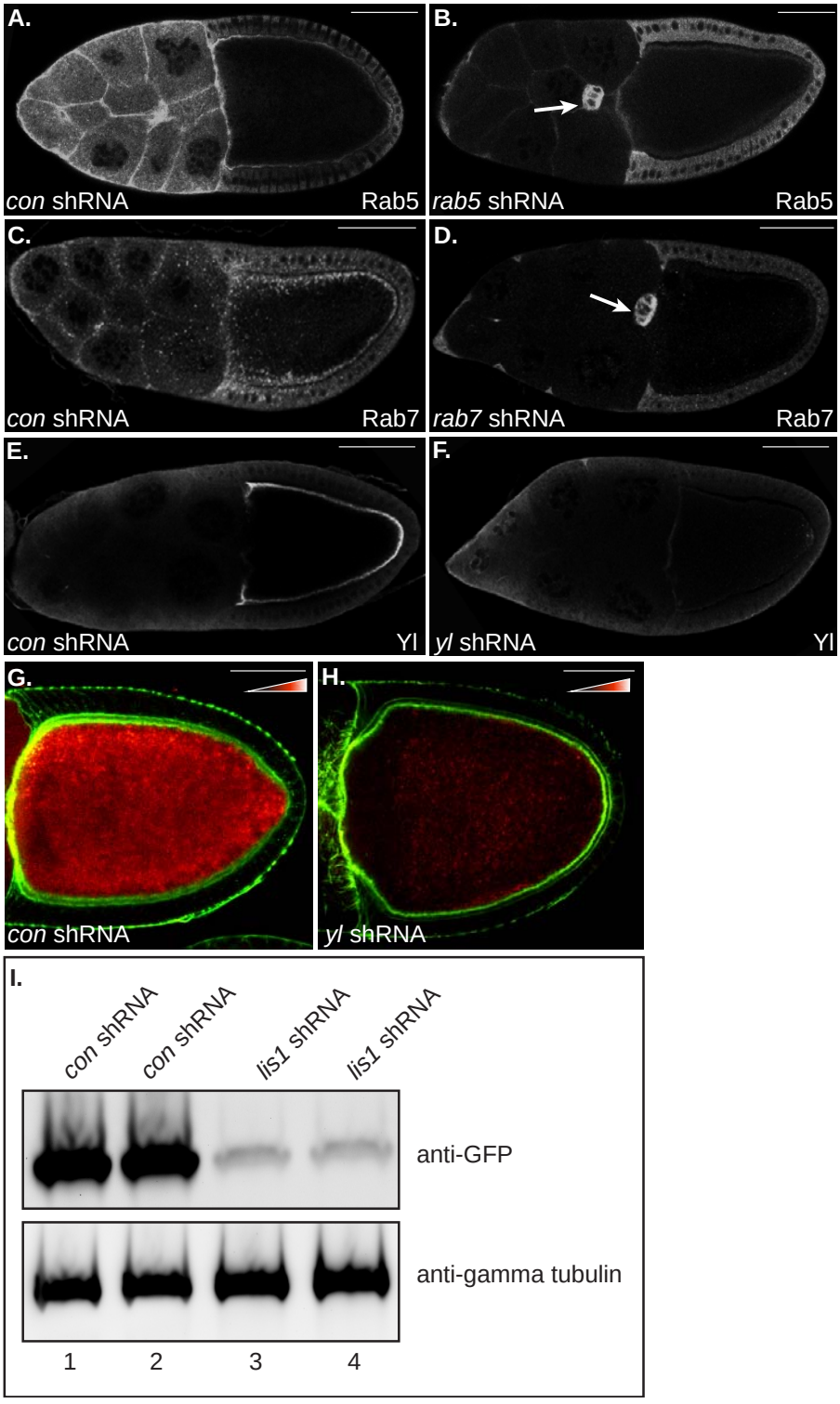

Figure S1 Depletion of Rab5, Rab7, Yl, and Lis1.

