Vacaru et al., http://www.jcb.org/cgi/content/full/jcb.200903152/DC1

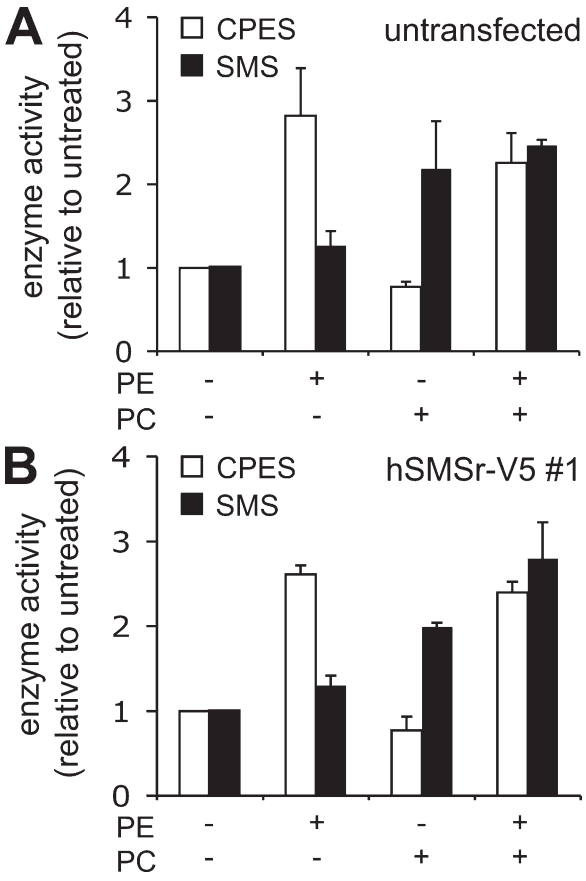

Figure S1. **Effect of externally added phospholipid on CPE production levels in HeLa cell lysates.** (A) Levels of CPES and SMS activity were determined by TLC analysis of reaction products formed when lysates of HeLa cells were incubated with NBD-Cer in the presence or absence of externally added PE or PC. (B) As described in A, except that incubations were with lysates of hSMSr-overexpressing HeLa cells (hSMSr-V5 #1), which have a sixfold higher level of CPES activity than untransfected cells. Enzyme activity levels were expressed relative to control (no phospholipid added). Error bars indicate range, $n = 2$.

