# Live Cell Visualization of Multiple Protein-Protein Interactions with BiFC Rainbow

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## Supporting Information



**Reference**

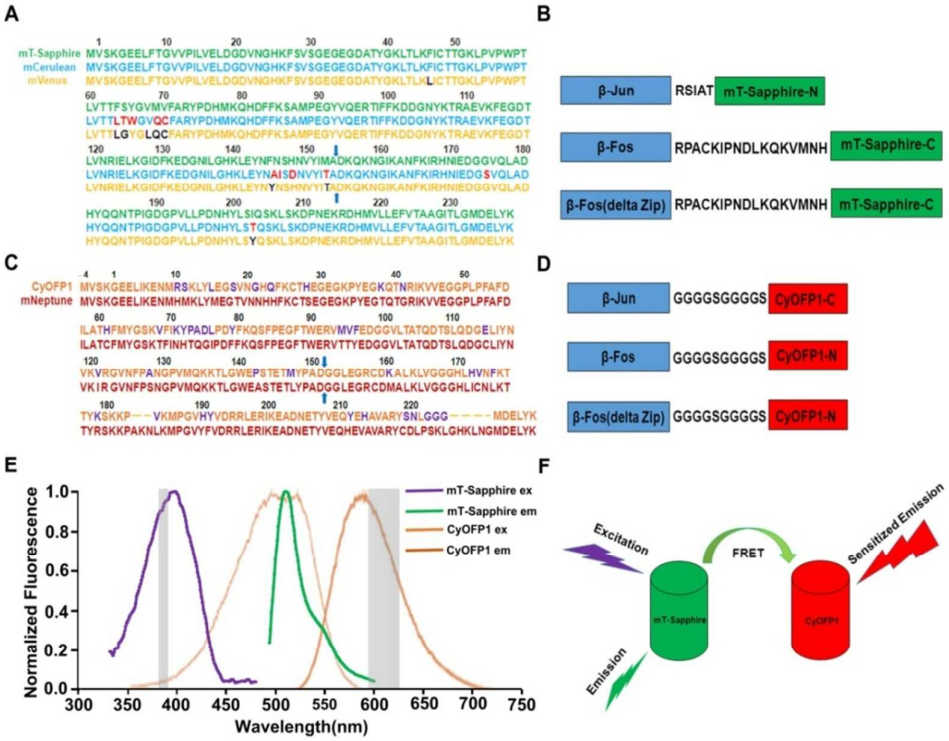


**Figure S1. Design of LSS FP-based BiFC assays and FRET by mT-Sapphire and CyOFP1.**

(A) Sequence alignment of mT-Sapphire to mCerulean and mVenus. The sequence of mT-Sapphire, mCerulean and mVenus are highlighted in green, cyan, and yellow, respectively. The amino acids differences of mCerulean to mT-Sapphire are highlighted in red while the amino acids differences of mVenus to mT-Sapphire are highlighted in black. The blue arrows indicate the splitting sites for BiFC analysis. (B) The plasmid constructs used in establishing mT-Sapphire-based BiFC assay. (C) Sequence alignment of CyOFP1 to mNeptune. The sequence of CyOFP1 is highlighted in orange while the sequence of mNeptune is highlighted in dark red. The amino acids differences of CyOFP1 to mNeptune are highlighted in violet. The blue arrows indicate the splitting sites for BiFC analysis. (D) The plasmid constructs used in establishing CyOFP1-based BiFC assay. (E) The excitation and emission spectra of mT-Sapphire and CyOFP1. The shaded rectangular regions indicate the transmission bandpasses of the excitation and emission filters used in this study. mT-Sapphire and CyOFP1 spectra data are from references.[1, 2] (F) FRET by donor mT-Sapphire and acceptor CyOFP1

