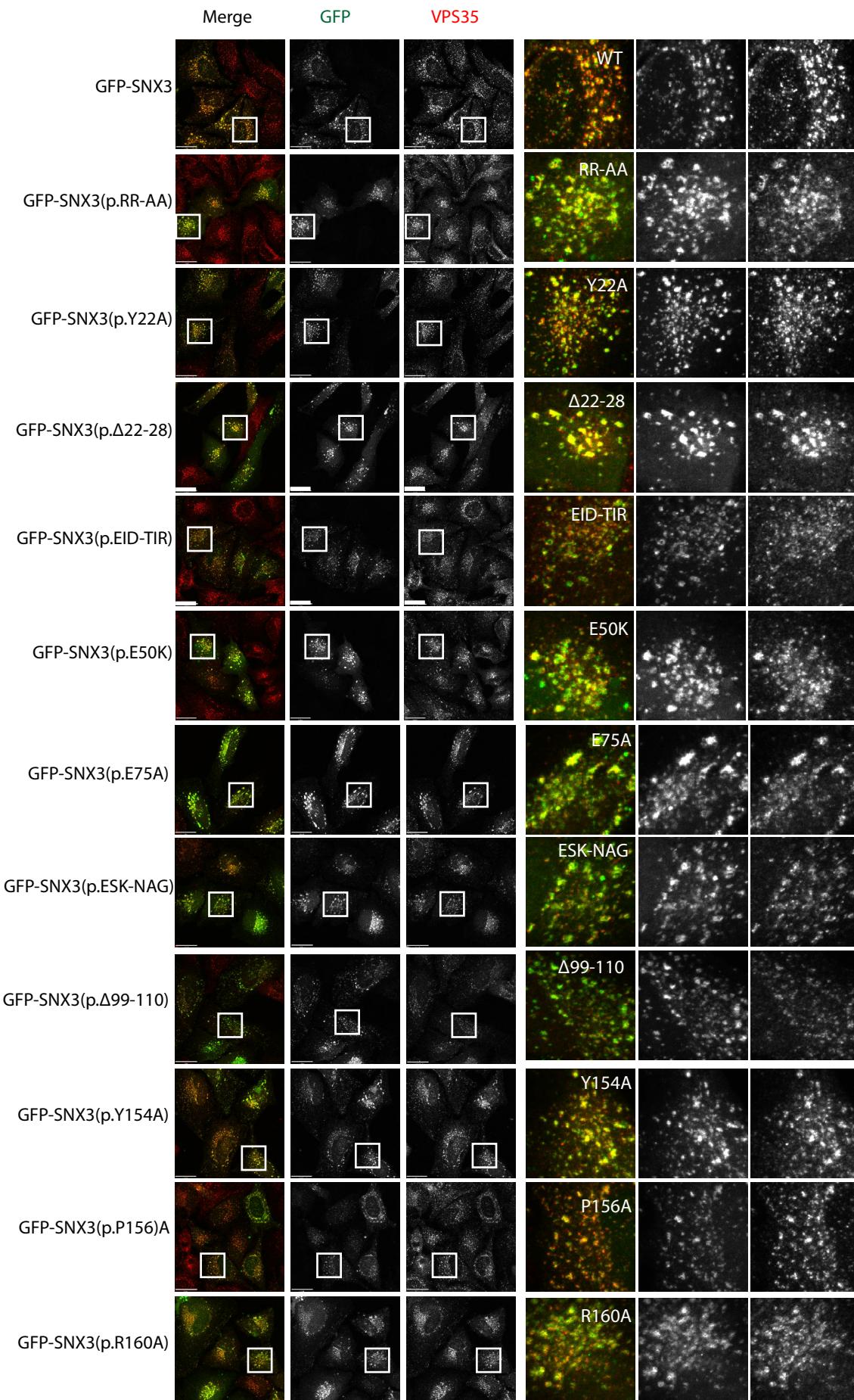


SNX3-retromer requires an evolutionary conserved MON2:DOPEY2:ATP9A complex to mediate Wntless sorting and Wnt secretion.

