Supplemental Materials for

Deep protein methylation profiling by combined chemical and immunoaffinity approaches reveals novel PRMT1 targets

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Long SCX gradient

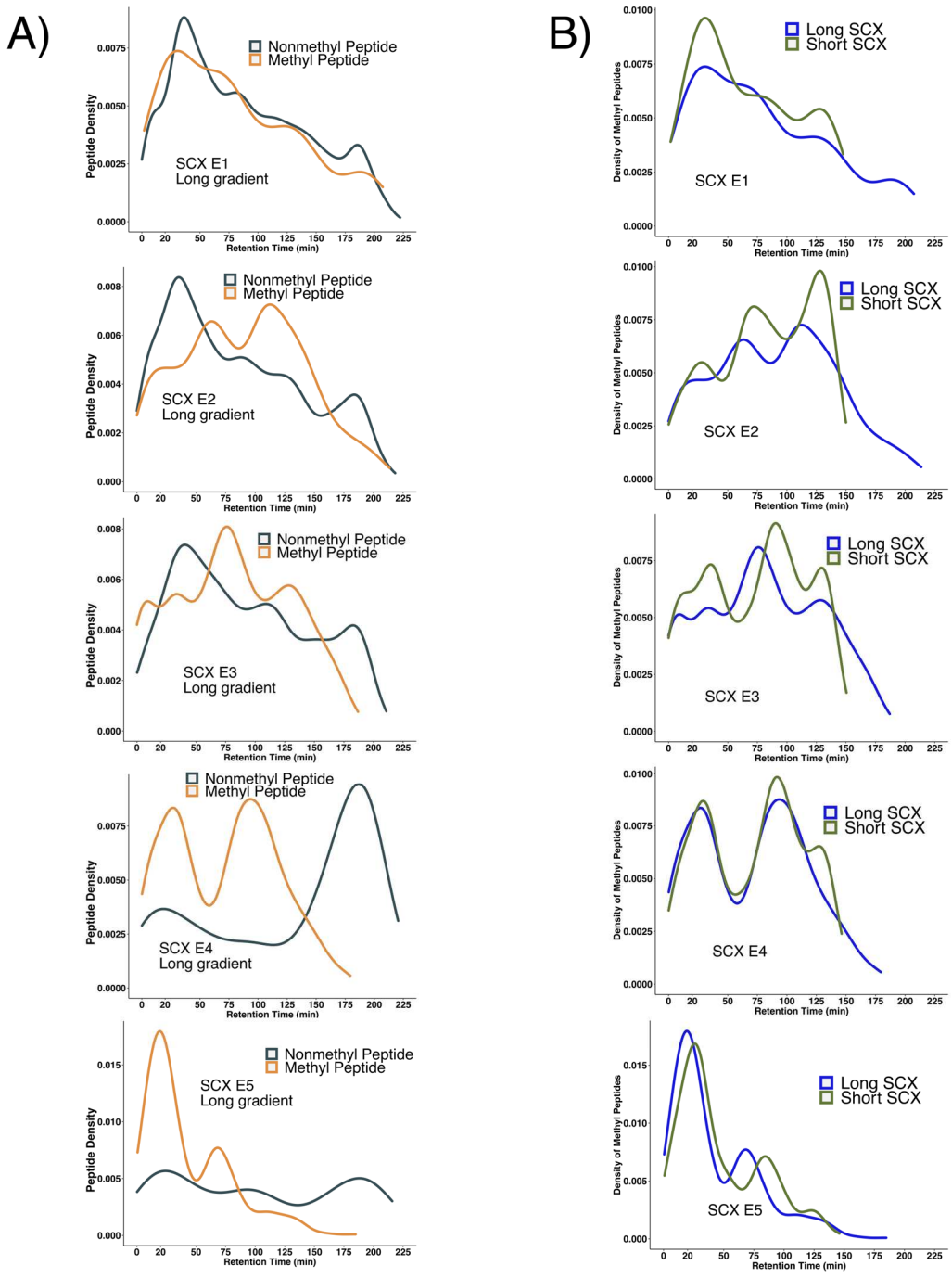
Time (min)	Buffer A (%)	Buffer B (%)	
0	98	2	Peptide Loading (spectra not collected)
20	98	2	
25	95	5	Peptide Separation
175	75	25	
205	55	45	
215	10	90	Wash
225	10	90	

Short SCX gradient

Time (min)	Buffer A (%)	Buffer B (%)	
0	100	0	Peptide Separation
50	93	7	
142	72	29	
152	10	90	Wash
155	10	90	

Supp. Fig. 1. Gradients for high pH SCX enrichment.

