

Differential RPA-1 and RAD-51 recruitment *in vivo* throughout the *C. elegans* germline, as revealed by laser microirradiation

Supplementary Figure Legends, and Tables with Table Legends

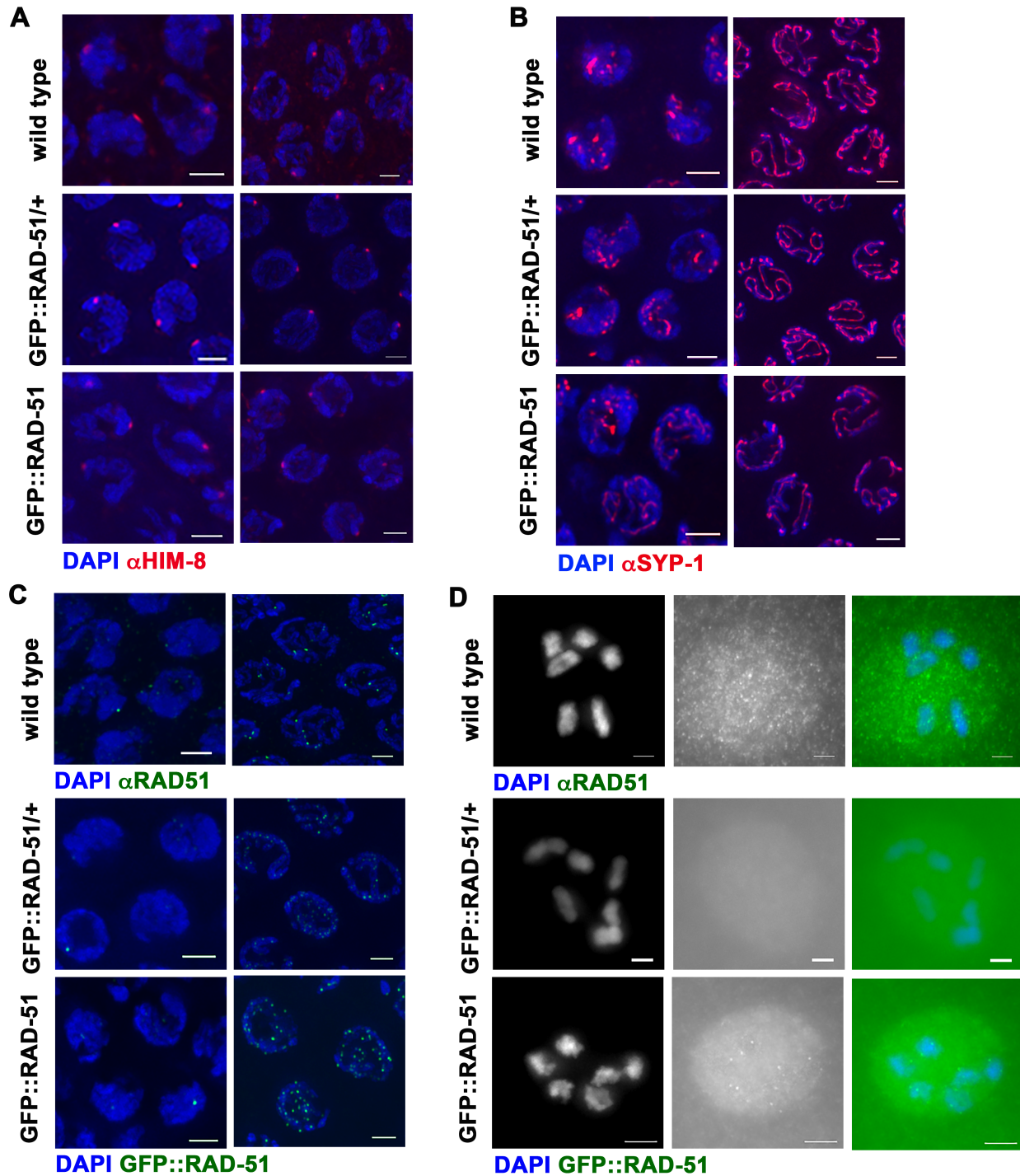


Figure S1. GFP::RAD-51 does not effect early meiotic events. (A) HIM-8 localization in wild type, GFP::RAD-51/+ (GFP::RAD-51/nT1) heterozygotes, and GFP::RAD-51 homozygotes. HIM-8 foci are paired at meiotic entry (TZ, left) and remain paired in pachytene (MP example right), (B) SYP-1 localization in wild type, GFP::RAD-51/+ (GFP::RAD-51/nT1) heterozygotes, and GFP::RAD-51 homozygotes. SYP-1 initiates synapsis at meiotic entry (TZ, left) and chromosomes are fully synapsed at pachytene (MP example, right), (C) RAD-51 localization in wild type, GFP::RAD-51/+ (GFP::RAD-51/nT1) heterozygotes, and GFP::RAD-51

