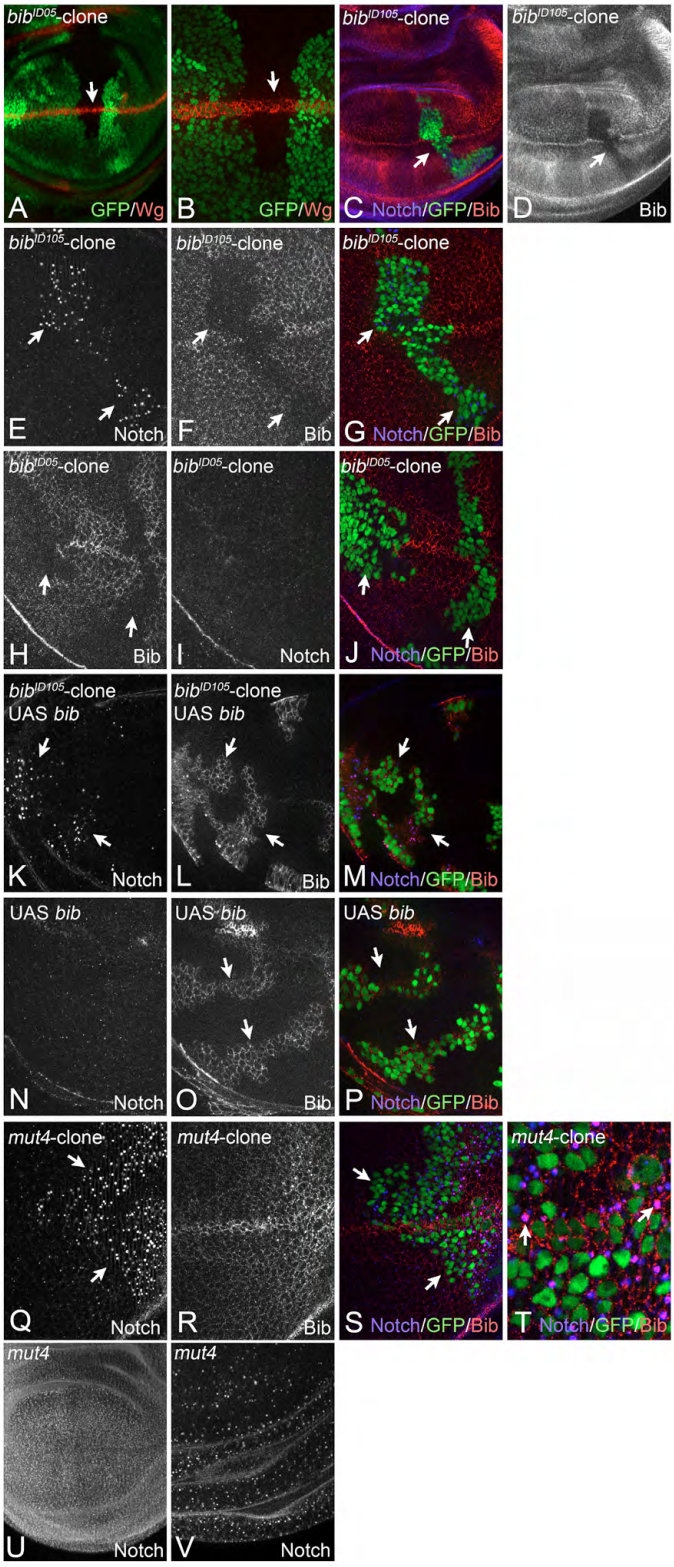

Fig. S1. **The *bib*^{ID05} chromosome carries a second mutation that causes the formation of increased Notch positive vesicles in imaginal disc cells.** (A, B) *bib* function is not required for Notch signalling along the D/V boundary of the wing. The arrow highlights the region where *bib* function is lost (labelled by loss of GFP). However, the expression of the Notch target gene *wg* is not affected in mutant cells. (C-G) Clone of the *bib*^{ID105} chromosome induced by MARCM. Arrow points to the mutant region, which is labelled through expression of GFP and shown in higher magnification in (E-G). In the clone area Bib is absent (D, F, G, arrows). The cells contain large vesicles that contain Notch (E, G). In contrast, in clones induced with the *bib*^{ID05} chromosome (arrows in I, J), Bib is still absent in mutant territories (H), but the cells do not contain large Notch positive vesicles (I). Expression of UAS *bib* in *bib*^{ID05} clones induced by MARCM, does not prevent formation of the large Notch positive vesicles (K-P). (Q-T) Clones induced with the separated *mut4* mutation. The cells contain the large Notch positive vesicles (Q, S, T), but do express Bib normally (R, S). Note, that a fraction of Bib is located in the large vesicles of *mut4* cells (T, arrows). (U, V) Wing imaginal disc of a *mut4* mutant fly. All cells contain the large Notch positive vesicles.

