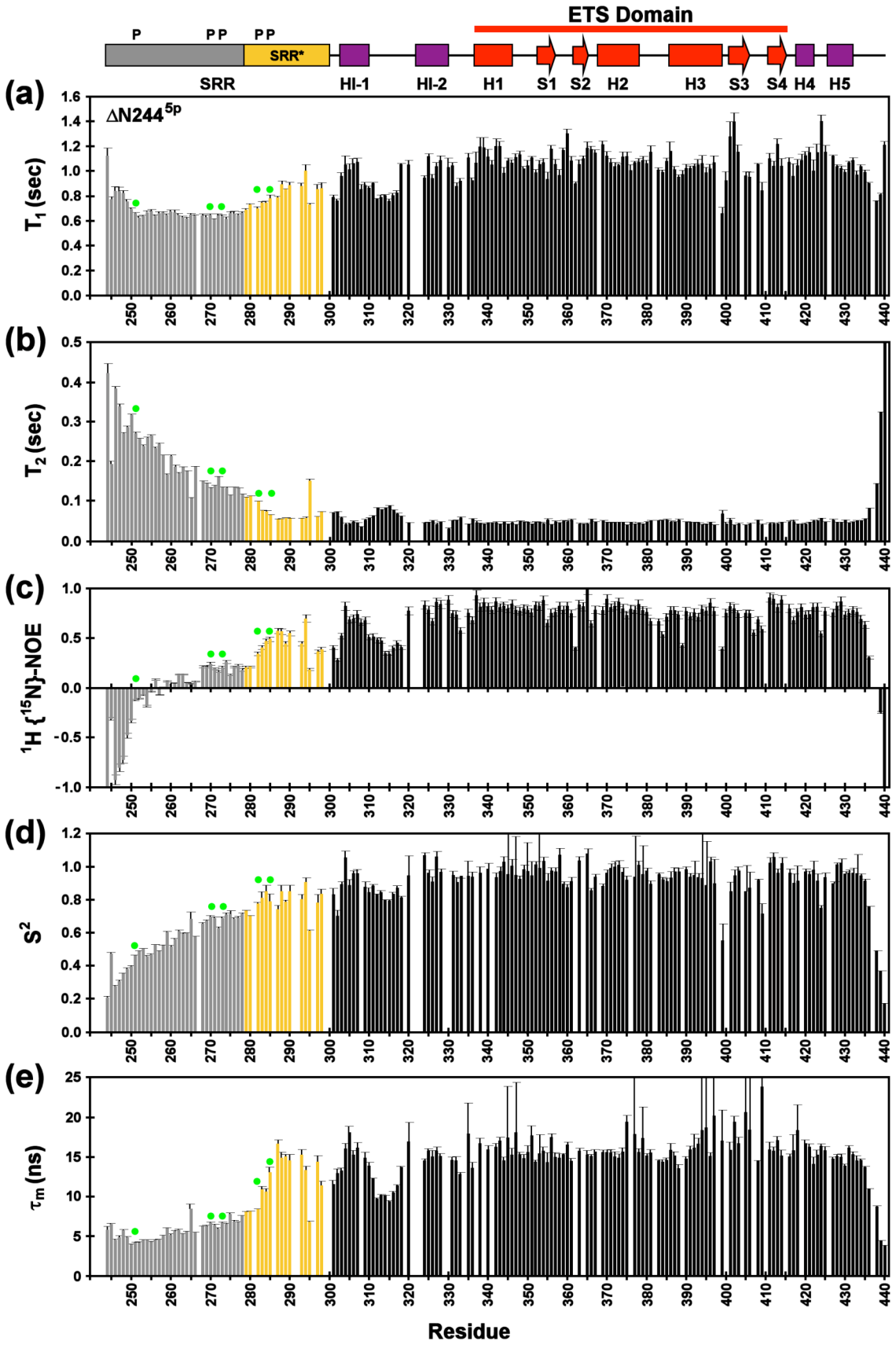
