**Supplemental Material of Burkalter, Roberts, Havener, and Ramsden**

**Supplemental Fig. 1.** Typical cell cycle profiles obtained by propidium iodide staining and flow cytometry after cell synchronization in (*A*) G1/G0 or (*B*) S phase. Shown are profiles of wild type (K1) cells.

**Supplemental Fig. 2.** Wild type (K1) cells were irradiated with 8 Gy or left untreated. At indicated time points after irradiation cells were fixed and probed for cleaved caspase-3, a marker for apoptotic cells (anti-cleaved Caspase-3 (Asp175) (5A1, Cell Signaling, 1:200) and Cy3-conjugated donkey anti-rabbit (Jackson ImmunoResearch; 1:1000)) as well as DAPI (5 µg/ml). No cells positive for cleaved Caspase 3 were detected after irradiation, indicating that apoptosis is not induced by irradiation during the analyzed time frame. As a positive control, cells were treated with 1µg/ml (f.c.) Staurosporine (Sigma) to induce apoptosis, which led to detection of cells positive for cleaved Caspase-3 after 2.5 h. Furthermore, staurosporine treatment induced fragmentation of nuclei at time points 5 h, 7.5 h, and 10 h, what was not observed in untreated or irradiated cells

**Supplemental Fig. 3.** Analysis of Rad51 focus formation by immunofluorescence during S phase. Quantification of Rad51 focus formation in R2-positive, S/G2 wild type (K1) and NHEJ-deficient (Xrs6) cells, averaged from three independent experiments.

**Supplemental Fig. 4.** Effect of p53R2 overexpression on nucleotide pools. Wild type (K1) or NHEJ-deficient (Xrs6) CHO cells were compared to subclones of these lines that stably overexpress p53R2 (K+P and X+P, respectively). Whole cell dATP levels (average

of two experiments +/- SD) were measured in G1/G0 or S phase enriched populations. We show the ratio of dATP in the p53R2 overexpressing subclone relative to the matching parental line. Cellular dNTP levels were measured essentially as previously described [1]. Synchronized cells were counted and washed twice with PBS. Cells were extracted by resuspension in 60% chilled ethanol containing 10 mM Tris pH7.5 at a density of $5x10^4$ cells per µl and incubated on ice for 2 hours followed by incubation for 3 minutes at 95˚C. Extracts were then clarified by centrifugation at 4˚C for 15 minutes at 16000 x g. The supernatant was transferred into a new tube, lyophilized, and resuspended in 25 mM Tris-HCl pH 7.5 at a concentration of $2.5x10^5$ cells/µl. Extracts were added to 25 µl reactions with 20 mM Tris 7.5, 5 mM DTT, 10 mM $MgCl_2$, 10 ng/µl BSA, 24-96 nM annealed oligonucleotide template (5'-CCGCCTCCACCGCC and 5'-AAATAAATAAATAAATAAATGGCGGTGGAGGCGG), and 6.6 nM [$\alpha$-$^{32}$P]TTP (along with 1.6 – 5 nM corresponding cold dTTP). Reactions were started with 0.25U Klenow fragment (exo$^-$) and stopped after 20 minutes at 37$^o$C by addition of EDTA to 20 mM. Reaction mixtures were then transferred onto DE80 paper (Whatman), washed 5 times with 5% $Na_2HPO_4$, and intensities analyzed by phosphor imager and ImageQuant as software. Averages and standard deviation of two experiments are shown.

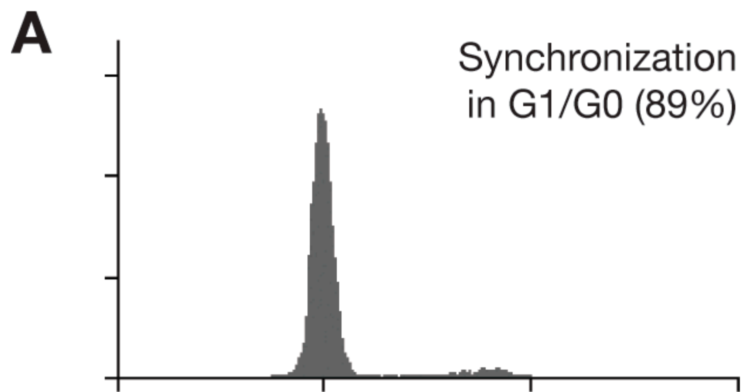
**Supplemental Fig. 5.** Repair of a targeted double strand break by homologous recombination. (*A*) Effect of p53R2 overexpression on recombination after 48 hours. The cell line K-DR was assessed for GFP expression after transfection with empty vector alone or with I-SceI expression. GFP expression was further analyzed after transient expression of wild type p53R2 or a catalytic mutant of p53R2 (p53R2-CM), respectively,

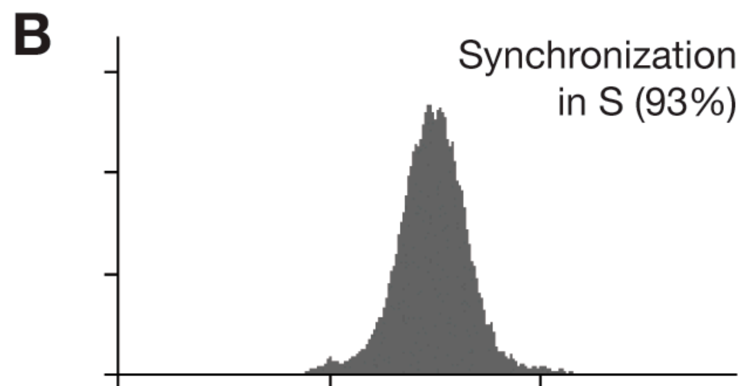
with or without simultaneous expression of I-SceI. Shown are the absolute levels of GFP positive cells 48 hours after transfection. *p53R2 overexpression stimulated GFP expression significantly; p=0.0372. The mean and the standard error of the mean of three independent experiments were calculated with Prism 4.0c (Graphpad).

