Supplemental Information Inventory:

Supplemental Data contains Figures S1-S4 and Table S1.

List of Supplemental Data Files:
File S1, related to **Figure 1**. Validation report for the A1 structure.
File S2, related to **Figure 1**. Validation report for the C3 structure.
File S3, related to **Figure 6**. Alignment used for isoform conservation ratio (ICR) analysis.
File S4, related to **Figure 6**. Spreadsheet containing the ICR data.
File S5, related to **Figure 7**. Alignment used for EVCouplings.
File S6, related to **Figure 7**. Coupling scores from EVcouplings

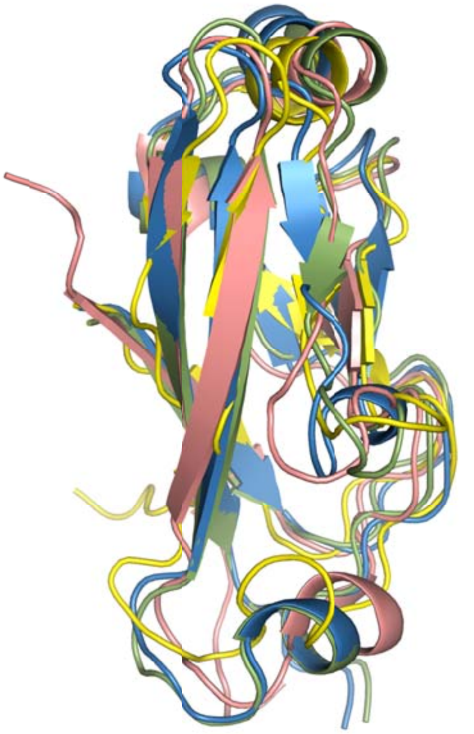

Figure S1, related to **Figure 1.** Superpositions indicate similar structures of clustered Pcdh EC1. Alignment of PcdhγA1 (blue), PcdhγC3 (green), Pcdhα4 (pink; 1wuz), and Pcdhβ14 (yellow; 1wyj). Pairwise backbone root-mean-square deviations (rmsds) range from 1.05 to 2.01 Å, indicating structural conservation among all subfamilies (α-, β-, γ-, and C-type) of clustered Pcdhs.