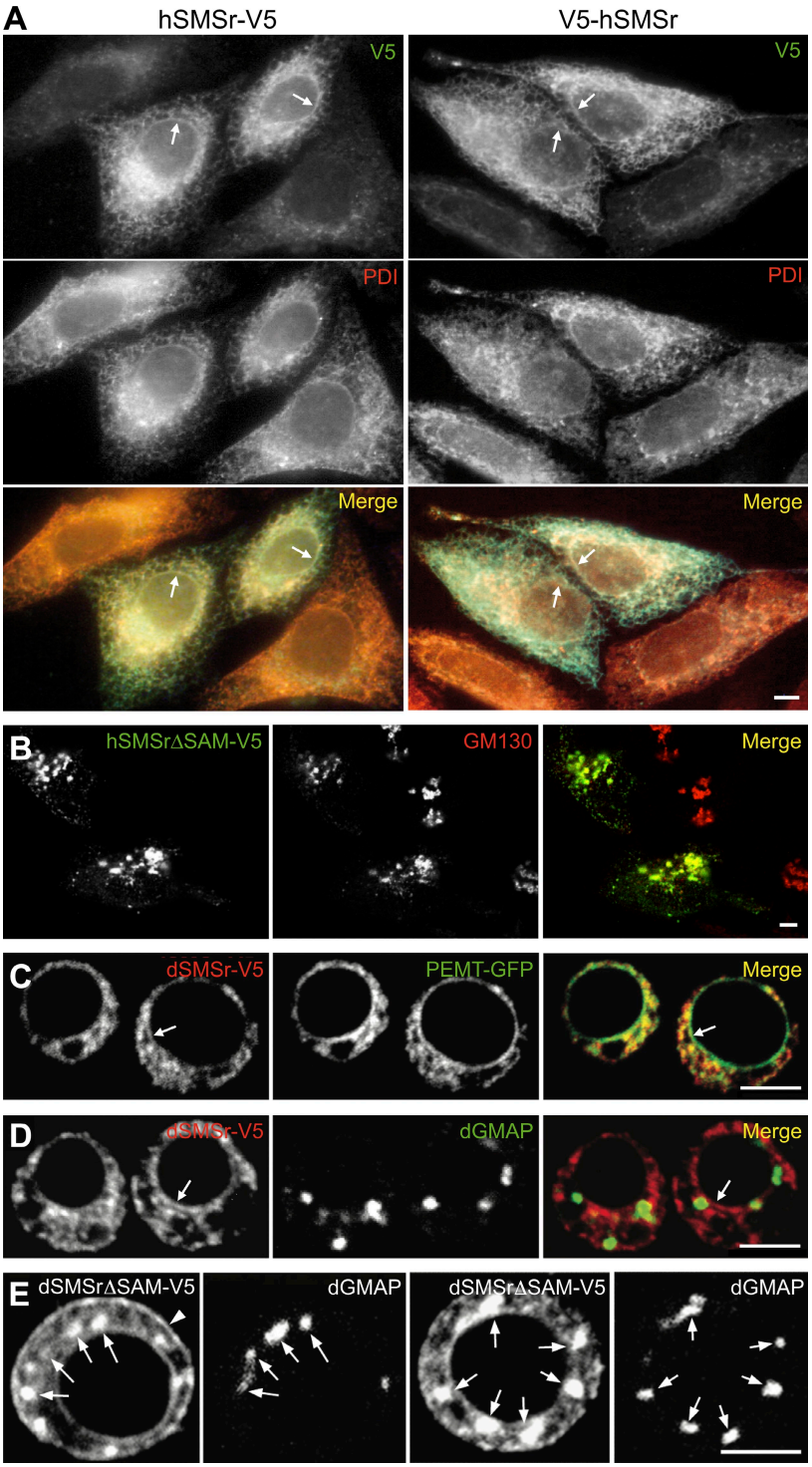

Figure S2. **The SAM domain containing N terminus of SMSr is required for ER retention.** (A) HeLa cells expressing hSMSr tagged with a V5 epitope at either the carboxy (hSMSr-V5) or amino terminus (V5-hSMSr) were double labeled for V5 and the ER marker protein disulfide isomerase (PDI) and visualized by conventional immunofluorescence microscopy. Note the extensive colocalization between both versions of hSMSr and protein disulfide isomerase, which is also exemplified by the nuclear envelope staining. (B) Confocal sections of HeLa cells expressing an N-terminal hSMSr-V5 truncation mutant lacking the SAM domain and double labeled for V5 and medial Golgi marker GM130. Note that SAM-deficient hSMSr fails to be retained in the ER and localizes to the Golgi. (C) Confocal sections of *Drosophila* S2 cells double transfected with V5-tagged dSMSr and the ER marker PEMT-GFP and immunostained for V5. (D) Confocal sections of dSMSr-V5–expressing S2 cells double labeled for V5 and the cis-Golgi marker dGMAP. (E) Confocal sections of S2 cells expressing an N-terminal dSMSr-V5 truncation mutant lacking the SAM domain and double labeled for V5 and cis-Golgi marker dGMAP. Note that, as for hSMSr, SAM-deficient dSMSr fails to be retained in the ER and localizes to the Golgi (arrows) and PM (arrowhead). (A, C, and D) Arrows indicate nuclear envelope staining. Bars, 5 µm.

