Supplemental Information:

Cooperative dynamics across distinct structural elements regulate PTP1B activity

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Contents:

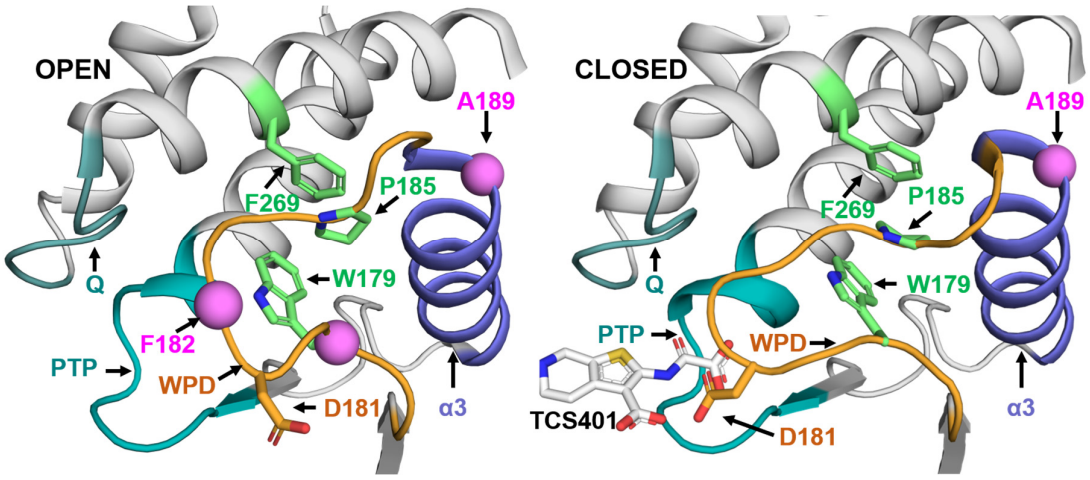


Figure S1: A CH/π switch enables the WPD loop closure in PTP1B. The open (left; PDB 5K9V) and closed (right; PDB 5K9W) state of PTP1B; the closed state is stabilized by adding saturating amounts of the PTP1B-specific inhibitor TCS-401 (sticks). Key structural components of PTP1B are highlighted, including the PTP and Q-loop (teal), the WPD-loop (orange) and helix α3 (lavender). Residues that form the CH/π switch that facilitates WPD loop closure during the catalytic cycle are labeled (F269, P185 and W179). Residues used previously for ^{15}N ct-CPMG relaxation dispersion dynamics analysis are shown as pink spheres.

