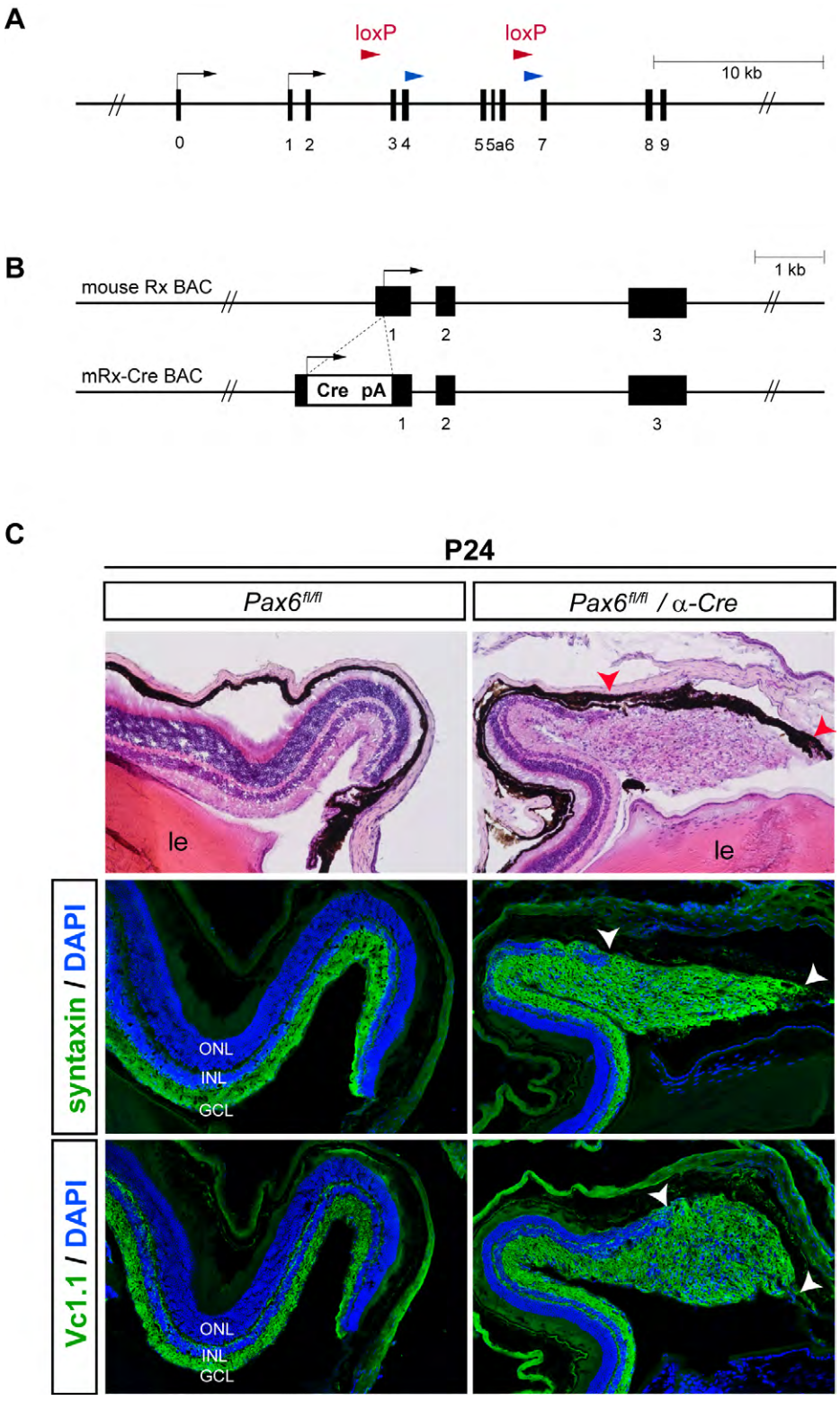
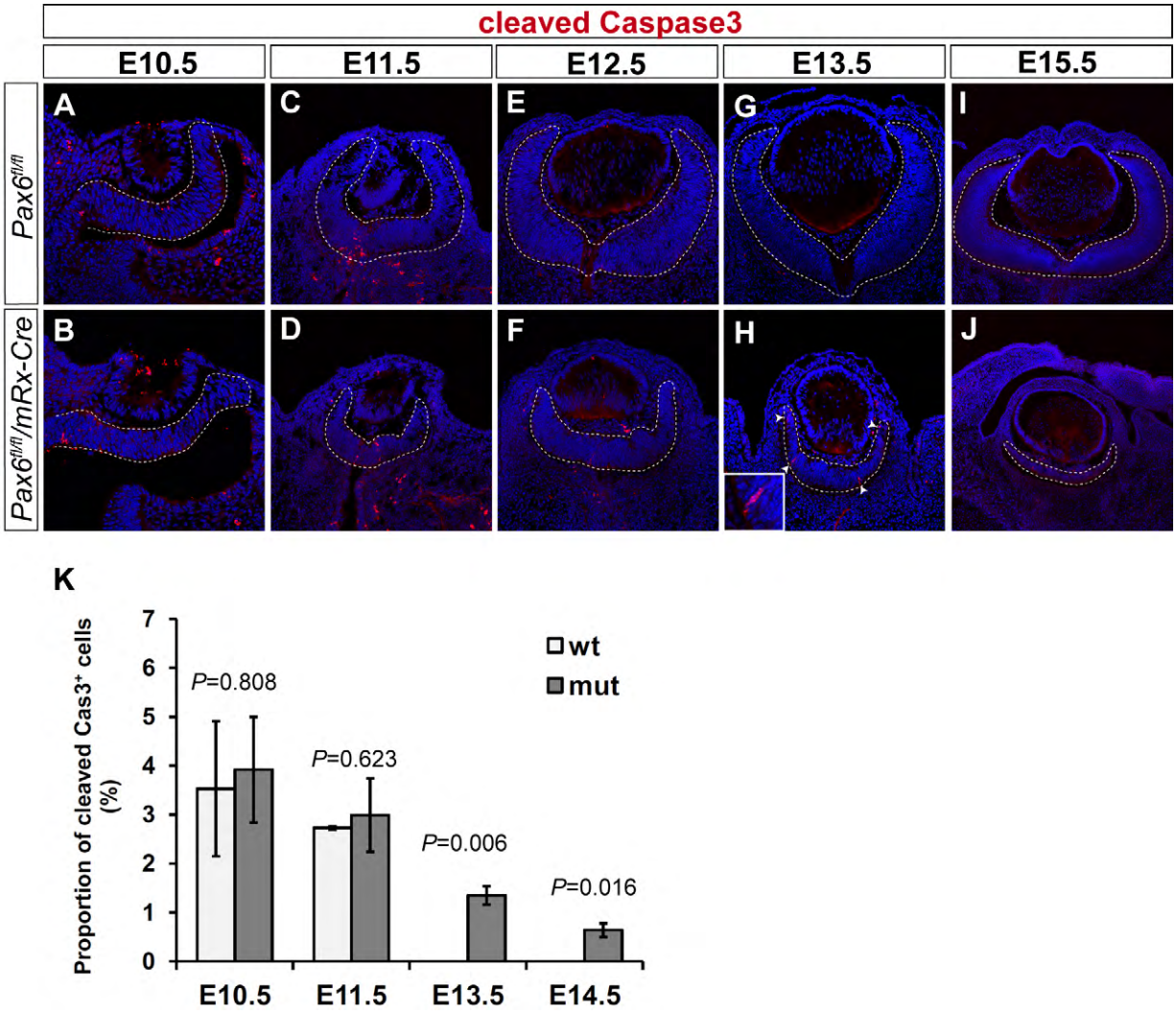
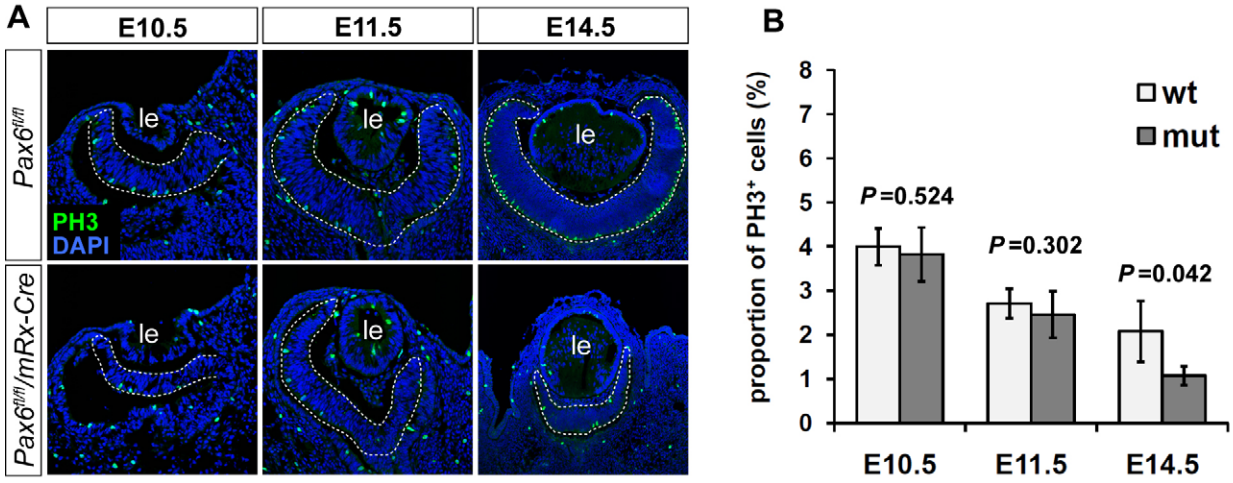


