SUPPLEMENTAL INFORMATION

Autoinhibition of ETV6 DNA-binding is established by the stability of its inhibitory helix

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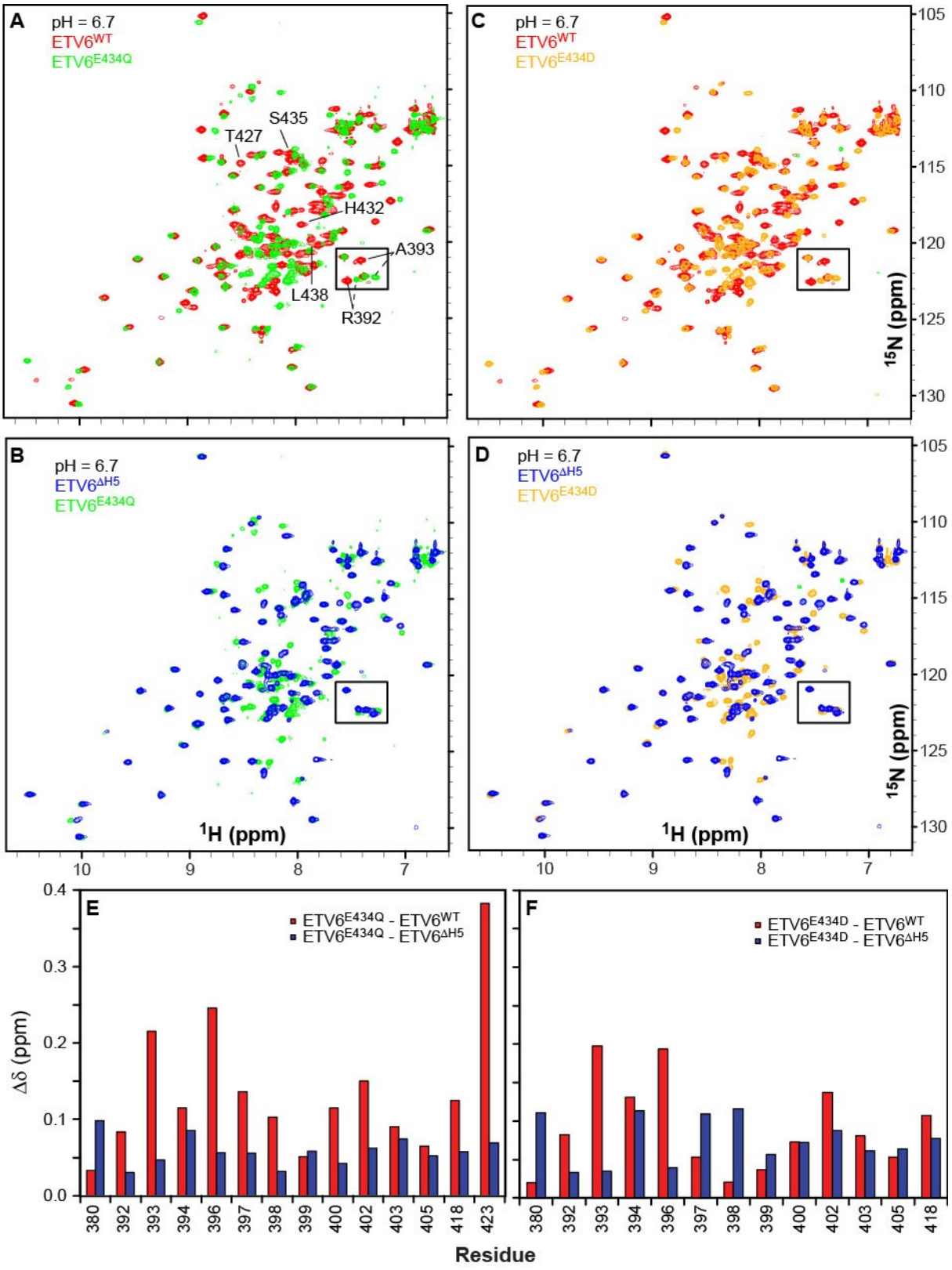
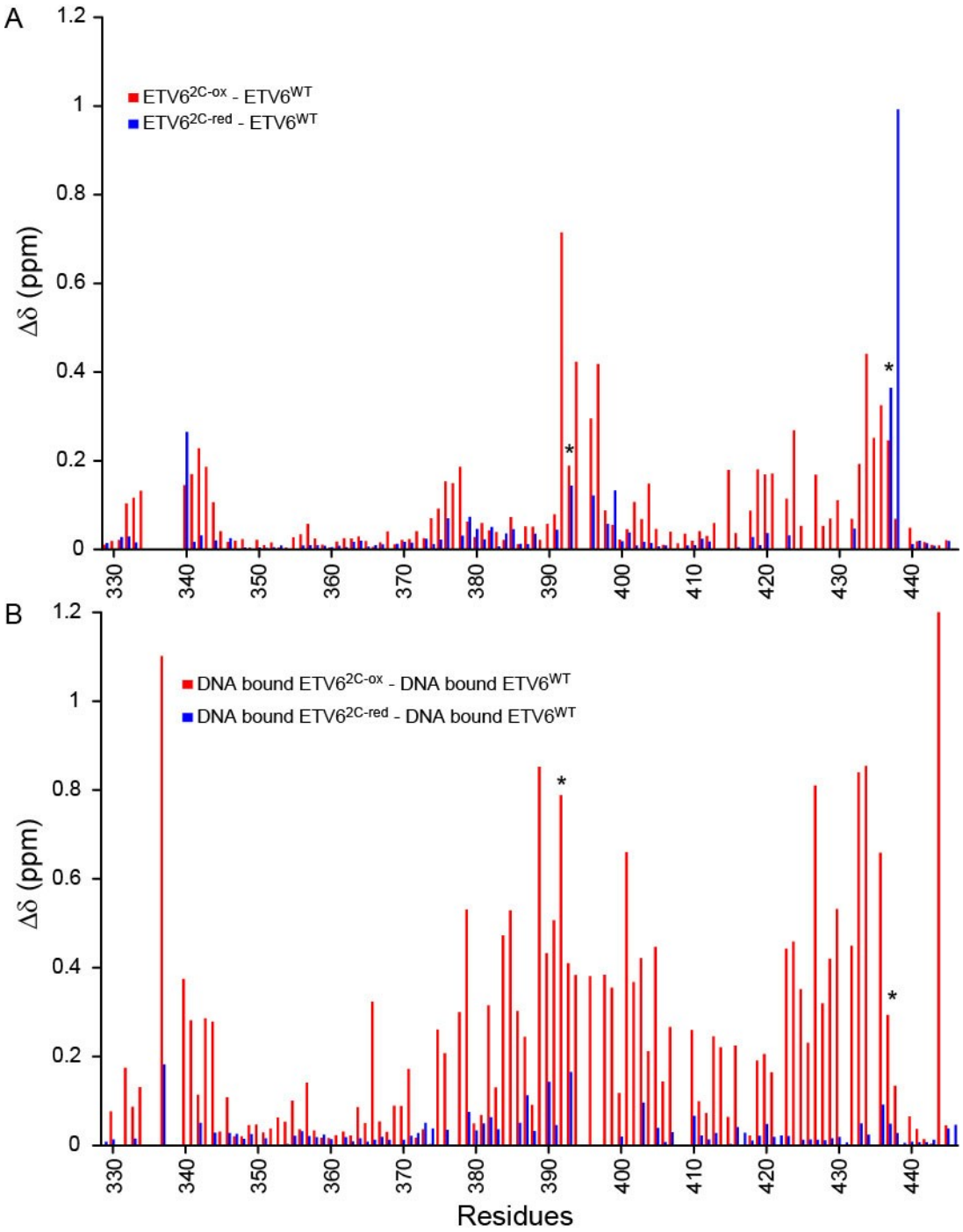
Table S1: Conditions for HX experiments

