Supporting Information

Genetic evidence for partial redundancy between the arginine methyltransferases CARM1 and PRMT6

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Figure S1. Validation of CARM1 MEFs and histone H3 arginine methyl-specific antibodies. (A) Total lysates from CARM1 WT, KO, and KI (enzyme-dead) MEF cells were probed with anti-CARM1 antibody. *In vitro* methylation was performed using the indicated cell lysates as the enzyme source with GST-PABP1 in the presence of [^{3}H] SAM (bottom panel). **(B)** 5 mg of histone H3 peptides that contain a specific methylated arginine site were separated by SDS-PAGE and immunoblotted using indicated histone H3 arginine methyl-specific antibodies. **(C)** Purified wild-type and arginine mutant GST-H3 tail were incubated with PRMT6 that was purified from Baculovirus-infected Sf9 in an *in vitro* methylation assay. Methylation status was analyzed by immunoblot using anti-H3R17me2a antibody.

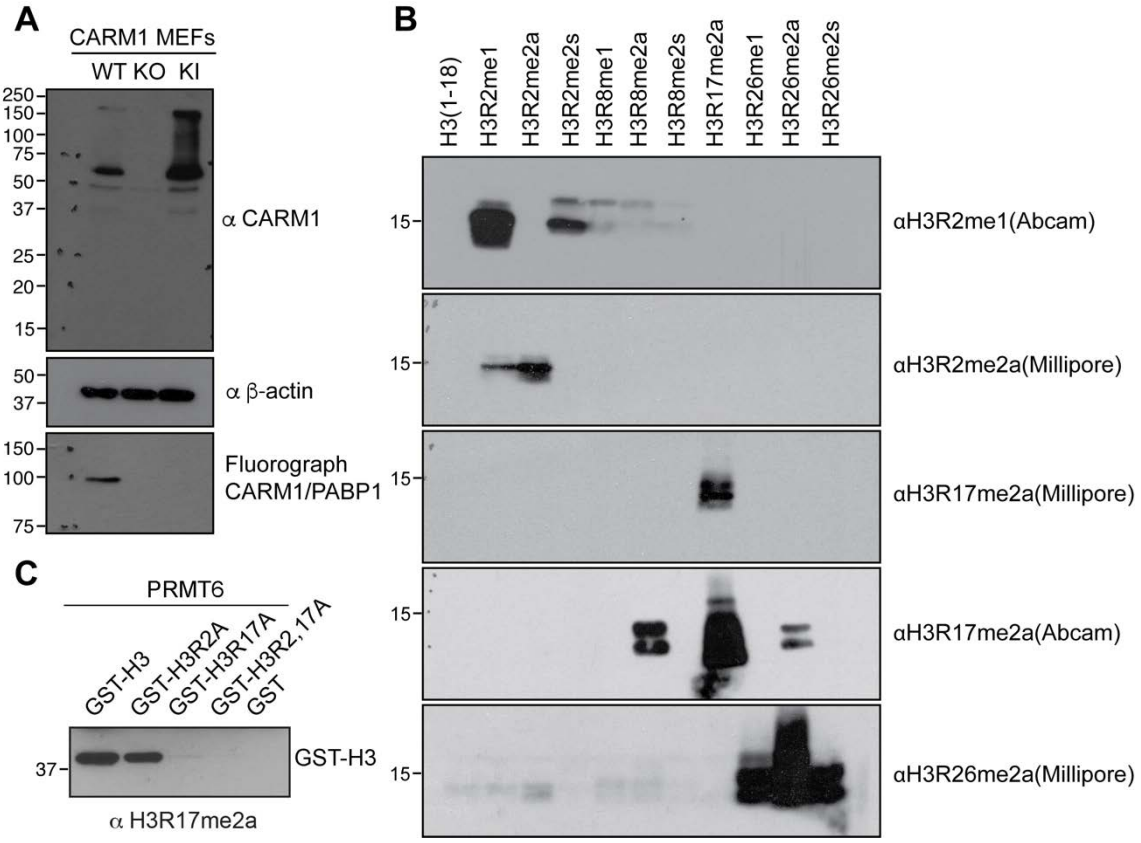

Figure S2. Histone H3R17me2a signal is generated by the nuclear expression of CARM1 and PRMT6. (A) HeLa cells were transfected with GFP-CARM1 and GFP-PRMT6 plasmids. Then the cells were stained with DAPI, and the GFP and DAPI signals were taken under a microscope. **(B)** HeLa cells were transfected with GFP-NLS vector and GFP-NLS-CARM1. Then the cells were stained with DAPI, and the GFP and DAPI signals were taken under a microscope. **(C)** HEK293T cells were transfected with GFP-NLS or GFP-NLS-CARM1. Then the cells were separated into 3 fractions: cytoplasm, nucleus and core histones. The levels of CARM1 and H3R17me2a were detected by Western blot. **(D)** HEK293T cells were transfected with ER*-flag-PRMT6 and then treated with 4-hydroxytamoxifen for 48 hrs. Then the cells were fractionated and the levels of PRMT6 and H3R17me2a were detected by Western blot.

