

Supplementary Figure legends

Fig. S1: Representative portion of the 2Fo-Fc map of the SHARPIN complex. The electron density map (grey) is contoured at 1.0σ . The dimer halves are shown as a stick model in orange and blue, respectively. The perpendicular orientation of the two helices is clearly visible.

Fig. S2: Interface of subunit I and IV. His25 (deprotonated at pH 7.7 in the crystallization solution) forms a stacking interaction which is sandwiched between a salt bridge formed by Lys43 and Glu108.

Fig. S3 Comparison of the electrostatic potentials of SHARPIN (A) with a typical phospholipid binding PH domain (β -spectrin; PDB accession number: 1BTN) bound to its ligand Ins(1,4,5)P₃ (B). The charge distributions are contoured at 1 kT and displayed in the same orientation as the ribbon presentations. The IP3 binding site of β -spectrin coincides clearly with a strong positive electrostatic potential (blue). The corresponding position in SHARPIN is predominantly negatively charged (red). The charge distributions at pH 7.4 were calculated using the program ABPS (1).

Fig. S4A Comparison of an EVH1 domain (Mena; PDB accession number 1EVH) (green) bound to a polyproline peptide (FPPPP) (magenta) in a polyproline II (PPII) conformation with the PH domain of SHARPIN (orange). (B) Hydrophobic contacts to the the proline rich sequence are primarily mediated by an “aromatic triad”, which is conserved in EVH1 domains but is absent in SHARPIN. A hypothetical complex between the poly proline peptide and SHARPIN shows the corresponding side chains of the PH domain.

References

1. Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 10037-10041.

Figure S1

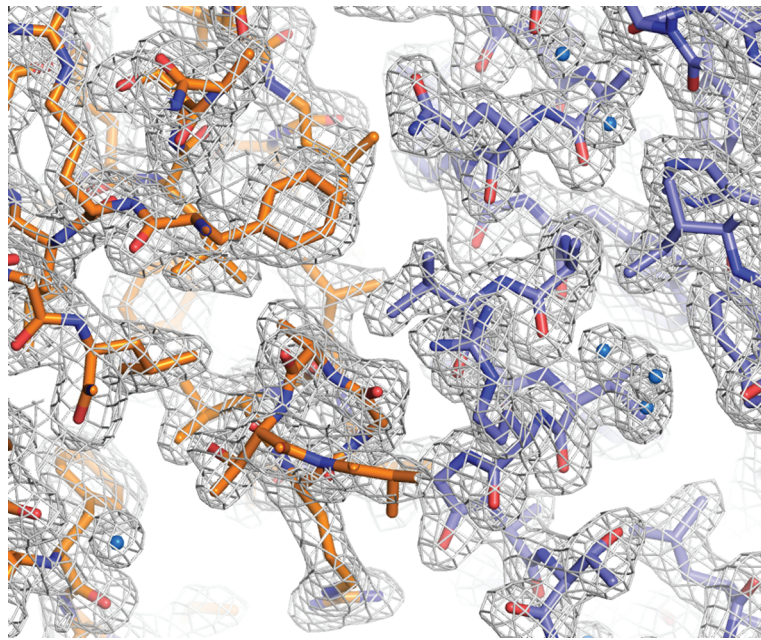


Figure S2

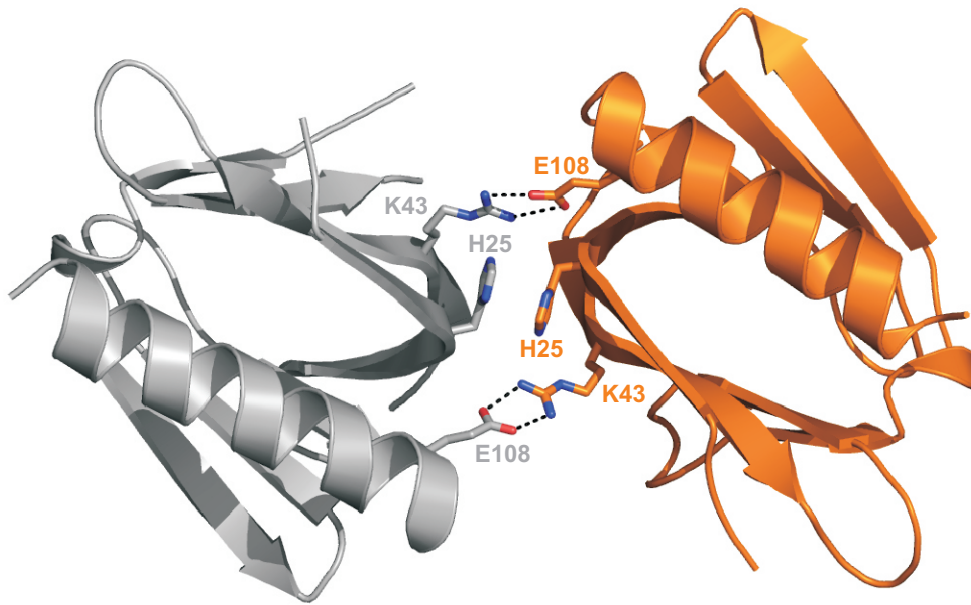
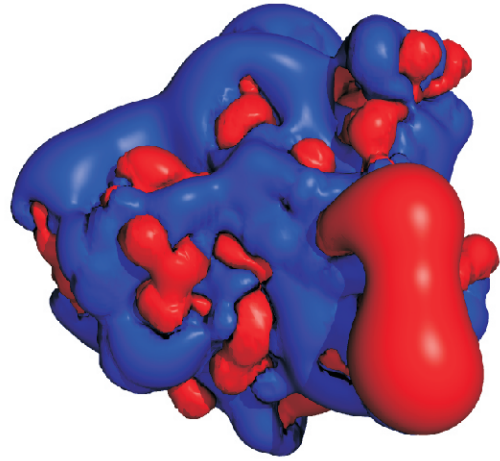


Figure S3

A



B

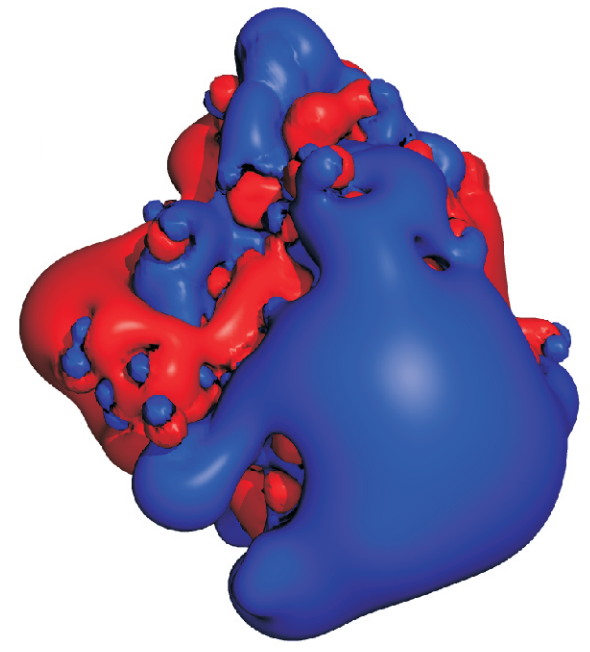


Figure S4

