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Supplemental Information

Therapeutic Targeting of RNA Splicing Catalysis through Inhibition of Protein Arginine Methylation

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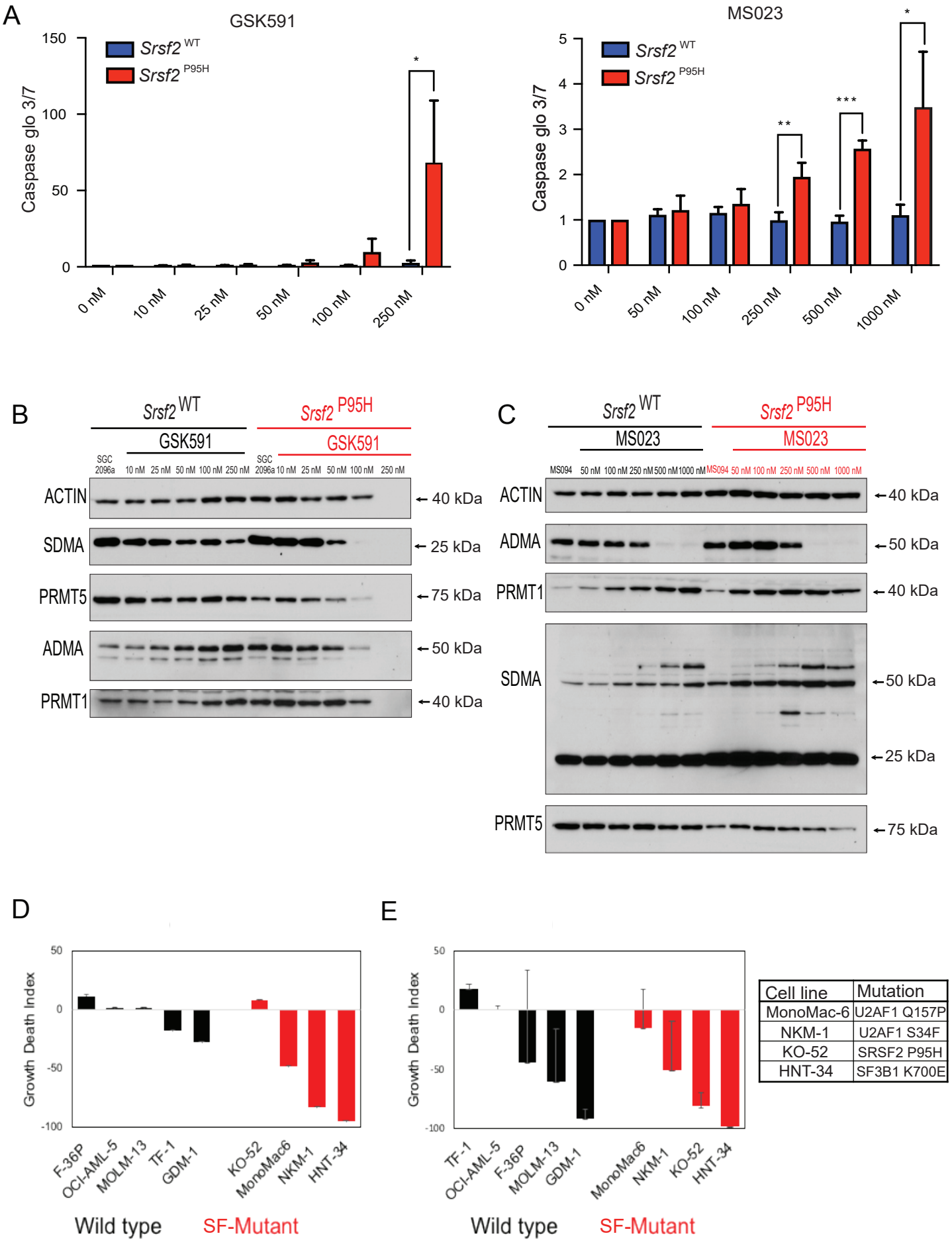
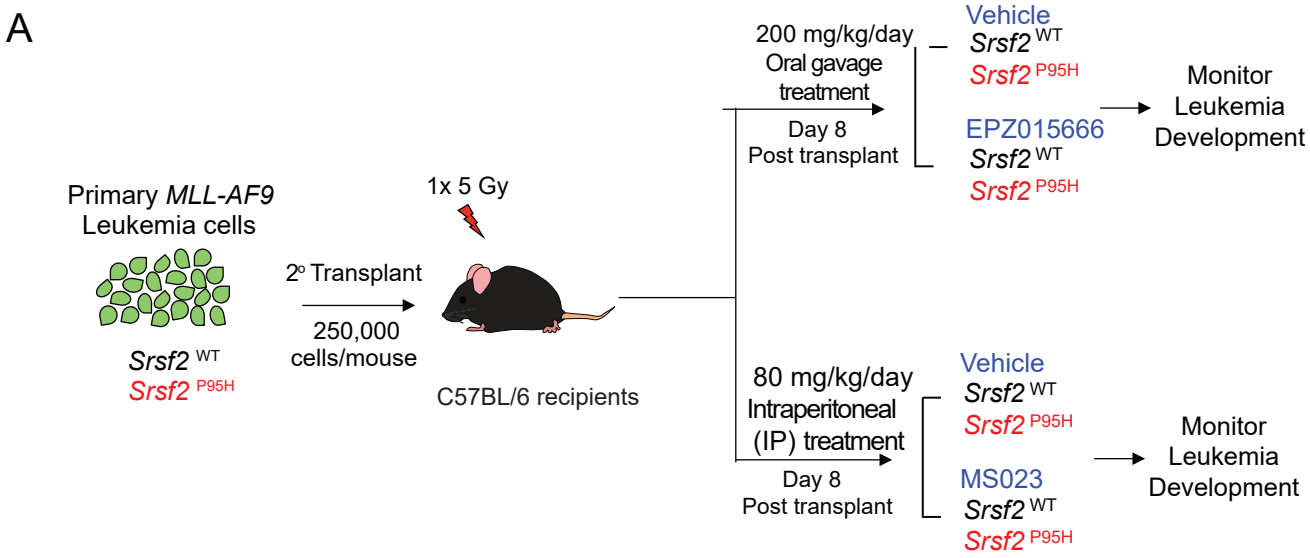
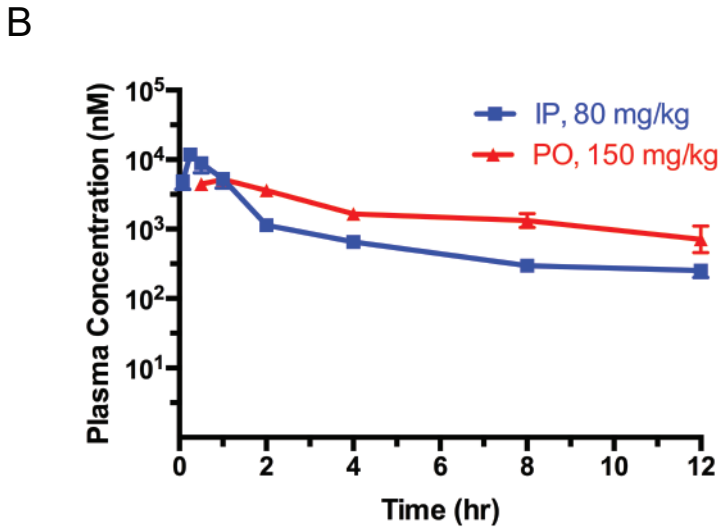
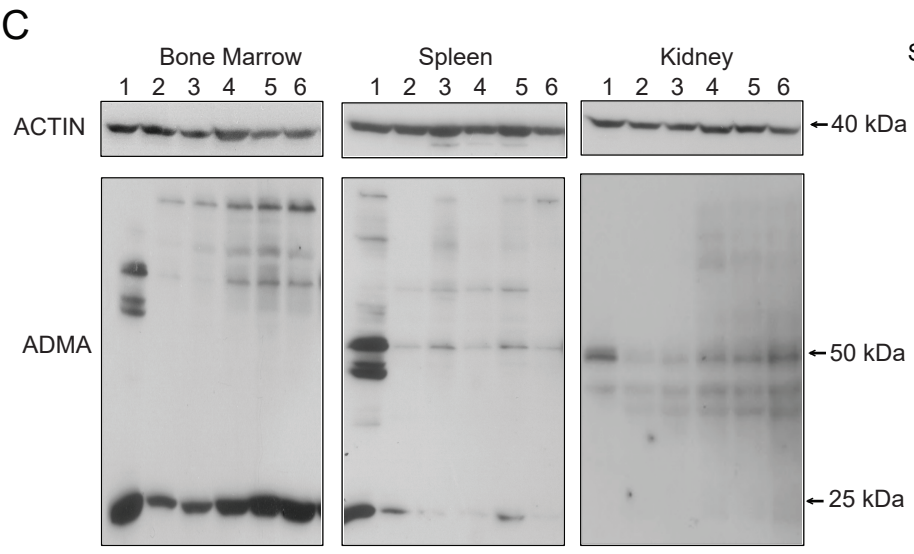

