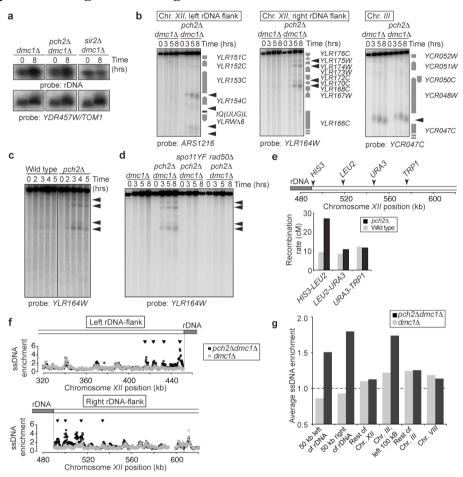
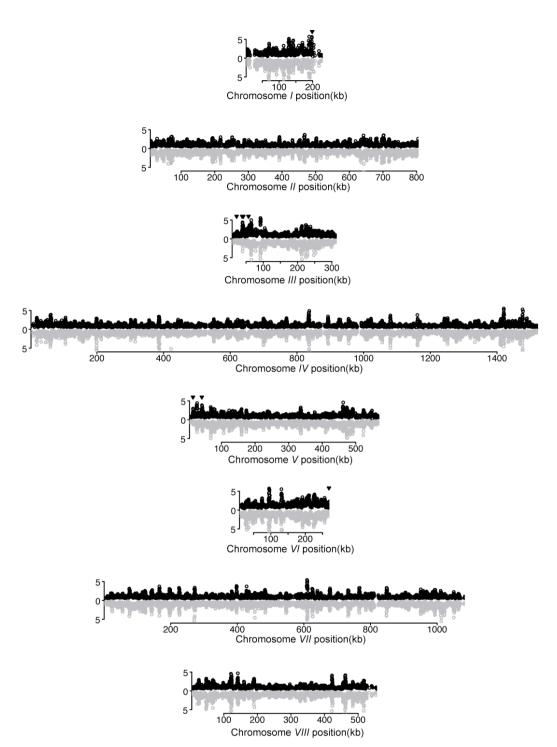
Supplemental Figures and Legends



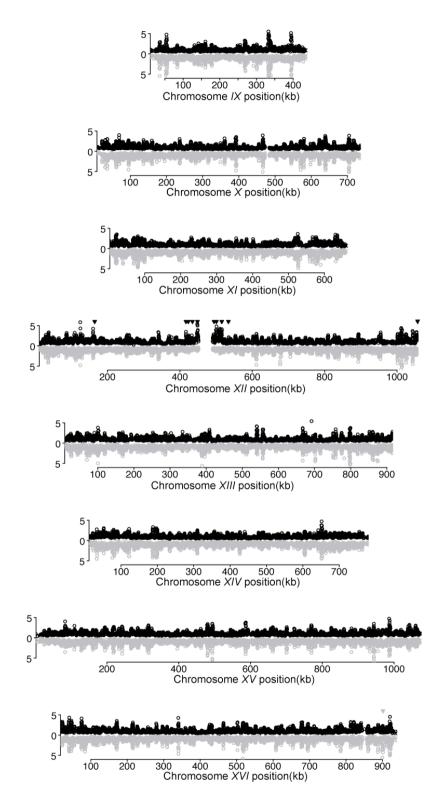
Supplementary Figure 1. Pch2-dependent suppression of DSBs at the rDNA borders.

