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1 **Exo1 recruits Cdc5 polo kinase to MutL γ to ensure efficient meiotic**
2 **crossover formation**

3

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1 **Abstract**

2 Crossovers generated during the repair of programmed meiotic double-strand
3 breaks must be tightly regulated to promote accurate homolog segregation
4 without deleterious outcomes such as aneuploidy. The Mlh1-Mlh3 (MutL γ)
5 endonuclease complex is critical for crossover resolution, which involves
6 mechanistically unclear interplay between MutL γ and Exo1 and Polo kinase Cdc5.
7 Using budding yeast to gain temporal and genetic traction on crossover regulation,
8 we find that MutL γ constitutively interacts with Exo1. Upon commitment to
9 crossover repair, MutL γ -Exo1 associate with recombination intermediates,
10 followed by direct Cdc5 recruitment that triggers MutL γ crossover activity. We
11 propose that Exo1 serves as a central coordinator in this molecular interplay,
12 providing a defined order of interaction that prevents deleterious, premature
13 activation of crossovers. MutL γ associates at a lower frequency near centromeres,
14 indicating that spatial regulation across chromosomal regions reduces risky
15 crossover events. Our data elucidate the temporal and spatial control surrounding
16 a constitutive, potentially harmful, nuclease. We also reveal a critical, non-catalytic
17 role for Exo1, through non-canonical interaction with Polo kinase. These
18 mechanisms regulating meiotic crossovers may be conserved across species.

19

20 **Significance**

21 Meiotic crossovers are essential for the production of gametes with balanced
22 chromosome content. MutL γ (Mlh1-Mlh3) endonuclease is a mismatch repair
23 heterodimer that functions also during meiosis to generate crossovers. Its activity
24 requires Exo1 as well as the MutS γ heterodimer (Msh4-Msh5). Crossovers also
25 require the polo kinase Cdc5, in a way that is poorly understood. We show that
26 Exo1 directly interacts with Cdc5, and that this promotes the activation of MutL γ
27 for crossovers. Since Cdc5 also controls other key meiotic processes, including
28 kinetochores mono-orientation and disassembly of the synaptonemal complex,
29 this direct interaction with the crossover proteins may be a way to coordinate
30 meiotic chromosome segregation events, to avoid untimely activation of the
31 MutL γ endonuclease once recruited to future crossover sites.

32

33

1 **Introduction**

2 Meiotic recombination provides crossovers that allow homologous chromosomes
3 to be transiently physically attached together and then properly segregated (1). A
4 lack or altered distribution of meiotic crossovers is a major source of aneuploidies,
5 leading to sterility or disorders such as Down syndrome, providing strong impetus
6 for understanding how crossovers are controlled during meiosis. Meiotic
7 recombination is triggered by the formation of programmed DNA double-strand
8 breaks (DSBs), catalyzed by Spo11 together with several conserved protein
9 partners (2). Following Spo11 removal, 5' DSB ends are resected and the 3' ends
10 invade a homologous template, leading to formation of D-loop intermediates.
11 After DNA synthesis, many of these intermediates are dismantled by helicases and
12 repaired without a crossover (3). However, a subset are stabilized, and upon
13 capture of the second DSB end, mature into a double Holliday junction (dHJ),
14 which is almost exclusively resolved as a crossover (4, 5). Essential to this
15 crossover pathway are a group of proteins, called ZMMs, which collectively
16 stabilize and protect recombination intermediates from helicases (3, 6-8). The
17 mismatch repair MutL γ (Mlh1-Mlh3) heterodimer is proposed to subsequently act
18 on these ZMM-stabilized dHJs to resolve them into crossovers (9). Accordingly,
19 MutL γ foci reflect the number and distribution of crossovers on both mammalian
20 and plant meiotic chromosomes (reviewed in (10)).

21 In a distinct process, eukaryotic mismatch repair, a MutS-related complex (MutS α
22 or MutS β) recognizes a mismatch, and recruits a MutL-related complex, to initiate
23 repair together with PCNA and Exo1 (reviewed in (11)). MutL α represents the
24 major mismatch repair activity, which involves its endonuclease activity (12, 13).
25 The related MutL γ is instead essential to meiotic crossover, a process that remains
26 relatively ill-defined, though MutL γ endonuclease activity is required for
27 crossover (9, 14-16). Despite its importance, and cross-species relevance, the
28 mechanism of dHJ resolution and crossover formation by MutL γ remains
29 unknown. MutL γ alone does not resolve HJs *in vitro* (15-17), and may need
30 additional partners, meiosis-specific post-translational modifications and/or a
31 specific DNA substrate to promote specific nuclease activity and hence crossover
32 formation.

1 Based on recent *in vitro* experiments and genome-wide analysis of meiotic
2 recombination, it seems that rather than acting as a canonical resolvase, MutLy
3 may nick the DNA, which could result in dHJ resolution and crossover formation
4 if two closely spaced nicks on opposite strands are made (17, 18). *In vitro* analyses
5 with recombinant proteins also suggest that MutLy may need to extensively
6 polymerize along DNA in order to activate its DNA cleavage activity (17).

7 MutLy partners with the ZMM MutSy heterodimer (Msh4-Msh5), proposed to first
8 stabilize recombination intermediates and then to work together with MutLy for
9 crossover resolution (reviewed in (8, 11)). In addition, the exonuclease Exo1
10 interacts *in vitro* with Mlh1. Previous work showed that Exo1's ability to interact
11 with Mlh1 is essential for MutLy function in crossover formation (19). This leaves
12 the mechanism and role of Exo1 in meiotic crossovers unclear, although broad
13 genetic analysis suggests a non-catalytic role given that *Exo1 null* yeast show
14 reduced crossovers and reduced viable gametes, but catalytic mutants of Exo1 do
15 not (9, 20). Likewise, Exo1-null mice are sterile, but not Exo1 catalytic mutants,
16 highlighting the conservation of this function (21, 22). How Exo1 is required for
17 activating MutLy is not known. Finally, the polo-like kinase Cdc5 (homolog of
18 human PLK1) is required for meiotic crossovers (23). Cdc5 expression is induced
19 by the Ndt80 transcription factor at the pachytene stage of meiotic prophase, once
20 homolog synapsis and recombination intermediates formation are achieved (24-
21 27). Cdc5 promotes multiple steps during exit from pachytene, including dHJ
22 resolution, disassembly of the synaptonemal complex, and sister kinetochore
23 mono-orientation (28-30). Among other targets, Cdc5 activates the Mus81-Mms4
24 structure-specific nuclease, by phosphorylating Mms4 (31), but this produces
25 only a minority of crossovers. It is not known how Cdc5 promotes the remaining
26 crossovers thought to come from the major, MutLy-dependent, pathway.

27 In order to define how these factors come together to control crossovers, we
28 undertook a comprehensive analysis of MutLy activation and distribution
29 genome-wide in budding yeast. We found that MutLy and Exo1 form a constitutive
30 complex that associates with recombination sites stabilized by ZMM proteins.
31 Exo1 then interacts with the Cdc5 kinase through a direct, specific contact that is
32 necessary for crossover formation by MutLy. Finally, we find that MutLy-

1 dependent crossover formation is regulated at multiple levels to avoid regions
2 around centromeres where deleterious outcomes could result.

3

4 **Results**

5 **Mlh3 foci on yeast meiotic chromosomes are distinct from ZMM foci**

6 To follow how MutL γ is initially recruited, we examined its relationship to ZMM
7 proteins, Zip3 and Msh4. A recent crystal structure of the C-terminal domain
8 (Cter) of Mlh1-3 shows that the last residues of Mlh1 are part of the Mlh3
9 endonuclease active site, rendering it impossible to C-terminally tag Mlh1 (Fig.
10 1A) (32). To preserve Mlh1-3 functionality *in vivo*, we introduced an internal tag
11 at an unstructured loop in Mlh3 that is predicted not to affect Mlh1-3 function and
12 catalytic activity (Fig. 1A). Tags inserted at this site had no major effect on
13 mismatch repair and no effect on spore viability or meiotic crossover frequency,
14 and are therefore suitable for further *in vivo* studies (Fig. S1).

15 As in plants and mammals, Mlh3 formed foci on yeast meiotic chromosomes (Fig.
16 1B-E). Importantly, Mlh3 foci were largely absent in *zip3 Δ* and in substantially
17 reduced numbers in *msh4 Δ* mutants (Figs. 1F, 1G and 1H), in agreement with
18 genetic data showing that MutL γ acts downstream of ZMM proteins. Surprisingly,
19 although Zip3 and to a lesser extent Msh4 are important for Mlh3 foci, Mlh3
20 showed very little colocalization with either Zip3 or Msh4 (Figs. 1B, 1C and 1I),
21 suggesting that Mlh3 coexists only briefly with the other two components at the
22 same intermediates. We conclude that Zip3 and Msh4 ZMM are required for
23 normal Mlh3 foci formation, and propose that Zip3 and Msh4 form earlier foci on
24 recombination intermediates, before Mlh3 binding, which may reflect their
25 function for stabilization of intermediates.

26

27 **Mlh3 associates with meiotic DSB hotspots at a late step of recombination**

28 We next examined Mlh3 association with specific loci by chromatin
29 immunoprecipitation. Mlh3 associated with the three tested DSB hotspots during
30 meiosis, reaching a maximum at the expected time of crossover formation (4-5 h)
31 (Figs. 2A and S2A) (5), with kinetics very similar to the ZMM proteins (33, 34).
32 Mlh3 also weakly associated with the chromosome axis, where DSB sites
33 transiently relocate during recombination (35, 36) (Fig. 2A). Mlh3 association

1 with DSB hotspots required DSB formation, since it was not observed in a *spo11Δ*
2 mutant (Figs. 2A and S2B), but was independent of its nuclease activity (*mlh3-*
3 *D523N* mutant, Figs. 2A and S2C). Mlh3 binding also required the ZMM protein,
4 Mer3 (Figs. 2A and S2D). In *msh4Δ* mutants, Mlh3 recruitment was strongly
5 reduced (Figs. 2A and S2E), consistent with the reduced number of Mlh3 foci in
6 *msh4Δ* (Figs. 1G and 1H). By contrast, Mlh3 bound at hotspots at nearly wild-type
7 levels in *exo1Δ* mutants (Figs. 2A and S2F). Finally, to understand how and when
8 it functions, we assessed the role of Cdc5 polo kinase, required for meiotic
9 crossover, in recruiting Mlh3 to recombination sites. We found that in a *ndt80Δ*
10 mutant, where Cdc5 is not expressed, Mlh3 was still recruited at DSB sites (Figs.
11 2A and S2G) and also formed foci on pachytene chromosomes (Fig. 1J). We
12 conclude that the lack of dHJ resolution in the absence of Cdc5 is not due to a
13 failure to recruit Mlh1-3 to recombination sites, but possibly a failure to activate
14 Mlh1-3 once recruited.