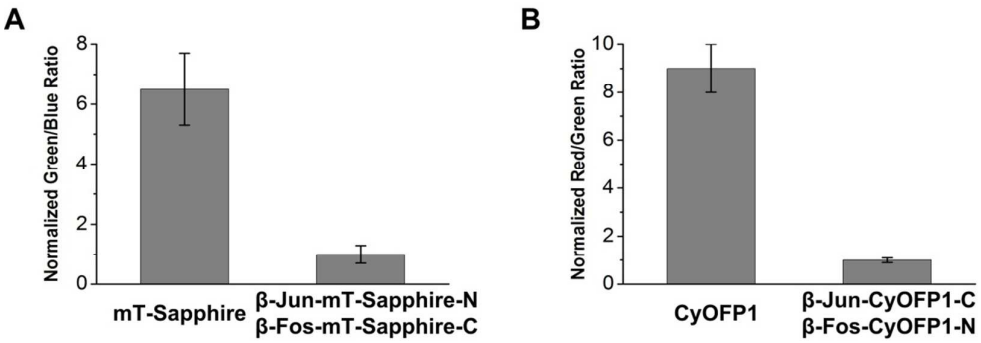


**Figure S2. Comparison of the brightness of full length fluorescent proteins and their derived BiFC reporters.**

(A) Normalized Green/Blue ratio comparison between mT-Sapphire and complemented mT-Sapphire. (B) Normalized Red/Green ratio comparison between CyOFP1 and complemented CyOFP1. The data were from three independent measurements. TagBFP or EGFP was used as internal control to normalize transfection efficiency.

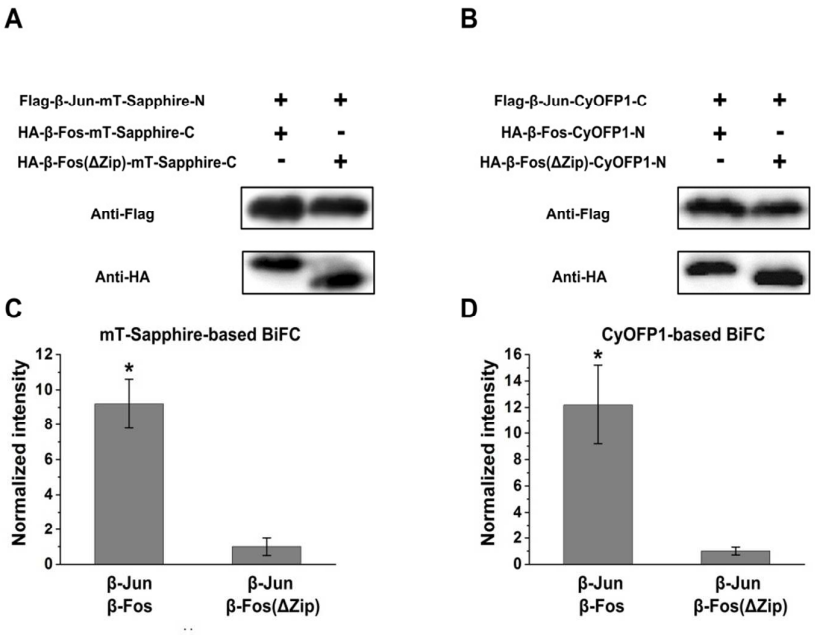


**Figure S3. Comparison of the brightness of positive and negative interaction pairs using mT-Sapphire and CyOFP1-based BiFC reporters.**

The protein expression levels of positive interaction pair ((β-Jun/β-Fos) and negative interaction pair (β-Jun/β-Fos(ΔZip)) using mT-Sapphire-based BiFC reporter (A) or CyOFP1-based BiFC reporter (B) were quantified by western-blotting and the ensemble normalized fluorescence intensities were compared in (C) and (D) respectively, $*p<0.01$. The data were from three independent measurements. The fluorescence of complemented CyOFP1 is measured after 30℃ incubation for 24 hours.

