Supplementary Data for

The HRP3 PWWP domain recognizes the minor groove of double-stranded DNA and recruits HRP3 to chromatin

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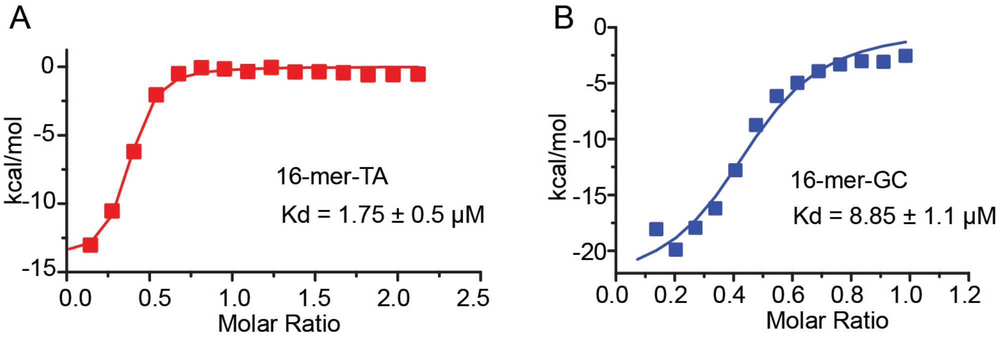
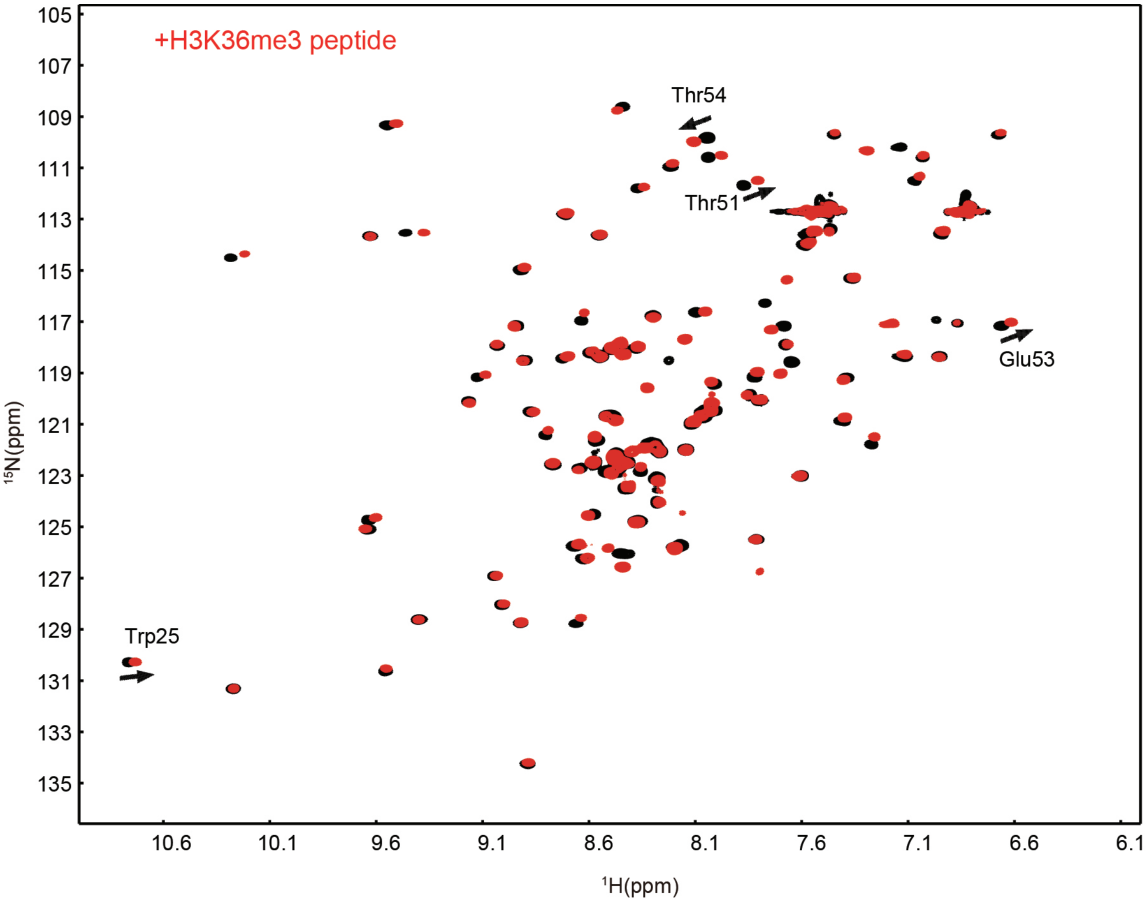
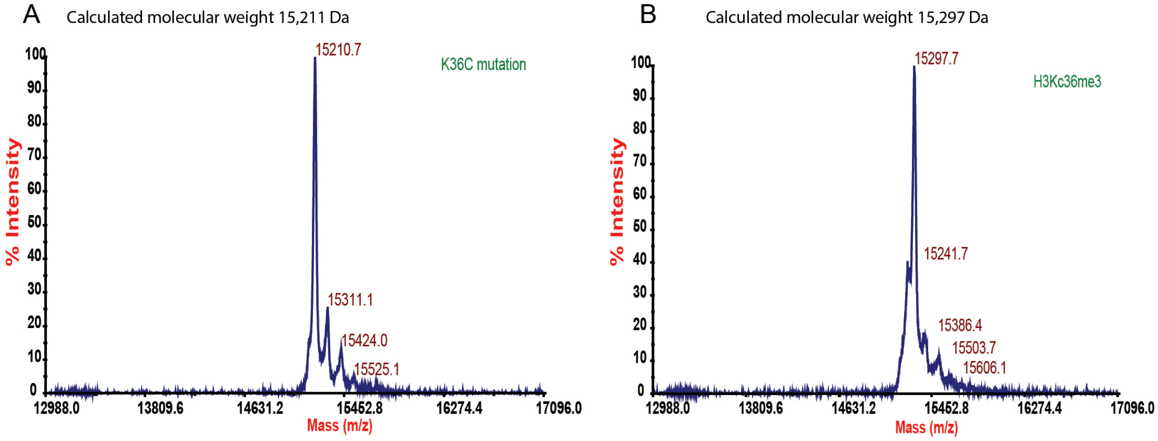
Supplementary Table S1