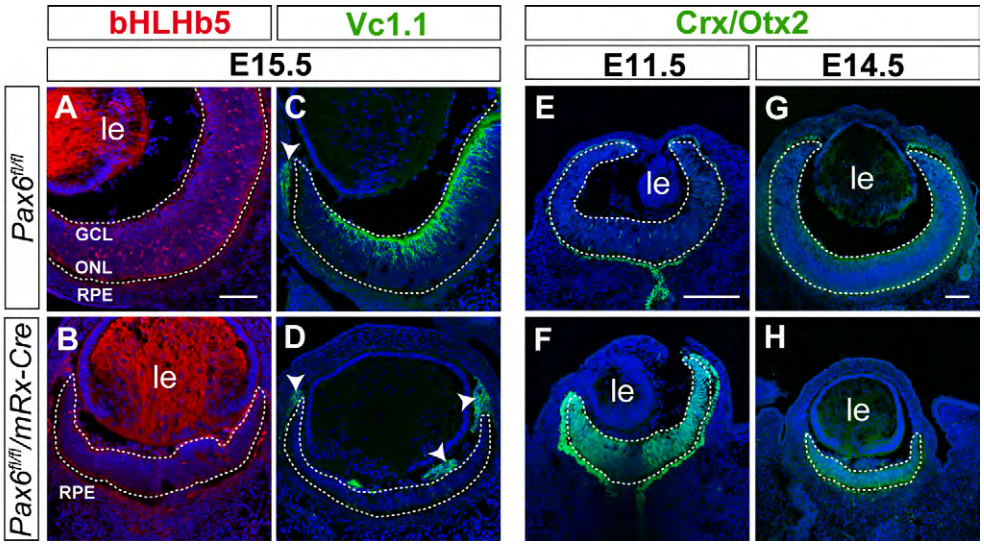
Supplementary Fig. S1. Schematic representation of mouse lines *Pax6*$^{fl/fl}$ and *mRx-Cre* used in this study. (A) To generate *Pax6*$^{fl/fl}$, loxP sites flanking exons 3-6 (red arrowheads) were introduced into *Pax6* locus by homologous recombination in embryonic stem cells. The paired domain of Pax6 is encoded by exons 5, 5a, 6 and 7. Blue arrowheads represent positions of loxP sites in *Pax6*$^{fl/fl}$ mice generated previously (Ashery-Padan et al., 2000). Details of gene targeting are available upon request. (B) To generate *mRx-Cre*, BAC containing 200kb covering the *Rx* locus was modified by BAC recombineering. The *Cre* coding region (Cre-pA) was inserted into the *Rx* translational initiation start site (ATG). Exons are indicated by black boxes. (C) *Pax6*$^{fl/fl}$ mice generated in this study were crossed with *α-Cre* to show that amacrine cells are generated in *Pax6*$^{fl/fl}$/*α-Cre* mutants as previously reported (Marquardt et al., 2001). Adult retinal sections were stained with antibodies against amacrine cell markers syntaxin and Vc1.1 (HNK-1 epitope); retinal areas with amacrine cells are indicated with arrowheads. le, lens; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer.



