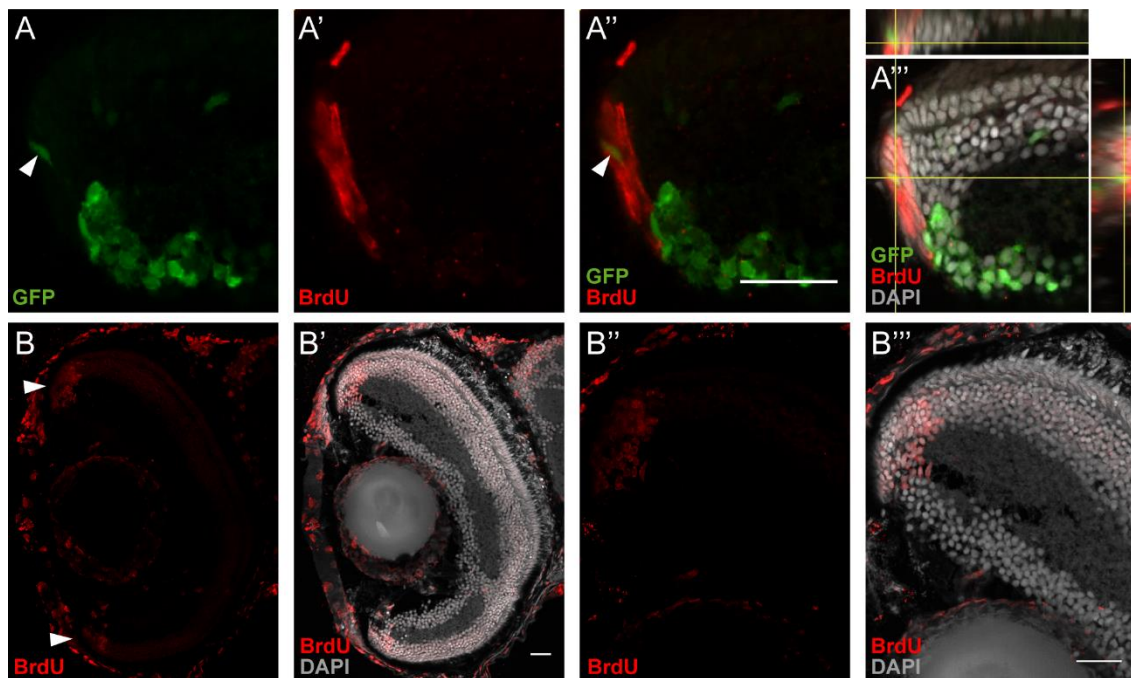**Supplemental Figures**

Figure S1. Proliferation is restricted to the CMZ where *atoh7* is expressed.

(**A-A'''**) A short pulse of BrdU (16 hours) marks proliferating cells in the CMZ. *Atoh7*-driven

EGFP (green) expression is co-localizing with BrdU (red) (arrowheads).

(**B-B'''**) A long pulse of BrdU (7 days) marks only proliferating cells in the CMZ (arrowheads).

