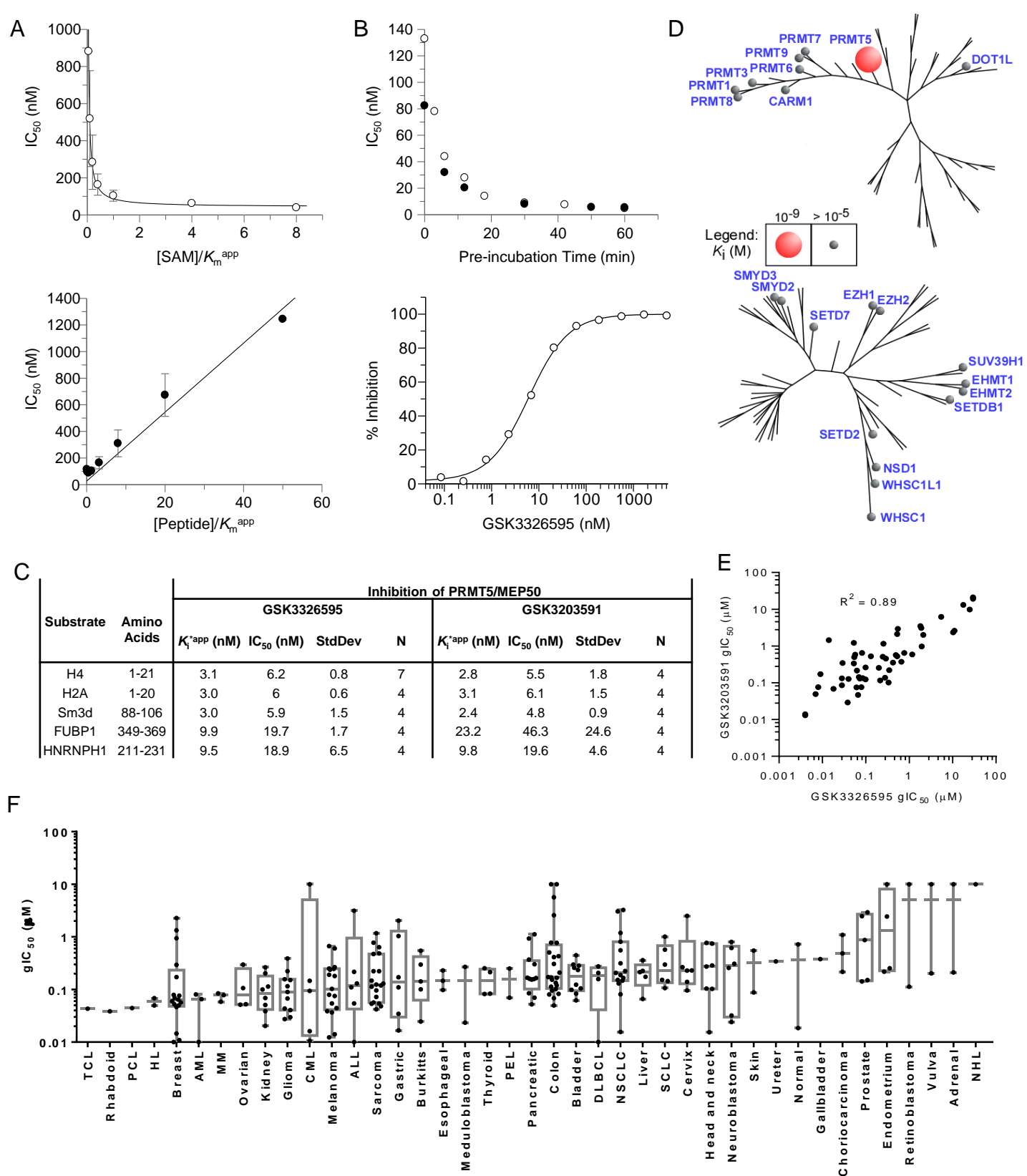
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Supplementary Figure S1. (A) Plots indicating the mode of inhibition against PRMT5/MEP50 by GSK3362595 according to Cheng-Prusoff relationship where IC_{50} values are graphed as a function of substrate concentration relative to K_m^{app} (top panel, SAM; bottom panel, H4 1-21 peptide). Data points (closed vs. open circles) represent two independent experiments. **(B)** IC_{50} values plotted as a function of Enz:SAM:GSK3326595 preincubation time. The open circles represent data generated using 0.8 nM PRMT5/MEP50 while closed circles represent data with 4 nM PRMT5/MEP50 (top panel). The bottom panel shows a representative IC_{50} curve for GSK3362595 inhibition of PRMT5/MEP50 activity following a 60 minute Enz:SAM:Inh preincubation fit to a 3-parameter dose-response equation. **(C)** Averaged IC_{50} values determined following a 60 minute Enz:SAM:Inh preincubation. IC_{50} values were determined by fitting inhibition data to a 3-parameter dose-response equation. K_i^{*app} values were calculated from IC_{50} values using the Cheng-Prusoff equation for a competitive inhibitor. **(D)** Phylogenetic tree highlighting the methyltransferases tested in the selectivity panel. GSK3326595 showed much greater potency for PRMT5 (10^{-8} M) than for any other tested enzyme (\bullet , $> 10^{-5}$ M). **(E)** Correlation plot comparing glC_{50} of tool PRMT5 inhibitor, GSK3203591, and candidate PRMT5 inhibitor, GSK3326595, in a panel of cell lines of various tumour types in a 6-day proliferation assay. R^2 value was calculated using the linear regression fitted to these data. **(F)** Box and whisker plots comparing average glC_{50} values across tumour types following 10 days of treatment with GSK3203591 (TCL- T-cell lymphoma, PCL- plasma cell leukemia, HL- Hodgkin's lymphoma, AML – acute myeloid leukemia, MM- multiple myeloma, CML- chronic myelogenous leukemia, ALL- acute lymphoblastic leukemia, PEL- primary effusion lymphoma, DLBCL- diffuse large B-cell lymphoma, NSCLC- non-small cell lung cancer, SCLC – small cell lung cancer, NHL- non-Hodgkin's lymphoma). Dots represent an average of values for individual cell lines.

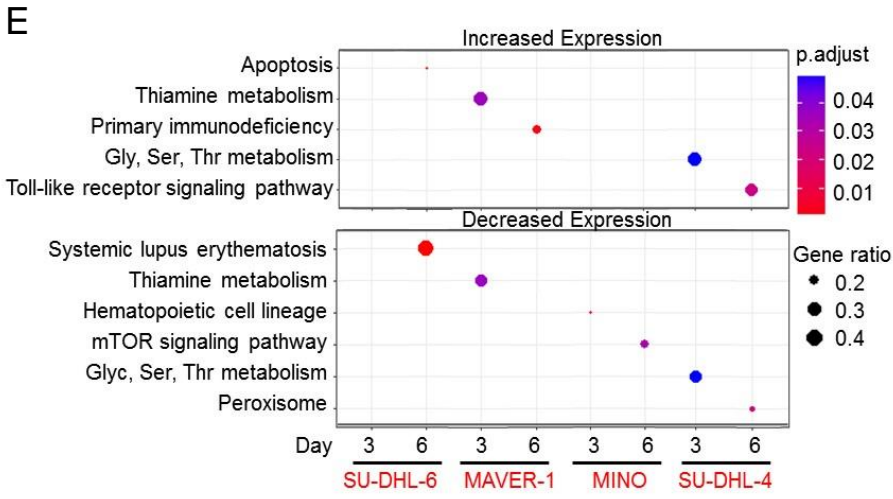
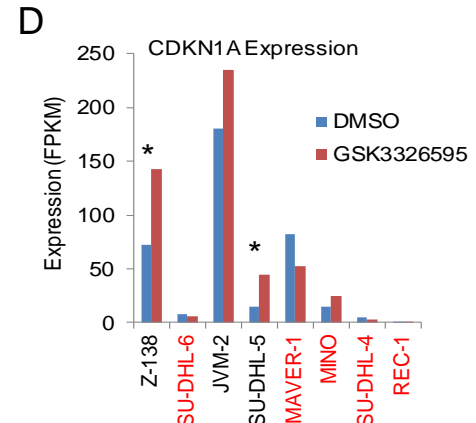
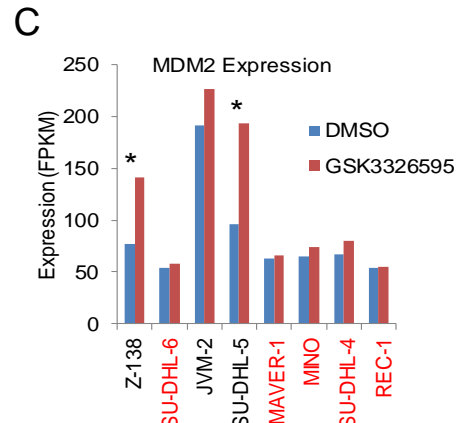
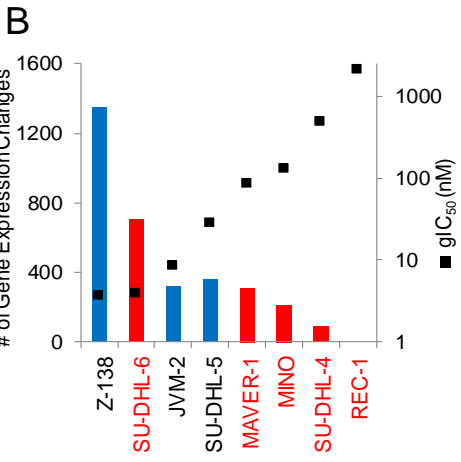
A

GSK3203591/DMSO Fold Change	Protein Name	Function
-141.0	LSM4	Splicing
-22.7	ZNF193	Unknown
-16.8	snRNP B1	Splicing
-14.5	snRNP B1	Splicing
-8.3	N-CoR1	Transcription
-8.2	snRNP B1	Splicing
-7.8	KHSRP	Splicing, RNA stability
-6.8	FUBP1	Splicing, RNA stability
-6.6	FUBP1	Splicing, RNA stability
-6.3	KHSRP	Splicing, RNA stability
-5.7	eEF1A1	Translation
-5.3	hnRNP H1; hnRNP H2	Splicing
-5.2	NEURL4	Centriole organization
-5.1	SACM1L	Unknown
-5.0	hnRNP H1; hnRNP H2	Splicing
-4.4	hnRNP H1; hnRNP H2	Splicing
-4.0	FUBP1	Splicing, transcription
-3.8	WDR33	mRNA processing
-3.8	G3BP-2	RNA binding protein
-3.4	hnRNP H1; hnRNP H2	Splicing
-2.8	KHSRP	Splicing, RNA stability
-2.8	TOX	T-cell development

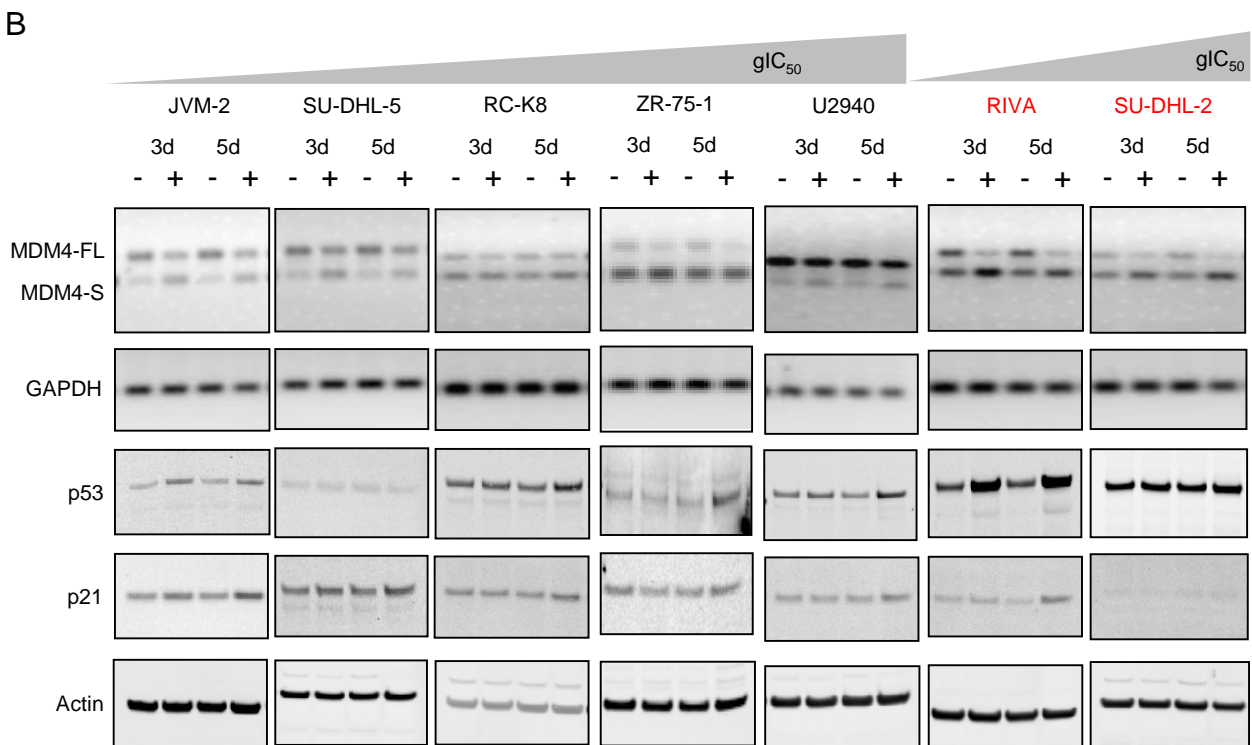
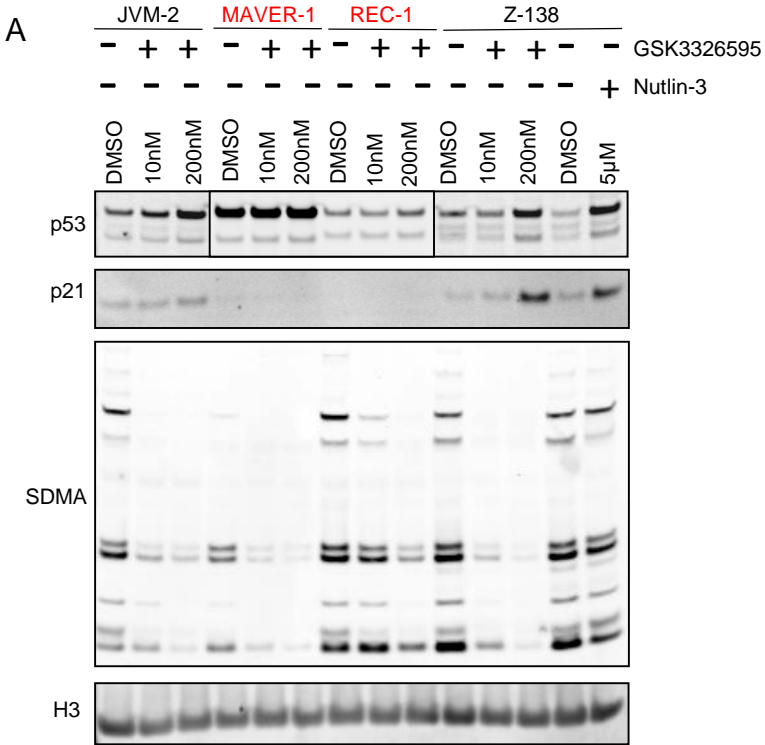
Supplementary Figure S2. (A) PRMT5 target proteins were identified using MethylScan™ technology in Z-138. More negative fold change values correspond to the greatest effect.

