Supporting Information

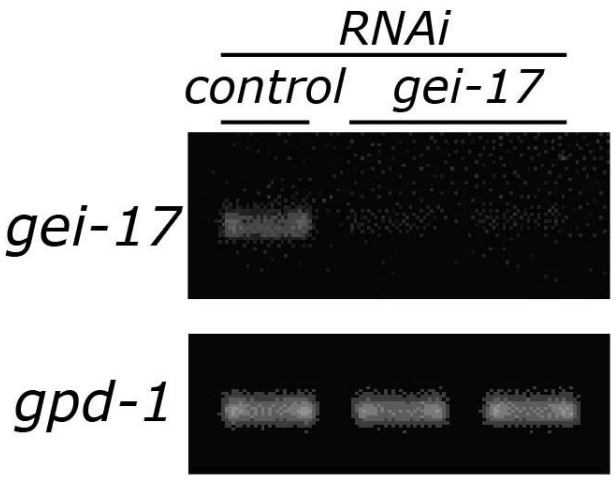

Figure S1. RT-PCR analysis of RNA expression after *RNAi* depletion of *gei-17*.
Single worm lysates were used for each reaction. The primer pairs used in amplification reactions span introns in *gei-17* or *gpd-1* to exclude genomic DNA contamination.

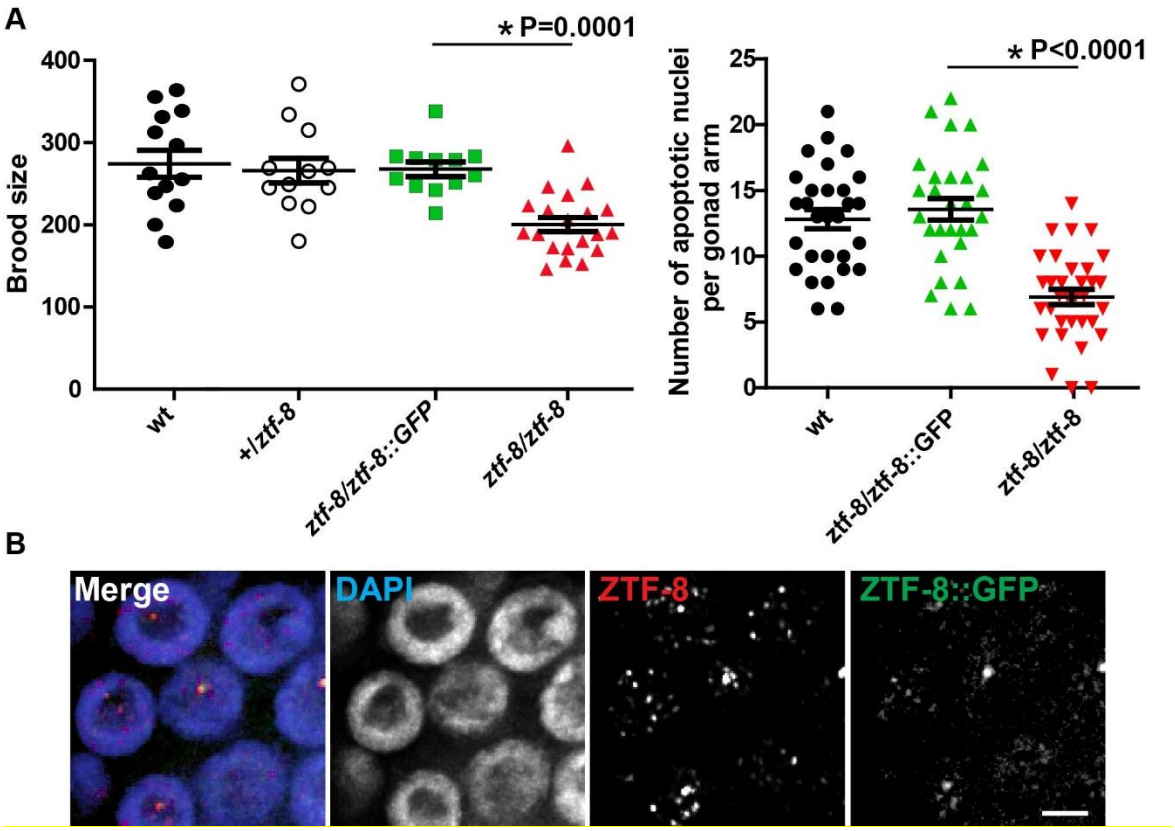

Figure S2. GFP::FLAG-tagged ZTF-8 (*rj22*) is able to rescue the *ztf-8* null mutant phenotypes.
A. Quantification of brood size and germline apoptosis in the indicated genotypes. Worms were treated with γ-IR (80 Gy) for apoptosis analysis. Asterisks indicate statistical significance compared to control by the Mann-Whitney test, 95% C.I. **B.** Co-staining with DAPI (blue) and an anti-ZTF-8 antibody (red) combined with detection of the endogenous GFP signal (green) in the ZTF-8::GFP::FLAG expressing worms. Of note, the endogenous GFP signal in this line is weaker than what is observed with an anti-GFP antibody. Bar, 2μm.

