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Supplemental Information

Crystal Structure of the Double Homeodomain of DUX4 in Complex with DNA

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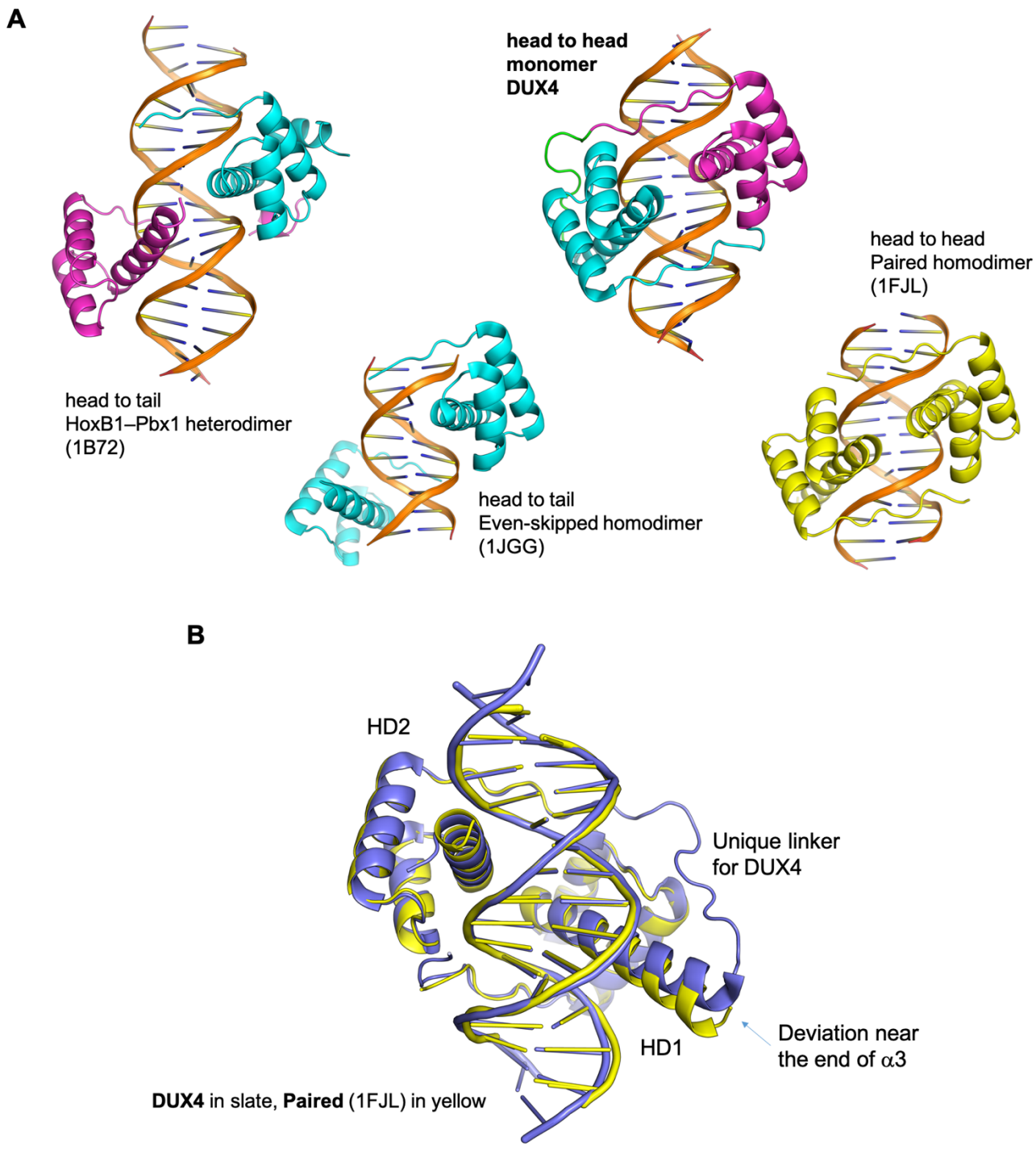

Figure S1. Structures of various homeodomain-DNA complexes. Related to Figures 1 and 4.

(A) Comparison of the homo-dimeric (Paired (Wilson et al., 1995), Even-skipped (Hirsch and Aggarwal, 1995)) or hetero-dimeric (HoxB1-Pbx1(LaRonde-LeBlanc and Wolberger, 2003; Piper et al., 1999)) homeobox proteins with the DUX4 double-homeodomain. (B) Superposition of the *Drosophila* Paired homodimer-DNA complex (PDB ID: 1FJL) (Wilson et al., 1995) with the DUX4-DNA complex.

