Supplemental Information

Supplemental Figure legends

Figure S1. Cell-type specific knock down of E-cadherin. Related to Figure 1. (A) Ecad expression (red) in a cluster expressing GFP specifically in polar cells (*), not outer cells, using UpdGal4. (A') a schematic representation of the image in A. (B-C') Normal distribution of Ecad (green in B and C', pseudo-colored in Rainbow RGB in C). (D-E') Ecad distribution following polar cell-specific Ecad RNAi. In B and D, UpdGal4 drives nuclear dsRed to mark polar cells. (F) Nurse cell-specific expression of GFP by the TripleGal4 driver. Border cells are labeled with SN (red) and nuclei with DAPI (blue). (F') Schematic of image shown in F. (G, H) Ecad (white) distribution in control (G) and following germ cell specific RNAi (H). Border cell cluster positions are indicated (arrows).

Figure S2. Functional characterization of the E-cadherin tension sensor and control. Related to Figure 3. (A-C) Confocal micrographs of stage 10 egg chambers labeled with Armadillo (red) and DAPI (blue). Border cell migration defects and β-catenin loss caused by germline knockdown of E-cadherin (A) can be rescued by Cad^{TS} (B) or the load-insensitive control (C). (D) Expression of Cad^{TS} and control in the front and back of the cluster. Error bars show SEM. (E) Time-lapse imaging of border cells expressing the GCaMP5 calcium indicator following addition of ionomycin. (F) Quantification of GCaMP5 intensity increase after addition of ionomycin (n=5) compared with baseline level (n=3). Error bars show SEMs. Cad^{TS} FRET index of the front (G) or back (H) of the cluster after

treatment with ionomycin or Y-27632. Error bars show SEMs. *p<0.05. (I) FRET indices for the back of the cluster comparing slboGal4 (+) to slboGal4; UASRac^{DN} for the tension sensor vs the load-insensitive control. (J) Histograms showing Cad^{TS} FRET index of the front or back of the cluster after DMSO or cytochalasin D treatment. Error bars show SEMs. (K) Front to back ratio of Cad^{TS} FRET after DMSO or cytochalasin D treatment. Error bars show SEMs. (L) Venus and mTFP Images before and after Venus photobleaching. ROI shows region of bleaching. Scale bar: 20μm. (M) Relative mTFP intensity before and after Venus photobleaching. ***p<0.001. Error bar shows SEM.

Figure S3. Defining boundary displacements in consecutive frames for morphodynamic profiling. Related to Figure 4. (A) GFP and RFP channels overlaid by initial segmentation using image smoothing and thresholding. Note that weaker pseudopodial regions can be detached from the cluster body. (B) Convex hull around all mask fragments. (C) Blue region indicates the difference map between convex hull and initial masks. (D) Refined mask (see text), defining the cluster outline in this particular frame. (E) Morphing boundary outlines between consecutive frame pairs. Black boundary displacement vectors are calculated by simple propagation along the boundary normal, without topological constraints (i.e. displacement vectors are allowed to cross). Yellow circles indicate intersections between the boundary at t (red) and the boundary at t+1 (green). (F–H) Zoom-up of a boundary sector with a salient protrusion (top row) or retraction (bottom row) event. Blue boundary displacement vectors are

calculated by minimizing Eq. S1 subject to the topological constraint that vectors cannot intersect (Eq. S2). The three scenarios illustrate effect of balancing boundary displacement vs. boundary strain. (I) Application of the topologically constrained minimization to morph between the boundaries in (E).

Figure S4. Border cell clusters deficient in expression of E-cadherin exhibit morphodynamic shifts identical to border cell clusters expressing dominant negative guidance receptors. Related to Figure 5. Morphodynamic activity maps of EcadRNAi (A) and EcadRNAi+RTK^{DN} (B). Comparison of fractions of protruding and retracting sectors at cluster front and back between border cell clusters expressing RTK^{DN}+EcadRNAi vs. EcadRNAi (C-F) and RTK^{DN} vs. RTK^{DN}+EcadRNAi (G-J). P-values of randomization test indicated in green, on the same axis as the fractions.

Figure S5. Identification of components in border cell-border cell communication. Related to Figure 7. (A-U) Confocal images of border cells before (A, D, G, J, M, P, S) and after 30 minutes of Rac photo-inactivation (B, E, H, K, N, Q, T). (C, F, I, L, O, R, U) Overlay of two images. UAS-PA-RacT17N and UAS-RNAi are driven by slboGal4 in (A-O) and hsFlp, AyGal4 in (P-U). The photo-treated region is shown as a dotted circle and white arrows indicate the normal direction of border cell migration.

