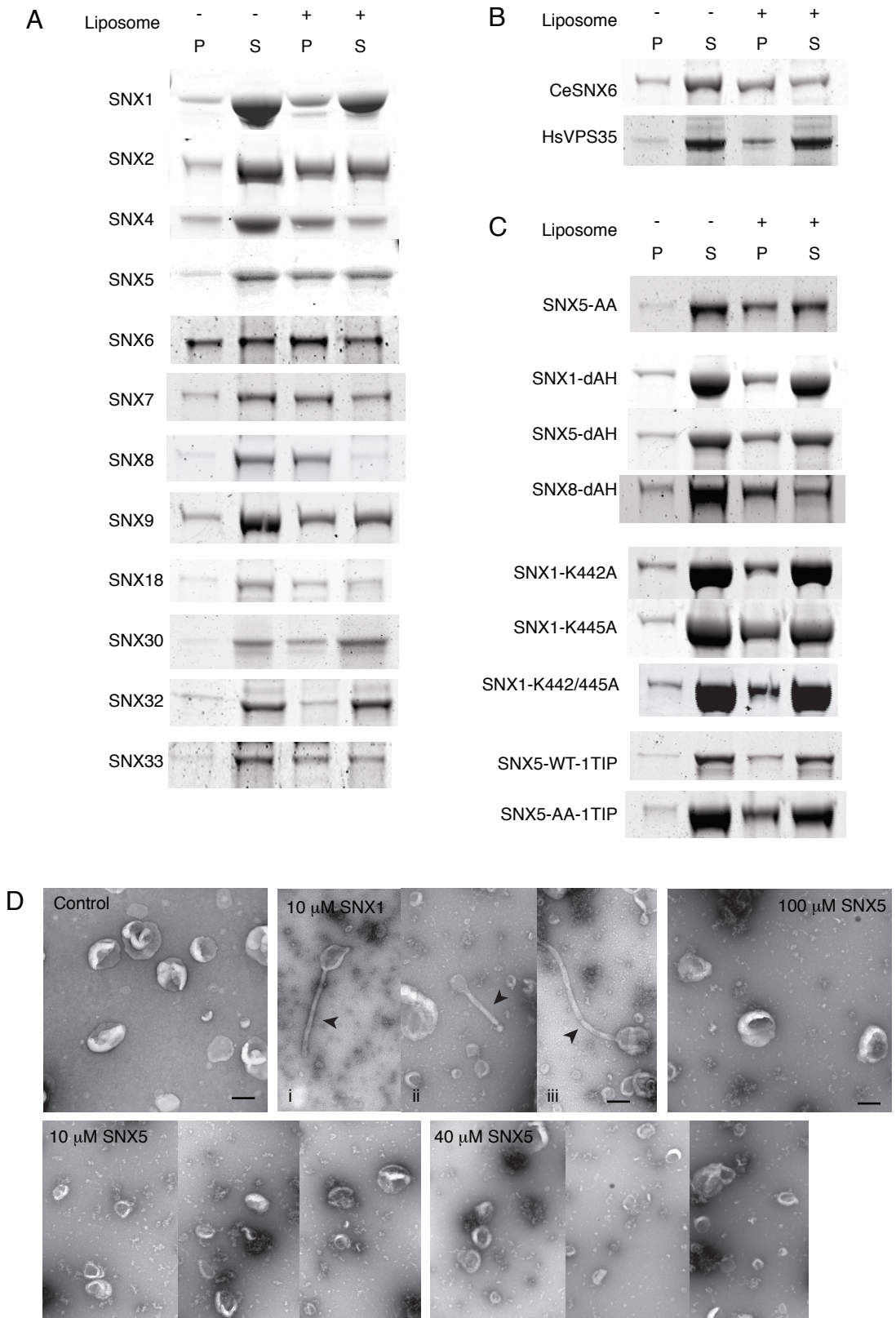