homozygotes. Few RAD-51 foci are observed at meiotic entry (TZ, left) in all genotypes and increase at MP example (right), **D**) RAD-51 localization in wild type, GFP::**RAD-51**/+ (GFP::**RAD-51**/nT1) heterozygotes, and GFP::**RAD-51** homozygotes at diakinesis -1. Few RAD-51 foci are observed at GFP::**RAD-51** homozygotes, but are completely absent from the other genotypes. Bivalent morphology I distorted at GFP::**RAD-51** homozygotes. Scale bar for all: 2 μ .

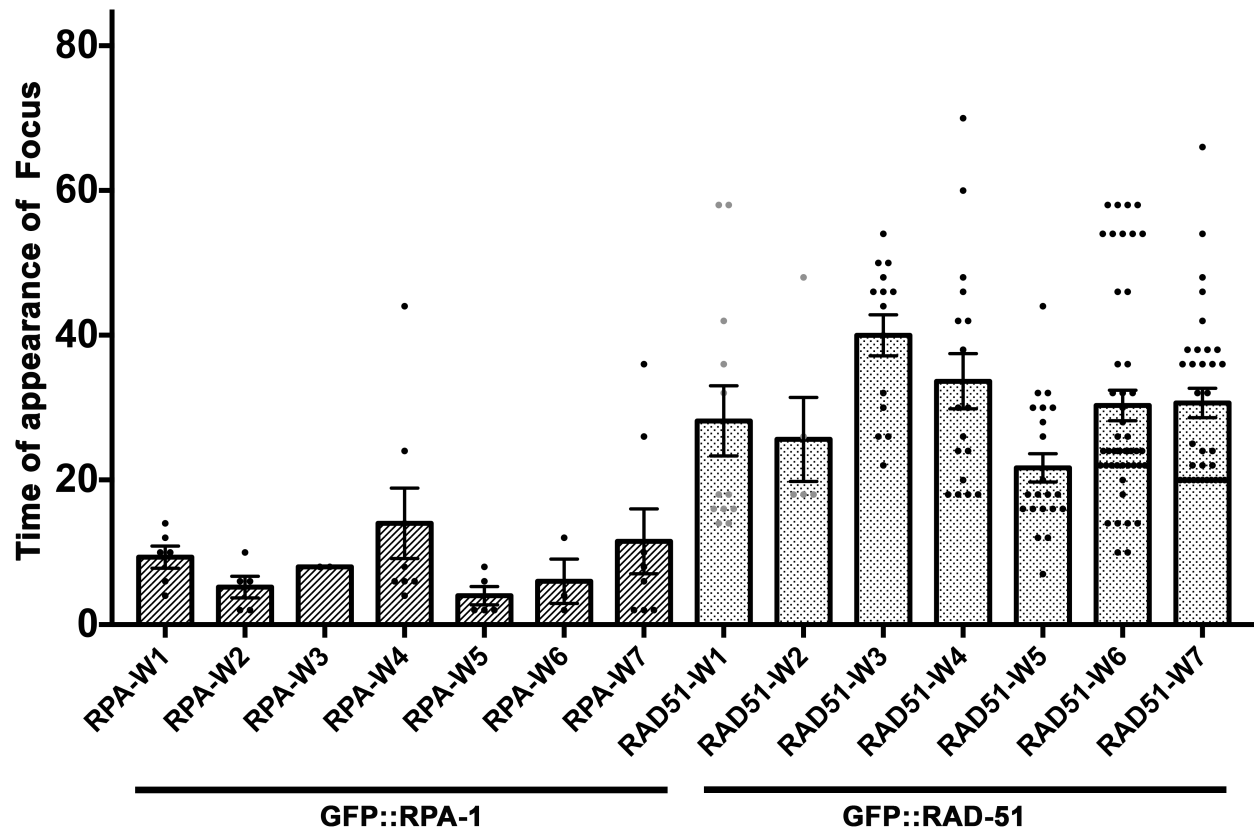


Figure S2. Variability between worms in microirradiation experiments. The data in figure 1C (15%) and IF (15%) represented for individual worms.

