Supplemental Files – Stemerdink et al., *Deciphering the largest disease-associated transcript isoforms in the human neural retina with advanced long-read sequencing approaches*

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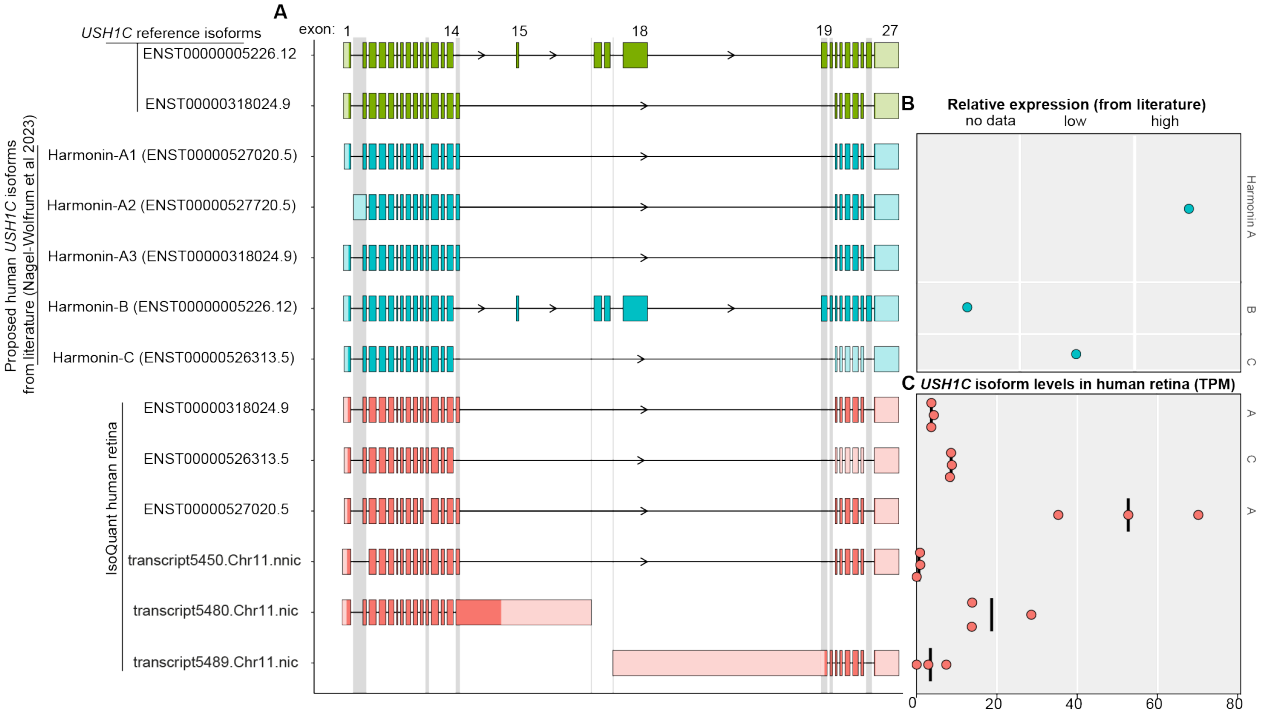
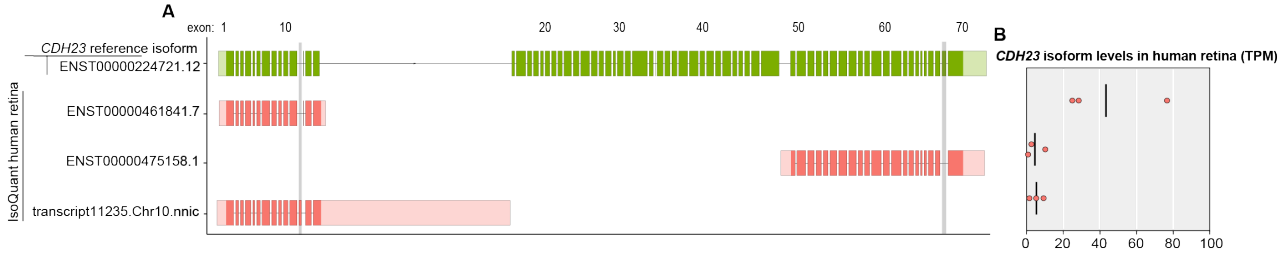
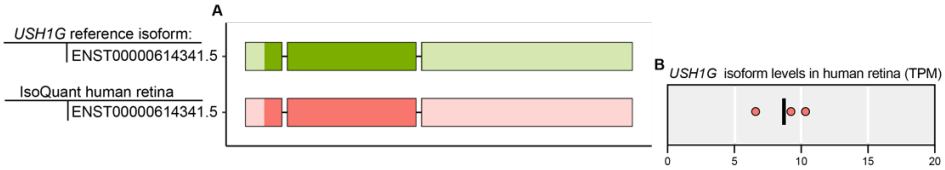