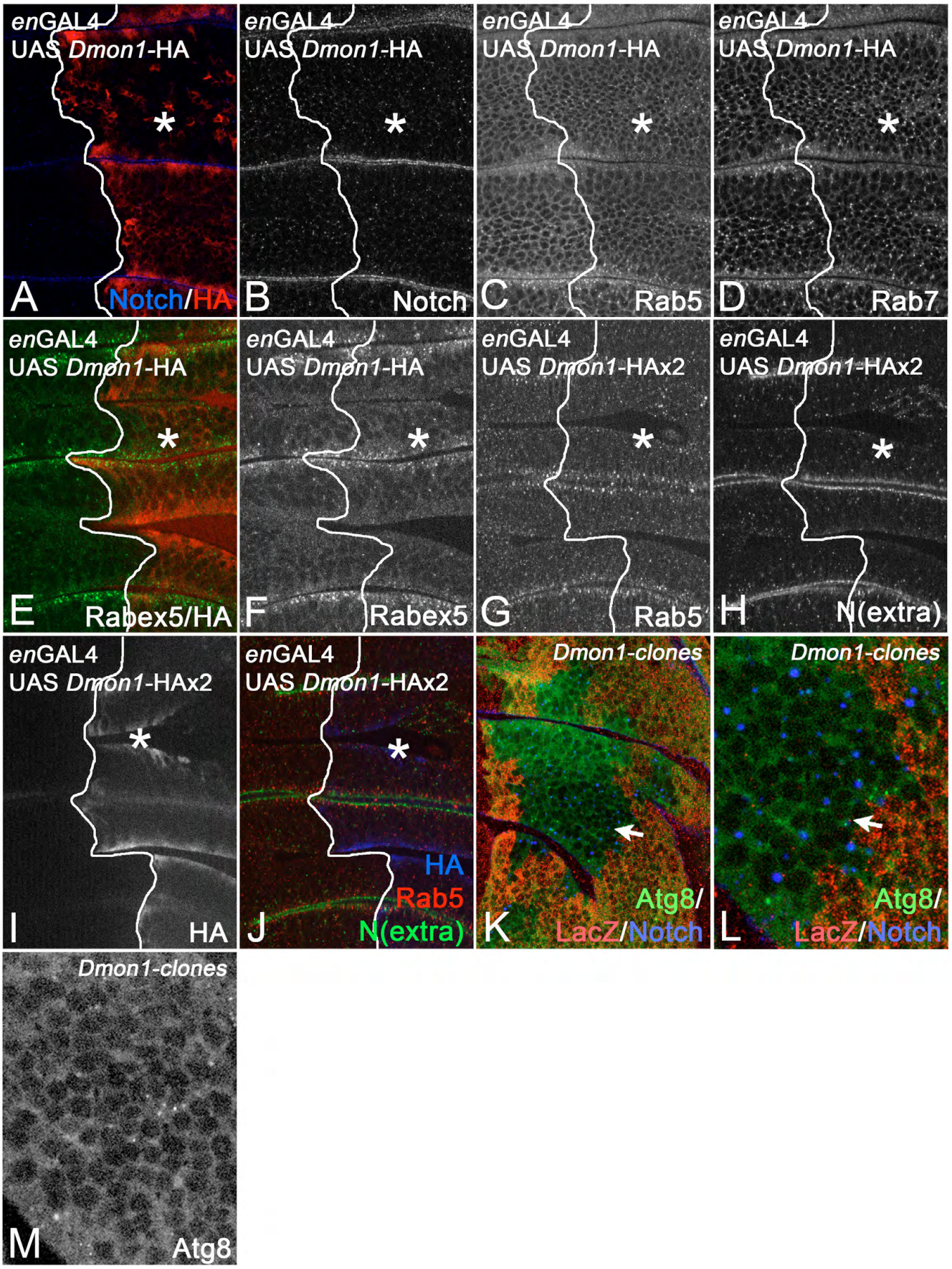

Fig. S2. Over-expression of UAS Dmon1-HA has no effect on distribution of endosomal key proteins. (A-F) Expression of one copy and (G-J) of two copies of UAS *Dmon1-HA*. The distribution of Notch (B, H), Rab5 (C, G), Rab7 (D) and Rabex5 (F) is not affected.

