Figure S1 (related with Fig. 1)
Lbx1 induces Pax2 in Tlx1/3-negative cells.

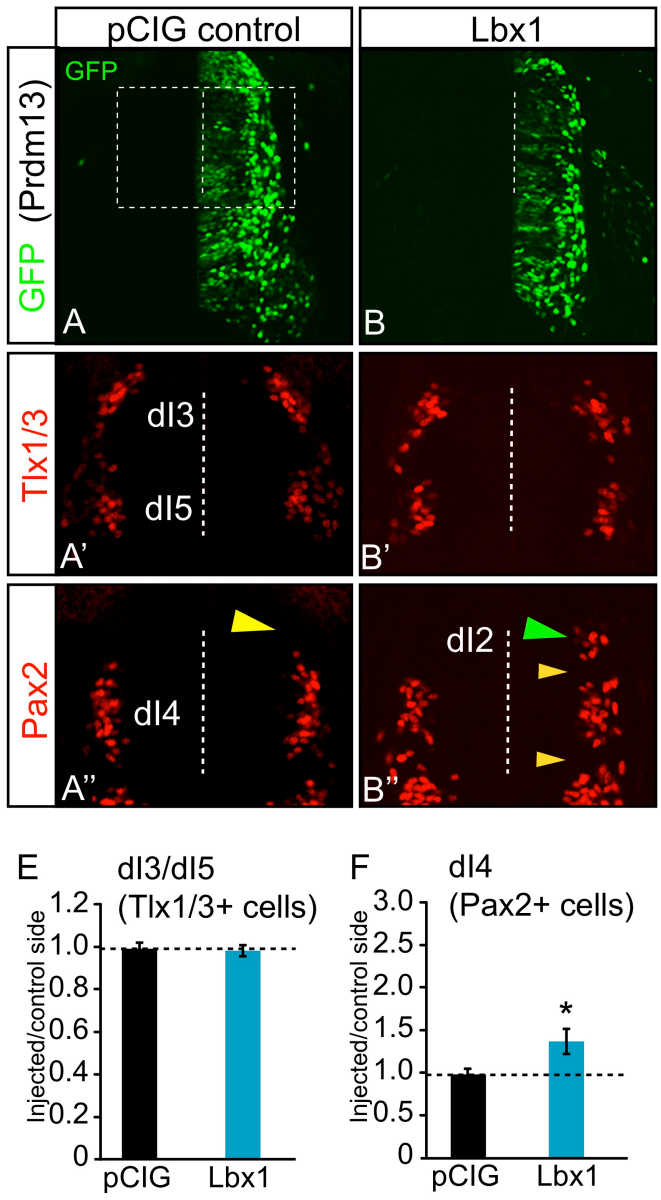
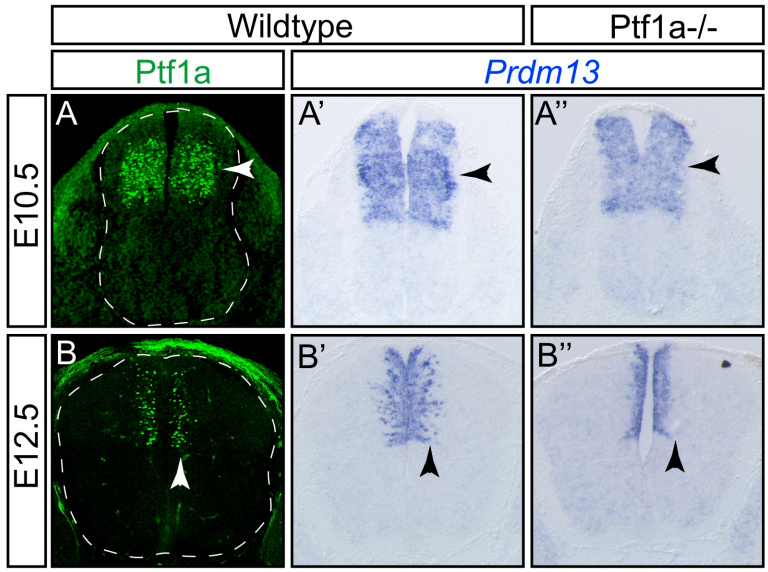


Figure S2 (related with Fig. 2)
Prdm13 expression in the dorsal neural.



