



Figures and figure supplements

Spatio-temporal regulation of concurrent developmental processes by generic signaling downstream of chemokine receptors

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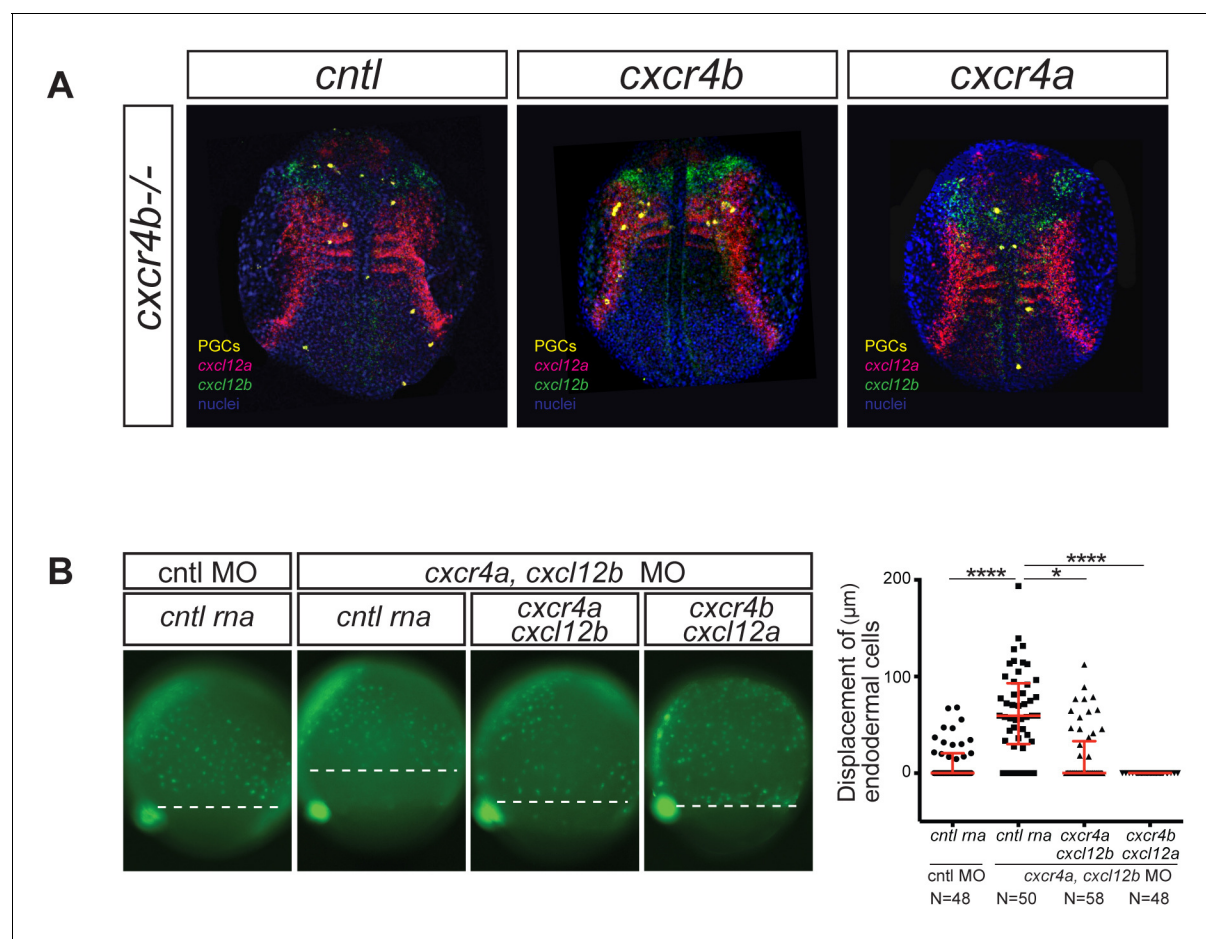


Figure 1. The signaling cascades initiated by Cxcr4a and Cxcr4b are functionally equivalent. (A) The position of PGCs (detected by *nanos3* RNA expression, yellow) relative to tissues expressing *cxcl12a* (magenta) and *cxcl12b* (green) RNAs in 12 hpf *cxcr4b*^{-/-} embryos. PGCs express control RNA (*cntl*, left panel) or RNAs encoding for Cxcr4b (middle panel) or Cxcr4a (right panel). 20 pg of control RNA and RNA encoding for Cxcr4a and Cxcr4b were injected. (B) Epifluorescence image of transgenic *sox17::gfp* embryos at 8 hpf. The embryos were injected with control (*cntl*) morpholino and control RNA (left panel). Experimental embryos were knocked down for Cxcr4a and Cxcl12b and the effect of control RNA, *cxcr4a* and Cxcl12b or *cxcr4b* along with *cxcl12a* mRNAs was examined. The quantitation of the endoderm displacement with respect to the forerunner cells is presented in the graph on the right. See also **Figure 1—figure supplement 1**. 0.8 pmol of each morpholino was used. 100 pg of receptor and 50 pg of ligand encoding mRNA was used, as well as equimolar amounts of control RNA.

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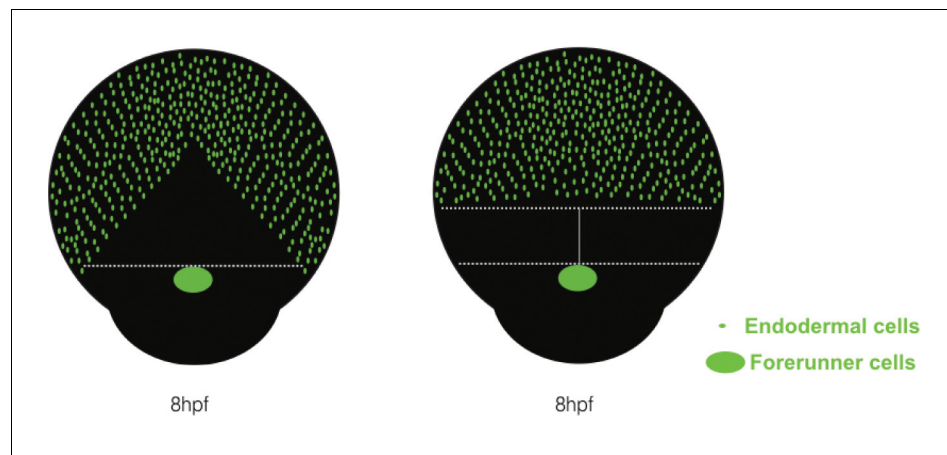


Figure 1—figure supplement 1. Cartoon showing the procedure employed for measuring the displacement between endoderm and forerunner cells.

