SUPPORTING INFORMATION

Tuning Inner-Ear Tip-Link Affinity Through Alternatively Spliced Variants of Protocadherin-15

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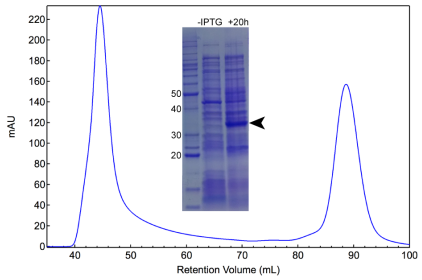
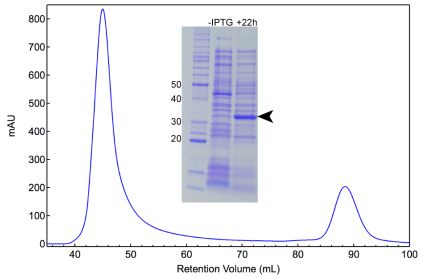
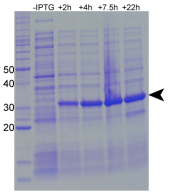
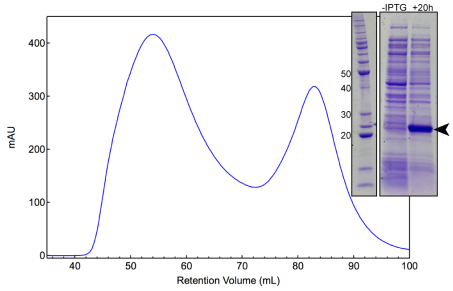
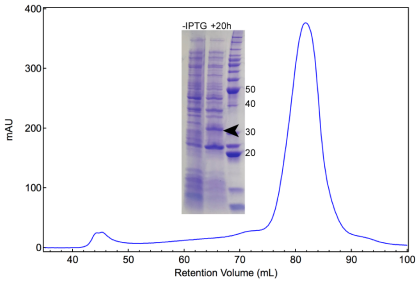
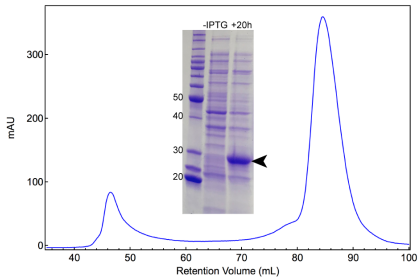
VARIANT	AMINO ACID SEQUENCE	SEC/SDS GEL
N1 UniProt entry: Q99PJ1-1 Accession no.: AAG53891 MW=26.2 kDa; 233 aa	QYDDDWQYEDCKLARGGPPATIVAID EESRNGTILVDNMLIKGTAGGPDPTIE LSLKDNVDYWVLLDPVKQMLFLNSTG RVLDRDPPMNIHSIVVQVQCVNKKVG TVIYHEVRIVVRDRNDNSPTFKHESYY ATVNELTPVGTTIFTGFSGDNGATDID DGPNGQIEYVIQYNPEDPTSNDTFEIP LMLTGNVVLRKRLNYEDKTRYYVIIQA NDRAQNLNERRTTTTTLTVD	
N2 UniProt entry: Q99PJ1-2 Accession no.: DQ354396 MW=25.5 kDa; 228 aa	QYDDDCKLARGGPPATIVAIDEESRN GTILVDNMLIKGTAGGPDPTIELSLKD NVDYWVLLDPVKQMLFLNSTGRVLD RDPPMNIHSIVVQVQCVNKKVGTVIY HEVRIVVRDRNDNSPTFKHESYYATV NELTPVGTTIFTGFSGDNGATDIDDGP NGQIEYVIQYNPEDPTSNDTFEIPLML TGNVVLRKRLNYEDKTRYYVIIQAND RAQNLNERRTTTTTLTVD	
N3 UniProt entry: Q99PJ1-8 Accession no.: DQ354402 MW=23.3 kDa; 206 aa	QYDDGTILVDNMLIKGTAGGPDPTIELS LKDNVDYWVLLDPVKQMLFLNSTGRV LDRDPPMNIHSIVVQVQCVNKKVGTVI YHEVRIVVRDRNDNSPTFKHESYYATV NELTPVGTTIFTGFSGDNGATDIDDGP NGQIEYVIQYNPEDPTSNDTFEIPLMLT GNVVLRKRLNYEDKTRYYVIIQANDRA QNLNERRTTTTTLTVD	
N4 UniProt entry: Q99PJ1-7 Accession no.: DQ354401 MW=21.1 kDa; 191 aa	QYDDDCKLARGGPPATIVAIDEESRN GTILVDNMLIKGTAGGPDPTIELSLKD NVDYWVLLDPVKQMLFLNSTGRVLD RDPPMNIHSIVVQVQCVNKKVGTVIY HEVRIVVRDRNDNSPTFKHESYYATV NELTPVGTTIFTGFSGDNGATDIDDGP NGQIEYVIQYNPEDPDRAQNLNERRT TTTTLTVD	
N5 Accession no.: DQ354407 MW=28.3 kDa; 260 aa	MTMAEQDNGHPLPAFASLHIEILDEN NQSPYFTMPSYQGYILESAPVGATISE SLNLTTPLRIVALDKDIEDVPPGGVPT KDPELHLFLNDYTSVFTVTPTGITRYL TLLQPVDREEQQTYTFLITAFDGVQE SEPVVVNIRVMDANDNTPTFPEISYD VYVYTDMSPGDSVIQLTAVDADEGSN GEISYEILVGGKGDFVINKTTGLVSIAP GVELIVGQTYALTVQASDNAPPAERR HSICTVYIEVLPPNNQSPPRF	
N6 UniProt entry: Q99PJ1-24 Accession no.: AAY24693 MW=25.1 kDa; 228 aa	QYDDHSICTVYTEVLPPNNQSPPRFP QLMYSLEVSEAMRIGAILLNLQATDRE GDPITYAIENGDPQRVFNLSETTGILS LGKALDRESTDRYILIVTASDGRPDGT STATVNIVVTDVNDNAPVFDPYLPRNL SVVEEEANAFVGQVRATDPDAGING QVHYSLGNFNNLFRITSNGSIYTAVKL NREARDHYELVVVATDGAVHPRHSTL TLYIKVLDIDDNSPVF	

