Figure S1. pcDNA3.1-CNGA3 plasmid mapping. The cloning vector was pcDNA3.1(+), which contains ori, human CMV promoter and enhancer, SV40 promoter, AmpR, Neo/KanR, multiple restriction endonuclease sites, bGH poly A signal, and SV40 poly A signal components. Insertion of the CNGA3 coding sequence was achieved using *Hin*dIII and *Bam*HI cloning sites, with the addition of a 3xFLAG tag after the Kozak sequence. The pcDNA3.1-CNGA3 plasmid contained 7,576 bases. p.S334F-F and p.S334F-R correspond to positions 1,984-2,015 and 1,968-2,000 in the plasmid, and include T and A as mutated bases, respectively. p.R189fs-F and p.R189fs-R correspond to positions 1,550-1,580 and 1,530-1,565 of the plasmid, and include T and A in as mutated bases, respectively. CNGA3, cyclic nucleotide-gated channel subunit α 3; ori, origin of replication; CMV, cytomegalovirus; AmpR, ampicillin resistance gene; Neo/KanR, neomycin/kanamycin resistance gene; bGH poly A, bovine growth hormone polyadenylation; SV40 poly A, SV40 polyadenylation; p.S334F, serine at position 334 of the protein is replaced by phenylalanine; p.R189fs, frameshift mutation where arginine is inserted at position 189; F, forward; R, reverse.

