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

McGough et al., 2018

Supplementary Information

	Merge	GFP	VPS35		
GFP-SNX3				WT	
GFP-SNX3(p.RR-AA)		** **		RR-AA	
GFP-SNX3(p.Y22A)				¥22A	
GFP-SNX3(p.Δ22-28)				Δ22-28	
GFP-SNX3(p.EID-TIR)				EID-TIR	
GFP-SNX3(p.E50K)		*		E50K	
GFP-SNX3(p.E75A)	/ * Ø.			E75A	
GFP-SNX3(p.ESK-NAG)				ESK-NAG	
GFP-SNX3(p.Δ99-110)				∆ 99-110	
GFP-SNX3(p.Y154A)				Y154A	
GFP-SNX3(p.P156)A				P156A	
GFP-SNX3(p.R160A)				R160A	

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 μ m.



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 μ m. (C) RPE-1 cells, stably expressing HA-WLS, were fixed and immuno-stained for HA and endogenous VPS35. The scale bar indicates 11 μ m.

Supplementary Figure 4: Uncropped blots



Figure 2D



Figure 2E



Figure 2F

aMon2

aGFP

Figure 2G



Figure 2H

aGFP



Figure 2I

aFlag





Supplementary Figure 4 (continued): Uncropped blots

a-VPS35

Figure 2	J	Figure 3A	
a-GFP		a-VPS35 a-SNX27	a-SNX2 a-SNX5
a-MON2		a-SNX1	a-HA a-GAPDH
a-VPS35		a-SNX3	a-SNX6
Figure 3	B	Figure 3C	
<mark>a-HA</mark> a-GAPDH		a-MON2	a-DOPEY2
a-VPS35 a-SNX3		a-HA a-GAPDH	a-DOPEY1
Fig	ure 3D	Figure 4H	
<mark>a-HA</mark> a-GAPDH		aGFP	aMon2
a-MON2			



Figure 5A

