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p53 Null Fluorescent Yellow Direct Repeat (FYDR) Mice have Normal Levels of Homologous Recombination

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Abstract

The tumor suppressor p53 is a transcription factor whose function is critical for maintaining genomic stability in mammalian cells. In response to DNA damage, p53 initiates a signaling cascade that results in cell cycle arrest, DNA repair or, if the damage is severe, programmed cell death. In addition, p53 interacts with repair proteins involved in homologous recombination. Mitotic homologous recombination (HR) plays an essential role in the repair of double-strand breaks (DSBs) and broken replication forks. Loss of function of either p53 or HR leads to an increased risk of cancer. Given the importance of both p53 and HR in maintaining genomic integrity, we analyzed the effect of p53 on HR *in vivo* using Fluorescent Yellow Direct Repeat (FYDR) mice as well as with the sister chromatid exchange (SCE) assay. FYDR mice carry a direct repeat substrate in which an HR event can yield a fluorescent phenotype. Here, we show that p53 status does not significantly affect spontaneous HR in adult pancreatic cells *in vivo* or in primary fibroblasts *in vitro* when assessed using the FYDR substrate and SCEs. In addition, primary fibroblasts from p53 null mice do not show increased susceptibility to DNA damage-induced HR when challenged with mitomycin C. Taken together, the FYDR direct repeat assay and SCE analysis indicate that, for some tissues and cell types, p53 status does not greatly impact HR.

Keywords

homologous recombination; p53; genomic stability; fluorescence; mitomycin C

1. Introduction

The p53 tumor suppressor gene is a transcription factor that plays an essential role in maintaining genomic stability. Indeed, greater than half of all tumors have lost p53 function [1–5]. Inherited mutations in p53 cause Li-Fraumeni syndrome, a genetic disorder characterized by an early incidence of cancer [6, 7], and p53 null mice develop tumors at an accelerated rate [8]. Together, these data indicate that p53 is a key inhibitor of tumor formation, and loss of p53 function provides cells with critical selective advantages for tumor formation.

p53 mediates cell cycle arrest and/or apoptosis in response to DNA damage. Cells are constantly exposed to endogenous and exogenous agents that can damage DNA [9]. Of the many lesions that form, DNA double-strand breaks (DSBs) are among the most cytotoxic and mutagenic, and mitotic homologous recombination (HR) provides a critical pathway for their repair [9]. In addition, HR provides the only pathway for accurate repair of broken replication forks [10]. Thus, HR is a critical for preventing tumor-promoting sequence rearrangements.

As with inherited mutations in p53, germline mutations in genes that modulate HR are also associated with an increased risk of cancer [11–15]. Exchanges between misaligned sequences can lead to tumorigenic rearrangements and loss of heterozygosity. While too much HR can be problematic [15–17], too little HR can also lead to genomic instability. In the absence of HR, misrepair can lead to large-scale sequence rearrangements. Indeed, germline mutations that suppress HR (*i.e.*, BRCA1 [18], BRCA2 [19], FANCC [20]) increase the risk of cancer [15, 21, 22]. Thus, maintaining the accuracy and the rate of HR is critical for preventing tumor formation.

Given the importance of both p53 and HR in maintaining genomic stability, a number of studies have focused on the effect of p53 status on HR. While some *in vitro* studies suggest that p53 suppresses HR [23–29], many other studies do not show any effect of p53 on HR [30–35]. Although the number of studies performed *in vivo* is limited, several investigators have nevertheless studied HR in *p53* null mice *in vivo*. These studies show either no effect or a slight suppressive effect of p53 on HR [11, 36, 37]. Taken together, there is limited information about the *in vivo* effect of p53 on HR in adult tissues.

Here, we investigate the impact of p53 on HR. We used the Fluorescent Yellow Direct Repeat (FYDR) mice, in which an HR event at an integrated transgene yields a fluorescent signal [38]. Analysis of pancreata from *Fydr*$^{y/y}$;*p53*$^{+/+}$ and *Fydr*$^{y/y}$;*p53*$^{-/-}$ mice shows that the frequency of recombinant cells is not affected by p53 *in vivo*. Since HR plays a critical role in the repair of broken replication forks, we further analyzed the effect of p53 on HR in rapidly dividing primary fibroblasts. However, p53 had no effect on the rate of spontaneous or damage-induced HR *in vitro*. Taken together, loss of p53 does not significantly impact SCEs or HR at the FYDR substrate *in vitro* or *in vivo*. These data call attention to the possibility that the impact of p53 on HR is cell type-dependent.

2. Materials and Methods

2.1 Animals

FYDR [38] and p53 mice [39] were described previously. 9 week old *Fydr*$^{y/y}$;*p53*$^{+/+}$ and *Fydr*$^{y/y}$;*p53*$^{-/-}$ littermates were compared in a sex-matched fashion.

2.2 Flow Cytometry

Pancreatic cells were disaggregated as described previously [40]. Disaggregated pancreatic cells were pelleted and resuspended in 350 µl OptiMEM (Invitrogen), filtered (35 µm), and analyzed with a Becton Dickinson FACScan flow cytometer (excitation 488 nm, argon laser). On average, ~1 million cells were analyzed per sample.

2.3 Imaging

Pancreata were imaged as described previously [40]. Briefly, whole pancreata were compressed to 0.5 mm and imaged using a fixed aperature time (1× objective). Filters included: visible light; UV-2E/C (Ex:330–380 nm, Em:420 nm); Red (Ex:540/25 nm, Em: 605/55 nm); and EYFP (Ex:460–500 nm, Em:510–560 nm). Foci were counted in a blinded

fashion. Pancreata surface area was determined using Scion Image Beta 4.02 Win (Scion Corporation).

2.4 Isolation of Ear Fibroblasts

Ears were isolated, minced, and incubated at 37°C in 4 mg/ml collagenase/dispase (Roche Applied Sciences). After 1 hour, two volumes of fibroblast medium were added [Dulbecco's Modified Eagle's Medium, 15% FBS, 100 units/ml penicillin, 100 µg/ml streptomycin, 0.3 mg/ml L-glutamine, 0.1mM Non-Essential Amino Acids, 5 µg/ml amphotericin B (Sigma)]. After 24 hours at 37°C and 5% CO_2, cells were triturated, filtered (70-µm mesh; Falcon), and seeded into dishes.