A
GSK591
Srsf2 WT
Srsf2 P95H
Caspase glo 3/7
150
100
50
0
0 nM
10 nM
25 nM
50 nM
100 nM
250 nM
*
MS023
Srsf2 WT
Srsf2 P95H
Caspase glo 3/7
5
4
3
2
1
0
0 nM
50 nM
100 nM
250 nM
500 nM
1000 nM
**
***
*
B
Srsf2 WT
Srsf2 P95H
GSK591
GSK591
SGC 2096a
10 nM
25 nM
50 nM
100 nM
250 nM
ACTIN
SDMA
PRMT5
ADMA
PRMT1
40 kDa
25 kDa
75 kDa
50 kDa
40 kDa
C
Srsf2 WT
Srsf2 P95H
MS023
MS023
MS094
50 nM
100 nM
250 nM
500 nM
1000 nM
ACTIN
ADMA
PRMT1
SDMA
PRMT5
40 kDa
50 kDa
40 kDa
50 kDa
25 kDa
75 kDa
D
Growth Death Index
50
0
-50
-100
F-36P
OCI-AML-5
MOLM-13
TF-1
GDM-1
KO-52
MonoMac6
NKM-1
HNT-34
Wild type
SF-Mutant
E
Growth Death Index
50
0
-50
-100
TF-1
OCI-AML-5
F-36P
MOLM-13
GDM-1
MonoMac6
NKM-1
KO-52
HNT-34
Wild type
SF-Mutant
Cell line | Mutation
MonoMac-6 | U2AF1 Q157P
NKM-1 | U2AF1 S34F
KO-52 | SRSF2 P95H
HNT-34 | SF3B1 K700E

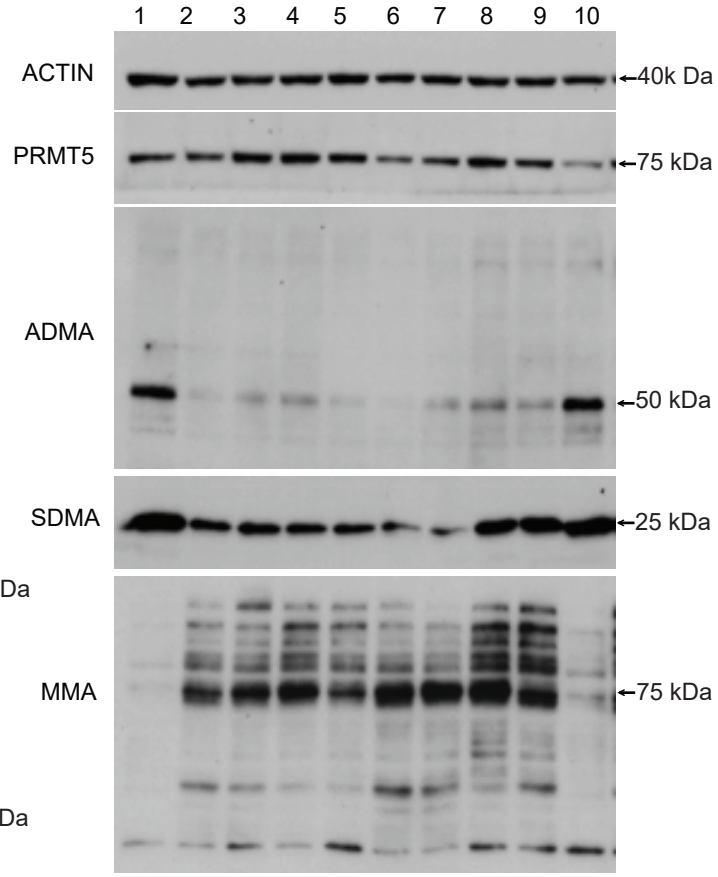
Figure S1. Effects of PRMT5 and type I PRMT inhibit on arginine methylation levels, Related to Figure 2. (A) Quantification of caspase-3/7 cleavage using a Caspase-Glo® 3/7 luminescence assay in *MLL-AF9/Vav*-cre *Srsf2*^{WT} and *MLL-AF9/Vav*-cre *Srsf2*^{P95H} upon exposure to GSK591 (left) or MS023 (right) normalized to cell viability readout from Cell-titre glo assay. (B-C) Western blot of actin, SDMA, PRMT5, ADMA, PRMT1 in *MLL-AF9/ Vav*-cre *Srsf2*^{WT} and *MLL-AF9/Vav*-cre *Srsf2*^{P95H} treated with increasing concentrations of (B) GSK591 or (C) MS023 (or indicated controls: SGC2096a for GSK591 or MS094 for MS023). (D-E) Growth Death Index (GDI) of human AML cell lines treated for 10 days with either GSK591 (D) or Type I PRMT inhibitor, GSK3368712 (E). All error bars represent SD. * p= 0.01-0.05; ** p = 0.001-0.01, *** p= 0.0001-0.001, **** p<0.0001 .







