PRMT5 regulates IRES-dependent translation via methylation of hnRNP A1

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Experimental Procedures

GST Pull-down Assay – GST or GST-tagged Tudor domain proteins were expressed in E. coli strain BL21 by induction of IPTG for 4 hours at 30°C. Total bacteria were solicited and the lysate was incubated with Glutathione Sepharose 4B (GE Healthcare) at 4°C overnight. The sepharose bound proteins were washed with PBS for 3 times and eluted with 25mM reduced glutathione. PRMT5$^{flox/flox}$ MEFs were lysed with mild buffer and incubated with the recombinant GST or GST-SMN Tudor domain at 4°C overnight. Then sepharose were added and incubated for 1 hour. The beads were then pelleted and washed with PBS for 3 times and the bound proteins were eluted with SDS loading buffer and subjected to Western blotting for the detection of indicated proteins.

Antibodies – PRMT1 antibody (a gift from Dr. Stephane Richard), pan-ADMA substrate antibody (collaboration with Cell Signaling Technologies), anti-LDH (Santa Cruz) and anti-Lamin A/C (Santa Cruz), LC3B, eIF2α and phosphorylated eIF2α (S51) antibodies (from Cell Signaling Technologies).

Immunofluorescence Staining for hnRNP A1 – PRMT5$^{flox/flox}$ MEFs were treated with or without 4-hydroxytamoxifen for 7 days and plated onto coverslips and cultured for 24 hours. Cells were washed, fixed with 4% paraformaldehyde for 10 min at room temperature, permeabilized with PBS containing 0.2% Triton and 0.8% FBS and washed three times with PBS. The cells were blocked with PBS containing 1% FBS for 1 hour, and anti-hnRNP A1 antibody was added to the coverslips and incubated for 2 hours. The cells were stained with Alexa Fluor 647-conjugated anti-mouse antibody followed by staining with 0.25μg/ml DAPI.

Primers used for qPCR:
Primers for both mouse and human:
PRMT5: TCAAAGCAGCCATTCTCCCCAC (For.) and TGGTTGGTGCCTGTGATGATGAAC (Rev.)
MEP50: AGACACTTATTGTCAGCAAG (For.) and AATCTGTGATGCTGGCTTG (Rev.)

Primers for human only:
CCND1: CTCCTGTGCTGCGAAGTGG (For.) and CTTCTGTTCCTCGCAGACCTCC (Rev.)
MYC: AGCGACTCTGAGGAGGAACA (For.) and CCCTCTTGGCAGCAGGATAG (Rev.)

HIF1a: ATCACCCTCTTCGTCGCTTC (For.) and ACTTATCTTTTTCTTGTCGTTCGC (Rev.)
ESR1: ACAAGCGCCAGAGAGATGAT (For.) and AAGGTTGGCAGCTCTCATGT (Rev.)
GAPDH: AGCCACATCGCTCAGACAC (For.) and GCCCAATACGACCAAATCC (Rev.)

Primers for mouse only:
CCND1: CCAACAACTTCCTCTCCTGCT (For.) and GACTCCAGAAGGGCTTCAATC (Rev.)
MYC: TCTCCACTCACCAGCACAACTACG (For.) and ATCTGCTTCAGGACCCT (Rev.)
HIF1a: GCGAGAACGAGAAGAAAAAGATGA (For.) and GCCGTCATCTGTTAGCACCA (Rev.)
ESR1: CTTCAGTGCCAACAGCCT (For.) and GACAGTCTCTCTCGGCCAT (Rev.)
GAPDH: CCCACTAACATCAAATGGGG (For.) and CCTTCCACAATGCCAAAGTT (Rev.)