2.5 SCE analysis

Primary ear fibroblasts from age and sex-matched $Fydr^{y/y};p53^{+/+}$ and $Fydr^{y/y};p53^{-/-}$ littermates were seeded at 2×10^5 cells per well. After 24 hours, cells were cultured in10 µM BrdU in McCoys media. After ~22 hr, cells were incubated for ~2 hr in 0.1 µg/ml Colcemid, and SCEs were analyzed as previously described [41]. SCEs from 25–30 metaphase spreads per genotype were counted in a blinded fashion. Analysis was performed on passage-matched samples (<5 passages).

2.6 Calculation of Rate in Primary Fibroblasts

Primary ear fibroblasts were isolated from sex-matched $Fydr^{y/y};p53^{+/+}$ and $Fydr^{y/y};p53^{-/-}$ littermates and rate experiments were performed in parallel. For rate experiments, ~10^4 cells were seeded into each of 24 independent cultures. Cultures were expanded to ~10^6 cells prior to flow cytometry. Freshly isolated fibroblasts were used for each experiment (passage 2). The MSS Maximum Likelihood Method was used to calculate the rate of recombination, as described [42]. Rate experiments were repeated with fibroblasts from seven different pairs of mice.

2.7 Quantification of spontaneous and DNA damage-induced recombinant cell frequency

Primary ear fibroblasts from sex-matched $Fydr^{y/y};p53^{+/+}$ and $Fydr^{y/y};p53^{-/-}$ littermates were seeded at 5×10^5 cells per 100 mm dish. After 24 hours, quadruplicate samples were cultured in Dulbecco's Modified Eagle's Medium or exposed to medium supplemented with 0.5 µg/ml mitomycin-C (CAS No 50-07-7) for 1 hour. After 72 hours, samples were analyzed by flow cytometry. Population growth was determined using a Coulter counter. Experiments were repeated with fibroblasts from three different cohorts.

3. Results

3.1 Effect of p53 status on spontaneous homologous recombination in pancreatic cells

To study HR *in vivo*, we previously developed FYDR mice that carry a direct repeat recombination substrate containing two differently mutated copies of the coding sequence for enhanced yellow fluorescent protein (EYFP). An HR event can restore full length *EYFP* coding sequence (Fig. 1) [38]. One method for measuring the *in vivo* frequency of fluorescent recombinant cells is to analyze disaggregated tissue by flow cytometry [40]. To determine the effect of p53 status on HR *in vivo*, we analyzed recombinant cells in pancreata of 9 week-old $Fydr^{y/y};p53^{+/+}$ and $Fydr^{y/y};p53^{-/-}$ mice. The median frequency of recombinant pancreatic cells is not significantly different between the two cohorts (Fig. 2A).

Analysis of FYDR pancreata by flow cytometry requires tissue disaggregation; thus, the contribution of clonal expansion versus independent HR events on recombinant cell frequency cannot be determined [40, 43, 44]. We previously developed *in situ* imaging

techniques that enable the direct detection of fluorescent recombinant foci (recombination events; see Fig. 1 [40]). For the $Fydr^{y/y};p53^{+/+}$ and $Fydr^{y/y};p53^{-/-}$ mice, the median number of recombinant foci is not significantly different between the two cohorts (Fig. 2B). Additionally, because the sizes of pancreata vary among mice within each cohort (data not shown), we analyzed the number of foci per unit surface area, and again found that the frequencies of recombinant foci per cm^2 are not significantly different (Fig. 2C). Although there is significant variation among individual animals, previous studies show that relatively subtle differences in the median value can nevertheless be detected. For example, a ~3 fold difference in recombination frequency was observed using fewer mice than in this study [40]. Thus, these data indicate that p53 status does not significantly impact the susceptibility of adult pancreatic cells to HR *in vivo*.

3.2 Effect of p53 Status on HR in Primary Fibroblasts in vitro

HR activity is most active during S phase and late S/G2 [45], however, in the pancreas, the vast majority of cells are not in S phase [46]. To explore the effect of p53 status on HR in rapidly dividing cells, we created primary ear fibroblast cultures from $Fydr^{y/y};p53^{+/+}$ and $Fydr^{y/y};p53^{-/-}$ mice, and analyzed genome-wide HR events using the sister chromatid exchange (SCE) assay [47]. There is not a significant difference in the frequency of spontaneous SCEs in $Fydr^{y/y};p53^{+/+}$ and $Fydr^{y/y};p53^{-/-}$ primary ear fibroblasts (Fig 3A). Primary fibroblasts were also analyzed via flow cytometry. No difference was observed in the frequency of fluorescent recombinant cells among $p53^{+/+}$ and $p53^{-/-}$ cultures (Fig. 3B).

As another independent approach to explore p53's potential effects on HR, we measured the rate of HR per cell division. For each experiment, 24 independent cultures were allowed to expand prior to analysis and the recombination rate was calculated using the MSS Maximum Likelihood Method [42]. We found that the rate of homologous recombination is not significantly different between $Fydr^{y/y};p53^{-/-}$ and $Fydr^{y/y};p53^{+/+}$ fibroblasts (Fig. 3C). Thus, taken together, these studies of HR using the FYDR system and the traditional SCE assay show that p53 status does not affect the spontaneous frequency or rate of HR in primary fibroblasts *in vitro*.

To determine if p53 impacts HR induced by an exogenous agent, we treated primary fibroblasts with a potent recombinogen, mitomycin-C (MMC), and assayed both cell proliferation and HR frequency. Consistent with being resistant to cell cycle arrest and to apoptosis, untreated $p53^{-/-}$ cells had a proliferative advantage (compare black bars in Fig. 3D) [48]. When challenged with MMC, $Fydr^{y/y};p53^{+/+}$ and $Fydr^{y/y};p53^{-/-}$ fibroblasts both show significant growth inhibition (see black versus gray bars in Fig. 3D). We next queried MMC-induced HR. For both $Fydr^{y/y};p53^{+/+}$ and $Fydr^{y/y};p53^{-/-}$ fibroblasts, there is a statistically significant increase in the frequency of recombinant cells for MMC-treated cultures (compare black and gray bars in Fig. 3E). However, $p53^{-/-}$ cells did not show an increase in susceptibility to MMC-induced HR events.

