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Supplemental Information

Network Rewiring of Homologous Recombination Enzymes during Mitotic Proliferation and Meiosis

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Figure S1

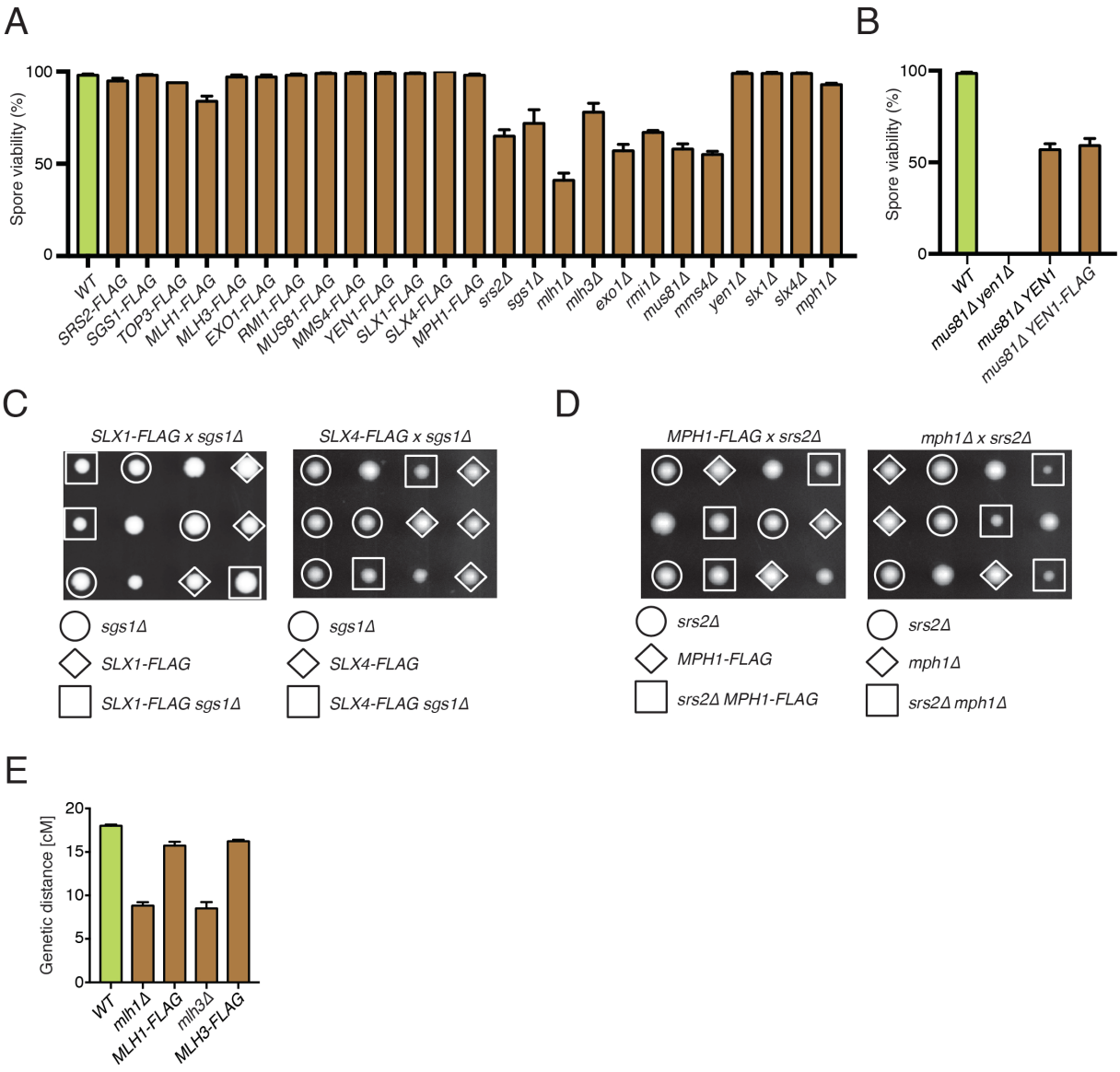


Figure S1. Functional analysis of FLAG-tagged RIPE factors. (Related to Figure 1, Table S1)

(**A**) The spore viability of strains with the indicated genotypes was determined after tetrad microdissection. With the exception of *MLH1-FLAG*, which showed a minor reduction in viability, all *FLAG* fusions supported normal spore growth. Gene deletions were used as positive controls for phenotypes arising from loss-of-function. (**B**) Given that deletion of *YEN1* did not lead to a noticeable reduction in spore viability, we monitored the functionality of the respective fusion in a sensitized background (*mus81Δ*). *YEN1-FLAG mus81Δ* and *YEN1 mus81Δ* showed comparable spore viability, which was significantly higher than in *yen1Δ mus81Δ* strains. (**C**) To monitor the functionality of Slx1-FLAG and Slx4-FLAG in a sensitized

