Table S1. Rescue of the lethality of *sec3* mutants by wild-type *sec3* transgene

Experiment	Actin-GAL4/Cyo; $sec3^{GT}$/TM6B,Tb X UAS-sec3/Cyo; $sec3^{GT}$/TM6B,Tb	Actin-GAL4/Cyo; $sec3^{PBac}$/TM6B,Tb X UAS-sec3/Cyo; $sec3^{PBac}$/TM6B,Tb
Number of total progeny	182	169
Number of homozygous mutant progeny	6	24
Rescue efficiency	23%	100%

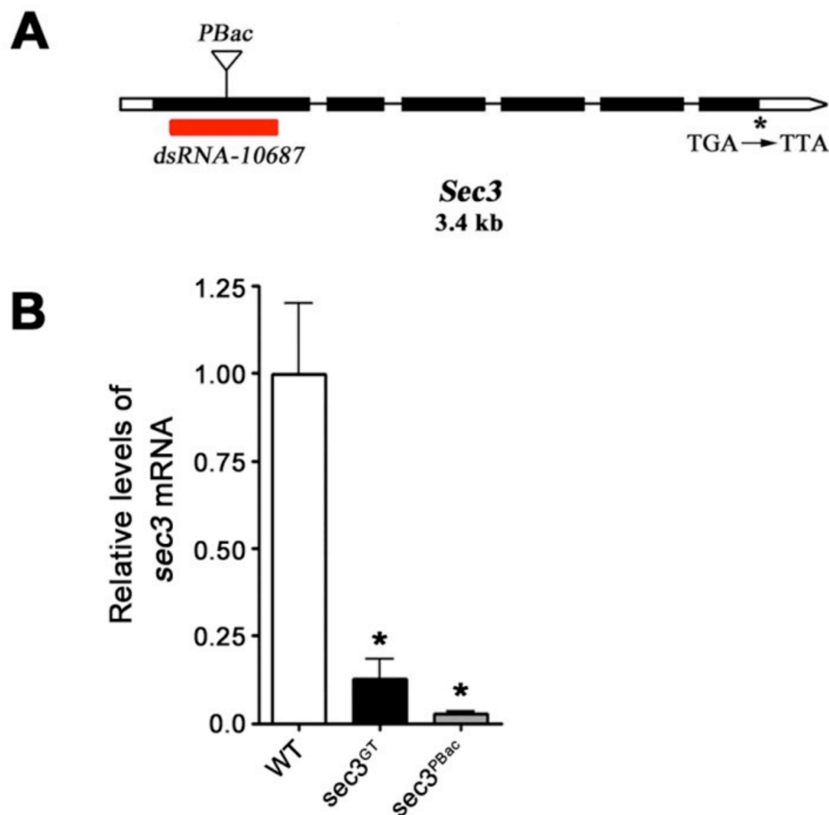

Figure S1. Characterization of the *sec3* mutations. (A), Gene structure and mutant alleles of *sec3*. Black boxes represent the CDS, and white boxes represent untranslated regions. The red box represents the target sequence region of RNAi. $sec3^{PBac}$ is a mutation with *piggyBac* transposon inserted in the first exon of the gene. $sec3^{GT}$ is a point mutation in stop codon. (B), The RNA levels of *sec3* is reduced in *sec3* mutants compared with the wild type flies. Experiments were repeated independently three times with consistent results and three replicates were used per time point.

