SUPPLEMENTAL MATERIAL

Novel Degenerative and Developmental Defects in a Zebrafish Model of Mucolipidosis Type IV

Li H, Pei W, Vergarajauregui S, Zerfas PM, Raben N, Burgess SM, and Puertollano R

The Supplemental material includes eight Supplemental Figures:

Supplemental Figure S1. Skeletal muscle pathology in adult *mcoln1ab*$^{-/-}$ mutants

Supplemental Figure S2. MicroCT images of skeleton structure of adult WT and *mcoln1ab*$^{-/-}$ zebrafish for both male (M) and female (F)

Supplemental Figure S3. Eye pathology in *mcoln1* mutants at 3 dpf

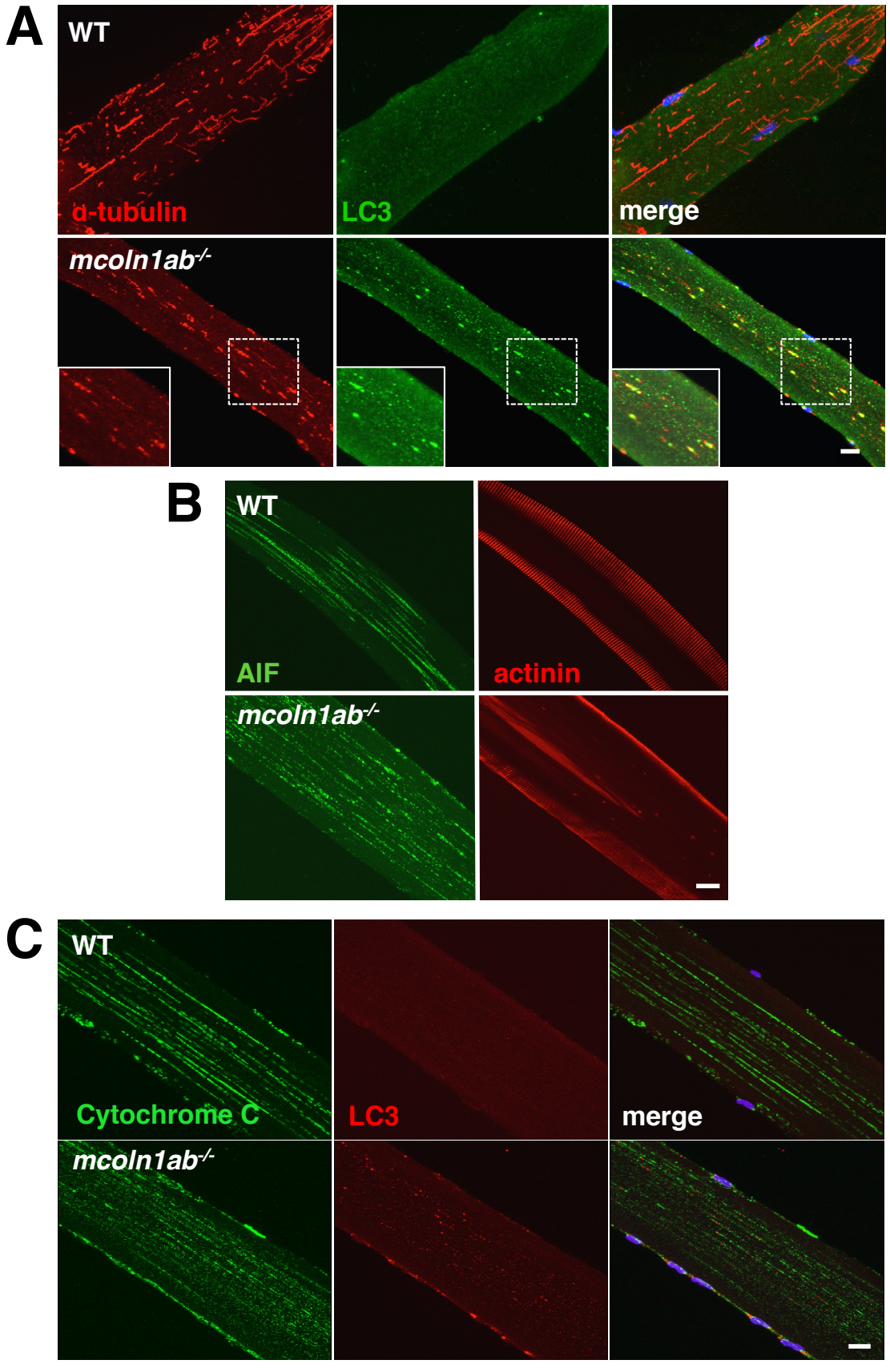
Supplemental Figure S4. Retina structure in *mcoln1* mutants at 3 dpf

