Replication fork maintenance pathways preserve chromosomes, but their faulty application at nonallelic repeats could generate rearrangements causing cancer, genomic disorders and speciation \(^9,10\). Potential causal mechanisms are homologous recombination and error-free postreplication repair (EF-PRR). Homologous recombination repairs damage-induced DNA double-strand breaks (DSBs) and single-ended DSBs within replication. To facilitate homologous recombination, the recombinase RAD51 and mediator BRCA2 form a filament on the 3’ DNA strand at a break to enable annealing to the complementary sister chromatid \(^11\) while the RecQ helicase, BLM (Bloom syndrome mutated) suppresses crossing over to prevent recombination \(^12\). Homologous recombination also stabilizes \(^6,7\) and restarts \(^8,9\) replication forks without a DSB \(^10,11\). EF-PRR bypasses DNA incongruities that impede replication by ubiquitinating PCNA (proliferating cell nuclear antigen) using the RAD6–RAD18 and UBC13–MMS2–RAD5 ubiquitin ligase complexes \(^12\). Some components are common to both homologous recombination and EF-PRR such as RAD51 and RAD18 \(^10,11\). Here we delineate two pathways that spontaneously fuse inverted repeats to generate unstable chromosomal rearrangements in wild-type mouse embryonic stem (ES) cells. Gamma-radiation induced a BLM-regulated pathway that selectively fused identical, but not mismatched, repeats. By contrast, ultraviolet light induced a RAD18-dependent pathway that efficiently fused mismatched repeats. Furthermore, TREX2 (a 3’–5’ exonuclease) suppressed identical repeat fusion but enhanced mismatched repeat fusion, clearly separating these pathways. TREX2 associated with UBC13 and enhanced PCNA ubiquitination in response to ultraviolet light, consistent with it being a novel member of EF-PRR. RAD18 and TREX2 also suppressed replication fork stalling in response to nucleotide depletion. Interestingly, replication fork stalling induced fusion for identical and mismatched repeats, implicating faulty replication as a causal mechanism for both pathways.

The identical and mismatched repeat reporters (IRR and MRR, Fig. 1a, b) were designed to investigate pathways that rearrange chromosomes through repeat fusion. Both reporters contain a 313-base-pair major satellite repeat (MSR) at each junction of an inversion in miniHPRT. These repeats are indirect so repeat fusion restores miniHPRT to enable survival in hypoxanthine, aminopterin, thymidine (HAT)-selecting media by a potential mechanism shown in Fig. 1c. The only difference between these reporters is that the MRR 3’ repeat contains seven mismatches with the longest contiguous homology being 67 bases. The IRR and MRR were stably transfected into wild-type AB2.2 and IB10 ES cells. About the same number of HAT-resistant colonies spontaneously grew for both reporters (Fig. 1d, P > 0.85, Student’s t-test), indicating that spontaneous repeat fusion occurred in wild-type cells.

The fused 5’ repeat for the MRR was sequenced to determine the switch location (Fig. 1e, Extended Data Fig. 1). Strand exchange in fusion yeast predominantly occurred at the palindromic centre after replication forks were induced to stall, an event called a U turn \(^9\). We found 6 of 14 switches had this U-turn at the base of a putative hairpin (all green), whereas two occurred at the apex (all orange) and six occurred in the stem (green-orange). Thus, strand exchange occurred at multiple locations.

It is possible that the switched strand replicated to the telomere, forming a dipericentric (Fig. 1c). Two-colour fluorescence in situ hybridization (FISH) was performed on clones with the IRR and MRR using a pericentromeric and telomeric probe. Dipericentrics and chromosomes with extra pericentromeres and telomeres (EPTs) \(^13\) were observed for cells with both reporters (Extended Data Fig. 2a and Extended Data Tables 1 and 2). EPTs seemed unstable because the pericentromere number and location varied between metaphase spreads from the same clone, implicating secondary events consistent with breakage–fusion–bridge cycles \(^17\). Spectral karyotyping on three MRR clones showed multiple fusion points confirming rearrangement complexity (Extended Data Table 3). Duplications of chromosome 1 (Fig. 1f, left) and translocations between chromosomes 14 and 11 (Fig. 1f, right) were frequently observed from the same clone and even in the same metaphase spread, indicating a role in genome topology \(^17\). Two-colour FISH was performed on a single clone (clone 18 from Extended Data Tables 2 and 3) with the MRR probe and either chromosome 1 or 14. This analysis revealed unstable structures because the MRR could be found at either chromosomes 1 or 14 (Extended Data Fig. 2b), indicating faulty DNA synthesis \(^13\). Furthermore, the MRR pattern changed from a discrete dot to multiple dots interspersed with chromosomal sequences similar to segmental duplications described during evolution \(^18\). Thus, both reporters caused unstable and complex rearrangements, yet the causal pathways are not known.

Complex genomic rearrangements could arise from faulty chromosome maintenance. Therefore, we tested whether γ-irradiation or ultraviolet light enhanced repeat fusion for wild-type AB2.2 cells with the IRR or MRR. Exposure to 4 Gy γ-irradiation induced repeat fusion for the IRR (Fig. 2a, left, P = 0.017, Student’s t-test) but not the MRR (Fig. 2a, right, P = 0.16), whereas exposure to 20 J m \(^{-2}\) ultraviolet light had the opposite effect on the IRR (Fig. 2b, left, P = 0.35) and MRR (Fig. 2b right, P = 0.006). This contrast suggests different pathways fused identical and mismatched repeats.

