SUPPLEMENTARY DATA

Histone Benzoylation Serves as an Epigenetic Mark for DPF and YEATS Family Proteins

Xiangle Ren[1], Yang Zhou[1], Zhaoyu Xue[2], Ning Hao[1], Yuanyuan Li[1], Xiaohuan Guo[1], Daliang Wang[1], Xiaobing Shi[2] and Haitao Li[1,3,*]

[1] MOE Key Laboratory of Protein Sciences, Beijing Advanced Innovation Center for Structural Biology, Beijing Frontier Research Center for Biological Structure, Department of Basic Medical Sciences, School of Medicine, Tsinghua University, Beijing 100084, China

[2] Center for Epigenetics, Van Andel Institute, Grand Rapids, MI 49503, USA

[3] Tsinghua-Peking Center for Life Sciences, Beijing 100084, China

* To whom correspondence should be addressed: lht@tsinghua.edu.cn

Supplementary Tables S1-S3

Supplementary Table S1. List of peptides used in this study.

Peptides	Sequence
$H3_{2\text{-}16}$K9bz	ac-RTKQTARKbzSTGGKAP-NH2
$H3_{2\text{-}16}$un	ac-RTKQTARKSTGGKAP-NH2
$H3_{2\text{-}16}$K9ac	ac-RTKQTARKacSTGGKAP-NH2
$H3_{2\text{-}16}$K9cr	ac-RTKQTARKcrSTGGKAP-NH2
$H3_{1\text{-}25}$K14bz	ARTKQTARKSTGGKbzAPRKQLATKAA-NH2
$H3_{1\text{-}25}$un	ARTKQTARKSTGGKAPRKQLATKAA-NH2
$H3_{1\text{-}25}$K14ac	ARTKQTARKSTGGKacAPRKQLATKAA-NH2
$H3_{1\text{-}25}$K14cr	ARTKQTARKSTGGKcrAPRKQLATKAA-NH2
$H3_{1\text{-}25}$K18bz	ARTKQTARKSTGGKAPRKbzQLATKAA-NH2
$H3_{1\text{-}25}$K18ac	ARTKQTARKSTGGKAPRKacQLATKAA-NH2
$H3_{1\text{-}25}$K18cr	ARTKQTARKSTGGKAPRKcrQLATKAA-NH2
$H3_{15\text{-}39}$K27bz	ac-APRKQLATKAARKbzSAPATGGVKKPH-NH2
$H3_{24\text{-}31}$K27bz	ac-AARKbzSAPA-NH2
$H3_{15\text{-}39}$un	ac-APRKQLATKAARKSAPATGGVKKPH-NH2
$H3_{15\text{-}39}$K27ac	ac-APRKQLATKAARKacSAPATGGVKKPH-NH2
$H3_{15\text{-}39}$K27cr	ac-APRKQLATKAARKcrSAPATGGVKKPH-NH2

Supplementary Table S2. Thermodynamic parameters of representative ITC titrations.

