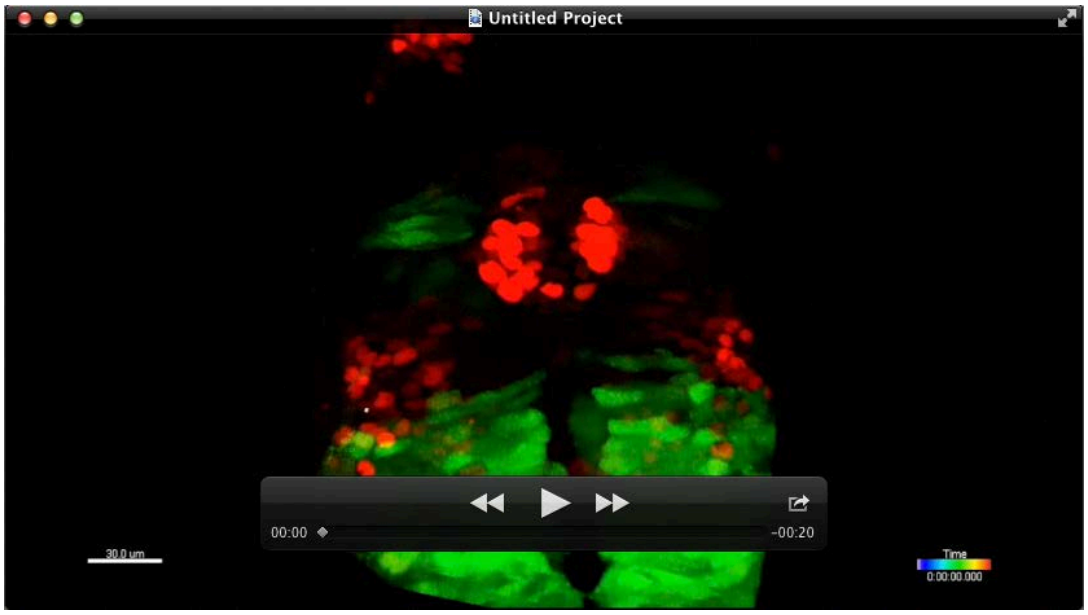


Movie 1: Time lapse imaging of *dbx1b*$^{+}$ and *cxcr4b*$^{+}$ labeled populations.

Time lapse imaging of a doubly labeled *TgBAC(dbx1b:GFP)* and *TgBAC(cxcr4b:nls-tdTomato)* embryo between 27 and 48 hpf. Labeling of the GFP channel is subtracted to facilitate visualization of the dTomato population. dTomato^{+} cells are visible in the region of the left habenula at 37 hpf (Time = 9:00:00).