4. Discussion

As guardian of the genome, p53 function is important for maintaining genomic integrity and loss of p53 results in an early onset and increased frequency of many types of cancers [6, 7, 39]. In response to DNA damage, p53 promotes the repair of DNA damage by inducing cell cycle arrest and activating DNA repair proteins. However, in the presence of extensive DNA damage, p53 signals for the elimination of damaged cells through apoptosis [49]. Due to p53's critical role in suppressing cancer, we set out to understand the mechanisms through which loss of p53 promotes genomic instability.

A number of studies have shown the direct involvement of p53 in HR. Specifically, p53 may act at stalled replication forks to prevent breakdown [50, 51]. Additionally, p53 can directly interact with and inhibit the function of HR proteins, including RPA [52], Rad51 [53, 54], BLM and WRN [55–57]. Finally, p53 has been shown to bind to Holliday Junctions [57–59]. Thus, a clear interaction between p53 and HR exists and some of its activities suggest that p53 would suppress HR. However, using FYDR mice we found that p53 status has no effect on HR *in vivo* in pancreatic cells or *in vitro* in primary fibroblasts, suggesting that the role of p53 in modulating spontaneous HR *in vivo* is minimal in at least some cell types. Thus, p53's tumor suppressor function may be more reliant on suppression of other forms of genomic instability and/or on other attributes of p53, rather than on its potential to suppress HR.

Using two different mouse models, previous studies examining the effect of p53 status on HR *in vivo* show different results. In pink-eyed unstable (*p^{un}*) mice, HR at a direct repeat during embryonic development can give rise to a dark spot on the fur or on the retinal epithelium [60, 61]. Using the fur spot assay, p53 had no effect on HR *in vivo* [36]. However, another study using the more sensitive *p^{un}* eye-spot assay showed that loss of p53 increased HR events, specifically during early embryonic development [11]. Because clonal expansion of recombined cells is required to detect spots on fur and retinal epithelium, *p^{un}* mice can only be used to detect recombination events that occur during embryogenesis. In contrast, FYDR mice can be used to detect recombination events that occur in adult tissues *in vivo*. Therefore, p53 may be less important in suppressing HR in adult versus embryonic tissues.

In addition to possible differences among stages of development, the effect of p53 on HR may be cell type specific. Indeed, studies using the adenine phosphoribosyltransferase (*Aprt*) mouse model to detect recombination events *in vivo* showed that while fibroblasts derived from *p53^{−/−}* mice had ~2 fold increase in spontaneous mutation events due to mitotic recombination; there was no difference in *p53^{−/−}* compared to *p53^{+/+}* T lymphocytes [37, 62]. Together, these data show that p53 may have different effects on HR in different cell types.

When considering the *in vivo* data, it is important to consider the limitations of the assay used here. It is noteworthy that there is a significant amount of variability from mouse to mouse. Some of this variation is due to stochastic factors, such as the timing of the recombination event, which can influence the total number of recombinant cells as a consequence of clonal expansion. Despite this variance, in older animals and in animals exposed to DNA damaging agents, a change in the median value has been observed, indicating that this approach can be used effectively to detect endogenous and exogenous factors that impact homologous recombination [40, 43]. Given that we did not detect an impact of p53 status on HR, even when analyzed with multiple approaches, we conclude that p53 has at most a subtle impact on HR in adult animals, at least in some tissues.

The effect of p53 function on HR has also been examined *in vitro* in multiple cell types. In studies using systems that detect HR events at specific loci (*e.g.*, *Tk*, EGFP, SV40), the majority suggest that p53 suppresses HR *in vitro* [23–26, 28, 29, 63–65]. In contrast, virtually all studies analyzing the effect of p53 on HR by examining SCEs show no effect of p53 function on HR [32, 34, 35, 66–70]. Additional SCE studies reveal that p53 does not significantly impact damage-induced HR when cells are exposed to a variety of agents (mitomycin-C, nitric oxide, low dose radiation and low dose UV) [66–70]. Thus, the observation here that p53 does not impact SCEs is consistent with previous studies.

It is clear that HR is dependent on multiple factors, including stage of development, cell type, and detection method. Interestingly, Slebos and Taylor found that p53 status influenced one type of HR reporter system, but not another [31]. Furthermore, p53 appears to act differently in different types of recombination events, depending on the amount of homology between the sequences [23, 28, 71, 72]. Finally, different types of mutations in the p53 gene (*e.g.*, null versus a point mutation) have different effects on HR [23, 29, 64, 73]. Here, we have shown that in cells and tissues of mice lacking p53, there is not a significant effect on recombination *in vivo* or *in vitro* when assessed at a direct repeat or via SCEs. Taken together, previous studies and the results presented here suggest a rather complex relationship between p53 and HR, which is consistent with p53's highly pleiotropic roles in cell behavior. Finally, this study, in combination with the results from several others [34–37, 72], emphasizes the extent to which HR is independent of p53 in many normal cells and tissues.

Highlights
► p53 does not impact homologous recombination at a direct repeat in mouse pancreata
► p53 null fibroblasts show normal levels of homologous recombination and sister chromatid exchanges. ► p53 does not appear to be a major factor in modulating HR in some cell types in vivo.

