Table S1: Distributions of motility coefficients of taste bud cells. See also Fig. **S2**. Shapiro-Wilk test was performed to assess normal distribution of the coefficients. Normality was rejected when p-value<0.05.

Click here to Download Table S1

Table S2: Comparison of the distributions of motility coefficients of taste bud cells. See also Fig. **S2**. If the distributions of both parameters were not deviating from normality, the Welch t-test was used. Otherwise the non-parametric two sample Kolmogorov-Smirnov test was applied.

Click here to Download Table S2

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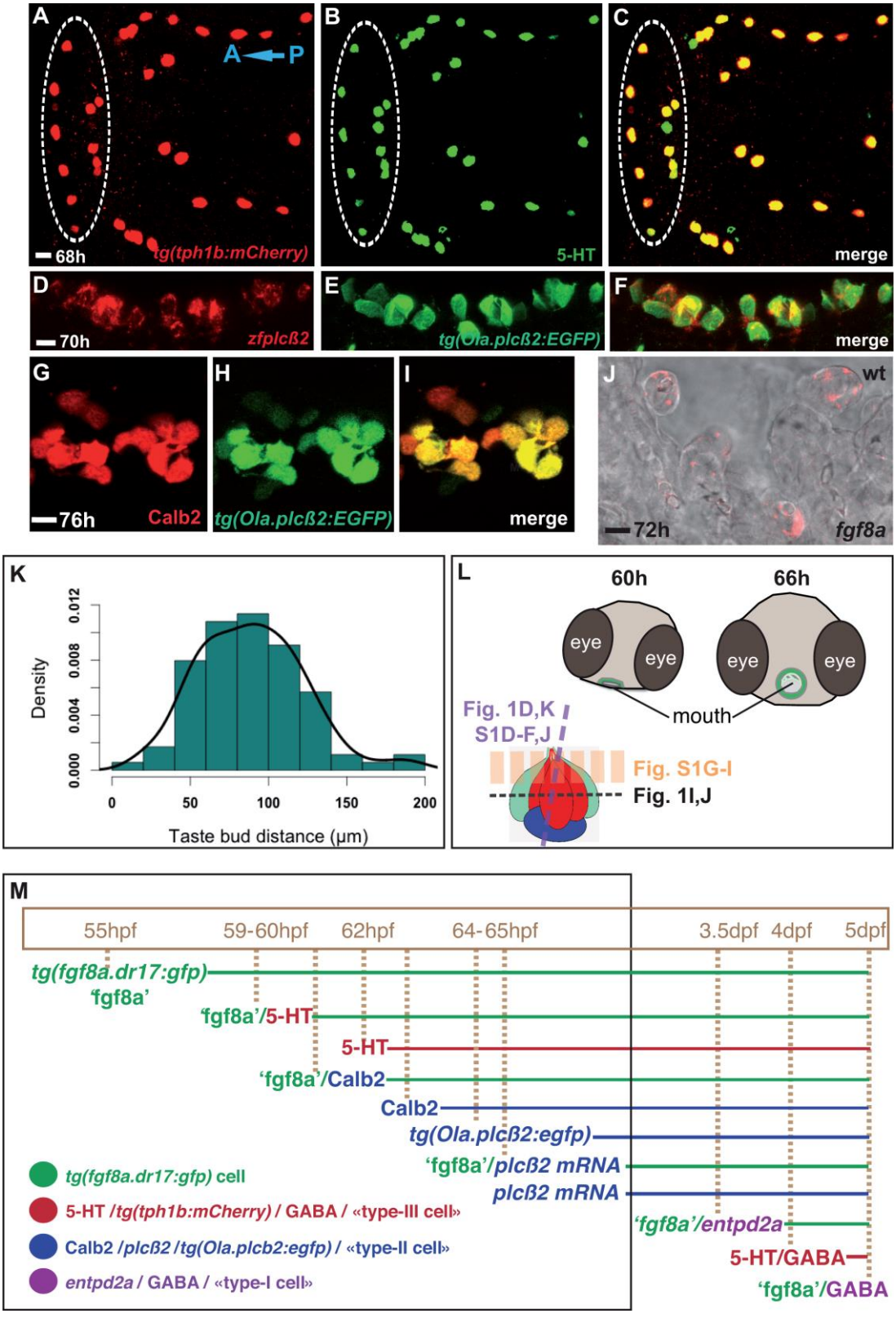

Fig. S1. Expression patterns of taste bud markers used in this study. A-C: Expression of *tg(tph1b:mCherry)* in the developing oropharyngeal 5-HT cells. Anterior to the left. 1µm confocal optic section showing cells co-expressing *tg(tph1b:mCherry)* and 5-HT in the oropharynx of an embryo processed for immunohistochemistry with anti-mCherry and anti-5-HT antibodies. Within the white dotted-line oval are located the 5-HT cells of the mouth; otherwise, 5-HT cells located on anterior pharyngeal arches. **D-I:**

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Expression of zebrafish *plcß2* and Calb2 in taste bud *tg(Ola.plcß2:egfp)* expressing cells. Anterior to the top. 5µm confocal optic sections in the mouth area, showing cells co-expressing *tg(Ola.plcß2:egfp)* (**E,H,** green, anti-GFP) and *plcß2* mRNA (**D,** red, cRNA probe) or Calb2 (**G,** red, anti-Calb2), in the oropharynx of two zebrafish larvae. **J: *fgf8a* mRNA is expressed in taste bud cells.** To validate the *tg(fgf8a.dr17:gfp)* line as a tool for taste bud development, we checked whether *fgf8a* mRNA expression persists in developing taste bud cells and not only expressed in the oropharyngeal epithelium as previously shown (Kapsimali et al., 2011). 2µm optic section showing *fgf8a* mRNA (fast red fluorescent signal) in taste-bud cells detected by transmission light. **K: Normal distribution of the distance (µm) between neighbouring taste buds in the wild-type mouth epithelium at 5dpf.** Mean value=89µm, sd=34µm, n=87 pairs from 9 larvae. Shapiro-Wilk normality test : W = 0.9809, p-value = 0.2217. **L:** Top: Cartoon showing the changing position of the mouth during the developmental period we performed imaging. The mouth is displaced from ventral (shown in the slightly oblique larval head schema at 60hpf) to dorsal and anterior position. The epithelium surrounding the mouth opening is coloured in green and we imaged the anterior part of it. Bottom: Schema of a forming taste bud. The dotted line shows the levels of z-optical sections in Fig.1 and S1. **M: Cartoon showing the (co-) expression of taste bud cell markers during developmental timing (hours or days post fertilization) in the zebrafish anterior mouth.** This study focused on tools required to observe early differentiating (*tg(fgf8a.dr17:gfp)* referred also as 'fgf8a' for clarity, 5-HT (Type-III), or Type-II (Calb2, *plcb2*) cells (black-lined rectangle). Markers for the same cell type, are shown in the same colour. The coloured lines indicate expression throughout this developmental time-period.

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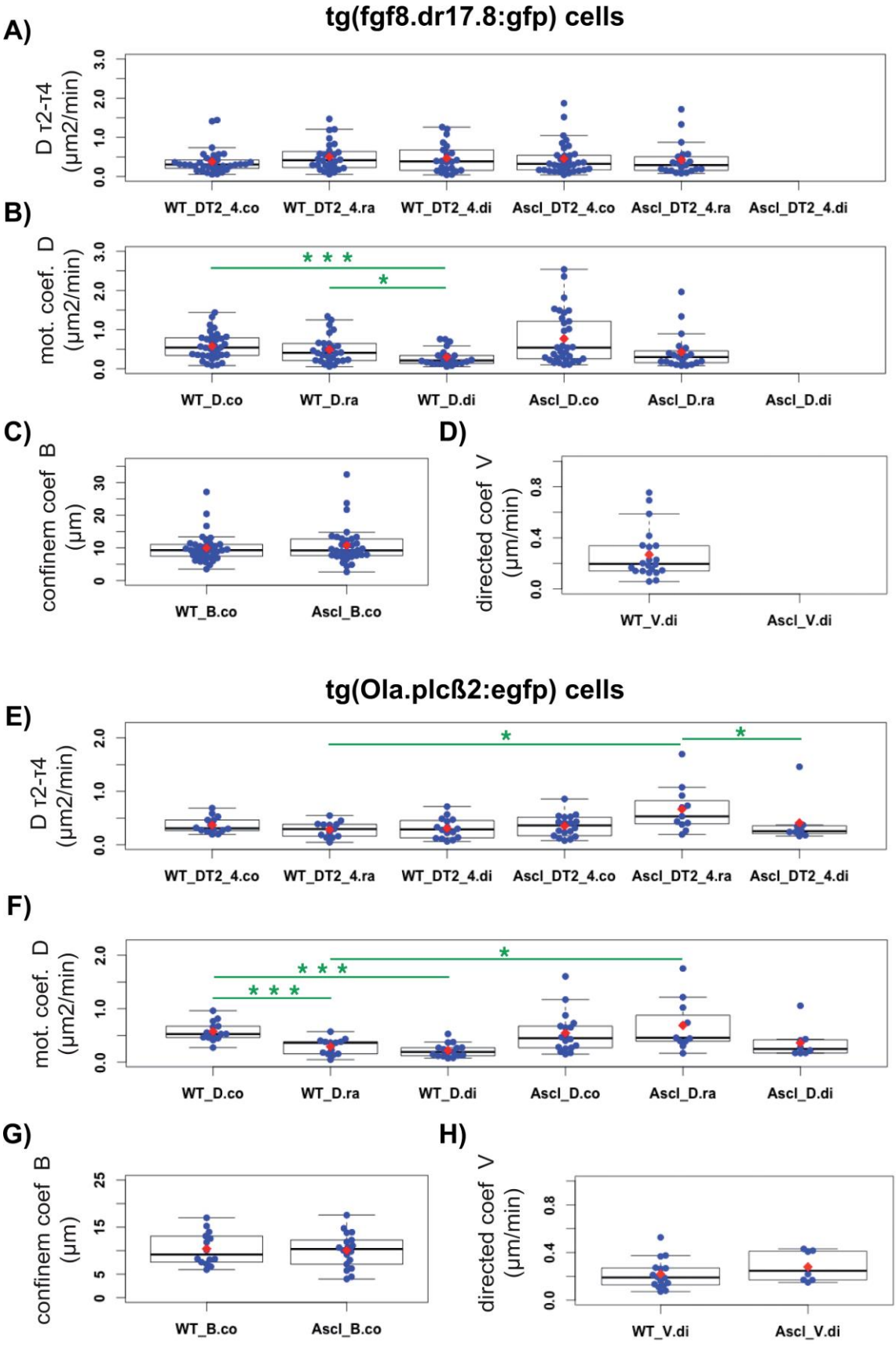

Fig. S2. Coefficients of relative cell displacement during wild-type and *ascl1a-/-* taste bud development. For the definition of each parameter see Materials and Methods and main text. 'Wild-type (wt)' cell data include

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data from wild-type larvae and wild-type siblings obtained from heterozygous *ascl1a*+/- crosses (n=2/6 experiments for *tg(fgf8.dr17:gfp)*, n=1/4, for *tg(Ola.plcß2:egfp)*. For number of samples, analysis of value distributions and statistical tests, see Tables **S1-S2.** Significant differences are shown with asterisks. **A-H:** boxplots of the distributions of coefficients D(τ2-τ4), D, B and V that characterize relative wild-type and *ascl1a-/- tg(fgf8.dr17:gfp)* or *tg(Ola.plcß2:egfp)* cell motility. **A,B,D**: *ascl1a-/- tg(fgf8.dr17:gfp)* cells are devoid of directed motility and corresponding coefficients are absent from the plots (Ascl_DT2_4.di, Ascl_D.di, Ascl_V.di). Wild-type *tg(fgf8.dr17:gfp)* cell relative directed motility (WT_DT2_4.di, WT_D.di, WT_V.di): data from 22 cells with relative directed motility for relative trajectories from the epithelium towards a taste bud (11/22) or from one organ to another (11/22) (see also Table **1**). **C,G**: The confinement coefficients B (B_co) came from the same continuous distribution in the case of wild-type and *ascl1a-/-* , for either *tg(fgf8.dr17:gfp)* or *tg(Ola.plcß2:egfp)* cells. **H:** The directional coefficients V (velocity) came from the same continuous distribution in the case of wild-type and *ascl1a-/- tg(Ola.plcß2:egfp)* cells.

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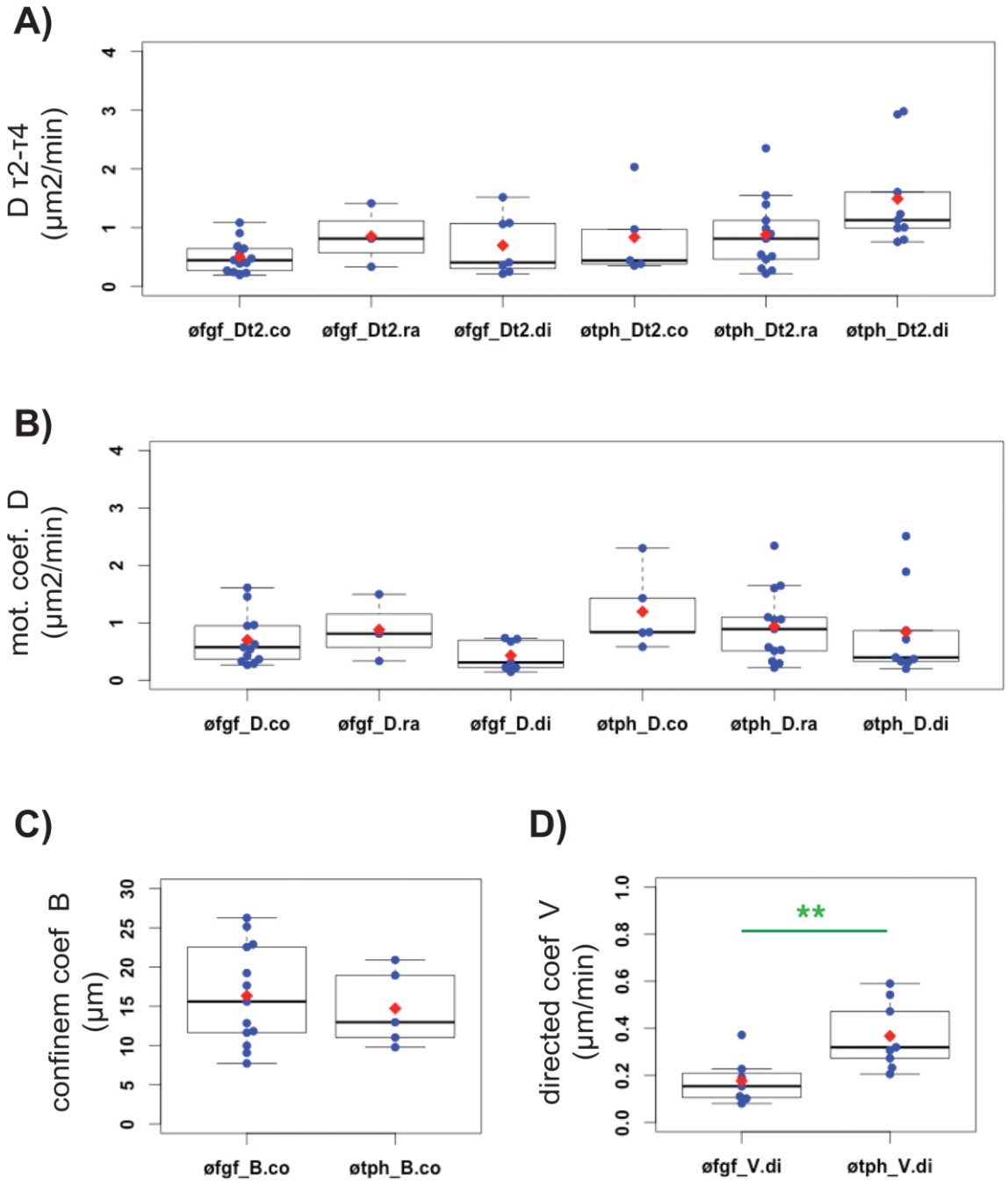
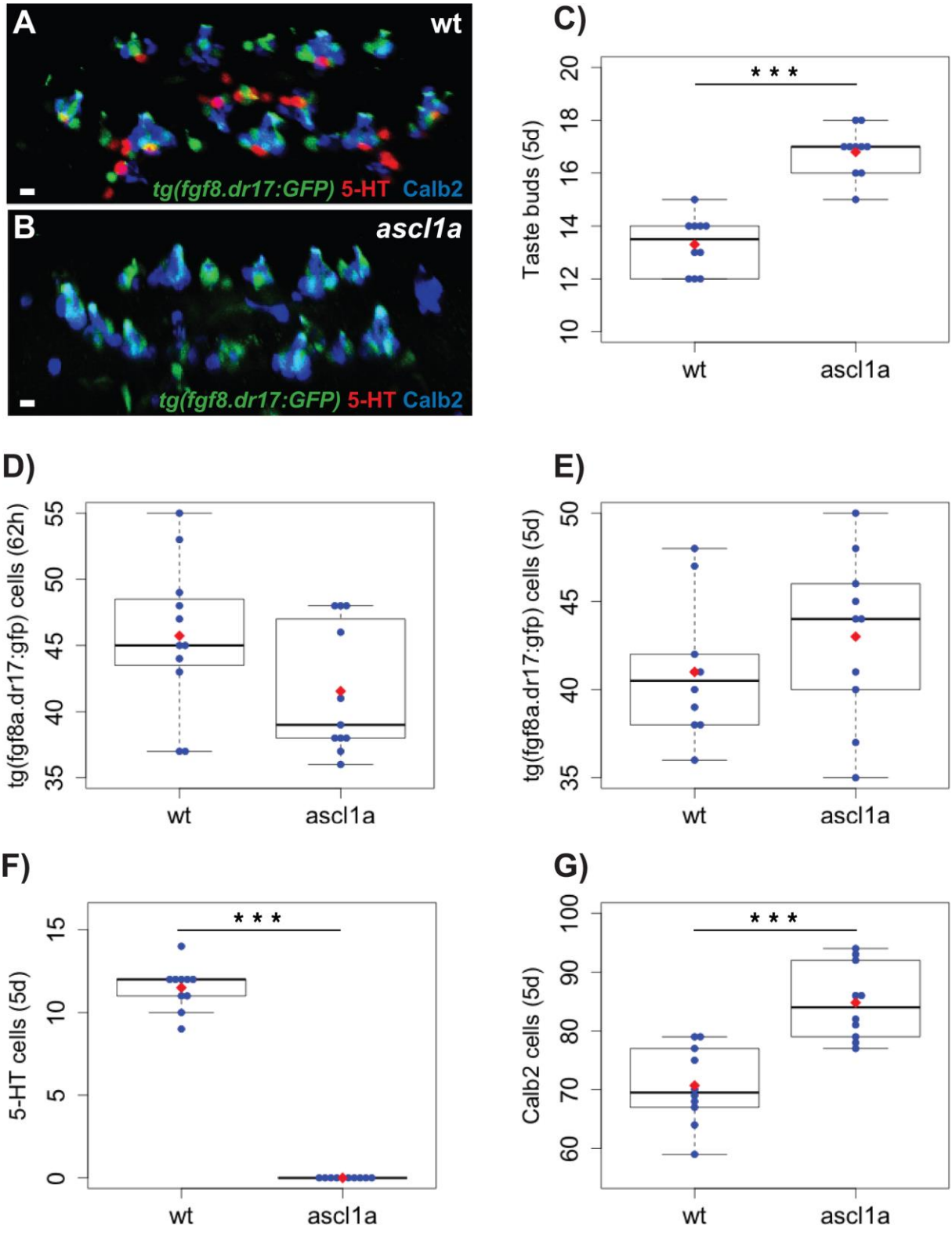


Fig. S3. Motility coefficients of taste bud cells after multiphoton laser ablation of a single cell of the organ. Øfgf signifies *tg(fgf8.dr17:gfp)* cell ablation; Øtph, *tg(tph1b:mCherry)* cell ablation. Data from: *tg(fgf8.dr17:gfp)* cell ablations (n=10 cells in 10 embryos): confined (co) relative displacement, 13/23 cells; random (ra) relative displacement, 3/23 cells; directed (di) relative displacement, 7/23 cells (from and towards the targeted organ). *tg(tph1b:mCherry)* cell ablations (n=10 cells in 10 embryos): confined (co) relative displacement, 5/27 cells; random (ra) relative displacement, 13/27 cells; directed (di) relative displacement, 9/27 cells, none joining the targeted organ; only quitting towards another one. The velocity V of these *tg(fgf8.dr17:gfp* cells is significantly different from that of *tg(fgf8.dr17:gfp)* cells with directed motion after *tg(fgf8.dr17:gfp)* cell ablation.
Shapiro.test: øfgf_V.di: W = 0.8798, p-value = 0.2256, øtph_V.di: W = 0.9195, p-value = 0.388. Welch two sample t.test : øfgf_V.di, øtph_V.di : t = -3.2131, df = 13.976, p-value = 0.006267.