background (*sgs1Δ*), haploid strains carrying the *SLX1-FLAG* or *SLX4-FLAG* and *sgs1Δ* were crossed to generate heterozygous diploids. After sporulation, tetrad dissection was performed to assess spore viability and colony growth. Relevant genotypes in the haploid spores are highlighted and show that both the Slx1-FLAG and Slx4-FLAG fusions support normal growth in the absence of Sgs1. (**D**) Haploid strains carrying the indicated alleles of *MPH1* and *srs2Δ* were analyzed as in (C). (**E**) To evaluate the functionality of Mlh1-FLAG and Mlh3-FLAG in supporting meiotic crossing-over, meiosis was induced in strains with the indicated genotypes for 48 hr at 30°C. Genetic distances at the *CEN8-THR1* interval were determined using the fluorescent markers described in Figure 5B. > 600 tetrads were analyzed in three independent experiments. Plotted values indicate mean +/- SD. The raw data is in Table S1.

Figure S2

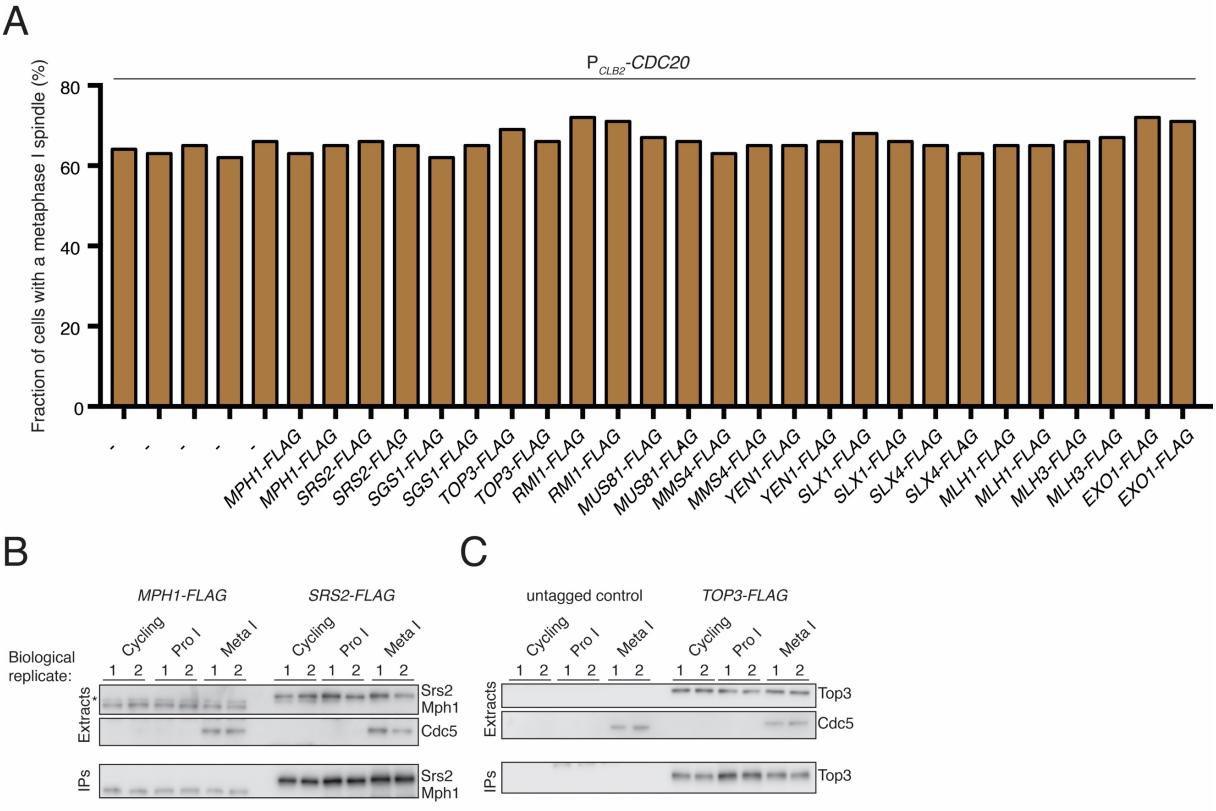


Figure S2. Fraction of cells in metaphase I, western blot and MS analyses of immuno-affinity purified RIPE components. (Related to Figure 2)

(**A**) The accumulation of cells in metaphase I (%), in large cultures of the indicated genotypes synchronously released to undergo meiosis, was evaluated by *in situ* immunofluorescence analysis of spindle morphology by α-tubulin staining. >200 cells were inspected per strain. (**B**) Protein extracts and immuno-affinity purified material from large mitotic (asynchronous cycling) or meiotic (prophase I or metaphase I) cultures expressing Mph1-FLAG or Srs2-FLAG were analyzed by western blotting for the indicated proteins. Two independent biological replicates were analyzed per condition. IPs: immuno-affinity purified material. *cross-reacting band. (**C**) As in (B) for untagged strains or strains expressing Top3-FLAG.

