Supplemental Data for:

The E3 ubiquitin ligase and protein phosphatase 2A (PP2A) binding domains of Alpha4 are both required for Alpha4 to inhibit PP2A degradation

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Running Title: Protection of PP2Ac requires both domains of Alpha4
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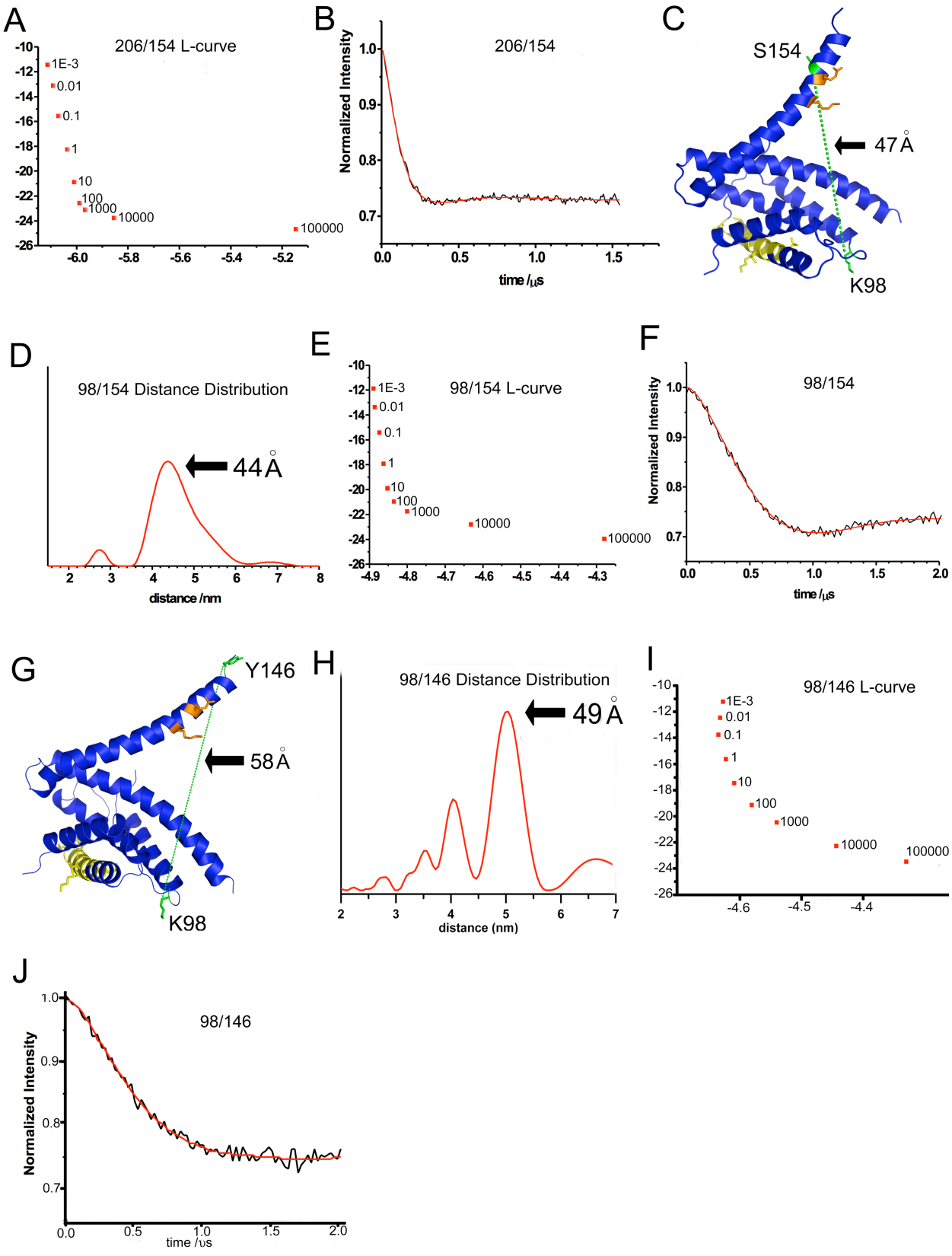
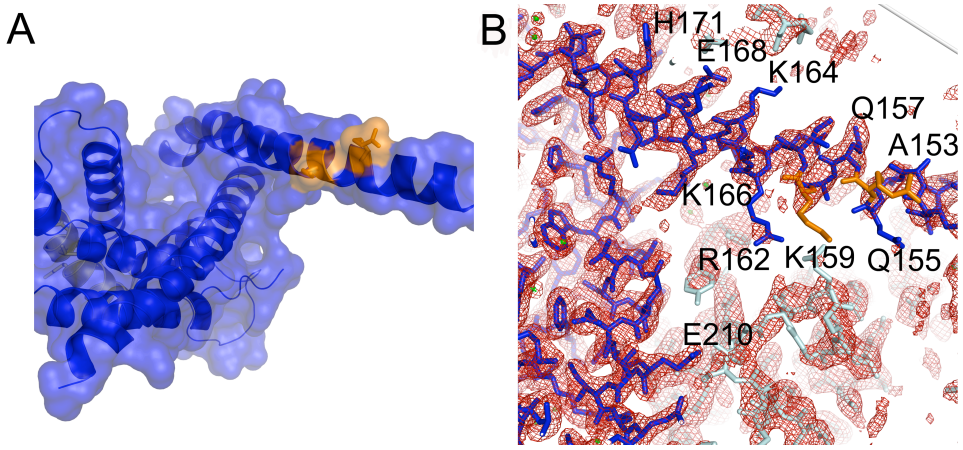
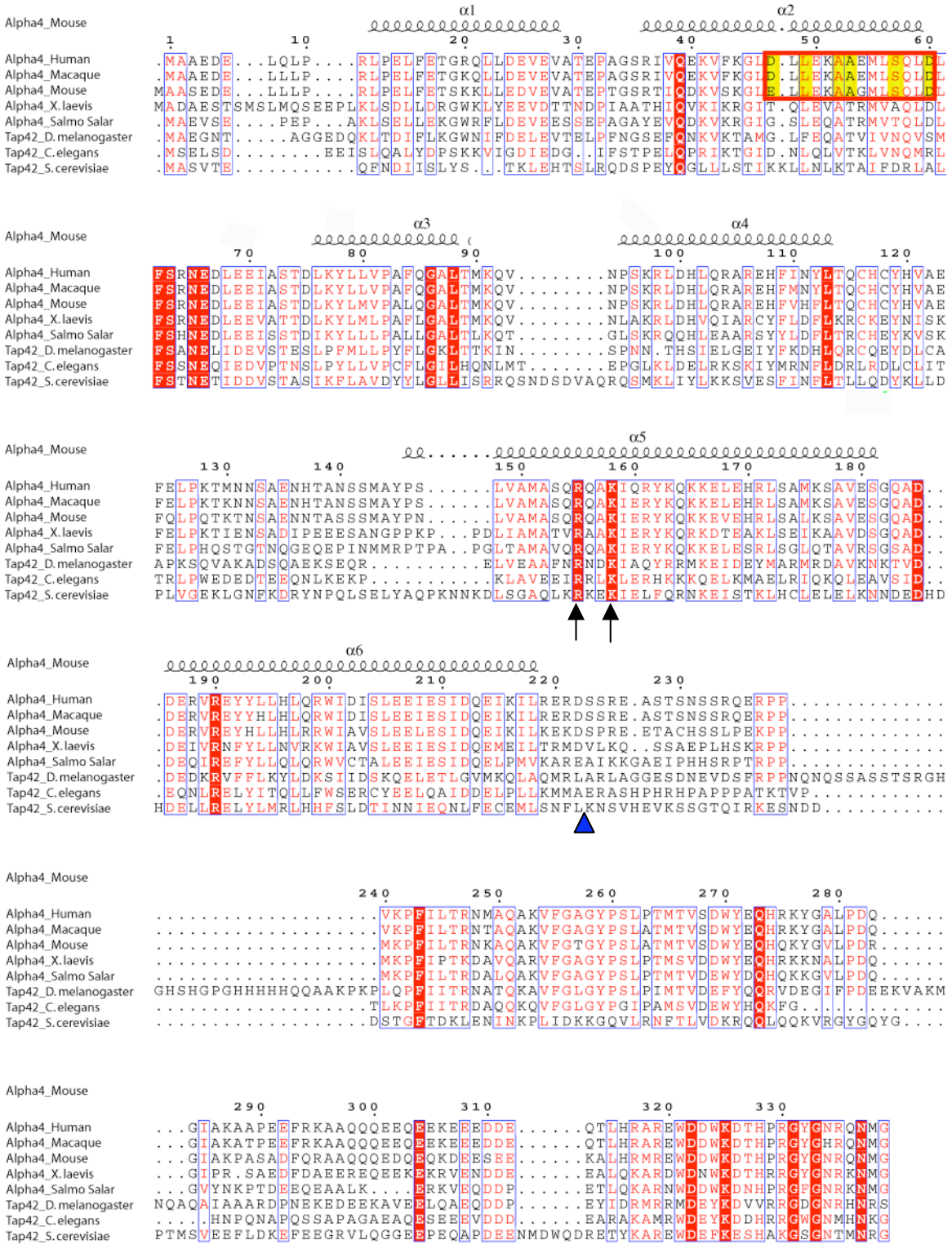
SUPPLEMENTARY FIGURES