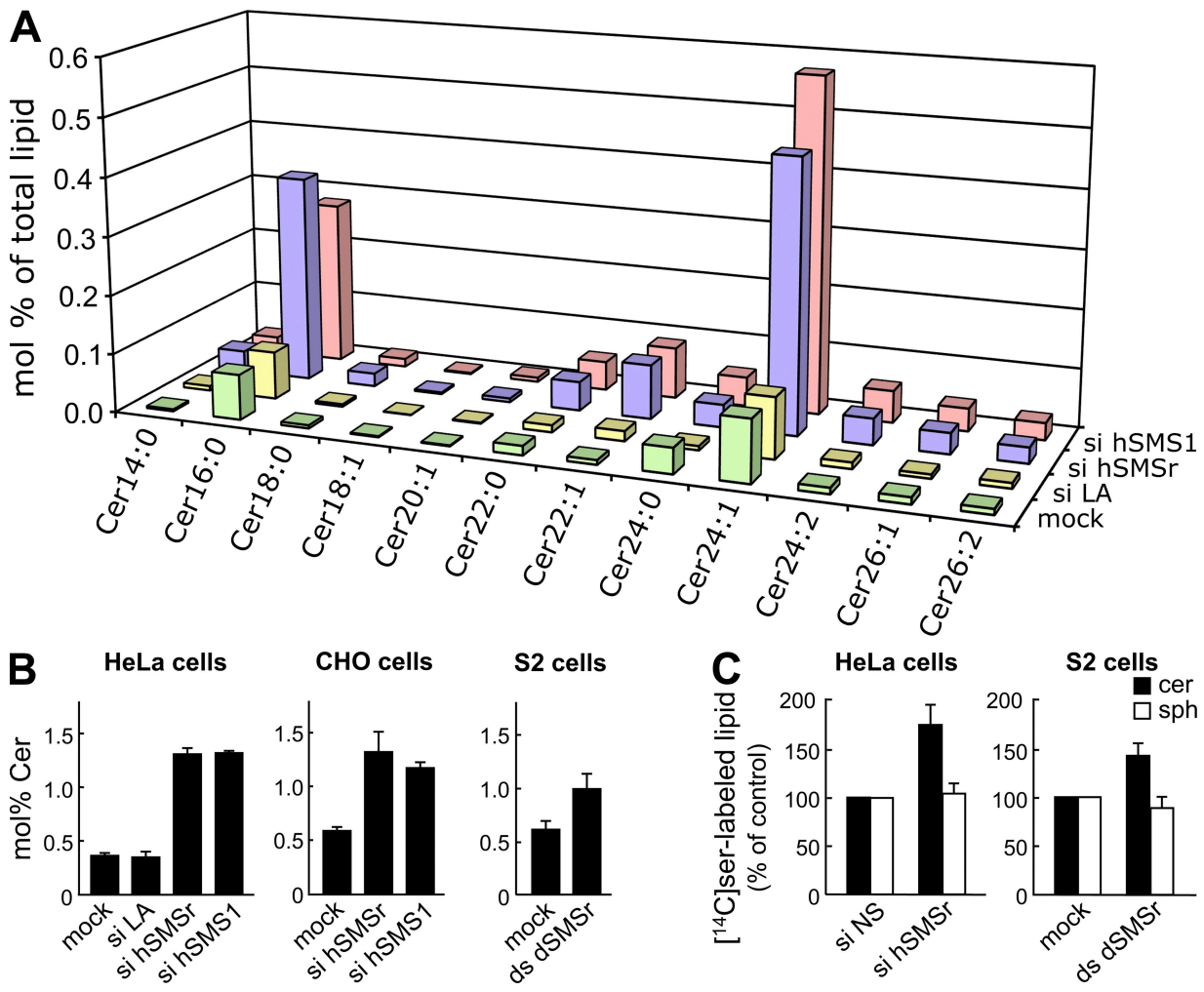

Figure S3. **SMSr-depleted cells accumulate all major species of ceramide but not sphingosine.** (A) HeLa cells were treated for 3 d with si LA, hSMSr (si hSMSr #2), or hSMS1 (si hSMS1), subjected to lipid extraction, and analyzed by MS/MS as described in Materials and methods. Levels of ceramide species are given in mole percentages of total lipids analyzed. Data shown are representative of three independent experiments (Table S1). (B) MS/MS quantification of ceramides in lipid extracts of mock-treated, LA-depleted, SMSr-depleted, or SMS1-depleted cells (HeLa, CHO-K1, and S2). Error bars indicate SD, $n = 3$. (C) Control (si nonsilencing [si NS] treated or mock treated) and SMSr-depleted cells were labeled with [^{14}C]serine for 5 (S2) or 8 h (HeLa). Levels of radiolabeled ceramides (cer) and sphingoid bases (sph) were determined by TLC and autoradiography and expressed as a percentage of control. siRNA treatment (HeLa and CHO) was for 3 d, whereas dsRNA treatment (S2) was for 7 d. SMSr depletions were with si hSMSr #2 or ds dSMSr #2. Error bars indicate range, $n = 2$.

