Supplementary File:

Endogenous Expression and Subcellular Localization of Core Apoptosis Regulators Reveal Key Differences Between Embryonic and Germline Apoptosis in *C. elegans*

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Figure S1

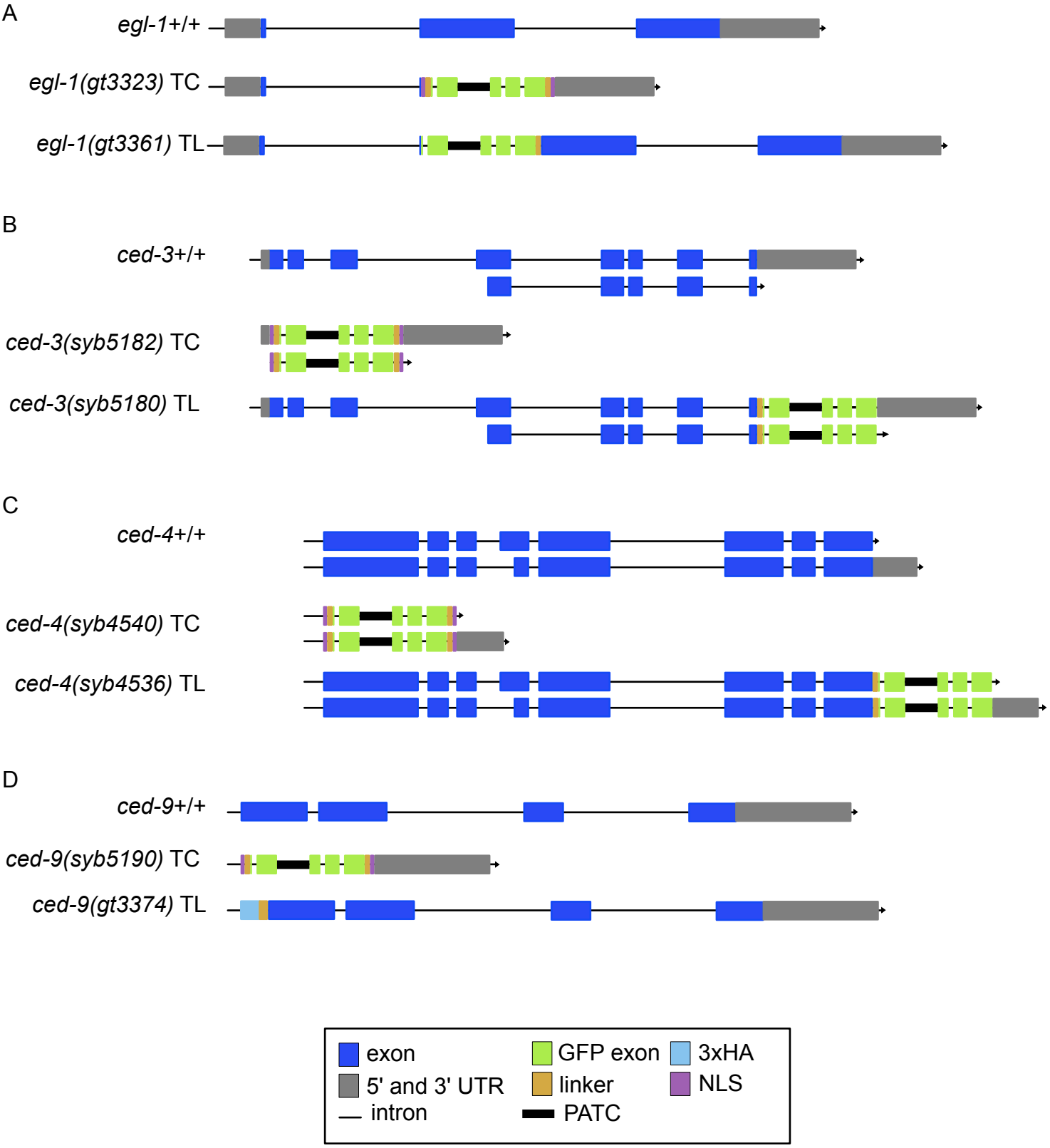
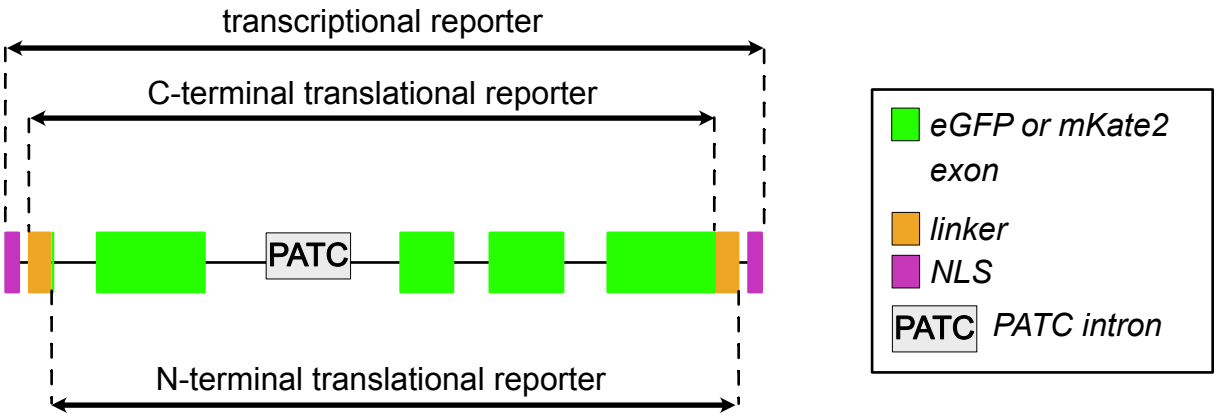

A
egl-1+/+
egl-1(gt3323) TC
egl-1(gt3361) TL
B
ced-3+/+
ced-3(syb5182) TC
ced-3(syb5180) TL
C
ced-4+/+
ced-4(syb4540) TC
ced-4(syb4536) TL
D
ced-9+/+
ced-9(syb5190) TC
ced-9(gt3374) TL
exon
GFP exon
3xHA
5' and 3' UTR
linker
NLS
intron
PATC

Figure S1: Transcriptional and translational reporters of the *C. elegans* apoptotic pathway generated in this study. Schematics of the transcriptional (TC) and translational (TL) reporters for **(A)** *egl-1,* **(B)** *ced-3,* **(C)** *ced-4,* and **(D)** *ced-9*. Their exons, introns, 5' and 3' UTR sequences are indicated. The exons of the fluorescent protein and the 3xHA tag, the linkers, the NLS, and the PATC intron (to ensure expression in the germline) are also indicated.

Figure S2

A



B

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CAAGGGTGGCACAGGAGGAACGGGCGGTAGTGGAGGCACTGGTTCCCGTCGTCGTA

AGGCCAACCCAACCAAGCTCTCCGAGAACGCCAAGAAGCTCGCCAAGGAGGTCGAG
AACTAG

NLS
Linkers
eGFP
mKate2
Introns
PATC intron
crRNA lower strand
crRNA upper strand

PAM site

C

ATGCCAAAGAAGAAGCGTAAGGTGAGTACTAGCGGTGGCAGTGGAGGTACCGGCGG
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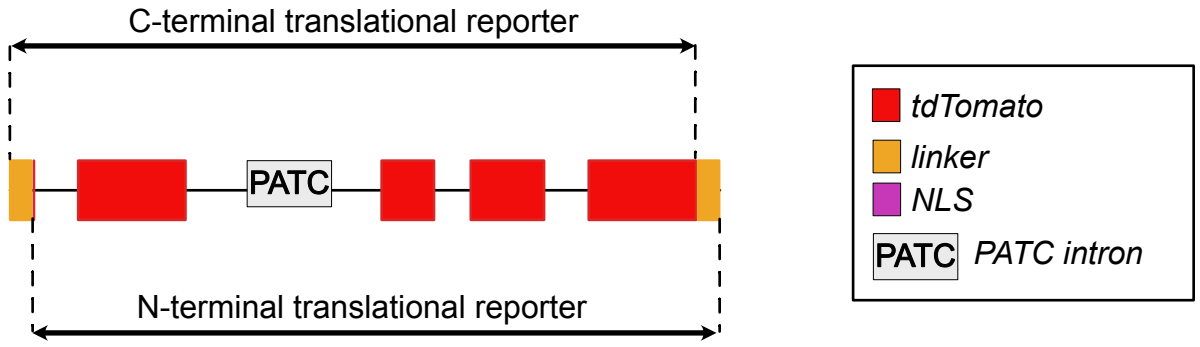
GAACGGGCGGTAGTGGAGGCACTGGTTCCCGTCGTCGTAAGGCCAACCCAACCAAG
CTCTCCGAGAACGCCAAGAAGCTCGCCAAGGAGGTCGAGAACTAG

Figure S2: Sequence of our eGFP and mKate2 constructs. (A) Schematic of our eGFP construct as an example, which can be used to generate transcriptional and translational reporters (N-and C-terminal). **(B)** Sequence of the eGFP construct (green), with NLS (purple), linkers (yellow), introns (black), PATC intron (italic black). crRNAs are indicated by arrows and their corresponding PAM site by a red box. **(C)** Sequence of the mKate2 construct (red), with NLS (purple), linkers (yellow), introns (black), PATC intron (italic black). crRNAs are indicated by arrows and their corresponding PAM site by a red box.

