Supplemental Figure Legends

Supplemental Figure S1. Expression of *Isl1* and *Isl2* during retina development. (A-D) In situ hybridization of wild type retina sections with *Isl1* and *Isl2* probes. Compared to *Isl1* expression in RGCs and amacrine cells (arrows) (A,C), *Isl2* is only expressed in selective 30% RGCs in the GCL (B,D). (E-J) Immunolabeled retinal sections at E13.5 (E-G) and E15.5 (H-J) show the overall co-expression (arrows) of ISL1 (red) and Brb3b (green) in *Isl2*-null mice with occasional BRN3B+-only cells (open arrowhead). The anti-ISL1 immunolabeling shows an identical ISL1 expression pattern in wild type (see Fig. 1) and *Isl2*-null retina. Inserts show the enlarged views of the corresponding boxed regions. NBL, neuroblast layer; GCL, ganglion cell layer. Scale bar: 100 μm.

Supplemental Figure S2. *Isl1*-lacZ reporter expression recapitulates the expression pattern of endogenous *Isl1*. In situ hybridization, anti-ISL1 immunolabeling, and X-Gal staining of $Isl1^{lacZ/+}$ retina sections at E14.5 show the similar expression profile of *Isl1* mRNA (A), ISL1 protein (B), and *Isl1*-lacZ (C). Inserts show the enlarged views of the corresponding boxed regions. Scale bar: 100 μm.

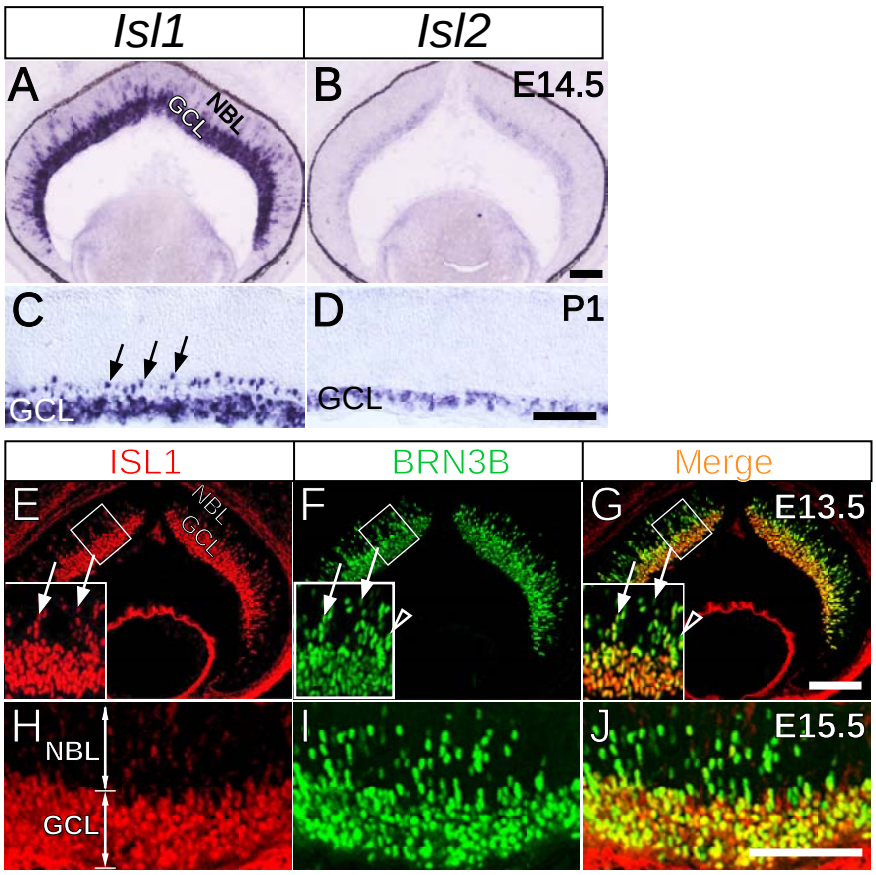
Supplemental Figure S3. Co-occupancy of ISL1 and BRN3B on RGC promoters in E13.5 WT retina but not in *Brn3b*- null or *Isl1*- null tissues. (A) Anti-BRN3B and anti-ISL1 antibodies co-precipitate with the promoters of *Brn3b, Shh, Brn3a* and *Isl2* in E13.5 WT retina. Both antibodies do not precipitate with these promoters in *Brn3b*-null retina. Anti-ISL1 antibody does not precipitate with these promoters in cerebellum. (B) Western blot with anti-ISL1 antibody using cerebellum and retina tissue preparation. The antibody primarily recognizes a single band of ISL1 in retina but not in cerebellum, suggesting that ISL1 is not expressed in cerebellum and that the anti-ISL1-precipitated chromatin is specifically ISL1-bound DNA.

