Supporting Information

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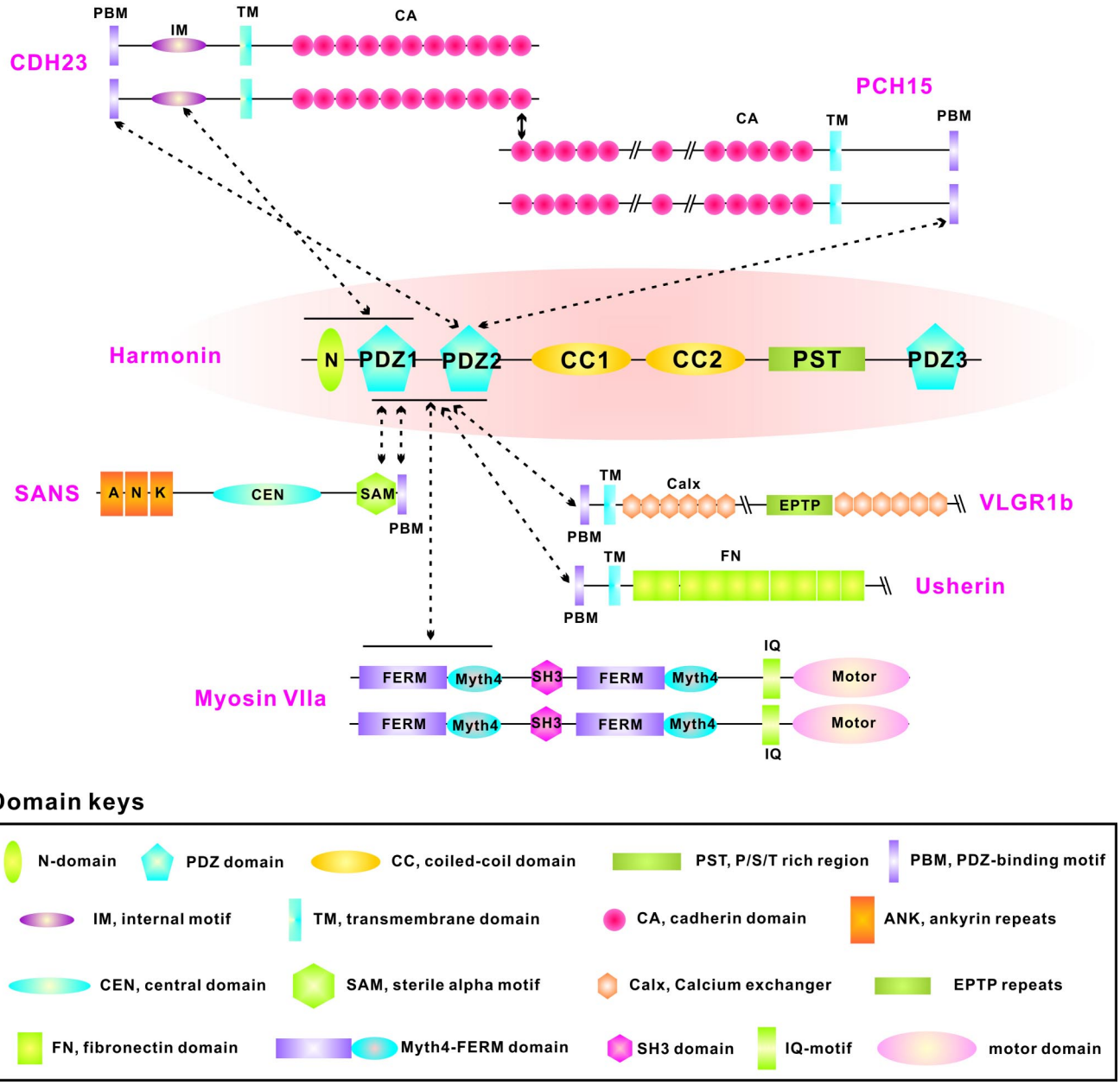


Fig. S1. The interaction network of the identified Usher proteins. In this presentation, the interactions that were previously known are indicated by black arrows and the newly-defined harmonin N-domain is also included. The figure also highlights that harmonin functions as the organization center for the assembly of the Usher protein interactome.

