CRISPR/Cas9 screenings unearth protein arginine methyltransferase 7 as a novel driver of metastasis in prostate cancer

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Supplemental information

The supplemental information contain supplemental methods, figures and uncropped images of the Western blot results presented in this manuscript.

1- Supplementary methods.

2- Supplementary figures 1-7.

3-Uncropped western blot files.

Supplementary methods

CRISPR/Cas9 library screening viral production

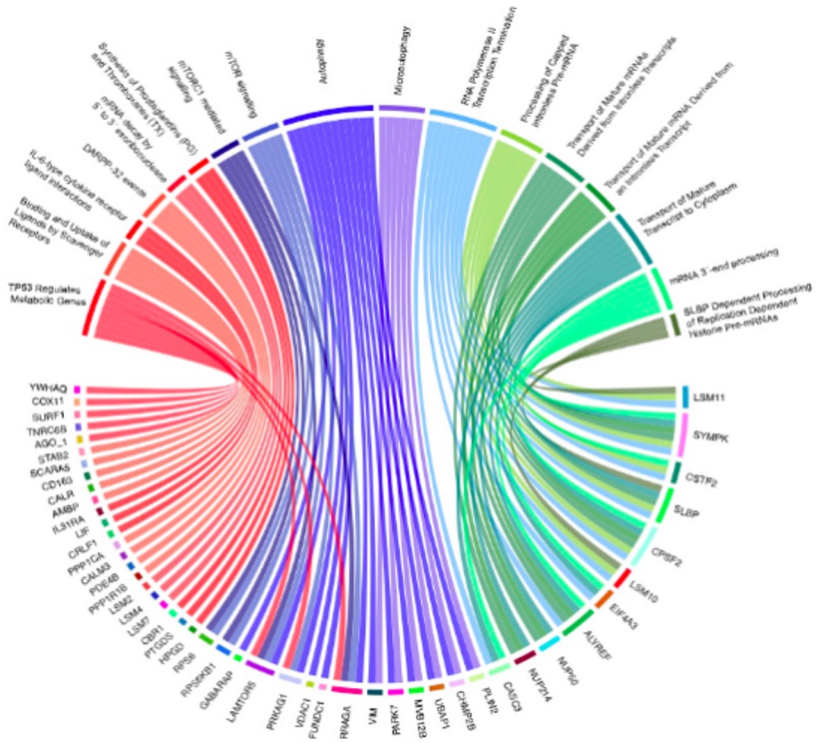
Lenti-Cas9 viral production was performed in HEK293T cells. A mixture of the 3 transfection plasmids (Packaging and enveloping plasmids psPAX2 (addgene#12260) and pMD2.VSVg (addgene#8454) together with lentiCas9-Blast plasmid (addgene#52962) was prepared and mixed with lipofectamine 2000 (Invitrogen) in a 1:3 ratio and transferred to the HEK293T cells. After 72h, the medium was collected, centrifuged at 2000 rpm for 5 min, and filtered with a 0.45 mm pore syringe filter. PC3 and DU145 cells were transduced with lenti-Cas9 virus to induce the expression of Cas9 in presence of 8 mg/mL of polybrene. 48h later, blasticidine selection (10 mg/ml) was performed for 5 days. Individual clones were expanded and the Cas9 presence was verified by western blot using Cas9 anti-mouse IgG (14697S, CST) and proliferation and migration tests were performed to ensure no clonal effect (data not shown). Subsequently, a mixture of the 3 transfection plasmids (Packaging and enveloping plasmids psPAX2 (addgene#12260) and pMD2.VSVg (addgene#8454) together with the sgRNA from library A (targeting 20,000 genes, including 1,864 sgRNA microRNA and 1,000 non-targeting controls) was prepared and mixed with lipofectamine 2000 in a 1:3 ratio and transferred to the HEK293T cells. After 72h, the medium was collected, centrifuged at 2000 rpm for 5 min and filtered with a 0.45 mm pore syringe filter.

Exogenous PRMT7 transfection to cells

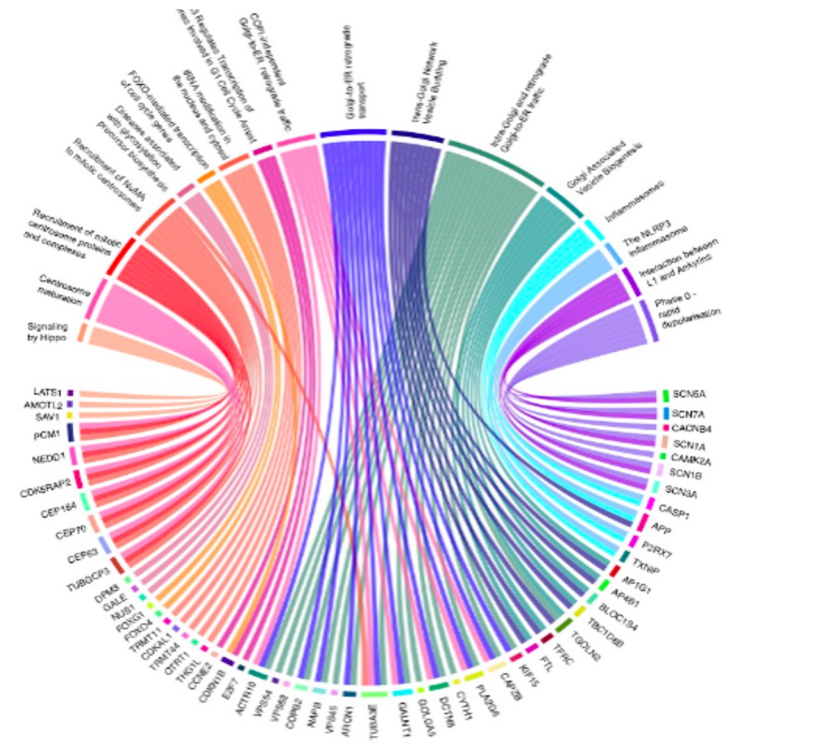
For exogenous PRMT7 expression pCDH1-PRMT7-GFP plasmid was obtained from addgene[37]. 4 ug of the plasmid were transfected to PRMT7 depleted and control PC3 using lipofectamine 2000 in a 1:3 ratio. Subsequently, proteins were extracted and levels of ITGb4 and ITGa1 were analyzed as described in Materials and methods of this study.

