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Supplemental information

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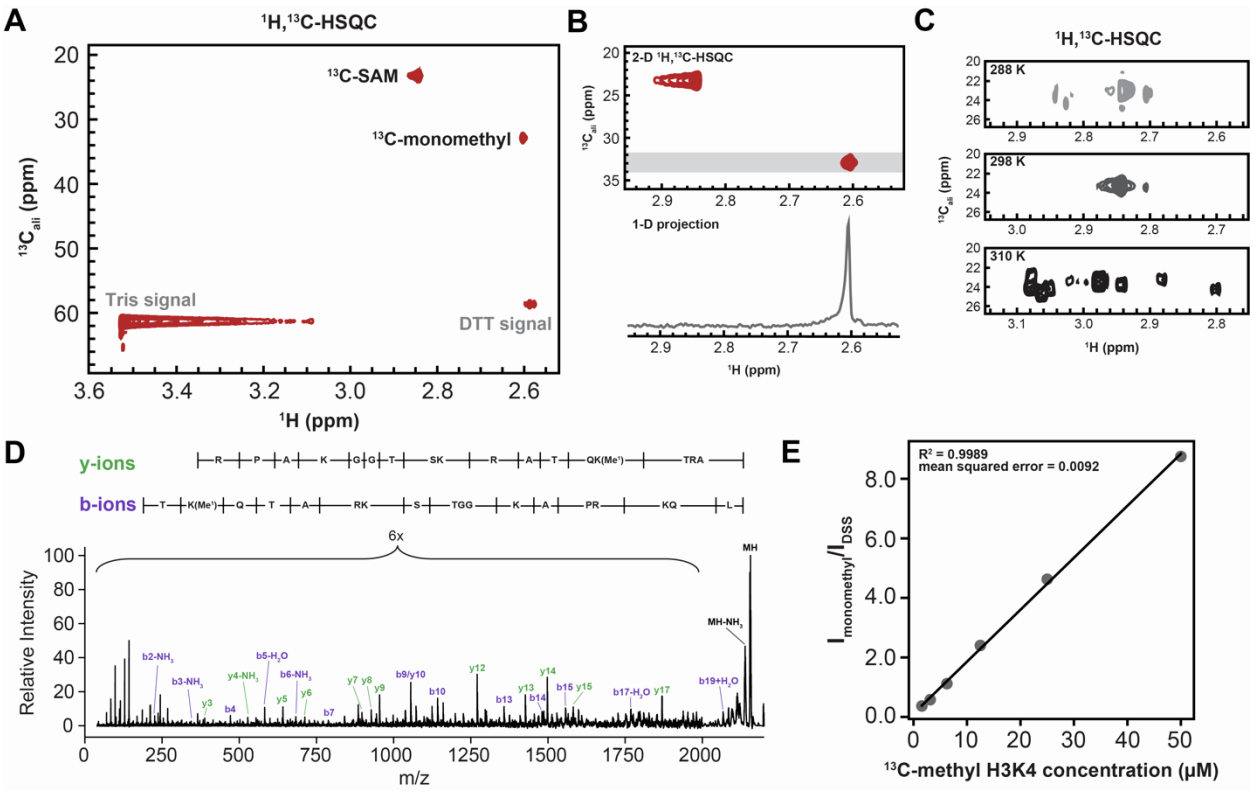
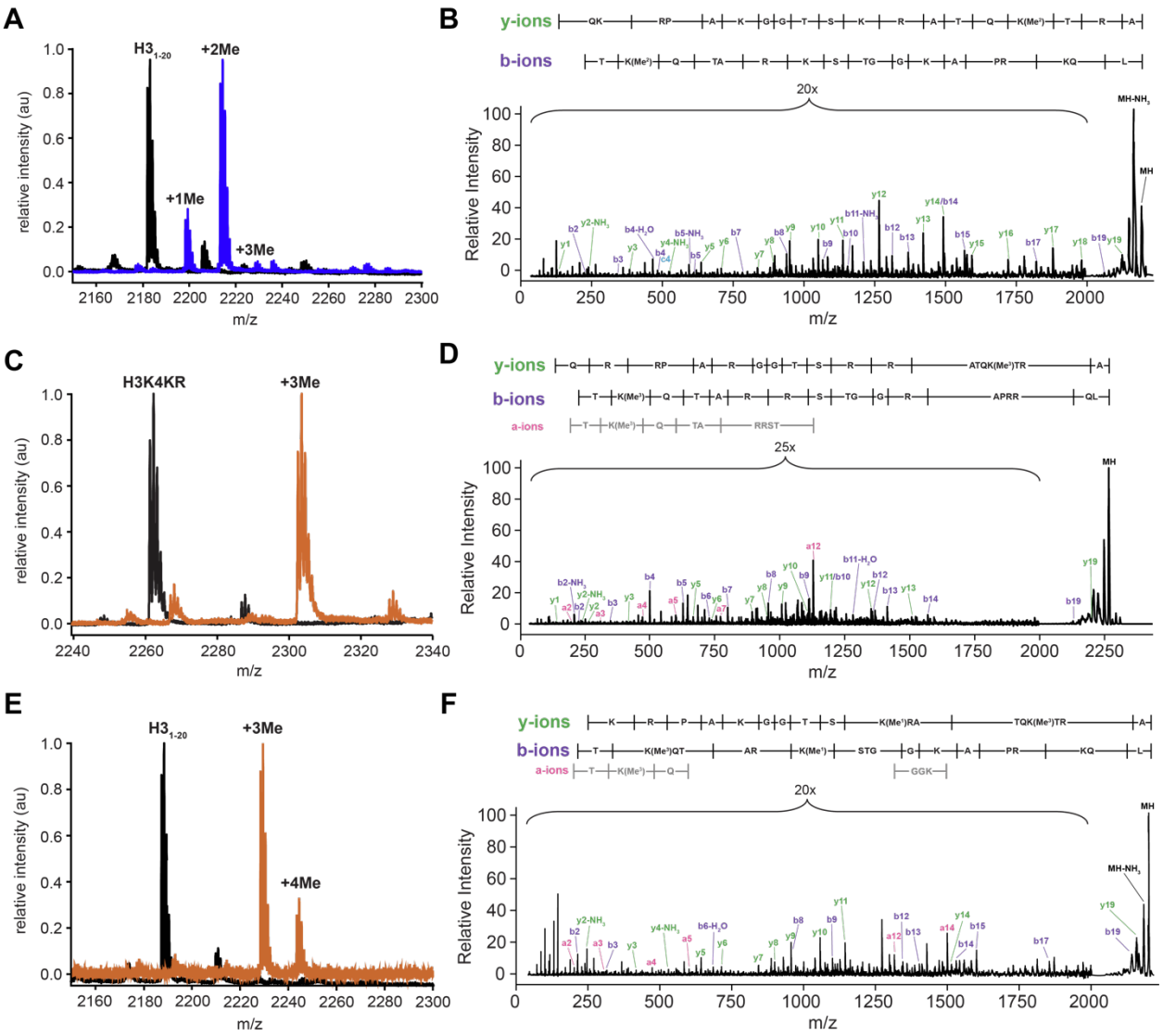


