LETTER

HELQ promotes RAD51 parologue-dependent repair to avert germ cell loss and tumorigenesis

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Repair of interstrand crosslinks (ICLs) requires the coordinated action of the intra-S-phase checkpoint and the Fanconi anemia pathway, which promote ICL incision, translesion synthesis and homologous recombination (reviewed in refs 1, 2). Previous studies have implicated the 3′–5′ superfamily 2 helicase HELQ in ICL repair in Drosophila melanogaster (MUS301 (ref. 3)) and Caenorhabditis elegans (HELQ-1 (ref. 4)). Although in vitro analysis suggests that HELQ preferentially unwinds synthetic replication fork substrates with 3′ single-stranded DNA overhangs and also disrupts protein–DNA interactions while translocating along DNA 3′–5′, little is known regarding its functions in mammalian organisms. Here we report that HELQ helicase-deficient mice exhibit subfertility, germ cell attrition, ICL sensitivity and tumour predisposition, with HELQ heterozygous mice exhibiting a similar, albeit less severe, phenotype than the null, indicative of haploinsufficiency. We establish that HELQ interacts directly with the RAD51 parologue complex BCDX2 and functions in parallel to the Fanconi anemia pathway to promote efficient homologous recombination at damaged replication forks. Thus, our results reveal a critical role for HELQ in replication-coupled DNA repair, germ cell maintenance and tumour suppression in mammals.

To examine the effect of HELQ deficiency in vertebrates, we generated a Helq−/− deficient mouse strain that is truncated at the carboxy terminus of HELQ (Fig. 1a, b and Extended Data Fig. 1a, b) and results in loss of detectable HELQ protein (Fig. 1d and Extended Data Fig. 1c). Although Helq−/− mice are viable (Fig. 1c), are born in normal Mendelian ratios and lack growth or developmental abnormalities (Extended Data Fig. 1d, e), breeding experiments with Helq−/− mutant pairs revealed a fertility defect. Eight heterozygous and 8 homozygous pairs were mated continuously for 5–6 months, resulting in 320 offspring in the case of heterozygotes (an average of 6.1 litters and 40 pups each) but only 38 pups in the case of homozygotes (1.4 litters and 4.7 pups per pair). Matings of mutants to control animals revealed that females contribute more to this phenotype than males (Fig. 1e).

Consistent with a fertility defect, Helq−/− testes were smaller than those of wild-type males (0.58% of body weight for wild type versus 0.38% for mutants (Fig. 1f)). Histological analysis of testes revealed many normal tubules but also regions of atrophy in the mutants (Fig. 1g and Extended Data Fig. 1g–i). Dysgenesis/atrophy was even more pronounced in Helq−/− ovaries (Fig. 1g and Extended Data Fig. 1f). A possible stem cell origin was investigated as no particular subset of spermatocytes appeared affected (Extended Data Fig. 1g–i). Indeed, Helq−/− adults had significantly fewer c-KIT (also known as KIT) + spermatogonia than controls (Extended Data Fig. 2a, b). As atrophy was not linked to ageing (Extended Data Fig. 2c), a developmental origin was examined; tubules from 5-day-old wild-type mice contained sixfold more spermatogonia than mutants (Fig. 1h), indicating that atrophic tubules in mutant adults may primarily arise from reduced spermatogonial stem cell pools during development.

The effect of HELQ deficiency during organismal ageing revealed that tumour-free survival was significantly reduced in Helq mutants (Fig. 1i and Extended Data Fig. 2d), with twice as many Helq+/− mice developing two or more primary tumours in comparison to controls (Fig. 1j). Ovarian tumours (resembling granulosa and other sex cord stromal tumours; Extended Data Fig. 3b–f) and pituitary adenomas (Extended Data Fig. 3g–j) were the most prominent tumour types in female mice, with incidences of 40% in the case of ovarian tumours and 30% in the case of pituitary tumours (Fig. 1k). Unexpectedly, Helq−/− heterozygous females also presented with ovarian pathology similar to that of younger mutant females (Extended Data Fig. 2d). Pathology included cystic (4 out of 7 mice) and dysgenetic/atrophic (5 out of 7 ovaries with few or no maturing follicles (7 out of 7) and luteinized stroma (2 out of 7). Helq−/− heterozygous females also frequently displayed pituitary (5 out of 7 mice), Harderian gland (3 out of 7) and gastrointestinal (3 out of 7) adenomas, polyps and hyperplasias. Although these phenotypes are less severe than observed in the Helq−/− homozygous mice, the data reveal that loss of a single allele of Helq confers haploinsufficiency in mice.

The phenotype of Helq−/− mice is similar to that observed in mouse models of Fanconi anemia (FANC) defects and sensitivity to ICLs are also hallmarks of Fanconi anemia and were therefore examined in Helq mutants. Although bone marrow HSPCs from Helq−/− mice exhibit hypersensitivity to the ICL agent mitomycin C (MMC; Extended Data Fig. 4a), Helq−/− HSPCs were not compromised in numbers (Extended Data Fig. 4b, c), proliferative capacity (Extended Data Fig. 4d, e) or engraftment (Extended Data Fig. 4f–i). HELQ-deficient cells exhibited hypersensitivity to replication blocking agents such as MMC and camptothecin (CPT; Fig. 2a, b), but not to ionizing radiation or ultraviolet radiation (Fig. 2c, d). Helq−/− cells also exhibited significantly more chromatid breaks and radial chromosomes than control cells upon treatment with mitomycin C (MMC; Fig. 2e, f). Silencing of HELQ by short interfering RNA (siRNA) in human cells resulted in similar phenotypes (Extended Data Fig. 4j).

To examine the phenotypic relationship between HELQ and the Fanconi anemia pathway we generated Helq−/− Fancd2−/− double-mutant mice (the Fancd2−/− strain is described in ref. 8). Double mutants were born in Mendelian ratios (Extended Data Fig. 4k) and growth and appearance were normal. Surprisingly, testes from double mutants were significantly smaller than single mutants and all tubules were atrophic, containing only Sertoli cells (Fig. 2f, g). Similarly, double-mutant cells exhibited greater sensitivity to MMC and CPT than either single-mutant (Fig. 2h, i) and spontaneous and MMC-induced chromosomal aberrations were significantly increased over the Helq single mutant...
in the Fanconi anaemia pathway (Fig. 3a, b and Extended Data Fig. 5a). Interaction partners identified by mass spectrometry were confirmed via immunoprecipitation/western blot (Fig. 3c). Reciprocal immunoprecipitation of RAD51C confirmed its association with Flag–HELQ (Fig. 3d), and endogenous HELQ was detected in RAD51C immunoprecipitates from 293T cells and vice versa (Fig. 3e). Recombinant BCDX2 proteins purified from either insect cells or bacteria also bound to purified Flag–HELQ but not to ALC1–Flag or Flag controls (Fig. 3f). Intriguingly, XRCC3, a component of the RAD51C–XRCC3 (CX3) RAD51 parologue complex, was not detected by either mass spectrometry or western blotting of Flag–HELQ immunoprecipitates (Fig. 3c). Furthermore, HELQ was not found in reciprocal immunoprecipitates with endogenous XRCC3 (Extended Data Fig. 5c). These data indicate that HELQ interacts directly with the BCDX2 complex but not with the CX3 complex.