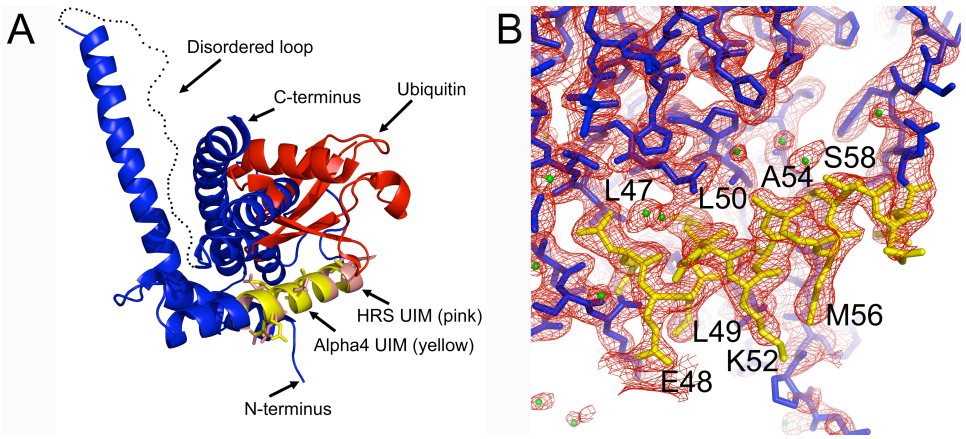
Supplementary Figure 1: Additional DEER data. A) Plot of the L-curve for L206C/S154C shows α = 100 as the optimal value for computing the distance distribution. B) Background-subtracted dipolar modulation echo curves for spin-labeled L206C/S154C Alpha4ΔC. Red line shows the best solution using Tikhonov regularization analysis. C) Ribbon diagram showing location of spin labels K98C and S154C (green) and distance between β-carbons. UIM consensus motif is shown in yellow and PP2Ac binding residues are shown in orange. (D) Distance distribution profile for K98C/S154C, corresponding to the best fit, shows a major distance distribution of ~44Å, compared to ~47Å in the crystal structure. E) Plot of the L-curve for K98C/S154C shows α = 100 as the optimal value for computing the distance distribution. F) Background-subtracted dipolar modulation echo curves for spin-labeled K98C/S154C Alpha4ΔC. Red line shows the best solution using Tikhonov regularization analysis. G) Ribbon diagram showing location of spin labels K98C and Y146C (green) and distance between β-carbons. UIM consensus motif is shown in yellow and PP2Ac binding residues are shown in orange. (H) Distance distribution profile for K98C/Y146C, corresponding to the best fit, shows a major distance distribution of ~49Å, compared to ~58Å in the crystal structure. I) Plot of the L-curve for K98C/Y146C shows α = 10 as the optimal value for computing the distance distribution. J) Background-subtracted dipolar modulation echo curves for spin-labeled K98C/Y146C Alpha4ΔC. Red line shows the best solution using Tikhonov regularization analysis. PYMOL was used to depict all molecular structures (*1*).



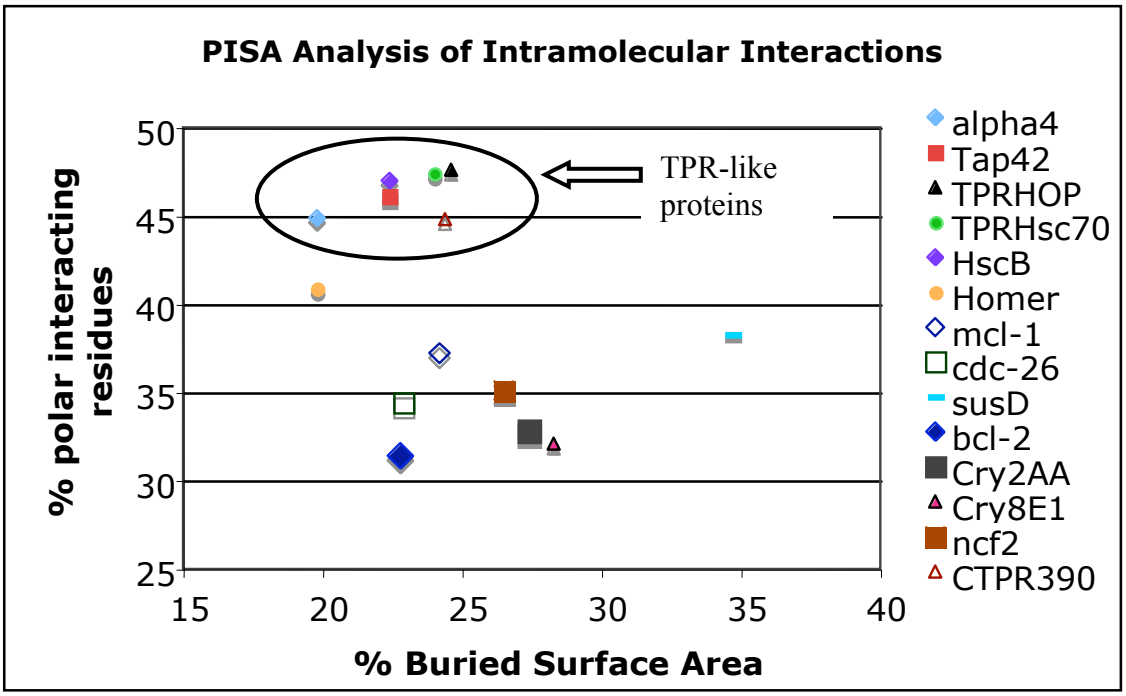
Supplementary Figure 2: Orientation of PP2Ac binding residues. A) Accesible surface rendering showing orientation of PP2Ac binding residues (orange) in Alpha4 (blue). B) 2mFo-DFc electron density map contoured at 1σ highlighting the PP2Ac binding residues (orange) within Alpha4 (blue). Symmetry molecules are shown in light blue and waters are shown in green.



