Supporting Information

Distal Mutations Rewire Allosteric Networks to Control Substrate Specificity in PTP1B

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SI Table 1. Sequences of the oligonucleotides used in site-directed mutagenesis.

Mutation	Oligonucleotide sequences
Y153A	FR: 5' GAAGATATTAAAAGCTACGCTACCGTTCGTCAGCTGG 3' RE: 5' CCAGCTGACGAACGGTAGCGTAGCTTTTAATATCTTC 3'
I275A	FR: 5' GCTACCTGGCGGTTGCCGAAGGTGCGAAG 3' RE: 5' CTTCGCACCTTCGGCAACCGCCAGGTAGC 3'
M282A	FR: 5' GTGCGAAGTTTATTGCGGGCGACAGCAGC 3' RE: 5' GCTGCTGTCGCCCGCAATAAACTTCGCAC 3'
E297A	FR: 5' AAGAACTGAGCCATCGGATCTGGAGCCGT 3' RE: 5' ACGGCTCCAGATCCGCATGGCTCAGTTCTT 3'

SI Table 2. Pre-steady-state and steady-state kinetic results for wild-type and allosteric alanine mutants of PTP1B. Enzymes with the highest k_{cat}/K_m value are colored red.

Substrate		k_2 (s^{-1})	k_3 (s^{-1})	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M ($\mu M^{-1}s^{-1}$)	Rank
pNPP	WT	120 ± 6	15 ± 0.1	68 ± 1.5	$(1.0 \pm 0.1) \times 10^3$	$(67 \pm 7) \times 10^{-3}$	2
	Y153A	62 ± 3	7.0 ± 0.04	46 ± 0.3	$(2.4 \pm 0.1) \times 10^3$	$(19 \pm 0.5) \times 10^{-3}$	5
	I275A	124 ± 4	12.1 ± 0.1	73 ± 1.0	$(1.0 \pm 0.1) \times 10^3$	$(75 \pm 5) \times 10^{-3}$	1
	M282A	111 ± 5	12 ± 0.1	64 ± 0.9	$(1.2 \pm 0.1) \times 10^3$	$(54 \pm 3) \times 10^{-3}$	3
	E297A	71 ± 7	8.6 ± 0.11	49 ± 0.4	$(2.0 \pm 0.05) \times 10^3$	$(26 \pm 0.8) \times 10^{-3}$	4
IR	WT	—	—	65 ± 0.7	35 ± 0.8	1.9 ± 0.05	3
	Y153A	—	—	43 ± 0.3	25 ± 0.5	1.7 ± 0.03	4
	I275A	—	—	47 ± 0.3	25 ± 0.4	1.9 ± 0.03	1
	M282A	—	—	44 ± 0.3	24 ± 0.4	1.8 ± 0.03	2
	E297A	—	—	50 ± 0.4	33 ± 0.7	1.5 ± 0.03	5
Mutated IR	WT	—	—	83 ± 1.0	27 ± 0.8	3.1 ± 0.10	4
	Y153A	—	—	73 ± 0.5	22 ± 0.4	3.3 ± 0.07	2
	I275A	—	—	81 ± 0.5	30 ± 0.4	2.7 ± 0.04	5
	M282A	—	—	92 ± 0.7	29 ± 0.6	3.2 ± 0.07	3
	E297A	—	—	62 ± 0.4	16 ± 0.4	3.8 ± 0.09	1
Jak2	WT	—	—	105 ± 1.0	149 ±3	0.7 ± 0.01	2
	Y153A	—	—	71 ± 0.3	127 ± 1	0.6 ± 0.005	4
	I275A	—	—	69 ± 0.4	132 ± 2	0.5 ± 0.01	5
	M282A	—	—	80 ± 0.3	95 ± 0.7	0.8 ± 0.01	1
	E297A	—	—	85 ± 0.7	120 ± 2	0.6 ± 0.01	3
$p62^{dok}$	WT	—	—	88 ± 0.5	18 ± 0.4	4.9 ± 0.01	3
	Y153A	—	—	72 ± 0.5	13 ± 0.4	4.6 ± 0.12	4
	I275A	—	—	56 ± 0.4	16 ± 0.4	3.6 ± 0.10	5
	M282A	—	—	69 ± 0.4	11 ± 0.3	6.4 ± 0.2	2
	E297A	—	—	66 ± 0.3	8.3 ± 0.3	7.9 ± 0.3	1

