Appendix for

Tudor-based Proteomic Strategy Pan-specifically Enriches and Identifies Protein Arginine Methylation

Lingzi Lu[#,[a]], Ting Li[#,[a]], Rou Zhang[#,[a]], Yutong Wang[[a]] , Xiaoping Ye,[[a]] Yixin Luo,[[a]] Lingyu Sun[[b]], Liang Qi[[a]], Zilu Ye*[[c]], Yang Mao*[[a], [d]], Yanqiu Yuan*[[a]]

#These authors contributed equally

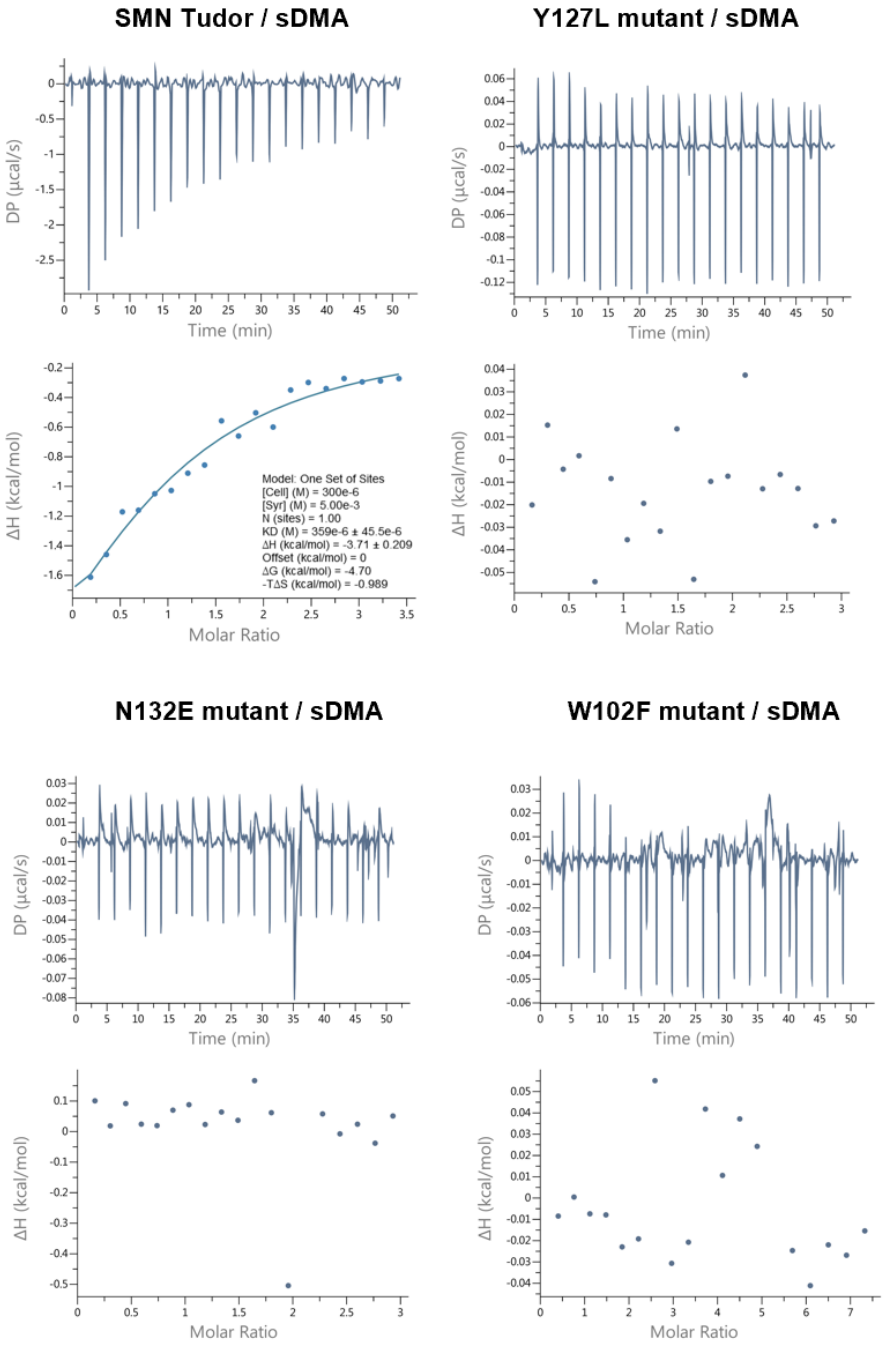
[a] L. Lu[#], T. Li[#], R. Zhang, X. Ye, Y. Wang, Y. Luo, Prof. Y. Mao, Prof. Y. Yuan
State Key Laboratory of Anti-Infective Drug Discovery and Development, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China
Email: maoyang3@mail.sysu.edu.cn (Y. Mao); yuanyq8@mail.sysu.edu.cn (Y. Yuan)