Protein	Peptide	ΔH(cal/mol)	-TΔS(cal/mol)	K$_a$ (M^{-1})	N	Chi2/DoF
AF9$_{\text{YEATS}}$	H3$_{2\text{-}16}$K9bz	-1.750E4 ± 124.4	10603.92	1.69E5 ± 6.12E3	0.872 ± 0.00464	8765
	H3$_{2\text{-}16}$un	N.D.				
	H3$_{2\text{-}16}$K9ac	-1.784E4 ± 144.5	10747.995	2.37E5 ± 1.20E4	0.929 ± 0.00563	1.85E4
	H3$_{2\text{-}16}$K9cr	-1.750E4 ± 109.0	9998.805	4.94E5 ± 2.74E4	0.995 ± 0.00451	1.988E4
ENL$_{\text{YEATS}}$	H3$_{15\text{-}39}$K27bz	-6378 ± 103.0	829.872	1.61E4 ± 715	0.920 ± 0	1675
	H3$_{15\text{-}39}$un	N.D.				
	H3$_{15\text{-}39}$K27ac	-5380 ± 209.3	128.5149	9.60E3 ± 837	0.910 ± 0	3257
	H3$_{15\text{-}39}$K27cr	-6990 ± 69.95	1138.1925	2.74E4± 950	0.930 ± 0	1556
GAS41$_{\text{YEATS}}$	H3$_{15\text{-}39}$K27bz	-5584 ± 719.7	102.2933	1.43E4 ± 2.06E3	0.984 ± 0.0936	2175
	H3$_{15\text{-}39}$un	N.D.				
	H3$_{15\text{-}39}$K27ac	-9151 ± 355.1	3284.91	2.84E4 ± 1.93E3	0.973 ± 0.0272	3745
	H3$_{15\text{-}39}$K27cr	-9556 ± 97.90	3140.835	7.21E4 ± 2.41E3	1.02 ± 0.00757	2180
YEATS2$_{\text{YEATS}}$	H3$_{15\text{-}39}$K27bz	-1.078E4 ± 376.3	4668.03	4.17E4 ± 3.67E3	1.02 ± 0.0257	1.486E4
	H3$_{15\text{-}39}$un	N.D.				
	H3$_{15\text{-}39}$K27ac	-6706 ± 120.4	1593.47	7.53E3 ± 273	0.950 ± 0	846.4
	H3$_{15\text{-}39}$K27cr	-1.075E4 ± 346.4	4927.365	2.53E4 ± 1.49E3	1.02 ± 0.0237	4184
MOZ$_{\text{DPF}}$	H3$_{1\text{-}25}$K14bz	-5022 ± 33.52	-1806.789	1.01E5 ± 3.46E3	1.09 ± 0.00518	932.8
	H3$_{1\text{-}25}$un	-1741 ± 118.4	-4174.1	2.14E4 ± 3.35E3	1.14 ± 0.0458	690.5
	H3$_{1\text{-}25}$K14ac	-3349 ± 56.15	-3577.8	1.17E5 ± 1.05E4	1.05 ± 0.0127	2884
	H3$_{1\text{-}25}$K14cr	-6014 ± 33.17	-1293.971	2.28E5 ± 8.55E3	0.937 ± 0.00382	1578
MORF$_{\text{DPF}}$	H3$_{1\text{-}25}$K14bz	-8596 ± 56.54	1693.492	1.14E5 ± 3.86E3	1.00 ± 0.00486	2453
	H3$_{1\text{-}25}$un	-7084 ± 102.0	1189.6185	2.08E4 ± 533	0.916 ± 0.00918	299.4
	H3$_{1\text{-}25}$K14ac	-7093 ± 39.26	527.7255	6.45E4 ± 1.26E3	0.905 ± 0.00362	445.3
	H3$_{1\text{-}25}$K14cr	-1.076E4 ± 26.21	3607.615	1.77E5 ± 2.45E3	0.819 ± 0.00149	561.4
DPF1$_{\text{DPF}}$	H3$_{1\text{-}25}$K14bz	-5154 ± 43.62	-3458.54	2.06E6 ± 2.93E5	1.05 ± 0.00549	1.059E4
	H3$_{1\text{-}25}$un	-303.0 ± 14.75	-7215.23	3.30E5 ± 1.45E5	1.14 ± 0.0403	643.7
	H3$_{1\text{-}25}$K14ac	-3890 ± 39.23	-4531.88	1.46E6 ± 2.22E5	1.11 ± 0.00726	8502
	H3$_{1\text{-}25}$K14cr	-9422 ± 35.06	123.7323	6.54E6 ± 6.48E5	1.04 ± 0.00203	8158
DPF2$_{\text{DPF}}$	H3$_{1\text{-}25}$K14bz	-5417± 19.43	-3607.615	4.10E6 ± 2.87E5	0.957 ± 0.00204	1731
	H3$_{1\text{-}25}$un	-937.3 ± 60.8	-6618.93	3.43E5 ± 1.89E5	1.12 ± 0.0534	8625
	H3$_{1\text{-}25}$K14ac	-4958 ± 19	-4144.285	4.61E6 ± 3.60E5	0.937 ± 0.00213	1664
	H3$_{1\text{-}25}$K14cr	-8764 ± 36.5	-998.8025	1.43E7 ± 2.02E6	0.924 ± 0.00212	6956
DPF3$_{\text{DPF}}$	H3$_{1\text{-}25}$K14bz	-4540 ± 30.00	-4233.73	2.65E6 ± 3.09E5	0.979 ± 0.00395	4435
	H3$_{1\text{-}25}$un	-368.6 ± 31.82	-6917.08	2.23E5 ± 1.72E5	1.45 ± 0.0897	2902
	H3$_{1\text{-}25}$K14ac	-5725 ± 24.56	-3160.39	3.25E6 ± 2.66E5	0.966 ± 0.00248	3055
	H3$_{1\text{-}25}$K14cr	-8097 ± 24.42	-1419.19	9.44E6 ± 8.42E5	0.885 ± 0.00140	3043
PHF10$_{\text{DPF}}$	H3$_{1\text{-}25}$K14bz	-4083 ± 31.05	-4263.545	1.33E6 ± 1.22E5	1.05 ± 0.00543	2377
	H3$_{1\text{-}25}$un	-1547 ± 65.44	-5933.185	3.08E5 ± 8.30E4	1.02 ± 0.0319	4254
	H3$_{1\text{-}25}$K14ac	-4894 ± 49.72	-3041.13	6.53E5 ± 5.89E4	1.02 ± 0.00748	4171
	H3$_{1\text{-}25}$K14cr	-6149 ± 40.71	-2352.4	1.69E6 ± 1.38E5	0.904 ± 0.00406	3618
BRD3$_{\text{BRD2}}$	H3$_{1\text{-}25}$K18bz			N.D.		
	H3$_{1\text{-}25}$un			N.D.		
	H3$_{1\text{-}25}$K18ac	-8530 ± 295.7	3190.205	8.25E3 ± 421	1.04 ± 0.0245	973.9
	H3$_{1\text{-}25}$K18cr			N.D.		
BRD9$_{\text{BRD}}$	H3$_{15\text{-}39}$K27bz			N.D.		
	H3$_{15\text{-}39}$un			N.D.		
	H3$_{15\text{-}39}$K27ac	-2611 ± 66.11	-2197.37	3.35E3 ± 163	1.02 ± 0	172
	H3$_{15\text{-}39}$K27cr			N.D.		

