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RESEARCH ARTICLE

Figures and figure supplements

Compensatory growth renders Tcf7l1a dispensable for eye formation despite its requirement in eye field specification

Rodrigo M Young *et al*

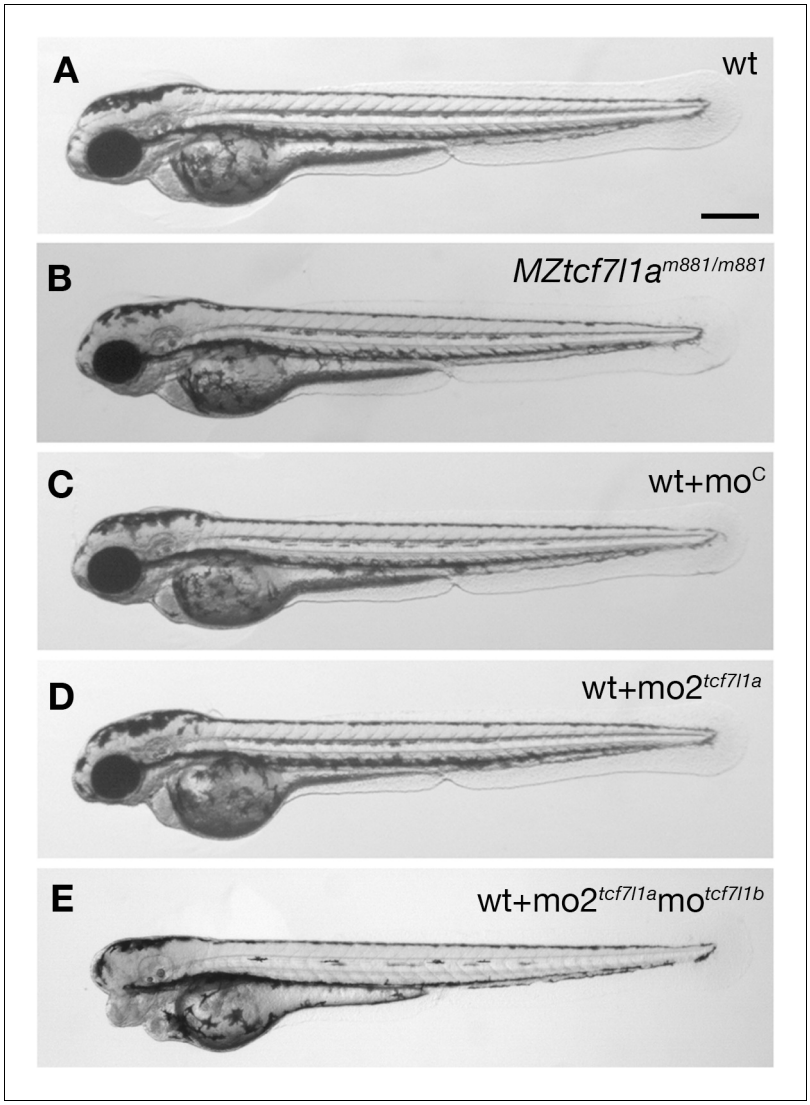

Figure 1. *Tcf7l1a* maternal zygotic (MZ) mutants and *tcf7l1a* morphants have no overt eye phenotype. Lateral views of typical wildtype (**A**) *MZtcf7l1a*$^{-/-}$ (**B**) wildtype injected with control morpholino (**C**) *tcf7l1a* morphant (**D**) and *tcf7l1a/tcf7l1b* double morphant (**E**) embryos at 2 days post fertilisation. All conditions n > 100 and over three independent experiments except when specified. Dorsal up, anterior to the left. Scale Bar = 250 μm.
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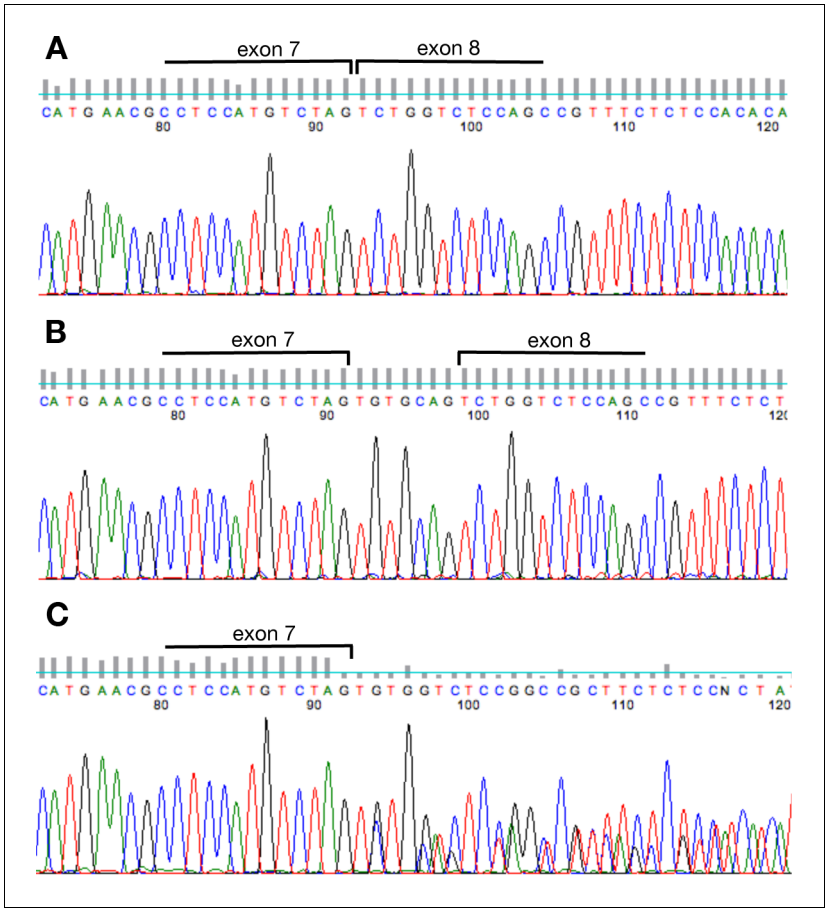

Figure 1—figure supplement 1. Sequence of the *tcf7l1a* exon7/8 boundary. RT-PCR chromatogram sequence of *tcf7l1a* exon7/8 fragment showing the expected intron splice in wildtype embryos (**A**). *tcf7l1a*$^{-/-}$ mutants show an unambiguous inclusion of 7 nucleotides from intron 7 (**B**) and a mixed read in mRNA coming from heterozygous siblings (**C**).

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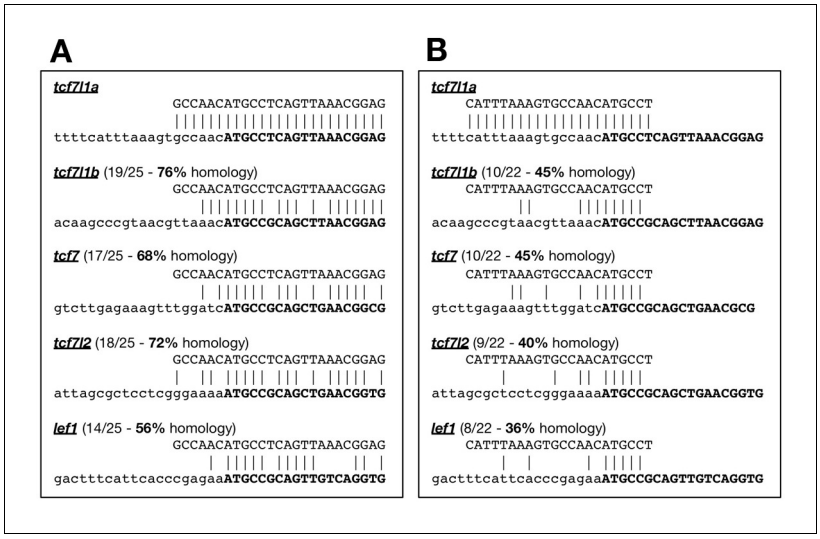

Figure 1—figure supplement 2. Alignment of *tcf7l1a*, morpholinos to *lef1* and other *tcf* genes. Alignment of the previously published *tcf7l1a* morpholino (**A**) mo1^{tcf7l1a}, ***Dorsky et al., 2003***), and mo2^{tcf7l1a} (**B**) with less homology to other *lef/tcf* genes. Target genes in bottom sequence, 5'UTR in lowercase, gene open-reading frame in bold uppercase.

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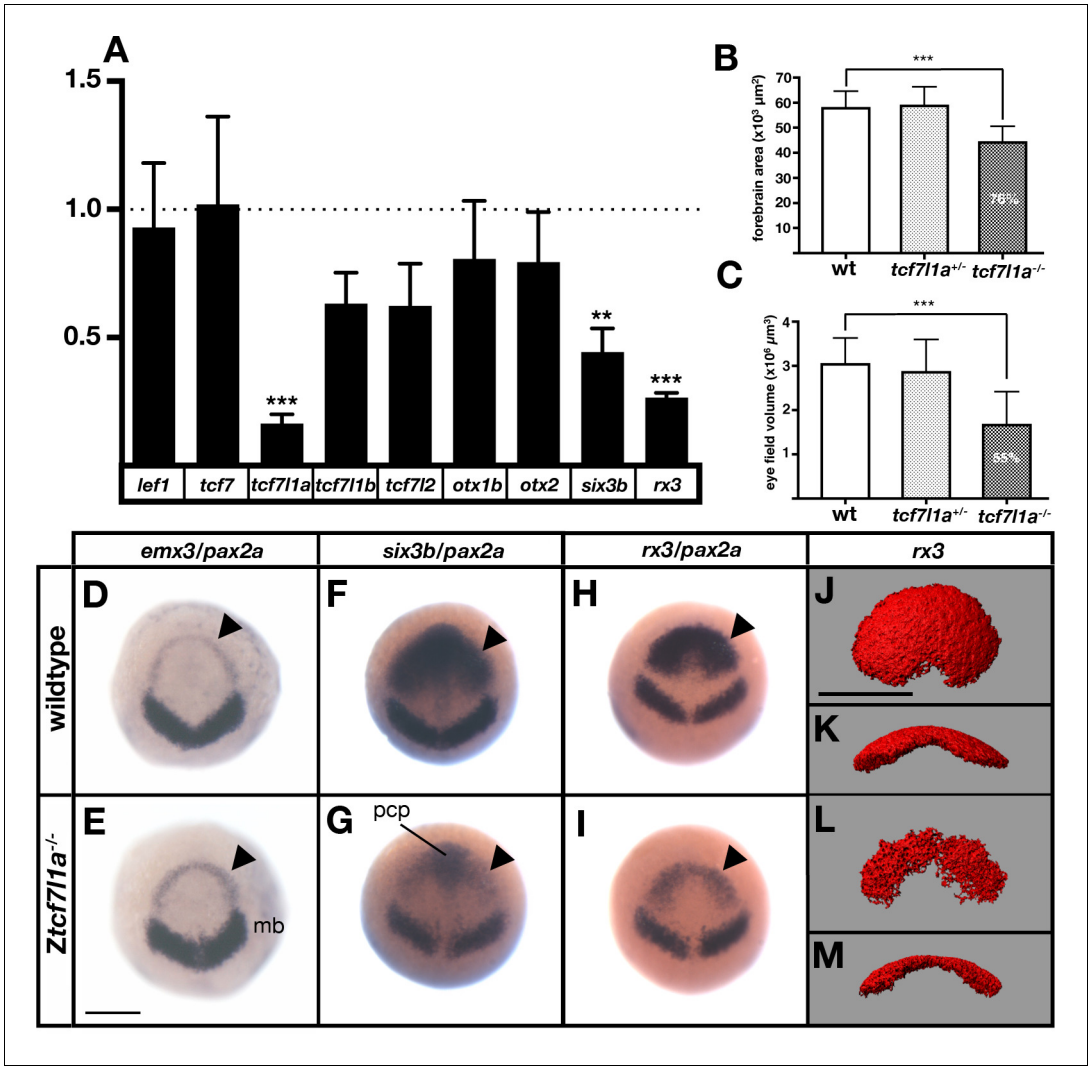

