**Supplementary Figure Legends**

**Figure S1. Differential IR sensitivity in CGC and McGill laboratory N2 strains**

Quantification of brood size in N2 lines from the indicated laboratories and the Caenorhabditis Genetics Center. Following IR treatment at the L1 stage, the CGC N2 line exhibits the same reduction in brood size as the N2 line from the Zetka lab (*p*>0.05) while the Roy laboratory at McGill University exhibits a brood size significantly higher brood than either one (*p*<0.001 for both comparisons), although still lower than in unirradiated controls (*p*<0.01).

**Figure S2. Adults irradiated at L1 do not show germline defects or elevated embryonic lethality**

**(A-B)** Representative images of DAPI (DNA) and anti‐HTP‐3 (meiotic chromosome axis) antibody staining of germline stem cells (mitotic zone) and meiotic prophase cells from germlines of (A) N2[R] or (B) N2[S] adults irradiated with 75 Gy of IR at the L1 stage. No gross cytological defects are visible in mitotic nuclei, meiotic prophase events, or the appearance of 6 well-formed bivalents in diakinesis nuclei. Scale bars, 5 μm. **(C**) N2 [S] hermaphrodites irradiated at L1 do not exhibit an increased embryonic lethality in comparison to the broods of unirradiated mothers, suggesting that the reduced brood size of IR‐treated N2 [S] animals is not caused by impaired or mutagenic DNA repair in the germline. The broods of N2 [R] mothers irradiated with 75 Gy at L1 show significantly higher embryonic lethality than either the progeny of unirradiated N2 [R] mothers or the progeny of N2 [S] mothers irradiated at the same dose (*p*<0.001 for both comparisons). Increased post-IR embryonic lethality in the N2 [R] background is consistent with the presence of a low level of NHEJ activity in the PGCs, which may result in mutagenic repair of some IR-induced DSBs, while the rest are repaired by HR. In the N2 [S] background which lacks NHEJ, DSBs are repaired by HR, resulting in high fidelity repair and no embryonic lethality. **(D)** Irradiation with 75 Gy at the L4 stage induces higher embryonic lethality in the progeny in both N2 genetic backgrounds in comparison to unirradiated controls (*p*<0.001 for both N2 [R] and N2 [S]). However, there is no significant difference in the level of embryonic lethality among the progeny of irradiated N2 [R] and N2 [S]. All statistical comparisons shown in the figure are to N2 [S] at the equivalent IR dose (Chi‐squared test, Bonferroni corrected for multiple comparisons to α = 0.01). ns = not significant (*p*>0.01); \*\*\* = *p*<0.001

**Figure S3. *nhj-1* gene structure and molecular lesions in mutant alleles**

**(A)** The coding region of *nhj-1*/*H19N07.3* consists of four exonic regions and three introns. In the *vv148* allele of N2 [S], exon 3 of *nhj-1* has been disrupted by a deletion of 5 nucleotides and a 115 bp insertion (composed of 107 nucleotides of unknown origin and 8 nucleotides duplicated from the exonic sequence). CRISPR mutagenesis was used to delete 7 nucleotides from Exon 3 and create the *nhj-1(vv144)* allele. **(B)** Predicted protein sequences of NHJ-1. The predicted long isoform of the NHJ-1 protein is 168 amino acids, with no known conserved domains; the shorter isoform is 130 a.a. residues in length (not shown). The *nhj-1(vv148)* mutation results in a truncated protein product with a frameshift producing 3 missense residues, followed by a premature stop codon (indel). The *nhj-1(vv144)* deletion results in a frameshift which creates a downstream sequence of 22 missense residues before terminating in a premature stop codon.

**Figure S4. The *nhj-1(vv148)* mutation is present in the Zetka lab N2 line**

**(A)** PCR of the *nhj-1* locus using flanking primers (Materials and Methods) using genomic DNA from N2 [R], N2 [S], and the Zetka laboratory N2 line. The reaction only yields the predicted product (1086 bp) from the N2 [R] template, likely because of the hairpin present in the *nhj-1(vv148)* insertion which may inhibit the PCR reaction. **(B)** PCR of the *nhj-1* locus using the forward flanking primer and an insertion-specific *nhj-1(vv148)* primer (Materials and Methods) using genomic DNA from N2 [R], N2 [S], and the Zetka laboratory N2 line. Both N2 [S] and the Zetka lab N2 templates give the expected band (497 bp), while the N2 [R] template does not, indicating the presence of the *vv148* indel in N2 [S] and Zetka lab N2, but not in N2 [R].

**Figure S5. *lig-4* gene structure and molecular lesions in mutant alleles**

**(A)** The coding region of *lig-4* consists of 11 exons and 10 introns. In the *vv134* allele, created in the N2 [R] background, and the *vv141* allele, created in the N2 [S] background, arginine in position 18 has been replaced by a stop codon. **(B)** Predicted protein sequences of *lig-4* and the mutant alleles. The wild-type LIG-4 protein is predicted to be 741 amino acids in length, while the *lig-4(vv134)* and *lig-4(vv141)* mutations are predicted to result in a truncated protein product of 17 a.a. There are no alternate translation start sites in the *lig-4* locus.

**Figure S6. OLLAS tags do not perturb *nhj-1* or *lig-4* function**

Quantification of the characteristic growth delay and vulval defects post-IR treatment at the L1 stage shows that OLLAS tagging of *nhj-1* or *lig-4* does not detectably affect the cNHEJ function of these genes. Three days **(A)** and four days **(B)** post-IR, *nhj-1:ollas* and *lig-4:ollas* animals show the same IR resistance as N2 [R] animals, while N2 [S] animals exhibit a high incidence of slow growth and vulval phenotypes.

**Figure S7. NHJ-1 and LIG-4 localize to somatic nuclei and are excluded from the germline precursor cells in L1 larvae**

Immunofluorescence micrographs of control and IR-treated L1 larva stained with α-OLLAS detecting **(A)** NHJ-1 or **(B)** LIG-4 from the endogenous locus, and α-HTP-3 detecting the two primordial germ cell nuclei (yellowboxes) in the indicated genotypesRepresentative nuclei are shown in insets (white boxes). The loss of *cku‐80* or *lig‐4* does not detectably affect the localization of NHJ‐1::OLLAS or LIG-4::OLLAS, nor does radiation treatment in either the control or *cku‐80* or *lig‐4* mutant backgrounds. Scale bars, 15 μm and 5 μm (insets).

**Figure S8. NHJ-1 and LIG-4 localize to late meiotic prophase nuclei independently of cNHEJ components**

Immunofluorescence micrographs of meiotic prophase nuclei of adult germlines stained with α-HTP-3 marked meiotic chromosome axes and α-OLLAS detecting NHJ-1 or LIG-4 from the endogenous locus in wild types and in NHEJ mutants. **(A)** NHJ‐1 and **(B)** LIG-4 localization is nuclear, punctate, and not detectably chromatin enriched. Adult intestinal cells are shown for comparison. LIG‐4 becomes reliably detectable in pachytene while NHJ-1 appears more enriched in comparison to LIG-4 at diplotene. **(C-D)** The loss of *cku‐80* or **(E-F)** *lig‐4 or nhj-1* does not detectably affect the localization of NHJ‐1 or LIG-4. Scale bars, 5 μm.