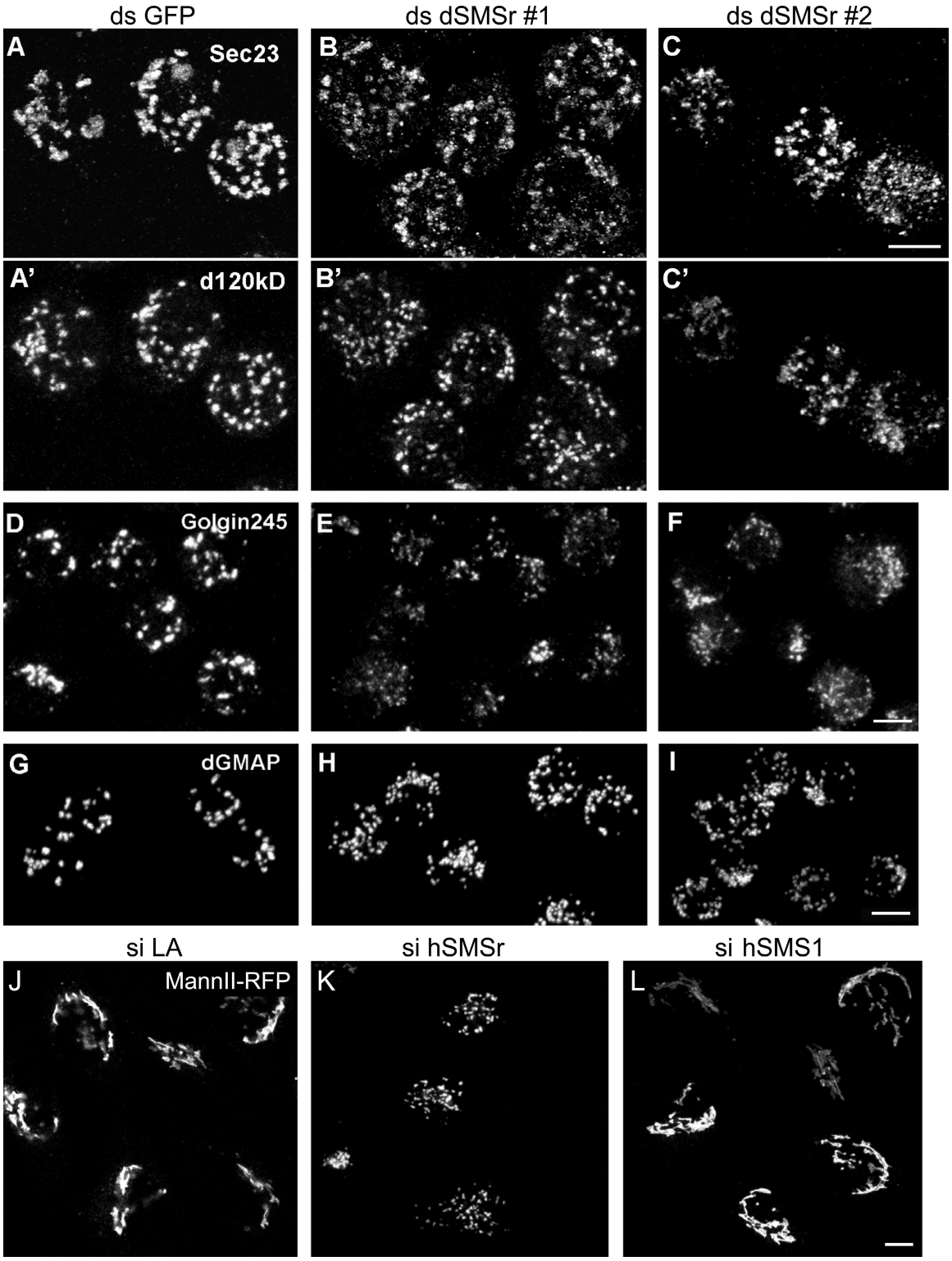

Figure S4. **SMSr depletion causes the disorganization of tER–Golgi units.** (A–C′) S2 cells treated for 7 d (see Materials and methods) with ds GFP or dSMSr (ds dSMSr #1 or #2) were double labeled for dSec23 (tER sites) and d120kd (Golgi stacks). Confocal projections of the single channels corresponding to Fig. 6 A are presented. (D–I) Confocal projections of S2 cells treated as in A–C′ and labeled for two additional Golgi markers, Golgin 245 and dGMAP. Note the disorganization of the tER–Golgi units in dSMSr-depleted cells, which is characterized by more numerous and smaller Golgi spots and a hazy staining pattern. (J–L) HeLa cells stably expressing mannosidase II–RFP were treated with si LA, hSMSr (si hSMSr #2), or hSMS1 (si hSMS1) for 3 d. Confocal projections of the single channels corresponding to Fig. 6 G are presented. Bars, 5 µm.