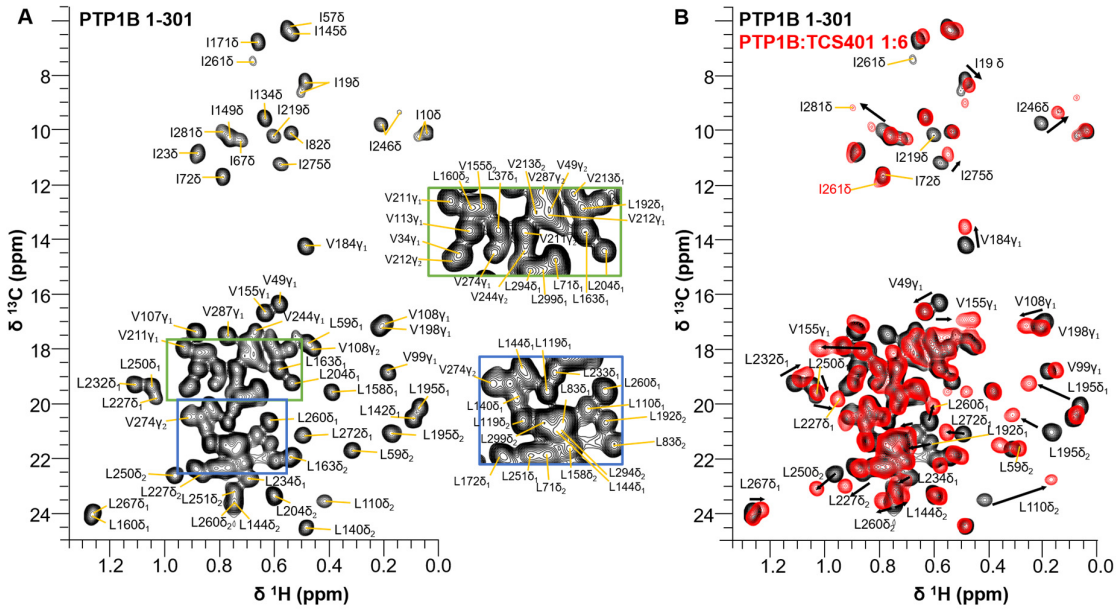


Figure S2: ^{13}C ILV assignment of PTP1B. (A) Fully annotated 2D [^{1}H,^{13}C] HSQC spectrum of PTP1B (residues 1-301). We did not perform a stereospecific assignment of Leu δ1/2 and Val γ1/2 residues; rather, the cross-peak with the lowest ^{13}C ppm value was always labeled 1. **(B)** Overlay of the 2D [^{1}H,^{13}C] HSQC spectrum of PTP1B (black) with that of TCS401-saturated PTP1B (red; 1:6 ratio). Chemical shift perturbations (CSPs) are shown using arrows. All data measured at 18.8 T.

