**SUPPLEMENTARY INFORMATION**

**Fig. S1. Relative gene expression of 27 genes of interest selected for the phenotypic screen.**

Relative gene expression ($log_2$ fold change) of 27 significant (FDR *p*-value <0.05) differentially expressed genes (DEGs) selected from top upregulated genes in RPE cells at 2 days post-injury (dpi) compared to 7 days post-fertilization (dpf) from a publicly available bulk RNA sequencing dataset (GSE174538; Lu, F., Leach, L. L. & Gross, J. M. mTOR activity is essential for retinal pigment epithelium regeneration in zebrafish. *PLoS Genet.* **18**, e1009628 (2022)).

**Fig. S2. Phenotypic screening results of additional positive regulators of RPE regeneration.**

(A-G) RpEGEN output plots showing median pixel intensity distributions (bin size = 5 angular degrees) within RPE regions of interest (ROIs) and statistical comparisons of dorsal-to-ventral median pixel intensity (bin size = 1 angular degree) between: (A) scrambled and *nrg1* groups, (B) scrambled and *fosl1b* groups, (C) scrambled and *ogflr1* groups, (D) scrambled and *ccn1l1* groups, (E) scrambled and *lipib* groups, (F) scrambled and *cidec* groups, and (G) scrambled and *il11a* groups. All plots represent comparisons between larval groups at 4dpi. Light blue rectangles indicate the region(s) with significantly higher (lighter) pixel intensity values spanning more than twenty angular degrees. Gray rectangles indicate the distal-most dorsal (0 to 30 angular degrees) and distal-most ventral (150 to 180 angular degrees) peripheral RPE areas omitted from analyses. Dashed black lines indicate a 95% confidence interval (CI). Exact regions with significant differences compared to scrambled controls can be found in Table S2. Abbreviation: dpi, days post-injury.

**Fig. S3. Phenotypic screening results of additional negative regulators of RPE regeneration.**

(A-F) RpEGEN output plots showing median pixel intensity distributions (bin size = 5 angular degrees) within RPE regions of interest (ROIs) and statistical comparisons of dorsal-to-ventral median pixel intensity (bin size = 1 angular degree) between: (A) scrambled and *zgc:153911* groups, (B) scrambled and *cpa4* groups, (C) scrambled and *adamtsl7* groups, (D) scrambled and *dkk1a* groups, (E) scrambled and *lepb* groups, and (F) scrambled and *serpine1* groups. All plots represent comparisons between larval groups at 4dpi. Light blue rectangles indicate the region(s) with significantly lower (darker) pixel intensity values

spanning more than twenty angular degrees. Gray rectangles indicate the distal-most dorsal (0 to 30 angular degrees) and distal-most ventral (150 to 180 angular degrees) peripheral RPE areas omitted from analyses. Dashed black lines indicate a 95% confidence interval (CI). Exact regions with significant differences compared to scrambled controls can be found in Table S2. Abbreviation: dpi, days post-injury.

**Fig. S4. Phenotypic screening results of additional GOIs that did not show an RPE regeneration phenotype.**

(A-J) RpEGEN output plots showing median pixel intensity distributions (bin size = 5 angular degrees) within RPE regions of interest (ROIs) and statistical comparisons of dorsal-to-ventral median pixel intensity (bin size = 1 angular degree) between: (A) scrambled and *ptx3a* groups, (B) scrambled and *ccl34a.4* groups, (C) scrambled and *clcf1* groups, (D) scrambled and *cxcl18a.1* group, (E) scrambled and *il11b* groups, (F) scrambled and *epcam* groups, (G) scrambled and *ptgs2a* groups, (H) scrambled and *ptgs2b* groups, (I) scrambled and *met* groups, (F) scrambled and *cxcl8a* groups, and (J) scrambled and *edn2* groups. All plots represent comparisons between larval groups at 4dpi. Gray rectangles indicate the distal-most dorsal (0 to 30 angular degrees) and distal-most ventral (150 to 180 angular degrees) peripheral RPE areas omitted from analyses. Dashed black lines indicate a 95% confidence interval (CI). Abbreviation: dpi, days post-injury.

**Fig. S5. The RPE layer appears phenotypically normal in unablated scrambled and *cldn7b* F0 knockout larvae.**

Representative immunofluorescence images of unablated (MTZ-) 9dpf (A-B’’’; n=6) scrambled and (C-D’’’; n=6) *cldn7b* F0 knockout larvae. Nuclei (white), eGFP (green), ZPR2 (magenta). (B,D) Digital zooms highlight (B’,D’; red asterisk) photoreceptor lamination, (B’,D’; cyan arrow) basal nuclei in the RPE, and distinct apical microvilli in RPE co-labeled with (B’’,D’’; white arrowhead) eGFP and (B’’’,D’’’; white arrowhead) ZPR2. Scale bars = 50µm. Abbreviations as follows: MTZ, metronidazole; dpf, days post-fertilization.

**Fig. S6. *cldn7b* F0 knockout does not impact cell proliferation in the RPE layer from 3dpi to 4dpi.**

(A-D) Representative immunofluorescence images showing the BrdU-labeled proliferative cells in the unablated (MTZ-) and ablated (MTZ+) scrambled and *cldn7b* F0 knockout larval eyes. Nuclei (white), BrdU (red). (E) Box plots showing significant increases in the number of BrdU-labeled cells in the RPE layer of ablated scrambled and *cldn7b* F0 knockout larvae when compared to the corresponding unablated larvae, and no significant differences of unablated or ablated *cldn7b* F0 knockout larvae when compared to scrambled controls. Scale bars = 50μm; ****$P$-value ≤0.0001, ns = not significant. Exact $p$-values, numbers of independent experiments (N), and numbers of biological replicates (n) can be found in Table S3. Abbreviations as follows: BrdU, bromodeoxyuridine; MTZ, metronidazole; dpi, days post-injury.

**Fig. S7. *cldn7b* F0 knockout does not affect localization of macrophages/microglia in unablated larvae.**

(A-F) Representative immunofluorescence images of mCherry signals in the unablated (MTZ-) *cldn7b* F0 knockout larval eyes at (A-B') 7dpf, (C-D') 8dpf, and (E-F') 9dpf. (A'-F') Single-channel images of mCherry signal with RPE regions of interest (ROIs). Nuclei (white), eGFP (green), mCherry (magenta). (G-I) Box plots showing no significant differences in mCherry signal between scrambled and *cldn7b* knockout groups at (G) 7dpf, (H) 8dpf, and (I) 9dpf. Scale bars = 50μm; ns = not significant. Exact $p$-values, numbers of independent experiments (N), and numbers of biological replicates (n) can be found in Table S3. Abbreviations as follows: MTZ, metronidazole; dpf, days post-fertilization.

**Fig. S8. *cldn7b* F0 knockout does not influence cell death in the RPE layer at 3dpi and 4dpi.**

(A-D) Representative immunofluorescence images of TUNEL+ puncta in (A,B) 8dpf unablated (MTZ-) and (C,D) 3dpi ablated (MTZ+) scrambled and *cldn7b* F0 knockout larval eyes. (F-I) Representative immunofluorescence images of TUNEL+ puncta in (F,G) 9dpf unablated (MTZ-) and (H,I) 4dpi ablated (MTZ+) scrambled and *cldn7b* F0 knockout larval eyes. Nuclei (white), TUNEL (red). (E,J) Box plots showing no significant differences in TUNEL signal between scrambled and *cldn7b* knockout groups at (E) 8dpf/3dpi and (J) 9dpf/4dpi. Scale bars = 50μm; ns = not significant. Exact $p$-values, numbers of independent experiments (N), and numbers of biological replicates (n) can be found in Table S3. Abbreviations as follows: MTZ, metronidazole; dpf, days post-fertilization; dpi, days post-injury.

**Fig. S9. Original images of cropped electrophoresis gels presented in Figure 1.**

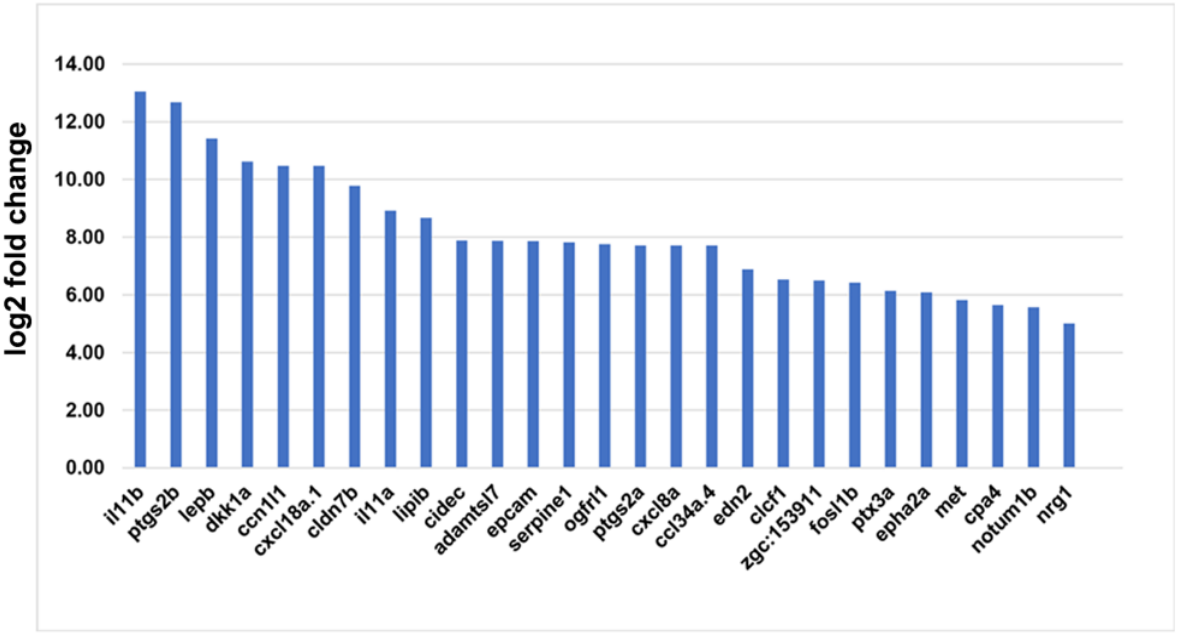
Original gel electrophoresis results for (A; red dotted box) *zgc:153911* headloop PCR and standard PCR shown in Fig. 1C (top gel), (B; teal dotted box) *nrg1* headloop PCR and standard PCR shown in Fig. 1C (bottom gel), and (C; blue dotted box) *cldn7b* genotyping shown in Fig. 1F.