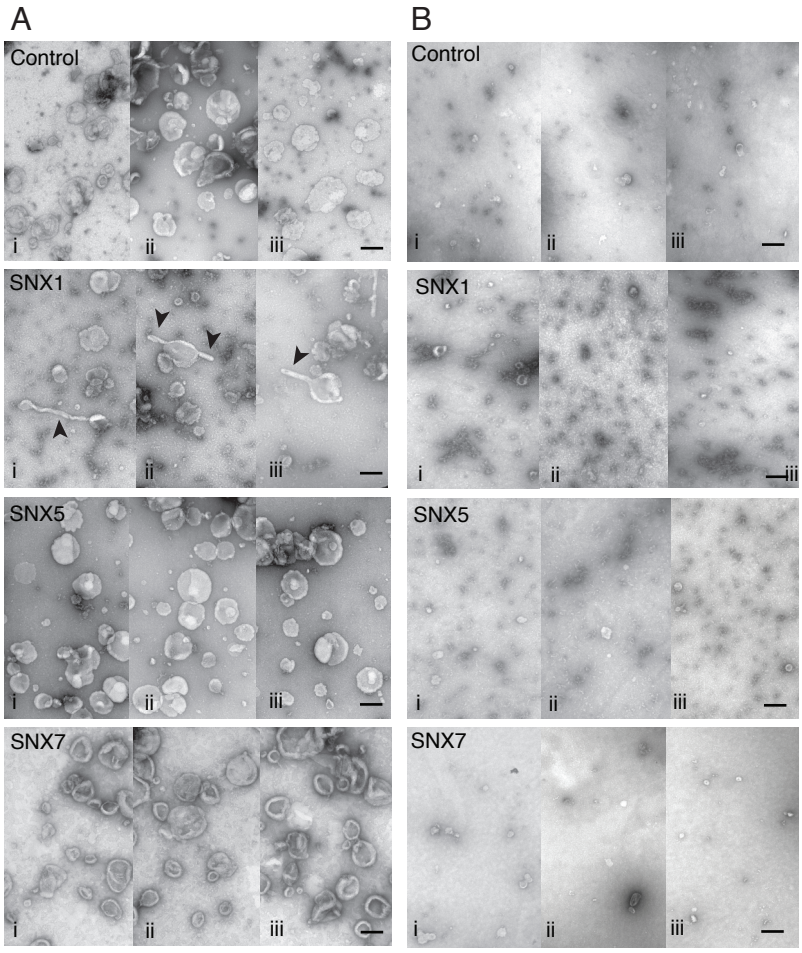


