Caenorhabditis elegans germline development requires *brap-2*

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Background. Mutations in *C. elegans* can produce visible and quantifiable defects in morphology, lifespan, and development. BRAP2/IMP (BRCA1-associated binding protein 2) has been characterized as an E3 ubiquitin ligase, a general cytoplasmic retention factor, a potential scaffold protein, and is found to be widely expressed throughout various mammalian tissues, most highly in testes. However, its role in the development or health of these tissues has not been addressed.

Results. The focus of this study is to determine the role of BRAP-2 in *C. elegans* germline development. We determined that *brap-2* mutants display defects in germline morphology and a reduction in brood size. We also found that chromosomal abnormalities and embryonic lethality are elevated in *brap-2* mutants following DNA damage, suggesting a potential role for BRAP-2 in facilitating DNA repair.

Conclusions. Our findings indicate that BRAP-2 is required for *C. elegans* germline health and identifies a novel role for BRAP-2 in germline development.

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Abstract

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Results. The focus of this study is to determine the role of BRAP-2 in *C. elegans* germline development. We determined that *brap-2* mutants display defects in germline morphology and a reduction in brood size. We also found that chromosomal abnormalities and embryonic lethality are elevated in *brap-2* mutants following DNA damage, suggesting a potential role for BRAP-2 in facilitating DNA repair.

Conclusions. Our findings indicate that BRAP-2 is required for *C. elegans* germline health and identifies a novel role for BRAP-2 in germline development.

Introduction

C. elegans is a powerful model organism due to its simplicity and genetic conservation with mammalian systems. It is a tiny, transparent hermaphroditic nematode that possesses an invariant number of somatic cells (Corsi, Wightman & Chalfie, 2015). Some of the benefits of this model is that it has a short generation time, reaching adulthood in 3 days, they produce a large number of progeny (~300) in every generation, and they can live up to 2 weeks (Corsi, Wightman & Chalfie, 2015). Genetic screens have uncovered the function of many conserved human genes, dissecting the genetic order of signaling cascades that execute stress responses, programmed cell death, and development (Ahringer, 2006). Novel gene mutations can alter *C.*

elegans development or produce visible phenotypes in morphology, which can implicate their involvement in disease-associated signaling pathways.

The germline is the only continuously proliferative tissue in *C. elegans*, where mitotically proliferating cells progress through stages of meiosis beginning in the transition zone (TZ) and undergo meiotic recombination in pachytene for arrest in diakinesis before becoming oocytes (Lui & Colaiácovo, 2013). The germline also employs mitotic arrest, DNA repair, and apoptosis in spatially distinct areas to protect nuclei from the threat of external and internal stressors for successful meiotic progression, gametogenesis, and self-fertilization (Gartner, Boag, Peter & Blackwell, 2008; Craig et al., 2012). Mutations in genes involved in coordinating and executing the DNA damage response (DDR) can exhibit many defects in embryonic lethality, chromosome morphology, and radiation sensitivity (Craig et al., 2012). For example, BRCA1 (Breast cancer susceptibility gene 1) is a tumor suppressor that participates in several DNA damage surveillance mechanisms including DNA repair, transcription, and cell cycle arrest (Jang & Lee, 2004). Although worms appear wildtype, loss of BRC-1 (the BRCA1 ortholog) causes an increase in germline apoptosis, increased RAD-51 (RAD51 ortholog) foci formation at double strand break (DSBs) sites, increased embryonic lethality, as well as increases the incidence of males (Boulton et al., 2004; Adamo et al., 2008). Thus, the continued maintenance and protection of the germline is vital for *C. elegans* development and survival.

BRAP2 (BRCA1-associated binding protein 2 or BRAP according to the HUGO database) is an E3 ubiquitin ligase and a cytoplasmic retention protein due to its ability to prevent BRCA1 nuclear import (Li et al., 1998). BRAP2 also interacts with other proteins and prevents their nuclear localization, most notably p21, CDC14, and PHLPP1 (Asada et al., 2004; Chen et al., 2009; Davies et al., 2013a; Fatima et al., 2015). BRAP2 has also been identified as a

RAS-responsive E3 ubiquitin ligase that upon activation of RAS is auto-ubiquitinated, releasing KSR1 for increased stimulus-dependent ERK activation (Chen, Lewis & White, 2008; Matheny et al., 2004; Matheny & White, 2006). Previously, we characterized the *C. elegans* BRAP2 ortholog BRAP-2 and found that *brap-2* mutants experience BRC-1 dependent L1 developmental arrest when exposed to oxidative stress (Koon & Kubiseski, 2010). In addition, we found that BRAP-2 is a negative regulator of SKN-1 (*C. elegans* Nrf2 ortholog) a transcription factor and master regulator of the phase II detoxification response (Hu et al., 2017). We also determined that BRAP-2 promotes DNA damage induced germline apoptosis, a vital component of the DDR (D'Amora et al., 2018).

The focus of this study was to determine the effects a loss of BRAP-2 has on *C. elegans* germline integrity. We found that *brap-2* mutants have reduced brood size and sperm quality, and following DNA damage induced by ionizing radiation (IR), and has increased mitotic nuclei radiation sensitivity. Overall, these results indicate that BRAP-2 is required for proper *C. elegans* germline health.

