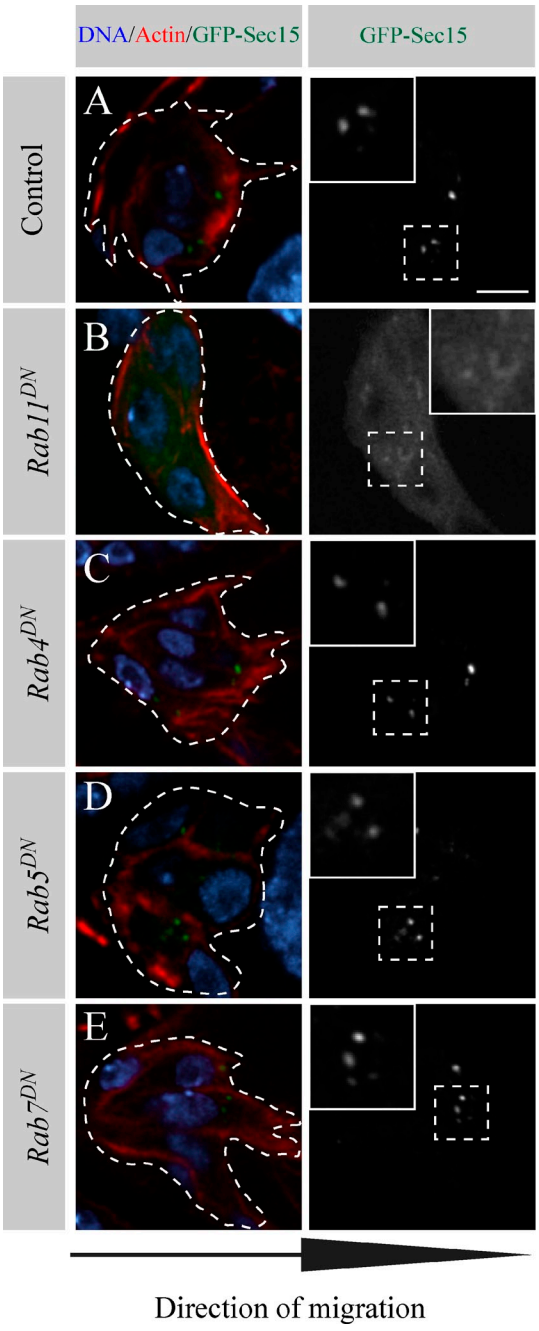
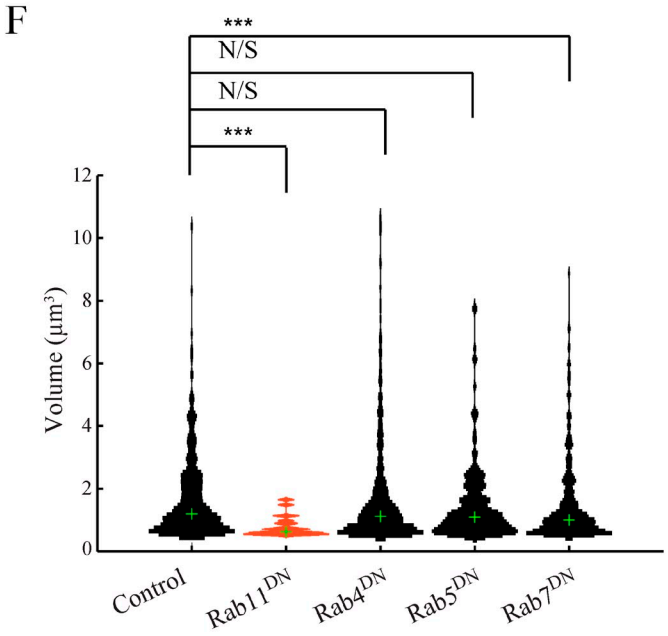
Laflamme et al., http://www.jcb.org/cgi/content/full/jcb.201112114/DC1