A

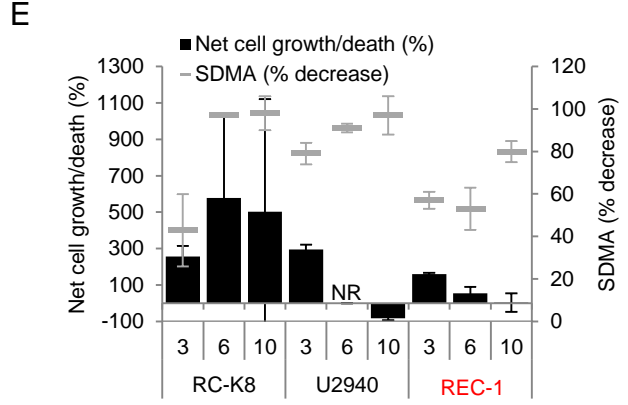
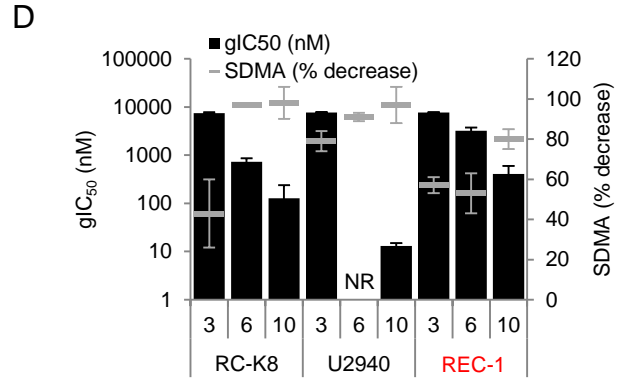
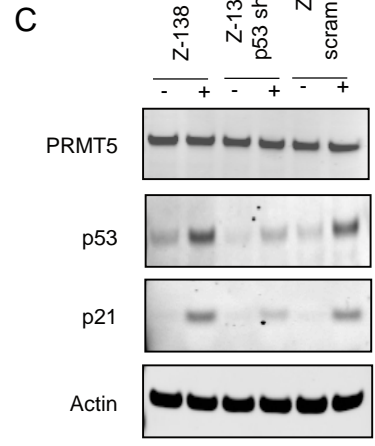
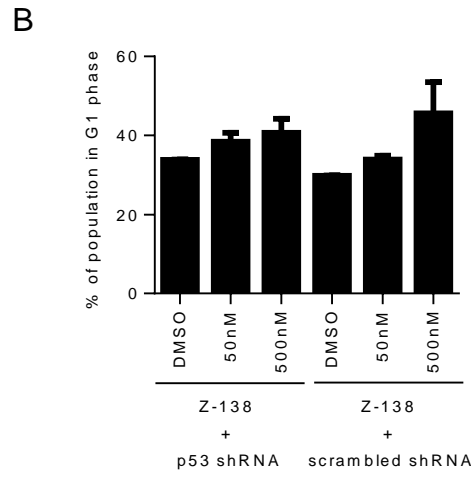
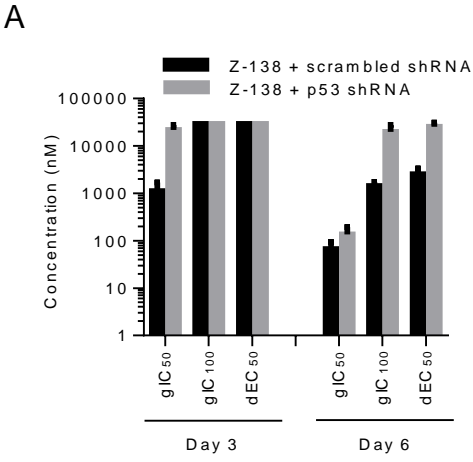
	<u>MDM4 splicing</u>	<u>Z-138</u>	<u>SU-DHL-6</u>	<u>JVM-2</u>	<u>SU-DHL-5</u>	<u>MAVER-1</u>	<u>MINO</u>	<u>SU-DHL-4</u>	<u>REC-1</u>
Day 3 FDR		1.0E-11	7.3E-03	3.8E-04	2.4E-03	1.5E-03	9.6E-02	6.9E-02	4.5E-01
Day 6 FDR		1.1E-10	1.6E-01	2.4E-07	1.3E-10	4.6E-02	1.2E-05	0.0E+00	8.5E-01



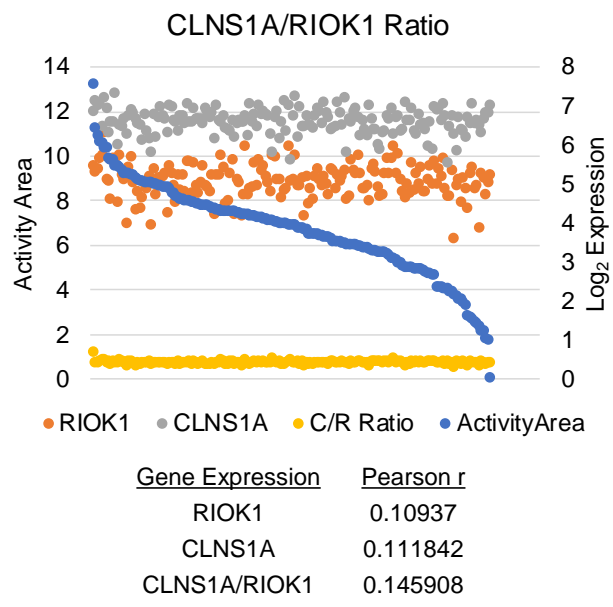
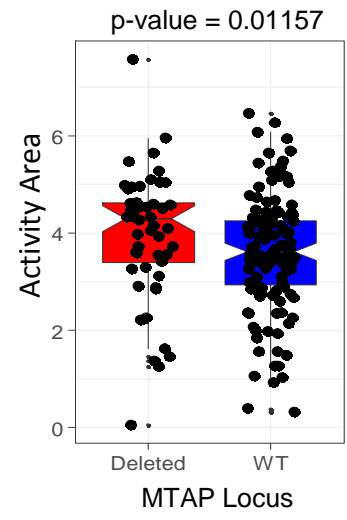
Supplementary Figure S3. **(A)** FDR values from rMATS alternative splicing output of MDM4 exon 6 skipping event upon treatment with GSK3326595 for 3 or 6 days. p53 mutant cell lines are highlighted in red. **(B)** The total number of significantly changed genes from days 3 and 6 are plotted against the GI_{50} for that cell line. The Spearman and Pearson correlations demonstrate a correlation between number of gene expression changes and sensitivity to PRMT5 inhibition. p53 mutant cell lines are highlighted in red. The FPKM values reported from CuffDiff outputs for **(C)** MDM2 and **(D)** CDKN1A. p53 mutant cell lines are highlighted in red. **(E)** Significantly changed genes in p53 mutant cell lines were submitted for MsigDB enrichment analysis (Broad) and the most significantly changed gene set for each is reported.



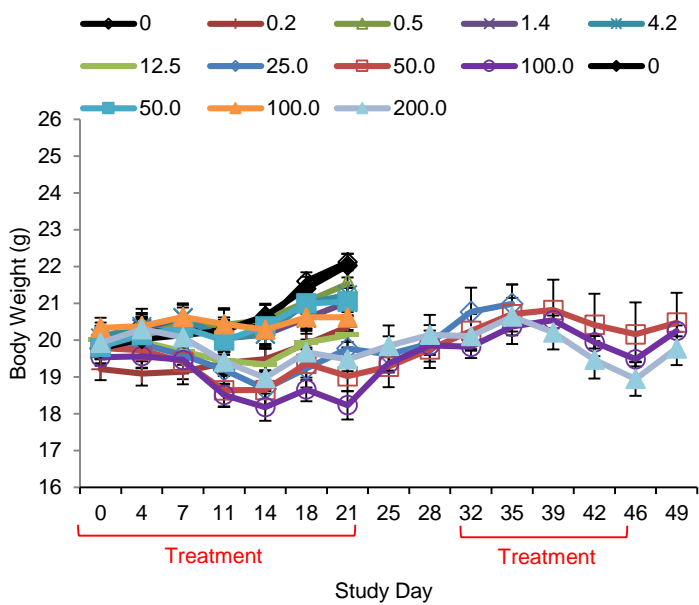
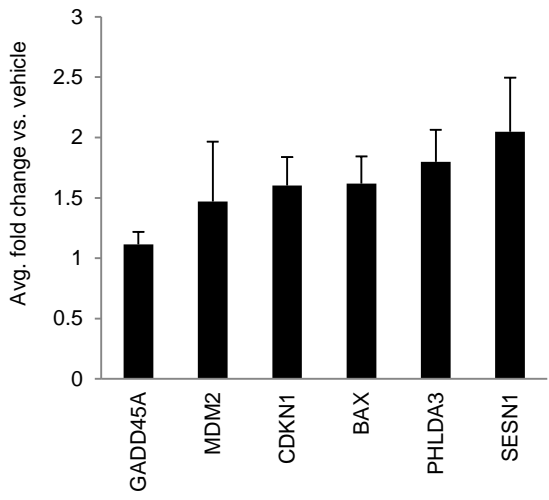
Supplementary Figure S4. (A) Western blot showing a p53 and p21 dose response in a panel of MCL cell lines following 3 days of treatment with 10 nM or 200 nM GSK3326595 in a panel of mantle cell lymphoma cell lines. A 5 μ M Nutlin-3 treatment condition was also included as a positive control for p53 activation. **(B)** MDM4 splicing and p53/p21 induction in an additional panel of p53 wild-type (black text) and mutant (red text) lymphoma cell lines treated with DMSO (-) or 200nM GSK3326595 (+) for 3 or 5 days arranged in order of increasing gIC_{50} value in a 6-day proliferation assay with GSK3326595.



Supplementary Figure S5. (A) Effect of p53 knockdown via lentiviral transduction of p53 shRNA on proliferation in Z-138 cells following 3 or 6 days of GSK3203591 treatment with respect to gIC_{50} , gIC_{100} , and dEC_{50} parameters. Transduction of a scrambled, non-targeting shRNA was used as a negative control. **(B)** Effect of p53 knockdown via lentiviral transduction of p53 shRNA on G1 cell cycle phase in Z-138 cells following 4 days of treatment with DMSO, 50 nM, or 500 nM GSK3203591. **(C)** Western blot showing the effect of p53 knockdown via lentiviral transduction of p53 shRNA on p53 and p21 protein levels in Z-138 cells following 4 days of 1 μ M GSK3203591 (+) or DMSO (-) treatment. **(D)** gIC_{50} and **(E)** net cell growth/death values from a 6-day growth/death assay with GSK3326595 (black bars) are plotted with % decrease in SDMA (gray rectangles) for 3-, 6-, and 10-day treatment time points. p53 mutant cell lines in red.

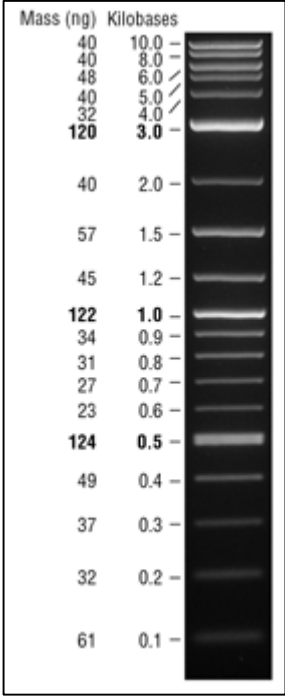
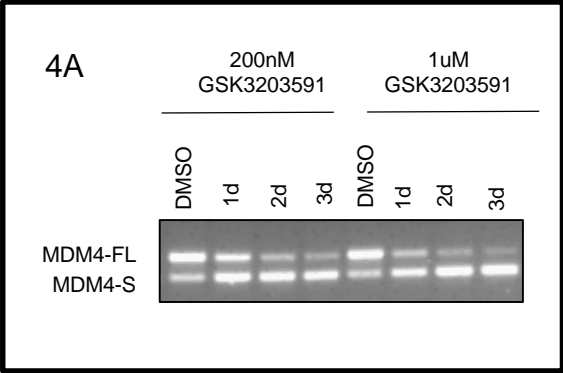
A**B**

Supplementary Figure S6. (A) Gene expression of CLNS1A, RIOK1, and the ratio of the two in 184 cell lines plotted by decreasing sensitivity. Pearson correlations with sensitivity are also shown for each parameter. **(B)** Activity area from proliferation assays are compared between MTAP homozygous deleted cell lines and those with at least one functional copy of MTAP. The result of a Wilcoxon rank sum test is shown.

A**B**

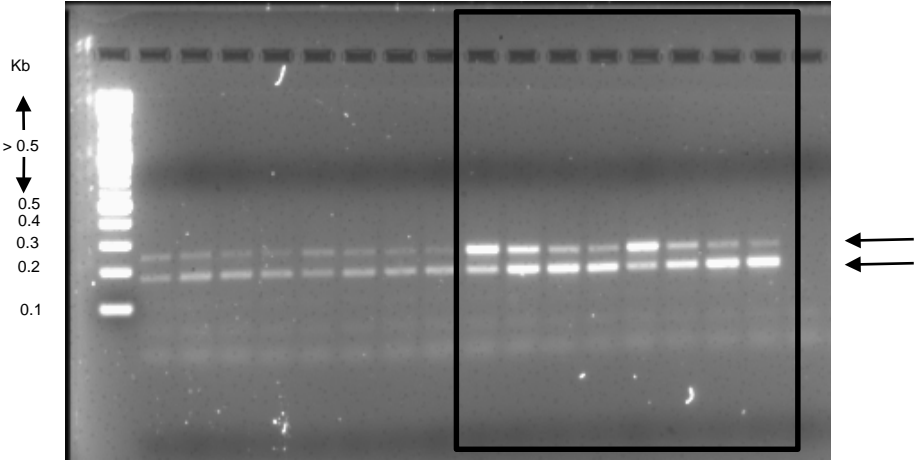
Supplementary Figure S7. (A) Body weight changes in S.C. Z-138 tumour bearing mice treated with GSK3326595. Treatments that resulted in significant decreases in tumour volume were placed on a treatment holiday for 10 days. Treatments were reinstated on day 32 for 2 weeks followed by a one week holiday. The 25 mg/kg dose group were euthanized on day 35 due to tumour burden. Day 53 data not available for this data set. **(B)** p53 target gene expression change in Z-138 tumor xenografts after 7 days of 100 mg/kg BID GSK3326595 treatment compared to vehicle control.

Full scans to accompany Figure 4A

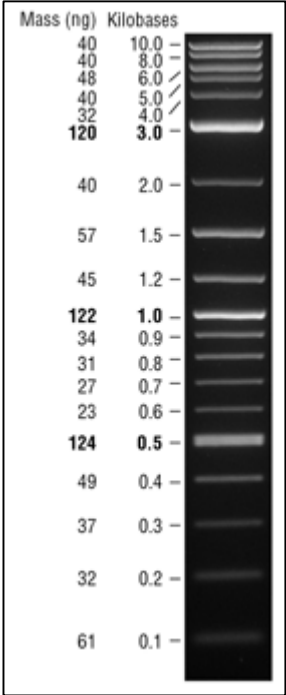
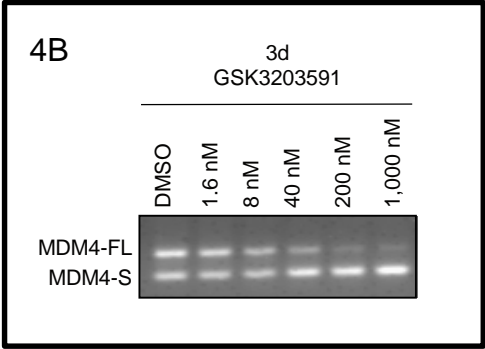


Quick-Load 2-Log DNA Ladder
(New England BioLabs #N0469S)

MDM4

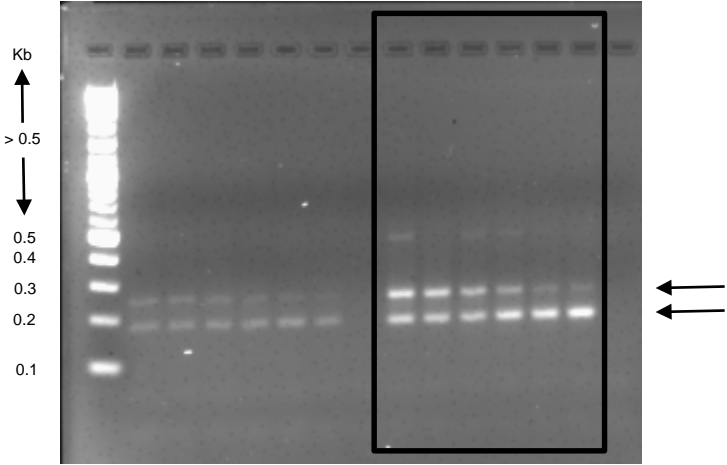


Full scans to accompany Figure 4B

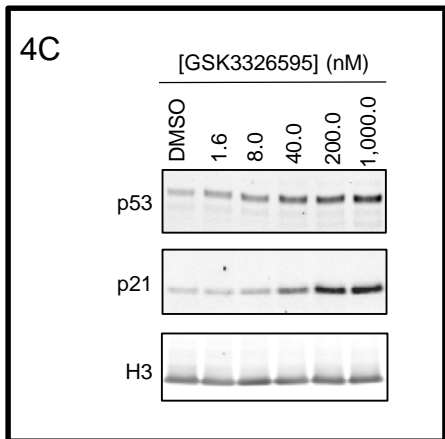


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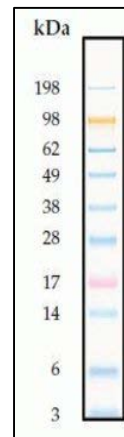


Full scans to accompany Figure 4C



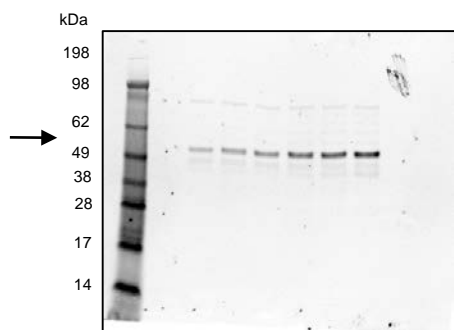
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Phosphorylase	148	105	98	97	111
BSA	98	78	62	64	71
Glutamic Dehydrogenase	64	55	49	51	55
Alcohol Dehydrogenase	50	45	38	39	41
Carbonic Anhydrase	36	34	28	28	n/a
Myoglobin Red	22	17	17	19	n/a
Lysozyme	16	16	14	14	n/a
Aprotinin	6	7	6	n/a	n/a
Insulin, B Chain	4	4	3	n/a	n/a

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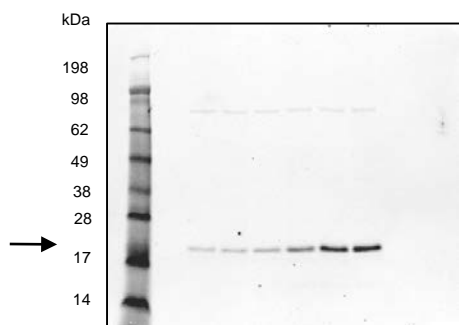


