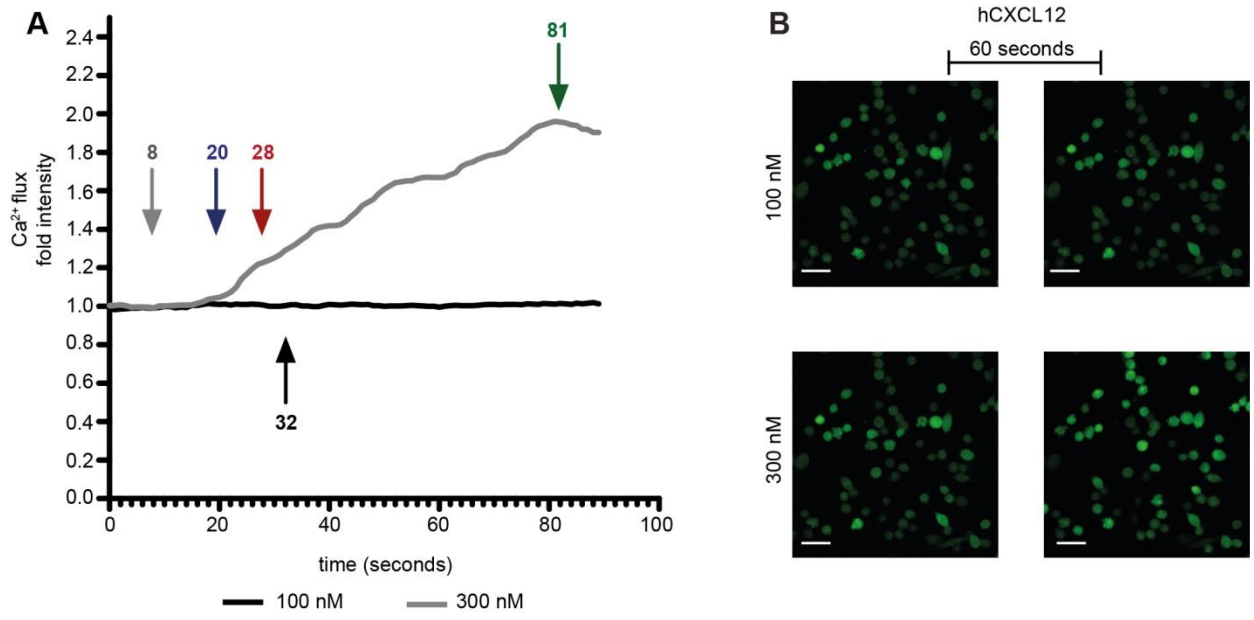
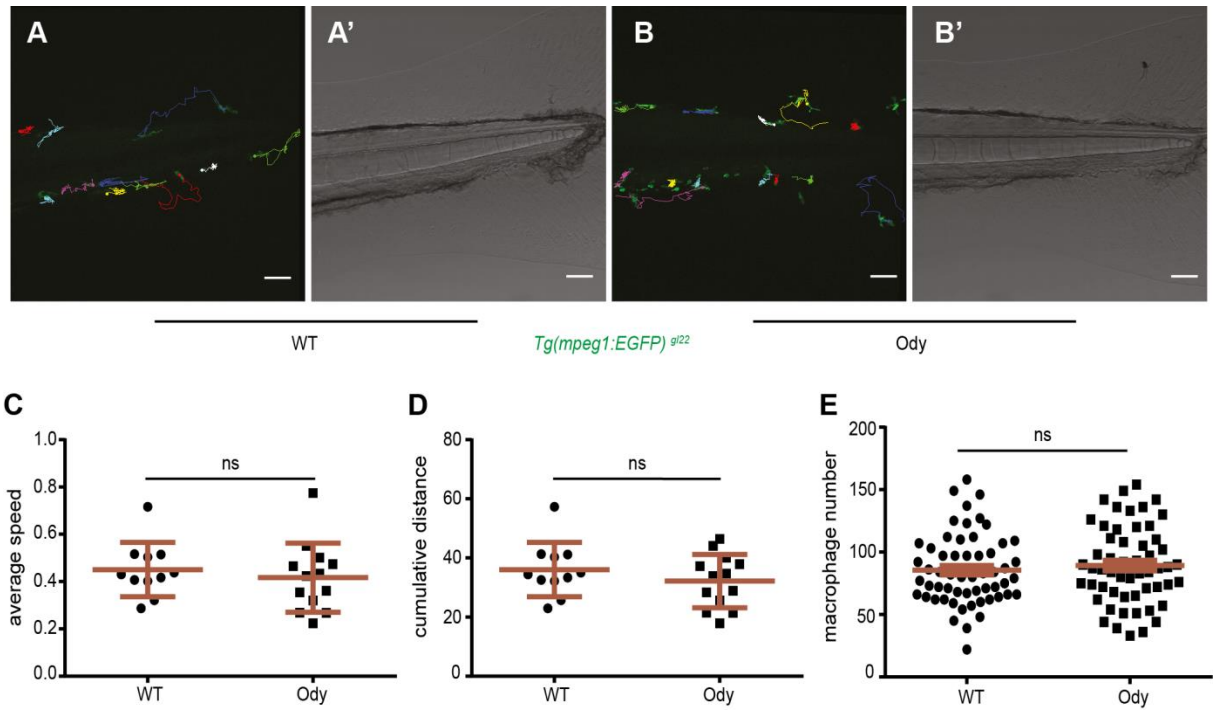