a, Example of a CHEF gel/Southern blot of $dmcl\Delta$ (H5217), $dmcl\Delta$ $pch2\Delta$ (H5216), and $dmcl\Delta$ $sir2\Delta$ (H2933) cells harvested at the indicated time points as analysed in Figure 1a. rDNA was released from chromosome XII by in-gel digest with XhoI. Blot was probed for rDNA (NTSI) sequence and YDR457W/TOM1, a DSB coldspot that served to correct for copy number changes associated with DNA replication and as loading control. The ratio of full-length fragments after DSB formation (8h after meiotic induction) over unbroken (0h) was calculated. See supplementary methods for electrophoresis conditions and probe information. **b**, Meiotic time course analysis of $dmc1\Delta$ (H118) and $dmc1\Delta$ $pch2\Delta$ cells (H2629). Southern blots of the left rDNA flank (HindIII; probe: ARS1216), the right rDNA flank (ApaLI; probe: YLR164W), and chromosome III (HindIII; probe: YCR047C). Grey boxes indicate the positions of open reading frames; black arrows highlight major DSB sites. c, Southern blot analysis of the right rDNA flank in wild-type (H119) and $pch2\Delta$ (H2817) cells. **d**, Southern blot of right rDNA flank in $dmc1\Delta$ (H118), $dmc1\Delta pch2\Delta$ (H2629), $dmc1\Delta pch2\Delta spo11-Y135F$ (H3102), and $dmc1\Delta pch2\Delta rad50\Delta$ cells (H3239). e, Crossover recombination in single-copy regions flanking the right border of the rDNA in wild-type (H3037, n=207) and $pch2\Delta$ (H3065; n=164) cells. Recombination rates are provided in centiMorgans (cM). Schematic indicates the chromosomal locations of analysed genetic markers. f, ssDNA profiles of $dmc l\Delta$ (H118, grey), and $dmc1\Delta$ pch2 Δ (H2629, black) cells in the regions directly abutting the rDNA array on Chromosome XII. Significantly different peaks are indicated with black triangles ($dmc1\Delta pch2\Delta$ over $dmc I\Delta$). Definition of significance and coordinates of significantly different peaks are indicated in Table S1. g, Average enrichment for $dmc1\Delta$ (H118, grey), and $dmc1\Delta$ $pch2\Delta$ (H2629, black) is plotted for the indicated chromosomal regions. The genome-wide average is indicated by the dotted line. See Supplementary Methods for chromosomal coordinates of the analysed regions.



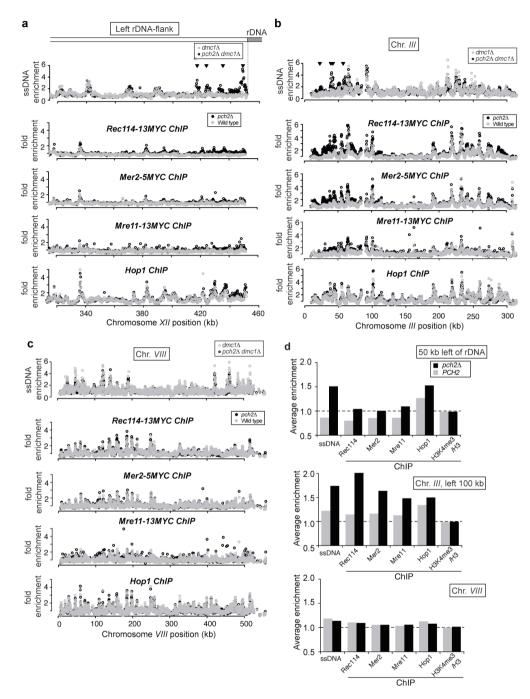
Supplementary Figure 2. Effects of Pch2 on DSB formation across the genome.

ssDNA profiles of the 16 budding yeast chromosomes in $dmc1\Delta$ (H118, grey) and $dmc1\Delta$ $pch2\Delta$ cells (H2629, black) cells. Chromosomes 1-8 are shown; see Figure S2-continued (next page) for chromosomes 9-16. Significantly different peaks are marked with black $(dmc1\Delta \ pch2\Delta)$ over $dmc1\Delta$) and grey arrowheads $(dmc1\Delta)$ over $dmc1\Delta$ $dmc1\Delta$ 0. Definition of significance and coordinates of significantly different peaks are indicated in Table S1.



