Electronic Supplementary Material (ESI) for Analyst.


# Supplementary Information

## Spying on Protein Interactions in Living Cells with Reconstituted Scarlet Light

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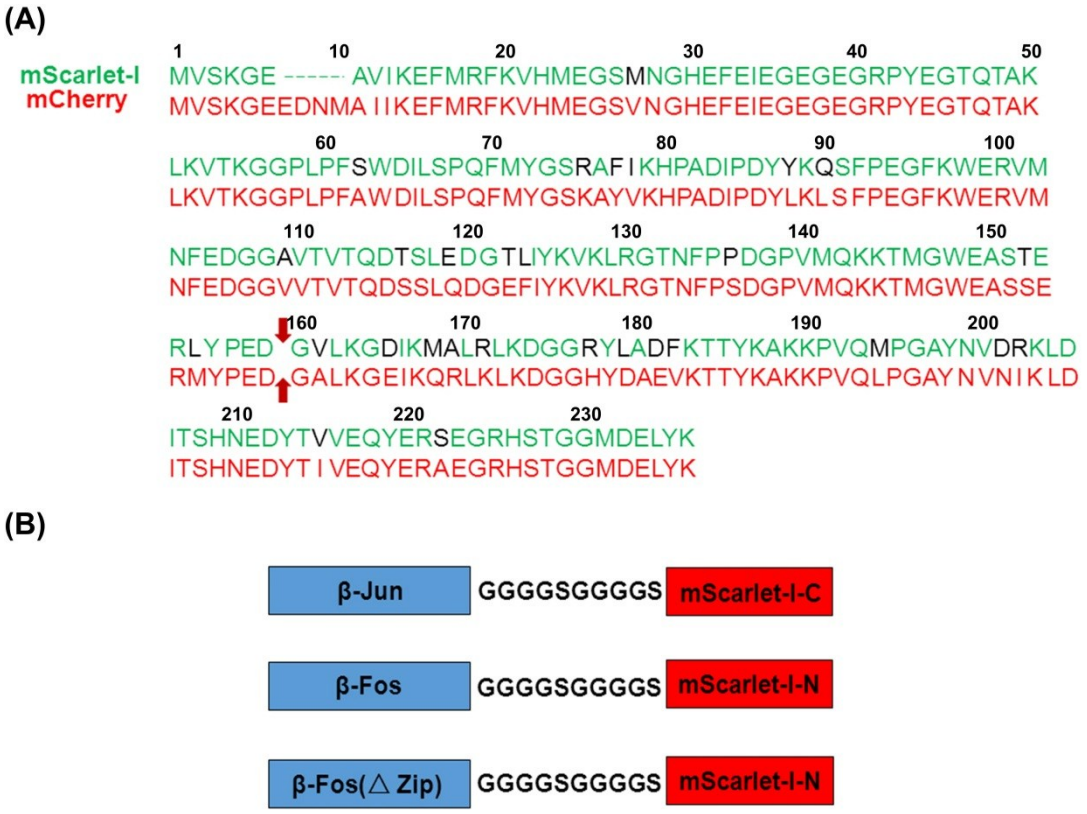


**Fig. S1 Design of mScarlet-I-based BiFC assay.** (A) Sequence alignment of mScarlet-I to mCherry. The sequences of mScarlet-I and mCherry are highlighted in green and red respectively. The amino acids differences of mScarlet-I to mCherry are highlighted in black. The dark red arrows indicate the splitting sites for BiFC analysis. (B) The plasmid constructs used in establishing mScarlet-I-based BiFC assay.

