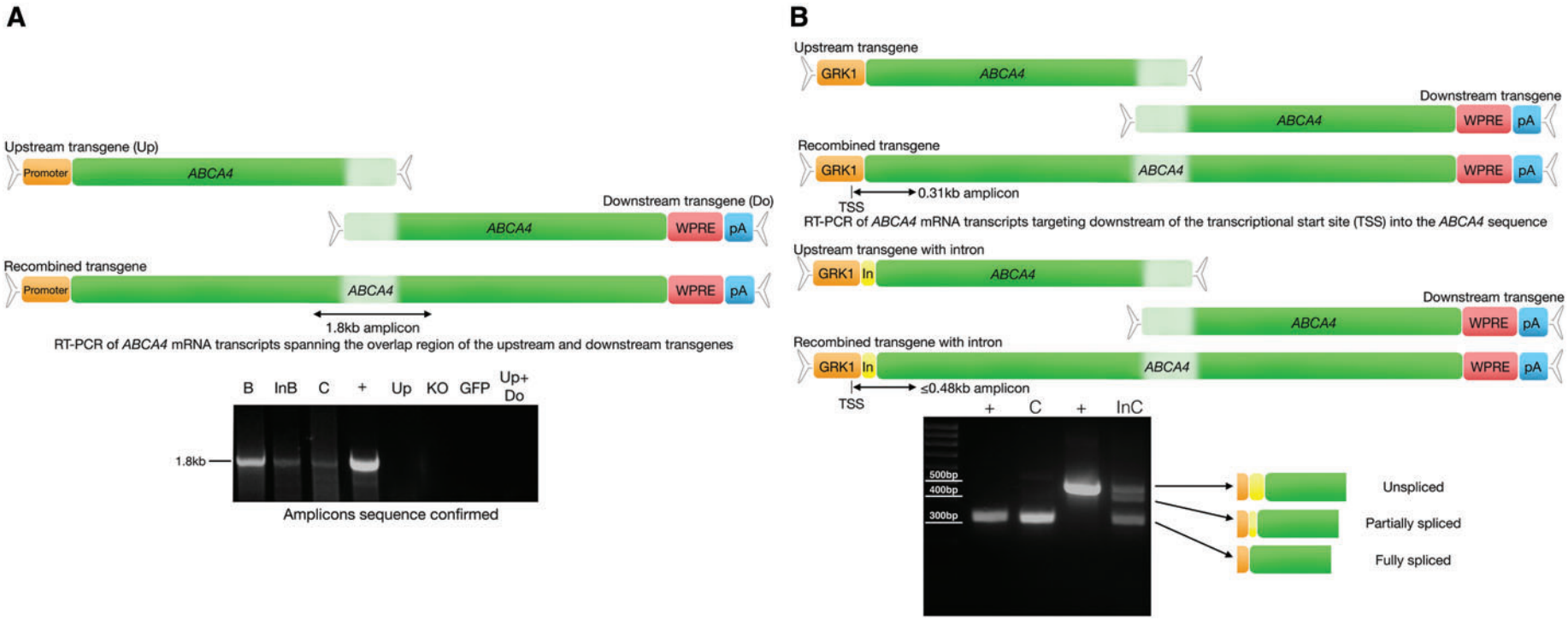

Supplementary Figure S4. RT-PCR of *ABCA4* mRNA from injected *Abca4*$^{-/-}$ eyes (n=4, pooled) confirmed transcripts from recombined transgenes had the correct coding sequence at the overlap region with successful splicing of the intron. **(A)** A forward primer binding *ABCA4* coding sequence within the upstream transgene was paired with a reverse primer binding the *ABCA4* coding sequence from the downstream transgene therefore a PCR product from the mRNA would only be seen after stable recombination. Amplicons were sequenced to confirm the correct *ABCA4* CDS was contained across the overlap regions of the transcripts. **(B)** In order to assess the splicing efficiency of the intron within the promoter, a forward primer binding downstream of the predicted *GRK1* transcriptional start site and a reverse primer binding within the upstream *ABCA4* coding sequence were used to assess transcript forms from dual vector C–injected eyes with and without the intron. *ABCA4* transcripts from dual vector C–injected eyes generated a single amplicon representing the original reference sequence. Transcripts from dual vector "Intron-C" (InC)–injected eyes generated three defined products that were sequenced and confirmed to be unspliced, partially spliced, and fully spliced variants. B/C, eyes injected with dual vector variants B or C (see Table 1); GFP, eyes injected with GRK1.GFP.pA AAV2/8 Y733F; InB/C, eyes injected with dual vector variant B or C in which the upstream transgene contains an intron; ITR, inverted terminal repeat; pA, polyA signal; WPRE, woodchuck hepatitis virus post-transcriptional regulatory element; Up, eyes injected with upstream vector only; Up+Do, pooled cDNA from upstream vector only injected eyes and downstream vector only injected eyes; +, ABCA4 plasmid control.