Figure 2. The prospective forebrain and eye field domains of the neural plate are reduced in Z*tcf7l1a*$^{-/-}$ mutants. (**A**) Graph showing RT-qPCR quantification of the mRNA levels of *lef1*, *tcf7*, *tcf7l1a*, *tcf7l1b*, *tcf7l2*, *otx1b*, *otx2*, *six3b* and *rx3* in Z*tcf7l1a*$^{-/-}$ mutants relative to wildtype embryos at 10hpf. Biological and technical triplicates, two independent experiments. (**B, C**) Quantification of the forebrain domain of the anterior neural plate (**B**) enclosed by *emx3* up to *pax2a* (**D, E**) expression by *in situ* hybridisation (reduction to an average of 76%, n = 11, one experiment, data in ***Supplementary file 1B***), and eye field volume (**C**) by *rx3* fluorescent *in situ* hybridisation confocal volume reconstruction (**J–M**) (reduction to an average of 55%, n = 10, one experiment, data in ***Supplementary file 1C***). (**D–I**) Expression of *emx3* (arrowhead)/*pax2a* (**D, E**), *six3b* (arrowhead)/*pax2a* (**F, G**) and *rx3* (arrowhead)/*pax2a* (**H, I**) in wildtype (**D, F, H**) and Z*tcf7l1a*$^{-/-}$ (**E, G, I**) embryos detected by *in situ* hybridisation at 10hpf. Reduction of *six3b* and *rx3* expression 100%, n > 40, three experiments. (**J–M**) Confocal volume reconstruction of *rx3* fluorescent *in situ* hybridisation in wildtype (**J, K**) and Z*tcf7l1a*$^{-/-}$ (**L, M**) mutants at 10hpf. (**J, L**) Dorsal view, anterior to top, and (**K, M**) transverse view from posterior, dorsal up. Abbreviations: mb, midbrain; pcp, prechordal plate Scale Bars = 250 µm.

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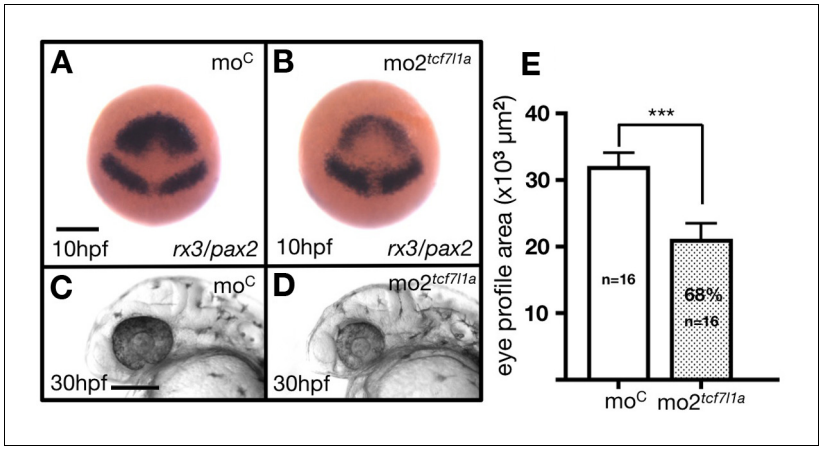

Figure 2—figure supplement 1. *tcf7l1a* morpholino ($mo2^{tcf7l1a}$) phenocopies the $Ztcf7l1a^{-/-}$ mutant. (**A, B**) Dorsal views of *rx3/pax2a in situ* hybridisation at 100%epibly and (**C, D**) lateral views of 30hpf wildtype embryos injected with 400pmols of (**A, C**) control morpholino or (**B, D**) $mo2^{tcf7l1a}$. Scale bar = 250 µm. (**E**) Plot showing the quantification of the eye profile area in wildtype embryos injected with control morpholino (first bar) or $mo2^{tcf7l1a}$ (second bar) at 30hpf. Scale bar = 200 µm.

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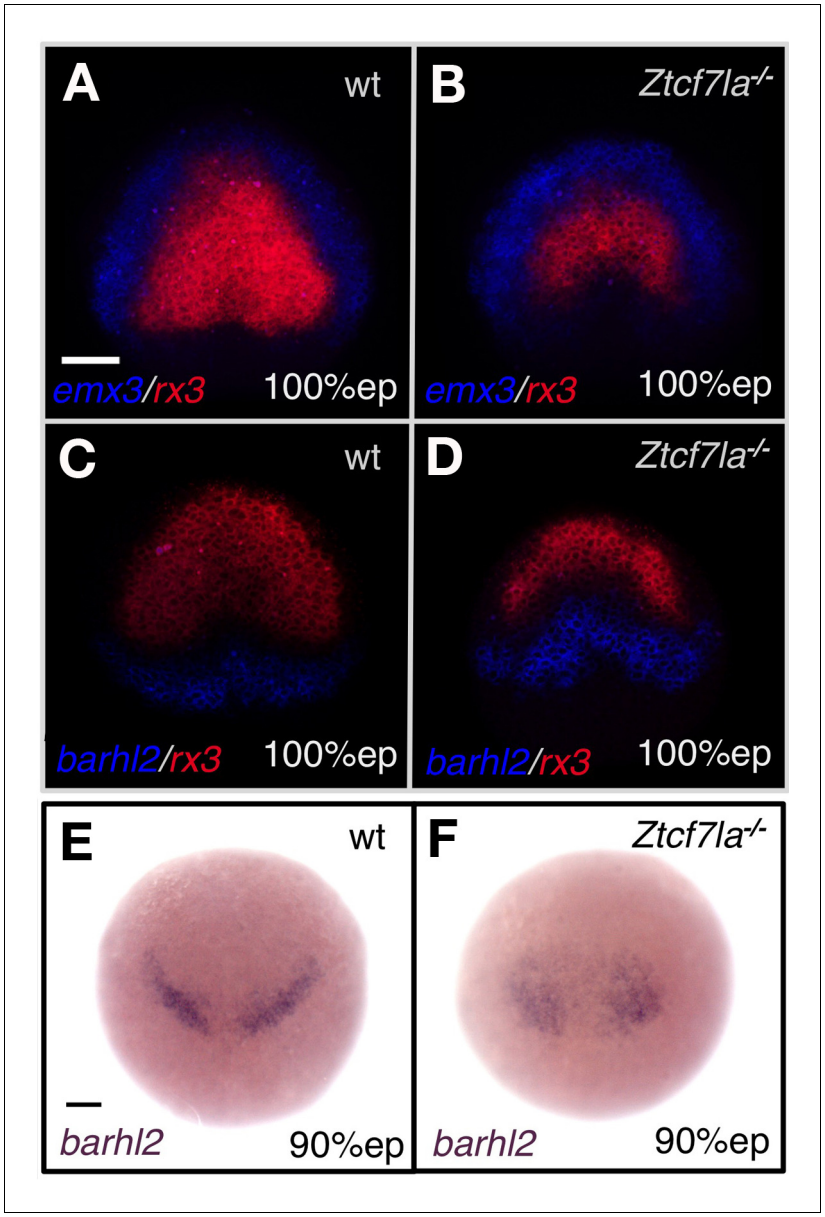

Figure 2—figure supplement 2. The eye field domain of the anterior neural plate is caudalised in *Ztcf7l1a* mutants. Dorsal views of anterior neural plates, anterior up. (**A–D**) Double fluorescent *in situ* hybridisation of prospective telencephalic marker *emx3* (blue) and prospective eye field marker *rx3* (red) (**A, B**), and prospective diencephalic marker *barhl2* (blue) and *rx3* (red) (**C, D**) in wildtype (**A, C**) and *Ztcf7l1a*$^{-/-}$ (**B, D**) embryos at 10hpf. Scale bar = 100 μm. (**E, F**) *In situ* hybridisation of *barhl2* in wildtype (**E**) and *Ztcf7l1a*$^{-/-}$ (**F**) embryos at 9hpf. All conditions n = 5, one experiment each. Scale bars = 100 μm.

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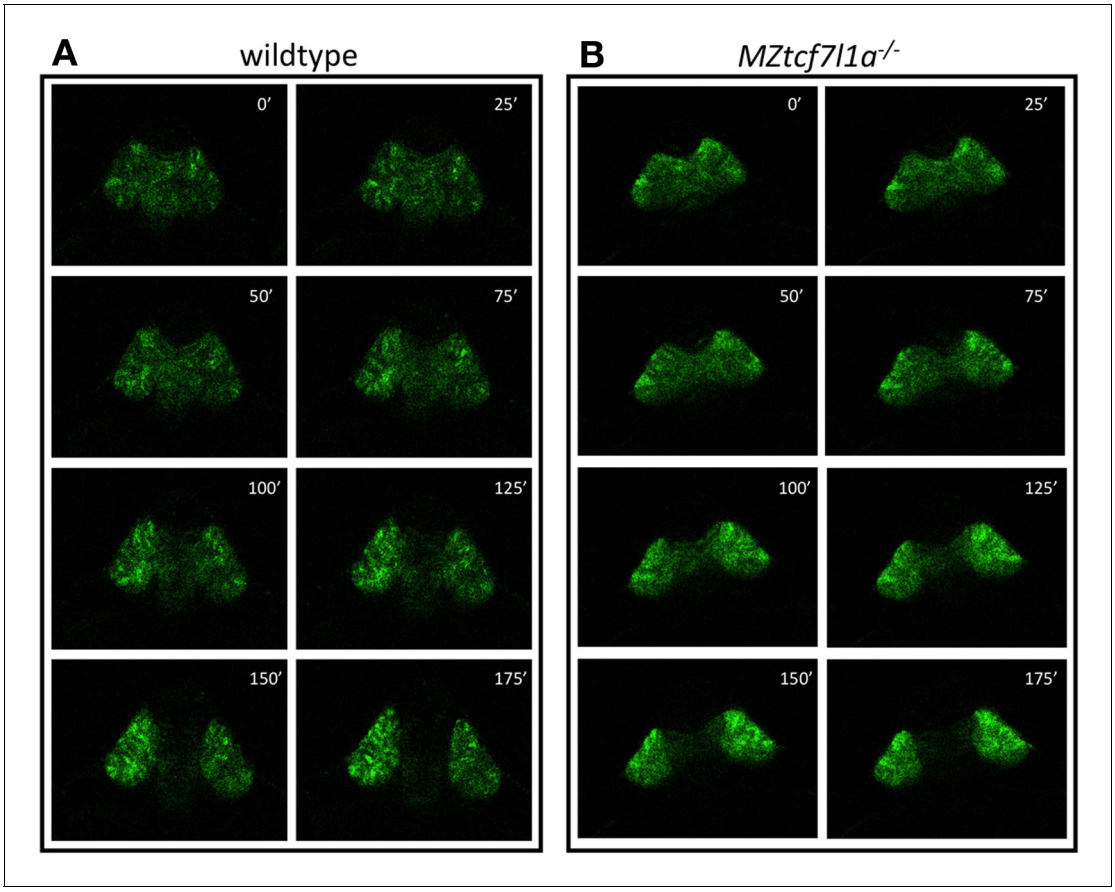