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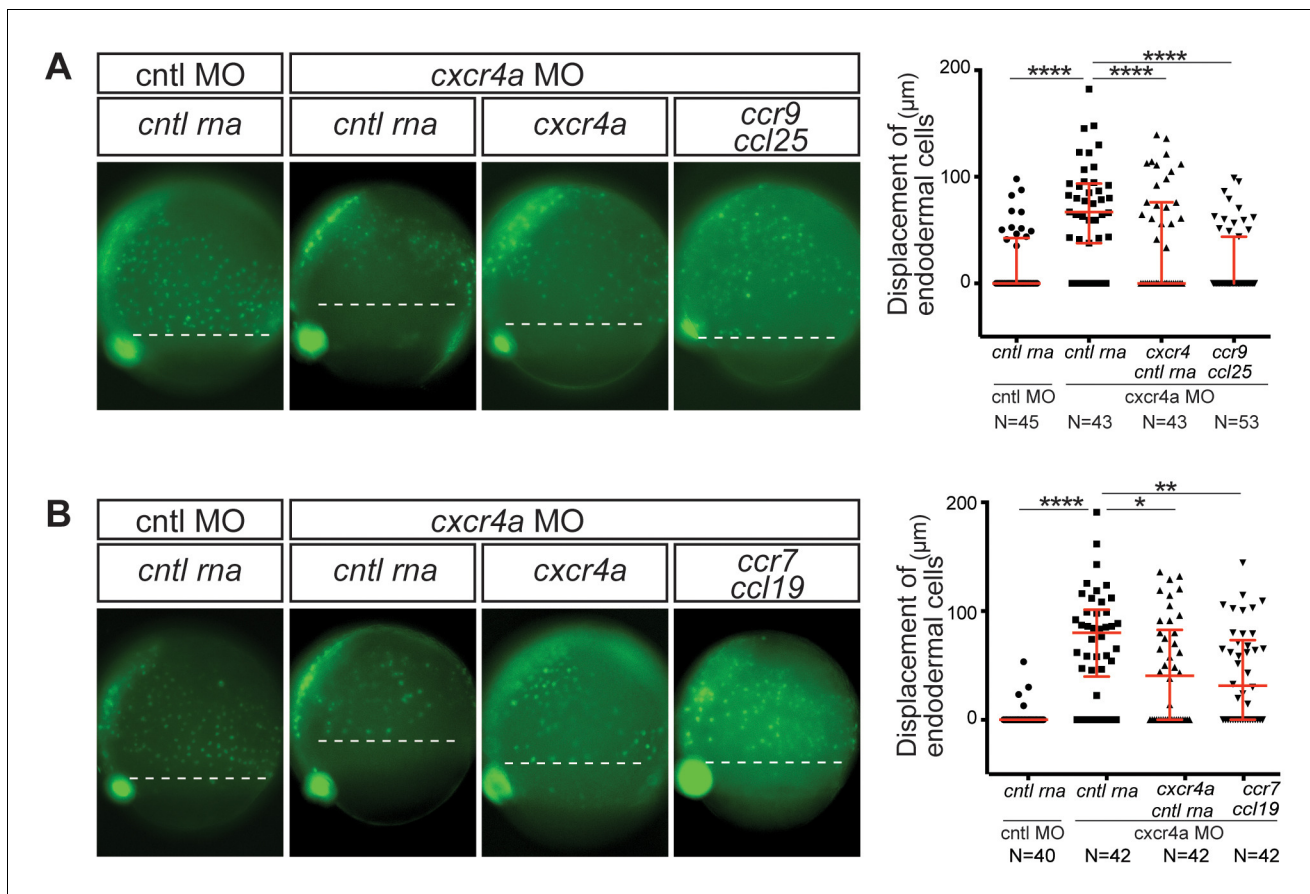


Figure 2. CC receptors can control a process regulated by CXC receptors. (A) Epifluorescence image of *sox17::gfp* transgenic control embryos (left panel) and embryos knock down for Cxcr4a. The Cxcr4a knocked down embryos were injected with control RNA (*cntl*) or with *cxcr4a* RNA, or co-injected with *ccr9* RNA and its ligand *ccl25* RNA. The quantitation of the endoderm displacement with respect to the forerunner cells is presented in the graph on the right. See also **Figure 1—figure supplement 1**. (B) Epifluorescence image of *sox17::gfp* transgenic control embryos (left panel) and of embryos knocked down for Cxcr4a. The embryos were injected with control RNA, with *cxcr4a* RNA, or co-injected with *ccr7* RNA and its ligand *ccl19* RNA. The quantitation of the endoderm displacement with respect to the forerunner cells is presented in the graph on the right. 0.8 pmol of each morpholino was used. 100 pg of receptor and 50 pg of ligand encoding mRNA was used, as well as equimolar amounts of control RNA.

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Cxcr4a	MAYYEHIVFEDDLSADNSSEFGSGDIGANFEVPCDVEVSHDFQRIFLPTVYGIIFVLGLI
Cxcr4b	MEFY-----DSIILDNSSDSGSGDYD--GEELCDLSVSNDFQKIFLPTVYGIIFVLGII
	* : * * . : *** : *** . * * : . * : *** : ***** : *
Cxcr4a	GNGLVVLVMGCQKKSRMTDKYRLHLSVADLLFVLTLPFWAVDAAKDWYFGGFMCVAVHM
Cxcr4b	GNGLVVLVMGFQKKSKNMTDKYRLHLSIADLLFVLTLPFWAVDAVSGWHFGGFLCVTVNM
	***** *** : . ***** : ***** : ***** . . . * : *** : * : *
Cxcr4a	IYTVNLYSSVLILAFISLDRYLAVVRATNSQGPRKLLANRIIYGVWLPALLTVPDLVF
Cxcr4b	IYTLNLYSSVLILAFISLDRYLAVVRATNSQNLKLLAGRVIIYGVWLPATFFTIPDLVF
	*** : ***** : ***** . *** . * : * : *** : : : * : *****
Cxcr4a	AKAESSAIRTFCEIYPQDSFVTWVVAFRFQHILVGFVLPGLVILICYCIIISKLSRGSK
Cxcr4b	AKIHNSMGITICELTYPQEANVIWKAVFRFQHIIIGFLLPGLIILTCYCIIISKLSKNSK
	** . . * : : * : ** *** : : * * . . ***** : : * : *** : ** ***** : . **
Cxcr4a	GT-QKRKALKTTTVVLIVCFVCWLPYCGGILLDTLMMLEVIPHSCLEQGLQKWIFVTEA
Cxcr4b	GQTLKRKALKTTVILILCFICWLPYCAGILVDALTMLNVISHSCFLEQGLEKWIFFTEA
	* ***** : * : * : ***** . *** : * : * * : * * * * : * : * . ***
Cxcr4a	LAYFHCCCLNPILYAFLGVKFKKSARSALSPSRGSSLKFLSKKRTGMSSVSTESESSSFHS
Cxcr4b	LAYFHCCCLNPILYAFLGVRFKSARNALSISSRSSHKMLTKRGPISSVSTESESSSALT
	***** : * . ***** . *** * * * : * : * : ***** :
Cxcr4a	S
Cxcr4b	S
	*

Figure 2—figure supplement 1. Protein sequence alignment of Cxcr4a and Cxcr4b using Clustal omega. (*)=Fully conserved residue. (.)=Conservation among groups of strongly similar properties, (.)=Conservation among group of weakly similar properties.