Supplementary Figure 1

A

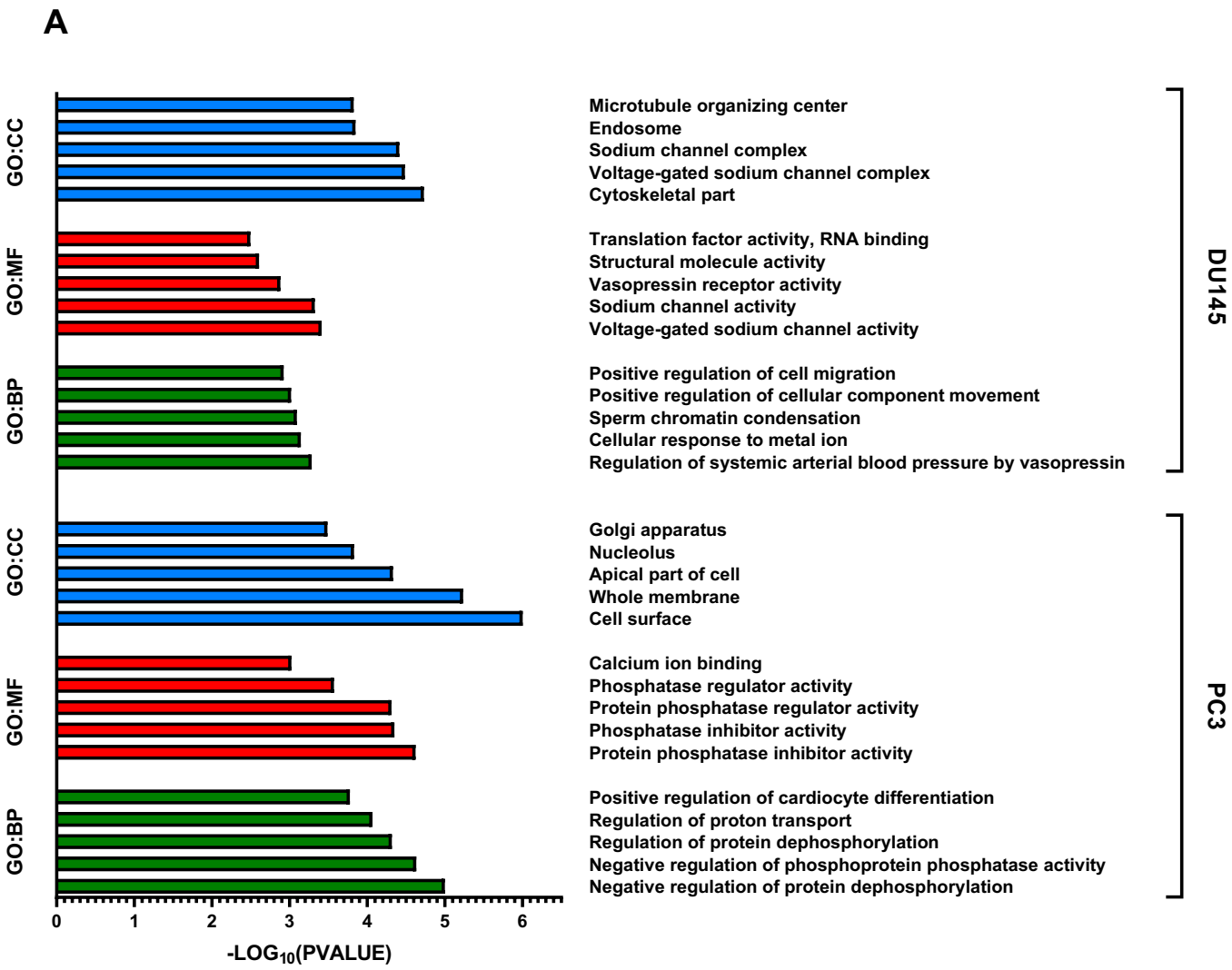


B



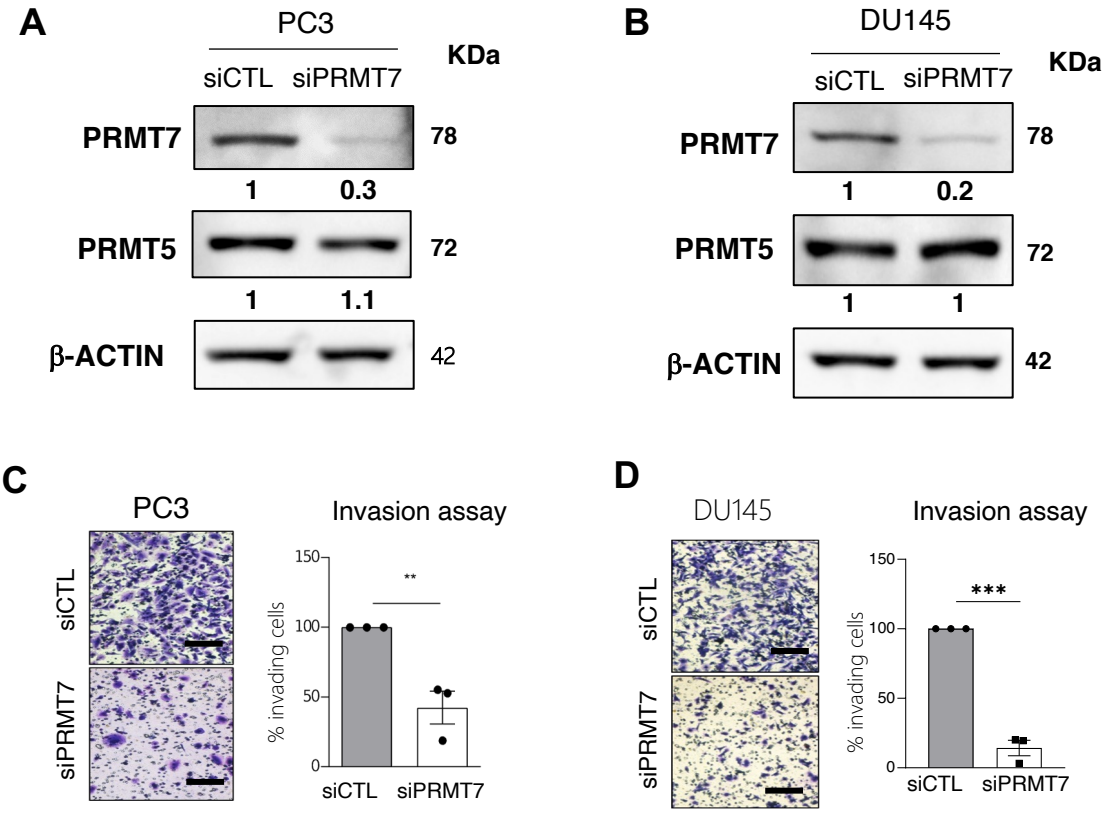
Supplementary Figure 1. Gene set enrichment analysis (GSEA) using Reactome database. A-B Results of GSEA Reactome analysis of **A,** PC3, **B,** DU145 screening results.

Supplementary Figure 2