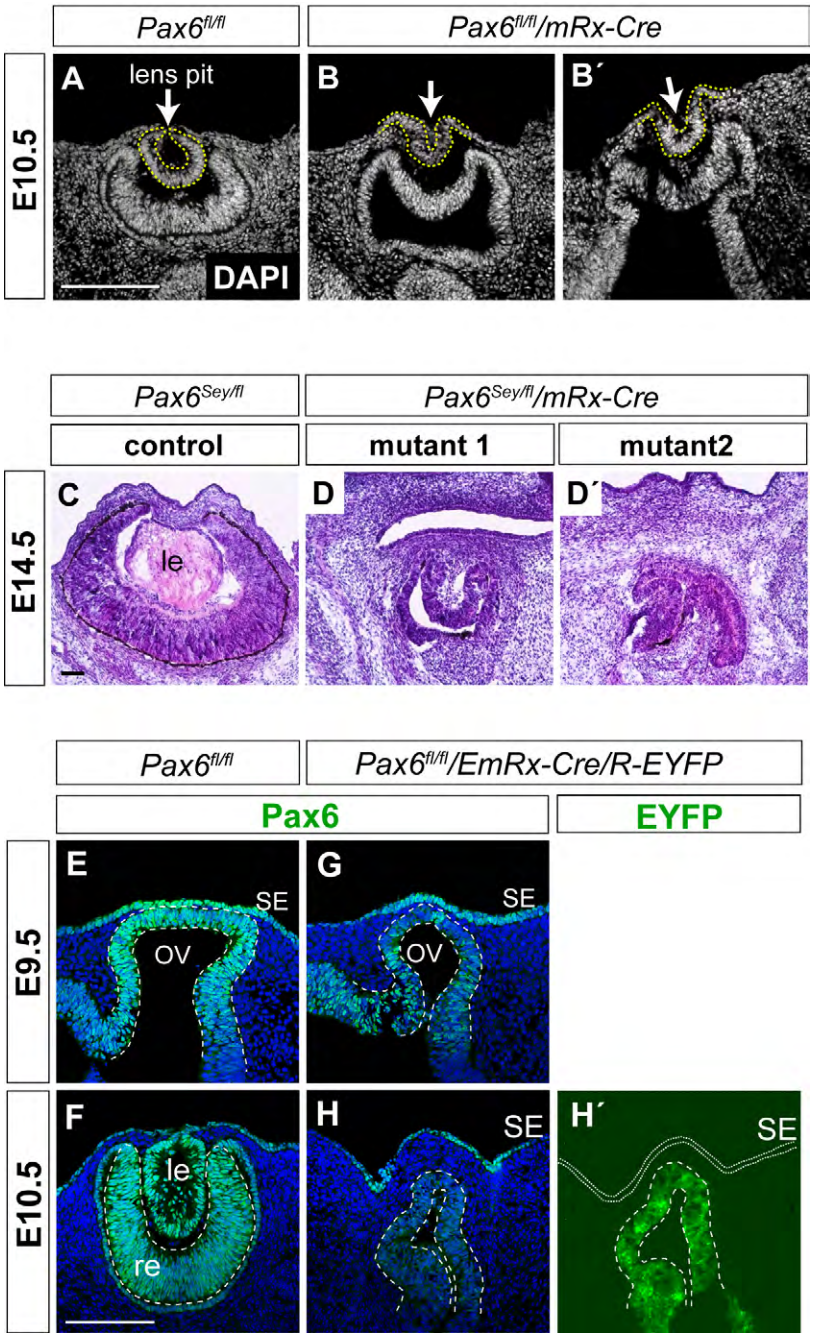
Supplementary Fig. S2. Apoptosis in *Pax6*-deficient retina. (A-J) Retinal sections of wild-type (*Pax6*$^{fl/fl}$) and mutant (*Pax6*$^{fl/fl}$*/mRx-Cre*) embryos were stained with antibody against cleaved Caspase3 (Cas3) at indicated stages. Retina is indicated with dashed line. (K) Quantification of apoptotic cells determined as proportion of Cas3^{+} cells versus DAPI^{+} cells in wild-type (wt) and *Pax6*-deficient (mut) retinae. Error bars indicate s.d. *P*-values are by Student's *t*-test.