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Cxcr4a	-----MA-----YYEHIVFEDDLSADNSSEFGSGDIGANFEVPCDVEV
Cxcr4b	-----ME-----FY-----DSIILDNSSDSGSGDYD--GEELCDLSV
Ccr7	MHAYTVFCPVLLIWSCHIKKSWSNMTEH-QMGEKATTEYDYTTGTVDY-DIYEQSCNKTN
Ccr9	-----MMNVTELITSLTP----QTSEYEEYGDFSTTSDYGD TDDGFCPSK
	. : . : *
Cxcr4a	SHDFQRIFLPTVYGIIFVLGLIGNGLVVLVMG-CQKKSRTMTDKYRLHLSVADLLFVLT
Cxcr4b	SNDFQKIFLPTVYGIIFVLGIIGNGLVVLVMG-FQKKSRTMTDKYRLHLSIADLLFVLT
Ccr7	NRTFRAWFLPTIYTIICLLALMGNFLVILTYLYF-KRLKTMVDVYLFNLAMADLLFAISL
Ccr9	VREFRMYEPMYWMIVILGAIGNTLVWVIYTHFKNRLKAMTDVYLLNLALADLFFLCTL
	. *: : * : * : * : * : * : * : * : * : *
Cxcr4a	PFWAVDAAKDWYFGGFMCAVHMIYTVNLYSSVLILAFISLDRYLAVVRATNSQGPR--K
Cxcr4b	PFWAVDAVSGWHFGGFLCVTVNMIYTLNLYSSVLILAFISLDRYLAVVRATNSQNLR--K
Ccr7	PFWAASFMTWHLGLYPCKAMFTIYKVSFFSGMFLLCISIDRYFSITKAUSAHRCSSA
Ccr9	PFWAADSIYGWAFGSLCKVVSAYKINFFSSMFLLCISVDRIYIVQTTKAQNSKRSR
	****. . * : * * . : : * : : * : : * : : * : : * : : *
Cxcr4a	LLANRIIYGVWLPAAALLTVPDLVFAKAESS-AIRTFCEIYYPQDSFVTWVAVFRFQHIL
Cxcr4b	LAGRVIYIGVWLPATFFTIPDLVFAKIHNS-SMGTCIELTYPQEANVIWKAUFQHI
Ccr7	VYQGQVSSLVTWVIAIVFSVPDMVFAVINSRGTCANV-----NYQDYVVKMVISQMV
Ccr9	LLYSKLICVLVWLLAALMSIPECLFARSKEDDESNTFCTMVYWNENNRTKILVLALQIC
	: . : : . * : : : * : : : * : : : * : : : *
Cxcr4a	VGFLVPLGLVILICYCIIISKLSRSGSGT-QKRKALKTTVVLIVCFVVCWLPYCGGILLDT
Cxcr4b	IGFLPGLIILTCYCIIISKLSKNSKGQTLKRKALKTTVILILCFVICWLPYCAVILVDA
Ccr7	LGFIIPAVVMGFCYICIIKTLMQAKN--FERNKAFKVIIVVVVFVFSQLPYNVVMGVSV
Ccr9	MGFCIPLVVMIFCYTNIIRTLLKTRN--FQKHKALRVILAVVAVFVLSQLPYNGMLVFEA
	: * : * : : * * * * . : : : : * : : : * : : * : : *
Cxcr4a	LMMLEVIPHSCLEQLQKWIFVTEALAYFHCCNLPILYAFLGVKFKKSARSALSPSRGS
Cxcr4b	LTMLNVISHSCFLEQGLEKWIFFTEALAYFHCCNLPILYAFLGVRFKSARNALSISSRS
Ccr7	QQT-----TDCAKDNTRLFALDVTMAVAFLRCCVNPFLYAFVGVFRNDLLKLLKDLGCL
Ccr9	TQAANMTVTNCTESIRFDIAGQVMKSIAYMHSCNPNFLYAFVGVFRKDVIRIFQNIQCV
	. * . . . : : : : * : : * : : * : : *
Cxcr4a	SLKFLSKK--RTGMSSVSTESSESSSFHSS
Cxcr4b	SHKMLTKK--RGPISVSTESSESSSALTS
Ccr7	RTSHLMYTHCGRRRSSVGLDTETTTTFSP
Ccr9	SNLKNSKLAGIPHRSSVMSD TDTTNALSL
	*** : : : : . :

Figure 2—figure supplement 2. Protein sequence alignment of Cxcr4a, Cxcr4b, Ccr7 and Ccr9 using Clustal omega. (*)=Fully conserved residue. (:) =Conservation among groups of strongly similar properties, (.)=Conservation among group of weakly similar properties.

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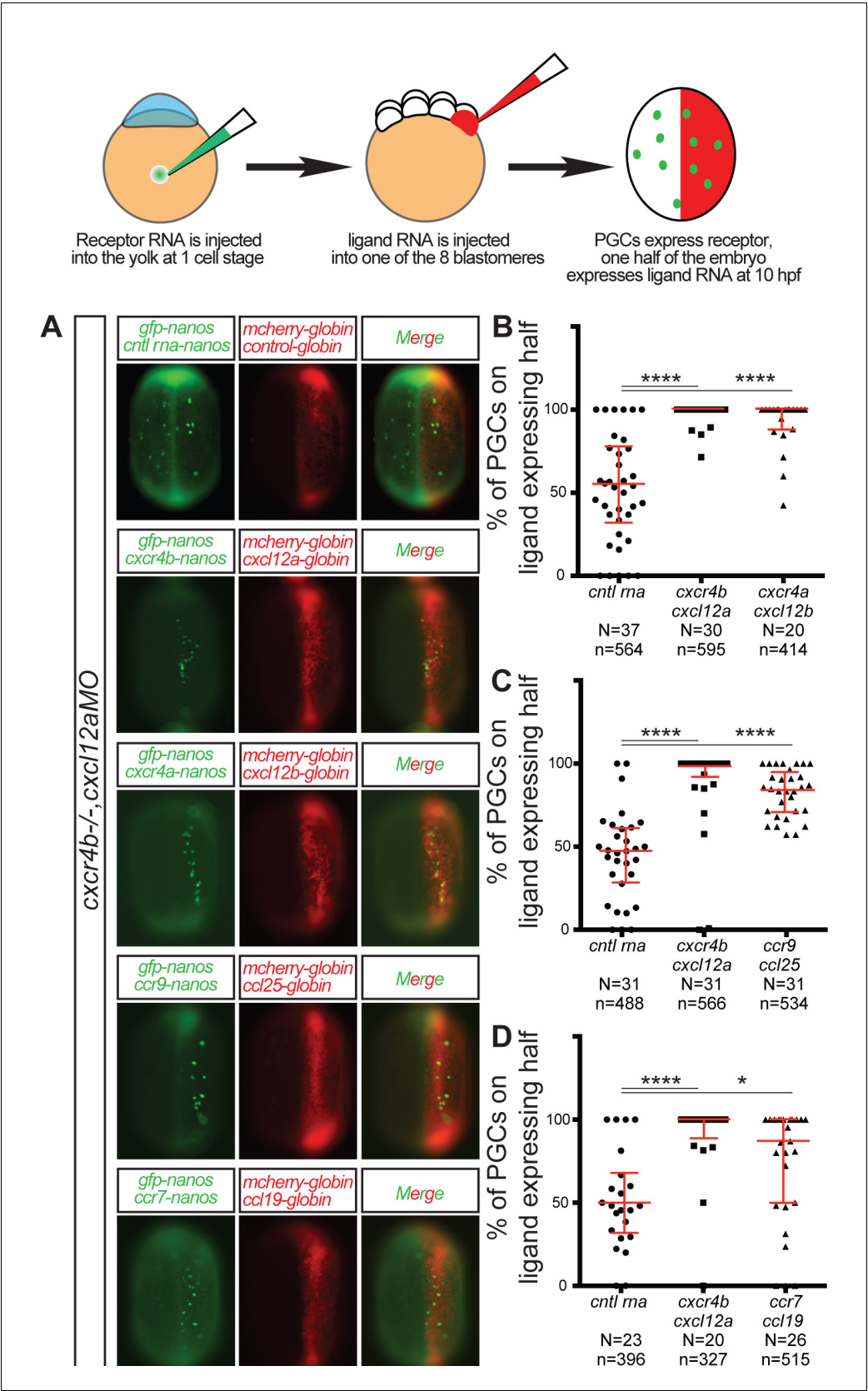