Acknowledgments

References

1. Hollstein M, Shomer B, Greenblatt M, Soussi T, Hovig E, Montesano R, Harris CC. Somatic point mutations in the p53 gene of human tumors and cell lines: updated compilation. Nucleic Acids Res. 1996; 24:141–146. [PubMed: 8594564]

2. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science. 1991; 253:49–53. [PubMed: 1905840]

3. Agirre X, Novo FJ, Calasanz MJ, Larrayoz MJ, Lahortiga I, Valganon M, Garcia-Delgado M, Vizmanos JL. TP53 is frequently altered by methylation, mutation, and/or deletion in acute lymphoblastic leukaemia. Mol Carcinog. 2003; 38:201–208. [PubMed: 14639659]

4. Hurt EM, Thomas SB, Peng B, Farrar WL. Reversal of p53 epigenetic silencing in multiple myeloma permits apoptosis by a p53 activator. Cancer Biol Ther. 2006; 5:1154–1160. [PubMed: 16855375]

5. Bello MJ, Rey JA. The p53/Mdm2/p14ARF cell cycle control pathway genes may be inactivated by genetic and epigenetic mechanisms in gliomas. Cancer Genet Cytogenet. 2006; 164:172–173. [PubMed: 16434325]

6. Srivastava S, Zou ZQ, Pirollo K, Blattner W, Chang EH. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. Nature. 1990; 348:747–749. [PubMed: 2259385]

7. Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CM, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH. Germline p53 mutations in a familial syndrome of breast cancer, sarcomas and other neoplasias. Science. 1990; 250:1233–1238. [PubMed: 1978757]

8. Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT, Weinberg RA. Tumor spectrum analysis in p53-mutant mice. Current Biology. 1994; 4:1–7. [PubMed: 7922305]

9. Friedberg, EC.; Walker, GC.; Siede, W.; Wood, RD.; Schultz, RA.; Ellenberger, T. DNA Repair and Mutagenesis. Second ed.. Washington, DC: ASM Press; 2006.

10. Helleday T, Lo J, van Gent DC, Engelward BP. DNA double-strand break repair: from mechanistic understanding to cancer treatment. DNA Repair (Amst). 2007; 6:923–935. [PubMed: 17363343]

11. Bishop AJ, Hollander MC, Kosaras B, Sidman RL, Fornace AJ Jr, Schiestl RH. Atm-, p53-, and Gadd45a-deficient mice show an increased frequency of homologous recombination at different stages during development. Cancer Res. 2003; 63:5335–5343. [PubMed: 14500365]

12. Saintigny Y, Makienko K, Swanson C, Emond MJ, Monnat RJ Jr. Homologous recombination resolution defect in Werner syndrome. Mol. Cell. Biol. 2002; 22:6971–6978. [PubMed: 12242278]

13. Nakayama H. RecQ family helicases: roles as tumor suppressor proteins. Oncogene. 2002; 21:9008–9021. [PubMed: 12483516]

14. D'Andrea AD, Grompe M. The Fanconi anaemia/BRCA pathway. Nat. Rev. Cancer. 2003; 3:23–34. [PubMed: 12509764]

15. Thompson LH, Schild D. Recombinational DNA repair and human disease. Mutat. Res. 2002; 509:49–78. [PubMed: 12427531]

16. Prince PR, Emond MJ, Monnat RJ Jr. Loss of Werner syndrome protein function promotes aberrant mitotic recombination. Genes Dev. 2001; 15:933–938. [PubMed: 11316787]

17. Chaganti RSK, Schonberg S, German J. A manyfold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes. Proc. Natl. Acad. Sci. USA. 1974; 71:4508–4512. [PubMed: 4140506]

18. Moynahan ME, Chiu JW, Koller BH, Jasin M. Brca1 controls homology-directed DNA repair. Mol. Cell. 1999; 4:511–518. [PubMed: 10549283]

19. Moynahan ME, Pierce AJ, Jasin M. BRCA2 is required for homology-directed repair of chromosomal breaks. Mol. Cell. 2001; 7:263–272. [PubMed: 11239455]

20. Niedzwiedz W, Mosedale G, Johnson M, Ong CY, Pace P, Patel KJ. The Fanconi anaemia gene FANCC promotes homologous recombination and error-prone DNA repair. Mol Cell. 2004; 15:607–620. [PubMed: 15327776]

21. Hahn SA, Greenhalf B, Ellis I, Sina-Frey M, Rieder H, Korte B, Gerdes B, Kress R, Ziegler A, Raeburn JA, Campra D, Grutzmann R, Rehder H, Rothmund M, Schmiegel W, Neoptolemos JP, Bartsch DK. BRCA2 germline mutations in familial pancreatic carcinoma. J Natl Cancer Inst. 2003; 95:214–221. [PubMed: 12569143]

22. Couch FJ, Johnson MR, Rabe K, Boardman L, McWilliams R, de Andrade M, Petersen G. Germ line Fanconi anemia complementation group C mutations and pancreatic cancer. Cancer Res. 2005; 65:383–386. [PubMed: 15695377]

23. Akyuz N, Boehden GS, Susse S, Rimek A, Preuss U, Scheidtmann KH, Wiesmuller L. DNA substrate dependence of p53-mediated regulation of double-strand break repair. Mol. Cell. Biol. 2002; 22:6306–6317. [PubMed: 12167722]

24. Bertrand P, Rouillard D, Boulet A, Levalois C, Soussi T, Lopez BS. Increase of spontaneous intrachromosomal homologous recombination in mammalian cells expressing a mutant p53 protein. Oncogene. 1997; 14:1117–1122. [PubMed: 9070661]

25. Mekeel KL, Tang W, Kachnic LA, Luo CM, DeFrank JS, Powell SN. Inactivation of p53 results in high rates of homologous recombination. Oncogene. 1997; 14:1847–1857. [PubMed: 9150391]

26. Wiesmuller L, Cammenga J, Deppert WW. In vivo assay of p53 function in homologous recombination between simian virus 40 chromosomes. J Virol. 1996; 70:737–744. [PubMed: 8551610]

27. Willers H, McCarthy EE, Alberti W, Dahm-Daphi J, Powell SN. Loss of wild-type p53 function is responsible for upregulated homologous recombination in immortal rodent fibroblasts. Int. J. Radiat. Biol. 2000; 76:1055–1062. [PubMed: 10947118]

28. Yun S, Lie ACC, Porter AC. Discriminatory suppression of homologous recombination by p53. Nucleic Acids Res. 2004; 32:6479–6489. [PubMed: 15601996]

29. Peng Y, Zhang Q, Nagasawa H, Okayasu R, Liber HL, Bedford JS. Silencing expression of the catalytic subunit of DNA-dependent protein kinase by small interfering RNA sensitizes human cells for radiation-induced chromosome damage, cell killing, and mutation. Cancer Res. 2002; 62:6400–6404. [PubMed: 12438223]