**Table S1. crRNAs and primers used in this study.**

**Table S2. RPE regions (>20 continuous angular degrees) with significant differences.**

**Table. S3. Statistics.**

For box plots, the line and plus within the box represent the median and mean, respectively; the top and bottom whiskers represent the maximum and minimum values, respectively; and each dot represents a biological replicate (one eye from one larva). For statistical tests, D'Agostino-Pearson omnibus normality test was first performed to determine whether data obeyed normal (Gaussian) distributions. For comparisons between two groups, unpaired Student's t-tests with Welch's correction were performed on datasets with normal distribution and non-parametric Mann–Whitney tests were performed on datasets that were not normally distributed. For multiple comparisons, Kruskal–Wallis ANOVA followed by Dunn's multiple comparisons tests were used to determine the significance between groups. Exact *p*-values, numbers of independent experiments (N), and numbers of biological replicates (n) for each dataset can be found in Table S3.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ccn1l1
cxcl18a.1
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cidec
adamtsl7
epcam
serpine1
ogfrl1
ptgs2a
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ccl34a.4
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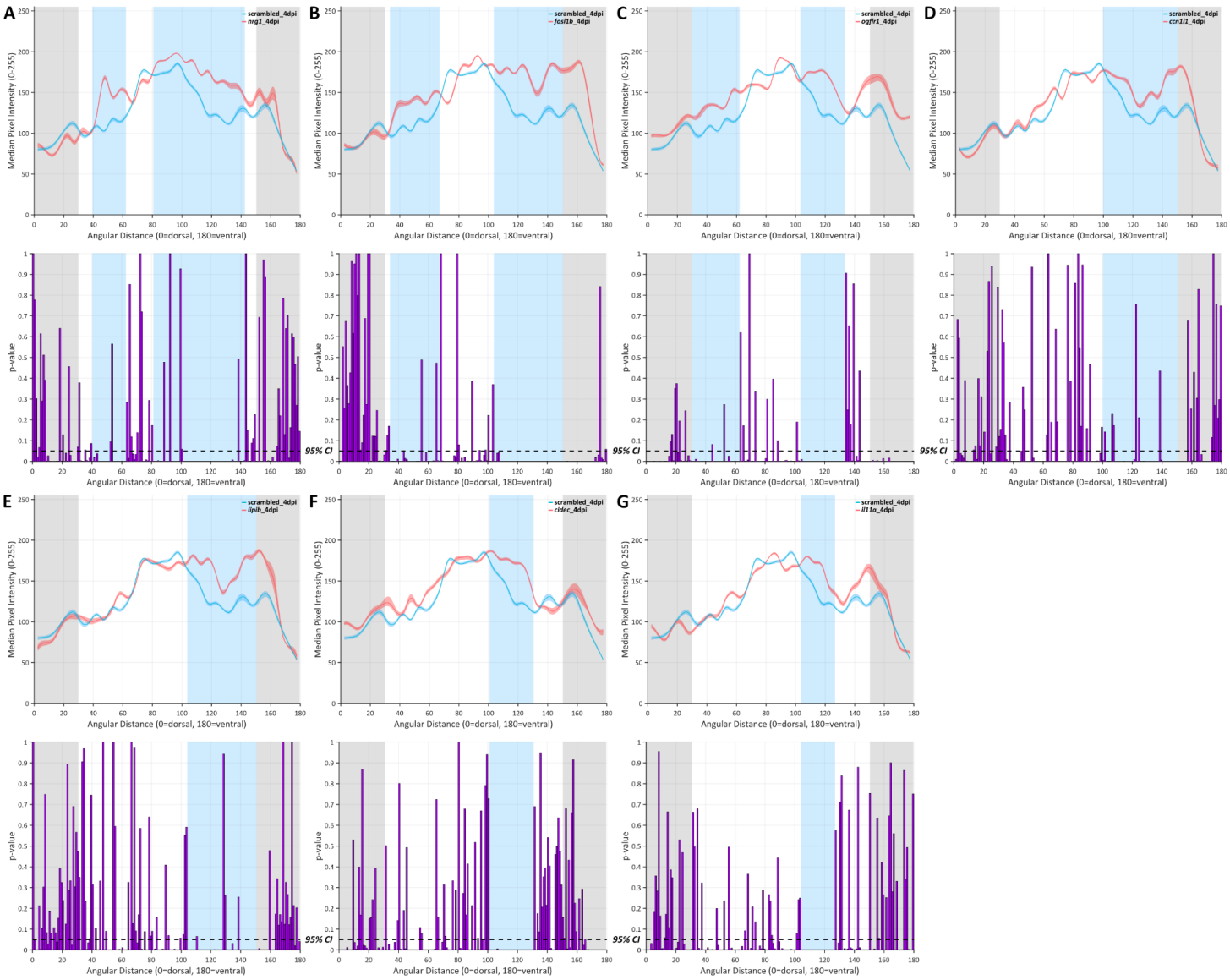
FIGURE S1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scrambled_4dpi
ogfrl1_4dpi
D
scrambled_4dpi
ccn1l1_4dpi
E
scrambled_4dpi
lipib_4dpi
F
scrambled_4dpi
cidec_4dpi
G
scrambled_4dpi
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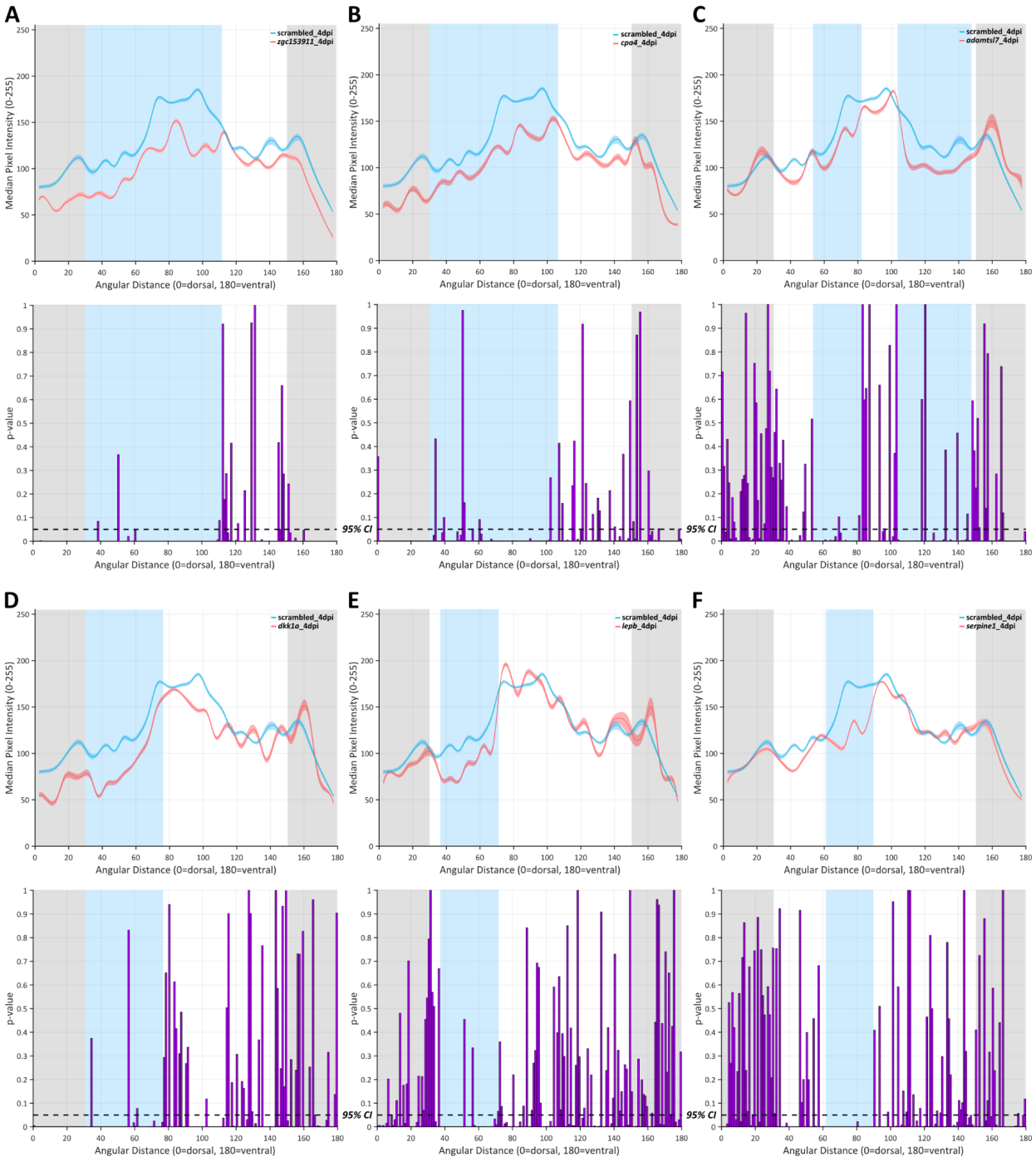
Median Pixel Intensity (0-255)
p-value
95% CI
Angular Distance (0=dorsal, 180=ventral)

FIGURE S2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C
D
E
F
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cpa4_4dpi
adamtsl7_4dpi
dkk1a_4dpi
lepb_4dpi
serpine1_4dpi
Median Pixel Intensity (0-255)
p-value
95% CI
Angular Distance (0=dorsal, 180=ventral)