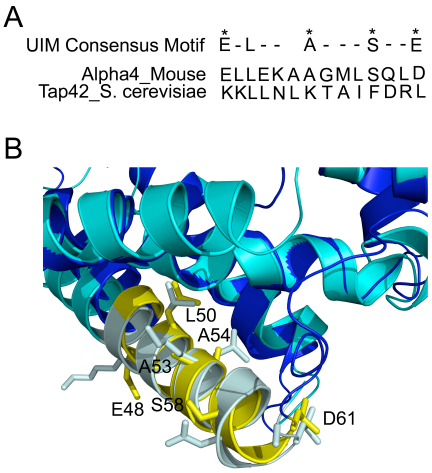
Supplementary Figure 3: Multiple sequence alignment of Alpha4/Tap42 showing conservation of UIM consensus in mammalian species with conserved residues highlighted in yellow and the motif boxed in red. Conserved residues (as determined by %Equivalent set at 0.7 in ESPRIPT (*2*)) are colored red and invariant residues are white on a red background. The black arrows indicate the residues essential for binding to PP2Ac. The C-terminus of the Alpha4ΔC construct is marked by a blue triangle. Figure was created using CLUSTALW (*3*) and ESPRIPT (*2*). Sequence database IDs are as follows: *H.sapiens* - CAG33063.1, *M. mulatta* - *NP_01182718*, *M. musculus* - EDL14183.1, *X. laevis* - NP_001084735, *S. salar* - NP_001140137, *D. melanogaster* - NP_723811.1, *C.elegans* - NP_497591.1, and *S. cerevisiae* - NP_013741.1.



Supplementary Figure 4: Position of the UIM consensus motif. A) Overlay of the Alpha4ΔC structure (blue) with the UIM colored in yellow and the crystal structure of Hrs bound to ubiquitin (2D3G) with the UIM in pink and ubiquitin in red showing that the UIM of Alpha4ΔC would need to move in order for ubiquitin to bind in a similar configuration. B) 2mFo-DFc electron density map contoured at 1σ showing residues within the UIM consensus region in yellow, Alpha4 in blue, and waters in green.



Supplemental Figure 5: Structural analysis of buried surface area and polar interacting residues using PISA(*4*). Proteins were analyzed by creating a separate chain for each helix for analysis by PISA. Total surface area of all helices was calculated and compared to the total buried surface area. Only interactions that were not part of secondary structures were counted as interacting residues. TPR-like proteins hae a higher percentage of polar interacting residues than alpha-helical proteins in general and trend towards a lower percentage of buried surface area.



Supplementary Figure 6: UIM consensus region not found in yeast homolog Tap42. A) Sequence comparison of UIM consensus sequence, UIM motif in Alpha4, and aligned area of TAP42 showing no UIM consensus motif in Tap42. Asterisks denote residues which when mutated in HRS-UIM have been shown to have a detrimental effect on ubiquitin binding (*5*). **B)** Overlay of Alpha4 (blue) with UIM consensus (yellow) and Tap42 (cyan)(2V0P) with aligned sequence residues (light cyan) showing dissimilarity of aligned sequences. UIM conserved residues (and comparable aligned residues in Tap42) shown as sticks.

SUPPLEMENTARY REFERENCES

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5. Fisher, R. D., Wang, B., Alam, S. L., Higginson, D. S., Robinson, H., Sundquist, W. I., and Hill, C. P. (2003) Structure and ubiquitin binding of the ubiquitin-interacting motif, *J Biol Chem 278*, 28976-28984.