PRMT5-mediated histone arginine methylation antagonizes transcriptional repression by polycomb complex PRC2

Fan Liu[1,4], Ye Xu[2,4], Xiaoqing Lu[2,4], Pierre-Jacques Hamard[4], , Daniel L. Karl[3,4], Na Man[4], Adnan Kasim Mookhtiar[4], Concepcion Martinez[4], Izidore S. Lossos[2,4], Jun Sun[4] and Stephen D. Nimer[1,2,4] *

Figure S1: PRMT5 doesn't directly affect PRC2 activity. A). HA-tagged PRMT5 was overexpressed with or without MEP50 and purified by HA agarose beads from 293T cells. In vitro methylation assay was performed using either the purified HA-PRMT5/MEP50 (lane 3 to 6) or commercially available recombinant PRMT5/MEP50 complex (lane 8 and 9). Recombinant EZH2 or histone H2A protein was used as substrates. Left panel: fluorography of the methylated proteins. Right Panel: Coomassie blue staining of the proteins. **B).** In vitro methylation assay using recombinant PRMT5/MEP50 as methyltransferase and core PRC2 complex, including EZH2, EED and SUZ12 as substrates. Histone H3 was used as a positive control. Left panel: fluorography of the methylated proteins; Right panel: Coomassie blue staining of the proteins. **C).** Weston blot shows the protein level of EZH2, SUZ12 and EED in PRMT5 inhibitor-treated MV4-11 cells. **D)**. Immunoprecipitation was performed in DMSO or PRMT5 inhibitor-treated MV4-11 cells using normal rabbit IgG or anti-EZH2 antibody. Precipitated EZH2 and co-precipitated SUZ12 were shown here. **E).** MV4-11 cells were either untreated or treated with DMSO, PRMT5 inhibitor (at 1uM or 5 uM) or EZH2 inhibitor (1uM) for 4 days. The expression level of JARID2, UTX, RBBP4 was determined by Western blotting. The reduced level of cellular symmetric di-methylated arginine (SDR) was confirmed in PRMT5 inhibitor-treated cells. **F).** EZH2 complex was immunoprecipitated from untreated MV4-11 cells, or MV4-11 cells treated with DMSO or PRMT5 inhibitor (0.5 uM or 1 uM), by antibody specific for EZH2. Purified EZH2 complex was subject to in vitro methylation assays with recombinant histone H3 as substrate. The recombinant EZH2 complex was used as a positive control in the last lane.

Figure S2: PRMT5-mediated H3 arginine methylation impairs the deposition of methylation on H3K37 by PRC2. A). Recombinant histone H3.2 and H3.3 proteins were first methylated by PRMT5/MEP50 complex for 2 hours, and then recombinant active EZH2 complex was added to the reaction for another 2 hours. Methylation of H3R8 and H3K27 was determined by antibodies specific for H3R8me2s and H3K27me1, respectively. Level of EZH2 in the reaction was determined by Weston blot, while levels of PRMT5 and H3 were determined by staining the membrane with Coomassie blue. **B).** In vitro methylation showing the auto-methylation of EZH2 in the presence or absence of PRMT5/MEP50 complex. **C).** Dot blot confirmed the methylation status of the H3 peptides. **D).** Biotinylated recombinant H3 was first methylated by PRMT5/MEP50 complex for 4 hours in 30ul of methylation buffer. Reaction was then diluted to 1 ml and recombinant PRC2 containing EZH2, EED and SUZ12 was added. After 1 hour of incubation, magnetics

streptavidin beads were used to pull-down H3 and H3 associated proteins, beads were then washed with buffers containing increased concentration of salt.

Figure S3: PRMT5-mediated histone arginine methylation antagonizes the transcriptional repression by PRC2. A). Confirmation of RNA-seq result by Real-time PCR. Three representative gene expression profiles (SPRY1, STC2 and CHAC1) are shown here. Left: Representative RNA-seq tracks. Right: Real-time PCR using Taqman Gene Expression Assays, expression of the indicated genes was normalized to that of HPRT1. n=4. **B)**. Heat map shows all H3K27me3 peaks in DMSO-, EZH2i- and PRMT5i-treated Molm13 cells. PC: peak center. **C)**. Heat map shows the H3K27me3 peaks at the TSS region in DMSO-, EZH2i- and PRMT5i-treated Molm13 cells.

