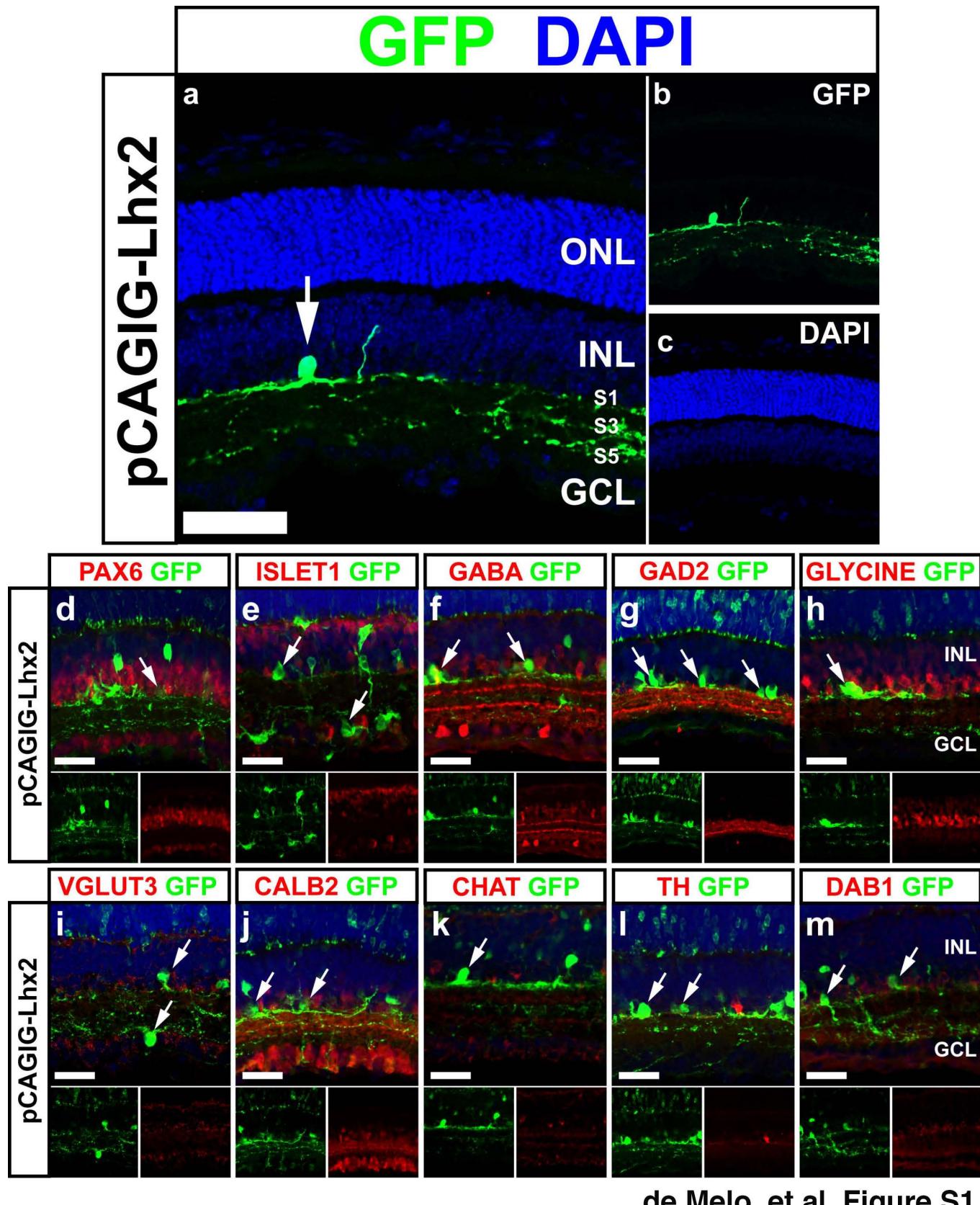
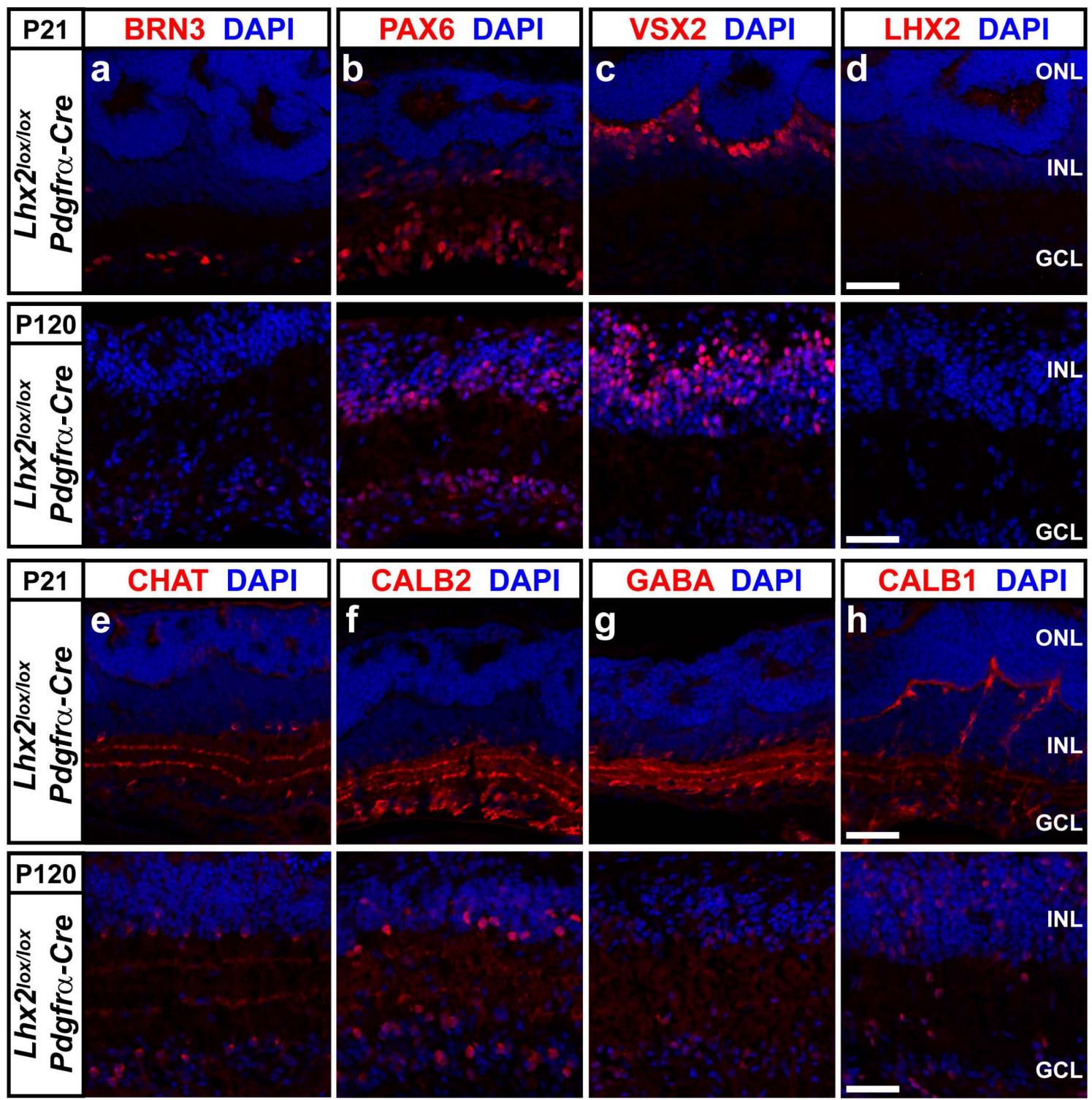