Figure S3

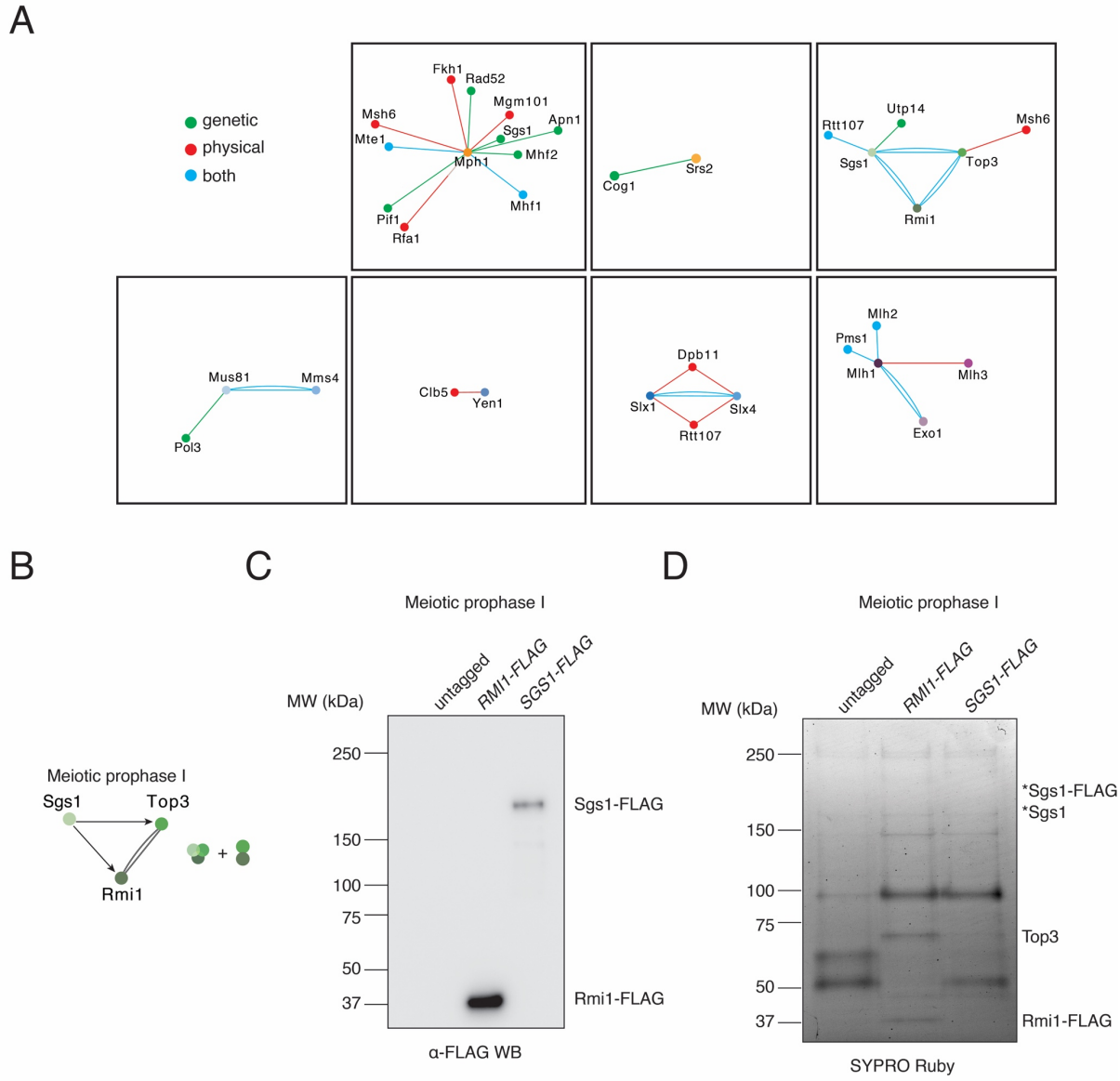


Figure S3. Previously identified physical and genetic interactions that could be captured in the current affinity proteomics RIPE network. (Related to Figure 3)

(**A**) Interactions identified in this study that were previously reported in BioGRID: green – genetic interaction; red – physical interaction; blue – both genetic and physical. (**B**) MS analysis of Sgs1-FLAG, Top3-FLAG and Rmi1-FLAG purifications (Figure 3C) suggest that, at least during prophase I, Sgs1 may exist in lower amounts than Top3 and Rmi1. Hence, Top3-Rmi1 may form complexes that lack Sgs1. (**C**) Immuno-affinity purified Rmi1-FLAG and Sgs1-FLAG, from *ndt80Δ* cultures, were analyzed by western blotting. (**D**) FLAG-affinity purifications from (C) were analyzed by SYPRO Ruby staining. *Sgs1 and Sgs1-FLAG levels are below the detection limits.

