Giagtzoglou et al., http://www.jcb.org/cgi/content/full/jcb.201106088/DC1

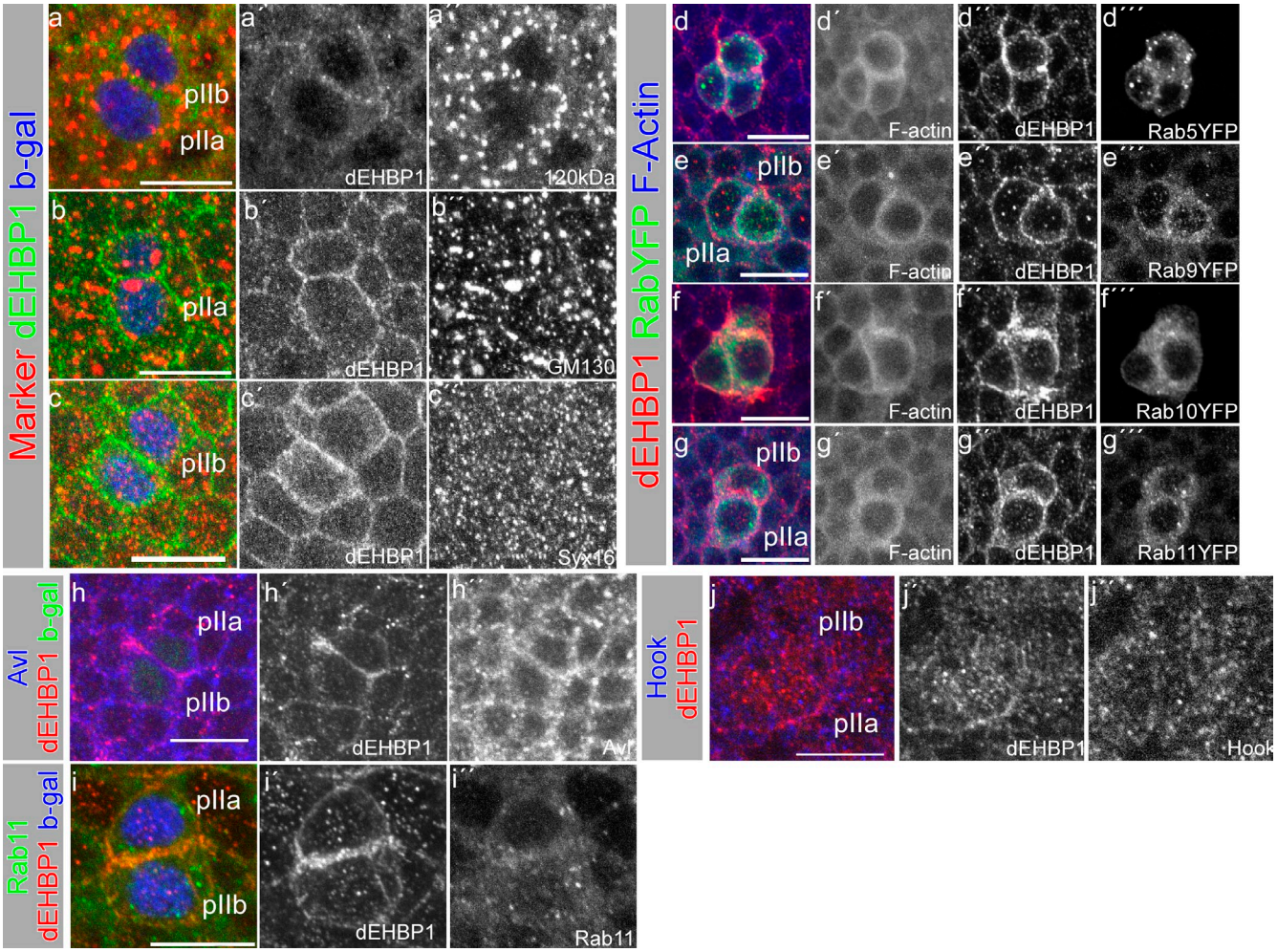


Figure S1. **dEHBP1 does not colocalize with markers of Golgi and early, late, or recycling endosomes (related to Figs. 3 and 4).** (a–c′′) dEHBP1 (a′, b′, and c′) does not colocalize with Golgi markers, p120kD (a′′), GM130 (b′′), or Syx16 (c′′). d-g′′′) dEHBP1 (d′′, e′′, f′′, and g′′) colocalizes with F-actin (d′, e′, f′, and g′) at the interphase of pIIb/pIIa cells (e and g), but it does not colocalize with Rab5YFP (d′′′), Rab9YFP (e′′′), Rab10YFP (f′′′), or Rab11YFP (g′′′), overexpressed in ESO lineages by *neur*^{Gal4}. (h–j′′) dEHBP1 (h′, i′, and j′) does not colocalize with Avl (h′′), Rab11 (i′′), and Hook (j′′), markers of early, recycling, and late endosomes, respectively. Bars, 10 µm.

