Loss of the major Type I arginine methyltransferase PRMT1 causes substrate scavenging by other PRMTs

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SUPPLEMENTAL INFORMATION

Figure S1. Expression analysis of PRMTs in the absence of PRMT1. PRMT1$^{fl/-}$ ER-Cre MEFs were untreated or treated with 4-hydroxytamoxifen (OHT) for 4 and 8 days. Whole cell extracts were prepared and subjected to Western analysis with αPRMT1, αPRMT3, αPRMT4, αPRMT5, αPRMT6, αPRMT7 and MMA1 antibodies. The β-actin control is shown for equal loading.

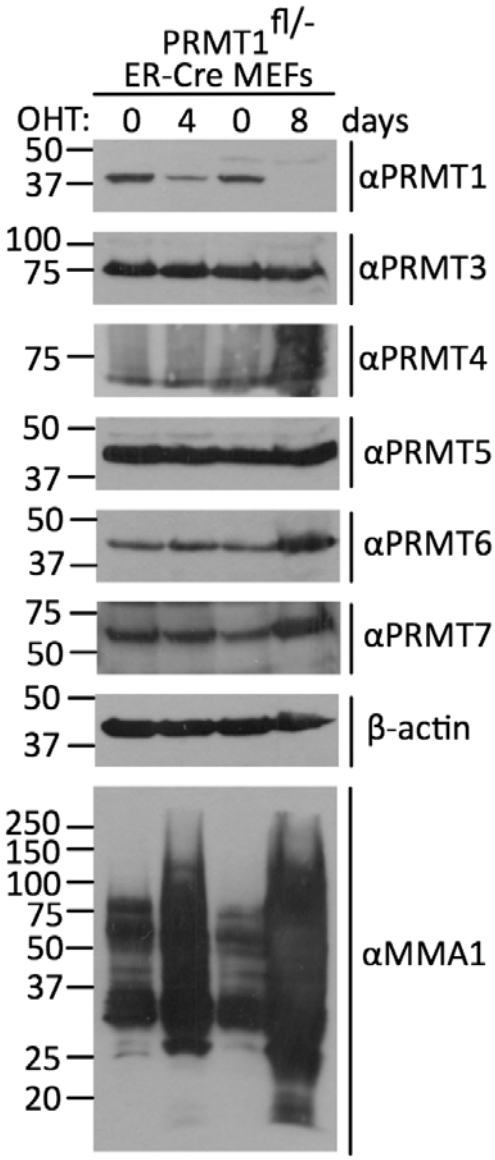

Figure S2. Co-immunoprecipitation of GFP-PRMTs with endogenous PRMT1. HEK293 cells transiently expressing GFP or GFP-PRMT [1-9] fusion proteins were immunoprecipitated with anti-GFP or anti-IgG antibodies and subjected to Western analysis with αPRMT1 (a) or αGFP (b) antibodies. PRMT1 co-immunoprecipitates with itself and with PRMT8 (indicated with solid white dots). (c) Western analysis of the input samples using αGFP antibody shows the expression of GFP-PRMT [1-9] fusion proteins, which are marked with solid white dots.

