

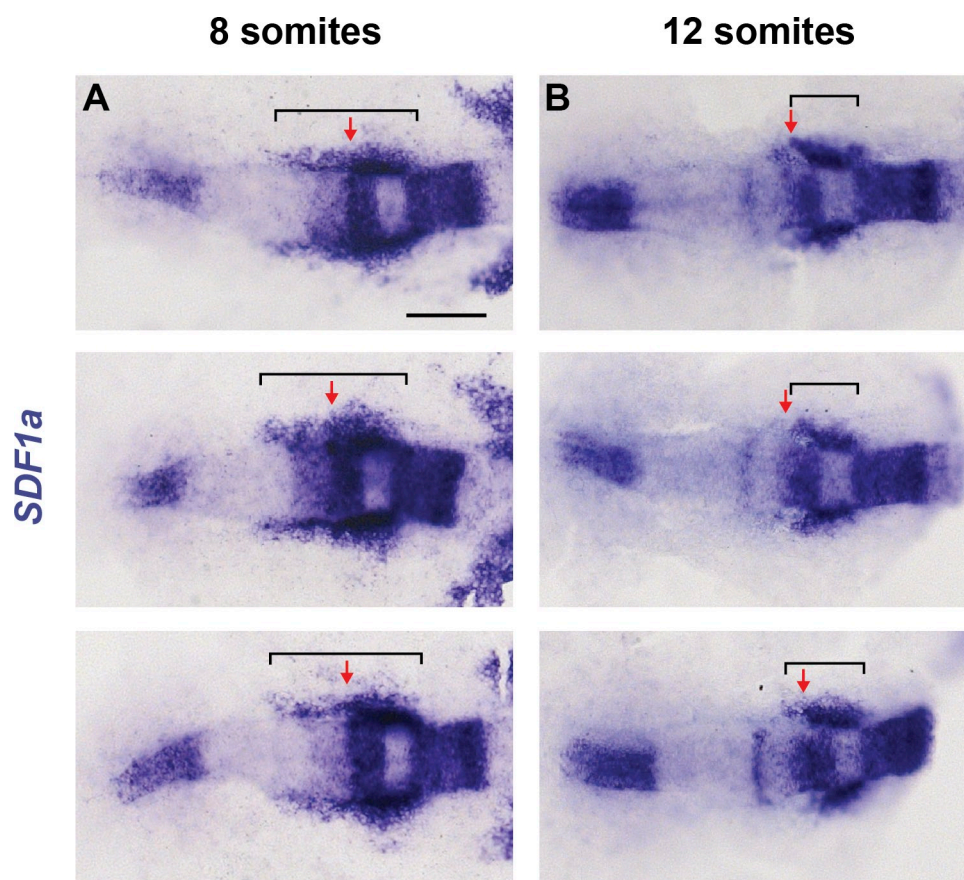
Lewellis et al., <http://www.jcb.org/cgi/content/full/jcb.201207099/DC1>

Figure S1. **Examples of the refinement of the *SDF1a* mRNA expression domain along the migratory route of the TgSNs.** This figure contains three pairs of embryos that show refinement of the *SDF1a* mRNA expression domain that delineates the migratory route of the TgSNs. (A) Eight-somite-stage embryos hybridized with an *SDF1a* antisense probe only so that TgSNs do not obscure the chemokine expression domain. The anterior border of the *SDF1a* expression domain that delineates the migratory route of the TgSNs (bracket) extends  $\sim 100\ \mu\text{m}$  past the anterior border of rhombomere 2 (red arrows). (B) Similarly treated 12-somite-stage sibling embryos. By this stage, the anterior border of the *SDF1a* expression domain has refined posteriorly to the site of trigeminal sensory ganglion assembly. Dorsal view, anterior to the left. Bar,  $100\ \mu\text{m}$ .

