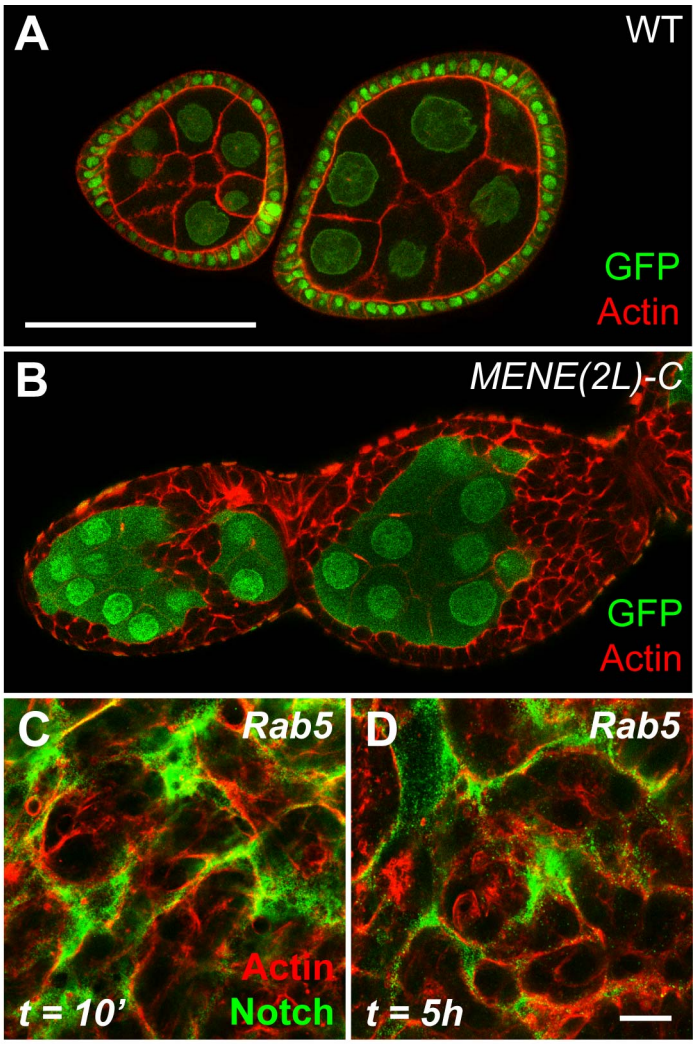
SUPPLEMENTAL FIGURE LEGENDS

Figure S1. *rbsn* and *Rab5* epithelial phenotypes. A-B: In adult ovaries, WT (GFP-positive, green in A) follicle cells form an epithelial monolayer, while clones of *MENE(2L)-C* (*rbsn*, GFP-negative in B) often multilayer and invade the wild-type germ cell cluster. Cells are stained with phalloidin (red) to mark cortical actin. C-D: Notch trafficking assay in *Rab5* mutant eye imaginal discs. After 10 minutes high levels of Notch (green) are present at the cell periphery (C) and persist after 5 hours (D). Cell outlines are labeled by staining for actin (red). Follicle cell clones were generated as in {Bilder, 2000 #3}. Scalebars, 100 μm in A-B, 10 μm in C-D.

Figure S2. Rbsn and Avl remain distinct from activated Rab4 and Rab11. Expression of YFP-tagged versions of constitutively active Rab4 (A, green) or Rab5 (B, green) in larval muscle cells under control of *MEF2-Gal4*. L3 larvae were dissected and stained for Avl (red) and Rbsn (blue). Despite some colocalization between Avl and Rbsn and Rab4 or Rab11 (arrowheads), most Avl and Rbsn does not localize to Rab4- or Rab11-positive structures (arrows). *UAS-Rab4QL-YFP* and *UAS-Rab11QL-YFP* were obtained from the Bloomington Stock Center. Scale, 10 μm.

Figure S3. Subcellular localization and RNAi phenotypes suggest that Syntaxin16 does not function in early endocytosis. A: Syx16 (green, arrows) is not found in the enlarged endosomes (labeled by Avl, red) of wing imaginal discs expressing an activated form of Rab5 (Rab5DA, {Entchev, 2000 #142}). B-C: RNAi-mediated knockdown using *en-GAL4* to drive expression of *UAS-Dicer2* and *UAS-Syx16-IR* transgenes ({Dietzl, 2007 #137} produces adult wings (C) that are indistinguishable from wild-type (B). D-F': Identical coexpression of *UAS-Vps45-IR* and *UAS-Dicer2* produces defects at larval stages: wing imaginal discs lose epithelial architecture (D) and express Mmp1 (A') in addition to accumulating high levels of Notch (E') and Crb (F'). Actin (red, D-F) outlines cells, and GFP marks the e*n-Gal4* expression domain. Note the expanded Crb domains marked by yellow arrows in C'. Scalebars, 5 μm in A, 0.5 mm in B-C, 100 μm in D-E, 10 μm in F.

