**Supplementary Figure 1. Analyses of subcellular F-actin signals and actin flows in migrating border cell groups.**

**a.** Definition of protrusions, cables and the cell-to-cell contacts in representative balanced, loose or tight border cell groups expressing LifeAct-GFP. Different colors marking either cables in one border cells or cell-cell contacts between two border cells. Yellow arrows marking protrusions, yellow arrowheads marking broken cables. **b, c.** Quantifications of protrusion number (b) and border cell area (c) in the indicated border cell groups. **d.** Ratio quantification of total peripheral F-actin signals distributed at the front, middle or rear cells in the indicated border cell groups. **e.** Representative PIV and divergence analyses of actin flows in balanced border cell group expressing LifeAct-GFP and Sqh-mCherry for F-actin and Myosin-II signals. PIV analysis performed on the LifeAct-GFP signals to highlight the direction and magnitude of actin flows. Divergence calculated from the actin flowfield to highlight the region where actin flows from different directions converge and thus Myosin-II signals accumulate at cables or protrusion-cable boundaries. a.u. means arbitrary unit for divergence level. White arrowheads (cables) or stars (protrusions) marking high PIV regions, while red arrowheads (cables) or stars (protrusions) marking negative divergence regions. **f.** Quantification of relative Myosin-II signals accumulated at cables in the indicated border cell groups. **g, h.** Scatter plot comparing a random sample of points in the actin flowfield for relative actin flow speed and relative Myosin-II intensity (g), or for divergence and relative Myosin-II intensity (h). Note the positive relationship between negative divergence and relative Myosin-II intensity (n = 6156 random points, 9 biologically independent samples); but no significant relationship between actin flow speed and relative Myosin-II intensity (n = 5876 random points, 9 biologically independent samples). Scale bars are 10 μm in e. Boxplot shows medians, 25th and 75th percentiles as box limits, minimum and maximum values as whiskers; each datapoint is displayed as a dot (from n biologically independent samples for each border cell group), in b, c, d and f. P values by two-sided Mann–Whitney test have been listed in Supplementary Note 1. Source data are provided as a Source Data file.
Supplementary Figure 2. Control experiments for the binding specificity of GTP-loaded dRac1 to PAK3RBD-GFP.

GTPγS- or GDP-loaded His-dRac1 was incubated with GST or GST-PAK3RBD-GFP recombinant proteins purified from *E. coli*. Input and pull-down samples were analysed by immunoblotting with antibodies against His (top panel) or GST (bottom panel). Asterisk marks non-specific signals. The results have been successfully repeated from the at least 3 independent experiments.
Supplementary Figure 3. Control experiments for subcellular Rac1 activity in border cells.

a. Representative PAK3RBD-GFP and F-actin images in border cell groups expressing Rac1DN, Cdc42DN and control (yw; named as WT), together with LifeAct-RFP to discriminate and label different regions enriched with subcellular F-actin. Since the expression of Rac1DN and Cdc42DN in border cells led to the defect in border cell detachment, we chose all these three border cell
groups during detachment for the comparison. Green arrows marking protrusions, green arrowheads marking cables, while green stars marking border cell-cell contacts. 

b. Quantification of relative PAK3RBD-GFP intensity located at contacts, cables or protrusions in the indicated border cell groups. 

c. Representative GFP and F-actin images in border cell groups expressing Rac1-GFP, Rac2-GFP and Rac3-GFP (endogenous patterns), together with LifeAct-RFP to monitor subcellular F-actin signals. Rac1-GFP vs. Rac2-GFP in border cells (but not two internal polar cells): 1+/−0.168 vs. 0.24+/−0.096, and Rac1-GFP vs. Rac3-GFP in border cells: 1+/−0.168 vs. 0.17+/−0.071, from n=35 independent samples. 

d. Representative PAK3RBD-GFP and F-actin images in border cell groups expressing Rac1 RNAi, Rac3 RNAi or Cdc42 RNAi, or with Rac2 LOF mutant, together with LifeAct-RFP to discriminate different regions enriched with subcellular F-actin. 

e. Quantification of relative PAK3RBD-GFP intensity located at contacts, cables or protrusions in the indicated border cell groups. 

f. Representative GFP and RFP images in border cell groups expressing Rac1-GFP (and Rac1 RNAi or control), or Cdc42-RFP (and Cdc42 RNAi or control), from fixed imaging. 