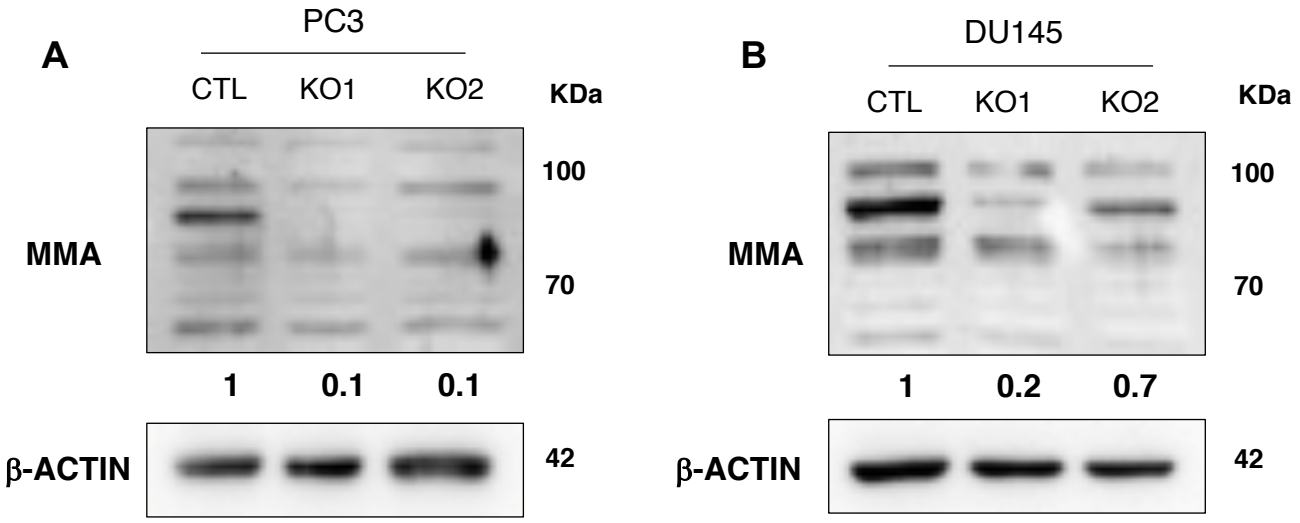
Supplementary Figure 2. Gene ontology enrichment analyses. A, Graphical representation of gene ontology enrichment analysis of gene ontology cellular component (GO:CC), gene ontology molecular function (GO:MF) and gene ontology biological pathway (GO:BP) analyses, for DU145 and PC3, respectively.