As ATR, RPA70, the BCDX2 complex and FANC D2–FANC I all respond to stalled replication forks, we examined the localization of green fluorescent protein-tagged HELQ (HELQ–GFP) expressed in NIH 3T3 cells. Subcellular fractionation revealed that HELQ–GFP is enriched on chromatin in response to replication fork stalling with either MMC or aphidicolin, and this is compromised by ATR inhibition (Fig. 3g and Extended Data Fig. 5e–g).

To determine the possible underlying cause of the defect in HELQ-deficient cells we examined replication dynamics, indices of checkpoint activation, Fanconi anaemia pathway activation, double-stranded break (DSB) formation, and the integrity of homologous recombination. Replication fork extension rates in $Helq^{+/+}$ cells were significantly lower than in wild-type cells (Extended Data Fig. 6d, e) and this was exacerbated by treatment with CPT (Extended Data Fig. 6d). Replication

![Figure 2](https://example.com/image2.png)

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![Figure 1](https://example.com/image1.png)

Figure 1 | A mouse model of HELQ deficiency. a, Helq genomic locus. Base pairs are indicated above. Introns are not to scale; exons are roughly to scale with black bar indicating 1 kilobase. Location of the β-geo gene trap and genotyping primers are shown. b, HELQ domain architecture: amino acids are indicated, red bar spans epitope recognized by the HELQ antibody used for western blotting. c, Helq genotype PCR. d, Lysates from ear fibroblasts of Helq mice probed for HELQ. e, The number of litters (black) and pups (pink) generated per 21-day gestational interval. f, Helq tests images and weights. g, Histological sections of Helq gonads. ‘A’ denotes atrophic tubules; asterisks denote developing ovarian follicles. h, Left, 5-day-old neonatal testes labelled with the Sertoli marker WT1 (brown) and haematoxylin (blue), to reveal spermatogonia (asterisks). Right, quantification of spermatogonia (SG). i, epithelial and stromal tumour-free survival of Helq mice. j, k, Frequency of mice with two or more primary tumours (j) and female-specific pathology (k). These results suggest that HELQ and FANC D2 act in parallel ICL repair pathways.
fork tract asymmetry was also evident in mutants relative to controls indicative of increased fork stalling/collapse (Extended Data Fig. 6f, g).

Checkpoint activation as measured by phosphorylation of ATM, CHK1 and CHK2 (also known as CHEK1 and CHEK2, respectively) and γH2AX in response to DNA damage was unaffected by loss of HELQ in either mouse or human cells (Extended Data Fig. 7a–d). Furthermore, HELQ-deficient cells exhibited constitutive FANCDD2 monoubiquitination, indicating that HELQ is dispensable for this modification (Fig. 4a). Assessment of RAD51 recruitment to damaged replication forks revealed that RAD51 is enriched on chromatin in response to MMC treatment in HELQ-deficient mouse and human cells (Fig. 4b and Extended Data Fig. 7e, 8a) and RAD51, RPA (also known as RPA1) and γH2AX accumulate in repair foci (Fig. 4c and Extended Data Fig. 7e–g). However, RAD51 and γH2AX persisted on chromatin and remained present in repair foci at later time points in HELQ-deficient mouse and human cells (Fig. 4b, c and Extended Data Fig. 7e, f), suggesting that the defect in the absence of HELQ occurs at a step downstream of RAD51 recruitment to damaged replication forks. Pulsed field gel electrophoresis revealed that DSBs form in Helq and Fancd2 single and double mutant cells after MMC treatment, but that these lesions persist at later time points, indicating that DSBs induced at ICs are not efficiently repaired (Fig. 4d). siRNA-induced depletion of HELQ resulted in a two- to threefold decrease in homologous recombination efficiency, implicating HELQ in promoting homologous recombination (Fig. 4e and Extended Data Fig. 8b). Furthermore, clonogenic survival of HELQ-deficient mouse and human cells were significantly compromised in response to poly-ADP ribose polymerase (PARP) inhibition, which is a hallmark of homologous recombination-deficient cells10 (Fig. 4f and Figure 3 | HELQ interacts with DNA replication stress response factors. a, Unique and total peptides identified by mass spectroscopy analysis of HELQ–Flag co-immunoprecipitates isolated from 293 cells. b, HELQ interaction network based on the mass spectroscopy results (coloured lines) and reported interactions from BIOGRID, STRING and MINT databases (dashed lines). c, Western blots of input and Flag immunoprecipitates (IP) from HELQ–Flag and Flag control samples. d, Reciprocal immunoprecipitates of endogenous RAD51C with HELQ–Flag. e, Endogenous HELQ and RAD51C immunoprecipitates from 293T cells. f, Purified, His-tagged BCDX2 complexes (top) were incubated with Flag-complexed beads (bottom) to test for a direct interaction. Flag and ALC1–Flag are shown as negative controls. NT, non-treated. g, Western blot analysis of whole cells extracts (WCE) and chromatin fractions (Chr) from NIH3T3 HELQ–GFP-expressing cells treated with or without 100 ng ml −1 MMC for 24 h.

Extended Data Fig. 8c). It is notable that the HELQ interacting protein and BCDX2 complex factor, RAD51D, is also required for PARP inhibitor resistance11.

In summary, our results uncover a critical role for HELQ in germ cell maintenance and tumour suppression in mammals, which we attribute to a role in replication-coupled DNA repair. The interaction between HELQ and the RAD51 parologue BCDX2 complex may provide molecular insight into the HELQ phenotype and its role in tumorigenesis, as the BCDX2 complex functions to promote replication-coupled homologous recombination, RAD51C has been implicated in a Fanconi anaemia-like disorder12, and mutations in RAD51B and RAD51D are associated with hereditary ovarian cancer in humans11,13–18. These findings suggest HELQ as a strong candidate for screening in human cancers and also explain why mutations in D. melanogaster homologues of HELQ, RAD51, and the two RAD51 paralogues (MUS301 (also known as SPN-C), SPN-A, SPN-B and SPN-D, respectively) confer a very similar phenotype19. Finally, our findings help to explain the prevalence of non-synonymous variants in HELQ, which are significantly associated with upper aerodigestive tract cancers, particularly amongst smokers20; and variants in HELQ associated with early menopause21, which may reflect the germ cell defects and ovarian dysgenesis observed in HELQ-deficient mice.

