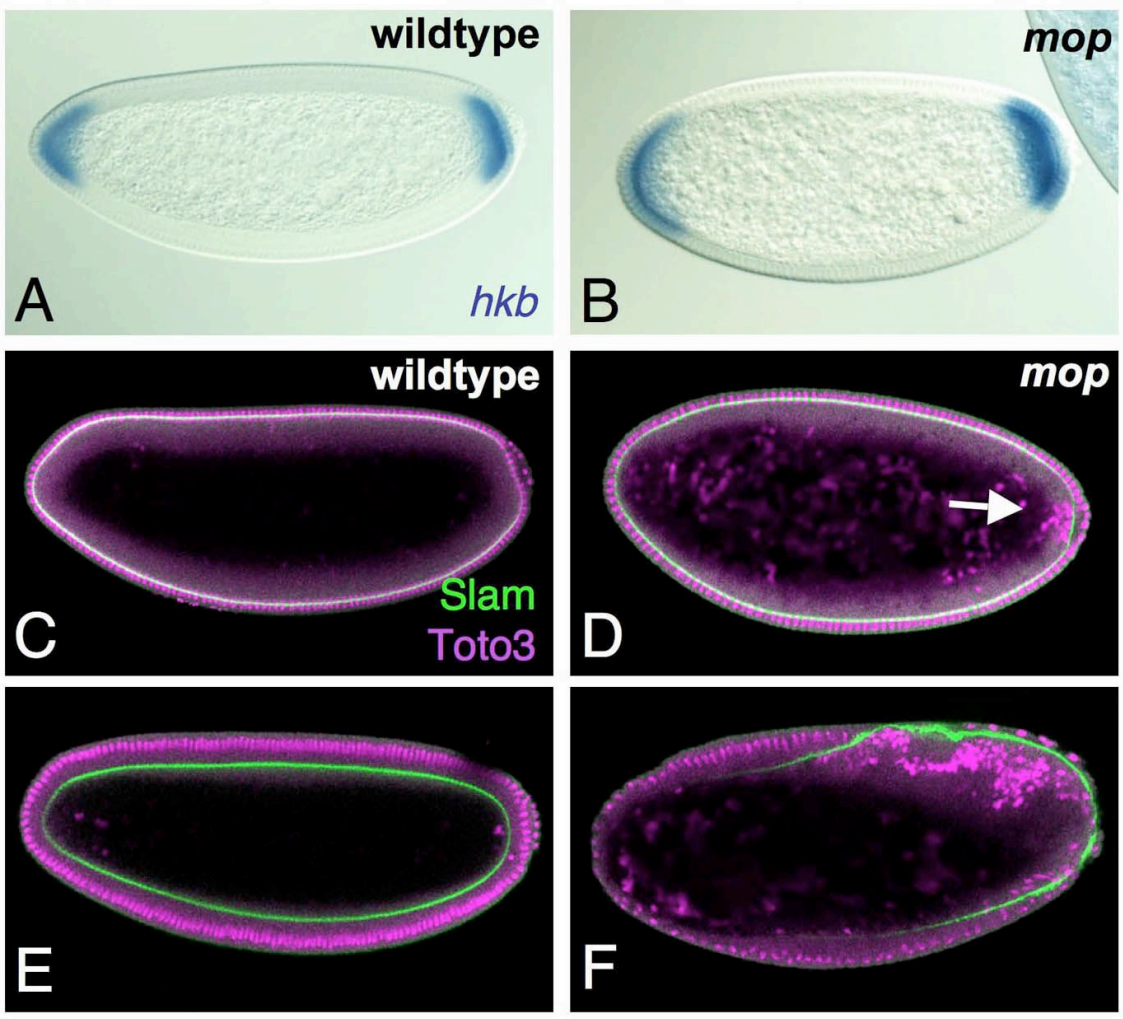
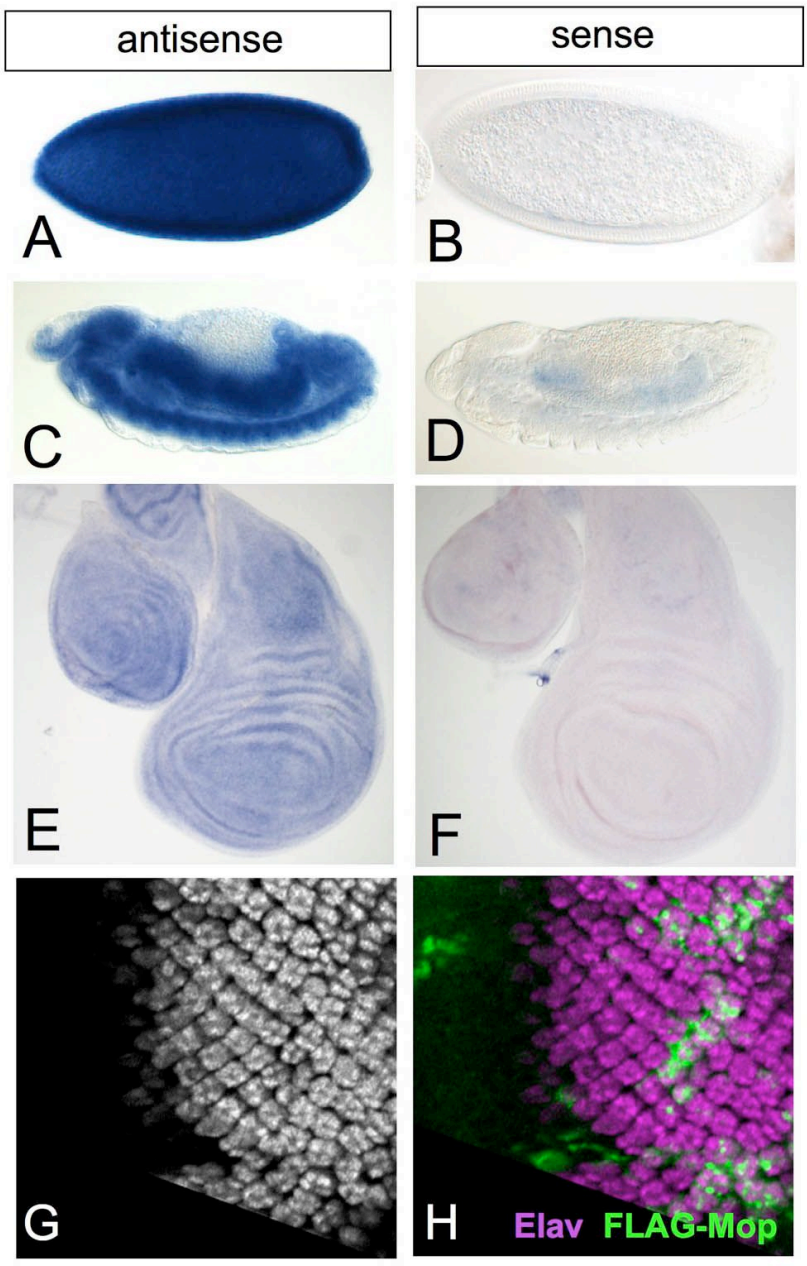
