Supplementary material:

Figure 1: Weak occurrence of post-mitotic nuclei in drug-treated synchronised NHF.
Percent of cells in mitosis or with aberrant post-mitotic nuclei (PMN) in non-treated (Ct), ICRF-193- and bleomycin-treated synchronised NHF (cf. Figure 4). For each situation, experiment has been done in duplicate and at least 500 cells were scored.

Figure 4: ICRF-193-treated synchronised E6 cells enter mitosis after a brief arrest in G2. Time-lapse analysis of E6 cells synchronised at G1/S transition and then released in the presence of bleomycin and ICRF-193. Mitotic cells were scored each hour.

Figure 5. DNA damage leads to irreversible cell cycle exit in G2.
The cells were synchronised at G1/S boundary and released in the absence (Ct) or the presence of drugs (treat.). After 24 hours, the drugs were removed by extensive washing (the same conditions were used as for release from hydroxyurea) and cell cycle re-entry was assessed after a given period by monitoring the BrdU incorporation (by indirect immunofluorescence of fixed cells). For the first time-point, BrdU was added when the control cells entered mitosis and BrdU incorporation was scored 24 hours later. For other time points, BrdU was added 24 hours prior to fixing cells. At least 500 cells were scored for each situation.

A series of experiments was carried out to test whether NHF can re-enter mitosis after shorter exposure to the drugs. We applied similar protocol as used previously by Kaufmann & Kies (Kaufmann & Kies, 1998). We treated the cells for 15 min and 2 hours with either drug and monitored entry into mitosis for the next five hours after washing the drugs (in the presence of nocodazole to trap mitotic cells). Whereas after shorter treatment with ICRF-193, a significant
population of NHF could re-enter mitosis (25-50% compared to the control), most of the cells exposed to bleomycine failed to enter mitosis during the same period. This suggested that damage inflicted after 15 min by this drug was sufficient to block mitotic entry.

Figure 6. Active pocket proteins are required for cell cycle exit in G2 after genotoxic stress. Kinase activity and Western blot analysis of cyclin A immunoprecipitates isolated from non-treated (Ct), ICRF-193- and bleomycin-treated (6hr) asynchronously growing wild-type (WT) and E7 cells. Arrows point to hyper-phosphorylated (3) and hypo-phosphorylated (1) cyclin A-associated Cdk1 isoforms. Note that, in comparison to cyclin B1-associated kinase, cyclin A-associated kinase is affected only modestly after 6 hr of treatment. This repeatedly observed phenomenon is probably due to cyclin A-Cdk2 complexes present in S-phase cells that are little affected by drug-treatment.