Supporting Information for

Crystal structure of TCPTP unravels an allosteric regulatory role of helix α7 in phosphatase activity

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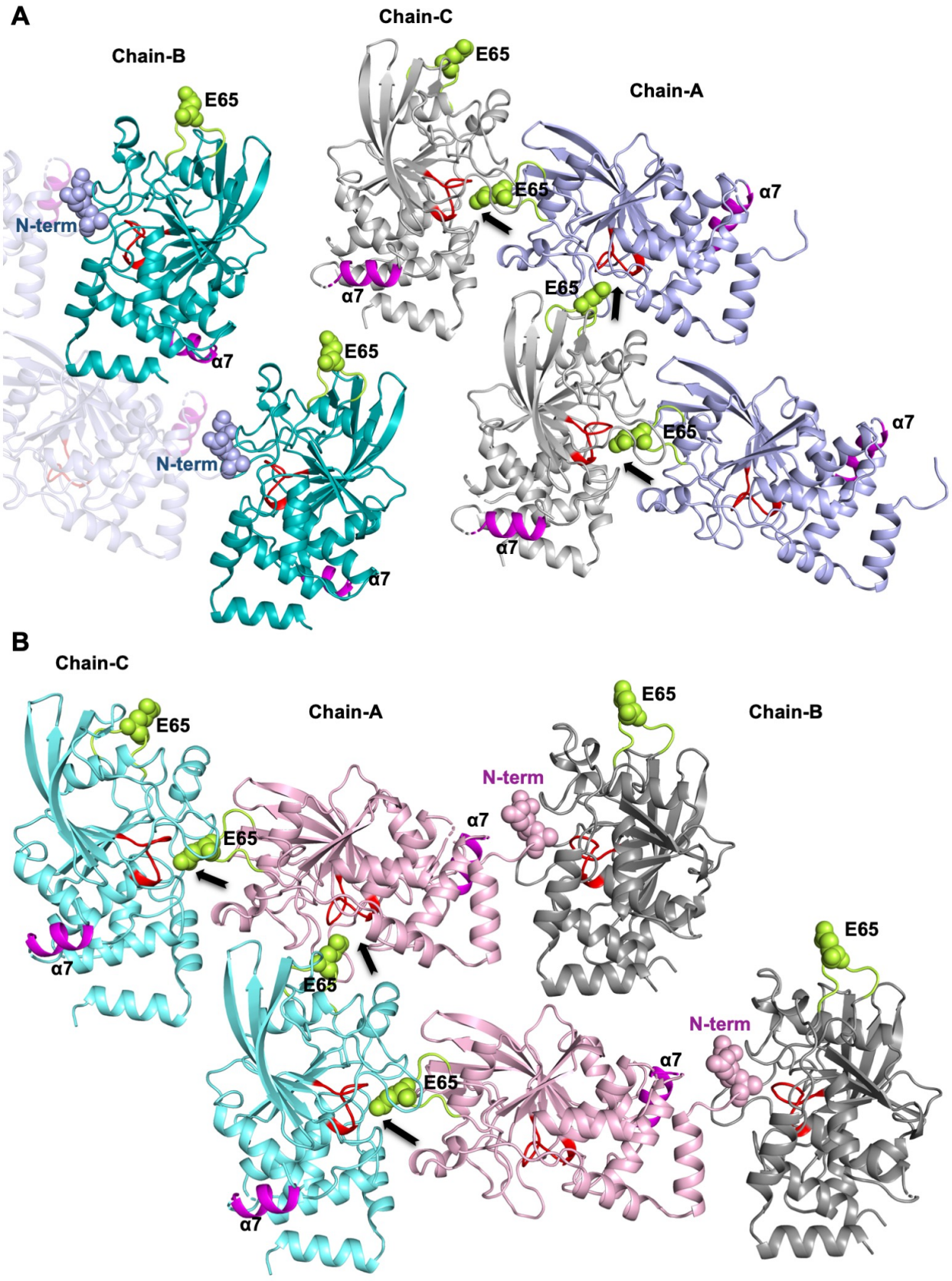
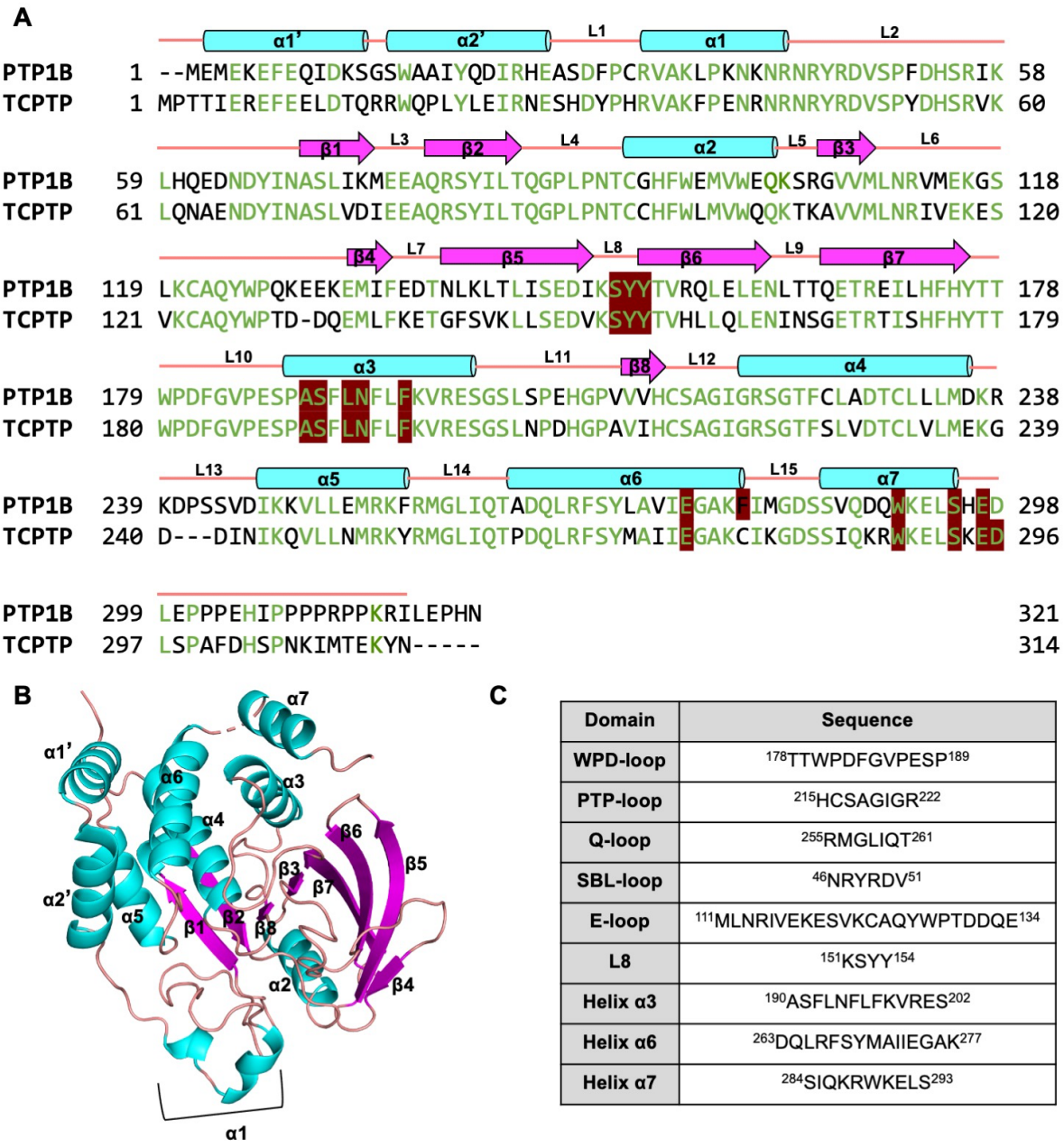