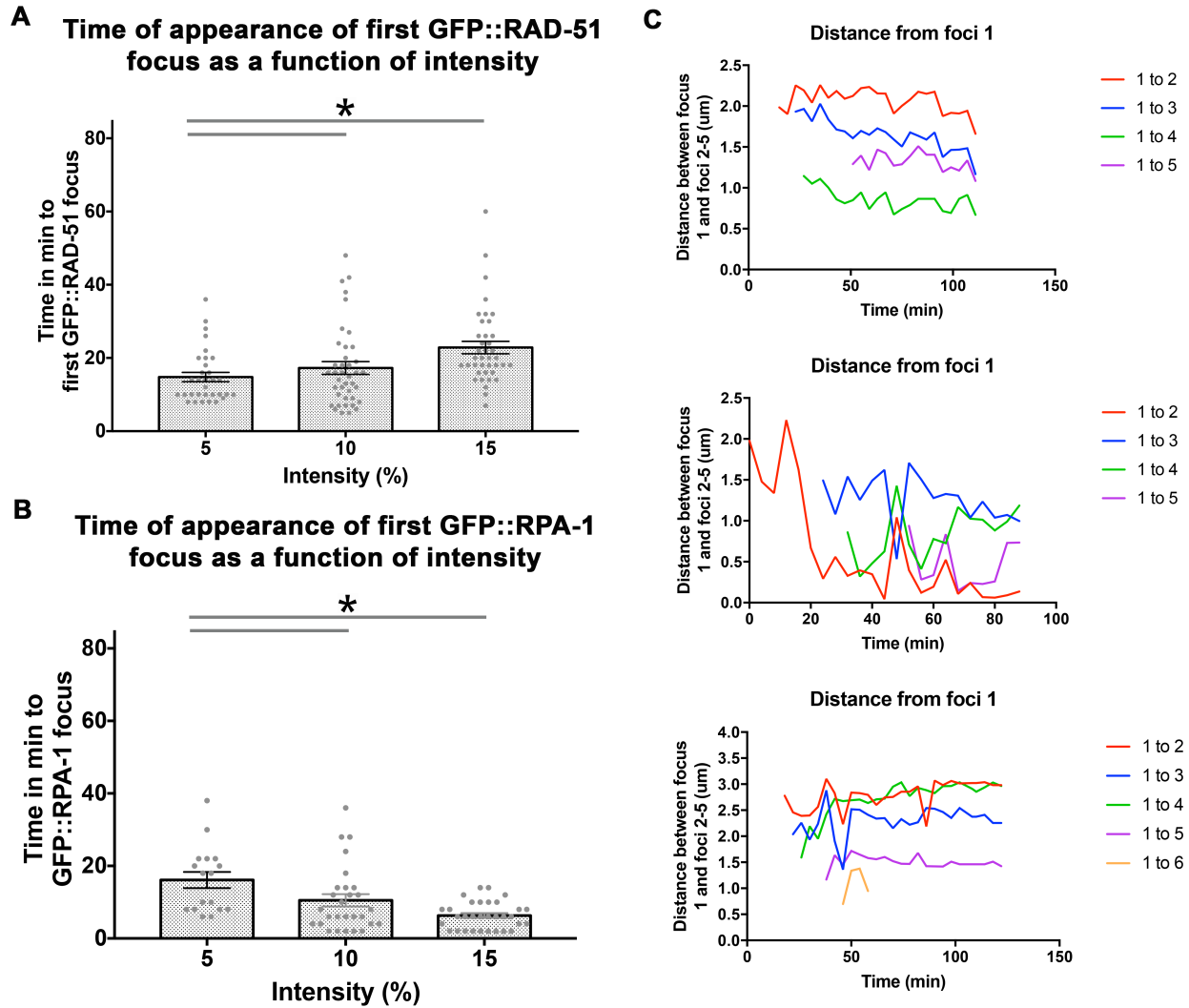


Figure S3. Appearance of GFP::RAD-51 foci after induction of a DSB via microirradiation. (A) Each data point represents the first focus generated in a nucleus for each of the corresponding intensities. Time of appearance (in minutes) of the first GFP::RAD-51 focus that appeared in each nucleus analyzed as a function of the pulse intensity applied. n foci SCN-PMT=23, FM-PMT=34, TZ=37, MP=32, LP=38. (B) Same analysis for GFP::RPA-1. n foci SCN-PMT=38, FM-PMT=39, TZ=43, MP=39, LP=46. (C) Movement of foci within two different mid-pachytene nuclei was quantified by measuring the distance (in μm) between each focus and the focus designated focus number one every four minutes through experiment run-time. Examples are for FM-PMT (top), TZ (middle) and MP (bottom).

