WRN helicase is a synthetic lethal target in microsatellite unstable cancers

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Synthetic lethality—an interaction between two genetic events through which the co-occurrence of these two genetic events leads to cell death, but each event alone does not—can be exploited for cancer therapeutics. DNA repair processes represent attractive synthetic lethal targets, because many cancers exhibit an impairment of a DNA repair pathway, which can lead to dependence on specific repair proteins. The success of poly(ADP-ribose) polymerase 1 (PARP-1) inhibitors in cancers with deficiencies in homologous recombination highlights the potential of this approach. Hypothesizing that other DNA repair defects would give rise to synthetic lethal relationships, we queried dependencies in cancers with microsatellite instability (MSI), which results from deficient DNA mismatch repair. Here we analysed data from large-scale silencing screens using CRISPR–Cas9-mediated knockout and RNA interference, and found that the RecQ DNA helicase WRN was selectively essential in MSI models in vitro and in vivo, yet dispensable in models of cancers that are microsatellite stable. Depletion of WRN induced double-stranded DNA breaks and promoted apoptosis and cell cycle arrest selectively in MSI models. MSI cancer models required the helicase activity of WRN, but not its exonuclease activity. These findings show that WRN is a synthetic lethal vulnerability and promising drug target for MSI cancers.

Defects in DNA mismatch repair (MMR) promote a hypermutable state with frequent insertion and/or deletion mutations that occur in nucleotide repeat regions—which are known as microsatellites. This class of hypermutations—MSI—contributes to occurrence of several cancers, predominantly in colon (15%)4, gastric (22%)5, endometrial (20–30%)6 and ovarian (12%)7 cancers. MSI can arise from Lynch syndrome4, which is caused by germline mutations in the MMR genes MSH2, MSH6, PMS2 or MLH1. More commonly, MSI cancers arise after somatic MMR inactivation, typically through hypermethylation of the MLH1 promoter4. Although MSI has been associated with notable responses to immune checkpoint blockade, 45–60% of such cancers do not respond to immune checkpoint blockade, and the use of immune checkpoint blockade can be limited by toxicity8,9. Thus, novel therapies are needed for tumours with MSI.

Hypothesizing that MSI and MMR deficiency may create vulnerabilities, we analysed two independent large-scale cancer dependency datasets, project Achilles and project DRIVE, for genes that are selectively essential in cancer cells with MSI (Fig. 1a). Project Achilles screened 517 cell lines with a genome-scale CRISPR–Cas9 library and project DRIVE analysed 398 cell lines using an RNA interference library to define genes that were essential for proliferation and survival of individual cancer cell lines10,11. We ascertained MSI status using next-generationsequencing12 to quantify the number and fraction of deletions that were located within microsatellite regions, identifying three groups: MSI, microsatellite stable (MSS) and indeterminate lines (Fig. 1b and Supplementary Table 1). These classifications were highly concordant with PCR-based MSI phenotyping13 and with predicted MMR deficiency (Extended Data Fig. 1a). In total, 51 unique MSI and 541 unique MSS cell lines (excluding those lines marked as indeterminate) were represented by one or both screening datasets.

Projects Achilles (using CRISPR–Cas9) and DRIVE each independently identified WRN, which encodes a RecQ DNA helicase, as the top preferential dependency in MSI compared to MSS cell lines (Q = 4.8 × 10−24 and 1.5 × 10−45, respectively; Fig. 1c). These findings remained true with PCR-based MSI classifications (Extended Data Fig. 1b). By contrast, none of the four other RecQ DNA helicasess were preferentially essential in MSI cell lines (Extended Data Fig. 1c). We then evaluated MSI as a biomarker for WRN dependency, demonstrating that the MSI–WRN relationship compared favourably to other strong biomarkers for vulnerabilities such as the relationships between activating KRAS and BRAF mutations and KRAS and BRAF dependencies, respectively (Extended Data Fig. 1d, e).

MSI is most commonly observed in colorectal, endometrial, gastric and ovarian cancers. MSI cell lines from these four lineages (n = 37) showed greater dependence on WRN than their MSS counterparts (n = 91; P = 4.2 × 10−13; Wilcoxon rank–sum test; Extended Data Fig. 2a). We also identified 14 MSI cell lines from lineages in which MSI was less common (six leukaemia lines, two prostate cancer lines and single models of other lineages). However, these MSI cell lines were distinct and contained a median 0.56-fold fewer deletion mutations in microsatellite regions compared to typical lineages (P = 1.7 × 10−9; Extended Data Fig. 2b). The lines from lineages in which MSI was less common were also less dependent on WRN (P = 1.1 × 10−5; Extended Data Fig. 2c), despite possessing events that are predictive of MMR deficiency (Supplementary Table 1). Correspondingly, the specificity of MSI as a biomarker for WRN dependency was improved by delineating MSI within MSI-predominant lineages (Extended Data Fig. 1d, e).

These observations suggest that WRN dependency is not simply a result of MMR deficiency but may require specific lineages and/or a stronger mutation phenotype. Indeed, WRN dependency correlated with the number of microsatellite deletions within all MSI cell lines and in MSI-predominant lineages (Spearman’s ρ = −0.74, n = 54, P < 2.2 × 10−16;
Spearman’s \( \rho = -0.57, n = 37, P = 3.3 \times 10^{-4} \), respectively; Extended Data Fig. 2c, d).

To further assess WRN dependency, we validated three single-guide RNAs (sgRNAs) that targeted WRN using immunoblots (Extended Data Fig. 3a) and evaluated WRN knockout in five MSS and five MSI cell lines, all of which were from MSI-predominant lineages, with an eight-day viability assay. Effects of WRN knockout were comparable to ‘pan-essential’ controls in MSI cell lines. WRN silencing in MSS models instead approximated negative controls, in which intergenic regions were targeted (Fig. 2a). Similarly, WRN depletion impaired the viability of MSI cells despite negligible effects in MSS cells in a 10-day competitive growth assay (Extended Data Fig. 3b). Complementing these CRISPR – Cas9 data, WRN silencing with short hairpin RNA (shRNA) impaired cell viability in MSI, but not MSS, cells (Extended Data Fig. 3c, d). To validate that these phenotypes were attributable to WRN inactivation, we developed an sgRNA that targets a WRN exon-intron junction (WRN EIJ sgRNA), which silenced endogenous WRN, but not exogenous WRN cDNA (Fig. 2b). Correspondingly, WRN cDNA rescued Cas9-expressing KM12 cells from WRN EIJ sgRNA, but not WRN sgRNA 2, which targets endogenous and exogenous WRN (Fig. 2c). These data indicate that the viability loss in MSI cells is attributable to WRN inactivation.

These findings suggest that WRN dependence with MSI could be exploited by WRN inhibition. WRN functions as both a 3′–5′ exonuclease and 5′–3′ helicase in processes such as DNA repair, DNA replication and telomere maintenance.\(^1\) To determine which enzymatic function is essential, we attempted to rescue WRN EIJ sgRNA using exonuclease-dead (E84A), helicase-dead (K577M) or dually exonuclease- and helicase-dead (E84A/K577M) versions of WRN cDNA in KM12 cells. Exonuclease inactivation did not attenuate rescue, suggesting this function is dispensable. By contrast, helicase inactivation prevented rescue (Fig. 2b, c), indicating that the helicase domain is a candidate therapeutic target.

Next, we validated WRN dependency in vivo using xenografts of KM12 cells transduced with doxycycline-inducible shRNAs that target WRN (WRN shRNA 1) or control (WRN-C911 shRNA 1) in which nucleotides 9–11 of WRN shRNA 1 were mutated to the complementary nucleotides, thus maintaining the ‘seed’ sequence associated with shRNA off-target effects.\(^6\) Induction of WRN shRNA 1 but not WRN-C911 shRNA 1 significantly impaired tumour growth (Fig. 2d). We observed substantial depletion of WRN after induction of WRN shRNA 1 in vivo. However, WRN levels recovered by three weeks (Fig. 2e), mirroring tumour growth recovery, suggesting that WRN re-expression was required to overcome induction of WRN shRNA 1. We further demonstrated that an shRNA that targets WRN impaired the viability of a newly generated patient-derived organoid that was generated from a colon cancer with MSI (Fig. 2f, g). By demonstrating the synthetic lethal relationship in vivo and in a patient-derived model, these data support that WRN is potential a therapeutic target for cancers with MSI.

To pursue the basis of WRN dependency, we first performed cell cycle analyses. WRN silencing reduced the proportion of MSI cells in S phase and increased the number of cells in G1 or G2/M phases, suggesting cell cycle arrest at either G1 or G2/M phases (Extended Data Fig. 4a, b). Furthermore, analyses of annexin V and propidium iodide staining show the induction of apoptosis and cell death in MSI cells after WRN silencing (Extended Data Fig. 4a, c, d). By contrast, MSS cell lines showed no significant evidence of increased cell cycle arrest nor cell death after WRN silencing. Consistent with the cell cycle and apoptosis assays, mRNA-sequencing analysis of WRN silencing in MSI cells revealed downregulation of genes associated with G2/M checkpoint progression and E2F target signatures, and upregulated signatures of apoptosis. This analysis additionally revealed p53 activation in WRN-depleted MSI cells (Fig. 3a). Immunofluorescence imaging of proteins in MSI models after WRN silencing showed an increase in phosphorylated (phospho-)p53 (S15), which indicates activation of p53.\(^7\) By contrast, we observed
substantially weaker changes in phospho-p53 intensity in WRN-depleted MSI models (P < 2 × 10^{-16}, contrast mean of test of mean fold change in MSS versus MSI lines; Fig. 3b, c and Extended Data Fig. 5a, b). WRN depletion in TP53 wild-type MSI cells increased protein levels of the cyclin-dependent kinase inhibitor p21, which is another indication of p53 activation. By contrast, there were significantly weaker changes in p21 expression after WRN silencing in MSS and TP53-mutant MSI cells (P < 2 × 10^{-16}, fold change in MSI TP53 wild-type versus MSS or TP53-null cells; Extended Data Fig. 5c–f). We then re-evaluated our dependency data, stratifying by MSI and p53 status. Whereas p53-intact MSI cell lines (n = 23) were more sensitive to WRN loss than their p53-impaired (n = 13) counterparts (P = 0.02, Wilcoxon rank-sum test; MSI cell lines from lineages in which MSI were common only), both wild-type and mutant TP53 MSI cell lines were dependent on WRN (Extended Data Fig. 5g). These data suggest that although WRN loss leads to p53 induction, p53 activity contributes to, but is not solely responsible for, WRN dependence.

