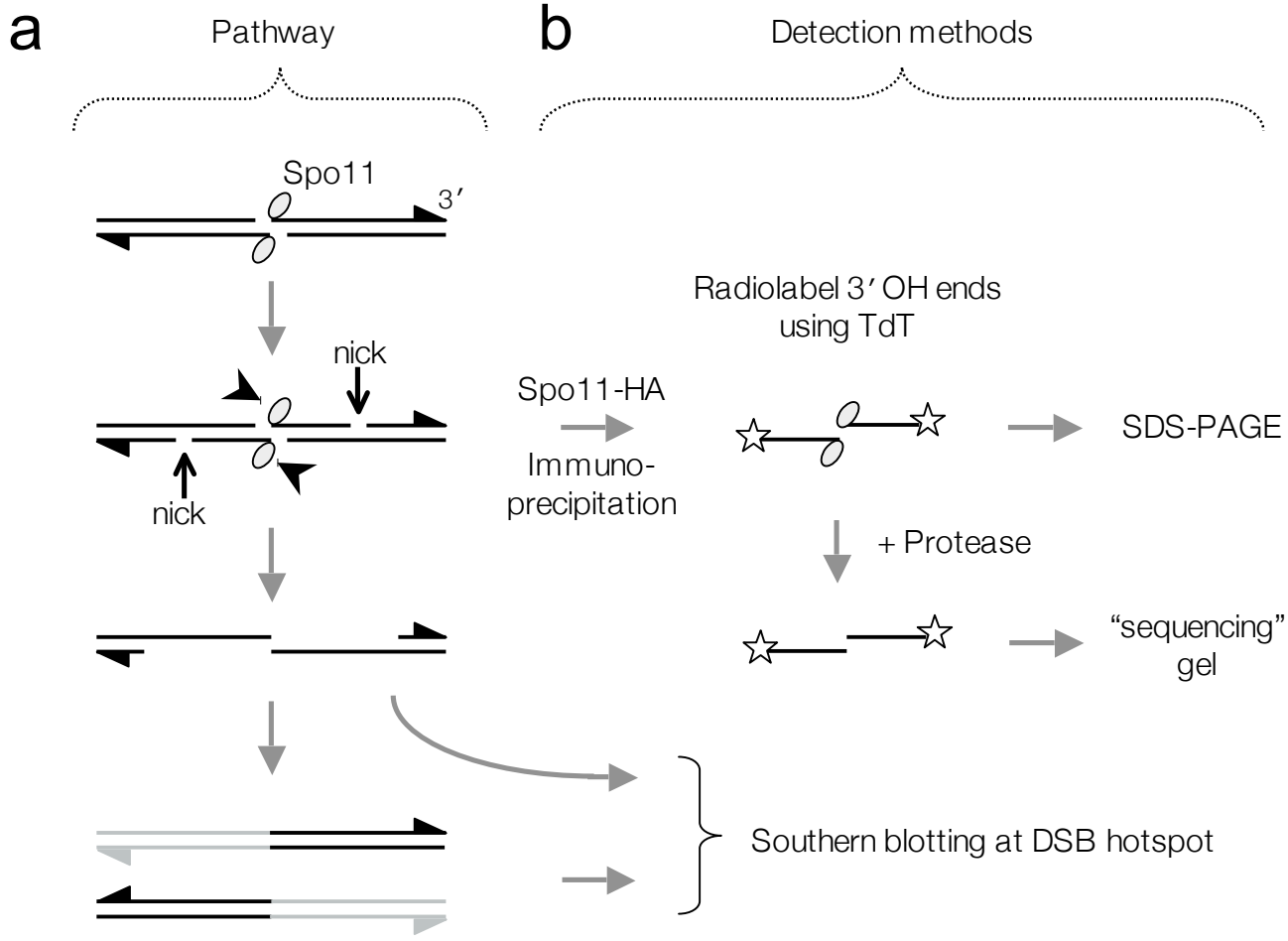

Figure S1. Overview of meiotic DSB end processing and detection methods used.
a, Meiotic DSBs are created by a Spo11 dimer (grey ellipses), which becomes covalently attached to both 5' DSB ends. Mre11/Sae2-dependent nicking creates Spo11-oligonucleotide complexes (arrowheads), and permits ssDNA resection flanking the DSB. Resected DSBs are repaired as noncrossovers, or as crossovers (shown). Strand polarity is indicated with black triangles. **b,** Spo11 oligonucleotide complexes are purified from denatured cell extracts by immunoprecipitation and end-labelled with ^{32}P-labelled chain-terminating nucleotide (stars) using terminal deoxynucleotide transferase (TdT). Spo11-oligo complexes are detected directly after fractionation on standard SDS-PAGE, or on a high-resolution denaturing urea/polyacrylamide "sequencing" gel after proteolysis (to measure more accurately the length of DNA attached to Spo11). Resected DSBs and crossovers are detected by Southern blotting and probing for a region close to a strong DSB hotspot (e.g. *HIS4*::*LEU2* or *ARE1*; see Figure S3 and S4 for details).

