Supplementary Figure 1. Comparison of BiFC efficiency between fragments derived from enhanced YFP and Venus. (A) Plasmids encoding FLAG-bJun fused to residues 1-172 of enhanced YFP (bJunYN) or Venus (bJunVN), or HA-bFos fused to residues 155-238 of enhanced YFP (bFosYC) or Venus (bFosVC), were cotransfected into COS-1 cells and incubated at 37°C for 12 h. Cells were continuously incubated at 37°C for another 4 h (left panels) or switched to 30°C for 4 h (right panels) before images were acquired. (B) COS-1 cells were cotransfected with plasmids as indicated in A with a plasmid encoding enhanced CFP. BiFC efficiency was calculated as YFP/CFP ratio and the fold increase of median ratio is shown. Note that the median of YFP/CFP ratios derived from Venus fragments decreased after exposure to 30°C for 4 h due to the increase in fluorescence intensity in CFP though the absolute yellow fluorescence intensity was not changed as shown in A. (C) Cells were harvested after image capturing and lysed in Laemmli loading buffer. One tenth of the total lysates was resolved in 10% SDS-PAGE and subjected to immunoblot analysis. The indicated fusion proteins were detected using anti-FLAG and anti-HA antibodies, respectively. The left two lanes show the expression of fusion proteins at 37°C and the right two lanes show the expression of fusion proteins after exposure to 30°C for 4 h.