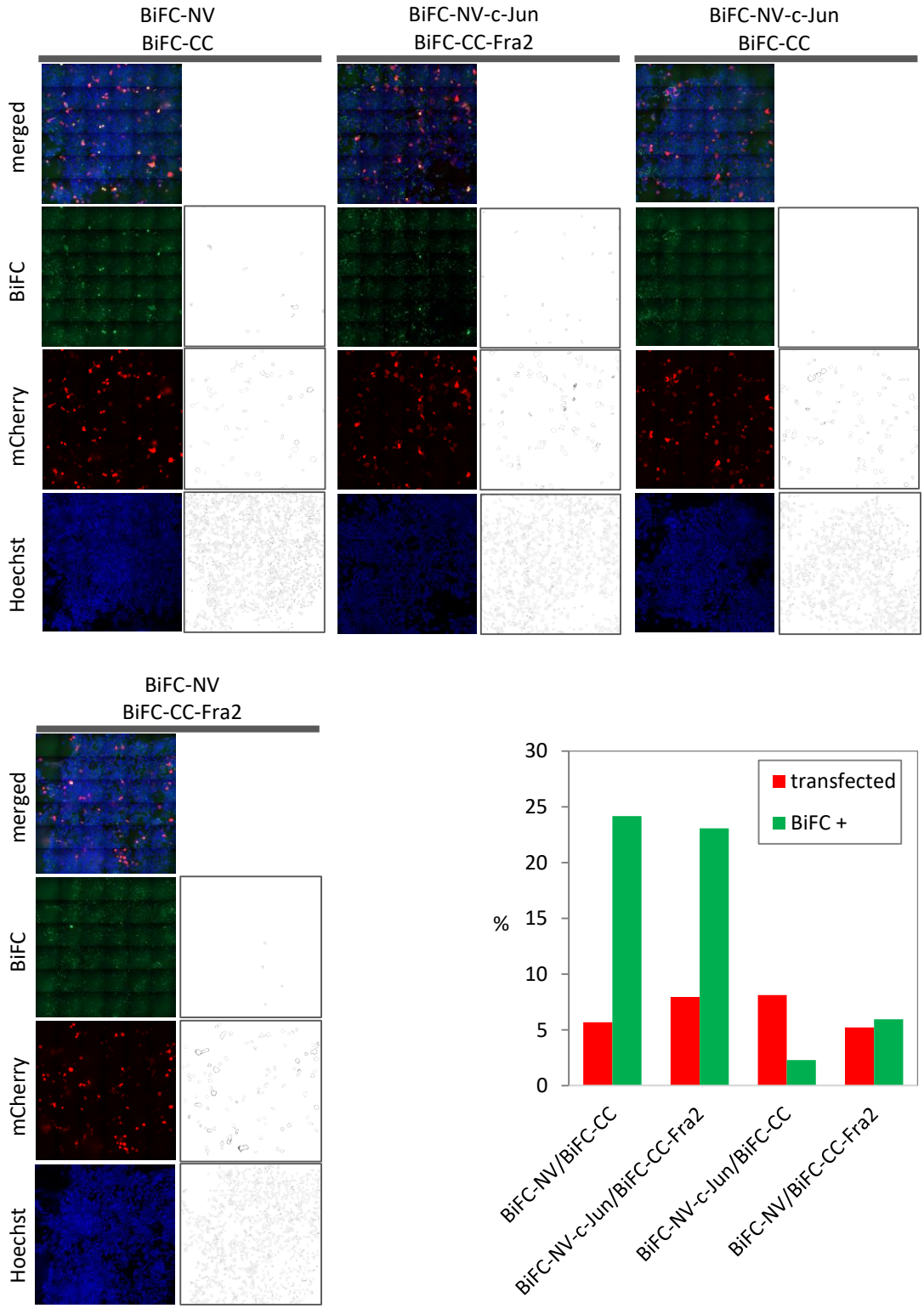
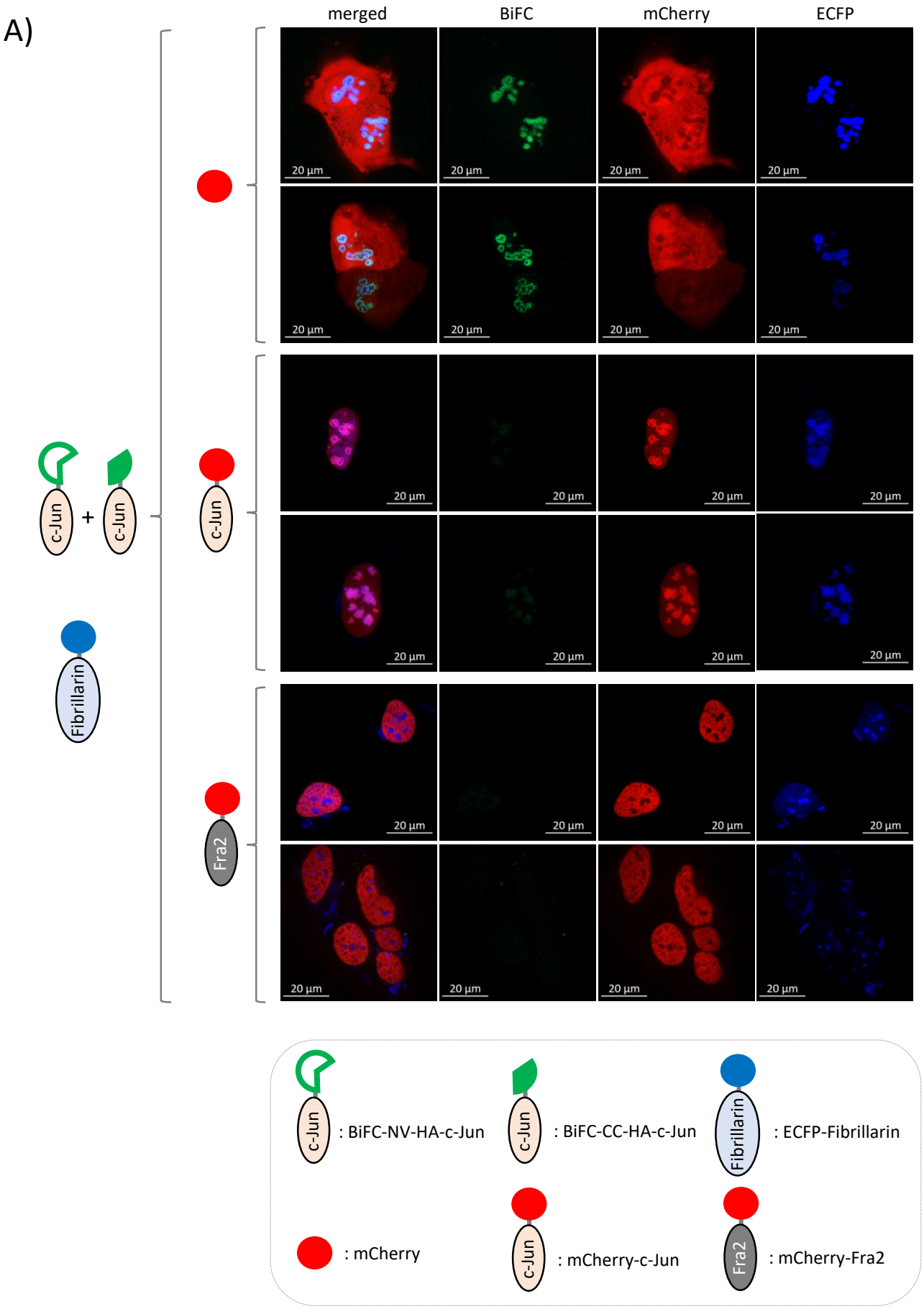