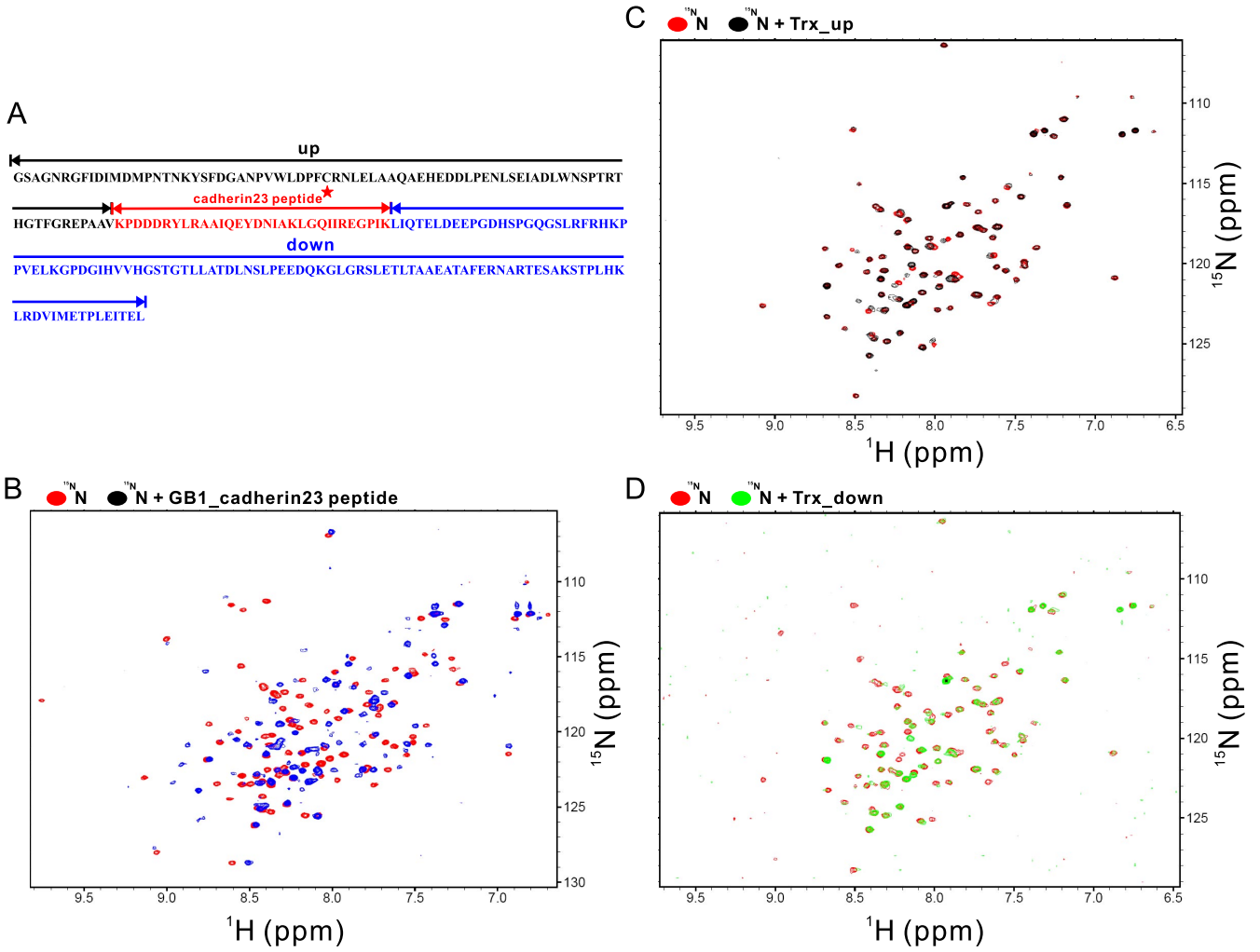


Fig. S2. Mapping of the exact harmonin N-domain binding region of cadherin 23. (*A*) The cadherin 23 cytoplasmic domain was divided into 3 regions, the up region (black), the middle region (red), and the down region (blue), to test their potential interaction with the harmonin N-domain. (*B*) Superposition plot of the ^{1}H-^{15}N HSQC spectra of the free-form N-domain and the protein in the presence of a saturating amount of the GB1-tagged cadherin 23 peptide (the middle region). The gross difference of the 2 spectra indicates the specific interaction between the harmonin N-domain and the cadherin 23 peptide fragment. (*C* and *D*) Superposition plot of the ^{1}H-^{15}N HSQC spectra of the free N-domain and the protein domain in the presence of excess amounts of the cadherin 23 up fragment (*C*) or down fragment (*D*), showing no specific interaction between the harmonin N-domain and these two cadherin 23 fragments.

