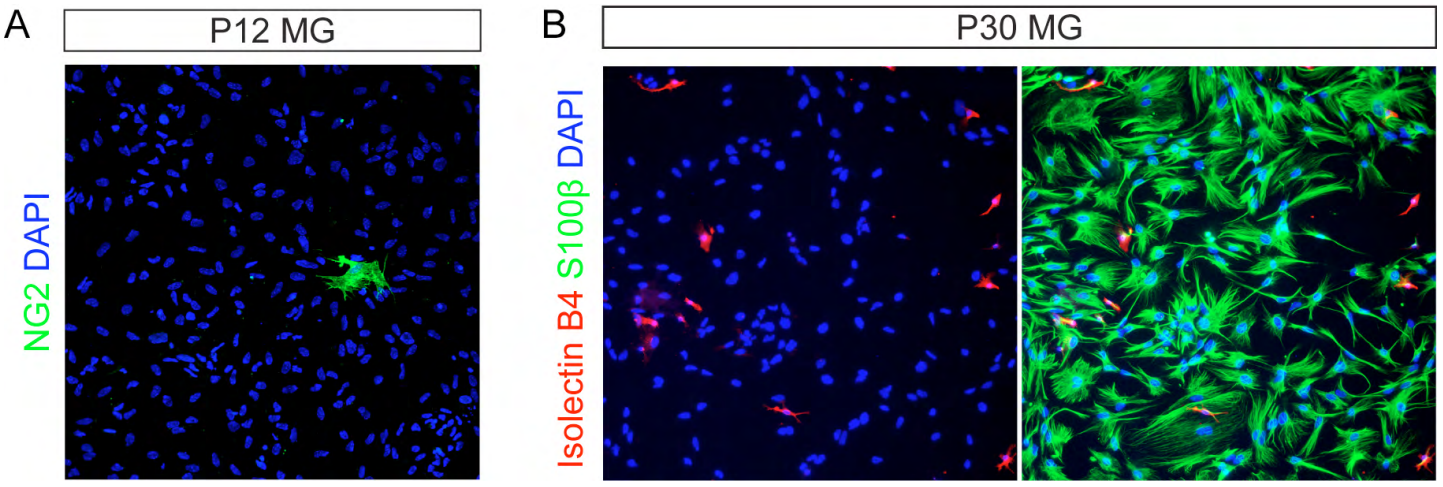


Fig. S1. P12 and P30 MG cultures have low contamination of other cell types. Related to Figs 1, 2. (**A**) Less than 1% of P12 MG labeled with the pericyte marker NG2 after 1 week in culture. (**B**) NMDA-damaged P30 retinas were dissociated 2 days after damage and cultured for one week. The majority of cells label with glial marker S100β, and few cells express the endothelial marker isolectin B4.

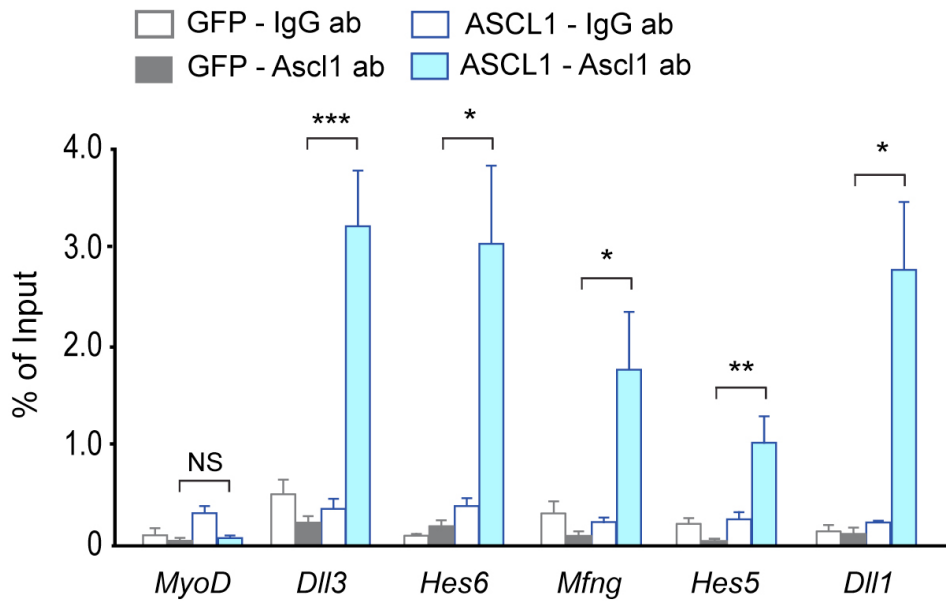


Fig. S2. ASCL1 does not bind predicted targets in GFP-infected MG. Related to Fig. 2. ChIP for Ascl1 or control IgG antibodies at the 5′ promoter region of genes indicated. Significant enrichment of Ascl1 in ASCL1-infected P12 MG compared with GFP-infected P12 MG was observed. Data are mean ± s.e.m. **P*<0.05, ***P*<0.01, ****P*<0.001 by Student's *t*-test.

