Figure S1. Sites C and D are unresponsive to p53 regulation. MCF7 cells were treated with 0.5 μM doxorubicin. The chromatin immunoprecipitates were obtained using p53 antibody, mouse normal IgG or without antibody, and subjected to PCR analysis. Chromatin inputs from doxorubicin-treated or non-treated MCF7 cells were also used as positive controls for PCR, while double-distilled H_2O was used as a PCR template for negative control. The PCR products were resolved on a 2% agarose gel. Data are presented as the mean ± standard deviation of triplicate transfections, and are representative of 3 independent experiments. PCR, polymerase chain reaction; Dox, doxorubicin.

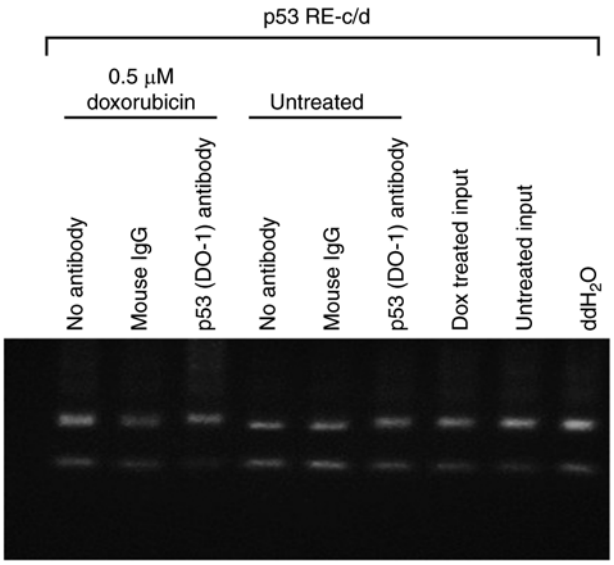

Figure S2. Mutations of sites A and B can rescue the inhibition of anillin promoter luciferase activity induced by Dox treatment. β-galactosidase expression plasmids were co-transfected with pGL3 vector or reporter plasmids A or B (A), or with pcDNA3, wild-type p53, R175H or R248W (B) into both HCT116/p53$^{+/+}$ and HCT116/p53$^{-/-}$ cells for 24 h. Cells were then treated with 0.5 μM doxorubicin for an additional 24 h. Luciferase reporter activity was measured and normalized to β-galactosidase activity in the same sample, and presented as fold reduction. Data are presented as the mean ± standard deviation of triplicate transfections, and are representative of 3 independent experiments. wt, wild-type.

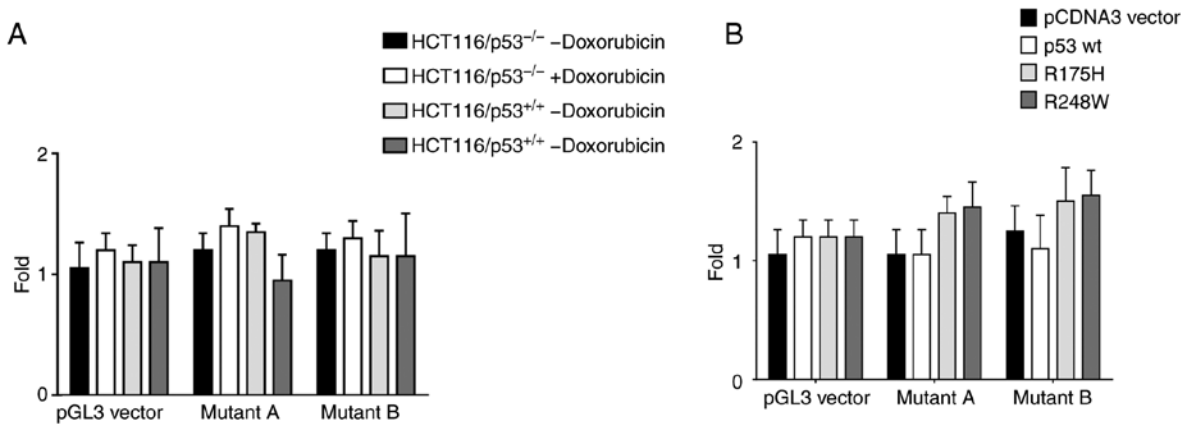

Figure S3. Transfection efficiency validation via western blotting. β-galactosidase expression plasmids were co-transfected with either pcDNA3, wt p53, R175H or R248W into HCT116/p53$^{-/-}$ cells for 48 h. Whole cell lysates were collected and p53 protein expression levels were determined by p53 DO-1 antibody using an immunoblotting assay. β-actin was used as a loading control. The experiment was repeated 3 times. wt, wild-type.