Supplementary Information

Westendorp et al. "E2F7 represses a network of oscillating cell cycle genes to control S-phase progression."

Supplementary materials and methods

Immunofluorescence staining

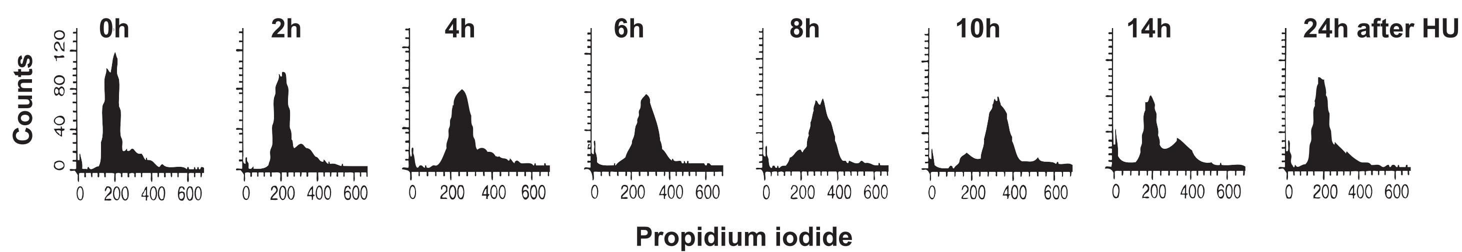
Asynchronously growing cells were cultured on glass coverslips and treated with doxycycline or vehicle for 24 hours. Then cells were washed twice with PBS and fixed with 4% formaldehyde in PBS for 10 minutes. After washing, cells were permeabilized with 0.1% Triton-X in PBS for 10 minutes. Subsequently, cells were pre-incubated with 5% bovine serum albumin (BSA) for 30 minutes, followed by incubation with an antibody against phosphorylated histone H3 (PH3, Millipore 06-570) for 1 hour at room temperature, in a 1:100 dilution in PBS containing 1% BSA. Cells were washed and incubated with an anti-rabbit IgG conjugated to Alexa Fluor 568 (1:500, A-11011, Invitrogen). Nuclei were stained by 20 minutes incubation with TO-PRO-3 (1:200 Invitrogen) and coverslips were mounted to microscopic slides with FluorSave reagent (Merck). Images were taken on a Bio-Rad 2100MP laser scanning system connected to a NIKON TE300 microscope, and percentages of PH3 positive nuclei were determined.

Transient transfections

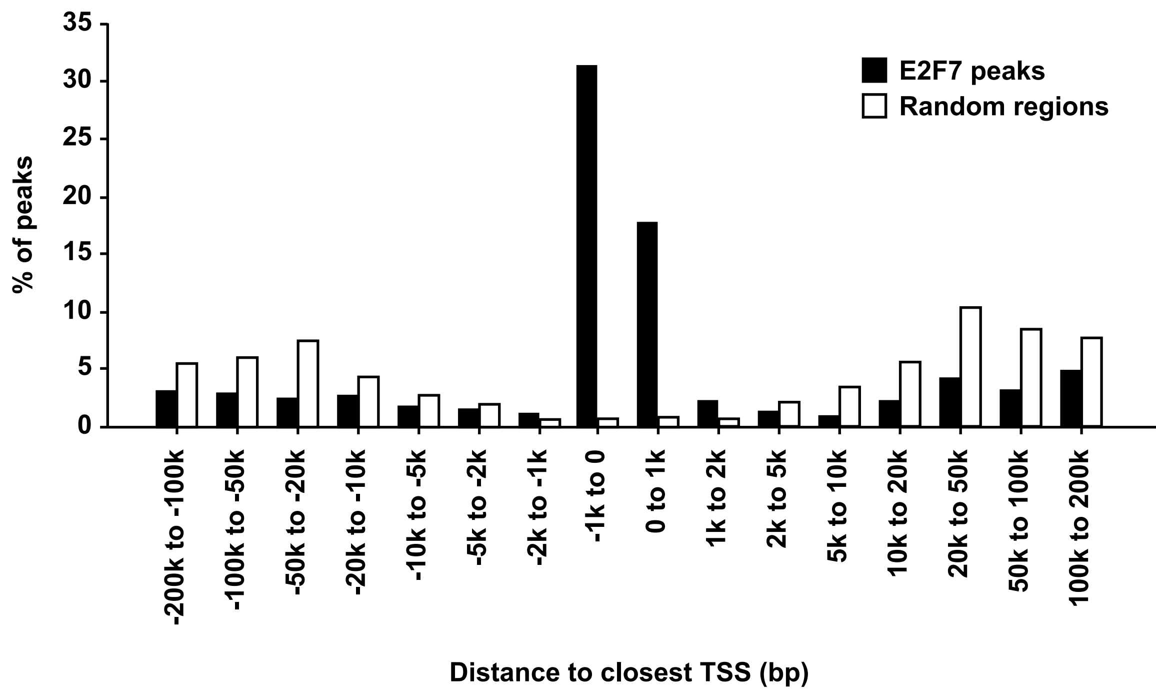
The 293T kidney epithelial cell line was used for transient transfections as described before (1). Briefly, cells were cultured with DMEM containing 10% FBS in 100 mm dishes. When the cells were ~30% confluent, calcium-phosphate mediated transfections were done with 12.5 μg pEGFP N3 plasmid (Invitrogen) containing cDNA encoding either EGFP-tagged wild type E2F7 or DNA-binding domain mutant E2F7.Cloning strategy is described in the main paper. After 48 hours, the near-confluent cells were processed for flow cytometry analysis using propidium iodide.

