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Supplemental information

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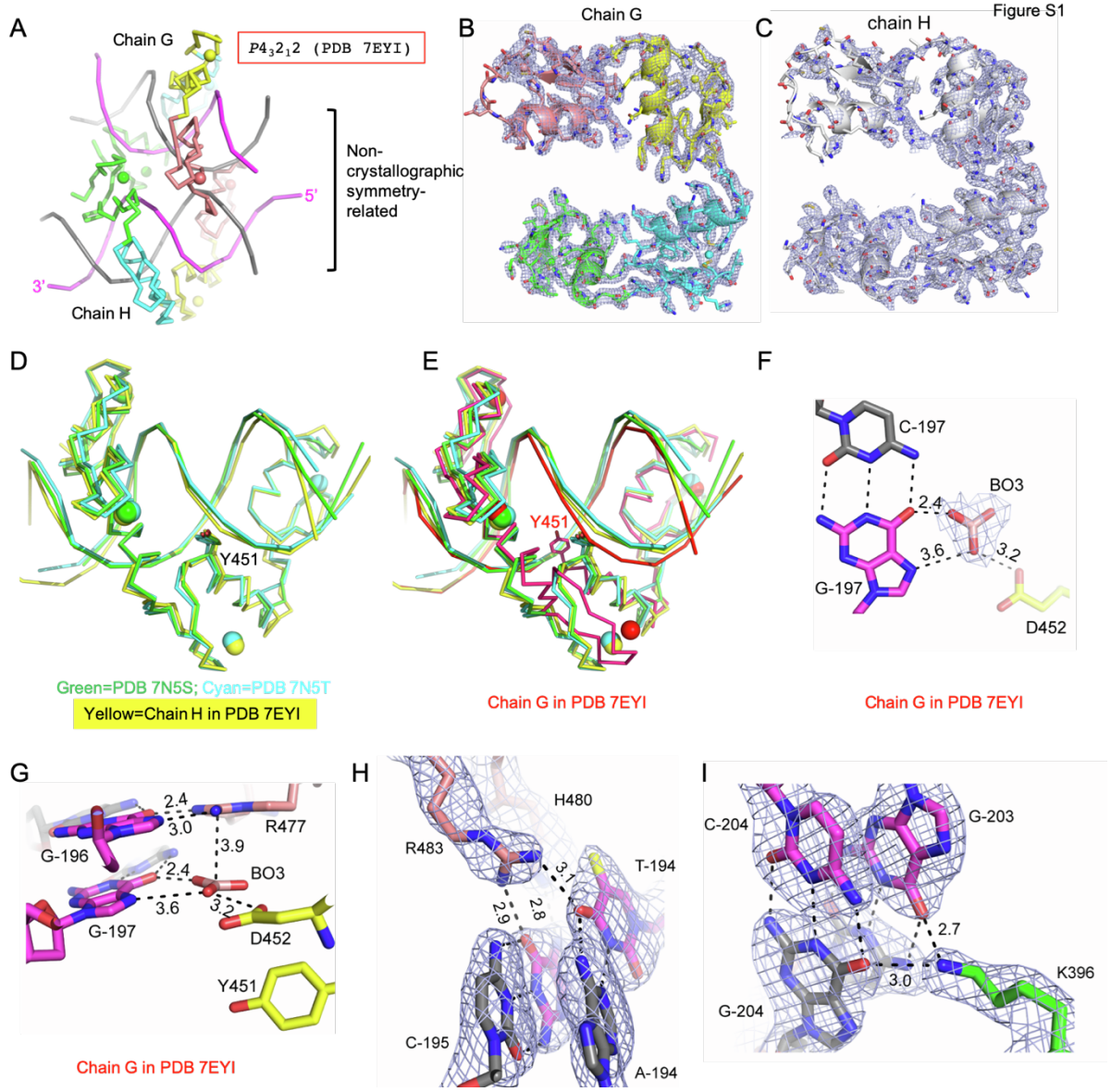
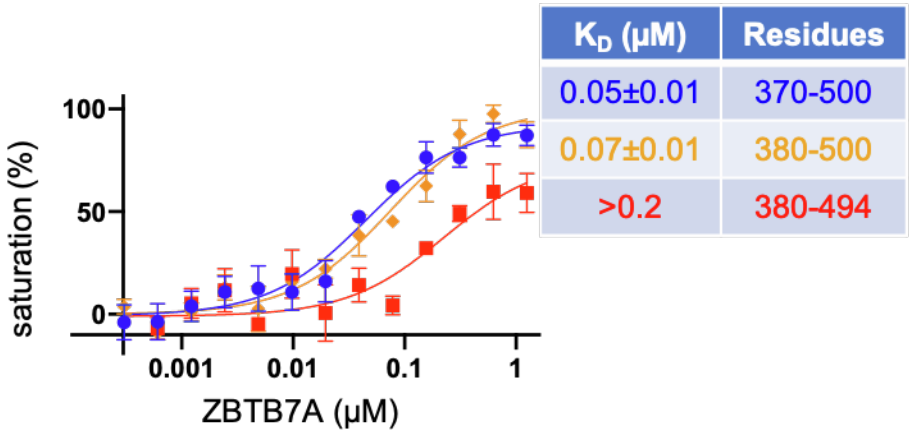