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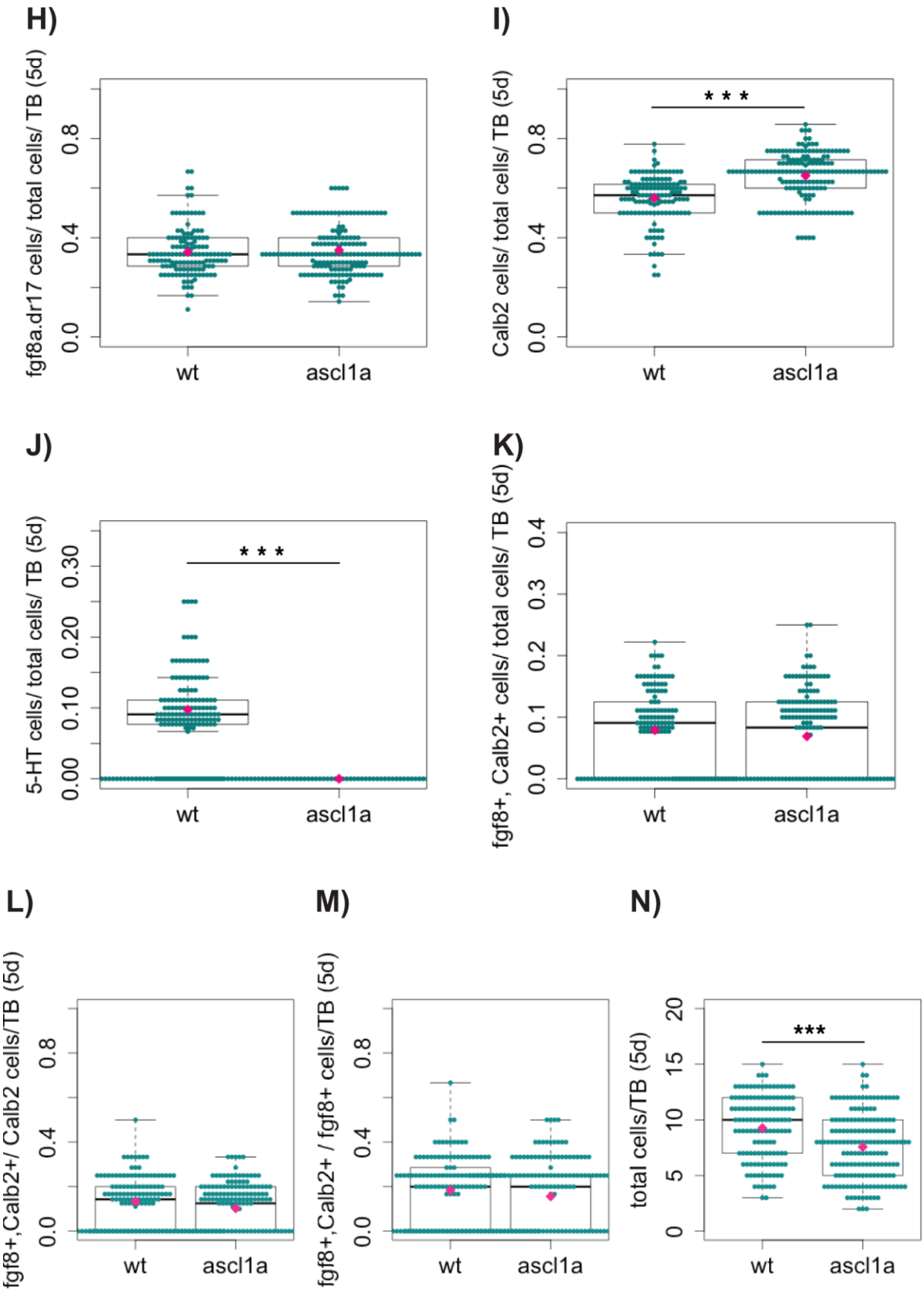

Fig. S4. Comparison of taste buds (A-B), their number (C), total number of cells of each type (D-G) and ratios of cells (per cell type and per taste bud, H-M) in wild-type and *ascl1a-/-* larvae mouth lips. The phenotype of taste buds and general larval morphology is indistinguishable between wild-type and heterozygous

ascl1a+/- larvae and therefore, these data were grouped as 'wild-type'. Data from 10 wild-type (wt: grouped 6 wild-type and 4 wild-type siblings from heterozygous *ascl1a*+/- crosses, n=133 taste buds) and 10 *ascl1a*-/- larvae (n=169 taste buds, 5dpf, fixed tissue), except **D** (n=11, 'wild-type'= 6 wild-type and 5 wild-type siblings from heterozygous *ascl1a*+/- crosses, 62hpf, fixed tissue). In the boxplots, black lines show median values and red spots, mean values. **A,B** confocal projections showing the taste buds of the mouth lips of a wild-type and an *ascl1a*-/- larva. *ascl1a*-/- larvae were devoid of 5-HT cells (**B,F,J**), had non significant difference in the number of *tg(fgf8a.dr17:gfp)* expressing cells (**D,E,H**) but had increased number of taste buds (**C**) and Calb2 (Type II) expressing cells (**G,I**) compared to the wild-type. **K** shows the ratio of cells per taste bud that coexpress *tg(fgf8a.dr17:gfp)* and Calb2. **L** shows the ratio of Calb2/Type II cells that co-express *tg(fgf8a.dr17:gfp)* within a taste bud. **M** shows the ratio of *tg(fgf8a.dr17:gfp)* cells that co-express Calb2/Type II cells within a taste bud. 5-HT cells co-expressing *tg(fgf8a.dr17:gfp)* at 5dpf were rare (n=7/115 cells 5-HT cells). **N:** The number of total cells varies from one developing taste bud to another at 5dpf. A significant difference was found between the distributions of the total number of cells per taste bud between wild-type and *ascl1a* mouth lips. Statistical tests: **C**: Welch two-sample t-test : t = -8.2832, df = 17.384, p-value = 1.923e-07. **D**: Two-sample Kolmogorov-Smirnov : D = 0.4545, p-value = 0.2058. **E**: Welch two-sample t-test : t = -1.4604, df = 16.982, p-value = 0.1624. **F**: Welch two-sample t-test : t = 26.8582, df = 9, p-value = 6.655e-10. **G**: Welch two-sample t-test : t = -4.8152, df = 17.968, p-value = 0.0001394. **H**: Two-sample Kolmogorov-Smirnov : D = 0.0799, p-value = 0.7283. **I**: Two-sample Kolmogorov-Smirnov : D = 0.4844, p-value = 1.332e-15. **J**: Two-sample Kolmogorov-Smirnov : D = 0.8647, p-value < 2.2e-16. **K**: Two-sample Kolmogorov-Smirnov : D = 0.1216, p-value = 0.2211. **L**: Two-sample Kolmogorov-Smirnov : D = 0.1282, p-value = 0.1732. **M:** Two-sample Kolmogorov-Smirnov : D = 0.1098, p-value = 0.3313 **N**: Two-sample Kolmogorov-Smirnov : D = 0.2529, p-value = 0.0001469.

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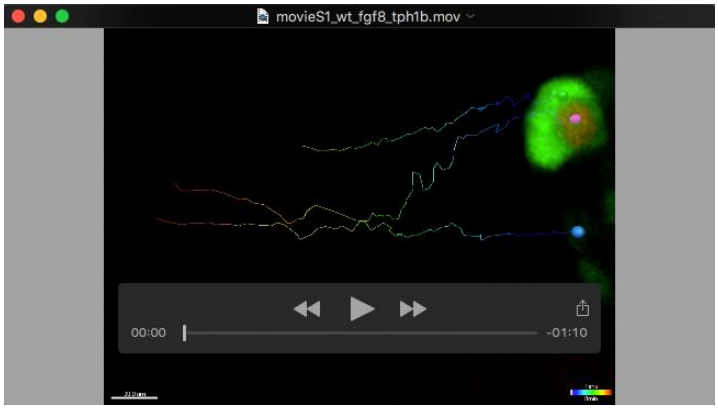

Movie 1. Time-lapse of spinning disk z-stack projections showing the behaviour of *tg(tph1b:mCherry)* and *tg(fgf8a.dr17:gfp)* expressing cells during taste bud formation, in the anterior-medial mouth of a wild-type embryo. Anterior to the left, imaging started at approximately 60hpf, time interval 8min. Snapshots of this movie are provided in Fig.3A-H.

Examples of cells are colour-coded with dots and corresponding tracks are displayed. Representative cases illustrating the diversity of taste bud cell displacement, that is, tracks of cells relative to the reference cells and MSD(τ) plots for time periods of interest are presented as raw data in Appendix S1 (p.1-5). The entire analysis of cell displacement is summarized Table 1, S1-S2 and Fig. S2.

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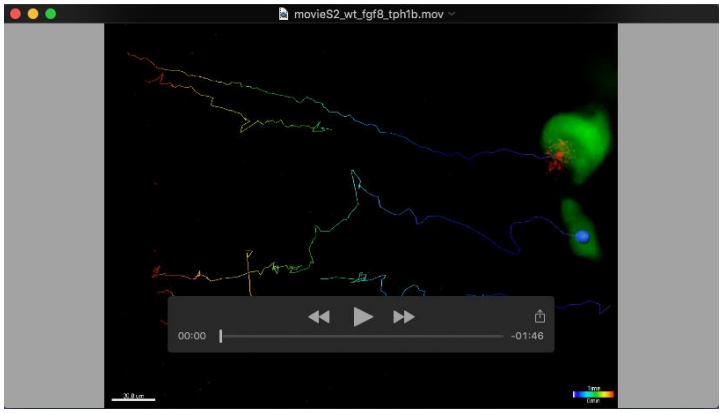
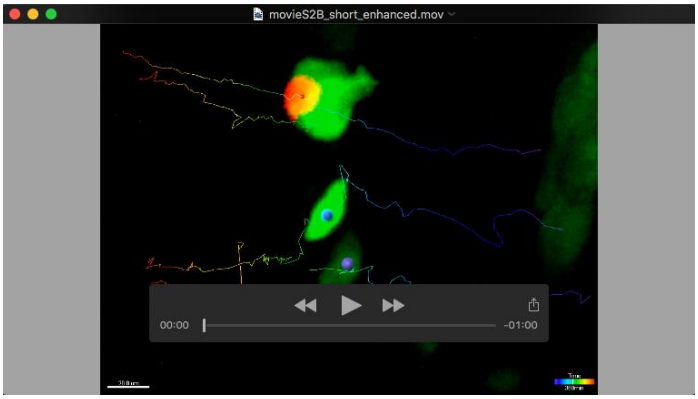


Movie 2. Time-lapse of spinning disk z-stack projections showing the behaviour of *tg(tph1b:mCherry)* and *tg(fgf8a.dr17:gfp)* expressing cells during taste bud formation, in the anterior-medial mouth of a wild-type embryo. Anterior to the left, imaging started at approximately 65hpf, time interval 8min. Snapshots of this movie are provided in Fig. 4A-O.

Examples of cells are colour-coded with dots and corresponding tracks are displayed. Representative cases illustrating the diversity of taste bud cell displacement, that is, tracks of cells relative to the reference cells and MSD(τ) plots for time periods of interest are presented as raw data in Appendix S1 (p.6-10). The entire analysis of cell displacement is summarized in Tables 1, S1-S2 and Fig. S2.



Movie 2B. A highly contrasted version of Movie **2** to better visualize the extra cell joining the taste bud at the bottom ('semi-circular' track, p.9 in Appendix S1).

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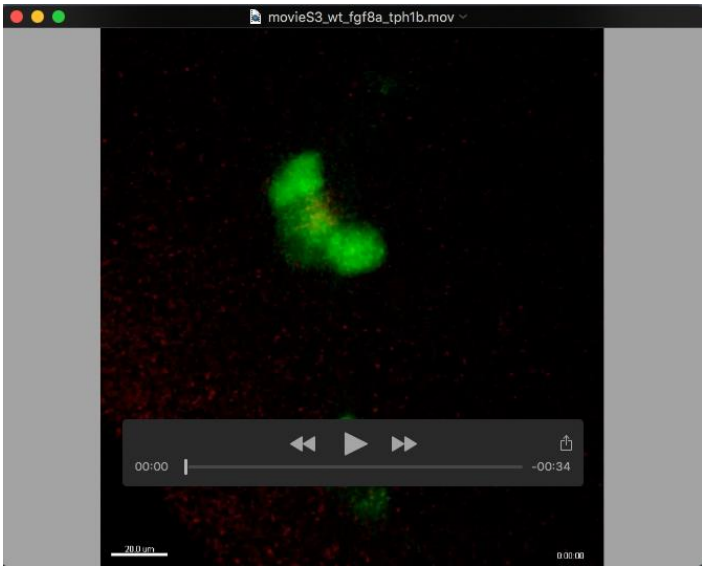
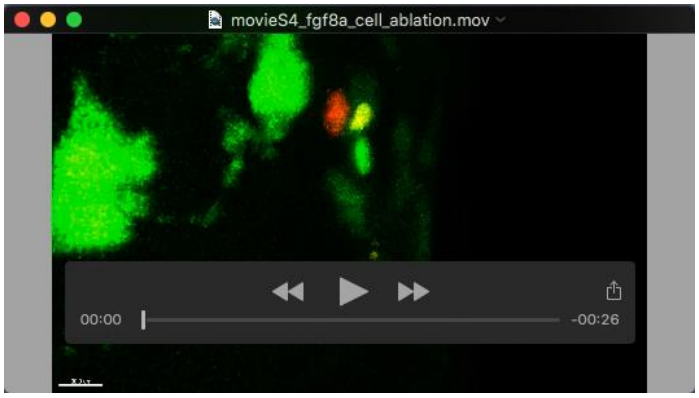


Movie 3. **Time-lapse of spinning disk z-stack projections showing the behaviour of *tg(tph1b:mCherry)* and *tg(fgf8a.dr17:gfp)* expressing cells during taste bud formation, in the anterior-lateral mouth of a wild-type embryo.** Anterior to the top left, imaging started at approximately 65hpf, time interval 9min. Snapshots of this movie are provided in Fig. 4P-U. Images are highly contrasted in order to visualize the double *tg(tph1b:mCherry); tg(fgf8a.dr17:gfp)* expressing cell and slightly rotated compared to Fig. 4P-U so that the displacement of additional *tg(fgf8a.dr17:gfp)* cells can be observed. The entire analysis of cell displacement is summarized in Tables 1, S1-S2 and Fig. S2.



Movie 4. Time-lapse of multi-photon laser z-stack projections showing the behaviour of *tg(tph1b:mCherry)* and *tg(fgf8a.dr17:gfp)* expressing cells after ablation of a *tg(fgf8a.dr17:gfp)* cell in the lateral mouth of a wild-type embryo. Anterior to the top left, embryonic stage, 64-65hpf, time interval 8min (one movie frame). The targeted cell before ablation is shown in Fig. 5A. Snapshots of this movie are provided in Fig. 5C-J. The green fluorescence rostral to taste bud cells (left side of the movie) is due to skin auto-fluorescent cells. The entire analysis of cell displacement is summarized in Fig. S3.

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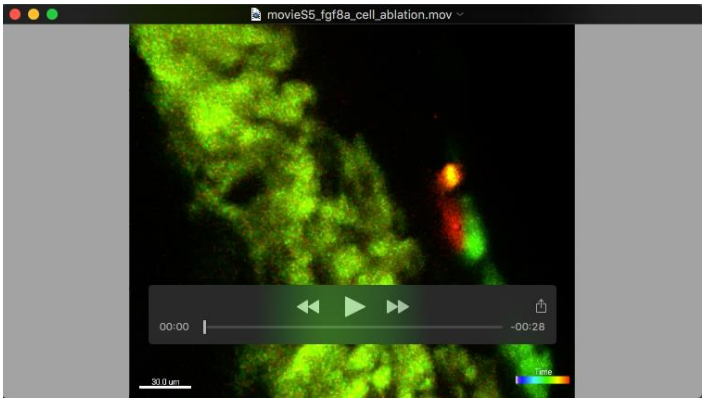
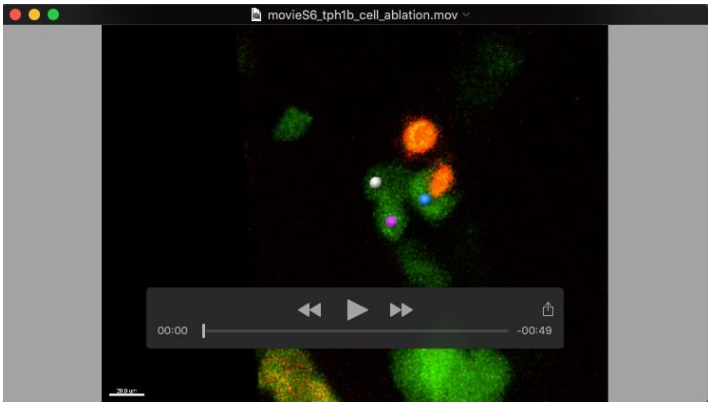


Movie 5. Time-lapse of multi-photon laser z-stack projections showing the behaviour of *tg(tph1b:mCherry)* and *tg(fgf8a.dr17:gfp)* expressing cells after ablation of a *tg(fgf8a.dr17:gfp)* cell in the lateral mouth of a wild-type embryo. Anterior to the top left, embryonic stage, 64-65hpf, time interval 8min (one movie frame). The targeted cell before ablation is shown in Fig. 5A'. Snapshots of this movie are provided in Fig. 5C'-G'. The green fluorescence rostral to taste bud cells (left side of the movie) is due to skin auto-fluorescent cells. The entire analysis of cell displacement is summarized in Fig. S3.



Movie 6. Time-lapse of multi-photon laser z-stack projections showing the behaviour of *tg(tph1b:mCherry)* and *tg(fgf8a.dr17:gfp)* expressing cells after ablation of a *tg(tph1b:mCherry)* cell in the anterior-lateral mouth, of a wild-type embryo. Anterior to the left, embryonic stage, 64-65hpf, time interval 8min (one movie frame). The targeted cell before ablation is shown in Fig. 5K. Snapshots of these movies are provided in Fig. 5M-U. The green fluorescence rostral to taste bud cells (left side of the movie) is due to skin auto-fluorescent cells. The entire analysis of cell displacement is summarized in Fig. S3.

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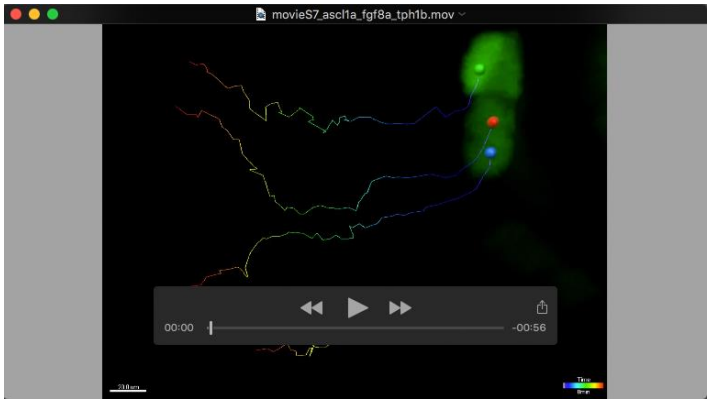
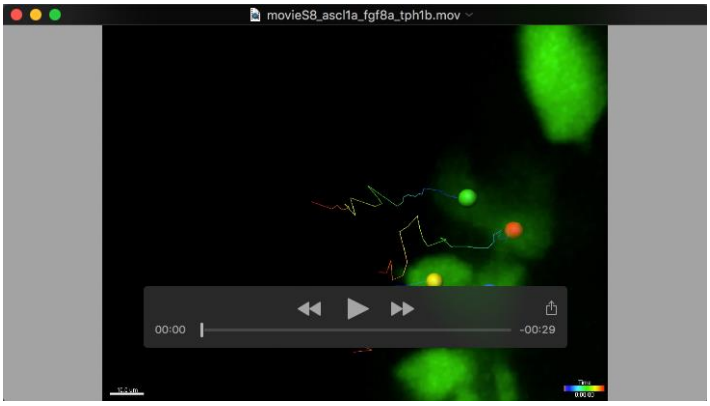


Movie 7. Time-lapse of spinning disk z-stack projections showing the behaviour of *tg(fgf8a.dr17:gfp)* expressing cells during taste bud formation in the anterior-medial mouth of an *ascl1a-/-* embryo. Anterior to the left, imaging started at approximately 60hpf, time interval 8min. The taste bud shown is located at similar position (most anterior and medial part of the mouth) to the taste bud showed in Movie 1 (wild-type embryo). Snapshots are provided in Fig. 3I-O.
Examples of cells are colour-coded with dots and corresponding tracks are displayed. Representative cases illustrating the diversity of taste bud cell displacement, that is, tracks of cells relative to the reference cells and MSD(τ) plots for time periods of interest are presented as raw data in Appendix S1 (p.11-14). The entire analysis of cell displacement is summarized in Table 1, S1-S2 and Fig. S2.



Movie 8. Time-lapse of spinning disk z-stack projections showing the behaviour of *tg(fgf8a.dr17:gfp)* expressing cells during taste bud formation in the anterior-medial mouth of an *ascl1a-/-* embryo. Anterior to the left, imaging started at approximately 65hpf, time interval 8min. See also appendix S1 (p. 15-16). The entire analysis is summarized in Table 1, S1-S2 and Fig. S2.

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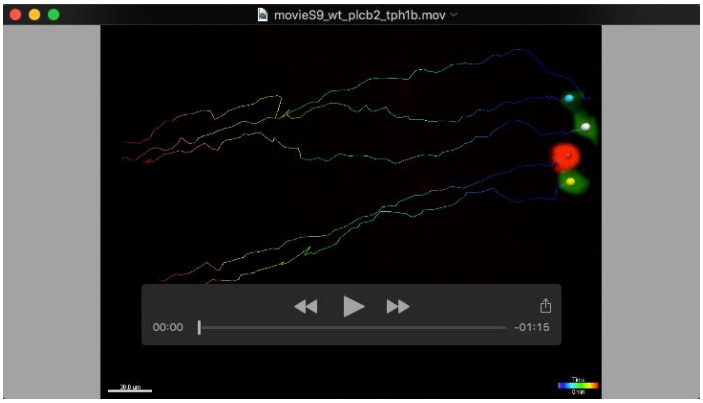
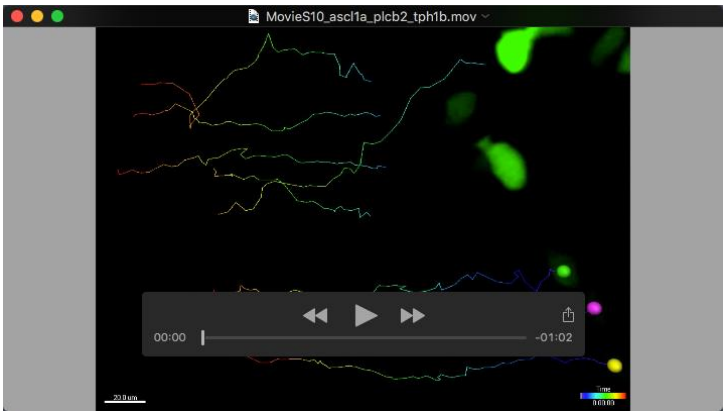


Movie 9. Time-lapse of spinning disk z-stack projections showing the behaviour of *tg(tph1b:mCherry)* and *tg(Ola.plcß2:egfp)* expressing cells during taste bud formation, in the anterior-medial mouth of a wild-type embryo. Anterior to the left, imaging started at approximately 64hpf, time interval 8min. Snapshots of this movie are provided in Fig.6A-H.

