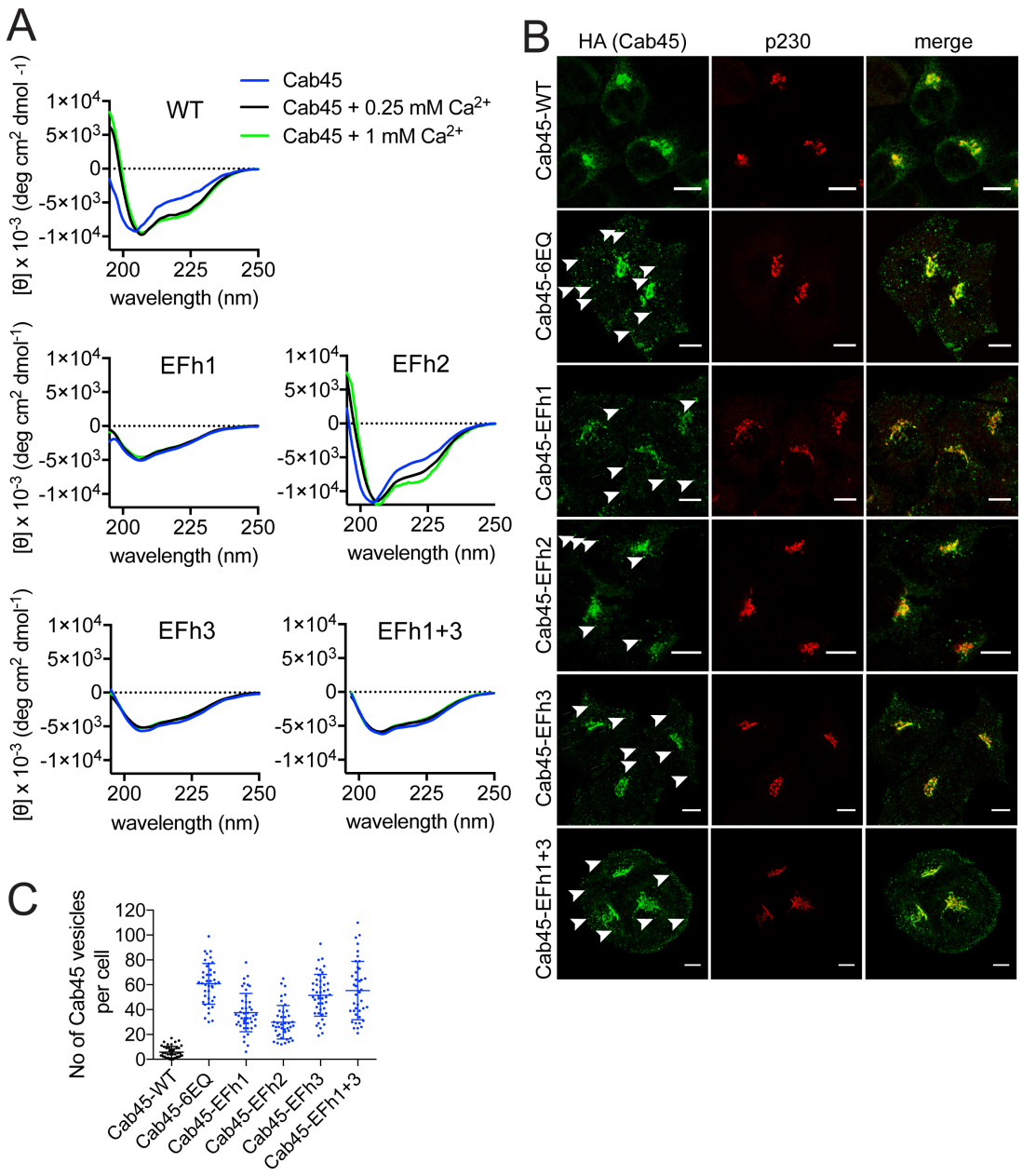

Figure S1. Cab45 EFh mutant expression shows vesicular localization, Related to Figure 2. (A) Far-UV CD analysis of recombinant Cab45 and the Cab45-EFh1, Cab45-EFh2, Cab45-EFh3 and Cab45-EFh1+3 mutants in the presence and absence of 0.25 mM or 1 mM Ca^{2+}. **(B)** HeLa Cab45-KO cells were stably transfected with either Cab45-WT or the mutants Cab45-6EQ, Cab45-EFh1, Cab45-EFh2, Cab45-EFh3 and Cab45-EFh1+3. Cells were visualized by anti-HA for Cab45 (green) and anti-p230 as a TGN marker (red)

and analyzed by confocal microscopy. Arrowheads point to cytoplasmic vesicles. Bars, 10 μm. **(C)** The number of Cab45 vesicles per cell was quantified for Cab45-KO cells stably expressing Cab45-WT, and the mutants Cab45-6EQ, Cab45-EFh1, Cab45-EFh2, Cab45-EFh3 and Cab45-EFh1+3.

