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Bcl11a is required for neuronal morphogenesis and sensory circuit formation in dorsal spinal cord development

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SUMMARY

Dorsal spinal cord neurons receive and integrate somatosensory information provided by neurons located in dorsal root ganglia. Here we demonstrate that dorsal spinal neurons require the Krüppel-C_2H_2 zinc-finger transcription factor Bcl11a for terminal differentiation and morphogenesis. The disrupted differentiation of dorsal spinal neurons observed in *Bcl11a* mutant mice interferes with their correct innervation by cutaneous sensory neurons. To understand the mechanism underlying the innervation deficit, we characterized changes in gene expression in the dorsal horn of *Bcl11a* mutants and identified dysregulated expression of the gene encoding secreted frizzled-related protein 3 (sFRP3, or Frzb). *Frzb* mutant mice show a deficit in the innervation of the spinal cord, suggesting that the dysregulated expression of Frzb can account in part for the phenotype of *Bcl11a* mutants. Thus, our genetic analysis of *Bcl11a* reveals essential functions of this transcription factor in neuronal morphogenesis and sensory wiring of the dorsal spinal cord and identifies Frzb, a component of the Wnt pathway, as a downstream acting molecule involved in this process.

KEY WORDS: Spinal cord, Transcription factor, Neuronal differentiation, Bcl11a (CTIP1), Mouse

INTRODUCTION

The ability of the nervous system to integrate and relay information relies on the coordinated development of neuronal circuits. An immense diversity of neuron types is created during development. Extrinsic and intrinsic mechanisms control their subsequent maturation and the establishment of functional connectivity. Connectivity relies on the presence of dendrites in postsynaptic neurons and on the cues that allow navigation of the axons of presynaptic neurons, both of which have to be precisely orchestrated to establish a functional neuronal circuitry (Marmigere and Ernfors, 2007; Dasen, 2009).

Somatosensory neurons and their target, the spinal cord, provide a model for the molecular analysis of neuronal circuit development (Vrieseling and Arber, 2006; Yoshida et al., 2006; Pecho-Vrieseling et al., 2009). During development of the dorsal spinal cord, different neuron types are generated from defined progenitor domains and subsequently become positioned in a highly organized laminar structure, the dorsal horn (Goulding et al., 2002; Helms and Johnson, 2003). Somatosensory neurons with cell bodies in dorsal root ganglia (DRG) innervate these spinal neurons, and distinct sensory neuron types project to different spinal cord layers. For instance, nociceptive neurons project to the upper layers of the dorsal horn, whereas mechanosensory and proprioceptive neurons innervate deeper layers (Brown, 1981).

Transcriptional networks are essential for spatiotemporally correct neuronal differentiation and wiring in the spinal cord (reviewed by Dasen, 2009). For example, members of the Paired related, LIM homeobox or TLX families of transcription factors are involved in cell fate decisions, morphogenesis, positioning and wiring of dorsal spinal neurons (Chen et al., 2001; Qian et al., 2002; Cheng et al., 2004; Ding et al., 2004; Marmigere et al., 2006). The signals provided by dorsal horn neurons that guide sensory axons from DRG neurons to their spinal targets are incompletely defined. Several members of the Wnt pathway are expressed in the spinal cord. Wnts have been extensively demonstrated to possess axon guidance activity and to control neural circuit formation (reviewed by Salinas and Zou, 2008), making this pathway an attractive candidate regulator in the process of sensory wiring within the dorsal horn.

In a screen designed to identify novel candidate genes that control development of the somatosensory circuitry, we identified *Bcl11a* as a gene that is expressed in the dorsal horn of the spinal cord and in sensory neurons. *Bcl11a* (also known as *Evi9*, *Ctip1*) encodes a C_2H_2 zinc-finger transcription factor that acts as a transcriptional regulator through its interaction with COUP-TF proteins and through direct, sequence-dependent DNA binding (Avram et al., 2000). Bcl11a is expressed in lymphohematopoietic cells, in which it controls the development of B- and T-lymphocytes (Liu et al., 2003b) and the maintenance of mature erythroid cells (Sankaran et al., 2008; Sankaran et al., 2009). The close relative, Bcl11b, is required for differentiation of striatal neurons and the corticospinal tract (Arlotta et al., 2005; Arlotta et al., 2008). However, functions of Bcl11a in the somatosensory system have not been defined.

Here we show that Bcl11a is an important regulator of terminal neuronal differentiation and wiring. Mutation of *Bcl11a* in spinal neurons disrupts their maturation and morphogenesis,

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which is accompanied by defective innervation of the dorsal horn by somatosensory neurons. Among the genes dependent on Bcl11a for expression, we identified the Wnt antagonist secreted frizzled-related protein 3 (*sFRP3*, or *Frzb*) as a transcriptional target. Interestingly, we observe similar innervation deficits in the dorsal spinal cord of *Bcl11a* and *Frzb* mutants. Our findings show that Bcl11a is essential for sensory circuit formation and implicate Frzb in one aspect of the changes observed in *Bcl11a* mutant mice.

MATERIALS AND METHODS

Animals

To generate a conditional knockout allele (*Bcl11a*^{flox}) a neomycin resistance cassette was introduced at the 3′ end of exon 1 of the *Bcl11a* gene. Exon 1 and the neomycin resistance cassette were flanked by loxP sites using previously described strategies (Liu et al., 2003a). Upon Cre-mediated recombination, exon 1 and the neomycin resistance cassette were excised from the *Bcl11a* locus, resulting in a null allele. To obtain mice with tissue-specific ablation of the *Bcl11a* allele, *Bcl11a*$^{flox/flox}$ mice were crossed to either Deleter-Cre (Schwenk et al., 1995), Brn4-Cre (Bcre-32 pedigree) (Wine-Lee et al., 2004) or Ht-PA-Cre (Pietri et al., 2003) transgenic mice. Homozygous mutants were compared with heterozygous littermates harboring a *Cre* allele. *Frzb* and *Sox10* mutant mice were described previously (Britsch et al., 2001; Lories et al., 2007). Mice were genotyped by PCR. All animal experiments were carried out in accordance with German law and were approved by the respective governmental offices in Berlin, Göttingen and Tübingen.

In situ hybridization, antibodies and histology

For in situ hybridization, spinal cords were dissected from mouse embryos at E14.5-18.5, fixed in 4% PFA and embedded in OCT compound (Sakura). Hybridizations were performed with DIG-labeled riboprobes on 18 μm cryosections.

For immunofluorescence staining, tissue was fixed with 4% PFA in 0.1 M sodium phosphate buffer (pH 7.4). Cryosections (14 μm) were obtained from matched cervical spinal cords. Stained sections were examined on a Zeiss LSM510 or Leica SP5II confocal microscope.

The following antibodies were used: rabbit and guinea pig anti-Lbx1 (Thomas Mueller, Berlin), rabbit anti-Ebf1 (H. Wildner and C. Birchmeier, Berlin), guinea pig anti-Lmx1b (T. Jessel, New York), rabbit anti-TrkA (L. Reichardt, San Francisco), rabbit anti-CGRP (Chemicon), rabbit anti-parvalbumin (Chemicon), rabbit anti-aquaporin 1 (Chemicon), rabbit anti-MAP2 (Chemicon), mouse monoclonal anti-HuC/D (Invitrogen), mouse monoclonal anti-tubulin (Sigma), and fluorophore-conjugated secondary antibodies (Dianova).

To generate an anti-Bcl11a antiserum, a 486 bp fragment of murine *Bcl11a* cDNA encoding amino acids 501-662 (NM_016707) was amplified by PCR and cloned into the bacterial expression vector pET-14b (Novagen), which provides the coding sequences for a His6 tag. His6-Bcl11a was propagated in BL21(DE3)pLysS cells, affinity purified on TALON metal resin (BD Biosciences) and injected into rabbits and guinea pigs (Charles River).

For anterograde labeling of sensory axons, segments of the vertebrate column with the spinal cord and DRG in loco were dissected from mutant and control embryos and fixed overnight in 4% PFA. DiI crystals (Invitrogen) were placed directly onto DRG, matched for their axial levels. DiI-loaded tissues were incubated at 37°C for up to 5 days. DiI tracings were examined on 80 μm vibratome sections using a confocal microscope.

BrdU labeling of neurons and Golgi staining of spinal cord were carried out as described (Heimrich and Frotscher, 1991; Gross et al., 2002).

Cell counting

Cervical spinal cords matched for axial level from at least three mutant and control animals were sectioned serially (14 μm). Cell numbers were counted on every fourth section. A total of three sections were evaluated per animal. Values are presented as mean±s.e.m. Differences were considered significant at $P<0.05$ by Student's *t*-test.

Neuron cultures

Primary cultures from dorsal spinal cord neurons were established according to a modified previously published protocol (Banker and Goslin, 1998). Dorsal spinal horns were dissected from control and mutant embryos at E18.5. Cells were plated on poly-lysine-coated slides at 6-8×10^4 cells/ml. Neurons derived from the superficial dorsal horn were identified by expression of Lmx1b and cultured for up to 14 days in serum-free medium in the presence of 10 ng/ml NGF. For Sholl analysis (Sholl, 1953), images of individual neurons were overlaid with a grid of concentric circles, and the length and number of neurite branches were determined at 2.5 μm intervals from the soma. Neuron cultures from three individual mutant and control animals and at least ten neurons per neuron culture were analyzed.

Electrophysiology

Whole-cell patch-clamp recordings of evoked excitatory postsynaptic currents (EPSCs) were performed as described (Liebel et al., 1997; Schmidt et al., 2007).