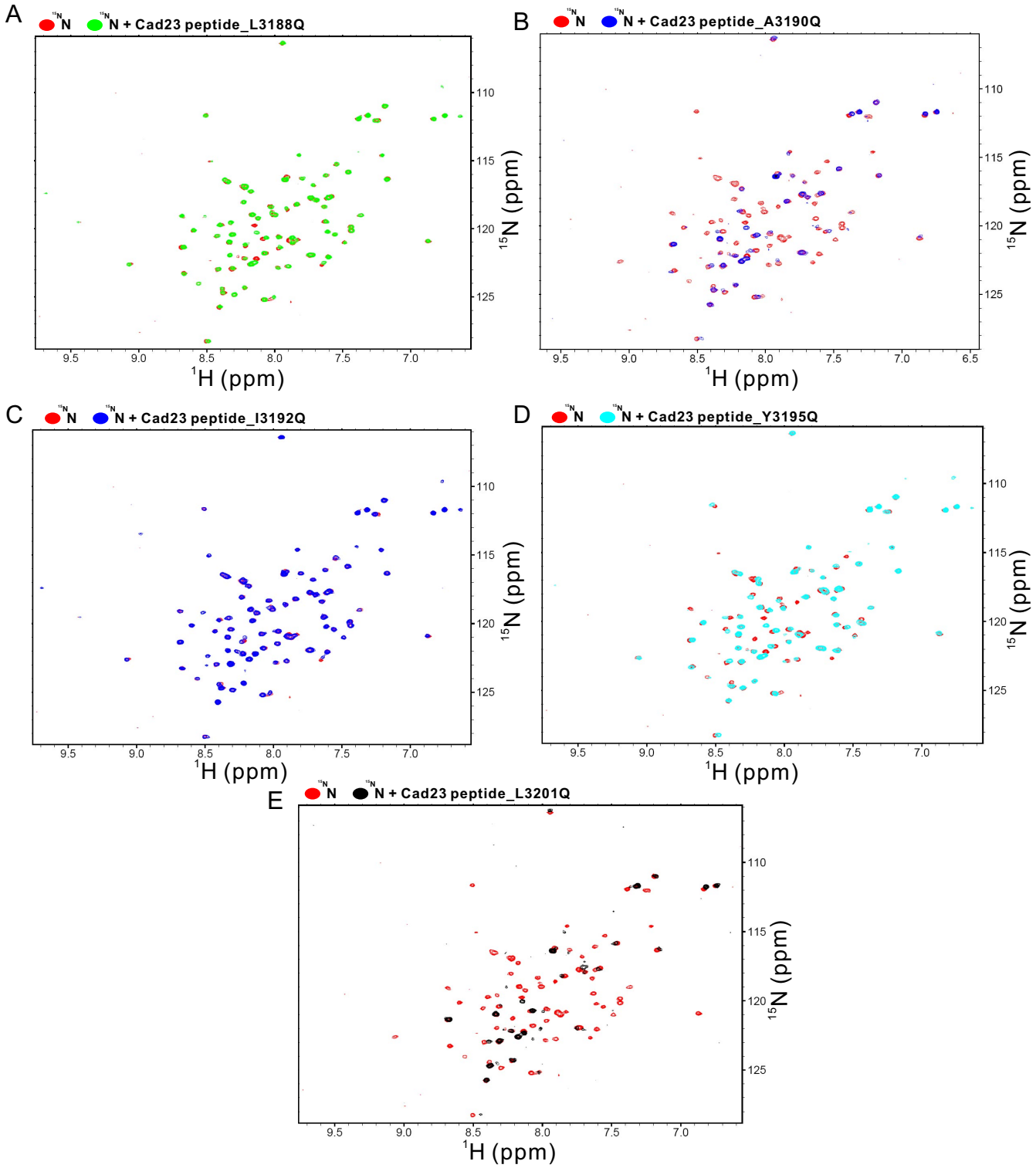
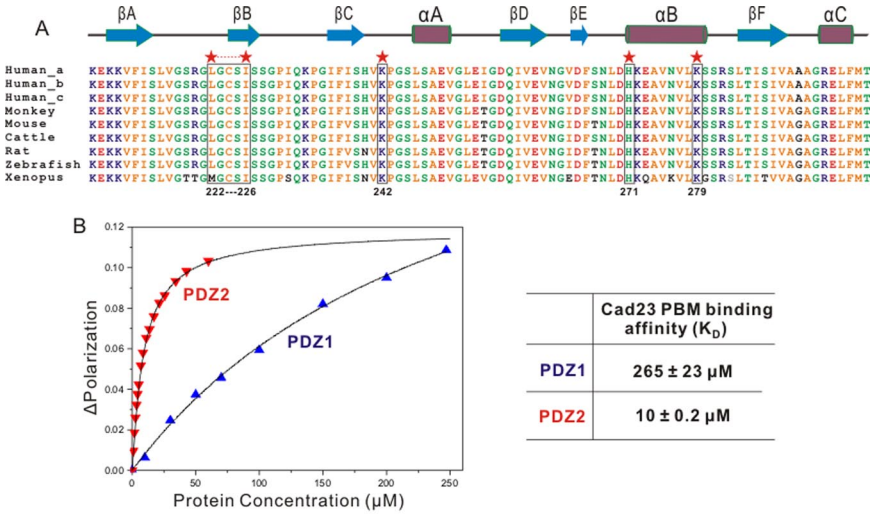