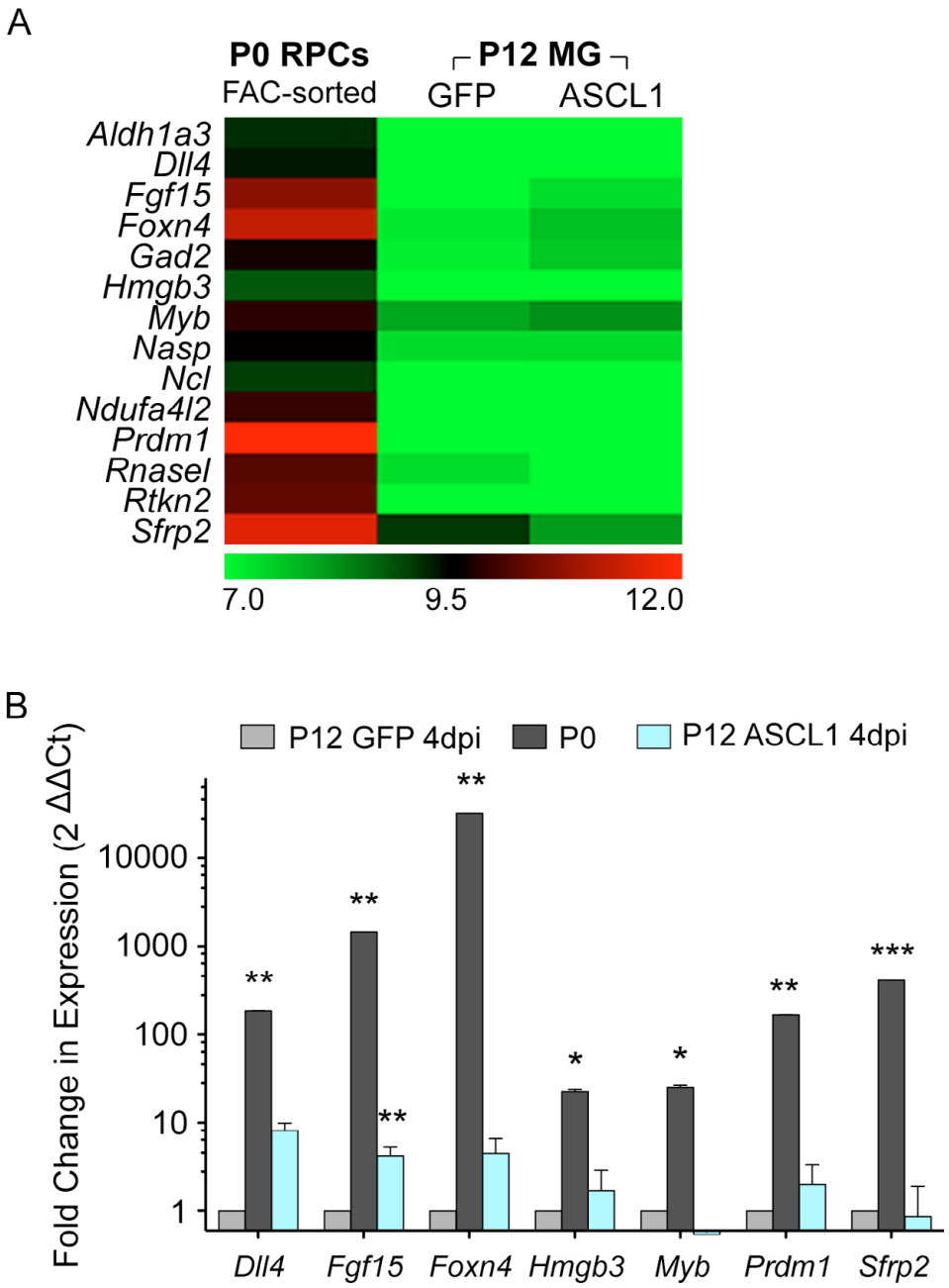


Fig. S3. A subset of progenitor-specific genes are not reactivated in MG by ASCL1 alone. Related to Fig. 2. (**A**) A subset of genes on microarray analysis at 4 dpi are highly expressed (log transformed normalized values) in P0 progenitors but do not upregulate after ASCL1 infection of MG. (**B**) Expression patterns were confirmed by qPCR. Data are mean ± s.e.m. **P*<0.05, ***P*<0.01, ****P*<0.001 by Student's *t*-test.

