A

```
         10          20          30          40          50          60
↓Exon 1
MAQGFAVGFD PLGLGDLSSG SLSSLSSRGH LGSDSGSTAT RYLLRKQQRL LNGPPRGIRA

         70          80          90         100         110         120
                   ↓Exon 2
SSPMGRVILI NSPIEANSDE SDIIHSVRVE KSPAGRLGFS VRGGSEHGLG IFVSKVEEGS

        130         140         150         160         170         180
  ↓Exon 3
SAERAGLCVG DKITEVNGLS LESTTMGSAV KVLTSSSRLH MMVRRMGRVP GIKFSKEKTT

        190         200         210         220         230         240
 ↓Exon 4
WVDVVNRRLV VEKCGSTPSD TSSEDGVRRI VHLYTTSDDF CLGFNIRGGK EFGLGIYVSK

        250         260         270         280         290         300
↓Exon 5                                                    ↓Exon 6
VDHGGLAEEN GIKVGDQVLA ANGVRFDDIS HSQAVEVLKG QTHIMLTIKE TGRYPAYKEM

        310         320         330         340         350         360
          ↓Exon 7
VSEYCWLDRL SNGVLQQLSP ASESSSSVSS CASSAPYSSG SLPSDRMDIC LGQEEPGSRG

        370         380         390         400         410         420
PGWGRADTAM QTEPDAGGRV ETWCSVRPTV ILRDTAIRSD GPHPGRRLDS ALSESPKTAL

        430         440         450         460         470         480
                          ↓Exon 8
LLALSRPRPP ITRSQSYLTL WEEKQQRKKE KSGSPGEKGA LQRSKTLMNL FFKGGRQGRL

        490         500         510         520         530         540
                                ↓Exon 9               ↓Exon 10
ARDGRREAWT LDSGSLAKTY PRLDIEKAGG VGPVQKFVTW RLRRDQERGR ALLSARSGSP
                                EMG VSPCCPG            Alu-specific 517 aa isoform
                                ---  -----------  ------------  ------------

        550         560         570         580         590         600
                                                          ↓Exon 11
SSQLPNVDEQ VQAWESRRPL IQDLAQRLLT DDEVLAVTRH CSRYVHEGGI EDLVRPLLAI
----------  ----------  ----------  ----------  ---VCARGRH RGPGEAPAGH

        610         620         630         640         650         660
                  ↓Exon 12                              ↓Exon 13
LDRPEKLLLL QDIRSVVAPT DLGRFDSMVM LVELEAFEAL KSRAVRPPAL RPARQDTPPK
PRQAGEAATA AGHQECGGPH RPGPLRQHGD ACGAGGF              561 aa isoform (exclusion of exon 9 + 10)

        670         680         690         700         710         720