Figure S1. Expression and purification of pcdh15 variants. For each of the variants studied, the UniProt entry code, accession number, and predicted molecular weight are provided in the leftmost column. The amino acid sequence of the two N-terminal EC repeats included in the construct, excluding the start codon (Met) and the hexahistidine tag (including any additional residues due to cloning) are provided in the center column. The sequence is color coded to highlight the end of exon 2 in red, exon 3 in cyan, and exon 4 in purple. The right column shows SDS gels before and after inducing protein expression, and SEC chromatograms of refolded pcdh15 variants. Peaks of aggregated protein are expected near ~45-50 mL and homogenous, refolded two EC repeat constructs are expected at a retention volume between 80-90 mL (Superdex 200 16/600 column).

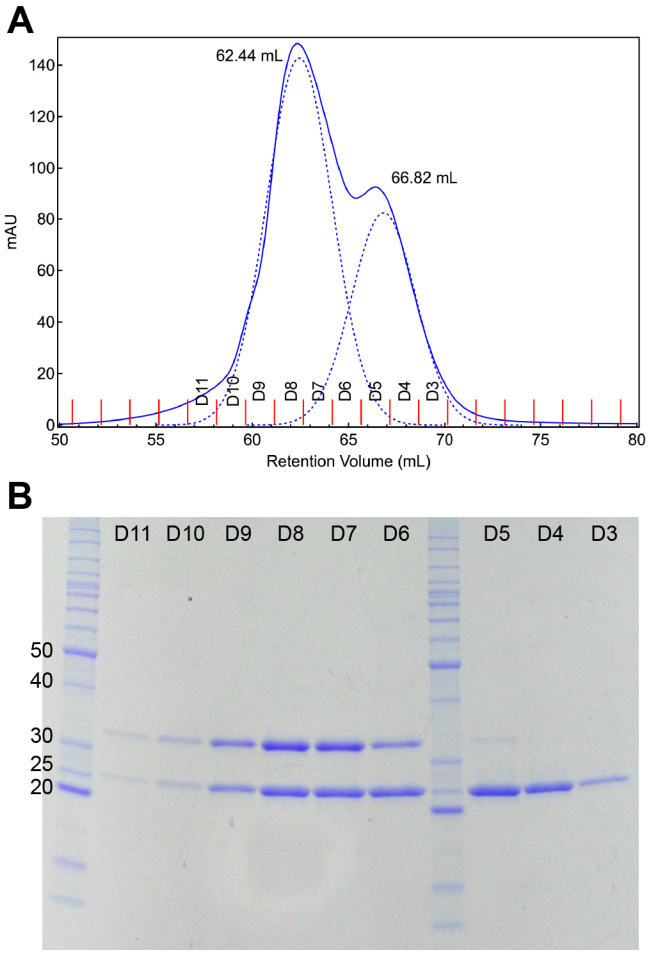


Figure S2. Purification and validation of cdh23-pcdh15(N2) complex. (A) SEC chromatogram displays a new peak at 62.44 mL, which represents the cdh23-pcdh15(N2) complex. The peak at 66.82 mL represents excess cdh23. Dotted lines are theoretical fits used to estimate retention volumes. The sample was analyzed on a Superdex 75 16/600 column. (B) Fractions from the SEC run were analyzed by SDS PAGE. Fractions D9-D6 were combined, concentrated, and utilized for crystal growth.

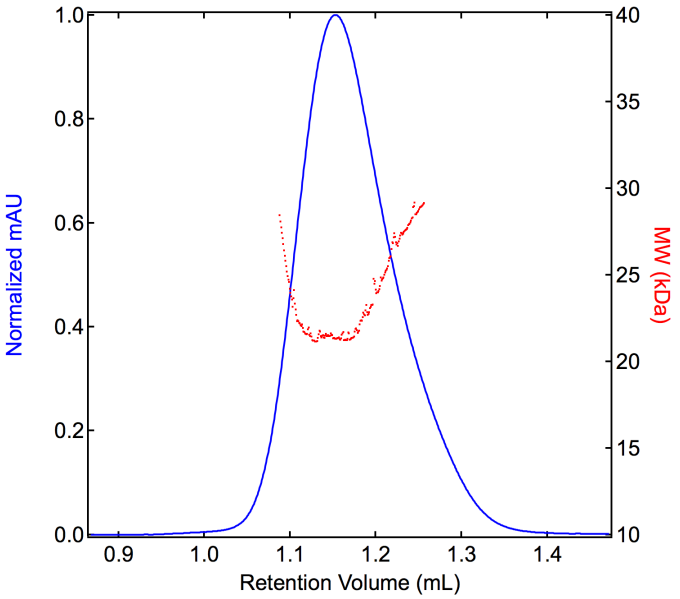
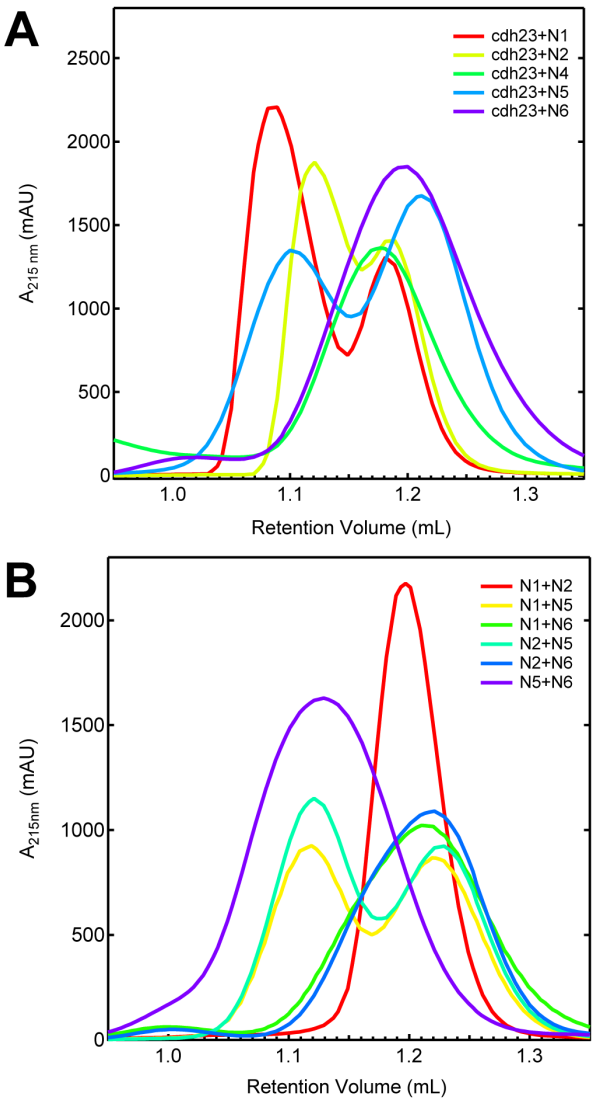


Figure S3. MALS analysis verifies monomeric state of pcdh15(N5). The refolded sample of pcdh15(N5) was analyzed on the AKTAMicro system connected in series with a Wyatt miniDAWN TREOS system. Protein absorbance at 280 nm was monitored (blue trace) as was light scattering from the sample, which was subsequently converted into molecular weight using a rod-like model (red dots). The predicted monomer mass of variant N5 is 28.3 kDa, while the measured value was ~26.0 kDa, which does not approach the molecular weight of a dimeric protein.



C

Sample	Retention Volume (mL)
pcdh15(N1)	1.22
pcdh15(N2)	1.22
pcdh15(N4)	(0.87); 1.14
pcdh15(N5)	1.11
pcdh15(N6)	1.18
cdh23	1.19

D

Sample	Retention Volume (mL)
cdh23+N1	1.09; 1.18
cdh23+N2	1.12; 1.18
cdh23+N4	(0.90); 1.18
cdh23+N5	1.10; 1.21
cdh23+N6	1.20

E

Sample	Retention Volume (mL)
N1+N2	1.20
N1+N5	1.12; 1.22
N1+N6	1.21
N2+N5	1.12; 1.23
N2+N6	1.21
N5+N6	1.13

Figure S4. Analytical SEC analysis of pcdh15 variants and complexes. (A) Heterophilic mixtures of cdh23 and pcdh15. (B) Pseudo-heterophilic mixtures of pcdh15 variants. (C-E) Tables of peak retention volumes for individual proteins (C), heterophilic complexes (D), and pseudo-heterophilic complexes (E) of pcdh15 variants. Peaks in parentheses are present in the chromatogram but not visible in the plots due to the axis scale.