For slice preparation, cervical spinal cords from control and *Bcl11a* mutant animals were microdissected at E18.5 and embedded in agarose (2.5% SeaPlaque agarose, CAMBREX). Transverse slices (200 μm) were prepared on a vibratome. During recordings, slices were perfused with a bath solution of 125 mM NaCl, 4 mM KCl, 10 mM glucose, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 mM $MgCl_2$.

Numerical data are reported as mean±s.e.m. Statistical analysis was performed using StatView (SAS Institute). Student's *t*-test (two-tailed, unpaired; decay time, rise time, evoked EPSC) or χ^2 test (success rates, response rates) were used for statistical comparisons.

Microarray analysis

Microarray experiments were performed according to MIAME standards (Brazma et al., 2001) (http://www.mged.org/Workgroups/MIAME/miame.html). For each time point analyzed (E14.5 and E16.5), dorsal spinal cords from ten age-matched *Bcl11a* mutant (*Bcl11a*$^{flox/flox}$*;Brn4-Cre*) and ten control (*Bcl11a*$^{flox/+}$*;Brn4-Cre*) animals were used. Segments of the cervical spinal cord were dissected in ice-cold PBS and matched for axial level. To isolate their dorsal halves, we used the sulcus limitans as an anatomical landmark. After dissection, dorsal spinal cord segments were transferred to Trizol (Invitrogen). For RNA isolation, mutant and control tissue were pooled separately. cDNA synthesis and biotinylation of cRNA probes were carried out according to standard Affymetrix protocols with three technical replicates of mutant and control RNA, starting with 5 μg total RNA for each replicate. From each replicate, 15 μg of labeled cRNA were hybridized for 16 hours at 45°C to mouse Affymetrix MOE430 2.0 microarrays in a GeneChip hybridization oven 645. After hybridization, microarrays were washed and stained in a GeneChip fluidics station 450, and scanned on a GeneChip scanner 3000 7G. Raw data analysis was performed using Bioconductor software (www.bioconductor.org). Quality of hybridization results was assessed with the affyPLM Bioconductor tool. After GC-RMA normalization of data, $\log_2$ signal intensity was used for value definition.

Quantitative real-time RT-PCR

Total RNA was prepared from dorsal spinal cord of controls and *Bcl11a* mutants using the RNeasy Mini kit (Qiagen). RNAs were reverse transcribed with Superscript II reverse transcriptase (Invitrogen) and quantitative real-time PCR (qRT-PCR) was performed using the QuantiTect SYBR Green PCR kit (Qiagen) in a LightCycler 480 system (Roche). The following oligonucleotides (5′-3′) were used: *Frzb*, GCTGAGAA-GTGGAAGGATCG and AACTGTGCGGTTTTCCTTTG; *Gapdh*, CCAGAGCTGAACGGGAAG and TGCTGTTGAAGTCGCAGG. The relative copy number of *Gapdh* RNA was quantified and used for normalization. Data were analyzed using the 2–ΔΔCt method (Livak and Schmittgen, 2001).

Chromatin immunoprecipitation (ChIP) on embryos

ChIP assays were performed on wild-type dorsal spinal cords collected from ten embryos at E14.5 using the EpiQuik Tissue Chromatin Immunoprecipitation kit (Epigentek, Framingdale, NY, USA) including

specific antibodies recognizing RNA polymerase II and mouse IgG as controls. Every ChIP was replicated at least three times. The precipitated DNA was analyzed by quantitative PCR using oligonucleotides (5′-3′) that recognize the promoter regions of: *Gapdh*, AAGGCTGGTGCT-GTGGAGAAACTG and GTCCCCTTGCAACATACATAACTG; *Frzb* promoter region 1 (Frzb1), GAGCAGCCAGGACTAGCAAC and ACCTGGGAGCACTTGGATC; *Frzb* promoter region 2 (Frzb2), TTGAGTTTTCAGGGGACTGG and TCTCTGTGGTGCGTTGAGAC; *Frzb* promoter region 3 (Frzb3), ACCATTGAGCAGAGGGATTG and TTCCCTAAGTGCCATTGAGC; *Drg11*, TCAAATGCCCTACTGCTCCT and GGGACTGCCCAGAATTGATA. All primer sets were tested by PCR using input and genomic DNA (supplementary material Fig. S4). Promoter regions for *Frzb* and *Drg11* were selected using ensembl.org, avoiding known RNA polymerase II binding sites. The promoter fragment for *Gapdh* specifically flanks the TATA box of this gene (Iankova et al., 2006). ChIP-qPCR results were analyzed by determining the amount of precipitated DNA as a percentage of input DNA. Data of three independent ChIP assays were pooled, mean±s.e.m. was calculated and statistical analysis performed using Student's *t*-test.

RESULTS

Expression and conditional mutation of *Bcl11a* in the spinal cord

In mice, at least three different isoforms, termed Evi9a, b and c, are generated from the *Bcl11a* gene (Nakamura et al., 2000). To analyze expression of Bcl11a, we generated antibodies against a 161 amino acid fragment of Bcl11a that is present in the Evi9a and b isoforms, but not in Evi9c (Fig. 1D-F; supplementary material Fig. S1), and performed in situ hybridizations with pan-specific (Fig. 1A-C) and isoform-specific (Fig. 1G-I) riboprobes. *Bcl11a* transcript and protein were first detected at E11.0 and E11.5, respectively, in the mantle zone of the most superficial dorsal neural tube, and, albeit at much lower levels, within a few cells of the ventricular zone (Fig. 1A,D). With ongoing development, Bcl11a was expressed by the majority of postmitotic dorsal spinal neurons, and at E18 81.47±2.41% of cells expressing the pan-neuronal marker HuC/D co-express Bcl11a within the dorsal horn (Fig. 1B,C,E,F,K; supplementary material Fig. S1). All three isoforms of *Bcl11a* were detected in the spinal cord. Unlike *Evi9b*, *Evi9a* and *Evi9c* expression appeared in almost identical patterns restricted to the dorsal spinal cord (Fig. 1G-I). In addition, Bcl11a was expressed by the majority of primary sensory neurons in DRG, as well as in a few cells in the ventral neural tube, some of which co-express Isl1 and appear to represent differentiating motoneurons (Fig. 1D,E; data not shown).

Mice with a null mutation in *Bcl11a* show defects in lymphopoiesis and die shortly after birth (Liu et al., 2003b). To define functions of Bcl11a in neurons and to distinguish functions in presynaptic primary sensory neurons from those in postsynaptic dorsal spinal neurons we employed the Cre/loxP system (Fig. 1L-N). Homozygous mutant offspring from floxed *Bcl11a* mice crossed to a Deleter-Cre (*Del-Cre*) mouse strain (Schwenk et al., 1995) died within a few hours after birth and were indistinguishable from *Bcl11a* null mutants (Liu et al., 2003b). Bcl11a protein expression was undetectable in such mice (Fig. 1N). In situ hybridization with isoform-specific probes demonstrated the absence of *Evi9c* transcripts in the *Bcl11a* mutants (Fig. 1J). When floxed *Bcl11a* mice were crossed to *Brn4-Cre* mice that express *Cre* in neurons of the central but not of the peripheral nervous system (Wine-Lee et al., 2004), Bcl11a expression was eliminated from spinal neurons but not in primary sensory neurons within DRG (Fig. 1L). Crossing floxed *Bcl11a* mice to an *Ht-PA-Cre* mouse line, in which *Cre* is active in neural crest cells and their derivatives (Pietri et al., 2003; Yoshida et al., 2006), completely abolished Bcl11a expression in primary sensory neurons, whereas Bcl11a expression was preserved in most of the dorsal spinal neurons (Fig. 1M).

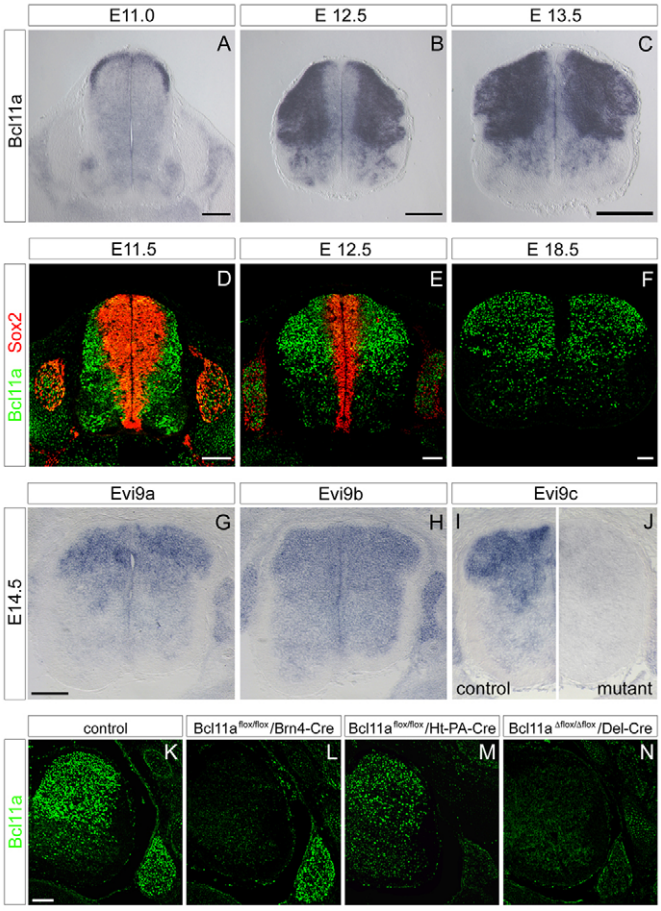

Fig. 1. Expression analysis and conditional mutation of *Bcl11a* in the spinal cord. (**A-F**) In situ hybridization (A-C) and immunohistological analysis (D-F) of Bcl11a expression in the embryonic spinal cord at different developmental stages using Bcl11a-specific probes and antibodies, respectively. The ventricular zone was visualized with antibodies against Sox2 (D,E). (**G-J**) In situ hybridizations using *Evi9a* (G), *Evi9b* (H) and *Evi9c* (I,J) specific probes on wild-type (G-I) or *Bcl11a* mutant (J) spinal cord sections of E14.5 embryos. (**K-N**) Immunohistological analysis of spinal cord and dorsal root ganglia (DRG) on transverse sections of control (K), *Bcl11a*$^{flox/flox}$;*Brn4-Cre* (L), *Bcl11a*$^{flox/flox}$;*Ht-PA-Cre* (M) and *Bcl11a*$^{\Delta flox/\Delta flox}$;*Del-Cre* (N) mouse embryos at E15.5 using antibodies against Bcl11a. Scale bars: 100 μm.