Examples of cells are colour-coded with dots and corresponding tracks are displayed. Representative cases illustrating the diversity of taste bud cell displacement, that is, tracks of cells relative to the reference cells and MSD(τ) plots for time periods of interest are presented as raw data in Appendix S1 (p.17-21). The entire analysis of cell displacement is summarized in Table 1, S1-S2 and Fig. S2.



Movie S10. Time-lapse of spinning disk z-stack projections showing the behaviour of *tg(Ola.plcß2:egfp)* expressing cells during taste bud formation, in the anterior-medial mouth of an *ascl1a-/-* embryo. Anterior to the left, imaging started at approximately 64hpf, time interval 8min. Snapshots of this movie are provided in Fig.6I-M.

Examples of cells are colour-coded with dots and corresponding tracks are displayed. Representative cases illustrating the diversity of taste bud cell displacement, that is, tracks of cells relative to the reference cells and MSD(τ) plots for time periods of interest are presented as raw data in Appendix S1 (p.22-27). The entire analysis of cell displacement is summarized in Table 1, S1-S2 and Fig. S2.

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Appendix S1. Analysis of taste bud cell displacement : examples of MSD (τ) plots

In the following pages, it is mentioned : 1) a brief summary of the key points illustrated by the selected tracks in a particular Movie. 2) the Movie name, the time period, a brief message about the cell behaviour. 3) the cell identity and the colour code of the dots to identify them in the Movie.

A-C: the trajectory of the cell of interest (A, green), of the reference cell (B, red, see Methods for definition of Reference cell) and the calculated trajectory of A relative to B (C, blue line). All trajectories are flattened, shown in 2D. In A and B the x,y,z coordinates are raw numbers, obtained from the microscope.

D: shows the MSD(τ) plot (square displacement (μm^2), time interval (min)). Each boxplot represents the values of square displacement for a particular time interval (τ1=one interval, τ2=two intervals…, each time interval 8min). Red dot = mean value in boxplot. Curves: black line corresponds to a linear fit through the initial τ2-τ4 values, used to determine the motility coefficient D(τ2-τ4) that is independent of cell motility type. The blue/red line corresponds to a fit according to equations (4), (5) or (6) (see Methods) that describe random (diffusion), confined (slower than diffusion) and directed (faster than diffusion) motion, respectively.

E: shows the relative deviation (RD), that is, for each time interval, the ratio of the fitted MSD (values obtained by the equation fit (blue/red line)) to the τ2-τ4 values (black line fit). High density RD around value 1 indicates random motility; >1, directed and <1, constrained / confined displacement (calculated according to (Kusumi et al., 1993)).

Inbox at left bottom: The α value was determined by plotting MSD(τ) $\propto \tau^{\alpha}$ for (τ > τ 4) (that is the initial points of the curve that correspond to random displacement were excluded) and its deviation from value 1, that is characteristic of random motility, was examined (see also equation (7) in Methods, for review (Saxton and Jacobson, 1997)). α>1 (threshold set here at ⩾1.1) or α<1 (threshold <0.9) correspond to directed (faster) or confined (slower) motility, respectively. The values of coefficients (D,B,V) characterize the motility of the cell according to the fits for τ2-τ4 and corresponding equation for random, directed or confined displacement. To assess the goodness of fit, first, we checked residuals for non constant variance and normality by examining residuals versus fitted values and quantile plots, respectively. r^2 = (1-SSres /SStot), where SSres is the sum of square of the residuals (the value minimized by the nonlinear regression) and SStot = sum[(y-mean(y))^2] (the sum of the squared differences between the data points and the average of the data points). The closest the r^2 to 1 the better the fit (Motulsky and Ransnas, 1987).

In some cases, (i.e. p.3,24), the MSD(τ) plot cannot be analysed with a single equation suggesting that the cell changes displacement mode over time. Therefore, it is analysed for one or more particular time periods through which the cell of interest is in or devoid of contact with the reference cell (or the cell group that contains it).

MOVIE S1
WT; fgf8a.gfp; tph1b.mCherry

The displacement of the epithelial fgf8a cell (blue dot) and rearrangement of the tph1b cell (pink dot) within the taste bud results in integration of the fgf8a cell (blue dot) into the taste bud.

Pg.2: 0-256min: time period of blue-dot fgf8a cell addition into the taste bud: the relative displacement of this cell to the tph1b cell is directed.

Pg.3: While the fgf8a cell (blue dot) is maintained into the taste bud, it keeps being rearranged relative to tph1b cell (pink dot) in a complex manner until the end of the movie (264-552min).

Pg.4: Their relative displacement is directed during the early period of maintenance (264-384min) without being associated to any other obvious behaviour.

Pg.5: During the process of fgf8a cell (blue dot) 'addition' (0-400min), cell rearrangements occur within the taste bud (i.e. the relative displacement of fgf8a cell (green dot) to the tph1b cell (pink dot) is directed).

The newly arrived fgf8a cell (blue dot) and the other taste bud cells are maintained within the taste bud until the end of the movie.

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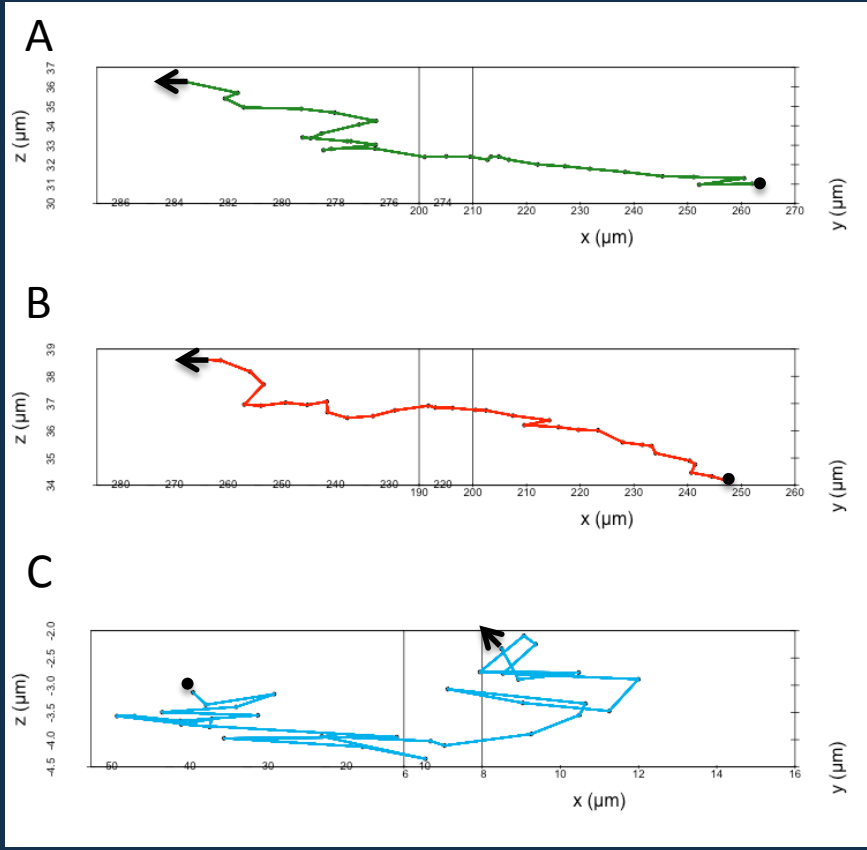
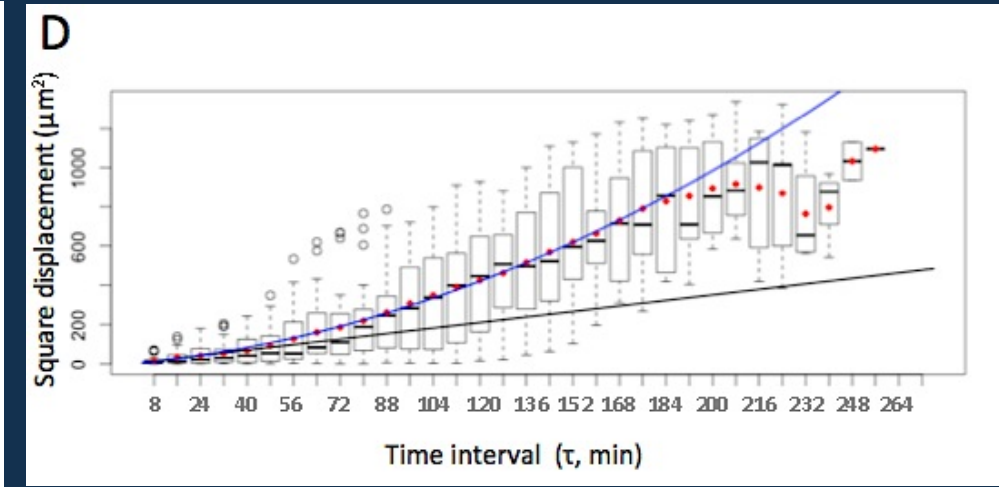
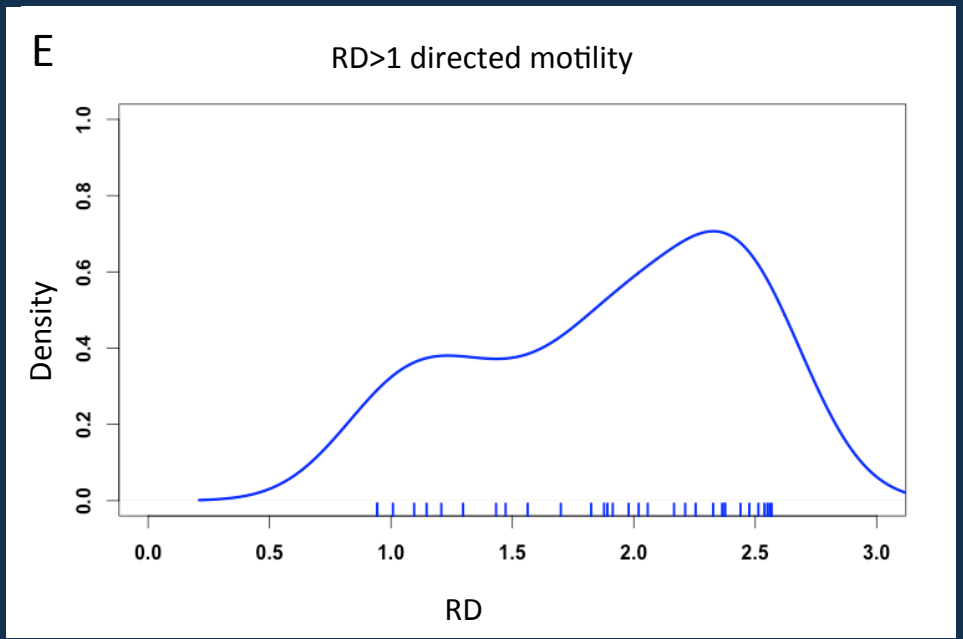
MovieS1, 0-256min (*cell joining the taste bud)*

A) fgf8a.gfp cell (blue dot) B) tph1b.mCherry (pink dot) C) Relative track A) to B)

D) MSD(τ) plot : Directed displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.29228	0.01814	16.11	0.00383**

############

α=1.3452

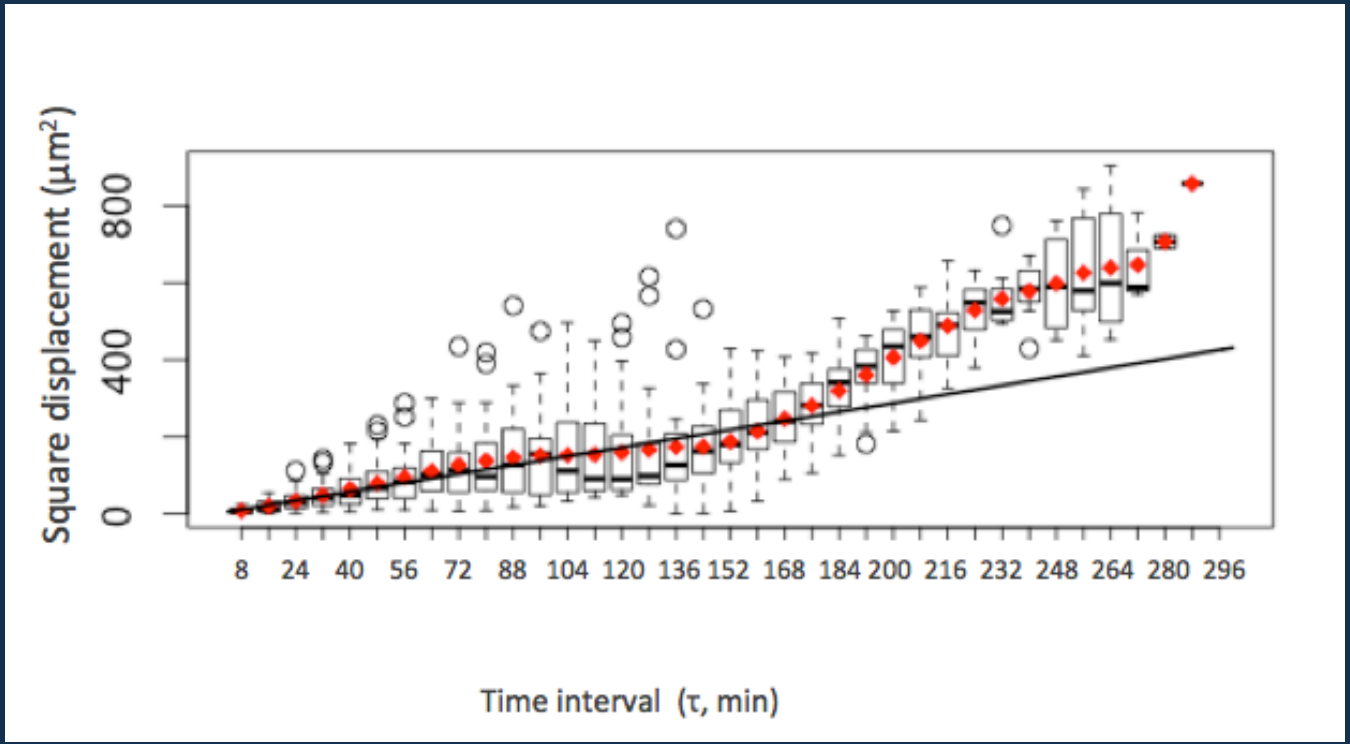
MSD ~ {6*D*T+V^2*T^2} (blue line)

	Estimate	Std. Error	t value	Pr(>|t|)
V	0.133252	0.001844	72.25	< 2e-16 ***
D	0.22890	0.01142	20.05	1.03e-14 ***

r^2 = 0.9996075

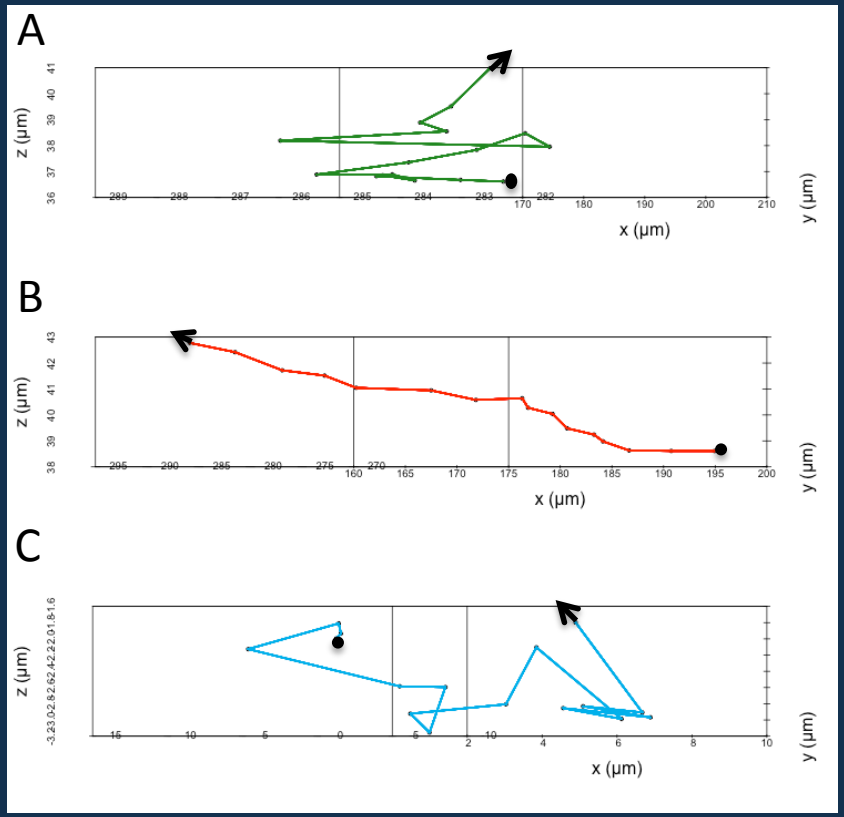
Significance codes: '***' 0.001 '**' 0.01 '*' 0.05

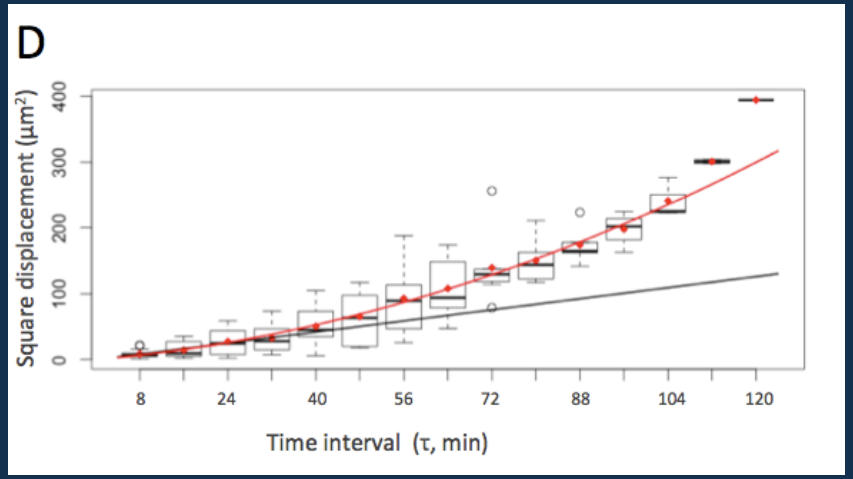
MovieS1, 264-552min (*cell that joined the taste bud)*
MSD(τ) plot of fgf8a.gfp cell (blue dot) displacement using tph1b.mCherry (pink dot) as reference.
No evident pattern, it hides more than one modes (see next slide).

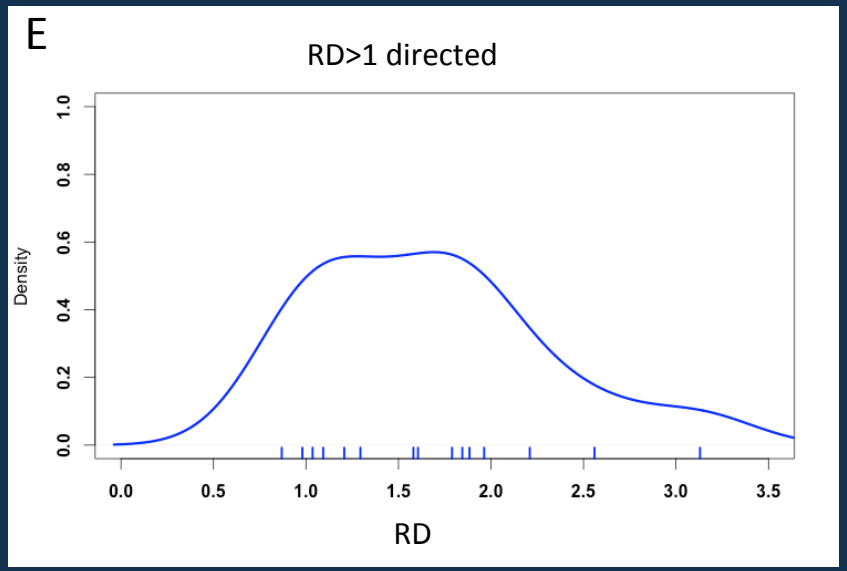


Development • Supplementary information

MovieS1, 264-384min *(cell that joined the taste bud)*
A) fgf8a.gfp cell (blue dot) B) tph1b.mCherry (pink dot) C) Relative track A) to B)
D) MSD(τ) plot : Directed displacement.