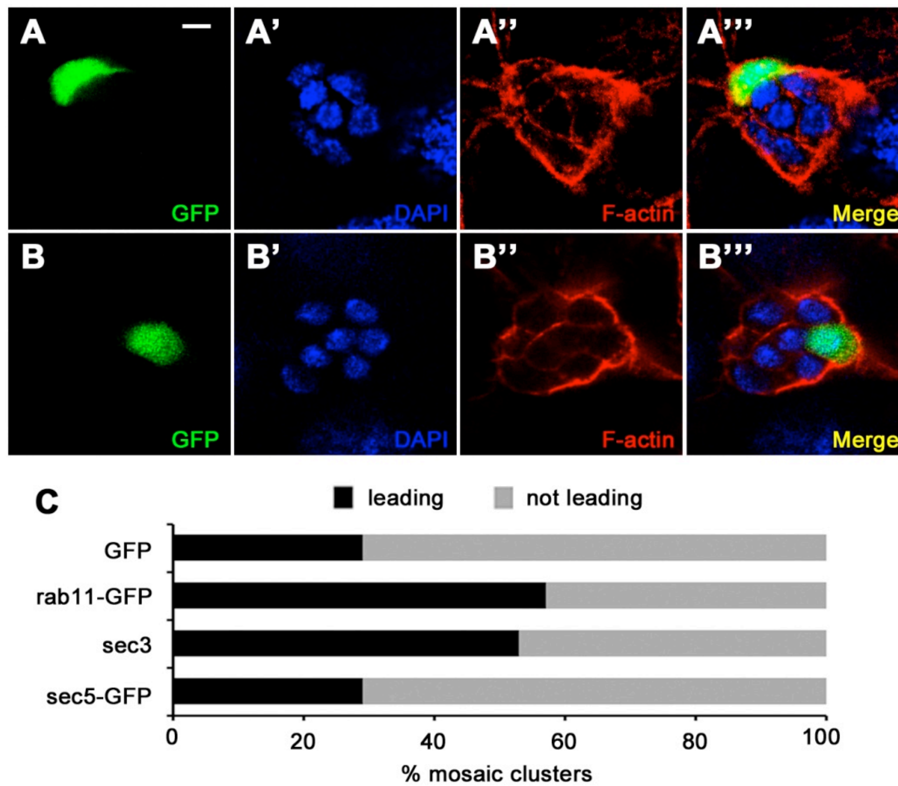

Figure S2. Local increase in Sec3 or Rab11-GFP level in single border cell (BC) biases it to become leading cell for BC cluster. (A-A''') An example of mosaic clusters containing 1-cell clone expressing UAS-*GFP* at the non-leading position. (B-B''') An example of mosaic clusters containing 1-cell clone expressing *UAS-sec3* at the leading position. (C) Cells in clones expressing only the GFP marker became leading cells in 29% of mosaic clusters (n=100), whereas cells expressing Sec3 and Rab11-GFP lead in 53% (n=45) and 57% (n=76) of mosaic clusters, respectively. Expressing Sec5-GFP does not change the frequency of BC becoming leading cell (n=51). Sale bar: 5um.