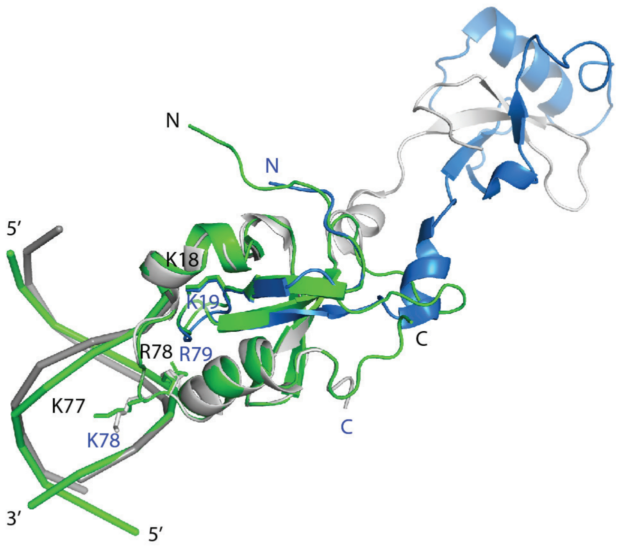
Supplementary Figure S1. ITC-based measurements of the binding affinities of HRP3 PWWP and various DNAs. (A, B) ITC-based binding curves of HRP3 PWWP titrated with the 16-mer-TA DNA (A) or the 16-mer-GC DNA (B).