We tested whether homologous recombination proteins fused identical repeats because homologous recombination corrects damage caused by γ-irradiation but not ultraviolet light \(^9\). We tested BLM-defective ES cells (blm\(^{–/–}\)) simply called blm\(^{–/–}\) \(^9\) because BLM regulates homologous recombination through Holliday junction dissolution \(^9\). Repeat fusion was significantly higher in blm\(^{–/–}\) cells as compared to AB2.2 cells for the IRR (Fig. 2c, compare lanes 1 and 2, P < 0.0001), but not the MRR (Fig. 2c, compare lanes 6 and 7, P = 0.47). Next we tested blm\(^{+/–}\) cells haploinsufficient for RAD51 or BRCA2 because BRCA2 enables RAD51 filament formation on DNA single stands to mediate strand annealing and Holliday junction formation. We found blm\(^{+/–}\) RAD51\(^{+/–}\) cells

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**LETTER**

Two replication fork maintenance pathways fuse inverted repeats to rearrange chromosomes

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(Extended Data Fig. 3) and blm<sup>−/−</sup> brca2<sup>+/+ex27−/−</sup> cells (Extended Data Fig. 4a) showed reduced repeat fusion (Fig. 2c, compare lane 2 to 3 and 4, P < 0.0001). Deleting the remaining Brca2 exon 27 copy (Extended Data Fig. 4b) further reduced repeat fusion (Fig. 2c, compare lanes 4 and 5, P = 0.049). Thus, BLM suppressed RAD51/BRCA2-mediated identical repeat fusion consistent with an homologous-recombination-based pathway (these data do not address the potential role of RAD51/BRCA2 in mismatch repeat fusion).

We tested if EF-PRR fused mismatched repeats because ultraviolet light, but not γ-radiation, induced PCNA ubiquitination in mammalian cells<sup>2</sup>. IB10 ES cells deleted for RAD18 (ref. 22) were analysed. These cells showed slightly lower levels of repeat fusion for the IRR as compared to IB10 control cells (Fig. 2d, compare lanes 1 and 2, P = 0.06). This reduction could reflect the nonessential participation of RAD18 in homologous recombination<sup>14</sup>. By contrast, RAD18-deletion significantly

Figure 2 | Two pathways enable repeat fusion that depend on sequence identity. Shown is the ratio of HAT-resistant colonies transfected with IRR in control cells displayed in Fig. 1d. a, Gamma radiation (4 Gy) increases fusion for the IRR (left) but not MRR (right). Survival fraction, ~10%. Biological replicates for lanes 1–4: 19, 11, 19 and 11, respectively. b, Ultraviolet light (20 J m<sup>−2</sup>) enables fusion for the MRR (right) but not IRR (left). Survival fraction, ~0.6%. Biological replicates for lanes 1–4: 19, 11, 19 and 11, respectively. c, BLM suppressed repeat fusion for the IRR but not MRR. blm<sup>−/−</sup> cells deleted for one copy of Rad51 exons 2–4 (blm<sup>−/−</sup> Rad51<sup>+/−</sup>ex2–4 cells), one copy of Brca2 exon 27 (blm<sup>−/−</sup> brca2<sup>+/−ex27</sup>) or two copies of Brca2 exon 27 (blm<sup>−/−</sup> brca2<sup>+/−ex27−/−</sup>), Biological replicates for lanes 1–7: 19, 23, 12, 12, 19 and 23, respectively. d, RAD18 enabled fusion for the MRR more than IRR. Biological replicates: 18 for all lanes. e, f, TREX2 suppressed fusion for the IRR (e) but enabled fusion for the MRR (f). Examined are trex2<sup>+</sup> cells that express human wild-type TREX2 (hTREX2) or human TREX2 mutated in the DNA binding domain (R167A) or catalytic domain (H188A). Biological replicates for lanes 1–4 in e: 19, 19, 20, and 23, respectively, and for lanes 1–5 in f: 19, 21, 21, 21 and 23, respectively. Error bars show s.e.m. throughout.
lowered fusion of mismatched repeats (Fig. 2d, compare lanes 3 and 4, \(P = 0.0005\)). The reduction of mismatched repeat fusion is greater than identical repeat fusion (\(P < 0.0001\)), demonstrating that the role of RAD18 in fusing mismatched repeats is more prominent than identical repeats. These results are consistent with EF-PRR fusing mismatched repeats. Yet, RAD18 is an E3 ubiquitin ligase so it could have broad function; therefore, mutations in other genes in the poorly understood EF-PRR pathway should be observed.

TREX2 could be a novel member of EF-PRR. Previously, we analysed \(\text{trex}^{2\text{null}}\) cells and cells that expressed wild-type human TREX2 (TREX2(WT)) and human TREX2 mutated in the catalytic domain (TREX2(H188A)) and DNA-binding domain (TREX2(R167A)), ~85% reduction in DNA binding\(^2\text{3,24}\). We found TREX2 deletion elevated levels of spontaneous isochromatid breaks and chromosomal rearrangements\(^2\text{3,25}\). TREX2(WT) rescued the null phenotype whereas TREX2(H188A) exacerbated this phenotype, suggesting a dominant effect\(^2\text{4}\). These observations suggested defective DSB repair. However, \(\text{trex}^{2\text{null}}\) cells exhibited increased DSB repair and normal BLM-regulated sister chromatid exchanges (SCEs)\(^2\text{6}\). Therefore, we proposed that TREX2 did not repair DSBs but instead suppressed DSB formation through an unknown pathway, possibly EF-PRR. In support, \(\text{trex}^{2\text{null}}\) cells had reduced levels of spontaneous SCEs\(^2\text{2,27}\).

TREX2-altered cells were tested for fusion of identical and mismatched repeats. \(\text{trex}^{2\text{null}}\) and TREX2(H188A)-expressing cells had elevated levels of identical repeat fusion as compared to control cells (AB2.2 and \(\text{trex}^{2\text{WT}}\) cells) (Fig. 2e, compare lanes 1 and 3 to 2 and 4, \(P < 0.05\)), corroborating our previous observations that homologous recombination is elevated in \(\text{trex}^{2\text{null}}\) cells and that an homologous-recombination-based pathway fuses identical repeats. A similar anti-recombination effect on identical repeats was seen for the 3’ exconucleases Exo1 and ExoVII in \(E.\) coli, suggesting that 3’ exonuclease activity inhibits these fusions\(^2\text{8}\). We also found \(\text{trex}^{2\text{null}}\) and TREX2(H188A)-expressing cells had very low levels of mismatch repeat fusion as compared to AB2.2, \(\text{Trex2}^{\text{WT}}\) and \(\text{Trex2(R167A)}\) cells (Fig. 2f, compare lanes 1, 3 and 4 to 2 and 5, \(P < 0.0006\)). Furthermore, TREX2-mediated ultraviolet-light-induced fusion of mismatched repeats (Fig. 2b right panel, \(P = 0.003\)). These data clearly separate the pathways that mediate identical and mismatch repeat fusion and demonstrate sequence identity determined pathway choice. These data also demonstrate the importance of the catalytic activity of TREX2 in mediating repeat fusion. Exonuclease activity would predictably remove intermediate 3’ mismatches or flaps that could occur at the DNA incongruity or during strand exchange and strand displacement. Furthermore, these data are consistent with TREX2 being part of the EF-PRR machinery.