The finding of increased p53 (S15) phosphorylation, a phosphorylation-target of DNA-damage response kinases ATR and ATM, suggested that WRN loss with MSI leads to DNA damage. This hypothesis is consistent with the roles of p53 and WRN in responding to DNA damage and preserving DNA integrity. Indeed, biallelic germline inactivation of WRN causes Werner syndrome, which is characterized by premature aging and an increased incidence of cancer due to impaired DNA damage repair and telomeric shortening, which leads to chromosomal aberrations. Next we show that WRN silencing in MSI, but not MSS, cells substantially increased γ-H2AX and 53BP1 foci, which are markers of double-stranded DNA breaks (DSBs) (Fig. 4a–c and Extended Data Fig. 6a–h). These findings were corroborated by increased formation of phospho-ATM (S1981) and phospho-CHK2 (T68) phosphorylation, which are indicative of DSB responses that are known to activate p53 and anti-proliferative signalling pathways (Extended Data Fig. 7a–e). We confirmed increased γ-H2AX in MSI cells treated with an shRNA against WRN in vitro and in vivo, suggesting that DSBs are not just a consequence of CRISPR–Cas9 activity (Fig. 2e and Extended Data Fig. 7e). These observations also explain why p53-impaired MSI cells are sensitive to WRN depletion as DSBs are toxic to cells, independent of p53 status.

To evaluate chromosomal integrity with WRN silencing, we examined metaphase spreads of two MSI and two MSS cell lines with telomere fluorescence in situ hybridization (FISH), 96 h after induction of WRN shRNA. First, we investigated whether a telomere defect precipitates the synthetic lethal relationship given the role of WRN in protection of the telomeres. WRN silencing induced DSBs and fragmentation throughout chromosomes in MSI cells, but not in MSS cells. However, we did not observe specific telomeric defects such as increased chromosomal end-to-end fusions or telomeric signal loss. Although we observed many MSI cells with chromosomal fragmentation after WRN silencing, we also identified MSI cells that had only a few DSBs (Fig. 4d, e and Extended Data Fig. 8a). Because this assay requires viable cells to cycle into metaphase, these data suggest that DSBs cause the lethal effects of WRN loss and are not merely a consequence of cell death.

Because our FISH data revealed diffuse DSBs in WRN-depleted MSI cells, we hypothesized that loci throughout chromosomes require WRN to maintain genomic stability with MSI. To support this hypothesis, we

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**Fig. 2 | WRN is a synthetic lethal partner with MSI.** a, Relative viability 8 days after sgRNA transduction. Negative controls targeting chromosome 2 intergenic sites: Chr.2-2 sgRNA, Chr.2-4 sgRNA. Pan-essential controls: POLR2D sgRNA, MYC sgRNA. WRN sgRNAs: WRN sgRNA 1, WRN sgRNA 2, WRN sgRNA 3. The following cell lines were used: ES2 (ovary, MSS), SW620 (colon, MSS); SW837 (colon, MSS); GCIY (stomach, MSS); HEC50B (endometrium, MSS); OVK18 (ovary, MSI); KM12 (colon, MSI); SW48 (colon, MSI); SNU1 (stomach, MSI); SNGM (endometrium, MSI). P values compare indicated sgRNAs against negative controls (Chr.2-2 and Chr.2-4 sgRNAs). b, Left, immunoblot. WRN and GAPDH levels in KM12 cells expressing the indicated WRN cDNA. WRN(E84A), exonuclease-dead mutant; WRN(K577M), helicase-dead mutant; WRN(E84A/K577M), double mutant. Right, immunoblot. WRN levels following sgRNA transduction. c, Relative viability 9 days after sgRNA transduction in KM12 cells expressing Cas9 and GFP or the indicated WRN cDNA. KM12 xenograft growth with or without doxycycline induction of WRN shRNA 1 or seed control (WRN-C911 shRNA 1). d, Representative images (f) and viability (g) of CCLF_CORE_0001_T 9 days after shRNA induction relative to no doxycycline treatment. Scale bar, 200 μm. Data are mean ± s.e.m. (a, c, d, g). n = 3 (a) or 6 (c) biological replicates; 5 (4 from day 15–18) and 5, 4 and 4 tumours for WRN shRNA 1 without and with doxycycline, WRN-C911 shRNA 1 without and with doxycycline, respectively (d), 2 biological replicates with 3 technical replicates each (g). P values were analysed using two-way analysis of variance (ANOVA) between WRN sgRNAs and negative controls (a), two-tailed Student’s t-test for WRN EIJ sgRNA values between mock and indicated WRN cDNA (c), likelihood ratio test comparing growth rates with and without doxycycline (d), two-tailed Student’s t-test between WRN shRNA and its corresponding seed control (g). Representative data from one experiment are shown. All experiments were performed three times, except for experiments in e–g and d, which were performed twice and once, respectively.
analysed the localization of WRN using immunofluorescence microscopy. After demonstrating the specificity of WRN immunofluorescence staining (Extended Data Fig. 8b), we observed predominantly dispersed WRN staining across the nucleoplasm in MSI cells, whereas greater co-localization of WRN with the nucleolar marker fibrillarin (Extended Data Fig. 8b–d) and less nucleoplasmic staining was found in MSS cells. Because WRN has been demonstrated to respond to DNA damage by disseminating from the nucleolus towards the nucleoplasm\(^{20}\), these data suggest that WRN is recruited to maintain genomic integrity in MSI cells.

We next evaluated the relationship between MMR deficiency and WRN dependency. We first investigated whether MSI leads to recurrent mutations and inactivation of another gene, creating a dependency on WRN. The second most significant dependency with MSI was RPL22L1, a dependency described in MSI that is attributable to an inactivating mutation of its parologue, RPL22\(^{21}\) (Fig. 1c). Analysing our screening dataset, we found no gene for which the loss could account for the preferential dependency on WRN of cells with MSI (Extended Data Fig. 9a). We also found no increased dependence on WRN in cell lines with hypermutations that are due to mutations in polymerase epsilon (POLE)\(^{22}\) (Extended Data Fig. 9b and Supplementary Table 1), suggesting that hypermutability alone cannot account for WRN dependency.

We then explored whether MMR deficiency contributes to WRN dependence using a model in which MMR activity of the MSI colorectal cell line HCT116—in which MLH1 and MSH3 are mutated—was restored by introducing chromosomes 3 and 5 (Chr.3+5), which include MLH1 and MSH3, respectively\(^{22}\) (Extended Data Fig. 10a). WRN knockdown led to γH2AX accumulation and impaired viability of parental HCT116 and control cells in which an additional chromosome 2 was introduced. By contrast, Chr.3+5 transfer suppressed

Fig. 3 | WRN depletion in MSI cells induces cell cycle arrest, apoptosis and a p53 response. a, Enrichment or depletion scores from gene set enrichment analyses in WRN-depleted OV18 cells plotted against WRN-depleted SW48 cells. Signature enrichment plots for hallmark gene sets are shown for WRN-depleted OV18 and SW48 cells. n = 2 biological replicates. b, Phosphorylated p53 (S15) immunofluorescence analysis after sgRNA transduction. Scale bar, 50 μm. c, Nuclear phospho-p53 (S15) staining intensity per cell. The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively; the bar in the middle of the box represents the median value and the whiskers represent the 1st and 99th percentiles. Outliers are represented as dots. Mean log(change in intensity) following WRN knockout compared to control sgRNA in MSI versus MSS cells; \(P < 2 \times 10^{-16}\), contrast test of least-squares means. Mean fold change (calculated as log(change in intensity)): 0.21 (KM12), 0.10 (SW48), 0.034 (SW620). n indicates the number of cells (treated with Chr.2-2 sgRNA, WRN sgRNA 2, WRN sgRNA 3, respectively) for KM12 (7,080, 14,410, 15,921), SW48 (15,329, 9,491, 13,196), SW620 (27,374, 23,898, 28,808). Representative data from one experiment are shown. All experiments were performed twice, except for experiments in a, which were performed once.

Fig. 4 | WRN depletion in MSI cells leads to accumulation of DSBs. a, Immunoblot. γH2AX, phospho-CHK2 (T86) and total CHK2, WRN and GAPDH levels following WRN knockout. Etoposide and hydroxyurea were used to generate DSBs and replication stress, respectively. b, γH2AX immunofluorescence following sgRNA transduction. Scale bar, 50 μm. c, Nuclear γH2AX staining intensity per cell. The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively; the bar in the middle of the box represents the median value and the whiskers represent the 1st and 99th percentiles. Outliers are represented as dots. Mean log(change in intensity) following WRN knockout compared to control in MSI compared with MSS cells; \(P < 2 \times 10^{-16}\), contrast test of least-squares means. Mean fold change (calculated as log(change in intensity)): 0.39 (KM12), 0.33 (SW48), −0.10 (SW620). b indicates the number of cells (treated with Chr.2-2 sgRNA, WRN sgRNA 2, WRN sgRNA 3, respectively) for KM12 (3,029, 8,880, 6,887), SW48 (13,246, 4,553, 7,216), SW620 (9,071, 5,174, 3,853). d, Telomere PNA-FISH of metaphase spreads with and without doxycycline induction of WRN shRNA 1. Hollow arrowhead indicates a chromosomal break. Filled arrowheads indicate chromosomal fragments. e, Metaphase spread of the pattern of DNA damage per cell. n = 2 independent experiments presented in tandem. f, Relative viability of HCT116 with and without MMR restoration 7 days after shRNA transduction. Negative control: RFP shRNA. Pan-essential controls: PSM2d shRNA, RPS6 shRNA. WRN shRNA: WRN shRNA 1, WRN shRNA 2. Data are mean ± s.e.m. (n = 6 biological replicates). P values were analysed by two-tailed Student’s t-test for the percentage of cells with DNA damage (e), two-way ANOVA between WRN shRNAs and RFP shRNA (f). In a–d, f, representative data from one experiment are shown. In e, data from two independent experiments are shown. All experiments were performed twice.
DBS accumulation and partially rescued viability from WRN shRNA treatment in a seven-day viability assay (Fig. 4f and Extended Data Fig. 10b). The rescue was indeed attributable to MMR restoration as CRISPR–Cas9 knockout of MLH1 sensitized HCT116 cells that possess additional Chr.3–5 to WRN silencing and induced DSBs after WRN silencing (Extended Data Fig. 10c, d). Longer-term clonogenic assays with HCT116 cells established that rescue of WRN dependency is modest with MMR restoration, but abolished with MLH1 knockout (Extended Data Fig. 10e, f).