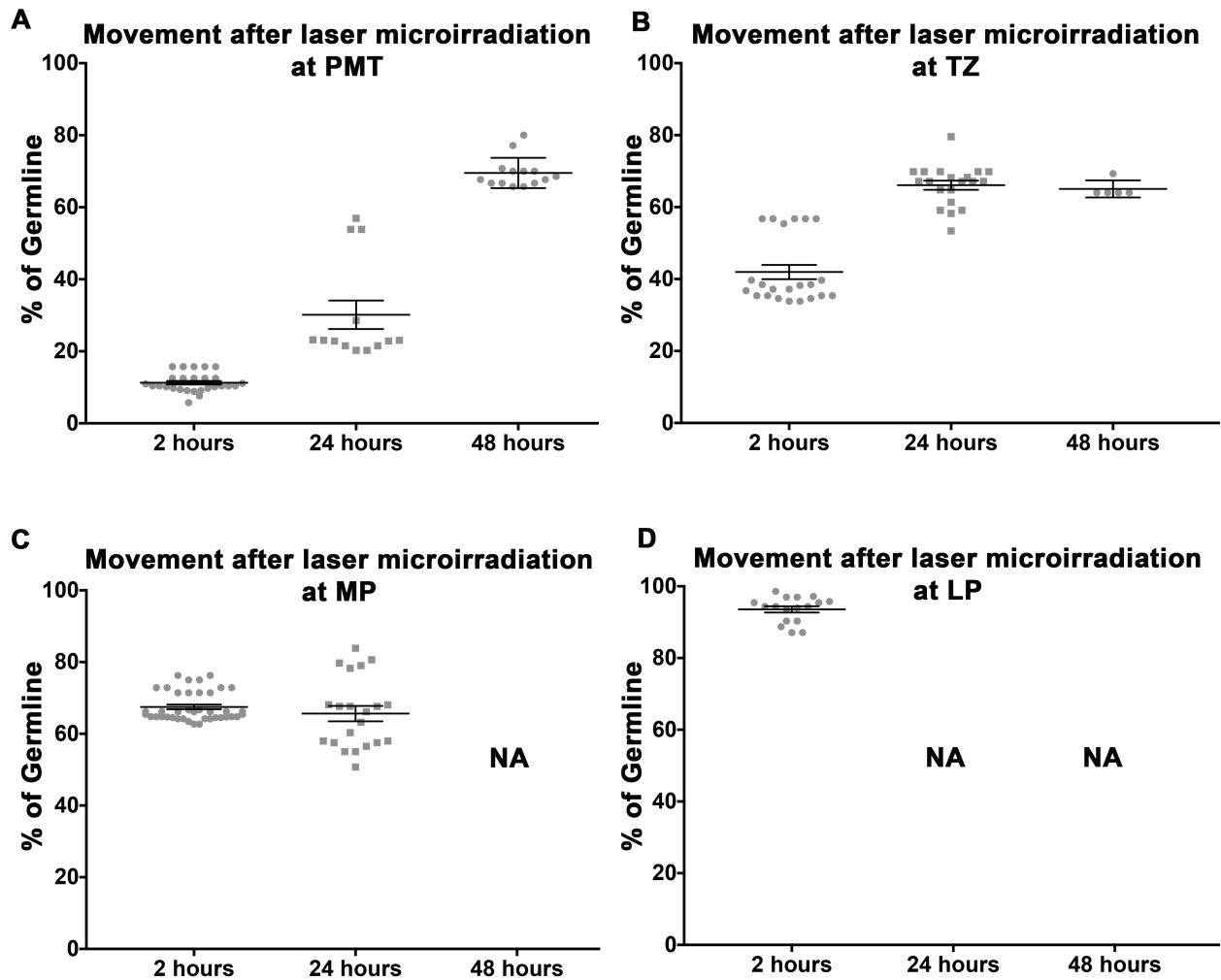


Figure S4. Movement of irradiated nuclei through the germline over time. Post-IR, worms were dissected at three different time points and foci were counted throughout the gonad. **(A, B, C, and D)** Each data point represents a focus in all four graphs. Foci were plotted as a function of the percentage of the gonad that they were located in with 1% being closest to the distal tip cell and 100% being closest to the last row in pachytene. The data indicates movement of nuclei that were irradiated in PMT, TZ, MP, and LP which correspond to A, B, C, and D, respectively, at the three chosen time points.