**References of Supplemental Material**

[1] P.A. Sherman, J.A. Fyfe. Enzymatic assay for deoxyribonucleoside triphosphates using synthetic oligonucleotides as template primers, Anal Biochem 180 (1989) 222-226.

Supplemental Figure 1:



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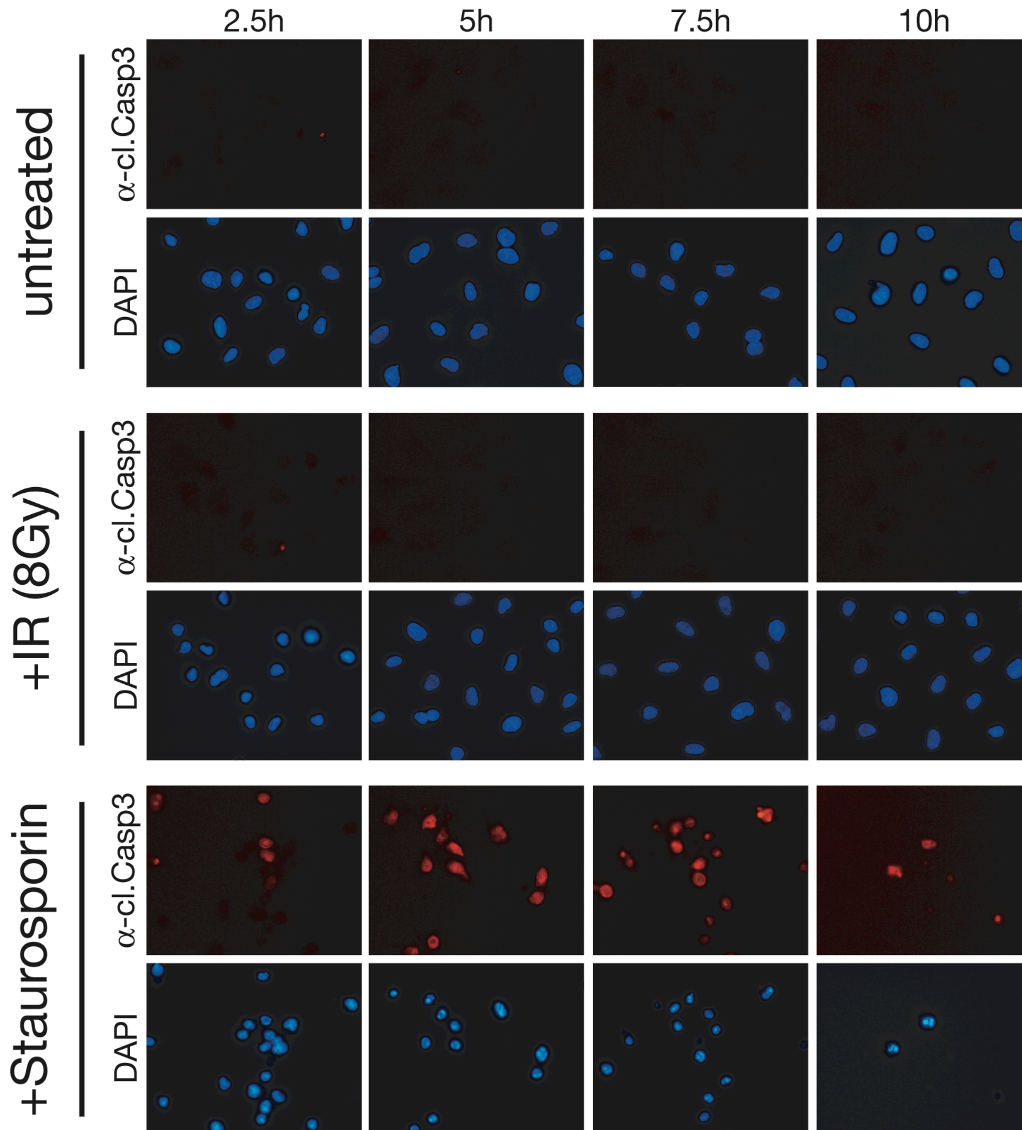

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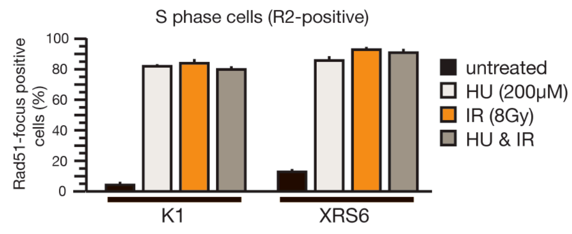
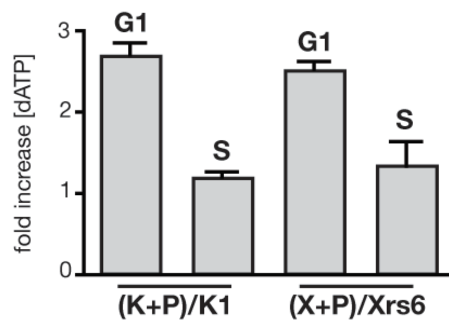


Supplemental Figure 4:



Supplemental Figure 5: