PNAS
www.pnas.org

Supplementary Information for

Rhizobium induces DNA damage in _Caenorhabditis elegans_ intestinal cells.

Marina Kniazeva and Gary Ruvkun

Marina Kniazeva
Email: kniazeva@molbio.mgh.harvard.edu

This PDF file includes:

Figs. S1 to S10
Tables S1
Captions for movies S1 to S3

Other supplementary materials for this manuscript include the following:

Movies S1 to S3

www.pnas.org/cgi/doi/10.1073/pnas.1815656116

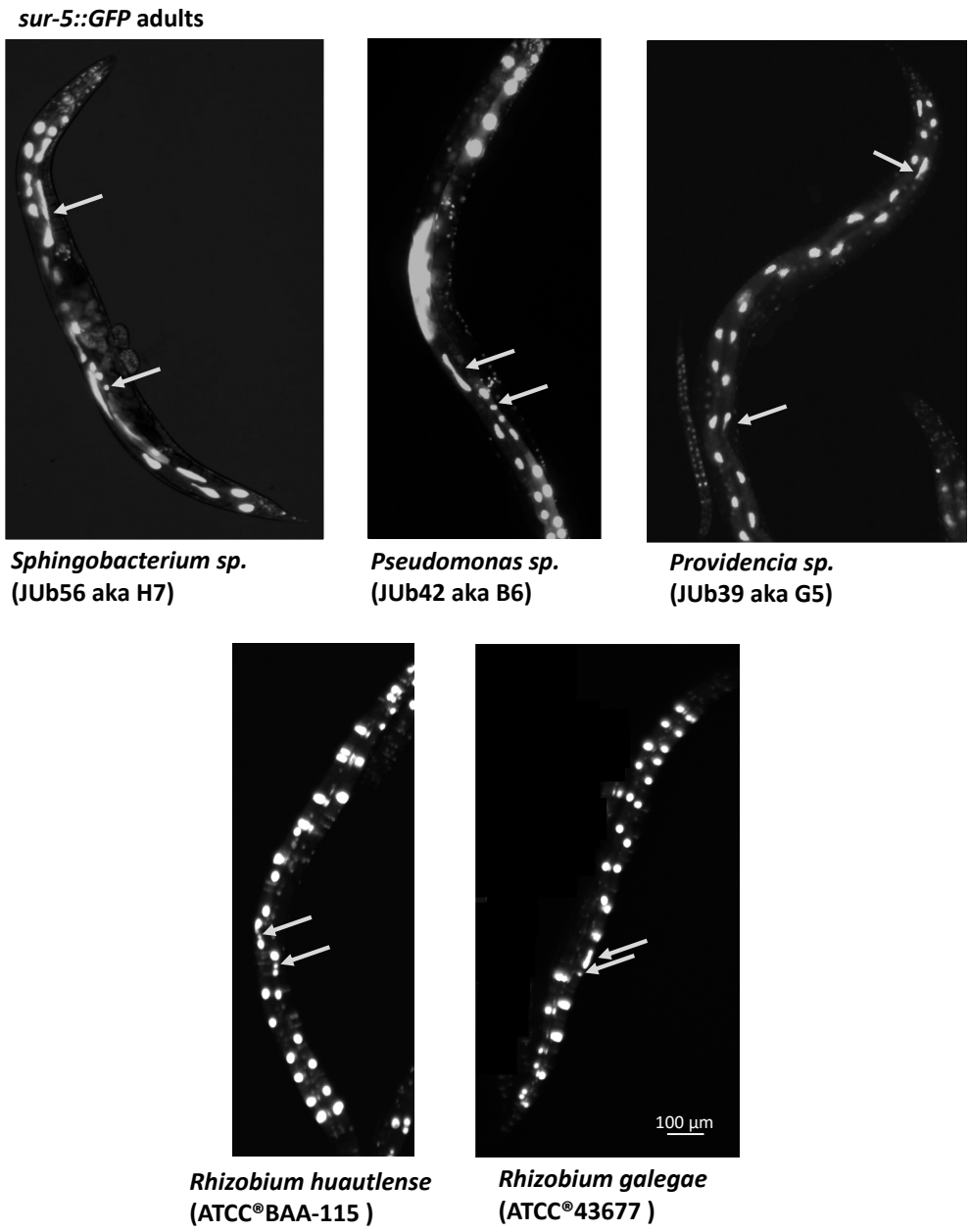


Fig. S1. **Wild bacterial isolates from the JUb library** (5, 7) **and *Rhizobium* species from ATCC collection alter karyokinesis in the *C. elegans* intestinal cells**. Fluorescence images of *sur-5::GFP C. elegans* fed on the indicated bacteria. Arrows point to abnormally shaped nuclei which are elongated or fragmented.