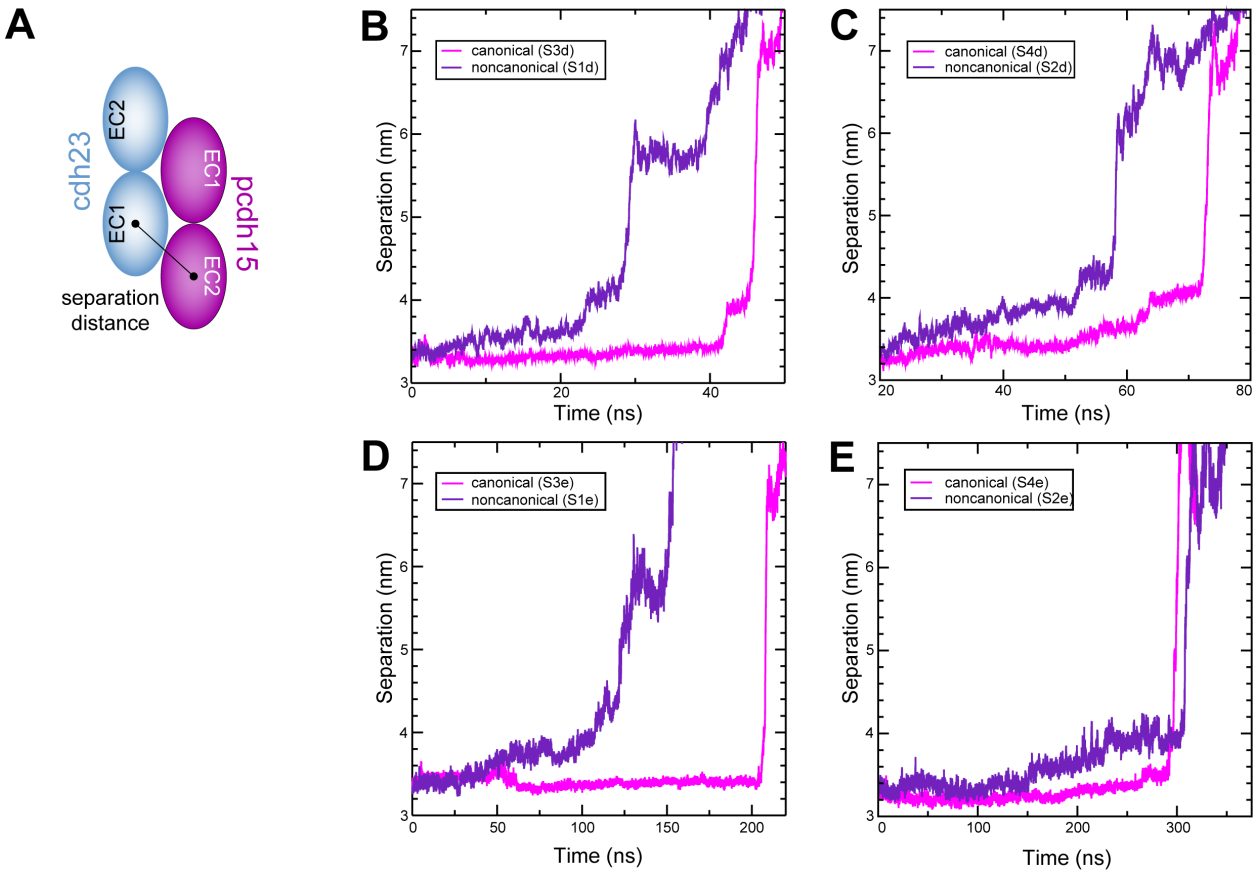


Figure S5. Unbinding trajectory paths for canonical and noncanonical complexes. (A) Separation distance between the centers of mass of cdh23 EC1 and pcdh15 EC2 plotted as a function of time for pulling at 0.1 nm/ns after the complexes were equilibrated for (B) 1 ns, and (C) 10 ns. Similar data are shown when the complexes were pulled at 0.02 nm/ns and equilibrated for (D) 1 ns, and (E) 10 ns. Simulations with low unbinding force (S1d and S1e) follow a distinct unbinding path in which EC1 of cdh23 separates earlier from pcdh15.

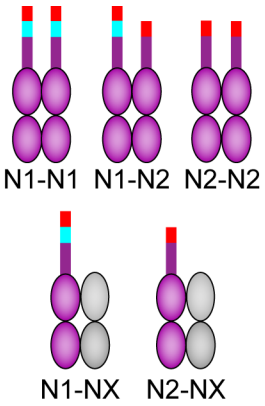


Figure S6. Scheme depicting potential pcdh15-pcdh15 dimers that may interact with cdh23. Five different combinations of PCDH15 may be observed in hair cells. NX represents other pcdh15 variants, such as N3, which could also bind with N1 or N2. These dimers could form heterotetrameric complexes with cdh23.

Table S1. Summary of SMD simulations.

Label	PDB	t_{sim} (ns)	Type	Start	Speed (nm/ns)	Average Peak Force (pN)[b]	Size (#atoms)	Size (nm^3)
S1a	4XXW	1	EQ[a]	–	–		153,825	27.9 x 8.5 x 7.0
S1b		2	SMD	S1a	10	1409.5		
S1c		6.5	SMD	S1a	1	669.8		
S1d		98.9	SMD	S1a	0.1	474.3		
S1e		222.3	SMD	S1a	0.02	397.1		
S2a	4XXW	20	EQ[a]	S1a	-		153,825	27.9 x 8.5 x 7.0
S2b		4	SMD	S2a	10	1259.5		
S2c		20.2	SMD	S2a	1	765.9		
S2d		127.0	SMD	S2a	0.1	694.7		
S2e		357.8	SMD	S2a	0.02	634.3		
S3a	4AQ8	1	EQ[a]	–	–		161,038	28 x 8.2 x 7.5
S3b		4	SMD	S3a	10	1246.8		
S3c		20	SMD	S3a	1	685.6		
S3d		111.2	SMD	S3a	0.1	794.4		
S3e		221.8	SMD	S3a	0.02	774.9		
S4a	4AQ8	20	EQ[a]	S3a	–		161,038	28 x 8.2 x 7.5
S4b		4	SMD	S4a	10	1340.8		
S4c		19.4	SMD	S4a	1	802.0		
S4d		91.7	SMD	S4a	0.1	579.1		
S4e		343.0	SMD	S4a	0.02	591.3		
Total:		1846.1						

[a]EQ indicates equilibration on the system where the backbone was unconstrained.

[b]Average peak force is calculated from the peak force measured on both C-terminal atoms used (using 50-ps running averages).