A-B. Egg chambers expressing a control shRNA (A) or shRNA against *rab5* (B) were fixed and processed for immunofluorescence using an antibody against Rab5. Representative egg chambers are shown. The arrow indicates somatic border cells. Neither the driver nor the shRNA is expressed in these cells. The border cells therefore represent a good control for the specificity of the depletion.

C-D. Egg chambers expressing a control shRNA (C) or shRNA against *rab7* (D) were fixed and processed for immunofluorescence using an antibody against Rab7. Representative egg chambers are shown. The arrow indicates somatic border cells.

E-F. Egg chambers expressing a control shRNA (E) or shRNA against *yl* (F) were fixed and processed for immunofluorescence using an antibody against Yl. Representative egg chambers are shown.

G-H. Egg chambers expressing a control shRNA (G) or shRNA against *yl* (H) were fixed and stained to reveal the actin cytoskeleton (green). Auto-fluorescent yolk particles are displayed using a color-coded range indicator.

I. Lysates were prepared from *Drosophila* S2 cells co-expressing either a control shRNA against *sh3px1* and GFP-Lis1 (Lanes 1, 2) or *lis1* shRNA-A and GFP-Lis1 (Lanes 3, 4). The lysates were run on an SDS-PAGE gel and examined by western blotting using an antibody against GFP. The blot was subsequently stripped and re-probed using an antibody against gamma-tubulin.

The scale bar on these images represents 50 microns.