Figure 4 | HELQ influences DNA repair and homologous recombination efficiency. a, Lysates from immortalized mouse cells, grown under physiological O2 and treated with or without 3 μM aphidicolin (APH) for 6 h, were probed for FANCDD2. Wild-type (WT), HELQ-deficient (HQ), unmodified (S) and ubiquitinated (L) forms of FANCDD2 were used. b, Chromatin fractions from immortalized mouse cells, probed for RAD51, histone H3 and α-tubulin at the indicated time points (hours) following treatment with or without 100 ng ml −1 MMC. c, Left, representative images of RAD51 focus formation in immortalized mouse cells at the indicated time points (hours) following treatment with 1 μM MMC. Right, quantification of RAD51 foci at the indicated time points. d, Pulsed field gel electrophoresis of genomic DNA from immortalized cells treated with or without 1 μM MMC for 1 h and recovered for the indicated number of hours. un, undamaged. Wells, intact DNA; arrow, band containing large chromosomal fragments (10–45 megabases); below the arrow, smaller fragments resolved by size. e, Homologous recombination frequencies in direct repeat (DR)–GFP reporter cells treated with the indicated siRNAs. LUC, luciferase. *P<0.05; **P<0.001. f, Clonogenic survival assays of immortalized mouse cells exposed to PARP inhibitor (PARPi). Error bars represent s.e.m.
METHODS SUMMARY

HELQ-deficient mice were generated from a commercially available embryonic stem cell clone (clone ID: RFF112, Bay Genomics, University of California, Davis). All strains were maintained on a mixed B6/129 background. Tests for histology were fixed in Bouin’s solution and periodic acid-Schiff/haematoxylin stained; all other tissues were fixed in neutral buffered formalin and stained with haematoxylin and eosin. Bone marrow cells were collected and analysed by FACS, colony formation and transplantation as described previously. HELQ–Flag, Flag, and ALCL–Flag cell lines were generated using the HEK293 Fip-In system according to the manufacturer’s protocol (Invitrogen). HELQ–GFP-expressing NIH3T3 cells were generated according to the bacterial artificial chromosome recombinering method described previously. Pulse field gel electrophoresis was carried out similar to the method described in ref. 24. Isolation of HELQ–Flag immunocomplexes entailed lysis of cells in the presence of benzonase to prevent non-specific DNA-bridging of proteins. Flag immunoprecipitates for mass spectrometry analysis were eluted by boiling in SDS–PAGE sample buffer and processed using standard methods. The biological General Repository for Interaction Data sets (BioGRID, http://thebiogrid.org/), the molecular interaction database (MINT, http://mint.bio.unizurich.ch/mint) and Search Tool for the Retrieval of Interacting Genes/Proteins database (STRING, http://string-db.org/) were used to compile the interaction network. For assays of in vitro binding to purified His-tagged BCDX2, Flag-tagged proteins were purified by washing immune-complexed beads four times with buffer containing 1 M NaCl. Purified Flag proteins were then incubated with recombinant BCDX2 complex, washed and eluted for analysis. Chromatin fractionation was carried out using modified versions of previously established methods. For siRNA transfections of U2OS cells, cells were subjected to two rounds of reverse transfections using siGENOME siRNA and Dharmafect1 (Thermofisher) according to the manufacturer’s protocol. Histology/immunohistochemistry, primary cell line derivation and immortalization, immunofluorescence, assays for clonogenic survival, metaphase aberrations, micronuclei and DNA combing were carried out using standard procedures.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Kim, H. & D’Andrea, A. D. Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. Genes Dev. 26, 1393–1408 (2012).
2. Kottemann, M. C. & Smogorzewska, A. Fanconi anemia and the repair of Watson and Crick DNA crosslinks. Nature 493, 356–363 (2013).
3. Boyd, J. B., Golino, M. D., Shaw, K. E., Os gode, C. J. & Green, M. M. Third-chromosome mutagen-sensitive mutants of Drosophila melanogaster. Genetics 97, 607–623 (1981).
4. Muzzoni, D. M., Plevani, P., Boulton, S. J., Cassata, G. & Marini, F. Cenabornublin elegans POLQ-1 and HELQ-30B function in two distinct DNA interstrand cross-link repair pathways. DNA Repair 7, 941–950 (2008).
5. Richards, J. D. et al. Structure of the DNA repair helicase hel308 reveals DNA binding and autoinhibitory domains. J. Biol. Chem. 283, 5118–5126 (2008).
6. Ward, J. D. et al. Overlapping mechanisms promote postsynaptic RAD-51 filament disassembly during meiotic double-strand break repair. Mol. Cell 37, 157–158 (2010).
7. Parmar, K., Lessi, A. & Niederhofer, L. J. Mouse models of Fanconi anemia. Mutat. Res. 668, 133–140 (2009).
8. Parmar, K. et al. Hematopoietic stem cell defects in mice with deficiency of FancD2 or Usp1. Stem Cells 28, 1186–1196 (2010).
9. Poser, I. et al. BAC Transgeneomics: a high-throughput method for exploration of protein function in mammals. Nature Methods 5, 409–415 (2008).
10. Huehls, A. M., Wagner, J. M., Hunton, C. J. & Karnitz, L. M. Identification of DNA repair pathways that affect the survival of ovarian cancer cells treated with a poly(ADP-ribose) polymerase inhibitor in a novel drug combination. Mol. Pharmacol. 82, 767–776 (2012).
11. Lovey, C. et al. Germline mutations in RAD51D confer susceptibility to ovarian cancer. Nature Genet. 43, 857–882 (2011).
12. Zaz, F. et al. Mutation of the RAD51C gene in a Fanconi anemia-like disorder. Nature Genet. 42, 406–409 (2010).
13. Meindl, A. et al. Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. Nature Genet. 42, 410–414 (2010).
14. Lovey, C. et al. Germline RAD51C mutations confer susceptibility to ovarian cancer. Nature Genet. 45, 475–476 (2012).
15. Vuorela, M. et al. Further evidence for the contribution of the RAD51C gene in hereditary breast and ovarian cancer susceptibility. Breast Cancer Res. Treat. 130, 1003–1010 (2011).
16. Pelton, L. M. et al. RAD51C is a susceptibility gene for ovarian cancer. Hum. Mol. Genet. 20, 3278–3288 (2011).
17. Wickramanyake, A. et al. Loss of function germline mutations in RAD51D in women with ovarian carcinoma. Gynecol. Oncol. 127, 552–555 (2012).
18. Osher, D. J. et al. Mutation analysis of RAD51D in non-BRCA1/2 ovarian and breast cancer families. Br. J. Cancer 106, 1460–1463 (2012).
19. Gonzalez-Reyes, Y., Elliott, H. & St Johnston, D. Oocyte determination and the origin of polarity in Drosophila: the role of the spindle genes. Development 124, 4927–4937 (1997).
20. McKay, J. D. et al. A genome-wide association study of upper aerodigestive tract cancers conducted within the INHANCE consortium. PLoS Genet. 7, e1001333 (2011).
21. Stolik, L. et al. Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. Nature Genet. 44, 260–268 (2012).
22. Cecchidi, R. et al. Bone marrow failure in Fanconi anemia is triggered by an exacerbated p53/p21 DNA damage response that impairs hematopoietic stem and progenitor cells. Cell Stem Cell 11, 36–49 (2012).
23. Ploemacher, R. E., van der Sluijs, J. P., van Beurden, C. A., Baert, M. R. & Chan, P. L. Use of limiting-dilution type long-term marrow cultures in frequency analysis of marrow-repopulating and spleen colony-forming hematopoietic stem cells in the mouse. Blood 78, 2527–2533 (1991).
24. Hanada, K. et al. The structure-specific endonuclease Mus81–Eme1 promotes conversion of interstrand DNA crosslinks into double-strands breaks. EMBO J. 25, 4921–4932 (2006).
25. Aygun, O., Sjesstrup, J. & Liu, Y. A. RECQ–RNA polymerase II association identified by targeted proteomic analysis of human chromatin. Proc. Natl. Acad. Sci. USA 105, 8580–8584 (2008).
26. Mizovea, O. K. & Petrini, J. H. DNA replication-dependent nuclear dynamics of the Mre11 complex. Mol. Cancer Res. 1, 207–218 (2003).