Figure S3

A



B

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ATGAGCTTTACAAGGGTGGCACAGGAGGAACGGGCGGTAGTGGAGGCACTGGTTAA

Linkers
tdTomato
3xHA
Introns
PATC intron and intron
crRNA lower strand
crRNA upper strand

PAM site

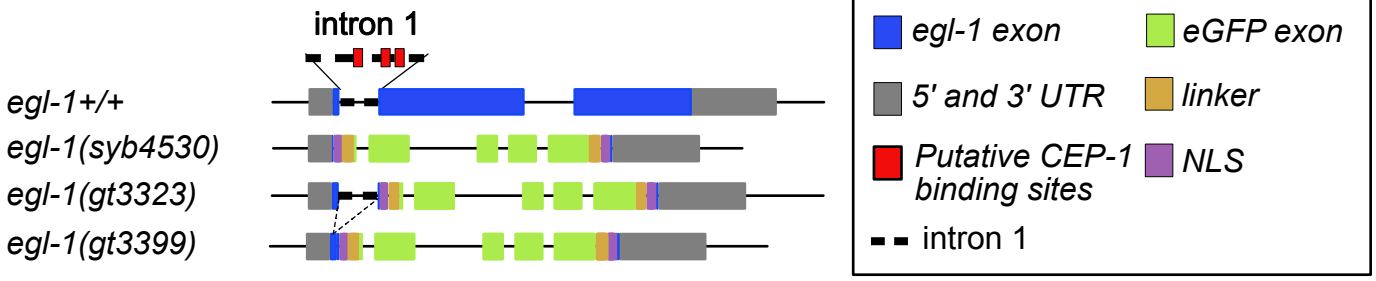
C

TACCCATACGACGTCCCAGACTACGCCTACCCATATGATGTCCCGGATTACGCTTACCCATA
CGATGTTCCAGATTACGCTGGTGGCACAGGAGGAACGGGCGGTAGTGGAGGCACTGGT

Figure S3: Sequence of our tdTomato and 3xHA constructs. (A) Schematic of our tdTomato construct as an example, which can be used to generate transcriptional and translational reporters (N- and C-terminal). **(B)** Sequence of the tdTomato construct (red), with NLS (purple), linkers (yellow), introns (black), PATC intron (italic black). crRNAs are indicated by arrows and their corresponding PAM site by a red box. **(C)** Sequence of the 3xHA construct (blue) with linker (yellow) for N-terminal tagging. This tag cannot be swapped, but the linker can be moved to the N-terminus for C-terminal tagging. This construct can be ordered as an ssODN from IDT.