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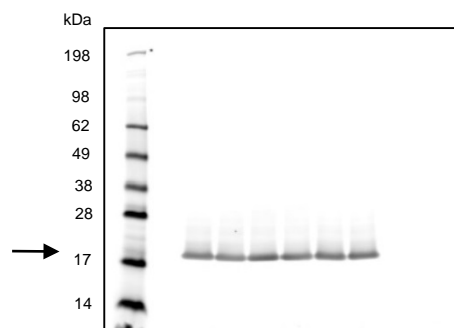
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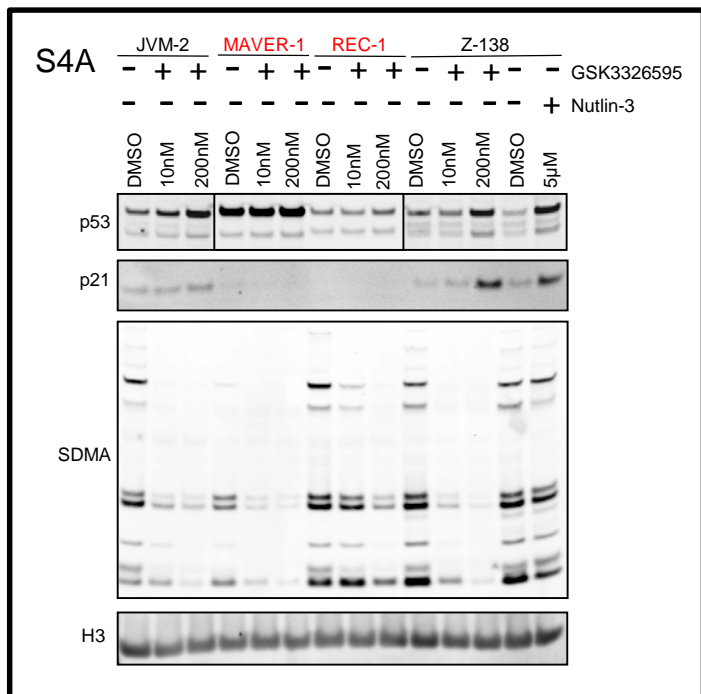
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H3

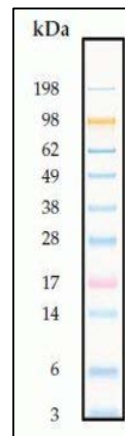


Full Scans to accompany Supplementary Figure S4A



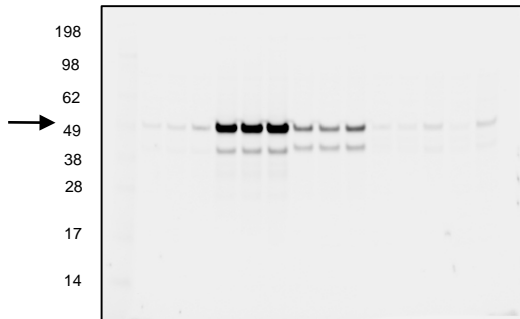
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Phosphorylase	148	105	98	97	111
BSA	98	78	62	64	71
Glutamic Dehydrogenase	64	55	49	51	55
Alcohol Dehydrogenase	50	45	38	39	41
Carbonic Anhydrase	36	34	28	28	n/a
Myoglobin Red	22	17	17	19	n/a
Lysozyme	16	16	14	14	n/a
Aprotinin	6	7	6	n/a	n/a
Insulin, B Chain	4	4	3	n/a	n/a

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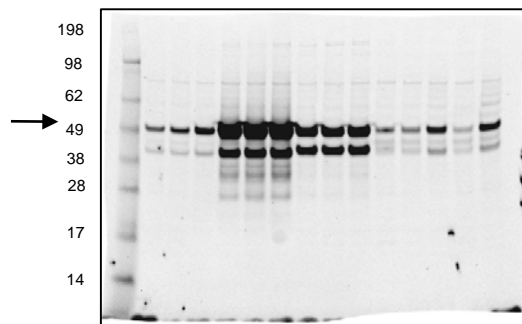


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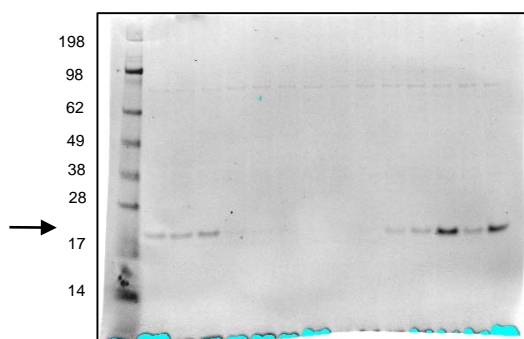
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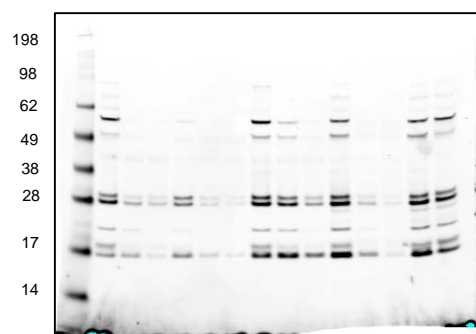
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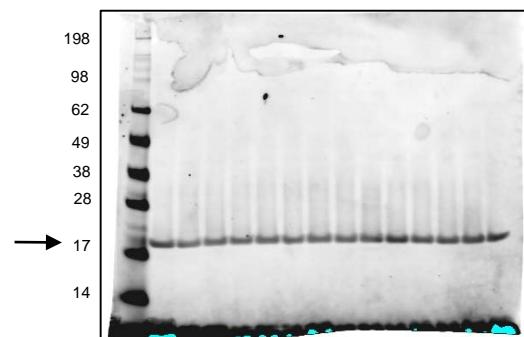
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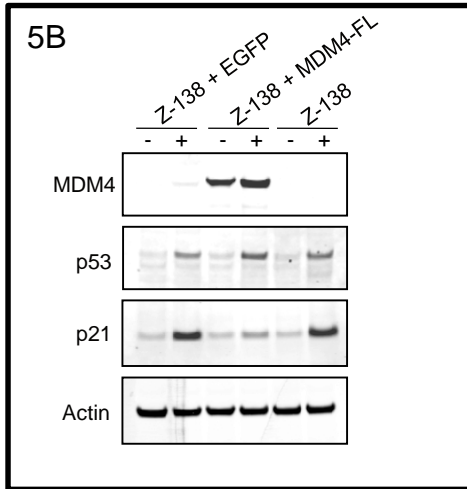
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H3



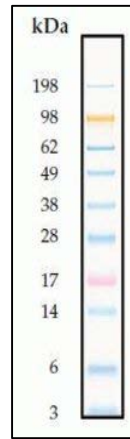
Full scans to accompany Figure 5B



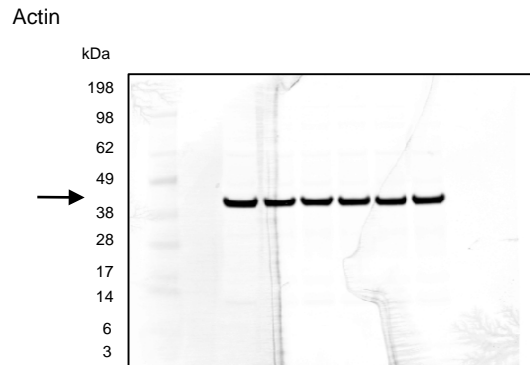
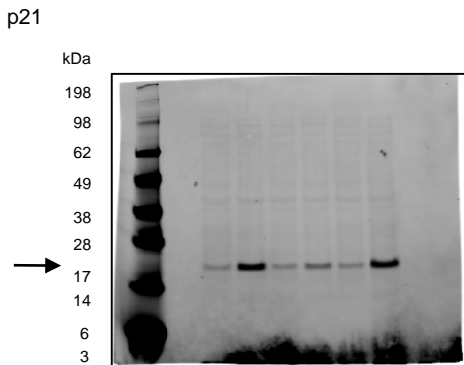
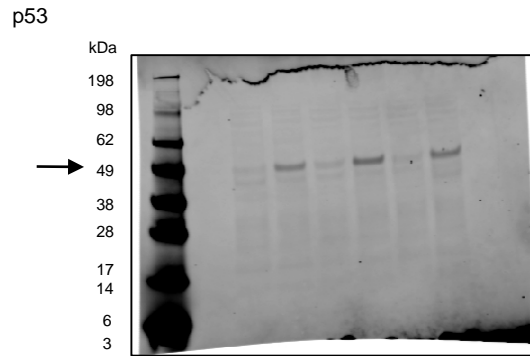
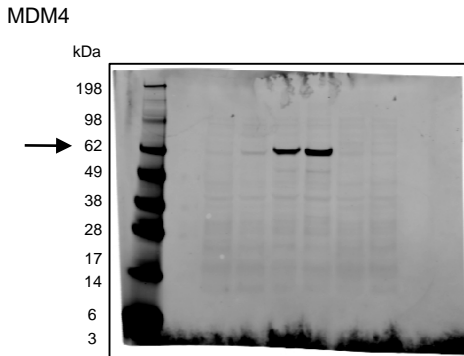
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Phosphorylase	148	105	98	97	111
BSA	98	78	62	64	71
Glutamic Dehydrogenase	64	55	49	51	55
Alcohol Dehydrogenase	50	45	38	39	41
Carbonic Anhydrase	36	34	28	28	n/a
Myoglobin Red	22	17	17	19	n/a
Lysozyme	16	16	14	14	n/a
Aprotinin	6	7	6	n/a	n/a
Insulin, B Chain	4	4	3	n/a	n/a

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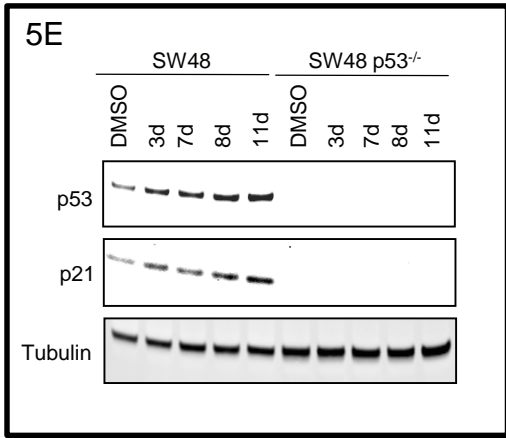
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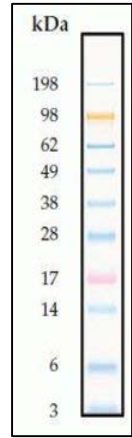


Full scans to accompany Figure 5E



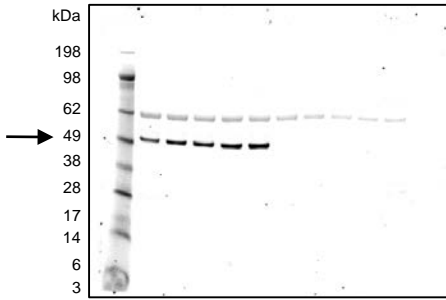
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Phosphorylase	148	105	98	97	111
BSA	98	78	62	64	71
Glutamic Dehydrogenase	64	55	49	51	55
Alcohol Dehydrogenase	50	45	38	39	41
Carbonic Anhydrase	36	34	28	28	n/a
Myoglobin Red	22	17	17	19	n/a
Lysozyme	16	16	14	14	n/a
Aprotinin	6	7	6	n/a	n/a
Insulin, B Chain	4	4	3	n/a	n/a

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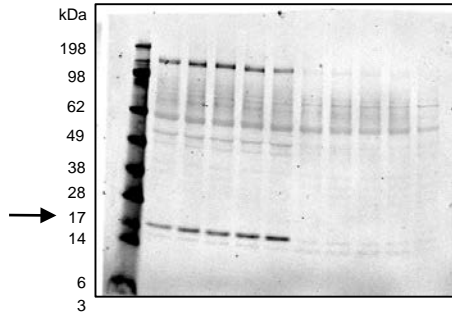


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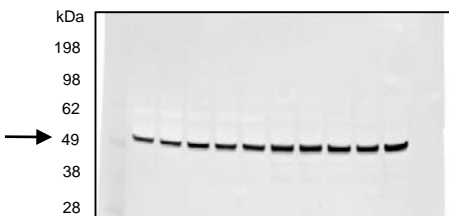
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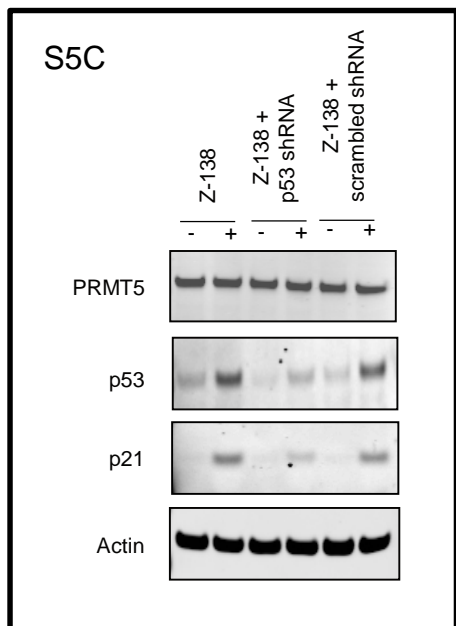
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Tubulin

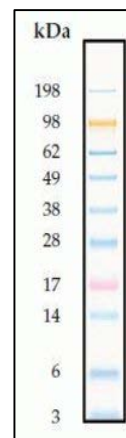


Full Scans to accompany Supplementary Figure S5C

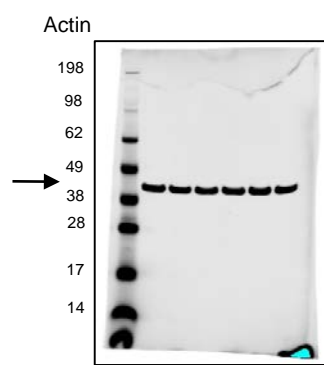
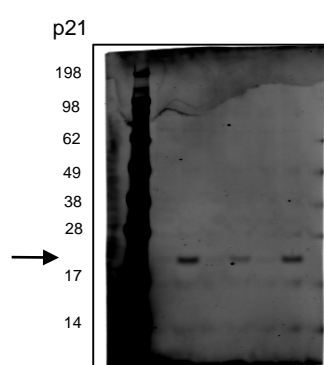
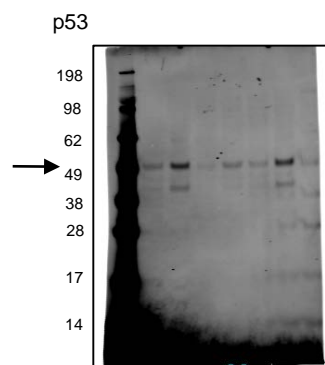
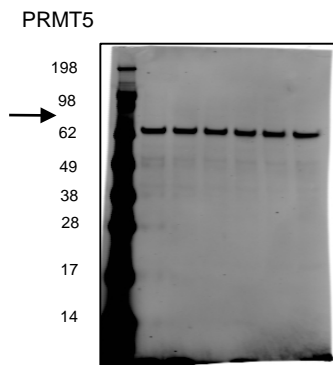


Protein	Approximate Molecular Weights (kDa)				
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Myosin					
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BSA	98	78	62	64	71
Glutamic Dehydrogenase	64	55	49	51	55
Alcohol Dehydrogenase	50	45	38	39	41
Carbonic Anhydrase	36	34	28	28	n/a
Myoglobin Red	22	17	17	19	n/a
Lysozyme	16	16	14	14	n/a
Aprotinin	6	7	6	n/a	n/a
Insulin, B Chain	4	4	3	n/a	n/a

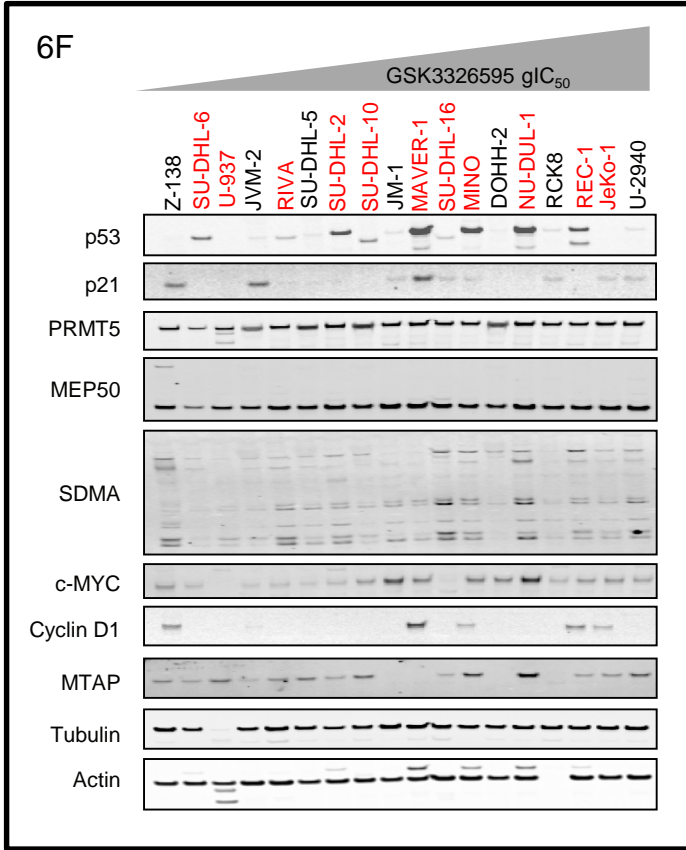
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SeeBlue Plus2 Pre-stained Protein Standard (Invitrogen #LC5925)