Figure 3. CC and CXC receptors can direct the migration of primordial cells. The experimental scheme is provided at the top. (A) Epifluorescence image of 10 hpf embryos expressing the *control* RNA or RNA encoding

Figure 3 continued on next page

Figure 3 continued

for the indicated ligand (Cxcl12a, Cxcl12b, Ccl25 and Ccl19) in one half of the embryo and *control* RNA or Receptor-encoding RNA (Cxcr4b, Cxcr4a, Ccr9 or Ccr7) in PGCs. Merged images show the position of PGCs with respect to control or ligand-expressing domains (red). (B–D) Graphs show the quantitation of the migration of PGCs as the percentage of GFP-labeled cells located within the ligand-expressing part of the embryos. 60 pg of *mGFP-nanos* was used to label PGCs in green and 40 pg of *m-cherry* mRNA was used for labeling the ligand expressing half of the embryo. 20 pg of receptor-encoding RNA was used and 30 pg of ligand-encoding RNA was used. 0.2 pmol of Cxcl12a morpholino was used. Equimolar amounts of control RNA were used. For raw data see **Figure 3—source data 1**.

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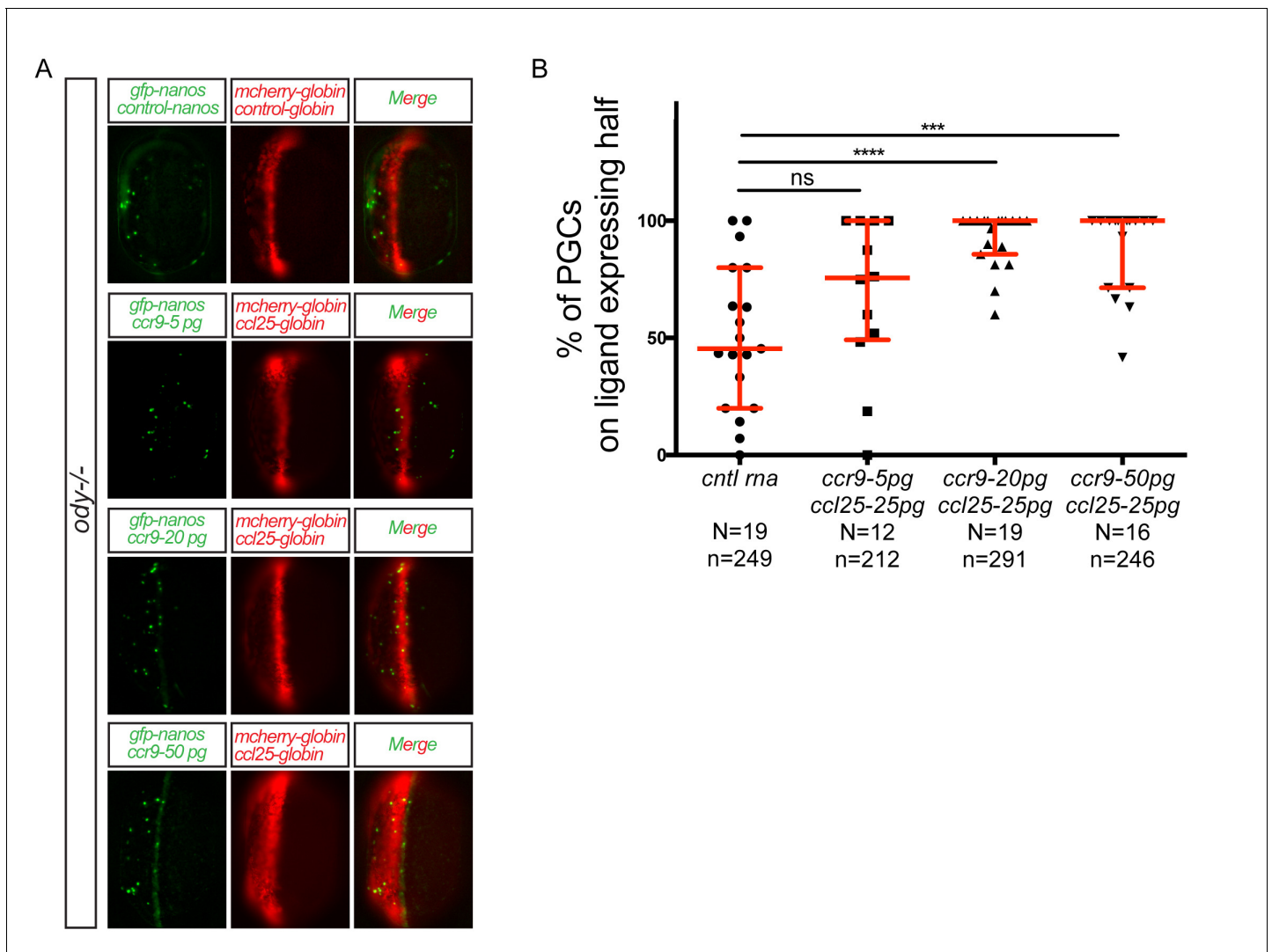


Figure 3—figure supplement 1. Different levels of chemokine receptor signaling leads to functionally same response. (A) Epifluorescence images of 10 hpf embryos. Embryos express the control RNA or the RNA encoding for Ccl25 in one half of the embryo and control RNA or different concentrations of RNA encoding for Ccr9 in PGCs. Merged images show the position of PGCs with respect to control or ligand-expressing domains (red). (B) Graphs show the quantitation of the migration of PGCs as the percentage of GFP-labeled cells located within the ligand-expressing domain of the embryos. 60 pg of mGFP-nanos was used to label PGCs in green and 40 pg of m-cherry mRNA was used for labeling the ligand expressing half of the embryo. For raw data see **Figure 3—figure supplement 1—source data 1**.

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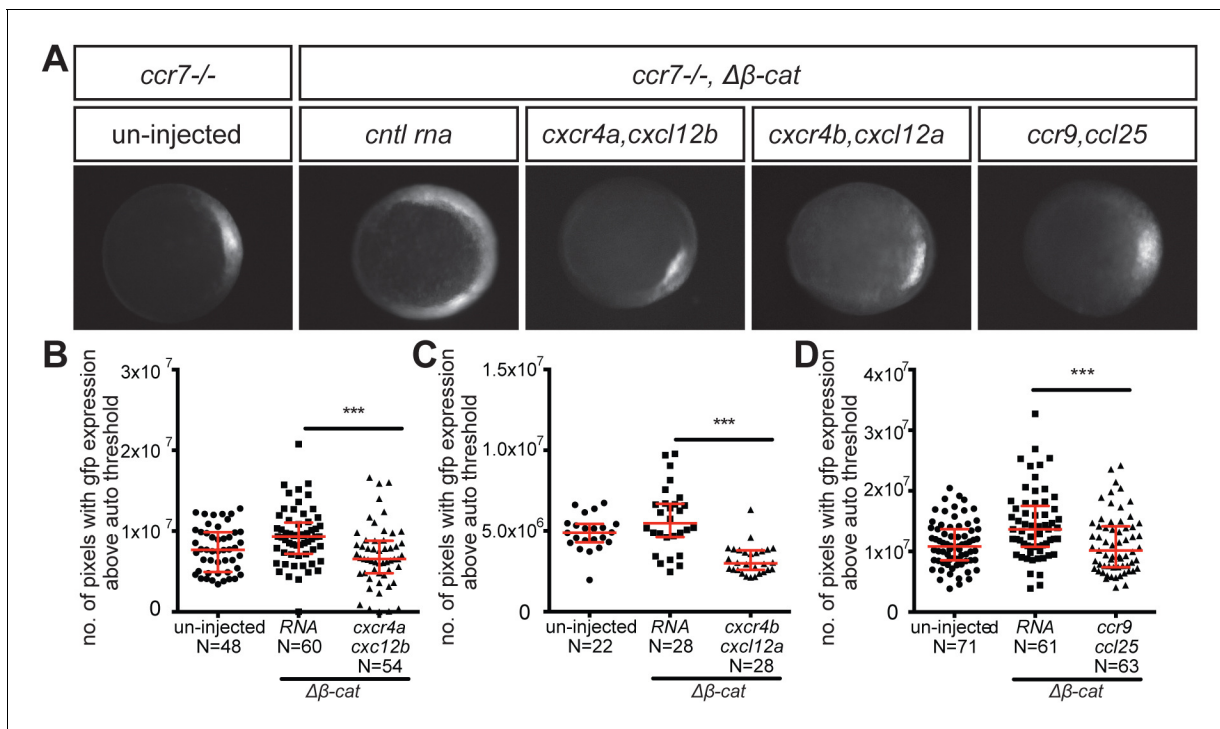


