

Supplementary Figure 1.

Oligonucleotides used for ChIP assays

BID p53 bs	F: 5' -TTAAAGAATCCTTTGCGGC-3' R: 5' -GTGATTCTCCTGCTTCAG-3'
Bax p53 bs	F: 5' -TAATCCCAGCGCTTTGGAG-3' R: 5' -TGCAGAGACCTGGATCTAGCAA-3'
PUMA p53 bs	F: 5' -GTACATCCTCTGGGCTCTGC-3' R: 5' -GGACAGTCGGACACACAC-3'
p21 ^{WAF1/CIP} p53 bs	F: 5' -GTGGCTCTGATTGGCTTTCTG-3' R: 5' -CTGAAAACAGGCAGCCCAAG-3'
BID HRE	F: 5' -GACTACTCTGGGCACCCC-3' R: 5' -GGAACACACATTTGCACA-3'
Epo HRE	F: 5' - GACCCAGCTACTTTGCGGAACTCAGC-3' R: 5' - TGGCCAGGGACTCTGCGGCTCTGG-3'
VEGF HRE	F: 5' -GTAGGTTTGAATCATCACGCAGG-3' R: 5' -GCACCAAGTTTGTGGAGCTGA-3'

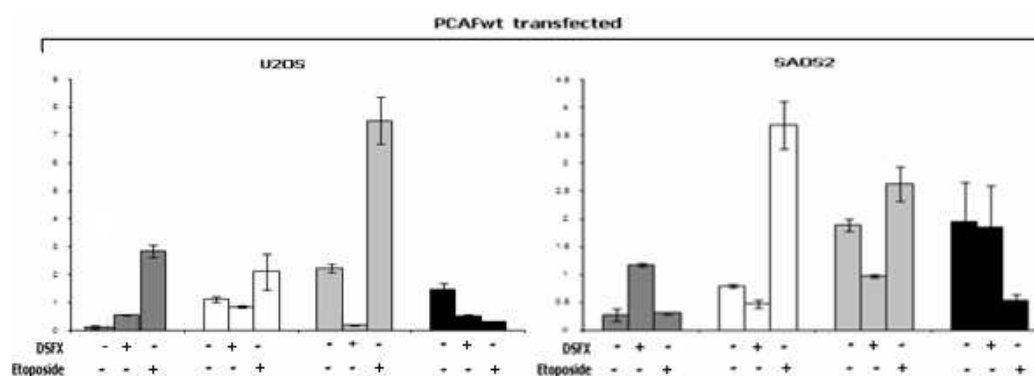
Primers used for the chromatin immunoprecipitation experiments

Supplementary Figure 2

A Oligonucleotides used for qRT-PCR

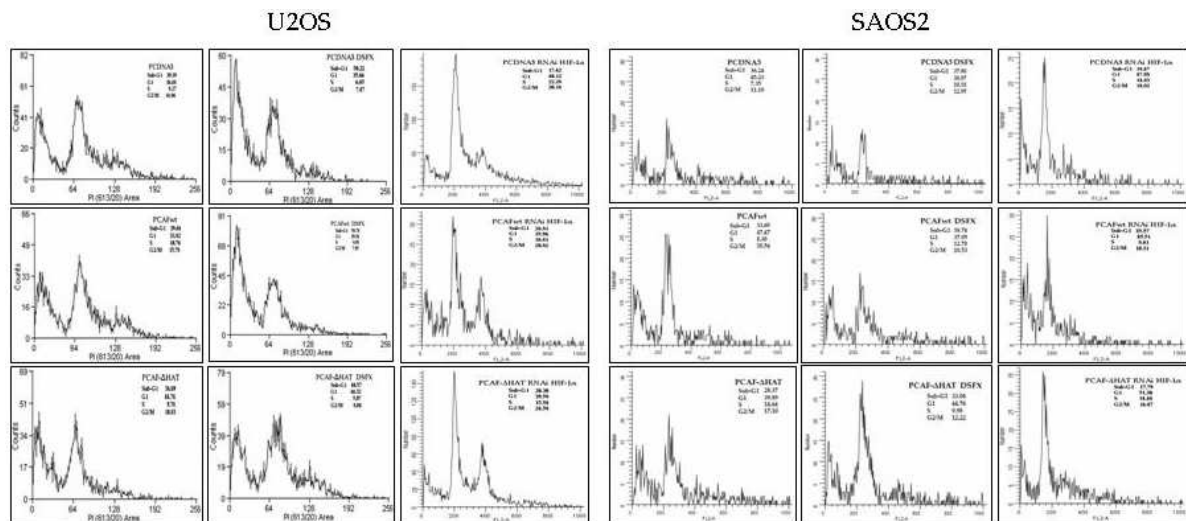
BID	F: 5' - TGTGAGGTCAACAACGGTTC - 3' R: 5' - AGCTCTCTGCGGAAGCTGT - 3'
PUMA	F: 5' - GAGCAGCACCTGGAGTCG - 3' R: 5' - GGCCCACTGTTCTCTCTC - 3'
Bax	F: 5' - TTTGCTTCAGGGTTTCATCC - 3' R: 5' - ATCCTCTGCAGCTCCATGTT - 3'
p21 ^{WAF1/CIP}	F: 5' - GGCCCACTGGACAGCGAGCA - 3' R: 5' - CCCAGGCGAAGTCACCCTCC - 3'
Rpl19 Used as internal standard	F: 5' - ATGTATCACAGCCTGTACCTG - 3' R: 5' - TTCTTGGTCTCTTCTCTCTTG - 3'