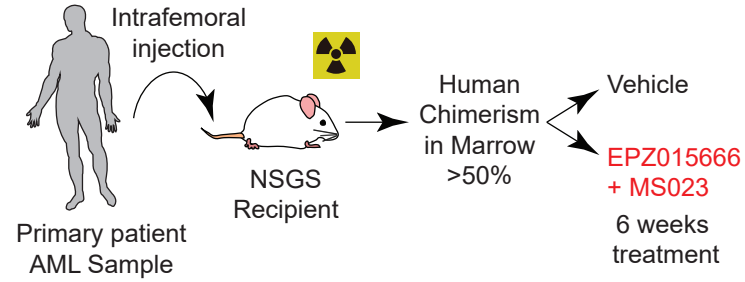
Lane	Mouse treatment info
1	Con
2	MS023 80 mg/kg , single dose per day, treated 5 d, sac 3 h after 5th dose
3	MS023 80 mg/kg , single dose per day, treated 4 d, sac 24 h after 4th dose
4	MS023 60 mg/kg , single dose per day, treated 5 d, sac 3 h after 5th dose
5	MS023 60 mg/kg , single dose per day, treated 4 d, sac 24 h after 4th dose
6	MS023 50 mg/kg , single dose per day, treated 5 d, sac 3 h after 5th dose



1 – con/veh
2 - MS023 (80 mg/kg) + EPZ (200 mg/kg)
3 – MS023 (80 mg/kg) + EPZ (150 mg/kg)
4 - MS023 (60 mg/kg) + EPZ (200 mg/kg)
5 - MS023 (60 mg/kg) + EPZ (150 mg/kg)
6 – MS023 (60 mg/kg) + EPZ (120 mg/kg)
7 – MS023 (50 mg/kg) + EPZ (200 mg/kg)
8 – MS023 (50 mg/kg) + EPZ (150 mg/kg)
9 – MS023 (40 mg/kg) + EPZ (200 mg/kg)
10 – con/veh

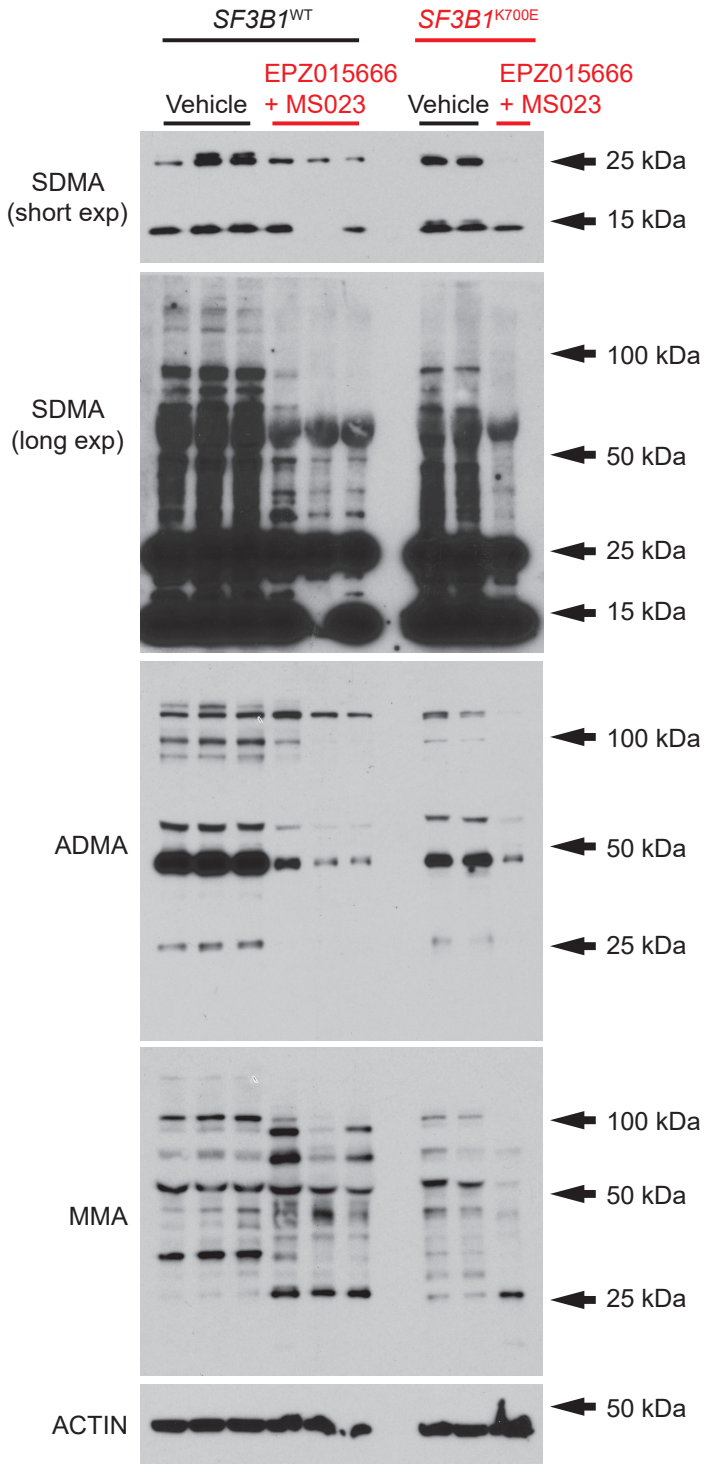
Figure S2. *In vivo* evaluations of PRMT inhibitors in mice. Related to Figure 3 and 4. (A) Schematic of secondary transplantation experiment to evaluate the efficacy of PRMT5 inhibition with EPZ015666 or Type I PRMT inhibition with MS023 *in vivo*. (B) Plasma exposure of MS023 in male Swiss Albino mice. Plasma concentrations of MS023 following a single 80 mg/kg intraperitoneal (IP) injection or 150 mg/kg oral (PO) administration over 12 hours. Plasma concentrations of MS023 reported at each of the 6 time points (0.5, 1, 2, 4, 8, and 12 h post dosing) are the average values from 3 test animals. Error bars represent SD. (C) Western blot of ADMA using tissue cells from normal C57BL/6 mice dosed *in vivo* with control vehicle ("con/veh") or MS023. ADMA: asymmetric dimethyl arginine. (D) Western blot of SDMA, ADMA and MMA using splenocytes from normal C57BL/6 mice dosed *in vivo* with control vehicle ("con/veh") or any of the combined doses of MS023 or EPZ015666 ("EPZ").