Three experiments were performed to test if TREX2 is a member of EF-PRR. First, TREX2 located to the nascent replication strand after ultraviolet light exposure (Extended Data Fig. 5a); thus, it was at the right place at the right time. Second, TREX2 associated with UBC13, but not MMS2, by glutathione-S-transferase (GST) pull-down (Extended Data Fig. 5b); UBC13–MMS2 is the E2 heterodimer that polyubiquitinates PCNA\(^1\text{2,21}\). In addition, TREX2 associated with UBC13 after ectopic expression in HeLa cells that was enhanced by ultraviolet light (Extended Data Fig. 5c); thus, it associated with the PCNA ubiquitination machinery. Third, we tested the impact TREX2 and RAD18 had on PCNA ubiquitination. As a control we found ultraviolet light, but not \(\gamma\)-radiation, enhanced PCNA ubiquitination as previously seen in human cells\(^2\text{3}\) (Extended Data Fig. 6a). TREX2 and RAD18 were needed for efficient PCNA ubiquitination after exposure to ultraviolet light (Extended Data Fig. 6b–d). In addition, cells deleted for both RAD18 and TREX2 (Extended Data Fig. 7) showed no further reduction in PCNA ubiquitination, indicating that they are epistatic (Extended Data Fig. 6b–d). These observations are consistent with TREX2 being part of the EF-PRR machinery and imply RAD18 and TREX2 in replication fork maintenance.

Potential mechanisms for repeat fusion are faulty DNA repair and faulty DNA replication\(^2\). Repeat fusion could manifest from faulty DNA repair since \(\gamma\)-radiation and ultraviolet light increased fusion. However, the odds that damage actually occurred in or near the reporter sequences is small (even after exposure to agent); thus, the agents could cause a compensatory increase in repair pathways. RAD51, BRCA2 and BLM are involved in both DSB repair and replication fork maintenance\(^6,7,10,11,15,29\) so either are possible while direct evidence that RAD18 and TREX2 maintain replication forks is lacking in mammalian cells. Therefore, \(\text{rad}^{18\text{–/–}}\) and \(\text{trex}^{2\text{null}}\) cells were exposed to a brief pulse of low concentration hydroxyurea (0.5 mM, 90 min) that depletes nucleotides to stall replication forks without causing DSBs\(^6,7,10,29\). We found \(\text{rad}^{18\text{–/–}}\) and \(\text{trex}^{2\text{null}}\) cells had elevated levels of stalled replication forks compared to control cells (Fig. 3a, \(P < 0.0001\)) similar to depletion of the RAD5 orthologue, HLF\(^\text{28}\). We further tested faulty replication as causal for repeat fusion by exposing cells with the IRR or MRR to this mild hydroxyurea concentration (Fig. 3b). This exposure increased repeat fusion for the IRR (\(P = 0.00025\), Student’s t-test) and MRR (\(P = 0.0037\)). Our observations suggest a BLM-regulated pathway consistent with homologous recombination fused identical repeats whereas a RAD18/TREX2-dependent pathway consistent with EF-PRR fused mismatched repeats during replicative stress. These pathways are good candidates for causing complex rearrangements found in cancer and genomic disorders in people and chromosomal variation that leads to species diversification.

METHODS SUMMARY

Repeat fusion assay: the reporters were randomly integrated into ES cells, selected in HAT and colonies counted (colonies were also counted without selection to control for seeding efficiencies). The percentage of HAT-resistant colonies was determined by dividing the number of HAT-resistant colonies by the number of cells electroporated multiplied by the seeding efficiency.

Figure 3 | Hydroxyurea-induced nucleotide depletion. a, RAD18 and TREX2 maintain replication forks. The percentage of stalled replication forks after hydroxyurea exposure is shown. Experimental design: cells were cultured in IdU (5-ido-2’-deoxyuridine) (20 min) to label nascent strand and then exposed to hydroxyurea (0.5 mM, 90 min) to stall replication and then cultured in CldU (5-chloro-2’-deoxyuridine) (20 min) to label restart. Fibre number observed without and with hydroxyurea: IB10 (1,943, 657), \(\text{rad}^{18\text{–/–}}\) (1,180, 1,460), AB2.2 (452, 510), \(\text{trex}^{2\text{null}}\) (705, 448). b, The impact of hydroxyurea (0.5 mM, 90 min) on repeat fusion for the IRR (left) and MRR (right). The ratio of HAT-resistant colonies as compared to AB2.2 cells transfected with the IRR (0.05%) is shown. Survival fraction is 100%. Error bars, s.e.m. Biological replicates, 6 for all lanes.
1. Hastings, P. J., Lupski, J. R., Rosenberg, S. M. & Ira, G. Mechanisms of change in gene copy number. *Nature Rev. Genet.* 10, 551–564 (2009).

2. Carr, A. M., Paek, A. L. & Weinert, T. DNA replication: failures and inverted fusions. *Semin. Cell Dev. Biol.* 22, 866–874 (2011).

3. Lee, J. A., Carvalho, C. M. & Lupski, J. R. A. DNA replication mechanism for gene amplification and HSR generation. *Mol. Cell. Biol.* 30, 233–243 (2005).

4. Schlacher, K. Regulation of post-translational modifications of the eukaryotic replication fork. *Mol. Cell. Biol.* 22, 866–874 (2011).

5. Wu, L. & Hickson, I. D. The Bloom’s syndrome helicase suppresses crossing over tolerance during DNA replication. *Nature Struct. Mol. Biol.* 290–299 (2013).

6. Schlacher, K., Wu, L. & Jasin, M. A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. *Cancer Cell* 22, 106–116 (2012).