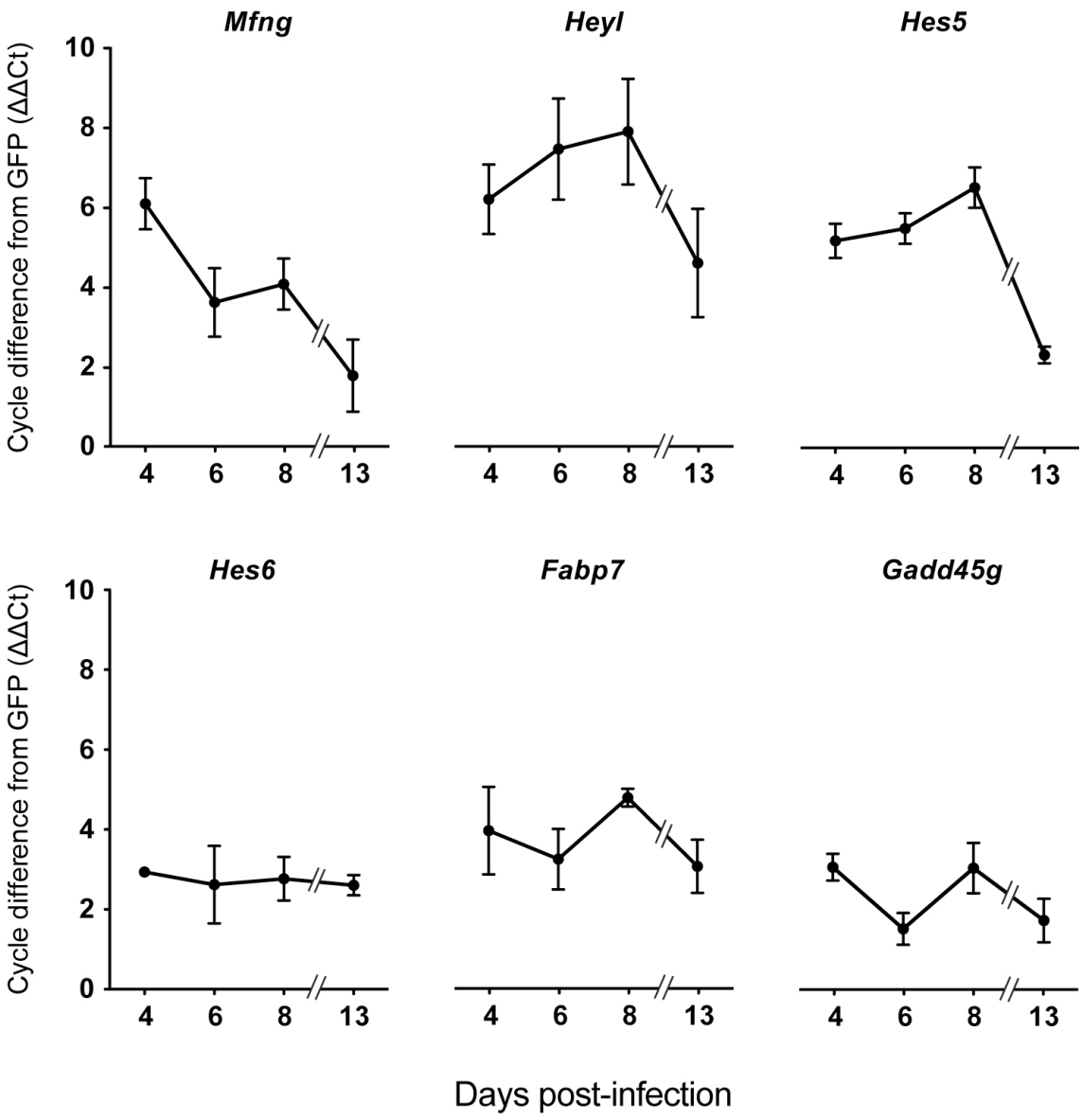


Fig. S4. Timecourse of expression of retinal progenitor genes. Related to Fig. 2. Progenitor genes are enriched in P12 ASCL1-infected MG at 4, 6 and 8 dpi compared with GFP-infected controls from paired data. By 13 dpi, *Mfng*, *Heyl* and *Hes5* are greatly decreased. Data are mean ± s.e.m.

