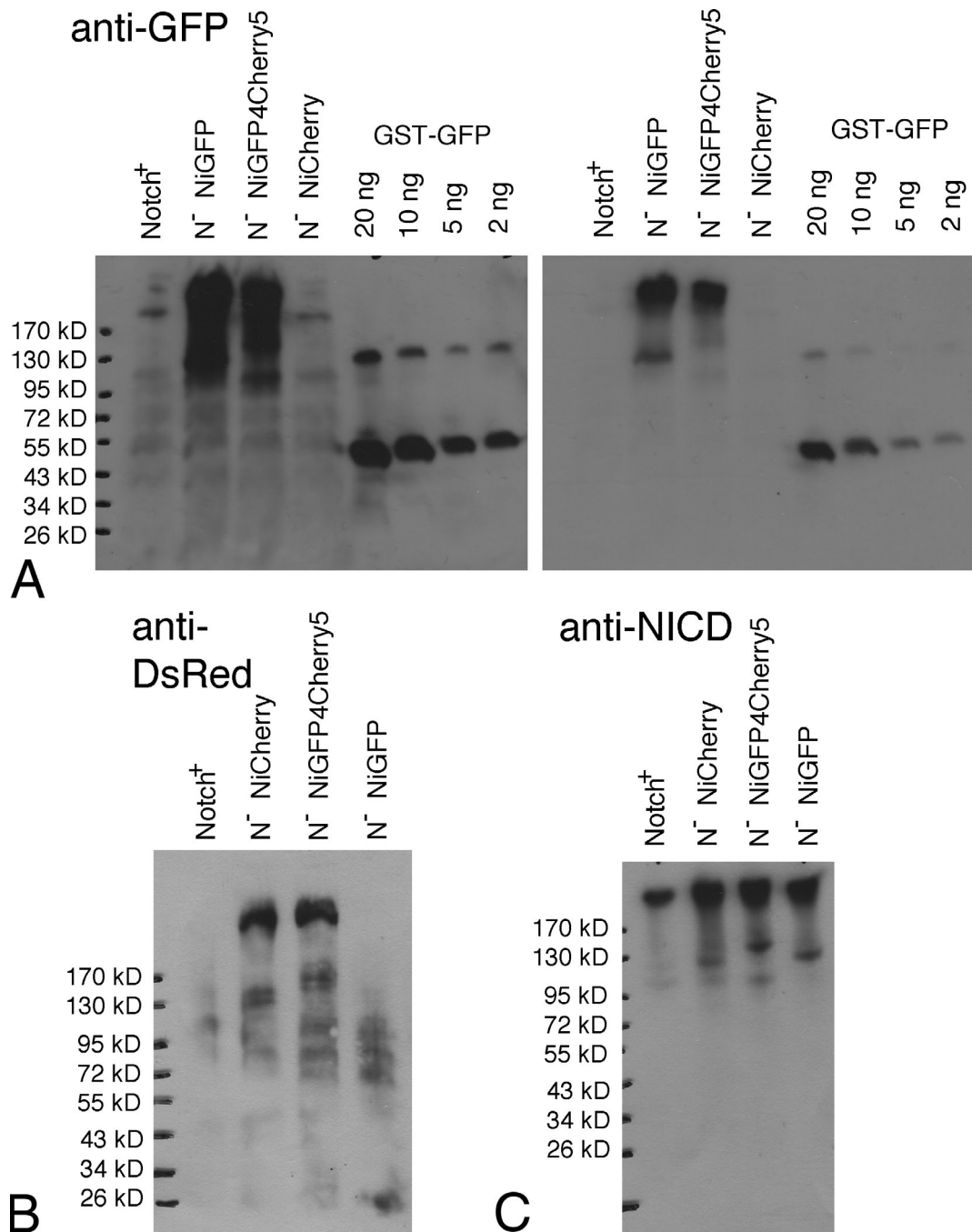
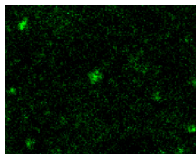