Supplementary Figure 1. All SNX-BAR proteins are associated with liposomes.
Coomassie-gel analysis of (A) all full-length mammalian SNX-BAR proteins, (B) *Caenorhabditis elegans* SNX6 (CeSNX6) and *Homo sapiens* VPS35 (HsVPS35), and (C) all mutant SNX-BAR proteins used in this study in the pellet (P) and supernatant (S) fractions after sedimentation in the presence or absence of liposomes.
(D) Example micrographs of liposomes incubated with control buffer, 10 μM SNX1-WT, 10 μM SNX5-WT, 40 μM SNX5-WT or 100 μM SNX5-WT final concentration for each protein (i-iii show three different example views), scale bar represents 200 nm, tubules are indicated by arrowheads.

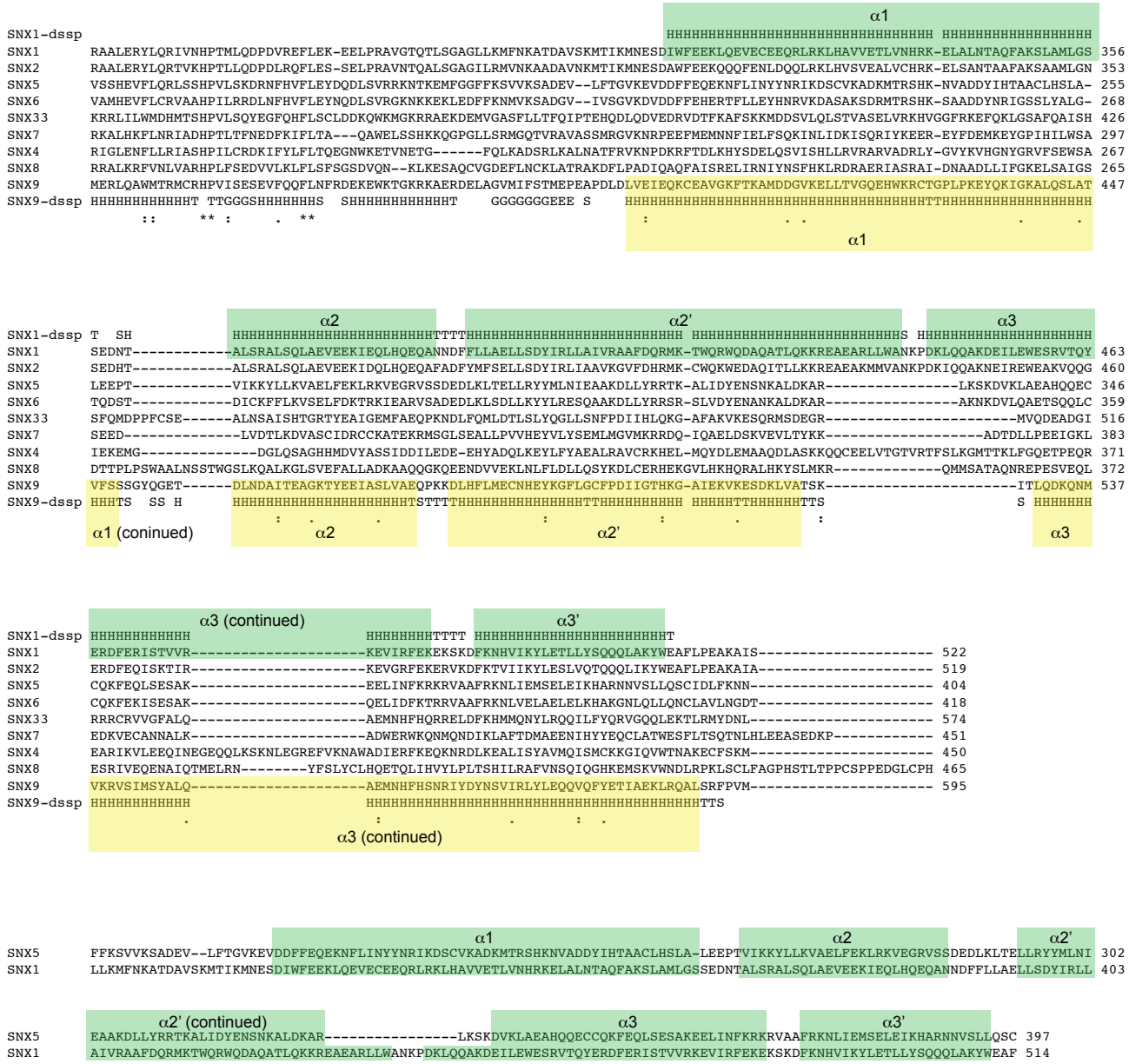


Supplementary Figure 2: Liposomes incubated with SNX5 or SNX7 do not contain more small vesicle production compared to SNX1 or buffer control.
Example micrographs of liposomes incubated with control buffer, SNX1-WT, SNX5-WT or SNX7-WT (A) before sedimentation and (B) the supernatant after 15 minute spin at 250,000 x g (i-iii show three different example views). Scale bar represents 200 nm. Tubules are indicated by arrowheads.