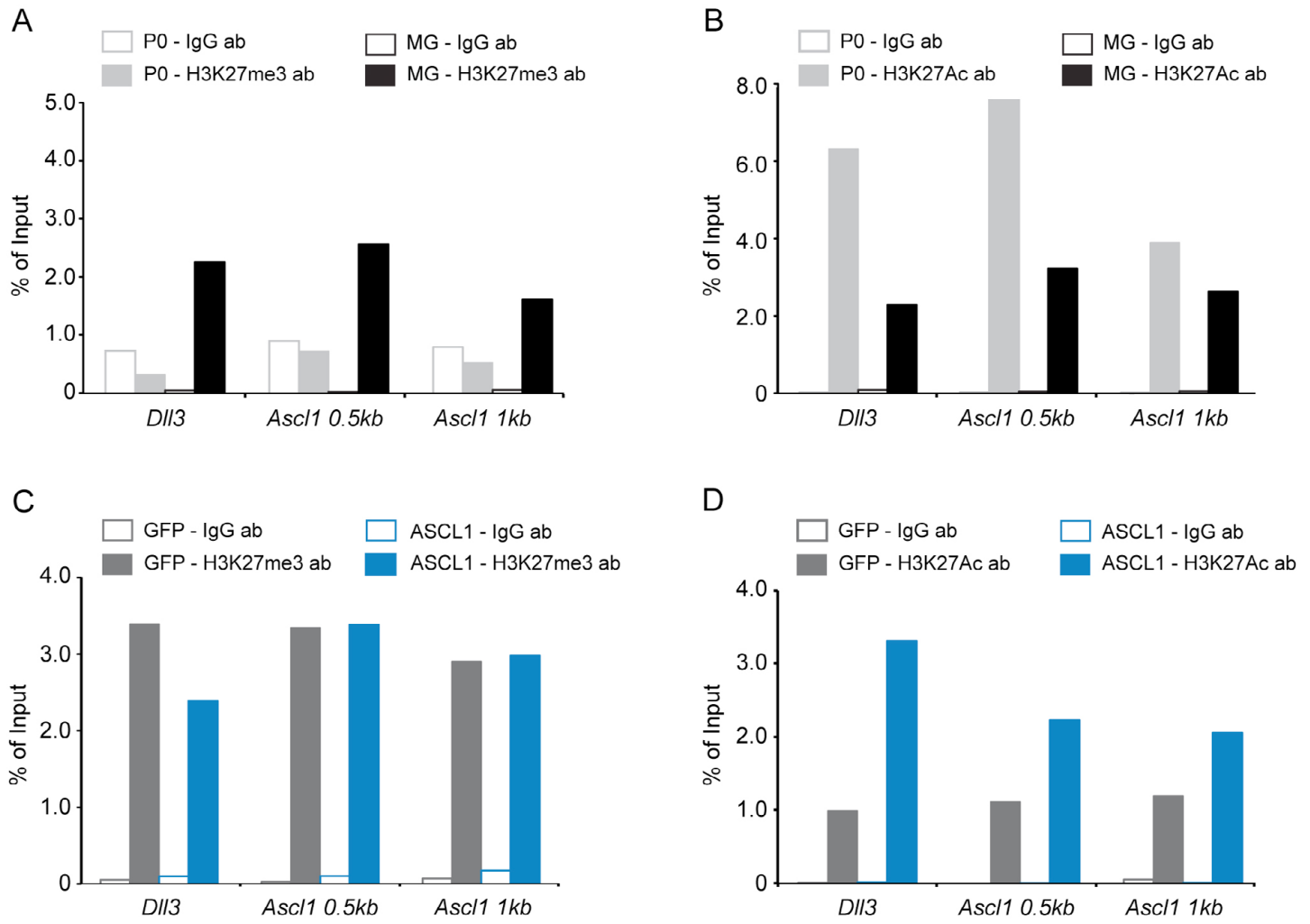


Fig S5. The *Ascl1* promoter is repressed during MG development and partially remodeled by *ASCL1* expression. Related to Fig. 5. ChIP for H3K27me3 or H3K27Ac versus IgG control antibodies at two locations in the *Ascl1* 5′ promoter, 500 bp and 1 kb upstream of the transcription start site. *Dll3* is included as a control progenitor gene locus consistent with Fig. 5. (**A**,**B**) The Ascl1 promoter shows an increase in tri-methylation (A) and a decrease in acetylation (B) between P0 progenitors and P12 MG. (**C**,**D**) ASCL1 infection of MG at 4 dpi reverses the acetylation (D), but not the tri-methylation (C) trend.

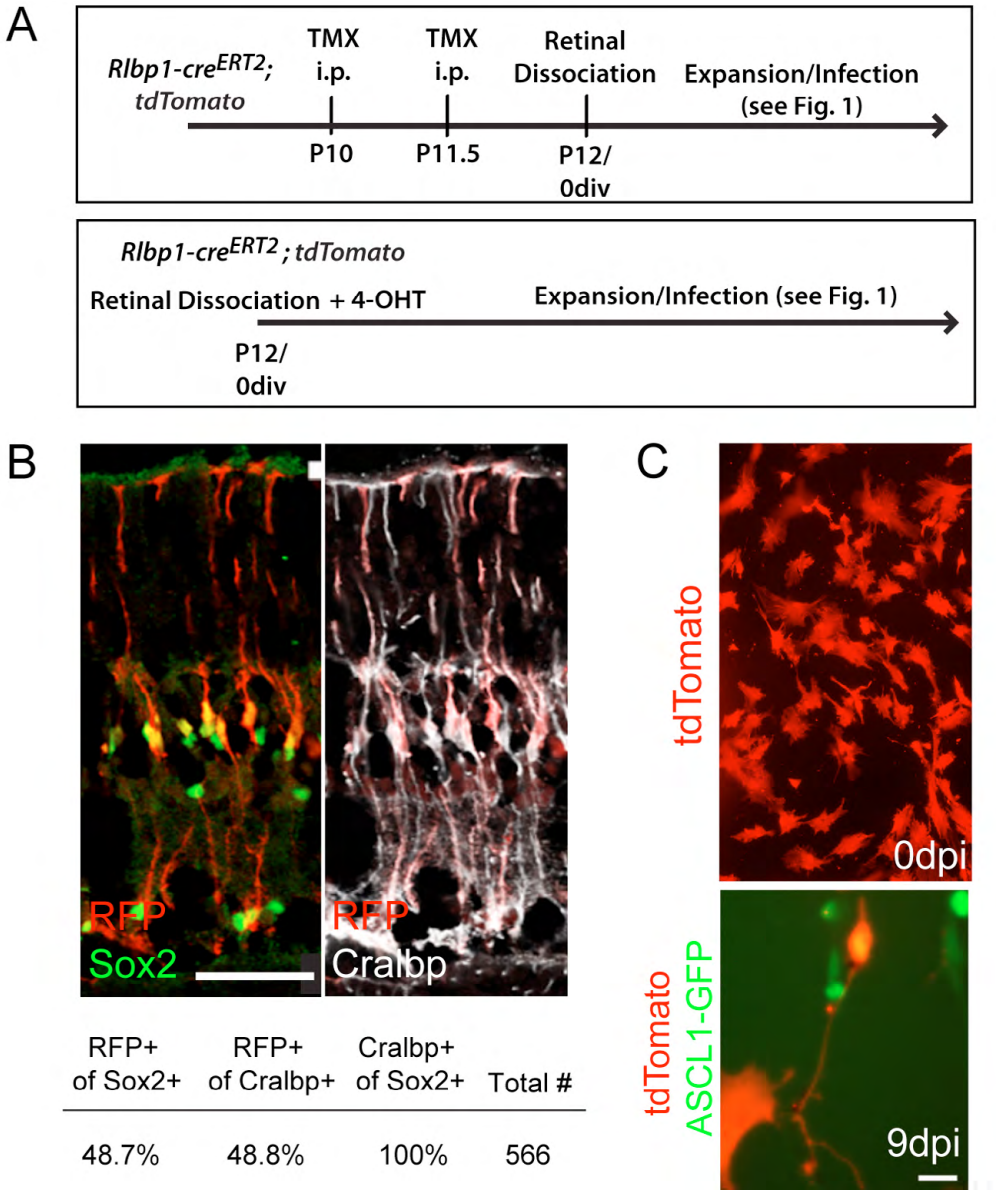


Fig. S6. Genetic fate mapping of cells derived from *Rlbp1-cre^{ERT2};tdTomato* P12 MG. Related to Fig. 7. (**A**) Experimental diagram. *Rlbp1-cre^{ERT2};R26-flox-stop-tdTomato* retinas express tdTomato in Cralbp+ MG after tamoxifen (TMX) administration. Tamoxifen was injected at P10 and P11.5 or 4-hydroxy-tamoxifen (4-OHT) was added to MG cultures to drive cre into the nucleus. MG were grown and infected as described in Fig. 1. (**B**) RFP+ cells in P12 *Rlbp1-cre^{ERT2};tdTomato* retinal cryosections colabel with glial markers Sox2 and Cralbp. Approximately 50% of RFP+ cells label with glial markers when TMX is administered *in vivo* (A, upper panel). (**C**) Dissociated P12 *Rlbp1-cre^{ERT2};tdTomato* ASCL1-infected MG cultures. By 9 dpi, ASCL1-GFP+tdTomato+ cells with a neuronal appearance are observable. Scale bars: 20 μm (C); 50 μm (B).