7. Muzzo, K., Lambert, S., Baldacci, G., Murray, J. M. & Carr, A. M. Nearby inverted repeats fuse to generate acentric and dicentric palindrome chromosomes by a replication template exchange mechanism. *Genes Dev.* 23, 2876–2886 (2009).

8. Muzo, K., Miyabe, I., Schlabetter, S. A., Carr, A. M. & Murray, J. M. Recombination restarted replication makes inverted chromosome fusions at inverted repeats. *Nature* 493, 246–249 (2013).

9. Petermann, E., Orta, M. L., Issaeva, N., Schultz, N. & Helleday, T. Hydroxurea-stalled replication forks become progressively inactivated and require two different RAD51-mediated pathways for restart and repair. *Mol. Cell* 37, 492–502 (2010).

10. Carr, A. M. & Lambert, S. Replication stress-induced genome instability: the dark sections appear only in the online paper.

11. Harada, S., Sekiguchi, N. & Shimizu, N. Amplification of a plasmid bearing a mammalian replication initiation region in chromosomal and extrachromosomal contexts. *Nucleic Acids Res.* 39, 958–969 (2011).

12. Horvath, J. E. et al. Using a pericentromeric interspersed repeat to recapitulate the phylogeny and expansion of human centromeric segmental duplications. *Mol. Biol. Evol.* 20, 1463–1479 (2003).

13. Falbo, K. B. Bloom mice. *Nature Genet.* 26, 424–429 (2000).

14. Motegei, A. et al. Polyubiquitination of proliferating cell nuclear antigen by HLTF and SHPRH prevents genomic instability from stalled replication forks. *Proc. Natl Acad. Sci. USA* 105, 12411–12416 (2008).

15. Tateishi, S. et al. Enhanced genomic instability and defective postreplication repair in RAD18 knockout mouse embryonic stem cells. *Mol. Cell. Biol.* 23, 474–481 (2003).

16. Chen, M. J., Ma, S. M., Dumitrache, L. C. & Hasty, P. Biochemical and cellular characteristics of the 3′ → 5′ exonuclease TREX2. *Nucleic Acids Res.* 35, 2682–2694 (2007).

17. Dumitrache, L. C., Hu, L. & Hasty, P. TREX2 exonuclease defective cells exhibit double-strand breaks and chromosomal fragments but not Robertsonian translocations. *Mutat. Res.* 662, 84–87 (2009).

18. Chen, M. J. et al. Cisplatin depletes TREX2 and causes Robertsonian translocations as seen in TREX2 knockout cells. *Cancer Res.* 67, 9077–9083 (2007).

19. Goldfuss, S. J., Morag, A. S., Belisle, K. A., Sutera, V. A. Jr & Lovett, S. T. DNA repeat rearrangements mediated by Dnak-dependent replication fork repair. *Mol. Cell* 21, 595–604 (2006).

20. Dutra, B. E. & Lovett, S. T. cis and trans-acting effects on a mutational hotspot involving a replication template switch. *J. Mol. Biol.* 356, 300–311 (2006).

21. Sirbu, B. M. et al. Analysis of protein dynamics at active, stalled, and collapsed replication forks. *Genes Dev.* 25, 1320–1327 (2011).

22. Bösl, A., Hajdu, L., Unk, I. & Haracska, L. Role of double-stranded DNA translocase activity of human HLTF in replication of damaged DNA. *Mol. Cell. Biol.* 30, 684–693 (2010).

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Author Contributions L.H., T.M.K., P.R.Y., C.M., L.C.D. and P.H. designed experiments. L.H., T.M.K., P.R.Y., C.M., L.C.D. and P.H. performed experiments. S.T. provided the results. L.H., T.M.K., P.R.Y., C.M., L.C.D. and P.H. designed experiments. L.H., T.M.K., M.Y.S., S.-AK., C.L.H., D.H.K. and PH performed experiments.

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METHODS

Construction of the IRR and MRR. The IRR and MRR contain a puromycin phosphotransferase (puro) selection cassette and an hprt mini-gene [miniHPRT]. Purom was positioned 5′ to miniHPRT and used to select for stable transfectants. mi

Two-colour FISH with the pericentromeric and telomeric probes. Perform two-colour FISH (Extended Data Fig. 2a) on HA

Spectral karyotyping. Perform spectral karyotyping (Fig. 1b) as described44 with commercial spectral karyotyping paint probes from Applied Spectral Imaging. Define rearrangements with nomenclature rules from the International Committee on Standard Genetic Nomenclature for Mice45.

Generation of mouse Rad51 targeting vector. Construct mouse Rad51 targeting vector (Extended Data Fig. 3) as described46. Amplify left (5′) and right (3′) homologous arms with high-fidelity PCR using genomic DNA extracted from AB2.2 ES cells and iPo

Repeat fusion assay. Repeat fusion is seen in cells transfected with the IRR or MRR (Figs 1d, 2 and 3b). Transfect ES cells (5 × 10^6, 800 μl PBS) with 5 μg of uncut IRR or MRR by electroporation (Bio-Rad Gene Pulser at 230 V, 500 μF). Seed cells onto 3–6 3.5-cm plates with mitotically inactivated MEFs. Each well is a replicate because they remain separate. Add puromycin (3 μg ml⁻¹) next day. About 100–200 puromycin-resistant colonies grow for each well. Seven days later, pool puromycin-resistant colonies for each well and plate onto a 3.5-cm plate precoated with gelatin. Days after passage onto a 10-cm plate precoated with gelatin. SEE below for cell exposure to DNA-damaging agents. For unexposed cells, next day seed 10^6 cells onto a gelatin-coated 10-cm plastic plate in M15 supplemented with 1× HAT (1 mM sodium hypoxanthine, 4 μM aminopterin and 160 μM thymidine). Count HAT-resistant colonies 10 days later. To control for seeding efficiencies, seed 2000 cells for each replicate onto a gelatin-coated 3.5-cm plastic plate and culture in M15 without selection. Select colonies from the plates and use them to isolate genomic DNA. Screen for targeted clones with PCR (Extended Data Fig. 3b).