Figure S4

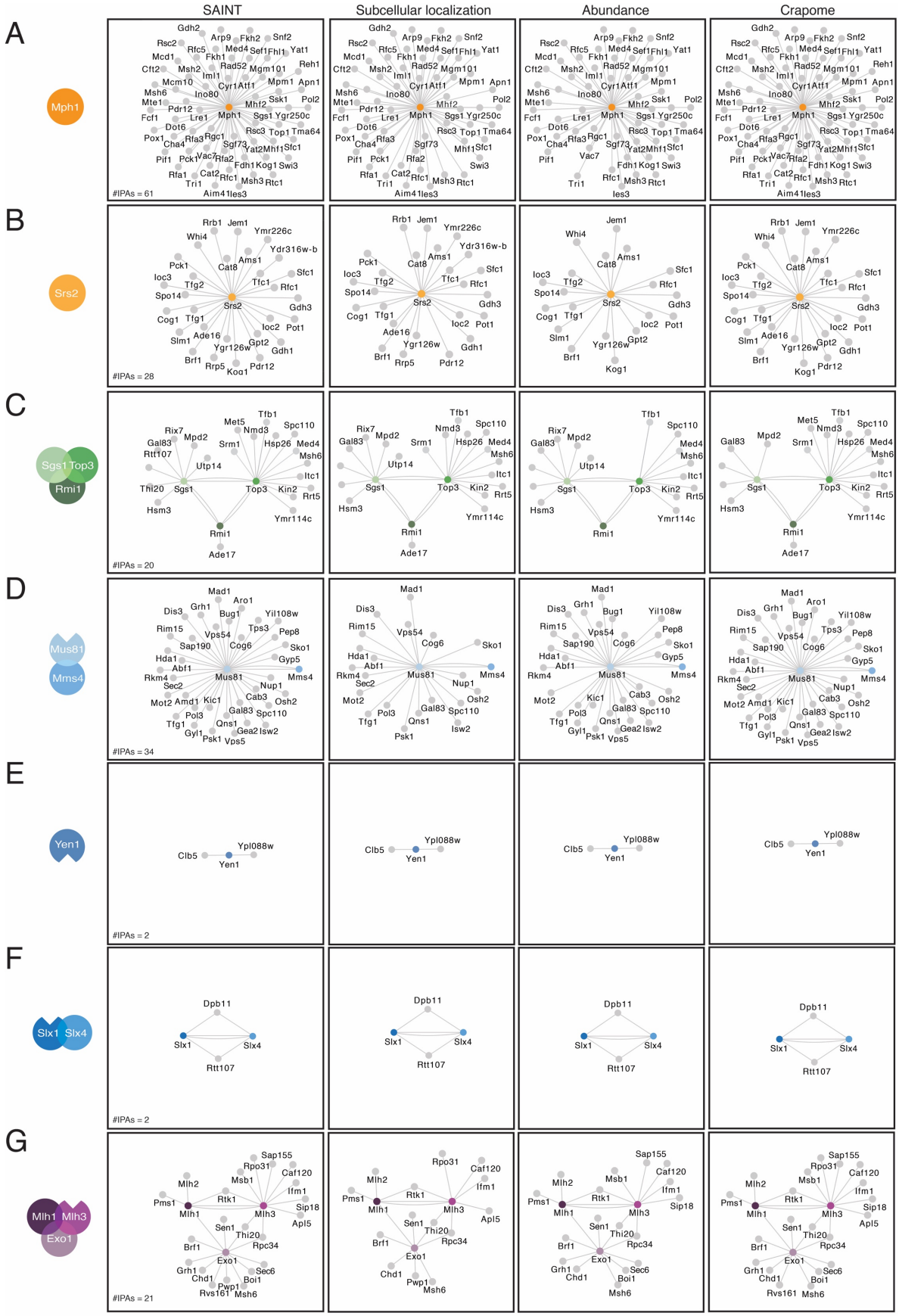

SAINT
Subcellular localization
Abundance
Crapome
A
Mph1
B
Srs2
C
Sgs1 Top3 Rmi1
D
Mus81 Mms4
E
Yen1
F
Slx1 Slx4
G
Mlh1 Mlh3 Exo1
#IPAs = 61
#IPAs = 28
#IPAs = 20
#IPAs = 34
#IPAs = 2
#IPAs = 2
#IPAs = 21

Figure S4. SAINT interactomes filtered by localization, abundance or common contaminants. (Related to Figure 3, Table S6)

(**A**) Putative interactors for Mph1 with a SAINT score ≥ 0.9 (left panel) subtracted on the basis of either their subcellular localization, protein abundance or prevalence in the Contaminant Repository for Affinity Purification (middle and right panels). IPAs, number of interaction partners. Subcellular localization: proteins with annotated localization to the cytoplasm, but not to the nucleus, are omitted; Protein abundance: highly abundant proteins (top 20%) are omitted; Crapome: proteins reported as common contaminants in anti-GPF and anti-HA purifications are omitted.

(**B**) As in (A) for Srs2.