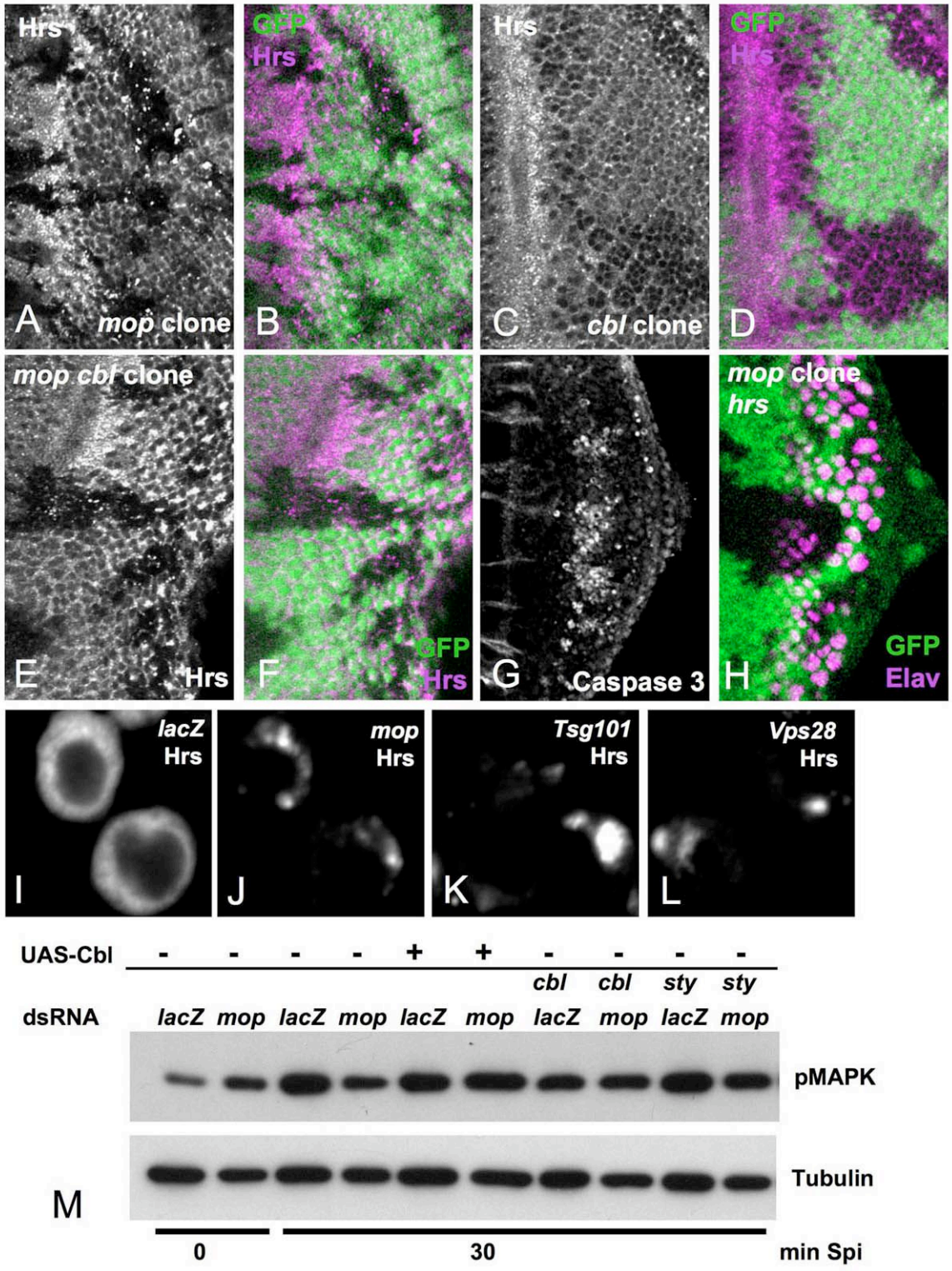


