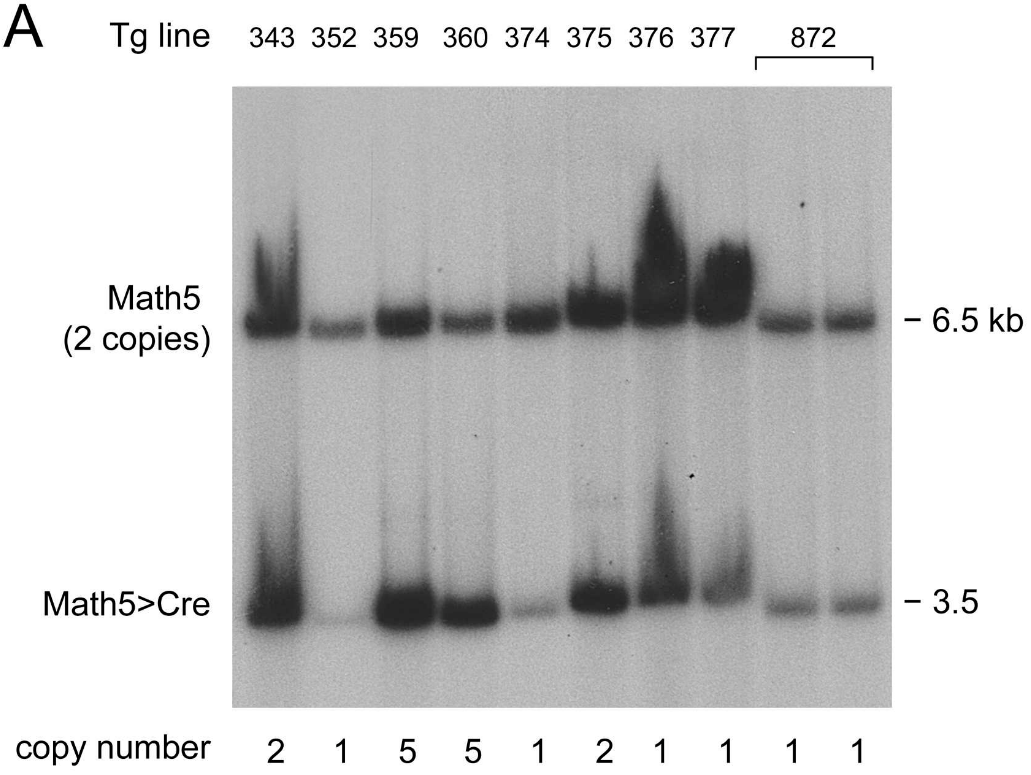
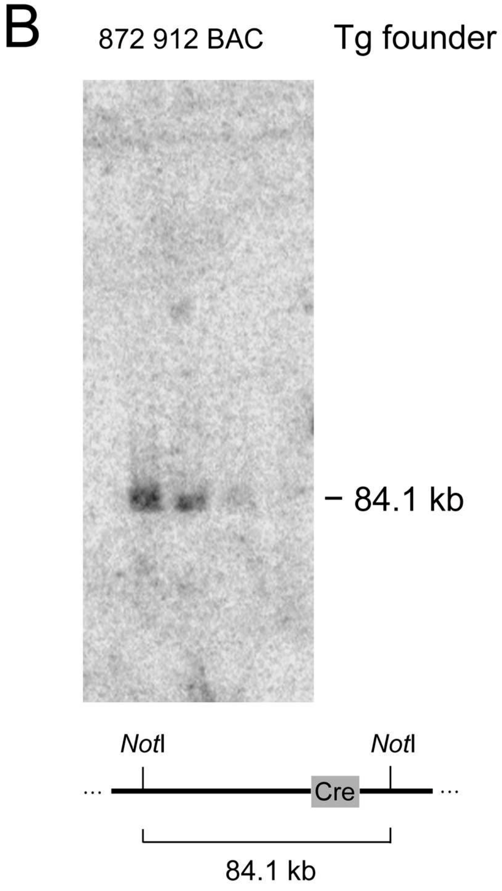
Suppl. Fig. 1. Copy number and integrity in Math5>Cre transgenes. (A) Southern blot of *Eco*RI-digested DNA from nine independent transgenic lines, hybridized with a 5' *Math5* genomic probe labeled with ^{32}P. The Math5>Cre BAC fragment (3.5 kb) is smaller than the endogenous *Math5* fragment (6.5 kb) due to the presence of an *Eco*RI site in the Cre cassette. Transgene copy number was estimated from the relative intensity of these fragments, as determined by densitometry. (B) Pulsed-field gel Southern analysis. Genomic DNA from Math5>Cre transgenic founders 872 and 912, and purified episomal DNA from BAC clone RP23-328P3-D1-68 were digested with *Not*I, separated by PFGE, and hybridized with a Cre probe. Both founders had the predicted 84.1 kb *Not*I fragment, indicating that this segment is intact. Tg, transgenic.

