SUPPLEMENTAL DATA

METHODS

NMR experiments. Measurements were done in a buffer containing 20 mM Na_2HPO_4 pH 6.5 and 100 mM NaCl on a Bruker DRX-600 spectrometer. Spectra were processed with program NMRPipe (Delaglio et al. 1995) and analyzed with NMRView (Johnson and Blevins 1994). Partial backbone chemical shift assignments of the AICD-C32/Fe65-PTB2 complex were obtained using standard methods (Sattler et al. 1999).

REFERENCES

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J., and Bax, A. (1995). NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J Biomol NMR *6*, 277-293.

Johnson, B.A., and Blevins, R.A. (1994). NMRview: A computer program for the visualization and analysis of NMR data. J Biomol NMR *4*, 603-614.

Sattler, M., Schleucher, J., and Griesinger, C. (1999). Heteronuclear multidimensional NMR experiments for the structure determination of proteins in solution employing pulsed field gradients. Prog NMR Spectroscop *32*, 93-158.

Figure S1 Superpositions of NMR spectra of Fe65-PTB2 with different AICD peptides. The superpositions of the ^{15}N-^{1}H heteronuclear single-quantum coherence (HSQC) spectra of the following peptide/protein complexes are shown in the given AICD/Fe65-PTB2 ratios: **(A)** Fe65-PTB2 (blue) with AICD-C32/Fe65-PTB2 (black, ratio 2:1). **(B)** AICD-C32/Fe65-PTB2 (black) and AICD-C50/Fe65-PTB2 (magenta, ratio 2:1). The two spectra superpose exactly. The AICD-C32 includes the sequence $D^{\underline{665}}$AAVTPEERHLSKMQQNGYENPTYKFFEQMQN. **(C)** AICD-C32/Fe65-PTB2 (black) with AICD-11mer/Fe65-PTB2 (green, ratio 6:1). The AICD-11mer includes the consensus PTB-binding sequence ($N^{\underline{680}}$GYENPTYKFF) as bound to other PTB domains (X11, PDB code 1x11; mDab1, PDB code 1oqn). **(D)** Free Fe65-PTB2 (blue) and both complexes with Fe65-PTB2 bound to either AICD-C32 (black) or AICD-11mer (green).