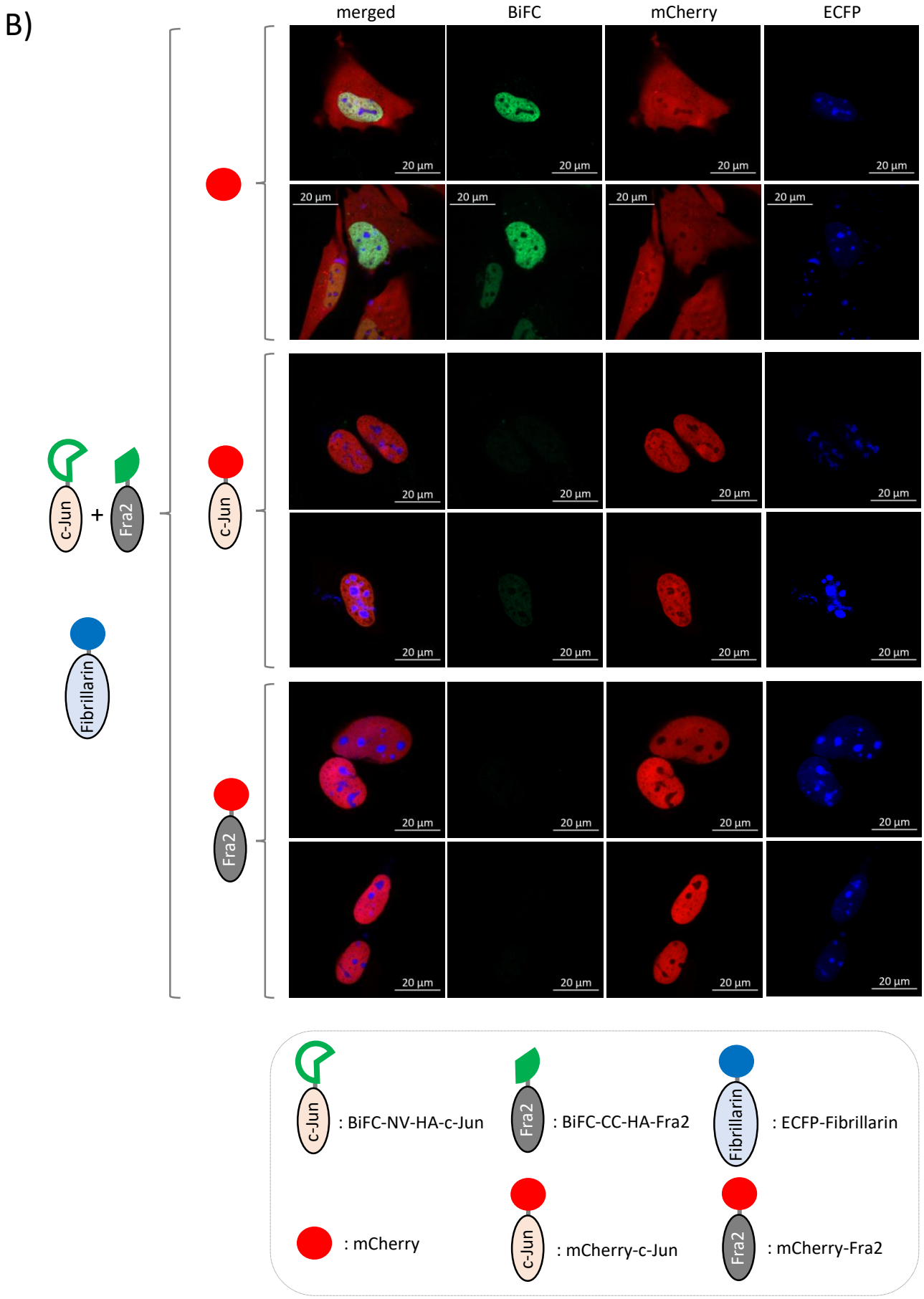


