Supplemental Material

The Structural Signature of the MYPT1:PP1 Interaction

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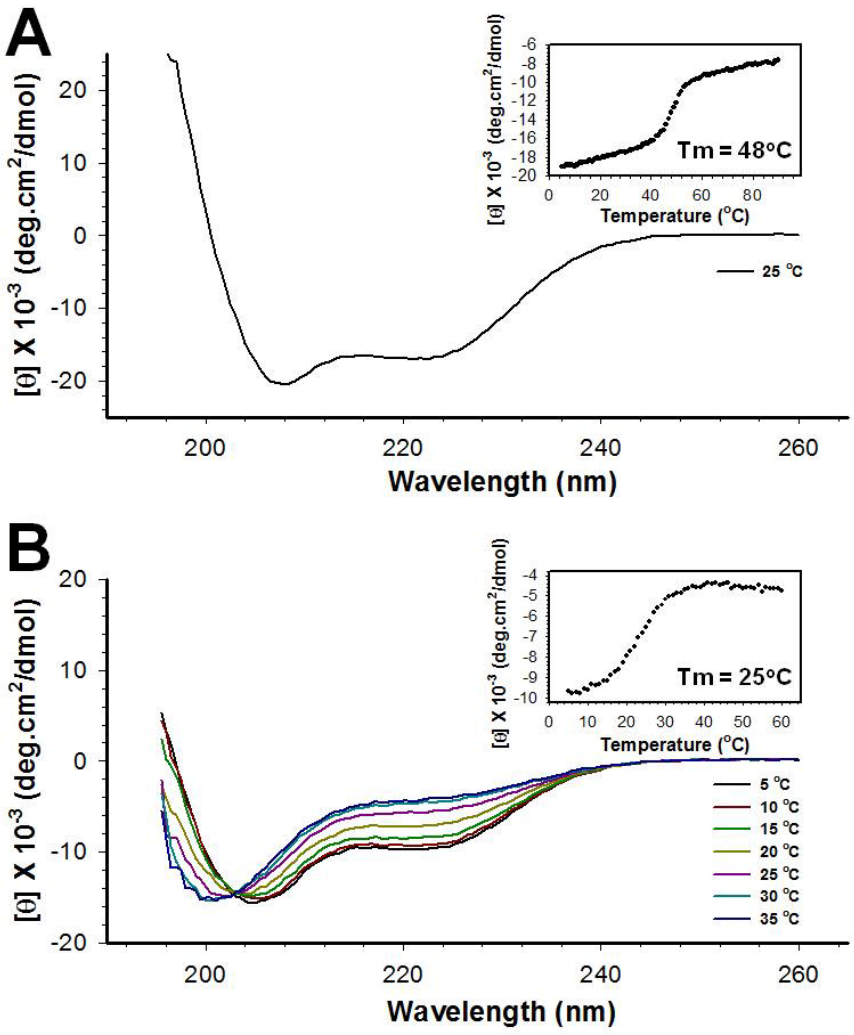
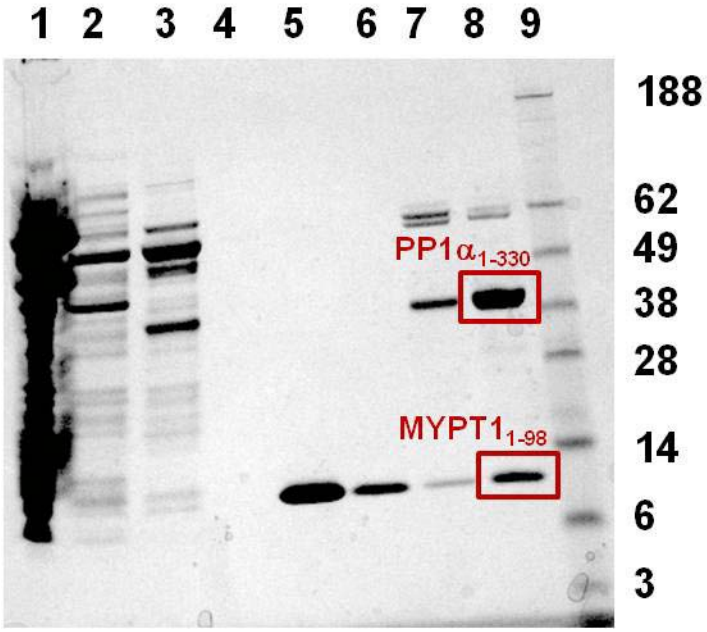
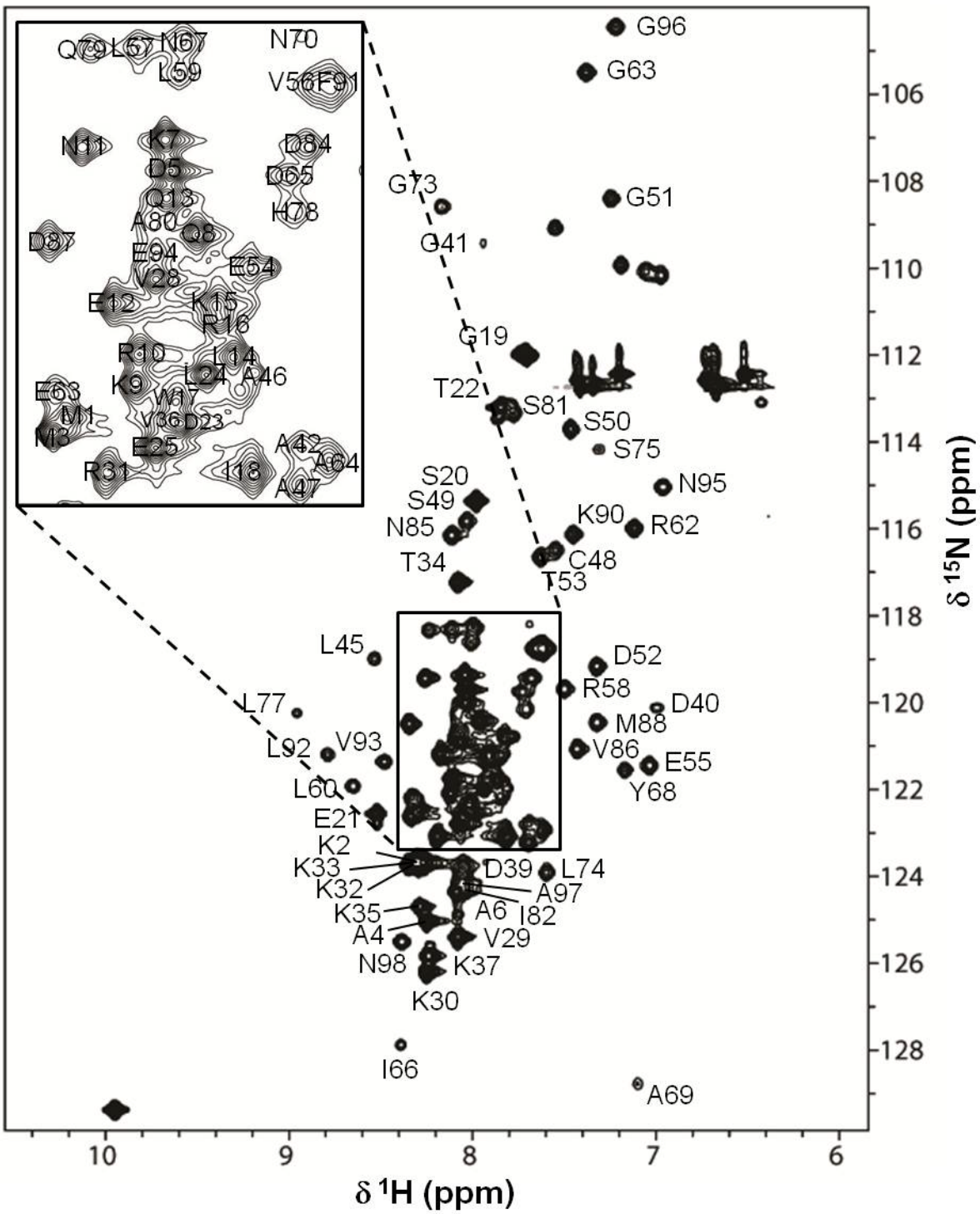


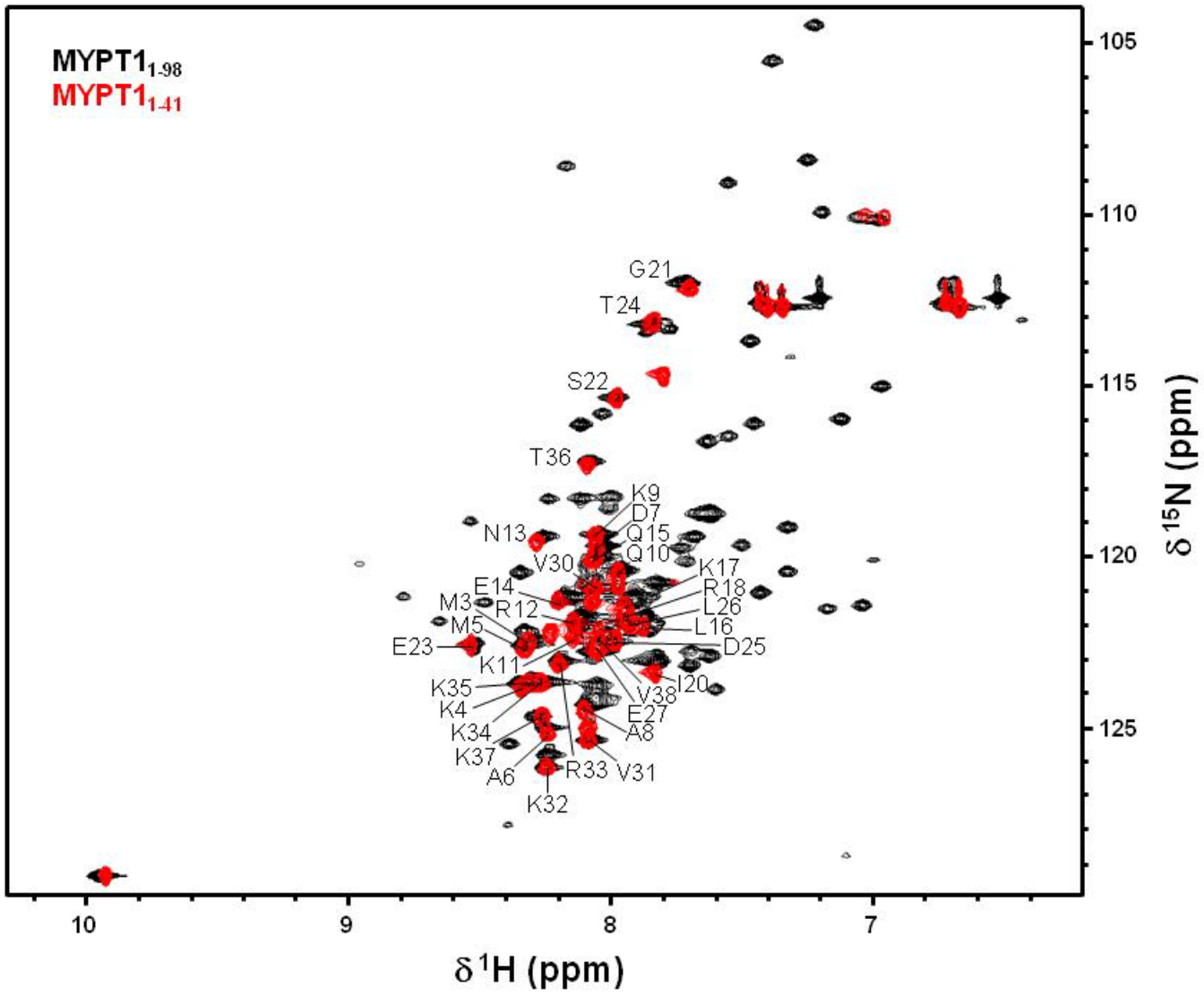
Supplemental Figure S1: Thermodynamic stability of MYPT1 constructs used in the studies. (A) Circular dichroism (CD) spectrum of $MYPT1_{1-299}$ recorded at 25°C (20 mM Na-phosphate pH 6.8, 50 mM NaCl, 0.5 mM TCEP; sample concentration 5 μM). The spectrum shows that expected hallmarks for an α-helical protein, with negative peaks at 222 and 208 nm. The insert shows a melting