MG cells do not incorporate BrdU in the hatchling medaka retina (n = 12 fish, data obtained
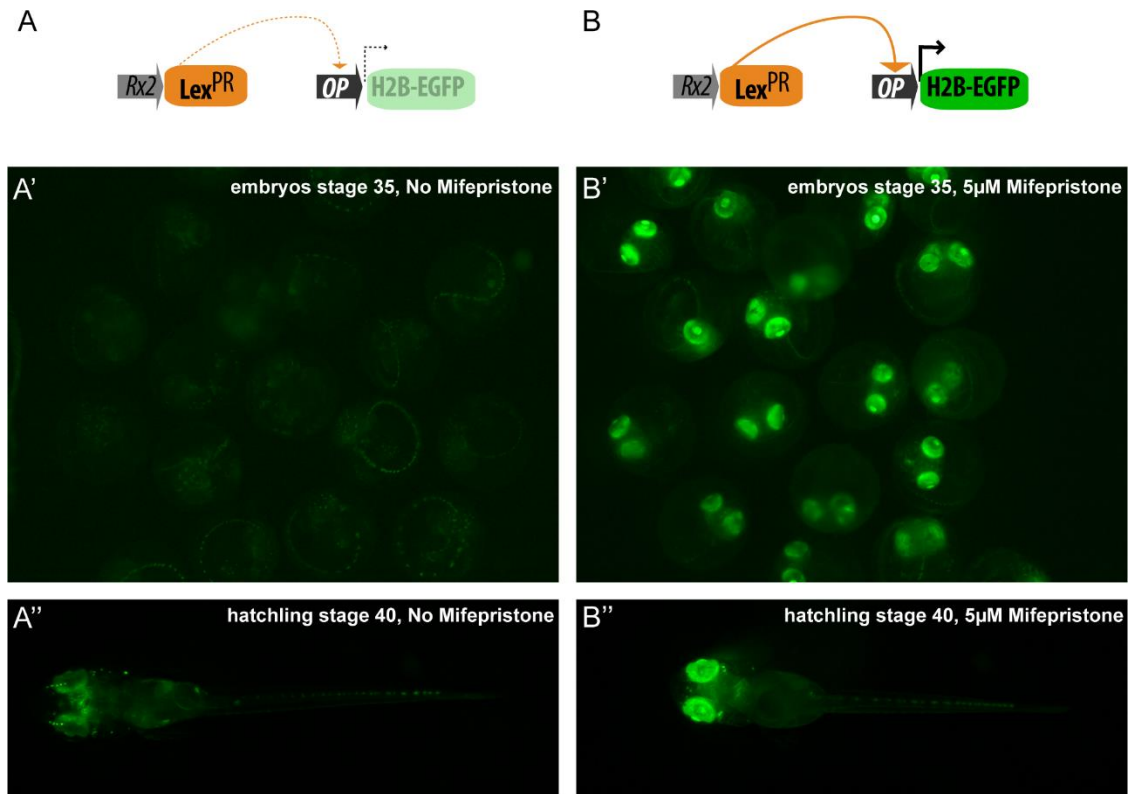
from 4 independent experiments). Scale bars are 20 μm.

Figure S2. Lex^PR inducible gene expression in medaka.

The Lex^PR system allows targeted and inducible gene expression in medaka embryos and hatchling fish (**A, B**). Transgenic *rx2*::Lex^PR *OP*::EGFP stage 35 embryos (**A'**) and hatchling (**A''**) show no EGFP expression. Upon addition of the drug Mifepristone, EFGP is detected in the *rx2* domain of embryos (**B'**) and hatchlings (**B''**).
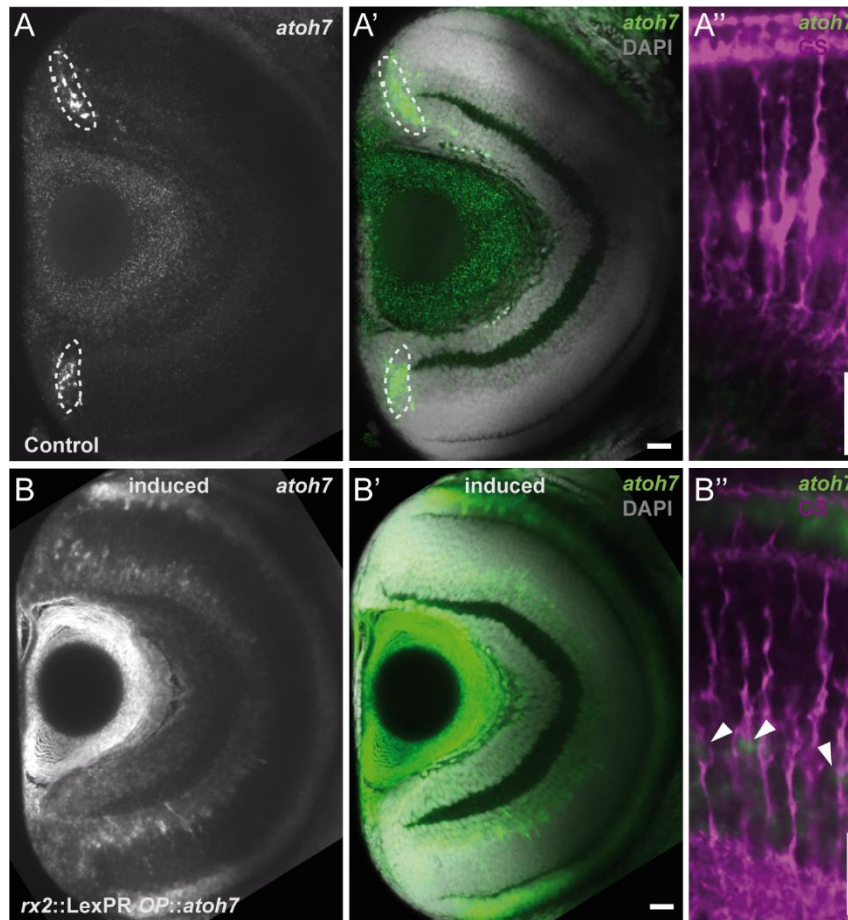
Figure S3. Targeted expression of *atoh7* expression to Müller glia cells.