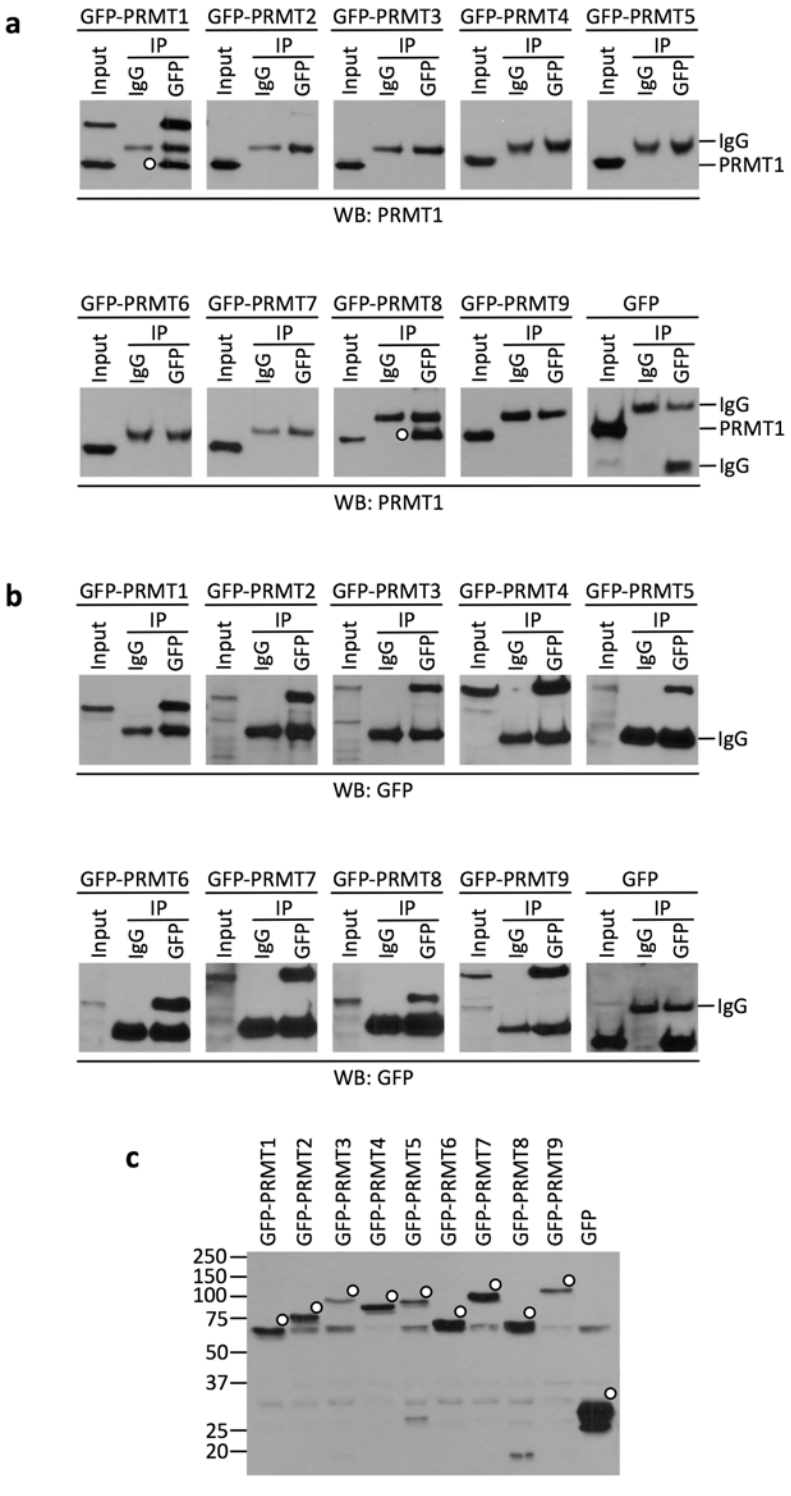

Figure S3. Hyper-monomethylation of SmB/SmB' upon PRMT5 loss. PRMT5 control and knockdown HeLa cells were immunoprecipitated (IP) with Y12 antibody and subjected to Western analysis with monomethylarginine (MMA5) antibody.

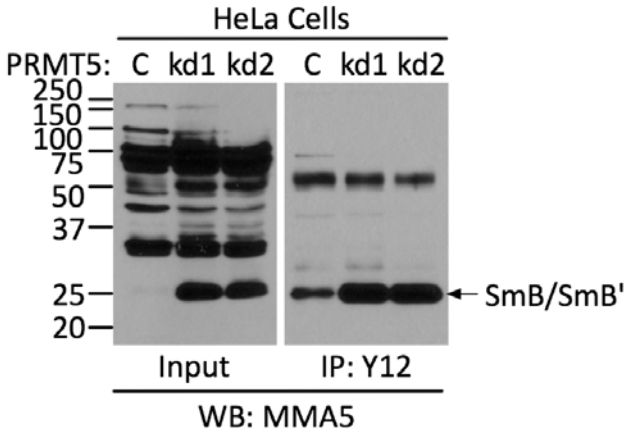

Figure S4. Workflow for the two-dimensional quantification of MMA, ADMA, SDMA, and arginine in MEFs.

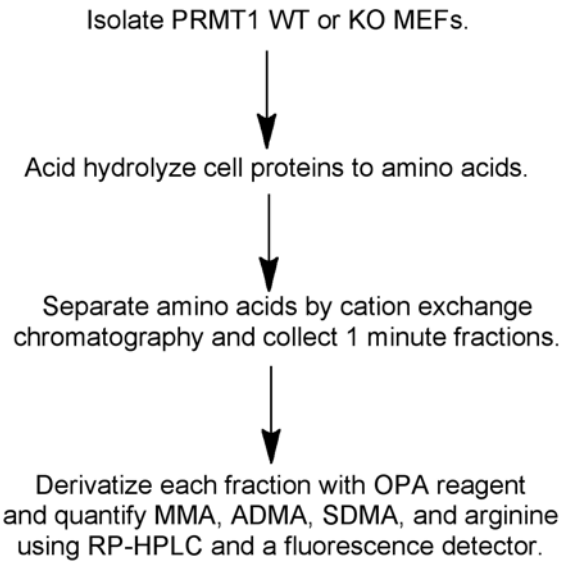

Figure S5. Reverse-phase HPLC methods optimized to quantify OPA-derivatives of arginine (R), MMA, ADMA, and SDMA.