Reference

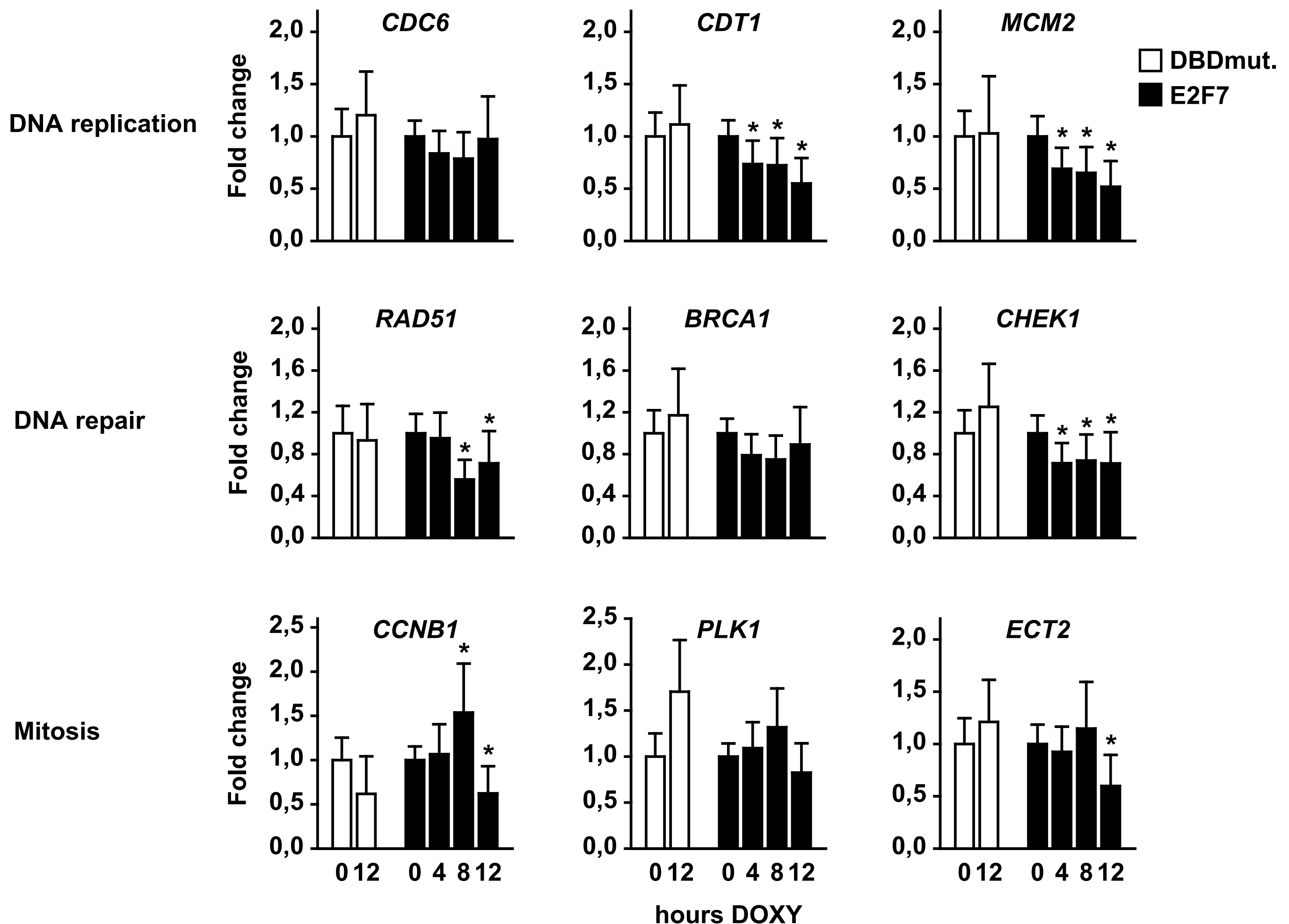
1. Li,J., Ran,C., Li,E., Gordon,F., Comstock,G., Siddiqui,H., Cleghorn,W., Chen,H.Z., Kornacker,K., Liu,C.G., et al. (2008) Synergistic function of E2F7 and E2F8 is essential for cell survival and embryonic development. *Dev. Cell.,* **14,** 62-75.