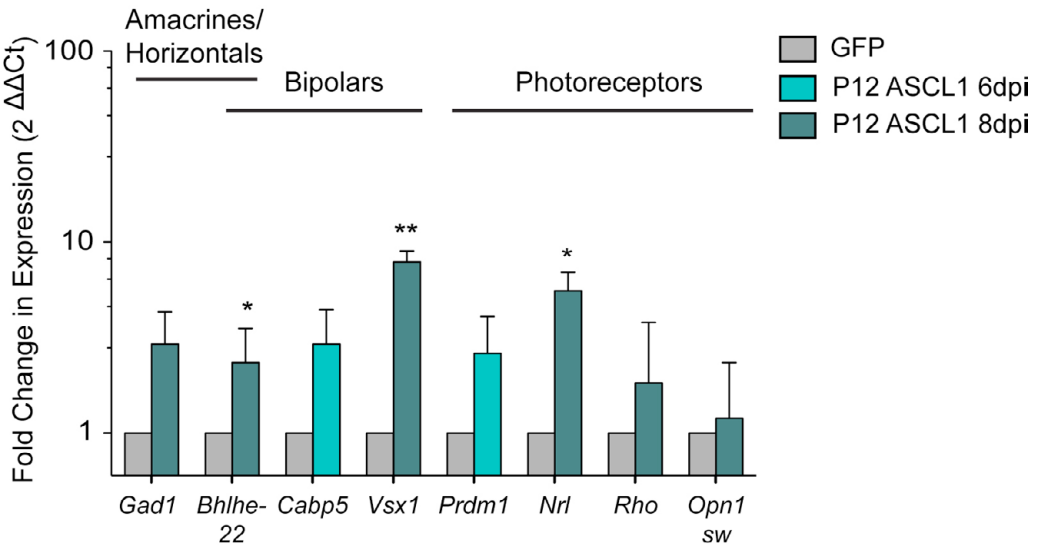


Fig. S7. Expression of late retinal neuronal genes. Related to Fig. 8. Genes specific to differentiated bipolar and amacrine cells are upregulated in P12 ASCL1-infected MG by qPCR. Data are mean ± s.e.m. Student's *t*-test, **P*<0.05, ***P*<0.01.

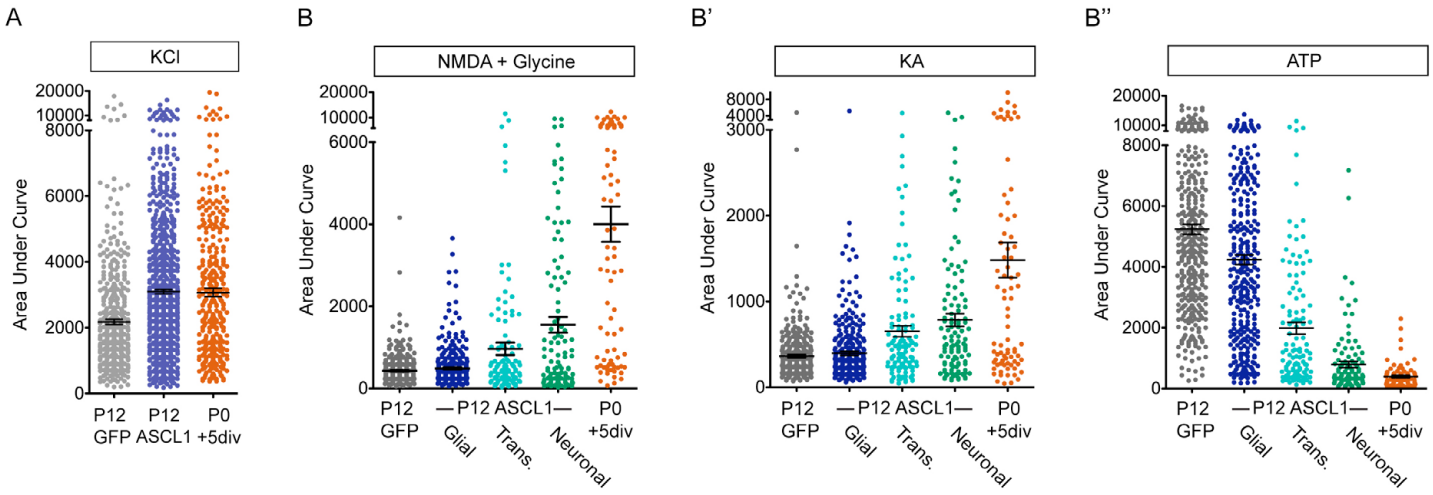


Fig. S8. Morphology of ASCL1-infected MG is associated with calcium responsiveness. Related to Fig. 9. (**A**) ΔF340/380 responses to KCl application, as measured by area under the curve. (**B-B''**) Classification of ASCL1-infected MG from Fig. 9 by morphology (glial, transitional or neuronal). Neuronal- and transitional-like cells were more responsive to NMDA (B) and KA (B') and less responsive to ATP (B'') compared with cells with a glial appearance.