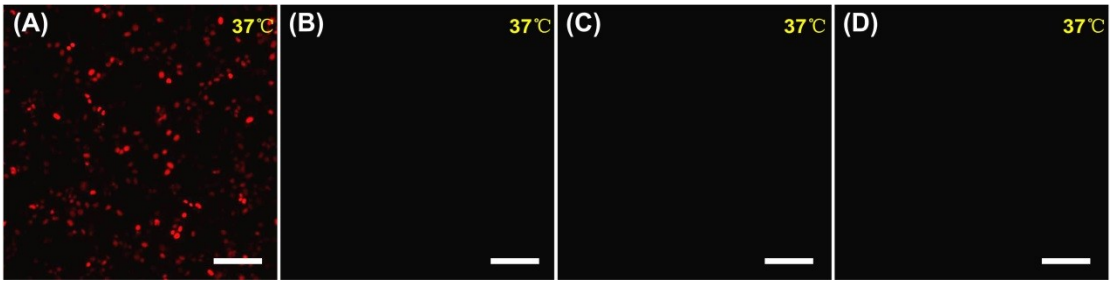


**Fig. S2 Detection and visualization β-Fos/ β-Jun heterodimerization in live HeLa cells directly at 37℃ with four red BiFC assays.** Detection and visualization β-Fos/ β-Jun heterodimerization with (A) mScarlet-I-based BiFC assay or (B) mRFP1(Q66T)-based BiFC assay or (C) mCherry-based BiFC assay or (D) mNeptune-based BiFC assay after same amount of plasmids transfection; Images were acquired with the same imaging conditions and displayed with the same intensity scale range. Scale bar: 200 μm

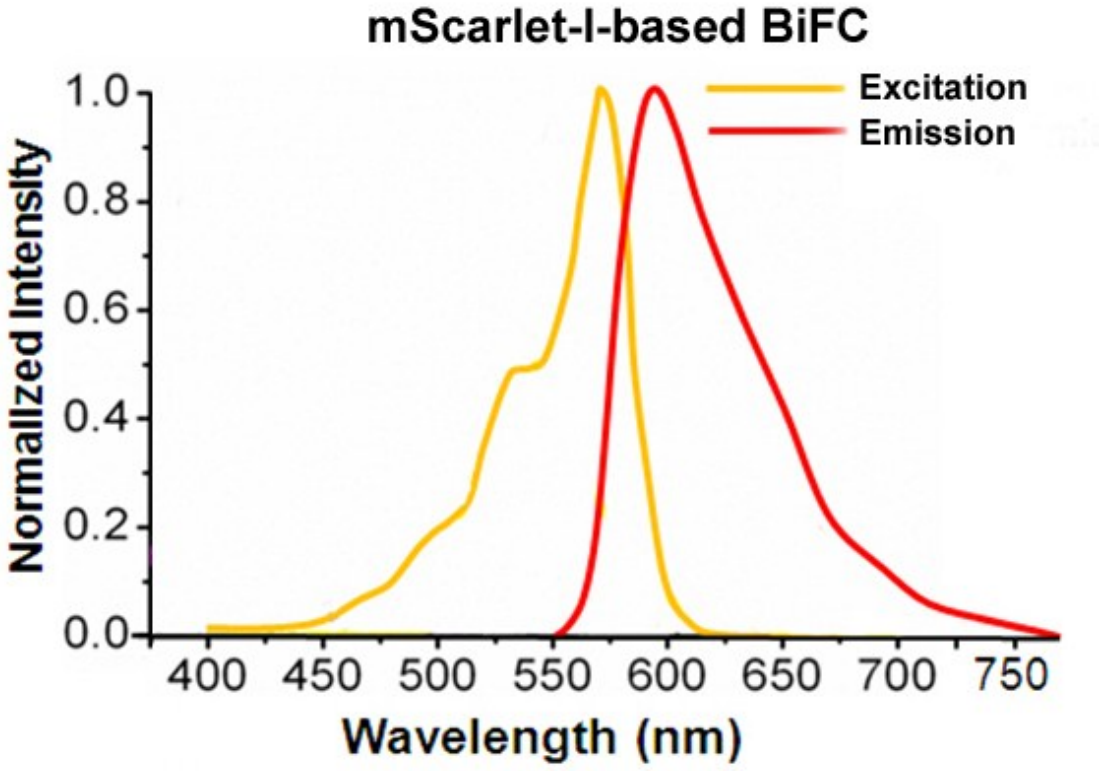


**Fig. S3 Spectra of complemented mScarlet-I mediated by β-Jun/β-Fos heterodimerization.** The spectra of live HeLa cells coexpressing β-Fos-mScarlet-I-N and β-Jun-mScarlet-I-C was measured by fluorescence spectrophotometer.