Supplemental_Fig_S1: *USH1C* isoforms identified by IsoQuant analysis compared to known isoforms from the literature. A. The GENCODE reference transcripts are depicted at the top in green, followed by the known human *USH1C* isoforms in blue (Nagel-Wolfrum *et al.*, 2023). The *USH1C* IsoQuant transcripts are depicted in red. The light green, blue and red colors indicate the untranslated regions (UTR) and the dark green, blue and red colors indicate the open reading frame (ORF) of each transcript. Differences between the IsoQuant isoforms and the GENCODE reference transcript are highlighted in grey boxes. IsoQuant predicts the absence of the Harmonin-B isoform in the human retina. **B.** Relative expression of *USH1C* isoforms based on literature in either the retina or the cochlea. **C.** The Transcripts Per Million (based on dataset 1) for each IsoQuant identified isoform are presented for the three individual samples.

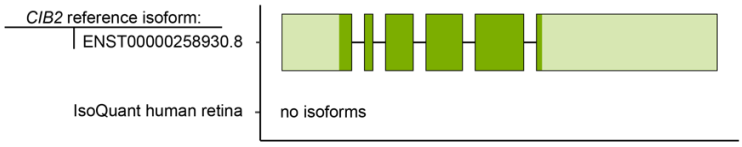


Supplemental_Fig_S2: *CDH23* isoforms identified by IsoQuant analysis. A. The GENCODE reference transcript is depicted at the top in green, followed by *CDH23* IsoQuant transcripts depicted in red. The light green and red colors indicate the untranslated regions (UTR) and the dark green and red colors indicate the open reading frame (ORF) of each transcript. Differences between the IsoQuant isoforms and the GENCODE reference transcript are highlighted in grey boxes. IsoQuant analysis indicates skipping of exon 69, which is in line with findings of Siemens *et al.* (2002) that indicate the presence of this penultimate exon in inner ear transcripts, and its absence in other tissues such as the retina. **B** The Transcripts Per Million (based on dataset 1) for each IsoQuant identified isoform are presented for the three individual samples.

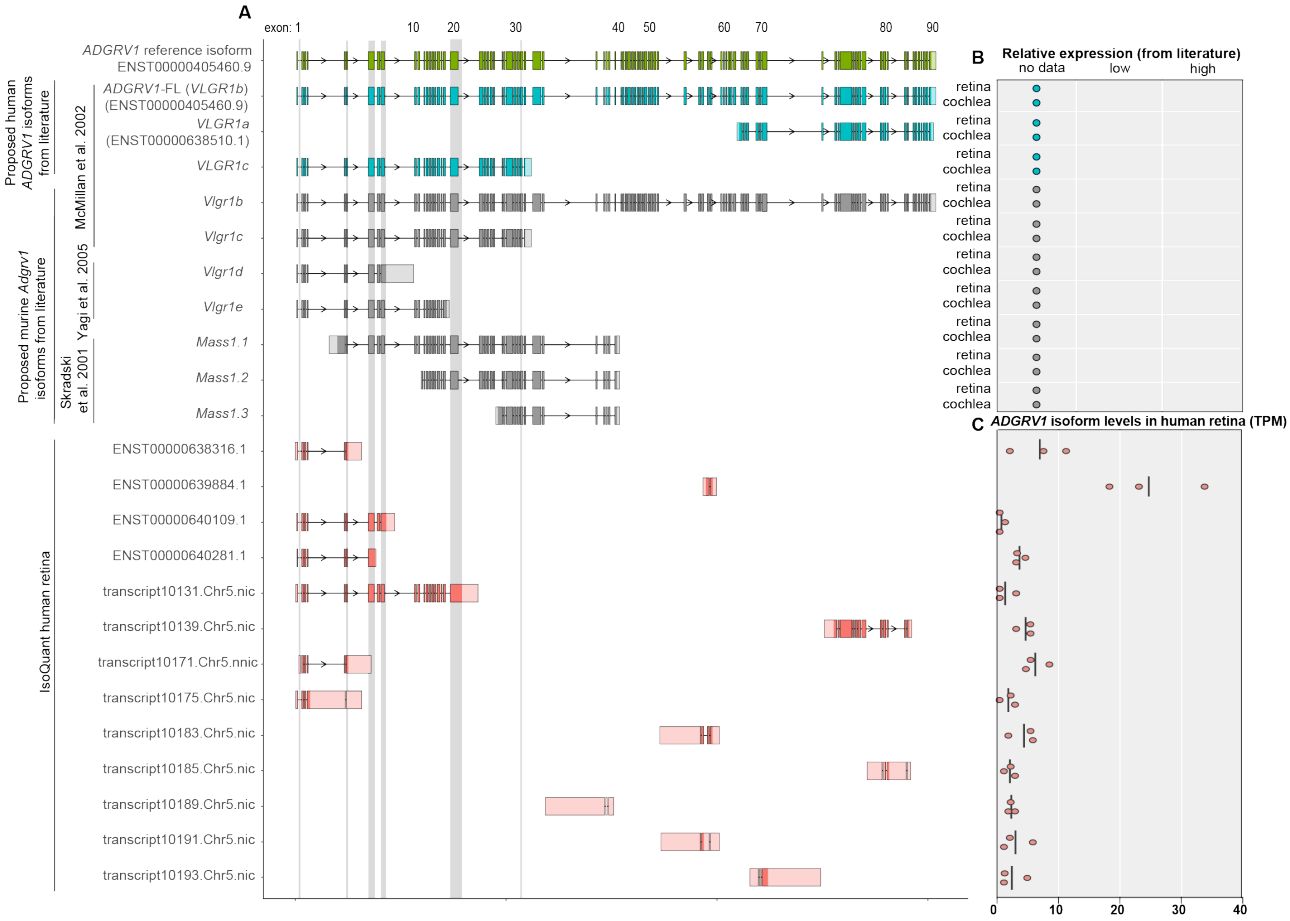
Supplemental_Fig_S3: *PCDH15* isoforms identified by IsoQuant analysis compared to known isoforms from the literature. A. The GENCODE reference transcripts are depicted at the top in green, followed by the known human *PCDH15* isoforms in blue (Ahmed *et al.*, 2008). The *PCDH15* IsoQuant transcripts are depicted in red. The light green, blue and red colors indicate the untranslated regions (UTR) and the dark green, blue and red colors indicate the open reading frame (ORF) of each transcript. Differences between the IsoQuant isoforms and the GENCODE reference transcript are highlighted in grey boxes. **B.** Relative expression of *PCDH15* isoforms based on literature in either the retina or the cochlea. **C.** The Transcripts Per Million (based on dataset 1) for the IsoQuant identified isoforms are presented for the three individual samples.