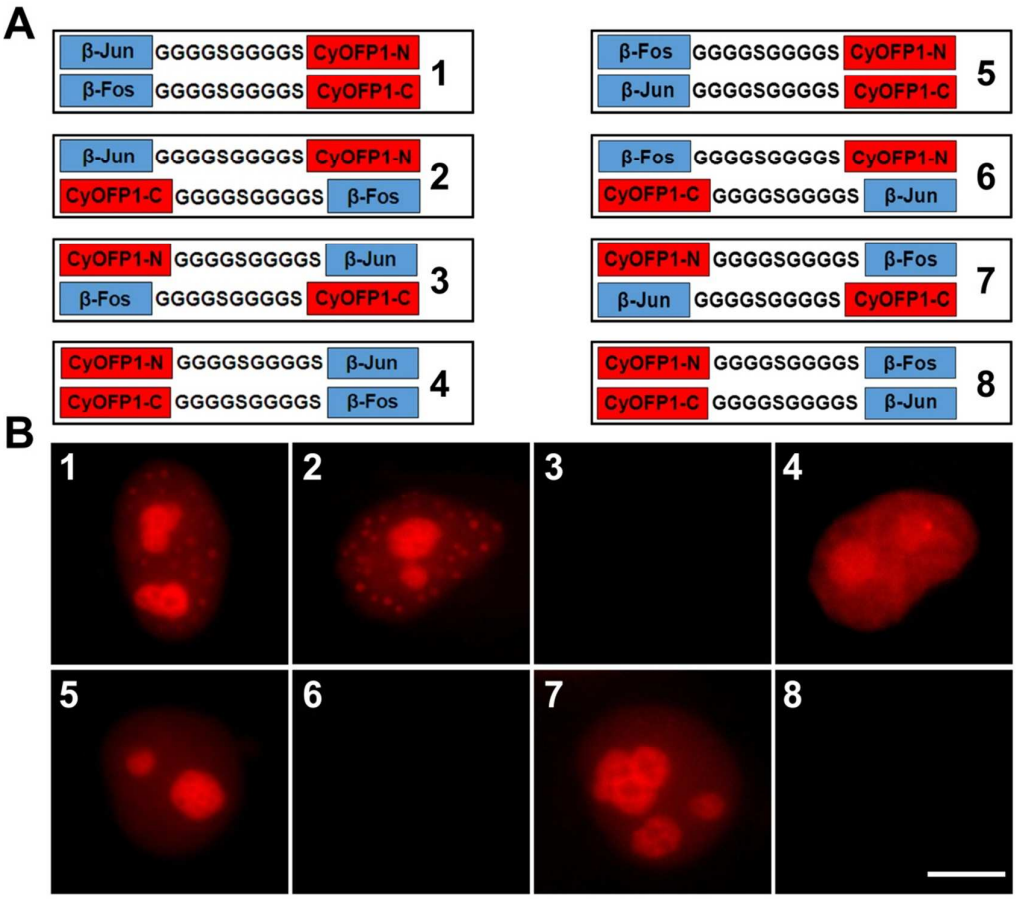


**Figure S4. Optimizing the BiFC efficiency of fragments from CyOFP1 for β-Jun/ β-Fos heterodimerization detection and imaging.**

(A) Design of plasmid constructs for optimizing the BiFC efficiency of fragments from CyOFP1 for imaging β-Jun/ β-Fos heterodimerization in live HeLa cells. (B) 8 combinations were tested, 5 combinations showed bright fluorescence. The expression of protein was also confirmed by Western blot (data not shown). Scale bar, 5μm.