Supplemental Figures

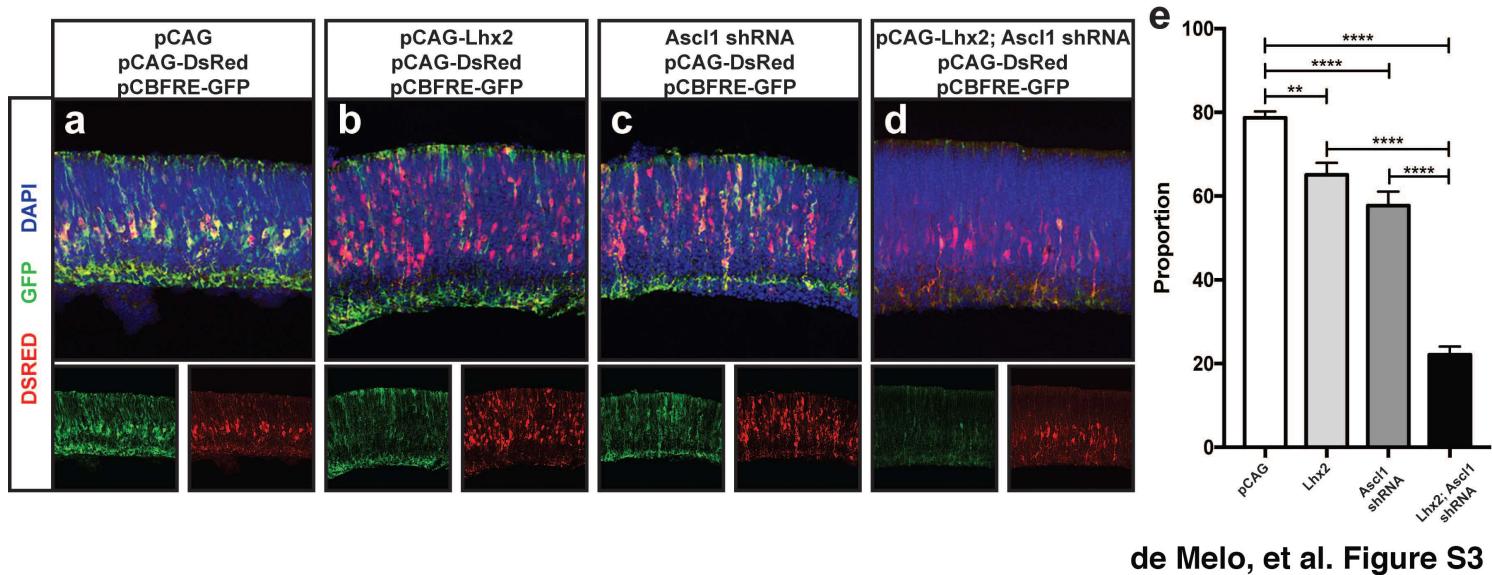


Supplementary Figure 1. Electroporation of *Lhx2* promotes the formation of wfACs. (a-c) Morphology of a wfAC generated following electroporation of *Lhx2*. (d) Generated wide field amacrine cells co-express the pan-amacrine marker PAX6. (e-m) Co-labeling with amacrine cell subtype selective markers reveals that amacrine cells generated by *Lhx2* electroporation do not fall within any well-established molecular category. ISLET1, CHAT- cholinergic starburst amacrine cells; GABA, GAD2- GABAergic amacrine cells; GLYCINE- glycinergic amacrine cells; VGLUT3- glutamatergic amacrine cells; CALB2- mixed population primarily All amacrine cells, A19 amacrine cells, and non-All glycine immunoreactive amacrine cells; TH- dopaminergic wide field amacrine cells; DAB1- All amacrine cells. Arrows indicate wfACs throughout. GCL, ganglion cell layer; INL, inner nuclear layer; outer nuclear layer; s inner plexiform layer sublamina. Scale bars, 50 µm (all panels).



