

SUPPLEMENTARY DATA

Molecular basis for bipartite recognition of histone H3 by the PZP domain of PHF14

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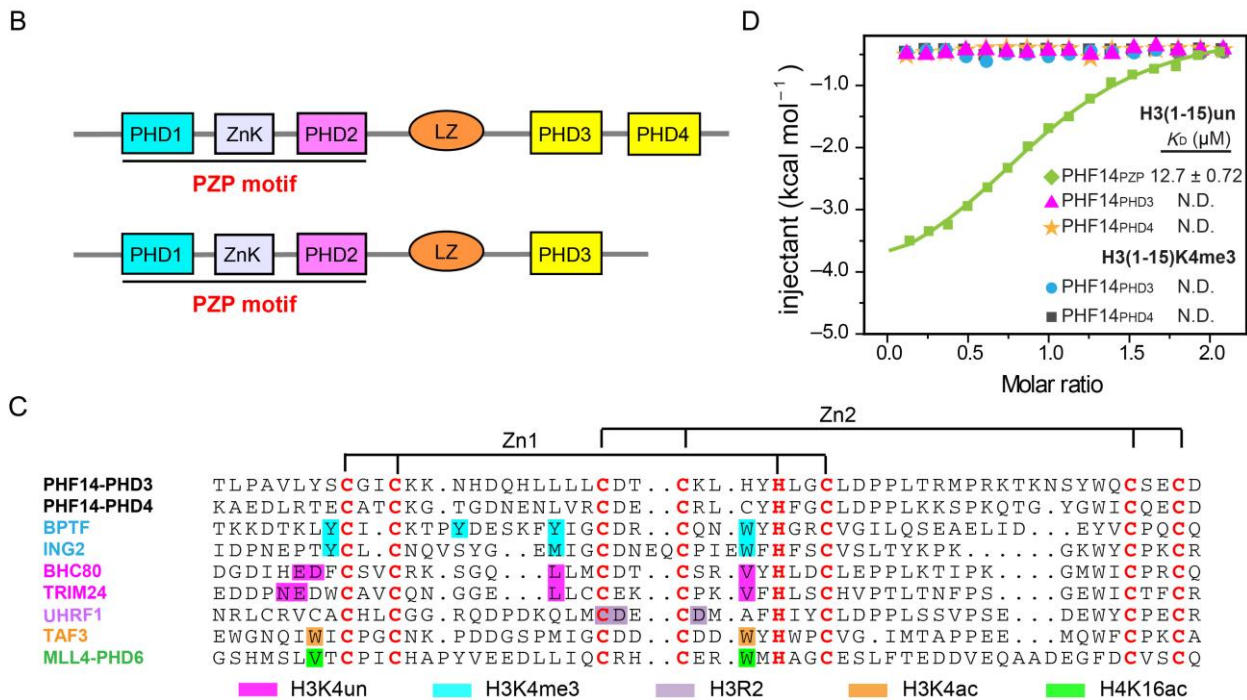