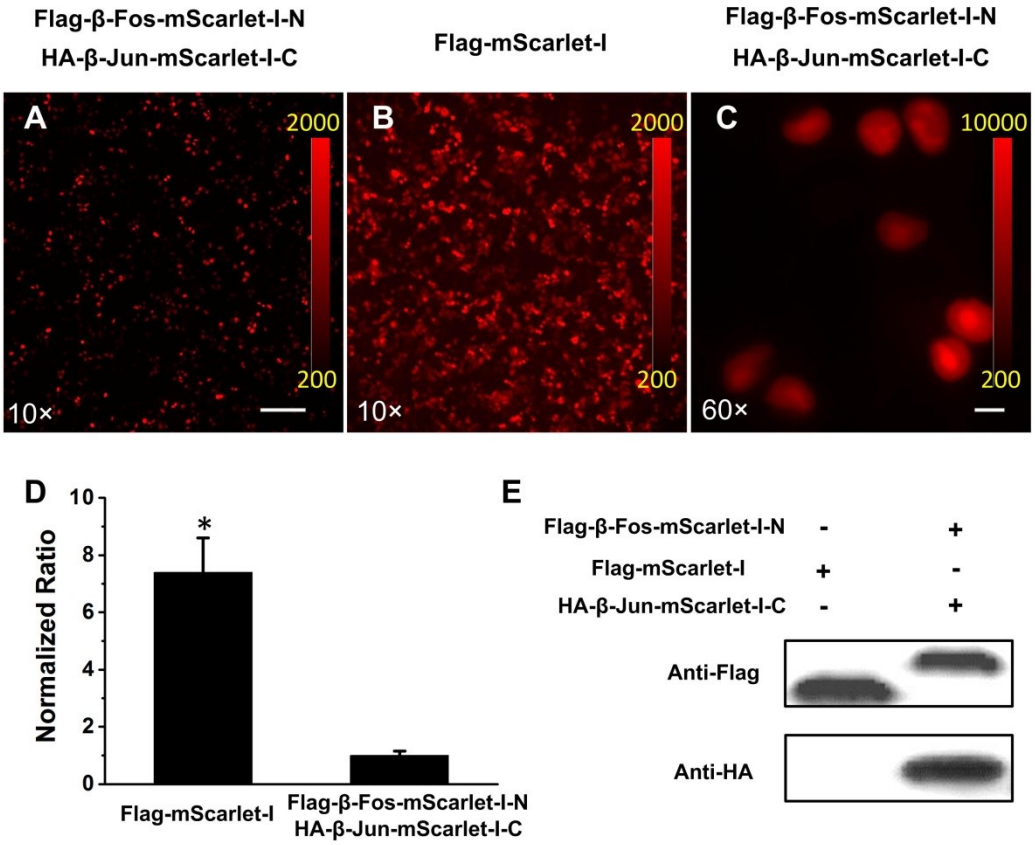


**Fig. S4 Comparison of the brightness of full length fluorescent protein and its derived BiFC reporter using constitutive β-Jun/β-Fos heterodimerization model.** (A-C) fluorescence images of HeLa cells expressing indicated constructs were recorded under the same imaging conditions. mCerulean fluorescent protein was used as a co-transfection marker to normalized different transfection and expression levels between different experimental groups. Scale bar: 200μm in (A) and (B) with10× objective lens and 20μm in (c) with 60×oil objective lens. (D) Normalized ensemble red-to-cyan ratios of HeLa cells expressing indicated constructs. *p<0.01 compared with cells coexpressing indicated BiFC constructs. (E) Comparable protein expression level of indicated constructs was determined by western blotting with Anti-Flag and Anti-HA antibody. Scale bar: 200 μm in (A) and (B), 20 μm in (C).