Figure S4: Splicing defects in PRMT5 inhibited cells. A). Aberrant splicing events in PRMT5i-treated cells were shown in the bar graph here. SE: Skipping Exons; RI: Retained Introns; MXE: Mutually Exclusive Exons; A5SS: Alternative 5' splicing sites; A3SS: Alternative 3' splicing sites. **B).** Venn diagram shows the overlap between the differentially expressed genes and the aberrant spliced genes in PRMT5i-treated cells vs DMSO control cells.

Figure S5: PRMT5-mediated repression of H3K27 tri-methylation contributes to its cell cycle effects. Synergy maps for PRMT5i and EZH2i-treated leukemia cell lines and primary AML samples. In the synergy maps, numbers in X axis and Y axis are the concentrations of EZH2 inhibitor and PRMT5 inhibitor, respectively. Green area represents antagonizing effect at the given drug concentrations, and red area is the synergistic effect at the given drug concentrations.

Table S1: List of Top 20 differentially expressed genes in PRMT5i-treated, compared to DMSO-treated Molm13 cells.

Table S2: List of top 20 differentially expressed genes in EZH2i-treated, compared to DMSO-treated Molm13 cells.

Table S3: List of top 20 differentially expressed genes in PRMT5i and EZH2i double-treated, compared to DMSO-treated Molm13 cells.

Table S4: Information of the patient samples. 1: the percentage listed indicates the frequency of the detected mutant allele; 2: "yes" means the mutation is known to occur in cancers; 3: "UK" means the mutation has not been linked to cancer, i.e the pathogenic function of the mutant allele is unknown.

