Supplementary Materials for

Using migrating cells as probes to illuminate features in live embryonic tissues

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The PDF file includes:

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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/49/eabc5546/DC1)

- Movies S1 to S11
Supplementary Methods

Landscape software
Landscape is an efficient processing pipeline that uses mathematical imaging methods for automated processing and registration of 3D image data derived from embryos. Furthermore, Landscape allows to automatically generate 2D cell distribution maps based on the registered 3D image data, which can be used for statistical analysis. The software performs 3D image registration accurately (see Fig. 1D, movie S3 and movie S11), while being fast enough to allow for a high data throughput, thus contributing to more reliable statistics. To allow for additional control Landscape presents the results of automated data processing and 3D registration for each embryo in a clear graphical user interface (GUI) that allows the user to evaluate each dataset concerning the registration results.

In the following we describe the methodology for the different stages of the processing pipeline implemented in Landscape. The proposed processing pipeline can be subdivided in preprocessing, segmentation, shape estimation, normalization, registration, and conformal mapping; each step depends on the result of the preceding stage (see Fig. 1C, fig. S1E).

Data Processing – In the first step all imaged microscopy channels are preprocessed. Our aim is to enhance the imaging contrast, which alleviates the complexity of the subsequent segmentation step.

For preprocessing the raw microscopy data, we first normalize all signal intensities to a fixed interval $[0, I_{\text{max}}]$ to guarantee that the imaged signals of different embryos are comparable. Subsequently, 3D morphological filters are employed to the normalized data. As filtering strategy, we observed that white top-hat filtering is most effective in our case. Here, one computes the morphological opening of the image data using a disk element filter and then computing the difference of the result to the original data (66), i.e.,

$$f_{\text{filtered}} = f_{\text{raw}} - d \circ f_{\text{raw}}$$  \hspace{1cm} (1)$$

where $f_{\text{raw}}$ is the normalized raw microscopy data, $d \circ$ is the morphological opening operation with filter $d$, and $f_{\text{filtered}}$ is the resulting image after morphological filtering. This morphological filtering operation removes background signals and significantly reduces the impact of image noise. In particular, we use a disk element filter as this turned out to yield the best processing results on our data. For each microscopy channel we optimized the size of the respective morphological filter with respect to the overall image quality.

Segmentation – In the second step of the processing pipeline we extract the relevant information from each microscopy channel via 3D image segmentation. Due to the signal characteristics that are induced by the imaging modality, one has to deal with photon counting noise in the image data, especially in regions with low imaging contrast. This image noise induces artifacts in the results of image segmentation if not taken properly into account. For this reason, sophisticated segmentation methods combine locally-weighted data fidelity terms with non-differentiable regularization techniques (67). Although these methods deliver the best results in terms of segmentation accuracy, their application to the acquired 3D image data is limited by the high computational complexity involved in minimizing the respective variational energy functionals. To allow for high-throughput analysis of large amounts of 3D image data, we solely considered segmentation methods that are fast to compute. The most efficient methods for segmentation are simple thresholding schemes. However, manually choosing an optimal threshold for all imaged data respectively is cumbersome and hinders the aim of an automated processing pipeline. For this reason, Landscape uses an unsupervised clustering method known as “k-means algorithm” (68) that performs 3D image segmentation by grouping each voxels signal intensity via the following energy minimization.
The overall minimization problem is then given by:

\[
\begin{align*}
\min_{\{c_i, \mu_i\} \mid i = 1, \ldots, k} \sum_{i=1}^{k} \sum_{x \in c_i} \|x - \mu_i\|^2
\end{align*}
\]  

(2)

where \( k \) is the estimated number of clusters to be found, \( C_i \) and \( \mu_i \) are the unknown clusters and their respective cluster centers (i.e., the mean value), and \( x_i \) are the signal intensities. This method minimizes the variances for each signal cluster, which can be interpreted as least squares estimator. As this method groups voxels according to their respective signal intensities without regarding any neighborhood information, one can typically observe outliers and artifacts in the resulting 3D segmentation. To remove these artifacts, we perform a subsequent postprocessing step on the segmentation result, again using morphological opening and closing operations (see section - Data Processing).

