Supporting Information

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SI Materials and Methods

Dominant Negative Constructs. To generate dominant negative Rab proteins the following mutations were generated by quickchange mutagenesis: *rab4* (EST LD16736), Ser22 to Asn; *rab7* (EST GH03685), Thr22 to Asn; and *rab11* (EST LD14551), Ser25 to Asn. The resulting constructs were cloned into pUAST.

Details of Image Acquisition and Quantitative Analysis. Images from fixed tissues were acquired using an LSM 510 META inverted confocal microscope (Zeiss), using either a 20× objective or a 63× objective. Images were acquired by sequential scans in multiple channels. For figure assembly, images were processed with Photoshop (Adobe), using only the "gaussian blur" and the "level" functions. For a better rendering of the blue channel, a posttreatment with the "selective color" function was performed. Quantifications of fluorescence intensities were performed on the original images using the Image J program (National Institutes of Health, Bethesda). Mean intensities were determined in an area following the plasma membrane at both the leading edge [posterior, $F(P)$] and the trailing edge [anterior, $F(A)$] of border cell clusters in three consecutive frames from a *z*-scan separated by 1 μm. Only images with signal in the linear range were considered for quantification. The central plan was determined as the one containing the two polar cells, because they do not express UAS constructs with the same intensity as the other cells of the cluster. Furthermore, only clusters that have not yet penetrated into the egg chamber, but were detached from other follicle cells, were considered. When possible, GFP was used to determine the limit of the cluster. Each value was corrected by subtracting the background determined outside the egg chamber. In the figures, we represented the ratio between the posterior and the anterior mean fluorescence $[F(P)/F(A)]$. In the case of pTyr, the same measurements were made, while excluding the leading edge of the polar cells. For Sec15, we counted the number of vesicles in the posterior half of the cluster $[V\#(P)]$ and in the anterior half $[V\#(A)]$ at different moments of migration. Again, we represented the ratio of posterior vs. anterior vesicle number $[V\#(P)/V\#(A)]$.

Live Imaging (Acquisition). Image acquisition was acquired at room temperature with a 20× objective and a cool Snap HQ_2 camera on an inverted Sweptfield microscope (Nikon Instruments). *Z*-scans were acquired every 30 s for up to 6 h with exposures not exceeding 200 ms per frame. Imaging was performed through a Greiner Lumox membrane (Sigma-Aldrich). Images were acquired using the program "NIS- Elements AR 3.0" (Nikon Instruments). They were processed with the same program and with Image J (National Institutes of Health).

1. Jékely G, Sung HH, Luque CM, Rørth P (2005) Regulators of endocytosis maintain localized receptor tyrosine kinase signaling in guided migration. *Dev Cell* 9:197–207.

Migration and Completion Indexes. The migration index (M.I.) was calculated with the formula

$$\text{M.I.} = \frac{1^{*}n(100\%) + 0.75^{*}n(75\%) + 0.5^{*}n(50\%) + 0.25^{*}n(25\%) + 0^{*}n(0\%) + 0.5^{*}n(\text{splitclusters})}{n(\text{total})},$$

where $n(100\%)$ corresponds to the number of egg chambers where the cluster reached the oocyte, and $n(75\%)$ is the number of chambers where the cluster migrated to 75% of the final distance, etc. We also considered in our quantification clusters that split and where three or more cells did not migrate whereas the rest of the cluster reached the oocyte. We gave a 0.5 coefficient to these clusters in our migration index because approximately half the cells completed only migration. A M.I. of 1 means 100% of the cluster completed migration, whereas a value of 0 means that none of the clusters in the examined egg chambers migrated. A value of 0.5 could be obtained by various combinations of migration defects, including all of the egg chambers displaying 50% migration or half of the chambers displaying a 100% migration and half of the chambers where the clusters did not migrate. The completion index (C.I.) corresponds to the number of egg chambers where the migration was completed $[n(100\%)]$ divided by the total number of egg chambers $[n(\text{total})]$:

$$\text{C.I.} = \frac{n(100\%)}{n(\text{total})}.$$

In the figures, we represented M.I. and C.I. normalized to the proper controls (nM.I. and nC.I.).