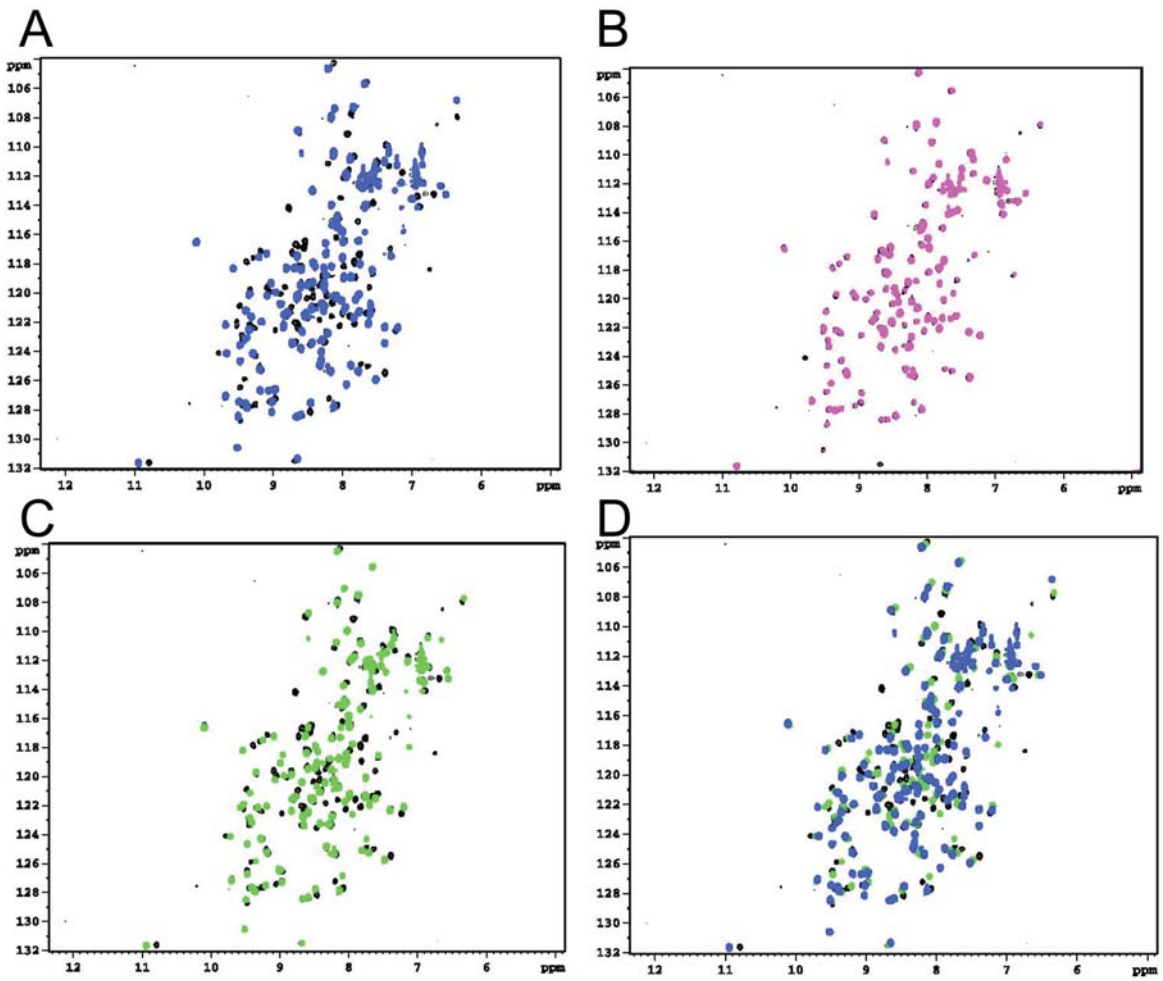


Figure S2 Structure-based sequence alignment of Dab-like PTB domains. The sequence alignment is based on the superposition of the respective PTB domain structures (APBB3, Fe65L2: green, PDB code 2dyq; X11, red, PDB code 1x11; mDab1, blue, PDB code 1oqn). Secondary structure and numbering above the sequences corresponds to Fe65-PTB2, with the exception of helix α1 (gray) of the mDab1 structure. All secondary structures are boxed within the respective sequences.