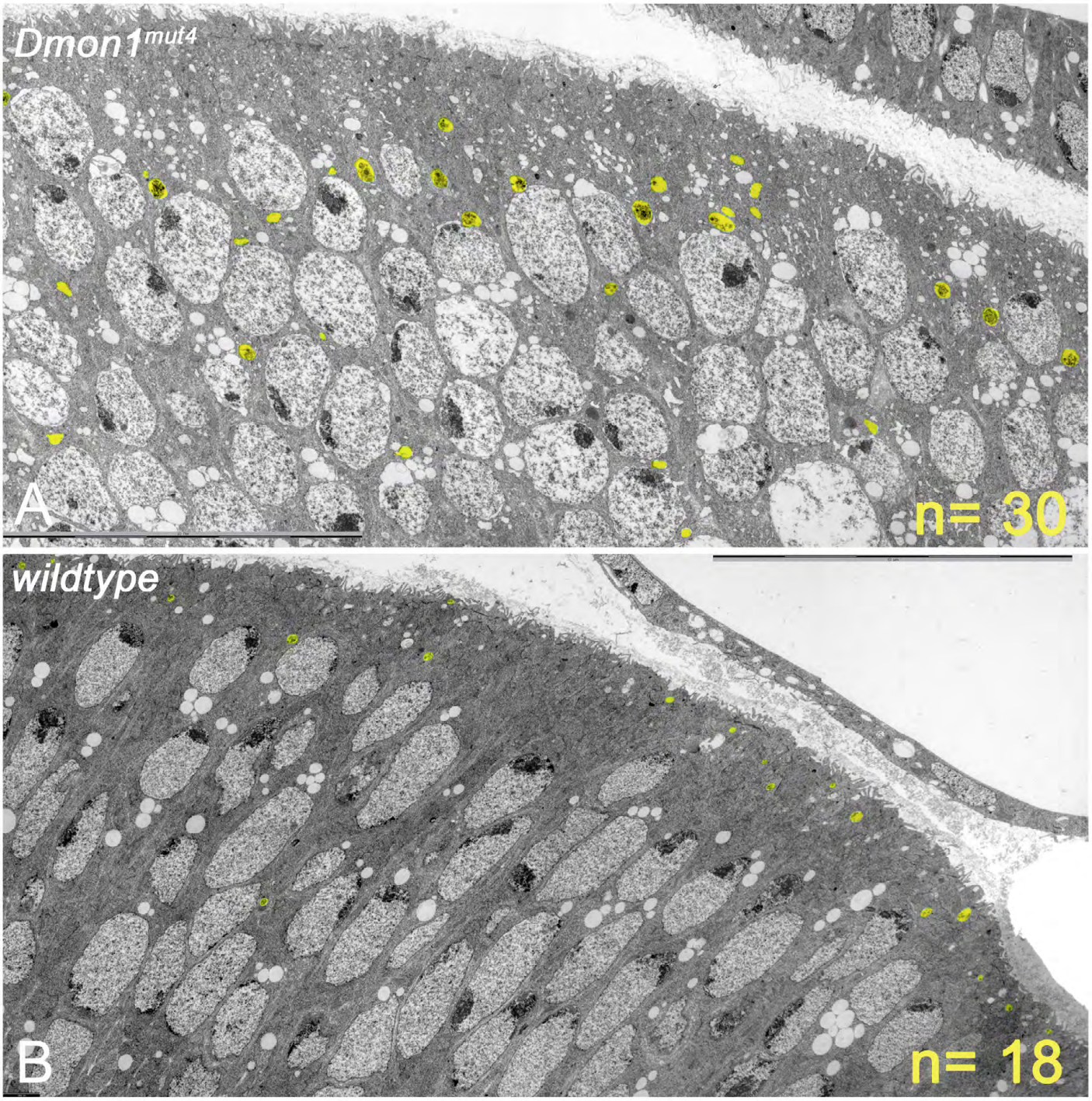

Fig. S3. Loss of *Dmon1* function results in the accumulation of MVBs in cells. (A, B) wildtype and mutant areas are shown at the same magnification. The MVBs are highlighted in yellow colour. While *Dmon1* cells have 30, wildtype cells have only 18 MVBs in the same area. Thus, *Dmon1* cells have more MVBs than their wildtype counterparts indicating that the turnover of endosomes is disturbed.