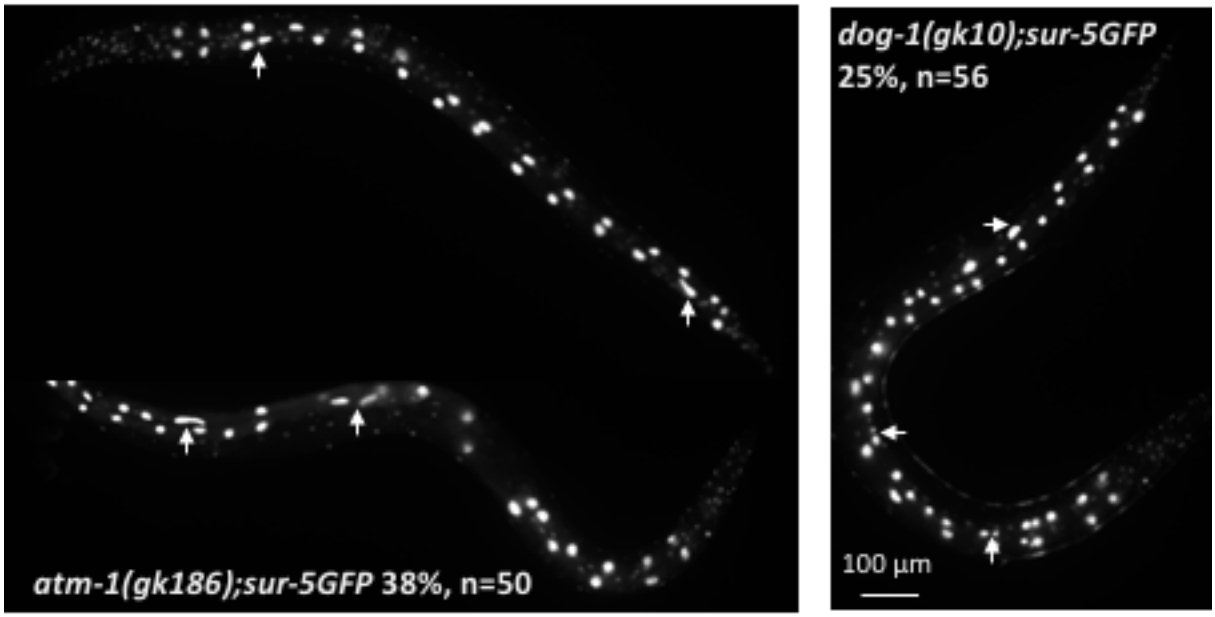


Fig. S2. ***C. elegans*** **mutants from Caenorhabditis Genetic Center (CGC),** ***atm-1 and dog-1,*** **crossed with** ***sur-5::GFP*** **reporter strain show abnormal karyokinesis in the gut.** Bright light and fluorescence images of the standard mutant alleles for these genes obtained from the *Caenorhabditis* Genetics Center (CGC). Arrows point to the elongated and double nuclei.

A

RNAi target gene	cosmid name	% affected worms	n, scored
control L4440 empty vector		8	80
Double strand break repair			
mus-101	F37D6.1	36	105
rad-51	Y43C5A.6	28	83
Chromatid cohesion/Chromatin factors			
lin-40 (former erg-1)	T27C4.4	72	91
pgn-85	Y43H11AL.3	6	80
Mitosis/Meiosis			
ani-2	K10B2.5	10	80
Protein degradation			
wwp-1	Y65b4br.4	30	44
RNA processing and trafficking			
npp-15	C29E4.4	6.25	
nola-3 (RNP complex)	C25A1.6	89	18
other			
GMP reductase	F32D1.5	7	27
sex-1	F44A6.2	44	78

