Supplementary Information

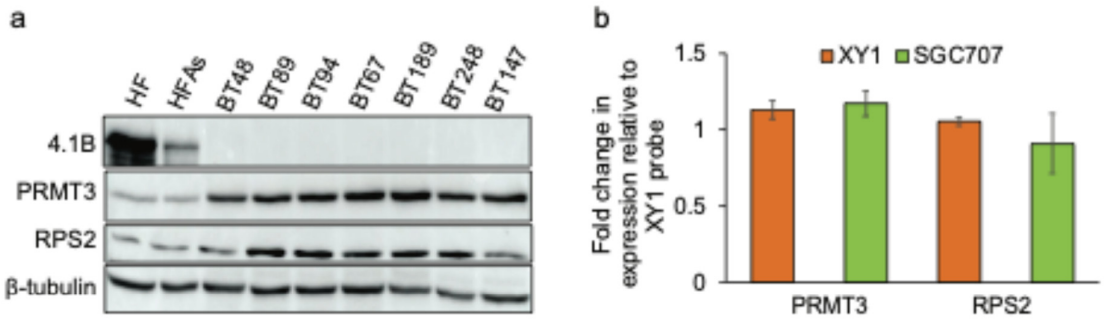
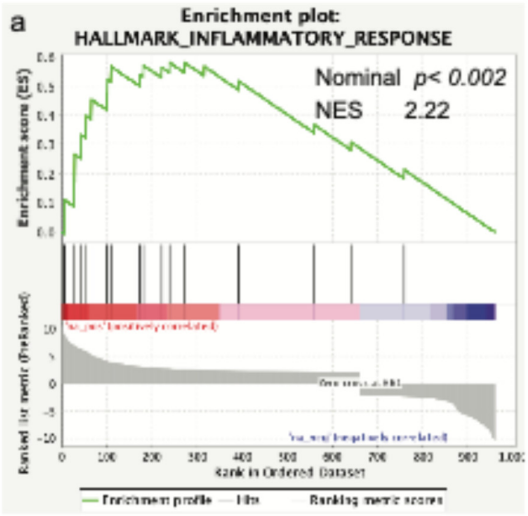

a
HF
HFAs
BT48
BT89
BT94
BT67
BT189
BT248
BT147
4.1B
PRMT3
RPS2
β-tubulin
b
XY1
SGC707
Fold change in expression relative to XY1 probe
1.5
1
0.5
0
PRMT3
RPS2

Figure S1: a Protein levels of 4.1B, PRMT3 and RPS2 in BTSC cell lines relative to HF and HFAs (n=3). b qPCR showing the fold change in expression of PRMT3 and RPS2 following treatment with SGC707 relative to XY1 negative probe (n=3). human fetal stem cells (HFs), human fetal astrocytes (HFAs).


a
Enrichment plot:
HALLMARK_INFLAMMATORY_RESPONSE
Nominal p< 0.002
NES 2.22
Enrichment score (ES)
Ranked list metric (PreRanked)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

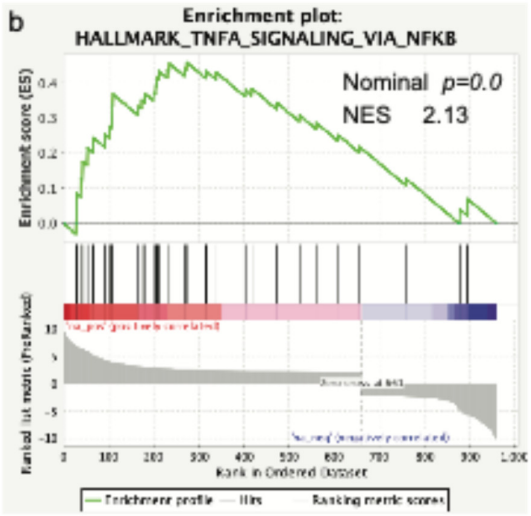

b
Enrichment plot:
HALLMARK_TNFA_SIGNALING_VIA_NFKB
Nominal p=0.0
NES 2.13
Enrichment score (ES)
Ranked list metric (PreRanked)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Figure S2: a, b Gene set enrichment analysis showing the enrichment plots for gene sets enriched for inflammatory response (a) TNF-α and NFK-β signaling pathways (b) in response to 72 hours treatment with SGC707 relative to the XY1 negative probe (n=3).