15 Having determined that Mlh3 binds at a late step of recombination, depending on
16 ZMM proteins, makes it a good candidate for being a marker for crossovers, as in
17 other organisms.

18

19 **Mlh3 binding levels correlate well with DSB frequencies, except in** 20 **centromeric and late replicating regions**

21 Whether Mlh1-3-dependent crossover has regional specificity across
22 chromosomal territories is an important question, knowing that crossovers mis-
23 localization can lead to aneuploidies (37). To address this, we mapped Mlh3
24 binding sites by ChIP-seq from highly synchronized meiotic cultures thanks to a
25 copper-inducible *pCUP1-IME1* construct (38). We used the 5 and 5.5 h time-points,
26 when crossovers appear (Fig. S3). We also mapped DSB sites by sequencing Spo11
27 oligonucleotides from *pCUP1-IME1* synchronized cultures, at 5 h when DSB levels
28 are maximal (Fig. S3). The Mlh3 binding map was highly similar to that of the Mer3
29 and Zip4 ZMMs: Mlh3 formed peaks around DSB hotspots, in addition to being
30 weakly associated with axis binding sites (Figs. 2B, 2C, S4A and S4B).

31 To get insight into the mode of action of Mlh1-3 once on recombination
32 intermediates, we next asked which DNA region Mlh3 occupies on individual DSB
33 hotspots. Mlh3 peaks at DSB hotspots had a width (2.2 kb) similar to that

1 previously determined for the length of resection tracts at DSB hotspots (1.6 kb)
2 (39) (Fig. S4C), which is predicted to result in recombination intermediates of
3 roughly similar size (18). Furthermore, in an *exo1-D173A* (*exo1-nd*) catalytic
4 mutant that shows a 2.2 fold reduction in resection tracts lengths (19, 39), Mlh3
5 peaks at DSB hotspots were clearly less wide than in wild-type (Figs. S4C and S4D),
6 consistent with the detectable Mlh1-3 distribution *in vivo* being mostly defined by
7 the limits of recombination intermediates.

8 Among the 1155 Mlh3 peaks identified (Table S1), 484 overlapped at least one of
9 the strongest 1000 DSB hotspots (Table S2), and among the strongest 200 DSB
10 hotspots, 164 had a detected Mlh3 peak. In addition, the signal intensities of DSB
11 and Mlh3 at the DSB hotspots were highly correlated (Fig. S4E). Despite this high
12 global correlation between DSB and Mlh3 binding frequencies, we observed a
13 significant reduction of Mlh3 binding per DSB in pericentromeric regions, (Figs.
14 2D and 2E and Figs. S5A and S5B) . By contrast, the ZMM Mer3 and Zip4 were not
15 reduced at pericentromeric regions, but were reduced in subtelomeric regions
16 (Figs. 2D and S5A), indicating a differential regulation than Mlh3.

17 Finally, we asked if the binding of ZMM and Mlh3 on DSB hotspots is modulated
18 by replication timing. Whereas ZMM binding was not affected, Mlh3 binding per
19 DSB was reduced in late replicating regions (Fig. 2F); this was seen in samples
20 from both 5 h (Fig. S5C) and 5.5 h (Fig. 2F) suggesting that this effect is not due to
21 temporal differences in Mlh3 loading between early and late replicating regions.
22 Altogether, our results indicate a regional control of Mlh3 binding to DSBs, that
23 operates negatively in centromeric and in late replicating regions.

24

25 **MutLy forms a complex with Exo1 and interacts with MutSy *in vivo***

26 To further gain a mechanistic handle on the function of key players in meiotic
27 crossover, we examined the *in vivo* interacting partners of MutLy. We
28 immunoprecipitated Mlh3 4 h after initiating meiosis, when crossovers appear
29 (5), followed by proteomics mass spectrometry analysis (Fig. 3A). Mlh3 pulled
30 down its MutLy partner Mlh1, as expected, but also Exo1, providing *in vivo* support
31 for previous proposals that it assists Mlh1-3 in crossover resolution (19) (Fig. 3A
32 and Table S3). We confirmed this interaction by Western blot analysis of the
33 reverse pull down of Mlh3 by Exo1 (Fig. 3B). Interestingly, Exo1 pulled down Mlh1

1 and Mlh3 throughout meiosis, and is therefore a constitutive partner of MutLy
2 (Fig. 3C). This interaction required the Mlh1 E682 residue that mediates the direct
3 interaction between Exo1 and Mlh1 (Fig. 3B), implying that the interaction
4 between Exo1 and MutLy is mediated by Mlh1. Consistent with these results, an
5 *mlh1E682A* mutant displays meiotic crossover defects (19). We noticed that the
6 Mlh1E682A protein is reproducibly present at lower levels than the wild-type
7 Mlh1 protein, although previous studies showed that it is still proficient for
8 mismatch repair. This may be due to its loss of interaction with several partners
9 (Exo1, but also Sgs1, Ntg2) (40).

10 Mlh3 immunoprecipitates also contained Pms1, the partner of Mlh1 in the MutL α
11 heterodimer (Fig. 3A, Table S3), and we confirmed this interaction in reverse
12 Pms1 pulldowns (Fig. 3D). This possible MutL α -MutLy interaction prompted us to
13 ask if MutLy-MutLy interactions also occur, possibly as a result of the proposed
14 MutLy polymerization needed to cleave DNA *in vitro* (17). However, we failed to
15 detect any Mlh3-Mlh3 interaction in coimmunoprecipitation assays (Fig. 3D,
16 lower panel and S5), suggesting either that MutLy does not interact with itself *in*
17 *vivo* or that this may be regulated and occur only at the time of CO formation,
18 making it difficult to detect.

19 Surprisingly, we did not recover any peptides from the Msh4-Msh5 (MutS γ)
20 heterodimer or from other ZMM proteins in the Mlh3 immunoprecipitates (Table
21 S3). A weak coimmunoprecipitation of Mlh1 and Mlh3 by Msh4 was detected, but
22 the fraction of Mlh1 and Mlh3 was very low (Fig. 3B), consistent with the lack of
23 visible co-localization of Mlh3 and Msh4 foci. This Mlh3-Msh4 interaction, even
24 transient *in vivo*, supports recent *in vitro* data showing that the activity of the
25 human MutLy complex is strongly stimulated by both Exo1 and MutS γ (41, 42).

26

27 **Cdc5 kinase interacts with both MutLy and Exo1 bound to recombination** 28 **sites**

29 We have shown that Cdc5 is not required for Mlh3 binding to DSB hotspots, and
30 moreover its targets relevant for crossover formation by the Mlh1-Mlh3 pathway
31 are still unknown (23). We did not detect Cdc5 in our meiotic Mlh3 pulldowns
32 (Table S3), but to address this issue, we examined if Cdc5 interacts with MutLy
33 and Exo1 in highly synchronous meiotic cells. At the time when crossovers start

1 to appear (5.5 h, Fig. S3), Cdc5 robustly immunoprecipitated Mlh1, Mlh3 and Exo1
2 (Fig. 4A), raising the possibility that it may directly activate MutL γ . Indeed, Cdc5
3 associated with recombination sites during meiosis, at the same time as Mlh3 (Fig.
4 4B). Since MutL γ associates with recombination sites in *ndt80 Δ* mutants, where
5 Cdc5 is absent (Fig. 2A), we propose that Cdc5 is recruited to a pre-existing Exo1-
6 MutL γ complex bound at CO sites.

7 To know which of the MutL γ -Exo1 components is a direct target of Cdc5, we
8 assessed their interdependency for *in vivo* coimmunoprecipitation with Cdc5.
9 Strikingly, Cdc5 still associated at normal levels with Mlh1 and Mlh3 in the
10 absence of Exo1 (Fig. 4C), suggesting that MutL γ itself or another of its interacting
11 partners may be a phosphorylation target of Cdc5. Moreover, Exo1 still
12 coimmunoprecipitated with Cdc5 in the absence of interaction with MutL γ (Fig.
13 4D, *mlh1E682A* mutant, where Exo1 interaction with Mlh1-3 is abrogated),
14 demonstrating that Cdc5 also interacts with Exo1.

15

16 **Cdc5 directly interacts with Exo1 to promote crossover formation**

17 We tested whether Cdc5 interacts directly with the above factors. Using a yeast
18 two-hybrid assay, we found that the Cdc5 polo-box domain (PBD), known to be
19 involved in interaction with Cdc5 targets, did interact with Exo1, whereas it did
20 not with either Mlh1 or Mlh3 (Fig. S7A). Cdc5 PBD usually binds a priming
21 phosphate, often formed by CDK1, in the context of a consensus motif, S-
22 S(Phos)/T(Phos)-P (43, 44). However, recombinant purified Exo1 strongly
23 interacted with purified Cdc5 (Figs. 4E and S7B), even after pre-treating Exo1 with
24 phosphatase, ruling out that this direct interaction is mediated through Exo1
25 phosphorylation.

26 A few other cases of phosphorylation-independent Cdc5 interactions have been
27 described, including that between Cdc5 and Dbf4 (45-47). Domain mapping
28 defined a small region of Exo1 (531 to 591) necessary and sufficient for
29 interaction with Cdc5 PBD using two-hybrid assays (Figs. 5A and S7C). This region
30 contains a motif (570-575) conserved among yeast species, resembling the
31 RSIEGA motif found in Dbf4 (Fig. 5B) (47). The Cdc5 PBD structure has recently
32 been solved, and shows a specific binding surface for the Dbf4 motif, opposite from
33 the canonical phosphopeptide binding site (Fig. 5Ci) (48). Interestingly, the Exo1

1 RSIEGA-like motif could be modeled interacting exactly with the same Cdc5
2 surface as Dbf4 (Fig. 5Cii). Importantly, mutation of the key residue of this surface
3 (S630Q mutation) abolished interaction with Exo1, as it did for Dbf4, but kept
4 intact the interaction with Spc72, a canonical interacting substrate of Cdc5 (Figs.
5 5D and S7D) (48). Conversely, mutation of the WHK motif of Cdc5 involved in
6 phosphopeptide recognition (44) did not alter interaction with Exo1 or Dbf4, but
7 reduced interaction with Spc72 (Figs. 5D and S7D) (47, 48). Furthermore,
8 mutation of the equivalent of the key residues of Dbf4 of this motif in Exo1 (*R570E*
9 *I572D G574A*, hereafter called *exo1-cid*, Cdc5 interaction-deficient) abolished the
10 two-hybrid interaction between Exo1 and Cdc5 (Figs. 5A and S7C), confirming
11 that Exo1 and Dbf4 bind in a similar way to Cdc5.

