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## **Supplemental Information**

## *In Vivo* Quantitative Imaging Provides Insights

## into Trunk Neural Crest Migration

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**Figure S1. Virally infected cells express the neural crest marker HNK-1. Related to Figure 1.** After time-lapse imaging (Figure 1D-F), the tissue slice (A, mCherry, red) was stained with anti-HNK-1 antibody (B, cyan), which recognizes migrating neural crest cells. C is the merged image. Scale bar: 50 µm



Figure S2. Neural crests targeted by plasmid electroporation display biased random walk. Related to Figure 2. (A) One selected time frame of a time-lapse movie on a tissue slice expressing Sox10E1-GFP (green), NC2-mCherry (red), PGK-NLS-iRFP (magenta). (B) Migrating cells were spot-segmented with the white dots representing their centers at t=0 and the corresponding cellular trajectories were color-coded according to time. (C-E) Track displacement lengths over time along x (C), y (D) and z (E) axes with each line standing for the trajectory of one cell (n = 22). (F-H) Total track displacement length as a function of cell initial position along x (F), y (G) z (H) axes with each dot standing for one cell. The random distribution ( $R^2 = 0.0356$ ) along the y axis (G) suggests a lack of correlation. (I) Averaged MSD curve of the migrating cells expressing H2B-GFP-T2A-mCherry (Figure 2) showed that cell motion was generally directed ( $\alpha > 1$ ). (J) The 3D distance of sister cells expressing H2B-GFP-T2A-mCherry (Figure 2) by the end of imaging were measured and the mean value was plotted. Scale bars: 15 µm



**Figure S3. Cell-cell contact-repulsion and adhesion force inside inter-cellular bridges. Related to Figure 5.** Cells virally expressed membrane-YFP (green). (A) Representative snapshots of contact-repulsion. As the filopodia touched each other, it collapsed and the cells moved away from each other. The signal intensity was adjusted from the corresponding movie to present cell morphologies more clearly. (B) Laser ablation experiments suggest adhesion in the inter-cellular bridge during contact-attraction. Representative snapshots showing that after laser ablation (red box), the two cells were detached to each other, suggesting strong adhesion force between cells. The signal intensity was adjusted from the corresponding movie to present cell morphologies more clearly. Scale bars: 5 µm. Also see Video S9.