Supplemental figure 1. Complementation of BiFC by formation of c-Jun/Fra2 heterodimer

HEK293T cells were seeded onto 12-well plates. 10ng mCherry and 30ng BiFC expression construct DNA were prepared per condition. The cells were transfected, and next day, after addition of Hoechst 33342 into the cell culture media, the cells were visualized by confocal fluorescent technique. To compare different conditions, A constant laser power and the same exposure time were used for acquisition of all images. Hoechst (nuclei), GFP (BiFC complemented), and mCherry (transfected) signals were recorded as images generated from tiling 6X6 field of views using Zen-blue software (Zeiss). The number of nuclei (total), BiFC positive, and mCherry positive (transfected) were outlined based on the size and signal intensity and counted using ImageJ software (NIH). The percentages of transfected cells (mCherry positive/total (Hoechst positive)*100) and of BiFC complementation (BiFC positive/mCherry positive *100) were calculated using n≥6 of the 6X6-tiled images and averaged values of each conditions were shown as a graph.

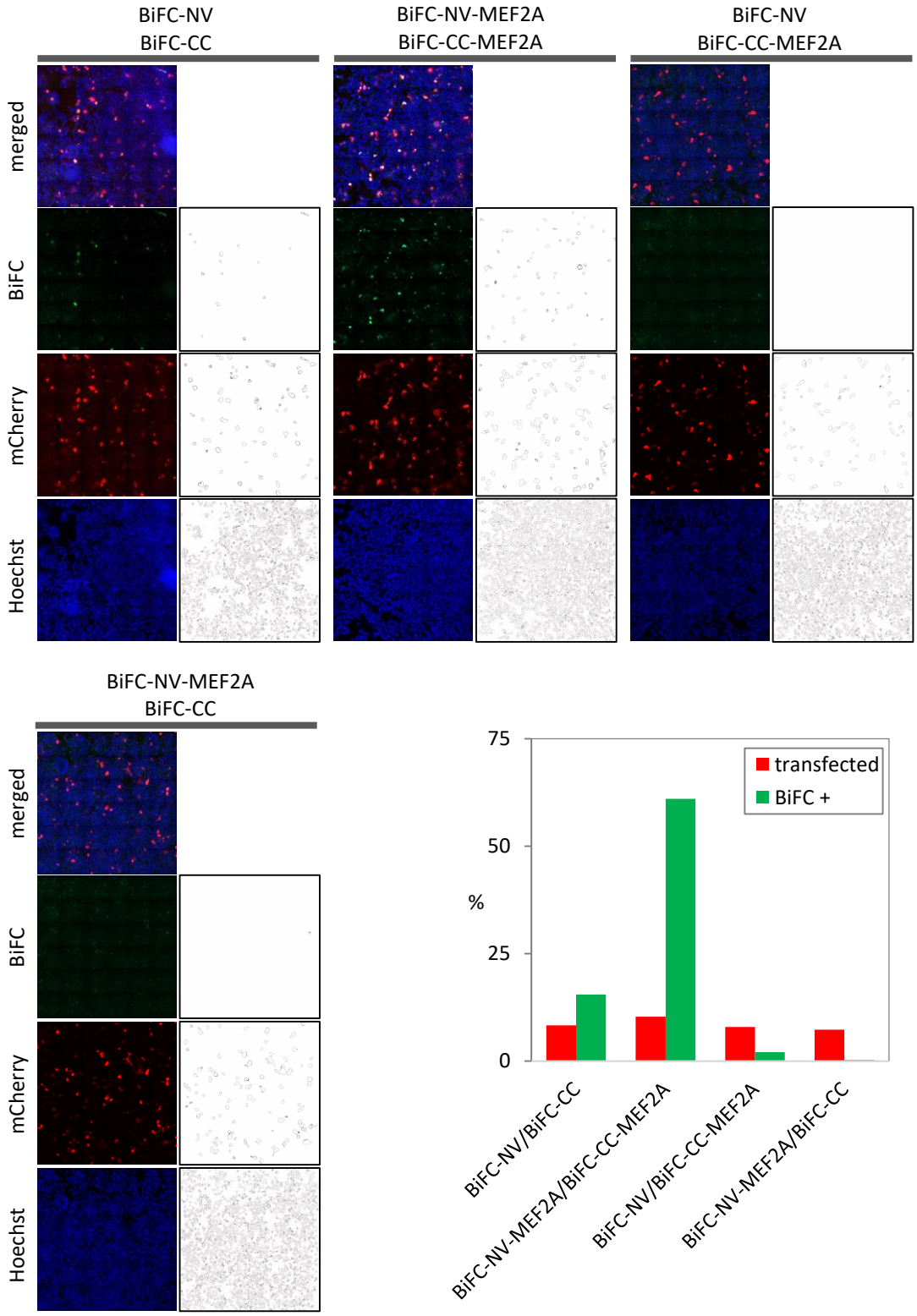


Supplemental figure 2 (Figure 1B extended)