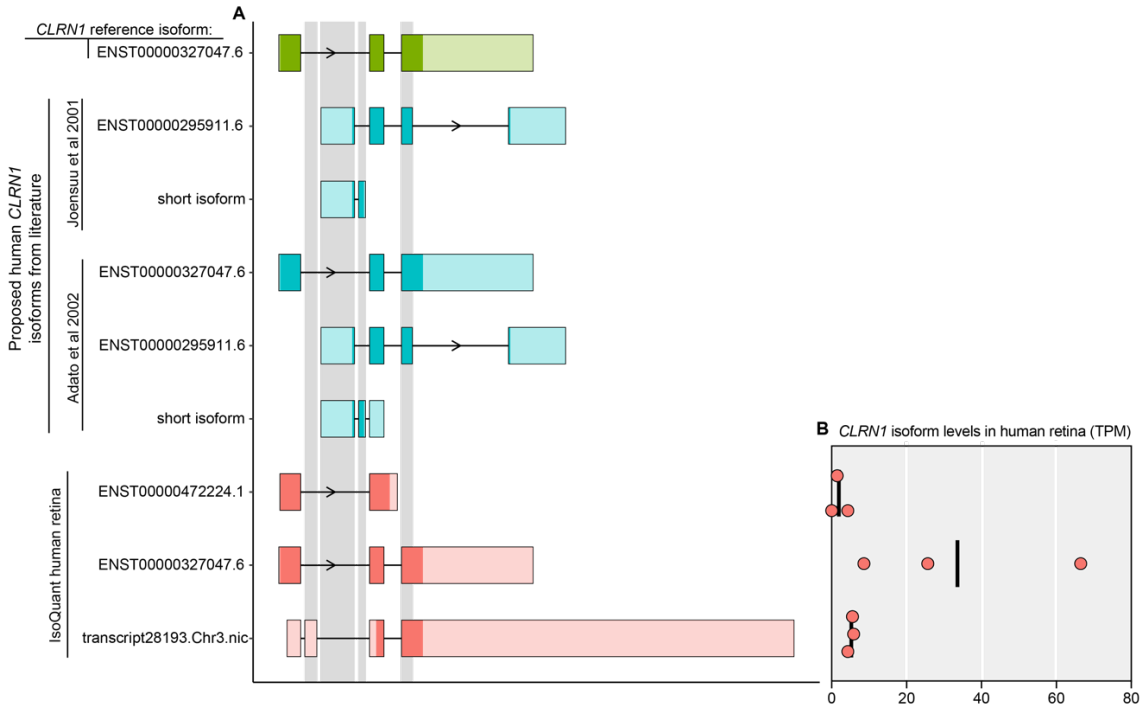
Supplemental_Fig_S4: *SANS* isoform identified by IsoQuant analysis. A. The GENCODE reference transcript is depicted at the top in green, followed by the *SANS* IsoQuant transcript depicted in red. The light green and red colors indicate the untranslated regions (UTR) and the dark green and red colors indicate the open reading frame (ORF) of each transcript. **B.** The Transcripts Per Million (based on dataset 1) for the IsoQuant identified isoform are presented for the three individual samples.