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Author Contributions C.A.A., R.L.L. and S.J.B. designed the study, performed experiments and wrote the manuscript unless otherwise stated. G.S. performed mouse post-mortem analyses and advised on histopathology; O.M. and A.A.S. performed and supervised DR-GFP homologous recombination assays, K.M. performed RAD51 foci experiments, Z.H. performed human clonogenic survival assays, K.P. and A.D. performed and supervised mouse bone marrow experiments, V.B. assisted with mouse tumour watch monitoring, J.M.S. supervised mass spectrometry, N.J.B. and C.S. advised on experiments and manuscript revisions, and all authors contributed to revision of the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.J.B. (simon.boulton@cancer.org.uk).
METHODS

Mouse strains, maintenance, localization of genetraps, genotyping. All mice were housed and maintained according to the Home Office guidance outlined in the Animals Scientific Procedures Act. All strains were maintained on a mixed B6/129 background.

A commercially available embryonic stem cell clone (clone ID: RRF112, Bay Genomics, University of California, Davis) harbouring the trapped allele of Helq was obtained. The position of the β-gene trap cassette from the pGT0Lfx vector was determined via splinkerette PCR29 using a modified version of the method described in ref. 28. In brief, 3 µg genomic DNA from Helq gene-trapped embryonic stem cells was digested overnight at 37 °C with BfaI. Splinkerette primers were annealed in SuRE Buffer M (Roche) by heating to 95 °C, followed by cooling by 1 °C increments for 15 s each. A total of 40 pmol of the annealed splinkerette adaptor was ligated to 600 ng of BfaI-digested genomic DNA, followed by passage over a PCR purification column (Qiagen). The splinkerette-adapted genomic DNA was re-digested with BfaI for 1 h (eliciting potential background due to splinkerette ligation to partially digested genomic DNA fragments) and re-purified.

Primary and nested PCRs were performed using genetrap- and splinkerette-specific primers, with 0.4% of the primary PCR used in the nested round. Nested PCR products were gel purified and sequenced using standard methods.

The Helq+/− mouse strain was generated using standard transgenic technology. For morphological and molecular characterization of the transgenic mice, see elsewhere in the Methods and Extended Data. FANCDC-deficient mice were described previously4. Mice were identified using standard ear snip methods, and mice were genotyped in a single reaction using the following primers: pGT0LxF_F2-CA GGGTTTCTCCAGTCACGAC (genetraper-specific primer), mHELQint11_F8-GT CCTTGTGCCAAAGTACAG (wild-type-specific primer), mHELQint11_R5-CC TAGGTGGCCTTTA1CTCTCTCTC (common primer). Fancd2 mice were genotyped according to the previously described method6.

Breeding data are presented as continuous data, individually housed pairs. The HelqFancd2 double-mutant strain was established from mating of double heterozygous Helq+/−Fancd2+/− mice. Weights of Helq mice were measured weekly starting at 10 days post-partum. The tumour watch cohort was established using littersmate controls wherever possible and mice were regularly monitored for signs of deterioration using a scoring system that will be described in a separate publication. Mice were euthanized before terminal end points were reached.

Statistics. For survival study, sample size was estimated using standard power calculation methods in order to measure a difference of 3–4 months in survival between mutant and control groups. For Helq and HelqFancd2 matings, deviations from expected Mendelian ratios were tested by Chi-squared analysis (P > 0.25 for both strains). For fertility analysis, the number of litters and pups were tested using Kruskal–Wallace analysis of variance. The Gaussian approximation was used to calculate the indicated P value (P < 0.0001). Dunn’s multiple comparison post-test was used to compare specific sample pairs. Results of P value calculations for MMC-damaged samples are indicated on the graph (P < 0.01 for wild-type versus single and double mutants, P < 0.01 for Helq mutants versus double mutants, and P = not significant for Helq versus Fancd2 single mutants and for Fancd2 mutants versus double mutants). For HEQL–GFP chromatin recruitment upon ATR inhibitor treatment, Student’s t-test was performed to determine whether the observed differences were statistically significant. For MMC and DNA damage, results were generated as follows: mice were euthanized and ear tissue was collected using sterile scissors or forceps, ear fragments were rinsed twice in 70% ethanol followed by two rinses in PBS supplemented with 100 µg ml−1 kanamycin. Tissue was transferred into 0.3 ml of protease solution (4 mg ml−1 each of collagenase D and dispase in DMEM; filter sterilized), and incubated at 37 °C for 45 min. In total, 1.5 ml DMEM containing 10% FBS, 1x glutamine and 5x antibiotic–antimycotic solution were added to protease solution containing ear fragments, and samples were incubated at 37 °C for 2 h. Cells were collected by centrifugation through a 40-µm cell strainer, and plated in DMEM as above except using 1x antibiotic–antimycotic solution. Cells were passaged upon reaching confluence to five dishes, and upon reaching confluence, cells were frozen at passage 1 or used immediately for immortalization or experiments.

Cell line derivation. Ear fibroblasts for primary and SV40 immortalized cultures were generated as follows: mice were euthanized and ear tissue was collected using sterile scissors, ear fragments, were rinsed twice in 70% ethanol followed by two rinses in PBS supplemented with 100 µg ml−1 kanamycin. Tissue was transferred into 0.3 ml of protease solution (4 mg ml−1 each of collagenase D and dispase in DMEM; filter sterilized), and incubated at 37 °C for 45 min. In total, 1.5 ml DMEM containing 10% FBS, 1x glutamine and 5x antibiotic–antimycotic solution were added to protease solution containing ear fragments, and samples were incubated at 37 °C for 2 h. Cells were collected by centrifugation through a 40-µm cell strainer, and plated in DMEM as above except using 1x antibiotic–antimycotic solution. Cells were passaged upon reaching confluence to five dishes, and upon reaching confluence, cells were frozen at passage 1 or used immediately for immortalization or experiments.

Fibroblasts were immortalized via transfection with a vector expressing SV40 large T antigen. Constitutively expressed HELQ–Flag, Flag, and ALC1–Flag cell lines were generated using the 293 Flp-In system according to the manufacturer’s protocol (Invitrogen). NIH3T3 cells stably expressing GFP-tagged mouse HELQ (consisting of a bacterial artificial chromosome (BAC) containing the entire Helq promoter and genomic locus) were generated according to the BAC recombineering protocol (Invitrogen). The vector was electroporated into NIH3T3 cells followed by overlaying with a 40-µm cell strainer, and plated in DMEM as above except using 1x antibiotic–antimycotic solution. Cells were passaged upon reaching confluence to five dishes, and upon reaching confluence, cells were frozen at passage 1 or used immediately for immortalization or experiments.

