

Supplemental Information

Structural Basis for Substrate Preference of SMYD3, A SET Domain-containing Protein Lysine Methyltransferase

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Running Title: Substrate preference of lysine methyltransferase SMYD3

Supplemental Experimental Procedures

Methyltransferase assay

The methyltransferase reactions in Fig. S4A using full-length histone H3 or nucleosome as substrates were performed in a 30 μ l reaction mixture containing 1 μ M 3 H-labeled S-adenosyl-L-methionine (PerkinElmer life science), 10 μ g recombinant SMYD3, 3 μ g recombinant histone H3 or 12 μ g histone octamers or 12 μ g oligonucleosomes in a methyltransferase assay buffer (20 mM Tris-HCl 8.5, 5 mM MgCl₂, 10 mM DTT). The reaction was stopped after 3 hours' incubation at 30 °C by addition of SDS sample buffer and reaction products were separated by 13% SDS-PAGE. Separated proteins were transferred onto PVDF membranes and then subjected to autoradiography. For quantification, the membranes were stained by Coomassie Blue G250 followed by liquid scintillation counting for each stained band.

Supplemental Figure Legends:

Figure S1. Superimposition of the reported SMYD3 structures. (A) The SMYD3-MAP3K2-SAH structure is shown in the same coloring scheme as in Fig. 2A. Apo structure of SMYD3 (PDB: 3QWP) is shown in white, SMYD3-SAM structure (PDB: 3MEK) is shown in light grey and SMYD3-Sinfungin structure (PDB: 3PDN) is shown in grey. (B) An enlarged view of co-factors binding to the same SAM binding pocket of SMYD3 formed by three loop regions.

Figure S2. Topology diagram of the arrangement of secondary structure elements of SMYD3. Number of the residues at the boundary of the secondary elements are labeled.

Figure S3. Electron density maps surrounding the substrate peptides of (A) MAP3K2 and (B) VEGFR1. A section of 2Fo-Fc map is displayed at 1.5 σ level.

Figure S4. (A) *In vitro* MTA results of SMYD3 catalyzing methyltransferase reactions on full length histone H3 (rH3), histone octamers (rOct) and nucleosome arrays (rON). dsDNA additives containing 5'-CCCTCC-3 affect the HMTase activity of SMYD3 on histone H3. (B) Analysis of the folded regions of MAK3K2 by FoldIndex (<http://bip.weizmann.ac.il/fldbin/findex>). Lys260 lies on a long loop region between the two folded domains.