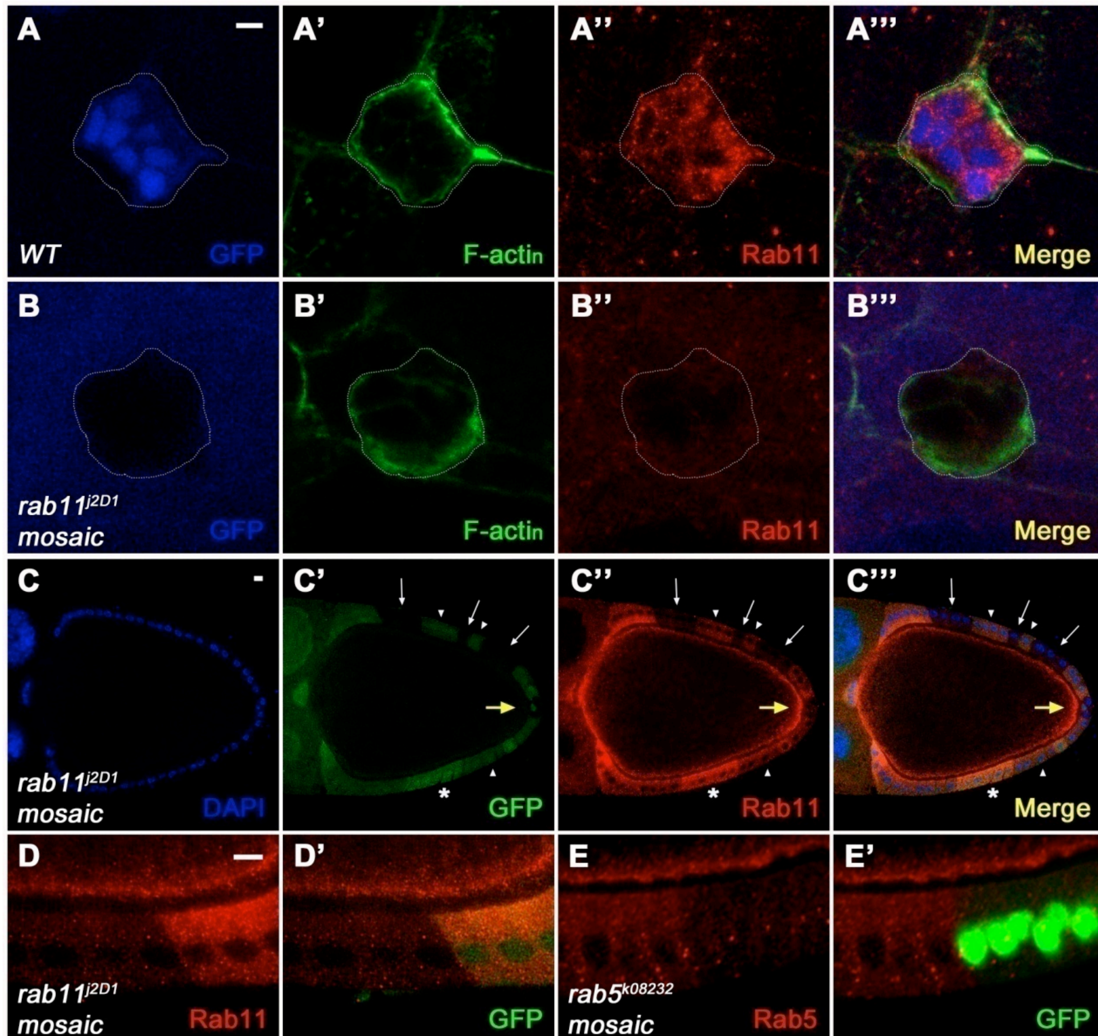

Figure S3. The specificity of Rab11 and Rab5 antibodies. (A-A''') The Rab11 staining in wild type BCs. (B-B''') Rab11 signal is strongly reduced in *rab11*^{j2D1}(Jankovics et al., 2001; Alone et al., 2005) BCs. Mutant cells are marked by absence of GFP. (C-C''') Rab11 signal is strongly reduced in *rab11*^{j2D1} follicle cells (FCs). White arrow shows homozygous mutant cells, arrowhead shows heterozygous cells, * shows wild type cells. Yellow arrow shows specific accumulation of Rab11 at the posterior pole of oocyte as previously reported (Dollar et al., 2002). (D-D'), High magnification view of Rab11 staining in one of the mutant clones shown in (C-C"'). (E-E') Rab5 signal is markedly reduced in a *rab5*^{k08232}(Wucherpfennig et al., 2003) FC clone. Mutant cells are positively marked by GFP. Sale bar: 5um.

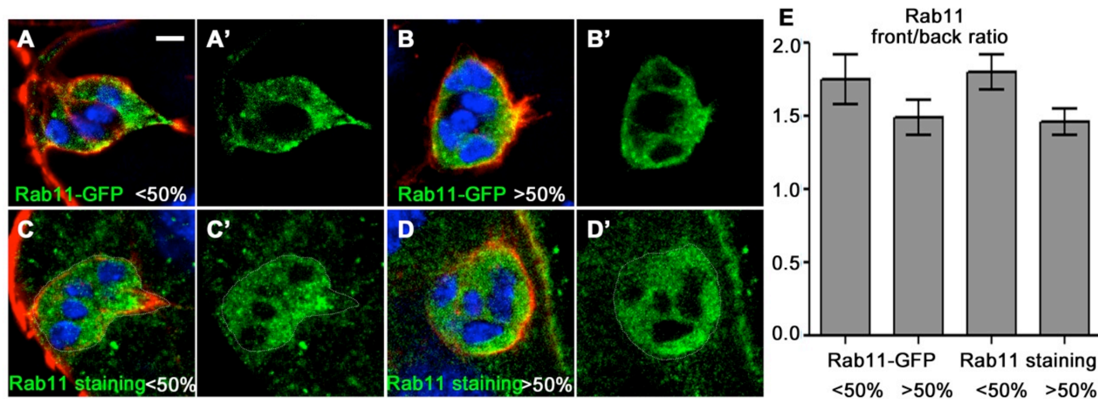

Figure S4. Rab11-GFP is polarized during border cell migration. (A-B'), Representative images showing the distribution of Rab11-GFP at 0%-50% and 50%-100% of the migration process respectively. Blue shows DAPI, red shows F-actin. (C-D') Representative images showing the distribution of Rab11 antibody staining signal at 0%-50% and 50%-100% of the migration process. (E) Fluorescence intensity was measured for Rab11-GFP or Rab11 antibody staining signals in both front[F(F)] and back[F(B)] regions of BC clusters, and the values were used to calculate the F(F)/F(B) ratios ($8<n<16$). Error bars indicate s.e.m. Scale bar: 5um.

