Supplemental Figure Legends

Supplemental Fig. 1. Etoposide-mediated p53R2 induction is defective in HCT116 cells. HCT116 and RKO colorectal adenocarcinoma cells were treated with 50 μM etoposide (VP16) and cells were harvested 6 and 24 hours post-treatment. *A, p53R2* expression was measured by Northern blot analysis. *GAPDH* mRNA is shown as a loading control. *B*, p53 protein levels were measured by Western blot analysis, using the same set of conditions described in *A*. α-tubulin protein levels were measured as a loading control.

Supplemental Fig. 2. Sodium bisulfite sequencing analysis of two additional regions of the p53R2 5' CpG island in RKO, HCT116, and LOVO colorectal cancer cell lines. *A*, 5'end of the p53R2 5' CpG island. *B*, 3' end of the p53R2 5' CpG island. In both *A* and *B*, the nucleotide positions relative to the NCBI-predicted p53R2 TSS are indicated. Open and filled circles represent unmethylated and methylated CpG sites, respectively, and rows correspond to individually sequenced clones. SssI modified DNA was analyzed as a positive control for DNA methylation in both regions.

Supplemental Fig. 3. siRNA knockdown of DNMT1. RKO cells were treated with the indicated concentrations of wildtype or mutant DNMT1 siRNA for five days, and DNMT1, p53R2, and Lamin B (loading control) protein expression were measured by Western blot analysis. LF2000 indicates cells treated with only the transfection reagent.

Supplemental Fig. 4. Loss of soluble nuclear DNMT1 parallels *p53R2* mRNA induction in decitabine-treated RKO cells. *A*, DNMT1 expression in soluble nuclear protein extracts was measured by Western blot analysis. Nuclear protein extracts were harvested five days post-treatment with the indicated concentrations of decitabine (DAC). Non-specific (NS) bands observed on the Western blot confirmed equivalent protein loading. *B*, *p53R2* mRNA expression in RNA extracts harvested in parallel with the samples described in *A*. *p53R2* expression was measured by Northern blot analysis and Ethidium Bromide (Etbr) staining of total RNA confirmed equivalent RNA loading.

Supplemental Fig. 5. The effect of RG108 treatment on genomic DNA methylation in RKO cells. RKO cells were treated with RG108 as described in Fig. 3*D*, and DNA extracts were harvested 5 days post treatment for analysis of (*A*) LINE-1 methylation by bisulfite pyrosequencing and (*B*) 5mdC levels by liquid chromatography-mass spectrometry, as described in the *Materials and Methods*.

Supplemental Fig. 6. siRNA-mediated knockdown of p53R2 in RKO cells. RKO cells were treated with 50nM p53R2 or 50nM control siRNA for 24 hours, at which time the cells were treated with the indicated concentrations of decitabine (DAC). Three days later, protein extracts were harvested and used to measure p53R2 expression. The experiment was performed in triplicate wells of a six-well plate, and Western blot results from each of the triplicate wells are shown. Western blot analysis of β-actin confirmed equivalent protein loading. The average fold decrease (relative to siRNA control treated cells) in p53R2 expression in p53R2 siRNA-treated cells is shown.

Supplemental Fig. 7. Decitabine treatment induces *p53R2* expression in MDS/AML patient bone marrow samples in the absence of DNA methylation changes at the p53R2 promoter. *A*, *p53R2* expression values (raw copy number values normalized to *GAPDH*) plotted over the entire patient group, separating pre- and post-treatment samples. Bars indicate the means of pre- and post-treatment samples. The difference between pre- and post-treatment samples was statistically significant (Student's T-test, one-tailed, p=0.017). *B*, Sodium bisulfite sequencing analysis of the central region of the 5' CpG island of p53R2 in two patients prior to treatment (upper) and in post-treatment samples (lower) (pt. #8, 8c; pt. #14, 8c) displaying induction of *p53R2* mRNA and/or protein following decitabine treatment. Open and filled circles represent unmethylated and methylated CpG sites, respectively, and rows correspond to individually sequenced clones. Numbers on the upper left sample indicate nucleotide positions relative to the p53R2 transcriptional start site, and are equivalent in all four sequenced samples.