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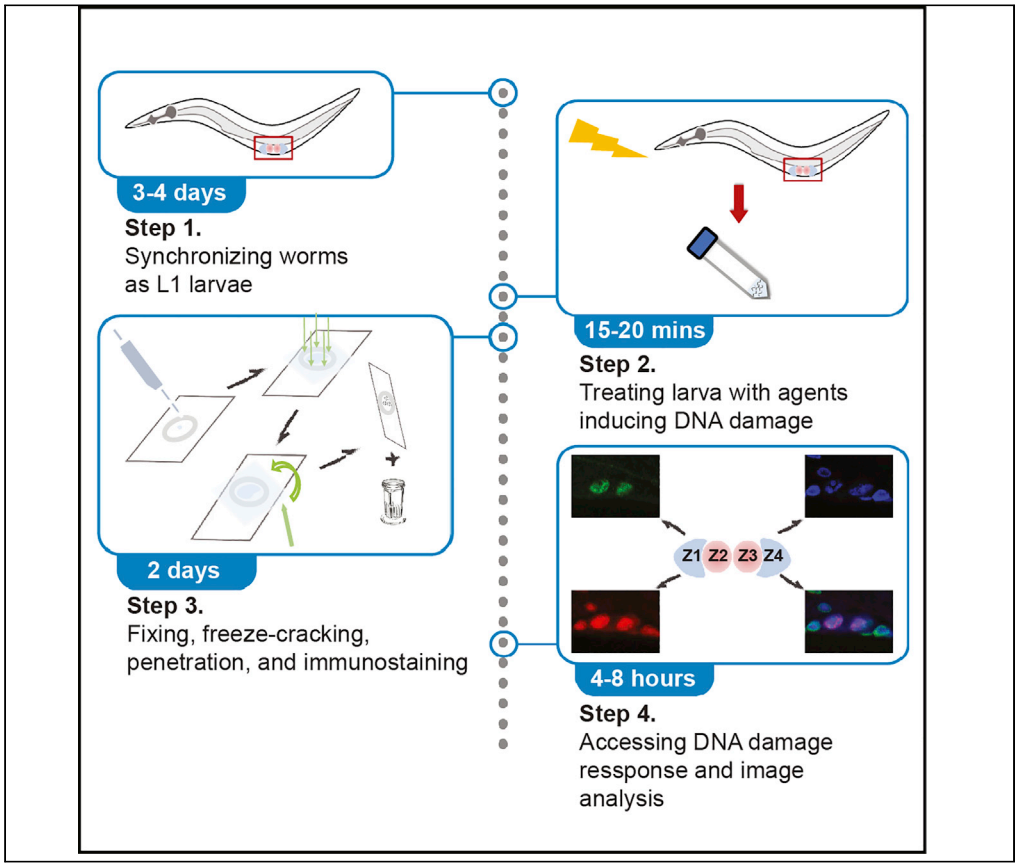
Evaluating DNA damage response through immunofluorescence staining of primordial germ cells in *Caenorhabditis elegans* L1 larva



C. elegans L1 larvae have two well-defined primordial germ cells embedded in a niche comprising two somatic gonad precursor cells. Thus, *C. elegans* provides an ideal model for studying intercellular signaling in response to DNA damage. However, existing staining protocols are focused on worms in later developmental stages and are not optimized for the L1 larvae. Here, we present a revised protocol for assessing the DNA damage response utilizing immunofluorescence staining specifically in *C. elegans* L1 larva.

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Highlights

Primordial germlines in *C. elegans* L1 larva as ideal stem cell-niche interaction models

Evaluating the intercellular DDR within microenvironment by PGC/SGP immunostaining

Optimized staining and quantification protocols for *C. elegans* L1 larva

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Evaluating DNA damage response through immunofluorescence staining of primordial germ cells in *Caenorhabditis elegans* L1 larva

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SUMMARY

***C. elegans* L1 larvae have two well-defined primordial germ cells embedded in a niche comprising two somatic gonad precursor cells. Thus, *C. elegans* provides an ideal model for studying intercellular signaling in response to DNA damage. However, existing staining protocols are focused on worms in later developmental stages and are not optimized for the L1 larvae. Here, we present a revised protocol for assessing the DNA damage response utilizing immunofluorescence staining specifically in *C. elegans* L1 larva.**

For complete details on the use and execution of this protocol, please refer to Ou et al. (2019).