Materials and Methods

C. elegans strains and genetics

All worm strains were cultured under standard conditions, as previously described (Brenner, 1974). All strains were obtained from the *Caenorhabditis* Genetics Center at the University of Minnesota and double mutant strains were generated using standard techniques. The N2 Bristol strain was used as the wild type, and unless noted otherwise and all experiments were conducted at 20°C. The following strains were used in this study: N2, *brap-2(ok1492) II* (YF15), *brc-1(tm1145) III* (DW102), and *brap-2(ok1492) II; brc-1(tm1145) III* (YF64).

Brood size and embryonic lethality

To assess brood size, 5 adult worms for each genotype were plated on a normal growth media (NGM) plate, in triplicate. Adult worms were allowed to lay eggs for 5 hours at 3 permissive temperatures (16°C, 20°C, and 25°C) and then removed. Eggs were scored, and the following day the number of un-hatched eggs and L1 larvae were counted to assess both brood size, number of males and embryonic lethality.

Phenotypic Analysis

For each genotype, worms were synchronized and when they reached the first day of adulthood were mounted on 2% agarose in 2 mM Levamisole (Sigma L9756) in M9 Buffer. To examine immunofluorescence, germlines, and male sensory rays images were taken using a Zeiss LSM 700 Confocal Laser-Scanning Microscope with Zen 2010 Software. Gonad length was calculated using the line measurement tool in Zen 2010 Lite Software.

DNA Staining

To quantify germline nuclei, whole worms were stained with DAPI as previously described (Craig et al., 2012). The ImageJ (National Institute of Health) cell counter plugin was used to determine the total number of nuclei per zone in one gonad arm per worm per genotype imaged and scored. To compare meiotic progression, germlines were dissected and stained with DAPI (VECTOR Labs H-1200). For chromosomal fragmentation, z-stack images of terminal oocytes at the -2 and -1 positions at the proximal end where examined for manual counting of condensed chromosome bodies.

Gonad Dissection

Gonad dissection and immunostaining was performed as previously described by (Lawrence, Chau & Engebrecht, 2015), with the following changes: Approximately 20–30 synchronized 1-day old adults were mounted onto 20 µL of 2mM Levamisole in M9 buffer on Superfrost Polylysine (Fisher 12-55505-15) coated slides in an area made with Immaedge Hydrophobic Pen (VECTOR Labs H-4000). The heads or tails were removed using 27G syringe needles in a "scissor-like motion". Then 2% paraformaldehyde was added, coverslip was mounted, and slides were allowed to sit for 10 minutes and then frozen on an aluminum block on dry ice for 10 minutes. Slides were then freeze-cracked using a razor blade and submerged in -20°C methanol for fixation for 1 minute.

Immunofluorescence in worms

Dissected Germlines: Following methanol fixation, slides were washed with 1X PBST (PBS, 0.1% Triton X-100) 3X for 5 minutes, followed by incubation for 1 hour in 2% BSA for 1 hour in a humid chamber. Primary antibody (prepared in 2% BSA) was added overnight at room temperature. Slides were washed 3X with 1X PBST and then secondary antibody was added dropwise and incubated for 1 hour at room temperature in a humid chamber in the dark. Slides were washed 4X with 1X PBST for 5 minutes and in the third wash 1 µg/ml DAPI was added. Before mounting, 3 µL of Prolong Gold Anti-Fade Reagent (Life P36930) was added and coverslips were sealed using clear nail polish. Images of 10 fluorescent worms per genotype were taken. Antibodies used were: rabbit anti-BRAP-2 (1:100), goat anti-rabbit Alexa Fluor 488 (1:1000) (Invitrogen A11001) and DAPI (1 µg/mL). Rabbit polyclonal anti-sera against *C. elegans* BRAP-2 (EEED8.16) was generated by the Toronto Recombinant Antibody Centre of the University of Toronto using a GST fusion protein with BRAP-2 antigen corresponding to residues 108 to 134, which lie in the N terminus of the protein.

Cell transfection and immunofluorescence

Human Embryonic Kidney (HEK) 293T cells (American Type Culture Collection, ATCC, Manassas, VA, USA) used to examine BRAP-2 cellular localization were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% Fetal Bovine Serum. Cells were co-transfected for 24 hours, with 1 µg DNA of mammalian expressing constructs using polyethylenimine (PEI) (Sigma 408727-100 mL) and Optimem (Clontech 31985). Cells were washed with PBS and then fixed with 4% paraformaldehyde for 15 minutes. Cells were permeabilized with 0.1 % Triton-X 100 for 5 minutes and then washed 3X with 1X PBST. Cells were incubated with 4% BSA for 1 hour in a humid chamber at room temperature. Cells were then incubated with primary antibody (in 4% BSA) for 1 hour at room temperature, and then washed 3X with 1X PBST. Secondary antibody (in 4% BSA) was then added for 1 hour at room temperature in the dark and then wash 3X with 1X PBST. In the third wash, 1 µg/mL DAPI was added. Coverslips were mounted onto plain glass slide with 3 µL of Pro-long Gold Anti-Fade Reagent and edges were sealed with clear nail polish. Z-stack images of cells were taken using a Zeiss LSM 700 confocal microscope at 40X. The following antibodies were used: rabbit anti-GST (1:100) and goat anti-rabbit Alexa Fluor 555 (1:200) for fluorescence visualization.