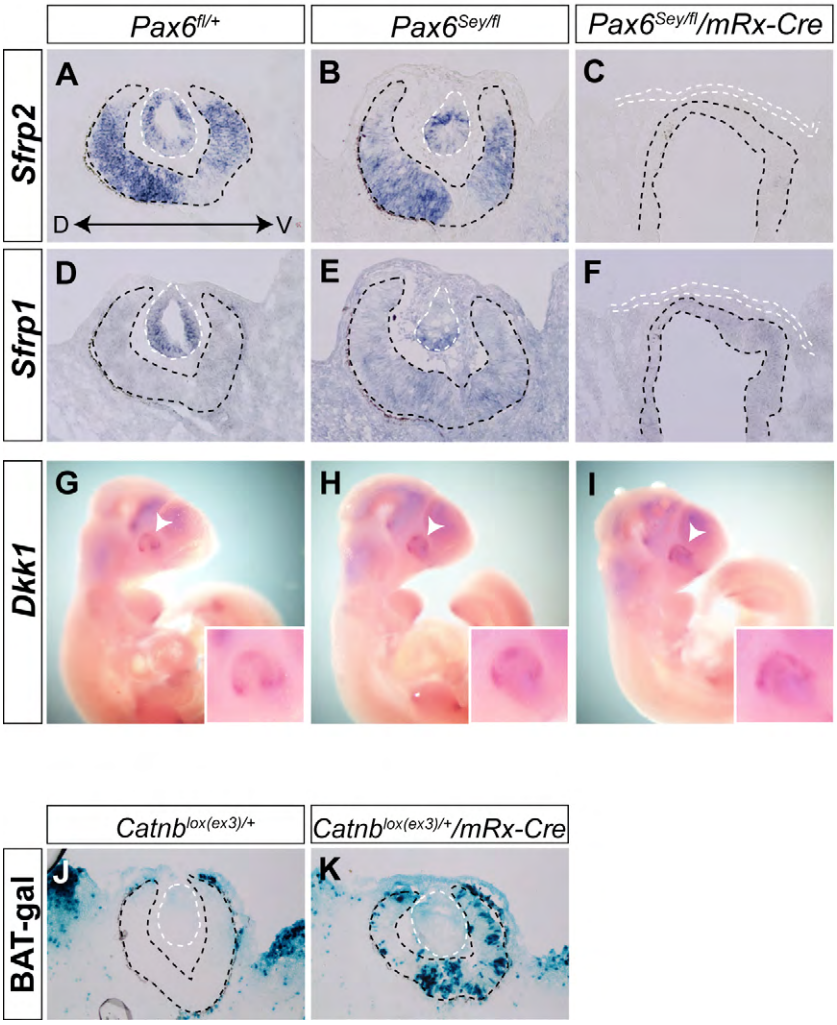
Supplementary Fig. S3. M-phase cell cycle arrest does not contribute to early proliferation phenotype in *Pax6*-deficient retina. (A) Sections stained with antibody against phosphorylated histone H3 (PH3) at E10.5, E11.5 and E14.5. (B) Quantification of M-phase cells determined as proportion of $PH3^+$ cells versus $DAPI^+$ cells in wild-type (wt) and *Pax6*-deficient (mut) retinae (indicated with dashed lines). Error bars indicate s.d. *P*-values are by Student's *t*-test. le, lens