Supplemental Figures

Figure S1

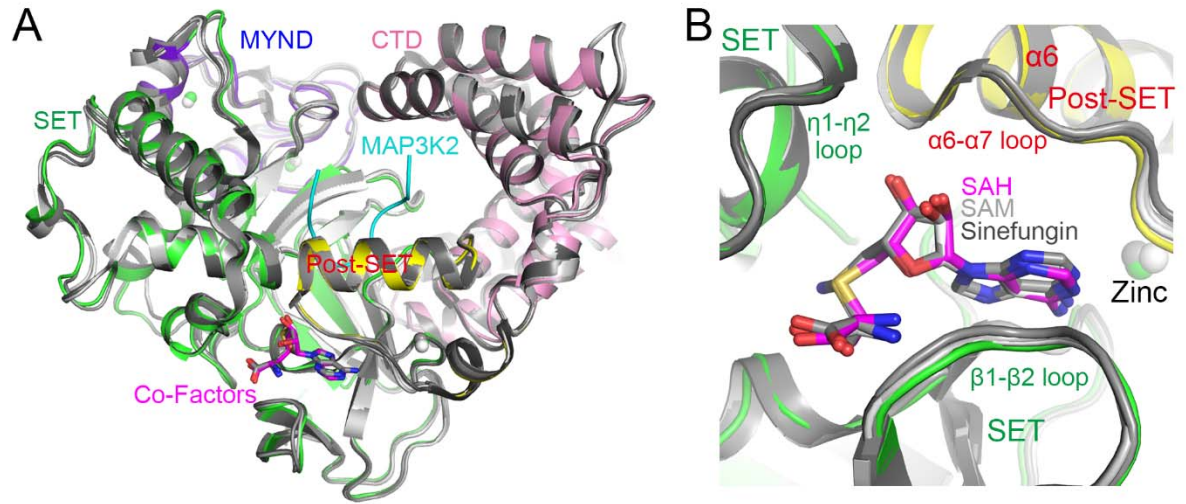


Figure S2

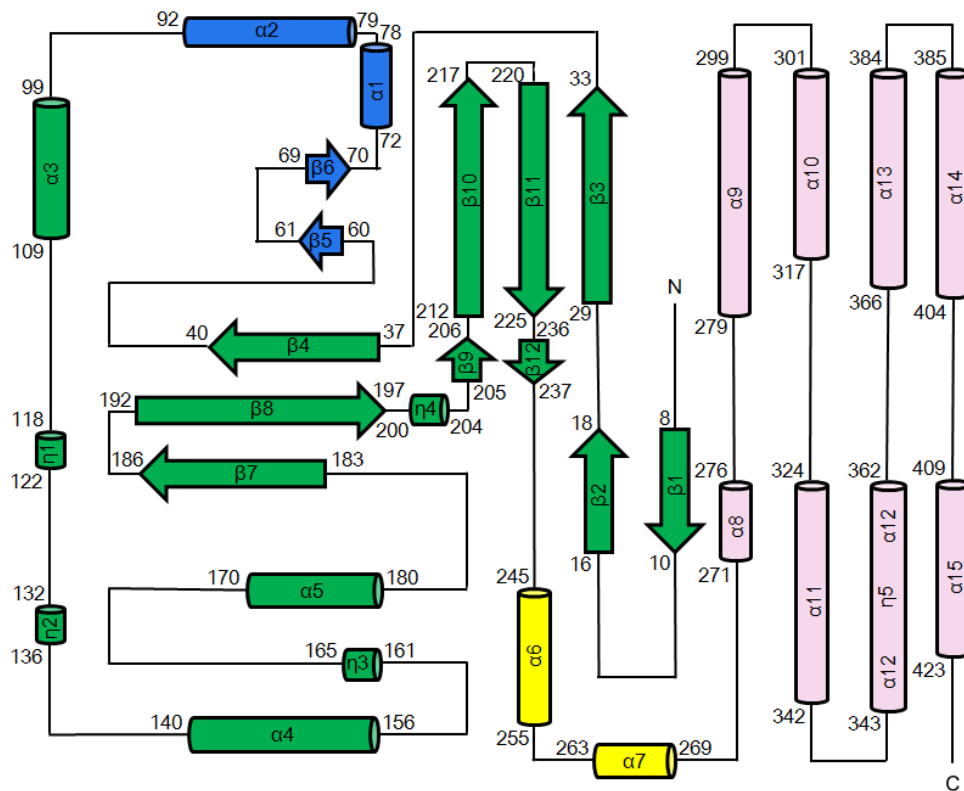


Figure S3

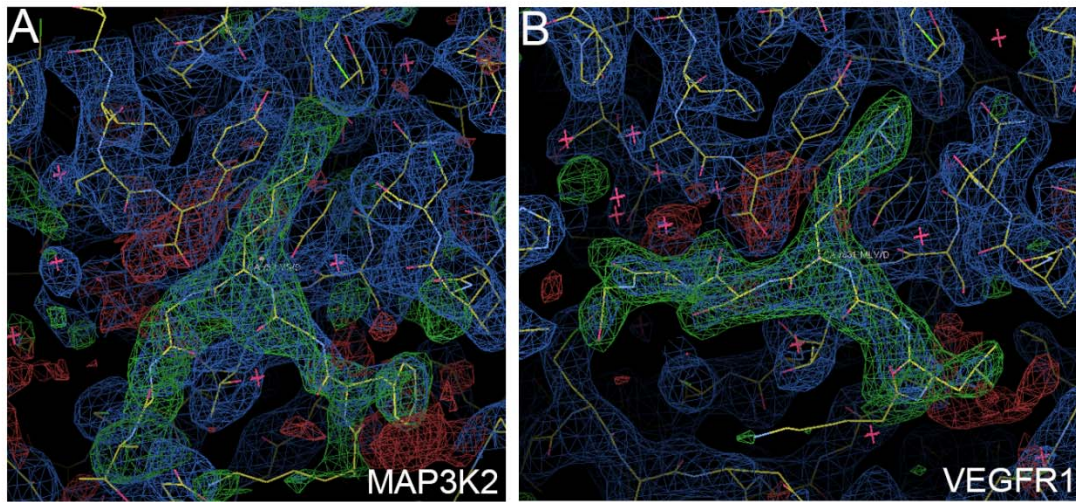


Figure S4

