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**PRMD9 cellular assay-Methods**

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# HEK293T cells were seeded in 12-well plates (2e5/ml) in DMEM supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 µg/mL). Next day cells were co-transfected with 0.1 µg/well FLAG-tagged wild type PRDM9, PRDM9 catalytic mutant (Y357A), 0.1 µg/well GFP-H3 and 0.8 µg/well empty vector using jetPRIME® transfection reagent (Polyplus-Transfection), following manufacturer’s instructions. After 3 h media were removed and cells were treated with inhibitor or DMSO control. After 20 h, media were removed and 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 1 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 1.5 h (80 V) onto PVDF membrane (GE Healthcare Amersham™ Hybond™, Fisher Scientific) in Tris-Glycine transfer buffer containing 20% MeOH and 0.05% SDS. Blots were blocked for 1 h in blocking buffer (5% milk in PBST: 0.1% Tween 20 PBS) and incubated with primary antibodies: anti-GFP (1:3000, Clontech # 632381), anti-H3K4me3 (1:2000, Millipore, #04-745) and anti-FLAG (1:5000, Sigma, #F1804) in blocking buffer overnight at 4 ºC. After five washes with PBST, 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 PBST. The signal was read on an Odyssey scanner (LiCor) at 800 nm and 700 nm.

# Constructs:

**PRDM9 (Q9NQV7) cDNA sequence, codon optimized, was cloned into**pcDNA3.1+N-DYK vector.

CCATTAATTAAGGATCCAATGAGCCCAGAGAAAAGCCAGGAAGAAAGCCCAGAAGAGGACACCGAGAGAACAGAAAGGAAACCCATGGTCAAGGACGCATTCAAGGATATCAGCATCTACTTCACCAAGGAGGAATGGGCAGAAATGGGAGACTGGGAGAAAACTAGATACAGGAATGTGAAGCGGAACTATAATGCCCTGATCACCATTGGCCTGCGGGCTACAAGACCCGCCTTCATGTGCCACCGGAGACAGGCCATCAAGCTGCAGGTGGACGATACCGAGGATTCCGACGAGGAATGGACACCCAGGCAGCAGGTGAAACCCCCTTGGATGGCTCTGCGAGTCGAGCAGCGGAAGCACCAGAAAGGCATGCCTAAAGCAAGCTTCTCCAACGAAAGCTCCCTGAAGGAGCTGTCTCGCACCGCCAACCTGCTGAATGCTTCTGGGAGTGAACAGGCCCAGAAGCCCGTGTCACCTAGCGGGGAGGCTTCCACTTCTGGACAGCATAGCCGGCTGAAACTGGAACTGAGAAAGAAAGAAACCGAGAGGAAGATGTACTCACTGCGCGAGCGAAAAGGCCACGCCTATAAGGAAGTGAGCGAGCCTCAGGACGATGACTACCTGTATTGCGAGATGTGCCAGAACTTCTTTATCGATTCCTGCGCAGCTCATGGACCACCCACCTTCGTGAAAGATTCTGCAGTCGACAAGGGGCACCCAAATAGAAGTGCCCTGTCACTGCCTCCAGGACTGAGGATCGGACCAAGCGGGATTCCCCAGGCAGGACTGGGGGTGTGGAACGAGGCATCCGATCTGCCTCTGGGCCTGCACTTTGGGCCATACGAAGGAAGAATCACCGAGGACGAGGAAGCAGCCAACAATGGCTATTCTTGGCTGATTACAAAGGGGAGGAATTGCTACGAGTATGTGGATGGCAAGGACAAAAGTTGGGCTAACTGGATGCGCTATGTGAATTGTGCACGGGATGACGAGGAACAGAACCTGGTCGCCTTCCAGTACCACAGGCAGATCTTTTATAGGACCTGCCGCGTGATTCGACCCGGCTGTGAGCTGCTGGTCTGGTACGGCGATGAATATGGGCAGGAGCTGGGAATCAAGTGGGGCAGCAAATGGAAGAAAGAGCTGATGGCCGGCCGCGAACCAAAGCCCGAGATTCACCCTTGCCCAAGCTGCTGTCTGGCTTTCTCTAGTCAGAAATTTCTGTCCCAGCATGTGGAGAGAAACCACTCAAGCCAGAATTTCCCAGGACCTAGCGCCAGGAAGCTGCTGCAGCCTGAAAACCCATGTCCCGGGGACCAGAATCAGGAGCAGCAGTACCCTGATCCACACTCCCGCAACGACAAGACAAAAGGCCAGGAAATCAAGGAGCGGAGTAAACTGCTGAATAAGAGAACTTGGCAGAGGGAGATTTCACGCGCTTTTTCCTCTCCCCCTAAAGGACAGATGGGCTCTTGCCGAGTGGGAAAGCGGATCATGGAGGAAGAGAGTCGCACTGGCCAGAAAGTCAACCCCGGGAATACCGGAAAGCTGTTCGTGGGAGTCGGCATCAGCCGGATTGCAAAGGTGAAATACGGGGAGTGTGGGCAGGGCTTCAGCGTGAAATCCGACGTGATTACCCACCAGAGAACCCATACAGGAGAAAAGCTGTACGTGTGCAGAGAGTGTGGCAGGGGGTTCTCTTGGAAAAGTCACCTGCTGATCCATCAGAGGATTCACACCGGAGAAAAGCCATACGTGTGCCGCGAGTGTGGACGAGGCTTTTCATGGCAGAGCGTCCTGCTGACACATCAGCGCACTCACACCGGCGAAAAGCCCTACGTGTGCCGGGAGTGTGGGAGAGGATTCTCCAGGCAGTCTGTCCTGCTGACTCATCAGAGGCGCCACACCGGGGAAAAGCCTTACGTGTGCAGGGAATGTGGCCGAGGGTTTAGTCGGCAGTCAGTCCTGCTGACCCACCAGCGACGGCACACTGGCGAGAAGCCATACGTGTGCAGGGAATGCGGAAGGGGCTTCAGCTGGCAGTCCGTCCTGCTGACCCATCAGAGAACACACACTGGCGAAAAACCTTACGTGTGCCGAGAGTGTGGACGAGGATTTTCTTGGCAGAGTGTCCTGCTGACCCACCAGAGAACCCACACAGGGGAGAAGCCTTACGTGTGCCGCGAATGCGGCAGAGGGTTCTCAAATAAGAGCCATCTGCTGCGGCACCAGAGAACTCATACCGGAGAAAAACCATACGTCTGCAGAGAGTGTGGAAGGGGCTTTCGCGATAAGTCCCACCTGCTGCGACATCAGCGGACCCATACTGGCGAAAAACCCTACGTGTGCAGGGAGTGTGGCAGGGGATTCAGGGACAAGAGTAACCTGCTGTCACATCAGCGGACCCACACAGGGGAAAAACCTTACGTCTGCAGGGAGTGTGGCCGCGGGTTTTCCAATAAGTCTCATCTGCTGCGCCACCAGCGAACTCATACCGGCGAAAAACCATACGTGTGCAGGGAGTGCGGACGGGGCTTCAGAAACAAGTCTCACCTGCTGAGGCATCAGCGCACCCACACCGGAGAGAAGCCCTACGTGTGCAGAGAATGTGGACGAGGCTTCAGCGATAGAAGTTCACTGTGCTATCACCAGCGGACCCATACCGGAGAAAAGCCTTACGTCTGTCGGGAAGATGAGTAATCTAGA

