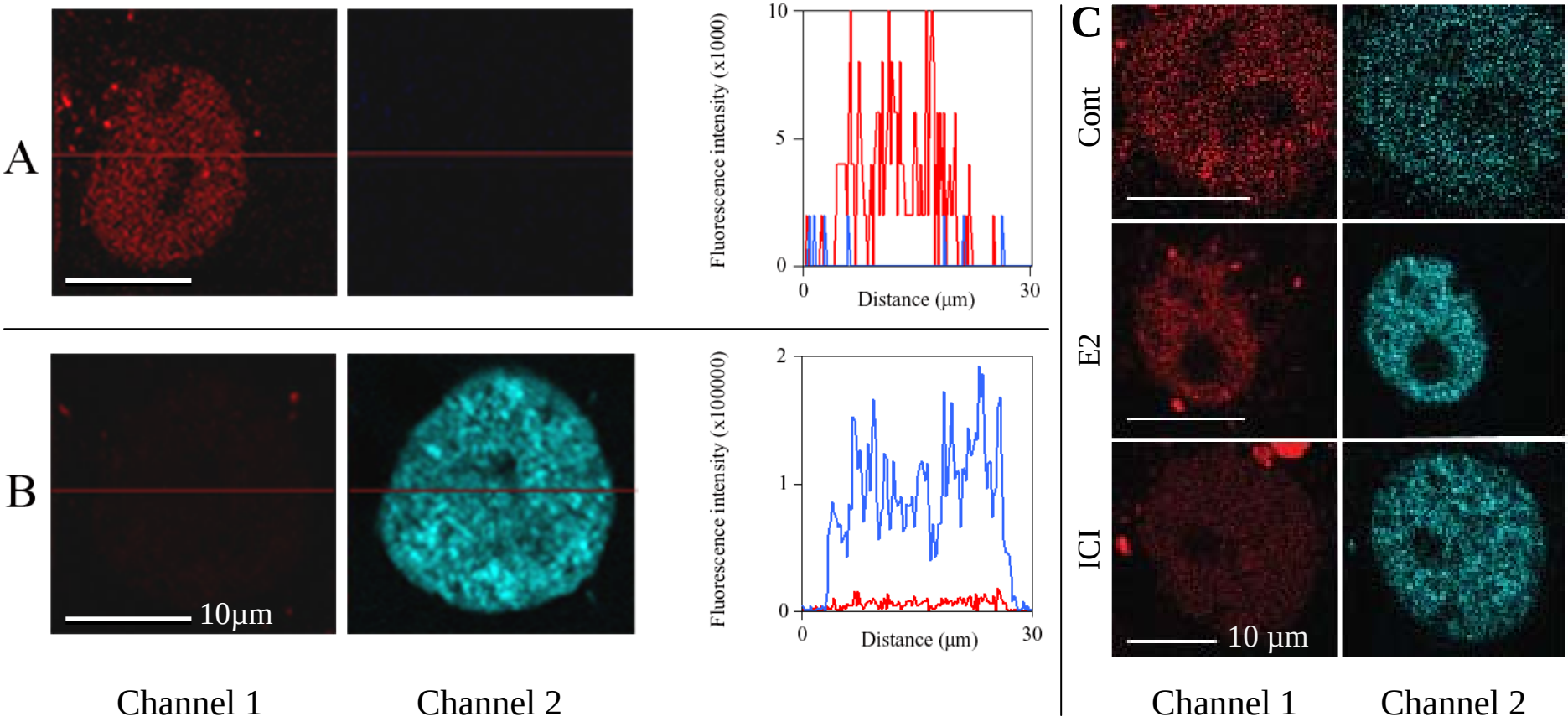
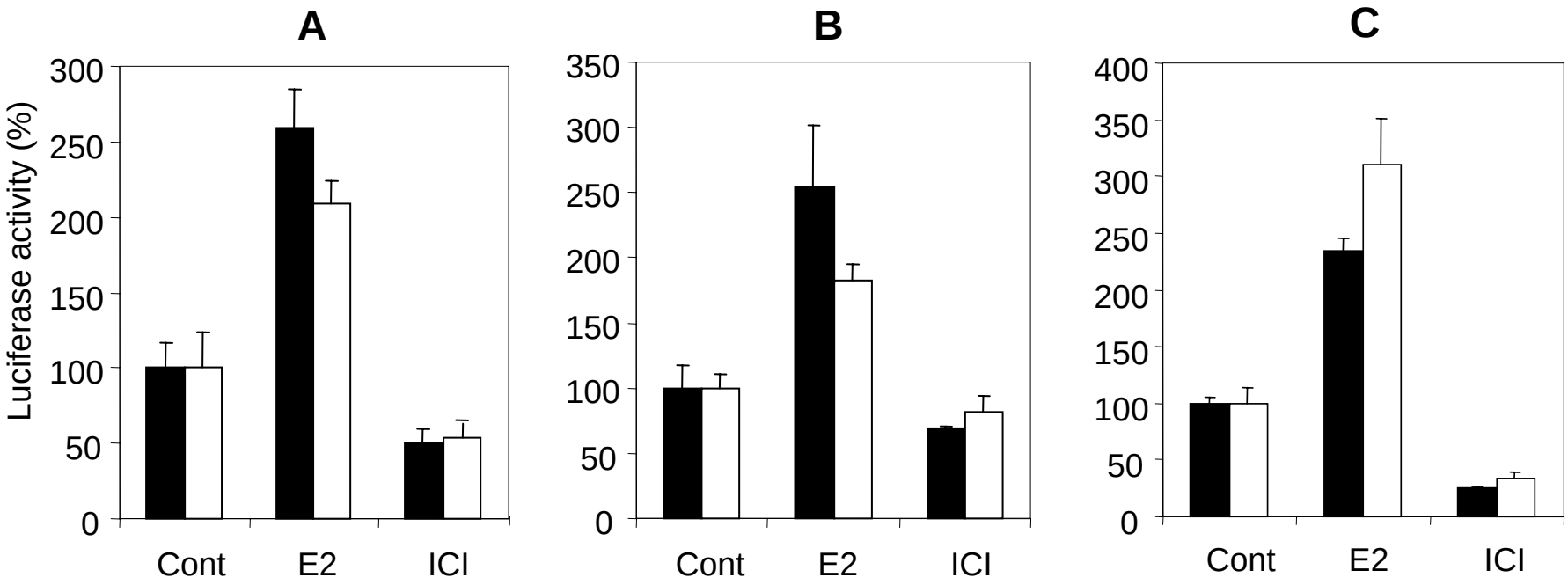
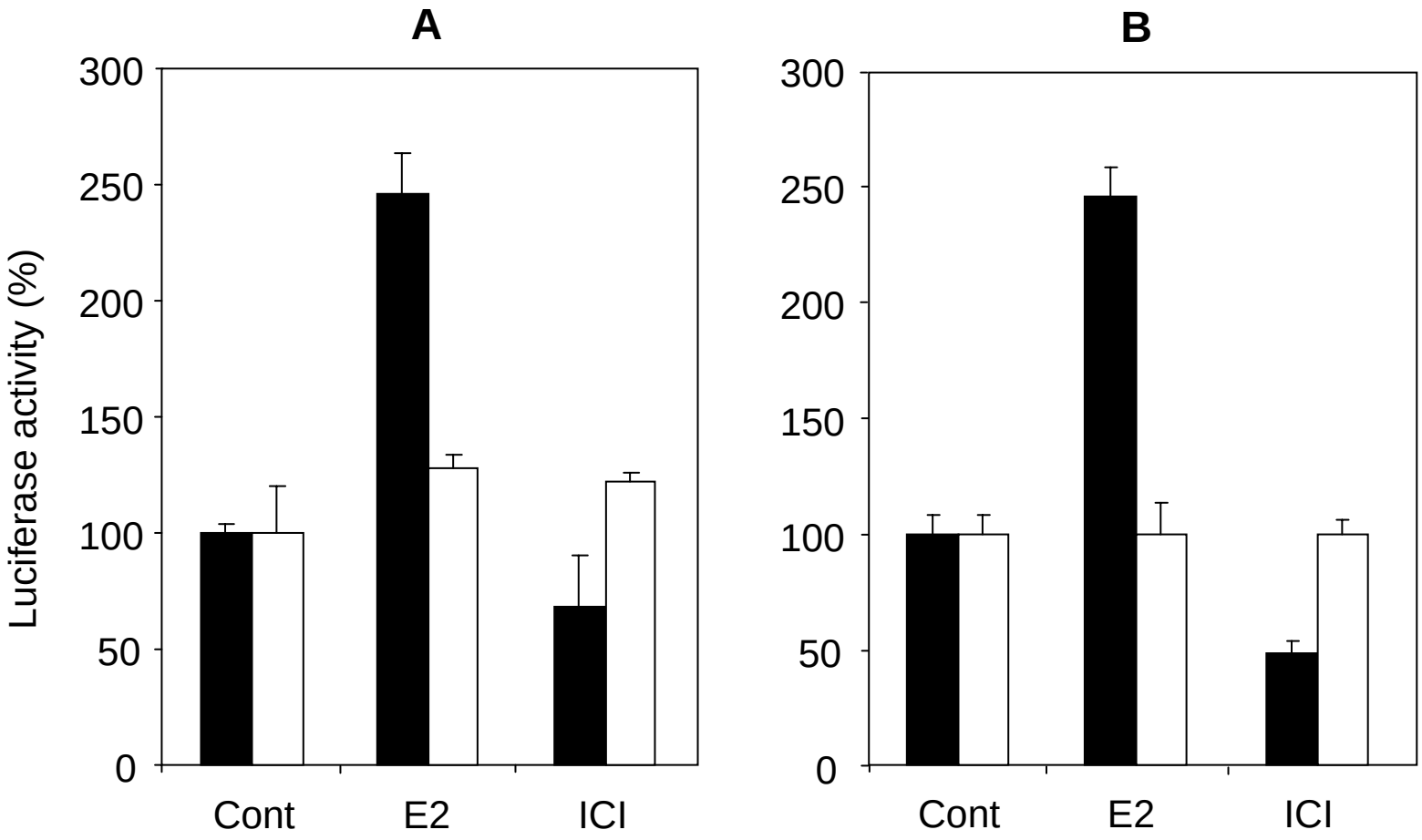



Supplementary Figure 1. **Cross talk control**. Two photon false colours images at 950 nm and fluorescence intensity along the red line of a nucleus of a COS-7 cell expressing **A** : only mCherry-TIF2 and **B** : only cer-ERβ. There is no significant cross talk between channel 1 and channel 2. The images from the two channels are shown at the same threshold, lightness and contrast. The central panel shows a plot of the two intensity values across the image along the red line shown in the left panels. **C** Two photon false colour images of the nucleus of cells co-expressing cer-ERα (channel 2) and mCherry-TIF2 (channel 1).