curve of $MYPT1_{1-299}$ recorded as a function of increasing temperature (measured @ 222 nm). $MYPT1_{1-299}$ has a melting temperature of ~48°C. (B) Circular dichroism spectra of $MYPT1_{1-98}$ recorded at different temperatures ranging from 5 to 35°C (identical conditions as A; sample concentration: 10 μM). The stability of $MYPT1_{1-98}$ increases with decreasing temperature as shown by the migration of the negative peak in the CD spectrum from ~201 (random coil) to ~208 nm (α-helical). $MYPT1_{1-98}$ has a melting temperature of ~25°C as shown in the insert.



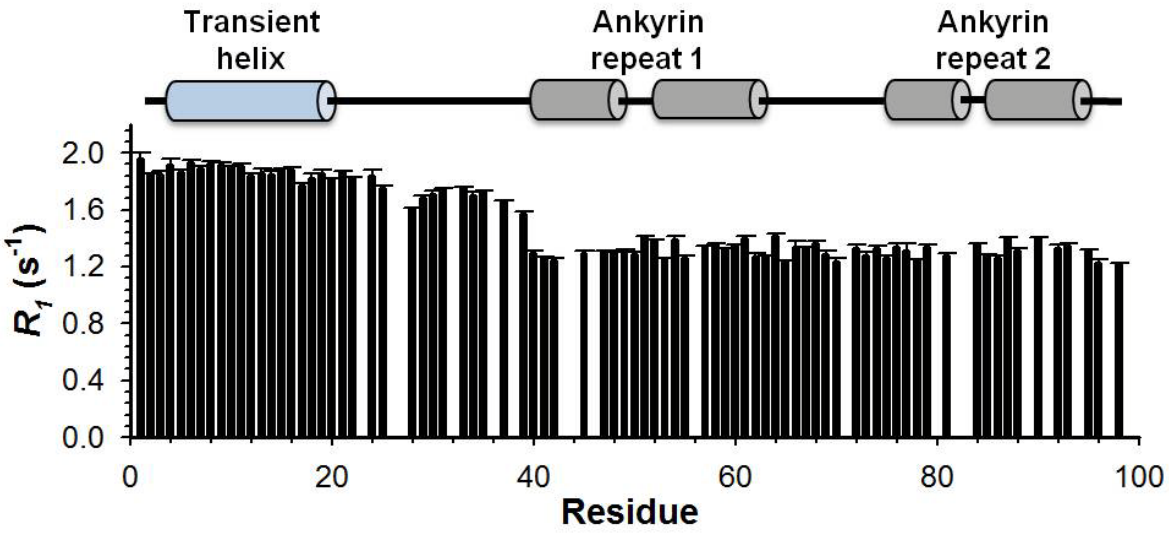
