Supplemental Material

Distinctive structural basis for DNA recognition by the fission yeast Zn_2Cys_6 transcription factor Pho7 and its role in phosphate homeostasis

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Supplemental Table S1

Supplemental Figures S1, S2, S3, S4, S5, S6, S7, and S8

Table S1

Crystallographic Data and Refinement Statistics

Data collection	**Pho7-DNA / remote**	**Pho7-DNA / Zn peak**
Beamline	24ID-E	24ID-C
Wavelength (Å)	0.97918	1.2827
Space group	$P2_1$	$P2_1$
a, b, c (Å)	50.83, 40.68, 58.23	52.69, 43.97, 57.49
α, β, ϒ (°)	90.00, 105.8, 90.00	90.00, 106.6, 90.00
Resolution (Å)	50.00-1.70 (1.75-1.70)	50.00-2.05 (2.10-2.05)
R_{pim}	0.040 (0.134)	0.036 (0.088)
$I/\sigma I$	24.1 (2.5)	39.8 (5.3)
Completeness (%)	96.1 (87.4)	88.3 (38.4)
Redundancy	5.5 (3.6)	5.0 (3.2)
Unique reflections	24169 (1086)	14424 (312)
$CC_{1/2}$	0.932 (0.953)	0.999 (0.993)
Heavy atom sites		Zn (2)
Figure of merit		0.658
Refinement		
R_{work}/R_{free} (%)	18.81/21.52	
Number of atoms	1545	
DNA	814	
Protein	460	
Zn	2	
Water	269	
B-factors (mean/Wilson)	35.1/23.0	
r.m.s. deviations		
Bond lengths (Å)	0.006	
Bond angles (°)	0.886	
Ramachandran favored (%)	96.5	
Ramachandran outliers (%)	0	
PBD ID code	6E33	