Transfect targeting vector (5 μg, cut with PacI) into bm1'/− ES cells (5 × 10^6 cells in 800 μl PBS) by electroporation (Gene Pulser Cuvettes with a 0.4 cm electrode gap at 230 V, 500 μF with a Gene Pulser Apparatus from Bio-Rad). After electroporation, seed cells onto 10 cm plates with mitotically inactivated MEFs. Next day, add M15 medium containing 1× HAT (0.1 mM hypoxanthine, 0.0004 mM aminopterin and 0.016 mM thymidine). Pick HAT-resistant colonies 7 days later onto a 96-well plate and maintain in HAT selection. Replace plate to freeze one plate and use the other to isolate genomic DNA.2 12 Screen for targeted clones with PCR (Extended Data Fig. 3).

H13F (in miniHPRT): 5′-GTAATGAAAAATTCTTTAATCTTTACCAACGAG CATTAG-3′ SR3 (outside the right arm): 5′-AGCCAGGTTATAGCTCTCAA GGAATCTGAAATCC-3′

PCR conditions: 1 cycle: 98 °C for 5 min; 53 cycles: 98 °C for 1 min, 67 °C for 1 min, 72 °C for 1 min 30 s; and 1 cycle: 72 °C for 10 min.

CRE-mediated deletion of SAgeo and 5′ miniHPRT. Delete SAgeo and 5′ half of miniHPRT using Cre recombinase to generate Rad51−/− ES cells (Extended Data Fig. 3c). Expand targeted ES cells in 1× HAT to remove HPR- negative cells that survived due to cross feeding. Removed HAT selection 2 days before transfection and cultured in 1× HT (1 mM sodium hypoxanthine and 160 μM thymidine); electroporate 5 × 10^6 cells in 800 μl DPBS with 10 μg of pPGKcreA using a Bio-Rad Gene Pulser at 230 V, 500 μF. After electroporation, seed 200 μl onto a 10-cm feeder plate without selection for 2–4 days to allow time for miniHPRT removal
and time for degradation of HPRT mRNA and protein. Then seed 4 × 10^6 cells onto a 10-cm feeder plate in 10 μM 6-thioguanine. Pick 6-thioguanine-resistant colonies 10 days later. Expand cells in 10 μM 6-thioguanine and replica plate. Freeze one plate and use the other to isolate genomic DNA. Confirmed Cre-mediated deletion with PCR (1.4 kb fragment).

PCR primers. RcFl1 (in RAD51 intron 1), 5'-GTGGTCTAATCTTCTAGAA CTG-3', AS2 (in exon 3-8 of miniHPRT), 5'-TGTCCTCGGTGTAGGTCG AGC-3'. Targeting mouse Brca2 exon 27. Replace the first copy of Brca2 exon 27 with PGKneoAP by cloning PGKneoAP into the SfiI sites of the Brca2 exon 27 deletion targeting vector (Extended Data Fig. 4a). Transfect as described for Rad51. Use PCR to detect targeted clones (Extended Data Fig. 4a).

PCR primers: NF (in neo), 5'-AGGCGATCTGGCTATGCTGCTTTAC AGG-3'; Brca2 intron 27 reverse, 5'-CCCCCGAGGAGGAAGATGAAGT CCTTACTCCAAGG-3'. Conditions: 35 cycles of 98°C for 1 min, 65°C for 1 min, 72°C for 1 min and 30 s.

Replace the second copy of Brca2 exon 27 with floxed miniHPRT (Extended Data Fig. 4b). Use PCR to detect targeted clones (Extended Data Fig. 4b).

PCR primers: HJ3F, 5'-GTAATGGAAAAATTCTTTTAAACACAGACG ACTATTAG3'-; B27R, 5'-CCCGTGCAGCGGAGGAGATGAAGT GCTTAC TCCAAG-3'. Conditions: 35 cycles of 98°C for 1 min, 65°C for 1 min, 72°C for 1 min and 30 s.

Removed the 5′ half of miniHPRT by Cre-mediated recombination to generate Brca2^/floxed;Anp2^+/-. Use PCR to detect removal (Extended Data Fig. 4b).

PCR primers: Bi26, 5'-TCAATTAAGGAAAGTAAGTCTTAAACCACAGC for 1 min and 30 s. 3 cycles: 98°C for 5 min, 45°C for 1 min, 72°C for 2 min 30 s. 1 cycle: 72°C for 10 min.

Detection of PCNA ubiquitination with chromatin-bound fraction. RAD18 and TREX22 participate in ultraviolet-induced PCNA ubiquitination (Extended Data Fig. 5a). Experiment performed as described10 with minor modifications. Transfected HeLa cells with 5 μg Myc-TREX2 using FuGENE6 (Roche). Replace right arm integrations with micrococcal nuclease (10 min, 37°C). Incubate reaction solution with 20 μg Protein G Sepharose beads for 3 h at 4°C. Wash beads with NETN buffer (50 mM Tris, 250 mM NaCl, 5 mM EDTA, pH 7.5, and 0.1% NP40) and subject to SDS–PAGE gel and blot with anti-Myc or anti-HA antibody.

Deletion of whole murine HPRT gene and protein. Then seed 4 × 10^6 cells in 10 μM 6-thioguanine. Pick 6-thioguanine-resistant colonies 10 days later. Expand cells in 10 μM 6-thioguanine and replica plate. Freeze one plate and use the other to isolate genomic DNA. Confirmed Cre-mediated deletion with PCR (1.4 kb fragment).

PCR primers. RcFl1 (in RAD51 intron 1), 5'-GTGGTCTAATCTTCTAGAA CTG-3', AS2 (in exon 3-8 of miniHPRT), 5'-TGTCCTCGGTGTAGGTCG AGC-3'. Targeting mouse Brca2 exon 27. Replace the first copy of Brca2 exon 27 with PGKneoAP by cloning PGKneoAP into the SfiI sites of the Brca2 exon 27 deletion targeting vector (Extended Data Fig. 4a). Transfect as described for Rad51. Use PCR to detect targeted clones (Extended Data Fig. 4a).

