Supplemental Data

RTEL1 Maintains Genomic Stability by Suppressing Homologous Recombination

Louise J. Barber, Jillian L. Youds, Jordan D. Ward, Michael J. McIlwraith, Nigel J. O'Neil, Mark I.R. Petalcorin, Julie S. Martin, Spencer J. Collis, Sharon B. Cantor, Melissa Auclair, Heidi Tissenbaum, Stephen C. West, Ann M. Rose, and Simon J. Boulton

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

***C. elegans* strains.** FX1866 *rtel-1(tm1866)*, FX1298 *fcd-2(tm1298)*, FX424 *rcq-5(tm424)* and FX1937 *mus-81(tm1937)* were generated by Shohei Mitani and the National Bioresource Project of Japan. VC13 *dog-1(gk10)*, VC193 *him-6(ok412)*, VC174 *wrn-1(gk99)* were generated by the International *C. elegans* Gene Knockout Consortium. Additional strains used include KR3499 *dpy-11(e224) unc-42(e270)*, KR180 *dpy-17(e164) unc-36(e251)*, LM99 *smn-1(ok355)* I/*hT2*[*bli-4(e937)Is48*] (I;III), BC2200 *eT1[unc-36]/dpy-18(e364); eT1[unc-36]/unc-46(e177)*, KR4145 *nT1/+*(IV)*; nT1/dpy-11*(V) and VC508 *acl-3(ok726) IV/nT1[qIs51]* (IV;V).

***C. elegans* screen for helicase mutants that confer synthetic lethality when combined with *him-6* (*sgs1/BLM*).** One of the characteristic phenotypes of both budding and fission yeast *srs2* mutants is that growth is severely impaired by the additional loss of *sgs1/rqh1*, a homologue of the human Bloom's Syndrome helicase (*BLM*) (Lee *et al*., 1999; Wang *et al*., 2001). It is believed that the growth defect and increased lethality results from an inability to either resolve HR or regress the strand invasion intermediate. We exploited this concept to identify potential antagonists of recombination in the nematode, screening for helicase genes that when mutated result in severely impaired progeny viability when combined with the *C. elegans BLM* homolog, *him-6* (Wicky *et al*., 2004). Ideally we would have liked to conduct an unbiased screen using a genome-wide RNAi library. However, in pilot studies we found that a significant number of genes involved in the maintenance of genomic stability are not effectively knocked down by RNAi in the worm. That is, RNAi did not recapitulate known phenotypes of gene deletion mutants. Furthermore, yeast *sgs1* is known to be synthetic lethal or sick with more than 100 other genes, and this number could reasonably be expected to be higher in *C. elegans*. We therefore worked on the premise that an anti-recombinase with functional similarity to Srs2 would possess a helicase domain. Of the synthetic growth defects known for *sgs1*, fewer than 10% concern a gene containing helicase domains. Therefore we performed a candidate-based screen using deletion mutant strains that was restricted in the first instance to helicases of the Rad3-like, Pif1-like, and RecQ families (20 helicase-encoding genes). We chose these families either for their slight sequence similarity to Srs2 (Rad3, Pif1) or as *in vitro* data has suggested a role in the negative regulation of HR (RecQ). The available deletion mutants in these families were crossed to *him-6*, and the viability of the double mutant progeny was assessed. From this screen *rtel-1* and to a lesser extent *dog-1* mutants were found to exhibit synthetic lethality when combined with *him-6*. *rtel-1* and *dog-1* were therefore considered to have a synthetic growth defect akin to *srs2 sgs1* in yeast and were further analyzed for defects in

regulating HR. In contrast to *rtel-1*, *dog-1* mutants did not exhibit any measurable defect in regulating HR; *dog-1* mutants are not hypo or hyper-recombinogenic (Table 2). Although small scale and non-exhaustive, this screen identified a novel uncharacterised gene, *rtel-1*, that when mutated, confers phenotypes predicted of an anti-recombinase.

