Supplementary Materials for

Identification of nonhistone substrates of the lysine methyltransferase PRDM9

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Other Supplementary Material includes the following:

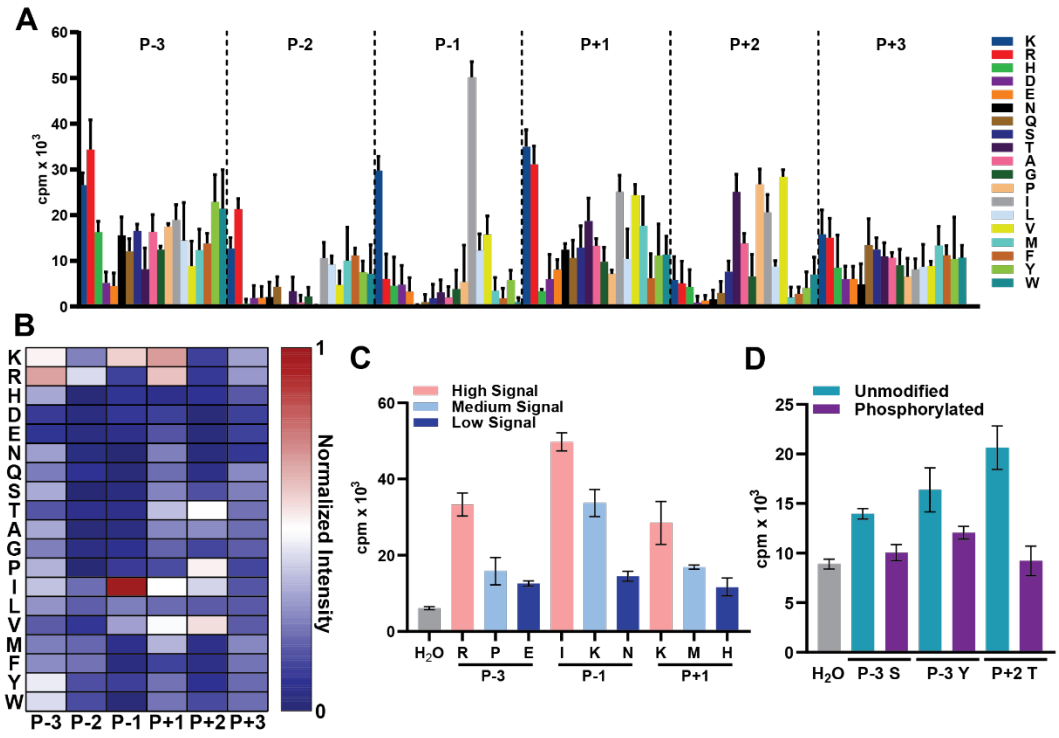

Figure S1. Analysis of PRDM9 substrate selectivity using a K-OPL. *A,* Data for PRDM9 K-OPL selectivity profile showing average signal for reactions with each K-OPL set (n=3). *B,* Heatmap normalized globally to the set with the highest signal. *C,* Validation of PRDM9 K-OPL selectivity profile on select K-OPL sets (n=2). *D,* PRDM9 reactions on phosphorylated K-OPL sets (n=3). Bar graphs display mean ±SD.