Continued

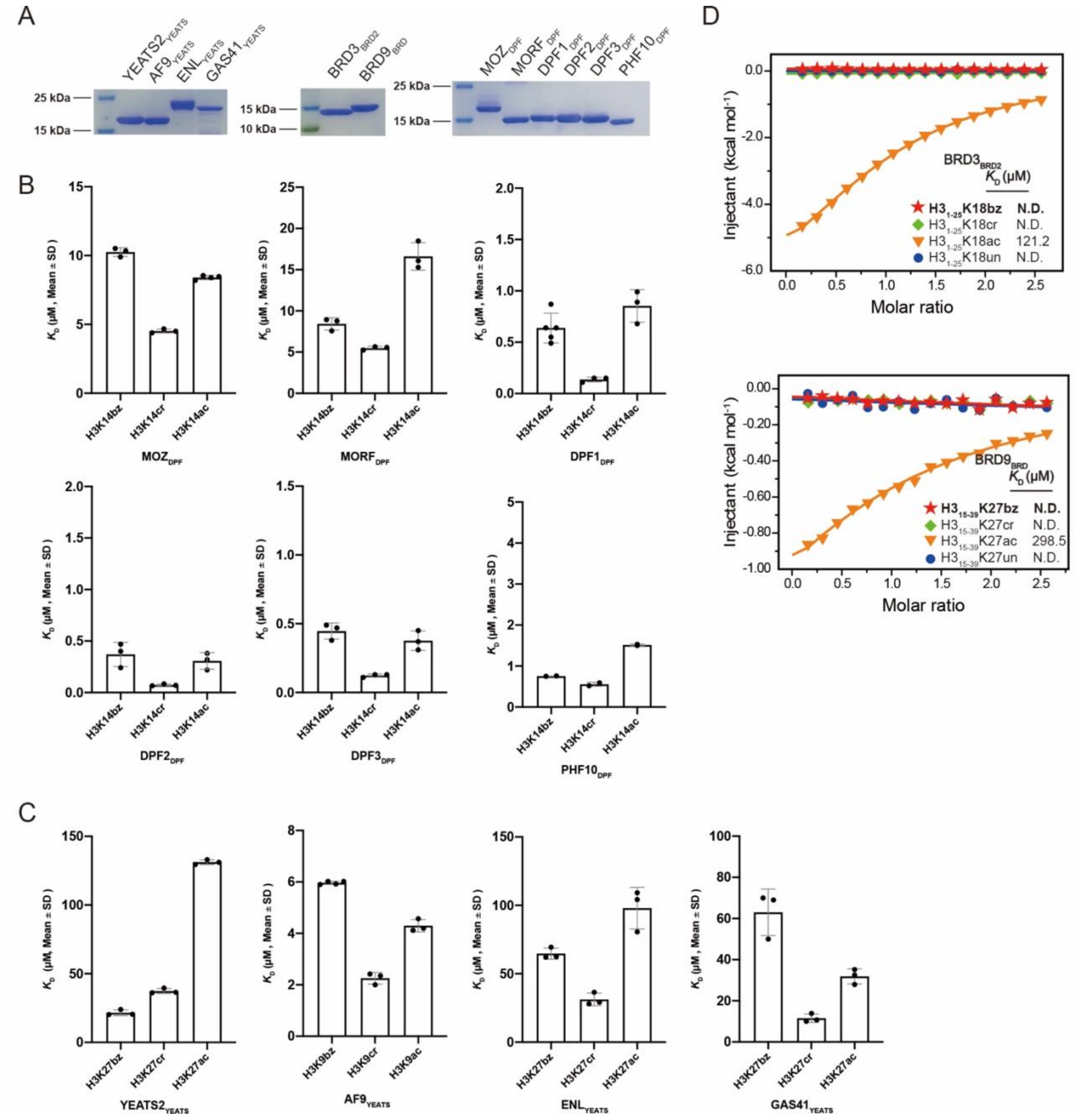
Protein	Peptide	ΔH(cal/mol)	-TΔS(cal/mol)	Ka (M-1)	N	Chi2/DoF
MOZ$_{DPF}$ L242I	H3$_{1-25}$K14bz	-8334 ± 48.52	1019.673	2.29E5 ± 6.50E3	0.974 ± 0.00420	1251
	H3$_{1-25}$K14ac	-3751 ± 120.7	-2644.5905	4.87E4 ± 3.03E3	0.976 ± 0.0232	638.5
	H3$_{1-25}$K14cr	-8181 ± 133.9	1067.377	1.63E5 ± 9.87E3	0.843 ± 0.0102	4517
MOZ$_{DPF}$ N235S	H3$_{1-25}$K14bz	-4875 ± 56.05	-2006.5495	1.10E5 ± 6.30E3	1.00 ± 0.00840	2223
	H3$_{1-25}$K14ac	-2785 ± 32.89	-4352.99	1.73E5 ± 1.24E4	0.972 ± 0.00849	1154
	H3$_{1-25}$K14cr	-5852 ± 41.72	-1520.565	2.53E5 ± 1.24E4	0.876 ±0.00461	2114
AF9$_{YEATS}$ F28S	H3$_{2-16}$K9bz	-1.625E4 ± 187.5	9047.91	2.85E5 ± 1.97E4	0.825 ± 0.00711	2.242E4
YEATS2$_{YEATS}$ S230F	H3$_{15-39}$K27bz	-6762 ± 243.8	795.294	3.34E4 ± 2.65E3	1.03 ± 0.0266	4022
YEATS2$_{YEATS}$ S230F	H3$_{15-39}$K27ac	-7015 ± 401.8	1518.5505	1.47E4 ± 1.13E3	1.08 ± 0.0444	1483
YEATS2$_{YEATS}$ S230F	H3$_{15-39}$K27cr	-9051 ± 194.6	2694.2025	6.61E4 ± 4.31E	0.892 ± 0.0142	6494
YEATS2$_{YEATS}$ W282A	H3$_{15-39}$K27bz	N.D.				
YEATS2$_{YEATS}$ Y262A		N.D.				