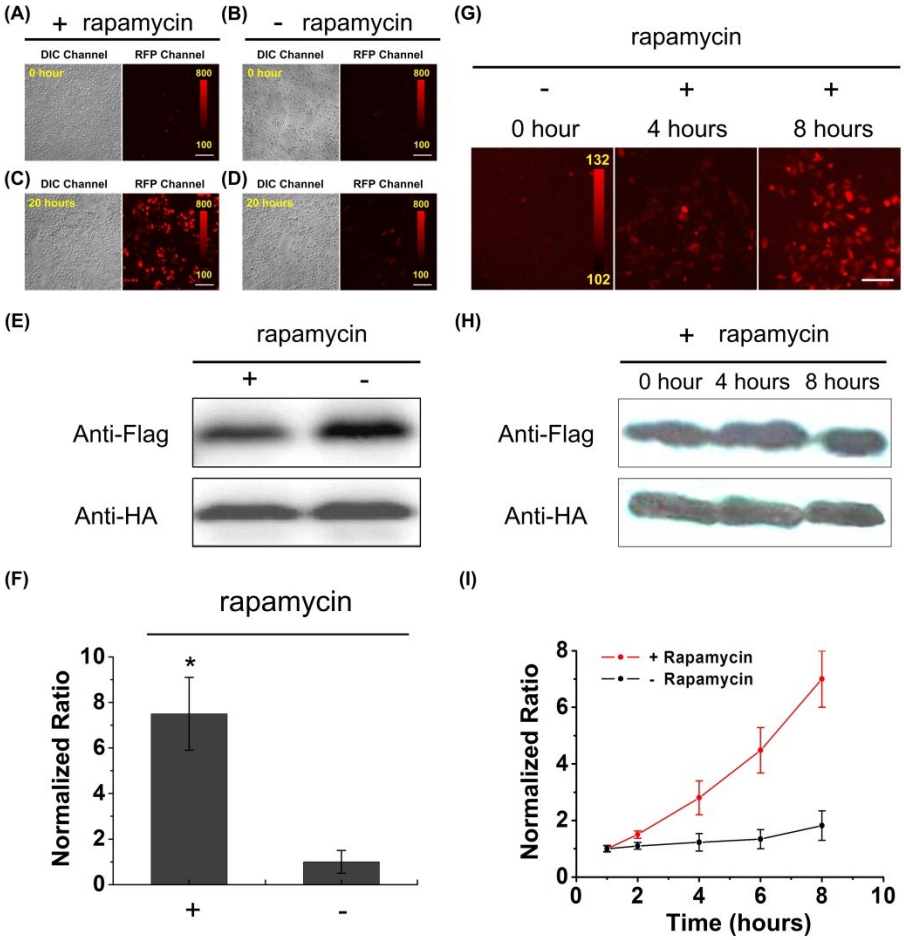


**Fig. S5 Characterization of mScarlet-I-based BiFC assay by rapamycin-inducible FRB/FKBP interaction system.** Two dish of HeLa cells co-transfected parallelly with the same amount of mScarlet-I-N-FKBP and FRB-mScarlet-I-C and mCerulean plasmids (internal control) were incubated at 37°C for 24 hours, and then the two dish of HeLa cells (A, B) were imaged with the same imaging conditions in both DIC and RFP channels. The intensity scale was set to the same from 100 to 800 for RFP channel in (A) and (B). After taking images of (A, B), 100 nM rapamycin was added to only one dish (A) and then the two dish of HeLa cells (A, B) were maintained at 37°C for the next 20 hours and imaged with the same imaging conditions in (C) and (D) respectively. The intensity scale was set to the same from 100 to 800 for RFP channel in (C) and (D). Scale bar: 200 μm. (E) After 20 hours rapamycin induction, comparable expression level of the fusion proteins in (C) and (D) was determined by western blotting with anti-Flag and anti-HA antibodies and (F) the ensemble red and cyan fluorescence intensity were measured by fluorescence spectrophotometer and the normalized red-to-cyan ratios were calculated. *$p<0.01$* compared with cells without rapamycin induction. The data were from three independent measurements and expressed as means±SD values. (G) In a typical independent experiment, the dynamic fluorescence images of the same live cell sample at 0 hour, 4 hours and 8 hours after rapamycin addition were taken using the same imaging conditions and the intensity scale was set to the same from 102 to 132. (H) Comparable expression level of the fusion proteins during 8 hours was quantified by western-blotting with anti-Flag and anti-HA antibodies indicating that the fluorescence increase was mainly due to rapamycin-induced complementation of fluorescent protein fragments. (I) Dynamic normalized red-to-cyan ratio changes with or without rapamycin induction within 8 hours. All images were taken using a 10× NA=0.40 objective lens.