Figure 2—figure supplement 3. Eye vesicle evagination in heterozygous and *Ztcf7l1a*$^{-/-}$ mutants. Confocal time lapse movie snapshots (1 frame every 25 min) of heterozygous sibling (**A**) and *Ztcf7l1a*$^{-/-}$ mutants (**B**) expressing the *Tg(rx3:GFP)*^{zf460Tg} transgene. First frame taken at 11hpf. Minutes after movie has started indicated in each frame. From two independent experiments n = 6 for both conditions.

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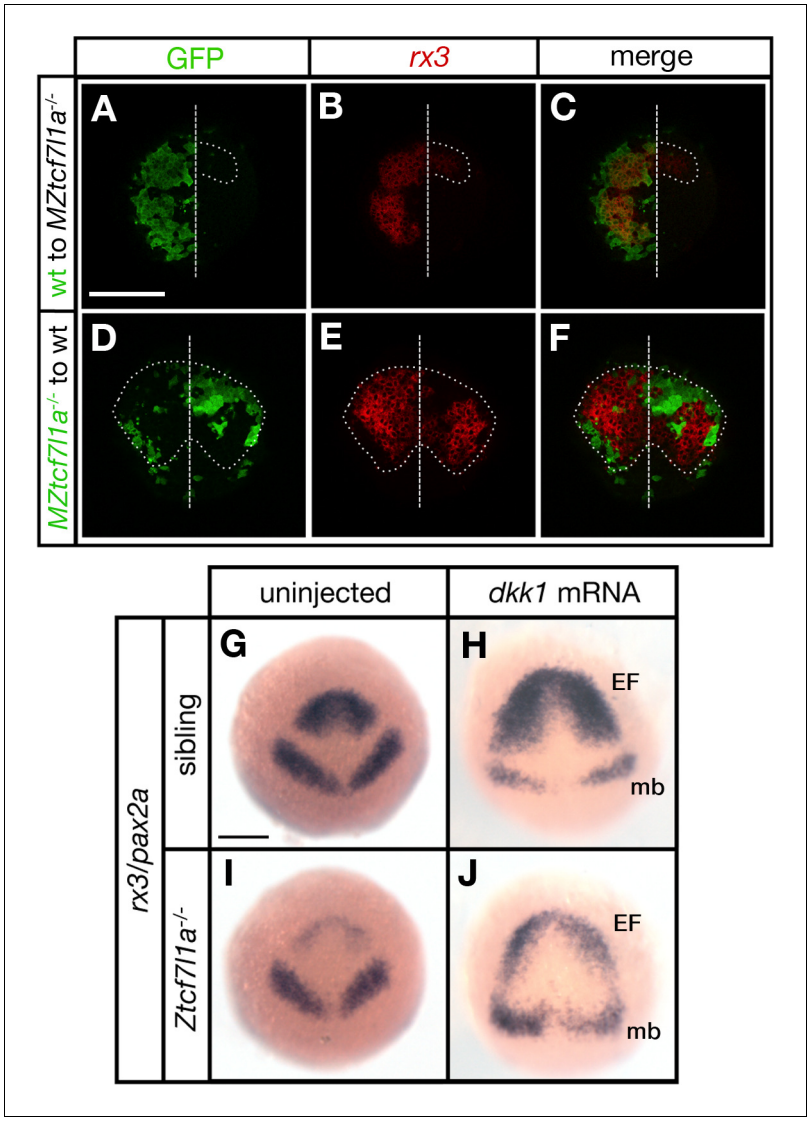

Figure 3. Tcf7l1a cell autonomously promotes *rx3* expression in the eye field. (**A–F**) Dorsal views of confocal images of *rx3* mRNA expression (red) detected by fluorescent *in situ* hybridisation at 10hpf in the anterior neural plates of chimeric embryos containing transplants of (**A–C**) wildtype (GFP+) donor cells in *MZtcf7l1a*$^{-/-}$ host embryos (100%, n = 13), and (**D–F**) *MZtcf7l1a*$^{-/-}$ (GFP+) donor cells in wildtype host embryos (100%, n = 9). Dotted line outlines eye fields; note in A-C that *rx3* expression extends considerably caudal to the reduced mutant eye field on the side of the neural plate containing wild-type cells. Dashed line marks the embryo midline. (**G–J**) *In situ* hybridisation of *rx3* and *pax2a* in sibling (**G, H**) and *Ztcf7l1a*$^{-/-}$ (**I, J**) 9hpf embryos, uninjected (**G, I**) or injected with 50 pg of *dkk1* mRNA (**H, J**). Abbreviations; EF, eyefield; mb, midbrain Scale Bars = 200 µm.

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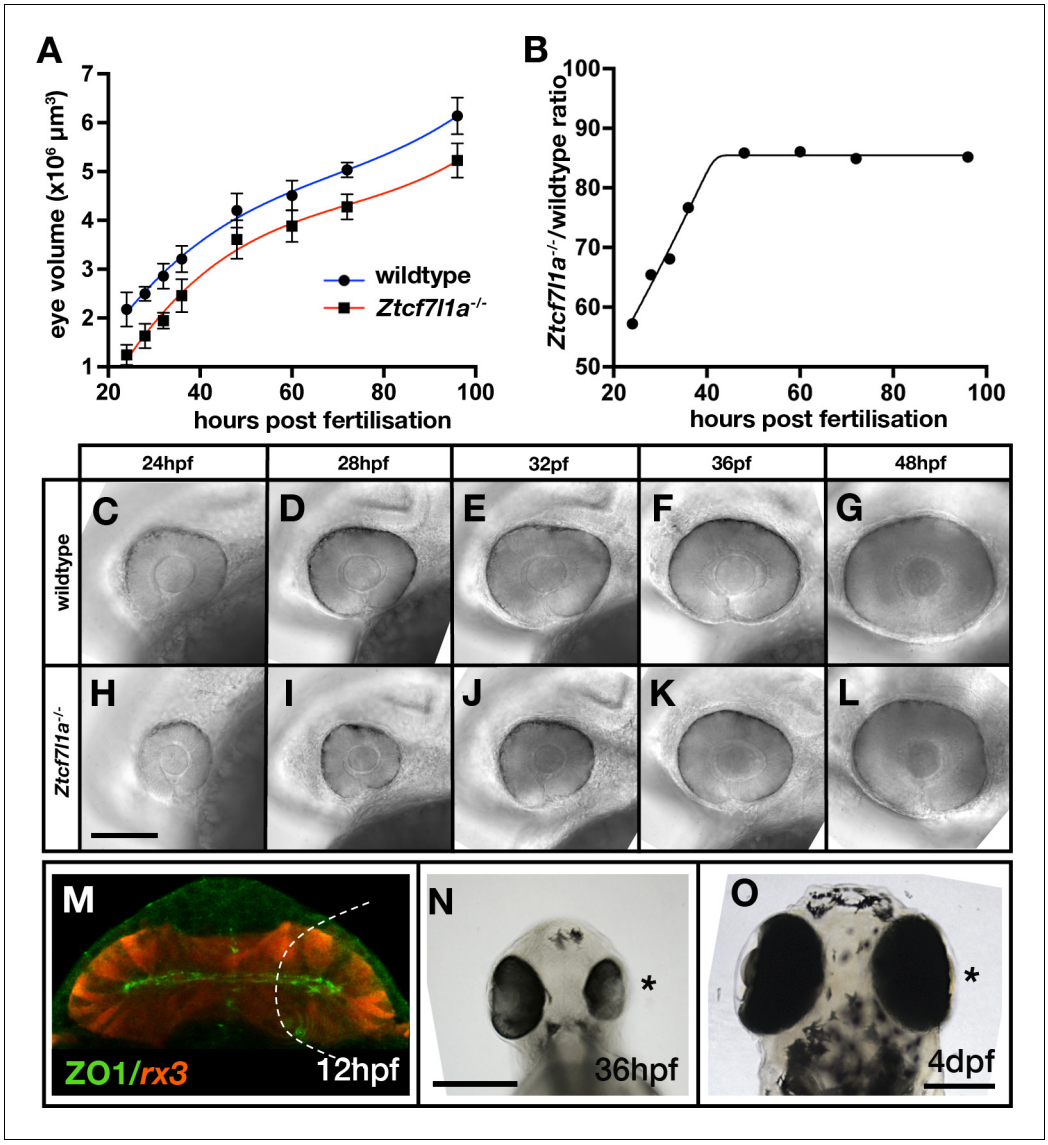

Figure 4. Eye size recovers in Z*tcf7l1a*$^{-/-}$ mutant and eye vesicle-cell removed embryos. (**A**) Growth kinetics of the eye in wildtype (blue line) and *Ztcf7l1a*$^{-/-}$ (red line) embryos at stages indicated (data in ***Supplementary file 1F***, one experiment, 24hpf, wt n = 12, *Ztcf7l1a*$^{-/-}$ n = 14; 28hpf, wt n = 15, *Ztcf7l1a*$^{-/-}$ n = 12; 32hpf, wt n = 13, *Ztcf7l1a*$^{-/-}$ n = 15; 36hpf, wt n = 16, *Ztcf7l1a*$^{-/-}$ n = 14; 48hpf, wt n = 11, *Ztcf7l1a*$^{-/-}$ n = 19; 60hpf, wt n = 11, *Ztcf7l1a*$^{-/-}$ n = 14; 72hpf, wt n = 13, *Ztcf7l1a*$^{-/-}$ n = 19; 96hpf, wt n = 13, *Ztcf7l1a*$^{-/-}$ n = 15). (**B**) Plot showing the ratio of *Ztcf7l1a*$^{-/-}$ to wildtype eye volume from data in (**A**). (**C–L**) Lateral views (dorsal up, anterior to left) of wildtype (**C–G**) and *Ztcf7l1a*$^{-/-}$ (**H–L**) eyes at stages indicated above panels. (**M–O**) Eye development following partial ablation of the optic vesicle in wildtype embryos at five somite stage. (**M**) Coronal confocal section of evaginating optic vesicles (red) in a wildtype *Tg(rx3:RFP)* five somite stage embryo. Dashed line indicates the approximate extent of ablations performed. 36hpf (**N**) and 4dpf (**O**) eyes in embryos in which cells were unilaterally removed from one optic vesicle (from n = 20). Asterisk indicates the eye that develops from the partially ablated optic vesicle. ZO1, zona occludens 1. Scale bars = 200 µm.