Supplemental Movie Legends:

Movie S1 Normal border cell migration in slboGal4, UASdsRed; UASmCD8GFP. The egg chamber is outlined in green.

Movie S2 Off-track border cell migration in slboGal4, UASdsRed; UASEcadRNAi/UASmCD8GFP.

Movie S3 Cluster disassembly in UpdGal4, UASEcadRNAi, UASdsRed, slboLifeact-GFP.

Movie S4 Normal border cell migration in TripleGal4, slboLifeact-GFP.

Movie S5 Off-track migration in TripleGal4, UASEcadRNAi, slboLifeact-GFP

Movie S6 A second example of off-track migration in TripleGal4, UASEcadRNAi, slboLifeact-GFP.

Movie S7 Hyper-polarized border cells in TripleGal4, UASEcad, slboLifeact-GFP.

Movies S8 Illustration of the construction of a morphodynamic activity map based on the example of a wild type border cell cluster in slboGal4, UASdsRed, mCD8GFP. Top panel: border cell cluster overlaid with computationally tracked cluster outline. Bottom panel: morphodynamic profile; red colors indicate fast protrusion velocity; blue colors indicate fast retraction velocity.

Supplemental tables

Table S1. List of Primers Used

#	**Primer sequence**
1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCCACCAGTGTCCAGC
2	GGGGACCACTTTGTACAAGAAAGCTGGGTGCTAGATGCGCCAGCCCTG
3	ACGACATTCGCGAGACGATCGGTACCTCAGGCGGCCGCTATTAATTACGAGGACGAGGGTG
4	CACCCTCGTCCTCGTAATTAATAGCGGCCGCCTGAGGTACCGATCGTCTCGCGAATGTCGT
5	ATGTGGTACCAGTGTGGTGGAATTCATGGTG
6	TCGAGCGGCCGCATACTTGTACAGCT
7	CAGGGCTGGCGCATCGGTACCTAGCACCCAGCTTTCTTGTAC
8	GTACAAGAAAGCTGGGTGCTAGGTACCGATGCGCCAGCCCTG
9	AGGGCTGGCGCATCGGTACCGGTGGAGGTAGTGTGGTGGAATTCATGGTG
10	AAAGCTGGGTGCTAGGCCGCATACTTGTACAGCT
11	TCGAGCGGCCGCTGGCCCCTTGTACAGCT
12	ATGTGGTACCATGGTGAGCAAGGGCGA
13	AGTCGGTACCATGGGTGTCGCAGATTTGATC
14	AGTCGGTACCTTACTTGTACAGCTCGTCCATGCC
15	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGGGTGTCGCAGATTTGATC
16	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAGCGCCTGTGCTATGTCTGCCC

Table S2. Summary of Movies for each genotype

Genotype	Number of Movies
WT	17
$EGFR^{DN}$	16
PVR^{DN}	16
RTK^{DN}	9
Rac^{DN}	12
RTK^{DN}, Rac^{DN}	17
RTK^{DN}, EcadRNAi	11
EcadRNAi	8
Tie^{DN}	15
RTK^{DN}, Tie^{DN}	8
PVR^{DN}, Tie^{DN}	9

Table S3. 26 Morphodynamic Profiling Features (MPF)