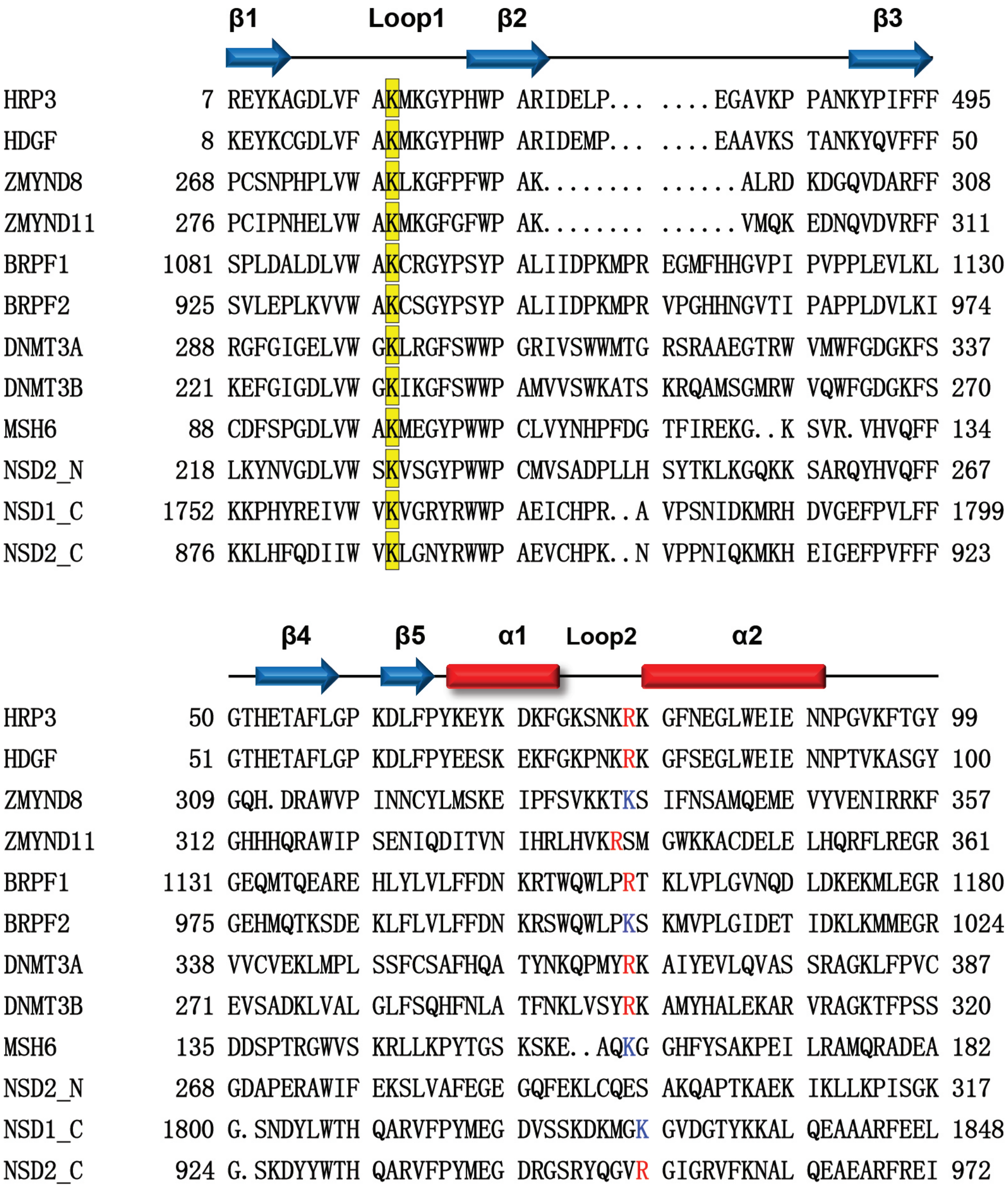
Supplementary Figure S2. Changes induced in the H^1-N^{15} HSQC NMR spectra of HRP3 PWWP upon binding of 4 mM H3K36me3 peptide. Superposition of the H^1-N^{15} HSQC NMR spectra of HRP3 PWWP in the absence (black) and presence (red) of 4 mM H3K36me3 peptide.



