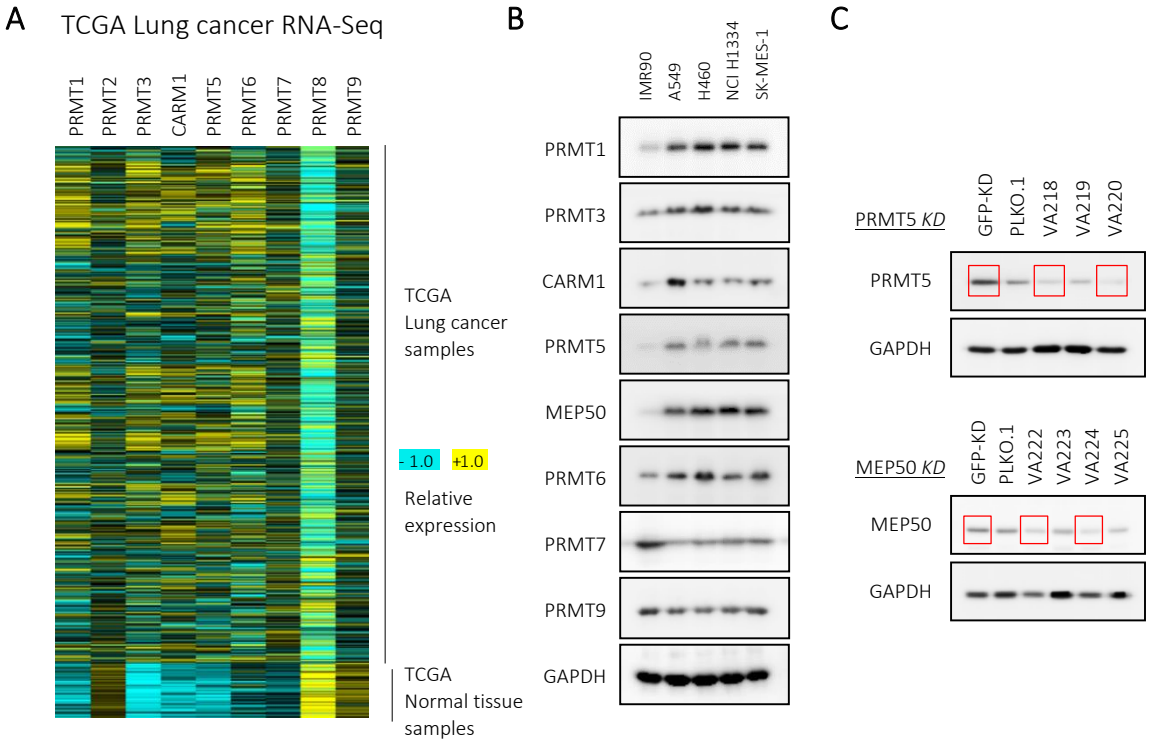
# A TGFβ-PRMT5-MEP50 Axis Regulates Cancer Cell Invasion through Histone H3 and H4 Arginine Methylation Coupled Transcriptional Activation and Repression

Hongshan Chen*°, Benjamin Lorton°, Varun Gupta†, and David Shechter°*

°Department of Biochemistry, †Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461

*For correspondence: david.shechter@einstein.yu.edu; hongshan.chen@einstein.yu.edu




**Supplementary Figure S1, Related to Figure 1.** PRMTs expression profile in lung cancer cell line and PRMT5 and MEP50 shRNA knockdown.

**A.** Heatmap of gene expression of PRMT 1-9 from TCGA lung cancer RNA-Seq data in normal and patients with lung adenocarcinoma (LUAD) or squamous cell carcinoma (LUSC), ranked by sample type (tumor or normal tissue).

**B.** Whole cell lysate immunoblots for PRMTs and MEP50 in the lung fibroblast cell line (IMR90) and corresponding lung cancer cell lines. GAPDH is a loading control.

**C.** Whole cell lysate immunoblots for PRMT5 and MEP50 in A549 cells (Lung adenocarcinoma). GAPDH is a loading control. Different shRNAs against PRMT5 or MEP50 are indicated. shRNAs against GFP or empty vector (PLKO.1) were used as control.