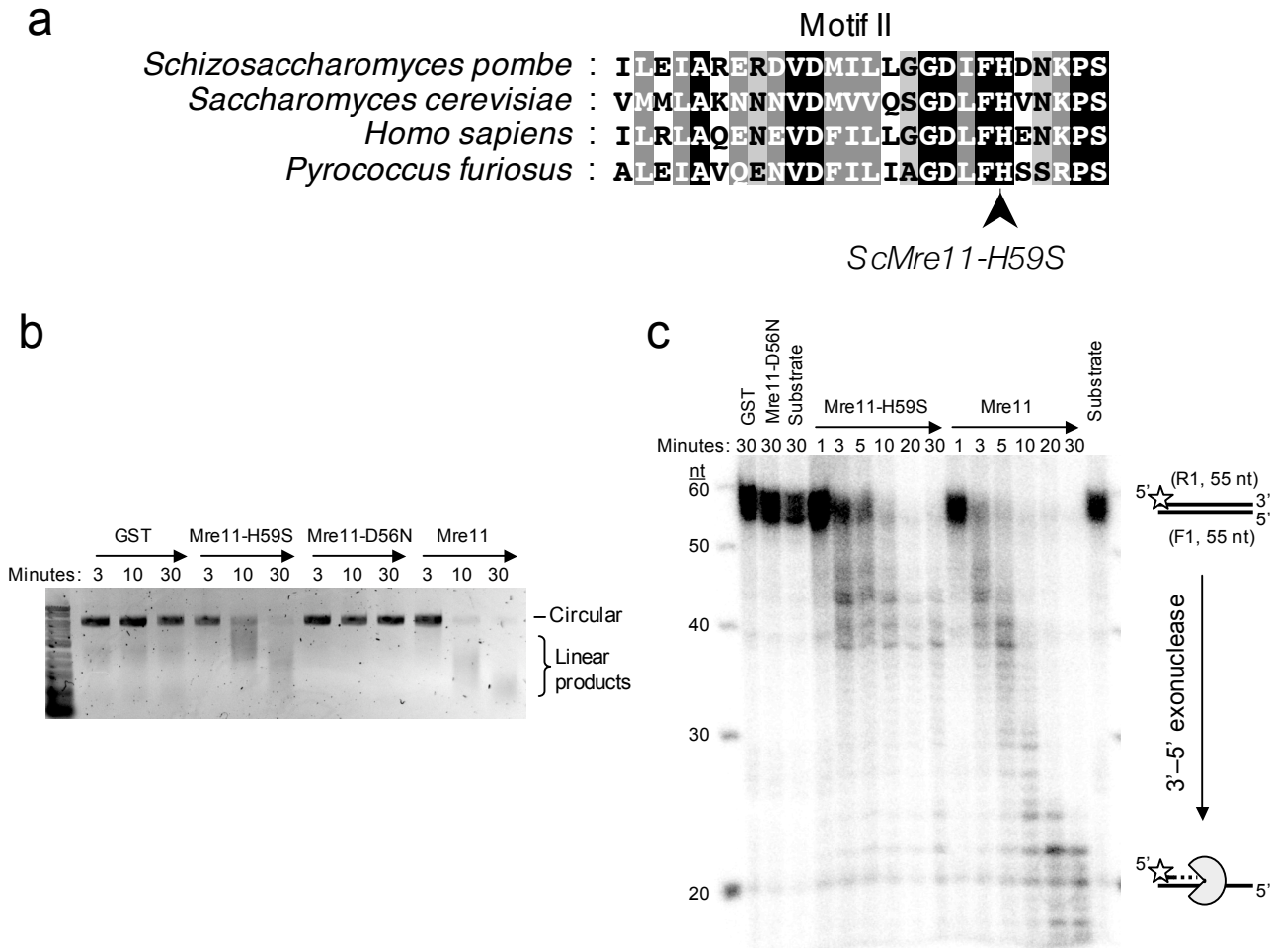

Figure S2. In vitro nuclease activity of Mre11 and Mre11-H59S.
a, Protein sequence alignment of Mre11 nuclease motif II in various species. The conserved *S.cerevisiae* histidine residue 59 mutated to serine in this study is indicated. Identical (black), conservative (dark grey), and semi-conservative (light grey) residues are indicated. **b,** ssDNA endonuclease activity. Circular single-stranded M13mp18 (0.05 pmoles; NEB) was incubated in 25 mM MOPS pH 7.0 / 60 mM KCl / 0.2 % Triton / 2 mM DTT / 5 mM MnCl2 at 37 ℃ for the indicated length of time with ~0.13 pmoles recombinant protein (GST only, GST-Mre11-H59S, GST-Mre11-D56N or GST-Mre11) overexpressed and prepared from yeast lysates of strain JEL1 (mre11Δ) bearing derivatives of plasmid pEGKT-MRE11[10]. Reactions were stopped by adding SDS to 0.3 % and Proteinase K to 0.5 mg/ml, and separated through 1 % agarose in 1x TAE stained with ethidium bromide. **c,** dsDNA 3'-5' exonuclease assay. A 55 bp dsDNA (2.8 pmol; 5'-labelled oligonucleotide R1: GTCTGTAGCACTGTGTAACACAGGCCAGATCCCATGCTGTCCTACGTGCCAGGTC annealed to its unlabelled reverse complement, F1) was incubated at 37 ℃ for the indicated length of time with ~0.13 pmoles of recombinant protein prepared as described in (b). Reactions were stopped as in (b), denatured by boiling with 2 volumes of loading dye (90 % formamide/ 10 mM EDTA / 0.01 % xylene cyanol / 0.005 % bromophenol blue), separated through 6 M urea / 12 % polyacrylamide in 1x TBE and imaged using a phosphor screen (Fuji). Cartoon depicts the exonuclease reaction being measured. Mre11-H59S exonuclease is less processive compared to wild-type Mre11.