Supplemental Figure S5. Cornea structure in adult *mcoln1* mutants

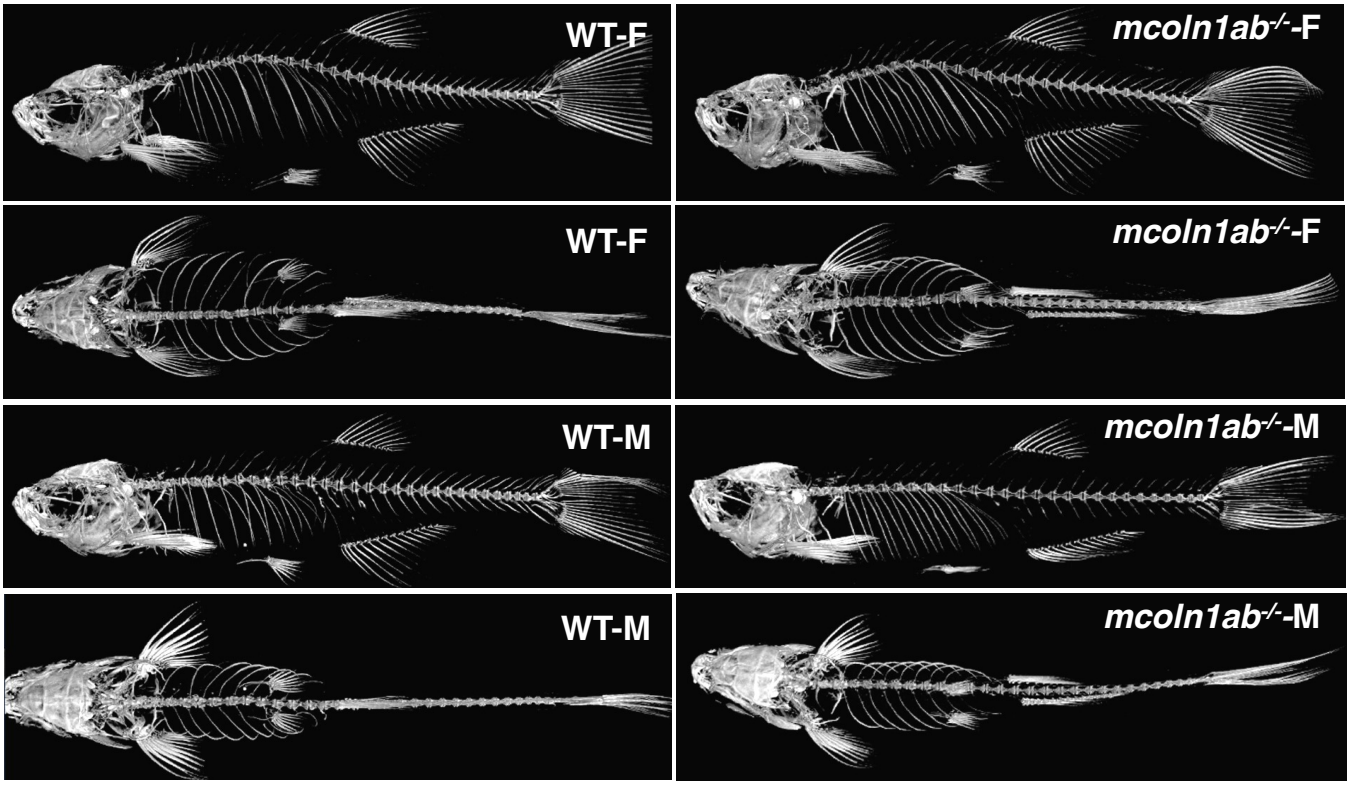
Supplemental Figure S6. Hair cell viability and morphology in *mcoln1a*$^{-/-}$ *and mcoln1b*$^{-/-}$ larvae

Supplemental Figure S7. Hair cell regeneration and sensitivity to antibiotics in *mcoln1ab*$^{-/-}$ larvae at 5 dpf