**Shape estimation and normalization** – Based on the segmented embryo nuclei, we perform a shape estimation for each imaged embryo individually. For this we assume that the overall embryo shape can be approximated by a three-dimensional ellipsoid. Given that those cells are located close to the embryo hull, we formulate an optimization problem to estimate the ellipsoid parameters that define the smallest ellipsoid enclosing those cells. In particular, our optimization scheme aims to compute the center coordinates \( c = (c_1, c_2, c_3) \) and the radii \( r = (r_1, r_2, r_3) \) associated with the ellipsoids major axes that describe the best fitting ellipsoidal shape.

Based on the implicit ellipsoid function \( f_{\text{ellipsoid}} \) an arbitrary point \( x = (x_1, x_2, x_3) \) in three-dimensional space can be classified as follows

\[
f_{\text{ellipsoid}}(x, c, r) = \frac{(x_1 - c_1)^2}{r_1^2} + \frac{(x_2 - c_2)^2}{r_2^2} + \frac{(x_3 - c_3)^2}{r_3^2} - 1 \quad \begin{cases} 
> 0, & \text{if } x \text{ is outside the ellipsoid} \\
= 0, & \text{if } x \text{ is on the boundary of the ellipsoid} \\
< 0, & \text{if } x \text{ is inside the ellipsoid}
\end{cases}
\]

(3)

With this function, we define three different terms to build up the minimization problem. To enforce the ellipsoid boundary to be close to the segmented cells we use a data fidelity term that aims to minimize the Euclidean distance between the boundary and the segmented cells' centroids \( x \) as:

\[
f_{\text{data}}(c, r) = \sum_x \left(f_{\text{ellipsoid}}(x, c, r)\right)^2
\]

(4)

We add an additional regularization term

\[
f_{\text{outliers}}(c, r) = \sum_x \max\left(0, f_{\text{ellipsoid}}(x, c, r)\right)
\]

(5)

that penalizes parts of the segmented cells lying outside of the estimated ellipsoid quadratically in distance to the ellipsoid boundary. To also take into account the possibility of outliers and segmentation artifacts we do not strictly enforce all segmented cells to lie within the estimated ellipsoid. As we are aiming to compute the minimal enclosing ellipsoid, we finally add a regularization term on the volume of the ellipsoid by penalizing large radii of the major axes via:

\[
f_{\text{radius}}(r) = r_1^2 + r_2^2 + r_3^2
\]

(6)

The overall minimization problem is then given by

\[
(\hat{c}, \hat{r}) = \arg \min_{c, r, \mu_0} (f_{\text{data}}(c, r) + \mu_1 f_{\text{outliers}}(c, r) + \mu_2 f_{\text{radius}}(r))
\]

(7)
with a global normalization factor $\mu_0 > 0$ and regularization parameters $\mu_1, \mu_2 > 0$ which we tuned on a large set of test data. For optimization we use convex approximations of the non-convex terms in the minimization functional and we implemented a conjugate gradient descent method (69) in Landscape to solve the minimization problem efficiently. After optimization of the ellipsoid parameters, we use the determined radii and center coordinates to calculate a linear transformation from the estimated ellipsoid to a reference system. In particular, we use the unit sphere as the reference system. The determined transformation matrix is used to transform the segmentation results of all microscopy channels to the new reference coordinate system resulting in normalized embryos of the same size and same shape for all data sets.

**Data registration** – After normalization of each embryo to the unit sphere all image data still has to be aligned with respect to their respective orientation. Using the signal of the reference structure, i.e., the axial mesoderm tissue in case of the zebrafish embryos (see Fig. 1B), we calculate for each embryo the correct rotation of the unit sphere in order to have all data equally oriented. To this end, we developed a novel geodesic registration algorithm that is significantly more efficient to compute than a possibly nonrigid 3D image registration of each voxel in the original image data. As we cannot expect that the segmentation of the reference structure is perfectly aligned with the surface of the unit sphere, we project it to this two-dimensional manifold first. Then, we parametrize the surface of the unit sphere using polar coordinates with a fixed discretization step width and determine points $y_i \in S^2, i = 1, ..., n$ corresponding to the segmented reference structure. We then compute the optimal great circle $\hat{G}$ that minimizes the geodesic distance with respect to the surface points $y_i$. This can be written as a geodesic least-squares regression model of the form:

$$\hat{G} = \arg\min_G \sum_{i=1}^n d_{geo}^2(y_i, G)$$

(8)

where $d_{geo}(y_i, G)$ denotes the minimum geodesic distance between a surface point $y_i \in S^2$ and the great circle $G$. We use sequential quadratic programming (70) to compute a minimizer of the optimization problem (8). Once the optimal great circle $\hat{G}$ approximating the reference structure is found, we use a reference point along $\hat{G}$, e.g., the center point of the reference structure, to rotate the unit sphere and thus register the image data accordingly.