Bcl11a is required for terminal differentiation and morphogenesis of dorsal spinal neurons

Analysis of dorsal spinal neurons of *Brn4-Cre;Bcl11a* mice revealed changes in differentiation that were first apparent at E14.5. In control mice, neurons of the superficial dorsal horn express the transcription factors Lmx1b, Ebf1, Drg11 (Prrxl1 – Mouse Genome Informatics) and Pax2, with a few Lbx1-positive cells intermingled (Fig. 2A,C,E; supplementary material Fig. S2). This region corresponds to layers I and II in the adult spinal cord and is innervated by cutaneous sensory afferents from DRG. During this period, Bcl11a is expressed by the majority of postmitotic neurons of the dorsal spinal horn, with Evi9a and Evi9c being more restricted to the superficial zone, and overlaps with Lmx1b

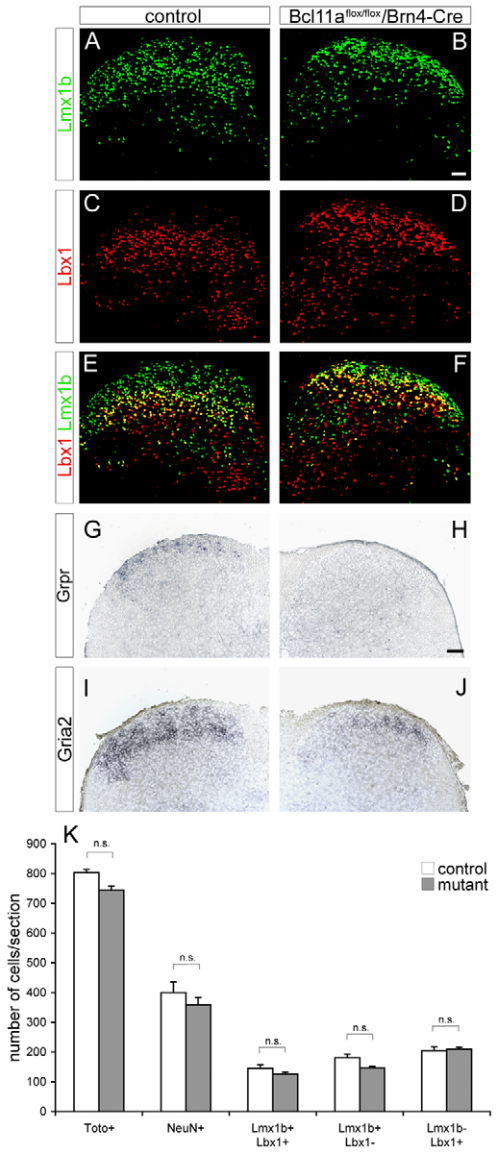

Fig. 2. Analysis of neuronal differentiation in the *Bcl11a* mutant dorsal spinal cord. (**A-F**) Immunohistological analysis on dorsal spinal cord sections from control (A,C,E) and *Bcl11a*$^{flox/flox}$;*Brn4-Cre* (B,D,F) mice at E18.5 using antibodies against Lmx1b (A,B,E,F) or Lbx1 (C-F). (**G-J**) In situ hybridization analysis on dorsal spinal cord sections from control (G,I) and *Bcl11a* mutant (H,J) animals at E18.5, with probes specific for *Grpr* (G,H) and *Gria2* (I,J). (**K**) Numbers of cells (Toto$^+$), neurons (NeuN$^+$) and of marker-defined neuron populations in the dorsal horn of *Bcl11a* mutant and control embryos. Mean±s.e.m. n.s., not significant. Scale bars: 50 μm.

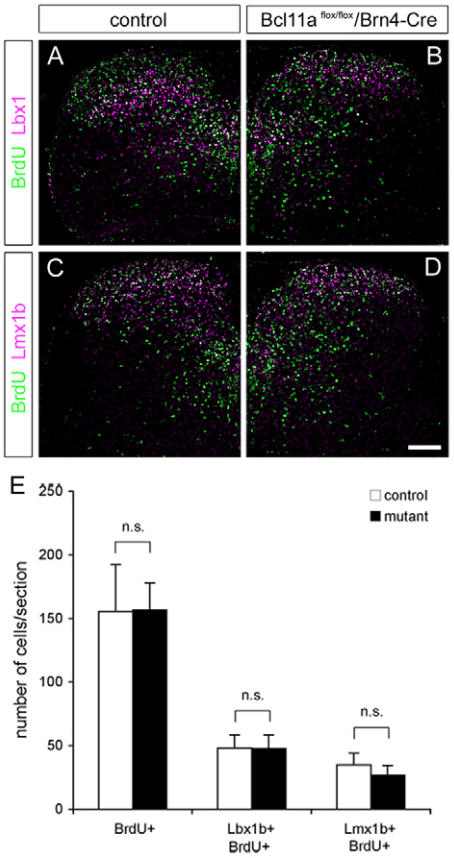

Fig. 3. Birthdating and tracing of *Bcl11a* mutant dorsal spinal neurons. (**A-D**) Immunohistochemical analysis of dorsal spinal cord on transverse sections of control (A,C) and *Bcl11a*$^{flox/flox}$;*Brn4-Cre* (B,D) mice that received a single dose of BrdU at E11.5 and were sacrificed at E16.5 using antibodies against BrdU (A-D), Lbx1 (A,B) and Lmx1b (C,D). (**E**) Numbers of BrdU$^+$, Lbx1$^+$ BrdU$^+$ and Lmx1b$^+$ BrdU$^+$ cells in the spinal cord of *Bcl11a* mutant and control embryos. Mean±s.e.m. n.s., not significant. Scale bar: 100 μm.

expression (Fig. 1C,F,G,I; supplementary material Fig. S1). In *Brn4-Cre;Bcl11a* mutants the superficial zone was invariably compressed, with the positions of the deeper layers shifted superficially. Lmx1b$^+$, Ebf1$^+$ or Drg11$^+$ nuclei within this zone appeared compacted when compared with controls (Fig. 2B,D,F; supplementary material Fig. S2A-D). To explore whether this phenotype is caused by changes in the size of specific neuron populations we determined the numbers of Toto$^+$, NeuN$^+$, Lbx1$^+$ or Lmx1b$^+$ cells within the dorsal horn. Total neuron numbers, as well as those of individual neuron populations, were unchanged in mutants (Fig. 2K). Pulse labeling with BrdU during late phase neurogenesis (Gross et al., 2002; Muller et al., 2002) revealed normal birth rates and migratory capacities of *Bcl11a* mutant dorsal spinal neurons (Fig. 3A-E). With ongoing development, neurons of the superficial dorsal horn express late differentiation markers, such as gastrin releasing peptide receptor (*Grpr*), galanin and *Gria2* (Baccei and Fitzgerald, 2004; Sun and Chen, 2007; Xu et al., 2008). Expression of these genes was greatly reduced (*Gria2*) or undetectable (*Grpr*, galanin) in mutants (Fig. 2G-J; data not shown), suggesting that neurons within the superficial dorsal horn require Bcl11a for terminal differentiation.

The compaction of neurons within the superficial dorsal horn might be caused by a reduced neuropil. To test this, we defined the expression of the microtubule-associated protein MAP2 (Mtap2 – Mouse Genome Informatics) (Ding et al., 2004). Compared with controls, MAP2 expression was greatly reduced in the superficial dorsal horn of *Bcl11a* mutant mice (Fig. 4A,B). At E18.5, Golgi-stained neurons in the dorsal horn of controls frequently display complex neurite trees (Fig. 4C). In homozygous mutants, neurites were severely reduced, and remaining structures often appeared misshapen (Fig. 4D). To quantify neurite formation in spinal neurons, we cultured primary neurons from superficial dorsal horn tissues and carried out a Sholl analysis (Fig. 4E-G). Viability and plating efficiency of mutant and control spinal neurons were

