SUPPLEMENTARY MATERIALS

MLL1 is essential for retinal neurogenesis and horizontal inner neuron integrity

Diana S. Brightman[1, 3], Rachel L. Grant[5], Philip A. Ruzycki[4], Ray Suzuki[5], Anne K. Hennig[1] and Shiming Chen[1, 2*]

[1]Department of Ophthalmology and Visual Sciences, [2]Department of Developmental Biology, [3]Molecular Cell Biology and [4]Molecular Genetics and Genomics graduate programs, Division of Biology & Biomedical Sciences, [5]College of Arts and Sciences, Washington University, Saint Louis, Missouri, USA

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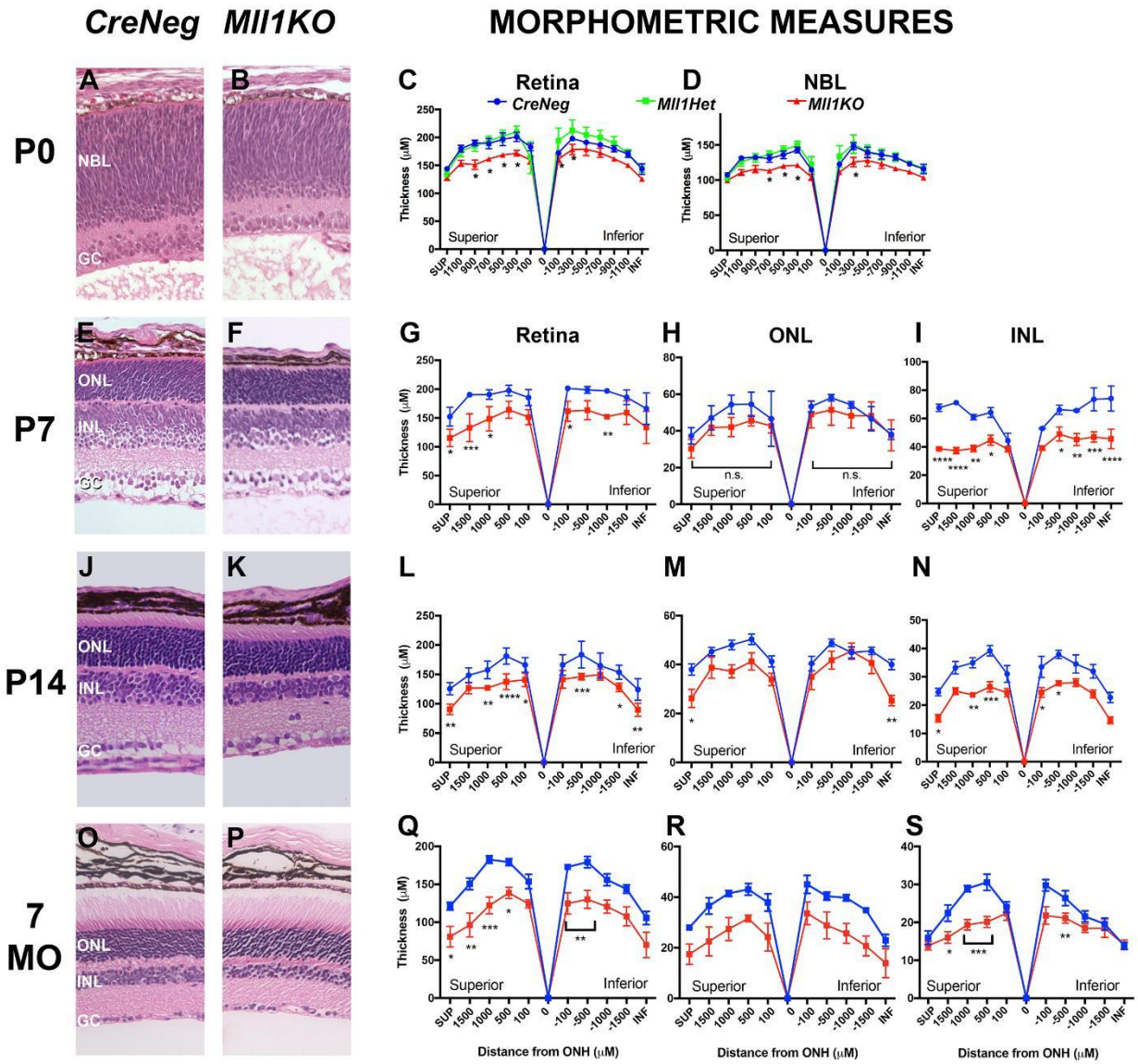
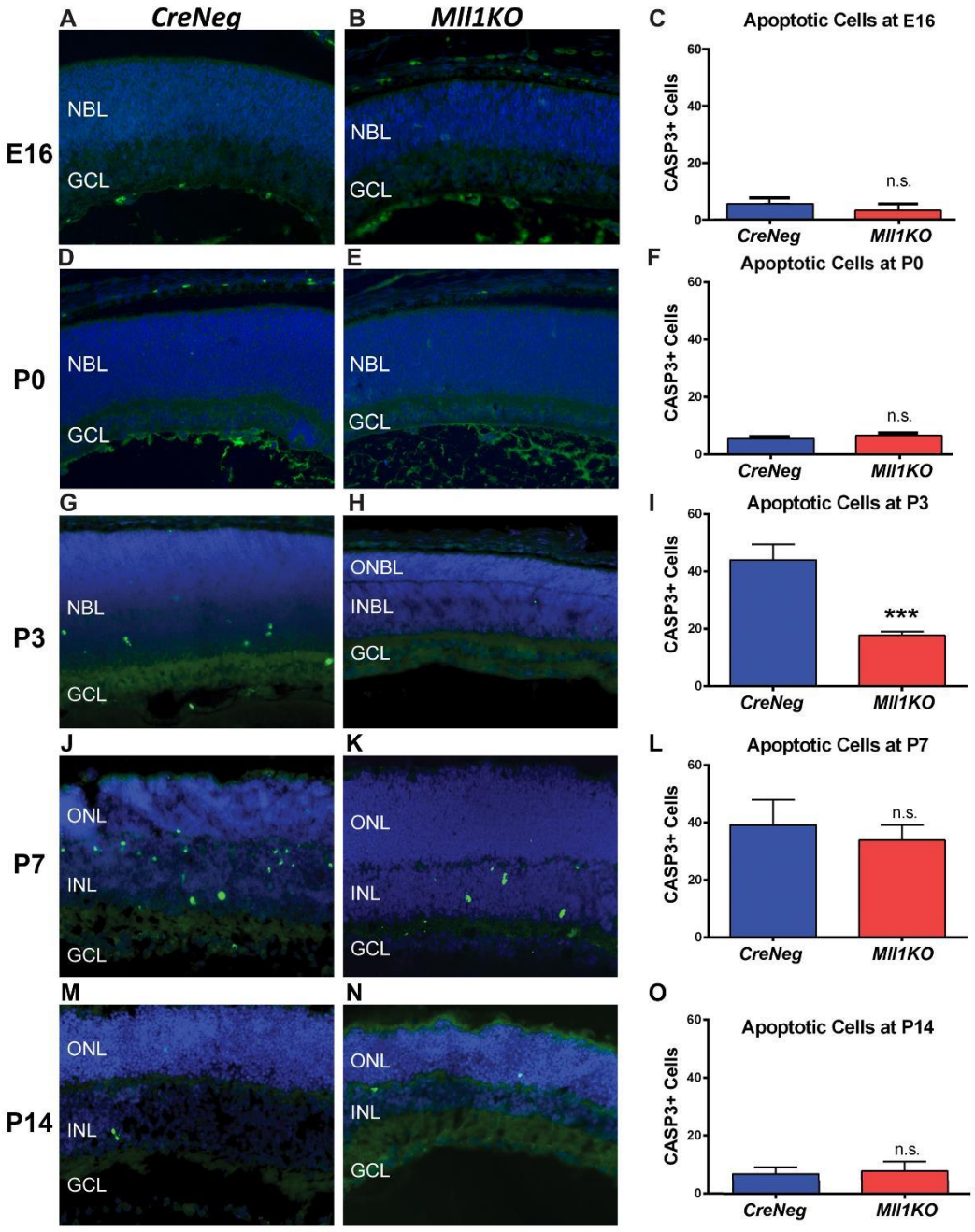
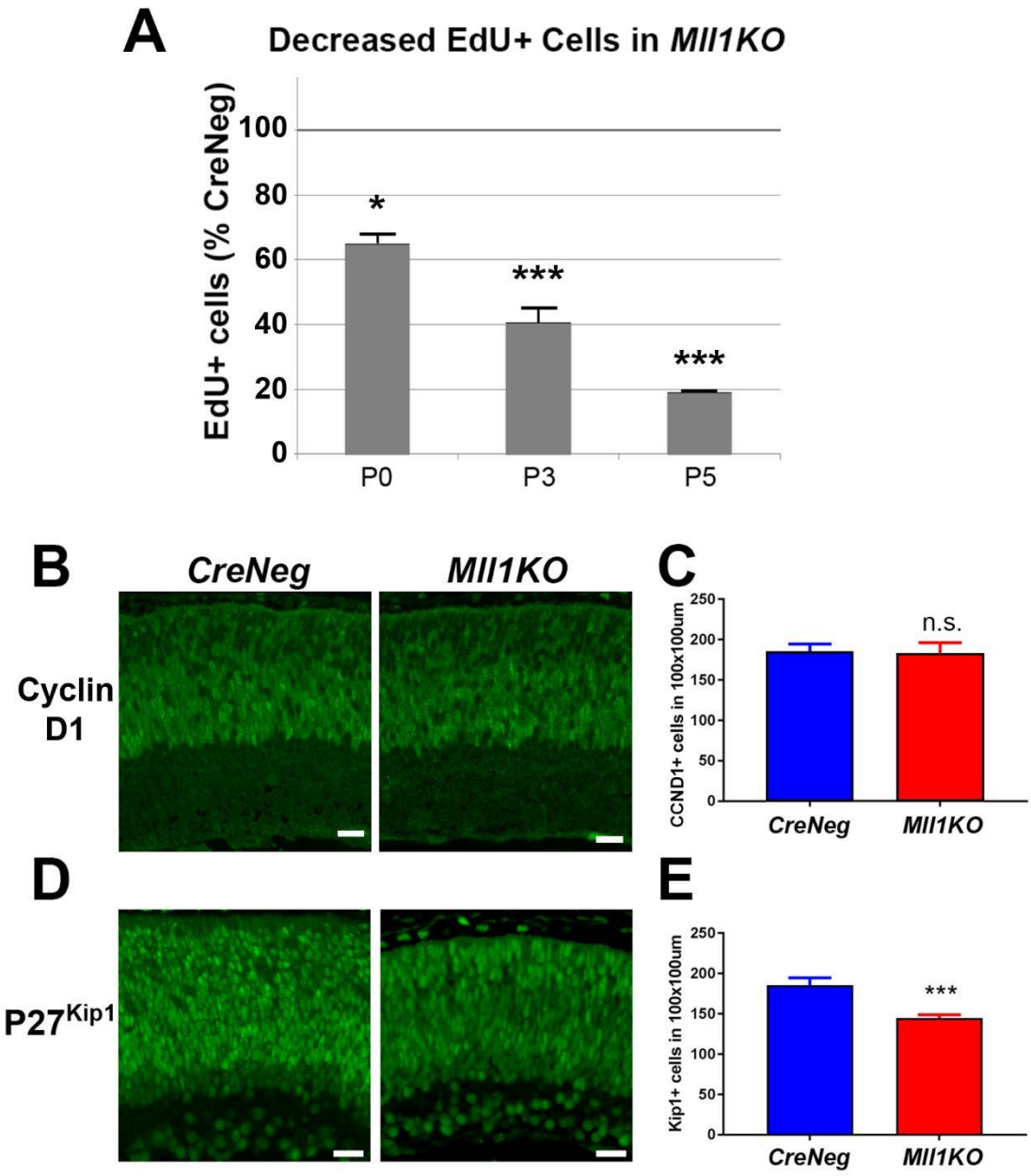


