SUPPLEMENTARY MATERIAL

TABLES

Table S1. List of candidate variants (SNVs). This list resulted after a prioritization process obtained with the different bioinformatics tools described in Materials and Methods. Positions are related to the "Genome Reference Consortium GRCh37". NR = not reported.

CHR	POS	GENE	PAVAR	PHEVOR	EXOMISER	MAF
16	23999898	**PRKCB**	6	10	1	NR
16	46991017	DNAJA2	6	10	0.86	0.0001
2	37586852	QPCT	7	8	0.81	0.0006
2	15615887	NBAS	6	33	1	NR
6	151161707	PLEKHG1/ MTHFD1L	6	23	0	0.0002
7	102952524	PMPCB/ SLC26A5	7	14	0	NR
13	86368804	SLITRK6	6	6	0.86	4.15E-05
17	64738865	PRKCA	7	16	1	NR

Table S2. Protein coding transcripts of the PRKCB human gene. Annotated protein coding transcripts of the PRKCB human gene with their Ensembl IDs (http://www.ensembl.org/index.html).

Name	Transcript ID	Bp	Protein
PRKCB-002	ENST00000303531	7969	673aa
PRKCB-001	ENST00000321728	2689	671aa
PRKCB-010	ENST00000466124	536	122aa
PRKCB-007	ENST00000472066	556	130aa
PRKCB-003	ENST00000498058	480	57aa
PRKCB-004	ENST00000498739	569	119aa

Table S3. Summary table for image analysis data (Mean Density (MD) / Area (μm^2) ± SEM)

Cochlear Turns	Apical MD	±	SEM	N	Middle MD	±	SEM	N	Basal MD	±	SEM	N	Average MD	±	SEM	N	Areas
Inner Border Cells	88.75	±	3.522	3	90.2	±	0.262	12	80.78	±	0.177	3	88.39	±	0.2688	18	929
Inner Hair Cells	47.37	±	0.524	6	26.90	±	0.298	17	19.57	±	0.234	6	27.91	±	0.3225	29	5335
Outer Hair Cell 1	41.63	±	1.198	5	77.84	±	1.323	10	48.49	±	1.312	6	60.83	±	1.290	21	1584
Outer Hair Cell 2	38.69	±	1.110	5	42.89	±	1.218	7	51.52	±	1.156	7	44.96	±	1.1665	19	1467
Outer Hair Cell 3	37.10	±	1.248	5	31.46	±	1.080	5	56.94	±	1.426	8	44.35	±	1.2805	18	1439
OHCs	39.14	±	0.381	15	56.18	±	0.407	22	52.72	±	0.415	21	50.52	±	0.4031	58	4490
Deiter Cell 1	25.45	±	0.707	1	88.19	±	1.571	11	50.05	±	1.266	8	69.80	±	1.4058	20	3604
Deiter Cell 2	34.28	±	0.764	3	47.36	±	1.077	10	66.10	±	1.242	10	53.80	±	1.1079	23	4058
Deiter Cell 3	35.01	±	1.030	1	30.06	±	0.781	9	61.87	±	1.502	9	45.39	±	1.1354	19	3563
DCs	32.66	±	0.270	5	57.14	±	0.378	30	59.93	±	0.430	27	56.38	±	0.3921	62	11225
Tectal Cells	61.67	±	1.733	4	50.31	±	1.756	8	12.22	±	0.781	3	45.72	±	1.5547	15	1408

Crista	**MD**	±	**SEM**	**N**	**Areas**
Vestibular SE - P	2.81	±	0.103	4	6949
Vestibular SE - C	3.53	±	0.153	2	4868
Vestibular SE	3.05	±	0.120	6	11817
Transitional Epith	5.86	±	0.238	4	2761
Dark Cell Epith	4.93	±	0.225	3	1112

***t*-tests**			
IBCs vs IHCs	p< 0.0000005	VSE vs TE	p< 0.0125
IBCs vs OHCs	p< 0.0001	VTE&DCE vs IBCs	p< 0.00000008
IBCs vs TCs	p < 0.00015	VTE&DCE vs TCs	p< 0.000014
aIHCs vs bIHCs	p< 0.0809	VSE vs IHCs	p< 0.0000013
IHCs vs OHCs	p< 0.0053	VSE vs OHCs	p< 0.000000000076
aDCs vs mDCs	p< 0.0227	VSE vs IBCs	p< 0.00000000083
aDCs vs bDCs	p< 0.005	VSE vs TCs	p< 0.000025
aTCs vs bTCs	p< 0.0012		
mTCs vs bTCs	p< 0.0049		

Values are given as Mean ± SEM, *N*s are the number of measurements for each cell type or region. *t*-tests with significant values comparing differences between cell types or regions are given in box at lower right. Other comparisons were non-significant. *Table Abbreviations: IBC*s, inner border cells; *IHC*, inner hair cell; *OHC*, outer hair cell; *DCs,* Deiters cells; *TCs,* tectal cells; *a, m, b,* apical, middle and basal cochlear turns; *SE*, sensory epithelium; *VSE*, vestibular sensory epithelium; *C*, vestibular central zone; *P*, vestibular peripheral zone; *TE,* transitional epithelium; *DCE,* dark cell epithelium.