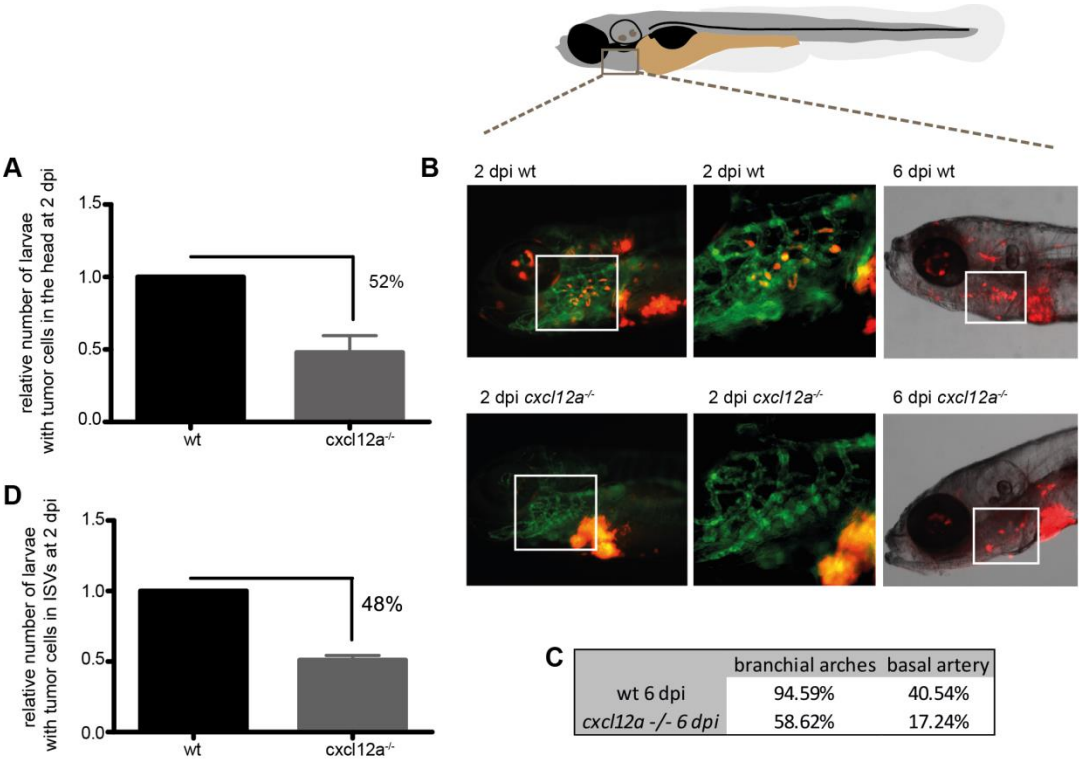