(**C**) As in (A) for Sgs1, Top3 and Rmi1.

(**D**) As in (A) for Mus81 and Mms4.

(**E**) As in (A) for Yen1.

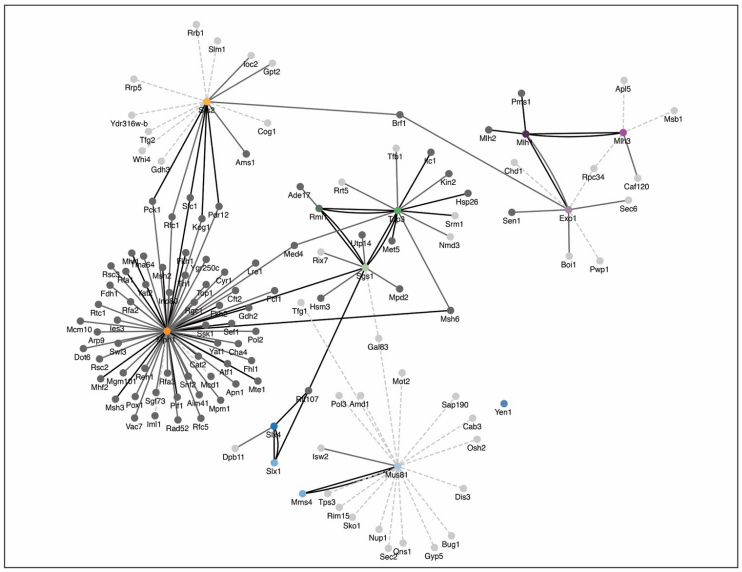
(**F**) As in (A) for Slx1-Slx4.

(**G**) As in (A) for Mlh1, Mlh3 and Exo1.

Figure S5

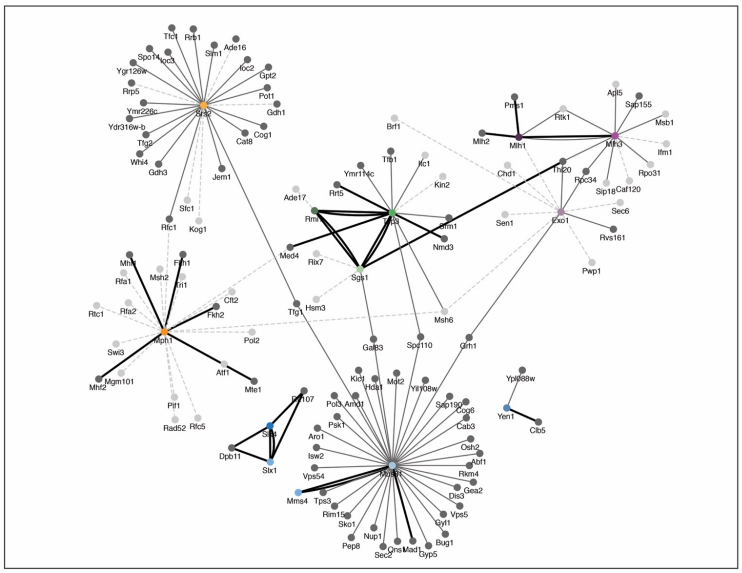
A

Mitotic proliferation



B

Meiotic prophase I



C

Meiotic metaphase I

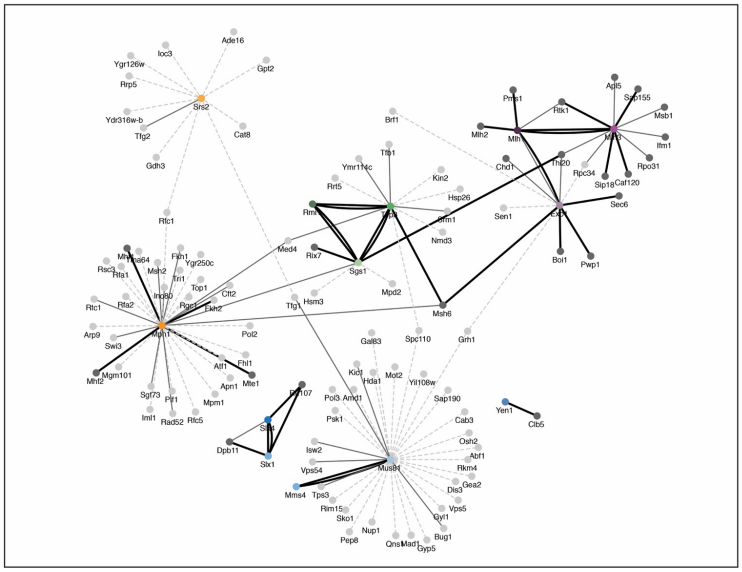


Figure S5. Semi-quantitative analysis of interaction strength across cellular contexts. (Related to Figure 4, Tables S2-4)

The context-specific strength of the bait-prey interactions for the RIPE network components was analyzed using the number of spectral counts detected by MS. (**A**) Subset of the RIPE network components detected in mitotically proliferating cells. The color and thickness of the edges reflects the fold-change in the number of spectral counts above background. Fold-change < 1, not shown; fold-change 1-2, dashed grey lines; fold-change 2-5, filled grey lines; fold-change > 5, thick black lines. Nodes that did not pass the 0.9 SAINT cutoff in cycling cells are in light grey, nodes that passed the cutoff are in dark grey. (**B**) Interactions detected in meiotic prophase I cells, as in (A). (**C**) Interactions detected in meiotic metaphase I cells, as in (A).