12 Interaction between Exo1-cid and Cdc5 was also strongly reduced in *in vivo*
13 coimmunoprecipitation assays, to around 35% of wild type levels (Fig. 6A). In
14 addition, *exo1-cid* mutants showed decreased crossovers, on two different tested
15 chromosomes, at the *HIS4LEU2* hotspot on chromosome III (Fig. 6B), and in the
16 *CEN8-ARG4* interval on chromosome VIII (Fig. 6C). Moreover, crossovers were
17 especially reduced by the *exo1-cid* mutation combined with a triple nuclease
18 mutant where only MutL γ -dependent crossovers remain (Figure 6B, *mms4-md*
19 *yen1 Δ slx4 Δ* , (9)), or with the single *mms4-md mutant* (Fig. 6C). In all cases
20 examined, crossover levels in *exo1-cid* were greater than in the *exo1 Δ* mutant, but
21 decreased to the same extent as in mutants that impair Exo1 interaction with
22 MutL γ (*exo1-FF477AA* and *mlh1E682A* mutants (19)), highlighting that the *in vivo*
23 crossover function of Exo1 is lost in the *exo1-cid* mutant. To ensure that the
24 crossover defects of Exo1-cid are due to its loss of interaction with Cdc5, we asked
25 if forced recruitment of Cdc5 to this mutant protein restores crossovers. We
26 therefore built strains where one copy of *exo1-cid* is fused to *CDC5*, behind the
27 endogenous *EXO1* promoter. To avoid confounding effects of expressing *CDC5* at
28 the same time as *EXO1*, and therefore throughout meiosis, we made a control
29 where *CDC5* was expressed from the *EXO1* promoter without fusion to *EXO1* (Fig.
30 6D). Furthermore, to avoid premature activation of the structure-specific
31 nucleases due to early expression of *CDC5* (31, 49), we conducted the experiment
32 in the triple nuclease background. Remarkably, only the fusion of Cdc5, but not its
33 kinase-dead version, to Exo1-cid increased crossovers, to levels approaching wild-

1 type Exo1 (Fig. 6D). Our results therefore demonstrate the direct function of Exo1
2 in recruiting Cdc5, for downstream phosphorylation of targets relevant for
3 crossover.

4 Since Exo1 directly interacts with Cdc5, we assessed if Exo1 phosphorylation is
5 important for crossovers. We mapped the *in vivo* Exo1 phosphorylation sites by
6 mass spectrometry of Exo1-TAP purified from meiotic cells after induction of
7 Cdc5, and identified S664 (within the CDK consensus SSP motif of Exo1) as being
8 phosphorylated, along with eight other sites, including three previously described
9 as resulting from DNA damage checkpoint activation (50) (Figs. S8A and B).
10 However, mutation of all nine phosphorylated residues of Exo1 (mutant *exo1-9A*)
11 did not affect crossovers (Fig. S8C). These results converge to suggest that Cdc5
12 does not phosphorylate Exo1 itself, or if it does, it is not important for crossover
13 formation. Instead, our data suggest that Exo1 interacts with Cdc5, to activate *in*
14 *situ* the kinase activity of Cdc5 towards its targets, MutL γ or its partners.

15

16 **Discussion**

17 In this study, we provide the first thorough *in vivo* characterization of MutL γ
18 complex function in meiotic crossovers. We show that MutL γ forms foci on meiotic
19 chromosomes and that globally, its binding to recombination sites is repressed
20 close to centromeres and in late replicating regions. This predicts that the
21 outcome of meiotic recombination is specifically modulated at specific
22 chromosomal regions. Relevant to MutL γ activation, we show a weak, possibly
23 very transient physical interaction with MutS γ during meiosis and a robust,
24 constitutive interaction with Exo1. We reveal a new non-catalytic function of Exo1
25 in interacting with Cdc5 to activate crossover formation. Our data highlight a non-
26 canonical, direct interaction, which does not seem to be accompanied by Cdc5-
27 mediated Exo1 phosphorylation. We propose that Exo1 serves as a matchmaker
28 and that direct binding of Cdc5 to Exo1 allows Cdc5 to be active and locally
29 phosphorylate MutL γ (Fig. 7).

30

31 **The map of Mlh3 binding sites is highly correlated with that of DSBs except**
32 **in specific chromosome regions**

1 The choice to make a crossover during meiotic DSB repair may be modulated
2 along chromosomes, but a precise and high resolution comparison of DSB and
3 crossover levels is missing (reviewed in (51)). In mice, studies of a few hotspots
4 suggest that crossover/noncrossover ratios can vary from one hotspot to another
5 (52-55). Recent data comparing a genome-wide DSB map with existing genetic
6 maps have shown that in female mice, crossover formation is repressed at
7 subtelomeric regions (56). Improper placement of crossovers in the vicinity of
8 centromeres is infrequent and negatively influences meiotic chromosome
9 segregation (37, 57, 58). Although recombination close to centromeres is already
10 reduced at the level of DSB formation, residual DSBs increase the risk of
11 aneuploidy (59). One way to prevent crossover formation by pericentromeric
12 DSBs is to repair them from the sister chromatid and/or as noncrossovers (60,
13 61). We found that Mlh3/DSB ratios are lower in pericentromeric regions than
14 along chromosome arms, which may also contribute to the lower crossover levels
15 per DSB observed in pericentromeres. Since ZMM binding is not significantly
16 reduced, we propose that there is a deselection of ZMM-bound at pericentromeric
17 recombination sites, which are then preferentially repaired without MutL γ
18 binding, as noncrossovers. In this regard, the phosphatase PP2A associated to
19 Shugoshin has been shown to counteract cohesin kinases, including Cdc5, in
20 pericentromeric regions (62). It may also prevent Cdc5-driven MutL γ activation in
21 these regions.

22 Prior to our study, it was not clear if replication timing also influenced the choice
23 to repair DSBs as a crossover (63, 64). The fact that Mlh3 binding per DSB is less
24 frequent in late replicating regions suggests there may be a preferred time
25 window for loading MutL γ onto recombination intermediates, such that DSBs
26 occurring late as a consequence of late replication would be less likely to load
27 MutL γ . It remains to be determined if these DSBs are repaired by the other
28 nucleases able to generate crossovers (such as Mus81-Mms4) or if they are
29 repaired through the noncrossover pathway. Recent cytological data in tomato
30 suggested that the different crossover promoting nucleases (MutL γ and the
31 structure-specific nucleases) are differently distributed along chromosomes (65).

32

1 **Mlh3 associates with DSB sites, at a late step of recombination, and forms**
2 **foci distinct from ZMM-bound foci**

3 MutL γ is predicted to act on recombination intermediates that have been
4 stabilized by ZMM proteins, consistent with our finding that, in their absence,
5 Mlh3 binding is absent (*zip3 Δ* and *mer3 Δ*) or reduced (*msh4 Δ*). The fact that we
6 did not see colocalization between ZMM and Mlh3 foci suggests that they are two
7 temporally distinct species that form on recombination intermediates, at least as
8 visible entities. Indeed, we detect much less (16 on average) Mlh3 foci than the
9 estimated Mlh1-Mlh3 dependent crossovers (around 80). Zip3 and Msh4 foci
10 would precede Mlh3 foci, to preserve the dHJs and hand them over to MutL γ ,
11 thereby positioning and activating MutL γ on the recombination intermediates,
12 without staying on chromatin as visible foci when the Mlh3 focus forms (Fig. 7).
13 Our results are consistent with those in mice, where early co-localization between
14 Msh4 and Mlh1 foci disappears at mid-pachytene (66, 67).

15 In budding yeast, ZMM foci (around 45 per nucleus) are proposed to all give rise
16 to crossovers (around 75 ZMM-dependent crossovers per meiosis) (51, 68),
17 contrary to plants, mammals and *Sordaria* where only a subset of ZMM foci result
18 in crossovers (69-72). However, the fact that we detect less Mlh3 foci (16 per
19 nucleus) than ZMM suggests that a similar selection of a subset of ZMM-bound
20 sites may operate in budding yeast, to become bound by MutL γ , the remaining
21 being dismantled by helicases into noncrossover products. In *Sordaria*, Msh4 foci
22 numbers diminish between early and mid-pachytene, a time frame in which Mlh1-
23 Mlh3 is believed to be recruited (73). An alternative hypothesis is that Mlh3
24 residence time on late crossover intermediates may be limited and therefore Mlh3
25 is not detected as a focus within a single nucleus on all crossover sites. Finally, we
26 cannot exclude that the foci we detect, which are much fewer (16 on average) than
27 predicted MutL γ -dependent crossovers (75), represent only a subset
28 (unproductive, or at a particular recombination stage) of all the Mlh3 bound sites.

29

30 **MutL γ forms a constitutive complex with Exo1 in meiotic cells but interacts**
31 **only transiently with MutS γ**

32 In many organisms (budding yeast, mice and plants) *msh4* or *msh5* mutants have
33 an earlier meiotic defect than MutL γ mutants, but it is attractive to propose, by

1 analogy to mismatch repair, that MutS γ also works directly with MutL γ -Exo1 for
2 crossover formation. Consistent with an early role for MutS γ , we show that most
3 Msh4 foci do not colocalize with Mlh3, but that Msh4 is required for the
4 association of Mlh3 with recombination sites. In addition, the detected interaction
5 between a small fraction of MutS γ with MutL γ in yeast meiotic cells fits also with
6 a late function for MutS γ through direct activation of MutL γ . A similar interaction
7 was also described for the cognate mouse proteins (67). A recent study reports
8 that human MutS γ strongly stimulates human MutL γ endonuclease *in vitro* (41).
9 Together with our cytological and interaction data, we can then propose that *in*
10 *vivo*, MutS γ contributes by creating the proper substrate where the MutL γ -Exo1
11 ensemble loads, and that a subset of MutS γ may subsequently activate MutL γ
12 catalysis by direct interaction with MutL γ (Fig. 7). However, it is worth noting that,
13 in *C. elegans*, MutS γ promotes crossovers and remains bound to crossover sites,
14 although crossover resolution is not achieved by MutL γ , suggesting that, in this
15 organism, MutS γ cooperates, directly or indirectly, with a nuclease other than
16 MutL γ (74, 75). Further work aiming at genetically separating these two functions
17 of MutS γ will clarify these issues.