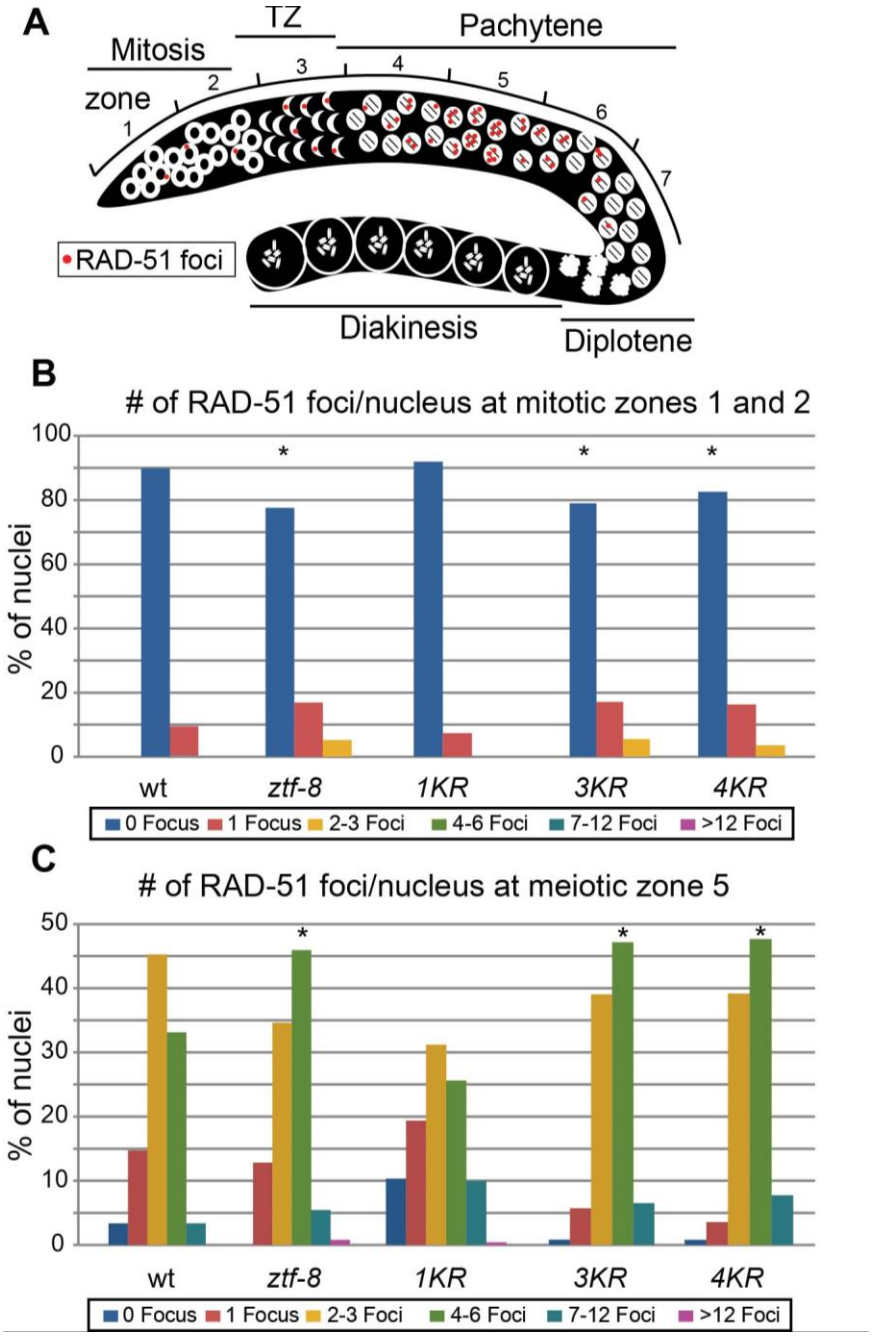

Figure S3. Assessing DSBR progression by quantitation of RAD-51 foci.
A. Schematic representation of a *C. elegans* germline indicating the position of the seven zones scored for RAD-51 foci. **B-C.** Graphs depict the percentage of nuclei carrying from 0 to >12 RAD-51 foci (y-axis) either in the mitotic germline region (**B**, zones 1 and 2) or at mid-pachytene during meiosis (**C**, zone 5) for the indicated genotypes (x-axis). Asterisks indicate statistical significance compared to wild type by the Mann-Whitney test, 95% C.I.

