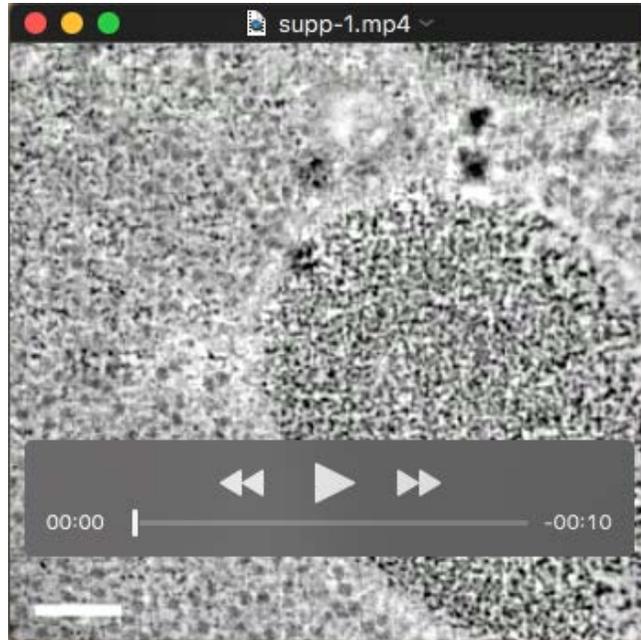
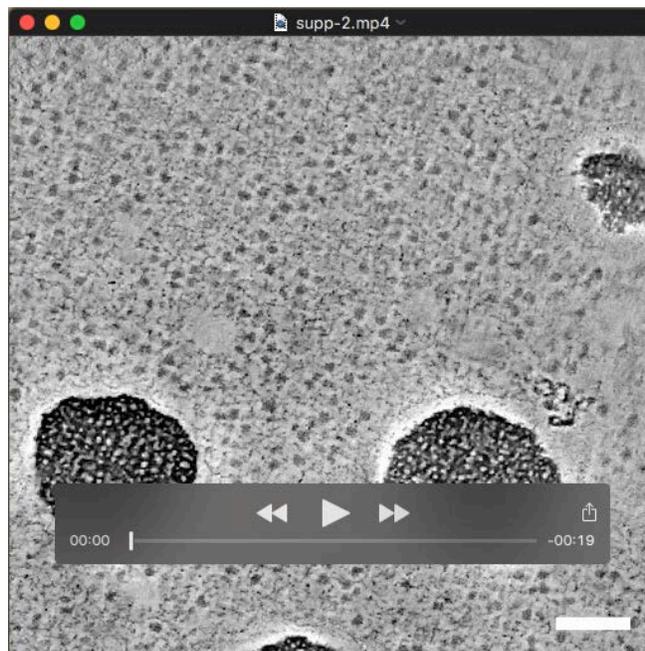


SUPPLEMENTARY INFORMATION



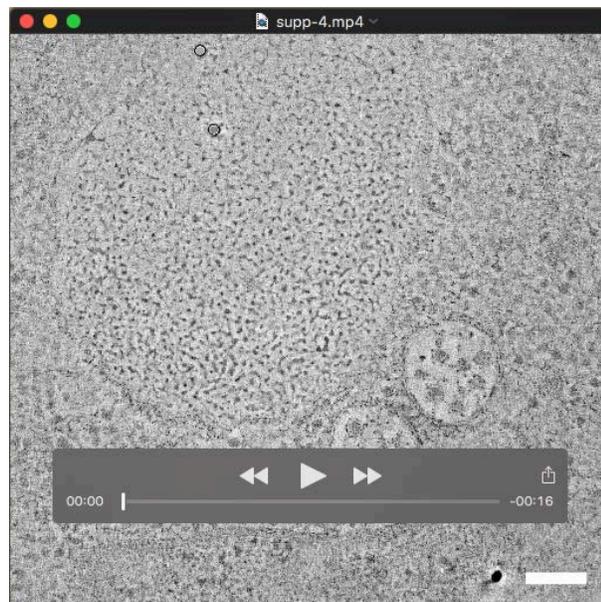
Movie 1. Representative example of the tomographic analysis of wild-type yeast transformed with an empty 2μ vector, as depicted in Fig. 3A.



Movie 2. Representative example of the tomographic analysis of wild-type yeast transformed with 2μ *DOA4*, as depicted in Fig. 3B.