BEFORE YOU BEGIN

The two PGCs (named Z2 and Z3) in *C. elegans* L1 larva are of special interest when it comes to developmental studies. The intercellular crosstalk within the microenvironment consisting of two SGPs (named Z1 and Z4) and PGCs can be revealed through immunofluorescence staining of proteins involved in the DNA damage response using worms synchronized at early L1 larval stage (Figure 1). To ensure optimized visualization of PGCs in L1 larva, P granule components found in the germ line cytoplasm such as PGL-1 and PGL-3 are frequently utilized as markers when performing co-staining (Kawasaki et al., 1998, 2004; Strome and Wood, 1982). In addition, the experiment should include appropriate strains as negative control of target proteins to help discriminate genuine signals from unexpected background noises.

Synchronizing worms at the L1 larval stage

Timing: 3–4 days

1. Obtain and grow worms on nematode growth medium (NGM) agar plates (e.g., around 1500 worms on a 10-cm NGM agar plate) seeded with OP 50 *Escherichia coli* lawn until half of the population are one-day or two-day adult worms.
2. Harvest worms by rinsing with M9 buffer (3–5 mL is sufficient for a 10-cm dish) in a sealed 15-mL tube and leave on the bench top for 10 min.

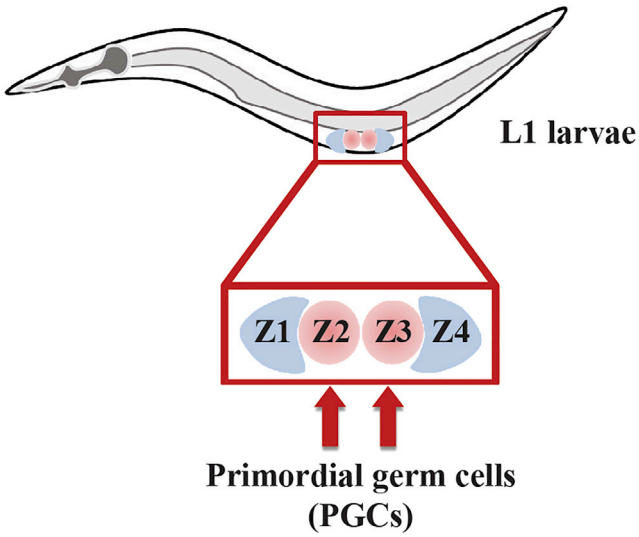

Figure 1. Scheme of primordial germ cells (PGCs) in *C. elegans* L1 larvae
Z1/Z4, somatic gonad precursor cells; Z2/Z3, PGCs.

3. Remove the supernatant carefully pipetting, avoid disturbing the worms settled at the bottom of the tube.
4. Wash worms with M9 buffer once or twice to eliminate any remaining bacteria.

   Note: Clear, not cloudy resuspension suggests an efficient bacteria removal after washing.

5. Resuspend settled worms with one volume of M9 buffer and one volume of 2× bleaching buffer.

   ⚠ CRITICAL: Bleaching buffer should be prepared freshly prior to the procedure in a fume hood and used within 4 h.

6. Vortex the mixture at the maximum strength (e.g., scale 10 using Vortex-Genie 2, Scientific Industries) until worms are lysed and embryos are released.
   a. Check the ruptured worms using stereomicroscope after 3 min vortex.
   b. Should any intact worm remain, continue vortexing for a few minutes, but no more than 6 min in total.

   ⚠ CRITICAL: Bleaching over 6 min may increase the incidence of dead embryos. Therefore, the bleaching procedure should ideally be completed in 5 min.

