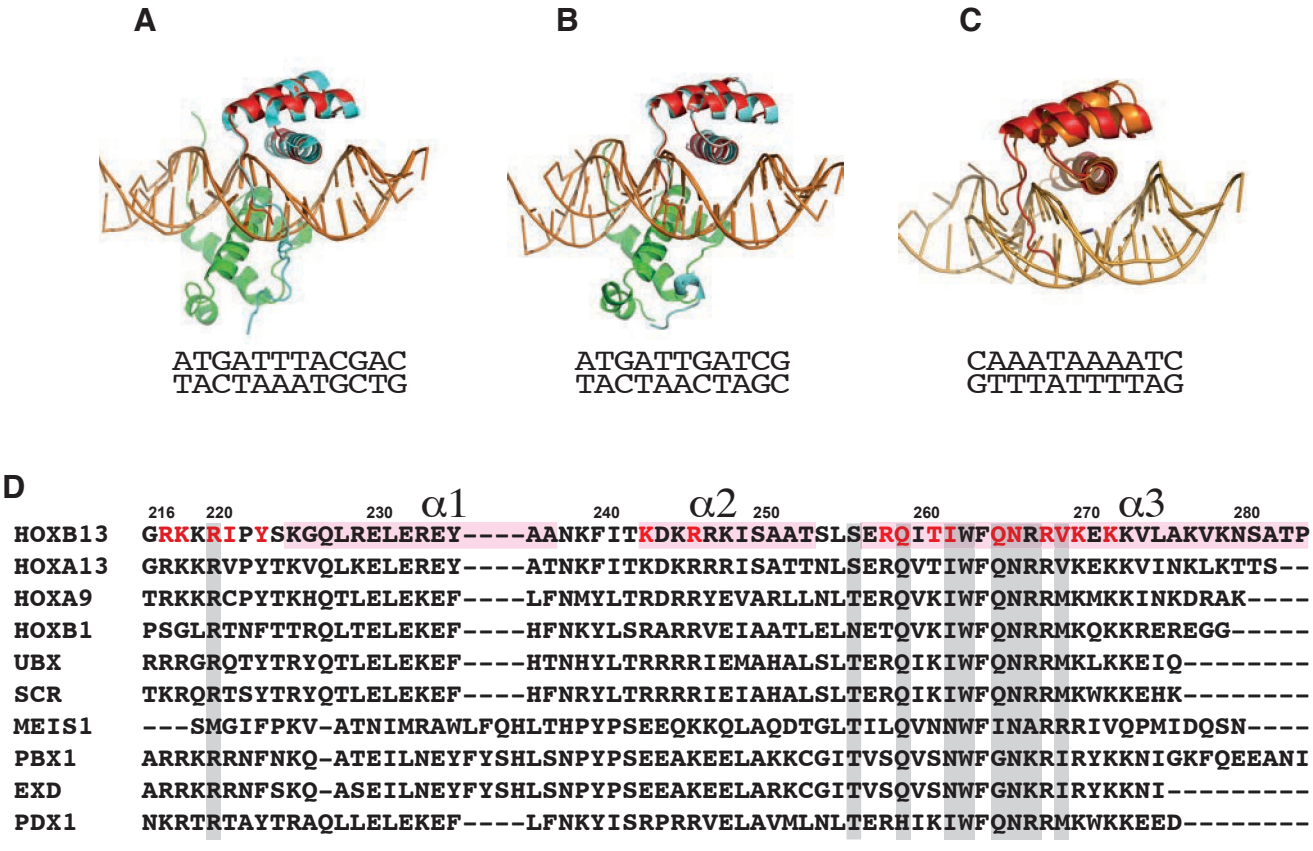
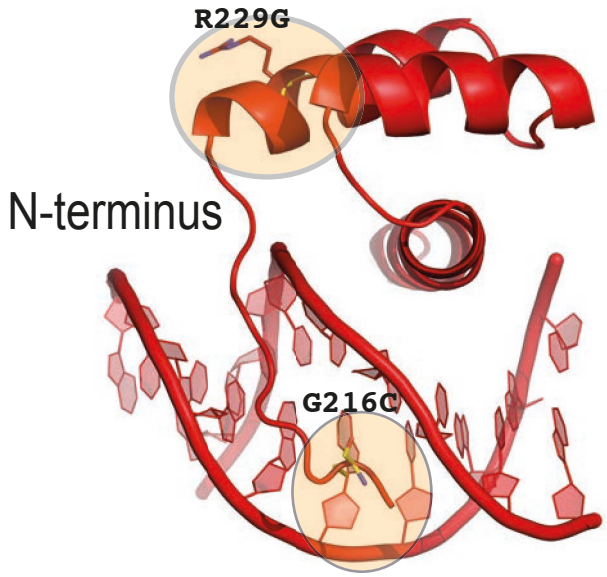


