Essential functions of MLL1 and MLL2 in retinal development and cone cell maintenance

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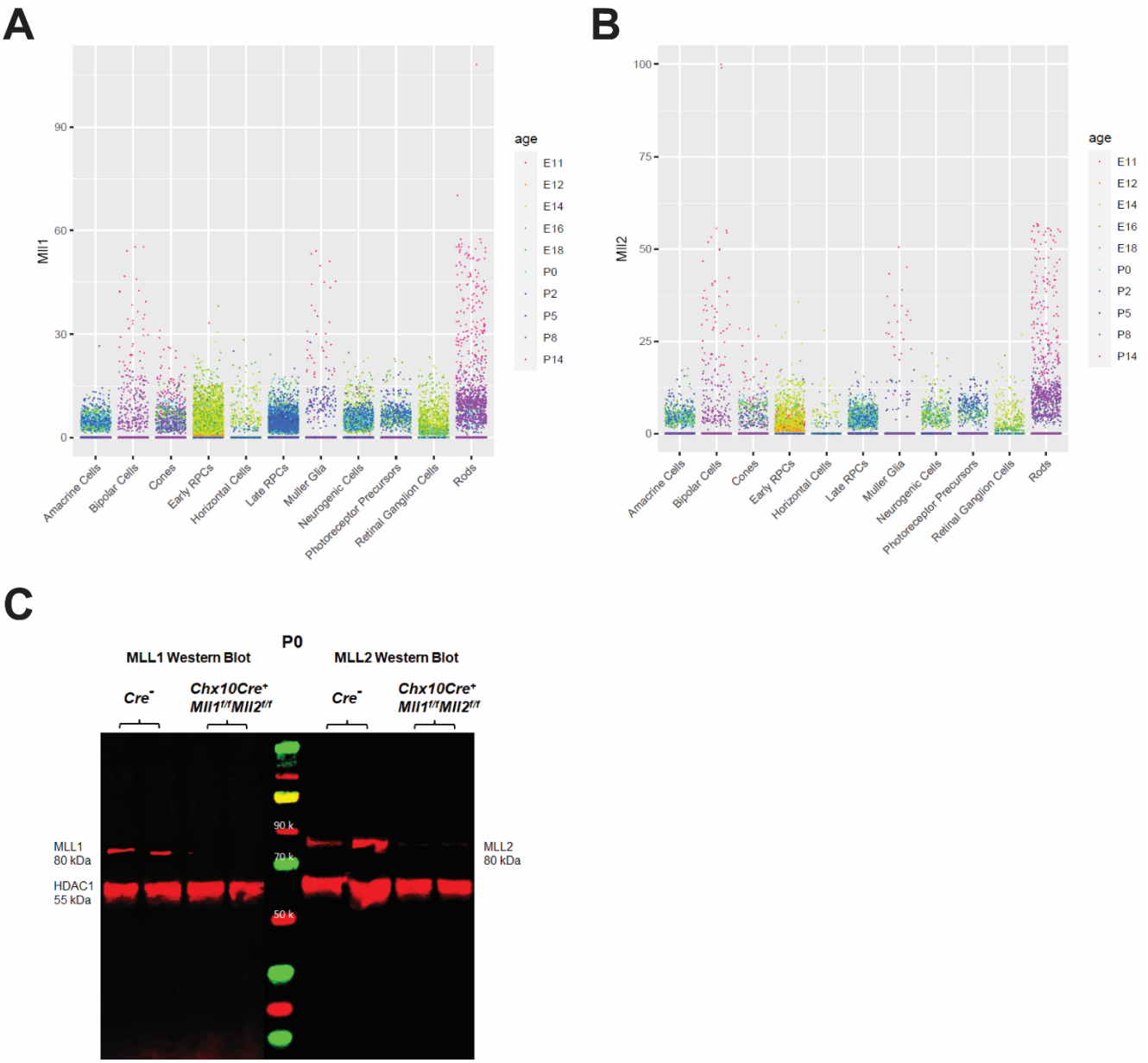
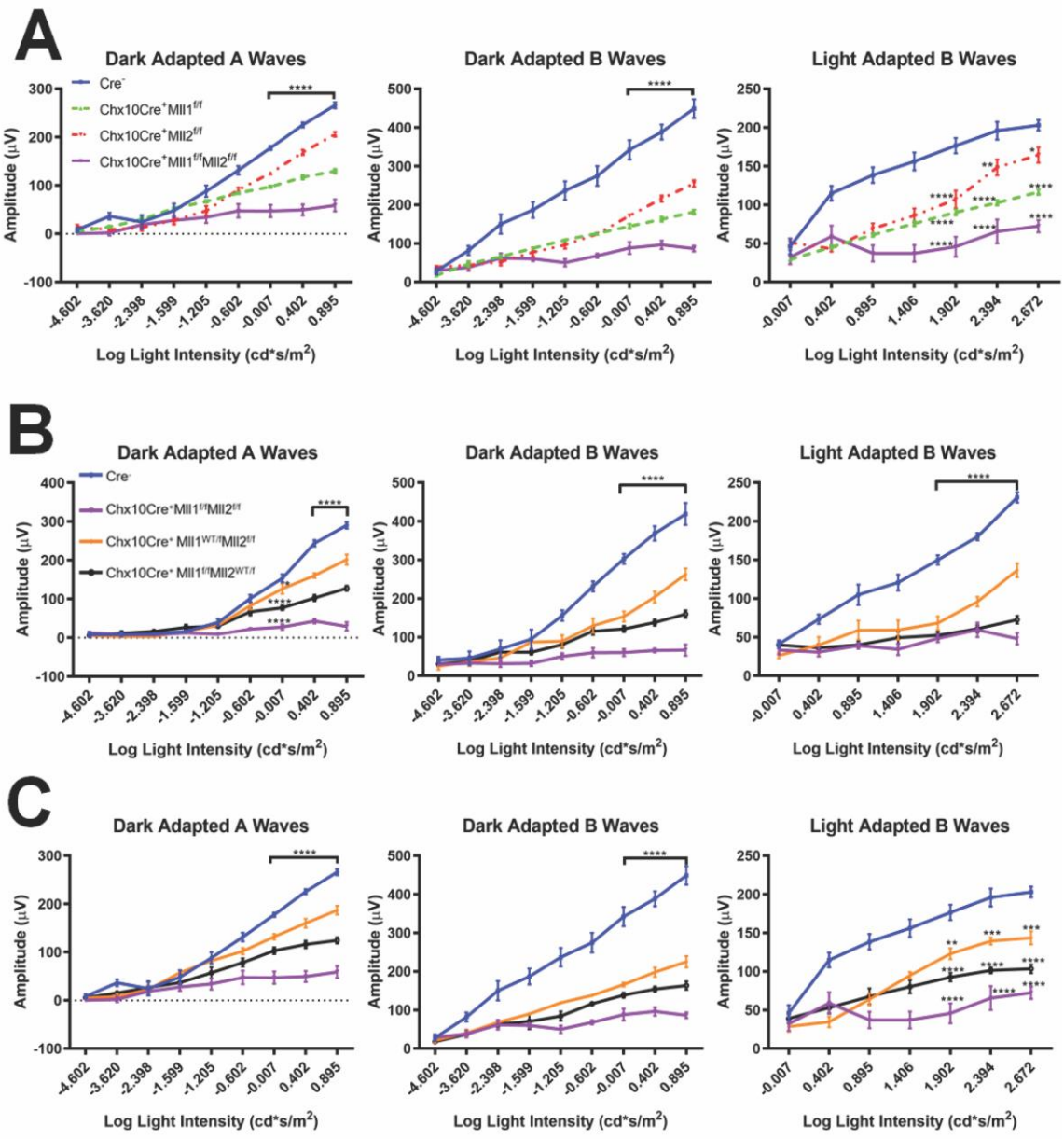
Supplemental Materials