FIGURE S3

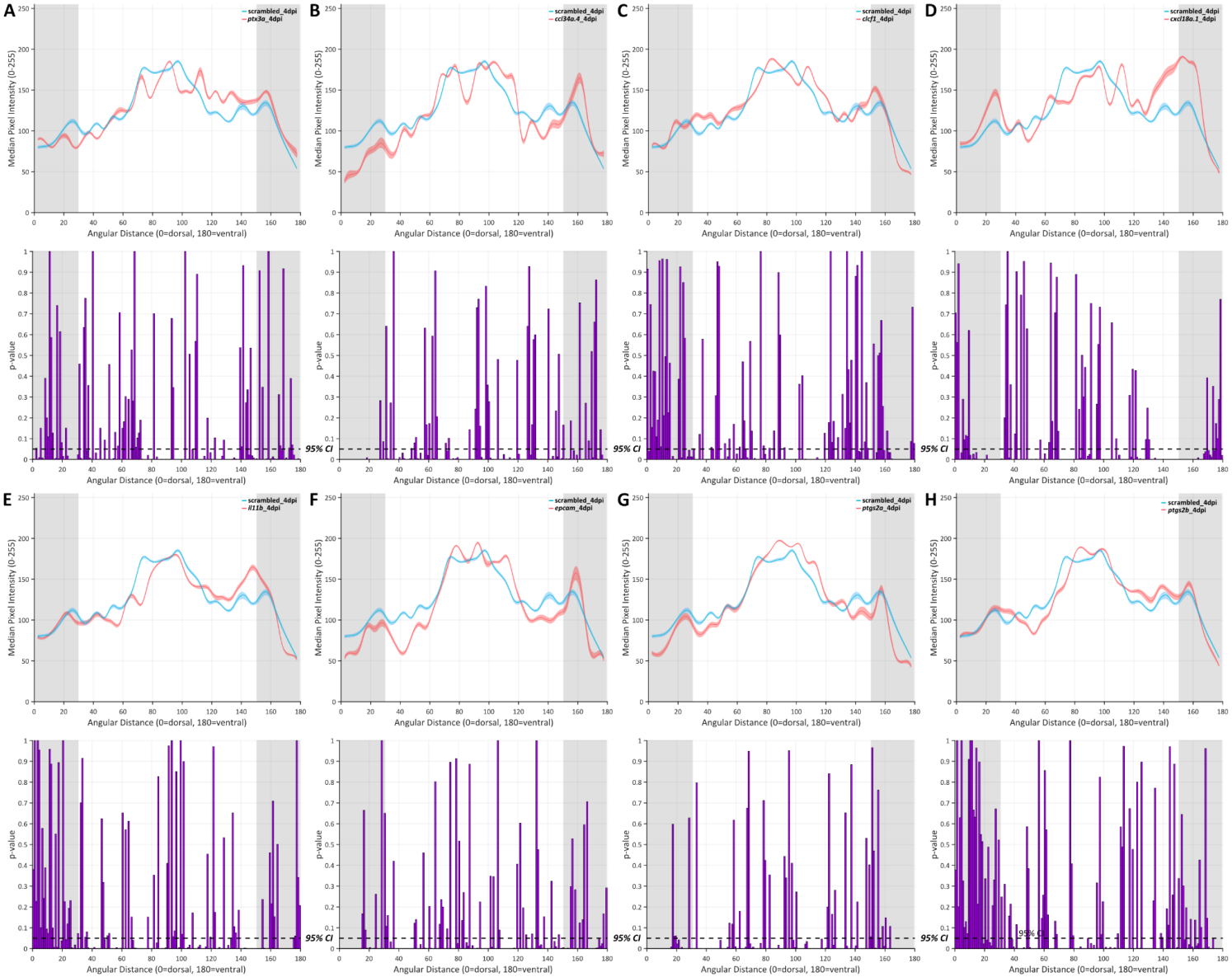



FIGURE S4 (continued on next page)

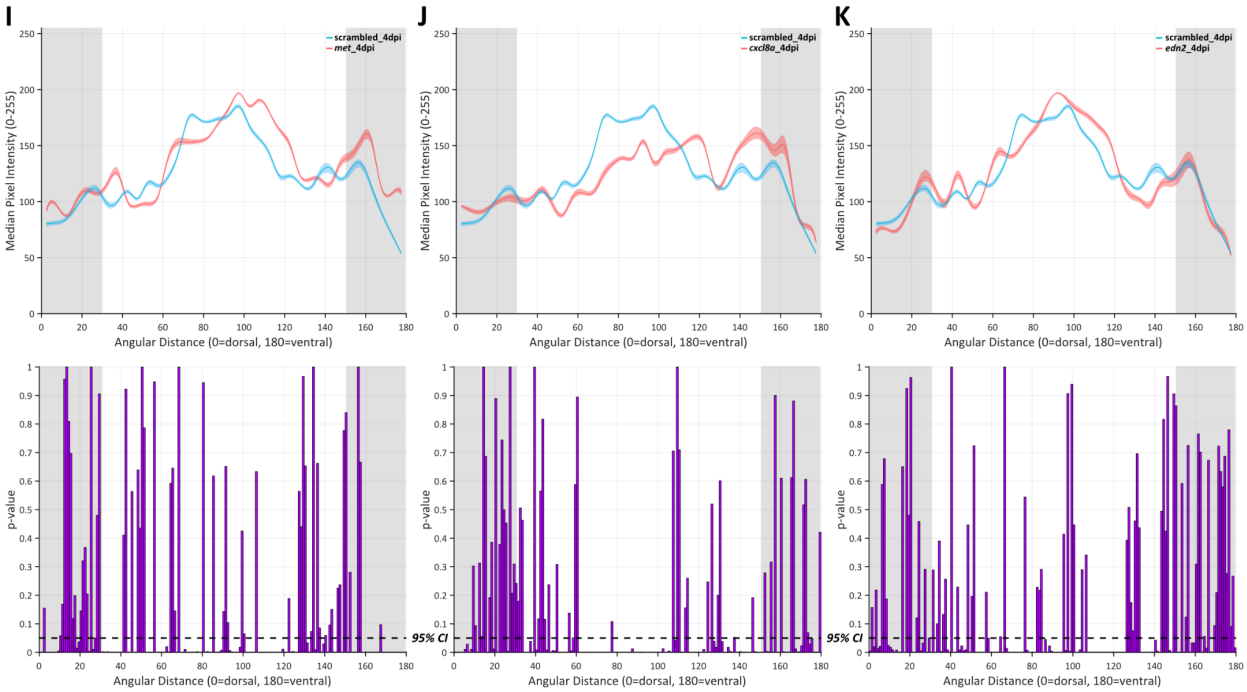
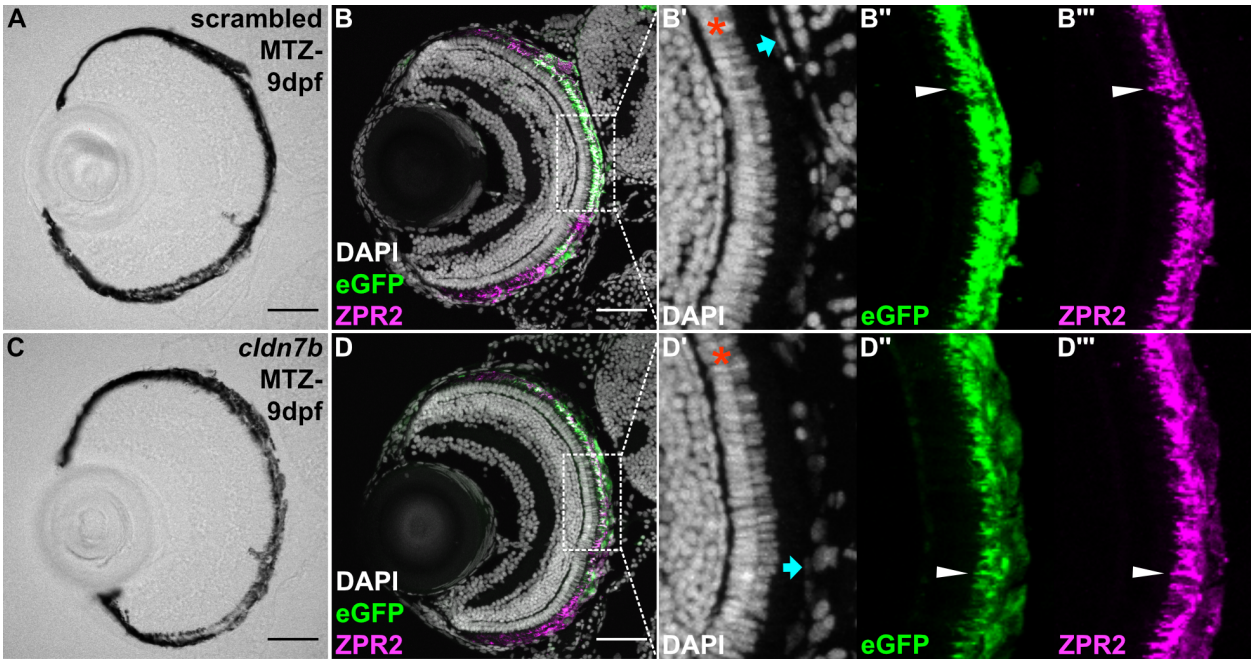



