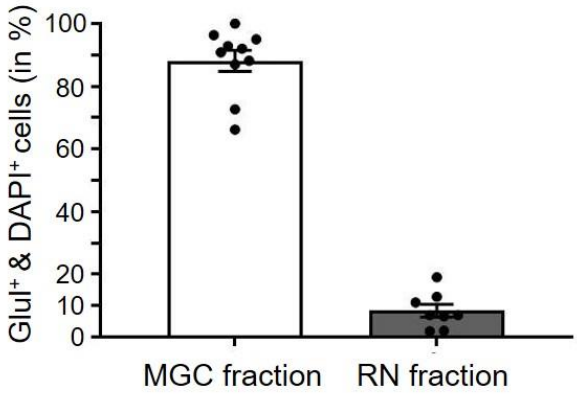
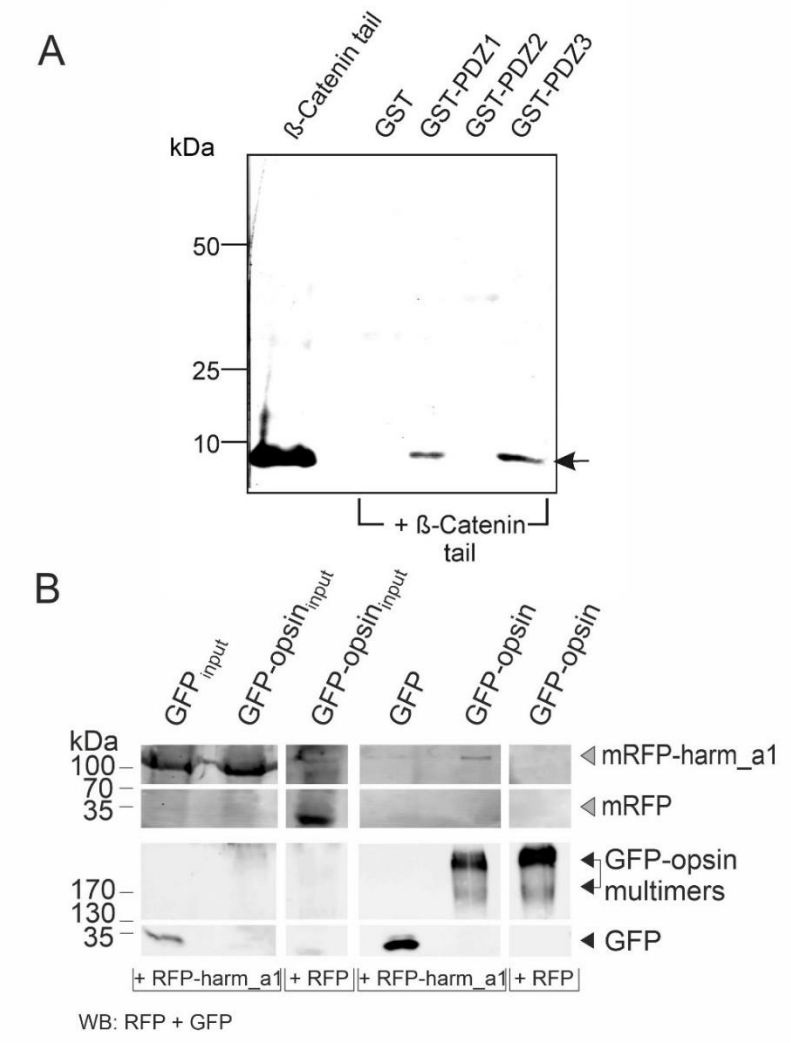
Supplemental material: Nagel-Wolfrum et al. *USH1C*/harmonin in human retina