          ↓Exon 14
RHLITPVPDS RGGFYLLPVN GFPEEEDNGE LRERLGALKV SPSASAPRHP HKGIPPLQDV

        730         740         750         760         770         780
PVDAFTPLRI ACTPPPQLPP VAPRPLRPNW LLTEPLSREH PPQSQIRGRA QSRSRSRSRS

        790         800         810         820         830         840
RSRSSRGQGK SPGRRSPSPV PTPAPSMTNG RYHKPRKARP PLPRPLDGEA AKVGAKQGPS

        850         860         870         880         890         900
                                      ↓Exon 15
ESGTEGTAKE AAMKNPSGEL KTVTLSKMKQ SLGISISGGI ESKVQPMVKI EKIFPGGAAF

        910         920         930         940         950         960
      ↓Exon 16
LSGALQAGFE LVAVDGENLE QVTHQRAVDT IRRAYRNKAR EPMELVVRVP GPSPRPSPSD

        970         980         990        1000        1010        1020
SSALTDGGLP ADHLPAHQPL DAAPVPAHWL PEPPTNPQTP PTDARLLQPT PSPAPSPALQ

TPDSKPAPSP RIP    1040
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B

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ctgcagaggagaagcagcagcggaagaaggagaagtcggggtcccctggggagaagggtg Exon 8
     ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

ccctgcagcgctccaagacgctgatgaacctcttcttcaagggagggcggcaggggaggc
     ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

tagcgcgggacgggcgcagagaggcctggacactggacagcgggagcctggccaaaactt
     ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

accctcgcctggacatagagaaaggtaagtagtgggctgatcaaaca...(433 bp)...
     ^    *    ^    *    ^    *    ^    *    ^    *    ^    *
                                transcribed Alu
agctaatttttttgcatcttttgtagagatgggggtttcaccctgttgcccaggctagt  AluSx
     ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

ctcaaattcctggactgaagctatctacccgccttagtctcccaaagtgctaggattaca
     ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

gcgtgatctaccacacctggccaatcccaactactctggaagctgaagcagcaagatcac  FLAM_C
     ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

ttgagcccaggagtttaagaccagcctgggcaacatagcaagaccctgtctctaaagaaa
     ^    *    ^    *    ^    *    ^    *    ^    *    ^    *

aaaaaaaagtaaaagaaaaataaagcacgtcttggctcagctgctgatttaagctgggtc
     ^    *    ^    *    ^    *    ^    *    ^    *    ^    *
```
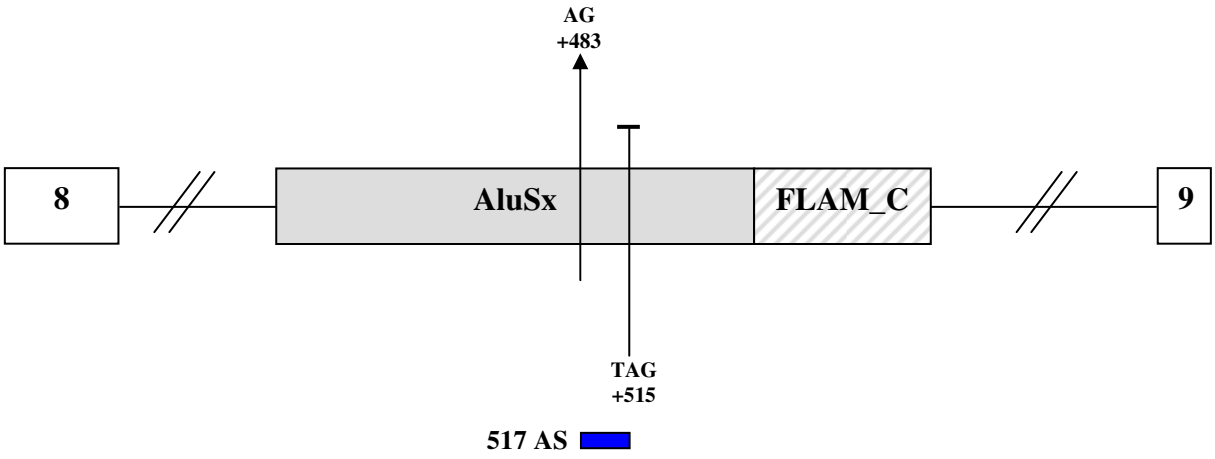
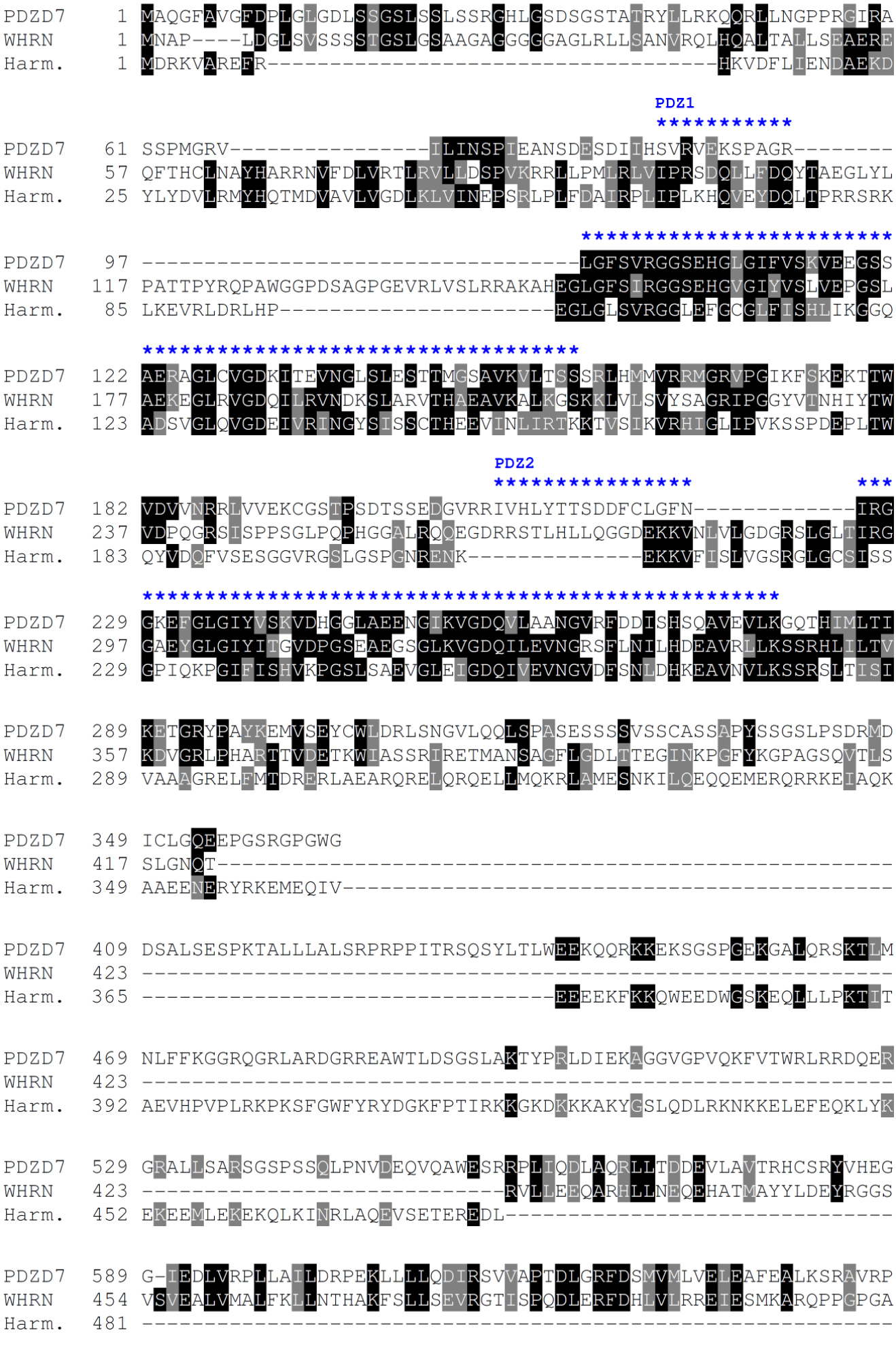
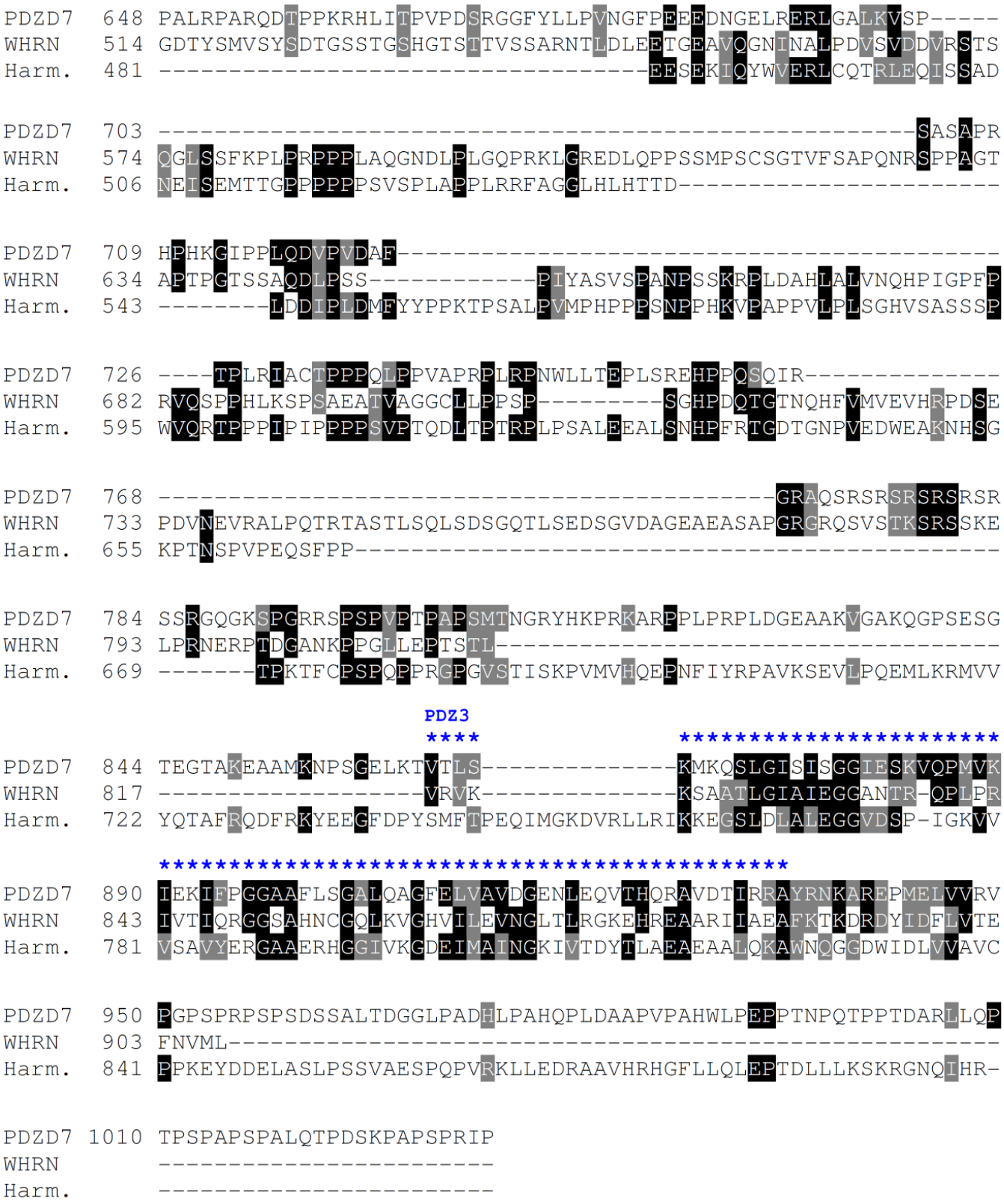