FIGURE S4 (continued from previous page)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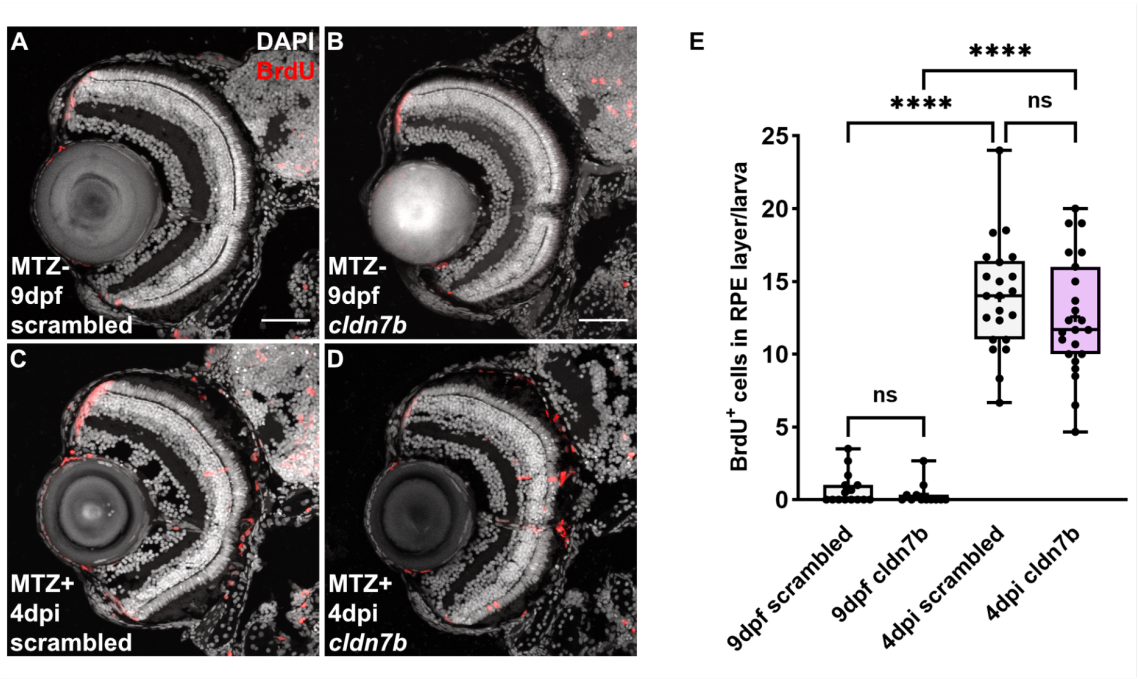
B
DAPI
eGFP
ZPR2
B'
DAPI
B''
eGFP
B'''
ZPR2
C
cldn7b
MTZ-
9dpf
D
DAPI
eGFP
ZPR2
D'
DAPI
D''
eGFP
D'''
ZPR2

FIGURE S5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MTZ-
9dpf
scrambled
B
MTZ-
9dpf
cldn7b
C
MTZ+
4dpi
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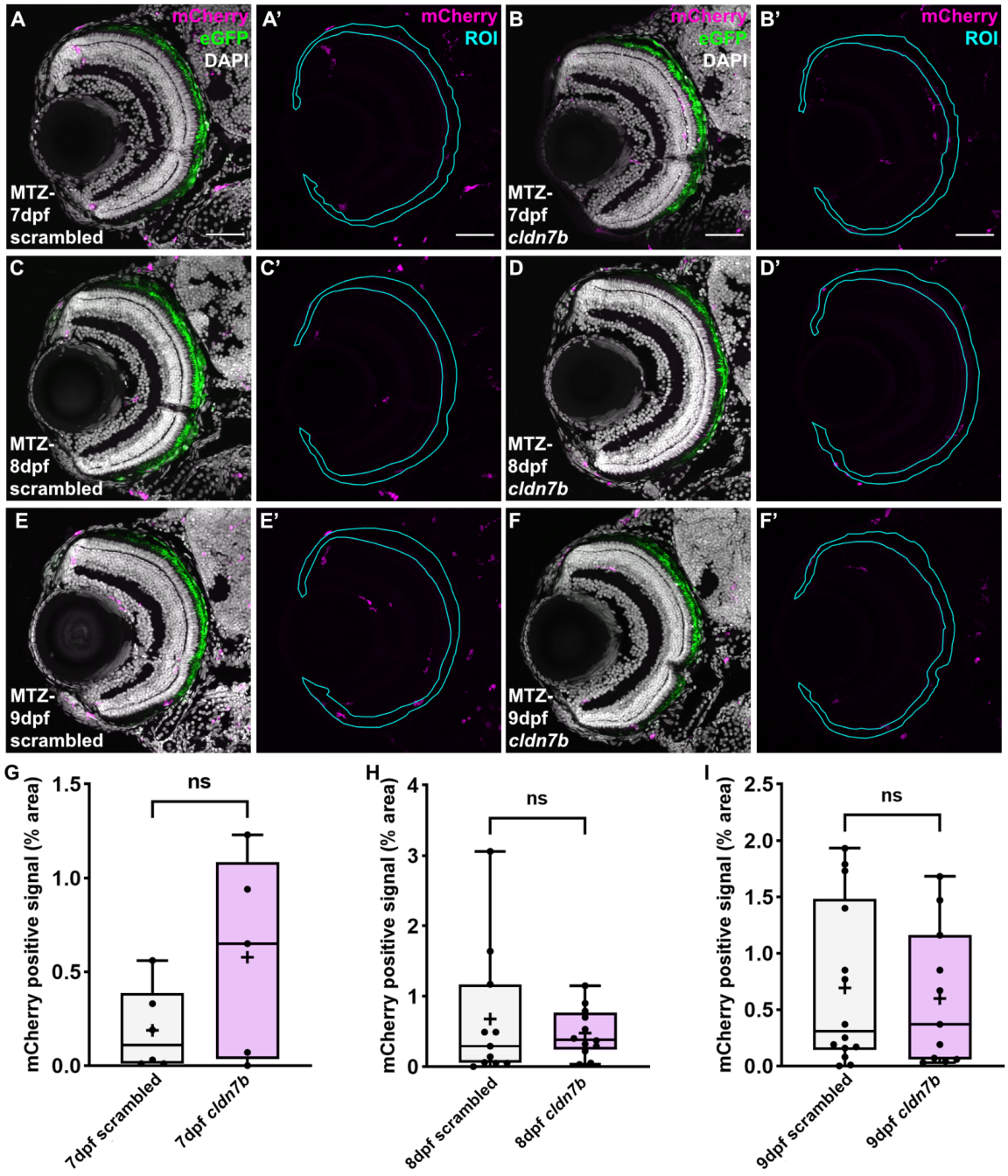
D
MTZ+
4dpi
cldn7b
E
****
****
ns
ns
25
20
15
10
5
0
BrdU+ cells in RPE layer/larva
9dpf scrambled
9dpf cldn7b
4dpi scrambled
4dpi cldn7b

FIGURE S6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B
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eGFP
DAPI
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7dpf
cldn7b
B'
mCherry
ROI
C
MTZ-
8dpf
scrambled
C'
D
MTZ-
8dpf
cldn7b
D'
E
MTZ-
9dpf
scrambled
E'
F
MTZ-
9dpf
cldn7b
F'
G
mCherry positive signal (% area)
ns
7dpf scrambled
7dpf cldn7b
H
mCherry positive signal (% area)
ns
8dpf scrambled
8dpf cldn7b
I
mCherry positive signal (% area)
ns
9dpf scrambled
9dpf cldn7b

FIGURE S7

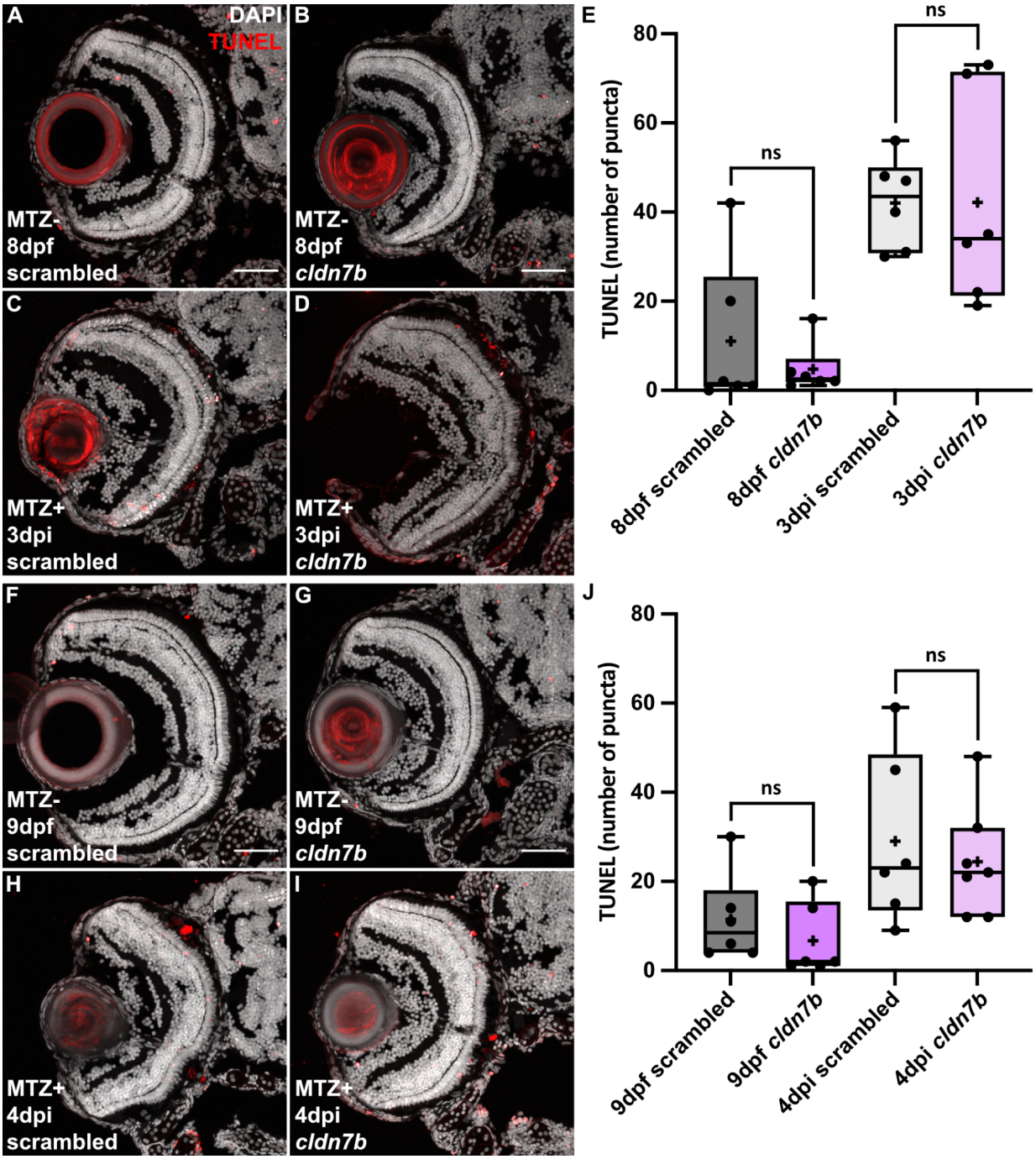
A
DAPI
TUNEL
MTZ-
8dpf
scrambled
B
MTZ-
8dpf
cldn7b
C
MTZ+
3dpi
scrambled
D
MTZ+
3dpi
cldn7b
E
TUNEL (number of puncta)
80
60
40
20
0
ns
ns
8dpf scrambled
8dpf cldn7b
3dpi scrambled
3dpi cldn7b
F
MTZ-
9dpf
scrambled
G
MTZ-
9dpf
cldn7b
H
MTZ+
4dpi
scrambled
I
MTZ+
4dpi
cldn7b
J
TUNEL (number of puncta)
80
60
40
20
0
ns
ns
9dpf scrambled
9dpf cldn7b
4dpi scrambled
4dpi cldn7b