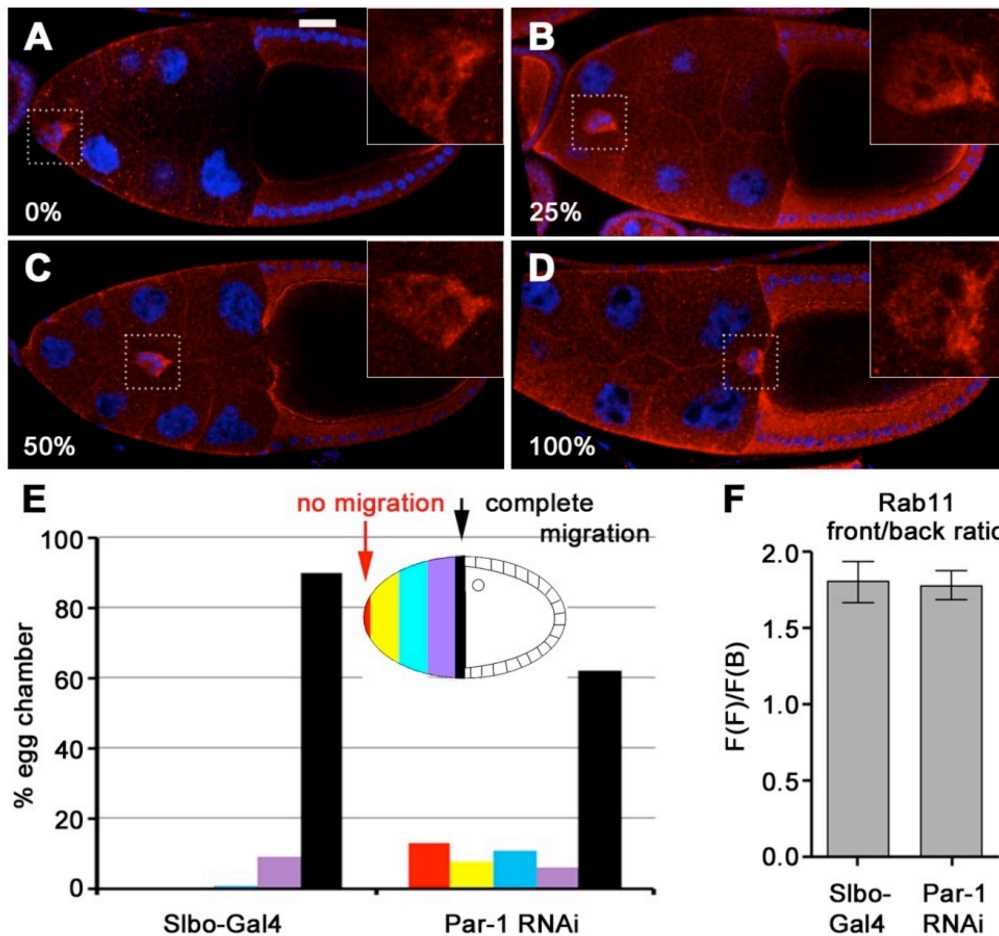

Figure S5. Expression of Par-1 RNAi delays BC migration but does not affect Rab11 polarity. (A-D) Representative images of BC clusters with migration defects or with normal migration all show Rab11 polarity. (E) Quantification of BC migration defects in control or in egg chambers expressing *Par-1 RNAi* driven by *Slbo*-GAL4 (n>80). (F) Quantification of Rab11 front/back ratio. Error bars indicate s.e.m. Scale bar: 20um.