**Conformal mapping** – In the final step of the processing pipeline all image data is normalized with respect to the unit sphere and registered. Based on a user-specified discretization step width we pool the center coordinates of the segmented cells from all registered embryo specimen in bins. Subsequently, we convolve the accumulated data with a binary spherical kernel, whose diameter corresponds to the cell width of the segmented cells. To compute statistical distribution maps from this accumulated data we first subdivide the unit sphere (and hence also the registered data) into shell layers of a user-specified thickness (e.g. see Fig. 2C). All signals within a shell layer are projected to its respective outer boundary, thus providing a number of two-dimensional manifolds that represent the accumulation of intensity signals within each shell layer. To gain a meaningful 2D representation of these signal distributions given as 3D shells, we parametrize the surface of each shell using polar coordinates with a user-specified discretization step width and compute a conformal mapping (71). This is comparable of transforming a three-dimensional globe to a two-dimensional world map. At the end of the automated processing pipeline implemented in Landscape the user obtains statistical heatmaps corresponding to the underlying empirical probability cell distribution at different depths within the reference embryo. These heatmaps allow to easily determine domains and embryonic layers showing preferentially more, or less cells within them (e.g. see Fig. 2A).
Computational Modelling of cell-barrier interaction

The cell is modelled as an ellipse with an aspect ratio of 1.5 with a number of positions $i$ on its boundary, each characterized by an $(x_i, y_i)$-coordinate with respect to the cell center. Furthermore, each position contains a certain intensity $I_{fm,i}$ of front marker and $I_{bm,i}$ back marker. To simulate the movement of the cell, the center of mass is moved stepwise. To this end, the sum is taken of all positions, multiplied by their front marker intensities:

$$(X, Y) = \left( \sum x_i I_{fm,i}, \sum y_i I_{fm,i} \right)$$

where the ratio of $\frac{Y}{X}$ determines the direction of movement and cell orientation, while the magnitude $\sqrt{X^2 + Y^2}$ determines the speed of movement. After every step, the front and back marker density is redistributed, according to the following rules:

- Positions with a high density of front (back) marker bind more front (back) marker than positions with a low density, according to a Hill equation with coefficient $n = 2$:

$$\frac{dI_{fm,i}}{dt} = \beta \frac{I_{fm,i}^n}{K^n + I_{fm,i}^n}$$

where $K$ and $\beta$ are the activation coefficient and the maximal expression level, respectively.

- A high front (back) marker density inhibits the back (front) marker:

$$\frac{dI_{bm,i}}{dt} = -\alpha I_{fm,i}$$

where $\alpha$ is a dimensionless parameter that sets the interaction strength.

- The front (back) marker can diffuse along the cell periphery

$$\frac{dI_{fm,i}}{dt} = D \frac{d^2I_{fm,i}}{dx^2}$$

where $D$ is the diffusion coefficient of front (back) marker along the cell periphery.

- The front (back) marker intensity at any position cannot be negative, and the total intensity in the entire cell is conserved

$$\sum I_{fm,i} \leq 1$$

Furthermore, as soon and as long as the center of mass of the cell is within a radius 1 of the barrier, $I_{fm,i}$ is set to zero to a certain depth within the cell. This forces the front marker to find a new equilibrium position, which in turn causes the cell to rotate and move away from the barrier.