These data suggest that MMR deficiency alone contributes to the synthetic lethal interaction that we found, although it does not fully explain this interaction, suggesting that genomic lesions that accumulate with MSI promote WRN dependence. The potential relevance of accumulation of genomic lesions is consistent with our observation that MSI cells with fewer microsatellite deletions are less dependent on WRN. Accumulating MSI defects could cooperate with MMR deficiency to form genomic structures that require WRN to resolve. Such structures could include insertion–deletion loops and/or displacement loops (D-loops) between homologous DNA sequences, which are known substrates for MMR machinery. Indeed, MMR deficiency in yeast creates a dependency on Sgs1, which is a homologue of WRN and BLM, to resolve homologous D-loops that are normally rejected by MMR. Beyond the potential role of WRN in preventing DNA damage, loss of WRN in non-homologous end joining and/or homologous recombination could further contribute to the accumulation of DSBs.

Our observations reveal that WRN inactivation induces DSBs and activates DSB responses to promote cell death and cell cycle arrest preferentially in MSI cells. Although WRN and other DNA helicases have been nominated as therapeutic targets, our study highlights WRN as a synthetic lethal target in cancer cells with MSI, defining a population in which a WRN-based therapeutic could be used. Further studies will be needed to further explore the intersecting roles of MMR deficiency and genomic lesions in MSI with WRN dependence. Although systemic WRN inhibition could induce complications akin to Werner syndrome, the manifestations of this syndrome require decades to emerge, suggesting that therapeutic benefits would greatly outweigh risks. Results from this study and the companion study by Behan et al. support efforts aimed at designing WRN helicase inhibitors to exploit WRN dependency in MSI cancers. More broadly, our findings highlight the power of large-scale cancer profiling efforts to identify cancer vulnerabilities and therapeutic biomarkers, illustrating how a cancer dependency map can accelerate the development of precision therapy for patients with cancer.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-1102-x.

Received: 7 August 2018; Accepted: 27 February 2019; Published online 10 April 2019.

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Acknowledgements We thank C. R. Boland, A. Goel, M. Koi, R. J. Monnat and R. Weissleder for reagents; A. Tang for graphical assistance; and P. Montgomery for the website. This work was funded by The Carlos Slim Foundation/Slim Initiative for Genomic Medicine, Broad Institute, Team MG, NIH (2T32CA009172-42A1 to E.M.C.) and the Irving W. Janickow Fellowship (to E.M.C.).

Author contributions E.M.C., T.S., F.V. and A.J.B. initiated the project, and designed and supervised the research plan. J.M.M., M. Ghandi, Y.L. and Y.E.M. performed computational analysis of the CCLE and cancer dependency datasets under the supervision of D.E.R., J.S.B., G.G., T.R.G., A.T., F.V. and E.M.C. T.S., B.G. and J.S.M., performed the viability experiments to validate the cancer dependency dataset findings with help from M.S., A.A., S.O.A. and L.L. The rescue experiments with WRN overexpression were performed by E.M.C. and B.G. The HCT116 viability experiments were performed by T.S. and analysed by Y.E.M. and M.I. M.I., J.S.M. and T.S. performed and analysed the cell cycle and apoptosis assays. Immunoblotts were performed by T.S., E.M.C. and J.S.M. Immunofluorescence experiments were performed by T.S., E.M.C., J.B.L., J.-B.A.R., H.A.R. and E.R. and analysed by T.S., E.M.C. and J.B.L. J.-B.A.R. performed, M.A. and A.D.A. performed the telomere PNA-FISH experiment. Z.D.N. and C.G.P. performed the fluorescence-based flow-cytometric host-cell reactivation assay. E.M.C., T.S., J.M.M., F.V. and A.J.B. wrote the manuscript. All the authors edited and approved the manuscript.

Competing interests A.J.B. receives research funding from Merck and Novartis. D.E.R. receives research funding from the Functional Genomics
Consortium (Abbvie, Janssen, Merck and Vir) and is a director of Addgene. A.T. consults for Tango Therapeutics. T.R.G. has advised Foundation Medicine, GlaxoSmithKline, Sherlock Biosciences and Forma Therapeutics. A.D.A. consults for Lilly Oncology, EMD Serono, Intellia Therapeutics, Sierra Oncology, Formation Biologics and Cyteir Therapeutics, consults and holds stock in Ideaya, and co-founded and holds stock in Cedilla Therapeutics. G.G. receives research funding from IBM and Pharmacyclics and is an inventor on multiple patent applications related to bioinformatics tools, including applications related to MuTect, ABSOLUTE, MSMuTect, MSMutSig and MSIClass. Y.E.M. is an inventor on patent applications related to the bioinformatics tools MSMuTect, MSMutSig and MSIClass. The Broad Institute, on behalf of E.M.C., T.S., J.M.M., M. Ghandi, F.V. and A.J.B., filed a US patent application related to the target described in this manuscript.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41586-019-1102-x.
Supplementary information is available for this paper at https://doi.org/10.1038/s41586-019-1102-x.
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METHODS

Genetic dependency data. CRISPR dependency data were taken from the 18Q4 Avana dataset. These data contain gene dependencies estimated for each gene and cell line using the CERES algorithm. RNA interference (RNAi) dependency data were derived from project DRIVE (Novartis) and were reprocessed using the DEMETER2 algorithm, which can be downloaded from the DEMETER2 figshare repo. For some analyses (for example, Extended Data Figs. 1e, 2c, d, 5g, 9b), we computed aggregate WRN dependency scores for each cell line by averaging together RNAi and CRISPR dependency scores (which are both normalized so that the median score of pan-essential genes is set at −1) .

For Extended Data Figs. 1c, 2a, 5g, 9b, the lower and upper parts of the boxes correspond to first and third quartiles (25th and 75th percentiles). The upper and lower whiskers extend to the largest and smallest value within 1.5 × the interquartile range (IQR) from the box. Gene dependency scores are normalized such that a value of 0 represents the median dependency score of negative control genes and −1 represents the median dependency score of sgRNAs that target pan-essential genes.

Genomics data. Cancer cell line genomic data used in the analysis, including gene-level mRNA expression, gene-level relative copy number and mutation calls, were taken from the DepMap 18Q4 data release. Protein abundance data of reverse-phase protein arrays (RPPA) were taken from the Cancer Cell Line Encyclopedia (CCLE) . All cell line omics data can be downloaded at DepMap (https://depmap.org/portal/).

Cell line annotations. Annotations of primary disease site for each cell line can found in the DepMap 18Q4 data release. The functional status of TP53 in 966 cell lines was annotated based on a combination of the mutlin-3 sensitivity of the cell lines (Genomics of Drug Sensitivity in Cancer; http://cancerrxgene.org) and Cancer Target Discovery and Development (CTDD - https://ogc.cancer.gov/programs/cdd2/dataportal) data sets, along with a p33 target gene expression signature computed from CCLE data.

Microsatellite classification. MSI classifications were obtained from phase II of the CCLE project. These classifications were based on the total number of deletions detected in each cell line, and the fraction of deletions in microsatellite regions, using several different data sources (CCLE whole-exome sequencing, CCLE whole-genome sequencing, CCLE hybrid capture and Sanger whole-exome sequencing datasets). These features were then used to classify each cell line as MSI, MSS or indeterminate. Unless otherwise indicated, our analysis excluded cell lines classified as indeterminate. When plotting the number of microsatellite deletions and fraction of deletions in microsatellite regions (Fig. 1b and Extended Data Fig. 1a), we averaged these values across the data sources available for each cell line after normalizing for systematic differences between data sources. Specifically, we used linear regression models to estimate and remove scale and offset differences between data sources so that the normalized number of deletions (and number of deletions in microsatellite regions) measured in each data source was equal on average across the average number of microsatellite deletions and fraction of deletions in microsatellite regions are provided in Supplementary Table 1.

MMR status. MMR status was determined based on omics data for the genes MSH2, MSH6, MLH1 and PMS2. For each gene, we determined whether it was mutated (any detected mutation classified as deleterious), deleted (relative log2 copy number −1) or lowly expressed (log2 (mRNA expression in transcripts per million) < 1). A gene was classified as inactivated if any of the above criteria were met, and cell lines in which any of these MMR genes were inactivated were classified as having ‘MMR loss’. The MMR status for cell lines for which we did not have either gene expression or copy number data available were classified as ‘NA’. In addition, we annotated MSI cell lines as having low protein expression of MSH2 and MSH6 if they had protein levels −1 in the RPPA data (Supplementary Table 1). However, RPPA data were not used for calling MMR loss, as we were missing RPPA data for many cell lines, and MMR loss was detectable from other omics data in most cases. We also classified the mutation status of POLE for all cell lines as ‘damaging’, ‘hotspot missense’ or ‘other’ using the ‘Variant_Annotation’ column of the CCLE mutation file.

Differential dependency analysis. Genes that were preferentially dependent in MSI compared to MSS cell lines were identified using linear modelling performed in parallel across genes using the R package Limma. We estimated the difference in mean dependency between MSS and MSI cell lines for each gene, and associated P values were derived from empirical-Bayes-moderated t-statistics. Q values were computed using the Benjamini–Hochberg method.

Dependency and biomarker analysis. To compute the predictability of gene dependencies from different biomarkers, we first called each cell line as dependent or not, by thresholding the average of the CRISPR- and RNAi-based dependency scores at a value of −0.5. We then computed the positive predictive value and sensitivity of each biomarker and associated P values and areas under the curve. To assess the independent contribution of any particular gene could account for WRN dependency, we determined the functional status of each gene (loss or not) in the same way as described above for MMR gene loss. For each gene, we then used a linear regression model to assess the contribution of both MSI status and loss of the gene to predicting WRN dependency (average of CRISPR and RNAi dependency scores). In Extended Data Fig. 9a, we plot the linear model coefficients, and associated P values, for MSI status accounting for loss of each gene.

mRNA sequencing. Cas9-expressing cells (SW48 and OVK18) were lentivirally transduced with the following sgRNAs: Chr.2-2 sgRNA, WRN sgRNA 2 and WRN sgRNA 3 (sequences provided below). Cells were selected with puromycin and RNA was purified 72 h after transduction. This was performed in duplicate before cDNA library preparation and subsequent RNA sequencing using the Illumina NextSeq 500 performed by the Molecular Biology Core Facilities at the Dana–Farber Cancer Institute (DFCI). This experiment was performed once with two biological replicates.

Differential expression analysis. We first excluded genes that had less than one count per million in more than half of the samples. The weighted trimmed mean of M values method was used to normalize the library-wide, using the calcNormFactors function from the R package edgeR. To estimate the fold change effect of WRN knockout (calculated as log(knockout/control)) on each gene in each cell line, we used the R package Limma. Specifically, we fit a linear model for the expression of each gene, using cell line and sgRNA (WRN versus control) as covariates. Read count data were transformed using the Limma function ‘voom’ before model fitting, in order to model the mean–variance relationship of the log(counts) data. We then extracted fold change effect sizes and empirical-Bayes-modulated t-statistics for the WRN knockout effect for each gene and cell line. Gene set enrichment analysis (GSEA) was run to test for gene sets that were up- or downregulated in each cell line after WRN knockout. In particular, we used the R package gsease to estimate normalized enrichment statistics, and associated P values, for each gene set in the Hallmark Collection from the Molecular Signatures Database. The GSEA algorithm was run using t-statistics as the gene-level statistics. 1 million random permutations for each cell line tested, and a ‘GSEA parameter’ of 1.