FIGURE S8

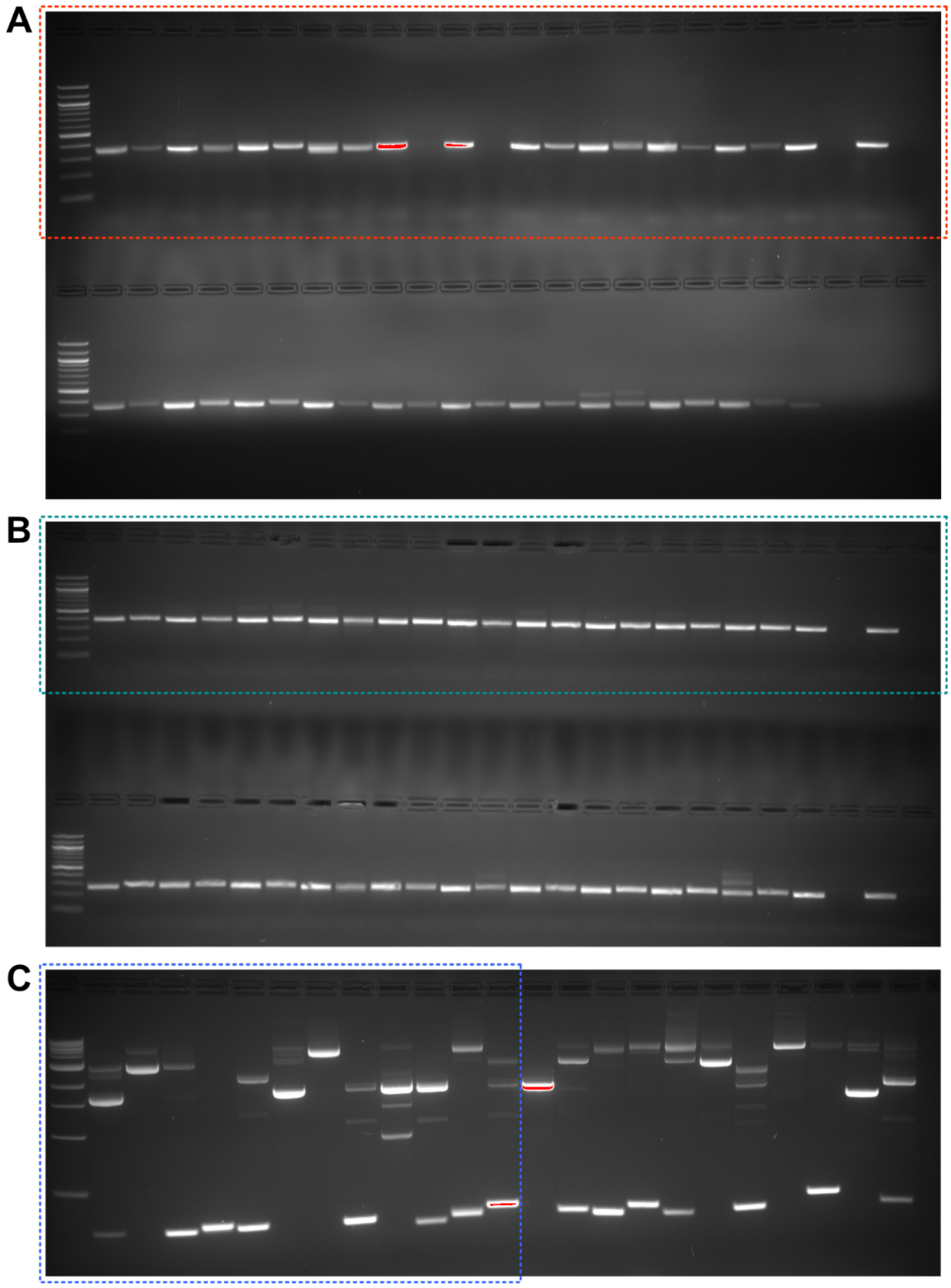


FIGURE S9

| Gene Names | crRNA loci | custom crRNA design/names given by IDT predesigned crRNA database | crRNA Sequences | PAMs | Forward(F) Primers for headloop PCR | Reverse(R) Primers for headloop PCR | Amplicon siz | Tagged Primers (F/R) for headloop PCR | | Mutagenic Rates(= H+ /S+ number of embryos) | F Primers for genotyping | R Primers for genotyping | Amplicon siz | Unavailibilit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | exon2 | | GGTTAGATACCACAAGATCC | TGG | GTGTGTTTGTATGTGTATCTGCAGGG | GGAGGAACATTCCATTGCAATTAGCC | 288 | F | **GGATCTTGTGGTATCTAACC**GTGTGTTTGTATGTGTATCTGCAGGG | 7/10 | | | | |
| *adamts17* | exon3 | custom | GGAGAATCCATCACTGCACC | TGG | GCTCTCAGCATGGCAACTGGTG | CGGCTGGCCATACACACAGACC | 491 | F | **GGTGCAGTGATGGATTCTCC**GCTCTCAGCATGGCAACTGGTG | 10/10 | N/A | | | 29.2% |
| | exon7 | | GGCAAATCACCTGACGATAC | TGG | GCAAGCAGTAGTGCATGCCT | CACTGCAGATCTTGAGCTGGC | 363 | R | **GTATCGTCAGGTTGATTTGCC**CACTGCAGATCTTGAGCTGGC | 7/10 | | | | |
| | exon2 | AA | GAAATCAGTTGCCTATAAC | CGG | GCTGCCATTGCTGTCATTATGTC | CCAGTGAATGCATTGTTCACCCTC | 353 | R | **GTTATAGGCAACGTGATTTCG**CTGCCATTGCTGTCATTATGTC | 10/10 | | | | |
| *ccl34a.4* | exon3 | AB | GTTATAGGCAACGTGATTTC | TGG | CTCCATGCGTCAAAGCAGTCATG | CAGCGGTGTAGAAACAGTGCTG | 322 | F | **GCTCTGGTTCCAGTGAATGC**CTCCATGCGTCAAAGCAGTCATG | 10/10 | GCTGCCATTGCTGTCATTATGTC | CAAGCTGACTTATTTCAGCAGAACCC | 696 | 18.7% |
| | exon4 | custom | GCACTCTGTCTACACTTCTG | AGG | GGGAGAAGATCCAGGAGCTCATAATC | CAAGCTGACTTATTTCAGCAGAACCC | 327 | F | **CAGAAGTGTAGACAGAGTGC**GGGAGAAGATCCAGGAGCTCATAATC | 10/10 | | | | |
| | exon2 | AC | AAGGACTGCGCTGCCACCTA | GGG | GCTGAAATGTTCTTGTCCTTCCACTC | GTCCATTTTCTTTGCACTTTTCCCATG | 499 | F | **TAGGTGGCAGCGCAGTCCTT**GCTGAAATGTTCTTGTCCTTCCACTC | 8/10 | | | | |
| *ccn1l1* | exon4 | AB | GCTTGACAGCCTATTCCAC | AGG | GCACACTGTGAAAAAGAGTCATG | GCTCTTACCTTCATTGATGGTGCAG | 314 | R | **GTGGAATAGGCGTGTCAAGC**GCTCTTACCTTCATTGATGGTGCAG | 7/10 | GCTGAAATGTTCTTGTCCTTCCACTC | GAAGAGTTCAGCTGGTGTGTTCAG | 4393 | 36.9% |
| | exon5 | custom | TGTGTGTCCATCTGCAACT | CGG | CCACAGAAACCTGTGCAGATCAG | GAAGAGTTCAGCTGGTGTGTTCAG | 246 | F | **AGTTGCAGATGGGACACACAC**CACAGAAACCTGTGCAGA | 9/10 | | | | |
| | exon3 | AA | GTTGATGACACGGAAGGGTC | TGG | CTGGGATTTCAAGCCATTTTGCG | CAGTCTTCACCTTGTGGTGCAG | 210 | R | **GACCCTTCCGTGTCATCAAC**AGTCTTCACCTTGTGGTGCAG | 8/10 | | | | |
| *cldec* | exon4 | AD | TGGAACTGGAATAGATACGC | AGG | GGCTCATTATTTTGCAATGTCGCTG | CTCCCAATGCGTCTGAGAATGTTTAC | 497 | F | **GCGTATCTATTCCAGTTCCA**GGCTCATTATTTTGCAATGTCGCTG | 10/10 | N/A | | | 27.9% |
| | exon6 | AB | GTTGCAATCAGGGTCTTTCT | TGG | CGAGTCGTTGACAATAGGAGTGTG | GTACATTCCATACAAGGTTGCCTGC | 243 | R | **AGAAAGACCCTGATTGCAAC**GTACATTCCATACAAGGTTGCCTGC | 10/10 | | | | |
| | exon2 | | ATACGAGCTAACCAAATACC | TGG | GTGTGACCGTTCATTTTTGGCTG | CGTCATCCTCACACGCTGATAAC | 287 | F | **GGTATTTGGTTAGCTCGTAT**GTGTGACCGTTCATTTTTGGCTG | 10/10 | | | | |
| *clcf1* | exon3 | custom | CTATCCCATCGTCTCACTCT | GGG | CCAAAGAAGAGGGAGAGAAGGAGG | CACCTTCCGCGAGAAGTCATTTG | 332 | F | **AGAGTGAGACGATGGGATAG**CCAAAGAAGAGGGAGAGAAGGAGG | 9/9 | GTGTGACCGTTCATTTTTGGCTG | CACCTTCCGCGAGAAGTCATTTG | 2857 | 24.9% |
| | exon3 | | ACACGTGTGGACTTATGGCG | CGG | CAATAAGGATGCACAGTGGATGGC | CCAGATATTGAGCCAAGCAACCC | 416 | F | **CGCCATAAGTCCACACGTGT**CAATAAGGATGCACAGTGGATGGC | 10/10 | | | | |
| | exon1 | AB | CGCACGACATCCAAAGGCC | TGG | CGCACGACATCCAAAGGCC | GTGAATAGTACGGCATGCGTG | 397 | R | **GGGCCTTGGATGTCGTGGG**GTGAATAGTACGGCATGCGTG | 10/10 | | | | |
| *cldn7b* | exon3 | AG | TTGACTTGTAAACCAGCCAC | AGG | CTCCTGTTGTGGCTGAAAAATGTC | GTTCATCCCAGGCCTGATGGTCATTG | 432 | R | **GTGGCTGGTTTACAAGTCAA**GTTCATCCCAGGCCTGATGGTCATTG | 8/8 | CGCACGACATCCAAAGGCCC | CCAGGTCGTGAACAAATGGCTC | 4251 | 17.0% |
| | exon4 | custom | ATCGAGAAATGCACCAGCCC | AGG | CAGCTAGCAACAACACTGTGAAGTG | CCAGGTCGTGAACAAATGGCTC | 354 | R | **GGGCTGGTGCATTTCTGAT**CCAGGTCGTGAACAAATGGCTC | 10/10 | | | | |
| | exon5 | AB | GTACATGGGACGGTTCTCGT | AGG | CGCCAAGATCTTGACTTTGCTG | CACCGTGCTGAACTGGAATC | 324 | R | **ACGAGAACCGTCCCATGTACC**ACCGTGCTGAACTGGAATC | 8/8 | | | | |
| *cpa4* | exon6 | AA | CACGGGTGGTGAGAACCGAC | CGG | GGACACCCTTGTTGCAAGTCAC | GAGGGATCGACGCCATAGTCTAAAG | 374 | F | **GTCGGTTCTCACCACCCGTG**GGACACCCTTGTTGCAAGTCAC | 8/9 | CGCCAAGATCTTCGACTTTGCTG | CAATCTAAAGACAGCCGTGATTGTTGC | 658 | 23.0% |
| | exon7 | AC | CCCTCCGTGACCTCTCTTCT | GGG | CAGGCTTCTGCTGTTTGGATCG | CAATCTAAAGACAGCCGTGATTGTTGC | 275 | F | **AGAAGAGAGGTCACGGAGGG**CAGGCTTCTGCTGTTTGGATCG | 9/10 | | | | |
| | exon1 | AB | AGTGTGAACAGACTGAGGAGC | AGG | CAAGGAGGACAGTTCAGTGCAC | CTCGGATGAATGTTGCAGCTGTC | 259 | R | **GCTCTCAGTCTGTTCACACT**CTCGGATGAATGTTGCAGCTGTC | 10/10 | | | | |
| *cxcl18a.1* | exon2 | AA | ATTTGAACCTTGTTGCAAAG | AGG | CTGCCAGATACTGGTCATGGAG | GACATGAGGGTGACGGATGATG | 593 | R | **CTTTGCAACAGGTTCAAAT**GACATGAGGGTGACGGATGATG | 10/10 | CAAGGAGGACAGTTCAGTGCAC | CTGCAGAGTATCTGCTGATTTCTGC | 1311 | 35.0% |
| | exon3 | AC | ACACTTCTTTATTTGACAAC | TGG | GTATCATTGCTCGCAAAGTAGTGCG | CTGCAGAGTATCTGCTGATTTCTGC | 267 | R | **GTTGTCAAATAAAGAAGTGT**CTGCAGAGTATCTGCTGATTTCTGC | 10/10 | | | | |
| | exon2 | | TTATGTGTTTTCCAATGCGT | CGG | CTGGCATTTCTGACCATCATTGAAGG | GTTGTCATCAAGGTGGCACTGTG | 400 | R | **AGCGATTGGAAAACACATAA**GTTGTCATCAAGGTGGCACTGTG | 10/10 | | | | |
| *cxcl8a* | exon3 | custom | TCTGGACCCCTCTGCTCCAT | GGG | GAAACAGAAAGCCGACGCATTGG | CGGGCATTCATGGTTTCTGCTG | 414 | F | **ATGGAGCAGAGGGGTCCAGA**GAAACAGAAAGCCGACGCATTGG | 10/10 | CTGGCATTTCTGACCATCATTGAAGG | CGGGCATTCATGGTTTTCTGCTG | 598 | 16.2% |