Supplementary Figure S3. Mass spectra to verify the incorporation of the trimethylation analogue of H3K36me3. (A, B) Mass spectra of H3 bearing the K36C mutation (A) and the trimethylation analogue of H3Kc36me3 (B). Calculated molecular weights of both peptides are indicated above the panels.



Supplementary Figure S4. Comparison of the DNA-bound structures of HRP3 PWWP and HDGF PWWP (PDB ID: 5XSK). HRP3 PWWP domain is coloured green. Swapped HDGF PWWP domains are coloured grey and blue, respectively. Key residues for the DNA recognition in HRP3 and HDGF are labelled in black and blue, respectively.



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HRP3           7 REYKAGDLVF AKMKGYPHWP ARIDELP... ....EGAVKP PANKYPIFFF 495
HDGF           8 KEYKCGDLVF AKMKGYPHWP ARIDEMP... ....EAAVKS TANKYQVFFF 50
ZMYND8       268 PCSNPHPLVW AKLKGFPFWP AK........ ......ALRD KDGQVDARFF 308
ZMYND11      276 PCIPNHELVW AKMKGFGFWP AK........ ......VMQK EDNQVDVRFF 311
BRPF1       1081 SPLDALDLVW AKCRGYPSYP ALIIDPKMPR EGMFHHGVPI PVPPLEVLKL 1130
BRPF2        925 SVLEPLKVVW AKCSGYPSYP ALIIDPKMPR VPGHHNGVTI PAPPLDVLKI 974
DNMT3A       288 RGFGIGELVW GKLRGFSWWP GRIVSWWMTG RSRAAEGTRW VMWFGDGKFS 337
DNMT3B       221 KEFGIGDLVW GKIKGFSWWP AMVVSWKATS KRQAMSGMRW VQWFGDGKFS 270
MSH6          88 CDFSPGDLVW AKMEGYPWWP CLVYNHPFDG TFIREKG..K SVR.VHVQFF 134
NSD2_N       218 LKYNVGDLVW SKVSGYPWWP CMVSADPLLH SYTKLKGQKK SARQYHVQFF 267
NSD1_C      1752 KKPHYREIVW VKVGRYRWWP AEICHPR..A VPSNIDKMRH DVGEFPVLFF 1799
NSD2_C       876 KKLHFQDIIW VKLGNYRWWP AEVCHPK..N VPPNIQKMKH EIGEFPVFFF 923