Cell lines and reagents. The ES2, OVK18, SW620, SW837, KM12, SW48, GCiY, SNU1, HEC50B and SNGM cell lines were collected by the CCLE before distri-bution for our use. The sources of the aforementioned cell lines can be found at DepMap.org and are as follows. The ES2, SW620, SW837, SNU1 and SW48 cell lines were originally obtained from the American Type Culture Collection (ATCC). The KM12 cell line was originally obtained from an academic laboratory and can be obtained as part of the NCI-60 cell lines. The GCiY and OVK18 cell lines were originally obtained from the RIKEN Cell Bank. The SNGM and HEC50B cell lines were originally obtained from the Health Science Research Resources Bank. Their identities were confirmed by SNP microarray. The HCT116 cell line and its various derivatives were provided by C. R. Boland, A. Goel and M. Ko. The aforementioned cell lines can be obtained from their respective sources. All cell lines were grown in medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 μg ml−1)–streptomycin (100 μg ml−1)–l-glutamine (292 μg ml−1; Gibco) unless otherwise stated. KM12, SW48, SW837, ES2, and SNU1 cells were cultured in RPMI-1640 (Gibco); OVK18 cells were cultured in MEM; GCiY cells were cultured in MEM supplemented with 15% FBS; SW620 cells were cultured in Leibovitz’s L-15 (Gibco); SNGM cells were cultured in Ham’s F12 with 20% FBS (Gibco); HEC50B cells were cultured in EEMEM (ATCC) supplemented with 15% FBS; HCT116 cells were cultured in McCoy’s 5A (Gibco); Stable Streptococcus pyogenes Cas9-expressing cell lines generated by lentiviral transduction of the pXPR_BRD111 construct were from project Achilles. The pXPR_BRD111 is available from Addgene (plasmid 78166).

CCLF_CORE_0001_T was obtained from a 58-year-old female patient with stage II MSI-H colon carcinoma (determined by loss of MLH1 and PMS2 with MLH1 promoter methylation using immunohistochemistry). All samples were obtained with informed consents of the patients at the DFCI. All procedures were conducted under a protocol approved by the DFCI Institutional Review Board. Samples were collected by translational research staff and labelled with a unique identifier before samples were processed and analysed in the laboratory. Per the DFCI IRB protocol, the link to protected health information was maintained within a database with access limited to select research staff. Samples were handled according to the medical ethical guidelines described by the Dana–Farber/Harvard Cancer
Center Office for Human Research Studies. Patient tumour resections were placed in a sterile conical tube containing DMEM medium (Thermo Fisher Scientific, 11995073) with 10% FBS (Sigma-Aldrich, F8317), 1% penicillin–streptomycin (Thermo Fisher Scientific, 15140163), 10 μg/mL of gentamicin and 250 ng/mL fungizone on wet ice during transport from the operating room to the research laboratory. Resections were placed in a 15-ml conical flask with 5 ml DMEM medium, 10% FBS, 1% penicillin–streptomycin and the digestion enzymes regular collagenase 1 mL (StemCell, 07912) and dispase 1 ml (StemCell Technologies, 07913). The flask was placed on a rotator and incubated at 37 °C for 1 h. The cells were then centrifuged at 1,000 r.p.m. for 5 min. Cell pellets were resuspended and later embedded into Matrigel (Corning, 356231) as per the previous published protocol44. Colorectal carcinoma organoids were passaged using iced-cold PBS and Gibco TrypLE Express (Thermo Fisher Scientific, 12604039) when the cells reached 80–90% confluence. CCLF_CORE_0001_T will be deposited to a third-party distributor at a later date.

All cell lines tested negative for mycoplasma.

Generation of ectopic WRN cDNA-expressing cell lines. The catalytically active version, exonuclease-dead (E48A), helicase-dead (K577M) and dually exonuclease and helicase-dead (E48A/K577M) versions of WRN cDNA were a gift from R. J. Monnat (Addgene, plasmids 46038, 46036, 46035 and 46037, respectively). The missense mutant forms of WRN have been previously demonstrated to lack their indicated enzymatic activity44. The WRN sequence was cloned into a modified lentiviral expression vector, pLX_TRC209, under an RRE promoter and modified to contain a neomycin-selectable marker. Sanger sequencing of the vectors and genomic DNA after integration were performed to confirm sequence identity. Lentiviruses were produced as described below and transduced into dual Cas9 Firefly-luciferase-expressing KM12 cells to create stable ectopic WRN cDNA-expressing cell lines.

Lentiviral production. Lentiviral production was performed using HEK293T cells as described on the GPP portal (https://portals.broadinstitute.org/gpp/public/).

sgRNAs. sgRNAs used in the validation studies were designed using the Broad Institute Genetic Perturbation Platforms sgRNA Designer (https://portals.broadinstitute.org/gpp/public/analysis-tools-sgna-design). sgRNAs targeting WRN include WRN sgRNA 1 (target sequence GTAAATGGAAAACCACCGG), WRN sgRNA 2 (ATCGTGAGGACACATCATG), WRN sgRNA 3 (GTACAGTAAGTTGACACG), sgRNA targeting the exon–intron junction (WRN EI) sgRNA were designed using the DESKGEN Cloud tool (https://www.deskgen.com/landing/cloud.html). The target sequence for WRN EI sgRNA is AGCACGTACATAAGCATCAG. Two negative controls targeting intergenic sites on chromosome 2 were used: Chr.2-2 sgRNA (GGTGTGCTATGAGCAGTG) and Chr.2-4 sgRNA (GGAGCTTCACCTCTGTACGT). Two pan-essential controls targeting POLR2D (AGAAGCTCGTCAATAGGCTCA) and MYC (AACAAGCTTGGACGCGCGCAG) were used. sgRNAs were inserted in the pXPR vector (BD Biosciences) under an RRE promoter and modified to contain a neomycin-selectable marker. Sanger sequencing of the vectors and genomic DNA after integration were performed to confirm sequence identity. Ectopic WRN cDNA-expressing cell lines were generated using 8-gene–modified lentiviral expression vector, pLX_TRC209, under an RRE promoter and modified to contain a neomycin-selectable marker. Sanger sequencing of the vectors and genomic DNA after integration were performed to confirm sequence identity. Lentiviruses were produced as described below and transduced into dual Cas9 Firefly-luciferase-expressing KM12 cells to create stable ectopic WRN cDNA-expressing cell lines.

Immunofluorescence. Immunofluorescence experiments were conducted essentially as described previously45 (except for the double staining for WRN and fibrillarin; see below). In brief, 2 days after lentiviral transduction, cells were seeded either on an eight-well Lab-Tek chamber slide (Thermo Fisher Scientific, 177402) or on a 96-well clear-bottom black polystyrene microplate (Thermo Fisher Scientific, 9904). The numbers of cells seeded per well for eight-well chambers (and 96-well plates) were as follows: 1 × 10^5 (5 × 10^4), 1.2 × 10^6 (6 × 10^5), 1.6 × 10^6 (8 × 10^5), 6 × 10^6 (3 × 10^5) and 8 × 10^6 (4 × 10^5) cells for SW620, KM12, SW48, ES2 and OV-K18 cells, respectively. Cells were fixed and stained 2 days later. Micrographic images were acquired using either epifluorescence microscopy (Fig. 3b and Extended Data Fig. 5a, c, e) or confocal microscopy (Fig. 4b and Extended Data Figs. 6b, e, g, 7a, c), which were performed on an Axio Observer Z1 microscope equipped with an Axiocam 506 monocamera and Apotome.2 (Zeiss) and a Zeiss LSM 700 laser scanning confocal system equipped with Axio Observer (Zeiss), respectively. These confocal microscopy images represent maximum intensity projections of five consecutive planes with a step size of 0.8 μm. For image quantification, images were acquired using an Opera Phenix High-Content Scanning System (PerkinElmer, HH14000000) and analyzed using Harmony High Content Imaging and Analysis Software (PerkinElmer, HH17000001). For phospho-p53, p21, -H2AX and phospho-ATM staining, signal intensities in the nucleus of at least 1,000 cells per sample were scored on background-subtracted images and presented as box plots. The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively, and the bar in the middle of the box represents the median value. The whiskers represent the 1st and 99th percentiles. Outliers are represented as dots. To score the patterns of nuclear staining, cells exhibiting a mean signal intensity of 12,000 or higher (for -H2AX, all cell types) (20,000 or higher for phospho-ATM, except for KM12 and SW48 cells, for which ‘40,000 or higher’ and ’10,000 or higher’ were used to identify cells with pan-nuclear phospho-ATM staining patterns, respectively) were first separated as cells with a pan-nuclear pattern of staining. For the rest of the cells, the number of foci within the nucleus was scored using a spot-detection program in the software. The relative abundance of cells that showed pan-nuclear staining and the relative abundance of cells that contained a specific number of foci were plotted. The numbers of nuclear foci that were observed in cells expressing the Apple–53BP1-truncu fluorescent marker were scored similarly (Extended Data Fig. 6f, h). To assess differences in pan-ATM staining between cell lines, intensity values in MSI versus MSS cell lines, we used a linear model approach. Specifically, linear models were fit to the log(intensity) values with guide and cell line intercept terms. Comparisons of the change in log(intensity) after WRN knockdown between cell lines were then obtained using the ‘contrast’ function of the R package lsmeans46.

Immunofluorescence analysis of WRN and fibrillarin was performed as previously described47. Images were obtained using the Zeiss LSM510 upright Confocal System (Extended Data Figs 8b, c). Weighted Pearson co-localization coefficients were calculated by obtaining z-stacks of five representative high-powered fields at 63 × magnification and scoring using the Zeiss Zen Blue software. Significance was calculated by two-tailed Student’s t-test for MSI cell lines compared to line - for the double staining for WRN and fibrillarin, the secondary antibody was goat anti-rabbit IgG, Alexa Fluor 488 (Thermo Fisher Scientific, A-11008) and goat anti-mouse IgG, Alexa Fluor 488 (Thermo Fisher Scientific, A-11001), which were used at a 1:2,000 dilution. For WRN and fibrillarin immunofluorescence experiments, goat-anti-mouse IgG, Alexa Fluor 488 (Thermo Fisher Scientific, A-11001) and goat anti-rabbit IgG, Alexa Fluor 555 (Thermo Fisher Scientific, A-21428) were used at 1:1,000 dilution. Following secondary antibody treatment, the nuclei were counterstained with 4′,6-diamidino-2′-phenylindole dihydrochloride (DAPI (Sigma-Aldrich, S9542), 1 μg mL–1). All immunofluorescence experiments were performed twice. Representative results from one experiment are shown.