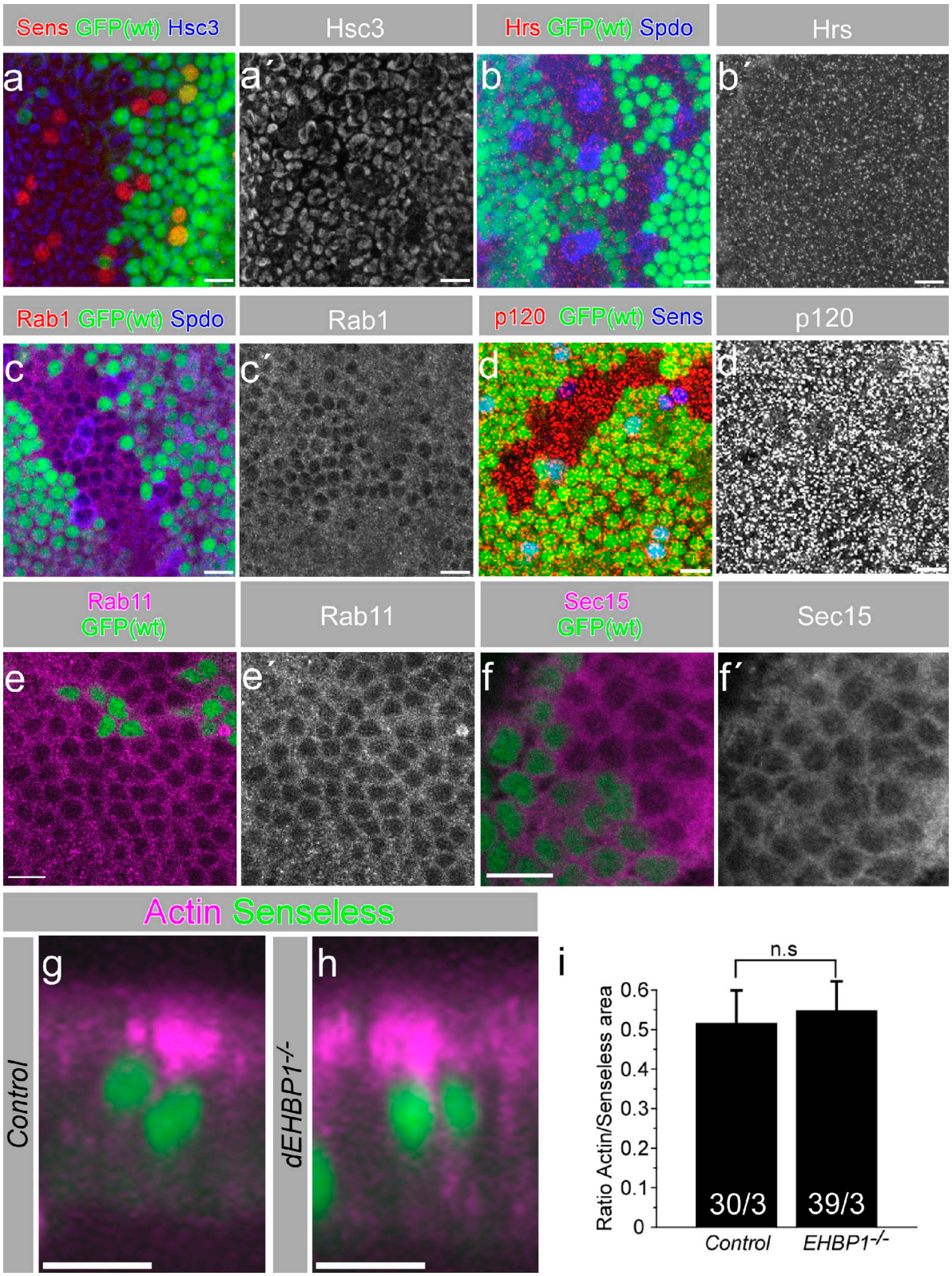


Figure S2. **The distribution of different subcellular compartments and the integrity of ARS are not affected in the absence of dEHBP1 (related to Fig. 5, a-f').** The endoplasmic reticulum marker Hsc3 (a–a'), the late endosomal marker Hrs (b–b'), the Golgi markers Rab1 (c–c') and p120 kD (d–d'), the recycling endosomal marker Rab11 (e–e'), and the exocyst member Sec15 (f–f') are unaffected in the absence of *dEHBP1*. Single channel representations for Hsc3, Hrs, Rab1, p120 kD, Rab11, and Sec15 are shown in a', b', c', d', e', and f', respectively. GFP marks the wild-type region in a, b, c, d, e, and f. (g and h) The characteristic ARS is formed properly between pII cells in the presence (g) or absence of *dEHBP1* (h). Bars (a–h): 10 µm. (i) Quantification of the ratio of the area of Actin to the area of Sens nuclei in the control and *dEHBP1*$^{-/-}$ ESO clusters. Numbers at the base of the bars represent the number of ESO clusters/thoraces used for quantification.