Figure S4. *rbsn, Vps45* and *Rab5* genetically interact with *avl*. Driving expression of a *UAS-Avl-IR* transgene {Lu, 2005 #27} with *en-GAL4* (A) produces a visible phenotype in the adult wing, which is enhanced by removing one copy of *avl* (B). Removing one genomic copy of *rbsn*, *Rab5*, or *Vps45* (C-E) also enhances the RNAi phenotype. Scale, 0.5 mm.



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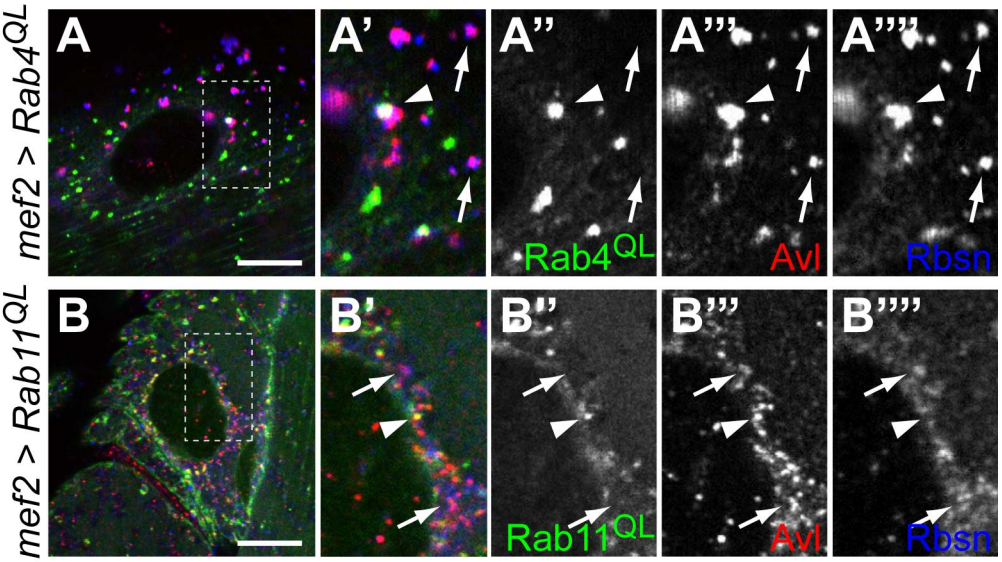

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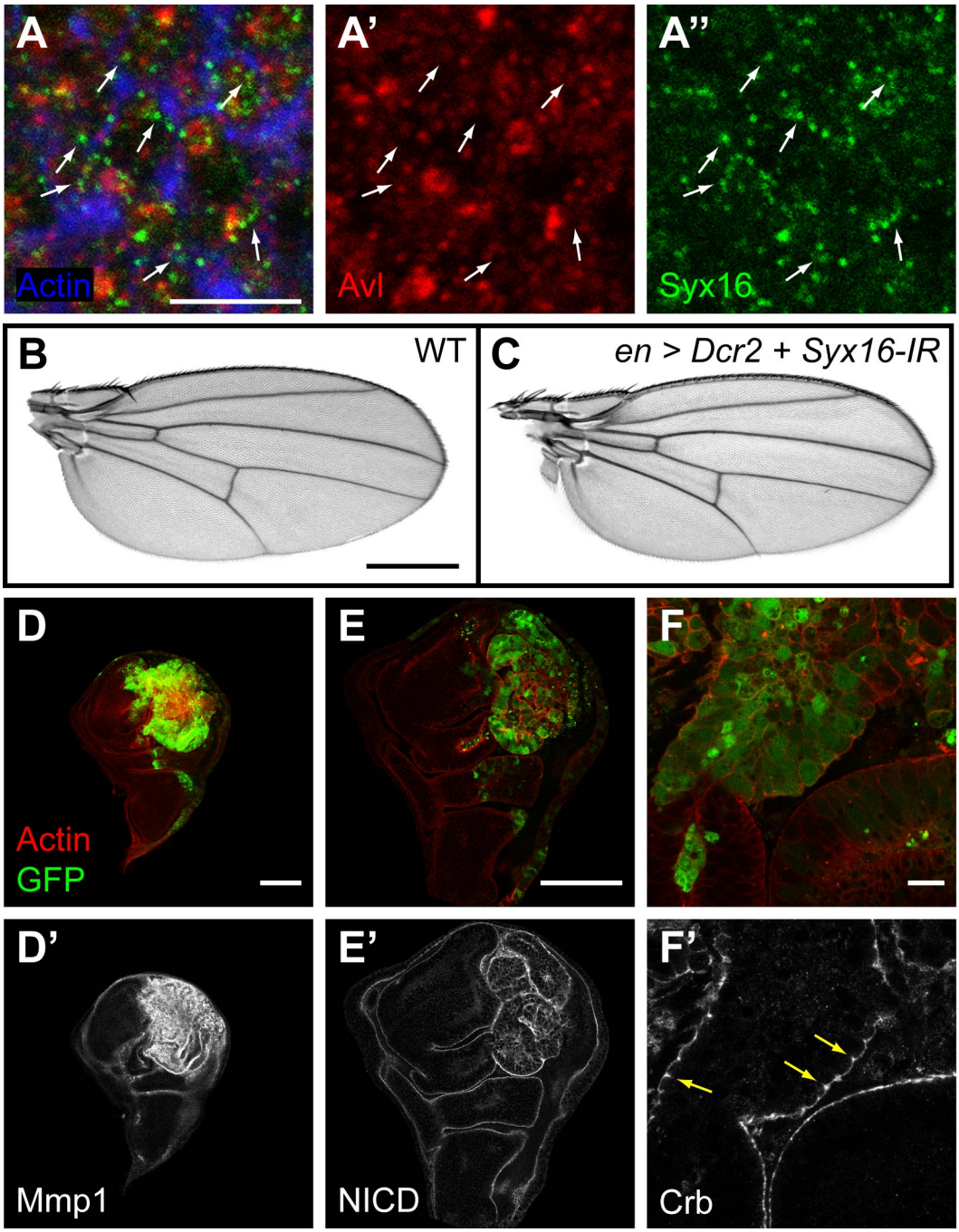