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

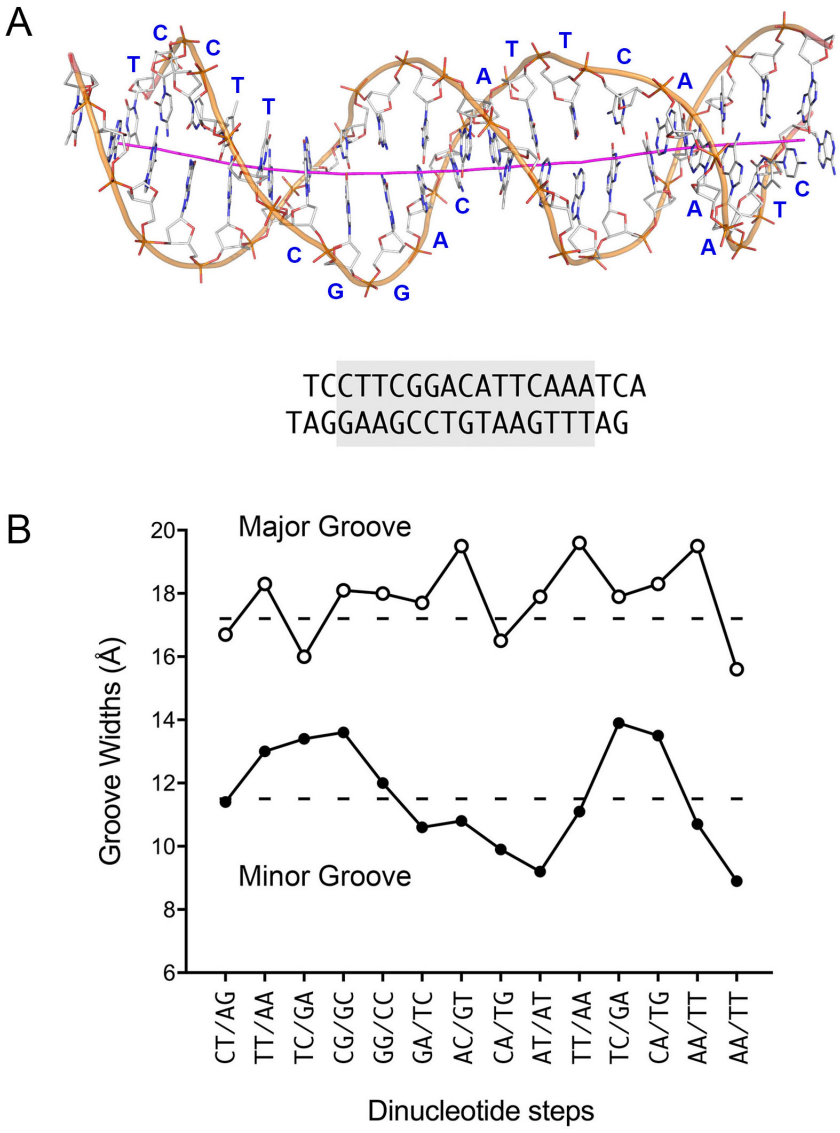

Figure S1. DNA conformation in the Pho7•DNA complex. (A) Stick model of the *tgp1* promoter DNA site in the Pho7•DNA complex, with a cartoon backbone trace (gold) through the phosphates. The magenta line shows the trajectory of the helical axis with a 9˚ bend, as calculated in CURVES+ (21). The nucleobases of the top DNA strand are indicated in blue bold fold. The sequences of the top and bottom strands of the DNA ligand are shown at bottom. (B) A plot of the major and minor groove widths at each indicated sequential dinucleotide step, as calculated in 3DNA (22). The groove widths of an idealized B-form DNA of the same sequence are denoted by the dashed lines.

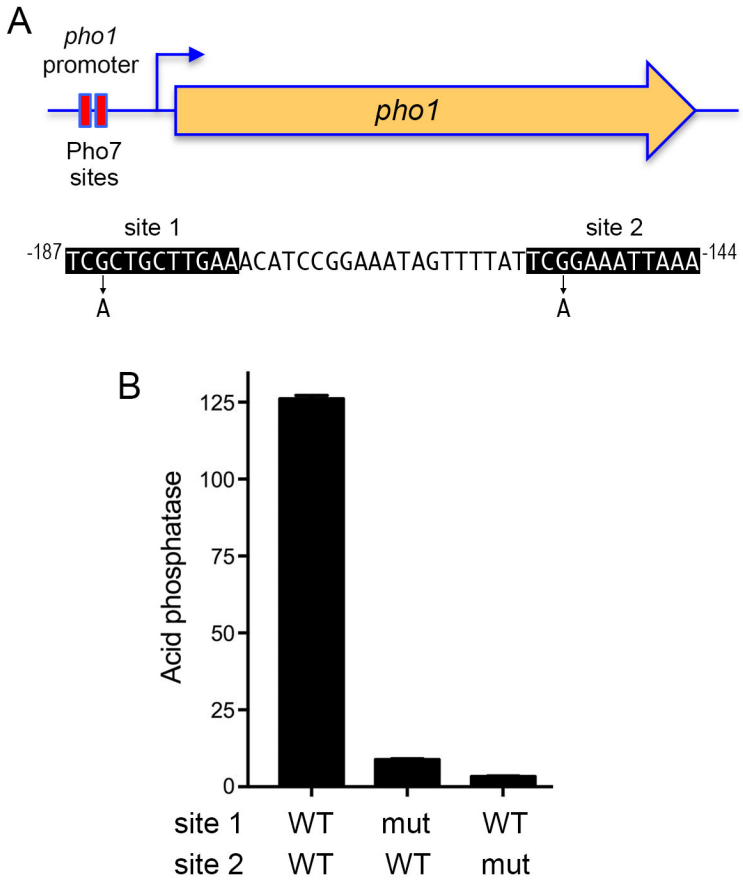

Figure S2. Effect of Pho7 site mutations in the *pho1* promoter on promoter activity *in vivo.* (A) The *pho1* reporter plasmid shown in cartoon form contains genomic DNA extending from 283-nt upstream of the *pho1* transcription start site (blue arrow) to 647-nt downstream of the *pho1* stop codon. Two Pho7 binding sites mapped within the *pho1* promoter are depicted as red boxes. The DNA sequence spanning sites 1 and 2 is shown below the cartoon. The Pho7 binding motifs are shown in white font on black background. Single G-to-A mutations were introduced into sites 1 and 2 at the positions indicated by vertical arrows. (B) *pho1* reporter plasmids with wild-type and the mutated Pho7 sites were introduced into a strain deleted for the endogenous *pho1* gene. Plasmid-containing cells were grown logarithmically in YES medium and assayed for acid phosphatase activity.

