Supplemental Information

The H1.0 C Terminal Domain is Integral For Altering Transcription Factor Binding Within Nucleosomes

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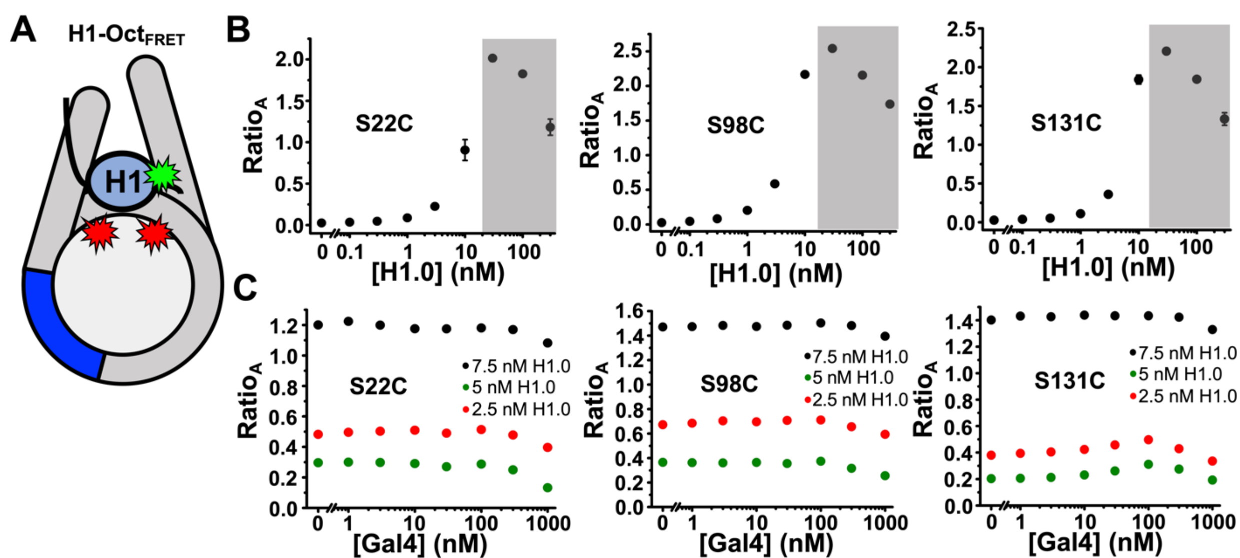
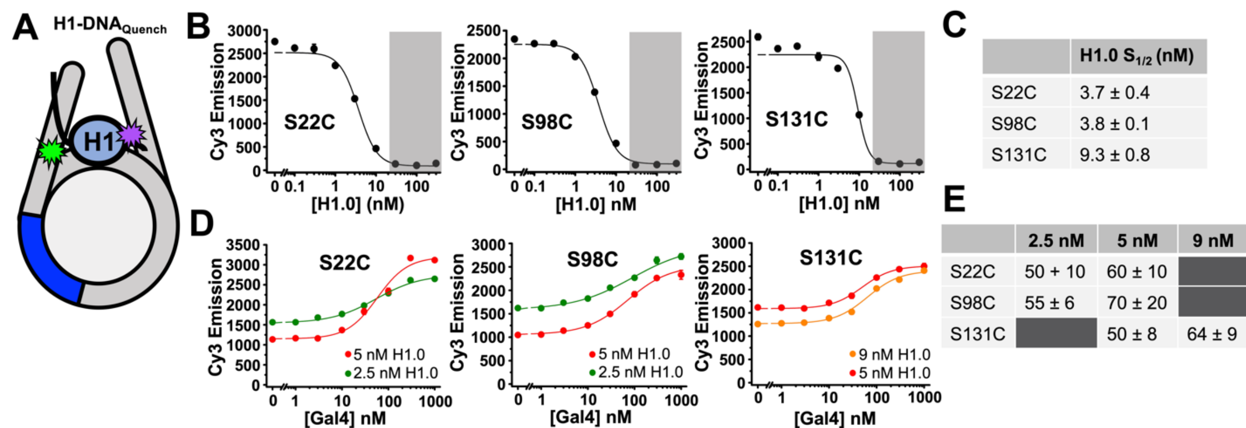


Figure S1: Complete fluorescence binding data for H1.0 and Gal4 to H1-Oct$_{FRET}$ nucleosomes. (**A**) Diagram of the H1-Oct$_{FRET}$ construct. (**B**) Measured Ratio$_A$ of Cy3 H1.0 S22C, S98C, or S131C binding to H1-Oct$_{FRET}$ nucleosomes. (**C**) Measured Ratio$_A$ of Gal4 binding to Cy3 H1.0 S22C, S98C, or S131C bound nucleosomes. Multiple concentrations of H1.0 used for each fluorophore location represented in black, green, and red.