Figure S1. **The comparison of HOXB13 structure with HOXB1 and HOXA9, Related to Figure 2**

The superposition of HOXB13-DNA^{CAA} complex (red) with: (**A**) HOXA9:PBX1-DNA complex (HOXA9 is in cyan, PBX1 is green, PDB entry 1PUF);(**B**) with HOXB1:PBX1-DNA complex (HOXB1 is in cyan, PBX1 is in green, PDB entry 1B72); (**C**) with HOXA13-DNA complex (HOXA13 is in orange, PDB entry 2LD5). The corresponding DNA sequences are presented under pictures.
(**D**) The sequence alignment of Hox proteins with known structures. The numbering corresponds to HOXB13. Three helices are labeled on the top and highlighted with light pink. The residues involved in interactions are highlighted in grey. The residues involved in interaction in HOXB13 are colored red.

A



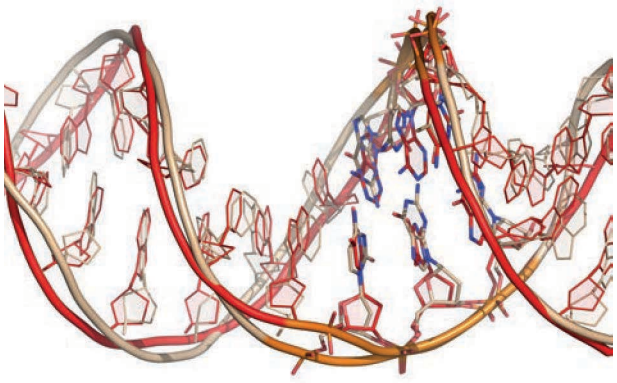
B

```
                216       220             230             240             250             260             270             280
HOXB13_  GRKKRIPYSKGQLRELEREYAANKFITKDKRRKISAATSLSERQITIWFQNRRVKEKKVLAKVKNSATP
HOXA13_  GRKKRVPYTKVQLKELEREYATNKFITKDKRRRISATTNLSERQVTIWFQNRRVKEKKVINKLKTTS--
HOXC13_  GRKKRVPYTKVQLKELEKEYAASKFITKEKRRRISATTNLSERQVTIWFQNRRVKEKKVVSKSKAPHLH
HOXD13_  GRKKRVPYTKLQLKELENEYAINKFINKDKRRRISAATNLSERQVTIWFQNRRVKDKKIVSKLKDTVS-
HOXC12_  SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLLRE----QA
HOXD12_  ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVLRE----QA
HOXA11_  TRKKRCPYTKYQIRELEREFFFSVYINKEKRLQLSRMLNLTDRQVKIWFQNRRMKEKKINRDRLQYYSA
HOXC11_  TRKKRCPYSKFQIRELEREFFFNVYINKEKRLQLSRMLNLTDRQVKIWFQNRRMKEKKLSRDRLQYFSG
HOSD11_  SRKKRCPYTKYQIRELEREFFFNVYINKEKRLQLSRMLNLTDRQVKIWFQNRRMKEKKLNRDRLQYFTG
HOXA10_  GRKKRCPYTKHQTLELEKEFLFNMYLTRERRLEISRSVHLTDRQVKIWFQNRRMKLKKMNRENRIRELT
HOXC10_  GRKKRCPYTKHQTLELEKEFLFNMYLTRERRLEISKTINLTDRQVKIWFQNRRMKLKKMNRENRIRELT
HOXD10_  GRKKRCPYTKHQTLELEKEFLFNMYLTRERRLEISKSVNLTDRQVKIWFQNRRMKLKKMSRENRIRELT
HOXA9 _  TRKKRCPYTKHQTLELEKEFLFNMYLTRDRRYEVARLLNLTERQVKIWFQNRRMKMKKINKDRAKDE--
HOXB9 _  SRKKRCPYTKYQTLELEKEFLFNMYLTRDRRHEVARLLNLSERQVKIWFQNRRMKMKKMNKEQGKE---
HOXC9 _  TRKKRCPYTKYQTLELEKEFLFNMYLTRDRRYEVARVLNLTERQVKIWFQNRRMKMKKMNKEKTDKEQS
HOXD9 _  TRKKRCPYTKYQTLELEKEFLFNMYLTRDRRYEVARILNLTERQVKIWFQNRRMKMKKMSKEKCPKGD-
CDX1  _  KDKYRVVYTDHQRLELEKEFHYSRYITIRRKSELAANLGLTERQVKIWFQNRRAKERKVNKKK------
CDX2  _  KDKYRVVYTDHQRLELEKEFHYSRYITIRRKAELAATLGLSERQVKIWFQNRRAKERKINKKKLQ----
```