***C. elegans* meiotic recombination.** The frequency of meiotic recombination was measured in the intervals between *dpy-11* and *unc-42*, and *dpy-17* and *unc-36*. Individual animals of genotype *dpy unc/+ +, rcq-5; dpy unc/++, dog-1; dpy unc/++* and *rtel-1; dpy unc/++* were plated and transferred daily for four days. In each of the broods, the number of wild-types, DpyUncs, Dpys and Uncs were scored. Recombination frequency (p) was calculated using the equation $p=1-(1-2(R/T))^2$, where: R=numbers of recombinants and T=total progeny. The map distance was calculated as px100. Confidence intervals for each recombination frequency were calculated using the statistics of Crow and Gardner (Crow, 1959).

Human cell culture, siRNA and drug treatments. HeLa and HEK293 cells (Cancer Research UK Cell Services, South Mimms, UK), FLP-In T-Rex-293 cells (Invitrogen), and SW480/SN3 cells (gift from Helen Bryant and Thomas Helleday, Sheffield, UK) were maintained as adherent monolayer cultures in appropriate media and atmosphere at 37°C.

Cloning of human *RTEL1*. Human *RTEL1* was cloned from cDNA as an EcoR1/Not1 fragment into pcDNA3-C-MYC-HIS. Site-directed mutagenesis was performed to produce the K48R mutation. Wt and K48R RTEL1-MYC-6HIS were subcloned into pDONR221 via a Gateway BP-reaction and then by LR-reactions into Gateway destination vectors for baculovirus expression (Baculodirect N-Term Linear DNA, Invitrogen), human cell transient expression (pCMV-FLAG), and FLP-In tetracycline-inducible stable expression (pDEST-Flag/FRT/TO, a gift from Janet Cronshaw, Cancer Research UK London Research Institute, UK).

Protein purification from human cells. Wild-type and K48R mutant RTEL1 fused to a FLAG epitope at the N-terminus and MYC-6HIS at the C-terminus were purified from HEK293 cells. FLAG-RTEL1-MYC-6HIS was transiently expressed in 400 ml HEK293 cells using lipofectamine plus (Invitrogen), and cells were collected 48 h after transfection. The cell pellet was lysed in extraction buffer (50 mM Tris HCl pH7.4, 400 mM NaCl, 10 % glycerol, 1 % Triton X-100, 0.1 % Igepal, protease inhibitor cocktail (Sigma P2714), phosphatase inhibitor cocktail (Sigma P5726), 50 units/ml benzonase). RTEL1 was purified to near homogeneity from the soluble fraction using anti-FLAG M2 agarose (Sigma) and eluted with an excess (500 μg/ml) of 3x FLAG peptide (Sigma) in elution buffer (50 mM Tris HCl pH7.4, 500 mM NaCl, 10 % glycerol). Purified protein was concentrated, quantified, confirmed, and stored as described for V5-RTEL1-MCY-6HIS. Purified proteins were tested and found to be devoid of detectable ssDNA and dsDNA exonuclease activity.

