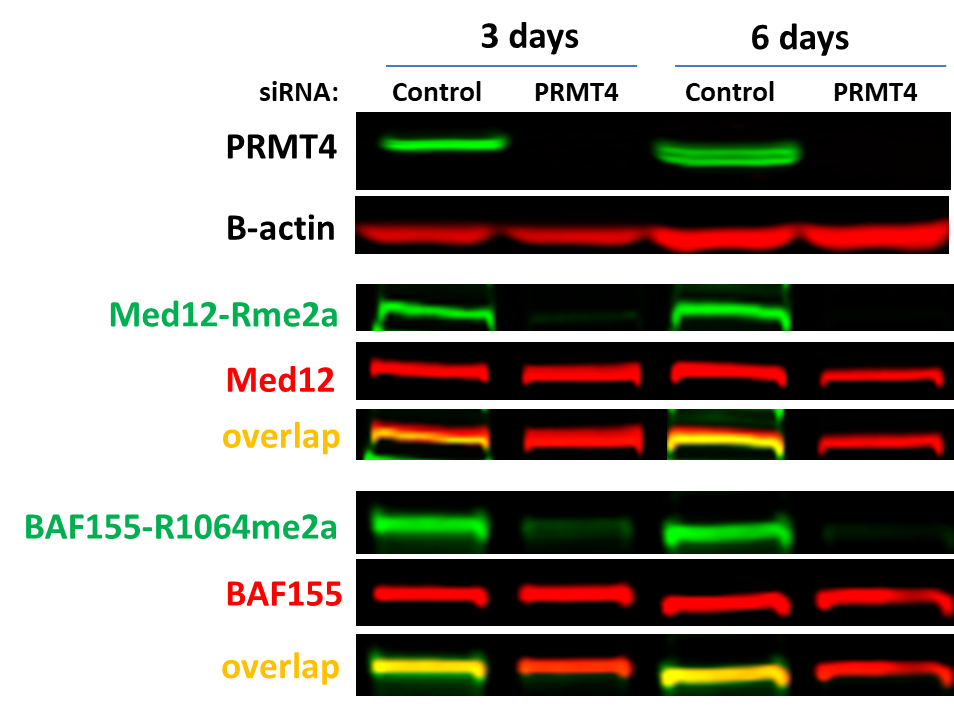
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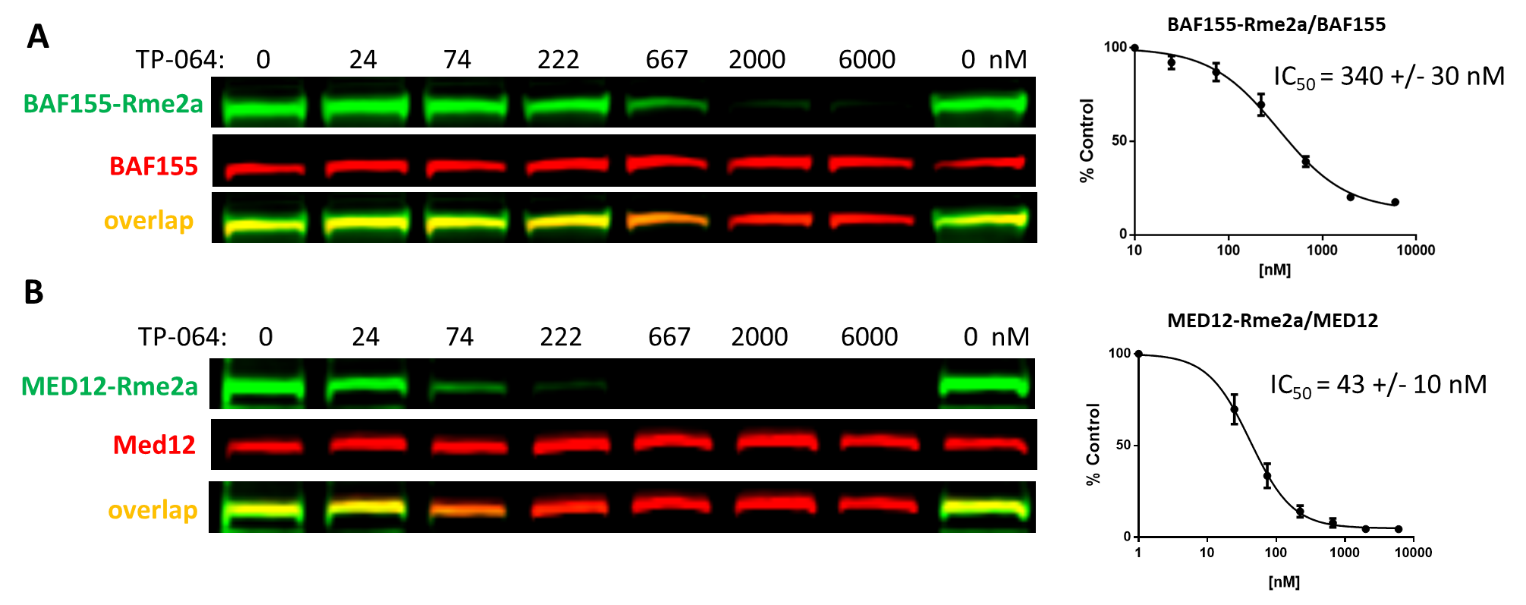
**PRMT4 (CARM1) cellular assay-Methods**

**Magdalena Szewczyk**

HEK293T 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 experiments, 40% confluent cells were transfected with 15 nM of either control siRNA or siRNA against PRMT4 (Dharmacon) using Lipofectamine™ RNAiMAX, following manufacturer instructions. Cells were harvested after 3 days or transfected again after 3 days for the 6-day experiment. For the inhibition experiment40% confluent cells were treated with different concentrations of PRMT4 selective inhibitor TP-064 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-Med12 (1:1000, Abnova # H00009968-A01), rabbit anti-Med12-Rme2a (1:1000, gift from Dr. Mark Bedford), anti-BAF155 (1:500, Santa Cruz, # sc-48350), anti-BAF155-R1064me2a (1:5000, Millipore Sigma, #ABE1339) 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. PRMT4 knock-down resulted in decrease of MED12 and BAF155 asymmetric dimethylation (Rme2a) levels.** HEK293T cells were treated with siRNA for 3 days and 6 days. The levels of methylation were analyzed in Western Blot.

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**Fig. 3. TP-064 decreases PRMT4 dependent MED12 and BAF155 asymmetric dimethylation (Rme2a) levels.**. HEK293T cells were treated with PRMT4 selective inhibitor (TP-064) at indicated concentrations for 48 h and BAF155-Rme2a (A) and MED12-Rme2a (B) levels were determined by Western blot. The graphs represent nonlinear fits of methyl signal intensities normalized to total protein levels. The results are mean +/- SEM of 3 replicates. MED12 Z factor = 0.6, and BAF155 Z factor = 0.76.