A



SF3B1^{WT} *MLL-ENL* *KRAS*^{G12C} *WT1*^{R370fs}
SF3B1^{K700E} inv3

B



C

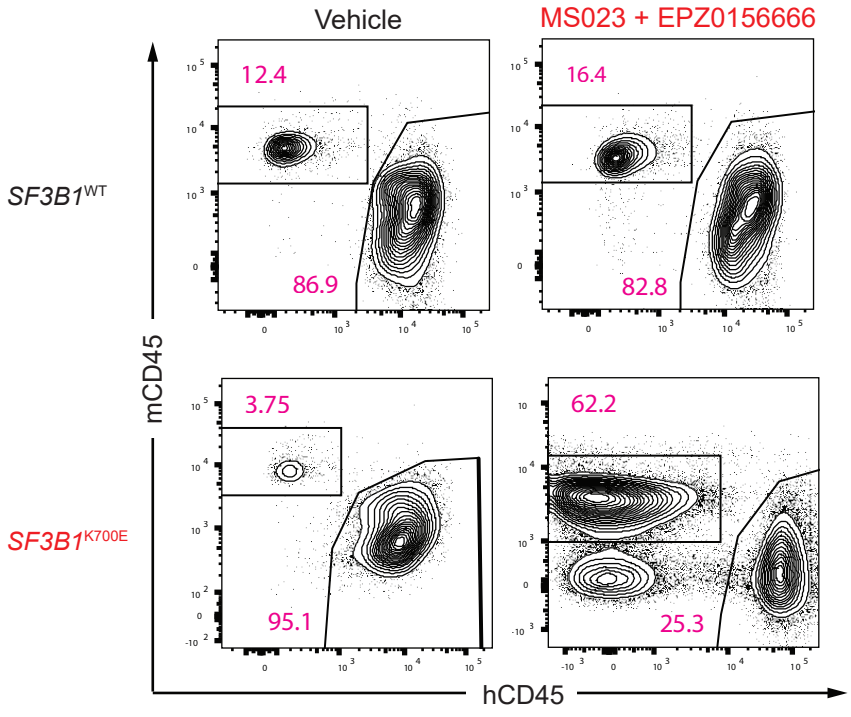


Figure S3. *In vivo* evaluations of PRMT inhibitors in PDX models of AML. Related to Figure 5. (A) Schematic of patient-derived xenograft (PDX) experiments using primary AML patient samples. *SF3B1* wildtype (WT) and *SF3B1*^{K700E} AML cells were injected intrafemorally into sub-lethally irradiated (200 Rad) NSGS mice. Following stable engraftment of leukemia cells in the bone marrow (indicated by the presence of human CD45), mice were subjected to vehicle or combined treatment of MS023 (60 mg/kg/day) and EPZ015666 (150 mg/kg/day) for 6 weeks. (B) Western blot analysis of SDMA, ADMA and MMA from bone marrow mononuclear cells of NSGS mice after 6 weeks of combined EPZ015666 and MS023 treatment. (C) Representative flow cytometry plots of human CD45 chimerism in the bone marrow of NSGS mice after 6 weeks of treatment.

