THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

doi: 10.1387/ijdb.140348mj

SUPPLEMENTARY MATERIAL

corresponding to:

Generation and characterization of mice harboring a conditional *CXCL12* allele

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Full text for this paper is available at: http://dx.doi.org/10.1387/ijdb.140348mj

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 AAG AAG ATA ATC CCA TC-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 GCA CTT TCA CTC T-3'; reverse, 5'-GAC GAC CTC GGC GTC CAT-3'; CXCL12 exon 2 forward, 5'-ACC AGT CAG CCT GAG CTA CC-3'; reverse, 5'-AAG GGC ACA GTT TGG AGT GT-3'; CXCL12 exon 3 forward, 5'-AGT GTG CAT TGA CCC GAA AT-3'; reverse, 5'-ATG ACC CCA GTC AGT GCT GT-3'.

RNA *in situ* hybridization and immunofluorescence

The embryonic brains were collected and fixed in 4% PFA overnight, 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 80*i* microscope camera.

References

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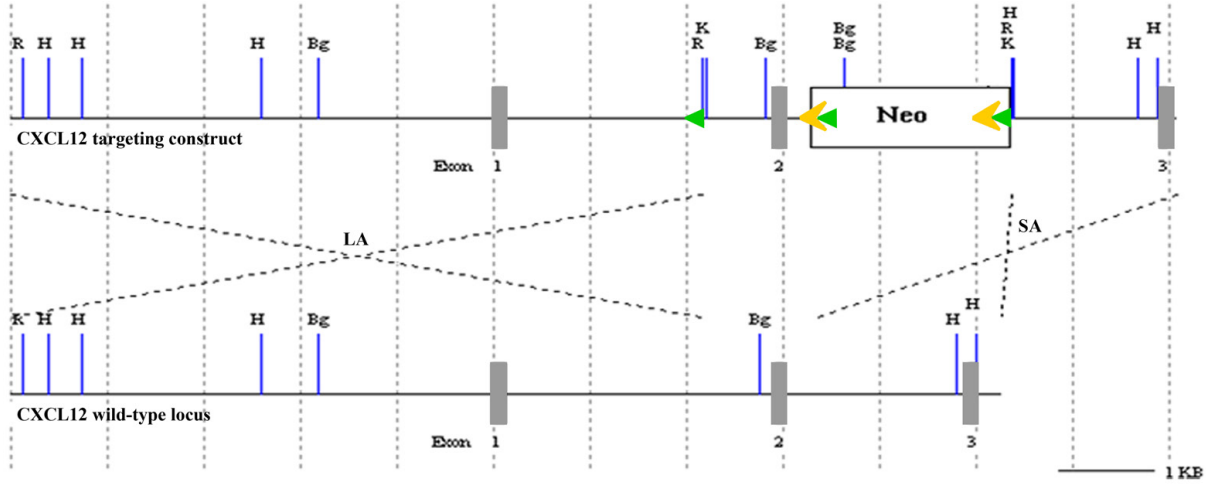
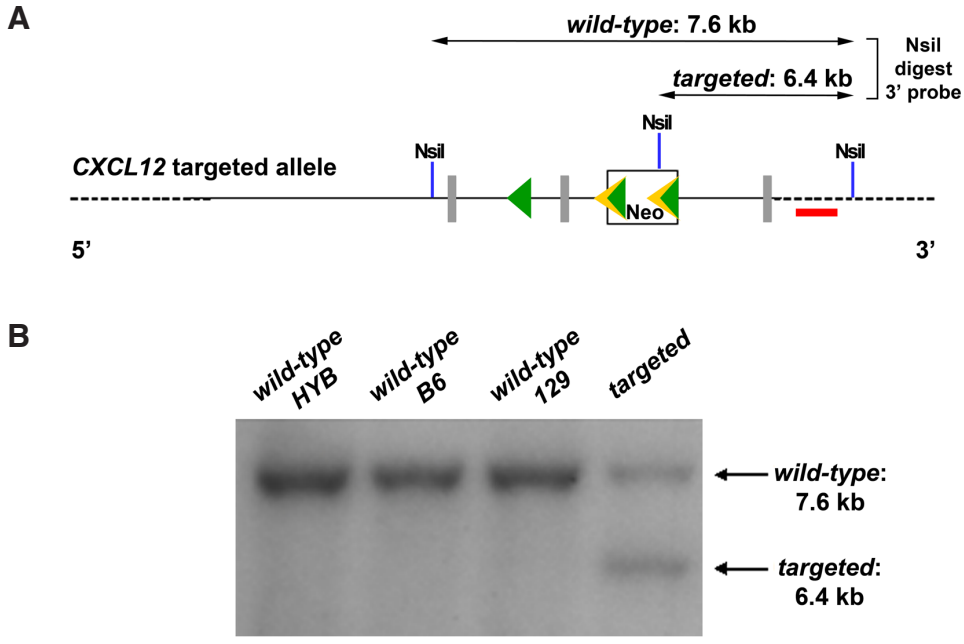
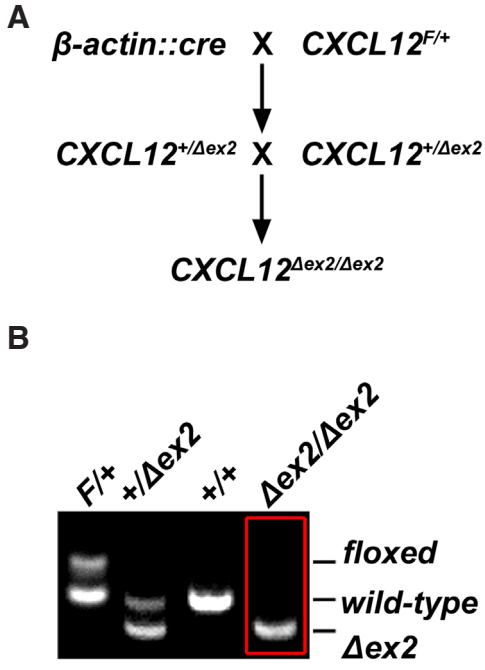
LU M, GROVE E, MILLER R. (2002). Abnormal development of the hippocampal dentate gyrus in mice lacking the CXCR4 chemokine receptor. *Proc Natl Acad Sci USA* 99: 7090-7095.