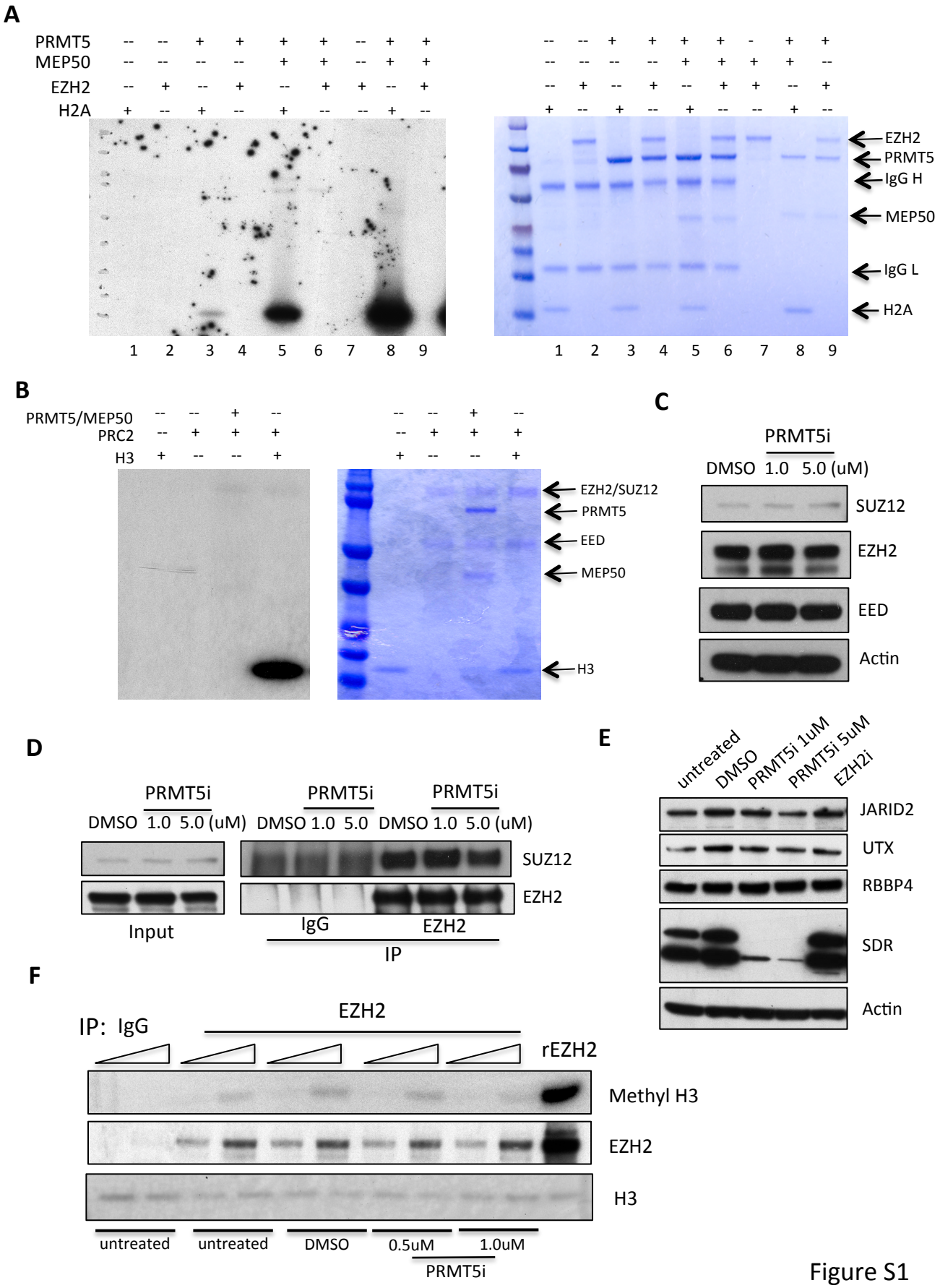

A
PRMT5
MEP50
EZH2
H2A
EZH2
PRMT5
IgG H
MEP50
IgG L
H2A
1 2 3 4 5 6 7 8 9
B
PRMT5/MEP50
PRC2
H3
EZH2/SUZ12
PRMT5
EED
MEP50
H3
C
PRMT5i
DMSO 1.0 5.0 (uM)
SUZ12
EZH2
EED
Actin
D
PRMT5i
DMSO 1.0 5.0 (uM)
SUZ12
EZH2
Input
IgG
EZH2
IP
E
untreated
DMSO
PRMT5i 1uM
PRMT5i 5uM
EZH2i
JARID2
UTX
RBBP4
SDR
Actin
F
IP: IgG
EZH2
rEZH2
Methyl H3
EZH2
H3
untreated
untreated
DMSO
0.5uM
1.0uM
PRMT5i