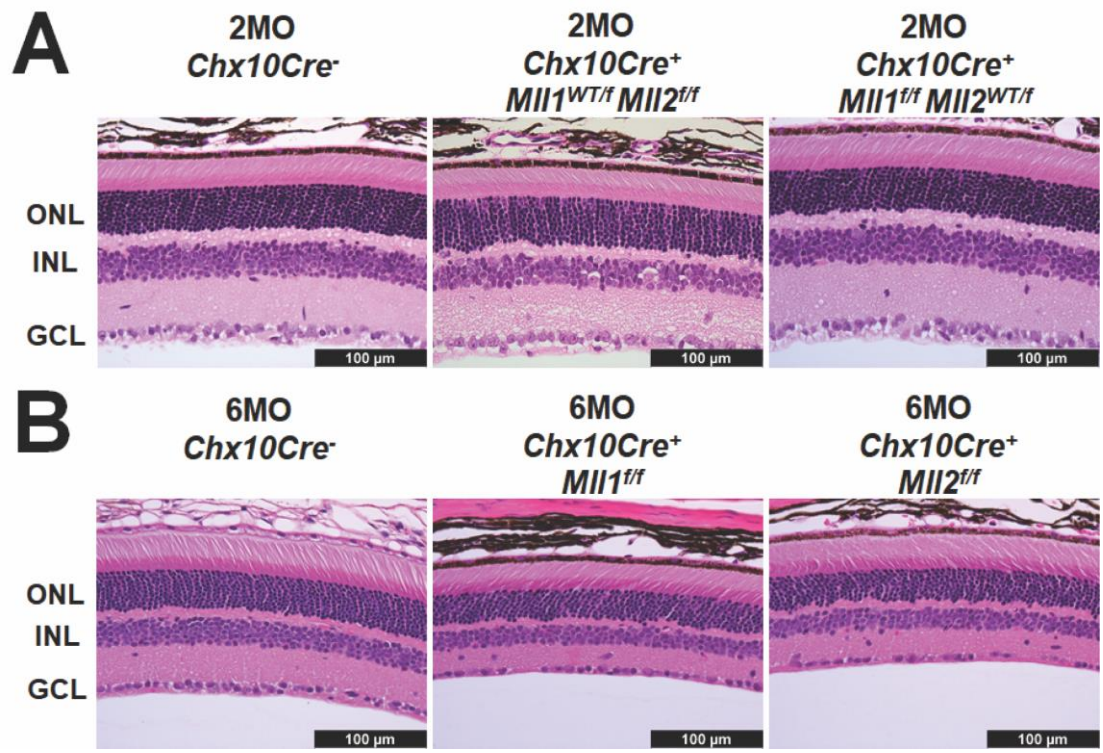
Supplemental Figures



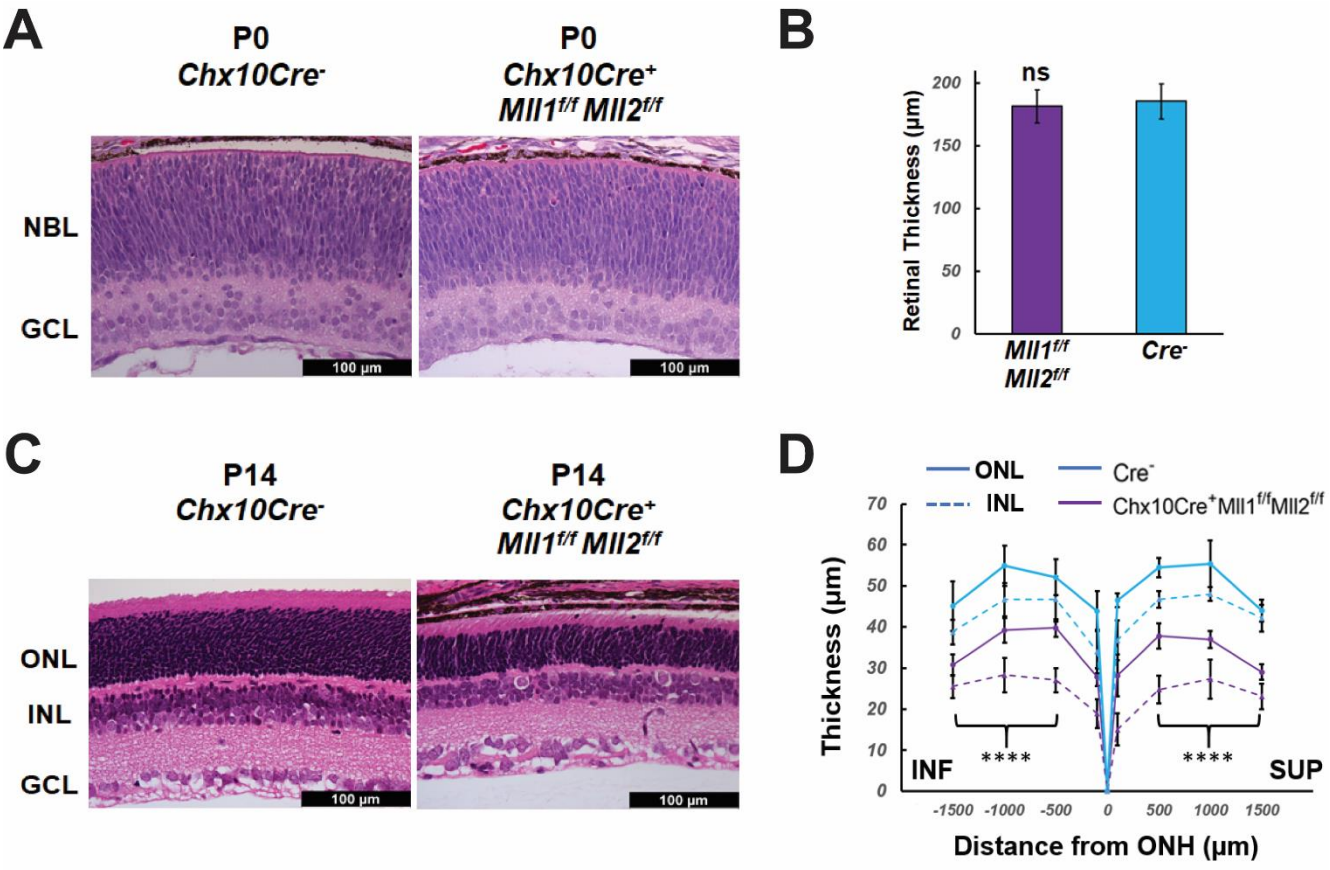
Supplemental Figure 1. Jitter plots of *Mll1* (**A**) and *Mll2* (**B**) expression in annotated cell types (x-axis) at various time stages (E11-P14) (Data retrieved from single-cell RNA-seq published in Clark B, 2019). Y-axis represents *Mll1* or *Mll2* transcripts per 10k transcripts. Each dot represents an individual cell. (**C**) Western blot of MLL1 (Left) and MLL2 (Right) in nuclear extracts of P0 retinal samples. HDAC1 (55 kDa) served as reference. Cleaved products of MLL1 and MLL2 were blotted at 80 kDa.



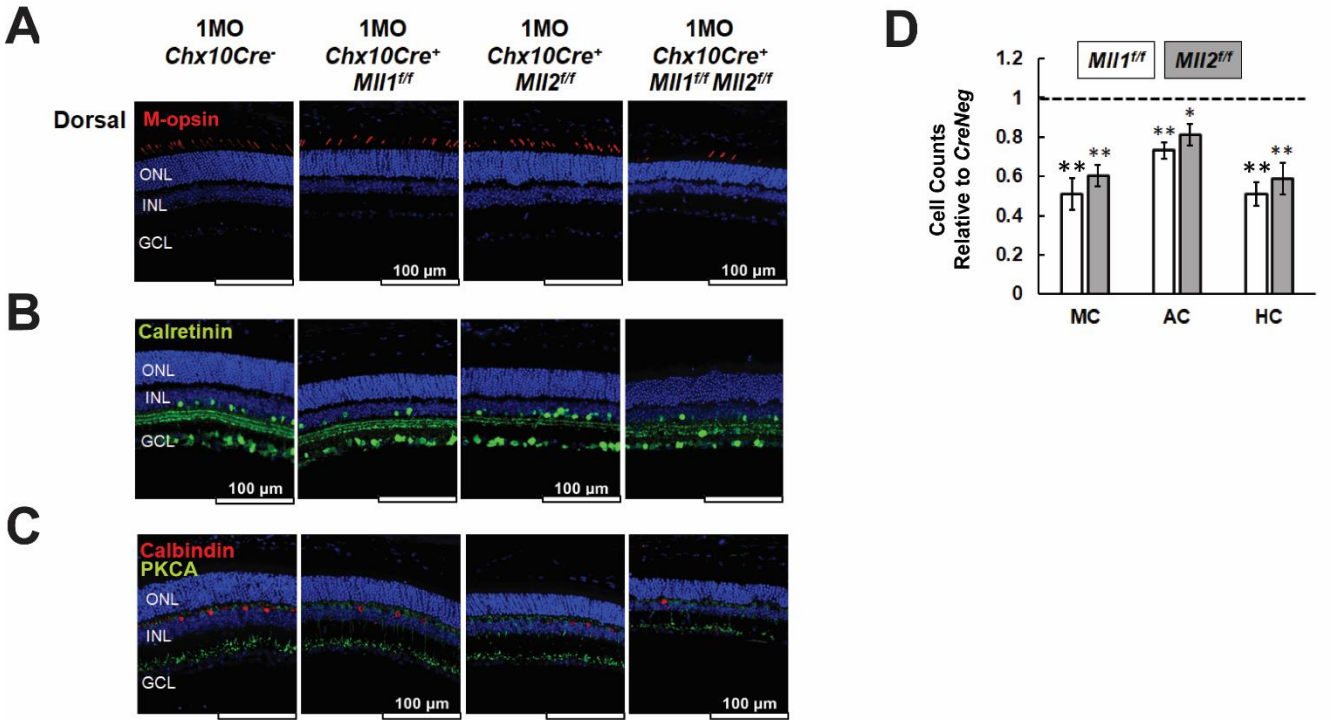
Supplemental Figure 2. (**A**) Dark adapted A-wave (left), dark adapted B-wave (center), light adapted B-wave (right) ERG of 2MO *Chx10Cre*$^{+}$*Mll1*$^{f/f}$, *Chx10Cre*$^{+}$*Mll2*$^{f/f}$, *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$, and *Chx10Cre*$^{-}$ mice. (**B**) Dark adapted A-wave (left), dark adapted B-wave (center), light adapted B-wave (right) ERG of 1MO *Chx10Cre*$^{+}$*Mll1*$^{WT/f}$*Mll2*$^{f/f}$, *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{WT/f}$, *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$, and *Chx10Cre*$^{-}$ mice. (**C**) Dark adapted A-wave (left), dark adapted B-wave (center), light adapted B-wave (right) ERG analysis of 2MO *Chx10Cre*$^{+}$*Mll1*$^{WT/f}$*Mll2*$^{f/f}$, *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{WT/f}$, *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$, and *Chx10Cre*$^{-}$ mice. Mean amplitudes (μV) are plotted against stimulus light intensity. Error bars represent SEM ($n \geq 4$). All statistics is done by two-way ANOVA and Tukey's multiple comparisons. Asterisks (*, **, ***, ****) denote $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$, respectively.



