SUPPLEMENTAL MATERIAL

Distinct Roles of Ape1 Protein in the Repair of DNA Damage Induced by Ionizing Radiation or Bleomycin

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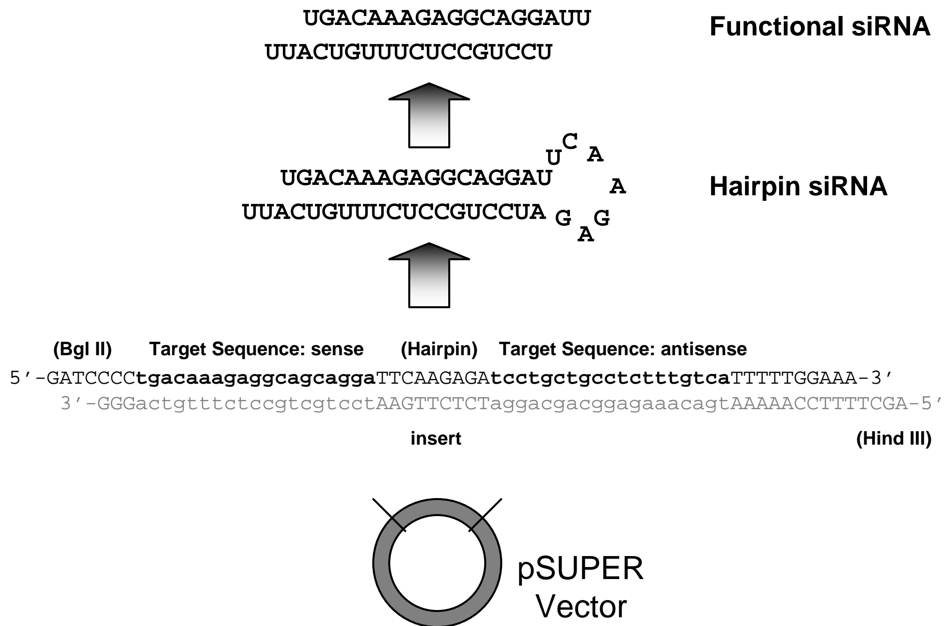
Supplemental Figure legends

Supplemental Figure 1. Design of shRNA sequences. The upper part of the figure shows the subcloned APE1 DNA insert, its placement in the vector, and processing of the shRNA to yield functional siRNA. The table shows the 64-bp sAPE1 and sLUC sequences placed in the vector.

Supplemental Figure 2. Titration of shRNA for Ape1 knockdown. The retroviral shRNA vectors (sLUC, the luciferase-specific shRNA vector, and sAPE1, the APE1-specific shRNA vector) were infected in varying amounts into TK6 cells (panel A) or HCT116 cells (panel B), and after 2 days, puromycin (1.5 μg/ml) was added to the culture medium for 5-7 d of selection. After an additional 3 d selection, cell samples were subjected to immunoblotting with an Ape1-specific antibody, which was quantified by phosphorimaging and normalized to immunoblotting for β-actin. The top panel shows a representative immunoblot, and the bottom panel the quantification. The data were quantified from two independent experiments. Standard deviations are shown, and * indicates a significant difference from sLUC control with $p<0.05$.

Supplemental Figure 3. Cytotoxicity induced by IR or BLM in Ape1-deficient TK6 and HCT116 cells by a colony-forming assay. Cells were untreated (Con) or infected with retroviral siRNA expression vectors for luciferase (sLUC; negative control) or APE1 (sAPE1). TK6 cells were treated with 1-4 Gy doses of 100 KeV X-rays (**Panel A**), or with 10-60 μg/ml BLM for 60 min (**Panel B**). HCT116 cells were treated with 2.5-10 Gy 100 KeV X-rays (**Panel C**), or for 60 min with 25-150 μg/ml BLM (**Panel D**). The treated cells were then reseeded into 60 mm dishes or 96-well plates, and the incubation continued in fresh medium for 10-15 days. The colonies formed were counted and normalized to results for untreated cells to determine plating efficiency. The data were quantified from two independent experiments. Standard deviations are shown, and * indicates a significant difference from the sLUC control with $p<0.05$.