RNA Isolation and Quantitative Real Time PCR (qRT-PCR)

Worm RNA Isolation and qRT-PCR was performed as previously described (Hu et al., 2017). Data were derived from 3 biological replicates and analyzed using the comparative method ($\Delta\Delta$Ct). mRNA expression level of each strain was compared relative to the wild type (N2) control, where the housekeeping gene *act-1* was used as the endogenous control for normalization. To verify equal primer amplification efficiency, a standard curve for each set of primers was constructed using a serial dilution of cDNA.

Radiation Sensitivity

Synchronized L4 worms were irradiated at 60 and 120 Gy. Approximately 18–20 hours later, five 1-day old hermaphrodites were transferred to NGM plates and allowed to lay eggs for 5 hours (with eggs laid corresponding to nuclei in the pachytene region) and were counted (Craig et al., 2012). The following day the number of unhatched eggs were scored. To determine L1 sterility, L1 stage worms were irradiated at 60 Gy, embryonic lethality of F1 progeny (corresponding to nuclei in the mitotic zone) was assessed to determine sterility of these IR treated animals (Ross et al., 2011).

Statistical Analysis

Statistical analysis was performed with GraphPad Prism® 7 Software. Student's t-test was performed when comparing two means. P values of <0.05 were taken to indicate statistical significance. Error bars represent +/- standard error of the mean.

Results

BRAP-2 is expressed in the *C. elegans* germline

Previous functional studies of BRAP2 in mammalian cell culture have determined that BRAP2 localizes to the cytoplasm, interacting with a variety of proteins to generally retain and prevent their nuclear localization (Asada et al., 2004; Davies et al., 2013a; Fatima et al., 2015). Another study found that BRAP2 is expressed in liver, heart, and lung tissues but is most highly expressed in testes (Fatima et al., 2015). Therefore, we wished to determine whether the tissue and cellular localization of BRAP-2 (BRAP2 ortholog) is conserved in *C. elegans*. To determine BRAP-2 subcellular localization *in vitro* we transfected a mammalian expressing construct with BRAP-2 in 293T HEK cells, and found that like mammalian BRAP2, BRAP-2 exhibited a

cytoplasmic localization (Fig 1A). We immuno-stained wild type germlines with α-BRAP-2 and found BRAP-2 to be localized in the cytoplasm surrounding germline nuclei, producing a "honeycomb effect" (Fig 1B). While examining BRAP-2 localization, we detected few germlines with BRAP-2 expression in the sperm sac (part of the somatic gonad) (Fig 1C). Therefore, the cytoplasmic localization and high expression within the germline is conserved in *C. elegans*. Therefore, it is possible that BRAP-2 function may be necessary for germline protection and development.

BRAP-2 is required for brood size and germline development

We next examined brood size and embryonic lethality in *brap-2* mutants at three temperatures: 16°C, 20ºC, and 25ºC. At all 3 temperatures, *brap-2(ok1492)* mutants displayed a 50% decrease in brood size compared to the wild type (Fig 2). While embryonic lethality appeared elevated in *brap-2* mutants at 20ºC, unexpectedly, it was observed to be most significantly increased at 16°C (Fig 2A), perhaps indicating that *brap-2* mutants do not adapt as well metabolically to lower temperatures as do wild type.

Having observed an overall reduction in brood size and elevated embryonic lethality, we examined *brap-2(ok1492)* mutant germlines for the presence of any abnormalities. Dissecting intact germlines of *brap-2(ok1492)* mutants followed by DAPI staining, revealed that *brap-2* mutants exhibited shortened mitotic and transition zones compared to the wild type (Fig 3). When we scored the number of nuclei diameters from the distal tip of the mitotic zone (MZ) to the beginning of the transition zone (TZ), we saw a more significant reduction at 0 Gy in *brap-2* mutants with an average of 16 nuclei, compared to 23 in the wild type (Fig 3B, C). This suggests that *brap-2* mutants may have reduced number of germline progenitor cells in the MZ. Specifically, we found *brap-2* mutants display similar numbers of germline nuclei in all zones

except the MZ, with a 50% reduction at 0 Gy and only a 20% reduction at 60 Gy (Fig 3D). This reduction in the number of mitotic nuclei may contribute to the 20% reduction in total germ cell number we observed previously in *brap-2* mutants (D'Amora et al., 2018). When we measured gonad length in *brap-2(ok1492)* mutants they displayed a 100 μm decrease compared to the wild type (Fig 3E). Interestingly, in addition to finding that *brap-2* mutants display a similar distribution of germline nuclei over surface area compared to the wild type, we also found that *brap-2* mutants displayed an increase in mitotically arrested nuclei, which may contribute to this reduction in cells in the MZ due to an interruption in cell cycle progression.

Previous work from our lab found that the levels of CKI-1 (p21 ortholog) *in vivo* in *brap-2* mutants increased in seam cells of the hypodermis, cells responsible for growth and development (Koon & Kubiseski, 2010). Using qRT-PCR, we measured the mRNA levels of the cyclin dependent kinase inhibitors *cki-1* and *cki-2* (p27 ortholog) and found that they increased 2-fold in *brap-2* mutants (Fig 4A, B) (Buck, Chiu & Saito, 2009). We also found that the G2 mitotic phase arrest associated gene *cyb-1* (Cyclin B1 ortholog) also increased 2-fold in *brap-2(ok1492)* (Fig 4C) (van der Voet et al., 2009). Interestingly, increased expression of all these genes in *brap-2* mutants was dependent on BRC-1. This indicates that there may be an increase in cell cycle arrest in the MZ, which may contribute to the increased mitotic arrest and reduction of mitotic cells in *brap-2* mutants. These results suggest that BRAP-2 is required for normal brood size, embryonic survival, as well as cell cycle regulation in the germline.