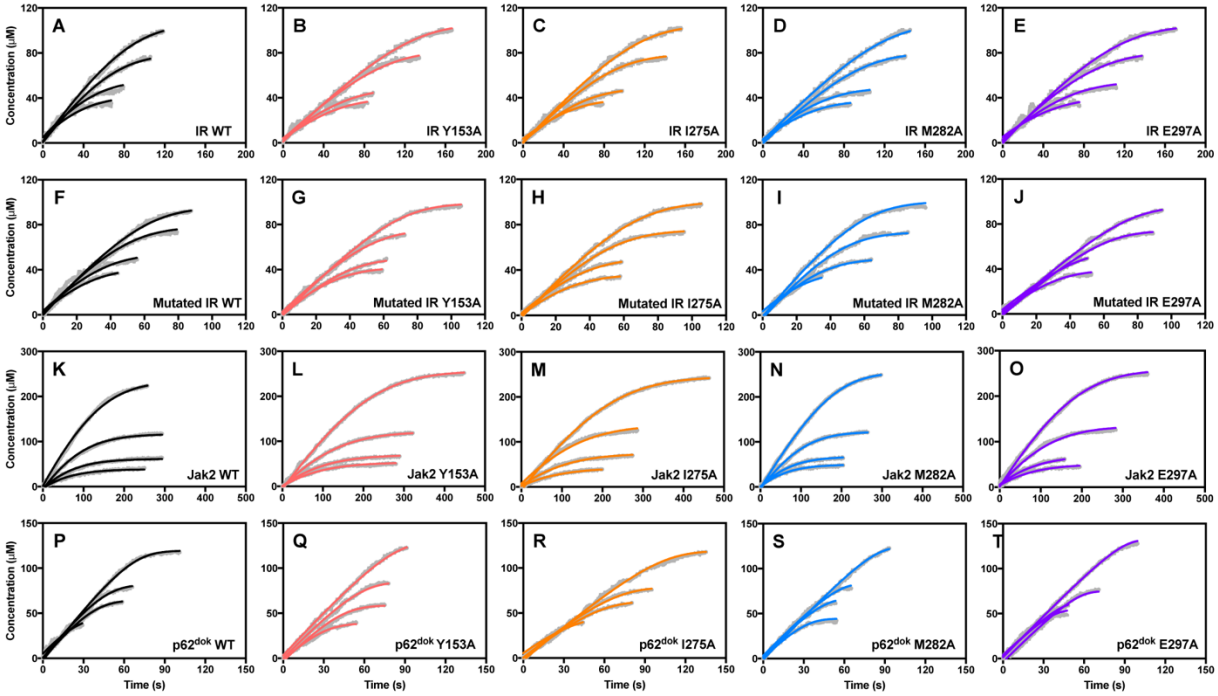
SI Table 3. ^{13}C methyl multiple quantum relaxation rates for τ_{cpmg} value = 0.41667 ms for apo PTP1B.

Residue	WT		I275A		E297A	
	R_2 (s^{-1})	Error (s^{-1})	R_2 (s^{-1})	Error (s^{-1})	R_2 (s^{-1})	Error (s^{-1})
I10CD1-HD1	25.53	0.20			25.93	0.13
I19CD1-HD1	24.89	0.09			21.46	0.05
I23CD1-HD1	26.54	0.17	29.16	0.30	26.37	0.09
V34CG1-HG1	28.93	0.72	28.37	0.70		
L37CD1-HD1	17.87	0.15	15.47	0.23	15.42	0.09
V49CG1-HG1	31.44	0.55	31.88	0.70	27.28	0.32
V49CG2-HG2	41.69	1.06				
I57CG1-HG1	27.47	0.17	28.65	0.21	26.46	0.11
L59CD1-HD1	32.51	0.49	30.05	0.70	32.62	0.27
L59CD1-HD2	31.97	0.65	32.86	0.59	32.69	0.37
I67CD1-HD1	31.59	0.50	30.86	0.58	28.83	0.28
L71CD1-HD1	26.56	0.13	21.40	0.22	24.80	0.09
L71CD2-HD2	28.02	0.20	23.22	0.23	23.83	0.10
I72CD1-HD1	31.01	0.11	29.39	0.13	27.64	0.06
I82CD1-HD1	29.24	0.22	26.98	0.28	28.24	0.13
L83CD1-HD1	35.32	0.27	29.87	0.29	28.80	0.15
V99CG1-HG1	29.83	0.51	28.31	0.66	28.30	0.29
V107CG1-HG1	27.80	0.23	27.74	0.28	26.32	0.14
V108CG1-HG1	35.70	0.60	34.16	0.78	32.06	0.35
V108CG2-HG2	27.22	0.57	26.28	0.64	27.26	0.31
L110CD1-HD1	63.31	1.51	53.87	1.13	34.96	0.30
L110CD2-HD2	75.47	3.63	67.83	2.42	39.52	0.38
V113CG1-HG1	34.86	0.29	33.51	0.40	31.77	0.17
L119CD1-HD1	22.01	0.13	17.79	0.18	16.60	0.06
L119CD2-HD2	18.89	0.09	15.91	0.14	16.86	0.05
I134CD1-HD1	17.43	0.05	16.12	0.07	15.95	0.03
L140CD1-HD1	34.84	0.42	32.26	0.44	32.27	0.25
L140CD2-HD2	35.19	0.56	44.77	0.70	39.61	0.35
L142CD1-HD1	25.19	0.26	24.81	0.32	24.96	0.15
L144CD1-HD1	24.67	0.26	25.36	0.33	23.13	0.15
L144CD2-HD2	36.57	0.34	30.95	0.33	29.74	0.16
I145CD1-HD1	15.89	0.07	17.16	0.10	13.21	0.04
I149CD1-HD1	15.99	0.07	16.80	0.10	14.07	0.05
V155CG1-HG1	38.94	0.56	39.96	0.67	22.59	0.13
V155CG2-HG2	34.06	0.66	41.10	0.71	31.00	0.26
L158CD1-HD1	25.83	0.41	23.23	0.50	25.54	0.25
L158CD2-HD2	33.90	0.32	29.26	0.34	33.21	0.19
L160CD1-HD1	48.96	0.95	47.95	0.95	37.92	0.40

