Generation of CXCL12 floxed mice

A CXCL12 floxed exon 2 mouse line was generated by inGenious Targeting Laboratory, Inc. (Ronkonoma, NY, USA). Briefly, a ~10.3 kb region used to construct the targeting vector was first subcloned from a positively identified C57BL/6 BAC clone using a homologous recombination based technique. The region was designed such that the short homology arm (SA) extended 2.0 kb to 5' of loxP/FRT flanked neomycin cassette. The long homology arm (LA) started on the 5' single loxP side. The single loxP site was inserted upstream of exon 2, and the loxP/FRT flanked neomycin cassette was inserted downstream of exon 2. The target region was inclusive of exon 2 (Fig. S1). The targeting vector was confirmed by restriction analysis and sequencing. The BAC was then subcloned into a 2.4 kb pSP72 (Promega) backbone vector containing an ampicillin selection cassette for retransformation of the construct prior to electroporation. A pGKgb2 loxP/FRT flanked neomycin cassette was inserted into the gene as described above. The targeting construct was linearized using NotI prior to electroporation into ES cells. Clones having integrated the targeting sequence were identified by PCR analysis and confirmed by Southern blot analysis (Fig. S2). These clones were then used to generate chimeric mice. Chimeras were crossed to mice harboring an ActinFLP germ line driver (Jackson Laboratories) to remove the neomycin cassette, and backcrossed for several generations to generate CXCL12 floxed/floxed (CXCL12 F/F) animals. All crosses were performed on a C57BL/6 background.

PCR and RT-PCR

Three sequence specific primers were used to distinguish between CXCL12 wild type, floxed and Δex2 alleles: CXCL12 Sense, 5'-AAA ATC CTC AAC ACT CCA AAC TG-3'; CXCL12 Antisense, 5'-CAG ACT AGC ACA GGA CAC ATC TC-3'; and MJ1, 5'-TTG CAG ACT ACA GGA CAC ATC TG-3'. Using these primers, wild type (609 bp), floxed (707 bp) and Δex2 (550 bp) amplicons were detected by PCR (Fig. S3B). Primers used in RT-PCR analyses include CXCL12 exon1 forward, 5'-GCT GCC GCC CCT ACG GTC CAT-3'; reverse, 5'-ACC AGT CAG CCT GAG GTA CAT-3', and CXCL12 exon 2 forward, 5'-ACC AGT CAG CCT GAG CTG CAT-3'; reverse, 5'-AGT GAG ACT TCA GCC CAT TGA CCC GAA AT-3'. Primers used in RT-PCR analyses include CXCL12 exon1 forward, 5'-GCT GCC GCC CCT ACG GTC CAT-3'; reverse, 5'-ACC AGT CAG CCT GAG GTA CAT-3', and CXCL12 exon 2 forward, 5'-ACC AGT CAG CCT GAG CTG CAT-3'; reverse, 5'-AGT GAG ACT TCA GCC CAT TGA CCC GAA AT-3'.

RNA in situ hybridization and immunofluorescence

The embryonic brains were collected and fixed in 4% PFA over night, embedded in OCT solution, and cryosectioned at 20–30μm. RNA in situ hybridization and immunofluorescence were performed as previously described (Joksimovic et al., 2009). A clone to generate the Barhl1 riboprobe was purchased from GE Healthcare (clone ID 6413504; accession number BC055731). The CXCL12 riboprobe encompassed all three exons (Fig. 1A) and was generously provided by Dr. Richard Miller (Lu et al., 2002). Antibodies used were mouse Pax6 (1:50, Developmental Studies Hybridoma Bank, Iowa City, IA), and either sheep TH (1:1500, Pel Freez) or rabbit TH (1:1500, Pel Freez). Images were taken using a Nikon Eclipse 80i microscope camera.

References

Supplementary Fig. S2. Southern blot analysis of selected embryonic stem (ES) cells. (A) The CXCL12 targeted allele is shown with the location of the Nsil restriction sites (vertical blue lines). After Nsil digestion, the DNA was hybridized with the 3’ probe (red box) for Southern blot analysis. Vertical gray boxes represent exons. loxP and FRT sites are indicated by green and orange arrowheads, respectively. (B) Southern blot analysis of the CXCL12 targeted allele. Control DNA was obtained from HYB (Hybrid, C57Bl/6 x 129/SvEv), B6 (C57Bl/6) and 129 (129/SvEv) wild-type animals.

Supplementary Fig. S3. A strategy to generate CXCL12Δex2/Δex2 mutant embryos. (A) A β-actin::cre line was used to recombine and remove exon 2 of the loxP-flanked CXCL12 gene. Initially, male β-actin::cre mice were crossed with female CXCL12F/+ mice to generate CXCL12+/Δex2 mice in which exon 2 of one CXCL12 allele is removed (Δex2). Next, we intercrossed CXCL12+/Δex2 mice to generate CXCL12Δex2/Δex2 mutant embryos deficient in exon 2. (B) PCR analysis of the wild type, floxed or Δex2 alleles. A homozygous, CXCL12Δex2Δex2 mutant is indicated by the red box.
Supplementary Fig. S4. CXCL12Δex2/Δex2 embryos are characterized by ectopic neuronal migration of pontine grey nucleus (PGN) neurons. Adjacent coronal sections of control (A,C,E) and mutant embryos (B,D,F) were subjected to Barhl1 RNA in situ hybridization (A–F) and Pax6 immunofluorescent labeling (insets) at E14.5. Black arrows and arrowheads depict an approximate position of the regions shown in insets for Pax6 immunolabeling in control and mutant embryos, respectively. White chevrons indicate derailed Pax6+ cells (inset in F). The upper image is a sagittal section labeled with DAPI in which white dotted lines indicate approximate positions of coronal sections shown in (A–F). Anterior is to the right, posterior to the left. Scale bars, 50 μm (insets) and 200 μm (A–F).

Supplementary Fig. S5. Severe reduction of the pontine grey nucleus (PGN) structure in CXCL12Δex2/Δex2 mutant embryos. E16.5 sagittal sections of control (A) and mutant (B) embryos were labeled with a Pax6 antibody. The prominent pontine grey nucleus (PGN) is marked by Pax6 expression in control embryos (A). In a sharp contrast, the PGN is drastically reduced in the CXCL12Δex2/Δex2 mutants (white arrowhead in B). Inset in (A) is a DAPI labeled sagittal section in which the white dotted box indicates the approximate region shown in (A,B) at higher magnification. Anterior is to the right, posterior to the left. Scale bar, 200 μm.