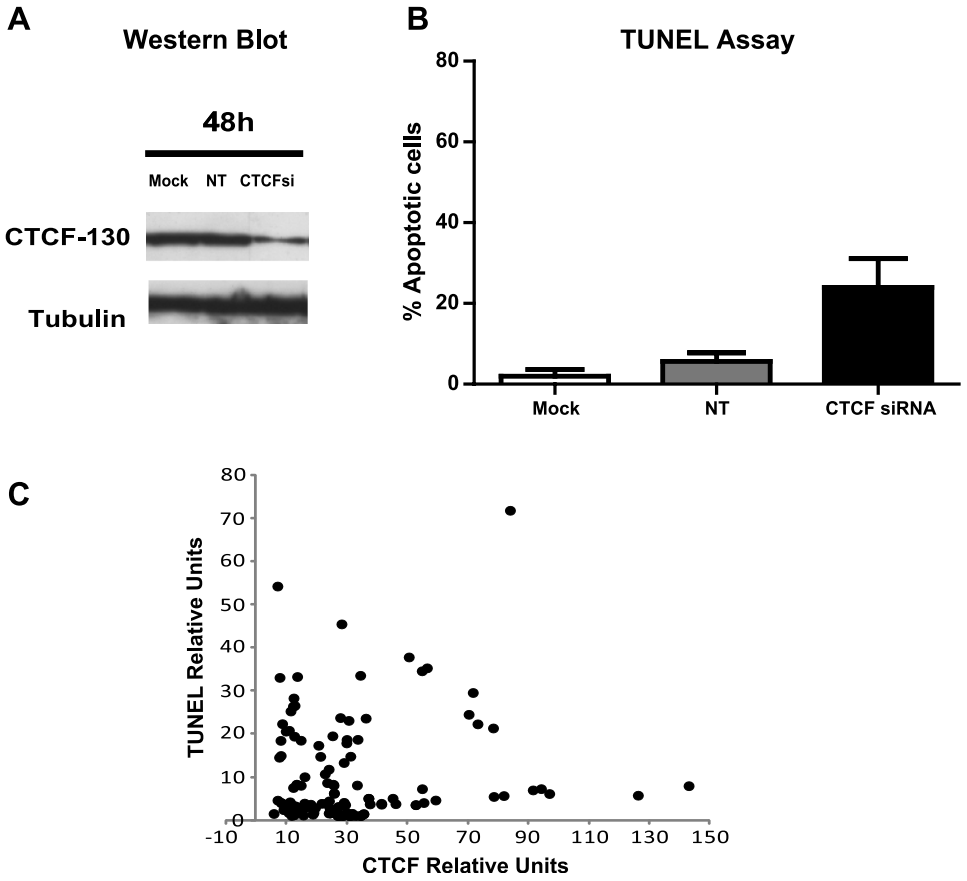


Figure W1. Transient knockdown of CTCF by CTCF siRNA induces apoptotic cell death in breast cancer cells, MCF-7. (A) Levels of CTCF are reduced in breast cancer cells, MCF-7, treated with the Hs_CTCF_4 siRNA as shown by Western blot analysis. Cells (2.5×10^5) were transfected with 50 pM target Hs_CTCF_4 siRNA (CTCF siRNA, "CTCF"), non-target siRNA ("NT"), or transfection reagent only ("Mock"). Forty-eight hours post-transfection, cells were collected, cellular extracts were prepared, and equal amounts (40 μg) of total protein were loaded onto SDS-PAGE. Samples were electrophoretically separated, blotted, and probed with the mouse monoclonal anti-CTCF antibody (BD Biosciences). The membrane was reprobed with the anti–α-tubulin antibody, which served as an internal control for protein loading. (B) Apoptotic cell death is induced by CTCF knockdown with the CTCF siRNA in breast cancer cells, MCF-7. Cells (2.5×10^5) were transiently transfected with 50 pM CTCF siRNA, non-target siRNA ("NT"), or transfection reagent only ("Mock"), and apoptotic cell death was assessed by TUNEL assay. The percentage of TUNEL-positive cells was calculated 48 hours after transfection. The results represent the mean values with the SDs (error bars) of three independent experiments. (C) Images were additionally analyzed using the ImageJ software [8]. The values were measured for CTCF and TUNEL staining in 100 cells; and the mean values for fluorescence were normalized by subtracting the background values. The results are presented as a scatter plot.

