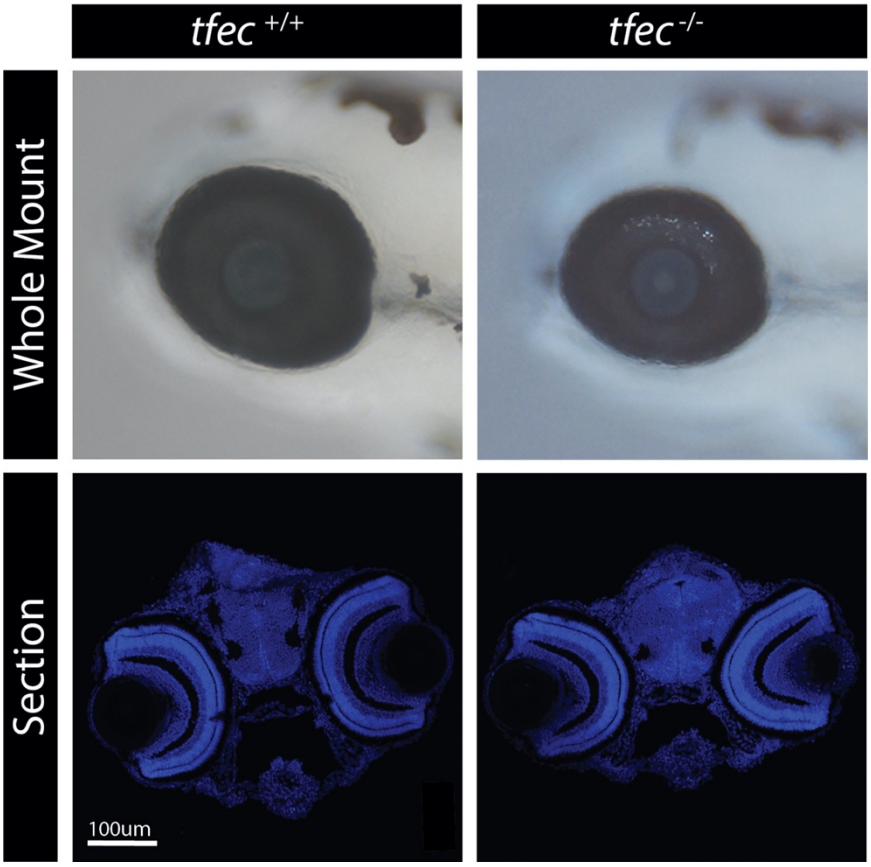


Figure S1. *tfec*$^{-/-}$ single mutants do not possess colobomas. Whole mount and transverse sections of 4dpf *tfec* $^{-/-}$ mutants reveals mild microphthalmia and pigmentation defects. No colobomas are detected. Dorsal is up in all images. Scale bar = 100μm.

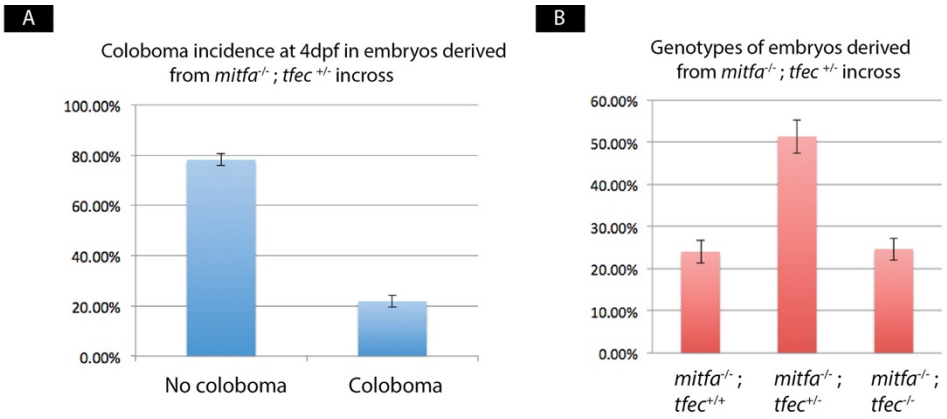


Figure S2. Quantification of colobomas in *mitfa* $^{-/-}$;*tfec* $^{+/-}$ incrosses. A) Coloboma incidence in embryos at 4dpf from *mitfa* $^{-/-}$;*tfec* $^{+/-}$ incrosses. On average 21% of embryos possessed colobomas of varying severity. (n=4 rounds of breeding) **B)** Genotypes of embryos in *mitfa* $^{-/-}$;*tfec* $^{+/-}$ incrosses. Expected Mendelian ratios of *mitfa* $^{-/-}$;*tfec* $^{+/+}$, *mitfa* $^{-/-}$; *tfec* $^{+/-}$, and *mitfa* $^{-/-}$;*tfec* $^{-/-}$ are observed (n= 4 rounds of breeding).