Supplementary Figure 1: Embryonic *mop* mutant phenotypes. All panels show early embryos with anterior to the left. (A, C, E) wildtype; (B, D, F) embryos derived from mop^{T612} germline clones. (A, B) show in situ hybridization with a *hkb* probe. Embryos lacking *mop* show normal *hkb* expression, indicating that Torso signaling is unaffected. (C-F) are stained for Slam, which marks the front of invaginating membranes (green) and for Toto-3 to label nuclei (magenta). The first visible abnormality in embryos lacking *mop* is a loss of association of nuclei with the cortex (arrow in D). As cellularization proceeds, membrane invagination becomes irregular and more nuclei fall into the embryo (F).

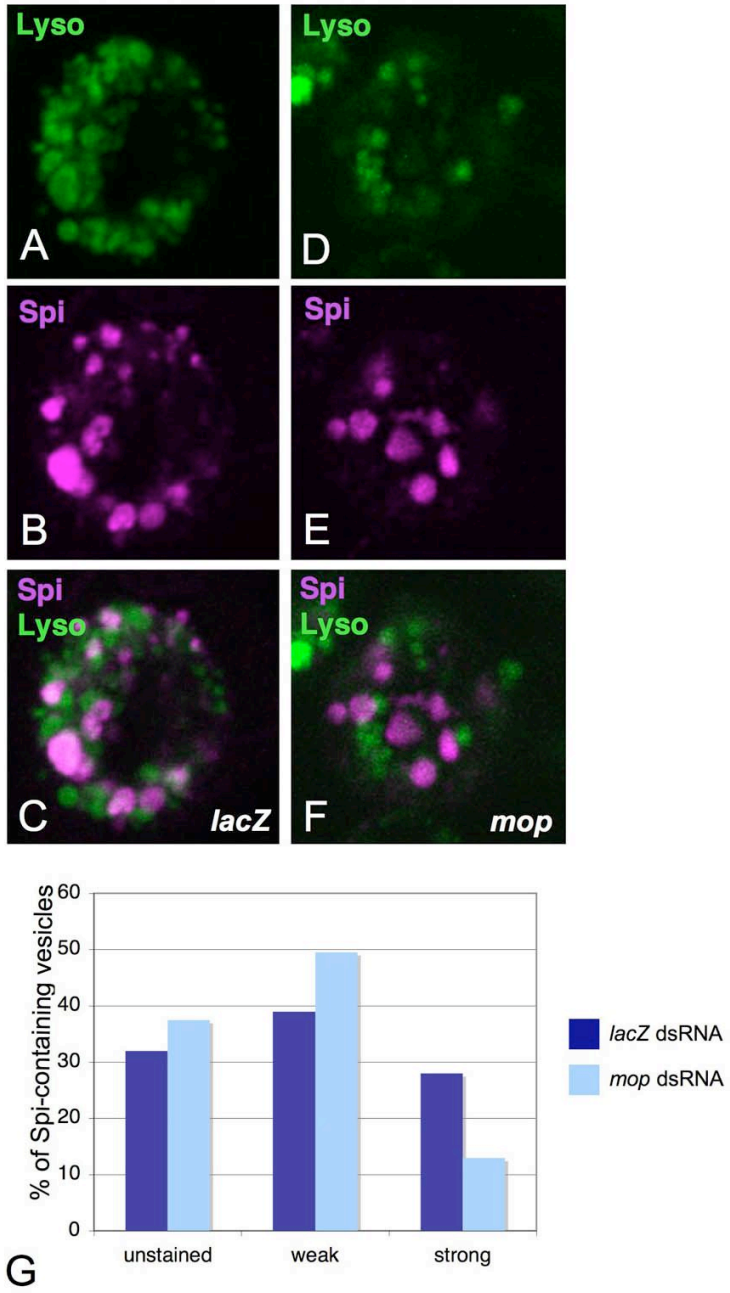