C Genomic coordinates of Ptf1a ChIP-seq peaks near Prdm13
(Mouse genome mm9)
Peak 1: chr4:21538810-21538960
Peak 2: chr4:21555570-21555720
Peak 3: chr4:21593210-21593360
Peak 4: chr4:21607350-21607500
Peak 5: chr4:21613025-21613175
Peak 6: chr4:21616240-21616390
Peak 7: chr4:21616795-21616945

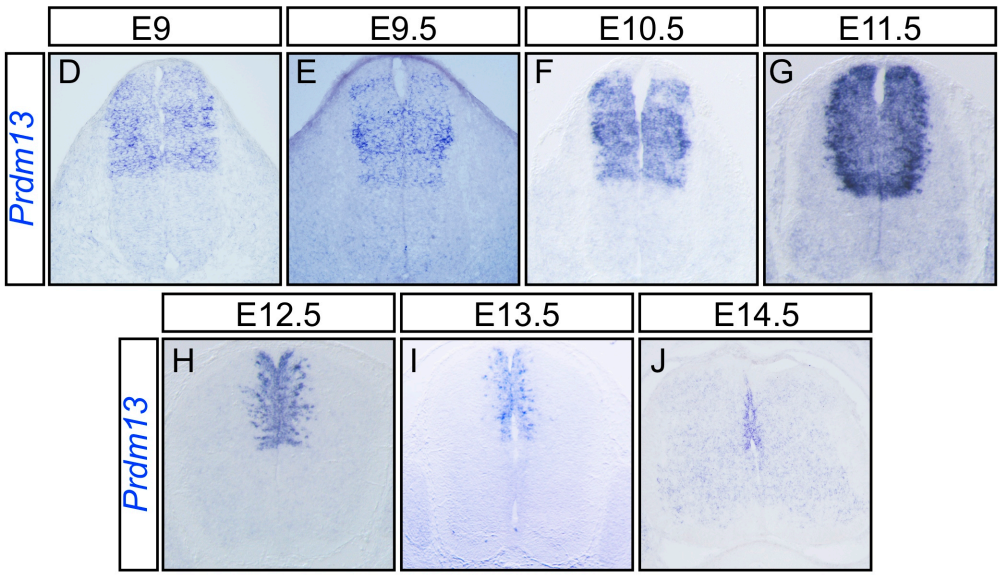
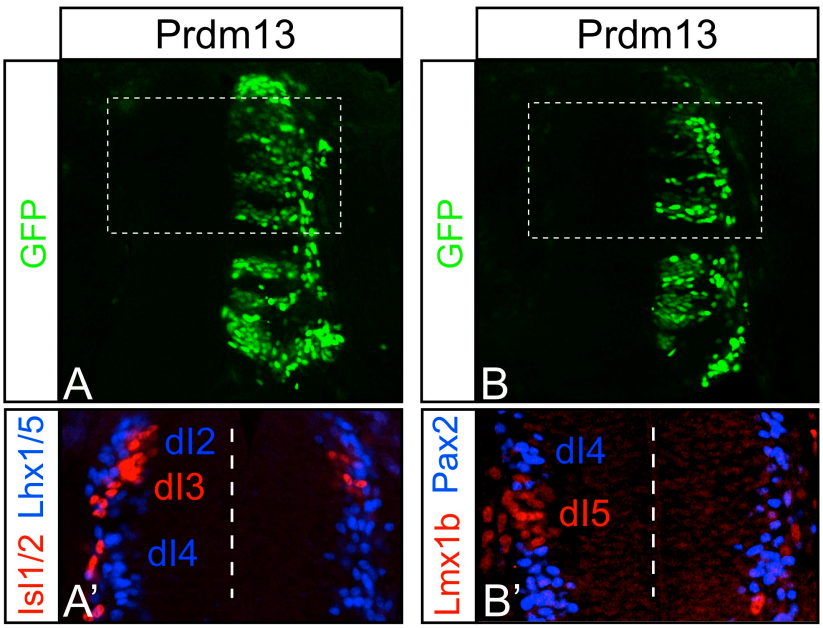


Figure S3 (related with Fig. 3)
Prdm13 induces inhibitory and suppresses excitatory neuronal markers in the dorsal neural tube.