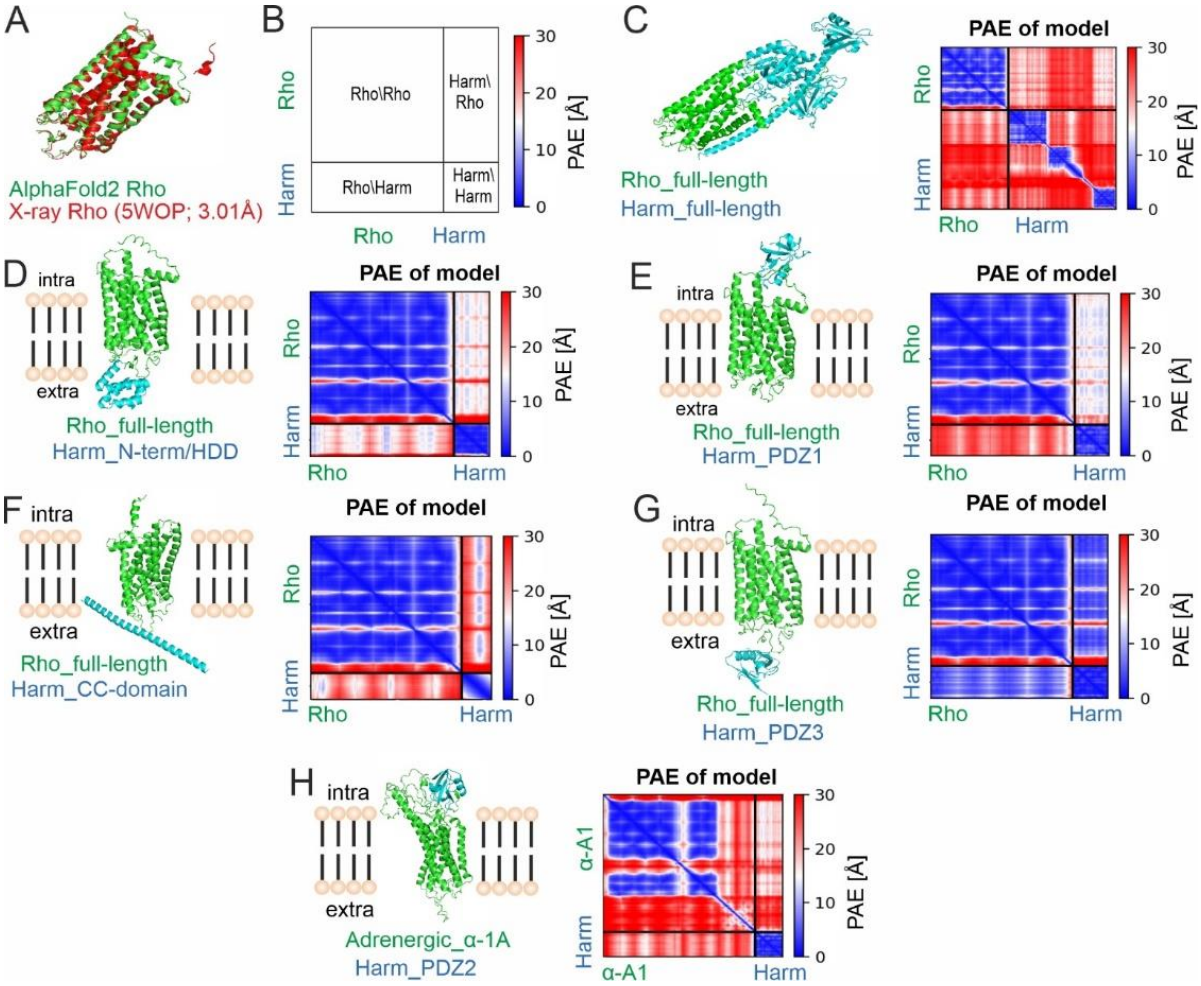
Supplemental Figures



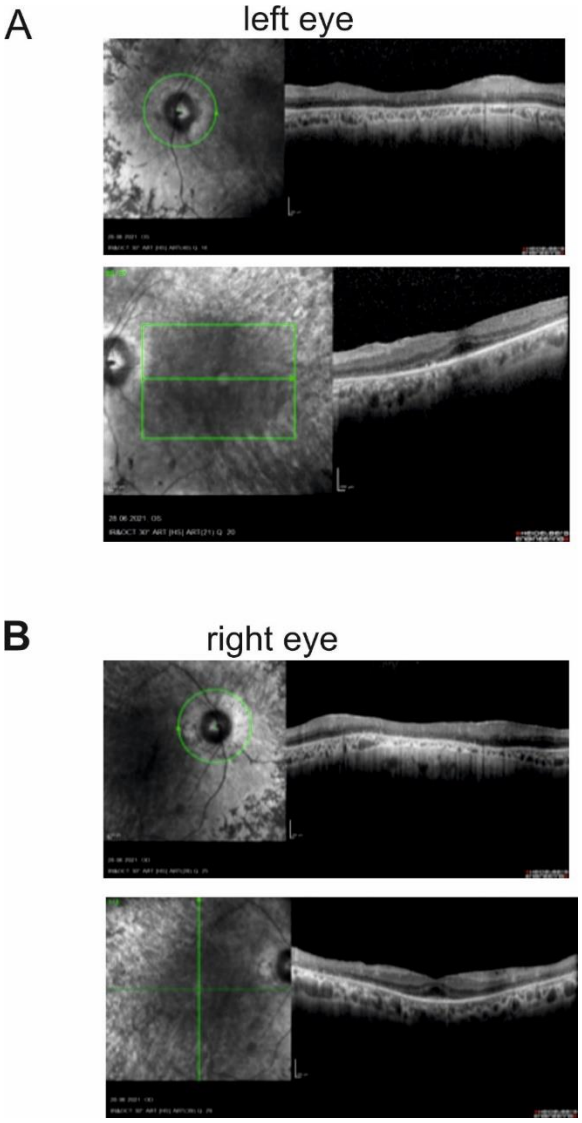
Supplemental Figure S1: Quantification of Müller glial cell content following magnetic-activated cell sorting (MACS) sorting of human retinae.
Drop samples from the Müller glial cell (MGC) fraction and retinal neuron (RN) fraction following magnetic-activated cell sorting (MACS) of human donor retinae were stained for the MGC marker glutamine synthetase (Glul) and DAPI as a nuclear marker. Percentage (%) of $Glul^+$ cells in each fraction was quantified revealing less than 10% contamination of the RN fraction with MGCs.