Figure S4

A



B

```
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C.bri:  egl-1_intron1 --------gtgagtttttctttttgatctacctagattttattcctcaaaaatgggttactgtagaacatgtttta     69
                                * ** *** ************ **  * *                   * * * *  * * *

C.el:   egl-1_intron1 ctccccctcctgagaaacaaatttttgtttttgtctgctcgcctcaccctcaacctctct--ctctctggatgtgtt    135
C.bri:  egl-1_intron1 tttcatcagaaaacaaaaacaaaaacagttttctctgcgtctctcgaaacagatttctctcgcatgcgagaagagga    146
                       * *  *     * *** * *       **** *****    ***       *  *****  *   *  ** * *

C.el:   egl-1_intron1 cgtggtgtagaaacaaaaacagatttttgtttttttgtttttgtttcttgtttagaacttgtatcctagtaattg    212
C.bri:  egl-1_intron1 ggaggcgcagagaagagcaaaactgtttttgtttttgtttc--tcttcttcttcttcttcttttt-----------tt    210
                       * ** * *** *  *  * *  * *** * *********     ***** ** *    *** *           *

C.el:   egl-1_intron1 ttagacatctccctactatctttcccctatataaacccccttcaaaaccttactaatttccag                 275
C.bri:  egl-1_intron1 ttcaacgtcttctaaatatcacg---------------tcttctag-----------------                 241
                      **  ** *** *  * ****                  **** *
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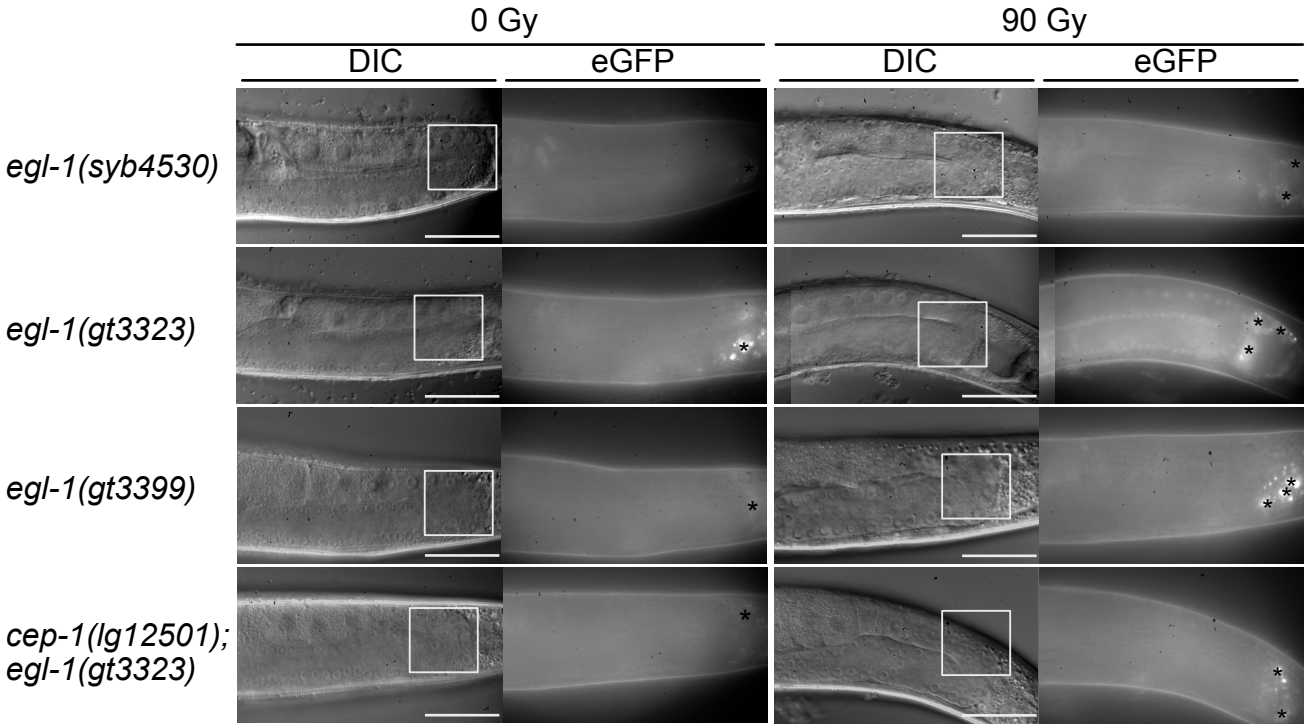
C



C'

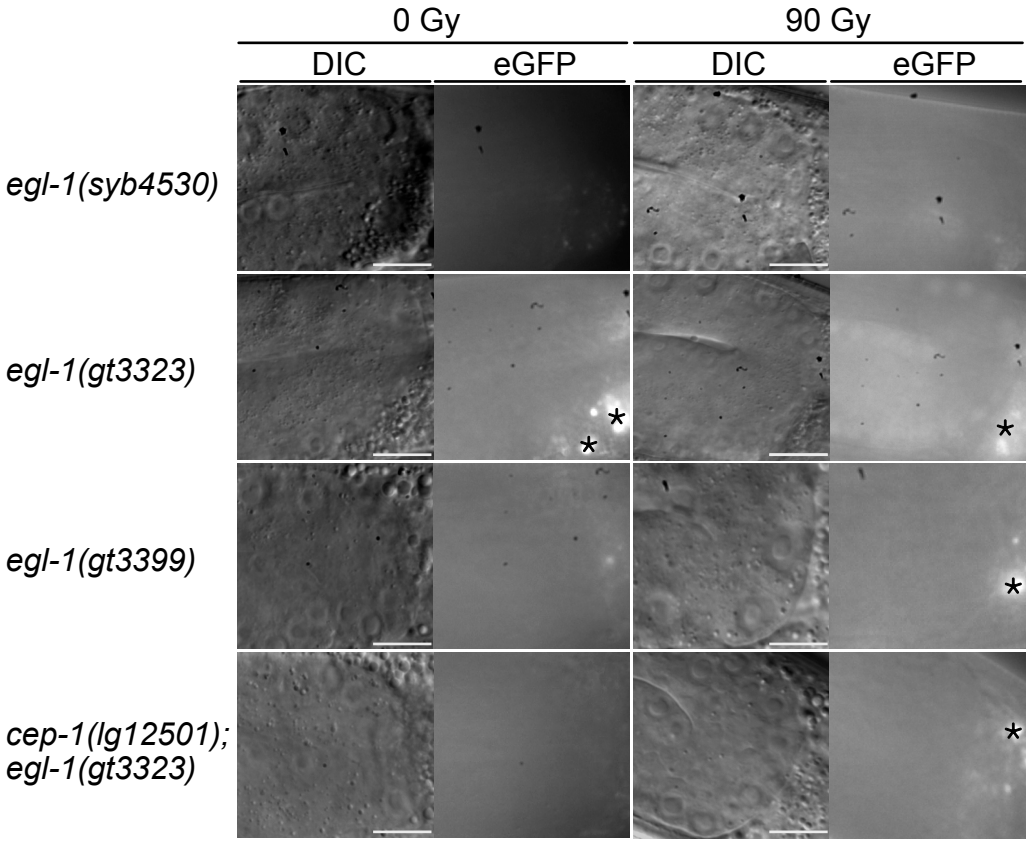


Figure S4: The *egl-1* intron1 is essential for the induction of *egl-1* transcription by IR. (A) Schematic of the transcriptional *egl-1* reporters, *egl-1(syb4530)*, *egl-1(gt3323)* and *egl-1(gt3399)*. The schematic of the *egl-1* wild-type gene (*egl-1*+/+) with intron1 (dotted line) and its putative CEP-1 binding sites is also indicated (red). The *egl-1(syb4530)* reporter was generated by replacing the entire coding sequence of *egl-1* with the *NLS::linker::eGFP::linker::NLS.