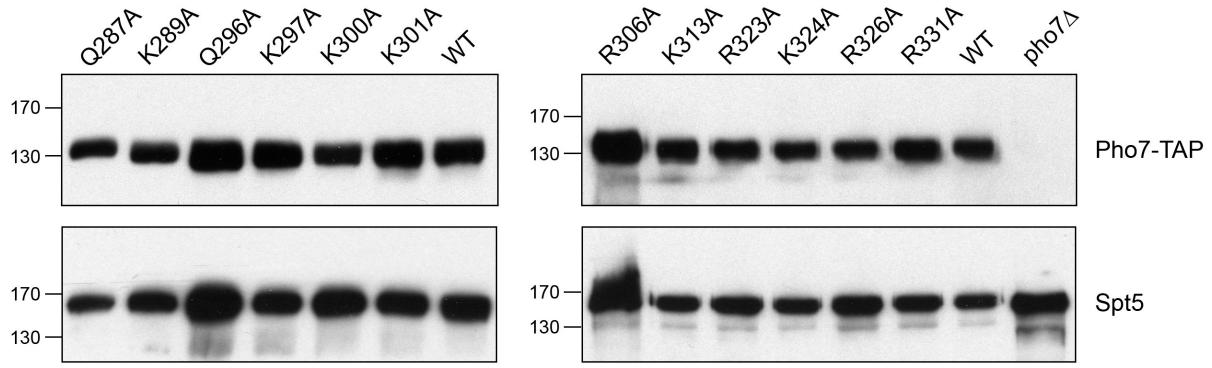

Figure S3. Western blots of whole cell extracts prepared from *S. pombe* strains with the indicated *pho7-TAP* or *pho7*Δ alleles. The blots were probed with antibodies recognizing the TAP tag (top panels) or the Spt5 protein (bottom panels). The positions of 170 kDa and 130 kDa size markers are indicated on the left.

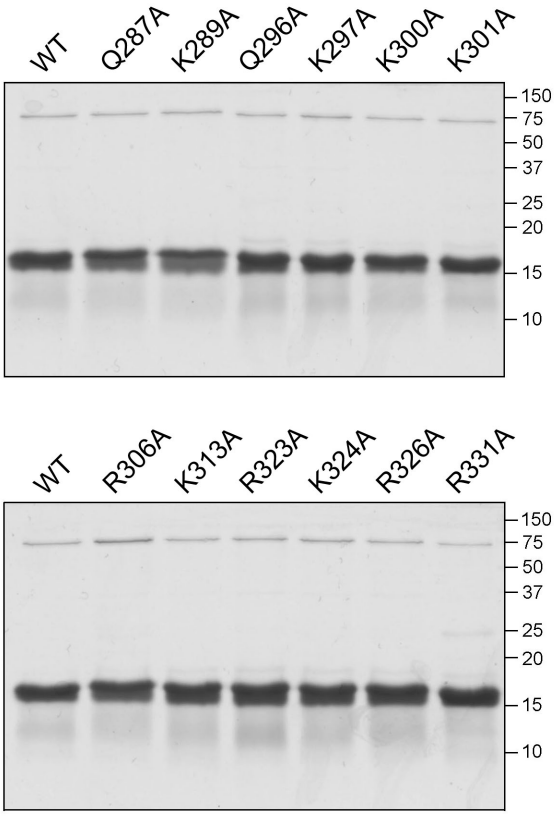

Figure S4. Pho7-DBD mutant proteins. Aliquots (5 µg) of the indicated wild-type and Ala-mutant DBD proteins were analyzed by SDS-PAGE. The Coomassie blue-stained gels are shown. The positions and sizes (in kDa) of marker polypeptides are indicated on the right.