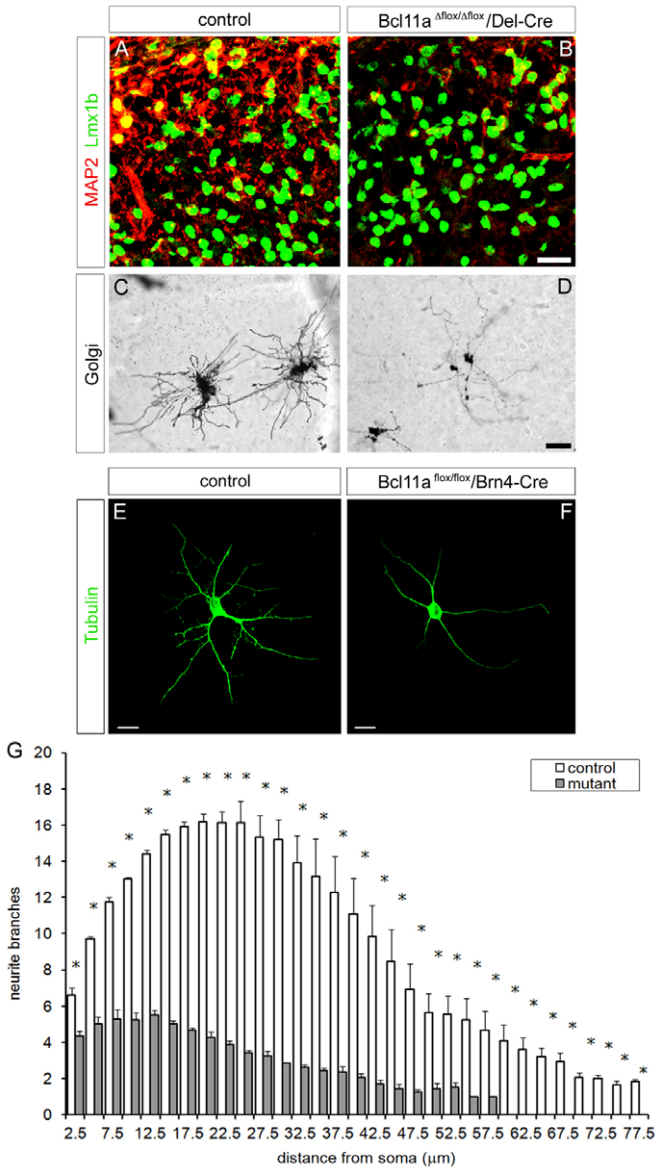

Fig. 4. Dorsal spinal neurons require Bcl11a for morphogenesis. (**A-D**) Transverse sections of the superficial dorsal horn of control (A,C) and Del-Cre recombined *Bcl11a* mutant (B,D) mice. Note that in mutants, Lmx1b-expressing neurons are more densely packed (B). Neurite morphology of control (C) and *Bcl11a* mutant (D) neurons in the superficial dorsal horn at E18.5 was visualized by Golgi staining. (**E**,**F**) Primary neuron cultures derived from dorsal spinal cord tissues of E18.5 control (E) and *Bcl11a* mutant (F) animals stained with anti-tubulin antibodies. (**G**) Sholl analysis of neurite length and arborization. Mean±s.e.m. *, $P<0.05$ (Student's *t*-test). Scale bars: 20 μm in B; 30 μm in D; 40 μm in E,F.

comparable. However, the overall number, length and branching frequency of neurites were significantly reduced in *Bcl11a* mutant neurons (Fig. 4G).

Primary sensory neurons require postsynaptic Bcl11a expression for correct wiring

Bcl11a is expressed in both presynaptic sensory neurons and postsynaptic spinal target neurons (Fig. 1). We next asked whether Bcl11a is required for correct wiring, and if so, on which site. Central axons of sensory neurons were labeled at E16.5 with DiI. In the superficial dorsal horns of *Brn4-Cre;Bcl11a* mutants, the density of DiI-positive fibers was greatly reduced and the remaining fibers appeared disorganized. Only a few axons crossing the midline or located in a dorsolateral region of the dorsal horn were detectable by DiI labeling in mutants (Fig. 5A,B). TrkA (Ntrk1 – Mouse Genome Informatics) -positive nociceptive fibers preferentially terminate in the superficial dorsal horn. Immunohistological analysis with antibodies against TrkA or aquaporin 1, a water channel protein that is expressed by small-diameter nociceptive fibers (Oshio et al., 2006), invariably revealed almost complete loss of such fibers in the dorsal horn of *Brn4-Cre;Bcl11a* mutants (Fig. 5C-F). Similar results were obtained with antibodies against CGRP (Calca – Mouse Genome Informatics), which marks a specific subset of peptidergic neurons terminating in the superficial dorsal horn (Fig. 5G,H). By contrast, parvalbumin-positive proprioceptive neurons projecting to the ventral spinal cord, where most neurons do not express Bcl11a (Fig. 1F,G,I), were unaffected by the mutation (Fig. 5I,J). Similar phenotypes were observed in spinal cords at E13.5 and E14.5, when cutaneous sensory afferents begin to grow into the dorsal horn (not shown). To exclude the possibility that the observed phenotype resulted from degeneration or aberrant differentiation of primary sensory neurons, we determined the numbers and apoptosis rates of TrkA-positive and parvalbumin-positive neurons within DRG. We did not observe any differences in apoptosis or in the size of individual neuron populations between *Brn4-Cre;Bcl11a* mutants and controls (Fig. 5K-P; data not shown).

To assess directly whether synaptic input from cutaneous sensory afferents to mutant dorsal spinal neurons was reduced, we recorded postsynaptic currents in dorsal horn neurons elicited by extracellular stimulation at the dorsal root entry zone (Fig. 5Q). Basic synaptic properties, such as amplitude, decay time constant (control: 3.86±0.70 mseconds, n=12; mutant: 3.55±0.41 mseconds, n=14) and rise time (control: 1.72±0.24 mseconds, n=12; mutant: 1.74±0.21 mseconds, n=14) were similar in mutant and control neurons (supplementary material Fig. S3A,B). In addition, paired-pulse properties, a commonly used criterion for presynaptic alterations, were unchanged in mutants (supplementary material Fig. S3C,D). However, the success rates of evoked excitatory postsynaptic currents (EPSCs) in dorsal horn neurons were significantly reduced (P<0.05) from 76.0% in controls to 52.4% in mutants (Fig. 5R).

When we analyzed *Ht-PA-Cre;Bcl11a* mutants at E18.5, TrkA-positive and aquaporin 1-positive axon projections were unchanged (Fig. 6C,D; data not shown). In addition, the primordial layer architecture in *Ht-PA-Cre;Bcl11a* mutants was indistinguishable from that of controls (Fig. 6A,B). Together, this indicates that Bcl11a is required in spinal target neurons, but is dispensable in presynaptic sensory neurons, for correct wiring and differentiation in the superficial dorsal horn.

The phenotype observed in *Bcl11a* mutant spinal cords does not exclude the possibility that impaired differentiation and morphogenesis of dorsal spinal neurons are caused indirectly by deficits in sensory innervation. To test this we analyzed *Sox10* mutant mice. In such mice, primary sensory neurons are eliminated before E12.5 by apoptosis (Britsch et al., 2001), allowing an assessment of dorsal horn development in the absence of sensory input (Fig. 6G,H). In *Sox10* mutants, expression of Lbx1, Lmx1b, Gria2 and Grpr, primordial layer architecture and neuron morphology as determined by Golgi and MAP2 staining were unchanged (Fig. 6E,F,I-N; data not shown). Thus, the defective