C



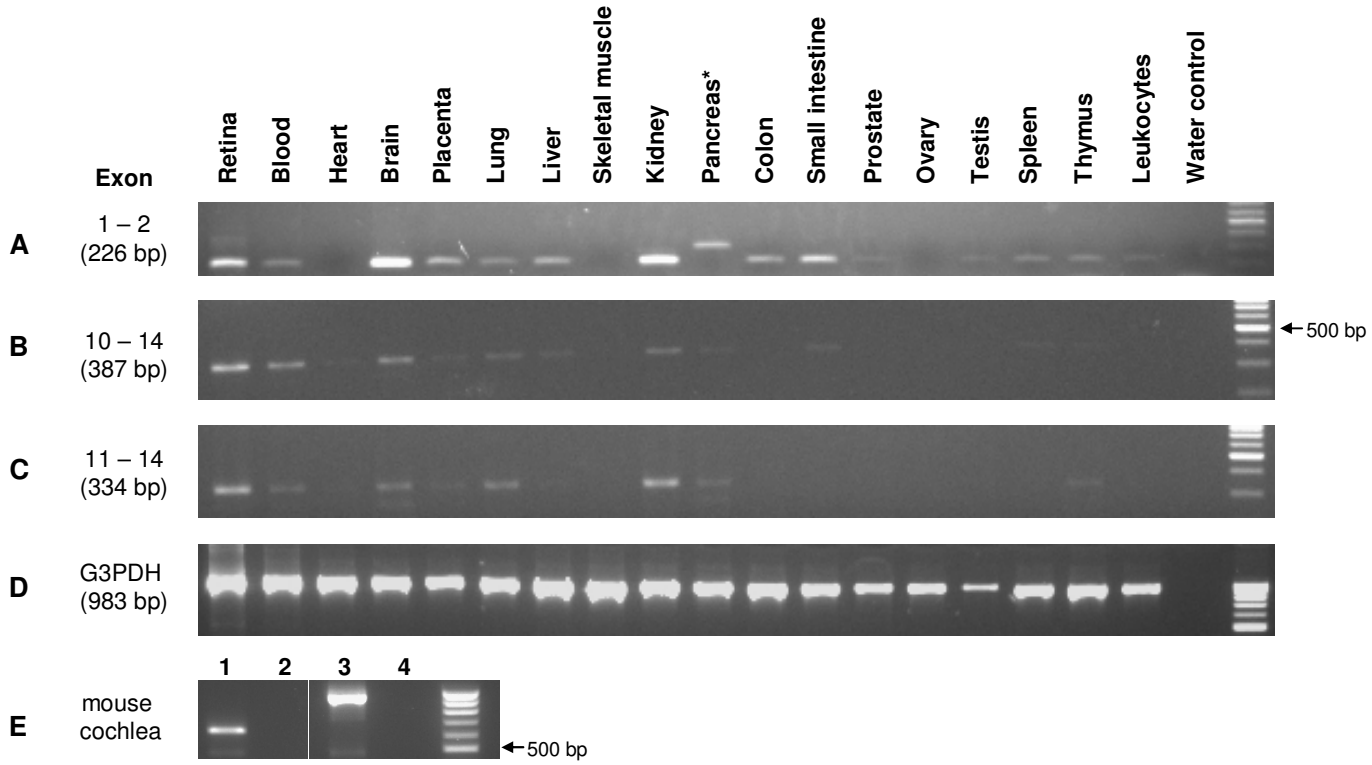
Supplemental Figure 1.

(A) Amino acid sequence of the long PDZD7 isoform (1,033 residues, GenBank acc. no. FJ617449), the Alu-derived 517 aa isoform (blue) and the 561 aa isoform that results from exclusion of exons 9 and 10 from the mRNA (grey). PDZ domains are highlighted in yellow. The proline-rich region is underlined. 5' ends of exons are indicated by arrows. The epitope chosen for production of the antibody is in green. **(B)** Genomic sequence of exon 8 (red) and intron 8. AluSx sequence in blue, transcribed Alu sequence in bold and underlined, FLAM_C in green. **(C)** Scheme of the Alu-specific splicing (517 aa isoform). Splicing occurs at an acceptor splice site located within the AluSx sequence (c.1522+483). Termination occurs at an *in-frame* stop codon (c.1522+515). The blue bar indicates the AluSx-derived sequence that is translated as C-terminus of the 517 aa *PDZD7* transcript.