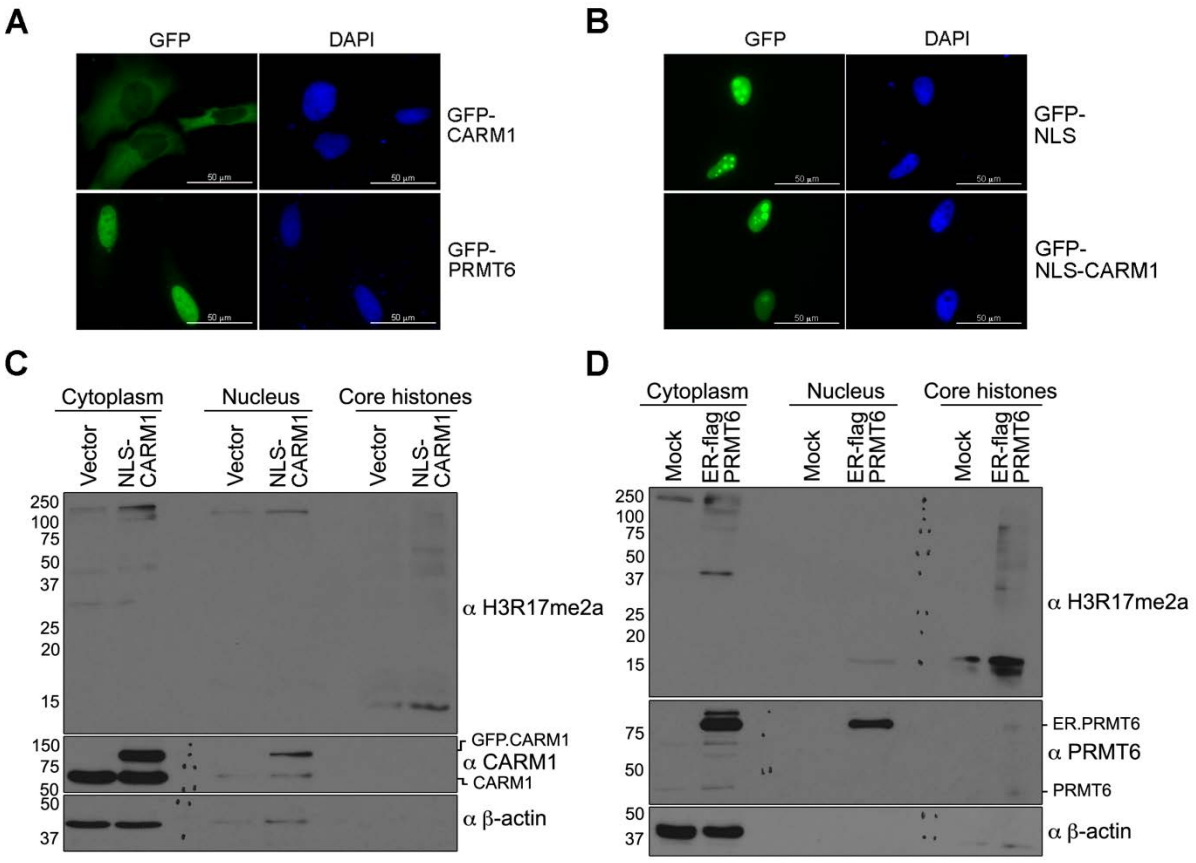

Figure S3. Genotype confirmation of the CARM1 and PRMT6 knockout embryos by PCR and Western blot. **(A)** Images of a representative litter of embryos from cross-breeding CARM1$^{+/-}$ PRMT6$^{-/-}$ mice**. (B)** Genomic DNA from the 10 embryos in (A) was extracted and applied to PCR for genotyping using primers described in "*Experimental Procedures*". DNA fragments derived from the wild-type (WT) and knockout (KO) alleles are indicated on the right. **(C)** Embryonic and primary cell lysates were separated by SDS-PAGE and subsequently immunoblotted with CARM1 and PRMT6 antibodies. "Controls" were genomic DNA from the original established PRMT6 KO and CARM1 Het mice.

