Fig. S1. The bib<sup>1005</sup> 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<sup>1005</sup> 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<sup>1005</sup> 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<sup>1005</sup> 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 Pl(3)P and Vps34 in recruitment of Rab7 to the endosome. (A-C) Expression of an UAS FYVE-GFP construct in wildtype discs, using ptcGal4. FYVE-GFP binds to Pl(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 enGal4 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 (Vps34kd), FYVE-GFP fails to be recruited to the endosomes. This phenotype indicates that the expression of Vps34kd efficiently prevents the formation of Pl(3)P. Nevertheless, cells expressing Vps34kd 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 Vps34kd 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^dm22. 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.