18

19 **Another example of non-canonical Cdc5 binding to its substrates, involving** 20 **the RSIEGA motif**

21 We found that Exo1 interacts with Cdc5 through the same mode as the only
22 example described so far, Dbf4. Besides the importance for the meiotic
23 recombination context, our study therefore adds another case where the recently
24 described interaction surface on Cdc5, distinct from the phosphopeptide
25 recognition surface, promotes interaction with a protein relevant for a biological
26 process (48). This surface is conserved in human PLK, and our work should open
27 new avenues to search for other partners of Cdc5 or human PLKs with similar
28 interacting motifs, and to inhibitors that may specifically target this new
29 interacting surface.

30

31 **Exo1 serves as a Cdc5 recruiting/activating platform for MutL γ crossover** 32 **formation**

1 Our study is the first to establish a functional molecular link between Cdc5 and the
2 MutLy-Exo1 complex, providing some explanation for why this kinase is
3 important for meiotic crossovers (23). We show that Exo1 is constitutively
4 associated with MutLy, and therefore the binding of Exo1 to MutLy is not sufficient
5 *in vivo* to activate resolution. Rather, our data, in particular the observation that
6 mutants blocking Exo1-Cdc5 interaction have crossover defects, suggest that
7 resolution is activated by Cdc5 through its direct interaction with Exo1. What
8 could be the downstream event triggered by Cdc5 binding to Exo1?
9 Cdc5 function in MutLy crossovers involves phosphorylation of at least a
10 substrate, because induction of a kinase-dead Cdc5 in meiosis is not sufficient to
11 promote crossover formation (23) and because the forced recruitment of Cdc5 to
12 Exo1 only stimulates crossover when Cdc5 kinase activity is intact (our results).
13 It is unlikely that Exo1 is the relevant phosphotarget, since mutation of Exo1's
14 meiotic phosphorylated residues that we have detected had no effect on
15 crossovers. Another obvious candidate for phosphorylation, once Cdc5 binds
16 Exo1, is the MutLy complex itself. However, attempts failed to detect Cdc5
17 activation-associated change in the mobility of Mlh1 or Mlh3 (this study, Fig. 3C,
18 and (76)), and published meiotic phosphoproteomes datasets do not contain
19 meiotic phosphorylation sites on Mlh1 or Mlh3 that would appear upon Cdc5
20 induction (77). Another possible target is the Chd1 protein, recently described as
21 a binding partner of Exo1 and important for MutLy crossovers (76). A proposed
22 function of this chromatin remodeler is to allow MutLy polymerization along DNA,
23 an event suggested important for *in vitro* nuclease activity on naked DNA (17, 76)
24 and that would require chromatin remodeling *in vivo*. Although we failed at
25 detecting indications that MutLy polymerizes (no MutLy-MutLy interaction, no
26 large spreading of Mlh3 peaks around DSB hotspots), we cannot exclude that once
27 loaded, MutLy may polymerize transiently when becoming active on a region
28 broader than recombination intermediates (Fig. 7). Further experiments
29 examining Mlh3 dynamics at the time of dHJ resolution and the influence of Cdc5
30 and Chd1 will be required to examine this possibility.
31 It is noteworthy that despite the reduced crossovers seen when Cdc5 no longer
32 directly interacts with Exo1, Cdc5 is able to coprecipitate with MutLy from meiotic
33 cells even in the absence of Exo1, and that the Exo1-cid mutant, although totally

1 deficient for two-hybrid interaction with Cdc5, also shows residual co-
2 precipitation with Cdc5 *in vivo*, likely via its interaction with MutLy. Therefore,
3 rather than being essential to bring Cdc5 to the MutLy-Exo1 complex, direct Exo1
4 binding may serve as the molecular switch to allow Cdc5 phosphorylate its targets
5 (Fig. 7).

6
7 As a whole, our study reveals that MutLy activation is tightly controlled *in vivo*,
8 both locally through the direct coupling of crossover formation with key meiotic
9 progression steps by the Cdc5 kinase, and globally by the underlying chromosome
10 structure. Much of this regulation is likely to operate in mammals, where the key
11 proteins are conserved. Our study provides an illustration of how nucleases at risk
12 of impairing genome integrity in the germline are kept in check through multiple
13 control levels.

14
15 **Materials and methods**

16 **Yeast manipulations**

17 All yeast strains are derivatives of the SK1 background except those used for 2
18 hybrid and mutation analyses (Table S4), and the strains used in specific figures
19 are listed in Table S5. All experiments were performed at 30°C. Growth conditions
20 and strain constructions are described in Supplemental Methods

21
22 **Analysis of crossover frequencies**

23 For crossover analysis at *HIS4LEU2*, cells were harvested from meiotic time
24 courses at the indicated time point. 2 µg genomic DNA was digested with *XhoI* and
25 analyzed by Southern blot using a labeled DNA probe A as described (5). The
26 radioactive signal was detected by a Phosphorimager (Typhoon, GE Healthcare)
27 and quantified using the Image Quant software as described (32). For genetic
28 distances on chromosome VIII, diploids were sporulated in liquid medium, and
29 recombination between fluorescent markers was scored after 24h in sporulation,
30 by microscopy analysis as described (78). Two independent sets of each strain
31 were combined and at least 600 tetrads were counted. Genetic distances in the
32 *CEN8-ARG4* interval were calculated from the distribution of parental ditype (PD),
33 nonparental ditype (NPD) and tetratype (T) tetrads and genetic distances (cM)

1 were calculated using the Perkins equation: $cM = (100 (6NPD + T))/(2(PD + NPD$
2 $+ T))$. Standard errors of genetic distances were calculated using Stahl Lab Online
3 Tools (<https://elizabethhousworth.com/StahlLabOnlineTools/>).

4

5 **Flag-affinity pull-down, proteomics mass spectrometry and co-** 6 **immunoprecipitation analyses**

7 2.10^{10} cells (for Flag pull-down and mass spectrometry analysis) or $1.2 \cdot 10^9$ cells
8 were harvested from synchronous meiotic cultures at the indicated time after
9 meiosis induction. Details are provided in the Supplemental Methods.

10

11 **Recombinant proteins and interaction assays**

12 Recombinant yeast Exo1-Flag and Cdc5 were purified from insect cells. Details of
13 protein purification and Exo1-Flag pull-downs are in the Supplemental Methods.

14

15 **Yeast two-hybrid assays**

16 Yeast two-hybrid assays were performed exactly as described (32). Cloning
17 details are in the Supplemental Methods.

18

19 **Modeling of Dbf4 and Exo1 peptides on Cdc5 PBD structure**

20 The RSIEGA motif of Exo1 was modeled based the structure of Cdc5 bound to a
21 Dbf4-derived peptide encompassing the RSIEGA motif of Dbf4 (PDB ID: 6MF6;
22 (48). The sequence was edited in Coot (79) and the rotamers of each residue
23 chosen to prevent clashes between Cdc5 and the modeled peptide.

24

25 **Cytology**

26 4×10^7 cells were harvested at the indicated time-point and yeast chromosome
27 spreads were prepared as described (80). Mlh3-myc18 was stained with primary
28 anti-myc rabbit antibody (Abcam, 1:600) and secondary FITC-conjugated anti-
29 rabbit (Thermo Fischer 1:600) for all cytological experiments except for the
30 colocalization with Zip3, for which primary 9E11 anti-myc mouse antibody was
31 used together with a secondary Cy3-conjugated anti-mouse (Jackson
32 ImmunoResearch, 1:600). Msh4-HA3 was stained with primary anti-HA mouse
33 antibody (Anopoli, 1:1000) and secondary Cy3-conjugated anti-mouse antibody

1 (Thermo Fischer 1:600) when used in parallel with rabbit based anti-myc. Zip3
2 was stained with a primary anti-Zip3 rabbit antibody (1:2000), a gift from Akira
3 Shinohara. The secondary antibody was Cy5-conjugated anti-rabbit (Amersham,
4 1:500). Fluorescent microscopy was carried out on a ZEISS AXIO Imager M2 with
5 a ZEISS Plan-Neofluar 100x, and a 2x additional magnification by a Zeiss optovar.
6 Images were taken at an exposure of 1 second for DAPI (BFP channel), Cy3 (Cy3
7 channel), FITC (FITC channel) and 2 seconds for Cy5 (AF660 channel). The Light
8 source: Sola SM II (Llumencor); camera: CoolSNAP HQ2 (Visitron Systems GmbH);
9 acquisition software: Visiview (Visitron Systems GmbH). Nuclei acquired with this
10 setup were analyzed by Fiji software and R-scripts.

11

12 **Chromatin immunoprecipitation, real-time quantitative PCR and ChIP-seq**

13 For each meiotic time point, $2 \cdot 10^8$ cells were processed as described (32). Details
14 are in the Supplemental Methods

15

16 **Spo11 oligonucleotide mapping**

17 Spo11-Flag oligonucleotides were purified and processed for sequencing library
18 preparation and analyzed as previously described (81), from synchronous *pCUP1-*
19 *IME1* strains (see Supplemental Methods).

20

21 **Sequencing data processing and bioinformatic analyses**

22 ChIP-seq and Spo11 oligo data were analyzed and normalized mostly as described
23 (33, 81). Details are provided in the Supplemental Methods.

24

25 **Data availability**

26 Sequencing data were deposited at the Gene Expression Omnibus (GEO) with the
27 accession numbers GSE133108 (Spo11 oligos in *pCUP1-IME1*), GSE132850 (Mer3
28 and Mlh3 ChIP-seq). Proteomic data were deposited on the Proteome Xchange
29 with the identifiers PXD014180 (Mlh3 pulldowns) and PXD014185 (Exo1
30 phosphosites).

31 All data are included in the article and SI Appendix (Table S6).

32

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15

16 **References**

- 17 1. N. Hunter, Meiotic Recombination: The Essence of Heredity. *Cold Spring*
18 *Harb Perspect Biol* **7**, a016618 (2015).
- 19 2. I. Lam, S. Keeney, Mechanism and Regulation of Meiotic Recombination
20 Initiation. *Cold Spring Harbor Perspectives in Biology* **7**, a016634 (2015).
- 21 3. A. De Muyt *et al.*, BLM helicase ortholog Sgs1 is a central regulator of
22 meiotic recombination intermediate metabolism. *Molecular Cell* **46**, 43-53
23 (2012).
- 24 4. T. Allers, M. Lichten, Differential timing and control of noncrossover and
25 crossover recombination during meiosis. *Cell* **106**, 47-57 (2001).
- 26 5. N. Hunter, N. Kleckner, The single-end invasion: an asymmetric
27 intermediate at the double-strand break to double-holliday junction
28 transition of meiotic recombination. *Cell* **106**, 59-70 (2001).
- 29 6. L. Jessop, B. Rockmill, G. S. Roeder, M. Lichten, Meiotic chromosome
30 synapsis-promoting proteins antagonize the anti-crossover activity of
31 Sgs1. *PLoS Genetics* **2**, e155 (2006).
- 32 7. S. D. Oh *et al.*, BLM ortholog, Sgs1, prevents aberrant crossing-over by
33 suppressing formation of multichromatid joint molecules. *Cell* **130**, 259-
34 272 (2007).
- 35 8. A. Pyatnitskaya, V. Borde, A. De Muyt, Crossing and zipping: molecular
36 duties of the ZMM proteins in meiosis. *Chromosoma* **128**, 181-198 (2019).
- 37 9. K. Zakharyevich, S. Tang, Y. Ma, N. Hunter, Delineation of joint molecule
38 resolution pathways in meiosis identifies a crossover-specific resolvase.
39 *Cell* **149**, 334-347 (2012).