PCR primers: NF (in neo), 5'-AGGCGATCTGGCTATGCTGCTTTAC AGG-3'; Brca2 intron 27 reverse, 5'-CCCCCGAGGAGGAAGATGAAGT CCTTACTCCAAGG-3'. Conditions: 35 cycles of 98°C for 1 min, 65°C for 1 min, 72°C for 1 min and 30 s.

Replace the second copy of Brca2 exon 27 with floxed miniHPRT (Extended Data Fig. 4b). Use PCR to detect targeted clones (Extended Data Fig. 4b).

PCR primers: HJ3F, 5'-GTAATGGAAAAATTCTTTTAAACACAGACG ACTATTAG3'-; B27R, 5'-CCCGTGCAGCGGAGGAGATGAAGT GCTTAC TCCAAG-3'. Conditions: 35 cycles of 98°C for 1 min, 65°C for 1 min, 72°C for 1 min and 30 s.

Removed the 5′ half of miniHPRT by Cre-mediated recombination to generate Brca2^/floxed;Anp2^+/-. Use PCR to detect removal (Extended Data Fig. 4b).

PCR primers: Bi26, 5'-TCAATTAAGGAAAGTAAGTCTTAAACCACAGC for 1 min and 30 s. 3 cycles: 98°C for 5 min, 45°C for 1 min, 72°C for 2 min 30 s. 1 cycle: 72°C for 10 min.

Detection of PCNA ubiquitination with chromatin-bound fraction. RAD18 and TREX22 participate in ultraviolet-induced PCNA ubiquitination (Extended Data Fig. 5a). Experiment performed as described10 with minor modifications. Transfected HeLa cells with 5 μg Myc-TREX2 using FuGENE6 (Roche). Replace right arm integrations with micrococcal nuclease (10 min, 37°C). Incubate reaction solution with 20 μg Protein G Sepharose beads for 3 h at 4°C. Wash beads with NETN buffer (50 mM Tris, 250 mM NaCl, 5 mM EDTA, pH 7.5, and 0.1% NP40) and subject to SDS–PAGE gel and blot with anti-Myc or anti-HA antibody.
40. Krijger, P. H. et al. HLTF and SHPRH are not essential for PCNA polyubiquitination, survival and somatic hypermutation: existence of an alternative E3 ligase. DNA Repair (Amst.) **10**, 438–444 (2011).

41. Friedrich, G. & Soriano, P. Promoter traps in embryonic stem cells: a genetic screen to identify and mutate developmental genes in mice. Genes Dev. **5**, 1513–1523 (1991).

42. Araki, K., Araki, M. & Yamamura, K. Targeted integration of DNA using mutant lox sites in embryonic stem cells. Nucleic Acids Res. **25**, 868–872 (1997).

43. Kim, T. M., Choi, Y. J., Ko, J. H. & Hasty, P. High-throughput knock-in coupling gene targeting with the HPRT minigene and Cre-mediated recombination. Genesis **46**, 732–737 (2008).

44. Donoho, G. et al. Deletion of Brca2 exon 27 causes hypersensitivity to DNA crosslinks, chromosomal instability, and reduced life span in mice. Genes Chromosom. Cancer **36**, 317–331 (2003).

45. Morimatsu, M., Donoho, G. & Hasty, P. Cells deleted for Brca2 COOH terminus exhibit hypersensitivity to gamma-radiation and premature senescence. Cancer Res. **58**, 3441–3447 (1998).

46. Moynahan, M. E., Pierce, A. J. & Jasin, M. BRCA2 is required for homology-directed repair of chromosomal breaks. Mol. Cell **7**, 263–272 (2001).

47. Terai, K., Abbas, T., Jazaeri, A. A. & Dutta, A. CRL4Cdt2 E3 ubiquitin ligase monoubiquitinates PCNA to promote translesion DNA synthesis. Mol. Cell **37**, 143–149 (2010).
Extended Data Figure 1 | Three locations for the switch within a hairpin.
There are seven mismatches located at positions 52, 111, 140, 178, 188, 204 and 246. This model shows the inverted repeats forming a hairpin to simply illustrate the location of the switch, although we do not know if hairpins form.

