Sikorska et al., http://www.jcb.org/cgi/content/full/jcb.201602010/DC1

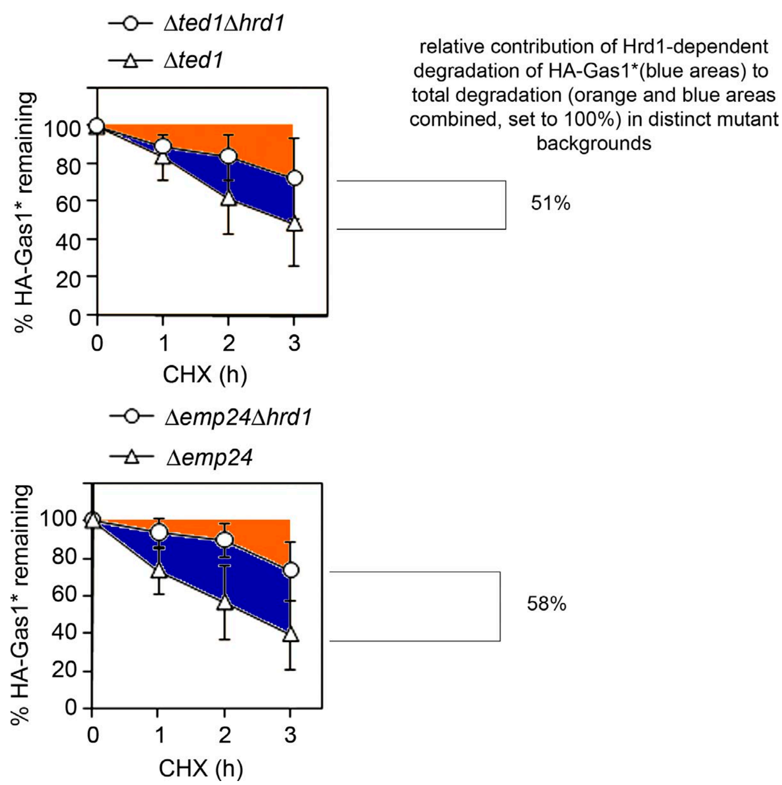

Figure S1. **ERAD is the major degradation pathway for HA-Gas1* in *Δted1* and *Δemp24* cells.** Graphical display of the degradation rates of HA-Gas1* in the indicated mutant strains from experiments described in Fig. 3 (E–G). The degradation rates of HA-Gas1* in the single and double mutants were determined by regression of linear trendlines. The degradation rate of HA-Gas1* in each individual single mutant was set to 100%. The extent of reduction in HA-Gas1* degradation in the double mutant compared with the single mutant is indicated in percent. Mean values and SDs from at least three individual experiments are shown.