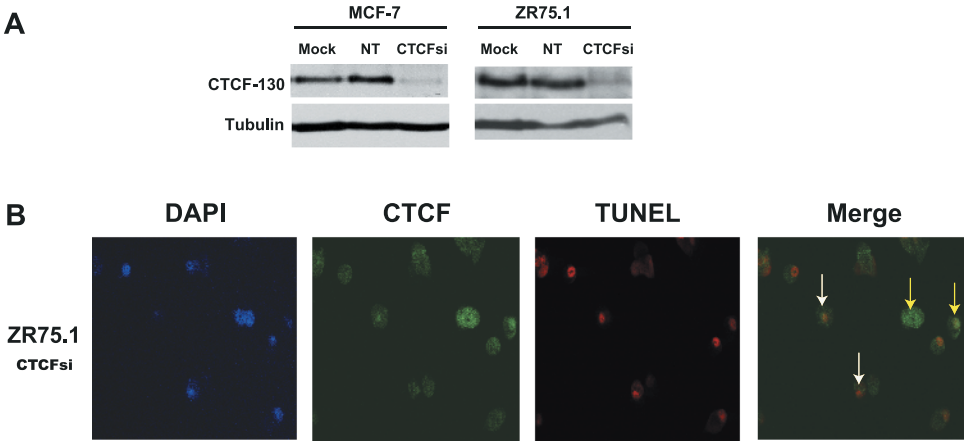


Figure W2. Transient knockdown of CTCF by SMARTpool CTCF siRNA induces apoptotic cell death in breast cancer cells. (A) Levels of CTCF are reduced in the breast cancer cells following CTCF knockdown, as shown by Western blot analysis. Breast cancer MCF-7 (2.5×10^5) and ZR75.1 (2.5×10^5) cells were transfected with 50 pM SMARTpool siRNA (CTCF siRNA, "CTCF"), 50 pM non-target siRNA ("NT"), or transfection reagent only ("Mock"). Forty-eight hours post-transfection, cells were collected, cellular extracts were prepared, and equal amounts (40 μg) of total protein were loaded onto SDS-PAGE. Samples were electrophoretically separated, blotted, and probed with the mouse monoclonal anti-CTCF antibody (BD Biosciences). The membrane was reprobed with the anti–α-tubulin antibody, which served as an internal control for protein loading. (B) Lower CTCF levels are associated with apoptotic death in breast cancer cells, ZR75.1. Cells (2.5×10^5) were transfected with 50 pM SMARTpool CTCF siRNA and analyzed by immunofluorescence staining with the anti-CTCF antibody (FITC, green fluorescence). Apoptotic cell death was assessed by TUNEL staining (TMR, red fluorescence). Nuclei were visualized by DAPI. Merge, overlay of the CTCF and TUNEL staining. TUNEL-positive (apoptotic) cells with low CTCF levels are indicated by white arrows. The TUNEL-negative cells with high levels of CTCF are indicated by yellow arrows.

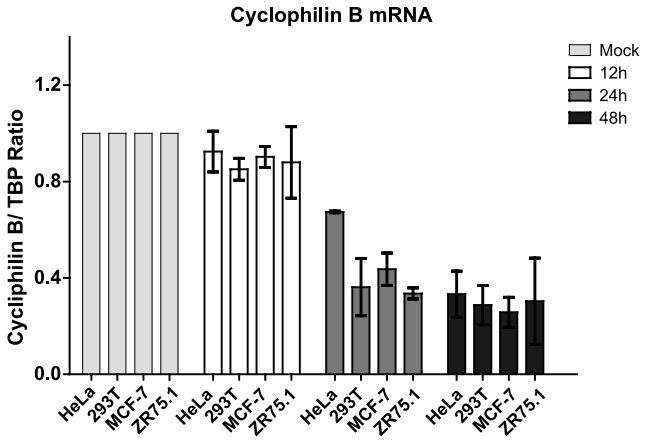
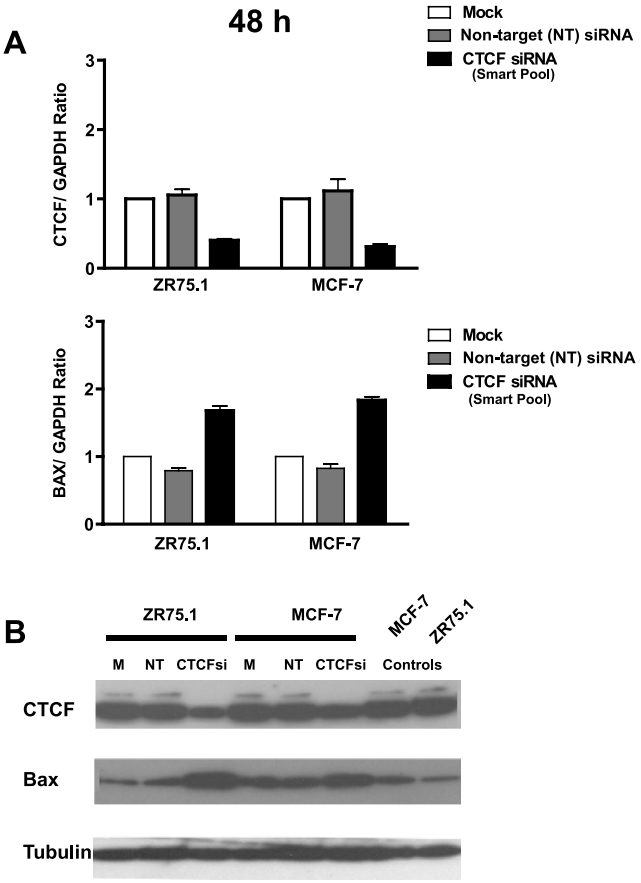