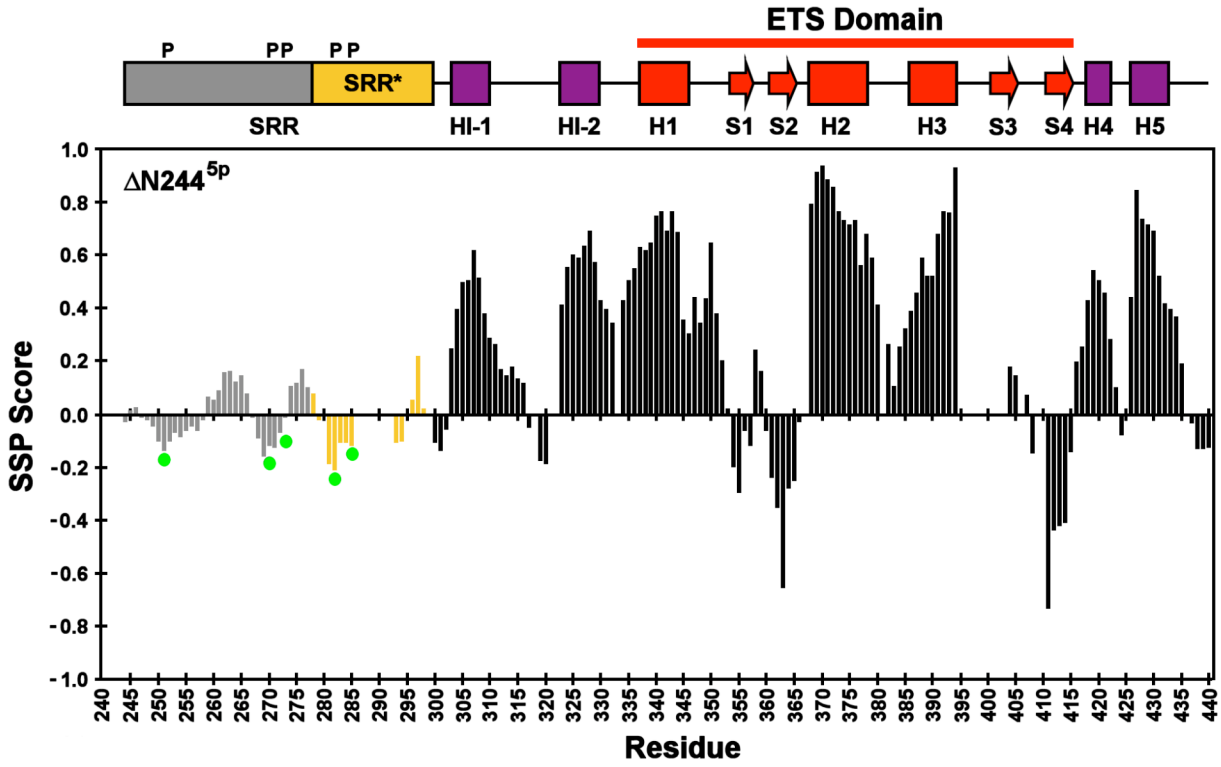
Supplemental Figure S1.