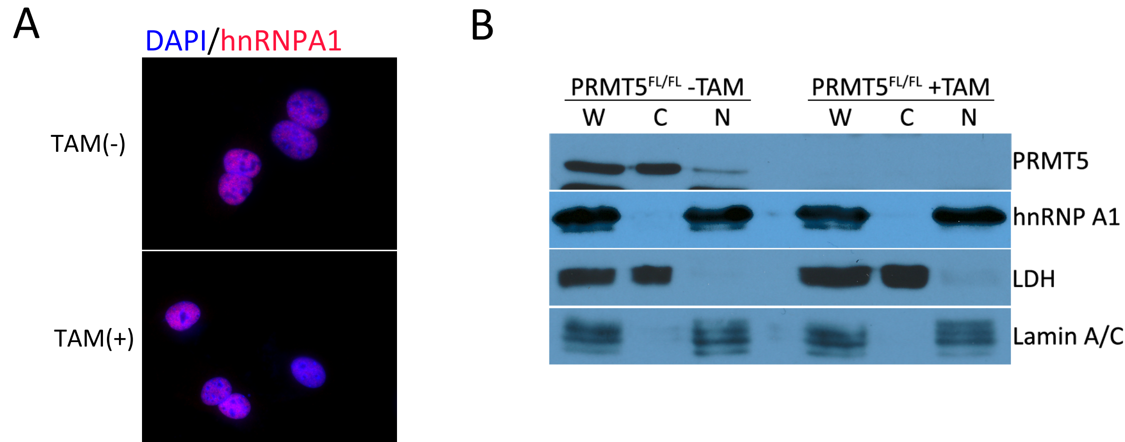

Figure S1: Methylation of hnRNP A1 by PRMT5 does not affect its subcellular localization. PRMT5$^{flox/flox}$ MEFs were treated with or without 2 μM tamoxifen for 8 days. **A.** Then half of the cells were fixed and stained with anti-hnRNP A1 antibody followed by Alexa Fluor 647 conjugated anti-mouse secondary antibody (red), and DAPI staining (blue). **B.** The other half were lysed with cytoplasmic/nuclear fractionation kit, and subjected to Western blotting for hnRNP A1. PRMT5 was also detected to control the knockout efficiency; LDH and Lamin A/C were for controlling fractionation.