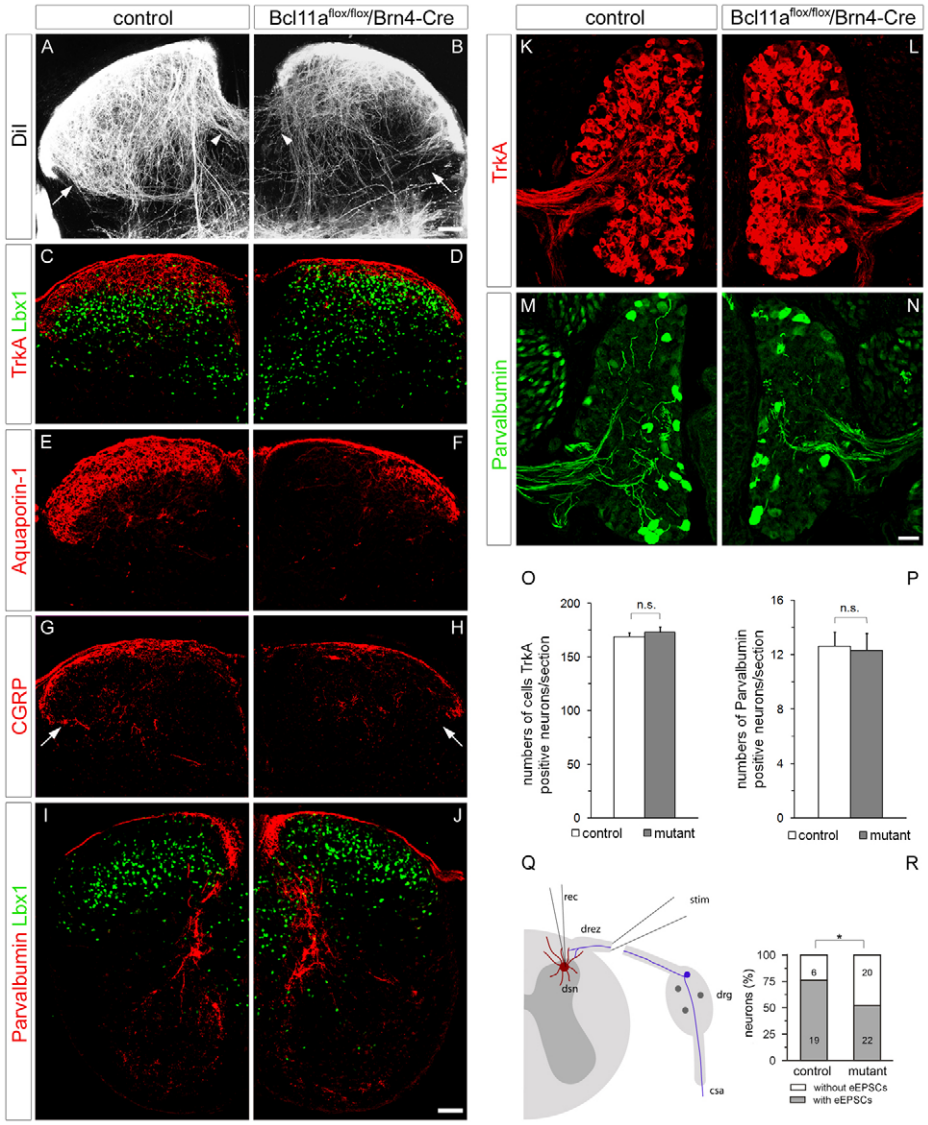

Fig. 5. Sensory axon projections in the *Bcl11a* mutant dorsal spinal horn. (**A-J**) Transverse sections of the dorsal horn of control (A,C,E,G,I) and Brn4-Cre recombined *Bcl11a* mutant (B,D,F,H,J) mice at E16.5 (A,B) and E18.5 (C-J). Sensory axons were traced with DiI (A,B). Axons crossing the midline are marked (arrowheads in A,B). Residual fibers are disorganized (arrows in A,B). A similar phenotype is detectable with anti-CGRP staining (arrows in G,H). (C-J) Immunohistological analysis of sensory axons projecting into the spinal cord with antibodies against TrkA (C,D), aquaporin 1 (E,F), CGRP (G,H) and parvalbumin (I,J). (**K-P**) Immunohistological detection (K-N) and quantification (O,P) of TrkA-positive (K,L,O) and parvalbumin-positive (M,N,P) sensory neurons in DRG. Mean±s.e.m. n.s., not significant. (**Q**,**R**) Electrophysiological analysis of evoked excitatory postsynaptic currents (eEPSCs) from dorsal horn neurons of control and Brn4-Cre recombined *Bcl11a* mutants at E18.5. *, $P<0.05$ (Fisher's exact test). rec, recording pipette; stim, stimulation pipette; dsn, dorsal spinal neuron; drez, dorsal root entry zone; drg, dorsal root ganglion; csa, central sensory axon. Scale bars: 50 μm.

neuronal differentiation and morphogenesis as observed in the *Bcl11a* mutant dorsal horn is independent of defective sensory innervation.

Dysregulated expression of Frzb accounts in part for the disrupted innervation of the *Bcl11a* mutant spinal cord

To identify genes that are differentially expressed in *Bcl11a* mutants, we performed microarray analyses on dorsal spinal cord tissues of E14.5 and E16.5 embryos. Analysis of E16.5 embryos revealed that the expression of 20 and 121 genes was significantly downregulated and upregulated, respectively (change $P\leq0.0001$; fold change ≤ -1.9 and ≥ 1.9; supplementary material Table S1). In accordance with a function in terminal differentiation (see above), *Gria2* and *Grpr* were significantly downregulated in mutants at E16.5 but not E14.5 (Table 1; see Fig. 2).

The gene encoding the extracellular signaling molecule secreted frizzled-related protein 3 (*Frzb*) was most consistently downregulated according to our microarray analyses (Table 1; supplementary material Table S1). As members of the sFRP family can act as guidance cues for navigating axons (Rodriguez et al., 2005), we focused on this candidate for further analysis. In situ hybridization verified downregulation of *Frzb* in the *Bcl11a* mutant spinal cord (Fig. 7A,B), and *Frzb* mRNA was 5.6-fold downregulated in qRT-PCR of *Bcl11a* mutant dorsal horn tissue ($P<0.01$; Fig. 7C). *Frzb* expression was unchanged in *Sox10* mutant dorsal horn, excluding downregulation of *Frzb* secondary to defective sensory innervation (Fig. 6O,P).

To test whether *Frzb* is directly regulated by Bcl11a, we performed ChIP on embryos; the specificity of the Bcl11a antibody was verified by western blot analysis (Jawaid et al., 2010). The Bcl11a antibody specifically precipitated two promoter fragments, corresponding to +623 to +849 (Frzb1) and –27 and +138 (Frzb2) relative to the transcription initiation site of *Frzb* (Fig. 7D,E). The amount of recovered Frzb1 and Frzb2 promoter DNA was significantly higher than with control IgG (Frzb1, Bcl11a antibody 0.233±0.02% and IgG 0.14±0.032% of input DNA; Frzb2, Bcl11a antibody 0.307±0.047% and IgG 0.12±0.03% of input DNA; $P<0.05$; Fig. 7D,E). No specific binding of Bcl11a to a distant fragment comprising –786 to –1008 was detected (Frzb3, Bcl11a antibody 0.009±0.0005% and IgG 0.03±0.02% of input DNA; Fig. 7D,E).

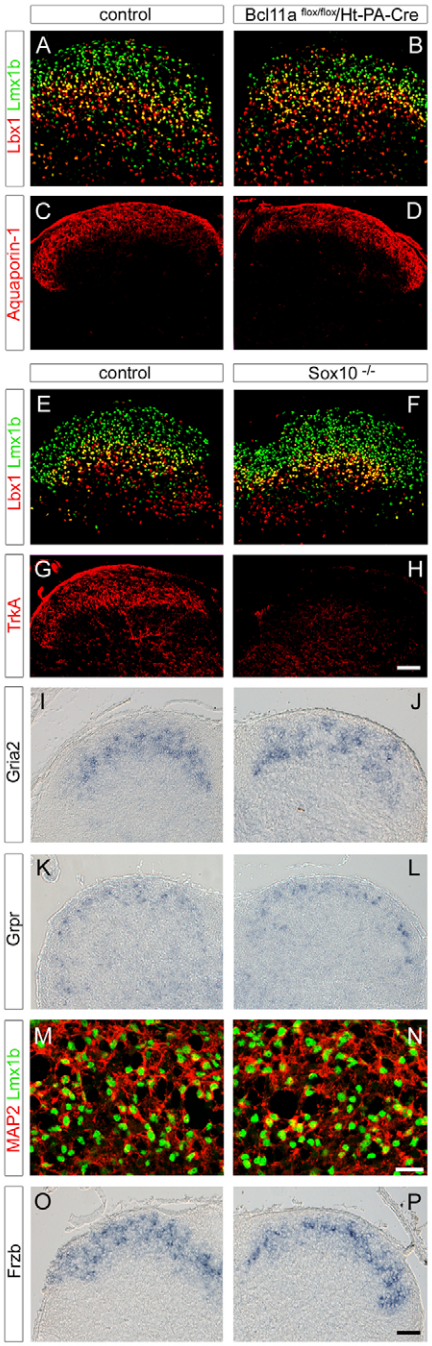

Fig. 6. Analysis of dorsal spinal cords of Ht-PA-Cre recombined *Bcl11a* mutants and of *Sox10* mutants. (**A-P**) Transverse sections of the dorsal horn from control (A,C,E,G,I,K,M,O), Ht-PA-Cre recombined *Bcl11a* mutant (B,D) and *Sox10* mutant (F,H,J,L,N,P) mice at E18.5, stained with antibodies against Lmx1b (A,B,E,F,M,N), Lbx1 (A,B,E,F), aquaporin 1 (C,D), TrkA (G,H) and MAP2 (M,N), or hybridized with probes against *Gria2* (I,J), *Grpr* (K,L) and *Frzb* (O,P). (M,N) High magnifications of the superficial zones of dorsal horns from controls (M) and *Sox10* mutants (N). Scale bars: 50 μm in H,P; 20 μm in N.

To validate the ChIP assays, an antibody against RNA polymerase II and a PCR strategy amplifying a *Gapdh* promoter fragment that flanks the TATA box were used (Iankova et al., 2006). We recovered significantly more *Gapdh* promoter DNA using the antibody directed against RNA polymerase II than with the IgG control; no significant enrichment of *Gapdh* promoter DNA was obtained with Bcl11a antibodies (*Gapdh*, RNA polymerase II antibody 0.51±0.038% and IgG 0.1±0.05% of input DNA, $P<0.05$; Bcl11a antibody 0.04±0.02% of input DNA; Fig. 7D,E). In addition, we used primers amplifying a promoter sequence of *Drg11*, which is expressed in dorsal horn neurons but is unaffected by the *Bcl11a* mutation (supplementary material Fig. S2). No significant recovery of *Drg11* promoter DNA was observed with Bcl11a antibodies when compared with the IgG control (Fig. 7E). Neither the Frzb1-3 nor the *Drg11* fragments used for ChIP contain a TATA box or other known RNA polymerase II binding sites. Accordingly, no significant precipitation of these fragments was observed with RNA polymerase II antibodies (Fig. 7E).

Analyzing the DNA sequence of the precipitated *Frzb* promoter region 1 revealed putative Bcl11a binding sites GGCCGG (starting at +824) and GGCCGC (starting at +796), both of which display high similarity to the previously described consensus binding motif of Bcl11a (Avram et al., 2002; Chen et al., 2009). Furthermore, a transgenic *Frzb* promoter fragment (indicated in Fig. 7D) lacking region 1 has been shown to recapitulate endogenous Frzb expression in mice, except in neural tube (Tylzanowski et al., 2004), supporting the assertion that interaction of Bcl11a protein with *Frzb* promoter region 1 is functional in neurons.

To assess whether Frzb is required for sensory innervation of the dorsal horn, we analyzed *Frzb* mutant mice (Lories et al., 2007) (Fig. 8A,B). In the dorsal horn of *Frzb* mutants we observed a reduction in TrkA and aquaporin 1 but not in parvalbumin expression (Fig. 8E-J). The reduction in the innervation of $TrkA^+$ and aquaporin 1^+ sensory neurons was less pronounced than observed in *Bcl11a* mutants. DiI labeling of primary sensory axons revealed reduced and disorganized sensory innervation of the dorsal horn of *Frzb* mutants (Fig. 8C,D). Frzb is also expressed in some sensory neurons (Fig. 8K,L). In mutants, the numbers and distribution of $TrkA^+$ and $parvalbumin^+$ neurons within DRG were normal (Fig. 8M-R). The primordial layer architecture, differentiation and morphology of dorsal spinal neurons were unchanged in *Frzb* mutants as assessed by expression of Lbx1, Lmx1b, Grpr and Gria2 and by Golgi and MAP2 staining (supplementary material Fig. S5A-J). We conclude that the dysregulated Frzb expression can in part account for the disrupted sensory innervation of the *Bcl11a* mutant spinal cord.