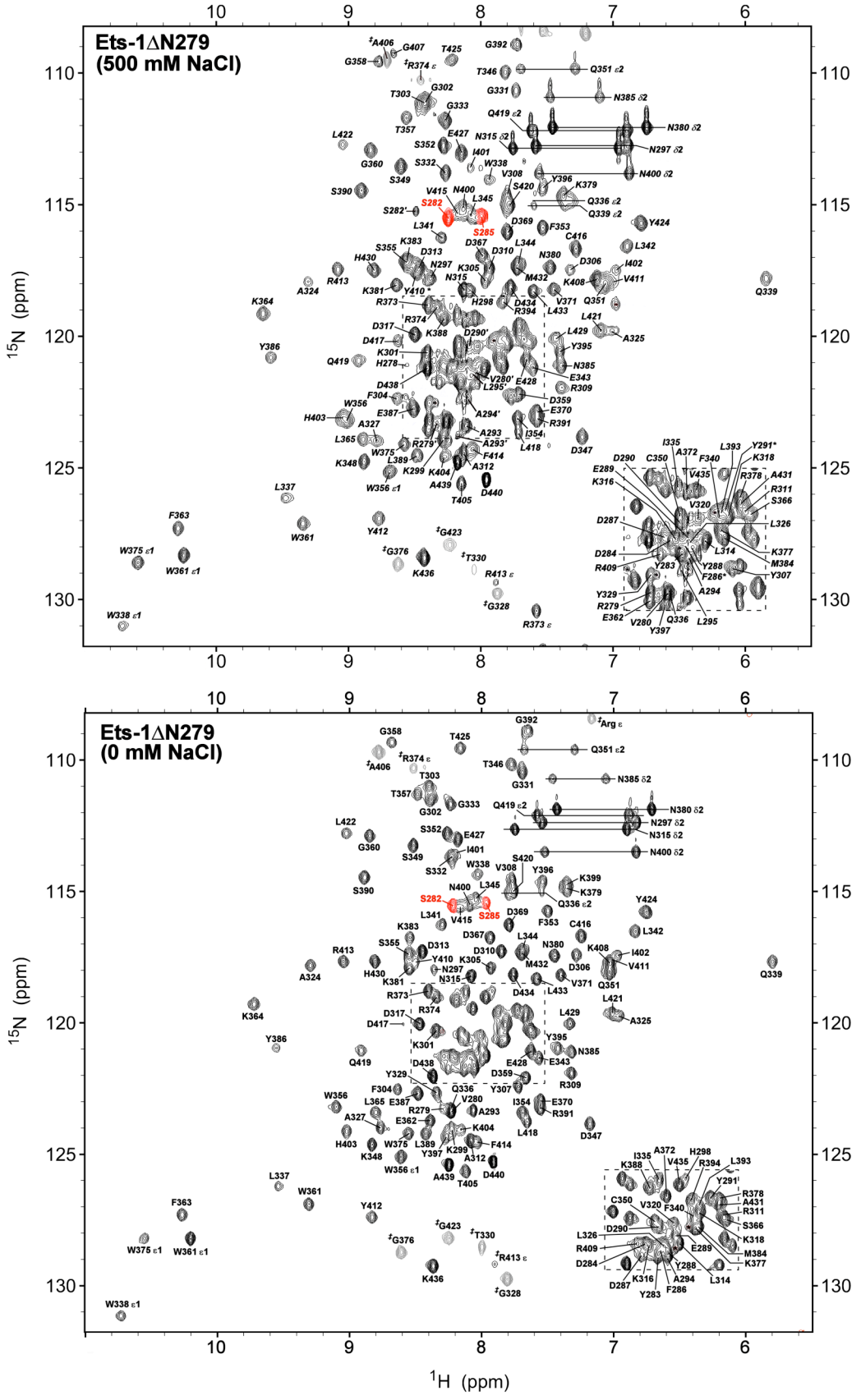
Supplemental Figure S1. The backbone dynamics of $\Delta N244^{5p}$ were characterized by amide ^{15}N (**a**) T_1, (**b**) T_2, and (**c**) $^{1}H\{^{15}N\}$-NOE measurements (500 mM NaCl, pH 6.5, 28 ^{o}C). Also shown are individually fit per-residue Lipari-Szabo model-free (**d**) order parameters S^2 and (**e**) correlation times τ_m. The regulatable unit (black) is well ordered with relatively uniform average heteronuclear NOE (0.78 ± 0.10), S^2 (0.92 ± 0.13), and τ_m (15.2 ± 2.7 nsec) values. In contrast, residues 244 to 278 of the SRR (gray) are very flexible on the nsec-psec timescale as evidenced by lower average heteronuclear NOE (-0.08 ± 0.22), S^2 (0.55 ± 0.11), and effective τ_m (5.7 ± 1.1 nsec) values. Residues 279 to 300 of the SRR (yellow) are also flexible, but show more restricted motion on this fast timescale (average NOE = 0.42 ± 0.15; S^2 = 0.79 ± 0.07; τ_m = 12.1 ± 3.2 nsec). Based upon this clear break in the relaxation properties of the SRR, we have focused on a deletion fragment, ΔN279, containing residues 279-300 (denoted SRR*). Similar relaxation profiles were observed for ΔN279 and $\Delta N279^{2p}$ (Fig. 5a,b), albeit with slightly more mobility for corresponding residues in the SRR* relative to those in $\Delta N244^{5p}$. This is consistent with a correlation of reduced dynamics and increased autoinhibition, as phosphorylation of ΔN244 at 5 serines dampens the flexibility of the SRR. The phosphoserines are indicated by green dots, and missing data points correspond to proline or residues with unassigned, overlapping, or weak NMR signals. A secondary structure schematic is shown above the graphs.

