Supplementary Data

Experimental Procedures

Zebrafish strains

Zebrafish (*Danio rerio*) embryos obtained from transgenic fish carrying the Tg(kop:mCherry-f-UTRnanos1) 1 or Tg(kop: EGFP-f- UTRnanos1) 2 were used as wild-type fish. The Zebrafish were handled according to the law of the state of North-Rhine-Westphalia, Germany.

Embryo embedding

The embedding was performed by putting the fixed deyolked embryos in disposable plastic molds (e.g., Tissue-Tek, cat no. 62352-07) that contained 1% low melting point agarose (e.g., Invitrogen, cat no. 16520-050)

Carbon-coated gridded coverslip

The carbon-coated gridded coverslip was generated using a BAL-TEC Med-020 coating system.

Imaging setup

Fluorescence imaging was performed using a Zeiss epifluorescence microscope (Axio-Imager.Z1). For the epifluorescence imaging, samples were visualized using 5× and 20× objectives for X/Y/Z positioning of the primordial germ cells in the embryo relative to the plane of the gridded carbon-coated coverslip. The ZEISS LSM-710 confocal microscope equipped with a pulsed 850-nm chameleon laser on a two-photon setup (Coherent) was used with 63× water immersion objective, NA = 1.0 for detailed three-dimensional (3D) imaging of the cells and laser marking. The confocal images were acquired with eight times averaging, 0.8-μm z-slice and 1 AU.

Image analysis and 3D reconstruction

The analysis of the images was performed using the ImageJ 2.0 software and the 3D reconstruction was done using the Imaris 7.2 software (Bitplane).

Supplementary References

1. Tarbashevich K, Reichman-Fried M, Grimaldi C, Raz E. Chemokine-dependent ph elevation at the cell front sustains polarity in directionally migrating zebrafish germ cells. Curr Biol 2015;25:1096–1103.
2. Blaser H, Eisenbeiss S, Neumann M, Reichman-Fried M, Thisse B, Thisse C, *et al.* Transition from non-motile behaviour to directed migration during early PGC development in zebrafish. J Cell Sci 2005;118:4027–4038.