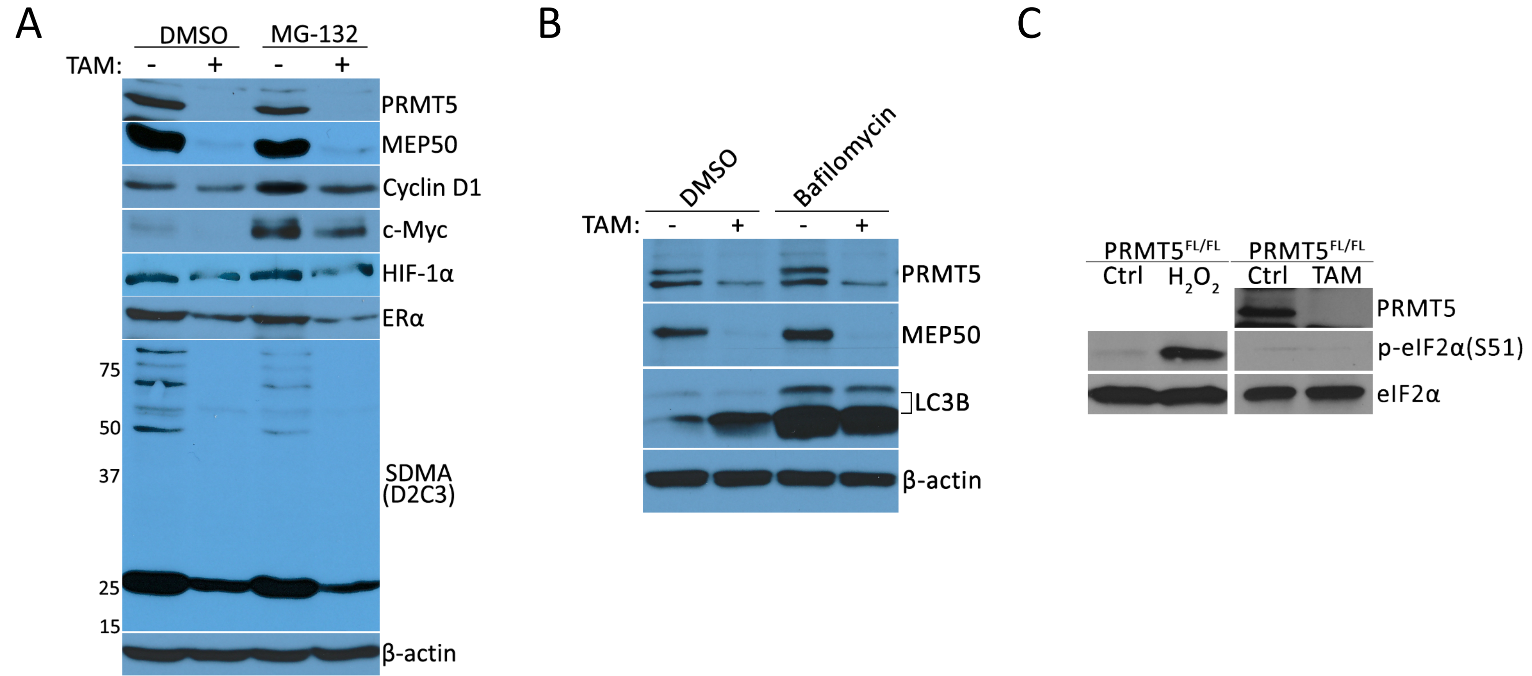

Figure S2: Proteins down-regulated by PRMT5 could not be rescued by proteasome inhibitor. **A.** PRMT5*flox/flox* MEFs were treated with or without 2 μM tamoxifen for 10 days, and then with DMSO or 20 μM of the proteasome inhibitor MG-132 for 4 hours. The protein levels of the indicated proteins were detected by Western blotting. **B.** PRMT5*flox/flox* MEFs were treated with or without 2 μM tamoxifen for 8 days, and then with DMSO or 200 nM of the lysosome inhibitor bafilomycin for 24 hours. The protein levels of the indicated proteins were detected by Western blotting. C. PRMT5*flox/flox* MEFs were either treated with 1 mM H_2O_2 for 2 hours or 2 μM tamoxifen for 8 days. The levels of total eIF2α and phosphorylated eIF2α were tested by Western blotting.