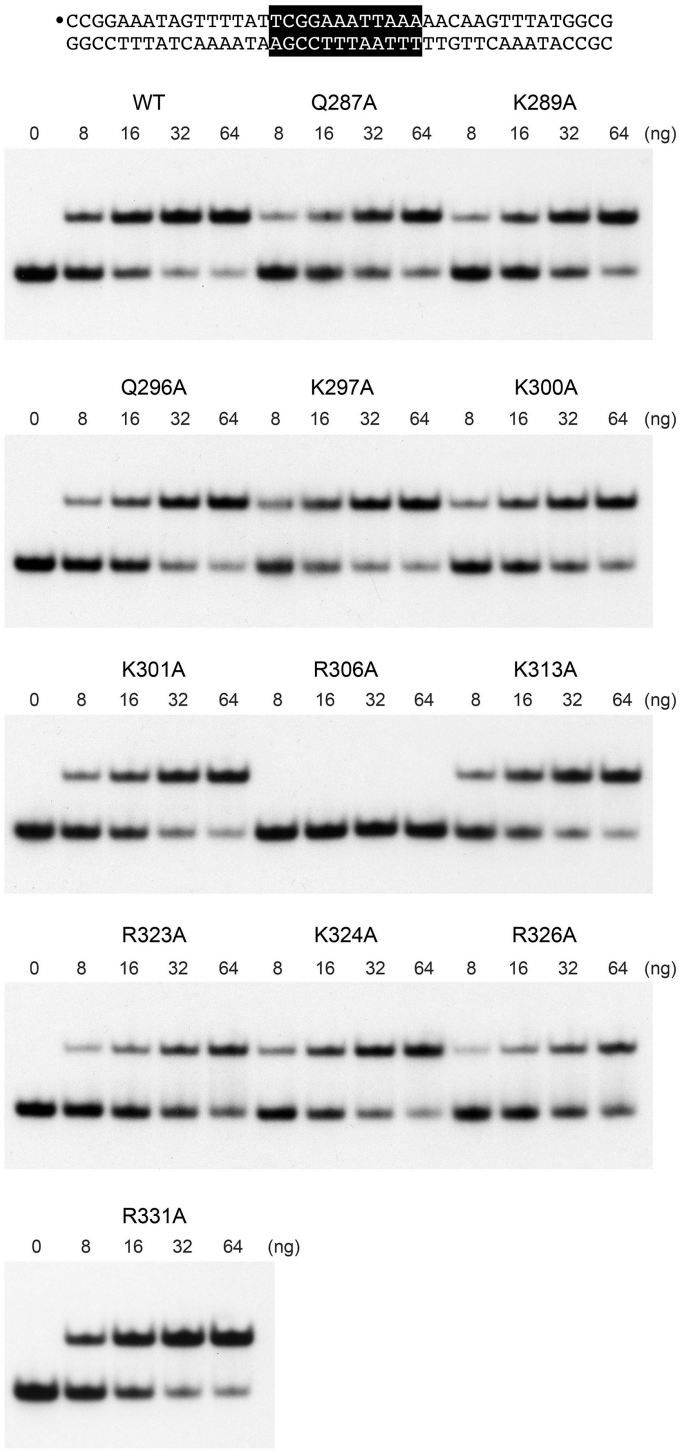

Figure S5. Binding of Pho7-DBD mutants to site 2 in the *pho1* promoter. EMSAs were performed using the ^{32}P-labeled site 2 DNA probe shown at the top. The ^{32}P label is indicated by ●. The Pho7 binding motif is shown in white font on black background. Reaction mixtures (10 µl) containing 0.24 pmol ^{32}P-labeled DNA, 0.34 µg poly(dI-dC), 50 mM NaCl, and 0, 8, 16, 32, or 64 ng Pho7-DBD were incubated for 10 min at room temperature. The mixtures were analyzed by native PAGE in 0.25x TBE. Autoradiographs of the dried gels are shown.