***brap-2* mutants possess increased mitotic radiation sensitivity and chromosomal abnormalities**

Having determined that BRAP-2 is expressed in the germline we asked whether BRAP-2, like BRC-1, is involved in DNA repair. To test this, we assessed whether *brap-2* mutants are

sensitive to DNA damage using two radiation sensitivity survival assays (Ross et al., 2011; Craig et al., 2012). First, sensitivity of meiotic pachytene nuclei was determined by scoring lethality of the progeny produced by L4 stage animals subjected to DNA damage. Following increasing doses of IR, *brap-2* mutants displayed similar sensitivity to DNA damage to that of the wild type (Fig 5A). We next examined the sensitivity of mitotic nuclei by scoring embryonic lethality of progeny produced by adult animals that were irradiated at the L1 stage. We found that *brap-2(ok1492)* mutants displayed a 20% increase in embryonic lethality following IR (Fig 5B). This indicates that BRAP-2 may be necessary to protect mitotic nuclei from DNA damage but is dispensable at the pachytene stage.

Following late pachytene, nuclei exit the loop region and move on to complete gametogenesis to become large oocytes arresting in diakinesis. Wild type oocytes possess 6 condensed bivalents, representing pairs of homologous chromosomes held together by chiasmata, the physical manifestation of DNA cross-over recombination (Lui & Colaiácovo, 2013). We scored the number of bivalents in *brap-2(ok1492)* oocytes and found that they possessed a similar number of bivalents to the wild type. However, following IR *brap-2* mutants exhibited a small increase in nuclei with greater than 6 bivalents compared to the wild type (Fig 5C). Overall, while examining the oocytes in *brap-2(ok1492)* mutants we also observed that approximately 30% of oocytes displayed chromosomes that appeared in very close proximity to each other or “aggregated”, making it more difficult to score (Fig 5D). These results indicate that BRAP-2 may be required to protect germline integrity, as a loss of *brap-2* increases mitotic nuclei sensitivity to radiation and may reduce the effectiveness of DNA repair seen with the appearance of abnormal oocyte chromosome morphology.

Discussion

Here we have shown that BRAP-2 expression is localized to the cytoplasm of the wild type hermaphrodite germline, surrounding germline nuclei suggesting that it may be required for its proper development and preservation of genetic integrity. Furthermore, we found that *brap-2* mutants displayed a 50% reduction in brood size across three different temperatures and observed a reduction in gonad length. We also found that *brap-2* mutants exhibit a significant reduction of nuclei in the MZ, as well as a reduction in the number of nuclei diameters from the distal tip of the MZ to the TZ, indicating that entry in the TZ may occur earlier in *brap-2* mutants.

Loss of mitotic nuclei has been observed in older worms and has been associated with defective mitotic cell cycle arrest, a decrease in mitotic index, and insulin and insulin-like growth factor-1 signaling (IIS) pathway activation, where elimination of IIS through *daf-2* knockdown or mutation elevated the number of stem cell progenitors in aging worms (Qin & Hubbard, 2015). We previously observed that *brap-2(ok1492)* mutants display enhanced mitotic arrest and potential attenuation of DAF-16, a downstream target of the IIS pathway (D'Amora et al., 2018). In addition, we observed a 2-fold increased expression of cycle arrest associated genes *cki-1*, *cki-2,* and *cyb-1* in *brap-2* mutants. It is possible that enhanced IIS and robust mitotic arrest could be contribute to the reduction of mitotic nuclei in *brap-2* mutant germlines, as well as contribute to the reduction in brood size. However, it is also possible that a loss of *brap-2* adversely affects other pathways that control meiotic progression or entry of pachytene nuclei into oogenesis such as the LET-60/MPK-1 (Ras/MPK-1 orthologs) pathway, that could decrease oocyte production and therefore lead to an overall reduction in brood size.

DNA repair mutants like *brc-1*, tend to display higher levels of embryonic lethality due to an accumulation of unrepaired DSBs induced during recombination (Craig et al., 2012).

Although not as high as *brc-1* mutants, *brap-2* mutants also display elevated levels of embryonic lethality, indicating it may be involved in DNA repair. After testing the radiation sensitivity of mitotic and pachytene nuclei, we found that a loss of *brap-2* reduced the resistance of only mitotic nuclei to IR. So far it has been observed that DDR mutants tend to increase damage sensitivity in either mitotic or pachytene nuclei not both, a trend consistent in *brap-2* mutants (Craig et al., 2012).

Protection of genomic integrity in the germline from both endogenous and exogenous stressors is essential, as DNA damage left unfaithfully repaired can lead to an accumulation of mutations, abnormal cells, compromised gametes, dysfunction and disease (Deng, 2006). Although it is unknown whether BRAP2 is directly involved in regulating development, DNA repair or meiosis, BRAP-2's high genetic, functional and tissue expression conservation to mammals, allows this study to serve as a starting point to determine if the role of BRAP-2 in *C. elegans* germline development and health is conserved in higher organisms. BRAP2 is conserved in the flowering plant model *Arabidopsis thaliana* which possesses conserved RING domain BRAP2 protein homologs BRIZ1 and BRIZ2 (BRAP2 RING ZnF-UBP domain-containing protein) that dimerize and exhibit conserved functional E3 ubiquitin ligase activity, required for seed germination and growth (Hsia & Callis, 2010). BRAP2 was also found to be highly expressed in mouse testis (Fatima et al., 2015). Future studies could focus on BRAP-2's role in proper sperm maturation that affects their capacity for fertilization.

