PRMT5 inhibition promotes PD-L1 expression and immuno-resistance in lung cancer

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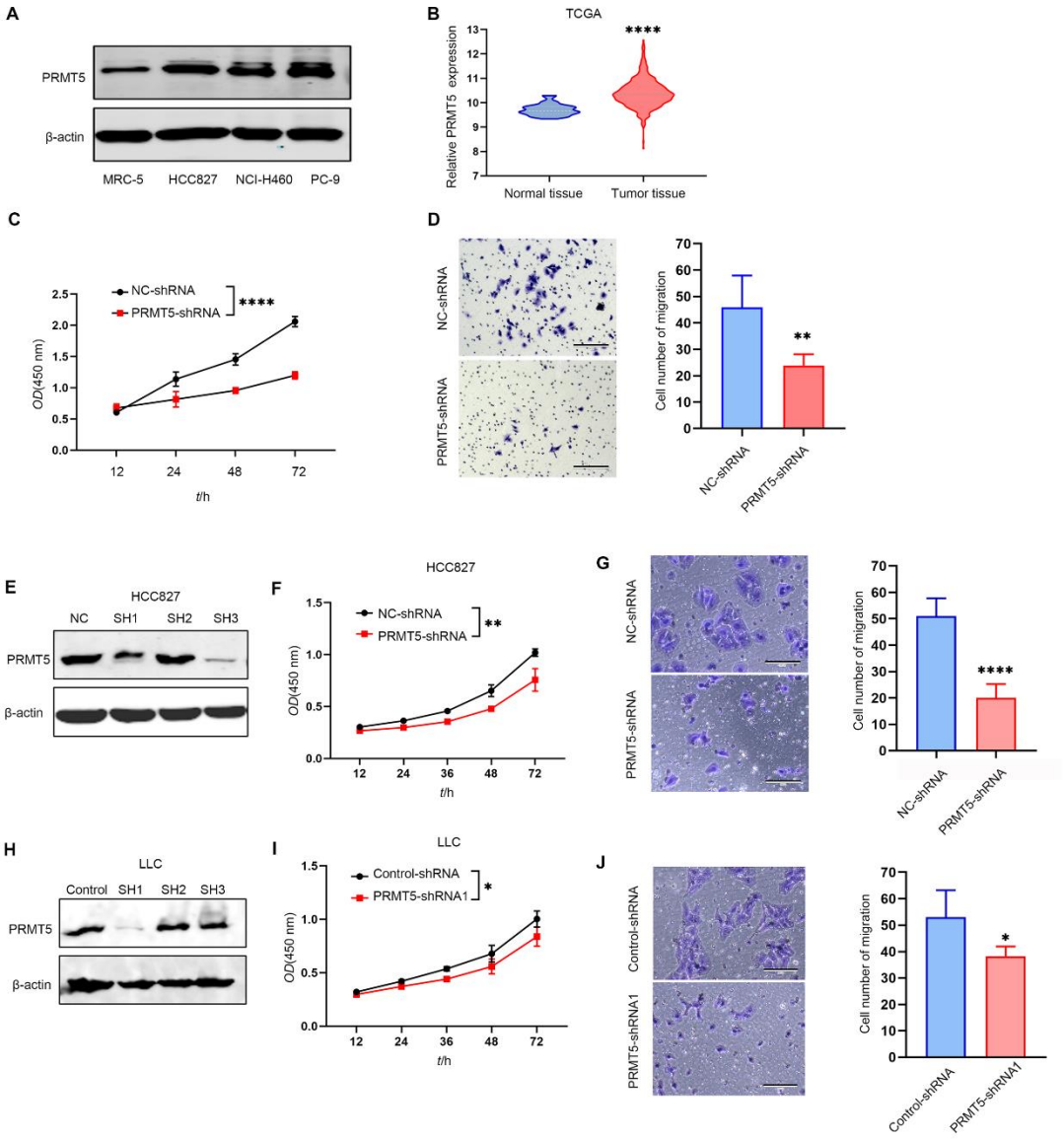
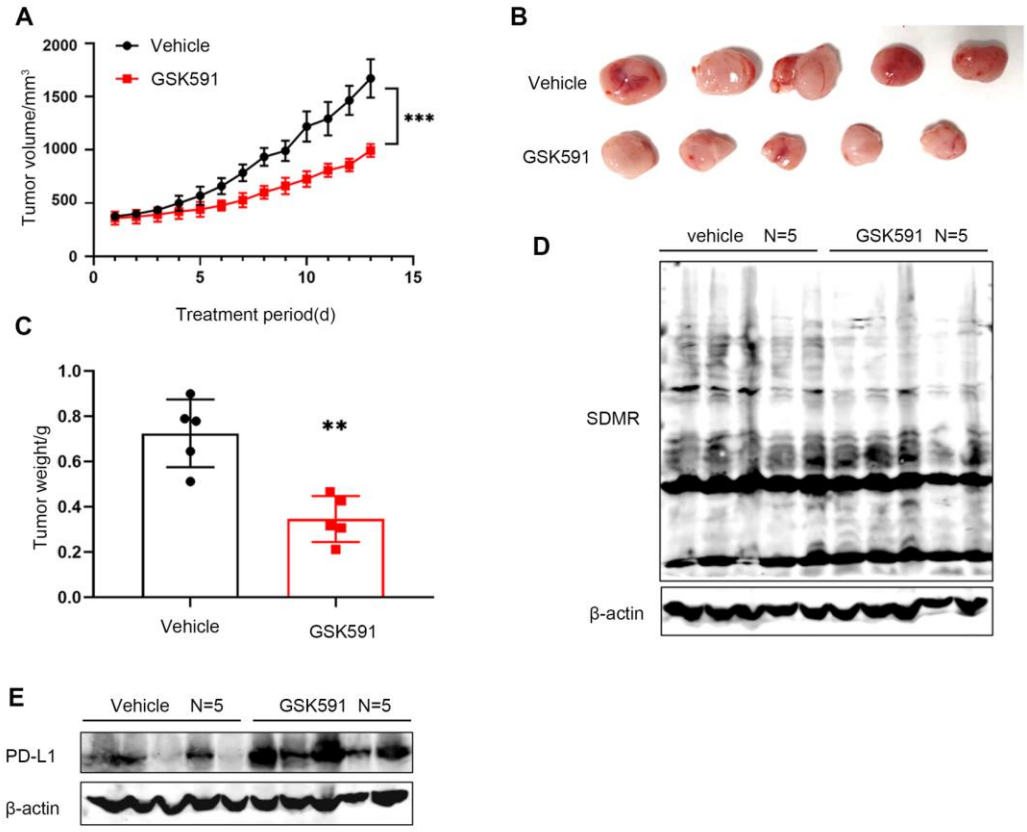