Figure S2. HOXB13 prostate cancer mutation

(**A**) Structural representation of two of three residues found mutated in single prostate families, Gly-216-Cys and Arg-229-Gly. The mutated residues are presented in ball-and-stick style and highlighted with orange rings. Note that the first mutation Gly-216-Cys belonging to the N-termini of HOXB13 DBD can affect the interactions forming by protein in narrow minor groove. The other mutation Arg-229-Gly is located at the beginning of helix 1 and because glycine residues is known as “helix-breaker” the mutation can effect the interaction between N-termini with DNA as well as the interaction between two helixes. (**B**) Sequence alignment of posterior members of HOX family. The cancer mutations found in HOXB13 are colored red. Light red columns highlight the residues involved in interactions with DNA. The numbering corresponds to HOXB13.

A



DNA^{TCG} + DNA^{CAA}
rmsd = 1.128

B

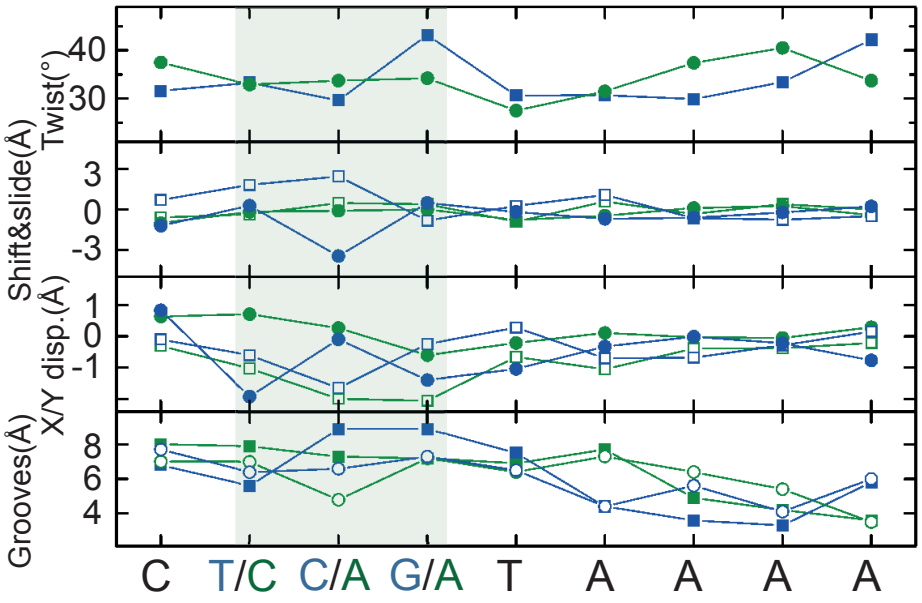


Figure S3. Paiwise comparison of two DNA molecules, Related to Figure 2

(**A**) Pairwise comparisons of DNA^{TCG} (wheat) and DNA^{CAA} (red); (**B**) Helicoidal parameters for HOXB13-DNA^{TCG} (blue) and HOXB13-DNA^{CAA} (green). Top: Helical twist; Middle top: shift (squares) and slide (circles); Middle bottom: X- (squares) and Y-displacements (circles); Bottom: Minor groove width (squares) and major groove depth (circles). The most pronounced differences are found for the TCGT and CAAT positions.