Conclusions

Here we have uncovered a novel role for BRAP-2 in *C. elegans* germline development and protection where its loss compromises brood size, embryonic viability, and affects germline development and morphology. Particularly, we found that BRAP-2 is required for the health and

protection of the mitotic progenitor cell pool. Although we have determined a new role for BRAP-2, further investigation is required to determine the mechanisms through which BRAP-2 may regulate meiotic germline development, DNA repair, as well as other possible signaling pathways that govern *C. elegans* development (Maduzia et al., 2002; Gumienny & Savage-Dunn, 2013).

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Figure 1

Germline expression and cellular localization of BRAP-2.

(A) BRAP-2 localizes to the cytoplasm of mammalian 293T HEK cells**.** BRAP-2::GST was transfected into 293T HEK cells for 24 hours, fixed, probed with α-GST, and stained with DAPI to detect BRAP-2 expression. Cells transfected with GST tag were used as a negative control. Representative images represent 3 independent trials. **(B)** BRAP-2 is expressed in the cytoplasm of the germline. Representative image of extruded wild type adult germlines stained with α-BRAP-2 antibody, found BRAP-2 to be highly expressed and exhibiting a "honeycomb" effect in the cytoplasm surrounding germline nuclei. Ten one-day old wild type adult worms were dissected and extruded germlines were examined. White arrows indicate the germline, while yellow arrows indicate the intestine of the worm. **(C)** BRAP-2 is expressed in the sperm sac of wild type hermaphrodite germlines. Figure displays a representative image depicting BRAP-2 expression in the sperm sac overlying nuclei in the wild type germline. BRAP-2 was detected in the sperm sacs of three extruded wild type adult germlines. White arrows indicate the sperm sac located at the proximal end of the gonad.

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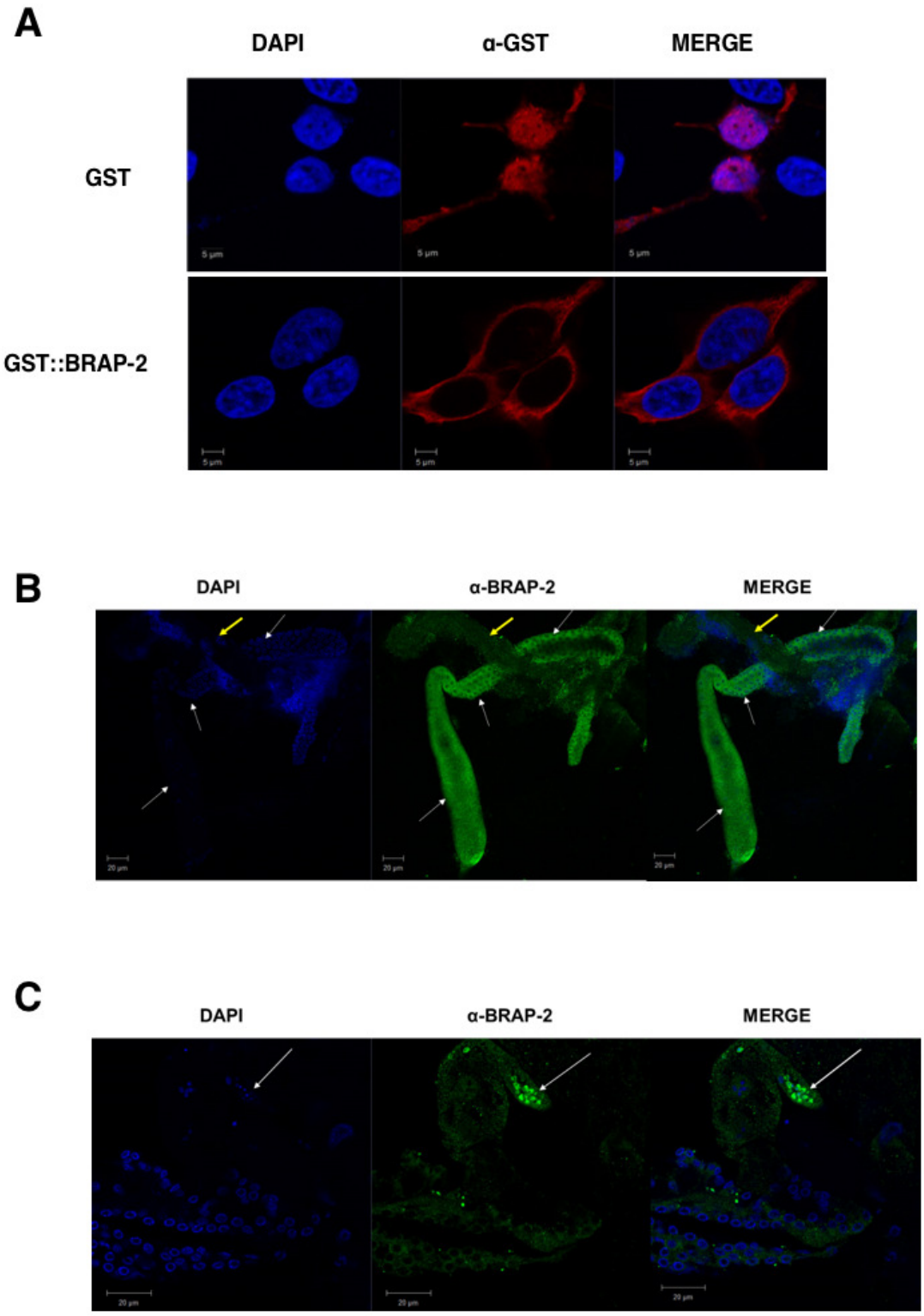
A
DAPI
α-GST
MERGE
GST
GST::BRAP-2
B
DAPI
α-BRAP-2
MERGE
C
DAPI
α-BRAP-2
MERGE

Figure 2

Brood size is reduced in *brap-2(ok1492)* mutants.