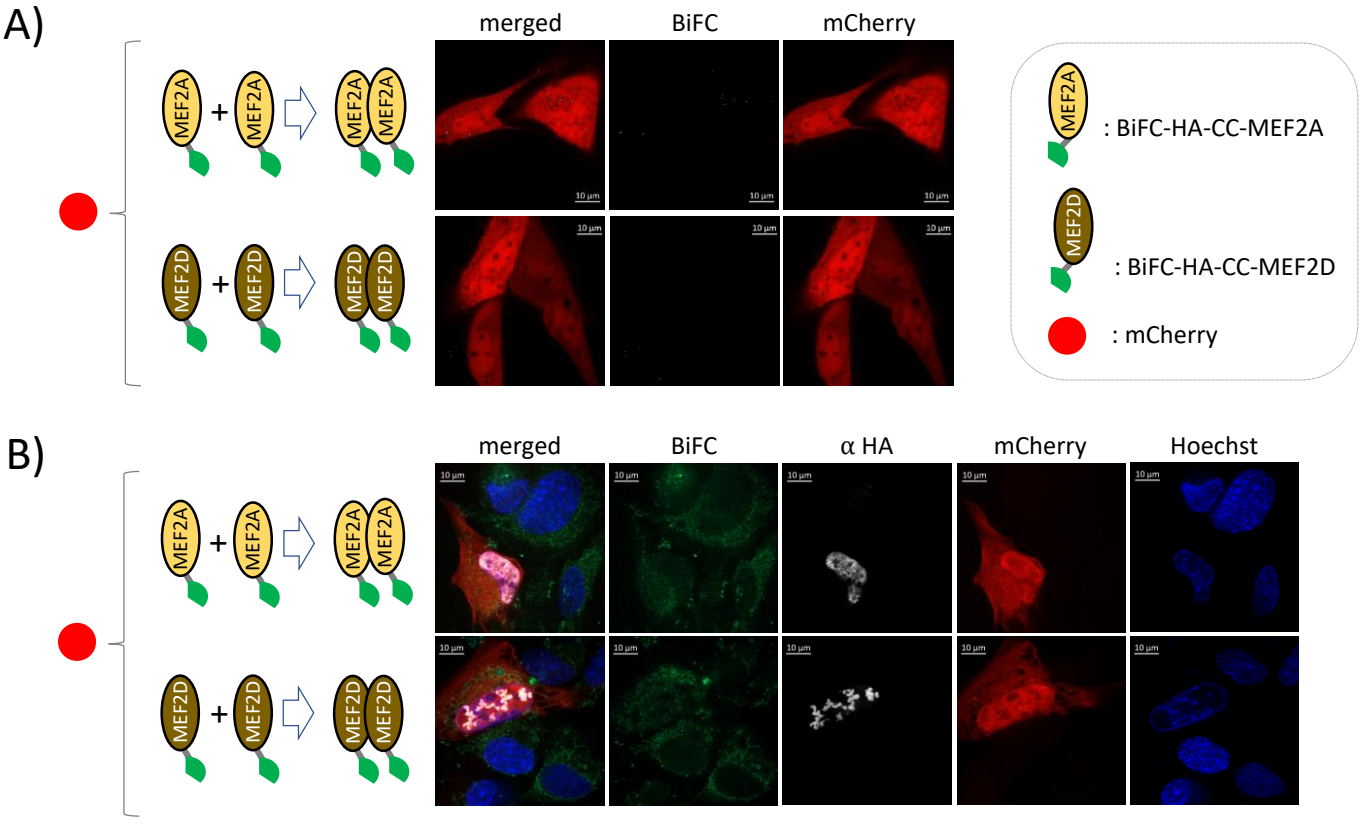
Supplemental figure 2 (Figure 1B extended)

Supplemental figure 2. Visualization of different sub-nuclear localization of a specific AP-1 dimer pair. C2C12 cells were transfected with indicated constructs and subjected to live cell confocal fluorescence microscopy analysis. The BiFC signal (green) was indication of formation of AP-1 dimer paring between BiFC tagged c-Jun/c-Jun homodimer (A) or c-Jun/Fra2 heterodimer (B). CFP signal marks nucleolus by fusing to nucleolar protein, Fibrillarin. Red signal depicts the cellular localization of mCherry, mCherry-c-Jun, or mCherry-Fra2 which competes with BiFC tagged c-Jun or Fra2 for AP-1 dimer formation.