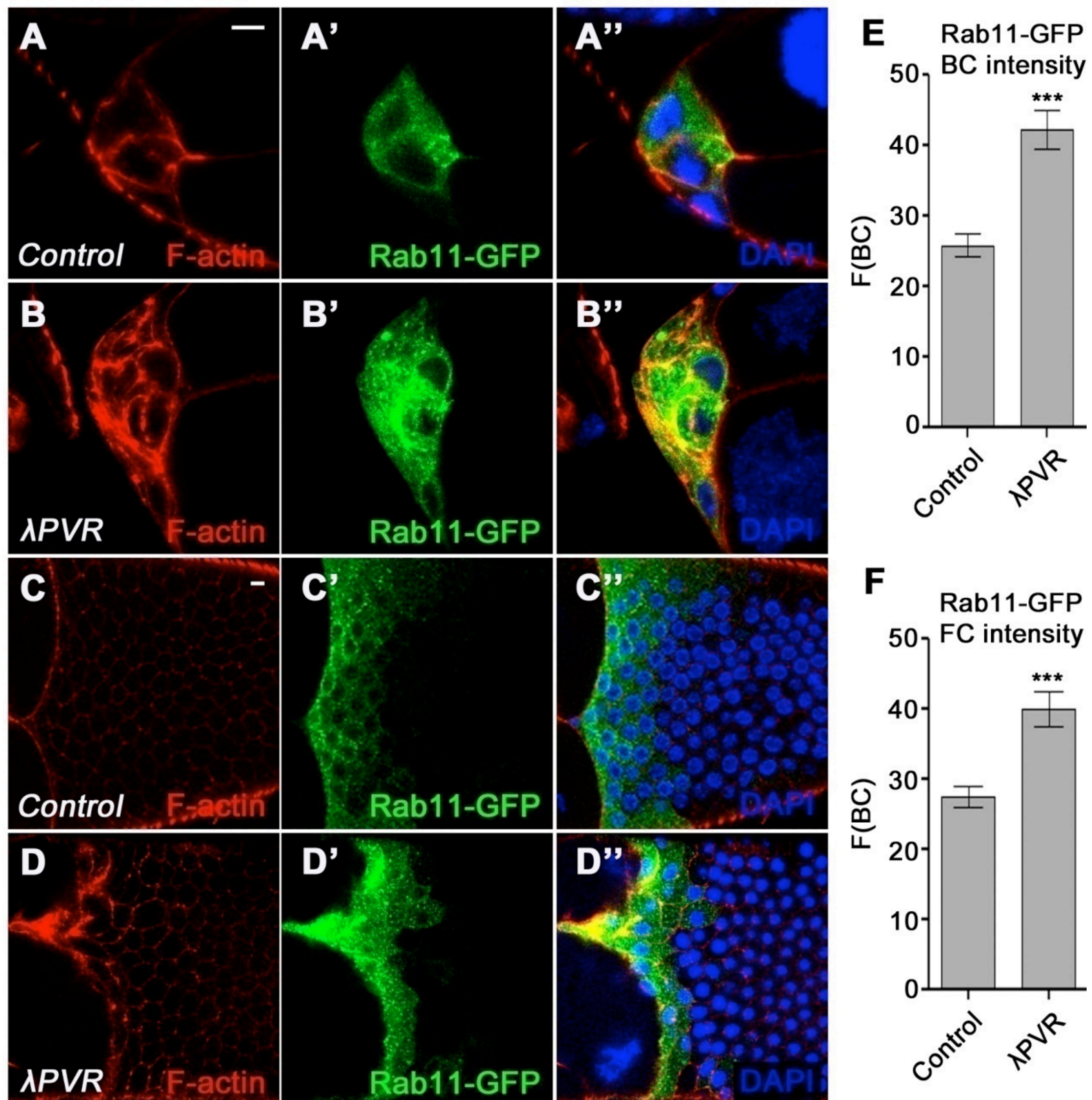

Figure S6. Rab11-GFP intensity is increased in BCs and FCs expressing λPVR. (A-B'',E) Expressing λPVR by Slbo-Gal4 increased Rab11-GFP intensity in BCs. (C-D'',F) Expressing λPVR by Slbo-Gal4 increased Rab11-GFP intensity in FCs. Error bars indicate s.e.m. Scale bar: 5um.