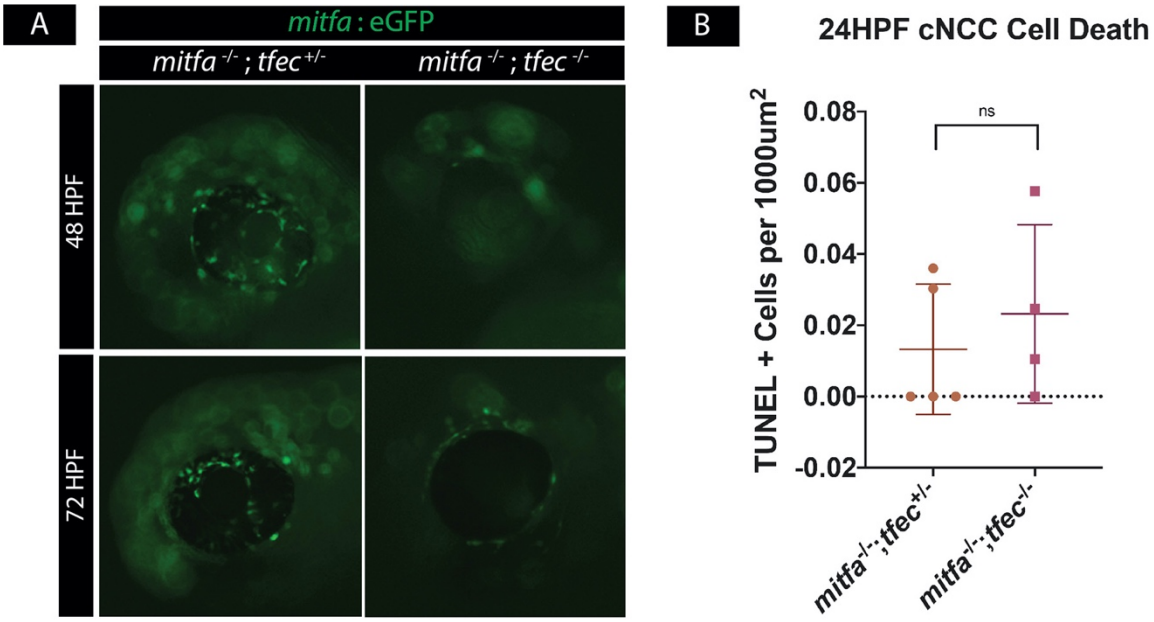


Figure S3. Loss of cNCCs in the POM is not the result cell death. A) Whole-mount images of *mitfa* $^{-/-}$*;tfec* $^{+/-}$ and *mitfa* $^{-/-}$*;tfec* $^{-/-}$ embryos reveals persistent loss of cNCC within the POM at 48hpf and 72hpf. **B)** Quantification of TUNEL^{+} cNCCs. While a trend of increased cell death is present, loss of cNCC numbers cannot be attributed solely to apoptosis at 24hpf (p=0.512, n=5 embryos).