- 1 10. S. Gray, P. E. Cohen, Control of Meiotic Crossovers: From Double-Strand
2 Break Formation to Designation. *Annual Review of Genetics* **50**, 175-210
3 (2016).
- 4 11. C. M. Manhart, E. Alani, Roles for mismatch repair family proteins in
5 promoting meiotic crossing over. *DNA Repair (Amst)* **38**, 84-93 (2016).
- 6 12. E. Gueneau *et al.*, Structure of the MutLalpha C-terminal domain reveals
7 how Mlh1 contributes to Pms1 endonuclease site. *Nature structural &*
8 *molecular biology* **20**, 461-468 (2013).
- 9 13. F. A. Kadyrov *et al.*, *Saccharomyces cerevisiae* MutLalpha is a mismatch
10 repair endonuclease. *J Biol Chem* **282**, 37181-37190 (2007).
- 11 14. K. T. Nishant, A. J. Plys, E. Alani, A mutation in the putative MLH3
12 endonuclease domain confers a defect in both mismatch repair and meiosis
13 in *Saccharomyces cerevisiae*. *Genetics* **179**, 747-755 (2008).
- 14 15. L. Ranjha, R. Anand, P. Cejka, The *Saccharomyces cerevisiae* Mlh1-Mlh3
15 heterodimer is an endonuclease that preferentially binds to Holliday
16 junctions. *J Biol Chem* **289**, 5674-5686 (2014).
- 17 16. M. V. Rogacheva *et al.*, Mlh1-Mlh3, a meiotic crossover and DNA mismatch
18 repair factor, is a Msh2-Msh3-stimulated endonuclease. *J Biol Chem* **289**,
19 5664-5673 (2014).
- 20 17. C. M. Manhart *et al.*, The mismatch repair and meiotic recombination
21 endonuclease Mlh1-Mlh3 is activated by polymer formation and can cleave
22 DNA substrates in trans. *PLoS Biol* **15**, e2001164 (2017).
- 23 18. M. C. Marsolier-Kergoat, M. M. Khan, J. Schott, X. Zhu, B. Llorente,
24 Mechanistic View and Genetic Control of DNA Recombination during
25 Meiosis. *Molecular Cell* **70**, 9-20 e26 (2018).
- 26 19. K. Zakharyevich *et al.*, Temporally and biochemically distinct activities of
27 Exo1 during meiosis: double-strand break resection and resolution of
28 double Holliday junctions. *Molecular Cell* **40**, 1001-1015 (2010).
- 29 20. H. Tsubouchi, H. Ogawa, Exo1 roles for repair of DNA double-strand breaks
30 and meiotic crossing over in *Saccharomyces cerevisiae*. *Mol Biol Cell* **11**,
31 2221-2233 (2000).
- 32 21. S. Schaetzlein *et al.*, Mammalian Exo1 encodes both structural and catalytic
33 functions that play distinct roles in essential biological processes. *Proc Natl*
34 *Acad Sci U S A* **110**, E2470-2479 (2013).
- 35 22. K. Wei *et al.*, Inactivation of Exonuclease 1 in mice results in DNA mismatch
36 repair defects, increased cancer susceptibility, and male and female
37 sterility. *Genes Dev* **17**, 603-614 (2003).
- 38 23. A. Sourirajan, M. Lichten, Polo-like kinase Cdc5 drives exit from pachytene
39 during budding yeast meiosis. *Genes & development* **22**, 2627-2632 (2008).
- 40 24. S. Chu *et al.*, The transcriptional program of sporulation in budding yeast.
41 *Science* **282**, 699-705 (1998).
- 42 25. S. Chu, I. Herskowitz, Gametogenesis in yeast is regulated by a
43 transcriptional cascade dependent on Ndt80. *Mol Cell* **1**, 685-696 (1998).
- 44 26. K.-S. Tung, G. S. Roeder, Meiotic chromosome morphology and behavior in
45 *zip1* mutants of *Saccharomyces cerevisiae*. *Genetics* **149**, 817-832 (1998).
- 46 27. L. Xu, M. Ajimura, R. Padmore, C. Klein, N. Kleckner, NDT80, a meiosis-
47 specific gene required for exit from pachytene in *Saccharomyces*
48 *cerevisiae*. *Mol Cell Biol* **15**, 6572-6581 (1995).

- 1 28. R. K. Clyne *et al.*, Polo-like kinase Cdc5 promotes chiasmata formation and
2 cosegregation of sister centromeres at meiosis I. *Nat Cell Biol* **5**, 480-485
3 (2003).
- 4 29. B. H. Lee, A. Amon, Role of Polo-like kinase CDC5 in programming meiosis
5 I chromosome segregation. *Science* **300**, 482-486 (2003).
- 6 30. B. H. Lee, A. Amon, Polo kinase--meiotic cell cycle coordinator. *Cell Cycle* **2**,
7 400-402 (2003).
- 8 31. J. Matos, M. G. Blanco, S. Maslen, J. M. Skehel, S. C. West, Regulatory control
9 of the resolution of DNA recombination intermediates during meiosis and
10 mitosis. *Cell* **147**, 158-172 (2011).
- 11 32. Y. Duroc *et al.*, Concerted action of the MutLbeta heterodimer and Mer3
12 helicase regulates the global extent of meiotic gene conversion. *Elife* **6**,
13 e21900 (2017).
- 14 33. A. De Muyt *et al.*, A meiotic XPF-ERCC1-like complex recognizes joint
15 molecule recombination intermediates to promote crossover formation.
16 *Genes & development* **32**, 283-296 (2018).
- 17 34. M. E. Serrentino, E. Chaplais, V. Sommermeyer, V. Borde, Differential
18 association of the conserved SUMO ligase Zip3 with meiotic double-strand
19 break sites reveals regional variations in the outcome of meiotic
20 recombination. *PloS Genetics* **9**, e1003416 (2013).
- 21 35. S. Panizza *et al.*, Spo11-accessory proteins link double-strand break sites to
22 the chromosome axis in early meiotic recombination. *Cell* **146**, 372-383
23 (2011).
- 24 36. D. Zickler, N. Kleckner, Meiotic chromosomes: integrating structure and
25 function. *Annual Review of Genetics* **33**, 603-754 (1999).
- 26 37. N. E. Lamb *et al.*, Characterization of susceptible chiasma configurations
27 that increase the risk for maternal nondisjunction of chromosome 21. *Hum*
28 *Mol Genet* **6**, 1391-1399 (1997).
- 29 38. M. Chia, F. J. van Werven, Temporal Expression of a Master Regulator
30 Drives Synchronous Sporulation in Budding Yeast. *G3 (Bethesda)* **6**, 3553-
31 3560 (2016).
- 32 39. E. P. Mimitou, S. Yamada, S. Keeney, A global view of meiotic double-strand
33 break end resection. *Science* **355**, 40-45 (2017).
- 34 40. C. Dherin *et al.*, Characterization of a highly conserved binding site of Mlh1
35 required for exonuclease I-dependent mismatch repair. *Molecular and*
36 *cellular biology* **29**, 907-918 (2009).
- 37 41. E. Cannavo *et al.*, Regulation of the MLH1-MLH3 endonuclease in meiosis.
38 *Nature* 10.1038/s41586-020-2592-2 (2020).
- 39 42. D. S. Kulkarni *et al.*, PCNA activates the MutLgamma endonuclease to
40 promote meiotic crossing over. *Nature* 10.1038/s41586-020-2645-6
41 (2020).
- 42 43. K. Y. Cheng, E. D. Lowe, J. Sinclair, E. A. Nigg, L. N. Johnson, The crystal
43 structure of the human polo-like kinase-1 polo box domain and its
44 phospho-peptide complex. *EMBO J* **22**, 5757-5768 (2003).
- 45 44. A. E. Elia *et al.*, The molecular basis for phosphodependent substrate
46 targeting and regulation of Plks by the Polo-box domain. *Cell* **115**, 83-95
47 (2003).
- 48 45. V. Archambault, P. P. D'Avino, M. J. Deery, K. S. Lilley, D. M. Glover,
49 Sequestration of Polo kinase to microtubules by phosphoprimering-

- 1 independent binding to Map205 is relieved by phosphorylation at a CDK
2 site in mitosis. *Genes Dev* **22**, 2707-2720 (2008).
- 3 46. A. M. Bonner *et al.*, Binding of Drosophila Polo kinase to its regulator
4 Matrimony is noncanonical and involves two separate functional domains.
5 *Proc Natl Acad Sci U S A* **110**, E1222-1231 (2013).
- 6 47. Y. C. Chen, M. Weinreich, Dbf4 regulates the Cdc5 Polo-like kinase through
7 a distinct non-canonical binding interaction. *J Biol Chem* **285**, 41244-41254
8 (2010).
- 9 48. A. W. Almawi *et al.*, Distinct surfaces on Cdc5/PLK Polo-box domain
10 orchestrate combinatorial substrate recognition during cell division. *Sci*
11 *Rep* **10**, 3379 (2020).
- 12 49. M. Arter *et al.*, Regulated Crossing-Over Requires Inactivation of
13 Yen1/GEN1 Resolvase during Meiotic Prophase I. *Dev Cell* **45**, 785-800
14 e786 (2018).
- 15 50. I. Morin *et al.*, Checkpoint-dependent phosphorylation of Exo1 modulates
16 the DNA damage response. *EMBO J* **27**, 2400-2410 (2008).
- 17 51. M. E. Serrentino, V. Borde, The spatial regulation of meiotic recombination
18 hotspots: Are all DSB hotspots crossover hotspots? *Experimental Cell*
19 *Research* **318**, 1347-1352 (2012).
- 20 52. F. Cole, S. Keeney, M. Jasin, Comprehensive, fine-scale dissection of
21 homologous recombination outcomes at a hot spot in mouse meiosis.
22 *Molecular Cell* **39**, 700-710 (2010).
- 23 53. E. de Boer, C. Heyting, The diverse roles of transverse filaments of
24 synaptonemal complexes in meiosis. *Chromosoma* **115**, 220-234 (2006).
- 25 54. E. de Boer, M. Jasin, S. Keeney, Local and sex-specific biases in crossover vs.
26 noncrossover outcomes at meiotic recombination hot spots in mice. *Genes*
27 *Dev* **29**, 1721-1733 (2015).
- 28 55. S. Sarbajna *et al.*, A major recombination hotspot in the XqYq
29 pseudoautosomal region gives new insight into processing of human gene
30 conversion events. *Hum Mol Genet* **21**, 2029-2038 (2012).
- 31 56. K. Brick *et al.*, Extensive sex differences at the initiation of genetic
32 recombination. *Nature* **561**, 338-342 (2018).
- 33 57. L. M. Kuhl, G. Vader, Kinetochores, cohesin, and DNA breaks: Controlling
34 meiotic recombination within pericentromeres. *Yeast* **36**, 121-127 (2019).
- 35 58. B. Rockmill, K. Voelkel-Meiman, G. S. Roeder, Centromere-proximal
36 crossovers are associated with precocious separation of sister chromatids
37 during meiosis in *Saccharomyces cerevisiae*. *Genetics* **174**, 1745-1754
38 (2006).
- 39 59. J. Pan *et al.*, A hierarchical combination of factors shapes the genome-wide
40 topography of yeast meiotic recombination initiation. *Cell* **144**, 719-731
41 (2011).
- 42 60. S. Y. Chen *et al.*, Global analysis of the meiotic crossover landscape.
43 *Developmental Cell* **15**, 401-415 (2008).
- 44 61. N. Vincenten *et al.*, The kinetochore prevents centromere-proximal
45 crossover recombination during meiosis. *eLife* **4**, e10850 (2015).
- 46 62. S. Galander, R. E. Barton, D. A. Kelly, A. L. Marston, Spo13 prevents
47 premature cohesin cleavage during meiosis. *Wellcome Open Res* **4**, 29
48 (2019).