Supplemental Figure 4. Colony-forming assay for BLM-sensitivity as a function of Ape1 and p53 status in TK6 and HCT116 cells. p53-deficient TK6 cells (see main text) or p53-null HCT116 cells (see main text) were treated with control siRNA-LUC (p53d-sLUC) or APE1-specific siRNA (p53d-sAPE1) or sAPE1 plus a p53 expression vector (p53d-sAPE1+p53). BLM treatment and the colony-forming assay were performed as described for Supplemental Figure 3. The data were quantified from two independent experiments. Standard deviations are shown, and * indicates significant difference from the sLUC control with $p<0.05$.



Design strategy for a 64-bp cassette encoding a hairpin RNA transcript that is processed to a functional siRNA. The cassette was inserted into the pSUPER vector (diagram after OligoEngine informational materials).

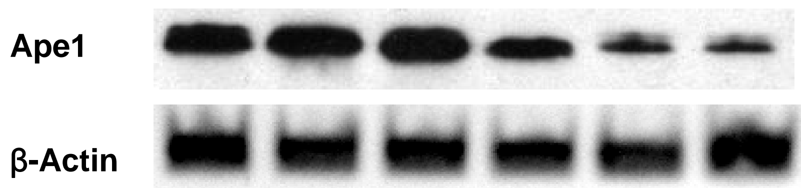
Sequences used for shRNA

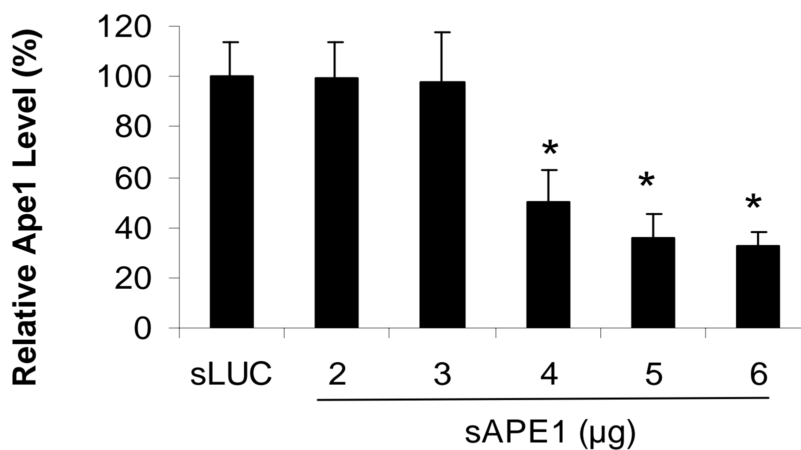
(sense and antisense target sequences are shown in lower case)

sAPE1	GATCCCCtgacaaagaggcagcaggaTTCAAGAGAtcctgctgcctctttgtcaTTTTTGGAAA
sLUC	GATCCCCcttacgctgagtacttcgaTTCAAGAGAtcgaagtactcagcgtaagTTTTTGGAAA

