

Insights into the recruitment of the H3K4me3 reader Spp1 by the meiotic double-strand break protein Mer2

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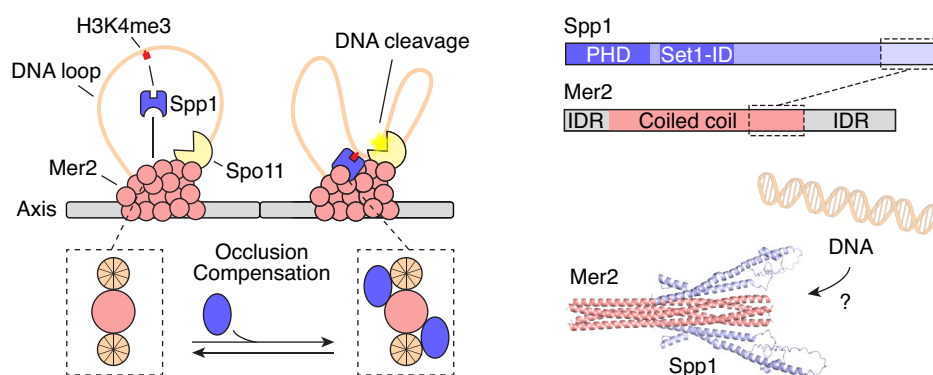
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Abstract

The formation of DNA double-strand breaks (DSBs) by Spo11 is tied to the loop-axis organization of meiotic chromosomes. Before DSB formation, chromatin loops marked by histone H3K4 trimethylation become tethered to the chromosome axis through interactions between Spp1 and Mer2. Mer2 is an essential partner of Spo11 thought to assemble the DSB machinery via biomolecular condensation, but these molecular assemblies remain poorly characterized. Here, using AlphaFold modeling, biochemical reconstitution, and biophysical validation, we explored the relationship between Mer2, Spp1, and their DNA-bound complexes. The tetrameric coiled-coil domain of Mer2 has four rotationally symmetrical sites that can bind a C-terminal α -helix of Spp1. However, Mer2-Spp1 complexes assemble with a 4×2 stoichiometry, perhaps through allosteric modulation or steric hindrance. Mer2 also accommodates multiple DNA duplexes, allowing the assembly of tripartite Mer2-Spp1-DNA complexes with branched DNA substrates and effective recruitment of Spp1 within nucleoprotein condensates. However, because the Spp1- and DNA-binding sites of Mer2 partially overlap, Spp1 recruitment reduces DNA binding by Mer2, which is compensated by positively charged residues within Spp1. Nevertheless, this DNA-binding motif appears to be dispensable for the meiotic function of Spp1. These findings provide insights into the structural organization of Mer2 and Spp1 and their role in the assembly of the meiotic DSB machinery.

Graphical abstract



Introduction

During meiotic prophase I, DNA double-strand breaks (DSBs) introduced by the topoisomerase-related Spo11 protein trigger a DNA repair mechanism by recombination, which is essential for the accurate segregation of homologous chromosomes [1, 2]. In *Saccharomyces cerevisiae*, Spo11 acts in concert with at least nine essential partners to catalyze break for-

mation and their activity is linked to chromosome organization [3]. Meiotic chromosomes form linear arrays of 10–20 kb DNA loops anchored along a proteinaceous axis [4]. Spo11 partners Rec114, Mei4, and Mer2 (RMM) are localized along the chromosome axis, but break formation happens within DNA loops [3, 5–7]. Breaks are induced within 100–300 bp hotspots located in nucleosome-depleted regions flanked by

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nucleosomes marked by histone H3 lysine 4 trimethylation (H3K4me3) [7–9]. The tethered loop-axis model suggests that cleavage by axis-immobilized Spo11 requires prior tethering of a DNA loop to the axis [5]. Spp1 was proposed to mediate this tethering through direct interactions with H3K4me3 and axis-bound Mer2, but the underlying molecular assemblies remain poorly defined [10, 11].

Spp1 is a member of the Set1 complex responsible for the deposition of H3K4me3 marks [12–14]. In the absence of Set1 or Spp1, overall DSB levels are reduced throughout the genome, although a subset of cold regions becomes hotter [8, 10, 11, 15]. The function of Spp1 in shaping the meiotic DSB landscape is independent of its interaction with Set1 but depends on direct binding to H3K4me3 marks and Mer2 [10, 11, 16].

In contrast to Spp1, which is dispensable for DSB formation, the absence of Mer2 abrogates meiotic DNA breaks [17]. In addition to binding Spp1, Mer2 also interacts with axis component Hop1 and other DSB proteins, including Rec114–Mei4, the Spo11 core complex, and the MRX complex [18–22]. Hence, Mer2 is thought to constitute an assembly hub for the meiotic DSB machinery.

Mer2 also has DNA-binding activity that is essential for DSB formation and undergoes DNA-dependent condensation *in vitro* [21, 23]. Based on these findings, Mer2, together with Rec114 and Mei4, was proposed to function as a scaffold to assemble chromatin sub-compartments [21]. Within those compartments, Mer2, Rec114, and Mei4 are thought to recruit Spo11 and other associated proteins, thereby controlling DSB induction [1, 21, 24, 25].

Here, we investigated the interaction between Mer2 and Spp1 and their relationship with the DNA-binding and condensation activities of Mer2. Our results suggest that the recruitment of Spp1 to Mer2 tetramers is controlled allosterically to assemble complexes with a 4×2 stoichiometry. Spp1 binding partially occludes the DNA-binding surface of Mer2. However, the cost associated with Spp1 recruitment is compensated for by direct interactions of Spp1 with DNA. This work provides molecular insights into the assembly of the meiotic DSB machinery.

Materials and methods

Preparation of expression vectors

Plasmids are listed in [Supplementary Table S3](#) and oligos are listed in [Supplementary Table S4](#). The vectors used to express HisSUMO-Mer2 (pCCB750) and HisSUMO-eGFP-Mer2 (pCCB777) from *Escherichia coli* were previously described [21]. The expression vector for HisMer2 was generated by PCR amplification of the MER2 coding sequence from pCCB750 with primers km001F and km001R and cloned into pET28b by Gibson assembly to yield pKM001. Expression vectors for Mer2 truncations were obtained by inverse PCR and self-ligation using template pCCB750 and primers cb1346 and cb1497 or cb1342 and dd121 to generate pDD057 (HisSUMO-Mer2^{41–314}) and pDD060 (HisSUMO-Mer2^{1–224}), respectively. Plasmid pDD057 was further used as template with primers cb1342 and dd121 to generate pDD065 (HisSUMO-Mer2^{41–224}).

The sequence of SPP1 was amplified from yeast genomic DNA using primers pl001 and pl002 and cloned into pSMT3 vector by Gibson assembly to yield pPL001 (HisSUMO-Spp1).

SPP1 with a Tev cleavage site was amplified using primers pl029 and pl030 and cloned by Gibson assembly in pMAL-c2x to yield pPL012 (MBP-Spp1). GST-Tev was amplified from pCCB407 using primers pl035 and pl036 and cloned into pEtDuet-1 to yield pPL015. The SPP1 sequence with a Tev site was then amplified from pPL012 with primers pl037 and pl038 and cloned into pPL015 to yield pPL016 (GST-Spp1). The expression vector for GST-HisSpp1 was created by amplifying the coding sequence of SPP1 with primers km004F and pl038 and cloning by Gibson assembly into a XhoI and KpnI digested product of pPL016, yielding pKM004.

Sequences coding for HisSUMO-Mer2 and MBP-Spp1 were amplified from pCCB750 and pPL012 with primers pl031 and pl032, and pl033 and pl034 and cloned by Gibson assembly into pETduet to yield pPL014.

The expression vector for wild-type MBP-Mer2 was obtained by amplification of MER2 from pCCB750 with primers pl027 and pl028 and cloning into pMAL-c2x by Gibson assembly to yield pPL011. The expression vector for the Mer2-V195D mutant was obtained by inverse PCR and self-ligation using template pPL011 and primers pl053 and pl054 to yield pPL036.

The expression vector for Spp1^{161–353} truncation was generated by inverse PCR and self-ligation of template pPL016 with primers pl145 and cb1486 to yield pPL094 (GST-Spp1^{161–353}). Expression vectors for full-length and truncated Spp1-R5K mutants were obtained using templates pPL016 and pPL094 and primers pl146 and pl147 to yield respectively pPL099 (GST-Spp1-R5K) and pPL095 (GST-Spp1^{161–353}-R5K).

Co-expression vectors for HisSUMO-Mer2^{138–224} and MBP-Spp1^{161–353} were made as follows. First, the sequences coding for the Mer2 and Spp1 fragments were amplified from pCCB750 and pPL012 using primers pl078 and pl079, and pl080 and pl081 and cloned into pSMT3 and pMAL-c2x by Gibson assembly to yield pPL041 and pPL042. Then, the sequences coding for HisSUMO-Mer2^{138–224} and MBP-Spp1^{161–353} were amplified from pPL041 and pPL042 using primers pl082 and pl083, and pl104 and pl105, respectively, and cloned separately into pEtDuet-1 by Gibson assembly to yield pPL045 and pPL053. Finally, the co-expression vector for HisSUMO-Mer2^{138–224} and MBP-Spp1^{161–353} truncations was obtained by restriction digestion of pPL045 and pPL053 using XhoI and HindIII and ligation of the fragments of interest to yield pPL054.

Point mutations for the analysis of the Mer2-Spp1 interaction surface were obtained by inverse-PCR and self-ligation using pPL054 (HisSUMO-Mer2^{138–224}, MBP-Spp1^{161–353}) as a template. PCR primers and resulting plasmids for each mutant are as follows: Spp1-Q317A (pl112 and pl113, pPL064), Spp1-Q318A (pl114 and pl115, pPL065), Spp1-S324A (pl134 and pl135, pPL066), Spp1-R328A (pl132 and pl133, pPL067), Spp1-L332A (pl118 and pl119, pPL068), Spp1-R336A (pl120 and pl121, pPL069), Spp1-Q339A (pl122 and pl123, pPL070), Spp1-I342A (pl124 and pl125, pPL071), Mer2-N184A (pl140 and pl141, pPL072), Mer2-Q188A (pl138 and pl139, pPL073), Mer2-E190A (pl136 and pl137, pPL074), Mer2-V195D (pl053 and pl054, pPL075), Mer2-D202A (pl116 and pl117, pPL076), Mer2-T208A (pl128 and pl129, pPL077), Mer2-R209A (pl126 and pl127, pPL078), Mer2-E214A (pl130 and pl131, pPL079), and Mer2-V195A (pl053 and pl144, pPL089).

The co-expression vector for Spp1^{307–353} and Mer2^{138–224} truncations was obtained by inverse PCR and self-ligation using pPL054 as a template and primers pl143 and cb1486 to yield pPL090.

The expression vector for Spp1 fused with mScarlet was obtained by amplifying mScarlet sequence from pCCB785 [21] with primers pl057 and pl058 and cloned in pPL016 by Gibson assembly to yield pPL032. Expression vector for wild-type Mer2 fused to eGFP was previously described [21] and V195D mutant was obtained using this construct (pCCB777) as a template by inverse PCR and self-ligation using pl053 and pl054 to yield pPL037.

Expression and purification of recombinant proteins

For the expression of Spp1 proteins, GST-tagged Spp1 (WT and R5K), untagged Spp1 (full-length and 161–353 truncations, WT and 5KR mutants), His-tagged Spp1, and mScarlet-Spp1 (WT and R5K), vectors were transformed in *E. coli* C41 cells and plated on LB plates containing the appropriate antibiotic. Cells were then cultured at 37°C to an optical density (OD₆₀₀) of 0.6. All the proteins were expressed as a fusion with an N-terminal GST tag cleavable with TEV protease. Expression was carried out at 18°C overnight with 0.25 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). Cell pellets were frozen in liquid nitrogen and kept at –80°C until use. All the purification steps were carried out at 0–4°C. Cells were then resuspended in lysis buffer (50 mM HEPES pH 7.5, 300 mM NaCl, 5% glycerol, 0.1% Triton, 5 mM β-mercaptoethanol, and 1 mM PMSF), lysed by sonication and centrifuged at 43 000 × *g* for 30 min. The cleared extract was loaded onto 1 ml of pre-equilibrated Glutathione sepharose resin (Cytiva). The column was washed extensively with lysis buffer. Proteins that retained the GST tag were then eluted with 5 ml of lysis buffer containing 10 mM reduced glutathione. For the versions without GST, the tag was cleaved on the column by overnight treatment with TEV protease. The flow-through was then loaded on a Superdex 200 Increase 10/300 GL column pre-equilibrated with gel filtration buffer (20 mM HEPES pH 7.5, 500 mM NaCl, 10% glycerol, and 0.1 mM dithiothreitol (DTT)). For all purifications, after gel filtration, fractions containing the protein were concentrated using 10-kDa cut-off Amicon centrifugal filters (Millipore). Aliquots were flash frozen in liquid nitrogen and stored at –80°C.

For the expression of Mer2 proteins [untagged Mer2 (WT and V195D), Alexa-labeled Mer2, truncations (Mer2^{138–224} and Mer2^{1–224}), eGFP-tagged Mer2 (WT and V195D)] and for co-expression of Mer2–Spp1 complexes, vectors were transformed in *E. coli* BL21 DE3 cells and plated on LB plates containing the appropriate antibiotic. Cells were then cultured in liquid medium at 37°C to OD₆₀₀ of 0.6. All constructs expressed Mer2 with an N-terminal 6His-SUMO tag, cleavable with Ulp1. Co-expression vectors also expressed Spp1 with an N-terminal MBP tag, cleavable by TEV protease. Expression was carried out at 30°C for 3 h with 1 mM IPTG for Mer2 and at 18°C overnight with 0.25 mM IPTG for the complexes. Cell pellets were frozen in liquid nitrogen and kept at –80°C until use. All the purification steps were carried out at 0–4°C. Cells were resuspended in nickel buffer (25 mM HEPES-NaOH pH 7.5, 1 M NaCl, 10% glycerol, 0.1 mM DTT, 40 mM imidazole, and 1 mM PMSF), lysed by sonication, and centrifuged at 43 000 × *g* for 30 min. The cleared extract was loaded onto

1 ml of pre-equilibrated Ni-NTA resin (Thermo Scientific). The column was washed extensively with nickel buffer then eluted in buffer containing 250 mM imidazole. To remove the His-SUMO tag of Mer2, complexes were incubated for 3 h with Ulp1. For Mer2 protein labeled with Alexa Fluor 488 (Invitrogen no. A10235), the Ulp1-treated protein was dialysed overnight in nickel buffer without imidazole, followed by fluorophore conjugation at room temperature for 1 h before gel filtration. Alexa Fluor 488 has a tetrafluorophenyl ester moiety that reacts with primary amines. All the proteins were then loaded either on a Superdex 200 Increase 10/300 GL column (Mer2) or on a Superose 6 Increase 10/300 GL column (Mer2–Spp1 complexes) pre-equilibrated with gel filtration buffer (25 mM HEPES-NaOH pH 7.5, 300 mM NaCl, 10% glycerol, 1 mM DTT, and 5 mM EDTA). For all purifications, after gel filtration, fractions containing the protein were concentrated using 10-kDa cutoff Amicon centrifugal filters (Millipore). Aliquots were flash frozen in liquid nitrogen and stored at –80°C.