Supplemental Figure S2: MYPT1:PP1 complex formation. To experimentally verify the activity of $MYPT1_{1-98}$, we tested its ability to form a complex with $PP1\alpha_{1-330}$ *in vitro.* Samples from different stages of the complex formation experiment were assayed by SDS PAGE as follows: (1) $PP1\alpha_{1-330}$ flow-through, (2) wash with high salt buffer (50 mM Tris pH 8.0, 700 mM NaCl, 5 mM imidazole, 1 mM $MnCl_2$), (3) wash of $PP1\alpha_{1-330}$ with 50 mM imidazole (50 mM Tris pH 8.0, 700 mM NaCl, 50 mM imidazole, 1 mM $MnCl_2$), (4) equilibration of the resin in low salt buffer (50 mM Tris pH 8.0, 50 mM NaCl, 5 mM imidazole, 1 mM $MnCl_2$), (5) flow-through of the incubation with purified $MYPT1_{1-98}$, (6) wash with low salt buffer, (7) wash with 250 mM imidazole (50 mM Tris pH 8.0, 50 mM NaCl, 250 mM imidazole, 1 mM $MnCl_2$), (8) elution of the MYPT1:PP1 complex with 500 mM imidazole (50 mM Tris pH 8.0, 50 mM NaCl, 500 mM imidazole, 1 mM $MnCl_2$), (9) molecular weight marker. Co-elution of His_6-$PP1\alpha_{1-330}$ (~ 40 kDa) and $MYPT1_{1-98}$ (~ 11 kDa), as indicated by the red boxes and corresponding labels, confirms successful complex formation.



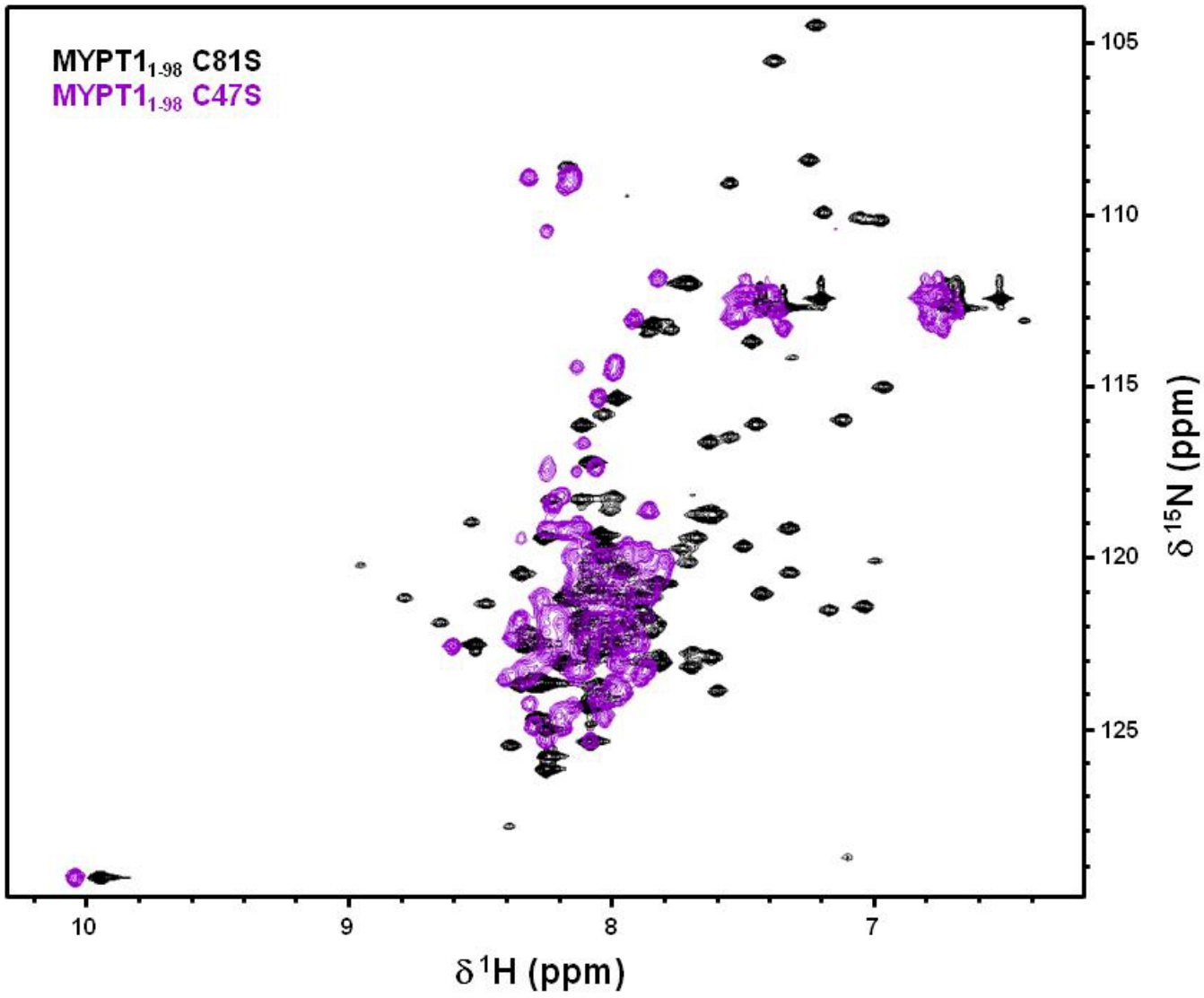
Supplemental Figure S3: Fully annotated 2D [^{1}H,^{15}N] HSQC spectrum of MYPT1$_{1\text{-}98}$. Residues were marked with their residue name (one letter code) and number in protein sequence.