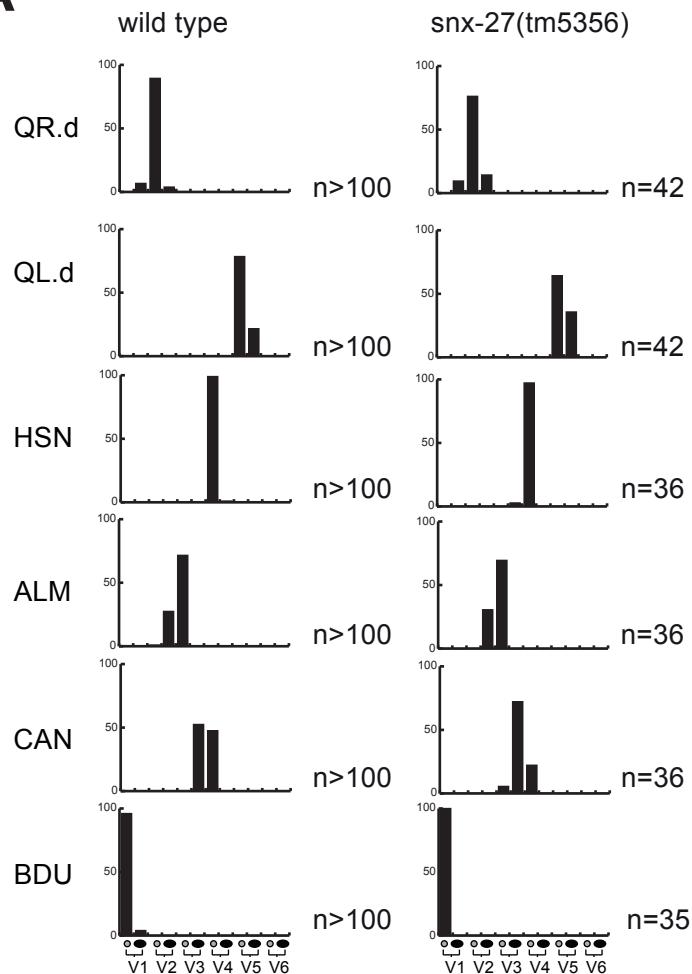
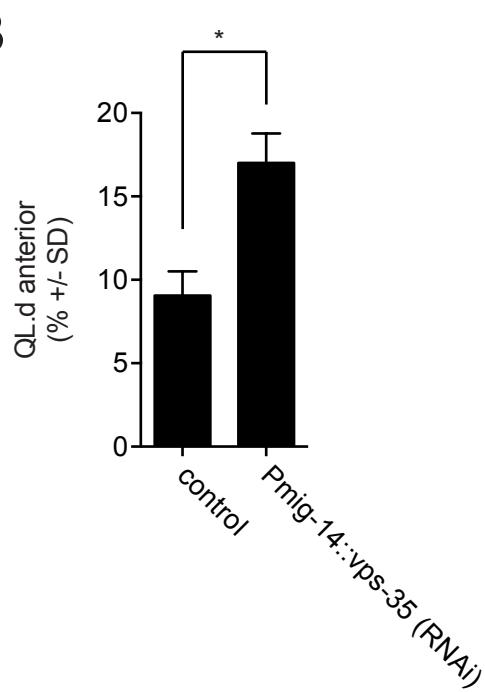
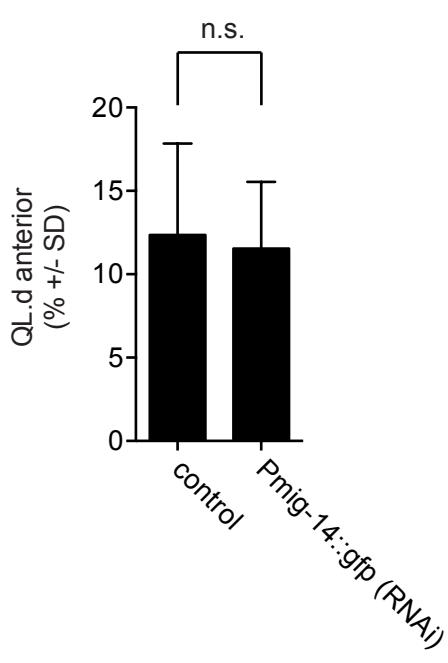
McGough et al., 2018

