

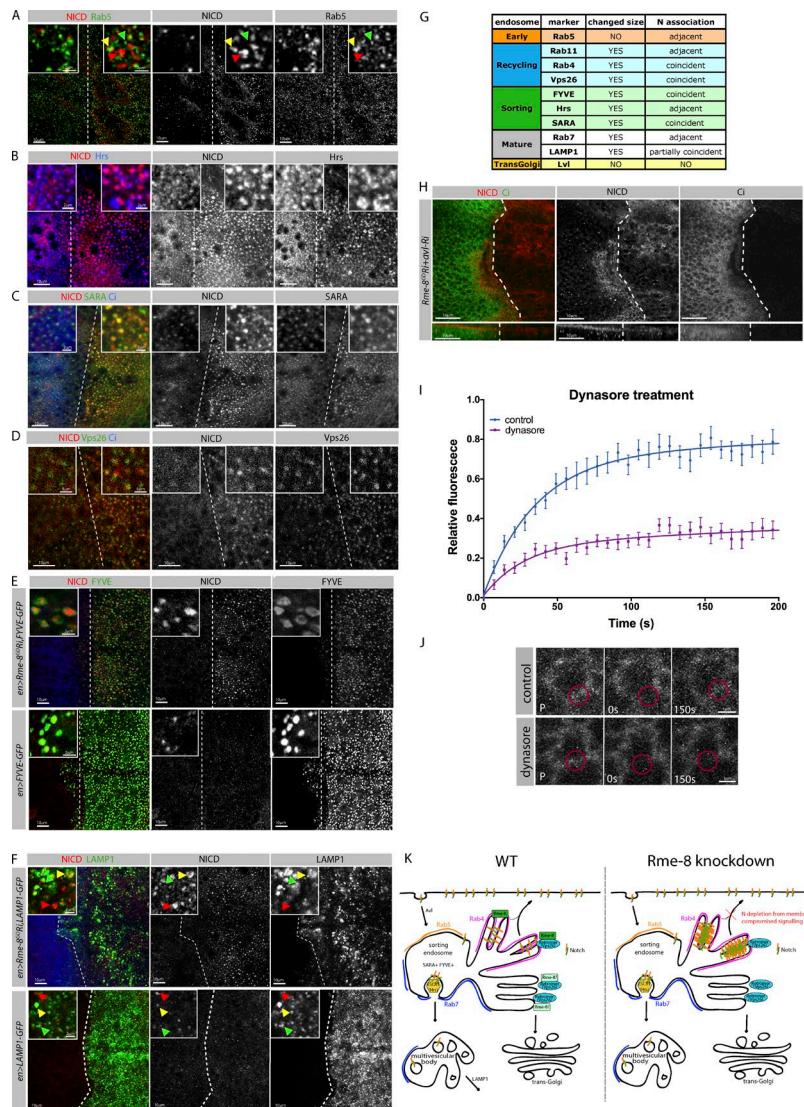
## Rme-8 depletion perturbs Notch recycling and predisposes to pathogenic signaling

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In Figure S3 J, the images of single FRAP examples were inadvertently removed during the production process. Below is the corrected version of the figure.

The supplemental material PDF has been corrected.



**Figure S3. Notch associates with enlarged endosomes.** (A–D) Relationship between Notch (NICD) and endocytic proteins in wing discs expressing *Rme-8-Ri* in the posterior compartment (right side of dashed line). (A) Enlarged puncta containing NICD but not Rab5 are detected in the *Rme-8*-depleted cells; NICD appears to accumulate away from Rab5 (see insets). (B) Enlarged puncta containing NICD and Hrs are detected in the *Rme-8*-depleted cells; NICD appears to accumulate beside Hrs (see insets). (C) Enlarged puncta containing NICD and SARA are detected in the *Rme-8* depleted cells; NICD appears to colocalize with SARA (see insets). (D) Enlarged puncta containing NICD and Vps26 are detected in the *Rme-8*-depleted cells; NICD colocalizes with Vps26 (see insets). (E) Enlarged NICD puncta appear surrounded by FYVE. Similar relationship is detected in WT tissues, where the puncta are smaller; FYVE-GFP expression alone does substantially alter the Notch accumulations (insets). (F) Enlarged puncta containing NICD and LAMP1 are detected in the *Rme-8*-depleted cells. Some puncta contain both proteins (insets, yellow arrowheads) others contain LAMP1 only (insets, green arrowheads), or NICD only (insets, red arrowheads). LAMP1 distribution is also disrupted in comparison to controls (bottom). Expression of LAMP1-GFP alone does not lead to altered accumulation of NICD. (G) Summary table with relevant information about the endosomal markers analyzed in *Rme-8* depleted cells. (H) *Avl* knockdown suppresses Notch accumulation in *Rme-8*-depleted cells. Distribution of NICD (left, red; right, white) and Ci (left, green; right, white) from wing discs expressing *avl-Ri+Rme-8-Ri*; xy section of discs at the subapical plane (top) and xz cross sections of the same specimens (bottom). (I and J) FRAP experiments, measuring recovery of Notch-GFP after photobleaching. (I) Plot of averaged recovery curves (mean  $\pm$  SEM) from Notch-GFP FRAP experiments with best-fit curves as solid lines of WT and dynasore-treated discs. (J) Single FRAP examples with red circles showing the bleach spots. P, prebleach. (K) Proposed model of *Rme-8* function in WT and subsequent *n* trafficking changes in *Rme-8*-depleted conditions.