Supplemental Information

Supplemental Material and Methods

Cell-cycle analysis and γH2AX staining

BrdU and anti-phospho-Histone H3 (Ser10) staining were performed as described (1). BrdU (10□M) was added in the culture medium for either 1hr or 0.5hr (for the pulse and chase experiment), before ethanol fixation. Staining was performed using an anti-BrdU-FITC (BD Pharmingen, #556028). For the γH2AX staining, samples were fixed overnight at -20°C in 70% ethanol, permeabilized in 0.25% Triton X-100 in PBS for 10 minutes on ice, incubated overnight at +4°C with the anti-γH2AX (clone JBW301, Upstate, #05-636) diluted 1:500 in 1%BSA in PBS and than 1hr at room temperature with a secondary antibody Alexa-488 (Invitrogen, #A21202) diluted 1:200. Samples were acquired using a FACSCalibur flow cytometer (Becton-Dickinson) and analyzed with the Flowjo software (Tree Star).

DNA repair assays

For the HR assay two million of U251 cells were transduced by electroporation using the Bio-Rad Gene Pulser II apparatus (Bio-Rad). 30 μg of the pDR-GFP reporter construct (2) were electroporated concomitantly with 30 μg of the pCβASce vector (3) that encodes for the I-*Sce*I endonuclease. The pNZE-CAG construct was used to monitor plasmid uptake in parallel electroporations. HR efficiency was monitored by flow cytometry for expression of GFP 24 hr after electroporation.

The Pem1-Ad2-EGFP reporter (4), kindly provided by Scott Kauffman, was used to test NHEJ efficiency. The Pem1-Ad2-EGFP vector has been digested overnight at 37°C with either *Hind*III or I-*Sce*I and the linearized constructs were purified using a

QIAquik gel purification kit (QIAGEN). U251 and T98G cells were then transduced by electroporation using 1 μg of the purified constructs. The pNZE-CAG construct was used to monitor plasmid uptake in parallel electroporations. NHEJ was monitored by flow cytometry for expression of GFP 48 hr after electroporation. To test the percentage of microhomology-mediated junctions (MMJ) and the distribution of microhomology lengths, genomic DNA was purified from the cells transduced with the linearized Pem1-Ad2-EGFP plasmids using a Miniprep purification kit (QIAGEN) and then electroporated into MAX Efficiency DH10B competent cells (Invitrogen). The plasmids recovered from the bacteria were digested with *Hind*III and analyzed by agarose gel electrophoresis.

U251 orthotopic GBM model and bioluminescence imaging

U251 cells were stably transduced with a HSV1-tk/ GFP/firefly luciferase (TGL) triple reporter construct (5) and GFP positive cells were purified by FACS. U251-TGL cells were than infected with pRetroSuper empty vector or pRetroSuper 53bp1 shRNA_A and subjected to puromycin selection. Two hundred fifty thousand cells were injected intracranially into athymic Nude-*Foxn1*^{nu} mice. For bioluminescence imaging mice were anesthetized with 3% isolflurane before retroorbital injection with 25 mg/kg body weight luciferin (10 mg/mL in water). One minute after injection of the luciferin, images were acquired for 10 seconds with an IVIS 200 (Xenogen).

pGIPZ lentiviral *53bp1* shRNAmirs:

Sh ID#	Clone ID	Sequence
1	V3LHS_318805	TGAGTCAGAATGATGACAA
2	V3LHS_318807	AGAAGTAGAAAGAAAAGTA
3	V3LHS_318808	TGATGCTTTCTACAAGTGA
4	V3LHS_318809	AGCAGCAACCCAGACTATA
5	V3LHS_644816	GGATCTTGTATATAGTTTT
6	V2LHS_56191	GACGGAGTACTAATAAGGA
7	V2LHS_56192	CAGATATCAGCTTAGACAA
8	V2LHS_56193	CCCTCTCGGAGGATGAGTA
9	V3LHS_635694	TGAGTCAGAATGATGACAA
10	V3LHS_635698	AGCAGCAACCCAGACTATA
11	V3LHS_635699	TGACAGTTGAGTGTTCTAA
12	Non-silencing ctrl	N.A.

Supplemental References

1. Theunissen JW, Petrini JH. Methods for studying the cellular response to DNA damage: influence of the Mre11 complex on chromosome metabolism. Methods Enzymol 2006;409:251-84.
2. Pierce AJ, Johnson RD, Thompson LH, Jasin M. XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev 1999 Oct 15;13(20):2633-8.
3. Richardson C, Moynahan ME, Jasin M. Double-strand break repair by interchromosomal recombination: suppression of chromosomal translocations. Genes Dev 1998 Dec 15;12(24):3831-42.
4. Seluanov A, Mittelman D, Pereira-Smith OM, Wilson JH, Gorbunova V. DNA end joining becomes less efficient and more error-prone during cellular senescence. Proc Natl Acad Sci U S A 2004 May 18;101(20):7624-9.
5. Ponomarev V, Doubrovin M, Serganova I*, et al.* A novel triple-modality reporter gene for whole-body fluorescent, bioluminescent, and nuclear noninvasive imaging. Eur J Nucl Med Mol Imaging 2004 May;31(5):740-51.