Supplementary Table S3. Summary of K_D values from independent ITC titrations and their statistical analyses.

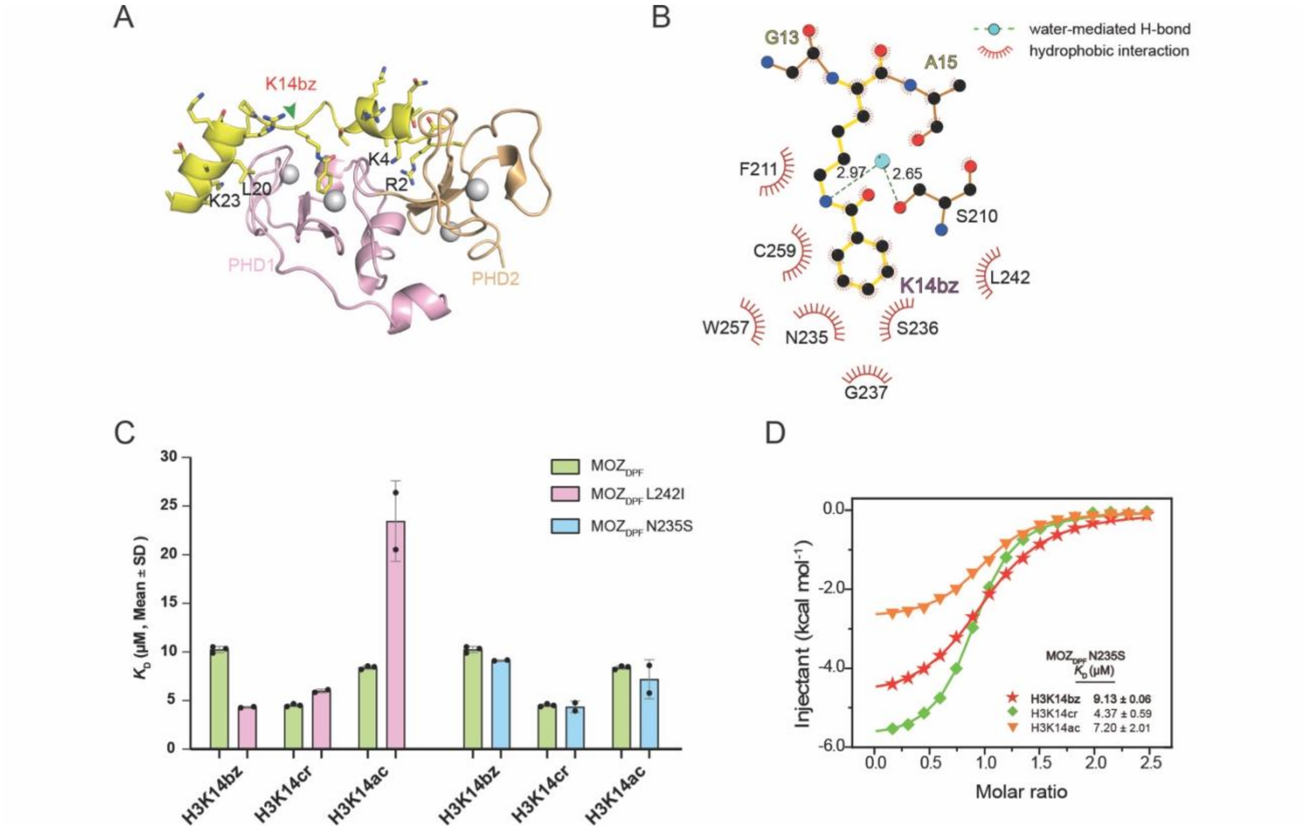
Protein	YEATS2$_{\mathrm{YEATS}}$				YEATS2$_{\mathrm{YEATS}}$ S230F				GAS41$_{\mathrm{YEATS}}$		
Peptide	H3K27bz	H3K27cr	H3K27ac		H3K27bz	H3K27cr	H3K27ac		H3K27bz	H3K27cr	H3K27ac
K_D (individual)	23.98	39.53	132.80		29.94	15.13	68.03		69.93	13.87	35.21
	20.24	36.50	129.53		29.67	13.55	74.63		50.00	10.71	27.93
	20.49	36.10	131.06						68.96	10.08	32.47
K_D (mean)	21.57	37.38	131.13		29.81	14.34	71.33		62.96	11.55	31.87
SD	2.09	1.88	1.64		0.19	1.12	4.67		11.24	2.03	3.68
Protein	AF9$_{\mathrm{YEATS}}$					AF9$_{\mathrm{YEATS}}$ F28S			ENL$_{\mathrm{YEATS}}$		
Peptide	H3K9bz	H3K9cr	H3K9ac			H3K9bz			H3K27bz	H3K27cr	H3K27ac
	5.92	2.02	4.22			3.51			62.11	36.50	104.17
K_D (individual)	6.02	2.42	4.12			3.83			62.50	29.15	80.60
	6.02	2.35	4.57			4.00			69.44	28.01	109.17
	5.92					3.85					
K_D (mean)	5.97	2.26	4.30			3.80			64.68	31.22	97.98
SD	0.06	0.21	0.24			0.21			4.12	4.61	15.26
Protein	MOZ$_{\mathrm{DPF}}$				MORF$_{\mathrm{DPF}}$				PHF10$_{\mathrm{DPF}}$		
Peptide	H3K14bz	H3K14cr	H3K14ac		H3K14bz	H3K14cr	H3K14ac		H3K14bz	H3K14cr	H3K14ac
K_D (individual)	9.90	4.39	8.55		8.77	5.65	15.50		0.75	0.59	1.53
	10.52	4.67	8.47		8.93	5.56	16.08		0.76	0.52	1.50
	10.34	4.50	8.20		7.58	5.24	18.48				
			8.40								
K_D (mean)	10.25	4.52	8.41		8.43	5.48	16.69		0.76	0.56	1.52
SD	0.32	0.14	0.15		0.74	0.22	1.58		0.01	0.05	0.02
Protein	DPF1$_{\mathrm{DPF}}$				DPF2$_{\mathrm{DPF}}$				DPF3$_{\mathrm{DPF}}$		
Peptide	H3K14bz	H3K14cr	H3K14ac		H3K14bz	H3K14cr	H3K14ac		H3K14bz	H3K14cr	H3K14ac
K_D (individual)	0.49	0.15	0.68		0.24	0.070	0.22		0.38	0.11	0.31
	0.87	0.15	0.89		0.47	0.066	0.38		0.49	0.13	0.37
	0.64	0.11	0.99		0.40	0.084	0.32		0.47	0.13	0.45
	0.55										
	0.64										
K_D (mean)	0.64	0.14	0.85		0.37	0.07	0.31		0.45	0.12	0.38
SD	0.14	0.02	0.16		0.12	0.01	0.08		0.06	0.01	0.07
Protein	MOZ$_{\mathrm{DPF}}$ L242I				MOZ$_{\mathrm{DPF}}$ N235S						
Peptide	H3K14bz	H3K14cr	H3K14ac		H3K14bz	H3K14cr	H3K14ac				
K_D (individual)	4.37	6.13	20.53		9.09	3.95	5.78				
	4.26	5.85	26.39		9.17	4.78	8.62				
K_D (mean)	4.32	5.99	23.46		9.13	4.37	7.20				
SD	0.08	0.20	4.14		0.06	0.59	2.01				