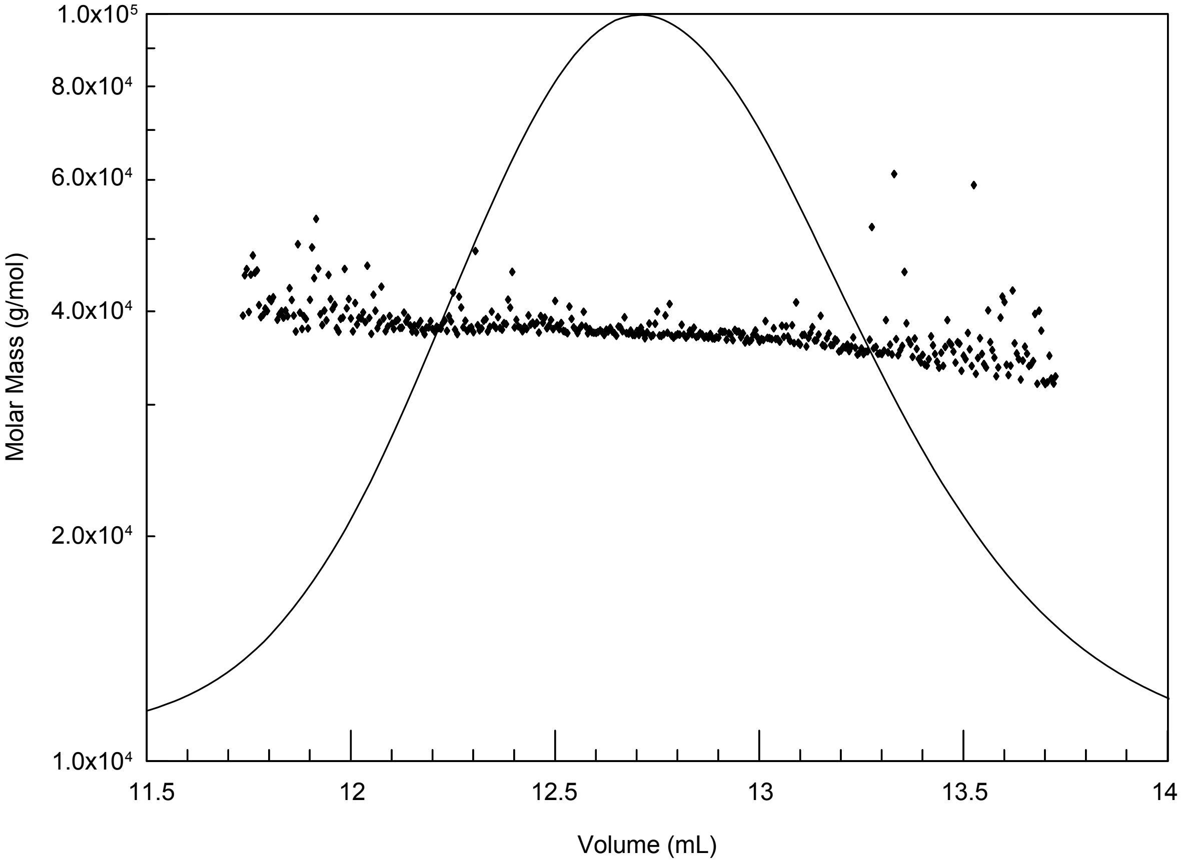

Figure S2, related to **Figure 2.** Multi-angle laser light scattering shows that A1 is a monomer in solution. The major peak at 12.86 mL corresponded to a molar mass of 37.7 kDa, compared to the predicted molar mass of 36.7 kDa.

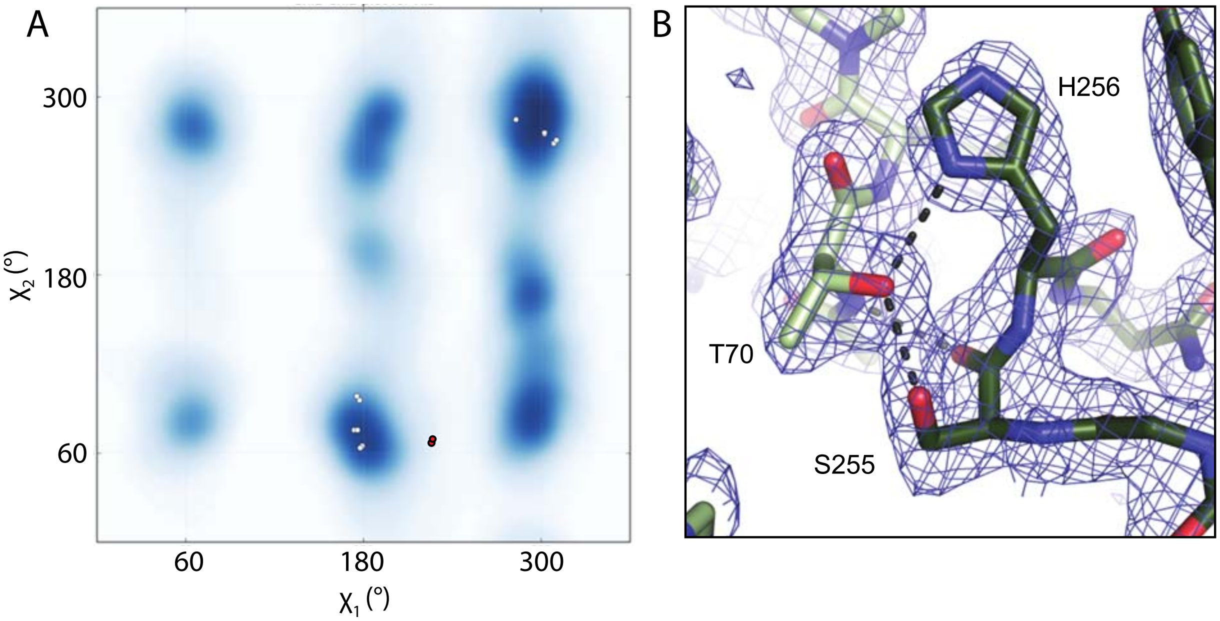

Figure S3, related to **Figure 3.** Histidine χ angle distributions for residues in C3 structure. (A) H256 from both subunits in the asymmetric unit (red symbols) are rotamer outliers with χ_1 of ~230° and χ_2 of ~60°. Blue shading corresponds to distribution of χ angles for histidine residues in the PDB, as generated by PHENIX (1). (B) Electron density at (final $2F_o$-F_c map at 1σ) of a hydrogen-bond network involving T70 from one protomer with S255 and H256 of another protomer from the C3 structure. Hydrogen bonds hold H256 is a stable rotamer outlier position.

