



Supplementary Figure S5. To explore further whether or not truncated ABCA4 protein was being generated by the upstream vector (but not detected with the polyclonal ABCA4 antibody), a FLAG tag was added to the N-terminus of the ABCA4 sequence. **(A)** HEK293T samples were transfected with plasmids carrying a FLAG-tagged version of the upstream transgene and were assessed for *ABCA4* transcript presence using primers directed to an upstream region of the *ABCA4* CDS (graph and panel above of RT-PCR). *ABCA4* transcripts were abundant ($n=3$), yet no truncated protein was detected by Western blot. pD, HEK293T cells transfected with the downstream transgene plasmid; pF, HEK293T cells transfected with a FLAG-tagged upstream transgene plasmid; UTC, untransfected HEK29T cells; +, positive control. **(B)** *Abca4*^{-/-} eyes were injected AAV2/8 Y733F vectors carrying a FLAG-tagged version of upstream transgene and were assessed for *ABCA4* mRNA transcript presence using primers directed to an upstream region of the *ABCA4* CDS (graph and panel above of RT-PCR). *ABCA4* transcripts were abundant in upstream vector only injected eyes ($n=2$), yet no truncated protein was detected by western blot. Error bars represent SEM. D, downstream vector only injected eyes; F, FLAG-tagged upstream vector only injected eyes; KO, uninjected *Abca4*^{-/-} eyes; -= empty lane; +, positive control.