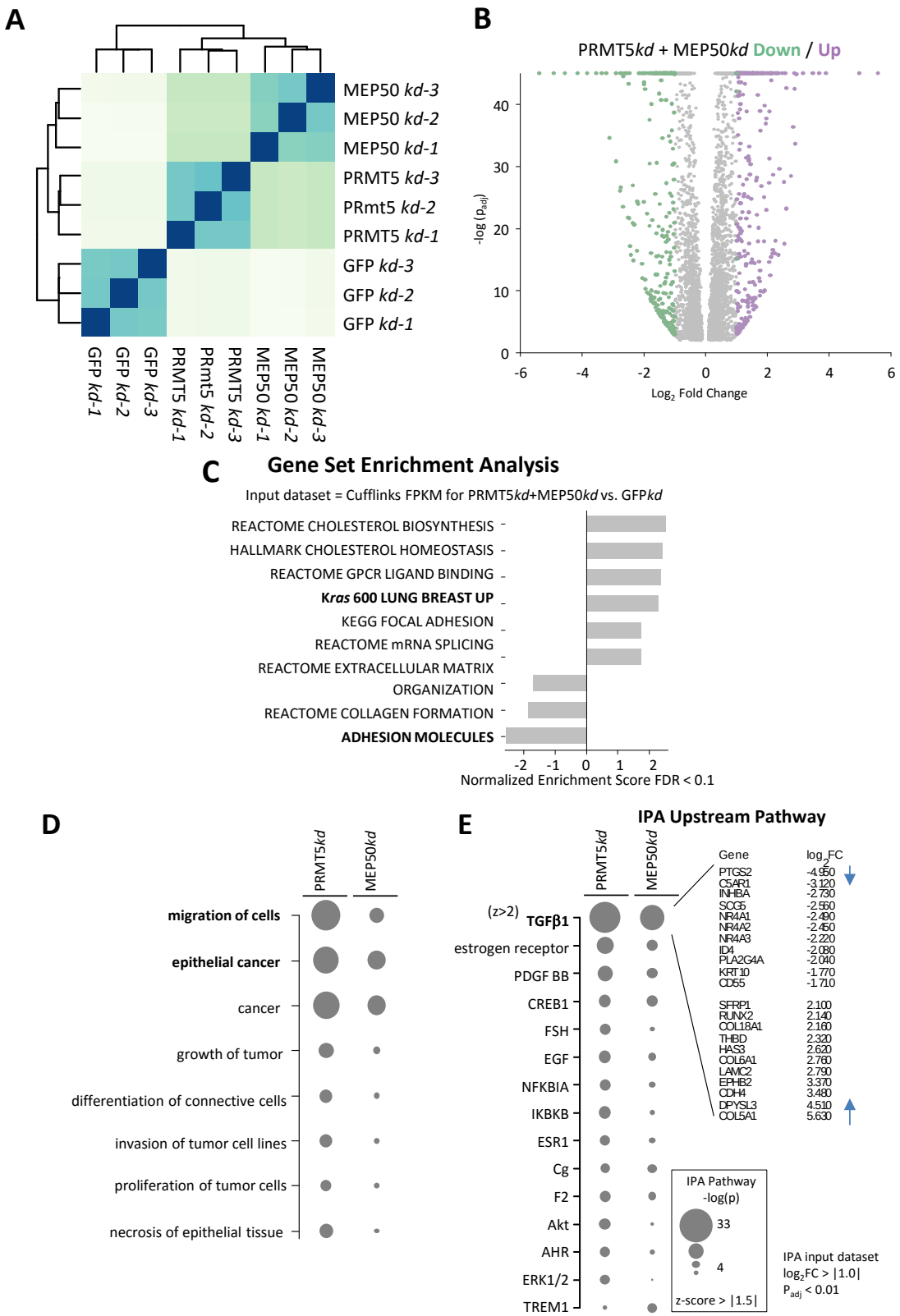

A
MEP50 kd-3
MEP50 kd-2
MEP50 kd-1
PRMT5 kd-3
PRmt5 kd-2
PRMT5 kd-1
GFP kd-3
GFP kd-2
GFP kd-1
GFP kd-1
GFP kd-2
GFP kd-3
PRMT5 kd-1
PRMT5 kd-2
PRMT5 kd-3
MEP50 kd-1
MEP50 kd-2
MEP50 kd-3
B
PRMT5kd + MEP50kd Down / Up
-log (padj)
Log2 Fold Change
C
Gene Set Enrichment Analysis
Input dataset = Cufflinks FPKM for PRMT5kd+MEP50kd vs. GFPkd
REACTOME CHOLESTEROL BIOSYNTHESIS
HALLMARK CHOLESTEROL HOMEOSTASIS
REACTOME GPCR LIGAND BINDING
Kras 600 LUNG BREAST UP
KEGG FOCAL ADHESION
REACTOME mRNA SPLICING
REACTOME EXTRACELLULAR MATRIX ORGANIZATION
REACTOME COLLAGEN FORMATION
ADHESION MOLECULES
Normalized Enrichment Score FDR < 0.1
D
PRMT5kd
MEP50kd
migration of cells
epithelial cancer
cancer
growth of tumor
differentiation of connective cells
invasion of tumor cell lines
proliferation of tumor cells
necrosis of epithelial tissue
E
IPA Upstream Pathway
PRMT5kd
MEP50kd
(z>2)
TGFβ1
estrogen receptor
PDGF BB
CREB1
FSH
EGF
NFKBIA
IKBKB
ESR1
Cg
F2
Akt
AHR
ERK1/2
TREM1
Gene
log2FC
PTGS2 -4.950
C5AR1 -3.120
INHBA -2.730
SCG5 -2.560
NR4A1 -2.490
NR4A2 -2.450
NR4A3 -2.220
ID4 -2.080
PLA2G4A -2.040
KRT10 -1.770
CD55 -1.710
SFRP1 2.100
RUNX2 2.140
COL18A1 2.160
THBD 2.320
HAS3 2.620
COL6A1 2.760
LAMC2 2.790
EPHB2 3.370
CDH4 3.480
DPYSL3 4.510
COL5A1 5.630
IPA Pathway -log(p)
33
4
z-score > |1.5|
IPA input dataset
log2FC > |1.0|
Padj < 0.01