For SEC-SAXS analyses, HisSUMO Mer2–MBP Spp1, Mer2–MBP Spp1, and Mer2^{138–224}–MBP Spp1^{161–353} complexes were obtained by co-transformation of the appropriate expression vectors, affinity chromatography with Ni-NTA resin, followed by size-exclusion chromatography using SAXS buffer (25 mM HEPES-NaOH pH 7.5, 500 mM NaCl, 5 mM EDTA, 5% glycerol, and 1 mM TCEP). Mer2^{41–224}–Spp1 and Mer2^{41–224}–Spp1^{161–353} complexes were obtained by mixing purified Mer2 and Spp1 subunits at a 4:6 ratio, followed by size-exclusion chromatography with SAXS buffer.

Pulldown assays

For Ni-NTA pulldowns made from cell lysates, tagged Mer2 and Spp1 truncations were co-expressed in 50 ml of *E. coli* BL21 cultures and purified by affinity chromatography on nickel resin following a procedure similar to that described above. Cells were lysed by sonication and centrifuged at 15 000 rpm for 15 min. The cleared extract was loaded onto 100 μl of pre-equilibrated Ni-NTA resin (Thermo Scientific) in nickel buffer containing 25 mM HEPES-NaOH pH 7.5, 1 M NaCl, 0.1% Triton X-100, 10% glycerol, 0.1 mM DTT, and 40 mM imidazole. After 30 min incubation on a rotating wheel at 4°C, the resin was loaded on a column, washed with 15 ml nickel buffer and then eluted in buffer containing 250 mM imidazole. Eluted proteins were boiled in 1× Laemmli buffer and analyzed by SDS–PAGE.

GST pulldowns were made with the indicated concentrations of purified proteins in 40 μl reactions in buffer containing 20 mM HEPES-NaOH pH 7.5, 300 mM NaCl, 5% glycerol, and 1 mM DTT. After a 2 h incubation on ice, the proteins were mixed with 100 μl of pre-equilibrated Glutathione sepharose resin (Cytiva) and left for 30 min on ice with occasional mixing. The resin was then centrifuged at 1500 × *g* for 1 minute and washed three times with 1 mL pulldown buffer. Both input and output were resuspended in the same volume of Laemmli buffer and analyzed by SDS–PAGE.

SEC-MALS

Light scattering data were collected using a Superose 6 increase 10/300 GL Size Exclusion Chromatography (SEC) column connected to an AKTA Pure Chromatography System (Cytiva). The elution from SEC was monitored by a differential refractometer (Optilab, Wyatt), and a static and dynamic,

multi-angle laser light scattering (LS) detector (miniDAWN, Wyatt). The SEC–UV/LS/RI system was equilibrated in 25 mM HEPES–NaOH pH 7.5, 300 mM NaCl, 1 mM DTT, and 5 mM EDTA at a flow rate of 0.4 ml/min. The weight average molecular masses were determined across the entire elution profile at intervals of 0.5 s from static LS measurement using ASTRA software.

Functionalization of AFM tips

AFM experiments were performed using Pyrex-nitride probe with a triangular gold-coated pyramidal silicon nitride PNP-TR-AU50 cantilever (Nanoworld) calibrated using the thermal noise method. For tip functionalization, the cantilevers were rinsed with ethanol, dried under a gentle nitrogen stream, and cleaned for 15 min by UV and ozone treatment (Jetlight). After that, tips were incubated overnight in a polyethylene glycol (PEG) solution prepared by mixing 0.05 mM HS-(CH₂)₁₁-EG₃-OH with 0.05 mM HS-(CH₂)₁₁-EG₃-NTA dissolved in ethanol in a 99:1 (v/v) ratio. The tips were then rinsed with ethanol, dried with nitrogen, and treated with 50 mM NiCl₂ for 30 min [26].

The tips were functionalized with Spp1 protein through an N-terminal His6 tag. The cantilevers were placed on Parafilm (Bemis NA), incubated with 40 µl of protein solution (0.1 mg/ml in buffer containing 20 mM HEPES–NaOH pH 7.5, 300 mM NaCl, 10% glycerol, 1 mM DTT, and 5 mM EDTA) for 1 h, and washed three times with protein binding buffer (20 mM Tris–HCl, 10 mM MgCl₂, and 100 mM NaCl).

Preparation of protein-coated model surfaces

Proteins were immobilized on gold-coated silicon surfaces (Platypus) using NHS-EDC chemistry [27]. The surfaces were first rinsed with ethanol, dried under nitrogen, and cleaned by UV-ozone treatment for 15 min. The surfaces were then incubated overnight in a solution containing alkanethiols (90% 1 mM 11-mercapto-1-undecanol and 10% 1 mM 16-mercaptohexadecanoic acid in ethanol). Next, the surfaces were rinsed with ethanol, dried under a gentle nitrogen stream, and immersed in a solution containing 25 mg/ml dimethylaminopropyl carbodiimide (EDC) and 10 mg/ml N-hydroxy succinimide (NHS) dissolved in H₂O. Finally, the chemically activated surfaces were incubated with 50 µl of the respective protein (15 µg/ml in buffer containing 20 mM HEPES–NaOH pH 7.5, 300 mM NaCl, 10% glycerol, 1 mM DTT, and 5 mM EDTA) on Parafilm for 1 h at 4°C, washed with protein binding buffer, and used on the same day.

Force–distance curve-based AFM

The acquisition of force–distance (FD) curves by AFM on model surfaces was carried out in protein binding buffer at room temperature, using the tips and surfaces described above. AFM tips were vertically approached and retracted from the surface while recording the force. A Multimode Nanoscope VIII (Bruker) was used. Areas of 25 µm² (1024 pixels/map) were scanned, the tip approach and retraction speeds were fixed at 1 µm/s, the ramp size was set to 500 nm, a setpoint force of 500 pN, and a resolution of 32 × 32 pixels. Curves were assessed for specific adhesion events individually, and the proportion of curves showing binding versus those that did not was used to calculate the binding frequency.

Dynamic force spectroscopy (DFS) experiments on model surfaces were conducted using a ForceRobot 300 (Bruker) un-

der the same conditions as the binding frequency measurements, with a varying retraction velocity of 0.1, 0.2, 1, 5, 10, and 20 µm/s. Force curves were analyzed using JPK Data Processing software (version 6.1.149). The results were plotted as DFS graphs and rupture force histograms for distinct loading rates ranges using Origin software (OriginLab), and the data were fitted to the Bell–Evans models as described elsewhere to extract the kinetic off rate [28, 29]. Briefly, the relationship between rupture force and loading rate is described by the following equation:

$$F(r) = \left(\frac{k_B T}{x_\beta} \right) \ln \left(\frac{r x_\beta}{k_{off} k_B T} \right)$$

where $F(r)$ is the most probable rupture force, k_B Boltzmann's constant, T the temperature in Kelvin, x_β the distance to the transition state, r the loading rate and k_{off} the off-rate constant at zero force.

For kinetic on-rate (k_{on}) analysis, the binding frequency was measured by varying the contact time (t) (the time the AFM tip is in contact with the surface). Briefly, the relationship between interaction time (τ) and binding frequency (BF) is described by the following equation:

$$BF = A * \left[1 - \exp \left(-\frac{t - t_0}{\tau} \right) \right]$$

where A represents the maximum BF and t_0 the lag time. Data fitting and τ extraction were performed using Origin software. The k_{on} value was subsequently calculated using the following equation:

$$k_{on} = \frac{4\pi r_{eff}^3 N_A}{3n_b \tau}$$

where, r_{eff} is the effective radius of the sphere, n_b is the number of binding partners, and N_A is the Avogadro constant. The effective volume is defined as $V_{eff} = (4\pi r_{eff}^3)/3$, representing the volume in which the interaction can take place. The parameters used for k_{on} calculation were as follows: $n = 1$ molecule of Spp1; $\tau = 75 \pm 12$ ms, extracted from the curve fit (Fig. 11); $r_{eff} = 14.5$ nm, calculated based on the AlphaFold model of Spp1 that yields a distance between the N-terminal His tag and C-terminal Mer2 binding site of about 11.5 nm, to which we added 3 nm for the PEG linker. Quantitative parameters for k_{on} and k_{off} are presented in [Supplementary Table S1](#).

SEC-SAXS

All experiments were performed at the SOLEIL BioSAXS beamline SWING (Gif-sur-Yvette, France). SEC-SAXS data were collected in HPLC mode using a Shodex KW404-4F column pre-equilibrated with SAXS buffer (25 mM HEPES–NaOH pH 7.5, 500 mM NaCl, 5 mM EDTA, 5% glycerol, and 1 mM TCEP). Samples were concentrated on-site to ~3–5 mg/ml using 10-kDa cutoff centrifugal filters (Amicon). 90 µl samples were injected and eluted at a flow rate of 0.2 ml/min while scattering data were collected with an exposure time of 990 ms and a dead time of 10 ms. The scattering of pure water was used to calibrate the intensity to absolute units. Data reduction was performed using FoxTrot. Data were processed using BioXTAS RAW [30] and analyzed using RAW and the ATSAS package [31]. The information on data collection and derived structural parameters is summarized in [Supplementary Table S2](#). *Ab initio* calculations were

performed with the DENSS suite [32] and DAMMIF [33], and P2 symmetry was imposed during shape reconstruction.

The molecular models of the various Mer2-Spp1 complexes were generated from the AlphaFold models obtained in this work and refined in Xplor-NIH v 2.49 [34]. In all refinement runs, the positions of the Mer2 coiled-coil domain and the interacting Spp1 helices 1 and 2 were kept fixed; SUMO globular domains treated as rigid body groups, while the intervening linkers and terminal tails given full torsional degrees of freedom. The computational protocol comprised an initial simulated annealing step followed by the side-chain energy minimization. The total minimized energy function consisted of the standard geometric (bonds, angles, dihedrals, and impropers) and steric (van der Waals) terms, a knowledge-based dihedral angle potential [34], and a SAXS energy term incorporating the experimental data [35]. In each refinement run, 100 structures were calculated, and 10 lowest-energy solutions retained for further analysis. The agreement between the experimental and calculated SAXS curves (obtained with the calcSAXS helper program, which is part of the Xplor-NIH package) was assessed by calculating the χ^2 :

$$\chi^2 = \frac{1}{n-1} \sum_{i=1}^n \left(\frac{I(q)_{calc, i} - I(q)_{exp, i}}{\delta I(q)_{exp, i}} \right)^2$$

where $I(q)_{calc, i}$ and $I(q)_{exp, i}$ are the scattering intensities at a given q for the calculated and experimental SAXS curves, $\delta I(q)_{exp, i}$ is an experimental error on the corresponding $I(q)_{exp, i}$ value, and n is the number of data points defining the experimental SAXS curve. The models were fitted into the *ab initio* densities with UCSF ChimeraX [36].

DNA substrates and gel shift assays

A fluorescent substrate that mimics a Holliday Junction was generated by annealing complementary oligos (cb923, cb924, cb925, cb934). Oligos were mixed in equimolar concentrations (10 μ M) in STE (100 mM NaCl, 10 mM Tris-HCl pH 8, and 1 mM EDTA), heated and slowly cooled on a PCR thermocycler (98°C for 3 min, 75°C for 1 h, 65°C for 1 h, 37°C for 30 min, and 25°C for 10 min).

Binding reactions (20 μ l) were carried out in 25 mM Tris-HCl pH 7.5, 10% glycerol, 100 mM NaCl, 2 mM DTT, and 1 mg/ml BSA. Reactions contained 10 nM fluorescently labeled substrate and the indicated concentration of protein. Complexes were assembled for 30 min at 30°C and separated by gel electrophoresis. Binding reactions were separated on 5% TAE-polyacrylamide gels at 150 V for 2.5 h, and gels were visualized using a Typhoon scanner (Cytiva).

In vitro condensation assays

Condensation was induced by threefold dilution of Spp1 and Mer2 proteins in buffer containing DNA and no salt to reach final 20 μ l reactions that contained 3 nM (100 ng) supercoiled plasmid DNA (pUC19), 25 mM Tris-HCl (pH 7.5), 10% glycerol, 100 mM NaCl, 2 mM DTT, 1 mg/ml bovine serum albumin (BSA), and 5% PEG 8000. After 30 min at 30°C with occasional mixing, 4 μ l was dropped onto a microscope slide and covered with a coverslip. Images were captured on a Zeiss Axio Observer with a 100 $\times$ /1.4 NA oil immersion objective. Images were analyzed with ImageJ using a custom-made script. In brief, a fixed threshold was applied to 129.24 $\times$ 129.24- μ m (2048 $\times$ 2048-pixel) images. The intensity in-

side the focus mask was integrated. Data were analyzed using GraphPad Prism 9.

Yeast strain construction

Yeast strains were from the SK1 background. All strains used in this study are listed in [Supplementary Table S5](#). The *SPP1-R5K* mutant was constructed by transformation of a DNA fragment obtained by overlap extension PCR. The 3' end of the *SPP1* gene containing the R5K mutation was amplified from plasmid pPL099 with primers pl160 and pl161. The KanMX6 cassette was amplified with primers pl162 and pl163. The two PCR products were purified by gel extraction and combined in the overlap extension PCR using primers pl160 and pl163. The resulting product was transformed into CBY006 cells and transformants selected with G418. The *SPP1-R5K* mutation was confirmed by PCR and sequencing of the *SPP1* genomic region and strains carrying this allele were subsequently produced by mating and tetrad dissection.