| | exon3 | | CTGTCCAGTTGTCATCAAGG | TGG | GAAACAGAAAGCCGACGCATTGG | CGGGCATTCATGGTTTTCTGCTG | 414 | R | **CCTTGATGACAACTGGACAG**CGGGCATTCATGGTTTTCTGCTG | 10/10 | | | | |
| | exon2 | AF | TTTGTCTGTCGTGCCGCAAG | CGG | GGTTAACCAGGTGGTTATGATGGCTG | GGAGAAATGAACCTTCATGCATGCTG | 283 | F | **CTTGCGGCACGACAGACAAA**GGTTAACCAGGTGGTTATGATGGCTG | 10/10 | | | | |
| *dkk1a* | exon3 | AB | CTGGTGTGGCTCTAACACGT | GGG | CCGATGCATCAACGGTACAAACG | GGAGATACGCAAGGAGAAACATGACAG | 361 | F | **ACGTGTTACAGGCACACAGG**CCGATGCATCAACGGTACAAACG | 10/10 | GGTTAACCAGGTGGTTATGATGGCTGGT | GTTTTTCTCTCTGGCCTCTGCAGG | 2507 | 21.9% |
| | exon4 | custom | CTGGTCACGGATCTGTAAGC | CGG | CCAGCACGTCTCTGAATGCAGATC | GTTTTTCTCTCTGGCCTCTGCAGG | 260 | F | **GCTTACAGATCCGTGACCAG**CCAGCACGTCTCTGAATGCAGATC | 10/10 | | | | |
| | exon2 | AD | ATTATCTAACCAGCTACTAC | AGG | GTTGGCAAATGTAGATTCCAGACAAG | GCAGGTTTGGAACAAATGAAAGGAG | 436 | R | **GTAGTAGCTGGTTAGATAAT**GCAGGTTTGGAACAAATGAAAGGAG | 10/10 | | | | |
| *edn2* | exon3 | AA | ACTGGAGCAGGTAGATGATCGG | CGG | CCTTTGTACAACAGTAAGATCACCCCG | GCCAATAGAGACAGTTTCTCTTTCTCTCCC | 324 | R | **CCGATCGTACCTTGCTCCAGT**GCCAATAGAGACAGTTTCTCTTTCTCTCCC | 8/8 | GTTGGCAAATGTAGATTCCAGACAAGG | GATTTGATCTTCAGGGTGGAGCG | 870 | 33.0% |
| | exon3 | AB | AAGATCACCCCGCTTGGTCT | TGG | CTCCTTTCATTTGTTCCAAACCTGC | GATTTGATCTTCAGGGTGGAGCG | 459 | F | **AGACCAAGCGGGGGTGATCTT**CTCCTTTCATTTGTTCCAAACCTGC | 10/10 | | | | |
| | exon3 | AA | TCCCACCCGATCTTGTGTCC | TGG | CACGAGCACAGACATGCCTAAC | CACTGTTGACGCACCAGCATAC | 302 | R | **GGACACAAGATCGGGGTGGG**ACACTGTTGACGCACCAGCATAC | 10/10 | | | | |
| *epcam* | exon4 | AC | CTCAGTTTGGTGGCATCAAT | GGG | GAACAGAAAGCGCGAGCCTGCG | CCAAACGTAACGCTGCTGTAG | 365 | R | **ATTGAGCAGAGGGGTCCAGA**GAACAGAAAGCGCGAGCGATTGG | 10/10 | CACGAGCACAGACATGCCTAAC | CAGCAATGATACCACCAGTCAGC | 1152 | 16.9% |
| | exon7 | custom | CCCGGAACATCAACCTCAAA | TGG | CGACCGTACTACAGATCTGTCCC | CAGCAATGATACCACCAGTCAGC | 293 | R | **TTTGAGGTTGATGTTCCGGG**CAGCAATGATACCACCAGTCAGC | 10/10 | | | | |
| *epha2a* | exon2 | AD | TAACCAACCTAATTCGCTCC | CGG | CGAAAGAACGTAAGTACACACCGTG | CAACGACACTGAACTGAAAGGACAC | 640 | R | **GGAGCGAATTAGGTTGGTTA**CAACGACACTGAACTGAAAGGACAC | 9/9 | | | | |
| | exon3 | AA | CCGCAAGAGCCAGTTTCGAA | AGG | CTGTAGAGCTGAGCTTCATCGTCC | GACAGGTGGTGTAGAACACCCTC | 321 | F | **TTCGAAACTGGCTCTTCGGG**CTGTAGAGCTGAGCTTCATCGTCC | 10/10 | N/A | | | 20.3% |
| | exon4 | custom | AACACCCAAGCTTGCGGACG | CGG | GGTCTGAAACGAGTAAAGGGTTCAGC | GGAAGAACTAGAGACAAGAGCAGTG | 396 | F | **CGTCCCGAACGTTGGGTGTTG**GGTCTGAAACGAGTAAAGGGTTCAGC | 7/7 | | | | |
| | exon1 | | GAGAGTCGTATTATGATTCC | CGG | GGCGTAAGGACACTGAAGAAAGC | CGTAGAGTCCTCAGTGTCATTCTGC | 506 | F | **GGAATCATAATACGACTCTC**GGCGTAAGGACACTGAAGAAAGC | 10/10 | | | | |
| *fosl1b* | exon2 | custom | CCACTGCTCCATTACAGGAC | GGG | GCAGAATGACACTGAGGACTCACG | CTACAGCACTGACTCACTCCTCC | 410 | R | **GTCCTGTAATGGAGCAGTGGC**TACAGCACTGACTCACTCCTCC | 10/10 | GGCGTAAGGACACTGAAGAAAAGC | GATCTGAACCAGCAGATTCAGCAC | 3635 | 35.6% |
| | exon4 | | AAAGAGATTGGCCACACTTGAA | AGG | GAAAGGAACAGCAGTAGCAGCTG | GATCTGAACCAGCAGATTCAGCAC | 330 | F | **TTCAAGTGTGGCAATCTCTT**GAAAGGAACAGCAGTAGCAGCTG | 10/10 | | | | |
| | exon2 | | TTGCTGGAAGAGACTTGAAC | CGG | CTGGTGGATGCCAGCAGTTTC | GGGTCTGATTGCAGCAAGACC | 367 | R | **GTTCAAGTCTCTTCCAGCAA**GGGTCTGATTGCAGCAAGACC | 9/9 | | | | |
| *il11a* | exon3 | custom | GACATGGTCTCCCACATCGG | GGG | CTCTCATGCGCAGGTCAAGCCTAC | CTTGCCTGATCACCCTAACCATCC | 501 | F | **CCGATGTGGGAGACCATGTC**CTCTCATGCGCAGGTCAAGCCTAC | 10/10 | CTGGTGGATGCCAGCAGTTTC | CCGGACTGACGATACAACTCATC | 2833 | 32.0% |
| | exon4 | | CGTCTGCCCCAACCTGAG | CGG | GACATAGTTGAAATGCCCTTGC | CCGGACTGACGATACAACTCATC | 462 | R | **CTCGAGTGTTGGGCAGGAGC**CCGGACTGACGATACAACTCATC | 6/8 | | | | |
| | exon1 | | CAAGCCATCAGGATGATGAG | GGG | GTTTTTGTGCTAACAGTGTCGCC | TGGGAAAACTAGCCCTCAAAATGAC | 284 | R | **CTCATCATCCTGATGGCTTG**TGGGAAAACTAGCCCTCAAAATGAC | 9/10 | | | | |
| *il11b* | exon3 | custom | CCAATTCGTCACTATTCCGT | TGG | CCTACCAATATGCCATAGTGGGTC | GTAGGTGACAGACAGCACAGTTG | 486 | R | **ACGGAATAGTGACGAATTGG**GTAGGTGACAGACAGCACAGTTG | 6/8 | GTTTTTGTGCTAACAGTGTCGCC | GCACCATAAAGATTGGCTGACGG | 2375 | 30.1% |
| | exon4 | | CTTCAGGGAACTGCTC | TGG | CTTACACTCGGACAGACAGCA | GCACATAAAGATTGGCTGACGG | 212 | R | **GAGCACTTGGATCCGTGAG**GCACATAAAGATTGGCTGACGG | 6/8 | | | | |
| | exon1 | AA | GACCCACGGCTCCCGAAGAC | AGG | GCTTGTTAATATCATCCCTGGTGGCC | GAAGTACATCATGCTAAACCAAGTGCCAAC | 397 | F | **GTCTTCGGGAGCCGTGGGT**CGCTTGTTAATATCATCCCTGGTGGCC | 7/10 | | | | |
| *lepb* | exon2 | AB | GGAGGAACTGGCCGTCTCAC | AGG | GTCTCCAGAGATTGATTTCGGCCC | GTTGAGGCAGAGCTTCTCCAGG | 301 | F | **GTGAGACGGCCAGTTCCTC**CGTCTCCAGAGATTGATTTCGGCCC | 10/10 | GCTGTTAATATCATCCCTGGTGGCC | GTTGAGGCAGAGCTTCTCCAGG | 1877 | 36.7% |
| | exon2 | AD | GACATCGACAACCCCATTGA | TGG | GGGAAAAAGGCTCTTGGTTCTTGGC | CCTCCAGTGTCCTCAGGAGAGTCTC | 383 | F | **TCAATGGGGTTGTCGATGTC**GGGAAAAAGGCTCTTGGTTCTTGGC | 6/6 | | | | |
| | exon2 | | GTGCACACCTGTAAGCAAGTGTC | CGG | GTGCACACCTGTAAGCAAGTGTC | CGATGTGGTTGATCCAGATTGGC | 269 | R | **TGCCAACCTCGAGTGTGGTC**CGATGTGGTTGATCCAGATTGGC | 10/10 | | | | |
| *lpib* | exon4 | custom | GTTGGATCGAGACGTTCCTC | AGG | GGCACAATGCCTTCCTCAGTTAG | GTGGGTGTCTCCTGTATTGTTTTTCC | 286 | R | **GAGGAACGTCTCGATCCAAC**GTGGGTGTCTCCTGTATTGTTTTTCC | 10/10 | GTGCACACCTGTAAGCAAGTGTC | GTATCTCCACCCTGACCTCTCC | 5080 | 40.5% |