HRP3          50 GTHETAFLGP KDLFPYKEYK DKFGKSNKRK GFNEGLWEIE NNPGVKFTGY 99
HDGF          51 GTHETAFLGP KDLFPYEESK EKFGKPNKRK GFSEGLWEIE NNPTVKASGY 100
ZMYND8       309 GQH.DRAWVP INNCYLMSKE IPFSVKKTKS IFNSAMQEME VYVENIRRKF 357
ZMYND11      312 GHHHQRAWIP SENIQDITVN IHRLHVKRSM GWKKACDELE LHQRFLREGR 361
BRPF1       1131 GEQMTQEARE HLYLVLFFDN KRTWQWLPRT KLVPLGVNQD LDKEKMLEGR 1180
BRPF2        975 GEHMQTKSDE KLFLVLFFDN KRSWQWLPKS KMVPLGIDET IDKLKMMEGR 1024
DNMT3A       338 VVCVEKLMPL SSFCSAFHQA TYNKQPMYRK AIYEVLQVAS SRAGKLFPVC 387
DNMT3B       271 EVSADKLVAL GLFSQHFNLA TFNKLVSYRK AMYHALEKAR VRAGKTFPSS 320
MSH6         135 DDSPTRGWVS KRLLKPYTGS KSKE..AQKG GHFYSAKPEI LRAMQRADEA 182
NSD2_N       268 GDAPERAWIF EKSLVAFEGE GQFEKLCQES AKQAPTKAEK IKLLKPISGK 317
NSD1_C      1800 G.SNDYLWTH QARVFPYMEG DVSSKDKMGK GVDGTYKKAL QEAAARFEEL 1848
NSD2_C       924 G.SKDYYWTH QARVFPYMEG DRGSRYQGVR GIGRVFKNAL QEAEARFREI 972
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Supplementary Figure S5. Sequence alignments of several PWWP domains. One or two human PWWP domains from each subfamily of PWWP-containing proteins are listed. Secondary structural elements from HRP3 PWWP are shown above the sequences. The conserved K18 in loop 1 of HRP3 PWWP and its counterparts in other PWWP domains are highlighted. R78 in loop 2 of HRP3 PWWP and its corresponding or neighboring basic residues in the other PWWP domains are color coded.

Supplementary Table S1. Data collection and refinement statistics

Crystal	Free PWWP(8-94)	PWWP(1-99) with 16-mer-TA	PWWP(1-99) with 10-mer-GC	PWWP(1-99) with DNA and H3(33-40)K36me3	PWWP(1-99) with DNA and H3(33-40)K36me2
PDB entry	6IIP	6IIQ	6IIR	6IIS	6IIT
Beam line	SSRF-BL19U1	SSRF-BL19U1	SSRF-BL19U1	SSRF-BL19U1	SSRF-BL17U1
Wavelength	0.91531	0.97853	0.97853	0.97853	0.97945
Space group	$P2_12_12_1$	$C2_1$	$P3_1$	$P3_1$	$P3_1$
Unit cell					
a, b, c (Å)	38.5, 39.9, 97.4	57.4, 33.6, 201.2	33.6, 33.6, 197.9	33.5, 33.5, 198.8	33.6, 33.6, 200.1
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 120.0	90.0, 90.0, 120.0	90.0, 90.0, 120.0
Resolution (Å)	50.0-0.95 (0.97-0.95)[a]	30.0-1.85 (1.89-1.85)	50.0-2.20 (2.24-2.20)	50.0-2.35 (2.39-2.35)	50.0-2.10 (2.14-2.10)
R_{sym}	0.072 (0.455)	0.084 (0.611)	0.111 (0.975)	0.087 (0.533)	0.135 (1.17)
I/σ (I)	52.6 (5.3)	22.3 (2.0)	35.9 (2.1)	16.0 (2.0)	36.5 (4.1)
Completeness (%)	97.4 (86.0)	94.3 (96.4)	99.9 (100)	91.6 (84.2)	100.0 (100.0)
Redundancy	11.9 (11.2)	4.2 (4.3)	7.5 (7.0)	3.6 (3.9)	7.2 (6.9)
Number of unique reflections	93005	31501	12832	9478	14978
R_{work}/R_{free} (%)	19.7/20.5	20.6/25.3	20.2/25.8	18.6/26.3	19.1/26.4
Number of non-H atoms					
Protein	1432	2432	1624	1632	1638
Peptide	/	/	/	72	70
DNA	/	615	410	410	410
Water	294	186	5	18	52
Average B factors (Å^2)					
Protein	10.4	36.5	79.3	47.4	43.5
Peptide	/	/	/	66.6	73.4
DNA	/	45.5	83.7	50.7	53.9
Water	19.2	46.3	70.6	44.1	45.4
R.m.s. deviations					
Bond lengths (Å)	0.006	0.008	0.009	0.010	0.008
Bond angles (°)	1.16	1.12	1.17	1.31	1.09

[a] Highest resolution shell (in Å) shown in parentheses.