Histology, immunohistochemistry and cell cycle analysis. For all histology, samples were perfused and embedded and sectioned at 4 µm. Tissues were fixed in 10% neutral buffered formalin (NBF) and processed for 1 week before sectioning. Histology, immunohistochemistry sample preparation and reagents.

For immunohistochemistry the NBF fixed sections were processed for paraffin embedding and rehydration in 3,3-diaminobenzidine (DAB) substrate (Vector) and counterstained in haematoxylin. Immunohistochemical analyses were carried out using standard methods. In brief, testes for immunohistochemistry were NBF fixed and sections from adult mice were generated as follows: mice were euthanized and testes were collected using sterile scissors, testes were rinsed twice in 70% ethanol followed by two rinses in PBS supplemented with 100 µg ml−1 kanamycin. Tissue was transferred into 0.3 ml of protease solution (4 mg ml−1 each of collagenase D and dispase in DMEM; filter sterilized), and incubated at 37 °C for 45 min. In total, 1.5 ml DMEM containing 10% FBS, 1x glutamine and 5x antibiotic–antimycotic solution were added to protease solution containing ear fragments, and samples were incubated at 37 °C for 2 h. Cells were collected by centrifugation through a 40-µm cell strainer, and plated in DMEM as above except using 1x antibiotic–antimycotic solution. Cells were passaged upon reaching confluence to five dishes, and upon reaching confluence, cells were frozen at passage 1 or used immediately for immortalization or experiments.

For MMC-induced transfections in HeLa cells (Extended Data Fig. 3d), human HELQ (cloned into the pcDNA6.2/C-EmGFP-DEST vector using Gateway technology (Invitrogen). The vector was transfected into HeLa cells with Lipofectamine 2000 using the manufacturer’s protocol (Invitrogen). Live or parafformaldehyde (PFA)-fixed cells (fixed cells were counterstained with DAPI (4',6-diamidino-2-phenylindole)) were visualized 48–96 h after transfection using epifluorescence using a Zeiss Axio Imager M1 microscope with an ORCA-ER camera (Hamamatsu), and images were acquired using the Velocity software (Improvision, Perkin Elmer). Clonogenic survival, metaphase spreads, growth and micronuclei analyses. For all experiments, fibroblast lines established from littermates or siblings were used wherever possible. Experiments involving primary cell lines were conducted in physiological O2 concentrations using cell lines of similar passage number.

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For clonogenic survival assays, SV40 immortalized mouse ear fibroblasts and siRNA-treated U2OS cells were plated in triplicate on 10-cm dishes at clonal density, allowed to adhere for 8–16 h, and damage treatments administered (CPT medium was changed after 24 h). After 8–10 days of growth, plates were rinsed, fixed/stained in 20% ethanol/4% crystal violet (w/v), rinsed in distilled water and colonies tabulated. All results were normalized to untreated to adjust for plating efficiency and determine percentage survival. Survival experiments were carried out on at least two independent sets of mutant and control mouse cell lines, and in most cases in experiments using lysates prepared from 293T cell lines treated with endogenous HELQ or RAD51C, common mutations in human breast cancer and other cancers. Results were obtained across all experiments and sets of cell lines.

For analysis of metaphase aberrations, SV40 immortalized cells were damaged as indicated and treated with colcemid (2 × 10⁻⁷ M) for 30 min, collected, swelled in hypotonic solution (0.075 M KCl) for 7 min at 37 °C, fixed and washed in ice-cold methanol-acetic acid (3:1), dropped on humid slides and briefly steamed over a 65 °C bath. Slides were dried, stained with Giemsa (Sigma) for 10 min, rinsed with distilled water, and coverslips were mounted (Permount, Fisher). For each sample 40 spreads were scored.

Growth kinetics of primary cells were determined using a modified 3T3 protocol to calculate cumulative population doublings. In brief, primary cell lines were counted and 150,000 cells reseeded in triplicate 10-cm dishes every third day. Cumulative results were obtained across all experiments and sets of cell lines. The more primitive haematopoietic stem cells with long-term repopulating ability were validated to compare with other functional assays. Specifically, day-7 and day-14 bone marrow in micro-cultures using the bone marrow stromal cell line FBMD-1 area-forming cell (CAFC) assay was performed by a limiting dilution analysis of bone marrow in micro-cultures using the bone marrow stromal cell line FBMD-1 (refs 8, 23). This assay quantifies a spectrum of haematopoietic cells that is well-validated to compare with other functional assays. Specifically, day-7 and day-14 CAFC correspond to early progenitor cells and to c.f.u.-spleen-day-12 cells, whereas the more primitive haematopoietic stem cells with long-term repopulating ability correspond to c.f.u.-spleen-day-28 CAFC.

Bone marrow transplantation was performed as described previously. In brief, bone marrow cells (5 × 10⁵) from control or HELQ/Flag-expressing cells (CD45.2⁺) were mixed with 2.5 × 10⁶ bone marrow supporting cells from CD45.1⁺ congenic mice and transplanted into lethally irradiated CD45.1⁺ congenic recipient mice. The donor cell engraftment efficiency in the recipient mice, after 17 weeks post-transplant, was determined by staining peripheral blood leukocytes with FITC-labelled anti-CD45.2 (clone 104) antibody. The percentage of donor-derived T cells, B cells and myeloid cells was determined by co-staining with PE-labelled anti-CD3ε (clone 145-2C11), anti-B220 (clone RA3-6B2) and anti-Mac-1/Gr-1 antibodies (clones M1/70 and RB6-8C5), respectively, and analysed on a FACScan instrument (Becton Dickinson). All antibodies were from BD Biosciences.

Mass spectrometry and proteomics. HELQ–Flag and Flag control cells were collected and lysed in benzoin buffer (20 mM Tris-Cl, pH 7.5, 75 mM NaCl, 10% glycerol, 2 mM MgCl₂, 0.5% NP40, 30 U/ml benzoin, protease inhibitors). NaCl concentration was adjusted to 150 mM, EDTA to 3 mM and lysates were cleared by centrifugation. Supernatants were pre-cleared with Protein G agarose beads for 30 min at 4 °C. Pre-cleared lysates were incubated with anti-Flag affinity agarose resin (Sigma) for 4 h at 4 °C. Beads were washed five times with wash buffer (20 mM Tris-Cl, pH 7.5, 150 mM NaCl, 3 mM EDTA, 0.5% NP40) and once with 1% SDS-PAGE sample buffer and eluates were resolved on NuPAGE Bis-Tris gels (Invitrogen) and stained with Sypro Ruby (Invitrogen). Gel slices were excised and processed for mass spectrometry using the Janus automated liquid handling system (PerkinElmer). Peptides were analysed by nanoscale capillary liquid chromatography–electrospray ionization/multi-stage mass spectrometry (LC–ESI MS/MS), data were processed using Mascot Distiller (Matrix Science) and exported to Scaffold for viewing (Proteme Software).

The Biological General Repository for Interaction Data sets (BioGRID, http://thebiogrid.org), the Molecular INTeraction database (MINT, http://mint.bio.uniroma2.it/mint), and Search Tool for the Retrieval of Interacting Genes/Proteins database (STRING, http://string-db.org) were used to compute the protein interaction network.