Tissue Staining and Antibodies. Egg chambers were prepared and stained using standard techniques (1). All antibodies are previously described and were used at the indicated dilutions: mouse monoclonal anti-phospho-Tyrosine 1:10 (4G10), mouse monoclonal anti-E-Cadherin 1:5 (DCAD2; Developmental Studies Hybridoma Bank, DSHB), and mouse monoclonal anti-integrin β-chain 1:10 (CF.6G11; DSHB). Secondary antibodies were from Invitrogen and coupled to Alexa dyes. A 20-μM solution of Rhodamine-labeled phalloidin (Sigma-Aldrich) was used at 1:100 dilution to visualize F-actin. DAPI (Sigma-Aldrich) was used to stain nuclei. Egg chambers were mounted in Mowiol 4–88 (Sigma-Aldrich).

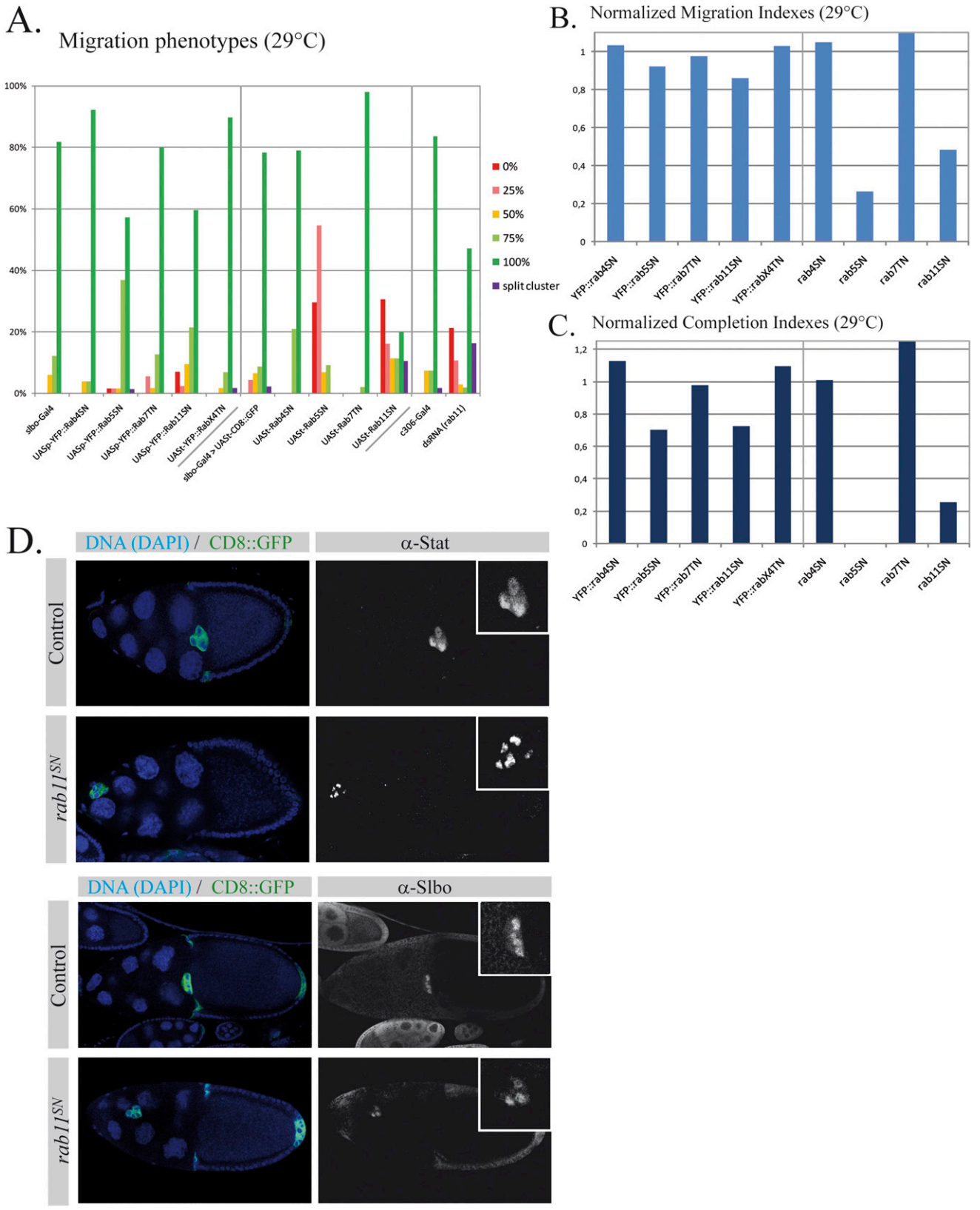


Fig. S1. Border cell migration requires Rab5 and Rab11 activities. (*A*) Quantification of border cell migration at stage 10 in control egg chambers, when dominant negative constructs of different Rab proteins are