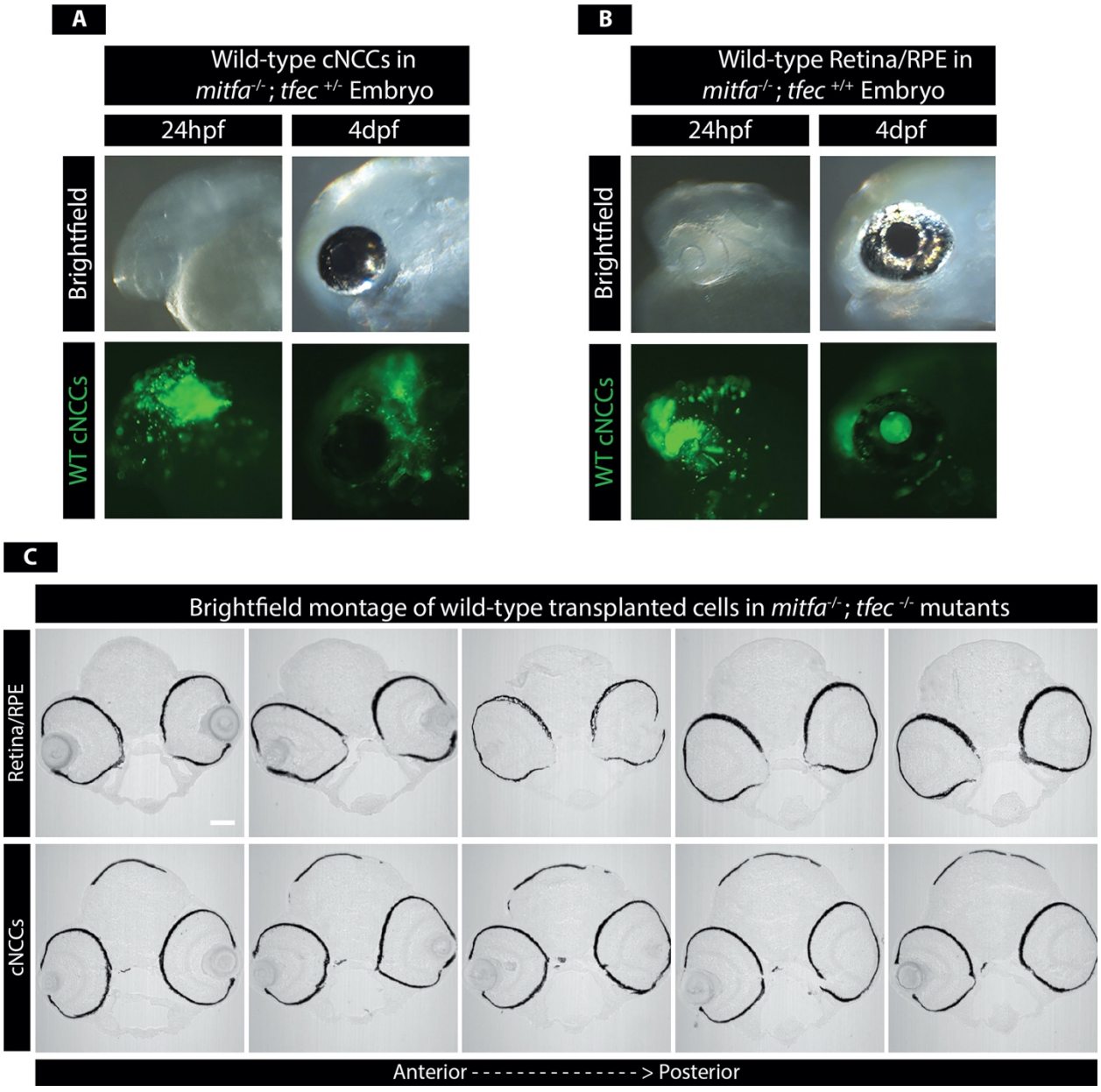
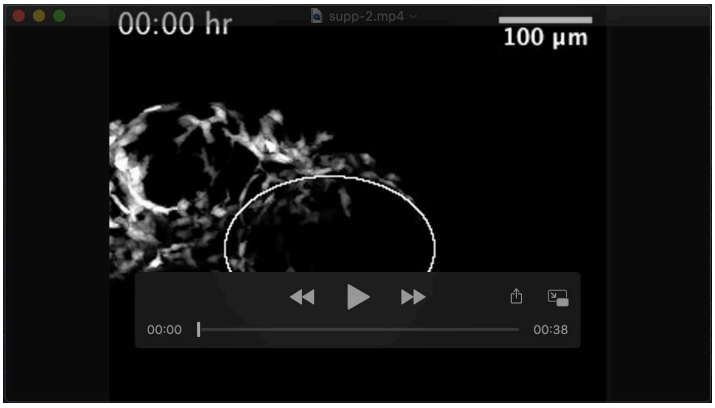
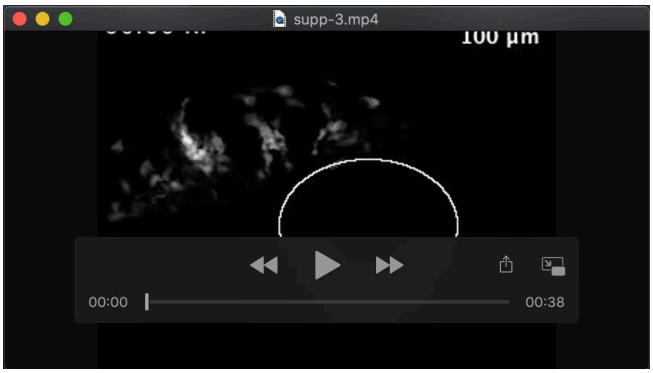


Figure S4. Transplantation of wild-type cells does not affect eye development. A) Wild-type cells transplanted into the region of cNCC origin in a *mitfa* $^{-/-}$*;tfec* $^{+/-}$ embryo shows no effect on eye development. **B)** Similarly, transplanted wild-types cells targeting the retina/RPE in *mitfa* $^{-/-}$*;tfec* $^{+/+}$ embryos show no effect on eye development. **C)** Serial sections of representative retina/RPE and cNCC transplanted embryos. CF closure phenotypes are only detected in retina/RPE cell transplanted embryos. Dorsal is up in all images. Scale bar = 100μm.



Movie 1: Wild-type embryos show normal migration of cNCCs starting at 25hpf. cNCCs can be seen migrating in and around the developing optic cup, eventually making their way around the entirety of the eye and into the choroid fissure.



Movie 2: Mutant embryos show remarkably fewer neural crest cells at 25hpf. Already, there is a lack of migration of the cells into and around the eye. These cNCCs never reach the CF during the 15-hour time-lapse movie and instead stay in the dorsal portion of the developing head. cNCCs seem to lack direction and at t=8:08-10:08, several dorsal cNCCs burst, implicating increased cNCC cell death in *mitfa* $^{-/-}$*;tfec* $^{-/-}$ embryos.