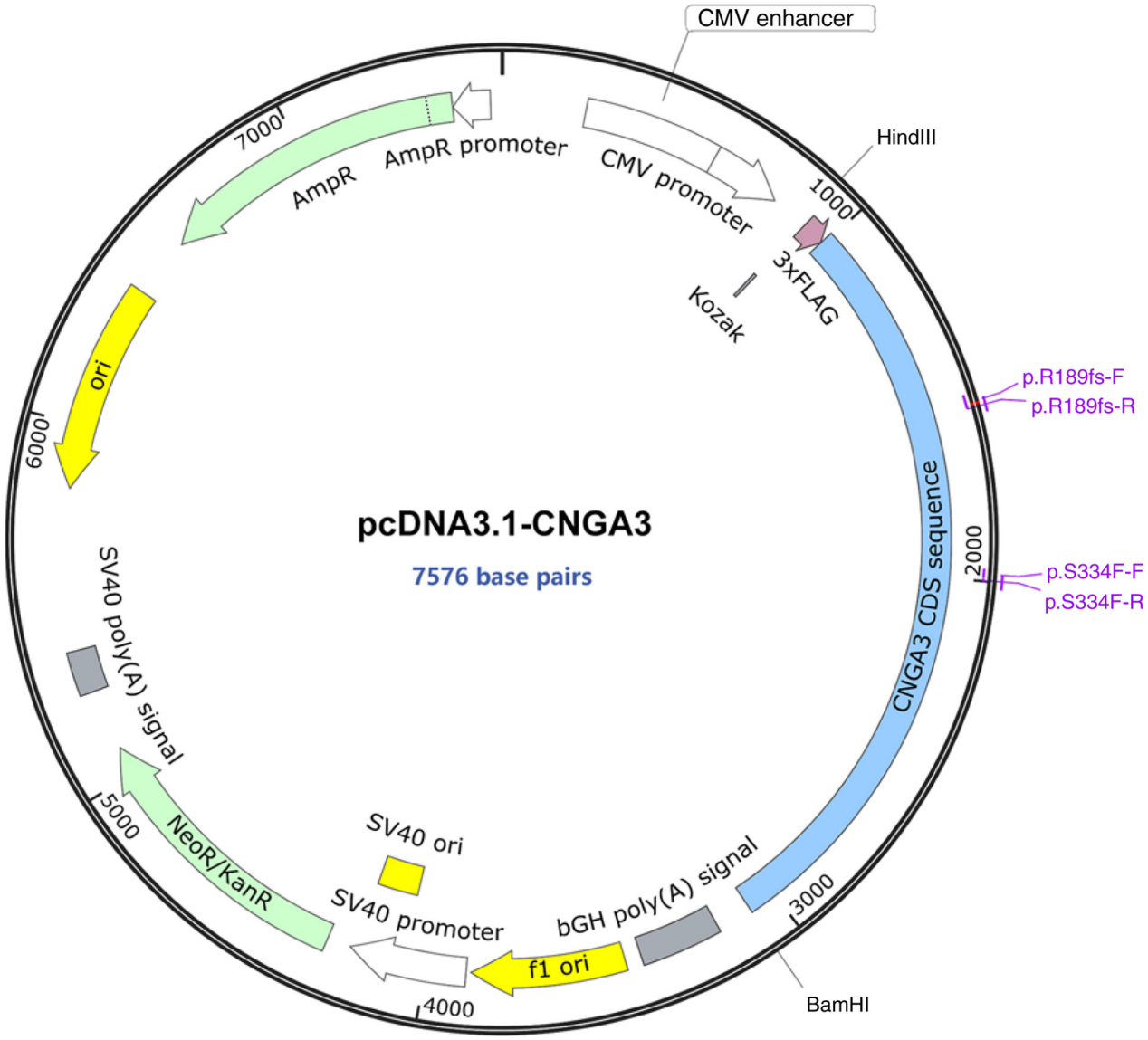

Figure S2. Retinal electroretinogram results during the follow-up visit. (A) Dark-adapted 0.01 ERG showing b-wave amplitudes (indicated by the green arrows) of 289.1 and 299.8 μV in the right and left eye, respectively. (B) Dark-adapted 3.0 ERG with a-wave amplitudes (indicated by the blue arrows) of 268.6 and 256.8 μV, and b-wave amplitudes (indicated by the red arrows) of 464.4 and 451.2 μV in the right and left eye, respectively. The b-wave amplitudes are below the normal range in both eyes (515-694 μV). (C) Oscillatory potential ERG indicates an approximately normal oscillatory potentials response. (D) Light-adapted 3.0 ERG demonstrates a pronounced reduction in the b-wave amplitude (indicated by the red arrow), with amplitudes of the right and left eye being 14.8 and 13.3 μV, respectively, which are markedly below the normal range (133-220 