Supplementary Information

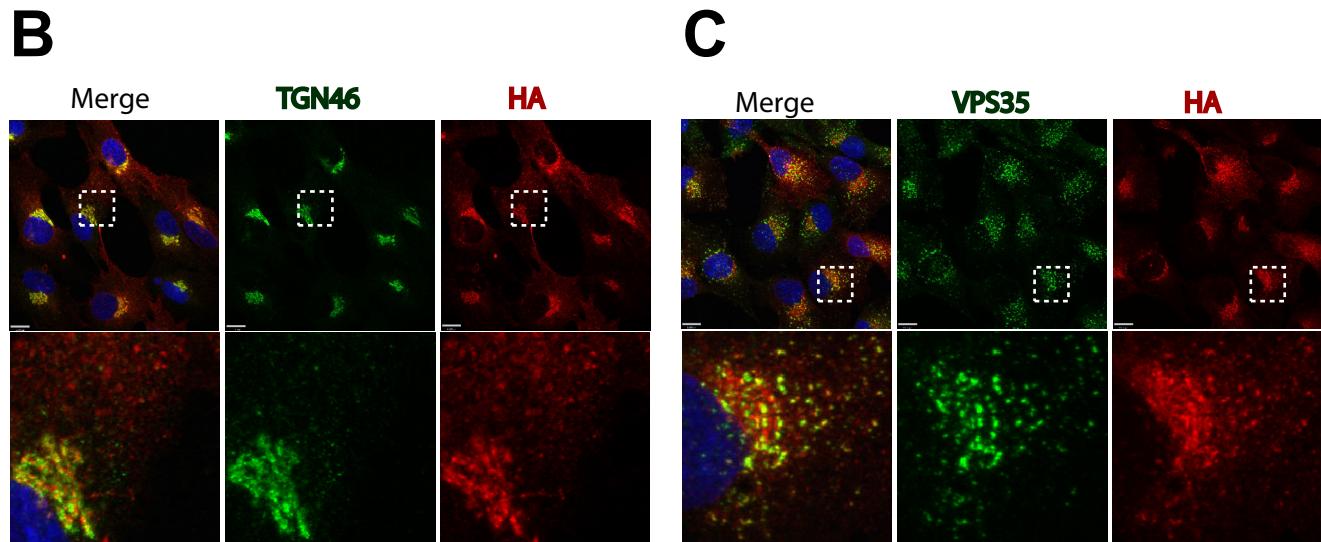
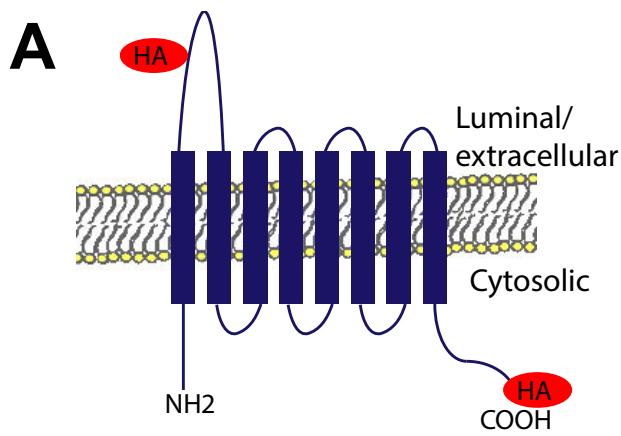


**Supplementary figure 1: Mutant GFP-SNX3 constructs localise to endosomes.**

Entire data set for Figure 1 C. Confocal images of HeLa cells transiently transfected to express wild type or mutated versions of GFP-SNX3 and subsequently stained for endogenous VPS26. Scale bar is 23  $\mu$ m.

**A****B****C****Supplementary figure 2.**

**(A)** Loss of *snx-27* does not affect the Wnt dependent cell migration of the HSN, ALM, CAN, BDU neurons and the Q neuroblasts. **(B)** Knock down of *vps-35* in Wnt producing cells enhances the Wnt signaling phenotype (anterior localization of the QL.d) of *vps-29* mutants. **(C)** Expression of gfp double stranded RNA has no effect on Wnt signaling in the *vps-29* mutant background.