Supplementary Figure.1 PRMT5 expression in Lung cancer cell and its effects on proliferation and migration of lung cancer cell lines.

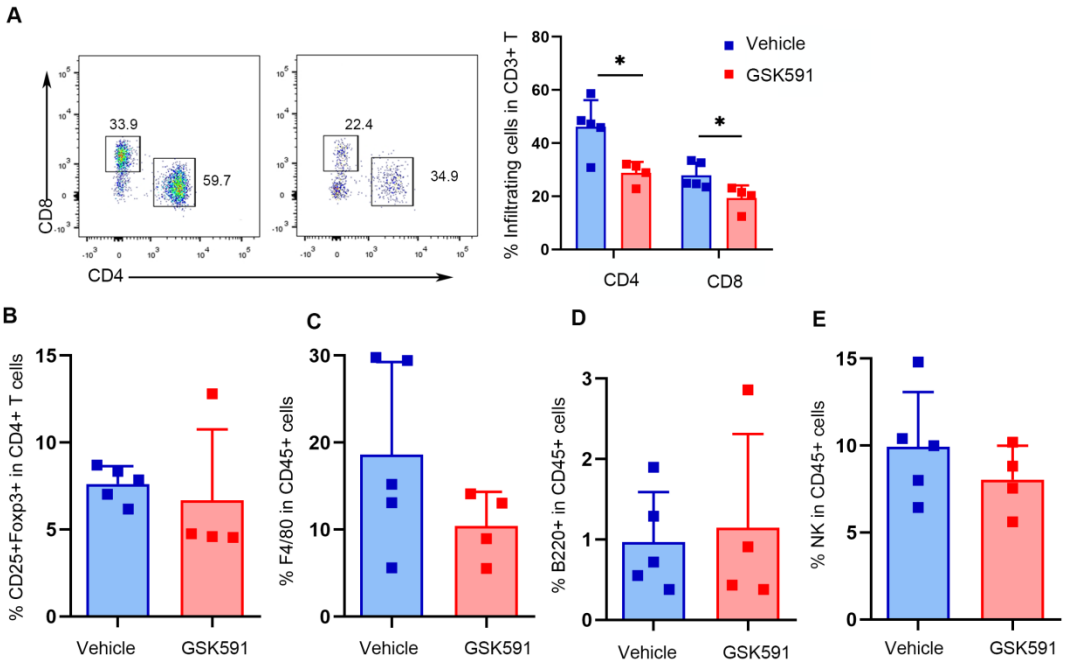
(A) PRMT5 expression in human embryonic lung fibroblasts and lung cancer cells lines was detected by western blot, β-actin as the loading control. (B) Analysis of TCGA RNA-seq data of human lung adenocarcinoma, violin plots showed the relative *PRMT5* expression between normal tissues and tumor tissues. Statistical differences were determined by two-tailed unpaired Student's t test, ****$P < 0.0001$. (**E,H**) Three sites of shRNA sequence targeting human PRMT5 or murine PRMT5 were designed, separately. *PRMT5* specific or control shRNA were transfected into human lung cancer line HCC827(**E**) and murine lung cancer cell line LLC(**H**).PRMT5 expression was measured by western blotting, β-actin as the loading control. (**C, F and I**)Effects of *PRMT5*-shRNA on the proliferation of NCI-H460(**C**), HCC827 (**F**) and LLC (**I**) cells were detected by CCK8 assay. Statistical differences were determined by two-tailed unpaired Student's t test, ****$P < 0.0001$. (**D, G, J**) Effects of *PRMT5*-shPRMT5 on the migration ability of NCI-H460 (**D**), HCC827 (**G**) and LLC

(**J**) cells. Scale bar 200μm. Data were the means ± SEM of three independent experiments. Statistical differences were determined by two-tailed unpaired Student's t test, $^{**}P < 0.01$.



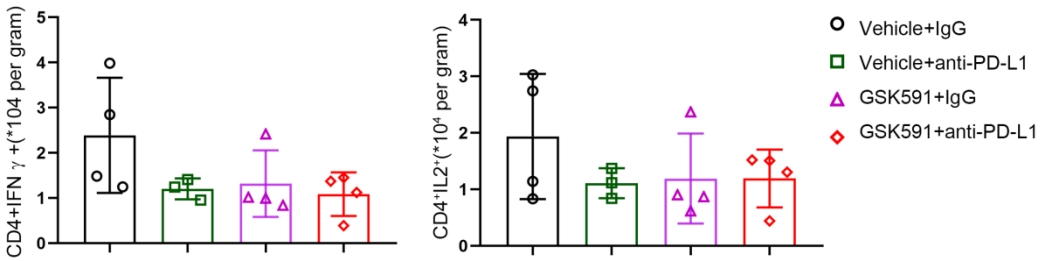
Supplementary Figure 2 Inhibition of PRMT5 suppresses lung cancer cell growth in nude mice.

(**A, B and C**) Nude mice bearing LLC tumors were treated with GSK591 (50mg/kg) or vehicle (n=5/group) for 12 days. Tumor growth curves (**A**), images of resected tumor (**B**), and tumor weights (**C**) were shown. Statistical differences were determined by two-tailed unpaired Student's t test, $^{**}P < 0.01$, $^{***}P < 0.001$. (**D and E**) SDMR (**D**) and PD-L1 (**E**) expression was analysis of tumor sections (N=5 each group) from vehicle and GSK591 treated group by western blot; β-actin served as loading control.