PDZD7 1 MAQGFAVGFDPLGLGDLSSGSLSSLSSRGHLGSDSGSTATRYLLRKQQRLLNGPPRGIRA
WHRN 1 MNAP----LDGLSVSSSSTGSLGSAAGAGGGGGAGLRLLSANVRQLHQALTALLSEAERE
Harm. 1 MDRKVAREFR----------HKVDFLIENDAEKD
PDZ1
PDZD7 61 SSPMGRV----------ILINSPIEANSDESDIIHSVRVEKSPAGR--------
WHRN 57 QFTHCLNAYHARRNVFDLVRTLRVLLDSPVKRRLLPMLRLVIPRSDQLLFDQYTAEGLYL
Harm. 25 YLYDVLRMYHQTMDVAVLVGDLKLVINEPSRLPLFDAIRPLIPLKHQVEYDQLTPRRSRK
PDZD7 97 ----------LGFSVRGGSEHGLGIFVSKVEEGSS
WHRN 117 PATTPYRQPAWGGPDSAGPGEVRLVSLRRAKAHEGLGFSIRGGSEHGVGIYVSLVEPGSL
Harm. 85 LKEVRLDRLHP----------EGLGLSVRGGLEFGCGLFISHLIKGGQ
PDZD7 122 AERAGLCVGDKITEVNGLSLESTTMGSAVKVLTSSSRLHMMVRRMGRVPGIKFSKEKTTW
WHRN 177 AEKEGLRVGDQILRVNDKSLARVTHAEAVKALKGSKKLVLSVYSAGRIPGGYVTNHIYTW
Harm. 123 ADSVGLQVGDEIVRINGYSISSCTHEEVINLIRTKKTVSIKVRHIGLIPVKSSPDEPLTW
PDZ2
PDZD7 182 VDVVNRRLVVEKCGSTPSDTSSEDGVRRIVHLYTTSDDFCLGFN----------IRG
WHRN 237 VDPQGRSISPPSGLPQPHGGALRQQEGDRRSTLHLLQGGDEKKVNLVLGDGRSLGLTIRG
Harm. 183 QYVDQFVSESGGVRGSLGSPGNRENK----------EKKVFISLVGSRGLGCSISS
PDZD7 229 GKEFGLGIYVSKVDHGGLAEENGIKVGDQVLAANGVRFDDISHSQAVEVLKGQTHIMLTI
WHRN 297 GAEYGLGIYITGVDPGSEAEGSGLKVGDQILEVNGRSFLNILHDEAVRLLKSSRHLILTV
Harm. 229 GPIQKPGIFISHVKPGSLSAEVGLEIGDQIVEVNGVDFSNLDHKEAVNVLKSSRSLTISI
PDZD7 289 KETGRYPAYKEMVSEYCWLDRLSNGVLQQLSPASESSSSVSSCASSAPYSSGSLPSDRMD
WHRN 357 KDVGRLPHARTTVDETKWIASSRIRETMANSAGFLGDLTTEGINKPGFYKGPAGSQVTLS
Harm. 289 VAAAGRELFMTDRERLAEARQRELQRQELLMQKRLAMESNKILQEQQEMERQRRKEIAQK
PDZD7 349 ICLGQEEPGSRGPGWG
WHRN 417 SLGNQT----------
Harm. 349 AAEENERYRKEMEQIV----------
PDZD7 409 DSALSESPKTALLLALSRPRPPITRSQSYLTLWEEKQQRKKEKSGSPGEKGALQRSKTLM
WHRN 423 ----------
Harm. 365 ----------EEEEKFKKQWEEDWGSKEQLLLPKTIT
PDZD7 469 NLFFKGGRQGRLARDGRREAWTLDSGSLAKTYPRLDIEKAGGVGPVQKFVTWRLRRDQER
WHRN 423 ----------
Harm. 392 AEVHPVPLRKPKSFGWFYRYDGKFPTIRKKGKDKKKAKYGSLQDLRKNKKELEFEQKLYK
PDZD7 529 GRALLSARSGSPSSQLPNVDEQVQAWESRRPLIQDLAQRLLTDDEVLAVTRHCSRYVHEG
WHRN 423 ----------RVLLEEQARHLLNEQEHATMAYYLDEYRGGS
Harm. 452 EKEEMLEKEKQLKINRLAQEVSETEREDL----------
PDZD7 589 G-IEDLVRPLLAILDRPEKLLLLQDIRSVVAPTDLGRFDSMVMLVELEAFEALKSRAVRP
WHRN 454 VSVEALVMALFKLLNTHAKFSLLSEVRGTISPQDLERFDHLVLRREIESMKARQPPGPGA
Harm. 481 ----------



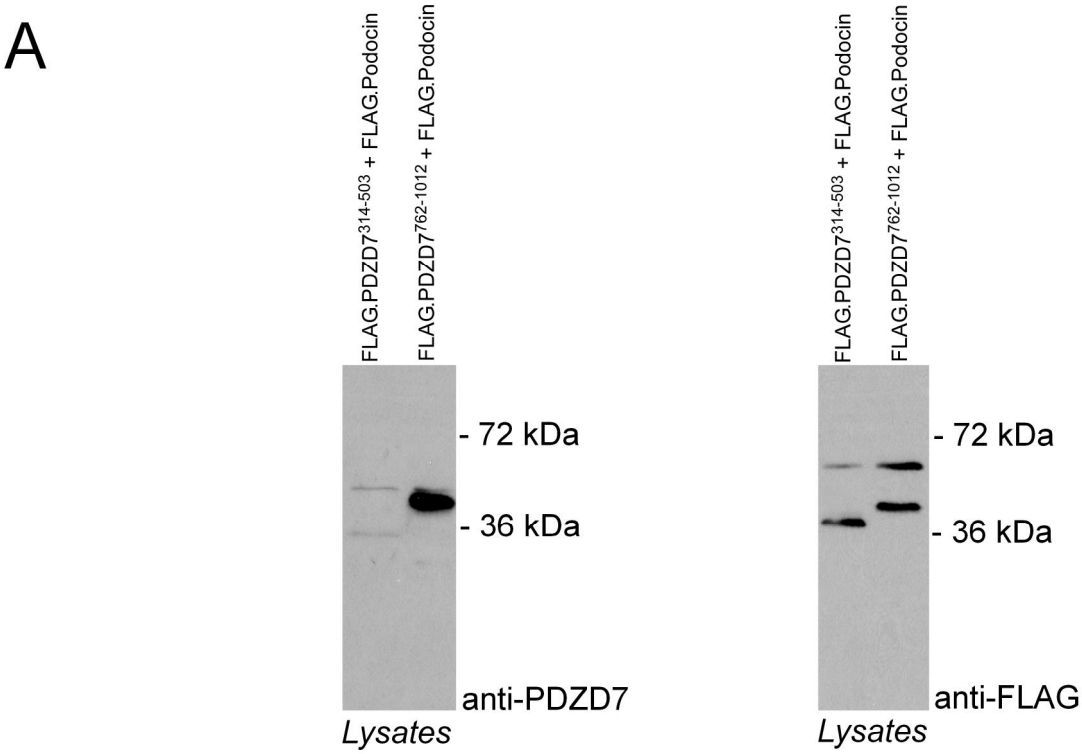
Supplemental Figure 2. Alignment of amino acid sequences of PDZD7 (1,033 residues isoform, GenBank acc. no. FJ617449), the USH2D protein whirlin (long isoform, GenBank acc. no. BC142614) and the USH1C protein harmonin (b3 isoform, GenBank acc. no. NM_153676).