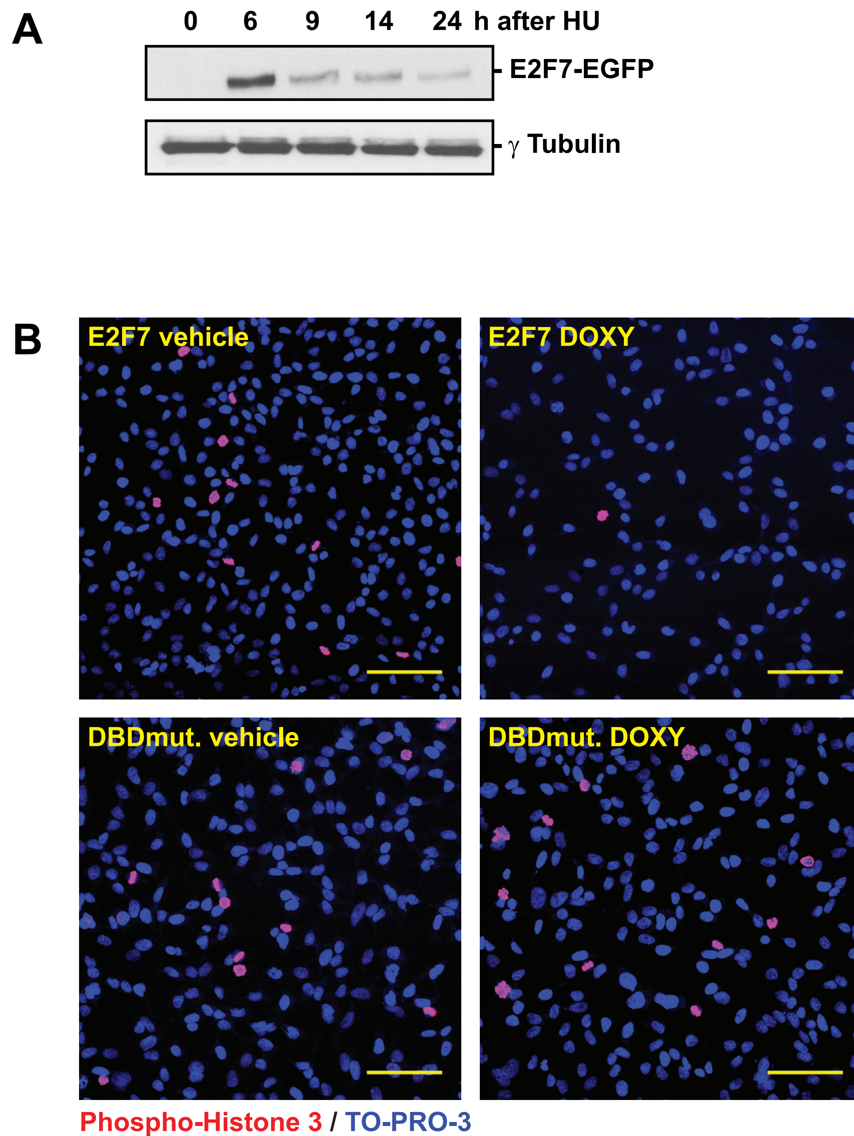
Supplementary Figure S1 DNA content of HeLa cells after release from HU synchronization, measured with propidium iodide staining and flow cytometry. The 2n peak was set to 200.