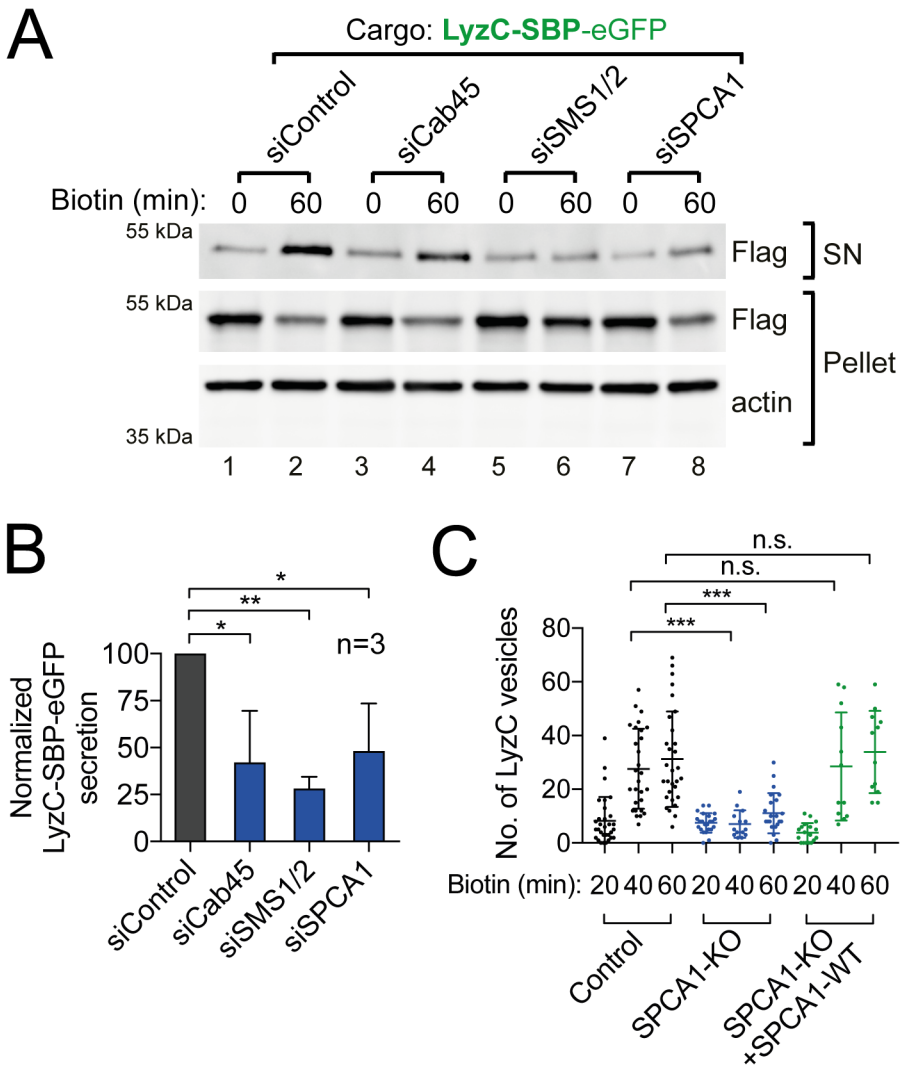

Figure S2. Depletion of SPCA1 impairs LyzC secretion and cargo sorting, Related to Figure 3 and Figure 4. **(A)** Depletion of SMS1/2, and SPCA1, cause retention of LyzC-SBP-eGFP within cells. HeLa cells transfected with control, Cab45, SMS1/2 and SPCA1 siRNA were transfected with LyzC-SBP-eGFP. Cell culture supernatants and whole cell lysates of cells were collected after 60 min incubation with Biotin and probed for FLAG epitope tagged LyzC-SBP-eGFP by immunoblotting. Actin was detected in the lysates as a loading control. **(B)** Semi-quantitative analysis of LyzC secretion by normalizing LyzC supernatant signals to their respective actin loading control. LyzC secretion was then determined by the ratio of 60 min to 0 min

samples after Biotin addition and normalized to control siRNA treated cells. The means (± s.d.) from 3 independent experiments are plotted. **(C)** The number of LyzC vesicles was quantified in HeLa control or SPCA1 null cells expressing either LyzC-SBP-eGFP alone, or co-transfected with SPCA1-WT. Vesicle counts (mean ± s.d.) from at least 12 cells per condition in 3 independent experiments are plotted.