Supplementary Figure 3



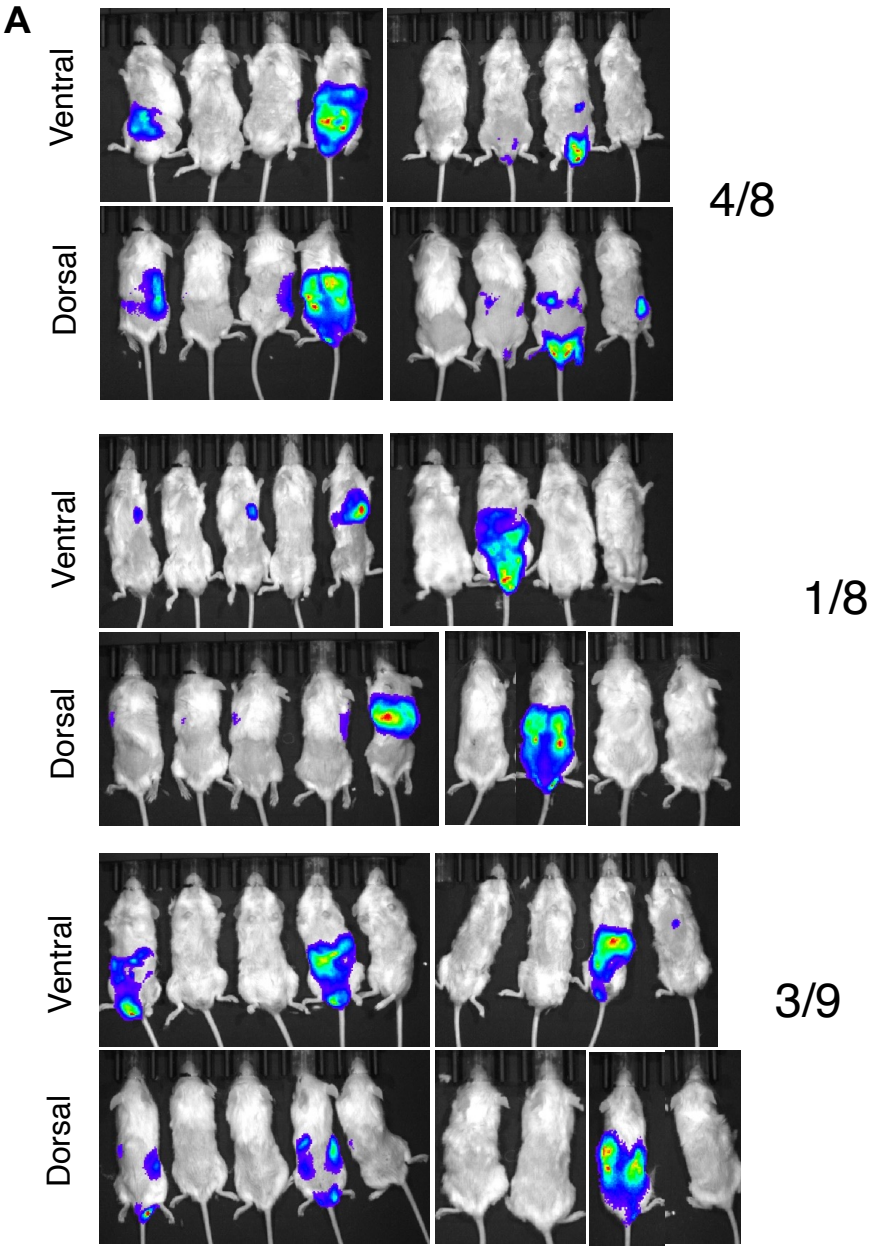
Supplementary Figure 3. Analysis of the effect of *PRMT7* inhibition by siRNA in PC3 and DU145 cells. A-B, Western blot analysis showing a reduction of PRMT7 expression levels while PRMT5 expression levels remain unaltered, for **A,** PC3, **B,** DU145 cells. **C-D,** Invasion assay using FBS as chemoattractant of siPRMT7 inhibited cells versus siCTL cells of **C,** PC3 and **D,** cell lines.

Supplementary Figure 4



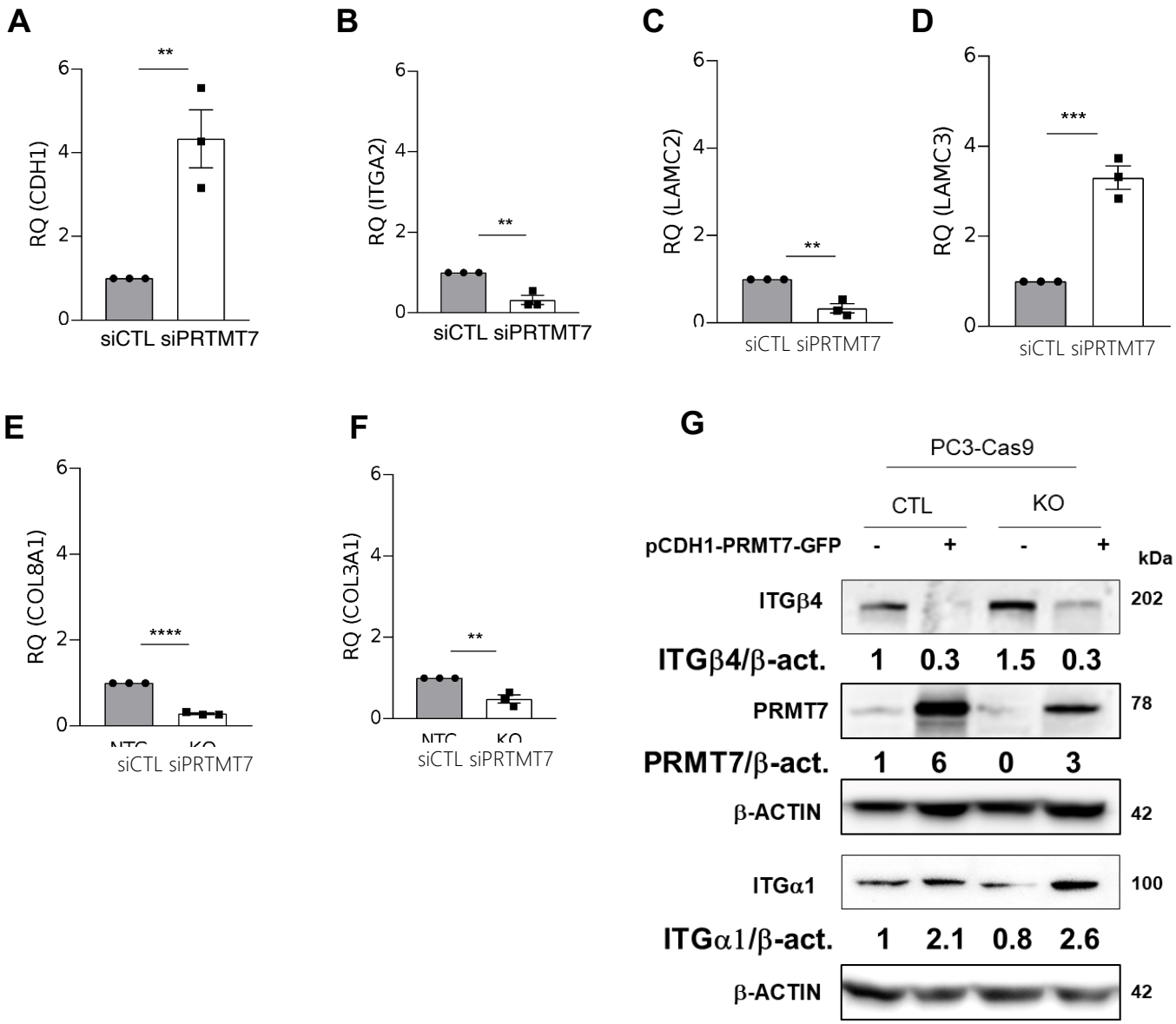
Supplementary Figure 4. PRMT7 depleted cells show reduced levels of MMA and lower viability. A-B, Western blot analyses of MMA levels upon PRMT7 genetic depletion in **A,** PC3-Cas9 and **B,** DU145-Cas9 cells.