Southern blot analysis of DSBs

Meiotic DSB analysis by Southern blotting was performed as described [37]. Briefly, diploid strains were grown overnight at 30°C on YPG plates (1% yeast extract, 2% peptone, and 3% glycerol) and then streaked on YPD plates (1% yeast extract, 2% peptone, and 2% dextrose). Two days later, diploid colonies were transferred to liquid YPD and cultured overnight with vigorous shaking. Saturated cultures were diluted in pre-sporulation (SPS) medium (0.5% Yeast Extract, 1% peptone, 0.67% yeast nitrogen base, 1% potassium acetate, 50 mM potassium phthalate, and antifoam) to a density of 1.3×10^6 cells/ml. After 7 h at 30°C, cultures reached a concentration of $1\text{--}1.3 \times 10^7$ cells/ml. Cells were diluted again in SPS to a final density of 3.25×10^5 cells/ml and incubated for 13–16 h until they reached a density of $3\text{--}4 \times 10^7$ cells/ml. Meiosis was induced by transferring the cells to 2% KOAc, supplemented with amino acids and 0.01% polypropylene glycol, and cells were harvested at the indicated time. After DNA extraction, 1–1.2 μ g of genomic DNA was digested either with HindIII-HF or KpnI-HF (NEB), for Southern blot analysis at CYS3 and PES4 hotspots, respectively, and separated on a 1% TBE-agarose SEA-KEM LE gel for 16 h at 70 V. DNA was transferred to a positively charged nylon membrane (Amersham Hybond-N+ from Cytiva) by vacuum transfer and hybridized with CYS3 probe (amplified with primers TTTGAAGGT-CACCGACATCC and CGATTAGTTGGTGGCTTGTT) or with the PES4 probe (amplified with primers AGCTTAT-CAAAGTGTGTGCA and GGCTGGGGATCTTGAAAGTA). Films were exposed for 3 days and developed by autoradiography using a Typhoon Biomolecular Imager (Cytiva).

Western blot analysis of H3K4me3 levels

For western blot analysis of H3K4me3 levels, cells were harvested 4 h into meiosis. Denaturing whole-cell extracts were prepared in 10% trichloroacetic acid with agitation in the presence of glass beads. Precipitated proteins were solubilized in Laemmli sample buffer and appropriate amounts of protein were separated by SDS-PAGE and analyzed by western blotting. Antibodies were mouse monoclonal anti-H3K4me3 (Invitrogen G.532.8, 1/10 000) and rabbit polyclonal anti-PGK1 antibody [22C5D8] (Abcam, ab113687, 1/10 000). Secondary antibodies were anti-mouse (Chemicon AP308P, 1/10 000)

and anti-rabbit (Dako P044801-2, 1/5000). Western blots were revealed using the Amersham imager 600 imaging system and quantified using ImageJ.

Results

Single-molecule probing of Mer2–Spp1 interaction by atomic force spectroscopy

Mer2 is a 314 amino-acid protein with a central coiled-coil domain (residues 41–224) flanked by N- and C-terminal intrinsically disordered regions (IDRs) (Fig. 1A). The coiled coil assembles as a parallel tetrameric bundle, the C-terminal half of which (138–224) is necessary and sufficient for tetramerization [23] and contains the Spp1 interaction domain [10] (Fig. 1B). Mutation of a conserved V195 residue within Mer2, predicted to be surface exposed, abolishes Mer2–Spp1 co-immunoprecipitation and yeast-two-hybrid interaction and recapitulates the meiotic phenotype of a *spp1Δ* strain [16].

Spp1 is a 353 amino-acid protein with an N-terminal plant homeodomain (PHD) finger that binds H3K4me3 modifications [38, 39] (Supplementary Fig. S1A), a Set1 interaction domain (Set1-ID) [16] (Supplementary Fig. S1B), and a C-terminal region predicted to contain two long α -helices, here referred to as helix 1 and 2, required for Mer2 binding [10] (Fig. 1A and B).

To verify that the *in vitro* interaction between Spp1 and Mer2 depends on Mer2–V195, as shown *in vivo* [16], we incubated GST-tagged Spp1 with wild-type Mer2 or a V195D mutant and pulled down proteins by glutathione affinity. SDS–PAGE analysis showed that ^{GST}Spp1 efficiently interacts with wild-type Mer2, but not the V195D mutant (Fig. 1C).

We set up a single-molecule interaction assay based on atomic force microscopy (AFM) [40, 41] to further characterize the interaction between Mer2 tetramers and Spp1 monomers. Spp1 was grafted to an Ni^{2+} -NTA-coated tip through an N-terminal His₆ affinity tag, and Mer2 was covalently immobilized to the surface by NHS-EDC chemistry (Fig. 1D) [42]. The interaction between Spp1 and Mer2 was measured by bringing the tip into contact with the surface and retracting it. Each approach–retraction cycle yields a FD curve, a subset of which reveals a binding event, detected by the deflection of the tip upon retraction (Fig. 1E). In the presence of Spp1 and Mer2, $7.6 \pm 1.8\%$ (mean $\pm$ SD, $n = 7$), of the curves showed interaction (Fig. 1F). When Mer2 was replaced by the V195D mutant, the binding frequency dropped to $4.5 \pm 1.3\%$. When either Spp1 was omitted from the tip, or Mer2 was omitted from the surface, the binding frequency was further decreased to about 2%. Hence, the assay captures specific single-molecule interactions between Spp1 and Mer2.

We extracted the force required to disrupt Spp1 and Mer2 interaction (rupture force). At standard velocity (1 $\mu\text{m/s}$, corresponding to a loading rate $> 10\,000$ pN/s), wild-type Mer2 yielded an average rupture force of 108.2 ± 3.1 piconewton (pN) (mean $\pm$ S.E.M.), which was reduced to 82.4 ± 4.4 pN for the Mer2–V195D mutant (Fig. 1F, right). This explains the strongly reduced interaction observed by pulldown, since weaker interactions will lead to faster dissociation during pull-down and washes.

To determine kinetic parameters of the interactions, we measured the rupture force at different loading rates (i.e. the force acting on the interaction over time). At a loading rate of 500 pN/s, the rupture force between wild-type Mer2 and

Spp1 peaks at about 36.7 ± 1.3 pN (Fig. 1G). Plotting the rupture force against the loading rate allows us to extract the free energy landscape under non-equilibrium conditions through application of a Bell–Evans model and deduce the dissociation rate (k_{OFF}) [42]. This revealed a $k_{\text{OFF}} = 0.19 \pm 0.15$ s^{−1} (Fig. 1H; Supplementary Fig. S2 and Supplementary Table S1). In addition, varying the contact time (t) allows us to estimate the interaction time, and thereby deduce the kinetic on-rate (k_{ON}), estimated at $10.2 \pm 0.8 \times 10^4$ M^{−1}·s^{−1} (Fig. 1I). Hence, a single Spp1 monomer binds to Mer2 tetramers with a dissociation constant ($K_{\text{D}} = k_{\text{OFF}}/k_{\text{ON}}$) of 1.9 ± 1.6 μM . This corresponds to a binding affinity about two orders of magnitude lower than the ensemble average affinity previously obtained by microscale thermophoresis (MST) [20], suggesting that the single-molecule interaction we characterized by AFM is rather different from that captured in bulk biochemical assays (see below).

Structural model of a minimal Mer2–Spp1 complex

Previous biochemical analyses indicated that Mer2 and Spp1 form a complex with a 4×2 (Mer2:Spp1) stoichiometry [20]. To gain further insights, we used AlphaFold3 [43] to predict the structure of a complex composed of Mer2 residues 138–224 and Spp1 residues 161–353. AlphaFold produced high-confidence models showing two Spp1 subunits bound to the surface of the Mer2 tetrameric coiled coil (Fig. 2A and Supplementary Fig. S3A).

Within the model, the C-terminal α -helix of Spp1 (helix 2, residues W307–R351) is bound anti-parallel to the Mer2 coiled coil. A long α -helix (helix 1, residues D169 to S233, interrupted by a kink at R181 and D182) folds back onto helix 2. In a pulldown assay, Spp1^{161–353} was sufficient for the interaction with Mer2^{138–224}, but Spp1^{307–353} was not (Fig. 2B), suggesting that helix 1 contributes to the stability of the Mer2–Spp1 interface. Consistently, in the absence of helix 1, AlphaFold could not accurately model the structure of the Mer2–Spp1 complex (Supplementary Fig. S3B).

In the AlphaFold model, Spp1 helix 2 interacts with two adjacent Mer2 helices (Fig. 2C). Spp1–Q317 contacts Mer2–R209 from one subunit and E214 from the other subunit; Spp1–Q318 contacts Mer2–T208; Spp1–S324 and R328 point inside the trimeric interface and contact Mer2–D202; Spp1–L332 contacts Mer2–V195; Spp1–R336 makes electrostatic interactions with Mer2–E190; Spp1–Q339 is in proximity to Mer2–Q188 and Spp1–I342 points toward Mer2–N184.

Sequence alignments of Spp1 and Mer2 homologs from seven species of the Saccharomycetaceae family indicate that most of the residues within the predicted Mer2–Spp1 interface are conserved (Fig. 2D and E). In particular, Spp1 residues R328, L332, R336, and Mer2 residues N184, Q188, R209, and E214 are invariant in the aligned sequences. Most other residues are also conserved or replaced by similar amino acids, including Mer2–V195 that is substituted to isoleucine in *Tetrapisispora phaffii*. Consistent with the structural conservation of the interface, AlphaFold yielded similar models across pairs of Mer2–Spp1 homologs from Saccharomycetaceae (Supplementary Fig. S4).

To test the AlphaFold model experimentally, we mutated the predicted interface residues to alanine and quantified the effect of the mutation on the interaction between Spp1 and Mer2 by pulldown. Wild-type and mutant ^{MBP}Spp1^{161–353} and

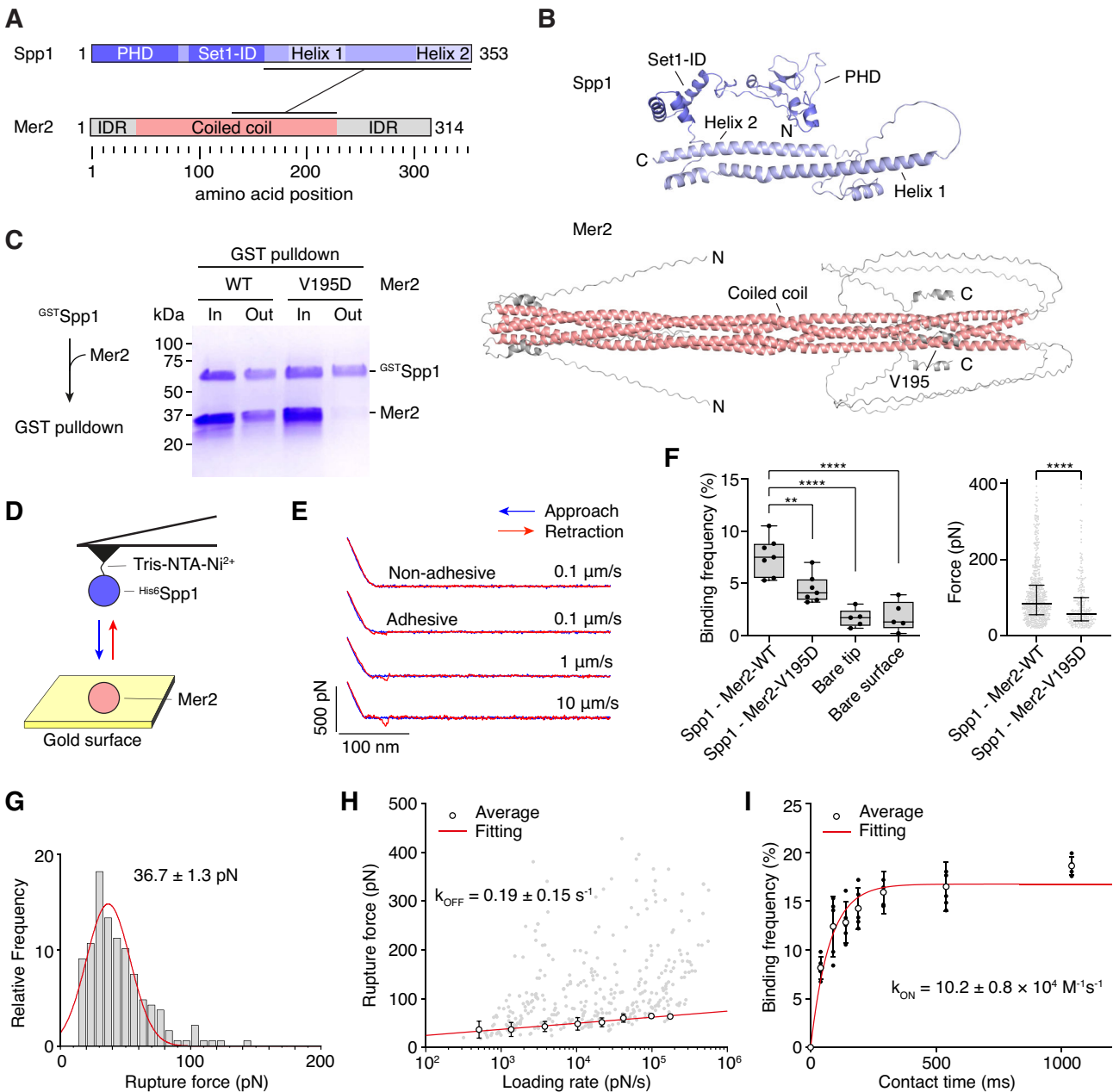


Figure 1. Single-molecule analysis of the interaction between Mer2 and Spp1. **(A)** Domain structure Spp1 and Mer2. PHD, plant homeodomain; Set1-ID, interaction domain. The Mer2-Spp1 interaction domain is indicated. **(B)** AlphaFold predictions of Spp1 (AF-Q03012-F1) and tetrameric Mer2. The position of the Spp1-interacting residue V195 is shown. **(C)** Pulldown of wild-type and mutant (V195D) Mer2 with GST-tagged Spp1. In, input; Out, output. **(D)** Setup of the AFM assay to quantify the kinetics and thermodynamics of the interaction between Spp1 and Mer2. Note that, while Spp1 is monomeric [20], a GST tag will artificially dimerize Spp1. **(E)** Examples of FD curves obtained upon approach and retraction of the tip from the surface at different loading rates. **(F)** (Left) Binding frequency between tip-immobilized Spp1 and surface-bound wild-type or mutant (V195D) Mer2, compared to controls without Spp1 or Mer2 (median and range of $n = 5-7$ independent experiments, $**P < 0.01$, $****P < 0.0001$ (one-way ANOVA)). (Right) Binding forces between Spp1 and wild-type or mutant Mer2 [median and range of $n = 587$ (WT) and 232 (V195D) individual measurements, $****P < 0.0001$ (Welch's t -test)]. **(G)** Histogram of the forces measured upon tip retraction (range of loading rate 350–900 pN/s). The data were fitted to a normal distribution ($R^2 = 0.88$). **(H)** Dynamic force spectroscopy analysis showing the rupture force as a function of loading rate for individual measurements (gray dots indicate individual unbinding events, $n = 6$ experiments). Open circles represent the mean rupture force obtained by fitting histograms at discrete loading rates and error bar represent the SD (see Supplementary Fig. S2). The red solid line represents the Bell-Evans fit extrapolated to zero-force. **(I)** Binding frequency as a function of contact time. Data points show the mean binding frequency $\pm$ SD from five adhesion maps, each consisting of 256 individual FD curves. Red solid line shows a non-linear curve fitted to the data.

