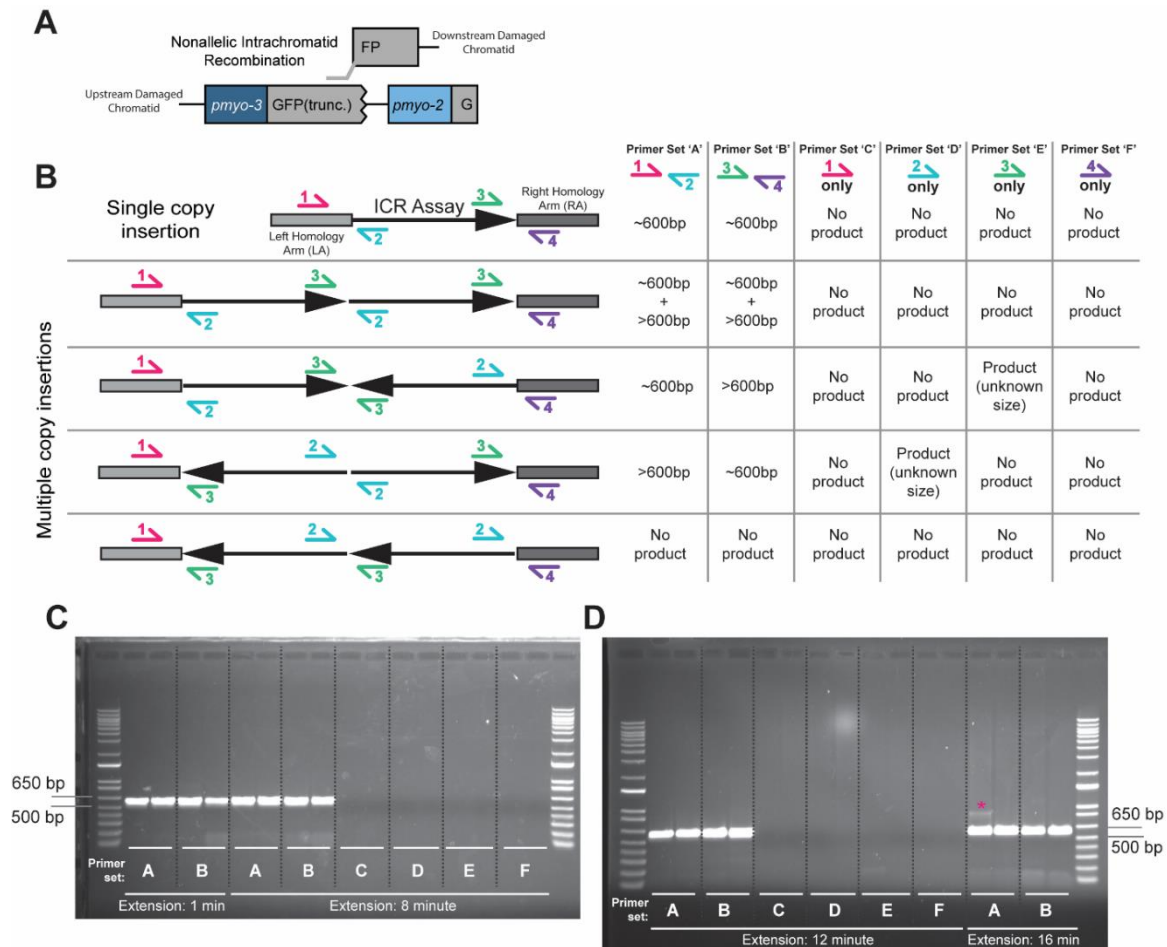


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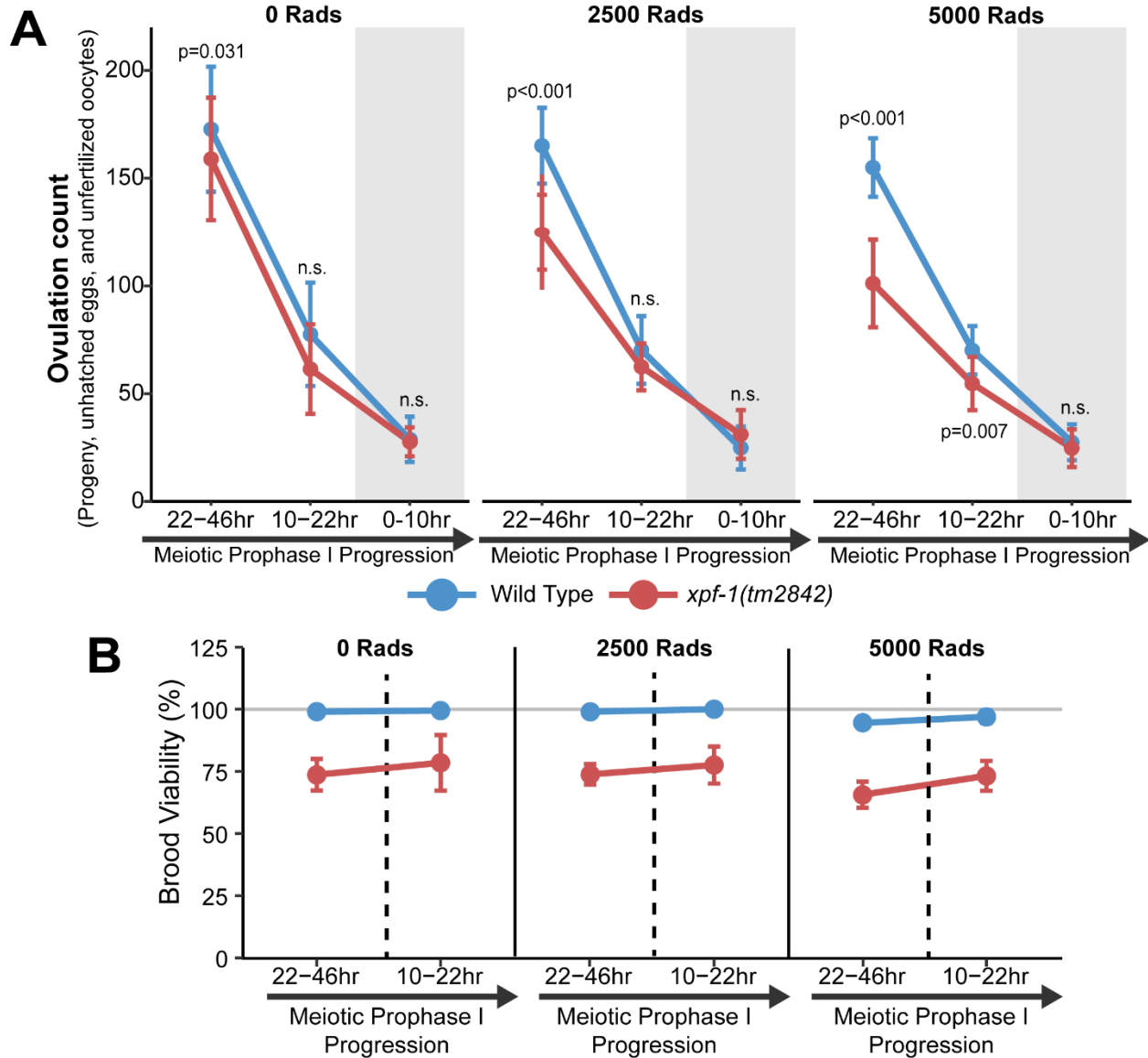
**Supplemental Information**

**Meiotic DNA break repair  
can utilize homolog-independent  