Cell viability assay. Cas9-expressing versions of the following cell lines were seeded in 100 μl of medium in 96-well plates (Corning 3904) excluding edge wells

**Research Letter**
at the following densities: ES2, 10^4 cells per well; OVK18, 1.5 × 10^4 cells per well; SW620, 2 × 10^4 cells per well; KM12, 2 × 10^4 cells per well; SW837, 2.5 × 10^4 cells per well; SW48, 2.5 × 10^4 cells per well; GCIY, 2 × 10^4 cells per well; SNU1, 1.5 × 10^4 cells per well; HEC50B, 1.75 × 10^4 cells per well; SN6G, 1.5 × 10^4 cells per well. All cell lines, except SNU1, were seeded the day before transduction. SNU1 cells—a line grown in suspension—were seeded on the day of transduction with 4 μg/ml polybrene. For the adherent cell lines, the medium was changed to medium containing 10 μg/ml polybrene. Viral concentrations were titrated to achieve >90% infection efficiency. Experiments were performed in triplicate by adding the appropriate amount of lentivirus to integrate vectors that encoded the desired sgRNA and the plates were spun at 93 g for 2 h at 30°C. The medium was changed the next day and every 3 days thereafter. Cell viability was assayed using CellTiter-Glo (Promega G7572) at 33 μl per well. Luminescence was read using a PerkinElmer EnVision 2105. Values were normalized to the average values from the negative control sgRNAs for each cell line. Experiments were performed three times. The triplicate results of one representative experiment are shown. Two-way ANOVA was used to test differences between the set of WRN sgRNAs or WRN shRNAs and negative control(s), while modelling individual sgRNA/shRNA differences (Figs. 2a and 4f and Extended Data Fig. 10c).

For CCLF_CORE_0001_T, 5 × 10^5 cells were seeded into 40 μl Matrigel domes in a 24-well plate in quadruplicate, with two wells with and two wells without 0.2 μg/ml of doxycycline. Medium was refreshed every 2–3 days. Nine days after doxycycline treatment, cell viability was assessed by first aspirating the medium, adding 300 μl of CellTiter-Glo 3D (Promega G9681) and incubating at room temperature for 45 min on a shaker. Then, three aliquots of 90 μl were taken from each well and were transferred to a 96-well plate (Corning) for three technical replicates each from two biological replicates. Luminescence was read as above. Values are presented as the mean ratio of luminescence signal from the doxycycline-treated condition compared to the condition without doxycycline with error bars representing the s.e.m. unless otherwise stated. Two-tailed Student’s t-tests were performed when comparing single sgRNAs or WRN shRNAs and negative control(s), while modelling individual sgRNA/shRNA differences (Figs. 2a and 4f and Extended Data Fig. 10c).

Luciferase competitive growth assay. Dual Cas9–Firefly-luciferase- and Renilla-luciferase-expressing cells were generated by transduction of Firefly-luciferase cDNA and Renilla-luciferase cDNA, respectively, in a PLX_TRC313 lentiviral expression vector containing a hygromycin-resistance gene. After hygromycin selection, these two versions of the cell line were co-seeded in a 96-well plate with cDNA and Renilla-luciferase cDNA, respectively, in a pLX_TRC313 lentiviral vector. PerkinElmer EnVision 2105. Values were normalized to the average values from the negative control sgRNAs for each cell line. Experiments were performed three times. The triplicate results of one representative experiment are shown. Two-way ANOVA was used to test differences between the set of WRN sgRNAs or WRN shRNAs and negative control(s), while modelling individual sgRNA/shRNA differences (Figs. 2a and 4f and Extended Data Fig. 10c).

Clonogenic assay. Cells were transfected with lentivirus expressing indicated shRNAs. After 24 h, the medium was replaced with medium containing 2 μg/ml puromycin. After 24 h of puromycin selection, lentivirally infected cells were detached with trypsin and reseeded onto a 24-well plate. The number of cells seeded per well were as follows: 3 × 10^4, 4 × 10^4, 6 × 10^4, 1 × 10^5, 8 × 10^4, and 1.2 × 10^5 cells for ES2, OVK18, SW620, SW837, KM12, SW48 and HCT116 cells, respectively. Cells were subsequently propagated for 2 weeks in puromycin-free medium, which was changed every 3 days. For crystal violet staining, cells were fixed with 10% formalin for 30 min at room temperature and subsequently stained with 250 μl per well of 0.1% crystal violet in 70% ethanol for 30 min at room temperature with constant shaking. To remove unbound crystal violet, cells were washed with deionized water three times for 5 min each. Quantification was performed by extracting the crystal violet dye with 250 μl of 0.1% acetic acid. Then, 50 μl was transferred into a 96-well format in triplicate. The experiments were performed three times (Extended Data Fig. 3c, d) or two times (Extended Data Fig. 10e, f). Crystal violet absorbance was determined using the Perkin Elmer EnVision 2105. The results of one representative experiment are shown with quantification, which shows the repeat measurements from a single experiment. Two-way ANOVA was performed for Extended Data Fig. 10f, in which HCT116 cells expressing Chr.3-5 and Chr.2-2 sgRNA vs HCT116 cells expressing Chr.2 and Chr.2-2 sgRNA and HCT116 cells expressing Chr.3-5 and MLHI sgRNAs were compared to HCT116 cells expressing Chr.3-5 and Chr.2-2 sgRNA.

Cell cycle analysis. Cas9-expressing cell lines were lentivirally transduced to deliver the desired sgRNAs or shRNAs. Medium was changed the next day to allow for antibiotic selection. Subsequently, 4 days after the lentiviral transduction, cells were labelled with EdU, collected and stained as per the Click-it Plus EdU Flow Cytometry Assay Kit recommendations. Stained cells were then examined using flow cytometry and results analysed with FlowJo. A representative result of two independent experiments is shown; each experiment was conducted in triplicate. Statistical analysis of the proportion of cells in S phase versus Chr.2-2 sgRNA was calculated by two-way ANOVA (Extended Data Fig. 4b).

Apoptosis assay. Cas9-expressing cell lines were lentivirally transduced to deliver vectors that encoded the desired sgRNAs or shRNAs. Medium was changed the next day without antibiotic selection. Cells were split 4 days after transduction. Then, 7 days after transduction, cells were collected and stained with annexin V–FITC and propidium iodide. Stained cells were then examined with flow cytometry and results analysed with FlowJo. A representative result of two independent experiments is shown; each experiment was conducted in triplicate. Significance was calculated for the sum of the proportions of cells in early apoptosis, late apoptosis and nonapoptotic death categories; in cells expressing a WRN sgRNA compared to cells expressing Chr.2-2 sgRNA. Extended Data Fig. 4c, d.

In vivo xenograft studies. Studies were approved by the Institutional Animal Care and Use Committee (IACUC) of the Broad Institute under animal protocol 0194-01-18. IACUC guidelines on the ethical use and care of animals were followed. For growth curves, 10^5 KM12 cells expressing WRN shRNA 1 or WRN-C911 shRNA 1 were inoculated subcutaneously into right hind flank of approximately 8-week old BALB/c-SCID interference Outbred (SHO) Charles River Laboratories. Tumours were measured twice weekly with callipers and the tumour volumes were calculated using the formula 1/2 × (width^2 × length). When primary tumours reached around 100 mm^3, the mice were randomized onto Control Teklad Global 18% Protein Rodent Diet or Teklad Global 18% Protein Rodent Diet containing 625 mg kg^-1 doxycycline hydrolyte. Mice remained on their respective diets throughout the remainder of the study. Animal body weights were recorded twice weekly during the course of the study for body condition scoring. For the growth curves, n = 5 (4 from days 15–18), 5, 4, 4 for WRN shRNA 1 without doxycycline, WRN-shRNA 1 with doxycycline, WRN-C911 shRNA 1 without doxycycline, WRN-C911 shRNA 1 with doxycycline treatment, respectively. One mouse treated with KM12 cells with WRN shRNA 1 without doxycycline was euthanized before day 15 because of tumour ulceration. No experiments exceeded the maximal tumour volumes of 2,000 mm^3 set forth by the IACUC. This study was not blinded. Sample size calculations were not performed. For target engagement, one mouse per time point was inoculated in the right and left flanks. To compare xenograft tumour growth curves across experimental conditions, we used a linear mixed model to describe the percentage increase in tumour volume as a linear function of time, with random slope terms per mouse, and an interaction term between growth rate and experimental conditions. Maximum likelihood models were fit using the package lme4. Reported P values assessing the significance of interactions between growth rate and experimental conditions were derived using a likelihood ratio test. Tumours were collected at the indicated time points. Experiments in Fig. 2d were conducted once. The immunoblot for this experiment (Fig. 2e) was performed twice from the same protein lysates.

Telomere PNA-FISH of metaphase spreads. In brief, 3 × 10^6 cells were plated in 10-cm dishes in the absence of doxycycline. For cells treated with doxycycline, 0.2 μg ml^-1 of doxycycline was added and refreshed every 48 h. Then, 96 h after adding doxycycline, cells were treated with 10 μg ml^-1 of colcemid for 3 h and then detached with trypsin. Half of the cell pellet was saved for immunoblot analysis. The other half of the cell pellet was treated with a hypotonic solution (60 mM KCl) and fixed with a 3:1 ratio of methanol:acetic acid. Metaphases were spread onto a slide and fixed with 3% formalin. The PNA-FISH probe (CCCTAA)_3 PNA probe (PNA Bio) was applied to the slides and denatured at 85°C for 3 min. The slides were washed with washing solution 1 (70% formamide, 0.1% Tween-20, 0.1% BSA in 10 mM Tris buffer, pH 7.5) twice and three times with washing solution II (150 mM NaCl, 0.1% Tween-20, 0.1% BSA in 50 mM Tris buffer, pH 7.5). Cy5–ATTGTTGGGAAAGGGA was used to label centromeres. Images were captured with a Nikon Eclipse 80i microscope and processed with the NIS-Elements BR software. In total, 30 to 60 metaphases were analysed for cells of the indicated conditions. Two-tailed Student’s t-tests were performed (Fig. 4e). These experiments were performed twice.