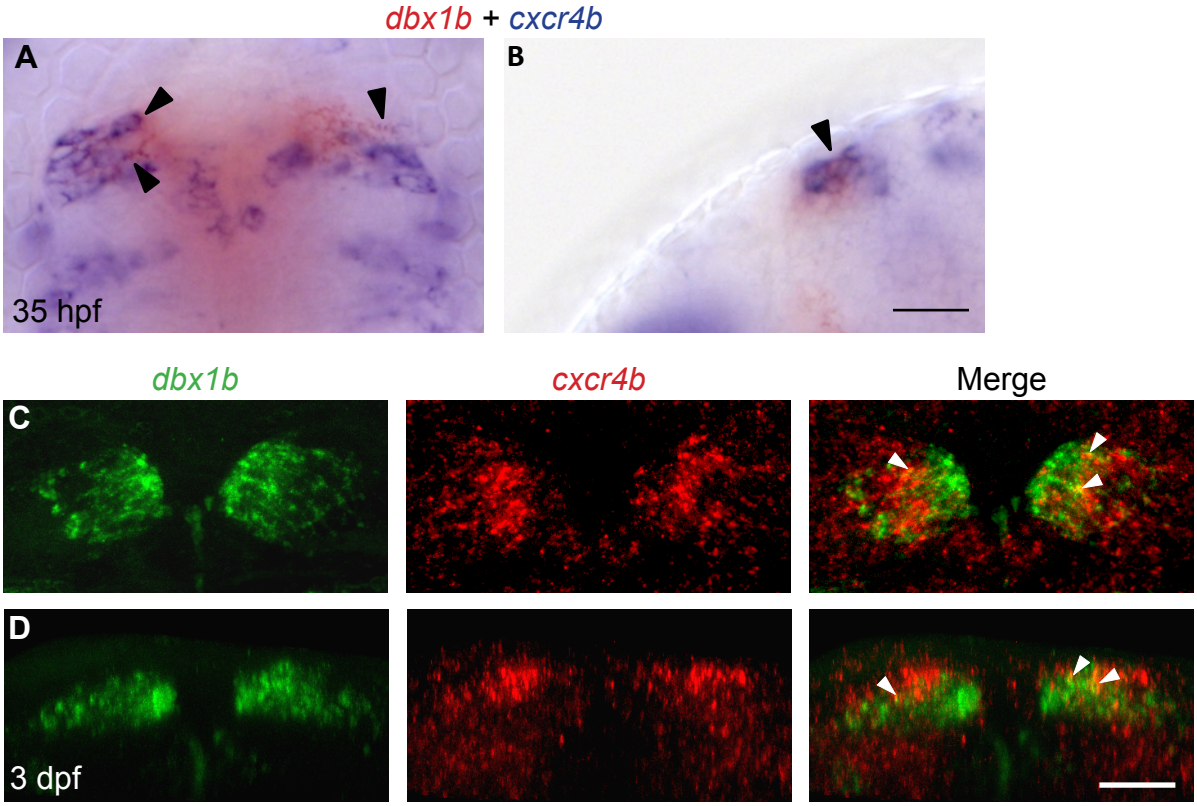
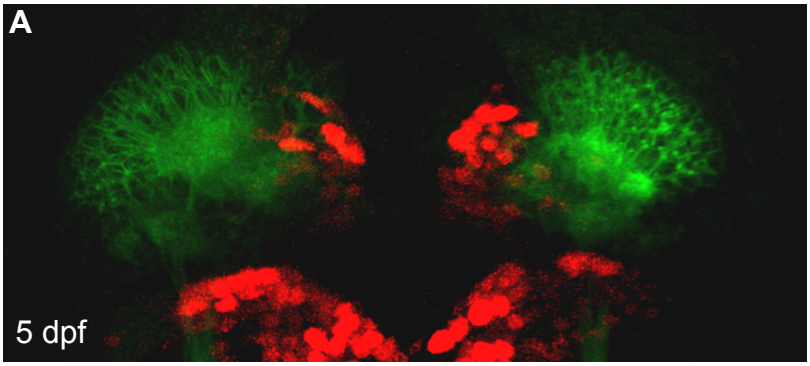


Fig. S1

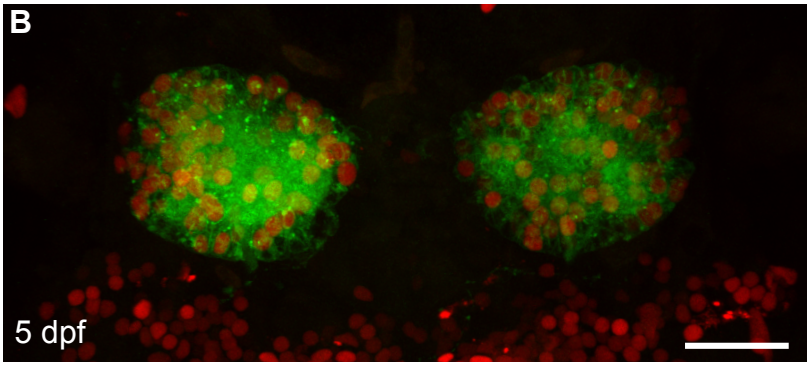
Fig. S1: Partial overlap in *cxcr4b* and *dbx1b* expression.

Visualization of *dbx1b* and *cxcr4b* transcripts by (A,B) colormetric *in situ* hybridization at 35 hpf or by (C,D) fluorescent *in situ* hybridization at 3 dpf. (A,C) Dorsal (B) lateral and (D) frontal views show partial co-localization of transcripts in a subset of dHb cells (arrowheads). Scale bar, 30 μm.