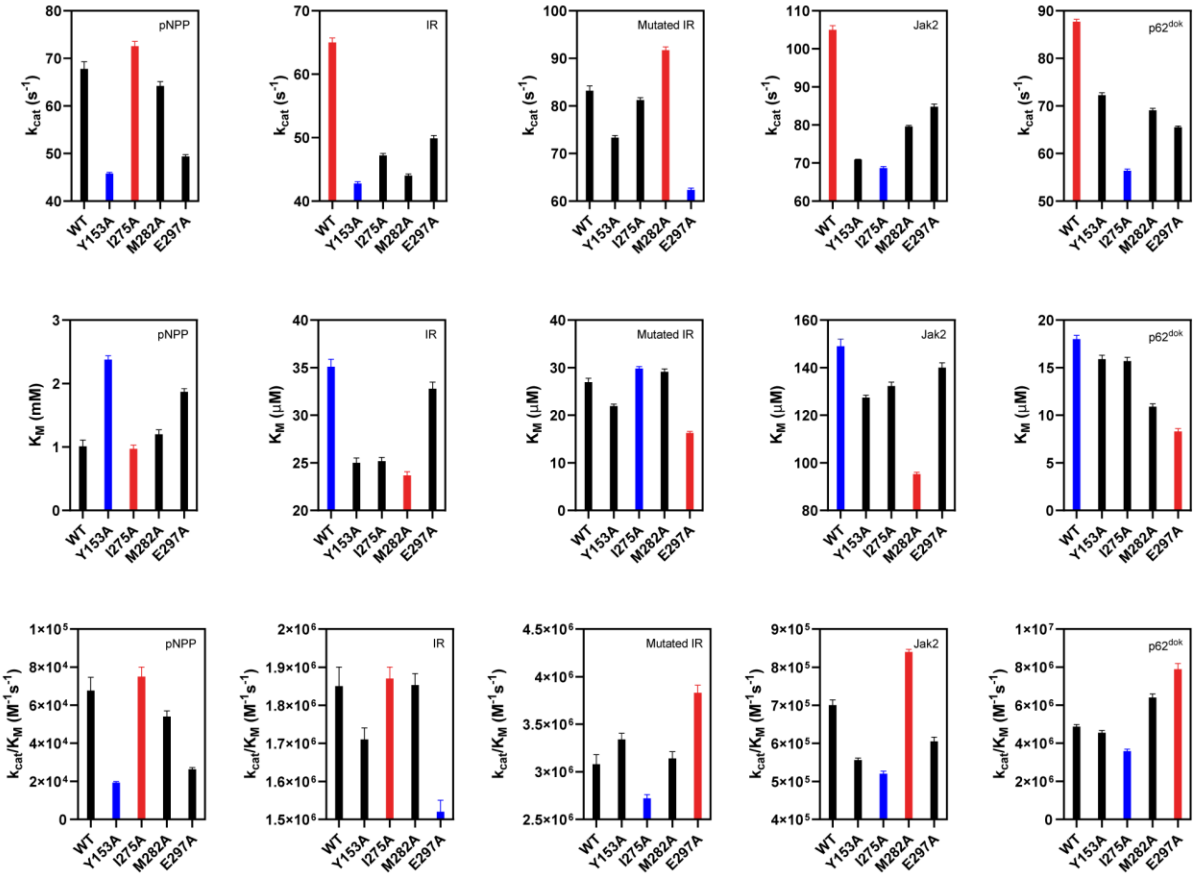
L160CD2-HD2	41.80	0.65	41.10	0.71	36.07	0.44
L163CD1-HD1	24.72	0.16	22.35	0.21	14.93	0.11
L163CD2-HD2	22.65	0.19	17.95	0.23	18.64	0.11
I171CD1-HD1	24.05	0.17	26.06	0.23	23.46	0.10
L172CD1-HD1	34.23	0.44	32.46	0.49	34.02	0.27
V184CG1-HG1	32.49	0.46	34.10	0.62	28.96	0.23
L192CD1-HD1	31.44	0.37	25.01	0.34	22.98	0.16
L195CD1-HD1	49.06	0.73	42.28	0.72	28.23	0.17
L195CD2-HD2	49.28	0.93	52.39	0.98	29.04	0.17
V198CG1-HG1	35.05	0.51	35.08	0.59	32.32	0.32
L204CD1-HD1	30.27	0.44	28.58	0.56	25.54	0.25
L204CD2-HD2	34.16	0.42	28.58	0.47	30.21	0.25
V211CG1-HG1	32.14	0.50	32.85	0.70	31.86	0.29
V211CG2-HG2	37.08	0.38	33.97	0.37	29.80	0.21
V212CG1-HG1	34.86	0.55	33.15	0.82		
V212CG2-HG2	31.88	0.43	34.96	0.47		
V213CG1-HG1	41.09	0.67	36.93	0.68	34.74	0.34
V213CG2-HG2	37.08	0.56	33.65	0.74	28.70	0.28
I219CD1-HD1	29.61	0.27	26.36	0.31	28.01	0.15
L227CD1-HD1	34.52	0.53	34.52	0.60	32.55	0.27
L227CD2-HD2	39.86	0.60	39.72	0.64	35.71	0.30
L232CD1-HD1	31.65	0.49	33.12	0.63	33.87	0.28
L233CD1-HD1	26.84	0.29	23.01	0.37	22.69	0.15
L234CD1-HD1	33.86	0.39	35.38	0.47	28.63	0.22
V244CG1-HG1	32.41	0.36	28.43	0.39	30.22	0.23
V244CG2-HG2	40.41	0.53			34.85	0.31
I246CD1-HD1	30.51	0.23	25.72	0.25	22.39	0.09
L250CD1-HD1	31.92	0.45	35.61	0.60	32.33	0.27
L250CD2-HD2	38.01	0.54	39.05	0.60	34.95	0.28
L251CD1-HD1	28.73	0.27	26.52	0.33	31.68	0.18
L251CD2-HD2	41.39	0.59	32.97	0.58	36.09	0.35
L260CD1-HD1	33.09	0.29	32.94	0.83	23.39	0.10
L260CD2-HD2	23.53	0.35			29.74	0.16
I261CD1-HD1	64.86	0.62	55.84	0.45	35.16	0.14
L267CD1-HD1	44.53	0.71	45.15	0.77	42.67	0.39
L272CD1-HD1	54.05	0.96			22.79	0.12
V274CG1-HG1	29.91	0.39	29.83	0.46	31.25	0.24
V274CG2-HG2	38.35	0.52	40.51	0.63	37.65	0.34
I275CD1-HD1	30.26	0.22			26.67	0.13
I281CD1-HD1	53.86	0.41	52.17	0.39	22.46	0.06
V287CG1-HG2	52.26	0.65	43.39	0.48	25.92	0.09
V287CG2-HG2	65.54	1.25			34.88	0.53