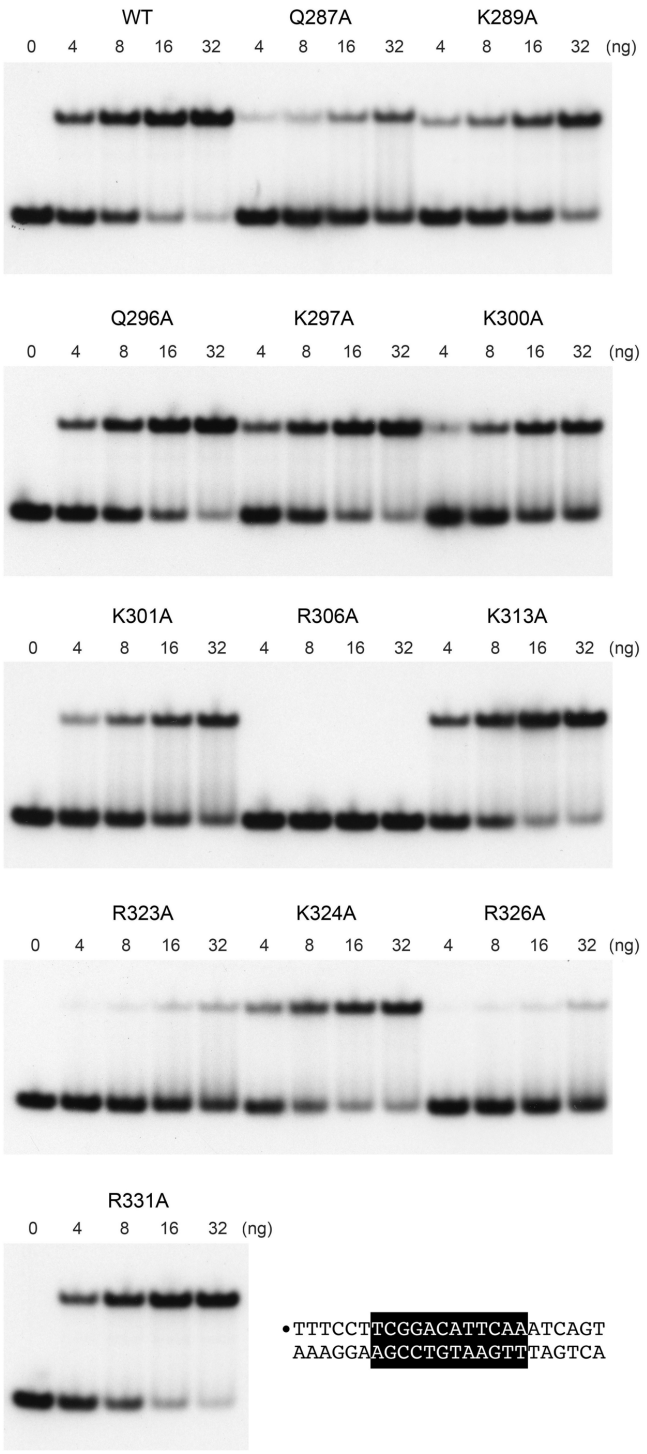

Figure S6. Binding of DBD mutants to the Pho7 site in the *tgp1* promoter. EMSAs were performed using the ^{32}P-labeled *tgp1* DNA probe shown at the bottom right. The ^{32}P label is indicated by ●. The Pho7 binding motif is shown in white font on black background. Reaction mixtures (10 µl) containing 0.5 pmol ^{32}P-labeled DNA, 0.34 µg poly(dI-dC), 200 mM NaCl, and 0, 4, 8, 16, or 32 ng Pho7-DBD were incubated for 10 min at room temperature. The mixtures were analyzed by native PAGE in 1x TBE. Autoradiographs of the dried gels are shown.