7. Precipitate released embryos by centrifuge at 1800 × *g* for 1 min, 20°C–22°C.

   Note: Centrifuge used in this protocol: Eppendorf 5810R with swing-bucket rotor.

8. Discard supernatant and wash embryos 4 times with M9 buffer, 10 mL each time.

   Note: Resuspend embryos by short vortexing at moderate speed for each wash.

9. After the final wash, resuspend embryos with 10 mL M9 buffer and leave on roller (20 rpm, Stuart™ SRT9D Roller Mixer) for at least 16-h hatching at 20°C.

   ⚠ CRITICAL: Instead of bleaching, L1 larvae can be obtained by filtering worm suspension through 11 μm Nylon Net filters; nevertheless, some late L1 worms might go through

the filter together with the desired early L1 worms, resulting in unsynchronized Z2/Z3 development in the worm population.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Goat anti-CEP-1 antibody	Generated and described by (Schumacher et al., 2005)	N/A
Rat anti-PGL-3 antibody	Provided by Prof. Susan Strome, University of California Santa Cruz, USA	N/A
Rabbit anti-histone H3 antibody	Abcam	Cat#ab1791 RRID: AB_302613
Rabbit anti-RAD-51 antibody	Novus Biologicals	Cat#29480002 RRID: AB_2284913
Donkey anti-goat IgG DyLight 488	Jackson ImmunoResearch	Cat#705-485-147
Donkey anti-goat IgG DyLight 594	Jackson ImmunoResearch	Cat#711-505-152
Donkey anti-rat IgG Alexa 488	Invitrogen	Cat#A21208 RRID: AB_141709
Donkey anti-rat IgG Alexa 594	Invitrogen	Cat#A21209
Donkey anti-rabbit IgG Alexa 594	Invitrogen	Cat#A21207 RRID: AB_141637
(store primary and secondary antibodies at −20°C and 4°C, respectively)		
Bacterial and virus strains		
OP50 *Escherichia coli*	Caenorhabditis Genetics Center	Ordering Strain Name: OP50-1
Chemicals, peptides, and recombinant proteins		
16% Paraformaldehyde (PFA)	Thermo Scientific	Cat#28906
Serva Agar	SERVA	Cat#11393.03
Bacto Peptone	Gibco	Cat#211820
CaCl2	Carl Roth	Cat#CN93.1
Cholesterol (dissolved in ethanol and stored at 4°C)	Sigma-Aldrich	Cat#C8503
EGTA	Millipore	Cat#324626
EDTA	Carl Roth	Cat#8043.2
HEPES	Sigma-Aldrich	Cat#H3375
KCl	Carl Roth	Cat# 6781.3
K_2HPO_4	Sigma-Aldrich	Cat#P8281
KH_2PO_4	Sigma-Aldrich	Cat#P0662
KOH	Sigma-Aldrich	Cat#221473
$MgSO_4$	Carl Roth	Cat# P027.3
NaCl	Sigma-Aldrich	Cat#S9888
$Na_2HPO_4 \cdot 2\ H_2O$	Carl Roth	Cat#4984.2
Nystatin (stored at −20°C)	Sigma-Aldrich	Cat#N9150
Sodium hypochlorite	Carl Roth	Cat#6846.2
Tween-20	Carl Roth	Cat#9127.1
(all solution dissolved in distilled water and stored at 20°C–22°C if not specified elsewhere)		
Experimental models: Organisms/strains		
C. elegans strain/BJS355 *xpc-1(tm3886)*	(Ou et al., 2019)	N/A
C. elegans strain/BJS68 *xpc-1(tm3886); cep-1(lg12501)*	(Ou et al., 2019)	N/A
C. elegans strain/BJS251 *xpc-1(tm3886); ife-4(tm684)*	(Ou et al., 2019)	N/A
C. elegans strain/BJS284 *xpc-1(tm3886); cep-1(lg12501); ife-4(tm684)*	(Ou et al., 2019)	N/A