[b] L. Sun
Guangdong Institute for Drug Control, Guangzhou, China

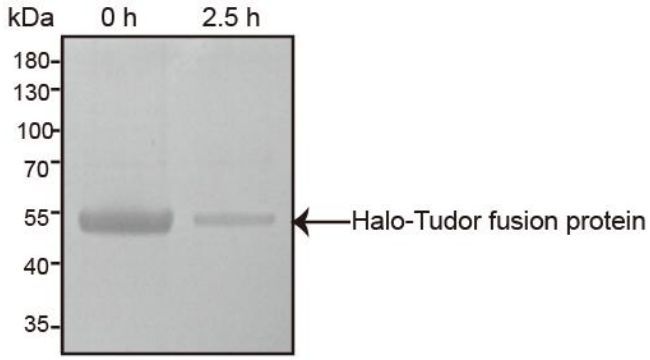
[c] Prof. Z. Ye
State Key Laboratory of Common Mechanism Research for Major Diseases, Suzhou Institute of Systems Medicine, Chinese Academy of Medical Sciences & Peking Union Medical College, Suzhou, China
Email: yzl@ism.pumc.edu.cn

[d] Prof. Y. Mao
Guangdong Provincial Key Laboratory of Drug Non-Clinical Evaluation and Research, Guangzhou, China

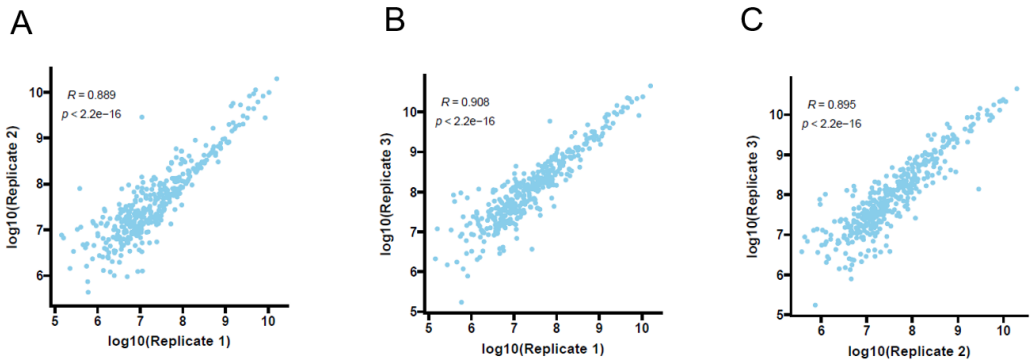
Table of contents



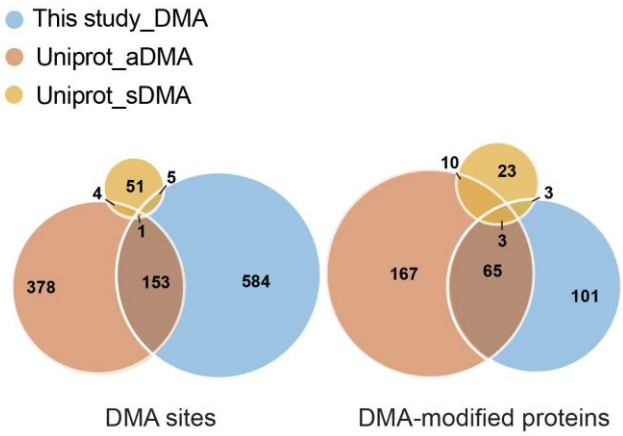
Appendix Figure S1. ITC titrations of wild type or mutant SMN Tudor with sDMA.