Finally, prior to cell movement, its distribution of front and back marker is iterated without advancing the cell until an equilibrium is reached. The parameters $\alpha$, $\beta$, $K$ and $D$ differ for the front and back marker, to reflect their asymmetric distribution along the cell periphery. Simulations were performed with a home-written Python code, which is available upon request.
Fig. S1. Differences in embryo size and imaging orientation. (A) Volume of the embryo. Embryo$^{\text{Half}}$ indicates the volume imaged, i.e., 400 µm from dorsal to ventral. Average volume of Embryo$^{\text{Half}}$ is 2.98 x 10$^7$ µm$^3$, N = 8 embryos. Data are presented as boxplots; error bars show the minimum to maximum range of the data points. (B) Volume of PGCs. Average volume of a PGC is 3.7 x 10$^3$ µm$^3$, N = 3 embryos, n = 56 PGCs. Data are presented as boxplots; error bars show the minimum to maximum range of the data points. (C) Overlay of two embryos that differ in size (see movie S2). Nuclei stained with Hoechst. Scale bar, 100 µm. (D) Overlay of two embryos that differ in imaging orientation (see movie S2) as determined by different position of the gsc:GFP expressing tissue. Scale bar, 100 µm. (E) The Landscape analysis framework. The nuclei, landmark and PGCs are segmented. The information from the nuclei channel is then used to estimate an ellipsoid shape; this ellipsoid is fitted into a sphere and all the data are transferred to a virtual, spherical embryo. Next, the landmark’s position is estimated and corrected and this correction is applied to the PGC positions (see corresponding method section – Landscape software for more details).
Fig. S2. Distribution of PGCs within germ layer derivatives. (A) Whole-embryo distribution heatmaps of nPGCs before (non-registered) and after registration (registered). The abundance value in the scale represents the proportion of all registered cells. N = 934 embryos, n = 21520 nPGCs. (B) Distribution maps of nPGCs in control and in cxcr4a KD embryos. Cxcr4a was knocked down with morpholino treatment. No differences in nPGC distribution are visible between the control and cxcr4a KD treatment. The abundance value in the scale represents the proportion of all registered cells. Control, N = 291 embryos, n = 6174 cells; cxcr4a KD, N = 294 embryos, n = 6255 cells. (C) The workflow for the generation of the germ layer-specific PGC distribution heatmaps. The signal pattern of RNA expressed within specific germ layers is segmented and registered onto a virtual embryo (as in Fig. 1C). Then, a mask is generated and overlaid on top of the virtual embryo containing PGC positions. The PGCs within the mask volume are later unfolded and projected (as in Fig. 1C). (D) Domains of RNA expression within the ectoderm (sox2 and tp63 expression domains), mesoderm (ntl, pcdh8 and pax8 expression domains) and the endoderm (sox17 expression domain) tissues (left panel, RNAscope). Scalebar, 70 µm. Segmented and 2D-projected germ layer area (middle panel). Germ layer-specific PGC distribution heatmaps for wild-type and cxcr4b mutant embryos (right panels). For the construction of the heatmaps the data presented in Fig. 1E (wild type) and Fig. 2B (cxcr4b mutant) were used. The dashed colored outlines represent germ layer masks used for the generation of the heatmaps.
Fig. S3. Validation of morpholino-induced noto knockdown. (A) Expression pattern of \textit{gsc}:GFP transgene in 10 hpf wild-type (left panel), \textit{noto} \textsuperscript{−−} (middle) and morpholino-induced \textit{noto} KD embryos (right). Nuclei stained with Hoechst. Scale bar, 100 µm. (B) Expression pattern of \textit{notail} (RNA) in 10 hpf wild-type (left panel), \textit{noto} \textsuperscript{−−} (middle) and morpholino-induced \textit{noto} KD embryos (right). (C) Notochord phenotype and \textit{gsc}:GFP expression in 24 hpf wild-type, \textit{noto} \textsuperscript{−−} and \textit{noto} KD embryos. Dashed boxes indicate the magnified region (right panels). Asterisks indicate notochord position. Scale bars, 100 µm (low magnification) and 20 µm (magnified panels). (D) RNAscope images of wild-type and \textit{noto} mutant embryos visualizing the expression of the RNA, encoding for the chemokine \textit{cxcl12a} (magenta) and the expression of the \textit{nanos} RNA as a marker of PGCs (white). Dashed boxes indicate the magnified regions. Scale bars, 150 µm.
**Fig. S4.** nPGC behavior upon interaction with the cell migration barrier. (A) nPGC approaching the notochord at a shallow impact angle (20-40°) migrate along it (see movie S7). Scale bar, 10 µm. (B) k-Means clustering analysis reveals three groups of cells that are characterized by their impact angle-dependent cell behaviors, i.e., shallow (cluster mean [40.1°, 0°]), intermediate (cluster mean [55.9°, 54.7°]) and steep (cluster mean [82.9°, 45.3°]). Angle of deflection was plotted against the angle of impact. N = 40 embryos, n = 47 PGCs. (C) The angle of deflection of cells interacting with the barrier at an intermediate angle (blue) and steep angle (orange). Intermediate, N = 24 embryos, n = 26 nPGCs; steep, N = 14 embryos, n = 13 nPGCs. The statistical significance was evaluated using the Mann–Whitney U-test (two-tailed, CI = 95%), p = 0.0001 (****). (D) The distribution along the front-rear cell axis and size of blebs in migrating nPGCs. The relative bleb size is the ratio between the bleb perimeter and the cell circumference. The rear of the cell (180 - 135° with respect to the front) is depicted in blue, the sides (135 - 45°) in grey and the front (45 - 0°) in orange. 65 blebs derived from 10 cells from 9 embryos were considered. (E) A minimal computational model that assumes distribution of units that confer front-rear cell identity, inhibition of front identity by the rear and inhibition of front identity by interaction with a barrier is sufficient for recapitulating the experimental observations. Front identity is manifested by protrusion formation and forward migration. Accordingly, in polarized cells the activity of the units is higher at the cell front and lower at the rear (upper panel). The graph presents the similarity between the behavior of the cells observed experimentally and the computed behavior. See movie S10 for simulated cell behavior in the case of shallow, intermediate and steep impact with the barrier. (F) Expression of mRNAs encoding for mechanosensitive ion channels (*trmp4a, trpm7, trpv4, piezo1, piezo2a2*), *odc* (a ubiquitously-expressed mRNA) and *nanos* (a germ-cell specific RNA) as assayed by RT-PCR on cDNA from PGCs.
A

