SUPPORTING INFORMATION

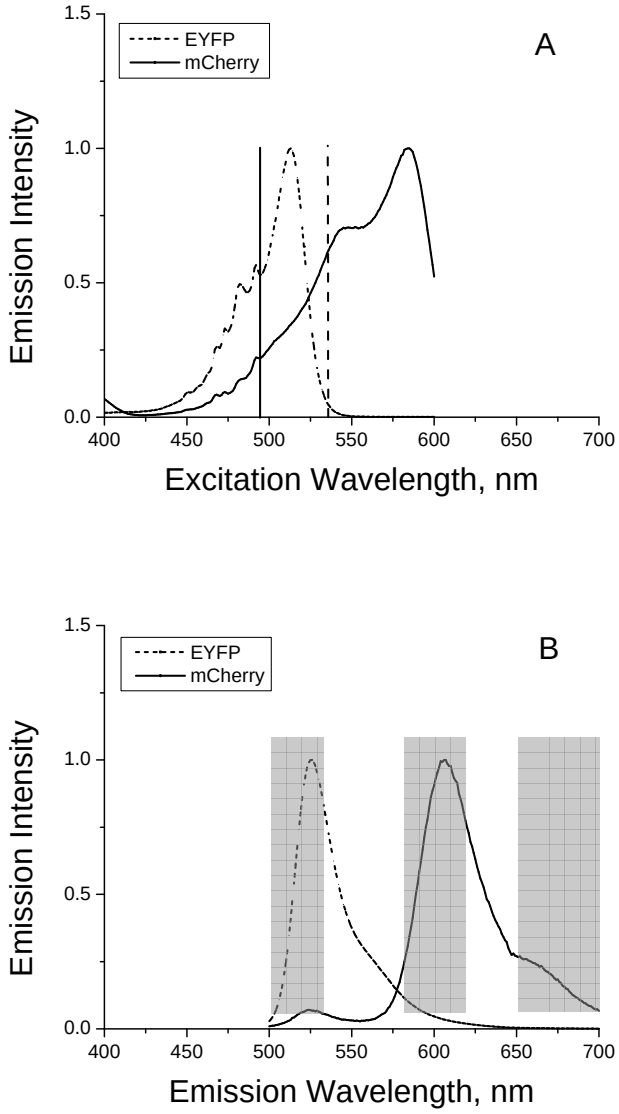

Figure S1. Excitation (A) and emission (B) spectra of EYFP and mCherry. EYFP and mCherry were expressed and purified as described in ref. [30]. Excitation scans were recorded by measuring the emission at 610 nm. Emission scans were carried out by exciting at 488 nm. Vertical lines in (A) show the laser wavelengths (488 nm and 543 nm) and bars in (B) show the emission channels (500-530 nm, 565-615 nm, >650 nm) used in the three scans (I_D, I_{FRET}, I_A).

EYFP/mCherry is a good FRET pair because the emission of EYFP overlaps with the excitation of mCherry.

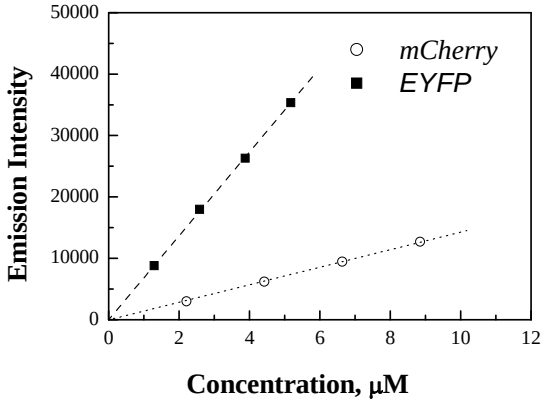

Figure S2. Calibration curves for EYFP and mCherry, obtained in the confocal microscope under conditions used for vesicle imaging. EYFP (squares) and mCherry (circles) were expressed, purified and resuspended in vesiculation buffer as described in ref. [30]. Protein concentrations were determined using 83,500 M^{-1} cm^{-1} and 72,000 M^{-1} cm^{-1} as the extinction coefficients for EYFP and mCherry, respectively. The slopes are the constants i_A and i_D, needed for GpA concentration calculations.