Figure S1

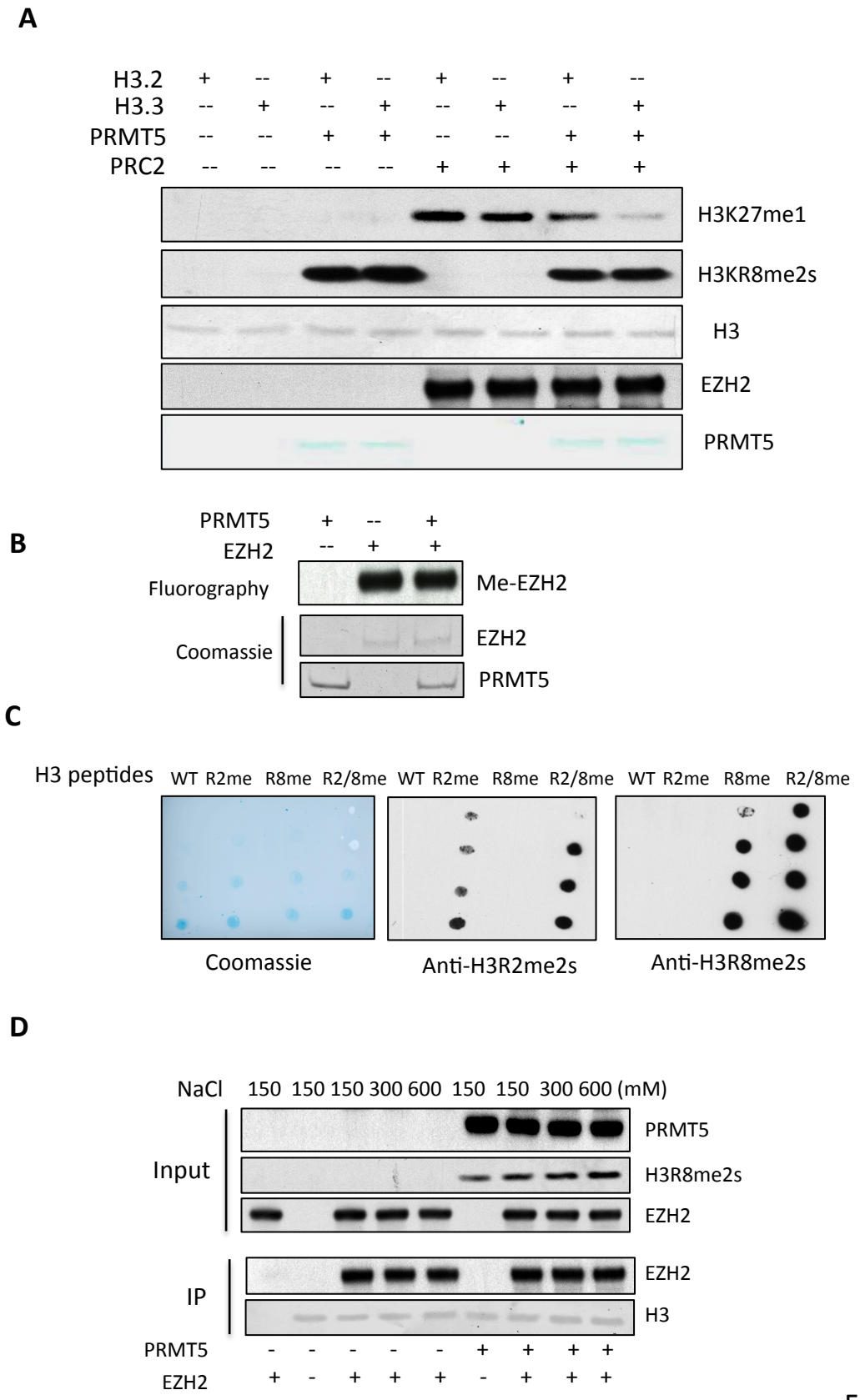

A
H3.2 + -- + -- + -- + --
H3.3 -- + -- + -- + -- +
PRMT5 -- -- + + -- -- + +
PRC2 -- -- -- -- + + + +
H3K27me1
H3KR8me2s
H3
EZH2
PRMT5
B
PRMT5 + -- +
EZH2 -- + +
Fluorography
Me-EZH2
Coomassie
EZH2
PRMT5
C
H3 peptides WT R2me R8me R2/8me WT R2me R8me R2/8me WT R2me R8me R2/8me
Coomassie
Anti-H3R2me2s
Anti-H3R8me2s
D
NaCl 150 150 150 300 600 150 150 300 600 (mM)
Input
PRMT5
H3R8me2s
EZH2
IP
EZH2
H3
PRMT5 - - - - - + + + +
EZH2 + - + + + - + + +