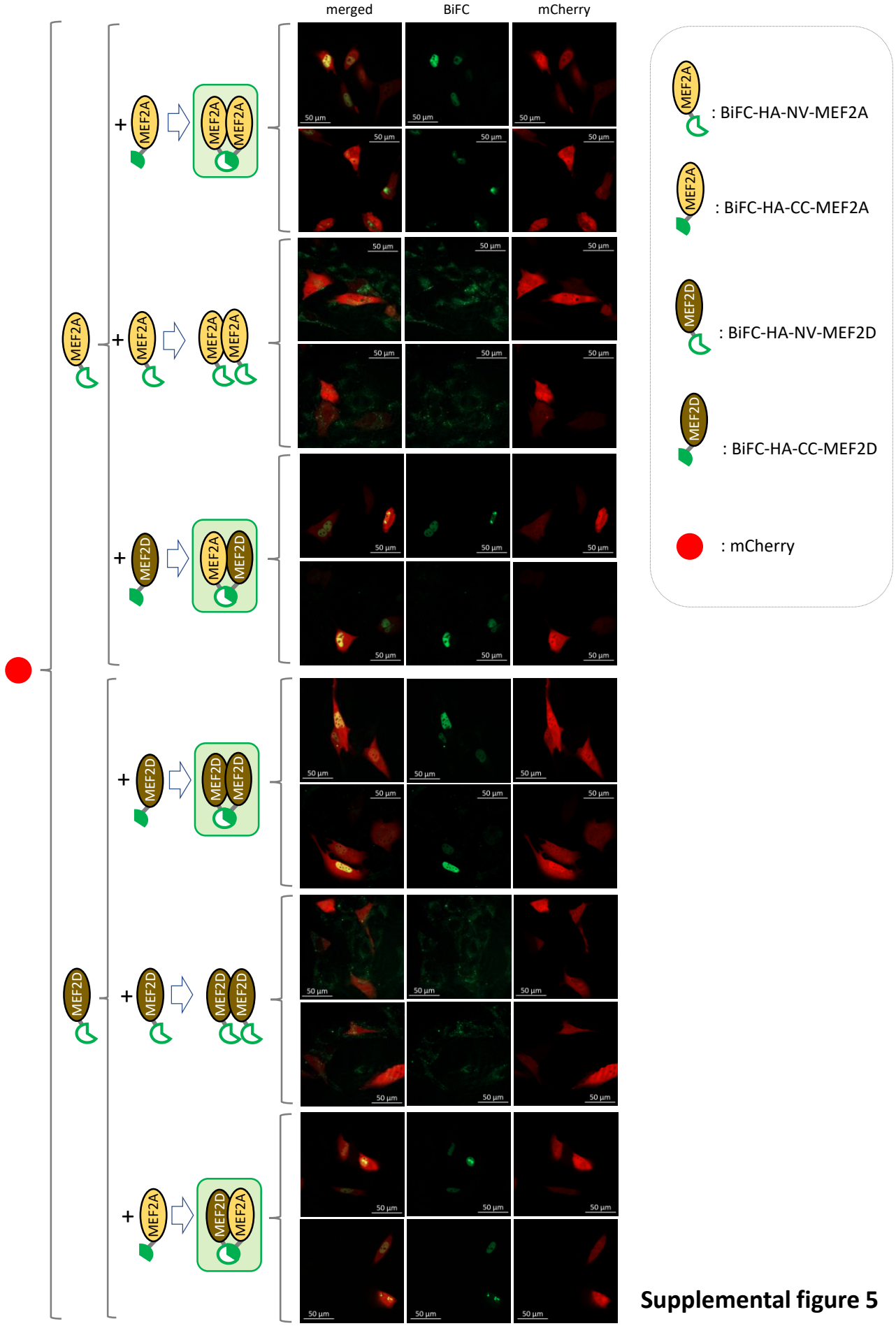
Supplemental figure 3. Complementation of BiFC by formation of MEF2A dimer

HEK293T cells were seeded onto 12-well plates. 10ng mCherry and 30ng BiFC expression construct DNA were prepared per condition. The cells were transfected, and next day, after addition of Hoechst 33342 into the cell culture media, the cells were visualized by confocal fluorescent technique. To compare different conditions, constant laser power and exposure time was used for acquisition of all images. Hoechst (nuclei), GFP (BiFC complemented), and mCherry (transfected) signals were recorded as images generated from tiling 6X6 field of views using Zen-blue software (Zeiss). The number of nuclei (total), BiFC positive, and mCherry positive (transfected) were outlined based on the size and signal intensity and counted using ImageJ software (NIH). The percentages of transfected cells (mCherry positive/total (Hoechst positive)*100) and of BiFC complementation (BiFC positive/mCherry positive *100) were calculated using 6 of 6X6-tiled images and averaged values of each conditions were shown as a graph.



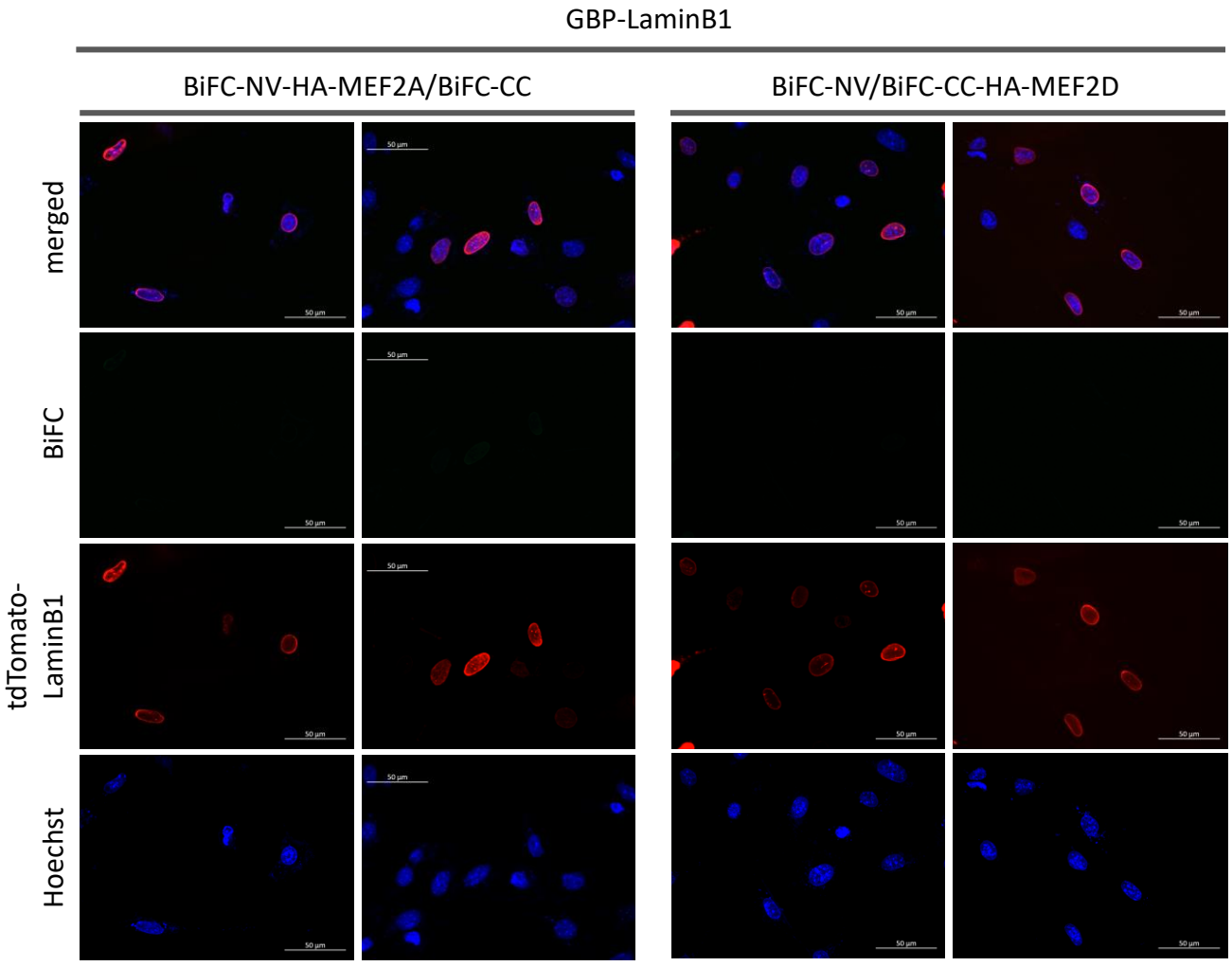
Supplemental figure 4. Visualization of MEF2 dimer pairs by BiFC technique

