SUPPLEMENTARY ONLINE MATERIAL

SUPPLEMENTARY FIGURES

Supplementary Figure S1: Coprecipitation assays showing binding of endogenous hIST1 to with CHMP1B, VPS4 and LIP5 fused to GST. 1% of the starting cell lysate and 10% of the volume eluted from the beads (pull-down) were analyzed by western blot with α-hIST1 antibody (top and middle panels respectively). Quantification of the amount of hIST1 protein bound to the GST fusion proteins via infrared imaging is given. The bottom panel shows the GST fusion protein expression with coomassie staining.

Supplementary Figure S2: siRNA treatment depletes all hIST1 isoforms. 293T cells were transfected with hIST1a, b, c or d fused to YFP and treated with siRNA against hIST1 or Luciferase. Cells were harvested and analyzed by western blot with α-GFP antibody and α-HSP90 as loading control.

Supplementary Figure S3: Highly conserved interaction of hIST1 and Ist1 proteins with the ESCRT pathway. Human and yeast IST1 proteins were tested against the indicated yeast (A) or human (B) ESCRT components in yeast two-hybrid assays. Error bars indicate the standard deviation from the mean of triplicate measurements. (C) Trafficking of CPY is not disrupted in *ist1Δ*or *vta1Δ* □□□□□, whilst *ist1Δvta1Δ* double mutants show a synthetic CPY sorting defect. Wild type yeast and *vps4Δ* yeast were used as controls. Secreted CPY was detected using a semi-quantitative filter assay. (D) Growth curves for wild type yeast or yeasts lacking Ist1, Vps4, Vps20 or Bro1. Optical densities at 600nm of cultures growing in rich medium were monitored over a period of 10 hours. Each point is the mean of duplicate samples.

Supplementary Figure S4: Trafficking of several MVB cargoes in in wild type, *ist1*Δ yeast, *vps4*Δ yeast, *vta1*Δ yeast and *ist1*Δ *vta1*Δ yeast. (A) CPS trafficking. Nomarski and fluorescence images of live yeast cells expressing GFP-CPS from a high copy (2μ) yeast plasmid. (B) Trafficking of Sna3p and Ste3p. Nomarski and fluorescence images of live yeast cells expressing GFP-Sna3p or GFP-Ste3p from low copy (CEN) plasmids.

Supplementary Figure S5: Salt tolerance assay. Dilutions of yeast cells were plated on YEPD supplemented with 1M, 1.5M or 2M sodium chloride.

Supplementary Table 1. Yeast strains used in this study. All yeast strains were derived from KEBY88. Yeast gene deletions with the kanamycin-resistance cassette (*Kan*^{r}) were constructed as described previously (Bowers *et al.*, 2004). The *ist1*Δ*::Kan*^{r} cassette was generated by PCR using yeast genomic DNA from Euroscarf strain Y01179 as a template. The PCR product (which included 500bp of the *IST1* 5' and 3' UTR) was subcloned into pCRblunt II-TOPO (Invitrogen Ltd.). To generate the *ist1*Δ*::TRP1* knockout construct, the *Kan*^{r} gene was removed by digestion with PacI and BglII and replaced by a BamHI/ PvuI *TRP1* fragment from pJJ248 (Jones and Prakash, 1990). The *vta1Δ::Kan*^{r} *ist1Δ::Hygro*^{r} strain was generated by transformation of JLY03 with a PCR product consisting of the hygromycin-resistance cassette from pAG32 (Euroscarf) with ends to allow recombination at the *IST1* locus. All *ist1* deletion strains were checked by PCR from genomic DNA.

Strain		Source
KEBY88	*MAT□ ura3-52 leu2-3,112 his3-Δ200*	(Bowers *et al.*, 2004)