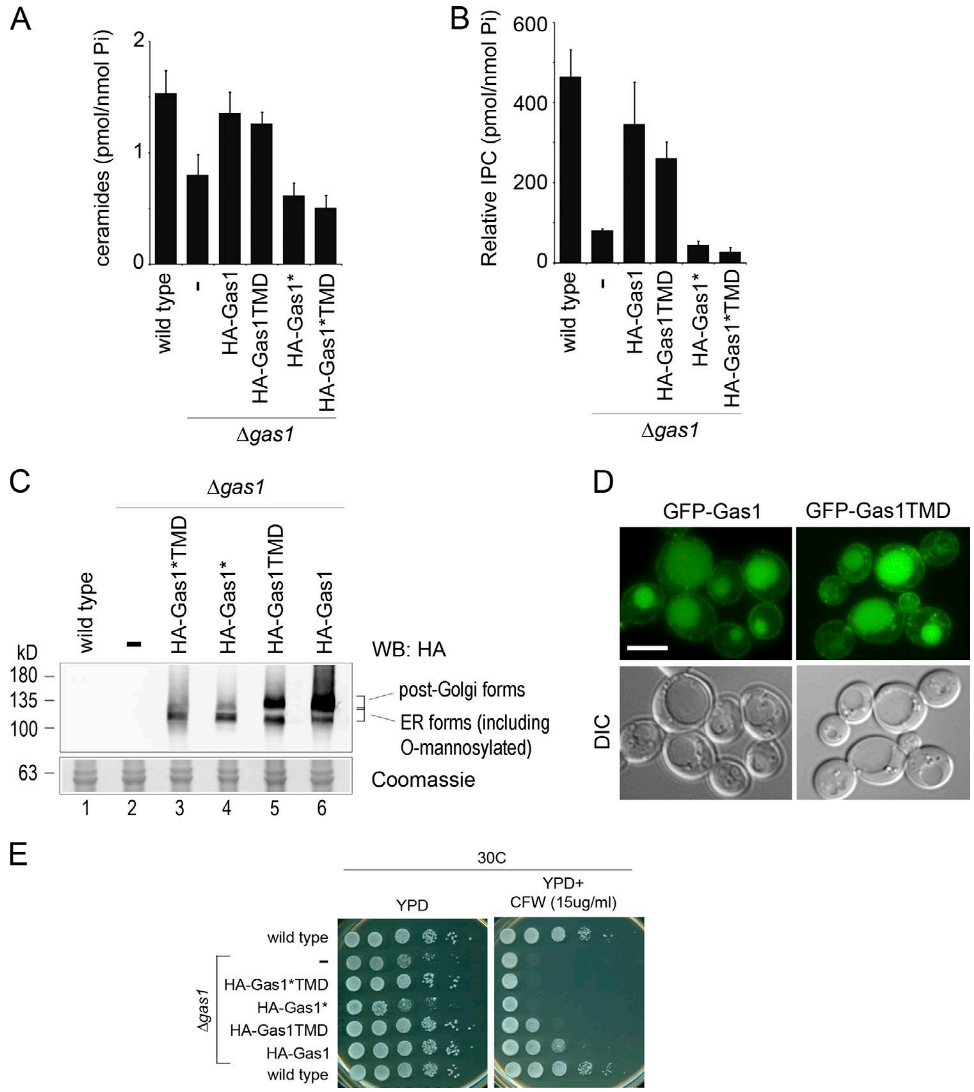

Figure S2. **Lack of evidence for a role of Gas1* in cellular ceramide cotransport.** (A) Ceramide profile of wild-type cells, *Δgas1* cells, and *Δgas1* cells expressing the indicated chromosomally integrated constructs. Three biological replicates were used for quantification, and mean values and SDs are indicated. *Δgas1* cells showed a drop in total cellular ceramide that could be partially reversed by expressing HA-Gas1 or HA-Gas1TMD but not HA-Gas1* or HA-Gas1*TMD. (B) Inositol phosphoceramide C (IPC) profiles of cells used in A. IPC levels were normalized to inorganic phosphate (see Materials and methods). Three biological replicates were used for quantification, and mean values and SDs are indicated. (C) Expression test for different Gas1 and Gas1* constructs in cells used in A and B. Exponentially growing cells were lysed and analyzed by SDS-PAGE and Western blotting (WB) with antibodies against HA. The lower part of the gel was separately stained with Coomassie as loading control. (D) Live cell fluorescence microscopy of wild-type cells expressing GFP-Gas1 and GFP-Gas1TMD. Both constructs are in part degraded inside the vacuole, likely because of tagging, in agreement with only partial complementation of cell wall defects (E). However, both constructs also show localization at the plasma membrane, demonstrating that Gas1TMD, despite lacking the GPI anchor, can be targeted to the plasma membrane, probably by bulk-flow mechanisms, explaining the observed post-Golgi glycosylation pattern of a fraction of the protein (C, lanes 5 and 6) and partial complementation of cell wall defects (E). DIC = Nomarski image. Bar, 3 µm. (E) Drop assay with cells used in A–C in the presence or absence of calcofluor white. Plates were imaged after 2 d (yeast extract peptone dextrose) or 4 d (yeast extract peptone dextrose plus calcofluor white). Gas1 can compensate in part for cell wall defects as well as ceramide and IPC alterations independent of the GPI anchor, suggesting that the observed changes in lipid profiles in *Δgas1* cells are not caused by a deficiency in (GPI-dependent) ceramide cotransport but rather by lack of Gas1 enzymatic activity. In any case, expression of Gas1* did not restore lipid profile to those found in wild-type cells.