Fig. S3. Identification of the critical residues of the cadherin 23 internal peptide required for binding to the harmonin N-domain. Superposition plot of the ^{1}H-^{15}N HSQC spectra of the free-form harmonin N-domain and the protein in the presence of excess amounts of various forms of GB1-tagged Cad23 peptide mutants. (*A*, *C*, and *D*) Substitutions of Leu-3188 (*A*), Ile-3192 (*C*), and Tyr-3195 (*D*) with Gln individually abolished the Cad23 peptide binding to the harmonin N-domain. (*B* and *E*) In contrast, replacement of Ala-3190 (*B*) or Leu-320 (*E*) with Gln had no obvious impact on its binding to the harmonin N-domain.



	Cad23 PBM binding affinity (K_D)
PDZ1	265 ± 23 μM
PDZ2	10 ± 0.2 μM

Fig. S4. Harmonin PDZ2 specifically binds to the carboxyl tail of cadherin 23. (*A*) Structure-based sequence alignment of harmonin PDZ2 from different species. In this alignment, the conserved hydrophobic residues are shown in orange, negatively-charged residues are in red, positively-charged residues are in blue, and the rest of the highly-conserved residues are in green. The residues that are directly involved in binding to the Cad23 PBM are boxed and highlighted by red stars. (*B*) Fluorescence polarization assay showing that the Cad23 PBM specifically binding to the PDZ2 domain of harmonin.