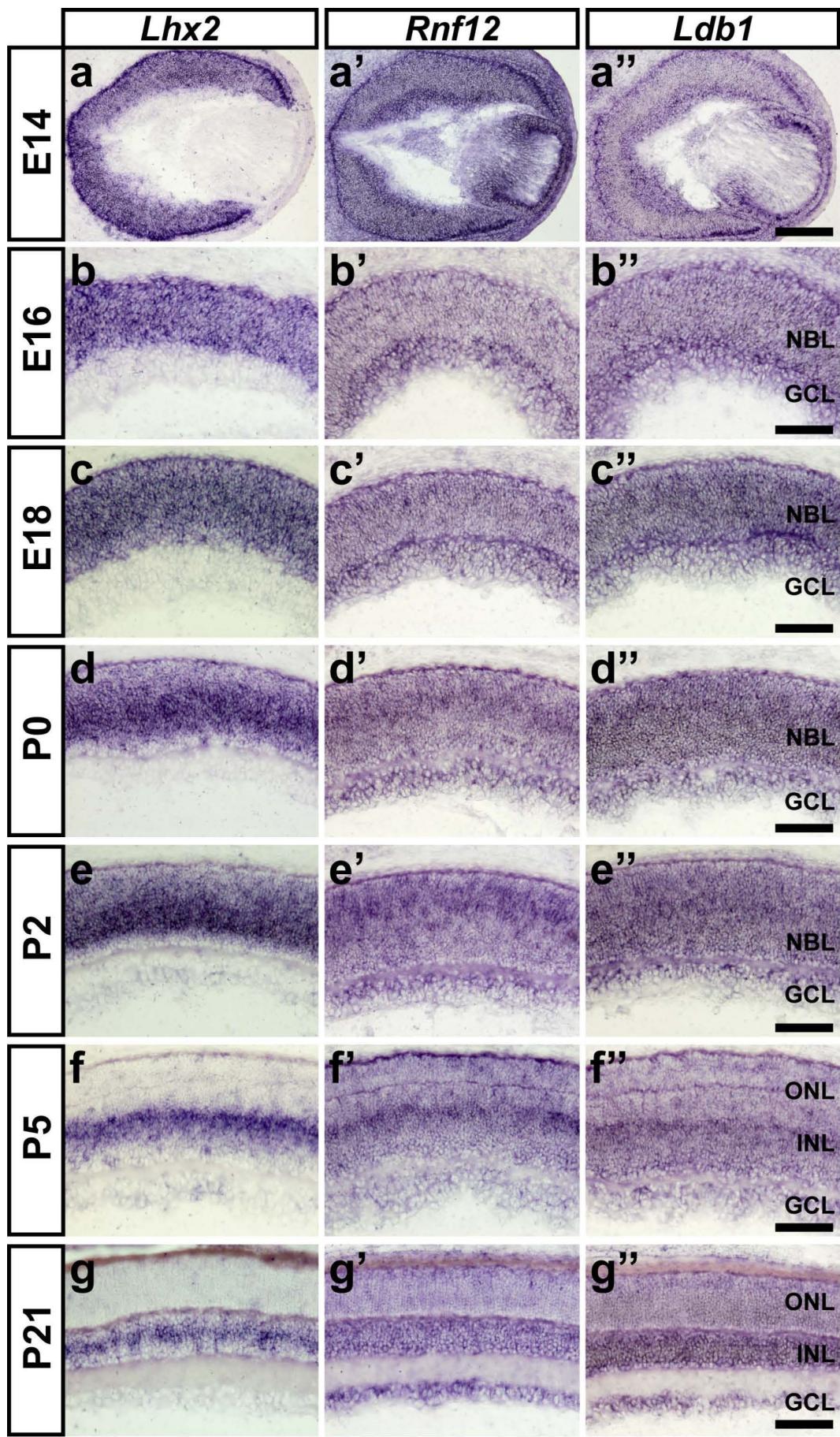
de Melo, et al. Figure S2

Supplementary Figure 2. (a-d) Expression of inner retinal cell class markers at P21 and P120 in *Pdgfra-Cre; R26YFP; Lhx2^{lox/lox}* retinas. Expression of the retinal ganglion cell marker Brn3 (a), retinal ganglion, amacrine, and horizontal cell marker Pax6 (b), and bipolar cell marker Vsx2 (c) are detectable at both P21 and P120. (d) Expression of Lhx2 is not detectable at both P21 and P120. (e-h) expression of amacrine cell subclass specific markers at P21 and P120 in *Pdgfra-Cre; R26YFP; Lhx2^{lox/lox}* retinas. Expression of choline acetyltransferase, Chat (e), calretinin, Calb2 (f), GABA, (g), and calbindin, Calb1 (h) are detectable at both P21 and P120. Scale bars, 50 μ m (d, h).