A
Chain-B
Chain-C
Chain-A
E65
N-term
α7
B
Chain-C
Chain-A
Chain-B
E65
N-term
α7

Figure S1. Crystal packing of TCPTP molecules in asymmetric unit illustrates multimerization of TCPTP. Chain-A and Chain-C are involved in pseudo substrate conformation. The pseudo substrate interaction at the active-site is pointed by a black arrow. Chain-B's active-site is blocked by extraneous residue of Chain-A coming from a vector. Extraneous residue is shown in sphere, which also weakly interacts with the PTP loop of Chain-B. (**A)** and **(B)** show multimerization of TCPTP in asymmetric unit of $TCPTP_{1-314}$ and $TCPTP_{1-302}$ crystal, respectively. Helix α7 is missing in Chain-B of $TCPTP_{1-302}$ crystal. PTP loop is shown in red, Helix α7 is shown in magenta, and loop L-2's E65 residue (sphere) that interacts with PTP loop is shown in lime color.



Domain	Sequence
WPD-loop	^{178}TTWPDFGVPESP^{189}
PTP-loop	^{215}HCSAGIGR^{222}
Q-loop	^{255}RMGLIQT^{261}
SBL-loop	^{46}NRYRDV^{51}
E-loop	^{111}MLNRIVEKESVKCAQYWPTDDQE^{134}
L8	^{151}KSYY^{154}
Helix α3	^{190}ASFLNFLFKVRES^{202}
Helix α6	^{263}DQLRFSYMAIIEGAK^{277}
Helix α7	^{284}SIQKRWKELS^{293}

Figure S2. Overview of TCPTP secondary structure elements, defined by the crystal structures of $TCPTP_{1-314}$ and $TCPTP_{1-302}$. **(A)** Amino acid sequences of TCPTP (1-314) and PTP1B (1-321) are aligned, identical residues are typed in fern color, interacting residues from helix α3, 6, 7 and loop 8 (L11 in PTP1B) in TCPTP and PTP1B are highlighted with dark red. The secondary structure elements are positioned over the residues responsible for respective formation of α-helices (shown in cyan) or β-sheets (shown in magenta), based on the $TCPTP_{1-314}$ and $TCPTP_{1-302}$ crystal

structures. **(B)** Cartoon representation and nomenclature of secondary structure elements in a 3D structure of TCPTP (derived from the $TCPTP_{1-314}$ crystal). **(C)** Important functional loops and helices of TCPTP denoted in this study are listed.

