Supplementary Methods

Preparation of Cell Lysates

Cells were washed with 1× PBS and resuspended in ice-cold 0.5% NP-40 EBC lysis buffer [0.5% NP-40, 50 mM Tris (pH 7.6), 120 mM NaCl, 1 mM EDTA, 1 mM Na_3VO_4, 50 mM NaF, and 1 mM β-mercaptoethanol] with a cocktail of protease inhibitors (Calbiochem, Billerica, MA). Cells were lysed by sonication and centrifuged at 14,000*g* for 10 minutes at 4°C. The resulting supernatant was collected as the total cell lysate and used for Western blot or co-IP as described [1].

DNA-PK Activity Assay

The SignaTECT DNA-PK Assay Kit was used to measure DNA-PK activity (Promega). First, the following reaction mixtures in the presence or absence of activator was prepared (i.e., 2.5 μl of DNA-PK activation buffer or control buffer, 5.0 μl of DNA-PK in 5× reaction buffer, 2.5 μl of DNA-PK biotinylated peptide substrate, 0.2 μl of BSA (10 mg/ml), and 5 μl of [γ-^{32}P]ATP). The mixture was pre-incubated at 30°C for 4 minutes. The whole-cell extract sample was diluted in the enzyme buffer and the reaction was initiated by adding the appropriate amount of enzyme sample and incubated at 30°C for 5 minutes. The reaction was stopped by addition of termination buffer. Sample was spotted onto the SAM^{2R} Biotin Capture membrane. The membrane was then washed and dried under a heat lamp for 10 minutes. DNA-PK enzyme activity was determined by scintillation counting. Each experiment was repeated three times and data represent the mean ± SD of three separate determinations.

In Vivo *DNA End-joining Assay*

The pGL3 plasmid (Promega), in which expression of the luciferase gene is controlled by the CMV promoter, was used to evaluate correct NHEJ activity that precisely rejoins broken DNA ends *in vivo* as described [2]. The pGL3 plasmid was completely linearized by the restriction endonuclease *Nar*I, which cleaves within the luciferase coding region as confirmed by agarose gel electrophoresis. The linearized DNA was purified and then dissolved in sterilized water. A 20:1 mixture of the linearized pGL3 plasmid and pTK Renilla control luciferase reporter vector (an internal control) was transfected into cells. After 48 hours, luciferase activity was measured using a dual luciferase assay system following the manufacturer's instruction (Promega). Because the pGL3 reporter plasmid was digested to completion with *Nar*I within the luciferase coding region, only precise DNA end-joining can restore the luciferase activity. Each experiment was repeated three times and data represent the mean ± SD of three separate determinations.

Electrophoretic Mobility Shift Assay

Ku DNA binding was analyzed by EMSA as described [3]. Briefly, two complementary 79-nucleotide oligonucleotides were denatured and then annealed to generate a 71-bp fragment with *Bam*HI overhangs at each end. These two oligonucleotides are given as follows: Oligonucleotide 1, GAT CCT CTG AGG ACA CAG CCT TGT ATT ACT GTG CAA GAC ACA CAA TGA GCA AAA GTT ACT GTG AGC TCA AAC TAA AAC C and Oligonucleotide 2, GAT CGG TTT TAG TTT GAG CTC ACA GTA ACT TTT GCT CAT TGT GTG TCT TGC ACA GTA ATA CAA GGC TGT GTA CTC AGA G. The fragments were labeled with T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP. Ku protein binding activity was measured by a nondenatural gel EMSA. Each reaction mixture of 20 μl contained 0.5 ng of radiolabeled probe, 1.5 μl of nuclear extract, and 1 μg of supercoiled competitor DNA (pJG4-5) in binding buffer [15 mM Hepes (pH 7.9), 50 mM NaCl, 5 mM MgCl, 1 mM DTT, 0.5 mM EDTA, 1 mM Tris-HCl (pH 7.9), and 5% glycerol]. For all binding assays, the protein was added last and the reaction mixture was incubated at room temperature for 20 minutes. The reaction mixture was resolved by nondenaturing electrophoresis through a 6% polyacrylamide gel in 0.53 Tris-borate-EDTA buffer. The gel was dried on Whatman 3M paper and exposed to Kodak film.

RNA Interference

H460 cells expressing endogenous c-Myc were transfected with c-Myc siRNA using Lipofectamine 2000 (Invitrogen). A control siRNA (nonhomologous to any known gene sequence) was used as a negative control. The levels of c-Myc expression were analyzed by Western blot analysis. Specific silencing of the targeted *c-Myc* gene was confirmed by at least three independent experiments.

Supplemental References

[1] Deng X, Gao F, Flagg T, Anderson J, and May WS (2006). Bcl2's flexible loop domain regulates p53 binding and survival. *Mol Cell Biol* **26**, 4421–4434.