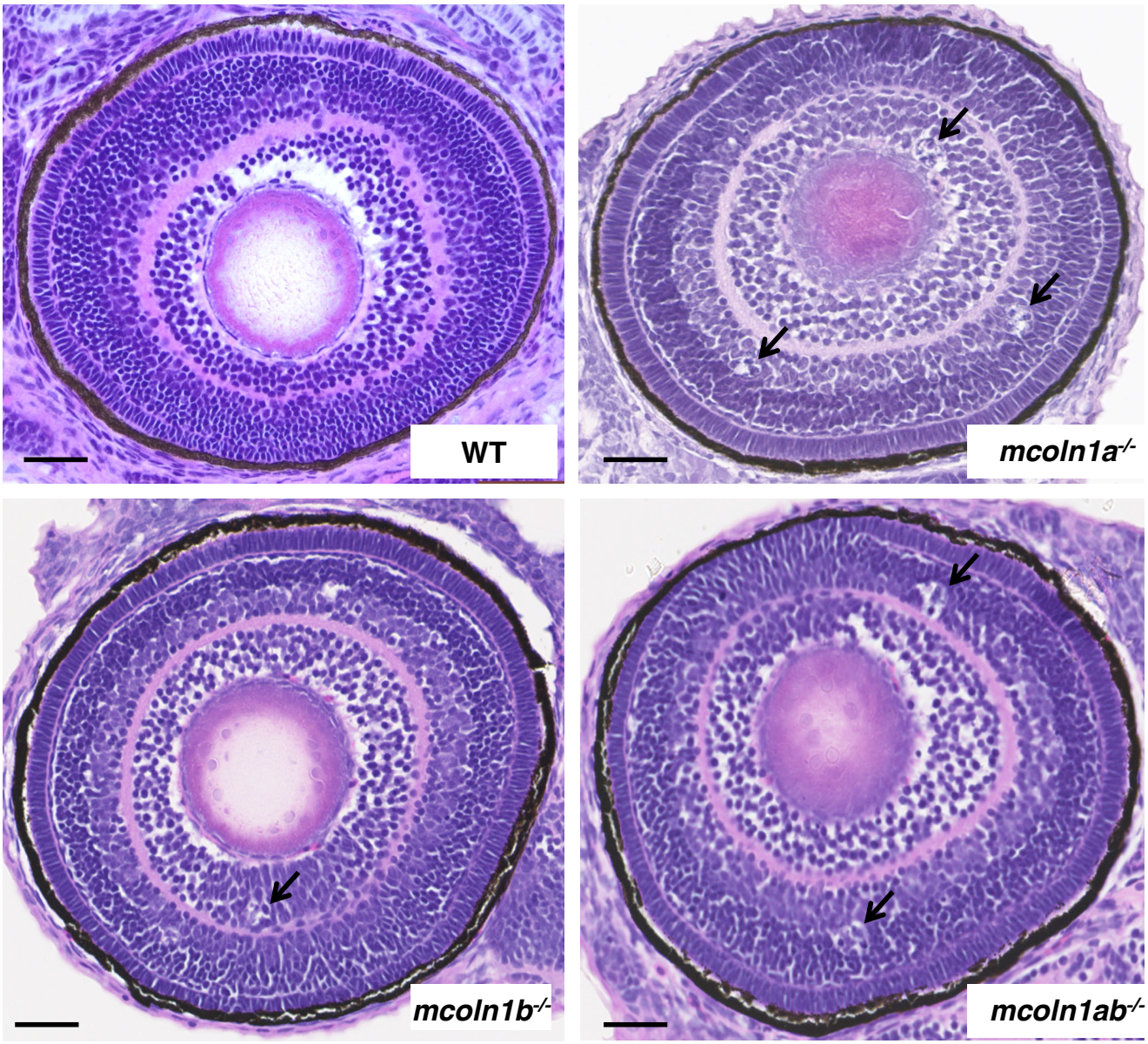
Supplemental Figure S8. Hair cell mitochondria in *mcoln1ab*$^{-/-}$ larvae at 5 dpf