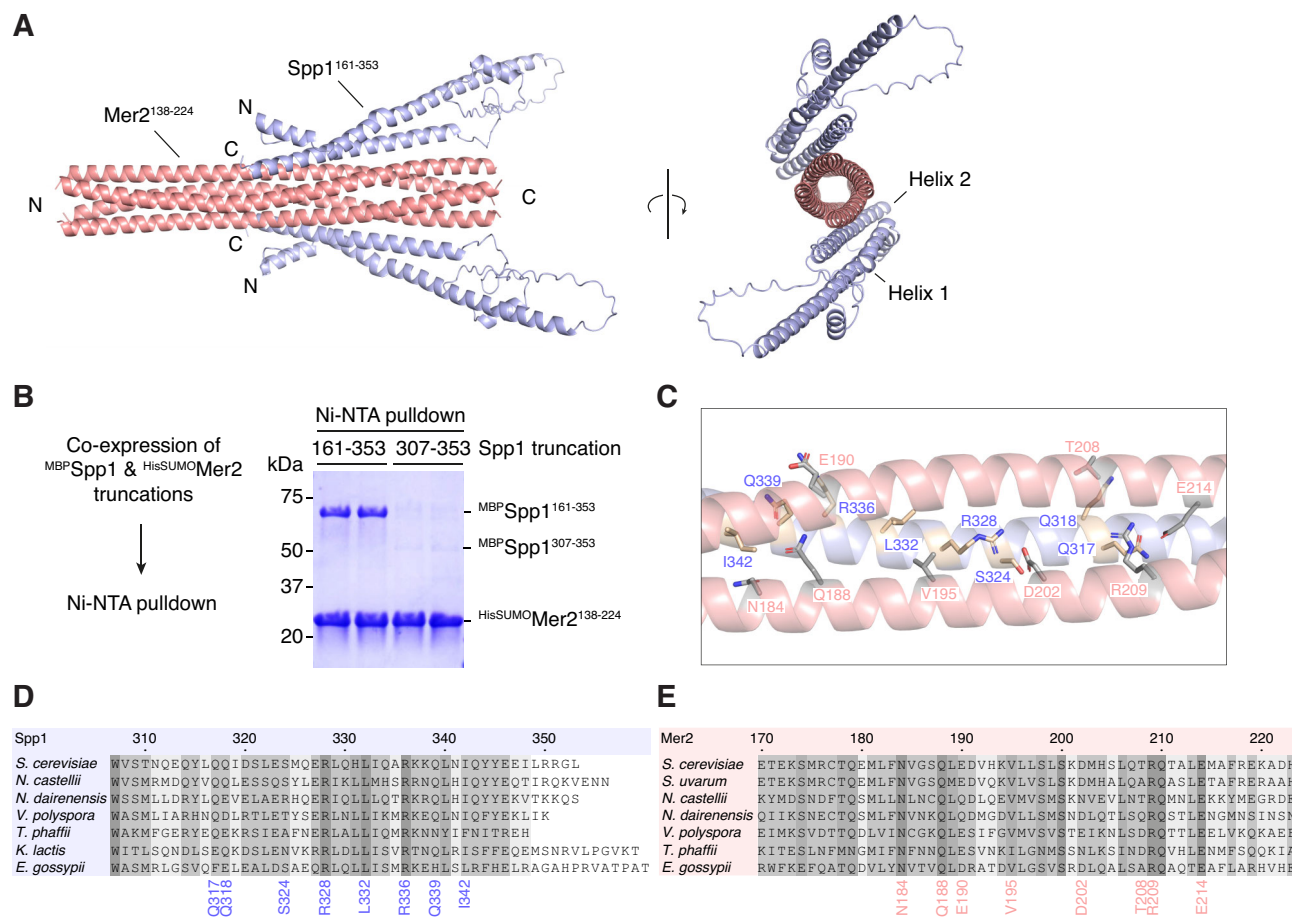


Figure 2. Model of a minimal Mer2-Spp1 complex. (A) AlphaFold prediction of a 4×2 complex between Mer2¹³⁸⁻²²⁴ and Spp1¹⁶¹⁻³⁵³. Input sequences are provided in [Supplementary Table S6](#). (B) Pulldown of MBP-tagged Spp1 truncations with HisSUMO-tagged Mer2¹³⁸⁻²²⁴. (C) Zoom of the interface between Mer2 and Spp1 with residues of interest highlighted. Mer2, pink; Spp1, blue. (D and E) Multiple sequence alignment of Spp1 (D) and Mer2 (E) with residues mutated in Fig. 3 highlighted.

HisSUMO-Mer2¹³⁸⁻²²⁴ were co-expressed in *E. coli* and pulled down by Ni-NTA affinity. The Mer2-V195D mutant was used as a control. As expected, Mer2-V195D and V195A strongly compromised the interaction with Spp1 (Fig. 3A). In addition, alanine substitution of Mer2 residues E190 and R209 significantly decreased the interaction with Spp1. On the Spp1 side, R328A and L332A mutations strongly decreased the interaction with Mer2, and S324A, R336A, and I342A had milder defects (Fig. 3B). Interestingly, the mutations that led to the strongest phenotypes were located towards the center of the predicted interface (Fig. 2C). Hence, the mutagenesis analysis supports the AlphaFold model of the Mer2-Spp1 interaction domain.

The stoichiometry of Mer2-Spp1 complexes

The AlphaFold model does not readily account for the reported 4×2 stoichiometry of the Mer2-Spp1 complex [20]. Indeed, given the four-fold radial symmetry of Mer2, one might expect Mer2 tetramers to accommodate up to four Spp1 subunits. Consistent with that idea, AlphaFold produces high-confidence 4×4 models without steric clashes between Spp1 subunits ([Supplementary Fig. S5A](#)). The binding sites are identical between 4×2 and 4×4 models, and the respective position of two Spp1 subunits to adjacent or op-

posite sides of the Mer2 coiled coil appears to be arbitrary ([Supplementary Fig. S5B](#)).

To clarify this, we sought to revisit the stoichiometry of Mer2-Spp1 complexes. We co-expressed MBP-tagged Spp1 and HisSUMO-tagged Mer2, purified a complex by Ni-NTA affinity chromatography and analyzed purified complexes by size-exclusion chromatography followed by multi-angle light scattering (SEC-MALS). This yielded a molecular mass of 365 ± 6 kDa, consistent with a 4×2 stoichiometry (expected MW = 370 kDa). We also produced a collection of tagged and untagged full-length and truncated complexes and analyzed the samples by SEC-MALS ([Supplementary Fig. S6](#)) and small angle X-ray light scattering (SAXS) ([Supplementary Table S2](#)). All the configurations yielded a 4×2 stoichiometry, regardless of the analysis method (Fig. 4A).

We considered the possibility that the purification protocol enriches for Mer2 compared to Spp1 subunits. However, when Spp1 was added in excess to Mer2, the complexes still yielded a 4×2 stoichiometry ([Supplementary Fig. S6C and D](#)). Hence, Mer2 tetramers only accommodate two Spp1 subunits and the underlying reason is not immediately evident from the AlphaFold models.

To further test the AlphaFold models, we used SAXS to determine the overall shape of the Mer2-Spp1 complexes. SAXS measurements of a 4×2 Mer2⁴¹⁻²²⁴-Spp1¹⁶¹⁻³⁵³ complex were in excellent agreement with the curve expected

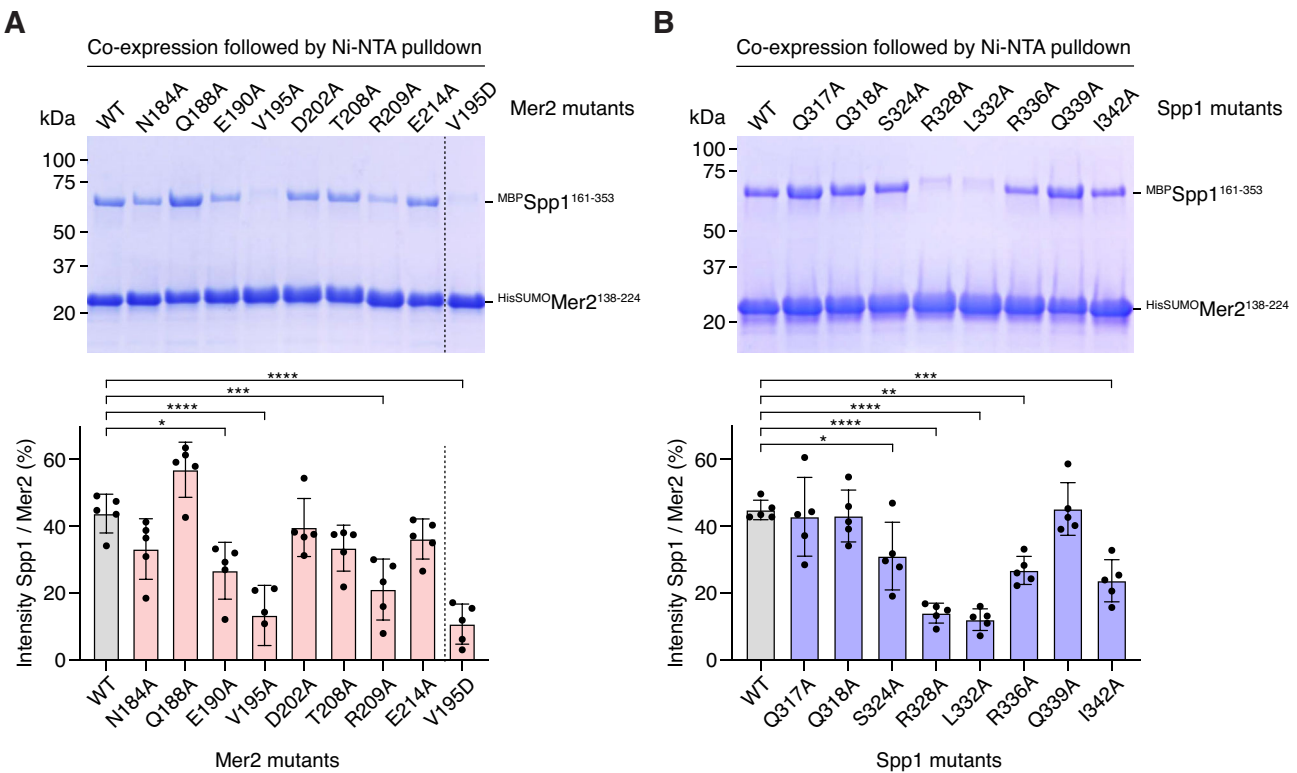


Figure 3. Mutational analysis of the Mer2-Spp1 interaction surface. **(A and B)** Ni-NTA pulldown analysis of MBP-tagged Spp1¹⁶¹⁻³⁵³ with HisSUMO-tagged Mer2¹³⁸⁻²²⁴. Panel (A) shows Mer2 mutants, panel (B) shows Spp1 mutants. Quantifications show the mean $\pm$ SD from five independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (one-way ANOVA).

based on structural models showing two Spp1 subunits bound to opposite sides of the Mer2 tetrameric coiled coil ($\chi^2 = 1.5$, Fig. 4B) and *ab initio* particle reconstruction from SAXS data were also consistent with the model (Fig. 4C). Similarly, a complex containing the Mer2 coiled coil and full-length Spp1, or a shorter truncation with MBP-tagged Spp1 were all consistent with AlphaFold models (Supplementary Fig. S7).

We consider two main scenarios that can account for the observed 4 $\times$ 2 stoichiometry. The first one is based on steric exclusion and proposes that Spp1 subunits cannot occupy adjacent binding sites on Mer2 (perhaps due to direct physical clashes or charge repulsions). The second is allosteric in nature and postulates that Spp1 binding induces positive cooperativity between opposite sites and perhaps negative cooperativity between adjacent sites (Fig. 4D). While mechanistically different, these models would be hard to distinguish from each other experimentally. However, an allosteric model would explain the low binding affinity obtained with our AFM assay, compared to the ones previously obtained by MST [20]. Indeed, our assay captures single-molecule interactions that, under this scenario, would correspond to a low-affinity primary contact between Spp1 and Mer2.

The full-length Mer2-Spp1 complex

To gain further insights into the Mer2-Spp1 complex, we used AlphaFold to model the structure of a full-length Mer2 tetramer and a 4 $\times$ 2 Mer2-Spp1 complex (Fig. 4E). AlphaFold consistently placed two Spp1 subunits on opposite sides of the Mer2 α -helical bundle, through the same interaction surface as for the truncated versions presented above. The position of the Spp1 PHD finger and Set1-ID varied between models

(Supplementary Fig. S8), and the C-terminal Mer2 IDR was placed loosely around the interaction domain.

To address whether the positioning of the Mer2 C-terminal IDR in these models was relevant, we asked whether the IDR contributes to the interaction between Mer2 and Spp1. By pulldown analysis, we found that the absence of the Mer2 C-terminal IDR led to a small but reproducible reduction in Spp1 binding (Supplementary Fig. S9A). AFM analysis also revealed a slight reduction in the binding frequency between Spp1 and Mer2¹⁻²²⁴, compared to the full-length control (Supplementary Fig. S9B), suggesting that the C-terminal Mer2 IDR may weakly contribute to the interaction.