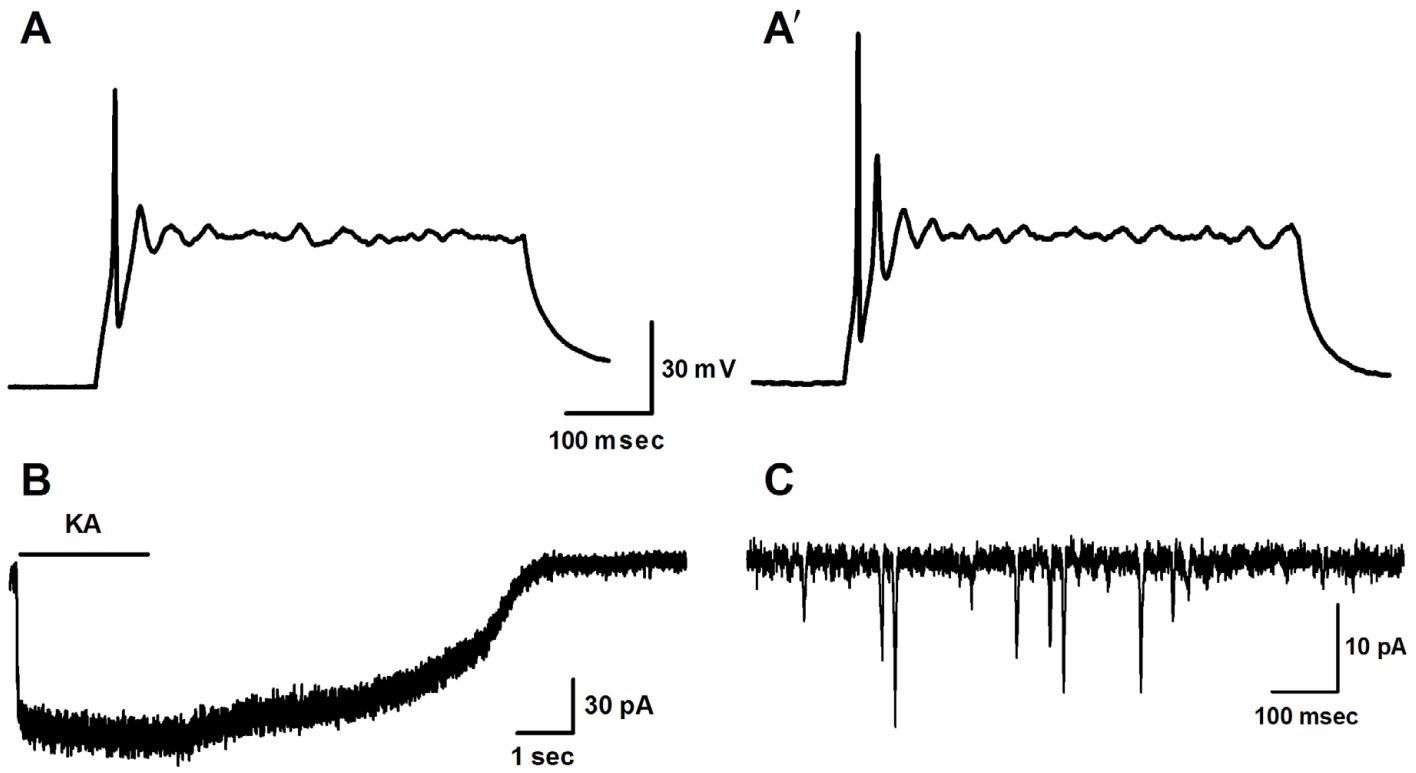
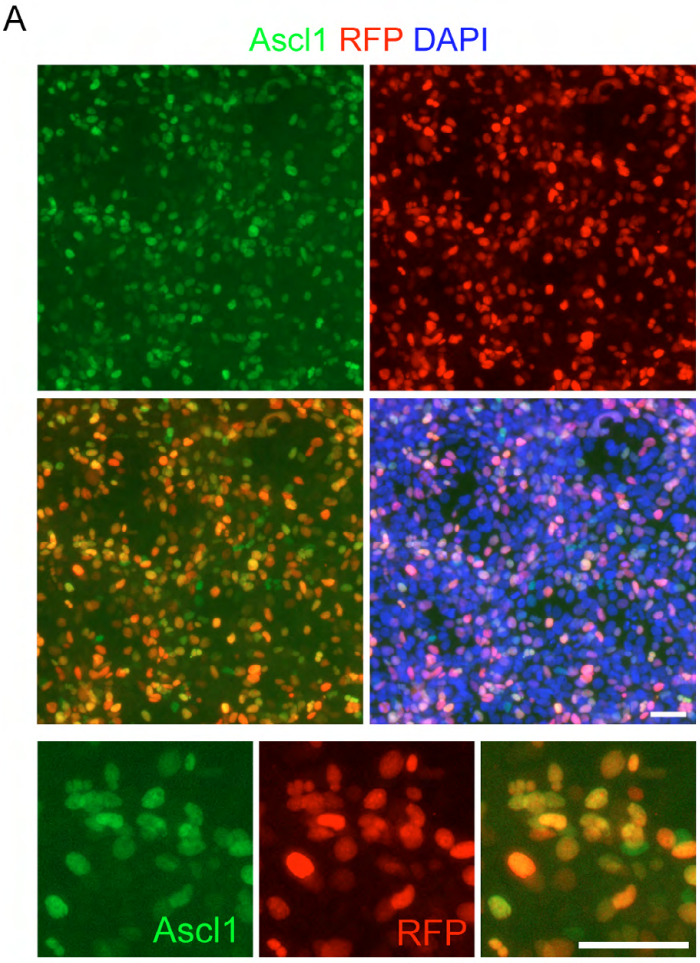
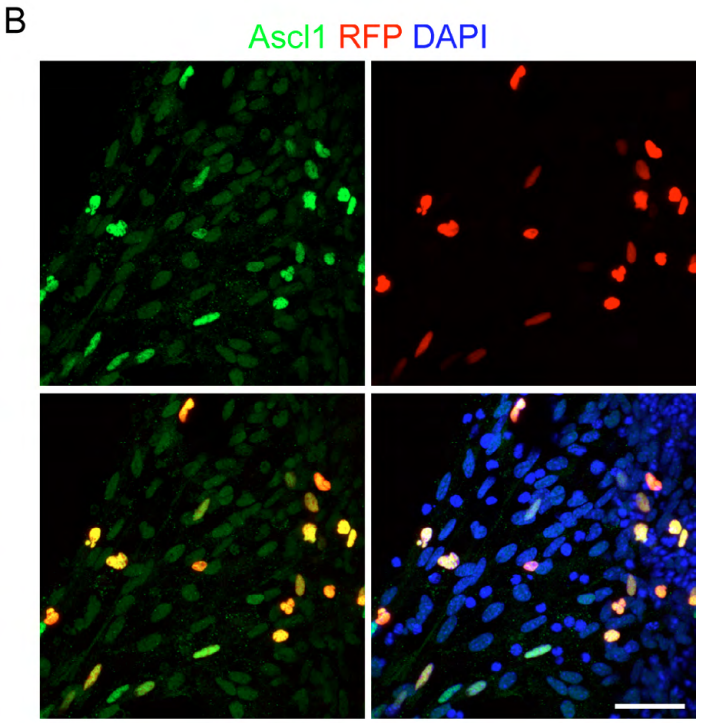