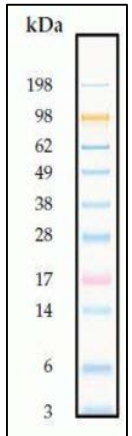


Full scans to accompany Figure 6F

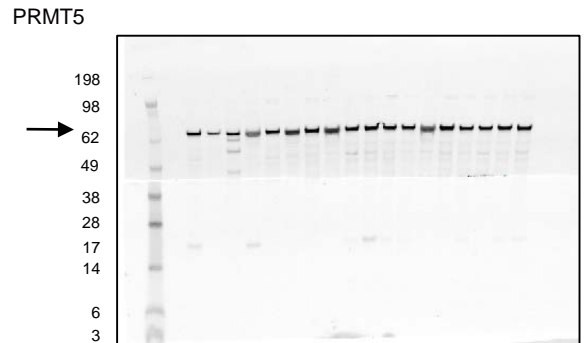
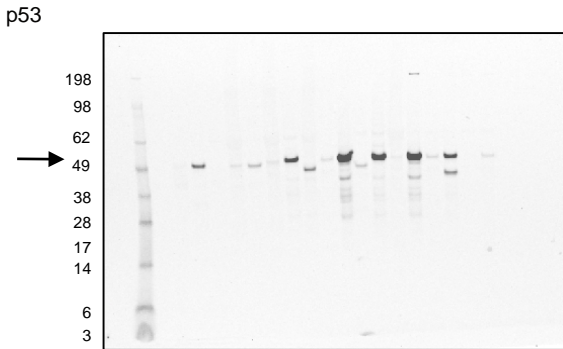


Protein	Approximate Molecular Weights (kDa)				
	Tris-Glycine	Tricine	NuPAGE [®] MES	NuPAGE [®] MOPS	NuPAGE [®] Tris-Acetate
Myosin	250	210	198	191	210
Phosphorylase	148	105	98	97	111
BSA	98	78	62	64	71
Glutamic Dehydrogenase	64	55	49	51	55
Alcohol Dehydrogenase	50	45	38	39	41
Carbonic Anhydrase	36	34	28	28	n/a
Myoglobin Red	22	17	17	19	n/a
Lysozyme	16	16	14	14	n/a
Aprotinin	6	7	6	n/a	n/a
Insulin, B Chain	4	4	3	n/a	n/a

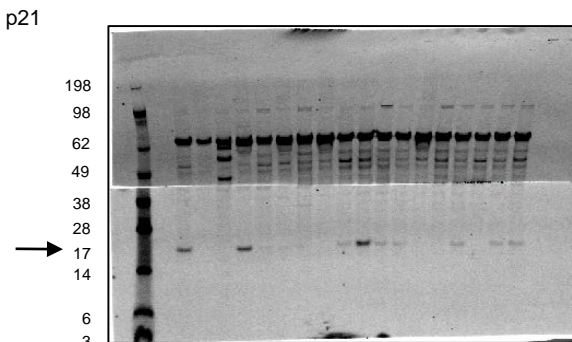
NuPAGE[®] Novex Bis-Tris 4-12% Gel
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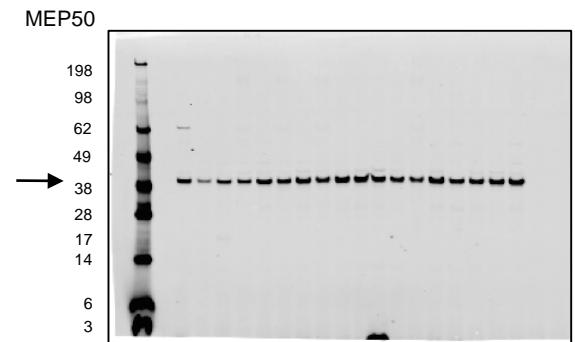
SeeBlue Plus2 Pre-stained Protein Standard (Invitrogen #LC5925)



Blot was cut between molecular weight markers 38kDa and 49kDa. Top portion was probed with PRMT5 antibody and bottom portion was probed with p21 antibody.

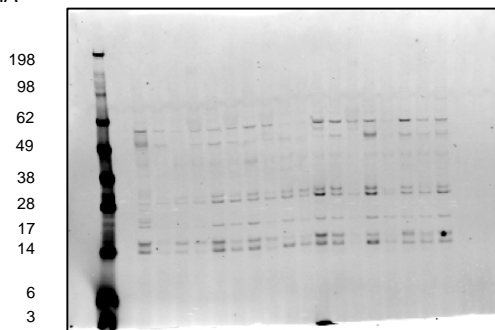


Blot was cut between molecular weight markers 38kDa and 49kDa. Top portion was probed with PRMT5 antibody and bottom portion was probed with p21 antibody.

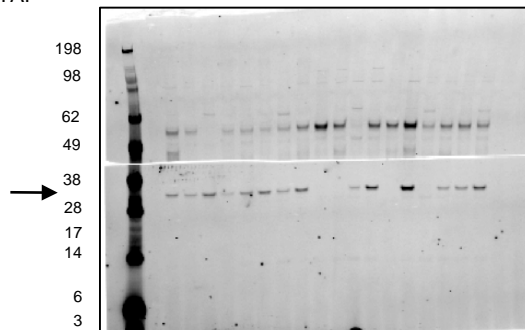


Full scans to accompany Figure 6F (continued)

SDMA

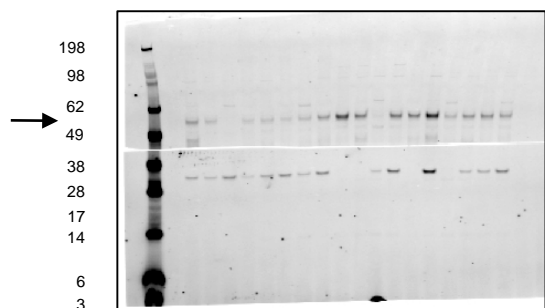


MTAP



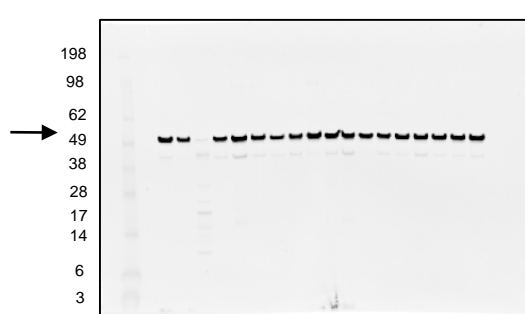
Blot was cut between molecular weight markers 38kDa and 49kDa. Top portion was probed with c-MYC antibody and bottom portion was probed with MTAP antibody.

c-MYC

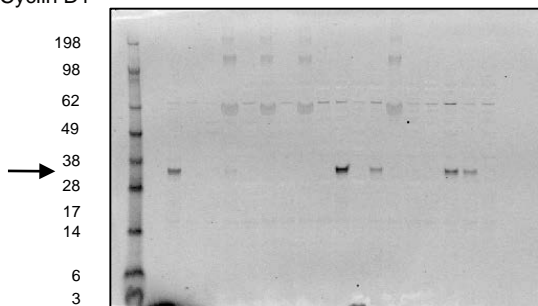


Blot was cut between molecular weight markers 38kDa and 49kDa. Top portion was probed with c-MYC antibody and bottom portion was probed with MTAP antibody.

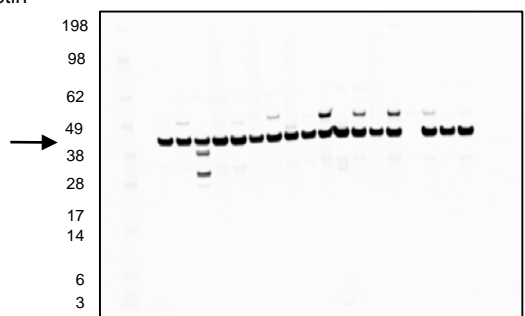
Tubulin



Cyclin D1



Actin



Supplementary Table 2: Correlation Between GSK3203591 and GSK3326595 6-day Proliferation Assay Data

Cell Line	gIC50 (uM)	
	GSK3326595	GSK3203591
5637	0.069	0.143
A172	1.167	0.590
BJAB	1.900	3.100
DOHH-2	0.213	0.116
HCC1143	0.246	0.509
HCC1806	0.098	0.261
HCC70	0.130	0.522
HT-1376	17.599	13.130
J82	29.326	20.947
JeKo-1	5.402	6.191
JM1	0.079	0.076
JVM-2	0.009	0.172
KMS-11	0.066	0.047
LN-229	0.254	1.150
LP1	1.945	0.975
MAVER-1	0.085	0.133
MCF-7	0.062	0.215
MDA-MB-231	0.544	2.928
MDA-MB-453	0.029	0.343
MDA-MB-468	0.057	0.585
MINO	0.130	0.536
NCI-H526	0.275	0.138
NCI-H526-R	0.344	0.219
NU-DHL-1	0.333	0.101
PER-403	0.038	0.029
PER-624	0.524	0.526
PER-704	0.658	0.375
Pfeiffer	0.421	0.352
RCK8	0.529	2.110
REC-1	2.108	1.994
RIVA	0.018	0.068
RL	0.014	1.434
ScaBER	0.198	0.255
SF295	24.722	9.843
SF539	0.061	0.075
SK-BR-3	0.073	0.131
SU-DHL-10	0.054	0.495
SU-DHL-16	0.099	0.124
SU-DHL-2	0.028	0.086
SU-DHL-4	0.492	0.571

SU-DHL-5	0.028	0.132
SU-DHL-6	0.004	0.013
SU-DHL-8	0.053	1.220
SW-780	0.290	0.451
T24	0.765	0.650
U251MG	1.798	3.491
U2932	0.053	0.336
U2940	10.176	2.349
U-87MG	0.040	0.127
U937	0.007	0.049
UM-UC-3	29.326	19.169
WSU-DLCL2	0.082	0.651
WSU-NHL	0.008	0.076
Z-138	0.004	0.014
ZR-75-1	10.974	2.615

Supplementary Table 3: gIC50 and Activity Area values for 10-day Proliferation Assay (Eurofins panel)

CellLine	gIC50 (nM)	ActivityArea	TumorType
SW-13	211	3.37	Adrenal
NCI-H295R	10000	1.23	Adrenal
MOLT-3	217	3.72	ALL
NALM-6	110	4.32	ALL
MOLT-16	54.2	4.95	ALL
CEM-C1	7.92	6.64	ALL
Jurkat	130	4.17	ALL
CCRFCEM	3140	1.25	ALL
MV-4-11	65.8	4.8	AML
HEL-92-1-7	9.02	6.46	AML
Thp1	80.5	4.51	AML
UM-UC-3	105	4.49	Bladder
TCCSUP	235	2.85	Bladder
T24	449	2.76	Bladder
BFTC-905	259	3.46	Bladder
SCaBER	123	4.22	Bladder
HT-1197	98.9	4.47	Bladder
J82	83.1	4.4	Bladder
647-V	62	4.91	Bladder
5637.000	285	3.5	Bladder
HT1376	297	3.48	Bladder
EFM-19	69.5	4.42	Breast
T47D	52.4	4.07	Breast
MDA MB 468	4.83	5.05	Breast
KPL-1	1330	1.92	Breast
MT-3	46.4	4.72	Breast
CAMA-1	78.9	4.27	Breast
MX1	294	3.29	Breast
BT474	172	3.41	Breast
SK-BR-3	76.4	4.28	Breast
AU565	14.6	5.44	Breast
MDA MB 453	64.5	4.23	Breast
MCF7	10.9	5.15	Breast
MDA-MB-436	49.7	5.04	Breast
MDA MB 231	56.7	5	Breast
BT-549	59.4	2.74	Breast
Hs 578T	947	2.06	Breast
BT20	2280	1.46	Breast
ST486	101	4.41	Burkitts
Daudi	142	4.05	Burkitts
Raji	24.5	5.64	Burkitts

EB-3	299	3.45	Burkitts
Ramos (RA 1)	546	2.79	Burkitts
DoTc2 4510	95.8	4.42	Cervix
C-4 I	139	4.13	Cervix
C-4 II	266	3.54	Cervix
HeLa	230	3.51	Cervix
C-33A	228	3.45	Cervix
SiHa	2510	1.55	Cervix
JEG-3	216	3.81	Choriocarcinoma
JAR	485	2.95	Choriocarcinoma
BeWo	1090	2.24	Choriocarcinoma
BV-173	10.6	6.26	CML
EM-2	94	4.54	CML
CML-T1	16.1	5.95	CML
MEG01	10000	0.05	CML
K562	146	3.97	CML
Colo 201	49.6	4.93	Colon
LS-174T	170	3.99	Colon
SW480	413	3.2	Colon
RKO-AS45-1	113	4.31	Colon
RKO	772	2.71	Colon
SW48	10000	1.59	Colon
HCT-116	166	3.91	Colon
NCI-H747	2580	1.56	Colon
SW620	5640	1.26	Colon
SW948	120	4.1	Colon
HT-29	95.2	4.32	Colon
Colo 205	10000	1.03	Colon
WiDr	505	2.38	Colon
SW837	102	4.23	Colon
Colo 320 HSR	80.3	4.49	Colon
DLD-1	134	4.13	Colon
RKOE6	210	3.66	Colon
SW403	153	3.67	Colon
HCT-15	68.4	4.77	Colon
Colo 320DM	82.5	4.65	Colon
LS1034	172	3.85	Colon
NCI-H508	307	3.48	Colon
HCT-8	430	3.18	Colon
SW1417	773	2.59	Colon
DOHH-2	157	3.95	DLBCL
DB	2.51	7.57	DLBCL
SR	275	3.44	DLBCL
HT	206	3.58	DLBCL
AN3 CA	254	3.37	Endometrium
HEC-1-A	2440	1.98	Endometrium
KLE	10000	1.05	Endometrium

RL95-2	218	3.47	Endometrium
OE21	146	3.99	Esophageal
OE19	98	4.45	Esophageal
OE33	231	3.7	Esophageal
OCUG-1	379	3.12	Gallbladder
SNU-16	34.4	5.36	Gastric
AGS	2030	1.49	Gastric
SNU-1	1040	2.25	Gastric
SNU-5	110	4.34	Gastric
KATO III	16.7	6.09	Gastric
HS 746T	171	3.86	Gastric
SW1088	40.5	5.09	Glioma
DBTRG-05MG	156	2.13	Glioma
A172	29.6	5.28	Glioma
U-87 MG	67.6	3.26	Glioma
DK-MG	89.5	4.52	Glioma
H4	151	3.91	Glioma
T98G	27.3	5.47	Glioma
SNB-19	223	3.27	Glioma
CCF-STTG1	115	4.32	Glioma
SW1783	389	2.92	Glioma
U-138MG	43.3	5.04	Glioma
L-428	330	2.98	HL
RPMI 6666	748	2.6	HL
Cal 27	103	4.46	HN
Detroit 562	104	4.36	HN
SCC-9	747	2.69	HN
SCC-4	286	2.85	HN
SCC-25	273	3.32	HN
SW1463	770	2.36	HN
FaDu	15.4	6.08	HN sq
Caki-1	68.8	4.79	Kidney
G-402	99.7	4.3	Kidney
Caki-2	114	4.32	Kidney
786-O	20.3	5.64	Kidney
A498	50.7	5.01	Kidney
ACHN	38.6	5.27	Kidney
769-P	203	3.75	Kidney
SK-NEP-1	264	2.87	Kidney
HuCCT1	225	3.52	Liver
HUH-6 Clone 5	361	3.09	Liver
SNU-423	66	4.77	Liver
HepG2	214	3.55	Liver
HLE	174	3.77	Liver
D283 Med	23.5	5.48	Meduloblastoma
Daoy	270	3.56	Meduloblastoma
COLO 829	54.4	4.09	Melanoma

Hs 695T	602	2.82	Melanoma
Hs 294T	12.3	5.68	Melanoma
SH-4	75.2	4.66	Melanoma
A7	36.7	5.29	Melanoma
C32	14.1	5.08	Melanoma
HMCB	666	2.33	Melanoma
C32TG	103	4.15	Melanoma
MeWo	249	3.7	Melanoma
SK-MEL-1	38.7	5.04	Melanoma
SK-MEL-3	181	3.38	Melanoma
SK-MEL-28	239	3.48	Melanoma
A101D	190	3.47	Melanoma
RPMI-7951	44.2	5.08	Melanoma
A375	79.6	4.05	Melanoma
CHL-1	247	3.6	Melanoma
MALME3M	265	3.55	Melanoma
RPMI 8226	58.4	4.58	MM
U266B1	78.5	4.18	MM
SKO-007	84.4	4.01	MM
CHP-212	24	5.24	Neuroblastoma
SK-N-FI	308	3.32	Neuroblastoma
MC-IXC	615	2.67	Neuroblastoma
SK-N-AS	31.9	3.45	Neuroblastoma
SK-N-DZ	257	3.65	Neuroblastoma
BE(2)C	803	2.42	Neuroblastoma
MHH-PREB-1	10000	0.38	NHL
Wi38	18.5	3.96	Normal
BPH1	718	2.74	Normal
ChaGoK1	1190	2.06	NSCLC
NCI-H460	224	3.53	NSCLC
A427	130	4.02	NSCLC
NCI-H596	188	2.89	NSCLC
COR-L105	314	3.49	NSCLC
NCIH441	556	2.88	NSCLC
Calu6	206	3.34	NSCLC
COR-L23	147	4.15	NSCLC
NCI-H292	166	3.25	NSCLC
SW900	3250	1.26	NSCLC
Calu1	3050	1.37	NSCLC
SKMES1	807	2.26	NSCLC
NCI-H661	152	3.94	NSCLC
A549	80.9	4.57	NSCLC
NCI-H520	15.6	5.93	NSCLC sq
ES-2	106	4.24	Ovarian
OVCAR3	51.4	5.03	Ovarian
CaOV3	298	3.4	Ovarian
SKOV3	52.6	4.21	Ovarian