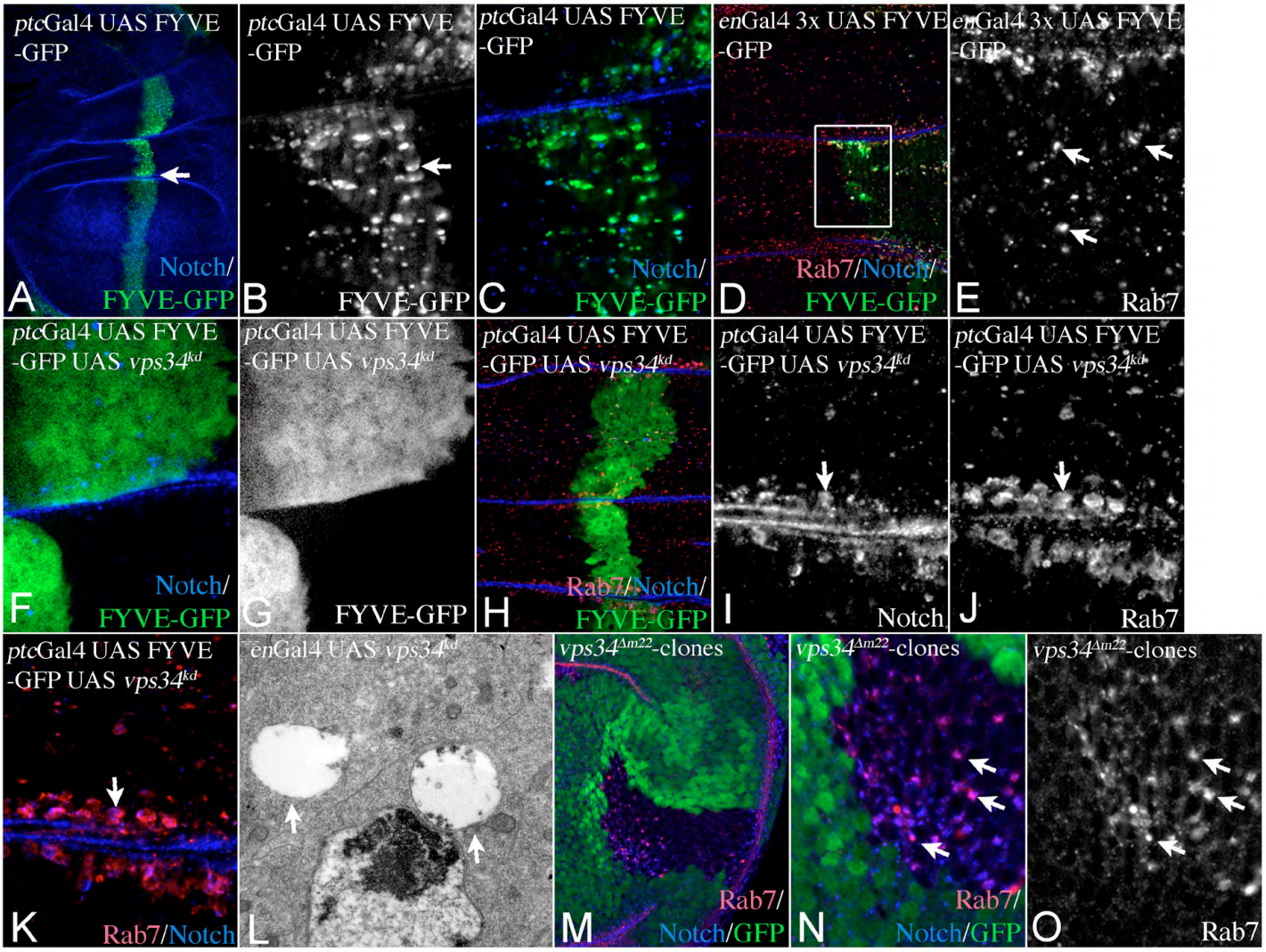

Fig. S4. **The role of PI(3)P and Vps34 in recruitment of Rab7 to the endosome.** (A-C) Expression of an UAS *FYVE-GFP* construct in wildtype discs, using *ptc*Gal4. FYVE-GFP binds to PI(3)P containing MEs (B), which are positive for Notch (B, C). The arrow in (B) points to one of the FYVE-GFP associated MEs. (D, E) Expression of three copies of the UAS *FYVE-GFP* construct with *en*Gal4 results in the formation of enlarged endosomes in cells of the posterior compartment, which are positive for Rab7 (arrows in E). (F, G) Upon co-expression of the kinase dead version of Vps34 (Vps34^{kd}), FYVE-GFP fails to be recruited to the endosomes. This phenotype indicates that the expression of Vps34^{kd} efficiently prevents the formation of PI(3)P. Nevertheless, cells expressing