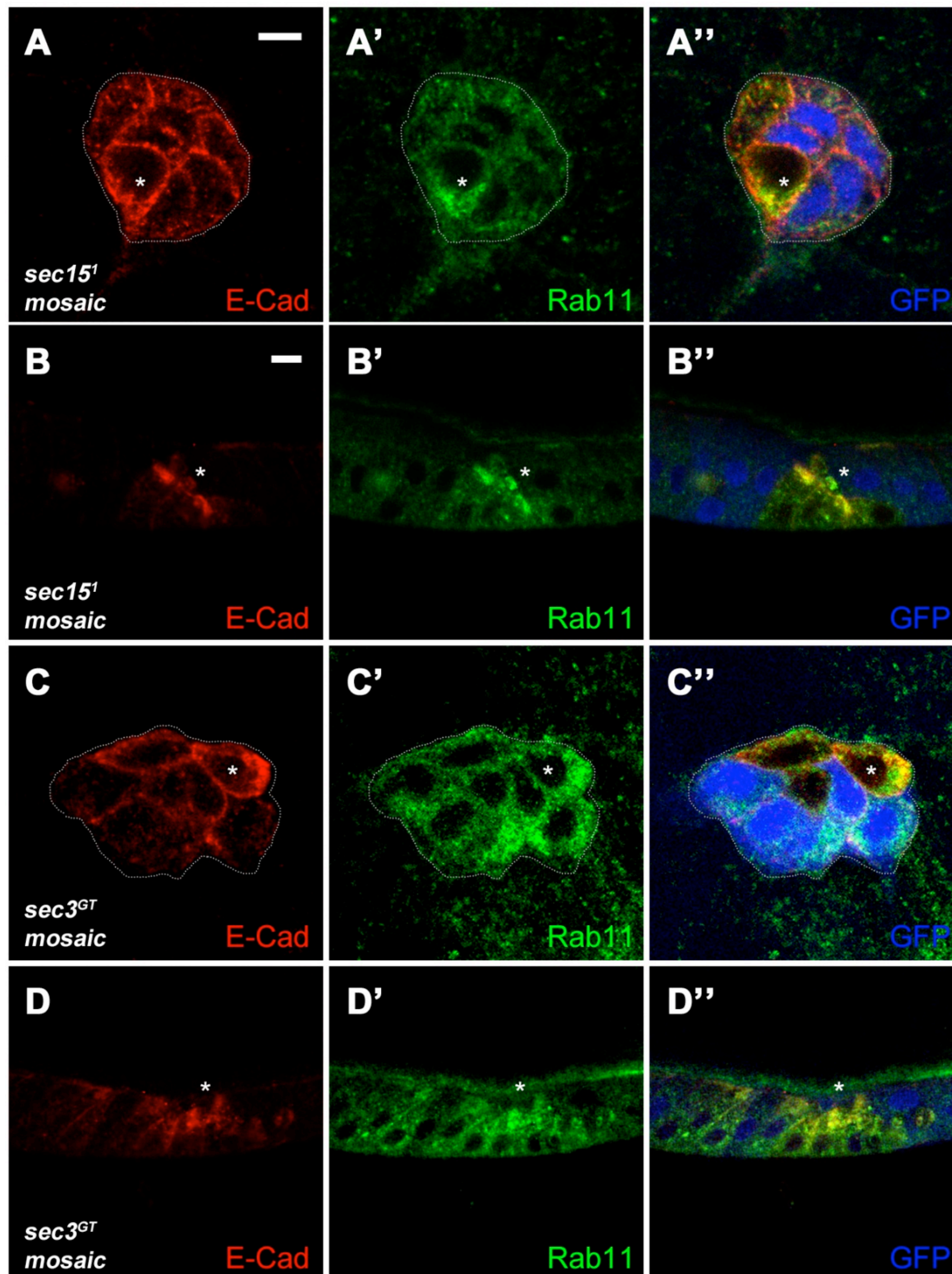

Figure S7. *sec15* mutant cells display similar phenotypes as *sec3* mutant cells. (A-B'') E-Cad accumulates in Rab11 marked vesicles in *sec15* ^{1}(Jafar-Nejad et al., 2005) BCs and FCs. (C-D'') E-Cad accumulates in Rab11 marked vesicles in *sec3*^{GT} BCs and FCs. Mutant cells are marked by * and absence of GFP. Sale bars: 5um.