DISCUSSION

The dorsal spinal cord processes somatosensory information and relays it to higher brain centers. In this study, we provide genetic evidence that the zinc-finger transcription factor Bcl11a is essential for the correct sensory innervation of the dorsal spinal cord. This function of Bcl11a is in part mediated by its role in the transcriptional control of *Frzb*.

Bcl11a is required for sensory circuit formation

Small-diameter cutaneous sensory afferents are greatly reduced in the dorsal horn of *Bcl11a* mutant mice. Similar phenotypes have been reported for mice with a mutation of the transcription factors *Lmx1b*, *Drg11* or *Tlx3*/*Tlx1* (Chen et al., 2001; Qian et al., 2002; Ding et al., 2004). These genes are expressed by the same neuron type and overlap in part with the expression of Bcl11a in the dorsal horn (supplementary material Fig. S1), raising the question of whether Bcl11a exerts part of its functions through a shared genetic program. However, the expression patterns of Lmx1b and Drg11

Table 1. Summary of microarray gene expression analysis in the dorsal spinal cord of control (*Bcl11a*$^{flox/+}$;*Brn4-Cre*) and *Bcl11a* mutant (*Bcl11a*$^{flox/flox}$;*Brn4-Cre*) mice at E14.5 and E16.5

		Fold change	
Gene symbol	Gene name	E14.5	E16.5
Bcl11a	B cell CLL/lymphoma 11A	–7.5***	–13.5***
Neurotransmission			
Grpr	gastrin releasing peptide receptor	nd	–6.2***
Gria2	glutamate receptor, ionotropic, AMPA2 (alpha 2)	nd	–1.9***
Signaling			
Frzb (*sFRP3*)	secreted frizzled-related protein 3	–3.0***	–2.2***

Average signal fold changes and change *P*-values (***, $P \leq 0.0001$) from three independent replicates are shown. Genes downregulated in *Bcl11a* mutant dorsal spinal cord are indicated by negative values. Only those genes verified by in situ hybridization, qRT-PCR and/or ChIP are shown.
nd, expression not detected.

as well as the number of Lmx1b-positive dorsal neurons were unchanged in *Bcl11a* mutants. Whereas we observed defects in the terminal differentiation of dorsal neurons in *Bcl11a* mutants, changes in neuron type composition and neuronal fate have been detected in the dorsal horns of *Lmx1b* and *Tlx3*/*Tlx1* mutant animals (Qian et al., 2002; Cheng et al., 2004; Ding et al., 2004).

Bcl11a is expressed in presynaptic sensory neurons as well as in their postsynaptic spinal targets. We ablated Bcl11a selectively in pre- and postsynaptic neurons to determine the relative contribution of pre- versus postsynaptic expression of Bcl11a to sensory circuit formation. Genetic data presented in this study demonstrate that expression of Bcl11a in postsynaptic dorsal spinal neurons is required for the ingrowth of sensory afferents and for providing synaptic input to their targets. By contrast, Bcl11a expression in primary sensory neurons is dispensable for this process. This does not, however, exclude additional, as yet undetermined functions of Bcl11a in primary sensory neurons.

Somatosensory afferents are not equally affected by the mutation of *Bcl11a* in spinal neurons. Although sensory afferents expressing TrkA, aquaporin 1 or CGRP are strongly reduced in the superficial dorsal horn, we do not detect major defects in proprioceptive fibers traveling to the ventral spinal cord. One explanation for this could be that only a few of their target neurons within the ventral spinal cord normally express Bcl11a. Thus, somatosensory neurons might differ in their dependence on Bcl11a. In addition, we detected all three major isoforms of Bcl11a, i.e. Evi9a, b and c, as being differentially expressed in the spinal cord. Whereas Evi9b is expressed throughout the spinal cord, Evi9a and c are predominantly expressed in the dorsal horn. Previous studies demonstrated that Evi9 isoforms differ in their subcellular localization and biological activity (Nakamura et al., 2000; Kuo et al., 2009), raising the possibility that ingrowth of sensory afferents to the dorsal horn might depend on Evi9a and/or c selectively.

In addition to the innervation deficit, neurons of the dorsal spinal cord of *Bcl11a* mutants displayed disrupted morphogenesis and terminal differentiation. This raised the question of whether dorsal horn neurons require sensory innervation for correct maturation. However, *Sox10* mutants lack sensory neurons by E12.5 but do not display major deficits in the differentiation and morphogenesis of

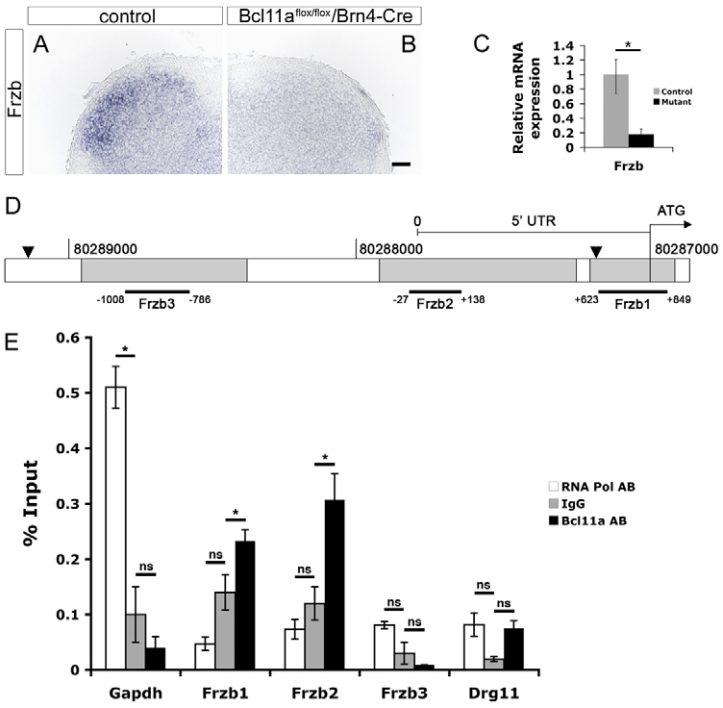

Fig. 7. Analysis of target gene expression in *Bcl11a* mutant dorsal spinal cord. (**A**,**B**) In situ hybridization analysis of *Frzb* mRNA expression in the dorsal horn of controls (A) and Brn4-Cre recombined *Bcl11a* mutants (B) at E14.5. Scale bar: 50 μm. (**C**) Determination of *Frzb* mRNA expression levels of control and mutant dorsal spinal cords by qRT-PCR at E14.5. Mean±s.e.m. *, $P<0.05$. (**D**) The *Frzb* promoter region indicating candidate regulatory regions (gray) as predicted by Ensembl Genome Browser and showing amplified fragments Frzb1-3 used in ChIP analysis. The 5′UTR and ATG are indicated. Black arrowheads mark the boundaries of a *Frzb* promoter fragment used previously to drive transgenic *Frzb* expression in mice (Tylzanowski et al., 2004). (**E**) ChIP assays on wild-type dorsal spinal cord tissue at E14.5 employing specific antibodies against Bcl11a or RNA polymerase II, and IgG as control. Binding of Bcl11a or RNA polymerase II to DNA was determined by qPCR using specific primer pairs for the *Frzb* promoter regions as indicated in D (region 1, +849 to +623; region 2, +138 to –27; region 3, –786 to –1008, relative to the transcription start site) as well as specific primer pairs amplifying the *Gapdh* and *Drg11* promoters. Pairwise comparison of ChIP data employing specific antibodies (RNA Pol AB, Bcl11a AB) versus IgG negative control was used for statistical analysis. Significantly more *Frzb* promoter DNA of regions 1 and 2 was recovered with the Bcl11a antibody than in controls Mean±s.e.m.; $n=3$. *, $P<0.05$ (Student's *t*-test); ns, not significant.