Supplementary Figure 5



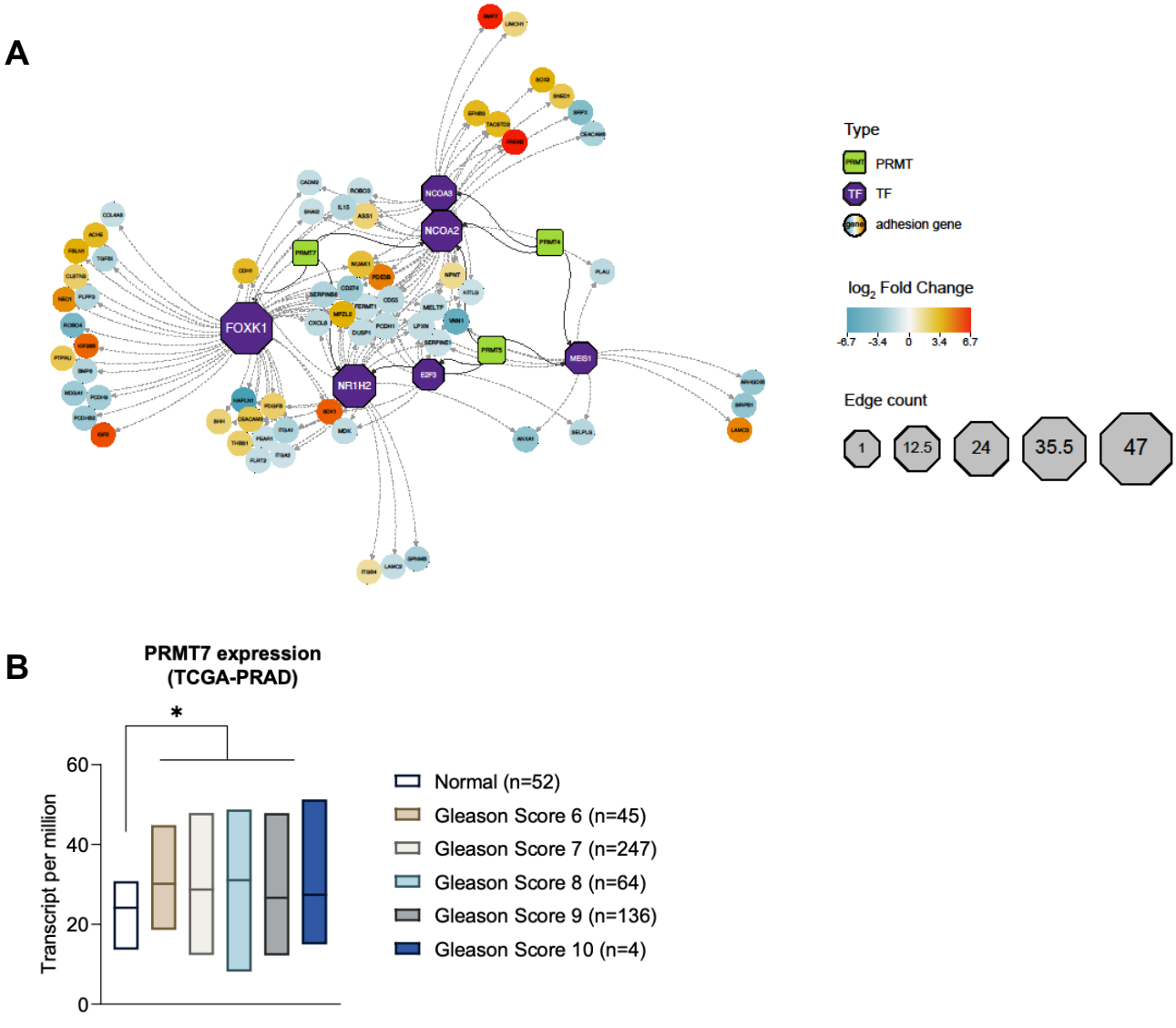
Supplementary Figure 5. Macrometastasis observed upon luciferine inoculation in mice A, Picture of mice in the IVIS imager **B,** Picture of liver macrometastasis in a mouse inoculated with CTL PC3 cells.