**Figure S2, Related to Figure 2.** Transcriptome is dramatically altered upon PRMT5-MEP50 knockdown.

**A.** The replicates of the PRMT5 and MEP50 knockdown RNA-Seq are highly correlated as illustrated by DESeq2 clustering.

**B.** Volcano plot of Spearman's correlation coefficient for the differentially regulated genes altered in both PRMT5 and MEP50 knockdowns versus the corresponding significance score ($-\log_2 (p_{adj})$ ).

**C.** Functional annotation by gene set enrichment analysis of genes changes upon PRMT5 and MEP50 knockdown in A549 cells. Enriched groups are ranked by the significance *p*-value.

**D.** Dotplot of gene ontology terms enriched on PRMT5 and MEP50 knockdown upregulated genes are shown in dots scaled by -log(p).

**E.** Dotplot of Ingenuity Pathway Analysis (IPA) diseases and functions enriched in both knockdowns are shown in dots scaled by –log(p).

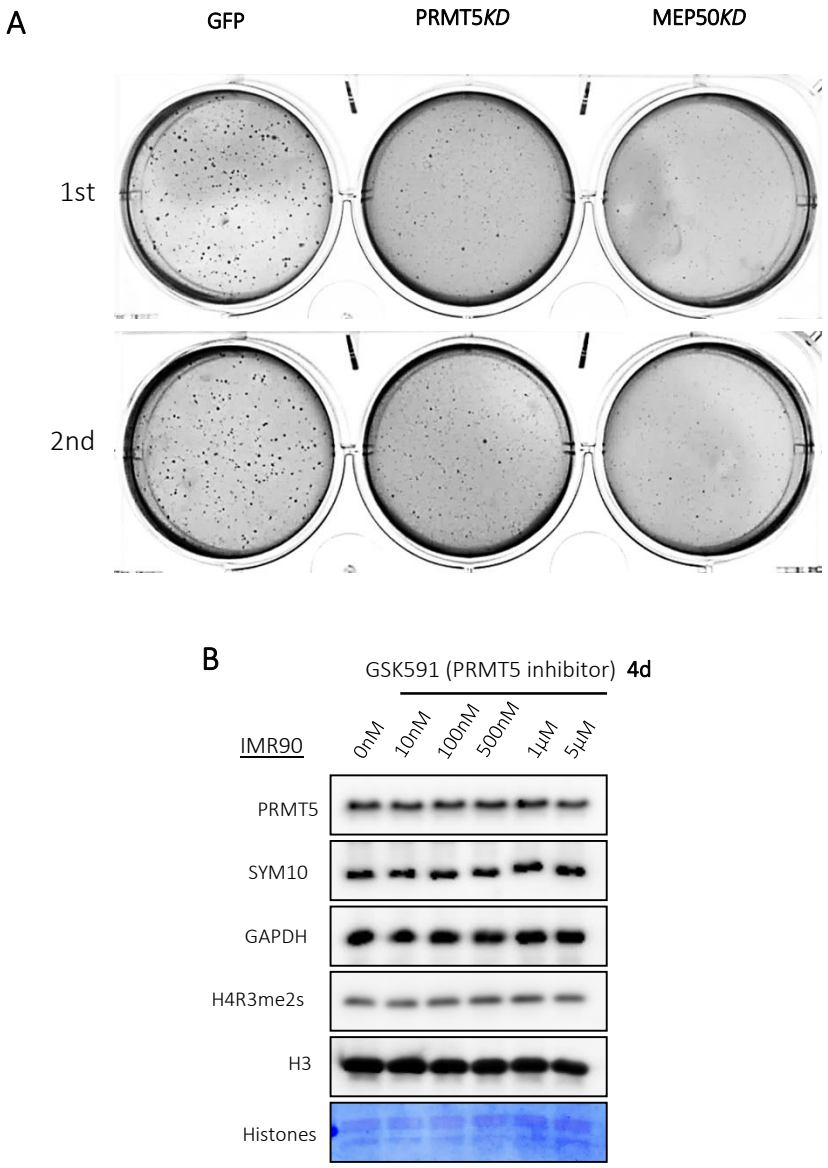



**Figure S3, Related to Figure 3.** PRMT5-MEP50 knockdown can prevent lung cancer cell mobility.

**A.** Representative crystal violet staining for colonies after 14d cell culture of A549 cells expressing shRNA targeted against GFP as a control (GFP*kd*), against PRMT5 (PRMT5*kd*) or against MEP50 (MEP50*kd*). 1st and 2nd represent 2 independently biological experiments.

**B.** Normal lung fibroblast cell line (IMR90) was treated with various indicated dosages of GS591 for 4 days and lysates or extracted histones were blotted for PRMT5, SYM10 (methylated SmD3), GAPDH (control), H4R3me2s, and H3 as indicated. DB71 stain of extracted histones is also shown.