	H1.0 $S_{1/2}$ (nM)
S22C	3.7 ± 0.4
S98C	3.8 ± 0.1
S131C	9.3 ± 0.8

	2.5 nM	5 nM	9 nM
S22C	50 + 10	60 ± 10	
S98C	55 ± 6	70 ± 20	
S131C		50 ± 8	64 ± 9

Figure S2: Complete fluorescence binding data for H1.0 and Gal4 to H1-DNA_{Quench} nucleosomes. (**A**) Diagram of the H1-DNA_{Quench} construct. (**B**) Background subtracted, non-normalized Cy3 emission of TQ3 H1.0 S22C, S98C, or S131C binding to H1-DNA_{Quench} nucleosomes. (**C**) Table of measured H1.0 $S_{1/2}$ values for each concentration of H1.0 and fluorophore location tested in panel B. (**D**) Background subtracted, non-normalized Cy3 emission of Gal4 binding to TQ3 H1.0 S22C, S98C, or S131C bound nucleosomes. Multiple concentrations of H1.0 used for each fluorophore location represented in orange, green, and red. (**E**) Table of measured Gal4 $S_{1/2}$ values for each concentration of H1.0 and fluorophore location tested. Dark grey represents concentration and fluorophore locations that weren't tested.

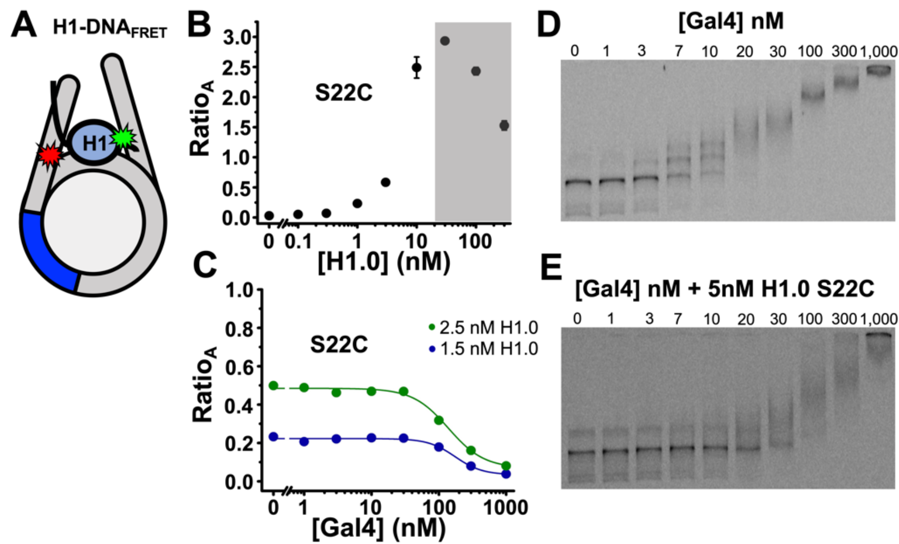


Figure S3: Fluorescence binding data for H1.0 and Gal4 to H1-DNA_{FRET} nucleosomes. (**A**) Diagram of the H1-DNA_{FRET} construct. (**B**) Measured $Ratio_A$ of Cy3 H1.0 S22C binding to H1-DNA_{FRET} nucleosomes. (**C**) Measured $Ratio_A$ of Gal4 binding to Cy3 H1.0 S22C bound nucleosomes. Gal4 $S_{1/2}$ with 1.5 nM H1.0 S22C is 174.2 ± 7.5 nM (blue). Gal4 $S_{1/2}$ with 2.5 nM H1.0 S22C is 138.4 ± 16.2 nM (green). (**D**) EMSA of Gal4 binding to H1-DNA_{FRET} nucleosomes in the absence of 5 nM H1.0 S22C. Estimated Gal4 $S_{1/2}$ of ~5-10 nM based on disappearance of the unshifted nucleosome band. (**E**) EMSA of Gal4 binding to H1-DNA_{FRET} nucleosomes in the presence of 5 nM H1.0 S22C. Estimated Gal4 $S_{1/2}$ of ~20-30 nM based on disappearance of the unshifted nucleosome band.