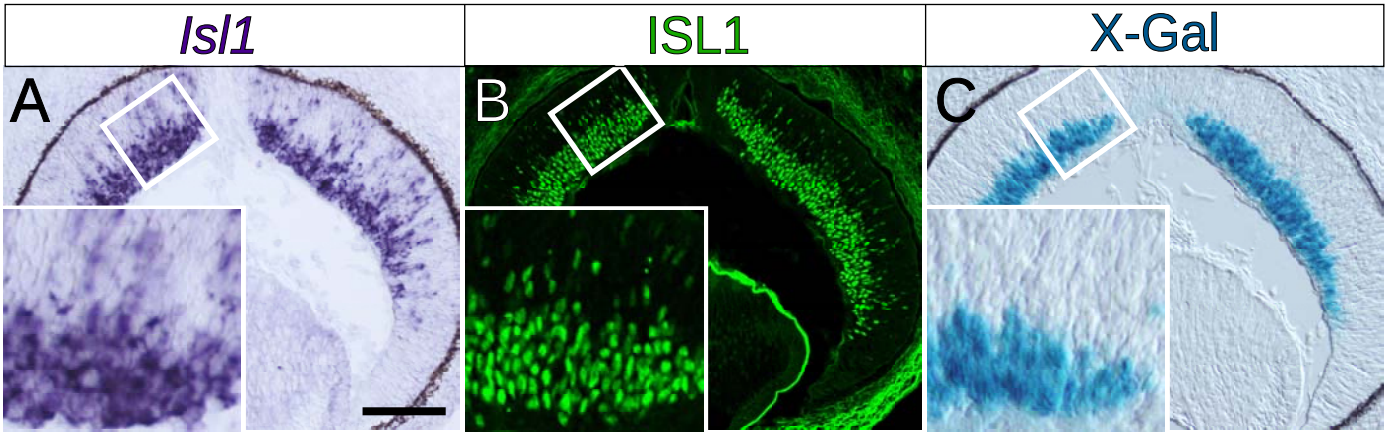
Supplemental Figure S4. Sequences of RGC-specific promoters occupied by both ISL1 and BRN3B during RGC differentiation. (A) Primer sets used to PCR amplify the precipitated promoter regions. (B) Sequences of