Reference:

10. Moreau, S., Ferguson, J. R. & Symington, L. S. The nuclease activity of Mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. *Mol Cell Biol* **19,** 556-566 (1999).

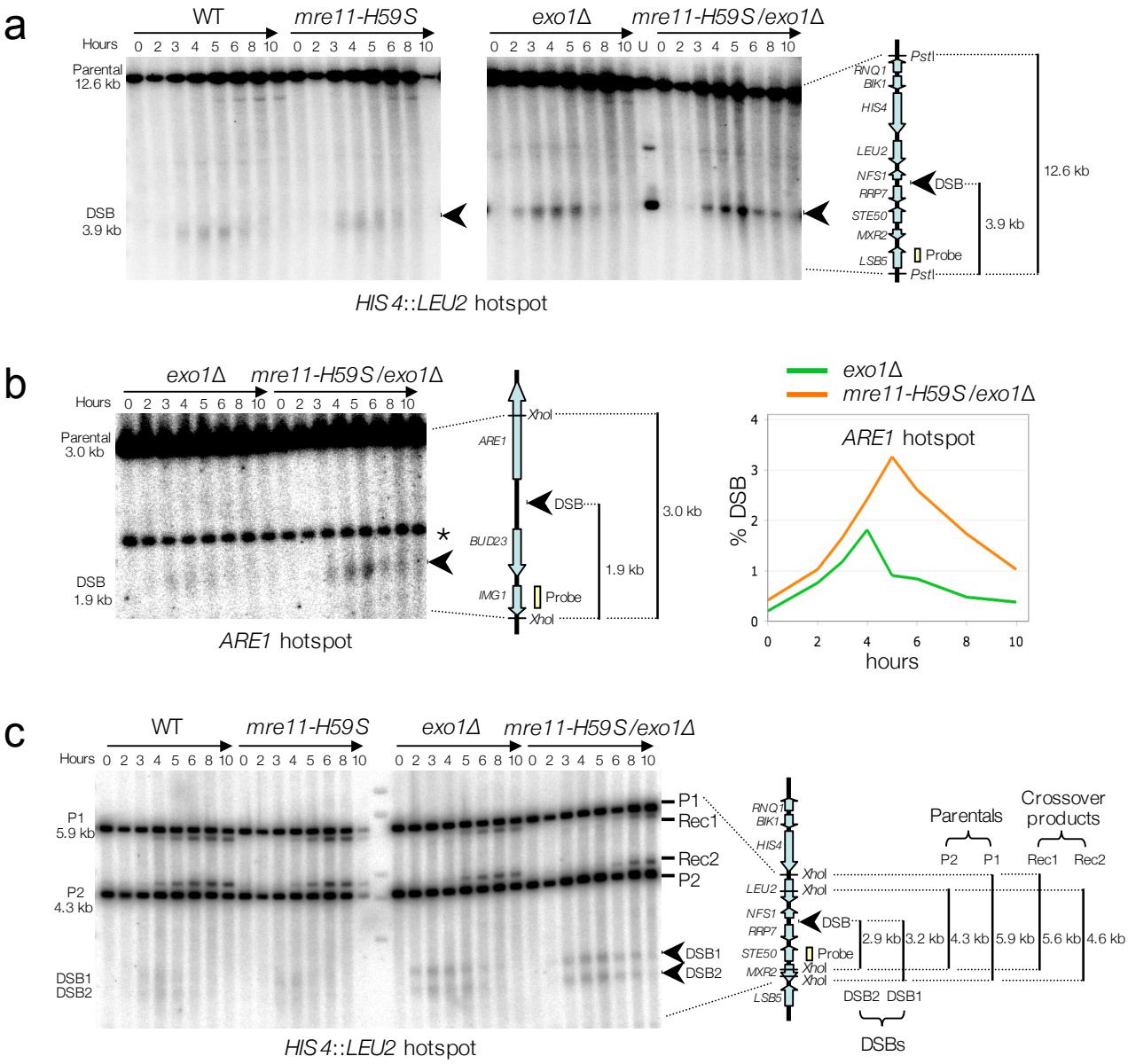

Figure S3. Southern blotting assays of meiotic DNA

Cartoons show restriction maps of relevant genomic regions. Cyan-coloured arrows indicate open reading frames. For all blots, DSB and crossover signals (expressed as a % of lane signal minus background) were quantified using Image Gauge software (Fuji). **a,** Detection of Spo11-DSBs at *HIS4*::*LEU2* recombination hotspot[28]. Genomic DNA isolated at the indicated times from synchronous meiotic timecourses of the indicated strains was digested with *Pst*I and separated through 0.7 % agarose in 1x TAE, blotted under vacuum to nylon membrane (Bio-Rad) in 0.5 M NaOH/1.5 M NaCl, and hybridised with a ^{32}P-labelled probe for the *LSB5* gene using standard conditions. (U, unresected control DNA from *sae2*Δ cells). **b,** Detection of Spo11-DSBs at *ARE1* recombination hotspot. As for (a), but DNA was digested with *Xho*I and separated through 0.6 % agarose in 1x TBE, and hybridised with a ^{32}P-labelled probe for the *IMG1* gene. **c**, Detection of crossover recombinants at *HIS4*::*LEU2* recombination hotspot[29]. As for (b), but DNA was hybridised with a ^{32}P-labelled probe for the *STE50* gene.