Vps34^{kd} still contain Rab7 positive endosomes (H-K). These endosomes are concentrated at the apical side of the imaginal disc epithelial cells (arrow in I-K). (L) TEM analysis of Vps34^{kd} expressing cells. The cells contain many enlarged MEs that contain only few ILVs (arrows). (M; O) Clonal analysis of the null allele of *vps34*, *vps34*$^{\Delta m22}$. Mutant cells contain Rab7 positive endosomes (arrows in N, O). Altogether, these results indicate that Rab7 is recruited to the endosome in a Vps34 independent manner.

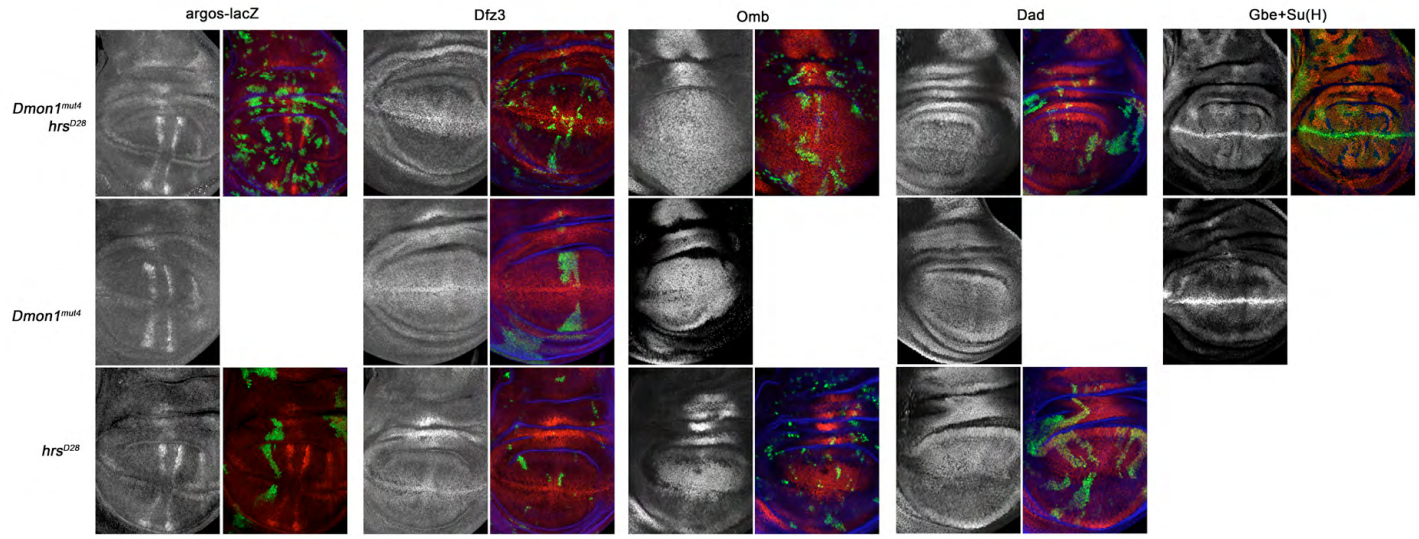

Fig. S5. **Concomitant loss of *hrs* and *Dmon1* function does not impact on signalling of RTK, Dpp, Wnt and Notch pathways.** The effect of *Dmon1* loss of function on the expression of the reporter genes is monitored either in *Dmon1* mutants or by clonal analysis.