* The *egl-1(gt3323)* reporter was generated by introducing the *NLS::linker::eGFP::linker::NLS* after the ATG of exon2 (exon1 and intron1 remain in this reporter). The *egl-1(gt3399)* reporter was generated by deleting intron1 from the *egl-1(gt3323)* reporter. **(B)** Alignment using Clustal Omega of intron1 of *egl-1* from *C. elegans* (*C.el*) and *C. briggsae* (*C.bri*). The putative CEP-1 binding sites, based on the consensus sequence are highlighted in red (73, 74) **(C)** Live imaging of the different reporter strains 24 hours post-IR with 0 Gy and 90 Gy. Insets show zoomed regions **(C')** outlined by white boxes (Scale bar 10 µm). As a control, we analyzed the *egl-1(gt3323)* reporter in the *cep-1(lg12501)* mutant background. Asterisks (*) mark intestinal autofluorescence.

Figure S5

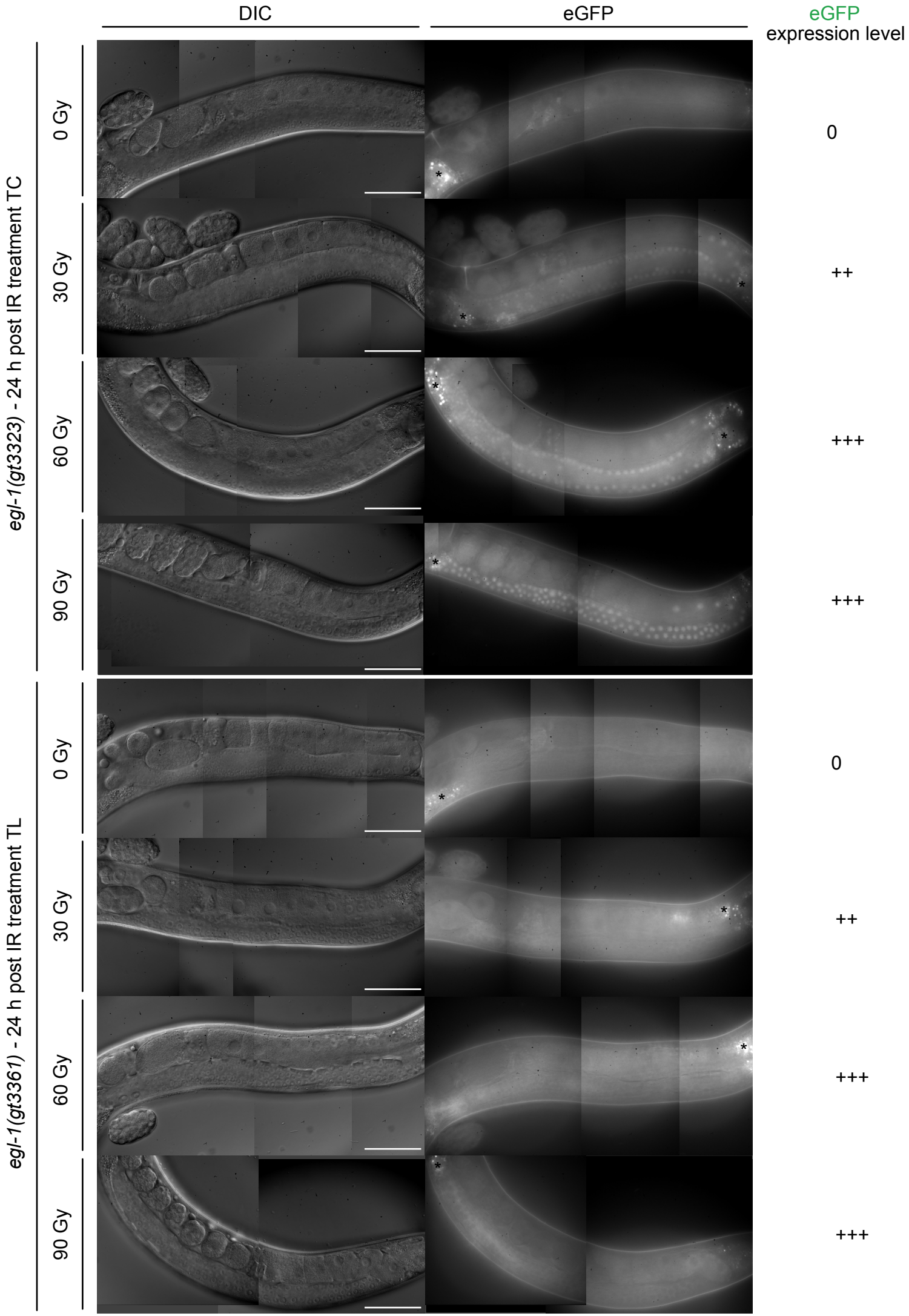

DIC
eGFP
eGFP expression level
egl-1(gt3323) - 24 h post IR treatment TC
0 Gy
30 Gy
60 Gy
90 Gy
0
++
+++
+++
egl-1(gt3361) - 24 h post IR treatment TL
0 Gy
30 Gy
60 Gy
90 Gy
0
++
+++
+++

Figure S5: The transcriptional *egl-1(gt3323)* TC and translational *egl-1(gt3361)* TL reporters are induced by IR in a dose-dependent manner. Representative DIC and eGFP images of germlines. Synchronized L4-staged worms were exposed to increasing doses of IR (0 Gy, 30 Gy, 60 Gy, and 90 Gy). Imaging was performed 24 hours post-irradiation. Scale bar 50 µm. Asterisks (*) indicate intestinal auto-fluorescence. Levels of eGFP expression are shown as: '0' = no, '+' = weak, '++' = medium, and '+++' = strong expression.

Figure S6

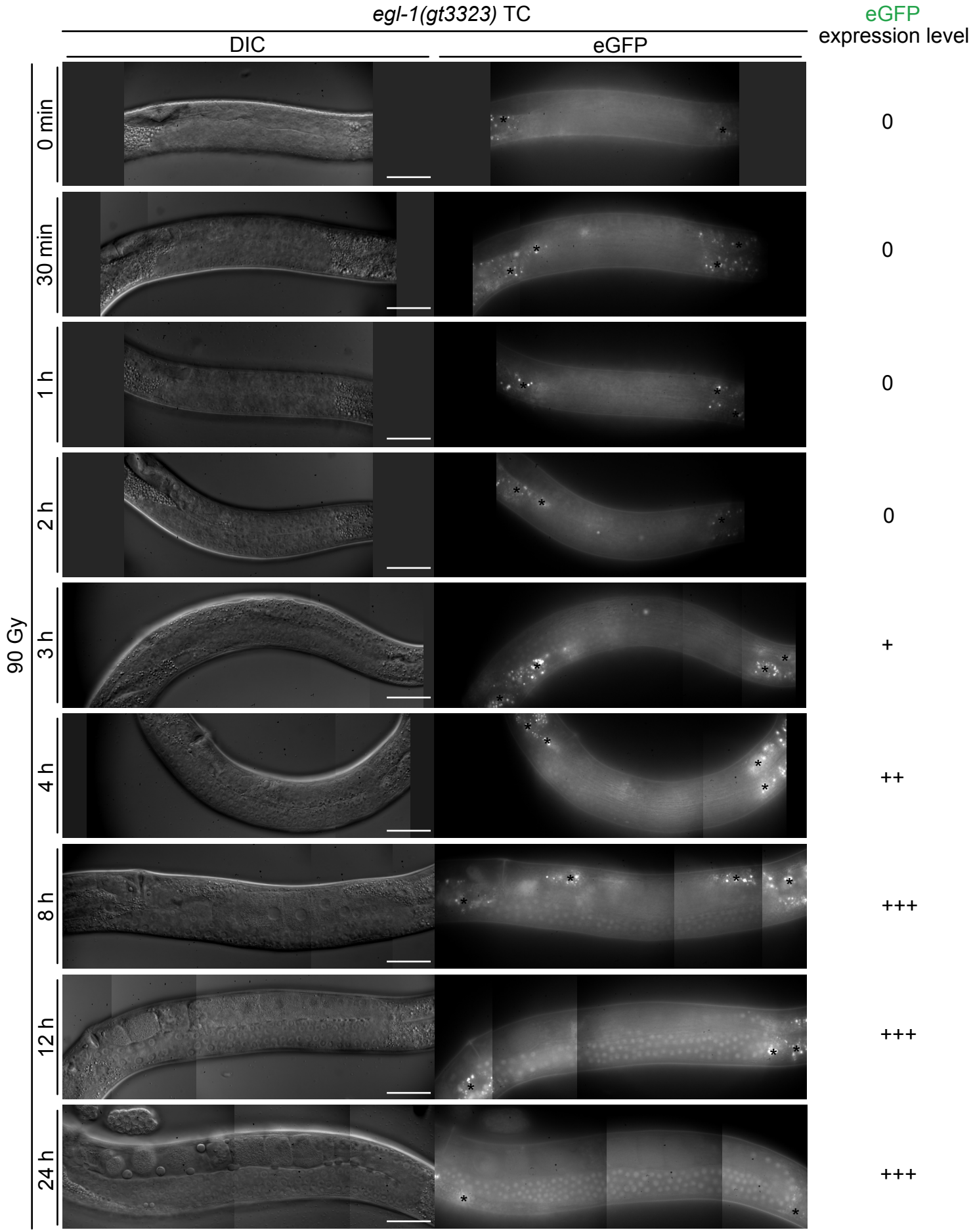

egl-1(gt3323) TC
DIC
eGFP
eGFP expression level
90 Gy
0 min
30 min
1 h
2 h
3 h
4 h
8 h
12 h
24 h
0
0
0
0
+
++
+++
+++
+++

Figure S6: The transcriptional *egl-1(gt3323)* TC reporter is induced by IR in a time-dependent manner. Representative DIC and eGFP images of germlines. Synchronized L4-staged worms were exposed to 0 Gy or 90 Gy dose of IR. Imaging was performed at different time points post-treatment (0.5, 1, 2, 3, 4, 8, 12, and 24 hours). Scale bar 50 µm. Asterisks (*) indicate intestinal auto-fluorescence. Levels of eGFP expression are indicted as: '0' = no , '+' = weak, '++' = medium and '+++' = strong expression