P<0.00001

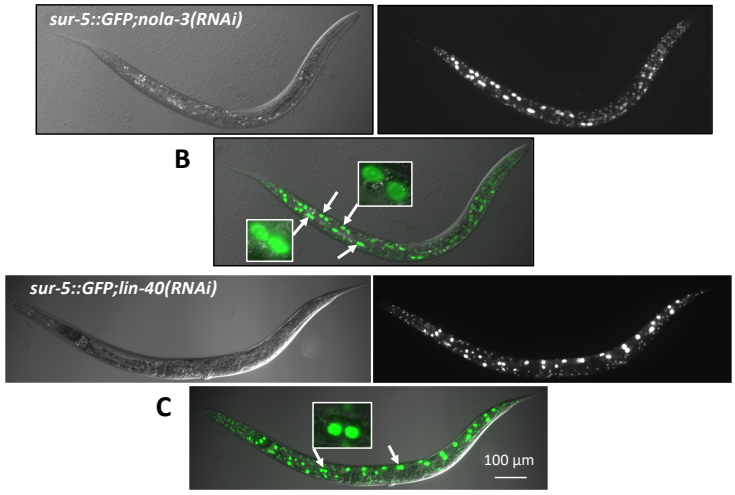
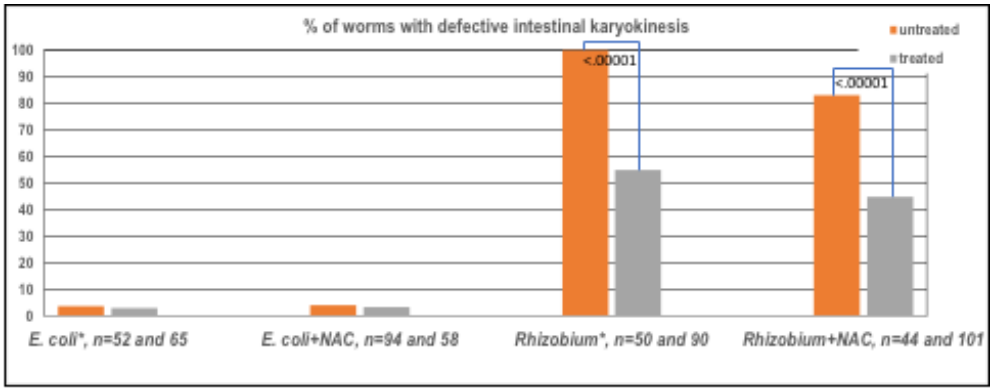
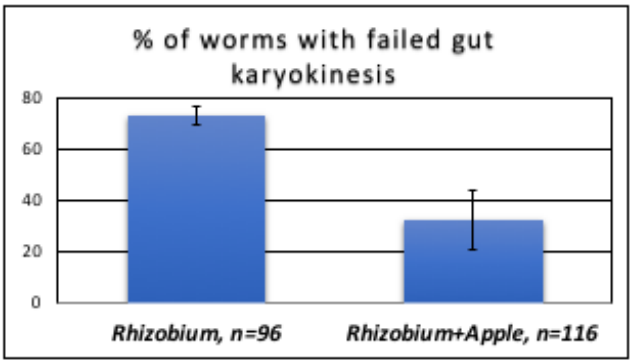


Fig. S3 **Inhibition of DDR genes representing several pathways by RNAi results in abnormal karyokinesis in gut.**

(A) Table showing tested RNAi target genes from the list of genes required for the normal DNA damage response (from van Haaften et al, 2006) and percent of *C. elegans* with affected karyokinesis in gut. The inhibition of genes in blue rows resulted in statistically significant (P<0.00001) increase in a number of *C. elegans* with abnormal karyokinesis. (B and C) Bright light, fluorescence and merged images illustrating failed karyokinesis in the gut of *nola-3(RNAi)* and *lin-40(RNAi)*. Arrows and inserts show elongated and double nuclei indicative of abnormal karyokinesis.