Figure S2

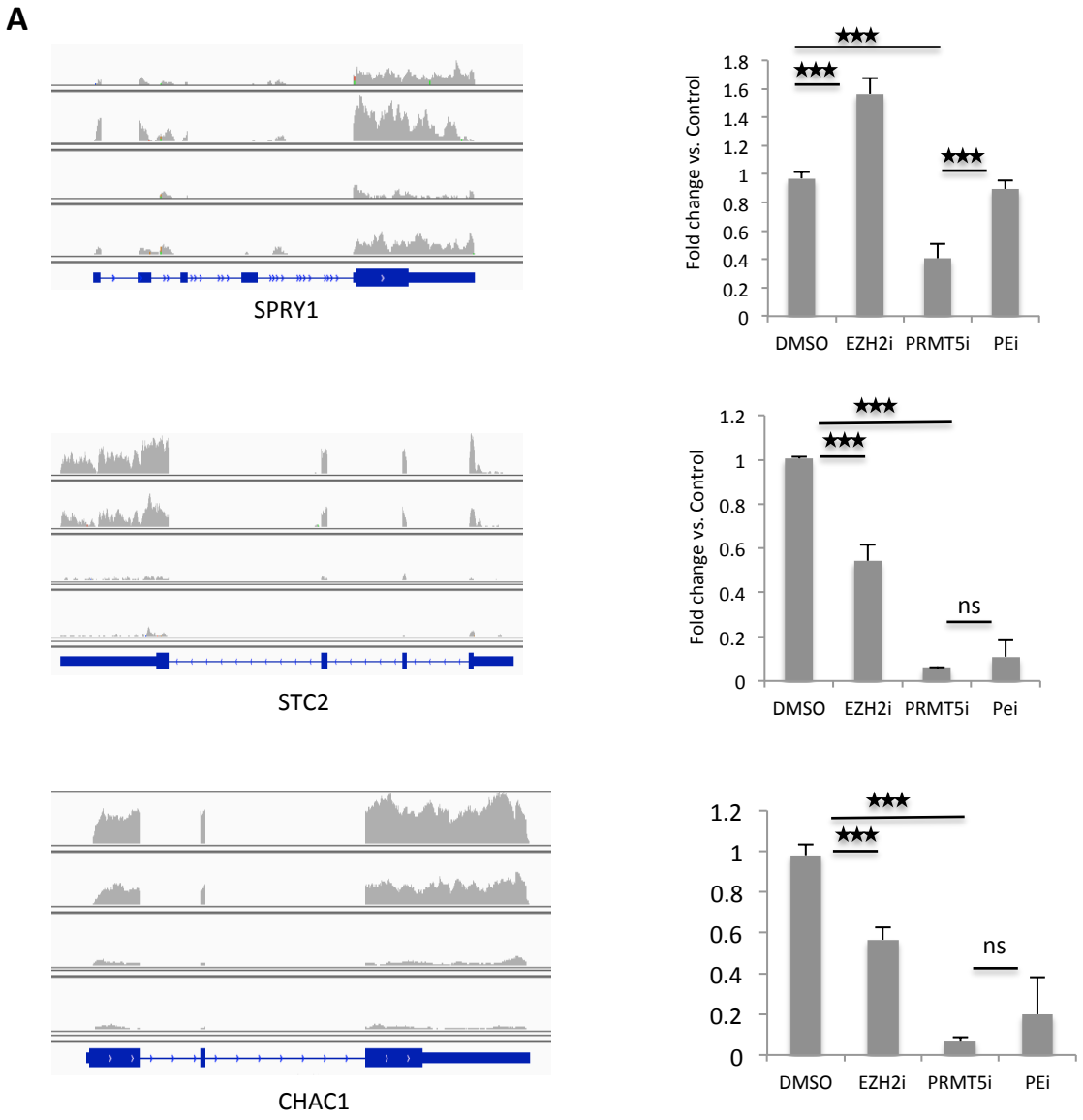
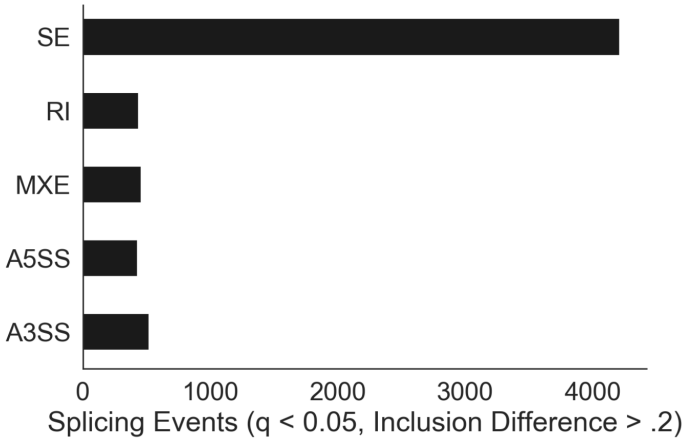