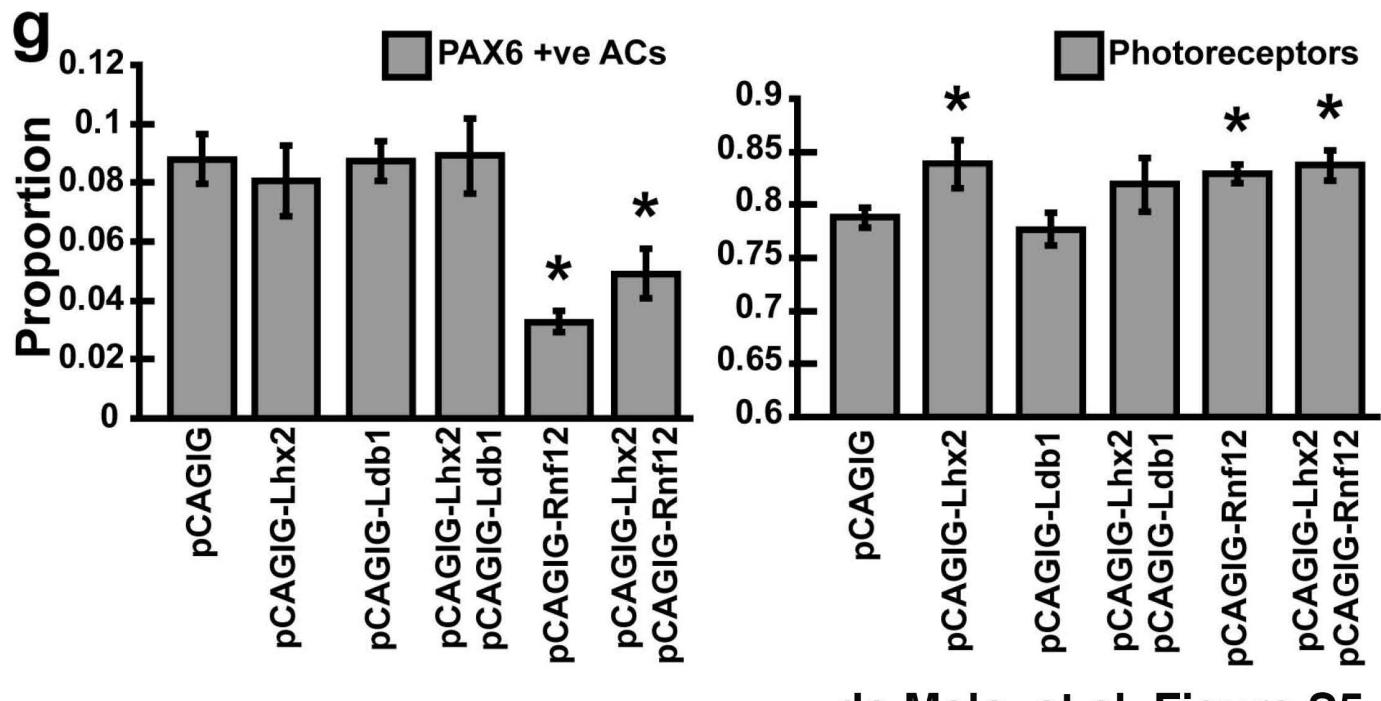
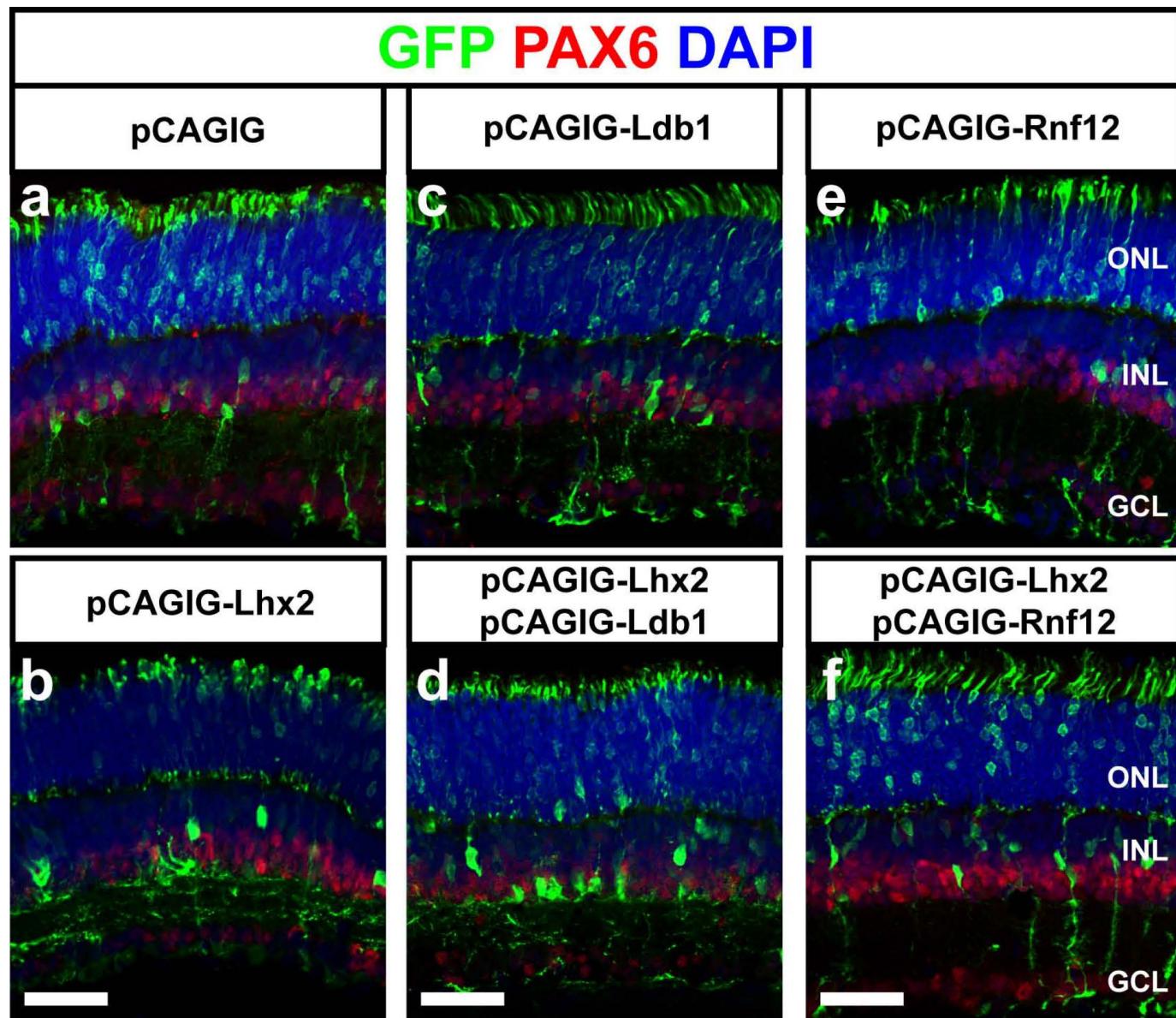
de Melo, et al. Figure S3

Supplementary Figure 3. Knockdown of *Ascl1* exacerbates *Lhx2*-mediated Notch inhibition. (a-d) Examples of *ex vivo* explant electroporations of P0 retinas cultured 2 days *in vitro* expressing the pCBFRE-GFP (Notch reporter), an electroporation control (pCAG-DsRED) and either: (a) pCAG control construct, (b) pCAG-Lhx2, (c) *Ascl1 shRNA* or (d) pCAG-Lhx2 and *Ascl1 shRNA*. (e) Quantification of the proportions of electroporated cells (red) that are expressing the pCBFRE-GFP transgene, with significant findings from a one-way ANOVA ($p < 0,0001$) analysis and Tukey multiple comparisons test indicated. *