L294CD1-HD1	12.90	0.04	14.01	0.05	15.10	0.02
L294CD2-HD2	24.90	0.08	23.23	0.19	15.58	0.03
L299CD1-HD1	12.90	0.04	25.19	0.11	10.72	0.03
L299CD2-HD2	13.67	0.04	15.82	0.06	10.14	0.02

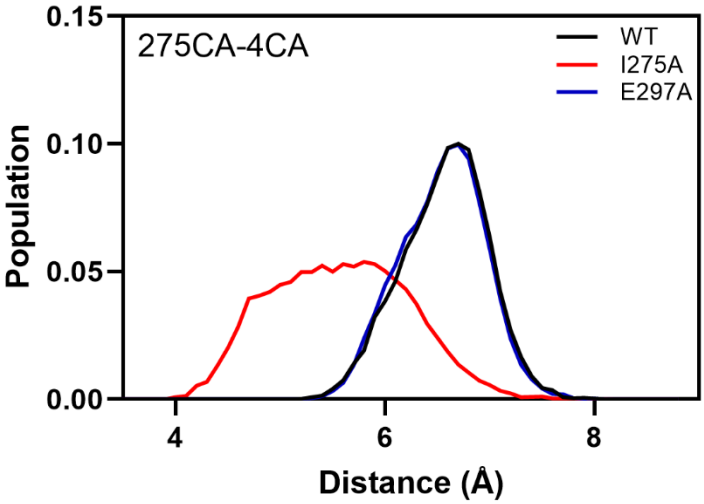
Empty cells indicate a missing unassigned residue in that mutant PTP1B.