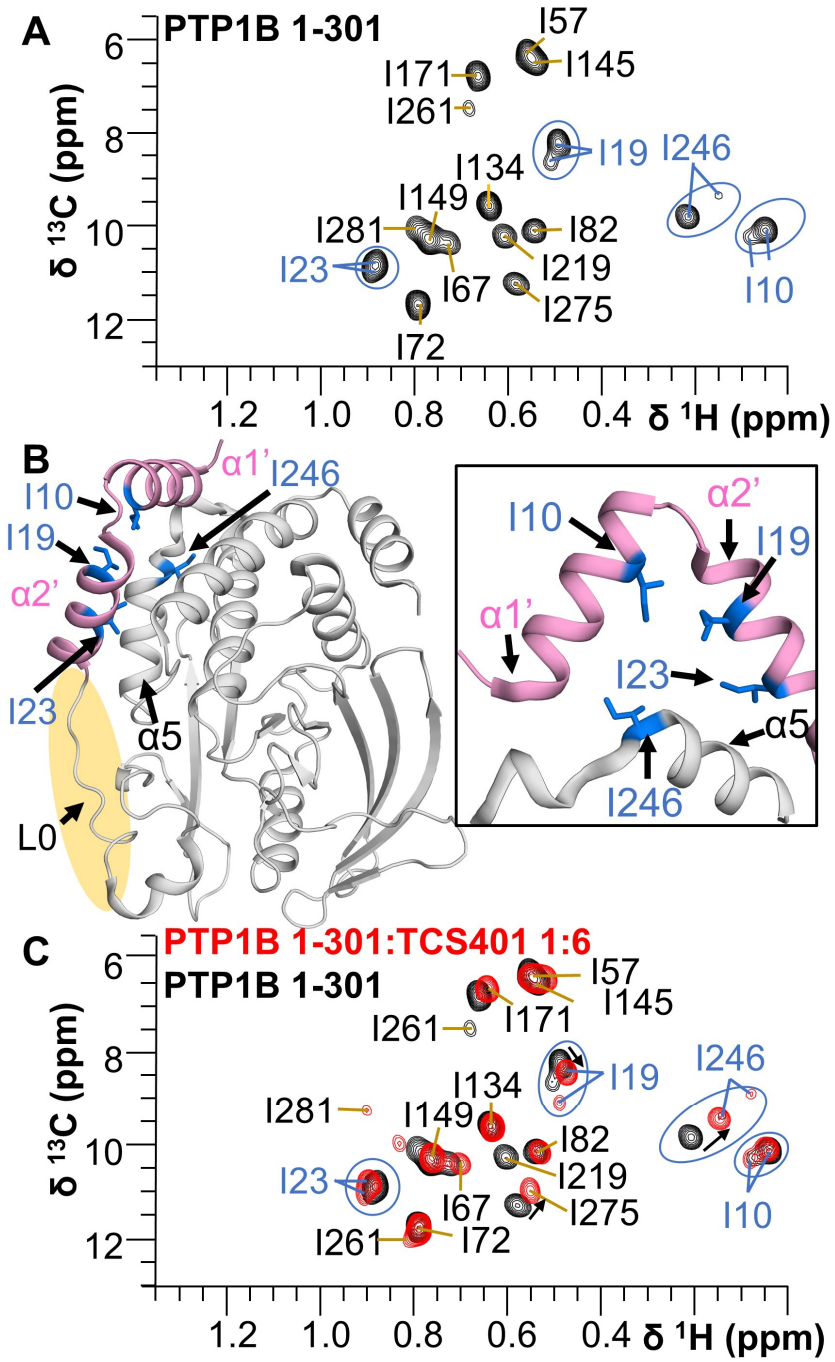


Figure S3: PTP1B helices α1' and α2' show slow exchange between a bound and unbound state. (A) 2D [^{1}H,^{13}C] HSQC of the ^{13}C methyl Ile δ region of PTP1B. Residues with duplicate peaks are highlighted by a blue circle. **(B)** PTP1B structure, with helices α1' and α2' colored pink, the Ile residues shown as sticks and colored blue and the linker L0 highlighted with a yellow ellipse. **(C)** Overlay of the 2D [^{1}H,^{13}C] HSQC of the ^{13}C methyl Ile δ region of PTP1B (black) with the 2D [^{1}H,^{13}C] HSQC of the ^{13}C methyl Ile δ region of PTP1B saturated with TCS401 (1:6 ratio; red). Ile residues that show duplicate peaks are highlighted with blue circles. All data measured at 18.8 T.

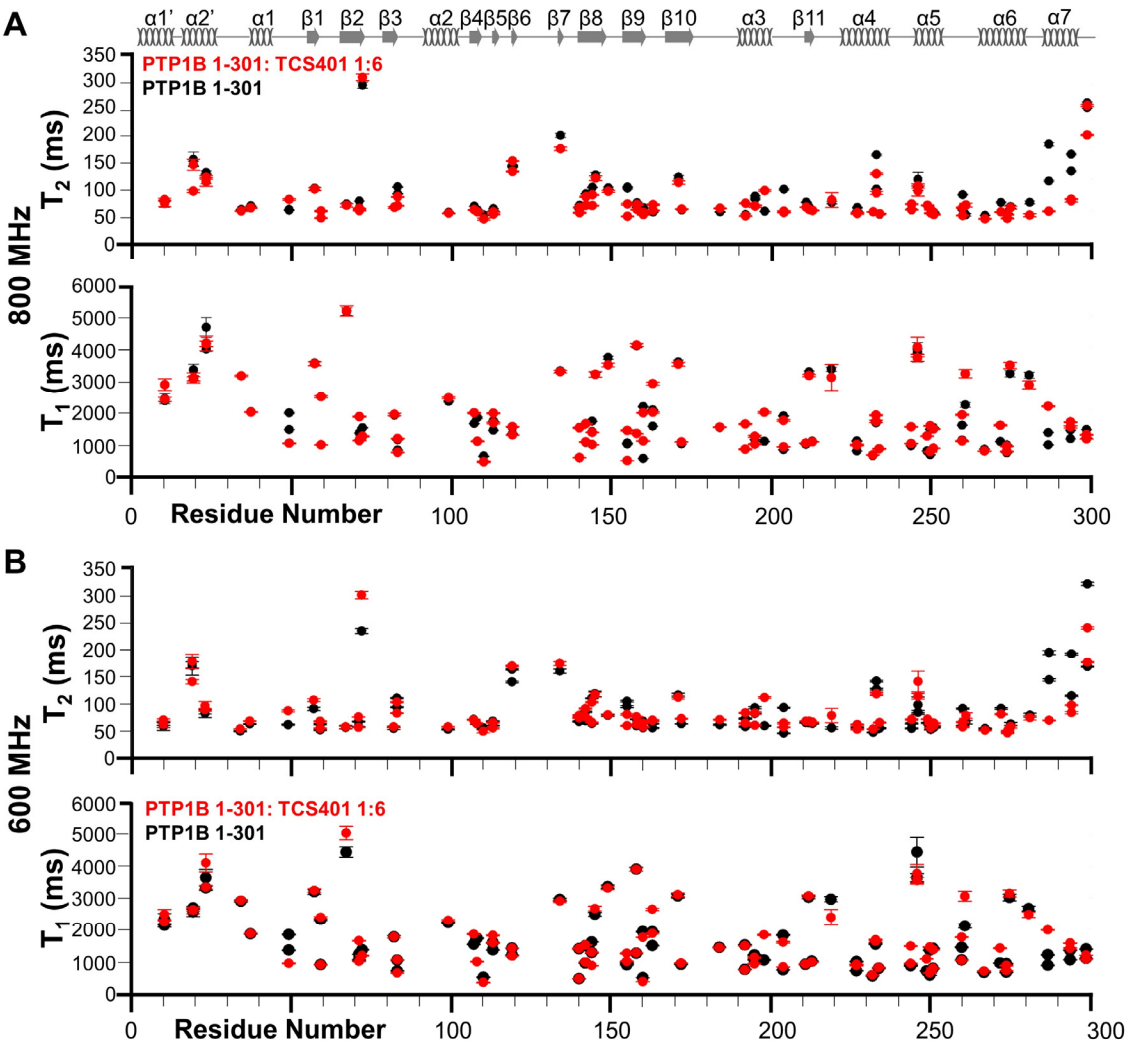
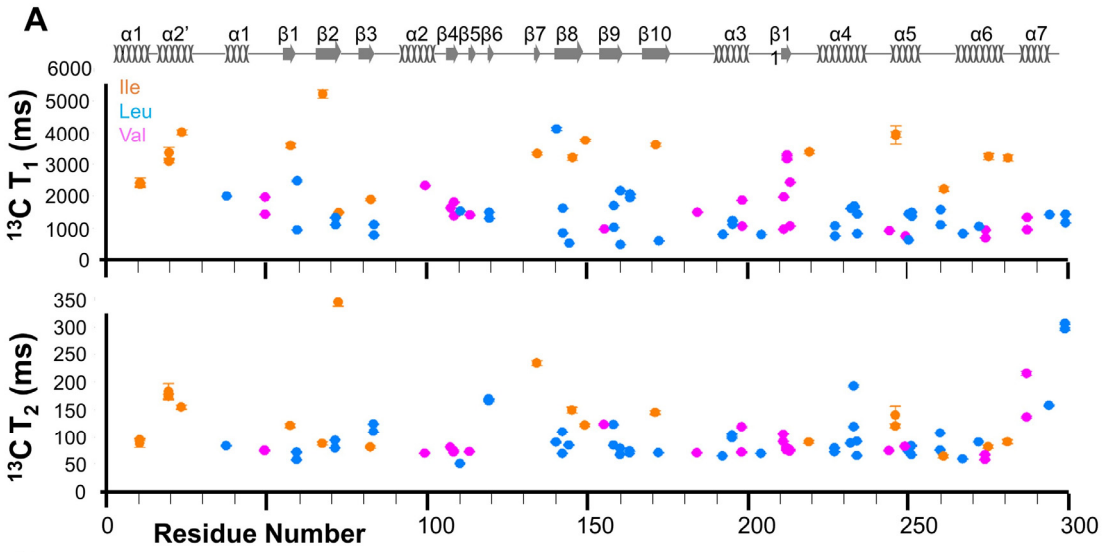