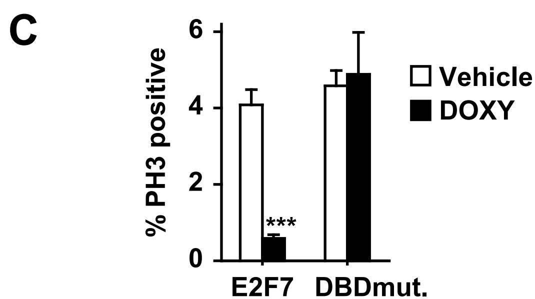


Supplementary Figure S2 Distribution of E2F7 ChIP-seq peaks according to proximity to closest transcription start site (TSS). For comparison, the distribution of randomly sampled DNA regions according to nearest TSS was taken.

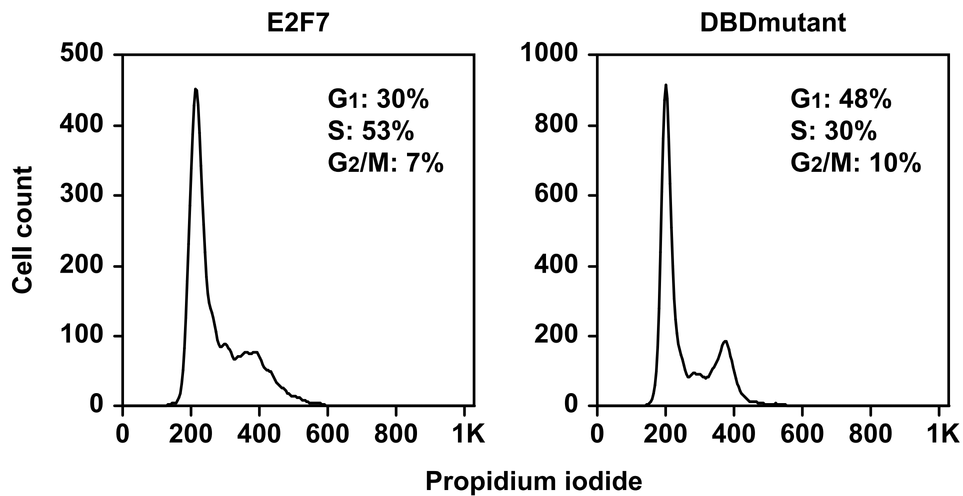


Supplementary Figure S3 Gene expression of G_1/S- and G_2/M-regulated genes involved in DNA replication, DNA repair and mitosis at different time point after doxycycline (DOXY) dependent induction E2F7-EGFP (E2F7) or E2F7-DBDmut-EGFP in asynchronously growing cells, analyzed by qPCR. Graphs represent average ± SEM (n=3). Asterisks indicate $P<0.05$ versus its corresponding 0h time point.





Supplementary Figure S4 Reduced mitotic index in HeLa cells with inducible overexpression of E2F7. (**A**) GFP immunoblots on lysates from HU-synchronized cells, where E2F7-EGFP was induced at the time of HU release (0h). Gamma-tubulin blots served as loading control. (**B**) Immunofluorescence staining of phospho-Histone H3 (PH3) on asynchronously growing cells, induced to express E2F7 or E2F7 DBDmutant for 24 hours. Scalebars correspond with 50 mm. (**C**) Quantification of percentage PH3 positive cells per field, Data represent average +/- SEM of 5 fields; triple asterisk indicates $P<0.001$ versus vehicle.



Supplementary Figure S5 S-phase accumulation after transient overexpression in 293T kidney epithelial cells. Cells were transfected with either EGFP-tagged wild type E2F7 or DBDmutant E2F7 using the calcium phosphate method. After 48 hours, cells were harvested, stained with propidium iodide, and DNA content was measured on a flow cytometer. Only EGFP-positive cells were analyzed. Plots indicate representative examples of three independent replicates.

