



**Supplementary Figure S5. The S283A mutation does not significantly affect the stability of Cdc25A in H1299 cells in G2.** H1299 inducible cells were blocked in G1/S by serum deprivation followed by thymidine block, then released in 10% serum-supplemented RPMI medium containing 1  $\mu\text{g/ml}$  doxycyclin. At 6 h (A) and 7.5 h (B) post-release when cells were predominantly in early and late G2, respectively, the protein synthesis inhibitor cycloheximide was added (100  $\mu\text{M}$  final concentration) for the indicated times. Cells were then subjected to Western blot analysis using an anti-HA antibody allowing the specific detection of the HA-tagged Cdc25A constructs. The obtained signals were quantified using actin as a normalization control. A representative experiment is shown.