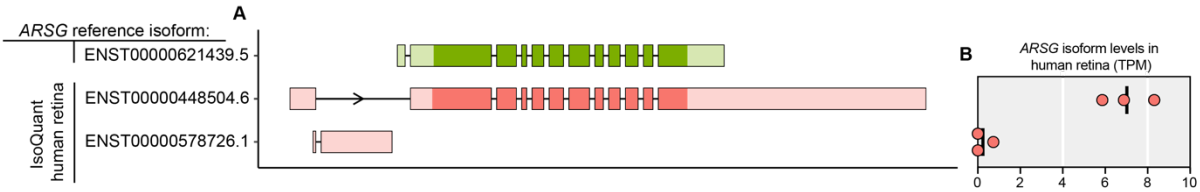
Supplemental_Fig_S5: No IsoQuant isoforms are identified for *CIB2* The GENCODE reference transcript is depicted at the top in green with light green colors indicating the untranslated regions (UTR) and the dark green colors indicating the open reading frame (ORF) the reference isoform. No IsoQuant isoforms are identified for *CIB2.*



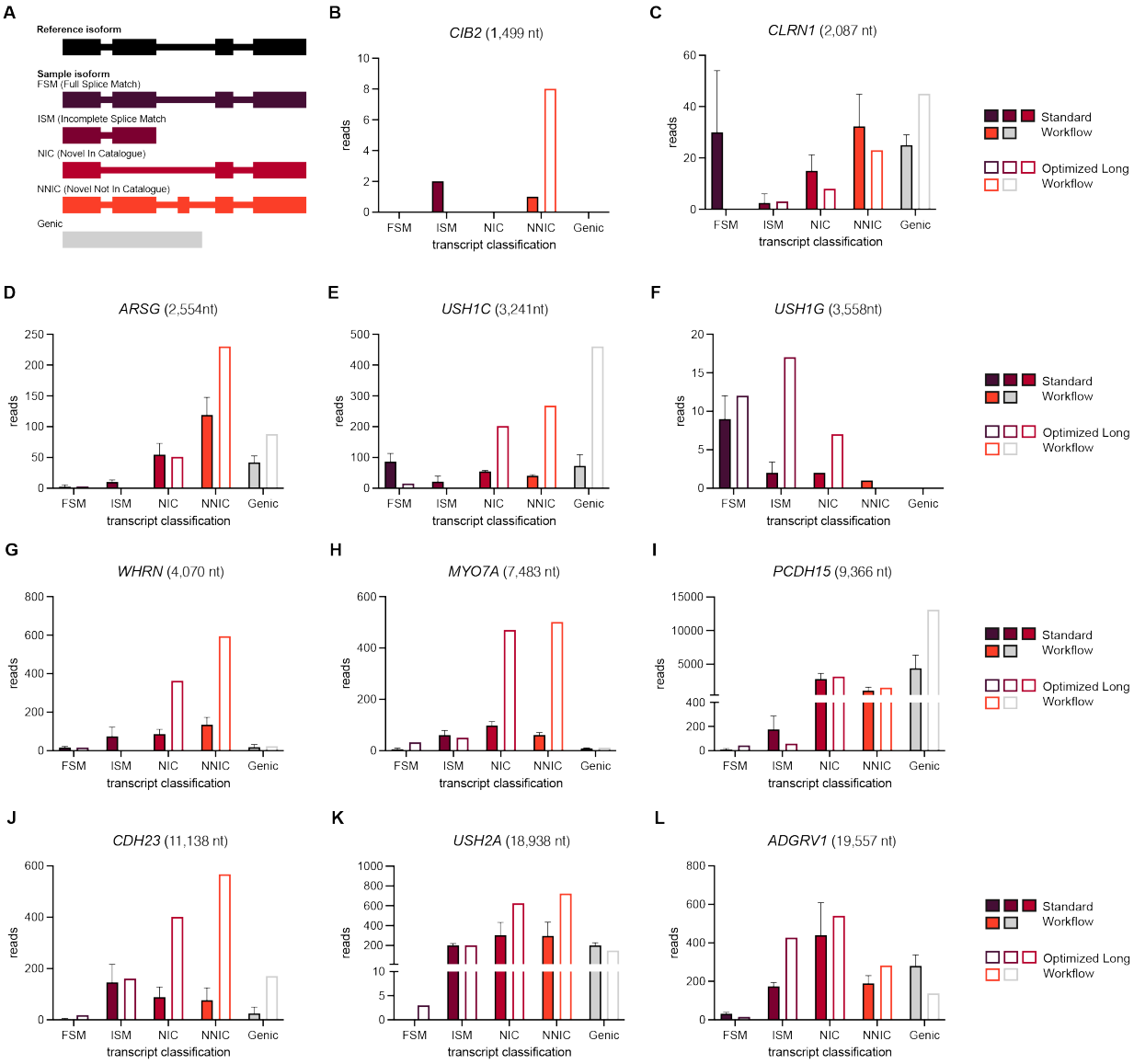
Supplemental_Fig_S6: *ADGRV1* transcript isoforms identified by IsoQuant analysis compared to known isoforms from the literature. A. The GENCODE reference transcript is depicted at the top in green, followed by the known human *ADGRV1* isoforms in blue (McMillan *et al.*, 2002), and the murine isoforms in grey (McMillan *et al.*, 2002; Skradski *et al.*, 2001; Yagi *et al.*, 2005). The *ADGRV1* IsoQuant transcripts are depicted in red. The light green, blue, grey and red colors indicate the untranslated regions (UTR) and the dark green, blue, grey and red colors indicate the open reading frame (ORF) of each transcript. Differences between the IsoQuant transcript isoforms and the GENCODE reference transcript are highlighted in grey boxes. **B.** Relative expression of *ADGRV1* isoforms based on literature in either the retina or the cochlea. **C.** The Transcripts Per Million (based on dataset 1) for each IsoQuant isoform are presented for the three individual samples.