Supplementary Figure 6



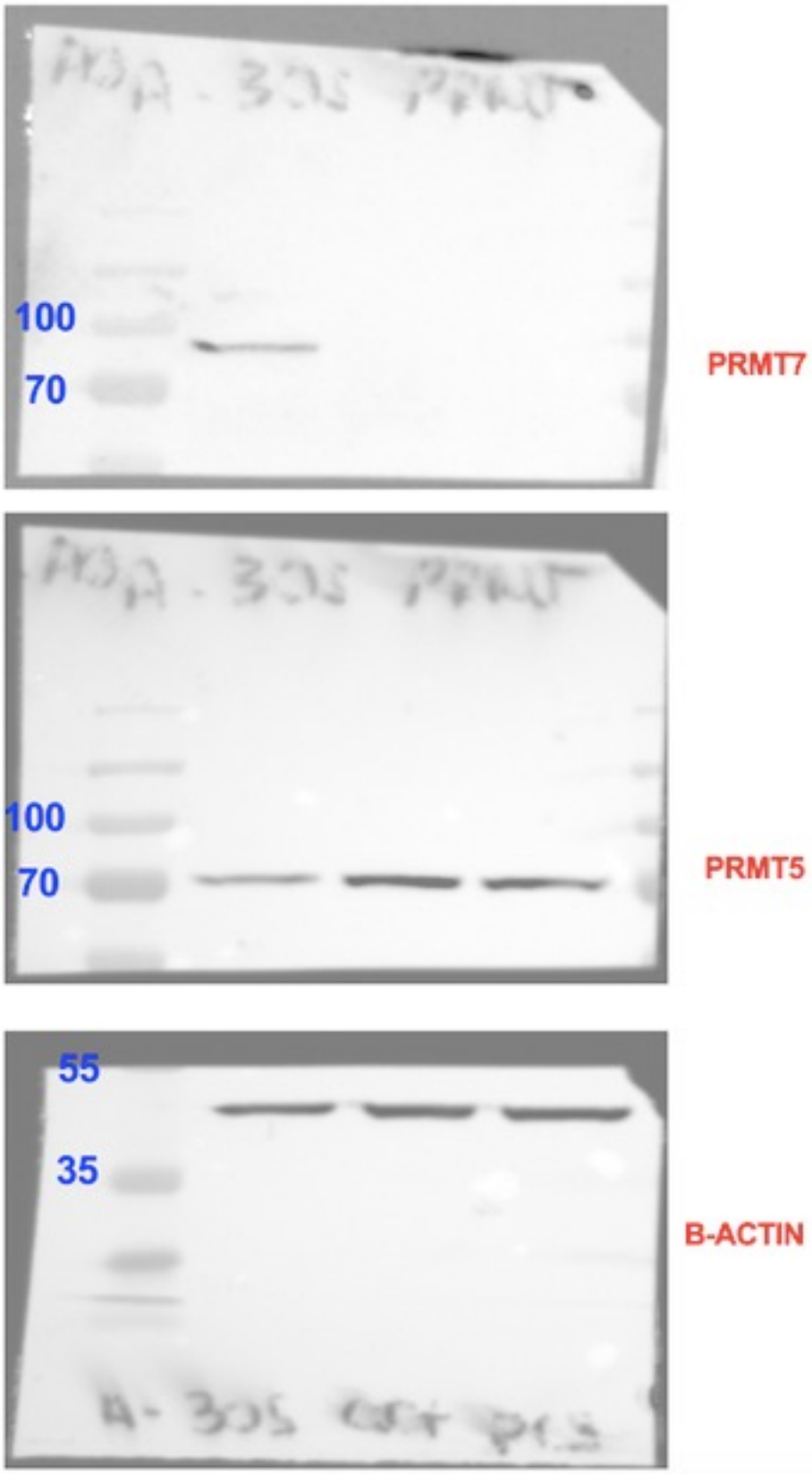
Supplementary Figure 6. PRMT7 produces a cell adhesion molecules gene expression switch in PC3 cells. A-F qPCR validation of **A** *CDH1*, **B** *ITGA2*, **C** *LAMC2*, **D** *LAMC3*, **E** *COL8A1*, **F** *COL3A1,* **G** Western blot showing ITGα1 and ITGβ4 protein levels upon exogenous PRMT7 overexpression in PC3 cells.

Supplementary Figure 7



Supplementary Figure 7. PRMT4, PRMT5 and PRMT7 transcription factor regulomes. A, Regulatory network of the three PRMTs (green squares) that methylate transcription factors (purple hexagons), and that bind to promoters of cell adhesion genes (circles) that are differentially expressed. Fold changes calculated in the differential gene expression analysis (PRMT7-KO vs. CTL cells) are represented by color. Edge count (size) indicates the number of connections per item of the network. **B,** *PRMT7* expression in PCa TGCA data base samples by Gleason score.

Uncroppeds of Figure 3B



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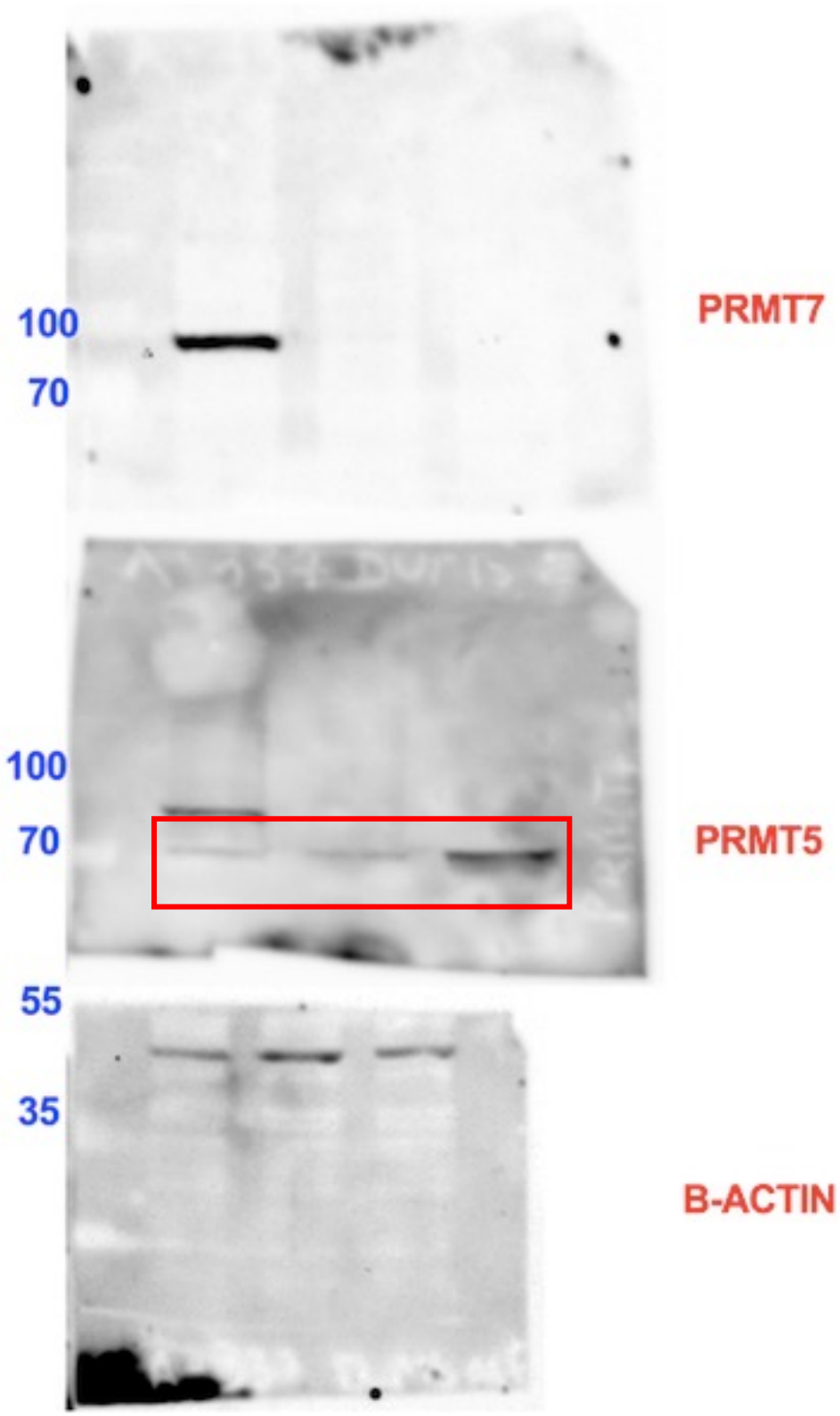