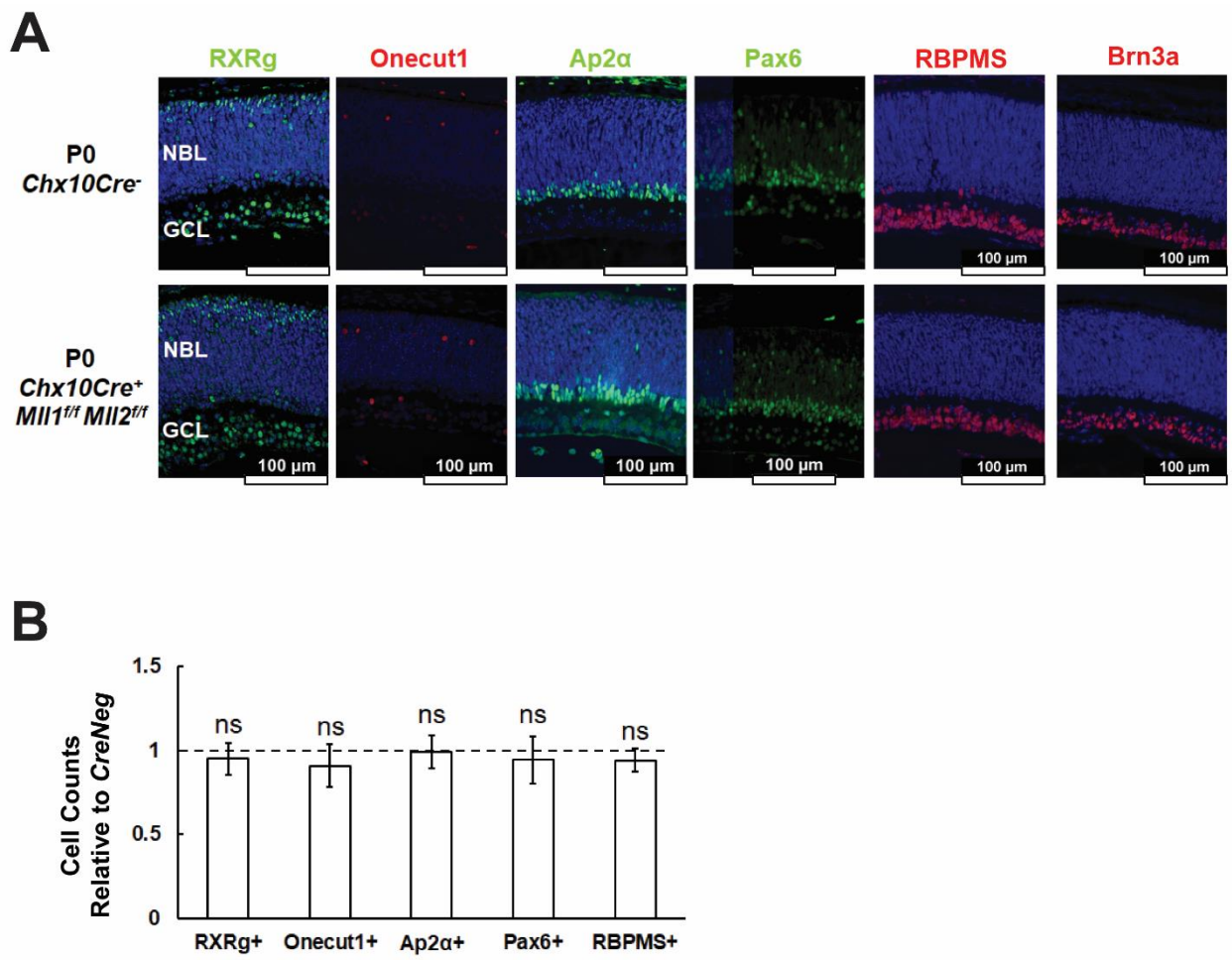
Supplemental Figure 3. Representative images of H & E-stained retinal cross-sections of the mice of indicated genotypes at 2MO (**A**) and 6MO (**B**). Scale bar = 100µm for all image panels.



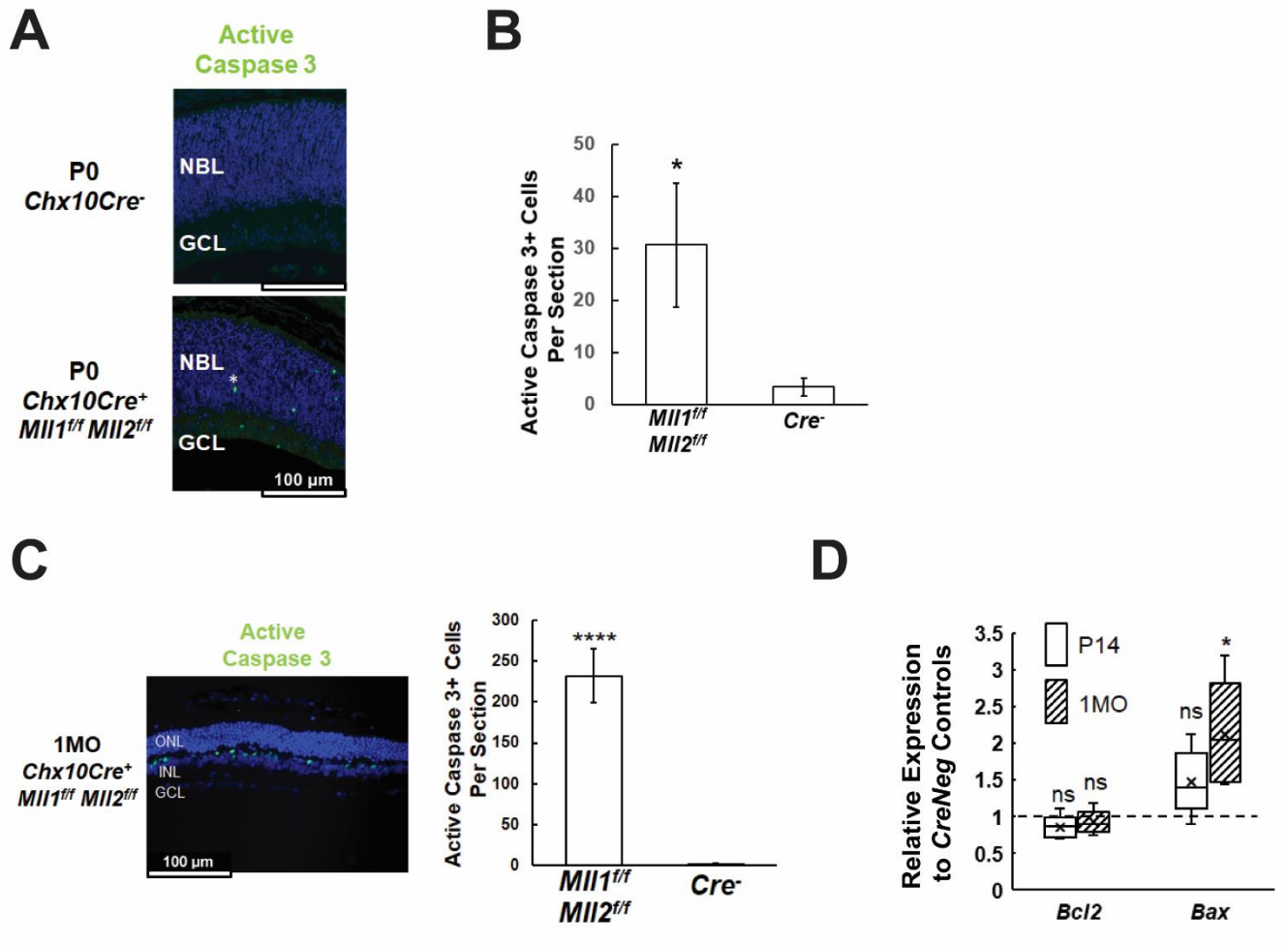
Supplemental Figure 4. (**A**) H & E -stained retinal cross-sections of P0 *Chx10Cre*$^{-}$ and *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. (**B**) Retinal thickness (µm) in P0 retinae at 500µm from the ONH. (**C**) H & E -stained retinal cross-sections of P14 *Chx10Cre*$^{-}$ and *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. Scale bar = 100µm for all image panels. (**D**) Plots of ONL (solid line) and INL (dotted line) thickness (µm) for P14 retinae at the indicated positions from the ONH. SUP and INF indicate superior and inferior sides of the retina. Error bars represent mean (SD) ($n \geq 4$). Results of statistical analysis for both ONL and INL thickness are shown. All statistics is done by Tukey's multiple comparisons. Asterisks (****) denote $P \leq 0.0001$, ns means not significant.