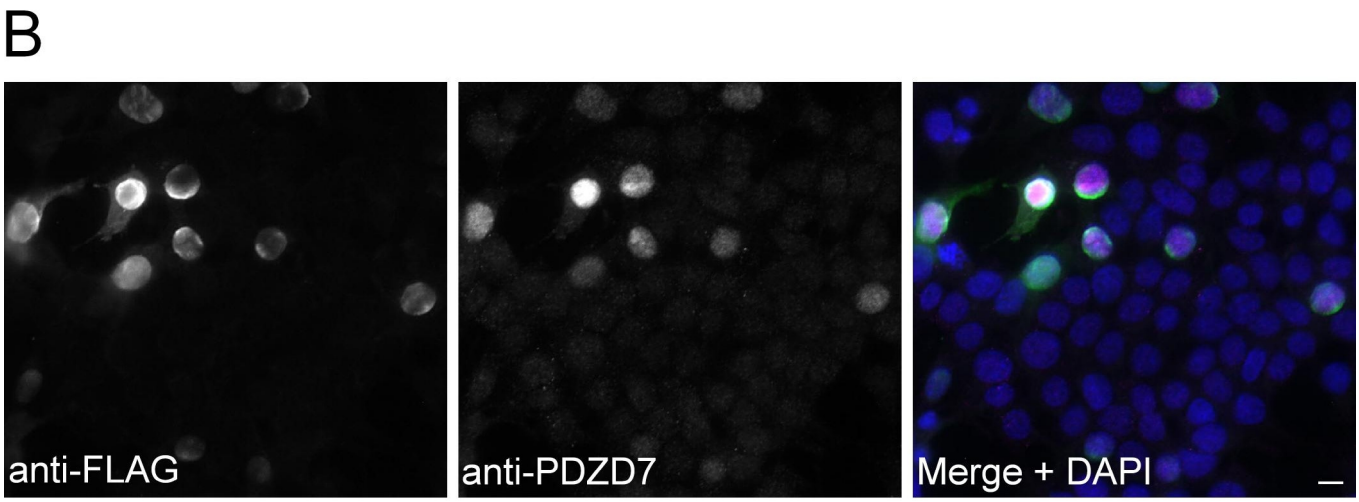


Supplemental Figure 3

Expression profile of human and mouse *PDZD7* (RT-PCR analyses).

Samples were taken after 30 cycles. Bands were verified by sequencing as *PDZD7* amplicons. Amplification was carried out with primers located in **(A)** exon 1/2, **(B)** exons 10/14, and **(C)** exons 11/14. **(D)** The same cDNA samples were used as a template for PCR with primers for a housekeeping gene (*G3PDH*). *Unspecific PCR product: Pancreatic carboxypeptidase A1, CPA1 (7q32.2). **(E)** Expression of *pdzd7* in the mouse cochlea. 1: mouse *pdzd7* primers c.11F (5'-GTACTAGCAGTCACTCGCCAC-3') vs. c.15R (5'-CCTGCGCCCAGGAGACTTGCCTTG-3'), 657 bp. 2: water control. 3: primers for a housekeeping gene (*G3PDH*). 4: water control. Lanes 1/2 and 3/4 were run on the same gel but noncontiguous.

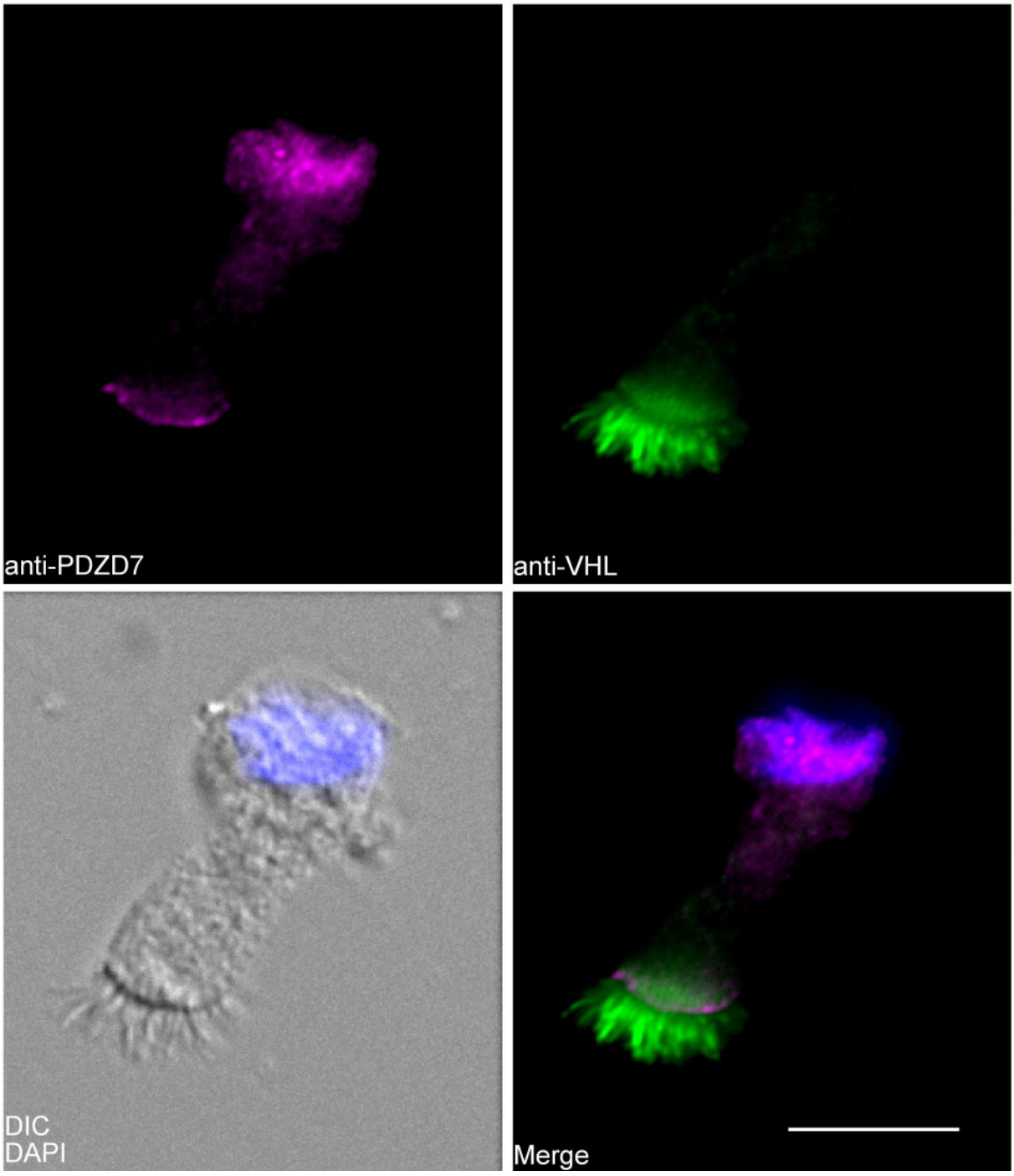




Supplemental Figure 4

A novel polyclonal antibody was raised against the newly predicted C-term of PDZD7.