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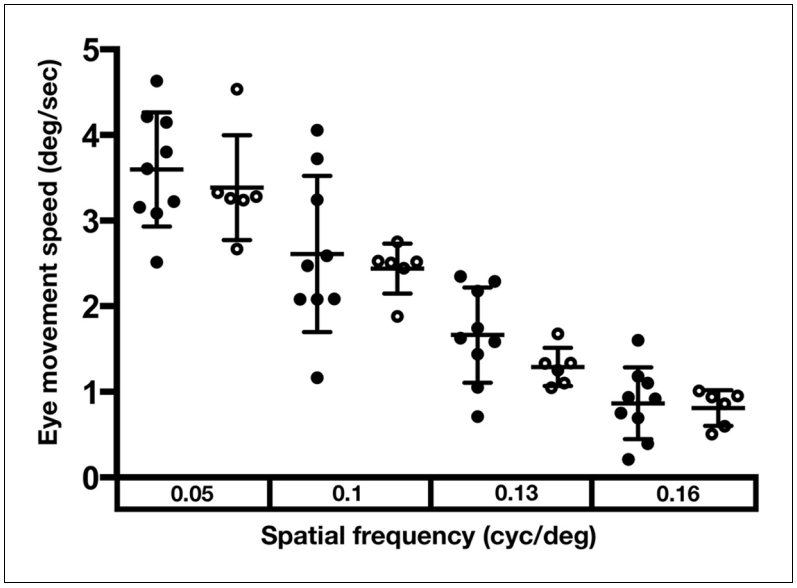

Figure 4—figure supplement 1. Optokinetic response analysis of wildtype and *Ztcf7l1a*$^{-/-}$ mutants. Average of left and right eye movement speed in degrees(deg)/second(sec), when wildtype (n = 9, filled circles) and *Ztcf7l1a*$^{-/-}$ mutants (n = 6, empty circles) embryos were presented with vertical moving stripes at increasing angular speeds, 0.05, 0.1, 0.13 and 0.16 cycles(cyc)/deg. Two independent experiments (data in ***Supplementary file 1E***).
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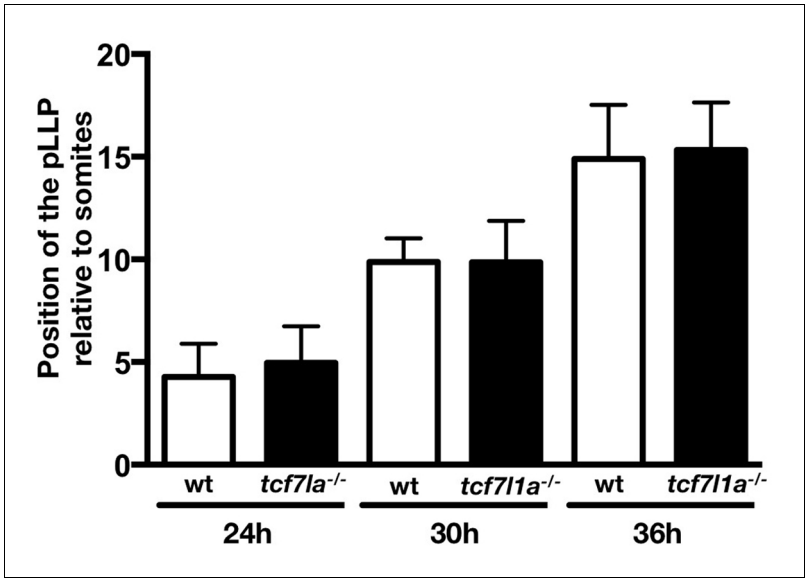

Figure 4—figure supplement 2. Quantification of the posterior lateral line primordium position in wildtype and *Ztcf7l1a*$^{-/-}$ mutants. Plot showing the position of the posterior lateral line primordium relative to somite number in wildtype (white bars) and *Ztcf7l1a*$^{-/-}$ mutants (black bar) at 24, 30 and 36hpf, (data in ***Supplementary file 1G***).
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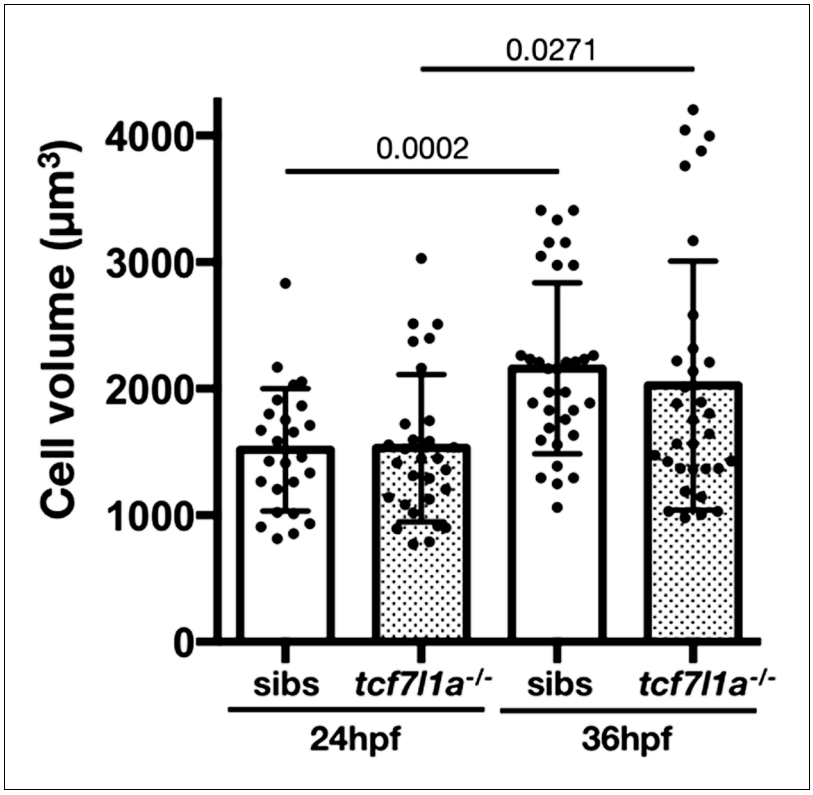

Figure 4—figure supplement 3. Cell volume quantification in *tcf7l1a* mutants and siblings. Plot showing individual cell volume quantification in siblings and *tcf7l1a*$^{-/-}$ mutants at 24 and 36hpf. Volume was measured in cells expressing GFP and mRFP, data in ***Supplementary file 1H***. Wildtype and heterozygous *tcf7l1a* embryos were pooled in a single sibling group at each timepoint. Three independent experiments, sibs 24hpf n = 25, *tcf7l1a*$^{-/-}$ 24hpf n = 29, p=0.935; sibs 36hpf n = 33, *tcf7l1a*$^{-/-}$ 36hpf n = 32, p=0.519, unpaired t-test.
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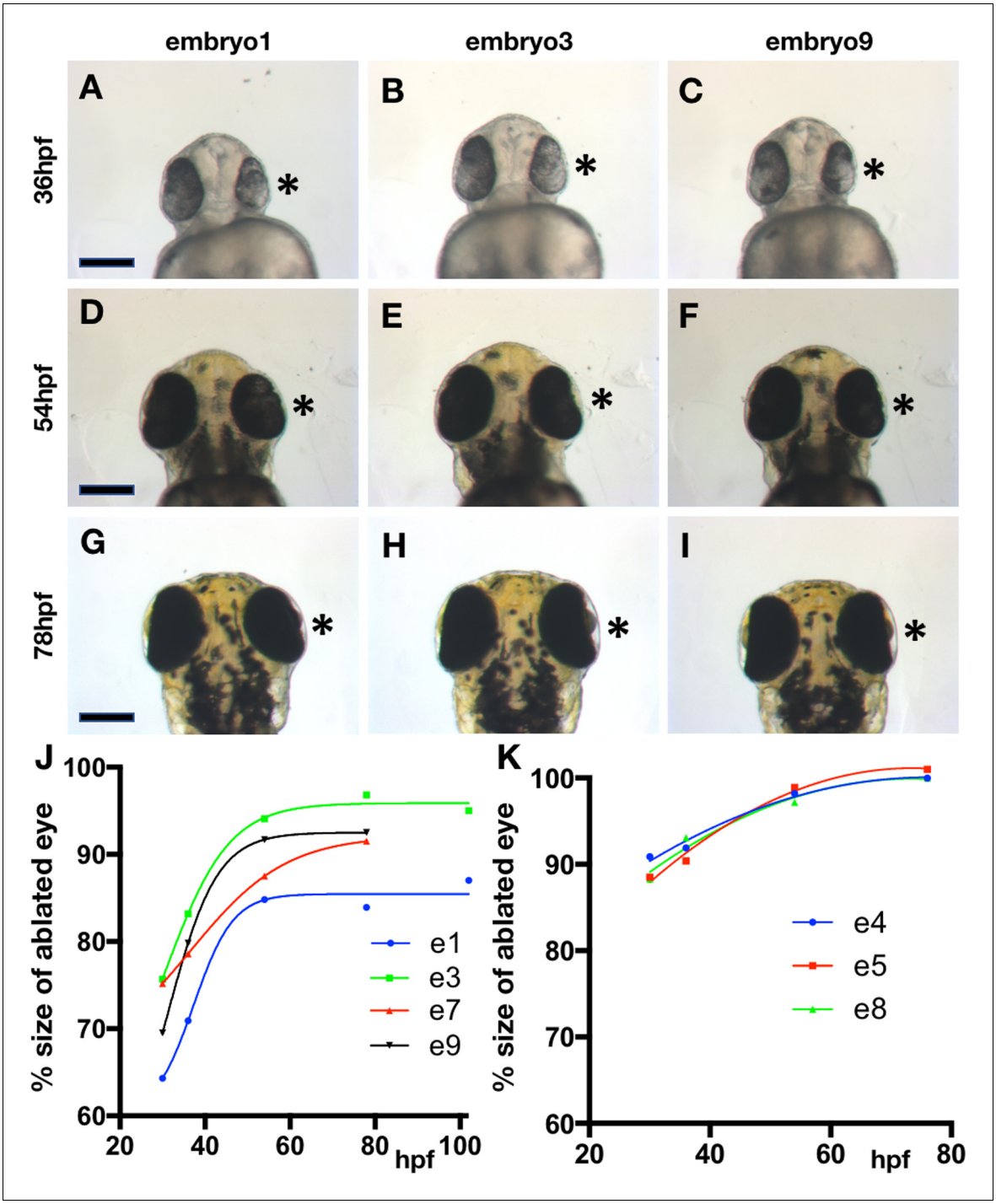

Figure 4—figure supplement 4. Growth kinetics of the eye from eye vesicle cell-removed embryos. (**A–I**) Wildtype embryo example 1 (**A, D, G**), 3 (**B, E, H**) and 9 (**C, F, I**) from which cells were removed from the right eye vesicle at 12hpf imaged at 36hpf (**A–C**), 54hpf (**D–F**) and 78hpf (**G–H**). Ventral view (**A–F**), dorsal view (**H–I**), anterior up. Images have been flipped such that the cell-removed eye is always on the right side of the embryo (asterisk). Scale bars = 200 μm. (**J, K**) Plots showing the ratio of control eye to cell-removed eye volume from data in ***Supplementary file 1I*** generated from lateral view imaging of both eyes in each embryo.