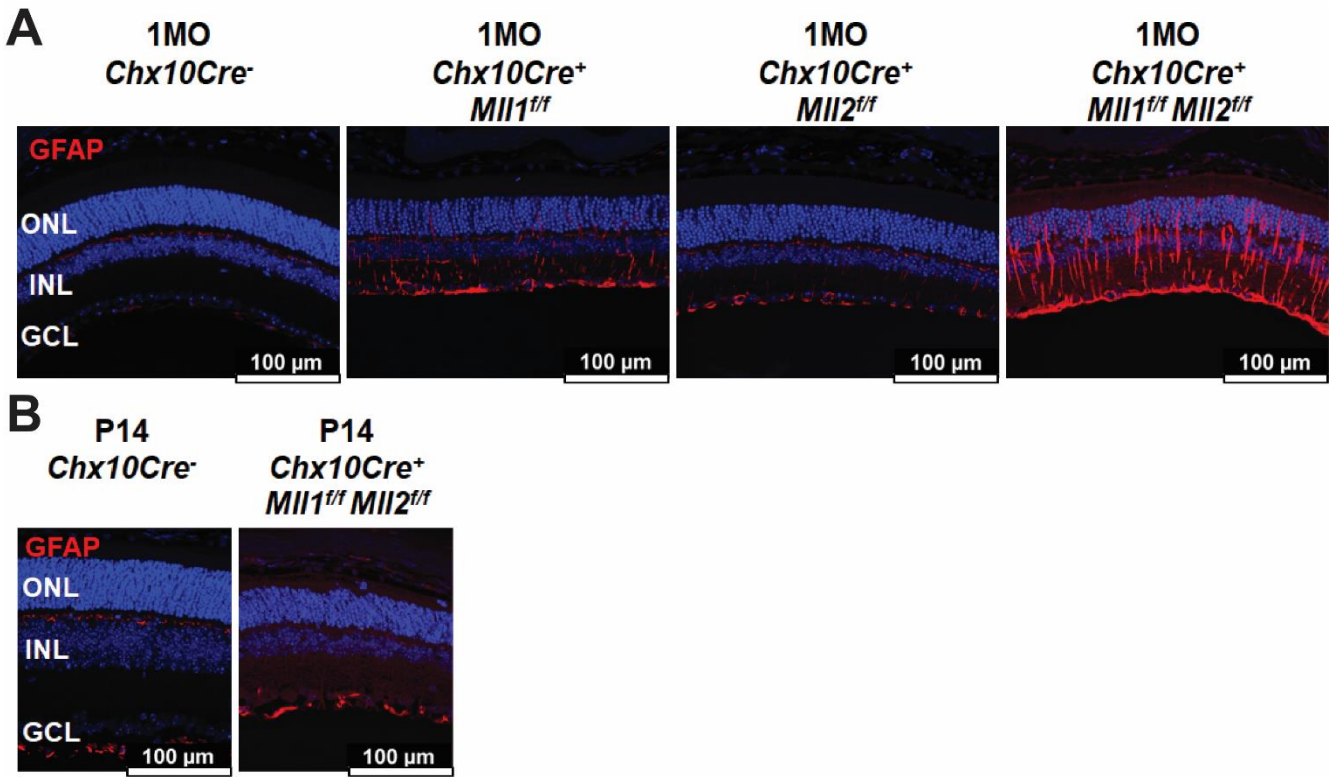
Supplementary Figure 5. Immunostaining of the following cellular markers in 1MO retinae of the indicated genotypes: Medium-wave-sensitive opsin 1 (M-opsin, red in **A**), Calretinin (green in **B**) for amacrine and ganglion cells, Calbindin D-28 (red in **C**) for horizontal cells. Nuclei are counterstained by DAPI (blue). Scale bar = 100µm for all image panels. (**D**) Cell counts for different retinal cell types in 1MO *Chx10Cre*$^{+}$*Mll1*$^{f/f}$ and *Chx10Cre*$^{+}$*Mll2*$^{f/f}$ retinae, normalized to counts from *Chx10Cre*$^{-}$ (*CreNeg*) littermates. Error bars represent mean (SD) (n≥3). Asterisks (*, **) denote $p \leq 0.05$ and $p \leq 0.01$ respectively, ns means not significant by one-way ANOVA. MC=M cone photoreceptors, AC=amacrine cells, HC=horizontal cells.



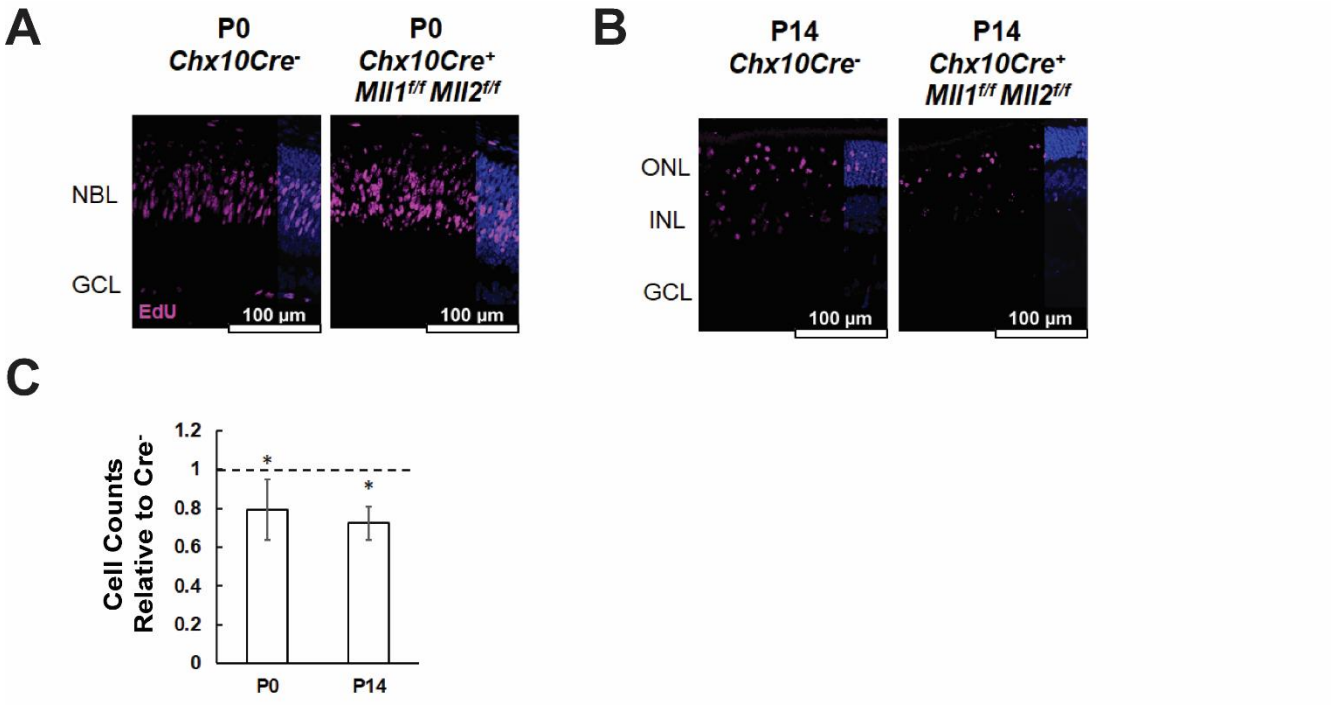
Supplemental Figure 6. (A) Immunostaining of the following cellular markers in P0 retinae of the indicated genotypes: (**A**) RXRg for fated cones and ganglion cells (green in 1st panel from left), Onecut1 for fated horizontal cells (red in 2nd panel), Ap2α for early-born amacrine cells (green in 3rd panel), Pax6 for early-born amacrine and other neurons (green in 4th panel), RBPMS and Brn3a for ganglion cells (red in 5th and 6th panels). Nuclei are counterstained by DAPI (blue). Scale bar = 100μm for all image panels. (**B**) Cell counts for different cell types in P0 *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae, normalized to counts from *Chx10Cre*$^{-}$ (*CreNeg*) littermates. Error bars represent mean (SD) (n≥3). ns means not significant by T-test.