expressed or after down-regulation of *rab11* by dsRNA ($26 < n < 105$). (*B* and *C*) Normalized migration and completion indexes after expression of different dominant negative Rab proteins. (*D*) Stat and Slbo labeling in control egg chambers and when cell migration is impaired by the expression of rab11SN.

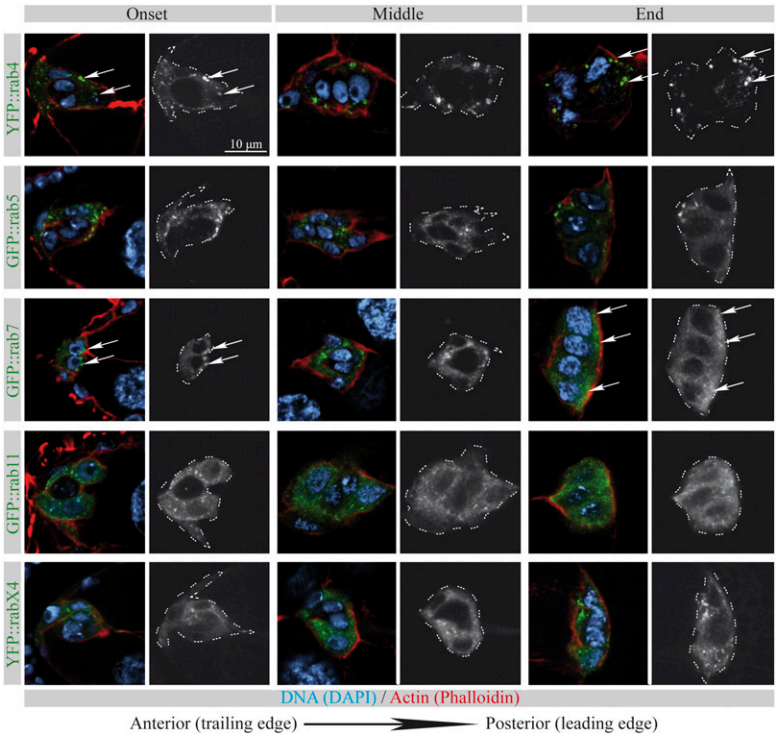


Fig. S2. Localization of different YFP/GFP fusions of Rab proteins during border cell migration. Representative images showing the distribution of YFP or GFP fusions of Rab proteins (green) at the onset, in the middle, and at the end of the migration process are shown. Egg chambers were additionally stained with phalloidin (red) and DAPI (blue). A grayscale image of the green channel is shown for every image. A dashed line delimits the border cell cluster in the grayscale image. Anterior is left and posterior is right. Arrows indicate polarized accumulation of Rab proteins.