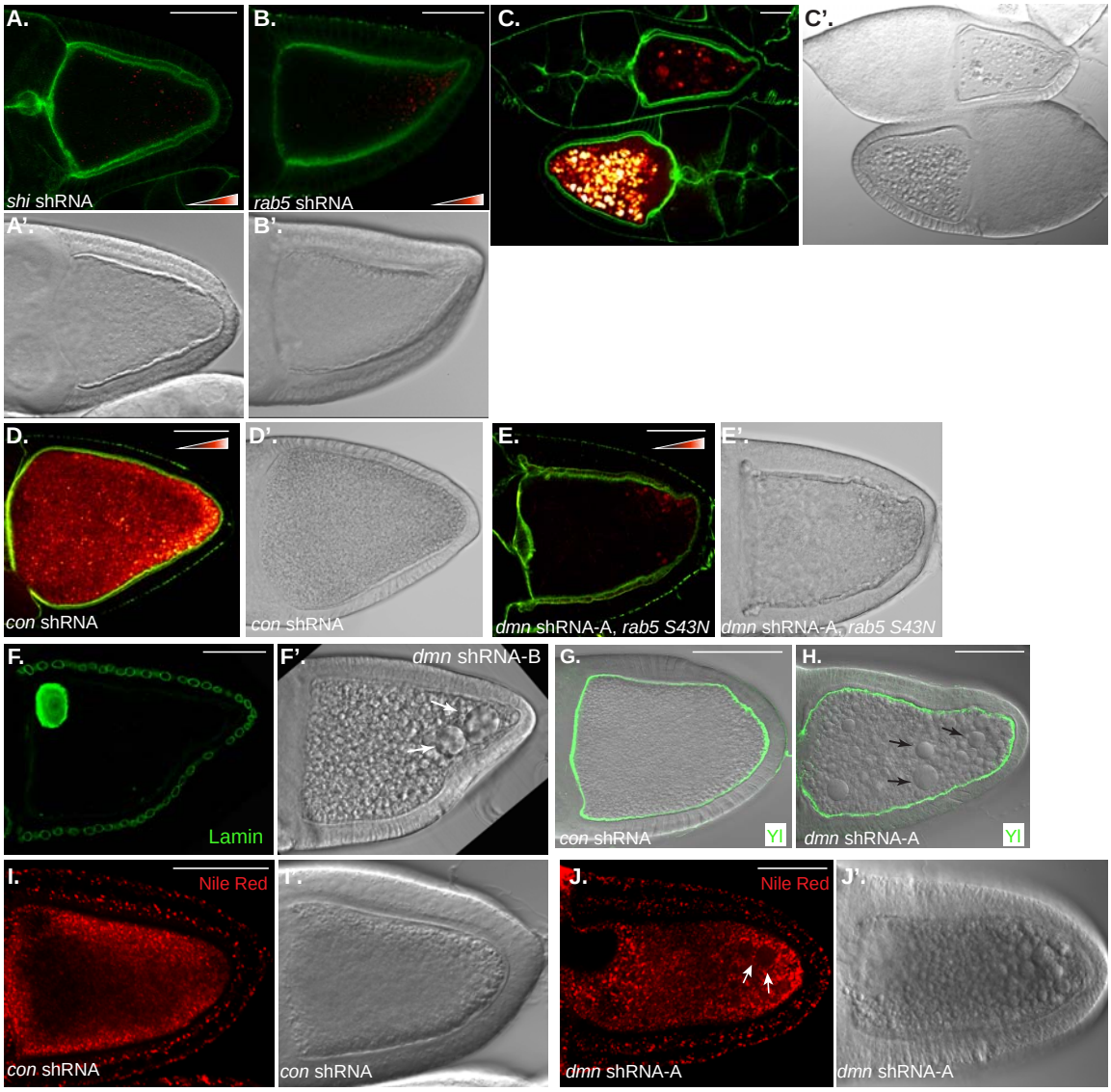

Figure S2 Phenotypes associated with shRNA-mediated depletion.

A-B. Egg chambers expressing an shRNA against *shibire* (A) or *rab5* (B) were fixed and stained to reveal the actin cytoskeleton (green). Auto-fluorescent yolk particles are displayed using a color-coded range indicator. DIC images are shown in A' and B'.

C. Egg chambers from a strain expressing *rab5* Q88L is shown. The egg chambers were fixed and stained to reveal F-actin (green). Yolk auto-fluorescent is displayed using a color-coded range indicator. A DIC image of these egg chambers is shown in C'.

D-E. Egg chamber expressing a control shRNA (D) or co-expressing *dmn* shRNA-A and *rab5S43N* (E) were fixed and stained to reveal the actin cytoskeleton (green). Auto-fluorescent yolk particles are displayed using a color-coded range indicator. DIC images are shown in D' and E'.

F. Egg chambers expressing *dmn shRNA-B* (F) were fixed and stained using an antibody against Lamin DmO (green). The DIC images is shown in F'.

G-H. Egg chambers expressing a control shRNA (G), or *dmn* shRNA-A (H) were fixed and processed for immunofluorescence using an antibody against Yl (green). The immunofluorescence signal was superimposed on the DIC image of the same egg chamber. Arrows indicate enlarged endosomes present in the Dmn depleted oocytes.

I-J. Egg chambers expressing a control shRNA (I) or *dmn* shRNA-A (J) were dissected and processed for Nile Red staining (red). DIC images are shown in I' and J'. The arrows indicate enlarged vesicles that are negative for Nile Red staining.

The scale bar on these images represents 50 microns.