chromatid templates in *C. elegans***

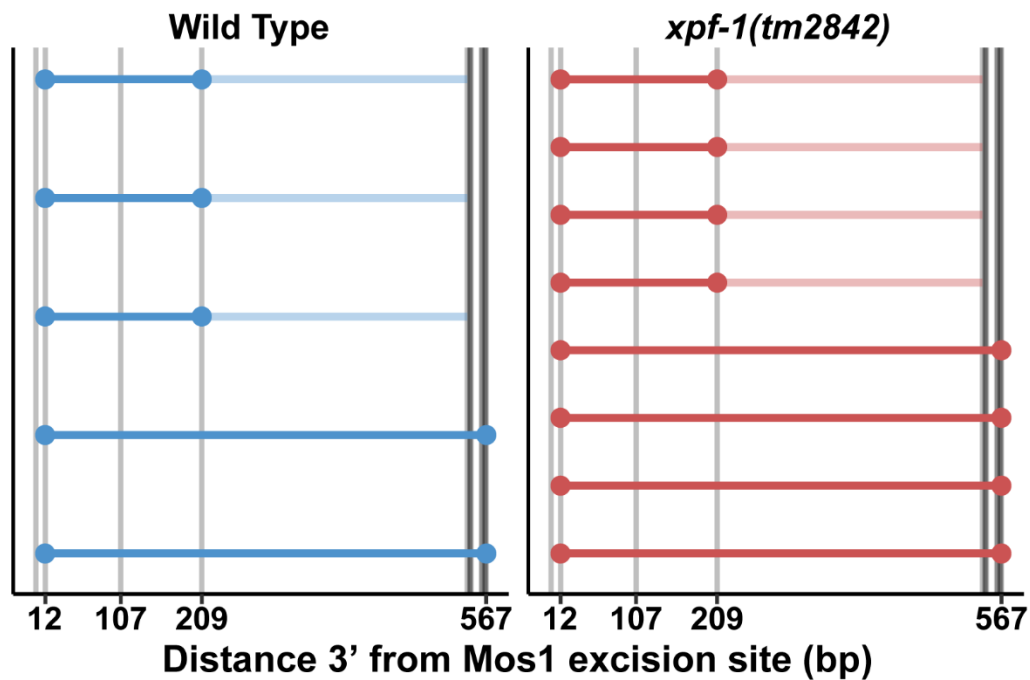
**Erik Toraason, Anna Horacek, Cordell Clark, Marissa L. Glover, Victoria L. Adler, Tolkappiyan Premkumar, Alina Salagean, Francesca Cole, and Diana E. Libuda**