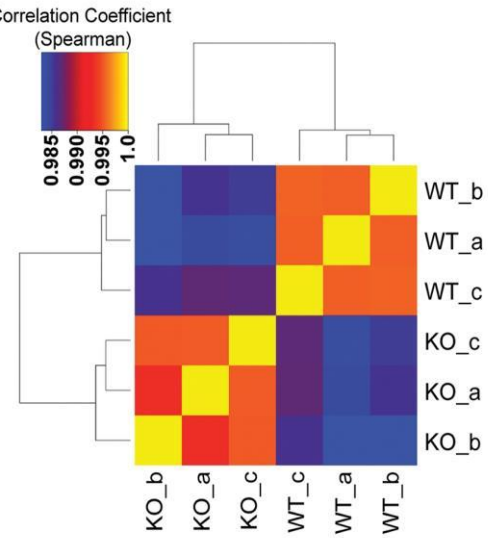
Supplementary Figure S1: Development of *Mll1KO* retina. Images of H&E-stained *CreNeg* (**A, E, J, O**) and *Mll1KO* (**B, F, K, P**) retinal cross-sections at the indicated ages, with morphometric measures comparing thickness of the whole retina (**C, G, L, Q**), the NBL (**D**), ONL (**H, M, R**) and INL (**I, N, S**), taken at the indicated positions from the ONH. SUP and INF indicate measurements taken 100um from the superior or inferior edge of the retina. **p*<0.05; ***p*<0.01; ****p*<0.001; *****p*<0.0001, by two-way ANOVA with repeated measures and Sidak's multiple comparisons test (n≥4).