μV). (E) Analysis by 30-Hz flicker ERG reveals an N1-P1 wave amplitude of 8.7 and 8.4 μV in the right and left eye, respectively, below the typical range (75-205 μV) for both eyes. This suggests the presence of anomalies in cone cells and their posterior retinal structures. ERG, electroretinography.

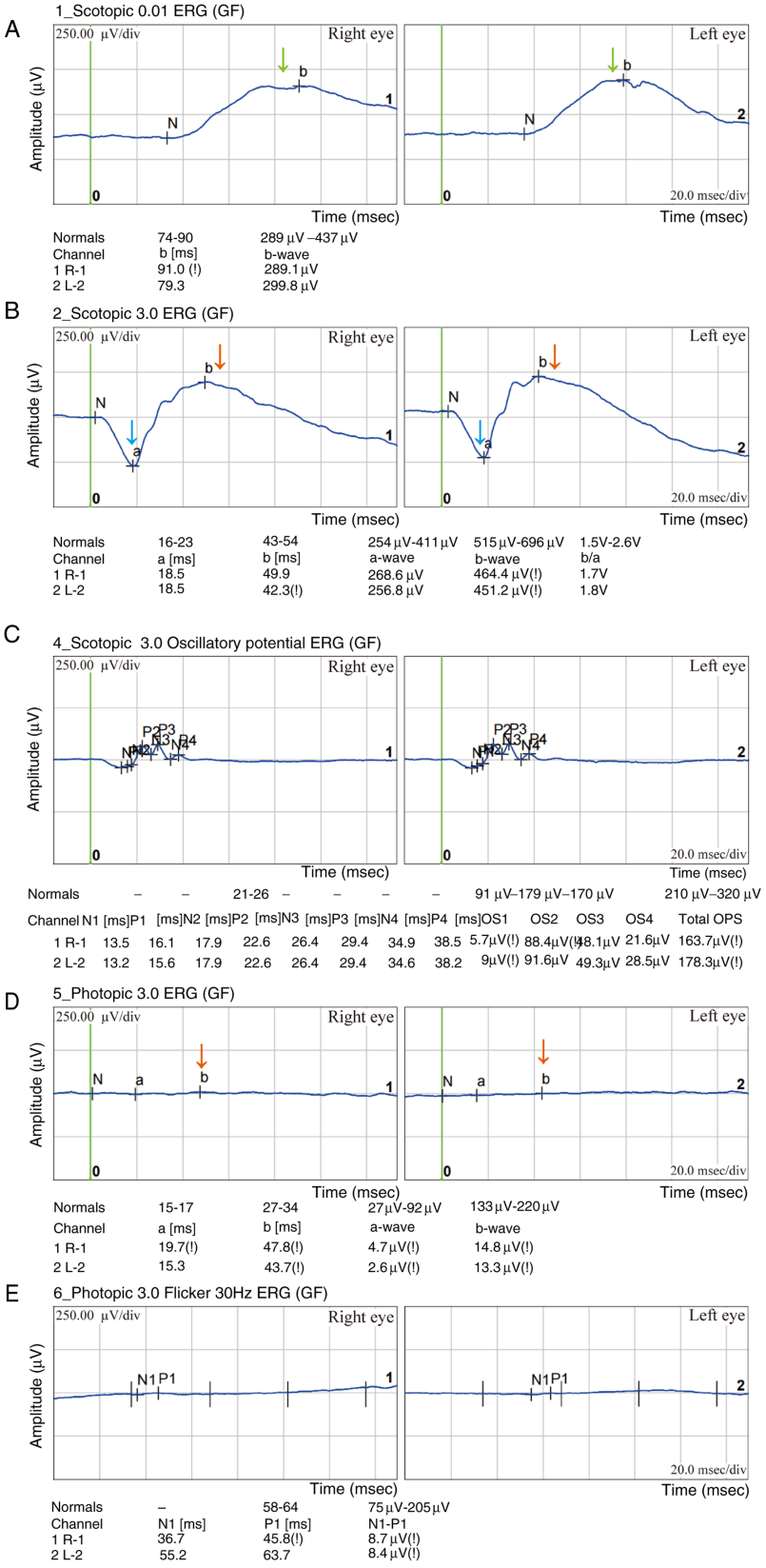

Figure S3. *CNGA3*:c.566_567insT mutation leads to amino acid changes. Nucleic acid sequence following *CNGA3*:c.566_567insT:p.R189fs mutation, with the shortened amino acid sequence (yellow arrow), and the insertion of base T between positions 566 and 567 in the cDNA sequence (marked in blue). This insertion results in the premature stop codon TGA at position 194 (marked in red) and a reduction in the number of amino acids encoded by the reading frame from 694 to 193, ultimately leading to a shorter and less stable protein. The *CNGA3*:c.566_567insT:p.R189fs mutation is located within the ion-trans domain of the *CNGA3* protein, as shown in Fig. 3F. CNGA3, cyclic nucleotide-gated channel subunit α 3.