Mia PaCa-2	158	3.75	Pancreatic
HPAF-II	154	4.05	Pancreatic
Hs 766T	1120	1.62	Pancreatic
BxPC-3	160	3.87	Pancreatic
AsPC-1	302	3.26	Pancreatic
Capan-2	69.7	4.63	Pancreatic
HuP-T4	84.9	4.21	Pancreatic
PANC-1	165	3.97	Pancreatic
CFPAC-1	52.3	4.98	Pancreatic
YAPC	168	3.88	Pancreatic
SU.86.86	934	2.2	Pancreatic
Capan-1	370	2.9	Pancreatic
BC-1	69.6	4.67	PEL
CRO-AP2	250	3.58	PEL
ARH-77	44.4	4.72	Plasma cell leukimia
PC-3	873	2.36	Prostate
BM-1604	142	4.01	Prostate
DU145	151	4.02	Prostate
22Rv1	2480	1.97	Prostate
LNCaP	2930	1.61	Prostate
Y79	113	3.36	Retinoblastoma
HT-3	10000	0.92	Retinoblastoma
G-401	38.4	5.26	Rhabdoid
RD	639	2.85	Sarcoma
SJRH30	472	3.11	Sarcoma
SW872	54	4.97	Sarcoma
MES-SA	72	4.58	Sarcoma
SK-UT-1	42.1	5.16	Sarcoma
MG-63	96	4.31	Sarcoma
SaOS2	48.2	5.07	Sarcoma
TE 381.T	147	3.97	Sarcoma
A204	223	3.73	Sarcoma
SW982	56.7	4.95	Sarcoma
SW1353	129	4.23	Sarcoma
SJSA1	773	2.34	Sarcoma
SK-LMS-1	113	4.24	Sarcoma
A-673	119	4.12	Sarcoma
HT-1080	53.5	4.05	Sarcoma
KHOS-240S	124	3.92	Sarcoma
HOS	223	3.72	Sarcoma
U2OS	485	3.08	Sarcoma
SW684	1170	1.84	Sarcoma
DMS273	298	3.1	SCLC
DMS53	106	4.19	SCLC
NCI-H69	144	3.22	SCLC
DMS114	1010	2.35	SCLC
SHP-77	152	2.98	SCLC

NCIH446	570	2.88	SCLC
A431	549	2.85	Skin
HLF	87.7	4.6	Skin
J-RT3-T3-5	43.2	5.09	TCL
CAL-62	253	3.65	Thyroid
CGTH-W-1	82.2	4.62	Thyroid
SW579	83.3	4.6	Thyroid
BHT-101	214	3.72	Thyroid
639-V	342	3.41	Ureter
SW962	203	3.58	Vulva
SW954	10000	0.31	Vulva

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0238	0238	0238	
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0242	0242	0242	
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0244	0244	0244	
0245	0245	0245	
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0249	0249	0249	
0250	0250	0250	
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0252	0252	0252	
0253	0253	0253	
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0255	0255	0255	
0256	0256	0256	
0257	0257	0257	
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0259	0259	0259	
0260	0260	0260	
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0274	0274	0274	
0275	0275	0275	
0276	0276	0276	
0277	0277	0277	
0278	0278	0278	
0279	0279	0279	
0280	0280	0280	
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0282	0282	0282	
0283	0283	0283	
0284	0284	0284	
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0287	0287	0287	
0288	0288	0288	
0289	0289	0289	
0290	0290	0290	
0291	0291	0291	
0292	0292	0292	
0293	0293	0293	
0294	0294	0294	
0295	0295	0295	
0296	0296	0296	
0297	0297	0297	
0298	0298	0298	
0299	0299	0299	
0300	0300	0300	

Year	Revenue	Operating Profit	Net Profit	Operating Profit Margin	Net Profit Margin	Operating Profit per Share	Net Profit per Share	Operating Profit per Share	Net Profit per Share	Operating Profit per Share	Net Profit per Share
2018	1,234,567	234,567	123,456	19.0%	10.0%	1.23	0.62	1.23	0.62	1.23	0.62
2019	1,345,678	245,678	134,567	18.2%	9.9%	1.34	0.63	1.34	0.63	1.34	0.63
2020	1,456,789	256,789	145,678	17.6%	10.0%	1.45	0.64	1.45	0.64	1.45	0.64

Supplementary Table 12: Association of 10-day Activity Area from Eurofins Cell Line Panel with Sanger Mutations

genes	p.value	mutatedCellLines	median_WT	median_MT	dMedian
ADC	0.000220695	141	3.55	4.29	-0.74
MEGF8	0.000671984	17	2.82	3.93	-1.11
COX4I2	0.000696504	6	2.155	3.81	-1.655
CAPN1	0.001074389	46	3.425	3.97	-0.545
INPPL1	0.001625844	17	2.85	3.89	-1.04
DIS3	0.001710364	30	3.34	3.95	-0.61
USP32	0.001834347	5	1.97	3.78	-1.81
ACIN1	0.002163315	23	3.25	3.945	-0.695
CCDC30	0.002401392	12	2.75	3.91	-1.16
TP53	0.002649119	49	3.47	3.96	-0.49
ANKRD52	0.002651865	24	3.245	3.92	-0.675
C20orf186	0.002731218	12	3.135	3.91	-0.775
CSPG4	0.002862413	16	3.365	3.92	-0.555
ATP1B2	0.002898047	4	1.525	3.75	-2.225
CNGA3	0.003008393	14	3.11	3.91	-0.8
TRH	0.003042461	5	1.84	3.78	-1.94
ANKRD26	0.003214683	30	3.34	3.94	-0.6
NCOR1	0.003466409	9	4.41	3.685	0.725
CASKIN1	0.00370116	8	2.655	3.87	-1.215
ENPEP	0.003787563	13	2.36	3.84	-1.48

Supplementary Table 12: Association of 10-day gIC₅₀ Values from Eurofins Cell Line Panel with Sanger Mutations

genes	p.value	mutatedCellLines	median_WT	median_MT	dMedian
MEGF8	0.000230006	17	602	149.5	452.5
TP53	0.000314354	49	257	143	114
C20orf186	0.00043074	12	336	152	184
COX4I2	0.000501276	6	1626.5	154	1472.5
CSPG4	0.000926814	16	308.5	152	156.5
CNGA3	0.00100639	14	490.5	152	338.5
CAPN1	0.00107443	46	270.5	147	123.5
ACIN1	0.001297018	23	299	147	152
ABCA8	0.001312394	36	258	147	111
USP32	0.001332233	5	1170	155	1015
LRRC4	0.00158154	20	365.5	152	213.5
ATP8A2	0.001674299	34	276	152	124
CASKIN1	0.001946257	8	760	153	607
CA8	0.001978389	40	248.5	146	102.5
BAIAP3	0.002440538	15	294	152	142
EPHB1	0.002497451	16	660	153	507
TRH	0.002505527	5	1170	155	1015
ANKRD52	0.002529885	24	290	147	143
BCLAF1	0.002663209	30	298	152	146
ATP1B2	0.00289814	4	2305	156	2149

Summary Table 14. Public Release Categories of Expenditures (USA) - Report from Activity Area

Activity Area	Category	Sub-Category	Code	Value	Count	Start Date	End Date	Release Date	Release Type	Release Status
ACQUISITION	ACQUISITION OF GOODS AND SERVICES	ACQUISITION OF GOODS	01000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ACQUISITION OF GOODS AND SERVICES	ACQUISITION OF SERVICES	02000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ACQUISITION OF GOODS AND SERVICES	ACQUISITION OF INFORMATION	03000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ACQUISITION OF GOODS AND SERVICES	ACQUISITION OF EQUIPMENT	04000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ACQUISITION OF GOODS AND SERVICES	ACQUISITION OF SUPPLIES	05000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ACQUISITION OF GOODS AND SERVICES	ACQUISITION OF MATERIALS	06000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ACQUISITION OF GOODS AND SERVICES	ACQUISITION OF CONTRACTS	07000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ACQUISITION OF GOODS AND SERVICES	ACQUISITION OF TECHNOLOGY	08000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ACQUISITION OF GOODS AND SERVICES	ACQUISITION OF INFRASTRUCTURE	09000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ACQUISITION OF GOODS AND SERVICES	ACQUISITION OF FACILITIES	10000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
OPERATIONS	OPERATIONS AND MAINTENANCE	OPERATIONS	11000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	OPERATIONS AND MAINTENANCE	MAINTENANCE	12000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	OPERATIONS AND MAINTENANCE	OPERATIONS AND MAINTENANCE	13000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	OPERATIONS AND MAINTENANCE	OPERATIONS AND MAINTENANCE	14000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	OPERATIONS AND MAINTENANCE	OPERATIONS AND MAINTENANCE	15000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	OPERATIONS AND MAINTENANCE	OPERATIONS AND MAINTENANCE	16000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	OPERATIONS AND MAINTENANCE	OPERATIONS AND MAINTENANCE	17000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	OPERATIONS AND MAINTENANCE	OPERATIONS AND MAINTENANCE	18000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	OPERATIONS AND MAINTENANCE	OPERATIONS AND MAINTENANCE	19000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	OPERATIONS AND MAINTENANCE	OPERATIONS AND MAINTENANCE	20000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
RESEARCH AND DEVELOPMENT	RESEARCH AND DEVELOPMENT	RESEARCH AND DEVELOPMENT	21000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	RESEARCH AND DEVELOPMENT	RESEARCH AND DEVELOPMENT	22000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	RESEARCH AND DEVELOPMENT	RESEARCH AND DEVELOPMENT	23000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	RESEARCH AND DEVELOPMENT	RESEARCH AND DEVELOPMENT	24000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	RESEARCH AND DEVELOPMENT	RESEARCH AND DEVELOPMENT	25000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	RESEARCH AND DEVELOPMENT	RESEARCH AND DEVELOPMENT	26000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	RESEARCH AND DEVELOPMENT	RESEARCH AND DEVELOPMENT	27000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	RESEARCH AND DEVELOPMENT	RESEARCH AND DEVELOPMENT	28000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	RESEARCH AND DEVELOPMENT	RESEARCH AND DEVELOPMENT	29000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	RESEARCH AND DEVELOPMENT	RESEARCH AND DEVELOPMENT	30000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
ADMINISTRATIVE	ADMINISTRATIVE	ADMINISTRATIVE	31000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ADMINISTRATIVE	ADMINISTRATIVE	32000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ADMINISTRATIVE	ADMINISTRATIVE	33000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ADMINISTRATIVE	ADMINISTRATIVE	34000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ADMINISTRATIVE	ADMINISTRATIVE	35000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ADMINISTRATIVE	ADMINISTRATIVE	36000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ADMINISTRATIVE	ADMINISTRATIVE	37000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ADMINISTRATIVE	ADMINISTRATIVE	38000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ADMINISTRATIVE	ADMINISTRATIVE	39000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final
	ADMINISTRATIVE	ADMINISTRATIVE	40000000	1000000000	1000000	2000-01-01	2000-12-31	2000-01-01	Public Release	Final

DESCRIPTION	UNIT	QUANTITY	UNIT PRICE	TOTAL PRICE	DATE
DESCRIPTION: [REDACTED]	1	1	1.00	1.00	1/1/2018
DESCRIPTION: [REDACTED]	2	2	2.00	4.00	1/1/2018
DESCRIPTION: [REDACTED]	3	3	3.00	9.00	1/1/2018
DESCRIPTION: [REDACTED]	4	4	4.00	16.00	1/1/2018
DESCRIPTION: [REDACTED]	5	5	5.00	25.00	1/1/2018
DESCRIPTION: [REDACTED]	6	6	6.00	36.00	1/1/2018
DESCRIPTION: [REDACTED]	7	7	7.00	49.00	1/1/2018
DESCRIPTION: [REDACTED]	8	8	8.00	64.00	1/1/2018
DESCRIPTION: [REDACTED]	9	9	9.00	81.00	1/1/2018
DESCRIPTION: [REDACTED]	10	10	10.00	100.00	1/1/2018
DESCRIPTION: [REDACTED]	11	11	11.00	121.00	1/1/2018
DESCRIPTION: [REDACTED]	12	12	12.00	144.00	1/1/2018
DESCRIPTION: [REDACTED]	13	13	13.00	169.00	1/1/2018
DESCRIPTION: [REDACTED]	14	14	14.00	196.00	1/1/2018
DESCRIPTION: [REDACTED]	15	15	15.00	225.00	1/1/2018
DESCRIPTION: [REDACTED]	16	16	16.00	256.00	1/1/2018
DESCRIPTION: [REDACTED]	17	17	17.00	289.00	1/1/2018
DESCRIPTION: [REDACTED]	18	18	18.00	324.00	1/1/2018
DESCRIPTION: [REDACTED]	19	19	19.00	361.00	1/1/2018
DESCRIPTION: [REDACTED]	20	20	20.00	400.00	1/1/2018
DESCRIPTION: [REDACTED]	21	21	21.00	441.00	1/1/2018
DESCRIPTION: [REDACTED]	22	22	22.00	484.00	1/1/2018
DESCRIPTION: [REDACTED]	23	23	23.00	529.00	1/1/2018
DESCRIPTION: [REDACTED]	24	24	24.00	576.00	1/1/2018
DESCRIPTION: [REDACTED]	25	25	25.00	625.00	1/1/2018
DESCRIPTION: [REDACTED]	26	26	26.00	676.00	1/1/2018
DESCRIPTION: [REDACTED]	27	27	27.00	729.00	1/1/2018
DESCRIPTION: [REDACTED]	28	28	28.00	784.00	1/1/2018
DESCRIPTION: [REDACTED]	29	29	29.00	841.00	1/1/2018
DESCRIPTION: [REDACTED]	30	30	30.00	900.00	1/1/2018
DESCRIPTION: [REDACTED]	31	31	31.00	961.00	1/1/2018
DESCRIPTION: [REDACTED]	32	32	32.00	1024.00	1/1/2018
DESCRIPTION: [REDACTED]	33	33	33.00	1089.00	1/1/2018
DESCRIPTION: [REDACTED]	34	34	34.00	1156.00	1/1/2018
DESCRIPTION: [REDACTED]	35	35	35.00	1225.00	1/1/2018
DESCRIPTION: [REDACTED]	36	36	36.00	1296.00	1/1/2018
DESCRIPTION: [REDACTED]	37	37	37.00	1369.00	1/1/2018
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DESCRIPTION: [REDACTED]	40	40	40.00	1600.00	1/1/2018
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DESCRIPTION: [REDACTED]	42	42	42.00	1764.00	1/1/2018
DESCRIPTION: [REDACTED]	43	43	43.00	1849.00	1/1/2018
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DESCRIPTION: [REDACTED]	50	50	50.00	2500.00	1/1/2018
DESCRIPTION: [REDACTED]	51	51	51.00	2601.00	1/1/2018
DESCRIPTION: [REDACTED]	52	52	52.00	2704.00	1/1/2018
DESCRIPTION: [REDACTED]	53	53	53.00	2809.00	1/1/2018
DESCRIPTION: [REDACTED]	54	54	54.00	2916.00	1/1/2018
DESCRIPTION: [REDACTED]	55	55	55.00	3025.00	1/1/2018
DESCRIPTION: [REDACTED]	56	56	56.00	3136.00	1/1/2018
DESCRIPTION: [REDACTED]	57	57	57.00	3249.00	1/1/2018
DESCRIPTION: [REDACTED]	58	58	58.00	3364.00	1/1/2018
DESCRIPTION: [REDACTED]	59	59	59.00	3481.00	1/1/2018
DESCRIPTION: [REDACTED]	60	60	60.00	3600.00	1/1/2018
DESCRIPTION: [REDACTED]	61	61	61.00	3721.00	1/1/2018
DESCRIPTION: [REDACTED]	62	62	62.00	3844.00	1/1/2018
DESCRIPTION: [REDACTED]	63	63	63.00	3969.00	1/1/2018
DESCRIPTION: [REDACTED]	64	64	64.00	4096.00	1/1/2018
DESCRIPTION: [REDACTED]	65	65	65.00	4225.00	1/1/2018
DESCRIPTION: [REDACTED]	66	66	66.00	4356.00	1/1/2018
DESCRIPTION: [REDACTED]	67	67	67.00	4489.00	1/1/2018
DESCRIPTION: [REDACTED]	68	68	68.00	4624.00	1/1/2018
DESCRIPTION: [REDACTED]	69	69	69.00	4761.00	1/1/2018
DESCRIPTION: [REDACTED]	70	70	70.00	4900.00	1/1/2018
DESCRIPTION: [REDACTED]	71	71	71.00	5041.00	1/1/2018
DESCRIPTION: [REDACTED]	72	72	72.00	5184.00	1/1/2018
DESCRIPTION: [REDACTED]	73	73	73.00	5329.00	1/1/2018
DESCRIPTION: [REDACTED]	74	74	74.00	5476.00	1/1/2018
DESCRIPTION: [REDACTED]	75	75	75.00	5625.00	1/1/2018
DESCRIPTION: [REDACTED]	76	76	76.00	5776.00	1/1/2018
DESCRIPTION: [REDACTED]	77	77	77.00	5929.00	1/1/2018
DESCRIPTION: [REDACTED]	78	78	78.00	6084.00	1/1/2018
DESCRIPTION: [REDACTED]	79	79	79.00	6241.00	1/1/2018
DESCRIPTION: [REDACTED]	80	80	80.00	6400.00	1/1/2018
DESCRIPTION: [REDACTED]	81	81	81.00	6561.00	1/1/2018
DESCRIPTION: [REDACTED]	82	82	82.00	6724.00	1/1/2018
DESCRIPTION: [REDACTED]	83	83	83.00	6889.00	1/1/2018
DESCRIPTION: [REDACTED]	84	84	84.00	7056.00	1/1/2018
DESCRIPTION: [REDACTED]	85	85	85.00	7225.00	1/1/2018
DESCRIPTION: [REDACTED]	86	86	86.00	7396.00	1/1/2018
DESCRIPTION: [REDACTED]	87	87	87.00	7569.00	1/1/2018
DESCRIPTION: [REDACTED]	88	88	88.00	7744.00	1/1/2018
DESCRIPTION: [REDACTED]	89	89	89.00	7921.00	1/1/2018
DESCRIPTION: [REDACTED]	90	90	90.00	8100.00	1/1/2018
DESCRIPTION: [REDACTED]	91	91	91.00	8281.00	1/1/2018
DESCRIPTION: [REDACTED]	92	92	92.00	8464.00	1/1/2018
DESCRIPTION: [REDACTED]	93	93	93.00	8649.00	1/1/2018
DESCRIPTION: [REDACTED]	94	94	94.00	8836.00	1/1/2018
DESCRIPTION: [REDACTED]	95	95	95.00	9025.00	1/1/2018
DESCRIPTION: [REDACTED]	96	96	96.00	9216.00	1/1/2018
DESCRIPTION: [REDACTED]	97	97	97.00	9409.00	1/1/2018
DESCRIPTION: [REDACTED]	98	98	98.00	9604.00	1/1/2018
DESCRIPTION: [REDACTED]	99	99	99.00	9801.00	1/1/2018
DESCRIPTION: [REDACTED]	100	100	100.00	10000.00	1/1/2018