Supplemental Figure S2.



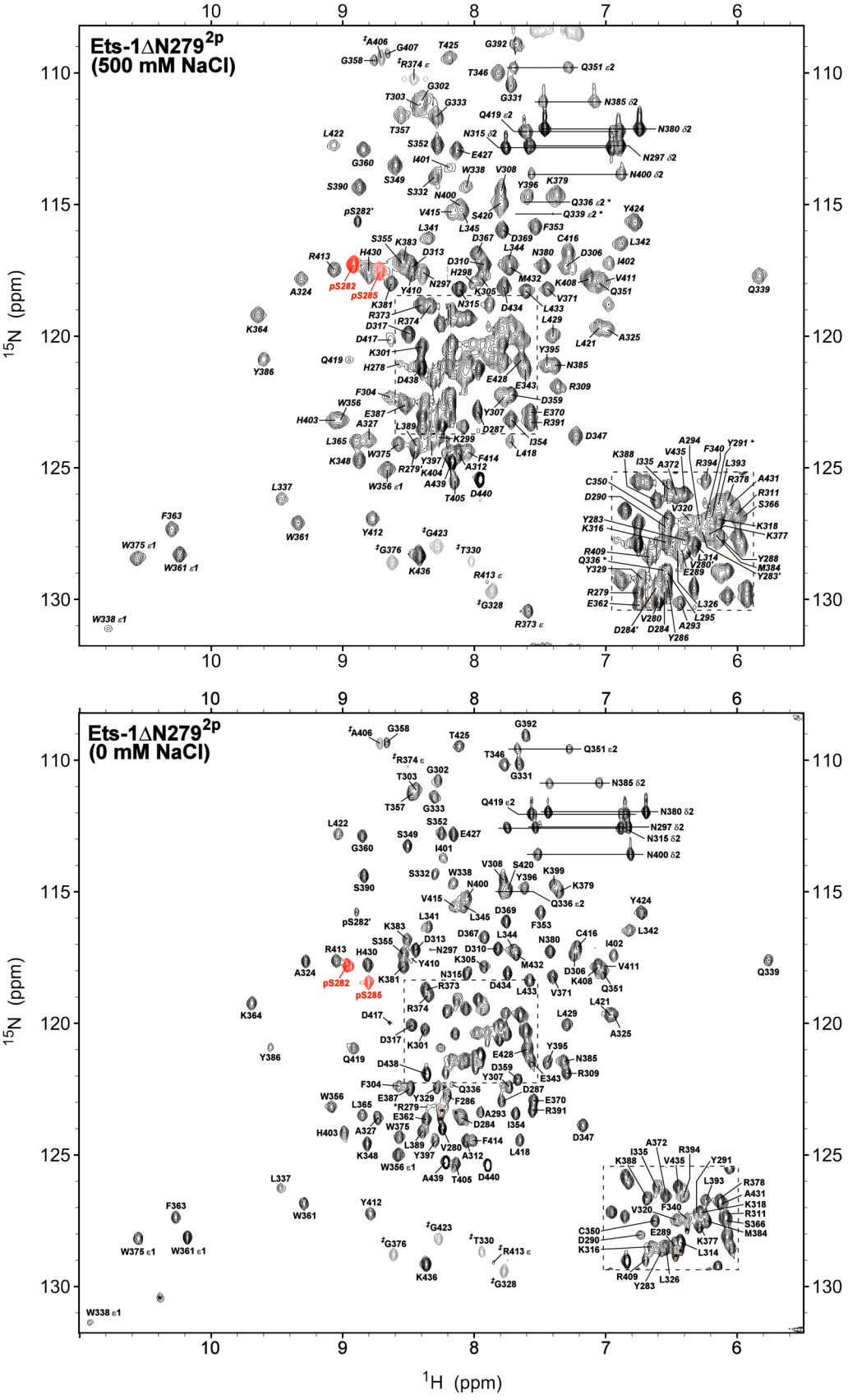
Supplemental Figure S2. The SRR of ΔN244^{5p} lacks any predominant secondary structure. Shown are secondary structural propensity (SSP) scores derived from available main chain ^{1}H, ^{13}C, and ^{15}N chemical shifts (using correction values derived from random coil phosphoserine chemical shifts[25]). Values approaching +1 and -1 are indicative of well defined α-helices and β-strands, respectively[24]. The SRR* is highlighted in yellow and phosphoserines are indicated by green dots. Missing data points correspond to prolines or residues with unassigned NMR signals.

Supplemental Figure S3.