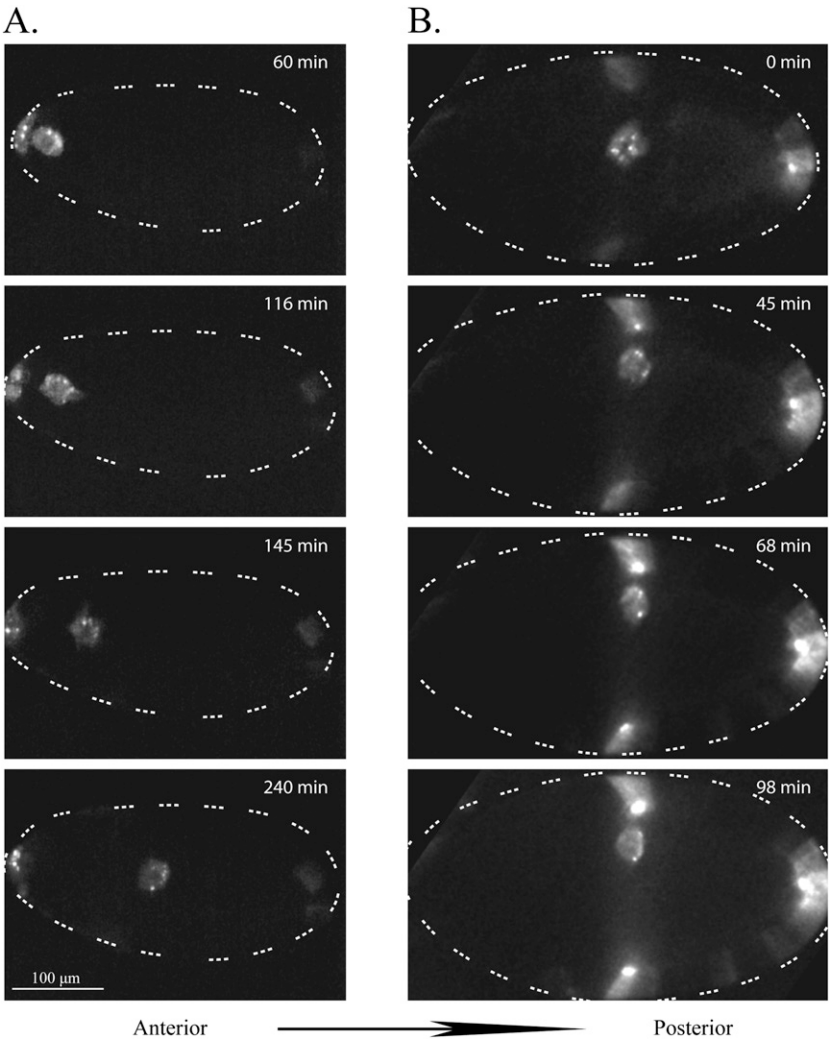
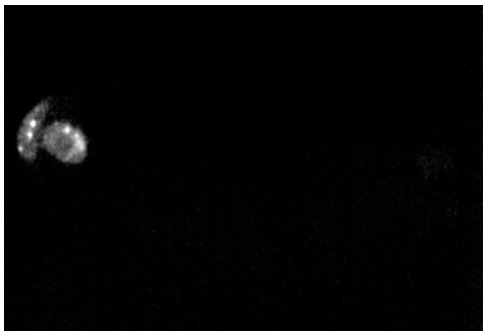
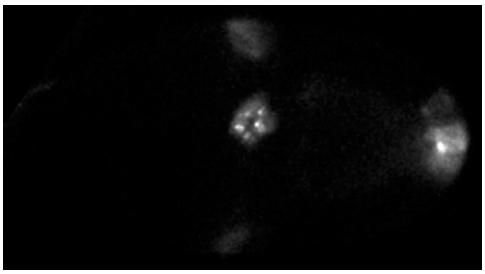


Fig. S3. Stills from movies showing the localization of Sec15 during border cell migration. Stills from movies showing GFP::sec15 during border cell migration at early phases (*A*, Movie S1) and at the end of the migration process (*B*, Movie S2) are shown. Stills where GFP::sec15 is clearly polarized are shown (except in *B*, time 0). Time is relative to the beginning of the movie.



Movie S1. Dynamics of GFP::sec15 at the beginning of border cell migration. Time-lapse movie shows the distribution of GFP::sec15 (expressed by *slbo-Gal4*) during the beginning of migration. At time 0, the cells have already detached from other follicle cells and have already migrated inside the egg chamber.

Movie S1



Movie S2. Dynamics of GFP::sec15 at the end of border cell migration. Time-lapse movie shows the distribution of GFP::sec15 (expressed by *slbo-Gal4*) during the dorso-ventral migration and the homing of the border cell cluster. Note that some structures can be observed for >90 min.

Movie S2