	trp1-901 lys2-801 suc2-Δ9 pep4-3	
KEBY106	*MAT□ ura3-52 leu2-3,112 his3-Δ200 trp1-901 lys2-801 suc2-Δ9 pep4-3 bro1Δ::Kan^r*	This study
KEBY94	*MAT□ ura3-52 leu2-3,112 his3-Δ200 trp1-901 lys2-801 suc2-Δ9 pep4-3 vps20Δ::Kan^r*	This study
KEBY92	*MAT□ ura3-52 leu2-3,112 his3-Δ200 trp1-901 lys2-801 suc2-Δ9 pep4-3 vps4Δ::Kan^r*	(Bowers *et al.*, 2004)
KEBY174	*MAT□ ura3-52 leu2-3,112 his3-Δ200 trp1-901 lys2-801 suc2-Δ9 pep4-3 ist1Δ::Kan^r*	This study
KEBY180	*MAT□ ura3-52 leu2-3,112 his3-Δ200 trp1-901 lys2-801 suc2-Δ9 pep4-3 ist1Δ::TRP1*	This study
KEBY178	*MAT□ ura3-52 leu2-3,112 his3-Δ200 trp1-901 lys2-801 suc2-Δ9 pep4-3 vta1Δ::Kan^r ist1Δ::Hygro^r*	This study
JLY03	*MAT□ ura3-52 leu2-3,112 his3-Δ200 trp1-901 lys2-801 suc2-Δ9 pep4-3 vta1Δ::Kan^r*	(Lottridge *et al.*, 2006)

SUPPLEMENTARY MATERIALS AND METHODS

Plasmids and yeast trafficking assay - The GFP-CPS, SNA3-GFP and STE3-GFP plasmids were gifts from David Katzmann, Hugh Pelham and Rob Piper, respectively (Odorizzi *et al.*, 1998; Urbanowski and Piper, 1999; Reggiori and Pelham, 2001). Yeast strains transformed with this plasmid were grown and viewed as described previously (Phelan *et al.*, 2006).

Salt tolerance assay - Yeast strains grown overnight in YEPD were diluted to 0.8 OD_{600nm} per ml and serially diluted. 2.5µl aliquots were spotted onto YEPD plates supplemented with additional 1M, 1.5M or 2M sodium chloride. Plates were incubated at 30°C.

Coprecipitation Assays - 293T cells were transfected with GST expression vectors (1 µg of each) and proceeded as described in the main text. The bead-bound proteins were analyzed by western blotting with α-hIST1 rabbit polyclonal antibody.

SUPPLEMENTARY REFERENCES

Bowers, K., Lottridge, J., Helliwell, S.B., Goldthwaite, L.M., Luzio, J.P., and Stevens, T.H. (2004). Protein-protein interactions of ESCRT complexes in the yeast Saccharomyces cerevisiae. Traffic *5*, 194-210.

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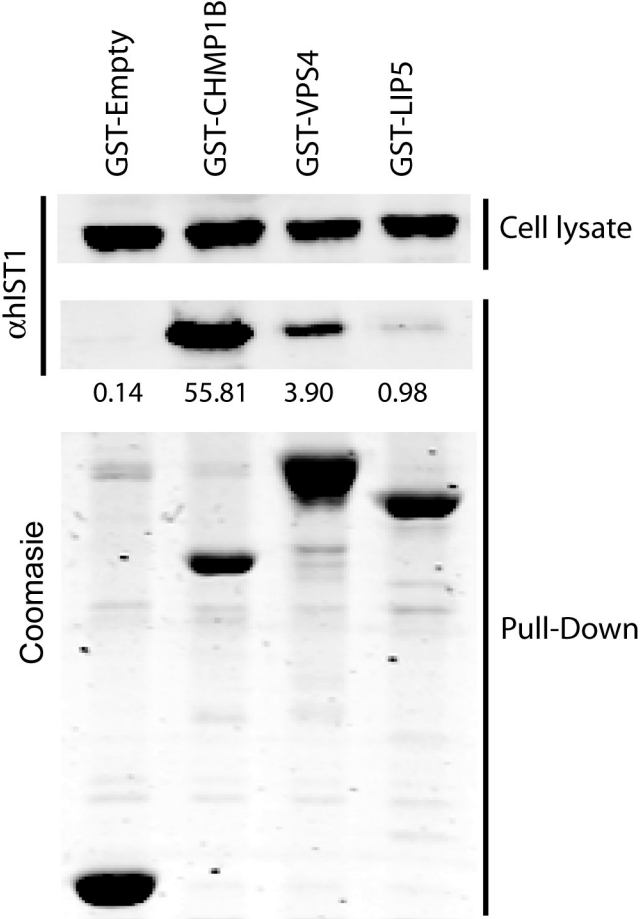
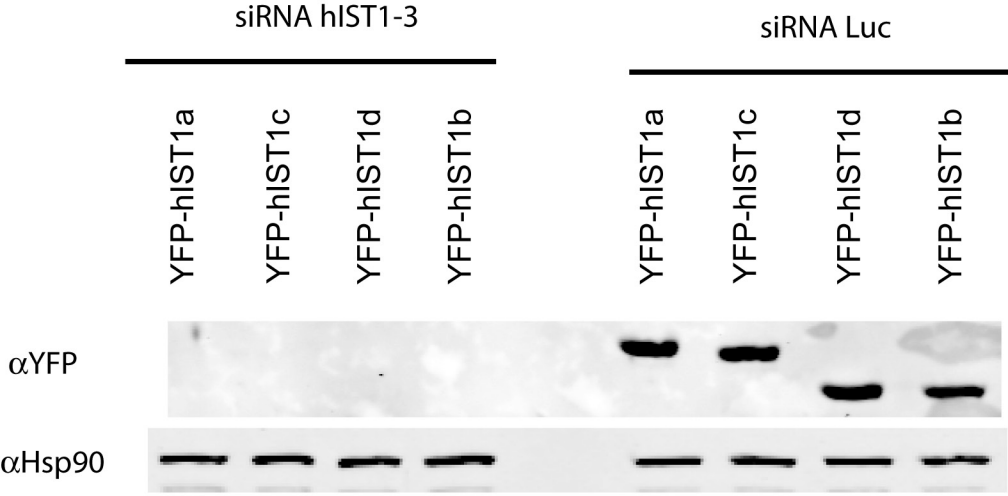