ɡ. Quantification of relative GFP/RFP intensity in total border cell groups in the indicated border cell groups. Scale bars are 10 μm in a, c, d, f. Boxplot shows medians, 25th and 75th percentiles as box limits, minimum and maximum values as whiskers; each datapoint is displayed as a dot (from n biologically independent samples for each border cell group), in b, e, g. P values by two-sided Mann–Whitney test have been listed in Supplementary Note 1. Source data are provided as a Source Data file.
Supplementary Figure 4. Control experiments for PA-Rac effects.

a. Representative time-lapse images of border cell groups expressing the indicated PA-Rac forms, together with LifeAct-RFP to monitor subcellular F-actin signals. Dotted blue circle labelling the PA regions with blue light illumination, either at cables in leader or rear border cells, or at border cell-to-cell contacts. PA means photo-activation.

b. Quantification of protrusion number before and after 18-25-minute photo-activation of the indicated PA-Rac forms at cables or border cell-to-cell contacts. Scale bars are 10 μm in a. Boxplot shows medians, 25th and 75th percentiles as box limits, minimum and maximum values as whiskers; each datapoint is displayed as a dot (from n biologically independent samples for each border cell group) in b. P values by two-sided Mann–Whitney test have been listed in Supplementary Note 1. Source data are provided as a Source Data file.
**Supplementary Figure 5. Quantifications of the PA-Rac effect.**

*a*-c, d-f, g-i, j-l. Quantifications of cable discontinuity (a, d, g, j), the ratio between cable F-actin signals and total peripheral F-actin signals (b, e, h, k), and total cell area (c, f, i, l) in the indicated border cell groups, before and after 18-25-minute photo-activation at the indicated regions.  

*m, n.* Quantifications of relative F-actin intensity located at the border cell-to-cell contacts in the border cell groups expressing either PA-RacT17N or PA-RacQ61L, before and after 18-25-minute photo-activation of PA-Rac at leader border cell cables (m) or protrusions (n).  

**o.** Time-lapse quantifications of mean migration speed (μm per minute) in the border cell groups expressing either PA-RacQ61L-LovC450M or PA-RacT17-LovC450M, before and after focal illumination of PA-Rac-LovC450M at leader border cell cables. Boxplot shows medians, 25th and 75th percentiles as box limits, minimum and maximum values as whiskers; each datapoint is displayed as a dot (from n biologically independent samples for each border cell group), in a-n. Data are presented as mean values +/- SD in o (from n biologically independent samples for each border cell group).  
P values by two-sided Mann–Whitney test have been listed in Supplementary Note 1. Source data are provided as a Source Data file.
**Supplementary Figure 6.** Rho1 signalling governs cable Rac1 activity to support supracellular cables.

**a, c.** Representative PAK3RBD-GFP and F-actin images in border cell groups with the expression of Rock-CA, Rock RNAi, Myosin-II RNAi or control (a), and with the treatment of Y27632 or DMSO control (c), together with LifeAct-RFP for different subcellular F-actin signals. Green arrows marking protrusions, green arrowheads marking cables, while green stars marking border cell-cell contacts.

**b, d.** Quantification of relative PAK3RBD-GFP intensity located at contacts, cables or protrusions in border cell groups with the indicated genetic backgrounds (b) and treatment (d).

**e, g.** Representative time-lapse Sqh-GFP and F-actin images in border cell groups expressing Opto-RhoGEF2 and LifeAct-RFP (e) and Opto-Rho1DN and LifeAct-RFP (g), before and after blue light illumination at the cable regions near border cell leading protrusions. Dotted blue circle labelling the PA regions with blue light illumination. PA means photo-activation.

**f, h.** Quantification of relative Myosin-II intensity before and after 120-second photo-activation of Opto-RhoGEF2 (f) and Opto-Rho1DN (h) at the cable regions near leading protrusions.

**i, k.** Representative time-lapse PAK3RBD-GFP and F-actin images in border cell groups expressing Opto-Rho1DN and LifeAct-RFP (i) and PA-RacT17N and LifeAct-RFP (k), before and after blue light illumination at the cable regions of one border cell.