The “long” SCX gradient was taken from Wang *et al.* (1). The first 20 minutes of the long gradient is used for sample loading, and MS/MS spectra were not collected during this phase in the original publication. The “short” SCX gradient starts with 0% acetonitrile to prevent early elution of hydrophilic peptides. Buffer A is 0.1% formic acid, and Buffer B is 80% acetonitrile, 0.1% formic acid.



Supp. Fig. 2. Short SCX gradient shows similar methyl peptide elution profile to Long SCX gradient.

A) Methyl and non-methyl peptide elution profiles on the Long SCX gradient. In all fractions methyl peptides began to drop off near 150 minutes into the Long gradient. In

later fractions methyl peptides showed high hydrophilicity and early elution time. B) Methyl peptide elution profile between Short and Long SCX gradients. The Short gradient shows a similar elution profile to the Long gradient but finishes earlier.

Standard gradient

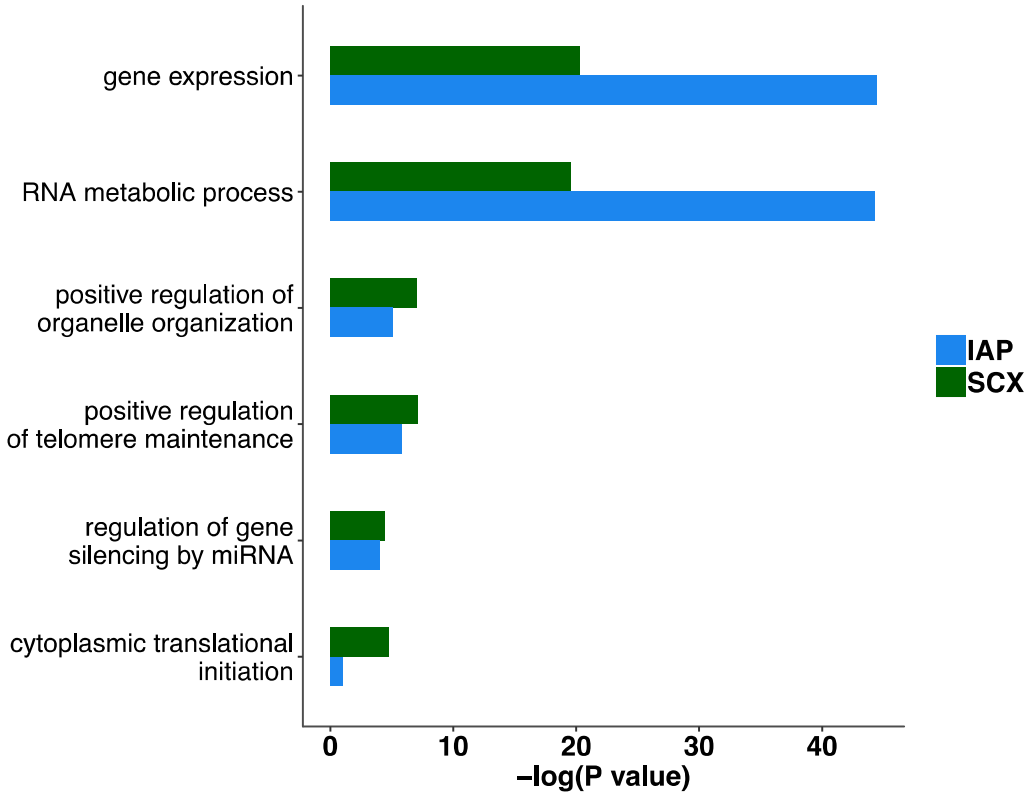
Time (min)	Buffer A (%)	Buffer B (%)	
0	97	3	Peptide Separation
110	62	38	
111	25	75	
121	15	85	Wash
130	15	85	

Slow gradient

Time (min)	Buffer A (%)	Buffer B (%)	
0	100	0	Peptide Separation
132	75	25	
142	10	90	Wash

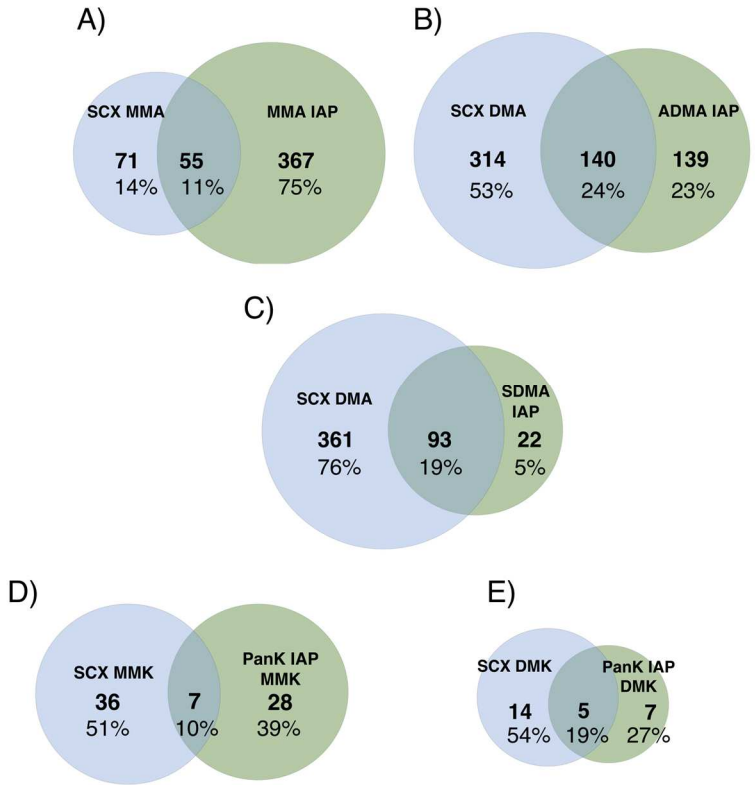
Supp. Fig. 3. Gradients for IAP enrichment.

The “standard” gradient is from an in-house proteomics method used to analyze complex cell lysates. The “slow” gradient ramps more slowly in acetonitrile in order to normalize elution times of hydrophilic methyl peptides.



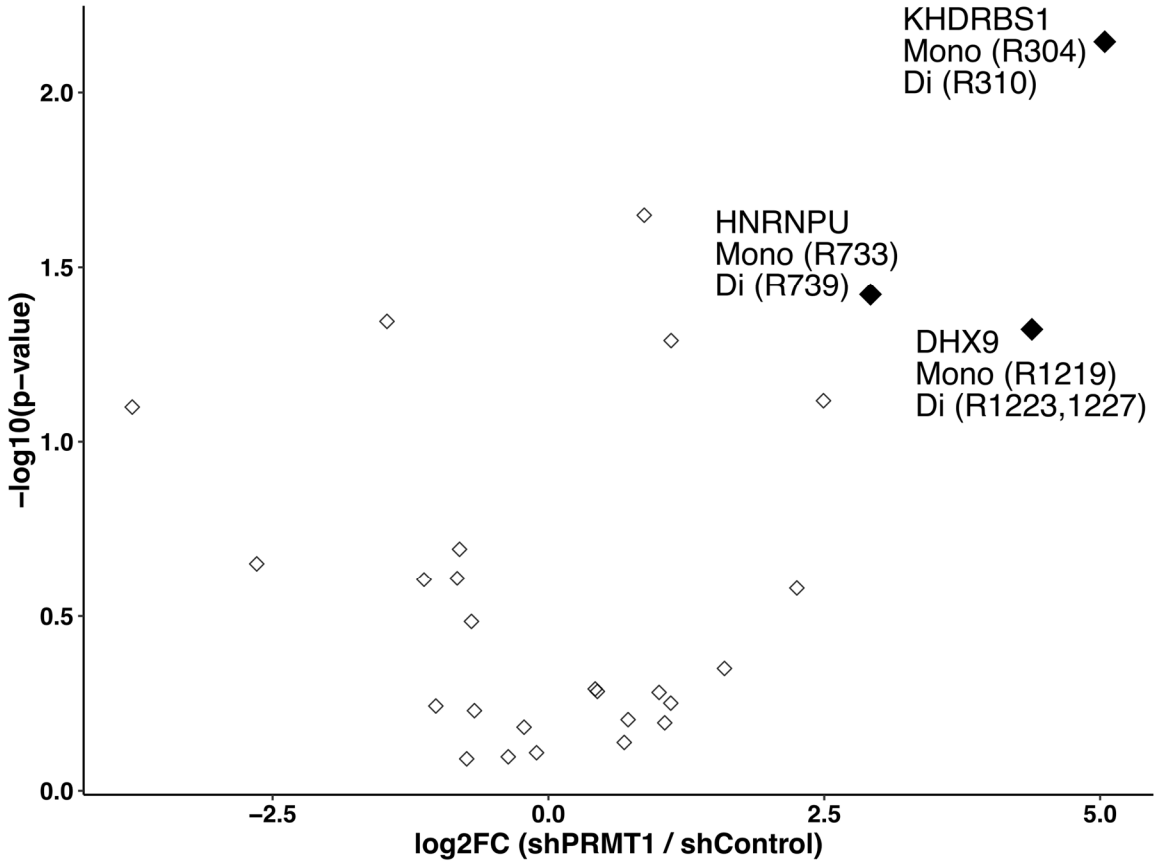
Supp. Fig. 4. SCX and IAP methyl-peptide enrichment techniques enrich for similar gene ontologies.