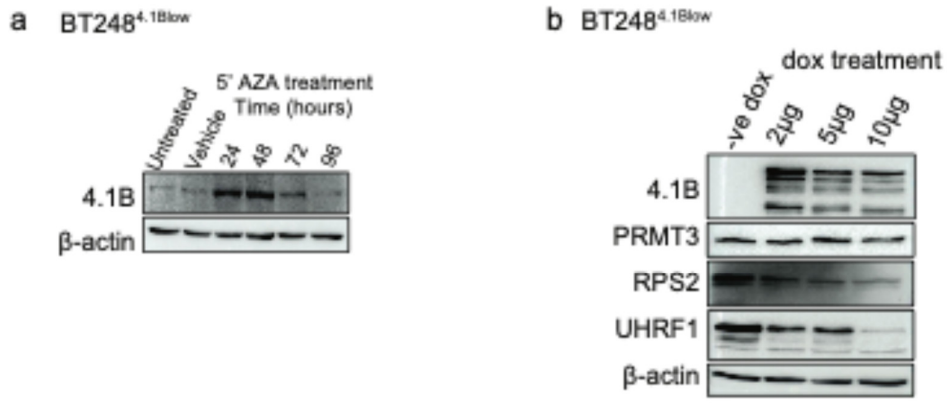

a
BT248^4.1Blow
5' AZA treatment
Time (hours)
Untreated
Vehicle
24
48
72
96
4.1B
β-actin
b
BT248^4.1Blow
dox treatment
-ve dox
2μg
5μg
10μg
4.1B
PRMT3
RPS2
UHRF1
β-actin

Figure S3: a Western blot analysis of induction of protein level of 4.1B following time course treatment of BT248$^{4.1Blow}$ with 5'-Aza relative to the vehicle control. (n=3)**.** b Testing of different doses of doxycycline for inducing the expression of 4.1B and its effect on PRMT3, RPS2 and UHRF1 proteins in BT248$^{EPB4.1L3low}$ cells relative to the negative control cells.

BT143$^{4.1Bhigh}$

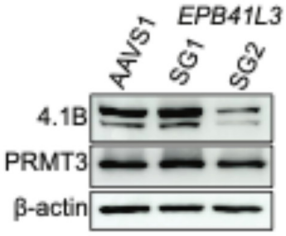


Figure S4: Western blot validation of *EPB4.1L3* KO in BT143$^{EPB4.1L3high}$ cells relative to the AAVS1 control cells.