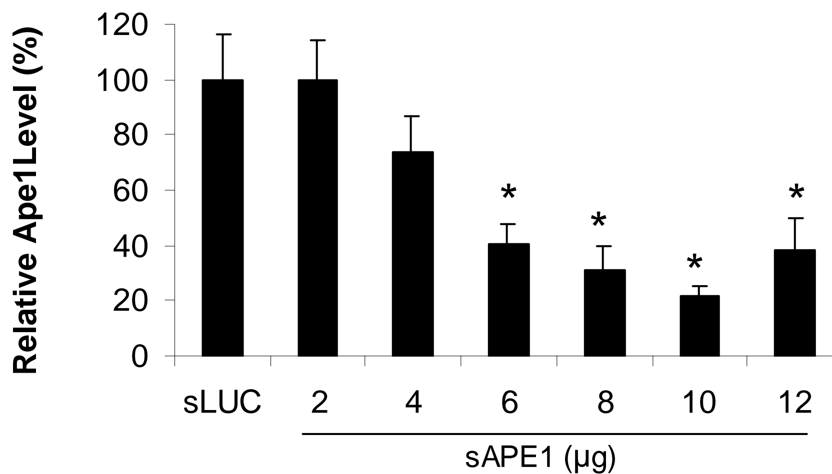
Supplemental Figure 1

A. TK6

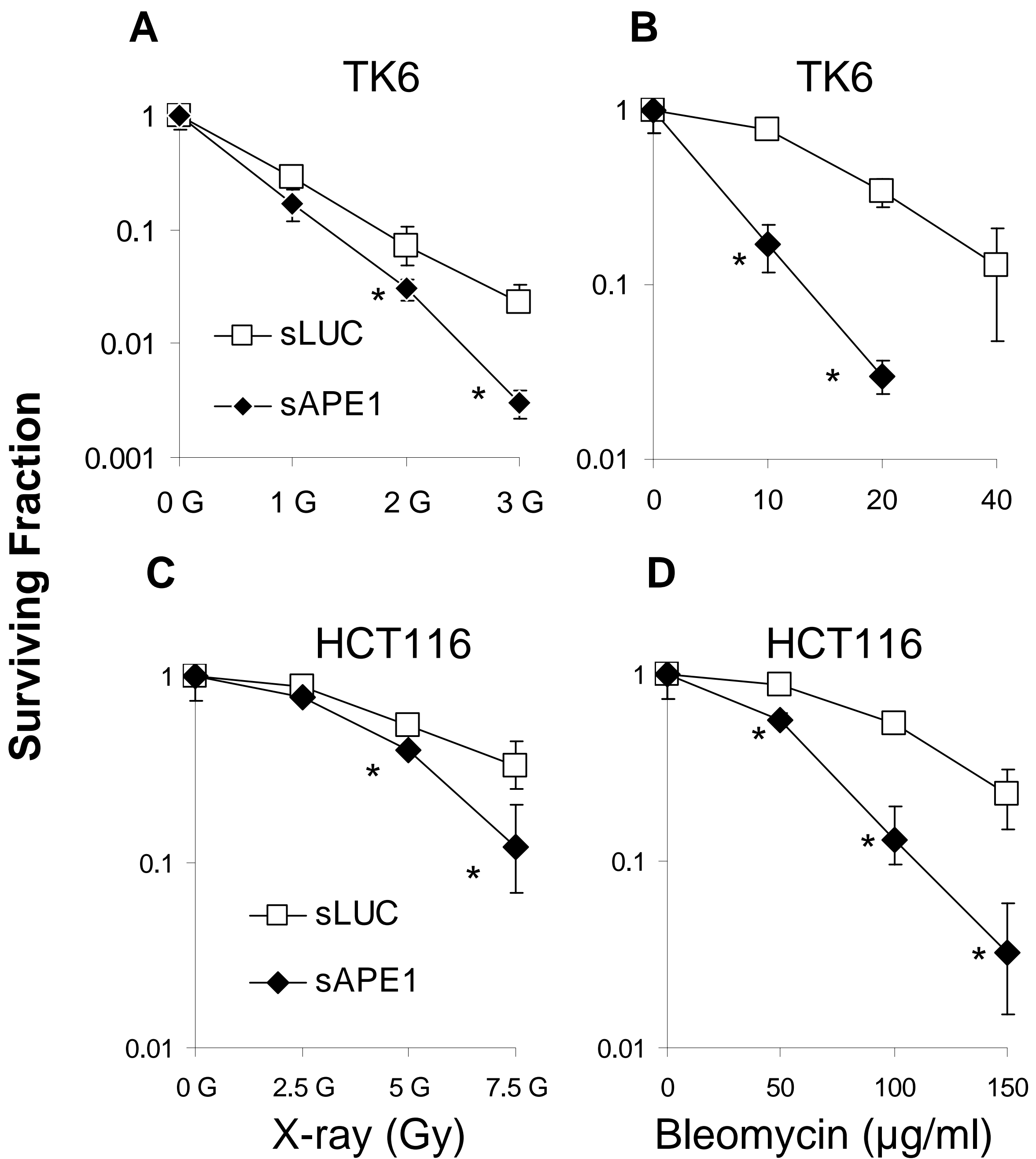