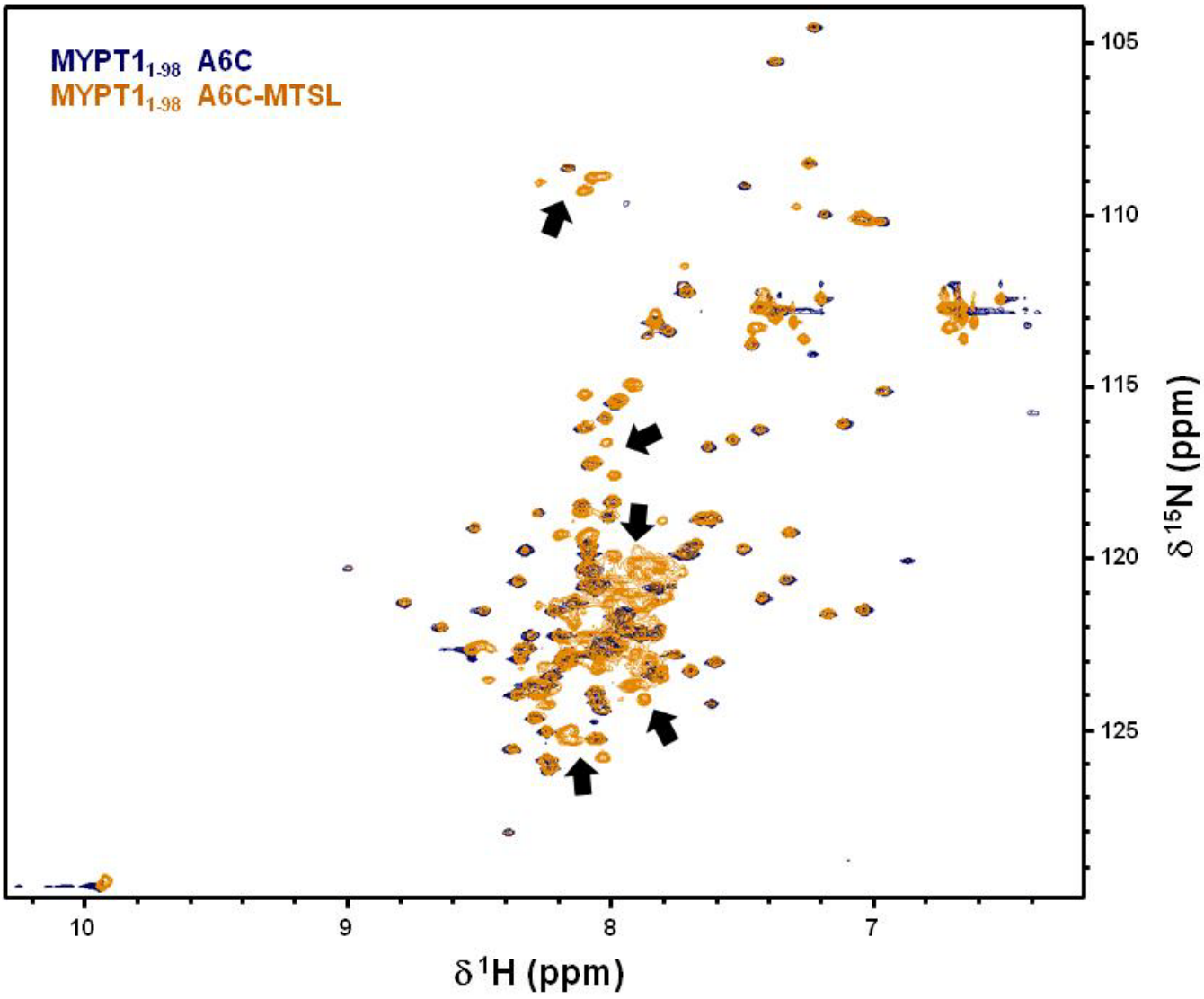
Supplemental Figure S4: Superposition of the 2D [^{1}H,^{15}N]-HSQC spectra of MYPT1$_{1-98}$ (black) and MYPT1$_{1-41}$ (red). Both spectra were acquired using identical sample concentration (0.5 mM), buffer (20 mM Na-phosphate pH 6.8, 50 mM NaCl, 0.5 mM TCEP) and temperature (278 K). H^{N}, N chemical shifts of MYPT1$_{1-41}$ overlap exceedingly well with those of MYPT1$_{1-98}$ confirming the independent behavior of these two domains. MYPT1$_{1-41}$ residues are annotated.



