SUPPLEMETARY INFORMATION

Supplementary Tables

Table S1. *S. cerevisiae* spore viability analysis

strains		WT	*hpr1Δ*
% Spore viability (n)		97 (90)	56.2 (121)
Viable spores per tetrad	4	81	20
	3	7	29
	2	2	45
	1	0	14
	0	0	13
% Sporulation (n)		98.5 (900)	74.7 (900)

Table S2. *S. cerevisiae* spore viability analysis with and without RNase H1 overexpression

strains		WT	WT RNaseH1	*hpr1Δ*	*hpr1Δ* RNase H1	WT (0.5%gal)
% Spore viability (n)		98.3 (90)	73.6 (90)	60 (90)	48.1 (90)	94.5 (54)
Viable spores per tetrad	4	85	22	10	5	43
	3	4	41	35	28	10
	2	1	25	31	20	1
	1	0	2	9	29	0
	0	0	0	5	8	0
% Sporulation (n)		98.5 (300)	98.5 (300)	74.5 (300)	79.7 (300)	96.1 (300)

Table S3. *C. elegans thoc-2* is an essential gene.

Genotype	n	Total eggs	Viable progeny	Percent lethality	Brood size[b]
N2 (wt)	10	2978	2932	1.6	297.8±10.5
*thoc-2(ok961)*heterozygous	20	2764	1029	62.8[c]	176.8±25.8
thoc-2(ok961) homozygous[a]	6	0	0	NA	
thoc-2(tm1310) heterozygous	20	2835	896	68.4[c]	192.05±27.8
thoc-2(tm1310) homozygous[a]	6	0	0	NA	

Embryonic lethality was scored by comparing the number of eggs that hatch to produce viable progeny versus the total number of eggs laid. [a] Hermaphrodites used were homozygous *thoc-2* mutants derived from *thoc-2* -/+ parents. [b]Average brood size ± SD ($p<0.001$; Student's t-Test). [c]The expected lethality for carrying the hT2 balancer chromosomes is 68.75%.

Supplementary Figures

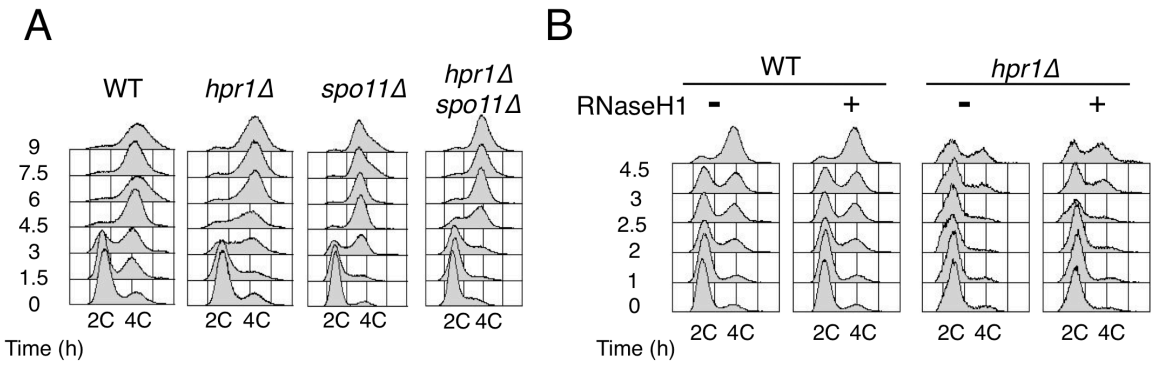

Fig. S1. *hpr1Δ* cell cycle profile. (**A**) FACS analysis of DNA contents after transfer to sporulation media for the indicated strains. (**B**) FACS analysis of DNA contents after transfer to sporulation media for the indicated strains in the presence of absence of RNase H1 overexpression.