nuclei | nPGCs

Anterior
Left → Right
Posterior

Relative cell density

1

nPGCs

Left

1

nuclei

0.0

0.5

0.0

0.5

0.2

0.4

0.6

0.8

1

2

Anterior

Posterior

nPGCs

nuclei

0.0

0.5

B

Brillouin shift

3 dpf embryo

Brillouin shift [GHz]

Distance [µm]

C

 tg(gsc:GFP) | anti-Laminin | merged

Wild type

nato

政协

nato

Wild type

nato

Prechordal plate

nato

nato

nato

Merger
Fig. S5. Biophysical characterization of the developing notochord region. (A) Nuclei and nPGC distribution maps at the level of the notochord tissue (left panels). Line plots show the density of nuclei (and thus of cells) and of nPGCs along the mediolateral (selected area 1) and anteroposterior (selected area 2) axis (upper right panels). Correlation tests between the distribution of nuclei and nPGCs for both areas employing a linear regression analysis (lower right panels). (B) Previous Brillouin studies revealed that the ECM surrounding the notochord shows increased Brillouin shift in 3 days post fertilization (dpf) embryos (46). To demonstrate that our measurement setup can detect such a change, we acquired Brillouin shift maps in the XZ plane of the notochord in a 3 dpf wild-type embryo. The image on the left shows the Brillouin shift close to the posterior end of the notochord. The shift across the middle of the notochord (N) is plotted on the right graph. The arrows point towards the increased shift at the border of the notochord. Importantly, as shown in (46), such an increased shift is symmetrical around the notochord and is visible only on the dorsal (D) and ventral (V) side of the notochord. Scale bar, 20 µm. (C) Representative confocal microscope images of immunofluorescent staining for pan-Laminin in wild-type and noto−/− embryos at 10 hpf. Embryos are dorsally oriented with anterior to the top. Arrowheads indicate notochord border. Scale bar, 20 µm.
A

\[ \text{tg}(gsc:GFP) \quad \text{Brillouin shift} \]