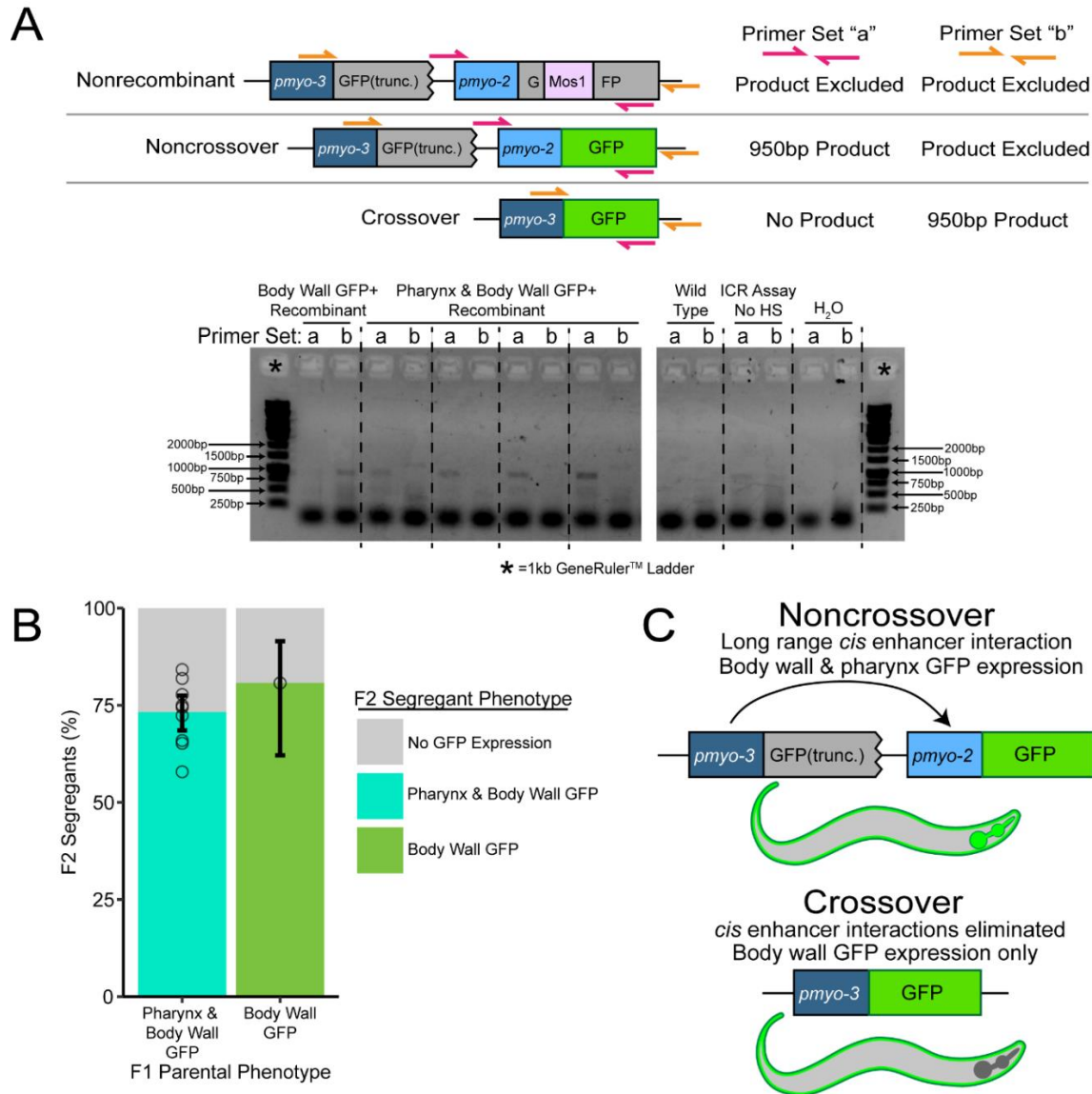
**FIGURE S1. Confirmation of single copy integration of the intersister/intrachromatid repair (ICR) assay. Related to Figure 1.** (A) Alternate mechanism for Figure 1A depicting DSB repair in the ICR assay by intrachromatid repair. If intrachromatid repair is engaged to resolve the DSB, the resected DNA strand would invade the GFP(trunc.) sequence upstream of the DSB within the damaged chromatid. (B) Diagram of the predicted ICR assay insertion targeted to *unc-5* on Chromosome IV of the *C. elegans* genome and schematics of possible single and tandem insertions of the ICR assay and PCR primer locations for detecting these possibilities. The grey boxes depict the upstream and downstream sequence used to target the ICR assay for integration at the *unc-5* locus, while the black arrow represents the ICR cassette. Primer 1 is a forward primer in the 3' end of the left homology arm on the *C. elegans* genome, and Primer 2 is a reverse primer positioned in the 5' end of the ICR cassette. Similarly, Primer 3 is a forward primer at the 3' end of the ICR cassette, while Primer 4 is a reverse primer positioned towards the 5' end of the right homology arm. Together, Primers 1+2 (Set 'A') and Primers 3+4 (Set 'B') span the genome-cassette junction on the 5' and 3' end of the cassette, respectively. Set 'A' amplicon's expected size is 596bp while set 'B' is 593bp. Reaction sets with primer(s) added in each set are detailed in the schematic table on the right. (C,D) PCR confirmation that ICR assay construct is integrated once with correct orientation at *unc-5*. PCRs were performed in duplicates with the indicated primer sets and extension times to detect any evidence of tandem insertions  $\leq 10$ kb apart. Irrespective of the amplification duration, only set A or B produced a product and the observed product is consistent with the expected size (~600bp). None of the single primers amplified a product. One non-specific band is sporadically observed (red asterisk). Thus, the ICR assay is likely inserted as a single copy and in the correct orientation.