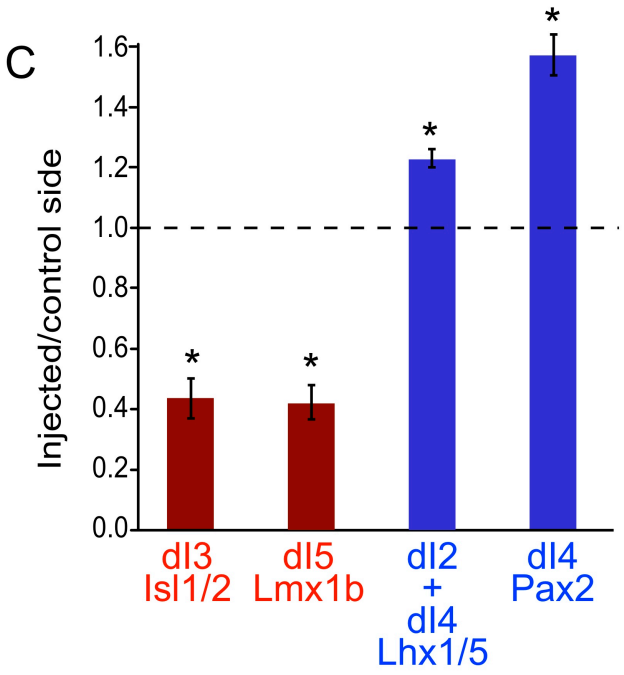
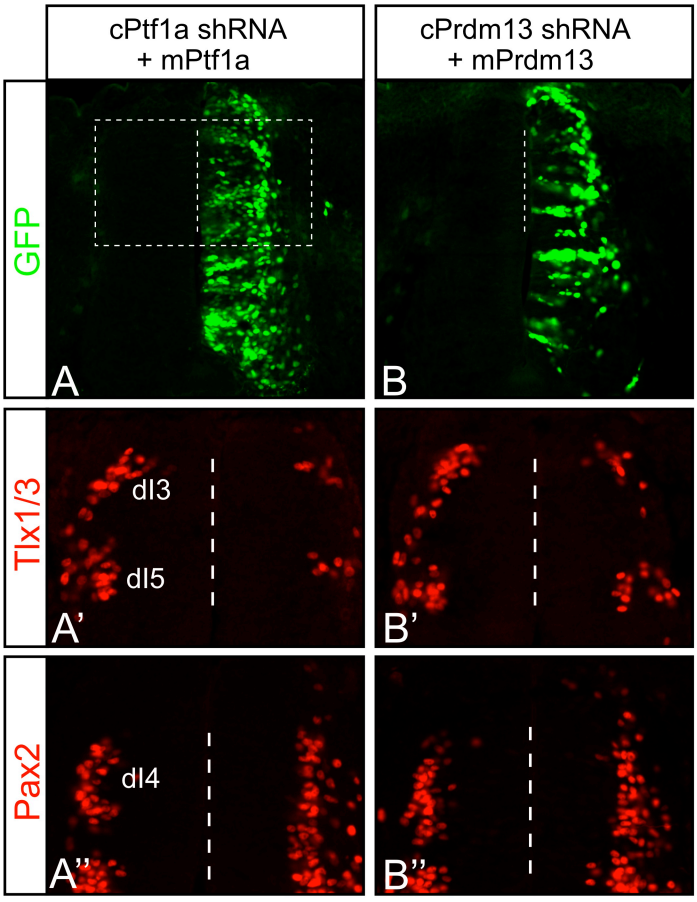
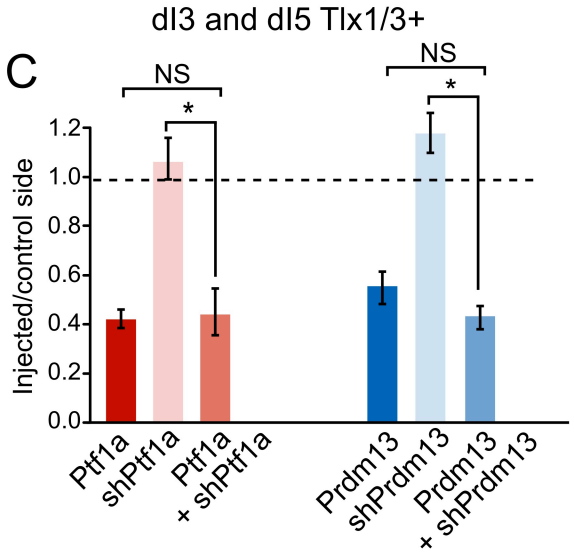


Figure S4 (related with Fig. 4)
Rescue of Ptf1a and Prdm13 shRNA knockdown phenotypes.