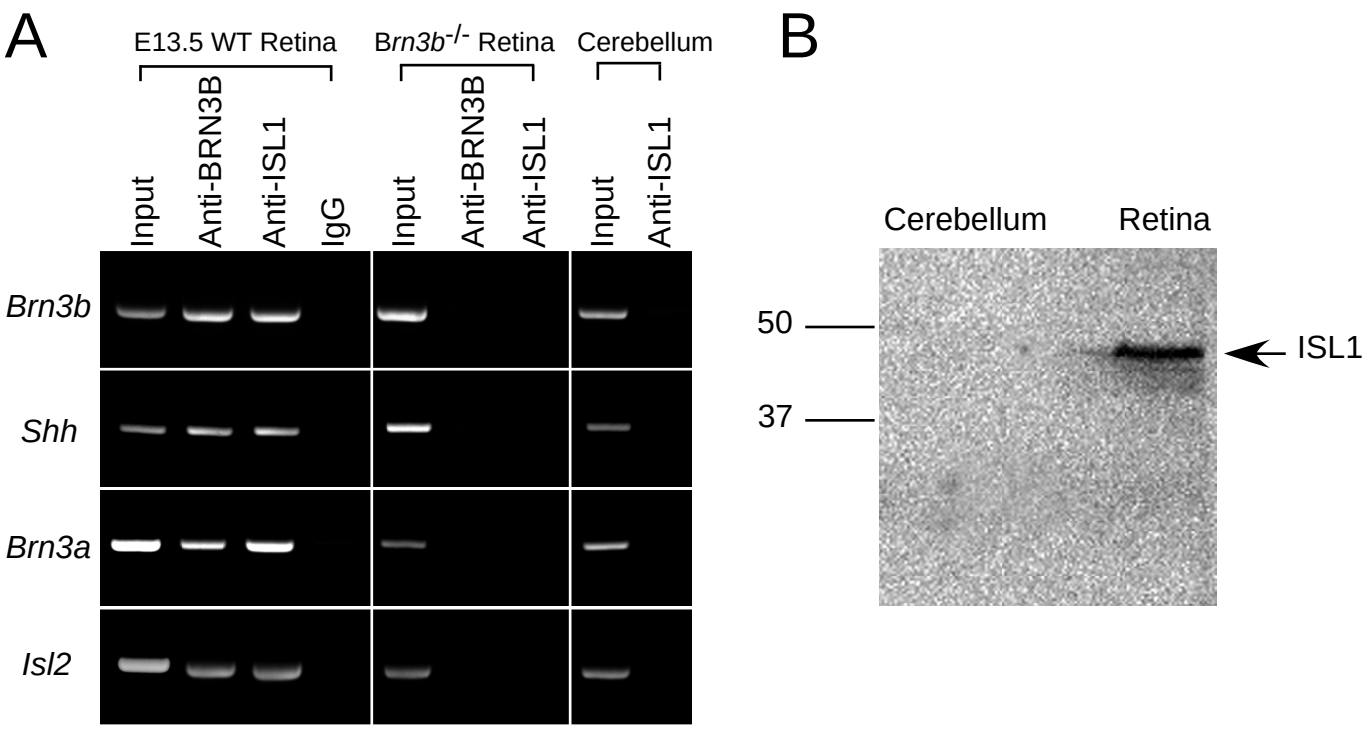
RGC-specific promoters flanking the Brn3-binding sites (red) and their distances from the transcription initiation sites, Potential ISL1-binding regions are indicated in blue. Identified ISL1-binding consensus is underlined. (C) Summary of the Brn3-binding sites in RGC-specific genes and the derived consensus sequence.

Supplemental Figure S5. Progenitor proliferation in E17.5 *Isl1*-null retina. *Isl1* -null retina displays a slight 17% decrease of M-phase proliferating cells as labeled by anti-pH3 (green, compare A to B; average number of pH3+ cells ± s.d.: controls: 184.25±9.19; *Isl1*-nulls: 152.38± 6.54, n=2, student t-test $p<0.05$.). C, D show the enlarged views of the boxed regions in A, B.

Supplemental Table S1. ISL1 and BRN3B regulate the expression of *Gap43* and *L1cam* in the retina. Real time PCR is performed with E14.5 retinas from *Isl1*-nulls, *Brn3b*-nulls and the controls. Relative expression of genes is calculated using the formula: $2^{\Delta MT- \Delta WT}$. ΔMT is the difference of cutoff cycles between the gene of interest and the control gene (β-actin) for mutants and ΔWT is that for controls. The numbers listed in the table are the average from three independent experiments.


Isl1
Isl2
A
NBL
GCL
B
E14.5
C
GCL
D
P1
GCL
ISL1
BRN3B
Merge
E
NBL
GCL
F
G
E13.5
H
NBL
GCL
I
J
E15.5


Isl1
ISL1
X-Gal
A
B
C


A
E13.5 WT Retina
B*rn3b*$^{-/-}$ Retina
Cerebellum
Input
Anti-BRN3B
Anti-ISL1
IgG
Input
Anti-BRN3B
Anti-ISL1
Input
Anti-ISL1
Brn3b
Shh
Brn3a
Isl2
B
Cerebellum
Retina
50
37
ISL1

A

	Forward primer	Reverse primer
Brn3b	5'-GGAAACCCCTGCTCTAATC	5'-GAAACGGTCCCCTTGTCTC
Brn3a	5'-AGACGCGAAACCAACAAAGAG	5'-CTGTGAGGCAGTCAGTCCAAG
Isl2	5'-TACTATCTGGCTTCATCTCCC	5'-TGGAGTAGAGTTCTCGGATGA
Shh	5'-CACAGAGTCGGGCTTGATTAG	5'-AGGATGGATAGGGTTTTGGAG
Brn3b ORF	5'-GAGCAGCAGTTCCAGCAGCAGTG	5'-GGTGGTGGTGGCTCTTACTCTGC

B

Brn3a 5' upstream sequence (-5651 to -5500)