TgBAC(dbx1b:nls-dTomato); *TgBAC(gng8:GFP-CAAX)*



dbx1b Cre lineage tracing; *TgBAC(gng8:GFP-CAAX)*



dbx1b Cre lineage tracing; *TgBAC(gng8:GFP-CAAX)*

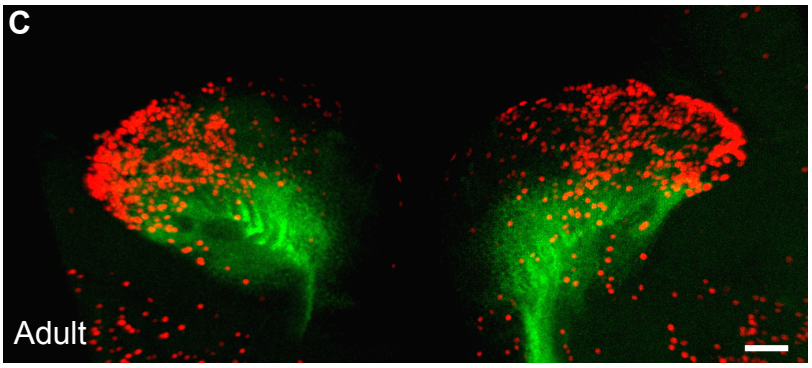


Fig. S2

Fig. S2: Medial *dbx1b*$^{+}$ cells give rise to all dHb neurons

TgBAC(gng8:CAAX-GFP) labels the cell membranes of dHb neurons (green). (A) *TgBAC(dbx1b:nls-dTomato)* labels cells medial and ventral to the GFP labeled neurons. Dorsal view, 5 dpf. (B,C) Lineage tracing using Cre recombinase under control of the *dbx1b* promoter [*TgBAC(dbx1b:Cre-mCherry)*] and a floxed reporter line [*Tg(β-actin:loxP-hmgb1-eCFP-loxP-H2B-mCherry)*] labels neurons throughout the dHb with nuclear mCherry at (B) 5 dpf and (C) in adult brain sections. Scale bars are (A,B) 30 μm and (C) 50 μm.

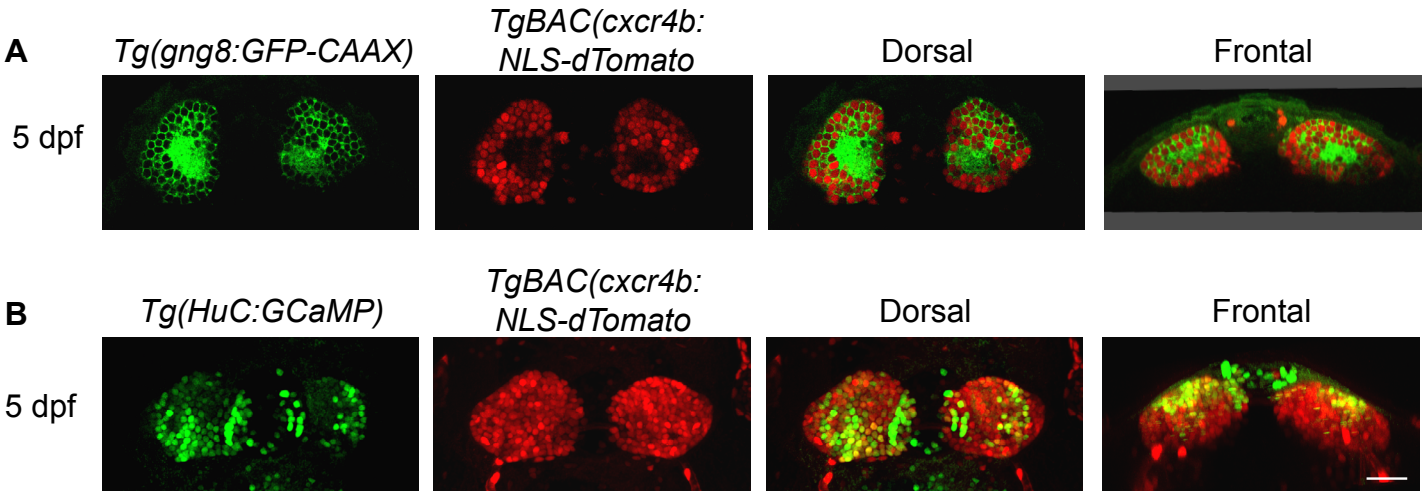


Fig. S3

Fig. S3: Persistence of fluorescent labeling in dHb neurons of transgenic larvae.