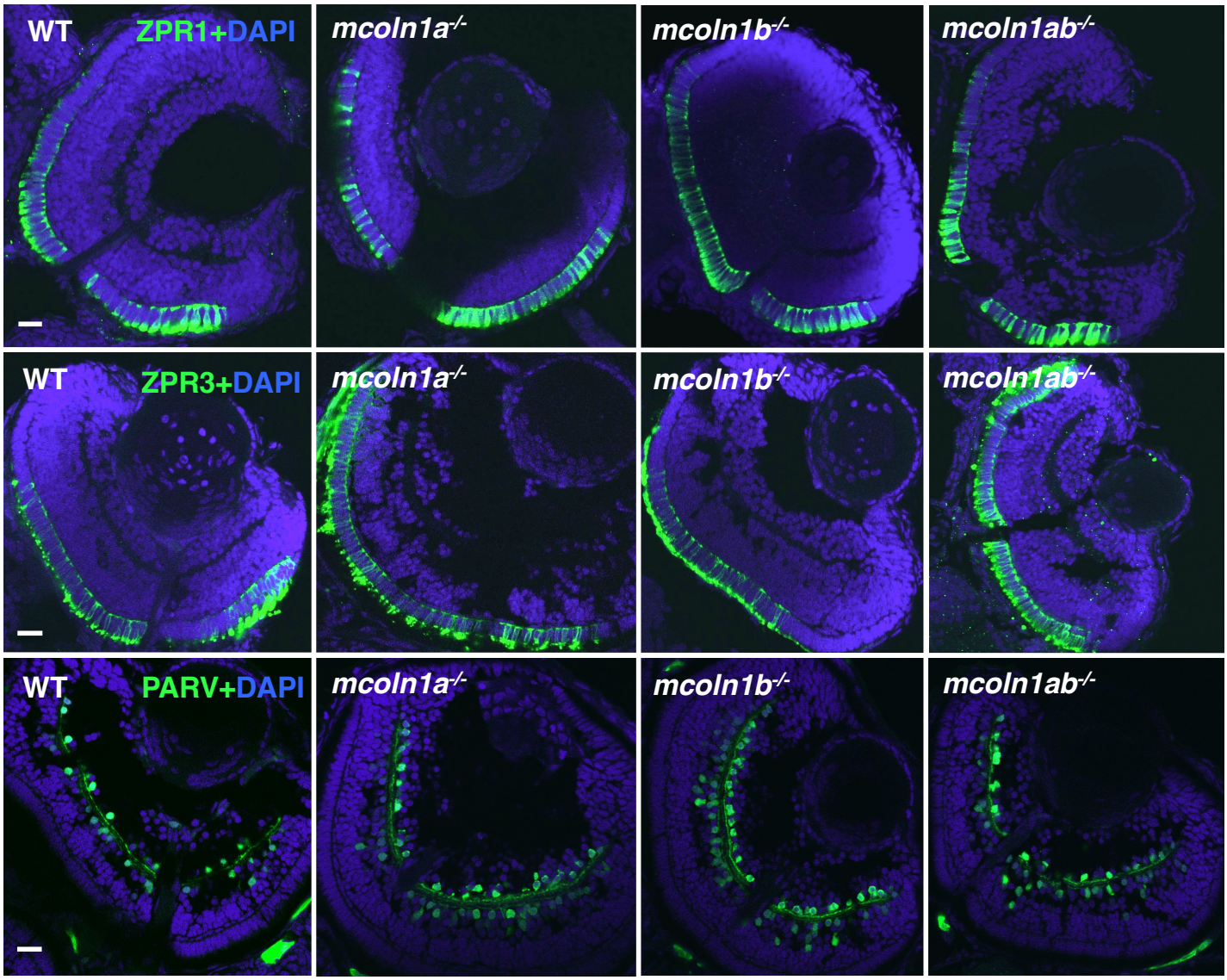
Supplemental Figure S1. Skeletal muscle pathology in adult *mcoln1ab*$^{-/-}$ mutants (**A**) Co-immunostaining for α-tubulin, LC3, and DAPI in single muscle fiber dissected from 8-month-old WT and *mcoln1ab*$^{-/-}$ zebrafish. (**B**) Co-immunostaining for AIF and actinin in single muscle fiber dissected from 12-month-old WT and *mcoln1ab*$^{-/-}$ zebrafish. Scale bar, 10 μm. (**C**) Co-immunostaining for cytochrome C, LC3, and DAPI in single muscle fiber dissected from 4-month-old WT and *mcoln1ab*$^{-/-}$ zebrafish. Scale bar, 10 μm.