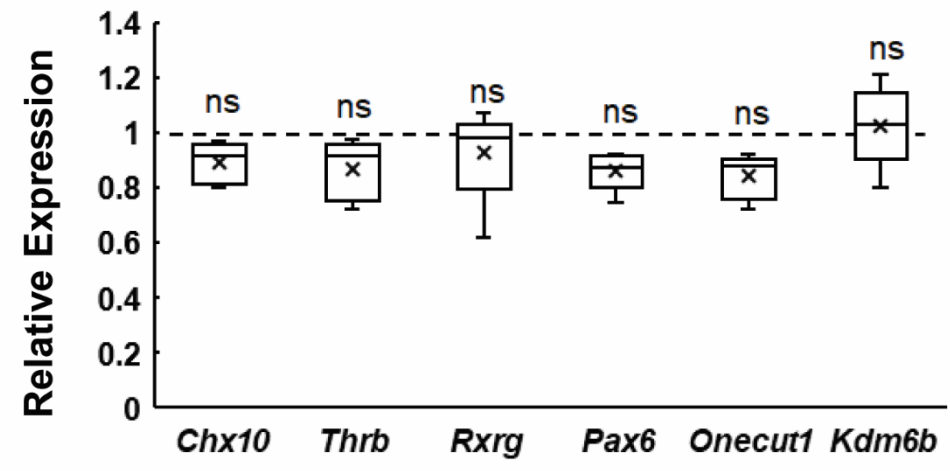
Supplemental Figure 7. (**A**) Active caspase 3 (green) immunostaining in P0 *Chx10Cre*$^{-}$ and *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. Active caspase 3 immunoreactivity labels cells undergoing apoptosis. (**B**) Cell counts for Active caspase 3+ cells in P0 retinae. (**C**) Active caspase 3 (green) immunostaining and Cell counts for active caspase 3+ cells in 1MO *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. Nuclei are counterstained by DAPI (blue). Scale bar = 100µm for all image panels. (**D**) qRT-PCR analysis of *Bax* and *Bcl2* in P14 and 1MO *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. Results are plotted as relative expression to *Chx10Cre*$^{-}$ (*CreNeg*) littermate controls (n≥4). Asterisks (*) denote $p \leq 0.05$ by one-way ANOVA, and ns means not significant.



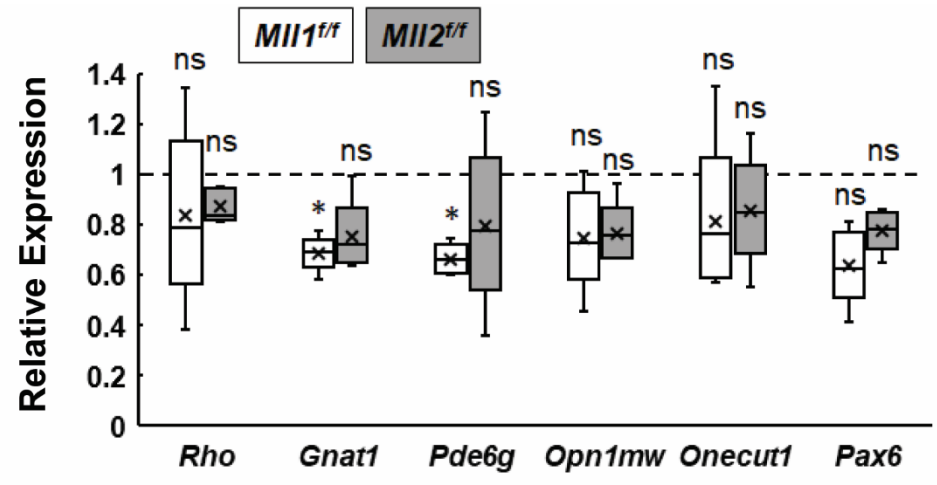
Supplemental Figure 8. Glia activation in *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$. (**A**) Glial fibrillary acidic protein (GFAP) immunostaining (in red) in 1MO mutant and control retinae. Nuclei are counterstained by DAPI (blue). Scale bar = 100µm for all image panels. (**B**) GFAP immunostaining in P14 mutant and control retinae.



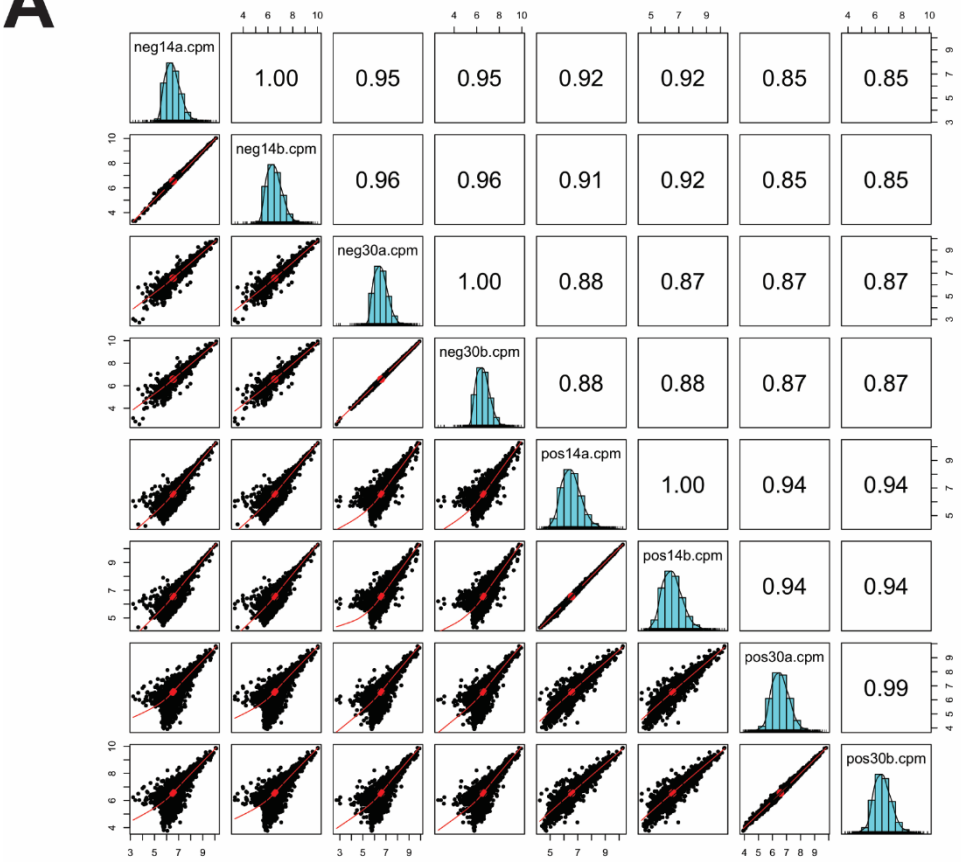
Supplemental Figure 9. EdU immunostaining (red) on P0 (**A**) and P14 (**B**) retinal cross-sections of *Chx10Cre*$^{-}$ and *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. Nuclei are counterstained by DAPI (blue). Scale bar = 100µm for all image panels. (**C**) Cell counts for EdU+ cells in P0 and P14 *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae, normalized to counts from *Chx10Cre*$^{-}$ littermates. Error bars represent SEM (n≥3). Asterisks (*) denote $p \leq 0.05$ by T-test.