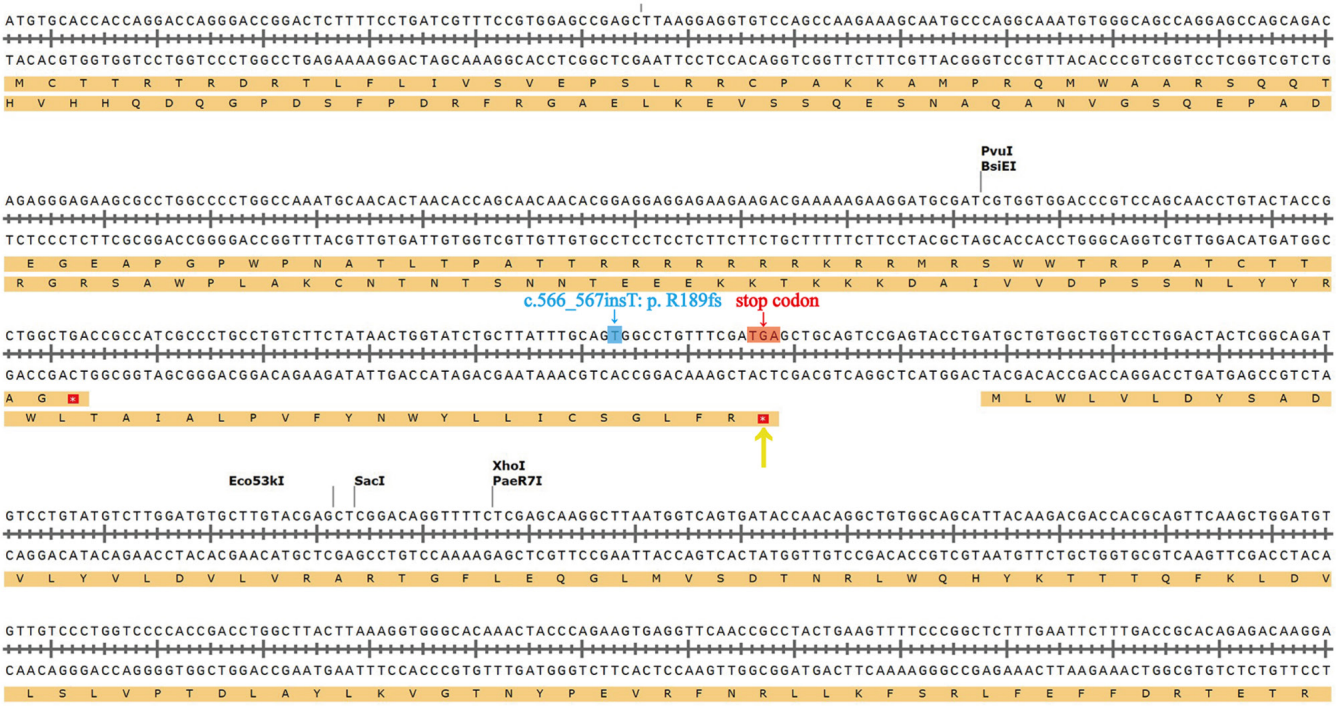

Figure S4. Sites of *CNGA3* expression. (A) CNGA3 expression in the brain. The brain image on the left highlights the regions of CNGA3 expression in the brain, with darker colors indicating higher levels. The data are based on the bulk RNA sequencing of micro-dissected brain regions and nuclei. Protein expression data are grouped into 13 major brain structures, with values representing the maximum expression in any region within each structure. The brain image on the right shows the color-coding used in the bar graph, which presents the expression of CNGA3 across brain regions. (B) Specific CNGA3 expression in body tissues. Elevated expression is observed in the brain, intestine, pituitary gland and retina. The bar graph shows normalized expression by tissue type, with different tissue groups shown in different colors. This RNA expression overview shows RNA data from two different sources: Internally generated Human Protein Atlas and Genotype-Tissue Expression project RNA-sequencing data, and a consensus dataset based on a combination of these two data sources. (C) Summary of normalized CNGA3 RNA expression across all single-cell types. The highest expression may be observed in cone cells. Color-coding represents cell-type groups, each consisting of different cell types with functional features in common. CNGA3, cyclic nucleotide-gated channel subunit α 3; nTPM, normalized parts per million.