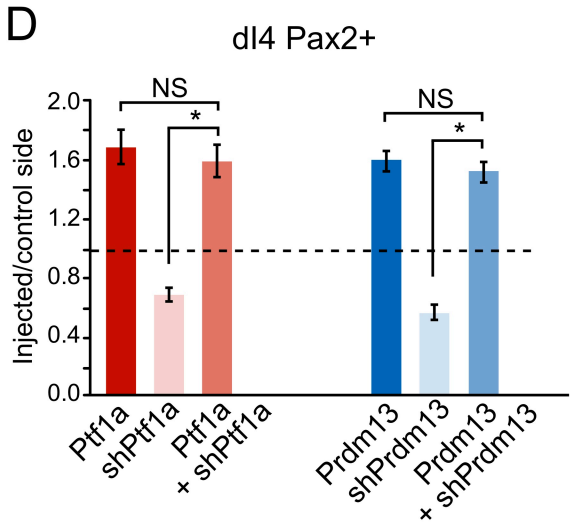
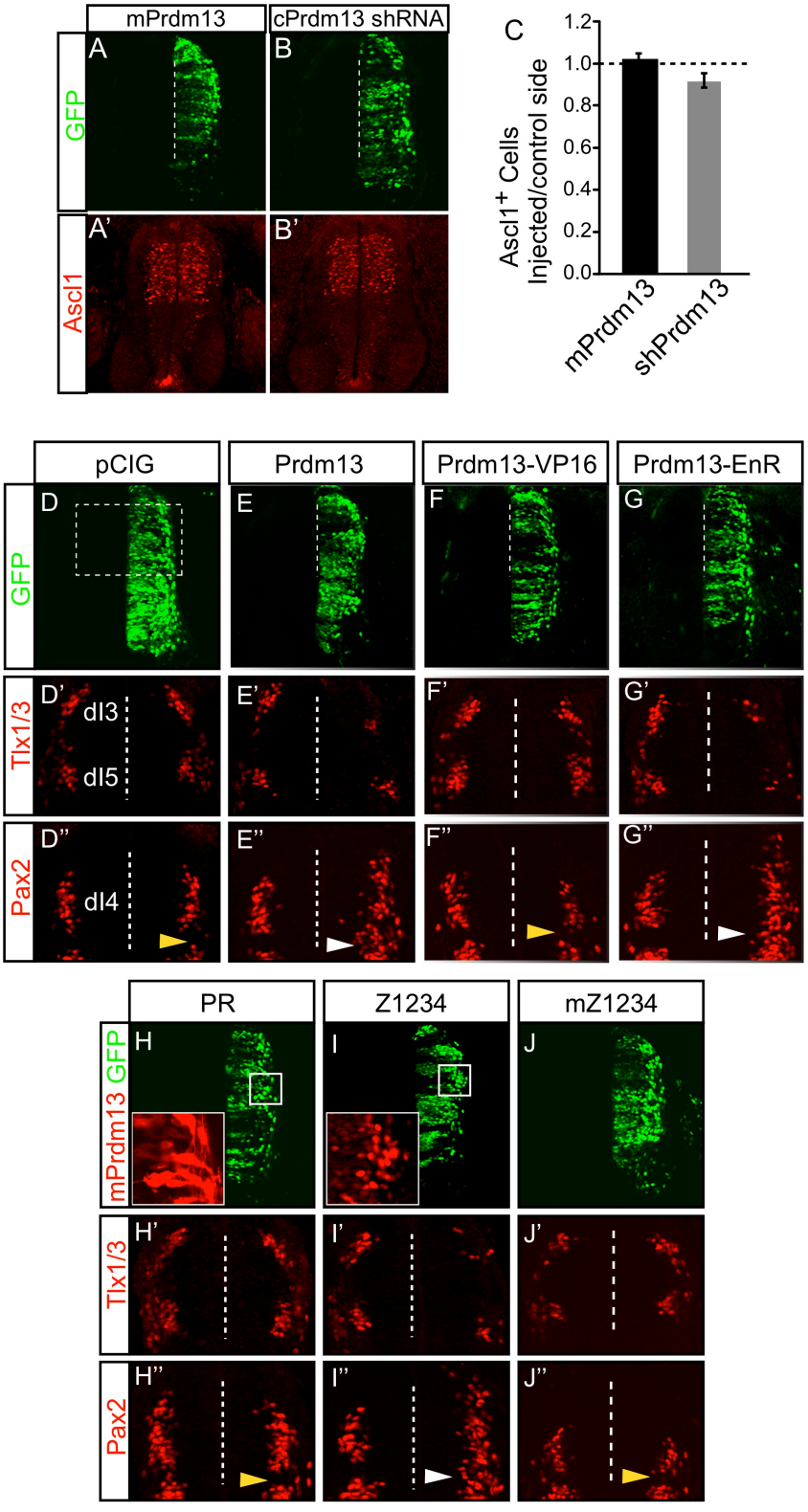


Figure S5 (related with Fig. 5)
The zinc fingers in Prdm13 are required for its activity.



Supplementary Figure Legends

Figure S1 (associated with Fig. 1). Lbx1 induces Pax2 in Tlx1/3-negative cells.

Stage HH12-13 chick embryos electroporated with either an empty pCIG vector as control (A), or an Lbx1 expression vector (B). Transverse sections through stage HH24-25 are shown with the electroporated side on the right. (A-B) GFP (green) indicates the electroporation efficiency along the dorsoventral axis. Dashed box in (A) is the region of the neural tube shown in (A'-B"). Dashed vertical line indicates the ventricle.

(A'-B', E) Dorsal neural tube section shows the dI3 and dI5 neuronal population marked by Tlx1/3 (red). The overexpression of Lbx1 does not affect the expression of Tlx1/3 (B' and E).

(A"-B", F) Dorsal neural tube section shows the dI4 neuronal population marked by Pax2 (red). The overexpression of Lbx1 causes ectopic $Pax2^{+}$ cells only in the dI2 domain where Tlx1/3 are not present (B", green arrowhead). This finding is consistent with Tlx1/3 repressing Lbx1 activity as reported (refCheng) (B", small yellow arrowheads). More than 6 embryos were quantified for each condition and error bars are reported as standard error of the mean. Asterisk indicates significant difference relative to the pCIG controls with p-value<0.001.