Reference:

28. Hunter, N. & Kleckner, N. The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. *Cell* **106,** 59-70 (2001).

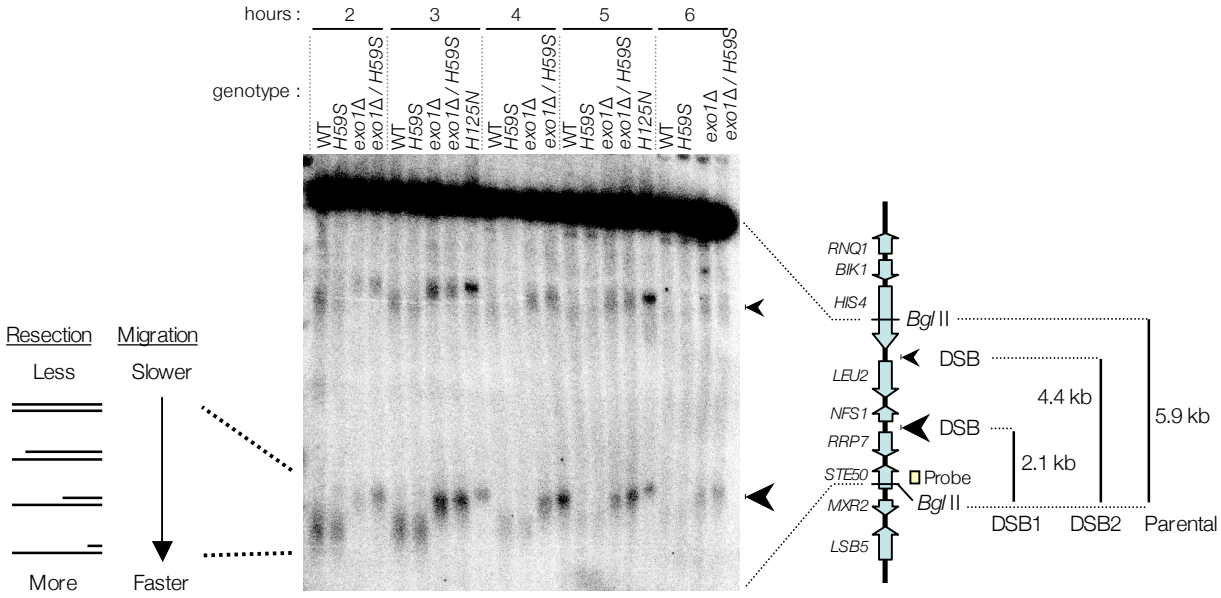

Figure S4. Detailed comparison of resection at *HIS4*::*LEU2* DSB hotspot in *mre11-H59S*

Genomic DNA isolated at the indicated times from synchronous meiotic timecourses of the indicated strains was digested with *Bgl*II and separated through 0.9 % agarose in 1x TAE, blotted under vacuum to nylon membrane (Bio-Rad) in 0.5 M NaOH/1.5 M NaCl, and hybridised with a ^{32}P-labelled probe for the *STE50* gene using standard conditions. DSBs were detected using an FLA5100 scanner and Image Gauge software (Fuji). Diagram on the left describes the relationship between the extent of resection and the rate of migration of the DSB fragments. Cartoon on the right depicts a map of *HIS4*::*LEU2* genomic region. Cyan-coloured arrows indicate open reading frames. *Bgl*II sites, probe (yellow box), and position of major and minor DSBs sites are indicated. At all timepoints, WT and *mre11-H59S* display very similar migration of DSB fragments, indicating very similar total resection profiles. Migration/resection is significantly reduced in *exo1*Δ, and reduced further in *exo1*Δ/ *mre11-H59S* double mutant, where DSB fragments migrate closer to that observed in *mre11-H125N* (where Spo11 protein is not removed and no ssDNA resection occurs).

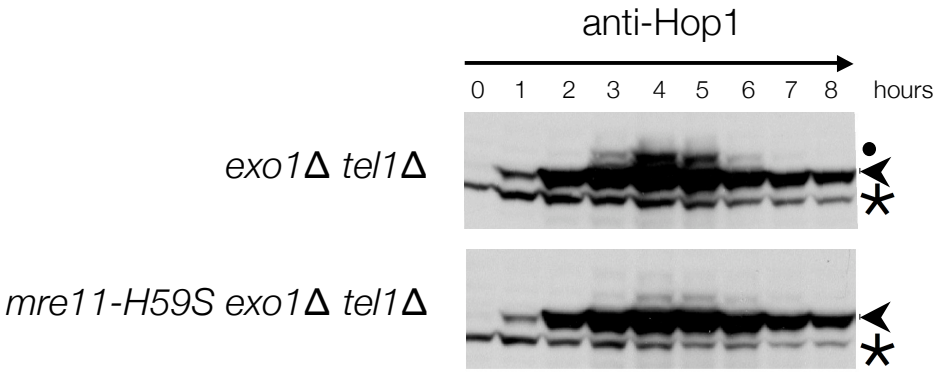

Figure S5. Tel1-independent Hop1 phosphorylation

Whole cell lysates were prepared at the indicated times from synchronous meiotic timecourses of the indicated strains, separated by 7.5 % SDS-PAGE, blotted to PVDF membrane and incubated with anti-Hop1 serum. Hop1 phosphorylation (dots) is reduced in *mre11-H59S*/e*xo1Δ*/*tel1Δ* compared to *exo1Δ*/*tel1Δ* indicating that in *exo1Δ* cells, Tel1-independent (Mec1) signalling requires the exonuclease activity of Mre11. Arrowhead is unmodified Hop1, asterisk is a cross-reacting band.