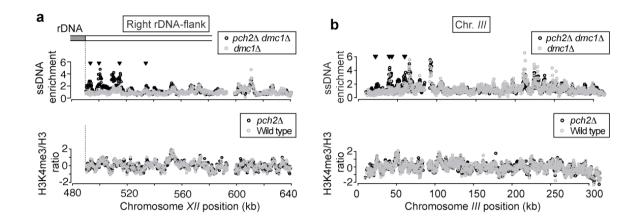
Supplementary Figure 2-continued. Effects of Pch2 on DSB formation across the genome.

ssDNA profiles of the Chromosomes 9-16 in $dmc1\Delta$ (H118, grey) and $dmc1\Delta$ $pch2\Delta$ cells (H2629, black) cells.



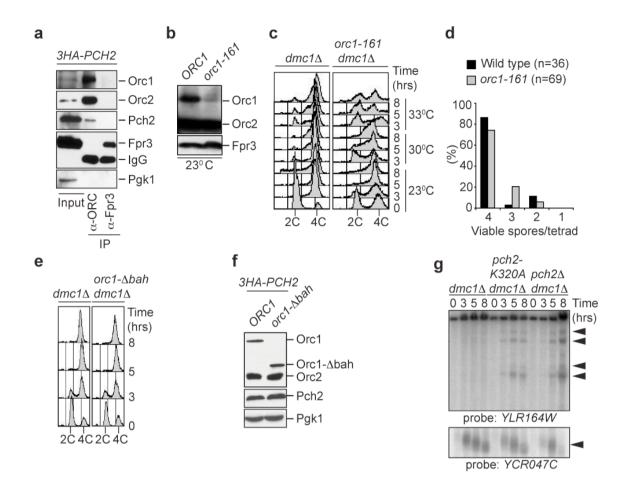
Supplementary Figure 3. Effects of Pch2 on DSB factor distribution at the left rDNA flank and on chromosomes III and VIII.

Analysis of ssDNA enrichment in $dmc1\Delta$ (H118, grey) and $pch2\Delta$ dmc1 Δ (H2629, black) cells (first panel) and ChIP-chip analysis for Rec114-13Myc (second panel, wild type:H4890; $pch2\Delta$:H4893), Mer2-5Myc (third panel, wild type:H5916; $pch2\Delta$:H5917), Mre11-13Myc (fourth panel, wild type:H5547; $pch2\Delta$:H5947) and Hop1 (fifth panel, wild type:H1556; $pch2\Delta$:H2817), in wild-type (grey) and $pch2\Delta$ cells (black) on the left rDNA flank on Chromosome XII (a), and along Chromosome III (b), and Chromosome VIII (c). d, Average enrichment for the different PCH2 and $pch2\Delta$ ssDNA and ChIP-strains (see a-c) in the indicated chromosomal regions is plotted. The genome-wide average is indicated by the dotted line. See Supplementary Methods for chromosomal coordinates of the analysed regions.



Supplementary Figure 4. Pch2 does not affect histone H3K4 trimethylation near the rDNA.

Analysis of ssDNA profiles (upper panel; $dmcl\Delta$ (H118, grey) and $dmcl\Delta$ $pch2\Delta$ cells (H2629, black)) and H3K4me3/H3 ratios for wild-type (H119, grey) and pch2Δ cells (H2817, black) in the region directly abutting the rDNA array on Chromosome XII (a), and along Chromosome *III* (b).