Figure S7

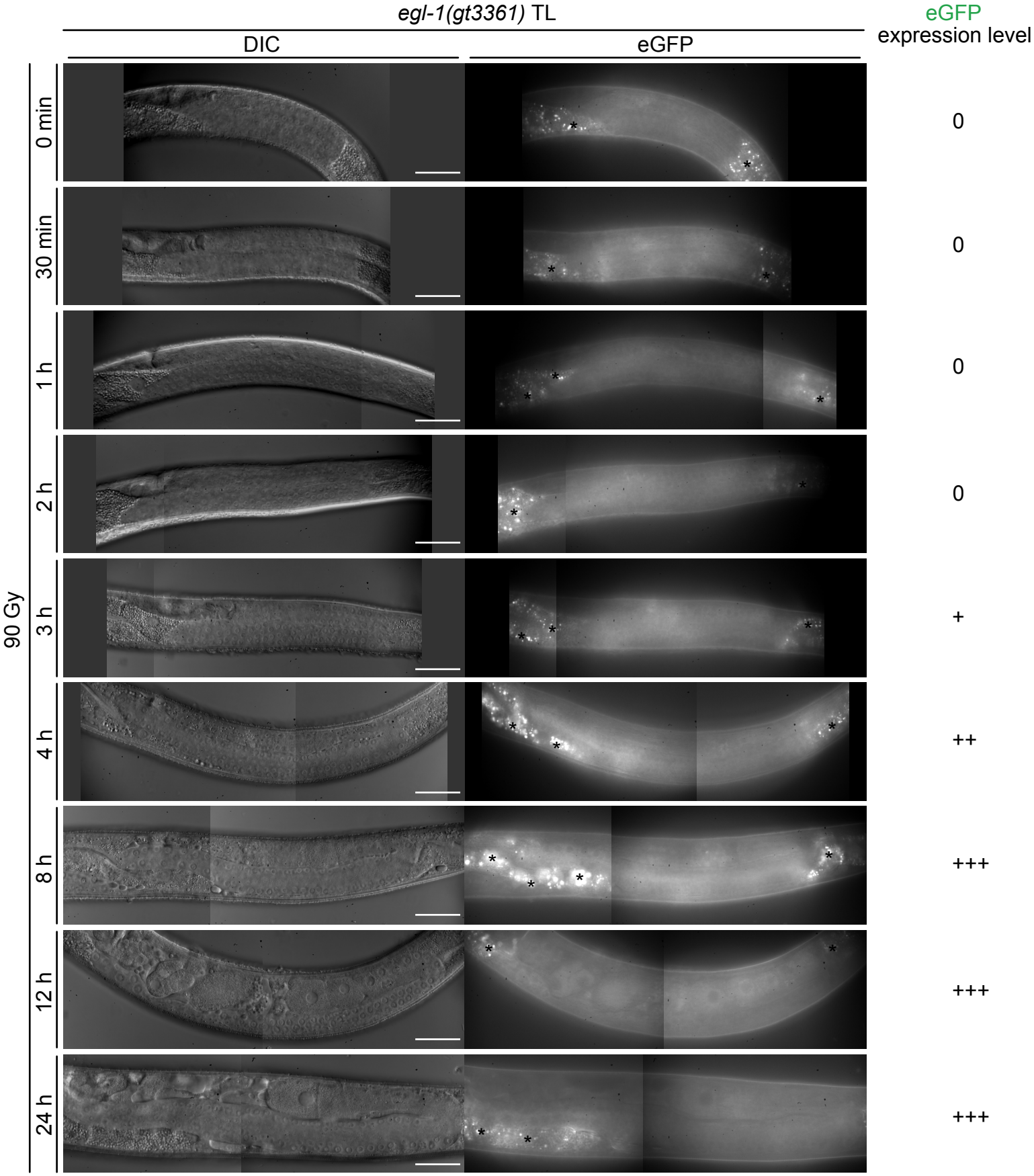

egl-1(gt3361) TL
DIC
eGFP
eGFP expression level
90 Gy
0 min
30 min
1 h
2 h
3 h
4 h
8 h
12 h
24 h
0
0
0
0
+
++
+++
+++
+++

Figure S7: The translational *egl-1(gt3361)* TL reporter is induced by IR in a time-dependent manner. Representative DIC and eGFP images of germlines. Synchronized L4-staged worms were exposed to 0 Gy or 90 Gy dose of IR. Imaging was performed at different time points post-treatment (0.5, 1, 2, 3, 4, 8, 12, and 24 hours). Scale bar 50 µm. Asterisks (*) indicate intestinal auto-fluorescence. Levels of eGFP expression are indicted as: '0' = no , '+' = weak, '++' = medium and '+++' = strong expression

Figure S8

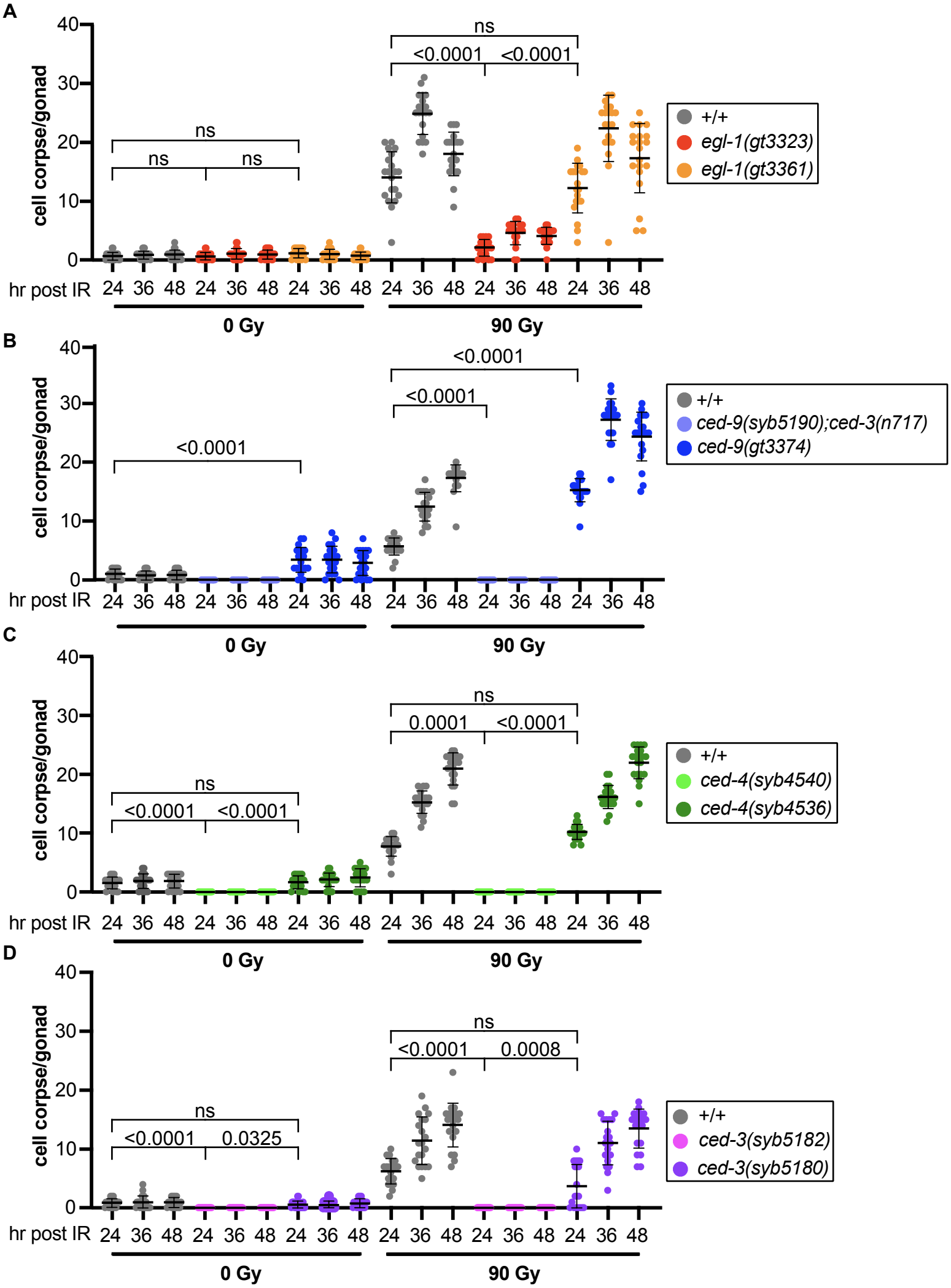

A
cell corpse/gonad
40
30
20
10
0
ns
ns
ns
ns
<0.0001
<0.0001
+/+
egl-1(gt3323)
egl-1(gt3361)
hr post IR
24 36 48 24 36 48 24 36 48 24 36 48 24 36 48 24 36 48
0 Gy
90 Gy
B
cell corpse/gonad
<0.0001
<0.0001
<0.0001
+/+
ced-9(syb5190);ced-3(n717)
ced-9(gt3374)
hr post IR
24 36 48 24 36 48 24 36 48 24 36 48 24 36 48 24 36 48
0 Gy
90 Gy
C
cell corpse/gonad
ns
<0.0001
<0.0001
ns
0.0001
<0.0001
+/+
ced-4(syb4540)
ced-4(syb4536)
hr post IR
24 36 48 24 36 48 24 36 48 24 36 48 24 36 48 24 36 48
0 Gy
90 Gy
D
cell corpse/gonad
ns
<0.0001
0.0325
ns
<0.0001
0.0008
+/+
ced-3(syb5182)
ced-3(syb5180)
hr post IR
24 36 48 24 36 48 24 36 48 24 36 48 24 36 48 24 36 48
0 Gy
90 Gy

Figure S8: Comparison of germline apoptosis in the different reporter strains. L4-staged worms were exposed to 0 Gy or 90 Gy of IR, and the number of germ-cell corpses per gonad arm was quantified at 24-, 36-, and 48-hours post-treatment using DIC optics. Wild type (+/+; gray) was included as a control in each experiment along with **(A)** the transcriptional *egl-1(gt3323)* (red) and translational *egl-1(gt3361)* (orange) reporters, **(B)** the transcriptional *ced-9(syb5190)* (light blue) and translational *ced-9(gt3374)* (blue) reporters, **(C)** the