Brood size and embryonic lethality (%) were scored at **(A)** 16°C **(B)** 20ºC and **(C)** 25ºC. At all temperatures, *brap-2(ok1492)* mutants displayed a 50% reduction in brood size compared to the wild type (N2). Elevated embryonic lethality was also observed in *brap-2(ok1492),* except at 25°C. At 20ºC, *brc-1(tm1145)* was used a positive control for embryonic lethality. Results represent 3 independent trials. $p<0.001$***, $p<0.05$*.

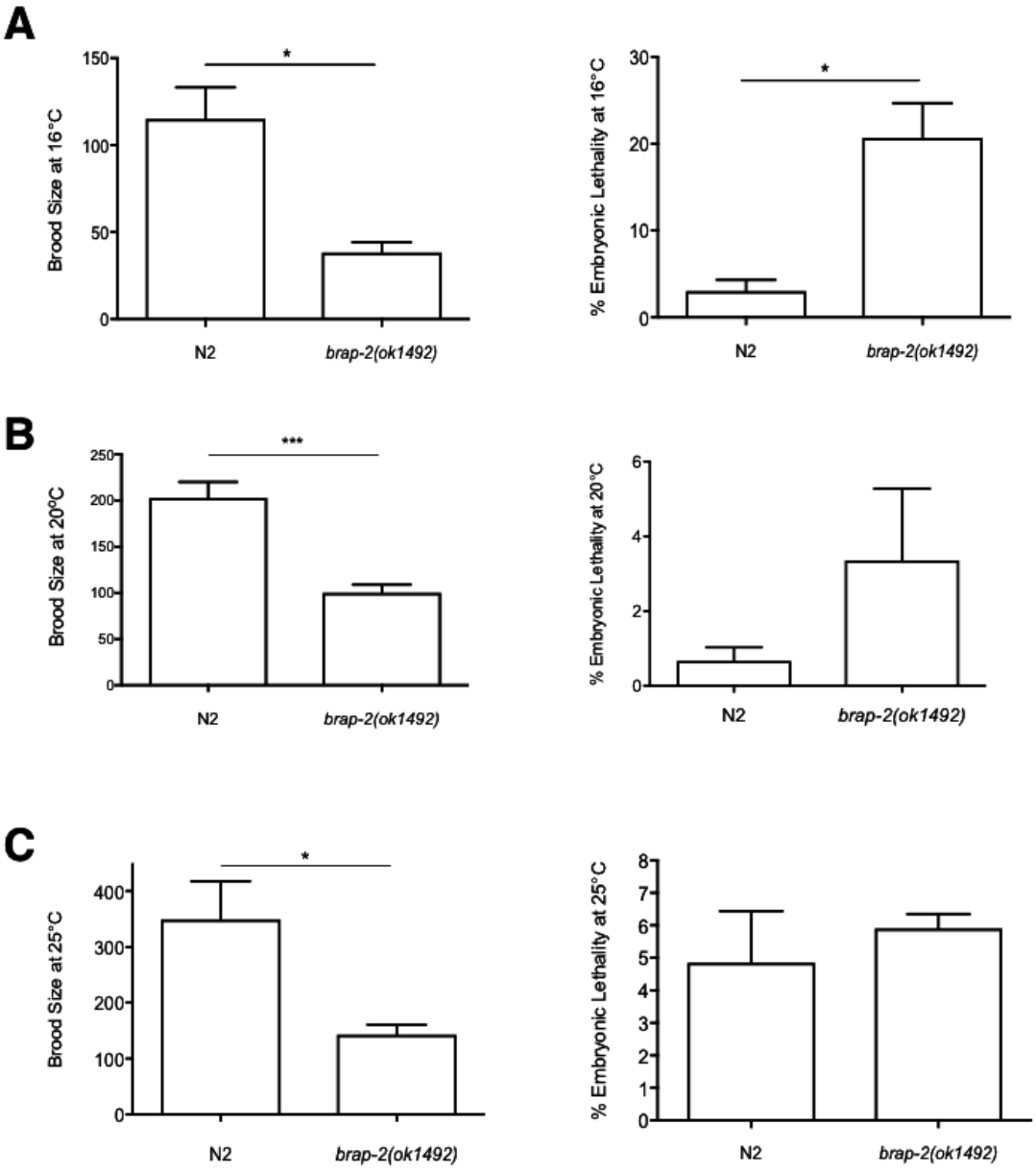

Figure 3

brap-2 mutants exhibit reduced gonad length and a shortened mitotic zone.