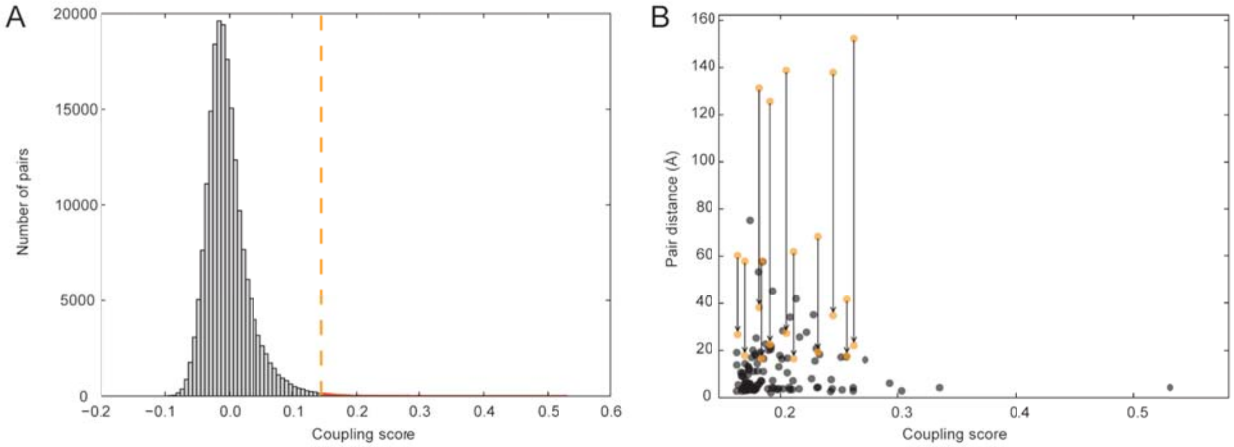

Figure S4, related to **Figure 7.** Top evolutionary correlations support antiparallel complex formation. (A) Histogram of correlated pair coupling scores from EVCouplings analysis. The vertical orange line indicates the significant coupling score cutoff of 1.6 times the background-normalized coupling scores. (B) The top 320 (alignment length/2) coupling scores plotted against the pair distance in the C3 monomer structure (black). Orange pairs are the 11 cross-EC pairs whose pair distance is minimized (shown by the dropped down arrows) in the A1 and C3 complexes. The minimized pair distance for the cross-EC pairs is the minimum of the pair distance when considering both the A1 and C3 complex structures.

Table S1, related to **Figure 4**. Cadherin structures used in analysis illustrated in **Figure 4**

PDB ID	Protein	Gene name	Species	#ECs	Group	Notes
1l3w	C-cadherin	CDH3	*Xenopus laevis*	5	Classical	only used EC1-3
2qvf	E-cadherin	CDH1	*Mus musculus*	2	Classical	
3q2v_a	E-cadherin	CDH1	*Mus musculus*	5	Classical	only used EC1-3
1edh	E-cadherin	CDH1	*Mus musculus*	2	Classical	
2qvI	N-cadherin	CDH2	*Mus musculus*	2	Classical	
3q2w	N-cadherin	CDH2	*Mus musculus*	5	Classical	only used EC1-3
3ubh	N-cadherin	CadN CG7100	*Drosophila melanogaster*	4	Classical	only used EC1-2
1ncj	N-cadherin	CDH2	*Mus musculus*	2	Classical	
3ppe_a	VE-cadherin	CDH5	*Gallus gallus*	2	Classical	
3lnd_a	Cadherin-6	CDH6	*Mus musculus*	2	Classical	W4A
2a62	Cadherin-8	CDH8	*Mus musculus*	3	Classical	
2a4e	Cadherin-11	CDH11	*Mus musculus*	2	Classical	
3k5s_a	T-cadherin	CDH13	*Gallus gallus*	2	Classical	
3mvs	Cadherin-23	CDH23	*Mus musculus*	2	Pcdh/non-classical	
2wcp	Cadherin-23	CDH23	*Mus musculus*	2	Pcdh/non-classical	
2whv	Cadherin-23	CDH23	*Mus musculus*	2	Pcdh/non-classical	all Ca^{2+}-binding sites bound
4apx_a	Cadherin-23	CDH23	*Mus musculus*	2	Pcdh/non-classical	
4apx_b	Pcdh-15	PCDH15	*Mus musculus*	2	Pcdh/non-classical	
4aq8_a	Cadherin-23	CDH23	*Mus musculus*	2	Pcdh/non-classical	form II
4aq8_c	Pcdh-15	PCDH15	*Mus musculus*	2	Pcdh/non-classical	form II
Pcdh15EC35	Pcdh-15	PCDH15	*Homo sapiens*	3	Pcdh/non-classical	unpublished; only used EC3-4
PcdhgC3	PcdhgC3	PcdhgC3	*Mus musculus*	3	clustered Pcdh	
PcdhgA1	PcdhgA1	PcdhgA1	*Mus musculus*	3	clustered Pcdh	