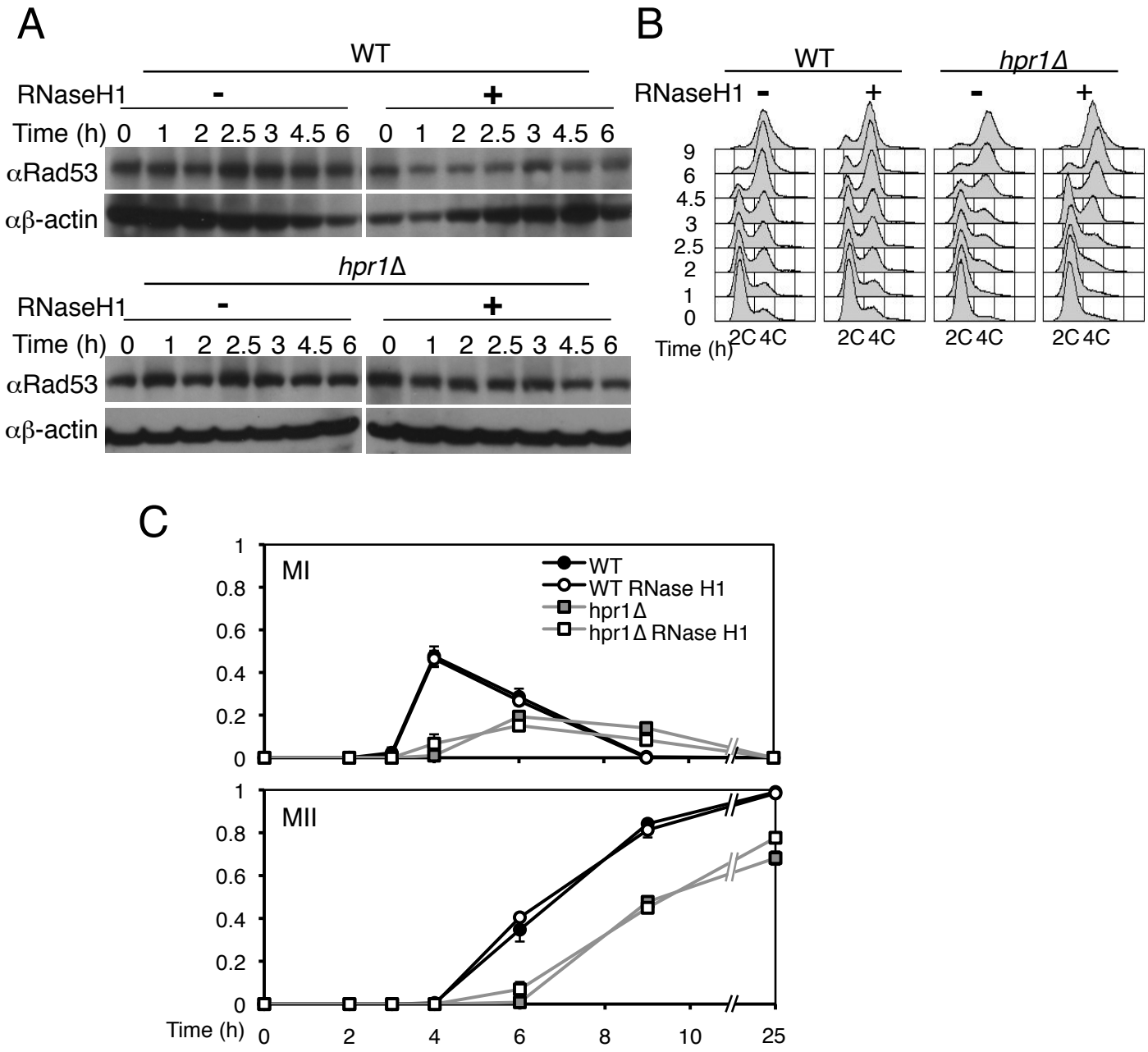

Fig. S2. Overexpression of RNase H1 under *tet* promoter. (**A**) Western analysis after transfer to sporulation media with and without RNase H1 overexpression. (**B**) FACS analysis of DNA contents after transfer to sporulation media for the indicated strains in the presence of absence of RNase H1 overexpression. (**C**) Fraction of cells that completed meiosis I (MI) and II (MII) in *HPR1* and *hpr1Δ* homozygous diploids with and without RNase H1 overexpression. The means of cells ± SD are shown.