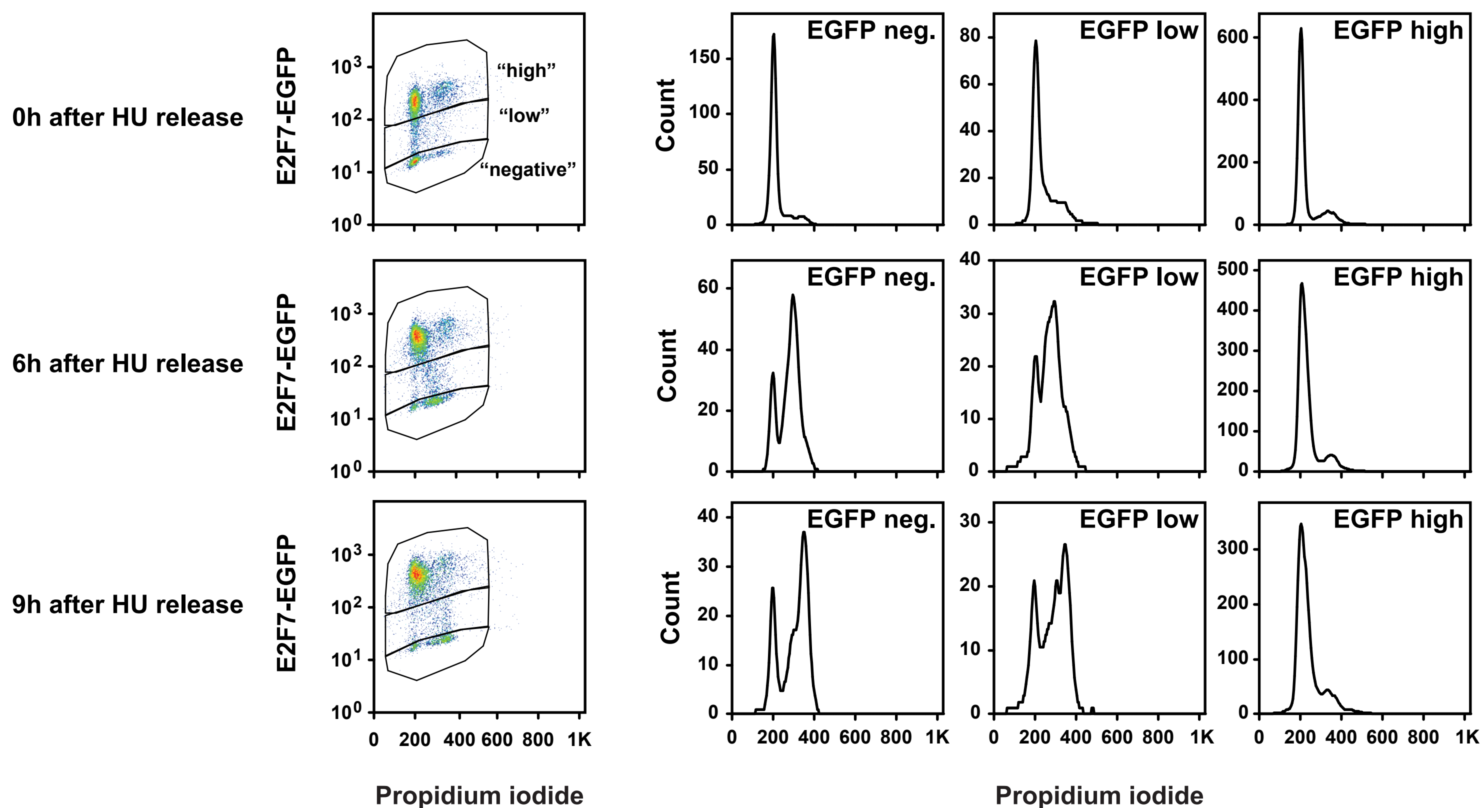


Figure S6 Levels of ectopic E2F7 correlate with severity of S-phase arrest. Cells were synchronized with HU and pretreated with doxycycline from 12 hours before HU release. Based on flow cytometric E2F7-EGFP signal, cells were gated into negative, low, and high E2F7-EGFP expression, and DNA content histograms at 0, 6, and 9 hours after release from hydroxyurea synchronization were compared.