A



B

Fig. S4. **Suppression of *Rhizobium* gut karyokinesis defect by free radical scavengers** (A) Graph shows % of *C. elegans* with defective intestinal karyokinesis. Eggs (untreated control) or L1 hatched in 5 mM NAC (treated sample) were placed on *E. coli* or *Rhizobium* with and without NAC supplement. P values for untreated vs. treated *C. elegans* on *Rhizobium* without and with NAC supplement were 4.1E-05 and 1E-05. *- 1 ml of water, a solvent for 1 ml NAC, was added to *E. coli* and *Rhizobium* lawns as a control. (B) Graph shows % of *C. elegans* with altered gut nuclei phenotype, which developed either on *Rhizobium* lawn or *Rhizobium* lawn supplemented with a fresh apple slice.

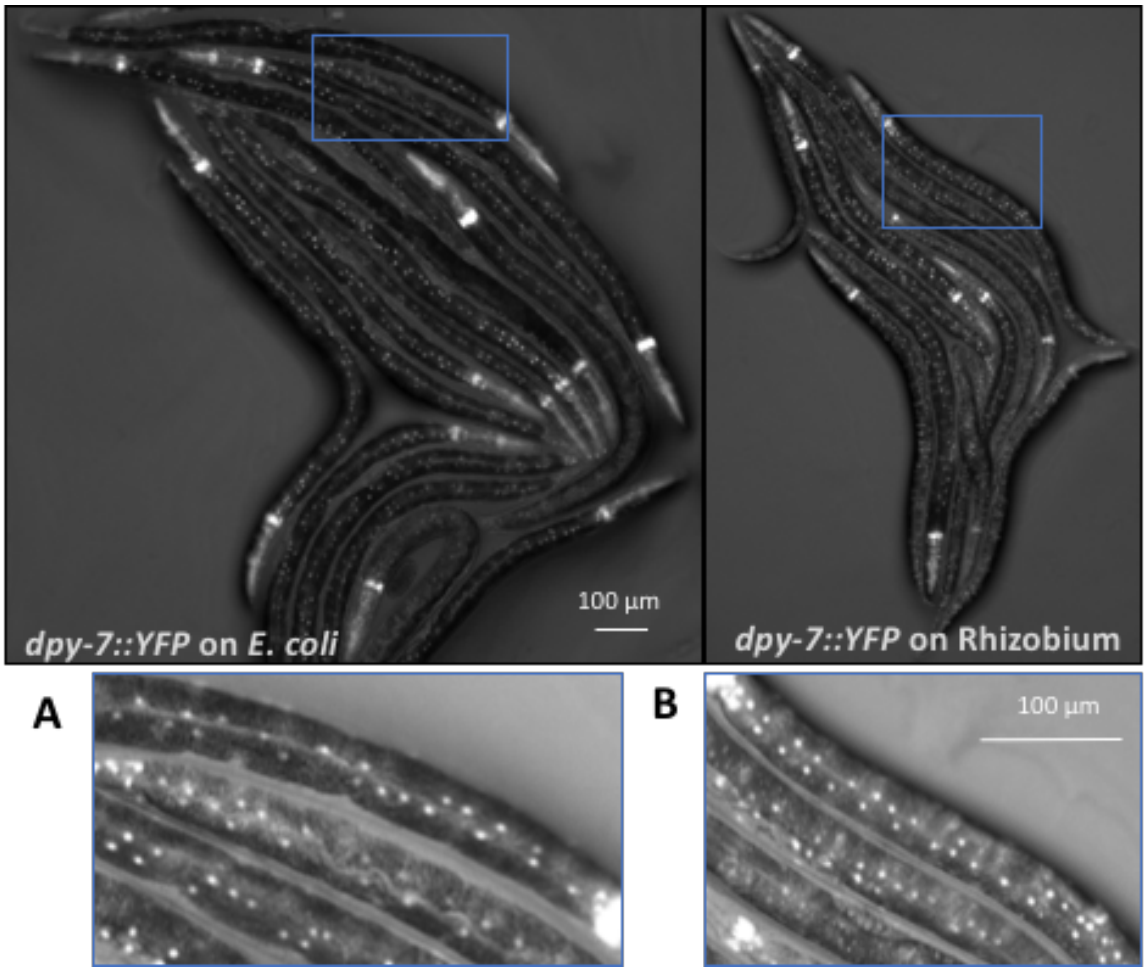


Fig. S5. **Karyokinesis of *C. elegans* hypodermal nuclei is not affected by feeding on *Rhizobium*.**

(A and B) Fluorescence images of *C. elegans* expressing *dpy-7p::2Xnls::YFP* reporter in hypodermal cells grown on *E. coli* and *Rhizobium*. Inserts illustrate similar shapes and distribution patterns of hypodermal nuclei in *C. elegans* fed with either bacteria.