**j, l.** Time-lapse quantification of relative PAK3RBD-GFP intensity located at cables or protrusions in one cell with Opto-Rho1DN photo-treatment (j) and in the whole groups with PA-RacT17N photo-treatment at one border cell cable (l), before and after photo-activation. Scale bars are 10 μm in a, c, i and k, 2 μm in e and g. Boxplot shows medians, 25th and 75th percentiles as box limits, minimum and maximum values as whiskers; each datapoint is displayed as a dot (from n biologically independent samples for each border cell group), in b, d, f and h. Data are presented as mean values +/- SD in j and l (from n
biologically independent samples for each border cell group). P values by two-sided Mann–Whitney test have been listed in Supplementary Note 1. Source data are provided as a Source Data file.
Supplementary Figure 7. Confirmation for the role of E-cadherin on controlling pulsed Myosin-II signal transmission at supracellular cables.

a. Time-lapse images of Myosin-II flows in one representative WT border cell group expressing Sqh-GFP but not mCD8-RFP signals. Yellow arrowheads marking Myosin-II pulsed flows.

b. Time-lapse images of Myosin-II flows in one representative Sqh-GFP expressing border cell group, in which 1-2 border cell clone(s) express E-cadherin RNAi (ShgRNAi) together with mCD8-RFP as the clone marker. Yellow arrowheads marking Myosin-II pulsed flows, while yellow stars marking

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**-diagram description here**
the clonal cell. **c.** Time-lapse images of Myosin-II flows in one representative Sqh-GFP expressing border cell group, in which 1-2 border cell clone(s) express E-cadherin RNAi ($Shg^{RNAi}$) together with mCD8-RFP as the clone marker. Yellow arrowheads marking Myosin-II pulsed flows, while yellow stars marking the clonal cell. The plane of time-lapse images between 900 seconds and 1060 seconds is 5 μm higher than the one between 0 seconds and 150 seconds. The results (a-c) have been successfully repeated from the at least 4 independent experiments. **d.** Representative time-lapse F-actin images of border cell groups expressing PA-RacQ61L and E-cadherin RNAi (upper), or PA-RacQ61L-LovC450M and E-cadherin RNAi (lower), together with LifeAct-RFP to monitor subcellular F-actin signals, before and after photo-activation of PA-RacQ61L at one border cell. Dotted blue circle labelling the PA regions with blue light illumination. PA means photo-activation. **e.** Quantifications of cable discontinuity, the ratio between cable F-actin signals and total peripheral F-actin signals, and protrusion number in the border cell groups expressing PA-RacQ61L and E-cadherin RNAi, before and after 20–minute photo-activation at one border cell. Scale bars are 10 μm in a-d. Boxplot shows medians, 25th and 75th percentiles as box limits, minimum and maximum values as whiskers; each datapoint is displayed as a dot (from n biologically independent samples for each border cell group) in e. P values by two-sided Mann–Whitney test have been listed in Supplementary Note 1. Source data are provided as a Source Data file.
Supplementary Figure 8. Confirmation for the role of Cdc42 on governing actin flows at protrusions and F-actin signal exchange between protrusion and cables for efficient border cell migration.

a. Representative PIV, divergence and retrograde/anterograde direction analyses of actin flows at leading protrusions in the border cell groups expressing PA-Cdc42Q61L/PA-RacQ61L, PA-Cdc42Q61L/PA-RacT17N, PA-Cdc42T17N/PA-RacQ61L or PA-Cdc42T17N/PA-RacT17N, together with LifeAct-GFP for F-actin signals. a.u. means arbitrary unit for divergence level. Dotted blue circle labelling the PA regions with blue light illumination. PA means photo-activation.

b. Quantification of mean flow speed (μm per minute) at leading protrusions in the indicated border cell groups. c. Quantification of the occurrence ratio of retrograde and anterograde actin flows at leading protrusions in the indicated border cell groups. d, e. Representative time-lapse images of border cell groups expressing PA-RacQ61L/PA-Cdc42T17N (d) or PA-RacT17N/PA-Cdc42T17N (e), together with LifeAct-RFP to monitor subcellular F-actin signals. Dotted blue circle labelling the PA regions with blue light illumination. PA means photo-activation. f, g. Time-lapse quantifications of cable discontinuity (f) and the ratio between cable F-actin signals and total peripheral F-actin signals (g) in the indicated border cell groups, before and after photo-activation of the indicated PA-Rac and PA-Cdc42 at leader border cell protrusions. h. Quantification of mean migration speed (μm per minute) in the indicated border cell groups, after photo-activation of the PA-Cdc42 or/and PA-Rac at leader border cell protrusions, compared with the photo-treated WT border cell groups. Scale bars are 2 μm in a, and 10 μm in d and e. Boxplot shows medians, 25th and 75th percentiles as box limits, minimum and maximum values as whiskers; each datapoint is displayed as a dot (from n biologically independent samples for each border cell group), in b, c and h. Data are presented as mean values +/- SD in f and g (from n biologically independent samples for each border cell group). P values by two-sided Mann–Whitney test have been listed in Supplementary Note 1. Source data are provided as a Source Data file.
Supplementary Figure 9. PVR and EGFR guide Rac1 activity and actin flows at protrusions and cables of migrating border cells.

