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**PRMT5 cellular assay**

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**Methods**

MCF7 cells were grown in 12-well plates in DMEM supplemented with 10% FBS, penicillin (100 units/mL) and streptomycin (100 µg/mL). For knock-down (KD) experiments 40% confluent cells were transfected with 15 nM of either control siRNA or siRNA against PRMT1, 3, 4, 5, 6, and 7 (Dharmacon) using Lipofectamine™ RNAiMAX, following manufacturer instructions. Cells were harvested after 3 days or transfected again after 3 days for 6 day KD experiment. For the inhibition experiment40% confluent cells were treated with different concentrations of GSK591 or DMSO control for 48 h.

Cells were lysed in 100 µL of total lysis buffer (20 mM Tris-HCl pH 8, 150 mM NaCl, 1 mM EDTA, 10 mM MgCl2, 0.5% TritonX-100, 12.5 U/mL benzonase (Sigma), complete EDTA-free protease inhibitor cocktail (Roche)). After 3 min incubation at RT, SDS was added to the final 1% concentration. Total cell lysates were resolved in 4-12% Bis-Tris Protein Gels (Invitrogen) with MOPS buffer (Invitrogen) and transferred in for 1.5h (80 V) onto PVDF membrane (GE Healthcare Amersham™ Hybond™ 0.2 µm, Fisher Scientific, #45-004-021) in Tris-Glycine transfer buffer containing 20% MeOH and 0.05% SDS. Blots were blocked for 1 h in blocking buffer (5% milk in TBST: 0.1% Tween 20 PBS) and incubated with primary antibodies: anti-smBB’ (1:200, Santa Cruz, #sc-130670), anti-Rme2s (1:2000, Cell Signalling #13222), anti-PRMT7 (1:1000, Abcam, #ab179822), anti-PRMT1 (1:5000, Millipore, #07-404), anti-PRMT3 (1:5000, Abcam, #ab191562), anti-PRMT4 (1:2000, Bethyl, #A300-421A), anti-PTMT5 (1:5000, Abcam, #ab109451), anti-PRMT6 (1:2000, Abcam, #ab47244), anti-β-actin (#ab3280,Abcam, 1:3000) in blocking buffer overnight at 4 ºC. After five washes with 0.1% TBST, the blots were incubated with goat anti-rabbit (IR800 conjugated, LiCor #926-32211) and donkey anti-mouse (IR 680, LiCor #926-68072) antibodies (1:5000) in Odyssey Blocking Buffer (LiCor) for 30 min at RT and washed five times with TBST. The signal was read on an Odyssey scanner (LiCor) at 800 nm and 700 nm.

 **Fig.2. PRMT5 knock-down results in decrease in SmBB’-Rme2s levels.** MCF7 cells were treated with siRNA targeted for different PRMTs and control siRNA for 6 (**A**) and 3 (**B**) days. **A.** Western blot analysis with an antibody recognizing symmetric arginine methylation (Rme2s) of various proteins. PRMTs expression after knock-down (KD) and β-actin controls are shown. Note that the loss of PTMT1 leads to decrease of Rme2a modification and the shared substrates become targets for PRMT5 leading to increase in Rme2s levels.**B.** Western blot analysis of the SmBB’-Rme2s levels after PRMT5 KD. **C.** Quantification of signal intensities of SmBB’-Rme2s normalized to total SmBB’ (n=1).



**Fig.3. PRMT5 chemical probe, GSK591, decreases SmBB’ symmetric dimethylation in a dose-dependent manner.** MCF7 cells were treated with inhibitor at indicated concentrations for 48 h and SmBB’-Rme2s and total SmBB’ levels were determined by Western blot. The graph represents nonlinear fits of SmBB’-Rme2s signal intensities normalized to total SmBB’. The results are mean +/- SEM of 3 replicates. The Z factor for the assay equals 0.67 (n=3).