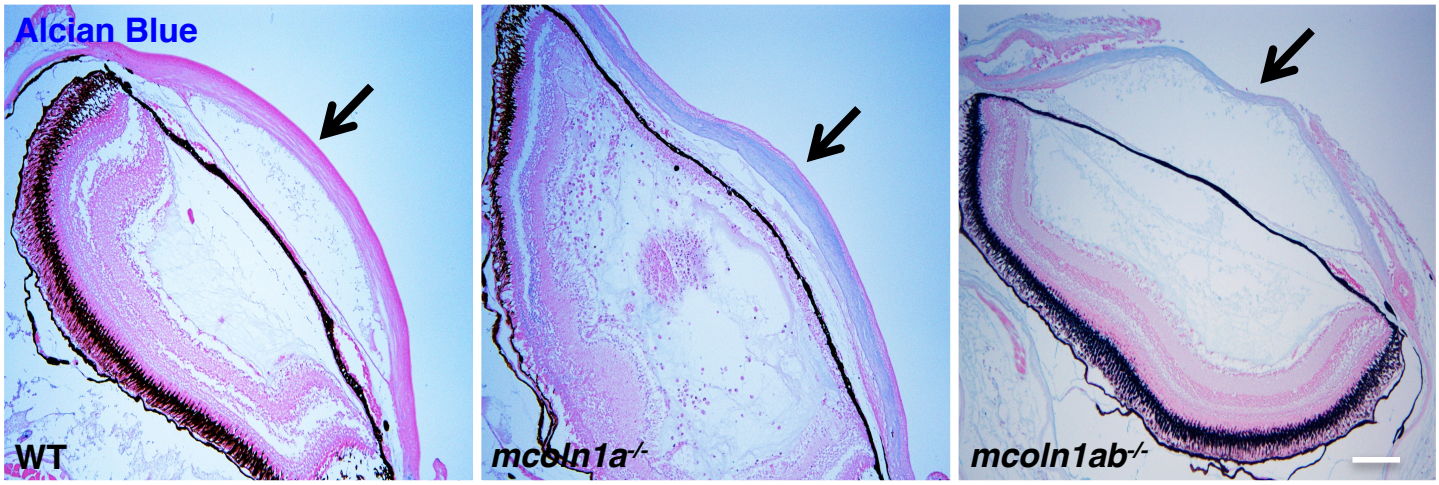
Supplemental Figure S2. MicroCT images of skeleton structure of adult WT and *mcoln1ab*$^{-/-}$ zebrafish for both male (M) and female (F).



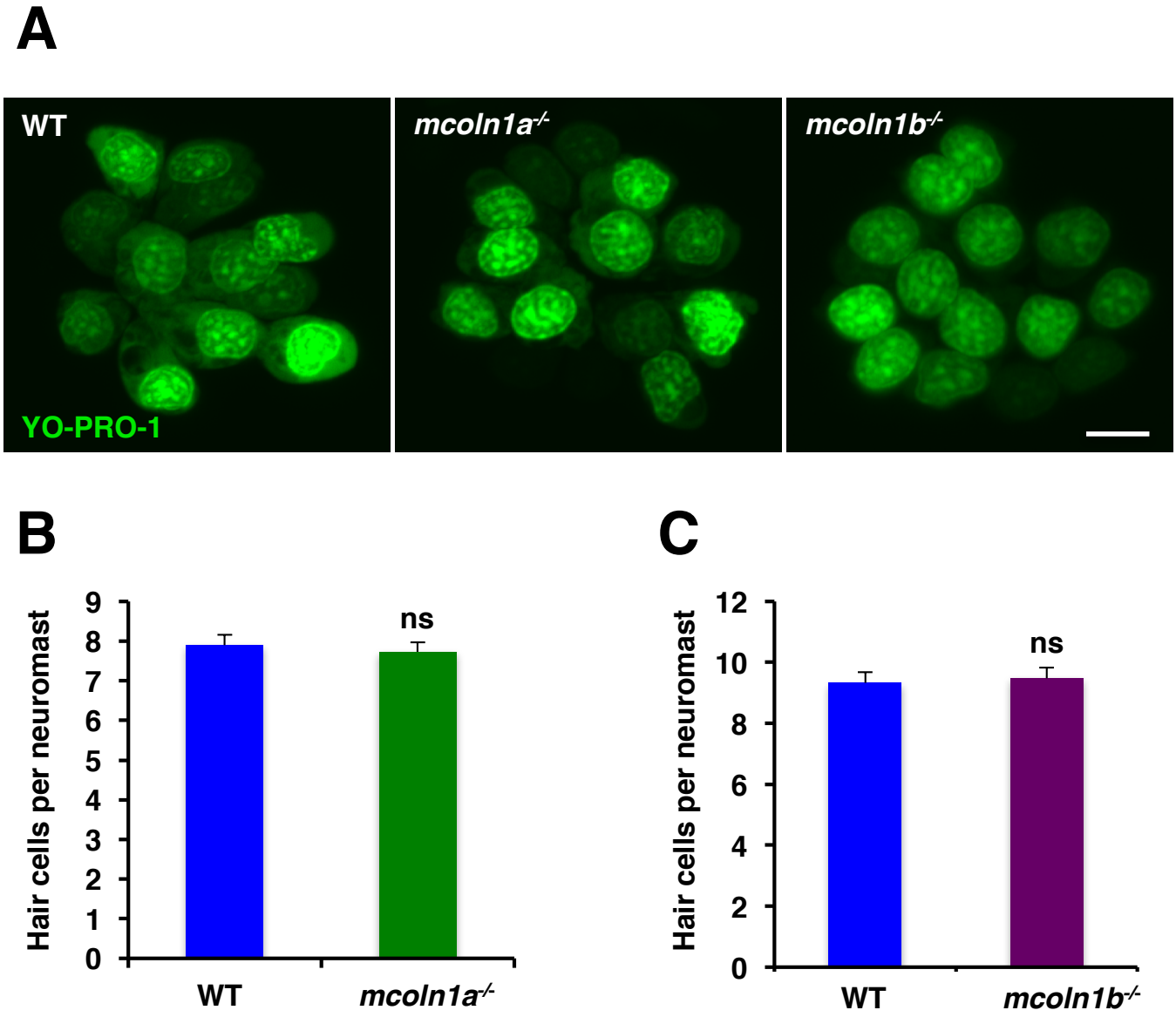
Supplemental Figure S3. Eye pathology in *mcoln1* mutants at 3 dpf. H&E staining on paraffin sections of 3 dpf zebrafish embryos showing the eye structure in WT and *mcoln1* mutants. Black arrows mark the apoptotic body-like structures. Scale bar, 50 μm.