Cell lysates, in vitro binding assay and fractionation for western blot analyses. Cell lines used in this study were short tandem repeat-profiled and tested for mycoplasma infection before use. All lysis buffers were supplemented with protease inhibitor cocktail (Roche) and phosphatase inhibitors (Sigma). For validation of mass spectrometry data, HELQ–Flag and Flag-expressing cells were used. (This was due to our inability to validate these interactions using endogenous HELQ, stemming from the fact that it is expressed at very low levels in most human cell lines, and no antibodies were found to reliably immunoprecipitate the human version. Validation using endogenous mouse HELQ was similarly hindered by a lack of reagents available for detection of the mouse RAD51 paralogues.) Cells were lysed in the presence of benzoin and 2 mg of total protein were immunoprecipitated with anti-Flag affinity resin as above. Beads were washed, bound proteins eluted with 1 × NuPAGE LDS sample buffer and analysed by western blot. Similar methods were employed for Western blotting to examine 293T cell lines to examine endogenous HELQ or RAD51C, common mutations in human breast cancer and other cancers.

For in vitro binding assays, HELQ–Flag, ALC1–Flag and Flag were lysed in the presence of benzoin and pre-cleared lysates was used for Flag immunoprecipitation as described above. Flag-immunocomplexed beads were then washed four times with a modified wash buffer containing 1 M NaCl to remove bound precipitates, and once with in vitro binding buffer (20 mM Tris-Cl, pH 7.5, 280 mM NaCl, 3 mM EDTA, 0.5% NP40). Washed beads were incubated with recombinant RAD51 parologue BCDX2 complex (gift of S. West’s laboratory) in binding buffer for 4 h at 4 °C and washed four times with the same buffer. Eluates were analysed by western blot.

GFP-tagged HELQ was stably expressed in NIH3T3 cells using a BAC recombineering method to C-terminally Flag/GFP-tag the BAC-containing full-length genomic HELQ, which included the endogenous promoter. This allowed HELQ–GFP to be expressed at physiological levels.

For chromatin fractionation of embryonic fibroblasts and siRNA-treated U2OS cells, cells were treated with or without 3 μM aphidicolin for 6 h or with or without 1 mM MMC for 24 h, collected and fractionated using a modified version of the method described in ref. 26: pellets were re-suspended in CSK buffer (10 mM PIPES, pH 6.8, 100 mM NaCl, 300 mM sucrose, 3 mM MgCl₂, 1 mM EGTA, pH 7, 0.5% Triton X-100), incubated for 10 min on ice (a small fraction of this was removed and SDS–PAGE sample buffer was added to obtain WCEs), pelleted at low speed and supernatants reserved as soluble fraction. Pellets were washed in CSK buffer and re-pelleted. Pellets were re-suspended in benzoin CSK buffer (10 mM PIPES, pH 7.5, 100 mM NaCl, 300 mM sucrose, 3 mM MgCl₂, 0.5% Triton X-100, 0.1 μl/μl benzoin), lysates were incubated for 30 min at 37 °C, pelleted and supernatants reserved for chromatin fraction. Pellets were re-suspended in high-salt CSK (recipe as above except NaCl was added to 500 mM), lysates were incubated for 10 min on ice, cleared at high speed and supernatants pooled with benzoin CSK lysates to yield chromatin extracts9. In total, 25 μg of soluble and 10 μg of chromatin proteins were analysed by western blotting.
Antibodies used for immunoprecipitation: RAD51C (R68 rabbit antibody), XRCC3 (10F1 mouse monoclonal antibody; 1:400). Antibodies used for immunofluorescence: RAD51 (1:200), XRCC2 (7B7 mouse monoclonal antibody; 1:400), XRCC3 (10F1 mouse monoclonal antibody; 1:400).

siRNA oligonucleotides: RAD51 (ref. 31) 5'-AAGGGAAUUAGUGAACCAAA-3', BRCA2 (ref. 32) 5'-AACAACAUUUGAGAACCAAA-3'. siRNA oligonucleotides used in DR–GFP and in HELQ_h (this study): 5'-GAAUUUCGCAAUUAUUU3', HELQ_3 (this study): 5'-AUGUGUGGUUAUGAAAGA-3', HELQ_hM (this study): 5'-CAAGGAGGATCTCTCAACTAA-3', HELQ_hO (this study): 5'-GUUUGAAGAAUUGGAAAGAACGAA-3', XRCC2: 5'-CAGGGTACTACGCAAGCCT-3', XRCC3: 5'-CAGAATATTGCTGCAATT-3', RAD51C: 5'-AAAGAAATGTCTCACAAAT-3', RAD51D: 5'-CTGGGTGAAATAGCTTA-3'.

siRNA transfection and ATR inhibition. U2OS cells were subjected to two rounds of reverse transfections using siGENOME siRNA and Dharmafect1 (Thermofisher) according to the manufacturer's protocol. Thirty-six hours after the second transfection using siGENOME siRNA and Dharmafect1 (Thermofisher) siRNA transfection and ATR inhibition.

siRNA oligonucleotides: RAD51 (ref. 31) 5'-AAGGGAAUUAGUGAACCAAA-3', BRCA2 (ref. 32) 5'-AACAACAUUUGAGAACCAAA-3'. siRNA oligonucleotides used in DR–GFP and in HELQ_h (this study): 5'-GAAUUUCGCAAUUAUUU3', HELQ_3 (this study): 5'-AUGUGUGGUUAUGAAAGA-3', HELQ_hM (this study): 5'-CAAGGAGGATCTCTCAACTAA-3', HELQ_hO (this study): 5'-GUUUGAAGAAUUGGAAAGAACGAA-3', XRCC2: 5'-CAGGGTACTACGCAAGCCT-3', XRCC3: 5'-CAGAATATTGCTGCAATT-3', RAD51C: 5'-AAAGAAATGTCTCACAAAT-3', RAD51D: 5'-CTGGGTGAAATAGCTTA-3'.

siRNA transfection and ATR inhibition. U2OS cells were subjected to two rounds of reverse transfections using siGENOME siRNA and Dharmafect1 (Thermofisher) according to the manufacturer's protocol. Thirty-six hours after the second transfection, cells were treated for 14 h with 3 μM aphidicolin. For ATR inhibition, 3 μM ATR inhibitor was added to culture media 30 min before aphidicolin treatment.