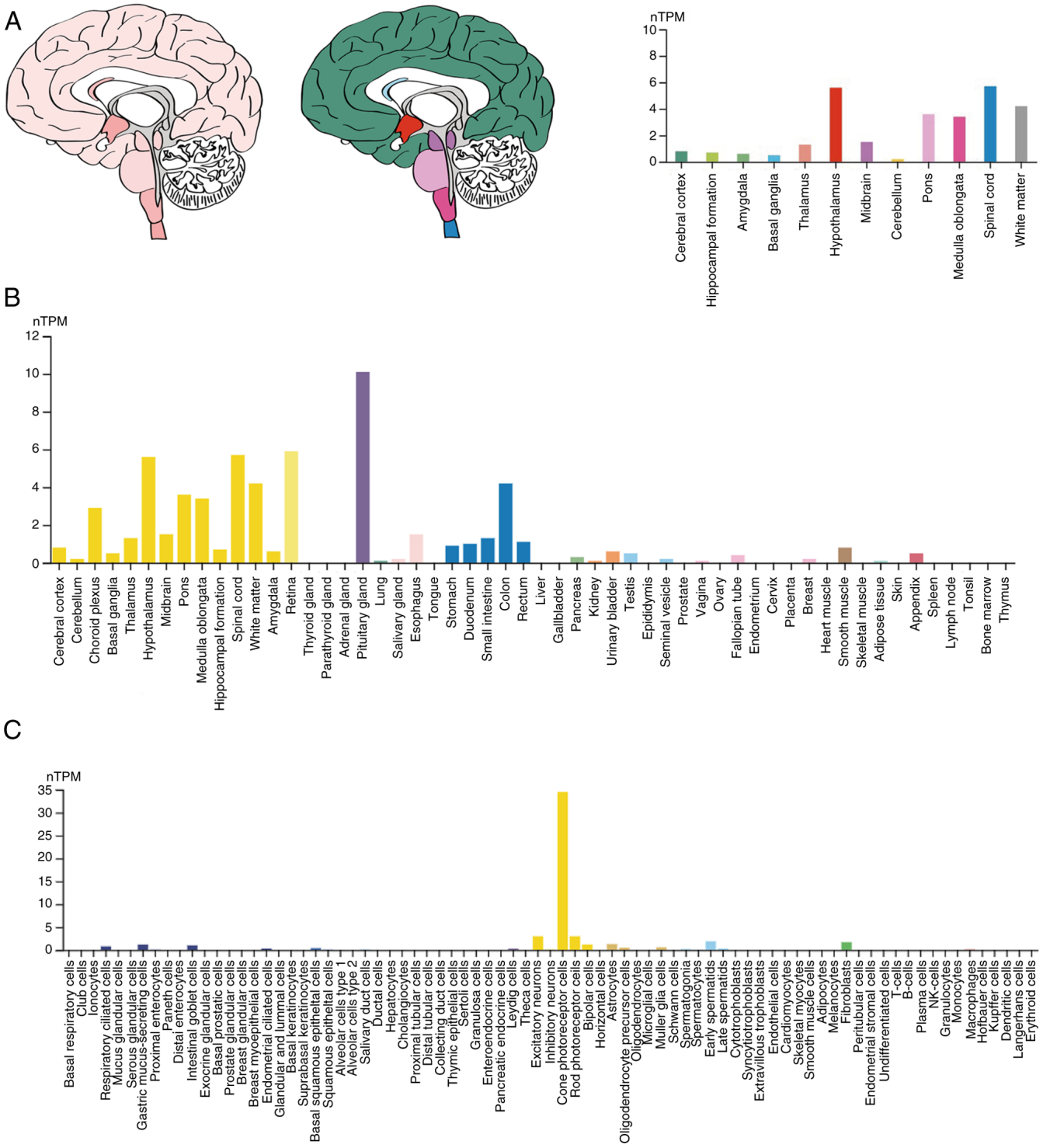

Figure S5. Original uncropped western blotting image of CNGA3 wild-type and mutant proteins in cells transfected with pcDNA3.1-CNGA3, pcDNA3.1-CNGA3-p.S334F and pcDNA3.1-CNGA3-p.R189fs plasmids. CNGA3, cyclic nucleotide-gated channel subunit α 3: IB, immunoblot.