Supplementary Fig. S4. Expression of bHLHb5, Vc1.1 and Crx in *Pax6*-deficient retina. (A-D) Confocal images showing bHLHb5 (A,B) and Vc1.1 (HNK-1 epitope) (C,D) immunoreactivity in wild-type (*Pax6*$^{fl/fl}$) and *Pax6*-deficient (*Pax6*$^{fl/fl}$*/mRx-Cre*) retina at E15.5. Arrowheads indicate Vc1.1 immunoreactivity in non-retinal tissue (B,C). (E-H) Crx protein expression assessed using Crx/Otx2 antibody at E11.5 (E, F) and E14.5 (G,H). Dashed lines indicate the position of retina. le, lens; GCL, ganglion cell layer; ONL, outer nuclear layer; RPE, retinal pigmented epithelium. Scale bar: 100 µm.



Supplementary Fig. S5. Pax6 elimination from early RPCs or OV neuroepithelium interferes with optic cup/lens pit morphogenesis. (A-B′) Transversal sections of E10.5 wild-type (*Pax6$^{fl/fl}$*) and mutant (*Pax6$^{fl/fl}$/mRx-Cre*) eyes of littermate embryos stained with DAPI (grey). Forming lens pit is indicated with dashed line. (C-D′) Section of control (*Pax6$^{Sey/fl}$*) and mutant (*Pax6$^{Sey/fl}$/mRx-Cre*) eyes of littermate embryos at E14.5 stained with hematoxylin-eosin. (E-H′) Sections of control (*Pax6$^{fl/fl}$*) and mutant (*Pax6$^{fl/fl}$/EmRx-Cre*) eyes stained with antibody against Pax6 at indicated stages. (H′) Expression of EYFP showing area of Cre-mediated deletion visualized using *R-EYFP* reporter mouse line. OV, optic vesicle; SE, surface ectoderm; le, lens; re, retina. Scale bar: 100 µm.