Figure S2 (associated with Fig. 2). *Prdm13* expression in the dorsal neural tube

(A-B") Transverse sections through mouse E10.5 and E12.5 spinal cord show Ptf1a expression by immunofluorescence, (A-B), and *Prdm13* mRNA by in situ hybridization in wildtype (A'-B') and *Ptf1a-/-* (A"-B"). Note that the expression of *Prdm13* is reduced (black arrowheads) in the *Ptf1a* null mutant specifically in the Ptf1a domain (white arrowheads).

(C) Genomic coordinates from the mouse genome build mm9 for the Ptf1a ChIP-seq peaks associated with the *Prdm13* gene locus.

(D-J) Transverse sections through mouse E9 to E14.5 spinal cords show *Prdm13* mRNA expression patterns by in situ hybridization in wildtype embryos. Prdm13 starts to express as early as E9 in the ventricular zone in the dorsal spinal cord with enrichment in regions lateral to the Ptf1a domain from E9.5 till E11.5 (E-G). By E14.5, *Prdm13* mRNA is almost gone, reflecting the diminished proliferative zone (J).

Figure S3 (associated with Fig. 3). Prdm13 induces inhibitory and suppresses excitatory neuronal markers in the dorsal neural tube

Stage HH12-13 chick embryos overexpressing Prdm13. Transverse sections through stage HH24-25 are shown with the electroporated side on the right. Dashed box in (A) is the region of

the neural tube shown in (A'-B"). Dashed vertical line indicates the ventricle. (A-B) GFP (green) indicates the expression of the ectopic Prdm13 and also the electroporation efficiency along the dorsoventral axis.

(A') Dorsal neural tube section shows the dI3 neuronal population marked by Isl1/2 (red), and dI2 and dI4 neuronal populations marked by Lhx1/5 (blue).

(B') Dorsal neural tube section shows the dI5 neuronal population marked by Lmx1b (red), and dI4 and dI6 neuronal populations marked by Pax2 (blue).

(C) The ratio of Isl1/2 (dI3) and Lmx1b (dI5) excitatory neurons (red) on the electroporated side versus the control side indicates that Prdm13 causes a dramatic decrease in the number of these neurons relative to empty vector control. The ratio of Lhx1/5 (dI2/dI4) and Pax2 (dI4) inhibitory neurons (blue) on the electroporated side versus the control side indicates that Prdm13 causes a significant increase in the number of these neurons relative to empty vector control. More than 6 embryos were quantified for each condition and error bars are reported as standard error of the mean. Asterisk indicates significant difference relative to controls with p-value<0.001.

Figure S4 (associated with Fig. 4). Rescue of Ptf1a and Prdm13 shRNA knockdown phenotypes

Stage HH12-13 chick embryos co-electroporated with a mouse Ptf1a expression vector and shRNA to chick Ptf1a (A), or a mouse Prdm13 expression vector and shRNA to chick Prdm13 (B). Transverse sections through stage HH24-25 are shown with the electroporated side on the right. (A-B) GFP (green) indicates the electroporation efficiency along the dorsoventral axis. Dashed box in (A) is the region of the neural tube shown in (A'-B'). Dashed vertical line indicates the ventricle.

(A'-B') Immunofluorescence for Tlx1/3 shows the phenotype of the knockdown constructs is rescued by the overexpression of Ptf1a or Prdm13 evident as a decrease in the number of Tlx1/3 neurons on the electroporated side.

(A"-B") Immunofluorescence for Pax2 shows the phenotype of the knockdown constructs is rescued by the overexpression of Ptf1a or Prdm13 evident as an increase in the number of Pax2 neurons on the electroporated side.

(C-D) Quantification of the rescue experiments shown in (A-B") relative to overexpression or shRNA experiments from Figs. 3,4. Error bars are reported as SEM. * indicates p-value<0.001. NS, not significant.

Figure S5 (associated with Fig. 5). The zinc fingers in Prdm13 are required for its activity.

Stage HH12-13 chick embryos were electroporated with the constructs indicated. Transverse sections through stage HH24-25 are shown with the electroporated side on the right.

(A-C) Endogenous Ascl1 expression is not altered when Prdm13 is either overexpressed (A') or knocked down (B') in chick neural tubes. (C) The number of $Ascl1^{+}$ cells does not change with manipulation of Prdm13 expression. Error bars are reported as SEM. p-value<0.001.

(D-J) GFP (green) indicates the electroporation efficiency along the dorsoventral axis. Dashed box in (D) is the region of the neural tube shown in (D'-J"). Insets in (H and I) are immunofluorescence for the ectopic mPrdm13 showing Z1234 contains information for nuclear localization of the protein.

(D'-J') Immunofluorescence for Tlx1/3 marks dI3/dI5 neurons. Quantified in Fig. 5B.

(D"-J") Immunofluorescence for Pax2 marks dI4 neurons. White arrowheads indicate ectopic Pax2 cells not found normally in the dI5 domain (marked by yellow arrowheads). Quantified in Fig. 5C.