(A) C2C12 cells were transfected with a combination of the indicated expression constructs in the left side panel. mCherry was included for monitoring transfection. Transfected cells were subjected to live cell imaging by confocal fluorescence microscopy. (B) The transfected cells were fixed and subjected to immunofluorescence analysis. Expression of BiFC-HA tag fusion proteins was confirmed using α HA antibody. Nucleus was stained with Hoechst 33342.

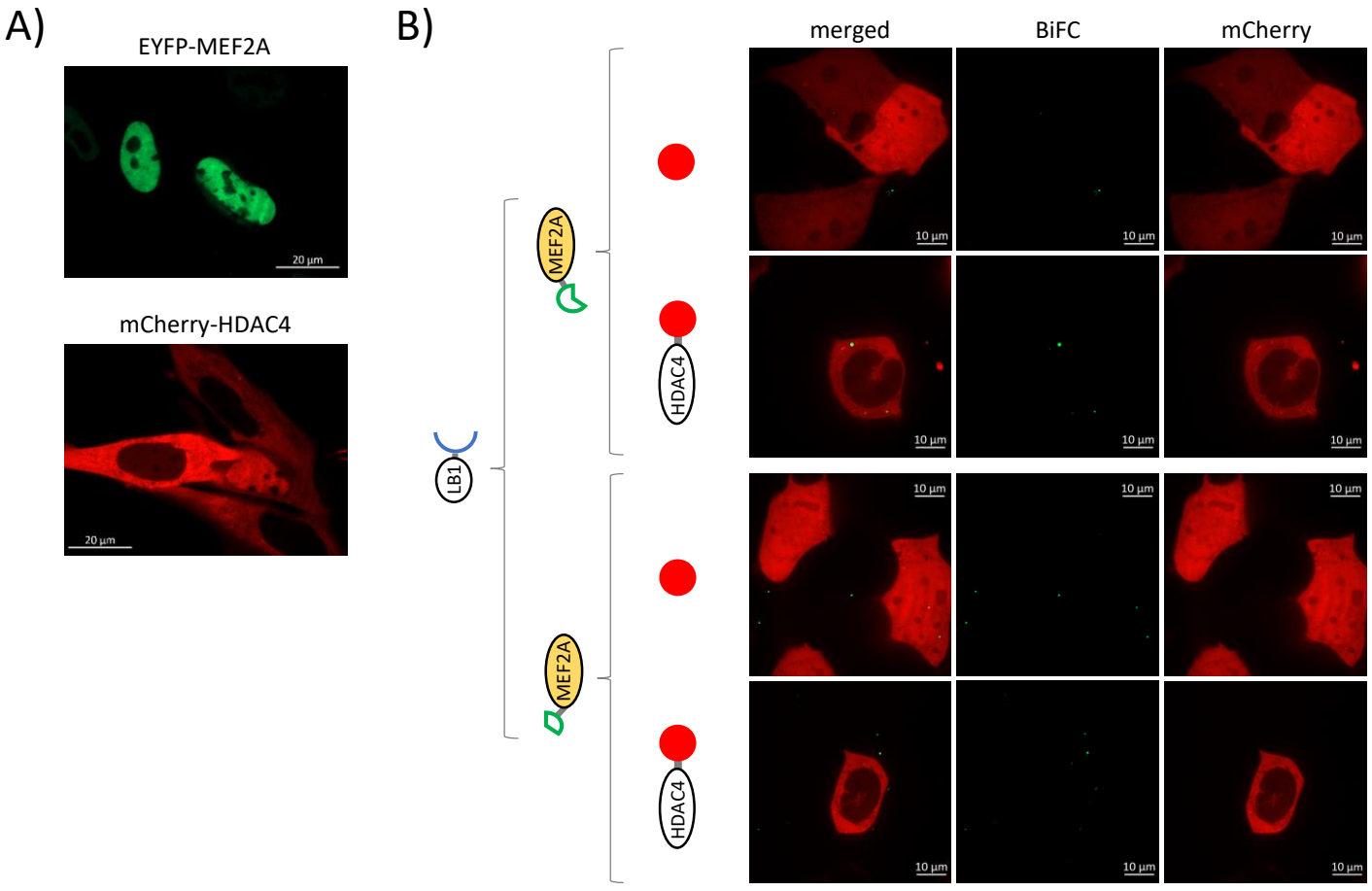


Supplemental figure 5

Supplemental figure 5. Visualization of a specific dimer paring by BiFC technique and 3-way PPI using BiFC and GBP-nanotrap techniques C2C12 cells were transfected with a combination of indicated expression constructs in the left side panel. mCherry was included for monitoring transfection. Transfected cells were subjected to live cell imaging by confocal fluorescence microscopy technique. BiFC signal (green) generation due to complementation and mCherry (red) were depicted in represented micrographs.