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
ImageJ	Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA	http://imagej.nih.gov/ij/, 1997-2015
GraphPad Prism 6	GraphPad Software, La Jolla California, USA	www.graphpad.com
Other		
UVB irradiation source	Philips; Waldmann; UVP	Philips UV6 bulbs; Waldmann UV236B irradiation device; UVX digital radiometer; UVX-31 probe
HistoBond®$^{+}$ adhesive microscope slide (with 1 ring white)	Marienfeld, Germany	REF#0810461
Hydrophobic PTFE printed microscope slides with 3 × 14 mm wells	Fisher Scientific	Cat#50-292-15
Centrifuge with swing-bucket rotor	Eppendorf	5810R
Stuart™ Roller Mixer	Sigma-Aldrich	SRT9D
Vortex-Genie™ 2 vortex mixer	Fisher Scientific	Vortex-Genie™ 2
DAPI Fluoromount-G® mounting medium	SouthernBiotech	Cat#0100-20
Nylon net filter, hydrophilic, 11 µm, 47 mm	Millipore	Cat#NY1104700

MATERIALS AND EQUIPMENT

NGM agar plate	Amount (for 1 liter)
Bacto Peptone	2.5 g
NaCl	3 g
Serva Agar	17 g
Distilled H_2O	until 945.5 mL autoclaved (e.g., 121°C for 20 min) and cooled down to 56°C before adding the rest ingredients
1 M $CaCl_2$	1 mL
1 M $MgSO_4$	1 mL
5 mg/mL Cholesterol	1 mL
1 M KPO_4	25 mL
Nystatin	2.5 mL

Note: 1 M KPO_4 is prepared by combining 1 M K_2HPO_4 with 1 M KH_2PO_4 in a ratio of 18:1.

Note: Store NGM agar plates at 20°C–22°C in sealed boxes for usage within one week; otherwise store plates in sealed boxes at 4°C for up to one month.

M9 buffer	Amount (for 1 liter)
KH_2PO_4	3 g
$NaHPO_4$	6 g
NaCl	5 g
Distilled H_2O	until 1 liter autoclaved and cooled down to room temperature before adding the rest ingredients
1 M $MgSO_4$	1 mL

Note: Prepare and store M9 buffer at 20°C–22°C for up to one month.

Bleaching buffer (2×)	Amount (for 50 mL)
1 M KOH	12.5 mL
Sodium hypochloride	10 mL
H_2O	until 50 mL

⚠ CRITICAL: Bleaching buffer should be prepared freshly prior to the procedure in a fume hood and used within 4 h.

Fixing buffer	Amount (for 50 μl)
10× Egg buffer	5.5 μl
10% Tween-20	0.5 μl
16% Paraformaldehyde (PFA)	9.5 μl
Distilled H_2O	34.5 μl

⚠ CRITICAL: Prepare the fixing buffer fresh at 20°C–22°C and use within one day.

10× Egg buffer	Amount (for 10 mL)
HEPES pH 7.4	250 mM
NaCl	1.18 M
KCl	480 mM
EDTA	20 mM
EGTA	5 mM
Distilled H_2O	until 10 mL

Note: Store the 10× Egg buffer at 20°C–22°C for constant use (up to 12 months) or store at −20°C for long-term storage (up to 3 years, avoiding freeze-thaw cycle).

PBST buffer	Amount (for 1 liter)
KH_2PO_4	240 mg
$NaHPO_4$	1.44 g
NaCl	8 g
KCl	200 mg
Distilled H_2O	until 1 liter autoclaved and cooled down to room temperature before adding the rest ingredient
Tween-20	1 mL

Note: Prepare and store the PBST buffer at 20°C–22°C for constant use (up to 6 months).

STEP-BY-STEP METHOD DETAILS

L1 larvae treatment and fixation

Timing: 15–20 min

The following procedure is optimized for investigating the early time window of PGC development with or without DNA damaging agents (e.g., UVB irradiation, 25 mJ/cm^2). In response to UV treatment, for instance, the growth of L1 worms may be retarded when nucleotide excision repair machinery is compromised as in the case of *xpc-1* mutant strain. In general, the more advanced the worm develop, the easier the fixative step.

