Shibuya et al., http://www.jcb.org/cgi/content/full/jcb.201408024/DC1

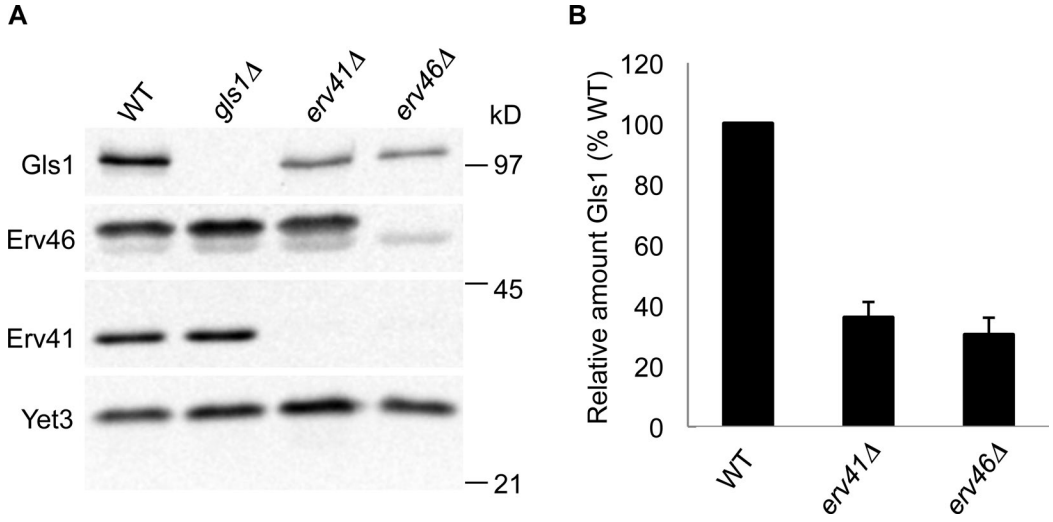

Figure S1. **The *erv46Δ* mutation reduces cellular levels of Gls1 similarly to *erv41Δ*.** (A) Cells grown to mid–log phase were lysed, resolved on a 10.5% polyacrylamide gel, and immunoblotted for Gls1, Erv46, Erv41, and Yet3 (loading control). WT (CBY740), *gls1Δ* (CBY1086), *erv41Δ* (CBY1168), and *erv46Δ* (CBY3612) strains were compared. The panel was assembled from a single immunoblot, and the molecular mass markers shown indicate relative positions across neighboring strips. (B) Relative amounts of Gls1 with standard error bars (n = 3). Gls1 levels were normalized with Yet3 as the loading control and plotted as a percentage relative to WT.

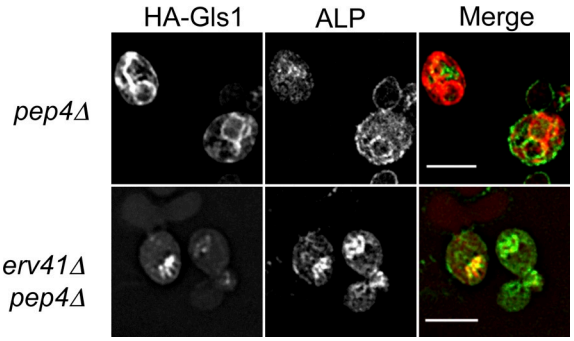

Figure S2. **Absence of Erv41 causes mislocalization of Gls1 to vacuoles.** Triple HA-tagged Gls1 was visualized by immunofluorescence microscopy using an anti-HA monoclonal antibody and an anti–mouse IgG Texas red–conjugated secondary antibody in *pep4Δ* (CBY3864) and *erv41Δ pep4Δ* (CBY3867) strains. The same cells were also stained for ALP as a vacuole marker using a polyclonal antibody against ALP and an anti–rabbit IgG FITC-conjugated secondary antibody. Bars, 5 µm.