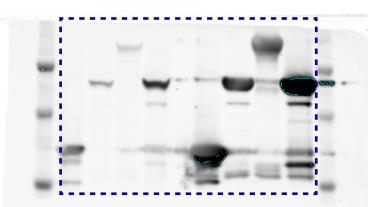
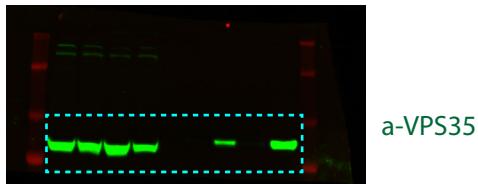


**Supplementary Figure 3: HA-WLS localises to the trans-golgi network and VPS35-positive endosomes**

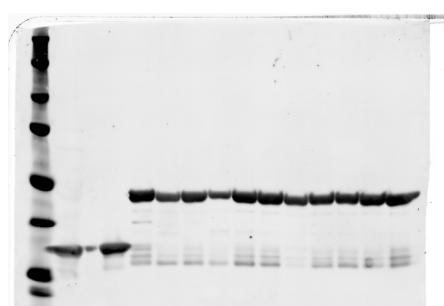
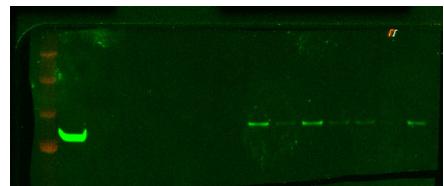
**(A)** Schematic representation of the HA-WLS construct: two HA-tags are cloned onto Wntless, one on the luminal-facing and one on the cytosolic-facing side of the protein. **(B)** RPE-1 cells, stably expressing HA-WLS, were fixed and immuno-stained for HA and endogenous TGN46. The scale bar indicates 11 µm. **(C)** RPE-1 cells, stably expressing HA-WLS, were fixed and immuno-stained for HA and endogenous VPS35. The scale bar indicates 11 µm.

Supplementary Figure 4: Uncropped blots

**Figure 1B**



**Figure 1D**

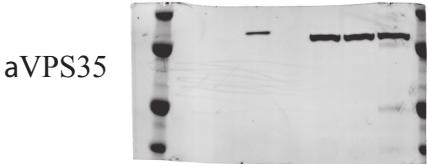
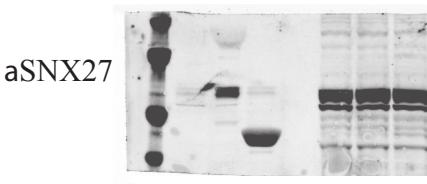


a-VPS35

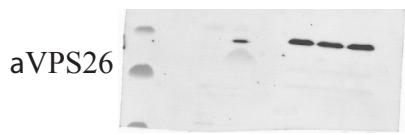


a-VPS35

**Figure 2D**

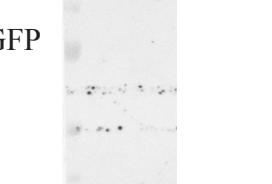


**Figure 2E**

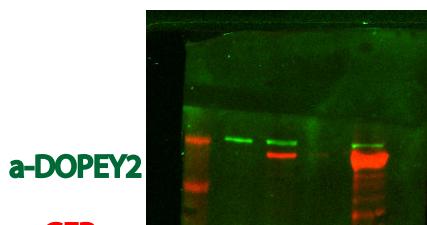


aGFP

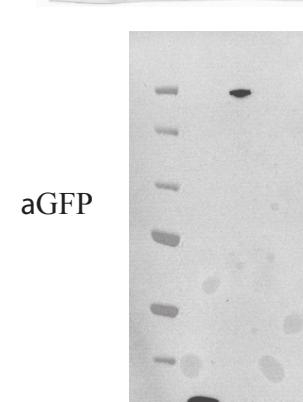
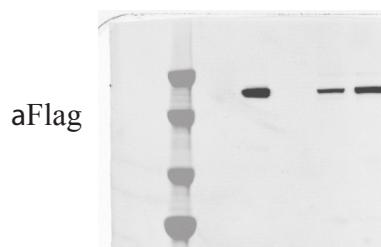
**Figure 2F**