Figure S6

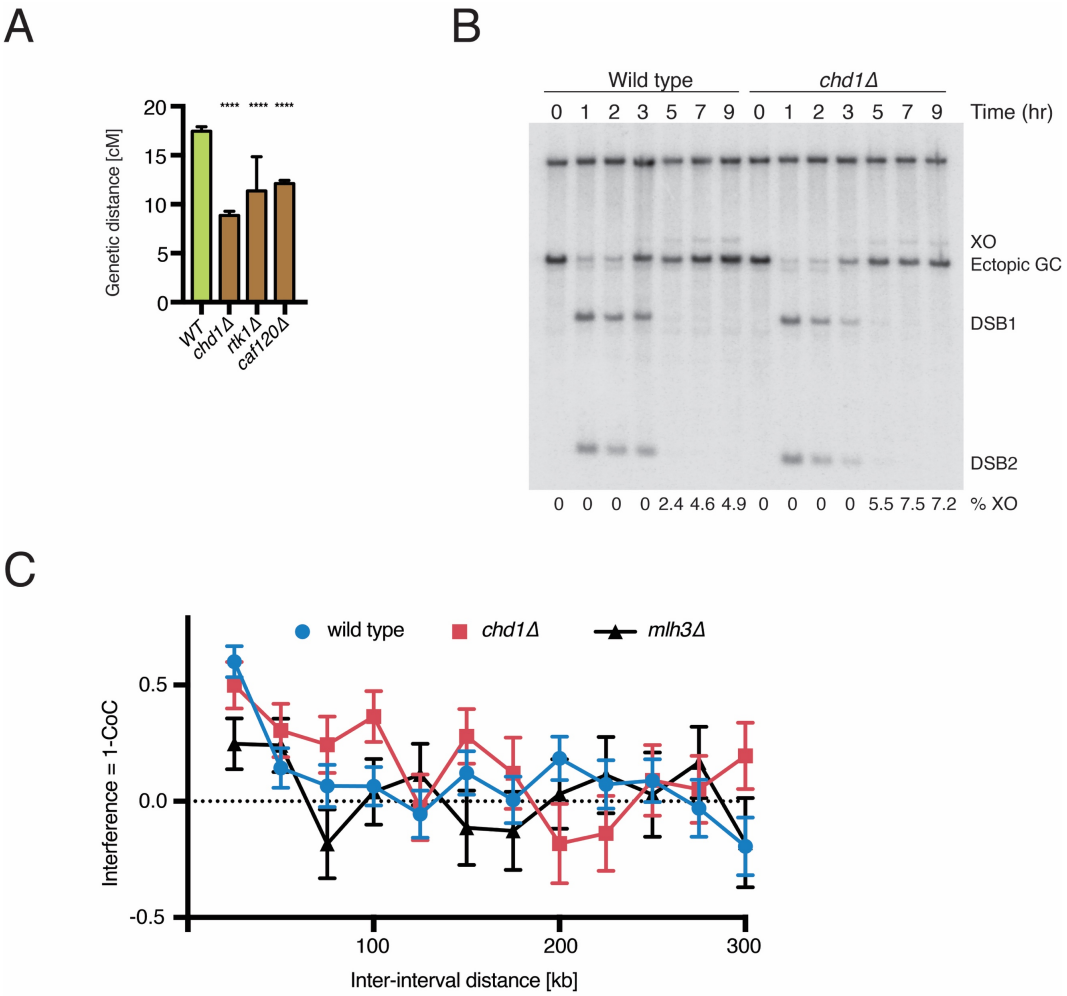


Figure S6. Functional analyses of RIPE network components during meiosis and physical analysis of DSB repair in *chd1Δ* cells during mitotic proliferation (Related to Figure 5 and Table S1)

(**A**) Meiosis was induced in strains with the indicated genotypes for 48 hr at 30°C. Genetic distances at the *CEN8-THR1* interval were determined using the fluorescent markers described in Figure 5B. > 1000 tetrads were analyzed in three independent experiments. Plotted values indicate mean +/- SD (two-tailed, unpaired t-test, ****p<0.0001). (**B**) Southern blot analysis of HO endonuclease-induced, ectopic DSB repair. EcoRI-digested DNA was run on an 0.8% agarose gel and analyzed with a *MAT*a probe. XO: gene conversion with crossover; GC: non-crossover gene conversion. After galactose induction of HO endonuclease, two cleavage products (DSB1 and DSB2) are seen. (**C**) Interference (1 – CoC: coefficient of coincidence) for COs in wild type*, chd1Δ and mlh3Δ* tetrads. For each inter-interval distance, the CoC was calculated individually for all possible interval pairs genome-wide, and the average +/- SEM is plotted.