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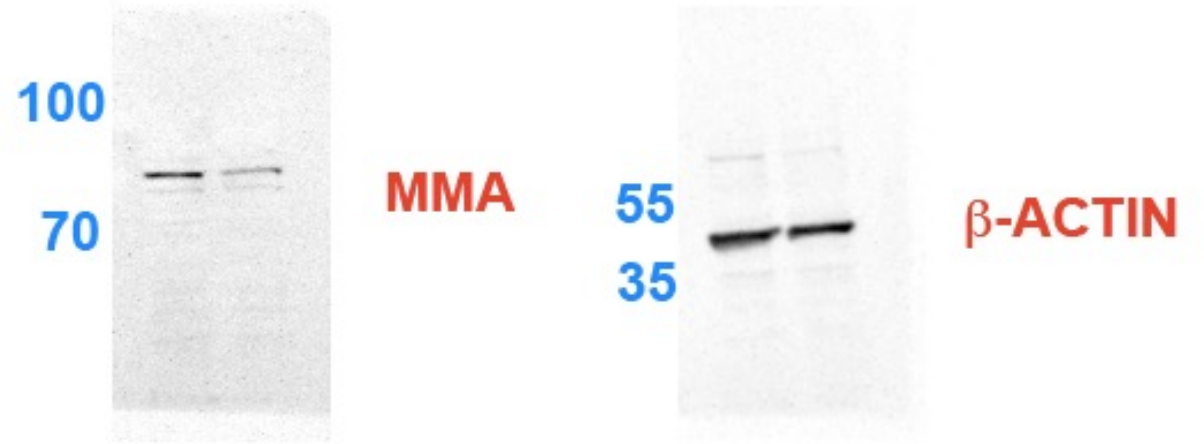

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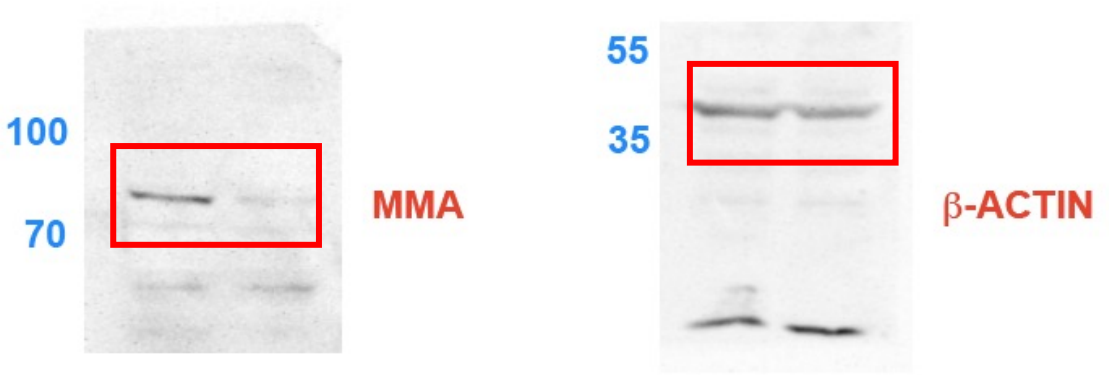

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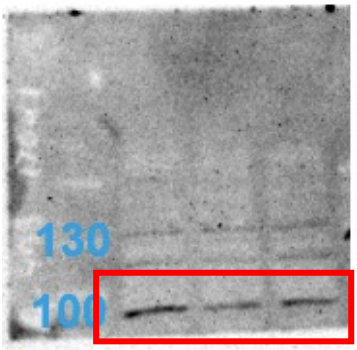
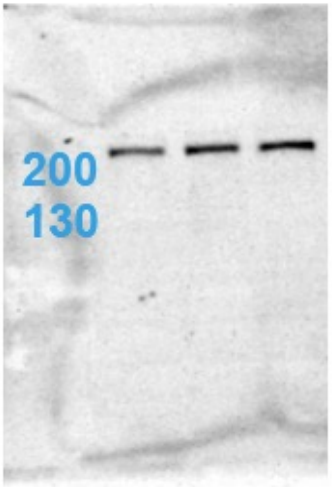
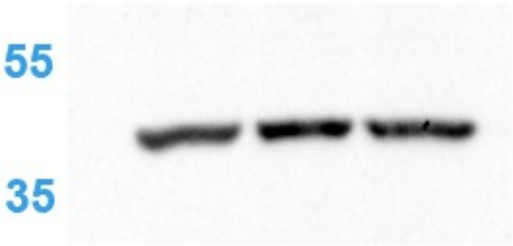


