Supplementary Table and Figure Legends

**Supplementary** **Table SI: Cell cycle distribution of control cultures and after the different treatments of HPV positive cell lines.**

**Supplementary** **Table SII: Cell cycle distribution of control cultures and after the different treatments of HPV negative cell lines.**

**Supplementary** **Table SIII: Values of the linear quadratic parameters, α and β, of both cervical cancer cell lines SiHa and HeLa after radiation alone and after radiation + hyperthermia.**

**Supplementary Figure S1**: **Expression of p53 with western blot in cell cultures and with immunohistochemistry in patient biopsies after radiation and hyperthermia treatment.** A, All HPV-positive cell lines show an induction of p53 after a treatment including hyperthermia (42⁰C for 60 min). B, In the p53wt HPV-negative cells lines radiation alone can already to cause an induction of p53. In the p53mut and p53null HPV-negative cells, no increase of p53 is observed. C and D, Additional patient biopsies, demonstrating the p53 induction after hyperthermia. E, Quantification of immunofluorescence staining of ten fields per condition. From left to right: SiHa (Fig. 1C, upper panel), HeLa (Fig. 1C, lower panel), Xenograft I (Fig. 1D), Patient E (Fig. 1E), Patient F (Fig. S1C), Patient G (Fig. S1D). Staining was significantly higher for all samples treated with HT compared to untreated samples (\*, P<0.001). HT, hyperthermia; RT, radiation.

**Supplementary Figure S2**: **Expression of p53 with western blot in SiHa cells after radiation and E6-siRNA treatment, and p53 and E6 after hyperthermia with immunohistochemistry in patient biopsies and xenograft tumors.** A, E6-siRNA in combination with RT induces p53 in SiHa and HeLa cells. B and C, Additional patient biopsies, demonstrating downregulation of E6 after HT. D, In untreated SiHa cells, the level of p53 is too low to be detected. After a 30-min treatment at 42⁰C, a small accumulation of p53 is noticed, while expression of E6 is less intense compared to control. After a 60-min treatment at 42⁰C, all cells are p53 positive, and cells no longer express E6. E, Quantification of immunofluorescence staining of ten fields per condition. From left to right: SiHa (Fig. 2A), HeLa (Fig. 2B), Xenograft I (Fig. 2C), Patient E (Fig. 2D), Patient F (Fig. S2B), Patient G (Fig. S2C), SiHa cells treated with 30 or 60 min HT (Fig. S2D). Green bars represent p53, red bars E6. Graph bars indicated with \* are significantly different from untreated samples (\*, P<0.001). HT, hyperthermia; RT, radiation.

**Supplementary Figure S3**: **Expression of p53 and E6 with immunohistochemistry and western blot in SiHa and HeLa cells after radiation, hyperthermia and cDDP treatment in the presence or absence of bafilomycin, chloroquine and MG132.** A, Immunofluorescence staining of DAPI (blue), E6 (red) and p53 (green) are shown for different conditions. High levels of p53 are observed after incubation with a proteasomal inhibitor (MG132), while there is no p53 seen after incubation with the lysosomal inhibitor (Bafilomycin) in HeLa cells. B, Western blot analysis of p53 in SiHa cells only show p53 detection after inhibition of the proteasomal pathway in untreated, radiation (RT) and cisplatin (cDDP) conditions. Any hyperthermia (HT) treatment, combined with RT or cDDP or HT alone, is seen to yield a p53 accumulation without the presence of any lysosomal or proteasomal inhibitor. C, Quantification of immunocytochemistry staining of ten fields per condition. From left to right: SiHa (Fig. 3C), HeLa (Fig. 3D), SiHa (Fig. 3E), HeLa (Fig. S3A). Green bars represent p53, red bars E6. All graph bars were significantly different from the untreated samples (\*, P<0.001). HT, hyperthermia; RT, radiation; cDDP, cisplatin.

**Supplementary Figure S4**: **Induction of apoptosis determined using Nicoletti assay in HPV-positive and HPV-negative cells after radiation, hyperthermia and combined treatment in the presence or absence of P53-siRNA,cDDP, chloroquine or MG-132.** A, Nicoletti assay showing apoptosis in SiHa and HeLa cells. Only after a treatment including hyperthermia, significantly higher percentages of apoptosis are observed. B, Nicoletti assay showing apoptosis in HPV-positive cells. Only after a hyperthermia or hyperthermia and radiation, significantly higher percentages of apoptosis are detected compared to the untreated situation. After transfecting cells with p53-siRNA prior to hyperthermia apoptotic levels almost return to the untreated levels. C, Nicoletti assay showing apoptosis in HPV-negative cells. In the p53wt cells, radiation alone already causes apoptosis. Induction of apoptosis is not observed in p53mut or p53null cells. (D) and Cleaved caspase-3 (E) analysis on SiHa (left) and HeLa (right) cells. Positive cells are detected after hyperthermia, or hyperthermia and radiation. Scrambled p53-siRNA prior to hyperthermia also induces apoptosis. F, Western blot analysis demonstrating only p53 induction of scrambled p53-siRNA prior hyperthermia. HT, hyperthermia; RT, radiation; RT+HT, radiation and hyperthermia. \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001