transcriptional *ced-4(syb4540)* (light green) and translational *ced-4(syb4536)* (green) reporters and **(D)** the transcriptional *ced-3(syb5182)* (pink) and translational *ced-3(syb5180)* (purple) reporters. (Mean ± SD is indicated, P values are indicated, ns: not significant. A two-tailed Mann-Whitney test was performed.)

Figure S9

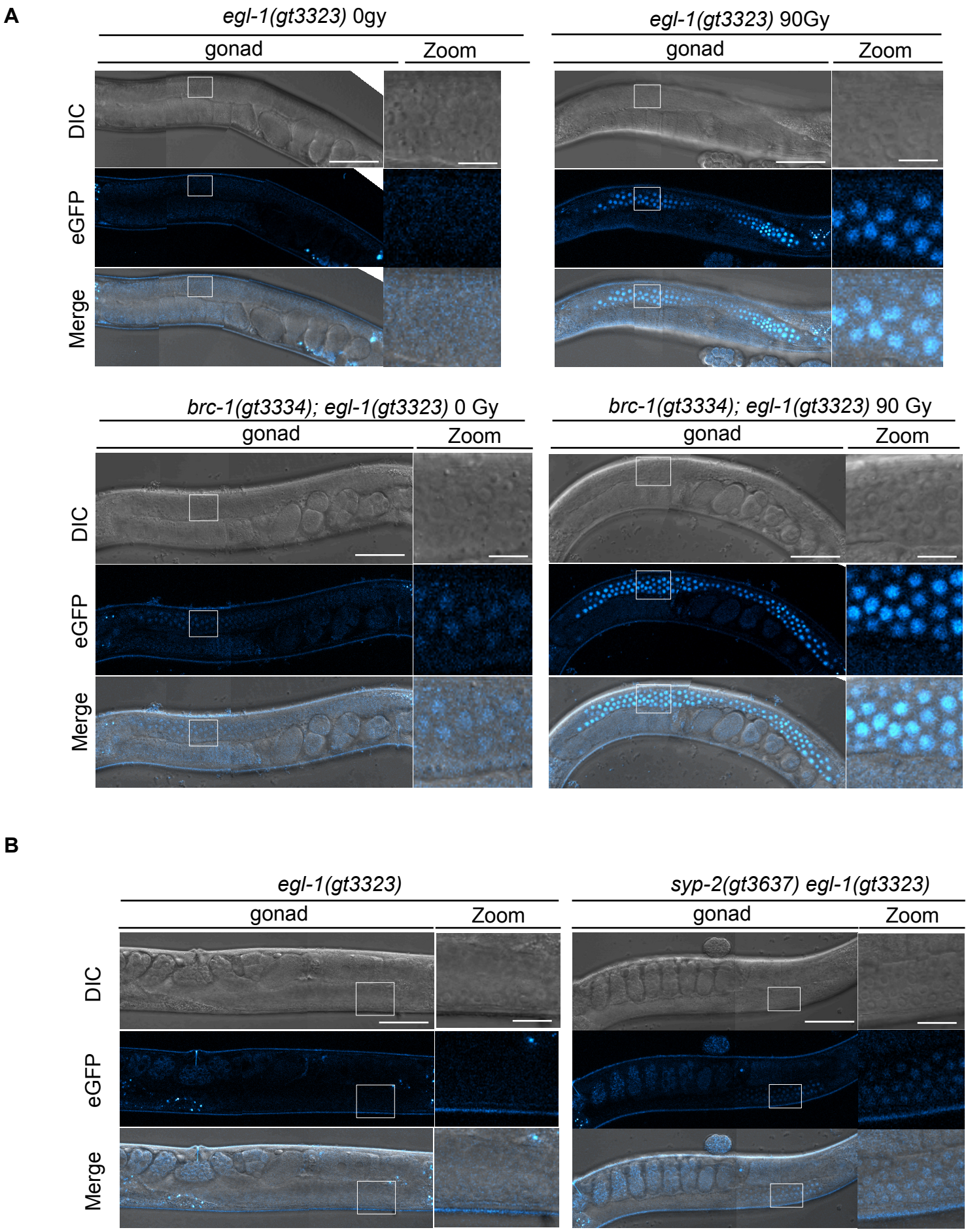

A
egl-1(gt3323) 0gy
gonad
Zoom
egl-1(gt3323) 90Gy
gonad
Zoom
DIC
eGFP
Merge
brc-1(gt3334); egl-1(gt3323) 0 Gy
gonad
Zoom
brc-1(gt3334); egl-1(gt3323) 90 Gy
gonad
Zoom
DIC
eGFP
Merge
B
egl-1(gt3323)
gonad
Zoom
syp-2(gt3637) egl-1(gt3323)
gonad
Zoom
DIC
eGFP
Merge

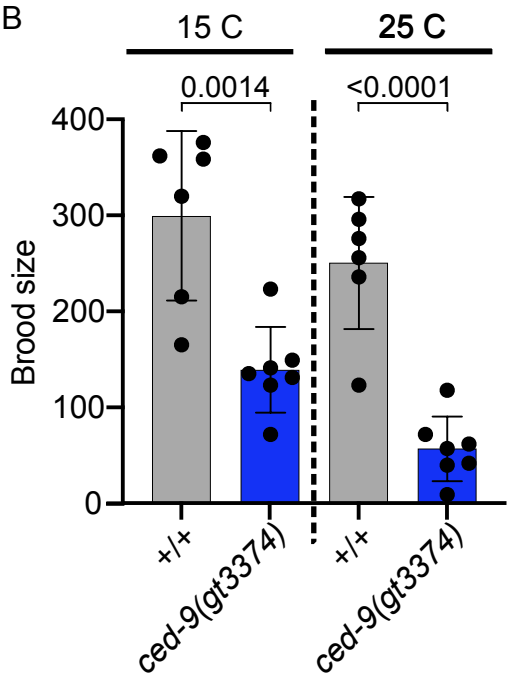
Figure S9: The transcriptional *egl-1(gt3323)* TC reporter is induced in the germline of *brc-1* and *syp-2* mutants. (A) Confocal live imaging of the *egl-1(gt3323)* and *brc-1(gt3323);egl-1(gt3323)* germlines, 24 hours post-IR treatment with 0 Gy or 90 Gy. IR treatment was performed at the L4 stage. **(B)** Confocal live imaging of the *egl-1(gt3323)* and *syp-2(gt3637) egl-1(gt3323)* germlines, 24 hours post-L4 stage, without IR treatment. Insets show zoomed regions outlined by white boxes in the main panels Scale bars: 10 µm (main panels), 2 µm (insets).

Figure S10

A

Genotype	cell death blocked in %	n
+/+	0	42
ced-3(n717)	100	31
egl-1(gt3323)	100	28
egl-1(gt3361)	0	28
ced-9(syb5190); ced-3(n717)	100	28
ced-9(gt3374)	0	28
ced-4(syb4540)	100	28
ced-4(syb4536)	0	28
ced-3(syb5180)	0	28

B



C

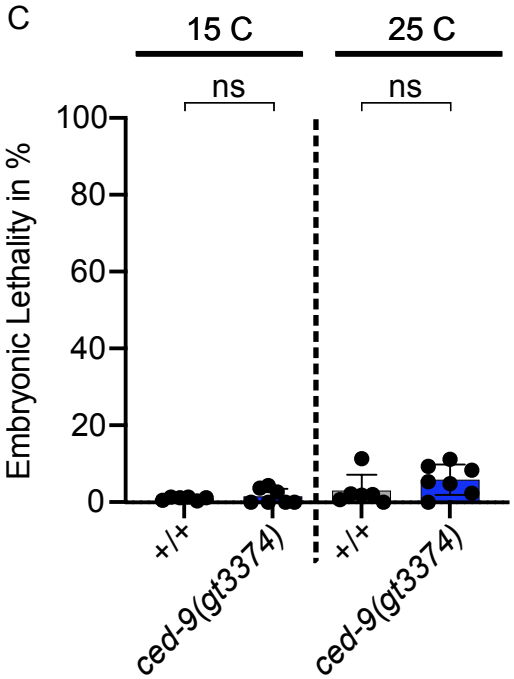
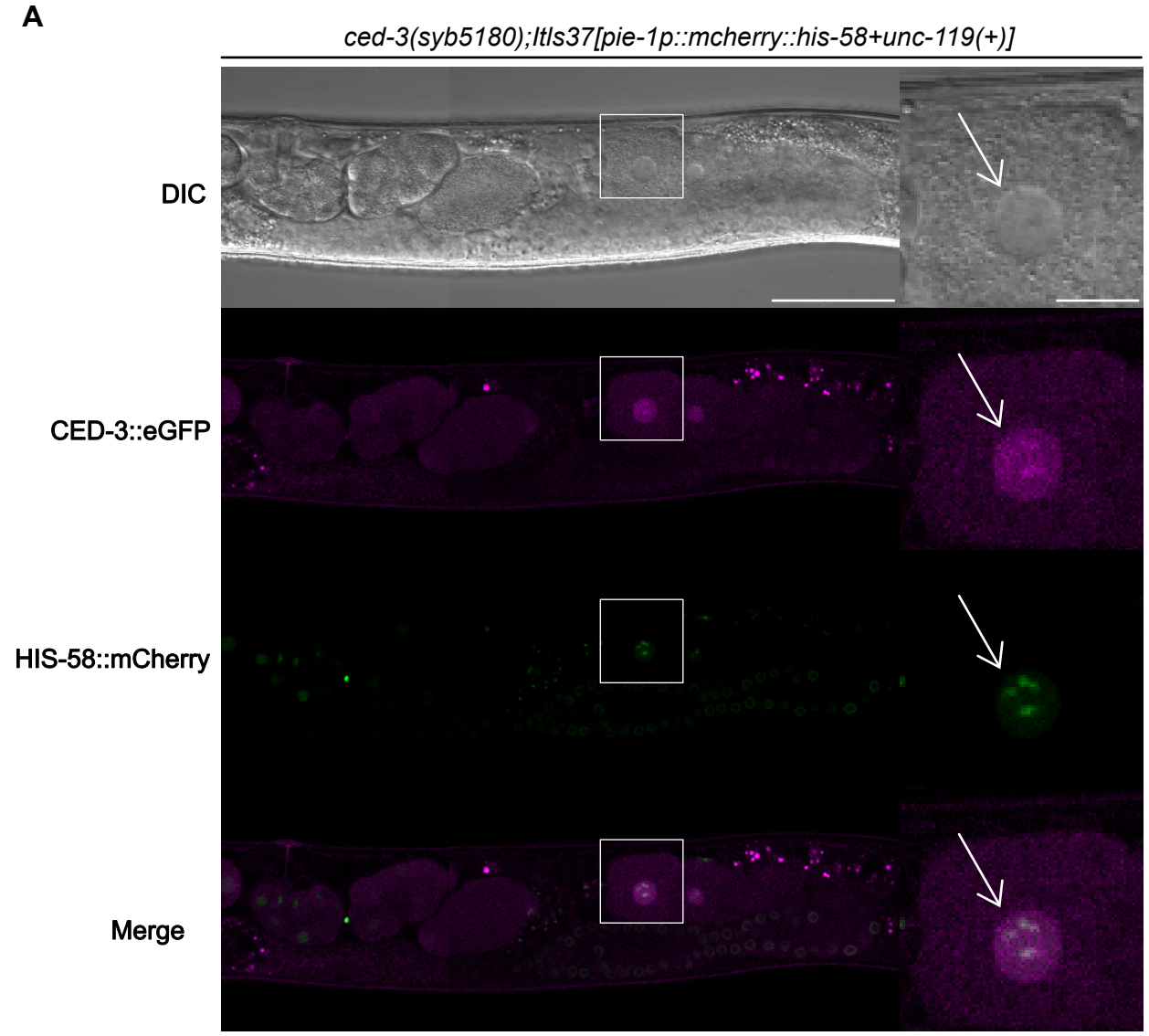


Figure S10: Lineage analysis of the first 14 apoptotic events in the *C. elegans* embryo. (A) The percentage of blocked apoptosis is indicated in the different genotypes. The number of cells analyzed is also indicated. For each reporter strain, two embryos were analyzed. **(B)** Brood size analysis of the translational *ced-9(gt3374)* TL reporter and wild type (+/+). **(C)** Embryonic lethality [%] of the translational *ced-9(gt3374)* TL reporter and wild type (+/+). (Mean ± SD is indicated. Statistical analyses of the brood size and embryonic lethality were performed using unpaired t-test and Mann-Whitney test, respectively).