Supplementary Figure S1. Ca^{2+} efflux in the parental line MDA-MB-231. Ca^{2+} mobilization was assessed upon stimulation with 100 and 300 nM hCXCL12. (A) After addition (32^{nd} second, black arrow) of 100 nM hCXCL12, no response was detected in MDA-MB-231. The same sample was subjected to stimulation with higher concentration (300 nM). In this case, the chemokine addition (8^{th} second, grey arrow) resulted in an initiation of response (20^{th} second, blue arrow), with the highest signal intensity registered 1 minute later (81^{st} second, green arrow). The red arrow indicates the expected time of highest signal intensity (8 seconds after signal initiation), which was observed upon stimulation of MDA-MB-231-B with 100 nM hCXCL12 (Figure 2D). (B) Signal intensity at ligand addition and 60 seconds after stimulation. No difference in signal intensity was observed with 100 nM hCXCL12, while fluorescence increase was registered with 300 nM hCXCL12. Scale bar: 50 µm.

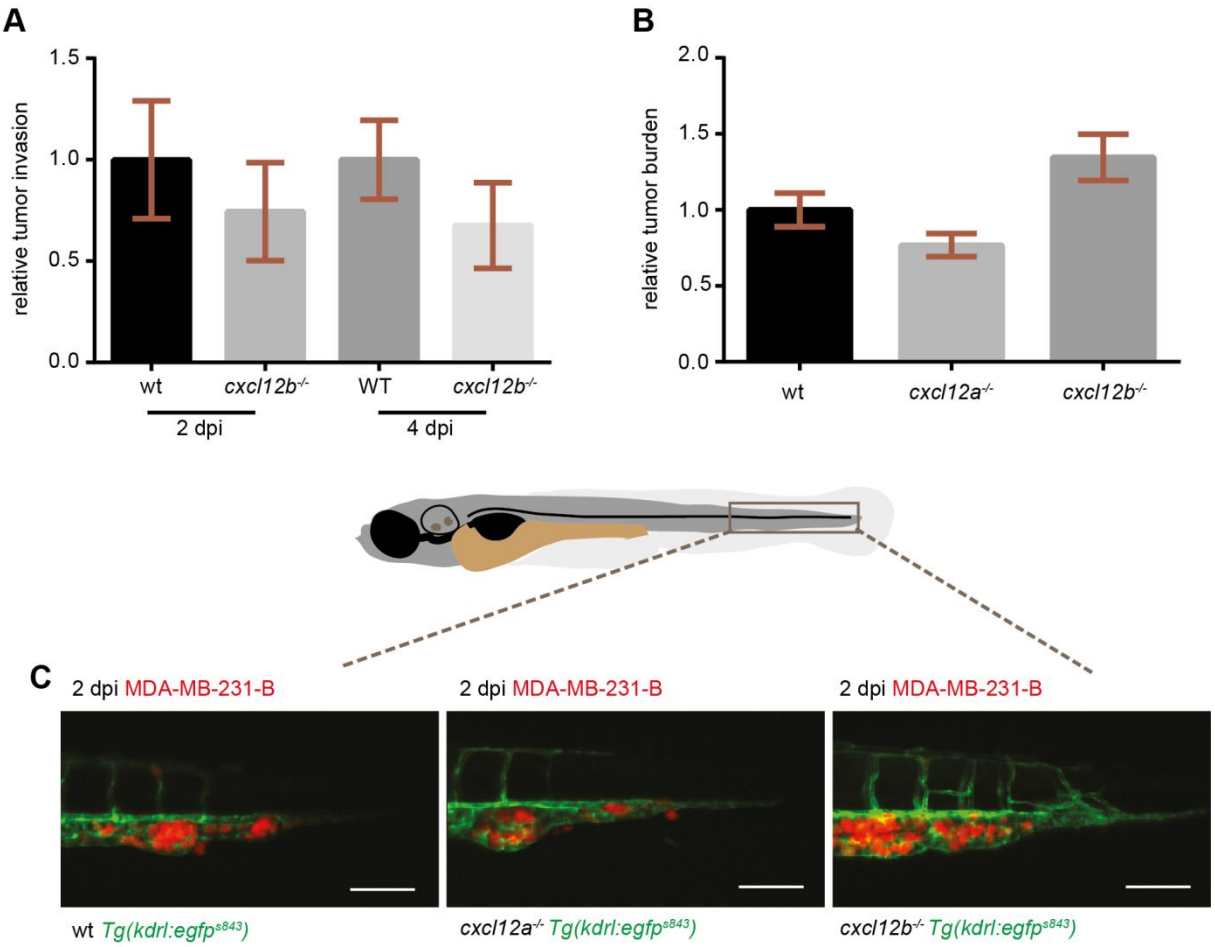