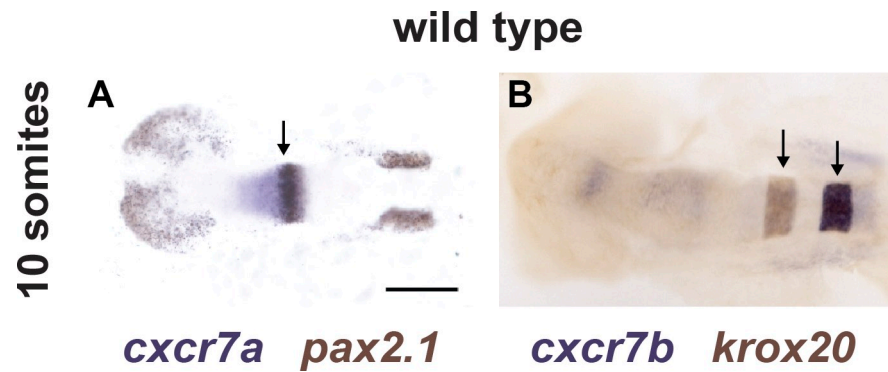


Figure S2. **Expression of *cxcr7a* and *cxcr7b* in the central nervous system.** This figure contains reference markers for identifying anatomical regions where *cxcr7a* and *cxcr7b* are expressed in the central nervous system. (A) 10-somite-stage wild-type embryos hybridized with *cxcr7a* (blue) and *pax2.1* (brown) antisense RNA probes. *pax2.1* is expressed in the MHB. The posterior part of the only *cxcr7a* expression domain present in the brain partially overlaps with *pax2.1* expression (arrow in A), confirming that *cxcr7a* is expressed in the anterior part of the MHB and the posterior part of the midbrain at this stage. (B) 10-somite-stage wild-type embryos hybridized with *cxcr7b* (blue) and *krox20* (brown) antisense RNA probes. *Krox20* is expressed in rhombomeres 3 and 5 at this stage. *Krox20* expression overlaps with two stripes of prominent *cxcr7b* expression (arrows in B), confirming that *cxcr7b* is expressed strongly in rhombomeres 3 and 5. Dorsal view, anterior to the left. Bar, 100  $\mu$ m.