The tripartite Mer2-Spp1-DNA complex

We previously showed that Mer2 binding to DNA involves both the Mer2 coiled-coil domain and the C-terminal IDR [21, 23]. Indeed, *in vitro*, the coiled-coil domain is necessary and sufficient for DNA binding. However, mutating IDR residues K265, R266, R267, and R268 (KRRR) to alanine severely compromises DNA-binding and condensation of Mer2 *in vitro* and abolishes meiotic DSB formation *in vivo* [21].

In the absence of N- and C-terminal IDRs, Mer2 does not assemble condensates *in vitro*, but retains a DNA-bridging activity and binds preferentially to branched DNA substrates compared to duplex DNA, indicating that Mer2 can bind simultaneously to multiple DNA duplexes [23]. Based on AFM imaging and comparisons with Mer2 orthologs (*Schizosaccharomyces pombe* Rec15 and *Mus musculus* IHO1), we previously proposed that the coiled-coil domain binds co-aligned DNA duplexes through electrostatic interactions spread along the length of the coiled coil [23]. Interestingly, AlphaFold pro-

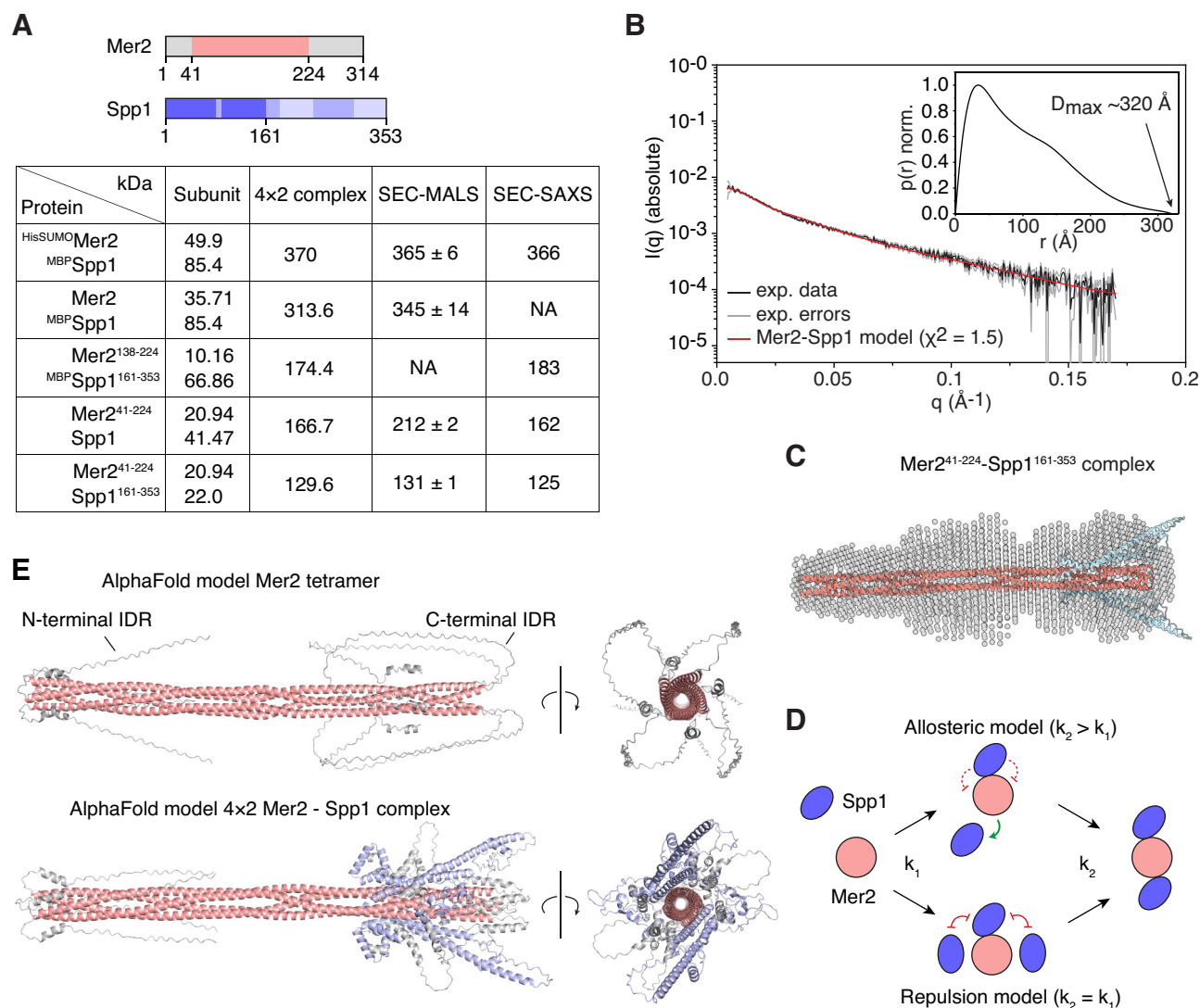


Figure 4. SEC-MALS and SAXS analyses of Mer2-Spp1 complexes. **(A)** Molecular weight measurements of Mer2-Spp1 complexes by SEC-MALS and SEC-SAXS (see [Supplementary Fig. S7](#) and [Supplementary Table S2](#)). **(B)** SEC-SAXS analysis of Mer2⁴¹⁻²²⁴-Spp1¹⁶¹⁻³⁵³ complexes. The main graph shows the experimental data (black), error margins (gray), and the fit of the AlphaFold model to the data (red). The inset shows a normalized probability distance distribution function obtained from the experimental SAXS data, with the approximate D_{max} value indicated. **(C)** Overlay of Mer2-Spp1 model with the *ab initio* reconstructed shape. **(D)** Allosteric and repulsion models can explain the preferential binding of two Spp1 subunits on opposite binding sites of the Mer2 coiled coil. The cartoon illustrates a cross-section perpendicular to the coiled-coil axis. **(E)** AlphaFold models of full-length Mer2 tetramer and 4x2 Mer2-Spp1 complexes.

posed a structural model of DNA-bound Mer2 tetramers entirely consistent with those expectations, with DNA duplexes aligned parallel to the coiled-coil axis and the KRRR motif contacting the DNA double helix (Fig. 5A).

Since the coiled-coil domain of Mer2, in particular the C-terminal half (residues 138–224), is both involved in binding Spp1 and DNA, we wondered how the presence of Spp1 and DNA influence each other and whether both binding partners could be accommodated simultaneously.

To address this, we modeled a 4x2 Mer2-Spp1 complex in the presence of two molecules of duplex DNA. Similar to the model without Spp1, the DNA duplexes were modeled parallel to the Mer2 coiled coil with the Mer2-KRRR motif bound to DNA (Fig. 5B).

Next, we sought to reconstitute a tripartite Mer2-Spp1-DNA complex *in vitro*. To do this, we performed gel shift analyses using a four-way branched DNA substrate

(Holliday Junction, HJ) assembled with partially overlapping 40 nt oligonucleotides. Mer2 forms a well-defined complex with HJ substrates, which is not observed with linear duplexes, presumably due to the tendency of Mer2 to form condensates [23]. In the presence of Spp1, we observed a complex of lower electrophoretic mobility compared to the Mer2-DNA complex formed in the absence of Spp1 (Fig. 5C). When reactions contained the Mer2-V195D mutant, the assembly of the tripartite Mer2-Spp1-DNA complex was abolished, but the Mer2-DNA complex was not affected. In addition, when the reactions contained the Mer2-KRRR mutant, both the Mer2-DNA and Mer2-Spp1-DNA complexes were absent (Fig. 5D).

Quantification of Mer2 titrations suggests that the presence of Spp1 mildly stimulates DNA binding ([Supplementary Fig. S10A](#)). Hence, despite occupying similar binding sites, we observe no competition between Spp1 and DNA for

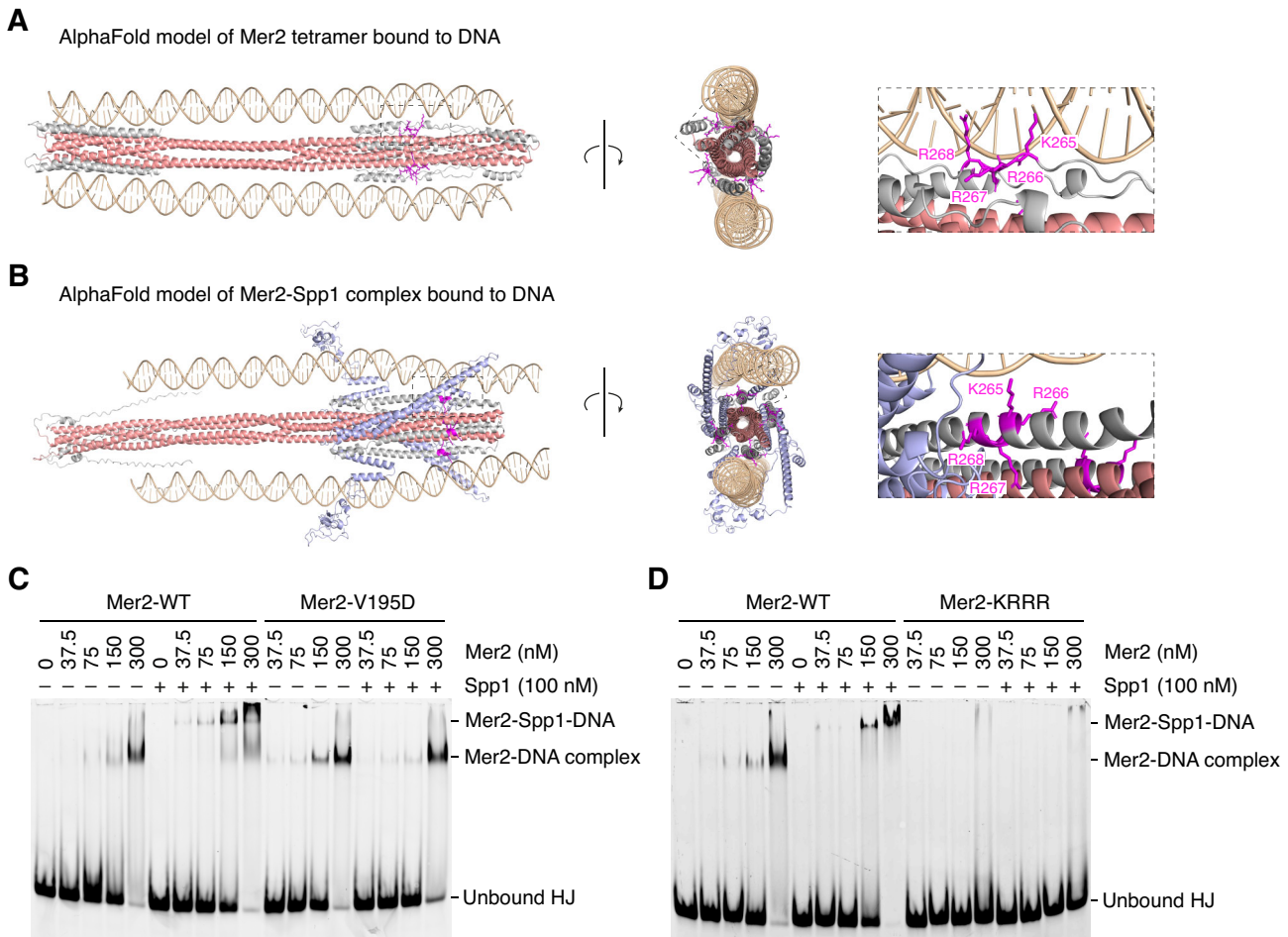


Figure 5. The tripartite Mer2-Spp1-DNA complex. **(A and B)** AlphaFold3 models of full-length Mer2 and Mer2-Spp1 complexes bound to two molecules of 80 bp double-stranded DNA. The position of the Mer2 DNA-binding residues (KRRR) is shown. While these models should be interpreted with caution, they recapitulate all of the known features of these complexes: (i) multiple DNA-binding sites, (ii) co-alignment of the coiled coil and DNA, and (iii) direct contacts between Mer2-KRRR and DNA [21, 23]. **(C and D)** Formation of a tripartite complex between wild-type or mutant Mer2, Spp1, and a branched DNA substrate (HJ, Holliday Junction with 20 bp arms).

access to Mer2. Consistently, in an order-of-addition experiment, the sequence in which Mer2 was incubated in the presence of Spp1 and DNA did not significantly impact the assembly of the complexes, further indicating that complex formation reached equilibrium before electrophoresis (Supplementary Fig. S10B). This equilibrium was reached very rapidly, since Mer2-Spp1-DNA complexes were formed efficiently even if Spp1 was added just before loading the gel (Supplementary Fig. S10C).

Spp1 is recruited to Mer2 condensates as a client

During meiosis, Spp1 has separable functions as part of the Set1 complex and as a Mer2 binding partner [16]. The diffusion rate of Spp1 is lower when it is associated with Mer2 compared to the Set1 complex [44], which can be explained by the recruitment of Spp1 within Mer2 condensates [21].

To investigate the relationship between Mer2 condensation and Spp1 recruitment, we mixed eGFP-tagged Mer2 and mScarlet-tagged Spp1 in the presence of plasmid DNA. This led to the formation of bright fluorescent foci with essentially perfect overlap between the two colors (Fig. 6A). When condensates were assembled with the V195D mutant, Mer2 foci formed efficiently but the Spp1 fluorescent signal was absent

(Fig. 6B). While Spp1 is not required for the formation of Mer2 foci (Fig. 6C), Mer2 is necessary for efficient formation of Spp1 foci (Fig. 6D). Nevertheless, Spp1 slightly stimulates Mer2 condensation. Hence, Spp1 is recruited to Mer2 foci essentially as a client, but it seems to contribute to DNA binding and condensation.

Spp1 directly contributes to DNA binding

We sought to address whether the weak stimulatory activity of Spp1 on Mer2 DNA binding and condensation are caused by direct interaction of Spp1 with DNA. We first asked whether Spp1 has DNA-binding activity, but a gel-shift analysis provided no evidence for DNA binding by full-length Spp1 (Fig. 7A). However, the C-terminal domain of Spp1 alone (residues 161–353) bound DNA quite efficiently (Fig. 7A). This suggests that the Spp1 N-terminal domain occludes a DNA-binding site within Spp1.