Supplementary Figure 5. Pch2 interacts with Orc1 and its ATPase activity is required to suppress DSBs.

a, Co-immunoprecipitation of 3HA-Pch2 with ORC. Co-IPs performed with α -ORC (using a pan-ORC antibody that recognises all 6 ORC subunits) or α -Fpr3 (as negative control) in 3HA-PCH2 cells (H3463). Samples were probed for: Orc1 (α-ORC), Orc2 (α-ORC), Fpr3, and Pgk1 as a cytoplasmic control. b, Western blot showing Orc1 and Orc2 protein levels in meiotic wild-type (H4962) and orc1-161 (H5033) cells grown at the permissive temperature (23°C). Samples were taken 3h after meiotic induction. Fpr3 was used as loading control. c, FACS analysis of $dmc1\Delta$ (H118) and orc1-161 $dmc1\Delta$ (H5137) cells at various times in meiosis, while incubated at 23°C, 30°C, and 33°C. d, Spore viability of wild-type (H13) and orc1-161 cells (H5033) sporulated at 23°C. e, FACS analysis of $dmc1\Delta$ (H5838) and $orc1-\Delta bah\ dmc1\Delta$ (H5865) cells at various times in meiosis while incubated at 30°C. f. Western blot showing protein levels of Orc1. Orc1-Δbah, Orc2, and 3HA-Pch2 in meiotic 3HA-PCH2 (H5772) and 3HA-PCH2 orc1-Δbah (H5894) cells. Samples were taken 3h after meiotic induction. Pgk1 was used as loading control. g, Meiotic time course in $dmc1\Delta$ (H118), pch2-K320A $dmc1\Delta$ (H4509), and pch2Δ dmc1Δ cells (H2629) analysed by Southern blotting of the right rDNA flank (ApaLI; probe: YLR164W) and of chromosome III (HindIII; probe: YCR047C). pch2-K320A was a kind gift of Akira Shinohara.

Supplementary Tables

Supplementary Table 1. Regions of ssDNA enrichment in pch2\Delta dmc1\Delta versus dmc1∆

Sites of differential ssDNA enrichment between $dmc1\Delta$ (H118) and $pch2\Delta$ $dmc1\Delta$ (H2629) strains are listed. Sites were selected at which the ssDNA signal was increased >2 fold at more than 3 data points within 500 bp of each other. To exclude false positives due to background fluctuations, regions were only considered significant when the region overlapped with an ssDNA peak within the enriched dataset. Using these criteria, we identified 173 enriched features in 18 regions that were significantly increased in $pch2\Delta$ $dmc1\Delta$ over $dmc1\Delta$ cells, of which 110 features (in 8 regions) were directly flanking the rDNA array (shown in bold).

						Distance	
					No. of	from	
	Start	End		Fold	features	^a rDNA,	
	enriched	enriched		enrichment	within	^b telomere or	Enriched
Chromosome	region (bp)	region (bp)	Peak (bp)	at peak	region	^c HML	Dataset
1	196719	198671	198671	4.61	6	30632 ^b	pch2∆dmc1∆
3	22131	24379	22524	3.42	7	7182°	$pch2\Delta dmc1\Delta$
3	37036	39665	38859	7.37	8	22087 ^c	pch2∆dmc1∆
3	40789	42066	41829	4.28	7	25840°	$pch2\Delta dmc1\Delta$
3	56386	60034	57095	3.40	11	41437°	$pch2\Delta dmc1\Delta$
5	13642	15812	15348	7.02	7	13642 ^b	pch2∆dmc1∆
5	41108	42923	41665	4.25	4	41108 ^b	$pch2\Delta dmc1\Delta$
6	267700	269851	268697	3.07	4	$0_{\rm p}$	$pch2\Delta dmc1\Delta$
12	164572	165524	164572	3.49	5	164572 ^b	$pch2\Delta dmc1\Delta$
12	416092	417652	416758	7.52	6	35278 ^a	pch2∆dmc1∆
12	423165	423965	423165	2.90	4	28205 ^a	pch2∆dmc1∆
12	431663	434643	434643	3.92	8	19707 ^a	pch2∆dmc1∆
12	444578	451090	448541	6.51	21	6792 ^a	pch2∆dmc1∆
12	491066	494638	494316	4.77	15	535 ^a	pch2∆dmc1∆
12	498803	511779	500878	6.72	37	8272 ^a	pch2∆dmc1∆
12	512872	517689	515671	3.78	15	22341 ^a	pch2∆dmc1∆
12	533975	535512	533975	2.14	4	43444 ^a	pch2∆dmc1∆
12	1057155	1057952	1057155	2.86	4	19942 ^b	$pch2\Delta dmc1\Delta$
16	899524	901317	901317	2.28	4	46379 ^b	$dmc1\Delta$