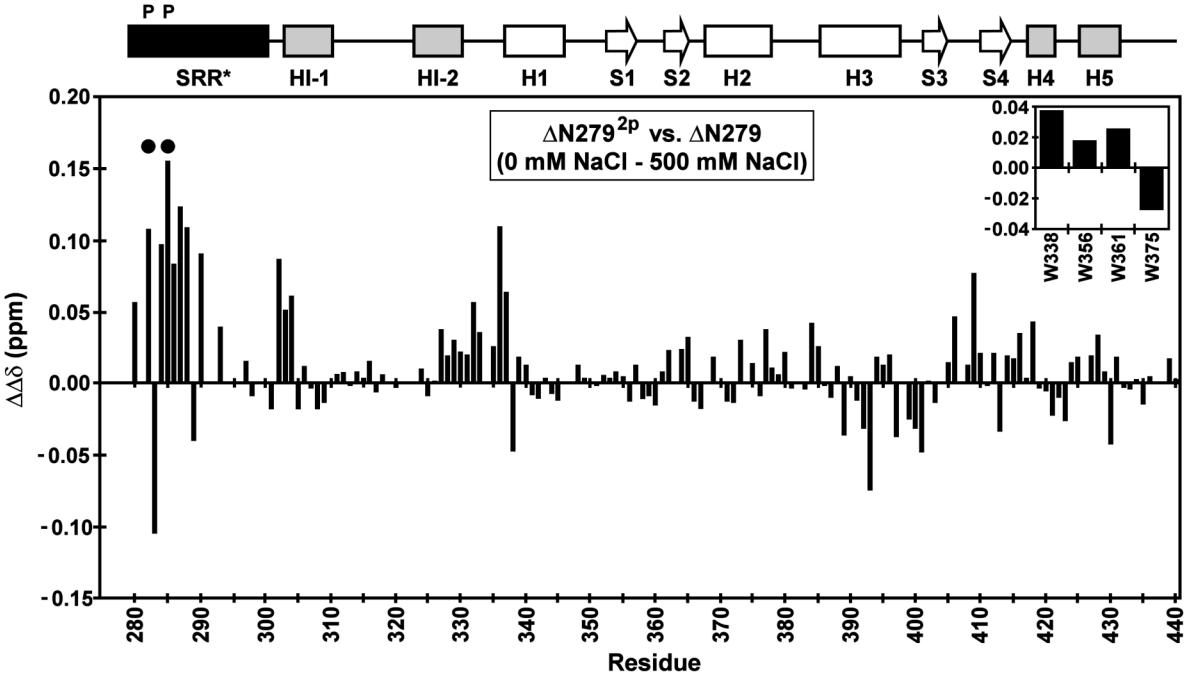
Supplemental Figure S3. The ^{1}H-^{15}N HSQC of ΔN279, acquired at 600 MHz and 28 °C in 20 mM phosphate (pH 6.5), 0.02% NaN_3, 5 mM dithiothreitol, 10% D_20 and either 500 mM NaCl (top panel) or 0 mM NaCl (bottom panel). Unassigned and tentatively assigned peaks are indicated by asterisks, while peaks folded in the ^{15}N-dimension are denoted by double daggers (‡). Peaks of residues in minor conformations are indicated by primes (′). The insets at the lower right of each panel correspond to the crowded region at the center of the spectrum. The signals of S282 and S285 are indicated in red.

Supplemental Figure S4.



Supplemental Figure S4. The ^{1}H-^{15}N HSQC of $\Delta N279^{2p}$, acquired at 600 MHz and 28 °C in 20 mM phosphate (pH 6.5), 0.02% NaN_3, 5 mM dithiothreitol, 10% D_20 and either 500 mM NaCl (top panel) or 0 mM NaCl (bottom panel). Unassigned and tentatively assigned peaks are indicated by asterisks, while peaks folded in the ^{15}N-dimension are denoted by double daggers (‡). Peaks of residues in minor conformations are indicated by primes (′). The insets at the lower right of each panel correspond to the crowded region at the center of the spectrum. The signals of phosphorylated serines pS282 and pS285 are indicated in red.

Supplemental Figure S5.



Supplemental Figure S5. A difference $^{1}H^{N}$ and ^{15}N chemical shift perturbation (ΔΔδ) plot for corresponding amides in ΔN279^{2p} versus ΔN279 in 0 mM versus 500 mM NaCl. The data are the differences between the red and black histogram bars of Figure 6b. Increasing ionic strength partially reduces the phosphorylation-dependent chemical shift perturbations of ΔN279. However, even at 500 mM NaCl, the ^{1}H-^{15}N HSQC spectrum of ΔN279 is altered upon phosphorylation (see Fig. 6a,b). This suggests that electrostatic interactions are not dominant in phosphorylation-dependent stabilization of the regulatable unit, and by inference, in DNA binding autoinhibition. The absence of a bar indicates that the ΔΔδ could not be unambiguously measured for a given residue due to spectral overlap or weak signals in at least one species or condition, and dark circles represent the phosphoacceptor serines.