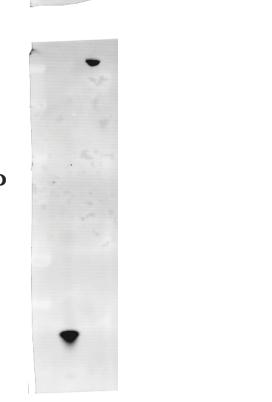
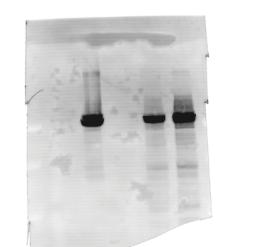
**Figure 2G**



**Figure 2H**

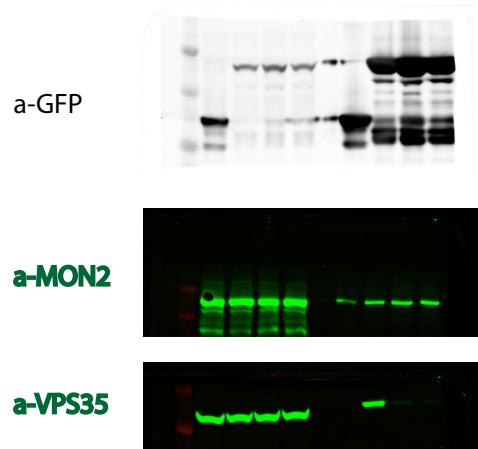


**Figure 2I**

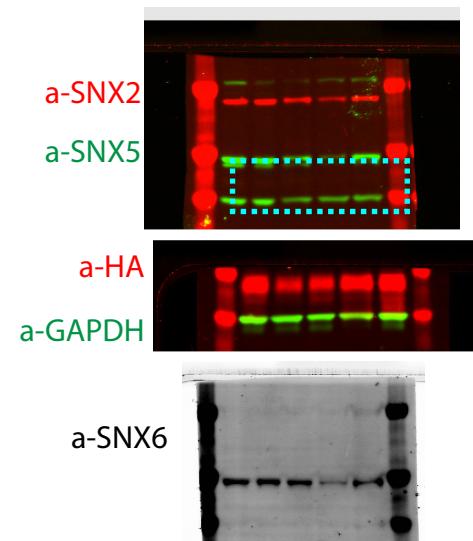
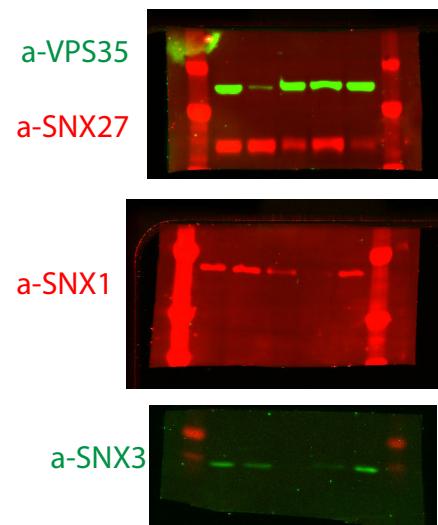


