

Hagedorn et al., <http://www.jcb.org/cgi/doi/10.1083/jcb.200602054>

## Supplemental materials and methods

### Fly genetics

All fly crosses were carried out at 25°C in standard laboratory conditions. For  $\alpha$ -Elav staining of eye imaginal discs,  $dAux^{J670K}/dAux^{J670K}$  and  $dAux^{J670K}/dAux^{w328X}$  were used. Colocalization analyses between Delta-positive structures and various subcellular structure markers were performed in *UAS-GFP-Rab5* (Wucherpfennig et al., 2003), *UAS-GFP-Rab7* (Entchev et al., 2000), *UAS-GFP-Rab11* (this study), and *UAS-Clc-EGFP* (Chang et al., 2002) mated individually to *GMR-GAL4*, *UAS-Dl-mRFP*.

### Immunohistochemistry

Immunostaining of eye discs was performed according to Wolff (2000). Rat  $\alpha$ -Elav 7E8A10 (Developmental Studies Hybridoma Bank) was used at 1:100, and the secondary antibody Alexa Fluor 594 (Invitrogen) was used at 1:200. All samples were mounted in VECTASHIELD Mounting Medium (Vector Laboratories) and imaged at 25°C with a 20x (.05) or 60x (1.25) lens using a confocal microscope (Nikon OPTIPHOT-2; MRC1024 system [Bio-Rad Laboratories]) with LaserSharp 3.0 software (Bio-Rad Laboratories). Images were processed in Volocity (Improvision) for 3D reconstructions, and  $\gamma$  levels and image size were adjusted using Photoshop (Adobe).