In 5 dpf *TgBAC(cxcr4b:nls-tdTomato)* larvae, dTomato labeling persists in dHb neurons distinguished by (A) *TgBAC(gng8:CAAX-GFP)* or (B) *Tg(HuC:H2B-GCaMP6s)* labeling. Scale bar is 30 μm.

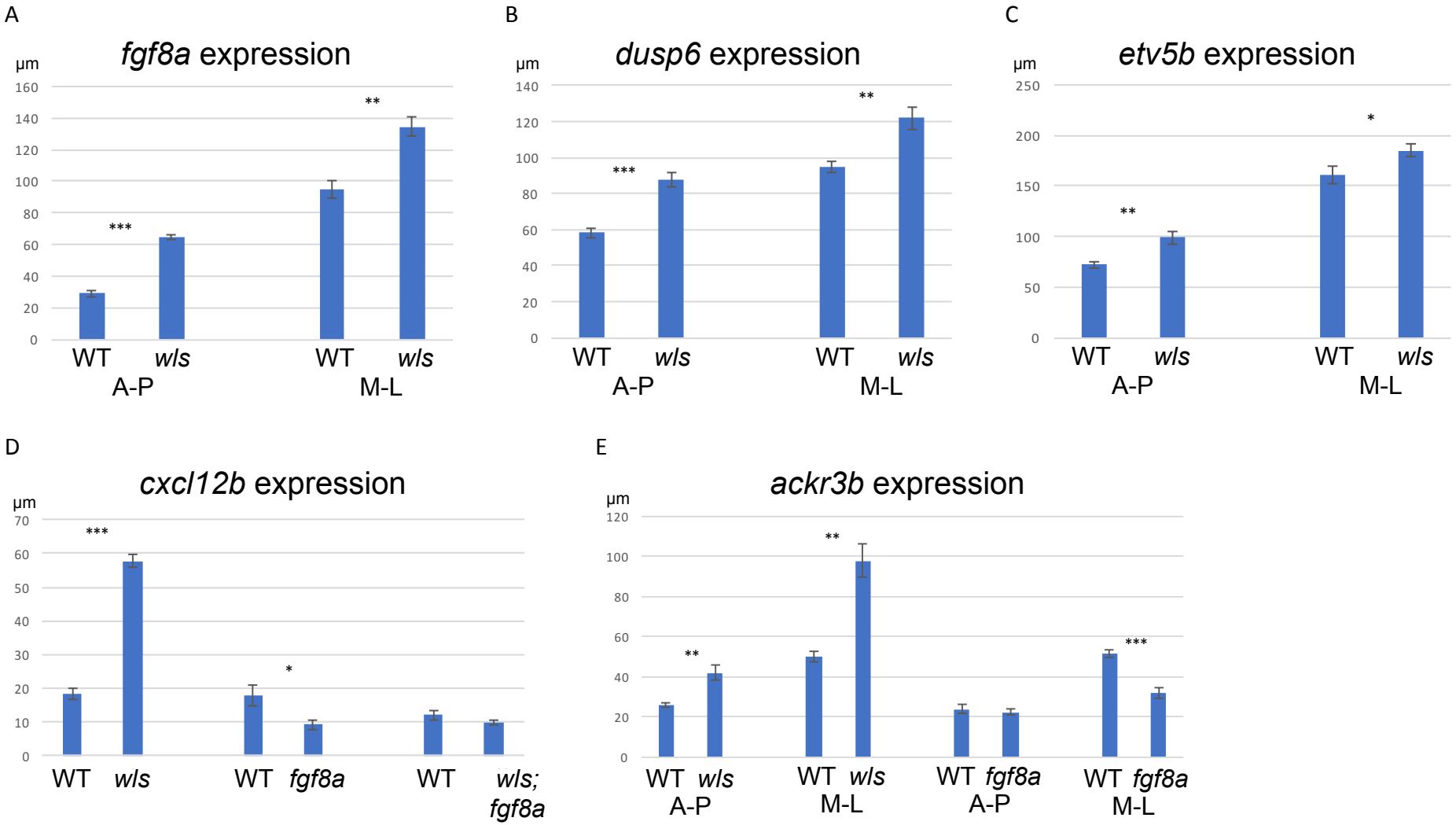


Fig. S4

Fig S4. Quantification of spatial domains of diencephalic gene expression.