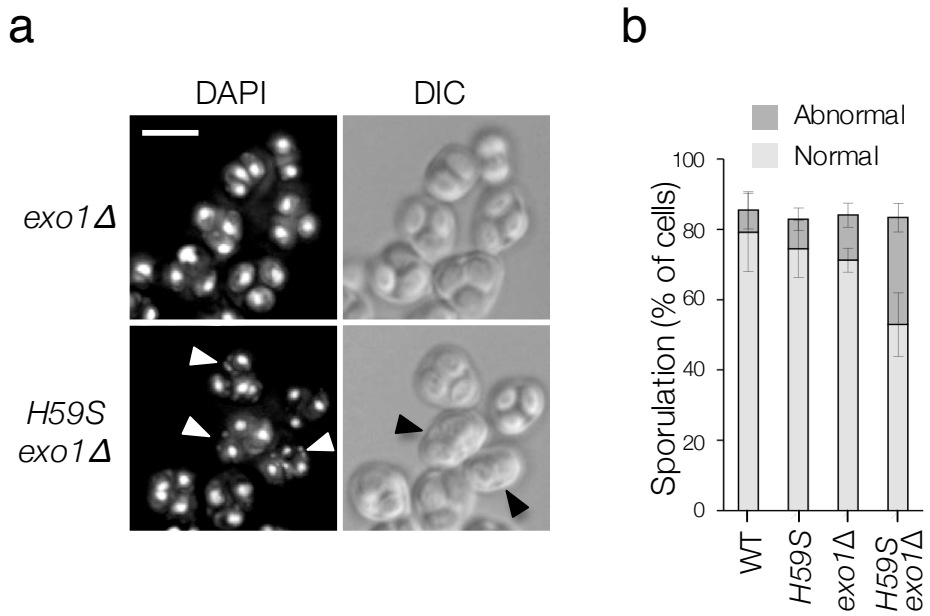

Figure S6. Frequency of abnormal tetrad formation is increased in *mre11-H59S*/*exo1*Δ

a, After 24 hours in sporulation conditions, the indicated strains were fixed with methanol and incubated with DAPI to stain DNA material. Abnormal sporulation (solid arrowheads) and fragmented chromosomes (open arrowheads) in *mre11-H59S*/*exo1*Δ are indicated. Scale bar is 5 μm. **b,** Chart shows sporulation frequencies as mean values ± s.d.