Suppl. Fig. 2. Birthdating curves for cones (top graphs) and rods (bottom graphs) in (A) wild-type Math5>Cre; Z/AP and (B) mutant Math5>Cre; R26*flox*GFP mice between E14.5 and E17.5 (see Suppl. Table 4). Single BrdU injections were given to pregnant dams on the indicated days, and photoreceptors were analyzed in the adult retinas. Curves show the percentage of each photoreceptor type that is BrdU+ for the hPLAP+ or GFP+ (red), hPLAP− or GFP− (blue), and combined (black) progenitor groups. The difference in scale between panels A and B reflects the relative deficiency of late-born rods (>E17) and relative excess of early-born cones (<E14) in *Math5* -/- mice.

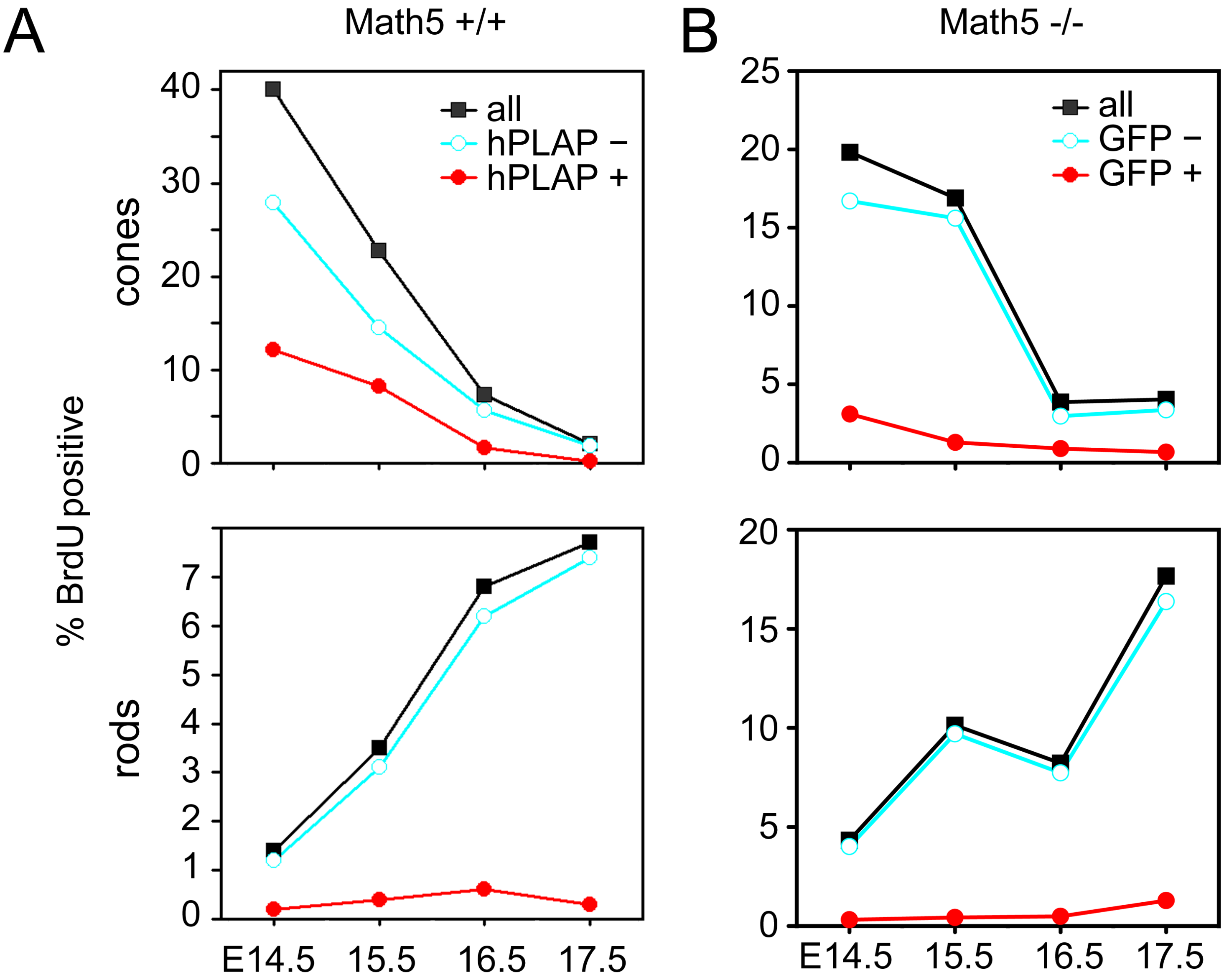
Suppl. Fig. 3. Proneural bHLH factors Neurod1 and Math5 are expressed in overlapping subsets of progenitor cells during early retinal neurogenesis. E11.5 *Math5-lacZ/*+ retinal sections were immunostained for βgal and Neurod1. Approximately half of the βgal+ cells co-express Neurod1 (arrows, inset). Scale bar, 50 μm.