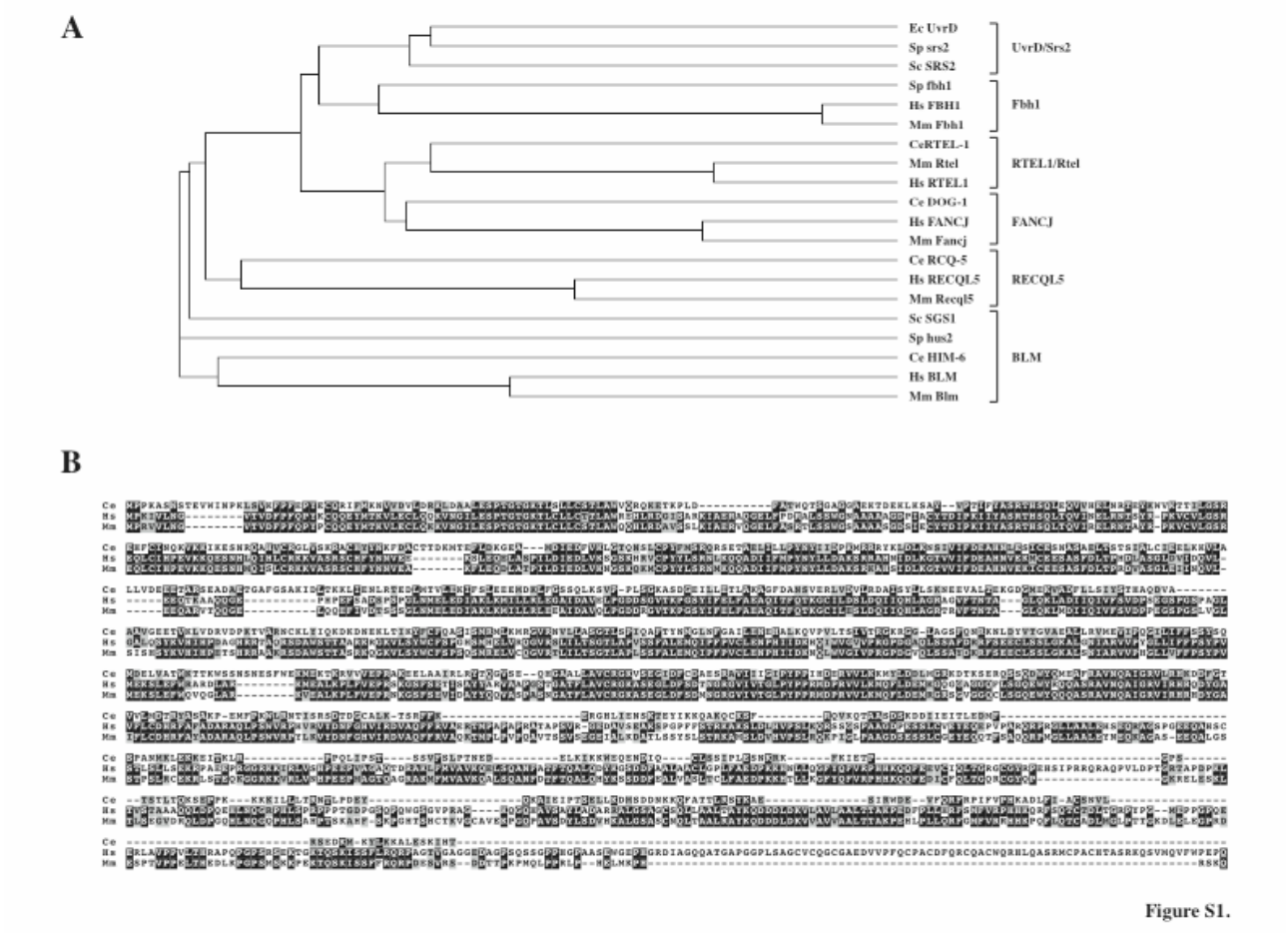

Figure S1. Cladogram and sequence alignment of RTEL1.

A. Protein sequences of the indicated proteins were obtained from GenBank. Phylogenetic analysis of the different helicase proteins from *S. cerevisiae*, *S. pombe*, *C. elegans*, *M. muscalaris*, and *H. sapiens* was performed using Phylip 3.65.