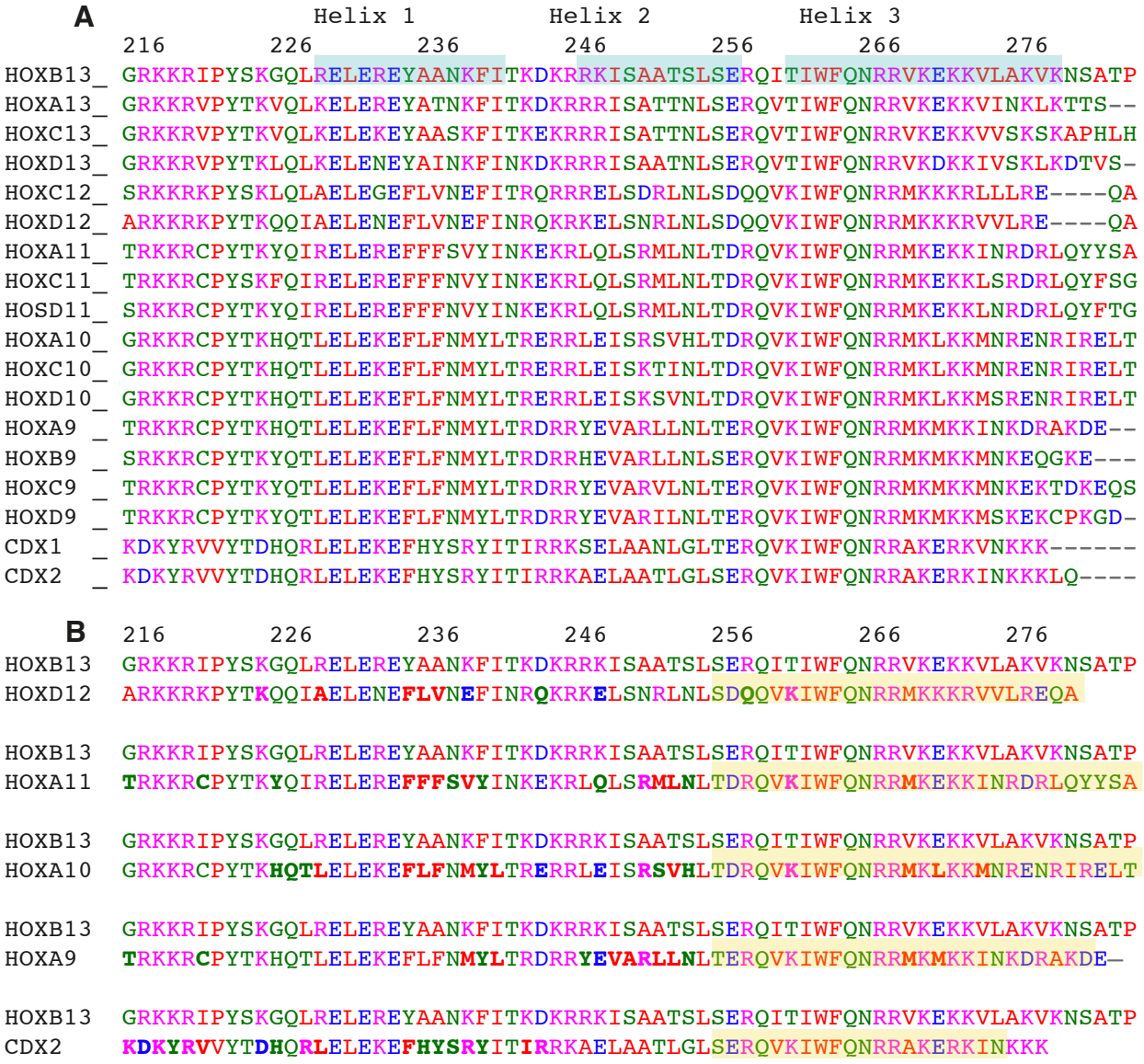


Figure S4. HOXB13 - HOXes/CDX mutations

(**A**) Sequence alignment of HOXB13 with other HOXes. Secondary structure (alpha-helixes) of HOXB13 is highlighed in cyan. (**B**) The pairwise alignment; note that in addition to single mutations there are combined mutations and replacement of Helix 3 (DNA-binding helix) to corresponding helix of other HOXes (highlighted in yellow). The numbering on the top of the sequences is HOXB13 numbering.

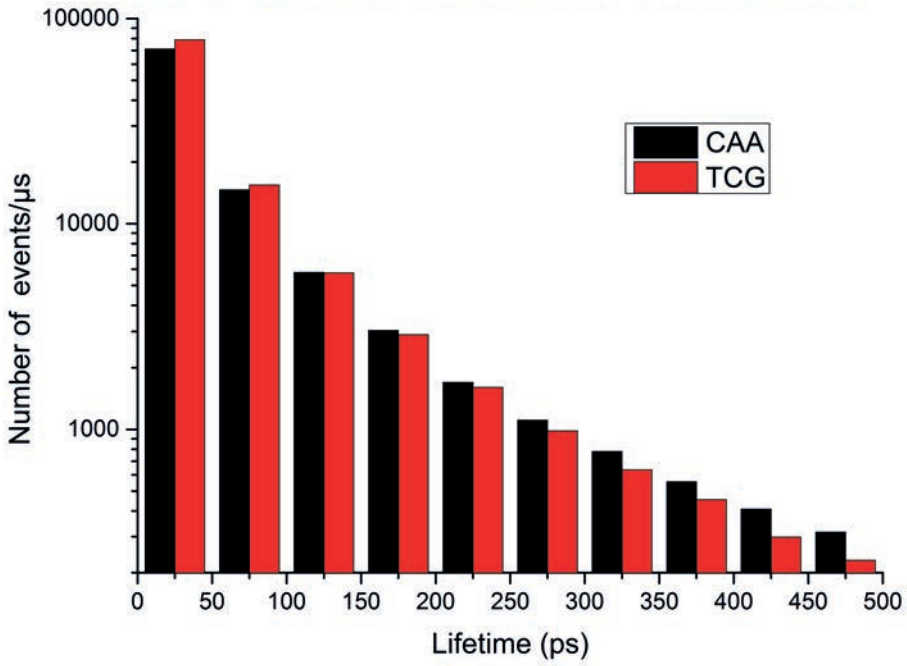


Figure S5. **Distribution of water-bridge lifetimes in HOXB13:DNA complexes**

Histogram showing the distribution of lifetimes of water bridges between the protein and the DNA for the HOXB13-DNA^{TCG} and HOXB13-DNA^{CAA} systems. The histogram is constructed by calculating the duration of each water bridge with 50 ps resolution from the molecular dynamics simulations; a water bridge is considered to exist when a water molecule is simultaneously hydrogen-bonded to one of the protein residues 255-272 and one of the DNA base pairs 5'-T(6)TTTACGAG(14)-3'.