Supplemental Table S1. Non-coding Protein Catalog of Escherichia coli. Export from UniProt

Accession	Gene	Protein	Length	MW	pI	Inst	CD	MD	MD2	MD3	MD4	MD5	MD6	MD7	MD8	MD9	MD10	MD11	MD12	MD13	MD14	MD15	MD16	MD17	MD18	MD19	MD20	MD21	MD22	MD23	MD24	MD25	MD26	MD27	MD28	MD29	MD30	MD31	MD32	MD33	MD34	MD35	MD36	MD37	MD38	MD39	MD40	MD41	MD42	MD43	MD44	MD45	MD46	MD47	MD48	MD49	MD50	MD51	MD52	MD53	MD54	MD55	MD56	MD57	MD58	MD59	MD60	MD61	MD62	MD63	MD64	MD65	MD66	MD67	MD68	MD69	MD70	MD71	MD72	MD73	MD74	MD75	MD76	MD77	MD78	MD79	MD80	MD81	MD82	MD83	MD84	MD85	MD86	MD87	MD88	MD89	MD90	MD91	MD92	MD93	MD94	MD95	MD96	MD97	MD98	MD99	MD100	MD101	MD102	MD103	MD104	MD105	MD106	MD107	MD108	MD109	MD110	MD111	MD112	MD113	MD114	MD115	MD116	MD117	MD118	MD119	MD120	MD121	MD122	MD123	MD124	MD125	MD126	MD127	MD128	MD129	MD130	MD131	MD132	MD133	MD134	MD135	MD136	MD137	MD138	MD139	MD140	MD141	MD142	MD143	MD144	MD145	MD146	MD147	MD148	MD149	MD150	MD151	MD152	MD153	MD154	MD155	MD156	MD157	MD158	MD159	MD160	MD161	MD162	MD163	MD164	MD165	MD166	MD167	MD168	MD169	MD170	MD171	MD172	MD173	MD174	MD175	MD176	MD177	MD178	MD179	MD180	MD181	MD182	MD183	MD184	MD185	MD186	MD187	MD188	MD189	MD190	MD191	MD192	MD193	MD194	MD195	MD196	MD197	MD198	MD199	MD200	MD201	MD202	MD203	MD204	MD205	MD206	MD207	MD208	MD209	MD210	MD211	MD212	MD213	MD214	MD215	MD216	MD217	MD218	MD219	MD220	MD221	MD222	MD223	MD224	MD225	MD226	MD227	MD228	MD229	MD230	MD231	MD232	MD233	MD234	MD235	MD236	MD237	MD238	MD239	MD240	MD241	MD242	MD243	MD244	MD245	MD246	MD247	MD248	MD249	MD250	MD251	MD252	MD253	MD254	MD255	MD256	MD257	MD258	MD259	MD260	MD261	MD262	MD263	MD264	MD265	MD266	MD267	MD268	MD269	MD270	MD271	MD272	MD273	MD274	MD275	MD276	MD277	MD278	MD279	MD280	MD281	MD282	MD283	MD284	MD285	MD286	MD287	MD288	MD289	MD290	MD291	MD292	MD293	MD294	MD295	MD296	MD297	MD298	MD299	MD300	MD301	MD302	MD303	MD304	MD305	MD306	MD307	MD308	MD309	MD310	MD311	MD312	MD313	MD314	MD315	MD316	MD317	MD318	MD319	MD320	MD321	MD322	MD323	MD324	MD325	MD326	MD327	MD328	MD329	MD330	MD331	MD332	MD333	MD334	MD335	MD336	MD337	MD338	MD339	MD340	MD341	MD342	MD343	MD344	MD345	MD346	MD347	MD348	MD349	MD350	MD351	MD352	MD353	MD354	MD355	MD356	MD357	MD358	MD359	MD360	MD361	MD362	MD363	MD364	MD365	MD366	MD367	MD368	MD369	MD370	MD371	MD372	MD373	MD374	MD375	MD376	MD377	MD378	MD379	MD380	MD381	MD382	MD383	MD384	MD385	MD386	MD387	MD388	MD389	MD390	MD391	MD392	MD393	MD394	MD395	MD396	MD397	MD398	MD399	MD400	MD401	MD402	MD403	MD404	MD405	MD406	MD407	MD408	MD409	MD410	MD411	MD412	MD413	MD414	MD415	MD416	MD417	MD418	MD419	MD420	MD421	MD422	MD423	MD424	MD425	MD426	MD427	MD428	MD429	MD430	MD431	MD432	MD433	MD434	MD435	MD436	MD437	MD438	MD439	MD440	MD441	MD442	MD443	MD444	MD445	MD446	MD447	MD448	MD449	MD450	MD451	MD452	MD453	MD454	MD455	MD456	MD457	MD458	MD459	MD460	MD461	MD462	MD463	MD464	MD465	MD466	MD467	MD468	MD469	MD470	MD471	MD472	MD473	MD474	MD475	MD476	MD477	MD478	MD479	MD480	MD481	MD482	MD483	MD484	MD485	MD486	MD487	MD488	MD489	MD490	MD491	MD492	MD493	MD494	MD495	MD496	MD497	MD498	MD499	MD500	MD501	MD502	MD503	MD504	MD505	MD506	MD507	MD508	MD509	MD510	MD511	MD512	MD513	MD514	MD515	MD516	MD517	MD518	MD519	MD520	MD521	MD522	MD523	MD524	MD525	MD526	MD527	MD528	MD529	MD530	MD531	MD532	MD533	MD534	MD535	MD536	MD537	MD538	MD539	MD540	MD541	MD542	MD543	MD544	MD545	MD546	MD547	MD548	MD549	MD550	MD551	MD552	MD553	MD554	MD555	MD556	MD557	MD558	MD559	MD560	MD561	MD562	MD563	MD564	MD565	MD566	MD567	MD568	MD569	MD570	MD571	MD572	MD573	MD574	MD575	MD576	MD577	MD578	MD579	MD580	MD581	MD582	MD583	MD584	MD585	MD586	MD587	MD588	MD589	MD590	MD591	MD592	MD593	MD594	MD595	MD596	MD597	MD598	MD599	MD600	MD601	MD602	MD603	MD604	MD605	MD606	MD607	MD608	MD609	MD610	MD611	MD612	MD613	MD614	MD615	MD616	MD617	MD618	MD619	MD620	MD621	MD622	MD623	MD624	MD625	MD626	MD627	MD628	MD629	MD630	MD631	MD632	MD633	MD634	MD635	MD636	MD637	MD638	MD639	MD640	MD641	MD642	MD643	MD644	MD645	MD646	MD647	MD648	MD649	MD650	MD651	MD652	MD653	MD654	MD655	MD656	MD657	MD658	MD659	MD660	MD661	MD662	MD663	MD664	MD665	MD666	MD667	MD668	MD669	MD670	MD671	MD672	MD673	MD674	MD675	MD676	MD677	MD678	MD679	MD680	MD681	MD682	MD683	MD684	MD685	MD686	MD687	MD688	MD689	MD690	MD691	MD692	MD693	MD694	MD695	MD696	MD697	MD698	MD699	MD700	MD701	MD702	MD703	MD704	MD705	MD706	MD707	MD708	MD709	MD710	MD711	MD712	MD713	MD714	MD715	MD716	MD717	MD718	MD719	MD720	MD721	MD722	MD723	MD724	MD725	MD726	MD727	MD728	MD729	MD730	MD731	MD732	MD733	MD734	MD735	MD736	MD737	MD738	MD739	MD740	MD741	MD742	MD743	MD744	MD745	MD746	MD747	MD748	MD749	MD750	MD751	MD752	MD753	MD754	MD755	MD756	MD757	MD758	MD759	MD760	MD761	MD762	MD763	MD764	MD765	MD766	MD767	MD768	MD769	MD770	MD771	MD772	MD773	MD774	MD775	MD776	MD777	MD778	MD779	MD780	MD781	MD782	MD783	MD784	MD785	MD786	MD787	MD788	MD789	MD790	MD791	MD792	MD793	MD794	MD795	MD796	MD797	MD798	MD799	MD800	MD801	MD802	MD803	MD804	MD805	MD806	MD807	MD808	MD809	MD810	MD811	MD812	MD813	MD814	MD815	MD816	MD817	MD818	MD819	MD820	MD821	MD822	MD823	MD824	MD825	MD826	MD827	MD828	MD829	MD830	MD831	MD832	MD833	MD834	MD835	MD836	MD837	MD838	MD839	MD840	MD841	MD842	MD843	MD844	MD845	MD846	MD847	MD848	MD849	MD850	MD851	MD852	MD853	MD854	MD855	MD856	MD857	MD858	MD859	MD860	MD861	MD862	MD863	MD864	MD865	MD866	MD867	MD868	MD869	MD870	MD871	MD872	MD873	MD874	MD875	MD876	MD877	MD878	MD879	MD880	MD881	MD882	MD883	MD884	MD885	MD886	MD887	MD888	MD889	MD890	MD891	MD892	MD893	MD894	MD895	MD896	MD897	MD898	MD899	MD900	MD901	MD902	MD903	MD904	MD905	MD906	MD907	MD908	MD909	MD910	MD911	MD912	MD913	MD914	MD915	MD916	MD917	MD918	MD919	MD920	MD921	MD922	MD923	MD924	MD925	MD926	MD927	MD928	MD929	MD930	MD931	MD932	MD933	MD934	MD935	MD936	MD937	MD938	MD939	MD940	MD941	MD942	MD943	MD944	MD945	MD946	MD947	MD948	MD949	MD950	MD951	MD952	MD953	MD954	MD955	MD956	MD957	MD958	MD959	MD960	MD961	MD962	MD963	MD964	MD965	MD966	MD967	MD968	MD969	MD970	MD971	MD972	MD973	MD974	MD975	MD976	MD977	MD978	MD979	MD980	MD981	MD982	MD983	MD984	MD985	MD986	MD987	MD988	MD989	MD990	MD991	MD992	MD993	MD994	MD995	MD996	MD997	MD998	MD999	MD1000	MD1001	MD1002	MD1003	MD1004	MD1005	MD1006	MD1007	MD1008	MD1009	MD1010	MD1011	MD1012	MD1013	MD1014	MD1015	MD1016	MD1017	MD1018	MD1019	MD1020	MD1021	MD1022	MD1023	MD1024	MD1025	MD1026	MD1027	MD1028	MD1029	MD1030	MD1031	MD1032	MD1033	MD1034	MD1035	MD1036	MD1037	MD1038	MD1039	MD1040	MD1041	MD1042	MD1043	MD1044	MD1045	MD1046	MD1047	MD1048	MD1049	MD1050	MD1051	MD1052	MD1053	MD1054	MD1055	MD1056	MD1057	MD1058	MD1059	MD1060	MD1061	MD1062	MD1063	MD1064	MD1065	MD1066	MD1067	MD1068	MD1069	MD1070	MD1071	MD1072	MD1073	MD1074	MD1075	MD1076	MD1077	MD1078	MD1079	MD1080	MD1081	MD1082	MD1083	MD1084	MD1085	MD1086	MD1087	MD1088	MD1089	MD1090	MD1091	MD1092	MD1093	MD1094	MD1095	MD1096	MD1097	MD1098	MD1099	MD1100	MD1101	MD1102	MD1103	MD1104	MD1105	MD1106	MD1107	MD1108	MD1109	MD1110	MD1111	MD1112	MD1113	MD1114	MD1115	MD1116	MD1117	MD1118	MD1119	MD1120	MD1121	MD1122	MD1123	MD1124	MD1125	MD1126	MD1127	MD1128	MD1129	MD1130	MD1131	MD1132	MD1133	MD1134	MD1135	MD1136	MD1137	MD1138	MD1139	MD1140	MD1141	MD1142	MD1143	MD1144	MD1145	MD1146	MD1147	MD1148	MD1149	MD1150	MD1151	MD1152	MD1153	MD1154	MD1155	MD1156	MD1157	MD1158	MD1159	MD1160	MD1161	MD1162	MD1163	MD1164	MD1165	MD1166	MD1167	MD1168	MD1169	MD1170	MD1171	MD1172	MD1173	MD1174	MD1175	MD1176	MD1177	MD1178	MD1179	MD1180	MD1181	MD1182	MD1183	MD1184	MD1185	MD1186	MD1187	MD1188	MD1189	MD1190	MD1191	MD1192	MD1193	MD1194	MD1195	MD1196	MD1197	MD1198	MD1199	MD1200	MD1201	MD1202	MD1203	MD1204	MD1205	MD1206	MD1207	MD1208	MD1209	MD1210	MD1211	MD1212	MD1213	MD1214	MD1215	MD1216	MD1217	MD1218	MD1219	MD1220	MD1221	MD1222	MD1223	MD1224	MD1225	MD1226	MD1227	MD1228	MD1229	MD1230	MD1231	MD1232	MD1233	MD1234	MD1235	MD1236	MD1237	MD1238	MD1239	MD1240	MD1241	MD1242	MD1243	MD1244	MD1245	MD1246	MD1247	MD1248	MD1249	MD1250	MD1251	MD1252	MD1253	MD1254	MD1255	MD1256	MD1257	MD1258	MD1259	MD1260	MD1261	MD1262	MD1263	MD1264	MD1265	MD1266	MD1267	MD1268	MD1269	MD1270	MD1271	MD1272	MD1273	MD1274	MD1275	MD1276	MD1277	MD1278	MD1279	MD1280	MD1281	MD1282	MD1283	MD1284	MD1285	MD1286	MD1287	MD1288	MD1289	MD1290	MD1291	MD1292	MD1293	MD1294	MD1295	MD1296	MD1297	MD1298	MD1299	MD1300	MD1301	MD1302	MD1303	MD1304	MD1305	MD1306	MD1307	MD1308	MD1309	MD1310	MD1311	MD1312	MD1313	MD1314	MD1315	MD1316	MD1317	MD1318	MD1319	MD1320	MD1321	MD1322	MD1323	MD1324	MD1325	MD1326	MD1327	MD1328	MD1329	MD1330	MD1331	MD1332	MD1333	MD1334	MD1335	
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Supplementary Table 16: Materials for Western Blotting and Gene Expression Studies

Primary Antibodies

Target	Application	Supplier	Catalog #
SDMA	Western, ELISA, Immunofluorescence	Cell Signaling Technology	13222S
SmD3	ELISA, Immunofluorescence	Abgent	AP12451A
MDM4	Western	Abcam	ab49993
p53	Western	Santa Cruz Biotechnology	sc-126
p21	Western	Sigma	P1484
p21	Western	Cell Signaling Technology	2946
H3	Western	Cell Signaling Technology	9715
Tubulin	Western	Sigma	T9026
Actin	Western	Thermo	PA1-46296
p53	Immunohistochemistry	Thermo	MA5-16387

Gene Expression Reagents

Target	Application	Supplier	Catalog #
HDMX, forward	PCR	Biosearch	SS294168-01
HDMX, reverse	PCR	Biosearch	SS294169-01
GAPDH	PCR	Qiagen	PPH00150F
CCL4	Taqman Assay	Thermo	Hs00605740_g1
CCL3	Taqman Assay	Thermo	Hs04194942_s1
GADD45A	Taqman Assay	Thermo	Hs00169255_m1
SESN1	Taqman Assay	Thermo	Hs00902787_m1
ACTA2	Taqman Assay	Thermo	Hs00426835_g1
MDM2	Taqman Assay	Thermo	Hs01066930_m1
PHLDA3	Taqman Assay	Thermo	Hs00385313_m1
TRIM22	Taqman Assay	Thermo	Hs01001179_m1
EGR2	Taqman Assay	Thermo	Hs00166165_m1
CDKN1A	Taqman Assay	Thermo	Hs00355782_m1
BAX	Taqman Assay	Thermo	Hs00180269_m1
Actin	Taqman Assay	Thermo	401846
GAPDH	Taqman Assay	Thermo	402869

Supplemental Materials and Methods

Compounds

Two PRMT5 inhibitors, GSK3203591 (tool compound, MW = 380.483) and GSK3326595 (clinical compound, MW = 452.549), were synthesized by GSK Medicinal Chemistry. These compounds are used interchangeably due to their similar profiles.