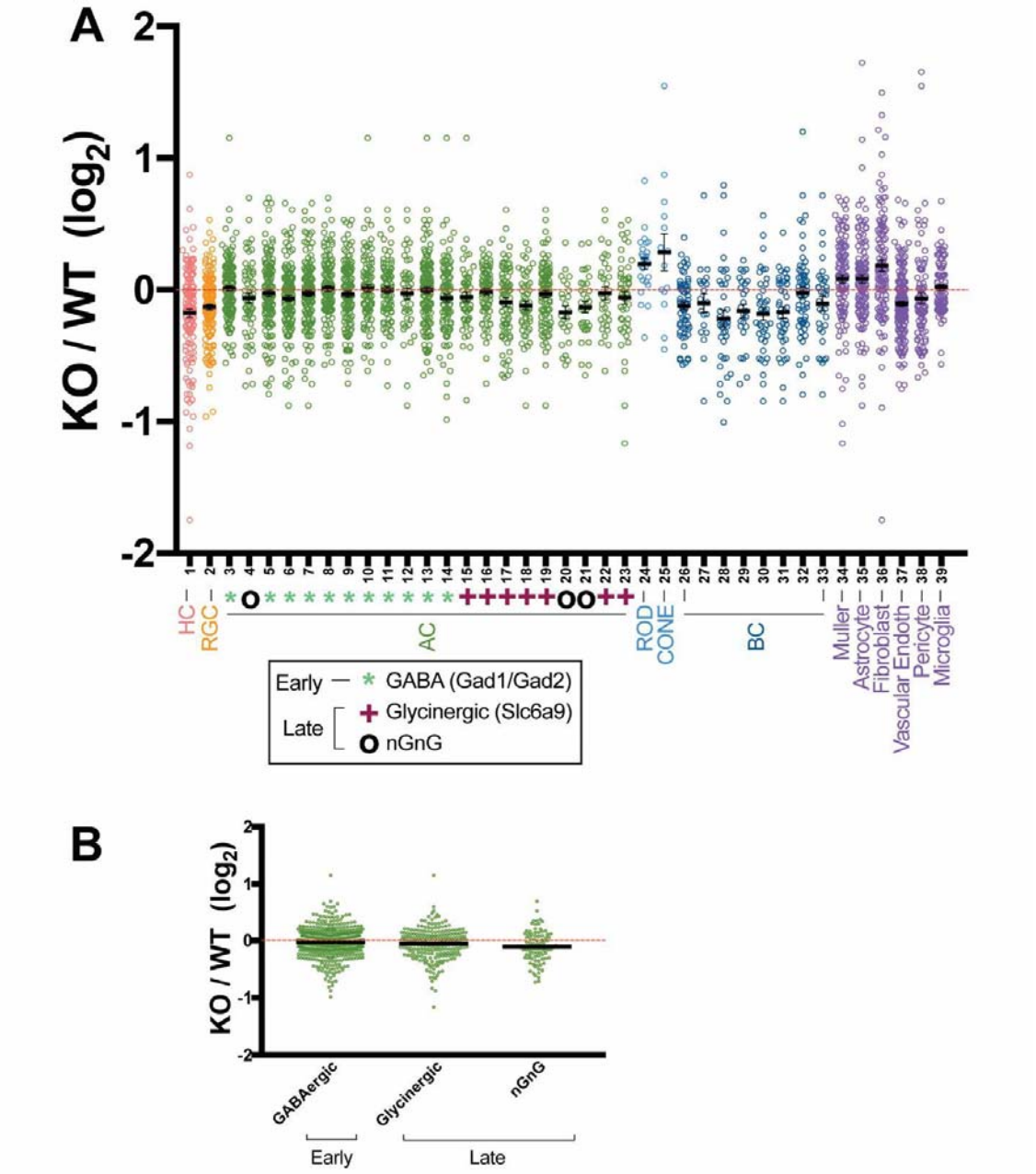
Supplementary Figure S2: *Mll1KO* retinas do not show increased apoptosis. Activated Caspase-3 immunostaining (green) with DAPI nuclear stain (blue) of retinal cross sections from *CreNeg* (**A, D, G, J, M**) *and Mll1KO* (**B, E, H, K, N**) mice at the indicated ages. (**C, F, I, L, O**) Quantification of CASP3+ cells per retinal section at each age. Three retinal cross-sections per sample and three samples per genotype were imaged, blinded and counted. The results are shown as mean + SEM. *p<0.05, n.s. = not significant by two-way ANOVA (n=3).