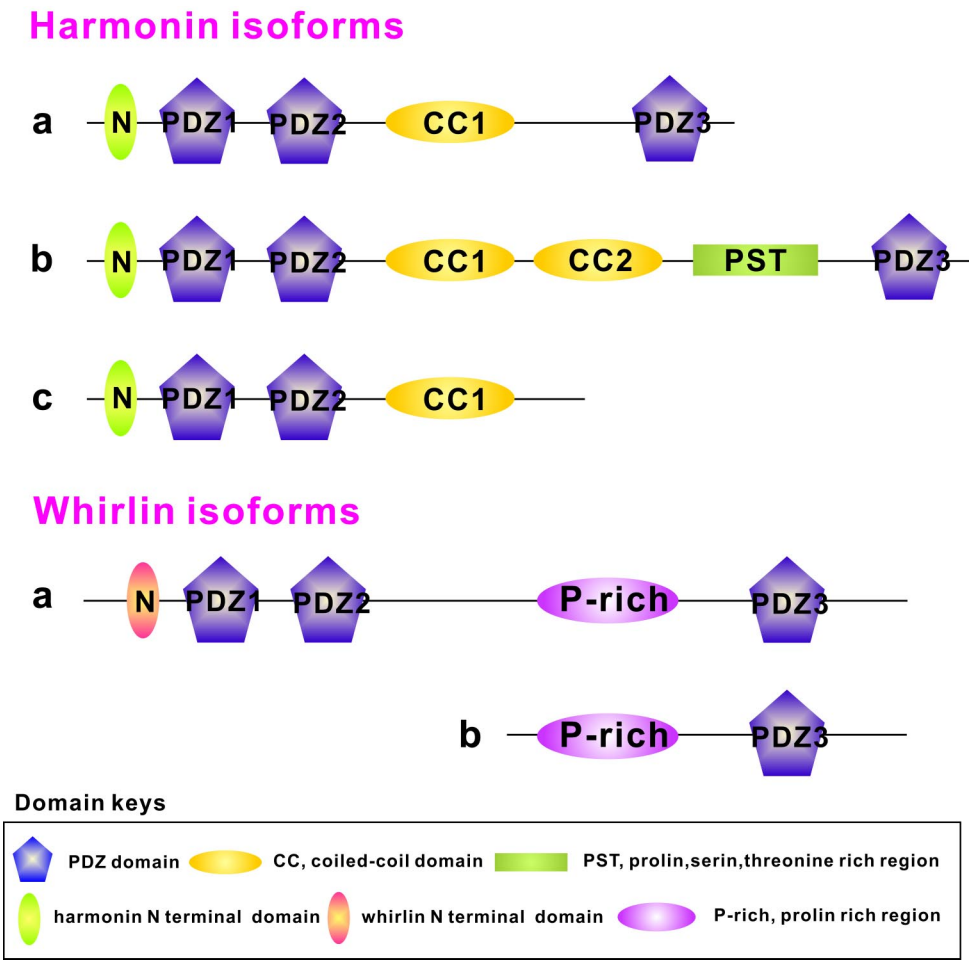


Fig. S5. Schematic diagram depicting the domain organizations of different isoforms of harmonin and whirlin.

Table S1. Structural statistics for the family of 20 NMR structures of the harmonin N-domain, the N-domain in complex with the Cad23 peptide, and the harmonin PDZ2/Cad23 PBM complex

Statistic	N-domain	N/Cad23 complex	PDZ2/Cad23 complex
Distance restraints			
Intraresidue ($i - j = 0$)	533	654	505
Sequential($\|i - j\| = 1$)	272	335	462
Medium range($2 \leq \|i - j\| \leq 4$)	228	266	318
Long range($\|I - j\| \geq 5$)	174	192	725
Hydrogen bonds	70	86	52
Total	1,277	1,533	2,062
Dihedral angle restraints			
Φ	57	72	48
Ψ	57	72	48
Total	114	144	96
Mean rmsd from the experimental restraints			
Distance, Å	0.001 ± 0.000	0.014 ± 0.000	0.003 ± 0.000
Dihedral, °	0.010 ± 0.008	0.235 ± 0.019	0.267 ± 0.048
Mean rmsd from idealized covalent geometry			
Bond, Å	0.001 ± 0.000	0.002 ± 0.000	0.001 ± 0.000
Angle, °	0.319 ± 0.005	0.355 ± 0.011	0.290 ± 0.004
Improper, °	0.125 ± 0.008	0.223 ± 0.011	0.134 ± 0.006
Mean energies, (kcal·mol^{-1})			
E_{NOE}*	2.98 ± 0.25	24 ± 1.43	1.1 ± 0.12
E_{cdih}*	0.00 ± 0.00	0.49 ± 0.08	0.43 ± 0.15
E_{L-J}	−280.6 ± 16.1	−342.0 ± 12.6	−322.8 ± 15.1
Ramachandran plot†			
% residues in the most favorable regions	90.4	93.3	82.9
Additional allowed regions	9.3	6.0	14.6
Generously allowed regions	0.0	0.7	2.0
Atomic rms differences, Å			
Backbone heavy atoms (N, C$^{\alpha}$, and C′)	0.48‡	0.43§	0.35¶
Heavy atoms	0.97‡	0.93§	0.83¶

None of the structures exhibits distance violations >0.3 Å or dihedral angle violations >4°.

*The final values of the square-well NOE and dihedral angle potentials were calculated with force constants of 50 kcal·mol^{-1}Å^{-1} and 200 kcal·mol^{-1}rad^{-1}.

†The PROCHECK-NMR program (www.biochem.ucl.ac.uk/~roman/procheck_nmr/procheck_nmr.html) was used to assess the overall quality of the structures.

‡Residues 4–77 of harmonin N-domain were used for superposition analysis.

§Residues 4–77 of N-domain plus the residues 3187–3197 of Cad23 peptide were used for superposition analysis.

¶Residues 209–216 and 222–298 of harmonin PDZ2 plus the last 5 residues of the Cad23 PBM were used for superposition analysis.