

## **Supplementary Information**

### **Cell lines**

Stable clones were selected and maintained in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum, in the presence of 250 $\mu$ g/ml G418 (Sigma), 1 $\mu$ g/ml Puromycin (Sigma) and 8 $\mu$ g/ml Tetracycline (Sigma). The parental (wt p53) and the somatic p53 knock-out (p53KO) HCT116 cell lines were grown in 10% FCS McCOY's 5A medium (GIBCO). MCF/7 cells were grown in 10% FCS DMEM and MDA-MB-231 cells in 10% FCS RPMI medium (GIBCO).

### **Cloning of Rad51 promoter**

Sequence from -948 to +1427 of the Rad51 promoter (GI: 7407070) was amplified by PCR using the following primers: forward (5'- TTACGCGTCACGTTGGCCAGGTTT ATCT-3') and reverse (5'- TTCTCGAGCACAGGAAGGCTTCTGAGGA- 3'). The PCR product was cloned into pCR-Blunt plasmid (Invitrogene) and the Rad51 promoter fragment of interest was obtained by KpnI and EcoRV digestion. The fragment was sub-cloned into pGL-3 Basic plasmid (Promega) at Kpn I/Sma I sites (Rad51-luc). Nucleotide sequence was confirmed by DNA sequence analysis with an ABI-PRISM 377 automatic sequencer.

The deletion of 42 bp encompassing the p53 binding sites (DEL Rad51-luc) was obtained by mutagenesis of the Rad51-luc plasmid (described above), following the procedure described by QuikChange® II site-directed mutagenesis kit (Stratagene). The following primers were used: (5'-GGGAAGAGGGCAGTCTGTAGCGC GCAGGGCGGAAGC-3') and (5'- GCTTCCGCCCTGCGCGCTACAGACTGCCCT CTTCCC-3'). Nucleotide sequence was further confirmed by DNA sequence analysis with an ABI-PRISM 377 automatic sequencer.

The mutant promoter (MUT Rad51-luc) was obtained performing two-step site-directed mutagenesis on the Rad51-luc plasmid (described above). Basically, we followed the protocol of the QuikChange® II site-directed mutagenesis kit (Stratagene). The following primers were used for the first mutagenesis: (5'-GGGCAGTCTGTAAATTCACGCAGGATTAAGTCTCGAGCTCCCG-3') and (5'-CGGGAGCTCGAGAGTTTAATCCTGCGTGAATTTACAGACTGCCC-3'). The second mutagenesis was performed with primers: (5'-CTCGAGCTCCCGTTTTAGGTTAGCGCGCAGG-3') and (5'-CCTGCGCGCTAACCTAAAACGGGAGCTCGAG-3'). Nucleotide sequence was confirmed by DNA sequence analysis with an ABI-PRISM 377 automatic sequencer.

### **Real-time qRT-PCR**

Total RNA was isolated with TRIzol reagent (GIBCO) and reverse-transcribed using the TaqMan Reverse Transcriptase Kit (Applied Biosystems). Quantitative PCR was performed on the ABI Prism 7700 Sequence detection System with Taq-Man specific primers and probes for RAD51 (Hs00153418\_m1) and human GAPDH (4333764F) (Applied Biosystems). Cycling conditions were: 2 minutes at 50°C and 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Error bars show SD from four experiments.

### **ChIP Assay**

ChIP experiments were performed following the protocol of Chromatin-Immunoprecipitation Assay Kit (Upstate Biotechnology) with minor modifications.  $2 \times 10^6$  Tet-Off inducible cells per experimental sample were plated in 100-mm culture dishes. Cells were cross-linked and incubated in 100  $\mu$ l of SDS lysis buffer per  $1 \times 10^6$  cells with a protease-inhibitor mixture, and sonicated to generate 200-500bp DNA fragments. After incubation with 2  $\mu$ g of anti-p53 antibody (DO-1) and cross-linking

reversal, bound DNA was obtained by phenol chloroform extraction and ethanol precipitation, and resuspended in 20µl of H<sub>2</sub>O. PCR was performed with 2 µl of immunoprecipitated target DNA. Input material corresponding to 1% of total sample was recovered prior to immunoprecipitation, and PCR was performed with 0.5 µl of purified DNA. Primer sets used were the following: 5'-CCTCGAACTCCTAGGCTCAGA-3' and 3'-TATGCAATGCAGCTGCGCC-5' for Rad51 promoter; and 5'-CTTTCCACCTTTCACCATTCC-3' and 3'-CGACCACCGATAAAACAGGAA-5, for the p21 promoter (kindly provided by S. Taylor). PCR cycling conditions, determined to be within the linear range, were the following: 5 minutes at 94°C followed by 34 cycles for ChIP samples and 30 cycles for input samples of 45 seconds at 95°C, 45 sec at 60°C and 45 seconds at 72°C, ending with one step of 10 minutes at 72°C.

#### **Luciferase reporter assay**

Cells were transfected by calcium phosphate method with pRc/CMV coding for p53 wt (a gift from Dr. A.Levine) or p53R280K mutant (obtained by site-directed-mutagenesis performed in p53wt pRc/CMV with QuikChange® II site-directed mutagenesis kit, Stratagene) together with the luciferase reporter plasmid containing the Rad51 promoter (Rad51-Luc) or the mutant RAD51 promoters together with pRL-CMV Renilla luciferase for internal transfection control. Luciferase activity was determined in protein extracts with DUAL-Luciferase assay following the manufacturer's protocol (Promega).

#### **Analysis of Rad51 foci formation**

Tet-Off inducible p53wt-B2I and p53R280K-A3 and HCT166 cells were fixed and permeabilized. After blocking with IFF (PBS-3% BSA-1%NGS), cells were treated with DNase I (1000u/ul Boehringer) for 2 hours at 37C° and stained with 1/100 dilution of the rabbit polyclonal anti-RAD51 antibody (BD-Bioscience). Cells were washed and

incubated with 1/1000 diluted ALEXA-555 conjugated anti-rabbit IgG in IFF for 40 minutes at 22°C. Finally, cells were incubated with TO-PRO-3 iodide (1: 10,000 Molecular Probes) to visualize nuclei. Rad51 *foci* were counted using a Leica TCS-SP2 confocal microscope.