Supplemental Figure S2. Analysis of novel interaction partners of harmonin.
(A) GST-pull down of harmonin PDZ domains and β-catenin tail. PDZ1 and PDZ3 of harmonin are interacting with the tail of β-catenin (β-Catenin tail). GST only and harmonin PDZ2 are not interacting with β-catenin. **(B)** GFP-Trap® demonstrate harmonin-RFP interaction with GFP-opsin. RFP-harmonin a1 was precipitated by immobilized GFP-opsin via GFP-beads, but not by GFP alone. RFP only did not interact with GFP-opsin.



Supplemental Figure S3. Structure predictions by AlphaFold2.
(A) The alignment of rhodopsin structures predicted by AlphaFold2 and determined by X-ray crystallography and NMR shows a high degree of concordance, indicating high accuracy of AlphaFold prediction for rhodopsin (Rho) structure based on amino acid sequences. **(B)** Schematic representation of the Predicted Alignment Error (PAE) of a model predicted by AlphaFold. As example: Rho-Harm protein complex. Rectangles lower left and upper right indicate Harm-Rod and Rho-Harm protein complexes, respectively. Heat map illustrates amino acid distances of the two analysed proteins in Å (red: large distance, blue: short distance, predicting protein-protein interaction). (**C**) Structure of the protein complex of full-length rhodopsin (Rho) and full-length harmonin_a1 predicted by AlphaFold2. Predicted Alignment Error (PAE) of the model is high in almost all regions indicating no interaction (red color in heatmap of Ham:Rho and Rho:Harm rectangles). (**D-H**) Structures of the protein complexes of (D) rhodopsin-harmonin_*N*-term/HHD, (**E**) rhodopsin-harmonin_PDZ1, (**F)** rhodopsin-harmonin_CC1, (**G**) rhodopsin-harmonin_PDZ3, and (**H**) full-length adrenergic receptor α-A1 and harmonin_PDZ2 predicted by AlphaFold. AlphaFold predicted low confidence of protein complexes for rhodopsin-harmonin_PDZ1and adrenergic receptor α-A1 and harmonin_PDZ2. For rhodopsin-harmonin_*N*-term/HHD, rhodopsin-harmonin_CC1, and rhodopsin-harmonin_PDZ3 complexes AlphaFold predicted binding of the intracellular protein harmonin to extracellular sites of rhodopsin.



Supplemental Figure S4. OCT of an USH1C patient.
(**A**, **B**) Optical coherence tomography of the left (A) and right (B) eye of a 47-year old male USH1C patients with confirmed mutations in *USH1C* (c.91C>T;.p.(R31*); c.238dupC;p.(Arg80Profs*69)) showing outer retinal atrophy with photoreceptor degeneration.

Supplemental Tables:

Supplemental Table S1. **Human retina donors.** An internal donor number (#) was assigned to all donors. All donors had no documented history of retinal disease. Abbreviations: M, male, F, female, MGCs, Müller glia cells; RNs, retinal neurons; RT-PCR, reverse transcriptase polymerase chain reaction; qRT, quantitative PCR; WB, Western blot; IF, immunofluorescence microscopy; EM, electron microscopy; age in years.

Donor #	Age	Gender	*hours post mortem*	Application
198-09	56	M	30	RT-PCR
199-09	66	F	31	RT-PCR, WB, IF, EM
205-09	57	F	21	RT-PCR, RNA-seq
220-09	44	F	31	RT-PCR, qRT
250-09	63	M	9 ½	RT-PCR, RNA-seq
252-09	68	F	11 ½	RT-PCR, qRT, IF
263-09	56	M	27	qRT, RNA-seq
269-09	73	F	29	RNA-seq
121-10	58	F	21	EM
16-0928-OS	76	M	9	Bulk RNA-seq
16-0928-OD	76	M	9	Bulk RNA-seq
16-0932-OS	70	F	9	Bulk RNA-seq
16-0932-OD	70	F	9	Bulk RNA-seq
19-0013-OD	89	M	30	WB – sorted MGCs/RNs
19-0015-OS	59	F	26	WB – sorted MGCs/RNs

Supplemental Table S2. RNA-seq analyses and bulk RNA-seq analyses of *USH1C*/harmonin transcripts in human retina. RNA-seq analyses of human retinae and bulk RNA-seq analyses in enriched Müller glia cells and retinal neurons of *USH1C*/harmonin transcripts in human retina. (see Excel sheets)

Supplemental Table S3. Primers for *USH1C*/harmonin class specific transcripts.

Isoform/ class	Forward Primer	Primer sequence 5´-> 3´	Reverse Primer	Primer sequence 3´-> 5´	Expected size (bp)
a	Exon 8	TCA TCA GCC TGG TAG GCT C	Exon 15	CAG GTT CCA CTC CCT GAT C	554
b	Exon 14	CAT CAC TGC TGA GGT ACA CC	Exon 18	CAG CAG AGG AAA TCT GCT C	349
c	Exon 14/22	CTT CGC AAG CCA AAG TGA TTT C	3´UTR	GGC TGA TCC GAG GCT TTG TG	458