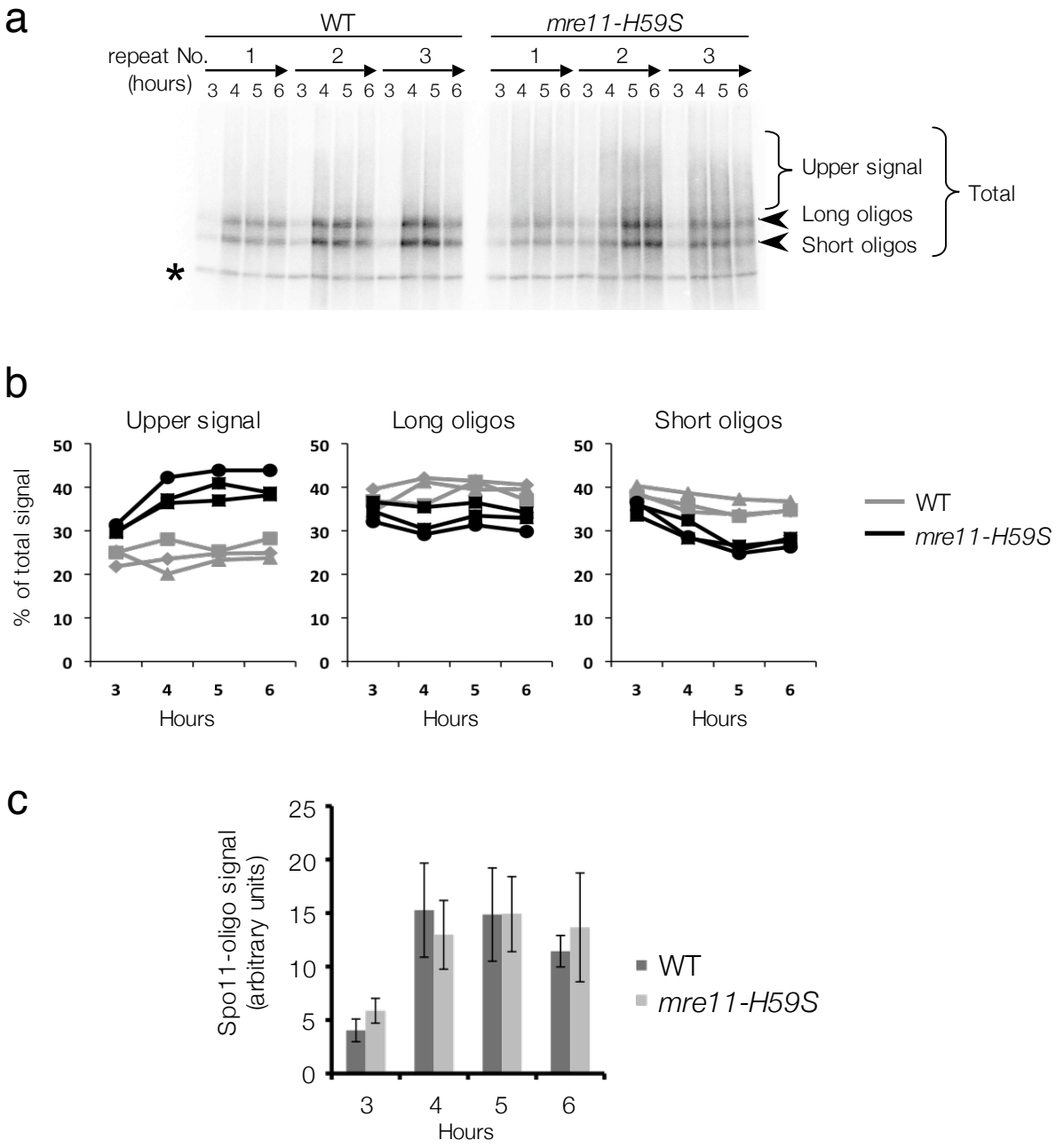

Figure S7. Identical timing of formation, but altered distribution, of Spo11-oligo complexes in WT and *mre11-H59S*

a, Spo11-oligo detection in wildtype (WT) and *mre11-H59S* during meiosis. Cultures were analysed in triplicate (1, 2 and 3), protein extracts prepared at the indicated times, and immunoprecipitated 3′-end labelled Spo11-complexes were fractionated by SDS-PAGE (see Methods). Asterisk marks an unrelated labeling artifact. **b,** Quantification of the proportion of signal present in the upper, long and short oligo species as a percentage of the total signal for the triplicate experiments shown in (a). **c,** Quantification of the total (upper+long+short) signal in (a). Quantifications were performed using ImageGauge software (Fuji). Bars in (c) show mean +/- S.D. No difference in the timing of total Spo11-oligo formation was observed between WT and *mre11-H59S*, whereas the distribution of molecules was reproducibly shifted towards longer molecules in *mre11-H59S*.