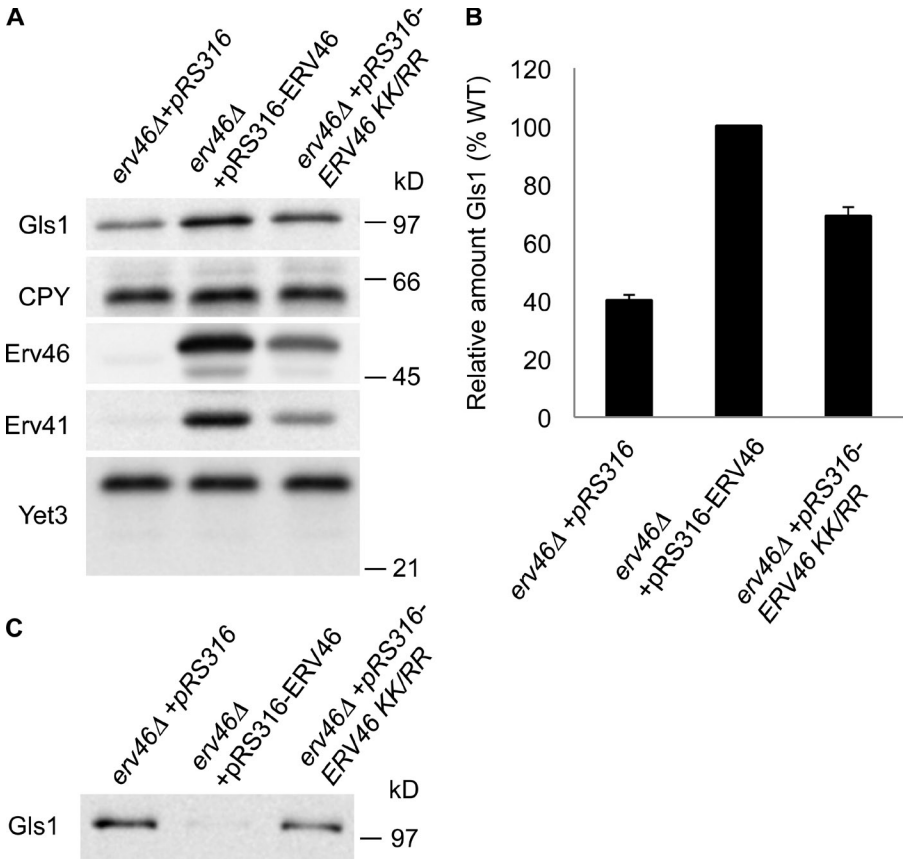

Figure S3. **Mutation of a COPI binding motif in the Erv46 tail mislocalizes Gls1.** (A) Cells grown to mid–log phase were lysed, resolved on a 10.5% polyacrylamide gel, and immunoblotted for Gls1, CPY, Erv46, Erv41, and Yet3 (loading control). *erv46Δ* + pRS316 (CBY4002), *erv46Δ* + pRS316-*ERV46* (CBY4003), and *erv46Δ* + pRS316-*ERV46 KK/RR* (CBY4004) strains were compared. The panel was assembled from a single immunoblot, and the molecular mass markers shown indicate relative positions across neighboring strips. (B) Relative amounts of Gls1 with standard error bars (n = 3). Gls1 levels were normalized with Yet3 as the loading control and plotted as a percentage relative to WT. (C) Proteins secreted to the extracellular medium were precipitated, resolved on a polyacrylamide gel, and immunoblotted for Gls1.

Table S1. **Plasmids used in this study**

Plasmid	Reference
pYEX4T-1-GLS1 (*URA3* 2μ plasmid, *CUP1* promoter for GST-Gls1[33–833] expression)	This study
pRS425-GLS1 (*LEU2* 2μ plasmid)	This study
pRS317-GLS1 (*LYS2 CEN* plasmid)	This study
pRS317-HA-GLS1 (*LYS2 CEN* plasmid)	This study
pGEX2T-FPR2 (*AmpR ori* plasmid, tac promoter for Gst-Fpr2 expression in *E. coli*)	This study
pRS424-ERV41 (*TRP1* 2μ plasmid)	Otte et al., 2001
pRS426-ERV46 (*URA3* 2μ plasmid)	Otte et al., 2001
pRS316-ERV46 (*URA3 CEN* plasmid)	Otte and Barlowe, 2002
pRS316-ERV46-KK/RR (*URA3 CEN* plasmid)	This study