B

timelapse analysis

\[ \begin{align*}
\text{region A} & \quad \text{speed [\mu m/min]} \\
\text{region B} & \quad \text{straightness index}
\end{align*} \]
Fig. S6. Analysis of the anterior nPGC-depleted embryonic regions. (A) Measurement of the Brillouin shift was acquired in the XZ planes at the border of the prechordal plate tissue (see schematics). Left panels show images of GFP signal and Brillouin shift in a representative embryo. Right graph shows the line plot of Brillouin shift, revealing no distinct change in Brillouin shift at the border of the prechordal plate. Green dashed lines label the prechordal plate border, as estimated by the GFP signal. Due to time acquisition difference, GFP signal does not perfectly overlap with the measured tissue (see corresponding method section). Scale bar, 20 µm. (B) To obtain insight into the behavior of nPGCs within the anterior regions lateral to the head (region A), we performed time-lapse imaging between 9.5 and 10.5 hpf. The nPGCs moving within region A were compared with nPGCs migrating in the posterior part of the embryo at the corresponding mediolateral longitude (region B). The plots on the right side present the comparison between speed and track straightness of nPGCs moving within these two regions. nPGCs that entered region A were able to move within it, with dynamic parameters similar to those nPGCs migrating within region B exhibited. N = 18 embryos, n = 27 cells for each group. The bars in plots show the mean ± the standard deviation. The statistical significance was evaluated using t-test (two-tailed, CI = 95%).
**Fig. S7. 3D registration of Drosophila embryos by Landscape.** (A) 3D image registration of *Drosophila* embryos by Landscape. Two images of the same embryo were acquired at different orientations, then the images were overlaid directly (no 3D image registration) and after correction 3D image registration by the software. The white area represents overlap of the signals between two embryo orientations (see movie S11). Scale bar, 50 µm. (B) Distribution of glial cell nuclei in a single *Drosophila* embryo (stage 14). Left panel shows the glial cell nuclei (Anti-Repo antibody, red). Embryo is ventrally oriented with anterior to the top. Scale bar, 50 µm. The right panel shows the resulting heatmap of glial cell nuclei distribution. Scaling shows proportion of all registered glial cell nuclei. (C) Quantitative heatmap of Repo-positive glial cell nuclei in *Drosophila* embryo (stage 14). 15 *Drosophila* embryos were used to generate a heatmap of glial cell nuclei positioning. Scaling shows abundance of glial cell nuclei distribution. (D) Representative image of a control *Drosophila* embryo (stage 15) used for the analysis of the tracheal branching by Landscape. Nuclei, required for the shape estimation, were labeled by DAPI staining (blue). As a landmark for proper 3D image registration and alignment of multiple datasets, neural tissue was visualized using Anti-HRP antibody (green). The tracheal branches were labeled using chitin staining (red). Right panel shows the overlay of all microscopy channels. The data presents the control embryo shown in Fig. 6B. Embryo is ventrally oriented with anterior pointing up. Scale bar, 50 µm.
Table S1. Distribution of PGCs within the different germ layers. The table presents the percentage of the germ cells that populate specific germ layer tissues in wild-type and in cxcr4b<sup>−/−</sup> embryos (first two columns) and the percentage of the embryo volume occupied by these tissues (third column). This data was used in the graph in Fig. 2C.

| germ layer derivatives | wild type | cxcr4b<sup>−/−</sup> | germ layer volume |
|------------------------|-----------|---------------------|------------------|
| ectoderm               | 9.78%     | 37.09%              | 49.45%           |
| mesoderm               | 32.30%    | 34.39%              | 32.43%           |
| endoderm               | 57.20%    | 15.96%              | 15.99%           |
Legends for movies

Movie S1. Distribution and motility of PGCs in wild-type embryos and in embryos lacking the Cxcr4b receptor. Time-lapse movies of PGCs in wild-type and cocr4b−/− embryos at 10 hpf. PGCs are labeled by Tg(kop:mCherry-F-3'nos) (red) and all nuclei (blue) are labeled by injection of h2a-tagBFP RNA. Movies optimized for contrast and processed with median filter using the Fiji software. Embryos are oriented dorsally, with anterior to the top. Frames captured at 1-min intervals. Scale bar, 100 µm.

Movie S2. Differences in embryo size and precise imaging orientation. Overlay of two different embryos that differ in size and imaging orientation. Nuclei stained with Hoechst (left). Landmark labeled by the gsc:GFP transgene (right). Embryos are oriented dorsally, with anterior to the top. The movie shows the data presented in fig. S1, C and D. Scale bar, 100 µm.

