Supplemental Materials

Molecular Biology of the Cell

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Supplemental information for
The Myopic-Ubpy-Hrs nexus enables endosomal recycling of Frizzled

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Figure S1: Mop is required for proper trafficking of Fz in the Drosophila wing disc.

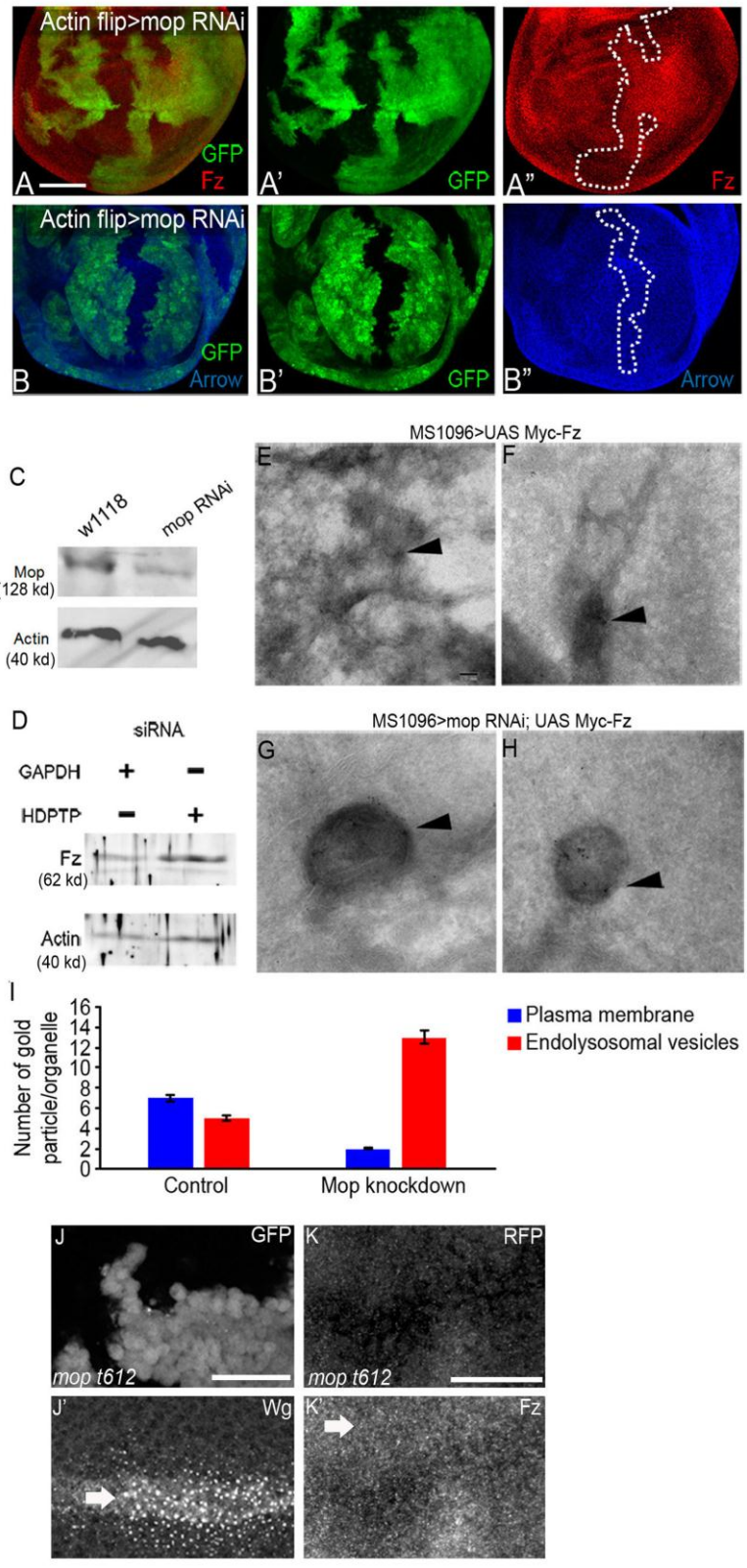

Figure S1: Mop is required for proper trafficking of Fz in the *Drosophila* wing disc. RNAi lines are expressed in flip out clones using *hs flp, actin Gal4*. Clones are marked by the presence of GFP. (A-A") Knockdown of a second set of mop^{RNAi} lines (VDRC ID: 14174) causes similar localization of Fz in puncta-like structures (in red). (B-B") *mop* knockdown causes no change in Arrow expression (in blue) in the *Drosophila* wing disc. (C) RNAi knockdown of *mop* (VDRC ID: 104860) in *Drosophila* wing imaginal disc causes reduction in Mop protein level compared to wild type control. Mop was knocked down using *MS1096Gal4*. (D) Knockdown of HDPTP causes accumulation of Fz protein in L cells compared to control GAPDH knockdown. (E-H) ImmunoEM of *Drosophila* wing imaginal disc expressing Myc Fz (E-F) and *mop RNAi; Myc Fz* (G-H) transgenes. Myc Fz normally localizes to the membrane (E, arrow) and endosomes (F, arrow). Overexpressing Myc Fz in the absence of Mop causes increased accumulation of Fz in endolysosomal structures (G-H, arrow). The transgenes were expressed with *MS1096Gal4*. *mop* was knocked down using VDRC line 104860. (I) Quantification of localization of Fz in control and *mop* mutant tissue. Bar graph shows that localization of Myc- Fz to the endolysosomal vesicles increases upon *mop* knockdown. (J-K) mop^{T612} clones causes punctate localization of Wg (J-J') and Fz (K-K') inside the clone. The clones are marked by the presence of GFP and RFP. Arrows indicates punctate localization of proteins inside the clones. Scale bars are 20 µm.

Figure S2: hrs loss phenocopies the endosomal trafficking defect of mop knockdown