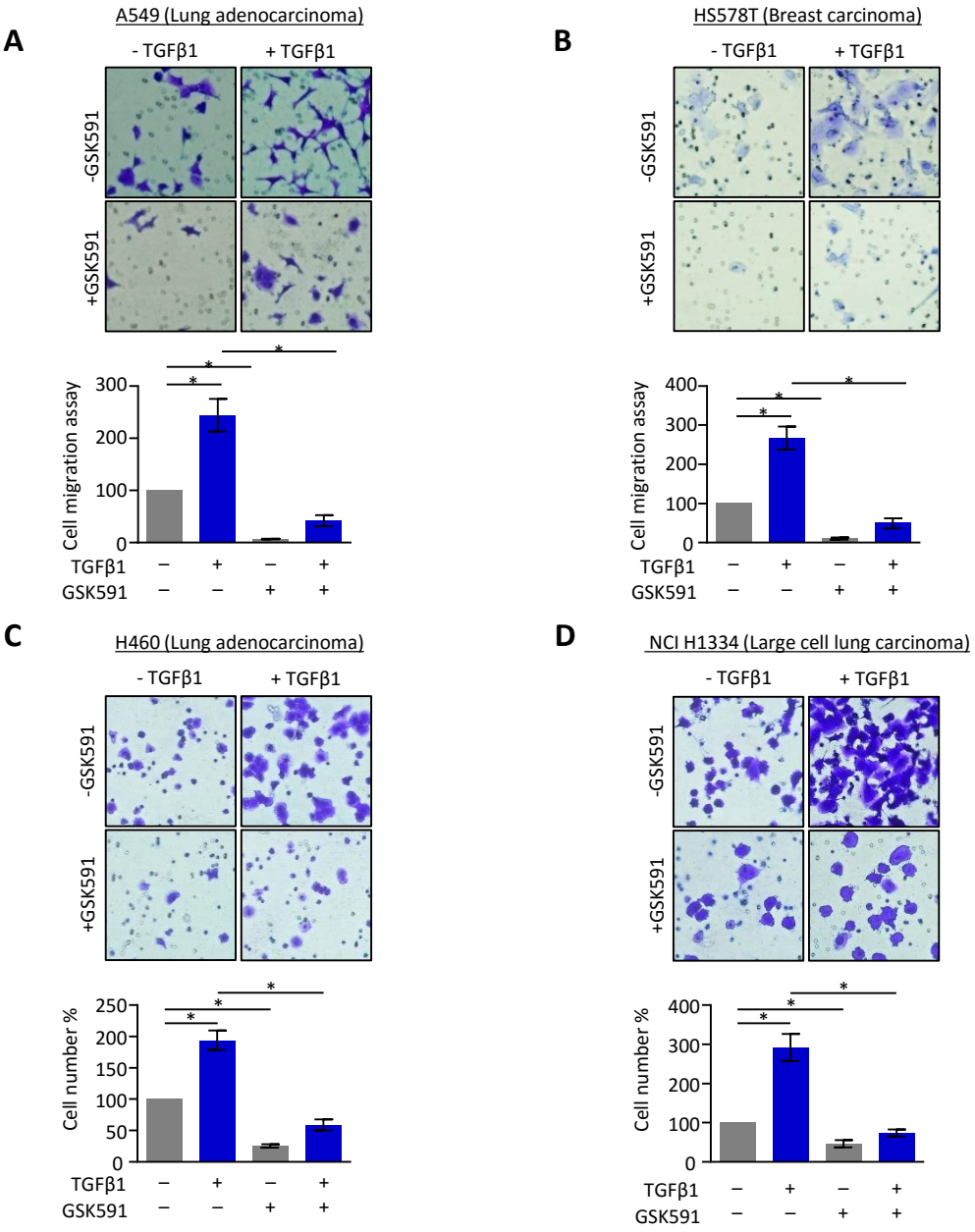



**Figure S4, Related to Figure 4.** PRMT5 inhibitor GSK591 blocked both lung and breast cancer cell lines TGFβ-stimulated cell mobility.

**A.** Cell migration assay for A549 and (**B**) HS578T cell lines. Migration of indicated cells treated with (+) or without (-) 500 nM GSK591 for 4 days and (+) or without (-) 10 nM TGFβ1 for 2 days.

**C.** Cell invasion assay for H460 and (**D**) NCI H1334 cell lines treated treated with (+) or without (-) 500 nM GSK591 for 4 days and (+) or without (-) 10 nM TGFβ1 for 2 days. Top: Representative crystal violet staining of invaded cells on the underside of the porous polycarbonate membrane through a phase-contrast microscope (20X) is