Biochemistry

Biochemical Studies

PRMT5/MEP50 was purified as described previously (1). In summary, Flag-PRMT5 (2-end) was coexpressed with his-MEP50 (2-end) in a baculovirus expression system and purified via NiNTA affinity. Flag-hu-PRMT9-2-845 (NP_612373) prep 3 was expressed in a baculovirus expression system and purified via anti-flag M2 chromatography and Superdex 75 gel filtration chromatography. The following reagents and consumables were purchased from commercial vendors: (1) [3H]-SAM, Adenosyl-L-Methionine, S-[methyl-3H]-, 1mCi (37MBq) PerkinElmer NET155H001MC, (2) SAM, Adenosyl-L-Methionine, New England BioLabs #B9003S, (3) Histone H4 1-21 peptide (SGRGKGGKGLGKGGAKRHRKV) AnaSpec, Inc. USA. Catalog #62499, (4) Histone H2A 1-20 peptide (SGRGKQGGKARAKAKTRSSRGG-K(Biotin)) AnaSpec, Inc. USA. Catalog #64842-1, (5) 96 well reaction plate, Costar #3884, (6) MSPH filter plate, Millipore MSPHNXB50, (7) MicroScint-20, PerkinElmer #6013621, (8) Arginine Binding Ysi SPA beads, PerkinElmer RPNQ0101. The following peptide substrates were custom ordered from 21st Century Biochemicals, USA.: (1) FUBP1 349-369 peptide (NPGGPGPGGRGRGRGQGNWNM-amide), (2) HNRNPH1 211-231 peptide (DRPGAGRGYNSIGRGAGFERM-amide), (3) SmD3 88-106 peptide (MKNKNQGSAGRGKAAILKAQ-amide), and (4) SAP145 496-516 peptide (NSVPVPRHWCFKRKYLQGKRG –amide). All other reagents were of commercial grade.

Data analysis converted cpm output to % inhibition. IC₅₀ values were determined using GraFit software by fitting the experimental % inhibition data to a 3-parameter model, where the upper limit was fixed at 100. To determine K^{app} , IC₅₀ data was fit to the Cheng-Prusoff equation for competitive or uncompetitive inhibition.

Formula to yield % inhibition:

$$\% \text{ inhibition} = \left(1 - \frac{cpm_{compd} - cpm_{min}}{cpm_{max} - cpm_{min}}\right) \times 100$$

Where cpm = counts per minute, $compd$ = signal in treated well, max = the positive signal control and min = the negative signal control

Formula to yield IC₅₀ values:

$$\% \text{ inhibition} = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{(1 + (IC_{50}/[I])^n)}$$

Where Top = inhibited value fixed to 100%, $Bottom$ = uninhibited value, $[I]$ = concentration of inhibitor, IC_{50} = half maximal inhibitory concentration and n = Hill Slope. The equation assumes that y (% inhibition) increases with increasing x ($[I]$).

Formula for Cheng-Prusoff:

$$\text{If Competitive; } K_i^{app} = \frac{IC_{50}}{1 + ([S]/K_m^{app})}$$

$$\text{If Uncompetitive; } K_i^{app} = \frac{IC_{50}}{1 + (K_m^{app}/[S])}$$

Where K_i^{app} is the binding affinity of the inhibitor, IC_{50} is half maximal inhibitory concentration, $[S]$ is the substrate concentration and K_m^{app} is the concentration of the substrate at which the enzyme activity is half maximal

Mode of Inhibition Studies

GSK3326595 (typically 11-point, 3-fold serial dilution spanning 0.09-5102 nM) was prestamped at 500 nL per well in the reaction plate. Titrating concentrations of SAM comprising of a mixture of ^3H -SAM and unlabeled SAM including a constant concentration of peptide (12 μL per well, at 2X in assay buffer) was added to the compound plate. The product inhibitor SAH (24 μL per well of 1 mM stock) was added to select wells for use as the minimum signal control. Enzyme mix (12 μL per well, 2X in assay buffer) was added to initiate the reaction. Final assay conditions consisted of 6 nM PRMT5/MEP50, 40 nM peptide, 50-8000 nM SAM in 50 mM Tris (pH 8.5), 0.002% Tween-20, 4 mM MgCl_2 and 1 mM DTT. Reactions were incubated for 30 min at room temperature and quenched with 24 μL per well of 1 mM SAH. Arginine Binding Ysi SPA beads at 3 mg/mL in 0.2M NH_4CO_3 (24 μL per well) were added, plates were sealed and equilibrated for ≥ 30 min, centrifuged and then read on a MicroBeta (PerkinElmer) following a ≥ 200 min delay to measure the amount of tritium incorporated into the peptide substrate, reported as counts per minute (CPM). The peptide substrate (positive charge) is captured on the bead (negative charge) through charge/charge interaction. Once in proximity to the radiolabel, the bead scintillant is stimulated and emits light. Above methods were used for the peptide mode of inhibition studies expect final assay conditions were 6 nM PRMT5/MEP50, 1.6-1000 nM peptide, 1000 nM SAM (250 nM ^3H -SAM and 750 nM unlabeled SAM) in 50 mM Tris (pH 8.5), 0.002% Tween-20, 4 mM MgCl_2 and 1 mM DTT.

Analysis of Slow Binding Inhibition

GSK3326595 (typically 11-point, 3-fold serial dilution spanning 0.09-5102 nM) was prestamped at 500 nL per well in the reaction plate. SAM comprising of a mixture of ^3H -SAM and unlabeled SAM (6 μL per well, 4X in assay buffer) followed by

PRMT5/MEP50 (12 μ L per well, 2X in assay buffer) were added to the compound plate and incubated for 3-60 min. The product inhibitor SAH (24 μ L per well of 1 mM stock) was added to select wells for use as the minimum signal control. Peptide mix (6 μ L per well, 4X in assay buffer) was added to initiate the reaction. For 0 min preincubation, SAM and peptide were added to the compound plate followed by enzyme addition to initiate the reaction. Final assay conditions consisted of 0.8 or 4 nM PRMT5/MEP50, 50 nM peptide (K_m^{app}), 1000 nM SAM (260 nM 3 H-SAM and 740 nM unlabeled SAM, K_m^{app}) in 50 mM Tris (pH 8.5), 0.002% Tween-20, 4 mM MgCl₂ and 1 mM DTT. Reactions were incubated for 30 min at room temperature and quenched with 24 μ L per well of 1 mM SAH. Arginine Binding Ysi SPA beads were added to the plate and processed as listed above.

IC₅₀ determinations with Enzyme: SAM: Inhibitor preincubation

GSK3326595 (typically 11-point, 3-fold serial dilution spanning 0.09-5102 nM) was prestamped at 500 nL per well in the reaction plate. SAM comprising of a mixture of 3 H-SAM and unlabeled SAM (6 μ L per well, 4X in assay buffer) followed by PRMT5/MEP50 (12 μ L per well, 2X in assay buffer) were added to the compound plate and incubated for 60 min. The product inhibitor SAH (24 μ L per well of 1 mM stock) was added to select wells for use as the minimum signal control. Peptide mix (6 μ L per well, 4X in assay buffer) was added to initiate the reaction. Reactions were incubated for 30 min at room temperature and quenched with 24 μ L per well of 1 mM SAH. Arginine Binding Ysi SPA beads were added to the plate and processed as listed above. Final assay conditions consisted of 2-6 nM PRMT5/MEP50, 1000 nM SAM (260 nM 3 H-SAM and 740 nM unlabeled SAM, K_m^{app}) in 50 mM Tris (pH 8.5), 0.002% Tween-20, 4 mM MgCl₂ and 1 mM DTT plus peptide. Assay was substituted with desired peptide substrate tested at respective K_m^{app} value, H4 1-21 (50 nM), H2A 1-20 (150 nM), SmD3 88-106 (230 nM), FUBPH1 349-369 (230 nM) or HNRNPH1 211-231 (230 nM).

Above methods were used for the PRMT9 inhibition studies expect final assay conditions were 3 nM PRMT9, 150 nM SAP145 peptide (K_m^{app}), 3000 nM SAM (510 nM 3 H-SAM and 3000 nM unlabeled SAM, K_m^{app}) in 25 mM Tris (pH 8), 0.002% Tween-20, 4 mM MgCl₂, 100 mM NaCl and 1 mM DTT. Following quench, a 30 μ L volume of the reaction was transferred to a premoistened MSPH filter plate followed by the addition of 150 μ L of 0.2M NH₄HCO₃. The peptide substrate (positive charge) is captured on the filter plate (negative charge) through charge/charge interaction. After an incubation time of 15 min, the plate was washed 4 times with 150 μ L per well of 0.2M NH₄HCO₃ and dried in an oven at 50°C. MicroScint-20 (35 μ L per well) was added, plates were sealed and equilibrated for \geq 30 min and then read on a TopCount (PerkinElmer) to measure the amount of tritium incorporated into the peptide substrate, reported as counts per minute (CPM).

Methyltransferase cross-screening panel

Cross-screening against a panel of 19 protein methyltransferase enzymes (shown in Figure 1D) was done according to general procedures as previously described (2).

Final assay conditions varied slightly depending on the enzyme tested. In summary, GSK3326595 (spanning 2-40,000 nM) was incubated for 30 min with enzyme in assay buffer. Substrate mix comprising of assay buffer with either nucleosome or biotin labeled peptide (10-330 nM) and between 8-2500 nM SAM (mixture of ³H-SAM and unlabeled) was added to initiate the reaction. The final substrate concentration was fixed at the K_m^{app} for each substrate (SAM, peptide or nucleosome) and was specific to the enzyme tested. Reactions were incubated for between 90-120 min at room temperature and quenched with unlabeled SAM. The minimum signal control contained the product inhibitor SAH (50-2000 μ M). After an incubation time of 1 hour to overnight, plates were washed with 0.1% Tween-20. Incorporation of radioactivity into substrate was measured by capturing substrate on a PerkinElmer Flashplate, and detecting on a PerkinElmer TopCount.

MethylScan[®]

MethylScan technology (Cell Signaling Technology) was used to evaluate the cellular targets of PRMT5 in the Z-138 cell line. Methylated peptide was immuno-affinity purified (IAP) with a symmetric dimethyl arginine antibody (Cell Signaling, # 13222S clone D2C3D6). Tandem mass spectrometry (LC-MS/MS) was used to analyze enriched symmetrically dimethylated peptides for qualitative sequence and site identification. Finally, quantitative analysis of methylated peptide fold-change allowed for comparison between DMSO and GSK3203591-treated samples. More negative fold change values correspond to the greatest effect.

Cellular proliferation assays

6-day, 384-well single agent proliferation assays (GSK)

Cells were plated at an optimized seeding density in 384-well format using the Hamilton STARlet automated liquid handling platform and were incubated for 24 hours. On assay Day 0, cells were treated in duplicate with a 20-point, two-fold dilution series of GSK3203591 or GSK3326595 ($\leq 29\mu$ M top dose) and $\leq 0.15\%$ DMSO, and incubated for 6 days. Cell growth was evaluated on Day 0 and Day 6 by measuring luminescence signal generated by addition of CellTiter-Glo reagent (Promega G8462).

Data were fit with a sigmoidal 4-point, 4 parameter, one-site dose response model using Assay Client software, where the following equation was used to generate a concentration response curve:

$$y (fit) = A + \frac{(B - A)}{1 + (C/x)^D}$$

Individual curves were QC checked for outlier data points, cell growth (population doubling ≥ 1.5), and proper curve fitting. Growth IC50 (gIC50) values correspond to the concentration intersecting the mid-point of the growth window (between DMSO and T0 values) and represent the concentration of compound required to inhibit the total cell growth observed during the assay by half. Growth IC100 (gIC100) values correspond to

the concentration at which 100% growth inhibition is achieved, based on the growth window. Net cell growth/death values were calculated by subtracting the T0 value (100%) from the Ymin value on the curve, and are a measure of net population cell growth or death. This value reflects the difference between the number of cells seeded at Day 0 (100%) and the number of cells remaining at the endpoint of the assay, such that negative values correspond to net cell death while positive values correspond to net cell growth. dEC50 values correspond to the concentration at which 50% net cell death is observed.

10-day, 384-well single agent proliferation assays (Eurofins panel)

Cells were grown in RPMI-1640, 10% FBS, 2 mM L-alanyl-L-Glutamine, 1mM Na Pyruvate or a special medium in a humidified atmosphere of 5% CO₂ at 37°C. Cells were seeded into 384-well plates and incubated in a humidified atmosphere of 5% CO₂ at 37°C. Compounds were added 24 hours post cell seeding. At the same time, a time zero untreated cell plate was generated. At 168 hours post cell seeding, the cell plates were briefly spun, and media was removed and replaced with fresh growth media. The plates were then re-dosed with the test compound. After a 240 hour incubation period, cells were fixed and stained with fluorescent nuclear dye to allow visualization of nuclei. Compounds were serially diluted 3.16-fold and assayed over 10 concentrations in a final assay concentration of 0.1% DMSO from the highest test concentration specified in the sample information chapter. Automated fluorescence microscopy was carried out using a Molecular Devices ImageXpress Micro XL high-content imager, and images were collected with a 4X objective.

16-bit TIFF images were acquired and analyzed with MetaXpress 5.1.0.41 software. EC50 and IC50 values were calculated using nonlinear regression to fit data to a sigmoidal 4-point, 4 parameter, one-site dose response model, where:

$$y (fit) = A + \frac{(B - A)}{1 + (C/x)^D}$$

Curve-fitting, EC₅₀ / IC₅₀ calculations and report generation was performed using a custom data reduction engine and MathIQ based software (AIM). The cell proliferation assay uses a cell image based analysis technique where cells are fixed and stained with fluorescent nuclear dye to visualize nuclei.

Cell proliferation is measured by the signal intensity of the incorporated nuclear dye. The cell proliferation assay output is referred to as the relative cell count. To determine the cell proliferation end point, the cell proliferation data output is transformed to percent of control (POC) using the following formula:

$$POC = \left(\frac{\text{relative cell count (compound wells)}}{\text{relative cell count (vehicle wells)}} \right) * 100$$

Relative cell count IC_{50} is the test compound concentration at 50% of maximal possible response. A relative cell count EC_{50} is the test compound concentration at the curve inflection point or half the effective response (parameter C of the fitted curve solution). GI_{50} is the concentration needed to reduce the observed growth by half. This is the concentration that inhibits the growth midway between untreated cells and the number of cells seeded in the well (Time zero value), and will be reported in this manuscript as gIC_{50} for consistency with GSK data. Activity area is an estimate of the integrated area above the curve. Activity area values generally range from 0-10, where a value of zero indicates no inhibition of proliferation at all concentrations, and a value of 10 indicates complete inhibition of proliferation at all concentrations. In rare instances, values <0 or >10 may be observed. In these instances, values <0 should be considered as equivalent to 0, whereas values >10 should be considered equivalent to 10.

Time zero non-treated plate is used to determine number of doublings in 72 hour assay period: Number of doublings in 72 hours = $\text{Log}_2[\text{Cell number (72 hrs end point)} / \text{Cell number (time zero)}]$.

10-day, 96-well single agent proliferation assays (GSK)

10-day growth-death assays were also performed on SW48 (Sigma CLL1008-1VL) and SW48 p53^{-/-} (Sigma CLL1013-1VL) cell lines to evaluate the effect of p53 knockout on proliferation. An extended time course was used in these experiments because Western blotting for SDMA suggested effective target engagement would require longer than 6-days (data not shown). Optimal cell seeding was determined by monitoring proliferation over a range of seeding densities in 96-well format and identifying the seeding density at which cells grow logarithmically at 3, 6, and 10-day time points. The PerkinElmer Zephyr workstation was used to seed cells in 96-well plates at the optimal seeding density in 100 μ L of culture media supplemented with 10% FBS. Seven plates were prepared per cell line (duplicate plates for Day 0, 3, and 6 reads) and incubated overnight at 37°C in 5% CO₂. The following day, cells were treated in duplicate on the Zephyr workstation with a 20-point, two-fold dilution series of GSK3326595 (26 μ M top dose) and $\leq 0.15\%$ DMSO. Plates were incubated for the indicated number of days at the conditions described above. Cell growth was measured using CellTiter-Glo reagent (Promega G8462) and luminescence signal was detected using an Envision microplate reader. A plate of untreated cells was read at the time of compound addition to determine the T=0 value representing the starting number of cells. Data were analyzed using XLfit at Graphpad Prism software.

SDMA Analysis

SDMA ELISA

In vitro treated lymphoma cells and frozen tumors powder samples were resuspended in RIPA buffer containing protease/phosphatase inhibitor cocktail (Cell Signaling

#5872S). Tumor powder was homogenized with a hand-held polytron (IKA T10 basic Ultra Turrax) on ice, followed by sonication with a hand-held polytron (IKA T10 basic Ultra Turrax) at setting 6, on ice (20 seconds/twice). Cell lysates were spun at 12,000 rpm for 10 minutes at 4°C and cleared lysate was transferred to a new tube.

Cell lysates were diluted with PBS Carbonate-Bicarbonate buffer pH 9.6 (Sigma, #C3041) and added to black high binding plates (Nunc Maxisorp #437111). A titration (serial 2-fold dilution from 960 to 2 ng/well) of vehicle treated control lysate was used to evaluate linear range. Lysate was incubated for 2 hours at room temperature. Lysate was removed and plates were washed 4 times with 200uL wash solution (PBST, Cell Signaling, #9809S), followed by a 2 hour block step with PBST with 1% bovine serum albumen (BSA; Alfa Aesar, #J61089). Prior to addition of the primary antibody, the plates are washed as previously described.