TTTAACAAATTAATTAATC**CTAATG**AATTAACACTTCATTTATTTAAACGCGCTACAGTCCATG
AATTAAATTTTCATGCAGTGATTAAAGGCTGTTAAAATATGCATGATTTCGTTAAAATTTTATAA
TAAATCAGGGCAATGTGTTAC

Shh 1st introns (+3761 ~ +3821)

CAGAGTCGGGCTTGATTAGCGGCACACGACCCAATGAATTAATAACCGGGCTGAGCTTCAA

Brn3b 5'upstream sequence (-3710 ~ -3580)

GCAAGACTGAAATGGGTTTTATTGATATGACCACGAGGATGTTGGTATTCTGAACACTCCTAA
AGGGAGAGATTGCAAGACTCCAAGTCTTGCCGATGAAGGAAGAACTGCATCTTTGCTCTAAT
GCAACA

Isl2 5'upstream sequence (-6464 ~ -6129)

CGAGCATTAGCCAGCCTCCTGGCACTGGGCTTGCCATGCTCTAGGTGTGTGATGAGGCTAG
TGGTGATAAATCTCTTCTGTCTGCAAGCAGCGAGGAGAGAGTGGCAGAGAGAGCTGCAACC
AGGGAGCATTATGATTTTGTTCTTAACTCCAAGGCATGTCTAAGGCACTATAAATAACACACA
TTGTCTATAATTCAAGTTGATCAAAACAGAACTCAGCCCAGTGATGTGGAGTGGAATCTGCT
GCCTCCAAACCAATTACGAATTTCCAACAGATGAGCGAGCGAGCGAGCGAGCGCCTGACTG
GAGTCCCTCCTGCAGTGGGCTAATGCCC

C

Brn3a	T**TAATTAAT**
	T**TAATT**C**AT**
	T**TAATT**C**AT**
	ATAATAA**AT**
Shh	T**TAATT**C**AT**
Brn3b	T**TT****ATTG****AT**
Isl2	**ATAATT**C**AA**

Consensus: A T ($^{A}/_{T}$) AT T N A T

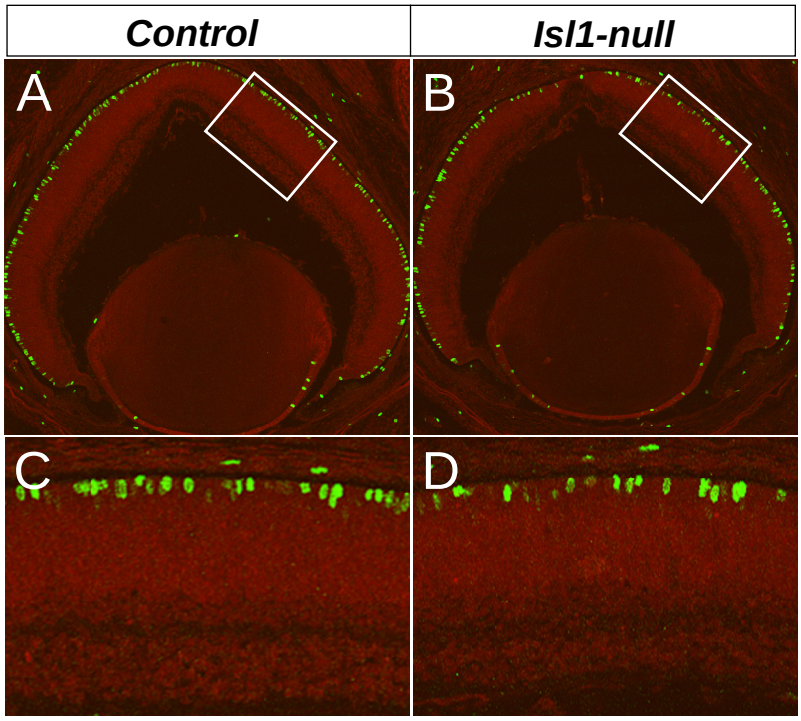

Control
Isl1-null
A
B
C
D

Table S1

	Isl1- null			*Brn3b*-null		
	Relative expression	Standard deviation	t-test	Relative expression	Standard deviation	t-test
Gap43	0.578	0.029	p<0.001	0.482	0.010	p<0.001
L1cam	0.690	0.017	p<0.01	0.727	0.082	p<0.05