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.17490	0.00896	19.52	0.00261 **

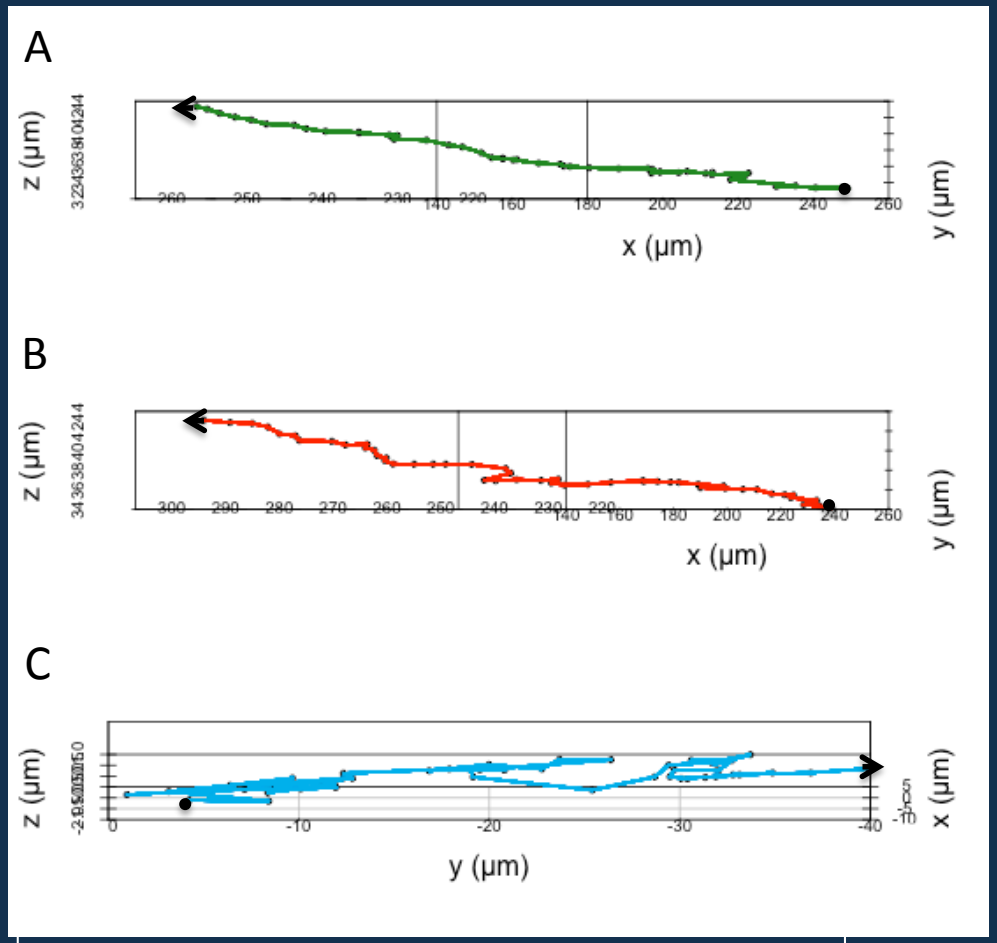
############
α= 1.286
MSD ~ {6*D*T+V^2*T^2} (red line)

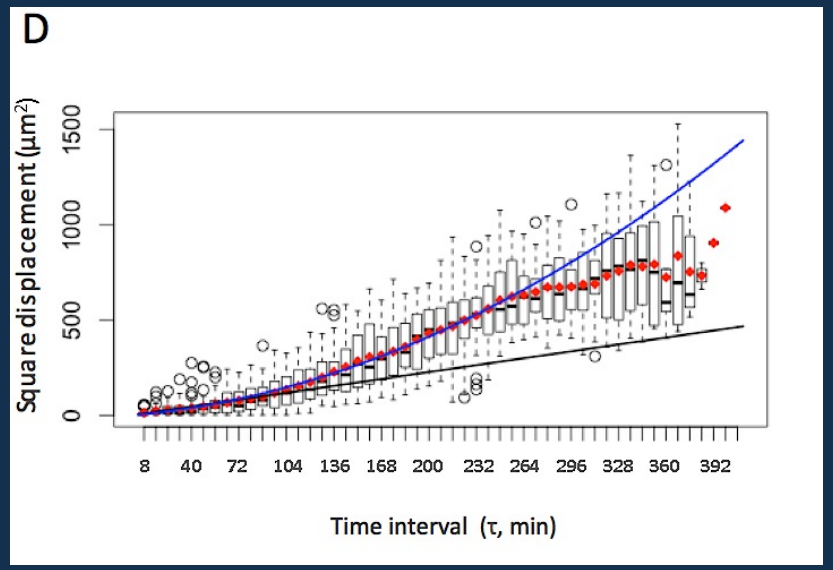
	Estimate	Std. Error	t value	Pr(>|t|)
V	0.121390	0.004701	25.821	3.40e-11 ***
D	0.12156	0.01589	7.651	9.96e-06 ***

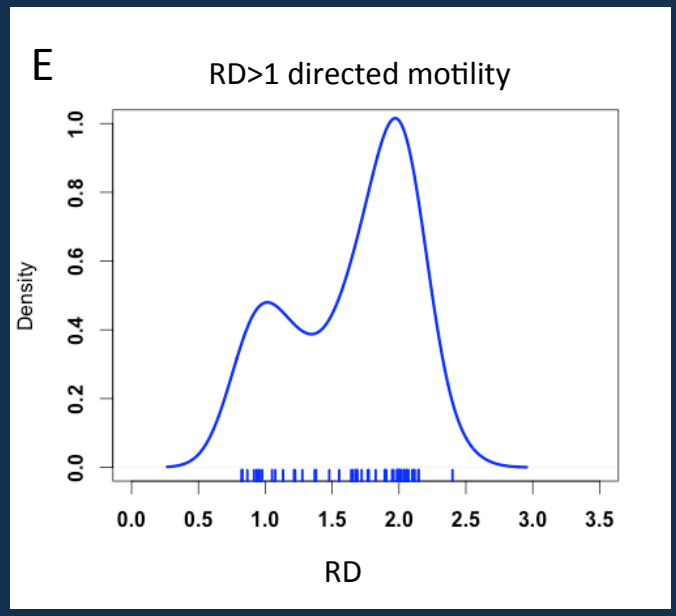
r^2 =0.9981782
Signif. codes: '***' 0.001 '**' 0.01 '*'0.05

Development • Supplementary information

MovieS1, 0-400min (*cell within the taste bud*)
A) fgf8a.gfp cell (green dot) B)tph1b.mCherry (pink dot) C) Relative track A) to B)
D) MSD(τ) plot : Directed displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.1889	0.0144	13.12	0.00576 **

########
α=1.596
MSD ~ {6*D*T+V^2*T^2} (blue line)

	Estimate	Std. Error	t value	Pr(>|t|)
V	0.082393	0.001426	57.79	< 2e-16 ***
D	0.117432	0.008126	14.45	2.56e-15 ***

r^2=0.9991391

MOVIE S2
WT; fgf8a.gfp; tph1b.mCherry

An fgf8a cell (blue dot) explores space between two taste buds, one rather well formed (top of the frame) and another early forming one (bottom of the frame).

Pg.7: The fgf8a cell blue-dot explores around the well-formed taste bud (obvious contact at 168min, quits at 312min). During this exploring period, it has directed displacement relative to the tph1b cell (red pixel).

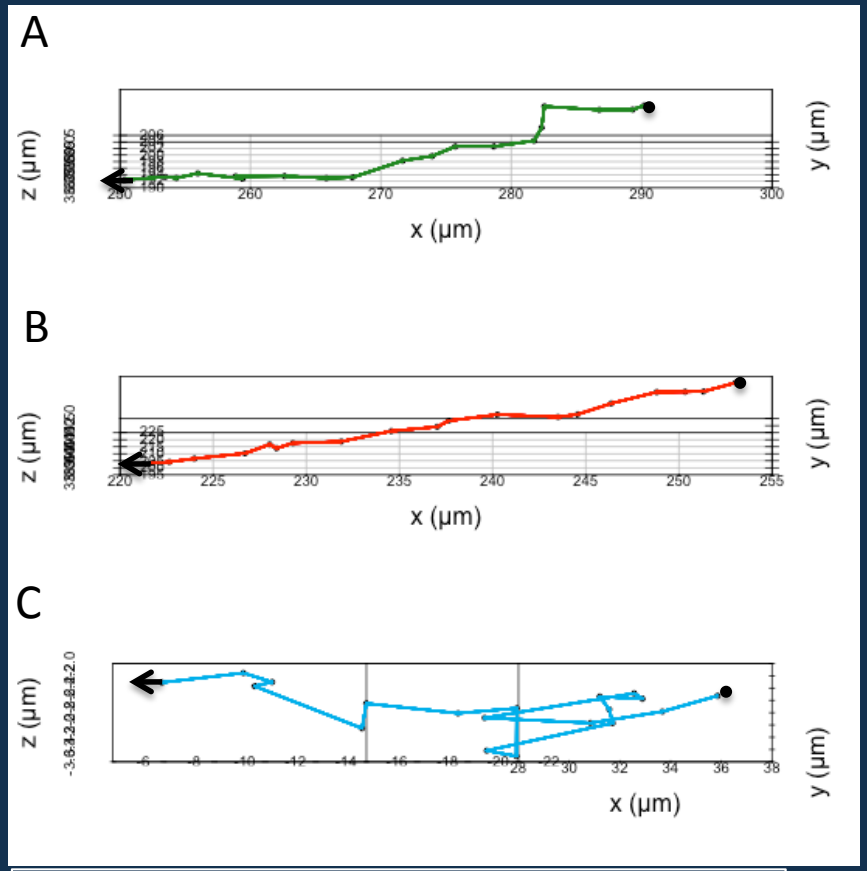
Pg.8: While fgf8a cell (blue dot) joins the forming taste bud (bottom frame, 312-408min), it is displaced in directed mode relative to the fgf8a cell (purple-dot) (or white-dot, not shown).
It is maintained within this taste bud until the end of the movie (840min) and makes contact with the late appearing tph1b cell (pink pixel).

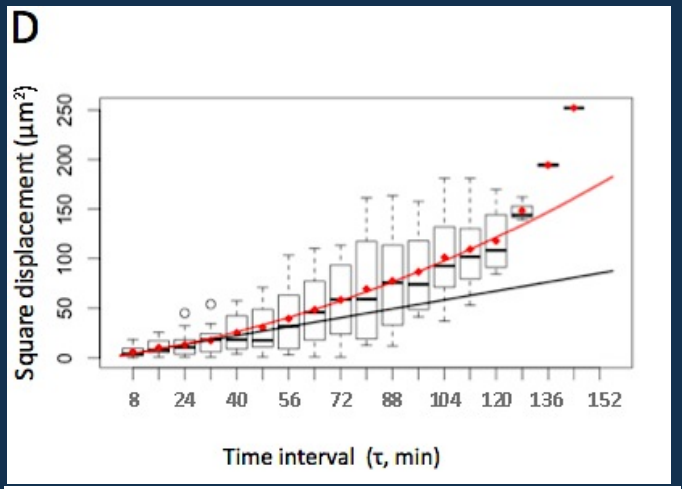
Pg.9: Directed relative displacement of another exploring fgf8a cell (green dot) while it joins the tph1b cell (pink pixel, 696-840min). See also contrasted images in Movie S2B.

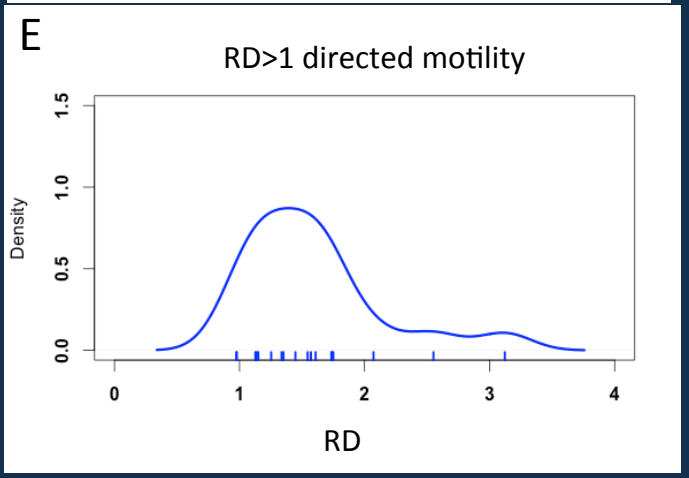
Pg.10: Example of an fgf8a cell (yellow dot) within the taste bud with confined displacement relative to the tph1b cell (red pixel, 376-840min).

Development • Supplementary information

MovieS2, 160-304min, (*cell moving between forming taste buds, exploring around upper taste bud*)
A) fgf8a.gfp cell (blue dot) B) tph1b.mCherry (red pixel) C) Relative track A) to B)
D) MSD(τ) plot : Directed displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.093384	0.003965	23.55	0.0018 **

############
α=1.453
MSD ~ {6*D*T+V^2*T^2} (red line)

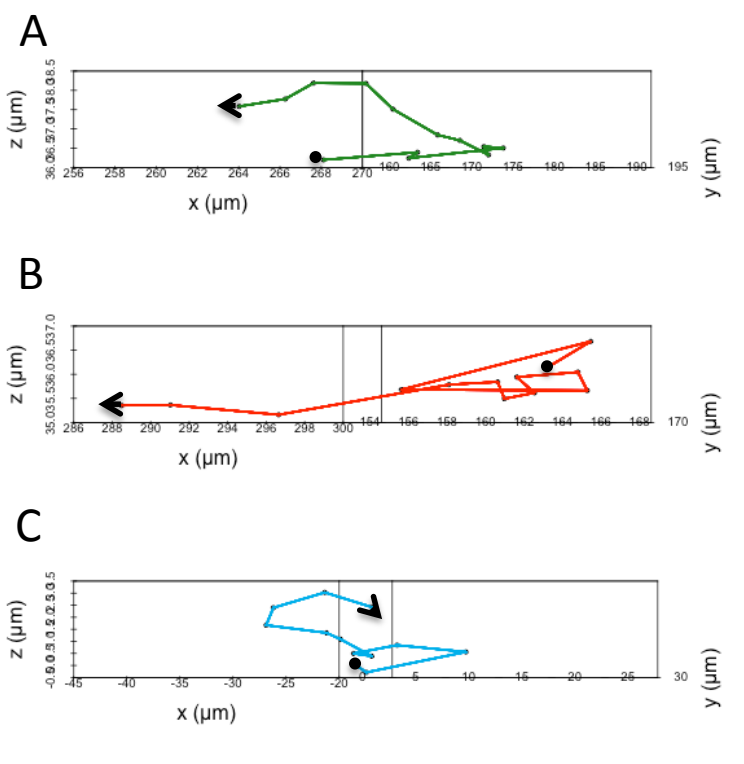
	Estimate	Std. Error	t value	Pr(>|t|)
V	0.066823	0.002279	29.32	2.91e-13 ***
D	0.07911	0.00487	16.25	5.14e-10 ***

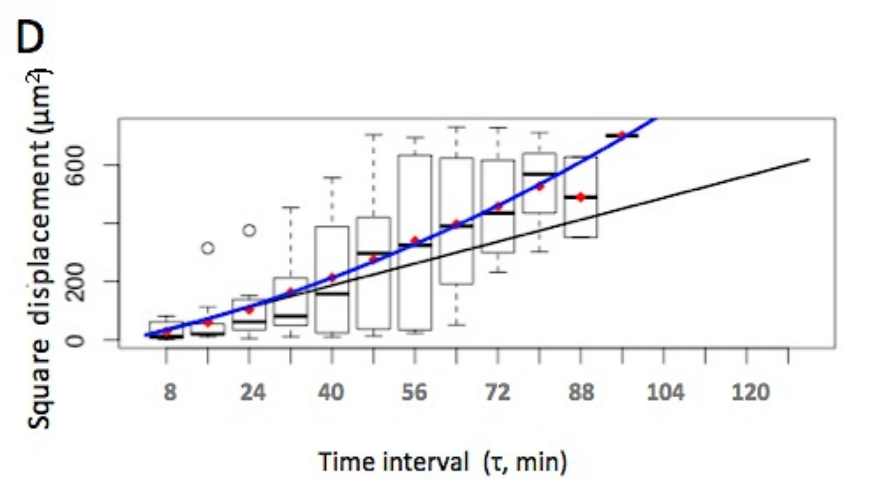
r^2=0.9992924

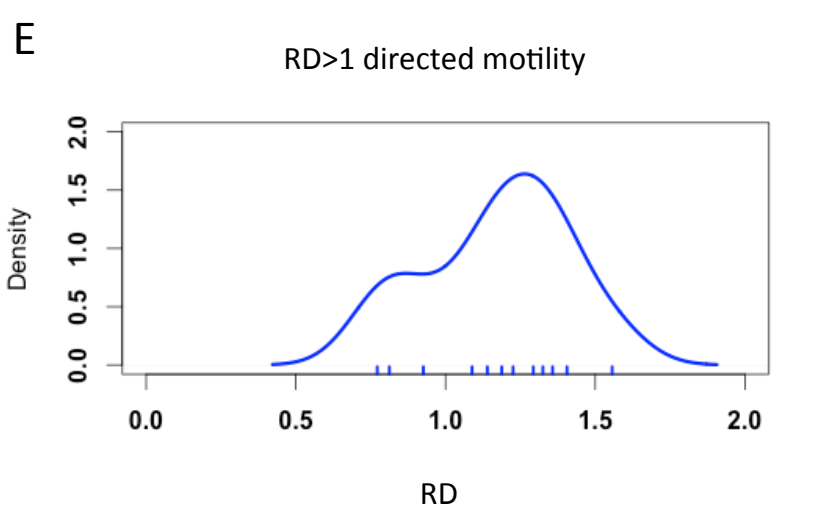
Movie S2, 312-408min (*cell moving between forming taste buds, directed to taste bud at the bottom of the frame*)

A) fgf8a.gfp cell (blue dot) B) fgf8a.gfp cell (purple dot) C) Relative track A) to B)

D) MSD(τ) plot : Directed displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.78113	0.05761	13.56	0.0054 **

############

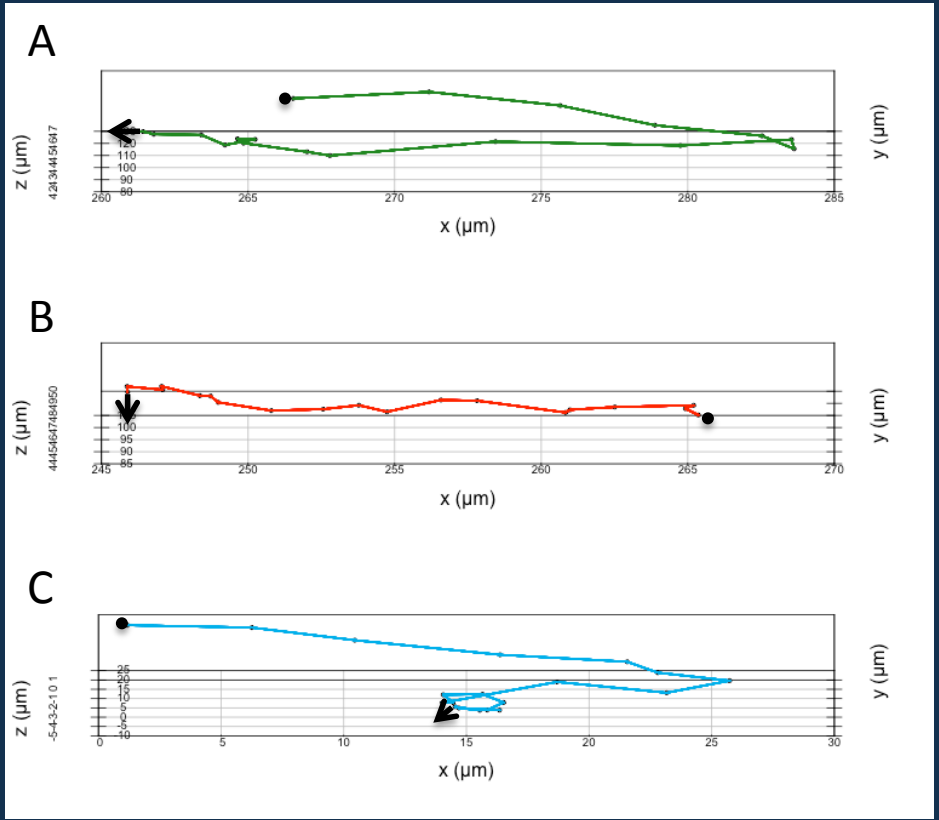
α=1.290

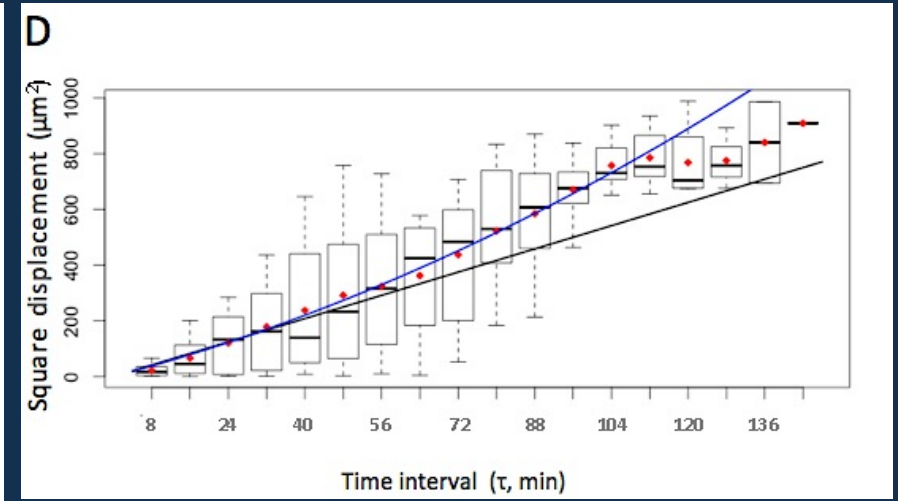
MSD ~ {6*D*T+V^2*T^2} (blue line)

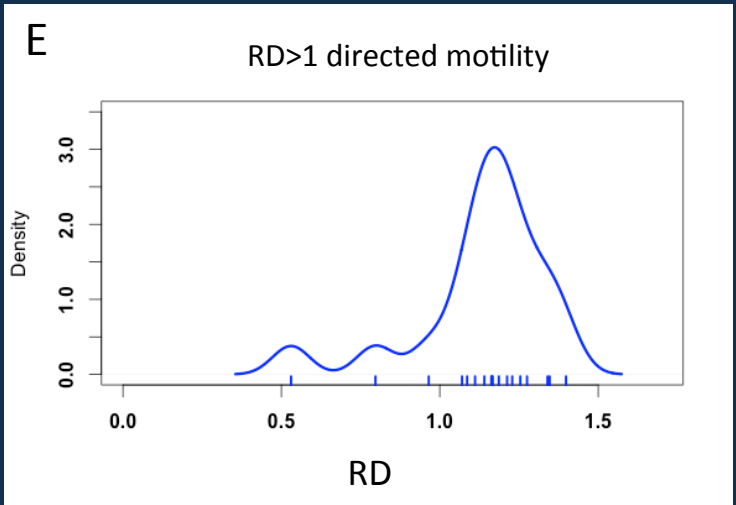
	Estimate	Std. Error	t value	Pr(>|t|)
V	0.182868	0.008733	20.94	2.84e-08 ***
D	0.666305	0.034543	19.29	5.41e-08 ***

r^2=0.9989044

Movie S2, 696-840min (*cell moving around group, reaches tph1b cell, bottom of the frame)*
A) fgf8a.gfp cell (green dot) B) tph1b.mCherry (pink pixel) C) Relative track A) to B)
D) MSD(τ) plot : Directed displacement