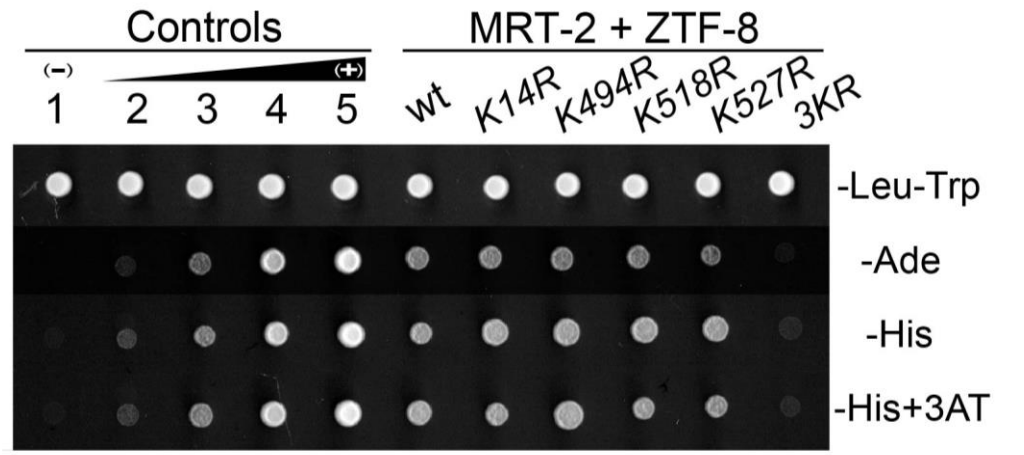

Figure S4. Yeast two-hybrid analysis of the interaction between MRT-2/Rad1 and ZTF-8.
Wild type full-length ZTF-8 as well as point mutants for the SUMOylation sites of ZTF-8 were tested for their interactions with MRT-2. K14R, K494R, K518R and K527R denote single point mutants, whereas 3KR denotes a construct carrying three mutations of K to R at amino acids 494, 518 and 527. Wild type and mutant ZTF-8 were fused to the DNA binding domain and full length MRT-2 was fused to the activation domain of GAL4. One negative (No. 1) and four positive controls (No. 2-5) were used as described in (37). Interactions were scored by growth on -Ade, -His and -His+3AT plates and compared to growth on -Leu-Trp control media.

Supplemental Table

Table S1. ZTF-8 interacting proteins identified by liquid chromatography-mass spectrometry (LC-MS) analysis.

Protein Name	ZTF-8::GFP::FLAG	4KR::GFP::FLAG	Control (GFP only)
UBC-9	8	Not detected	Not detected
SMO-1	36	6	Not detected
GEI-17	8	6	Not detected
MRT-2	8	Not detected	Not detected
HUS-1	7	Not detected	Not detected

Immunoprecipitations from ZTF-8::GFP::FLAG and 4KR::GFP::FLAG whole worm lysates with anti-GFP agarose beads were analyzed by LC-MS. Worm expressing only GFP under the *unc-17* promoter (*vsIS48[Punc-17::gfp*]) were used as a negative control. Experiment was performed in triplicate. The potential ZTF-8 interacting proteins that were identified in at least two of the experiments were listed. Numbers indicate the total mass spectra collected from three experiments.

File S1

Supplemental Experimental Procedures

RNA interference

Feeding RNAi experiments were performed at 20ºC as described in [2]. The entire coding sequence of *gei-17* cloned into the pL4440 feeding vector was used for RNAi experiments. HT115 bacteria carrying the empty pL4440 vector were used as the control RNAi.

cDNA was produced from single-worm RNA extracts using the One step RT-PCR kit (USB). The effectiveness of RNAi was examined by assaying the expression of the transcript being depleted in four individual animals subjected to RNAi by feeding. Expression of the *gpd-1(T09F3.3)* transcript was used as a control.

Quantitative analysis for RAD-51 Foci

Quantitative analysis of RAD-51 foci was performed as in [3]. Five to nine germlines were scored for each genotype. The average number of nuclei scored per zone for a given genotype was as follows, ± standard deviation: zone 1, n=151.3 ± 32.3, zone 2, n=148.0 ± 30.0 and zone 5=132.0 ± 37.3. Statistical comparisons between genotypes were performed using the two-tailed Mann-Whitney test, 95% confidence interval (C.I.).

Immunoprecipitation of mass spectrometry (LC-MS)

ZTF-8::GFP::FLAG transgenic *rj22* and control worms expressing only GFP under the *unc-17* promoter (*vsIS48[Punc-17::gfp]*) were lysed and prepared as described in the *In vivo* SUMOylation assay section. After incubating worm lysates with anti-GFP agarose beads (MBL International) over 12 hours at 4°C, binding proteins were immunoprecipitated and eluted as described in manufacturer's protocol and submitted for LC-MS/MS analysis at the Taplin MS Facility, Harvard Medical School (Dr. S. Gygi).

Supplemental References

1. Vidal, M., Brachmann, R.K., Fattaey, A., Harlow, E., and Boeke, J.D. (1996). Reverse two-hybrid and one-hybrid systems to detect dissociation of protein-protein and DNA-protein interactions. Proceedings of the National Academy of Sciences of the United States of America *93*, 10315-10320.
2. Timmons, L., Court, D.L., and Fire, A. (2001). Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. Gene *263*, 103-112.
3. 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.