Supplementary Figure 2**. Transactivation assay**. Relative luciferase activity for different proteins and their respective fusion proteins, with 100% activity for the control EtOH (CONT) and two ligand conditions : agonist E2 and antagonist ICI.
**A** : ERα (black) and cer-ERα (white); **B** : ERβ (black) and cer-ERβ (white); **C** : cer-ERα (black) and cer-ERα + mCherry-TIF2 (white).
Mean +SD of triplicates of two independent experiments. Mean of the relative luciferase activity for control versus agonist condition are significantly different for each protein (Student test, α=0.995 for proteins of A and C and ERβ in B, α=0.95 for cer-ERβ in B). The fold inductions for cer-ERα is lower than for ERα, as is often observed for fusion proteins.




Supplementary Figure 3. **Transactivation assays of mutants**. Relative luciferase activity for two fusion proteins and their mutant, with 100% activity for the control EtOH and two ligand conditions : agonist E2 and antagonist ICI.
**A** : cer-ERβ (black) and cer-ERβ AF2 - mutated ligand dependent Activation Function 2 - (white);
**B** : cer-ERα (black) and cer-ERα DBD - mutated DNA Binding Domain (white). Mean +SD of triplicates. Mean of the relative luciferase activity for control versus agonist condition are significantly different for each wild type protein (Student test, α=0.995) and significantly equal for cer-ERα DBD in B (α=0.95).