### Supplementary Figure 4 (continued): Uncropped blots

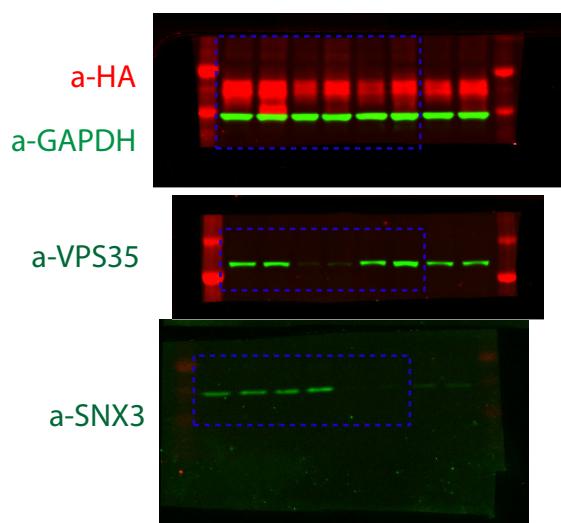
**Figure 2J**



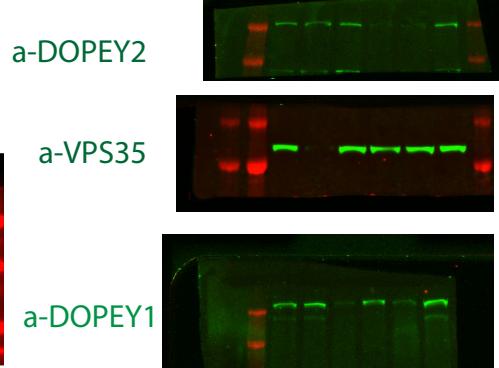
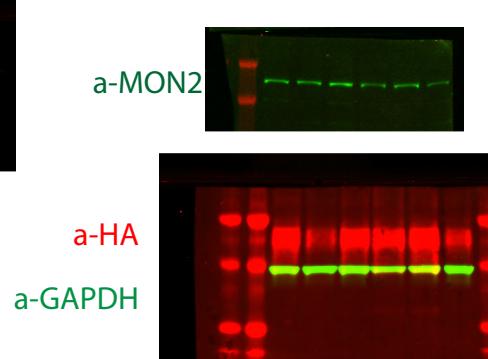
**Figure 3A**



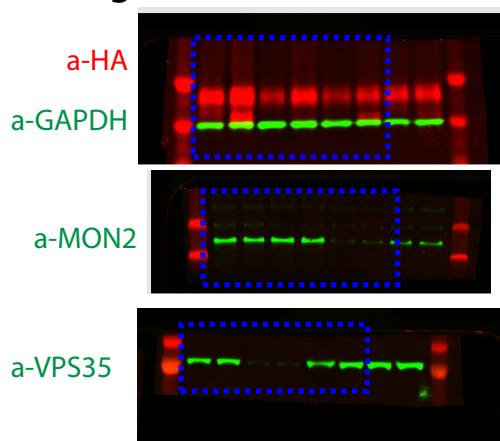
**Figure 3B**



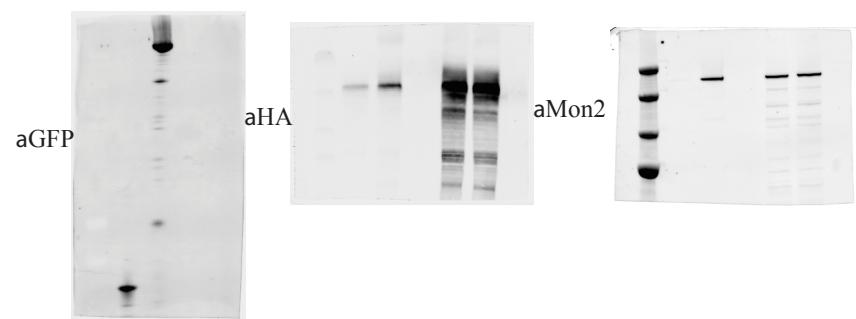
**Figure 3C**



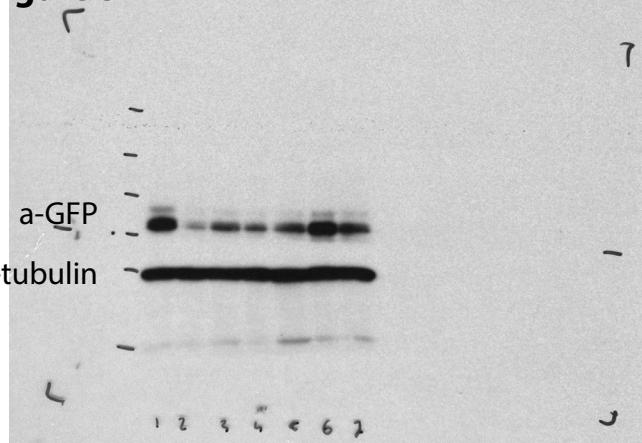
**Figure 3D**



**Figure 4H**



**Figure 3E**



**Figure 5A**

