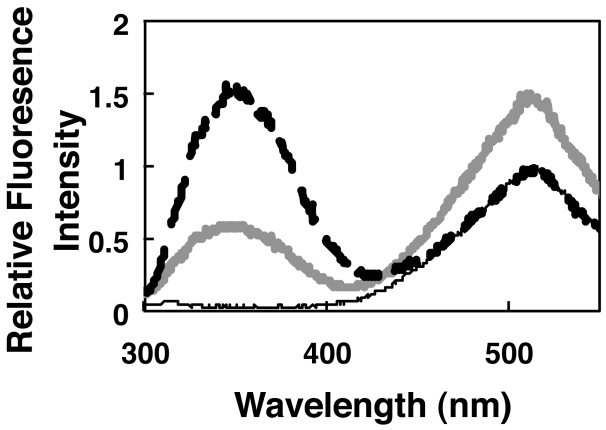

Figure S1: Qualitative FRET measurement of granuphilin C2A binding with vesicles containing PG. Emission spectra are shown of PM(−)PIP_x/PS/PI(+)PG vesicles prior to protein addition (thin black curve), after addition of 1 μM C2A (solid gray curve), and after addition of 8 mM IP_6 (dashed black curve). Experiment was performed as described in the main text, except that measurements were made using a PerkinElmer LS55 fluorescence/luminescence spectrometer. Differences in lineshape from Figs. 1-3 likely reflect differences in the wavelength dependence of detector sensitivity between the two instruments.

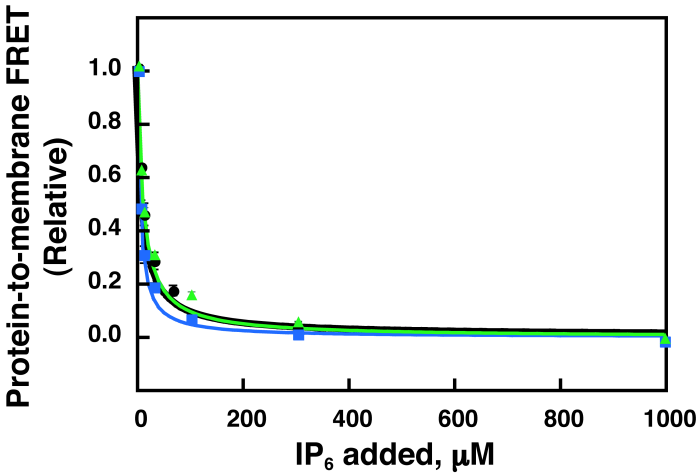

Figure S2: IP_6 competition measurement of C2A interaction with anionic background lipids. Protein-to-membrane FRET was monitored (excitation 284 nm, emission 512 nm) of 1 μM C2A in the presence of PC/PS/dPE (black circles and curve), PC/PI/dPE (blue squares and curve), or PC/PG/PE (green triangles and curve). Curves show best fits to eq. 2, and fit parameters are given in Table 2. Error bars are standard deviation of three independent replicate measurements, and where not visible are smaller than the data symbols.

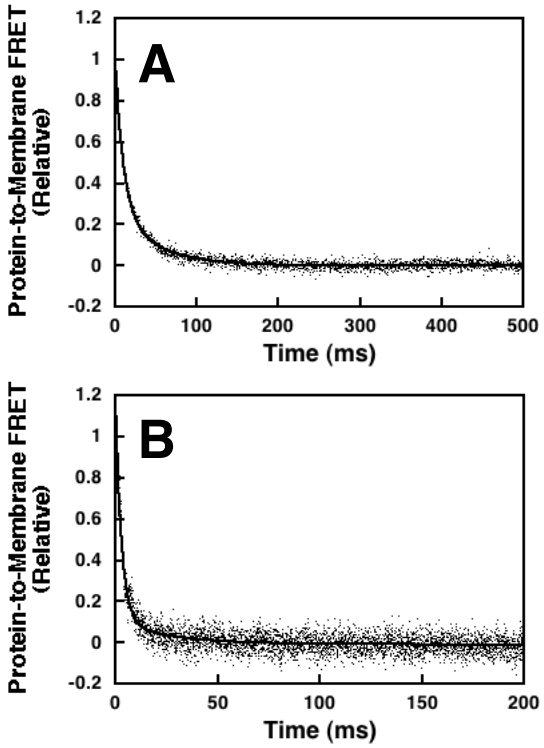

Figure S3: Rates of granuphilin C2 domain vesicle dissociation induced by IP_6. (**A**) C2A or (**B**) C2B domain (0.3 μM) was preincubated with PM(+)PIP_2 vesicles (75 μM total accessible lipid) in assay buffer containing 100 μM EDTA (see Methods). Dissociation was measured following rapid mixing with 8 mM IP_6 (all concentrations are after mixing). Both dissociation profiles were biexponential with the following rate constants. C2A: 96 ± 4 s^{-1} (amplitude 73 ± 2 %) and 20 ± 1 s^{-1} (amplitude 27 ± 2 %). C2B: 330 ± 10 s^{-1} (amplitude 92 ± 2%) and 36 ± 5 s^{-1} (amplitude 8 ± 2 %).