- 1 63. V. Borde, A. S. Goldman, M. Lichten, Direct coupling between meiotic DNA
2 replication and recombination initiation. *Science* **290**, 806-809 (2000).
- 3 64. C. Buhler, V. Borde, M. Lichten, Mapping meiotic single-strand DNA reveals
4 a new landscape of DNA double-strand breaks in *Saccharomyces*
5 *cerevisiae*. *PLoS Biol.* **5**, e324 (2007).
- 6 65. L. K. Anderson *et al.*, Combined fluorescent and electron microscopic
7 imaging unveils the specific properties of two classes of meiotic crossovers.
8 *Proc Natl Acad Sci U S A* **111**, 13415-13420 (2014).
- 9 66. B. Kneitz *et al.*, MutS homolog 4 localization to meiotic chromosomes is
10 required for chromosome pairing during meiosis in male and female mice.
11 *Genes & development* **14**, 1085-1097 (2000).
- 12 67. S. Santucci-Darmanin *et al.*, The DNA mismatch-repair MLH3 protein
13 interacts with MSH4 in meiotic cells, supporting a role for this MutL
14 homolog in mammalian meiotic recombination. *Human molecular genetics*
15 **11**, 1697-1706 (2002).
- 16 68. E. Mancera, R. Bourgon, A. Brozzi, W. Huber, L. M. Steinmetz, High-
17 resolution mapping of meiotic crossovers and non-crossovers in yeast.
18 *Nature* **454**, 479-485 (2008).
- 19 69. L. Chelysheva *et al.*, The Arabidopsis HEI10 Is a New ZMM Protein Related
20 to Zip3. *PLoS Genetics* **8**, e1002799 (2012).
- 21 70. A. De Muyt *et al.*, E3 ligase Hei10: a multifaceted structure-based signaling
22 molecule with roles within and beyond meiosis. *Genes & development* **28**,
23 1111-1123 (2014).
- 24 71. A. Reynolds *et al.*, RNF212 is a dosage-sensitive regulator of crossing-over
25 during mammalian meiosis. *Nature Genetics* **45**, 269-278 (2013).
- 26 72. X. Sun *et al.*, Transcription dynamically patterns the meiotic chromosome-
27 axis interface. *Elife* **4** (2015).
- 28 73. A. Storlazzi *et al.*, Recombination proteins mediate meiotic spatial
29 chromosome organization and pairing. *Cell* **141**, 94-106 (2010).
- 30 74. A. Woglar, A. M. Villeneuve, Dynamic Architecture of DNA Repair
31 Complexes and the Synaptonemal Complex at Sites of Meiotic
32 Recombination. *Cell* **173**, 1678-1691 e1616 (2018).
- 33 75. J. Zalevsky, A. J. MacQueen, J. B. Duffy, K. J. Kemphues, A. M. Villeneuve,
34 Crossing Over During *Caenorhabditis elegans* Meiosis Requires a
35 Conserved MutS-Based Pathway That Is Partially Dispensable in Budding
36 Yeast. *Genetics* **153**, 1271-1283 (1999).
- 37 76. P. Wild *et al.*, Network Rewiring of Homologous Recombination Enzymes
38 during Mitotic Proliferation and Meiosis. *Mol Cell* **75**, 859-874 e854 (2019).
- 39 77. R. T. Suhandynata, L. Wan, H. Zhou, N. M. Hollingsworth, Identification of
40 Putative Mek1 Substrates during Meiosis in *Saccharomyces cerevisiae*
41 Using Quantitative Phosphoproteomics. *PLoS One* **11**, e0155931 (2016).
- 42 78. D. Thacker, I. Lam, M. Knop, S. Keeney, Exploiting spore-autonomous
43 fluorescent protein expression to quantify meiotic chromosome behaviors
44 in *Saccharomyces cerevisiae*. *Genetics* **189**, 423-439 (2011).
- 45 79. P. Emsley, K. Cowtan, Coot: model-building tools for molecular graphics.
46 *Acta Crystallogr D Biol Crystallogr* **60**, 2126-2132 (2004).
- 47 80. M. Xaver, L. Huang, D. Chen, F. Klein, Smc5/6-Mms21 prevents and
48 eliminates inappropriate recombination intermediates in meiosis. *PLoS*
49 *Genet* **9**, e1004067 (2013).

- 1 81. I. Lam, N. Mohibullah, S. Keeney, Sequencing Spo11 Oligonucleotides for
2 Mapping Meiotic DNA Double-Strand Breaks in Yeast. *Methods Mol Biol*
3 **1471**, 51-98 (2017).
- 4 82. X. Zhu, S. Keeney, High-Resolution Global Analysis of the Influences of Bas1
5 and Ino4 Transcription Factors on Meiotic DNA Break Distributions in
6 *Saccharomyces cerevisiae*. *Genetics* **201**, 525-542 (2015).
- 7
- 8

1 **Figure legends**

2

3 **Fig. 1: Mlh3 forms foci on yeast pachytene meiotic chromosomes, distinct**
4 **from ZMM foci**

5 (A) Crystal structure of the C-terminal region of *S. cerevisiae* Mlh1-Mlh3
6 heterodimer showing the position of the internal tags in Mlh3. The Mlh1 and Mlh3
7 regions are colored in light and dark blue, respectively. The Mlh1 binding motif for
8 Exo1 and the endonuclease site of Mlh3 are colored in red and yellow, respectively.

9 (B) Comparison of Mlh3-Myc18 and Zip3 foci.

10 (C) Comparison of Mlh3-Myc18 and Msh4-HA foci.

11 (D) and (E) Quantification of Mlh3, Zip3 and Msh4 foci from (B) and (C).

12 (F) and (G): Mlh3-myc foci in *zip3Δ* (F) and *msh4Δ* (G).

13 (H) Quantification of Mlh3-Myc foci in *zip3Δ* and *msh4Δ* mutants from (B), (F) and
14 (G). 51 nuclei each examined.

15 (I) Co-localization quantification of Mlh3 with Zip3 or Msh4 foci. The % of Mlh3
16 foci co-localizing is indicated.

17 (J) Quantification of Mlh3 foci in pachytene-selected nuclei of wild-type (same as
18 in (B)) and *ndt80Δ*. 51 nuclei each examined.

19 (B) to (J): all experiments at 4 h in meiosis, except *ndt80Δ* (6 h in meiosis).

20 See also Fig. S1.

21

22 **Fig. 2: Mlh3 associates with meiotic DSB hotspots and its distribution is**
23 **influenced locally by specific chromosome features**

24 (A) Mlh3-Myc levels at the three indicated meiotic DSB hotspots and one axis-
25 associated site relative to a negative control site (*NFT1*) assessed by ChIP and
26 qPCR during meiotic time-courses. Values are the mean \pm S.E.M. from three (four
27 in WT) independent experiments at the time-point of maximum enrichment. The
28 full corresponding time-courses are in Fig. S2. The cartoon illustrates the position
29 of sites analyzed by qPCR relative to the meiotic chromosome structure.

30 (B) ChIP-seq binding of Mlh3 (at 5h30 in meiosis) compared to the binding of the
31 Mer3 and Zip4 (33) ZMMs, to DSBs (Spo11 oligos, (82)) and to axis sites (Red1
32 ChIP-seq, (72)). Normalized data are smoothed with a 200 bp window.

1 (C) Average ChIP-seq signal at the indicated features. Same data as in (B). The
2 Mer3 and Zip4 ChIP-seq signals are aligned on the Spo11 hotspots midpoints from
3 (82), and Mlh3 ChIP-seq signal on the *pCUP1-IME1* Spo11 hotspots midpoints (this
4 study). Lower panel, ChIP-seq signal is aligned on the Red1 peaks summits from
5 (72).

6 (D) ZMM and Mlh3 signals per DSB vary with the proximity to a centromere or a
7 telomere. The ChIP-seq signal of each protein divided by their corresponding
8 Spo11 oligo signal (Spo11 signal from (82) for Mer3 and Zip4, and *pCUP1-IME1*
9 Spo11 signal for Mlh3) was computed on the width plus 1 kb on each side of the
10 strongest corresponding 2000 Spo11 hotspots at the indicated chromosome
11 regions: interstitial (1805 and 1829 hotspots); 0 to 10 kb from a centromere (29
12 and 28 hotspots); 10 to 20 kb from a centromere (50 and 46 hotspots); 0 to 40 kb
13 from a telomere (117 and 97 hotspots). For each region, the corresponding
14 interstitial control was computed by randomly selecting groups of interstitial
15 hotspots regions with the same median Spo11 oligo level, this step was repeated
16 10000 times. Statistical differences (Mann-Whitney-Wilcoxon test) between
17 different regions and their interstitial control are indicated.

18 (E) Examples of DSB (*pCUP1-IME1* Spo11 oligo) and Mlh3 binding in an interstitial
19 region and two pericentromeric regions. The normalized signal smoothed with a
20 200 bp window size is indicated.