a, The switch occurs at the apex of the hairpin before the first mismatch at position 52 such that the 5' MSR has the same sequence as the orange repeat.
b, The switch occurs in the stem of the hairpin after the first mismatch at position 52 but before the last mismatch at position 246 such that the 5' MSR is a mixture of both the green and orange repeat.
c, The switch occurs at the base of the hairpin after the last mismatch in position 246 such that the 5' MSR has the same sequence as the green repeat.
Extended Data Figure 2 | Complex chromosomal rearrangements in wild-type cells with the IRR and MRR. a, Two-colour FISH on metaphase spreads stained with a telomeric probe (green), a MSR probe in the pericentromere (red) and counterstained with DAPI (blue). (1)–(3) Multipercentric chromosomes from cells with the IRR: (1) Typical dipericentric, (2) chromosome with extra pericentromeres and telomeres (EPT)\textsuperscript{15}, (3) segmental duplication with the extra pericentromeres on only one chromatid. (4)–(8) Multipercentric chromosomes from cells with the MRR: (4) typical dipericentric, (5)–(7) EPTs, (8) extra pericentromere on only one chromatid. Chromosomal abnormalities were found for 15/19 (P < 0.0001, Yates-corrected chi-square test) and 18/19 (P < 0.0001) HAT-resistant colonies transfected with the IRR and MRR, respectively, but none were found for non-transfected cells as previously described\textsuperscript{15}. b, Two-colour FISH on nuclei using the MRR as a probe (red) along with either chromosome 1 or 14 (green). For some nuclei the MMR associated with chromosome 14 (1) whereas for others it associated with chromosome 1 (2). Note the MRR is located to both chromosomes 14 but only one chromosome 1. Thus, the MRR moved to different altered chromosomes observed with spectral karyotyping, consistent with the notion that the MRR is the source of instability. In addition, the size of the red dot(s) varied, suggesting continuous nonallelic fusions that could expand or contract the number of MRR units. For some nuclei the MRR appeared as a discrete dot, indicating one contiguous array of reporter units (1 and 2, red insets) but for others it was speckled, suggesting arrays of MRR units were interspersed with chromosomal sequences (3, red inset). For one speckled cluster a fragment of chromosome 1 surrounded only one red dot, highlighting the complexity of this rearrangement (green inset). The MRR probe was also found protruding at the edge or outside of some nuclei, indicating these unstable structures could be extruded from the nucleus similar to micronuclei (4, red inset).
Extended Data Figure 3 | Targeting Rad51 exons 2–4. a, SAβgeo-miniHPRT is used for selection. SAβgeo (green) is a fusion of β-galactosidase and neomycin phosphotransferase and is capable of trapping promoters to improve targeting efficiency. A Right element (RE) mutant loxP is in the intron (blue green arrow). In addition, another RE mutant loxP is 5’ to SAβgeo. A FLP recombination target (FRT) is at the 3’ end of miniHPRT. b, Replacing Rad51 exons 2–4 (exon 2 is the first coding exon) with the SAβgeo-miniHPRT selection cassette. PCR is used to screen G418 HAT-resistant ES cell clones for gene targeting using primers H13F and SR3. c, Removal of SAβgeo, the 5’ half of miniHPRT and a RE mutant loxP by Cre-mediated recombination to generate Rad51+/Δex2-4 cells. Screen 6-thioguanine-resistant clones by PCR using primers RCF1 and AS2.
Extended Data Figure 4 | Targeting Brca2 exon 27. There were two gene targeting vectors so we could observe cells deleted for one (blm<sup>+/−</sup> Brca2<sup>Δex27-n</sup>) and two (blm<sup>−/−</sup> Brca2<sup>Δex27-h/Δex27-n</sup>) copies of Brca2 exon 27. a, The first targeting vector (Δex27-n) replaced Brca2 exon 27 with neomycin phosphotransferase (neo) and probably generated a severe defect because exon 27 was not replaced with a splice donor to ensure polyadenylation<sup>44</sup>. This means deletion of the first copy probably caused a haploinsufficiency. The Brca2 gene after targeting. NF and B27R are PCR primers used to screen for targeted clones. b, The second targeting vector (Δex27-h) replaced Brca2 exon 27 with miniHPRT that contains a splice donor and polyadenylation sequences. Previously we showed Brca2 exon 26 spliced into HPRT exon 3 to ensure polyadenylation. Cells mutated with this second targeting vector produced a truncated BRCA2 protein at normal levels and were hypersensitive to γ-radiation and deficient in homologous recombination<sup>36,45,46</sup> and replication fork maintenance<sup>6</sup>. Replacing the second copy of Brca2 exon 27 with a floxed miniHPRT<sup>36</sup> to make Brca2<sup>Δex27-h/Δex27-n</sup> cells. H13F and B27R primers were used to screen for targeted clones. Cre-mediated recombination removed the 5′ half of miniHPRT. Brca2 exon 26 splices into miniHPRT exons 3–8 (grey line) to generate a polyadenylated Brca2 transcript that is deleted for exon 27<sup>36,45</sup>. There is the addition of one amino acid followed by a stop codon and this transcript produces a protein at wild-type levels that associates with RAD51, presumably through the BRC motifs<sup>46</sup>. B26 and H3-8R PCR primers were used to screen for Cre-mediated deletion.
Extended Data Figure 5 | TREX2’s response to ultraviolet light and association with UBC13. 

**a**. Coimmunoprecipitation of IdU and Myc-TREX2 in HeLa cells after exposure to 20 J m\(^{-2}\) ultraviolet light. No treatment, NT.

**b**. GST pull-down of \(^{35}\)S-labelled short isoform wild-type (WT) TREX2.  

**c**. Coimmunoprecipitation with Myc-TREX2 and HA-UBC13 in HeLa cells before and 6 h after exposure to 20 J m\(^{-2}\) ultraviolet light.
Extended Data Figure 6 | RAD18 and TREX2 ubiquitinate PCNA.

a, Exposure of AB2.2 cells to ultraviolet light, but not γ-radiation, induced PCNA ubiquitination. Immunoprecipitate endogenous PCNA and immunoblot with anti-ubiquitin (Ub, left), then strip and immunoblot with anti-PCNA (right). PCNA–Ub1 and PCNA–Ub3 are visible; yet, IgG obscures PCNA–Ub2. In addition, the Ub blot, but not the PCNA blot, reveals a previously unidentified band between PCNA–Ub1 and PCNA–Ub2. Ultraviolet light, but not γ-radiation, increased levels of PCNA–Ub1 and PCNA–Ub3 as previously shown in human cells21 (the same was true for the unknown protein). Survival fraction: 20 J m⁻², 0.6%; 60 J m⁻², 0.06%; 5 Gy, 8%; 15 Gy, 0.001%.

b, Analysis of trex2null and rad18²/² cells and double-mutant cells. In response to 60 J m⁻² ultraviolet light, trex2null and rad18²/² cells had reduced levels of PCNA–Ub1 and PCNA–Ub3 and unknown protein as compared to IB10 cells. rad18⁻/⁻ cells exhibited a marginally greater reduction than trex2null cells, indicating that RAD18 has a greater role in PCNA ubiquitination. The double-mutant cells failed to show a further reduction, indicating that TREX2 and RAD18 are epistatic. Some ubiquitinated PCNA was present in mutant cells, indicating that other proteins ubiquitinate PCNA; similar observations were made for cells deleted for HLT and SHPRH40. For example, CRL4Cdt2, independent of RAD18, monoubiquinates PCNA with and without ultraviolet-light-induced damage27. c, Bar graph illustrating the reduction of PCNA–Ub1 and PCNA–Ub3 in IB10, trex2null, rad18⁻/⁻, and double-mutant cells as shown in b, left (immunoprecipitation-PCNA, blot-Ub), after band intensities were quantified with ImageJ and normalized for loading with short exposure PCNA. Statistics (t-test) for PCNA–Ub1 and PCNA–Ub3 using three experiments (lanes): 1 vs 2 (0.0016, 0.0058), 1 vs 3 (0.0036, 0.0026), 1 vs 4 (0.0064, 0.0001), 2 vs 3 (0.0214, 0.0774), 2 vs 4 (0.0310, 0.0486), 3 vs 4 (0.3169, 0.1209).