Figure S1. Related to Figure 3. Additional structural information in *P*4$_3$2$_1$2 space group (PDB 7EYI). (A) Two complexes (chains G and H) in the asymmetric unit. (**B-C**) 2Fo-Fc composite omit electron density maps (contoured at 1σ level) for chain G (panel D) and chain H (panel E) of the two complexes. The electron densities of ZF4 are less ordered than that of ZF1-3, and **we** did not observe electron densities of the C-terminus residues (491-506) though used in the crystallization. **(D)** Superimposition of three highly similar complexes as indicated. (**E**) Chain G in PDB 7EYI has the largest deviation (particularly ZF3) from the other three structures. (**F-G**) A boric acid binds in the DNA-protein interface of complex G in PDB 7EYI. An omit 2Fo-Fc electron density in light blue mesh (contoured at 1.0σ level above the mean) is shown for the bound boric acid. (**H-I**) The corresponding 2Fo-Fc omit map (contoured at 1.0σ with carve of 2.0Å) of (H) R483 bridging between two base pairs (-195 and -194) and (**I**) K396 bridging between two base pairs (-204 and -203).

Figure S2



K_D (μM)	Residues
0.05±0.01	370-500
0.07±0.01	380-500
>0.2	380-494

Figure S2. Related to Figure 1D. Binding affinities of ZBTB7A fragments with DNA oligo as measured by fluorescence polarization assays and plotted as percentage of saturation.

Figure S3

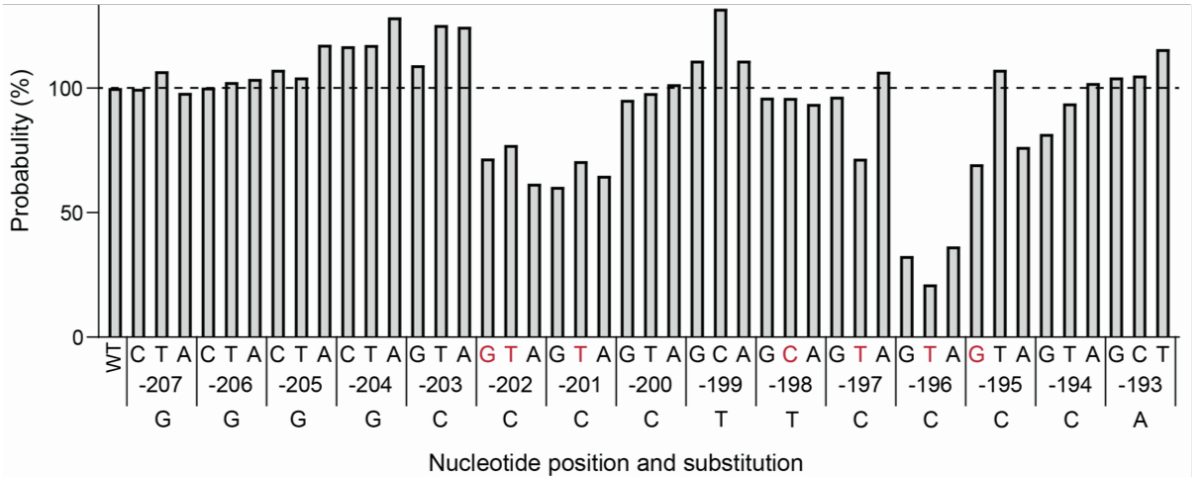


Figure S3. Related to Figure 4A. The quantification of shifted bands in the EMSA experiments.

Table S1. Related to Figures 2 and 3. X-ray data collection and refinement statistics