A

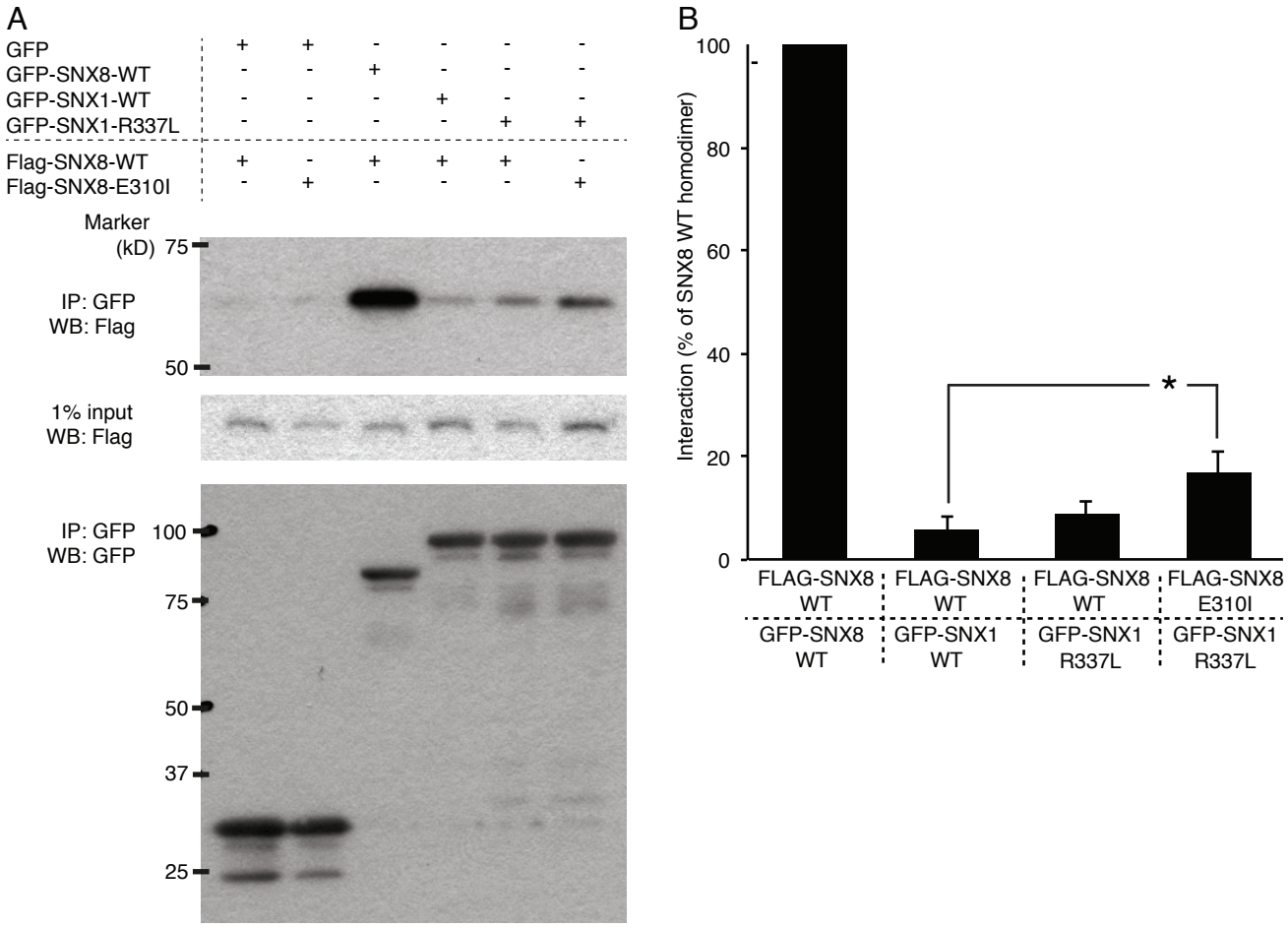
	Full length	SH3	PX	AH	BAR	NCBI
SNX1	522		147-255	281-298	284-517	NP_003090
SNX2	519		143-265	278-295	281-514	NP_003091
SNX4	450		59-184	194-211	209-440	NP_003785
SNX5	404		32-169	183-200	179-393	NP_689413
SNX6	418		45-182	194-211	203-416	NP_689419
SNX7	473		120-233	246-263	217-459	NP_057060.2
SNX8	465		78-178	180-197	200-444	NP_037453
SNX9	595	4-59	251-357	201-214	386-595	NP_057308
SNX18	628	4-58	276-403	225-238	415-621	NP_001096045
SNX30	437		91-206	216-233	207-429	NP_001013012
SNX32	403		27-163	176-193	185-403	Q86XE0
SNX33	574	4-58	240-334	179-192	373-554	ABV26009