a. Representative PAK3RBD-GFP and F-actin images in the indicated groups for signal mis-localization. b. Representative PAK3RBD-GFP and F-actin images in the indicated groups. Green arrows marking protrusions, green arrowheads marking cables, while green stars marking border cell-cell contacts. c. Quantification of relative PAK3RBD-GFP intensity located at contacts, cables or protrusions in the indicated groups. d. Representative PIV analyses of actin flows in the whole group and at protrusions, and retrograde/anterograde direction analysis of actin flows at protrusions in the indicated group. e, f, g. Quantification of mean flow speed (μm per minute) at protrusions (e), the occurrence ratio of protrusion retrograde and anterograde actin flows (f) and actin flows occurring at cables (g) in the indicated border cell groups. In g, RGB colour marking the time window; number at perimeter showing the angle degree where actin flows occur during the indicated time window. Number above the circle showing the occurrence number of actin flows at cable with mean values +/- SD. h, j. Representative time-lapse F-actin images of the indicated groups, before and after photo-activation of PA-RacQ61L at cables (h) or protrusions (j) of one border cell. Dotted blue circle labelling the PA regions with blue light illumination. PA means photo-activation. i, k. Quantifications of cable discontinuity, the ratio between cable F-actin signals and total peripheral F-actin signals, and total cell area in the groups expressing PA-RacQ61L, PVR-DN and EGFR-DN, before and after 20-30-minute photo-activation at cables (i) or protrusions (k) of one border cell. Scale bars are 1 μm in a, and 10 μm in b, d, h and j. Boxplot shows medians, 25th and 75th percentiles as box limits, minimum and maximum values as whiskers; each datapoint is displayed as a dot (from n biologically independent samples for each border cell group), in c, e, f, i and k. P values by two-sided Mann–Whitney test have been listed in Supplementary Note 1. Source data are provided as a Source Data file.
Supplementary Figure 10. Characterization of the intermediators between guidance receptors and Rac1, and of the effectors downstream of Rac1 in controlling border cell protrusions.

a. Representative PAK3RBD-GFP and F-actin images in border cell groups expressing Mbc RNAi, ELMO RNAi, Raf RNAi or control, together with LifeAct-RFP to discriminate and label different regions enriched with subcellular F-actin signals. 
b. Quantification of relative PAK3RBD-GFP intensity located at contacts, cables or protrusions in the indicated border cell groups. c, d. Quantification of cable discontinuity in border cell groups expressing PVR-DN, Mbc RNAi or ELMO RNAi (c), EGFR-DN or Raf RNAi (d). e, f. Quantification of protrusion number in border
cell groups expressing PVR-DN, Mbc RNAi or ELMO RNAi (e), EGFR-DN or Raf RNAi (f). g. Representative F-actin images of border cell groups expressing Abi RNAi, Scar RNAi, Arp3 RNAi, PAK1 RNAi or PAK3 RNAi, together with LifeAct-RFP to monitor subcellular F-actin signals. h. Quantification of protrusion number in border cell groups expressing Abi RNAi, Scar RNAi, Arp3 RNAi, PAK1 RNAi or PAK3 RNAi. i. Representative time-lapse images of border cell groups expressing Abi-GFP, driven by Slbo-Gal4/UAS genetic tool. The results of these time-lapse images for Abi-GFP distribution pattern at protrusion tips have been successfully repeated from the at least 3 independent experiments. Scale bars are 2 μm in i, and 10 μm in a and g. Boxplot shows medians, 25th and 75th percentiles as box limits, minimum and maximum values as whiskers; each datapoint is displayed as a dot (from n biologically independent samples for each border cell group), in b-f, h. P values by two-sided Mann–Whitney test have been listed in Supplementary Note 1. Source data are provided as a Source Data file.
Supplementary Figure 11. Original data of western blot analyses shown in supplementary figure 2. The results have been successfully repeated from the at least 3 independent experiments.
### Supplementary Note 1. Figures P value

**Figure 1. b**

| Condition       | P value  |
|-----------------|----------|
| balanced vs. loose | <0.0001 |
| loose vs. tight  | <0.0001 |
| balanced vs. tight | <0.0001 |

**Figure 1. c**

| Condition       | P value  |
|-----------------|----------|
| balanced vs. loose | <0.0001 |
| loose vs. tight  | <0.0001 |
| balanced vs. tight | <0.0001 |

**Figure 1. d**

| Condition       | P value  |
|-----------------|----------|
| balanced vs. loose | <0.0001 |
| loose vs. tight  | <0.0001 |
| balanced vs. tight | <0.0001 |