Figure W3. Transfection with the *cyclophilin B* siRNA leads to the reduction of the *cyclophilin B* mRNA levels in non–breast (HeLa and 293T) and breast cancer (MCF-7 and ZR75.1) cell lines. Cells were transfected with 50 pM *cyclophilin B* siRNA and collected 12, 24, and 48 hours post-transfection. The reagent-only ("Mock") reaction was used as control. The total RNA was extracted and analyzed by RT-qPCR. The expression of *cyclophilin B* was normalized to *TBP* mRNA expression, and the comparative C_t method ($\Delta\Delta C_t$) was used to calculate relative *cyclophilin B* mRNA levels. Columns represent fold change of *cyclophilin B*/*TBP* relative to mock-transfected cells (designated as 1.0). For each sample, measurements were performed at least in triplicates; error bars represent SDs.



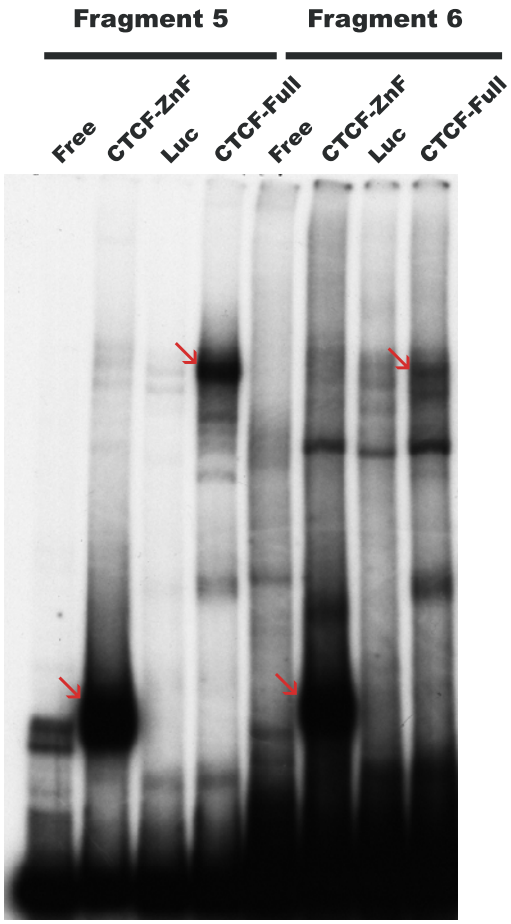


Figure W4. Levels of *Bax* mRNA and Bax protein increase in breast cancer cells following CTCF knockdown by the CTCF–SMARTpool siRNA. (A) Analysis of *Bax* mRNA. Breast cancer ZR75.1 (2.5×10^5) and MCF-7 (2.5×10^5) cells were transfected with 50 pM CTCF–SMARTpool siRNA, non-target siRNA (control), or transfection reagent only ("Mock") and harvested 48 hours post-transfection. Total RNA was prepared and analyzed by RT-qPCR. The expression levels of *CTCF* (upper panel) and *Bax* mRNA (lower panel) were calculated using the comparative C_t method ($\Delta\Delta C_t$) and normalized to *GAPDH* mRNA expression, representing fold change of *CTCF*/*GAPDH* mRNA or *Bax*/*GAPDH* levels relative to mock-transfected cells (designated as 1.0). For each sample, measurements were done at least in triplicates. Columns, mean mRNA/*GAPDH* relative level values. Bars, SDs. The difference in *CTCF* mRNA and *Bax* mRNA levels between cells treated with CTCF siRNA and control (non-target, NT) siRNA was statistically significant ($P \leq 0.01$). (B) Analysis of Bax protein levels. Following transfection as described above, cells were lysed, proteins separated by SDS-PAGE, blotted, and analyzed by Western blot analysis using CTCF, Bax, and α-tubulin (loading control) antibodies. Abbreviations: CTCFsi, CTCF siRNA; NT, non-target siRNA; M, transfection reagent only ("Mock"); two controls in the right lanes are extracts from untreated cells.

Figure W5. The full-length CTCF binds to fragments 5 and 6 within the promoter-proximal region of human *Bax* gene in the EMSA. EMSA analysis of CTCF binding to two overlapping fragments, 5 and 6, of the human *Bax* promoter. The DNA fragments were end-labeled with ^{32}P, and EMSA analyses were performed as described under Materials and Methods section. The *in vitro* translated 11-Zn-finger domain of CTCF (CTCF-ZnF), full-length CTCF (CTCF-Full), and luciferase (control, Luc) were used to assess specific and non-specific binding to the DNA. Free, free probe. Specific ZF-CTCF-DNA and CTCF-DNA complexes are indicated by red arrows.