In control fish, *atoh7* expression is only detectable in a small area next to the CMZ (dotted circle in **A, A'**, compare to Figure S1b). No *atoh7* expression is detected in GS positive MG cells (**A''**). Upon induction with Mifepristone, *atoh7* mRNA is detected in the ONL, in the INL and in the CMZ (**B, B'**). *atoh7* expression in the INL co-localizes with GS positive cells (arrowheads) (**B''**). The panels A-B'' show whole mount fluorescent *in situ* hybridizations for *atoh7* mRNA (green), antibody staining for GS (magenta) and staining for DAPI (gray). Scale bars are 20 μm.
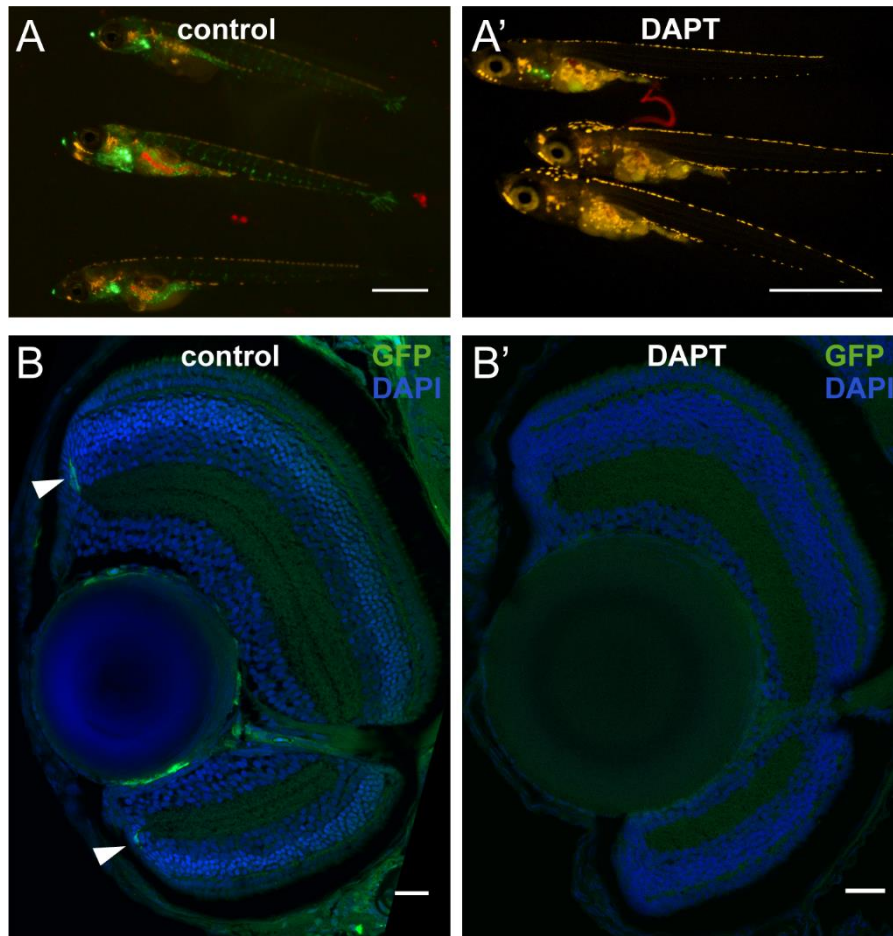
Figure S4. The *tp1-MmHbb*:d2GFP reporter line recapitulates Notch signaling in medaka.