Table S2. **Primers used in this study**

Primer	Sequence	Reference
GLS1-EcoRI	5′-GAGATCGAATTCGAAGAATATCAAAAGTTC-3′	This study
GLS1–SalI-1	5′-CTGCAGGTCGACTCAGAAGCGTCCAAG-3′	This study
GLS1-NotI	5′-ATAAGAATGCGGCCGCGAGATTCGAAGAAGACCCAT-3′	This study
GLS1–SalI-2	5′-TCCGAGGCGTCGACTGTGACATTTCATAATGCAT-3′	This study
GLS1-XhoI	5′-GAGTCCTCGAGATGCTTATTTCAAAATCTAAG-3′	This study
GLS1-IntF	5′-ATCCGGAATATGAAACCATGG-3′	This study
GLS1-IntR	5′-AGTGAAGGTACGCCCAACC-3′	This study
FPR2-BamHI	5′-CGGGATCCGGTTCCCTGTCAGATTTGGAAATCGGTATTATC-3′	This study
FPR2-EcoRI	5′-CGGAATTCCTAGGCGGCTGATTTCACGTCTACCAATTC-3′	This study
YAL042w-NotI	5′-ATAAGAATGCGGCCGCCTGCAGATGACATTGCGCTGC-3′	Otte et al., 2001
YAL042w-BamHI	5′-CGCGGATCCGCCATGATCTCTCGGGTTGG-3′	Otte et al., 2001
ERV46(KK/RR)-F	5′-CGATCTGGGGCAGGAGGAGCCAGTAGAGG-3′	This study
ERV46(KK/RR)-R	5′-CCTCTACTGGCTCCTCCTGCCCCAGATCG-3′	This study
ERV46-IntF	5′-TAAGGAACAGCACGGGCAG-3′	This study
ERV46-IntR	5′-CCCACTCGTTCACCAGCAG-3′	This study
Erv41–KO-F	5′-TTTTGCTGTAACGTACAGGCAGAGACTTCAGGAGTATATACGGATCCCCGGGTTAATTAA-3′	This study
Erv41–KO-R	5′-GTTATCTTGGGTATACTGCATTCTCTTTTCTTTTACATATGAATTCGAGCTCGTTTAAAC-3′	This study
Erv46–KO-F	5′-GACAGTGATATAACACACATAGCTAGACACAATAGACATCCGGATCCCCGGGTTAATTAA-3′	This study
Erv46–KO-R	5′-AGTATGTAGAAAGGGCTCTTCCCTATGACAGTCTCTTCCTGAATTCGAGCTCGTTTAAAC-3′	This study
Gls1–KO-F	5′-CAACTGTTAATAAGGAAGTAGTGGATAATAACGGTTCAGGTTGCTTCACACGGATCCCCGGGTTAATTAA-3′	This study
Gls1–KO-R	5′-ACATATCTGGTTACATATCTGTTTATATGACTGAAAATATCTACATAGATGAATTCGAGCTCGTTTAAAC-3′	This study

F, forward; R, reverse.

Table S3 shows the mass spectrometry peptide data for the *erv41Δ* and *erv46Δ* strains from SILAC experiments and proteome changes sorted from smallest to largest $\log_2$ (*erv41Δ*/control) scores and is provided online in an Excel spreadsheet.

References

Otte, S., and C. Barlowe. 2002. The Erv41p-Erv46p complex: multiple export signals are required in trans for COPII-dependent transport from the ER. *EMBO J.* 21:6095–6104. http://dx.doi.org/10.1093/emboj/cdf598

Otte, S., W.J. Belden, M. Heidtman, J. Liu, O.N. Jensen, and C. Barlowe. 2001. Erv41p and Erv46p: new components of COPII vesicles involved in transport between the ER and Golgi complex. *J. Cell Biol.* 152:503–518. http://dx.doi.org/10.1083/jcb.152.3.503