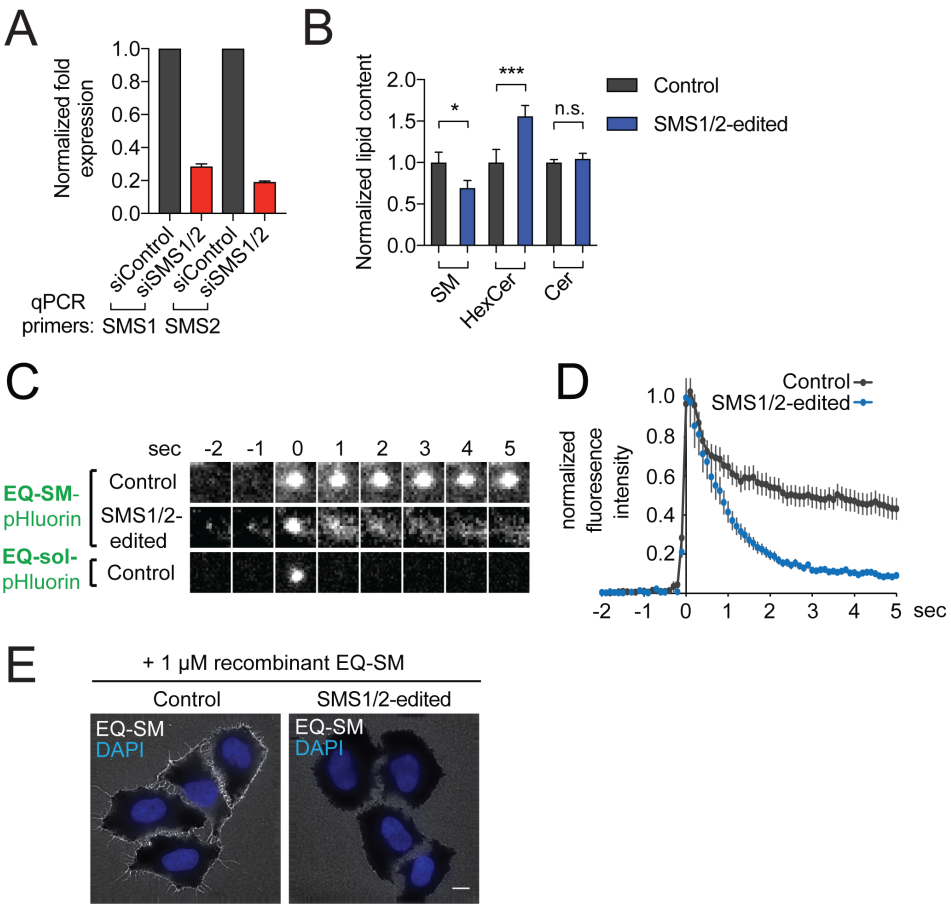

Figure S3. Characterization of SM depleted cells lines, Related to Figure 4. (A) Quantitative real-time PCR of cells treated with SMS1/2 siRNA confirmed a reduction of mRNA expression of SMS1 and SMS2 to 26.8% and 19% compared to cells treated with control siRNA. Samples were normalized to the housekeeping gene GAPDH and the data was presented as relative expression fold change normalized to control cells. A representative experiment with the means (s.e.m.) of technical triplicates is shown. **(B)** Sphingolipid analysis of SMS1 and SMS2 depleted cells. The relative amounts of the indicated sphingolipid species are shown. The values are the means (s.e.m.) of three replicate measurements. $P<0.05$ for SM, $P<0.01$ for HexCer. **(C)** Time-lapse TIRF micrographs of HeLa control or SMS1/2-edited cells expressing either EQ-SM-pHluorin or EQ-sol-pHluorin. Note that EQ-SM-

pHluorin remains bound to the plasma membrane at the site of exocytosis in unmodified control cells, but it dissipates from the site of exocytosis in SMS1/2-edited HeLa cells. A time-lapse gallery of micrographs showing dissipation of EQ-sol-pHluorin after exocytosis is shown for comparison. **(D)** Fluorescence intensity profiles of EQ-SM-pHluorin exocytosis. The normalized mean fluorescence intensities (s.e.m.) of 34 and 57 exocytic events (control and SMS1/2-edited, respectively) in TIRF micrographs are plotted. Fluorescence values were normalized to the peak intensity observed for each series. **(E)** HeLa control and SMS1/2-edited cells were incubated with 1 μM recombinant EQ-SM to probe SM at the plasma membrane. EQ-SM containing a FLAG epitope was visualized by immunofluorescence of anti-FLAG (white) and DAPI to label the nucleus. Bars, 10 μm.