The primary antibodies, SDMA (Cell Signaling, # 13222S clone D2C3D6, lot #2) and SMD3 (Abgent, #AP12451A), were diluted with 1% BSA buffer, 100uL added/well. Plates were incubated overnight with rocking at 4°C. Primary antibody was removed, plates were washed as previously described, and secondary HRP-conjugated anti-rabbit antibody (Cell Signaling Technology #7074) was added and incubated at room temperature for one hour. Following the final wash step, plates were tapped briefly on paper towels to remove any residual wash buffer and 100uL of Luminata Forte substrate (Millipore #WBKUF0500) was added to each well. Plates were incubated for 15 minutes with rocking and read on an Envision plate reader using a 96 well luminescence protocol. SDMA levels were normalized to SMD3 values and IC₅₀ values were determined using a 4 parameter curve fit.

SDMA Imaging Assay

The SDMA imaging assay was used to evaluate the potency of GSK3326595 in breast cancer cell lines. Cells were plated at 2,000 cells per well in an optical bottom 96-well cell culture plate (Nunc 1256670) and allowed to adhere overnight. Cells were treated for 72 hours with a 10-point 5-fold dilution series (dose range = 0.8 pM – 1500 nM), fixed with 4% formaldehyde (ThermoScientific 28908), permeabilized, and blocked with 10% goat serum (Gibco 16210) in immunofluorescence (IF) buffer (130mM NaCl, 7mM Na₂HPO₄, 3.5 mM NaH₂PO₄, 7.7mM NaN₃, 0.1% BSA, 0.2% Triton X, 0.05% Tween 20) prior to overnight incubation with antibodies specific for SDMA (Cell Signaling Technology # 13138BF, lot D2C3D6) and SMD3 (Abgent # AP12451A, lot 150508HF). Following washes with IF buffer, cells were further incubated with fluorescent secondary antibodies (Invitrogen Oregon Green Anti-rabbit #11038) and DAPI nuclear staining (Invitrogen #D357) prior to quantification of nuclear SDMA and SMD3 levels via Molecular Devices MetaExpress high-content fluorescent imager using a 10x objective to image a minimum of 4 sites per well. Image analysis was performed with ImageExpress software using Multiwavelength scoring to quantitate each fluorescent signal (FITC, and DAPI) in each well. SDMA levels were then normalized to SMD3 levels and IC₅₀, IC₉₀, EC₅₀ and EC₉₀ values were determined using a 4 parameter curve fit.

Cell Cycle and Cell Death Analysis

Cell cycle analysis

Cell cycle phase distribution was examined by flow cytometry of propidium iodide stained nuclei. Cells were treated with PRMT5 inhibitor or DMSO for the indicated period of time, depending on experiment. Pellets were processed using the BD Biosciences CycleTest kit according to the manufacturer's instructions and were evaluated using a FACSCalibur flow cytometer. FlowJo software was used to determine the cell cycle distribution.

Annexin V and 7AAD Staining

Early apoptosis and cell death were evaluated using Annexin V staining (APC Annexin V, BD Biosciences, #550475) and 7-AAD (BD Bioscience #559925) and performed as recommended by the manufacturer. Cells were treated with PRMT5 inhibitor for 3 or 6 days or Camptothecin, a positive control, for 12 hours. Compound treated cells were washed with cold PBS, and cell pellets were resuspended in 1X binding buffer (10X binding buffer, BD Bioscience #556454) to a concentration of a million cells per ml. Stain was added to 100 ul cells in duplicate, and incubated for 15 minutes at room temperature followed by further 5 fold dilution with 1 X binding buffer. Fluorescent events were collected on a FACSCalibur Becton Dickonson (BD Biosciences) and data were analyzed using FlowJo (Treestar, Ashland) to discriminate live cells (Annexin V (-) 7-AAD (-)) from apoptotic cells (AnnexinV (+)7-AAD (-)) from dead cells Annexin V (+/-) 7-AAD (+). Individual control samples unstained, and stained with each fluorochrome independently are used to establish spectral overlap. Compensation was calculated post data collection within the software.

RNA-Seq

RNA-Seq sample preparation and differential expression

Z-138, SU-DHL-6, JVM-2, SU-DHL-5, MAVER-1, MINO, SU-DHL-4, and REC-1 cell lines were treated with either DMSO (control) or 200 nM GSK3326595. Cells were collected for RNA-seq analysis post 3 and 6 days. Z-138, GRANTA-519, and DOHH-2 cell lines were treated with either DMSO (control) or 1000 nM GSK3203591. Cells were collected for RNA-seq analysis post 2 and 4 days.

RNA was extracted from cell pellets with Trizol using Qiagen's RNeasy Mini Kit. Libraries were prepared using Illumina TruSeq Stranded Total RNA with Ribo-Zero Gold Library Prep and sequenced on Illumina HiSeq2500 to 60-80 million PE 2 x 101bp. RNA-seq fastq files were aligned to human hg38 with the gencode GRCh38.v23 annotation using a 2-pass STAR-2.5.2b (3) and then ribosomal and mitochondrial alignments were removed. Differential gene expression was calculated using Cufflinks2 suite considering genes with q-value less than 0.05, and a minimum expression >1

FPKM, and a minimum 2-fold change in expression as significant (4). Individual gene expressions in FPKM values were taken from the normalized differential values from the cuffdiff output files.

Gene pathway analysis

Gene symbols were submitted for MsigDB comparing against the CP, CP:BIOCARTA, CP:KEGG, CP:REACTOME, and Hallmark gene sets (Broad), as well as using the R package clusterProfiler and ReactomePA (5,6), reporting only the most significant gene set.

Alternative splicing analysis

To calculate global splicing events, alignment files from STAR were used as input for rMATS and run using default parameters with the `-novelSS` flag to identify novel splicing events. Events were considered significant with $FDR < 0.01$, $SD < 0.2$, junction coverage > 10 for both the spliced in and excluded junctions, and $-0.2 < \text{IncLevelDifference} < 0.2$ for alternative splicing events from “ JunctionCountOnly” output files (6).

MDM4 splicing and p53 pathway analysis

MDM4 splicing and p53 pathway analysis

RNA isolation and RT-PCR for MDM4 splicing analysis

RNA isolated from cell pellets was converted to cDNA using ABI High Capacity Reverse Transcription Reagents (4274966). Reverse transcription reactions were incubated in a thermal cycler for 10 minutes at 25°C, 2 hours at 37°C, and 5 minutes at 85°C. Master mix was prepared using Thermo DreamTaq Master Mix (K1081), 20uM HDMX forward primer (Biosearch Technologies, catalog # SS294168-01), 20uM HDMX reverse primer (Biosearch Technologies, catalog #SS294169-01), and nuclease-free water. A control master mix was prepared similarly using GAPDH primers (Qiagen PPH00150F). PCR reactions were prepared by combining 10uL of 9-fold diluted cDNA with 40uL of master mix and were incubated on a thermal cycler as follows: 1 cycle of 95°C for 5 minutes; 26 cycles of 95°C for 40 seconds, 58°C for 30 seconds, and 72°C for 40 seconds; then 1 cycle of 72°C for 4 minutes. Reaction products were separated using agarose ethidium bromide gel electrophoresis and visualized using a Bio-Rad VersaDoc Model 5000 imaging system. Images were captured using Bio-Rad Quantity One software at various exposure times to optimize signal strength and avoid saturation. Images are shown using an inverted color scheme for easier visualization.

Western Blotting for p53 pathway analysis

Gel samples were prepared by combining whole cell lysates with NuPAGE LDS Sample Buffer (NP007), NuPAGE Sample Reducing Agent (NP009), and RIPA buffer (Sigma R0278). Samples were loaded onto NuPAGE Novex 4-12% Bis-Tris protein gels and run in MES SDS (NP002) buffer at 150V until bands were well resolved. Proteins were transferred onto nitrocellulose with an iBlot and probed membranes were developed

using a LiCOR Odyssey scanner. Blots were scanned at various exposures to optimize signal strength and avoid saturation. Antibodies against p53 (Santa Cruz sc-126), p21 (Sigma P1484 or Cell Signaling 2946), SDMA (Cell Signaling 13138BF, clone #D2C3D6), MDM4 (Abcam ab49993), H3 (Cell Signaling 9715), tubulin (Sigma T9026), and actin (Thermo PA1-46296) were used to probe protein expression levels in Western blotting experiments.

Sensitivity Correlations

A 10-point dose response growth assay was performed 10 days post GSK3203591 treatment in a panel of 240 cell lines representing various tumor types. Activity area was calculated for each cell line and used as the metric for sensitivity.

Whole exome-seq mutation data for 1000 cell lines was downloaded from the Genomics of Drug Sensitivity in Cancer (Sanger Institute's <http://www.cancerrxgene.org>) and used to calculate each gene's mutant versus wild type activity area using a Wilcoxon rank sum test from the 185 common cell lines. Waterfall plots of cell lines sorted by increasing activity area with mutant cell lines indicated in green were generated and p-value calculated using R.

CCLF microarray RMA normalized expression values across 1019 cell lines were used to correlate each gene's expression profile using a Pearson correlation with activity area in the 178 common cell lines. Genes were sorted by Pearson correlation and the sorted gene list submitted to GSEA pre-ranked analysis interrogating hallmark gene sets, canonical pathways, BioCarta, Kegg, and Reactome gene sets (Broad Institute). Increasing false discovery rates (FDR) are plotted against normalized enrichment scores of the gene sets. An example gene of the lowest p-value from each of "Reactome_mRNA_Splicing", "Reactome_nonsense_mediated_decay_by_the_exon_junction_complex", and "PID_p53_regulation_pathway" gene sets is plotted comparing cell lines sorted by activity area. Each of the CLNS1A, RIOK1, and the ratio of the two's gene expression, is plotted comparing cell lines sorted by activity area.

A heatmap of all genes within the Reactome_mRNA_splicing gene set (the lowest p-value) in the 20 most resistant and sensitive cell lines hierarchical clustered by euclidean distance.

MTAP gene level copy data was downloaded from the Genomics of Drug Sensitivity in Cancer for 1000 cell lines and the 185 cell lines in common had a Wilcoxon rank sum test performed to test the difference in the mean of sensitivities between MTAP homozygous deleted cell lines and those with at least one functional copy.

Lentiviruses

Lentiviruses were engineered to express MDM4-FL (generated in-house), EGFP (generated in-house), p53-targeting shRNA (Santa Cruz, sc-29435-V), or a scrambled non-targeting shRNA (Santa Cruz, sc-108080). Lentiviral particles were added at a multiplicity of transduction (MOT) of 20 to Z-138 cells that had been seeded into 6-well dishes, then polybrene was added to a final concentration of 50ug/mL. The dishes were centrifuged at 1,000xg for 90 minutes at 37°C. Cell pellets were collected, supernatant discarded, and pellets were resuspended in culture media. Each pellet was re-seeded into a fresh 6-well dish and then incubated at 37°C and 5% CO₂ for 48h. Puromycin selection was used to generate and maintain stable cell lines. An anti-MDM4 antibody (Abcam ab49993) was used to evaluate the success of the overexpression via Western blot. Proliferation assays were conducted in 384-well format at 3- and 6-day time points with these cell lines as described earlier.

Gene expression

Gene expression analysis in Z-138 cell line

Z-138 cells were obtained from ATCC, (CRL-3001) and maintained in RPMI-1640 supplemented with 10% horse serum. Cells were incubated at 37°C with 5% CO₂ and split once a week. Z-138 cells were treated with increasing doses of GSK3326595 in 96-well cell culture plates for 2 and 4 days, RNA was isolated using TurboCapture 96 Kit (Qiagen # 72251). Reverse transcription was performed with High Capacity Reverse Transcription reagents (ABI #4374966), followed by qPCR (ABI Taqman Fast Advanced Master mix, cat # 4444557) analysis on ABI ViiA7 real-time PCR machine using the Fast TaqMan Comparative Ct ($2^{-\Delta\Delta Ct}$) method (7). Taqman assays targeted the genes CCL4 (Hs00605740_g1), CCL3 (Hs04194942_s1), GADD45A (Hs00169255_m1), SESN1 (Hs00902787_m1), ACTA2 (Hs00426835_g1), MDM2 (Hs01066930_m1), PHLDA3 (Hs00385313_m1), TRIM22 (Hs01001179_m1), EGR2 (Hs00166165_m1), CDKN1A (Hs00355782_m1), BAX (Hs00180269_m1), actin (Thermo 401846), and GAPDH (Thermo 402869).

Xenograft studies

Z-138 efficacy and PD study

The Z-138 (human B Cell Non-Hodgkin's Lymphoma) cells from ATCC (ATCC No: CRL-3001, Lot No: 57634322) were maintained *in vitro* as a suspension culture in IMDM medium supplemented with 10% heat inactivated horse serum, 100 U/ml penicillin and 100 µg/ml streptomycin at 37 °C in atmosphere of 5% CO₂ in air. The tumor cells were routinely subcultured twice weekly with fresh medium. Cells growing in an exponential growth phase were harvested and counted for tumor inoculation. Each mouse was inoculated subcutaneously at the right flank with Z-138 tumor cells (5×10^6 cells/mouse, 50% Matrigel) in 0.2 ml mixture of base media and Matrigel (IMDM:

Matrigel = 100:100) for tumor development. The treatments were started when the tumor size reached 152.98 mm³ for the tumor efficacy study (day 14 post inoculation). Tumor bearing mice were block randomized into 13 groups and each group has 10 mice. Mice were dosed with either Vehicle [0.5% methylcellulose (Sigma #M0430, St. Louis, MO) or GSK3326595D in vehicle at 0.2, 0.5, 1.4, 4.2, 12.5, 25, 50, 100 mg/kg twice daily, and 50, 100, 200 mg/kg once daily. Twice daily doses were administered 12 hour between doses.

All the procedures related to animal handling, care and the treatment in this study were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai Chempartner following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). At the time of routine monitoring, the animals were checked for any effects of tumor growth and treatments on normal behavior such as mobility, food and water consumption (by looking only), and body weight gain/loss (body weights were measured twice weekly), corneaopacity, matted fur and any other abnormal effect. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset. Animals that were observed to be in a continuing deteriorating condition or their tumor size exceeding 3000 mm³ were euthanized prior to death or before reaching a comatose state.

Tumor size was measured twice weekly in two dimensions using a caliper, and the volume was expressed in mm³ using the formula: $V = 0.5 a \times b^2$ where a and b were the long and short diameters of the tumor, respectively. The tumor size was then used for calculations of TGI values. Tumor growth inhibition (TGI) was calculated according to the following equation:

$$TGI (\%) = \left(1 - \frac{(TV_{Treatment,Day 22} - TV_{Treatment,Day 0})}{(TV_{Control,Day 22} - TV_{Control,Day 0})} \right) * 100\%$$

Treatment groups with limited efficacy were euthanized on treatment day 22, and sampling was 2 hours post the last dose. Subsequently tumors were flash frozen and pulverized.

SDMA ELISA in Z-138 xenograft

SDMA inhibition in frozen tumor powder samples was analyzed by ELISA, as described earlier.

p53 IHC in Z-138 xenograft

Performed IHC detection by using HRP/DAB IHC detection kit (ABC kit, #ab64261) and following the manufacturer's protocol. Anti-p53 rabbit monoclonal, clone SP5 from Thermo Scientific # MA5-16387), used as primary antibody for IHC. Images were taken

with AxioVision software, and cell staining intensity was quantified using the Meta Imaging Series software from Molecular Devices.

Gene expression analysis in Z-138 xenograft

Z-138 xenograft tumors were treated with various doses of GSK3326595 QD (50, 100 and 200 mg/kg) and BID (3, 6, 12.5, 25, 50 and 100 mg/kg) and collected at 2 hours post last dose. Mouse tissue samples were resuspended in QIAzol (Qiagen #79306) lysis reagent and vortexed to completely homogenize lysate. Samples were pre-cleared by MaXtract High Density tubes (Qiagen #129056) and RNA was isolated by RNeasy Mini kit (Qiagen #74106). Then, RT-PCR was performed with High Capacity Reverse Transcription reagents (ABI #4374966), followed by qPCR (ABI Taqman Fast Advanced Master mix, cat # 4444557) analysis on ABI ViiA7 real-time PCR machine using the Fast TaqMan $\Delta\Delta C_t$ method. A panel of 11 GSK3326595-responsive genes (see “Gene Expression in Z-138 cell line” for gene list and catalog information) was analyzed and normalized to two housekeeping genes, GAPDH and ACTIN. The average fold change in the treatment group vs. the vehicle group as well as the number of genes with higher than 2-fold change in gene expression for each dose were calculated.

REC-1 efficacy study

We tested GSK3326595 anti-tumor activity in a xenograft model of REC-1, an MCL cell line that is not sensitive at 6 days of exposure to PRMT5 inhibition *in vitro*.

The study consisted of six groups (n = 10) of mice bearing subcutaneous REC-1 tumors on Day 1. Group 1 served as the primary control group and received vehicle 1 (0.5% methylcellulose), orally (p.o.) twice daily to the end of the study (bid to end), first day one dose. Group 2 was given GSK3326595 p.o., at 126.1 mg/kg (79.3% active compound), BID to end, first day one dose. Tumors were measured twice per week. The study endpoint was a mean tumor volume of 2000 mm³ in the control group or 25 days, whichever came first. The study reached the 2000 mm³ endpoint on Day 25. The control tumor growth in this study was in line with our historical data although tumors in this group reached endpoint with measurements taken by an alternate technician. For this reason TGI analysis was performed on Day 22.

Treatment outcome was based on percent tumor growth inhibition (%TGI), defined as the percent difference between Day 22 median tumor volumes (MTVs) of treated and control mice. MTV values for groups were compared with the non-parametric Mann-Whitney U test. The results were analyzed and were deemed statistically significant at $P \leq 0.05$. A treatment that produced at least 60% TGI was considered to have potential therapeutic activity. Animals were also monitored for partial regression (PR) and complete regression (CR) responses. Treatment tolerability was assessed by body Weight (BW) measurements and frequent observation for clinical signs of treatment-related (TR) side effects.

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