Supplemental Figures

Figure 1. Over-expression of Bap31 molecules with C-terminal YFP tags enhances expression of MHC class I molecules on the cell surface. The correlation between expression of our new constructs Bap31-YFP and Bap31-YFP-KKEE and MHC class I molecules at the cell surface is compared to the correlation of expression of our original N-terminal-tagged YFP-Bap31 or of free YFP and MHC class I molecules at the cell surface.

Figure 2.

FRET between YFP- and CFP-tagged molecules in the ER membrane.

A) The YFP- and CFP-tagged proteins used in this study: Bap31, Bap29 and the human MHC I molecule, HLA-A2. Proteins are arranged from left to right in order of their mention in the text. The C-terminus of Bap31-YFP is KKEE, and that of Bap29-YFP is KKRL. B) FRET between CFP and YFP inserted in tandem in the cytoplasmic tail of Bap31. The nominal ratio of donor to acceptor of this construct, our positive control for FRET, is 1:1. C) FRET negative control, the fluorophores in the nominal FRET pair, YFP-Bap31 and and Bap31-CFP are on opposite sides of the ER membrane bilayer and hence out of FRET range. For FRET measurement, three to six regions of interest (ROI) were placed on the ER of the cell. Each point represents the calculated FRET efficiency of an ROI as a function of the ratio of its YFP fluorescence before bleaching (acceptor concentration) to CFP fluorescence after bleaching YFP (donor concentration). Only ROI whose fluorescence intensities of YFP and CFP ranged between 500 and 3000 arbitrary units (a.u.) were used to calculate FRET.

B) Our positive control for FRET was Bap31-YFP connected to CFP with a 25-amino acid linker (Bap31-YFP-CFP in Figure 2A). After photobleaching of YFP, the CFP intensity increased by about 18% (Figure 2B). The spread of values seen for the tandem reflects some combination of the photophysics of the probes and the resolution of our microscopes.

C) Our negative control for FRET was Bap31 with N-terminal YFP (9), and Bap31 with C-terminal CFP (YFP-BAP31 and BAP31-CFP, respectively in Figure 2A). The acceptor and the donor are on opposite sides of the ER membrane, out of FRET range and indeed, no FRET was observed between YFP-Bap31 and Bap31-CFP co-expressed in HeLa cells (Figure 2C). The small negative values of FRET are probably due to slight donor (CFP) bleaching during acceptor (YFP) photobleaching.