shown. Bottom: Quantification of the invaded cells shown in. Values are mean ± S.E.M. of three independent experiments. *$p < 0.05$ from one-way ANOVA test.

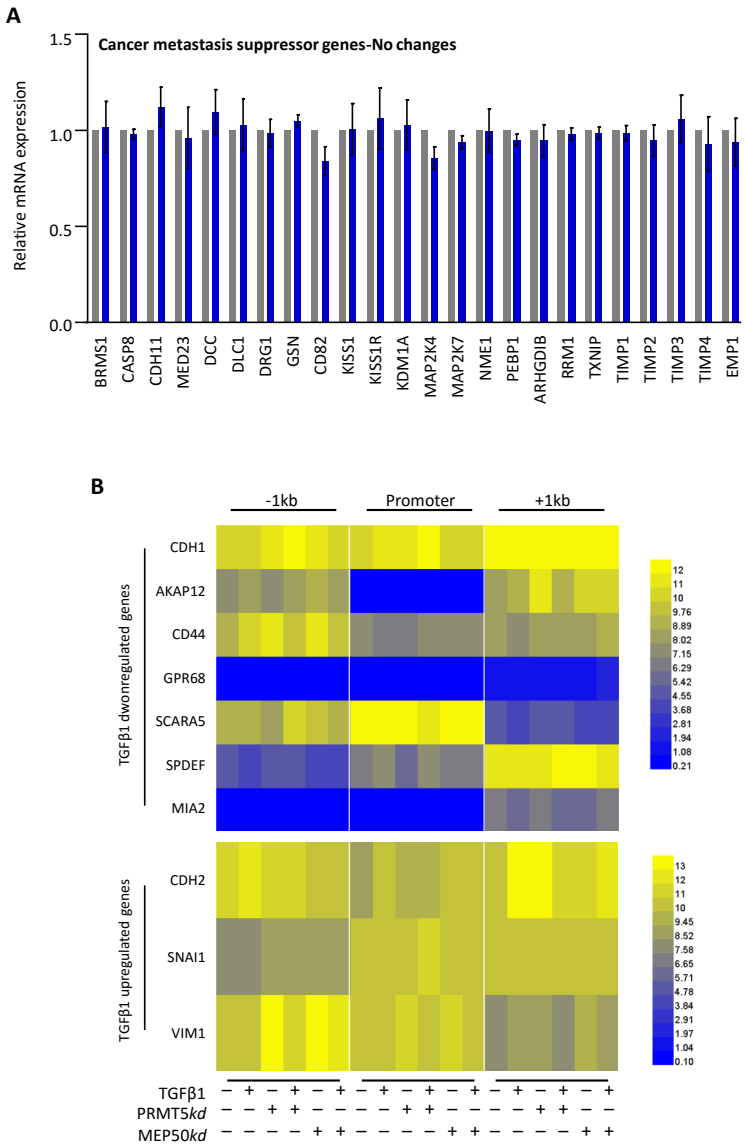



**Figure S5, Related to Figure 6.** Controls for qRT-PCR and ChIP experiments

**A.** Relative mRNA levels of indicated cancer suppressor genes in A549 cells treated with 10 nM of TGFβ1 for 2 days were determined by qRT-PCR. β-Actin was used as an internal control. Values are means ± S.E.M. of three independent experiments. To normal cells, there has no significant gene expression changes for the ones treated with 10 nM of TGFβ1 for 2 days for the genes listed. *p* value are measured with one-way ANOVA test.