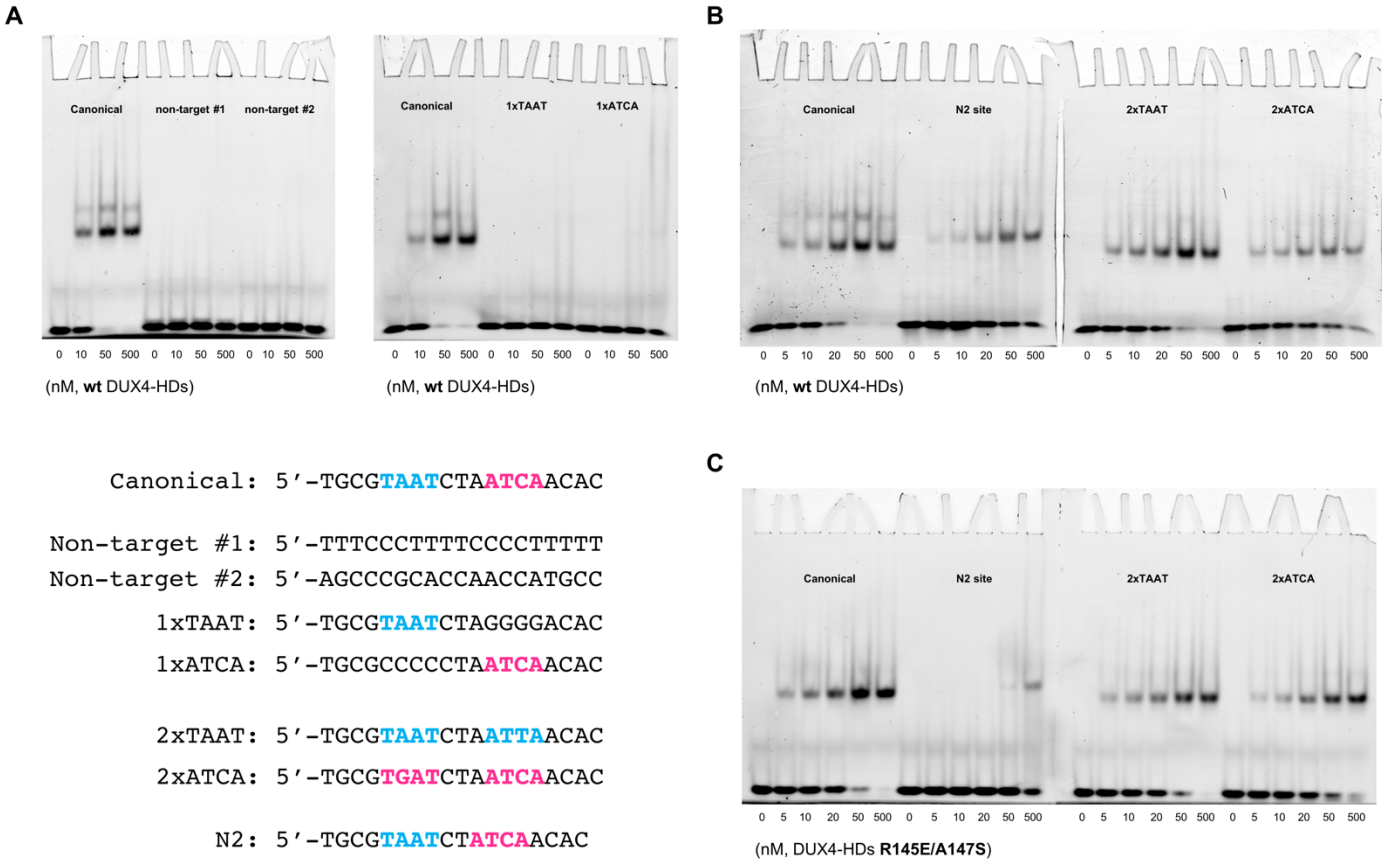

Figure S2. *In vitro* DNA-binding analyses by electrophoretic mobility shift assay (EMSA). Related to Figure 2. Proteins used were the crystallized wild-type DUX4(15-155) in (A, B) and DUX4(15-155) R145E/A147S mutant in (C). DNA sequences of the fluorescently labeled probes are shown on the lower left. They were annealed with unlabeled complementary oligos to generate double-stranded DNA substrates.