Feature Index	Feature Name	Feature Definition
MPF 1	Front Protrusion Percentage	Percentage of protruding segments at cluster front*
MPF 2	Front Retraction Percentage	Percentage of retraction segments at cluster front*
MPF 3	Back Protrusion Percentage	Percentage of protruding segments at cluster back*
MPF 4	Back Retraction Percentage	Percentage of retraction segments at cluster back*
MPF 5	Avg Front Protrusion Velocity	Average velocity of protruding segments at cluster front
MPF 6	25% Front Protrusion Velocity	25th percentile velocity of protruding segments at cluster front
MPF 7	50% Front Protrusion Velocity	50th percentile velocity of protruding segments at cluster front
MPF 8	75% Front Protrusion Velocity	75th percentile velocity of protruding segments at cluster front
MPF 9	Avg Front Retraction Velocity	Average velocity of retracting segments at cluster front
MPF 10	25% Front Retraction Velocity	25th percentile velocity of retracting segments at cluster front
MPF 11	50% Front Retraction Velocity	50th percentile velocity of retracting segments at cluster front
MPF 12	75% Front Retraction Velocity	75th percentile velocity of retracting segments at cluster front
MPF 13	Avg Back Protrusion Velocity	Average velocity of protruding segments at cluster back
MPF 14	25% Back Protrusion Velocity	25th percentile velocity of protruding segments at cluster back
MPF 15	50% Back Protrusion Velocity	50th percentile velocity of protruding segments at cluster back
MPF 16	75% Back Protrusion Velocity	75th percentile velocity of protruding segments at cluster back
MPF 17	Avg Back Retraction Velocity	Average velocity of retracting segments at cluster back
MPF 18	25% Back Retraction Velocity	25th percentile velocity of retracting segments at cluster back
MPF 19	50% Back Retraction Velocity	50th percentile velocity of retracting segments at cluster back
MPF 20	75% Back Retraction Velocity	75th percentile velocity of retracting segments at cluster back
MPF 21	Mean of Fragment Velocity at Cluster Front	Average velocity of all segments at cluster front
MPF 22	Standard Deviation of Fragment Velocity at Cluster Front	Standard deviation of all segments at cluster front
MPF 23	Mean of Fragment Velocity at Cluster Back	Average velocity of all segments at cluster back
MPF 24	Standard Deviation of Fragment Velocity of at Cluster Back	Standard deviation of all segments at cluster back
MPF 25	Average Protrusion Velocity (Whole Cluster)	Average velocity of protruding segments along cluster boundary
MPF 26	Average Retraction Velocity (Whole Cluster)	Average velocity of retracting segments along cluster boundary

* Each percentage feature is calculated with velocity threshold=2, when fragment with absolute protrusion velocity smaller than two pixels per frame was assigned with zero velocity.

Supplemental methods

1. Pharmacological treatments of live egg chambers.

Egg chambers were dissected in live imaging medium. For time-lapse imaging of slboGal4, UAS-GCaMP5 flies, calcium ionophore ionomycin (I24222, life techonologies) was added to a final concentration of 3μM, and images were taken immediately after, with an interval of 1min. To measure the response of Cad^{TS} to ionomycin (3μM), ROCK inhibitor Y-27632 (100μM, Y0503, Sigma-Aldrich) and actin polymerization inhibitor cytochalasin D (20μg/ml, C8273, Sigma-Aldrich), mTFP/Venus FRET images of border cells were taken 1 hour after addition of the drugs.

2. Construction of transgenic flies

2.1 Tension sensor fly construction:

Full length *Drosophila* E-cadherin cDNA was amplified with primers 1 and 2 (See Supplemental Table 1) and cloned into pDONR vector using the Invitrogen Gateway BP clonase. A short sequence GGTACCTCAGGCGGCCGCT with KpnI and NotI cutting sites was introduced after the transmembrane domain and before the β-catenin binding site of E-cadherin using Agilent Quickchange Lightning Site-Directed Mutagenesis Kit (primers 3 and 4). Tension sensor module with linkers (Addgene # 26021) was amplified with primer 5 and 6, and inserted between the KpnI and NotI sites. For the control construct, one KpnI site was introduced before the stop codon of cadherin using pirmer 7 and 8. The tension sensor fragment together with linker was amplified with primer 9 and 10,

and inserted into the KpnI site using ClonTech In-fusion HD kit. For the mTFP and Venus only controls, the mTFP or Venus fragment was inserted between the same KpnI and NotI sites like the Cadherin tension sensor construct (primer 5,11 for mTFP and 12, 6 for Venus). All of the constructs in pDONR vector were eventually put into fly Gateway expression vector pUWR (Drosophila Genomics Resource Center) using Invitrogen Gateway LR clonase. Transgenic flies were generated by Bestgene Inc.

2.2 LifeactGFP/ LifeactRFP fly construction:

For slboLifeactGFP, LifeactGFP was amplified by high fidelity PCR with primers 13 and 14 (See Supplemental Table 1). Then both PCR product and slbo promoter vector were digested with KpnI and products ligated with T4 DNA ligase. For UASt-LifeactRFP, we used primers 15 and 16 to amplify from Lifeact-dsRed construct, and cloned it into pUASt vector using the gateway recombination system. Transgenic flies were generated by Bestgene Inc.