d, Bar graph illustrating the reduction of PCNA–Ub1 in trex2null, rad18⁻/⁻, and double-mutant cells as shown in b, right (immunoprecipitation-PCNA, blot-PCNA), after band intensities were quantified with ImageJ and normalized for loading with short exposure PCNA. The stripping and re-probing leaves quantification unreliable for PCNA–Ub3 and further work is required to clarify the extent to which Ub modification is influenced in these backgrounds. Statistics (t-test) for PCNA–Ub1 using three experiments (lanes): 1 vs 2 (0.0021), 1 vs 3 (0.0061), 1 vs 4 (0.0460), 2 vs 3 (0.0212), 2 vs 4 (0.0163), 3 vs 4 (0.0604).
Extended Data Figure 7 | Deleting Trex2 in IB10 control and rad18−/− cells. A floxed MiniHPRTΔ36 was used to replace the entire Trex2 coding sequences (located on a single exon)25. Targeted clones were detected using PCR with TX2 LR55 and HATrev primers for the left arm and HATfor and TX2 RR33 primers for the right arm. Removal of the Trex2 coding sequence was verified by PCR using mTX2For and mTX2Rev primers.
Extended Data Table 1 | IRR summary

| clone | #MPS | DP | EPT | SD |
|-------|------|----|-----|----|
| 1     | 32   | 3  | 0   | 0  |
| 2     | 32   | 0  | 0   | 0  |
| 3     | 30   | 3  | 0   | 0  |
| 4     | 33   | 0  | 0   | 0  |
| 5     | 25   | 1  | 0   | 0  |
| 6     | 31   | 1  | 0   | 0  |
| 7     | 30   | 0  | 0   | 0  |
| 8     | 23   | 1  | 0   | 0  |
| 9     | 27   | 2  | 0   | 0  |
| 10    | 24   | 1  | 0   | 0  |
| 11    | 27   | 0  | 0   | 0  |
| 12    | 31   | 0  | 2   | 0  |
| 13    | 47   | 0  | 1   | 0  |
| 14    | 53   | 0  | 2   | 0  |
| 15    | 65   | 2  | 1   | 0  |
| 16    | 46   | 0  | 1   | 0  |
| 17    | 48   | 1  | 1   | 0  |
| 18    | 49   | 1  | 0   | 0  |
| 19    | 54   | 0  | 1   | 1  |

Dipericentric (DP), extra pericentromere and telomere (EPT), metaphase spread (MPS), segmental duplication (SD).
Extended Data Table 2 | MRR summary

| clone | #MPS | DP | EPT | SD |
|-------|------|----|-----|----|
| 1     | 27   | 1  | 0   | 0  |
| 2     | 22   | 3  | 0   | 0  |
| 3     | 22   | 0  | 1   | 0  |
| 4     | 35   | 1  | 2   | 0  |
| 5     | 31   | 1  | 3   | 0  |
| 6     | 31   | 1  | 1   | 0  |
| 7     | 29   | 0  | 0   | 0  |
| 8     | 26   | 0  | 2   | 0  |
| 9     | 28   | 3  | 0   | 0  |
| 10    | 22   | 0  | 2   | 1  |
| 11    | 26   | 2  | 1   | 0  |
| 12    | 22   | 2  | 2   | 1  |
| 13    | 47   | 0  | 24  | 0  |
| 14    | 34   | 0  | 1   | 0  |
| 15    | 35   | 1  | 11  | 0  |
| 16    | 36   | 1  | 1   | 0  |
| 17    | 44   | 2  | 0   | 0  |
| 18    | 38   | 0  | 28  | 0  |
| 19    | 36   | 1  | 3   | 0  |

Dipericentric (DP), extra pericentromere and telomere (EPT), metaphase spread (MPS), segmental duplication (SD).
### Extended Data Table 3 | Spectral karyotyping summary

| MRR clone | nuclei | Simple EPT          | Complex EPT          | other                                      |
|-----------|--------|---------------------|----------------------|--------------------------------------------|
| 13        | 1      | -                   | -                    | -                                          |
|           | 2      | Dup(1;1)            | T(14;13)             | -                                          |
|           | 3      | Dup(7;7)            | T(3;13)              | chromatid fusion (8;19)                   |
|           | 4      | Dup(1;1)            | T(14;13)             | chromatid break (16)                      |
|           | 5      | -                   | -                    | Del(1)                                     |
|           | 6      | -                   | T(14;13)             | Del(3)                                     |
|           | 7      | chr1-chr4-chr17     | T(14;13)             |                                            |
|           | 8      | Dup(1;1) Dup(7;7)   | T(14;13) T(2;?)      |                                            |
|           | 9      | Dup(1;1)            | -                    |                                            |
| 15        | 1      | Dup(1;1)            | -                    | T(11;14) T(14;11)                         |
|           | 2      | Dup(1;1)(1;)        | -                    | T(11;14) T(14;13) Del(13)                 |
|           | 3      | Dup(1;1)            | T(2;13)              | T(11;14) T(14;11)                         |
|           | 4      | Dup(1;1)            | -                    | T(11;3)                                    |
|           | 5      | Dup(1;1)            | -                    | Ins(3;11) T(11;3) Del(3)                  |
|           | 6      | Dup(1;1)            | -                    | T(11;14) T(14;11)                         |
|           | 7      | Dup(1;1)            | -                    | T(11;14) T(14;11)                         |
| 18        | 1      | Dup(1;1) Dup(7;7)   | T(14;11)             | -                                          |
|           | 2      | -                   | T(14;11)             | -                                          |
|           | 3      | Dup(1;1) Dup(12;12)| T(14;11)             | -                                          |
|           | 4      | Dup(1;1)            | T(14;11)             | -                                          |
|           | 5      | -                   | T(14;11)             | -                                          |
|           | 6      | Dup(1;1) Dup(7;7)   | T(14;11)             | -                                          |
|           | 7      | Dup(1;1) Dup(7;7)   | T(14;11)             | chromatid fusion (17;14)                  |
|           | 8      | Dup(1;1) Dup(12;12)| T(14;11)             | -                                          |

Simple extra pericentromere and telomere (EPT) involves one chromosome. Complex EPT involves more than one chromosome. Other has only one pericentromere.