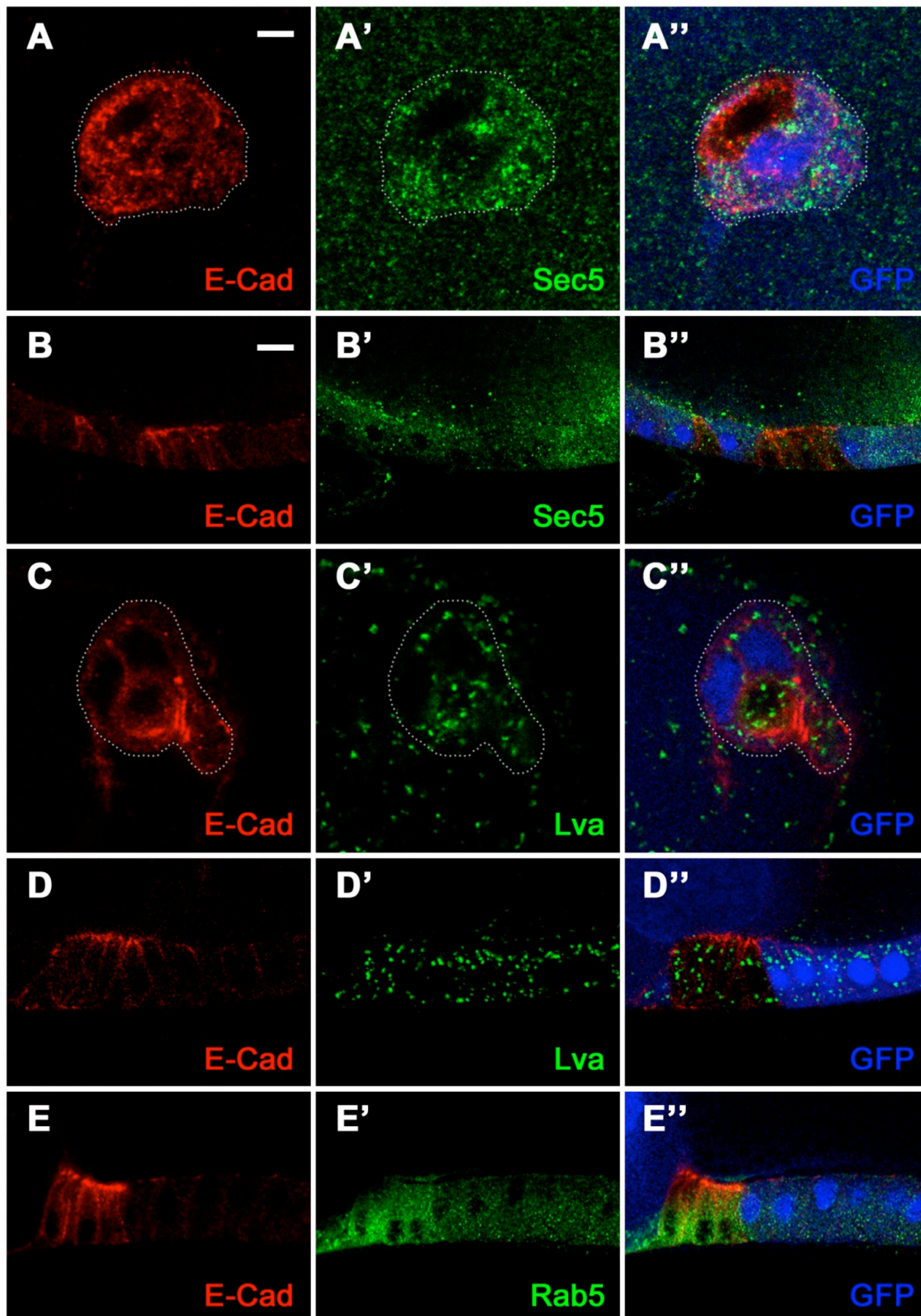

Figure S8. E-cad is not detected in other compartments labeled by Lva and Rab5. (A-B'') *sec3*^{GT} mutant BCs and FCs shows reduced Sec5 signal. (C-D'') *sec3*^{GT} mutant BCs and FCs shows normal distribution of Lva staining, which is and not colocalized with elevated E-Cad staining. (E-E') *sec3*^{GT} mutant FCs shows no siginficant colocalization between Rab5 and E-Cad. The Rab5 signal is elevated in the basal lateral region whereas E-Cad is strongly elevated at the apical cytoplasmic

region. Note that we were unable to obtain good Rab5 stainings in BCs due to antibody penetrability issues. Sale bars: 5um.