3. FRET image processing

Cad-Venus flies were used to determine excitation of Venus by 458nm laser, which was negligible and hence ignored (abt). Cad-mTFP flies were used to determine the bleed-through of mTFP to the (519-566nm) Venus channel, which was 40% of the mTFP signal (dbt). Corrected FRET (cFRET) image was calculated using formula:

$cFRET=FRET- dbt(I_D)I_D - abt(I_A)I_A$ (Grashoff et al., 2010)

(I_D: donor image intensity, I_A: acceptor image intensity)

cFRET image was registered with mTFP image using the TurboReg plugin. A Gaussian smooth filter was then applied to both mTFP and cFRET images. A background value was obtained from a region outside the egg chamber and subtracted from whole image. The cFRET image was thresholded and converted to a binary mask with the foreground set to 1. We used the cFRET mask image to multiply with cFRET image, and then divide by mTFP image to get the FRET ratio image. To measure mean FRET ratio at the front and back of migrating border cell clusters expressing either the Cad^{TS} or the tension insensitive control, we made 7μM x 7μM square regions of interest at the front and at the back (identified in the LifeactRFP channel) of the cluster in FRET ratio image, and recorded the peak value of the intensity histogram. The front to back FRET ratio was then calculated.

In order to measure FRET efficiency, we performed acceptor photobleaching experiments with 4% paraformaldehyde-fixed ubi-Cad^{TS} samples (to reduce recovery of Venus after photobleaching) using a Zeiss LSM 710 confocal microscope. After 2 rounds of mTFP and Venus imaging, we selected a region of interest (ROI) in the border cells, used 3 laser lines (514nm, 543nm, and 594nm) and looped 250 times to effectively bleach the acceptor Venus. mTFP and Venus images were taken immediately after acceptor photobleaching. Acceptor intensity decreased to about 20% of the original intensity, while donor intensity

jumped to 170% of the original value (Figures S2L and S2M). FRET efficiency was calculated using:

E= 1- ($\frac{DA}{D\Omega}$)

(D_A= donor intensity before photobleaching; D_Ω=donor intensity after photobleaching) (Bastiaens and Pepperkok, 2000)

Mean of E is 40.63% with standard deviation of 5.7% using samples from 6 different bleaching experiments. We also repeated the acceptor photobleaching experiments in live samples and mean E value was about 10%.

Expression levels of Cad^{TS} and control were determined by exciting samples with 514nm laser and acquiring images in the (519-566nm) Venus channel.

4. Morphodynamic profiling of border cell migration

To obtain an unbiased assessment of the roles various molecular components involved in guidance signaling play in directed border cell migration we implemented the morphodynamic profiling approach previously introduced for the analysis of single cell migration (Machacek and Danuser, 2006). Morphodyamic profiling uses live cell image sequences and characterizes the local movements of the cell edge rather than just the centroid movement to characterize motility. This offers a much refined description of the dynamics that lead to net movement, including spatiotemporal patterns that indicate the state of polarity and processes related to path finding. The method entails three steps described below in more detail: 1) Defining the outline of the border cell cluster in every

time point; 2) Assembly of morphodynamic activity map. This step consists of two sub-steps. First, cluster outlines are morphed between consecutive time points to determine the instantaneous displacement of every location on the cluster boundary. The data is then transformed into a morphodynamic activity map, which represents the spatiotemporal coordination of cell edge protrusion and retraction events in a shape invariant frame of reference. 3) Extraction of numerical features from the activity maps. These numbers then define the morphodynamic profile of a border cell cluster. Because of the shape invariance of the activity map morphodynamic profiles can be directly compared within and between genotypes.

4.1 Image Segmentation

To identify the outlines of border cell clusters, we first obtained in each frame an image mask covering the cluster by smoothing the GFP channel based on level set methods (Sumengen, 2004) and Gaussian filtering, followed by iterative thresholding (Ridler and Calvard, 1978) to separate foreground (cluster) from background (mostly autofluorescence from the surrounding cells in the oocyte). In some frames, the cell cluster extended thin pseudopodial structures with low signal at the front (Figure S3A). To avoid the division of the cluster and pseudopodial structures into several parts, we implemented a gap bridging approach. First we calculated the convex hull of the separate parts (Figure S3B). Subsequently, we obtained the difference map between the convex hull and the segmented parts (Figure S3C), and added pixels with intensities greater than the

40^{th} percentile of the difference map to the mask. The refined mask was further smoothed by morphological closing (Figure S3D).

For some movies, the border cell cluster stayed in contact with the ovary epithelium for the first few frames. In these frames the cluster movement was not representative of directed motion. On the other hand, in some movies, the cluster was masked by strong autofluorescence the last few frames, rendering the cluster outline inaccurate. To exclude such frames from the profiling, we evaluated the segmentation results by visual inspection and discarded some time points at the beginning and the end of movies. We also confirmed that slight changes in the segmentation methods and control parameters, which may dislocate the cell boundaries by several pixels, did not affect the overall boundary movement and downstream morphodynamic analysis.