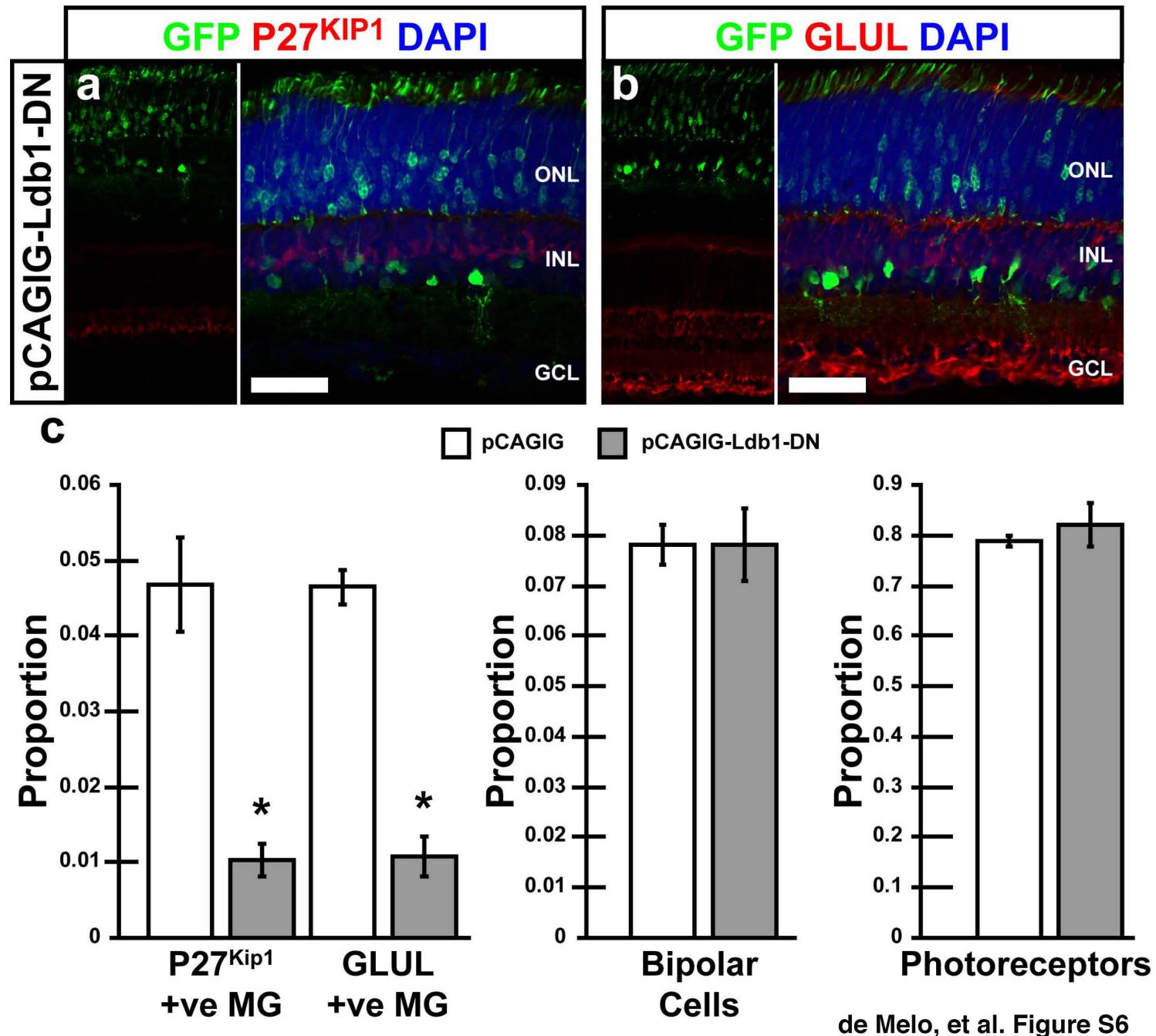


de Melo, et al. Figure S4

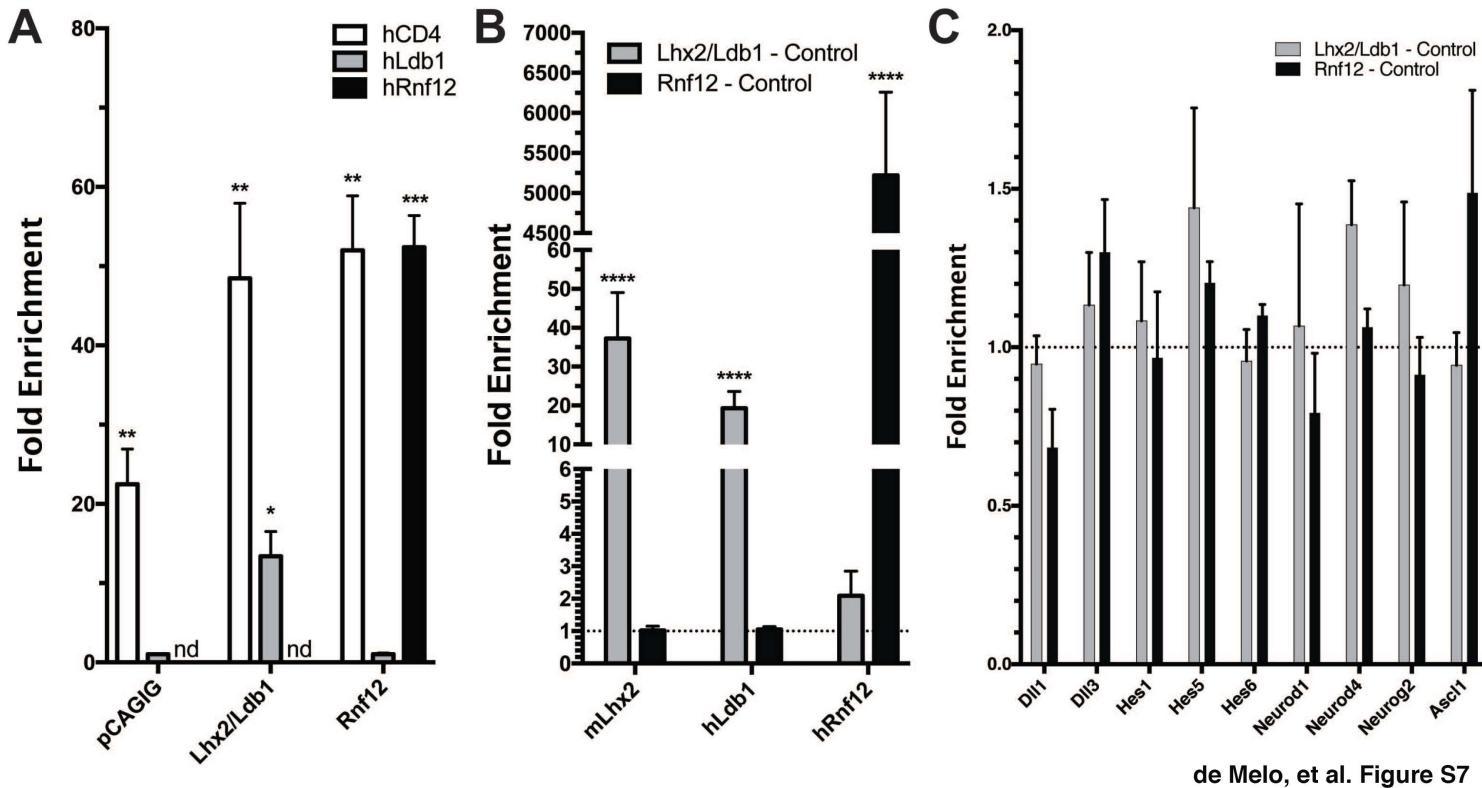
Supplementary Figure 4. RNA expression of *Rnf12* and *Ldb1* contrasted with *Lhx2* during mouse retinal development. (a-c) RNA expression of *Lhx2* is restricted to RPCs during embryonic time points with down regulation occurring in early-born neurons in the GCL. (d-g) Down regulation of *Lhx2* in newly generated neurons continues in postnatal retina, with *Lhx2* becoming restricted to MG and subsets of amacrine cells consistent with previous reports. (a'-c') Low levels of *Rnf12* RNA expression are seen in the NBL during embryonic time points, with higher expression detected in the GCL at E14 and E18. (d'-f') *Rnf12* expression is up regulated in the medial NBL at P0 and remains robustly expressed in the NBL and medial INL at P2 and P5. (g') Adult expression of *Rnf12* is located in all three retinal layers, with the INL showing the strongest labeling. (a''-c'') *Ldb1* expression was detected throughout the embryonic retina. (d''-e'') Neonatal expression of *Ldb1* is enriched in the NBL. (f'') At P5 enrichment of *Ldb1* in the medial INL is detected. (g'') Adult expression of *Ldb1* is localized in the INL and subsets of cells in the GCL. Weaker expression of *Ldb1* was also detected in the ONL. Scale bars, 200 μm (a''), 100 μm (b''-g'').



Supplementary Figure 5. Co-electroporation of *Lhx2* with *Ldb1* or *Rnf12* results in changes in neurogenesis. (a-c, g) electroporation of *Lhx2* or *Ldb1* does not alter the proportion of amacrine cells (PAX6 +ve) generated. (d, g) Co-electroporation of *Lhx2* with *Ldb1* generates an identical wide field amacrine cell phenotype as electroporation of *Lhx2* alone. (e-g) Electroporation of *Rnf12* inhibits the formation of amacrine cells, while co-electroporation of *Rnf12* with *Lhx2* blocks the formation of wide-field amacrine cells generated by electroporation of *Lhx2* alone ($P<0.05$; $N=6$; PAX6 +ve, pCAGIG-Rnf12 vs. pCAGIG; ($P<0.05$; $N=6$; PAX6 +ve, pCAGIG-Rnf12/Lhx2 vs. pCAGIG). (b, e-g) Electroporation of *Lhx2*, *Rnf12*, or *Lhx2* and *Rnf12* results in mild increases in photoreceptor numbers ($P<0.05$; $N=6$). *, indicates significant decrease. Scale bars, 50 μ m (all panels).