21 (F) The ZMM and Mlh3 signals per DSB vary with the timing of DNA replication.
22 Same legend as in (D) for the indicated chromosomal regions, except that early
23 and late regions were directly compared to each other.

24 See also Figs. S2, S3, S4 and S5.

25

26 **Fig. 3: MutLy forms a complex with Exo1 and transiently interacts with**
27 **MutSy *in vivo***

28 (A) Affinity pull-down of Mlh3-Flag from cells at 4 h in meiosis. Silver-stained gel
29 of pulled-down proteins. Table: mass-spectrometry analysis of selected proteins
30 reproducibly identified in all replicates and not in the controls (no tag strain). The
31 number of peptides of 4 independent experiments is shown (two with benzonase
32 treatment, two without). Detail of pulled-down proteins in Table S3.

1 (B) Coimmunoprecipitation by Exo1-TAP or Msh4-TAP from cells at 4 h in meiosis
2 analyzed by Western blot. The asterisk indicates the uncleaved Msh4-TAP, of which
3 the protein A region is weakly recognized by the anti-HA antibody. The Tev-cleaved
4 Msh4-TAP is no longer recognized by the anti-HA.

5 (C) Exo1-TAP pull-down throughout meiosis. The graph indicates the ratio of
6 immunoprecipitated Mlh1 and Mlh3 relative to Exo1 in the Tev eluates at the
7 indicated times in meiosis. Values are mean \pm SD of two independent experiments.

8 (D) Coimmunoprecipitation between Pms1-Flag and Mlh3-Myc (upper panel) or
9 between Mlh3-Flag and Mlh3-Myc (lower panel) from cells at 4 h in meiosis
10 analyzed by Western blot. Asterisks indicate a non-specific cross-hybridizing
11 bands.

12 See also Fig. S6.

13

14 **Fig. 4: The Cdc5 kinase interacts with both MutLy and Exo1 bound on**
15 **recombination sites**

16 (A) Coimmunoprecipitation by Cdc5-TAP of Mlh1-HA, Mlh3-Myc and Exo1-Myc
17 from *pCUP1-IME1* synchronized cells at 5 h 30 in meiosis analyzed by Western
18 blot.

19 (B) Mlh3-Myc and Cdc5-TAP association with the indicated DSB hotspots as
20 revealed by CHIP and qPCR at the indicated times of a *pCUP1-IME1* synchronized
21 meiotic time-course. Same normalization as in Fig. 2. Values are the average of
22 three (Mlh3-Myc) or four (Cdc5-TAP) independent experiments \pm S.E.M.

23 (C) Coimmunoprecipitation by Cdc5-TAP of Mlh1-HA and Mlh3-Myc is
24 independent of Exo1. Same conditions as in (A).

25 (D) Coimmunoprecipitation by Cdc5-TAP of Exo1 is independent of Exo1
26 interaction with MutLy. Same conditions as in (A).

27 (E) Direct, phosphorylation-independent interaction between recombinant Exo1
28 and Cdc5 proteins. Western Blot showing the pulldown of purified Cdc5-PM
29 (phosphomimetic, see Methods) by Exo1-Flag, in the presence or absence of CDK1
30 or lambda phosphatase.

31 See also Fig. S7.

32

33 **Fig. 5: Cdc5 directly interacts with Exo1 through a non-canonical site**

1 (A) Delineation of the Exo1 motif responsible for interaction with Cdc5 polo-box
2 domain (PBD) by two hybrid assays. The GAL4-BD fusions with indicated Exo1
3 fragments were tested in combination with a GAL4-AD-Cdc5-PBD fusion. +
4 indicates an interaction. Exo1-cid: Cdc5 interaction-deficient.
5 (B) Conservation of the Exo1 region interacting with Cdc5 PBD and illustration of
6 the Exo1-cid mutation. The Dbf4 motif interacting with Cdc5 PBD (47) is indicated
7 (consensus from 17 Saccharomycetaceae family species, see Methods).
8 (C) Modeling of Dbf4 and Exo1 motifs on the crystal structure of Cdc5 PBD. (i)
9 crystal structure of the Cdc5 PBD bound to a Dbf4-derived peptide encompassing
10 the RSIEGA motif (PDB ID: 6MF6) (48). The structural elements of the polo box
11 domain are color coded. The region of the domain where phosphorylated
12 substrates bind is labeled for reference. (ii) Model of the Cdc5-Exo1 interaction
13 based on the crystal structure of the Cdc5-Dbf4 complex with Cdc5 shown in the
14 same orientation and color scheme as in (i). The inset shows the residues
15 mediating the interaction between the RSIEGA motif of Exo1 (red labels) and Cdc5
16 (black labels).
17 (D) The same surface of Cdc5 used for interaction with Dbf4 is used for interaction
18 with Exo1. The GAL4-BD fusions with indicated fragments were tested in
19 combination with a GAL4-AD-Cdc5-PBD fusion with the indicated mutations
20 (WHK stands for W517F H641A K643M). + indicates an interaction.
21 See also Fig. S7 and S8.

22

23 **Fig. 6: Cdc5 direct interaction with Exo1 promotes crossover formation**

24 (A) Coimmunoprecipitation by Cdc5-TAP of Exo1-Myc in meiotic cells or in cells
25 growing mitotically. Same conditions as in Fig. 4A. Right: quantification of Exo1-
26 Myc levels in the Tev eluate relative to the input. Error bars represent S.D. of two
27 independent experiments.

28 (B) Meiotic crossover frequencies at the *HIS4LEU2* hotspot in the *exo1-cid* mutant.
29 Left: representative Southern blot analysis of crossovers in the indicated *exo1*
30 mutants, in an otherwise wild-type (*MMS4 YEN1 SLX4*) or triple nuclease mutant
31 (*mms4-md yen1Δ slx4Δ*) background. *mms4-md* stands for *pCLB2-mms4*. Right:
32 quantification of crossovers. Values are the mean \pm S.E.M. of 4 independent

1 experiments (wild-type background) or mean \pm S.D. of 2 independent experiments
2 (triple nuclease mutant), normalized to the corresponding *EXO1* value.

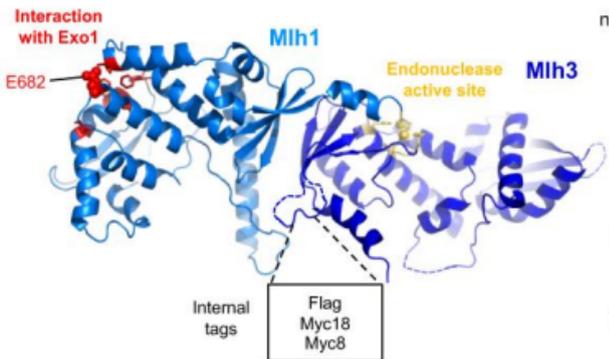
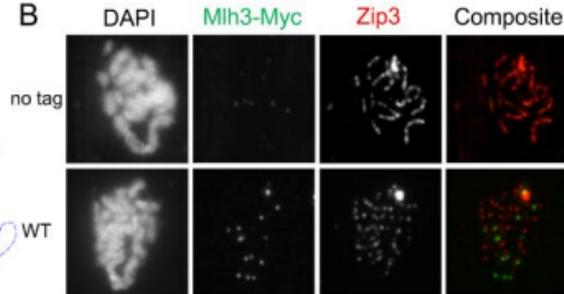
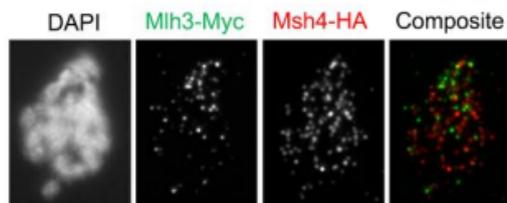
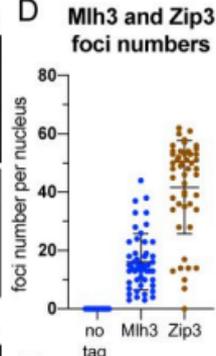
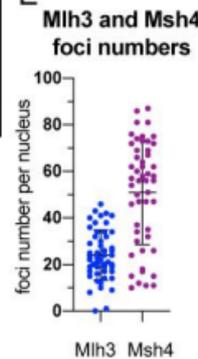
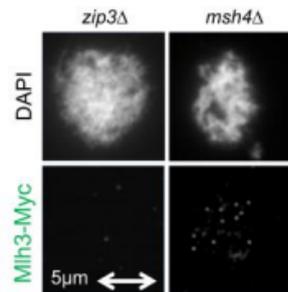
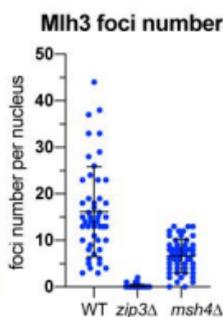
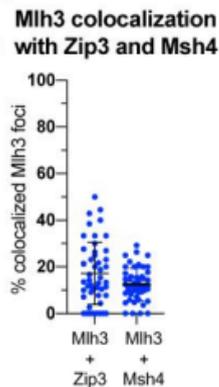
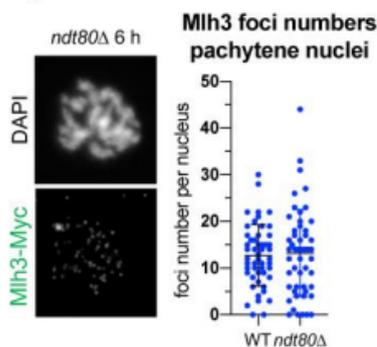
3 (C) Meiotic crossovers on chromosome VIII. Left: illustration of the fluorescent
4 spore setup (78). Right: genetic distances measured in the *CEN8-ARG4* genetic
5 interval, for each indicated genotype.

