



Supplementary Figure 2. Repression of the global level of Ser2P is associated neither with histone deacetylation and sumoylation, nor sumoylation of Cyc T1, Cyc H and MAT1. (a) Ectopically expressed PIAS1 inhibited global Ser2P of Pol II CTD in a sumoylation-dependent manner. HEK293T cells were transfected with wild-type PIAS1 or E3 ligase-deficient PIAS1m mutant in the presence or absence of co-expressed SUMO-1. Two days after transfection the cells were harvested and WB analyses were performed to examine the effect on Ser2P, Ser5P, total Pol II, and expression of HA-PIAS1 and HA-PIAS1m as indicated. (b) IF staining analysis showing that ectopic expression of PIAS1 or PIAS1m with or without SUMO-1 had no effect on acetylation of histones H3 and H4 in HeLa cells. (c) IF staining analysis showing that ectopic expression of PIAS1 or PIAS1m with or without SUMO-1 had no effect on Ser5P of Pol II CTD. (d) The same samples in (a) were analyzed for potential sumoylation on CycT1, Cyc H, MAT1 and HDAC1 by WB analysis. Arrow indicates the expected size of each of the unmodified protein. (e) Ectopic expression of PIAS1/SUMO-1 had no significant effect on H3 and H4 sumoylation. Core histones were prepared from HEK293T cells transfected with indicated expression constructs for two days and were analyzed by Western blot using H3 and H4 antibodies.