Supplemental Figure 10. qRT-PCR analysis of selected genes in P0 *Chx10Cre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. Results are plotted as relative expression to *Chx10Cre*$^{-}$ littermate controls (n≥4). ns means not significant by T-test.



Supplemental Figure 11. qRT-PCR analysis of selected genes in 1MO *Chx10Cre*$^{+}$*Mll1*$^{f/f}$ and *Chx10Cre*$^{+}$*Mll2*$^{f/f}$ retinae. Results are plotted as relative expression to *Chx10Cre*$^{-}$ littermate controls (n≥4). Asterisks (*) denote P ≤ 0.05 by T-test, ns means not significant.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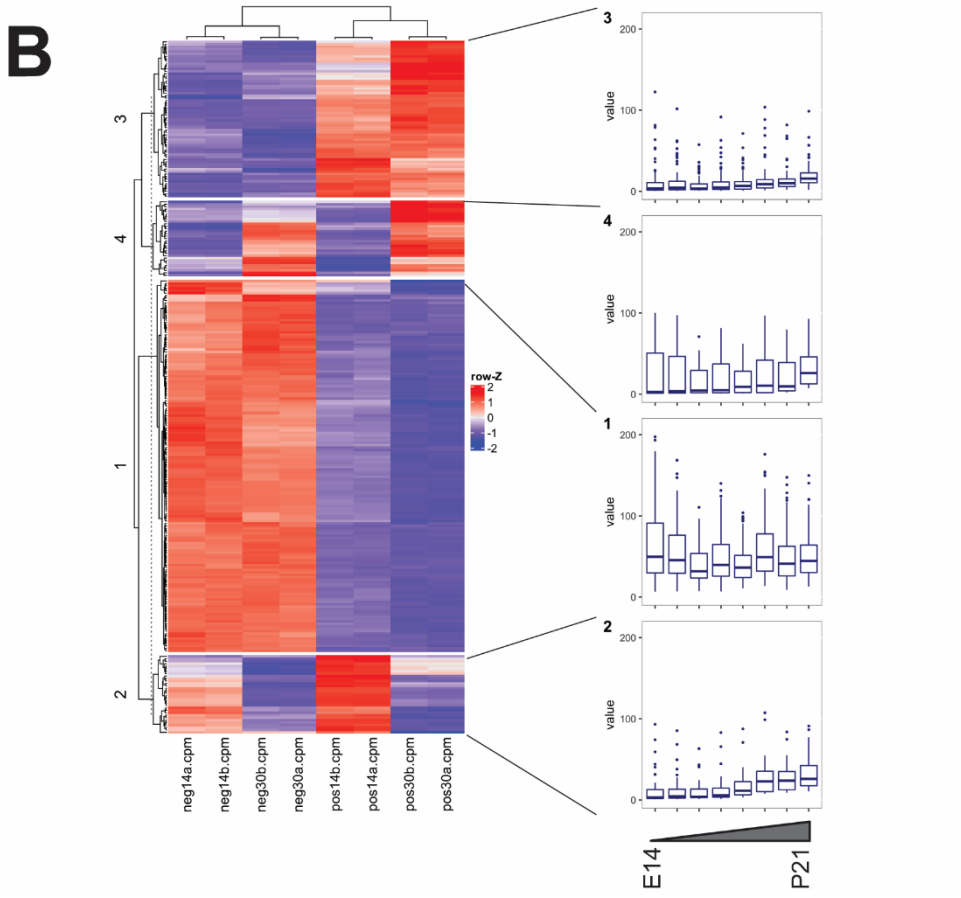
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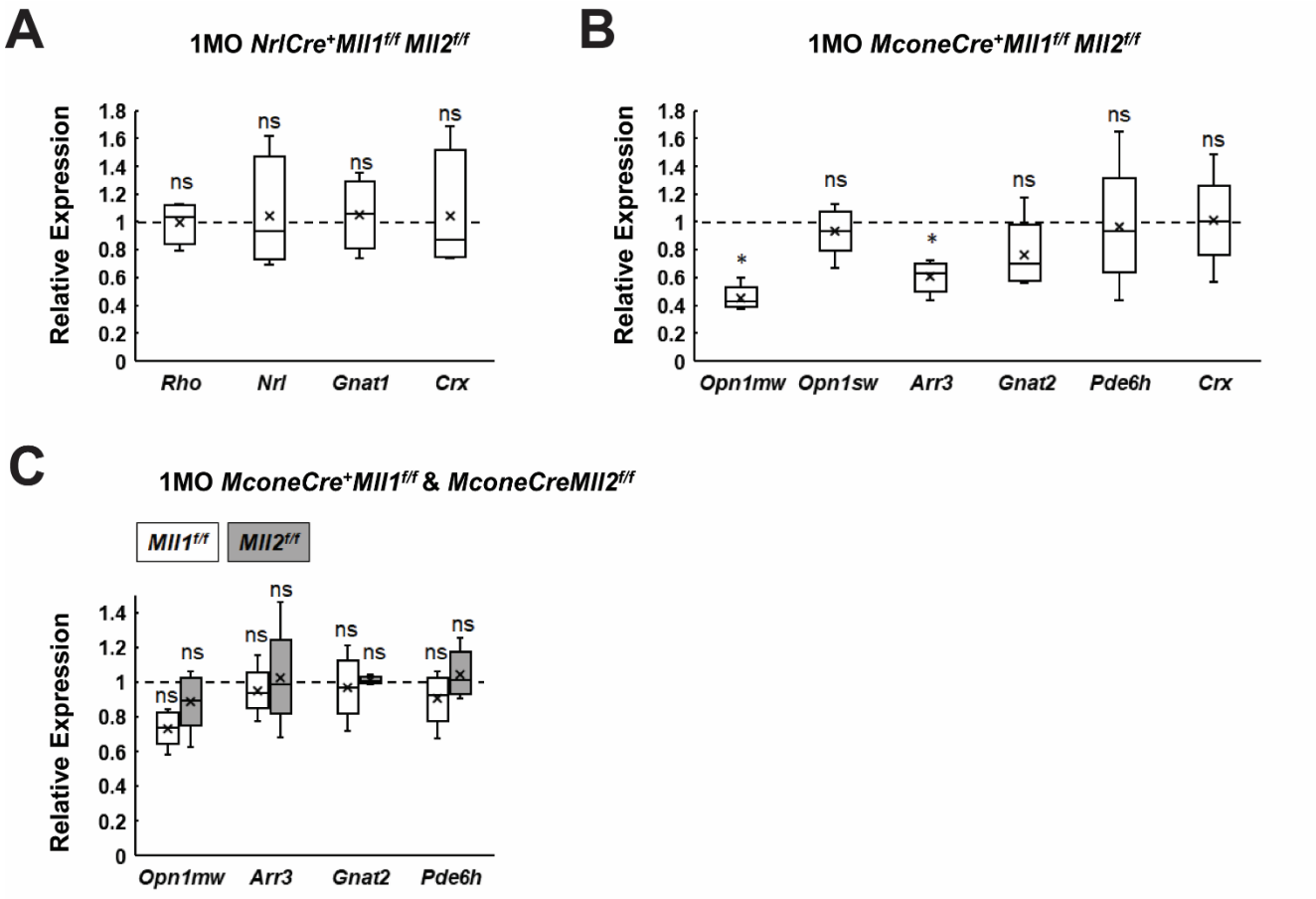
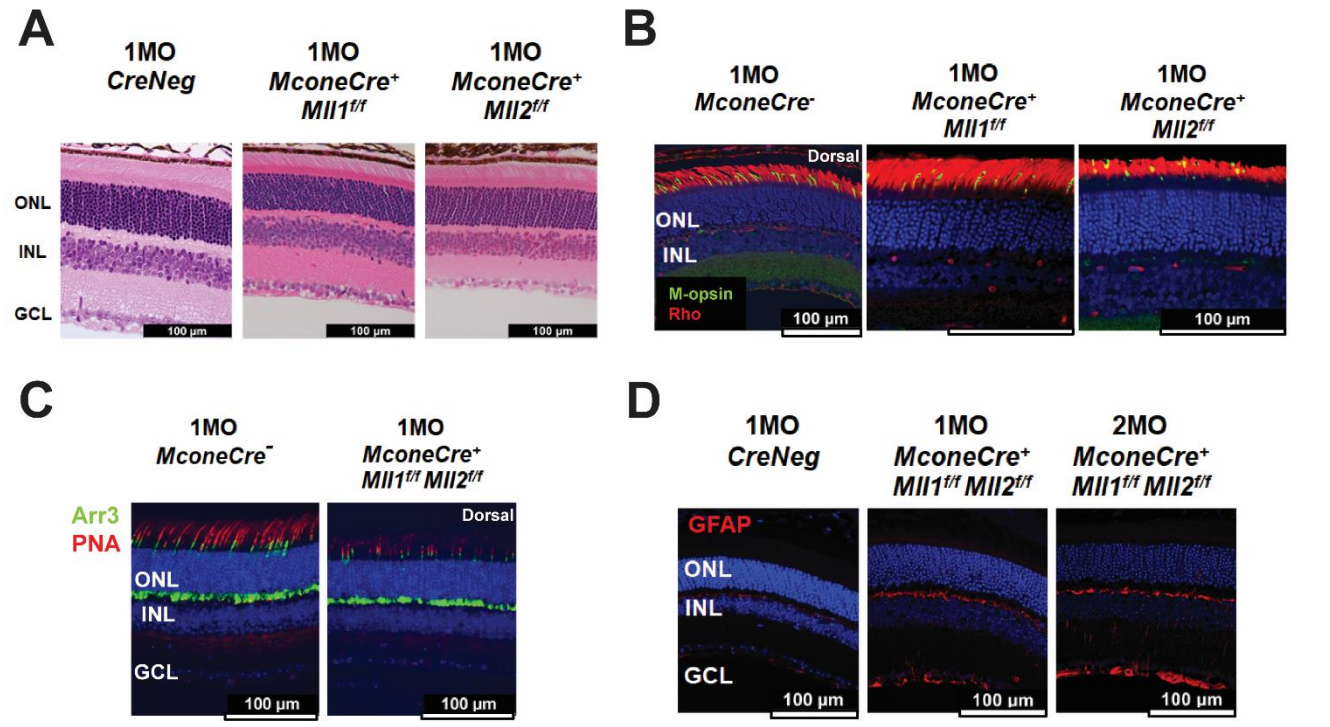
E14
P21