Table S4. PKCB-related signaling pathways

Top Signaling Pathways	Fisher's Exact Test	Ratio	z-score	Molecules
Axonal Guidance Signaling	p < 0.0000189	0.06	NaN	DPYSL2, ADAM22, FZD10, ECEL1, WNT3, MYL2, PDGFC, TUBB2B, NTNG1, TUBA8, NGFR, UNC5D, PRKAR1B, ADAM23, ABLIM2, ROBO2, SEMA3E, TUBB3, TUBB2A, L1CAM, EPHA3, GNG3, SEMA3A, ADAMTS6, PAK3, LINGO1, GNAL, PRKCB
α-Adrenergic Signaling	p < 0.00232	0.09	1.89	CAMK4, PYGM, ADCY1, PRKAR1B, SLC8A2, PYGL, GNG3, PRKCB
14-3-3-mediated Signaling	p < 0.0136	0.07	2	TUBB3, YWHAG, TUBA8, TUBB2A, MAPK10, VIM, TUBB2B, PRKCB
CXCR4 Signaling	p < 0.0216	0.06	1.89	RHOV, MYL2, RND3, PAK3, ADCY1, MAPK10, GNG3, GNAL, PRKCB

FIGURES

Figure suppl.1. Pipeline of the workflow with the number of variants filtered out at each stage. The first step is to identify common variants to all cases. After this, we filtered out variants by using our in-house control database and MAF<0.001. We scored SNVs using a seven point scoring system (Pathogenic Variant or PAVAR score), according to the effect on protein structure and phylogenetic conservation: (SIFT (Sort Intolerant from Tolerant), PolyPhen2 (Polymorphism Phenotyping v2), Grantham's Matrix, GERP+ (Genomic Evolutionary Rate Profiling), Mutation taster, PhastCons and PhyloP); and we keep SNVs with a score > 5. Finally, we take into account another bioinformatics tools that include phenotype information such as Exomiser v2 and Variant Annotation Analysis and Search Tool (VAAST) + Phevor to select the best candidate probably pathogenic SNV.