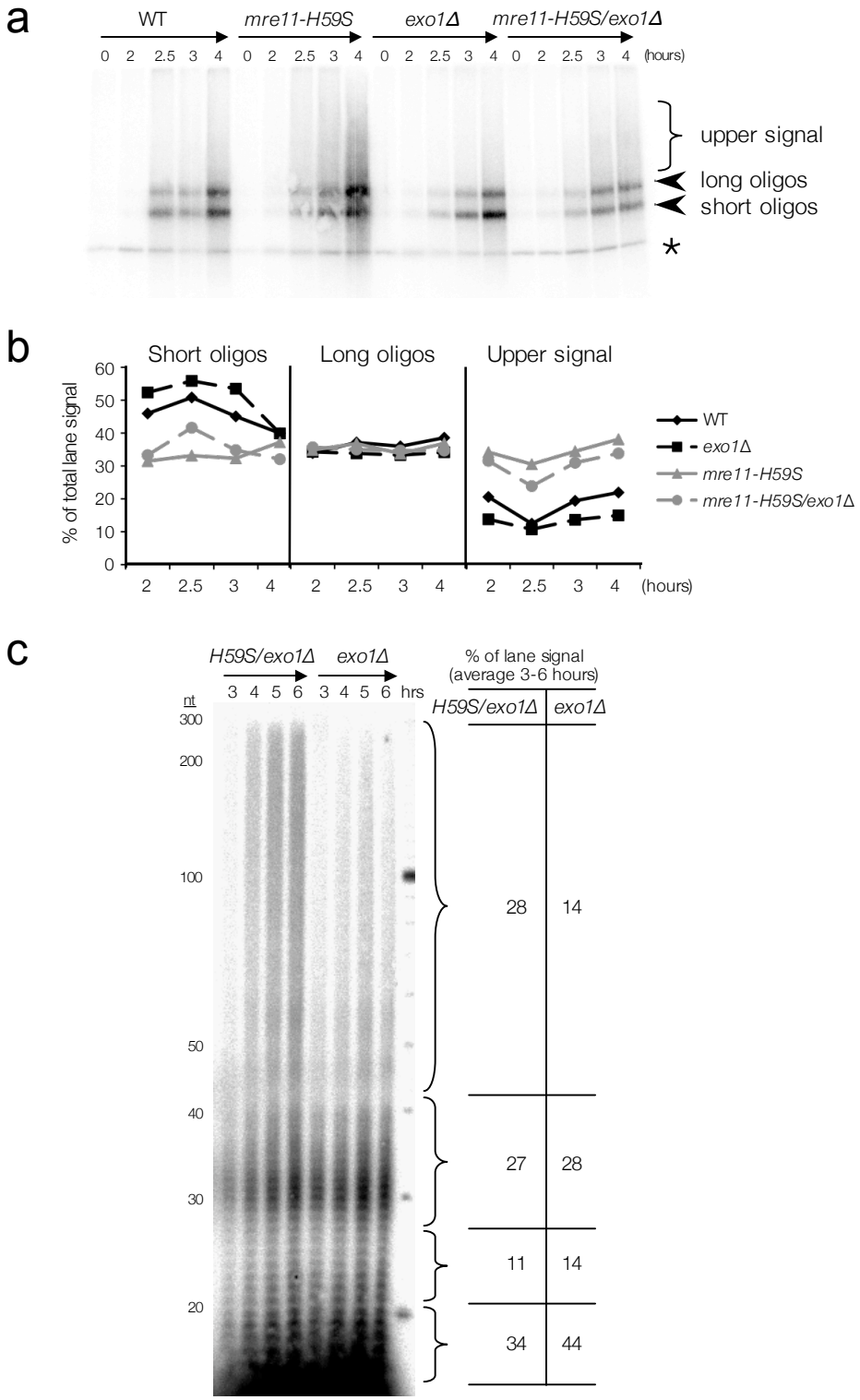

Figure S8. Altered distribution of Spo11-oligo length is independent of Exo1

a, Spo11-oligo detection in wildtype (WT), *mre11-H59S*, *exo1*Δ and *mre11-H59S/exo1*Δ during meiosis. Cell extracts were prepared at the indicated times and following 3'–end-labelling, Spo11-complexes were fractionated by SDS-PAGE. Asterisk marks an unrelated labeling artifact. **b,** Quantification of the percentage of: upper signal, long oligos, and short oligos (from a) was performed using ImageGauge software (Fuji). **c,** Nucleotide-resolution distribution of total deproteinised Spo11-oligonucleotides in *exo1*Δ and *mre11-H59S/exo1*Δ cells. Following 3'-end-labelling, Spo11-oligos were incubated with Proteinase K and fractionated by denaturing PAGE (see Methods for further details). The *mre11-H59S* mutation increases the abundance of Spo11-oligo signals 41-300 nt in length – and the effect is independent of Exo1.

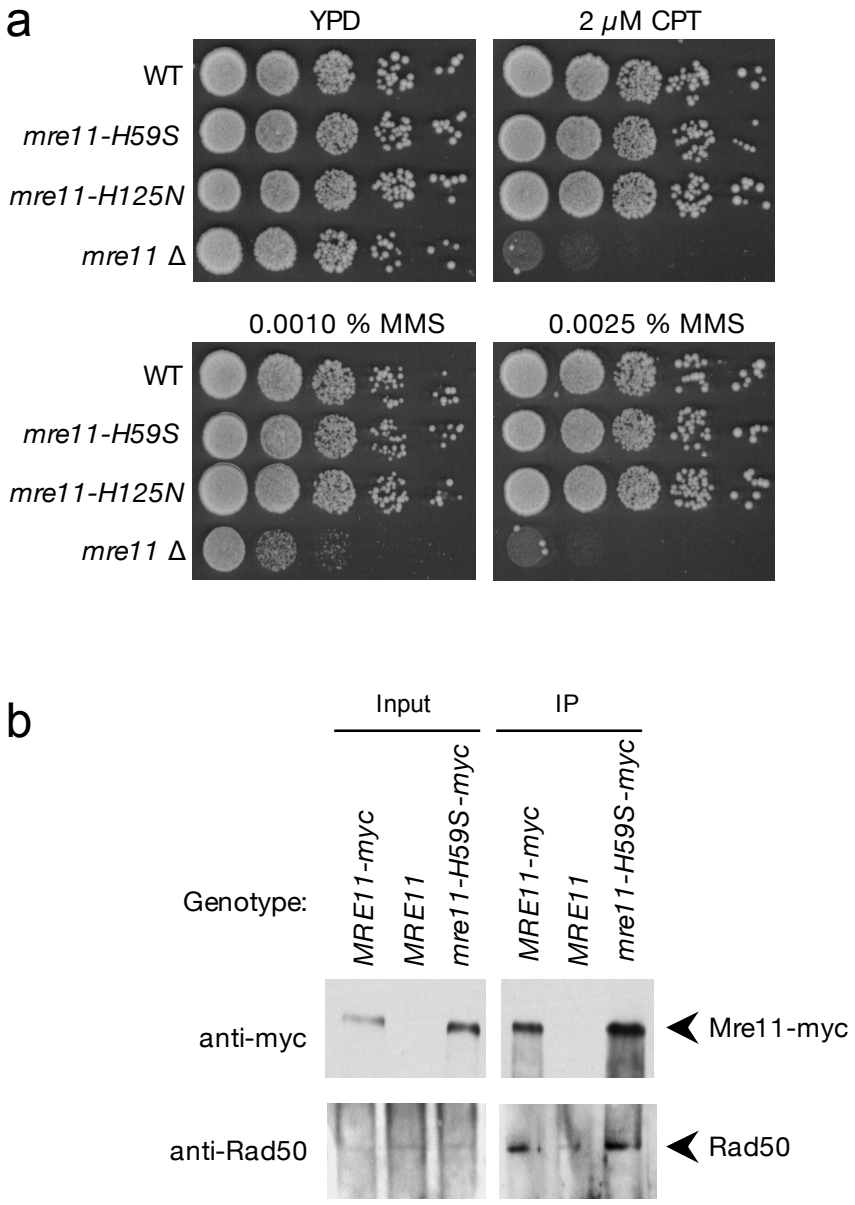

Figure S9. Mre11-complex formation in *mre11-H59S*

a, Ten-fold serial dilutions of the indicated strains were spotted onto solid media containing the indicated compounds and incubated for 2 days at 30°C. Like *mre11-H125N* (which is known to retain Mre11-complex formation[30]), *mre11-H59S* is not sensitive to low doses of DNA damage (CPT, camptothecin; MMS, methyl methanesulphonate). In addition, both mutants are far less sensitive compared to *mre11*Δ, strongly suggesting that other Mre11-complex functions are retained. **b,** Co-immunoprecipitation of Mre11 and Rad50. Protein extracts were prepared in 140 mm NaCl buffer from cells of the indicated genotype, immunoprecipitated (IP) with an anti-myc antibody (mouse monoclonal; 9E10 sc-40) and the western blot was probed with anti-myc (rabbit polyclonal; A14 sc-789) and anti-Rad50 antibody (a gift from J. Petrini).