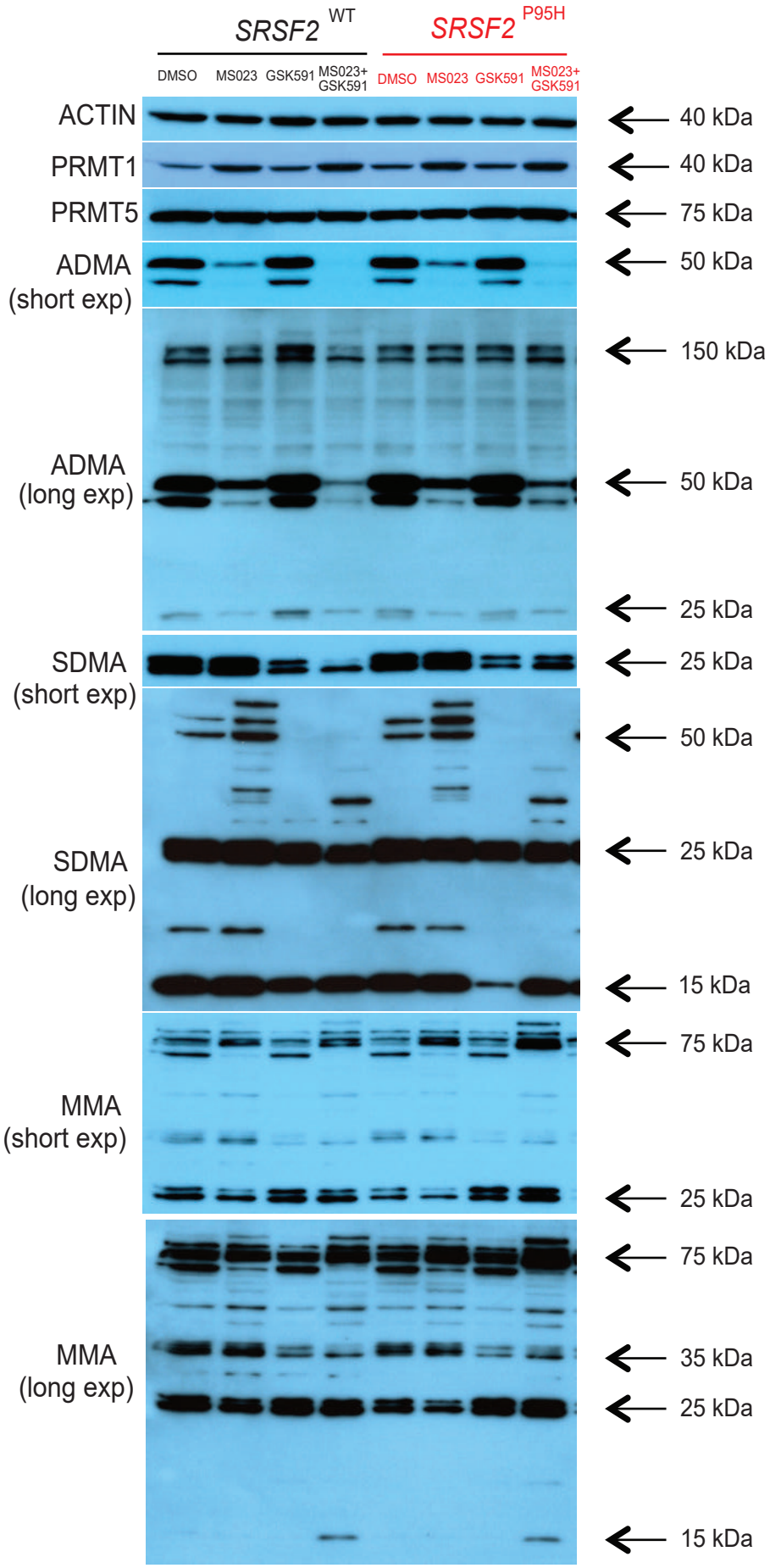
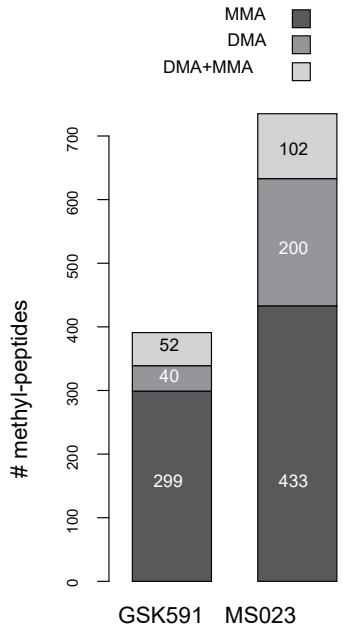
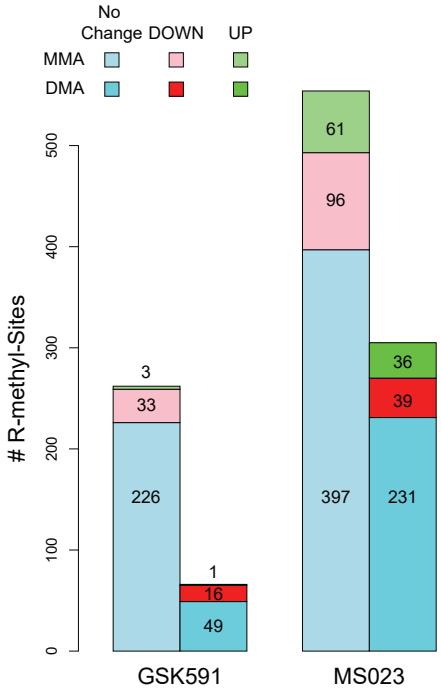


Figure S4. Inhibition of PRMTs in K562 cells. Related to Figure 5. Western blot showing changes in SDMA, ADMA and MMA in K562 cells with MS023 and GSK591 treatment *in vitro*.

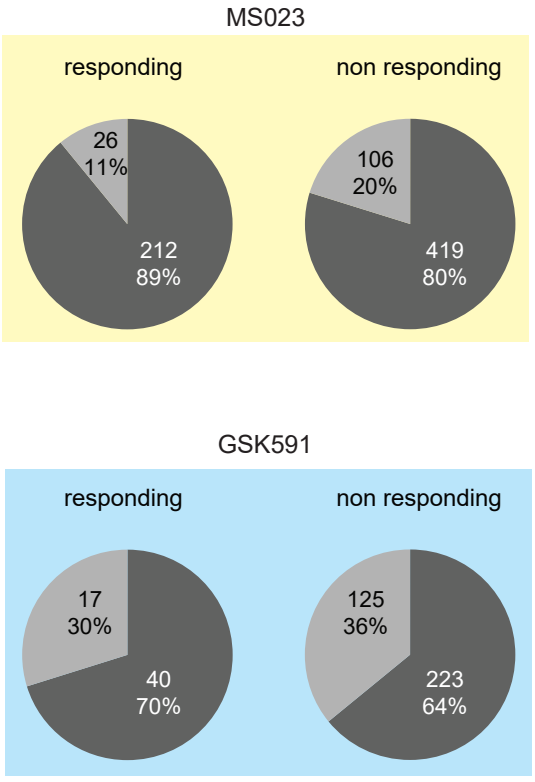
A


MMA
DMA
DMA+MMA
methyl-peptides
0
100
200
300
400
500
600
700
52
40
299
102
200
433
GSK591
MS023

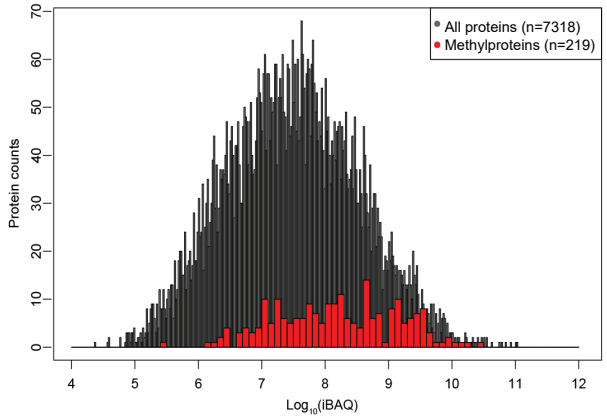
B


No Change
DOWN
UP
MMA
DMA
R-methyl-Sites
0
100
200
300
400
500
3
33
226
1
16
49
61
96
397
36
39
231
GSK591
MS023

C


MS023
responding
non responding
26
11%
212
89%
106
20%
419
80%
GSK591
responding
non responding
17
30%
40
70%
125
36%
223
64%

D


All proteins (n=7318)
Methylproteins (n=219)
Protein counts
0
10
20
30
40
50
60
70
4
5
6
7
8
9
10
11
12
Log10(iBAQ)