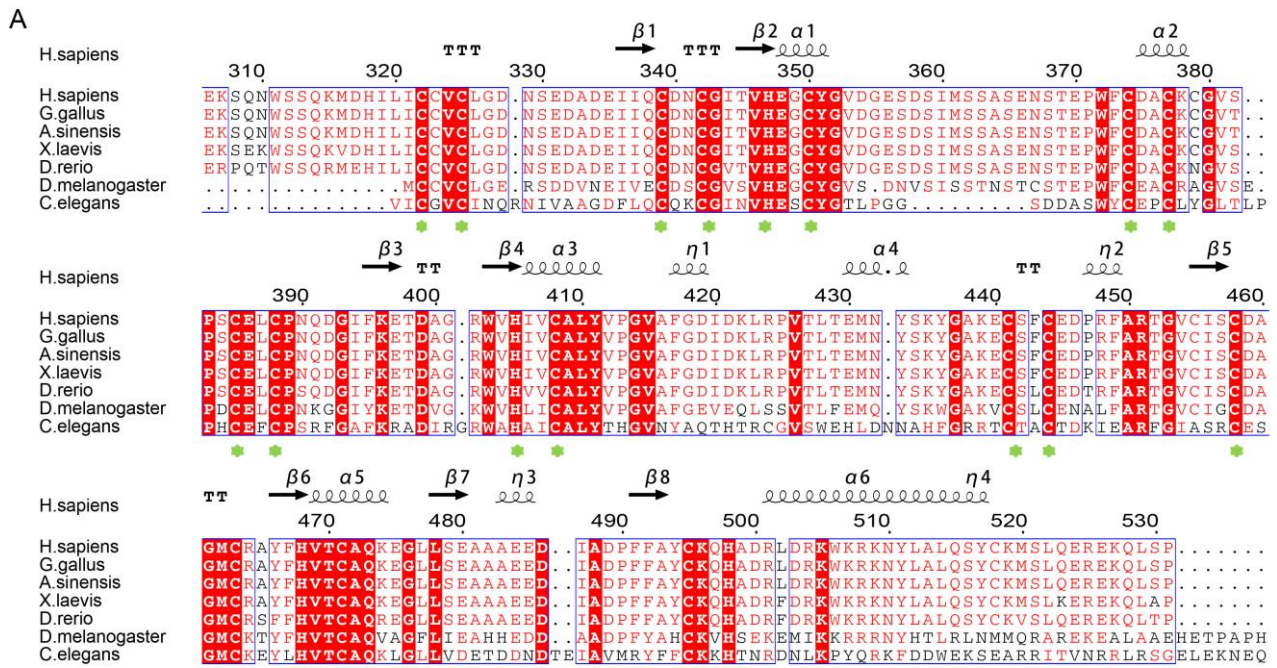
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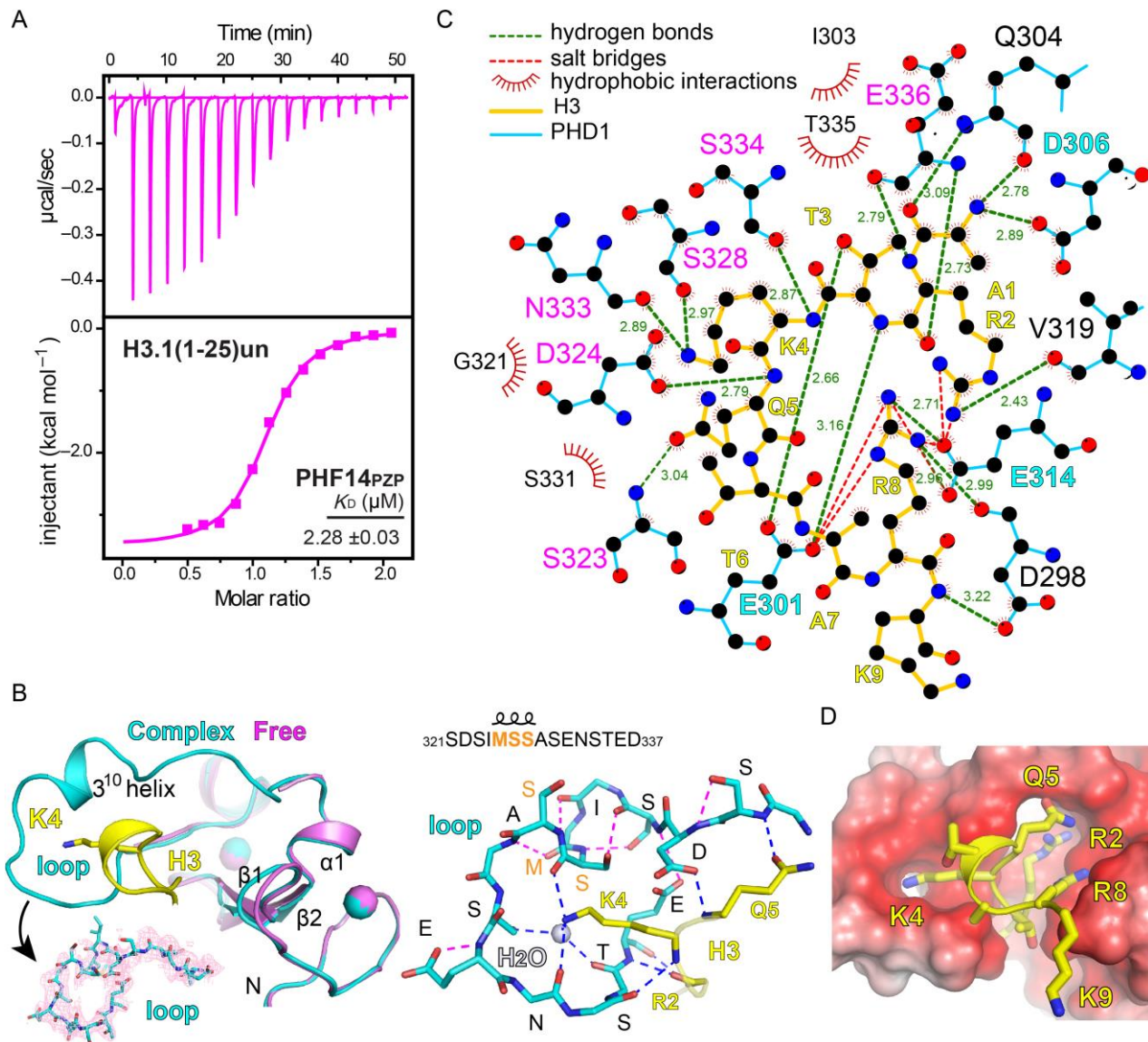
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Supplementary_Figures S1-6