Supplemental Experimental Procedures (SEP)

Antibodies used:

Name	Source	Use	Dilution		Ref
Ascl1	Johnson Lab generated	IHC	1:10000	guinea pig	(Kim et al., 2008)
Ptf1a	Johnson Lab generated	IHC	1:5000	guinea pig	(Hori et al., 2008)
Pax2	Zymed	IHC	1:1000	rabbit	
Tlx1/3	gift from T. Müller and C. Birchmeier	IHC	1:20,000	rabbit	
Islet1/2	gift from T. Jessell	IHC	1:5000	rabbit	(Tsuchida et al., 1994)
Lmx1b	gift from T. Müller and C. Birchmeier	IHC	1:2000	guinea pig	(Muller et al., 2002)
Lhx1/5	4F2, Developmental Studies Hybridoma Bank	IHC	1:100	mouse	
Brn3a	gift from E. Turner	IHC	1:500	guinea pig	(Quina et al., 2005)
Lbx1	gift from T. Müller and C. Birchmeier	IHC	1:10000	rabbit	
Caspase 3	Abcam ab2302	IHC	1:100	rabbit	
Ptf1a	gift from R. MacDonald	ChIP	2.4 µg/tube reaction	rabbit	(Beres et al., 2006)
Rbpj	Johnson lab generated using peptide sequence (CKKK)NSSQVPSNESNTNSE	ChIP	5 µL/tube reaction	rabbit	
Ascl1	BD Pharmigen, 556604	ChIP		mouse	
Prdm13	Johnson lab generated using	ChIP	10	rabbit	

	bacterially expressed full length GST-tagged Prdm13		µL/tube reaction		

Primers:

Primers for ChIP-qPCR:

Gene name	F	R
Kirrel2 ORF (Cont, Fig. 6B)	AGAGGACATGGTGGTGCTGTTGG	TGAGCAGAGACCAGCTCACCTG
eTlx1	GGAGCCTCCTCCCTCAATCGGT	CACCAGCGTCCGCTCTGCCA
eTlx3	TGCACCAGTAGCAGGTGCCAG	AGGCCGGCACCAGAAACAATCG
Dll1 M (eDll1)	GCGTGGCTGTCATTAAGG	GGTGCTGTCTGCATTACC
Dll1 N (Cont, Fig. 6C)	ATGACACGCCTTTAGACG	AGCTGTGGGAGTATAGAGAC

Oligonucleotides for generating the shRNA:

Target gene name	Target sequence
Ptf1a	1. AAGTGTAACTTAAGATTCGGA 2. AAGAAAATCATCATCTGCCAC
Prdm13	1. AAGAAGCCCTTGGGAACAAAG 2. AAGCTGTACTCCCGCAAGTAC
DSred control	AAGGTGAAGTTCATCGGCGTG

Primers for generating ISH probes:

Probe name	F	R
cPrdm13	TCGTCTGTTCCTGTACTGCGGC	CGTCGGTGAAGCAGACGTCCAC
mPrdm13	TACATCTGCTGGTACTGCTGG	GCGCTCCACAGGGAGCCCGGG

Primers for generating GFP reporter constructs:

Enhancer::GFP	F	R
eTlx1::GFP	GCACGCTTGCACAAGTAGTA	CACTCGGGTCACTGCACTT
eTlx3::GFP	GTACTGTCATTCACAGCAATAGTT	TTACCCCTACCCCTCACACC

Primers for genotyping Ptf1a null:

Genotyping	F	R	Band size
Wild-type	TGAGGAAGATTTCTTCACCGA CCAGTCCTC	CGGTAGCAGTATTCGTG TAGCTGGTG	133bp
Mutant	GGACATGTTCAGGGATCGCCA GGCG	GCATAACCAGTGAAACA GCATTGCTG	300bp

Experimental Procedures:

Chromatin Immunoprecipitation and Sequencing Library Preparation

Ptf1a, Rbpj and Prdm13 ChIPs

Solutions:

Buffer A (15 mM Hepes-Cl 7.6, 60 mM KCl, 15 mM NaCl, 0.2 mM EDTA, 0.2 mM EGTA, 0.34 M sucrose, 1 tab/10 mL Protease inhibitor tablet[a])

Buffer B (15 mM Hepes-Cl 7.6, 60 mM KCl, 15 mM NaCl, 2.1 M sucrose, 1 tab/10 mL Protease inhibitor tablet[a])

Buffer C (15 mM Hepes-Cl 7.6, 60 mM KCl, 15 mM NaCl, 0.34 M sucrose, 2 mM $MgCl_2$, 1 tab/10 mL Protease inhibitor tablet[a])

[a] Roche Complete, Mini cat #11836153001

ChIP sonication Buffer (1% Triton X-100, 0.1% Deoxycholate, 50 mM Tris 8.1, 150 mM NaCl, 5 mM EDTA). Just before use, add protease inhibitor tablet to each 10 ml.

