Supplementary Material

The PNT domain from *Drosophila* Pointed-P2 contains a dynamic N-terminal helix preceded by a disordered phosphoacceptor sequence

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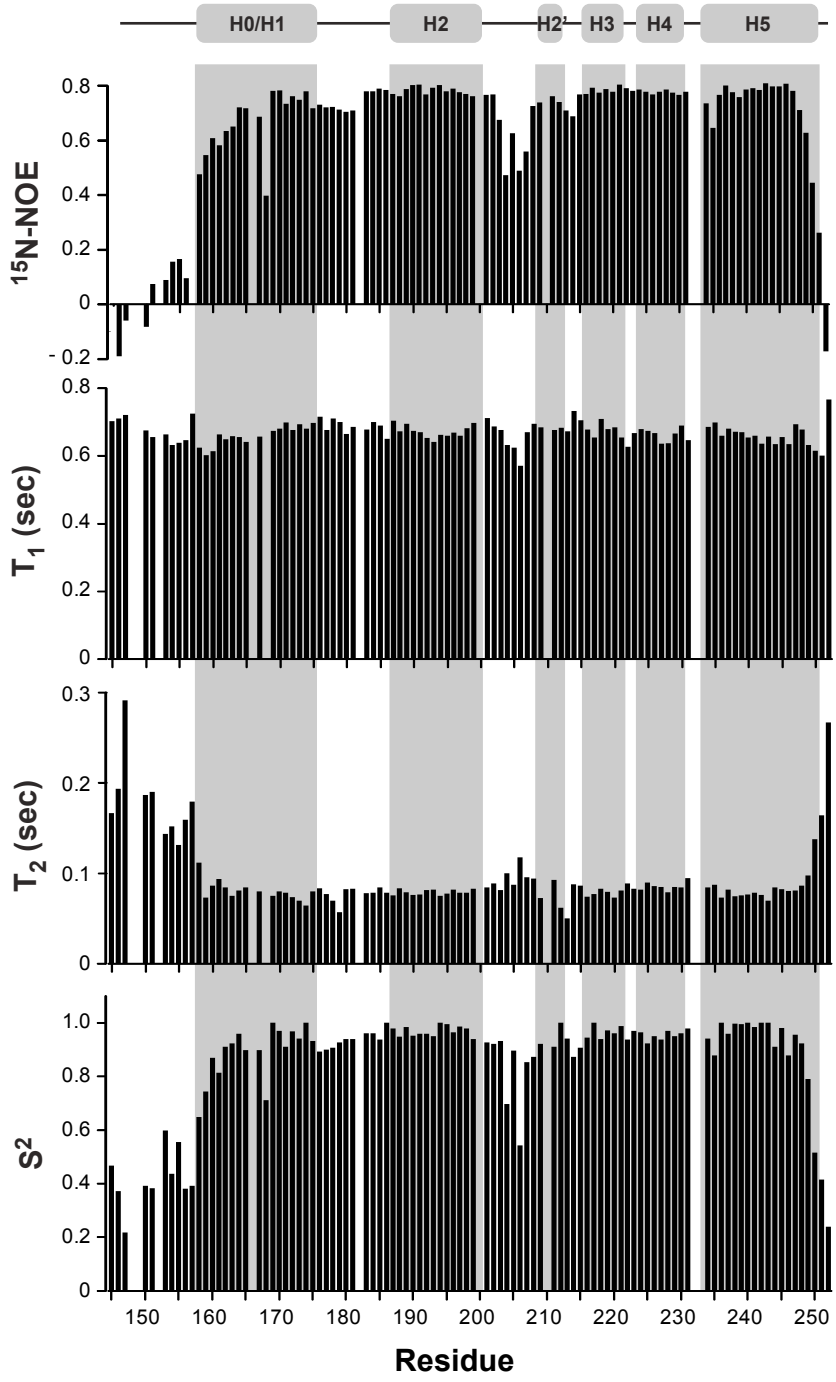
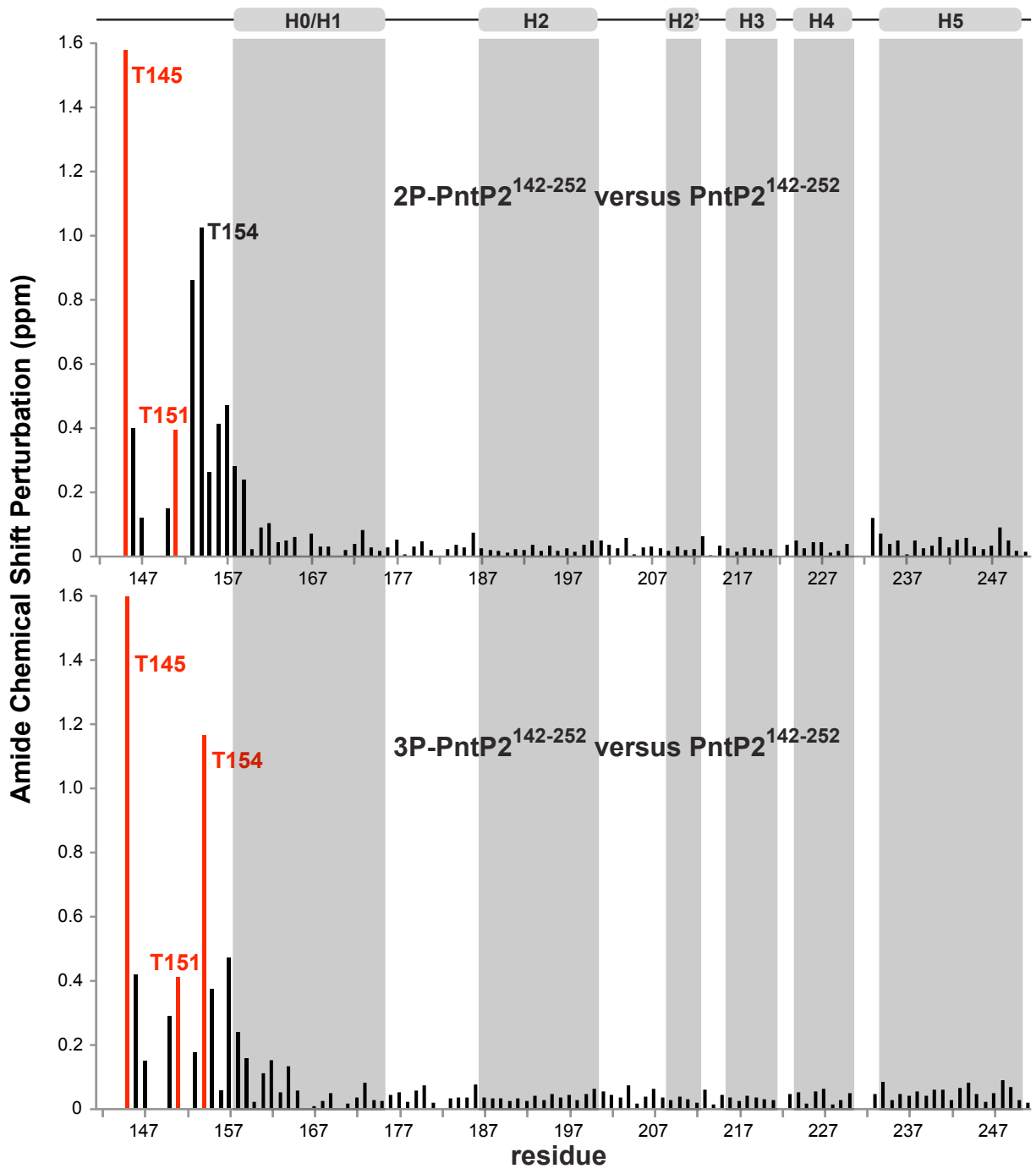