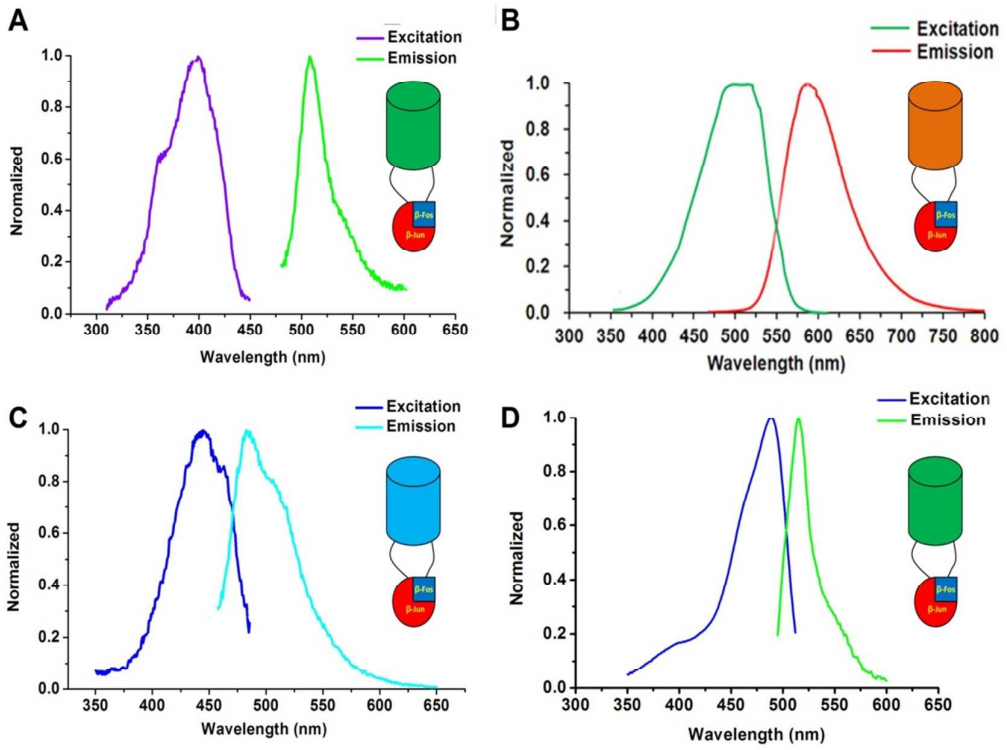


**Figure S5. Spectrum of complemented fluorescent protein fragments.**

Spectrum of complemented fluorescent protein mediated by optimized β-Jun and β-Fos tagged to (A) mT-Sapphire-N and mT-Sapphire-C or (B) CyOFP1-N and CyOFP1-C or (C) CrN and mT-Sapphire-C or (D) VN and mT-Sapphire-C fragments. Spectrums were measured by fluorescence spectrophotometer.