Agromayor *et al*, Figure S1



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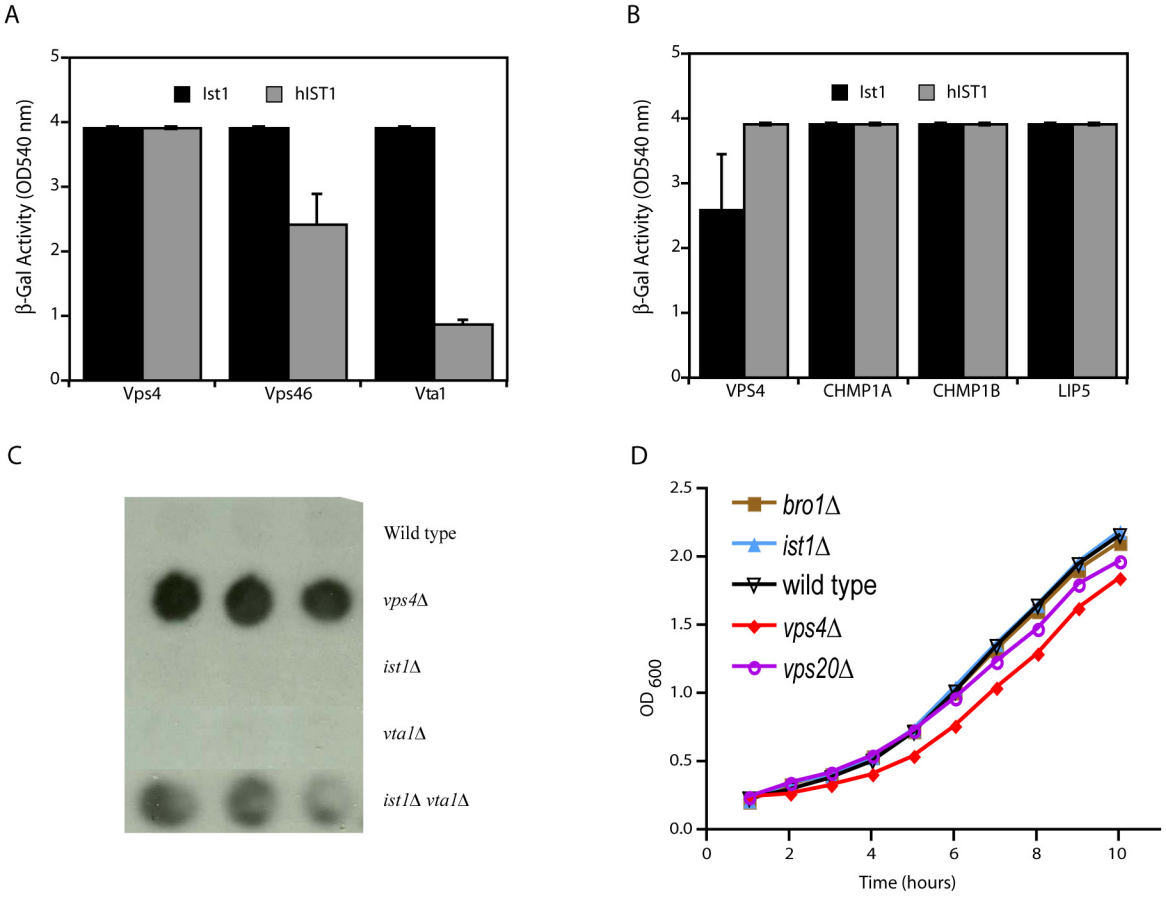