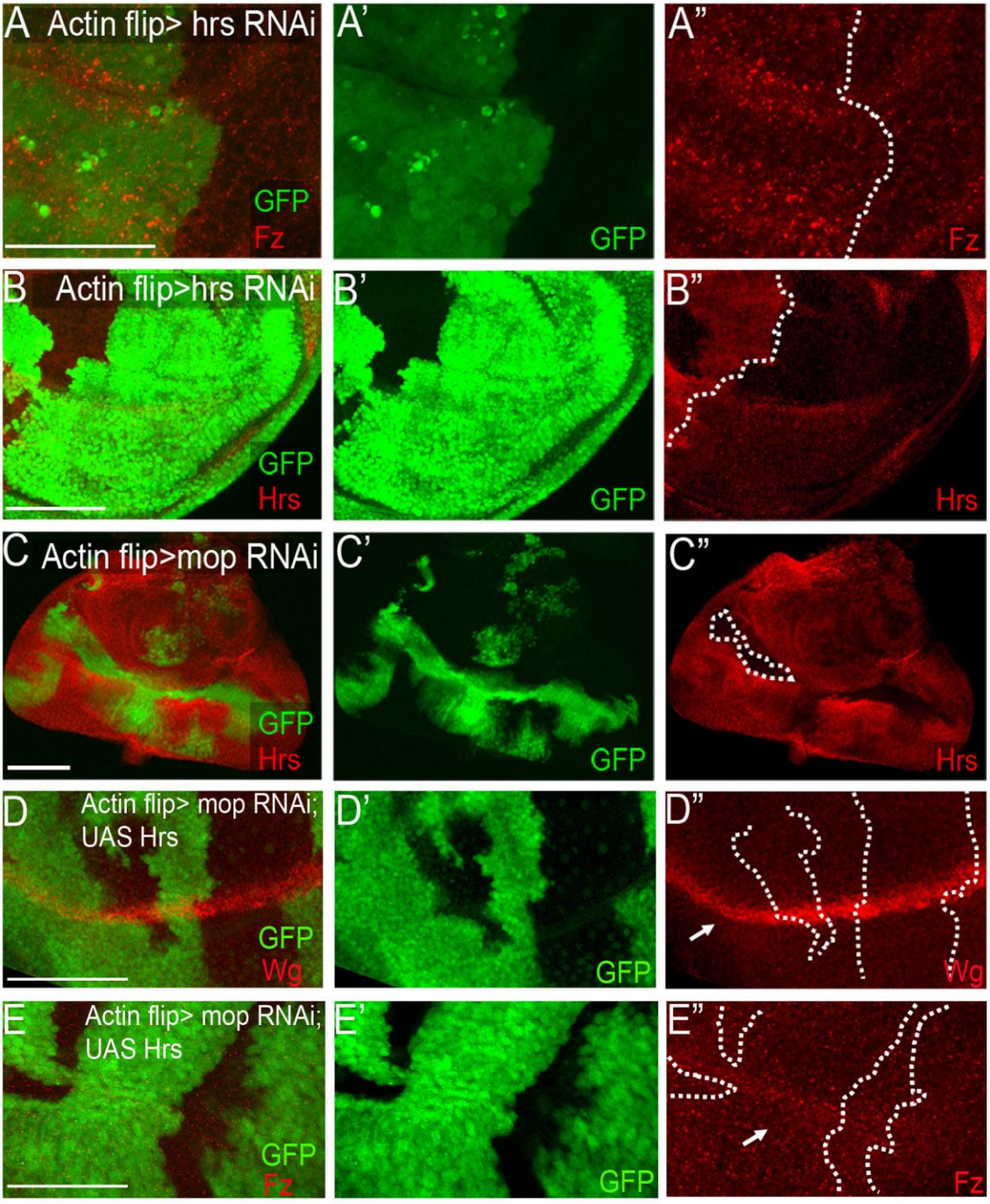

Figure S2: Hrs loss phenocopies the endosomal trafficking defect of *mop* knockdown in the *Drosophila* wing disc. RNAi lines were expressed in flipout clones using *hs flp, actin Gal4.* (A-A') Knockdown of a second set of hrs^{RNAi} line (Trip: 34086) causes similar localization of Fz in puncta like structures (in red) as that of *mop* ^{RNAi}. (B-B') Reduced Hrs levels in hrs^{RNAi} tissue

suggests the specificity of the RNAi line. (C-C') Knockdown of *mop* in eye discs causes reduction of Hrs. (D-E) Simultaneous overexpression of Hrs upon *mop* knockdown rescues the punctate localization of Wg (D-D") and Fz (E-E") inside the clone. Clones are marked by the presence of GFP. *mop* was knocked down using VDRC line 104860. Scale bars are 20 µm.

Figure S3: Mop recruits Fz to the membrane.