Supplemental Figure 1

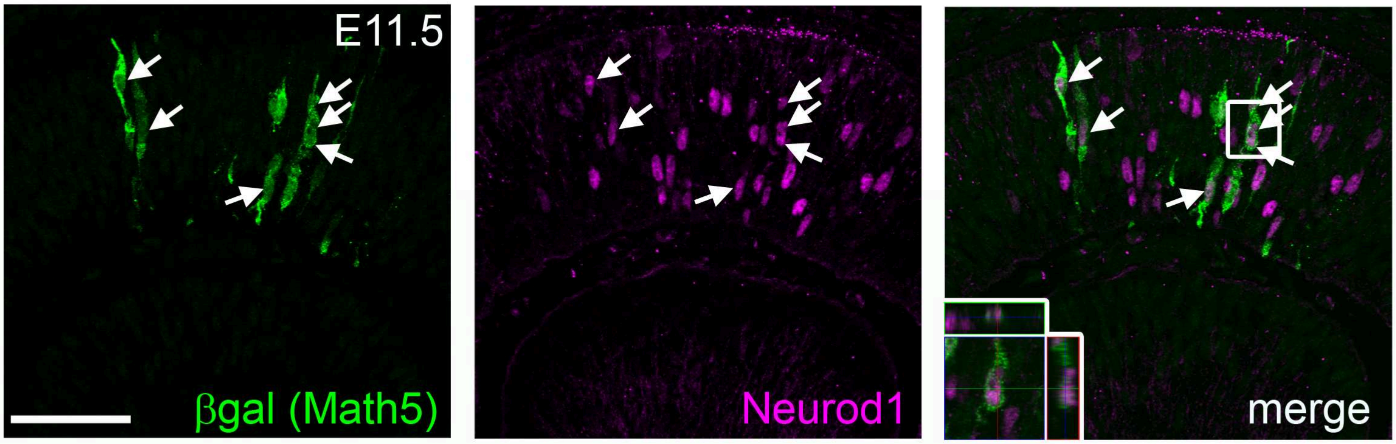




Supplemental Figure 2



Supplemental Figure 3



Suppl. Table 1. Cell type distribution of Math5 lineage descendants in Math5 mutant mice

	Math5 lineage cells counted	**cell type[‡] (% of Math5 lineage)**
CELL TYPE	**(*a*)**	**(*a*/*b*) x 100**
cone	189	21
horizontal	14	1.6
amacrine	295	33
INL	187	21
displaced	108	12
rod	358	40
bipolar	17	1.9
Müller glia	21	2.3
TOTAL	**894 (*b*)**	100

All labeled cells were identified and counted in 23 fields (200X, 6 eyes, Z/AP reporter). All cells in the GCL were assumed to be displaced amacrine cells, as no cells in Math5 mutant retinas were positive for RGC markers Brn3a or Brn3b.

[‡] These values are shown in Fig. 3I (right pie chart).

Suppl. Table 2. Dual reporter concordance for Math5>Cre labeled retinal cells

	cells counted[†]			concordance (%)[#]	
cell type	**hPLAP+ GFP+ (*d*)**	**total hPLAP (*a*)**	**total GFP (*g*)**	$\frac{d}{a + g - d}$	**SEM[‡]**
rods	334	376	390	77	3
cones	257	274	289	84	1
INL amacrines	330	376	368	80	4
GCL neurons*	257	287	284	82	3
TOTAL	1178	1313	1331	80	1

All single and double labeled cells were counted in 18 fields (200X) representing 4 eyes from 3 Math5>Cre; Z/AP; R26*flox*GFP mice. hPLAP, alkaline phosphatase; GFP, green fluorescent protein.

*RGCs and displaced amacrines.

[†] There was no significant difference in the number of cells labeled by each reporter in the fields surveyed (paired *t* test, $P > 0.1$).

[#] The incomplete concordance (<100%) in this experiment validates our assumption that Cre levels are not saturated.

[‡] Standard error of the mean (SEM) calculated from concordance for $n = 4$ eyes. There was no significant difference among cell types (one-way ANOVA, $P > 0.15$).