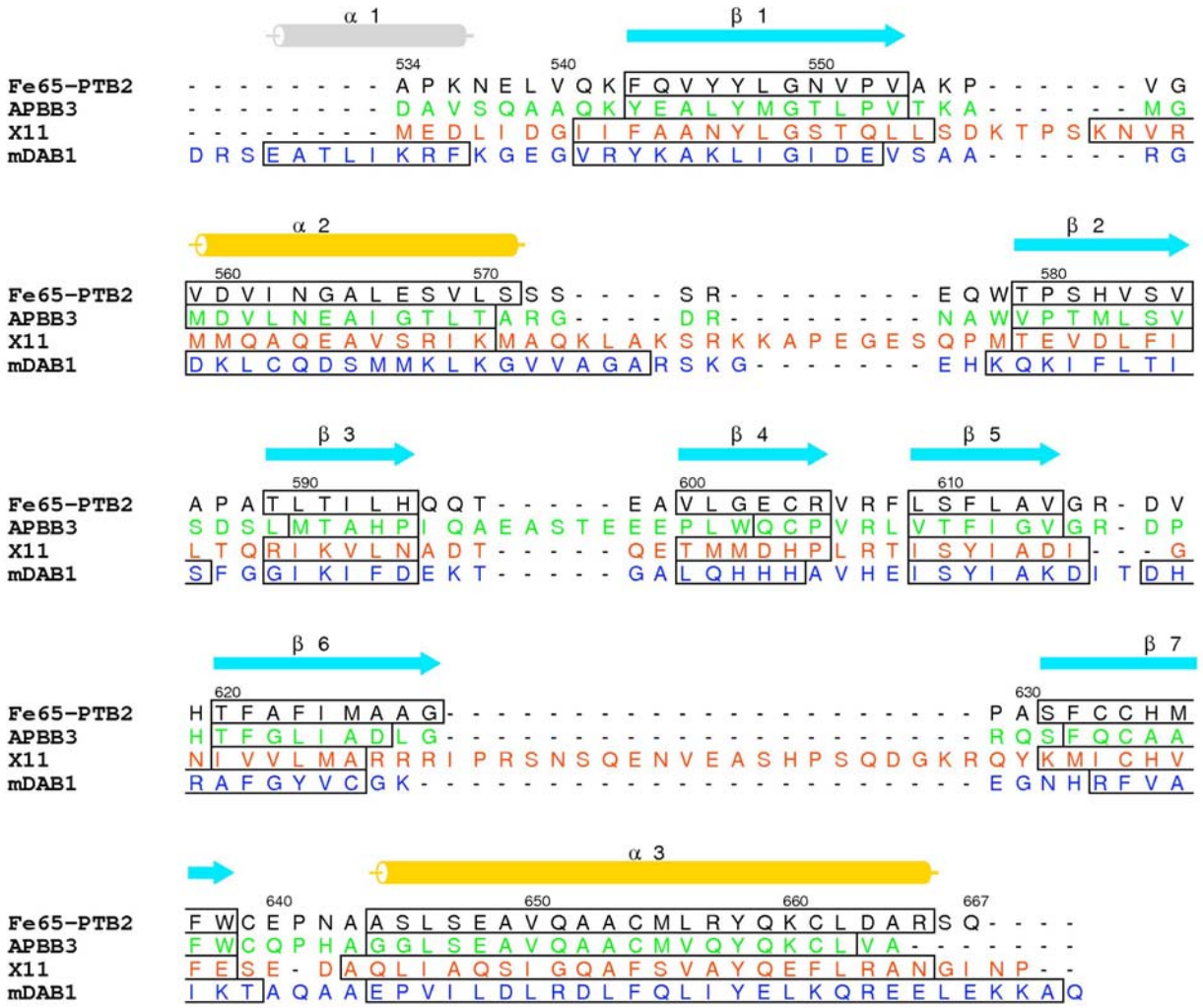


Figure S3 Superposition of Dab-like PTB domains**.** Fe65-PTB2 is shown in a cartoon representation with APBB3, X11, and mDab1 superposed and shown as ribbons. Colour coding is identical to Fig. 1. Termini and secondary structures are labelled.

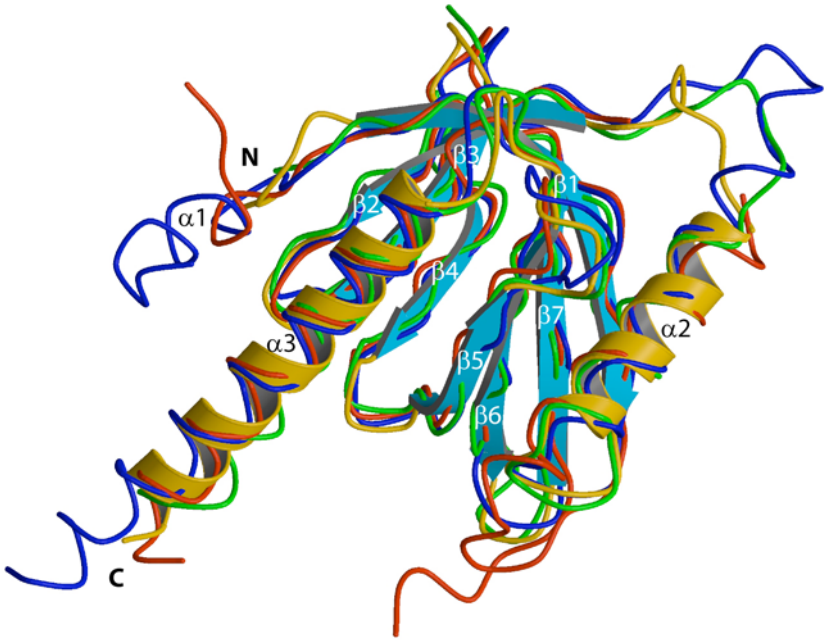
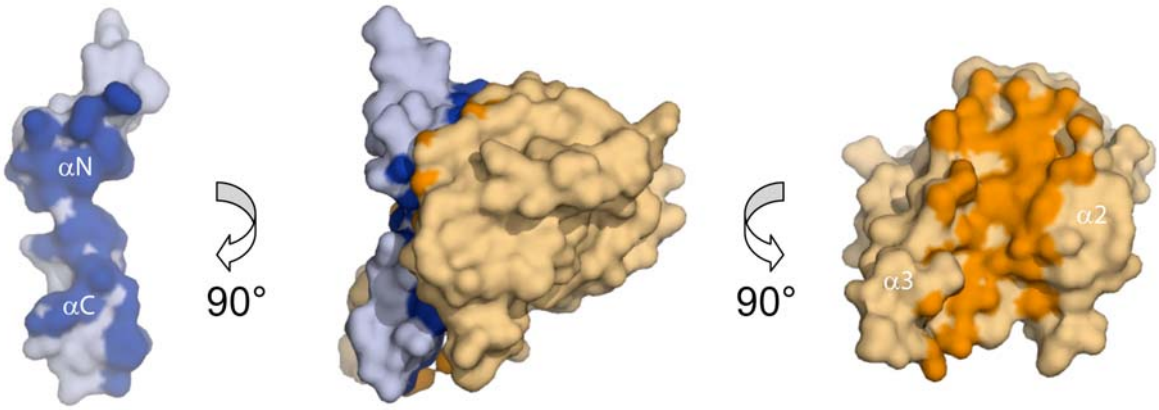


