



ARTICLE

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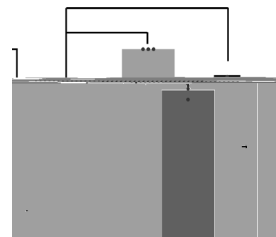
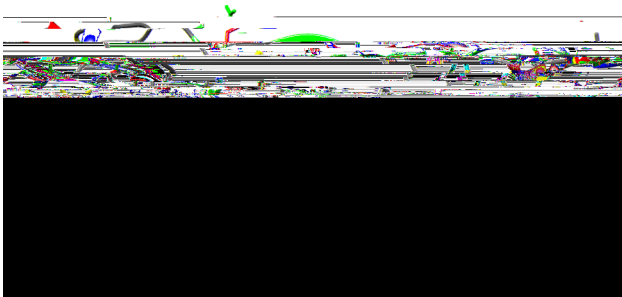
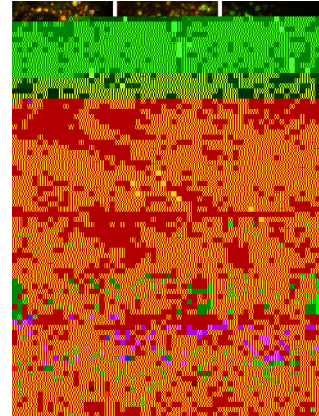
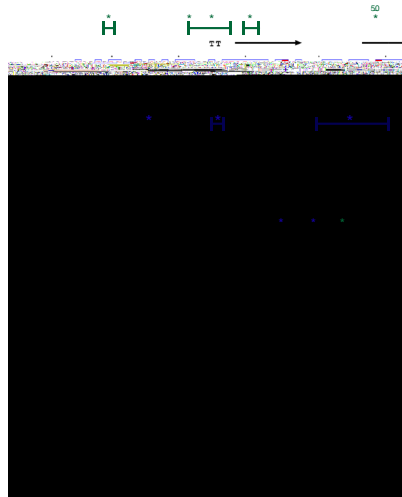
SNX3-retromer requires an evolutionary conserved MON2:DOPEY2:ATP9A complex to mediate Wntless sorting and Wnt secretion

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Wntless transports Wnt morphogens to the cell surface and is required for Wnt secretion and morphogenic gradients formation. Recycling of endocytosed Wntless requires the sorting nexin-3 (SNX3)-retromer-dependent endosome-to-Golgi transport pathway. Here we demonstrate the essential role of SNX3-retromer assembly for Wntless transport and report that SNX3 associates with an evolutionary conserved endosome-associated membrane remodelling complex composed of MON2, DOPEY2 and the putative aminophospholipid translocase, ATP9A. In vivo suppression of *Ce-mon-2*, *Ce-pad-1* or *Ce-tat-5* (respective MON2, DOPEY2 and ATP9A orthologues) phenocopy a loss of SNX3-retromer function, leading to enhanced lysosomal degradation of Wntless and a Wnt phenotype. Perturbed Wnt signalling is also observed upon overexpression of an ATPase-inhibited TAT-5(E246Q) mutant, suggesting a role for phospholipid flippase activity during SNX3-retromer-mediated Wntless sorting. Together, these findings provide in vitro and in vivo mechanistic details to describe SNX3-retromer-mediated transport during Wnt secretion and the formation of Wnt-morphogenic gradients.