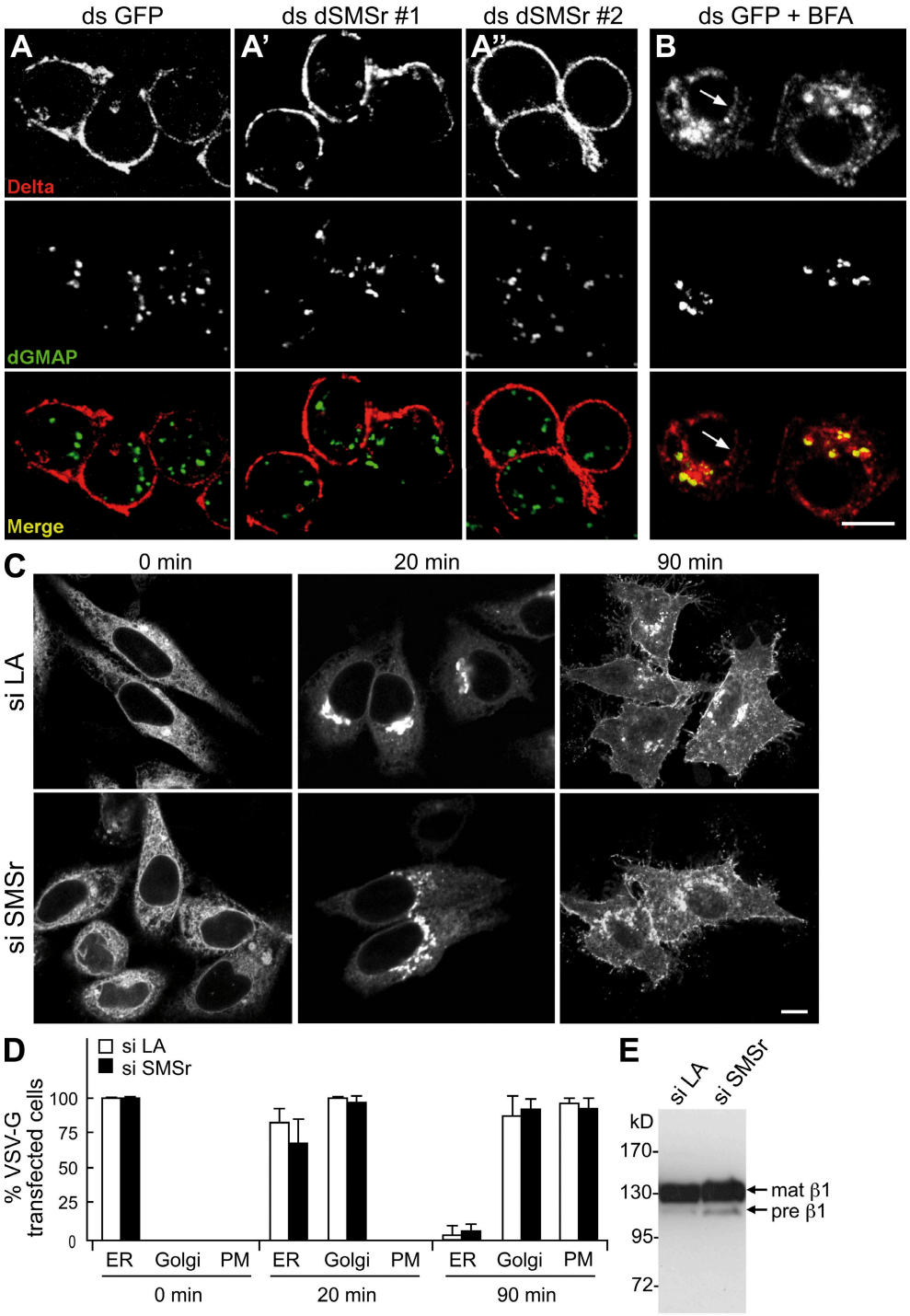

Figure S5. **Anterograde protein transport to the PM is not impaired in SMSr-depleted cells.** (A–B) Delta S2 cells were treated for 7 d with ds GFP (A), ds dSMSr #1 (A'), or ds dSMSr #2 (A'') and induced for 1 h with $CuSO_4$ followed by 90-min chase in the presence of cycloheximide in the absence (A–A'') or presence of BFA (B). The cells were double labeled for Delta and dGMAP. In contrast to BFA-treated cells, in which Delta is blocked in the ER (nuclear envelope staining marked by an arrow) and tER sites (partial colocalization with dGMAP), dSMSr depletion does not impair anterograde transport to the PM. Confocal sections are presented. (C) HeLa cells were treated with si LA or hSMSr (si hSMSr #2) for 7 d. After 6 d of treatment, cells were transfected with a plasmid-encoding GFP-tagged VSV-G ts045 protein. VSV-G ts045 was arrested in the ER at 39.5°C and chased out at 31.5°C. Confocal images of cells fixed after 0, 20, and 90 min of chase are shown. Bars, 5 µm. (D) Quantification of cells treated as in C and expressing VSV-G in a particular location (ER, Golgi, and PM). For each experimental condition, >50 VSV-G–positive cells were analyzed. Note that hSMSr depletion does not impair anterograde transport of VSV-G. (E) HeLa cells were treated with si LA or si hSMSr #2 for 7 d and subjected to immunoblot analysis using an antibody against β1-integrin. Note that hSMSr depletion does not significantly alter the ratio between fully glycosylated mature β1-integrin (mat β1) and its partially glycosylated precursor (pre β1). Error bars indicate SD, $n = 3$.