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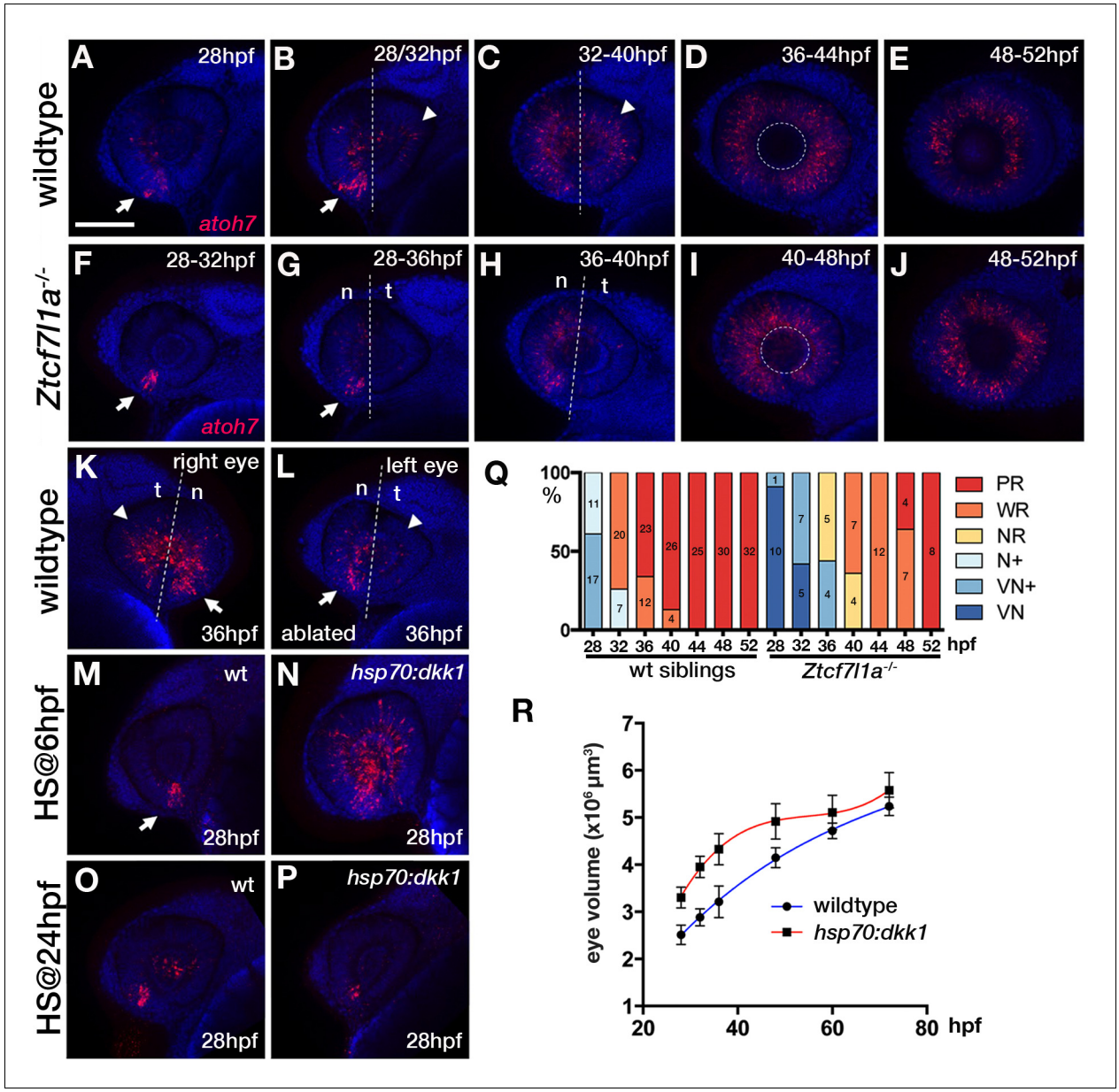

Figure 5. Neurogenesis is delayed in small *tcf7l1a*$^{-/-}$ eyes and accelerated in large eyes following *hsp70:dkk1* overexpression. (**A–P**) Lateral views of eyes showing *atoh7* fluorescent *in situ* hybridisation in typical wildtype (**A–E, M, O**), *Ztcf7l1a*$^{-/-}$ (**F–J**), wildtype left-side optic vesicle-ablated (**K, L**); from n = 5 embryos) and *Tg(HS:dkk1)*^{w32} (**N, P**) embryos at stages indicated. (**M–P**) Wildtype (**M, O**) and heterozygous sibling *Tg(HS:dkk1)*^{w32} embryos (**N, P**) heat-shocked at 6hpf (**M, N**); from n = 7/9 embryos) or 24hpf (**O, P**); from n = 10/10 embryos) for 45' at 37°C and grown to 28hpf. Anterior is to the left except in (**K**) in which anterior is to the right. Arrows indicate ventro-nasal retina; arrowheads indicate dorso-temporal retina; dashed line approximate the nasal-temporal division; dashed circle marks lens position. Abbreviations: n, nasal, t, temporal. Scale bar = 100 µm. (**Q**) Histogram showing the spatial distribution of *atoh7* expression in sibling and *Ztcf7l1a*$^{-/-}$ retinas at the indicated hours post-fertilisation (data in ***Supplementary file 1F***). VN, ventro nasal expression; VN+, ventro-nasal expression plus a few scattered cells; N+, nasal expression plus scattered cells covering the whole retina; NR, nasal retina expression; WR, whole retina expression; PR, expression localised to the peripheral retina. Numbers in bars represent the number of embryos scored for the particular category of *atoh7* expression. (**R**) Plot showing the growth kinetics of the eye in wildtype (blue line) and *Tg(HS:dkk1)*^{w32} (red line) embryos at times indicated (data in ***Supplementary file 1K***).

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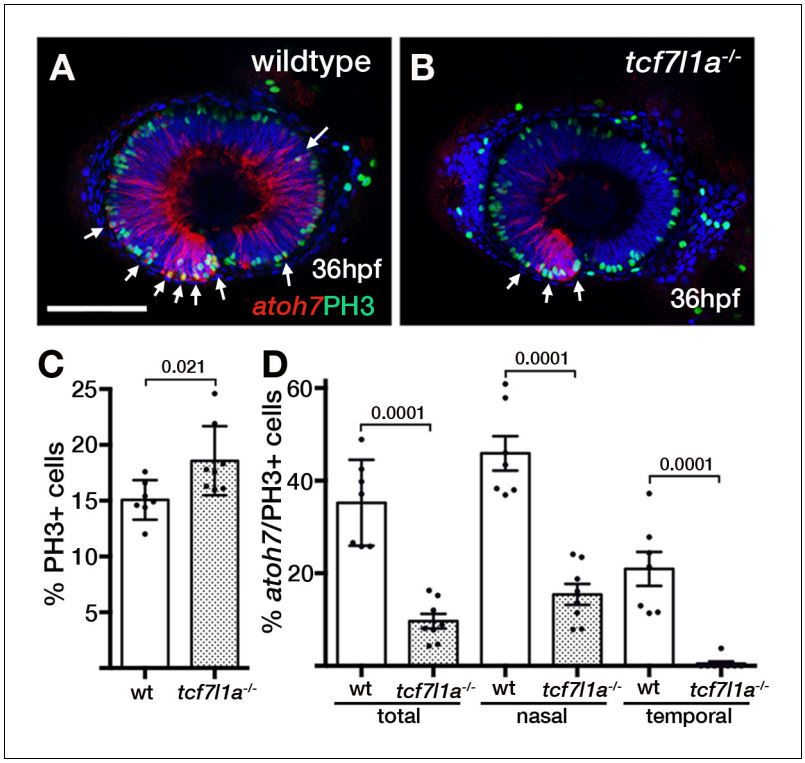

Figure 6. Z*tcf7l1a* mutants show more retinal progenitor cells undergoing proliferation. (**A–B**) Immunostaining detecting phosphohistone3 (PH3, green) and RFP (*Tg(atoh7:GAP-RFP)*$^{cu2Tg-}$, red) in wildtype (**A**) and Z*tcf7l1a*$^{-/-}$ (**B**) eyes at 36hpf . Arrows indicate selected double PH3/RFP positive cells. n, nasal; t, temporal. Scale bar = 100 μm. (**C–D**) Plot showing the percentage of PH3-positive cells (**C**) data in ***Supplementary file 1L***) and double PH3/RFP-positive cells (**D**), data in ***Supplementary file 1L***). Single experiment, wildtype n = 7, Z*tcf7l1a*$^{-/-}$ n = 8, figures over the bars show p-values from unpaired t-tests.

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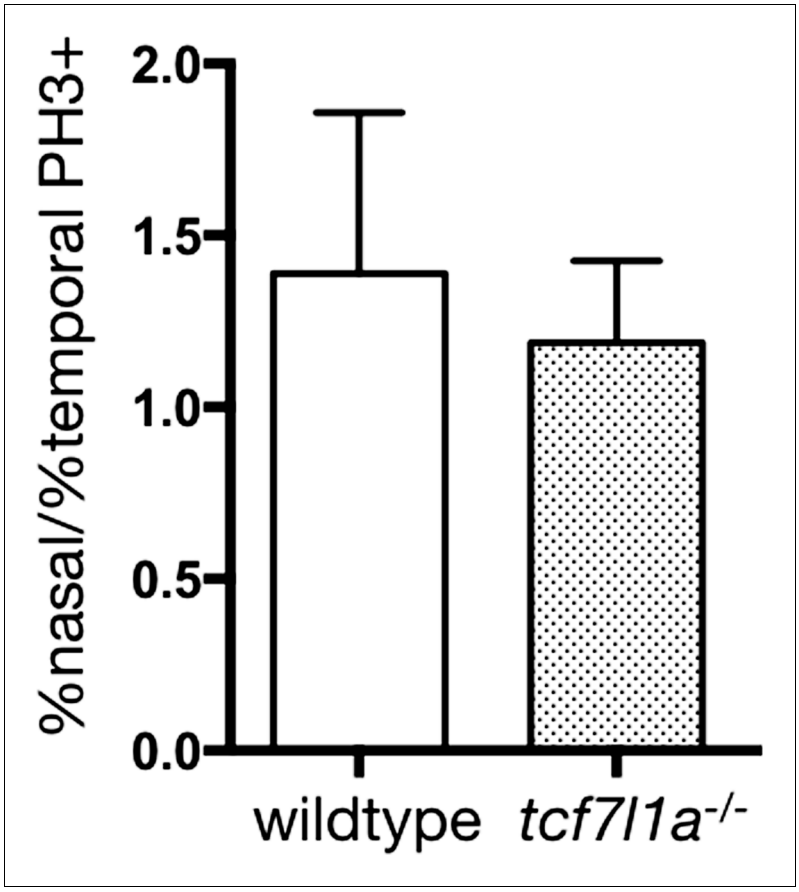

Figure 6—figure supplement 1. Ratio of double atoh7/phosphohistone3 cells in the nasal and temporal retina. Plot showing the ratio of atoh7/phosphohistone3 positive cells of nasal over temporal retina in wildtypes (n = 7) and *tcf7l1a*$^{-/-}$ mutants (n = 8). Data in ***Supplementary file 1K***. Difference in both conditions is not significant, p=0.3, unpaired t-test).