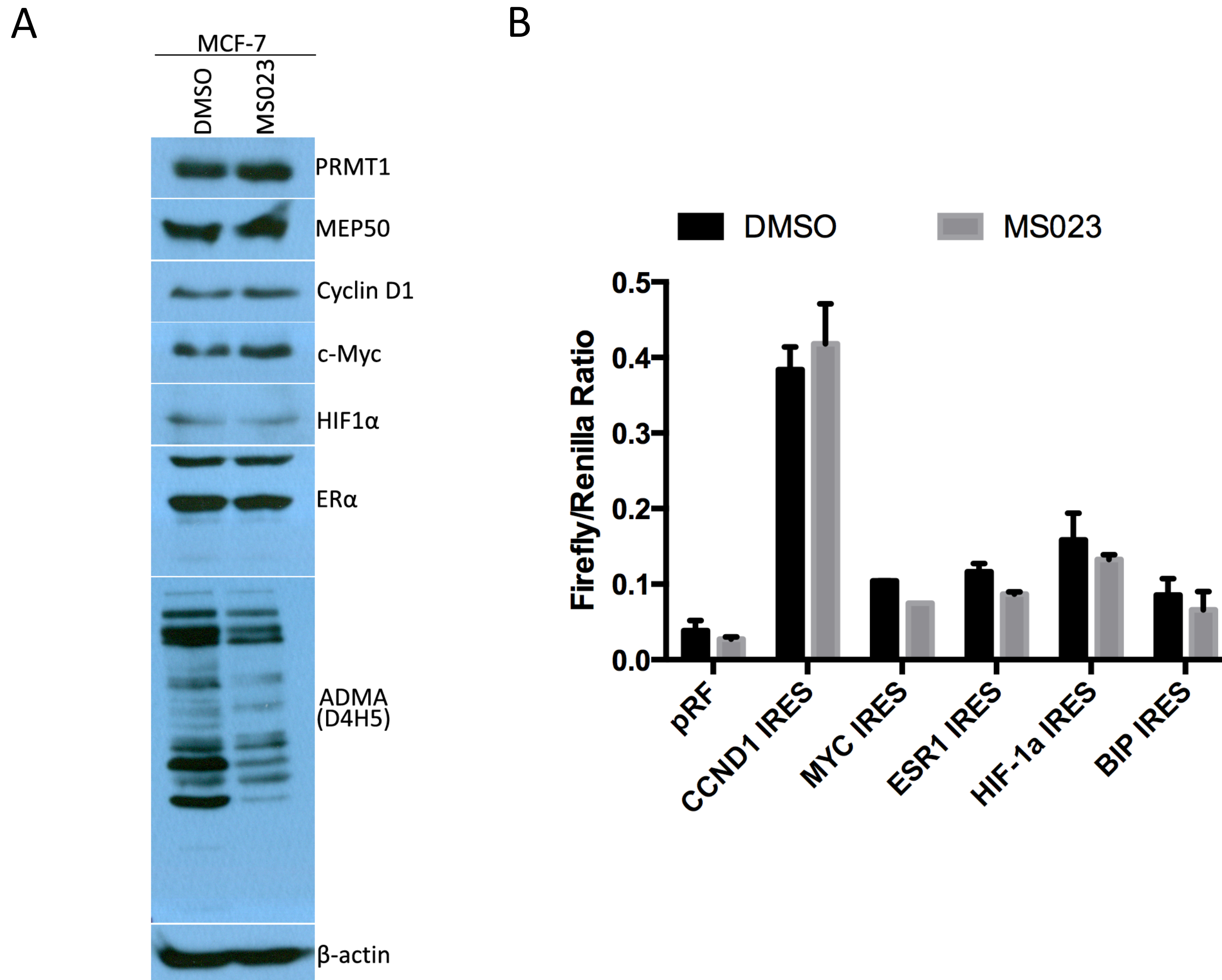

Figure S3: Inhibition of PRMT1 activity does not affect the IRES-dependent translation of indicated genes. A. MCF-7 cells were treated with 1 μM PRMT1 inhibitor (MS023) for 4 days. The protein levels of the indicated proteins were detected by Western blotting. **B.** MCF-7 cells were treated with 1 μM PRMT1 inhibitor for 4 days. On day 3, cells were transfected with indicated IRES-dependent reporter constructs. At the end of day 4, cells were harvested for luciferase activity assay. The ratios of firefly over renilla luciferase activity were calculated.

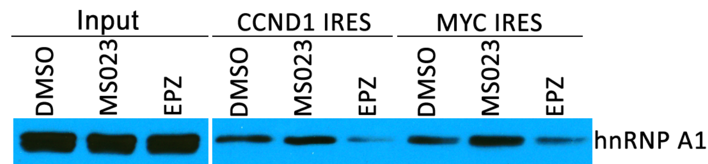

Figure S4: Inhibition of PRMT1 activity enhanced the binding between hnRNP A1 and its target IRESes. PRMT5$^{flox/flox}$ MEFs were treated with 1 μM PRMT1 inhibitor or 5 μM PRMT5 inhibitor for 4 days. RNA pulldown was performed with biotin-labeled CCND1 or MYC IRES. The protein levels of hnRNP A1 bound to IRESes were detected by Western blotting.