Fig. S9. ASCL1-infected MG-derived cells have neuron-like electrophysiological responses. Whole-cell patch clamp. (**A**,**A′**) Current clamp recordings of action potentials evoked in two different ASCL1– GFP+ cells with neuronal morphology (15 dpi) in response to a 40 pA current step lasting 500 mseconds. (**B**) Voltage clamp recording of response (same cell as in A) to a 3-second application of 50 µm kainate (KA). (**C**) Voltage clamp recording of spontaneous miniature postsynaptic potentials in a third ASCL1– GFP+ cell (20 dpi) co-cultured for 18 days with retinal neurons dissociated from P0 animals.





# RFP+ /Ascl1-	# Ascl1+ /RFP-	Total #	% RFP+ of Ascl1+	% Ascl1+ of RFP+
14	26	887	97.0	98.4

Fig. S10. Validation of *tetO-hASCL1-IRES-mCherryn* plasmid expression. Related to Fig. 10. (**A**) Stable rtTA-expressing HEK293T cells were infected with *tetO-hASCL1-IRES-mCherryn* lentiviral particles in the presence of 750 ng/ml doxycycline and fixed at 3 dpi. Ninety-seven percent of ASCL1+ cells immunolabeled for RFP and 98.4% of RFP+ cells labeled with Ascl1, indicating that mCherry/RFP can reliably be used as a marker for ASCL1 expression. (**B**) Ascl1 and mCherry markers colocalize in *αPax6-cre;R26-stop-flox-rtTA* P12 retinal explants infected with *tetO-hASCL1-IRES-mCherryn.* Scale bars: 50 μm (A); 100 μm (B).

Table S1. Primer sequences used for qPCR and ChIP analyses

qPCR primers

Gene name	F sequence (5′ to 3′)	R sequence (5′ to 3′)
Actb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
ASCL1 (human)	CATCTCCCCCAACTACTCCA	CAGTTGGTGAAGTCGAGAAGC
Atoh7	ATCACCCCTACCTCCCTTTCC	CGAAGAGCCTCTGCCCATA
Bhlhe22	TGAACGACGCTCTGGATGAG	GGTTGAGGTAGGCGACTAAGC
Cabp5	ATGAGAACGATGGGTTACATGC	CACGGCCACCAAGGTTCATT
Cralbp	GGCACTTTCCGCATGGTTC	CCGGGTCTCCTCCTTTTCAT
Crx	TCTCTCACCTCAGCCCCTTAT	ACCCACTGAAATAGGAACTTGGA
Dll1	CAGGACCTTCTTTCGCGTATG	AAGGGGAATCGGATGGGGTT
Dll4	TTCCAGGCAACCTTCTCCGA	ACTGCCGCTATTCTTGTCCC
Fabp7	GGACACAATGCACATTCAAGAAC	CCGAACCACAGACTTACAGTTT
Fgf15	TCACATGGACCCTGTTGTGT	CAGCAGCCTCCAAAGTCAGT
Foxn4	CATGAAGGAGCACTTCCCCTA	TTTCCGGGCGGTCTGAGAT
Gad1	CACAGGTCACCCTCGATTTTT	ACCATCCAACGATCTCTCTCATC
Gadd45g	GGGAAAGCACTGCACGAACT	AGCACGCAAAAGGTCACATTG
Hes5	AGTCCCAAGGAGAAAAACCGA	GCTGTGTTTCAGGTAGCTGAC
Hes6	ACCACCTGCTAGAATCCATGC	GCACCCGGTTTAGTTCAGC
Heyl	CAGCCCTTCGCAGATGCAA	CCAATCGTCGCAATTCAGAAAG
Hmgb3	AGGTGACCCCAAGAAACCAAA	GCAAAATTGACGGGAACCTCTG
Isl1	TATCCAGGGGATGACAGGAAC	GCTGTTGGGTGTATCTGGGAG
Mfng	ATGCACTGCCGACTTTTTCG	CCTGGGTTCCGTTGGTTCAG
Myb	GAGCACCCAACTGTTCTCG	CACCAGGGGCCTGTTCTTAG
Mycn	ACCATGCCGGGGATGATCT	ATCTCCGTAGCCCAATTCGAG
Neurod1	ATGACCAAATCATACAGCGAGAG	TCTGCCTCGTGTTCCTCGT
Neurod4	AGCTGGTCAACACACAATCCT	GTTCCGAGCATTCCATAAGAGC
Neurog2	AACTCCACGTCCCCATACAG	GAGGCGCATAACGATGCTTC
Nrl	TCCCAGTCCCTTGGCTATGG	CACCGAGCTGTATGGTGTG
Olig2	GCTCACCAGTCGCTTCATCT	GCGCGAAACTACATCCTCAT
Opn1sw	CAGCATCCGCTTCAACTCCAA	GCAGATGAGGGAAAGAGGAATGA
Opn2	CCCTTCTCCAACGTCACAGG	TGAGGAAGTTGATGGGGAAGC
Otx2	TATCTAAAGCAACCGCCTTACG	AAGTCCATACCCGAAGTGGTC
Prdm1	TTCTCTTGGAAAAACGTGTGGG	GGAGCCGGAGCTAGACTTG
Prox1	AGAAGGGTTGACATTGGAGTGA	TGCGTGTTGCACCACAGAATA
Sfrp2	CGTGGGCTCTTCCTCTTCG	ATGTTCTGGTACTCGATGCCG
Slc1a3	ACCAAAAGCAACGGAGAAGAG	GGCATTCCGAAACAGGTAACTC
Tubb3	TAGACCCCAGCGGCAACTAT	GTTCCAGGTTCCAAGTCCACC
Turbo-Gfp	GACCAAGACTGGGGAGATCA	ACAGCCACAATGGTGTCAAA
Vsx1	GAGGCACAGGACGGTTTTCA	AGCTCTGTTTTCGCAGCCA