**FIGURE S2. Ovulation rate and brood viability of wild type and *xpf-1* following ionizing radiation treatment. Related to Figure 4.** Reproductive statistics of  $n=15$  parent hermaphrodites of each respective genotype were scored for each irradiation treatment dose. (A) Mean ovulation counts (living progeny, unhatched eggs, and unfertilized oocytes) of young adult hermaphrodites exposed to 0, 2500, or 5000 Rads of ionizing radiation. Ovulation counts of  $n=15$  parent hermaphrodites of each respective genotype were scored for each irradiation treatment dose. Shaded timepoints indicate timepoints not included in the interhomolog or non-interhomolog windows. Error bars represent standard deviations.  $p$  values were calculated by Mann-Whitney U test. (B) Mean brood viability of young adult hermaphrodites exposed to 0, 2500, or 5000 Rads of ionizing radiation. Vertical dashed lines delineate between timepoints representing the interhomolog window (22-46 hrs) and timepoints representing the non-interhomolog window (10-22hrs). Error bars represent standard deviations.



**FIGURE S3. Intersister/intrachromatid crossover conversion tracts. Related to Figure 3.** Converted polymorphisms within ICR assay wild-type and *xpf-1(tm2842)* ICR assay crossover recombinant loci. Each horizontal line represents the sequenced locus of a single recombinant. High opacity lines connect contiguous converted polymorphisms within a single tract and represent minimum tract length, while the low opacity lines represent the range between converted and the most proximal non-converted polymorphism.