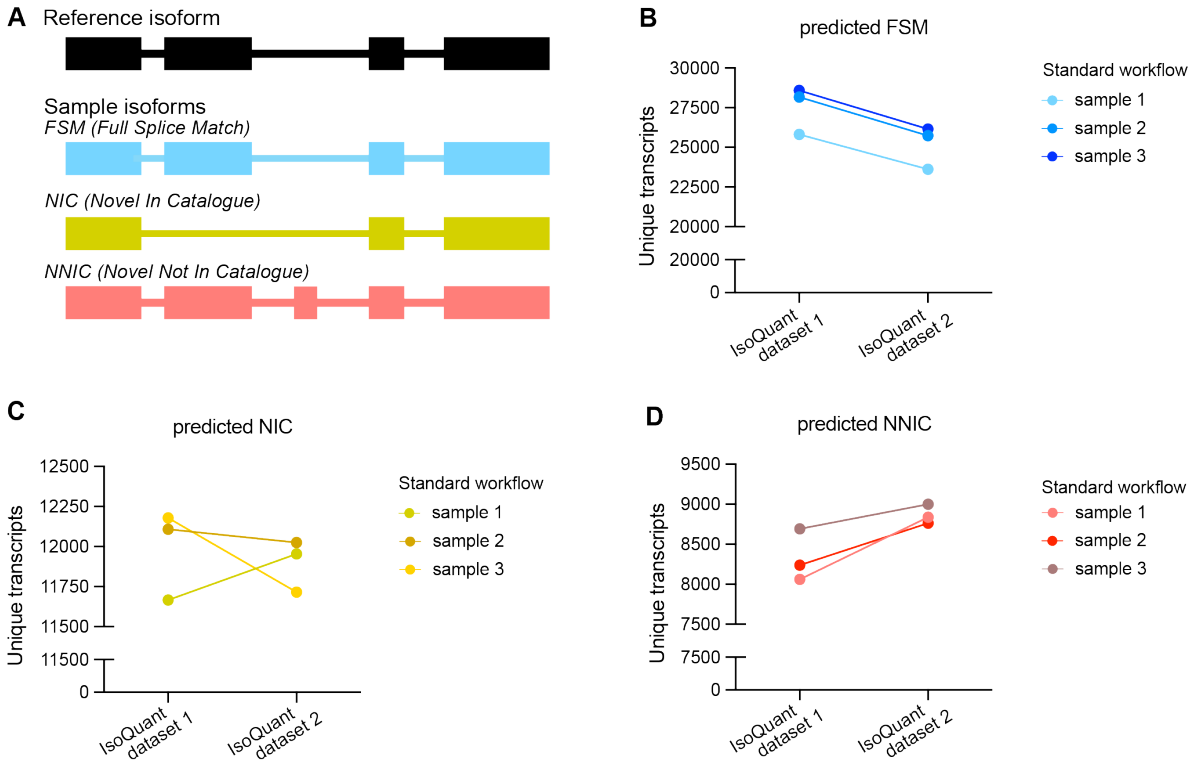
Supplemental_Fig_S7: *CLRN1* isoforms identified by IsoQuant analysis compared to known isoforms from the literature. A. The GENCODE reference transcript is depicted at the top in green, followed by the known human *CLRN1* isoforms in blue (Adato *et al.*, 2002; Joensuu *et al.*, 2001). The *CLRN1* IsoQuant transcripts are depicted in red. The light green, blue and red colors indicate the untranslated regions (UTR) and the dark green, blue and red colors indicate the open reading frame (ORF) of each transcript. Differences between the IsoQuant isoforms and the GENCODE reference transcript are highlighted in grey boxes. **B.** The Transcripts Per Million (based on dataset 1) for the IsoQuant identified isoforms are presented for the three individual samples.



Supplemental_Fig_S8: *ARSG* isoforms identified by IsoQuant analysis. A. The GENCODE reference transcript is depicted at the top in green, followed by the *ARSG* IsoQuant transcripts depicted in red. The light green and red colors indicate the untranslated regions (UTR) and the dark green and red colors indicate the open reading frame (ORF) of each transcript. **B.** The Transcripts Per Million (based on dataset 1) for the IsoQuant identified isoforms are presented for the three individual samples.