Supporting Figure 1: Detection of monomethylated H3 peptide by NMR and mass spectrometry. (A) 2-D ^{1}H, ^{13}C-HSQC of an endpoint reaction of natural-abundance $H3_{1-20}$ and Set7 with ^{13}C-SAM as the cofactor. The region of the spectrum that contains the peaks of interest (^{13}C-SAM and ^{13}C-methyllysine) is distinct from the region that contains signals from the buffer components. (B) Zoomed-in and cropped view of the HSQC in (A) (top). The grey shaded region represents the slice of the 2-D experiment used to calculate the 1-D projection (bottom) for the purposes of tracking the monomethyl peak over >100 experiments. (C) The ^{13}C-SAM resonance(s) at the final timepoint of a real-time NMR experiment at three temperatures. The presence of multiple distinct peaks at the end of a methylation reaction conducted at 310 K, but not the lower temperatures, suggests a temperature-dependent instability of the ^{13}C-SAM cofactor. (D) MALDI-TOF-TOF MS/MS spectrum of the +Me1 peak from (D) confirms that $H3_{1-20}$ was monomethylated on Lys4. (E) Plot of ratio of monomethylated H3 and DSS standard intensities versus H3 concentration. Linear regression demonstrates linearity of monomethyl peak intensity with respect to monomethyl peptide concentration between 1 and 50 μM.