Supplementary Table 2. rDNA recombination in pch2∆ and wild-type cells

Recombination frequencies as measured by tetrad dissection using strains that carry different *URA3* integrations within the rDNA array (see Figure 1c,d). Strain numbers are indicated. CO-associated repeat number changes were determined by CHEF gel analysis of XhoI-digested chromosomes (probe: *NTS1/2*).

Number of rDNA repeats between left flank and <i>URA3</i> insertion	Genotype	Total array length (repeats)	Tetrads analysed	Cross- over events HIS3- URA3	Cross- over events URA3- TRP1	Fraction of total crossovers in <i>HIS3-URA3</i> interval	Fraction of total rDNA repeats in HIS3-URA3 interval	CO-associated repeat number changes for <i>HIS3-URA3</i> interval
1	<i>pch2Δ</i> (H4611)	103	465	8	46	15%	1%	2 of 5 (+1, 0, +6, 0, 0)
3	pch2Δ (H4613)	99	533	12	36	25%	3%	9 of 12 (-1, 0, +4, 0, +2, +1, +15, +8, +1, +3, 0, +3)
10	<i>pch2Δ</i> (H3823)	110	494	20	33	38%	9%	6 of 11 (+3, 0, +2, 0, -2, +13, 0, -7, 0, +6, 0)
12	<i>pch2∆</i> (H4612)	110	367	19	30	39%	11%	16 of 19 (0, - 5, +2, 0, +6, +7, +2, -4, -3, +5, +9, 0, +3, -4, +13, +12, - 2, -7, +19)
29	<i>pch2Δ</i> (H3820)	108	503	20	26	44%	27%	
49	pch2Δ (H3821)	99	308	27	24	53%	50%	
12	wild type (H4881)	110	2350	4	10	30%	11%	3 of 4 (+1, +2, 0, -3)

Supplementary Table 3. Genotypes of yeast strains used in this study.