B



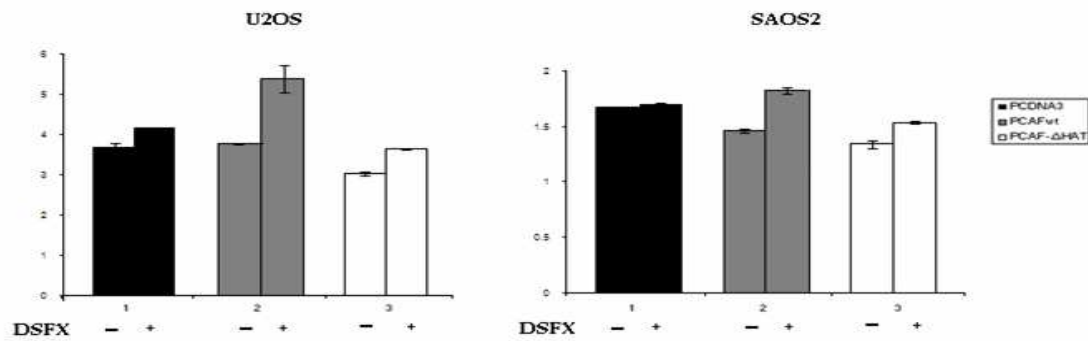
(A) Primers used for qRT-PCR for the p53 target genes BID, PUMA, Bax and p21^{WAF-1/CIP-1}. The mRNA levels of these genes were normalized to Rpl19 mRNA which is not affected by DSFX treatment. (B) The RNeasyPlus mini Kit (QIAGEN) was used to extract total RNA from U2OS and SAOS2 cells previously transfected with PCAFwt expression vector and treated with 250 μ M DSFX or 10 μ M etoposide for 20h or left untreated as specified in the Figure. The RNA concentrations were determined and cDNA synthesis was performed according to the two-step protocol (ABgene) using an oligo dT primer (ABgene) and 1 μ g of total RNA. cDNA was diluted 1:4 and used for qPCR analysis performed with SYBR® Green JumpStart™ Taq ReadyMix™ (Sigma) in DNA Opticon 3 system (Bio-Rad). Each reaction was performed in duplicates and each value was standardized against Rpl19 values and plotted.