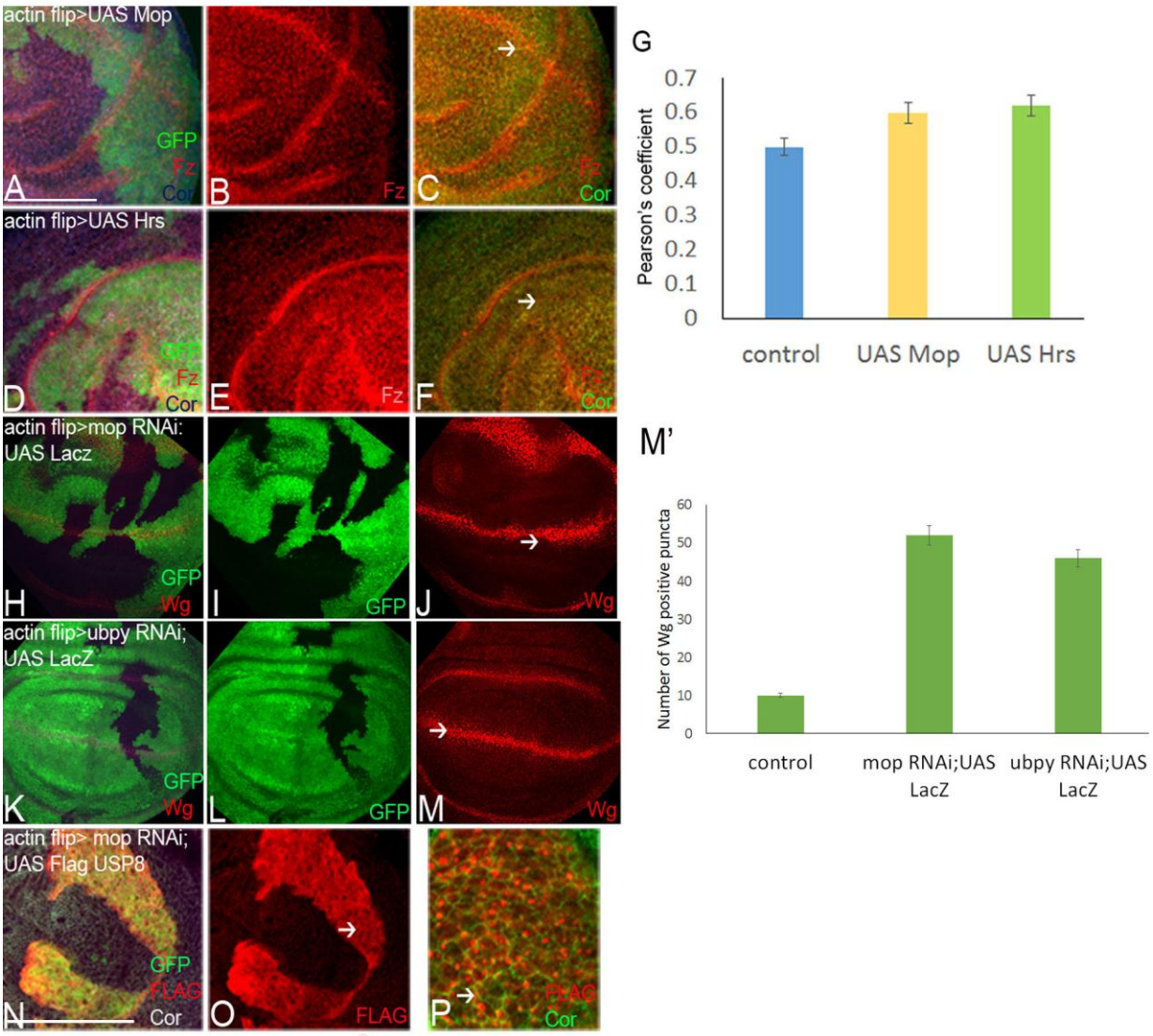

Figure S3: Mop recruits Fz to the membrane. (A-C) Overexpression of Mop causes increased accumulation of Fz at the membrane, as marked by Coracle. (D-F) Hrs overexpression causes a similar increase in membrane localization of Fz. (G) Quantification of colocalization of Fz and Cor was done by measuring Pearson's coefficient in a control and affected tissue. Bar graph shows that localization of Fz to the membranes increases upon the overexpression of Mop and Hrs. The p value was 0.0001 for co-localization of Fz and Cor in UAS Mop, and 0.0019 in UAS Hrs genotypes. Simultaneous overexpression of UAS LacZ in mop^{RNAi} (VDRC ID: 14174) (H-J) and $ubpy^{RNAi}$ (K-M) knockdown tissue does not rescue the punctate expression of Wg in the

signal receiving cells. (M') Quantification of Wg puncta across the dorsal ventral boundary. (N-P) The loss of Ubpy upon *mop* knockdown (VDRC ID: 104860) can be rescued by simultaneous expression of UAS Flag-USP8. (P) Flag-USP8 colocalizes with Coracle. Arrow in O denotes the region which is zoomed in P. The transgenes were overexpressed in the *Drosophila* wing disc using *hs flp, actin Gal4.* GFP marks the region of overexpression.