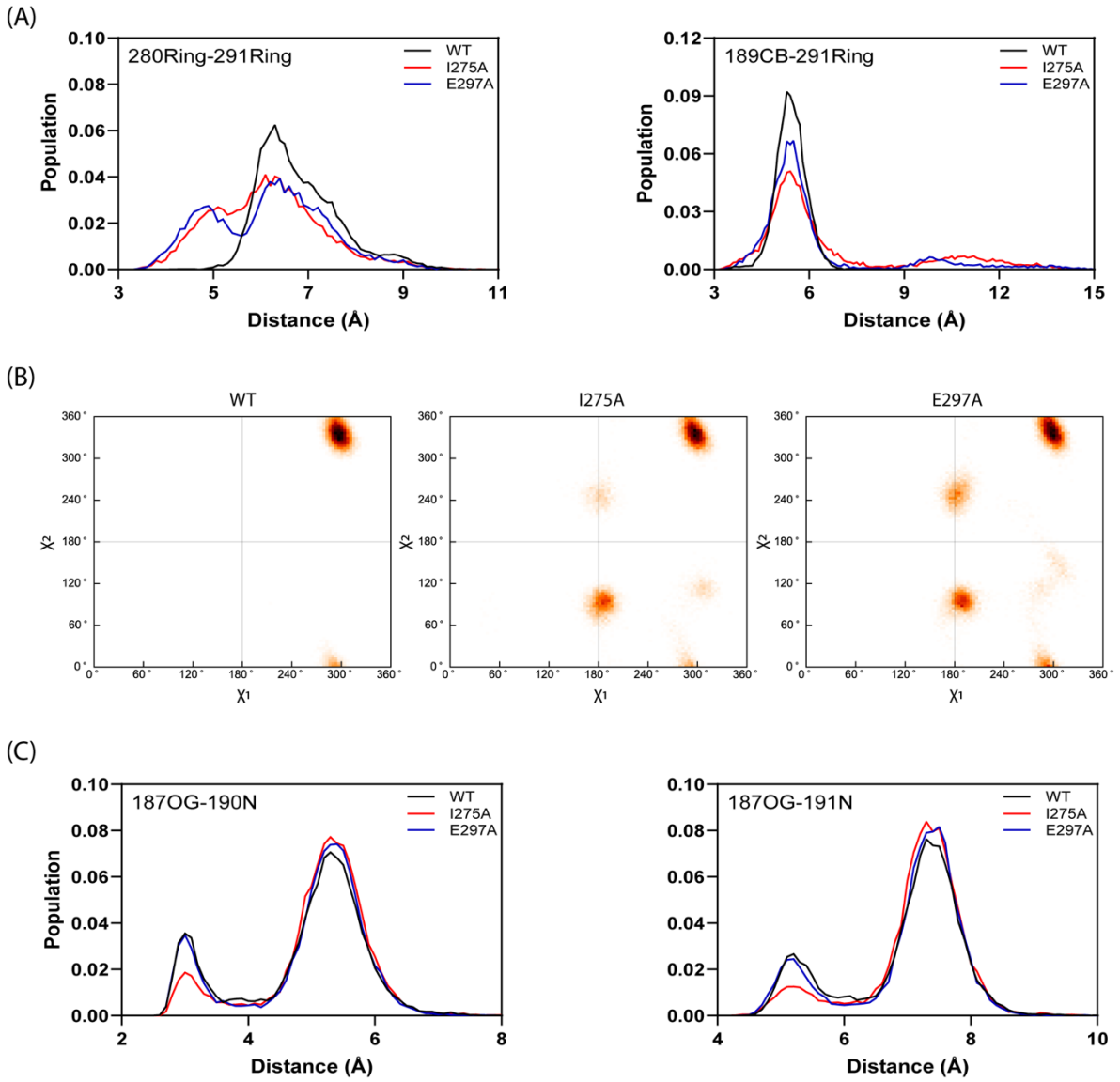
SI Figure 1. Progress curves representing the formation of dephosphorylated peptides as a function of time with WT PTP1B. Solid lines are the non-linear least squares fit to the gray data points with (A – E) IR, (F – J) Mutated IR, (K – O) Jak2, and (P – T) $p62^{dok}$ peptides. The experiments were performed at 30 ^{o}C with 25 nM of enzyme at pH 6.0 and the absorbance was recorded every 0.2 second at 282 nm. The curves from WT, Y153A, I275A, M282A, and E297A are colored in black, pink, orange, blue, and purple, respectively.