	Protium – Deuterium Exchange		CLEANEX-PM	
	Temperature	pH*[a]	Temperature	pH
ETV6^{WT}	20°C	6.5	20°C	6.7, 7.7
			30°C	6.5, 7.2
			35°C	No result
ETV6^{H432R}	20°C	6.2	20°C	6.7, 7.7
			30°C	6.4, 7.2
			35°C	7.8
ETV6^{RAE}	20°C	6.0	20°C	6.7, 7.7
			30°C	6.3, 7.2
			35°C	8.2
ETV6$^{2C\text{-}ox}$	20°C	6.5	20°C	6.7, 7.7
			30°C	6.3, 7.2, 8.6
			35°C	8.6

[a] pH measurement without isotope effect correction.


A
pH = 6.7
ETV6WT
ETV6E434Q
S435
T427
H432
A393
L438
R392
C
pH = 6.7
ETV6WT
ETV6E434D
15N (ppm)
B
pH = 6.7
ETV6ΔH5
ETV6E434Q
1H (ppm)
D
pH = 6.7
ETV6ΔH5
ETV6E434D
15N (ppm)
1H (ppm)
E
ETV6E434Q - ETV6WT
ETV6E434Q - ETV6ΔH5
F
ETV6E434D - ETV6WT
ETV6E434D - ETV6ΔH5
Δδ (ppm)
Residue

Supplemental Figure S1. Substitution of Glu434 with Gln or Asp leads to the unfolding of helix H5. Overlaid ^{15}N-HSQC spectra of the mutant ETV6^{E434Q} with **(A)** ETV6^{WT} and **(B)** ETV6$^{\Delta H5}$ at pH 6.7. Similarly, overlays for the mutant ETV6^{E434D} with **(C)** ETV6^{WT} and **(D)** ETV6$^{\Delta H5}$ at pH 6.7 are shown. Well-resolved peaks for several residues (Thr427, His432, Ser435 and Leu438) in helix H5 of ETV6^{WT} are identified in **A**. Peaks corresponding to Arg392 and Ala393 in helix H3, which are sensitive to the presence of folded H5, are also indicated by solid and broken lines in the wild type and mutant proteins, respectively. Combined amide chemical shift differences (Δδ) versus ETV6^{WT} and ETV6$^{\Delta H5}$ are shown for **(E)** ETV6^{E434Q} and **(F)** ETV6^{E434D}. Along with the appearance of additional signals with random coil chemical shifts for the mutant proteins, the closer similarity of their spectra with that of ETV6$^{\Delta H5}$ than with ETV6^{WT} indicates that helix H5 is predominantly unfolded when Glu434 is replaced with Gln or Asp.



Supplemental Figure S2. (A) The disulfide bond between Cys393 (*) in the DNA recognition helix H3 and Cys437 (*) in the inhibitory helix H5 modestly perturbs the ^{15}N-HSQC spectrum of ETV6$^{2C\text{-}ox}$ relative to ETV6^{WT}. Reduction of the disulfide to generate ETV6$^{2C\text{-}red}$ largely restores wild-type chemical shifts. The histogram of chemical shift perturbations corresponds to the

overlaid spectra shown in Figure 2C and 2D. **(B)** The spectra of DNA-bound ETV6^{WT} and ETV6$^{2C\text{-}red}$ are very similar, indicating that the A393C and V437C mutations do not significantly perturb DNA binding by the ETS domain. In contrast, the spectra of bound ETV6^{WT} and ETV6$^{2C\text{-}ox}$ are dramatically different. This reflects the weak binding of ETV6$^{2C\text{-}ox}$ to DNA via a displaced interface, as shown in Figure 7. The original ^{15}N-HSQC spectra of ETV6^{WT} and ETV6$^{2C\text{-}red}$ bound to DNA duplex containing the ETS recognition sequence $^{5'}$-GGAA-$^{3'}$ are not shown herein.