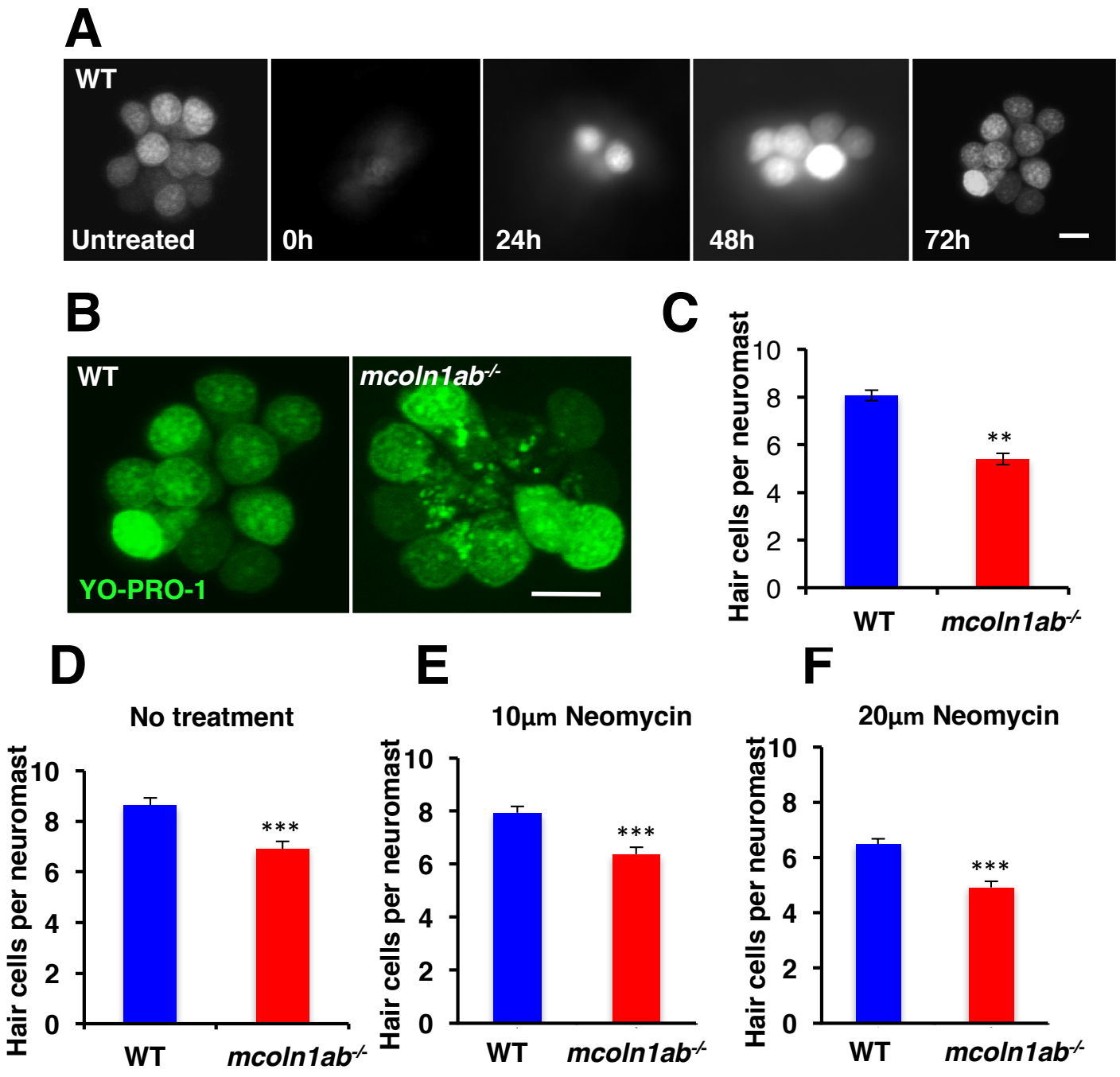
Supplemental Figure S4. Retina structure in *mcoln1* mutants at 3 dpf. Immunostaining on cryostat sections of 3 dpf zebrafish eyes showing the cone photoreceptors (ZPR1), the rod photoreceptors (ZPR3) and amacrine cells (PARV) in WT and *mcoln1* mutants. Scale bar, 20 µm.