30. Willers H, McCarthy EE, Hubbe P, Dahm-Daphi J, Powell SN. Homologous recombination in extrachromosomal plasmid substrates is not suppressed by p53. Carcinogenesis. 2001; 22:1757–1763. [PubMed: 11698336]

31. Slebos RJ, Taylor JA. A novel host cell reactivation assay to assess homologous recombination capacity in human cancer cell lines. Biochem. Biophys. Res. Commun. 2001; 281:212–219. [PubMed: 11178982]

32. Bunz F, Fauth C, Speicher MR, Dutriaux A, Sedivy JM, Kinzler KW, Vogelstein B, Lengauer C. Targeted inactivation of p53 in human cells does not result in aneuploidy. Cancer Res. 2002; 62:1129–1133. [PubMed: 11861393]

33. Honma M, Zhang LS, Hayashi M, Takeshita K, Nakagawa Y, Tanaka N, Sofuni T. Illegitimate recombination leading to allelic loss and unbalanced translocation in p53-mutated human lymphoblastoid cells. Mol. Cell Biol. 1997; 17:4774–4781. [PubMed: 9234733]

34. Sengupta S, Linke SP, Pedeux R, Yang Q, Farnsworth J, Garfield SH, Valerie K, Shay JW, Ellis NA, Wasylyk B, Harris CC. BLM helicase-dependent transport of p53 to sites of stalled DNA replication forks modulates homologous recombination. EMBO J. 2003; 22:1210–1222. [PubMed: 12606585]

35. So S, Adachi N, Koyama H. Absence of p53 enhances growth defects and etoposide sensitivity of human cells lacking the Bloom syndrome helicase BLM. DNA Cell Biol. 2007; 26:517–525. [PubMed: 17630856]

36. Aubrecht J, Secretan MB, Bishop AJ, Schiestl RH. Involvement of p53 in X-ray induced intrachromosomal recombination in mice. Carcinogenesis. 1999; 20:2229–2236. [PubMed: 10590213]

37. Shao C, Deng L, Henegariu O, Liang L, Stambrook PJ, Tischfield JA. Chromosome instability contributes to loss of heterozygosity in mice lacking p53. Proc. Natl. Acad. Sci. USA. 2000; 97:7405–7410. [PubMed: 10861008]

38. Hendricks CA, Almeida KH, Stitt MS, Jonnalagadda VS, Rugo RE, Kerrison GF, Engelward BP. Spontaneous mitotic homologous recombination at an enhanced yellow fluorescent protein (EYFP) cDNA direct repeat in transgenic mice. Proc. Natl. Acad. Sci. USA. 2003; 100:6325–6330. [PubMed: 12750464]

39. Jacks T, Shih TS, Schmitt EM, Bronson RT, Bernards A, Weinberg RA. Tumour predisposition in mice heterozygous for a targeted mutation in Nf1. Nature Genetics. 1994; 7:353–361. [PubMed: 7920653]

40. Wiktor-Brown DM, Hendricks CA, Olipitz W, Rogers AB, Engelward BP. Applications of fluorescence for detecting rare sequence rearrangements in vivo. Cell Cycle. 2006; 5:2715–2719. [PubMed: 17172860]

41. Sobol RW, Kartalou M, Almeida KH, Joyce DF, Engelward BP, Horton JK, Prasad R, Samson LD, Wilson SH. Base excision repair intermediates induce p53-independent cytotoxic and genotoxic responses. J. Biol. Chem. 2003; 278:39951–39959. [PubMed: 12882965]

42. Rosche WA, Foster PL. Determining mutation rates in bacterial populations. Methods. 2000; 20:4–17. [PubMed: 10610800]

43. Wiktor-Brown DM, Hendricks CA, Olipitz W, Engelward BP. Age-dependent accumulation of recombinant cells in the mouse pancreas revealed by in situ fluorescence imaging. Proc. Natl. Acad. Sci. USA. 2006; 103:11862–11867. [PubMed: 16882718]

44. Wiktor-Brown DM, Kwon HS, Nam YS, So PT, Engelward BP. Integrated one- and two-photon imaging platform reveals clonal expansion as a major driver of mutation load. Proc Natl Acad Sci U S A. 2008; 105:10314–10319. [PubMed: 18647827]

45. Takata M, Sasaki MS, Sonoda E, Morrison C, Hashimoto M, Utsumi H, Yamaguchi-Iwai Y, Shinohara A, Takeda S. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. Embo J. 1998; 17:5497–5508. [PubMed: 9736627]

46. de Dios I, Urunuela A, Pinto RM, Orfao A, Manso MA. Cell-cycle distribution of pancreatic cells from rats with acute pancreatitis induced by bile-pancreatic obstruction. Cell Tissue Res. 2000; 300:307–314. [PubMed: 10867825]

47. Sonoda E, Sasaki MS, Morrison C, Yamaguchi-Iwai Y, Takata M, Takeda S. Sister chromatid exchanges are mediated by homologous recombination in vertebrate cells. Mol. Cell. Biol. 1999; 19:5166–5169. [PubMed: 10373565]

48. Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell. 1993; 74:957–967. [PubMed: 8402885]

49. Helton ES, Chen X. p53 modulation of the DNA damage response. J Cell Biochem. 2007; 100:883–896. [PubMed: 17031865]

50. Kumari A, Schultz N, Helleday T. p53 protects from replication-associated DNA double-strand breaks in mammalian cells. Oncogene. 2004 in press.