Methylated peptides identified by SCX and IAP from LN229 cells were analyzed for enrichment in molecular function gene ontologies (FDR < 0.05). The methyl proteins enriched by SCX and IAP share common ontologies even though they target different methyl sites within those ontologies. GO enrichment was determined using Panther DB (http://www.pantherdb.org) (2)



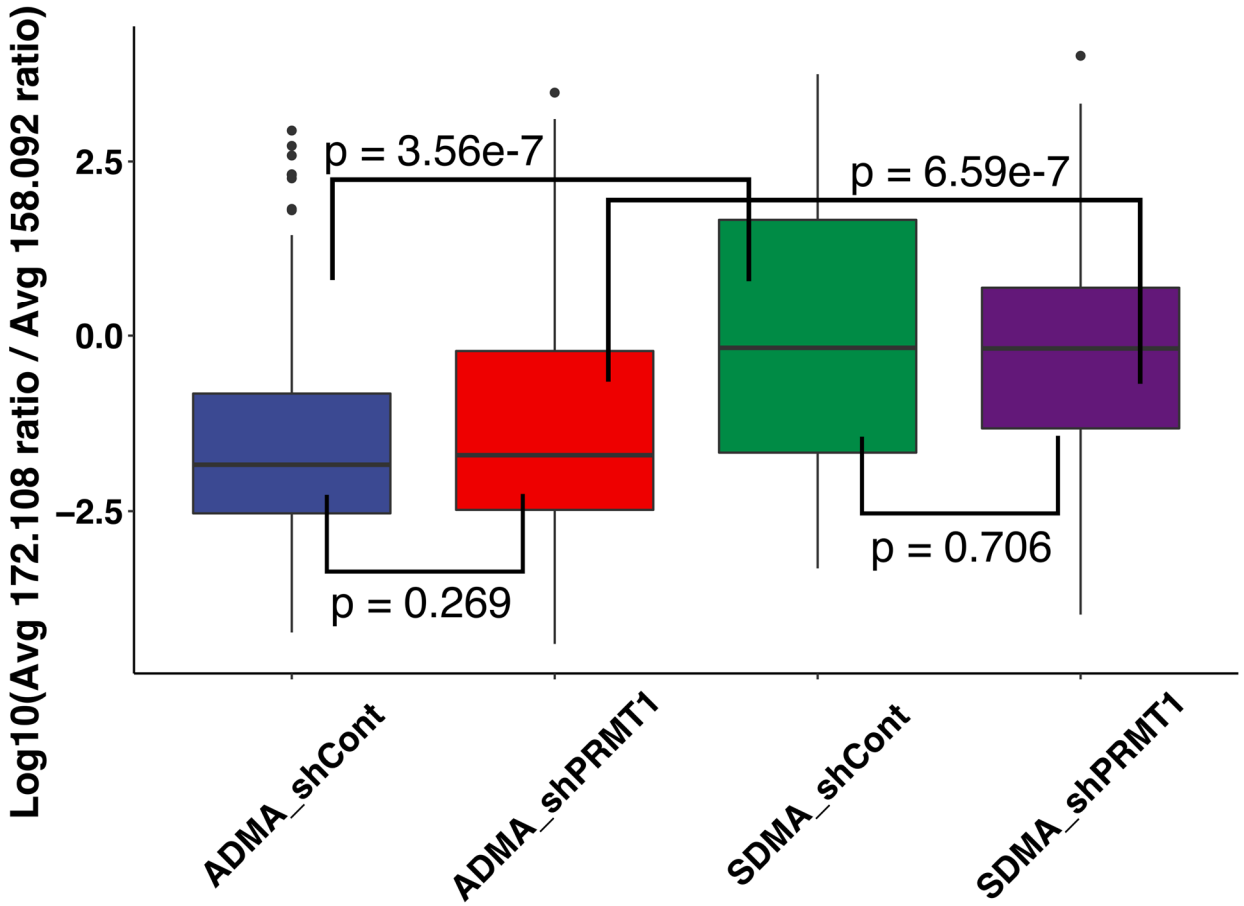
Supp. Fig. 5. Overlap of quantified methyl arginine and lysine sites are low between SCX and IAP across 293T experiments.