(**A**) The *tp1-MmHbb*:d2GFP Notch transcriptional reporter is activated in various tissues such as blood vessels, brain and intestine in hatchling medaka fish. (**B**) Expression can also be observed in the peripheral retina (arrowheads). (**A', B'**) Upon treatment with 50 µM DAPT for 2 days the expression is downregulated (n=6 fish, data obtained from 2 independent experiments). Scale bars are 20 µm.

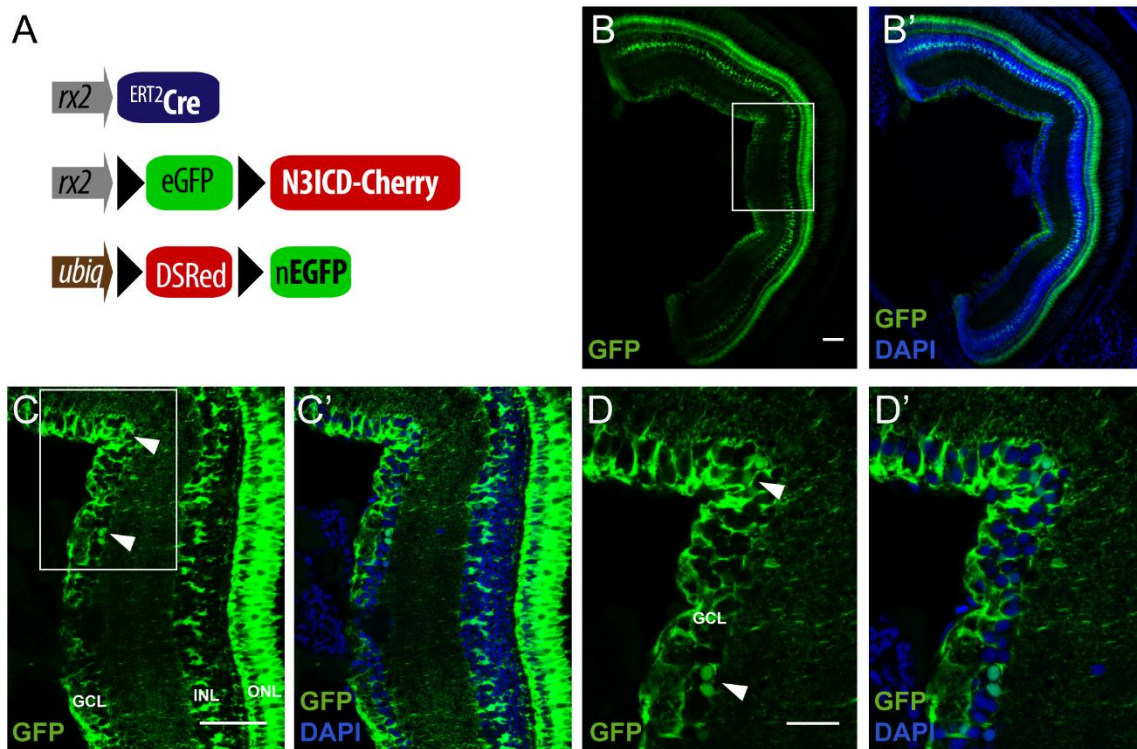**Figure S5. Differentiation of MG cells upon targeted NICD expression.**