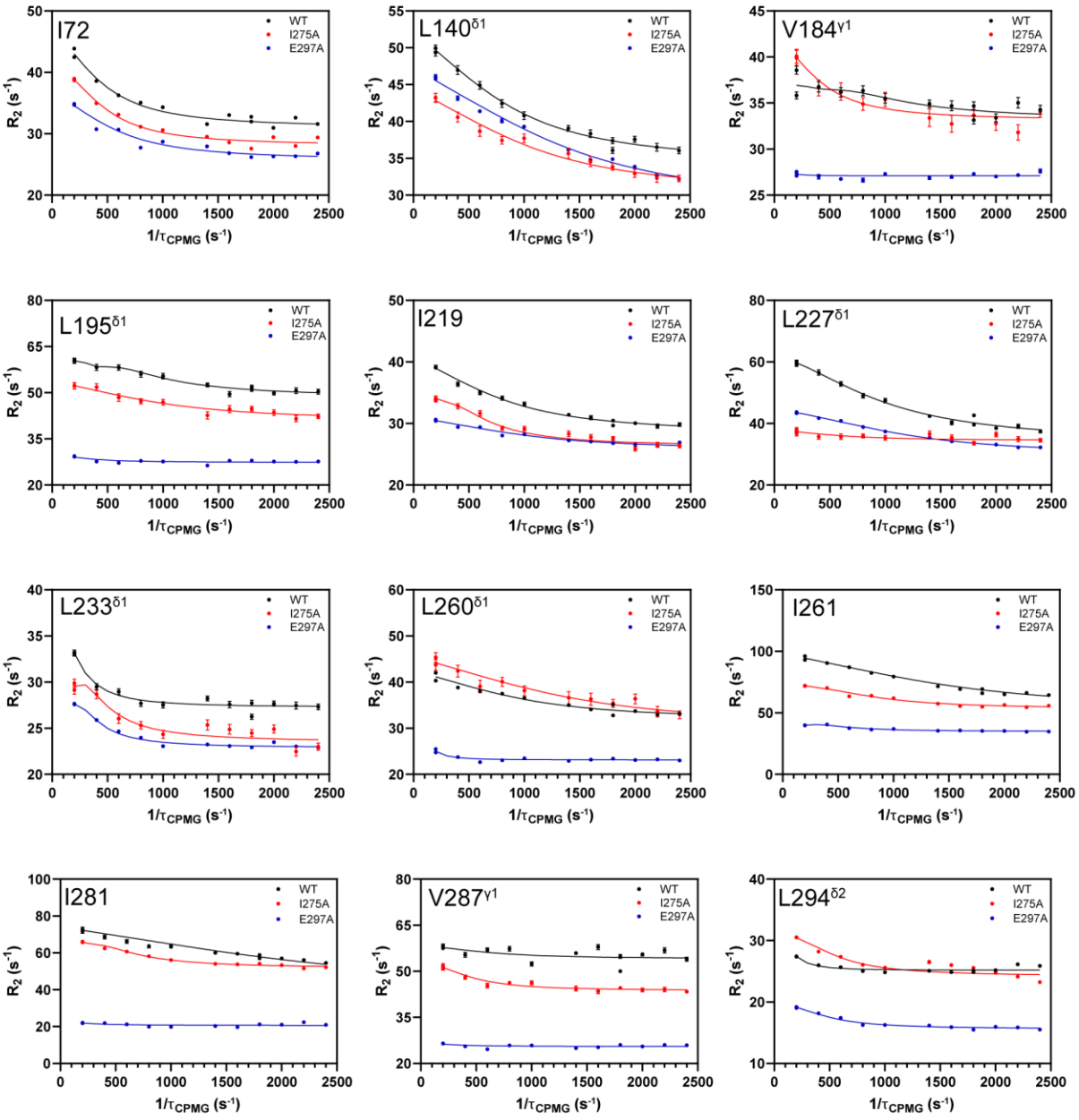
SI Figure 2. Bar graph representation of the catalytic efficiency of k_{cat}/K_m with different PTP1B peptide and pNPP substrates. The substrate is indicated at the top of each panel and highest (lowest) k_{cat}/K_m values are indicated in red (blue). The mutant PTP1B enzyme is indicated at the bottom of each panel.