Supplementary_Table S1-3

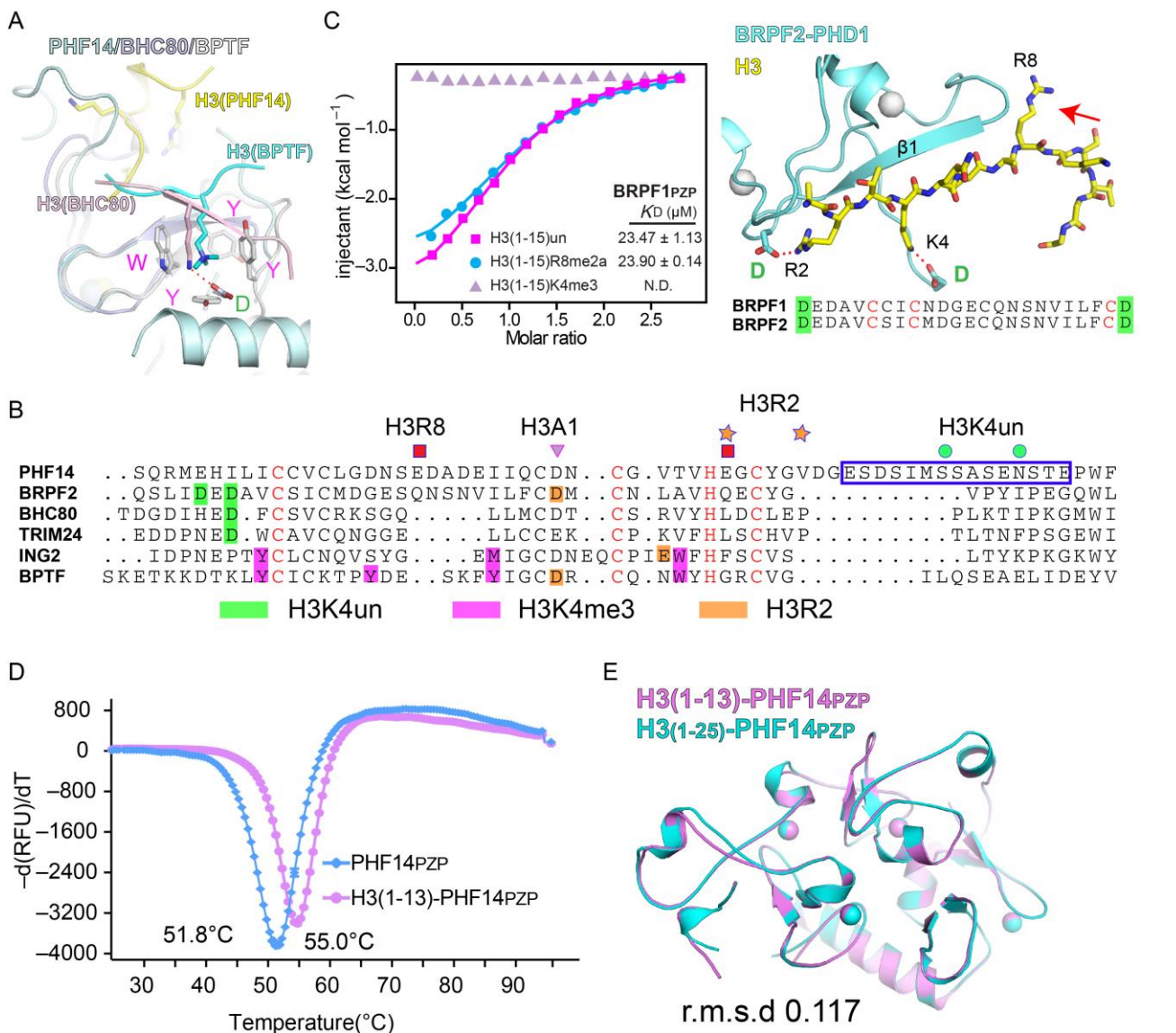


Supplementary Figure S1. PHF14_{PZP} is an unmodified histone H3₍₁₋₃₄₎ reader. (A) Sequence alignment of PHF14_{PZP} orthologues. (B) Domain architectures of two isoforms of PHF14. (C) Sequence alignment of PHF14_{PHD3}, PHF14_{PHD4} and other PHD-containing histone reader proteins. Key residues recognizing H3K4me3, H3K4un, H3R2un, H3K4ac and H4K16ac are shaded in cyan, magenta, purple, orange and green background, respectively. (D) ITC fitting curves of PHF14_{PHD3}, PHF14_{PHD4} and PHF14_{PZP} titrated with unmodified H3₍₁₋₁₅₎ and H3₍₁₋₁₅₎ K4me3 peptides, respectively.