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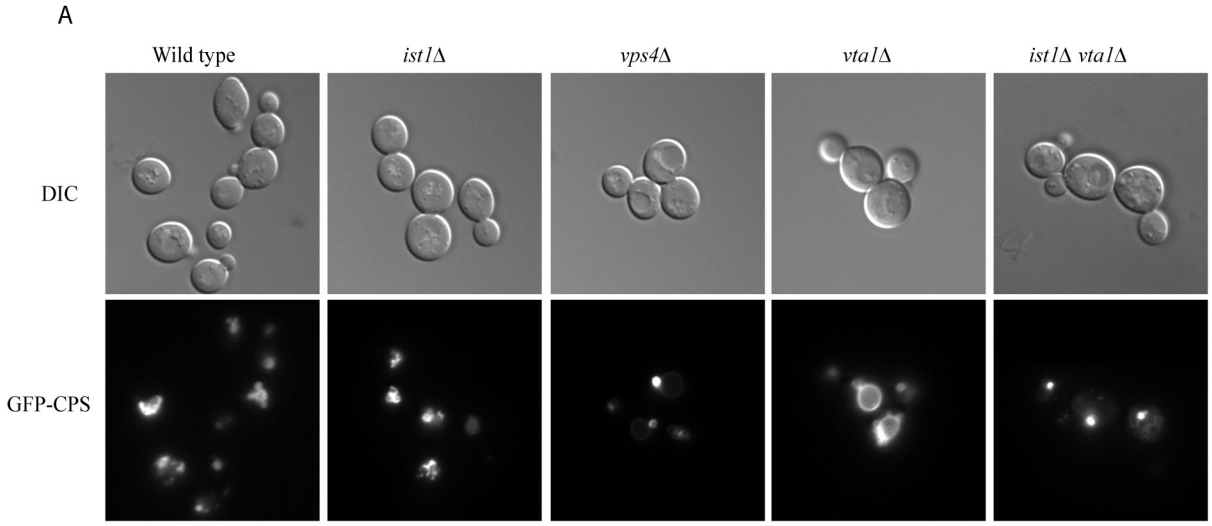

