

Supplementary Figure S3. Detection of full-length ABCA4 6 weeks postinjection in  $Abca4^{-/-}$  eyes treated with different dual vector combinations. (A) Dual vector overlap variants were injected into  $Abca4^{-/-}$  mouse eyes with a faint band of full length ABCA4 visible at 245kDa 6 weeks postinjection for all variants (n=3 eyes per lane). As predicted, no ABCA4 protein band was seen in uninjected control eyes (K0) and dual vector X injected eyes, which carried no overlap between upstream and downstream vectors. Truncated ABCA4 was detected around 135 kDa in eyes injected with variants A and B. (B) Including an intron in the upstream vector ("In" annotated samples) of dual vector overlap variants had a significant influence on the levels of full length ABCA4 detected 6 weeks postinjection (two-way ANOVA, n=3-6, intron \*\*p=0.004, overlap p=0.04, interaction ns). (C) Representation of the ABCA4 amino acid length with the regions targeted by the commercially available antibodies used for Western blot (Abcam) and immunohistochemistry (AntibodiesOnline) indicated.