B www.biochemj.org



Supplementary Figure 1 Visualization of interactions between CK2 α and CK2 β in living cells by BiFc

(A) YFP fluorescence emission and indirect immunofluorescence images of HeLa cells transfected with plasmids expressing the YFP fragments fused to CK2 α or CK2 β as indicated in each panel. Cells were directly observed for YFP fluorescence (panels a, d, g, j, m) or immunostained for YFP (panels b, e, h, k, n) with monoclonal anti-GFP antibody followed by Cy3-labelled secondary antibody to enhance the signal as described in Experimental procedures. The diagrams on the left of the images represent the experimental strategies used. (**B**) Western blot analysis of the levels of protein expression. Cells extracts corresponding to panels m, n, o in Figure 1A were analysed by Western blotting using anti-CK2 α (lane 1) and CK2 β (lane 2) antibodies.

YFP raw fluorescence (FITC) is approximatively 20 times lower [31] in conditions of YFP bimolecular complementation (panel m) than with full-length YFP (panels a and g). Since expression levels of YN-CK2 α and YC-CK2 β are lower than endogenous CK2 α and CK2 β (Figure 1B), this low fluorescence emission could be explained by competition of the endogenous CK2 subunits for bimolecular complex formation. Note that the monoclonal anti-GFP antibody used in this study recognizes only full-length YFP (panels b, h) but not YC or YN fragments (panels e, k) (unpublished observation). Therefore, indirect immunofluorescence with this mouse anti-GFP antibody can be used to enhance the detection of bimolecular complex formation (panel n).



Supplementary data Figure 1