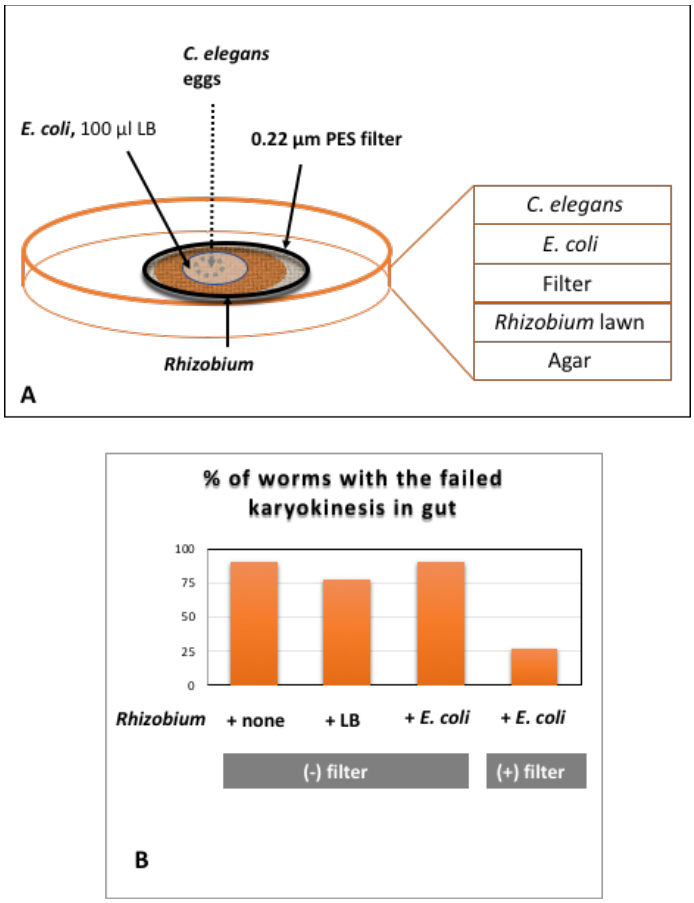
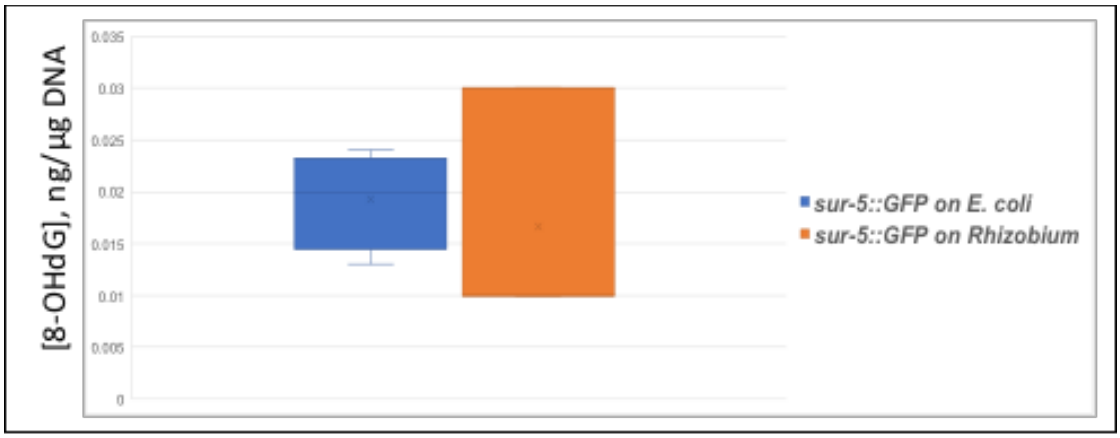


Fig S6. **A close contact between *Rhizobium* cells and *C. elegans* is required for intestinal karyokinesis defects.**