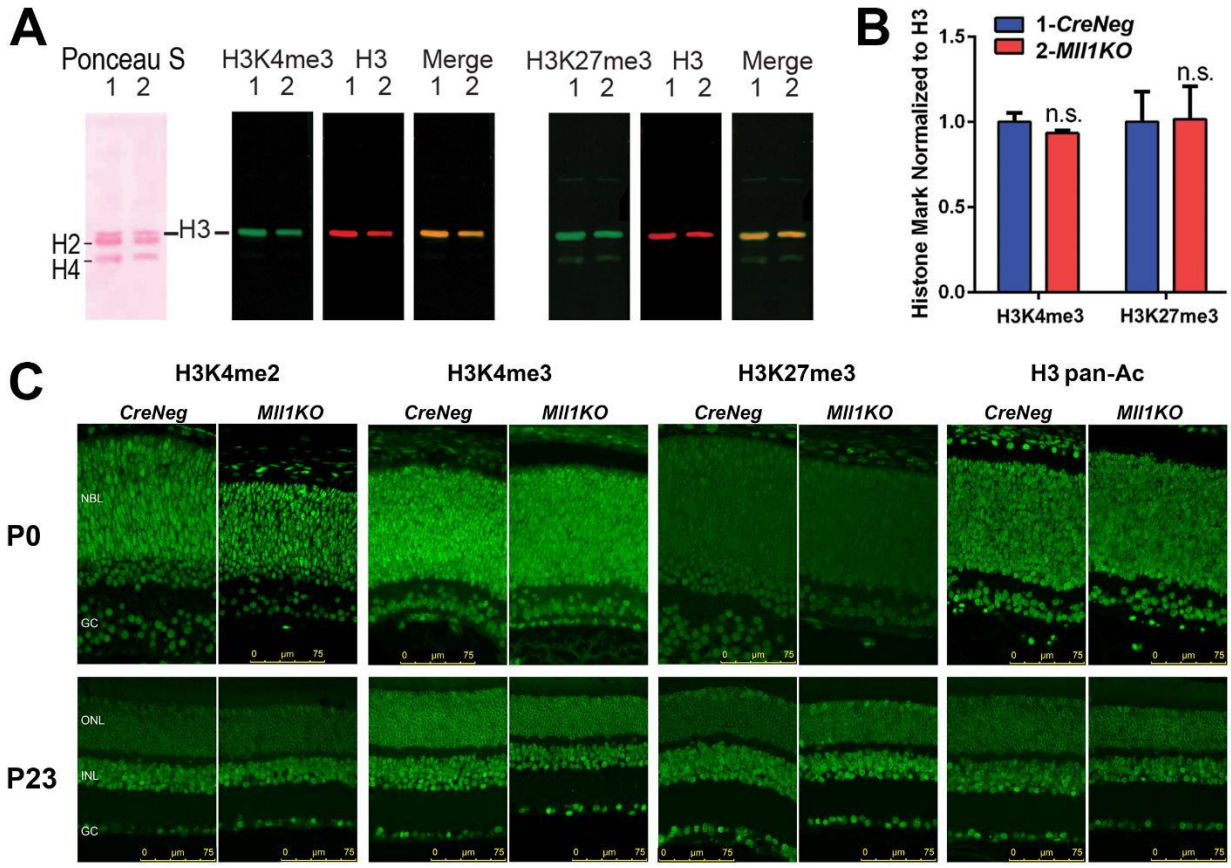
Supplementary Figure S3: Proliferation of retinal progenitor cells (RPCs) decreases in postnatal *Mll1KO* mice. (**A**) *Mll1KO* retinas show progressively fewer EdU-labeled cells after a 4-hour pulse than *CreNeg* littermates. Results are shown as mean + SEM. **p*<0.05; ****p*<0.0001 by two-way ANOVA with Tukey's multiple comparisons (n=3). (**B**) P0 retinal cross sections of *CreNeg* and *Mll1KO* retinas were immunolabeled with anti-Cyclin D1 (CCND1) (green) for proliferating cells. (**C**) Quantification of labeled cells in 100umX100um areas across each section, shown as mean + SEM. No significant differences (n.s.) were detected between the two genotypes. (**D, E**) Retinal cross-sections from the same mice as in Panel B were immunolabeled for the cyclin-dependent kinase inhibitor $p27^{Kip1}$ and quantified. Scale bar = 20um. ****p*<0.001 by two-way repeated-measures ANOVA with Sidak's multiple comparisons test (n=3).