Supplemental_Fig_S9: Comparison between Usher syndrome isoform transcripts obtained from PacBio standard workflow and optimized long workflow datasets. A: Schematic representation that compares retinal transcripts to the GENCODE reference transcriptome: Full Splice Matches (FSMs), Incomplete Splice Matches (ISM), Novel In Catalog (NIC) and Novel Not In Catalog (NNIC). Genomic contamination is indicated in grey. **B-L:** Number of reads for each USH gene distributed per isoform category. Filled boxes are depicting the mean (±SD) for human neural retina samples 1-3 prepared according to the PacBio standard workflow. The empty boxes indicate the number of reads identified in the sample prepared following an optimized PacBio long transcript workflow.



Supplemental_Fig_S10: Number of unique transcripts associated with each transcript class, as predicted by IsoQuant for each standard workflow sample. A. Schematic representation that compares IsoQuant transcript classifications to the GENCODE reference isoform. Transcript isoforms that fully match the reference are classified as Full Splice Matches (FSMs). Novel transcripts are those that either align to the reference splice junctions, in which case they are designated as Novel In Catalog (NIC), or contain novel splice junctions and are termed Novel Not In Catalog (NNIC). **B, C, D.** Number of unique transcripts associated with each transcript class, as predicted by IsoQuant for each standard workflow sample. Based on the input per dataset, classifications differ for IsoQuant dataset 1 (analysis of 3 Standard Workflow samples) or IsoQuant dataset 2 (combined analysis of 3 standard workflow samples with the optimized long transcript workflow sample). **B.** Number of unique FSM predicted transcripts per dataset, **C.** Number of unique NIC predicted transcripts per dataset, **D.** Number of unique NNIC predicted transcripts per dataset.

Supplemental_Fig_S11: Examination of IsoQuant algorithm outputs across different datasets, highlighting the influence of including or excluding the PacBio long transcript workflow sample on the IsoQuant-predicted *MYO7A* isoforms. The *MYO7A* gene serves as an example to illustrate the divergence in identified isoforms by the IsoQuant algorithm. The green annotation represents the *MYO7A* reference isoform. In red are the *MYO7A* isoforms resulting from the IsoQuant analysis of standard workflow samples 1-3, as identified in dataset 1 (Riepe *et al.*, 2024). This includes both Full Splice Matches (FSMs) to the reference isoform, as well as Novel In Catalogue (NICs) and Novel Not In Catalogue (NNICs) isoforms. In blue is the *MYO7A* isoform following the IsoQuant analysis of the combined standard and long transcript workflow samples in dataset 2. Despite the increased input data, this analysis only yielded a single NNIC isoform, with no FSMs or NICs detected.

Supplemental_Fig_S12: Oxford Nanopore Technologies (ONT) long-read sequencing dataset of three independent human retina samples confirmed the use of alternative 5' transcription start site in *MYO7A* transcripts. Snapshot of Integrative Genomics Viewer (IGV), zoomed in on the canonical and alternative start site region of *MYO7A* (exons 1 – 4). Sequencing reads of ONT long-read sequencing of three independent human neural retina samples (MYO7A_ONT_Hs1.bam - MYO7A_ONT_Hs3.bam) confirm the usage of both the know start site (starting at canonical exon 1) and the usages of an alternative start exon located in intron 2. The usage of both the canonical- and the alternative start site has also been observed across the sequencing reads of all PacBio long-read sequencing samples, as exemplified by reads shown for the human neural retina sample prepared following the standard PacBio workflow (MYO7A_PacBio_Hs1.bam).

Supplemental_Table_S1: Overview of observed events following manual curation of reads in BAM-files of Samples 1-4.