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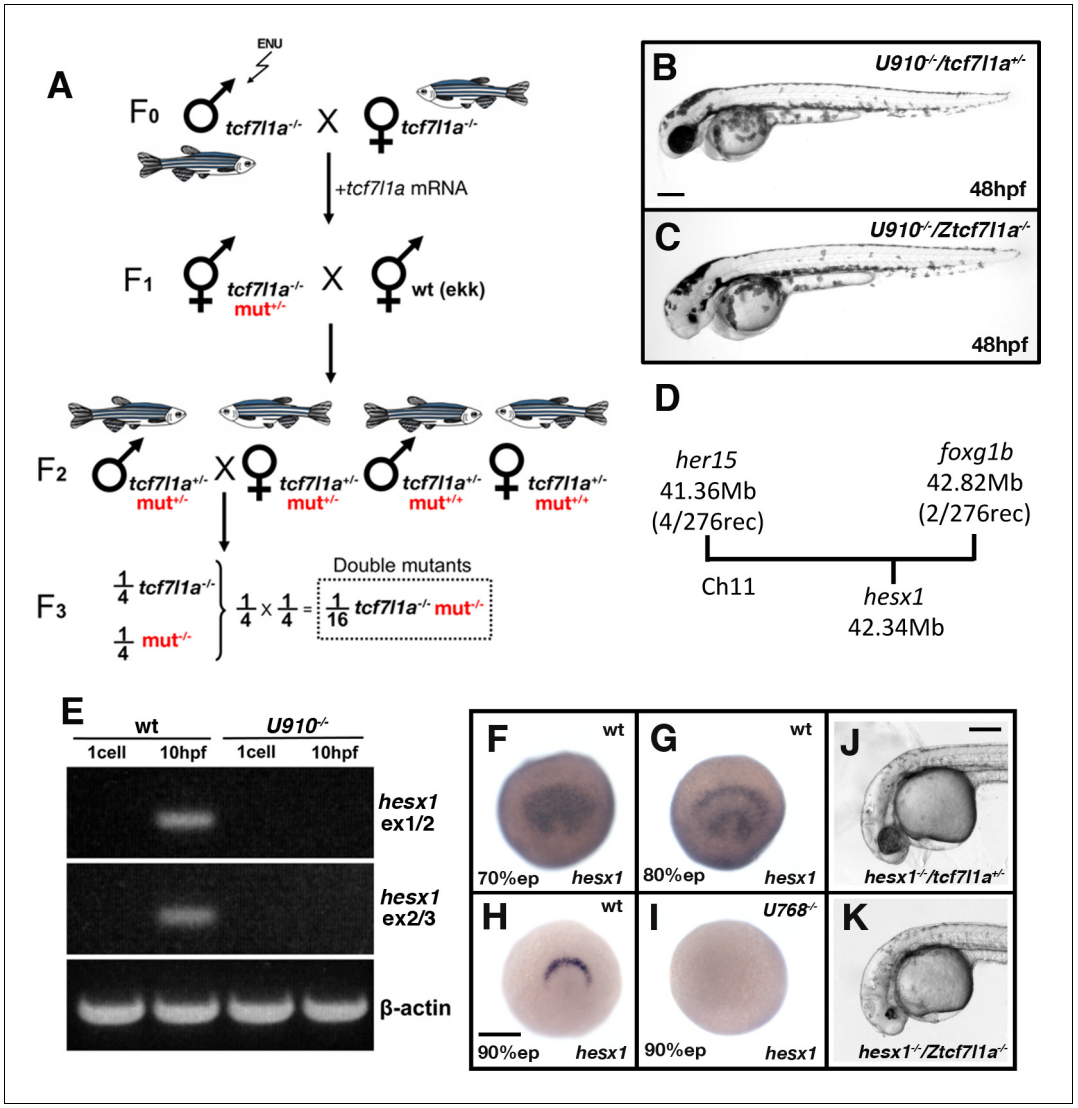

Figure 7. *Ztcf7l1a*$^{-/-}$ mutants lacking Hesx1 function fail to form eyes. (**A**) Schematic of the genetic strategy to isolate mutations that modify the *tcf7l1a*$^{-/-}$ mutant phenotype. (**B–C**) *U910* modifier of the *tcf7l1a*$^{-/-}$ mutant phenotype. Lateral views of homozygous *U910* embryos that are heterozygous (**B**) or homozygous (**C**) for the *tcf7l1a* mutation. (**D**) Representation of SSLP segregation linkage analysis mapping of U910 modifier of *tcf7l1a* to a 1.46 megabase (Mb) interval on chromosome 11 (Ch11; rec, recombinants). (**E**) RT-PCR for *hesx1* spanning exons 1–2 (top panel), exons 2–3 (middle panel) and β-actin (bottom panel) on wildtype (lanes 1 and 2) and *U910*$^{-/-}$ (lanes 3 and 4) embryo cDNA from 1 cell stage (lanes 1 and 3) and 10hpf (lanes 2 and 4). Single experiment. (**F–I**) *hesx1 in situ* hybridisation on wildtype (**F–H**) and *U910*$^{-/-}$ (**I**) embryos at epiboly (ep) stages indicated. Dorsal views, anterior up. (**J, K**) Lateral views of *hesx1*$^{-/-}$ (Δex1/2)/*tcf7l1a*$^{+/-}$ (**J**) and *hesx1*$^{-/-}$ (Δex1/2)/*Ztcf7l1a*$^{-/-}$ (**K**) embryos. Four independent experiments, n = 53. Scale bars = 200 μm.

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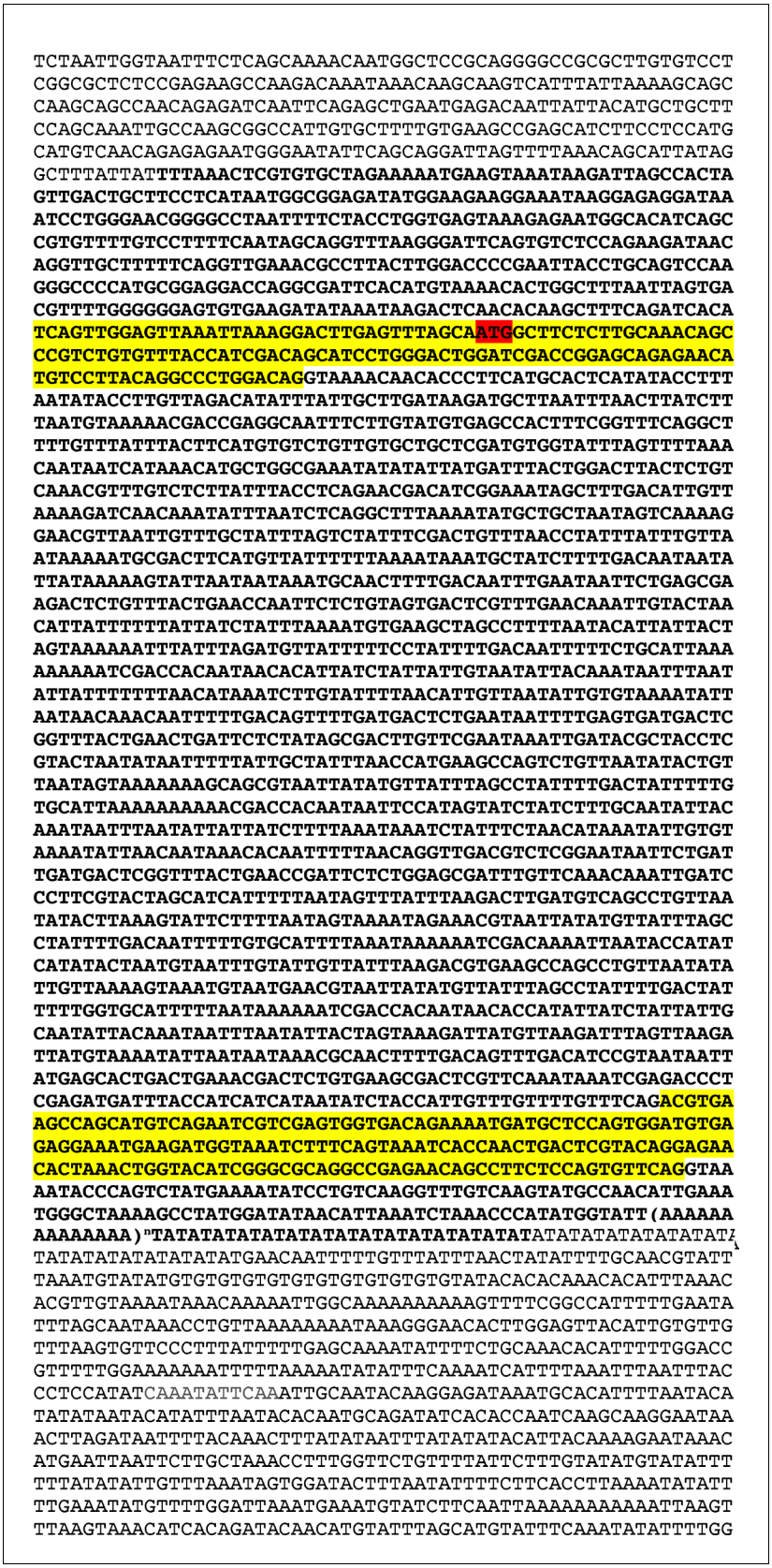