B. Protein sequence alignment of the indicated proteins was performed using ClustalW 1.83.

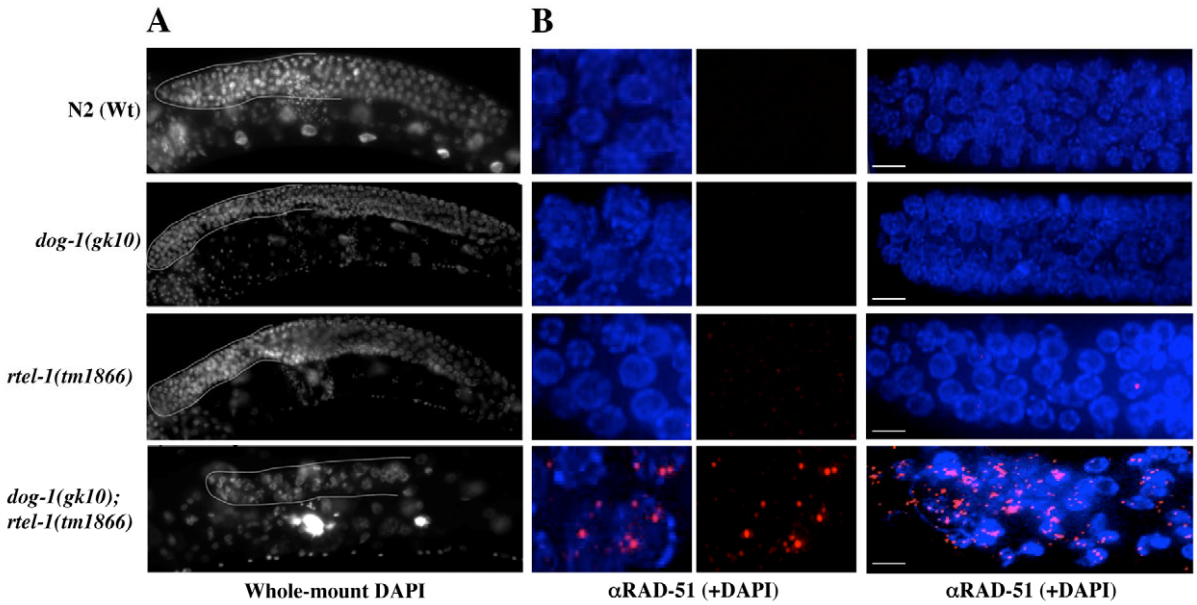

Figure S2.

Figure S2. Mitotic catastrophe and increased incidence of RAD-51 foci in *rtel-1 dog-1* mutants.

A. Representative images of whole mount DAPI-stained worms of the indicated genotypes. The germ line has been outlined in white. The distal end of the germ line is on the left. Notice the abnormal nuclear size and shape in the *rtel-1 dog-1* mutant compared to N2 (Wt) and the single mutants (a close up of the mitotic compartment is shown in B).

B. Representative images of nuclei within the mitotic compartment of the germ line immunostained for RAD-51 (red) and counterstained with DAPI (blue) in worms of the indicated genotype. Scale bar = 5 μm.