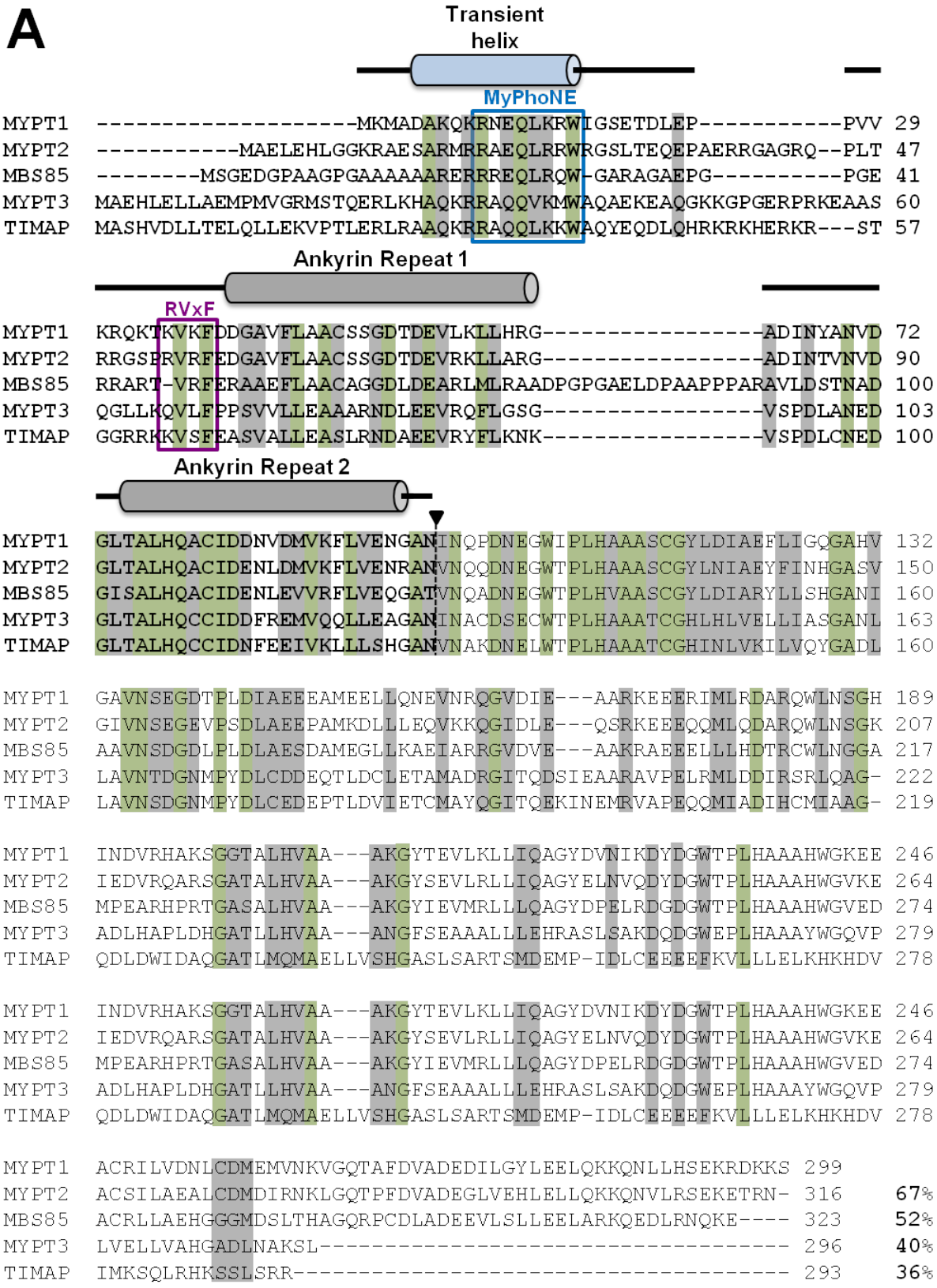
Supplemental Figure S5: MYPT1$_{1\text{-}98}$ ^{15}N R_1 relaxation rates plotted as a function of the protein sequence. The two-domain behavior showed by MYPT1$_{1\text{-}98}$ is reflected by the relaxation rates. The N-terminal PP1-binding domain shows less restricted backbone motions than the C-terminal ankyrin-repeat domain, as indicated by higher R_1 values. Secondary structural elements, based on the MYPT1:PP1 complex structure is shown as cartoon representations.