Supplementary Figure S2. Cxcr4b does not affect macrophage basal motility and total number. (A) Macrophage tracks in a wild-type *Tg(mpeg1:EGFP)* ^{gl22} sibling. (B) Macrophage tracks in a cxcr4b $^{-/-}$ *Tg(mpeg1:EGFP)* ^{gl22} sibling. (A', B') Bright field images of (A) and (B), respectively show the orientation of the tail fins. Scale bar: 50 µm. (C) Average speed and (D) cumulative distance of macrophages do not differ in WT and Ody larvae at 4 dpf. Statistical significance is assessed using unpaired t-test, ns $p > 0.05$. (E) Total number of macrophages detected by immunohistochemistry is the same in Ody and WT siblings at 2 dpf. Statistical significance is assessed using Mann-Whitney test, ns $p > 0.05$. WT: n=57, Ody: n=55.

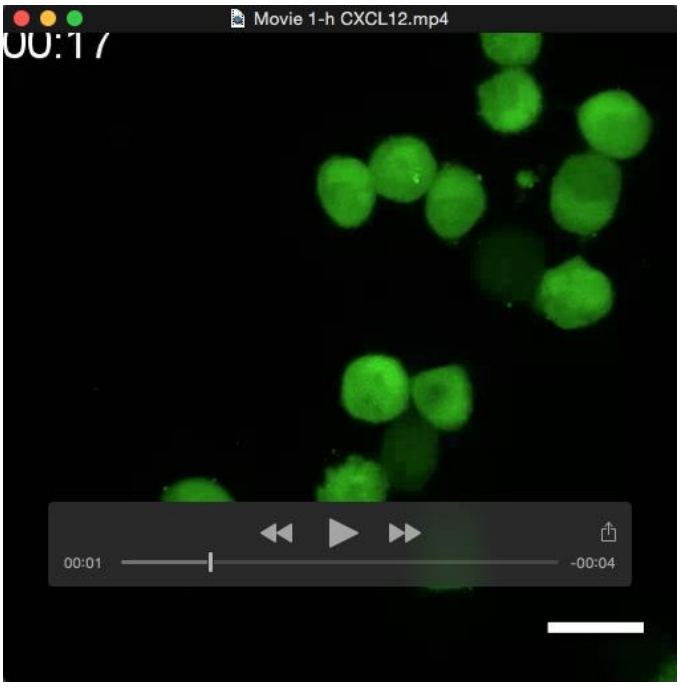


	branchial arches	basal artery
wt 6 dpi	94.59%	40.54%
cxcl12a -/- 6 dpi	58.62%	17.24%

Supplementary Figure S3. Lack of functional ligand affects MDA-MB-231-B cell positioning. (A) MDA-MB-231-B cell localization in the head region is reduced in *cxcl12a* $^{-/-}$ mutants, in particular in branchial arches (B, C) and basal artery (C). Localization in the intersegmental vessels is also diminished (D).