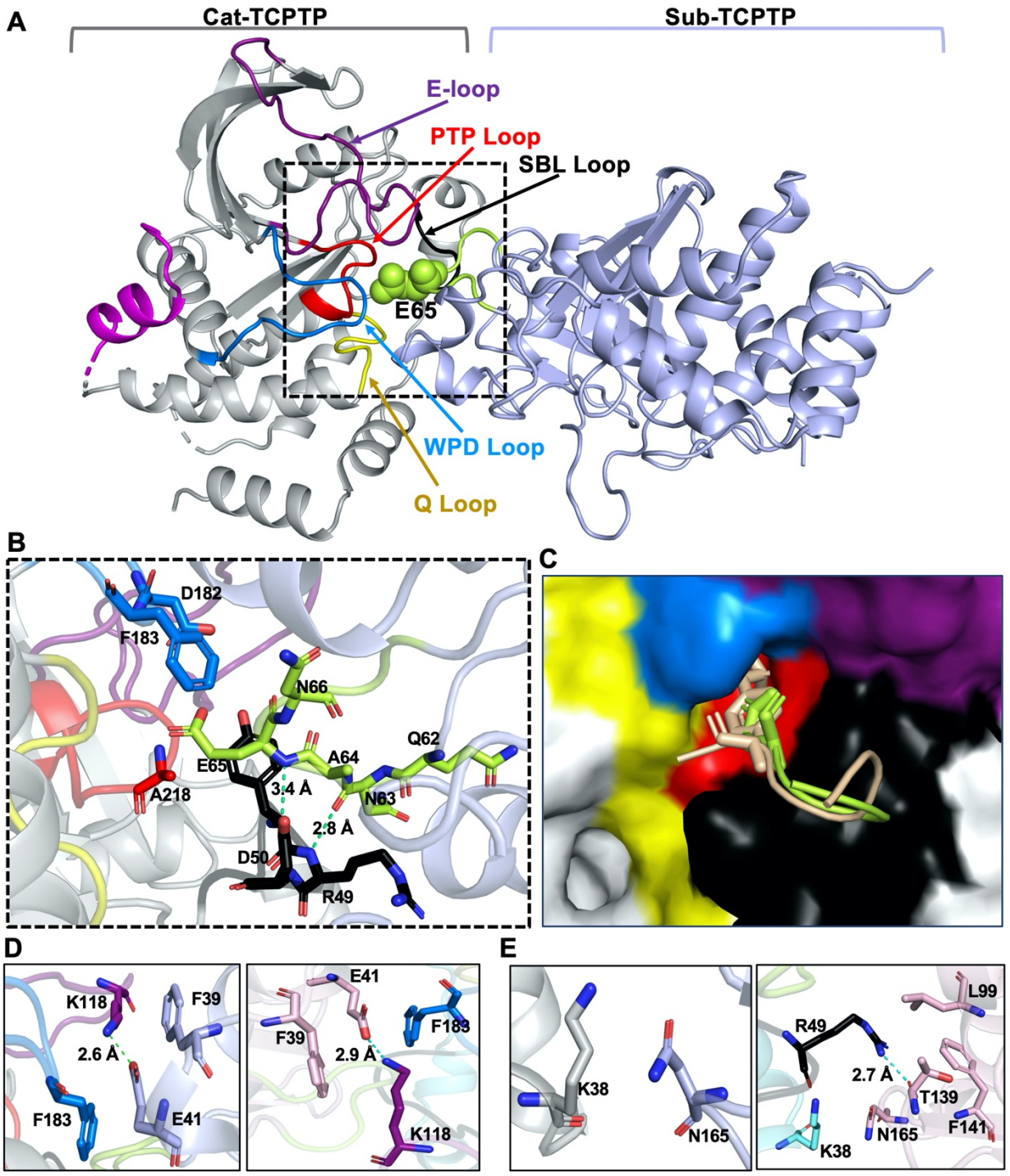
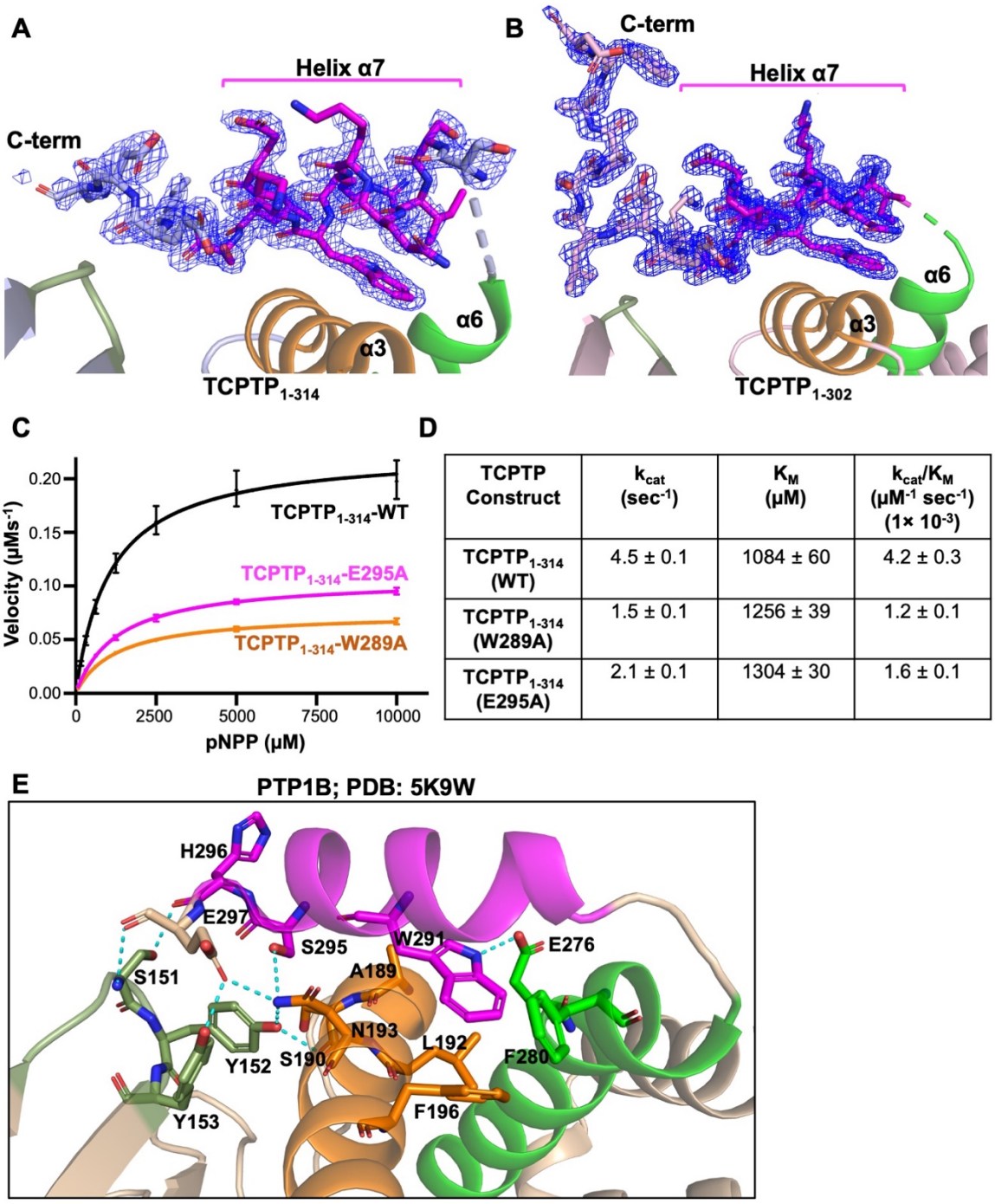