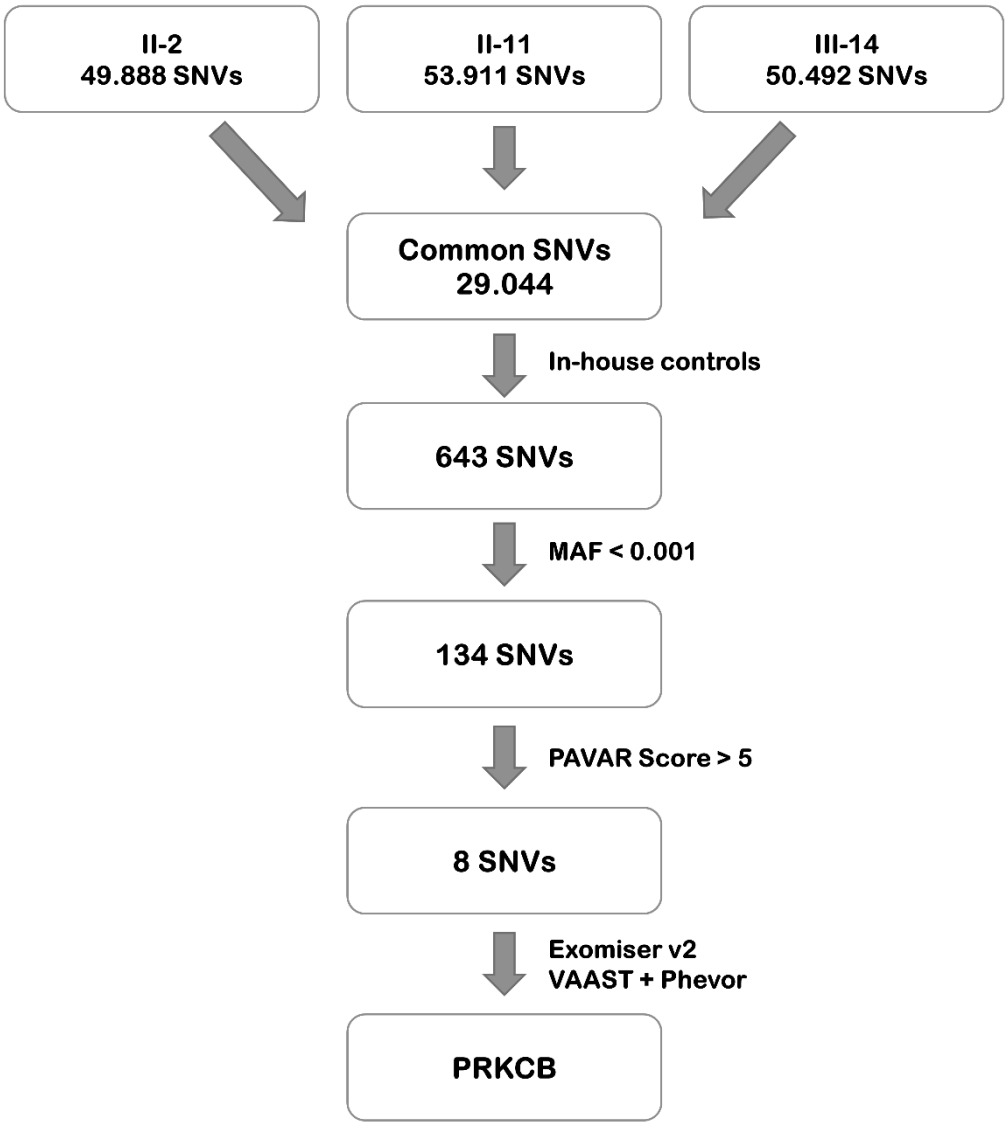

Figure suppl.2. qPCR of PRKCB in human cochlea and semicircular canals (SC). (**A**) Validation of the expression using primers for PRKCB (246bp) and a housekeeping gene, HPRT1 (92bp). (**B**) ΔCt values (ΔCt cochlea = 2.83 ± 0.40; ΔCt sc = 2.49 ± 0.38) calculated after performing qPCR using SYBR® Green RT-PCR techniques.

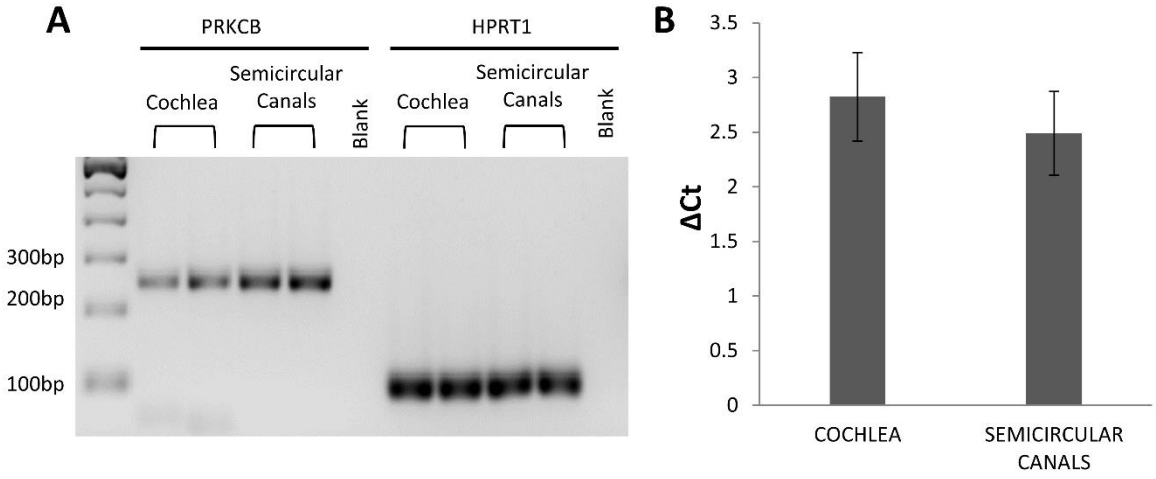

Figure suppl.3. Image analysis, cochlear example. In this confocal section from the basal turn of the cochlea, individual cochlear cells of different types were traced and the mean density/area measured in ImageJ. Cell nuclei were defined in all cells in which they could be distinguished and their densities were individually subtracted from each cell's total density to obtain a more homogeneous measurement across cell types. The intensity scale in the top upper right of the figure shows mean density levels from 0 to 255. Scale bar = 20 μm.