E

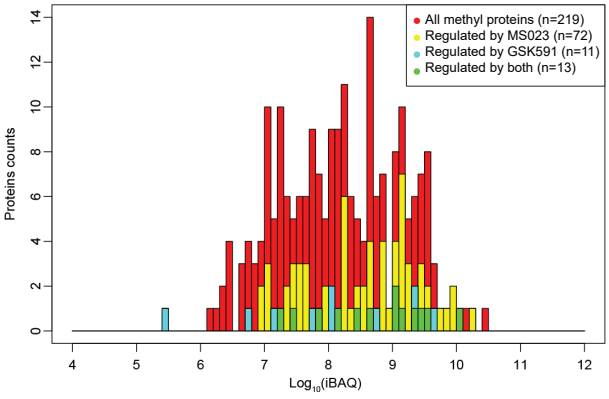

All methyl proteins (n=219)
Regulated by MS023 (n=72)
Regulated by GSK591 (n=11)
Regulated by both (n=13)
Proteins counts
0
2
4
6
8
10
12
14
4
5
6
7
8
9
10
11
12
Log10(iBAQ)

Figure S5. Proteomic identification of methyl-arginine substrates in leukemia cells. Related to Figure 6. (A) Peptides carrying monomethyl and/or dimethyl arginine identified and quantified in cells treated with GSK591 or MS023 (Andromeda score ≥ 25 and PTM localization probability ≥ 0.50). (B) Methyl sites identified in cells treated with GSK591 or MS023, respectively, divided by methylation degree and regulation state. (C) Pie charts of the methyl-peptides responding or not to GSK591 and MS023 which were orthogonally-validated by intersection with a high-quality methyl-proteome dataset (Ong et al., 2004) identified through the heavy methyl SILAC labeling strategy. (D) iBAQ intensity of the quantified methyl-proteins over the whole proteome. (E) iBAQ intensity of the methyl-proteins responding to the indicated PRMT inhibitors, over the all quantified methyl-proteins.

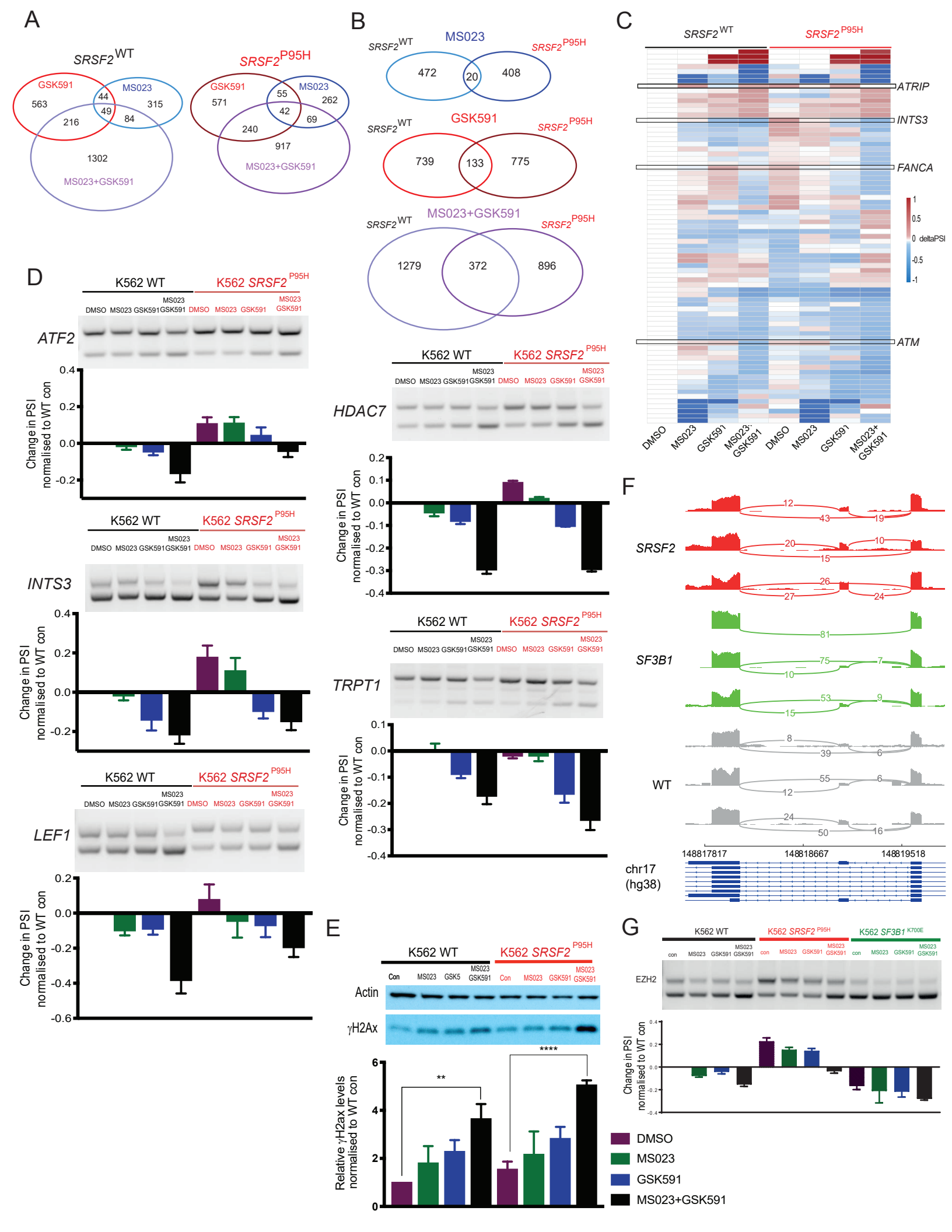
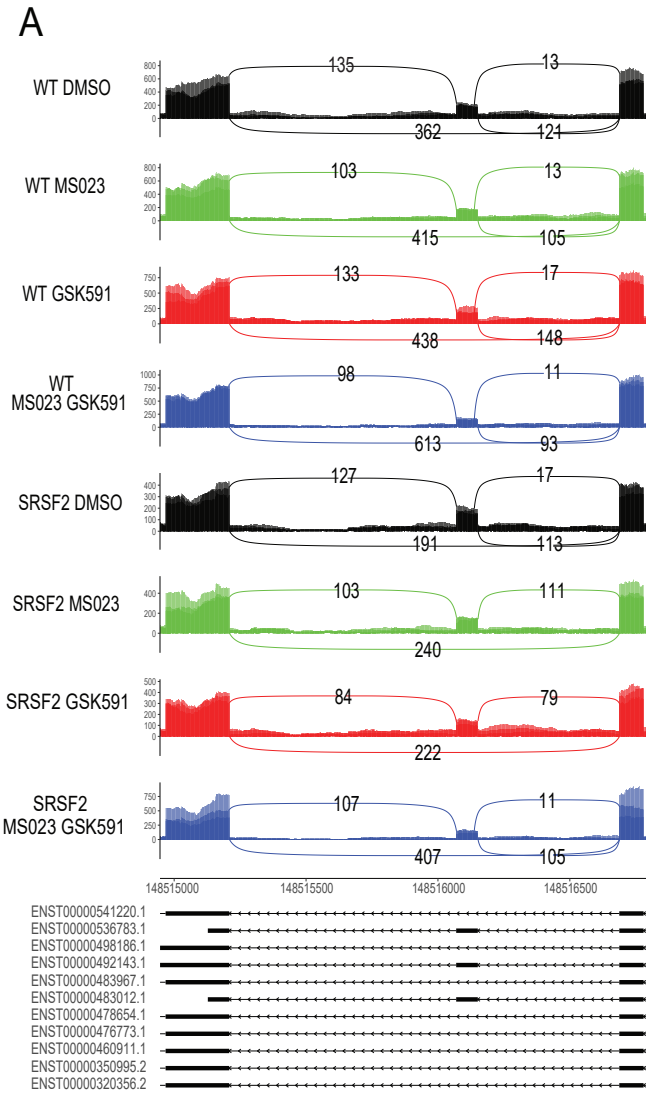