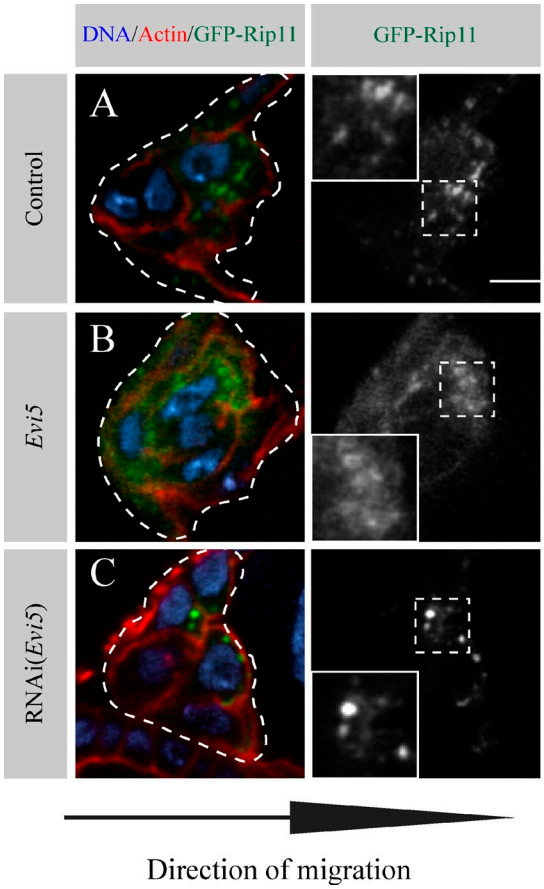


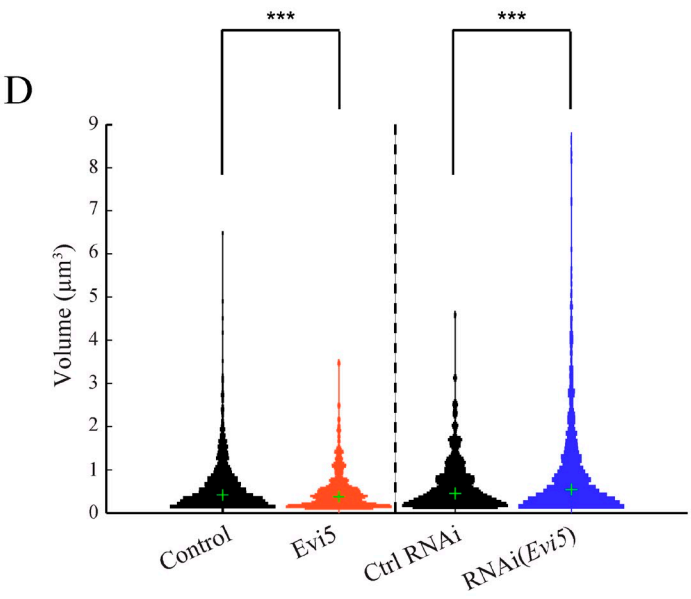


G

Condition #1	Condition #2	KS-test >0.5µm^3	Ranksum test >0.5µm^3
Control	Rab11^{CA}	0.0031	0.0072
Control	Rab11^{DN}	6.9E-05	1.6E-06
Control	Evi5	0.0055	0.0050
Control	Rab4^{DN}	0.17	0.072
Control	Rab5^{DN}	0.13	0.054
Control	Rab7^{DN}	0.0024	0.00096
Control RNAi	RNAi(*evi5*)	2.7E-15	1.4E-20

Figure S1. **Analysis of GFP-Sec15 compartment volumes.** (A–E) Representative images showing the distribution of GFP-Sec15 at the onset of migration (stage 9) for the indicated conditions. The dotted lines outline BC clusters as determined by the GFP signal. The insets show a higher magnification of the regions marked by dashed line squares. Bar, 5 µm. (F) Computational analysis of the conditions (A–E). Violin plots are vertical, side-by-side displays of histograms that represent the relative distribution of vesicle counts for a given volume. To facilitate comparison of the histogram shapes, we normalized each histogram by its maximum bin size. Thus, within each histogram, relative bin size reflects relative numbers of vesicles, but bin sizes across histograms are not comparable. The distributions were thresholded at 0.5 µm^3. Green crosses indicate the medians of the thresholded distribution (458 < *n* vesicles < 1,472). The orange distribution highlights the strong phenotype induced by *Rab11*^{DN} expression. ***, P < 0.05 for both the KS test and the rank sum test. (G) Statistical analysis of the distribution (KS test) and the median (rank sum test) of the GFP-Sec15 compartment population under different conditions. P-values are indicated for each described statistical test. P-values are significant when lower than 0.05.





E

Condition #1	Condition #2	KS-test >0.1µm³	Ranksum test >0.1µm³
Control	Evi5	0.0070	0.0011
Control RNAi	RNAi(*evi5*)	0.00093	0.00033

Figure S2. **Analysis of the distribution of GFP-Rip11 after modulating Rab11 activity.** (A–C) Representative images showing the distribution of GFP-Rip11 at the onset of migration (stage 9) for the indicated conditions. The dotted lines outline BC clusters as determined by the GFP signal. The insets show a higher magnification of the regions marked by dashed line squares. Bar, 5 µm. (D) Computational analysis of GFP-Rip11 vesicles of the conditions (A–C) as in Fig. S1 F. The distributions were thresholded at 0.1 µm³ (732 < *n* vesicles < 1,334; ***, P < 0.05; KS test and rank sum test). Green crosses indicate the medians of the thresholded distribution. (E) Statistical analysis of the distribution (KS test) and the median (rank sum test) of the GFP-Rip11 vesicle population under different conditions. P-values are indicated for each described statistical test. P -values are significant when lower than 0.05.