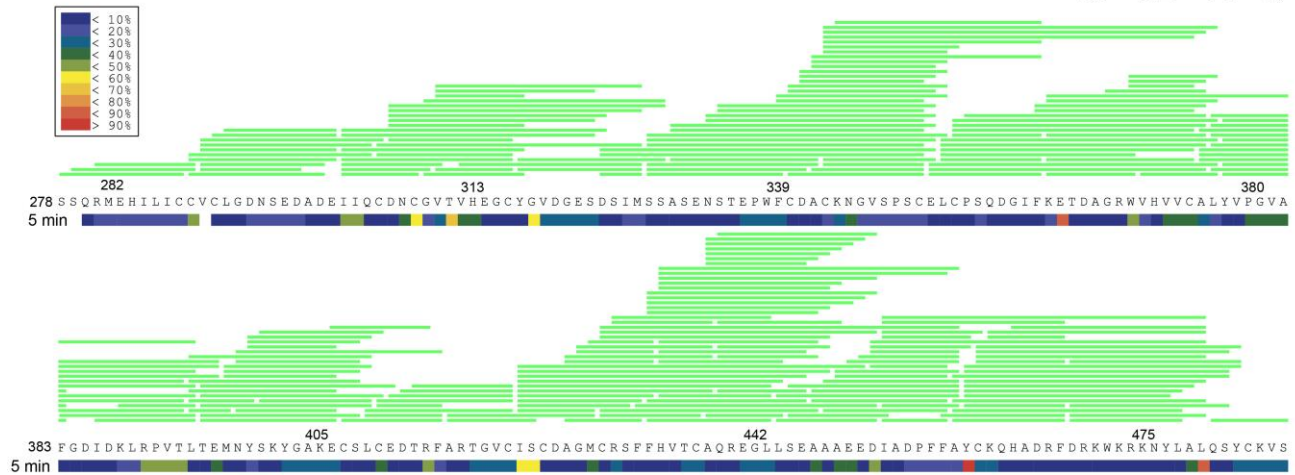
Supplementary Figure S3. Interaction analyses for H3₍₁₋₂₅₎ readout by PHF14_{PZP}. (A) ITC titration and fitting curves of H3₍₁₋₂₅₎-PHF14_{PZP} interaction pair. (B) Left, structural alignment of free PHF14_{PZP} (magenta) and H3₍₁₋₂₅₎-PHF14_{PZP} complex (yellow/cyan). 2Fo-Fc omit map of the reordered insertion loop is contoured at 0.5 σ level and shown as pink meshes; Right, hydrogen binding network of between H3 (yellow) and the insertion loop (cyan). Bluewhite sphere, water; magenta dashes, intra-chain hydrogen bonds within the insertion loop; blue dashes, inter-chain hydrogen bonds. Note the 3^{10} helix (η) formation of the “MSS” motif of the insertion loop. (C) LIGPLOT diagram listing critical contacts between PHF14_{PZP} and the modelled H3₍₁₋₉₎ peptide. (D) Intimate engagement of H3₍₁₋₉₎ by an acidic cleft of PHD1 of PHF14_{PZP}. The electrostatic potential surface is colored as a spectrum from blue (≤ 5 kT/e) to red (≥ 5 kT/e). Side chains of H3 R2, K4, Q5, R8 and K9 are shown as yellow sticks.

