

LEGENDS TO SUPPLEMENTAL FIGURES

Supplemental Figure 1. NONOate reduces the effects of MCTP and c-PTIO on C5-ceramide uptake and localization in the Golgi. PAEC cultures were treated with MCTP or c-PTIO for 4 days as indicated in the legend to Fig. 1 together with the inclusion of NONOate (400 μ M) in the culture medium. Panel A: phase-contrast images. Panel B: BODIPY TR C5-ceramide uptake (loading for 30 min at 37°C in DMEM followed by washout and imaging in serum-containing medium at 37°C over the next 10-15 min). Scale bars = 50 μ m.

Supplemental Figure 2. NONOate reduces the effects of MCTP and c-PTIO on Golgi enlargement and dispersal. In an experiment similar to that in Supplemental Fig. 2 the cultures were fixed, permeabilized and immunostained for giantin and then for DAPI (Panel A; scale bar = 50 μ m). The cell images obtained were enumerated for Golgi enlargement/dispersal (mean % of cells per image with enlarged/dispersed Golgi \pm SE; n = 6 or more 40X epifluorescence images per variable)(Panel B). *, $P < 0.05$ in comparisons with the untreated control group, and #, $P < 0.05$ in comparison with the respective group not treated simultaneously with NONOate.

Supplemental Figure 3. Functional retrograde uptake and internalization is inhibited after MCTP and c-PTIO. Respective fluorescent ligand uptake and internalization assays were carried out in PAEC cultures 4 days after commencement of the MCTP or c-PTIO treatment using procedures summarized in Materials and Methods with variations as indicated in the figure. Multiple epifluorescence images (40X objective, n = 4-6 per variable) were collected in each experiment. Panels A-E illustrate the full 40X objective image frames corresponding to those in Fig. 5. Scale bars = 50 μ m.