Figure 4. Control of dorsoventral axis specification by different chemokine receptors. (A) Epifluorescence image of 5 hpf *ccr7*^{-/-} mutant embryos carrying the *gsc::gfp* transgene. Uninjected embryo (left panel) and embryos sensitized by injection of $\Delta\beta$ -Catenin encoding RNA (right panels) are presented. The embryos were also injected with control RNA (*cntl*) or with RNA encoding for different chemokine receptors (*Cxcr4a*, *Cxcr4b* or *Ccr9*) and their cognate ligands (*Cxcl12b*, *Cxcl12a* or *Ccl25*). (B–D) Graphs showing the area of the *gooseoid* expression domain determined by quantifying the number of pixels with GFP signal above the auto threshold in the different treatments. 100 pg of mRNA encoding for the receptors was injected and 60 pg of RNA encoding for the ligands. 2.5 pg of $\Delta\beta$ -Catenin-encoding RNA was inject to sensitize the embryos. Equimolar amounts of control RNA were used. For raw data see **Figure 4—source data 1**.

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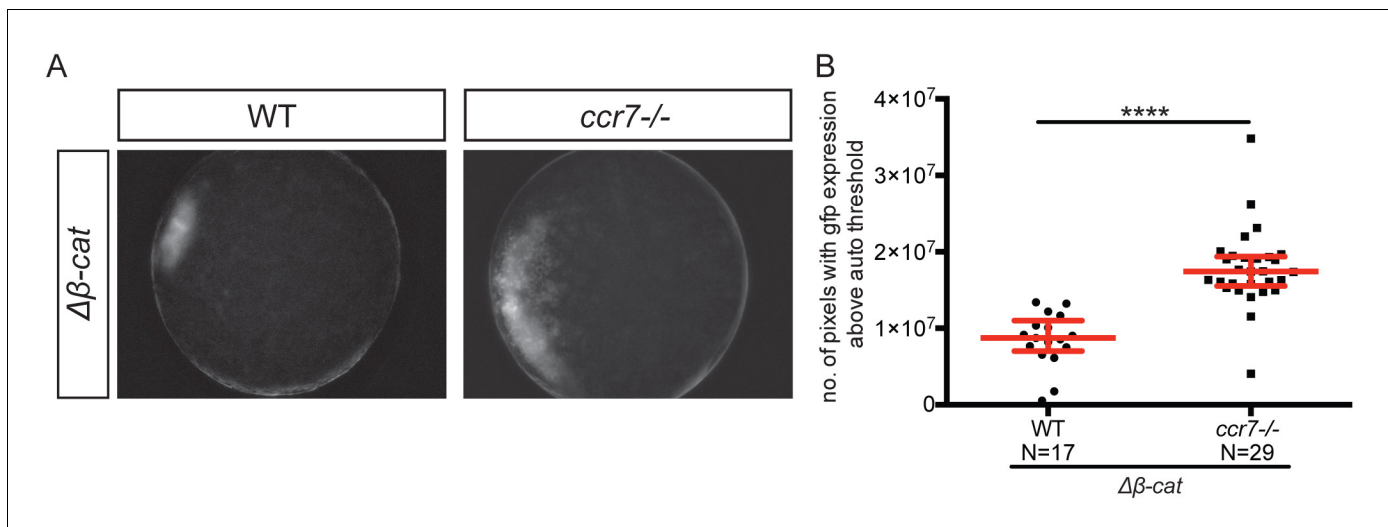


Figure 4—figure supplement 1. Embryos lacking Ccr7 are hypersensitive to very low dose of $\Delta\beta$ -catenin. (A) Expansion of the *goosecoid* expression domain in early embryos. Epifluorescence image of wild-type (WT) tg *gsc::gfp* and *ccr7*^{-/-} *gsc::gfp* embryos sensitized by injection of 2.5 pg of $\Delta\beta$ catenin RNA. (B) Graph showing the area of *goosecoid* domain measured by quantifying the number of pixels with GFP signal above the auto threshold.

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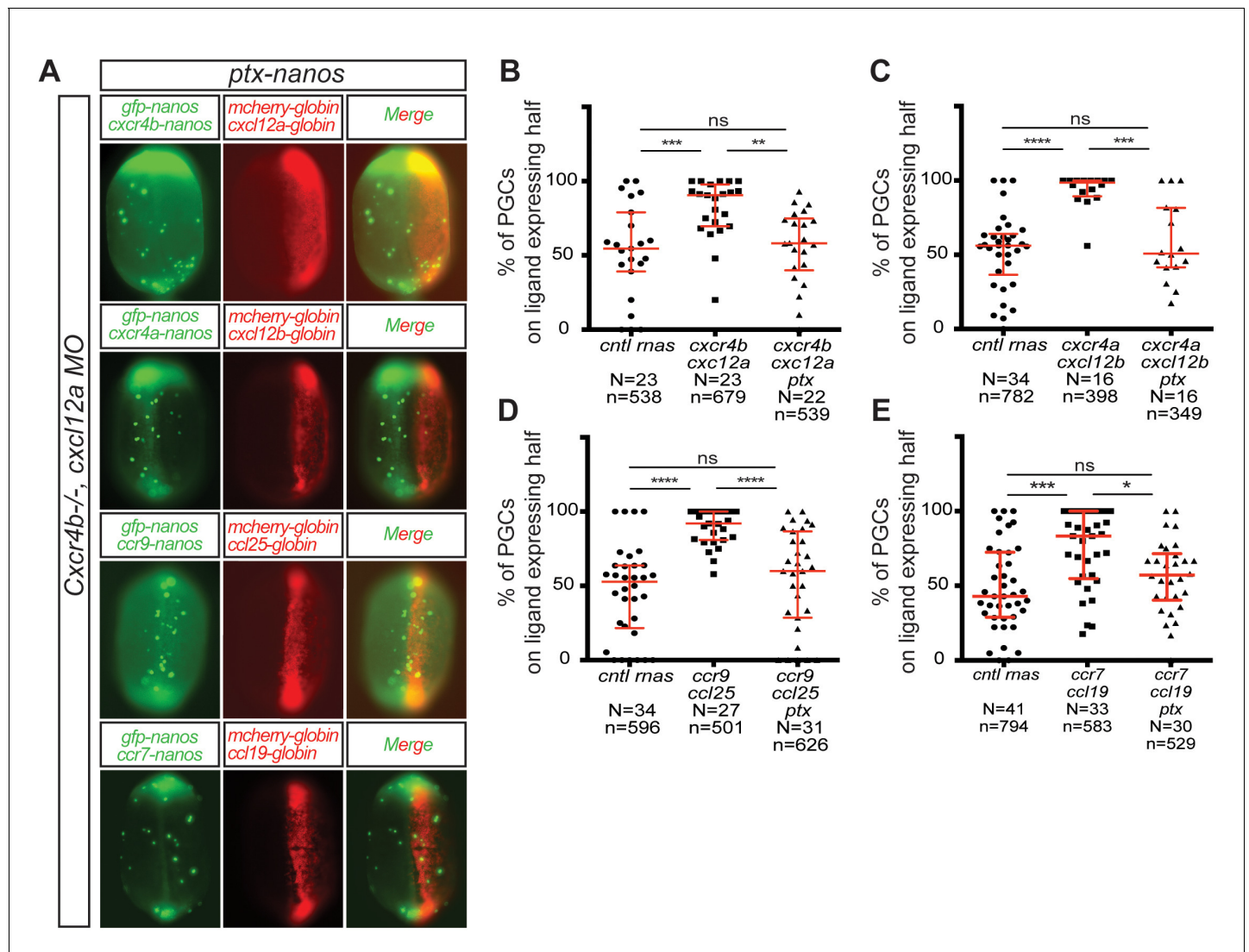