4.2 Assembly of Morphodynamic Activity Map

Morphodynamic activity maps were constructed in two steps: 1) Identification of the displacement of cluster boundaries between consecutive frames. 2) Mapping of the displacements into a kymograph revealing the spatiotemporal coordination of protrusion and retraction events. The morphing of two cluster boundaries between consecutive frames is equivocal and thus requires some arbitrary yet reasonable assumptions that guide the mapping. It is noted that a simple orthogonal mapping of the cell edges usually violates the constraint that the topology of boundary points must be maintained (Figure S3E; regions with stronger protrusion or retraction activity where displacement vectors of

neighboring points cross). In (Machacek and Danuser, 2006) we demonstrated the application of the Level Set Method as a mathematically rigorous approach to achieve a mapping with correct boundary topology. Here, we used a computationally less expensive and comparably accurate approach. A B-form spline was fitted to the edge pixel positions of the two boundaries, with nodes corresponding to each edge pixel. The spline representations of the boundaries were then divided into sectors between intersections (Figure S3E). For each segment we then determined the boundary displacements by minimizing the objective function:

$$(\hat{o}_1,\ldots,\hat{o}_n) = \underset{(o_1,\ldots,o_n)}{\arg\min}[\underbrace{\sum_{i=1}^{n}\left(x(t+1,o_i)-x(t,p_i)\right)^2}_{SUM_A} + \omega \underbrace{\sum_{i=2}^{n}\left(\frac{o_i(t+1)-o_{i-1}(t+1)}{p_i(t)-p_{i-1}(t)}\right)^2}_{SUM_B}]$$

(S1)

subject to $e_1 = o_1 < o_2 < \ldots < o_n = e_n$

(S2)

Here, the variable *n* denotes the number of nodes in the particular sector, which is equal to the number of edge pixels. $p_{1,2,\ldots n}(t)$ are the parameters of the spline at time *t* defining equally spaced edge nodes $x(t, p_i)$. The goal of Eq. S1 & S2 is to identify *n* spline parameters $o_{1,2,\ldots n}(t+1)$ in between the intersection points e_1 and e_n that define non-equally spaced nodes $x(t+1, o_i)$ at *t+1*. Equation S2 enforces the constraint that the order of the mapped points must be maintained. The first sum in Eq. S1, SUM_A, determines the total displacement of the vectors in

the sector. SUM_B determines the total change in node spacing and therefore the strain the sector experiences between two frames. The two sums have different physical units. To balance them correctly we introduce a factor ω as follows:

$$\omega = w * \left[\frac{SUM_A}{SUM_B} \right]_{iteration=1} = w * \left[\frac{\sum_{i=1}^{n} \left(x(t+1, o_i) - x(t, p_i) \right)^2}{\sum_{i=2}^{n} \left(\frac{o_i(t+1) - o_{i-1}(t+1)}{p_i(t) - p_{i-1}(t)} \right)^2} \right]_{iteration=1}$$

The factor ω is calculated only in the first iteration of the minimization, as the unit conversion by the ratio SUM_A/SUM_B changes insubstantially thereafter. The parameter *w* is a free user-control that allows the definition of the trade-off between minimal edge displacement and minimal lateral strain. If w is set close to 0 the mapping vectors are determined such that overall displacement is minimized without considering the edge strain. This could yield drastic local expansions of the cell edge in areas of high protrusion activity (w ~ 0, Figure S3F). If instead w is set to values much greater than 1 minimization of Eq. S1 is equivalent to minimizing the strain, i.e. the mapping vectors are determined such the distance between points along the edge stays constant. As a result large displacements over an entire sector may be generated (w >> 1, Figure S3G). In general the best compromise between these extreme scenarios is found by giving the displacement and the strain equal weights (w = 1, Figure S3H). All morphodynamic profiles recorded for this work were computed with w = 1 (Figure S3I).

After mapping the boundary points for all sectors, we calculated the displacement projection onto the normal of the boundary to obtain a measurement of the local boundary movement. If the displacement had the same direction as the normal, the movement was classified as a protrusion event, otherwise it was classified as a retraction event. We then divided the cluster outline into 30 segments and calculated the mean displacement per segment. The number of segments was chosen such that the width of each segment approximately matches the finest length scale over which protrusion and retraction events alternate, without considering undulations only due to noise (Welch et al., 2011). The mean displacements of all segments in one time point were pasted into a column of the kymograph. This procedure was repeated for each time point of the movie resulting in a matrix representation of the boundary movement called morphodynamic activity map (Figures 4B-4E, Figure S4A and S4B).