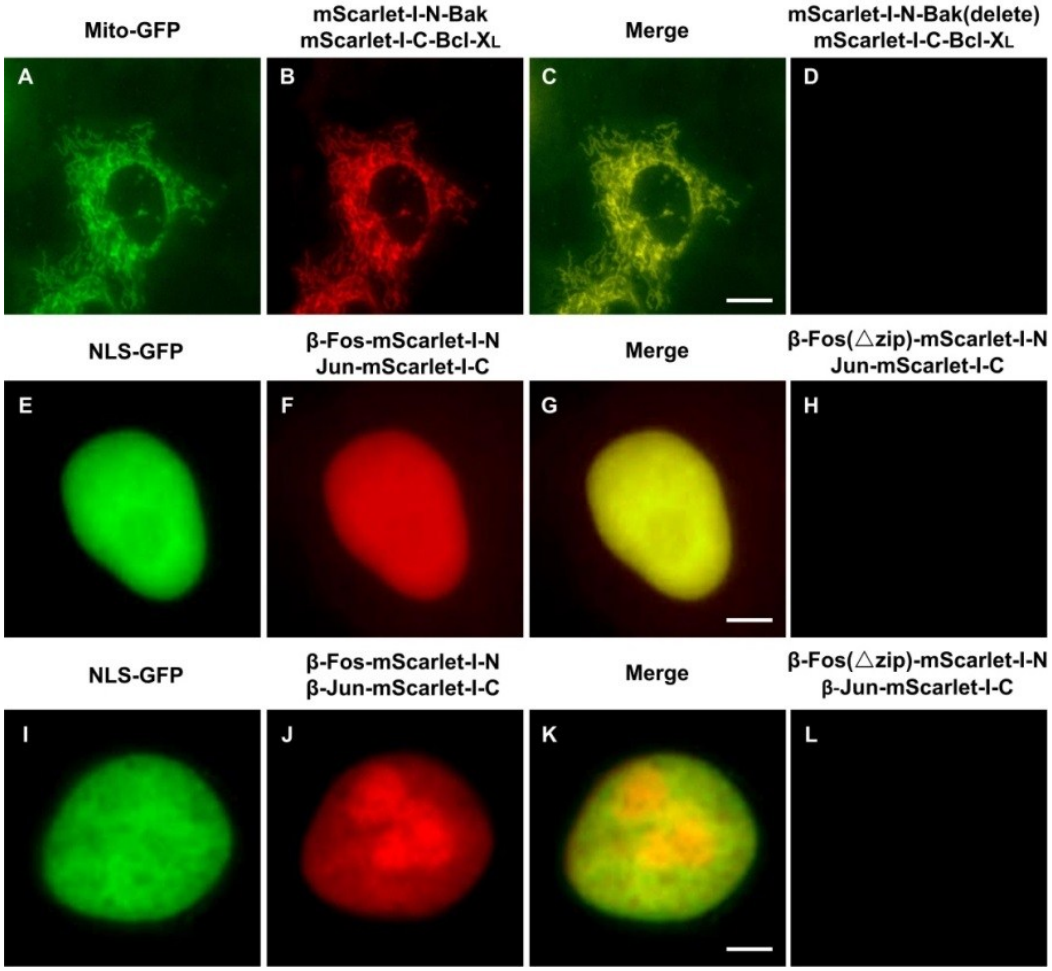


**Fig.S6 Detection, visualization and validation of various PPIs with different subcellular localizations by mScarlet-I-based BiFC assay in living HeLa cells.** Typical images of specific detection of Bak/Bcl-$X_L$ heterodimerization (B, D) and validation of its subcellular localizations by Mito-GFP (A, C) in live HeLa cells or specific detection of β-Fos/Jun heterodimerization (F, H) and validation of its subcellular localizations by NLS-GFP (E, G) in live HeLa cells or specific detection of β-Fos/β-Jun heterodimerization (J, L) and validation of its subcellular localizations by NLS-GFP (I, K) in live HeLa cells. The images from non-interacting negative controls were taken and displayed under the same conditions as their interacting counterpart. Scale bar: 20μm in (C) and 5μm in (G) and (K).