Couturier et al., <http://www.jcb.org/cgi/content/full/jcb.201407071/DC1>

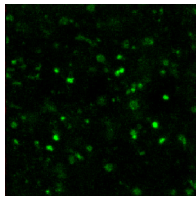
**Figure S1. Western blot analysis of tagged Notch.** (A–C) Anti-GFP (A), anti-DsRed (B; recognizing Cherry), and anti-NICD (C) Western blot analysis of total wing imaginal disc extracts. (A) A purified GST-GFP protein was loaded to evaluate the amount of GFP-tagged Notch in these extracts. (A) Anti-GFP specifically recognized full-length NiGFP and NiGFP4Cherry5 as well as ~140 (NiGFP)- and ~160 (NiGFP4Cherry5)-kD molecular species. These likely correspond to S1-cleaved receptors with the increase in size being attributable to the fluorescent protein tags. Note the absence of signal at the expected size for GFP. (B) Similarly, anti-DsRed specifically recognized full-length NiCherry and NiGFP4Cherry5 and smaller ~140/~160-kD species. No proteolytically resistant GFP and/or Cherry moieties or other unexpected proteolytic fragments were detected (A and B), even on overexposed autoradiograms (A). Anti-NICD recognized full-length Notch, NiGFP, NiCherry, and NiGFP4Cherry5 as well as a ~140 (NiGFP and NiCherry)- and ~160 (NiGFP4Cherry5)-kD molecular species. N, Notch.

Table S1. Genotypes used in this paper

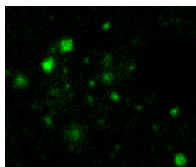
Genotypes	Figures
$N^{55e11} w^{1118}/Y; M[3 \times P3\text{-RFP.attP.w}^*.NiGFP]51D/+$	Fig. 1, B and B'
$N^{55e11} w^{1118}/Y; M[3 \times P3\text{-RFP.attP.w}^*.NiCherry]51D/+$	Fig. 1, C and C'
$N^{55e11} w^{1118}/Y; M[3 \times P3\text{-RFP.attP.w}^*.NiGFP]51D/M[3 \times P3\text{-RFP.attP.w}^*.NiCherry]51D$	Fig. 1, D and D'
$N^{55e11} w^{1118}/Y; ; PB[y^*.attP.w^*.NiGFP4Cherry5]68D/+$	Fig. 1, E–F' and Fig. 3, K and L
$P[pRab\text{-}5\text{-Cherry-Rab5}] w^{1118}/N^{55e11} w^{1118} P[neur\text{-}Histone2B\text{-RFP}]619; ; M[3 \times P3\text{-RFP.attP.w}^*.NiGFP]51D/+$	Fig. 3, B and B'
$N^{55e11} w^{1118} P[neur\text{-}Histone2B\text{-RFP}]619/Y; ; M[3 \times P3\text{-RFP.attP.w}^*.NiCherry]51D/GFP\text{-Rab5}[KI]$	Fig. 3 C
$N^{55e11} w^{1118} P[Ubx\text{-}flp] P[neur\text{-}Histone2B\text{-RFP}]619/Y; ; M[3 \times P3\text{-RFP.attP.w}^*.NiCherry]51D/+; ; P[pTub\text{-}YFP\text{-}Rab11]/+$	Fig. 3 D
$N^{55e11} w^{1118} P[neur\text{-}Histone2B\text{-RFP}]619/Y; ; M[3 \times P3\text{-RFP.attP.w}^*.NiCherry]51D/+; ; P[pTub\text{-}GFP\text{-}Lamp1]/+$	Fig. 3 E
$N^{55e11} w^{1118} P[Ubx\text{-}flp] P[neur\text{-}Histone2B\text{-RFP}]619/Y; ; M[3 \times P3\text{-RFP.attP.w}^*.NiCherry]51D/+; ; P[pTub\text{-}YFP\text{-}Rab7]/+$	Fig. 3, F and G
$N^{55e11} w^{1118}/Y; ; ap\text{-}Gal4 P[pTub\text{-}Gal80^{ts}]/+; ; PB[y^*.attP.w^*.NiGFP4Cherry5]68D/+$	Fig. 3 H
$w^{1118}/Y; ; ap\text{-}Gal4/+; ; PB[y^*.attP.w^*.NiGFP4Cherry5]68D/P\{TRiP.HMSO1287\}attP2 (rbcn3A^{RNAi})$	Fig. 3 I
$N^{55e11} w^{1118} P[Ubx\text{-}flp] P[neur\text{-}Histone2B\text{-RFP}]619/Y; ; P[neo, ry FRT40A] Igd^{d7}/P[neo, ry FRT40A] P[pTub\text{-}nlsRFP]; ; PB[y^*.attP.w^*.NiGFP4Cherry5]68D/+$	Fig. 3, M and M'
$CadGFP (KI)/CadCherry (KI)$	Fig. 5, A–A''
$w^{1118}/Y; ; P[y^*.attP.w^*.SpdoCherry2GFP3]68A4 spdo^{ZZ27}/spdo^{G104}$	Fig. 5 C
$w^{1118}/Y; ; P[y^*.attP.w^*.SpdoCherryGFP]99F8$	Fig. 5, D–D''; Fig. 6 D; and Fig. 7 E
$N^{55e11} w^{1118}/Y; ; P[pneur\text{-}nlsFP670]/+; ; PB[y^*.attP.w^*.NiGFP4Cherry5]68D/+$	Fig. 6 and Fig. 7, A–C''
$P[Ubx\text{-}flp]/Y; ; P[neo, ry FRT40A] numb^{15}/P[neo, ry FRT40A] P[pTub\text{-}nlsGFP]; ; P[y^*.attP.w^*.SpdoCherryGFP]99F8/+$	Fig. 7 F