4.3 Morphodynamic Features for Directed Cell Migration

After construction of the morphodynamic activity map the segments were assigned to cluster front, cluster back, or cluster side. Several statistics of the segment velocities associated with front protrusion, front retraction, back protrusion and back retraction were calculated, including percentage features, percentile features and moment (average and standard deviation) features (summarized in Supplemental Table 3). All segments across all frames were pooled to form a velocity distribution for feature calculation. For example, morphodynamic features (MPF1-MPF4) considered the percentage of protruding (or retracting) segments at cluster front/back across all frames, where protruding

(or retracting) segments were defined as those segments with velocity greater (or smaller) than a protrusion velocity threshold of 2 pixels/frame. This choice of threshold excluded fluctuating segments of small velocities. Other percentile features (MPF6-8,10-12,14-16,17-19) and the remaining moment features provided complimentary measurement of the protrusion and retraction at cluster front, back or all boundaries. All features were calculated for each movie and statistical significance was evaluated by a randomization test (Cardillo, 2008) at significance levels 0.05 or 0.001 to assess difference in the mean values of the feature distributions between any pairwise genotypes.

As the percentage features are highly dependent on the chosen protrusion velocity threshold, thresholds between 2-4 pixels/frame were examined to ensure that the observed morphodynamic phenotypes are robustly similar or different across different thresholds (Figures 5E-5H, Figure S4C-S4J). Thresholds greater than 4 pixels/frame were not further investigated as the majority of the protrusion and retraction activities happened below 4 pixels/frame.

5. Photomanipulation of PA-Rac

To photoactivate, the 458 nm laser was set at 10% power for 0.1 ms per pixel in a 7micron spot and the photoactivation scan took approximately 25 seconds. After 30 seconds, border cells were imaged using 568nm laser excitation. This series of steps was repeated for the duration of the timelapse experiment. Where

indicated, 15-20 Z planes separated by 1.5 μm were obtained before and after photoactivation (samples were illuminated every 80 seconds for one hour).
Cell protrusions were counted as follows: a circle corresponding to the average cluster diameter was drawn and any extension more than 2 μm beyond that was considered a protrusion. Analysis and quantification were done with ImageJ and MATLAB, as previously described (Wang et al., 2010).

6. Immunohistochemistry:

Anti-E-cadherin (rat DCAD2) and anti-Singed (mouse sn7c) antibodies were obtained from Developmental Studies Hybridoma Bank and diluted 1:25 in PBTx (1xPBS+0.5% TritonX-100). Secondary antibodies conjugated with Alexa-488 or Alexa-568 (Molecular Probes) were diluted 1:400 in PBTx. Fixed images were captured with a Zeiss LSM 510 microscope.

References:

Bastiaens, P.I.H., and Pepperkok, R. (2000). Observing proteins in their natural habitat: the living cell. Trends Biochem Sci *25*, 631-637.

Cardillo, G. (2008). Rndttest: An alternative to Student t-test assessing difference in means., (http://www.mathworks.com/matlabcentral/fileexchange/20928).

Grashoff, C., Hoffman, B.D., Brenner, M.D., Zhou, R.B., Parsons, M., Yang, M.T., McLean, M.A., Sligar, S.G., Chen, C.S., Ha, T.*, et al.* (2010). Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. Nature *466*, 263-U143.

Machacek, M., and Danuser, G. (2006). Morphodynamic profiling of protrusion phenotypes. Biophys J *90*, 1439-1452.

Ridler, T., and Calvard, S. (1978). Picture Thresholding Using an Iterative Selection Method. Systems, Man and Cybernetics, IEEE Transactions on *8*, 630-632.

Sumengen, B. (2004). A matlab toolbox implementing level set methods. (http://barissumengen.com).

Wang, X., He, L., Wu, Y.I., Hahn, K.M., and Montell, D.J. (2010). Light-mediated activation reveals a key role for Rac in collective guidance of cell movement in vivo. Nat Cell Biol *12*, 591-597.

Welch, C.M., Elliott, H., Danuser, G., and Hahn, K.M. (2011). Imaging the coordination of multiple signalling activities in living cells. Nat Rev Mol Cell Biol *12*, 749-756.