Supplementary Figure 2: *mop* expression. (A-F) In situ hybridization of embryos (A-D) or imaginal discs (E, F) with antisense (A, C, E) or sense (B, D, F) *mop* probes. *mop* RNA is maternally provided to early embryos (A) and expression is strongest in the nervous system and gut at later stages (C). *mop* is ubiquitously expressed in imaginal discs (E). (G, H) show an eye disc with mop^{T482} clones expressing a UAS-FLAGMop transgene. Clones are positively marked by FLAG staining (green in H) and stained with anti-Elav (G, magenta in H). FLAG-tagged Mop rescues photoreceptor differentiation.



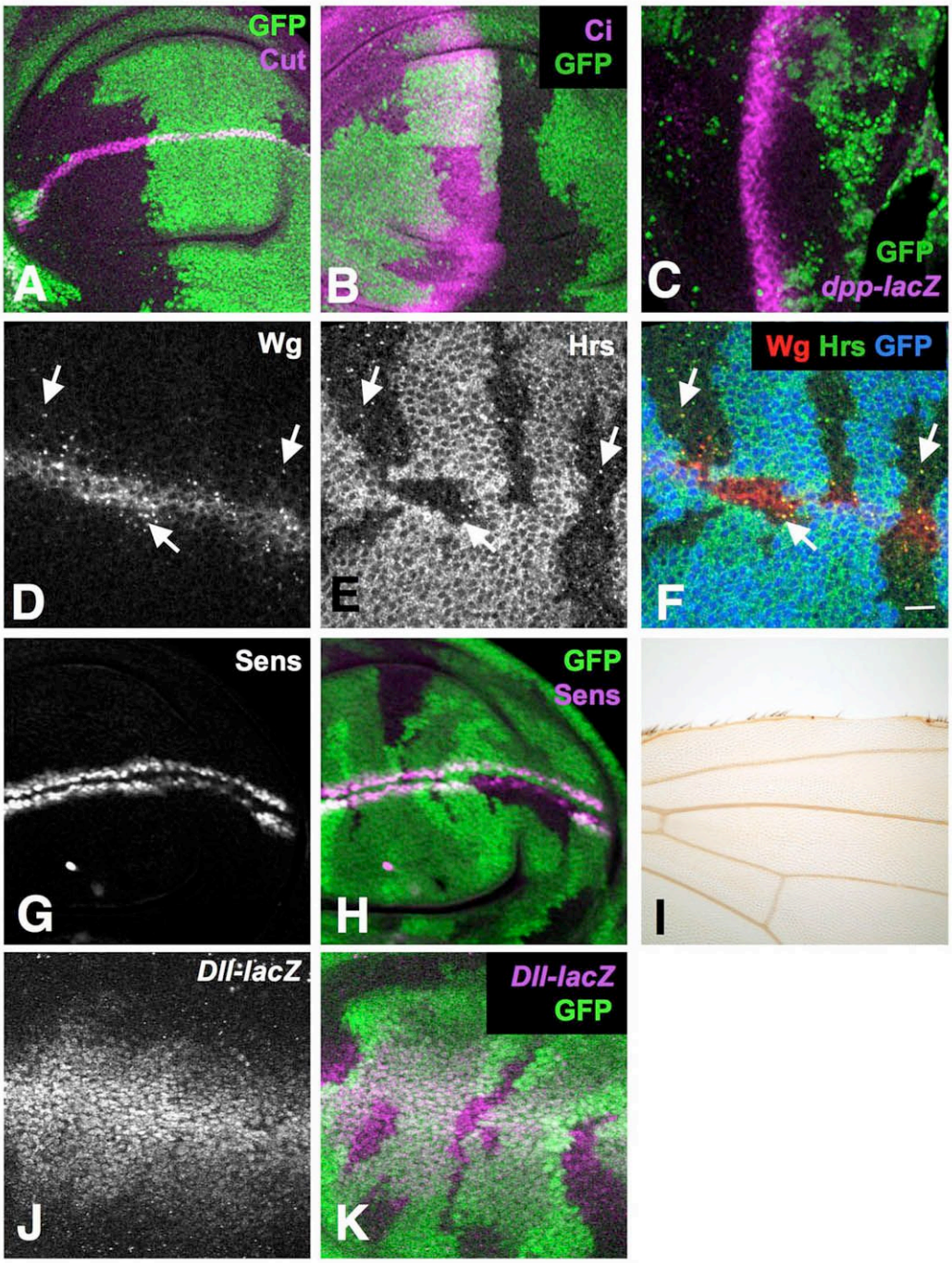
Supplementary Figure 3: Interactions between *mop, cbl, hrs* and *sty*. (A-F) show eye discs stained with anti-Hrs (A, C, E, magenta in B, D, F). mop^{T612} clones (A, B), cbl^{F165} clones (C, D)