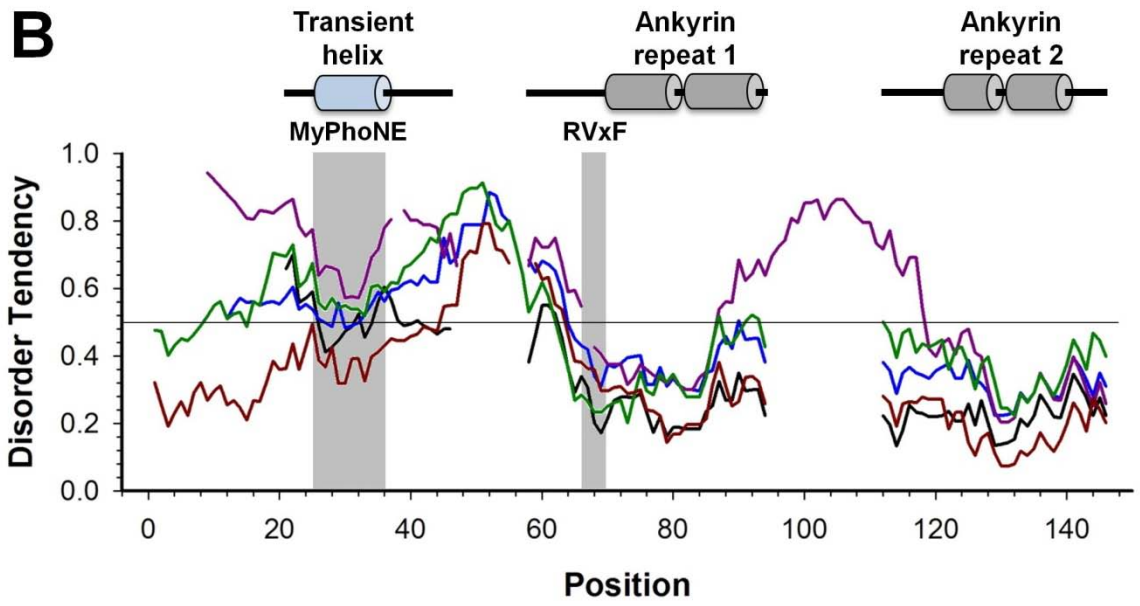


Supplemental Figure S6: Effect of the mutation of cysteine residues on the overall structure of MYPT1$_{1-98}$. Superposition of the 2D [^{1}H,^{15}N]-HSQC spectra of MYPT1$_{1-98}$ C81S (black) and MYPT1$_{1-98}$ C47S (purple). Experimental conditions are identical to those described in **Supplemental Figure S4**. Mutation of Cys47 to Ser disrupts the packing and thus folding of the ankyrin repeats, as shown by the disappearance of the well-dispersed NH cross-peaks in the MYPT1$_{1-98}$ C47S spectrum (purple).



Supplemental Figure S7: Superposition of the 2D [^{1}H,^{15}N]-HSQC spectra of MYPT1$_{1-98}$ A6C (blue) and MYPT1$_{1-98}$ A6C-MTSL (orange). Experimental conditions are identical to those described in **Supplemental Figure S4**. Labeling of MYPT1$_{1-98}$ A6C with MTSL leads to the appearance of a new set of cross-peaks in the 2D [^{1}H,^{15}N]-HSQC spectrum, indicated by multiple arrows. This suggested the existence of an additional conformation in solution and made the use of the paramagnetic label impossible.