Supporting Figure 2: Extent of methylation and location of modifications for MLL1 and PRDM9 validated by MALDI-TOF MS and TOF-TOF MS/MS. (A) The MALDI-TOF mass spectrum of unmodified $H3_{1-20}$ (black, 2183 m/z) compared with $H3_{1-20}$ following treatment with MLL1 and ^{13}C-SAM (blue) shows primarily dimethylation (+Me2, 2213 m/z). Congruent with the final NMR timepoint, there was still some remaining monomethylated peptide (+Me1, 2198 m/z). We also observed a very small population of trimethylated peptide, which is consistent with previous reports on MLL1 activity. (B) MS/MS spectrum of the +Me2 peak from (A) shows that both methyl marks were placed on Lys 4. (C) Mass spectrum of unmodified H3K4KR peptide (black, 2267 m/z) compared with H3K4KR after treatment with PRDM9 and ^{13}C-SAM (orange) shows complete trimethylation (+Me3, 2312 m/z). (D) MS/MS spectrum of the +3Me peak from (C) shows that all three methyl marks were directed to Lys 4. (E) Mass spectrum of unmodified $H3_{1-20}$ (black, 2183 m/z) compared with $H3_{1-20}$ after treatment with PRDM9 and ^{13}C-SAM (orange) shows that trimethylation (+Me3, 2228 m/z) was the major species. A peak at 2243 m/z suggests that a minor population of $H3_{1-20}$ was tetramethylated (+Me4, 2243 m/z). (F) MS/MS analysis of the +4Me peak from (E) shows that PRDM9 placed an aberrant methyl mark on Lys 9 in addition to the expected three on Lys 4. This justified our switch to a lysine-deficient mutant peptide (H3K4KR) for PRDM9 analysis by RT-NMR.

Supporting Table 1: Chemical shifts of peaks of interest for each methyltransferase.

	Set7†		MLL1		PRDM9	
	δ ^{1}H (ppm)	δ ^{13}C (ppm)	δ ^{1}H (ppm)	δ ^{13}C (ppm)	δ ^{1}H (ppm)	δ ^{13}C (ppm)
SAM	2.842	23.229	2.779	23.110	2.777	23.130
monomethyl	2.603	32.860	2.536	32.751	2.533	32.811
dimethyl	--	--	2.684	42.655	2.680	42.646
trimethyl	--	--	--	--	2.940	52.717

†Set7 experiments were conducted at 298 K whereas MLL1 and PRDM9 experiments were collected at 288 K.

Supporting Table 2: Key reagents and resources used in this study.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
BL21(DE3) *E. coli*	New England BioLabs	Cat# C2527I
Rosetta(DE3) *E. coli*	MilliporeSigma	Cat# 70954
Rosetta(DE3) pLysS *E. coli*	MilliporeSigma	Cat# 70956
Chemicals, Peptides, and Recombinant Proteins		
L-methionine (methyl-^{13}C, 99%)	Cambridge Isotope Laboratories, Inc.	Cat# CLM-206-PK
$H3_{1-20}$: ARTKQTARKSTGGKAPRKQL	Genscript	
H3K4KR: ARTKQTARRSTGGRAPRRQL	Genscript	
Recombinant DNA		
pET28a-Set7 (109-366)	Dr. Cheryl Arrowsmith	RRID:Addgene_40746
pET28-PRDM9 (195-415)	Dr. Cheryl Arrowsmith	RRID:Addgene_162257
pMAL-C2-TEV S219V	(1)	RRID:Addgene_19893
pST44-MWRA	Dr. Song Tan	(2)
pHis-DPY-30 (1-99)	(3)	N/A
pET19b-SAM Synthetase	Dr. Squire J. Booker	(4)
Software and Algorithms		
ProteinProspector, MS-Product	https://prospector.ucsf.edu/prospector/mshome.htm	RRID:SCR_014558
Spyder (Python)	https://www.spyder-ide.org	RRID:SCR_017585
TopSpin	https://www.bruker.com/products/mr/nmr/nmr-software/nmr-software/topspin/overview.html	RRID:SCR_014227
MATLAB	https://www.mathworks.com/products/matlab.html	RRID:SCR_001622
NMRFAM_Sparky	https://nmrfam.wisc.edu/nmrfam-sparky-distribution/	(5)

Supporting References

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