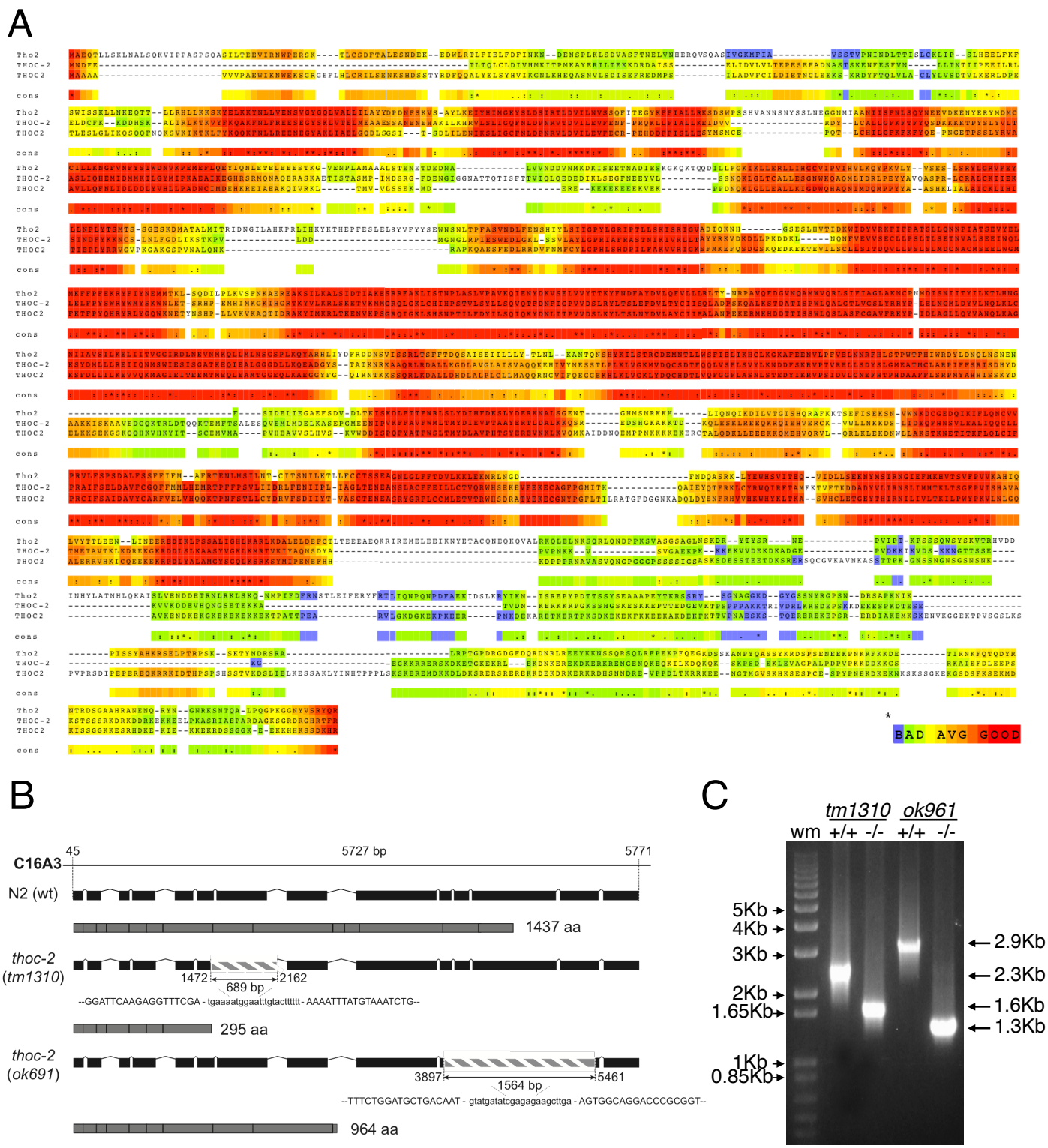

Fig. S3. Characterization of *C. elegans thoc-2* deletion alleles. (**A**) Alignment of S. cerevisiae Tho2, C. elegans THOC-2 and H. sapiens THOC2. (**B**) Scheme of *thoc-2* gene structure and the predicted protein product for wild-type and the *thoc-2(tm1310)* and *thoc-2(ok691)* deletion proteins. *thoc-2(tm1310)* carries a 689-bp deletion in exon 6, while *thoc-2(ok691)* carries a 1564-bp deletion in exon 10, and both generate a premature stop codon. Numbers indicate the nucleotide positions in cosmid C16A3.8. (**C**) Nested single worm PCR with thoc-2 specific primers for both deletions. Wilde-type N2 (lanes 2 and 4), and *thoc-2 (tm1310)* (lane 3) and *thoc-2 (ok691)* (lane 5) mutant worms showing the 689-bp and 1564-bp deletions.