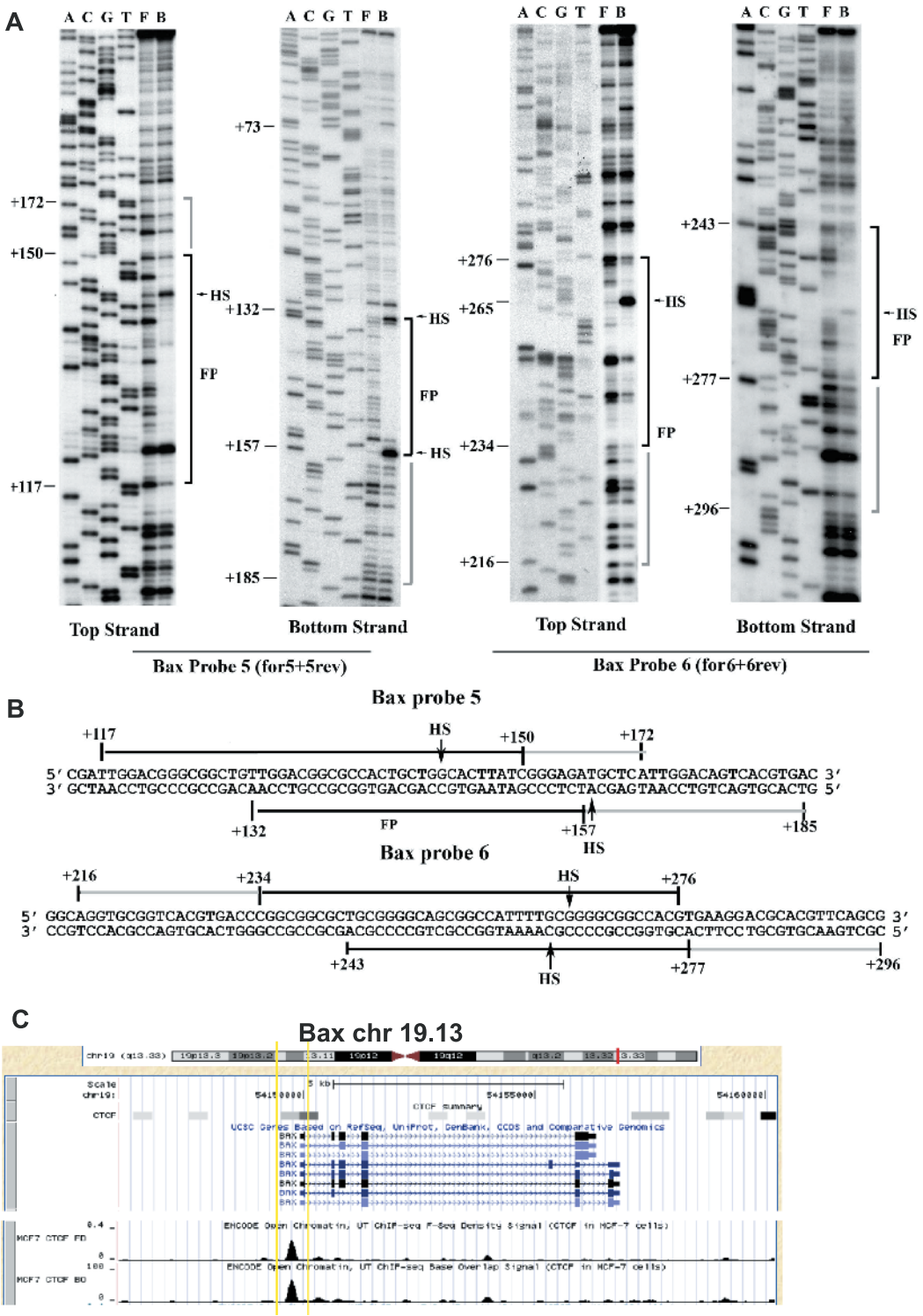


Figure W6. Footprinting analysis of CTCF binding to fragments 5 and 6 of the *Bax* gene promoter. (A) The 5′-^{32}P end-labeled fragments containing promoter regions between +24 and +226 (fragment 5) and +174 and +358 (fragment 6) downstream of the transcription start site were used as probes in the binding reaction with *in vitro* translated full-length CTCF. ACGT, the Maxam-Gilbert sequencing reactions; F, free probe; B, bound probe (reaction with CTCF). The strongly and weakly protected regions are indicated by black and gray lines, respectively. HS, hypersensitive sites; FP, footprint. Coordinates of the binding sites: chr19: 54,149,742 to 54,149,922. Coordinates are from the UCSC genome browser. (B) The sequences of the two CTCF bound fragments 5 and 6 within *Bax* promoter region. The lines on the top and bottom of double-stranded DNA sequences summarize the CTCF footprint on the top and bottom strands, respectively. (C) Analysis of the ChIP-seq data for CTCF binding in MCF-7 cells in the UCSC genome browser [http://genome.ucsc.edu/ (NCBI36/ hg18)]. Enrichment in CTCF is detected in the regions where CTSs in the *Bax* promoter were mapped (boxed in yellow). Coordinates mapped by ChIP-Seq: chr19: 54,149,575 to 54,149,958. (D) The maps depicted in C are shown at higher resolution. The positions of the transcription start site and translation start are indicated by the green and red arrows, respectively; the positions of the sites are given in brackets. Locations of fragments 5 and 6 are shown on the expanded maps below.