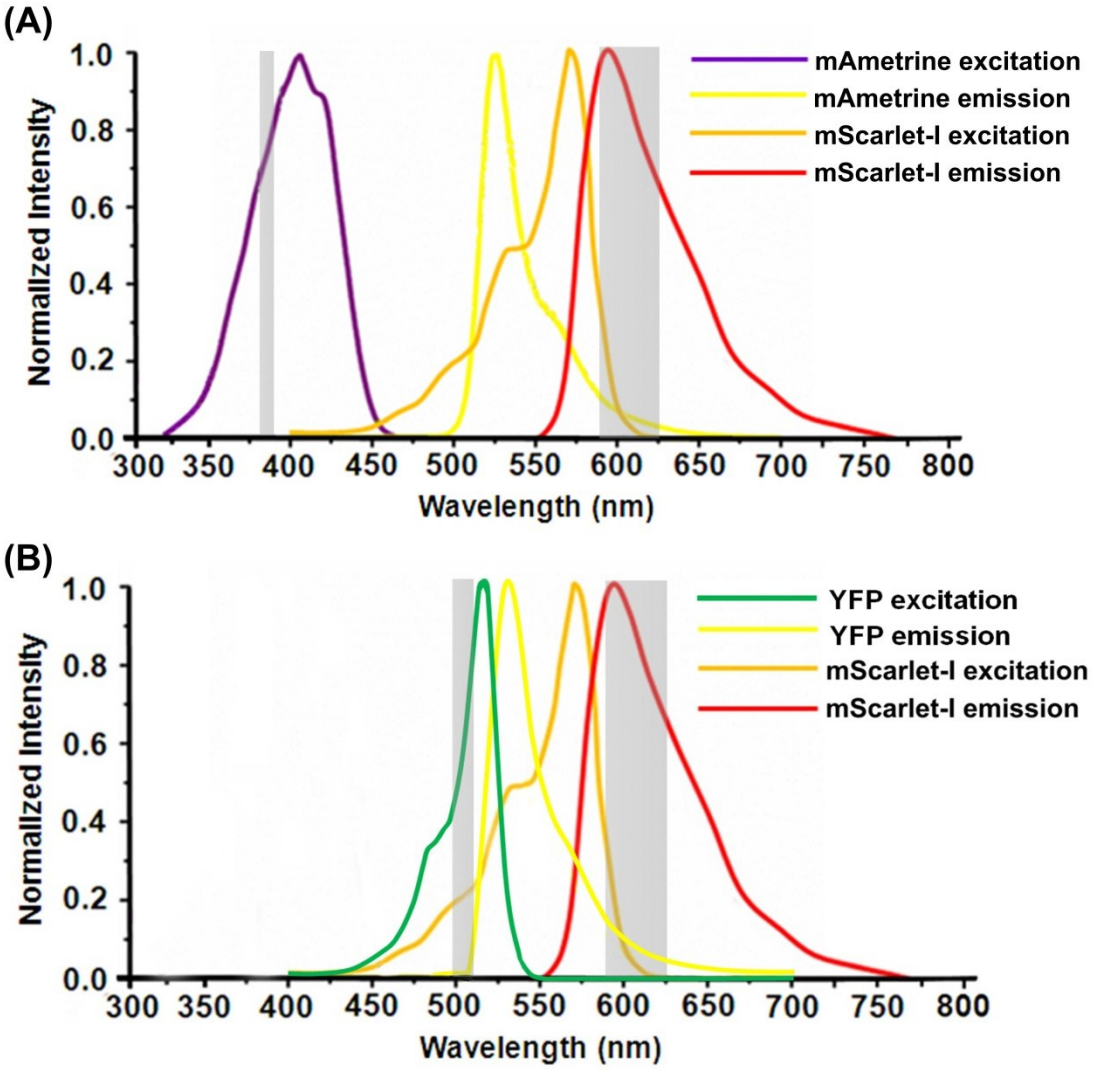


**Fig. S7 Spectra of mAmetrine/YFP and mScarlet-I for FRET measurement.** The excitation and emission spectra of mAmetrine and mScarlet-I (A) or excitation and emission spectra of YFP and mScarlet-I (B) for FRET measurement. The shaded rectangular regions indicate the transmission band passes of the excitation and emission filters used in this study.