Supplementary Figure 3 RMT5 inhibition reduced T cell infiltration into lung tissue.

C57BL/6 mice bearing LLC tumor were treated with GSK591 (50mg/kg, n=4) or vehicle (n=5) for 12 days. Mice were sacrificed on day 20 after implantation. (**A**) Flow cytometry analysis of tumor infiltrating $CD8^+$ T and $CD4^+$ T cells. The representative plots were shown (Left). Bar graph was the means ± SEM (Right). Statistical differences were determined by two-tailed unpaired Student's t test, $*P < 0.05$.(**B-E**) Flow cytometry analysis of tumor infiltrating Treg cells (**B**), macrophages (**C**), B cells (**D**) and NK cell (**E**).



Supplementary Figure 4 Combined PRMT5 inhibition and anti-PD-L1 antibody treatment had no impact on CD4 T cells cytokines expression.
C57BL/6 mice bearing LLC tumors following treatment with IgG (10mg/kg), anti-PD-L1 (10mg/kg), GSK591 (50mg/kg) and the combination (n=4 per group) (day 18 post tumor cell implantation). Quantification of CD4 T cells expressing effector markers (IFNγ and IL-2) as per gram of tumor.

Supplementary Table 1. List of antibodies used for flow cytometry

antibody	Company	Cat#
PE Anti-mouse PDL1	Biolegend	124307
BV421 Anti-human PDL1	BD	563738
APC-cy7 Anti-human CD8	Biolegend	344714
Percpcy5.5 Anti-mouse CD8	BD	
FITC Anti-Mouse CD4	BD	557307
Annexin V PE Apop Dtec Kit	BD	559763
BV421 Anti-human IFNγ	BD	562988
APC Anti-human TNF-α	Invitrogen	17-7349-82
PE Anti-human Granzyme B	Biolegend	372208
BV421 Anti-mouse TNFα	Invitrogen	48-7321-82
APC Anti-mouse IFNγ	Invitrogen	17-7311-81
PE-Cy™7 Anti-Mouse CD45	BD	552848
APC-cy7 Anti-mouse CD3	BD	557596
PerCP/Cyanine5.5 Anti-human CD4	Biolegend	317428
BV421 Anti-mouse CD8a	Biolegend	100737
PE Anti-mouse IL-2	Invitrogen	12-7021-82

Supplementary Table 2. Primers sequences

Genes	Species	Sequence(5’-3’)
Prmt5-F	Mouse	CTGAATTGCGTCCCCGAAATA
Prmt5-R	Mouse	AGGTTCCTGAATGAACTCCCT
Actb -F:，	Mouse	TGTCCACCTTCCAGCAGATGT
Actb -R:	Mouse	AGCTCAGTAACAGTCCGCCTAG
CD274-F	Human	ACAGCTGAATTGGTCATCCC
CD274-R	Human	TGTCAGTGCTACACCAAGGC
GAPDH-F	Human	GGAGCGAGATCCCTCCAAAAT
GAPDH-R	Human	GGCTGTTGTCATACTTCTCATGG
STAT1-F	Human	CGGCTGAATTTCGGCACCT
STAT1-R	Human	CAGTAACGATGAGAGGACCCT
Cd274-F	Mouse	AAGCCTCAGCACAGCAACTTCAG
Cd274-R	Mouse	TGTAGTCCGCACCACCGTAGC
CD274-Promoter-F	Human	AACCAATGCAAGGGCTATCTC
CD274-Promoter-R	Human	GTGCCTGTGTGCTCCCTTTTC
GAPDH-Promoter-F	Human	CTGAGCAGACCGGTGTCACATC
GAPDH- Promoter-R	Human	GAGGACTTTGGGAACGACTGAG
Gapdh-Promoter-F	Mouse	CTCAGGGCGCGAAAGTAAAG
Gapdh-Promoter-R	Mouse	CGGCCCGGAGTCTTAAGTAT
Cd274-Promoter-F	Mouse	CACCCCTGCTTTCACTGTTG
Cd274-Promoter-R	Mouse	GTGAAGTTTCCGCAGACCAC