

**Supplementary Figure 3**. SUMO-2/3 and some but not all PIAS family proteins can inhibit global Ser2P of Pol II CTD. (a) Like SUMO-1, SUMO-2 and SUMO-3 could also enhance inhibition of Ser2P by PIAS1. HeLa cells were transfected with or without PIAS1 and SUMO-1, SUMO-2, and SUMO-3 as indicated. Two days after transfection the cells were processed for IF staining analysis of Ser2P. Transfected cells were indicated by arrows. (b) Experiments were performed as in (a) except Ser5P of Pol II CTD was examined. (c) The ability of various PIAS family proteins to inhibit Ser2P was tested by ectopic expression of various PIAS proteins with or without SUMO-1 in HeLa cells, followed by IF staining analysis using anti-Ser2P antibody. Note that inhibition of Ser2P was observed for PIASy and PIASx $\beta$ , but not for PIAS3 and PIASx $\alpha$ .