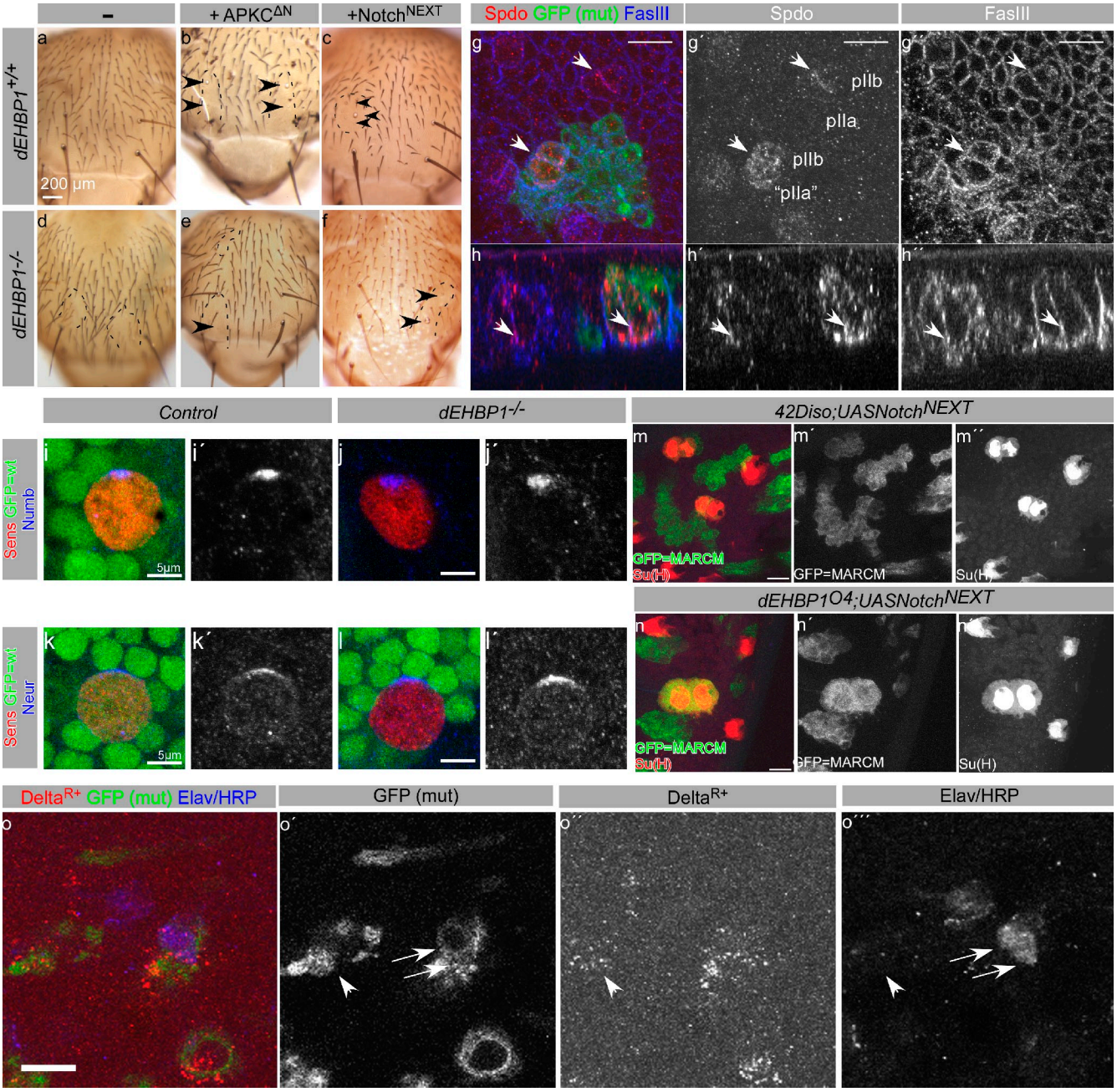
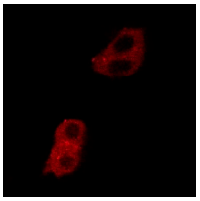
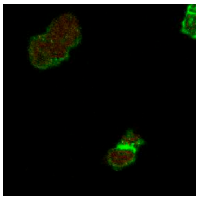