**Figure 1. e**

| Condition       | P value  |
|-----------------|----------|
| balanced vs. loose | 0.04     |
| loose vs. tight  | 0.0476   |
| balanced vs. tight | 0.9487  |

**Figure 1. f**

| Condition       | P value  |
|-----------------|----------|
| balanced vs. loose | <0.0001 |
| loose vs. tight  | <0.0001 |
| balanced vs. tight | <0.0001 |

**Figure 1. g**

| Condition       | P value  |
|-----------------|----------|
| front vs. middle | <0.0001 |
| front vs. rear   | <0.0001 |
| middle vs. rear  | 0.0012   |

**Figure 1. j**

| Condition       | P value  |
|-----------------|----------|
| balanced vs. loose | <0.0001 |
| loose vs. tight  | <0.0001 |
| balanced vs. tight | <0.0001 |

**Figure 2. b**

| Condition       | P value  |
|-----------------|----------|
| balanced vs. loose | 0.2359   |
| loose vs. tight  | 0.1462   |
| balanced vs. tight | 0.8545  |

**Figure 2. c**

| Condition       | P value  |
|-----------------|----------|
| front vs. middle | 0.8545 |
| front vs. rear   | 0.665  |
| middle vs. rear  | 0.3354 |

**Figure 2. d**

| Condition       | P value  |
|-----------------|----------|
| front vs. middle | 0.0003 |
| front vs. rear   | 0.0084 |
| middle vs. rear  | 0.1973 |

**Figure 3. b**

| Condition       | PA-RacT17N (PA at leader cell cables) | PA-RacT17N (PA at leader cell protrusions) | PA-RacQ61L (PA at leader cell cables) | PA-RacQ61L (PA at leader cell protrusions) |
|-----------------|---------------------------------------|---------------------------------------------|----------------------------------------|---------------------------------------------|
| before PA vs. after PA | <0.0001                              | <0.0001                                     | <0.0001                                | 0.0005                                     |

**Figure 3. c**

| Condition       | PA-RacT17N (PA at leader cell cables) | PA-RacT17N (PA at leader cell protrusions) | PA-RacQ61L (PA at leader cell cables) | PA-RacQ61L (PA at leader cell protrusions) |
|-----------------|---------------------------------------|---------------------------------------------|----------------------------------------|---------------------------------------------|
| before PA vs. after PA | 0.0005                                | 0.0003                                      | 0.0084                                 | 0.0005                                      |
**Figure 4. d**

| Time Comparison          | Nearby | Far Away |
|--------------------------|--------|----------|
| 0 min vs. 5 min          | 0.0001 | 0.0029   |
| 5 min vs. 10 min         | <0.0001| 0.0001   |
| 10 min vs. 15 min        | <0.0001| <0.0001  |

**Figure 4. h**

| Time Comparison          | Nearby | Far Away |
|--------------------------|--------|----------|
| 0 min vs. 5 min          | <0.0001| <0.0001  |
| 5 min vs. 10 min         | <0.0001| <0.0001  |
| 10 min vs. 15 min        | 0.0029 | 0.0232   |

**Figure 5. b**

| Time Comparison          | Contacts | Cables | Protrusions |
|--------------------------|----------|--------|-------------|
| Contl vs. Rho1DN         | 0.8137   | <0.0001| 0.0002      |
| Contl vs. Rho1CA         | <0.0001  | <0.0001| 0.0602      |

**Figure 5. d**

| Time Comparison          | Cables | Protrusions |
|--------------------------|--------|-------------|
| 0 sec vs. 180 sec        | 0.0002 | 0.0002      |

**Figure 5. f**

| Time Comparison          | Cables | Protrusions |
|--------------------------|--------|-------------|
| 0 sec vs. 180 sec        | >0.9999| 0.7209      |

**Figure 5. h**

| Time Comparison          | Nearby | Far Away |
|--------------------------|--------|----------|
| 0 min vs. 12 min         | 0.003  | 0.0002   |

**Figure 5. J**

| Time Comparison          | Nearby | Far Away |
|--------------------------|--------|----------|
| 0 min vs. 12 min         | 0.6454 | 0.8785   |

**Figure 6. b**

| Treatment Comparison     |          |
|--------------------------|----------|
| Contl vs. PA-RacQ61L     | 0.7103   |
| Contl vs. PA-RacT17N     | 0.3494   |
| Contl vs. PA-Cdc42Q79L   | <0.0001  |
| Contl vs. PA-Cdc42T17N   | <0.0001  |

