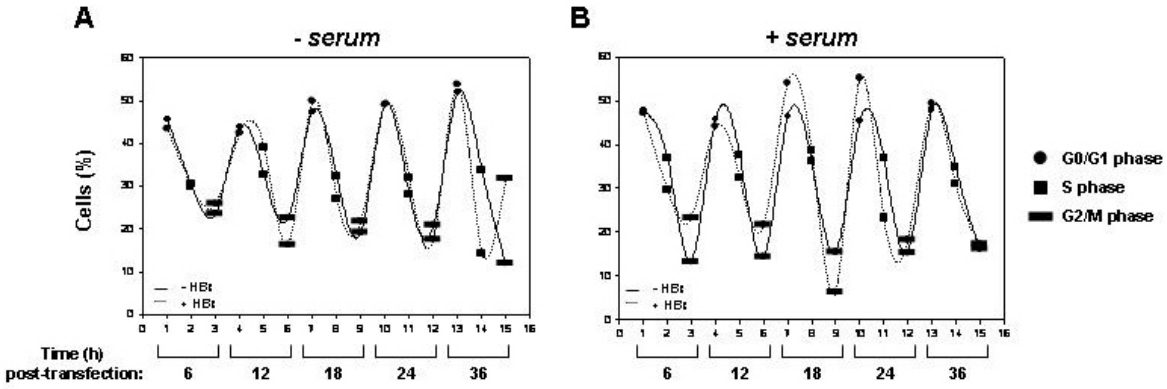
Supplementary data

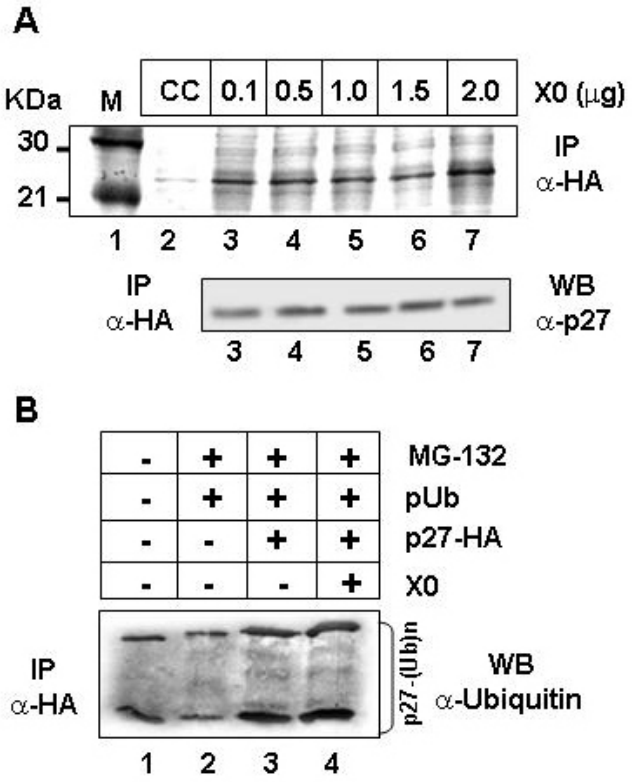
Suppl. 1

Pattern of cell cycle in the presence of HBx. 293 cells were transfected with X0-IRES-EGFP vector and grown in the absence (A) or presence (B) of 10% serum for indicated time periods. The EGFP positive cells were gated and analyzed by flow cytometry. Cell cycle analysis was performed as described in materials and methods and plotted using Sigmaplot



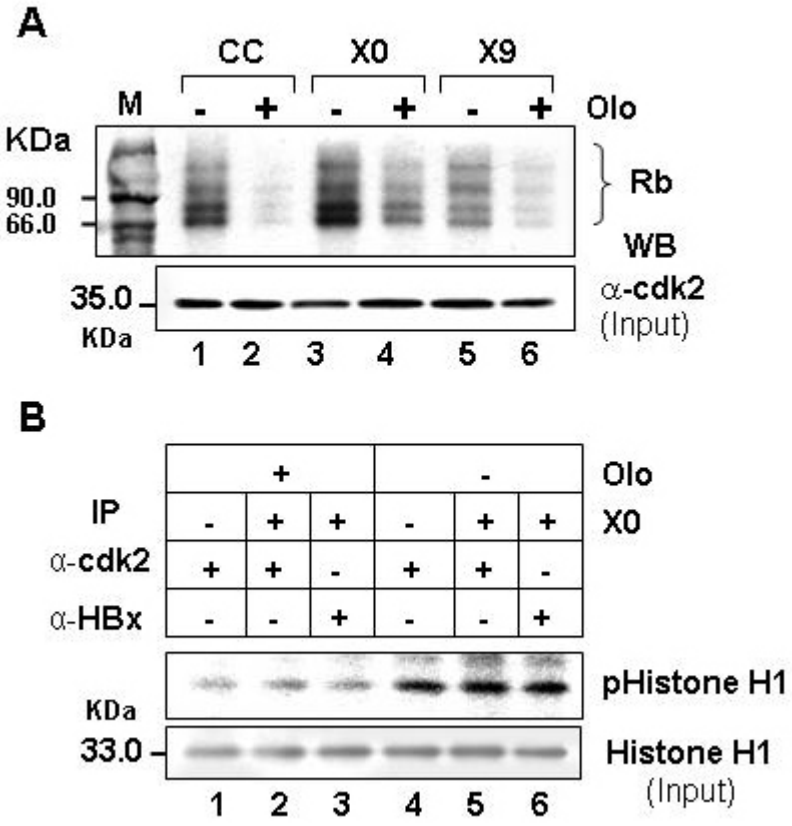
Suppl. 2

Translational and post-translational stability of $p27^{Kip1}$ (Mass - 27 KDa) in the presence of HBx. (A) The co-translational stability of p27 was determined by transfecting Huh7 cells with p27-HA (1 μg) and increasing amounts (0.1-2 μg) of X0. After pulse labeling for 30 min, the cell extracts were immunoprecipitated with anti-HA antibody and autoradiographed. (B) To investigate the level of ubiquitinated p27, cells were transfected with p27-HA and/or X0 and pUb and grown in the presence of MG132 for 3h. Cell extracts were immunoprecipitated with anti-HA antibody followed by western blotting with anti-ubiquitin antibody.



Suppl. 3

Induction of cellular cdk2 activity by HBx. X0- and X9-transfected and synchronized Huh7 cells were harvested 10 h after serum stimulation and the extracts were immunoprecipitated with anti-cdk2 or anti-HBx antibodies and used for *in vitro* phosphorylation of different substrates in the absence (-) or presence (+) of olomoucine (Olo). (A) phosphorylation of recombinant GST-Rb by cdk2 immunoprecipitates. The cdk2 level (input) was measured by western blotting. (B) phosphorylation of histone H1 with cdk2 and HBx immunoprecipitates. CC, cell control; M, molecular weight markers.



Suppl. 4

Signaling pathways in the regulation HBx interaction with cyclin-cdk complex. Huh7 cells were transfected either with X0 alone or with the expression vectors for physiological inhibitors of Src kinases, PI3K and MAPK, i.e., CSK, DN-PI3K or N17Ras respectively. After 48h, cell extracts were immunoprecipitated (IP) either with anti-HBx antibody or anti-cdk2 anti-serum followed by western blotting for cyclin E. Total levels of cyclin E and cdk2 were also measured in these extracts. One-tenth of cell extracts was also probed for active and/or total Erk 1/2, PI3-Akt.