Figure 5. CC and CXC receptors can regulate the migration of primordial cells employing the same downstream signaling pathway. (A) Epifluorescence image of 10 hpf embryos expressing different ligands (Cxl12a, Cxl12b, Ccl25 and Ccl19) in one half of the embryo (mCherry-expressing cells) and PGCs (mGFP) expressing different chemokine receptors (Cxcr4b, Cxcr4a, Ccr9 and Ccr7) along with PTX. Merged images show the position of PGCs with respect to control (*cntl*), or ligand-expressing cells. (B–E) Graphs showing the quantitation of directed cell migration, by presenting the percentage of PGCs located within the ligand-expressing domains. 60 pg of *mGFP-nanos* was used to label PGCs in green and 40 pg of *m-cherry-globin* mRNA was used for labeling the ligand-expressing half of the embryo. 10 pg of PTX-encoding RNA were injected to inhibit the Gi protein. 20 pg of receptor-encoding RNA was used and 30 pg of ligand-encoding RNA was used. 0.2 pmol of cxcl12a morpholino was used. Equimolar amounts of *cntl* RNA were used. For raw data see **Figure 5—source data 1**.

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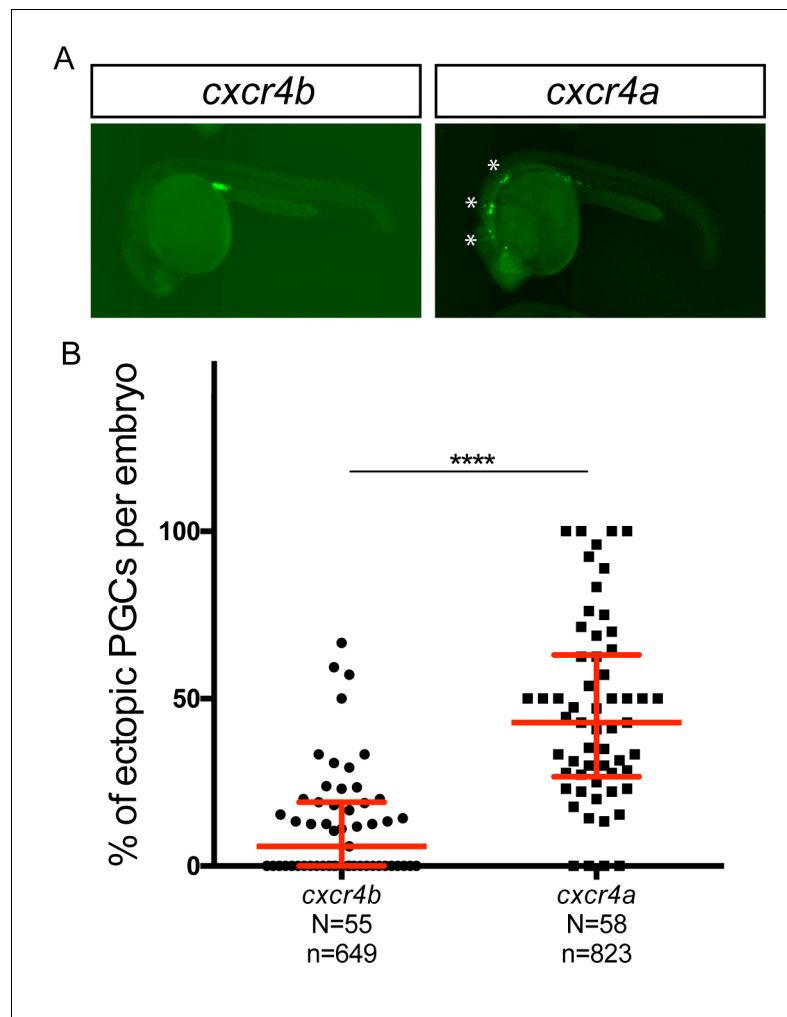


Figure 5—figure supplement 1. Overexpression of Cxcr4a in wild-type PGCs leads to increase in number of ectopic PGCs. (A) Wild-type embryos expressing Cxcr4b (left) or Cxcr4a (right) in their PGCs. (B) Graph showing percentage of ectopic PGCs per embryo. 80 pg of RNA encoding for either Cxcr4a or Cxcr4b was injected into the embryo. The inclusion of the *nanos* 3'UTR leads to preferential expression of the proteins in the PGCs. 60 pg of *mGFP-nanos* RNA was used to label PGCs in green. For raw data see Source data file **Figure 5—figure supplement 1**.

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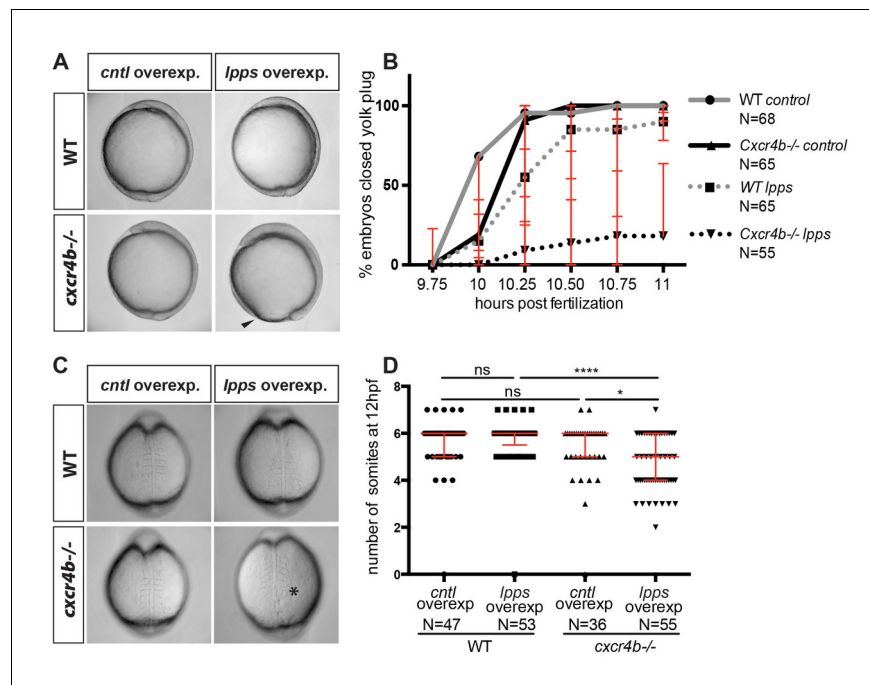