**B.** Heatmap generated by ChIP-qPCR values for histone H3 values at -1kb, the promoter, or +1kb of the indicated genes demonstrate the H3 was pulled down evenly as the ChIP control for the various histone PTMs in Figure 6. ChIP-qPCR of TGFβ1-downregulated genes are arrayed on the top and TGFβ1-upregulated genes are on the bottom. The heatmap is arrayed from blue (no enrichment) to yellow (maximal enrichment). Between different groups there is no significant H3 enrichment changes. *p* value are measured with one-way ANOVA test.

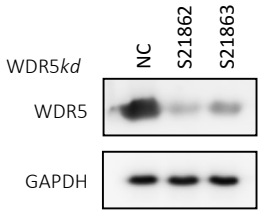



**Figure S6, Related to Figure 7.** Confirmation of depletion of WDR5 in A549 cell line.

Immunoblots for WDR5 and GAPDH as control, from A549 cells infected with siRNA targeted against negative control (NC) or against 2 independent WDR5 siRNA (ID s21862 and s21863) for 3 days.

**Table S1.** Antibodies used in this study.

| Factor or PTM | Vendor | Cat Number | Dilution For blotting | Vol.for ChIP (µl) |
|---|---|---|---|---|
| PRMT1 | Millipore | 07-404 | 1:5,000 | |
| PRMT3 | Gene Tex | GTX116478 | 1:5,000 | |
| CARM1 | Cell Signaling | 4438 | 1:5,000 | |
| PRMT5 | Millipore | 07-405 | 1:5,000 | |
| MEP50 | LPBio | AR-0145-S | 1:5,000 | |
| PRMT6 | Cell Signaling | 14641 | 1:5,000 | |
| PRMT7 | Pierce | PA5-30748 | 1:10,000 | |
| PRMT9 | Homemade | N/A | 1:5,000 | |
| E-cadherin | Cell signaling | 3195 | 1:3,000 | |
| Vimentin | Cell signaling | 5741 | 1:3,000 | |
| Snail | Cell signaling | 3879 | 1:2,000 | |
| SYM10 | Millipore | 07-412 | 1:5,000 | |
| SNRPD3 | Abcam | ab157118 | 1:10,000 | |
| WDR5 | Millipore | 07-706 | 1:5,000 | |
| GAPDH | Abcam | ab9484 | 1:10,000 | |
| H3R2me1 | Abcam | ab15584 | 1:5,000 | 8 |
| H3R2me2s | Abcam | Ab194684 | 1:5,000 | 6 |
| H3R8me2s | Novus Biologicals | NB21-1063 | 1:5,000 | 10 |
| H4R3me1 | Novus Biologicals | NB21-2011 | 1:10,000 | 10 |
| H4R3me2a | Active Motif | 39705 | 1:10,000 | 8 |
| H4R3me2s | Abcam | ab5823 | 1:10,000 | 3 |
| H3K4me3 | abcam | Ab8580 | 1:10,000 | 5 |
| H3 | Abcam | ab1791 | 1:100,000 | 4 |
| HRP-conjugated anti-rabbit secondary antibody | GE | NA834V | 1:30,000 | |
| HRP-conjugated anti-mouse secondary antibody | GE | NA931V | 1:30,000 | |

**Table S2.** ChIP primers used in this study.

The ChIP primers with red bold are the ones used for genes indicated in Figure 6.