Immunofluorescence. Cells were first washed in PBS and then fixed with 2% PFA at room temperature (18 °C) for 15 min, and then washed three times in PBS. The fixed cells were further permeabilized with 3% BSA in PBS plus 0.1% Triton X-100, secondary antibodies (provided by Jackson ImmunoResearch) were applied and incubated for 1 h in the dark. The stained coverslips were mounted for 24 h at 50 °C and then allowed to recover for 16–48 h. Cells were collected and processed for pulsed field gel electrophoresis (PFGE) analysis similar to previously described methods41. In brief, cell suspensions were placed on ice, cell numbers counted and equivalent cell numbers or each genotype were embedded in agarose plugs in duplicate. Cells were digested by incubating plugs in proteinase K overnight at 50 °C, plugs were washed 4 × 1 h, sealed with low-melting-point agarose into the well of a 1% agarose/0.5× Tris/borate/EDTA (TBE) pulsed field gel, and run for 24 h on a Gene Navigator PFGE apparatus (Amersham) using the following conditions: running temperature: 13 °C, running angle: 120° (hex electrode); connection setup: 1, 2, 3: N/S 5 s, E/W 5 s; power program: 180 V for 15 min, 170 V 30 min, 160 V 1 h, 150 V 2 h, 140 V 4 h, 130 V 8 h, 120 V 7 h. Gels were post-stained with ethidium bromide and washed in 0.5 × TBE. PFGE was carried out on two independent sets of mutant and control cell lines and results were repeated two or more times for each set of cell lines. Similar results were obtained across all experiments and sets of cell lines.

Pulsed field gel electrophoresis analysis. immortalized embryonic fibroblasts were treated with or without MMC for 1 h and then allowed to recover for 16–48 h. Cells were collected and processed for pulsed field gel electrophoresis (PFGE) analysis similar to previously described methods41. In brief, cell suspensions were placed on ice, cell numbers counted and equivalent cell numbers or each genotype were embedded in agarose plugs in duplicate. Cells were digested by incubating plugs in proteinase K overnight at 50 °C, plugs were washed 4 × 1 h, sealed with low-melting-point agarose into the well of a 1% agarose/0.5× Tris/borate/EDTA (TBE) pulsed field gel, and run for 24 h on a Gene Navigator PFGE apparatus (Amersham) using the following conditions: running temperature: 13 °C, running angle: 120° (hex electrode); connection setup: experimental values obtained for both experiments.

Carotenoid recombination reporter assays. DSB repair efficiency by homologous recombination was measured in DR–GFP U2OS cells as described previously41. In brief, 48 h after the first round of siRNA transfection (40 nM) using Lipofectamine RNAiMAX (Invitrogen), cells were either mock-transfected (pcDNA3.1) or transfected with 0.6 μg of an I-SceI expression plasmid (pCBASce) together with siRNA (20 nM) using 3.6 μg of Lipofectamine 2000 (Invitrogen). The media was replaced 3 h after I-SceI transfection and cells were analysed for GFP expression by flow cytometry on a Cyan ADP (Dako) 72 h after I-SceI transfection. To confirm siRNA efficiency, western blotting was carried out on 50 μg of NP-40 lysates plus sonication run on 4–15% Precast SDS–PAGE gels (Bio-Rad).

DNA combing. Replication tracts were labelled for 20 min with 20 μM iododeoxyuridine (IdU) (in atmospheric O2 experiments, cells were treated with or without 2.5 μM CPT for the final 15 min of IdU labelling to test replication fork stalling/restart), washed with PBS and labelled for 20 min with 200 μM chlorodeoxyuridine (CIdU). Cells were washed, collected on ice, counted and embedded in agarose. Cells were digested in 2–3 changes of proteinase K buffer for 24 h at 50 °C. Plugs were washed for 5 × 10 min in Tris-EDTA followed by β-agarase digestion overnight at 42 °C. Genomic DNA was combed onto silanized coverslips (Genomic Vision) using a Molecular Combing System instrument (Genomic Vision), dried and stained using previously described methods42. Experiments were conducted on two separate sets of mutant and control cell lines with similar results obtained for both experiments.

LETTER

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Extended Data Figure 1 | Allele and subfertility of HELQ deficiency.

**a**, Schematic of the β-geo gene trap and its approximate location within the Helq genomic locus. **b**, Sequence and traces showing the exact location of the gene trap insertion as determined by sequencing of splinkerette PCR products. **c**, HELQ western blot from wild-type (+/+ ) and Helq mutant (ΔC/ΔC) mouse cells, showing loss of HELQ and absence of a HELQ–βgal fusion protein (which, if present, would be evident in the region of the blot marked with the red bar). **d**, Table of observed and expected Mendelian ratios calculated from heterozygous matings. Chi-squared analysis was used to test for deviation of observed from expected. **e**, Average weights of Helq mice tracked between 2 and 12 weeks of age. Means of 5–13 mice for each group are shown, and for clarity, s.d. is not plotted. Differences are not significant. **f**, Table of ovarian pathology in 30-week-old Helq control and mutant females (black text) and heterozygous females within the tumour watch study (blue text, 17–21 months old). **g–l**, Histological sections of testes from HelqΔC/ΔC males showing various degrees of atrophy, including: normal tubules (g), mild atrophy (h, i), pockets of atrophy (arrows), pyknotic nuclei (asterisks); moderate atrophy (j, k), missing spermatogenic layer (arrowheads) and severe atrophy (l), with only Sertoli cells present.
Extended Data Figure 2 | HELQ-deficient germ cell and tumour phenotypes. a, Immunohistochemical analysis of adult testes labelled with the stem cell marker c-KIT (brown) to highlight spermatogonia, and counterstained with haematoxylin to visualize remaining cells in the tubule (blue). Two representative images from wild-type (left) and mutant (right) mice are shown, with red circles indicating c-KIT+ cells. The number of c-KIT+ cells for each panel is indicated in the bottom right corner. Boxed regions in top panels are magnified in bottom panels to demonstrate staining. S, Sertoli cells; SG, c-KIT+ spermatogonia. b, Tabulation of average c-KIT+ cells per tubule normalized to the number of Sertoli cells. c, Testes weights plotted by mouse age in days. Linear regression used to generate slope of best-fit line; tested best-fit line for deviation of slope from 0: \( R^2 \) and \( P \) values are indicated, revealing no correlation between age and testes weight for Helq mutants. d, Table of tumour frequency and tumour spectrum of Helq mutant and control mice showing data for all mice, females, and males in the tumour watch cohort. 129/B6 background phenomena are coloured in grey text, Helq mutant-specific effects are in black, and female-specific pathology is highlighted in pink.

|                        | all mice                  | Female          | Male           |
|------------------------|---------------------------|-----------------|----------------|
|                       | HeLo\(^{+/+}\) | HeLo\(^{AC/AC}\) | HeLo\(^{+/+}\) | HeLo\(^{AC/AC}\) |
| mice in cohort         | 41                        | 44              | 27             | 26              | 14             | 18             |
| total tumors (non-lymphoma) | 34                        | 63              | 16             | 43              | 18             | 21             |
| % with tumors (non-lymphoma) | 46.3                      | 63.6            | 37.0           | 69.2            | 64.3           | 55.6           |
| lymphoma               | 29.3                      | 20.5            | 37.0           | 19.2            | 14.3           | 22.2           |
| lung tumor              | 17.1                      | 13.6            | 11.1           | 7.7             | 28.6           | 22.2           |
| liver tumor             | 9.8                       | 4.5             | 3.7            | 3.8             | 21.4           | 5.6            |
| Harderian gland tumor   | 7.3                       | 25.0            | 3.7            | 19.2            | 14.3           | 33.3           |
| other glandular tumors\(^1\) | 9.6                       | 18.0            | 14.8           | 23.1            | 0              | 11.1           |
| epithelial              |                           |                 |                |                 |                |                |
| gastric tumour          | 19.5                      | 29.5            | 11.1           | 26.9            | 35.7           | 33.3           |
| pituitary tumour        | -                         | -               | 3.7            | 28.0*           | 0              | 0              |
| ovarian tumour          | -                         | -               | 7.4            | 40.0*           | -              | -              |
| other tumors\(^2\)      | 4.9                       | 0               | 3.7            | 0               | 7.1            | 0              |