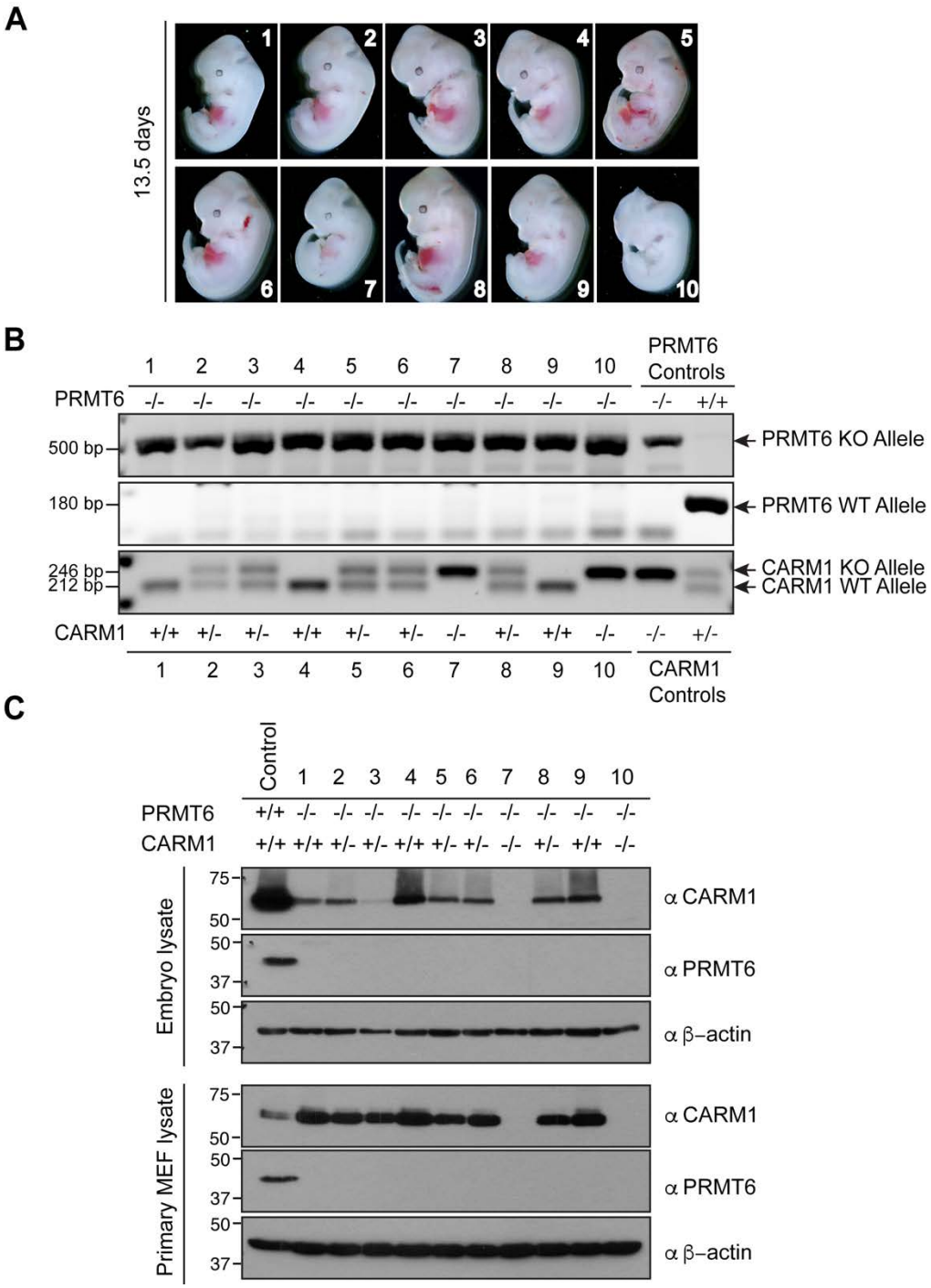

Figure S4. Cell growth curve with the treatment of CARM1 and/or PRMT6 inhibitors. Wild-type MEFs were treated with 3 μM CARM1 inhibitor alone, 3 μM PRMT6 inhibitor alone or the inhibitors together, for a total of 6 days. Cells were counted using CellTiterGlo® kit and the luminescence was recorded every 2 days.

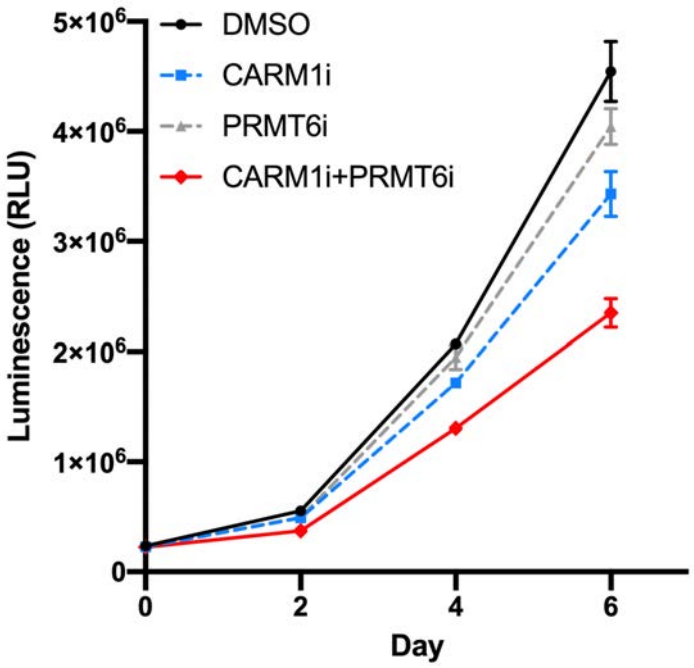

Figure S5. The specificity of pan CARM1 substrate antibody. HEK293T cells were treated with inhibitors to type I PRMT (MS023), CARM1 (TP-064) or PRMT6 (EPZ020411) with increasing concentrations, for a total of 4 days. The levels of CARM1 substrates were monitored by Western blot using the pan CAMR1 substrate antibody (αCARM1^{sub}).