Supplementary Fig. S6. Wnt/β-catenin inhibitors *Sfrp1* and *Sfrp2* are downregulated upon OV-specific *Pax6* inactivation but Wnt signaling is not responsible for the arrested lens development. (A-F) *Sfrp2* (A-C) and *Sfrp1* (D-F) mRNA expression in control (*Pax6*^{fl} *and Pax6*$^{Sey/fl}$) and mutant (*Pax6*$^{Sey/fl}$*/mRx-Cre*) E11.0 eyes. (G-I) *Dkk1* mRNA expression at E10.5; eye region indicated with arrowheads. (J,K) Activity of Wnt/β-catenin signaling assessed using a BAT-gal reporter mouse in control (*Catnb*$^{lox(ex3)/+}$) (J) and retinal mutant (*Catnb*$^{lox(ex3)/+}$*/mRx-Cre*) (K) with activated Wnt/β-catenin pathway in developing neuroretina. Retina is indicated with black dashed line; lens or the corresponding tissue with white dashed line.

Table S1. Primary antibodies and RNA probes

Primary antibodies

Antibody	Host	Dilution	Source
Pax6	Rabbit	1:500	Covance (PRP-278P)
Sox2	Goat	1:400	Santa-Cruz (sc-17320)
cyclin D1	Mouse	1:500	Santa-Cruz (sc-450)
$p27^{Kip1}$	Mouse	1:1000	BD Biosciences (610241)
$p57^{Kip2}$	Goat	1:70	Santa-Cruz (sc-1039)
Tuj1	Mouse	1:1500	R&D Systems (MAB1199)
cleaved caspase 3	Rabbit	1:300	Cell Signaling (D175)
PH3	Rabbit	1:1000	Upstate (06-570)
Lef1	Rabbit	1:500	Cell Signaling (C12A5)
Pcna	Mouse	1:3000	Sigma, P8825
Crx/Otx2	Rabbit	1:500	Kind gift from Dr Craft (Zhu and Craft, 2000)
Six3	Rabbit	1:2000	Kind gift from Dr P. Bovolenta (unpublished)
Otx2	Rabbit	1:300	R&D Systems (BAF1979)
Blimp1	Rat	1:300	Santa-Cruz (sc-47732)
Rxrγ	Rabbit	1:1500	Santa-Cruz (sc-555)
Nr2e3	Rabbit	1:100	Kind gift from Dr S. Chen (Chen et al., 2005)
bHLHb5	Goat	1:400	Santa-Cruz (sc-6045)
Vc1.1 (HNK-1)	Mouse	1:100	Sigma (C6680)
Lhx2	Goat	1:1000	Santa-Cruz (sc-19344)
Hes1	Rabbit	1:1000	Kind gift from Dr N. Brown (Lee et al., 2005)
Chx10	Sheep	1:800	Exalpha (X1180P)
Foxe3	Rabbit	1:1000	Kind gift from Dr Carlsson (Blixt et al., 2007)
Prox1	Rabbit	1:2000	Chemicon (AB5475)
BrdU	Rat	1:100	Abcam (AB6326)
pErk1/2	Rabbit	1:3000	Cell Signaling (9101S)
pSmad1/5	Rabbit	1:2000	Invitrogen (700047)

RNA probes

Gene	Source
Neurod1	Open Biosystems
$p57^{Kip2}$	Open Biosystems
Rx	Open Biosystems
Trβ2	Open Biosystems
Crx	Open Biosystems
Atoh7	Kindly provided by Dr Brown (Brown et al., 1998)
Math3	Kindly provided by Dr Brown (Farah et al., 2000)
Mash1	Kindly provided by Dr Brown (Brown et al., 1998)
Bmp4	Kindly provided by Dr Hogan (Jones et al., 1991)
Bmp7	Kindly provided by Dr Hogan (Lyons et al., 1995)
Ngn2	Kindly provided by Dr J. Rubenstein (Sommer et al., 1996)
Sfrp1	Kindly provided by Dr S. Pleasure (Rattner et al., 1997)
Sfrp2	Kindly provided by Dr S. Pleasure (Rattner et al., 1997)
Dkk1	Kindly provided by Dr S. Krauss (Diep et al., 2004)