Figure 7—figure supplement 1. Genomic DNA sequence of *hesx1* locus spanning exons 1 and 2. Genomic DNA sequence deleted in *U910* fish in bold. *hesx1* exons 1 and 2 highlighted in yellow. Open reading frame first codon in exon one is highlighted in red.
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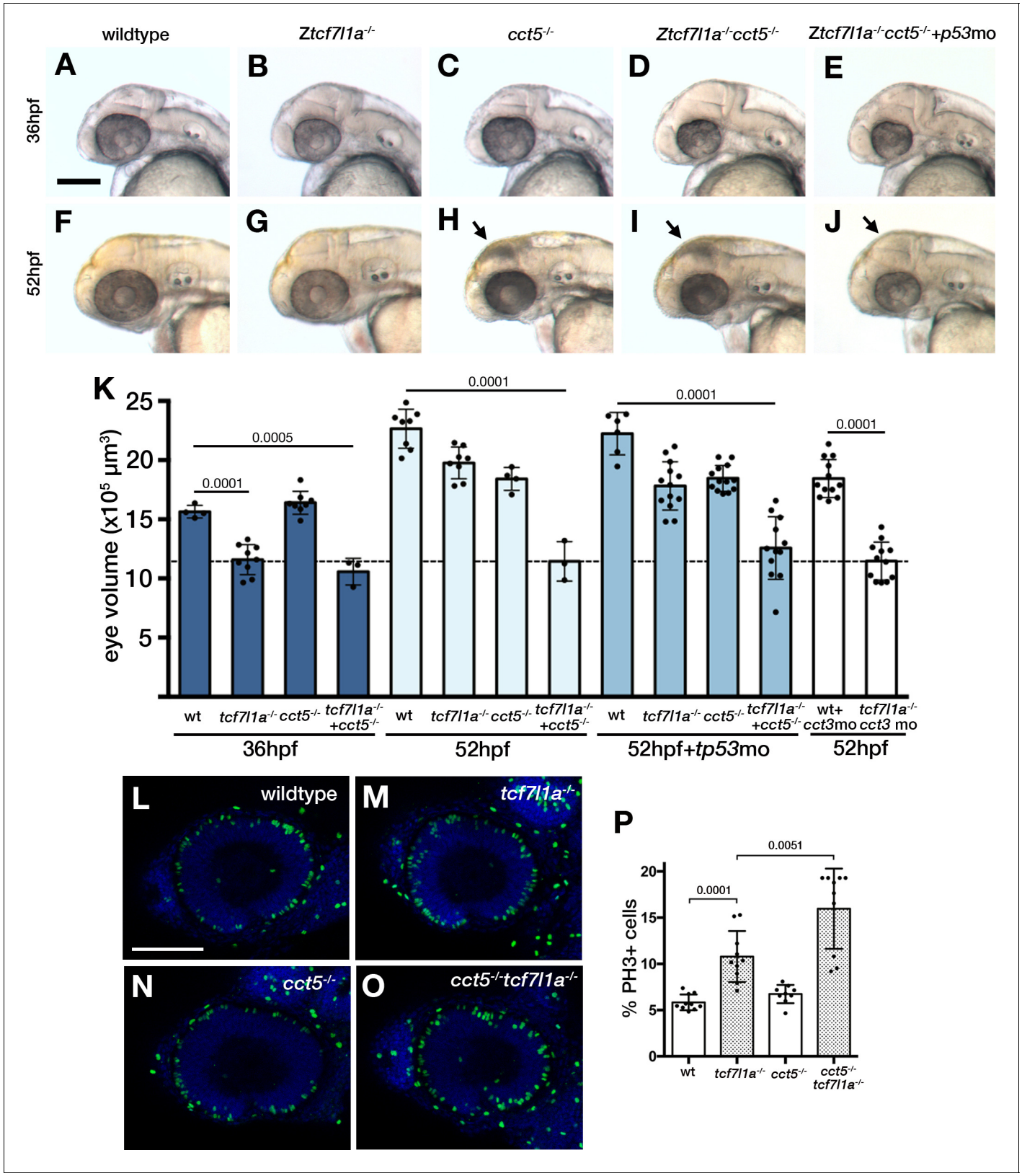

Figure 8. Loss of *tcf7l1a* modifies the *cct5*^{u762} mutant eye phenotype. (**A–J**) Lateral views of wildtype (**A, F**), *Ztcf7l1a*$^{-/-}$ mutant (**B, G**) *cct5*$^{U762/u762}$ mutants (**C, H**), double *cct5*$^{U762/u762}$/*Ztcf7l1a*$^{-/-}$ mutants (**D, I**) and double *cct5*$^{U762/u762}$/*Ztcf7l1a*$^{-/-}$ mutants injected with 0.8 pmol of *cct3* morpholino (**E, J**) at indicated stages. Scale bar = 100 µm. Full data in ***Supplementary file 10O***, single experiment, 36hpf, wt n = 4, *Ztcf7l1a*$^{-/-}$ n = 9, *cct5*$^{-/-}$ n = 8, *cct5/*

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Figure 8 continued

Ztcf7l1a$^{-/-}$ n = 3; 52hpf, wt n = 8, *Ztcf7l1a*$^{-/-}$ n = 8, *cct5*$^{-/-}$ n = 4, *cct5/Ztcf7l1a*$^{-/-}$ n = 3; 52hpf + 2 pmol *tp53* morpholino, wt n = 6, *Ztcf7l1a*$^{-/-}$ n = 13, *cct5*$^{-/-}$ n = 13, *cct5/Ztcf7l1a*$^{-/-}$ n = 12; 52hpf + 0.8 pmol *cct3* morpholino, wt n = 12, *Ztcf7l1a*$^{-/-}$ n = 12. (**K**) Eye volume quantification at the indicated timepoints and conditions shown in **A–J**) (data in ***Supplementary file 1O***). Unpaired t-test. (**L–O**) Immunostaining detecting phosphohistone3 (PH3, green) in wildtype (**L**), *Ztcf7l1a*$^{-/-}$ (**M**), *cct5*$^{-/-}$ (**N**), *cct5*$^{-/-}$/*Ztcf7l1a*$^{-/-}$ (**O**) eyes at 32hpf. (**P**) Plot showing the percentage of PH3 positive cells in the eyes shown in **L–O**) (data in ***Supplementary file 1Q***) Single experiment, wildtype n = 10, *Ztcf7l1a*$^{-/-}$ n = 10, *cct5*$^{-/-}$ n = 9, *cct5/Ztcf7l1a*$^{-/-}$n = 10, unpaired t-tests.

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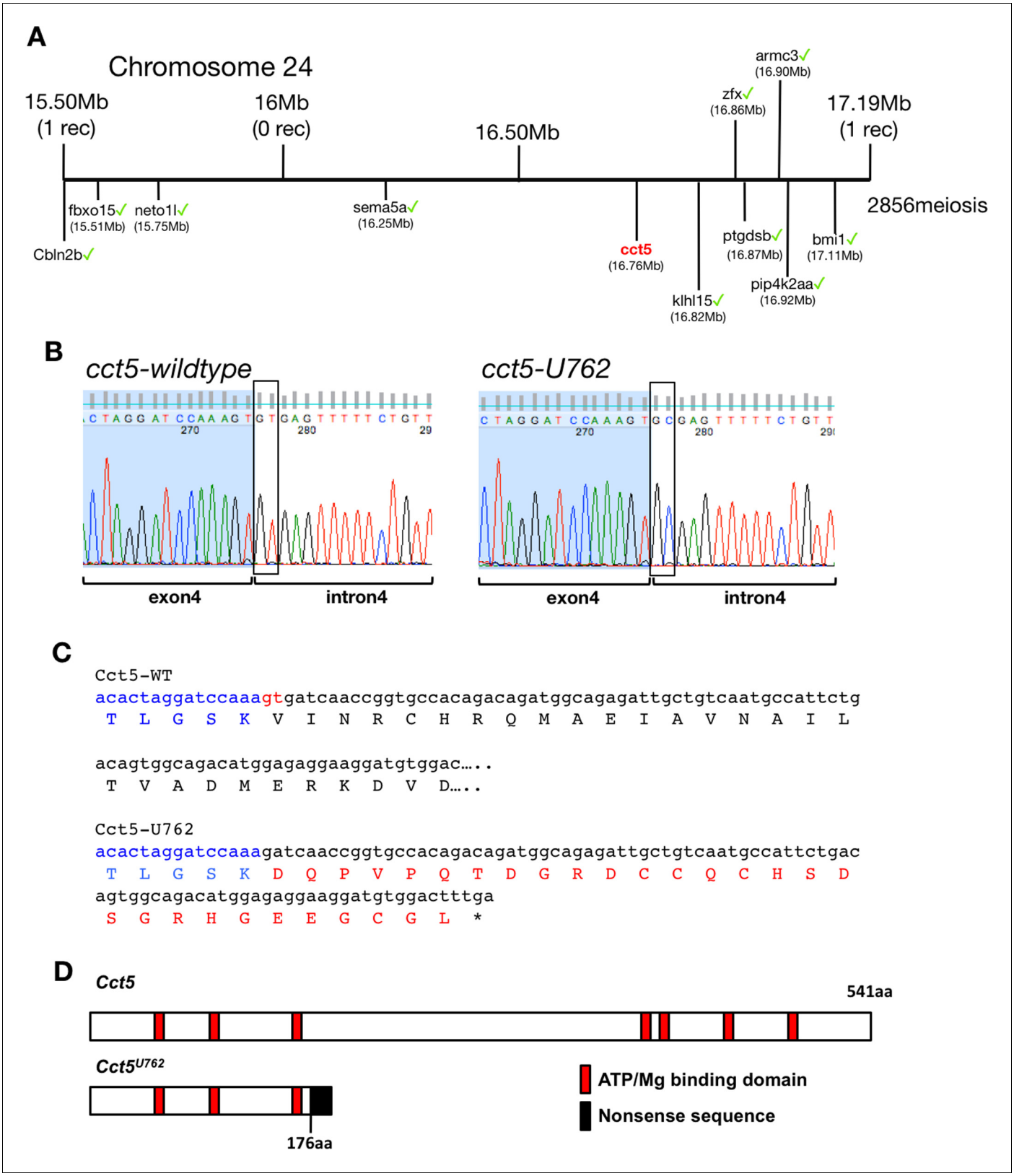

Figure 8—figure supplement 1. Genetic mapping of *U762* and description of the *cct5*^{U762} mutation. (**A**) Representation of the SSLP segregation linkage analysis mapping of U762) modifier of *tcf7l1a* to a 1.69 Mb interval on chromosome 12, between 15.50 Megabases (Mb) with one recombinant (rec) and 17.19 Mb with one rec. Green ticks highlight sequenced genes in the interval that show no mutations. (**B**) DNA sequencing chromatograms of

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Figure 8—figure supplement 1 continued

the genomic fragment encompassing the 3′ end of *cct5* exon 4 and 5′ end of intron four from wildtype (left) and $cct5^{U762/762}$(right) embryos. Boxes show the splice donor nucleotides in intron 4. (**C**) Nucleotide and protein sequence of wildtype (top alignment) and $cct5^{U762}$ (bottom alignment). *cct5* exon four nucleotides and amino acids in blue. The last two 3′ nucleotides in exon four that are used in $cct5^{U762}$ as a splice donor in red. Nonsense amino acid sequence in $cct5^{U762}$ in red. (**D**) Cartoon of wildtype and $Cct5^{U762}$ protein product. Red boxes show Mg^{2+}/ATP binding domains, black box indicates nonsense mutant protein stretch.