Figure S3. TCPTP crystal packing reveals a pseudo substrate-bound conformation, mimicking the interaction of phosphatase-substrate complex **(**complex composed by a phosphotyrosine peptide and a catalytic domain of PTP). **(A)** Structure shows interaction of TCPTP dimmer in asymmetric

unit of $TCPTP_{1-314}$ crystal packing. One molecule offering an active-site in the interaction was named Cat-TCPTP (catalytic TCPTP), while another molecule posing loop L-2's E65 residue in interaction was named Sub-TCPTP (substrate TCPTP). Catalytically important loops are colored (PTP loop: red, WPD loop: marine blue, Q-loop: yellow, SBL loop: black and E-loop: purple). **(B)** pTyr mimicking interaction between the loop L2 residues from Sub-TCPTP and the catalytically important loops surrounding the active-site from Cat-TCPTP. Residues from Cat-TCPTP involved in the interaction are colored (PTP loop: red, WPD loop: marine blue, Q-loop: yellow, SBL loop: black and E-loop: purple). Loop 2 residues from Sub-TCPTP are shown in lime color. **(C)** Surface representation shows orientation of E65 residue (limon color) from Sub-TCPTP's interacting loop and a real substrate pTyr (wheat color, from PTP1B-pTyr peptide complex structure; PDB: 1PTU) in the active-site of TCPTP. The active-site surface is colored (PTP loop: red, WPD loop: marine blue, Q-loop: yellow, SBL loop: black and E-loop: purple). **(D)** Residues involved in intermolecular interaction at the interface formed by the WPD loop and the E-loop from Cat-TCPTP and helix-1 residues from Sub-TCPTP in crystal packing, (Left panel shows interaction at the interface of $TCPTP_{1-314}$ crystal while right panel is from $TCPTP_{1-302}$ crystal). **(E)** Residues involved in intermolecular interaction at the interface formed by helix-α1 and SBL Loop from Cat-TCPTP and loop- L7, L9, helix-α2 and betta sheet-5 from Sub-TCPTP in crystal packing (Left panel shows interaction at the interface of $TCPTP_{1-314}$ crystal, while right panel shows details of $TCPTP_{1-302}$ crystal).



TCPTP Construct	k_{cat} (sec^{-1})	K_M (μM)	k_{cat}/K_M (μM^{-1} sec^{-1}) (1×10^{-3})
$TCPTP_{1-314}$ (WT)	4.5 ± 0.1	1084 ± 60	4.2 ± 0.3
$TCPTP_{1-314}$ (W289A)	1.5 ± 0.1	1256 ± 39	1.2 ± 0.1
$TCPTP_{1-314}$ (E295A)	2.1 ± 0.1	1304 ± 30	1.6 ± 0.1

Figure S4. (A) Electron density map (2Fo-Fc, σ=1.0) of helix α7 in $TCPTP_{1-314}$ crystal structure **(B)** Electron density map (2Fo-Fc, σ=1.0) of helix α7 in $TCPTP_{1-302}$ crystal structure **(C)** Michaelis-Menten curves show the catalytic activity of TCPTP variants. Data are presented as means ± SE from three independent assays of pNPP dephosphorylation. **(D)** Summary of kinetic

parameters determined by Michaelis-Menten kinetics. Data are presented as means ± SE. $TCPTP_{1-314}$-WT data is also shown in main figure 4B. **(E)** Intramolecular interactions of helix α7 and abutting residues from its C-terminus with helix α3 (Orange), helix α6 (Green) and loop L-11 (Smudge green) in PTP1B crystal (PDB: 5K9W). It shows similar patterns as observed in the TCPTP crystal structures presented by the current study.