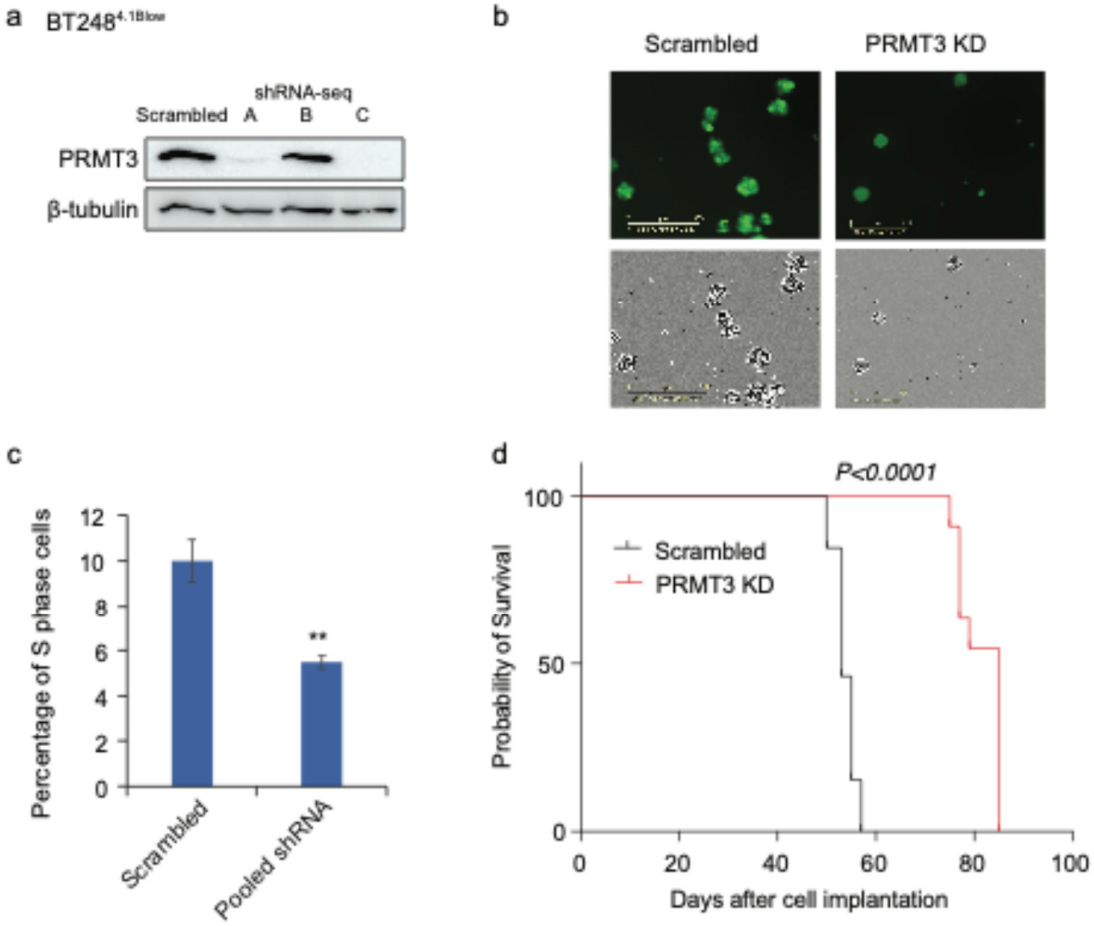

a
BT248 4.1Blow
shRNA-seq
Scrambled A B C
PRMT3
β-tubulin
b
Scrambled
PRMT3 KD
c
Percentage of S phase cells
12
10
8
6
4
2
0
**
Scrambled
Pooled shRNA
d
P<0.0001
100
50
0
Probability of Survival
Scrambled
PRMT3 KD
0 20 40 60 80 100
Days after cell implantation

Figure S5: a Validation of shRNA mediated KD of PRMT3 in BT248 cells relative to the Scrambled control cells. b Representative fluorescent and brightfield images showing PRMT3 KD in BT248 cells along with scrambled control images. (Reporter gene; GFP). Scale bar: 300μm. (n=3). c EdU cell cycle analysis of PRMT KD BT248 cells relative to the Scrambled control cells. Significance was determined using unpaired two-tailed t-test at 95% confidence intervals, $^{**}p<0.01$; data are represented as mean ± SEM; n=3. d Kaplan-Meier survival curves for mice orthotopically xenografted with PRMT3 KD BT248 cells compared to mice xenografted with scrambled control cells (Log-rank (Mantel-Cox) method, $^{****}p<0.0001$, n=10).


a
BT248 4.1Blow
XY1
SGC707
β-thuj
PRMT3
UHRF1
β-actin
b
BT143 4.1Bhigh
XY1
SGC707
β-thuj
PRMT3
UHRF1
β-actin

Figure S6: a, b Validating the on-target effect of β-thujaplicin in parallel with SGC707 treatment on the protein levels of PRMT3 and UHRF1 in (a) $BT248^{EPB4.1L3low}$ and (b) $BT143^{EPB4.1L3high}$ cells relative to their respective XY1 treated control cells.

Supplementary Tables

Table S1: gRNA used in this study.

gRNA	Sequence
PRMT3-1	CCTGTTACAGAACAGGCAGG
PRMT3-2	TGTCATTGCGGACCTCCAGG
UHRF1-1	CGCCGACACCATGTGGATCC
UHRF1-2	ACACCATGTGGATCCAGGTT
EPB4.1L3-1	AGCGGCGGCGAACTGCTCCA
EPB4.1L3-2	TGCAAAGTGATACTTCTCGA
AAVS1	ATCCTGTCCCTAGTGGCCC

Table S2: qPCR primers used in this study.