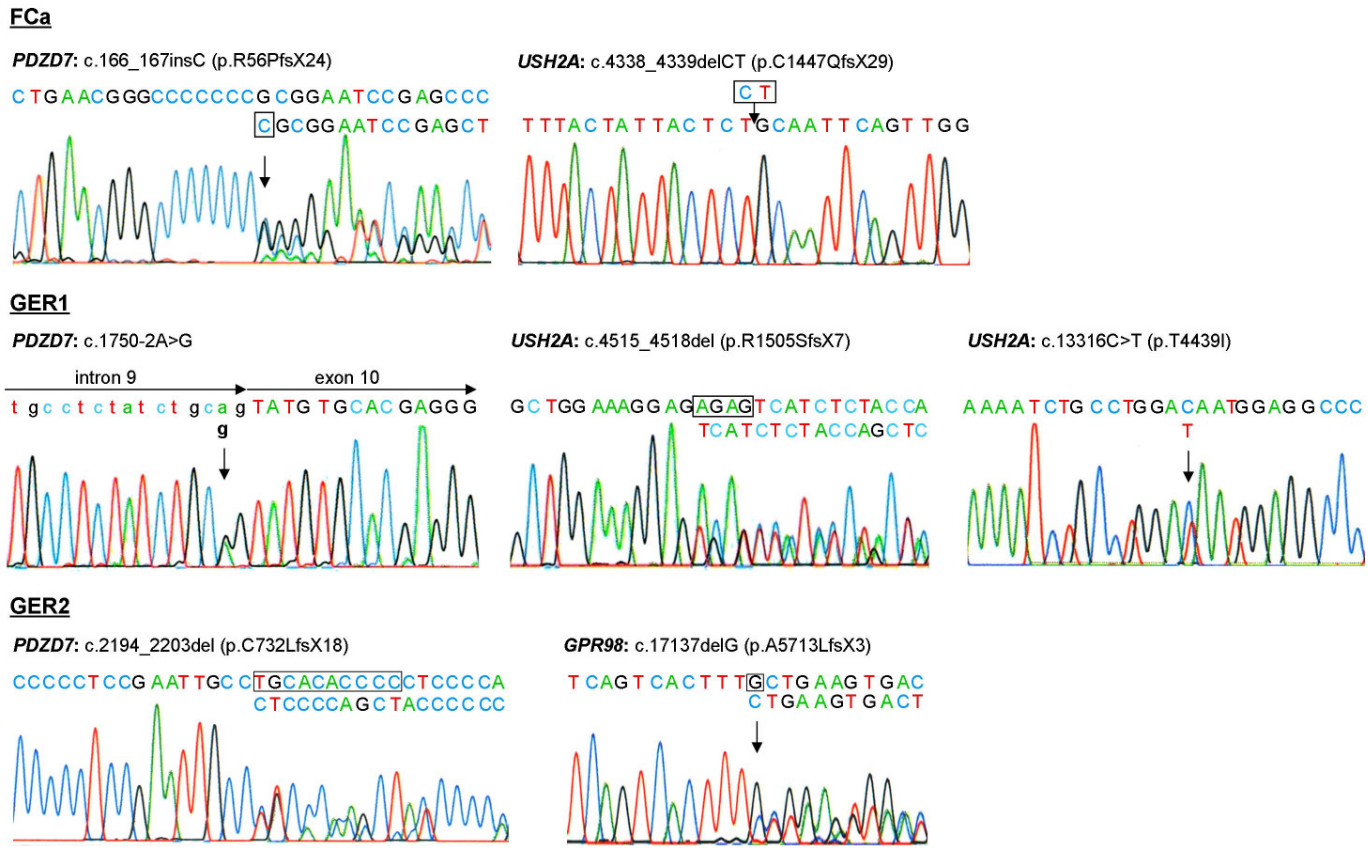
(A) To prove specificity of the antibody, HEK293T cells were co-transfected with FLAG.Podocin and either FLAG.PDZD7$^{314-503}$ or FLAG.PDZD7$^{762-1012}$. Western blot analysis of cell lysates revealed that the antibody only recognizes the targeted protein truncation but neither FLAG.Podocin nor a control truncation of PDZD7. Comparable protein expression was proven by an anti-FLAG staining of the same lysates. **(B)** For further validation of the antibody, HEK293T cells were transfected with FLAG.PDZD7$^{762-1012}$. This PDZD7 truncation could be detected with either an anti-FLAG or an anti-PDZD7 antibody, resulting in an overlapping staining pattern. Scale bar: 10 µm.



Supplemental Figure 5

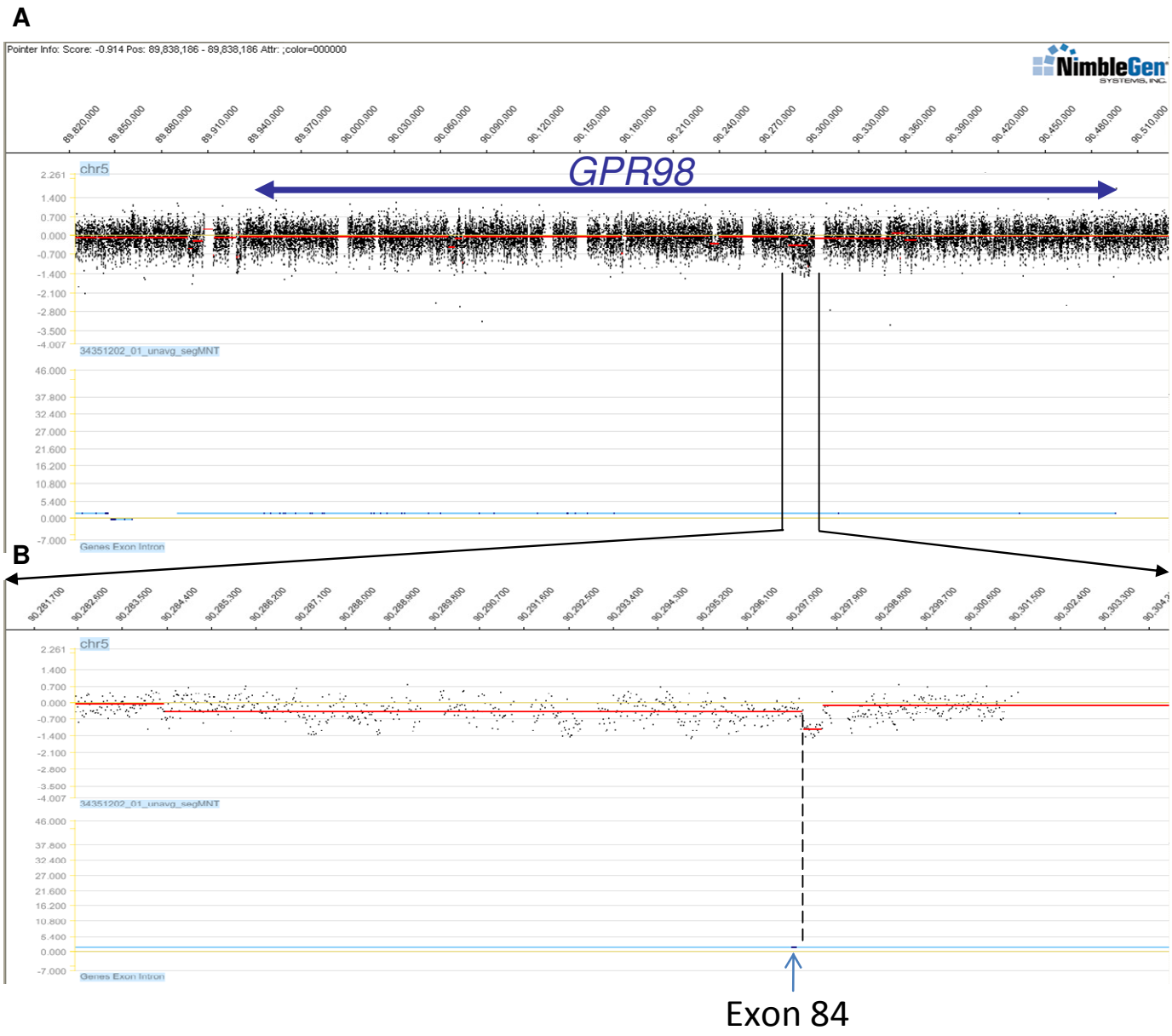
PDZD7 localizes to the ciliary base.