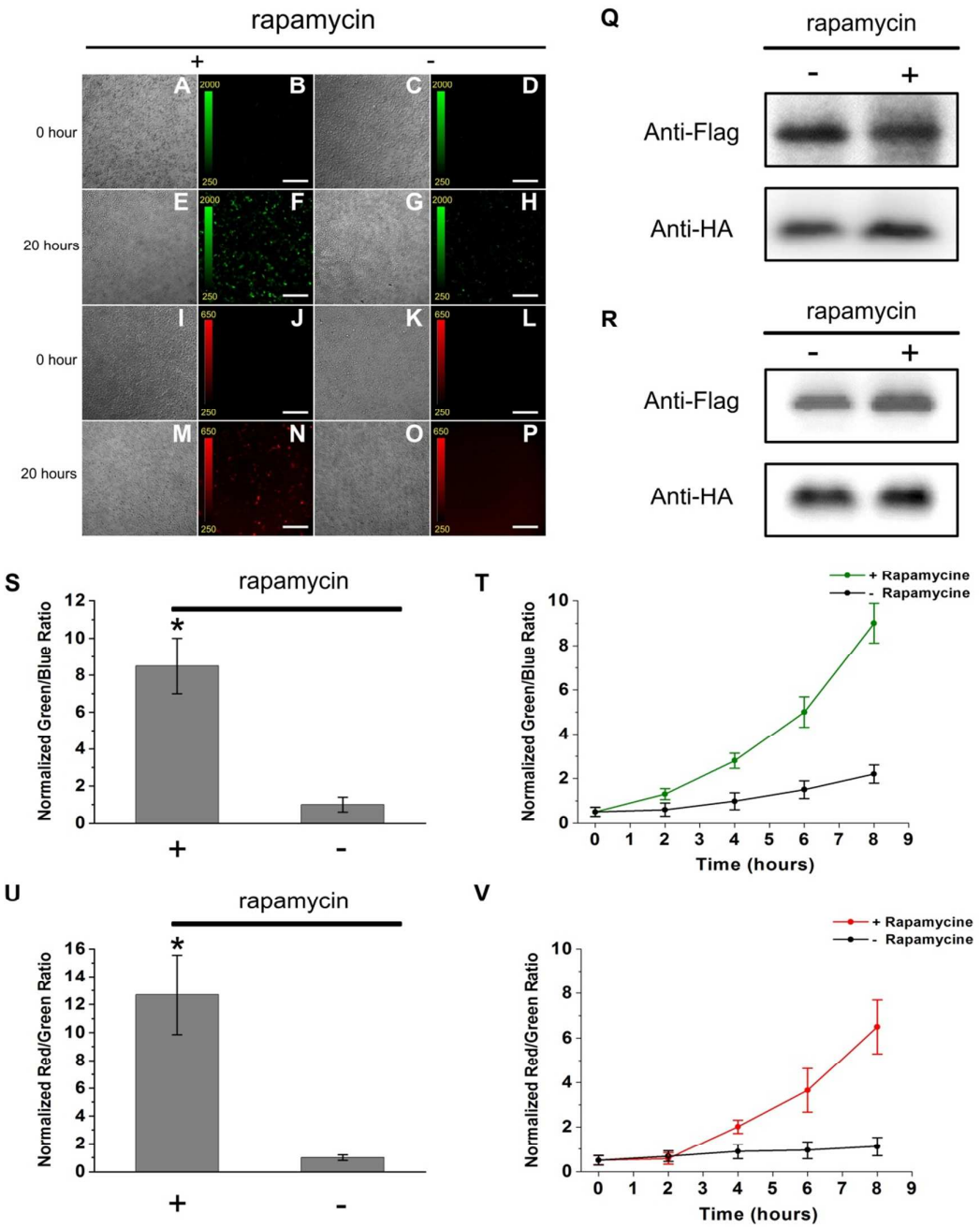


**Figure S6. Characterization of mT-Sapphire-based BiFC assay and CyOFP1-based BiFC assay by rapamycin-inducible FRB/FKBP interaction system.**

Two dish of HeLa cells co-transfected parallelly with the same amount of FKBP-mT-Sapphire-N, FRB-mT-Sapphire-C and pcDNA3.1(+)-TagBFP plasmids (internal control) were incubated at 37°C for 24 hours, and then the cells were imaged with the same imaging conditions (A,B,C,D).(A,C) DIC channel,(B,D) mT-Sapphire channel, the intensity scale was set to the same from 250 to 2000 for (B) and (D).

After taking images of (A,B,C,D), 100 nM rapamycin was added to only one dish (A,B) and then the two dish of HeLa cells were maintained at 37°C for the next 20 hours and imaged with the same imaging conditions (E,F,G,H). (E, G) DIC channel, (F, H) mT-Sapphire channel, the intensity scale was set to the same from 250 to 2000 for (F) and (H). Scale bar: 300μm. Similarly, two dish of HeLa cells co-transfected parallelly with the same amount of pcDNA3.1 (+)-Flag-FKBP-CyOFP1-N and pcDNA3.1 (+)-HA-FRB-CyOFP1-C and pcDNA3.1(+)-EGFP plasmids (internal control) were incubated at 37°C for 24 hours, and then the cells were imaged with the same imaging conditions (I,J,K,L).(I,K) DIC channel,(J,L) CyOFP1 channel, the intensity scale was set to the same from 250 to 650 for (J) and (L). After taking images of (I,J,K,L), 100 nM rapamycin was added to only one dish (I,J) and then the two dish of HeLa cells were maintained at 30°C for the next 20 hours and imaged with the same imaging conditions (M,N,O,P). (M, O) DIC channel, (N, P) CyOFP1 channel, the intensity scale was set to the same from 250 to 650 for (N) and (P). Scale bar: 300μm. (Q, R) Comparable expression level of the fusion proteins in (F) and (H) or (N) and (P) determined by western blotting with anti-Flag and anti-HA antibodies. (S, U) After 20 hours rapamycin induction, the ensemble green and blue or red and green fluorescence were measured by fluorescence spectrophotometer and the normalized green-to-blue ratios (S) and red-to-green ratios (U) were calculated. *$p<0.01$ compared with cells without rapamycin induction. The data were from three independent measurements. The ensemble fluorescence ratio dynamics were also monitored and calculated every 2 hours with or without 100 nM rapamycin addition for mT-Sapphire-based BiFC assay (T) at 37℃ or CyOFP1-based BiFC assay (V) at 25℃