Figure S11


A
ced-3(syb5180);ltls37[pie-1p::mcherry::his-58+unc-119(+)]
DIC
CED-3::eGFP
HIS-58::mCherry
Merge

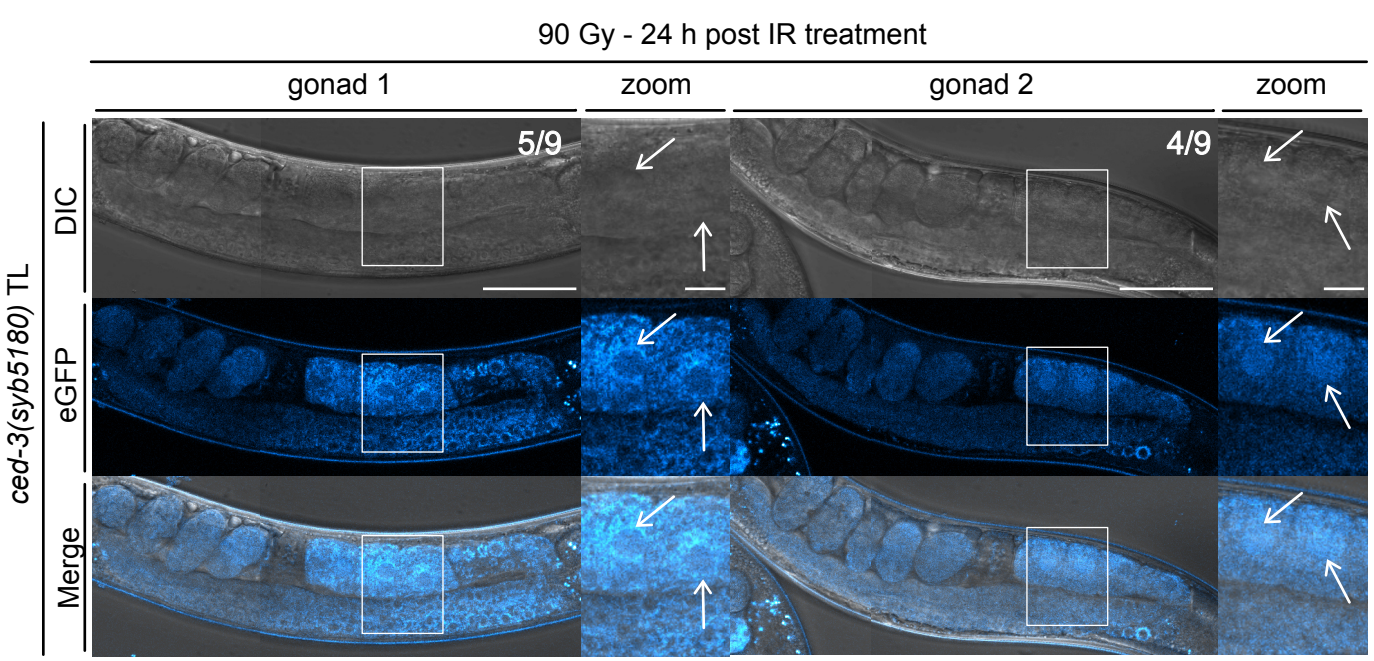

B
90 Gy - 24 h post IR treatment
gonad 1
zoom
gonad 2
zoom
5/9
4/9
ced-3(syb5180) TL
DIC
eGFP
Merge

Figure S11: Subcellular localization of CED-3. (A) Confocal imaging of the *Itls37[pie-1p::mcherry::his-58+unc-119(+)*]; *ced-3(syb5180)* TL reporter in the germline. The merged panel indicates the co-localization of CED-3 (magenta) with HIS-58 (green). The white box indicates the zoomed region, and a white arrow marks the nucleus of an oocytes. Scale bars: 50 µm (main panels), 10 µm (insets). **(B)** Representative confocal images of two *ced-3(syb5180*) animal germlines following IR treatment. The white box indicates the magnified region shown in the inset. White arrows mark nuclei of oocytes. The numbers correspond to the number of germlines analyzed. Scale bars: 50 µm (main panels), 10 µm (insets).

Figure S12

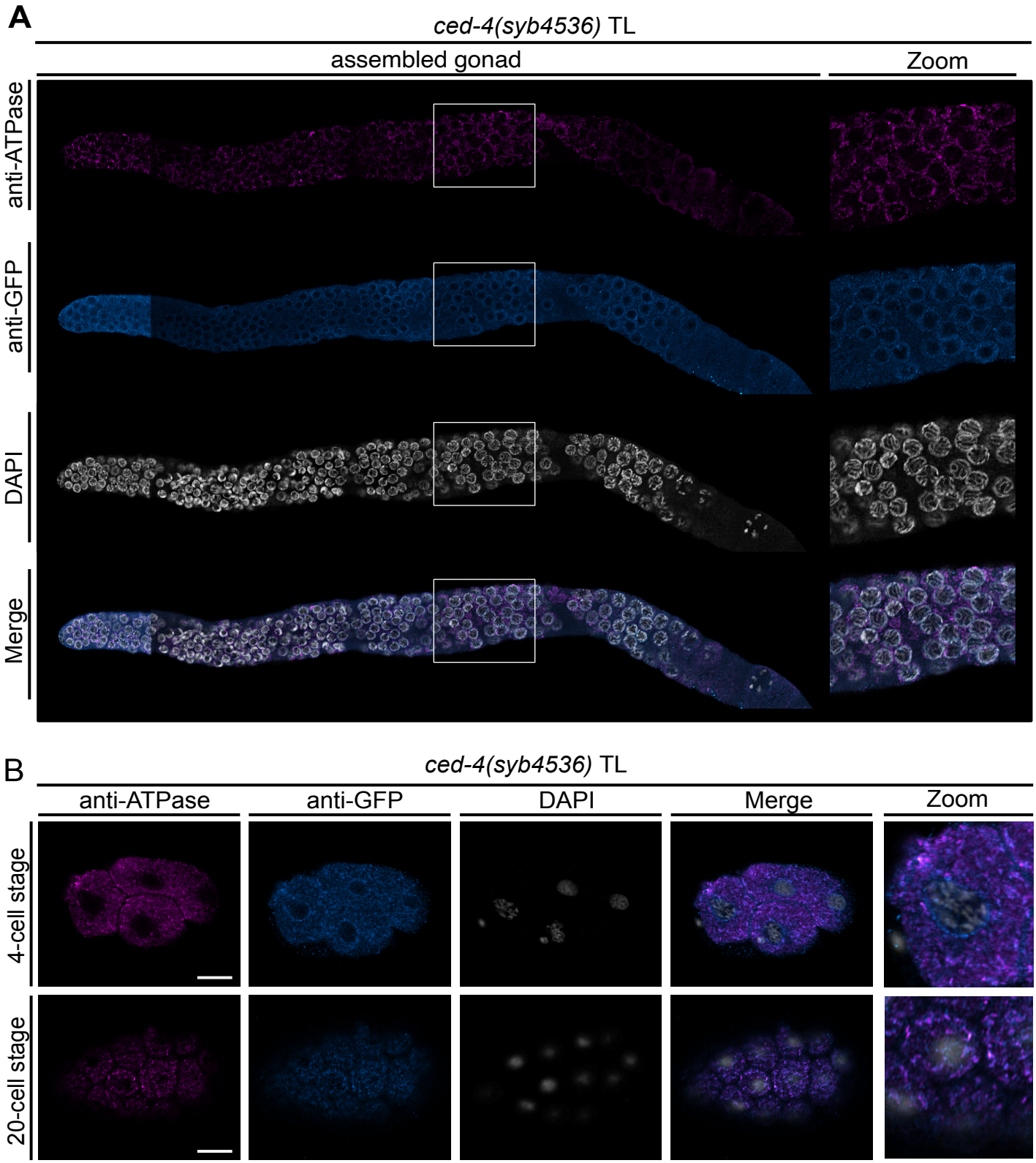

A
ced-4(syb4536) TL
assembled gonad
Zoom
anti-ATPase
anti-GFP
DAPI
Merge
B
ced-4(syb4536) TL
anti-ATPase
anti-GFP
DAPI
Merge
Zoom
4-cell stage
20-cell stage

Figure S12: Subcellular localization of CED-4. (A) Airyscan imaging of immuno-stained germlines of the translational *ced-4(syb4536)* TL reporter with anti-GFP, anti-ATPase and DAPI. **(B)** Airyscan imaging of immuno-stained embryos at different stages of the translational *ced-4(syb4536)* TL reporter with anti-GFP, anti-ATPase and DAPI. Scale bars: 10 µm (main panels), 2 µm (insets).

Figure S13

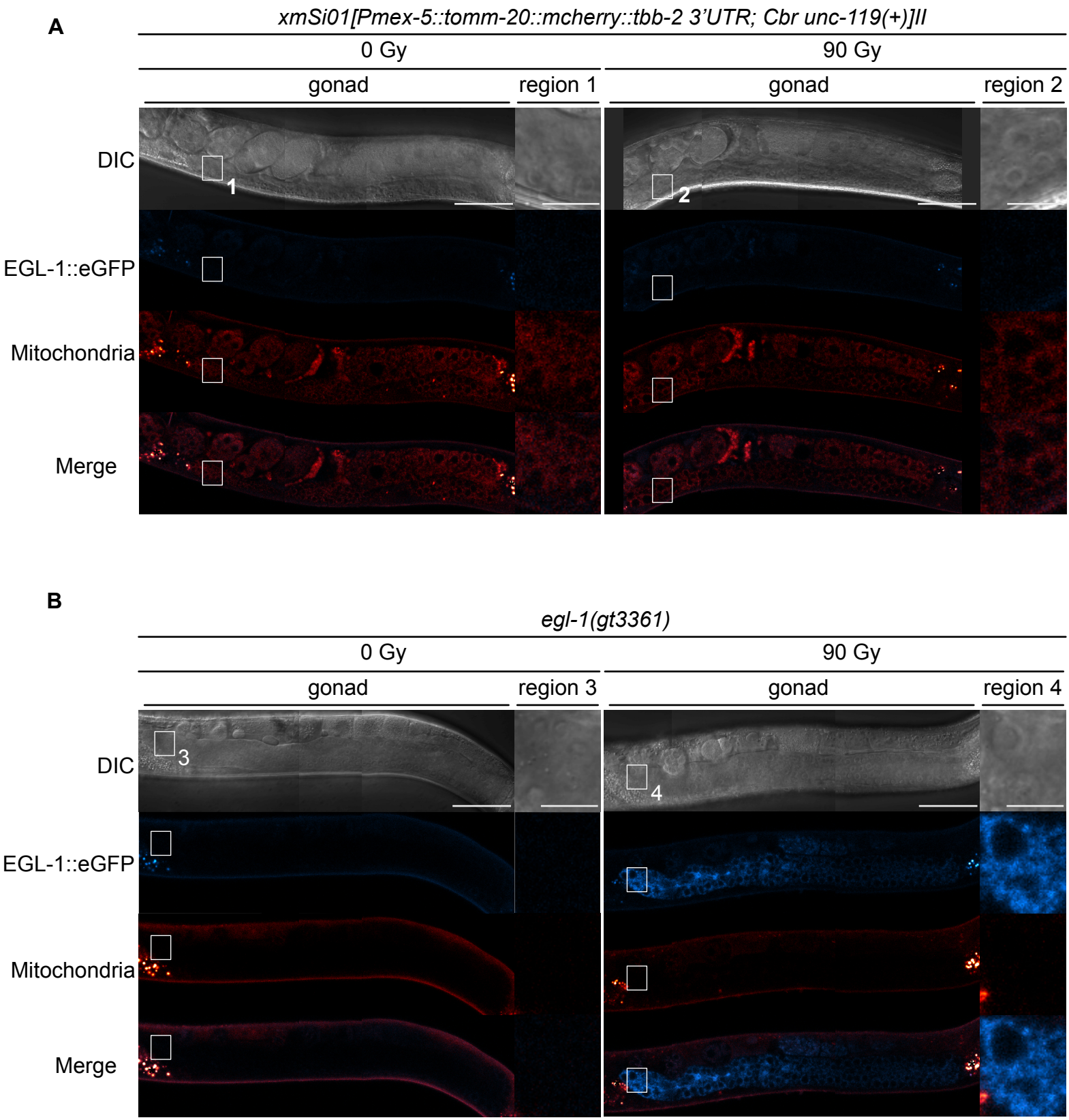

A
xmSi01[Pmex-5::tomm-20::mcherry::tbb-2 3'UTR; Cbr unc-119(+)]II
0 Gy
90 Gy
gonad
region 1
gonad
region 2
DIC
EGL-1::eGFP
Mitochondria
Merge