Shuangping_SuppFig.4

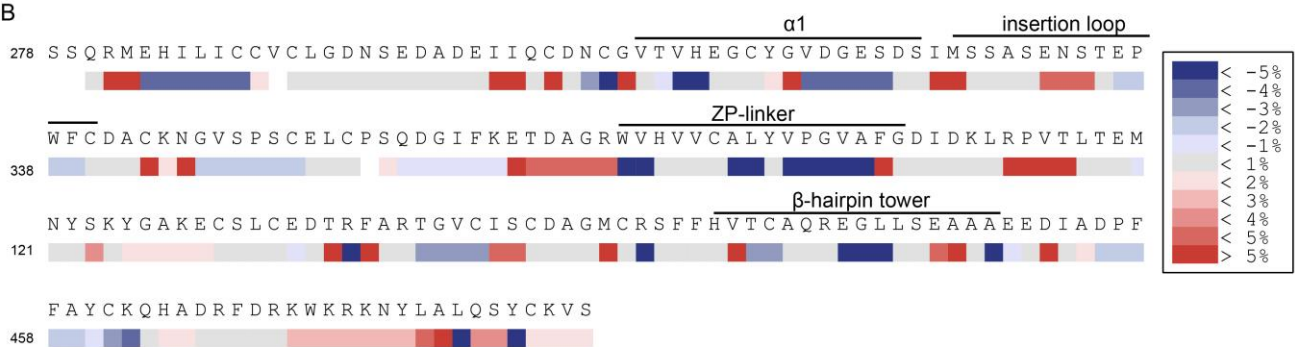


Supplementary Figure S4. (A) Structural alignment of H3-bound PHD fingers of PHF14 (pale cyan), BHC80 (blue white) and BPTF (grey). Key residues of BHC80 and BPTF responsible for H3K4un and H3K4me3 recognition are highlighted green and magenta, respectively. (B) Sequence alignment of representative H3-binding PHD fingers. Key residues responsible for H3R2, H3K4un and H3K4me3 recognition of BRPF2, BHC80, TRIM24, ING2, and BPTF are shaded in orange, green and magenta, respectively. Orange stars, green circles, red squares and violet triangles denote H3R2, H3K4, H3R8 and H3A1 binding residues of PHF14, respectively. (C) ITC fitting curves of BRPF1 titrated with H3 peptides(left); Structure analysis of H3₍₁₋₁₃₎ recognition by BRPF2_{PHD1} (PDB, 2L43) and sequence alignment of BRPF1_{PHD1} and BRPF2_{PHD1}(right). Key residues are shown as sticks. (D) TSA melting curves of PHF14_{PZP} and H3(1-13)-PHF14_{PZP}. (E) Structural alignment of H3(1-13)-PHF14_{PZP} (violet) and H3₍₁₋₂₅₎-PHF14_{PZP} complex(cyan).

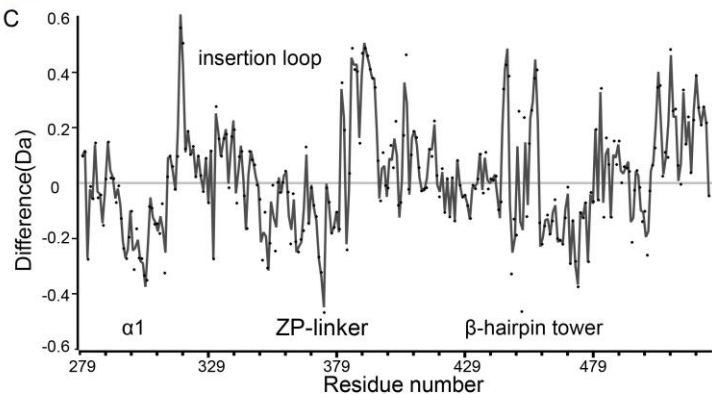
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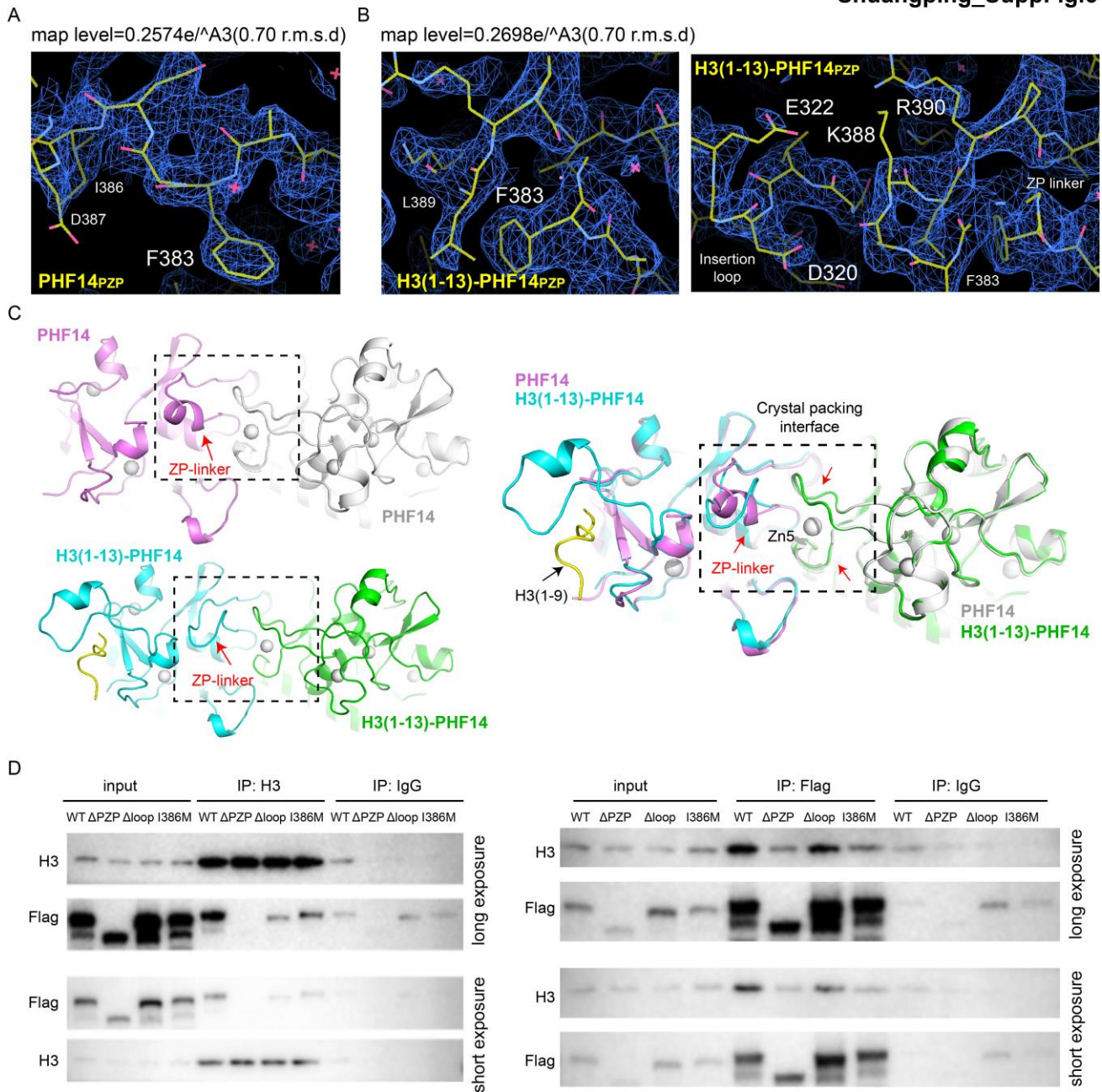
B