Gene	**Curated transcripts***	**Novel exons** (in-frame)**	**Novel exon** (out of frame)**	**In-frame skipping events**	**Out of frame skipping** events	**Alternative/novel splice junctions**	**Intron retentions**
MYO7A	ENST00000409709.9	Alternative transcription start site 5' of canonical exon 3, Exon 30A, 31A, 31B	Exon 2A, 2B, 2C, 2D, 4A, 15A, 27A, 44A, 46B	Exon 3, 11, 26	Exon 9, (9+10 combined), 14	5' extension of exon 8, 12, 17, 28, 42, 44 3' extension of exon 12 5' truncation of exon 35, 40, 44, 16	Intron 8, 9, 11, 28, 30 (**observed in ~25% of reads**), 33, 37 (**observed in ~25% of reads**), 38, 40, 41, 42, 43, 44
USH1C	ENST00000005226.12	Exon 15A, 20A	Exon 15B, 18A, alternative penultimate exon	Exon 2, 11, 15	Exon 19, 20	5' extension of exons 6, 12, 16 3' extension of exon 5 3' truncation of exon 14	Intron 3, 4, 7, 10, (10 + 11 combined), (22+23 combined), 23, 23 (with internal fragment spliced out), 24 (with internal fragment spliced out)
CDH23	ENST00000224721.12	Exon 11A, 31A, 66A	Exon 1A, 25A, 26A, 44A, 44B, 44C, 45A, 48A, 48B, 48C, 48D	Exon 12, 34, 46, (56-57 combined skip), **exon 69 skip observed in ~80% of reads**	-	5' extension of exons 6, 12, 46, 49, 55, 60, 65, 70 3' extension of exons 16, 55, 60, 63 5' truncation of exons 58, 60 3' truncation of exons 58, 59, 60	Intron 7, 23 (45-46 combined), 46, 50, 64, 65, (65-66 combined)
PCDH15	ENST0000064397	Exon 1A, 2A, 5A, 9A, 9B, 18A, 18B	Exon 1B, 9C, 9D, 9E, 13A, 13B, 15A, 16A, 17A, 18C, 26A, 26B, 26C, 32A, 32B, 36A	Exon 19, 21, 22 27, 31, 33 (exon 33, 34, 35, 36 combined skip)	Exon 10, 14, 16, 17 23, 26	5' truncation of exon 11 3' truncation of exon 36	-
SANS	ENST00000614341.5	-	-	-	-	-	-
CIB2	ENST00000258930.8	-	-	-	-	-	-
USH2A	ENST00000307340.8	Exon 3A	Exons 4A, 5A, 8A, 9A, 9B, 9C, 11A, 11B, 11C, 12A, 13A, 13B, 13C,	Exon 8, (exon 6,7,8 combined skip) 28, (exon 33-37 combined skip)	Exon 3, (exon 5, 6, 7, 8, 9 combined skip) exon 6, 9, 12, (exon 12-13 combined	5' extension of exon 65, 70 3' extension of exon 14, 15	Intron 5, 6

			14A, 15A, 16A, 20A, 20B, 20C, 20D, 22A, 32A, 32B, 35A, 37A, 41A, 43A, 44A, 44B, 45A, 49A, 50A, 58A, 61A, 64A, 64B, 65A, 67A, 70A, 71A		skip), exon 14, (exon 15-19 combined skip), (exon 15-22 combined skip) exon 26, (exon 33-34 combined skip) , exon 42, 52, 55, (exon 65-67 combined skip),	5' truncation of exon 8, 19, 46, 64 3' truncation of exon 1, 6, 10, 13, 25, 46,	
ADGRV1	ENST00000405460.9	Exon 1A, **39A inclusion observed in ~50% of reads**, 86A	Exon 77A, 77B, 77C, 79A, 79B, 86A	Exon 4	Exon 67, 76, (exon 76-77 combined skip), (exon 88-89 combined skip)	5' extension of exon 28, 58, 89 3' extension of exon 2, 25, 43, 58, 69, 88 5' truncation of exon 26, 42, 52 3' truncation of exon 6, 49	Intron 4, 39, 52, 56, 64, 88
WHRN	ENST00000362057.4	Exon 2A, **7B inclusion observed in ~80% of reads** (7B + 7G combined)	(Exon 7A + 7B combined), 7C, 7D 7E, 7F, 7G, 7H	Exon 3, 7	Exon 4, (exon 5+6 combined skip)	5' extension of exon 5 3' extension of exon 4 5' truncation of exon 7, 10 3' truncation of exon 9	**Intron 4 retention observed in ~50% of reads** Intron 6, (combined intron 4 + 9 retention)
CLRN1	ENST00000327047.6	-	-	-	-	-	-
ARSG	ENST00000621439.5	Exon 8A, 10A, alternative terminal exon	Alternative transcription start site 5' of canonical exon 1, Exon 2A, 10B, 10C, 10D	-	(Exon 7+8 combined skip), (exon 9+10 combined skip)	5' extension exon 9 3' extension exon 2, 3, 5, 6, 11,	-

*Curated MANE select transcripts that were used to annotate observed events.

** Novel identified exons are designated with alphabetical labels (e.g., 'A', 'B') to denote their relative positioning. For instance, '30A' indicates a novel exon observed downstream of the canonical exon 30.

Supplemental_Table_S2: Descriptive sample statistics.

Sequencing sample	**S1** (m64167e_210819_012015)	**S2** (m64167e_210902_121011)	**S3** (m64167e_210903_121024)	**S4** (m64037e_211216_160915)
Retina sample	Sample 1	Sample 2	Sample 3	Sample 2
Library preparation method	Standard	Standard	Standard	Long transcript enrichment
Sequel II output				
Polymerase reads	5,063,614	7,011,790	6,942,704	6,354,053
Mean subread length	3,060	1,848	2,113	4,514
CCS3 reads	3,353,662	4,216,550	4,044,519	4,017,245
CCS0 reads				5,573,627
Reads with intron retention (%)*	28.45	26.16	20.37	42.9 (CCS3) 42.8 (CCS0)
Isoquant dataset 1				
Total unique transcripts#	45,539	48,514	49,456	
FSM	25,809	28,166	28,583	
NNIC	8,063	8,240	8,694	
NIC	11,667	12,109	12,109	
Isoquant dataset 2				
Total unique transcripts#	44,411	46,525	46,877	41,019
FSM	23,619	25,733	26,161	21,880
NNIC	8,838	8,766	9,000	7,866
NIC	11,954	12,026	11,716	11,273
Isoquant dataset 3				
Total unique transcripts#				44,646
FSM				23,070
NNIC				9,226
NIC				12,350

* Percentage of intron retention calculated from the read assignment file produced by isoquant.