B

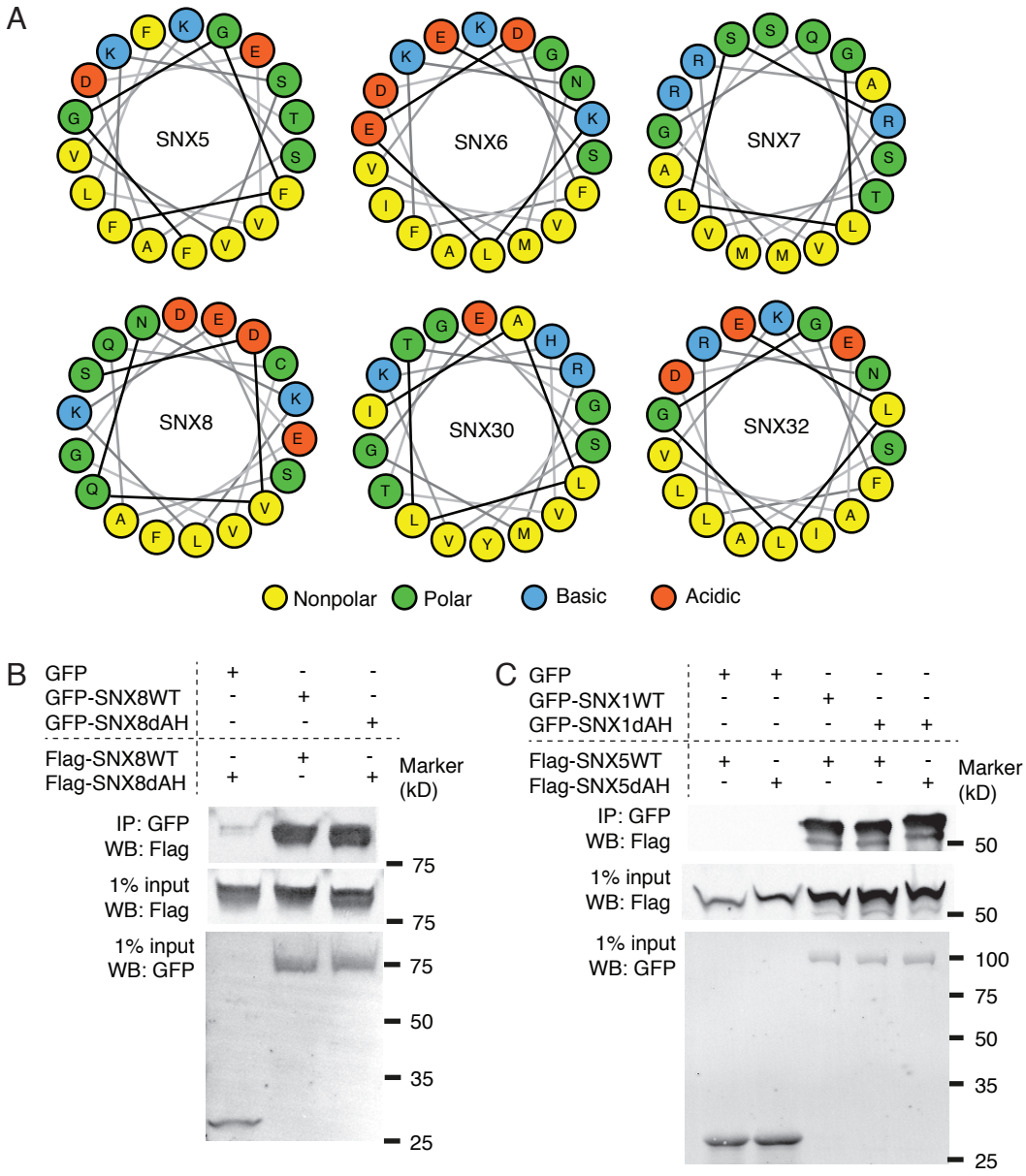


C

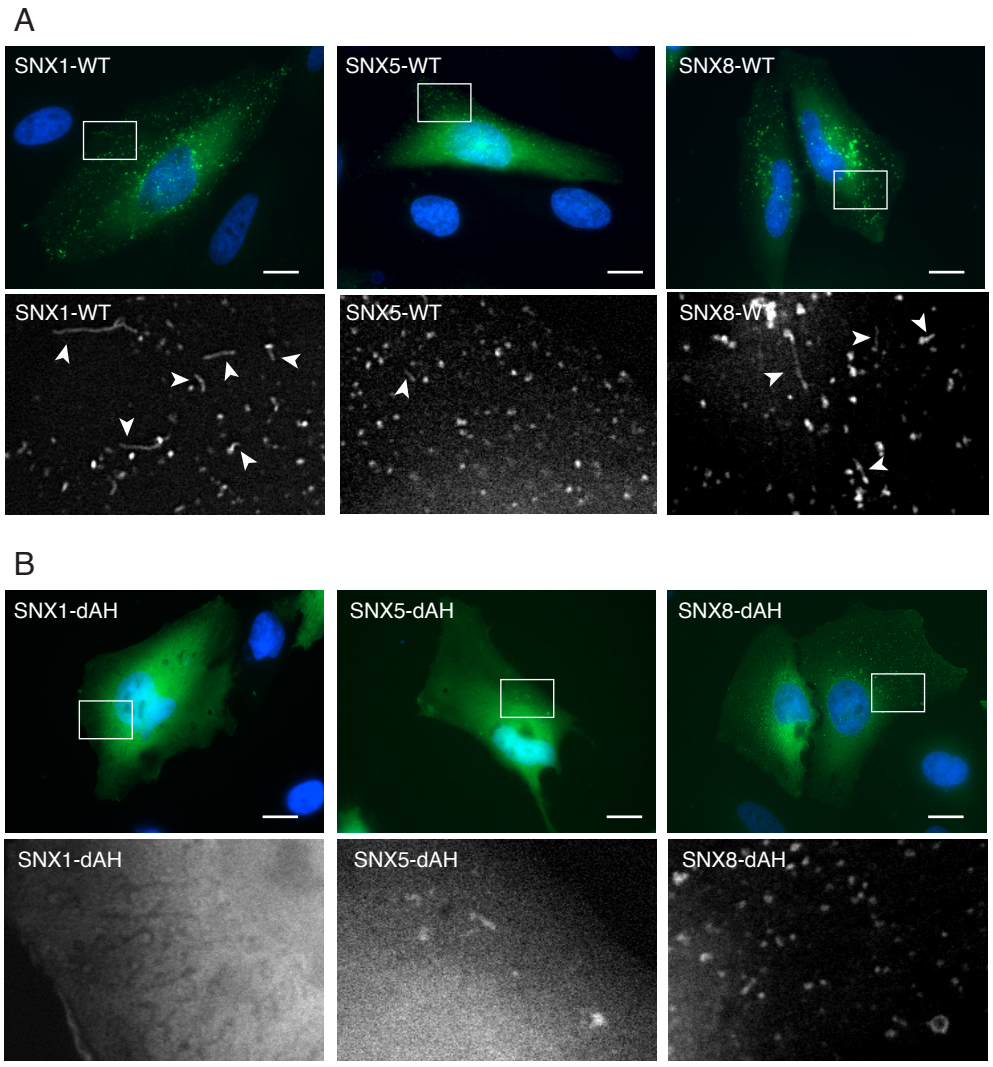
Supplementary Figure 3: Sequence alignments used to construct the homology model of SNX5 based on SNX1. (A) Overview of the domain structure of the *Homo Sapiens* SNX-BAR proteins. Numbers indicate amino acids in the sequence.
(B) Multi-alignment of SNX1, SNX2, SNX4, SNX5, SNX6, SNX7, SNX8, SNX9 and SNX33. SNX1-dssp and SNX9-dssp indicate the secondary structure per residue (H = α-helix, E = β-sheet, G = 3-helix S = bend, T = hydrogen bonded turn), based on SNX1 crystal structure in this study and the SNX9 structure published elsewhere (Pylypenko et al., 2007). α-Helixes of the BAR domain are indicated for SNX1 (green) and SNX9 (yellow).
(C) The sequence alignment used for the homology model of SNX5 based on the SNX1 structure. This alignment was extracted from the multi-alignment in (B).



Supplementary Figure 4: Removal of charged residues in the BAR interface of SNX1 and SNX8 allows dimerization of these two proteins.
(A) Immunoblots of expressed Flag-SNX8-WT or Flag-SNX8-E310I co-expressing GFP control, GFP-SNX8-WT, GFP-SNX1-WT or GFP-SNX1-R337L precipitated using GFP nanotrap.
(B) Quantitative analysis of the SNX1-SNX8 interaction as depicted in A, expressed as percentage of the SNX8-WT:SNX8-WT interaction. Intensity of fluorescent secondary antibodies was detected by an Odyssey membrane scanner and quantified in ImageJ. Values are average of 4 independent experiments, error bars indicate SEM. Asterisk indicates significant difference as tested by t-test, $p > 0.05$.