| Primer set name | Location in the genome | Forward sequence | Reverse sequence |
|---|---|---|---|
| AKAP12 -1kb | chr6:151238642+151238737 | TCAAAATGGGGGAAATGTGT | CACGAAGGCCAATTTCTCTG |
| AKAP12 P | chr6:151239706+151239799 | TCCGGTAACAGCCTCATTTC | ATCCTCCGGAACAAGTGATG |
| AKAP12 +1kb | chr6:151240812+151240886 | AGAGAACTTCCCAGCCCATC | ACCCTGAAAAGCCTCCAAAG |
| CD44-1kb | chr11:35138161+35138345 | ATGGTGGATGGTTGTGGTTT | CATCCTCCTGTCCATCCACT |
| CD44 P | chr11:35138742+35138891 | AACCCAGAGATCTTGCTCCA | GAGACGCACTGGCTTTCTTC |
| CD44 +1kb | chr11:35139817+35140003 | TGGTCACAGAAGGGATCACA | TCATGGAGACCCAAGACCTC |
| MED23-1kb | chr6:131585753+131585847 | TTTTTCCCCTCTGCTGTATTCT | AATTTCCCTGGATAGGCTTTT |
| MED23 P | chr6:131586621+131586745 | TAGGCCAATGTCAGTCACCA | TGATTGTTTCCCGACTCCAT |
| MED23 +1kb | chr6:131587945+131588058 | GGAGTGTCGTGGGGTAGATG | GGGTGTGTGTGAGGGAGAAC |
| GPR68-1kb | chr14:91231464+91231541 | CAGATGAATTCCAGGGATGG | TCTTGGCTCGTTGCTTTTCT |
| GPR68 P | chr14:91232216+91232332 | CTGGGCTCTGTCCTCAAAAG | AGAGCCTTCTCTCCCTCCAC |
| GPR68 +1kb | chr14:91233661+91233767 | CACAAACTCTGCAGGAAGCA | GAATAAGCCGTGTCCTCTGC |
| SCARA5-1kb | chr8:27869187+27869300 | GCTCTTCTCCATGGAACCTG | GGAAATCCACAAAGGAAGCA |
| SCARA5 P | chr8:27869627+27869703 | TCTTGTAAGATGGGCCTTGG | TGGCTCAAGCCTTATTTGGT |
| SCARA5 +1kb | chr8:27870841+27870986 | AGAGGCTTGGAAGATGCAAA | GGAGGAGAGCTGATGTGGAG |
| SPDEF-1kb | chr6:34536714+34536828 | CAAGGGAGTGAGACACAGCA | TGGCAGTGAGCAAATAGCAC |
| SPDEF P | chr6:34537854+34537970 | AACTCAGGGGTGCAGATGTC | AGGGCAGTGACTCGACAAAG |
| SPDEF +1kb | chr6:34538618+34538715 | GGTGTAGTTGCGGTGAGGTT | TACCGGGAGGTTTGTGACTC |
| MIA2-1kb | chr14:39233121+39233300 | CCAACGGAAATCACACTGTA | TCTGCCATTGAAAGAGGTCA |
| MIA2 P | chr14:39234123+39234200 | ATTTGGCGTTCACAGAATCC | AGGTCTGCCAGCAGTTTTGT |
| MIA2 +1kb | chr14:39234900+39235017 | TGCAAAATAAATTACATCCCAAT | AAGCAATTTTTGATTCTGCTGAG |
| CDH1 -1kb | chr16:68735940+68736048 | TGCCTGGCCCTATTGTTACT | CCCTATGCTGTTGTGGGACT |
| CDH1 P | chr16:68737215+68737321 | GTGAACCCTCAGCCAATCAG | TCACAGGTGCTTTGCAGTTC |
| CDH1 +1kb | chr16:68738469+68738561 | AGAAATTGCACTCCCACACC | GATCCCCAAATCTGCGTAAA |
| CDH2 -1kb | chr18:27950018+27950116 | CAGCATGGAGGCACAGTCTA | GAGCTTGCCTCTGGGAATTT |
| CDH2 P | chr18:27951910+27952010 | GGCACATAAAATCCCAGTGC | TGGGCTCAGAGGGAATATCA |
| CDH2 +1kb | chr18:27951682+27951787 | TGGTCTCATCCCCCAAGATA | TGCTTCAACACGCTTTTGTT |
| VIM -1kb | chr10:17226963+17227105 | ATGCCTTGTCCTCCTTTTCC | GTGTGCCTGGAACCCTTAGA |
| VIM P | chr10:17228395+17228494 | GGCCCAGCTGTAAGTTGGTA | CCTAGCGGTTTAGGGGAAAC |
| VIM +1kb | chr10:17229220+17229290 | GAGGGGACCCTCTTTCCTAA | GAGAGTGGCAGAGGACTGGA |
| SNAI1 -1kb | chr20:49981560+49981683 | CGGCACCAAGTGACTAAACA | CACAGGTCTCACCGTTCTTG |
| SNAI1 P | chr20:49983118+49983246 | GCGAGCTGCAGGACTCTAAT | GTGACTCGATCCTGGCTCA |
| SNAI1 +1kb | chr20:49983626+49983698 | GGGGTCCTACGTGTGAGAGA | TCCACAGGACAGACCAGGTT |