(A) An experimental setup to separate *Rhizobium* cells and *C. elegans* on NGM agar plates covered the *Rhizobium* lawn with a 0.22 µm pore-size filter. An aliquot of *E. coli* suspension is added on the top of the filter to initiate postembryonic growth of *C. elegans*. (B) Graph shows percentage of *C. elegans* with defective karyokinesis under the indicated conditions. The *C. elegans* were maintained from the time of hatching on an untreated *Rhizobium* lawn, or the lawn treated with 100 µl liquid LB media or 100 µl suspension of *E. coli*, or 100 µl of suspension of *E. coli* on top of a filter over the *Rhizobium* lawn, n>50. The insert shows a vertical arrangement of layers on a plate.

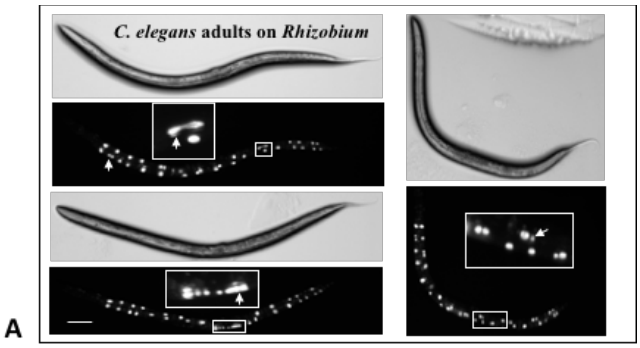


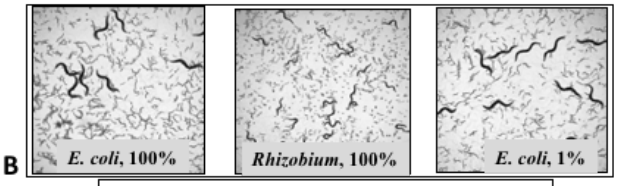
Concentration of [8-OHdG], ng/µg DNA

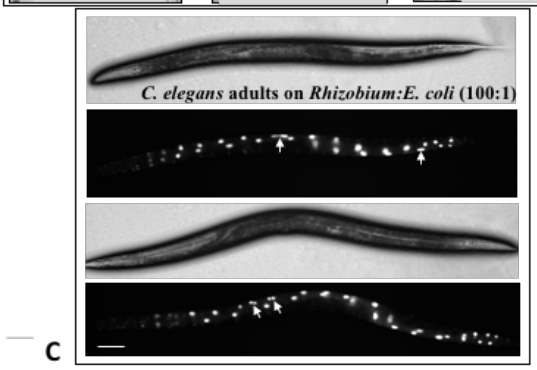
Replicates	*sur-5::GFP* on *E. coli*	*sur-5::GFP* on *Rhizobium*
#1	0.013	0.03
#2	0.019	0.01
#3	0.021	0.01
#4	0.024	n/a

Fig. S7. **No significant changes in 8-OHdG levels were detected in the chromosomal DNA isolated from *C. elegans* grown on *E. coli* and *Rhizobium*.**

Graph shows concentration of 8-OHdG per 1 µg of DNA. The data was obtained with OxiSelect Oxidative DNA damage ELISA Kit. The data shown on the graph are detailed on a list below.