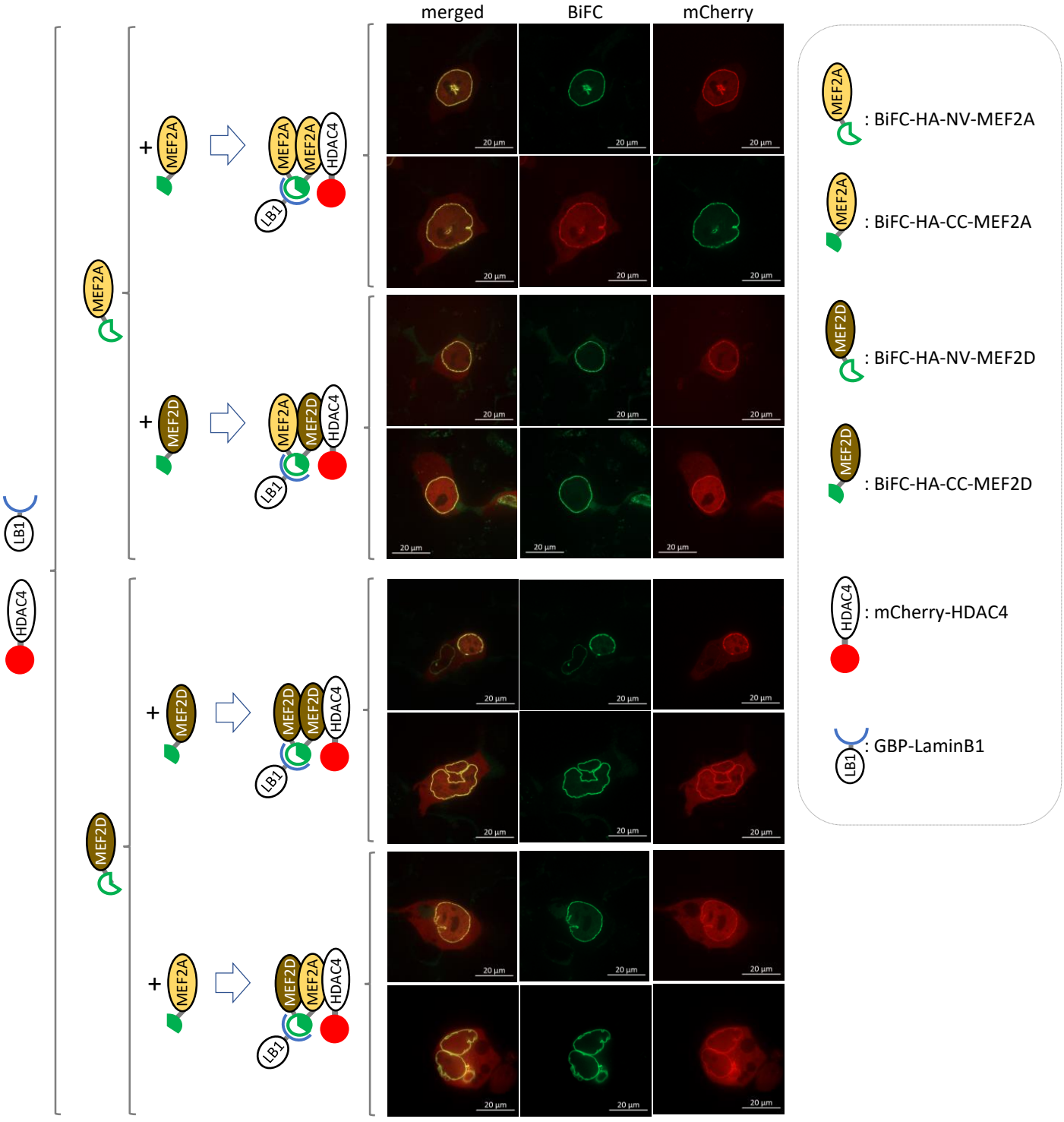


Supplemental figure 6. Visualization of a specific dimer paring by BiFC technique and 3-way PPI using BiFC and GBP-nanotrap techniques C2C12 cells were transfected with a combination of indicated expression constructs. tdTomato-LaminB1 was included for monitoring transfection and marking nuclear envelopes. Transfected cells were subjected to live cell imaging by confocal fluorescence microscopy technique. No BiFC signal (green) generation observed due to no complementation between BiFC tags.



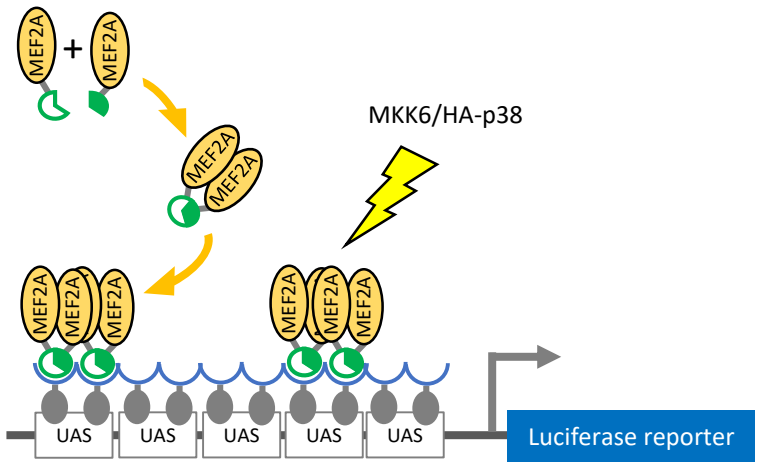
Supplemental figure 7. Complementation of BiFC is required for GBP recognition.

