

12. Kent, T., Chandramouly, G., McDevitt, S. M., Ozdemir, A. Y. & Pomerantz, R. T. Mechanism of microhomology-mediated end-joining promoted by human DNA polymerase  $\theta$ . *Nat. Struct. Mol. Biol.* **22**, 230–237 (2015).
13. Wyatt, D. W. et al. Essential roles for polymerase  $\theta$ -mediated end joining in the repair of chromosome breaks. *Mol. Cell* **63**, 662–673 (2016).
14. van Schendel, R., van Heteren, J., Welten, R. & Tijsterman, M. Genomic scars generated by polymerase  $\theta$  reveal the versatile mechanism of alternative end-joining. *PLoS Genet.* **12**, e1006368 (2016).
15. Koole, W. et al. A Polymerase  $\theta$ -dependent repair pathway suppresses extensive genomic instability at endogenous G4 DNA sites. *Nat. Commun.* **5**, 3216 (2014).
16. Mateos-Gomez, P. A. et al. Mammalian polymerase  $\theta$  promotes alternative NHEJ and suppresses recombination. *Nature* **518**, 254–257 (2015).
17. Ceccaldi, R. et al. Homologous-recombination-deficient tumours are dependent on Poltheta-mediated repair. *Nature* **518**, 258–262 (2015).
18. Shima, N., Munroe, R. J. & Schimenti, J. C. The mouse genomic instability mutation *chaos1* is an allele of Polq that exhibits genetic interaction with *Atm*. *Mol. Cell Biol.* **24**, 10381–10389 (2004).
19. Goff, J. P. et al. Lack of DNA polymerase  $\theta$  (POLQ) radiosensitizes bone marrow stromal cells in vitro and increases reticulocyte micronuclei after total-body irradiation. *Radiat. Res.* **172**, 165–174 (2009).
20. Higgins, G. S. et al. A small interfering RNA screen of genes involved in DNA repair identifies tumor-specific radiosensitization by POLQ knockdown. *Cancer Res.* **70**, 2984–2993 (2010).
21. van Schendel, R., Roerink, S. F., Portegijs, V., van den Heuvel, S. & Tijsterman, M. Polymerase  $\theta$  is a key driver of genome evolution and of CRISPR/Cas9-mediated mutagenesis. *Nat. Commun.* **6**, 7394 (2015).
22. Schimmel, J., Kool, H., van Schendel, R. & Tijsterman, M. Mutational signatures of non-homologous and polymerase  $\theta$ -mediated end-joining in embryonic stem cells. *EMBO J.* **36**, 3634–3649 (2017).
23. Hucl, T. et al. A syngeneic variance library for functional annotation of human variation: application to BRCA2. *Cancer Res.* **68**, 5023–5030 (2008).
24. Drean, A. et al. Modeling therapy resistance in BRCA1/2-mutant cancers. *Mol. Cancer Ther.* **16**, 2022–2034 (2017).
25. Edwards, S. L. et al. Resistance to therapy caused by intragenic deletion in BRCA2. *Nature* **451**, 1111–1115 (2008).
26. Behan, F. M. et al. Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. *Nature* **568**, 511–516 (2019).
27. Meyers, R. M. et al. Computational correction of copy number effect improves specificity of CRISPR-Cas9 essentiality screens in cancer cells. *Nat. Genet.* **49**, 1779–1784 (2017).
28. Noordermeer, S. M. et al. The shieldin complex mediates 53BP1-dependent DNA repair. *Nature* **560**, 117–121 (2018).
29. Mitra, A. K. et al. In vivo tumor growth of high-grade serous ovarian cancer cell lines. *Gynecol. Oncol.* **138**, 372–377 (2015).
30. Elstrodt, F. et al. BRCA1 mutation analysis of 41 human breast cancer cell lines reveals three new deleterious mutants. *Cancer Res.* **66**, 41–45 (2006).
31. Dev, H. et al. Shieldin complex promotes DNA end-joining and counters homologous recombination in BRCA1-null cells. *Nat. Cell Biol.* **20**, 954–965 (2018).
32. Findlay, S. et al. SHLD2/FAM35A co-operates with REV7 to coordinate DNA double-strand break repair pathway choice. *EMBO J.* **37**, <https://doi.org/10.15252/emboj.2018100158> (2018).
33. Gao, S. et al. An OB-fold complex controls the repair pathways for DNA double-strand breaks. *Nat. Commun.* **9**, 3925 (2018).
34. Zimmermann, M. et al. CRISPR screens identify genomic ribonucleotides as a source of PARP-trapping lesions. *Nature* **559**, 285–289 (2018).
35. Mirman, Z. et al. 53BP1-RIF1-shieldin counteracts DSB resection through CST- and Polalpha-dependent fill-in. *Nature* **560**, 112–116 (2018).
36. Ghezraoui, H. et al. 53BP1 cooperation with the REV7-shieldin complex underpins DNA structure-specific NHEJ. *Nature* **560**, 122–127 (2018).