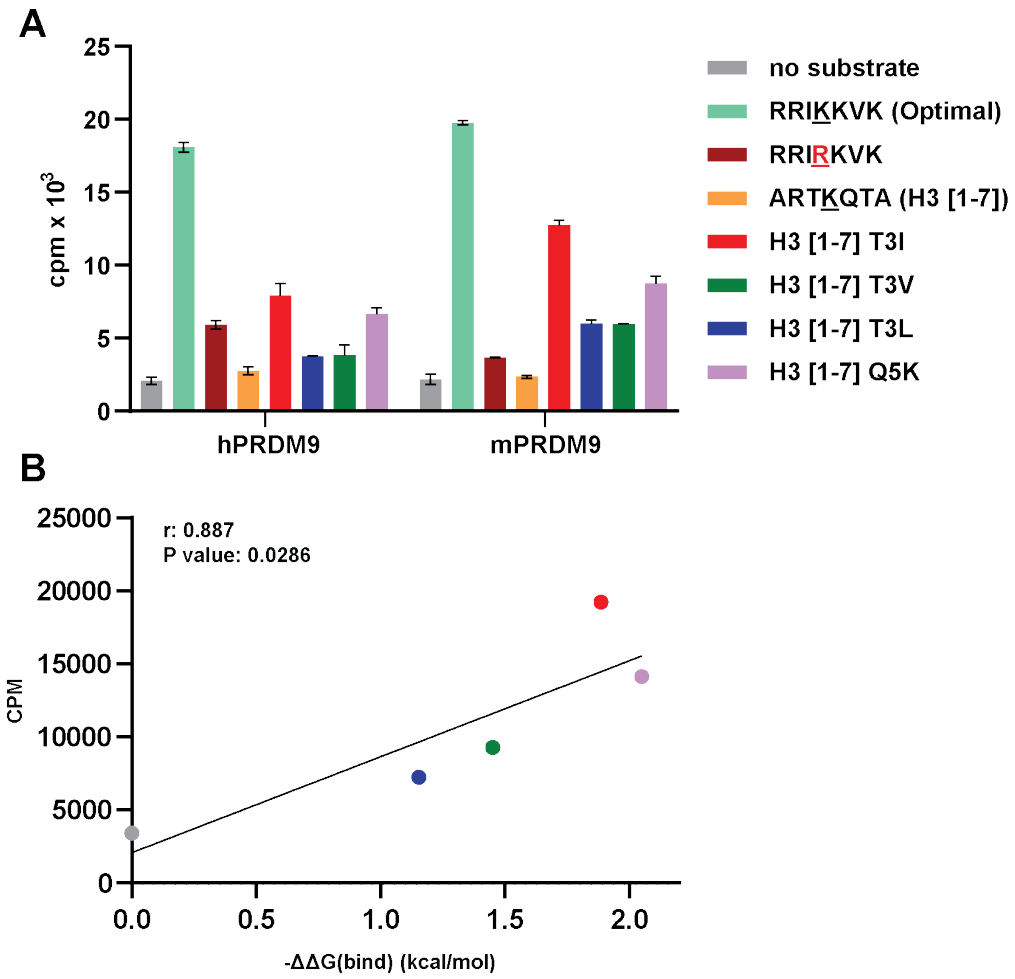

Figure S2. Analysis of PRDM9 substrate selectivity using a K-OPL *A,* Comparison of human PRDM9 and mouse Prdm9 on peptide substrates shows the highly homologous PR/SET domain have the same substrate selectivity on peptides used in this study. Graph depicts mean (n=2) ±SD signal from scintillation proximity methyltransferase assays (SPA) with biotin labeled peptides. *B,* Correlation of changes in relative binding free energies determined from MSλD for H3 peptides substituted at critical positions with experimental changes in methyltransferase activity for PRDM9. On-tailed Pearson (r) correlation was calculated using GraphPad Prism 9.

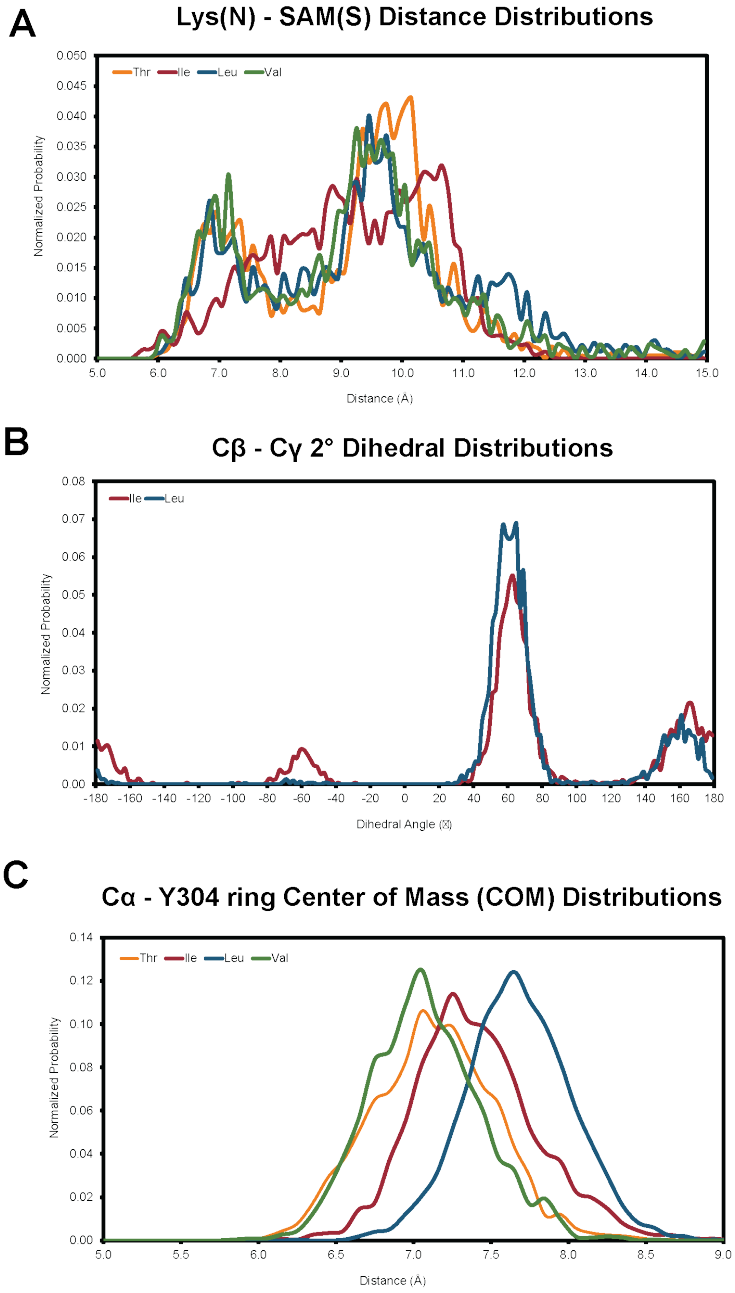

Figure S3. Additional MSλD trajectory analyses of PRDM9 substrate selectivity. *A,* Lys(N) - SAM(S) Distance Distributions. *B,* Cβ - Cγ 2° Dihedral Angle Distributions. *C,* Cα - Y304 ring Center of Mass (COM) Distance Distributions.

