Supplementary materials and methods

Cell lines
Wild-type (MCF-7 and HBL100) and mutated (MDA-MB-231, R280K and T47D, L194F) P53 breast tumor cell lines were purchased from ATCC (American Type Culture Collection, Manassas, VA). Cells were routinely grown at 37°C with 5%CO2 in RPMI1640 medium supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA).

Full-length cDNA synthesis and cloning
The full-length of Survivin-3B coding sequences were obtained and cloned as described previously (Végran et al. 2006).

Stable transfections
5.10^5 cells were grown in a medium without antibiotics in 12-well plates. Two days later, cells were transfected with 1µg of plasmid containing Survivin-3B insert or YFP. Stable transfections were performed by LipofectAMINE2000 (Invitrogen) according to manufacturer’s instructions. The colonies were selected by 10µg/ml Blasticin (Invitrogen). The presence of YFP and YFP coupled Survivin-3B was controlled by Western blot with anti-GFP (ab6556), Survivin^full length (ab2050) and Survivin^1-12 (ab469) antibodies (Supplementary Figure S2A) and by microscopy (Supplementary Figure S2B). For siRNA transfection, 8.10^5 cells were transfected with 300pmol of Survivin-3B siRNA. Two days later, experiments were performed.

Assessment of apoptosis cell death by flow cytometry and in situ cell death detection
Apoptosis was induced by the combination of S-fluorouracil/epirubicin/cyclophosphamide (FEC) (190µg/ml, 1µg/ml, 5mg/ml, respectively) in MDA-MB-231, MCF-7, and T47D and HBL100 (45µg/ml, 0.1µg/ml, 2mg/ml) cell lines corresponding to the IC50 of parental cells. Apoptotic cell death detection by flow cytometry was performed as previously described (Végran et al. 2006) and confirmed by using the in situ cell death detection kit TMR red (Roche), in accordance with the manufacturer’s protocol. This technique allows detecting DNA strand breaks in apoptotic cells.

Sequencing of PCR products
The specificity of all the PCR amplifications was verified by the sequencing of PCR-products. Mutations in exons 2-10 of the p53 gene were analyzed as described elsewhere (Végran et al. 2007).

References