Supporting Information

Characterization of SETD3 methyltransferase mediated protein methionine methylation

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The Supporting Information contains Figures S1-S3 and Tables S1-S2.

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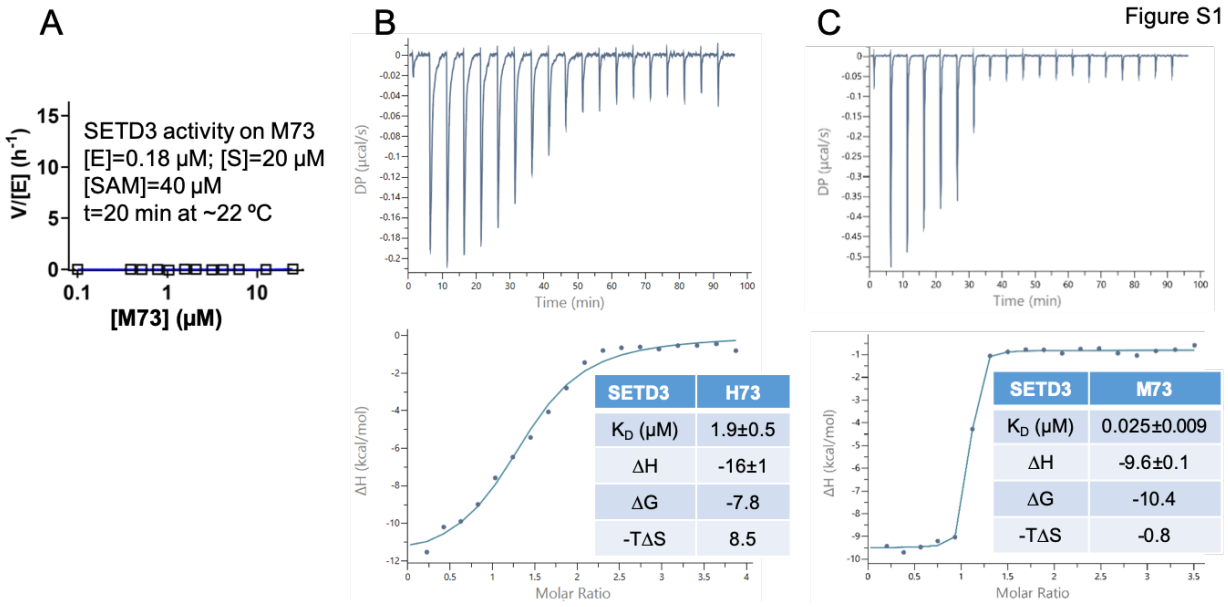


Figure S1. Related to Figure 2.

(**A**) Under the indicated reaction conditions, SETD3 activity on Met^{73} is negligible; but Met^{73} inhibits SETD3 activity on His^{73} (see Fig. 2*G*). (**B-C**) ITC experiments of SETD3 against His^{73} (B) and Met^{73} (C). The derived binding parameters are inserted.

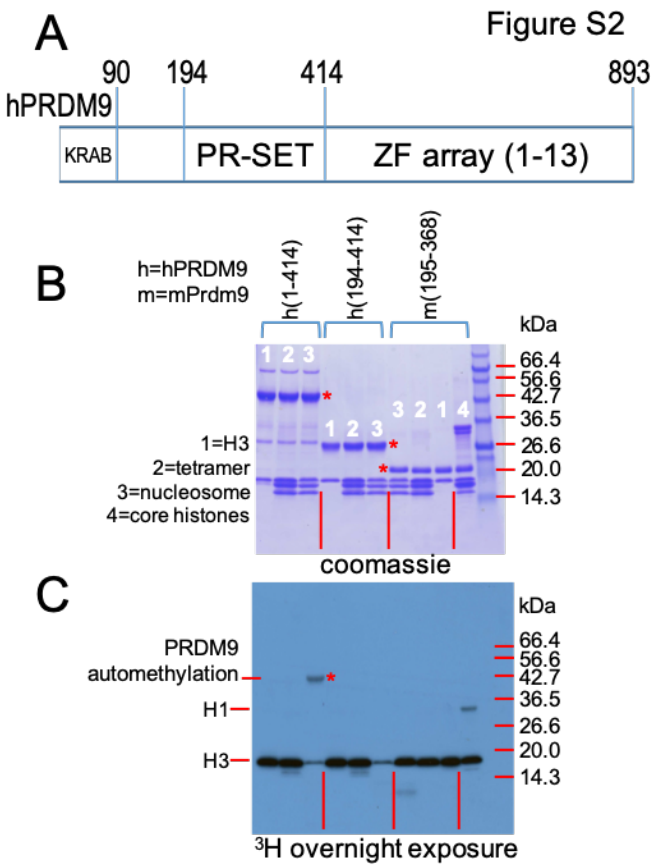
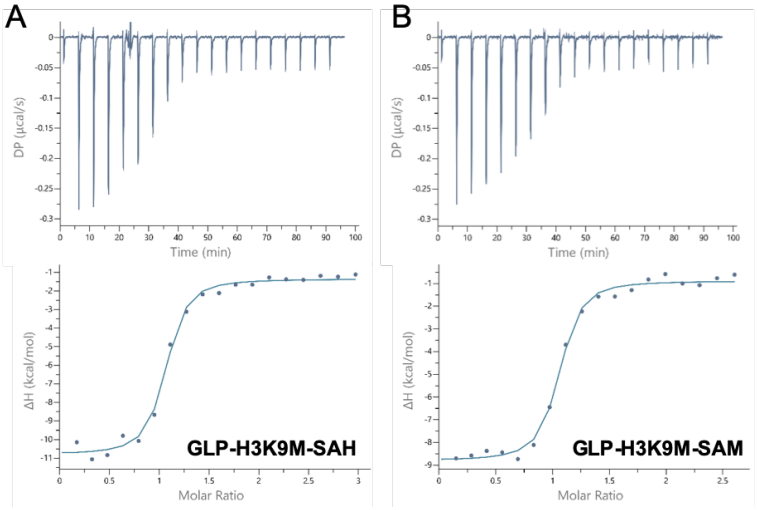


Figure S2. Related to Fig. 4*D*. Human PRDM9 automethylation.