Suppl. Table 3. Cumulative BrdU labeling experiment (E10.5 to P0)

	Math5 lineage cells counted		
cell type	**BrdU+**	**total**	**% BrdU+**
rods	70	70	100
cones	53	53	100
INL neurons	62	65	95
GCL neurons	53	53	100
TOTAL	238	241	99

Suppl. Table 4. Birthdates of Math5 lineage retinal descendants

Math5 +/+ mice

time of pulse		cones counted (% of combined total)			rods counted (% of combined total)		
		hPLAP+	**hPLAP−**	**combined**	**hPLAP+**	**hPLAP−**	**combined**
E14.5	**BrdU+**	136 (12.1)	314 (27.9)	450 (40.0)	64 (0.2)	455 (1.1)	519 (1.3)
	BrdU−	243 (21.6)	431 (38.4)	674 (60.0)	315 (0.8)	38,700* (97.8)	39,100* (98.7)
	total	379 (33.7)	684 (66.3)	1,124 (100.0)	379 (1.0)	39,200* (98.9)	39,600* (100.0)
E15.5	**BrdU+**	79 (8.3)	141 (14.5)	220 (22.7)	140 (0.4)	1,073 (3.1)	1,213 (3.5)
	BrdU−	214 (22.0)	537 (55.3)	751 (77.3)	317 (0.9)	32,600* (55.3)	33,000* (96.5)
	total	293 (30.2)	678 (69.8)	971 (100.0)	457 (1.3)	33,700* (69.8)	34,000* (100.0)
E16.5	**BrdU+**	28 (1.6)	100 (5.7)	128 (7.3)	383 (0.6)	3,881 (6.2)	4,264 (6.8)
	BrdU−	513 (29.1)	1,124 (63.6)	1,637 (92.7)	480 (0.8)	57,400* (92.4)	57,900* (93.2)
	total	541 (30.7)	1,224 (69.3)	1,765 (100.0)	863 (1.4)	61,300* (98.6)	62,100* (100.0)
E17.5	**BrdU+**	2 (0.2)	20 (1.9)	22 (2.1)	122 (0.3)	2,729 (7.4)	2,851 (7.7)
	BrdU−	300 (28.5)	732 (69.4)	1,032 (97.9)	264 (0.7)	34,000* (91.6)	34,300* (92.3)
	total	302 (28.7)	752 (71.3)	1,054 (100.0)	386 (1.0)	36,700* (99.0)	37,100* (100.0)

For the E14.5 pulse, 16 fields (200X) were counted (3 eyes, 3 mice). For the E15.5 pulse, 14 fields (200X) were counted (4 eyes, 2 mice). For the E16.5 pulse, 24 fields (200X) were counted (5 eyes, 4 mice). For the E17.5 pulse, 15 fields (200X) were counted (4 eyes, 3 mice).

* Combined rod totals were estimated by multiplying the combined cone counts by 35.2 (Jeon *et al.* 1998).

Math5 -/- mice

time of pulse		cones counted (% of combined total)			rods counted (% of combined total)		
		GFP+	**GFP−**	**combined**	**GFP+**	**GFP−**	**combined**
E14.5	**BrdU+**	21 (3)	113 (17)	134 (20)	26 (0.3)	326 (4)	352 (4)
	BrdU−	87* (13)	455* (67)	542* (80)	83* (1)	7,699* (95)	7,782* (96)
	total	108 (16)	568* (84)	676* (100)	109 (1.3)	8,025* (99)	8,134* (100)
E15.5	**BrdU+**	8 (1)	97 (16)	105 (17)	32 (0.4)	1,066 (14)	1,098 (15)
	BrdU−	27* (4)	490* (79)	517* (83)	40* (0.5)	6,337* (85)	6,377* (85)
	total	35 (6)	587* (94)	622* (100)	72 (1.0)	7,403* (99)	7,475* (100)
E16.5	**BrdU+**	10 (1)	33 (3)	43 (4)	65 (0.5)	692 (5)	757 (6)
	BrdU−	14* (1)	1,054* (95)	1,068* (96)	58* (0.4)	12,548* (94)	12,606* (94)
	total	24 (2)	1,087* (98)	1,111* (100)	123 (0.9)	13,240* (99)	13,363* (100)
E17.5	**BrdU+**	5 (1)	25 (3)	30 (4)	115 (1)	1,461 (16)	1,576 (18)
	BrdU−	73* (10)	639* (86)	712* (96)	138* (2)	7,212* (81)	7,350* (82)
	total	78 (11)	664* (89)	742* (100)	253 (3)	8,673* (97)	8,926* (100)

For the E14.5 pulse, 14 fields (200X) were counted (2 mice). For the E15.5 pulse, 7 fields (200X) were counted (2 mice). For the E16.5 pulse, 9 fields (200X) were counted (2 mice). For the E17.5 pulse, 10 fields (200X) were counted (2 mice).

* Combined rod and cone totals were estimated by multiplying the measured number of inner nuclear layer nuclei by 0.326 for cones or 3.92 for rods. These ratios were determined empirically in Math5 -/- mice (data not shown).