1. Synchronize worms with genetic background of interest at the L1 larval stage before subjected to treatment, e.g., UVB irradiation.
   a. Plate fixed number of L1 worms (suspended in M9 buffer) in NGM plates corresponding to the area of plates. For example, a 3.5-cm NGM plate can reside 6000 L1 worms.
   b. Once the buffer dries and worms settled, initiate treating process as required and incubate worms at 20°C after feeding worms with concentrated OP 50.

   Note: Nutritional cues are essential for initiating the development of PGCs (Fukuyama et al., 2006; Sulston et al., 1983).

2. Harvest worms at the desired time point by rinsing plates with M9 buffer and collect in a sealed 15-mL tube.
3. Centrifuge at 800–1000 × *g* for 2–3 min to spin down worms.

   ⚠ CRITICAL: Centrifuge at lower speed/shorter time may lead to lower harvest of worms/samples while centrifuge at higher speed/longer time may retain too much bacteria that interferes with further procedure and causes strong background after staining.

4. Remove supernatant as much as possible without disturbing settled worms.
5. Prepare Fixing buffer according to volume required and aliquot 5 μl in a 0.2-mL tube.

   ⚠ CRITICAL: Prepare the fixing buffer fresh at 20°C–22°C and use within one day.

   Alternatives: 10× Egg buffer can be replaced with aforementioned M9 buffer.

6. Transfer 5 μl of worms to the 0.2-mL tube containing Fixing buffer and mix gently with pipetting 5 times.
7. Transfer 5 μl of mixture to a HistoBond®$^{+}$ Adhesion Microscope slide (with 1 ring white) (Figure 2A, left panel).

   Note: We recommend using slides with printed rings or wells in order to avoid crashing worms when applying pressure on specimens in the following step.

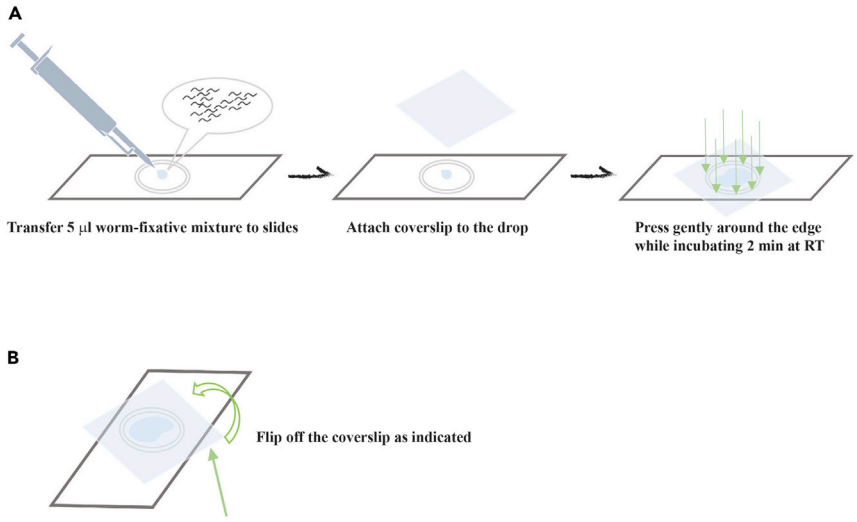

Figure 2. Demonstration of critical steps in immunostaining of L1 larva

(A) Fixation of L1 larva. Once applying the coverslip, press gently around the edge as indicated by the green arrows.

(B) Permeabilization of specimen with freeze-cracking. The slide should be frozen for performing freeze-cracking. Flip off the coverslip in the direction indicated using fingers.

Alternatives: This protocol also works with hydrophobic PTFE printed microscope slides with 3 × 14 mm wells (Fisher Scientific) that are pretreated with 0.3% poly-L-lysine.

8. Attach a 24 × 24-mm coverslip to the drop, preferably unparallel to the slide (Figure 2A, middle panel), and press around the edge (white ring) while incubating 2 min at 20°C–22°C (Figure 2A, right panel).