**Figure 6. c**

| Treatment Comparison     | Retrograde Flow | Anterograde Flow |
|--------------------------|-----------------|------------------|
| Contl vs. PA-RacQ61L     | 0.4119          | 0.5516           |
| Contl vs. PA-RacT17N     | 0.5573          | 0.6539           |
| Contl vs. PA-Cdc42Q79L   | 0.0197          | 0.0127           |
| Contl vs. PA-Cdc42T17N   | 0.0159          | 0.0430           |

**Figure 6. d**

| Treatment Comparison     |          |
|--------------------------|----------|
| Contl vs. PA-Cdc42Q79L   | <0.0001  |
| Contl vs. PA-Cdc42T17N   | <0.0001  |
| PA-Cdc42Q79L vs. PA-Cdc42T17N | <0.0001 |

**Figure 7. b**

| Treatment Comparison     |          |
|--------------------------|----------|
| Contl vs. PVR-DN         | 0.4689   |
| Contl vs. EGFR-DN        | 0.5310   |

**Figure 7. e**

| Treatment Comparison     |          |
|--------------------------|----------|
| Contl vs. PVR-DN         | 0.0029   |
| Contl vs. EGFR-DN        | 0.9048   |
| PVR-DN vs. EGFR-DN       | 0.0001   |

**Figure 7. f**

| Treatment Comparison     | Retrograde Flow | Anterograde Flow |
|--------------------------|-----------------|------------------|
| Contl vs. PVR-DN         | 0.0001          | 0.0001           |
| Contl vs. EGFR-DN        | 0.8421          | 0.4967           |

**Figure 7. h**

| Treatment Comparison     |          |
|--------------------------|----------|
| Cable Discontinuity      | <0.0001  |
| Actin Cable/Periphery    | <0.0001  |
| Protrusion Number        | <0.0001  |

**Figure 7. j**

| Treatment Comparison     |          |
|--------------------------|----------|
| Cable Discontinuity      | <0.0001  |
| Actin Cable/Periphery    | <0.0001  |
| Protrusion Number        | <0.0001  |
## Supplementary Figures P value

### Supplementary Figure 1. b

| Comparison          | p value |
|---------------------|---------|
| balanced vs. loose  | <0.0001 |
| loose vs. tight     | <0.0001 |
| balanced vs. tight  | <0.0001 |

### Supplementary Figure 1. c

| Comparison          | p value |
|---------------------|---------|
| balanced vs. loose  | <0.0001 |
| loose vs. tight     | <0.0001 |
| balanced vs. tight  | <0.0001 |

### Supplementary Figure 1. d

| Comparison          | p value |
|---------------------|---------|
| front vs. middle    | <0.0001 |
| front vs. rear      | <0.0001 |
| middle vs. rear     | 0.2301  |

### Supplementary Figure 1. e

| Comparison          | p value |
|---------------------|---------|
| balanced vs. loose  | <0.0001 |
| loose vs. tight     | <0.0001 |
| balanced vs. tight  | <0.0001 |

### Supplementary Figure 3. b

| Treatment          | contacts | cables     | protrusions |
|--------------------|----------|------------|-------------|
| Contl vs. Rac1DN   | 0.3489   | <0.0001    | < 0.0001    |
| Contl vs. Cdc42DN  | <0.0001  | 0.9109     | < 0.0001    |

### Supplementary Figure 3. c

| Treatment          | contacts | cables     | protrusions |
|--------------------|----------|------------|-------------|
| Contl vs. Rac2 LOF | 0.6228   | 0.786      | 0.9007      |
| Contl vs. Rac3 RNAi| 0.7635   | 0.9824     | 0.9007      |
| Contl vs. Rac RNAi | 0.4147   | < 0.0001   | 0.0015      |
| Contl vs. Cdc42 RNAi| < 0.0001| 0.9124     | < 0.0001    |

### Supplementary Figure 3. d

| Treatment          | p value |
|--------------------|---------|
| PA-Rac17N          | 0.9727  |
| PA-RacQ61L         | 0.9099  |
| PA-RacT17N-LovC450M| 0.9105  |
| PA-RacT17N         | 0.0002  |
| PA-RacQ61L         | 0.001   |

### Supplementary Figure 5. a-c

| Treatment          | p value |
|--------------------|---------|
| before PA vs. after PA | 0.8126  |
| after PA            | 0.6987  |