Figure S4 The AICD/Fe65-PTB2 interface. **(A)** Surface representation of the AICD/Fe65-PTB2 complex with the AICD in blue and Fe65-PTB2 in orange (middle panel). Interacting regions are highlighted in darker colours. The outer panels are rotated and the molecules separated to have a view inside the binding interface. The view of the right panel corresponds approximately to Fig. 1a in the manuscript. **(B)** Detailed view of the AICD/Fe65-PTB2 interaction including all involved residues and given together with a semitransparent Fe65-PTB2 surface.

A



B

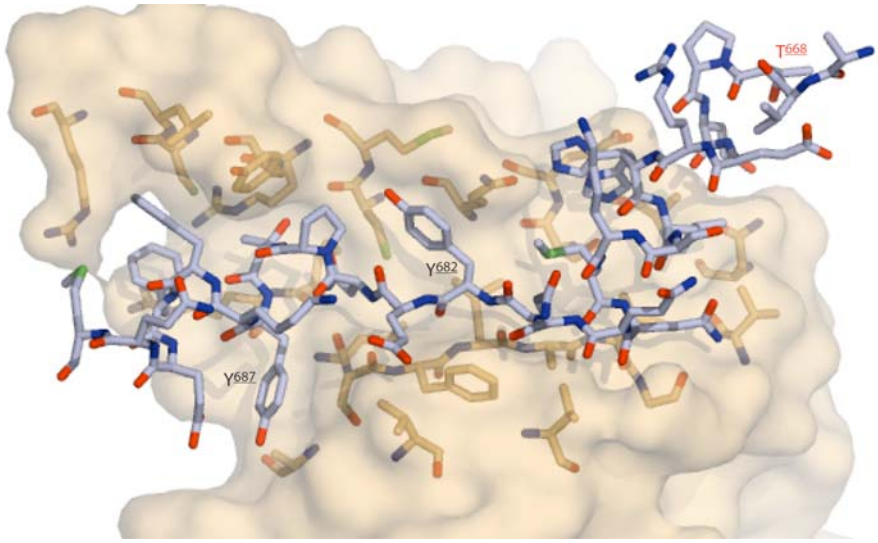


Figure S5 Superposition of the AICD peptide bound to different PTB domains. Stereo view of the AICD peptide (residues N^{680} to K^{688}) in complexes with three different phosphotyrosine binding domains: Fe65-PTB2 (yellow), X11 (magenta), PDB code 1x11, and mDab1 (black), PDB code 1oqn. In the AICD/Fe65-PTB2 complex, the peptide bond of G^{681} is flipped in respect to the other structures. In the AICD/X11 complex, Y^{682} reveals a different sidechain conformation. In all structures the two tyrosines Y^{682} and Y^{687} are not phosphorylated.