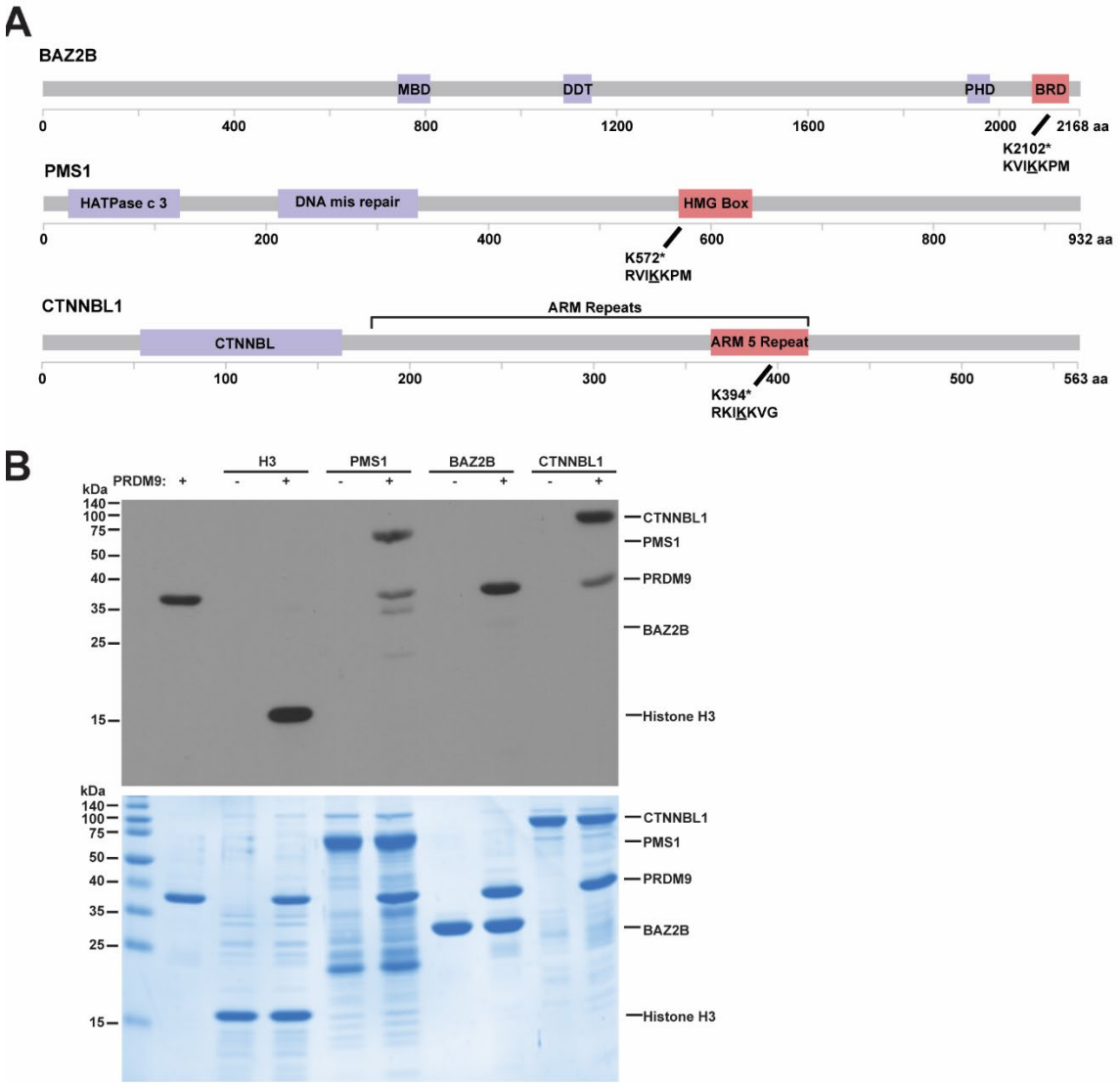

Figure S4. Domain maps of putative PRDM9 non-histone substrates and *in vitro* methyltransferase assays using recombinant proteins. *A*, The predicated PRDM9 methylation site and 7-mer motif is displayed. Data for domain boundaries was derived from UniProt. *B,* PRDM9 activity on recombinant non-histone substrates detected by fluorography (top panel). Bottom panel shows total protein stained with Coomassie after separation by SDS-PAGE. Representative of two independent experiments.