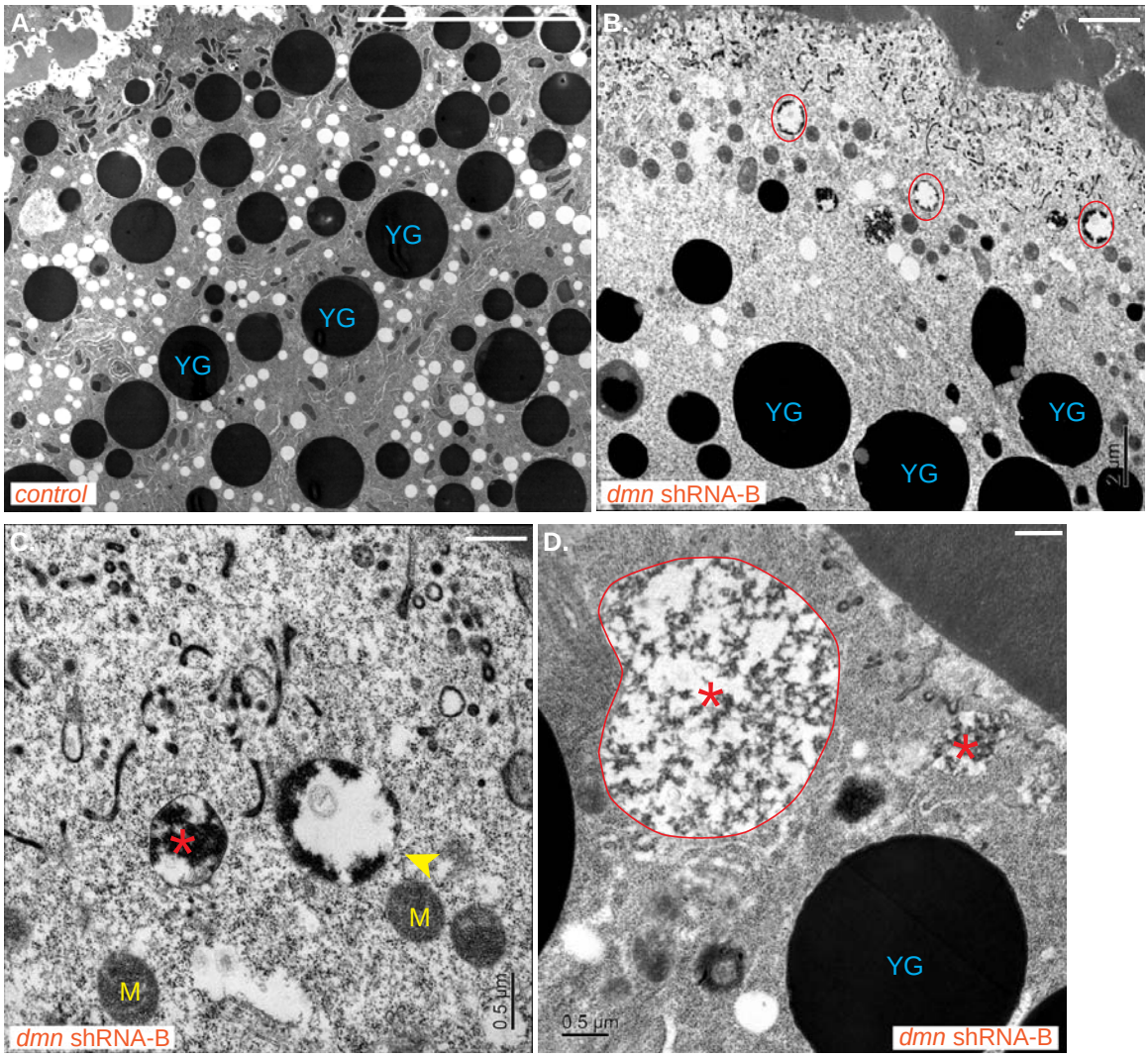

Figure S3 Ultra-structural images of Dmn depleted oocytes.
A. The ultra-structure of an oocyte expressing a control shRNA is shown. The scale bar is 2 microns.

B. The ultra-structure of an oocyte expressing a *dmn* shRNA-B is shown. The scale bar is 2 microns.

C-D. The ultra-structure of oocytes expressing a *dmn* shRNA-B is shown. The scale bar is 0.5 microns.

YG indicates condensed yolk granules. M indicates mitochondria. The red circles indicate endocytic intermediates. The arrowhead indicates endocytic intermediate structures containing some yolk and intraluminal vesicles. The asterisk indicates endocytic vesicles with partially condensed yolk proteins.