PDZD7 staining of human nasal cells demonstrates localization of PDZD7 at the ciliary base and at the nucleus. Cilia were visualized with an anti-VHL antibody, nuclei with DAPI. Scale bar: 10 µm.



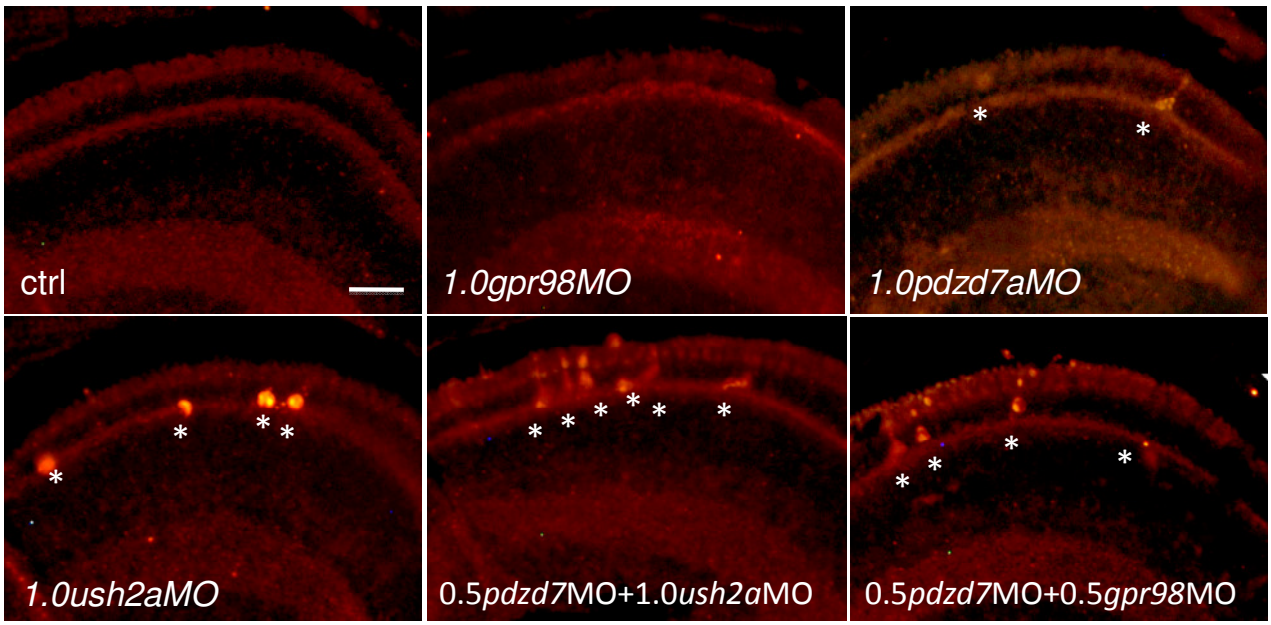
Supplemental Figure 6

Electropherograms of *PDZD7*, *USH2A* and *GPR98* mutations identified in this study.



Supplemental Figure 7

Exclusion of large deletions or duplications affecting coding parts of *GPR98* by high-resolution array CGH using a customized NimbleGen array (black dots indicate oligonucleotide probes that densely cover the genomic *GPR98* region). Array CGH with a 6.0 Affymetrix SNP array likewise did not indicate any potentially deleterious copy number variation (not shown). **(A)** Overview of the array CGH data from the NimbleGen customized array. **(B)** Enlargement of a region with a questionable 12 kb deletion that would encompass *GPR98* exon 84. PCR experiments based on multiple primer combinations did not amplify junction fragments that would be expected if the 12 kb deletion was present.



Supplemental Figure 8

Anti-active Caspase-3 labeling of control and morpholino-treated retinas.

Exon	Forward primer	Reverse primer	Coding sequence (bp)	Fragment size (bp)
1	ggagctcacacttctgagaggc	cctgccacctccctgtgactc	226	520
2	cttagaaatgggctgacctgc	gtccctgacagcagcatcc	141	384
3	gctctgacaatcctcactctg	ctctccttattttgaggtcag	175	453
4+5	ccagctaatgaacctatgc	ctaccttctagtggctgtg	177/148	858
6	gcatgatgaatttggaggac	ctgtactcaagcgatcctc	61	341
7	cagcatttcggagcagcggg	gtctgagcagcctggagcttg	396	694
8	ggaagaggaaccgcactctcac	ctcatgaggaaactgaggctc	198	489
9	cctcagggccaggcctcttg	cataccattctagctgcctg	51	330
10	ggagcatataggctaggctag	cactagcctcctggtgtcaag	176	414
11-13	gaacctttcccaaggcacgcag	gtggacaagagctttccttc	92/92/72	848
14.1	gcgattctaatagaacatgtc	cctggagacttgccttgac	612	511
14.2	ctggctgctgacagaaccc	ggtgtgagcctctgcgcctg		471
15+16	gagtagtaacagaatgtagg	gatgctggagtcagtgggtg	101/384	1058