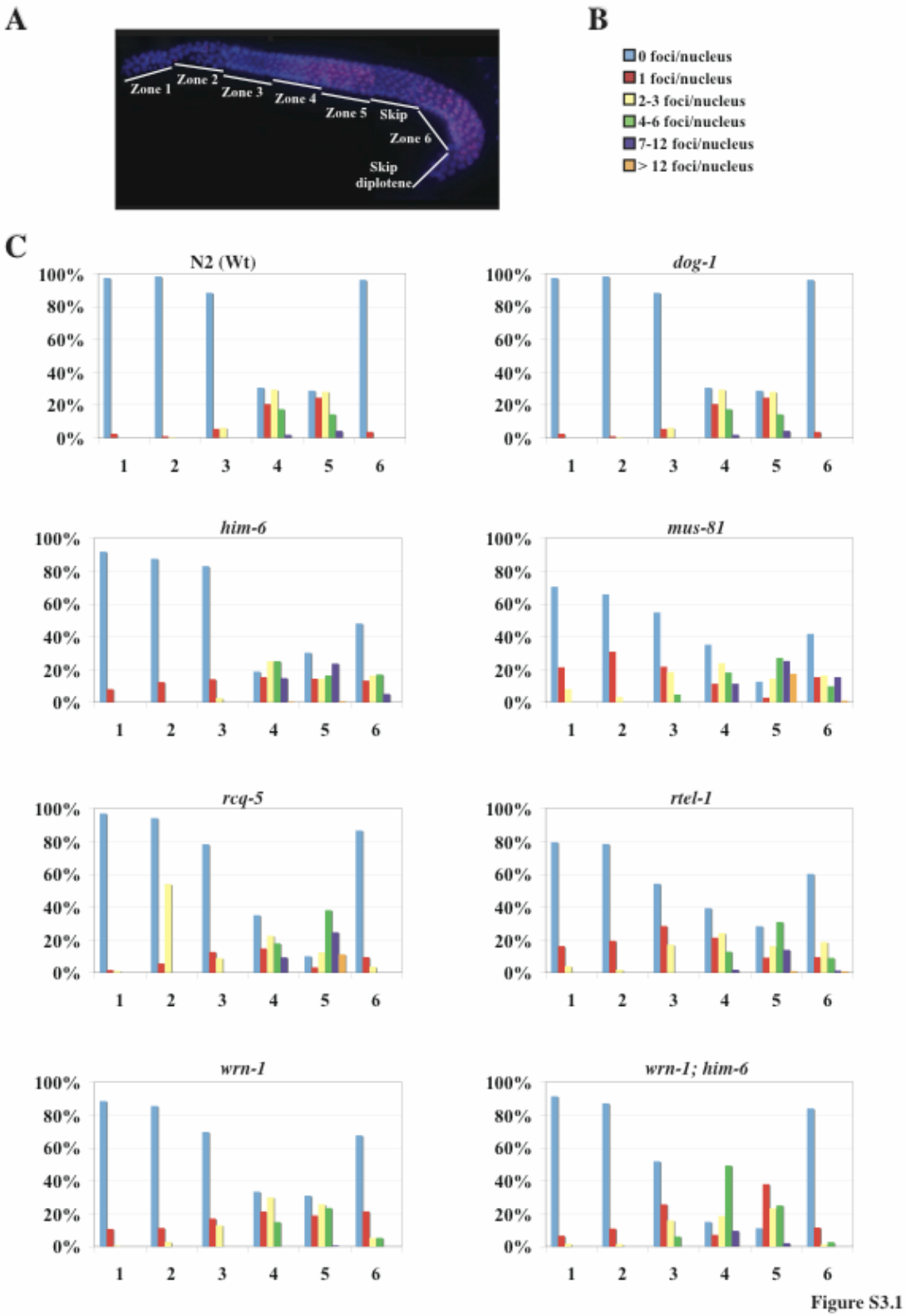
A
Zone 1
Zone 2
Zone 3
Zone 4
Zone 5
Skip
Zone 6
Skip diplotene
B
0 foci/nucleus
1 foci/nucleus
2-3 foci/nucleus
4-6 foci/nucleus
7-12 foci/nucleus
> 12 foci/nucleus
C
N2 (Wt)
dog-1
him-6
mus-81
rcq-5
rtel-1
wrn-1
wrn-1; him-6
100%
80%
60%
40%
20%
0%
1
2
3
4
5
6