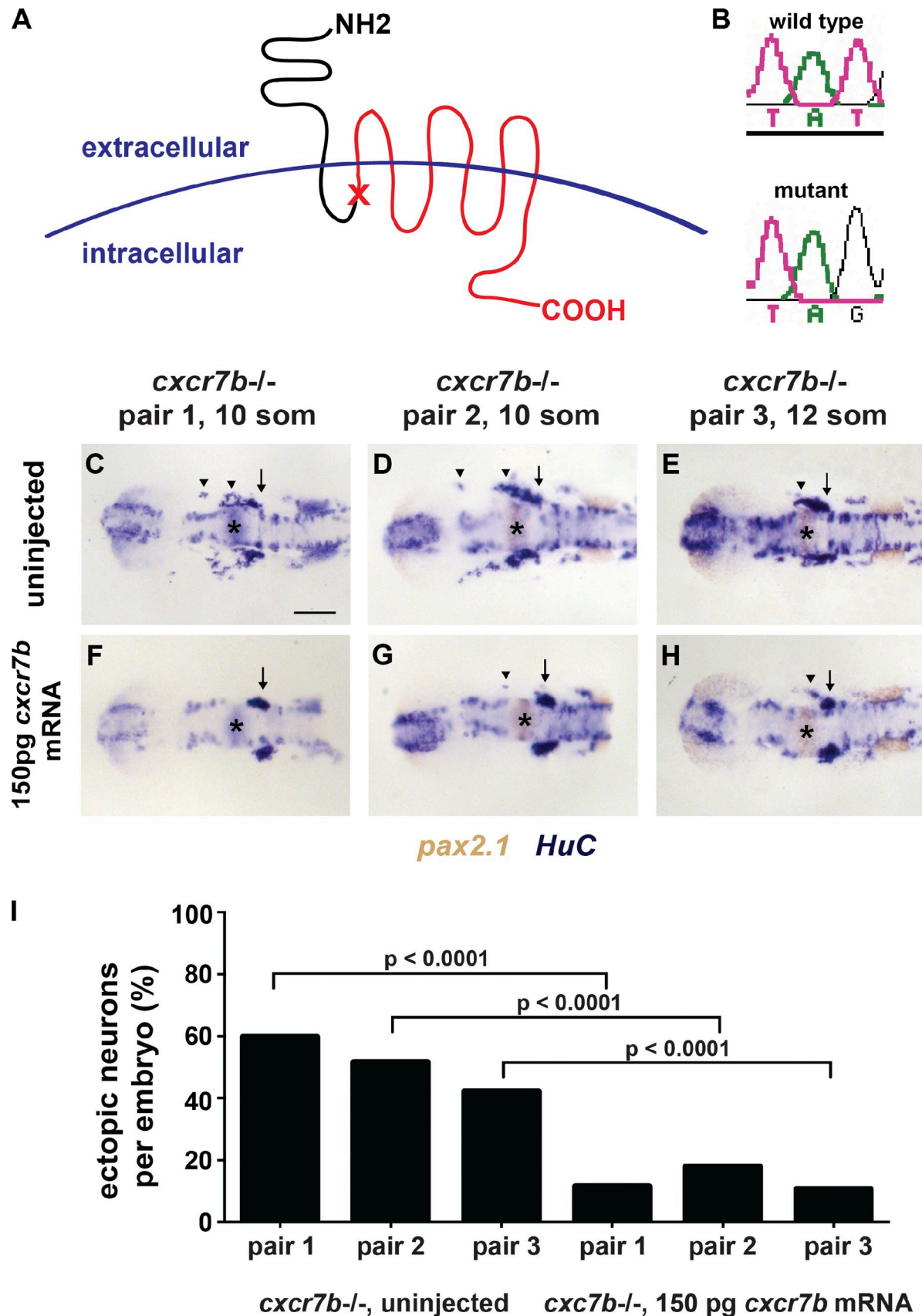


Figure S3. **Ubiquitous *cxcr7b* expression rescues neuron migration in *cxcr7b* mutant embryos.** This figure contains information about the *cxcr7b* mutation and the ability of *cxcr7b* mRNA to rescue the neuron positioning defect in *cxcr7b*<sup>-/-</sup> embryos. (A) The mutation in *cxcr7b* introduces a premature stop codon at amino acid position 76 that truncates almost 80% of the protein. (B) The sequencing trace at the position that encodes amino acid 76 for a wild-type embryo and a *cxcr7b* homozygous mutant embryo. (C–E) Representative images of *cxcr7b*<sup>-/-</sup> embryos from three separate sets of parents fixed at the 10- or 12-somite stage and stained with *HuC* and *pax2.1* antisense RNA probes to visualize TgSNs (blue) in relation to the MHB (brown or blue, asterisks). (F–H) Injection of 150 pg of *cxcr7b* mRNA in *cxcr7b*<sup>-/-</sup> embryos from clutches derived from the same three sets of parents used for C–E. Arrows and arrowheads in C–H denote correctly positioned and mispositioned neurons, respectively. (I) Summary of neuron positioning defects.  $n \geq 9$  embryos for each column. Contingency table analysis using Fisher's exact test was applied to determine statistical significance. In all three cases,  $P < 0.0001$ . Dorsal view, anterior to the left. Bar, 100  $\mu$ m. See also Table 6.