MSD ~ {6*D*T} (black line)

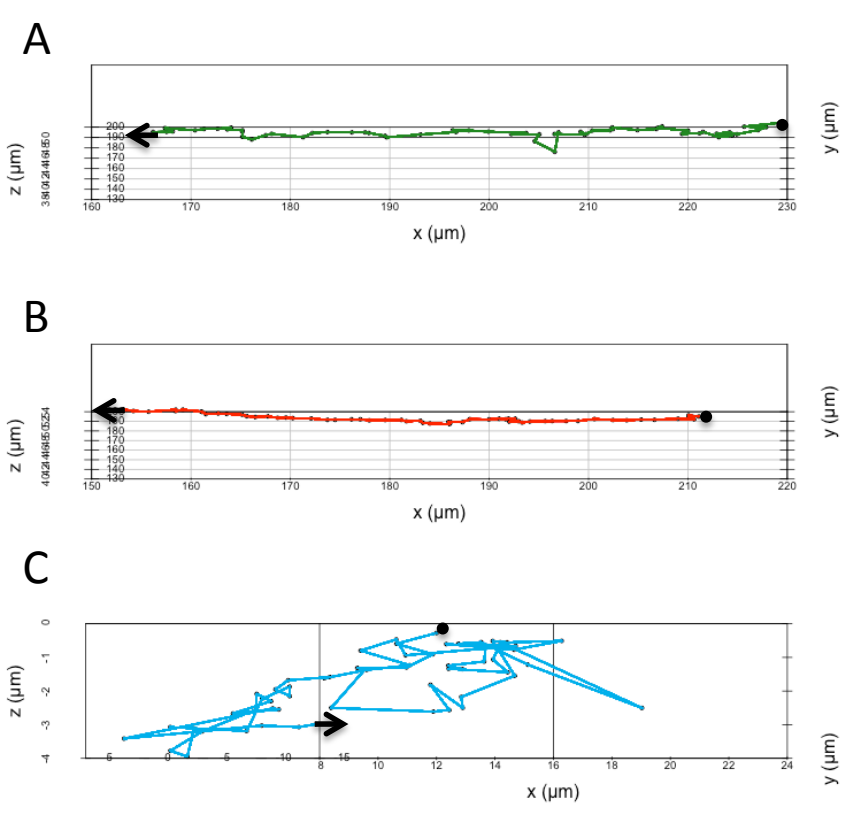
	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.86751	0.05749	15.09	0.00436 **

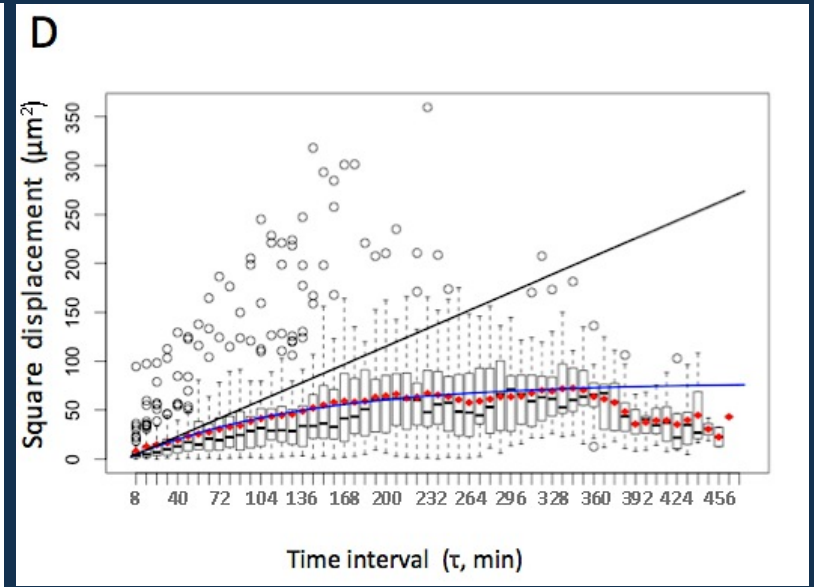
############
α=1.2163
MSD ~ {6*D*T+V^2*T^2} (blue line)

	Estimate	Std. Error	t value	Pr(>|t|)
V	0.15437	0.00971	15.90	1.99e-09 ***
D	0.75867	0.04483	16.92	9.71e-10 ***

r^2=0.9975611

MovieS2, 376-840min (*cell inside the taste bud*)
A) fgf8a.gfp cell (yellow dot) B)tph1b.mCherry (red pixel) C) Relative track A) to B)
D) MSD(τ) plot : Confined displacement





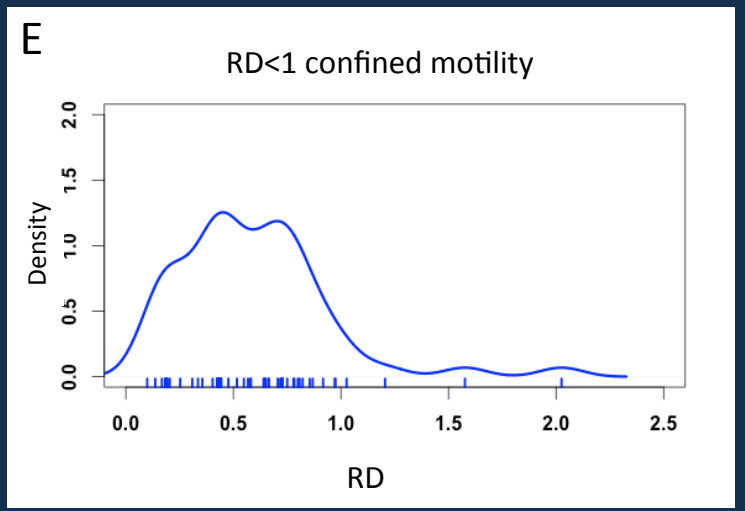
MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.09588	0.01087	8.822	0.0126 *

############
α=0.6033
MSD ~ {B^2*(1-exp(-6*D*T/B^2))} (blue line)

	Estimate	Std. Error	t value	Pr(>|t|)
B	8.83838	0.08067	109.56	<2e-16 ***
D	0.096951	0.002378	40.78	<2e-16 ***

r^2=0.9911777



MOVIE S7
ascl1a-/-;fgf8a.gfp;tph1b.mcherry

The forming ascl1a-/-;fgf8a expressing taste bud shown at the medial part of the frame, is located at similar position (most anterior-medial mouth) to the wild-type organ shown in movie S1 and time-lapse started at the same embryonic stage (60hpf).

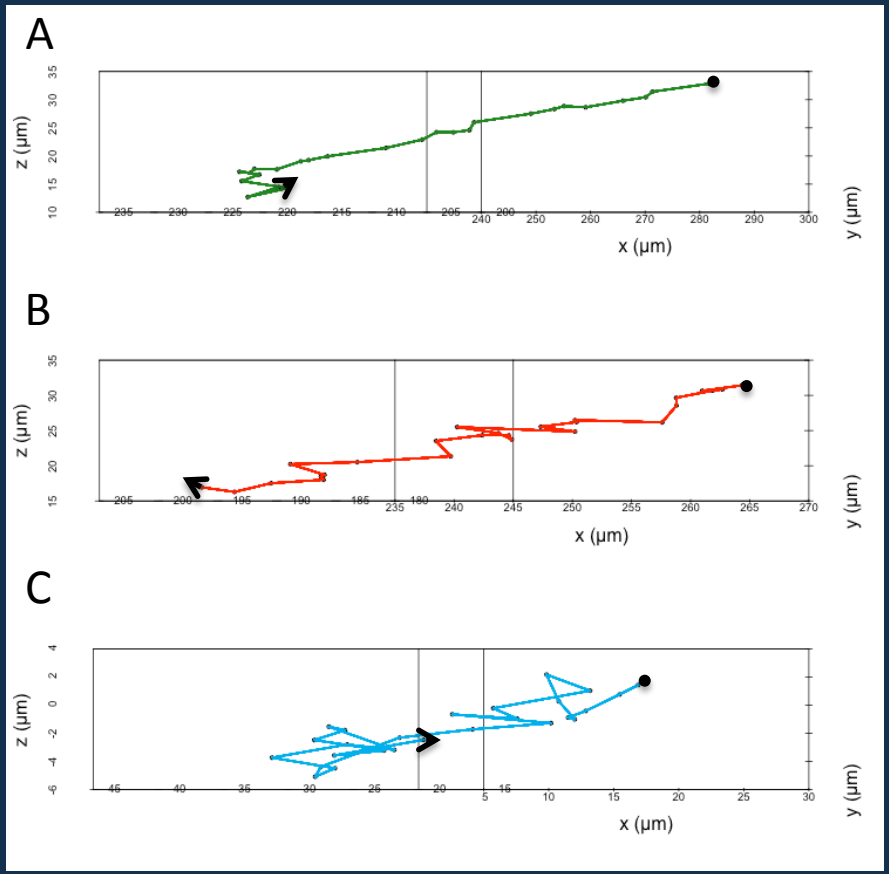
In contrast to wild-type taste buds (i.e shown in Movies S1 and S2), in the ascl1a-/- larva, the fgf8a cells form taste buds but cell groups also split and cells regroup.
As a result, the reference cell (closest at the center of the mass of the group) changes and it is redefined according to the cell relationships within the group where the cells assemble (join to form an organ).

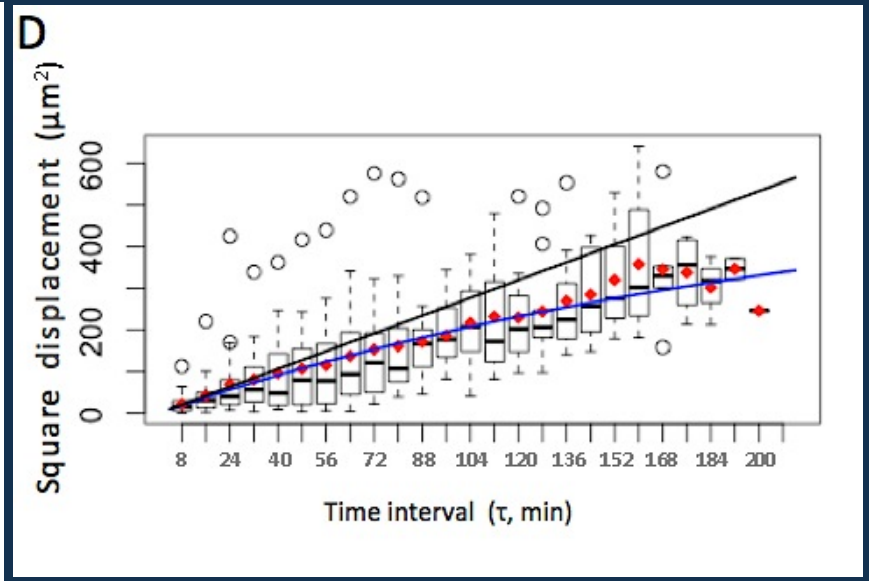
Pg.12: fgf8a cells (green dot and red dot) initially belong to the same cell group, then separate and regroup. During the period (136-336min) that the fgf8a cell (red dot) quits and regroups with the fgf8a cell (green dot) cluster, the relative displacement of the red and green-dot cells is confined.

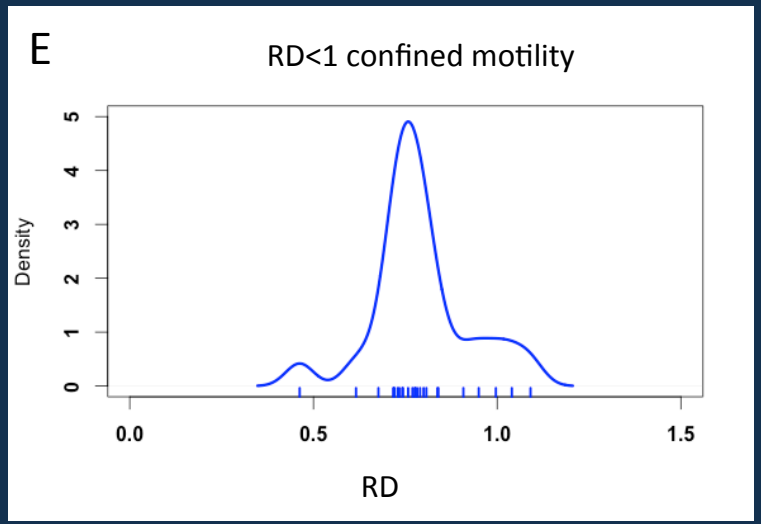
Pg.13: (0-288min) The fgf8a cell (blue dot) is displaced in a confined mode relative to the reference fgf8a cell (red dot). After they split, the fgf8a cell (blue dot) is displaced towards the group at the bottom of the frame (yellow and pink dots, it approaches fgf8a cell (yellow dot) at 400-408min). The relative displacement of the fgf8a cells (blue and yellow dots) during this period (296-400min) is random (pg.14). Same case if other cells of the group used as reference (i.e. pink dot, not shown).

Development • Supplementary information

Movie S7, 136-336min *(time during which cell-red-dot quits cluster containing cell-green-dot and re-joins it)* A) fgf8a.gfp cell (red dot) B) fgf8a.gfp cell (green dot) C) Relative track A) to B)
D) MSD(τ) plot : Confined displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)	
DT2-T4	0.44492	0.019762	2.52	0.00197	**

#############

α=0.8655

MSD ~ {B^2*(1-exp(-6*D*T/B^2))} (blue line)

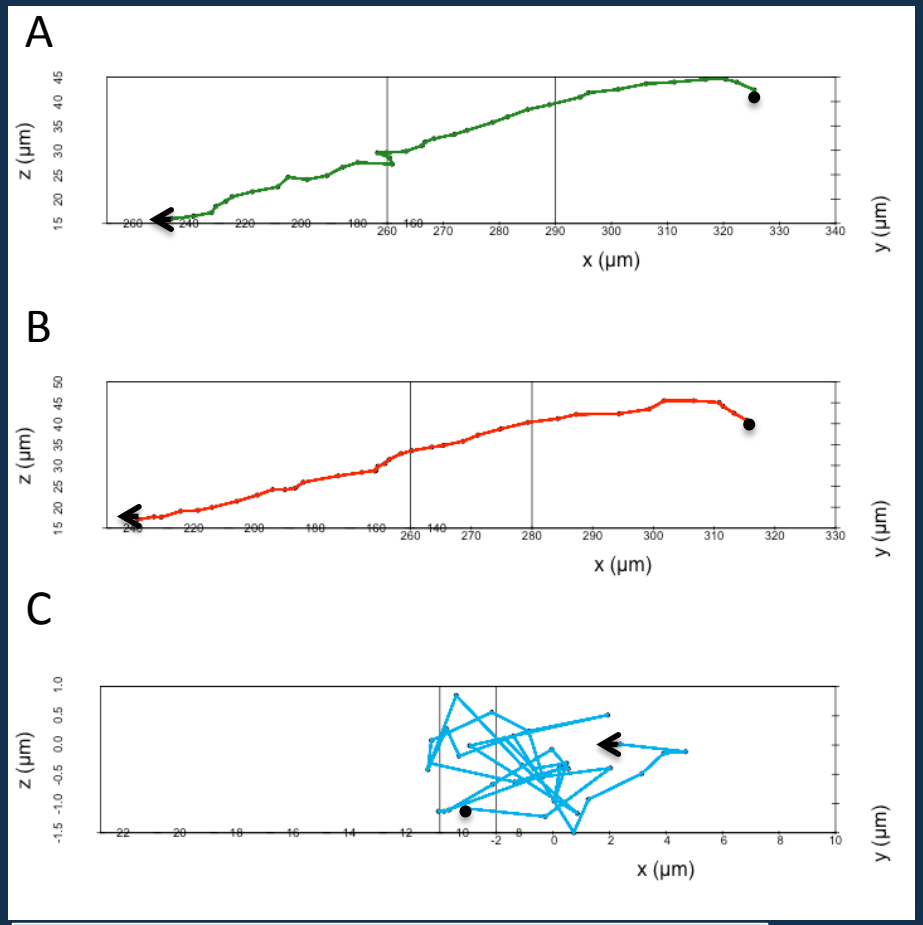
	Estimate	Std. Error	t value	Pr(>|t|)	
B	23.715	2.157	10.99	2.85e-08	***
D	0.41703	0.01925	21.66	3.63e-12	***

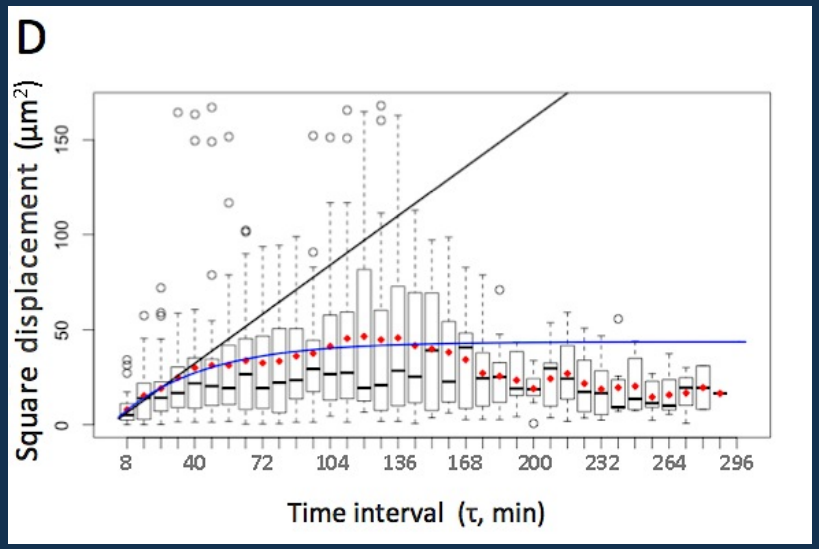
r^2=0.9969937

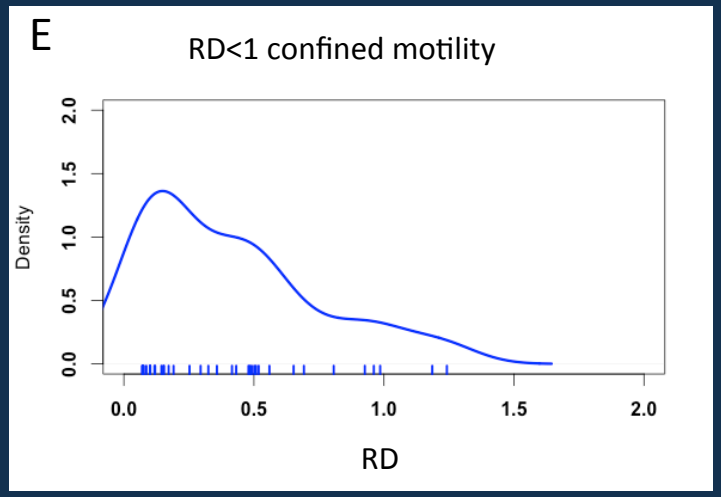
MovieS7, 0-288min (*cells moving together, medial part of the frame)*

A) fgf8a.gfp cell (blue dot) B) fgf8a.gfp cell (red dot) C) Relative track A) to B)

D) MSD(τ) plot : Confined displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.134664	0.007166	18.79	0.00282 **

############

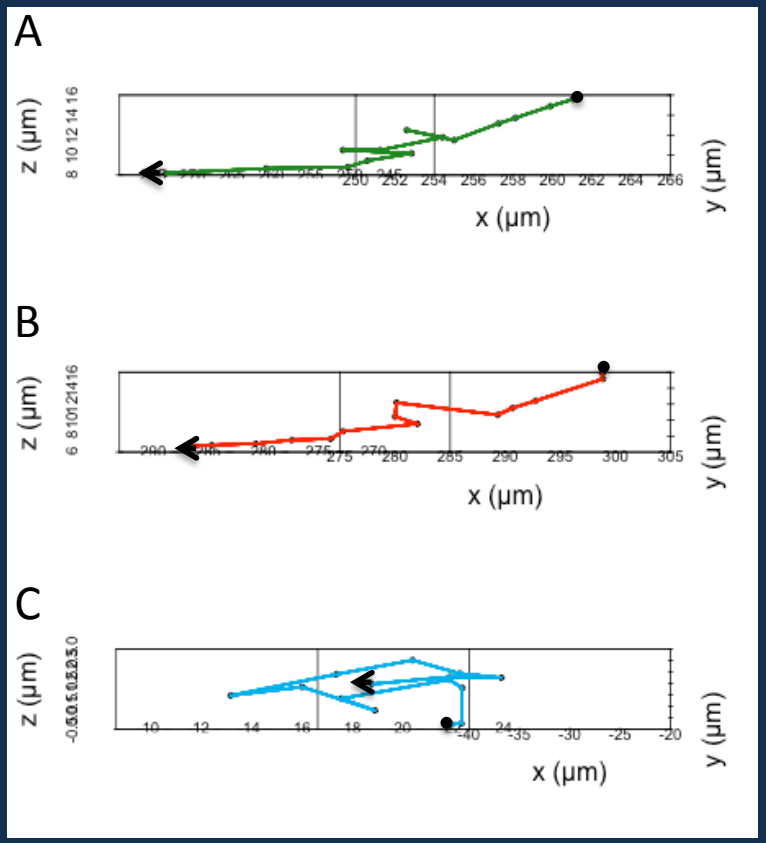
α=0.5068

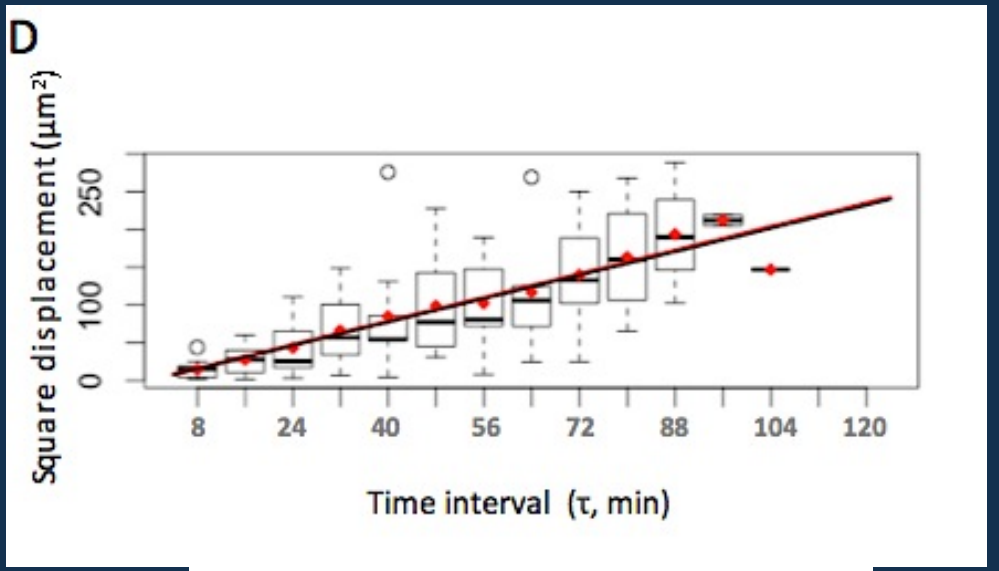
MSD ~ {B^2*(1-exp(-6*D*T/B^2))} (blue line)