Supplementary Figure S4: Hierarchical cluster analysis of RNAseq results (shown as heatmap representing Spearman's Rank Correlation Coefficient) shows a clear distinction between the *Mll1KO* ("KO") and *C57Bl/6J* ("WT") libraries sequenced, as well as reproducibility between the three biological replicate libraries ("a", "b" and "c") representing each genotype.



Supplementary Figure S5: Expression changes of cell type-specific gene sets. (**A**) Gene expression (*Mll1KO* vs *WT* [$\log_2$]) of cell-type-specific genes from 39 different retinal cell types, determined by drop seq (Macosko 2015) confirms overrepresentation of photoreceptor and glial gene expression. Subtype expression of Bipolar (BC) and Amacrine cell (AC) genes support a complex phenotype with some subtypes being more affected than others. (**B**) Overall, early-born GABA-ergic ACs are less affected than late-born non-GABA/non-Glycinergic (nGnG) ACs.



Supplementary Figure S6: Comparison of histone marks in *CreNeg* and *Mll1KO* retinas. (**A**) Histones were acid-extracted from P24 *CreNeg* (Lane 1) or *Mll1KO* (Lane 2) retinas as previously described (Hennig 2013). Western Blots were stained with Ponceau S for protein bands, then with the antibodies indicated (see Supplementary Table S2 for antibody information). "Merge" shows both channels together. (**B**) Quantification of band intensities, normalized to Histone H3. Results are presented as the mean+SEM of three experiments. (**C**) Histologic sections from P0 and P23 *CreNeg* and *Mll1KO* retinas were immunolabeled for the histone marks indicated. No obvious differences were seen between the different genotypes in the intensity, distribution, or nuclear pattern of staining at either age.

Supplementary Table S1: *Mll1* PCR Primer Sequences

Designation		Sequence	Location in NM_001081849
Primer Set 1	qRT-PCR Forward	5'- AGGAAGCCCAAGAAAGGACTC -3'	nt 10969-10989
	qRT-PCR Reverse	5'- AATCCCCAGCATCCGCAAAC-3'	nt 11148-11129
Primer Set 2	Forward deletion	5'-TGAGTACAACCCTAACGATGAGGAA -3'	nt 11349-11373
	Reverse deletion	5'- CGGAATCTCATGGGCATTG -3'	nt 11444-11426
Genotyping Primers	Mll1-Flox_F	5'- TCTCTGAAGTAAGCCTTTCTTAG -3'	(not in CDS)
	Mll1-Flox_R	5'- CAGTGGACATTCCAACTCTTCAA -3'	(not in CDS)

