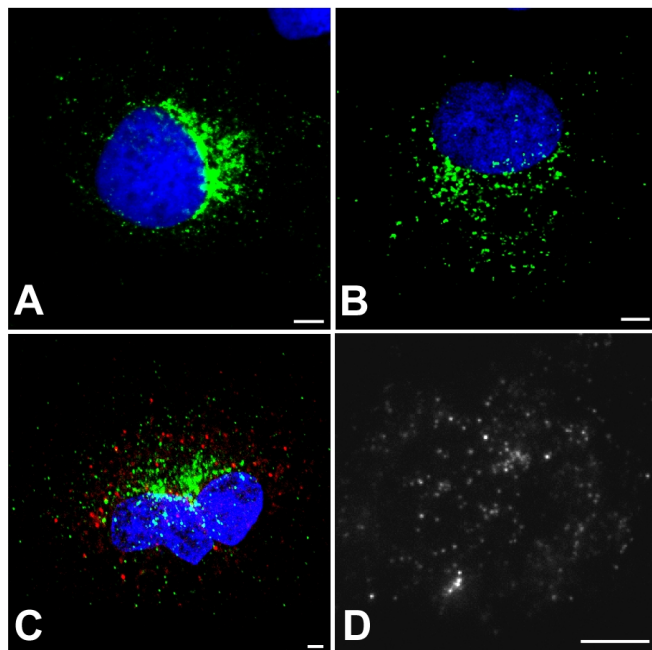
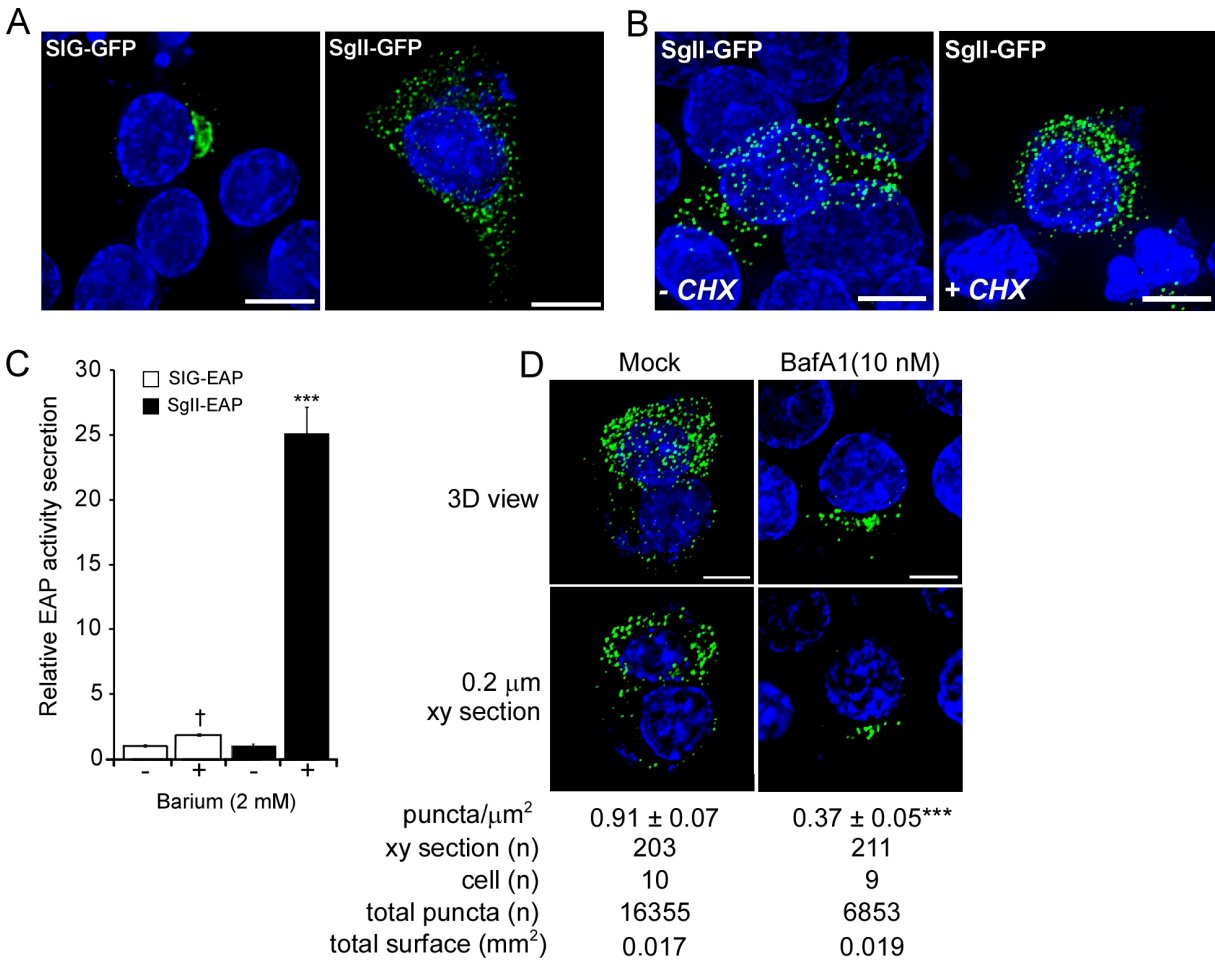