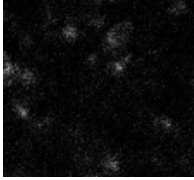
Video 1. **Localization of NiGFP and Cherry-Rab5 in notum epithelial cells.** Live imaging of GFP-tagged Notch (green) and Cherry-tagged Rab5 (red) in an  $N^{55e11} H2B\text{-RFP}/Cherry\text{-Rab5}; NiGFP/+$  pupa at 16 h APF showing a subapical confocal section. A large NiGFP-positive, Cherry-Rab5 endosome is indicated with an arrow. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 580; Carl Zeiss). Time is in minutes and seconds.



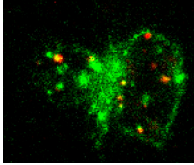
Video 2. **Localization of NiGFP and Cherry-Rab4 in notum epithelial cells.** Live imaging of GFP-tagged Notch (green) and Cherry-tagged Rab4 (red) in an  $N^{55e11}/Y; Cherry\text{-Rab4}; NiGFP/+$  pupa at 16 h APF. A subapical confocal section is shown. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 580; Carl Zeiss). Time is in minutes and seconds.



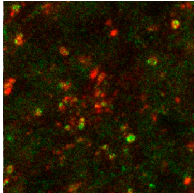
Video 3. **Localization of NiCherry and GFP-Rab5 in notum epithelial cells.** Live imaging of Cherry-tagged Notch (red) and GFP-tagged Rab5 (green) in an  $N^{55e11} H2B\text{-RFP}/Y; NiCherry/GFP\text{-}Rab5$  pupa at 16 h APF. A subapical confocal section is shown. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 580; Carl Zeiss). The arrow points to a Rab5-positive endosome that contains NiCherry. Time is in minutes and seconds.



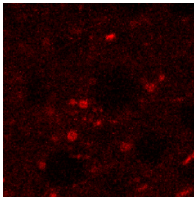
Video 4. **Localization of NiCherry and YFP-Rab7 in notum epithelial cells.** Live imaging of Cherry-tagged Notch (red) and YFP-tagged Rab7 (green) in an  $N^{55e11}$   $H2B-RFP/Y$ ;  $NiCherry/YFP-Rab7$  pupa at 16 h APF. A basal confocal section is shown. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 580; Carl Zeiss). Time is in minutes and seconds.