C



Supplementary Figure S5. HDX-MS analyses of H3₍₁₋₃₄₎ recognition by PHF14_{PZP}. (A) Sequence coverage of peptic digested PHF14_{PZP}. (B) Deuterium uptake profiles. Incorporation of deuterium at 5 min are indicated by color-coded blocks underlining the amino acid sequence. The color legend shows the deuterium uptake levels. Regions not covered are colored grey. (C) Difference plot of the HDX-MS data from PHF14_{PZP} in the presence and absence of H3₍₁₋₃₄₎. Negative values mean less exchange and more protection upon and positive values mean more exchange and less protection upon.



Supplementary Figure S6. (A) The 2fo-fc electron density around F383 residue in free PHF14_{PZP} structure. (B) The 2fo-fc electron density around F383 residue (left) and D320, E322, K388 and R390 residues (right) in H3(1-13)-PHF14_{PZP} complex structure. Map level is indicated in each panel. (C) Crystal packing analysis of PHF14_{PZP} (violet ribbon-grey ribbon) and H3(1-13)-PHF14_{PZP} (cyan ribbon-green ribbon). Crystal packing presentation of PHF14_{PZP} and H3(1-13)-PHF14_{PZP} (left) and structural alignment of PHF14_{PZP} and H3(1-13)-PHF14_{PZP} (right). Black dotted bordered rectangle indicate the crystal packing interface. ZP-linker region and other corresponding region have been labelled (D) Uncropped images of Co-IP assays under long and short exposure time.

Supplementary Table S1. The list of peptides

Peptides	Sequence
H3.1(1-34)un	ARTKQTARKSTGGKAPRKQLATKAARKSAPATGG-NH2
H3.3(1-34)un	ARTKQTARKSTGGKAPRKQLATKAARKSAPSTGG-NH2
H3.1(1-25)un	ARTKQTARKSTGGKAPRKQLATKAA-NH2
H3 (1-15)un	ARTKQTARKSTGGKA-NH2
H3 (1-15)R2me2a	AR(me2a)TKQTARKSTGGKA-NH2
H3 (1-15)R2me2s	AR(me2s)TKQTARKSTGGKA-NH2
H3 (1-15)T3ph	ART(ph)KQTARKSTGGKA-NH2
H3 (1-15)K4me3	ARTK(me3)QTARKSTGGKA-NH2
H3 (1-15)R8me2a	ARTKQTAR(me2a)KSTGGKA-NH2
H3 (1-15)R8me2s	ARTKQTAR (me2s)KSTGGKA-NH2
H3 (1-15)K9me3	ARTKQTARK(me3)STGGKA-NH2
H3.1(14-34)un	KAPRKQLATKAARKSAPATGG-NH2
H3.3(14-34)un	KAPRKQLATKAARKSAPSTGG-NH2
H3.3(14-34)K18ac	KAPRK(ac)QLATKAARKSAPSTGG-NH2
H3.3(14-34)K23ac	KAPRKQLATK(ac)AARKSAPSTGG-NH2
H3.3(14-34)K27ac	KAPRKQLATKAARK(ac)SAPSTGG-NH2
H3.3(14-34)K27me3	KAPRKQLATKAARK(me3)SAPSTGG-NH2
H3 (20-28)un	LATKAARKS