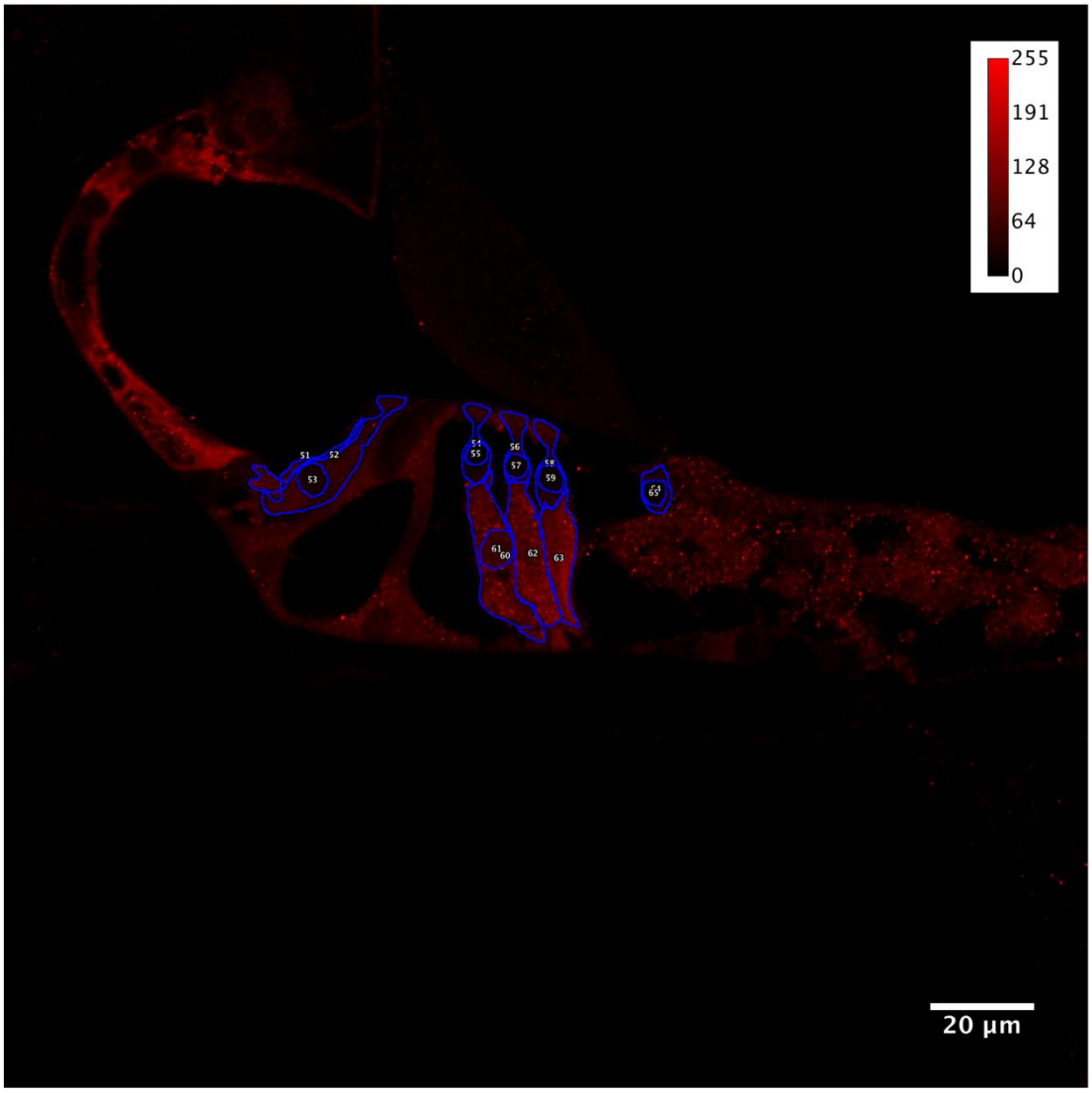

Figure suppl.4. Image analysis, vestibular example. In this confocal section from a vestibular crista ampullaris, individual regions (VSEc, VSEp, TE and DCE) were traced and the mean density/area measured in ImageJ. Individual hair cells or supporting cells could not be distinguished because their overall intensity levels were so dim and their borders were indistinct. Cell nuclei were defined in all cells in which they could be distinguished and their densities were subtracted in aggregate from the region's total density to obtain more homogeneous measurements across regions. The intensity scale in the top upper right of the figure shows mean density levels from 0 to 255. Scale bar = 20 μm.