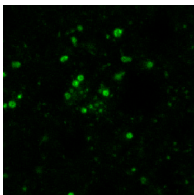
Video 5. **Localization of SpdoCherryGFP in pIIa/pIIb cells.** Live imaging of SpdoCherryGFP (red and green) in a  $SpdoCherryGFP/+$  pupa at 16 h APF. An endosomal fusion event seen in pIIb is highlighted using a 4x magnification. pIIa/pIIb cell pairs were imaged at  $t = 20$  min after mitosis. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 580; Carl Zeiss). Time is in minutes and seconds.



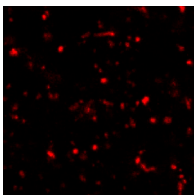
Video 6. **Localization of NiGFP and Cherry-Rab5 in pIIa/pIIb cells.** Live imaging of GFP-tagged Notch (green) and Cherry-tagged Rab5 (red) in an  $N^{55e11}$   $H2B-RFP/Cherry-Rab5$ ;  $NiGFP/+$  pupa at 16 h APF. The pIIa and pIIb cells were identified on apical (weak NiGFP signal; left image) and basal ( $H2B-RFP$ -positive nuclei; central image). NiGFP-positive Cherry-Rab5 endosomes are indicated with a green arrow in pIIb at  $t = 20$  min after mitosis. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 580; Carl Zeiss). Time is in minutes and seconds.



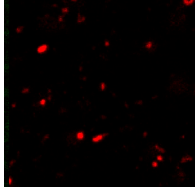
Video 7. **Localization of SpdoGFP1 and Cherry-Rab5 in pIIa/pIIb cells.** Live imaging of GFP-tagged Spdo (green) and Cherry-tagged Rab5 (red) in a  $Cherry-Rab5/+$ ;  $SpdoGFP1/+$  pupa at 16 h APF. SpdoGFP1 colocalized with Cherry-Rab5 in pIIb 20 min after mitosis (Spdo was used to identify pIIa/pIIb cells). A subapical confocal section is shown. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 580; Carl Zeiss). Time is in minutes and seconds.



Video 8. **Localization of NiCherry and GFP-Rab5 in pIIa/pIIb cells.** Live imaging of Cherry-tagged Notch (red) and GFP-tagged Rab5 (green) in an  $N^{55e11}$   $H2B-RFP/Y$ ;  $NiCherry/GFP-Rab5$  pupa at 16 h APF. The pIIa and pIIb cells were identified using  $H2B-RFP$  (left). A subapical confocal section is shown in the video. NiCherry did not colocalize with GFP-Rab5 in pIIb at  $t = 20$  min after mitosis. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 580; Carl Zeiss). Time is in minutes and seconds.



Video 9. **Localization of NiCherry and SpdoGFP1 in pIIb.** Live imaging of Cherry-tagged Notch (red) and GFP-tagged Spdo (green) in an  $N^{55e11}$   $H2B-RFP/+$ ;  $NiCherry/+$ ;  $SpdoGFP1/+$  pupa at 16 h APF. The pIIa and pIIb cells were identified using  $H2B-RFP$  and SpdoGFP1. A subapical confocal section is shown in the video. NiCherry did not colocalize with SpdoGFP1 in pIIb at  $t = 20$  min after mitosis. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 580; Carl Zeiss). Time is in minutes and seconds.



Video 10. **Localization of NiCherry and NumbGFP in p11b.** Live imaging of Cherry-tagged Notch (red) and GFP-tagged Numb (green) in an *NiCherry/+; NumbGFP/+* pupa at 16 h APF. The p11a and p11b cells were identified using the unequal segregation of Numb at mitosis. A subapical confocal section is shown in the video. NiCherry did not colocalize with NumbGFP in p11b at  $t = 20$  min after mitosis. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 580; Carl Zeiss). Time is in minutes and seconds.