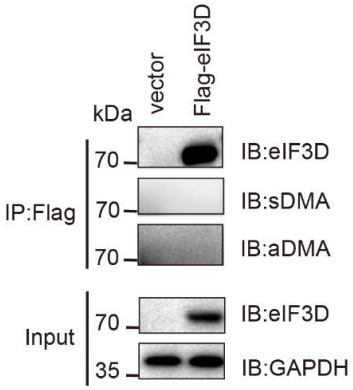
Appendix Figure S2. Coomassie blue staining after incubating 100 µL of Halo-link agarose resin with 360 µg of Halo-Tudor fusion protein at room temperature for 2.5 hours.



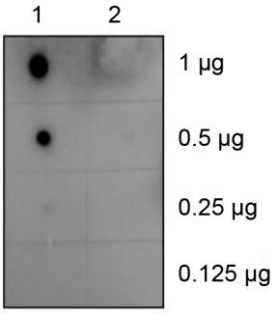
Appendix Figure S3. Correlation of the peptide precursor abundances. The Pearson correlation coefficient was used as a measure of linear correlation.



Appendix Figure S4. Venn diagram illustrates the overlapping sites or proteins containing DMA (both sDMA and aDMA) between our study and the UniProt database (2023_01).

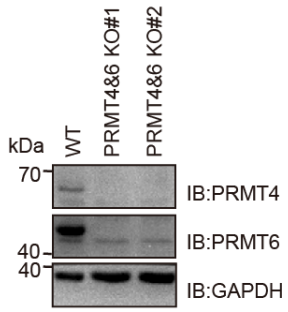


Appendix Figure S5. Immunoblot analysis of arginine dimethylation of immunoprecipitated Flag-eIF3D in HEK293T cells. Anti-sDMA and anti-aDMA antibodies were purchased from cell signaling technology with catalog numbers CST-13222 and CST-13522, respectively.



Appendix Figure S6. Dot blot analysis of anti-R99me2a antibody generated in the study. Lane 1: antigen Cys-RNRMR$_{me2a}$FAQRNL at different concentrations; Lane 2: antigen Cys-RNRMRFAQRNL at different concentrations. Antibody was diluted 1:4000.

A



B

	PRMT4 genomic DNA	PRMT6 genomic DNA
Wild type	GGACATCATCATCTCGGAGCCCATGGGCTACATGCTCT	CGCCTGCGCGTGCTGCTGCGCTACAAAGTGGGAGACCA
PRMT4&6 KO#1	GGACATCATCATCTCGGAG----------TCT (-16)	CGCCTGCGCGTGCTGCTGCGCTACAAAAGTGGGAGACCA (+1)
	GGACATCATCATCTCGGAGCC-ATGGGCTACATGCTCT (-1)	
PRMT4&6 KO#2	GGACATCATCATCTCGG----------CTACATGCTCT (-10)	CGCCTGCGCGTGCTGCTGCGCTAC----------CA (-12)
	GGACATCATCATCTCGGAGC---------TACATGCTCT (-8)	CGCCTGCGCGTGCTGCTGCGCTACAAAAGTGGGAGACCA (+1)

Appendix Figure S7. (A). Western blot analysis of PRMT4 and PRMT6 in both PRMT4 and PRMT6 double knockout HEK293T cell clones. GAPDH was used as a loading control. (B). Genomic DNA sequence of gRNA targeting site in both PRMT4 and PRMT6 double knockout HEK293T cell clones.