Table S1. **Levels of ceramide species in control and SMS-depleted HeLa cells**

Cer species	Mole percentage of total lipid analyzed			
	Mock	**si LA**	**si hSMSr**	**si hSMS1**
Cer14:0	0.003 ± 0.001	0.008 ± 0.003	0.035 ± 0.016	0.027 ± 0.005
Cer16:0	0.077 ± 0.002	0.081 ± 0.002	0.353 ± 0.121	0.279 ± 0.009
Cer18:0	0.005 ± 0.000	0.004 ± 0.000	0.022 ± 0.007	0.014 ± 0.003
Cer18:1	0.002 ± 0.001	0.000 ± 0.000	0.003 ± 0.001	0.002 ± 0.000
Cer20:1	0.000 ± 0.000	0.000 ± 0.000	0.006 ± 0.003	0.007 ± 0.000
Cer22:0	0.016 ± 0.000	0.012 ± 0.000	0.049 ± 0.021	0.049 ± 0.001
Cer22:1	0.008 ± 0.002	0.017 ± 0.001	0.092 ± 0.025	0.087 ± 0.005
Cer24:0	0.043 ± 0.006	0.007 ± 0.001	0.039 ± 0.020	0.049 ± 0.003
Cer24:1	0.105 ± 0.008	0.103 ± 0.004	0.464 ± 0.079	0.572 ± 0.030
Cer24:2	0.010 ± 0.001	0.011 ± 0.000	0.045 ± 0.009	0.054 ± 0.002
Cer26:1	0.011 ± 0.002	0.007 ± 0.000	0.036 ± 0.008	0.039 ± 0.001
Cer26:2	0.010 ± 0.000	0.009 ± 0.000	0.027 ± 0.006	0.029 ± 0.004
Sum	0.292 ± 0.015	0.260 ± 0.008	1.170 ± 0.030	1.206 ± 0.010

Cer, ceramide. HeLa cells were treated for 3 d with si LA, si hSMSr #1, or si hSMS1, subjected to lipid extraction, and analyzed by MS/MS as described in Materials and methods. Levels of ceramide species are given in mole percentages of total lipid analyzed. Data shown are means ± SD; $n = 3$.