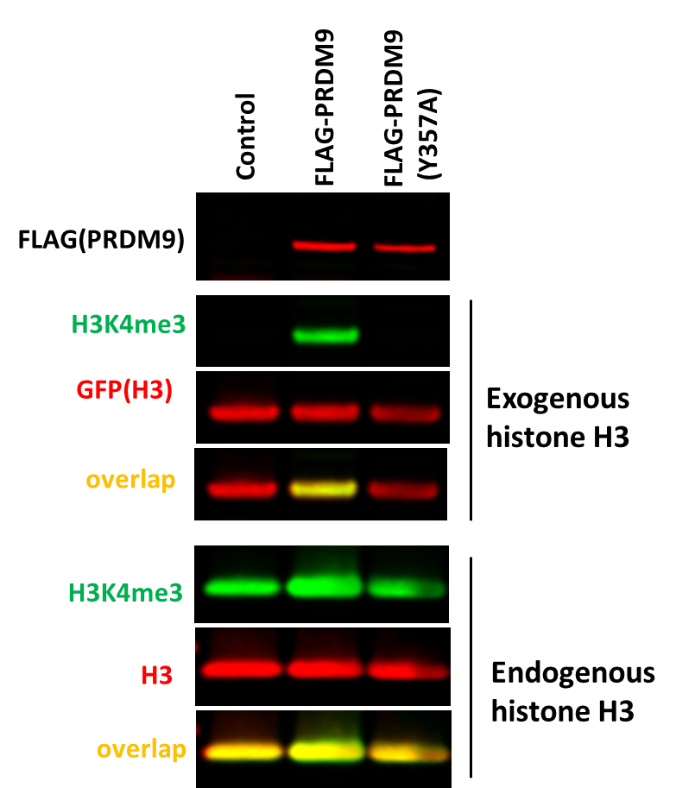
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**PRDM9 Y357A mutant** was madeusing Q5® Site-Directed Mutagenesis kit (NEB), following the manufacturer’s instructions.

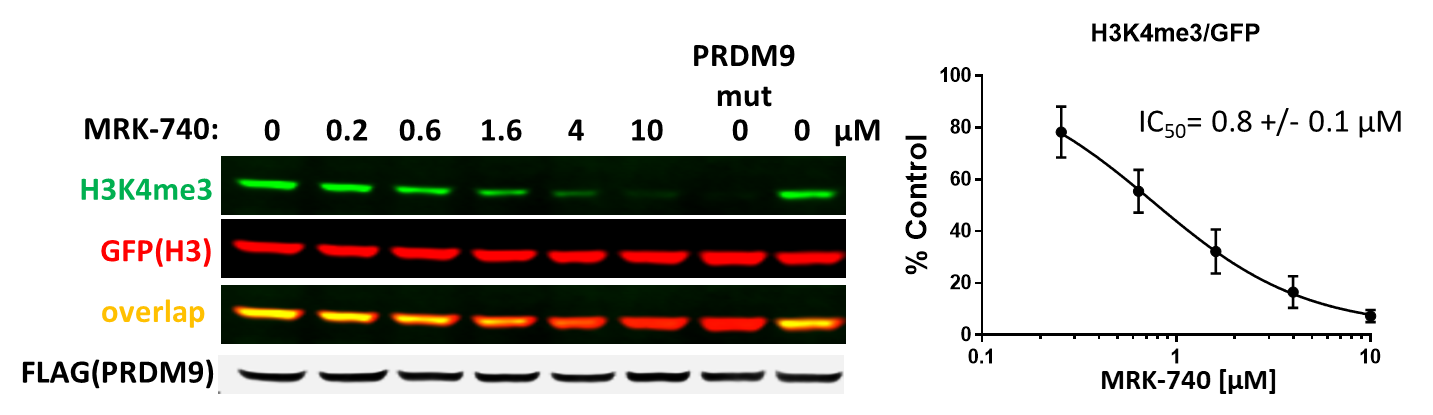
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**Histone H3.1** **(P68431)** was cloned into pAcGFP1-N3 vector.

MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEACEAYLVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA

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**Fig.1. The wild type but not catalytic mutant of PRDM9 methylated exogenous and endogenous histone H3K4.** HEK293T cells were co-transfected with GFP-tagged histone H3 and empty vector(control), FLAG-tagged wild type, or Y357A catalytic mutant PRDM9 24 h**.** The methylation levels were analyzed in Western Blot. (Shawna Organ)

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**Fig.2. MRK-740 decreases PRDM9 dependent H3K4 trimethylation in cells**. HEK293T cells were co-transfected with FLAG-tagged PRMT9 (wild type or Y357A mutant) and GFP-tagged histone H3 and treated with inhibitor for 20 h. H3K4me3 levels were determined by Western blot. The graph represents the nonlinear fit of H3K4me3 signal intensities normalized to GFP. The results are mean +/- SEM of 3 replicates.(Magdalena Szewczyk)