Movie 3. Representative example of the tomographic analysis of *snf7*^{ΔMM1} yeast transformed with 2μ *DOA4*, as depicted in Fig. 3C.



Movie 4. Representative example of the tomographic analysis of wild-type yeast transformed with 2μ *doa4*¹⁻³⁴⁸, as depicted in Fig. 3D.

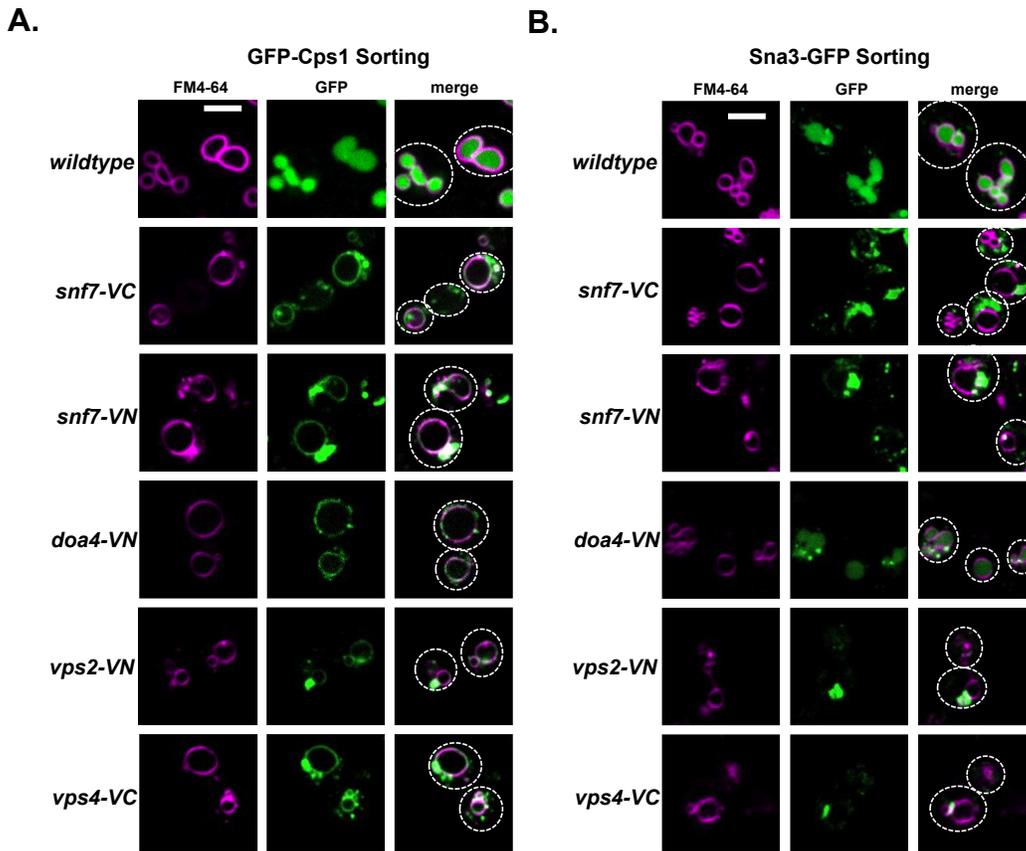


Figure S1. ILV cargo sorting in yeast expressing C-terminal VN- or VC-tagged ESCRT-III subunits

Live-cell fluorescence microscopy of GFP-Cps1 (A) or Sna3-GFP (B) localization in yeast strains expressing the indicated BiFC fusion proteins used in this study. Vacuolar and endosomal membranes are stained with FM4-64. Cell outlines are traced with the dashed lines. Bar, 4 μ M.

Table S1. Yeast strains and plasmids used in this study.