Figure S7

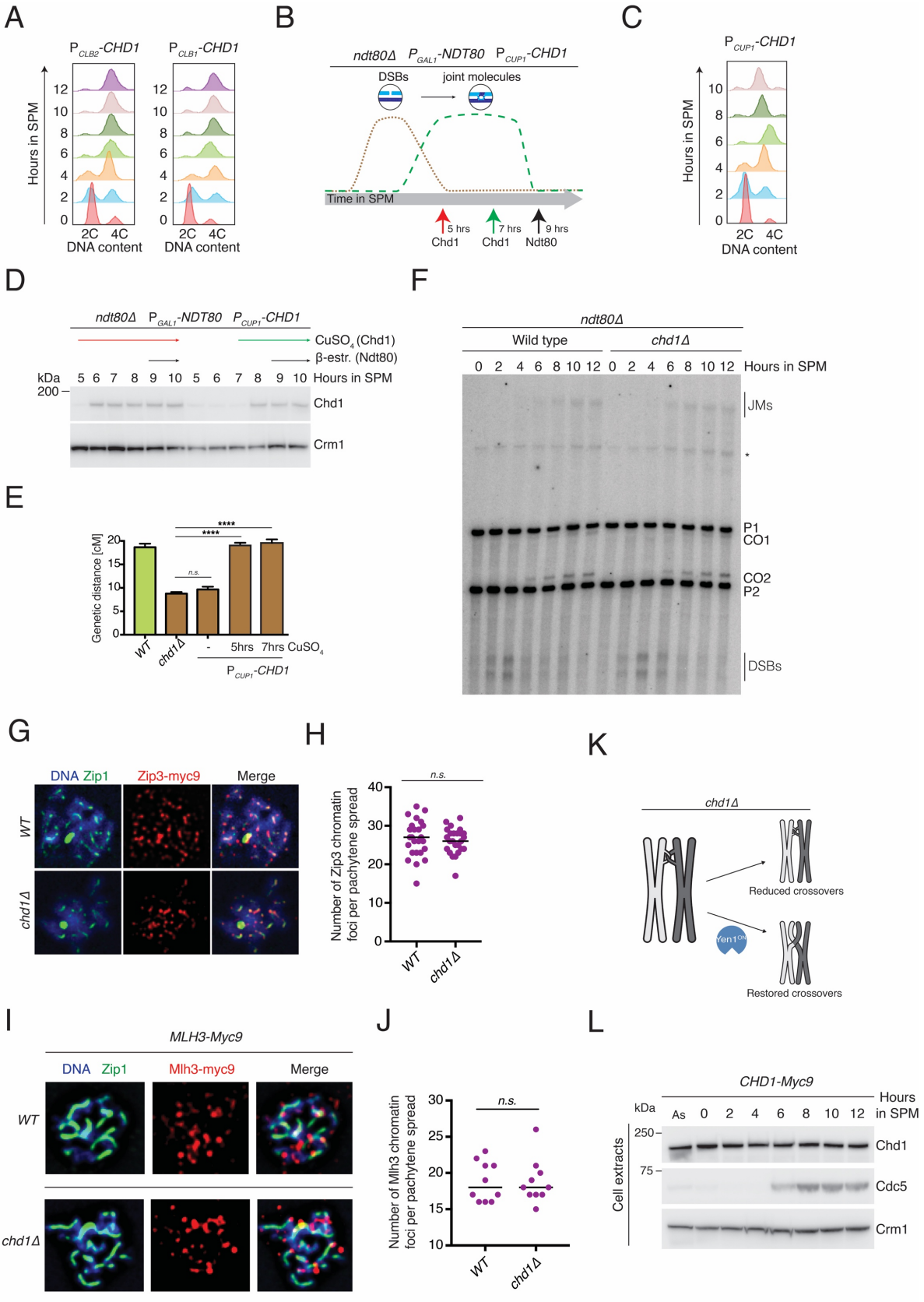

A
P CLB2-CHD1
P CLB1-CHD1
Hours in SPM
12
10
8
6
4
2
0
2C 4C
DNA content
B
ndt80Δ P GAL1-NDT80 P CUP1-CHD1
DSBs
joint molecules
Time in SPM
5 hrs
Chd1
7 hrs
Chd1
9 hrs
Ndt80
C
P CUP1-CHD1
Hours in SPM
10
8
6
4
2
0
2C 4C
DNA content
D
ndt80Δ P GAL1-NDT80 P CUP1-CHD1
CuSO4 (Chd1)
β-estr. (Ndt80)
kDa
200
5 6 7 8 9 10 5 6 7 8 9 10 Hours in SPM
Chd1
Crm1
E
Genetic distance [cM]
20
15
10
5
0
****
****
n.s.
WT
chd1Δ
-
5hrs
7hrs CuSO4
P CUP1-CHD1
F
ndt80Δ
Wild type
chd1Δ
0 2 4 6 8 10 12 0 2 4 6 8 10 12 Hours in SPM
JMs
*
P1
CO1
CO2
P2
DSBs
G
DNA Zip1
Zip3-myc9
Merge
WT
chd1Δ
H
n.s.
Number of Zip3 chromatin foci per pachytene spread
40
30
20
10
0
WT
chd1Δ
K
chd1Δ
Reduced crossovers
Yen1GN
Restored crossovers
I
MLH3-Myc9
DNA Zip1
Mlh3-myc9
Merge
WT
chd1Δ
J
n.s.
Number of Mlh3 chromatin foci per pachytene spread
30
25
20
15
10
WT
chd1Δ
L
CHD1-Myc9
kDa
As 0 2 4 6 8 10 12 Hours in SPM
250
75
Cell extracts
Chd1
Cdc5
Crm1

Figure S7. Chd1 remodels chromatin during meiosis to facilitate crossover recombination. (Related to Figure 6, Table S1)

(**A**) FACS analysis of DNA content from a meiotic time course of cells expressing P_{CLB2}-*ha3-CHD1* (left panel) or P_{CLB1}-*ha3-CHD1* (right panel). Samples were collected at 2-hr intervals after induction of meiosis by transfer into SPM. (**B**) Scheme of Chd1 induction from P_{CUP1} at different time points during meiosis in an *ndt80Δ* strain containing P_{GAL1}-*NDT80*. After DNA replication is completed, 5 hr after induction of meiosis, one third of the culture is treated with 1 μM $CuSO_4$, to induce Chd1 expression as cells form joint molecules. Another third is treated with $CuSO_4$ two hours later (7 hr after induction of meiosis), to induce Chd1 expression after cells accumulate in pachytene (green curve). The remaining third of the culture was left untreated, to control for the potential effects of leakiness in Chd1 expression. β-estradiol is added to the 3 cultures 9 hr after induction of meiosis to trigger Ndt80 expression and exit from pachytene. (**C**) FACS analysis of DNA content from a meiotic time course of P_{GAL1}-*NDT80* P_{CUP1}-*CHD1* cells treated with β-estradiol 9 hr after transfer to SPM. Samples were collected at 2-hr intervals. Samples are from the experiment described in (B). (**D**) Western blot