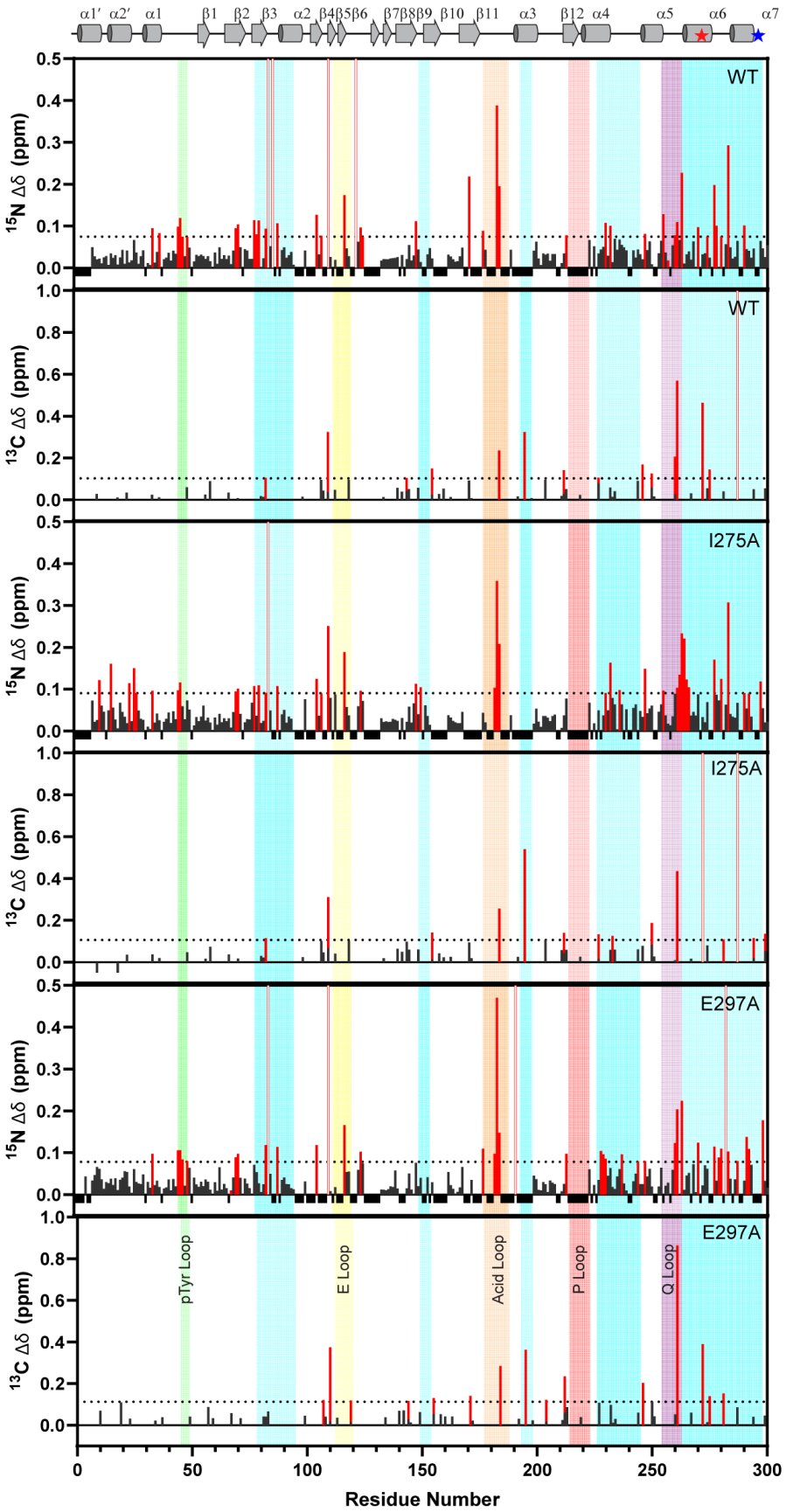
SI Figure 3. Distance profiles from MD simulations between Cα of Residue 275 and Cα of E4.