A
Transient helix
MyPhoNE
Ankyrin Repeat 1
RVxF
Ankyrin Repeat 2
MYPT1 ----------MKMADAKQKRNEQLKRWIGSETDLEP----------PVV 29
MYPT2 ----------MAELEHLGGKRAESARMRRAEQLRRWRGSLTEQEPAERRGAGRQ--PLT 47
MBS85 --------MSGEDGPAAGPGAAAAAARERRREQLRQW-GARAGAEPG----------PGE 41
MYPT3 MAEHLELLAEMPMVGRMSTQERLKHAQKRRAQQVKMWAQAEKEAQGKKGPGERPRKEAAS 60
TIMAP MASHVDLLTELQLLEKVPTLERLRAAQKRRAQQLKKWAQYEQDLQHRKRKHERKR---ST 57
MYPT1 KRQKTKVKFDDGAVFLAACSSGDTDEVLKLLHRG----------ADINYANVD 72
MYPT2 RRGSPRVRFEDGAVFLAACSSGDTDEVRKLLARG----------ADINTVNVD 90
MBS85 RRART-VRFERAAEFLAACAGGDLDEARLMLRAADPGPGAELDPAAPPPARAVLDSTNAD 100
MYPT3 QGLLKQVLFPPSVVLLEAAARNDLEEVRQFLGSG----------VSPDLANED 103
TIMAP GGRRKKVSFEASVALLEASLRNDAEEVRYFLKNK----------VSPDLCNED 100
MYPT1 GLTALHQACIDDNVDMVKFLVENGANINQPDNEGWIPLHAAASCGYLDIAEFLIGQGAHV 132
MYPT2 GLTALHQACIDENLDMVKFLVENRANVNQQDNEGWTPLHAAASCGYLNIAEYFINHGASV 150
MBS85 GISALHQACIDENLEVVRFLVEQGATVNQADNEGWTPLHVAASCGYLDIARYLLSHGANI 160
MYPT3 GLTALHQCCIDDFREMVQQLLEAGANINACDSECWTPLHAAATCGHLHLVELLIASGANL 163
TIMAP GLTALHQCCIDNFEEIVKLLLSHGANVNAKDNELWTPLHAAATCGHINLVKILVQYGADL 160
MYPT1 GAVNSEGDTPLDIAEEEAMEELLQNEVNRQGVDIE---AARKEEERIMLRDARQWLNSGH 189
MYPT2 GIVNSEGEVPSDLAEEPAMKDLLLEQVKKQGIDLE---QSRKEEEQQMLQDARQWLNSGK 207
MBS85 AAVNSDGDLPLDLAESDAMEGLLKAEIARRGVDVE---AAKRAEEELLLHDTRCWLNGGA 217
MYPT3 LAVNTDGNMPYDLCDDEQTLDCLETAMADRGITQDSIEAARAVPELRMLDDIRSRLQAG- 222
TIMAP LAVNSDGNMPYDLCEDEPTLDVIETCMAYQGITQEKINEMRVAPEQQMIADIHCMIAAG- 219
MYPT1 INDVRHAKSGGTALHVAA---AKGYTEVLKLLIQAGYDVNIKDYDGWTPLHAAAHWGKEE 246
MYPT2 IEDVRQARSGATALHVAA---AKGYSEVLRLLIQAGYELNVQDYDGWTPLHAAAHWGVKE 264
MBS85 MPEARHPRTGASALHVAA---AKGYIEVMRLLLQAGYDPELRDGDGWTPLHAAAHWGVED 274
MYPT3 ADLHAPLDHGATLLHVAA---ANGFSEAAALLLEHRASLSAKDQDGWEPLHAAAYWGQVP 279
TIMAP QDLDWIDAQGATLMQMAELLVSHGASLSARTSMDEMP-IDLCEEEEFKVLLLELKHKHDV 278
MYPT1 INDVRHAKSGGTALHVAA---AKGYTEVLKLLIQAGYDVNIKDYDGWTPLHAAAHWGKEE 246
MYPT2 IEDVRQARSGATALHVAA---AKGYSEVLRLLIQAGYELNVQDYDGWTPLHAAAHWGVKE 264
MBS85 MPEARHPRTGASALHVAA---AKGYIEVMRLLLQAGYDPELRDGDGWTPLHAAAHWGVED 274
MYPT3 ADLHAPLDHGATLLHVAA---ANGFSEAAALLLEHRASLSAKDQDGWEPLHAAAYWGQVP 279
TIMAP QDLDWIDAQGATLMQMAELLVSHGASLSARTSMDEMP-IDLCEEEEFKVLLLELKHKHDV 278
MYPT1 ACRILVDNLCDMEMVNKVGQTAFDVADEDILGYLEELQKKQNLLHSEKRDKKS 299
MYPT2 ACSILAEALCDMDIRNKLGQTPFDVADEGLVEHLELLQKKQNVLRSEKETRN- 316 67%
MBS85 ACRLLAEHGGGMDSLTHAGQRPCDLADEEVLSLLEELARKQEDLRNQKE---- 323 52%
MYPT3 LVELLVAHGADLNAKSL---------- 296 40%
TIMAP IMKSQLRHKSSLSRR---------- 293 36%



Supplemental Figure S8: (A) Primary sequence alignment of human MYPT1 (residues 1-299) and its isoforms MYPT2, MYPT3, MBS85 and TIMAP (order is based on sequence identity). Identical residues are highlighted in green, similar residues are shaded in gray. The two consensus PP1 binding motifs, MyPhoNE and RVxF, are depicted by blue and violet boxes, respectively. MYPT1$_{1\text{-}98}$ and corresponding primary sequences in MYPT2/3, MBS85 and TIMAP are highlighted in bold. Secondary structural elements, based on the MYPT1:PP1 crystal structure, are shown using cartoon representations. (B) Comparison of disorder tendency among members of the MYPT family. The disorder tendency was predicted using the program IUPred. Values of disorder tendency were plotted according to the primary sequence alignment in Figure S8A. In the graph: MYPT1 (black), MYPT2 (blue), MBS85 (violet), MYPT3 (green) and TIMAP (red). A disorder tendency above 0.5 suggests regions with higher propensity for disorder. The two PP1 consensus binding motifs, MyPhoNE and RVxF, are highlighted by gray boxes. Secondary structural elements, based on the MYPT1:PP1 crystal structure, are shown using cartoon representations.