Supplementary Figure 1. Representative genotyping data of \textit{Clrn1}^{-/-} and \textit{Clrn1}^{N48K/N48K} mice in A/J background. (A) Schematic diagram illustrating WT, \textit{Clrn1}, and \textit{Clrn1}^{N48K} alleles. Top row: WT \textit{Clrn1} allele contains 4 exons. Middle row: In \textit{Clrn1}^{-} allele, exon 1 was removed by Cre-loxP recombination which left one loxP site. Bottom row: In \textit{Clrn1}^{N48K} allele, exon 1 of \textit{Clrn1} gene was replaced with a mutated exon 1 (with the G mutation replacing N at the 48th codon with K). In this allele, one loxP site is located downstream of exon 1. (B) Genotyping of \textit{Clrn1}^{-/-} mice. Genomic DNA isolated from tail snips was analyzed by two sets of PCR reactions. Primer pair P3/P4 (left panel) effectively produces a 2066 bp product from WT allele, but a product of about 700 bp from \textit{Clrn1}^{-} allele. A second primer pair, C1/P4 (right panel) amplifies a product at the size of 1035 from the WT allele only. Taken together, P3/P4 and C1/P4 are used to distinguish between WT, \textit{Clrn1}^{+/-}, and \textit{Clrn1}^{-/-}. (C) Genotyping of \textit{Clrn1}^{N48K/N48K} mice. PCR reaction with primer pair 2L/2R produced a 275 bp product for \textit{Clrn1}^{N48K} allele and a 230 bp product for WT allele.

Supplementary Figure 2. CLRN1 deficiency does not affect the protein expression levels of outer segment markers rhodopsin and peripherin/rds, and Müller glia marker GFAP. Immunoblotting analyses were conducted on retinas of WT (black bar) and \textit{Clrn1}^{-/-} (grey bar) mice at 4.5-month-old when progressive functional changes are anticipated to be dramatic in \textit{Clrn1}^{-/-} mice based on the time course of decay in ERG amplitudes (see Fig. 2). Expression levels were calculated by normalizing the band intensity of rhodopsin, peripherin/rds or GFAP to the band intensity of tubulin. The data are represented by mean ± SE (n = 3). There is no significant difference between WT and \textit{Clrn1}^{-/-} in rhodopsin, peripherin/rds and GFAP protein expression level (p > 0.42).
Supplementary Figure 3. *Clrn1* deficiency did not affect the synapse structures. Sections of fixed eye cups of 9-month-old WT and *Clrn1*−/− mice were probed with antibodies against Go α (A), Calbindin (B), Calretinin (C), Bassoon (D), RIBEYE (E). Sections were probed with an antibody against myosin VIIA (green, F), fluorescently labeled phalloidin (green, G) and Hoechst 33342 (blue). In (F), RPE microvilli (arrows) are labeled by anti-Myosin VIIA antibody. Asterisks indicate where choroidal IgG was labeled by the secondary antibody, Alexa 488 conjugated anti-mouse IgG, and do not represent signals originated from anti-Myosin VIIA. Based on bassoon labeling, number and size of synaptic ribbons were compared between WT and *Clrn1*−/− mice. The number of ribbons were counted in areas spanning 93.75 μm in the horizontal axis, ~1 mm from optic nerve head, and determined to be 104.24 ± 5.49 for WT and 98.75 ± 8.95 for *Clrn1*−/−, respectively (mean ± SD, n = 4 retinas, p=0.399 by test); Diameters of horseshoe shaped ribbons were measured for WT and *Clrn1*−/− and determined to be 0.82 ± 0.04 μm and 0.86 ± 0.07 μm, respectively (mean ± SD, n = 69 ribbons from 4 *Clrn1*−/− retinas and n=92 ribbons from 4 WT retinas, p=0.429 by t test). Phalloidin and Hoechst 33342 were used to reveal F-actin and nuclei, respectively. OS: outer segment; IS: inner segment; ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; RPE: retinal pigment epithelium. The synapse markers, myosin VIIA and F-actin showed similar distribution patterns in WT and *Clrn1*−/− retina. Scale bars, 5 μm.
Supplemental Figure 1, Tian et al.
Supplemental Figure 2, Tian et al
Supplemental Figure 3, Tian et al