K_D values in red are from representative ITC fitting curves. SD, standard deviation.

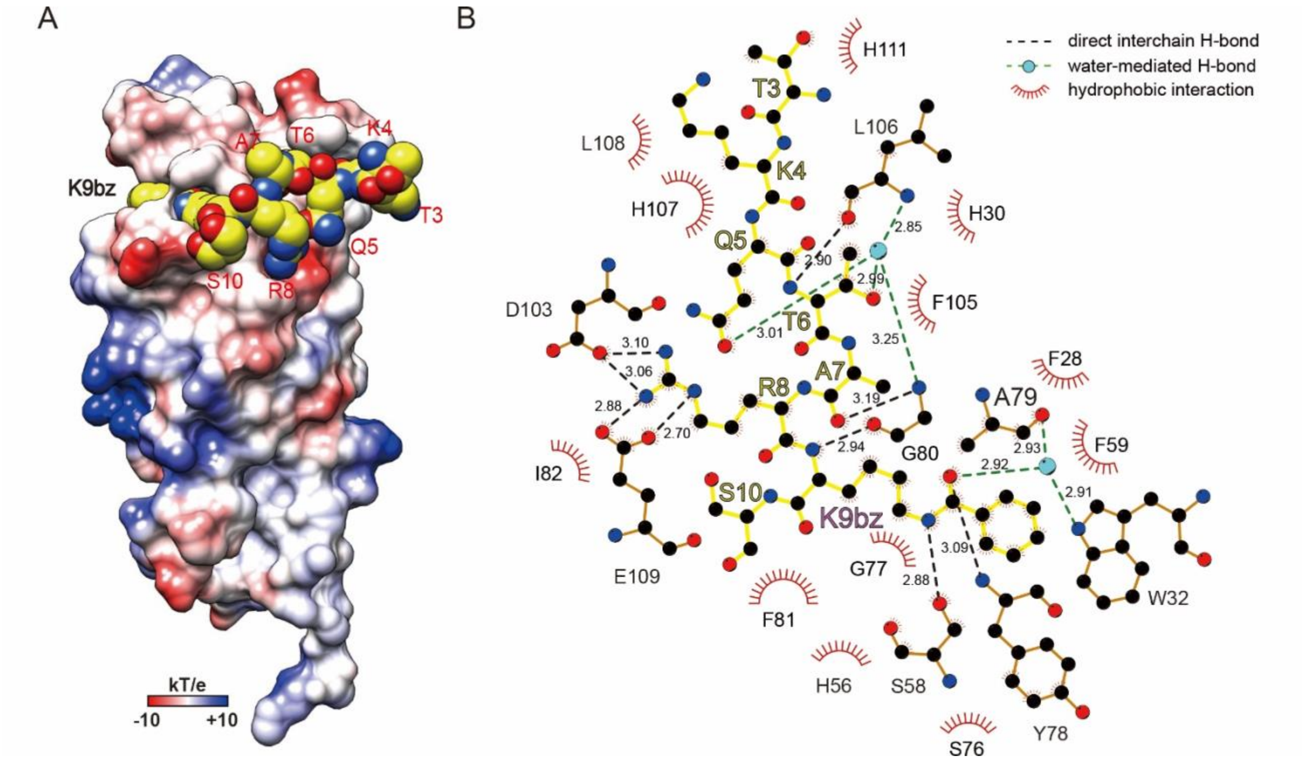
Supplementary Figures S1-S8



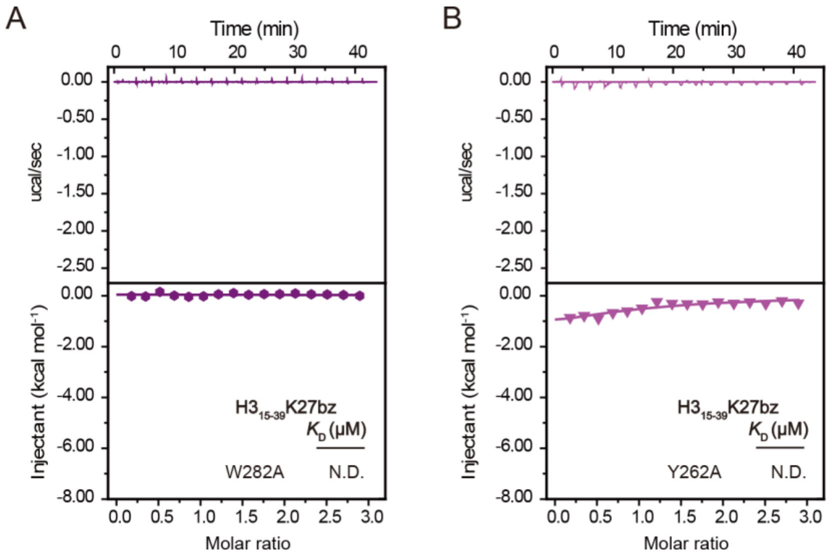
Supplementary Figure S1. Recognition of histone benzoylation by acylation reader proteins. (A) Coomassie blue staining of twelve purified human histone acylation readers. (B) Binding affinities of Kbz, Kcr and Kac titrated to DPF family members. Each black dot represents one ITC titration experiment. Error bar, standard deviation. (C) Binding affinities of Kbz, Kcr and Kac titrated to YEATS family members. (D) ITC fitting curves of the indicated histone peptides titrated into BRDs. N.D., not detectable.



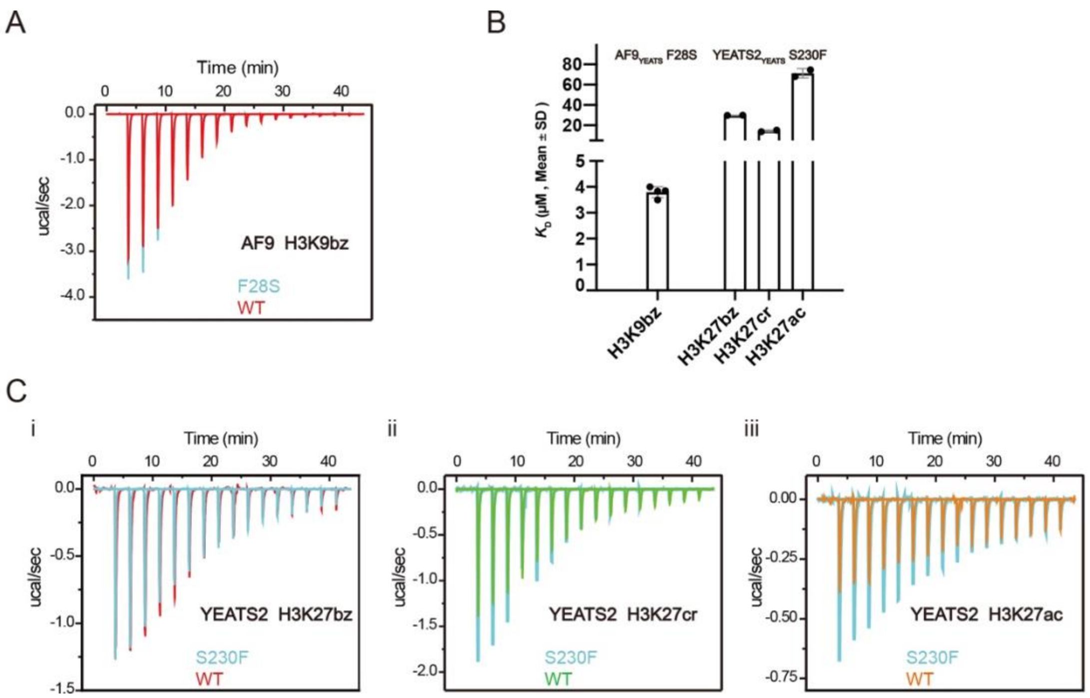
Supplementary Figure S2. Molecular basis for H3K14bz binding by MOZ_{DPF} and mutagenesis studies. (A) Overall structure of MOZ_{DPF} bound to $\text{H3}_{1\text{-}25}$K14bz peptide. PHD1, PHD2, and histone peptide are colored pink, orange, and yellow, respectively. (B) LigPlot diagram listing critical interactions between K14bz and MOZ_{DPF}. Black ball, carbon; Blue ball, nitrogen; Red ball, oxygen; Cyan ball, water; Dashes, hydrogen bond; Curved brushes, hydrophobic interaction. Hydrogen bond distances are labeled Angstroms. (C) Binding affinity comparison of MOZ_{DPF} and its mutants in recognition of H3K14bz, H3K14cr and H3K14ac. (D) ITC fitting curves of the indicated histone peptides titrated into MOZ_{DPF} N235S mutant. Mean K_D and standard deviation are shown (N = 2).