A
SRSF2WT
GSK591
MS023
563
44
315
49
216
84
1302
MS023+GSK591
SRSF2P95H
GSK591
MS023
571
55
262
42
240
69
917
MS023+GSK591
B
MS023
SRSF2WT
SRSF2P95H
472
20
408
GSK591
739
133
775
MS023+GSK591
1279
372
896
C
SRSF2WT
SRSF2P95H
ATRIP
INTS3
FANCA
ATM
1
0.5
0 deltaPSI
-0.5
-1
DMSO
MS023
GSK591
MS023+GSK591
D
K562 WT
K562 SRSF2P95H
DMSO MS023 GSK591 MS023+GSK591
ATF2
Change in PSI normalised to WT con
INTS3
LEF1
HDAC7
TRPT1
E
Con
MS023
GSK5
Actin
γH2Ax
Relative γH2ax levels normalised to WT con
**
****
F
SRSF2
SF3B1
WT
148817817
148818667
148819518
chr17 (hg38)
G
K562 SRSF2P95H
K562 SF3B1K700E
EZH2
DMSO
MS023
GSK591
MS023+GSK591

Figure S6. Validation of splicing events from RNA-seq analysis. Related to Figure 7. (A) Venn diagram showing the overlap of cassette exon splicing events in K562 wild-type and *SRSF2*^{P95H} cells upon MS023, GSK591, or combination treatment. (B) Venn diagram showing overlap of cassette exon splicing events for K562 wild-type vs *SRSF2*^{P95H} cells upon MS023, GSK591, or combination treatment. (C) Heatmap showing change in PSI of cassette exon splicing events normalized to PSI of WT control within the "DNA Repair" GO category. (D) RT-PCR and quantification showing validation of cassette exon skipping events (*ATF2, HDAC7, LEF1, TRPT1, INTS3*) identified from RNA-seq analysis of K562 cells described in (A). (E) Western blot showing changes in gH2Ax levels upon MS023 and/or GSK591 treatment of K562 wild-type and *SRSF2*^{P95H} *in vitro*. (F) Sashimi plots of the *EZH2* poison exon in nine distinct MDS patient samples from (Pellagatti et al., 2018) three of which were SRSF2 mutant, SF3B1 mutant, and three lacking any mutation in an RNA splicing factor. (G) RT-PCR data showing changes in PSI levels of *EZH2* poison exon inclusion in K562 cells with or without knockin of *SRSF2*^{P95H} or *SF3B1*^{K700E} upon MS023 and GSK591 treatment *in vitro*. All error bars represent SD. * p= 0.01-0.05; ** p = 0.001-0.01, *** p= 0.0001-0.001, **** p<0.0001 Student's t-test used for statistical analysis.