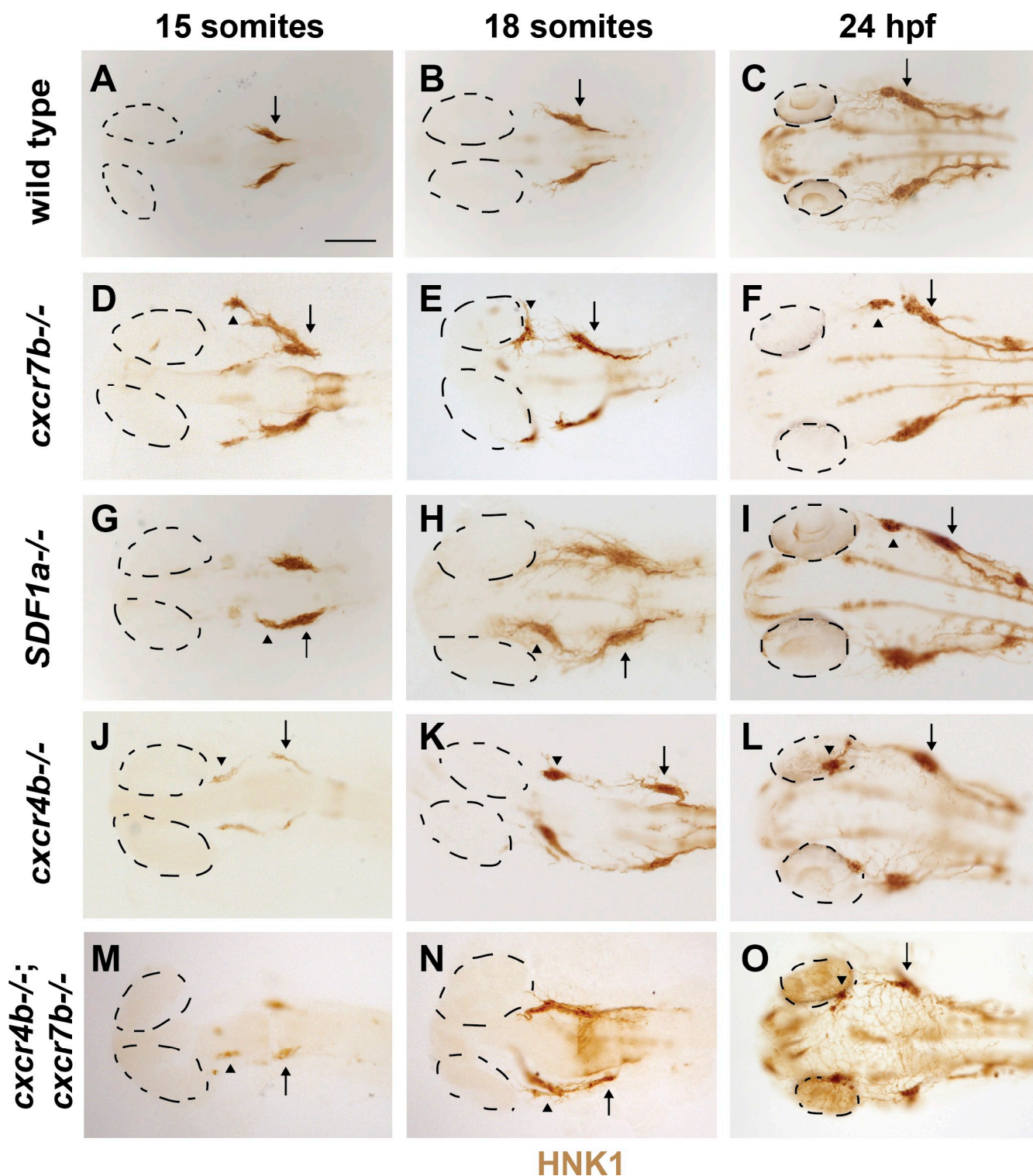


Figure S4. **TgSN positioning defects in chemokine signaling mutants at later stages.** This figure shows the TgSN positioning defects through 24 hpf in different chemokine signaling mutants. Embryos were immunostained with anti-HNK1 at stages that occur well after TgSN migration is complete in wild-type embryos. Arrows and arrowheads denote correctly positioned and mispositioned neurons, respectively. Broken lines outline the eye fields. Dorsal view, anterior to the left. Bar, 100  $\mu$ m. (A–C) Wild-type embryos fixed and stained at the 15-somite stage, 18-somite stage, and 24 hpf. (D–F) Similarly treated stage matched *cxcr7b*<sup>-/-</sup> embryos. (G–I) Similarly treated stage matched *SDF1a*<sup>-/-</sup> embryos. (J–L) Similarly treated stage matched *cxcr4b*<sup>-/-</sup> embryos. (M–O) Similarly treated stage matched *cxcr7b*<sup>-/-</sup>; *cxcr4b*<sup>-/-</sup> embryos.

**An image-processing script is available for download as a text file.**