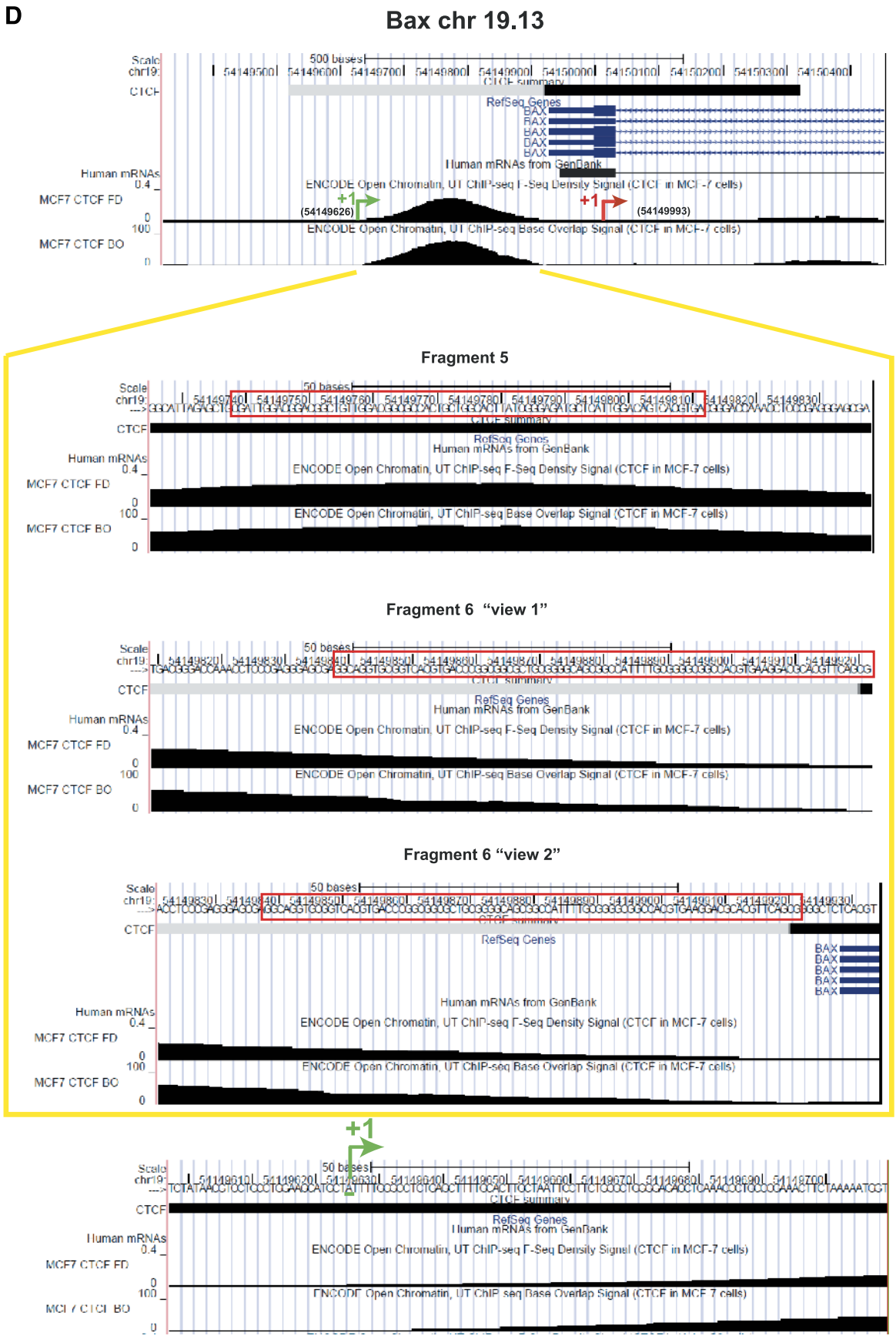


Figure W6. (*continued*).