| | exon5 | | CGGCTGATCTAATCCTCCAT | TGG | CCTATGCTCTGTTTCCTGTGGC | CCTATGCTCTGTTTCCTGTGGC | 162 | R | **ATGGAGGATTAGATCAGCCG**GTATCTCCACCCTGACCTCTCC | 10/10 | | | | |
| | exon2 | AA | TCGAGTTGCATTCTGAGGT | TGG | CGTTGCGAACTCTGAACCTCTGAG | GTTGAAAACCACTTGAGCCGAACG | 358 | R | **ACGCGAAGGTGGCAACTCGAG**GTTGAAAACCACTTGAGCCGAACG | 10/10 | | | | |
| *met* | exon2 | AB | TAAGACTGGACCGGTCCATG | AGG | CTCCAAACTCGACCTCTCAGTGAC | CCTCTAACACATGACGACTGCAGAC | 341 | F | **CATGGACCGGTCCAGTCTTA**CTCCAAACTCGACCTCTCAGTGAC | 10/10 | N/A | | | 20.7% |
| | exon11 | custom | CGTGGAAGATCCCAAATTTG | AGG | GACCTGCATGATGATGAGGATAAG | TGACACTATGCGTGACAATGCATAC | 317 | F | **CAAATTTGGGATCTTCCACG**GACCTGCATGATGATGAGGATAAG | 9/10 | | | | |
| | exon1 | AB | TCCTCTGTCACCTGTAATGA | CGG | GAGTTTCCTCTGGACTTCACAGC | GGAGTGAAGTTCGAACCTTCAGC | 264 | F | **TCATTACAGGTGACAGAGGA**GAGTTTTCCTCTGGACTTCACAGC | 7/7 | | | | |
| *notum1b* | exon2 | AA | GATTCTGTCTCCACACCAG | AGG | GACCAACCCAGTCAGTTAGTAGGTG | CCCATGACAGCATAGTCATCTGAAC | 563 | F | **CTGGTTGTGGAGACAGAATC**GACCAACCCAGTCAGTTAGTAGGTG | 8/10 | GAGTTTTCCTCTGGACTTCACAGC | CTCCTTGATGACCTCCTGAATGATC | 4259 | 32.3% |
| | exon3 | custom | CAAACGTCACTGGACGAGTA | CGG | CTGAGACAACATATGCTGGATATGGG | CTCCTTGATGACCTCCTGAATGATC | 343 | R | **TACTGCTCCAGTGACGTTTG**CTCCTTGATGACCTCCTGAATGATC | 8/10 | | | | |
| | exon2 | | CCATGGCAAGTGCTTCACGC | TGG | GCTCTGCAGTAAAACCTCTCTC | CATGTAGAACGACAGTGTGACGG | 276 | F | **GCGTGAAGCACTTGCCATGG**GCTCTGCAGTAAAACCTCTCCTC | 10/10 | | | | |
| *nrg1* | exon5 | custom | TCCACCGGAAACCTGCAGC | TGG | GCAATGTCCTCCGTCTAAGAG | GTAAGCGTCAGCTAGCTAAGCG | 382 | F | **GCTGCAGGTTTTCCGGTGG**GCAATGTCCTCCGTCTAAGAG | 10/10 | N/A | | | 30.3% |
| | exon6 | | TCTGTCATATGAGCTGGGAC | AGG | GCCCCATTTTGGTGTCTTTGTC | GGCTAGATGAGTGAGTGATGGC | 277 | R | **GTCCCAGCTCATATGACAGG**GGCTAGATGAGTGAGTGATGGC | 10/10 | | | | |
| | exon2 | AB | ACATGATGGAGTCTTCCACC | AGG | CGTCAGTGTTTCAACATCACAAGAGG | GAATGTGTTTCTGCTGACTGTACAGC | 413 | F | **GGTGGAGGACTCCATCATGT**CGTCAGTGTTTCAACATCACAAGAGG | 9/10 | | | | |
| *ogfrl1* | exon3 | AA | CGATATTCATTCGGCCTGCG | AGG | GCTGTACAGTCAGCAGAAACACATTC | GCAGCTTTGTACTGTGAAAGAGCTTCC | 337 | R | **CGTCAGCCGAATGAATATCGG**CAGCTTTGTACTGTGAAAGAGCTTCC | 10/10 | N/A | | | 13.3% |
| | exon7 | AD | ACTCGCATTCTGAAGAGCT | TGG | GCGTCCTTTCATGTTTGGCATTG | GTGTGAAGACGAAGTATTCCAGTGC | 261 | F | **AGACTCTTCAGAATGCGAGT**GCGTCCTTTCATGTTTGGCATTG | 10/10 | | | | |
| | exon4 | AA | CCGACGTACAATGCTGATTA | TGG | CAGCATCTCTTACCTGAGGGATGG | CATGAGACTGCTCCTCTGTGGATC | 373 | F | **TAATCAGCATTGTACGTCGGC**AGCATCTCTTACCTGAGGGATGG | 7/10 | | | | |
| *ptgs2a* | exon2 | AB | CCTTGGATAAACACACGCCC | TGG | CTCGACGAGAGTAACGCATCTCTG | GGCGTGTGAGAAGCTCAGCTGTAAG | 452 | R | **GGGCGTGTGTTTATCCAAGGGC**GTGTGAGAAGCTCAGCTGTAAG | 10/10 | CTCGACCAGAGTAACGCATCTCTG | CGTCAACGTATAAATCGCACCCTG | 1237 | 28.4% |
| | exon5 | AF | GCATATTTATGGAGAGACGC | TGG | CTCTCACAGTTCTTCAAGTCGGA | CGTCAACGTATAAATCGCACCCTG | 309 | F | **GCGTCTCTCCATAAATATGC**CTCTCACAGTTCTTCAAGTCGGA | 9/9 | | | | |
| | exon4 | AA | ATTGGCGTTGGGCAATCACG | CGG | GCCCATCTGATCGGATAGTCCTCC | GCTTATCCACACACCCAAGGCTC | 256 | R | **CGTGATTGCCCAACGCCAAT**GCTTATCCACACACCCAAGGCTC | 10/10 | | | | |
| *ptgs2b* | exon2 | AB | CGAGGTGTCTGTACGGAAAT | GGG | GGTTTGCACAGGAGGTAAGCCTAC | GCCAAAAGCAGAAATGGGCAGAG | 328 | F | **ATTTCCGTACAGACACCTCGG**GGTTTGCACAGGAGGTAAGCCTAC | 10/10 | GGTTTGCACAGGAGGTAAGCCTAC | CAAATACGCACCCATGGTTCAG | 2344 | 24.4% |
| | exon5 | AD | AGATTGGTGCGTTGAGGATC | TGG | CCTTGGGTGTGTGGATAAGCAAC | CAAATACGCACCCATGGTTCAG | 420 | R | **GATCCTCAACGCACCAATC**TCAAATACGCACCCCATGGTTCAG | 10/10 | | | | |
| | exon1 | | AATACCTGATGAGGACCAGT | TGG | CTCATATTAACACATGGCCAGCAAGTC | GTCACTTCATCGATTTCTGTTTGGCAG | 417 | F | **ACTGGTCCTCATCAGGTATT**CTCATATTAACACATGGCCAGCAAGTC | 10/10 | | | | |
| *ptx3a* | exon2 | custom | TGTGAAGAGCTTATCCCATT | TGG | GTGGATAGCATGTTGACGCAATGAC | CAGATGCGCAAGATCCACCATAC | 435 | R | **AATGGGATAAGCTCTTCACA**CAGATGCGCAAGATCCACCATAC | 10/10 | N/A | | | 31.8% |
| | exon3 | | ATTGGTCCTCAATCAAGTCTGG | GGG | GCCTGACACTTCATTCAAGTCTGG | GATGCACTCTGTCCACTCAACAC | 307 | R | **ATGGATTGCTTAGGACCAAT**GATGCACTCTGTCCACTCAACAC | 10/10 | | | | |
| | exon1 | AC | CAGAGAGTAGCCCATCTTGG | AGG | CAGTCTGCTCCGGATCGAAATCTG | GCTACCTCCACTCCATCTTCACTG | 308 | R | **CCAAGATGGGCTACTCTCTGG**CTACCTCCACTCCATCTTCACTG | 10/10 | | | | |
| *serpine1* | exon2 | AD | GGTTGACCGGAAGATCATCT | TGG | GGTTGACCGGAAGATCATCT | CATCAGTTGGTCAGACGTCCAG | 379 | F | **AGATGATCTTCCGGTCAACC**GCTACTCTAAAACTGCTCGCCTCC | 9/10 | CAGTCTGCTCCGGATCGAAATCTG | GCCATCCTTAGACACGAACTCACC | 903 | 23.5% |
| | exon3 | AB | GAATGCCCTGCACTTCCACG | GGG | CTGGACGTCTGACCACTGATG | GCCATCCTTAGACACGAACTCACC | 463 | F | **CGTGGAAGTGCAGGGCATTC**CTGGACGTCTGACCACACTGATG | 10/10 | | | | |
| | exon2 | AA | ATTCCCTGTGGATTCTTCGT | GGG | CTGCTGCCAACAAACTGATCATTC | CTGCTGCCAACAAACTGATCATTC | 305 | F | **ACGAAGAATCCACAGGGAAT**CTGCTGCCAACAAACTGATCATTC | 10/10 | | | | |
| *zgc:153911* | exon3 | AC | GACGGATGGAGTCAATCTGC | TGG | ACCTGCTCCATCAGCACAAACTC | CAGCTGGACACGATGTAAAGTCC | 350 | F | **GCAGATTGACTCCATCCGTC**ACCTGCTCCATCAGCACAAACTC | 8/10 | CTGCTGCCAACAAACTGATCATTC | CTGTGACAAACACACCAGATATCTGAC | 1368 | 33.3% |
| | exon4 | custom | CAGACTTTTAGTTTCACTGG | AGG | CAGACTTTTAGTTTCACTGG | CTGTGACAAACACACCAGATATCTGAC | 358 | R | **CCAGTGAAACTAAAAGTCTG**CTGTGACAAACACACCAGATATCTGAC | 9/10 | | | | |
| | N/A | #1 | CGTTAATCGCGTATAATACG | | | | | | | | | | | |
| **scrambled** | N/A | #2 | CATATTGCGCGTATAGTCGC | NA | | | | | | | | | | 6.0% |
| | N/A | #3 | GGCGCGTATAGTCGCGCGTA | | | | | | | | | | | |