A) Overlap of MMA sites quantified by SCX and MMA IAP. Low overlap indicates both techniques enrich different subsets of methyl sites. B) Overlap of dimethyl sites enriched by SCX and ADMA IAP. C) Overlap of dimethyl sites enriched by SCX and SDMA IAP. Dimethyl are preferentially enriched by SCX due to missed cleavages occurring on modified arginines leading to more positive charge. D) Overlap of monomethyl lysine (MMK) between SCX and PanK IAP. E) Overlap of dimethyl lysine (DMK) between SCX and PanK IAP. Trimethyl lysine was not confidently observed in PanK IAP and 7 trimethyl lysine sites were localized in SCX.



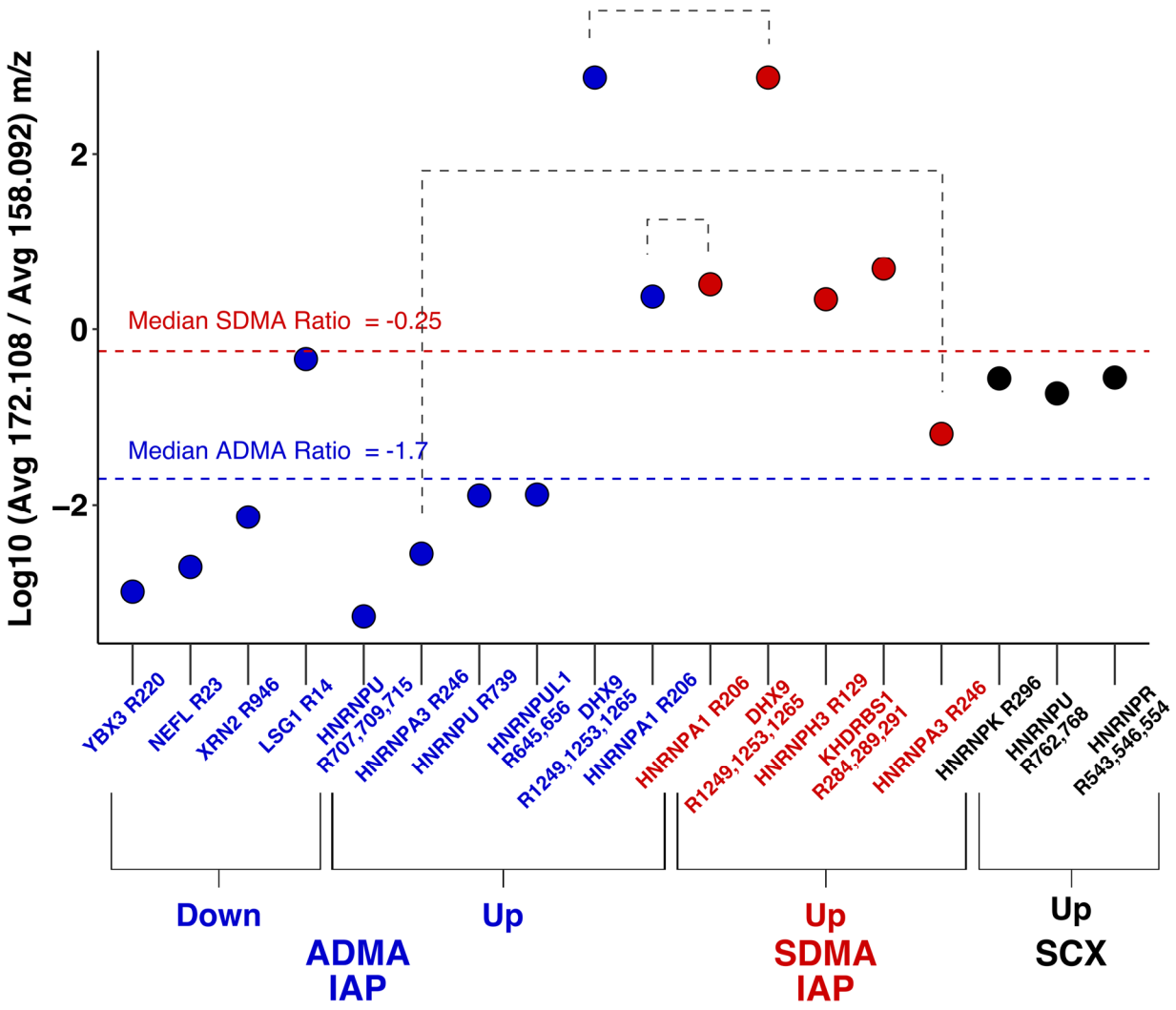
Supp. Fig. 6. Analysis of mixed methylation by SCX in 293T cells.

A volcano plot of SCX methyl peptides containing mixed monomethyl and dimethyl arginine sites within the same peptide (FDR < 0.05) from 293T cells expressing shRNA against PRMT1 or a scrambled control. Open diamonds are methyl peptides with mixed mono- and di-methyl R sites, and filled in diamonds are significantly changing FDR < 0.05 sites. SCX enriches for all missed cleavages of arginine and therefore produces a mixture of methyl peptides with multiple MMA and DMA sites.



Supp. Fig. 7. Ratio of 172.108 (SDMA characteristic ion) to 158.092 (ADMA characteristic ion) for ADMA_shControl, ADMA_shPRMT1, SDMA_shControl, and SDMA_shPRMT1 datasets.