or *mop*^{T612} *cbl*^{F165} double mutant clones (E, F) are marked by the absence of GFP (green in B, D, F). Loss of *cbl* does not restore normal Hrs localization to *mop* mutant clones. (G, H) show an *hrs*^{D28}*/Df(2L)Exel6277* eye disc in which *mop*^{T612} clones are marked by the absence of GFP (green in H), stained with anti-active Caspase 3 (G) and anti-Elav (magenta in H). Loss of *hrs* does not rescue photoreceptor differentiation or cell survival in *mop* mutant clones. (I-L) show anti-Hrs stainings of the dsRNA-treated D2F cells used in Fig. 6. Enlarged endosomes are seen on depletion of *mop, Tsg101* or *Vps28*. (M) shows a Western blot with antibodies to diphospho-MAPK and Tubulin of lysates from D2F cells treated with *lacZ*, *mop*, *cbl* or *sty* dsRNA and transfected with *actin*-GAL4, UAS-*cblL* as indicated, and incubated with Spi for 0 or 30 min. MAPK phosphorylation in Mop-depleted cells was partially rescued by overexpressing Cbl or by depleting Cbl or Sty.



Supplementary Figure 4: Mop promotes lysosomal entry of Spi. (A-F) show examples of D2F cells treated with Alexa-labeled Spi and imaged 4 hours later, with Lysotracker shown in green (A, C, D, F) and Spi in magenta (B, C, E, F). (A-C) were treated with *lacZ* dsRNA and (D-F) with *mop* dsRNA. (G) is a quantification of the percentage of vesicles containing Alexa-labeled Spi between 3 and 4 hours following Spi treatment that do not stain with Lysotracker or show weak or strong uptake of Lysotracker. Dark blue bars are from D2F cells treated with *lacZ*

dsRNA and light blue bars are from D2F cells treated with *mop* dsRNA. n = 452 vesicles for *lacZ* and 368 vesicles for *mop*, taken from two independent experiments.



Supplementary Figure 5: Effects of *mop* on other signaling pathways. (A, B, D-H, J, K) show third instar wing discs, (C) shows a third instar eye disc and (I) shows an adult wing. *mop* mutant clones are marked by the absence of GFP (green in A-C, blue in F, green in H, K) or unmarked (I). mop^{T612} was used for (A, C-F, I J,K) and mop^{T482} for (B, G-H). Clones in (A-C) were generated in a *Minute* background. (A) is stained with anti-Cut (magenta). Normal Cut

expression suggests that Notch signaling is unaffected. (B) is stained with anti-Ci (magenta) and (C) is stained with anti-β-galactosidase reflecting *dpp-lacZ* expression (magenta). Ci upregulation and *dpp* expression are normal in *mop* mutant clones, suggesting that Hh signaling is unaffected. (D-F) are stained with anti-Wg (D, red in F) and anti-Hrs (E, green in F). In *mop* mutant clones, Wg accumulates in large punctae that often colocalize with Hrs (arrows in F). (G-H) are stained with anti-Sens (G, magenta in H) and (J-K) are stained with anti-β-galactosidase reflecting *Dll-lacZ* expression (J, magenta in K). Sens expression is reduced, consistent with loss of the adult wing margin (I), but *Dll* expression is not significantly affected, suggesting that Wg signaling is only weakly reduced. Scale bars are 10 μm.