SUPPLEMENTARY FIGURES



Supplementary Figure 1

Figure S1. Subcellular distribution of SIG-GFP and SgII-GFP expressed in non-neuroendocrine COS-7 cells. COS-7 transiently transfected (48 h) with expression plasmids encoding SIG-GFP (**A**) or SgII-GFP (**B**) were processed for photoprotein fluorescence and 3D imaging by confocal microscopy. Shown are the 3D views of 9 to 13 optical xy sections acquired along the z axis. Nuclei were visualized with Hoechst 33342 (blue). (**C**) COS-7 expressing SgII-GFP were processed for immunocytochemistry using a polyclonal rabbit anti-LGP110 and secondary Alexa Fluor 594-conjugated goat anti-rabbit antibodies (red). (**D**) TIRFM image of a characteristic COS-7 cell expressing SgII-GFP. Scale bars: 5 μm .



Supplementary Figure 2

Figure S2. Trafficking of SgII fusion proteins in PC12 cells. (A) Subcellular distribution of SIG-GFP and SgII-GFP. Cells were transfected for 48 h with expression plasmids encoding SIG-GFP or SgII-GFP and examined by deconvolution microscopy. Shown are the 3D views of 9 to 13 optical xy sections acquired along the z axis. Nuclei were visualized with Hoechst 33342 (blue). Scale bar, 5 μm. (B) PC12 cells expressing SgII-GFP were exposed for 3 h to either DMSO (- CHX) or cycloheximide (+ CHX; 10 g/mL) before processing for photoprotein fluorescence and imaging. (C) Chemiluminescence detection of secretagogue-evoked secretion of EAP chimeras. Cells transiently expressing SIG-EAP, or SgII-EAP were exposed for 15 min to Calcium Saline buffer (mock) or to 2 mM Barium (Ba²⁺). EAP secretion was assayed as described in experimental procedures section. Release of EAP is expressed relative to enzymatic activity release in the absence of secretagogue. Values are given as the means ± SEM. of triplicate determinations. †, p > 0.05; ***, p < 0.001 as compared with control (mock), ANOVA with Dunnett's post test. (D) Effect of the vacuolar H⁺-V-ATPase inhibitor Bafilomycin A1 on the subcellular distribution of SgII-GFP in PC12 cells. PC12 transfected with SgII-GFP and exposed (22 h) to mock (DMSO) or BafA1 (10 nM) were analyzed by deconvolution microscopy. Shown are representative 3D images or xy section (0.2 μm) views. Nuclei were visualized with Hoechst 33342 (blue). Scale bars: 5 μm. ***: p<0.001, t-test.