In the AlphaFold model of the Mer2-Spp1-DNA complex, a patch of positively charged residues located between helix 1 and helix 2 (K246, R247, K248, K249, K250, K251, here referred to as R5K) is shown to interact with DNA (Fig. 7B). To test whether these residues are responsible for the DNA-binding activity of Spp1, we mutated this stretch of residues to

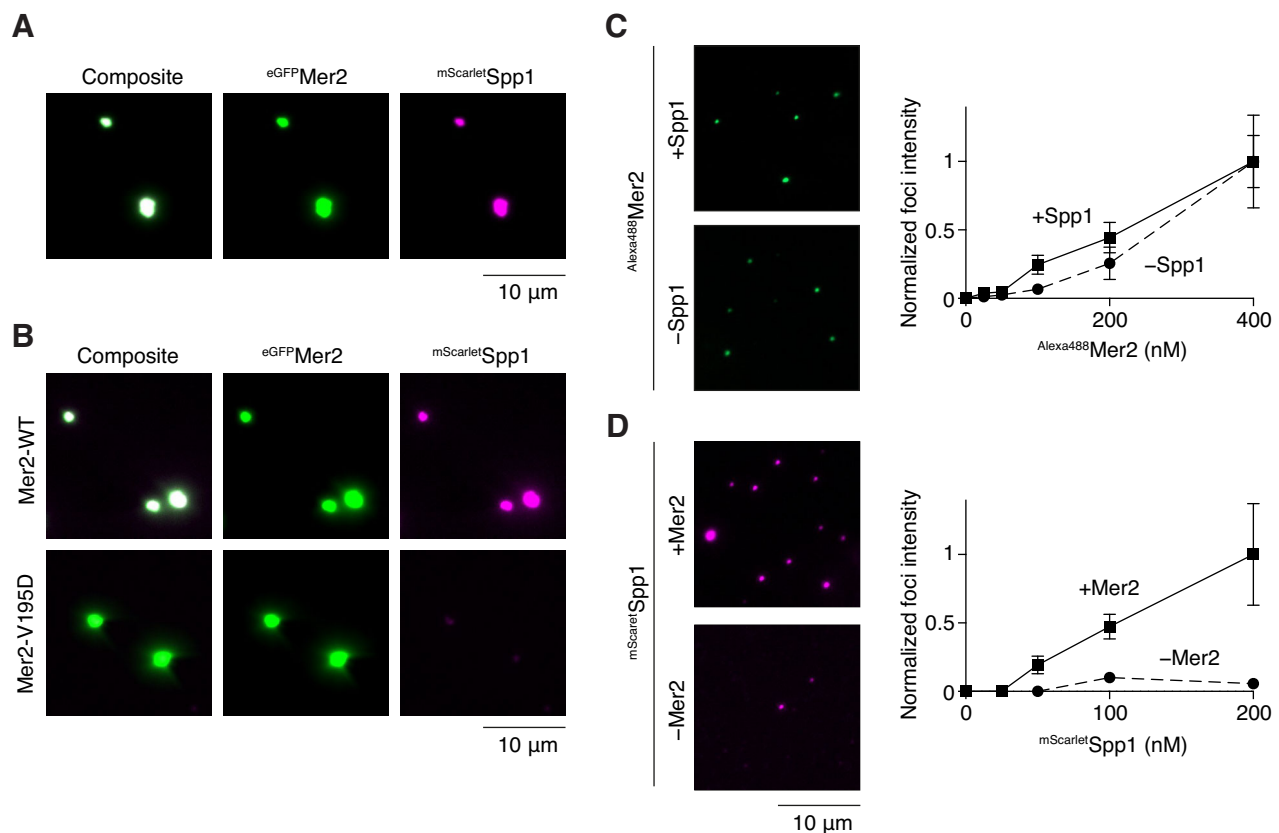


Figure 6. Mer2-Spp1 nucleoprotein condensates. **(A)** Visualization of nucleoprotein condensates by epifluorescence microscopy using tagged Mer2 and Spp1. **(B)** Effect of the Mer2-V195D mutation on the colocalization of Mer2 and Spp1 condensates. **(C)** Impact of Spp1 on the formation of Mer2 foci. Quantifications show the average focus intensity (normalized to 400 nM Mer2-Spp1). **(D)** Impact of Mer2 on the formation of Spp1 foci. Quantifications show the average focus intensity (normalized to 200 nM Spp1 + Mer2). Error bars show mean $\pm$ SD from ten fields of view.

alanine and established the impact of the mutation on DNA binding by a gel shift assay. In the context of truncations that only included the minimal Mer2-Spp1 interaction domains, Mer2^{138–224} alone bound very weakly to the HJ substrate and Spp1^{161–353} strongly promoted DNA binding. As expected, the Spp1-R5K mutation completely abolished this stimulatory effect (Fig. 7C).

In the context of full-length proteins, wild-type Spp1 did not significantly bind DNA by itself but enabled the assembly of a tripartite complex in the presence of Mer2. Mutation of the R5K motif strongly decreased the assembly of the tripartite complex and Mer2-DNA complexes were formed instead (Fig. 7D and E). The transition from Mer2-Spp1-DNA to Mer2-DNA complex could be explained if the R5K mutation affects Mer2-Spp1 interaction. However, this was not the case (Fig. 7F).

These results show that, even though full-length Spp1 does not bind DNA by itself, it does so in the context of a complex with Mer2. However, mutating a DNA-binding site within a complex is expected to reduce the overall affinity of the complex for DNA. This is not what we observed, and the Spp1-R5K mutation only marginally reduced DNA binding. Instead, the Spp1-R5K mutation drove the formation of Mer2-DNA complexes at the expense of Mer2-Spp1-DNA assemblies, suggesting an alternative explanation. We propose that the presence of Spp1 inherently reduces the DNA-binding activity of Mer2, which is counter-balanced by binding of the Spp1-R5K motif to DNA. When the R5K motif is mutated, the

cost associated with Spp1 binding to Mer2 drives the equilibrium in favor of free Mer2 complexes (Fig. 8A).

A prediction of this model is that the Spp1-R5K mutant would be excluded from Mer2 condensates, even though the protein-protein interaction between Mer2 and Spp1 is unaffected. Consistently, we found that mutation of the R5K motif severely reduced the recruitment of Spp1 to Mer2 condensates *in vitro* (Fig. 8B).

In vivo, the absence of Spp1 leads to a severe reduction of DSB levels at some hotspot (e.g. CYS3 and DEP1) and an increase in DSBs at others (e.g. PES4) [11]. We sought to evaluate the relevance of the Spp1 DNA-binding motif in shaping the meiotic DSB landscape. Since the R5K mutation leads to exclusion of Spp1 from Mer2 condensates *in vitro*, we hypothesized that the mutation may recapitulate the phenotype of a *spp1* Δ strain, even though the H3K4me3 and Mer2-binding functions of Spp1 are unaffected. However, this was not the case. Southern blot analysis of DSB formation in repair-defective *sae2* Δ or *dmc1* Δ backgrounds indicated that the *SPP1-R5K* mutation does not reduce DSB formation at CYS3 and DEP1 hotspots, and does not stimulate DSB formation at the PES4 hotspot, in contrast to the *spp1* Δ mutation (Fig. 8C and Supplementary Fig. S11A).

The absence of Spp1 also leads to severely reduced H3K4me3 levels in vegetative and meiotic cells [11–14]. To address whether the DNA-binding motif of Spp1 is required for this function, we measured global H3K4me3 levels during meiosis by western blot analysis. However, mutation of

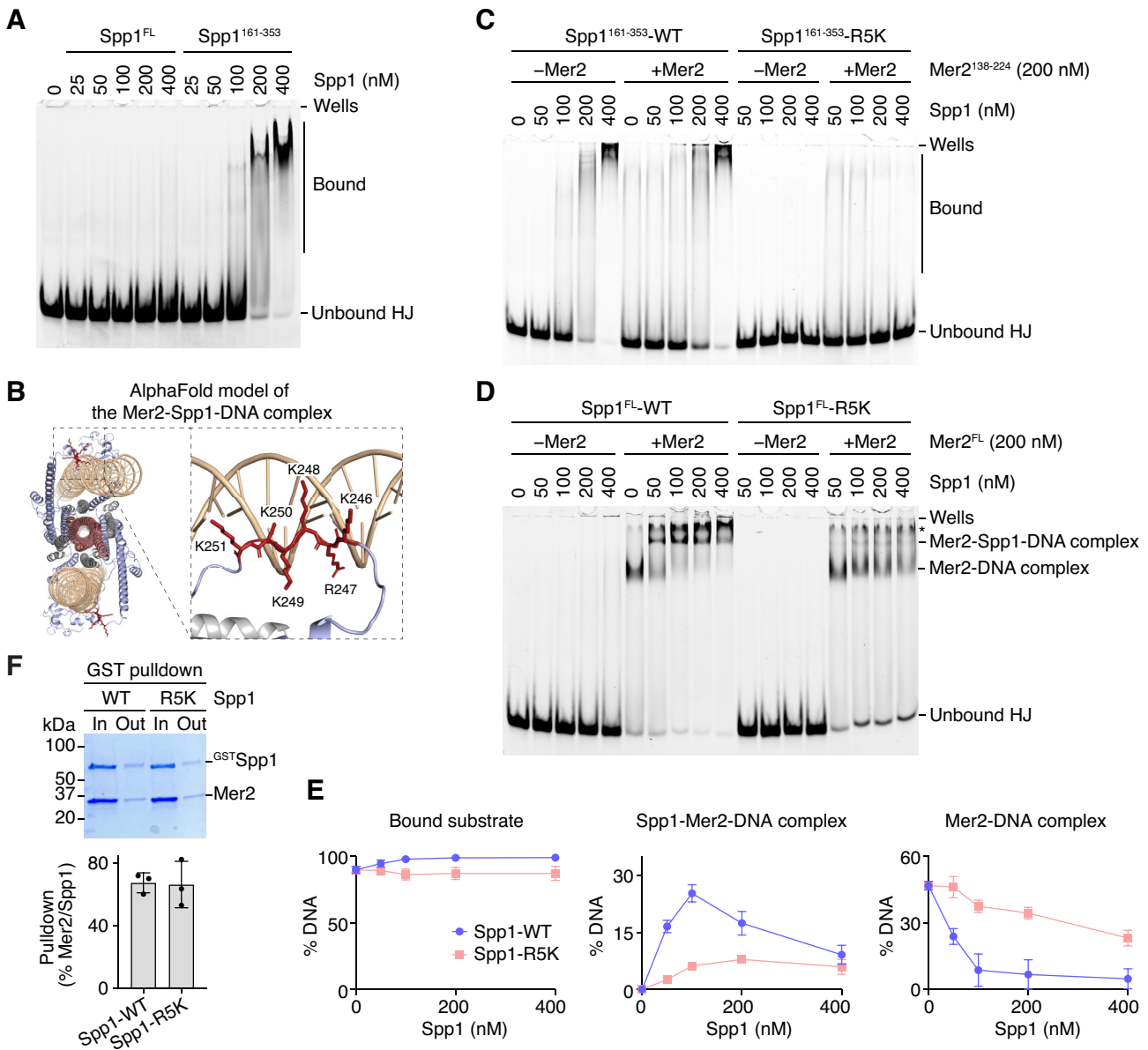


Figure 7. DNA-binding activity of Spp1. **(A)** Gel shift assay of full-length or truncated Spp1 binding to a branched DNA substrate (HJ, Holliday Junction with 20 bp arms). **(B)** AlphaFold3 model of Mer2-Spp1-DNA complexes. The position of the Spp1-R5K motif is highlighted. **(C)** Gel shift assay of wild-type (WT) and mutant (R5K) Spp1¹⁶¹⁻³⁵³ truncations on a HJ substrate in the presence or absence of Mer2¹³⁸⁻²²⁴ truncation. The Spp1-R5K mutant has residues K246, R247, K248, K249, K250, and K251 mutated to alanine. **(D)** Gel shift assay of wild-type and mutant full-length Spp1 DNA binding in the presence or absence of full-length Mer2. The band labeled * corresponds to a higher-order Mer2-(Spp1)-DNA assembly, which we interpret as reflecting the condensation property of Mer2. The detection of this band varied somewhat between experiments. **(E)** Quantification of the gel shift assay in panel (D) (mean $\pm$ range from two experiments). **(F)** Pull-down of Mer2 with wild-type and mutant (R5K) GST-tagged Spp1. In, input; Out, output. Quantifications show the mean $\pm$ SD from three replicates.

the Spp1-R5K motif did not reduce H3K4me3 levels relative to the wild type, in contrast to Spp1 deletion (Supplementary Fig. S11C).

Hence, although the R5K motif promotes DNA binding by Spp1 *in vitro*, it does not seem to be required for the meiotic functions of Spp1 *in vivo*.

Discussion

Here, we used biochemical reconstitution, single-molecule analyses, and AlphaFold modeling to explore the interaction between Mer2 and Spp1 and their relationship with DNA.

We determined thermodynamic and kinetic parameters of the Mer2-Spp1 interaction and presented structural models of *S. cerevisiae* Mer2-Spp1 complexes, supported by SAXS and mutagenesis. In addition, we show that Spp1 is recruited to Mer2 condensates and identified a motif within Spp1 that fosters the interaction of Mer2-Spp1 complexes with DNA. Our results have implications for the role of Mer2 and Spp1 in the assembly of the meiotic DSB machinery.