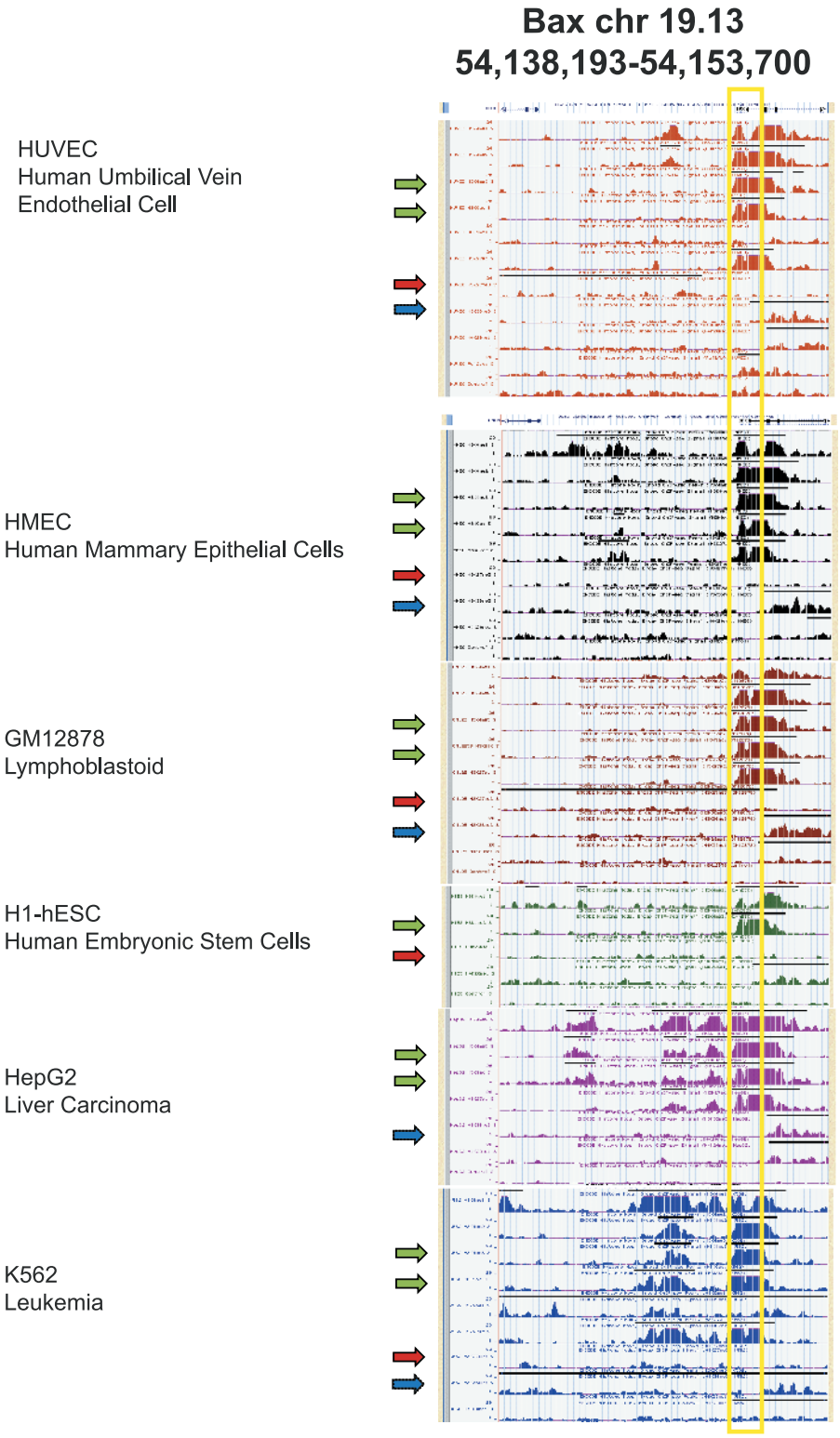


Figure W7. Open chromatin configuration is detected in the regions containing CTSs in the *Bax* promoter (boxed in yellow, coordinates are shown on top of the diagram) in a variety of breast and non–breast cells: analysis of the ChIP-seq data in the UCSC genome browser [http://genome.ucsc.edu/ (NCBI36/hg18)]. Typical examples are shown; they include HUVEC (human umbilical vein endothelial cell), HMEC (human mammary epithelial cells), GM12878 (lymphoblastoid), H1-hESC (human embryonic stem cells), HepG2 (liver carcinoma), and K562 (leukemia). Green arrows, histone marks associated with open chromatin; red arrows, histone marks associated with closed chromatin; blue arrows, dual histone marks (open and close chromatin).