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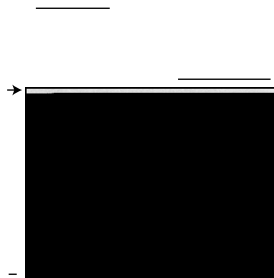
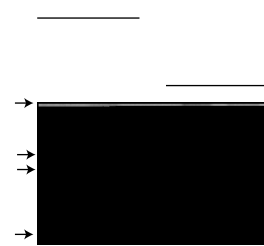
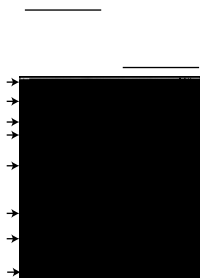
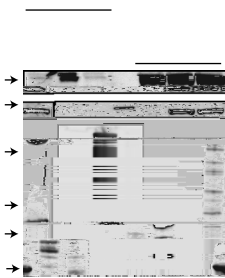
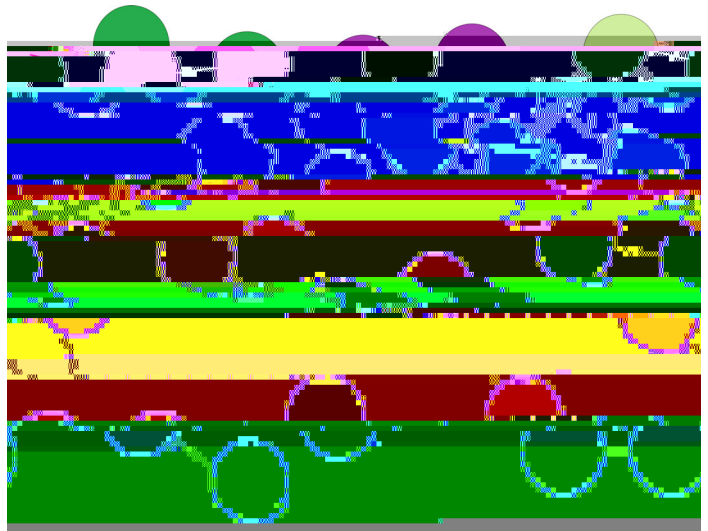
$C = -3(h - 256 - 22A)$ $(= 202)$ P $a f a$ SN a
 s $-20/$ $(\dots I)$ A 3
 3 35 3 3 3
 (-1) 3



(0 0)
(6 4) % %
39
40-43 1,854 fi
1), fi
>3- 3
2).
106
(2)
14,15,30-32
3 26A, 29
35 (2) 1, 2).
1, 2, 5, 6 (32) BA
14,15 %
41,44,45 27, 27-
(2) C -27
2A). 3

46- (s) (3B) 35
 A- fi A- 3
 35 (3) A- 3
 fi 35 A- 1 2
 (3) A- 1 2
 (BA) 23,24

27 (27-1) 41
 fi A-
 %
 3- 2. A- fi
 A- 3 35 (3)
 2.



fr. A- A- 9A A-
A- A- 9A
fr. (. 5 ,). A 9A
3- %
C. S. A

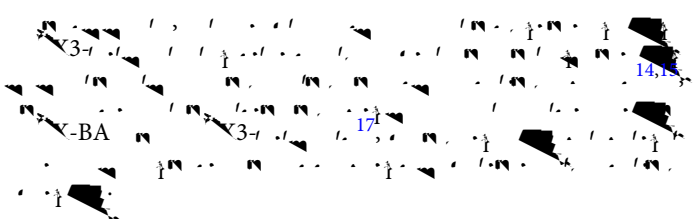
C-35 (2B-). 2 A 9A
 A 5 2
 2 AP 1- A 5
 A 9A, A 5 L
 C. A 5
 L fl. 53
 fl. 53
 P. 2, P. 2 (342) A
 E₂ 62.63
 A 5
 (. 5) A 5 (246) -29
 (m1320)
 (. 5) A 5 A
 fl.
 2- AP 1-
 A 5
 C.

Discussion

B 35
 C.
 3
 %
 2, 2, 3
 fl. L 48
 2, 2 A 9A
 L)
 3
 %

14. 2-AP1 3-1

$$C_5 = -27^{67} \cdot \dots \cdot 1 \cdot 1 \quad \text{---} \quad 3-1 \cdot 1 \quad \dots$$



Methods

Cell culture.

293 cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) (AA). Cells were then cultured in DMEM supplemented with 1% FBS (AA).

Immunofluorescence analysis.

Cells were fixed with 4% (v/v) paraformaldehyde (PFA) for 10 min. Cells were permeabilized with 0.1% (v/v) Triton X-100 (TX-100) for 10 min. Cells were then blocked with 0.5% (w/v) BSA for 30 min. Cells were incubated with primary antibody (A) for 1 h at room temperature. Cells were then incubated with secondary antibody (A B - 2) for 1 h at room temperature.

Immunoprecipitation and western blotting analysis.

293 cells were cultured in DMEM supplemented with 10% (v/v) FBS (AA). Cells were then cultured in DMEM supplemented with 1% FBS (AA) for 48 h. Cells were then harvested and lysed in RIPA lysis buffer. Cell lysates were immunoprecipitated with anti-A antibody (A) for 2 h at 4°C. Immunoprecipitates were then analyzed by western blotting.

53. ... & ... *J. Biol. Chem.* **291**, 15727–15739 (2016).
54. ... A. A. & ... *C. L.* **6**, 1228791 (2016).
55. ... B ... & ...