**Table S3.** RT-qPCR primers used in this study.

| Gene name | Forward sequence | Reverse sequence |
|---|---|---|
| ACTB | AGCTACGAGCTGCCTGAC | AAGGTAGTTTCGTGGATGC |
| PRMT5 | TTGCCGGCTACTTTGAGACT | ACAGATGGTTTGGCCTTCAC |
| MEP50 | AGCACTGCCTCTCTCTCACC | ACACGGCCAATTCCTCATAG |
| BRMS1 | GAAGGCACCTCTGGTTTCTG | CTGCCCTAGCCTTTTTGATG |
| CASP8 | TCCCCAAACTTGCTTTATGC | GACCCCAGAGCATTGTTAGC |
| AKAP12 | TGGCAGGAAGACATTCTGTG | GCGGGTGGAATTTAAACAAA |
| CDH1 | CAAGTGCCTGCTTTTGATGA | GTTTTCTGTGCACACCTGGA |
| CDH2 | TGCTTCAACACGCTTTTGTT | TGGTCTCATCCCCCAAGATA |
| CDH11 | CACGGCTCCTCCTTATGACT | CGAGGTCCCCAGTTCTGTAG |
| CD44 | AAGGAACCTGCAGAATGTGG | TCCAACGGTTGTTTCTTTCC |
| MED23 | AGCCATGAACAGTGGGTCTC | AATTTGGAACCCTTGCTGTG |
| DCC | GGGACCACTTTGGAAATGAA | AGAAGTGGCGATGATGGAAC |
| DLC1 | ATCATTCCAAGGCCAAACTG | GAGAATCTCCGTGCTTCCTG |
| DRG1 | GCTGGATGCTGAAACTGTGA | TGTTTCCTTCCACCACATCA |
| GAS1 | AGATTGTGGCCAGTGAGGAT | GGCGCAGATACAAACAGTGA |
| GSN | ACGGCTGAAGGACAAGAAGA | TGAGCTCACCAGGAACCTCT |
| CD82 | GATGGTCCTGTCCATCTGCT | TCAGTACTTGGGGACCTTGC |
| KISS1 | GACCTGCCTCTTCTCACCAA | TGGGTCTAGAATTCCCCACA |
| KISS1R | TTGGTCTCTTGTGACGTTCG | TGATCCAGAAAGTCCTGTGTTG |
| KLF17 | GGGAGAGGAAGGGACATAGC | TCAGGAACCTGGAAGTCACC |
| KDM1A | TCTTCTTTGCGGGAGAACAT | CCCAAAAACTGGTCTGCAAT |
| MAPK14 | TGTATTTGGGCCAAGGTGTT | CACCCTGTCCCTCTGGAGTA |
| MAP2K4 | CCCCCACGGTATCCTAAACT | CAGATGGGAAGTTGACAGCA |
| MAP2K7 | GGGCTGCCTGGTTTTATTTT | AGGGCTCCCCACTTAACACT |
| NME1 | AGAAAGGATTCCGCCTTGTT | GGCCCTGAGTGCATGTATTT |
| PEBP1 | TGTTGGGACATGGCAATCTA | CATTCAGCAACTCCAGACCA |
| ARHGDIB | CTCGGCCTGAGGAGTATGAG | TGGTCTTGCTTGTCATCGTC |
| RRM1 | CATCCACATTGCTGAGCCTA | GATTAGCCGCTGGTCTTGTC |
| TXNIP | CTTGCCCACTGTGACTTCAA | TCCTAACACAGGGCAGGAAG |
| GPR68 | CTGGGTCAGTGACATTGGTG | TGGGAAGCCAGTGTTTAAGG |
| TIMP1 | TGACATCCGGTTCGTCTACA | TGCAGTTTTCCAGCAATGAG |
| TIMP2 | CTCTCCATTTGGCATCGTTT | TTTGAGTTGCTTGCAGGATG |
| TIMP3 | GGAGAGCTGCAGAGTGTCCT | AGCTAGGGAAAGGGAACCAA |
| TIMP4 | GCCAGGACTATTCCCTTTCC | ATGACATTCGCCATTTCTCC |
| MMP2 | AGCGTGAAGTTTGGAAGCAT | CCTCCGGGTCCTTCTCTAGT |
| MMP9 | AAGCTGGACTCGGTCTTTGA | CCTGTGTACACCCACACCTG |
| SCARA5 | GGAGGAGAGCTGATGTGGAG | AGAGGCTTGGAAGATGCAAA |
| SPDEF | GACCAGTGAGGAGAGCTGGA | CATAGCTGTGGGGCTTGAGT |
| MIA2 | CAGAGCACATTCCCAAACCT | GCCCTGTATCCTCATCTCCA |
| EMP1 | GGCTCCTAGGCTCAGTGGTA | GCCTGTTGTTTGGTTTTGGT |
| ARHGAP29 | TAAACCACATGCTCCCATCA | CTTTGTCTGGGTCTGGCATT |
| SNAI1 | GCTCCACAAGCACCAAGAGT | ATTCCATGGCAGTGAGAAGG |