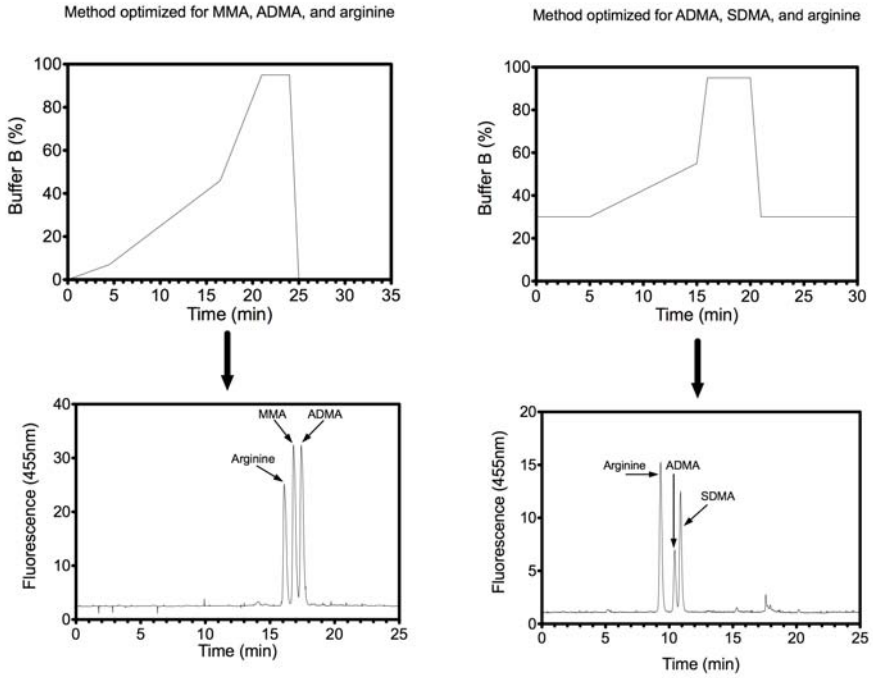

Table S1. Comparison of the relative levels of MMA, ADMA, and SDMA in different mammalian cells. Values from rat brain and liver were taken from Matsuoka (1972)[1] as translated by Paik and Kim (1980)[2], and converted from μ mol per g protein assuming an average arginine residue content of 5% in proteins.

Cells	MMA	ADMA	SDMA	Reference
	Average number of residues per 1000 arginine residues			
Wild-type MEFs	0.12	4.01	0.34	This study
PRMT1 KO MEFs	0.57	2.13	0.94	This study
Rat brain	2.04	3.60	0.99	[1, 2]
Rat liver	0.65	2.95	0.35	[1, 2]
Rat brain nuclear fraction	1.98	10.1	1.10	[1, 2]

1. Matsuoka, M. [Epsilon-N-methylated lysine and guanidine-N-methylated arginine of proteins. 3. Presence and distribution in nature and mammals]. *Seikagaku* **44**, 364-70 (1972).
2. Paik, W.K. & Kim, S. Natural occurrence of various methylated amino acid derivatives. (ed. Meister, A.) (John Wiley & sons, New York, 1980).

Table S2. Antibodies used in this study. The monomethylarginine (MMA1-5), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) antibodies tested in this study were generated by Cell Signaling Technology®.

Antibody Name	Company	Antibody #	Catalog #	Antibody type	Host Species	WB Dilution	Results Location
MMA1	Cell Signaling	D5A12A3	8711	Monoclonal	Rabbit	1:2000	Figure 1A-E
MMA2	Cell Signaling	D3C4A6	8015	Monoclonal	Rabbit	1:1000	Figure 1A-E Figure 2A Figure S1
MMA3	Cell Signaling	D2F4E5		Monoclonal	Rabbit	1:1000	Figure 1A-E
MMA4	Cell Signaling	D1C6D1		Monoclonal	Rabbit	1:1000	Figure 1A-E
MMA5	Cell Signaling	D7B7F1		Monoclonal	Rabbit	1:1000	Figure 1A-E Figure S3
ADMA	Cell Signaling	BL8241	Not yet commercially released	Polyclonal	Rabbit	1:500	Figure 1A-E Figure 2B
SDMA	Cell Signaling	BL8243	Not yet commercially released	Polyclonal	Rabbit	1:1000	Figure 1A-E Figure 2C
H3R17me2a	Millipore	N/A	07-214	Polyclonal	Rabbit	1:1000	Figure 1A-E
Y12	A gift from Robin Reed	N/A	N/A	Monoclonal	Mouse	1:100	Figure S3
PRMT1	A gift from Stephane Richard	N/A	N/A	Polyclonal	Rabbit	1:1000	Figure 1A Figure 2 Figure S1 Figure S2A-B
PRMT3	N/A	N/A	N/A	Polyclonal	Rabbit	1:500	Figure 1B Figure S1
CARM1	Bethyl	N/A	A300-421A	Polyclonal	Rabbit	1:2000	Figure 1C Figure S1
PRMT5	Active Motif	N/A	61001	Polyclonal	Rabbit	1:2000	Figure 1D Figure S1
PRMT6	Bethyl	N/A	A300-929A	Polyclonal	Rabbit	1:1000	Figure 1E Figure S1
PRMT7	A gift from Said Sif	N/A	N/A	Polyclonal	Rabbit	1:500	Figure S1