Figure S2  The lysosomes and Golgi/TGN system are not involved in disulfide bond reduction of the folate-FRET reporter. Panel A: Neither the folate-FRET reporter nor its reduced fragments localize in lysosomes. KB cells were treated with LysoSensor Yellow/Blue DND-160 (1 µM) and the folate-FRET reporter (100 nM) for 45 min. After washing and further incubation for 12 h to allow reduction of the folate-FRET disulfide bond to reach completion, cells were imaged for BODIPY, rhodamine and LysoSensor blue fluorescence using the proper excitation and emission settings (488/520 nm, 545/600 nm and 363/520 nm, respectively). Panel B: Effect of brefeldin A (BFA) on disulfide reduction of the folate-FRET conjugate. KB cells were first treated with BFA (2.5 ug/ml) for 1 h followed by 0.5 h incubation with the folate-FRET (100 nM). After washing cells with fresh medium to remove unbound folate-FRET, BFA was added back and remained present throughout the entire observation period. Cells were incubated for another 12 h before imaging for BODIPY, FRET and rhodamine fluorescence (488/520 nm, 488/600 nm, 545/600 nm, respectively). Panel C: Folate-FRET conjugate does not traffic to the Golgi/TGN system. KB cells were treated with a thioether (nonreducible) analog of the folate-FRET conjugate for 30 min. After washing and further incubation for 2 h, cells were stained with BODIPY FL C₅-ceramide complexed to BSA (5 ug/ml) according to the manufacturer’s instructions. Cells were finally imaged for BODIPY and FRET-induced fluorescence (488/520 nm and 488/600 nm, respectively). Virtually the same distribution pattern was seen using a simpler folate conjugate, namely folate-rhodamine. Note the lack of coincident labeling in the overlay panel on the right. Bar = 10 µm.