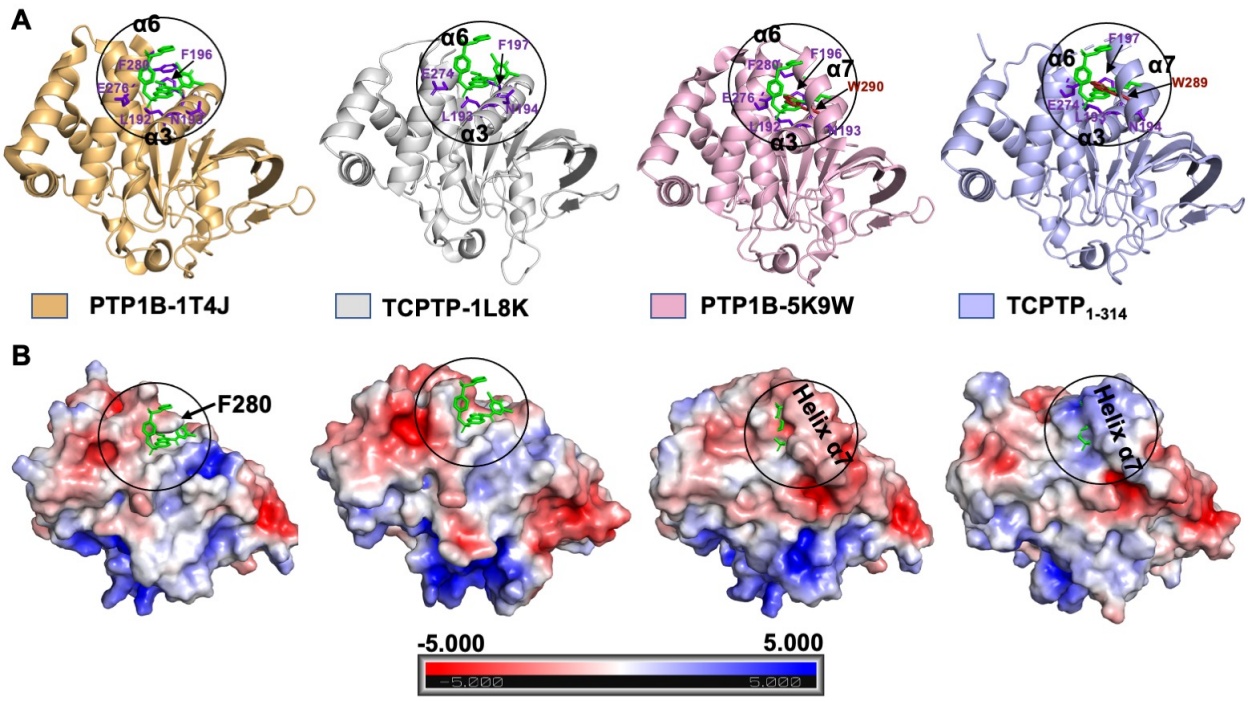


Figure S5. The allosteric binding sites at the interface of helix α3, - α6 and - α7 show distinct structural variations in TCPTP and PTP1B due to absence of phenylalanine in helix α6 of TCPTP and surrounding surface charge developed by helix α7 as well as abutting residues. Black circles highlight the intersection of helix α3, - α6 and - α7 where allosteric compound binds. The key residues from helix α3, α6 lining the binding pocket of the allosteric compound are shown as stick in purple. Helix α7 residue Tryptophan , key for intramolecular interaction is shown in dark red. **(A)** Comparative visualization of allosteric inhibitor compound (green, BBR) at the allosteric binding site of TCPTP and PTP1B. ***From left to right:*** crystal structure of PTP1B without helix α7 in complex with allosteric inhibitor compound (light orange; PDB:1T4J); allosteric compound overlaid in allosteric binding pocket of TCPTP without helix α7 (light grey; PDB: 1L8K); allosteric compound overlaid in allosteric binding pocket of PTP1B with helix α7 (light pink; PDB: 5K9W); and allosteric compound overlaid in allosteric binding pocket of TCPTP with helix α7 (light blue; $TCPTP_{1-314}$ crystal). **(B)** Comparative surface electrostatic potential view shows that

surface charge near the allosteric compound binding site in PTP1B and TCPTP is different. TCPTP has positive potential while PTP1B has negative potential. ***From left to right:*** crystal structure of PTP1B without helix α7 in complex with allosteric inhibitor compound (PDB: 1T4J); allosteric compound overlaid in allosteric binding pocket of TCPTP without helix α7 (PDB: 1L8K); allosteric compound overlaid in allosteric binding pocket of PTP1B with helix α7 (PDB: 5K9W); and allosteric compound overlaid in allosteric binding pocket of TCPTP with helix α7 ($TCPTP_{1-314}$ crystal).