Supplementary Figure 6. Electroporation of a dominant-negative *Ldb1* construct (pCAGIG-Ldb-DN) phenocopies *Lhx2* loss of function in postnatal retina. (a, b) Electroporation of pCAGIG-Ldb1-DN at P0 by electroporation resulted in a significant decrease at P14 of MG ($P27^{Kip1}$ and GLUL +ve). (c) Quantification of MG ($P27^{Kip1}$ and GLUL +ve), bipolar cells, and photoreceptors in pCAGIG vs. pCAGIG-Ldb1-DN electroporated retinas. Scale bars, 50 μ m (a, b).



de Melo, et al. Figure S7

Supplementary Figure 7. (a) qRT-PCR assessing fold enrichment of electroporated cells after hCD4 immunosorting. Fold enrichments indicated transcripts enrichment from CD4+ RNA extractions compared to CD4- fractions. (b) qRT-PCR comparing normalized expression of *mLhx2*, *hLdb1* and *hRnf12* transcript expression in CD4+ RNA fractions to control pCAGIG CD4+ fractions in Lhx2-Ldb1 or Rnf12 overexpression experiments. (c) qRT-PCR comparing normalized expression of Notch-pathway and neurogenic gene transcript expression in CD4+ RNA fractions in Lhx2-Ldb1 or Rnf12 overexpression experiments to control pCAGIG CD4+ electroporations. Analysis of significance was determined by (a + b) paired, two-tailed t-Tests on ΔCT values or (c) one-way ANOVA followed by Tukey's multiple comparisons tests. * indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$; **** indicates $p < 0.0001$. nd – not detected.

Supplemental Table 1: Reduced expression of neurogenic bHLH factors in P0.5***Lhx2* cKO retina**

Gene	Control	<i>Lhx2</i> cKO	Fold-change
Ascl1	181.0	63.4	0.35
Hes6	409.2	229.8	0.56
Olig2	33.3	19.5	0.59
Neurod1	306.0	196.7	0.64
Neurod4	196.4	130.4	0.66
Neurog2	55.2	42.6	0.77

RNA-Seq data from P0.5 *Pdgfra-Cre;Lhx2*^{lox/lox} retina was previously described in

(1). RPKM values for each gene are listed.

Supplemental Table 2: Primers used for qRT-PCR analysis.

Table ST2: qRT-PCR Primer Table *				
Gene	5' Primer	3' Primer	Source	PrimerBank ID
hCD4	TGCCTCAGTATGCTGGCTCT	GAGACCTTGCCCTCCTGTTC	Harvard Primer Bank	343790968c1
mLhx2	TGGCAGTAGACAAGCAATG	TGAAGCAGTTGAGGTGATAAA		
hLdb1	GACGACATGATGCCGATAAA	GGTAGTTGAGAGTGGATTGG		
hRnf12	ACCGATTGGATCGAGAAGAACG	TGTAGTCGTCAGCAACTCT	Harvard Primer Bank	223717979c1
DII1	GACCTCGAACAGAAAAACCCA	TTCTCCGTAGTAGTGCTCGTC	Harvard Primer Bank	164565442c3
DII3	CTGGTGTCTCGAGCTACAAAT	TGCTCCGTATAGACCAGGGAC	Harvard Primer Bank	6681199a1
Hes1	CCAGCCAGTGTCAACACGA	AATGCCGGAGCTATCTTCT	Harvard Primer Bank	6680205a1
Hes5	AGTCCCAGGAGAAAAACCGA	GCTGTGTTCAAGGTAGCTGAC	Harvard Primer Bank	6754182a1
Hes6	ACCACCTGCTAGAACATGC	GCACCCGGTTAGTCAGC	Harvard Primer Bank	9506777a1
Neurod 1	ATGACCAAATCATACAGCGAGAG	TCTGCCTCGTGTTCCTCGT	Harvard Primer Bank	142387581c1
Neurod 4	AGCTGGTCAACACACAATCCT	GTTCCGAGCATTCCATAAGAGC	Harvard Primer Bank	158966711c1
Neurog 2	AACTCCACGTCCCCATACAG	GAGGCGCATAACGATGCTTCT	Harvard Primer Bank	34328159c1
Ascl1	GCAACCGGGTCAAGTTGGT	CAAGTCGTTGGAGTAGTTGGG	Harvard Primer Bank	141802882c1

* Primer sequences from Harvard Primer Bank are previously published in (Spandidos et al., 2008; 2010; Wang and Seed, 2003)

Supplementary References:

1. Spandidos A, Wang X, Wang H, Seed B (2010) PrimerBank: a resource for human and mouse PCR primer pairs for gene expression detection and quantification. *Nucl. Acids Res.* 38:D792-9.
2. Spandidos A, Wang X, Wang H, Dragnev S, Thurber T, Seed B (2008) A comprehensive collection of experimentally validated primers for Polymerase Chain Reaction quantitation of murine transcript abundance. *BMC Genomics*. 9:633
3. Wang X, Seed B (2003) A PCR primer bank for quantitative gene expression analysis. *Nucleic Acids Res.* 31(24): e154; pp.1-8.