Supplemental Table 1

Primers for *PDZD7* mutation screening.

cDNA	Forward primer	Reverse primer	Amplicon (bp)
human			
1 – 763	ATGGCGCAGGGTTTCGCAGTG	CCACCTTGATGCCATTCTCCTCG	763
512 – 1899	GCATCAAGTTCTCCAAGGAG	CTCCACAAGCATCACCATGC	1388
1753 – 3102+41 (3'-UTR)	GTGCACGAGGGAGGCATAGAGG	CACCCACTGACTCCAGCATC	1391
1391 – Alu in intron 8	CCAAGACGCTGATGAACCTC	GCTGGTCTTAAACTCCTGG	218/ 386
zebrafish			
100 bp of 5'-UTR – 247	CTCTGGACTGTAATCCTGCTAG	GTGAGGATGGCCATCTGG	347
1–646	ATGGCTCATTCGTCTGACACGG	CTTTGCCGCCACGGATGTTGAAG	646
175–938	CCTGTCGATGGTGGAGATGAC	GTGGAGGAGTATGAATCTGAGC	764
757–1248	GACATCACACACAGCAATGCAG	CAGAAACTAGTCGAATGGTAGG	413
1209–1738	GACAGCCCATTCCCGGACAAGG	CGACCAGAAACCTTTTACAGTGTC	530
1620–2237	GGAGCAAAGCCCGATGCCTAGC	CGTCCAGTGCGTTCTGTGCTG	619
2167–2660	CGCTCTTTGAGTCCTGCTCG	CTGCCCTTCAGGACATCAC	494
784-1930	TGGCGGCCTGGCAGAACAAAATG	GGGGCGCACTAGATCCTCAACCAC	1146
1989-2907	TCATTCCTGCCACAGATCTGG	CTGAAAGCCCTCCGAATGG	918
1989-3'UTR	TCATTCCTGCCACAGATCTGG	CTTCGCTGTGAGTAAATCAGG	1033

Supplemental Table 2

Primers for cloning human full-length *PDZD7* and the isoform derived from intron 8 Alu insertion.

Position	Exon/ IVS	Change	Frequency in patients	Rel. frequency in patients	Frequency in controls	Rel. frequency in controls	Rel frequency total	rs number; carrier(s)
c.1-86A>G	IVS 0	-	27/474	0.0570	21/508	0.0413	0,0479	
c.159G>C	exon 1	p.G59G	1/474	0.0021	0/508	0	0,0010	1 x USH1
c.367+7A>G	IVS 2	-	153/474	0.3228	n. d.	n. d.	n. d.	rs6584410
c.367+51delT	IVS 2	-	3/452	0.0133	n. d.	n. d.	n. d.	
c.368-54A>G	IVS 2	-	6/460	0.0130	n. d.	n. d.	n. d.	
c.542+48C>G	IVS 3	-	48/446	0.1076	n. d.	n. d.	n. d.	rs3740496
c.539C>T	exon 3	p.T180M	1/474	0.0021	n. d.	n. d.	n. d.	1 x USH2
c.559C>T	exon 4	p.R187W	1/474	0.0021	n. d.	n. d.	n. d.	1 x USH2D (Ebermann et al., 2007)
c.572T>A	exon 4	p.V191E	7/474	0.0148	n. d.	n. d.	n. d.	
c.719+136C>A	IVS4	-	9/474	0.0190	n. d.	n. d.	n. d.	rs41291480
c.928+20delC	IVS 6	-	254/474	0.5359	n. d.	n. d.	n. d.	
c.928+63A>C	IVS 6	-	90/248	0.3629	n. d.	n. d.	n. d.	rs7075685
c.928+110C>T	IVS 6	-	117/248	0.4718	n. d.	n. d.	n. d.	rs7075659
c.936C>T	exon 7	p.N312N	8/474	0.0169	n. d.	n. d.	n. d.	rs35038258
c.1008C>T	exon 7	p.P336P	1/474	0.0021	n. d.	n. d.	n. d.	1 x USH2
c.1011C>T	exon 7	p.Y337Y	10/474	0.0211	n. d.	n. d.	n. d.	rs34705415
c.1388G>C	exon 8	p.R463P	13/474	0.0274	n. d.	n. d.	n. d.	
c.1522+88G>C	IVS 8	-	168/438	0.3836	n. d.	n. d.	n. d.	rs11190793
c.1613G>A	exon 10	-	6/474	0.0127	n. d.	n. d.	n. d.	
c.1749+43delA	IVS 10	-	253/468	0.5406	n. d.	n. d.	n. d.	rs34125357
c.1934-55C>T	IVS 12	-	88/474	0.1857	n. d.	n. d.	n. d.	rs807020
c.1934-40C>G	IVS 12	-	1/514	0.0019	n. d.	n. d.	n. d.	1 x USH2
c.2006-79A>G	IVS 13	-	1/454	0.0022	8/852	0.0069	0,0074	
c.2006-26_37ins17bp	IVS 13	-	8/474	0.0169	23/852	0.0234	0.0263	
c.2011C>A	exon 14	p.R671S	1/474	0.0021	0/808	0	0.0001	1 x USH2
c.2049G>A	exon 14	p.P683P	8/474	0.0169	3/808	0.0037	0.0086	rs34693310
c.2132A>G	exon 14	p.H711R	10/474	0.0211	2/808	0.0025	0,0094	rs34616847
c.2144C>T	exon 14	p.P715L	4/474	0.0084	7/808	0.0087	0.0086	
c.2250G>T	exon 14	p.W750C	1/474	0.0021	4/808	0.0050	0.0039	
c.2319C>T	exon 14	p.R773R	88/474	0.1857	127/808	0.1572	0.1677	rs807022
c.2340_2341ins6bp	exon 14	p.S780_R781insRS	249/474	0.5253	363/808	0.4493	0.4773	
c.2368A>G	exon 14	p.K790Q	7/474	0.0148	n. d.	n. d.	n. d.	
c.2564C>A	exon 14	p.T855N	82/474	0.1730	n. d.	n. d.	n. d.	rs807023
c.2718+146G>T	IVS 15	-	1/474	0.0021	n. d.	n. d.	n. d.	1x USH2
c.3092G>A	exon 16	p.R1031H	30/474	0.0633	n. d.	n. d.	n. d.	

Supplemental Table 3

Non-pathogenic variations in *PDZD7*.

237 patients (474 alleles) were analyzed. Differences in control numbers are due to heterozygosity of deletion or insertion polymorphisms upstream of SNP positions or otherwise illegible sequence at the respective position. For variants detected only once, the USH subtype of the patient is given in the last column.