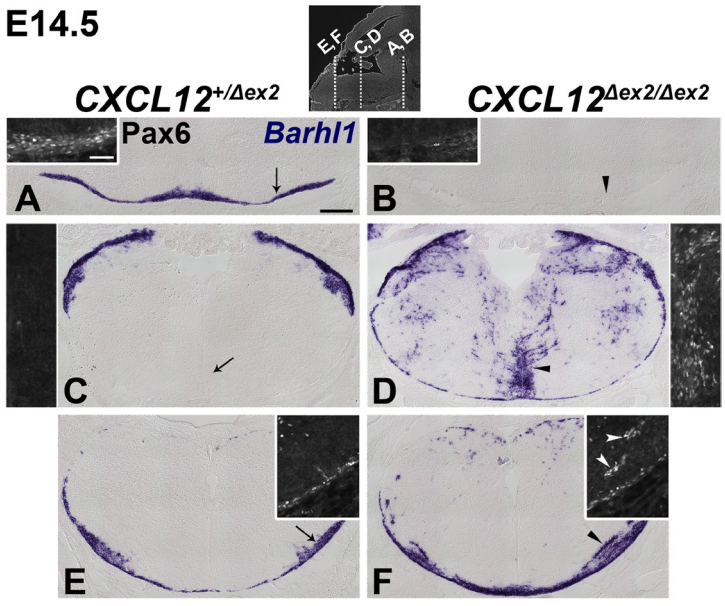
Supplementary Fig. S1. Conditional targeting construct of *CXCL12*. *The* CXCL12 *wild-type locus (lower) and targeting construct (upper) are shown. The 5' single* loxP *site was inserted in the intron 1 region upstream of exon 2 of the* CXCL12 *gene and the* loxP/FRT *flanked Neomycin (Neo) cassette was inserted in the intron 2 region downstream of exon 2. Exons and restriction digestion sites are depicted as vertical gray boxes and vertical blue lines, respectively. The Neo cassette is flanked by* loxP *(green arrowheads) and* FRT *(orange chevrons) sites. Bg, Bgl II; H, Hind III; K, Kpn I; LA, long homology arm; R, EcoR I; SA, short homology arm.*



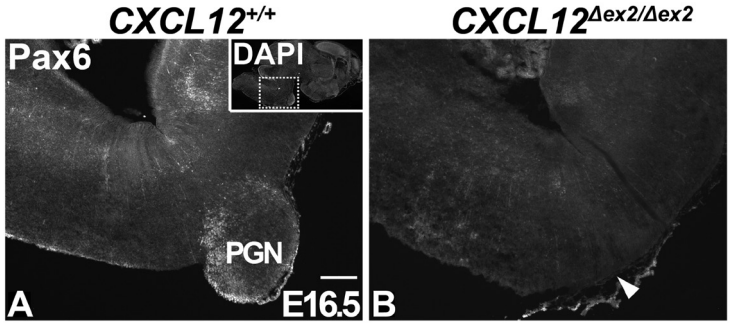
Supplementary Fig. S2. Southern blot analysis of selected embryonic stem (ES) cells. (A) *The* CXCL12 *targeted allele is shown with the location of the* NsiI *restriction sites (vertical blue lines). After* NsiI *digestion, the DNA was hybridized with the 3' probe (red box) for Southern blot analysis. 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 *CXCL12*$^{\Delta ex2/\Delta ex2}$ 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$^{+/\Delta ex2}$ *mice in which exon 2 of one* CXCL12 *allele is removed (*Δex2*). Next, we intercrossed* CXCL12$^{+/\Delta ex2}$ *mice to generate* CXCL12$^{\Delta ex2/\Delta ex2}$ *mutant embryos deficient in exon 2.* **(B)** *PCR analysis of the* wild type, floxed *or* Δex2 *alleles. A homozygous,* CXCL12$^{\Delta ex2/\Delta ex2}$ *mutant is indicated by the red box.*



Supplementary Fig. S4. *CXCL12*$^{\Delta ex2/\Delta ex2}$ 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 immunoflourescent 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 *CXCL12*$^{\Delta ex2/\Delta ex2}$ 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* CXCL12$^{\Delta ex2/\Delta ex2}$ *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.*