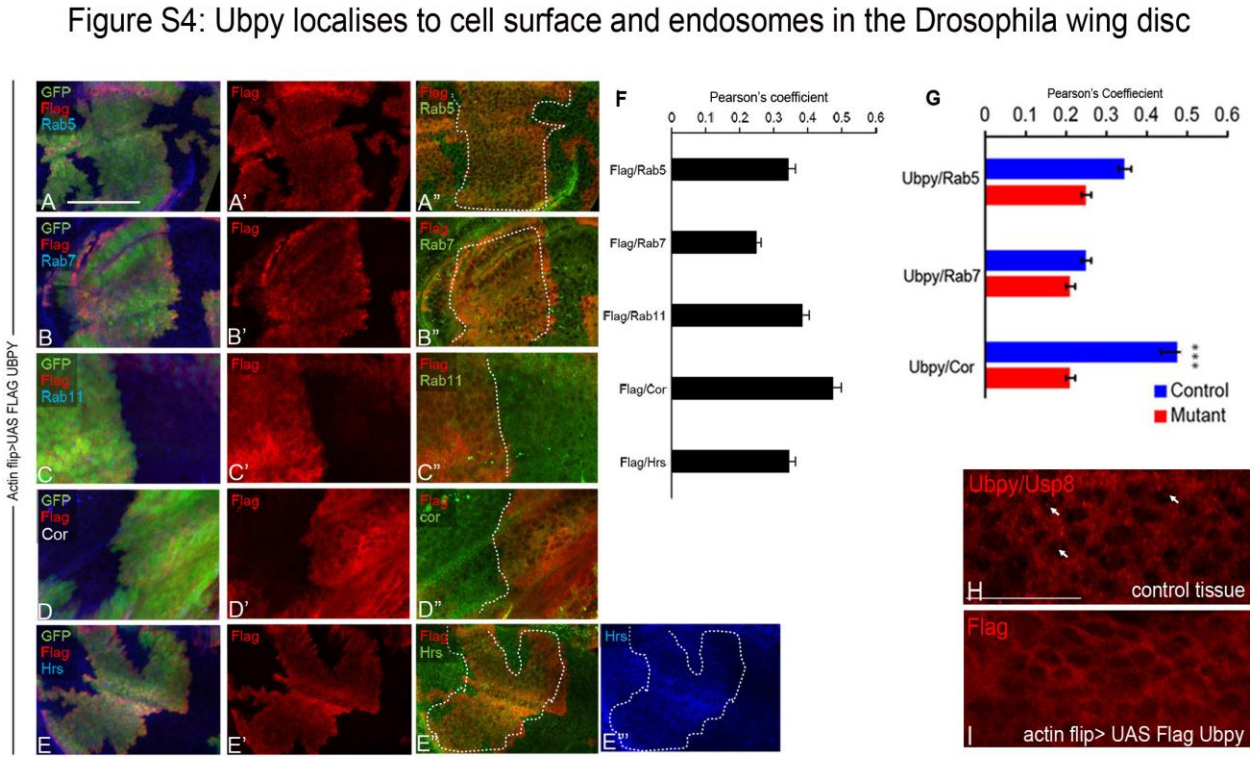

Figure S4: Ubpy localises to cell surface and endosomes in the *Drosophila* wing disc. (A-E) Flag Ubpy was overexpressed in the *Drosophila* wing disc using *hs flp, actin Gal4.* GFP positive tissue marks the region of overexpression. Colocalization with Rab5 (A-A"), Rab7 (B-B"), Rab11 (C-C"), Cor (D-D") and Hrs (E-E''') is shown. (F) Quantification of colocalization of Flag Ubpy and markers was done by measuring Pearson's coefficient in control and affected tissue. Bar graph shows that Ubpy mostly localizes to the membrane and recycling endosomes, followed by its localization to Rab5 and Rab7. Similarly Hrs and Ubpy also colocalize in the wing disc. The error bar represents error of percentage from six independent replicates. N=6. Scale bars are 20 µm. (G) Quantification of co-localization of Flag Ubpy in *mop* knockdown and control tissue. Ubpy localization to Rab5-containing early endosomes and membrane is significantly reduced upon *mop* knockdown. $p<0.003$ for Ubpy and Rab5, $p<0.02$ for Ubpy and Rab7 and $p<0.0001$ for Ubpy and Cor. (H-I) Endogenous Ubpy (H) and Flag-Ubpy (I) both localize to the cell membrane and in endosomes (puncta-like structure). Arrow indicates the endosomal accumulation of Ubpy in puncta.

Figure S5: The endosomal localization of Mop is not dependent on Ubpy.