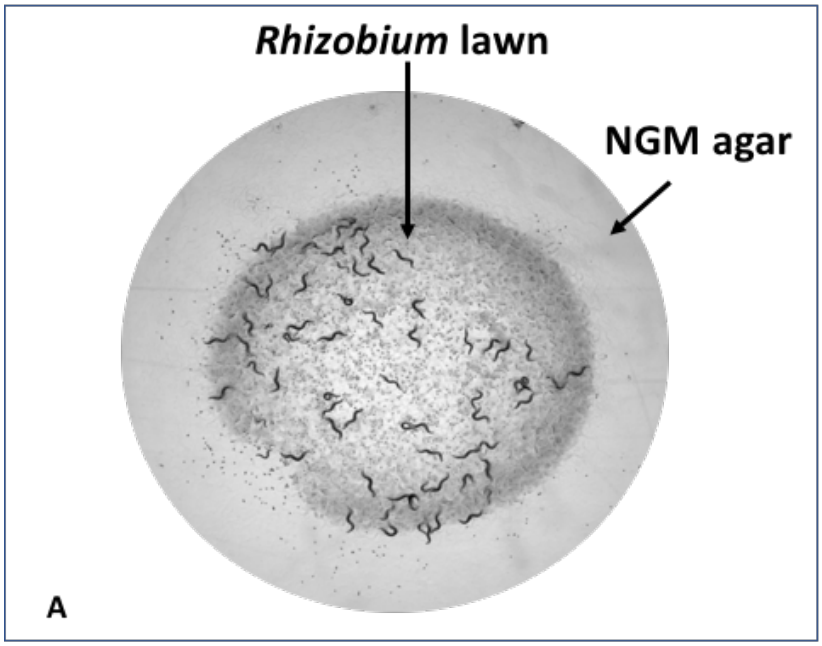


D

Eggs plated	% of affected worms	n,	Body size
E. coli , 100%	5.6	82	wt
Rhizobium:*E.coli*			
1:10	31	180	wt
1:1	41	148	wt
10:1	41	214	wt
100:1	55	125	wt
Rhizobium, 100%	79	76	small
L3 from Rhizobium on *E.coli*	75	50	wt

Fig. S8. **Small body size of *C. elegans* fed on *Rhizobium* is not correlated with the karyokinesis defect.**

(A-C) Fluorescence, DIC, and dissecting scope images of adult *C. elegans* grown on *E. coli* and *Rhizobium*. (A) Upper DIC images show scrawny phenotype of *C. elegans* fed on *Rhizobium*. The inserts on fluorescence images show elongated, double and fragmented nuclei of the gut in *C. elegans* grown on *Rhizobium* (arrows). The scrawny phenotype, but not abnormal gut nuclei morphology can be rescued with *E. coli* mixed with the *Rhizobium* (B-D).



	# with abnormal gut nuclei	number on lawn	n,
E. coli	2	35	52
Rhizobium	69	95	100

B

Fig. S9. ***C. elegans* do not avoid *Rhizobium* lawn.**

(A) Image of an NGM plate with *C. elegans* grown on 10x concentrated *Rhizobium* lawn from eggs. (B) Quantification of the phenotype on the *E. coli* and *Rhizobium* lawn.