Figure 6. Functional compensation between G-protein-coupled receptors from different groups. (A) Brightfield images showing 10 hpf embryos overexpressing RNA encoding for LPPs (lpp1-varX1, lpp3-varX1) or control RNA. Wild-type (WT) embryos overexpressing Lpps and embryos lacking Cxcr4b function (*cxcr4b*^{-/-}) developed normally. Cxcr4b-deficient embryos overexpressing Lpps exhibited a delay in gastrulation and failed to close the yolk plug. (B) Graph showing the percentage of embryos with closed yolk plug between 9.75 and 11 hpf. (C) Brightfield images of embryos at 12 hpf. WT embryos overexpressing Lpps and embryos lacking Cxcr4b function (*cxcr4b*^{-/-}) developed normally and formed six somites by this stage. Embryos lacking Cxcr4b function (*cxcr4b*^{-/-}) that overexpress Lpps developed only five somites that appear abnormal. (D) Graph showing the number of somites at 12 hpf in WT and *cxcr4b*^{-/-} embryos expressing control or Lpps encoding RNA. 75 pg of RNA encoding each Lpp was injected. Equimolar amounts of control RNA were used. For raw data see **Figure 6—source data 1**. DOI: <https://doi.org/10.7554/eLife.33574.022>

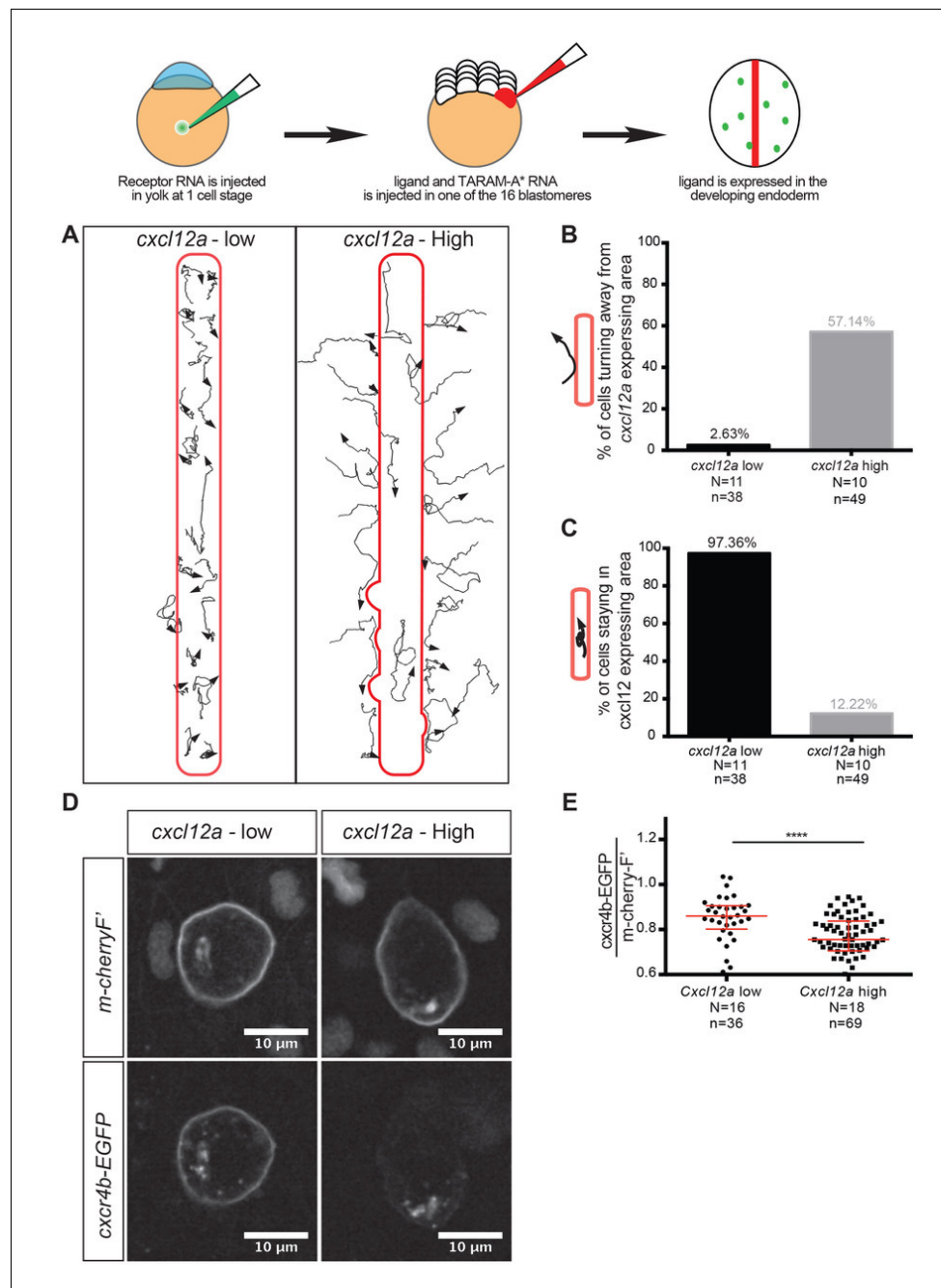


Figure 7. Regulation of reverse migration of PGCs by receptor internalization. (A) Tracks of PGCs with respect to domains in the embryo expressing low or high levels of *Cxcl12a*, in embryos lacking the endogenous ligand. PGCs migrated into domains expressing low levels of the attractant and remained within them, while the cells turned away when the domains expressed high levels of *Cxcl12a*. (B) Graph showing percentage of cells that moved away from the *Cxcl12a* expressing domains (2.6% in the case of low *Cxcl12a* expression and 57.1% in the case of high *Cxcl12a* levels). (C) Graph showing the percentage of cells that remained for 90 minutes or more within the *Cxcl12a* expressing area 97.4% in the case of the low expression of the chemokine and 12.2% in the case of high *Cxcl12a* expression). (D) High-magnification images of PGCs expressing EGFP-tagged *Cxcr4b* and farnesylated *mCherry* on their membranes. The cells interacted with low and high *Cxcl12a* expressing domains as in A. (E) Graph showing the level of *Cxcr4b* on the PGC membrane as a ratio between the EGFP signal and that of the farnesylated *mCherry* in cells exposed to low and high concentrations of the ligand. 20 pg of *cxcr4b-nanos*, 400 pg and 25 pg of *cxcl12a* RNA was injected to achieve high and low expression domains; 60 pg of *mGFP-nanos* was used to label the PGCs; 30 pg of *m-cherry-H2B* was used to label the cells expressing *Cxcl12*; and 2 pg of *TARAM-*

Figure 7 continued on next page

Figure 7 continued

A*. 101 pg of *cxcr4b*-EGFP-nanos was used in the receptor internalization assay and 60 pg of *m-cherry*-nanos was used to label the membrane of PGCs. 0.2 pmol of Cxcl12a morpholino was used. Equimolar amounts of *control* RNA were used. For raw data see **Figure 7—source data 1**.