ITGα1

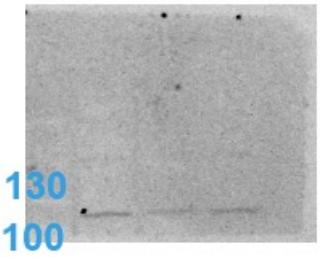


ITGβ4



β-ACTIN

Uncroppeds of Figure 6E



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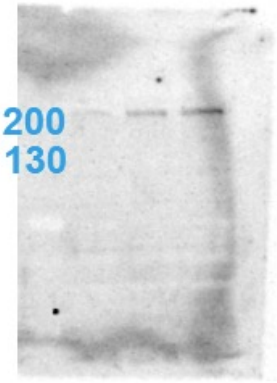

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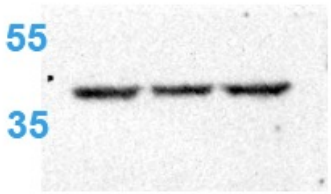
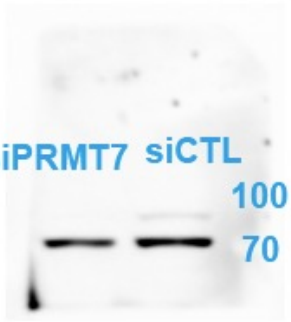


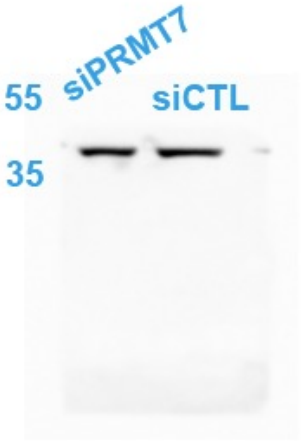
β-ACTIN

Uncroppeds of Supplementary Figure 2A

PRMT7



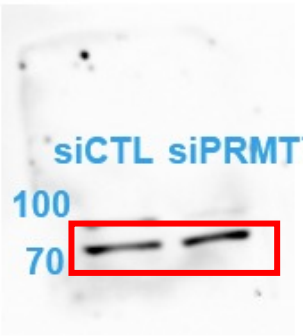
PRMT5



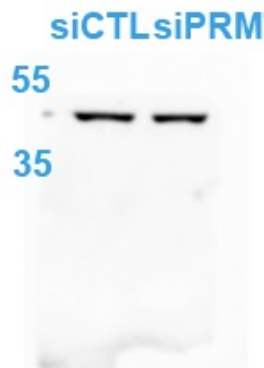
β-ACTIN

Uncroppeds of Supplementary Figure 2B

PRMT7



PRMT5



β-ACTIN

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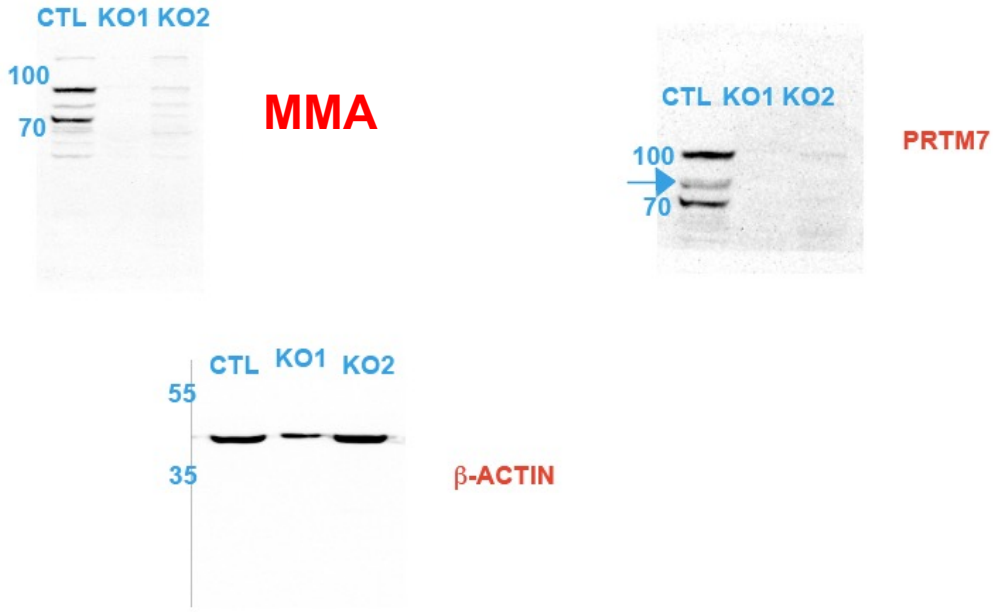

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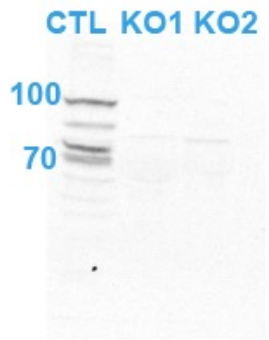
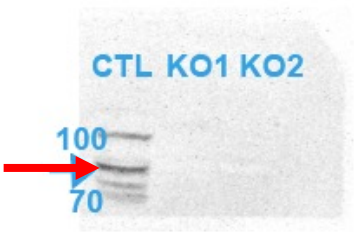
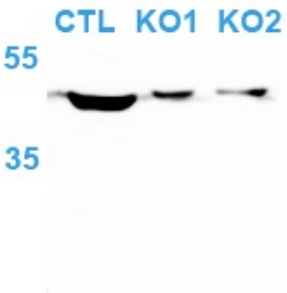


MMA



PRTM7



β-ACTIN

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