	0	1	5	10	15
1	>0.9999				
5	<0.0001 ***	<0.0001 ***			
10	<0.0001 ***	<0.0001 ***	0.0555		
15	<0.0001 ***	<0.0001 ***	0.5611	0.2991	
n nuclei	171	30	34	35	42
Mean	0.0117	0	0.5294	0.8857	0.881
Std. Deviation	0.1078	0	0.5633	0.5827	0.5927
Std. Error of Mean	0.0082	0	0.0966	0.0985	0.0915

Table S1. Number of GFP::RPA-1 foci as a function of intensity. All pairwise comparisons performed using the two-tailed Mann-Whitney test. “n nuclei” indicates the number of nuclei that were irradiated at the corresponding intensity. Standard deviation and standard error of mean are provided as well.

	5	10	15
10	0.0723		
15	0.0015**	0.2006	
n foci	18	31	37
Mean	15.78	12	9.189
Std. Deviation	9.046	9.947	9.327
Std. Error of Mean	2.132	1.786	1.533

Table S2. Time of appearance of GFP::*RPA-1* focus as a function of intensity. All pairwise comparisons performed using the two-tailed Mann-Whitney test. “n foci” indicates the number of foci that were generated by the corresponding pulse intensity, and which measurements of appearance time were taken. 1% pulse intensity was intentionally left out given that this intensity did not generate any foci.

	0	1	5	10	15
1	0.0622				
5	<0.0001 ***	<0.0001 ***			
10	<0.0001 ***	<0.0001 ***	<0.0001 ***		
15	<0.0001 ***	<0.0001 ***	<0.0001 ***	0.4229	
n nuclei	220	30	40	40	41
Mean	0.0318	0.3667	1.225	3.875	3.634
Std. Deviation	0.2002	0.6149	0.8619	2.544	2.844
Std. Error of Mean	0.0135	0.1123	0.1363	0.4022	0.4441

Table S3. Number of GFP::RAD-51** foci as a function of intensity.** All pairwise comparisons performed using the two-tailed Mann-Whitney test. “n nuclei” indicates the number of nuclei that were irradiated at the corresponding intensity. Standard deviation and standard error of mean are provided as well.

	5	10	15
10	<0.0001 ***		
15	<0.0001 ***	0.5066	
n foci	49	152	149
Mean	19.04	30	30.13
Std. Deviation	12.24	17.65	13.78
Std. Error of Mean	1.749	1.431	1.129

Table S4. Time of appearance of GFP::*RAD-51* focus as a function of intensity. All pairwise comparisons performed using the two-tailed Mann-Whitney test. “n foci” indicates the number of foci that were generated by the corresponding pulse intensity, and which measurements of appearance time were taken. 1% pulse intensity was intentionally left out given that this intensity only generated 2 foci, an n value too low for accurate statistical inclusion.

	0 to 1	1 to 2	2 to 3	3 to 4	4 to 5
1 to 2	0.0028				
2 to 3	<0.0001***	0.2174			
3 to 4	<0.0001***	0.0019**	0.0058**		
4 to 5	<0.0001***	0.0002**	0.0011**	0.0747	
n foci	62	30	28	19	9
Mean	22.08	29.8	32.93	41.47	51.33
Std. Deviation	8.959	12.18	12.12	11.23	13.08
Std. Error of Mean	1.138	2.223	2.29	2.577	4.359

Table S5. Time of appearance of next GFP::*RAD-51* focus as a function of order of appearance. All pairwise comparisons were performed using the two-tailed Mann-Whitney test. “n foci” indicates the number of foci that were either the first (0 to 1), second (1 to 2), third (2 to 3) and so on in that category of order of appearance.

Genotype	<i>GFP::<i>rpa-1</i></i>		<i>GFP::<i>rpa-1</i></i>		<i>GFP::<i>rad-51</i></i>	
Condition	wild type	<i>rad-51(ok2218)</i>	pL4440	<i>rad-51(RNAi)</i>	pL4440	<i>rpa-1(RNAi)</i>
p value	0.0979		0.2690		<0.0001***	
n nuclei	42	37	29	29	40	30
Mean	0.881	0.6486	1	0.8966	3.425	0.7
Std. Deviation	0.5927	0.484	0.378	0.5571	1.947	1.343
Std. Error of Mean	0.09146	0.07957	0.07019	0.1034	0.3078	0.2452

Table S6. Number of nuclei with GFP::*RPA-1* or GFP::*RAD-51* foci for RNAi and knockout experiments. The genotype indicates the strain that was subjected to the two conditions directly below, and the foci that were examined in that particular experiment. “n nuclei” indicates the number of nuclei that were irradiated in each of the corresponding experiments. P-values were calculated using the two-tailed Mann-Whitney test.