Fluorescence-based multiplexed host cell reactivation (FM-HCR) assays. Assays to assess MMR impairment in isogenic HCT116 cells were carried out as previously described. SW620, SW837, HCT116 and its derivatives were collected as per the FM-HCR reporter plasmids (50 ng pmxOrange_GG for mismatch repair with 50 ng pmx_mPlum as transfection control or 50 ng pmx_max_mOrange as undamaged control and 50 ng pmx_mPlum as transfection control) and carrier DNA in a 10-μl volume using
the ThermoFisher Neon transfection system (1.200 V, 20 ms, 2 pulses). Percentage reporter expression was calculated as previously reported for MMR reporters. Approximately 2 × 10^6 transfected cells were seeded into 12-well culture plates and analysed by flow cytometry at 24 h after transfection. These experiments were performed three times. Two-tailed Student's t-tests were performed when comparing HCT116 parental to HCT116 Chr.2 cells and HCT116 parental to HCT116 Chr.3+5 cells. Two-way ANOVA was performed when comparing HCT116 cells expressing an additional Chr.3+5 and Chr.2-2 sgRNA to HCT116 cells expressing an additional Chr.3+5 and MLH1 sgRNA 1 and HCT116 cells expressing an additional Chr.3+5 and MLH1 sgRNA 2 (Extended Data Fig. 10a).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability
Source Data for Figs. 2a–g, 3a, 3b, d, f, h, 6a, c, d, f, h, 7b, d, e, 8d, 10a–d, f are provided with online version of the paper. mRNA-seq data (shown in Fig. 3a) have been deposited in the Gene Expression Omnibus (GEO) repository under accession number GSE126464. DepMap omics and dependency data used for analyses are available as a FigShare repository: Cancer Data Science. DepMap datasets for WRN manuscript. (2019). https://doi.org/10.6084/m9.figshare.7712756.v1.

Code availability
Code used for analysis can be found at https://github.com/cancerdatasci/WRN_manuscript. All materials can be accessed at https://depmap.org/WRN.

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Extended Data Fig. 1 | Functional genomic screening identifies that MSI cancers are selectively dependent on WRN. a, Screened cell lines plotted by number of deletions and fraction of deletions occurring in microsatellite (MS) regions. Genes involved in MMR that are lost are indicated by different colours. None, no predicted loss of MMR genes. Multiple, loss of more than one MMR gene. n/a, not available. b, Using PCR-based MSI phenotyping, P values (empirical Bayes moderated t-test) were plotted against the mean difference of dependency scores between MSI and MSS cell lines for Achilles (n = 19 MSI; n = 291 MSS) and DRIVE (n = 23 MSI; n = 252 MSS). c, Dependency scores for each RecQ helicase plotted for MSI and MSS cell lines from Achilles (n = 32 MSI; n = 413 MSS) and DRIVE (n = 38 MSI; n = 337 MSS). Q values (Wilcoxon rank-sum test) for Achilles/DRIVE are 5.0 × 10⁻⁴/1.7 × 10⁻⁸, 0.73/0.52, 0.73/0.85, 0.25/0.73 and 0.08/not available for WRN, RECQL, BLM, RECQL5 and RECQL4, respectively. Centre lines indicate medians. Boxes indicate 25th and 75th percentiles; whiskers extend to 1.5 × IQR beyond the box and individual data points are represented by dots. d, Sensitivity and positive predictive value of indicated relationship between biomarker and genetic dependency. e, Dependency score distributions and associated biomarkers for example biomarker–genetic dependency relationships. Width of coloured regions represent density estimates. Horizontal dashed line: threshold used to separate dependent and non-dependent cell lines. n = 37, 14, 541 MSI cell lines from typical lineage, MSI cell lines from atypical lineage, MSS cell lines, respectively. n = 120/546 KRAS hotspot mutants/other; 65/601 BRAF hotspot mutants/other; 86/580 PIK3CA hotspot mutants/other.
Extended Data Fig. 2 | MSI cells from MSI-predominant lineages have a greater mutational burden and WRN dependency. a, WRN dependency scores plotted by lineage, sub-classified by MSI and MSS status. Boxes indicate 25th and 75th percentiles; whiskers extend to $1.5 \times$ IQR beyond the box and individual data points are represented by dots. b, Microsatellite deletions in cell lines classified as MSS ($n=541$), MSI from an infrequent MSI lineage ($n=45$), or MSI from an MSI-predominant lineage ($n=54$). *$P=1.7 \times 10^{-9}$, Wilcoxon signed-rank test. Width of coloured regions represent density estimates. c, MSI cell lines plotted by their average WRN dependency and number of microsatellite deletions. Lineages are colour-coded. d, MSI cell lines from MSI-predominant lineages are plotted by their average WRN dependency and number of microsatellite deletions. Lineages are colour-coded.
Extended Data Fig. 3 | WRN depletion preferentially impairs MSI cell viability. a, Immunoblot of WRN and GAPDH levels 4 days after sgRNA transduction. b, Relative viability following sgRNA transduction in a competitive growth assay. Data are mean ± s.e.m. (n = 6 biological replicates). Comparison between WRN sgRNAs and negative controls at day 10; two-way ANOVA; *P = 0.37, †P = 1.2 × 10⁻⁷, ‡P = 0.23, §P = 2.7 × 10⁻¹⁹. c, Clonogenic assay after shRNA transduction with a non-targeting negative control (RFP shRNA (shRFP)), a pan-essential control (PSMD2 shRNA (shPSMD2)) and two shRNAs against WRN (shWRN1 and shWRN2). d, Relative staining intensity of the clonogenic assay. Data are mean ± s.e.m. (n = 3 technical replicates). Representative data from one experiment are shown. All experiments were performed three times.
Extended Data Fig. 4 | WRN depletion preferentially induces cell cycle arrest and apoptosis in MSI cells. a, Gating strategy. For cell cycle analyses (top), debris and dead cells were excluded based on forward scatter-area (FSC-A) and side scatter-area (SSC-A) profiles. Subsequently, singlets were identified based on FSC-A and forward scatter-height (FSC-H) profiles. These singlets were then analysed for DAPI (DNA content) and EdU–Alexa Fluor 647 (EdU–647) staining intensities. EdU in cells exhibiting higher staining intensity than unstained cells (cells exhibiting higher staining intensity than unstained cells) were identified. On the basis of the positivity of these markers, cells were classified into one of the following three categories: viable (annexin-V–PI−), nonapoptotic death (annexin-V–PI+ and annexin-V−PI+). b, Cell cycle evaluation 4 days after sgRNA transduction. Comparison between Chr.2-2 sgRNA and WRN sgRNAs for the percentage of S-phase cells; two-way ANOVA; *P = 0.16, †P = 0.67, ‡P = 6.1 × 10−7, §P = 3.5 × 10−4, ¶P = 0.69, ‾P = 2.6 × 10−4. c, Annexin-V and propidium iodide staining evaluating early apoptosis and late apoptosis/non-apoptotic cell death 7 days after sgRNA transduction. Comparison between Chr.2-2 sgRNA and WRN sgRNAs for the percentage of dying/dead cells; two-way ANOVA; *P = 0.10, †P = 0.41, ‡P = 3.4 × 10−3, §P = 3.6 × 10−4, ¶P = 0.57, ‾P = 3.6 × 10−4. d, Annexin-V and propidium iodide staining 4 and 8 days after shRNA transduction. Comparison between RFP shRNA and WRN shRNAs; two-way ANOVA; 1.3 × 10−3 (SW837 day 4), 1.6 × 10−2 (SW837 day 8), 1.2 × 10−6 (KM12 day 4), 4.3 × 10−6 (KM12 day 8). Three biological replicates are presented in tandem for b–d. Representative data from one experiment are shown. All experiments were performed twice.
Extended Data Fig. 5 | WRN depletion activates a p53 response in MSI cells. a. Phosphorylated p53(S15) immunofluorescence images following sgRNA transduction in ovarian cell lines (ES2 and OVK18). Scale bar, 50 μm. b. Nuclear phosphorylated p53(S15) staining intensity per cell following WRN knockout compared to control sgRNA. Data were analysed as fold change (log(WRN sgRNA/control sgRNA)); mean = 0.059 (OVK18), mean = −0.037 (ES2). Difference in fold change between OVK18 and ES2; contrast test of least-squares means; \( P < 2 \times 10^{-16} \); \( n \) indicates the number of cells treated with Chr.2-2 sgRNA, WRN sgRNA 2, WRN sgRNA 3, respectively, for OVK18 (3,982, 1,143, 2,740) and ES2 (4,916, 3,072, 3,690). c. p21 immunofluorescence images following sgRNA transduction in colon cell lines (SW620, KM12 and SW48). KM12 is a p53-impaired MSI cell line. Scale bar, 50 μm. d. Nuclear p21 staining per cell. Data were analysed as fold change (log(WRN sgRNA/control sgRNA)); mean = 0.157 (OVK18), mean = −0.010 (ES2). \( n \) indicates the number of cells analysed following treatment with Chr.2-2 sgRNA, WRN sgRNA 2, WRN sgRNA 3, respectively, for SW620 (7,278, 13,768, 11,576) and KM12 (16,117, 14,200, 11,301). e. p21 immunofluorescence images following sgRNA transduction in ovarian cell lines. Scale bar, 50 μm. f. Nuclear p21 staining intensity per cell. Data were analysed as fold change (log(WRN sgRNA/control sgRNA)); mean = 0.016 (SW48 cells), mean = −0.032 (KM12), mean = −0.032 (KM12). \( n \) indicates the number of cells treated with Chr.2-2 sgRNA, WRN sgRNA 2, WRN sgRNA 3, respectively, for SW48 (16,203, 7,617, 13,257), SW620 (7,278, 13,768, 11,576) and KM12 (16,117, 14,200, 11,301). f. Centre line indicates the median, boxes indicate the 25 to 75th percentiles, whiskers indicate the 1st to 99th percentiles and dots indicate outliers. g. Box plots indicate 25th and 75th percentiles; whiskers extend to 1.5 × IQR beyond the box and individual data points are represented by dots. Representative data from one experiment are shown. a–f. Experiments were performed twice.
Extended Data Fig. 6 | WRN depletion preferentially induces DSBs in MSI cells. a, Nuclear γ-H2AX foci per cell following sgRNA transduction in colon cell lines. b, γ-H2AX immunofluorescence images following sgRNA transduction in ovarian cell lines. Scale bar, 50 μm. c, Nuclear γ-H2AX staining intensity per cell following sgRNA transduction. Difference in fold change between OVK18 and ES2; contrast test of least-squares mean; \( P < 2 \times 10^{-16} \). Mean = 0.147 (OVK18) and mean = 0.055 (ES2). \( n \) indicates the number of cells analysed following treatment with Chr.2-2 sgRNA, WRN sgRNA 2, WRN sgRNA 3, respectively, for OVK18 (2,612, 4,823, 6,164) and ES2 (6,429, 6,469, 6,388). Centre line indicates the median, boxes indicate the 25 to 75th percentiles, whiskers indicate the 1st to 99th percentiles and dots indicate outliers. d, Nuclear γ-H2AX foci per cell following sgRNA transduction in ovarian cell lines. e, Fluorescence of Apple–53BP1 foci in colon cell lines exogenously expressing Apple–53BP1(truncated). Scale bar, 50 μm. f, Nuclear Apple–53BP1 foci per cell following sgRNA transduction in colon cell lines. g, Fluorescence of Apple–53BP1 foci following sgRNA transduction in ovarian cell lines exogenously expressing Apple–53BP1(truncated). Scale bar, 50 μm. h, Nuclear Apple–53BP1 foci per cell in ovarian cell lines. Representative data from one experiment are shown. All experiments were performed twice.
Extended Data Fig. 7 | WRN depletion preferentially induces DSB responses in MSI cells. 