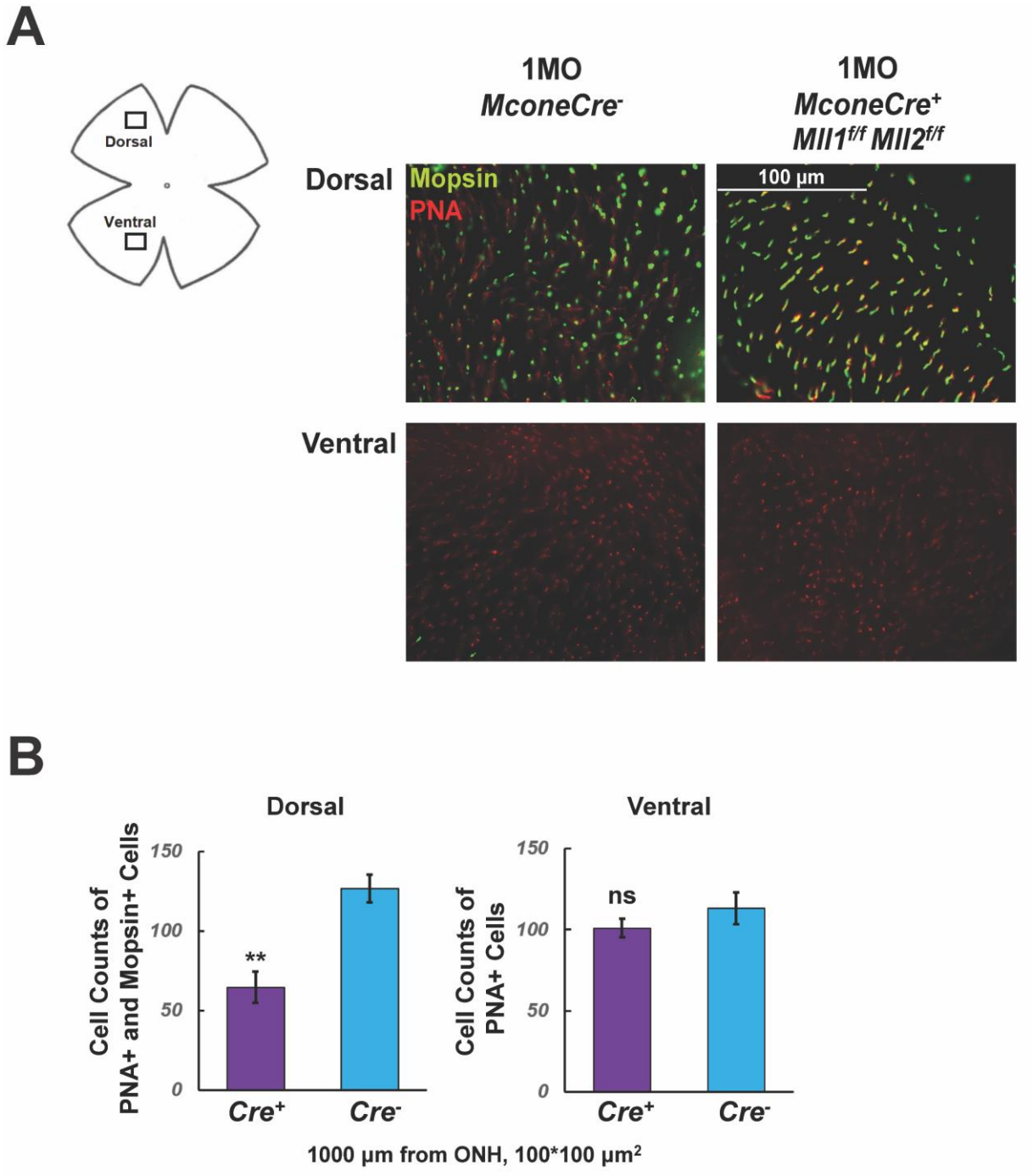
Supplemental Figure 12. (**A**) Correlation plot comparing each library by scatterplot and Pearson correlation value. (**B**) Clustered heatmap of all 279 H3K4me3 peaks that showed differential deposition in pairwise comparisons between all samples (>2 fold-change, P< 0.05). Boxplots display enrichment of indicated histones profiled over retinal development (E14-P21) in Aldiri et al., 2017.



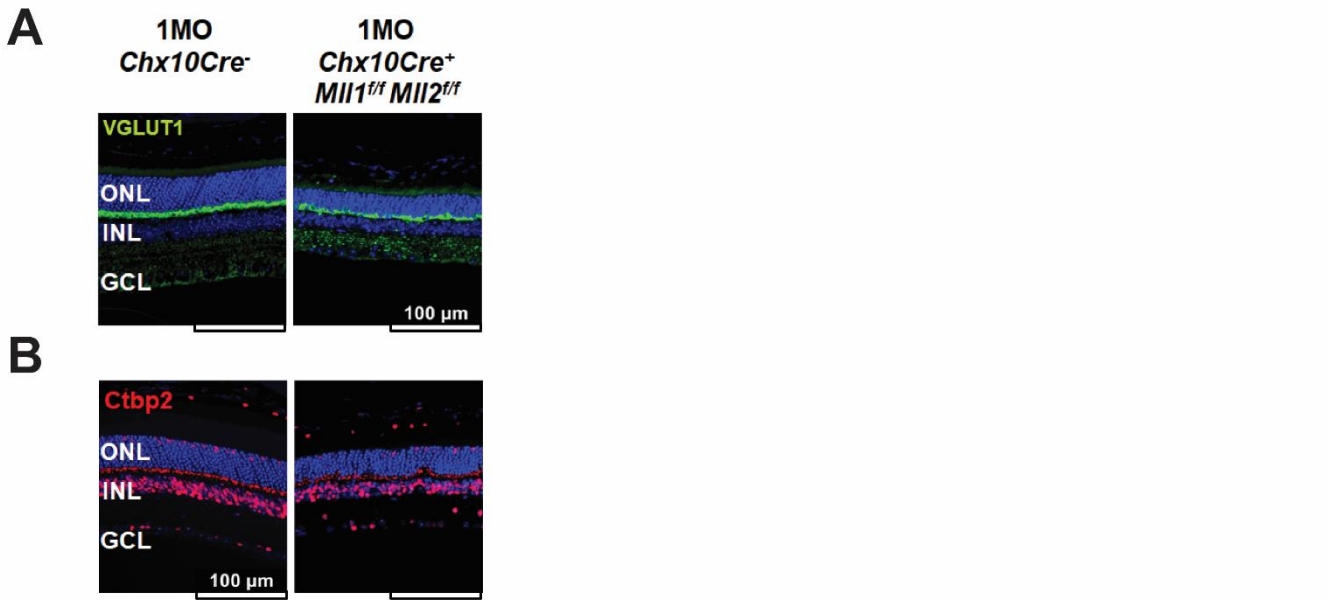
Supplemental Figure 13. (**A**) qRT-PCR analysis of selected genes in 1MO *NrlCre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. Results are plotted as relative expression to *NrlCre*$^{-}$ littermate controls (n≥4). (**B**) qRT-PCR analysis of selected genes in 1MO *MconeCre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. Results are plotted as relative expression to *MconeCre*$^{-}$ littermate controls (n≥4). (**C**) qRT-PCR analysis of selected genes in 1MO *MconeCre*$^{+}$*Mll1*$^{f/f}$ and *MconeCre*$^{+}$*Mll2*$^{f/f}$ retinae. Results are plotted as relative expression to *MconeCre*$^{-}$ littermate controls (n≥4). Asterisks (*) denote $p \leq 0.05$ by T-test, ns means not significant.