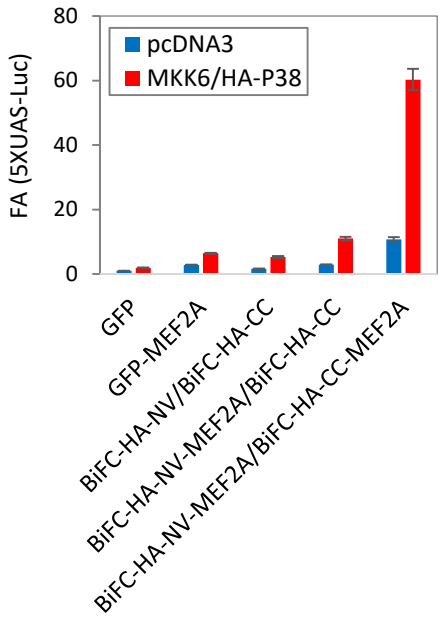
(A) C2C12 cells were transfected with either EYFP-MEF2A or mCherry-HDAC4. Transfected cells were subjected to live cell imaging by confocal fluorescence microscopy. (B) HEK293T cells were transfected with GBP-LaminB1 (GBP-LB1), BiFC-HA-NV-MEF2A or BiFC-HA-CC-MEF2A combined with mCherry or mCherry-HDAC4 expression constructs. Transfected cells were subjected to live cell imaging by confocal fluorescence microscopy. GBP did not recognize BiFC tag without complementation.



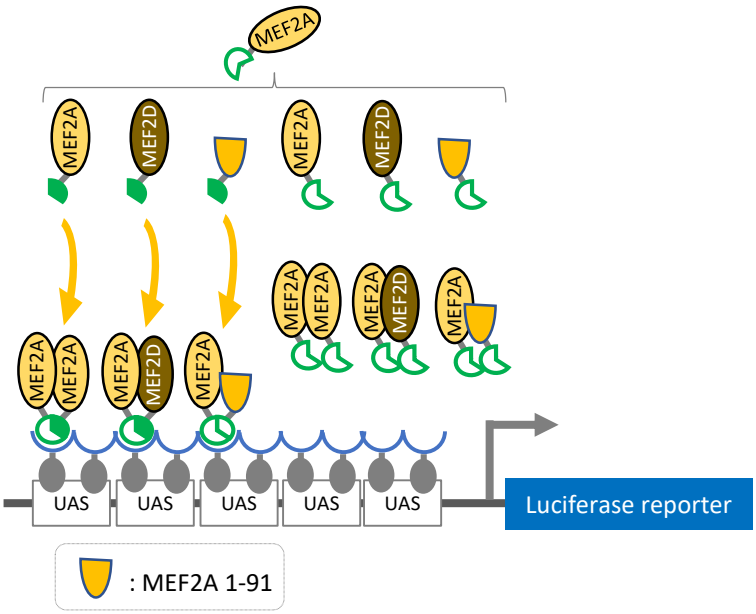
Supplemental figure 8. Visualization of a specific dimer paring by BiFC technique and 3-way PPI using BiFC and GBP-nanotrap techniques HEK293T cells were transfected with a combination of indicated expression constructs in the left side panel. Generation of BiFC signal (green) due to complementation of BiFC tags indicates a specific MEF2 dimer formation. Co-localization of BiFC signal and mCherry-HDAC4 but not mCherry was indicated PPI.

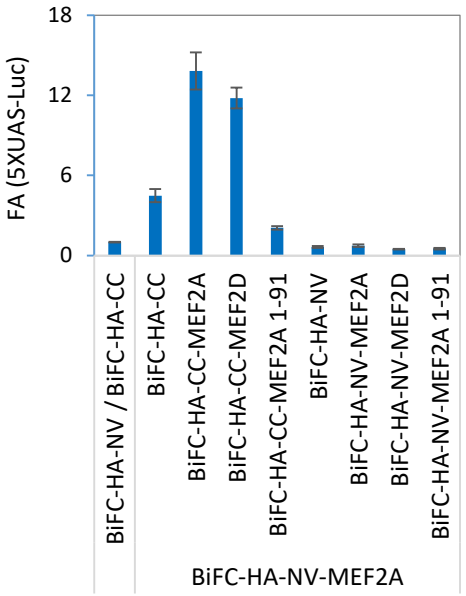
A.





B.





Supplemental figure 9. BiFC-MEF2A dimer was activated by P38MAPK and repressed in the presence of BiFC-MEF2A 1-91

C3H10T1/2 cells were transfected with the indicated constructs in addition to the promoter-less renilla luciferase construct in triplicate per condition. The cells were harvested and subjected to luciferase reporter gene assay. A constant volume of cell lysate was used to determine reporter gene activity (firefly luciferase) and transfection control (renilla luciferase), which was used for standardizing the transfection efficiency. An average of the standardized firefly luciferase values of each condition in triplicate was calculated and fold activation (FA) to control condition was graphed. Error bar = standard deviation, n=3. (A) BiFC-MEF2A dimer activation by MKK6/P38MAPK signing. (B) Repression of BiFC-MEF2 activity by BiFC-MEF2A 1-91.