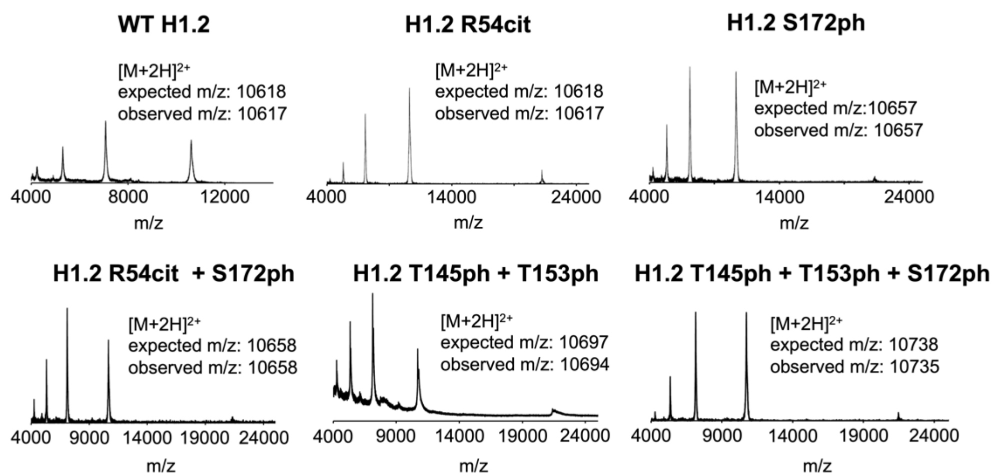


Figure S4: MALDI-TOF of final synthetic H1.2 containing PTMs. Expected and observed m/z for peak with two positive charges.

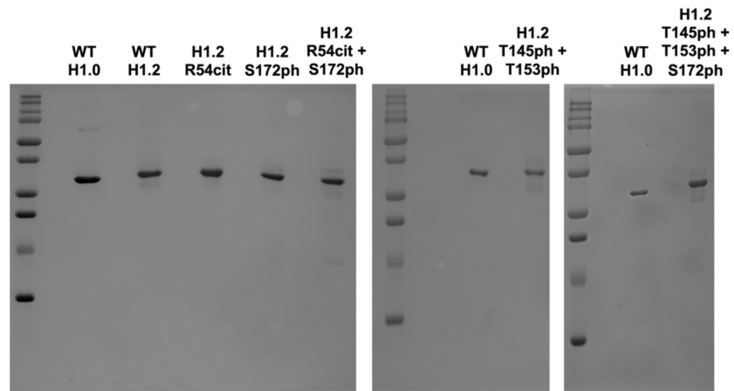


Figure S5: SDS acrylamide gels of final synthetic H1.2 containing PTMs.

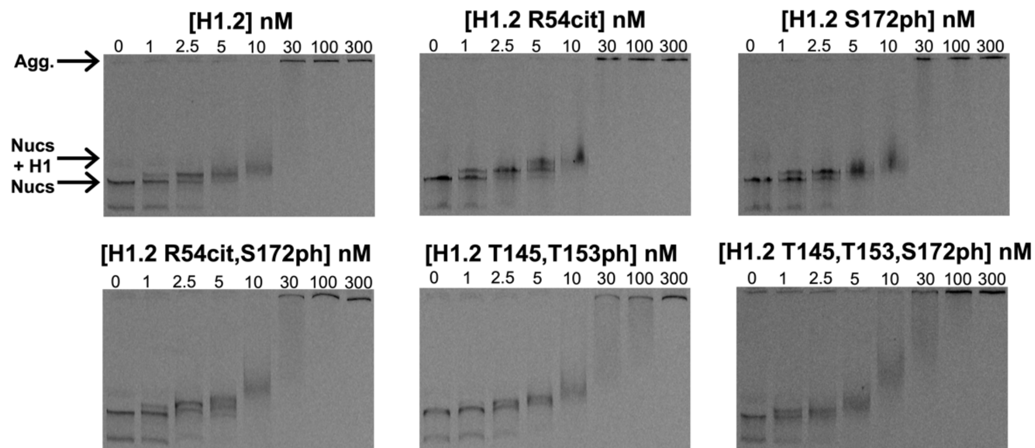


Figure S6: EMSAs of synthetic H1.2 with various PTMs binding to DNA-Oct$_{FRET}$ nucleosomes.

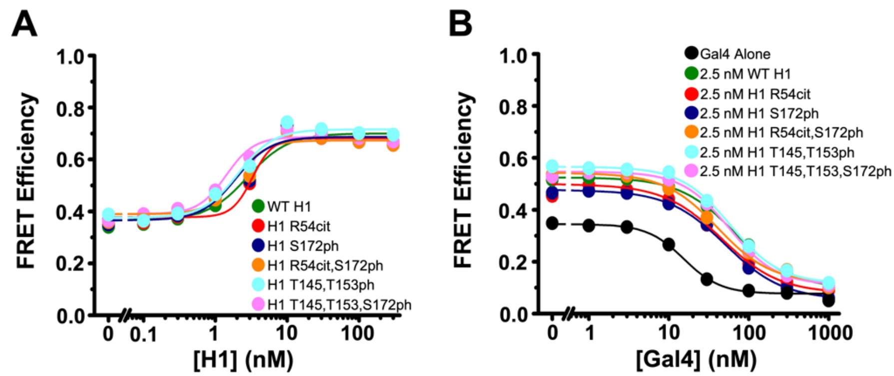


Figure S7: H1.2 and Gal4 binding to DNA-Oct$_{FRET}$ nucleosomes. (A) FRET Efficiency of H1.2 with various PTMs binding to nucleosomes. Data for all PTMs tested. (C) FRET efficiency of Gal4 binding to H1.2 bound nucleosomes using various PTMs on H1.2. Data for all PTMs tested.