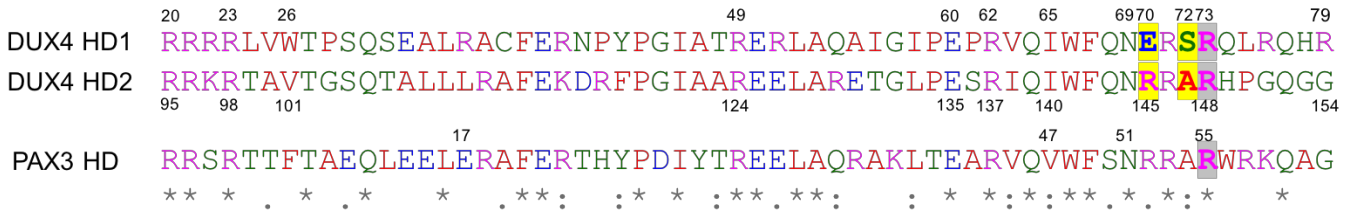

Figure S3. Amino acid sequence alignment between HD1 and HD2 of DUX4 and PAX3 HD. Related to Figure 2. The arginine residue that makes critical base contact (DUX4 HD2) or backbone contacts (DUX4 HD1 and PAX3) is highlighted in gray in each homeodomain. DUX4 residues highlighted in yellow play important roles in determining target DNA sequence selectivity as shown in Figure 2.

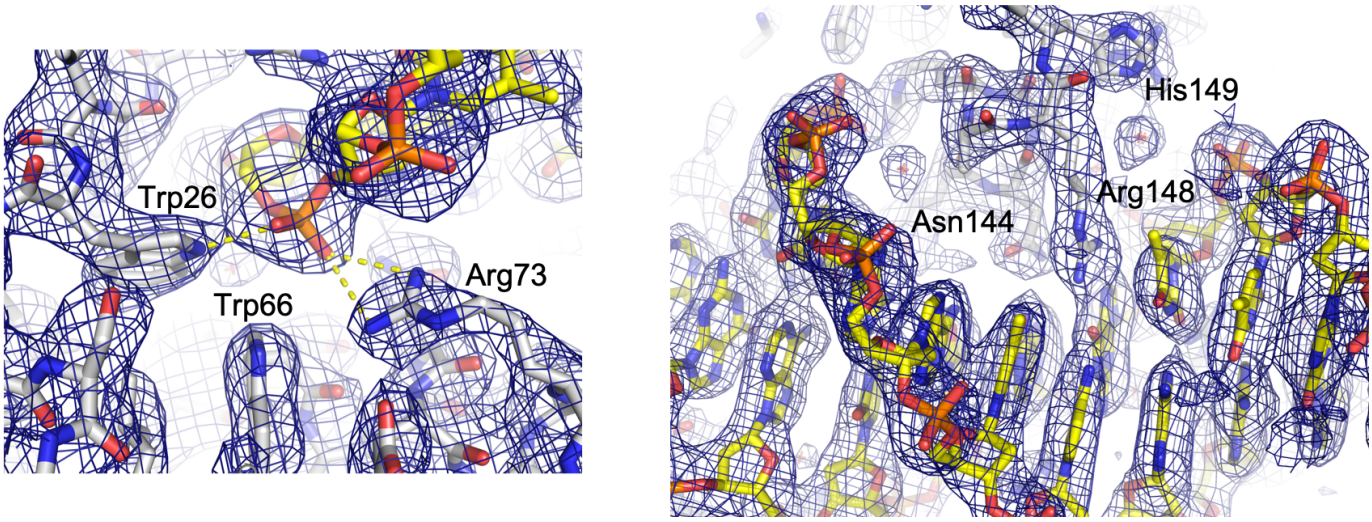

Figure S4. Representative electron density map for the DUX4(15-155)-DNA complex. Related to Figure 1. 2mFo-DFc electron density is shown by blue mesh, contoured at 1.0 σ.

Table S1. Data collection and refinement statistics. Related to Method Details.

Data collection	
Space group	$C222_1$
Cell dimensions	
a, *b*, *c* (Å)	67.19 73.14 108.56
Wavelength (Å)	0.9792
Resolution (Å)	36.57 - 2.12 (2.20 - 2.12)[a]
R_{merge}	0.073 (1.37)
$I / \sigma I$	20.2 (2.3)
Completeness (%)	97.2 (83.0)
Redundancy	17.2 (13.6)
Refinement	
Resolution (Å)	36.57 - 2.12 (2.20 - 2.12)
No. reflections	15091
R_{work} / R_{free} (%)	19.8 / 24.7
No. non-H atoms	1860
Protein	1117
DNA	691
Ligand	4
Solvent	48
B-factor	66.10
Protein	67.14
DNA	65.09
Ligand/Ion	90.09
Solvent	54.59
R.m.s. deviations	
Bond lengths (Å)	0.011
Bond angles (°)	1.12

[a]Values in parentheses are for highest-resolution shell.