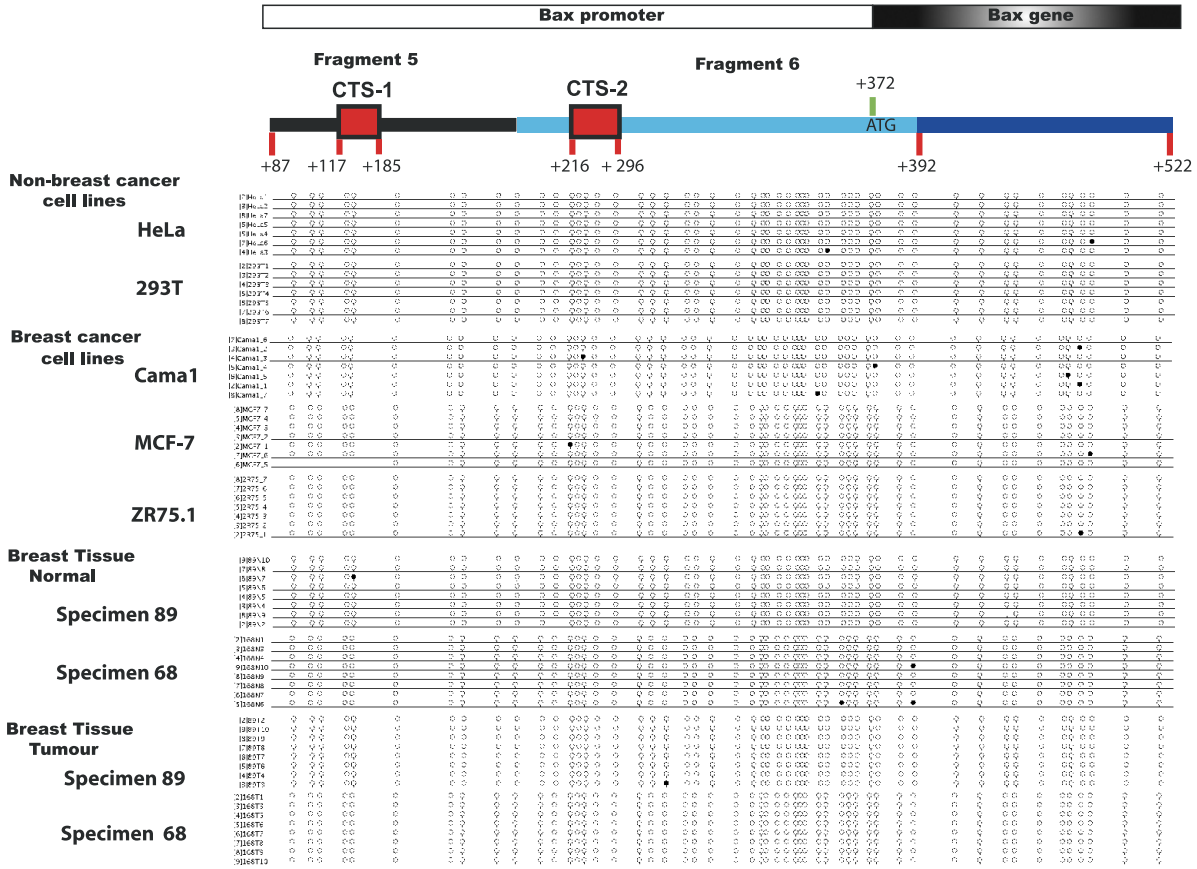


Figure W8. The *Bax* promoter is unmethylated in breast and non–breast cells and in normal and tumor breast tissues. As described under the Supplemental Materials and Methods section, genomic DNA (500 ng) from non–breast (HeLa and 293T) and breast (Cama1, MCF-7, and ZR.75.1) cell lines, two tumors (89T and 68T), and paired peripheral to tumors (89N and 68N) was purified, treated with bisulfate, amplified by PCR, and cloned. The plasmids from individual colonies were isolated and the *Bax* promoter was sequenced. DNA methylation status of CpG islands within fragments 5 and 6 is indicated as lollipop figures. Filled lollipops refer to methylated sites; open lollipops refer to unmethylated sites.

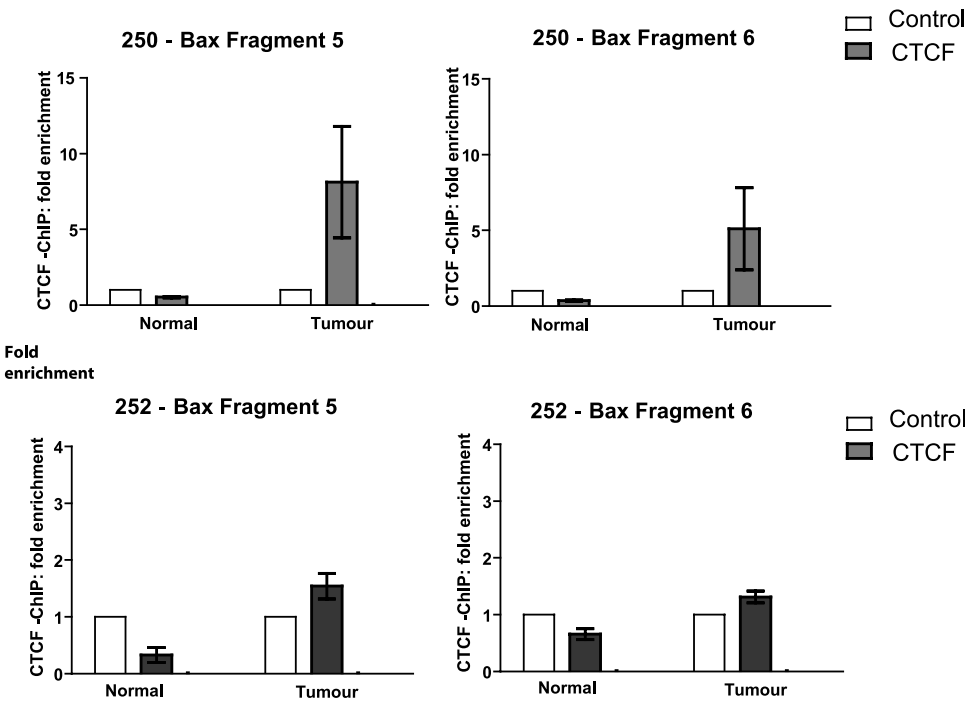


Figure W9. The CTSs are enriched with CTCF in breast cancer cells compared with normal breast and non–breast cancer cells. Analysis of CTCF binding to the *Bax* promoter in normal and tumor breast tissues (specimens 250 and 252, upper and lower panels, respectively) using ChIP assays. Cells were fixed with 1% formaldehyde to cross-link protein-DNA interactions and sonicated, and DNA-protein complexes were immunoprecipitated with the rabbit polyclonal anti-CTCF antibody (Abcam) [9]. The DNA was extracted and real-time PCR was performed using primers specific to *Bax* fragment 5 or fragment 6 as described under Materials and Methods section. Results were calculated as the percentage of input chromatin precipitated at the region of interest and presented as fold change relative to the control ChIP experiment with no antibody (designated as 1.0). Experiments were performed in triplicate, and the mean value is shown. Error bars indicate SDs.