High Salt Wash Buffer (1% Triton X-100, 0.1% Deoxycholate, 50 mM Tris-8.1, 500 mM NaCl, 5 mM EDTA)

LiCl Wash Buffer (250 mM LiCl, 0.5% NP-40, 0.5% Deoxycholate, 10 mM Tris-8.1, 1 mM EDTA)

TE Buffer (10mM Tris, 8.1, 1 mM EDTA)

Protein A/G Plus Agarose (Santa Cruz)

Proteinase K (19 mg/ml, Boehringer Mannheim # 1964372)

Elution Buffer (1% SDS, 0.1 M NaHCO3)

37% Formaldehyde (ACS reagent grade)

1.25 M glycine

ChIP protocol:

Neural tube, telencephalon, and limb tissues from E12.5 mouse embryos were dissected and placed in Buffer A on ice. Nuclei were liberated by dounce homogenization and purified by centrifugation through a sucrose gradient (Buffer A and Buffer B; 1:1). For the Prdm13 ChIP, the nuclei were incubated in 1.5 mM ethylene glycolbis (succinimidyl succinate) a long-arm cross-linker in Buffer C for 20 min at RT, prior to fixation in 1% formaldehyde for 10 minutes at 30 degrees C (Zeng et al., 2006). Fixation was terminated by adding glycine to a final concentration of 0.125M. Chromatin was sheared using a Diagenode Bioruptor for 28 minutes on high power with 30s:30s on:off cycles. 150-200 µg of neural tube or telencephalon/limb chromatin was immunoprecipitated with either 2.4 µg affinity purified rabbit anti-Ptf1a antibody (Beres et al., 2006), 5 µL of rabbit Rbpj antisera (laboratory generated, TX857), or 10 µL of rabbit Prdm13 antisera (laboratory generated, TX970), and 50 µL Protein A/G agarose beads (Santa Cruz). Captured bead:antibody complexes were washed twice with sonication buffer, three times with a high salt buffer, three times with LiCl buffer, and twice with TE buffer. Two 15 minute elutions were performed with 1% SDS, 0.1M $NaHCO_3$, 10mM Tris at room temperature. The immunoprecipitated chromatin was purified using Qiagen's PCR cleanup kit and re-suspended in 60 µL elution buffer. Prior to sequencing, ChIP quality was determined by qPCR for known targets and negative control regions.

Independent libraries were prepared from neural tube and telencephalon ChIPs according to Illumina's protocol (NEBNext ChIP-Seq Sample Prep Master Mix Set 1). Amplification of the libraries was conducted using Invitrogen's Platinum *Pfx* polymerase for 12 cycles of 94^{o}C for 15 seconds, 62^{o}C for 30 seconds, 72^{o}C for seconds. Single-end sequencing of 36 bp (Ptf1a) or 40 bp (Rbpj) were conducted on the Illumina GAIIx or 50 bp (Prdm13) was conducted on the Illumina HiSeq 2000 sequencer.

Ascl1 ChIP

Solutions:

Fix solution (11% Formaldehyde, 0.1 M NaCL, 1 mM EDTA pH 8.0, 0.05 mM EGTA 8.0, 1 M Hepes 8.0)

10x IP buffer (0.2 M Hepes 8.0, 0.2 M NaCl, 0.02 M EDTA 8.0)

Working IP Buffer (1x IP buffer, 0.1% Trition X-100, 1mg/ml BSA)

Wash Buffer (50 mM Hepes 7.6, 1 mM EDTA 8.0, 1.0% NP-40, 0.7% Deoxycholate, 512 mM LiCl)

Elution buffer (10 mM Tris 8, 1.0% SDS)

ChIP Protocol:

E12.5 Neural tube or limb tissue was dissected and placed in cold PBS. Nuclei were liberated by dounce homogenization, 1/10 volume of fixation solution was added to the nuclei for rotated for 10 minutes. Fixation was stopped by adding 1/20 volume of 2.5M glycine (non-buffered), and incubated for 5 minutes at RT. Nuclei were spun at 1.2K rpm at 4 degrees C or at the low speed sufficient to pellet the sample for 5 minutes. The nuclei pellet with rinsed with cold PBS and re-spun at 1.2K rpm at+4C or at the low speed sufficient to pellet the sample for additional 5 minutes. The pellet was then re-suspended in 300ul of IP buffer. Chromatin was sheared in the IP buffer using a Diagenode Bioruptor for 28 minutes on high power with 30s:30s on:off cycles. 250 µg of neural tube or limb chromatin was immunoprecipitated with 5.0 µg affinity purified mouse anti-Ascl1 (BD Pharmigen) antibody, and 50 µL and Sheep anti-Mouse IgG Dynabeads (Invitrogen) overnight at 4 degrees C. Captured bead:antibody complexes were washed seven times with wash buffer and twice with TE buffer. Chromaton elution was performed by adding 500ul of elution buffer to the bead:antibody complexes and rotated at 65 degrees. The immunoprecipitated chromatin was purified using Qiagen's PCR cleanup kit and re-suspended in 60 µL Qiagen's elution buffer.

mRNA Isolation and Sequencing Library Preparation:

Mouse neural tubes were dissected from E11.5 *12.4kbPtf1a::mcherrry* embryos either wildtype or null for Ptf1a and placed into DMEM/F12 on ice and dissociated in 0.25% trypsin for 15 minutes at 37 degrees C. Trypsin activity was quenched with 2% fetal bovine serum, and mcherry positive cells were purified from the resulting single cell suspension by fluorescence activated cell sorting (FACS). Total RNA from FACS sorted neural tube populations were extracted and purified with Zymo's Mini RNA Isolation Kit. mRNA was purified, reverse transcribed and amplified for sequencing with Illumina's

mRNA-Seq kit according to manufacturer's instructions. Two independent libraries were sequenced for each cell population.