Table S1. **Yeast strains used in this study**

Strain	Genotype	Source
VGY100	MATa; BY background	Laboratory strain
VGY302	MATx; BY background	Laboratory strain
VGY381	MATa; W303 background	Laboratory strain
VGY382	MATx; W303 background	Laboratory strain
VGY1204	MATa; W303 background, Δpep4::KanMX6, Δhrd1::HygrB, containing HA-Gas1* (pMF616, YIp, URA3)	This study
VGY1206	MATx; W303 background, Δpep4::KanMX6, containing HA-Gas1* (pMF616, YIp, URA3)	This study
VGY1242	MATa; W303 background, Δbst1::KanMX6	This study
VGY1403	MATa; W303 background, containing GFP-Gas1* (NSp19, YIp, URA3)	This study
VGY1487	MATa; W303 background, Δhrd1::HygrB, containing HA-Gas1*TMD (NSp23, YIp, URA3)	This study
VGY1489	MATa; W303 background, Δhrd1::HygrB, Δbst1::KanMX6, containing HA-Gas1* (pMF616, YIp, URA3)	This study
VGY1490	MATa; W303 background, containing HA-Gas1*TMD (NSp23, YIp, URA3)	This study
VGY1491	MATa; W303 background, Δemp24::HIS, containing Gas1*TMD (NSp23, YIp, URA3)	This study
VGY1492	MATa; W303 background, Δemp24::HIS, Δbst1::HygrB, containing HA-Gas1* (pMF616, YIp, URA3)	This study
VGY1737	MATa; W303 background, Δhrd1::HygrB, Δcwh43::HIS, containing HA-Gas1* (pMF616, YIp, URA3)	This study
VGY1746	MATa; W303 background, Δemp24::HygrB, Δcwh43::KanMX6, containing HA-Gas1* (pMF616, YIp, URA3)	This study
VGY2612	MATx; BY background, Δgas1::KanMX6	This study
VGY2617	MATx; BY background, Δgas1::HA-Gas1*TMD (URA3)	This study
VGY2620	MATx; BY background, Δgas1::HA-Gas1TMD (URA3)	This study
VGY2622	MATx; BY background, Δgas1::HA-Gas1 (URA3)	This study
VGY2626	MATx; BY background, Δgas1::HA-Gas1* (URA3)	This study
VGY2799	MATa; W303 background, Δcwh43::HIS, containing GFP-Gas1* (NSp19, YIp, URA3)	This study
VGY2894	MATa; W303 background, containing HA-Gas1*ΔTMD (VGp256, YIp, URA3)	This study
VGY2895	MATa; W303 background, Δhrd1::HygrB, containing HA-Gas1*ΔTMD (VGp256, YIp, URA3)	This study
VGY2897	MATa; W303 background, containing HA-CPY*GPI (clone392, CEN, TRP1)	This study
VGY2898	MATa; W303 background, Δhrd1::HygrB, containing HA-CPY*GPI (clone392, CEN, TRP1)	This study
VGY2900	MATa; W303 background, containing HA-CPY*TMD (LLp43, CEN, TRP1)	This study
VGY2901	MATa; W303 background, Δhrd1::HygrB, containing HA-CPY*TMD (LLp43, CEN, TRP1)	This study
VGY2925	MATx; W303 background, Δpep4::KanMX6, containing GFP-CPY*TMD (LLp45, CEN, TRP1)	This study
VGY2926	MATx; W303 background, Δpep4::KanMX6, containing GFP-CPY*GPI (LLp47, CEN, TRP1)	This study
VGY2934	MATa; W303 background, containing GFP-CPY*GPI (clone409, YIp, URA3)	This study
VGY2935	MATx; W303 background, Δpep4::KanMX6, containing GFP-CPY*GPI (clone409, YIp, URA3)	This study