Genotype	<i>GFP::<i>rpa-1</i></i>		<i>GFP::<i>rpa-1</i></i>		<i>GFP::<i>rad-51</i></i>	
Condition	wild type	<i>rad-51(ok2218)</i>	pL4440	<i>rad-51(RNAi)</i>	pL4440	<i>rpa-1(RNAi)</i>
p value	0.458		0.6106		0.1013*	
n foci	35	25	29	26	128	26
Mean	7.771	7.36	8.828	10.19	35.33	27.65
Std. Deviation	6.924	8.056	6.291	7.767	18.68	21.2
Std. Error of Mean	1.17	1.611	1.168	1.523	1.651	4.158

Table S7. Time of appearance of GFP::*RPA-1* or GFP::*RAD-51* foci in RNAi and knockout experiments. Genotype indicates strain subjected to the two conditions directly below, as well as the foci that were generated in that specific experimental condition. “n foci” indicates the number of foci that were produced after irradiation under each experimental condition. P-values were calculated using the two-tailed Mann-Whitney test.

	SCN-PMT	FM-PMT	TZ	MP	LP
FM-PMT	0.1309				
TZ	0.1474	>0.9999			
MP	0.055	0.5605	0.5648		
LP	0.0005**	0.0274*	0.0232*	0.124	
n nuclei	37	45	50	42	43
Mean	0.6216	0.8	0.82	0.881	1.07
Std. Deviation	0.4917	0.5045	0.5956	0.5927	0.5519
Std. Error of Mean	0.0808	0.0752	0.0842	0.0915	0.0842

Table S8. Number of GFP::RPA-1 Foci/nucleus by stage.** Specific stages include mitotic nuclei (G2/S and G2) as well as meiotic nuclei (TZ, MP, and LP). “n nuclei” indicates the number of nuclei that were irradiated. All pairwise comparisons of number of nuclei irradiated with foci were performed using the two-tailed Mann-Whitney test.**

	SCN-PMT	FM-PMT	TZ	MP	LP
FM-PMT	0.2638				
TZ	0.0377*	0.0003**			
MP	0.451	0.0344*	0.013*		
LP	0.6106	0.0607	0.071	0.7928	
n foci	23	36	41	37	46
Mean	11.3	15.31	9.756	9.189	11.65
Std. Deviation	9.431	12.93	17.17	9.327	12.85
Std. Error of Mean	1.967	2.155	2.682	1.533	1.895

Table S9. Time of appearance of GFP::RPA-1** focus by stage.** Specific stages include mitotic nuclei (G2/S and G2) as well as meiotic nuclei (TZ, MP, and LP). “n foci” indicates the number of foci that were generated after irradiation in each of the corresponding stages. All pairwise comparisons of number of foci irradiated foci were performed using the two-tailed Mann-Whitney test.

	SCN-PMT	FM-PMT	TZ	MP	LP
FM-PMT	0.5029				
TZ	0.1502	0.0134*			
MP	0.0081**	0.0012**	0.1915		
LP	0.7634	0.7517	0.0218*	0.0004**	
n foci	100	118	158	149	155
Mean	26.52	25.31	28.95	30.13	24.75
Std. Deviation	16.92	16.76	15.62	13.78	13.78
Std. Error of Mean	1.692	1.543	1.243	1.129	1.106

Table S10. Time of appearance of GFP::*RAD-51* focus by stage. Specific stages include mitotic nuclei (G2/S and S) as well as meiotic nuclei (TZ, MP, and LP). “n foci” indicates the number of foci that were generated after irradiation in each of the corresponding stages. All pairwise comparisons were performed using the two-tailed Mann-Whitney test.

Movie S1

GFP::RPA-1 spo-11 recording. The movie contains 45 (46 total including the frame prior to irradiation) frames taken every 2 minutes following microirradiation. 6 nuclei were irradiated

Movie S2

GFP::RAD-51 spo-11 recording. The movie contains 8 frames taken every 6 minutes. Frame 1 before microirradiation, the others following microirradiation. 7 nuclei were irradiated

Movie S3

GFP::RAD-51 spo-11 recording. The movie contains 8 frames taken every 6 minutes. No nuclei were irradiated