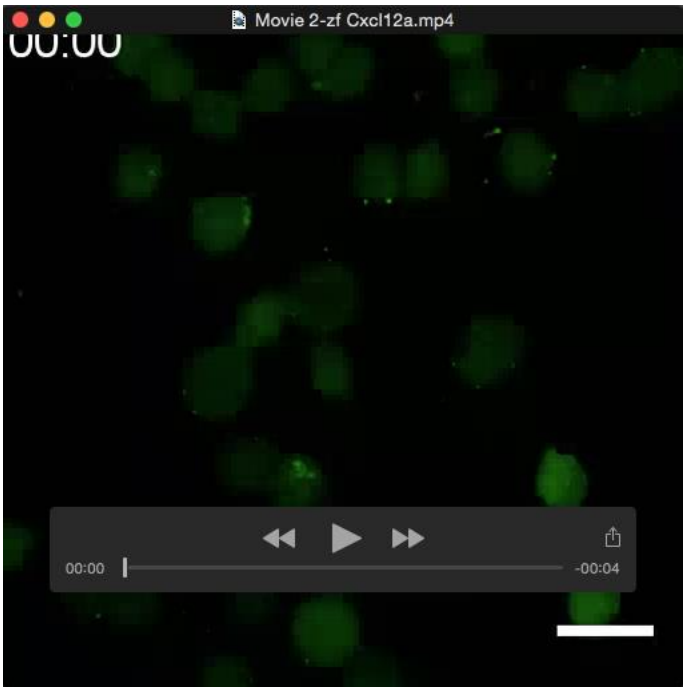


Supplementary Figure S4. Micrometastasis formation occurs in *cxcl12* single ligand mutants. Engraftment of MDA-MB-231-B cell line resulted in micrometastasis formation in the tail fin region, characterized by invading single cells in both the *cxcl12b* $^{-/-}$ mutant and wt siblings at 2 and 4 dpi (A) and tumor burden in wt, *cxcl12a* $^{-/-}$ and *cxcl12b* $^{-/-}$ larvae at 2 dpi (B and C). The trend was not statistically significant (One-way ANOVA and Bonferroni *post hoc* test). Number of larvae in (A): n=30 (wt 2 dpi), n=20 (*cxcl12b* $^{-/-}$ 2 dpi), n=19 (wt 4 dpi) and n=15 (*cxcl12b* $^{-/-}$ 4 dpi). Number of larvae in (B): n= 23 (wt), n=27 (*cxcl12a* $^{-/-}$) and n=27 (*cxcl12b* $^{-/-}$). Scale bars: 50 µm.