Strain No.	Relevant genotype	Reference
H13	MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG,	1
(=A4962)	trp1::hisG	
	MATalpha, ho::LYS2, lys2, ura3, leu2::hisG,	
	his3::hisG, trp1::hisG	
H118	H119, but $dmc1\Delta$:: $ARG4/dmc1\Delta$:: $ARG4$	2
(=NKY1455)		
H119	MATa, ho::LYS2, lys2, leu2::hisG, his4X::LEU2-URA3,	3
(=NKY1551)	ura3, arg4-Bgl2,	
	MATalpha, ho::LYS2, lys2, leu2::hisG, his4B::LEU2,	
***	ura3, arg4-Nsp	
H2629	H118, but $pch2\Delta$:: $KanMX4/pch2\Delta$:: $KanMX4$	This study
H2817	H119, but $pch2\Delta$:: $KanMX4/pch2\Delta$:: $KanMX4$	This study
H2953	H118, but <i>sir2::TRP1/sir2::TRP1</i>	This study
H3026	MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG,	This study
	trp1::hisG	
	MATalpha, ho::LYS2, trp1::hisG, his3::hisG,	
	leu2::hisG, ura3,	
	pch2Δ::KanMX/+, YLR162W-A::TRP1, ARS1216::HIS3	
H3027	H3026, but <i>pch2Δ::KanMX/ pch2Δ::KanMX</i>	This study
H3037	H13, but $pch2\Delta$:: $KanMX/+$, $MAS1$:: $HIS3$,	This study
	HMX1::URA3, IFH1::TRP1, ylr184w::LEU2	
H3038	H2953, but $pch2\Delta$:: $KanMX4/pch2\Delta$:: $KanMX4$	This study
H3065	H13, but $pch2\Delta$:: $KanMX/pch2\Delta$:: $KanMX$,	This study
	MAS1::HIS3, HMX1::URA3, IFH1::TRP1,	
	ylr184w::LEU2	
H3102	MATa, ho::LYS2, lys2, ura3::hisG, leu2::hisG, arg4-Bgl	This study
	II, his4B::LEU2,	
	MATalpha, ho::LYS2, lys2, ura3::hisG, leu2::hisG,	
	arg4-Bgl II, his4B::LEU2,	
	spo11-Y135F-HA-URA3/spo11-Y135F-HA-URA3,	
	$pch2\Delta$::KanMX4/pch2 Δ ::KanMX4, dmc1 Δ ::ARG4/	
	dmc1∆::ARG4	
H3239	MATa, ho::LYS2, lys2, ura3, leu2::hisG, his4B::LEU2,	This study
	MATalpha, ho::LYS2, lys2, ura3, leu2::hisG,	
	his4B::LEU2,	
	$dmc1\Delta$::ARG4/ $dmc1\Delta$::ARG4, rad50::URA3/	
777.0.61	rad50::URA3, pch2Δ::KanMX4/pch2Δ::KanMX4	
H3261	H3262, but leu2::SIR2::LEU2	This study
H3262	MATa, ho::LYS2, lys2, leu2::hisG, ura3, trp1::hisG,	This study
	his3::hisG,	
	MATalpha, ho::LYS2, lys2, leu2::hisG, ura3,	

	1.1.61.21.6	T
	trp1::hisG, his3::hisG,	
	pch2Δ::KanMX4/ pch2Δ::KanMX4, dmc1Δ::HIS3/	
	dmc1Δ::HIS3, sir2::TRP1/sir2::TRP1	
H3282	H3262, but <i>leu2::sir2-345::LEU2</i>	This study
H3463	MATa, ho::LYS2, lys2, ura3, leu2::hisG,his4B::LEU2,	This study
	arg4-Bgl II,	
	MATalpha, ho::LYS2, lys2, ura3, leu2::hisG,	
	his4B::LEU2, arg4-Bgl II,	
	pch2::URA3:pPCH2(300bp):3HA-	
	PCH2/pch2::URA3:pPCH2(300bp):3HA-PCH2	
H3820	MATalpha, lys2, ho::LYS2, trp1::hisG, his3::hisG,	This study
	leu2::hisG, ura3	
	ARS1216::HIS3, YLR162W-A::TRP1,	
	MATa, lys2, ho::LYS2, trp1::hisG, his3::hisG,	
	leu2::hisG, ura3, pch2∆::KanMX/ pch2∆::KanMX	
	rDNA::URA3 (@29 th repeat)	
H3821	H3820, but <i>rDNA::URA3 (@49th repeat)</i>	This study
H3823	H3820, but <i>rDNA::URA3</i> (@10 th repeat)	This study
H4509	H118, but <i>pch2-K320A/ pch2-K320A</i>	This study
H4611	H3820, but <i>rDNA::URA3</i> (@1 st repeat)	This study
H4612	H3820, but <i>rDNA::URA3</i> (@12 th repeat)	This study
H4613	H3820, but <i>rDNA::URA3</i> (@3 rd repeat)	This study
H4737	H2629,but $rdn\Delta\Delta$::HIS3/ $rdn\Delta\Delta$::HIS3, [pRDN-	This study
	hyg::URA3::leu2-8]/[pRDN-hyg::URA3::leu2-8], flo8	
	(unmarked)/+	
H4798	H2629, but t(Chr.2;Chr12) lys2::hphMX4::pGPD-loxP-	This study
	ADE2/t(Chr.2;Chr12) lys2::hphMX4::pGPD-loxP-	
	ADE2	
	t(Chr.12;Chr2) YLR162W-A::natMX4::loxP	
	/t(Chr.12;Chr2) YLR162W-A::natMX4::loxP	
H4890	MATa, ho::LYS2, TRP1, his3::hisG, ura3, lys2,	This study
	leu2::hisG,	
	MATalpha, ho::LYS2, trp1::hisG, his3::hisG, URA3,	
	lys2, LEU2,	
	REC114-13MYC::HIS3/REC114-13MYC::HIS3	
H4893	H4890, but $pch2\Delta$:: $KanMX/pch2\Delta$:: $KanMX$	This study
H4952	H118, but <i>pch2Δ::KanMX/+</i> , <i>orc1::orc1-161</i>	This study
	/orc1::orc1-161	
H5028	H13, but orc1::orc1-161/orc1::orc1-161	This study
H5033	H5033, but orc1::orc1-161/orc1::orc1-161	This study
H5137	MATa, ho::LYS2, lys2, trp1::hisG,leu2::hisG,	This study
	his4X::LEU2-URA3, his3::hisG, ura3, arg4-nsp(?),	
	$dmc1\Delta::ARG4, orc1::orc1-161$	
	MATalpha, ho::LYS2, lys2, ura3,	
	leu2::hisG,his4B::LEU2, arg4-Bgl II (?),	