Representative images comparing DAPI-stained extruded wild type (N2, **A**) germline nuclei progression to that of *brap-2(ok1492)* mutants **(B)**. Due to the large size, images presented are a composite of two images taken at of the same gonad. N= 10 per genotype. Asterisk indicates the distal tip. PCD (Programmed cell death) labels the loop region of the gonad were apoptosis occurs. **(C)** The number of nuclei diameters [scored from the distal tip of the mitotic zone (MZ) to the beginning of the transition zone (TZ)] are reduced in *brap-2* mutants before and after IR. **(D)** *brap-2(ok1492)* mutants displayed a more significant reduction in the number of mitotic nuclei in the MZ at 0 Gy, than at 60 Gy. **(E)** Representative images of the MZ for each genotype. Rows of nuclei were manually counted in whole worms stained with DAPI from the distal tip (marked by asterisk) to the beginning of the TZ (marked by the dotted line). White number represents the average length of the mitotic zone in nuclear diameters from the distal tip of the germline to the TZ. Whole worms were fixed and stained with DAPI. Nuclei of the MZ were counted from one slice of the Z-stack using ImageJ. **(F)** *brap-2* mutants exhibit a reduction in gonad length compared to the wild type (N2). One day old adult worms were fixed and stained with DAPI and images of whole worms were taken. Gonad length was measured for one gonad per worm from the distal tip to terminal oocyte at the -1 position. Results represent N=16–40 worms per genotype. $p<0.001$***, $p<0.01$**, $p<0.05$.

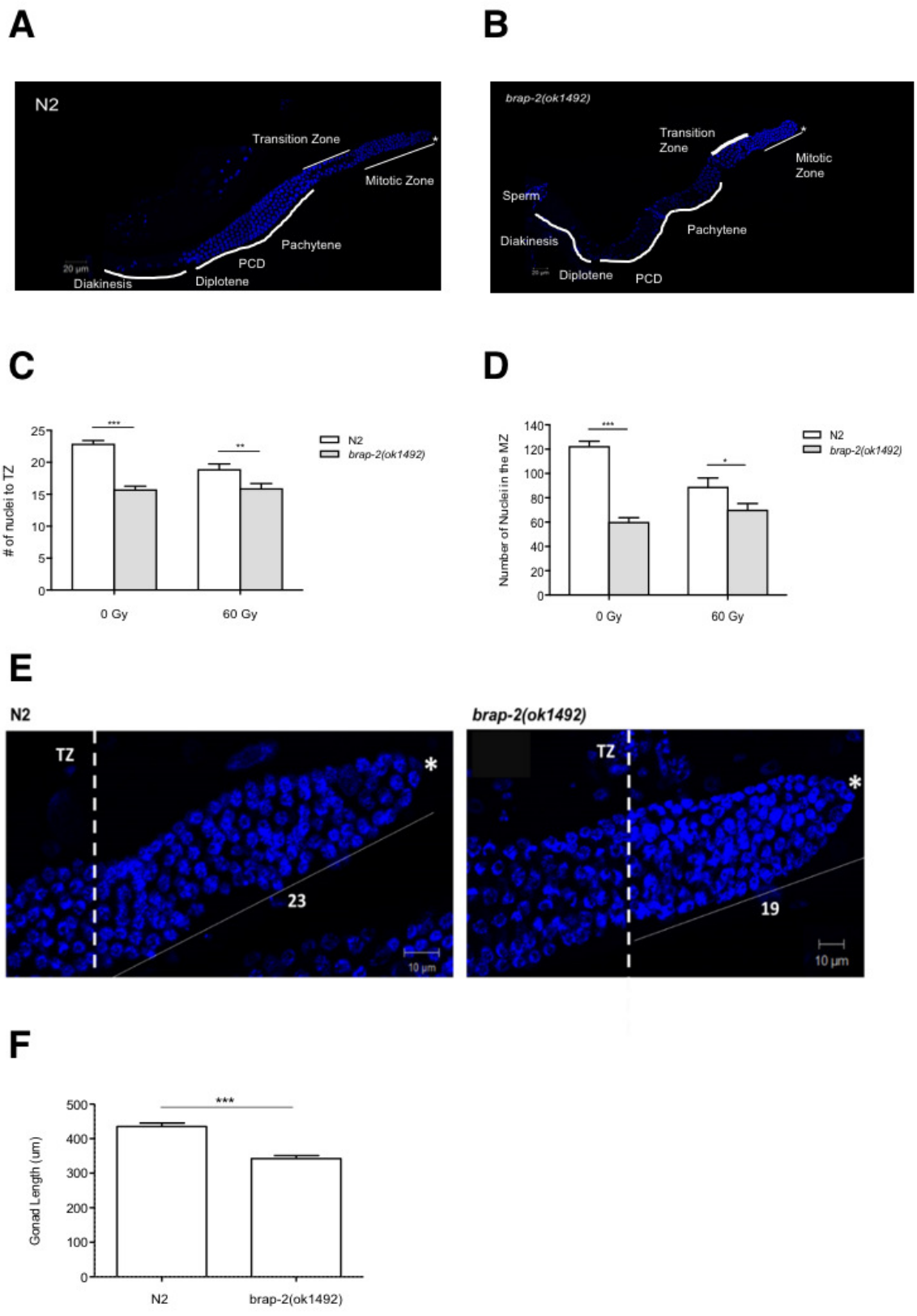

Figure 4

brap-2(ok1492) mutants display increased mRNA levels of *cki-1*, *cki-2* and *cyb-1.*

Using qRT-PCR, mRNA levels of **(A)** *cki-1* **(B)** *cki-2* and **(C)** *cyb-1* were quantified. Levels of *cki-1*, *cki-2,* and *cyb-1* all increased 2-fold with a loss of *brap-2*. Results represent 3 biological replicates, normalized to *act-1* of N2. $p<0.001$***, $p<0.01$**, $p<0.05$*.

