Table S1. **Specificity of the Sec16 antibody used in immunoelectron microscopy experiments**

Organelle	Observed number of gold particles	0.01-μm² squares	Labeling density[a]	Expected gold particles[b]	Relative labeling index[c]	χ² values
Nucleus	7	476	0.0147	4.75	1.475	1.07
Cytosol	3	1,227	0.0024	12.23	0.245	6.97
ER/ERES	16	409	0.0391	4.08	3.924[d]	34.86[d]
Mitochondria	0	231	0	2.30	0	2.3
Multivesicular body	0	65	0	0.65	0	0.65
Golgi	0	48	0	0.48	0	0.48
Plasma membrane	0	141	0	1.41	0	1.41
Other	0	11	0	0.11	0	0.11
Total	26	2,608	0.01	26	1	47.85[e]

The same procedure described in Table S2 was used to analyze labeling by the Sec16 antibody in the same electron micrographs.
[a]Number of gold particles divided by number of grid intersections.
[b]Number of grid intersections multiplied by total labeling density.
[c]Compartment labeling density divided by total labeling density.
[d]Number of gold particles significantly higher than the expected value ($P < 0.0001$).
[e]The distribution of gold particles significantly deviates from random ($P < 0.0001$).

Table S2. **Specificity of the GFP antibody used in Immunoelectron microscopy experiments**

Organelle	Observed number of gold particles	0.01 μm² squares	Labeling density[a]	Expected gold particles[b]	Relative labeling index[c]	χ² values
Nucleus	8	476	0.02	52.02	0.154[d]	37.25[d]
Cytosol	86	1,227	0.07	134.09	0.641[e]	17.24[e]
ER/ERES	142	409	0.35	44.70	3.177[f]	211.84[f]
Mitochondria	29	231	0.13	25.24	1.149	0.56
Multivesicular body	7	65	0.11	7.10	0.985	0
Golgi	5	48	0.10	5.25	0.953	0.01
Plasma membrane	8	141	0.06	15.41	0.519	3.56
Other	0	11	0.00	1.20	0	1.20
Total	285	2,608	0.11	285	1	270.46[g]

10 electron microscopy images (71,250 or 97,500 magnification) of cryosections of HeLa cells expressing FP-17 and incubated at 10°C were divided into 0.01-μm² squares by random superposition of a grid. Each square was classified for the presence of recognizable organelle membranes or cytosol/nucleus (indicated in Organelle column), and the number of gold particles within each square was counted. Since ERES could not always be clearly recognized on the basis of morphology, ER and ERES were considered together. Labeling densities and relative labeling index were then estimated for every organelle and the results were tested for randomness (Mayhew, T.M. 1992. *J. Neurocytol.* 21:313–328).
[a]Number of gold particles divided by number of grid intersections.
[b]Number of grid intersections multiplied by total labeling density.
[c]Compartment labeling density divided by total labeling density.
[d]Number of gold particles significantly lower than expected value ($P < 0.0001$).
[e]Number of gold particles significantly lower than expected value ($P = 0.0159$).
[f]Number of gold particles significantly higher than expected value ($P < 0.0001$).
[g]Distribution of gold particles significantly deviates from random ($P < 0.0001$).

Table S3. **Distribution of FP-17 and -22 between ER and ERES analyzed by immunoelectron microscopy after a 10°C block**

Organelle	Observed number of gold particles		Number of grid intersections		Labeling density[a]		Expected gold particles[a]		Relative labeling index[a]		χ² values	
	FP-17	FP-22	FP-17	FP-22	FP-17	FP-22	FP-17	FP-22	FP-17	FP-22	FP-17	FP-22
ER	409	161	1,196	644	0.342	0.250	400.66	171.30	1.02	0.94	0.174	0.62
ERES	8	20	48	36	0.167	0.556	16.08	9.58	0.50[b]	2.09[c]	4.06[b]	11.4[c]
Total	417	181	1,244	680	0.335	0.266	417	181	1	1	4.234[d]	11.97[e]