(**A**) Constructs used for 1 month-lineage analysis of MG cells upon N3ICD expression. Upon tamoxifen induction, the *rx2*::[ERT2]Cre mediates excisions resulting in the expression of N3ICD-mCherry and nuclear GFP in the recombined cells. (**B-D'**) One month after recombination, cells expressing nuclear GFP were observed in the GCL (arrowheads) via antibody staining for GFP (green) and staining for DAPI (blue) (n=5 out of 7 fish, data obtained from 2 independent experiments). Scale bars in B and C are 50 µm, in D 20 µm. The rectangle in B indicates the area magnified in C and the rectangle in C the area magnified in D.

Supplementary Table S1. List of target genes used in the MG proliferation assay.

Target gene	MG proliferation
Ascl1a	No
Atoh7	Yes
CyclinD	No
NeuroD	No
Neurogenin	No
Pax6	No
Rheb	No
Rx2	No
Yap	No

Supplementary Table S2. Vector sequences.

Vector name	Vector sequence
cmlc2::ECFP *OP*::EGFP	AAATTGTAAACGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCAT TTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGA GATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGA CTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACC ATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCT AAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAA GGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTC ACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCC CATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCG CTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAAC GCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAGCGCGTAGGGAT AACAGGGTAATGCGCGCGTAATACGACTCACTATAGGGCGAATTGGGAGCTTAAATC AGTTGTGTTAAATAAGAGACATTCAAAATAAATGTAAATGAGCTCTCCAAATCAGCAG ACTTAACATTCTTTAAAATGATTGATTCAATAGTGATAAAAATCAGGCATAGCCAGTTG TAACTTTAGATAAATTACAGAAAATGTCAAATACAGAGAACCGATTCTTTTTTATGATA CATCCAAGCACACATTTAACACAATCCAGGCAAACCCCGAATTTCACAGTCACAAGCA CTGTTTGTACAAGAGCTTTGCCTAAGGACACACAGTCTCTATAAGTCCAGGTCGTTGG TTTCACTCTTATTTTAAACATGTGACATTTTTCCTGCCATCCTGTCTTAGGCTGCTGTTT GCTTCATTCCATGTCACATTAAATTCCTCAGTAGCACCTTTTACACACACAGCCAATCTT TTCCAGAAAATTCAATTGCTTTGAAGAGATAATGTGTGAACAAATCCATTTAGAAAAG GAAAATTAAGAATTTGTAAAATCATCTGTAAATTGTTGGCATTCTTCTGTATATGAACA TCACATCATTTACAGGTAAAGGTCTGGTCATTAATTATATGACAATTTACTGGTATTAT TTTGTGAAAGGGGCTATTTTCAATGCGTTCATCCATCCTTTTCATCCCTCAAATCTCTCA TTCACGTCCCCCTCCCCATCTGCACACTTTATCTCATTTTCCACCCTGCTGGAATCTGAG CACTTGTGCAGTTATCAGGGCTCCTGTATTTAGGAGGCTCTGGGTGTCCATGTAGGGG ACGAACAGAAACACTGCAGACCTTTATAGAAGAACAATTGATAAGAGTCCTCATACAT AAAGACTCCATTAGTAAGCCAGTGACCCAGGAGCCCAGACCAACAGCAAAGCAGACA GTGACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGG GCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCC CGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTGGGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGG TGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACATCAGCCACAAC GTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGC CACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC ATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCC TGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCG CCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCAAACCCGCT GATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTG CCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAAT TGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGA CAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGC TCTATGGCTTCTGAGGCGGAAAGAACCAGCACGTGGCGCCTAGGCCGCCGATCGTCG ACTAGTTATAATTTAAATTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATA TAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATT TATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAA AACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTAC TAGATCGGGAATTGATCCCCCCTCGACAGCTTGCATGCCGCTTGGGCTGCAGGTCGA

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