(**A**) Schematic of human PRDM9, two fragments were generated, residues 1-414 and 194-414. (**B**) A 5-25% gradient gel run at 120 V. The reaction conditions were [E] = 5 µM, [^{3}H-SAM] = 5 µM, [S] = 3.6 µM at 50 mM Tris (pH 8.5), 150 mM NaCl. 5% glycerol, 2 mM DTT and 2 mM $MgCl_2$. The substrates include (1) recombinant H3, (2) H3-H4 and H2A-H2B tetramer, (3) reconstituted mononucleosome, and (4) calf thymus core histones. The reactions proceeded for 1 h at room temperature, and then mixed with 1X loading dye and run on a gradient SDS-PAGE gel. (C) The gel was soaked in an autoradiography enhancer solution for ~ 30 min, dried and exposed to film at -80°C for ~16-18 h. The automethylation (indicated by a red *) was observed for the long fragment (residues 1-414), and only when the lower activity on substrate nucleosome was observed.

Figure S3



C

GLP-H3M9 peptide binding
In the presence of SAH and SAM

GLP-H3K9M	SAH	SAM
K_D (μM)	0.13±0.04	0.12±0.03
ΔH (kcal/mol)	-9.4±0.2	-7.9±0.2
ΔG (kcal/mol)	-9.3	-9.4
-TΔS (kcal/mol)	-1.6	-1.5

Figure S3. ITC experiments of GLP against H3 K9M peptide in the presence of SAH (A) and SAM (B). (C) Summary of ITC parameters.

Table S1. Summary of X-ray data collection and refinement statistics

PDB ID	6WK1	6WK2
SETD3 complex	WT+Met^{73}+SAH	N255V+Met^{73}+SAM
Space group	$P2_1$	$P2_1$
Cell dimensions (Å)	60.3, 175.5, 66.7	60.3, 176.0, 66.3
(α=γ=90°)	β=92.3°	β=92.6°
Resolution (Å)	35.99-1.89 (1.96-1.89) *	36.65-1.76 (1.81-1.76)
[a] R_{merge}	0.072 (0.661)	0.062 (0.531)
R_{pim}	0.062 (0.445)	0.073 (0.740)
$CC_{1/2}$	0.986 (0.526)	0.993 (0.396)
[b] <I/σI>	10.2 (1.4)	13.8 (1.3)
Completeness (%)	93.6 (74.7)	94.7 (83.3)
Redundancy	4.7 (2.5)	5.2 (2.9)
Observed reflections	479,253	670,276
Unique reflections	102,262 (8146)	129,205 (11,330)
Refinement		
Resolution (Å)	1.89	1.76
No. reflections	102,199	129,062
[c] R_{work} / [d] R_{free}	0.194 / 0.228	0.182 / 0.206
No. Atoms	(Two complexes)	(Two complexes)
Protein	7767	7668
Peptide	308	292
SAH/SAM	52	54
Solvent	825	765
B Factors (Å^2)		
Protein	27.4	30.7
Peptide	32.6	38.1
SAH/SAM	18.8	20.3
Solvent	35.4	40.7
R.m.s. deviations		
Bond lengths (Å)	0.005	0.005
Bond angles (°)	0.8	0.8

* Values in parenthesis correspond to highest resolution shell.

[a] $R_{merge}=\Sigma|I-\langle I\rangle|/\Sigma I$, where I is the observed intensity and <I> is the averaged intensity from multiple observations.

[b] <I/σI> = averaged ratio of the intensity (I) to the error of the intensity (σI).

[c] $R_{work}=\Sigma|Fobs-Fcal|/\Sigma|Fobs|$, where Fobs and Fcal are the observed and calculated structure factors, respectively.

[d] R_{free} was calculated using a randomly chosen subset (5%) of the reflections not used in refinement.

Table S2. ETD parameters used in HPLC-MS/MS

	MS1	MS2 (1)	MS2 (2)
MS resolution setting	60,000	30,000	30,000
AGC target	5.0e5	5.0e5	5.0e5
Maximum injection time	200 ms	100 ms	100 ms
Scan range (*m/z*)	400-1500	150-2000	150-2000
ETD Reaction time (ms)	N/A	28.87 and 115.47	14
ETD target	N/A	charge-dependent	5.0e5
Max reagent injection	N/A	charge-dependent	200 ms
Data compliance review			
Name of peaklist-generating software		ProSightPC 4.0; ProteinProspector	
Name of the search engine		ProSightPC 4.0	
Name of sequence database searched		search was against a single peptide	
Number of entries in the database actually searched		no database matching; a single peptide analysis	
Specificity of all proteases used to generate peptides		no proteases were used; a synthesized peptide	
Number of missed and/or non-specific cleavages permitted		proteolysis not performed	
List of all fixed modifications (including residue specificity)		No fixed modifications	
List of all variable modifications (including residue specificity)		Methylation on lysines and methionines	
Mass tolerance for precursor ions		10 ppm	
Mass tolerance for fragment ions		20 ppm	
Threshold score/Expectation value for accepting individual spectra		Three spectra were manually selected based on precursor mass	
Estimation of false discovery rate (FDR) and how calculated (for large datasets)		This was not a large dataset as we only analyzed one peptide and searched for one peptide	