Strain	Genotype	Reference
SEY6210	<i>MATα leu2-3,112 ura3-52 his3Δ200 trp1-Δ901 lys2-Δ801 suc2-Δ9</i>	Robinson et al. 1988
BWCPL1270	W303a; <i>snf7-VC::KANMX6</i>	Webster et al. 2014
BWCPL1471	W303a; <i>vps4-VC::KANMX6</i>	Webster et al. 2014
CPL1284	W303a; <i>snf7-VN::KANMX6</i>	Webster et al. 2014
GOY437	SEY6210; <i>doa4-VN::HIS3</i>	This study
GOY461	SEY6210; <i>snf7-VC::KANMX6 doa4-VN::HIS3</i>	This study
MWY25	SEY6210; <i>snf7^{L231A;L234A}::KANMX6</i>	Wemmer et al. 2011
DCBY100	SEY6210; <i>snf7-VC::KANMX6 doa4-VN::HIS3 bro1Δ::TRP1</i>	This study
DCBY112	SEY6210a; <i>vps2-VN::HIS3</i>	This study
DCBY116	SEY6210; <i>vps2-VN::HIS3 snf7-VC::KANMX6</i>	This study
DCBY153	SEY6210a; <i>his2-VN::TRP1</i>	This study
DCBY159	SEY6210; <i>snf7-VC::KanMX6 his2-VN::HIS3</i>	This study
DCBY163	SEY6210a; <i>snf7^{L231A;L234A}-VC::TRP1</i>	This study
DCBY169	SEY6210; <i>snf7^{L231A;L234A}-VC::KANMX6 doa4-VN::HIS3</i>	This study
DCBY175	SEY6210a; <i>snf7^{L231A;L234A}-VN::HIS3</i>	This study
DCBY177	SEY6210a; <i>snf7^{L231A;L234A}::KANMX6 vps2-VN::HIS3</i>	This study
DCBY179	SEY6210; <i>snf7-VC::KANMX6 doa4¹⁻³⁴⁸-VN::TRP1</i>	This study
DCBY180	SEY6210a; <i>snf7^{L231A;L234A}::KANMX6 vps4-VC::TRP1</i>	This study
DCBY181	SEY6210; <i>snf7^{L231A;L234A}-VC::KANMX6 doa4¹⁻³⁴⁸-VN::TRP1</i>	This study
DCBY183	SEY6210; <i>snf7-VC::KANMX6 doa4¹⁻³⁴⁸-VN::TRP1 bro1Δ::HIS3</i>	This study
Plasmid	Genotype	Reference
pRS202	<i>URA3 Ap^R 2μ</i>	Christianson et al. 1992
pRS426	<i>URA3 Ap^R 2μ</i>	Christianson et al. 1992
pST39	<i>Ap^R</i>	Tan 2001
pCR44	<i>URA3 Ap^R 2μ (pRS426) GFP-Doa4¹⁰⁷⁻³⁴⁸</i>	This study
pCR52	<i>URA3 Ap^R 2μ (pRS426) GFP-Doa4¹⁻³⁴⁸</i>	This study
pCR58	<i>URA3 Ap^R 2μ (pRS426) GFP-Doa4</i>	This study
pCR152	<i>Ap^R (pST39) GST-Doa4¹⁻⁸⁰</i>	Richter et al. 2013
pCRQ2	<i>Ap^R (pST39) GST-Doa4¹⁻³⁴⁸</i>	This study
pDCB17	<i>LEU2 Ap^R 2μ (pRS415) GFP-Cps1</i>	This study
pDCB90	<i>URA3 Ap^R (pRS202) Doa4¹⁻³⁴⁸</i>	This study
pDN336	<i>TRP1 Ap^R (pDN614) PGK1_{pr}::RLuc CPS1_{pr}::FLuc-CPS</i>	Shideler et al., 2015
pGO289	<i>URA3 Ap^R (pRS202) DOA4</i>	Luhtala and Odorizzi 2004
pGO511	<i>Ap^R (pST39) VPS20</i>	Johnson et al. 2017
pGO515	<i>Ap^R (pST39) VPS24</i>	This study
pGO516	<i>Ap^R (pST39) VPS2</i>	Richter et al. 2013
pGO547	<i>Ap^R (pST39) SNF7</i>	Wemmer et al. 2011
pGO557	<i>Ap^R (pST39) Snf7¹⁻²²⁵</i>	This study
pGO560	<i>Ap^R (pST39) Snf7^{L231A,L234A}</i>	Richter et al. 2013
pGO613	<i>Ap^R (pST39) Snf7^{L199D,V202D}</i>	This study
pGO640	<i>URA3 Ap^R 2μ (pRS426) GFP-Doa4¹⁻⁸⁰</i>	Richter et al. 2013
pGST-DOA4 ^{RH}	<i>Ap^R (pST39) GST-Doa4¹⁰⁷⁻³⁴⁸</i>	This study