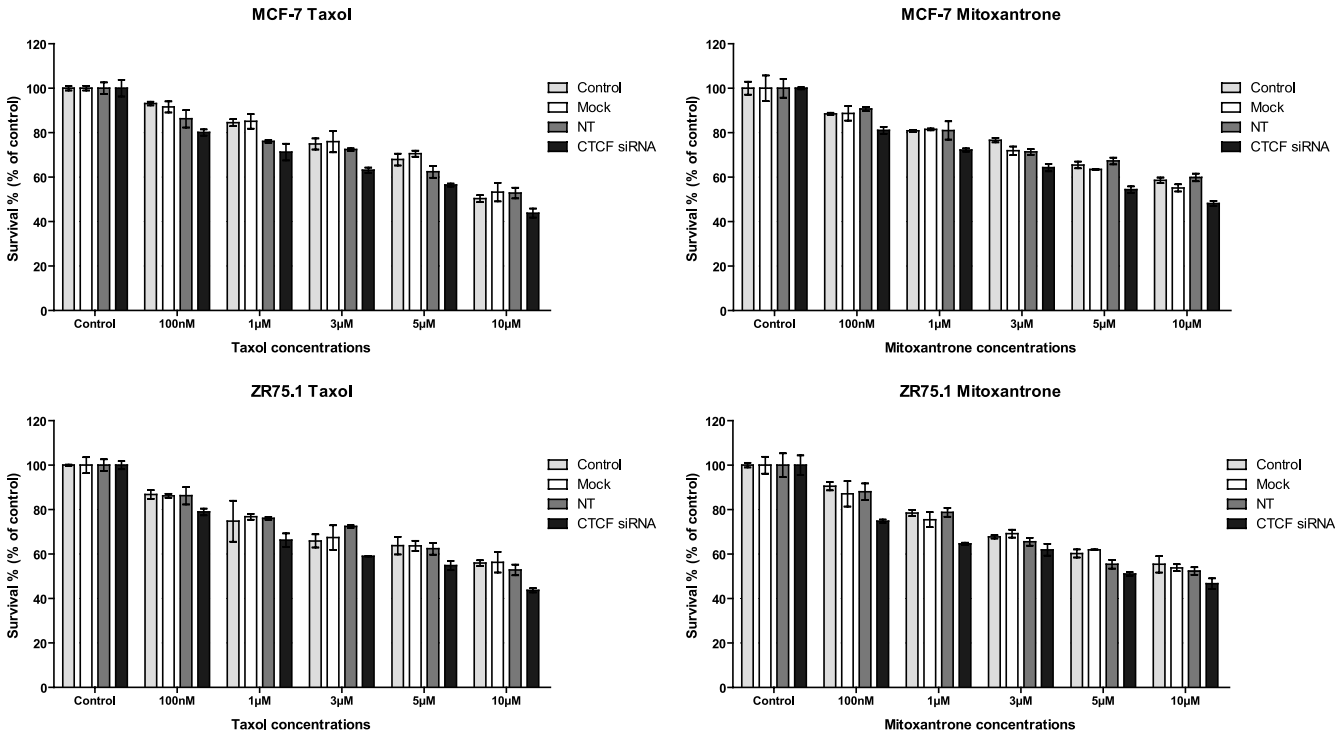


Figure W10. Cytotoxic effect of Taxol (paclitaxel) and Mitoxantrone in MCF-7 and ZR75.1 breast cancer cells. MCF-7 and ZR75.1 cells were transfected with 50 pM CTCF siRNA, 50 pM non-target siRNA, or transfection reagent only ("Mock"). Cells were treated with trypsin (24 hours post-transfection), counted, replated onto 96-well plates (5×10^4 per well), and incubated overnight. After a total of 48 hours post-transfection, the medium was changed to the phenol-free RPMI containing the drugs at the indicated concentrations and left for further 24 hours. Cell survival was measured using MTT assay (see Supplemental Materials and Methods section for details). Control cells were treated with DMSO (0.1%) or water alone. Cell survival was calculated as a percent of control (DMSO-treated in experiments with Taxol or water-treated in experiments with Mitoxantrone) cells as described previously [7]. The results represent the mean values with the SDs (error bars) of three independent experiments. The difference in the survival of cells treated with Taxol or Mitoxantrone at all concentrations was significantly less for cells transfected with the CTCF siRNA compared with other conditions ($P \leq 0.01$). Abbreviations: CTCFsi, CTCF siRNA; NT, non-target-siRNA; Mock, transfection reagent only; C, control (cells only).