Supplementary Figure 5: The amphipathic helixes of SNX-BAR proteins.
(A) Cartoons of the arrangement of the 18-residue AHs of SNX5, SNX6, SNX7, SNX8, SNX30 and SNX32 identified in this study (see Figure 6A).
(B) Immuno-blots of expressed wild type (WT) and amphipathic helix mutants (dAH) of Flag- and GFP-tagged SNX8 proteins in HEK-293T cells using GFP-nanotrap IP.
(C) Immuno-blots of expressed wild type (WT) and amphipathic helix mutants (dAH) of Flag- and GFP-tagged SNX1 and SNX5 in HEK-293T cells using GFP-nanotrap IP.



Supplementary Figure 6: Endosomal tubulation by SNX-BAR-dAH mutant protein expression in intact mammalian cells.
Confocal microscopy images of HeLa cells expressing (A) GFP-SNX1-WT, GFP-SNX5-WT or GFP-SNX8-WT or (B) GFP-SNX1-dAH, GFP-SNX5-dAH or GFP-SNX8-dAH in green, nucleus was stained by DAPI (blue). Colour images show the whole-cell distribution of the expressed protein, black-and-white images show the GFP-signal in the boxed area. Scale bar represents 10 µm, GFP-labeled tubules are indicated by arrowheads.

A

```
SNX5-WT        311    RRTKALIDYENSNKALDK-----ARL--KSKDVKLAEAHQQ---------ECCQKFEQLS 354
SNX5-1TIP      311    RRTKALIDYQDAQATLQKKREAEARLLWANKPDKLQQAKDEILEWESRVTQCCQKFEQLS 370
consensus             RRTKALIDY------L-K-----ARL----K--KL--A-------------CCQKFEQLS
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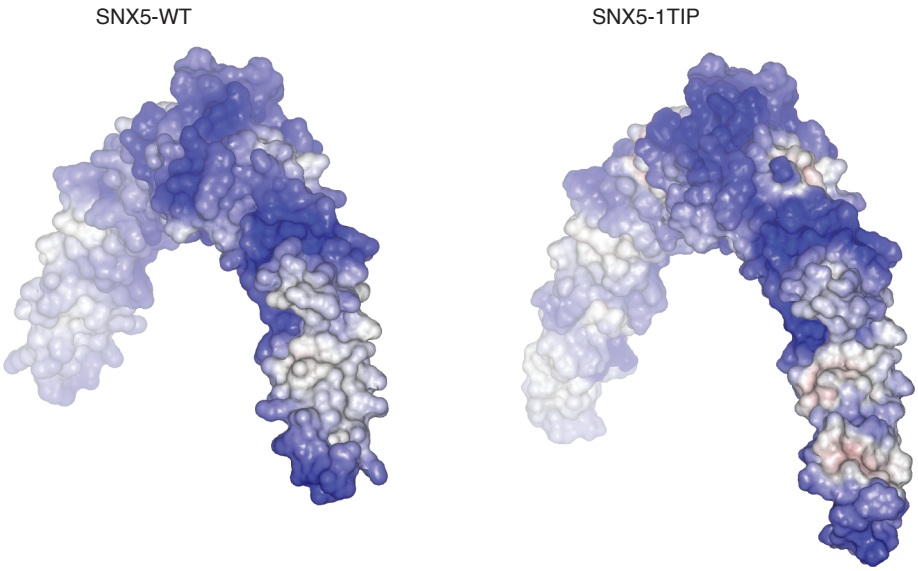
B



Supplementary Figure 7: The tip-loop region of SNX1 and SNX5.
(A) Sequence alignment of the tip-loop region of SNX5-WT and SNX5-1TIP. The highlighted lysines correspond to K442 and K445 of SNX1 present in SNX5-1TIP.
(B) Positive surface charge (blue residues) of the tip-loop region of the SNX5-WT homology model and (C) the SNX5-1TIP, including the SNX1 tip-loop region (Q421-Q462) taken from the SNX1 crystal structure.