A
SPRY1
Fold change vs. Control
DMSO
EZH2i
PRMT5i
PEi
STC2
Pei
ns
CHAC1
B
All called broad peaks
-10KB
PC
10KB
C
Gencode TSS
TSS

Figure S3

A


SE
RI
MXE
A5SS
A3SS
0
1000
2000
3000
4000
Splicing Events (q < 0.05, Inclusion Difference > .2)

B

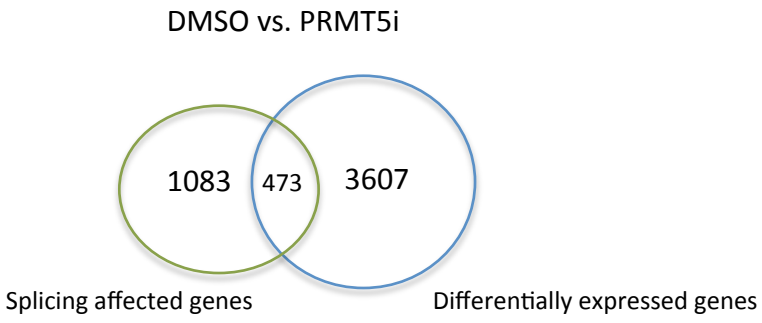
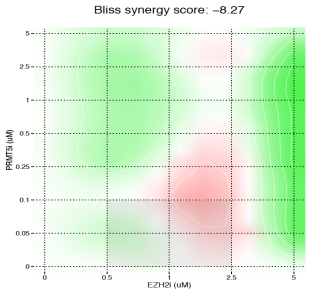
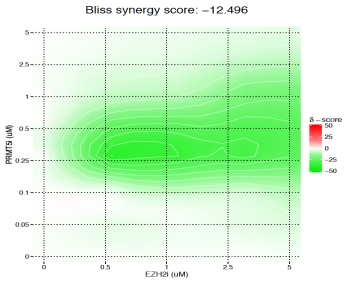

DMSO vs. PRMT5i
1083
473
3607
Splicing affected genes
Differentially expressed genes