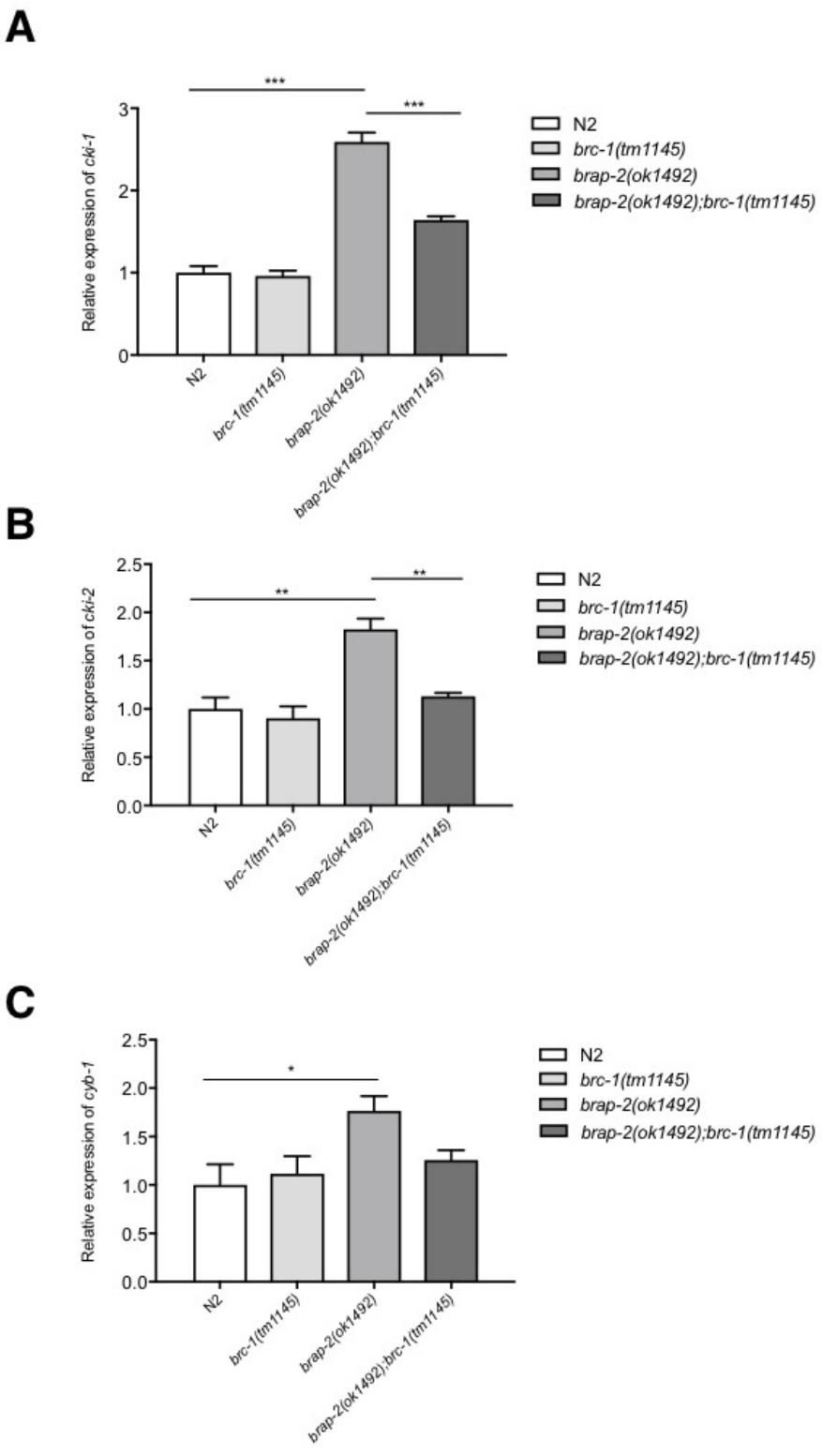

Figure 5

brap-2(ok1492) mutants are hypersensitive to IR during larval development.

(A) At increasing doses of IR, embryonic lethality in *brap-2(ok1492)* mutants is similar to the wild type (N2), suggesting that a loss of *brap-2* does not affect radiation sensitivity of pachytene nuclei. Results represent 3–6 independent trials. **(B)** An increase in embryonic lethality of progeny (arising from mitotic nuclei) is observed with the loss of *brap-2*, indicating that BRAP-2 is required by mitotic nuclei to resist DNA damage. Results represent 3 independent trials. $p<0.001$***, $p<0.01$**, $p<0.05$*. **(C)** We observed that *brap-2* mutants possess a small proportion of oocytes with greater than 6 bivalents before and after DNA damage (60 Gy) compared to the wild type (N2). **(D)** *brap-2(ok1492)* mutants also displayed oocytes that appeared aggregated compared to N2. Representative images of N2 and *brap-2(ok1492)* diakinesis nuclei. White arrow indicates abnormal chromosome. N=40 oocytes per genotype. The number of DAPI bodies was counted manually in -1 and -2 position oocytes.

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