[2] Shin KH, Kang MK, Dicterow E, Kameta A, Baluda MA, and Park NH (2004). Introduction of human telomerase reverse transcriptase to normal human fibroblasts enhances DNA repair capacity. *Clin Cancer Res* **10**, 2551–2560.

[3] Wu X and Lieber MR (1996). Protein-protein and protein-DNA interaction regions within the DNA end-binding protein Ku70-Ku86. *Mol Cell Biol* **16**, 5186–5193.

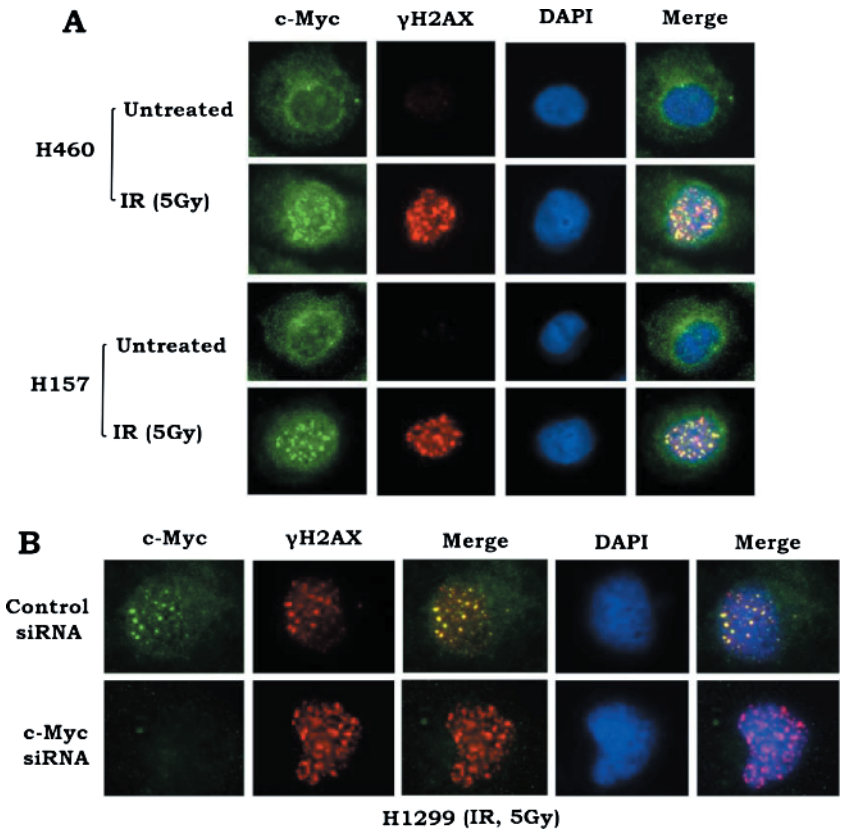

Figure W1. IR induces formation of c-Myc foci and DSBs in various types of human lung cancer cells. (A) H460 or H157 cells were treated with IR (5 Gy). c-Myc and γ-H2AX were analyzed immediately by immunofluorescent staining using primary anti–c-Myc (rabbit) and anti–γ-H2AX (mouse) antibodies as well as Alex 488 (green)–conjugated anti-rabbit or Alexa 594 (red)–conjugated anti-mouse secondary antibodies. (B) H1299 cells were transfected with c-Myc siRNA or control siRNA. Cells expressing c-Myc siRNA or control siRNA were treated with IR (5 Gy). c-Myc and γ-H2AX were analyzed by immunofluorescent staining as described in A.

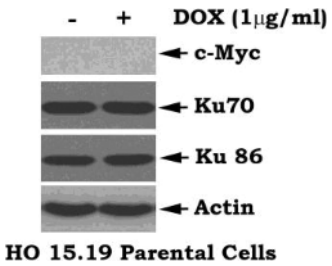

Figure W2. DOX has no effect on expression of c-Myc, Ku70, and Ku86 in HO15.19 parental c-Myc null cells. HO15.19 parental cells were treated with DOX for 24 hours. c-Myc, Ku70, and Ku86 were analyzed by Western blot.