37. Setiapatra, D. & Durocher, D. Shieldin—the protector of DNA ends. *EMBO Rep.* **20**, <https://doi.org/10.15252/embr.201847560> (2019).
38. Bunting, S. F. et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* **141**, 243–254 (2010).
39. Feng, W. et al. Genetic determinants of cellular addiction to DNA polymerase  $\theta$ . *Nat. Commun.* **10**, 4286 (2019).
40. Bouwman, P. et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. *Nat. Struct. Mol. Biol.* **17**, 688–695 (2010).
41. Jaspers, J. E. et al. Loss of 53BP1 causes PARP inhibitor resistance in Brca1-mutated mouse mammary tumors. *Cancer Disco.* **3**, 68–81 (2013).
42. Callen, E. et al. 53BP1 Enforces Distinct Pre- and Post-resection Blocks on Homologous Recombination. *Mol. Cell* **77**, 26–38 e27 (2020).
43. Zhou, Y., Caron, P., Legube, G. & Paull, T. T. Quantitation of DNA double-strand break resection intermediates in human cells. *Nucleic Acids Res.* **42**, e19 (2014).
44. Tomimatsu, N. et al. Exo1 plays a major role in DNA end resection in humans and influences double-strand break repair and damage signaling decisions. *DNA Repair (Amst.)* **11**, 441–448 (2012).
45. Mimitou, E. P. & Symington, L. S. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* **455**, 770–774 (2008).
46. Nimmonkar, A. V. et al. BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. *Genes Dev.* **25**, 350–362 (2011).
47. Zhu, Z., Chung, W. H., Shim, E. Y., Lee, S. E. & Ira, G. Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* **134**, 981–994 (2008).
48. Myler, L. R. et al. Single-molecule imaging reveals the mechanism of Exo1 regulation by single-stranded DNA binding proteins. *Proc. Natl Acad. Sci. USA* **113**, E1170–E1179 (2016).
49. Cejka, P. DNA end resection: nucleases team up with the right partners to initiate homologous recombination. *J. Biol. Chem.* **290**, 22931–22938 (2015).
50. Niu, H. et al. Mechanism of the ATP-dependent DNA end-resection machinery from *Saccharomyces cerevisiae*. *Nature* **467**, 108–111 (2010).
51. Zhou, C., Pourmal, S. & Pavletich, N. P. Dna2 nuclease-helicase structure, mechanism and regulation by Rpa. *Elife* **4**, <https://doi.org/10.7554/eLife.09832> (2015).
52. Mengwasser, K. E. et al. Genetic screens reveal FEN1 and APEX2 as BRCA2 synthetic lethal targets. *Mol. Cell* **73**, 885–899.e886 (2019).
53. Lord, C. J. & Ashworth, A. PARP inhibitors: synthetic lethality in the clinic. *Science* **355**, 1152–1158 (2017).
54. Farmer, H. et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434**, 917–921 (2005).
55. Bryant, H. E. et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* **434**, 913–917 (2005).
56. Ryan, C. J., Bajrami, I. & Lord, C. J. Synthetic lethality and cancer—penetrance as the major barrier. *Trends Cancer* **4**, 671–683 (2018).
57. Drean, A., Lord, C. J. & Ashworth, A. PARP inhibitor combination therapy. *Crit. Rev. Oncol. Hematol.* **108**, 73–85 (2016).
58. Takata, K., Shimizu, T., Iwai, S. & Wood, R. D. Human DNA polymerase  $\eta$  (POLN) is a low fidelity enzyme capable of error-free bypass of 5S-thymine glycol. *J. Biol. Chem.* **281**, 23445–23455 (2006).
59. Seki, M. et al. High-efficiency bypass of DNA damage by human DNA polymerase  $\eta$ . *EMBO J.* **23**, 4484–4494 (2004).
60. Walton, J. B. et al. CRISPR/Cas9-derived models of ovarian high grade serous carcinoma targeting Brca1, Pten and Nf1, and correlation with platinum sensitivity. *Sci. Rep.* **7**, 16827 (2017).
61. Lord, C. J., McDonald, S., Swift, S., Turner, N. C. & Ashworth, A. A high-throughput RNA interference screen for DNA repair determinants of PARP inhibitor sensitivity. *DNA Repair (Amst.)* **7**, 2010–2019 (2008).
62. Booi, T. H. et al. Development of a 3D tissue culture-based high-content screening platform that uses phenotypic profiling to discriminate Selective inhibitors of receptor tyrosine kinases. *J. Biomol. Screen* **21**, 912–922 (2016).
63. Di, Z. et al. Ultra high content image analysis and phenotype profiling of 3D cultured micro-tissues. *PLoS ONE* **9**, e109688 (2014).
64. Sandercock, A. M. et al. Identification of anti-tumour biologics using primary tumour models, 3-D phenotypic screening and image-based multi-parametric profiling. *Mol. Cancer* **14**, 147 (2015).

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