Assembly of the Mer2-Spp1 complex

Consistent with previous research [20], we have shown that Mer2 and Spp1 assemble a complex with a 4 $\times$ 2

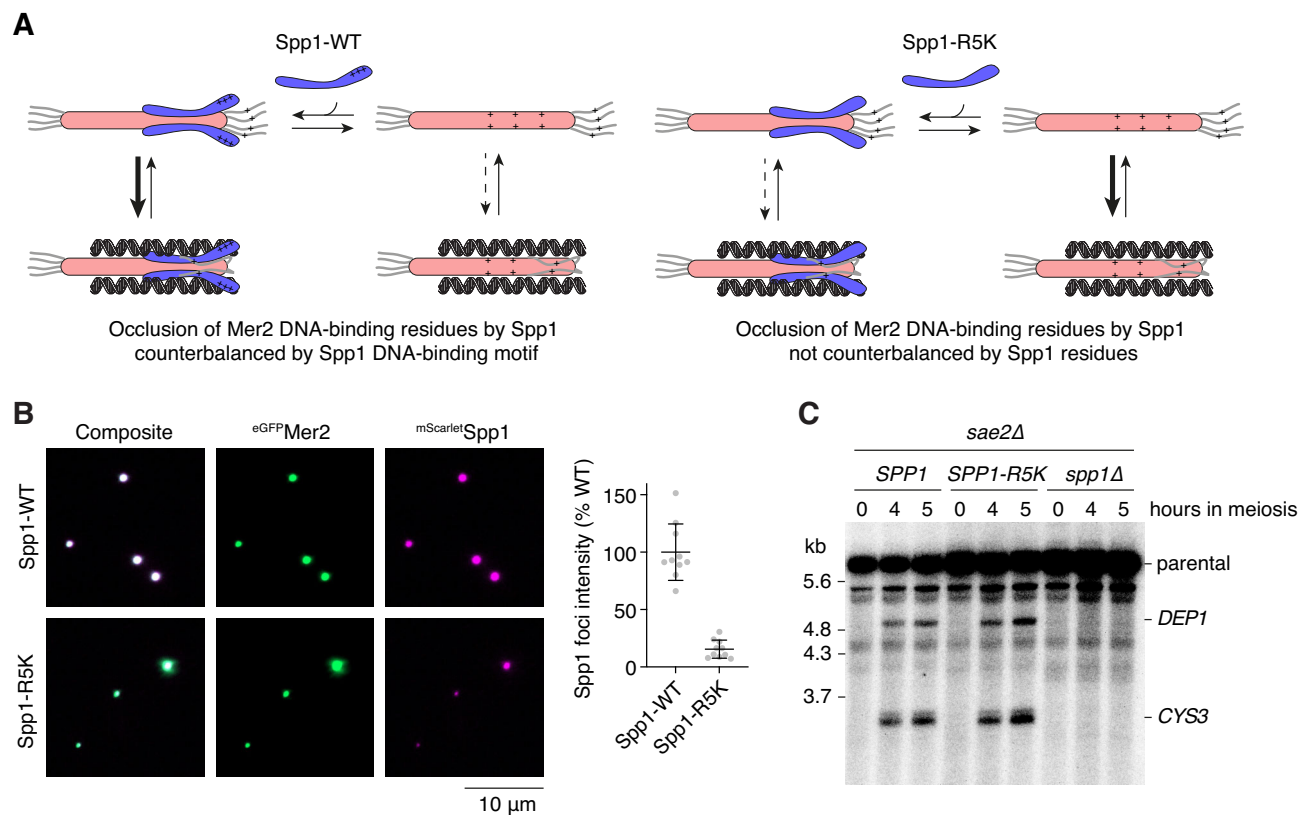


Figure 8. Interplay between Mer2, Spp1 and DNA binding. **(A)** Model for the role of Spp1 DNA binding function. (Left) We propose that Spp1 binding to Mer2 occludes DNA-binding residues (+) within Mer2. This is compensated by the Spp1-R5K motif that drives the equilibrium in favor of Mer2–Spp1–DNA complexes. (Right) When the R5K motif is mutated, the decreased DNA-binding activity of Mer2 caused by Spp1 binding drives the equilibrium toward Mer2–DNA complexes. **(B)** Effect of Spp1-R5K mutation on the recruitment of Spp1 within Mer2 condensates. Quantifications show the total focus intensity per field of view, normalized to Spp1-WT. Error bars are mean $\pm$ SD ($n = 10$ fields of view). **(C)** Southern blot analysis of meiotic DSB formation at the *CYS3* and *DEP1* hotspots in wild-type and Spp1 mutant strains. A representative image of three independent experiments is shown.

stoichiometry, where the C-terminal α -helix of Spp1 (helix 2, residues 307–351) binds anti-parallel to and interacts with two adjacent helices at the C-terminus of the Mer2 coiled coil (residues 180–224). Spp1 helix 1 (residues 169–233) folds on top of helix 2 and stabilizes this interface (Fig. 2). This model is supported by pulldown assays and mutagenesis (Fig. 3), SAXS experiments (Fig. 4), and is consistent with previous truncation [10] and mutagenesis [16] experiments in yeast.

Nevertheless, the structural model proposed by AlphaFold does not readily account for the 4 $\times$ 2 stoichiometry of the complex. Instead, the model suggests that the tetrameric coiled-coil domain of Mer2 has four binding sites for Spp1 that could be occupied simultaneously. It is possible that some aspects of the model are inaccurate and four Spp1 subunits would sterically clash or else repulse each other. However, we favor an alternative explanation, based on allosteric modulation of the Mer2–Spp1 interaction domain. We suggest that binding of one Spp1 subunit to Mer2 induces tension on the coiled coil that increases the affinity of the opposite Spp1-binding site, and perhaps reduces the affinity of the two adjacent sites (Fig. 4D). In other words, binding of Spp1 is positively cooperative at opposite sides of the coiled coil, but negatively cooperative at adjacent sites, leading to the symmetrical arrangement of two Spp1 subunits on opposite sides of the Mer2 coiled coil. Evidence supporting this model comes from our AFM-based force spectroscopy assay (Fig. 1) that revealed

a binding affinity of single Spp1 monomers to Mer2 tetramers about 100 $\times$ lower than those obtained from ensemble average experiments [20]. While these differences in affinity could be due to technical reasons and the steric exclusion model therefore remains plausible, the allosteric model would also explain why varying the ratio of Mer2 and Spp1 in a gel shift assay only reveals a single Mer2–Spp1–DNA complex rather than multiple complexes of distinct stoichiometries (Fig. 5 and Supplementary Fig. S10). Elucidating this mechanism will require high-resolution structural determination of Mer2–Spp1 complexes.

Assembly of the Mer2–Spp1–DNA complexes

AlphaFold modeling suggested that Mer2 tetramers can bind co-aligned DNA duplexes through interactions that involve the coiled-coil domain and the C-terminal IDR (Fig. 5). Models of Mer2–Spp1–DNA complexes showed similar configurations with Mer2 and Spp1 residues binding the DNA backbone. Although these structural models require experimental validation, they are fully consistent with current data. First, based on AFM imaging and mutagenesis of IHO1 and Rec15 (Mer2 orthologs in mice and *S. pombe*), we previously proposed that the Mer2 coiled coil bridges DNA duplexes through electrostatic interactions along its length [23], in agreement with the AlphaFold models. Second, the models

are consistent with the known function of the Mer2-KRRR motif in promoting Mer2 DNA binding, condensation and DSB formation [21]. Third, the AlphaFold models identified the Spp1-R5K motif as putative DNA-binding residues, which we validated *in vitro* (Fig. 7).

A DNA-binding activity of Spp1 was not anticipated based on its proposed loop-axis tethering function [10, 11]. However, it has previously been noticed that Spp1 binds DNA *in vitro*, and this activity was mapped to a fragment of Spp1 containing residues 170–255 [45]. Here, we identified the residues responsible for this activity.

In our hands, full-length Spp1 does not bind DNA by itself, but the C-terminal part of Spp1 alone does, suggesting that the N-terminus of Spp1 occludes the C-terminal DNA-binding domain. However, how this auto-inhibition may take place is not clear. Regardless, since mutation of the R5K motif compromises the assembly of Mer2–Spp1–DNA complexes *in vitro*, full-length Spp1 binds DNA in the context of its complex with Mer2, even if it does not interact with DNA by itself (Fig. 7).

An occlusion–compensation mechanism for Mer2–Spp1–DNA interactions

Based on truncation analyses, the C-terminal part of the Mer2 coiled coil had previously been implicated in binding Spp1 [10] and DNA [21]. We, therefore, wondered how the recruitment of Spp1 and DNA might affect each other. Three general scenarios can be envisioned. Spp1 and DNA could bind independently to Mer2, they could compete for access to Mer2, or they could bind cooperatively (Supplementary Fig. S12). First, independent and competition models are not compatible with a DNA-binding activity of Spp1. Second, titration experiments revealed no evidence for competition between Spp1 and DNA for Mer2 binding. In contrast, the presence of Spp1 appeared to slightly stimulate DNA binding and condensation, supporting a weak cooperativity model. The AlphaFold models fit with these observations, since Mer2 coiled coils can simultaneously accommodate Spp1 and DNA without major steric clashes, and interaction between Spp1 and DNA can account for the observed cooperativity. The cooperativity could be either reciprocal or unilateral (one-sided). The structural model fits best with a one-sided cooperativity model, where the presence of Spp1 facilitates DNA binding rather than vice versa (Supplementary Fig. S12E).

In addition to independent, competition, and cooperativity models, another scenario can be considered, encompassing both negative and positive effects of Spp1 on DNA binding. We refer to this as an “occlusion–compensation” model (Supplementary Fig. S12G). Although the AlphaFold models suggest a harmonious coexistence of Spp1 and DNA around the Mer2 tetramers, a comparison of Mer2–Spp1 and Mer2–DNA models suggests that Spp1 occludes putative DNA-binding residues found along the Mer2 coiled coil. This suggests a model where Spp1 recruitment reduces the DNA-binding activity of Mer2, but this is compensated by Spp1–DNA interactions. Our data indicates that this is likely to be the case. Indeed, under the one-sided (Spp1 → DNA) cooperativity model, mutation of the Spp1-R5K motif would be expected to reduce the DNA-binding activity of the complex but would not be expected to affect the ratio between Mer2–Spp1 and Mer2 nucleoprotein complexes. Instead, we found that mutating the Spp1-R5K motif does not strongly

decrease DNA binding, but rather favors the formation of Mer2–DNA complexes at the expense of Mer2–Spp1–DNA complexes.

Hence, our interpretation is that the cost associated with Spp1 binding is compensated by interactions between the Spp1-R5K motif and DNA, such that the binding equilibrium is driven toward the formation of tripartite Mer2–Spp1–DNA complexes (Fig. 8A). When the R5K motif is mutated, Spp1 recruitment leads to a net decrease in DNA binding, driving the equilibrium toward Mer2–DNA complexes. Consistent with this model, mutation of the Spp1-R5K motif drives the exclusion of Spp1 from Mer2 condensates *in vitro*.

Nevertheless, since mutating the R5K motif does not recapitulate the meiotic phenotype of a *spp1Δ* strain (Fig. 8C and Supplementary Fig. S11), the DNA-binding activity of Spp1 does not appear to be important for its meiotic function. Assuming that the R5K mutation also leads to a reduction of Spp1 binding to Mer2 condensates, as is the case *in vitro* (Fig. 8B), the implication is that the amount of Spp1 recruitment to Mer2 condensates is not a limiting factor in shaping the DSB landscape. Nonetheless, recruitment of Spp1 is important, since the Mer2–V195D mutation that strongly reduces the interaction with Spp1 does recapitulate the phenotype of the *spp1Δ* strain [16].

Potential functions of the Spp1 DNA-binding activity

The *SPP1-R5K* mutation did not affect meiotic DSB formation at the *PES4*, *CYS3*, and *DEP1* loci, and did not cause a global reduction of H3K4me3 levels (Fig. 8C and Supplementary Fig. S11A–C). Given the significant effect of the mutation on DNA binding *in vitro* (Fig. 7), the lack of meiotic phenotype *in vivo* may be somewhat surprising. Indeed, a multiple sequence alignment of Spp1 shows a conserved patch of positively charged residues in the corresponding loop throughout Saccharomycetaceae, suggesting that the R5K motif is functionally relevant (Supplementary Fig. S11D). However, it is possible that these residues have a subtle role that may have been obscured through functional redundancies.

For instance, in addition to Set1-dependent H3K4me3, meiotic DSB formation is influenced by other histone modifications, including Dot1-dependent H3K79 methylation [46]. Synthetic defect in DSB formation have been described between Set1 and Dot1 mutants [46]. The Paf1 transcription elongation complex (Paf1C) that promotes both H3K4 and H3K79 methylation, was also shown to shape the DSB landscape independently of Set1 and Dot1 [47], and synthetic defects have been observed between Paf1C components (Rtf1 and Cdc73) and myc-tagged versions of Rec114 and Mer2 [48]. Given these genetic interactions, it would be interesting to investigate whether mutation of the Spp1-R5K motif exhibits a meiotic phenotype in a sensitized background (e.g. *dot1*, *rtf1*, or *REC114myc*).

Recent evidence further indicates that Spp1 and other subunits of the Set1 complex, including Sgh1, are acetylated at their N-terminus [49]. Deletion of Sgh1, mutation of its N-terminal acetylation site, or deletion of the N-acetyltransferase NatA, cause synthetic defects on global H3K4me3 levels with *spp1Δ* mutants. It would be interesting to test whether a functional role for the Spp1 DNA-binding activity could be revealed in these mutant backgrounds.

More generally, the Set1 complex and H3K4 methylation exert diverse functions in transcription regulation and chromatin organization [50–54], and have been implicated in DNA replication [15, 55, 56] and repair [57, 58], the maintenance of telomere boundaries [50, 59], and chromosome segregation [60], all of which could be directly or indirectly impacted by Spp1. For instance, Spp1 was recently shown to be recruited behind stalled replication forks to prevent the accumulation of ssDNA by restricting access of Exo1 [61]. It would also be interesting to explore a putative function for the DNA-binding activity of Spp1 in this context.

In summary, our work reveals new molecular insights into the role of Mer2 and Spp1 in the assembly of the meiotic DSB machinery, and highlight an as-yet uncharacterized DNA-binding property of Spp1 that could have implications for its multiple functions during and beyond meiosis.

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Supplementary data

Supplementary data is available at NAR online.

Conflict of interest

The authors declare no competing interests.

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Data availability

SAXS data have been deposited to the Small Angle Scattering Biological Data Bank with identifiers SASDXW2, SASDXX2, SASDXY2, and SASDXZ2.