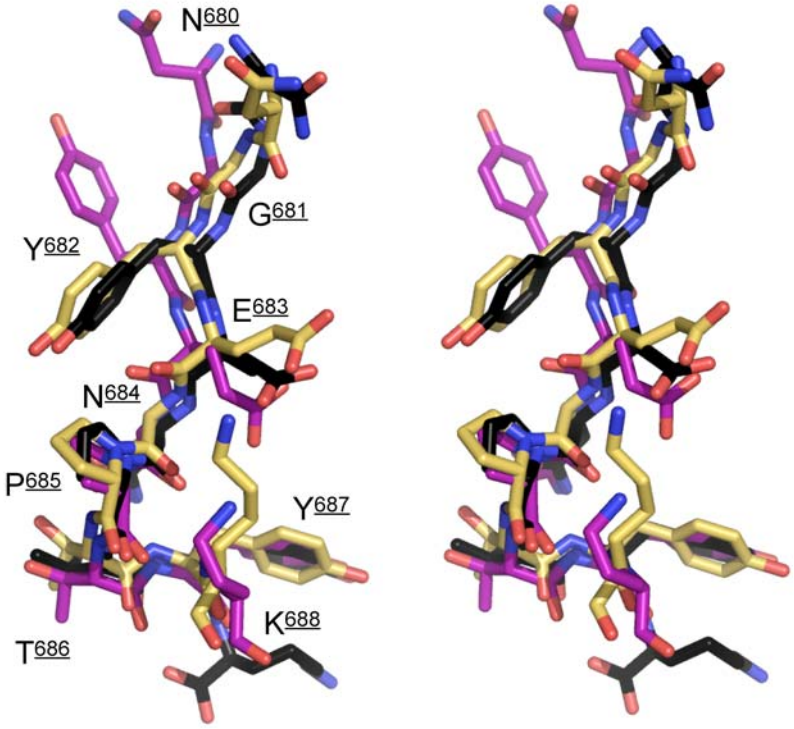


Figure S6 NMR spectra showing the structural changes upon T^{668} phosphorylation. **(A)** Superposition of ^{15}N-^{1}H heteronuclear single-quantum coherence (HSQC) spectra of Fe65-PTB2 with either AICD-C32 unphosphorylated (black) or with pT^{668} (red) added in a 1:2 or 1:4 ratio, respectively. The shifting Fe65-PTB2 residues (N-terminus of helix α3 that binds to the T^{668}PEE capping box) are labelled. **(B)** Surface representation of Fe65-PTB2 in complex with AICD-C32 (shown as ribbons). Fe65-PTB2 residues revealing structural changes in the comparison of the complexes with unphosphorylated and pT^{668} AICD-C32 are coloured in red.

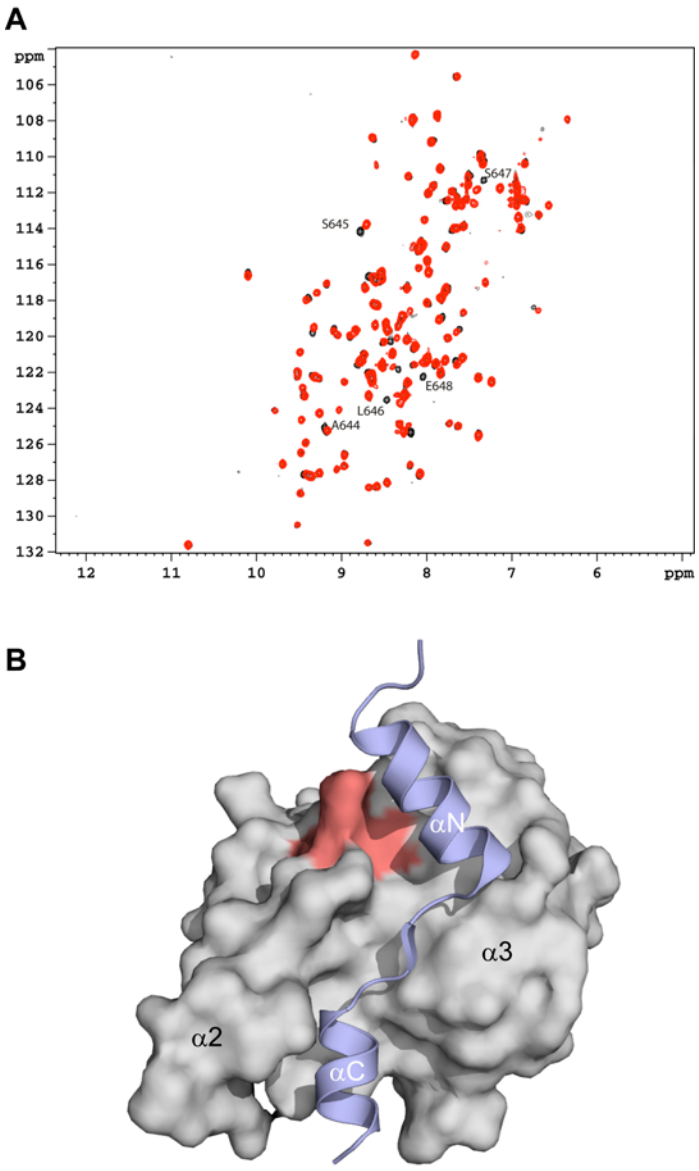


Table S1 Refinement statistics for AICD mutant structures

Complex of Fe65-PTB2 with	***AICD-T668E***	***AICD-T668A***
Resolution (Å)	33.7 – 2.2	19.3 – 2.0
No. reflections	27268	33505
R_{work} / R_{free}	20.2 / 24.7	20.6 / 24.1
No. atoms		
Protein	2441	2432
Water molecules	128	236
B-factors (Å^2)		
Overall	50.9	42.6
Fe65-PTB2	48.1	39.2
AICD-C32	62.0	52.8
Water	50.1	48.9
R.m.s. deviations		
Bond lengths (Å)	0.015	0.019
Bond angles (°)	1.400	1.569
Ramachandran plot quality (%)		
Most favoured	94.1	95.2
Additionally allowed	5.9	4.8