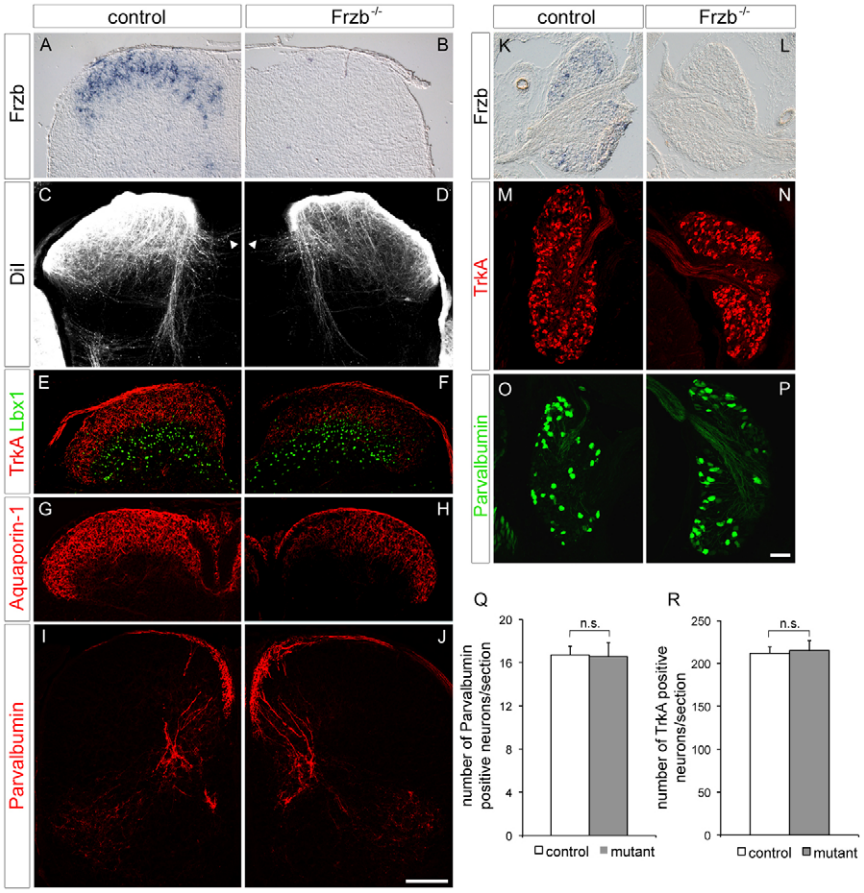

Fig. 8. Sensory axon projections in the *Frzb* mutant dorsal spinal horn. (**A-J**) Transverse sections of the dorsal horn of control (A,C,E,G,I) and *Frzb* mutant (B,D,F,H,J) mice at E16.5 (A-D) and E18.5 (E-J) hybridized with probes specific for *Frzb* (A,B) or stained with antibodies against TrkA (E,F), aquaporin 1 (G,H) or parvalbumin (I,J). Sensory axons entering the dorsal horn were traced with DiI (C,D). Axons crossing the midline are marked by arrowheads (C,D). (**K-P**) transverse sections of DRG of controls (K,M,O) and *Frzb* mutants (L,N,P) hybridized with *Frzb*-specific probes (K,L) or stained with antibodies against TrkA (M,N) and parvalbumin (O,P). (**Q**,**R**) Quantitative analysis of sensory neurons in DRG. Mean±s.e.m. n.s., not significant. Scale bars: 100 μm in J; 50 μm in P.

dorsal spinal neurons. Thus, the disrupted morphogenesis and differentiation of dorsal horn neurons in *Bcl11a* mutants is not caused by the deficit in sensory innervation.

Dendritic morphology and the presence of a particular transmitter receptor repertoire are important determinants of the number and type of presynaptic partners of a neuron (Hausser et al., 2000). Ingrowth of sensory afferents precedes terminal differentiation of spinal neurons (Ding et al., 2004). Thus, defective target differentiation, as observed in *Bcl11a* mutants, is unlikely to be the only cause for the deficit in dorsal horn innervation. We propose instead that Bcl11a regulates the expression of signals that guide somatosensory axons to their spinal targets. We identified such a guidance factor, Frzb, which was downregulated in the dorsal horn of *Bcl11a* mutants. The family of sFRPs function either as extracellular inhibitors of Wnt signaling or they can act independently of Wnt ligands. Wnt signals regulate multiple steps during the assembly of neural circuits (reviewed by Bovolenta et al., 2006; Salinas and Zou, 2008). In particular, sFRP1 acts as a guidance factor for retinal axons (Rodriguez et al., 2005). In addition, Wnts act as anterior-posterior guidance cues for commissural axons in the spinal cord, a function that is regulated by sFRPs (Lyuksyutova et al., 2003; Domanitskaya et al., 2010). Furthermore, neurite outgrowth of somatosensory neurons is controlled by the Wnt receptor Ryk (Lu et al., 2004).

We show that the disrupted Frzb expression is in part responsible for the deficit in sensory innervation of the dorsal horn of *Bcl11a* mutants. In particular, we observe reduced and disorganized cutaneous sensory innervation in the dorsal horn of *Frzb* mutants. Changes in the somatosensory system had not been noted previously in *Frzb* mutants (Lories et al., 2007). However, sensory innervation deficits in *Frzb* mutants are milder than those in *Bcl11a* mutants, and the differentiation and morphogenesis of dorsal horn neurons appeared unaffected. This indicates that, in addition to *Frzb*, other genes exist that control neuronal development and are expressed in a Bcl11a-dependent manner.

Several lines of evidence suggest that *Frzb* is a direct target of Bcl11a: (1) Frzb was downregulated at early and late time points (E14.5 and E16.5) during development of the dorsal spinal cord of *Bcl11a* mutants, and Frzb expression did not depend on sensory innervation and was unchanged in *Sox10* mutants; (2) two distinct promoter fragments of *Frzb* are precipitated by ChIP with a Bcl11a-specific antibody; and (3) consensus binding sites for Bcl11a are present in the Frzb1 fragment. These experiments thus support a direct role of Bcl11a in the control of *Frzb*. Bcl11a was originally described as a COUP-TF-interacting protein (CTIP1). Mice with a targeted deletion of *COUP-TF1* (*Nr2f1* – Mouse Genome Informatics) display aberrant projections of cranial nerve axons (Qiu et al., 1997). COUP-TF1 is expressed in the developing spinal cord, and it is thus possible that Bcl11a cooperates with COUP-TF1 to regulate the differentiation of spinal neurons and sensory circuit formation.

A role for Bcl11a in neuronal morphogenesis

An important aspect of Bcl11a function in the dorsal horn is its role in neuronal morphogenesis. In the absence of Bcl11a, dorsal spinal neurons initiate neuritogenesis but form hypoplastic neurites with severely reduced branching complexity in vivo. A similar phenotype is observed when dorsal spinal neurons of *Bcl11a* mutants are cultured. It was previously reported that knockdown of Bcl11a-L (which corresponds to Evi9a in mice) in cultured rat

hippocampal neurons results in enhanced axonal branching and dendrite outgrowth, an effect that was proposed to be mediated by transcriptional control of *Dcc* and *Map1b* (Kuo et al., 2009). We did not detect changes in Dcc and Map1b (Mtap1b – Mouse Genome Informatics) expression in the spinal cord of *Bcl11a* mutant mice (data not shown). In our mutants, all three isoforms of *Bcl11a* were ablated from the genome. Evi9a and Evi9b are the major isoforms expressed in the murine hippocampus during development (Sebastian Karl and S.B., unpublished). Thus, the increase in neurite branching after knockdown of Evi9a might reflect residual Evi9b functions in rat hippocampal neurons in vitro, suggesting that the biological activities of Bcl11a are isoform specific (Nakamura et al., 2000; Kuo et al., 2009); they might also be neuron type specific.

In summary, we provide here a genetic characterization of the functions of the transcription factor Bcl11a in neuronal differentiation and the formation of sensory circuits. By the identification of Frzb, we link the transcriptional function of Bcl11a to its role in the guidance of sensory neurons.

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Competing interests statement
The authors declare no competing financial interests.

Supplementary material
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Differential gene expression in *Bcl11a* mutant dorsal spinal cord

Affymetrix ID	Gene symbol	Genbank ID	Change *P*-value	Fold-change
Downregulated genes				
1447334_at	*Bcl11a*	BB526119	4.81E-08	–13.45414464
1419406_a_at	*Bcl11a*	NM_016707	4.73E-08	–8.116123948
1450260_at	*Grpr*	M57922	3.67E-05	–6.204484917
1457072_at	*Bcl11a*	BF731393	7.75E-08	–5.453025359
1456632_at	*Bcl11a*	BB424718	1.41E-08	–5.36405061
1446293_at	*Bcl11a*	BB471990	4.43E-05	–4.796960888
1428642_at	*Slc35d3*	AK018094	6.14E-05	–3.589984253
1422825_at	*Cart*	NM_013732	4.59E-05	–2.882588616
1457797_at	*Slc8a1*	AV340788	1.51E-05	–2.674092389
1446622_at	*A330068G13Rik*	BB191623	2.24E-05	–2.24557795
1416658_at	*Frzb*	U91905	9.01E-07	–2.239973937
1432825_at	*2900018N21Rik*	AK013552	2.44E-05	–2.239020383
1438239_at	*Mid1*	BG073178	6.4E-05	–2.153784217
1433701_at	*Mpped1*	BB371430	2.11E-05	–2.051349964
1452656_at	*Zdhhc2*	BB224658	7.02E-05	–1.96649857
1436035_at	*3830431G21Rik*	AV225683	1.06E-05	–1.962778283
1426047_a_at	*Ptprr*	AF129509	6.32E-05	–1.941226429
1460668_at	*Gal*	NM_010253	2.87E-05	–1.937069656
1455869_at	*Camk2b*	BG862223	5.72E-05	–1.913351264
1418161_at	*Jph3*	NM_020605	1.30E-05	–1.905919862
1445589_at	*Slc23a2*	BM940232	5.47E-05	–1.902432029
1434776_at	*Sema5a*	BQ176610	6.04E-05	–1.86572666
1437319_at	*D9Ertd414e*	BG069400	1.54E-05	–1.865635356
1453098_at	*Gria2*	AK014389	1.82E-05	–1.863601115
1437422_at	*Sema5a*	AV375653	7.67E-05	–1.855922895

Affymetrix ID	Gene symbol	Genbank ID	Change *P*-value	Fold-change
Upregulated genes				
1450736_a_at	*Hbb-bh1*	NM_008219	2.20E-09	35.61121791
1437990_x_at	*Hbb-bh1*	AV147727	9.03E-09	35.26825279
1437810_a_at	*Hbb-bh1*	AV311770	2.67E-07	34.62677676
1429517_at	*Zfyve20*	BC017622	3.19E-05	7.109685906
1432589_at	*Plcg1*	BF166706	2.81E-07	7.013992117
1430391_a_at	*St8sia4*	AK003690	2.91E-06	6.301004155
1420230_at	*AA414993*	AA414993	2.61E-06	6.009843385
1431196_at	*Atp2c1*	BG296252	2.40E-06	4.728163341
1449885_at	*Tmem47*	NM_138751	1.43E-07	4.722526295
1429385_at	*Wdr68*	BI082535	2.49E-06	4.272174849
1448716_at	*Hba-x*	M26898	6.16E-07	4.213009015
1452638_s_at	*Dnm1l*	BC027538	1.35E-05	4.004418365
1456255_at	*AI314180*	BB553400	1.01E-06	3.965965013
1437257_at	*Wdr47*	BB344753	1.50E-08	3.95664415
1451610_at	*ECM29l*	BC024561	6.51E-06	3.952606098
1427430_at	*AI848100*	BB148987	5.43E-05	3.894914242
1431064_at	*Dpp8*	BF119821	1.64E-05	3.802372114
1420652_at	*Ate1*	BE309332	3.60E-05	3.642381587
1448458_at	*Top2b*	BB166592	5.88E-07	3.634330961