Measurements (μM) of (A) *fgf8a*, (B) *dusp6,* (C) *etv5b*, (D) *cxcl12b* and (E) *ackr3b* gene expression domains in *wls*$^{-/-}$*, fgf8a*$^{-/-}$*,* and *wls*$^{-/-}$*; fgf8*$^{-/-}$ mutants and their WT siblings along the anterior-posterior (A-P) and medial-lateral (M-L) axes of the brain. Statistical analysis performed with Students t-test. * represents $p< 0.05$; ** $p< 0.0005$ and *** $p< 0.5 \times 10^{-5}$.

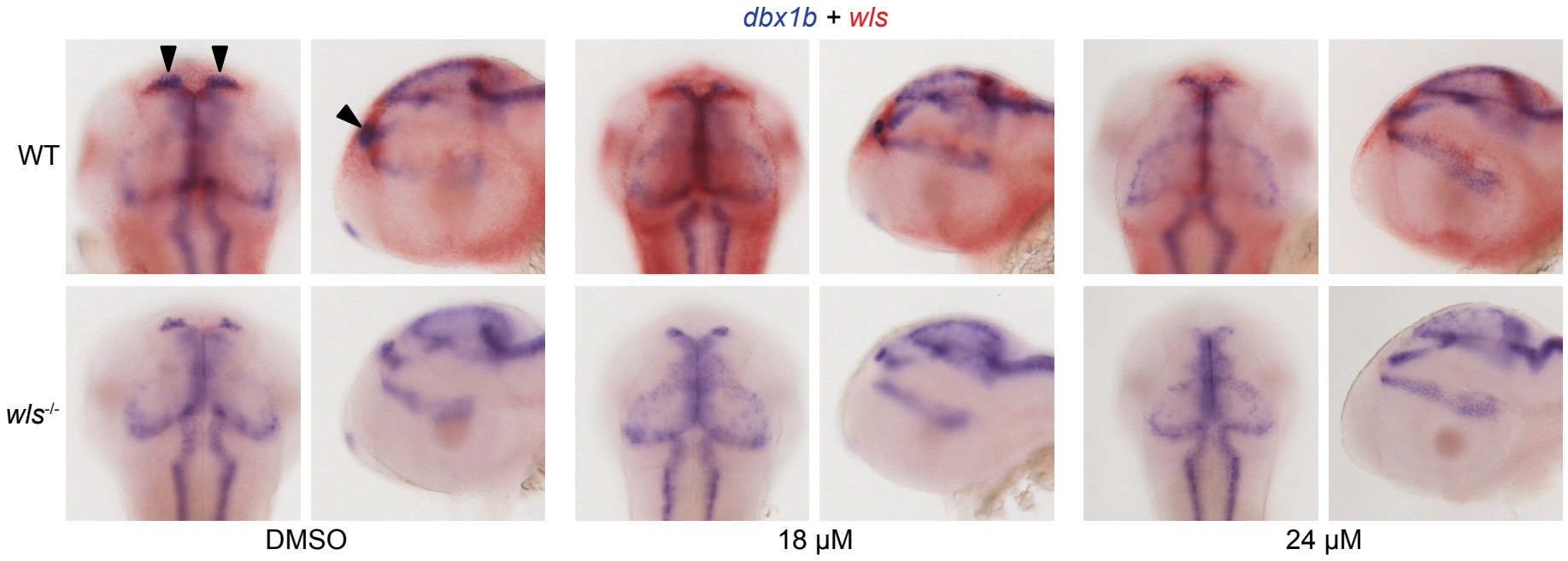


Fig. S5

Fig. S5: Inhibition of Fgf signaling reduces *dbx1b* and *cxcl12b* expression domains in a dose-dependent manner

Between 24 and 48 hpf, WT and *wls* mutant sibling embryos were treated with either 0.3% DMSO, 18 µM SU5402 + 0.3% DMSO, or 24 µM SU5402 + 0.3% DMSO. Diencephalic expression of *dbx1b* (arrowheads) is reduced in a dose-dependent manner in WT and *wls* mutants.