Figure S3.1

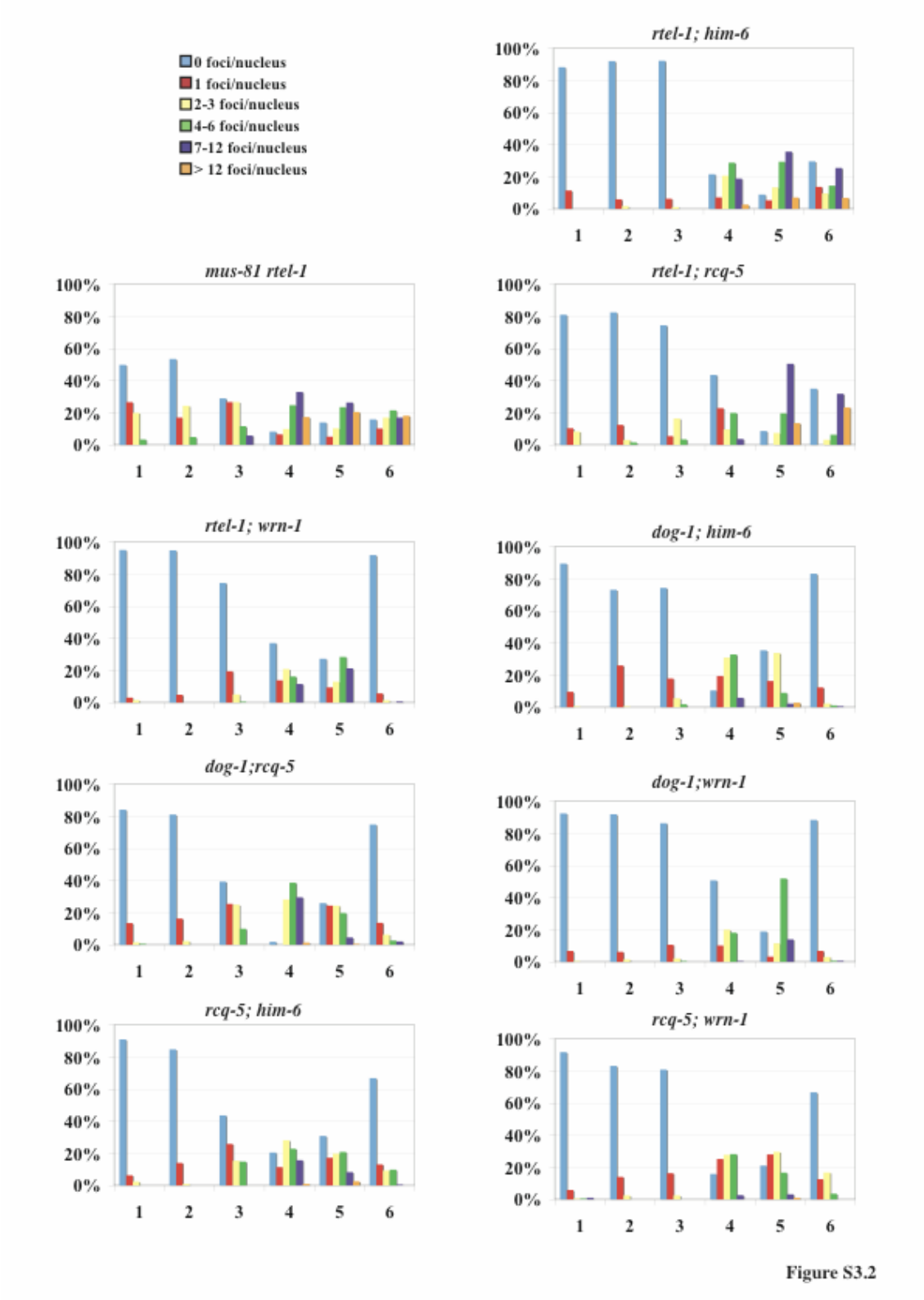

Figure S3. Detailed quantification of RAD-51 foci through the germ line.
A. A schematic of the *C. elegans* germ line (distal end is on the left) sub-divided into six zones for foci quantification, as previously described (Colaiacovo *et al*., 2003).
B. Colour key used in histograms classifying the number of RAD-51 foci per nucleus.
C. Histograms of the percentage nuclei exhibiting RAD-51 foci (as defined in B) in each of the six germ line zones. Each histogram represents a different genotype, as indicated. A minimum of five animals were quantified for each genotype.