51. Squires S, Coates JA, Goldberg M, Toji LH, Jackson SP, Clarke DJ, Johnson RT. p53 prevents the accumulation of double-strand DNA breaks at stalled-replication forks induced by UV in human cells. Cell Cycle. 2004; 3:1543–1557. [PubMed: 15539956]

52. Romanova LY, Willers H, Blagosklonny MV, Powell SN. The interaction of p53 with replication protein A mediates suppression of homologous recombination. Oncogene. 2004; 23:9025–9033. [PubMed: 15489903]

53. Sturzbecher HW, Donzelmann B, Henning W, Knippschild U, Buchhop S. p53 is linked directly to homologous recombination processes via Rad51/Reca protein interaction. Embo J. 1996; 15:1992–2002. [PubMed: 8617246]

54. Orre LM, Stenerlow B, Dhar S, Larsson R, Lewensohn R, Lehtio J. p53 is involved in clearance of ionizing radiation-induced RAD51 foci in a human colon cancer cell line. Biochem Biophys Res Commun. 2006; 342:1211–1217. [PubMed: 16516153]

55. Wang XW, Tseng A, Ellis NA, Spillare EA, Linke SP, Robles AI, Seker H, Yang Q, Hu P, Beresten S, Bemmels NA, Garfield S, Harris CC. Functional interaction of p53 and BLM DNA helicase in apoptosis. J Biol Chem. 2001; 276:32948–32955. [PubMed: 11399766]

56. Brosh RM Jr, Karmakar P, Sommers JA, Yang Q, Wang XW, Spillare EA, Harris CC, Bohr VA. p53 Modulates the exonuclease activity of Werner syndrome protein. J Biol Chem. 2001; 276:35093–35102. [PubMed: 11427532]

57. Yang Q, Zhang R, Wang XW, Spillare EA, Linke SP, Subramanian D, Griffith JD, Li JL, Hickson ID, Shen JC, Loeb LA, Mazur SJ, Appella E, Brosh RM Jr, Karmakar P, Bohr VA, Harris CC. The processing of Holliday junctions by BLM and WRN helicases is regulated by p53. J Biol Chem. 2002; 277:31980–31987. [PubMed: 12080066]

58. Prabhu VP, Simons AM, Iwasaki H, Gai D, Simmons DT, Chen J. p53 blocks RuvAB promoted branch migration and modulates resolution of Holliday junctions by RuvC. J Mol Biol. 2002; 316:1023–1032. [PubMed: 11884140]

59. Lee S, Cavallo L, Griffith J. Human p53 binds Holliday junctions strongly and facilitates their cleavage. J. Biol. Chem. 1997; 272:7532–7539. [PubMed: 9054458]

60. Brilliant MH, Gondo Y, Eicher EM. Direct molecular identification of the mouse pink-eyed unstable mutation by genome scanning. Science. 1991; 252:566–569. [PubMed: 1673574]

61. Gondo Y, Gardner JM, Nakatsu Y, Durham-Pierre D, Deveau SA, Kuper C, Brilliant MH. High-frequency genetic reversion mediated by a DNA duplication: the mouse pink-eyed unstable mutation. Proc. Natl. Acad. Sci. USA. 1993; 90:297–301. [PubMed: 8419934]

62. Liang L, Shao C, Deng L, Mendonca MS, Stambrook PJ, Tischfield JA. Radiation-induced genetic instability in vivo depends on p53 status. Mutat Res. 2002; 502:69–80. [PubMed: 11996974]

63. Willers H, McCarthy EE, Wu B, Wunsch H, Tang W, Taghian DG, Xia F, Powell SN. Dissociation of p53-mediated suppression of homologous recombination from G1/S cell cycle checkpoint control. Oncogene. 2000; 19:632–639. [PubMed: 10698508]

64. Xia F, Amundson SA, Nickoloff JA, Liber HL. Different capacities for recombination in closely related human lymphoblastoid cell lines with different mutational responses to X-irradiation. Molecular & Cellular Biology. 1994; 14:5850–5857. [PubMed: 8065318]

65. Janz C, Wiesmuller L. Wild-type p53 inhibits replication-associated homologous recombination. Oncogene. 2002; 21:5929–5933. [PubMed: 12185593]

66. Li CQ, Pang B, Kiziltepe T, Trudel LJ, Engelward BP, Dedon PC, Wogan GN. Threshold effects of nitric oxide-induced toxicity and cellular responses in wild-type and p53-null human lymphoblastoid cells. Chem. Res. Toxicol. 2006; 19:399–406. [PubMed: 16544944]

67. Smiraldo PG, Gruver AM, Osborn JC, Pittman DL. Extensive chromosomal instability in Rad51d-deficient mouse cells. Cancer Res. 2005; 65:2089–2096. [PubMed: 15781618]

68. Bouffler SD, Kemp CJ, Balmain A, Cox R. Spontaneous and ionizing radiation-induced chromosomal abnormalities in p53-deficient mice. Cancer Res. 1995; 55:3883–3889. [PubMed: 7641208]

69. Ishizaki K, Ejima Y, Matsunaga T, Hara R, Sakamoto A, Ikenaga M, Ikawa Y, Aizawa S. Increased UV-induced SCEs but normal repair of DNA damage in p53-deficient mouse cells. Int. J. Cancer. 1994; 58:254–257. [PubMed: 8026887]

70. Schwartz JL, Russell KJ. The effect of functional inactivation of TP53 by HPV-E6 transformation on the induction of chromosome aberrations by gamma rays in human tumor cells. Radiat Res. 1999; 151:385–390. [PubMed: 10190489]

71. Dudenhoffer C, Rohaly G, Will K, Deppert W, Wiesmuller L. Specific mismatch recognition in heteroduplex intermediates by p53 suggests a role in fidelity control of homologous recombination. Mol Cell Biol. 1998; 18:5332–5342. [PubMed: 9710617]

72. Honma M, Hayashi M, Sofuni T. Cytotoxic and mutagenic responses to X-rays and chemical mutagens in normal and p53-mutated human lymphoblastoid cells. Mutat Res. 1997; 374:89–98. [PubMed: 9067419]