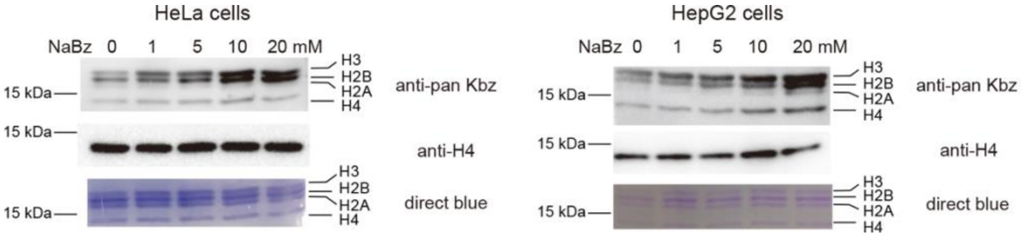
Supplementary Figure S3. Recognition of H3K9bz by AF9$_{\mathbf{YEATS}}$. (A) Overall structure of AF9$_{\mathrm{YEATS}}$ bound to H3K9bz peptide. AF9$_{\mathrm{YEATS}}$ is shown as electrostatic potential surface ranging from −10 (red) to +10 (blue) kT/e. H3K9bz is depicted in space-filling-surface view. (B) LigPlot diagram listing critical contacts between H3K9bz (yellow sticks) and AF9$_{\mathrm{YEATS}}$ (orange sticks). Symbols are the same as described for Supplementary Figure S2B.



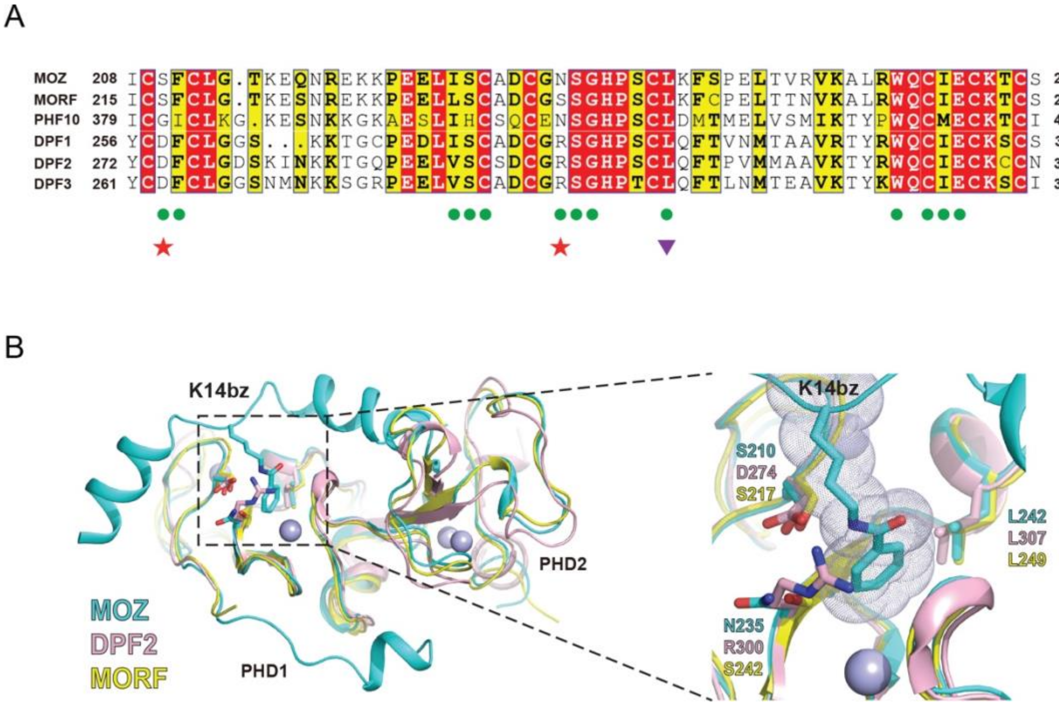
Supplementary Figure S4. The importance of aromatic sandwiching residues of YEATS2 in H3K27bz recognition. ITC fitting curves of H3K27bz peptide titrated into (A) W282A and (B) Y262A mutant YEAST2$_{\text{YEATS}}$. N.D., not detectable.



Supplementary Figure S5. ITC binding curves on "tip-sensor" residue mutants. (A) Binding curves of $\mathrm{AF9}_{\mathrm{YEATS}}$ and its F28S mutant titrated with H3K9bz peptide. (B) Binding affinities of indicated peptides titrated to $\mathrm{AF9}_{\mathrm{YEATS}}$ F28S and $\mathrm{YEATS2}_{\mathrm{YEATS}}$ S230F mutants. (C) Binding curves of $\mathrm{YEATS2}_{\mathrm{YEATS}}$ and its S230F mutant titrated with (i) H3K27bz, (ii) H3K27cr and (iii) H3K27ac peptides.