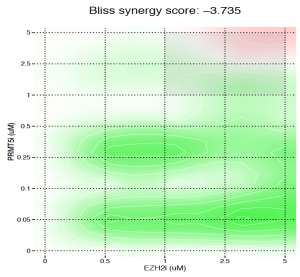
Figure S4



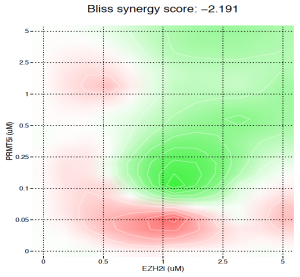
Nomo-1



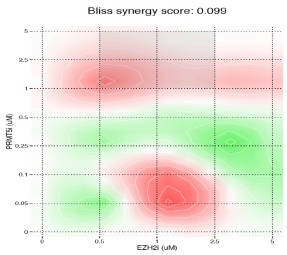
Molm13



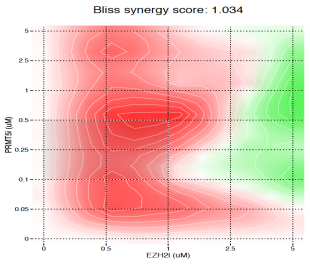
HEL



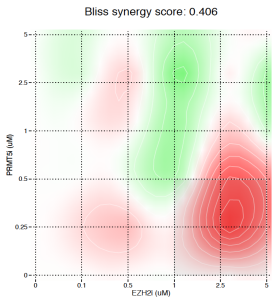
OCI-AML3



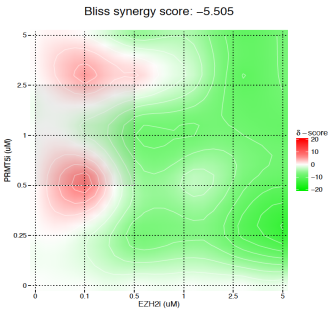
MV4-11



MonoMac6



Patient#1



Patient#2

Figure S5

	Gene Name	log2fold changes	Padj
1	SP100	-5.2	1.21379E-54
2	FUT1	-4.9	5.51586E-11
3	SH3RF3	-4.5	1.42285E-05
4	STC2	-4.1	9.69E-106
5	NRTN	-4.0	2.39097E-11
6	SLC6A9	-3.9	1.45763E-24
7	CHAC1	-3.9	2.44156E-71
8	INHBE	-3.6	2.44145E-46
9	NLRP12	-3.5	1.01108E-58
10	HIST3H2BB	-3.2	8.32314E-45
11	RHBDL3	4.3	2.3663E-24
12	SCHIP1	4.1	5.40123E-12
13	SERPINB2	3.9	4.78694E-24
14	CHRNA6	3.8	2.29273E-31
15	MMP2	3.8	8.28758E-14
16	CA6	3.7	6.83773E-15
17	SCN9A	3.5	3.83525E-06
18	KIT	3.5	1.97035E-14
19	ACPP	3.5	9.879E-166
20	MPEG1	3.4	5.6909E-19

Table S1

	Gene Name	log2fold changes	padj
1	CXCL10	-2.8	9.89609E-09
2	CCL2	-2.7	0.000914228
3	SIGLEC11	-2.2	0.016267844
4	ACP5	-2	0.044203784
5	IL1B	-1.86	0.007161772
6	PLA2G4C	-1.8	5.90055E-06
7	VCAN	-1.6	0.001232745
8	CD48	-1.4	7.72921E-05
9	FCER2	-1.4	7.95835E-05
10	CHST2	-1.3	0.000150651
11	FST	3.1	0.000307097
12	RGS13	2.9	0.012311318
13	SERPINB2	2.8	0.001504263
14	KLHL13	2.7	0.000334009
15	GJA1	2.7	0.000401762
16	AMOTL1	2.7	0.002278644
17	KIAA0125	2.6	0.01188846
18	ECI2	2.4	1.06544E-15
19	COL1A2	2.2	0.022027355
20	DES	2.1	9.2435E-06

Table S2

	Gene Name	log2fold changes	padj
1	CT45A6	-6.8	0.00027706
2	ST6GALNAC3	-4.8	1.7395E-05
3	S100P	-4.8	1.29089E-31
4	SLC6A9	-3.9	3.35842E-09
5	STC2	-3.9	3.43242E-14
6	GDAP1L1	-3.9	1.98127E-08
7	CHAC1	-3.6	0.00686391
8	NLRP12	-3.6	0.001249452
9	INHBE	-3.5	1.86805E-20
10	STX16-NPEPL1	-3.4	0.007317739
11	KLHL4	5.0	1.19555E-05
12	SERPINB2	5.0	4.51391E-30
13	SCHIP1	4.9	1.11409E-12
14	IGHM	4.4	4.9895E-07
15	CHRNA6	4.0	6.28969E-25
16	SCN9A	3.9	2.38157E-05
17	CD69	3.8	9.57939E-09
18	MT1H	3.6	2.92513E-22
19	SEPP1	3.6	3.11171E-09
20	BMP10	3.6	7.89416E-10

Table S3

Patient ID	Collection Date	Diagnosis	Karyotype	Mutations (VAF/ pathogenic)	Flow Markers	Drug Treatment
Patient#1	11/28/2018	AML	46,XX[20]	DNMT3A (17%[1]/yes[2]; 23%/UK[3]) ; PPM1D (17%/ yes); PHF6 (9%/ UK)	No immune-phenotypically abnormal B or T-cell populations identified. Blasts are not increased.	Proliferated in culture and treated with PRMT5 and EZH2 inhibitors for 6 days. Response to both inhibitors.
Patient#2	12/11/2018	AML	46,XY[20]	Not detected	Blasts are not increased, few polytypic B cells and T cells, without immune-phenotypical abnormalities	Proliferated in culture and treated with PRMT5 and EZH2 inhibitors for 6 days. Response to both inhibitors.

Table S4