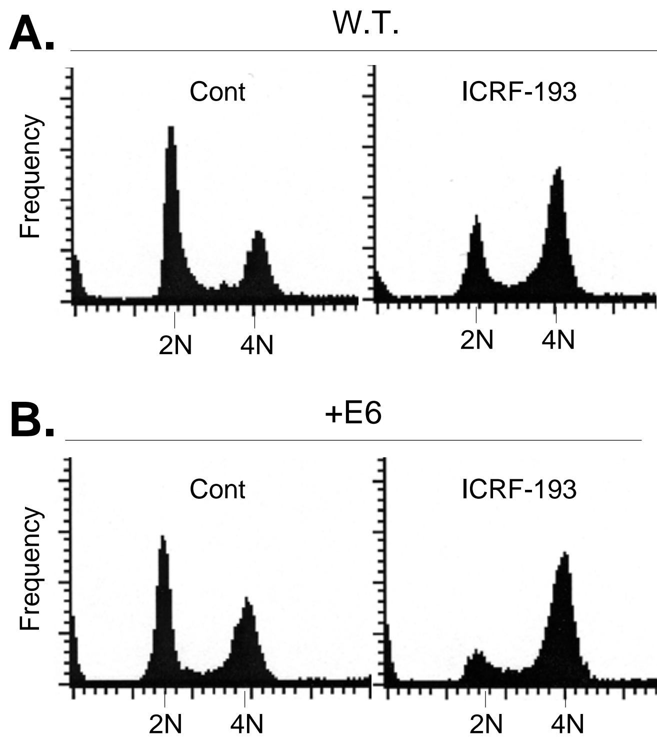
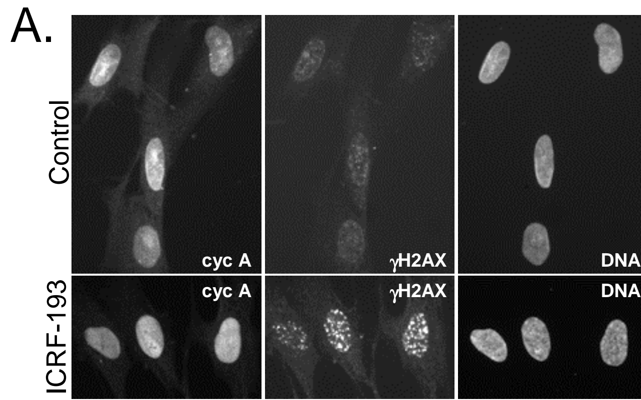
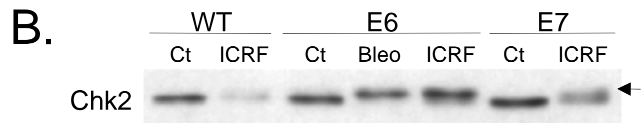
Baus, Suppl. Figure 1

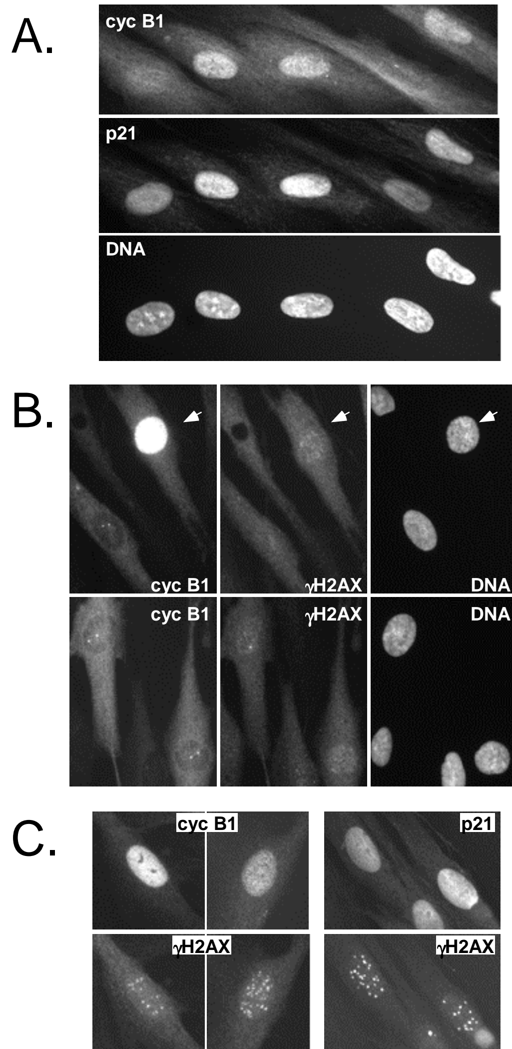


Baus, Suppl. Figure 2





Baus, Suppl. Figure 3



Supplementary material

Supplementary Figure 1: Cell cycle profile of the experiments presented in Figures 1 and 3.

Flow cytometric (FACS) analysis of synchronized wild-type (**A**) or HPV16-E6-expressing (**B**) fibroblasts that were used for immunofluorescence experiments shown in Figure 1 (wild-type) and Figure 3 (E6). Cells were pre-synchronised by a release from quiescence (G0) achieved by contact inhibition, synchronised at the G1/S boundary by incubation with hydroxyurea and the drug was added 2 hours after release from the G1/S block. Control cells were fixed in methanol 26 hours after replating at the time when most control cells had entered mitosis (hence pronounced peak of cells with 2N DNA content). ICRF-193-treated cells were fixed three hours later.

Supplementary Figure 2: Exposure to ICRF-193 results in γ-H2AX foci accumulation.

A. Co-localisation of cyclin A and γ-H2AX in untreated (control) and ICRF-193-treated NHF. For comparison, see Supplementary Figure 2B and C. The cells were fixed in para-formaldehyde and simultaneously stained with mouse monoclonal anti-γ-H2AX (Texas Red) and with rabbit polyclonal anti-cyclin A (FITC) antibodies.

B. Western blot showing Chk2 SDS-PAGE mobility shift in response to ICRF-193. The protein extracts were prepared from wild-type fibroblasts (WT) and NHF expressing HPV16-E6 (E6) or HPV16-E7 (E7) oncogenes, as described in the legend for Figure 6. The cultures were exposed to ICRF-193 (24h) and Bleomycine (12 hrs, only E6 cells).

Supplementary Figure 3: γ-irradiated NHF accumulate nuclear cyclin B1.

A. Co-localisation of cyclin B1 and p21 in γ-irradiated NHF. The cells were fixed in methanol and simultaneously stained with mouse monoclonal anti-cyclin B1 (Texas Red) and with rabbit polyclonal anti-p21 (FITC) antibodies.

B and **C**. Double immunofluorescence showing co-localisation of γ-H2AX and cyclin B1 (or p21) in untreated cells synchronized at G2/M boundary (**B**) and γ-irradiated (**C**) NHF. Arrows in **B** point at the cell in prophase. The cells were fixed in methanol and simultaneously stained with mouse monoclonal anti-cyclin B1 (Texas Red) and with rabbit polyclonal anti-γ-H2AX (FITC) antibodies.