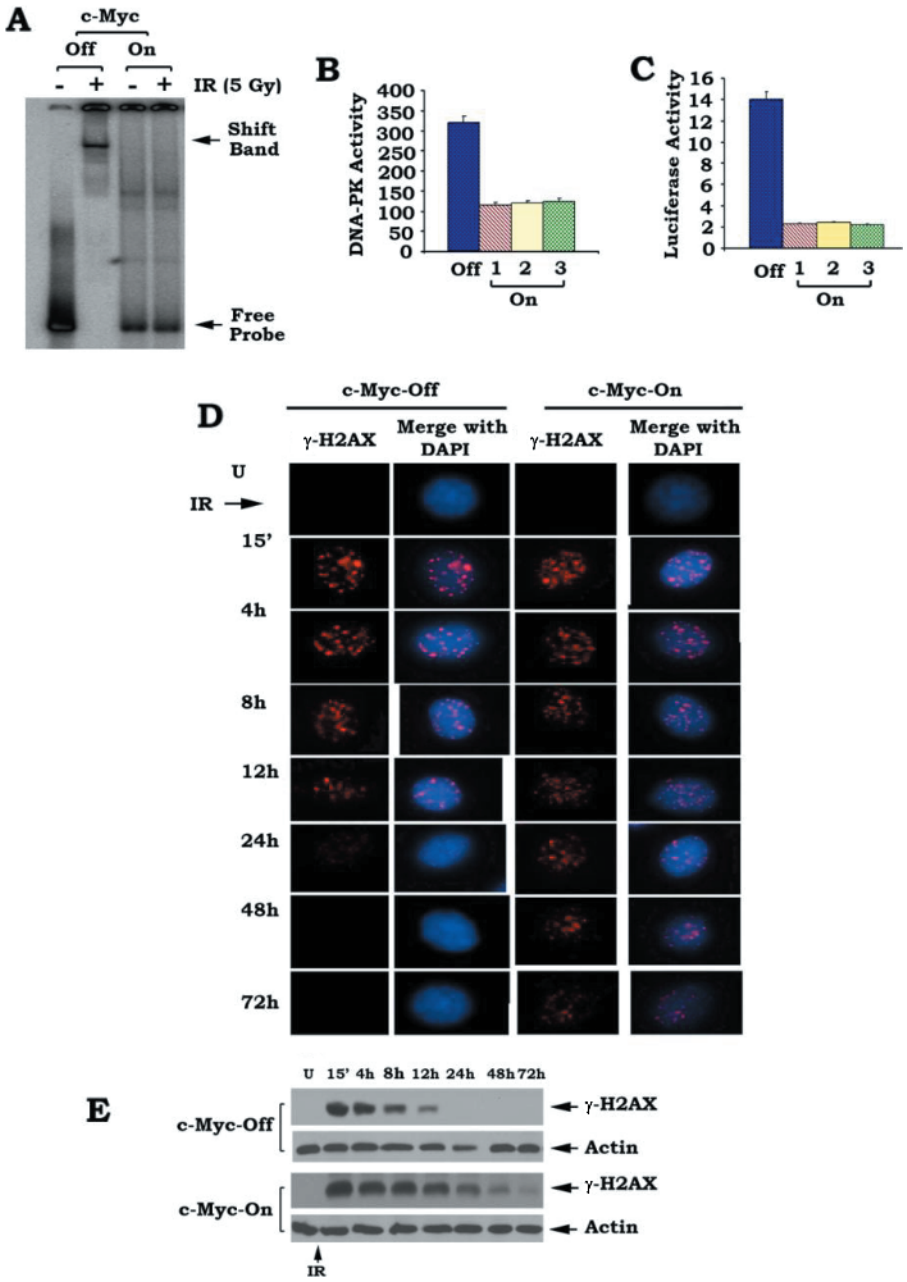

Figure W3. Conditional expression of c-Myc results in decreased DNA-PK, DNA end-joining, and DNA Ku binding activities in association with suppression of DSB repair. (A) The c-Myc–Off and c-Myc–On HO15.19 cells were exposed to 5 Gy of IR. Ku DNA binding was analyzed by EMSA. (B) DNA-PK activity was measured using the SignaTECT DNA-PK Assay Kit in c-Myc–Off or c-Myc–On cells. Error bars represent ± SD. (C) DNA end-joining activity was measured in c-Myc–Off or c-Myc–On HO15.19 cells. Error bars represent ± SD. (D and E) The c-Myc–Off and c-Myc–On HO15.19 cells were exposed to 5 Gy of IR. Cells were incubated in normal culture medium for various times. DSBs were determined by analysis of γ-H2AX by immunostaining and Western blot using γ-H2AX antibody.