**FIGURE S4. Intersister/intrachromatid repair assay recombinant progeny expressing GFP in multiple tissues are likely single noncrossovers events. Related to Figure 1, STAR methods.** The design of the ICR assay predicts that recombinant progeny should exhibit either body wall or pharynx GFP expression (Figure 1A). However, the majority of pharynx GFP+ progeny also expressed GFP in the body wall. (A) PCR screening of recombinant progeny demonstrates expected crossover and noncrossover products in body wall and body wall + pharynx GFP expressing recombinants. ‘PCR product restricted’ indicates primer sets unable to amplify the given sequence based on the extension time of the PCR cycle. Wells in ethidium bromide stained agarose gel marked with asterisks were loaded with 1kb GeneRuler DNA ladder. (B) Both body wall and body wall + pharynx expression phenotypes segregate in ratios consistent with dominant Mendelian traits arising from a single locus (Chi Square Test of Goodness of Fit  $p \gg 0.05$  for both parental phenotypes). Error bars represent 95% Binomial confidence intervals, bars indicate proportion of segregants of each phenotype across all broods scored. Circles represent the proportion of segregants with respective phenotypes from the broods of individual F1 recombinant hermaphrodites scored. (C) Body wall + Pharynx GFP expression likely arises from known long-range enhancer activity between *myo-2* and *myo-3* promoter sequences<sup>S1</sup>.

<b>Recombination Frequency Counts of ICR Assay in Wild-type</b>					
Timepoint post Heat Shock	Total Progeny	Noncrossover (Intersister/ Intrachromatid)	Crossover (Intersister/ Intrachromatid)	% Recombinant Progeny (95% CI)	% Crossovers (95% CI)
10-22 hours	3317	19	7	0.78% (0.54-1.15%)	26.9% (13.7-46.1%)
22-34 hours	2372	17	2	0.80% (0.51-1.25%)	10.5% (2.9-31.4%)
34-46 hours	3032	16	1	0.56% (0.35-0.90%)	5.8% (1.0-27.0%)
46-58 hours	2159	12	1	0.60% (0.35-1.03%)	7.7% (1.4-33.3%)
58-70 hours	1190	8	1	0.76% (0.40-1.43%)	12.5% (2.0-43.5%)
Interhomolog Window (22-58 hours)	7563	45	4	0.65% (0.49-0.86%)	8.1% (3.2-19.2%)
Total	10880	64	11	0.69% (0.55-0.86%)	14.7% (8.4-24.4%)

<b>Recombination Frequency Counts of Interhomolog Assay</b>				
Timepoint post Heat Shock	Total Progeny	Total Recombinant Progeny (Crossover:Noncrossover/)	% Recombinant (95% CI)	% CO (95% CI)
10-22 hours	1625	2 (0:2)	0.12% (0.03-0.45%)	0% (0-65.8%)
22-34 hours	1989	51 (3:38)	2.56% (1.96%-3.36%)	7.3% (2.5-19.4%)
34-46 hours	1721	92 (8:81)	5.35% (4.38-6.51%)	8.9% (4.6-16.7%)
46-58 hours	1447	78 (5:68)	5.40% (4.34%-6.68%)	6.8% (3.0-15.1%)
58-70 hours	710	19 (2:15)	2.68% (1.72%-4.14%)	11.8% (3.3-34.3%)
Interhomolog Window (22-58 hours)	5157	221 (16:187)	4.29% (3.77-4.87%)	7.9% (4.9-12.4%)
Total	6782	223 (16:189)	3.29% (2.9-3.7%)	7.8% (4.9-12.3%)

**TABLE S1. Recombination frequency counts of ICR assay and Interhomolog assay performed in wild-type animals. Related to Figure 1.**

<b>Recombination Frequency Counts of <i>xpf-1(tm2842)</i> ICR Assay</b>					
Timepoint post Heat Shock	Total Progeny	Noncrossover (Intersister/ Intrachromatid)	Crossover (Intersister/ Intrachromatid)	% Recombinant (95% CI)	% CO (95% CI)
10-22 hours	2618	4	2	0.23% (0.11-0.50%)	33.3% (9.7-70%)
22-34 hours	1793	9	2	0.61% (0.34-1.1%)	18.2% (5.1-47.7%)
34-46 hours	2400	9	2	0.46% (0.26-0.82%)	18.2% (5.1-47.7%)
46-58 hours	1819	11	0	0.61% (0.39-1.1%)	0% (0-25.9%)
58-70 hours	813	4	0	0.49% (0.19-0.13%)	0% (0-49.0%)
Interhomolog Window (22-58 hours)	6012	29	4	0.55% (0.39-0.77%)	12.1% (4.8-27.3%)
Total	8630	33	6	0.45% (0.33-0.62%)	15.4% (7.2-29.7%)

**TABLE S2. Recombination frequency counts of ICR assay performed in *xpf-1(tm8242)* animals. Related to Figure 2.**

#### **SUPPLEMENTAL REFERENCES**

- S1. Okkema, P.G., Harrison, S.W., Plunger, V., Aryana, A., and Fire, A. (1993). Sequence Requirements for Myosin Gene Expression and Regulation in *Caenorhabditis elegans*. *Genetics* 135, 385–404.