73. Saintigny Y, Rouillard D, Chaput B, Soussi T, Lopez BS. Mutant p53 proteins stimulate spontaneous and radiation-induced intrachromosomal homologous recombination independently of the alteration of the transactivation activity and of the G1 checkpoint. Oncogene. 1999; 18:3553–3563. [PubMed: 10380877]
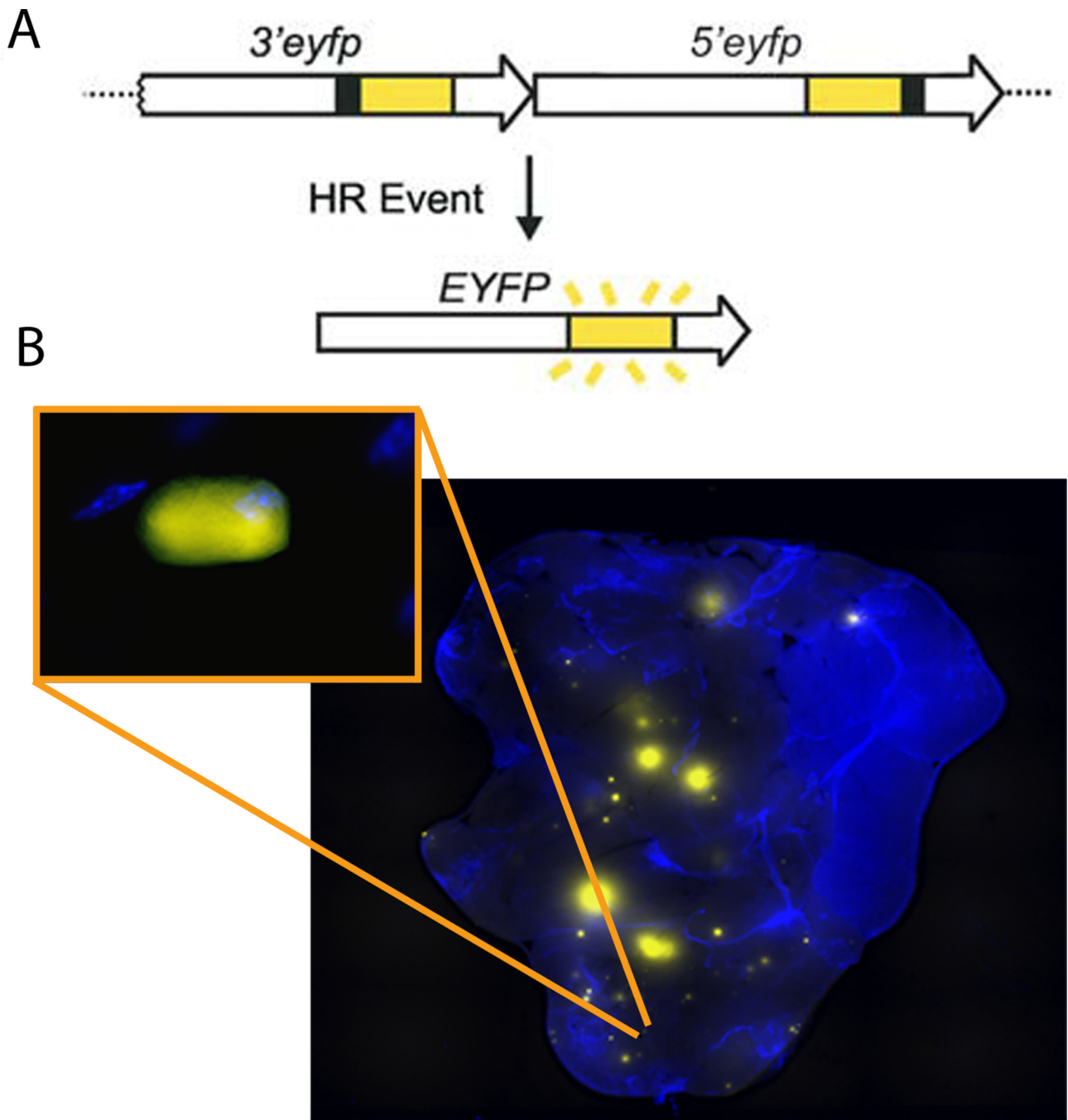
Fig 1.
FYDR system and analysis of the effect of p53 on HR *in vivo*. (A) Arrangement of the
FYDR recombination substrate: large arrows indicate expression cassettes; yellow boxes
show coding sequences; black boxes show positions of deleted sequences (deletion sizes not
to scale). (B) Compiled image for representative FYDR pancreata. Cell nuclei were stained
with Hoechst and imaged using a UV-2E/C filter (435–485 nm). Image was collected at 1×
using an EYFP filter (510–560 nm). Insert is an individual fluorescent cell.
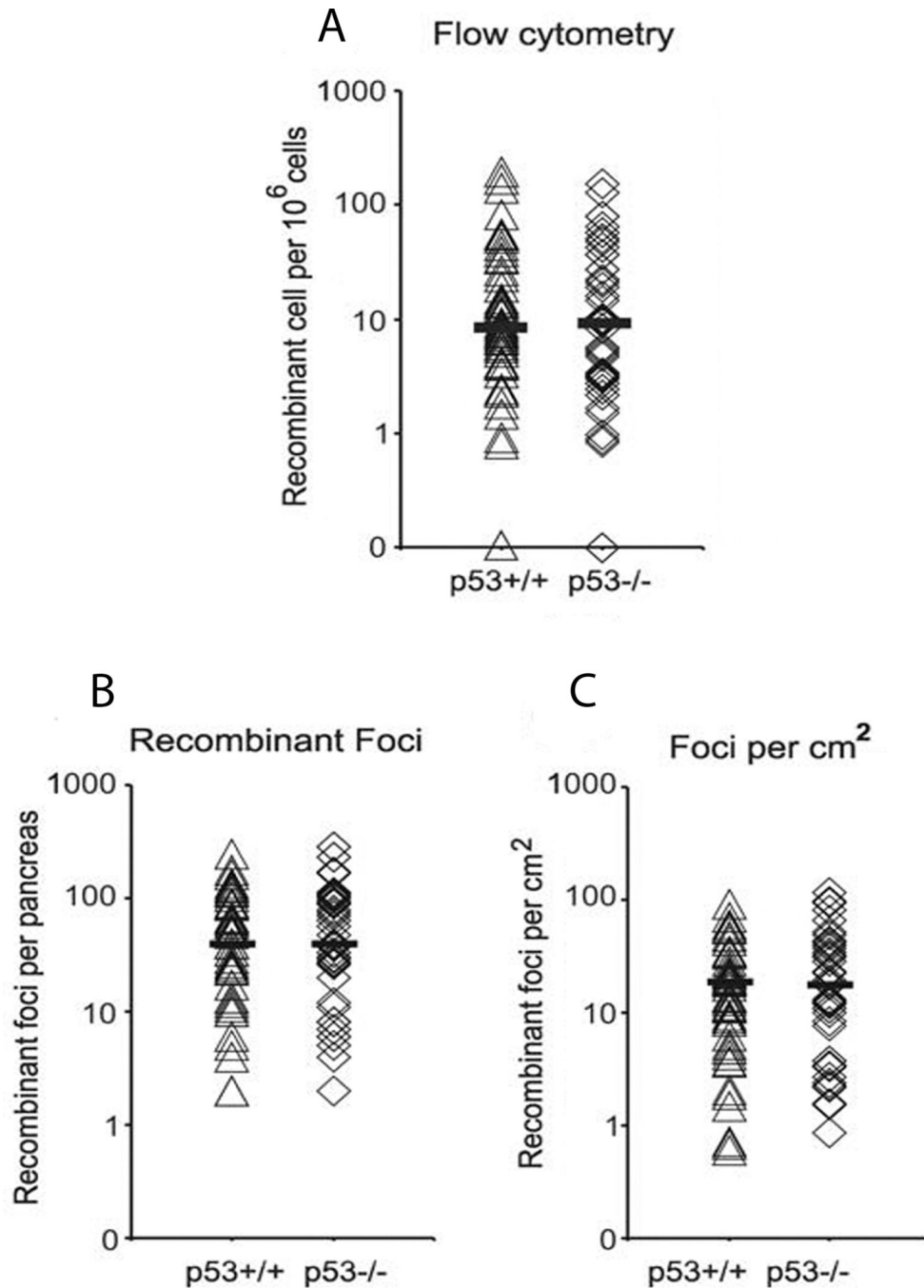
Fig 2.
Effects of p53 status on Homologous recombination *in vivo*. (A) Spontaneous frequency of recombinant pancreatic cells per million as determined by flow cytometry for $Fydr^{y/y};p53^{+/+}$ (n=49) and $Fydr^{y/y};p53^{-/-}$ (n=47) mice. Points on the x-axis indicate individual mice with zero recombinant cells. (B) Spontaneous recombinant foci per pancreas detected by *in situ* image analysis for $Fydr^{y/y};p53^{+/+}$ (n=48) and $Fydr^{y/y};p53^{-/-}$ (n=47) mice. (C) Spontaneous recombinant foci per cm^2 detected by *in situ* image analysis for $Fydr^{y/y};p53^{+/+}$ (n=47) and $Fydr^{y/y};p53^{-/-}$ (n=47) mice. Medians are indicated by black bars.