References

1. Yadav VK, Claeys Bouuaert C Mechanism and control of meiotic DNA double-strand break formation in *S. cerevisiae*. *Front Cell Dev Biol* 2021;9:642737. <https://doi.org/10.3389/fcell.2021.642737>
2. Zickler D, Kleckner N Meiosis: dances between homologs. *Annu Rev Genet* 2023;57:1–63. <https://doi.org/10.1146/annurev-genet-061323-044915>
3. Lam I, Keeney S Mechanism and regulation of meiotic recombination initiation. *Cold Spring Harb Perspect Biol* 2015;7:a016634. <https://doi.org/10.1101/cshperspect.a016634>
4. Ur SN, Corbett KD Architecture and dynamics of meiotic chromosomes. *Annu Rev Genet* 2021;55:497–526. <https://doi.org/10.1146/annurev-genet-071719-020235>
5. Blat Y, Protacio RU, Hunter N *et al.* Physical and functional interactions among basic chromosome organizational features govern early steps of meiotic chiasma formation. *Cell* 2002;111:791–802. [https://doi.org/10.1016/S0092-8674\(02\)01167-4](https://doi.org/10.1016/S0092-8674(02)01167-4)
6. Panizza S, Mendoza MA, Berlinger M *et al.* Spo11-accessory proteins link double-strand break sites to the chromosome axis in early meiotic recombination. *Cell* 2011;146:372–83. <https://doi.org/10.1016/j.cell.2011.07.003>
7. Pan J, Sasaki M, Kniewel R *et al.* A hierarchical combination of factors shapes the genome-wide topography of yeast meiotic recombination initiation. *Cell* 2011;144:719–31. <https://doi.org/10.1016/j.cell.2011.02.009>
8. Borde V, Robine N, Lin W *et al.* Histone H3 lysine 4 trimethylation marks meiotic recombination initiation sites. *EMBO J* 2009;28:99–111. <https://doi.org/10.1038/emboj.2008.257>
9. Tischfield SE, Keeney S Scale matters: the spatial correlation of yeast meiotic DNA breaks with histone H3 trimethylation is driven largely by independent colocalization at promoters. *Cell Cycle* 2012;11:1496–503. <https://doi.org/10.4161/cc.19733>
10. Acquaviva L, Szekvolgyi L, Dichtl B *et al.* The COMPASS subunit Spp1 links histone methylation to initiation of meiotic recombination. *Science* 2013;339:215–8. <https://doi.org/10.1126/science.1225739>
11. Sommermeyer V, Beneut C, Chaplais E *et al.* Spp1, a member of the Set1 Complex, promotes meiotic DSB formation in promoters by tethering histone H3K4 methylation sites to chromosome axes. *Mol Cell* 2013;49:43–54. <https://doi.org/10.1016/j.molcel.2012.11.008>
12. Schneider J, Wood A, Lee JS *et al.* Molecular regulation of histone H3 trimethylation by COMPASS and the regulation of gene expression. *Mol Cell* 2005;19:849–56. <https://doi.org/10.1016/j.molcel.2005.07.024>
13. Morillon A, Karabetsov N, Nair A *et al.* Dynamic lysine methylation on histone H3 defines the regulatory phase of gene

- transcription. *Mol Cell* 2005;18:723–34. <https://doi.org/10.1016/j.molcel.2005.05.009>
14. Dehé PM, Dichtl B, Schaft D *et al.* Protein interactions within the Set1 complex and their roles in the regulation of histone 3 lysine 4 methylation. *J Biol Chem* 2006;281:35404–12.
 15. Sollier J, Lin W, Soustelle C *et al.* Set1 is required for meiotic S-phase onset, double-strand break formation and middle gene expression. *Embo j* 2004;23:1957–67. <https://doi.org/10.1038/sj.emboj.7600204>
 16. Adam C, Guérois R, Citarella A *et al.* The PHD finger protein Spp1 has distinct functions in the Set1 and the meiotic DSB formation complexes. *PLoS Genet* 2018;14:e1007223. <https://doi.org/10.1371/journal.pgen.1007223>
 17. Rockmill B, Engebrecht JA, Scherthan H *et al.* The yeast MER2 gene is required for chromosome synapsis and the initiation of meiotic recombination. *Genetics* 1995;141:49–59. <https://doi.org/10.1093/genetics/141.1.49>
 18. Arora C, Kee K, Maleki S *et al.* Antiviral protein Ski8 is a direct partner of Spo11 in meiotic DNA break formation, independent of its cytoplasmic role in RNA metabolism. *Mol Cell* 2004;13:549–59. [https://doi.org/10.1016/S1097-2765\(04\)00063-2](https://doi.org/10.1016/S1097-2765(04)00063-2)
 19. Henderson KA, Kee K, Maleki S *et al.* Cyclin-dependent kinase directly regulates initiation of meiotic recombination. *Cell* 2006;125:1321–32. <https://doi.org/10.1016/j.cell.2006.04.039>
 20. Rousová D, Nivsarkar V, Altmannova V *et al.* Novel mechanistic insights into the role of Mer2 as the keystone of meiotic DNA break formation. *eLife* 2021;10:e72330.
 21. Claeys Bouuaert C, Pu S, Wang J *et al.* DNA-driven condensation assembles the meiotic DNA break machinery. *Nature* 2021;592:144–9. <https://doi.org/10.1038/s41586-021-03374-w>
 22. Priyadarshini P, Survi M, El Yazidi Mouloud W *et al.* Recruitment of Mre11 to recombination sites during meiosis. *Nat Commun* 2026;17:4937. <https://doi.org/10.1038/s41467-026-71310-5>
 23. Daccache D, De Jonge E, Liloku P *et al.* Evolutionary conservation of the structure and function of meiotic Rec114–Mei4 and Mer2 complexes. *Genes Dev* 2023;37:535–53. <https://doi.org/10.1101/gad.350462.123>
 24. Oger C, Claeys Bouuaert C SPO11 dimers are sufficient to catalyse DNA double-strand breaks *in vitro*. *Nature*;2025;639:792–9. <https://doi.org/10.1038/s41586-024-08574-8>
 25. Ait Bella H, Survi M, Urdian-Araiza J *et al.* Dimerization of the *S. cerevisiae* Spo11 core complex. *bioRxiv*, <https://doi.org/10.64898/2026.01.16.699991>, 17 January 2026, preprint: not peer reviewed.
 26. Pfreundschuh M, Alsteens D, Wieneke R *et al.* Identifying and quantifying two ligand-binding sites while imaging native human membrane receptors by AFM. *Nat Commun* 2015;6:8857. <https://doi.org/10.1038/ncomms9857>
 27. Koehler M, Ray A, Moreira RA *et al.* Molecular insights into receptor binding energetics and neutralization of SARS-CoV-2 variants. *Nat Commun* 2021;12:6977. <https://doi.org/10.1038/s41467-021-27325-1>
 28. Rankl C, Wildling L, Neundlinger I *et al.* Determination of the kinetic on- and off-rate of single virus-cell interactions. *Methods Mol Biol* 2011;736:197–210.
 29. Alsteens D, Newton R, Schubert R *et al.* Nanomechanical mapping of first binding steps of a virus to animal cells. *Nature Nanotech* 2017;12:177–83. <https://doi.org/10.1038/nnano.2016.228>
 30. Hopkins JB, Gillilan RE, Skou S BioXTAS RAW: improvements to a free open-source program for small-angle X-ray scattering data reduction and analysis. *J Appl Crystallogr* 2017;50:1545–53. <https://doi.org/10.1107/S1600576717011438>
 31. Manalastas-Cantos K, Konarev PV, Hajizadeh NR *et al.* ATSAS 3.0: expanded functionality and new tools for small-angle scattering data analysis. *J Appl Crystallogr* 2021;54:343–55. <https://doi.org/10.1107/S1600576720013412>
 32. Grant TD *Ab initio* electron density determination directly from solution scattering data. *Nat Methods* 2018;15:191–3. <https://doi.org/10.1038/nmeth.4581>
 33. Franke D, Svergun DI DAMMIF, a program for rapid *ab-initio* shape determination in small-angle scattering. *J Appl Crystallogr* 2009;42:342–6. <https://doi.org/10.1107/S0021889809000338>
 34. Schwieters CD, Kuszewski JJ, Tjandra N *et al.* The Xplor-NIH NMR molecular structure determination package. *J Magn Reson* 2003;160:65–73. [https://doi.org/10.1016/S1090-7807\(02\)00014-9](https://doi.org/10.1016/S1090-7807(02)00014-9)
 35. Schwieters CD, Clore GM Using small angle solution scattering data in Xplor-NIH structure calculations. *Prog Nucl Magn Reson Spectrosc* 2014;80:1–11. <https://doi.org/10.1016/j.pnmrs.2014.03.001>
 36. Meng EC, Goddard TD, Pettersen EF *et al.* UCSF ChimeraX: tools for structure building and analysis. *Protein Sci* 2023;32:e4792. <https://doi.org/10.1002/pro.4792>
 37. Murakami H, Borde V, Nicolas A *et al.* Gel electrophoresis assays for analyzing DNA double-strand breaks in *Saccharomyces cerevisiae* at various spatial resolutions. *Methods Mol Biol* 2009;557:117–42.
 38. Shi X, Kachirskaja I, Walter KL *et al.* Proteome-wide analysis in *Saccharomyces cerevisiae* identifies several PHD fingers as novel direct and selective binding modules of histone H3 methylated at either lysine 4 or lysine 36. *J Biol Chem* 2007;282:2450–5. <https://doi.org/10.1074/jbc.C600286200>
 39. He C, Liu N, Xie D *et al.* Structural basis for histone H3K4me3 recognition by the N-terminal domain of the PHD finger protein Spp1. *Biochem J* 2019;476:1957–73. <https://doi.org/10.1042/BCJ20190091>
 40. Müller DJ, Dumitru AC, Lo Giudice C *et al.* Atomic force microscopy-based force spectroscopy and multiparametric imaging of biomolecular and cellular systems. *Chem Rev* 2021;121:11701–25.
 41. Fernandez M, Shkumatov AV, Liu Y *et al.* AFM-based force spectroscopy unravels stepwise formation of the DNA transposition complex in the widespread Tn3 family mobile genetic elements. *Nucleic Acids Res* 2023;51:4929–41. <https://doi.org/10.1093/nar/gkad241>
 42. Alsteens D, Pfreundschuh M, Zhang C *et al.* Imaging G protein-coupled receptors while quantifying their ligand-binding free-energy landscape. *Nat Methods* 2015;12:845–51. <https://doi.org/10.1038/nmeth.3479>
 43. Abramson J, Adler J, Dunger J *et al.* Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* 2024;630:493–500. <https://doi.org/10.1038/s41586-024-07487-w>
 44. Karányi Z, Halász L, Acquaviva L *et al.* Nuclear dynamics of the Set1C subunit Spp1 prepares meiotic recombination sites for break formation. *J Cell Biol* 2018;217:3398–415. <https://doi.org/10.1083/jcb.201712122>
 45. Murton BL, Chin WL, Ponting CP *et al.* Characterising the binding specificities of the subunits associated with the KMT2/Set1 histone lysine methyltransferase. *J Mol Biol* 2010;398:481–8. <https://doi.org/10.1016/j.jmb.2010.03.036>
 46. Bani Ismail M, Shinohara M, Shinohara A Dot1-dependent histone H3K79 methylation promotes the formation of meiotic double-strand breaks in the absence of histone H3K4 methylation in budding yeast. *PLoS One* 2014;9:e96648. <https://doi.org/10.1371/journal.pone.0096648>
 47. Gothwal SK, Patel NJ, Colletti MM *et al.* The double-strand break landscape of meiotic chromosomes is shaped by the Paf1 transcription elongation complex in *Saccharomyces cerevisiae*. *Genetics* 2016;202:497–512. <https://doi.org/10.1534/genetics.115.177287>
 48. Zhang Y, Suzuki T, Li K *et al.* Genetic interactions of histone modification machinery Set1 and PAF1C with the recombination complex Rec114–Mer2–Mei4 in the formation of meiotic DNA double-strand breaks. *Int J Mol Sci* 2020;21:2679.

49. Woo H, Oh J, Cho YJ *et al.* N-terminal acetylation of Set1-COMPASS fine-tunes H3K4 methylation patterns. *Sci Adv* 2024;10:eadl6280. <https://doi.org/10.1126/sciadv.adl6280>
50. Nislow C, Ray E, Pillus L SET1, a yeast member of the trithorax family, functions in transcriptional silencing and diverse cellular processes. *MBoC* 1997;8:2421–36. <https://doi.org/10.1091/mbc.8.12.2421>
51. Pinskaya M, Morillon A Histone H3 lysine 4 di-methylation: a novel mark for transcriptional fidelity? *Epigenetics* 2009;4:302–6. <https://doi.org/10.4161/epi.4.5.9369>
52. Terzi N, Churchman LS, Vasiljeva L *et al.* H3K4 trimethylation by Set1 promotes efficient termination by the Nrd1–Nab3–Sen1 pathway. *Mol Cell Biol* 2011;31:3569–83. <https://doi.org/10.1128/MCB.05590-11>
53. Margaritis T, Oreal V, Brabers N *et al.* Two distinct repressive mechanisms for histone 3 lysine 4 methylation through promoting 3'-end antisense transcription. *PLoS Genet* 2012;8:e1002952. <https://doi.org/10.1371/journal.pgen.1002952>
54. Deshpande N, Bryk M Diverse and dynamic forms of gene regulation by the *S. cerevisiae* histone methyltransferase Set1. *Curr Genet* 2023;69:91–114. <https://doi.org/10.1007/s00294-023-01265-3>
55. Rizzardi LF, Dorn ES, Strahl BD *et al.* DNA replication origin function is promoted by H3K4 di-methylation in *Saccharomyces cerevisiae*. *Genetics* 2012;192:371–84. <https://doi.org/10.1534/genetics.112.142349>
56. Serra-Cardona A, Duan S, Yu C *et al.* H3K4me3 recognition by the COMPASS complex facilitates the restoration of this histone mark following DNA replication. *Sci Adv* 2022;8:eabm6246. <https://doi.org/10.1126/sciadv.abm6246>
57. Schramke V, Neecke H, Brevet V *et al.* The set1Delta mutation unveils a novel signaling pathway relayed by the Rad53-dependent hyperphosphorylation of replication protein A that leads to transcriptional activation of repair genes. *Genes Dev* 2001;15:1845–58. <https://doi.org/10.1101/gad.193901>
58. Faucher D, Wellinger RJ Methylated H3K4, a transcription-associated histone modification, is involved in the DNA damage response pathway. *PLoS Genet* 2010;6:e1001082. <https://doi.org/10.1371/journal.pgen.1001082>
59. Corda Y, Schramke V, Longhese MP *et al.* Interaction between Set1p and checkpoint protein Mec3p in DNA repair and telomere functions. *Nat Genet* 1999;21:204–8. <https://doi.org/10.1038/5991>
60. Zhang K, Lin W, Latham JA *et al.* The Set1 methyltransferase opposes Ipl1 aurora kinase functions in chromosome segregation. *Cell* 2005;122:723–34. <https://doi.org/10.1016/j.cell.2005.06.021>
61. Ghaddar N, Corda Y, Luciano P *et al.* The COMPASS subunit Spp1 protects nascent DNA at the Tus/Ter replication fork barrier by limiting DNA availability to nucleases. *Nat Commun* 2023;14:5430. <https://doi.org/10.1038/s41467-023-41100-4>