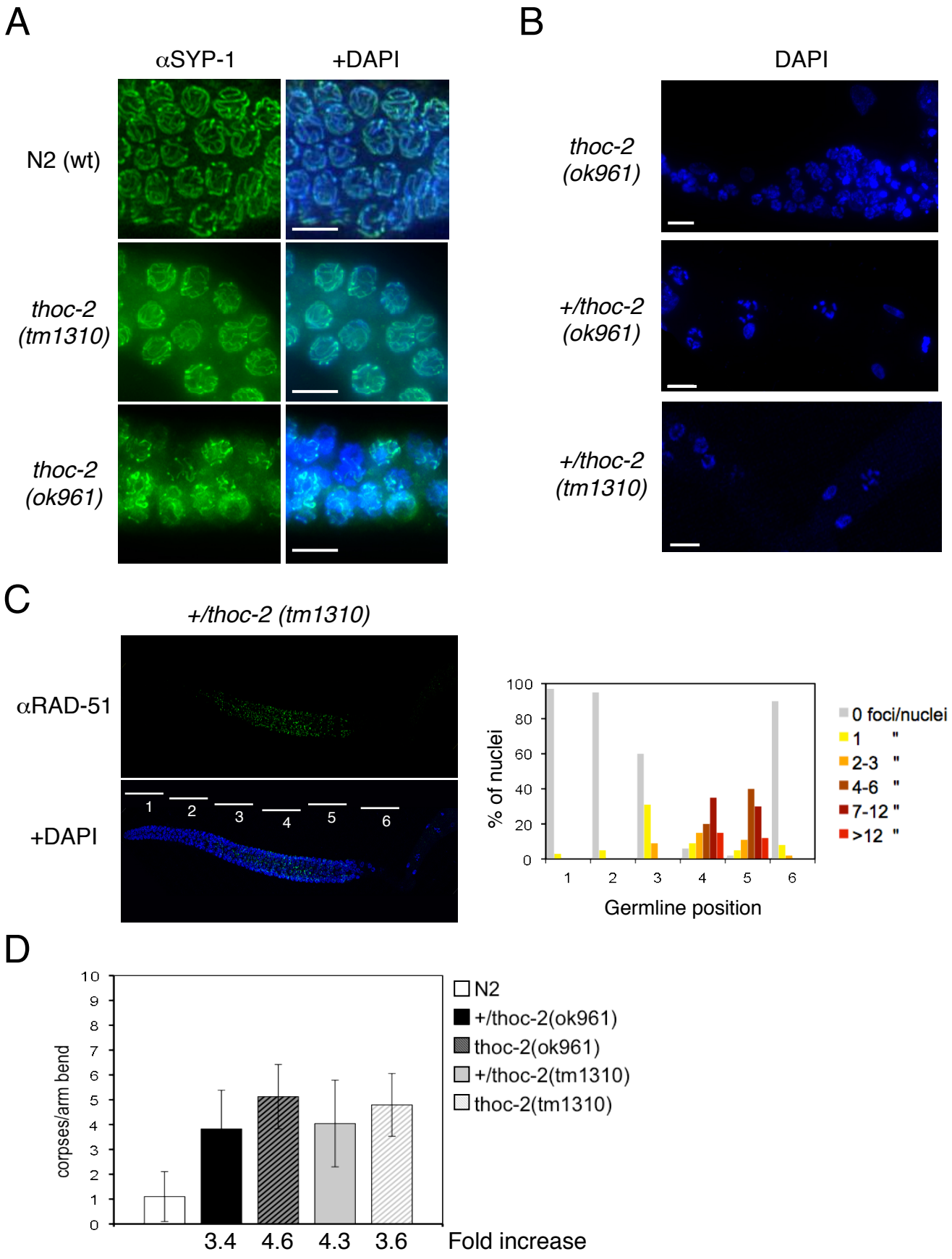

Fig. S4. *thoc-2* animals show defective meiosis and increased apoptosis. (**A**) Representative images of a pachytene germline region immunostained with SYP-1 and counterstained with DAPI from animals of the indicated genotype. (**B**) Representative

images of a diplotene-diakinesis germline region counterstained with DAPI from animals of the indicated genotype. (**C**) Representative image of heterozygous *thoc-2* mutants fixed germline immunostained with RAD-51 and quantification. (**D**) Quantification of apoptotic corpses in 24 hours post-L4 stage animals of the indicated genotypes. The means of apoptotic corpses per arm bend ±SEM are shown. Scale bars represent 10μm.