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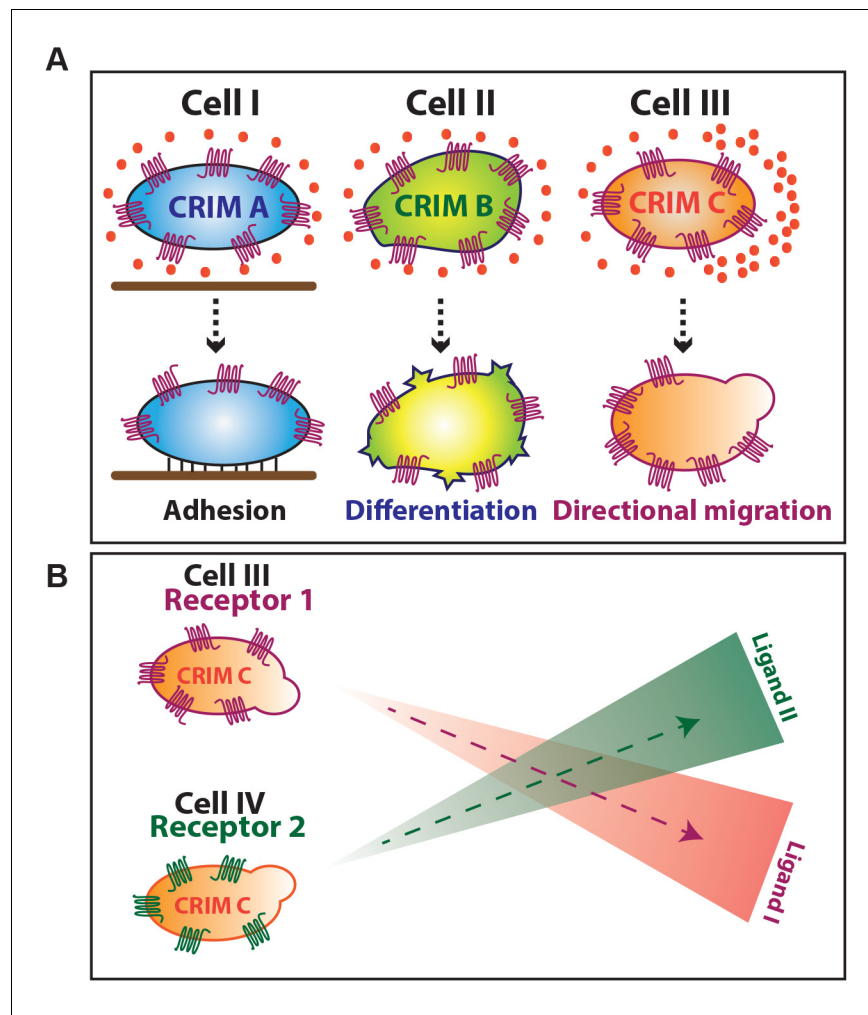


Figure 8. Graphical representation of Chemokine Receptor-signal Interpretation Module (CRIM). (A) Graphical summary of results. (B) Schematic representation of concurrent trafficking of different cell types possessing identical CRIM and expressing different chemokine receptors.

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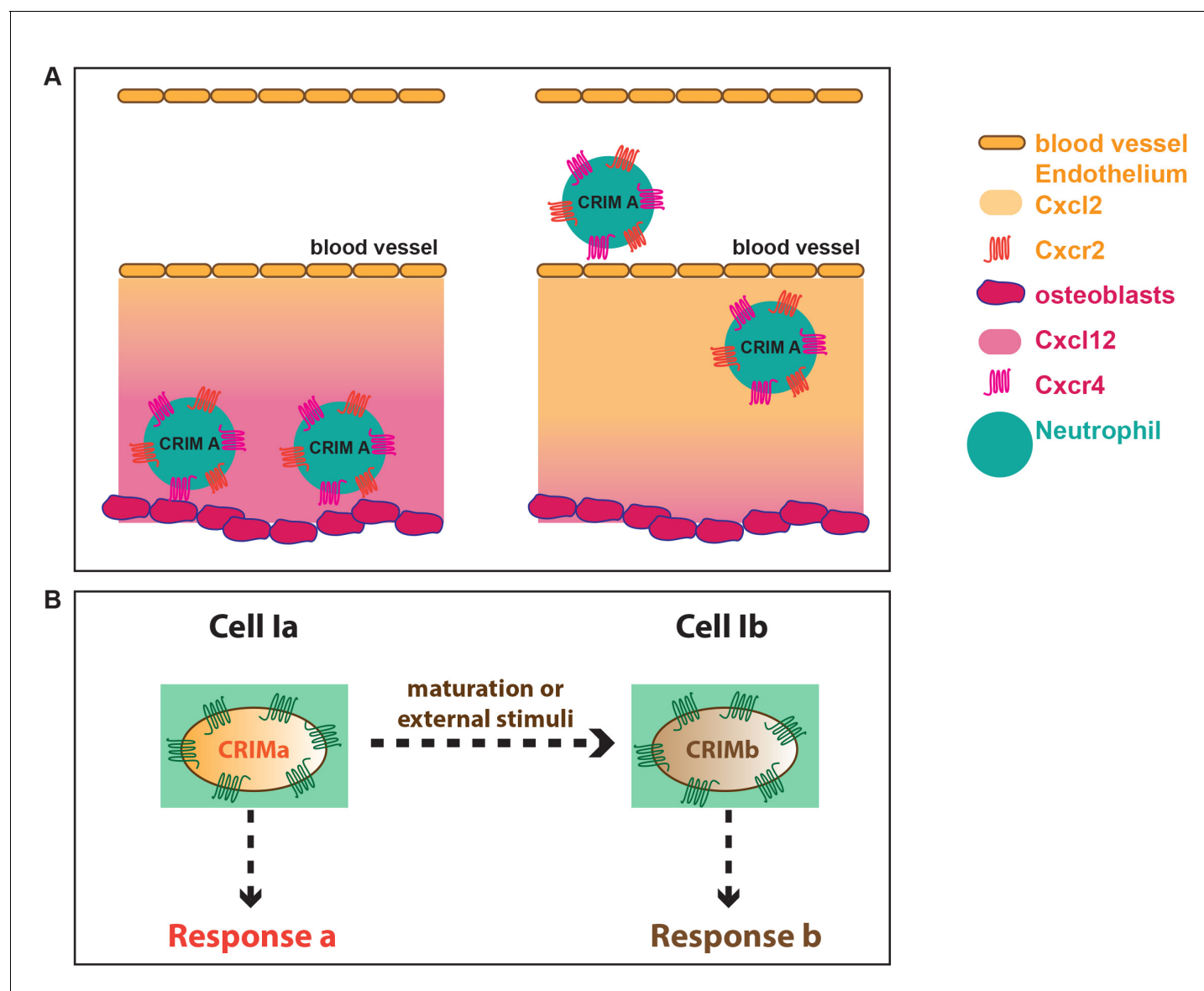


Figure 8—figure supplement 1. Graphical explanation of the effect of CRIM on cell behavior. (A) Positioning of cells by different ligands expressed in spatially distinct locations. (B) Schematic explanation of a scenario showing cells altering their CRIM following maturation.

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