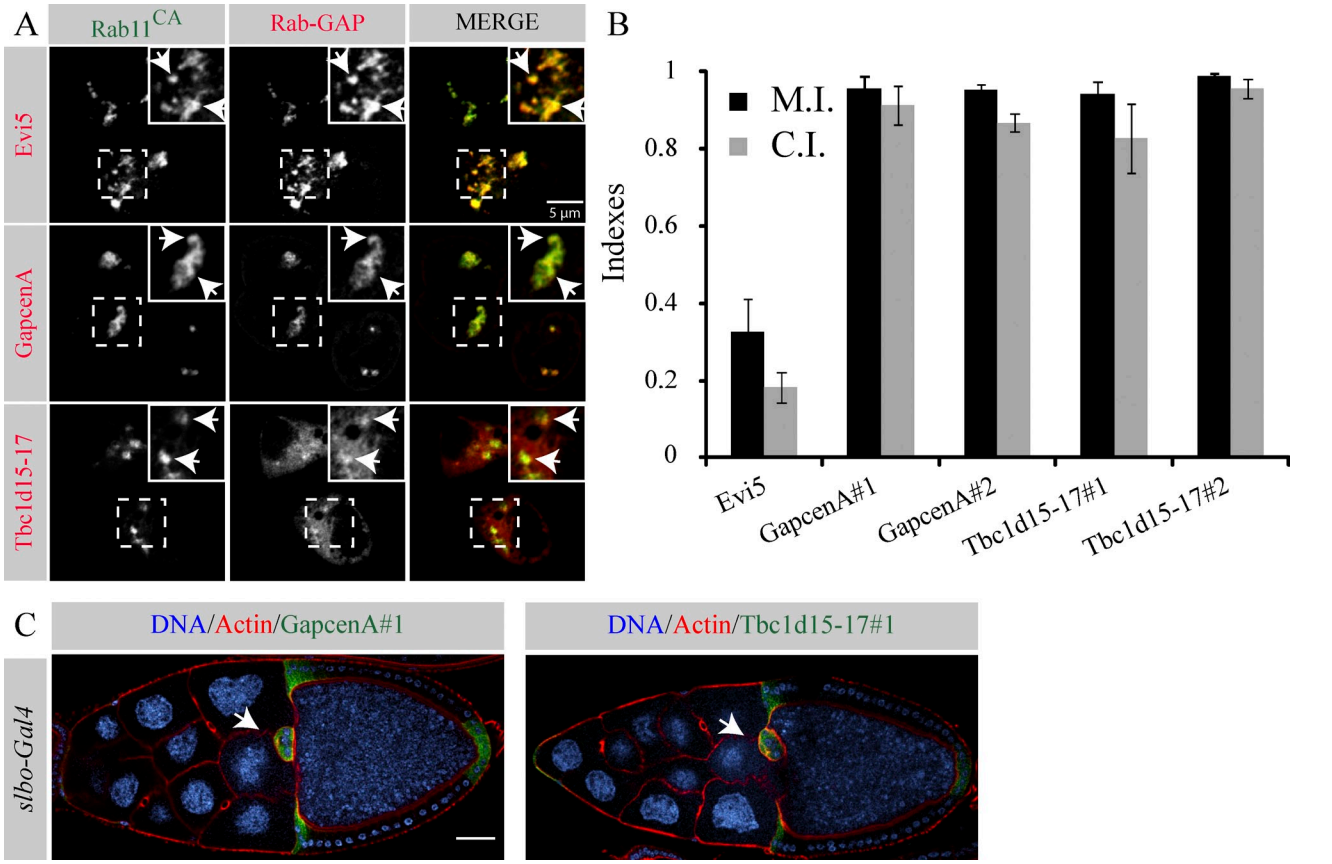


Figure S3. **GapcenA and Tbc1d15-17 are not involved in BC migration.** (A) Representative images showing the distribution of Evi5, GapcenA, and Tbc1d15-17 when coexpressed with *YFP-Rab11*^{CA} in S2 cells. A grayscale image of both the green and red channel is shown for every image. Arrows point to structures where a GAP protein and *Rab11*^{CA} colocalize. The insets show a higher magnification of the regions marked by dashed line squares. (B) M.I. and C.I. of BCs of different fly lines expressing *GapcenA-mcherry* and *Tbc1d15-mcherry* compared with the expression of *Evi5-mcherry* ($45 < n < 176$). Error bars are standard error of the mean. (C) Representative images of stage 10 egg chambers in which the BCs express the indicated constructs. Arrows point to structures where a GAP protein and *Rab11*^{CA} colocalize. Bar, 20 µm.