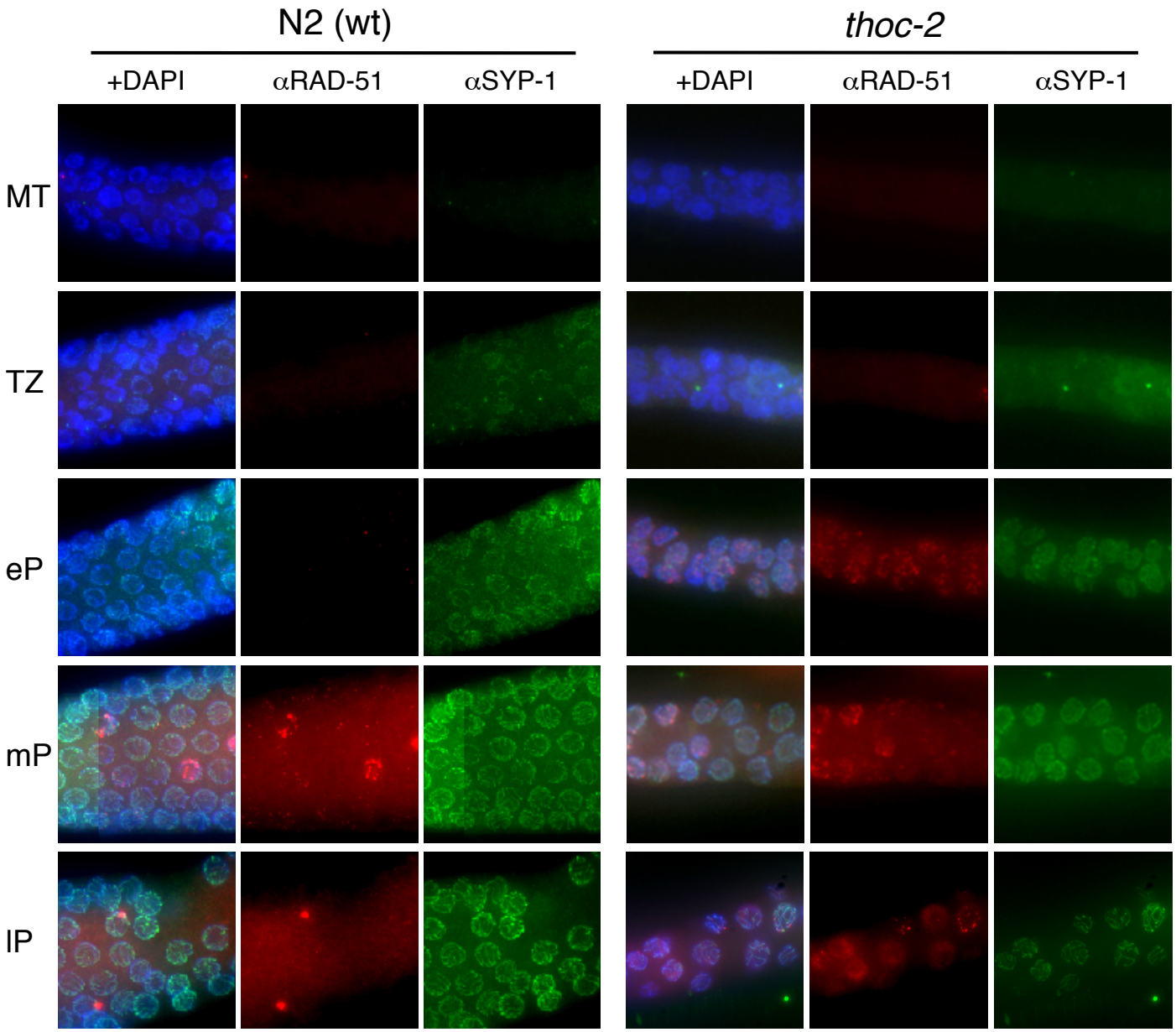

Fig. S5. *thoc-2* animals show increased RAD-51 foci. Representative images of mitotic (MT), transition zone (TZ), early, mid and late pachytene (eP, mP and lP) germline regions immunostained with SYP-1, RAD-51 and counterstained with DAPI from animals of the indicated genotype.