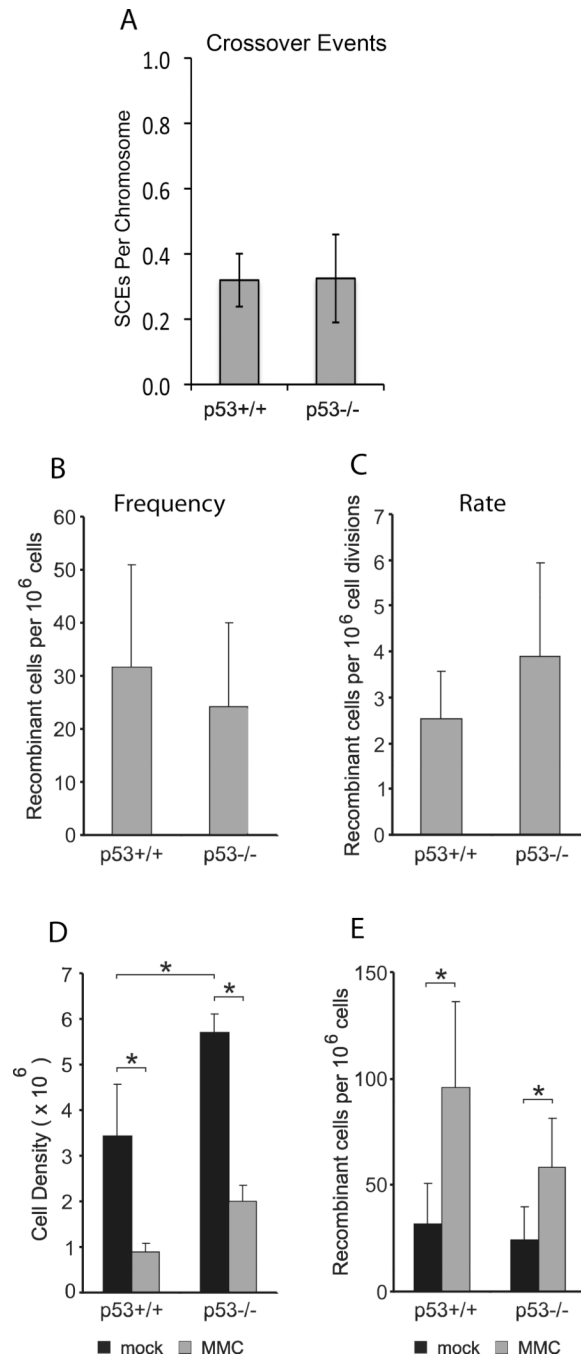
Fig 3.
Effects of p53 status on HR *in vitro* (A) Effect of p53 status on SCEs. Frequency of SCE events in primary mouse ear fibroblasts (p=0.35, 2-tailed Student's t-test). (B) Frequency of recombinant fluorescent primary fibroblasts. Three independent experiments were performed in quadruplicate, and the average of the three experiments is shown (p=0.32, 2-tailed Student's t-test). (C) Rate of HR in primary fibroblasts as determined by the MSS Maximum Likelihood Method [42] (p=0.16, 2-tailed Student's *t*-test). Each bar indicates the average of 7 independent experiments. (D) Cell density (black bars) and MMC-treated (gray bars) primary fibroblasts. Each bar indicates the average of three independent experiments, each performed in quadruplicate. * p<0.0001, 2-tailed Student's *t*-test. (E) Frequency of

recombinant cells for mock- (black bars) and MMC-treated (gray bars) fibroblasts. Each bar indicates the average of three independent experiments, each performed in quadruplicate. Note that data for the mocktreated samples is also shown in part B. * p<0.0005, 2-tailed Student's t-test. Error bars indicate 1 SD.