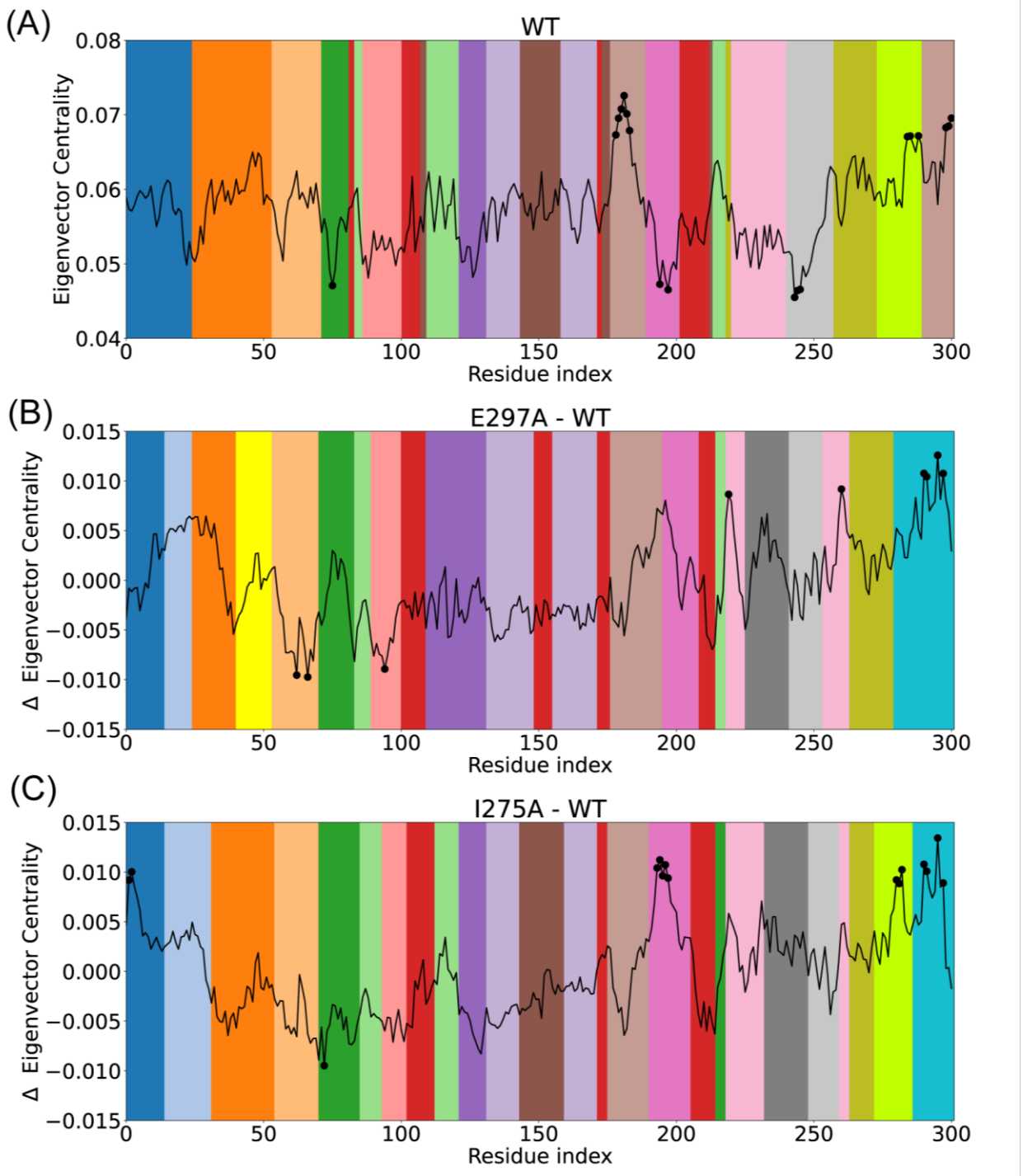
SI Figure 4. Perturbations to interactions between residues near the C-terminus of the acid loop as observed in MD simulations. (A) Distance profiles between the center of mass of the aromatic ring of W291 and the center of mass of the aromatic ring of F280 and distance profiles between the center of mass of the aromatic ring of W291 and the C^{β} of A189. (B) χ_1 and χ_2 distributions of the dihedral angles in W291. (C) Distance profiles between the O^{γ} atom of S187 and the N atoms of S190, and O^{γ} of S187 and N of F191.



SI Figure 5. ^{13}C methyl ILV Carr-Purcell-Meiboom-Gill relaxation dispersion of apo PTP1B collected at 800 MHz with a total relaxation time of 20 ms.


α1′ α2′ α1 β1 β2 β3 α2 β4β5β6 β7β8β9 β10 β11 α3 β12 α4 α5 α6 α7
WT
WT
I275A
I275A
E297A
E297A
pTyr Loop
E Loop
Acid Loop
P Loop
Q Loop
15N Δδ (ppm)
13C Δδ (ppm)
Residue Number

SI Figure 6. ^{15}N and ^{13}C(ILV) chemical shift perturbation plots of VO_4^{3-}-bound WT, I275A and E297A PTP1B with the secondary structure displayed on top. The red bars represent amino acid residues with CSP values above 1.5 times the standard deviation from the 10% trimmed mean, the gray ones are for residues with values below the threshold, and the black ones for residues that are missing or have ambiguous assignments. The background color scheme is the same as in **Figure 4**.



SI Figure 7. Communities and average eigenvector centralities for WT, E297A, and I275A. Communities are colored as in main text **Figure 9**. Eigenvector centralities were computed from the generalized correlation coefficient matrix and were averaged over eight replicas. (A) Community partitioning for WT PTP1B plotted with eigenvector centrality values. Residues with eigenvector centralities > 2 standard deviations from the mean are shown as black dots. (B, C) Community partitioning for (B) E297A and (C) I275A plotted with the change in eigenvector

centrality from WT. Residues with changes in eigenvector centrality > 2 standard deviations from the mean are shown as black dots.