A test for randomness was applied to ER and ERES as explained in Material and methods ($n = 25$ and 19 for FP-17 and -22, respectively). ERES were identified as portions of ER profiles within a distance of 60 nm from 12-nm gold particle (Sec16 antibody). Membrane surface area was estimated stereologically by randomly superimposing a 100 × 100-nm grid on the micrographs and counting the number of intersections between membranes assigned to different compartments and the grid's lines.
[a]See Table S2.
[b]Number of gold particles significantly lower than expected value ($P = 0.0439$).
[c]Number of gold particles significantly higher than expected value ($P = 0.0007$).
[d]The distribution of gold particles significantly deviates from random ($P = 0.0396$).
[e]The distribution of gold particles significantly deviates from random ($P = 0.0005$).

Table S4. **Plasmids and antibodies used in this study**

	Source	**Additional information**
Plasmids		
VSVG-GFP	J. Lippincott-Schwartz (National Institutes of Health, Bethesda, MD)	Presley et al. (1997)[a]; temperature- sensitive version of VSVG under the cytomegalo virus promoter
VSVG-YFP	A. De Matteis (Mario Negri Sud, S. Maria Imbaro, Italy)	GFP in VSVG-GFP replaced with YFP
VSVG-mCerulean	Our laboratory	GFP in VSVG-GFP replaced with mCerulean
pSar1p^{dn}CMUIV	B. Storrie (University of Arkansas for Medical Sciences, Little Rock, AR)	Storrie et al. (1998)[b]; dominant-negative (H79G) Sar1 cDNA
Rtn4a-myc	M. Strittmatter (Yale University Medical School, New Haven, CT)	GrandPre et al. (2000)[c]; myc-tagged human Rtn4a under the cytomegalo virus promoter
Sec23A-EYFP	R. Pepperkok (European Molecular Biology Laboratory, Heidelberg, Germany)	Human Sec23A tagged with EYFP under the cytomegalo virus promoter (Forster et al., 2006)[d]
Antibodies		
Anti–ERGIC-53 mouse monoclonals	H.P. Hauri (Biozentrum, Basel, Switzerland)	Schweizer et al. (1988)[e]
Anti-Sec16 sheep polyclonals	D. Stephens (University of Bristol, Bristol, UK)	Watson et al. (2006)[f]
Anti–ribophorin I rabbit polyclonals	G. Kreibich (New York University Medical School, NY, NY)	Yu et al. (1990)[g]
Mouse monoclonal anti-myc	Santa Cruz Biotechnology, Inc.	
Anti-Sec23 rabbit polyclonals	Affinity BioReagents	
Anti-GFP rabbit polyclonals	AbCam	
Cy5-conjugated secondary antibodies	Jackson ImmunoResearch Laboratories	
Gold-conjugated anti–sheep and anti–rabbit antibodies	Jackson ImmunoResearch Laboratories	
Alexa 568–conjugated secondary antibodies	Invitrogen	

[a]Presley, J.F., N.B. Cole, T.A. Schroer, K. Hirschberg, K.J. Zaal, and J. Lippincott-Schwartz. 1997. *Nature.* 389:81–85.
[b]Storrie, B., J. White, S. Rottger, E.H. Stelzer, T. Suganuma, and T. Nilsson. 1998. *J. Cell Biol.* 143:1505–1521.
[c]GrandPre, T., F. Nakamura, T. Vartanian, and S.M. Strittmatter. 2000. *Nature.* 403:439–444.
[d]Forster, R., M. Weiss, T. Zimmermann, E.G. Reynaud, F. Verissimo, D.J. Stephens, and R. Pepperkok. 2006. *Curr. Biol.* 16:173–179.
[e]Schweizer, A., J.A.M. Fransen, T. Bachi, L. Ginsel, and H.-P. Hauri. 1988. *J. Cell Biol.* 107:1643–1653.
[f]Watson, P., A.K. Townley, P. Koka, K.J. Palmer, and D.J. Stephens. 2006. *Traffic.* 7:1678–1687.
[g]Yu, Y., D.D. Sabatini, and G. Kreibich. 1990. *J. Cell Biol.* 111:1335–1342.