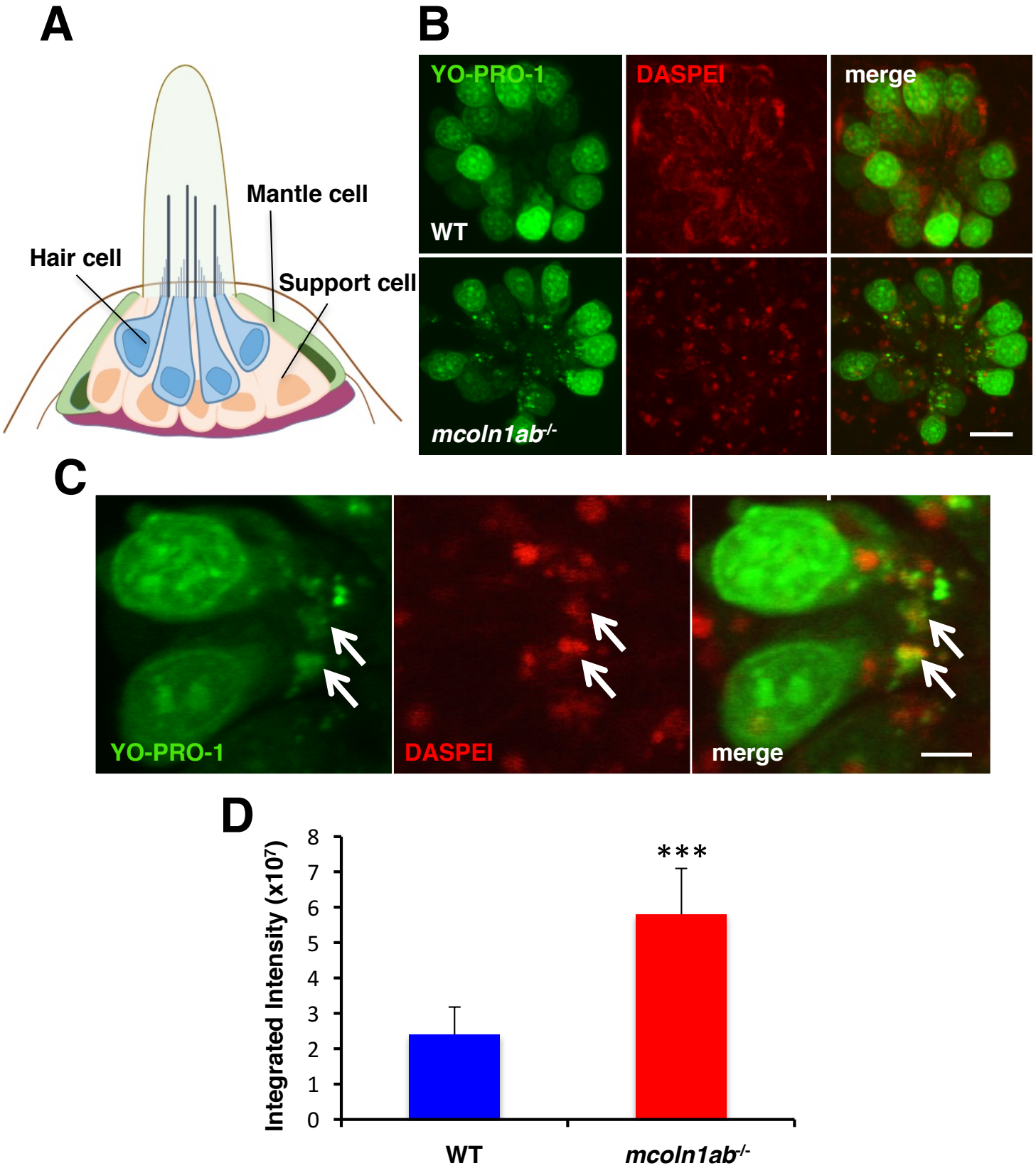
Supplemental Figure S5. Cornea structure in adult *mcoln1* mutants. Alcian Blue staining on paraffin sections of adult zebrafish eye for WT, *mcoln1a*$^{-/-}$ and *mcoln1ab*$^{-/-}$. Black arrows mark the cornea. Scale bar, 50 µm.