samples from cultures in (C) were collected at the indicated time points to monitor for expression of Chd1. A basal level of Chd1 can be detected prior to addition of $CuSO_4$. (**E**) Spore formation was allowed to occur for 48 hr in cells from (D). Genetic distances were determined and plotted values indicate mean +/- SD (two-tailed, unpaired t-test, *n.s.*, $p > 0.05$; ****$p < 0.0001$). (**F**) Physical analysis of recombination at the *HIS4-LEU2* locus in *ndt80Δ CHD1* or *ndt80Δ chd1Δ* mutants. Cells were collected at the indicated time intervals after transfer into sporulation medium (SPM). Psoralen-crosslinked DNA prepared from the meiotic time courses was analyzed by Southern blotting. JM: joint molecules; asterisk indicates ectopic crossovers. The image shown is representative of 2 independent experiments. P1, parental DNA 1; P2, parental DNA 2; CO1 and CO2, reciprocal recombinants from P1 and P2; DSBs, double-strand breaks. (**G**) Chromosome spreads from strains with the indicated genotypes and expressing Zip3-myc9 were prepared 7 hr after induction of meiosis and stained for DNA, Zip1 and Zip3-myc9. Representative images are shown. (**H**) Scatter plot with the analysis of Zip3 foci number in chromosome spreads from (G). Horizontal line depicts the median number of Zip3 foci per cell. 25 cells were analyzed per condition (two-tailed t-test, *n.s.* non-significant). The data shown is representative of 2 independent experiments. (**I**) Chromosome spreads from strains with the indicated genotypes and expressing Mlh3-myc9 were prepared 7 hr after induction of meiosis and stained for DNA, Zip1 and Mlh3-myc9. Prophase I cells were identified by Zip1 loading. Representative images are shown. (**J**) Scatter plot with the analysis of Mlh3-myc9 foci number in chromosome spreads from (I), as in (H). (**K**) Experimental design to test if defective

crossover formation in *chd1Δ* mutants is caused by the inability of MutLγ-Exo1 to process DNA joint molecules. Expression of active Yen1 resolvase ($Yen1^{ON}$ is resistant to inhibitory phosphorylation) should restore crossover formation in the absence of *CHD1*. (**L**) Western blot analysis of the indicated proteins in TCA extracts from a meiotic time course of cells expressing Chd1-myc9. Samples were collected at 2-hr intervals after induction of meiosis.