Supplementary Table S2. Thermodynamic parameters for ITC experiments

Protein	Peptide	ΔH (cal/mol)	$-\Delta S$ (cal/mol)	K_a (M^{-1})	N	χ^2/DOF
PHF14_{PZP}	H3.3(1-34)un	-4451±64.48	-4,651.14	4.71E6±1.02E6	1.06±0.00877	1.007E4
	H3.3(1-34)un	-4681±173.3	-4,472.25	5.11E6±1.97E6	1.03±0.0128	1.586E4
	H3.1(1-34)un	-4435±42.66	-5,038.735	8.60E6±1.31E6	0.963±0.00430	3829
	H3.1(1-34)un	-4538±94.29	-4,561.695	4.65E6±2.14E6	0.965±0.0114	4.711E4
	H3.1(1-34)un	-3635±93.58	-5,575.405	5.66E6±2.43E6	1.05±0.0134	2.732E4
	H3(1-15)un	-4744±87.16	-1899.215	7.38E4±3.96E3	1.01±0.0114	750.1
	H3(1-15)un	-5387±145.1	-1419.194	9.74E4±6.26E3	1.05±0.0159	1327
	H3(1-15)K9me3	-4322±300.7	-1,347.638	1.43E4±1.78E3	1.06±0.0395	990.4
	H3(1-15) K9me3	-4200±234.1	-1,420.522	1.20E4±1.33E3	1.00±0.0245	1000.5
	H3(1-25)un	-3508±61.31	-4,203.915	4.42E5±5.22E4	1.07±0.00976	3956
	H3(1-25)un	-3385±95.83	-4,293.36	4.33E5±9.02E4	1.03±0.0164	1.441E4
	H3.1(14-34)un	-1755±103.0	-5,992.815	4.69E5±1.26E5	0.974±0.0180	1838
	H3.1(14-34)un	-1639±48.95	-6,082.26	4.66E5±1.12E5	1.05±0.0138	2894
	H3.3(14-34)un	-1384±76.79	-6,350.595	4.69E5±1.31E5	0.978±0.0239	2300
	H3.3(14-34)un	-1413±95.71	-6,231.335	3.96E5±8.97E4	1.09±0.0279	1127
	H3(1-13)- PHF14_{PZP}	H3.1(14-34)un	-3785±51.75	-3846	3.98E5±3.67E4	0.974±0.00754
H3.1(14-34)un		-3773±48.87	-4,114.47	6.14E5±5.50E4	0.959±0.00715	2653
H3.3(14-34)un		-3579±81.91	-4,233.73	5.39E5±1.00E5	1.01±0.0133	1.070E4
H3.3(14-34)un		-3627±107.0	-4174.1	5.13E5±1.03E5	1.00±0.0151	1.122E4
H3.3(14-34)un		-3047±36.83	-4830	5.93E5±7.12E4	1.09±0.00851	3898
H3.3(14-34)K18ac		-2603±61.13	-4,092.19	7.86E4±7.56E3	1.06±0.0144	827.5
H3.3(14-34)K18ac		-2634±108.0	-4,054.84	8.06E4±1.34E4	0.877±0.0235	3102
H3.3(14-34)K27ac		-3550±65.69	-3,935.58	3.09E5±4.50E4	1.07±0.0139	9005
H3.3(14-34)K27ac		-3459±120.2	-3,935.58	2.65E5±5.88E4	0.993±0.0200	1.162E4
H3.3(14-34)K27me3		-3952±67.66	-3,846.135	5.08E5±6.08E4	1.01±0.00912	5195
H3.3(14-34)K27me3		-2894±87.97	-4,859.845	4.85E5±1.32E5	0.842±0.0185	1.252E4
H3(1-13)- PHF14_{PZP}-E322A		H3.3(14-34)un	-2074±33.28	-5,217.625	2.19E5±2.34E4	1.03±0.0113
H3(1-13)- PHF14_{PZP}-E320A	H3.3(14-34)un	-1143±52.65	-6,350.595	3.19E5±1.24E5	1.08±0.0347	7317
PHF14_{PZP}-Δloop	H3.3(14-34)un	-1127±61.96	-6,440.04	2.87E5±7.18E4	1.10±0.0220	1676
	H3.3(14-34)un	-1160±39.96	-6,469.855	3.88E5±8.00E4	0.973±0.0170	959.0
PHF14_{PZP}I386M V378M	H3(1-34)un	-1415±52.79	-6,141.89	3.48E5±8.02E4	1.04±0.0200	2907
	H3(1-34)un	-1464±89.03	-6,201.52	4.12E5±1.04E5	1.10±0.0286	1993
AF10_{PZP}	H3.3(1-34)un	-8998±66.32	-533.6885	1.60E6±1.81E5	1.07±0.00499	2.091E4
	H3.3(1-34)un	-8381±95.62	-94.51	1.63E6±2.70E5	1.07±0.00731	3.862E4
	H3(1-15)un	-6231±490.0	-354.7985	2.03E4±3.07E3	1.00±0.0512	7663
	H3(1-15)un	-7069±544.2	-1,144.896	2.19E4±3.39E3	0.965±0.0469	1.034E4
	H3.3(14-34)un	-4243±38.94	-3,100.76	2.40E5±1.57E4	0.983±0.00665	2359
	H3.3(14-34)un	-4185±32.13	-3,100.76	2.23E5±1.20E4	0.986±0.00554	1642
	H3(21-28)un	-3363±171.1	-2,838.388	3.50E4±4.90E3	1.02±0.0339	3368
	H3(21-28)un	-3288±225.2	-2,832.425	3.06E4±5.55E3	1.02±0.0453	3822
	H3.1(14-34)un	-3293±58.90	-3,965.39	2.09E5±2.51E4	1.01±0.0133	4460
	H3.1(14-34)un	-3103±73.16	-4,144.285	2.03E5±2.87E4	1.01±0.0171	5060
	H3.3(14-34)K23a	-3768±127.4	-2,528.312	4.13E4±3.79E3	0.990±0.0231	2224
	H3.3(14-34)K23ac	-3352±82.64	-2,921.87	3.97E4±2.66E3	0.965±0.0169	898.6