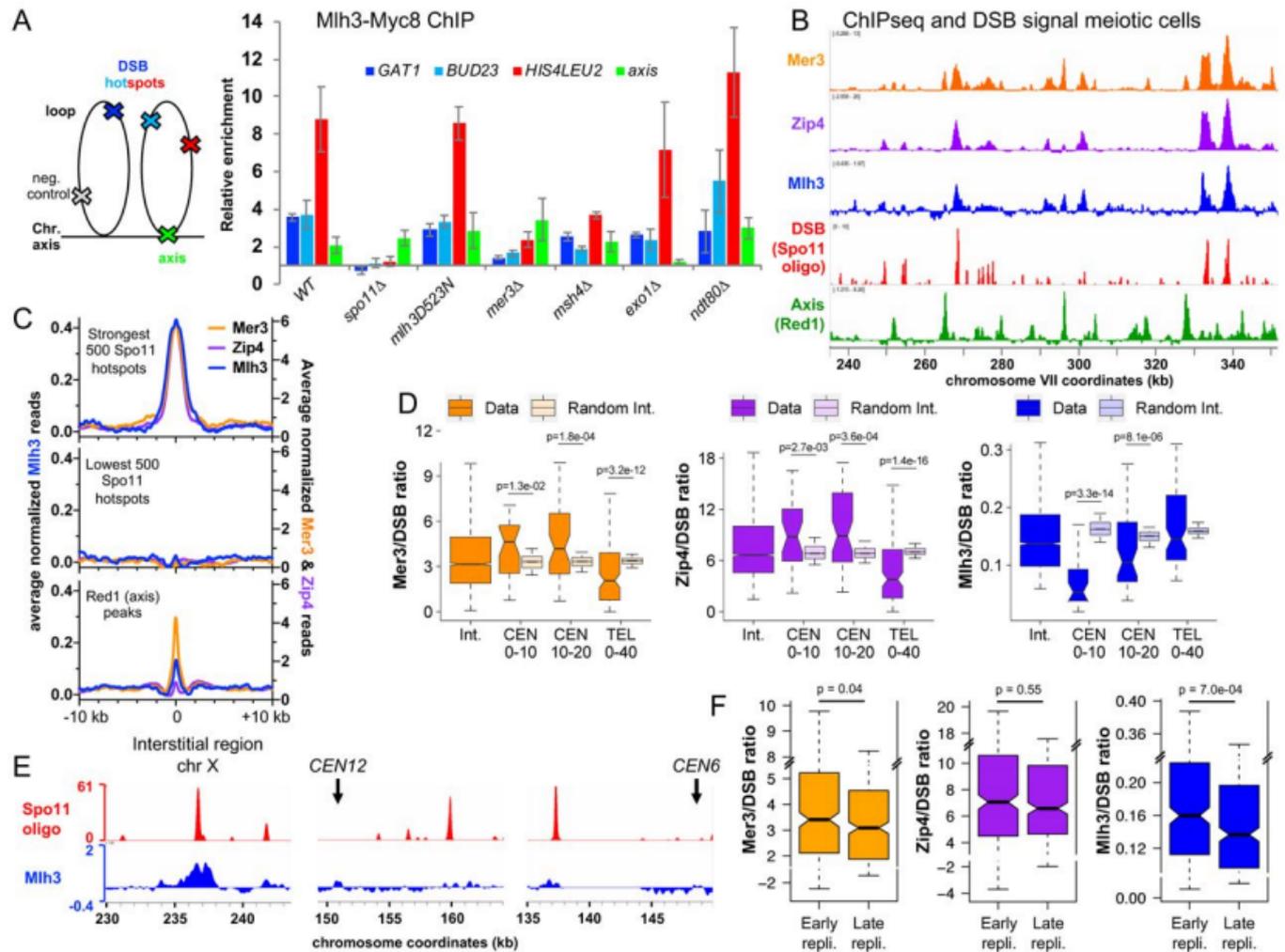
6 (D) Meiotic crossover frequencies at the *HIS4LEU2* hotspot with an Exo1-cid-Cdc5
7 fusion protein. Left: scheme illustrating the different experimental setups. In each
8 cell, proteins are expressed from the two allelic endogenous *EXO1* promoters (left)
9 or *CDC5* promoters (right) . Same legend as in (B). Values are the mean \pm S.D. of 2
10 independent experiments, normalized to the homozygous *exo1-cid-Myc* value

11

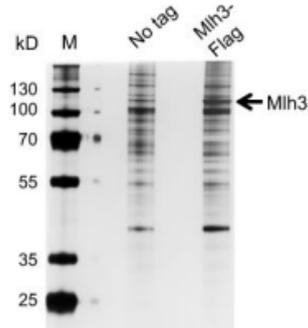
12 **Fig. 7: Model of MutLy binding to sites of recombination and activation for**
13 **crossover formation**

14 MutSy stabilizes dHJ intermediates and forms foci on chromosomes. MutLy, in
15 complex with Exo1, binds dHJ intermediates that have been stabilized by MutSy
16 and other ZMM proteins. For simplicity, MutSy is shown embracing both DNA
17 duplexes of the intermediates, but recent data also suggest it may embrace
18 separately each DNA duplex (74). Once bound on dHJ, MutLy may form a focus
19 when interacting with MutSy (model shown here), or later, in the process of its
20 activation (not shown). Upon *NDT80* activation, the Cdc5 kinase is induced, and
21 interacts with the MutLy-Exo1 complex through multiple interactions (green
22 cloud). Among these interactions, the direct interaction of Cdc5 with Exo1 is
23 important for MutLy-driven crossover formation. We propose that this interaction
24 allows Cdc5 to phosphorylate MutLy, which activates its nuclease function and
25 produces crossover formation. This may occur through transient MutLy
26 polymerization.

A MutL γ Cter structure**B****C****D****E****F****G****H****I****J**

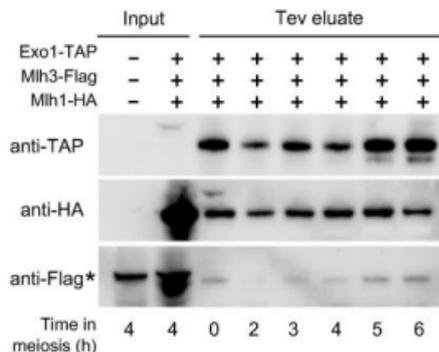


A Mlh3-Flag pull-down meiotic cells

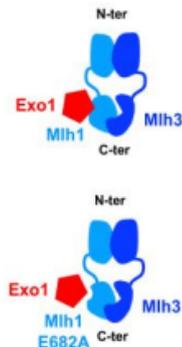
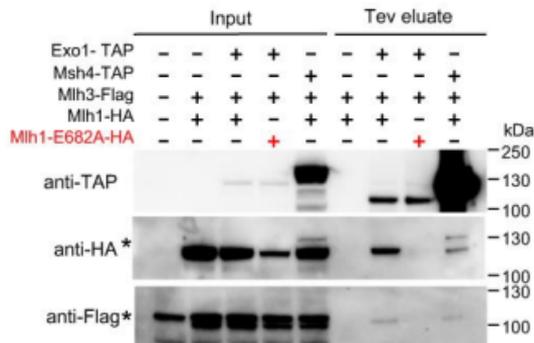


Name	Mean peptide #		% coverage
	No tag	Mlh3-Flag	
Mlh3	-	64	54
Mlh1	-	32	48
Exo1	-	4	12
Pms1	-	6	11

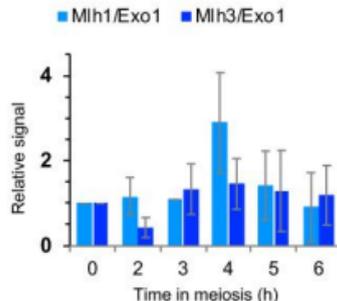
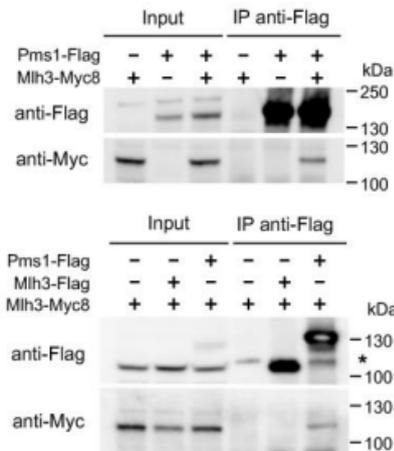
C Exo1-TAP pull-down during meiosis



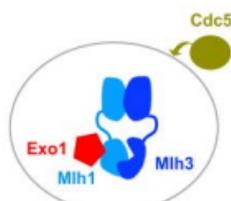
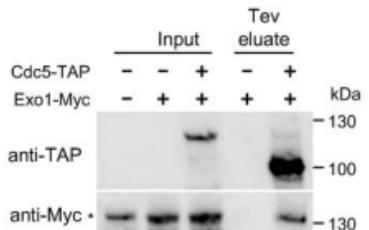
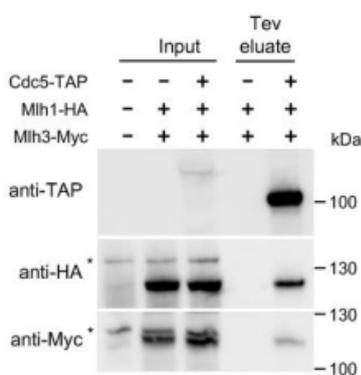
B Exo1-TAP and Msh4-TAP pull-down meiotic cells



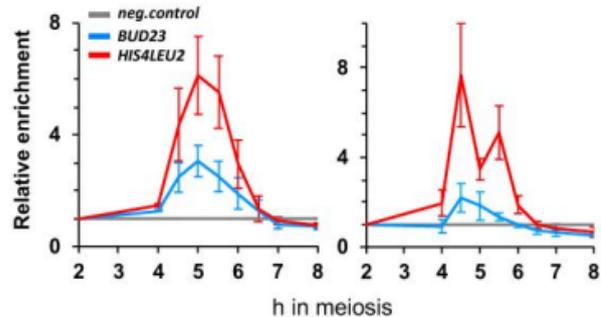
D Pms1-Flag pull-down meiotic cells



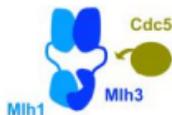
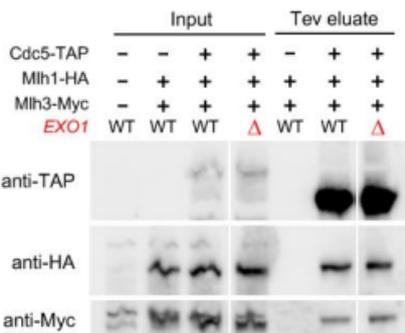
A Cdc5-TAP pull-down



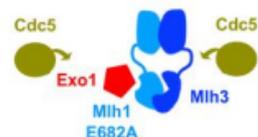
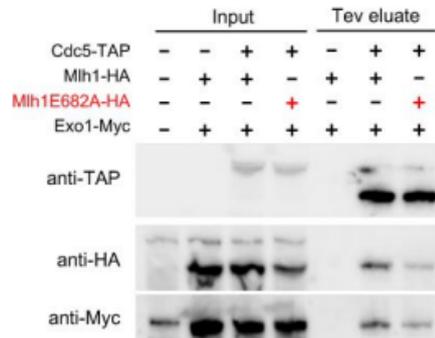
B Mih3-Myc8 ChIP Cdc5-TAP ChIP



C Cdc5-TAP pull-down



D Cdc5-TAP pull-down



E *in vitro* Exo1-Flag pull-down