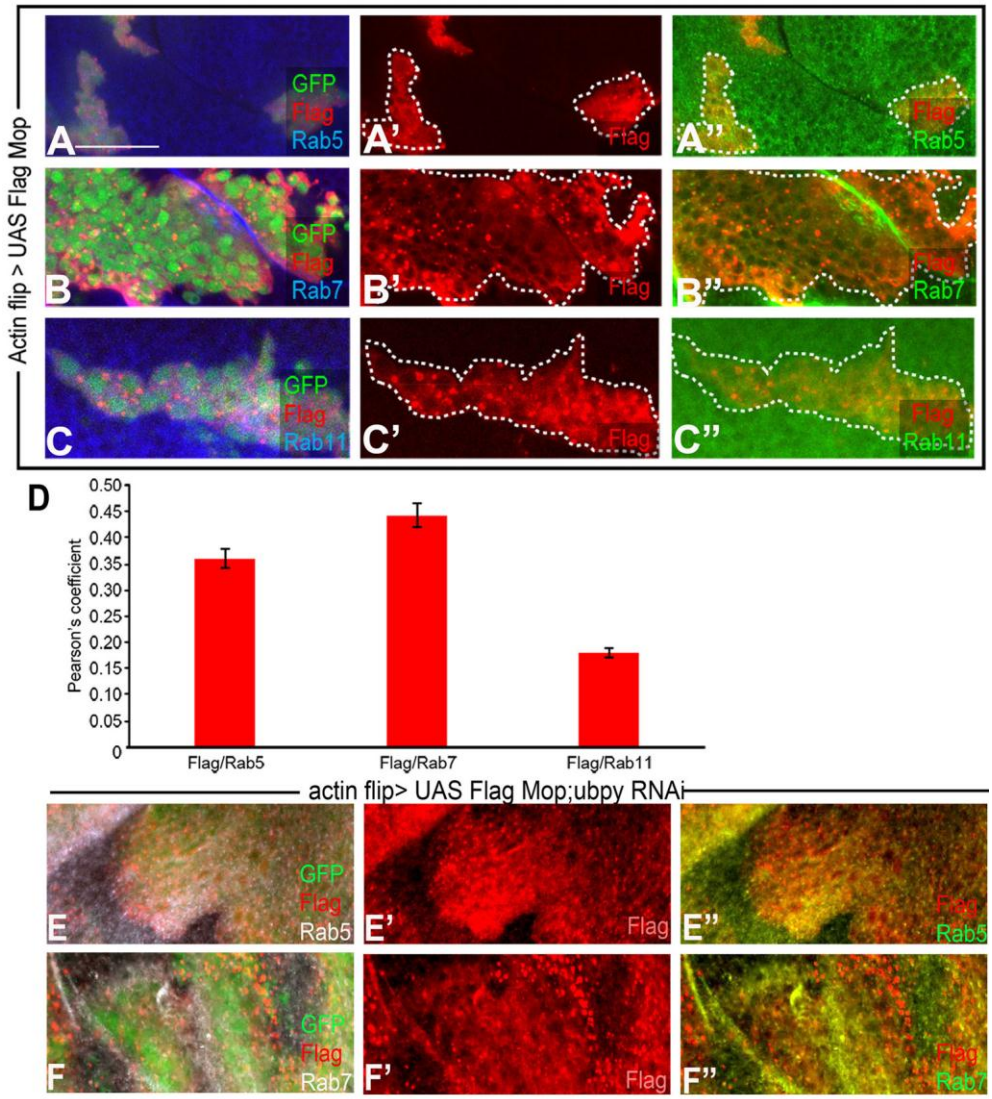

Figure S5: The endosomal localization of Mop is not dependent on Ubpy. (A-C) Flag Mop was overexpressed in the *Drosophila* wing disc using *hs flp, actin Gal4*. GFP positive tissue marks the region of overexpression. Colocalization with Rab5 (A-A"), Rab7 (B-B"), Rab11 (C-C") is shown. (D) Quantification of colocalization of Flag-Mop and markers was done by measuring Pearson's coefficient in control and affected tissue. Bar graph shows that Mop mostly localizes to Rab7-positive late endosomes, followed by its localization with Rab5. The error bar represents error of percentage from six independent replicates. N=6. Scale bars are 20 µm. (E-F) Levels of Flag-Mop are unchanged upon *ubpy* knockdown. Colocalization of Mop and Rab5 (E-E") and Mop and Rab7 (F-F") are not changed upon simultaneous knockdown of *ubpy*. Clones are marked by the presence of GFP.