⚠ CRITICAL: Apply the coverslip carefully to avoid air bubbles, which will reduce the contact between slide surface and worms, decreasing the worm attachment as well as the efficiency of permeabilization via freeze-cracking.

⚠ CRITICAL: Gently press the coverslip around the ring during the 2-min incubation. This help L1 worms to attach better to the slide, hence reducing potential loss during further steps. Do not let the coverslip-slide slip over one another, which twists the worms (Troubleshooting 1).

9. Move slides carefully into the box with dry ice and leave for at least 10 min or until thoroughly frozen and proceed to the next step.

Note: Do not replace dry ice with liquid nitrogen as bubbles generated in the latter might disturb the slides, resulting in cracking of the coverslips.

Note: Despite being a "Pause point" in the general practice of immunostaining worms in later developmental stages, we recommend strongly to proceed directly to the following steps, avoiding storing samples at −80°C, which might potentially compromise the sample.

Freeze-cracking and penetration

Timing: 40 min

Immunostaining relies on antibody recognizing and binding to specific antigens. The cuticle of nematodes, however, becomes a barrier for efficient antibody penetration to the tissue. Combining methanol treatment and freeze-cracking facilitates permeabilization while minimizing morphological disruptions when performing staining in L1 larva. For more detailed discussion and application of freeze-cracking in immunostaining, please refer to a comprehensive review for freeze-cracking (Duerr, 2013).

10. Prechill 100% methanol in a coplin jar (50–100 mL depending on the jar size) at −20°C.
11. Take out frozen slide (one each time), and flip the coverslip using fingertip (Figure 2B).

⚠ CRITICAL: Freeze-crack the slide when it is still frozen; defrosted slide is not optimal for this step (Troubleshooting 2).

12. Immediately immerse the slide in the ice cold methanol and incubate for 10 min.

Note: Methanol is toxic. Please handle under a fume hood with gloves.

Note: Carefully place following slides to prevent them from overlapping with one another.

13. Remove slides from the methanol and wash them in a staining container with PBST buffer for 10 min.
14. Repeat washing with refreshed PBST two more times to remove any residual methanol.

Blocking, antibody incubation, and mounting

Timing: 2 days

As soon as the fixation and permeabilization is completed, the slides are now ready for antibody incubation. To eliminate unspecific binding and thereby unfavorable background, a blocking step with normal serum depending on the host species of secondary antibodies, e.g., donkey serum in this protocol, is strongly recommended prior to incubation with primary antibodies (Troubleshooting 3).

15. Wipe slides with kimwipes around the white ring without disturbing the attached worms.

Note: This prevents the solution from dispersing all over the slide during the blocking procedure.

16. Prepare a humid chamber, ideally a light-protective chamber, with damped paper towels.
17. Place wiped slides in the chamber with specimen side facing up and block with 50 μl PBST containing 10% donkey serum for 30 min at 20°C–22°C.
18. Binding of primary antibodies:
    a. Dilute primary antibody in PBST with 10% donkey serum. In this case, CEP-1 antibody (Schumacher et al., 2005) is used for the detection of p53 homolog in worms with the dilution of 1:300 while PGL-3 is used as markers of PGCs with the dilution of 1:10000.
    b. Alternatively, RAD-51, a pivotal factor involved in homologous recombination (Baumann and West, 1998), can be used for detecting the presence of double-stranded breaks with the dilution of 1:350.
    c. Use 50 μl per slide for incubation in a humid chamber at 4°C for at least 16 h.
19. Wash slides in a staining container with PBST buffer for 10 min.
20. Repeat the washing with refreshed PBST two more times for 3 washes in total.
21. Wipe slides with kimwipes around the white ring to remove excessive solution.
22. Binding of secondary antibodies:
    a. Dilute secondary antibodies in PBST with 10% donkey serum according to the corresponding primary antibodies and detecting channels desired. For example, we use anti-goat IgG DyLight 594 and anti-rat IgG Alexa 488 for detecting CEP-1 and PGL-3, respectively.
    b. All secondary antibodies are used with the dilution of 1:350.
    c. Use 50 μl per slide for incubation in a humid and light-protective chamber at 20°C–22°C for 2 h.
23. Wash slides in a staining container with PBST buffer for 10 min × 3 times.
24. Wipe slides with kimwipes around the white ring to remove excessive solution.
25. Place a coverslip with thickness compatible for confocal microscopy [#1.5 (0.17 mm)] on a flat surface.
26. Transfer around 15 μl of DAPI Fluoromount-G® mounting medium on top of the coverslip laying on a steady rack.

