

Supplementary Figure 9. SUMO inhibits global transcription in various cells. (a) In addition to PIAS1, PIASy and PIAS α could also repress global transcription. HeLa cells were transfected with various PIAS proteins and SUMO-1 as indicated. One day after transfection, the cells were incubated with BrUTP for 30 min before being processed for analyzing the levels of BrU incorporated into RNAs by IF staining using an anti-BrU antibody. Note that PIAS3 and PIAS α failed to repress global transcription, consistent with their inability to inhibit Ser2P. (b) IF staining showing that knockdown of UBC9 in A549 cells resulted in elevated levels of BrU incorporation. The experiments were as in (a) except knockdown of UBC9 with two different shRNAs was performed. (c) Knockdown of UBC9 in A549 cells resulted in increased levels of global transcription as measured by total amount of RNAs from the same number of control and shRNA-transfected cells. (d) BrU incorporation assay showing reduced global transcription in Senp1-/- and Senp2-/- MEFs. The control, Senp1-/- and Senp2-/- MEFs were cultured in the presence of BrUTP for 30 min before being processed for IF staining using anti-BrU antibody.