Supplementary Material and Methods

Strains and maintenance

C. elegans thoc-2 deletions were backcrossed six times with wild type Bristol N2 and then balanced with JK2739. In order to generate the double mutant *thoc-2;spo-11*, previously the *spo-11* strain balancer was changed crossing with JK2663.

S. cerevisiae transformations were performed using the lithium acetate/polyethylene glycol method (Gietz and Woods, 2001).

S. cerevisiae synchronous meiotic cultures were prepared as described previously (Alani et al, 1990). Briefly, yeast cells were grown in liquid YPA (1% yeast extract, 2% Bacto Peptone, 1% Potassium Acetate) for 13 hours at 30ºC. Cells were harvested, washed, and resuspended in SPM (0,3% Potassium Acetate) pre-equilibrated at 30ºC. For RNase H overexpression under *GAL1* promoter, the appropriated strains were transformed with the pGAL::RNH1 (Huertas & Aguilera, 2003) and the SPM was complemented or not with galactose (0.5%). For RNase H overexpression under *tet* promoter, the appropriated strains were transformed with the ptet::RNH1 and the SPM was complemented or not with doxocyclin (5μg/ml).

S. cerevisiae tetrads were dissected on solid media containing 2%peptone, 1% yeast extract, 4% glucose, 0.004% adenine, 2% agar, and spores were germinated at 30ºC.

S. cerevisiae meiosis nuclear division profiles and DSB analysis

Aliquots were collected from synchronized meiotic cultures at various times, fixed with 70% (v/v) ethanol and stained with 0.1 μg/ml with 4´,6´-diamidino-2-phenylindole. Mononucleate, binucleate, and tetranucleate cells were scored by microscopy (at least 200 cells per time point).

For meiotic DSB detection genomic DNA was prepared in agarose plugs and digested with StuI (New England) and analyzed by Southern as described (Borde et al, 2004). Quantification with a Fuji FLA-5100 was performed according to standard procedures. Relative amount of the signal derived from each band was normalized with respect to the background of the respective line.

Plasmids

To analyze Rad52 foci formation during meiosis, plasmid pWJ1344 carrying Rad52-YFP was used and described previously (Lysby et al., 2001).

Centromeric plasmid pCM189 carrying the yeast RNH1 ORF under *tet* promoter was obtained by PCR amplification of RNH1 ORF from plasmid p*GAL*::RNH1 (Huertas et al., 2003) using primers 5´-ATGGCAAGGCAAGGGAACTT-3´ and 5´-TTATCGTCTAGATGCTCCTT-3´ and cloned into pGEM-T vector. A NotI fragment was sub-cloned into pCM189 vector.

Fluorescence microscopy

Leica DM6000B inverted microscope was used to examine the germlines with 40X HCXPL APO/1.25 OIL or 63X HCXPL APO/1.40 OIL lens, and images captured using Leica LAS AF computer software. For RAD-51 analysis a Confocal Leica TCS SP5 microscope with 40X HCXPL APO /1.5-0.75 OIL lens was used. Three-dimensional data sets were computationally deconvolved, and regions of interest projected into one dimension.

Cytological preparation and immunostaining

Gravid hermaphrodites were transferred to 30ml PBS on a poly-L lysine coated slide (slides were washed in 70% ethanol, then given 2 coats of 100% poly-L-lysine, air drying between each coat). The worms were washed in PBS before transferring to 50ml 10mM levamisole. Germlines were extruded by removing the head and tail using a fine gauge needle (27 G). Levamisole was replaced with 4% para-formaldehyde (PFA) in PBS for 10min and germlines were permeabilized for 5 min in TBSBT (TBS+0.5% BSA +0.1% Triton X-100), then washed in TBSB for at least 2 5min, followed by blocking for 30min. Primary antibodies were diluted in TBSB and incubated overnight at 4ºC in a humid chamber. Germlines were subsequently washed at RT at least 3x5 min in TBSB before incubation with the secondary antibody for 1–2h at room temperature. Finally, germlines were washed at least 3x5min in TBSB before mounting with a coverslip on Vectashield containing DAPI (Vector Laboratories). Dilutions used were the following: rabbit α-RAD-51 (1:800) and guinea pig α-SYP-1 (1:800). The appropriate secondary antibodies were diluted 1:5000.