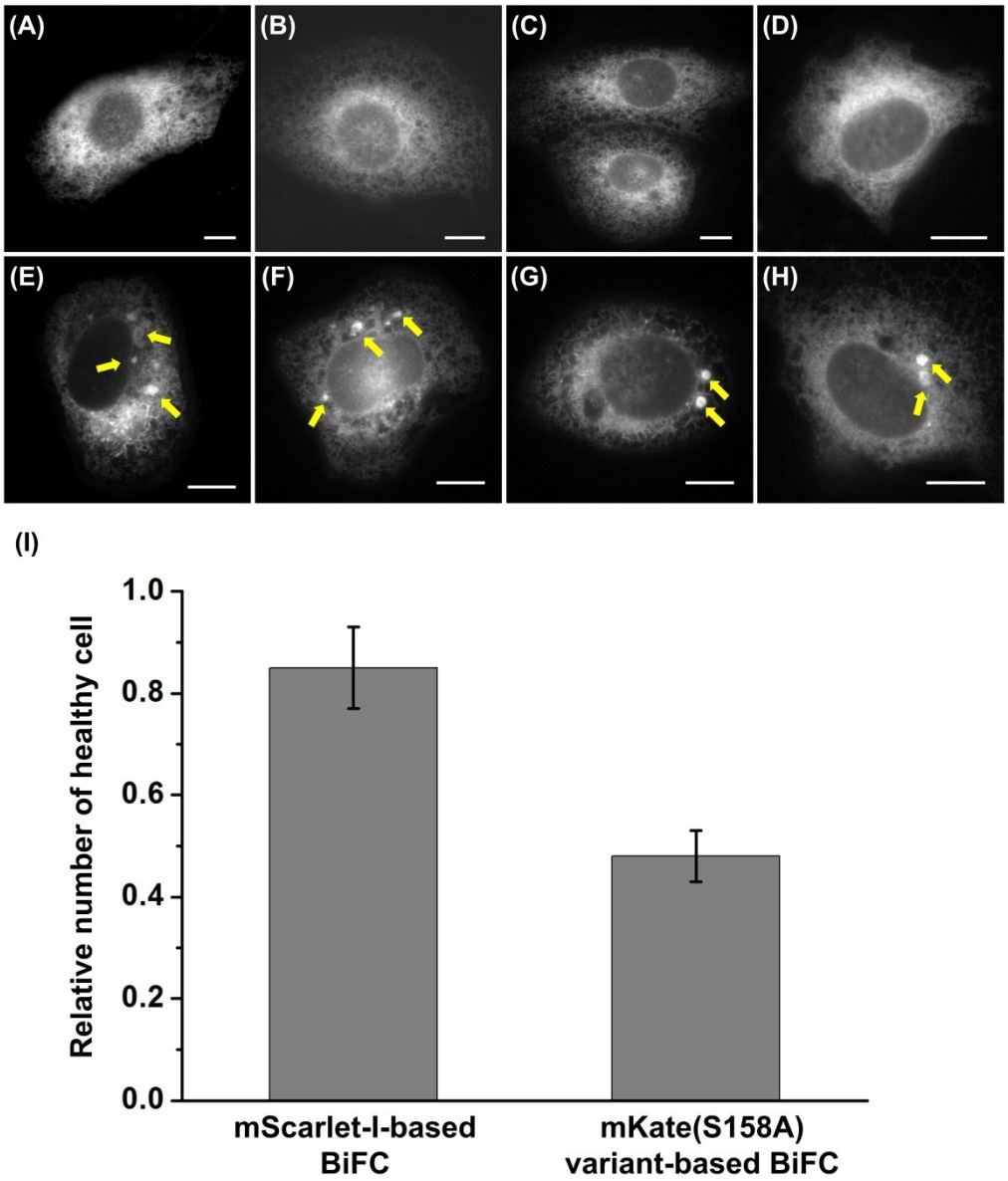


**Fig. S8 Visualizing protein interactions at subcellular structure with BiFC assays derived from mScarlet-I and mKate (S158A) variant.** Detection and visualization of FRB/FKBP interactions targeted at endoplasmic reticulum (ER) membrane with ER targeting sequence (CytERM) [1, 2] by mScarlet-I-based BiFC assay (A-D) or mKate (S158A) variant-based BiFC assay (E-H) in live U2OS cells. Typical images of U2OS cells with high expression levels of fusion proteins were displayed. Yellow arrows indicated OSER structures in live cells. FRB/FKBP interactions were induced by adding saturation concentration of rapamycin to cell culture medium. Images were taken under the same imaging conditions and displayed with the same intensity range scale. Scale bar: 20μm. (i) Cytotoxicity of both BiFC assays were quantified in live HeLa cells detecting Lifeact homodimerization after 48 hours to 72 hours of the same amount of plasmids transfection (3μg). Cell apoptosis or unhealthy cells were identified by Hoechst 33342 staining with typical fragmented or shriveled nucleus morphology. Individual relative number of healthy cell was then calculated accordingly from three independent experiments.

**Movie 1 Detecting and Time-lapse imaging Bcl-$X_L$ and Bak heterodimerization at mitochondria in a living HeLa cell with mScarlet-I-based BiFC assay.**

HeLa cells were cotransfected with mScarlet-I-N-Bak and mScarlet-I-C-Bcl-$X_L$ plasmids, after 24 hours incubation at 37℃, cells were directly imaged under a IX81 fluorescence microscope equipped with a 100× NA=1.45,oil-immersion objective lens. The time-lapse images were recorded with 500ms exposure time for each frame and totally 200 frames were taken at an interval time of 5s between each frame. The movie was then displayed with a speed of 20 frames per second.

## Reference


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2. D. S. Bindels, L. Haarbosch, L. van Weeren, M. Postma, K. E. Wiese, M. Mastop, S. Aumonier, G. Gotthard, A. Royant, M. A. Hink and T. W. Gadella, Jr., *Nat Methods*, 2017, **14**, 53-56.