	dmc1Δ::ARG4,orc1::orc1-161	
H5216	MATa, ho::LYS2, lys2, ura3, leu2::hisG, his4B::LEU2,	This study
110210	arg4-Bgl II	11110 50000
	MATalpha, ho::LYS2, lys2, ura3, leu2::hisG,	
	his4B::LEU2, arg4-Bgl II?,	
	$dmc1\Delta::ARG4/dmc1\Delta::ARG4$,	
	pch2Δ::KanMX4/pch2Δ::KanMX4	
H5217	MATa, ho::LYS2, lys2, leu2::hisG, trp1::hisG, ura3,	This study
	ARG4, his3::hisG?, his4B::LEU2,	,
	MATalpha, ho::LYS2, lys2, leu2::hisG, ura3, arg4-Bgl2,	
	$his 4B:: LEU2, dmc1\Delta:: ARG4/dmc1\Delta:: ARG4$	
H5547	MATa, ho::LYS2, TRP, his3::hisG, ura3, LEU2	This study
	MATalpha, ho::LYS2, trp1::hisG, his3::hisG, URA3,	
	leu2::hisG	
	MRE11-13MYC::HIS3	
	MRE11-13MYC::HIS3	
H5583	MATa, ho::LYS2, lys2, leu2::hisG, TRP1, his4B::LEU2,	This study
	ura3, ARG4,	
	MATalpha, ho::LYS2, lys2, leu2::hisG, TRP1,	
	$his 4B:: LEU2, ura 3, ARG 4, , dmc 1 \Delta:: ARG 4/$	
	$dmc1\Delta$::ARG4, rDNA::URA3 (@ 1^{st}	
	repeat)/rDNA::URA3 (@1 st repeat)	
H5622	H5583, but $pch2\Delta$:: $KanMX/pch2\Delta$:: $KanMX$	This study
H5636	H5622, but $rDNA::URA3$ (@3 rd repeat)/ $rDNA::URA3$	This study
	$(@3^{rd} repeat)$	
H5706	H5622, but $rDNA::URA3$ (@ 10^{th} repeat)/ $rDNA::URA3$	This study
	$(@10^{th} repeat)$	
H5772	MATa, ho::LYS2, lys2, ura3, leu2::hisG, TRP1, HIS3(?)	This study
	his4B::LEU2, pch2::URA3:pPCH2(300bp):3HA-PCH2	
	orc1::TRP1, ura3::ORC1::URA3	
	MATalpha, ho::LYS2, lys2, ura3, leu2::hisG, TRP1,	
	HIS3(?)	
	his4B::LEU2, pch2::URA3:pPCH2(300bp):3HA-PCH2	
*****	orc1::TRP1, ura3::ORC1::URA3	
H5838	MATalpha, ho::LYS2, lys2, leu2::hisG, TRP1,ura3,	This study
	$arg4-Bgl2, dmc1\Delta::ARG4, orc1::TRP1,$	
	ura3::ORC1::URA3	
	MATa, ho::LYS2, lys2, leu2::hisG, trp1::hisG,	
	his4B::LEU2,ura3, arg4-Bgl2, dmc1Δ::ARG4,	
115065	orc1::TRP1, ura3::ORC1::URA3	701 : 4 1
H5865	MATalpha, ho::LYS2, lys2, leu2::hisG, his4B::LEU2,	This study
	ura3, arg4-Bgl2(?), $dmc1\Delta$::ARG4, orc1::TRP1,	
	ura3::orc1deltaNTD(1-235)::URA3	
	MATa, ho::LYS2, lys2, leu2::hisG, his4B::LEU2, ura3,	