A
WT DMSO
135
13
362
121
WT MS023
103
13
415
105
WT GSK591
133
17
438
148
WT MS023 GSK591
98
11
613
93
SRSF2 DMSO
127
17
191
113
SRSF2 MS023
103
111
240
SRSF2 GSK591
84
79
222
SRSF2 MS023 GSK591
107
11
407
105
148515000
148515500
148516000
148516500
ENST00000541220.1
ENST00000536783.1
ENST00000498186.1
ENST00000492143.1
ENST00000483967.1
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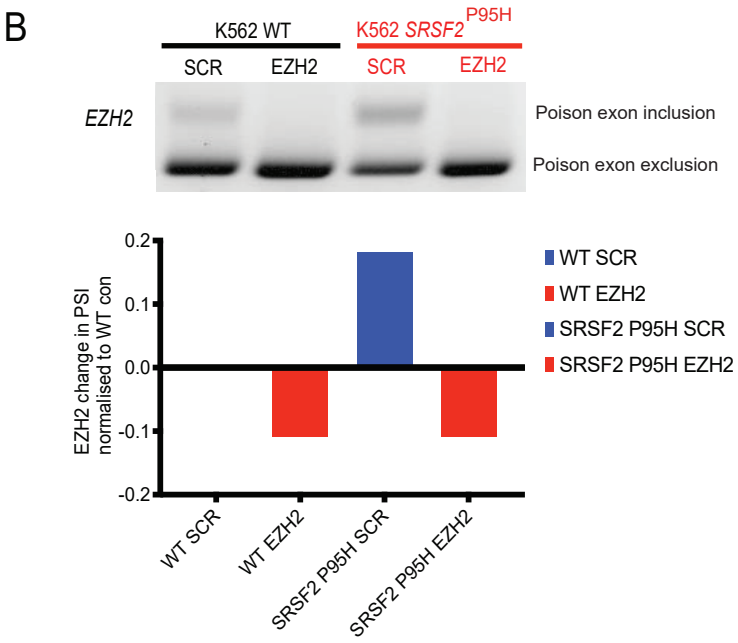
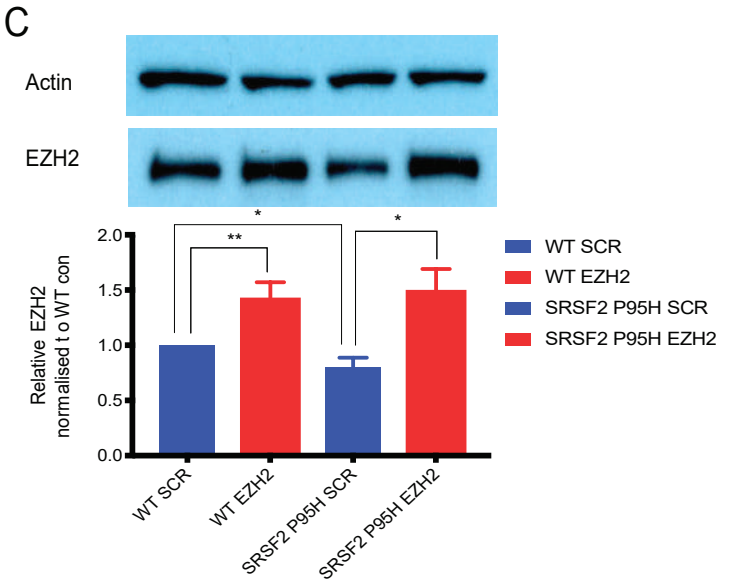
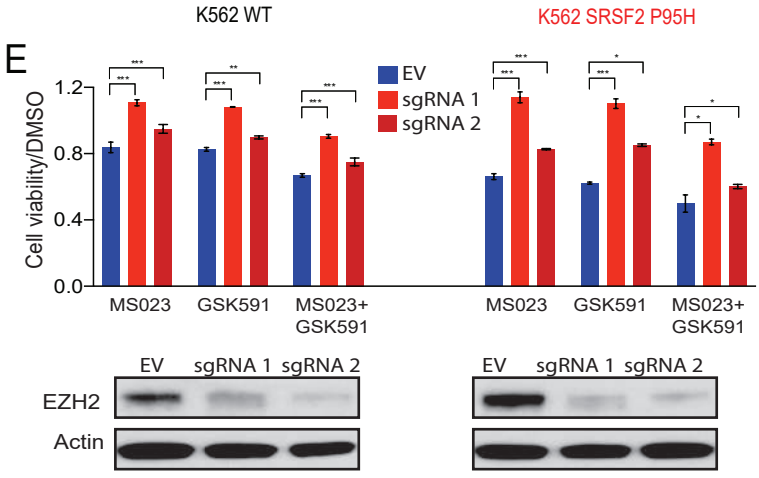
ENST00000460911.1
ENST00000350995.2
ENST00000320356.2


B
K562 WT
K562 SRSF2 P95H
SCR
EZH2
SCR
EZH2
EZH2
Poison exon inclusion
Poison exon exclusion
EZH2 change in PSI normalised to WT con
0.2
0.1
0.0
-0.1
-0.2
WT SCR
WT EZH2
SRSF2 P95H SCR
SRSF2 P95H EZH2
WT SCR
WT EZH2
SRSF2 P95H SCR
SRSF2 P95H EZH2


C
Actin
EZH2
Relative EZH2 normalised t o WT con
2.0
1.5
1.0
0.5
0.0
*
**
*
WT SCR
WT EZH2
SRSF2 P95H SCR
SRSF2 P95H EZH2
WT SCR
WT EZH2
SRSF2 P95H SCR
SRSF2 P95H EZH2


E
K562 WT
K562 SRSF2 P95H
Cell viability/DMSO
1.2
0.8
0.4
0.0
EV
sgRNA 1
sgRNA 2
MS023
GSK591
MS023+ GSK591
MS023
GSK591
MS023+ GSK591
EV
sgRNA 1
sgRNA 2
EV
sgRNA 1
sgRNA 2
EZH2
Actin

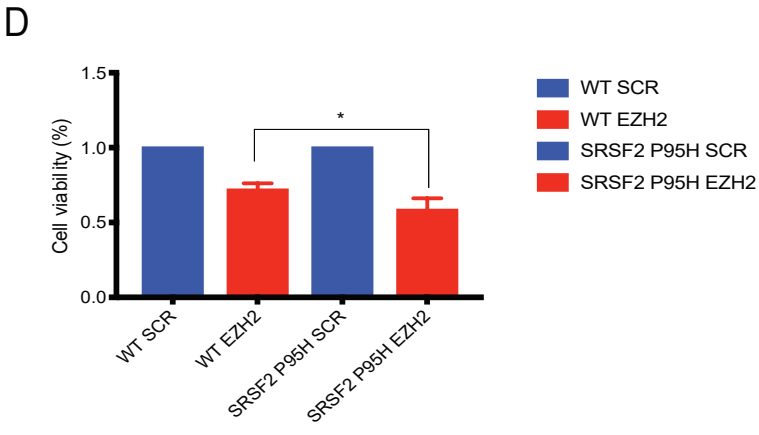

D
Cell viability (%)
1.5
1.0
0.5
0.0
*
WT SCR
WT EZH2
SRSF2 P95H SCR
SRSF2 P95H EZH2
WT SCR
WT EZH2
SRSF2 P95H SCR
SRSF2 P95H EZH2

Figure S7. PRMT5 and type I PRMTs inhibition affects *EZH2* splicing. Related to Figure 7. (A) Sashimi plots and splicing gel showing changes in PSI levels of EZH2 poison exon upon MS023 and GSK591 treatment *in vitro*. (B) Splicing gel showing changes in PSI levels of *EZH2* poison exon upon electroporation of K562 wild-type or *SRSF2*^{P95H} cells with a scrambled (SCR) antisense oligonucleotide (AON) or an AON targeting the poison exon of *EZH2* (EZH2).(C) Western blot showing changes in EZH2 protein levels upon electroporation of K562 cells with AON SCR or AON EZH2. (D) Percentage of viable K562 wild-type or *SRSF2*^{P95H} cells 48 hours after AON electroporation. (E) Percentage of viable K562 wild-type (left) or *SRSF2*^{P95H} (right) cells with or without CRISPR/Cas9 knock-out of EZH2 upon treatment with MS023, GSK591 or combination. Bottom panel: Western blot showing EZH2 levels to in cells with and without CRISPR/Cas9 EZH2 knock-out. All EZH2 protein bands were quantified using ImageJ, normalized to the actin bands and expressed in levels relative to WT EZH2 protein level. All error bars represent SD. * p= 0.01-0.05; ** p = 0.001-0.01, *** p= 0.0001-0.001, **** p<0.0001 Student's t-test used for statistical analysis.