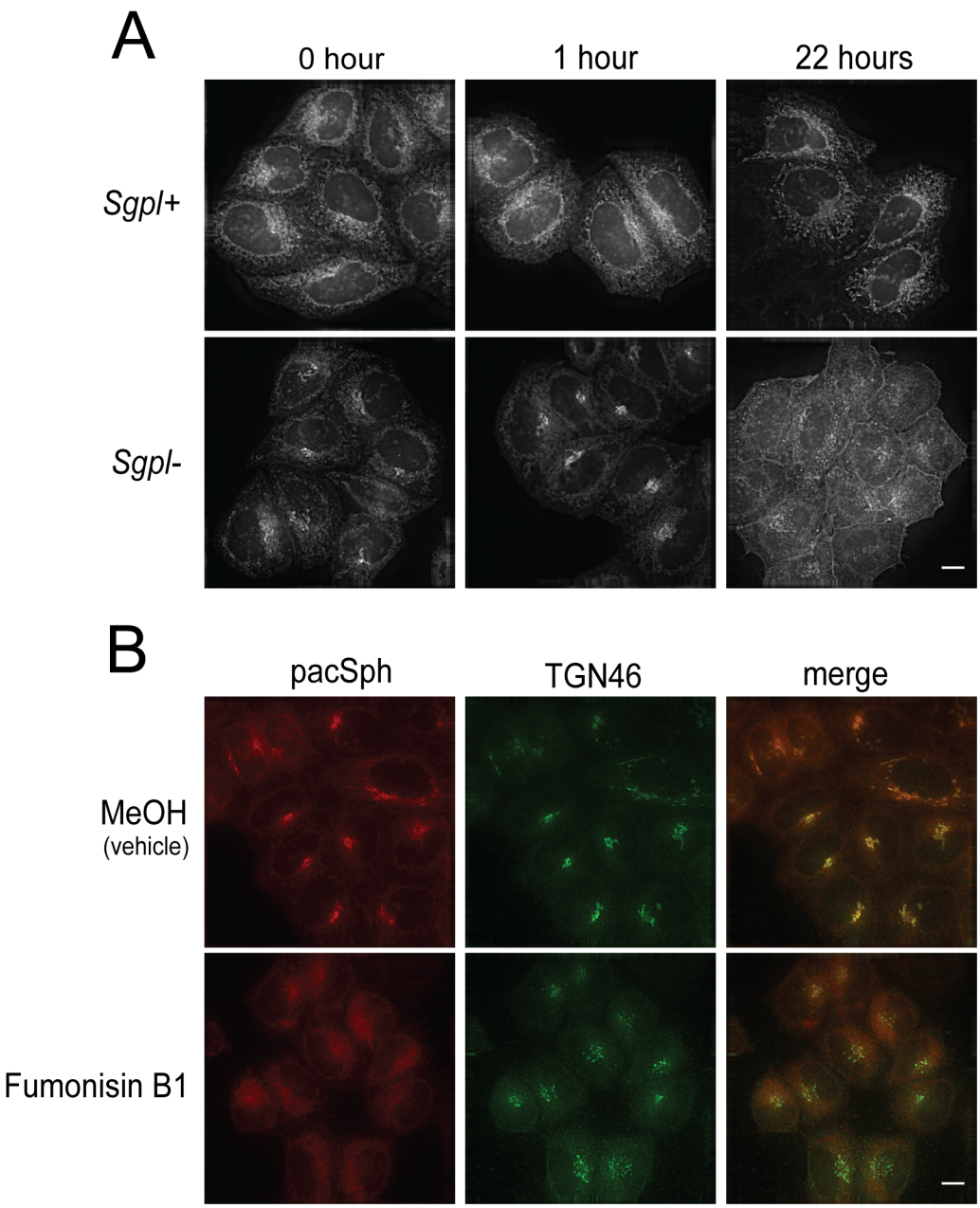

Figure S4. Pac-sphingosine-labeled sphingolipids are trafficked to the Golgi apparatus and then the plasma membrane, Related to Figure 5. (A) Time course of pacSph labeling of control (*SGPL1*+) and *SGPL1* null (*SGPL1*-) cells. *SGPL1*- HeLa cells and control parental cells (*SGPL1*+) were incubated with 0.6 μM pacSph for 30 minutes, followed by a chase period of 1 or 22 hours in medium lacking pacSph. Fixed, permeabilized cells were incubated with click chemistry reagents to covalently attached Alexa647