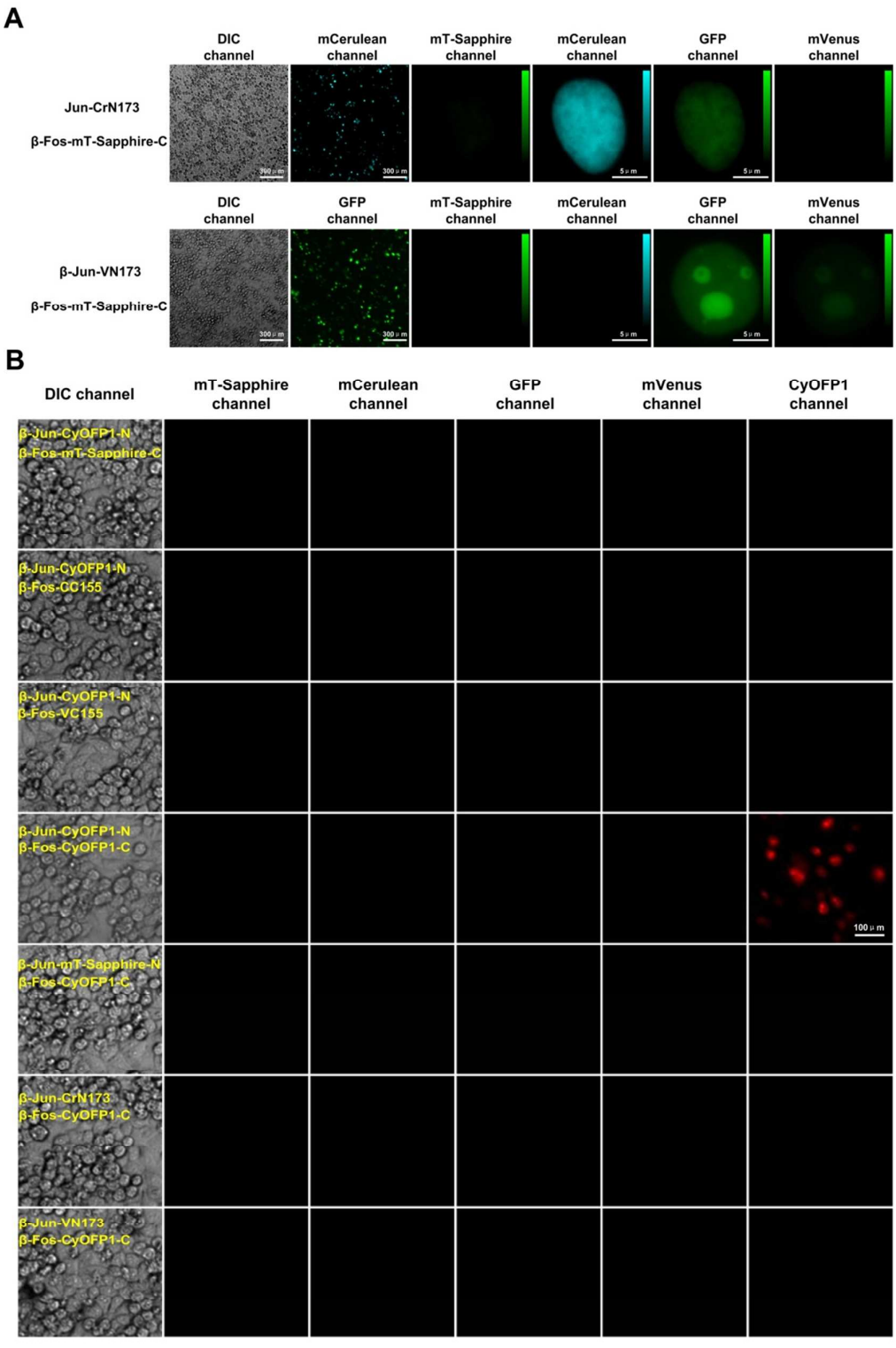
A
DIC channel
mCerulean channel
mT-Sapphire channel
mCerulean channel
GFP channel
mVenus channel
Jun-CrN173
β-Fos-mT-Sapphire-C
300 μm
300 μm
5 μm
5 μm
DIC channel
GFP channel
mT-Sapphire channel
mCerulean channel
GFP channel
mVenus channel
β-Jun-VN173
β-Fos-mT-Sapphire-C
300 μm
300 μm
5 μm
5 μm
B
DIC channel
mT-Sapphire channel
mCerulean channel
GFP channel
mVenus channel
CyOFP1 channel
β-Jun-CyOFP1-N
β-Fos-mT-Sapphire-C
β-Jun-CyOFP1-N
β-Fos-CC155
β-Jun-CyOFP1-N
β-Fos-VC155
β-Jun-CyOFP1-N
β-Fos-CyOFP1-C
100 μm
β-Jun-mT-Sapphire-N
β-Fos-CyOFP1-C
β-Jun-CrN173
β-Fos-CyOFP1-C
β-Jun-VN173
β-Fos-CyOFP1-C