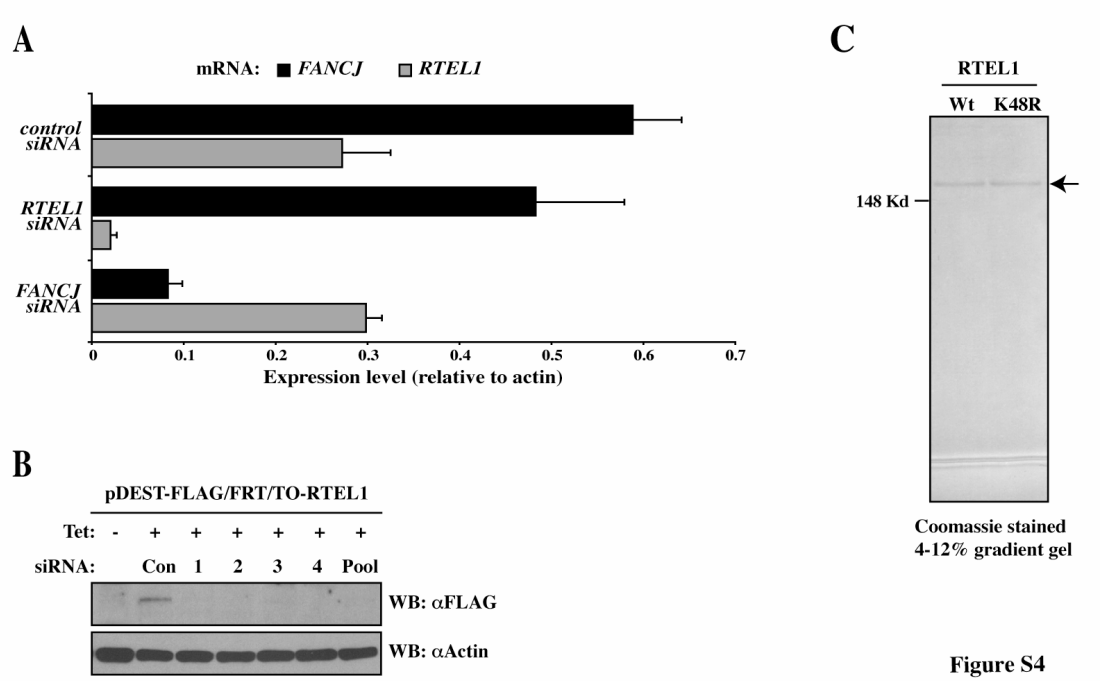

Figure S4. Confirmation of *RTEL1* knockdown by siRNA.
A. mRNA expression levels of RTEL1 and FANCJ, relative to actin, as determined by quantitative RT-PCR following treatment of HEK293 cells with the indicated siRNAs (non-targeting control, *RTEL1*, and *FANCJ*).
B. Western blot of whole-cell extracts obtained 48 h after siRNA knockdown of FLAG-RTEL1 expressed from an integrated Tetracycline-inducible construct in stable FLP-In T-Rex-293 cells.
C. Human Wt and K48R RTEL1 purified from Hi5 insect cells, resolved on a 4-12% gradient gel and stained with Coomassie.

SUPPLEMENTAL REFERENCES

Colaiacovo, M.P., MacQueen, A.J., Martinez-Perez, E., McDonald, K., Adamo, A., La Volpe, A., and Villeneuve, A.M. (2003). Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. Dev Cell *5*, 463-474.
Crow, E.L., and Gardner, R.S. (1959). Confidence intervals for the expectation of a Poisson variable. Biometrika *46*, 441-453.
Lee, S.K., Johnson, R.E., Yu, S.L., Prakash, L., and Prakash, S. (1999). Requirement of yeast *SGS1* and *SRS2* genes for replication and transcription. Science *286*, 2339-2342.
Wang, S.W., Goodwin, A., Hickson, I.D., and Norbury, C.J. (2001). Involvement of *Schizosaccharomyces pombe* Srs2 in cellular responses to DNA damage. Nucleic Acids Res *29*, 2963-2972.
Wicky, C., Alpi, A., Passannante, M., Rose, A., Gartner, A., and Muller, F. (2004). Multiple genetic pathways involving the *Caenorhabditis elegans* Bloom's syndrome genes *him-6*, *rad-51*, and *top-3* are needed to maintain genome stability in the germ line. Mol Cell Biol *24*, 5016-5027.