Movie S3. Zebrafish embryos registration by the Landscape software. The same zebrafish embryo was imaged at two orientations (original and rotation) and a computationally reduced version of it (size reduction) were tested for registration by Landscape. The nuclei (blue, orange) were stained with Hoechst and the landmark (green, magenta) was labeled by the gsc:GFP transgene. nPGCs (red, cyan) are labeled by Tg(kop:mCherry-F-3'nos). Embryos are dorsally oriented with anterior to the top. The movie shows the data presented in Fig. 1D. Scale bar, 100 µm.

Movie S4. Emergence of nPGC distribution patterns within the zebrafish embryo. Construction of distribution heatmaps made of different number of nPGCs, employing a random cell selection from the 21520 cells imaged (see also Fig. 2A). As the number of cells increases, more areas that nPGCs colonize to different extents become visible. The abundance value in the scale represents the proportion of all registered cells within each frame.

Movie S5. nPGC behavior at the midline in control embryos. nPGCs do not cross the embryo midline. Somatic nuclei (blue) labeled with h2a-tagBFP RNA, PGCs (red) by Tg(kop:mCherry-F-3’nos) and the landmark (green) labeled by the gsc:GFP transgene. Movie optimized for contrast and processed with median filter using the Imaris software. Embryo is dorsally oriented with anterior to the top. The movie shows the data presented in Fig. 4A. Cell of interest labeled with an arrowhead. Scale bar, 100 µm.

Movie S6. nPGC behavior at the midline in notochord-deficient embryos. nPGCs cross the midline of an embryo in which notochord development is inhibited (cxcr4b−/−, noto KD). Somatic nuclei (blue) are labeled with h2a-tagBFP RNA, PGCs (red) are labeled by Tg(kop:mCherry-F-3’nos) and the landmark (green) is labeled by the gsc:GFP transgene. Movie optimized for contrast and processed with median filter using the Imaris software. The movie shows the data presented in Fig. 4A. Cells of interest labeled with arrowheads. Scale bar, 100 µm.

Movie S7. nPGC approaching the notochord at a shallow impact angle. An nPGC approaching the notochord tissue at a shallow impact angle (20°). Actin labeled with Lifeact (red at the cell front), the cell membrane with EGFP-F (green) and the notochord by the gsc:GFP transgene. Time 0 is the time the cell interacts with the barrier. Frames captured at 1-min intervals. The movie shows data presented in fig. S4A. Scale bar, 10 µm.
**Movie S8. nPGC approaching the notochord at an intermediate impact angle.** An nPGC approaching the notochord tissue at an intermediate impact angle (55°). Actin labeled with Lifeact (red at the cell front), the cell membrane with EGFP-F (green) and the notochord by the gsc:GFP transgene. Time 0 is the time the cell interacts with the barrier. Frames captured at 1-min intervals. The movie shows data presented in Fig. 5A. Scale bar, 10 µm.

**Movie S9. nPGC approaching the notochord at a steep impact angle.** An nPGC approaching the notochord tissue at a steep impact angle (85°). Actin labeled with Lifeact (red at the cell front), the cell membrane with EGFP-F (green) and the notochord by the gsc:GFP transgene. Time 0 is the time the cell interacts with the barrier. Frames captured at 1-min intervals. The movie shows data presented in Fig. 5A. Scale bar, 10 µm.

**Movie S10. Computational modeling of the interaction of migrating cells with physical barriers.** Upon interaction of the cell with the barrier at a shallow angle, only few polarity units on the side of the cell form contact with the barrier. Such events do not strongly change the direction of migration, as the unaffected front units maintain the migration of the cells in a similar trajectory along the barrier (shallow scenario). In cells that interact with the barrier at an intermediate angle, the polarity units located at the front and side of the cell become less active. In this case, the unaffected units on the side opposite to the barrier direct the movement of the cell away from it (intermediate scenario). In cells approaching the barrier with a steep angle, almost all polarity units located at the cell front are affected. Averaging the strength of the remaining polarity units result in a migration direction that is shallower than that observed in the intermediate scenario (steep scenario). See also fig. 4E and corresponding method section for more details.

**Movie S11. Drosophila embryos registration by the Landscape software.** Demonstration of 3D image registration of two images of the same Drosophila embryo (original and rotated position). Nuclei of the embryo (Hoechst staining, blue). Landmark (green, magenta) labeled by Phalloidin staining. Anti-Repo antibody labels glial cell nuclei. Embryos are ventrally oriented with anterior to the top. The movie shows the data presented in fig. S7A. Scale bar, 50 µm.
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