Supplementary Table S2: Antibodies

Antibody	Source	Species	Marks
Activated Caspase-3	R&D Systems AF835	Rabbit	Apoptotic cells
AP-2-alpha (AP-2a)	DSHB 3B5	Mouse	Amacrine cells
Brn3a	Millipore AB5945	Rabbit	GC
Calbindin D-28K	Sigma C9848	Mouse	HC, AC
Calbindin D-28K	Sigma C7354	Rabbit	HC, AC
Calretinin	Millipore ab5054	Rabbit	AC, HC
Chx10	Exalpha X1180P	Sheep	Progenitors, pan-BP
CtBP2	BD Biosciences 612044	Mouse	Cones, ribbon synapses
Cyclin D1	Sigma C7464	Mouse	Proliferating cells
Cyclin D3	Sigma AV03038	Rabbit	Proliferating cells
Glutamine Synthetase	BD Biosciences 610517	Mouse	Mueller glia
GlyT1	Millipore AB1770	Goat	Glycinergic AC
H3K4me2	Abcam 7766	Rabbit	Histone H3 di-methyl K4
H3K4me3	Millipore 07-473	Mouse	Histone H3 tri-methyl K4
H3K9me3	Abcam 8898	Rabbit	Histone H3 tri-methyl K9
H3K27me3	Upstate 07-449	Rabbit	Histone H3 tri-methyl K27
H3 pan-Ac	Millipore 106-599	Rabbit	acetylated Histone H3
Ki67	BD Biosciences 550609	Mouse	Proliferating cells
Lim1 (4F2)	DSHB AB 531784	Mouse	HC
MLL1	Bethyl A300-086A	Rabbit	MLL1
Neurofilament (NF-M)	DSHB 2H3	Mouse	Axons
Onecut 1 (HNF6)	Santa Cruz sc-13050	Rabbit	HC, Cones
Onecut 2	R&D AF6294	Sheep	HC
p27Kip1	BD 610241	Mouse	Cycling cells, MG
Pax6	DSHB Pax6	Mouse	Progenitors, AC
Phospho-histone H3	Milllipore 06-570	Rabbit	Mitotic cells
PKCa	Sigma P5704	Mouse	Rod BP
PNA-Rhodamine	Vector Labs RL-1072	(lectin)	Cones
Prox1	Millipore MAB5652	Mouse	HC, AC, BP
Rhodopsin (RetP1)	Sigma O4886	Mouse	Rods
Sall3	Sigma HPA016656	Rabbit	Cones, HC
VGluT1	Millipore AB5905	Guinea Pig	PR synaptic terminals

Supplementary Table S3. HC Gene PCR Primer Sequences

Gene Name (Refseq #)	Designation	Sequence	Location
Cx57 **(NM_010289)** (gap jct a10; Gja10)	Cx57 F1 Cx57 R1	5'- GGTTTGGACAGACAGATTAGG -3' 5'- GTGATGGGCTATTTTCTCCG -3'	nt 1251-1270 Exon 2 nt 1405-1386 Exon 2
OC1 **(NM_008262)** (*Hnf6*)	Oc1 F1 Oc1 R1	5'- GGAAAGAGCAAGAACACGG -3' 5'- GATGAGGACGATGAACTGC -3'	nt 1491-1509; Exon 2 nt 1752-1694; Exon 2
Prox1 **(NM_008937)**	Prox1 F4 Prox1 R4	5'- CAGAAGGACTCTCTTTGTCAC -3' 5'- GCTGAACCACTTGATGAGC -3'	nt 2120-2140, Exon 2 nt 2355-2337, Exon 4

REFERENCES

Hennig, A. H., Peng, G.-H., & Chen, S. Transcription coactivators p300 and CBP are necessary for photoreceptor-specific chromatin organization and gene expression. *PLoS ONE* **8**, e69721, doi:10.1371/journal.pone.0069721 (2013).

Macosko, E. Z. *et al.* Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell* **161**, 1202-1214, doi:10.1016/j.cell.2015.05.002 (2015).