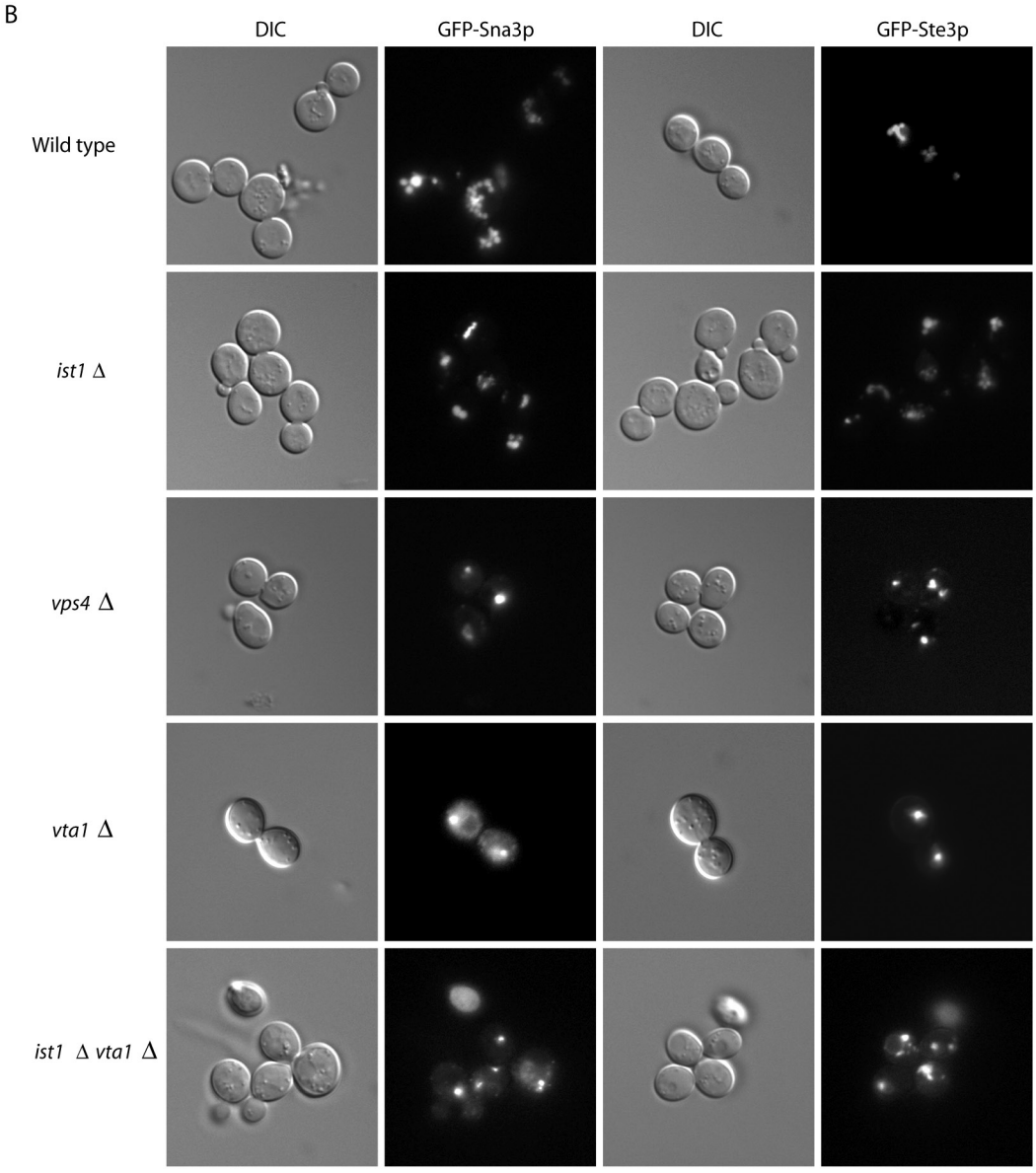

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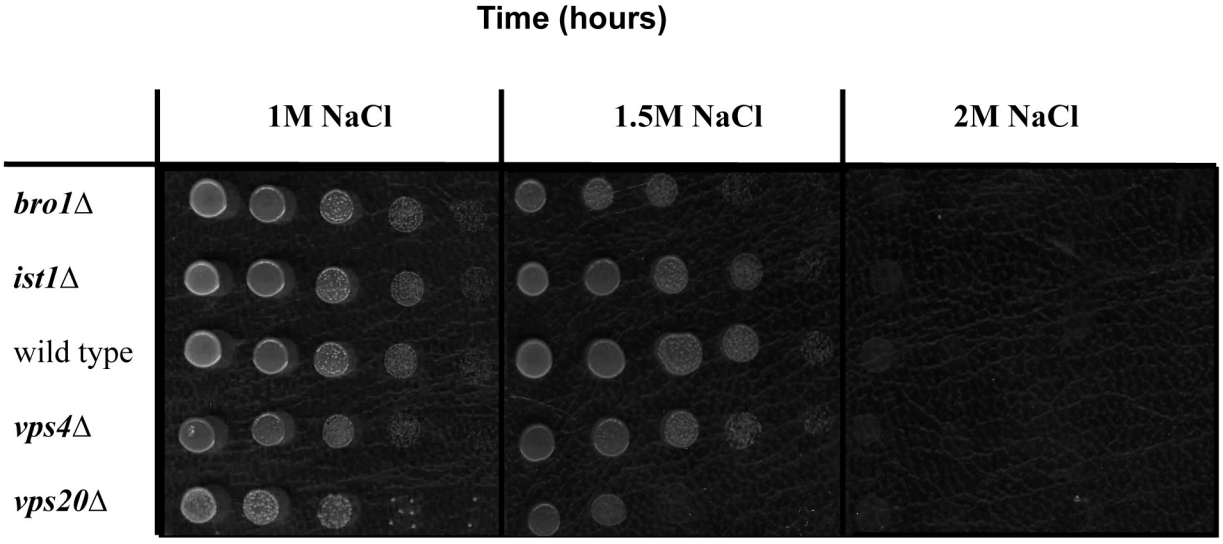

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