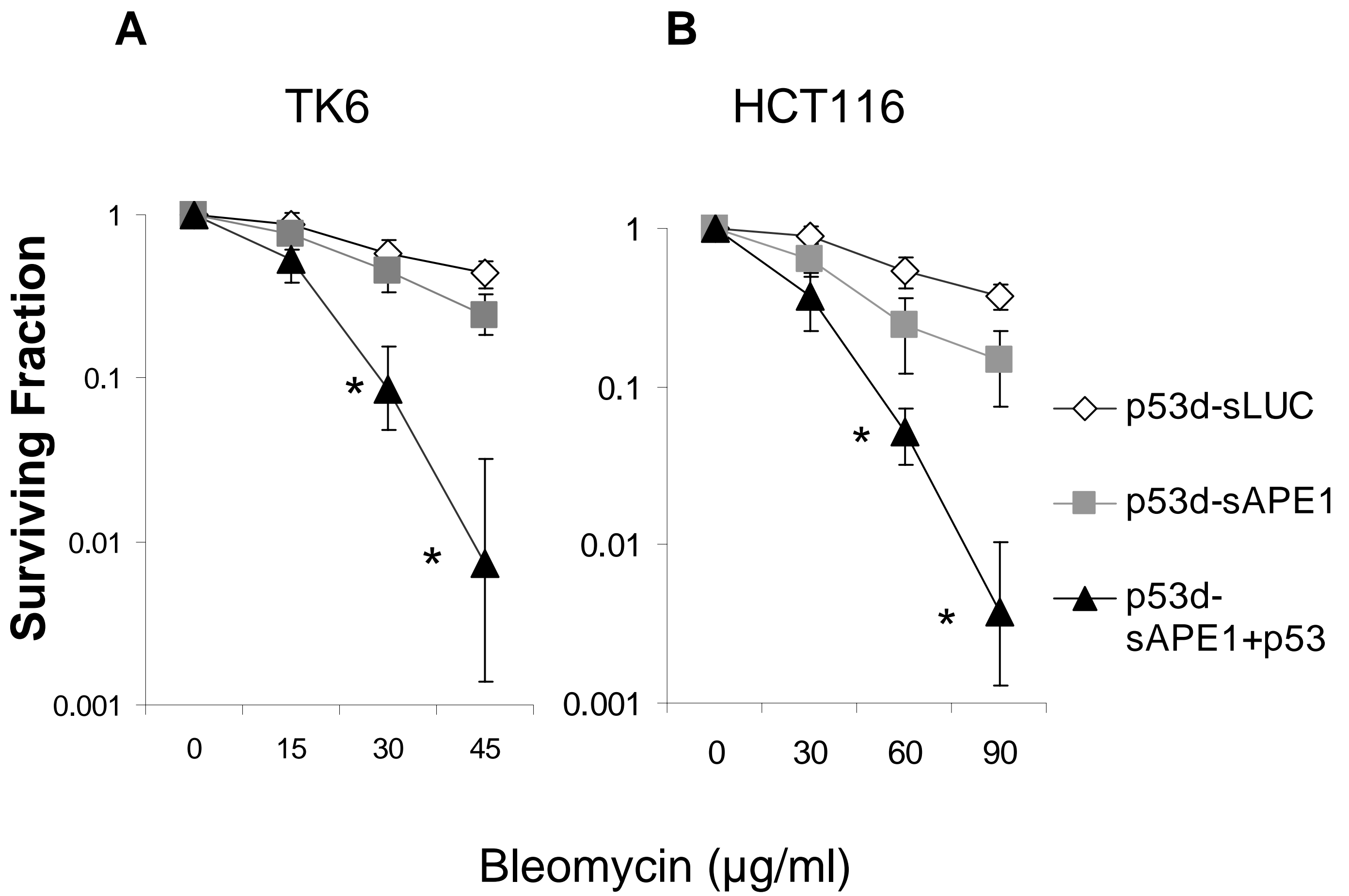
B. HCT116



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4