1426505_at	*Evi2b*	AI122415	8.92E-06	3.632934073
1424570_at	*Ddx46*	BF023426	2.40E-05	3.55541412
1416660_at	*Eif3s10*	AW701127	5.64E-05	3.506743664
1423325_at	*Pnn*	AV135835	1.05E-05	3.497019017
1444199_at	*Elk4*	AW046689	3.97E-05	3.477198429
1419519_at	*Igf1*	NM_010512	2.75E-05	3.421510667
1421248_at	*Syn3*	NM_013722	2.97E-07	3.301289723
1456343_at	*Slc35f1*	BB540579	2.48E-05	3.282755202
1450087_a_at	*Nolc1*	NM_053086	7.53E-06	3.226996335
1427830_at	*Zfp260*	L36316	7.67E-05	3.226275533
1435872_at	*Pim1*	BE631223	3.29E-05	3.216496521
1442766_at	*Ppp4r1*	AI846596	1.01E-05	3.206447054
1434419_s_at	*Tardbp*	AW538183	3.15E-05	3.189801662
1444904_at	*Cbfa2t1h*	BG068236	5.63E-05	3.177284101
1428017_at	*Pknox2*	AF487460	1.35E-06	3.162853048
1447871_at	*Mtx2*	BI714072	5.88E-07	3.061908909
1450321_at	*Zfp354c*	NM_013922	1.26E-05	3.057033256
1416019_at	*Dr1*	NM_026106	1.79E-06	3.046954246
1433804_at	*Jak1*	BQ032637	4.48E-05	2.907430036
1416748_a_at	*Mre11a*	NM_018736	2.58E-06	2.888036044
1460304_a_at	*Ubtf*	BB832806	2.10E-05	2.879600247
1444536_at	*AI462171*	AI462171	1.89E-05	2.878207922
1440984_at	*Baz2b*	BE943712	5.30E-05	2.869833884
1455658_at	*Cggbp1*	BI080272	1.05E-06	2.864477895
1431338_at	*Caskin1*	AK014376	2.90E-05	2.863331895
1419425_at	*Cnr1*	NM_007726	6.34E-08	2.851835278
1443522_s_at	*Phip*	BM221262	1.14E-05	2.850630874
1418889_a_at	*Csnk1d*	NM_139059	6.42E-05	2.82555789
1453590_at	*Arl5b*	BQ032239	4.07E-05	2.810921874
1445238_at	*Ccl21b*	BB239244	1.25E-06	2.772489491
1449504_at	*Kpna1*	U20619	3.21E-05	2.768349436
1419344_at	*Tcte1*	NM_013688	3.98E-05	2.761346663
1424325_at	*Esco1*	BB308198	9.93E-05	2.746473362
1425515_at	*Pik3r1*	M60651	3.54E-05	2.69283597
1450243_a_at	*Dscr1l1*	NM_030598	2.66E-07	2.684780535
1420899_at	*Rab18*	AW542340	6.42E-05	2.67333255
1449548_at	*Efnb2*	U30244	1.81E-05	2.669096203
1420816_at	*Ywhag*	NM_018871	3.83E-05	2.649034404
1444996_at	*Depdc5*	BG067666	1.30E-06	2.629950635
1446926_at	*Pycard*	AU040917	1.05E-06	2.617144887
1431274_a_at	*Hspa9a*	AA543265	2.13E-06	2.583065882
1446682_at	*Zswim6*	BB660139	6.46E-05	2.564815322
1449949_a_at	*Cxadr*	U90715	7.14E-05	2.542242336
1459648_at	*Rutbc3*	BE627992	1.14E-05	2.498150765
1432344_a_at	*Aplp2*	AK013376	2.28E-06	2.497216168
1446484_at	*Mef2c*	BB558401	8.13E-06	2.490409664
1415773_at	*Ncl*	BF118393	1.80E-06	2.484618366
1421477_at	*Cplx2*	NM_009946	6.42E-06	2.479666949
1435009_at	*Slc9a6*	BB611738	1.11E-05	2.455488305
1423214_at	*Plxnc1*	BB476707	1.93E-05	2.387979685
1424332_at	*Rab40c*	AF422144	8.06E-05	2.358015469
1456651_a_at	*Tpr*	BG067858	4.77E-06	2.356109997
1423329_at	*Gdap1*	AU017649	1.90E-05	2.350862988
1416043_at	*Nasp*	BB493242	3.65E-05	2.335816875

1455748_at	*Gpr178*	AI662241	2.74E-05	2.333911832
1417781_at	*Lass4*	BB006809	1.30E-05	2.333638981
1420982_at	*Rnpc2*	NM_133242	2.37E-05	2.310019202
1450522_a_at	*H1f0*	NM_008197	5.74E-05	2.306425231
1454304_at	*Epn2*	BB626346	2.64E-05	2.287035859
1460131_at	*Rnf170*	BG067721	1.48E-05	2.238187031
1450392_at	*Abca1*	BB144704	2.52E-05	2.212401088
1426714_at	*D11Ertd18e*	AK003278	3.14E-06	2.190147066
1427408_a_at	*Thrap3*	BC012655	2.24E-06	2.177392372
1426715_s_at	*D11Ertd18e*	AK003278	3.59E-05	2.150738751
1421789_s_at	*Arf3*	NM_007478	9.78E-07	2.147247301
1440228_at	*Ranbp6*	BB477637	4.80E-06	2.143338627
1427638_at	*Zbtb16*	Z47205	8.42E-06	2.141114995
1425597_a_at	*Qk*	AW060288	2.42E-05	2.126582508
1442872_at	*BC021438*	BB022993	5.72E-06	2.09856836
1451730_at	*Zfp62*	BC022935	4.90E-05	2.090004098
1425713_a_at	*Rnf146*	BC019182	3.43E-05	2.089779763
1425490_a_at	*Wdr13*	AK017549	1.85E-06	2.082986698
1438686_at	*Eif4g1*	BB531220	5.31E-05	2.074047996
1454107_a_at	*Kif2a*	AK016720	2.30E-05	2.070858853
1450762_s_at	*Zfp191*	BF780333	7.83E-05	2.051713553
1450407_a_at	*Anp32a*	AF022957	6.35E-06	2.040646039
1421339_at	*Extl3*	NM_018788	4.81E-05	2.006553153
1458676_at	*Nktr*	BB712791	3.42E-05	2.00603001
1431328_at	*Ppp1cb*	AK017392	2.73E-05	2.000893152
1425631_at	*Ppp1r3c*	U89924	2.86E-05	1.99903859
1448127_at	*Rrm1*	BB758819	9.49E-05	1.993870937
1443878_at	*Rapgef6*	BB306768	2.65E-05	1.99328962
1434282_at	*Ibtk*	BM250711	4.11E-05	1.988935323
1460426_at	*Pde4dip*	AI639670	3.06E-06	1.984632664
1416701_at	*Rnd3*	BC009002	1.64E-05	1.983916553
1449578_at	*Supt16h*	AW536705	2.11E-05	1.973119101
1431030_a_at	*Rnf14*	AK010162	2.65E-05	1.970928939
1425624_at	*Epm2aip1*	BC018474	9.24E-05	1.962687538
1460386_a_at	*Slc1a1*	AF087578	2.41E-05	1.955461765
1444333_at	*Strn3*	BB485260	1.93E-05	1.955034629
1458685_at	*Garnl1*	BB552367	1.65E-05	1.953421381
1442629_at	*Nt5c2*	AU017183	6.50E-05	1.951756982
1456104_at	*Psmd11*	BE949498	6.46E-05	1.950168902
1426744_at	*Srebf2*	BM123132	2.79E-05	1.943511478
1442404_at	*Ncl*	BB624790	2.61E-05	1.943410453
1415795_at	*Spin*	BM228780	1.83E-05	1.942326357
1447924_at	*Nucks1*	AW048468	1.07E-06	1.942215708
1417755_at	*Topors*	NM_134097	7.31E-05	1.939400843
1439042_at	*Adcyap1r1*	BB427884	6.38E-05	1.937250691
1452430_s_at	*Sfrs1*	X66091	6.39E-05	1.917936232
1453853_a_at	*Arhgef12*	AI481688	2.04E-05	1.913243672
1425329_a_at	*Cyb5r3*	AF332060	4.23E-05	1.90763443
1451762_a_at	*Kif1b*	AB023656	5.13E-05	1.904250658
1416732_at	*Top2b*	BB166592	1.20E-05	1.900368892

Systematic analysis of gene expression in control and Brn4-Cre recombined *Bcl11a* mutant spinal cords using Affymetrix oligonucleotide microarrays (MOE 430 2.0 Gene Chip). Expression profiles were determined at E16.5. The average signal fold change and the change *P*-value from three replicates are shown. Genes were selected based on the following cut-offs: fold change ≥ 1.9 for upregulated genes and ≤ -1.9 for those downregulated in mutants; change *P*-value ≤ 0.0001.