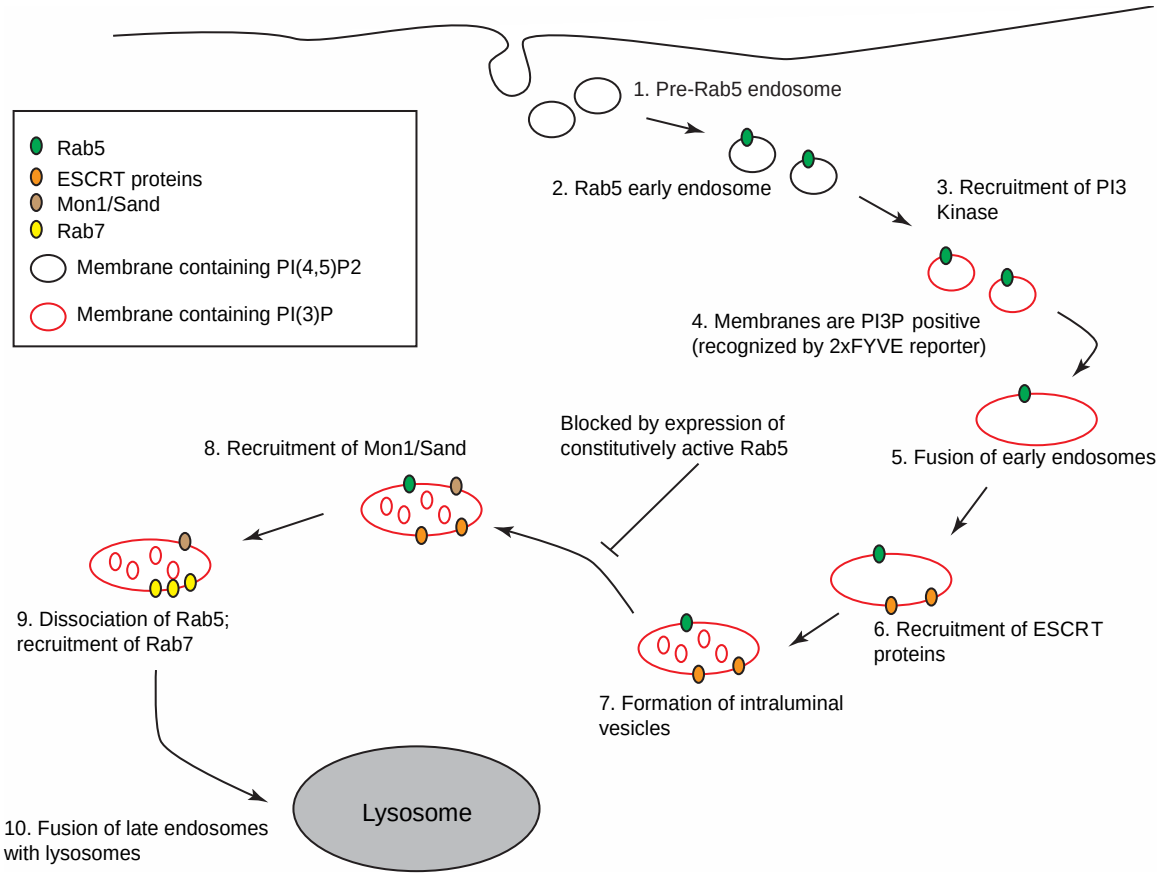

Figure S4 A model of endocytic maturation in mammalian cells.
Upon entry into the cell, some cargoes first traffic through a pre-Rab5 compartment. Subsequently, Rab5 associates with these vesicles. Often, these are referred to as 'early endosomes'. Next, the kinase Vps34 is recruited to these endosomes by Rab5, thus generating phosphatidylinositol 3-phosphate (PI3P) on the membrane of these vesicles. This phosphoinositide modification is recognized by the 2xFYVE reporter. These vesicles also have a tendency to fuse and become larger. Next, proteins of the ESCRT complex are recruited to early endosomes by binding PI3P present on the membrane of these vesicles. The ESCRT complex proteins are responsible for formation of intraluminal vesicles. Subsequently, the protein Mon1/Sand is recruited to these endosomes. The activity of Mon1/Sand results in displacement of Rab5 and in concomitant recruitment of Rab7. These Rab7-positive vesicles are often referred to as 'late endosomes'. Finally, Rab7 promotes the fusion of late endosomes with lysosomes.