VGY2936	MATa; W303 background, containing GFP-CPY*TMD (clone410, YIp, URA3)	This study
VGY2937	MATx; W303 background, Δpep4::KanMX6, containing GFP-CPY*TMD (clone410, YIp, URA3)	This study
LLY140	MATa; W303 background, containing GFP-Gas1 (LLp16, YIp, URA3)	This study
LLY143	MATa; W303 background, containing GFP-Gas1TMD (LLp17, YIp, URA3)	This study
LLY147	MATa; W303 background, containing GFP-Gas1*TMD (LLp18, YIp, URA3)	This study
LLY303	MATa; W303 background, containing GFP-CPY*TMD (LLp45, CEN, TRP1)	This study
LLY306	MATa; W303 background, containing GFP-CPY*GPI (LLp47, CEN, TRP1)	This study
NY283	MATa; W303 background, containing HA-Gas1* (pMF616, YIp, URA3)	This study
NY285	MATx; W303 background, Δted1::HIS, containing HA-Gas1* (pMF616, YIp, URA3)	This study
NY286	MATa; W303 background, Δbst1::KanMX6, containing HA-Gas1* (pMF616, YIp, URA3)	This study
NY287	MATa; W303 background, Δgup1::KanMX6, containing HA-Gas1* (pMF616, YIp, URA3)	This study
NY289	MATa; W303 background, Δcwh43::KanMX6, containing HA-Gas1* (pMF616, YIp, URA3)	This study
NY290	MATa; W303 background, Δemp24::HygrB, containing HA-Gas1* (pMF616, YIp, URA3)	This study
NY297	MATa; W303 background, Δhrd1::HygrB, containing HA-Gas1* (pMF616, YIp, URA3)	This study
NY302	MATa; W303 background, Δhrd1::HygrB, Δted1::HIS, containing HA-Gas1* (pMF616, YIp, URA3)	This study
NY381	MATa; W303 background, Δhrd1::HygrB, Δemp24::HIS, containing HA-Gas1* (pMF616, YIp, URA3)	This study
NY428	MATa; W303 background, Δted1::HIS, containing GFP-Gas1* (NSp19, YIp, URA3)	This study
NY430	MATa; W303 background, Δbst1::KanMX6, containing GFP-Gas1* (NSp19, YIp, URA3)	This study
NY443	MATx; W303 background, EMP24-TAP(HIS3), containing HA-Gas1* (pMF616, YIp, URA3)	This study
NY444	MATx; W303 background, EMP24-TAP(HIS3), Δted1::HIS, containing HA-Gas1* (pMF616, YIp, URA3)	This study
NY472	MATa; W303 background, containing HA-Gas1 (NSp17, YIp, URA3)	This study
NY474	MATa; W303 background, containing HA-Gas1TMD (NSp20, YIp, URA3)	This study
NY476	MATa; W303 background, containing HA-Gas1*TMD (NSp23, YIp, URA3)	This study
NY478	MATx; W303 background, EMP24-TAP(HIS3), containing HA-Gas1 (NSp17, YIp, URA3)	This study
NY480	MATx; W303 background, EMP24-TAP(HIS3), containing HA-Gas1TMD (NSp20, YIp, URA3)	This study
NY482	MATx; W303 background, EMP24-TAP(HIS3), containing HA-Gas1*TMD (NSp23, YIp, URA3)	This study
NY567	MATa; W303 background, Δhrd1::HygrB, containing GFP-Gas1* (NSp19, YIp, URA3)	This study
NY568	MATa; W303 background, Δemp24::HIS, containing GFP-Gas1* (NSp19, YIp, URA3)	This study

Chromosomal tagging of proteins and gene deletions and integrations were performed using PCR (if needed) in combination with standard homologous recombination techniques.