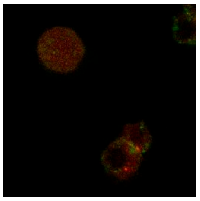
Figure S3. **Loss of dEHBP1 is epistatic to gain of function of Notch signaling, achieved by ectopic expression of constitutively active DaPKC$^{\Delta N}$ or Delta^{R+}, but not by activated Notch^{NEXT}.** (a and b) Loss of *dEHBP1* within thoracic clones results in bald patches of cuticle, devoid of mechanosensory bristles, as outlined by dashed line in b (compare a with b; also see Fig. 1). (b and c) Overexpression of *DaPKC$^{\Delta N}$* (b) or *Notch^{NEXT}* (c) in wild-type (*FRT42Diso*) clones within thoracic epithelia, outlined by dashed line, results in enlarged socket structures, indicated by the arrowheads. (e) Overexpression of *DaPKC$^{\Delta N}$* in the absence of *dEHBP1* within thoracic clones, outlined by dashed line, results in bald patches of cuticle, devoid of mechanosensory bristles, as indicated by the arrowheads. (f) Overexpression of *Notch^{NEXT}* in the absence of *dEHBP1* within thoracic clones, outlined by dashed line, still results in enlarged socket structures, indicated by the arrowheads. (g–h′′) XY sections (g–g′′) and a single XZ section (h–h′′) of thoracic epithelia bearing clones of *dEHBP1^{O4}* overexpressing *DaPKC$^{\Delta N}$*. Please note that Spdo is enriched in the absence of *dEHBP1* (bottom cluster in g′–g′′, right cluster in h′–h′′). Spdo (g′–h′) localizes at the interphase of pII cells, along with the lateral membrane marker FasIII (g′′–h′′), in the presence or absence of dEHBP1, as the arrows indicate. Bars, 10 µm. (i–l′) The localization of the cell fate determinants Numb (i–j′′) and Neur (k–l′′) in the presence (i–i′, k–k′) or absence (j–j′, l–l′) of dEHBP1. Single channel representations for Numb (i′–j′) and Neur (k′–l′) are shown in black and white. Bars, 5 µm. (m-n′′) Overexpression of *Notch^{NEXT}* in wild-type (m–m′′) or *dEHBP1^{O4}* (n–n′′) results in the development of extra Su(H)-positive socket cells. Single channel representations are shown for GFP (m–m′′) and Su(H) (n–n′′). Bars, 10 µm. (o–o′′) Overexpression of the variant *Delta $^{R+}$* in the absence of *dEHBP1* results in the development of extra Elav/HRP-positive neuronal cells, indicated by arrows. Overexpression of the variant *Delta $^{R+}$* is driven by the ubiquitous driver *tub^{Gal4}*, and therefore, we detect Delta in epithelial cells as well (left arrowhead). Single channel representations are shown for GFP (o′), Delta^{R+} (o′′), and Elav/HRP (o′′′). Bars, 10 µm.



Video 1. **mCherry-dEHBP1 is localized within intracellular vesicles and at the interface of pIIa and pIIb cells.** Dividing cells in ESO clusters expressing mCherry-dEHBP1 by neur^{Gal4} driver were imaged by time-lapse confocal microscopy using an inverted laser scanning confocal microscope (model TE2000U; Nikon) equipped with a C1 confocal imaging system (488-, 543-, and 633-nm lasers; Nikon). Frames were taken every minute. Images are shown at the medial level of the cluster (related to Fig. 4).



Video 2. **mCherry-dEHBP1 is localized partially with Spdo-GFP at the apical side of the interface of pIIa and pIIb cells.** Dividing cells in ESO clusters expressing Spdo-GFP and mCherry-dEHBP1 by neur^{Gal4} driver were imaged by time-lapse confocal microscopy using an inverted laser scanning confocal microscope (model TE2000U; Nikon) equipped with a C1 confocal imaging system (488-, 543-, and 633-nm lasers; Nikon). Frames were taken every minute. Images are shown at the apical level of the cluster (related to Fig. 4).



Video 3. **mCherry-dEHBP1 is localized partially with Spdo-GFP at the medial side of the interface of pIIa and pIIb cells.** Dividing cells in ESO clusters expressing Spdo-GFP and mCherry-dEHBP1 by neur^{Gal4} driver were imaged by time lapse confocal microscopy using an inverted laser scanning confocal microscope (model TE2000U; Nikon) equipped with a C1 confocal imaging system (488-, 543-, and 633-nm lasers; Nikon). Frames were taken every minute. Images are shown at the medial level of the cluster (related to Fig. 4).