Reference:

30. Krogh, Berit O, Bertrand Llorente, Alicia Lam, and Lorraine S Symington. "Mutations in Mre11 Phosphoesterase Motif I That Impair *Saccharomyces Cerevisiae* Mre11-Rad50-Xrs2 Complex Stability in Addition to Nuclease Activity." *Genetics* **171**, 1561-70 (2005)

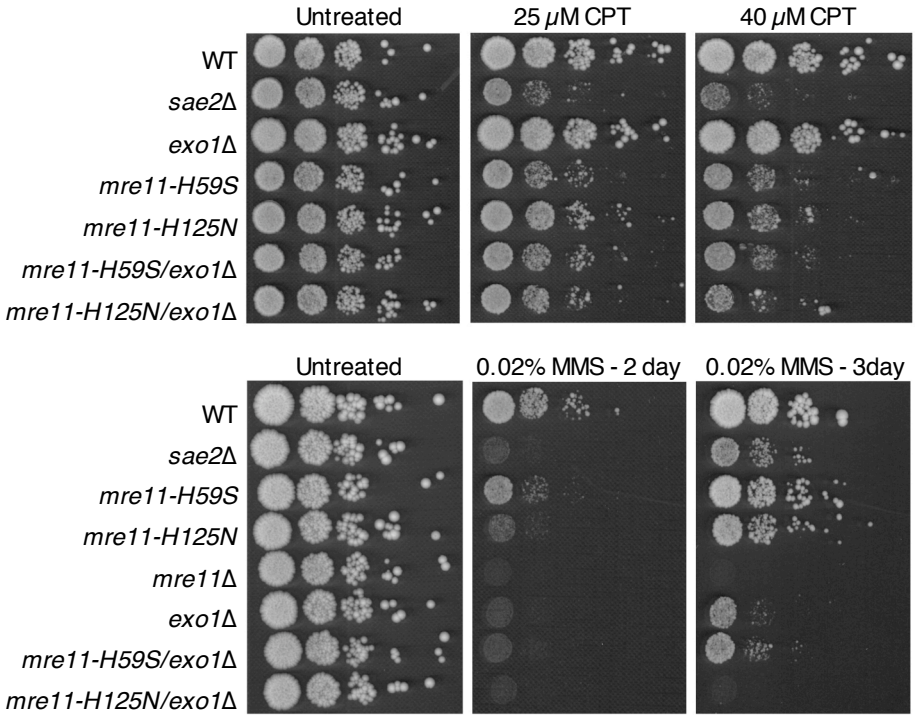

Figure S10. DNA damage sensitivity of *mre11* nuclease mutants in combination with *exo1*Δ

Ten-fold serial dilutions of the indicated strains were spotted onto solid media containing the indicated compounds and incubated for 2 or 3 days at 30°C (MMS, methyl methanesulphonate ; CPT, camptothecin). The nuclease mutants, *mre11-H59S* and *mre11-H125N* are slightly less sensitive to DNA damage than mutation of the accessory factor Sae2 (similar to human CtIP[31]). Combining *mre11-H59S* with a deletion of *EXO1* did not further sensitise cells to MMS, whereas *mre11-H125N/exo1*Δ was more sensitive than was either single mutant. This is consistent with Mre11 endonuclease activity (which is still present in *mre11-H59S*) permitting alternative exonuclease processing by the Sgs1-Dna2 complex[2,4,5]. This result, which is contrary to the synergistic effects seen in meiosis, is in fact expected because the Sgs1-Dna2 backup resection pathway does not function efficiently in meiosis[19,21]. No further sensitivity to camptothecin was observed in either double mutant consistent with factors other than Exo1 functioning to resolve topoisomerase I lesions (e.g. Tdp1).

References:

2. Mimitou, E. P. & Symington, L. S. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* **455,** 770-774 (2008).
4. 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).
5. Mimitou, E. P. & Symington, L. S. Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. *EMBO J* **29,** 3358-3369 (2010).
19. Manfrini, N., Guerini, I., Citterio, A., Lucchini, G. & Longhese, M. P. Processing of meiotic DNA double-strand breaks requires cyclin-dependent kinase and multiple nucleases. *J Biol Chem* **285,** 11628-11637 (2010).
21. Zakharyevich, K., Ma, Y., Tang, S., Hwang, P. Y., *et al*. Temporally and Biochemically Distinct Activities of Exo1 during Meiosis: Double-Strand Break Resection and Resolution of Double Holliday Junctions. *Mol Cell* **40,** 1001-1015 (2010).
31. Sartori, A. A., Lukas, C., Coates, J., Mistrik, M., *et al*. Human CtIP promotes DNA end resection. *Nature* **450,** 509-514 (2007).