Supplementary Figure 3



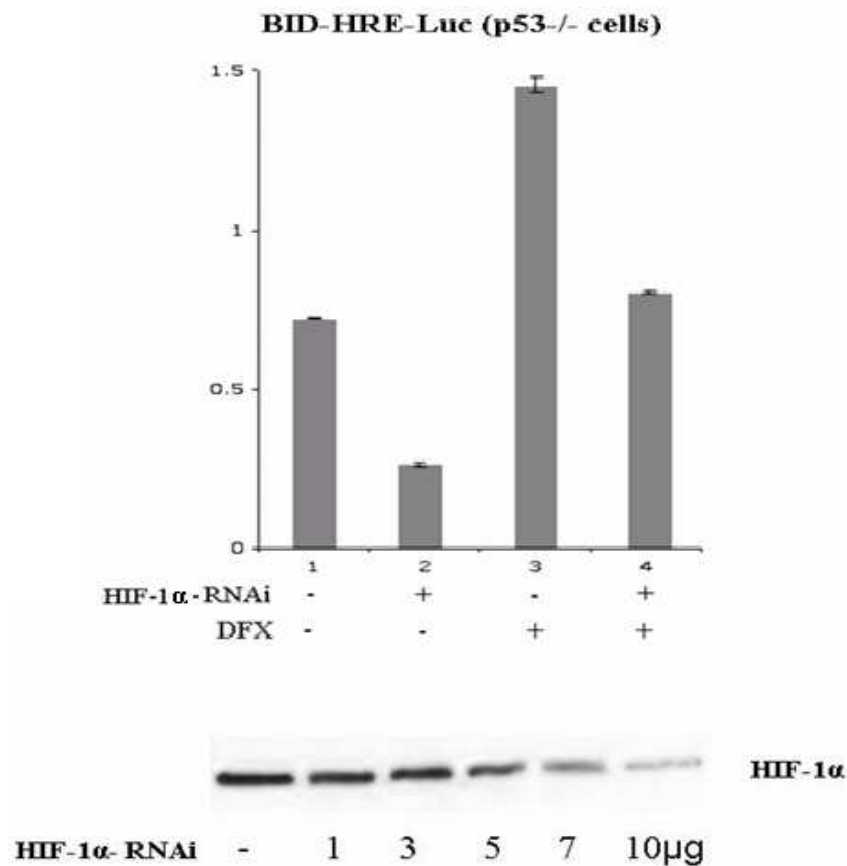
Cell cycle profiles of U2OS and SAOS2 cells transfected with either PCAFwt, PCAF- Δ HAT or empty vectors together with RNAi against HIF-1 α where indicated and a plasmid expressing CD20. Transfections were carried out using polyfect (QIAGEN) and the average transfection efficiency was 30% for U2OS and 12% for SAOS2 cells. DSFX (250 μ M) was added 24 h after transfection, cells were grown for an additional 20h and then harvested, stained with propidium iodide and CD20 and their cell cycle profile was determined. Only the cells transfected with CD20 were counted and the percentage of the CD20 positive cells in the different phases of the cell cycle is indicated in the insert within each panel. FACS experiments were performed two times and the profiles shown are from one experiment.

Supplementary Figure 4



Caspase 3 assay was performed according to the manufacturer recommendations (Alexis Biochemicals). In summary U2OS and SAOS2 cells were transfected with PCDNA3 empty vector, PCAFwt, or PCAF- Δ HAT and treated with DSFX as indicated. 48h after transfection cells were harvested in PBS, precipitated (500g for 10 min) and the cell pellet was lysed in 120 μ l of caspase 3 buffer consisting of 20mM Hepes (pH 7.4), 100mM NaCl, 0.1% CHAPS, 10mM DTT and 10% glycerol. Cell lysates were centrifuged at 10,000g for 10min at 4°C. Aliquots of the supernatant were used to determine protein concentration (Bio-Rad) and equal amounts of proteins were incubated with 250 μ M caspase 3 substrate in the caspase 3 buffer at 37°C for 30min. Fluorescence was measured using the FLUOstar OPTIMA plate reader (BMG labtech) by setting the appropriate excitation (380nm) and emission (460nm) wavelengths.

Supplementary Figure 5



BID-HRE-luc reporter was constructed by PCR amplification of a ~400bp fragment from the BID promoter upstream to the translation start site from human genomic DNA isolated from U2OS cells with the forward primer 5'-GGTACCATTAAACGCTAACTTGCC -3' and reverse primer 5'- GAGCTCGACCTAGAATTCAGC -3' using Pfu polymerase (Stratagene). The insertion of the correct fragment in the pGL3 promoter vector was confirmed by sequencing the construct. Luciferase reporter assays with the BID-HRE-Luc reporter were carried out in SAOS2 cells transfected with 10 μ g RNAi for HIF-1 α (bars 2, 4) and treated with DSFX for 20h (bars 3, 4), and luciferase activity was followed as described in Material and Methods (top panel). Bottom panel shows efficient inhibition of HIF-1 α expression after transfection of 10 μ g RNAi for this transcription factor. HIF-1 α induction 20h after DSFX treatment resulted in a two fold activation of the BID-HRE-Luc expression (compare bars 1 to 3). Silencing of HIF-1 α expression by HIF-1 α RNAi transfection consequently eliminated this induction to the basal levels (compare bars 3 to 4). Efficient silencing of the transcription factor upon transfection of HIF-1 α RNAi (10 μ g) is shown in the bottom panel.