BRPF1_{PZP}

H3.3(1-34)un	-3953±58.04	-3,130.575	1.56E5±1.23E4	1.02J±0.00992	2821
H3.3(1-34)un	-3424±54.90	-3,637.43	1.47E5±1.10E4	0.923±0.00971	1830
H3(1-15)un	-3944±67.48	-2,352.4035	4.12E4±2.12E3	1.04±0.0122	802.2
H3(1-15)un	-3998±155.2	-2,337.496	4.41E4±5.84E3	1.05±0.0273	4686
H3(1-15)R8me2a	-3704±57.58	-2,602.8495	4.20E4±1.85E3	0.955±0.0104	494.0
H3(1-15)R8me2a	-3650±20.32	-2,690.6354	4.16E4±5.65E3	0.980±0.0105	1028

Supplementary Table S3. Data collection and refinement statistics

	Zebrafish PHF14 _{PZP}	PHF14 _{PZP} &H3 ₍₁₋₂₅₎	H3(1-13)-PHF14 _{PZP}
Data collection	Native	Native	Native
Space group	P2 ₁	C222 ₁	C222 ₁
Wavelength (Å)	1.2818	0.9792	1.2818
Cell dimensions			
<i>a, b, c</i> (Å)	42.2, 41.9, 52.9	41.9, 151.0, 81.8	41.8, 150.4, 81.4
α, β, γ (°)	90, 99.0, 90	90, 90, 90	90, 90, 90
Resolution (Å)	50-1.85 (1.88-1.85)	50-2.1 (2.14-2.10)	50-2.0 (2.05-2.00)
No. of unique reflections	29,578 (1488)#*	15,363 (748)	16,737 (1282)
<i>R</i> _{sym} (%)	7.3 (72.5)	127.5 (88.9)	12.5 (91.4)
<i>I</i> / σ (<i>I</i>)	20.0 (1.9)	30.6 (2.3)	15.5 (3.6)
Completeness (%)	95.7 (95.4)	99.8 (99.9)	93.9 (99.6)
Redundancy	2.3 (2.3)	11.8 (8.7)	13.2 (12.9)
Refinement (F>0)			
Resolution (Å)	41.7-1.855	32.2-2.1	40.7-2.0
No. reflections	29,554	15,340	16,713
<i>R</i> _{work} / <i>R</i> _{free}	17.4/22.5	16.5/20.4	17.7/21.2
No. atoms			
Protein	1433	1552	1542
Peptide	-	73	73
Zn/Mg/Ca/EG	5/1/-/1	5/-/2/-	5/-/2/-
Water	92	88	116
<i>B</i> -factors			
Protein	45.7	58.0	47.4
Peptide	-	91.8	77.6
Zn/Mg/Ca/EG	37.6/33.0/-/47.8	38.4/-/53.0/-	31.7/-/41.6/-
Water	44.9	46.8	42.4
R.m.s. deviations			
Bond lengths (Å)	0.004	0.006	0.006
Bond angles (°)	0.644	0.732	0.765
Ramachandran plot (%)			
Favored	97.8	93.0	90.3
Allowed	2.2	7.0	9.7
Outliers	0	0	0

#This data set contains anomalous signal

*Outmost resolution shell in parenthesis