**Fig.S7. Cross-combinations of fragments from different fluorescent proteins used in BiFC assays.**

(A) HeLa cells cotransfected with Jun-CrN173 and β-Fos-mT-Sapphire-C were imaged through DIC channel, mT-Sapphire channel, mCerulean channel, GFP channel and mVenus channel 24 h after transfection (upper panel). The exposure time is constant (1 s) in each channel and the color bar indicate the constant gray scale (200-800). HeLa cells cotransfected with β-Jun-VN173 and β-Fos-mT-Sapphire-C were imaged through DIC channel, mT-Sapphire channel, mCerulean channel, GFP channel and mVenus channel 24 h after transfection (lower panel). The exposure time is constant (1 s) in each channel and the color bar indicate the constant gray scale (200-20000). (B) HeLa cells cotransfected with the indicated plasmids were imaged through DIC channel, mT-Sapphire channel, mCerulean channel, GFP channel, mVenus channel 24 h after transfection and CyOFP1 channel after another 8 hours in room temperature.

## Reference:

1. Chu, J.; Oh, Y.; Sens, A.; Ataie, N.; Dana, H.; Macklin, J. J.; Laviv, T.; Welf, E. S.; Dean, K. M.; Zhang, F.; Kim, B. B.; Tang, C. T.; Hu, M.; Baird, M. A.; Davidson, M. W.; Kay, M. A.; Fiolka, R.; Yasuda, R.; Kim, D. S.; Ng, H. L.; Lin, M. Z. A bright cyan-excitable orange fluorescent protein facilitates dual-emission microscopy and enhances bioluminescence imaging in vivo. *Nat Biotechnol* 2016, 34, 760-7.

2. Zapata-Hommer, O.; Griesbeck, O. Efficiently folding and circularly permuted variants of the Sapphire mutant of GFP. *BMC Biotechnol* 2003, 3, 5.