**Table S1. crRNAs and primers used in this study.**

**Table S2. RPE regions (>20 continuous angular degrees) with significant differences.**

| Gene name | Region(s) with significant difference (>20 angular degrees) compared to scrambled controls | Total region size (angular degrees) |
|---|---|---|
| *nrg1* | 40-63(+); 81-143(+) | 85 |
| *fosl1b* | 34-67(+); 105-150(+) | 78 |
| *ogflr1* | 30-62(+); 103-134(+) | 63 |
| *ccn1l1* | 100-150(+) | 50 |
| *lipib* | 104-150(+) | 46 |
| *cldn7b* | 101-133(+) | 32 |
| *cidec* | 101-131(+) | 30 |
| *il11a* | 104-126(+) | 22 |
| *zgc:153911* | 30-111(-) | 81 |
| *cpa4* | 30-106(-) | 76 |
| *adamtsl7* | 55-82(-); 104-147(-) | 70 |
| *dkk1a* | 30-76(-) | 46 |
| *epha2a* | 82-102(-); 120-143(-) | 43 |
| *lepb* | 37-71(-) | 34 |
| *serpine1* | 61-91(-) | 30 |

(+)/(-) indicate higher/lighter (+) or lower/darker (-) pixel intensity in the F0 knockouts than the scrambled controls.

## Table S3. Statistics

| Figures | Numbers of independent experiments (N) | Compared groups | Statistical tests | Biological replicates (n) | *p*-values |
|---|---|---|---|---|---|
| Fig. 3G | N=1, N=1 | 2dpi scrambled vs. 2dpi *cldn7b* | unpaired Student's t-test with Welch's correction | n=9, n=11 | $p$=0.2367 |
| | N=3, N=3 | 3dpi scrambled vs. 3dpi *cldn7b* | unpaired Student's t-test with Welch's correction | n=19, n=23 | $p$=0.0017 |
| | N=3, N=3 | 4dpi scrambled vs. 4dpi *cldn7b* | unpaired Student's t-test with Welch's correction | n=14, n=16 | $p$=0.0040 |
| Fig. S6E | N=3, N=3, N=3, N=3 | 9dpf scrambled vs. 9dpf *cldn7b* vs. 4dpi scrambled vs. 4dpi scrambled | Kruskal–Wallis one-way ANOVA | n=15, n=12, n=22, n=23 | H=51.29 $p$<0.0001 |
| | | 9dpf scrambled vs. 9dpf *cldn7b* | Dunn's multiple comparison test | | $p$>0.9999 |
| | | 4dpi scrambled vs. 4dpi *cldn7b* | Dunn's multiple comparison test | | $p$>0.9999 |
| | | 9dpf scrambled vs. 4dpi scrambled | Dunn's multiple comparison test | | $p$<0.0001 |
| | | 9dpf *cldn7b* vs. 4dpi *cldn7b* | Dunn's multiple comparison test | | $p$<0.0001 |
| Fig. S7G | N=1, N=1 | 7dpf scrambled vs. 7dpf *cldn7b* | Mann Whitney test | n=6, n=5 | $p$=0.3030 |
| Fig. S7H | N=3, N=3 | 8dpf scrambled vs. 8dpf *cldn7b* | Mann Whitney test | n=11, n=12 | $p$=0.7509 |
| Fig. S7I | N=3, N=3 | 9dpf scrambled vs. 9dpf *cldn7b* | unpaired Student's t-test with Welch's correction | n=14, n=11 | $p$=0.7285 |
| Fig. S8E | N=1, N=1 | 8dpf scrambled vs. 8dpf *cldn7b* | Mann Whitney test | n=6, n=6 | $p$=0.6970 |
| | N=1, N=1 | 3dpi scrambled vs. 3dpi *cldn7b* | Mann Whitney test | n=6, n=6 | $p$=0.8182 |
| Fig. S8J | N=1, N=1 | 9dpf scrambled vs. 9dpf *cldn7b* | Mann Whitney test | n=6, n=6 | $p$=0.1861 |
| | N=1, N=1 | 4dpi scrambled vs. 4dpi *cldn7b* | Mann Whitney test | n=6, n=7 | $p$=0.8065 |