ZBTB7A (PDB)	7EYI	7N5S	7N5T
DNA (5' – 3') (3' – 5')	ATAGGGCCCCTTCCCAAC ATCCCGGGGAAGGGTTGT	GGGGCCCCTTCCCCA CCCGGGGAAGGGGTC	AGGCCCCTTCCCCAC CCGGGGAAGGGGTGT
Synchrotron	BL19U1	SERCAT (22-ID)	SERCAT (22-ID)
Date collected	2020-12-05	2020-12-14	2020-12-14
Wavelength (Å)	0.979	1.0	1.0
Space group	$P4_32_12$	$P2$	$P222_1$
Cell dimensions (Å)	67.4, 67.4, 228.7	46.9 37.0 74.3	35.5, 36.4, 176.4
α, β, γ (°)	90, 90, 90	90, 100, 90	90, 90, 90
Resolution (Å)	40.00−2.40 (2.44−2.40)	36.32-2.85 (2.95-2.85)	36.37-2.89 (2.99-2.89)
R_{merge}	0.094 (1.38)	0.097 (1.67)	0.222 (1.27)
R_{pim}	0.019 (0.291)	0.030 (0.701)	0.033 (0.736)
$CC_{1/2}$, CC*	0.994 (0.799)	0.610 (0.871)	0.873 (0.966)
$I/\sigma I$	36.9 (2.3)	18.8 (1.4)	24.7 (2.1)
Completeness (%)	99.1 (98.8)	92.6 (66.0)	99.3 (97.0)
Redundancy	25.2 (23.1)	10.3 (4.1)	41.3 (17.4)
Observed reflections	539,565	56,976	228,897
Unique reflections	21,433 (1037)	5,550 (384)	5,536 (542)
Wilson B-factor (Å^2)	39	96	77
Mean FOM (SAD)	0.48 at 2.4 Å		(severe anisotropy)[1]
Density modification	0.51 at 2.4 Å		
Refinement (F>0)			
No. reflections (overall)	19,866	5,528	5,418
No. reflections (test)	1,029	251	269
R_{work} / R_{free}	0.222 / 0.267	0.226 / 0.272	0.247 / 0.310
Number of atoms			
Protein	1816	833	837
DNA	1394	577	592
ZN	8	4	4
Water	29	-	-
Boric acid	4	-	-
B factors (Å^2)			
Protein	47.1	114	116
DNA	50.7	113	106
ZN	48.7	115	125
Water	31.3	-	-
Boric acid	32.4	-	-
R.m.s. deviations			
Bond lengths (Å)	0.003	0.003	0.003
Bond angles (°)	0.5	0.4	0.6
Ramachandran plot (%)			
Favored	97.3	95.4	95.4
Allowed	2.7	4.6	4.6

[1] The anisotropic Wilson B tensor for 7N5T:
111.39 0.00 0.00
0.00 139.88 -0.00
0.00 -0.00 39.51
The values 111.39, 139.88, 39.51 relate to the principal axes of an ellipse. If numbers are about the same, there's no anisotropy or isotropic. If one number (axis) differs significantly from the others, there is a severe ellipse.)

Table S2. Related to Figures 4B and 4C. Summary of ITC data.

ZBTB7A	DNA	ΔH (kJ/mol)	−TΔS (kJ/mol)	ΔG (kJ/mol)	N	K_D (μM)
@150 mM NaCl						
	WT	−13.7±0.2	5.08	−8.66	1.10	0.35±0.05
	C−194A	−13.3±0.2	4.84	−8.50	1.09	0.47±0.07
@300 mM NaCl						
	WT	−11.1±0.2	2.98	−8.10	1.00	0.92±0.09
	G−204A	−13.2±0.5	6.00	−7.20	1.25	4.3±0.5
	C−203G	−11.3±0.6	4.55	−6.71	1.12	10±1
	T−198C	−13.7±0.4	6.52	−7.19	0.94	4.4±0.3
	C−197T	−9.6±0.4	2.70	−6.93	1.11	6.8±0.7
	C−196T	−12.0±0.5	5.06	−6.90	0.99	7.2±0.7
	C−195G	−11.0±0.7	4.28	−6.76	0.97	9±1

No binding was detected for C-202G, C-202T and C-201T mutations.

K_D, N, ΔH, −TΔS, ΔG stand for dissociation constant, stoichiometry, enthalpy, entropy and Gibbs energy, respectively. Each K_D value is presented as fitted value ± error.

Table S3. Related to Key Resources Table. Oligonucleotides used in this paper.

Oligonucleotides	Application
5′-GGCCCCTTCCCCAC -3′ 3′-CCGGGGAAGGGGTGT-5′-FAM	FP binding
5′-AGGCCCCTTCCCCAC-3′ 3′- CCGGGGAAGGGGTGT-5′	Crystallization
5′-GGGGCCCCTTCCCCA -3′ 3′- CCCGGGGAAGGGGTC-5′	Crystallization
5′-ATAGGGCCCCTTCCCAAC -3′ 3′- ATCCCGGGGAAGGGTTGT-5′	Crystallization
5′-GGCCCCTTCCCCA-3′ 3′-CCGGGGAAGGGGT-5′	IDT binding (WT)
5′-GACCCCTTCCCCA-3′ 3′-CTGGGGAAGGGGT-5′	IDT (G-204A)
5′-GGGCCCTTCCCCA-3′ 3′-CCCGGGAAGGGGT-5′	IDT (C-203G)
5′-GGCGCCTTCCCCA-3′ 3′-CCGCGGAAGGGGT-5′	IDT (C-202G)
5′-GGCTCCTTCCCCA-3′ 3′-CCGAGGAAGGGGT-5′	IDT (C-202T)
5′-GGCCTCTTCCCCA-3′ 3′-CCGGAGAAGGGGT-5′	IDT (C-201T)
5′-GGCCCCTCCCCCA-3′ 3′-CCGGGGAGGGGGT-5′	IDT (T-198C)
5′-GGCCCCTTTCCCA-3′ 3′-CCGGGGAAAGGGT-5′	IDT (C-197T)
5′-GGCCCCTTCTCCA-3′ 3′-CCGGGGAAGAGGT-5′	IDT (C-196T)
5′-GGCCCCTTCCGCA-3′ 3′-CCGGGGAAGGCGT-5′	IDT (C-195G)