Counts of total transcripts calculated from the isoquant transcript model grouped counts file. FSM = Full Splice Match, NNIC = Novel Not In Catalog, NIC = Novel In Catalog.

Supplemental_Table_S4: Mean qPCR CT values of cDNA samples before and after Samplix Xdrop Sort

Target	Mean CT value unenriched sample	Mean CT value enriched sample
USH2A 5' Front	25.8	13.4
USH2A Middle	25.7	15.7
USH2A 3' End	23.7	17.9
ADGRV1 5' Front	26.4	16.3
ADGRV1 Middle	24.5	16.5
ADGRV1 3' End	26.1	12.2

Supplemental_Table_S5: Details of human neural retina samples used for PacBio Iso-Seq

Sample ID	Sex	Age	Time until enucleation	RIN value
Sample 1	Male	59	2 hours 35 minutes	8.2
Sample 2	Male	63	9 hours 5 minutes	7.3
Sample 3	Female	58	11 hours 55 minutes	7.5

Supplemental_Table_S6: Composition of *USH2A* and *ADGRV1* detection sequence and validation sequence primers.

Primer ID	Forward sequence	Reverse Sequence	Targeted exons
USH2A Front dPCR	ACAACTGAGACTGCTGTTAACC	GACCATGGCACTGACATCTC	8-9
USH2A Front qPCR	GCAAAGCAAACGTTATTGGGCT	TACACTGGCAGGGCTCACAT	12-13
USH2A Middle dPCR	GAAAGCCTATAGTGAGGACAGCA	TCTCAGTACAGCCAGCCAAA	30-31
USH2A Middle qPCR	TGTTTCGGTTGGTTGCCTCC	GGTCCAGGTTTGTCCTCTGC	40-41
USH2A End dPCR	TGGAGTGACACCTTCCTCCT	CCTTCGTCAGTCGTGCAGAT	68-69
USH2A End qPCR	ACTGTGGGAAGCCATCATGG	TGTCTGTGAATGTGGTGCGT	71-72
ADGRV1 Front dPCR	ACTGAATGGCACTGGAGGAG	AGTTGGCAGTCTCACCTTCC	13-14
ADGRV1 Front qPCR	TGCTAGCAAGATTGGATGGGA	ACAATGGTGGCACTTCCCAA	16-17
ADGRV1 Middle dPCR	GGCTTTGTCTTCAGCCAATG	GCTGGTGTGAAGGAGTGGAT	50-51
ADGRV1 Middle qPCR	CTCCTTCACACCAGCCTCAG	CGCTCCGAATTCCAGCAGTA	49-50
ADGRV1 End dPCR	ATAAAGTGGACGTGGTGCCA	AAACGTCCACCGACAAGCAT	70-71
ADGRV1 End qPCR	GCAACATGACCCCAACACTG	CAACCAGGGCTAGGAAACGT	72-73

Supplemental_Table_S7: Details of human neural retina samples used for Oxford Nanopore Technology sequencing.

Sample ID	Time until enucleation	RIN value	Reads (passed)
Sample 1	< 20 hours	> 8	10,206,706
Sample 2	< 20 hours	> 8	18,463,865
Sample 3	< 20 hours	> 8	11,388,116

Supplemental_Table_S8: *MYO7A* and *WHRN* qPCR primer sequences

Primer ID	Forward sequence	Reverse Sequence	Targeted exons
MYO7A 5' canonical start	CTTCCTGAGTCCTCCGTGC	CACATGGTCCCCCTGCTG	1-2
MYO7A 5' alternative start	ACCAGGCGTTCAAGACCTAC	CATCATCCACCACCTGGACC	Alt.1-2
MYO7A 3' end	ACGTCACTGGGCTACAAGATG	CTCCCCGTGACTGTTCACTT	48-49
GUSB	AGAGTGGTGCTGAGGATTGG	CCCTCATGCTCTAGCGTGTC	2-3
WHRN exon 7B	GACCTGATGGAGAACACTCTG	GATCTGACATCATCCACGGAC	7B - 9
WHRN intron 4 retention	CTGGAGATGTGTGTCTTTCCC	CCCTTGTAAAATCCTGGCTTG	Intron 4 – exon 5-6
WHRN exon 8-9	TCTGGACCTGGAGGAAACTG	GGAGGTGGATCTGACATCATC	8-9

Reference list:

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