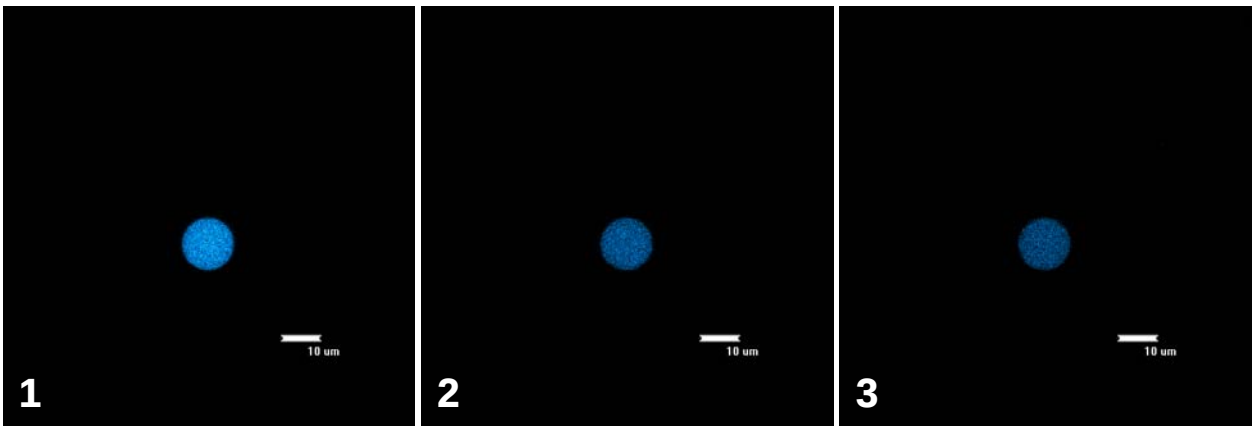

Figure S3. Determination of the gauge factor G_F for the FRET pair of EYFP and mCherry. Vesicles from CHO cells expressing a linked EYFP_mCherry construct were imaged to obtain the image intensities I_D, I_{FRET} and I_A using the donor scan (1), FRET scan (2) and acceptor scan (3). The gauge factor G_F was determined using equation (5).

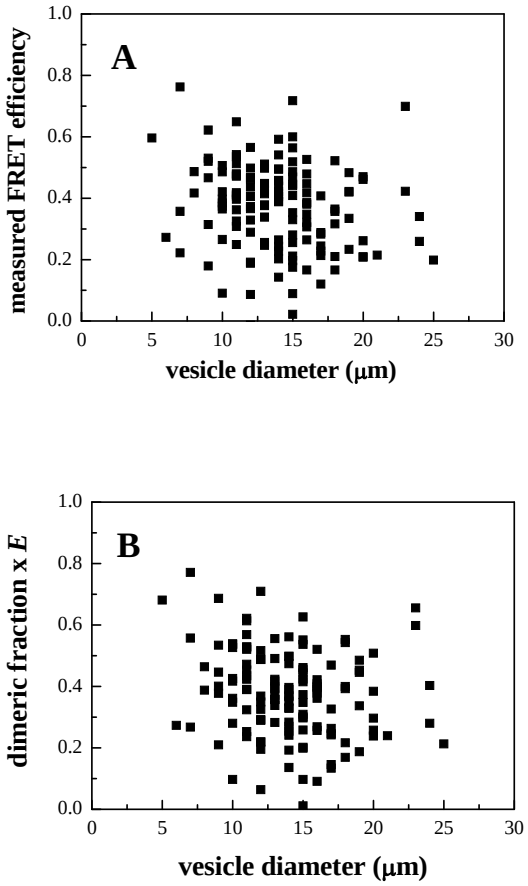

Figure S4. The vesicle size has no effect on (A) the measured FRET efficiency and (B) the calculated dimeric fraction.