ChIP primers

Gene name	F sequence (5′ to 3′)	R sequence (5′ to 3′)
MyoD	GGCTTTTAGGCTACCCTGGAT	TGGTGAAGAAAGCAGTCGTG
Hes5	TTCCCACAGCCCGGACATT	GCGCACGCTAAATTGCCTGTGAAT
Dll1	AGCTCTTTCTCTCCGCATTG	CTGTTATTGTGCGAGGCTGA
Hes6	CATGTCAATGCACCGATTGGC	GCCTAAGTGGCAGGAGGTC
Dll3	TGCCCGAAGACTGAAGACTAATT	TGGGCTCAGGAAGGTGTGA
Ascl1 0.5 kb	GCCACTCCTCTGAAAGATGC	TTTATTCCACACAGCCCACA
Ascl1 1 kb	CAGGGAAGGGTTTAGGCAGA	CTCTCCCCTCCTACCTTCCT

Table S2. GO analysis of most highly regulated genes after Ascl1 infection of P12 MGs

GO term	Description	*P*-value	FDRq-value	Enrichment
GO:0007399	nervous system development	1.14E-15	1.29E-11	4.95
GO:0060284	regulation of cell development	1.37E-14	7.71E-11	2.48
GO:0045664	regulation of neuron differentiation	1.81E-14	6.81E-11	2.97
GO:0050767	regulation of neurogenesis	3.41E-14	9.63E-11	2.76
GO:0023051	regulation of signaling	8.08E-14	1.82E-10	2.18
GO:0050804	regulation of synaptic transmission	8.42E-14	1.58E-10	5.32
GO:0051969	regulation of transmission of nerve impulse	9.50E-14	1.53E-10	4.98
GO:0010646	regulation of cell communication	9.96E-14	1.40E-10	2.17
GO:0048731	system development	1.08E-13	1.35E-10	3.29
GO:0050793	regulation of developmental process	2.17E-13	2.45E-10	2.04
GO:0051960	regulation of nervous system development	2.89E-13	2.97E-10	2.59
GO:0071840	cellular component organization or biogenesis	3.90E-13	3.67E-10	1.4
GO:0048869	cellular developmental process	1.03E-12	8.97E-10	2.49
GO:0007275	multicellular organismal development	1.26E-12	1.01E-09	2.98
GO:0031644	regulation of neurological system process	1.45E-12	1.09E-09	4.56
GO:0051239	regulation of multicellular organismal process	1.63E-12	1.15E-09	2.03
GO:0032502	developmental process	2.91E-12	1.93E-09	2.02
GO:0016043	cellular component organization	3.94E-12	2.47E-09	1.39
GO:0044708	single-organism behavior	4.43E-12	2.63E-09	3.89
GO:0045595	regulation of cell differentiation	5.65E-12	3.19E-09	2.19
GO:0042391	regulation of membrane potential	5.89E-11	3.17E-08	3.01
GO:0007610	behavior	8.25E-11	4.23E-08	3.42
GO:0022402	cell cycle process	1.94E-10	9.51E-08	1.85
GO:0048856	anatomical structure development	2.82E-10	1.33E-07	1.92
GO:2000026	regulation of multicellular organismal development	4.78E-10	2.16E-07	2
GO:0044057	regulation of system process	5.54E-10	2.41E-07	3.11
GO:0007219	Notch signaling pathway	7.24E-10	3.03E-07	14.14
GO:0048522	positive regulation of cellular process	8.41E-10	3.39E-07	1.57
GO:0030154	cell differentiation	1.34E-09	5.23E-07	2.41
GO:0007268	synaptic transmission	1.36E-09	5.11E-07	4.08
GO:0050806	positive regulation of synaptic transmission	5.72E-09	2.08E-06	8.07
GO:0031646	positive regulation of neurological system process	5.85E-09	2.06E-06	7.44
GO:0048646	anatomical structure formation involved in morphogenesis	6.14E-09	2.10E-06	3.27
GO:0051128	regulation of cellular component organization	6.88E-09	2.28E-06	1.54
GO:0044767	single-organism developmental process	1.17E-08	3.76E-06	1.96
GO:0007049	cell cycle	1.34E-08	4.20E-06	1.8
GO:0051971	positive regulation of transmission of nerve impulse	1.36E-08	4.14E-06	7.63
GO:0048518	positive regulation of biological process	1.67E-08	4.97E-06	1.61
GO:0006260	DNA replication	1.72E-08	4.99E-06	2.7