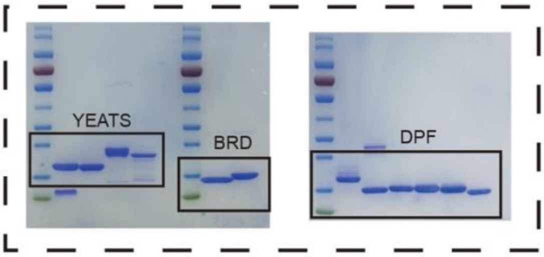


Supplementary Figure S6. The inducible feature of histone benzoylation in HeLa and HepG2 cells. Western blot analysis of core histone Kbz levels in response to the indicated concentrations of NaBz treatment in HeLa and HepG2 cells. Unmodified histone H4 was used as a loading control.

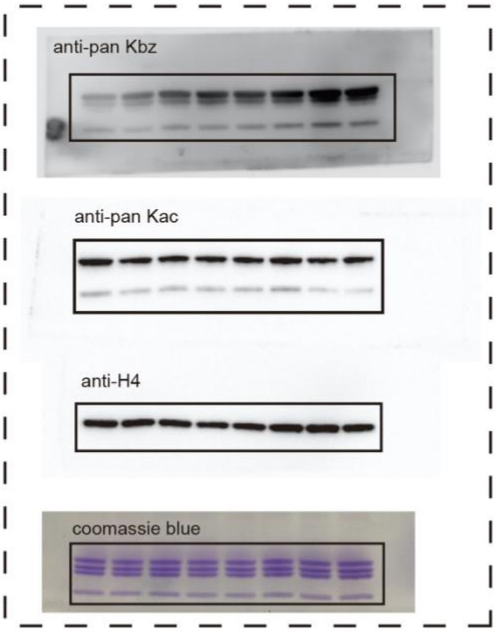


Supplementary Figure S7. Sequence and structural alignment of acylation reader pockets among human DPF family members. (A) The sequence alignment of human DPFs around the reader pocket. Green circles, pocket residues; Red stars, divergent pocket residues; Purple triangle, the conserved Leu residue that contacts Kbz. (B) The structural alignment of MOZ$_{DPF}$, DPF2$_{DPF}$ and MORF$_{DPF}$ with key residues highlighted as sticks. Grey spheres, zinc ions. Close-up view highlights the encapsulation of Kbz by conserved or divergent pocket residues among DPFs. Grey dots denote van der Waals surface of K14bz.

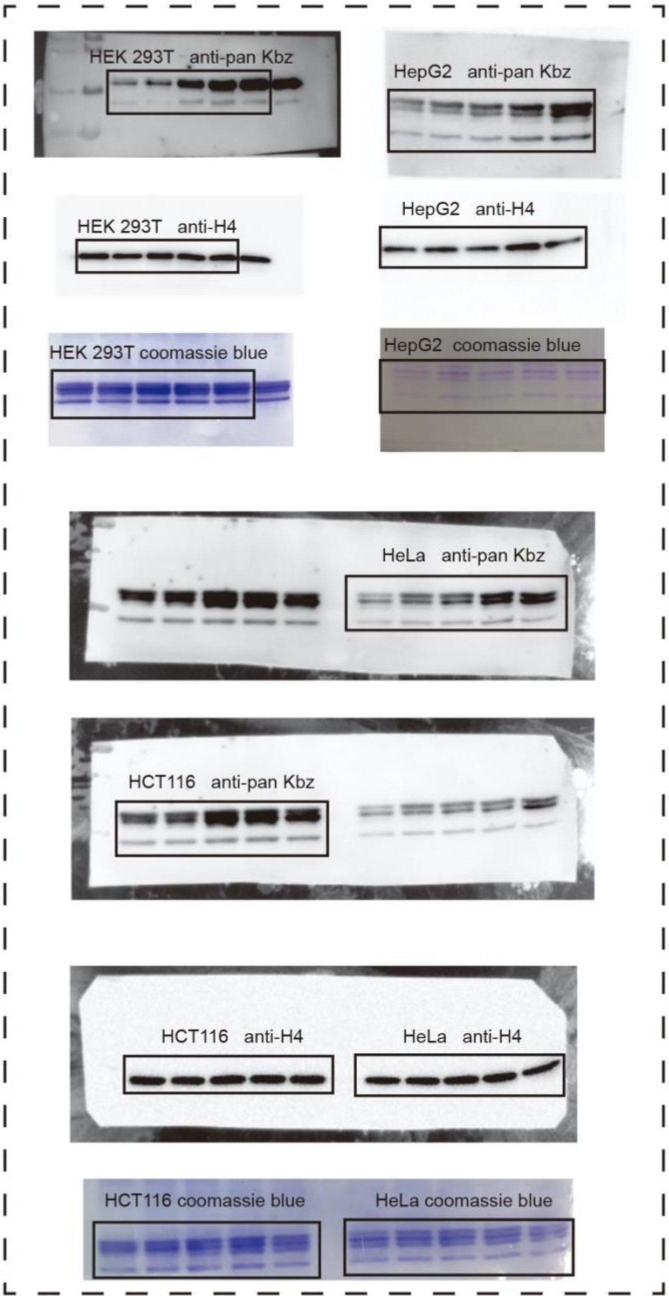
Related to Supplementary Figure S1A



Related to Figure 6C



Related to Figure 6A and Supplementary Figure S6



Supplementary Figure S8. Uncropped figures.