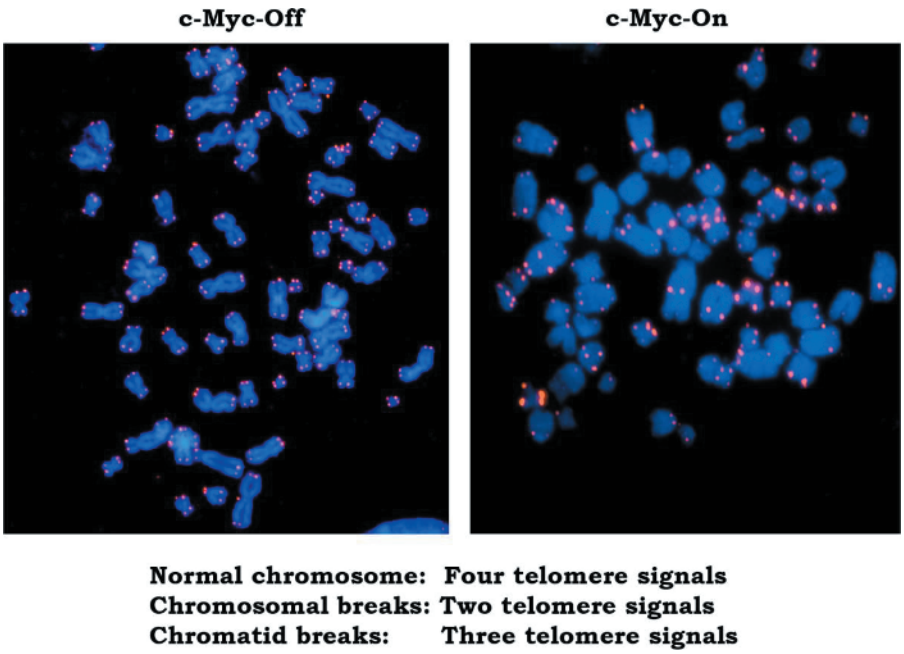

Figure W4. Conditional expression of c-Myc results in increased genetic instability. (A) Representative cytogenetic abnormalities were analyzed by T-FISH in c-Myc–Off and c-Myc–On HO15.19 cells (addition of DOX for 4 weeks). DAPI-stained chromosomes are shown in blue. Red dots come from telomere signals.

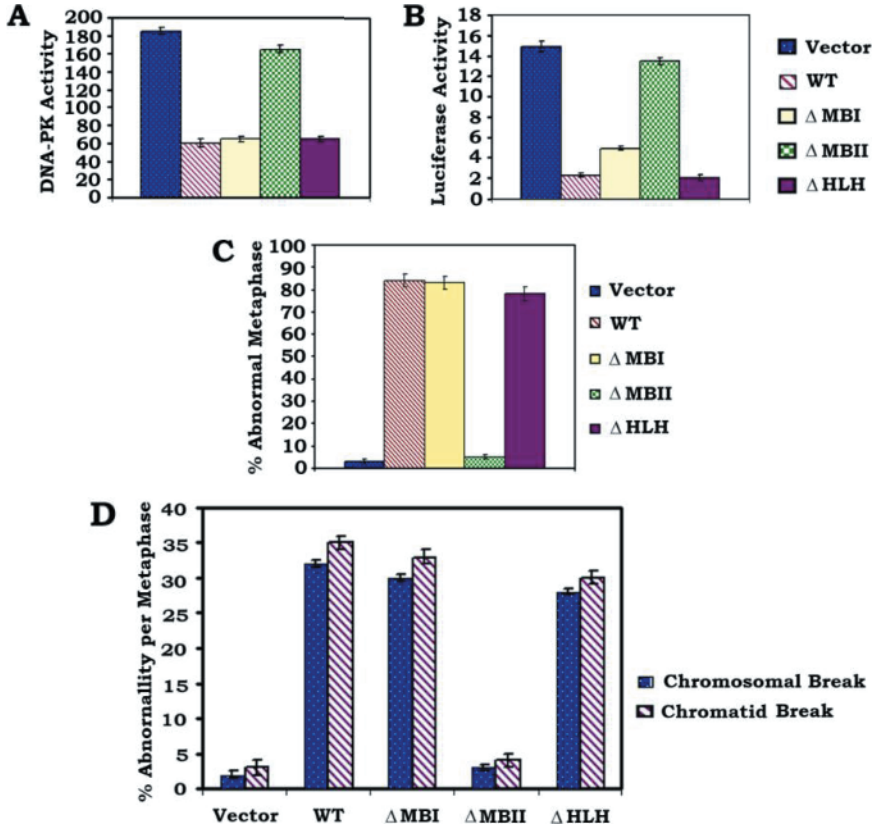

Figure W5. The ΔMBII domain of c-Myc is essential for c-Myc suppression of DNA-PK and DNA end-joining activities, as well as for enhanced genetic instability. (A) DNA-PK activity was measured using the SignaTECT DNA-PK Assay Kit in HO15.19 cells expressing WT or each of c-Myc deletion mutants. Error bars represent ± SD. (B) DNA end-joining activity was measured in HO15.19 cells expressing WT or each of c-Myc deletion mutants. Error bars represent ± SD. (C and D) Percentage of abnormal metaphases and frequency of cytogenetic abnormality per metaphase from HO15.19 cells expressing WT or each of c-Myc deletion mutants were quantified by T-FISH analysis. At least 30 metaphases per culture were analyzed. Each experiment was repeated three times and error bars represent ± SD.