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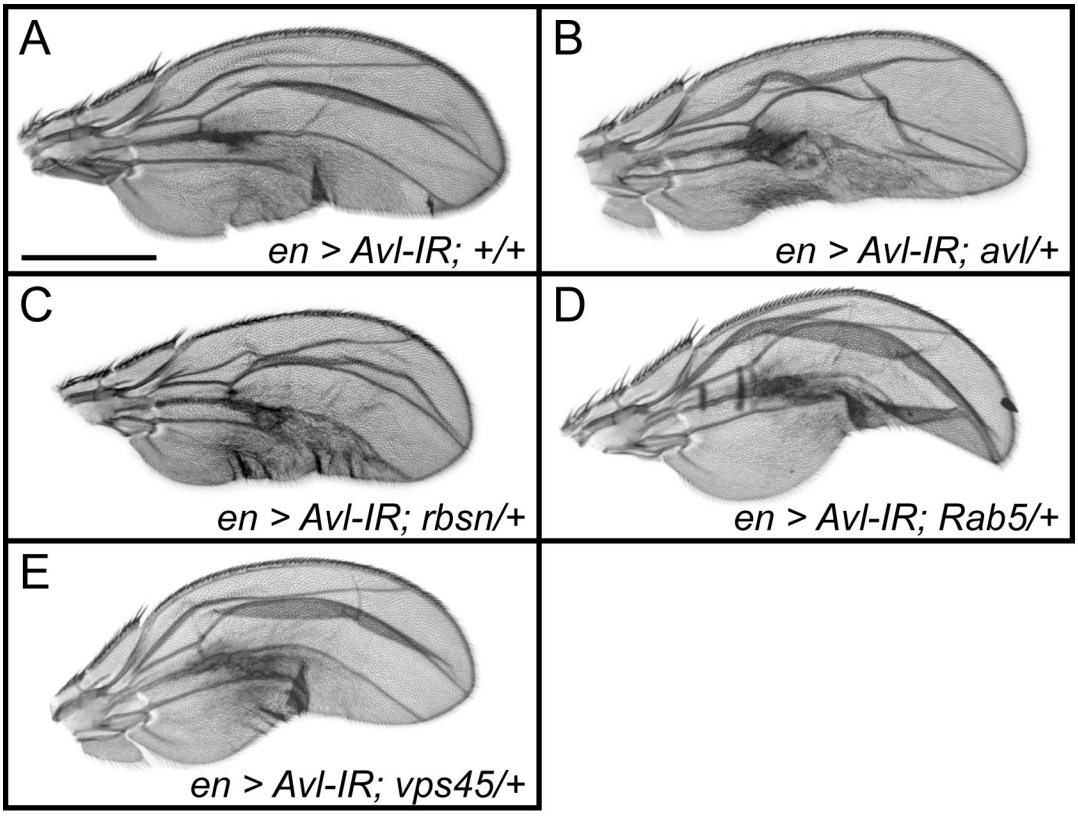

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