Alternatives: Histone H3 (1:100 dilution) can be used for replacing DAPI as a co-staining control for intensity normalization.

27. Apply the slide with specimen side gently on the mounting medium until the medium disperse over the whole area.
28. Place the mounted slide with specimen facing-up in a light-protective chamber or drawer. Leave for 24 h or until the mounting medium is dry.
29. Seal the slides with durable sealing reagent such as coverslip sealant or nail polish. The slides are then ready for microscopy imaging.

Note: To preserve the signals of immunostaining, store slides light-protected at 4°C if imaged in few days, or store slides at −20°C for extended storage.

EXPECTED OUTCOMES

As previously published, CEP-1 is specifically expressed in PGCs (Figure 3A, left panel). DAPI staining can be used for normalization. Alternatively, the ubiquitously expressed histone H3 allows assessing the penetrance of antibodies (Figure 3A) and provides an optimal intensity normalization. Co-staining with PGC marker - PGL-3 further facilitates the identification of PGCs (Figure 3B), when the damage signals, e.g. RAD-51, are not confined to single cell types. It is normal to have some ruptures of worms (Figure 3B, red arrow) that may sometimes foster antibody penetrance. However, these ruptures also generate unspecific, disturbing signals under circumstances where antibodies, either primary or secondary, interact with exposed tissues. In terms of high fluorescence background after staining, please refer to the troubleshooting section for solution (Troubleshooting 4).

QUANTIFICATION AND STATISTICAL ANALYSIS

For evaluating the results of the CEP-1 immunofluorescence staining assay, we first manually select PGCs using the polygon selection tool and measure the fluorescence intensity of both CEP-1 and