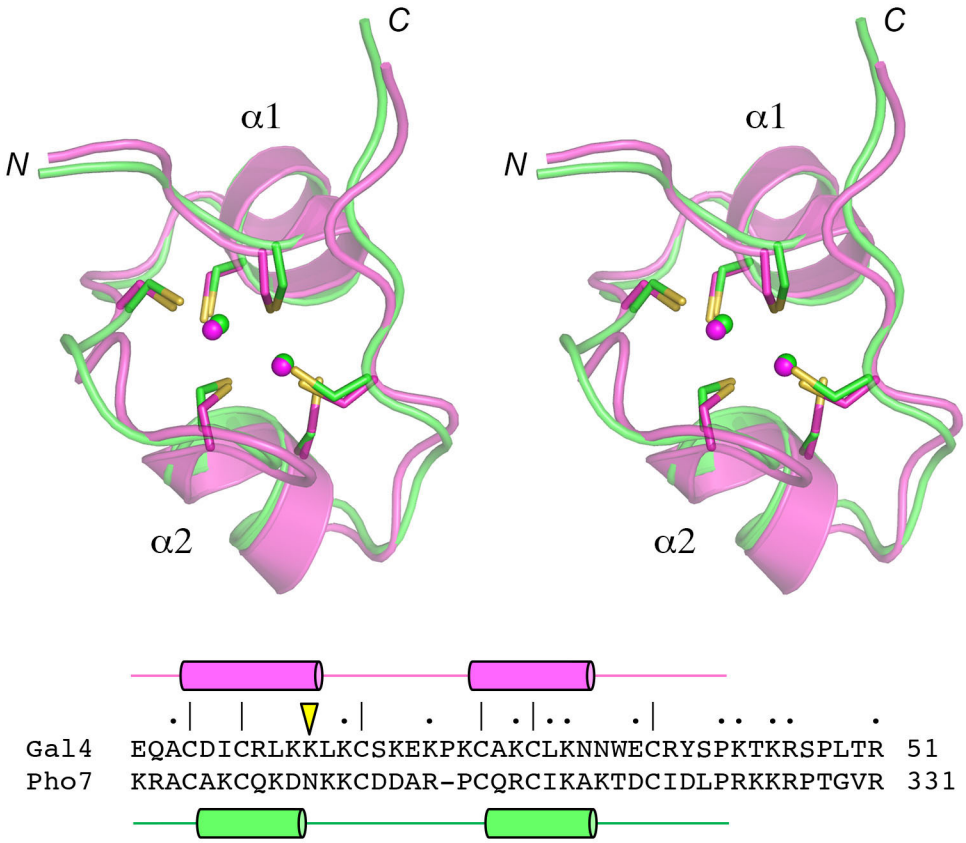

Figure S7. Structural alignment of the Pho7 and Gal4 zinc-binding modules. (Top panel) Stereo view of the superimposed zinc-binding modules of Gal4 (magenta) and Pho7 (green), with zinc atoms as spheres coordinated tetrahedrally to the six cysteines side chains (stick models). The N and C termini and the α1 and α2 helices of the modules are labeled. (Bottom panel) Alignment of the primary and secondary structures of the Gal4 and Pho7 zinc-binding modules. The zinc-binding cysteines are denoted by |. Other positions of amino acid side chain identity/similarity are indicated by dots. A key DNA specificity determinant (Lys18 in Gal4; Asn299 in Pho7) is highlighted by the yellow triangle.

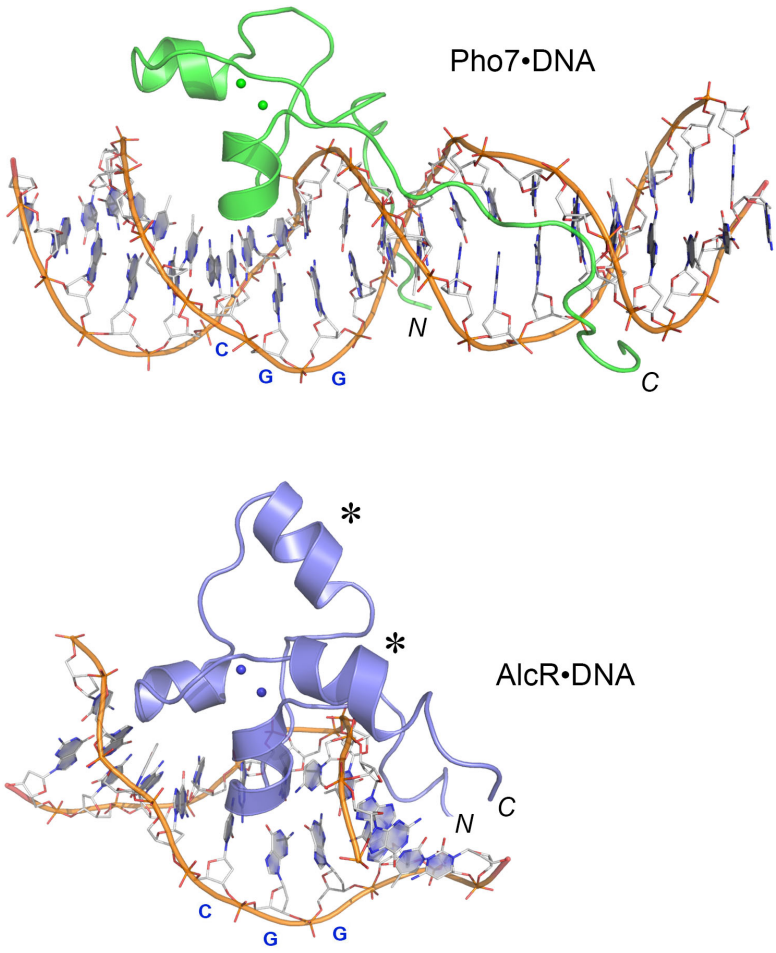

Figure S8. Comparison of Pho7 and AlcR DNA complexes. The Pho7•DNA and AlcR•DNA (pdb 1F4S) complexes were superimposed with respect to their zinc atoms and then offset vertically. The 5'-CGG base pair triplet that they recognize in the major groove is indicated. The Pho7 and AlcR proteins are colored green and blue, respectively. The AlcR DBD has two α-helices (indicated by ⁎) that are not present in Pho7.