Gene	Forward	Reverse
PRMT3	TCAGAGCACCAAAACACACTG	TTCAAGGCTTCACCTGCTTTA
RPS2	GGAAAAGCGACAAGAAGGAAC	AGCTGCTTCCCAGTAGGTCTC

Table S3: MS-PCR primers targeting CpG sites within 5' regulatory regions of *EPB4.1L3* gene.

Forward	Reverse
M-AGTCGTAGTTTAGGGTTGTTCGTC	M-GAAATTAACTTTCCTCGCACG
UM-GAAGTTGTAGTTTAGGGTTGTTTGTT	UM-ACAAAATTAACTTTCCTCACACAAT
M-AGAGTCGTTTTCGTAGATATTTTTC	M-GCCTAAATCGACCTACCGTA
UM-AGTTGTTTTTGTAGATATTTTTTGA	UM-CACCTAAATCAACCTACCAT
M-GAGGTTTAGTAGTTTTTAGCGTCGT	M-GTTACATCTTCCCGTAACTCGAC
UM-TGGGAGGTTTAGTAGTTTTTAGTGTT	UM-ACATTACATCTTCCCATAACTCAAC
M-TAGTCGAGTTACGGGAAGATGTAAC	M-AAAATACGAATAAAAAATCGAAACGT
UM-TTTATAGTTGAGTTATGGGAAGATGTAAT	UM-AATACAAATAAAAAATCAAAACATT
M-ATTTTTAAGGTTATTTAAGAATCGA	M-TCTATATAAAATCTCCTATATCGCA
UM-ATTTTTAAGGTTATTTAAGAATTGA	UM-ATCTATATAAAATCTCCTATATCACA

M- Methylation, UM-Unmethylation

Table S4: List of primary and secondary antibodies used in this study.

Antibodies	**Source**	**Catalog number**
Rabbit monoclonal to PRMT3 (EPR13279)	Abcam	Cat# ab191562
Mouse monoclonal to PRMT3	Invitrogen	Cat# 730020
Mouse monoclonal to EPB4.1L3	Sigma-Aldrich	Cat# SAB1404955
Rabbit Polyclonal to EPB4.1L3	Invitrogen	Cat# PA5-83023
Rabbit monoclonal to RPS2 (EPR10834(B))	Abcam	Cat# ab155961
Mouse monoclonal to DNMT1	Active Motif	Cat# ab39204
Mouse monoclonal to UHRF1	Santa Cruz	Cat# sc-373750
Rabbit polyclonal to DNMT3A	Santa Cruz	Cat# sc-20703
Rabbit monoclonal to ADMA	Cell signaling	Cat# 13522
Rabbit monoclonal to p21	Cell signaling	Cat# 2947
Rabbit monoclonal to CD133	Cell signaling	Cat# 5860
Rabbit monoclonal to p53	Cell signaling	Cat# 2527
Rabbit monoclonal to BNIP3 (EPR4034)	Abcam	Cat# ab109362
Mouse monoclonal to GLUT1	Abcam	Cat# ab238050
Normal mouse IgG	Millipore Sigma	Cat# 12-371
Normal rabbit IgG	Millipore Sigma	Cat# 12-370
Rabbit polyclonal to Beta tubulin	Cell signaling	Cat# 2146
Mouse monoclonal to Beta actin	Abcam	Cat# ab195475
Rabbit polyclonal to H3 nuclear marker	Abcam	Cat# ab1791
Rabbit monoclonal to H4 (EPR16599)	Abcam	Cat# 177840
Mouse monoclonal anti-rabbit IgG light chain (HRP)	Abcam	Cat# ab99697
Rat monoclonal anti-mouse kappa light chain (HRP)	Abcam	Cat# ab99632
Goat anti-rabbit IgG (H+L) Alexa Fluor™ 488+	Invitrogen	Cat# A32731
Goat anti-mouse IgG (H+L) Alexa Fluor™ 555+	Invitrogen	Cat# A32727