### Supplementary Figure 5. d-f

| Treatment          | p value |
|--------------------|---------|
| before PA vs. after PA | <0.0001|
| after PA            | <0.0001|
| before PA vs. after PA | <0.0001|
| after PA            | <0.0001|
| before PA vs. after PA | <0.0001|
| after PA            | <0.0001|
| before PA vs. after PA | <0.0001|
| after PA            | <0.0001|

| Treatment          | p value |
|--------------------|---------|
| before PA vs. after PA | 0.0147  |
| after PA            | 0.0001  |

### Supplementary Figure 5. g-i

| Treatment          | p value |
|--------------------|---------|
| before PA vs. after PA | <0.0001|
| after PA            | 0.0021  |
| before PA vs. after PA | <0.0001|
| after PA            | 0.0004  |
| before PA vs. after PA | <0.0001|
| after PA            | 0.0232  |
**Supplementary Figure 5. j-l**  
|          | PA-RacT17N | PA-RacQ61L |  
|----------|------------|------------|  
| before PA vs. after PA (cable discontinuity) | <0.0001 | 0.0001 |  
| before PA vs. after PA (F-actin cable/periphery ratio) | 0.0001 | <0.0001 |  
| before PA vs. after PA (BC area) | 0.0185 | 0.0232 |  

**Supplementary Figure 5. m, n**  
|          | PA-RacT17N | PA-RacQ61L |  
|----------|------------|------------|  
| before PA vs. after PA (leader cables) | 0.6305 | 0.469 |  
| before PA vs. after PA (leader protrusions) | 0.6305 | 0.5417 |  

**Supplementary Figure 6. b**  
|          | contacts | cables | protrusions |  
|----------|----------|--------|-------------|  
| Contl vs. ROCK-CA | 0.0017 | <0.0001 | 0.0809 |  
| Contl vs. ROCK RNAi | 0.7421 | <0.0001 | 0.0005 |  
| Contl vs. Sqh RNAi | 0.646 | <0.0001 | 0.0003 |  

**Supplementary Figure 6. d**  
|          | contacts | cables | protrusions |  
|----------|----------|--------|-------------|  
| Contl vs. Y27632 | 0.7421 | <0.0001 | 0.0005 |  

**Supplementary Figure 6. f**  
|          | 0 s vs. 120 s |  
|----------|----------------|  
|          | 0.002 |  

**Supplementary Figure 6. h**  
|          | 0 s vs. 120 s |  
|----------|----------------|  
|          | 0.002 |  

**Supplementary Figure 6 j**  
|          | cables | protrusions |  
|----------|--------|-------------|  
| 0 sec vs. 180 sec | 0.0002 | 0.0002 |  

**Supplementary Figure 6. l**  
|          | cables | protrusions |  
|----------|--------|-------------|  
| 0 min vs. 12 min | 0.0002 | 0.0006 |  

**Supplementary Figure 7. e**  
|          | cable discontinuity | F-actin cable/periphery ratio | protrusion number |  
|----------|---------------------|-----------------------------|------------------|  
| before PA vs. after PA | 0.7109 | 0.25 | >0.9999 |  

**Supplementary Figure 8. b**  
|          | PA-Cdc42Q79L vs. PA-Cdc42Q79L/PA-RacQ61L | 0.0002 |  
|----------|------------------------------------------|--------|  
|          | PA-Cdc42Q79L vs. PA-Cdc42Q79L/PA-RacT17N | 0.1457 |  
|          | PA-Cdc42T17N vs. PA-Cdc42T17N/PA-RacQ61L | 0.0435 |  
|          | PA-Cdc42T17N vs. PA-Cdc42T17N/PA-RacT17N | 0.6965 |  

**Supplementary Figure 8. c**  
|          | retrograde flow | anterograde flow |  
|----------|-----------------|-----------------|  
| PA-Cdc42Q79L vs. PA-Cdc42Q79L/PA-RacQ61L | 0.0266 | 0.0434 |  
| PA-Cdc42Q79L vs. PA-Cdc42Q79L/PA-RacT17N | 0.1011 | 0.122 |  
| PA-Cdc42T17N vs. PA-Cdc42T17N/PA-RacQ61L | 0.9682 | 0.4002 |  
| PA-Cdc42T17N vs. PA-Cdc42T17N/PA-RacT17N | 0.9654 | 0.8968 |  

**Supplementary Figure 8. f**  
|          | PA-Cdc42T17N/PA-RacQ61L | PA-Cdc42T17N/PA-RacT17N |  
|----------|--------------------------|--------------------------|  
| 0 min vs. 22.5 min | 0.7959 | 0.9116 |  