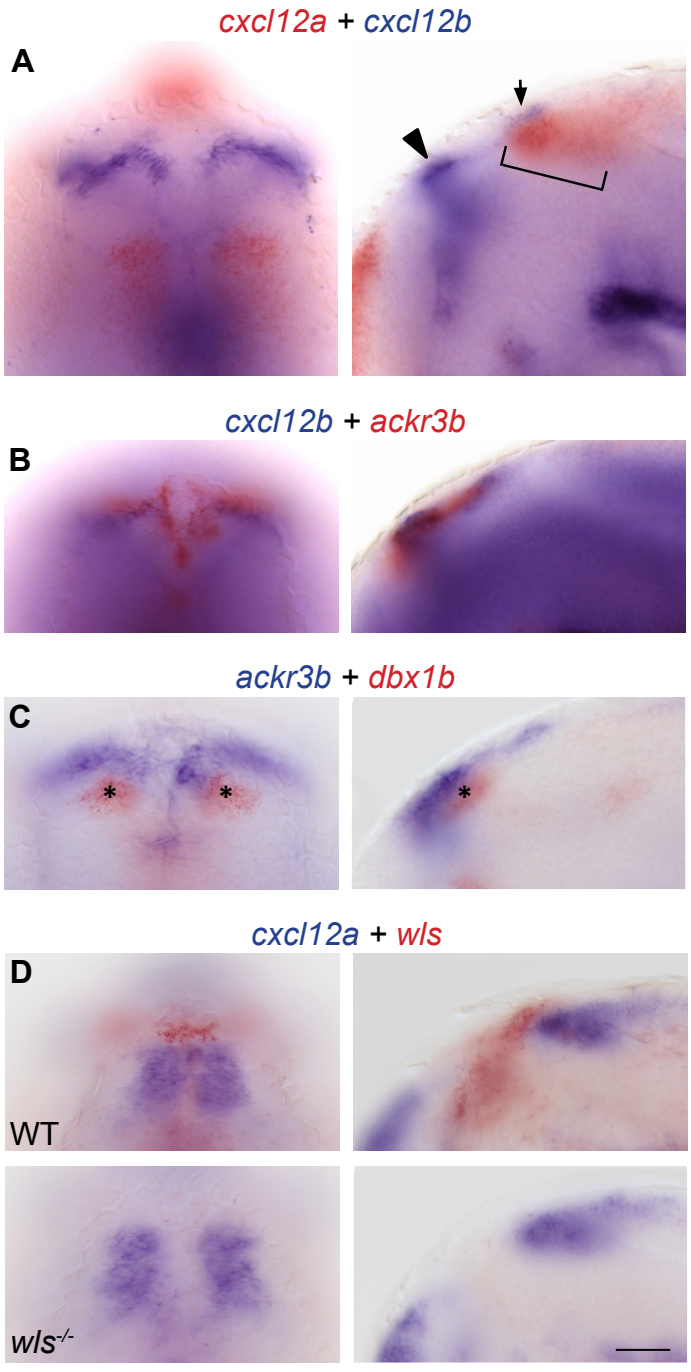


Fig. S6

Fig. S6: Diencephalic expression of genes in the Cxcr4-chemokine signaling pathway.

(A) The *cxcl12a* gene is expressed ventral and caudal (bracket) to the developing habenulae. *cxcl12b* transcripts are found in high levels rostral (arrowhead) and lower levels caudal (arrow) to the dHb. (B) Transcripts for *ackr3b* are found anterior and medial to the rostral domain of *cxcl12b* expression and (C) the *dbx1b*$^{+}$ progenitors (asterisks). (D) Expression of *cxcl12a* is not affected in *wls* mutants. Dorsal (left) and lateral (right) views. All embryos are 35 hpf. Scale bar is 50 μm.