Supplemental Figure 14. (**A**) H & E-stained retinal cross-section of 1MO *Cre*$^{-}$ (*CreNeg*), *MconeCre*$^{+}$*Mll1*$^{f/f}$, *MconeCre*$^{+}$*Mll2*$^{f/f}$ retinae. (**B**) M-opsin and Rho immunostaining in the dorsal region of 1MO *MconeCre*$^{-}$, *MconeCre*$^{+}$*Mll1*$^{f/f}$, and *MconeCre*$^{+}$*Mll2*$^{f/f}$ retinae. (**C**) Cone arrestin (Arr3) and PNA immunostaining in the dorsal region of 1MO *MconeCre*$^{-}$ and *MconeCre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. (**D**) GFAP immunostaining in 1MO *MconeCre*$^{-}$ (*CreNeg*), 1MO and 2MO *MconeCre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. Nuclei are counterstained by DAPI (blue). Scale bar = 100µm for all image panels.



Supplemental Figure 15. (**A**) Whole-amount images of Mopsin (green) and PNA (red) immunostaining in 1MO *MconeCre*$^{-}$ and *MconeCre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. Top panel contains images taken at the dorsal retinae; bottom panel contains images taken at the ventral retinae. (**B**) Cell counts for Mopsin and PNA co-labelled cells at dorsal (left) and ventral (right) in 1MO *MconeCre*$^{-}$ and *MconeCre*$^{+}$*Mll1*$^{f/f}$*Mll2*$^{f/f}$ retinae. Error bars represent SEM (n=3). Cell counting is taken within an area of 100*100 µm^2, 1000 µm from ONH. Asterisks (**) denote $p \leq 0.01$ by T-test, ns means not significant.



Supplemental Figure 16. Immunostaining of the following biomarkers in 1MO retinae of the indicated genotypes: (**A**) Vesicular glutamate transporter 1 (VGLUT1 in gren) and (**B**) C-terminal binding protein 2 (Ctbp2 in red) Nuclei are counterstained by DAPI (blue). Scale bar = 100µm for all image panels. VGLUT1 immunoreactivity labels the membrane of synaptic vesicles, Ctbp2/RIBEYE immunoreactivity labels ribbon synapses.

Supplemental Table 1: Antibodies

Antibodies	Source	Species
Active Caspase-3	R&D Systems AF835	Rabbit
Ap2α	DSHB 3B5	Mouse
Brn3a	MilliporeSigma MAB1585	Mouse
Calbindin D28K	MilliporeSigma C9848	Mouse
Calretinin	MilliporeSigma AB5054	Rabbit
Cone Arrestin	MilliporeSigma AB15282	Rabbit
Ctbp2	BD Biosciences 612044	Mouse
GFAP	Agilent z0334	Rabbit
Glutamine Synthetase	BD Biosciences 610517	Mouse
Iba1	WakoFujifilm 019-19741	Rabbit
Ki67	BD Biosciences 550809	Mouse
M-opsin (Opn1mw)	MilliporeSigma AB5405	Rabbit
Onecut1	Santa Cruz sc13050	Rabbit
Pax6	DSHB Pax6	Mouse
Phospho-Histone H3	MilliporeSigma 06-570	Rabbit
PKCA	MilliporeSigma P5704	Mouse
PNA-Rhodamine	Vector Labs RL-1072	lectins
Rhodopsin (RetP1)	MilliporeSigma O4886	Mouse
S-opsin (Opn1sw)	Santa Cruz sc-14363	Goat
RXRg	Santa Cruz sc-555	Rabbit
VGluT1	MilliporeSigma AB5905	Guinea pig
RBPMS	MilliporeSigma ABN1362	Rabbit
MLL1	Cell Signaling 14197	Rabbit
MLL2	Cell Signaling 38058	Rabbit
HDAC1	Santa Cruz sc-7872	Rabbit

Supplemental Table 2: Primer Sets for qPCR

Gene	Forward Primer (5' – 3')	Reverse Primer (5' – 3')
Actb	CCAACTGGGACGACATGGAG	TGGTACGACCAGAGGCATACA
Arr3	AAGTTTTCCATCTACCTGGGG	TCACATCCAAGTCATCACGG
Bax	GCAGAGGATGATTGCTGACG	TTCCAGATGGTGAGCGAGG
Bcl2	AAAATACAGCATTGCGGAGG	TCCAGCATCCCACTCGTAGC
Chx10	CTCCCAGAAGACAGGATACAGGTG	TGCCTCCAGCGACTTTTTGTG
Crx	TGTCCCATACTCAAGTGCCC	TGCTGTTTCTGCTGCTGTCG
Ctbp2	TGGTGGATGAGAAAGCCTTAGC	ATGCGACCTGTGATTGCTCG
Gfap	GAAGAAAACCGCATCACCATTC	CCAGAAGGAAGGGAAGTGCTG
Gnat1	ACGATGGACCTAACACTTACGAGG	TGGAAAGGACGGTATTTGAGG
Gnat2	AGTCAAGACAACAGGCATCATCG	TCACTTCGTCATCCTCCACCAG
Grm6	CCGCATCTACCGCATTTTCG	TCCACCGTCCTCTGTTCCTCATA
Kdm6b	GTCTGATGCCAAGAGGTGGAAG	GCTGATGGTCTCCCAATAGTGC
Mll1	TGAGTACAACCCTAACGATGAGGAA	CGGAATCTCATGGGCATTG
Mll2	AAAGACATCCAAAGAGGCTGTGG	TGTAGCACCCAATACCCTTCCC
Nr2e3	AGTCCCAGGTGATGCTAAGC	TTCTAAGATGTGCTGCCCC
Nrl	TTCTGGTTCTGACAGTGACTACG	AAGGCTCCCGCTTTATTTC
Onecut1	GGAAAGAGCAAGAACACGG	GATGAGGACGATGAACTGC
Onecut2	GATGTCTCACCTCAATGGC	TGGTTCTTGCTCTTTGCG
Opn1mw	GGTGGTGATGGTCTTCGCATAC	TTGGAGGTGCTGGAAAGTTCAG
Opn1sw	GCTGGACTTACGGCTTGTCACC	TGTGGCGTTGTGTTTGCTGC
Pax6	CCAGTGTCTACCAGCCAATCCC	GGTGAAATGAGTCCTGTTGAAGTGG
Pkca	CATTGCCCCAGAGATAATCGC	GGTGTTTGGTCATAAGTCCTTTGC
Pde6a	CACTCCTGAGAGATGAGAGCC	CAGGGTTTGGTGATGGCTG
Pde6b	CAAGAAAGTGGGCACAGAAG	ATAGGCAGAGTCCGTATGC
Pde6g	GCAAGGGTTTGGGGATGACA	CGTGCAGCTCTAGGTGATTGAA
Pde6h	ACGGGAACACATTCGGCTC	CCAGATGGGTGAACGCTTC
Prox1	CAGAAGGACTCTCTTTGTCAC	GCTGAACCACTTGATGAGC
Rho	GCTTCCCTACGCCAGTGTG	CAGTGGATTCTTGCCGCAG
RXRg	CGTGGAGAACTCAACAAATGACCC	TGGATAAACCCTTGGCATCTGG
Thrb	CAACCTGGATGACACTGAAGTCG	TCTAAGAACAGAGGCGGGAAGAG
Ubb	CAACATCCAGAAAGAGTCAACC	ATGTTGTAATCAGAGAGGGTGC