	Estimate	Std. Error	t value	Pr(>|t|)
B	6.6011	0.1087	60.71	< 2e-16 ***
D	0.17907	0.01455	12.31	3.35e-10 ***

r^2=0.9606285

MovieS7, 296-400min (*cell moving to cluster located at the bottom part of the frame)*
A) fgf8a.gfp cell (blue dot) B) fgf8a.gfp cell (yellow dot) C) Relative track A) to B)
D) MSD(τ) plot : Random displacement





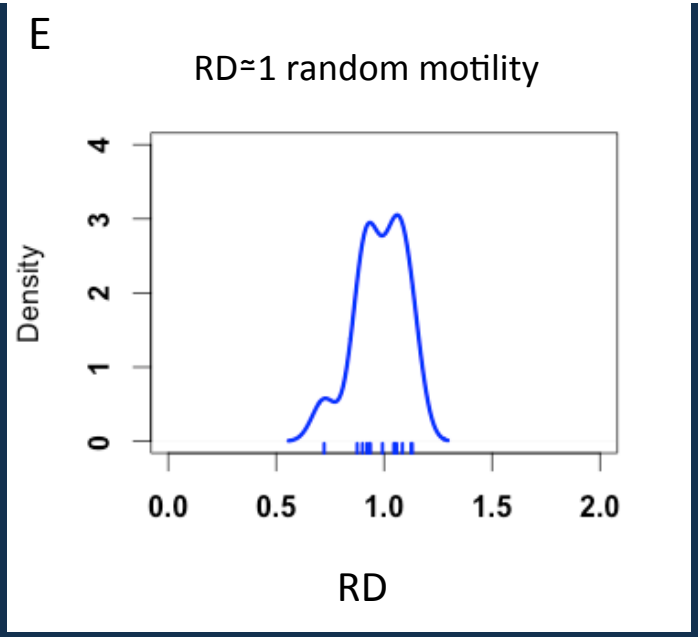
MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.325832	0.005924	55	1.09e-12 ***

##########
α=1.0727
MSD ~ {6*D*T} (red line)

	Estimate	Std. Error	t value	Pr(>|t|)
D	0.342860	0.007438	46.1	6.13e-14***

r^2=0.9938784

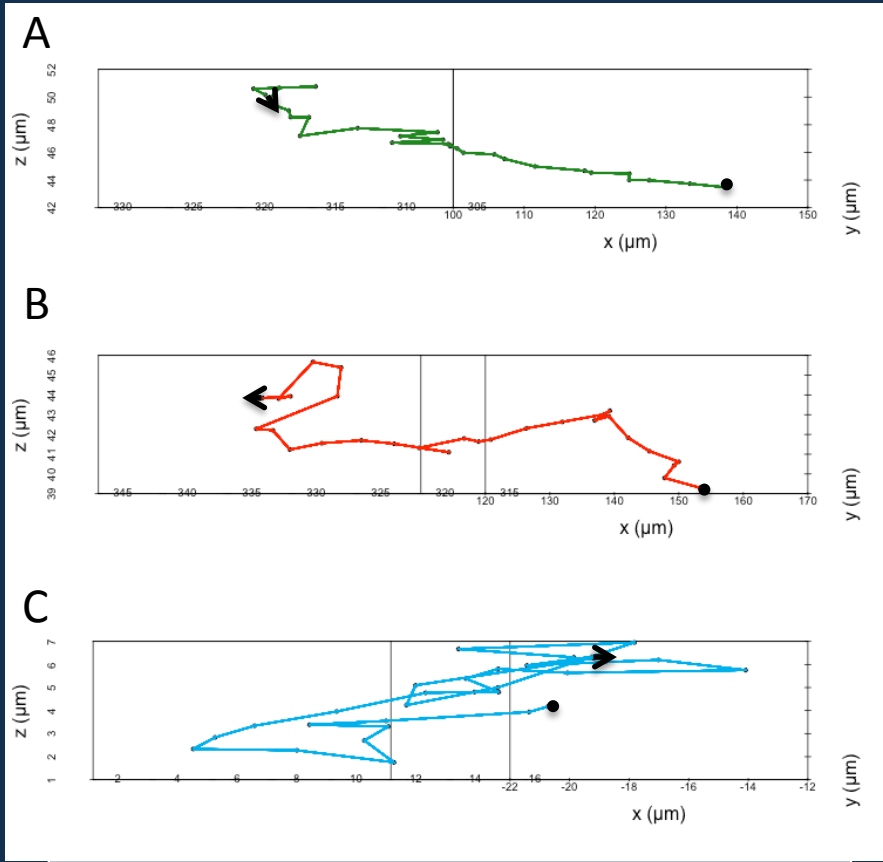


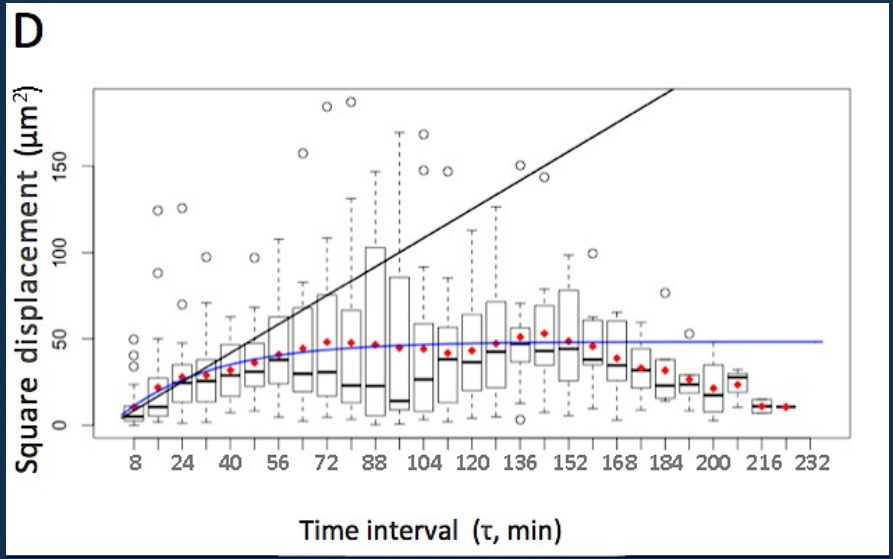
MOVIE S8
ascl1a-/-; fgf8a.gfp; tph1b.mCherry

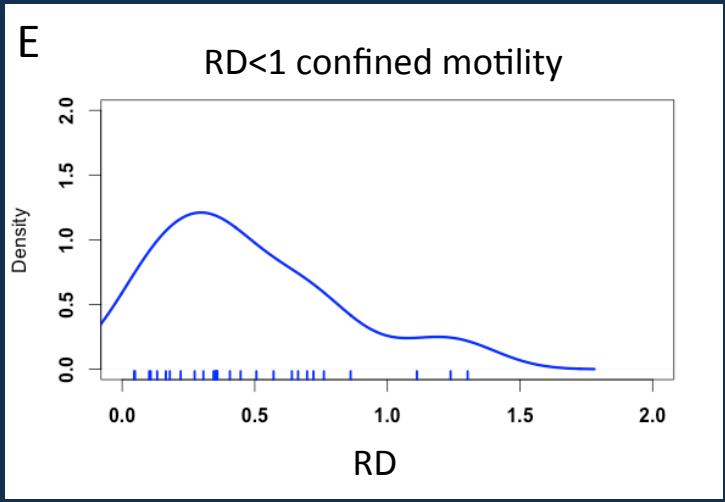
Representative examples of fgf8a cell tracks in the ascl1a-/- mutant. The relative displacement of these cells is confined. Pg.16 shows the relative displacement of fgf8a cell (red dot) and fgf8a cell (blue dot - reference cell).

Development • Supplementary information

Movie S8, 0min-3h44min *(fgf8a cell rearrangement in ascl1a-/- mouth epithelium).*
A) fgf8a.gfp cell (blue dot) B) fgf8a.gfp cell (red dot) C) Relative track A) to B)
D) MSD(τ) plot : Confined displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.17375	0.02022	8.592	0.0133 *

##############
α=0.4384
MSD ~ {B^2*(1-exp(-6*D*T/B^2))} (blue line)

	Estimate	Std. Error	t value	Pr(>|t|)
B	6.94216	0.08273	83.91	< 2e-16 ***
D	0.26033	0.02017	12.91	1.55e-10 ***

r^2=0.9632

MOVIE S9
WT; plcb2.gfp; tph1b.mCherry

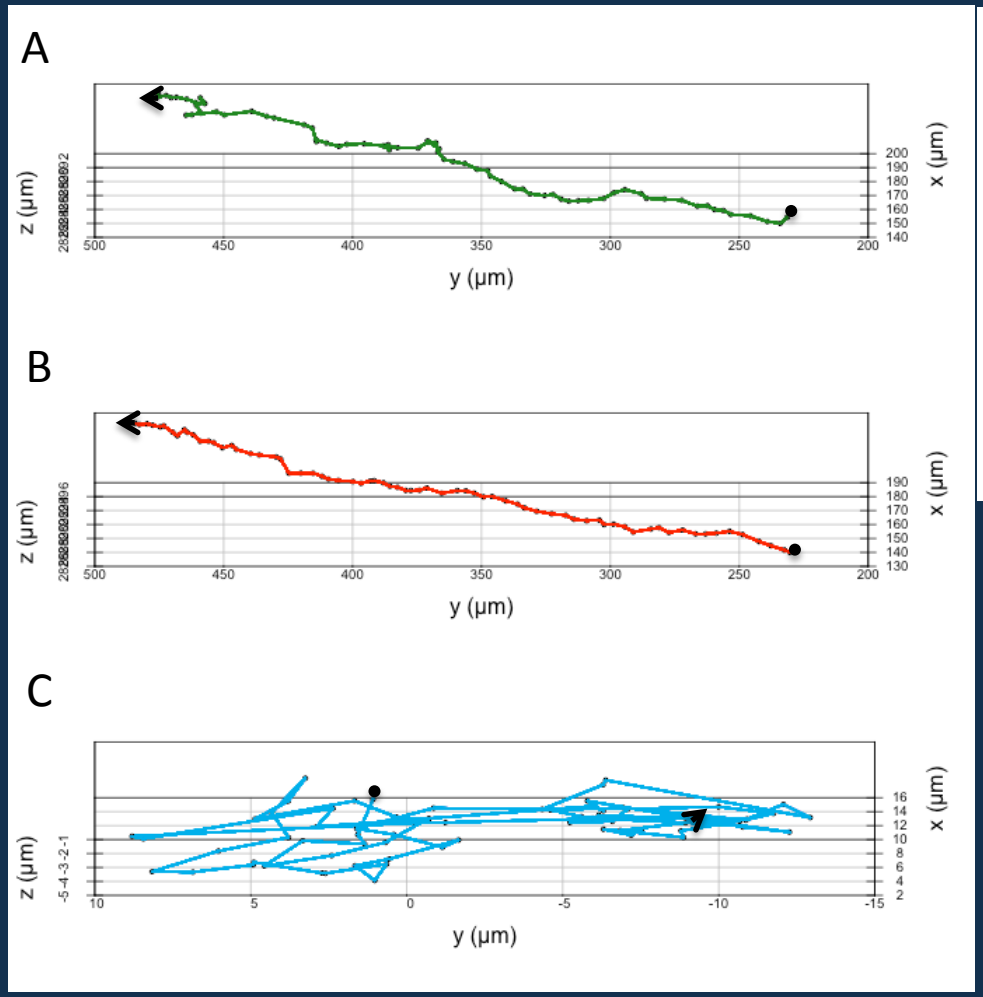
Pg.18: Plcb2 cell (yellow dot) maintaining contact with a tph1b cell (red dot); they have confined relative displacement as in the case of fgf8a cells (pg.10)

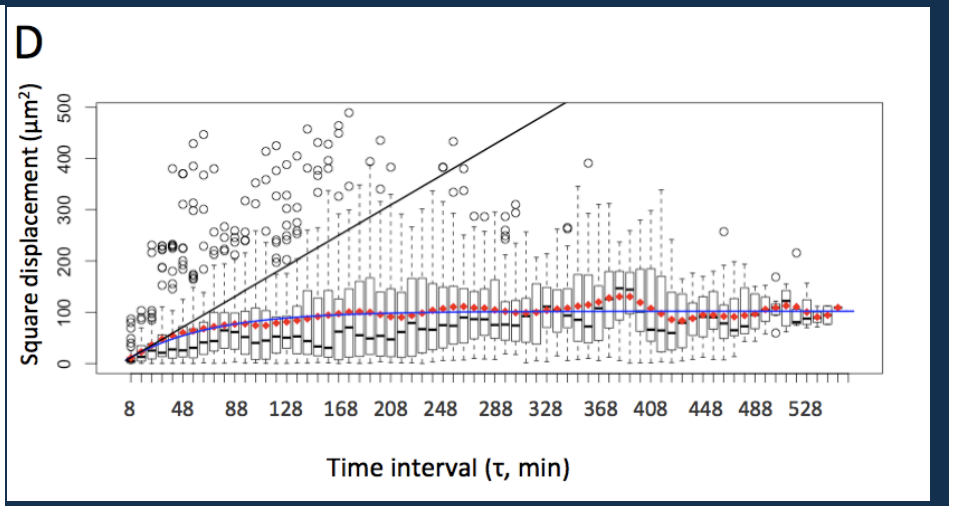
Pg.19: Plcb2 cell (white dot) explores space and joins a tph1b cell (pink dot) in a directed relative mode (256-368min). During the early period of contact maintenance (376-480min) their relative displacement was also directed (pg.20), as in the case of fgf8a/tph1b cells (pg. 4).

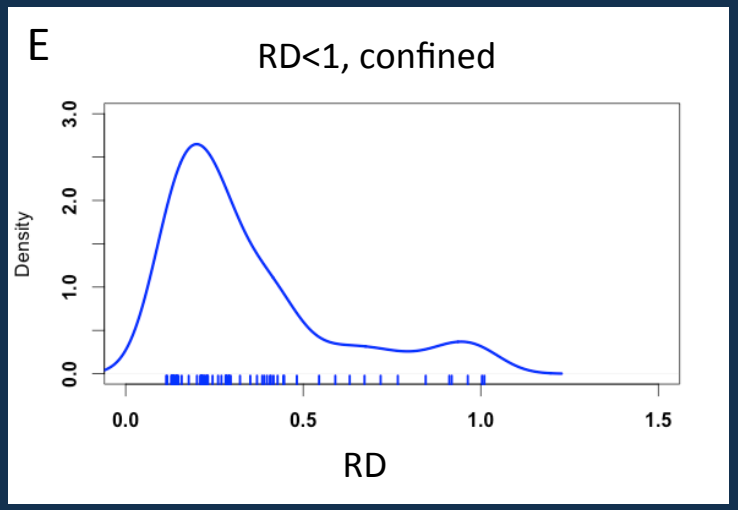
Pg.21: 16-312min: the plcb2 cell (blue dot) joins the tph1b cell (pink dot) in random relative displacement

Development • Supplementary information

Movie S9, 96-648min *(plcb2 cell in contact with tph1b cell constantly, additional images used, not shown in the movie)* A) plcb2.gfp cell (yellow dot) B) tph1b.mCherry cell (red dot)
C) Relative track A) to B) D) MSD(τ) plot : Confined displacement







MSD ~ {6*D*T} (black line)

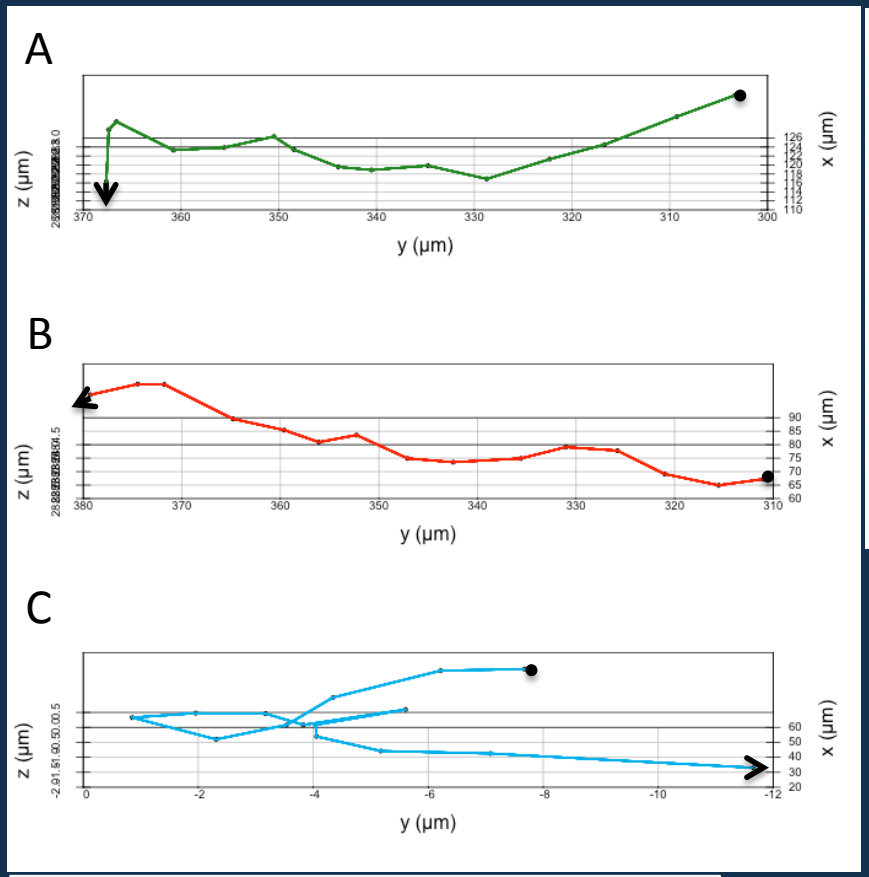
	Estimate	Std. Error	t value	Pr(>|t|)
D	0.247706	0.002614	94.76	0.000111 ***

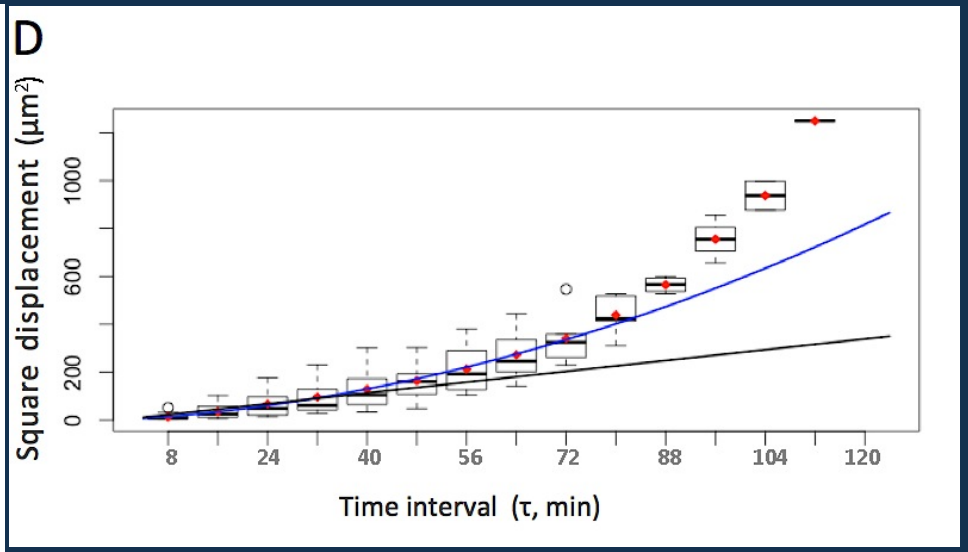
α=0.3195
MSD ~ {B^2*(1-exp(-6*D*T/B^2))} (blue line)

	Estimate	Std. Error	t value	Pr(>|t|)
B	10.11253	0.06293	160.69	<2e-16 ***
D	0.27190	0.01624	16.75	<2e-16 ***

r^2=0.886426

Movie S9, 256-368min *(plcb2.gfp cell (white dot) explores space and joins tph1b cell-pink dot)*
A) plcb2.gfp cell (white dot) B) tph1b.mCherry cell (pink dot) C) Relative track A) to B)
D) MSD(τ) plot : Directed displacement