Figure S4. PTP1B ^{13}C ILV fast timescale relaxation data at two magnetic fields. (A) 18.8 T (800 MHz ^{1}H Larmor) T_1 and T_2 ^{13}C ILV side chain relaxation data for PTP1B (black) and PTP1B:TCS401 (1:6 ratio; red) plotted against PTP1B sequence; corresponding PTP1B secondary structural elements shown above. **(B)** 14.1 T (600 MHz ^{1}H Larmor) T_1 and T_2 ^{13}C ILV side chain relaxation data for PTP1B (black) and PTP1B:TCS401 (1:6 ratio; red) plotted against PTP1B sequence; corresponding PTP1B secondary structural elements shown above.



B

	T_1 (ms)	T_{1p} (ms)	T_2 (ms)
All	**1840 ± 110**	**106 ± 6**	**88 ± 6**
Ile	**3100 ± 180**	**145 ± 16**	**100 ± 10**
Leu	**1370 ± 100**	**108 ± 7**	**91 ± 7**
Val	**1600 ± 150**	**82 ± 7**	**76 ± 6**

Figure S5. PTP1B ^{13}C ILV fast timescale relaxation data. (A) T_1 and T_2 ^{13}C ILV side chain relaxation data plotted against PTP1B primary sequence and colored based on amino acid type: Ile (orange), Leu (blue) and Val (pink); corresponding PTP1B secondary structural elements shown above. **(B)** Table reporting the T_1 and T_2 ^{13}C ILV side chain relaxation data by amino acid type. All data measured at 18.8 T.

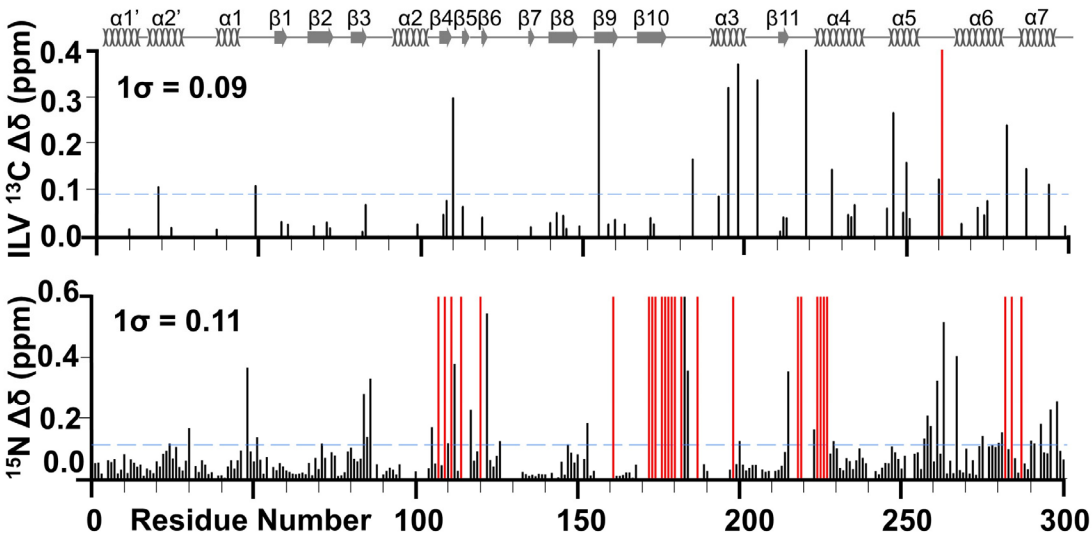


Figure S6. Comparison of ^{15}N backbone and ^{13}C ILV side chain chemical shift perturbation (CSP) data of PTP1B upon TCS401 binding. CSPs plotted against PTP1B residue number; corresponding PTP1B secondary structural elements shown above. Red lines indicate peaks that broadened beyond detection upon saturation with TCS401.