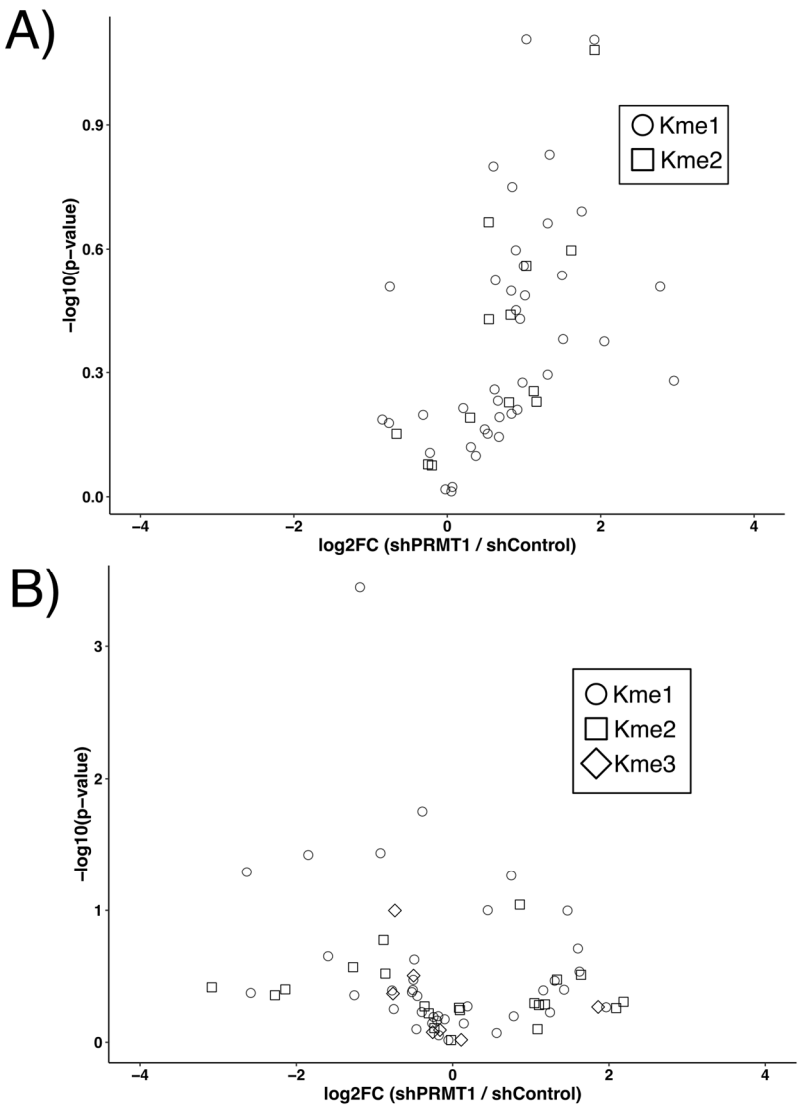
Log_{10} ratio of the averaged abundance of the 172.108 ion (SDMA characteristic ion) to the 158.092 ion (ADMA characteristic ion) separated by shControl or shPRMT1 samples. The ratio supports the idea that 158.092 is more abundant in ADMA peptides and 172.108 is more abundant in SDMA peptides. A 7 ppm mass window was used for the measurement of the intensity of the ions extracted from multiple MS2 spectra and averaged per peptide across samples. At least two unique MS2 spectra were required for each peptide. Statistical significance was determined using the Mann-Whitney test.



Supp. Fig. 8. Log10 Ratios of (172/158) m/z for significantly changing ADMA, SDMA, and SCX dimethyl sites.

Dotplot of the log10 ratio (172/158) for significantly changing dimethyl sites in ADMA, SDMA, and SCX experiments upon knockdown of shPRMT1. For ADMA downregulated sites, three of four sites show log10 (172/158) ratios below the median of the ADMA dataset, indicating true ADMA sites for YBX3 R220, NEFL R23, and XRN2 R946. Upregulated ADMA sites have ratios below the median in four of six sites, HNRNPU R707/709/715, HNRNPA3 R246, HNRNPU R739, HNRNPUL1 R645/656. SDMA upregulated sites show ratios above the median for SDMA in four of five site, HNRNPA1

R206, DHX9 R1249/1253/1265, HNRNPH3 R129, and KHDRBS1 R284/289/291. The upregulated SCX sites, HNRNPK R296, HNRNPU R762/768, and HNRNPR R543/546/554 are ambiguous as they have values between the medians of ADMA and SDMA, but trend towards SDMA. Repeated sites are connected by dotted gray line.



Supp. Fig. 9. Protein methyl-lysine sites by IAP and SCX are not significantly changed upon PRMT1 knockdown.

A) Volcano plot of mono-, di-, and tri-methyl lysine peptides enriched by PanK IAP. B) Volcano of mono-, di-, and tri-methyl lysine peptides enriched by SCX. No significantly changing peptides (FDR < 0.05) were identified when comparing 293T cells expressing shRNA against PRMT1 or a scrambled control. Circles are monomethyl lysine, squares are dimethyl lysine, and diamonds are trimethyl lysine.

Supplemental References

1. Wang, K., Dong, M., Mao, J., Wang, Y., Jin, Y., Ye, M., and Zou, H. (2016) Antibody-Free Approach for the Global Analysis of Protein Methylation. *Anal. Chem.* 10.1021/acs.analchem.6b02872
2. Mi, H., Huang, X., Muruganujan, A., Tang, H., Mills, C., Kang, D., and Thomas, P. D. (2017) PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. *Nucleic Acids Res.* **45**, D183–D189