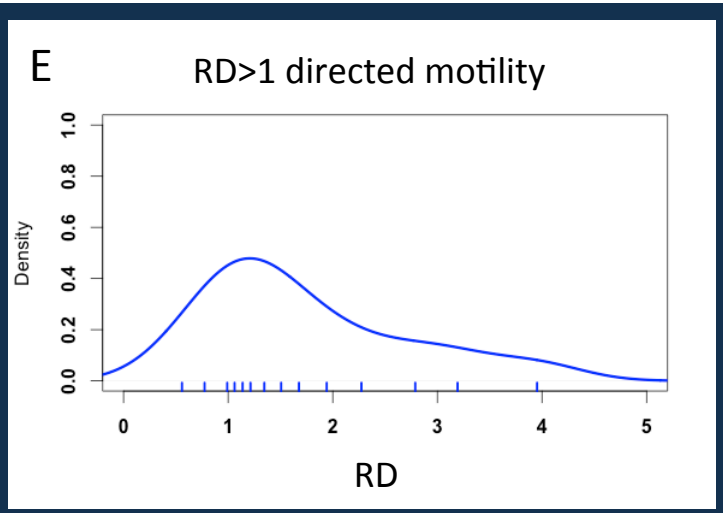
MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)	
DT2-T4	0.47040	0.03175	14.82	0.00452	**

##############
α=1.4801
MSD ~ {6*D*T+V^2*T^2} (blue line)

	Estimate	Std. Error	t value	Pr(>|t|)	
V	0.21135	0.00628	33.655	4.58e-08	***
D	0.24117	0.02620	9.206	9.27e-05	***

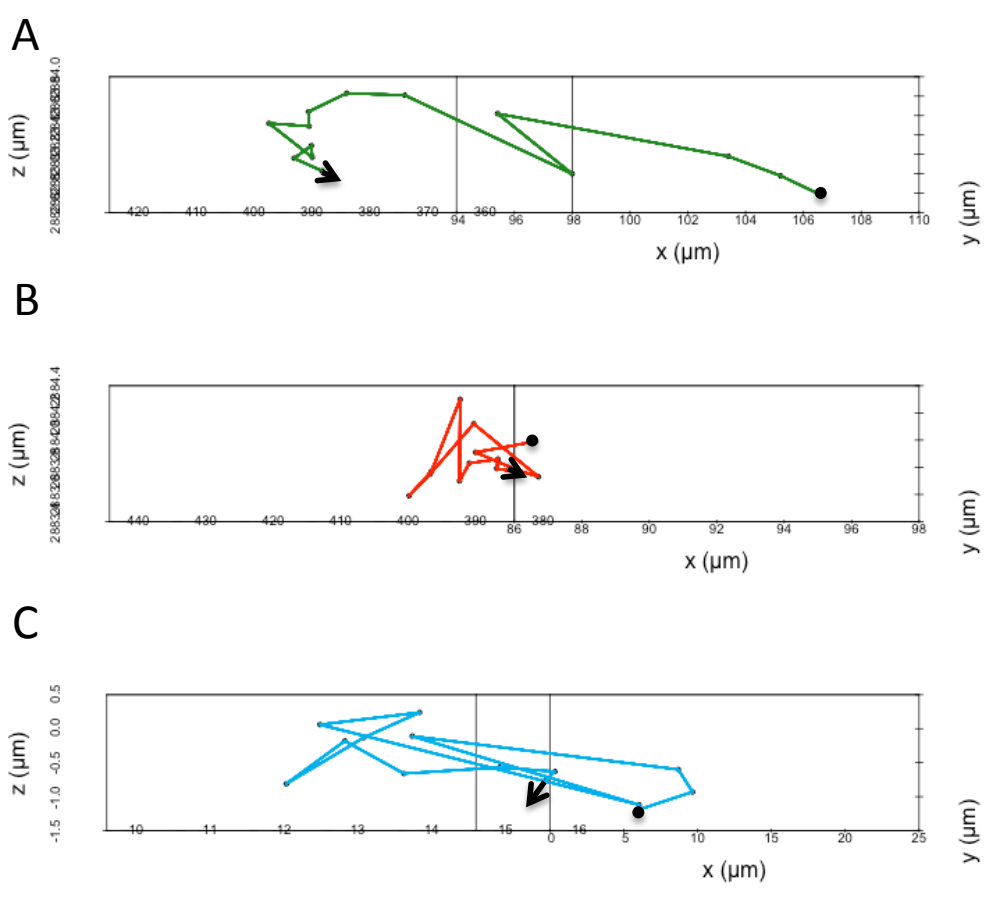
r^2=0.9999106

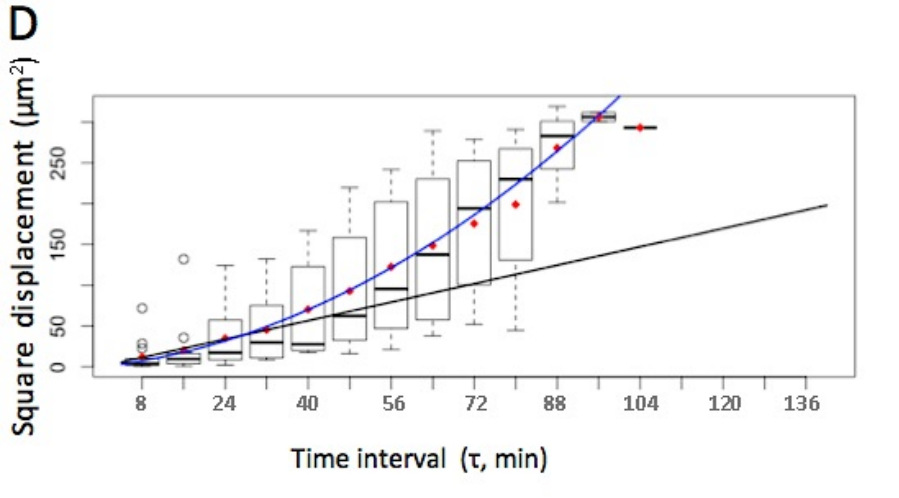


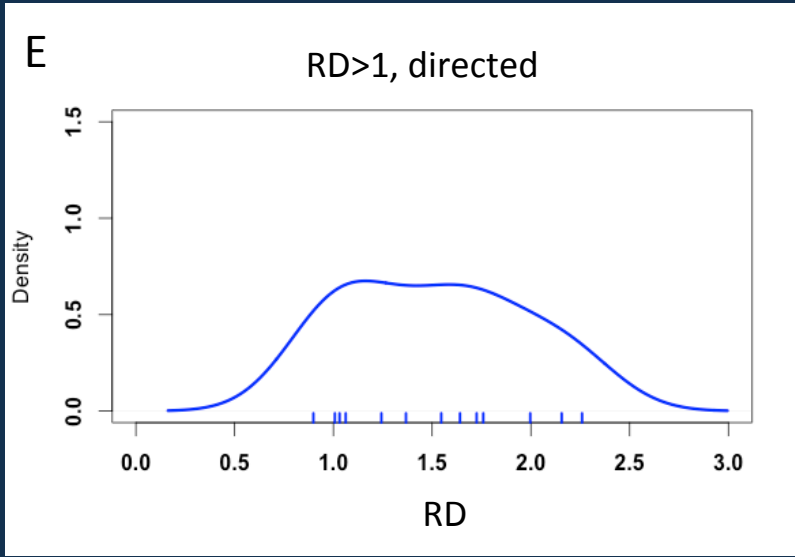
Movie S9, 376-480min *(plcb2.gfp cell -white dot in contact with tph1b cell -pink dot)*

A) plcb2.gfp cell (white dot) B) tph1b.mCherry cell (pink dot) C) Relative track A) to B)

D) MSD(τ) plot : Directed displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.235587	0.00696	33.82	0.000873 ***

##########

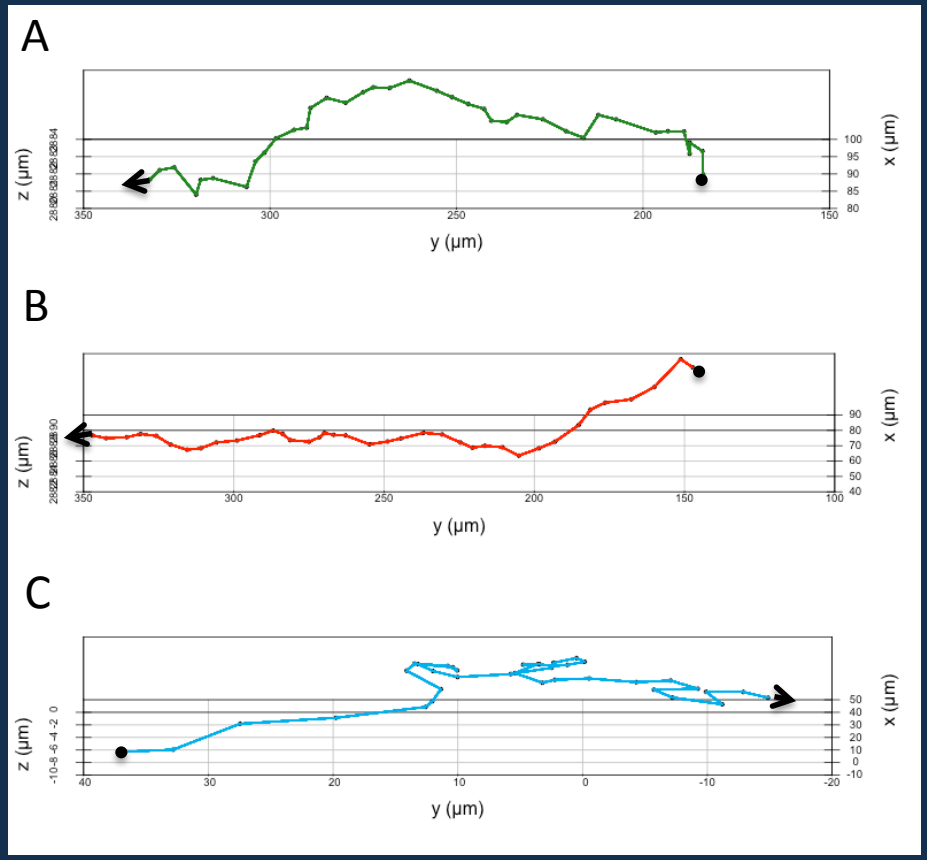
α=1.3522

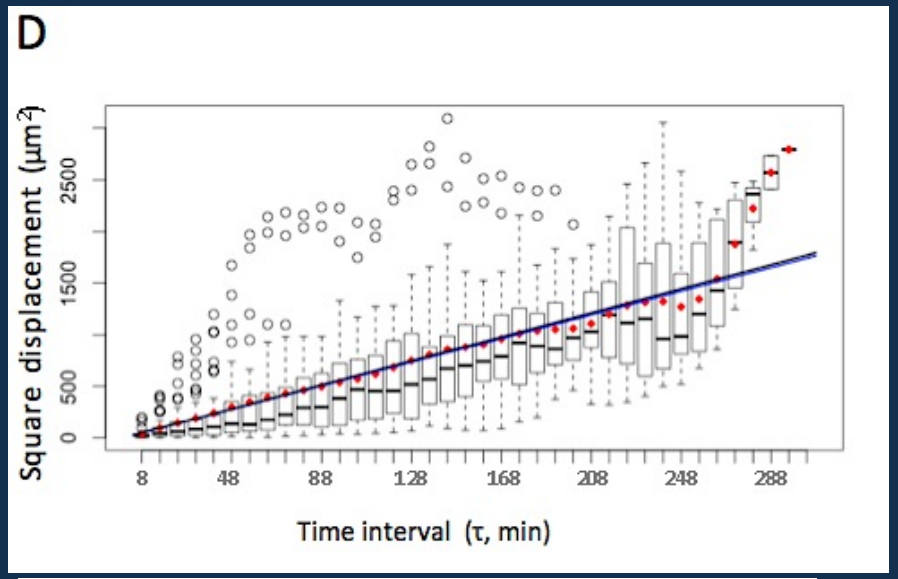
MSD ~ {6*D*T+V^2*T^2} (blue line)

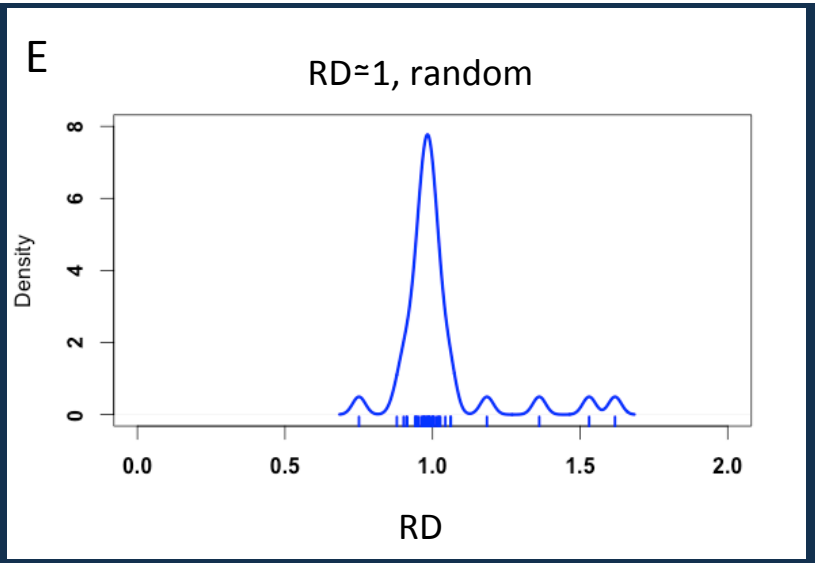
	Estimate	Std. Error	t value	Pr(>|t|)
V	0.161620	0.002585	62.51	4.77e-12 ***
D	0.11732	0.01081	0.85	4.60e-06 ***

r^2=0.9993496

Movie S9, 16-312min *(plcb2.gfp cell-blue dot joins tph1b.mCherry cell -pink dot)*
A) plcb2.gfp cell (blue dot) B) tph1b.mCherry cell (pink dot) C) Relative track A) to B)
D) MSD(τ) plot : Random displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.971573	0.003425	283.7	1.24e-05 ***

##########
α=1.026
MSD ~ {6*D*T} (blue line)

	Estimate	Std. Error	t value	Pr(>|t|)
D	0.955910	0.004272	223.8	<2e-16 ***

r^2=0.9996081

MOVIE S10
ascl1a-/-; plcb2.gfp; tph1b.mCherry

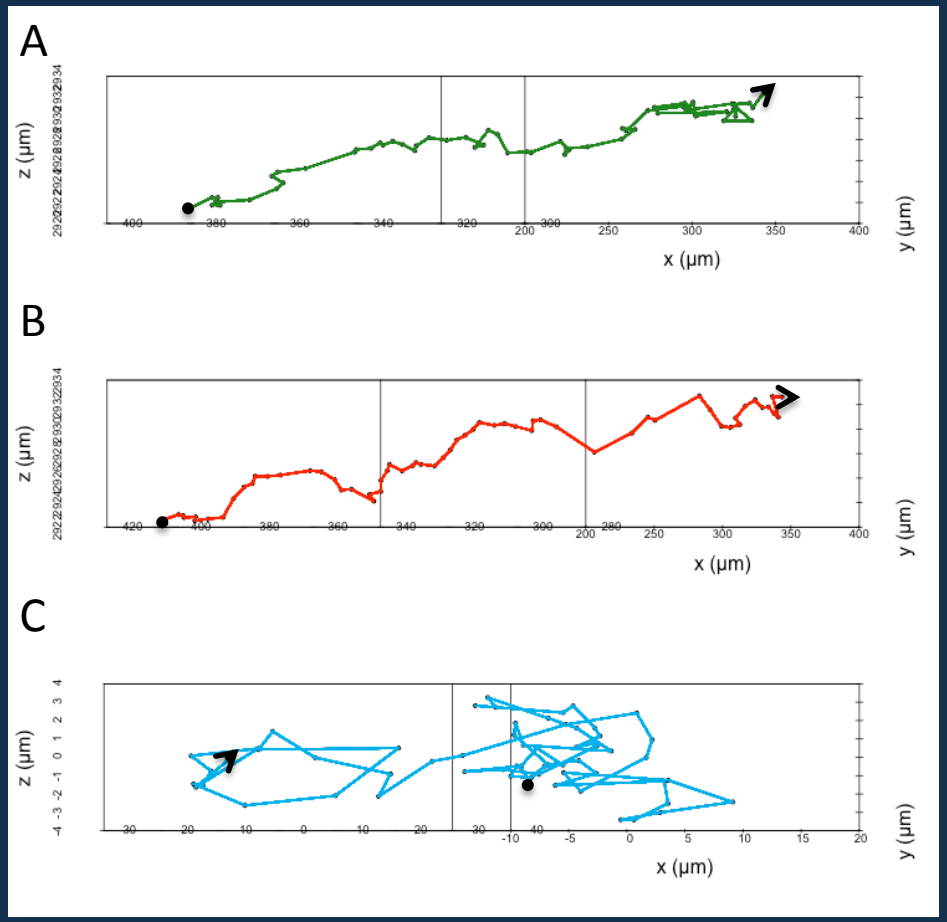
The plcb2 cells in the ascl1a-/- mutant have more complex patterns of displacement compared to the wild-type (see tracks on this Movie and pg.24). Four examples are analyzed here, showing profiles of displacement while the cells assemble.

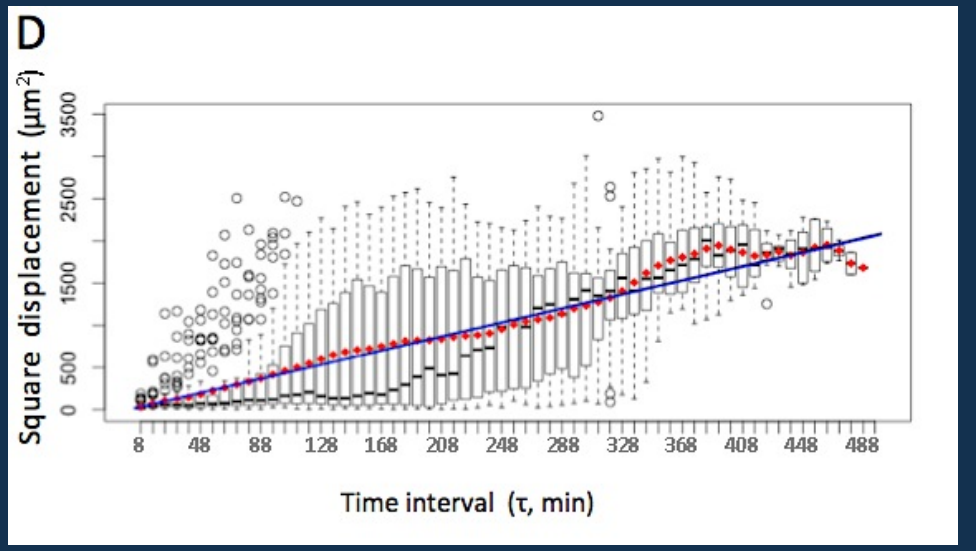
Group of plcb2 cells at the bottom of the frame:
Pg.23: 0-8h08min: The relative displacement of the plcb2 cells (green and pink dots) is random.
Pg.24: The plcb2 cell (yellow dot) has a complex displacement plot relative to the plcb2 cell (pink dot).
Pg.25: The first phase (0-4h56min) of plcb2 cells (yellow and pink dots) displacement is confined. Later, as the plcb2 cells (pink and green dots) swap positions, the plcb2 cell (green dot) becomes the reference cell (at the center of the group). The relative displacement of plcb2 cells (yellow and green dots) is random (5h52min-8h08min, pg.26).

Pg.27: The plcb2 cell (blue dot) quitted its neighbour and joins the plcb2 cell (red dot, reference) in a directed mode. Same case if the reference cell is the plcb2 white dot cell (not shown).
This is in contrast with the fgf8a cells (green and red dots) in Movie S7 pg.12.