Bioinformatics:

ChIP-Seq Peak Calling:

Sequence reads were mapped to the mm9 assembly of the mouse genome using Bowtie (Langmead et al., 2009). Reads that had greater than two base pair mismatches or aligned to more than one place in the reference genome were removed. In addition, duplicate reads were removed for further analysis. Unique read numbers were normalized to 10 million total reads for comparison across experiments. Peak calling was performed by Homer (Heinz et al., 2010), the FDR cutoff was set at 0.001 and peaks were required to have greater than a 4-fold read enrichment over local background and control sample. Control samples used for Ptf1a ChIP-Seq was a Ptf1a ChIP in the telencephalon, RbpJ ChIP-seq was compared to input sample, and Prdm13 was compared to both Prdm13 ChIP in the telencephalon and input sample.

RNA-Seq Analysis:

Sequence reads were aligned to the mm9 build of the mouse genome using TopHat v1.4.1 (Trapnell et al., 2009). Default settings were used with the following exceptions: -G option (instructs TopHat to initially map reads onto a supplied reference transcriptome) and –no-novel-juncs option, which ignores putative splice junctions occurring outside of known gene models. Expression levels were determined by the FPKM method using Cuffdiff v1.3.0 with the same reference gene annotation file (from RefSeq) used in the TopHat alignment (Trapnell et al., 2010). Upper quartile normalization (-N option) was performed to more accurately estimate levels of low-abundance transcripts, and multiple read correction (-u option) was selected to better distribute reads mapping to multiple genomic locations. The minimum number of alignments needed for significance testing was set to 1 (-c option). All other settings were left at default values.

References

Beres, T.M., Masui, T., Swift, G.H., Shi, L., Henke, R.M., and MacDonald, R.J. (2006). PTF1 is an organ-specific and Notch-independent basic helix-loop-helix complex containing the mammalian Suppressor of Hairless (RBP-J) or its paralogue, RBP-L. Mol Cell Biol *26*, 117-130.

Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y.C., Laslo, P., Cheng, J.X., Murre, C., Singh, H., and Glass, C.K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell *38*, 576-589.

Hori, K., Cholewa-Waclaw, J., Nakada, Y., Glasgow, S.M., Masui, T., Henke, R.M., Wildner, H., Martarelli, B., Beres, T.M., Epstein, J.A.*, et al.* (2008). A nonclassical bHLH Rbpj transcription factor complex is required for specification of GABAergic neurons independent of Notch signaling. Genes Dev *22*, 166-178.

Kim, E.J., Battiste, J., Nakagawa, Y., and Johnson, J.E. (2008). Ascl1 (Mash1) lineage cells contribute to discrete cell populations in CNS architecture. Mol Cell Neurosci *38*, 595-606.

Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol *10*, R25.

Muller, T., Brohmann, H., Pierani, A., Heppenstall, P.A., Lewin, G.R., Jessell, T.M., and Birchmeier, C. (2002). The homeodomain factor lbx1 distinguishes two major programs of neuronal differentiation in the dorsal spinal cord. Neuron *34*, 551-562.

Quina, L.A., Pak, W., Lanier, J., Banwait, P., Gratwick, K., Liu, Y., Velasquez, T., O'Leary, D.D., Goulding, M., and Turner, E.E. (2005). Brn3a-expressing retinal ganglion cells project specifically to thalamocortical and collicular visual pathways. J Neurosci *25*, 11595-11604.

Trapnell, C., Pachter, L., and Salzberg, S.L. (2009). TopHat: discovering splice junctions with RNA-Seq. Bioinformatics *25*, 1105-1111.

Trapnell, C., Williams, B.A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M.J., Salzberg, S.L., Wold, B.J., and Pachter, L. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol *28*, 511-515.

Tsuchida, T., Ensini, M., Morton, S.B., Baldassare, M., Edlund, T., Jessell, T.M., and Pfaff, S.L. (1994). Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. Cell *79*, 957-970.

Zeng, P.Y., Vakoc, C.R., Chen, Z.C., Blobel, G.A., and Berger, S.L. (2006). In vivo dual cross-linking for identification of indirect DNA-associated proteins by chromatin immunoprecipitation. Biotechniques *41*, 694, 696, 698.