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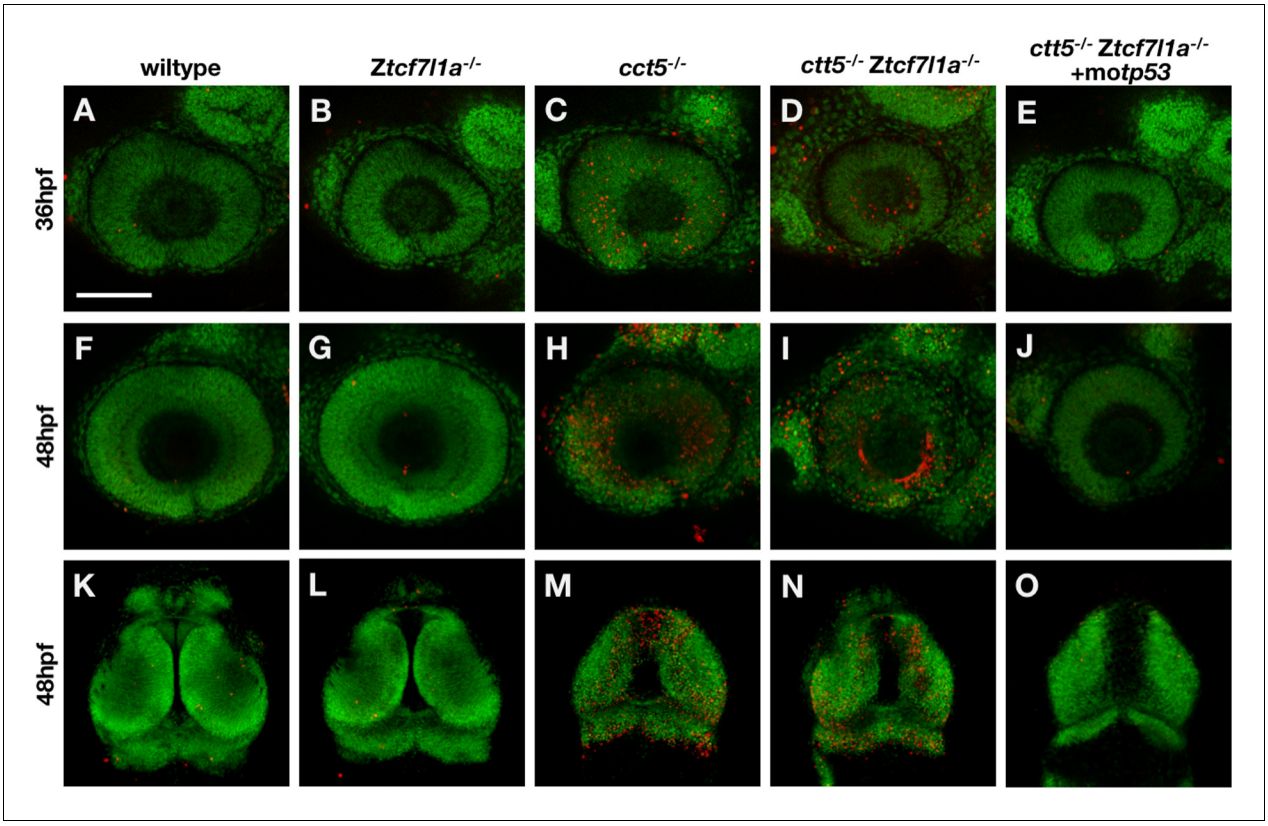

Figure 8—figure supplement 2. Whole mount Tunel cell death analysis in *cct5/tcf7l1a* mutants. Tunel assay labelling detecting apoptotic cells (red) performed in wildtype (**A, F, K,** 100% n = 8), *Ztcf7l1a*$^{-/-}$ (**B, G, L,** 100% n = 10), *cct5*$^{-/-}$ (**C, H, M,** 100% n = 9), *cct5/Ztcf7l1a*$^{-/-}$ (**D, I, N,** 100% n = 4) and *cct5/Ztcf7l1a*$^{-/-}$ + 2 pmol of *tp53* morpholino (**E, J, O,** 100% n = 5). DAPI pseudocoloured in green. Scale bar = 100 μm.

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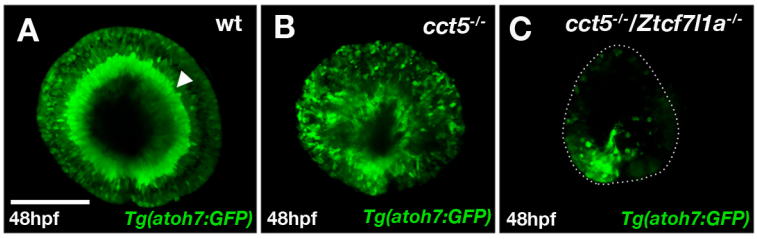

Figure 8—figure supplement 3. *atoh7* expression in *cct5/tcf7l1a* mutants. (**A–C**) Anti-GFP immunocytochemistry (green) showing Tg(*atoh7:GFP*)- labelled neurons in wildtype. (**A**) *cct5*$^{-/-}$ (*cct5*$^{U762/U762}$) (**B**) and *cct5*$^{-/-}$*/Ztcf7l1a*$^{-/-}$ (**C**) embryos at 48hpf. Dotted line in (**C**) outlines the eye profile. Arrowhead points to the retina ganglion cell layer. Scale bar = 100 μm.

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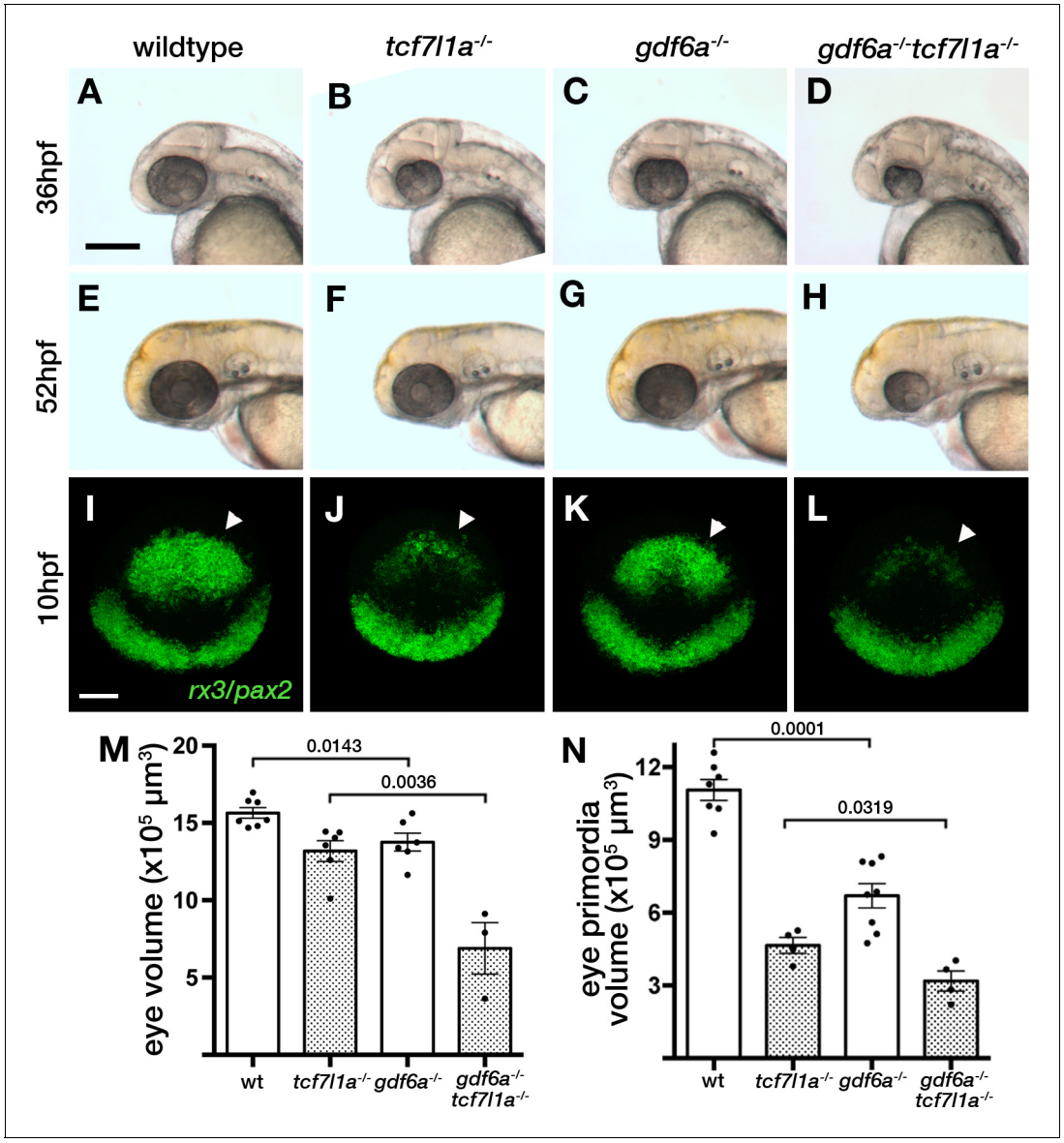

Figure 9. Loss of *tcf7l1a* modifies the *gdf6a*$^{U768/U768}$mutant eye phenotype. (**A–H**) Lateral views of eyes in wildtype (**A, E**), *Ztcf7l1a*$^{-/-}$ (**B, F**), *gdf6a*$^{U768/U768}$ (**C, G**) and double *gdf6a*$^{U768/U768}$/*Ztcf7l1a*$^{-/-}$ (**D, H**) embryos at 36hpf (**A–D**) and 52hpf (**E–H**). Dorsal up, anterior to left. Arrows indicate the lens. Scale bar = 200 µm. (**I**) Whole mount fluorescent in situ hybridisation for *rx3* and *pax2a* in wildtype (**I**), *Ztcf7l1a*$^{-/-}$ (**J**), *gdf6a*$^{U768/U768}$(**K**) and double *gdf6a*$^{U768/U768}$/*Ztcf7l1a*$^{-/-}$ (**L**) embryos at 10hpf. Dorsal view, anterior up. Arrows point to *rx3* eye field expression. Scale bar = 100 µm. (**M**) Eye volume quantification in wildtype (n = 7), *Ztcf7l1a*$^{-/-}$ (n = 6), *gdf6a*$^{-/-}$(n = 6) and *gdf6a*$^{U768/U768}$/*Ztcf7l1a*$^{-/-}$ double mutant siblings (n = 3) at 36hpf (data in ***Supplementary file 1R***). Single experiment, unpaired t-test. (**N**) Eye field volume quantification from *rx3* fluorescent *in situ* hybridisation shown in I-L in wildtype (n = 7), *Ztcf7l1a*$^{-/-}$ (n = 4), *gdf6a*$^{-/-}$(n = 8) and *gdf6a*$^{U768/U768}$/*Ztcf7l1a*$^{-/-}$ double mutant siblings (n = 4) at 10hpf (data in ***Supplementary file 1S***). Single experiment, unpaired t-test.

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