Development • Supplementary information

Movie S10, 0-8h08min *(cells at the bottom of the frame)*.
A) plcb2.gfp cell (green dot) B) plcb2.gfp cell (pink dot) C) Relative track A) to B)
D) MSD(τ) plot : Random displacement





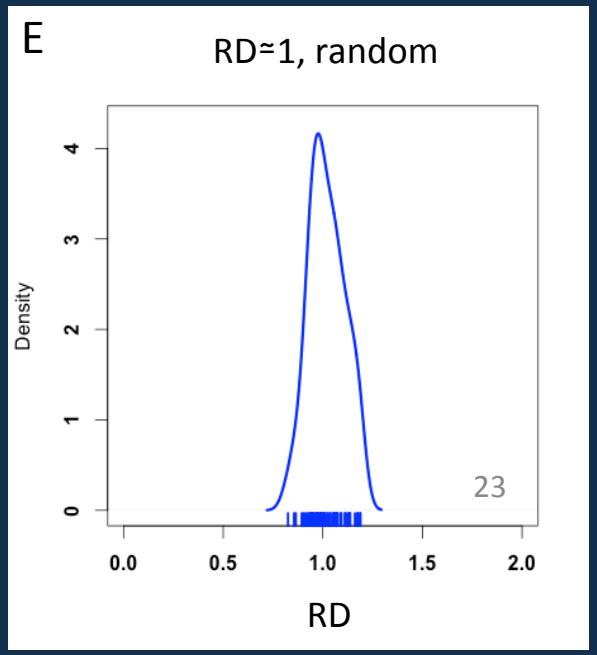
MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)	
DT2-T4	0.69617	0.03664	19	0.00276	**

########
α=1.004
MSD ~ {6*D*T} (blue line)

	Estimate	Std. Error	t value	Pr(>|t|)	
D	0.690830	0.004402	157	<2e-16	***

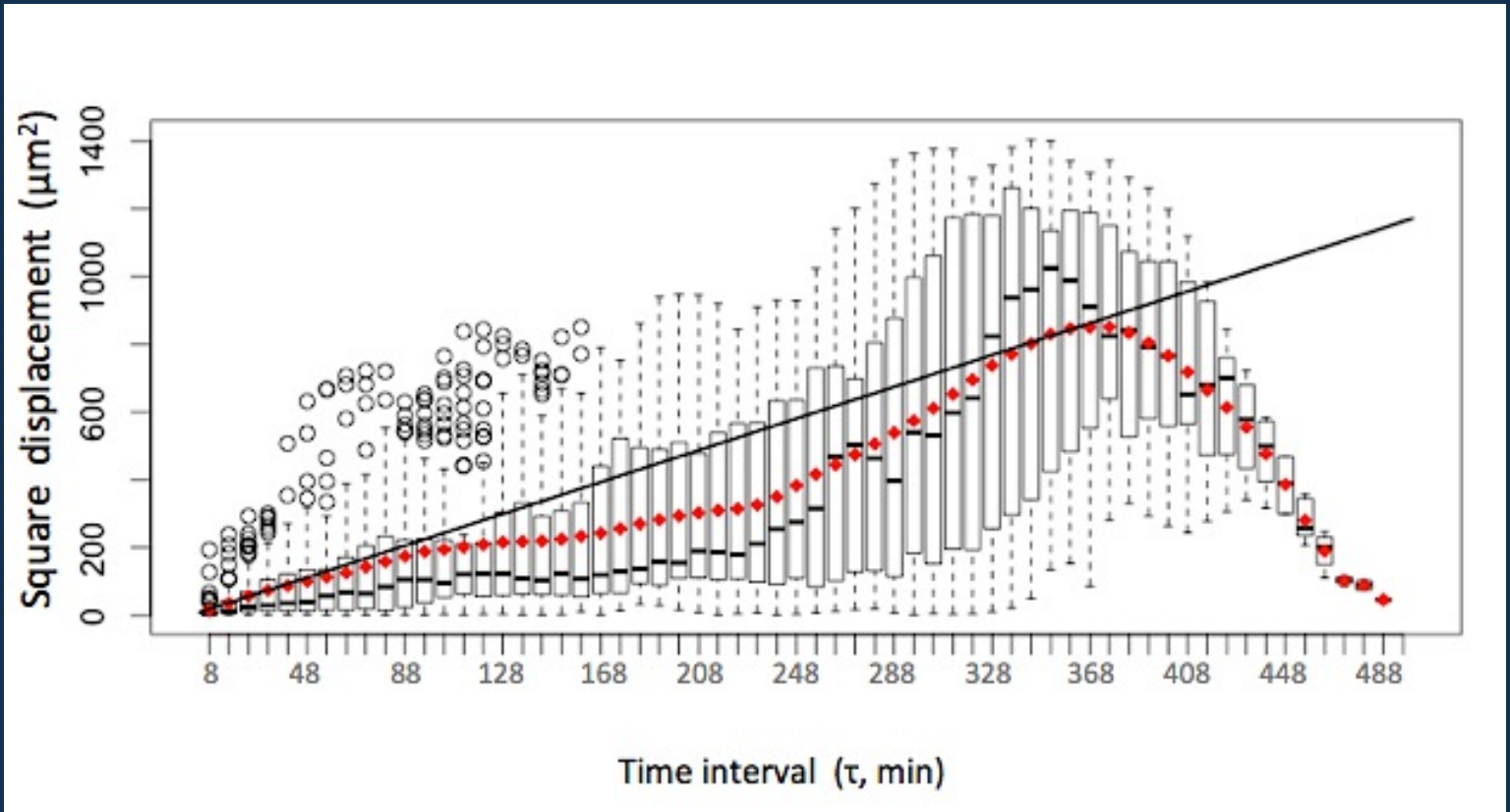
r^2=0.9960918



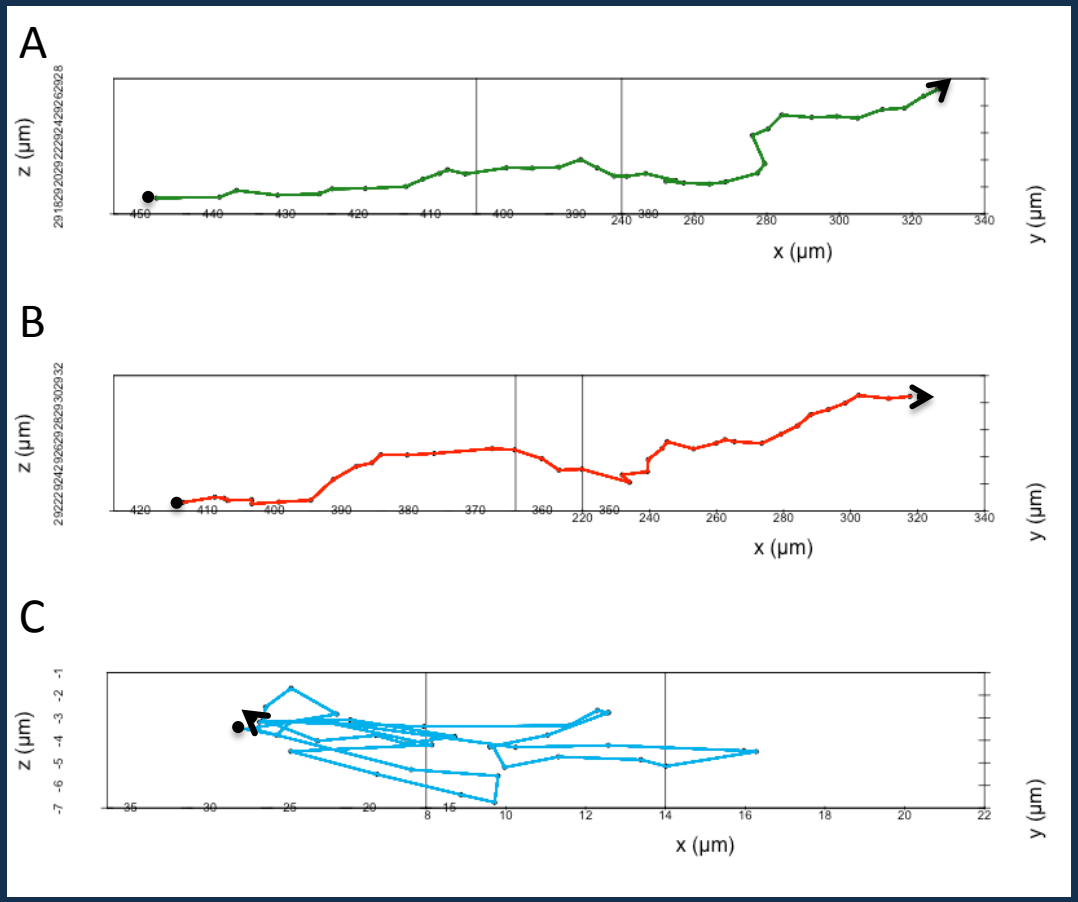
Movie S10, 0-8h08min *(cells at the bottom of the frame)*.

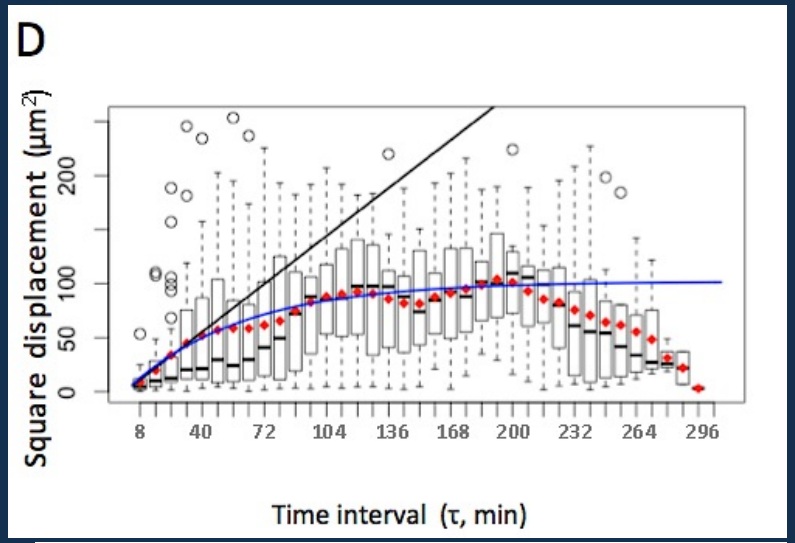
A) plcb2.gfp cell (yellow dot) B) plcb2.gfp cell (pink dot) C) Relative track A) to B)

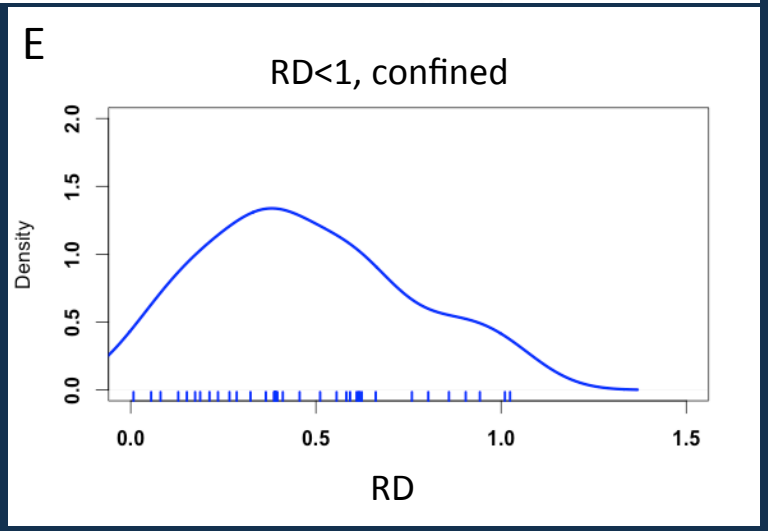
D) MSD(τ) plot : No evident pattern, rather hides more than one modes (see next slide).



Movie S10, 0-4h56min *(cells at the bottom of the frame)*.
A) plcb2.gfp cell (yellow dot) B) plcb2.gfp cell (pink dot) C) Relative track A) to B)
D) MSD(τ) plot : Confined displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.230607	0.006311	36.54	0.000748 ***

##########
α=0.3911
MSD ~ {B^2*(1-exp(-6*D*T/B^2))} (blue line)

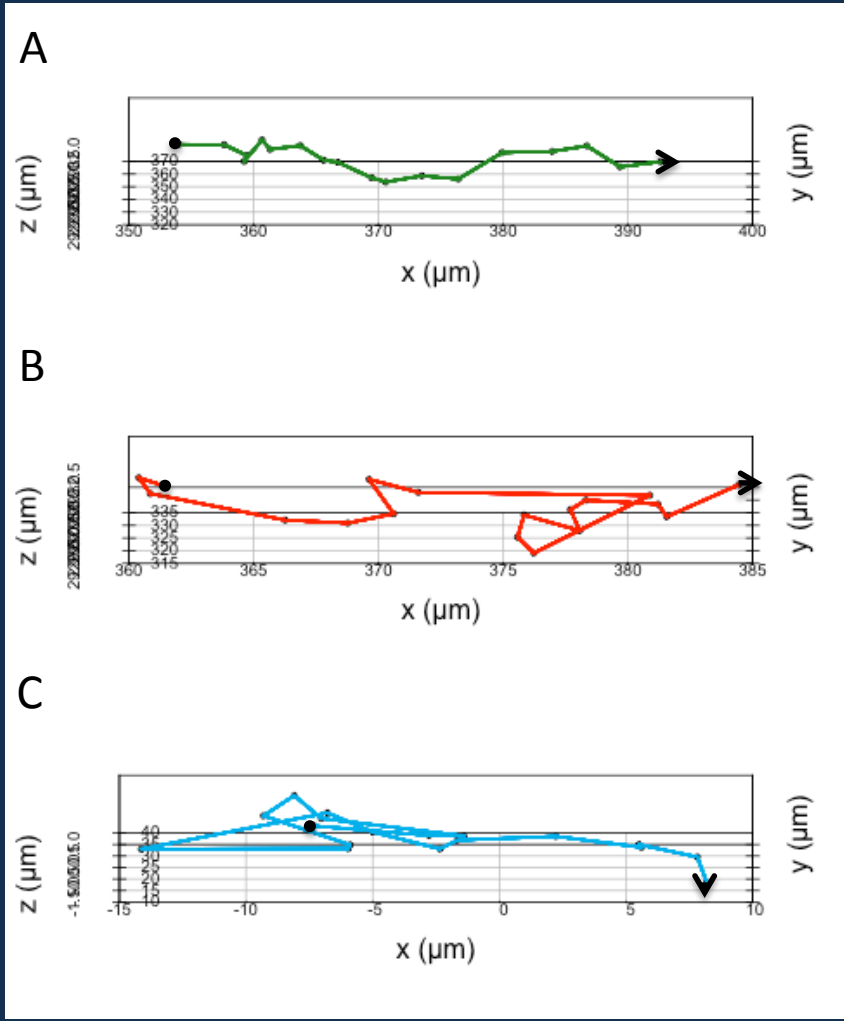
	Estimate	Std. Error	t value	Pr(>|t|)
B	10.0960	0.1018	99.14	< 2e-16 ***
D	2.2850	0.1022	22.37	1.38e-14 ***

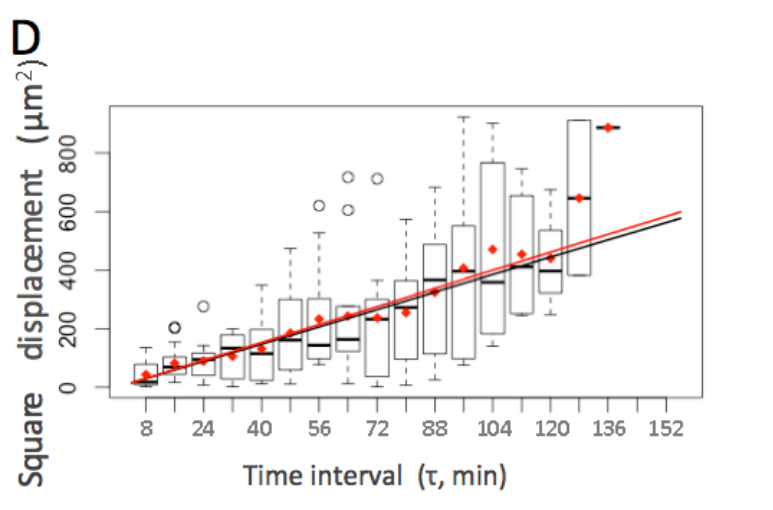
r^2= 0.9902818

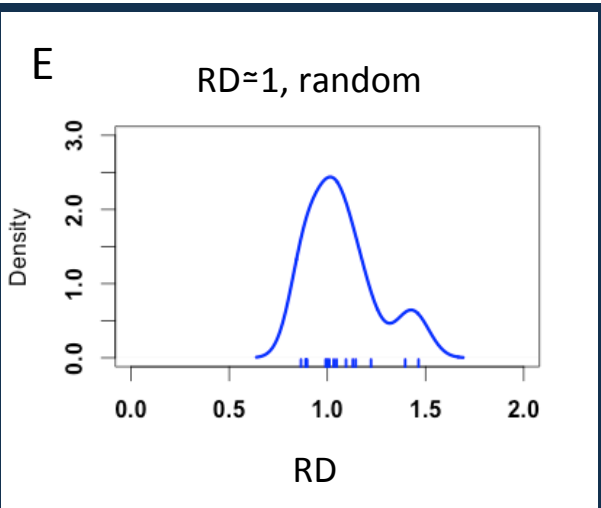
Movie S10, 5h52min-8h08min *(plcb2 cells at the bottom of the frame: cell-green dot and cell-pink dot swap position, cell-green dot in the center of the group and reference).*

A) plcb2.gfp cell (yellow dot) B) plcb2.gfp cell (green dot) C) Relative track A) to B)

D) MSD(τ) plot : Random displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)
DT2-T4	0.61669	0.07263	8.491	0.0136 *

α=1.076

MSD ~ {6*D*T}(red line)

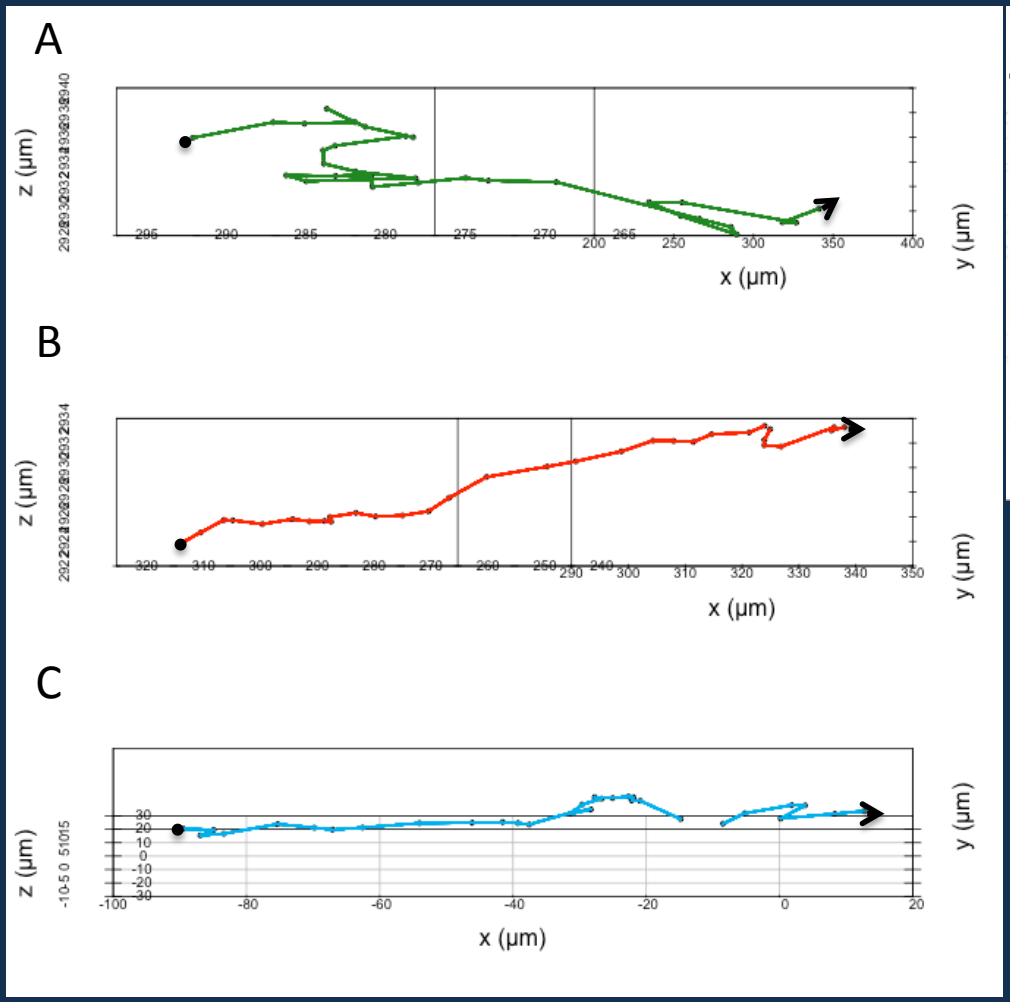
	Estimate	Std. Error	t value	Pr(>|t|)
D	0.64006	0.01466	43.67	1.35e-14 ***

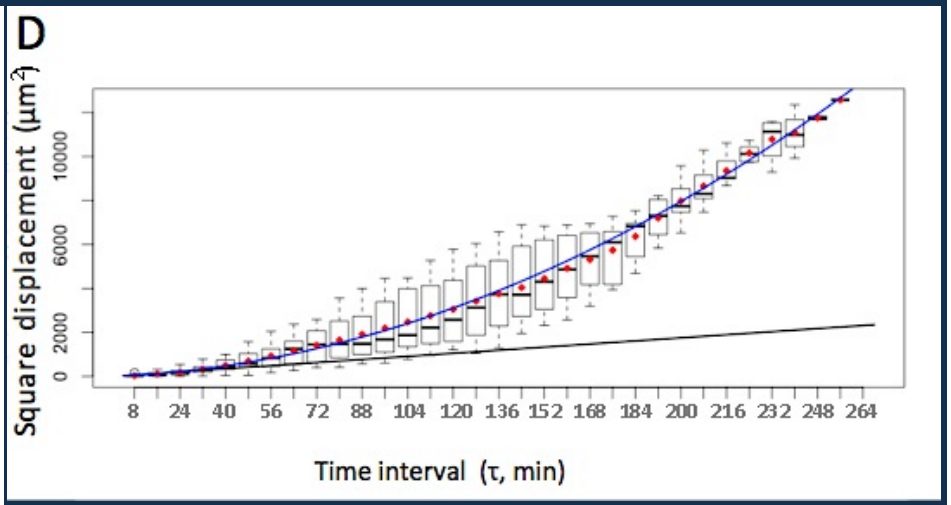
r^2=0.9927551

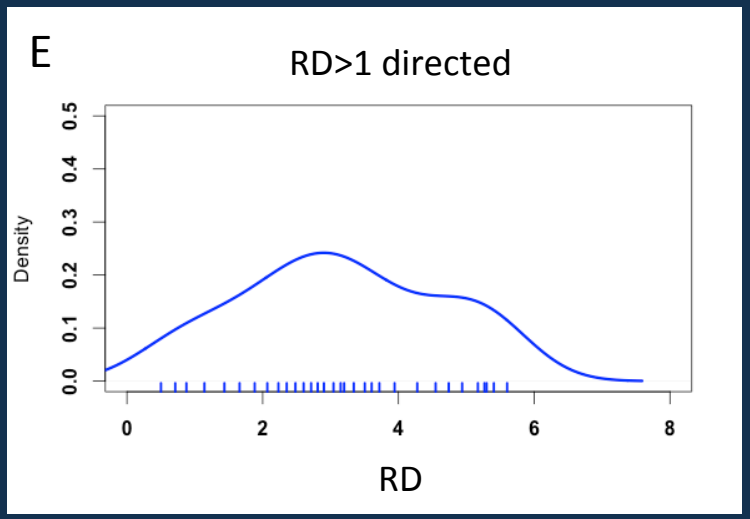
Movie S10, 2h32min-6h48min *(plcb2.gfp cell -blue dot joins plcb2.gfp cell-red dot)*

A) plcb2.gfp cell (blue dot) B) plcb2.gfp cell (red dot) C) Relative track A) to B)

D) MSD(τ) plot : Directed displacement







MSD ~ {6*D*T} (black line)

	Estimate	Std. Error	t value	Pr(>|t|)	
DT2-T4	1.4608	0.1709	8.547	0.0134	*

α=1.7320

MSD ~ {6*D*T+V^2*T^2} (blue line)

	Estimate	Std. Error	t value	Pr(>|t|)	
V	0.415714	0.002031	204.70	< 2e-16	***
D	1.054763	0.055705	18.93	2.26e-11	***

r^2=0.9999293

Development • Supplementary information