**Supplementary Figure 8. g**  
|          | PA-Cdc42T17N/PA-RacQ61L | PA-Cdc42T17N/PA-RacT17N |  
|----------|--------------------------|--------------------------|  
| 0 min vs. 22.5 min | 0.5167 | 0.9265 |  

**Supplementary Figure 8. h**  
|          | Contl vs. PA-Cdc42Q79L | 0.0021 |  
|----------|------------------------|--------|  
|          | Contl vs. PA-Cdc42Q79L/PA-RacQ61L | 0.0409 |  
|          | Contl vs. PA-Cdc42Q79L/PA-RacT17N | <0.0001 |  
|          | Contl vs. PA-Cdc42T17N | <0.0001 |  
|          | Contl vs. PA-Cdc42T17N/PA-RacQ61L | <0.0001 |  
|          | Contl vs. PA-Cdc42T17N/PA-RacT17N | <0.0001 |  
|          | PA-Cdc42Q79L vs. PA-Cdc42Q79L/PA-RacQ61L | 0.3599 |  

| Supplementary Figure 9. c | contacts  | cables  | protrusions |
|--------------------------|-----------|---------|-------------|
| Contl vs. PVR-DN/EGFR-DN | 0.6903    | < 0.0001 | 0.4891      |

| Supplementary Figure 9. e | Contl vs. PVR-DN/EGFR-DN | 0.0089    |

| Supplementary Figure 9. f | Contl vs. PVR-DN/EGFR-DN | 0.0052    |
|----------------------------|---------------------------|-----------|
| retrograde flow            | anterograde flow          |           |
| before PA vs. after PA     | < 0.0001                  | < 0.0001  |
| after PA vs. PVR-DN        | 0.1821                    | 0.8676    |

| Supplementary Figure 9. i | Contl vs. PVR-DN/EGFR-DN | 0.0185    |
|--------------------------|---------------------------|-----------|
| cable discontinuity      | F-actin cable/ periphery ratio | protrusion number |
| before PA vs. after PA    | < 0.0001                  | < 0.0001  |
| after PA vs. PVR-DN       | 0.1821                    | 0.8676    |

| Supplementary Figure 9. k | Contl vs. PVR-DN/EGFR-DN | 0.0007    |
|--------------------------|---------------------------|-----------|
| cable discontinuity      | F-actin cable/ periphery ratio | protrusion number |
| before PA vs. after PA    | 0.0007                    | 0.0021    |
| after PA vs. EGFR-DN      | 0.9705                    | 0.1655    |

| Supplementary Figure 10. b | Contl vs. Mbc RNAi | 0.9941    |
|----------------------------|--------------------|-----------|
| contacts                    |                    |           |
| Contl vs. ELMO RNAi        | 0.9357              | < 0.0001  |
| Contl vs. Raf RNAi         | 0.9824              | 0.0076    |

| Supplementary Figure 10. c | Contl vs. Mbc RNAi | 0.3954    |
|----------------------------|--------------------|-----------|
| Contl vs. PVR-DN/EGFR-DN   |                    |           |
| Contacts                   |                    |           |
| Contl vs. ELMO RNAi        | 0.8583              |           |
| Mbc RNAi vs. ELMO RNAi     | 0.6027              |           |

| Supplementary Figure 10. d | Contl vs. Mbc RNAi | 0.1257    |
|----------------------------|--------------------|-----------|
| Contl vs. PVR-DN/EGFR-DN   |                    |           |
| Cable discontinuity        |                    |           |
| Contl vs. Elmo RNAi        |                    |           |

| Supplementary Figure 10. e | Contl vs. Mbc RNAi | 0.4501    |
|----------------------------|--------------------|-----------|
| Contl vs. PVR-DN/EGFR-DN   |                    |           |
| Protrusion number          |                    |           |
| Contl vs. Elmo RNAi        |                    |           |

| Supplementary Figure 10. f | Contl vs. Mbc RNAi | 0.7091    |
|----------------------------|--------------------|-----------|
| Contl vs. PVR-DN/EGFR-DN   |                    |           |
| Protrusion number          |                    |           |
| Contl vs. Elmo RNAi        |                    |           |

| Supplementary Figure 10. h | Contl vs. Mbc RNAi | <0.0001   |
|----------------------------|--------------------|-----------|
| Contl vs. PAK1 RNAi        |                    |           |
| Contl vs. PAK3 RNAi        |                    |           |
| Contl vs. Abi RNAi         |                    |           |
| Contl vs. Scar RNAi        |                    |           |
| Contl vs. Arp3 RNAi        |                    |           |
| Contl vs. PAK1 RNAi        |                    |           |
| Contl vs. PAK3 RNAi        |                    |           |