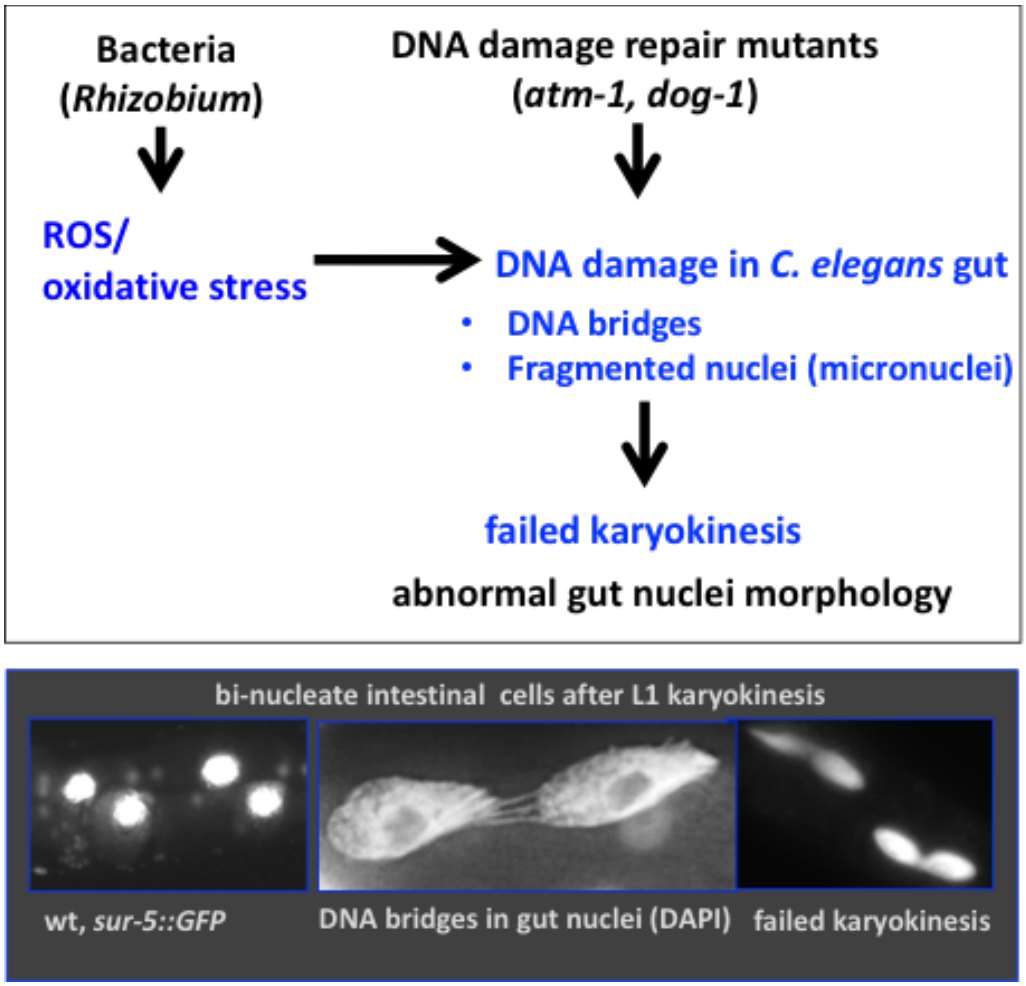


Fig. S10. **Proposed mechanism of *Rhizobium*-induced DNA damage, failed karyokinesis resulting in abnormal gut nuclei patterning.**

The scheme (A) explains how the abnormal *sur-5::GFP*-highlighted gut nuclei phenotype (B) can serve as a readout in screens for genes involved in oxidative stress and DNA damage response and repair.

Table S1. **Evaluation of aberrant karyokinesis phenotype in C. elegans wild type and mutants fed on various bacteria under indicated conditions.**

Feeding Bacterial Strains	Worm strains	Treatment*	Average % affected worms	Number of scored worms	Number of affected gut nuclei per worm
E. coli	*sur-5::GFP*	none	4	>1,000**	1-2
		Hydrogen peroxide	63	50	>10***
		1% oxygen	4	150	1-2
		21%, air	4	150	1-2
		100% oxygen	76	150	>2
		L1 in water o/n****	4	52	1-2
		L1 in NAC o/n	3	65	1-2
	*atm-1(mg665);sur-5::GFP^*	none	100	>1,000	>10
	*dog-1(mg667);sur-5::GFP^*	none	100	>1,000	>10
	*atm-1(gk186);sur-5::GFP^^*	none	38	50	>2
	*dog-1(gk10);sur-5::GFP^^*	none	25	56	>2
R. huautlense, JUb 45	*sur-5::GFP*	none	78	>1,000	>2
		1% oxygen	30	150	>2
		21%, air	67	150	>2
		50% oxygen	70	150	>2
		100% oxygen	100	150	>10
		L1 in water o/n	100	50	>2
		L1 in NAC o/n	55	90	>2
		Apple	30	116	>2
***R. huautlense,* ATCC BAA-115**	*sur-5::GFP*	none	46	50	>2
***R. galegae,* ATCC 43677**	*sur-5::GFP*	none	58	50	>2
***Sphingobacteriu sp.* JUb56**	*sur-5::GFP*	none	>25*****	50	>2
***Pseudomonas sp.* JUb42**	*sur-5::GFP*	none	>25	50	>2
***Providencia sp.* JUb39**	*sur-5::GFP*	none	>25	50	>2

*- treatments are described in Material and Methods

**- number of scored worms throughout the study

***- stopped count at the given number of affected nuclei

****- o/n, overnight

*****- stopped count after 10 affected worms out of 50 on a plate observed

^-EMS mutants from this study

^^- CGC mutants

Movie S1. **L1 larvae in a drop of S-basal buffer on NGM agar plate are treated with 3% hydrogen peroxide.**

Movie S2. **L1 larvae in a drop of S-basal buffer on *E. coli* lawn are treated with 3% hydrogen peroxide.**

Movie S3. **L1 larvae in a drop of S-basal buffer on *Rhizobium* lawn are treated with 3% hydrogen peroxide.**