Supplemental Figure S1. Amide ^{15}N T_1, T_2, and steady-state heteronuclear ^{15}N-NOE values for PntP2$^{142-252}$, recorded at 25 ^{o}C with a 600 MHz NMR spectrometer. Model-free analysis of with TENSOR2 yielded an isotropic correlation time for global tumbling of 8.7 ± 0.1 ns and the

illustrated anisotropic generalized S^2 order parameters. The latter were fit with the homology model of PntP2$^{142-252}$ generated using Ets1$^{29-138}$ (2JV3.pdb) as a template. However, isotropic order parameters are similar (not shown). The cartoon and grey rectangles indicate the helices based on the consensus MICS scores for the three PntP2$^{142-252}$ species (Fig. 3B-D), and missing data correspond to prolines or residues with overlapping signals. Note that a reduced ^{15}N-NOE value and increased T_2 lifetime reflects increased mobility of the amide ^{15}N-^{1}H^{N} bond vector on the nsec-psec timescale, and hence a lower order parameter.



Supplemental Figure S2. Amide chemical shift perturbations upon phosphorylation are localized to residues near the phosphoacceptor threonines. Shown are values for (top) 2P-PntP2$^{142-252}$ and (bottom) 3P-PntP2$^{142-252}$ versus unmodified PntP2$^{142-252}$. The cartoon and grey

rectangles indicate the helices based on the consensus MICS scores for the three PntP2$^{142-252}$ species (Fig. 3B-D), and the phosphoacceptors are highlighted in red. Missing data correspond to prolines or residues with overlapping signals. The amide chemical shift perturbations were calculated as $\{(\Delta\delta_{(1HN)})^2 + (\Delta\delta_{(15N)}/5)^2\}^{1/2}$.

Parenthetically, these data and the spectra of Figure 1 show that great caution must be exercised when using ^{15}N-HSQC spectra alone to identify sites of phosphorylation. For example, the perturbations of Thr154 presumably due to phosphorylation of Thr151 in 2P-PntP2$^{142-252}$ are comparable to those due to its own modification in 3P-PntP2$^{142-252}$. Similarly, Thr151 is also perturbed upon phosphorylation of Thr154. These effects might result in part from electrostatic interactions between the phosphate moieties. Also, Gly153 experiences compensating shift perturbations due to phosphorylation of Thr151 followed by Thr154 such that its signal almost overlaps in the ^{15}N-HSQC spectra of PntP2$^{142-252}$ and 3P-PntP2$^{142-252}$.