fluorophore to pacSph. Cells were visualized by deconvolution fluorescence microscopy. Bars, 10 μm. Note that pacSph-labeled sphingolipids are trafficked to the Golgi apparatus and then the plasma membrane. **(B)** An inhibitor of ceramide synthase, fumonisin B1 (FB1), prevents targeting of pacSph to the Golgi apparatus. *SGPL1*- HeLa cells were pre-incubated with FB1 (50 μM) for 24 hours, followed by labeling of cells with 0.6 μM pacSph. Cells were processed to label pacSph with Alexa647 and for immunofluorescence detection of TGN46 to identify the Golgi apparatus. Scale bars, 10 μm.

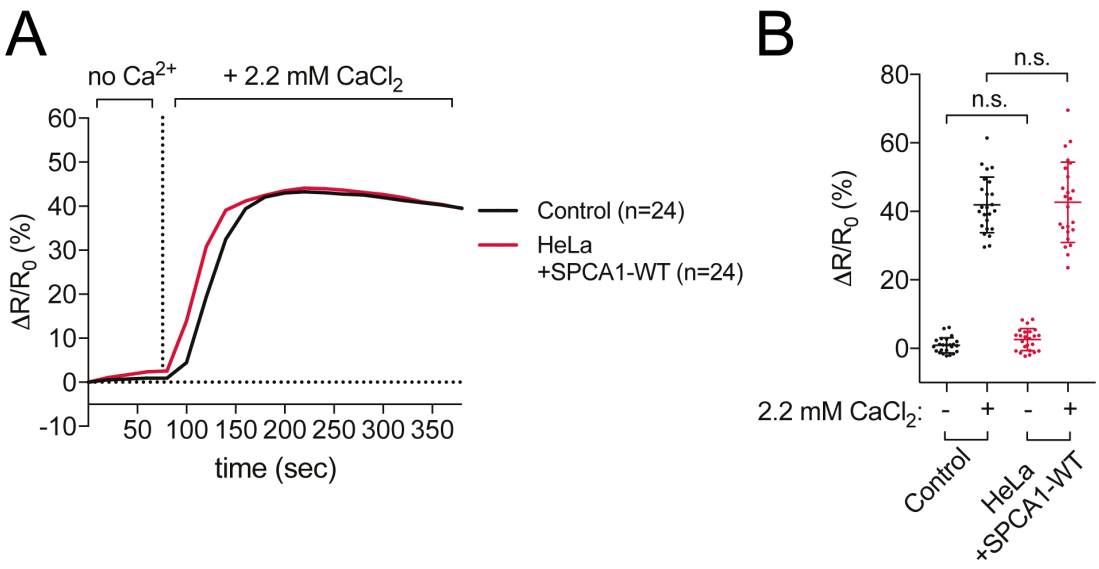

Figure S5. Overexpression of SPCA1 does not alter Ca^{2+} influx, Related to Figure 6. (A) Quantification of FRET images of HeLa control cells and cells that overexpress SPCA1-WT. Fluorescence signals reflecting TGN [Ca^{2+}] are presented as $\Delta R/R_0$. Data are plotted as the mean Ca^{2+} influx over time. **(B)** Data are plotted as the mean ± s.d. Ca^{2+} influx at before Ca^{2+} addition (at 80 sec) or after Ca^{2+} addition (at 300 sec). Data was acquired for at least 24 cells per condition in two independent experiments.

object 1	object 2	R =	n
SPCA1	GM130	0.58 ± 0.07	37
	p230	0.54 ± 0.08	38
	SMS1	0.76 ± 0.06	75
SMS1	GM130	0.33 ± 0.06	37
	p230	0.62 ± 0.09	38
pacSph	GM130	0.49 ± 0.08	32
	p230	0.76 ± 0.06	22

Table S2. Pearson's correlation analysis for Golgi residents, Related to Figure 5. The mean Pearson's correlation coefficients (R) (± s.d) were determined for the indicated pairs of proteins or pacSph-labeled lipids. The number of cells analyzed for each condition is indicated (n).