a, Phospho-ATM(S1981) immunofluorescence images following sgRNA transduction in colon cell lines. Scale bar, 50 μm.
b, Nuclear phospho-ATM(S1981) foci per cell following sgRNA transduction in colon cell lines.
c, Phospho-ATM(S1981) immunofluorescence images following sgRNA transduction in ovarian cell lines. Scale bar, 50 μm.
d, Nuclear phospho-ATM(S1981) foci per cell following sgRNA transduction in ovarian cell lines.
e, γH2AX, phospho-CHK2(T68), total CHK2, WRN and GAPDH levels following shRNA transduction. Representative data from one experiment are shown. All experiments were performed twice.
Extended Data Fig. 8 | WRN is preferentially recruited to DNA in MSI cells. a, Telomere PNA-FISH of metaphase spreads with or without doxycycline induction of WRN shRNA 1. Hollow arrowhead, chromosomal breaks. Filled arrowhead, chromosomal fragments. b, WRN immunofluorescence images following treatment with WRN shRNA 1 or control shRNA (WRN-C911 shRNA 1). c, WRN immunofluorescence images. Scale bar, 20 μm. d, Analyses of WRN co-localization with the nucleolar marker, fibrillarin, by Pearson’s co-localization coefficients. Data are mean ± s.e.m. (n = 5 biological replicates); two-tailed Student’s t-test; *P = 1.0 × 10^{-3}, †P = 4.3 × 10^{-5}, ‡P = 0.014. Representative data from one experiment are shown. All experiments were conducted twice.
Extended Data Fig. 9 | Paralogue dependencies and hypermutation alone cannot explain the WRN–MSI relationship. a, Estimated association between WRN dependency and MSI status after controlling for loss of indicated genes (effect size estimates for the linear model are plotted against significance). If loss of a gene can fully account for the MSI–WRN relationship, the difference in dependency and significance would be 0. Genes for which the loss are typically associated with insertion and deletion (indel) mutations (over half of loss events) are highlighted in red. \( n = 51 \) MSI, \( n = 541 \) MSS. b, Average WRN dependency score for MSS and MSI lines stratified by POLE status \( (n = 4, 5, 35, 497, 2, 12, 5, 10, 22 \) cell lines per category in order of left to right). Boxes indicate 25th and 75th percentiles; whiskers extend to \( 1.5 \times \text{IQR} \) beyond the box and individual data points are represented by dots.
Extended Data Fig. 10 | MMR deficiency contributes to WRN dependency. 

a, Flow-cytometric host-cell reactivation assay measuring the ability of the indicated cell lines to repair a G:G mismatch in a plasmid reporter, thus activating the fluorescence reporter and measuring MMR activity. Data are mean ± s.e.m. from three independent experiments; two-tailed Student’s t-test, *P = 5.5 × 10\(^{-2}\), †P = 2.3 × 10\(^{-5}\); two-way ANOVA, ‡P = 3.6 × 10\(^{-8}\). 
b, Immunoblot. γH2AX, WRN, MLH1, MSH3 and GAPDH levels following shRNA transduction in HCT116 derivatives. 

c, Relative viability of HCT116 derivatives 7 days after shRNA transduction. Data are mean ± s.e.m. (n = 6 biological replicates); two-way ANOVA; P = 5.7 × 10\(^{-20}\) (* compared to †), P = 3.3 × 10\(^{-12}\) († compared to §), P = 1.6 × 10\(^{-16}\) († compared to $). 
d, Clonogenic assay after shRNA transduction for 15 days. 

e, Relative staining intensity of the clonogenic assay. Data are mean ± s.e.m. (n = 3 biological replicates); two-way ANOVA; P = 3.6 × 10\(^{-6}\) (* compared to †), P = 8.5 × 10\(^{-8}\) († compared to §), P = 2.8 × 10\(^{-8}\) († compared to §). 

b–f, Representative data from one experiment are shown. All experiments were conducted twice except in a, for which experiments were conducted three times.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- The statistical test(s) used AND whether they are one- or two-sided
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  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | No software was used for collection of genomic data. |
| Data collection | Luminescence and absorbance measurements were obtained by the Perkin Elmer EnVision 2105. |
| Data collection | Data collection for quantifying immunofluorescence, other than that for WRN and fibrillarin, were acquired using an Opera Phenix High-Content Screening System (PerkinElmer, HH14000000). Micrographic images were acquired using either epifluorescence microscopy or confocal microscopy, which were performed on an Axio Observer.Z1 microscope equipped with an AxioCam 506 mono camera and Apoptome.2 (Carl Zeiss) and a Zeiss LSM 700 laser scanning confocal system equipped with Axio Observer (Carl Zeiss), respectively. For the WRN/Fibrillarin IF, images were obtained via the Zeiss LSM510 Upright Confocal System. |
| Data analysis | For PNA/Telomere FISH: Images were captured with a Nikon Eclipse 80i microscope and processed with software NIS-Elements BR. 30 to 60 metaphases were analyzed for cells of the indicated genotypes. |
| Data analysis | As described in the methods section, analysis of Project Achilles and Project DRIVE dependency datasets, as well as differential expression analyses, were performed with RStudio (R version 3.4.1), using the following R packages: “Limma”, “edgeR”, and “fgsea”. |
| Data analysis | For differential expression analysis, we first excluded genes which had less than 1 count per million in more than half of the samples. The weighted trimmed mean of M-values method was used to normalize the library size of each sample, using the calcNormFactors function from the R package: edgeR. To estimate the log-fold change (LFC) effect of WRN knockout on each gene in each cell line we used the R package Limma. Specifically, we fit a linear model for the expression of each gene, using cell line and sgRNA (WRN vs. control) as covariates. Read counts data were transformed using the Limma function ‘voom’ prior to model fitting, in order to model the mean-variance relationship of the log-counts data. We then extracted LFC effect sizes and empirical-Bayes moderated t-statistics for the WRN knockout effect for each gene and cell line. Gene set enrichment analysis (GSEA) was run to test for gene sets that were up- or down-regulated in each cell line after WRN knockout. In particular, we used the R package fgsea to estimate normalized enrichment statistics, and associated P values, for each gene set in the Hallmark Collection from the Molecular Signatures Database. The GSEA algorithm was
run using t-statistics as the gene-level statistics, 1 million random permutations for each cell line tested, and a “GSEA parameter” of 1.

For all IF other than that for WRN and fibrillarin, images were analyzed on Harmony High Content Imaging and Analysis Software (PerkinElmer, HH17000001). For WRN/Fibrillarin IF: Weighted Pearson colocalization coefficients were calculated by obtaining Z-stacks of 5 representative highpowered fields at 63x magnification and scored via the Zeiss Zen Blue software.

To assess differences between the effect of WRN knockout on IF intensity values (phospho-p53-, p21-, γH2AX- and phospho-ATMstaining) in MSI versus MSS cell lines, we used a linear model approach. Specifically, linear models were fit to the log-intensity values with guide and cell line intercept terms. Comparisons of the change in log-intensity after WRN knockout between cell lines were then obtained using the ‘contrast’ function of the R package lsmmeans.

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

As described in the methods section, all genomic data used in the analysis has been published and can be found in the Cancer Cell Line Encyclopedia (CCLE) portal (https://portals.broadinstitute.org/ccle/data). Cancer cell line genomic data used in the analysis, including gene-level mRNA expression, gene-level relative copy number, and mutation calls, were taken from the DepMap 18Q4 data release. CRISPR dependency data were taken from the 18Q4 Avana dataset Novartis' Project DRIVE be downloaded from (https://doi.org/10.6084/m9.figshare.6025238.v1).

CRISPR dependency data from Project Achilles can be downloaded from the Figshare repository (https://doi.org/10.6084/m9.figshare.5863776.v1). Source data for Figs. 2a-g, 3c, 4a, e, f, and Extended Data Figs. 3a, b, d, 4b-d, 5b, d, f, 6a, c, d, f, 7b, d, e, 8d, 10a-d, f are provided with the paper. mRNA-seq data (shown in Fig. 3a) have been deposited in the Gene Expression Omnibus (GEO) repository under accession number GSE126464. DepMap omics and dependency data used in analysis are available as a Figshare repository: Cancer Data Science. DepMap Datasets for WRN manuscript. (2019). doi:10.6084/m9.figshare.7712756.v1

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**

All cell lines included in the functional genomic datasets were analyzed unless they were excluded due to indeterminate MSI status. No sample size calculation was performed. The differential WRN dependency between MSI and MSS cell lines was highly significant in two independent large-scale functional genomic datasets, demonstrating that this sample size was sufficient. We validated these results using a smaller sample of these cell lines (6 MSI and 5 MSS cell lines representing at least one cell line from the 4 most common MSI lineages) to confirm that these differences were also present using a single-gene knockout approach, and 5 cell lines per group was more than sufficient for this purpose.

**Data exclusions**

Cell lines in which MSI status was indeterminate was excluded from the analyses. This exclusion criteria was pre-established.

**Replication**

With the exception of RNA-seq (which was performed with two biological replicates), all experiments were replicated at least once. All attempts at replication successfully confirmed our findings.

**Randomization**

Samples were allocated by MSI/MSS status. In the in vivo experiment, mice were randomized to a dox or no dox diet when the primary tumors reached ~100 mm³.