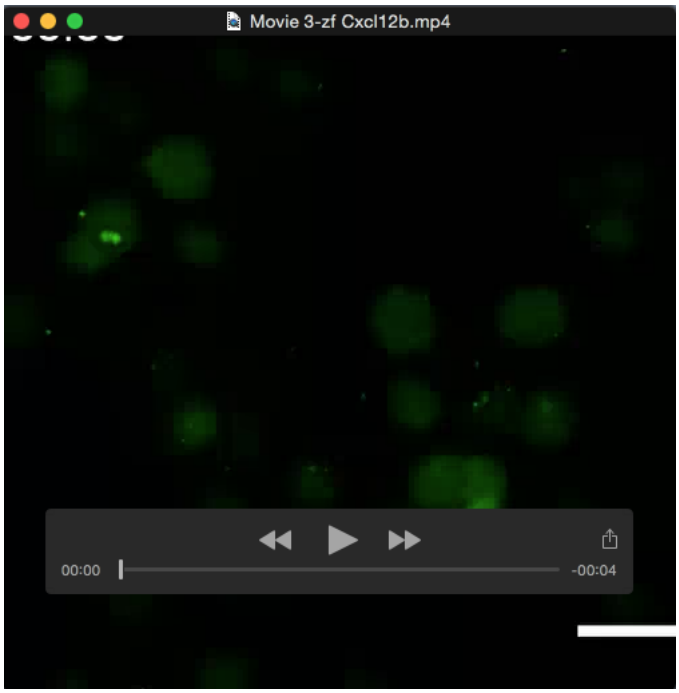


Supplementary Movie 1: Human CXCL12 induces Ca^{2+} mobilization in MDA-MB-231-B.

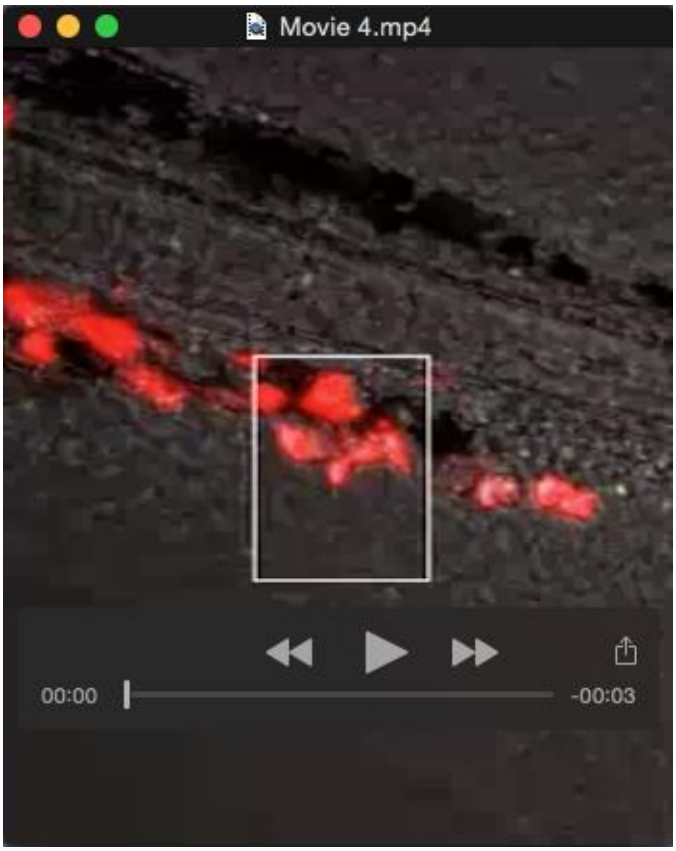
Tumor cells responded rapidly and simultaneously to ligand stimulation. Time is shown in seconds. Scale bar: 50 µm



Supplementary Movie 2: Zebrafish Cxcl12a administration results in Ca^{2+} flux in MDA-MB-231-B. The response appeared slower and not synchronized, compared to the human chemokine. Time is shown in seconds. Scale bar: 50 µm.



Supplementary Movie 3: Zebrafish Cxcl12b is able to activate Ca^{2+} release from intracellular storage in MDA-MB-231-B. The responsive pattern was comparable to the other zebrafish chemokine (Cxcl12a), therefore it was slower and not synchronized compared to human CXCL12. Time is shown in seconds. Scale bar: 50 µm



Supplementary Movie 4: Initiation of invasion via filopodium extension. MDA-MB-231-B dsRed cells localize in the CHT region in 4 dpi zebrafish embryo. The movie shows one tumor cell initiating invasion via filopodium extension in the tail fin. The area of interest is indicated. The movie has been recorded for 3 hours, with frame acquisition every minute. Time lapse has been performed with Nikon A1R confocal laser scanning microscope, using 488 and 561 lasers and a 20x lens (NA 0.75).