In situ detection of germline DNA synthesis

Direct incorporation of Cy3-dUTP into germline nuclei was performed as described (Jaramillo-Lambert et al, 2007), except that the injection mix consisted of 50 μM Cy3-dUTP (Amersham Bioscences) in PBS pH 7.2. After the appropriate exposure to the Cy3-dUTP, gonads were dissected, fixed and DAPI-stained. The total number of cells that incorporated Cy3-dUTP was determined for each dissected germline.
Commercial RNase H (Invitrogen) was co-microinjected at 0.2 U/μl.

Apoptosis assay

Apoptotic corpses were visualized with SYTO-12 (Invitrogen) as described (Gumienny et al, 1999), at 16-20h post-L4. The total number of germlines scored for the different genotypes were: N2 n=49, +/*thoc-2(ok961)* n=23, homozygous *thoc-2(ok961)* n=24, +/*thoc-2(tm1310)* n=23, homozygous *thoc-2(tm1310)* n=30.

Table of *Caenorhabditis elegans* strains		
Strain name	Genotype	Origin
N2	Bristol wild-type	CGC
AV106	*spo-11(ok79)* IV/ nT1[unc-?(n754) let-?](IV;V)	CGC
JK2739	*lin-6(e1466) dpy-5(e61)* I/ hT2[bli-4(e937) let-?(q782) qIs48](I;III)	CGC
JK2663	*dpy-11(e224) mes-4(bn67)* V/ nT1[qIs50](IV;V)	CGC
VC673	*thoc-2(ok961)* III/ hT2[bli-4(e937) let-?(q782) qIs48](I;III)	CGC
tm1310	*thoc-2(tm1310)* III	S. Mitani
GIN101	*thoc-2(tm1310)* III/ hT2[bli-4(e937) let-?(q782) qIs48](I;III) (X6 outcrossed)	This work
GIN102	*thoc-2(ok961)* III/ hT2[bli-4(e937) let-?(q782) qIs48](I;III) (X6 outcrossed)	This work
GIN103	*spo-11(ok79)* IV/ nT1[qIs50](IV;V)	This work
GIN104	*thoc-2(tm1310)* III/ *spo-11(ok79)* IV/ hT2[bli-4(e937) let-?(q782) qIs48] (I;III)/ nT1[qIs50] (IV;V)	This work

Table of *Saccharomyces cerevisiae* strains		
Strain name	Genotype	Origin
NKY1551	*MAT a/α ho::LYS2/" ura3/" lys2/" leu::hisG/" arg-4-Nsp/arg4-Bgl his4X::LEU2(Bam)-URA3/HIS4B::LEU2*	(Teresawa et al, 2007)
NKY1551 *hpr1Δ*	Same as NKY1515 but *hpr1ΔHYG/hpr1ΔKAN*	This work
NKY611	*ho::LYS2/ '', ura3/ '', lys2/ '', leu2::hisG/ ''*	(Alani et al, 1990)
NKY611 *hpr1Δ*	Same as NKY611 but *hpr1ΔHYG/hpr1ΔKAN*	This work
NKY644 x NKY646	*MAT a/a spo11Δ*	From N. Kleckner
NKY644/646 *hpr1Δ*	*MAT a/a spo11Δ hpr1Δ*	This work

Table of Antibodies			
Antibody	Use	Dilution	Reference
Mouse α-BetaActine (8224)	WB	1:1000	Abcam
Rabbit α-H2ASer129P (15083-100)	WB	1:400	Abcam
Rabbit α-RAD-51	IF	1:800	(Alpi et al, 2003)
Goat α-RAD-53 (yC-19)	WB	1:300	S.Cruz Btech.
Guinea pig α-SYP-1	IF	1:800	(MacQueen et al., 2002)

Supplementary References

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