Supplemental Figure S6. Hair cell viability and morphology in *mcoln1a*$^{-/-}$ *and* *mcoln1b*$^{-/-}$ larvae. (**A**) YO-PRO-1 staining in live zebrafish larvae showing hair cell morphology in WT, *mcoln1a*$^{-/-}$ and *mcoln1b*$^{-/-}$ larvae at 5 dpf. Scale bar, 5 µm. (**B,C**) Quantification of hair cell number per neuromast in YO-PRO-1 stained WT and *mcoln1a*$^{-/-}$ (B) or WT and *mcoln1b*$^{-/-}$ (C) 5 dpf larvae. Data are shown as mean±SD and represent 40 neuromast from 10 embryos per genotype counted in a representative experiment. The data were analyzed using paired t test (ns, not significant).



Supplemental Figure S7. Hair cell regeneration and sensitivity to antibiotics in *mcoln1ab*$^{-/-}$ larvae at 5 dpf. (**A**) YO-PRO-1 staining showing hair cell regeneration after 10 µM copper sulfate treatment in WT larvae at 5 dpf. Scale bar, 5 µm. (**B**) YO-PRO-1 staining showing hair cell morphology after 10 µM copper sulfate treatment at 5 dpf followed by 48 h recovery in WT and *mcoln1ab*$^{-/-}$ larvae. Scale bar, 5 µm. (**C**) Quantification of the experiment shown in (B). Data are shown as mean±SD and represent 30 neuromasts from 10 embryos per genotype counted in a representative experiment. The data were analyzed using paired t test (**p<0.01). (**D-F**) Quantification of the number of hair cells per neuromast in WT and *mcoln1ab-/-* mutants for non-treated (D), 10µm neomycin-treated (E) and 20µm neomycin-treated (F) 5 dpf larvae. Data are shown as mean±SD and represent 40 neuromasts from 10 embryos per genotype. The data were analyzed using paired t test (***p<0.001).



Supplemental Figure S8. Hair cell mitochondria in *mcoln1ab*$^{-/-}$ larvae at 5 dpf. (**A**) Cartoon for neuromast structure. (**B**) YO-PRO-1 and DASPEI staining in WT and *mcoln1ab*$^{-/-}$ larvae at 5 dpf. Scale bar, 5 μm. (**C**) Higher magnification showing co-staining of YO-PRO-1 and DASPEI in *mcoln1ab*$^{-/-}$ hair cells. Scale bar, 2 μm. (**D**) Quantification of hair cell mitoSOX staining histogram intensity at 5 dpf. Data are shown as mean±SD and represent 6 hair cells counted in a representative experiment. The data were analyzed using paired t test (***p<0.001).