	4 D 10	
	arg4-Bgl2,	
	$dmc1\Delta$::ARG4, orc1::TRP1, ura3::orc1deltaNTD(1-	
	235)::URA3	
H5894	MATa, ho::LYS2, lys2, ura3, leu2::hisG, TRP1(?),	This study
	HIS3(?), arg4-Bgl II (?),	
	pch2::URA3:pPCH2(300bp):3HA-PCH2	
	orc1::TRP1, ura3::orc1deltaNTD(1-235)::URA3	
	MATalpha, ho::LYS2, lys2, ura3, leu2::hisG, TRP1(?),	
	HIS3(?)	
	his4B::LEU2, ARG4,	
	pch2::URA3:pPCH2(300bp):3HA-PCH2	
	orc1::TRP1, ura3::orc1deltaNTD(1-235)::URA3	
H5916	MATa, ho::LYS2, lys2, ura3, leu2::hisG, TRP,	This study
110 / 10	his3::hisG	
	his4X::LEU2-(Bam)-URA3, arg4-Nsp	
	$pch2\Delta$::KanMX, mer2(rec107)::MER2-5myc	
	MATalpha, ho::LYS2, lys2, ura3, leu2::hisG, HIS3+,,	
	trp1::hisG,arg,	
	$pch2\Delta$::KanMX, $pch2$::KanMX4 mer2(rec107)::MER2-	
	5myc, mer2(rec107)::MER2-5myc,	
H5917	MATa, ho::LYS2, lys2, ura3, leu2::hisG, TRP,	This study
	his3::hisG	
	his4X::LEU2-(Bam)-URA3, arg4-Nsp (?),	
	mer2(rec107)::MER2-5myc	
	MATalpha, ho::LYS2, lys2, ura3, leu2::hisG, HIS3+,,	
	trp1::hisG,arg(?),	
	mer2(rec107)::MER2-5myc, pch2::KanMX4	
H5947	H5547, but $pch2\Delta$:: $KanMX4/pch2\Delta$:: $KanMX4$	This study
	, F :	J

Supplementary references

- Lee, B. H. and Amon, A., Role of Polo-like kinase *CDC5* in programming meiosis I chromosome segregation. *Science* **300** (5618), 482 (2003).
- Bishop, D. K., Park, D., Xu, L., and Kleckner, N., *DMC1*: a meiosis-specific yeast homolog of *E. coli* recA required for recombination, synaptonemal complex formation, and cell cycle progression. *Cell* **69** (3), 439 (1992).
- Storlazzi, A., Xu, L., Cao, L., and Kleckner, N., Crossover and noncrossover recombination during meiosis: timing and pathway relationships. *Proc Natl Acad Sci U S A* **92** (18), 8512 (1995).