1
2
B
egl-1(gt3361)
0 Gy
90 Gy
gonad
region 3
gonad
region 4
DIC
EGL-1::eGFP
Mitochondria
Merge
3
4

Figure S13: Germline images of the controls used for the analysis (for Figure 4A). Confocal live imaging of the **(A)** mitochondrial reporter *xmSi01[Pmex-5::tomm-20::mCherry::tbb-2 3'UTR]* and **(B)** the translational *egl-1(gt3361)* reporter separately to ensure no crosstalk between the two channels. L4-staged worms were irradiated with a 90 Gy dose of IR, and images were captured 24 hours post-treatment. The white box indicates the magnified region shown in the inset. Scale bars: 50 µm (main panels), 10 µm (insets).

Figure S14

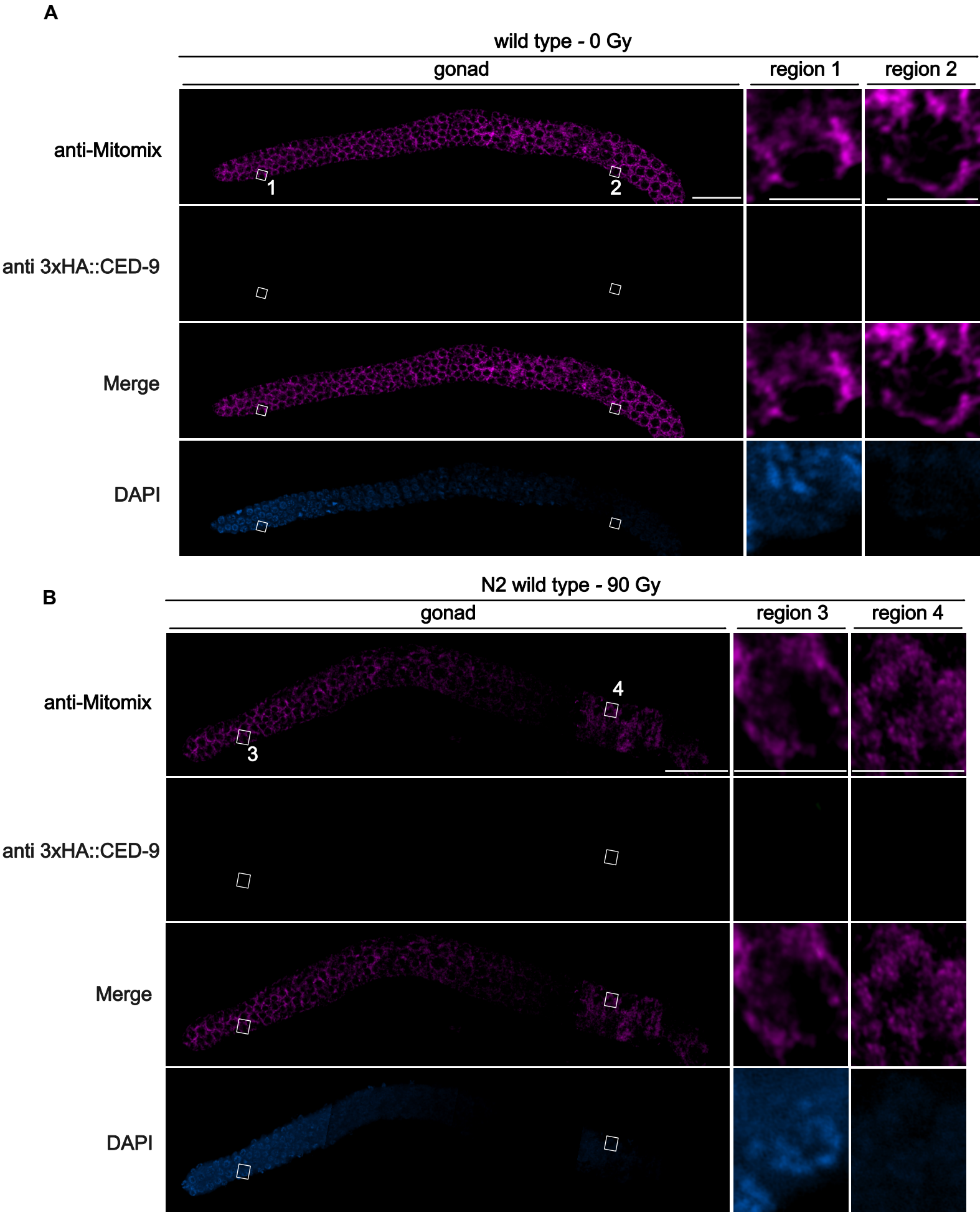

A
wild type - 0 Gy
gonad
region 1
region 2
anti-Mitomix
anti 3xHA::CED-9
Merge
DAPI
1
2
B
N2 wild type - 90 Gy
gonad
region 3
region 4
anti-Mitomix
anti 3xHA::CED-9
Merge
DAPI
3
4

Figure S14: Germline images of the N2 control used for the analysis (For Figure 7B). Airyscan images of the immuno-stained germlines of the N2 wild-type strain with anti-GFP, anti-HA, and DAPI. L4-staged worms were irradiated with a 90 Gy dose of IR, and images were captured 24 hours post-treatment. The white box indicates the magnified region shown in the inset. Scale bars: 50 µm (main panels), 10 µm (insets).

Supplementary Video 1

Time-lapse movie showing a small apoptotic corpse lacking EGL-1 entirely, consistent with physiological germ-cell apoptosis (as shown in Figure 2A,A')

Supplementary Video 2

Time-lapse movie showing a small apoptotic corpse with an increase in EGL-1, followed by a decrease (as shown in Figure 2B,B').

Supplementary Video 3

Time-lapse movie showing a large apoptotic corpse exhibiting a marked and sustained increase that culminates in EGL-1 hyperaccumulation (as shown in Figure 2C,C').

Supplementary Video 4

Time-lapse movie showing a large apoptotic corpse with moderate EGL-1 expression, which does not reach hyperaccumulated levels as in Supplementary Video 3 (as shown in Figure 2D,D').