\(^1\) including: salivary, thyroid, parathyroid, adrenal, mammary, and endometrial gland adenomas and cystic hyperplasias

\(^2\) including: leiomyosarcoma, spindle cell sarcoma

* Pituitary tumors not identified in 1 of 26 PMs, therefore tumor frequency calculated from 25 animals in the mutant cohort.
Extended Data Figure 3 | Tumour histology of HELQ deficiency. **a**, The frequency of liver steatosis in all mice, and inflammation and activated mammary tissue in female mice. **b**–**f**, Ovary sections showing normal wild-type ovary (**b**) and common ovarian pathology in mutant animals (**c**–**f**). Low-magnification (**c**) and high-magnification (**d**) images of dysgenic ovary from a **Helq** mutant exhibiting a sex cord stromal tumour containing tubular-like structures. Low-magnification (**e**) and high-magnification (**f**) images of large nodular granulosa cell tumour from a **Helq** mutant. Arrowheads indicate mitotic figures (**f**). **g–j**, Pituitary sections showing low-magnification (**g**) and high-magnification (**h**) images of normal wild-type pituitary. Low-magnification (**i**) and high-magnification (**j**) images of pituitary tumour from a **Helq** mutant mouse. Arrows indicate boundary where large, haemorrhagic pituitary adenoma compresses overlying brain (**h**).
Extended Data Figure 4 | Characterization of Helq<sup>ADC</sup> bone marrow and generation of Helq<sup>ADC</sup>Fancd2 double-mutant offspring. a, Helq<sup>ADC</sup> and control bone marrow cells were isolated and exposed to MMC at the indicated doses and clonogenic survival of haematopoietic progenitors was plotted as percentage of surviving cells relative to untreated. Means ± s.e.m. for three mice per genotype are shown. b–i, Bone marrow (BM) from mutant and control mice was isolated and subjected to various haematopoietic stem and progenitor cell analyses: tabulation of bone marrow LSK (lineage<sup>2</sup>Sca-1<sup>+</sup>c-Kit<sup>+</sup>) cell populations (b, c); bone marrow c.f.u.-c (colony-forming units in culture) assays (d); bone marrow day-28 cobblestone area-forming cells (CAFCs; e); and total donor-derived leukocyte (f), myeloid (g) and lymphoid (h, i) engraftment upon bone marrow transplantation. Raw data (symbols) and means (horizontal lines) from three mice are plotted (b, c, e); means ± s.e.m. for three mice per genotype (d); and means ± s.e.m. for 6–10 recipients for each genotype (f–i). j, siRNA-treated U2OS cells were plated for clonogenic survival and treated with the indicated reagents. k, Observed and expected Mendelian ratios calculated from Helq<sup>ADC</sup>Fancd2<sup>/+</sup> double heterozygous matings. Chi-square analysis was used to test for deviation of observed from expected.
Extended Data Figure 5 | HELQ mass spectrometry, its relationship with the RAD51 paralogues, ATR, and overexpression. **a**, HELQ purification scheme and SDS–PAGE gel showing proteins co-purified with HELQ–Flag and control Flag immunoprecipitates. **b**, Cells treated with the indicated siRNAs were collected and probed for HELQ and the RAD51 paralogues. **c**, XRCC3 immunoprecipitated from HELQ–Flag and Flag control cell lysates and probed for Flag, XRCC3 and RAD51C (positive XRCC3 interacting protein control). IgG was used as a negative control. **d**, HeLa cells transiently expressing recombinant HELQ–GFP (green panels) fixed and stained with DAPI (blue panels) to identify nuclei. Two examples of spontaneous nuclear aggregation patterns are shown: small focal aggregates (right) and large filamentous aggregates (left). **e**, Chromatin fractions of HELQ–GFP cells treated with or without 2 μM APH for 24 h. Cells treated as in **e**, with or without 3 mM ATR inhibitor (ATRI). Quantification of HELQ in chromatin fractions normalized to H3 (right). **g**, Cells treated with or without 100 ng ml⁻¹ MMC for 24 h and fractionated as in **e**.
Extended Data Figure 6 | Spontaneous defects, checkpoint indices, damage foci and clonogenic survival of HELQ-deficient cells. a, Primary \( \text{Hely} \) mutant and control cell lines were grown in physiological \( \text{O}_2 \) for the indicated number of days and passaged regularly to generate a cumulative population doubling (CPD) curve. Means ± s.d. of triplicate replicas are shown. b, c, \( \text{Hely} \) mutant and control cells grown on coverslips were formaldehyde fixed and DAPI stained to determine levels of spontaneous micronuclei formation: percentage of 100 cells exhibiting 1 or more micronucleus (b); representative images (c), micronuclei (arrows). d, e, DNA combing used to calculate replication fork rates of primary cells grown under atmospheric (d) or physiological (e) \( \text{O}_2 \). Cells in d were treated with or without 2.5 \( \mu \text{M CPT} \) for 15 min during labelling. f, Examples of origin containing IdU (green)- and CldU (red)-labelled fibres. g, Right versus left replication tract lengths to determine fork asymmetry (defined as tracts falling outside the interquartile lines).
Extended Data Figure 7 | Checkpoint and double-strand break repair function. a, b, Immortalized HelQΔCΔC and Helq<sup>−/−</sup> cells treated with or without 500 ng ml<sup>−1</sup> MMC (a) or 50 nM CPT (b) for 20 h and probed for the indicated checkpoint indices. c, Primary wild-type (WT), HELQ-deficient (HQ) and FANCD2-deficient (D2) cells were left untreated (−), or exposed to 5 Gy irradiation and collected 30 min later, or 3 h MAPT for 16 h and harvested 10 min later, and lysates were probed for the indicated checkpoint indices. d, Phospho-Ser 345 CHK1 levels in U2OS cells subjected to 1 μM MMC for 24 h. e, Immortalized mouse cells were treated with 1 μM MMC for 24 h, allowed to recover for the indicated times (in hours), and stained for γH2AX. f, Quantification of percentage of positive cells from e. g, RPA32 and RAD51 foci formation in U2OS cells ± 1 μM MMC for 24 h subjected to control and HELQ siRNA.
Extended Data Figure 8 | Homologous recombination dynamics and PARP inhibitor sensitivity. a, U2OS cells treated with or without 100 ng ml⁻¹ MMC for 24 h, allowed to recover for the indicated times (in hours), fractionated and probed for RAD51. H4 and α-tubulin are shown as controls for chromatin fractionation. b, DR–GFP reporter cells treated with the indicated siRNAs were probed for BRCA2, HELQ and RAD51; transcription factor IIH (TFIIH) is shown as a loading control. c, siRNA-treated U2OS cells were plated for clonogenic survival and treated with the indicated reagents.