**Blinding**

Blinding was not feasible in this study. Knowledge of the tested cell lines were necessary to technically perform the experiment given different culture conditions and different transduction efficiencies. Moreover, the morphology of the cells under microscopy would be telling. The in vivo experiment was also not blinded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.
Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑  | Antibodies            |
| ☑  | Eukaryotic cell lines |
| ☑  | Palaeontology         |
| ☑  | Animals and other organisms |
| ☑  | Human research participants |
| ☑  | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑  | ChIP-seq              |
| ☑  | Flow cytometry        |
| ☑  | MRI-based neuroimaging |

Antibodies

For immunoblotting, the following antibodies were used:
- **anti-phospho-Chk2 [T68]** (R&D Systems, AF1626, JBC0314111, 1:1000)
- **anti-(total) Chk2** (Cell Signaling Technology, 3440, 1:1000)
- **anti-γH2AX** (Cell Signaling Technology, 9718, 13, 1:1000)
- **anti-GAPDH** (Cell Signaling Technology, 5174, 1:1000)
- **anti-MLH1** (Cell Signaling Technology, 3515, 2, 1:1000)
- **anti-MSH3** (BD Biosciences, 611390, 7255501, 1:400)
- **anti-WRN** (Novus Biologicals, nb100-472, A2, 1:1000).
- Goat anti-Rabbit IRDye 800CW (LI-COR, 926-32211, C80718-15, 1:5000)
- Goat anti-Rabbit IRDye 680LT (LI-COR, 926-68021, C60531-05, 1:5000)
- Goat anti-Mouse IRDye 800CW (LI-COR, 926-32210, C60405-05, 1:5000)

For immunofluorescence, the following antibodies were used:
- **anti-γH2AX** (Millipore Sigma, 05-740, 2967311, 1:200)
- **anti-phospho ATM [S1981]** (Millipore Sigma, 05-740, 2967311, 1:200)
- **anti-phospho Chk2 [T68]** (R&D Systems, AF1626, JBC0314111, 1:1000)
- **anti-fibrillarin** (Abcam, ab5821, GR29348302, 1:500)
- **anti-phospho p53 [S15]** (Cell Signaling Technology, 9284, 15, 1:100)
- **anti-WRN** (Sigma, W0393, 037M4751V, 1:200).
- Alexa Fluor 488 goat anti-Rabbit IgG (Thermo Scientific, A11008, 1705869, 1:200)
- Alexa Fluor 488 goat anti-Mouse IgG (Thermo Scientific, A11001, 1907294, 1:200-1000)
- Alexa Fluor 555 goat anti-Rabbit IgG (Thermo Scientific, A21428, 1786491, 1:1000)

Validation

Antibodies were purchased from reputable sources as listed below and pre-validated for the following uses:
- **anti-phospho-Chk2 [T68]** (R&D Systems, Catalog # AF1626): immunoblotting, immunocytochemistry. We further validated this antibody by demonstrating that etoposide, which causes double strand breaks, increases phospho-Chk2 levels.
- **anti-(total) Chk2** (Cell Signaling Technology, 3440): immunoblotting
- **anti-γH2AX** (Cell Signaling Technology, 9718): immunoblotting. We further validated this antibody by demonstrating that etoposide, which causes double strand breaks, increases γH2AX levels.
- **anti-GAPDH** (Cell Signaling Technology, 5174): immunoblotting
- **anti-MLH1** (Cell Signaling Technology, 3515): immunoblotting. We further validated this antibody by CRISPR/Cas9 knockout of MLH1.
- **anti-MSH3** (BD Biosciences, 611390): immunoblotting.
- **anti-WRN** (Novus Biologicals, nb100-472): immunoblotting. We further validated this antibody by CRISPR/Cas9 and RNAi-mediated silencing WRN.
- Goat anti-Rabbit IRDye 800CW (LI-COR, 926-32211): immunoblotting
- Goat anti-Rabbit IRDye 680LT (LI-COR, 926-68021): immunoblotting
- Goat anti-Mouse IRDye 800CW (LI-COR, 926-32210): immunoblotting
- **anti-γH2AX** (Millipore Sigma, 05-636): immunofluorescence
- **anti-p21** (Santa Cruz Biotechnology, sc-6246, R2317, 1:100)
- **anti-phospho ATM [S1981]** (Millipore Sigma, 05-740, 2967311, 1:200)
- **anti-phospho Chk2 [T68]** (R&D Systems, AF1626, JBC0314111, 1:1000)
- **anti-fibrillarin** (Abcam, ab5821, GR29348302, 1:500)
- **anti-phospho p53 [S15]** (Cell Signaling Technology, 9284, 15, 1:100)
- **anti-WRN** (Sigma, W0393, 037M4751V, 1:200).
- Alexa Fluor 488 goat anti-Rabbit IgG (Thermo Scientific, A11008, 1705869, 1:200)
- Alexa Fluor 488 goat anti-Mouse IgG (Thermo Scientific, A11001, 1907294, 1:200-1000)
- Alexa Fluor 555 goat anti-Rabbit IgG (Thermo Scientific, A21428, 1786491, 1:1000)
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

ES2, OVK18, SW620, SW837, KM12, SW48, GCIY, SNU1, HEC50B, and SNGM were collected by the CCLE prior to distribution for our use. The sources of the aforementioned cell lines can be found at depmap.org and are as follows. ES2, SW620, SW837, SNU1, and SW48 were originally obtained from the American Type Culture Collection (ATCC). KM12 was originally obtained from an academic lab and can be obtained as part of the NCI-60 cell lines. GCIY and OVK18 were originally obtained from the RIKEN Cell Bank. SNGM and HEC50B were originally obtained from the Health Science Research Resources Bank (HSRRB).

HCT116 and their derivatives were gifts from Drs. Richard Boland, Ajay Goel and Minoru Koi. A patient-derived organoid, CCLF_CORE_0001_T, was obtained from a 58 year old female patient with stage II MSI-H (determined by IHC loss of MLH1/PMS2 with MLH1 promoter methylation) with her informed consent, at the Dana-Farber Cancer Institute (DFCI).

Authentication All cell lines were fingerprinted by SNP arrays except for the HCT116 which were provided by the collaborator.

Mycoplasma contamination Cell lines were tested negative for mycoplasma.

Commonly misidentified lines

(See ICLAC register)

Project Achilles performed fingerprinting to ensure the identity of the screened cell lines. All misidentified cell lines were removed from the final dataset.

See McDonald, E.R., et al., Cell. (2017) for details regarding the cell lines in Project DRIVE. Our experiments included no commonly misidentified cell lines as per ICLAC version 9.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals ~8 week old female SCID Hairless Outbred (SHO) mice obtained from Charles River Laboratories were used.

Wild animals This study did not involve wild animals.

Field-collected samples This study did not involve samples collected from the field.

Ethics oversight The Broad Institute’s Institutional Animal Care and Use Committee (IACUC) approved this study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics CCLF_CCLF_CORE_0001_T, was obtained from a 58 year old female patient with stage II MSI-H (determined by IHC loss of MLH1/ PMS2 with MLH1 promoter methylation). This was only a single patient but the patient was not previously treated for their cancer and underwent resection with curative intent. As this was only a single report and not a clinical trial with multiple participants, we cannot comment on any specific bias that is idiosyncratic to this individual tumor. However, this study was to determine whether the effects (dependence upon WRN across a large number of MSI cancer cell lines models) merely represented a cell line ‘artifact.’ We have utilized this model and the data generated from this model to present reassuring data that the observations from a diverse set of cell lines also holds in a novel patient derived model.

Recruitment Patients undergoing clinical care at our center (Dana-Farber Cancer Institute) are routinely (nearly universally) screened for enrollment to generate living cancer models from any residual tumor available from clinical procedures (e.g. surgical resection with curative intent). To ensure representation across genders, age, and racial groups, nearly all patients at the Dana-Farber Cancer Center are approached in the pre-operative center for enrollment. We do not anticipate any biases.

Ethics oversight All human research protocols were conducted under a Dana-Farber Cancer Institute Institutional Review Board (IRB) approved protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.
Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Cells were plated at the following densities in a 12 well plate:
- KM12-Cas9 - 4e5/well
- SW620-Cas9 - 3e5/well
- ES2-Cas9 - 2e5/well
- OVK18-Cas9 - 3e5/well
- SW48-Cas9 - 4e5/well
- SW837-Cas9 - 4e5/well

Cells were transduced in media containing polybrene at a final concentration of 4ug/mL. Once viral media was added to the cells, all cells (with the exception of ES2 311Cas9, which shows viral toxicity when spun) were spun for 2 hours at 2000 rpm and 37 degrees celsius. 24 hours post infection, cells were split and were put under puromycin selection (1ug/mL final concentration)

The EdU assay was completed on both day 4 and day 7 post infection using the following protocol, adapted from the protocol from the Click-iT™ Plus EdU Flow Cytometry Assay Kit.

Cells were pulsed with EdU (5-ethyl-2'-deoxyuridine) for 2-4 hours (KM12, SW620, ES2 and OVK18 for 2 hours, SW48 and SW837 for 4 hours) at a final concentration of 10uM. After EdU incubation, cells were washed with 1X PBS and trypsinized with 100uL of 0.25% trypsin per well. After ~5 minutes of incubation with trypsin, the appropriate media supplemented with 10% FBS was added to stop trypsinization. Cells were washed once with PBS, once with PBS+1%BSA, and then resuspended in 100uL of fixative and left in the dark for 10-15 minutes at room temperature. Cells were then washed with PBS+1%BSA and resuspended in 100uL of Saponin-based permeabilization and wash buffer.

Per sample:
- 469uL PBS
- 5uL Copper protectant
- 1.25uL fluorescent dye picolyl azide
- 25uL Reaction Buffer Additive (made from 10X Reaction Buffer Additive diluted in deionized water)

Sample was incubated in the dark for 30 minutes at room temperature. Samples were then washed with saponin based p&W buffer and then resuspended in 0.2% TritonX with Dapi diluted 1:100 in PBS. Left in the dark for 10 minutes at room temperature, washed with PBS and then resuspended in 500uL of PBS to be read out via flow cytometry.

Serum starvation samples were made by replacing media with serum free media 24 hours before EdU staining, and cells were prepared in the same way for flow cytometry. We also included a no EdU sample.

Instrument

Beckman-Coulter CytoFLEX S

Software

FlowJo v10

Cell population abundance

10,000 cells from each sample were analyzed from each experiment

Gating strategy

As per Extended Data Fig 4a: For cell cycle analysis, debris and dead cells were excluded based on forward scatter-area (FSC-A) and side scatter-area (SSC-A) profiles. Subsequently, singlets were identified based on FSC-A and forward scatter-height (FSC-H) profiles. These singlets were then analyzed for DAPI (DNA content) and EdU-Alexa Fluor 647 (EdU-647) staining intensities. EdU-647-positive cells (cells exhibiting higher staining intensity than unstained cells) were classified as ‘S-phase’. EdU-647-negative cells were classified either as ‘G1-phase’ or ‘G2/M-phase’ based on their DNA content. For apoptosis analyses, debris were excluded based on FSC-A and SSC-A profiles. The remaining samples were analyzed for Annexin V-FITC and propidium iodide (PI) staining intensities. Subsequently, Annexin V-FITC-positive cells and PI-positive cells (cells exhibiting higher staining intensity than unstained cells) were identified. Based on the positivity of these markers, cells were classified into either of the following three categories: Viable (Annexin V-negative, PI-negative), Early Apoptosis (Annexin V-positive, PI-negative), and Late Apoptosis/Nonapoptotic death (PI-positive).

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.