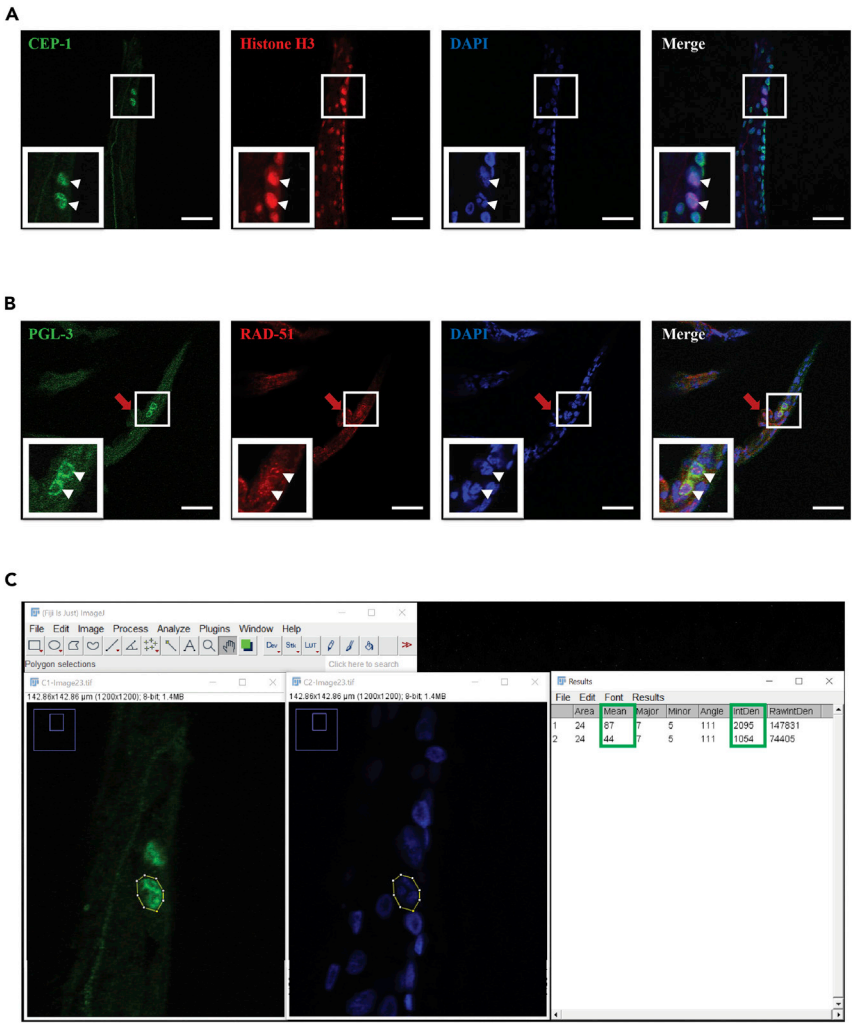

Figure 3. Expected results and data analysis

(A) Representative images of co-staining CEP-1 with histone H3 in *xpc-1;ife-4* mutant 16 h-post UV treatment.

(B) Representative images of co-staining PGC marker PGL-3 with RAD-51 in *xpc-1* mutant 16 h-post UV treatment.

(C) Demonstration of PGC quantification using ImageJ. Scale bars represent 20 μm; white triangles mark the PGCs; the red arrows in the panel (B) indicate the rupture of worm; green rectangles in the panel C indicate the intensity measurement for quantification and normalization.

DAPI (or the co-stained histone H3) with the analyze > measure function in ImageJ (Figure 3C, be aware that exact same area is selected in both channels, (Troubleshooting 5)). Final value is the calculated results by normalizing the CEP-1 fluorescence intensity with corresponding DAPI intensity (or the intensity of co-stained histone H3) before plotted using GraphPad Prism 6. For each group, 15–30 images (worms) are analyzed per experiment.

LIMITATIONS

The current protocol is suitable not only for the detection of target proteins using immunofluorescence staining, but also for the visualization of GFP- or mCherry-conjugated proteins. We suggest, however, to use an additional secondary antibody to amplify the signals of tagged proteins, especially for those generated via CRISPR-based method.

Due to the nature of this procedure, rupture of worms is sometimes unavoidable. Despite rupture of worms favoring antibody penetrance, occasionally the original organization of Z2/Z3 PGCs might be disturbed and thereby impedes their identification. Hence, co-staining with PGC markers such as PGL-1 or PGL-3 is highly recommended for facilitating the PGC identification.

In order to stain the cell markers with proteins of interest, it is desirable to perform a multi-staining at once. Nonetheless, in our hands, we achieved only double-staining successfully. An improved combination/availability of antibodies usable in the *C. elegans* larvae would further expand the potential use of this protocol for evaluating the DNA damage response.

TROUBLESHOOTING

Problem 1

Twisted/broken worms (step 8–11)

Potential solution

Any accidental movement between slide and coverslip will twist the worms, resulting in broken worms when imaging. First of all, use dry ice (ideally with a flat surface) as liquid nitrogen sparkles upon contacting with slides. Bubbles generated within may disturb the coverslip with shear forces. Second, continue with the following steps right after freezing slides on dry ice instead of storing specimens at −80°C for later use. This would reduce the possibility of movement/damage between slide and coverslip.

Problem 2

Few worms remained on the slide after freeze-cracking (step 11)

Potential solution

This might derive from several reasons. First ensure the washing step is complete because remaining bacteria decrease the contact of worms with the coated slide and thereby the attachment. When pressing the coverslip, make sure the pressure is sufficient (yet not too much to totally smash the worms), which shall foster the attachment of worms on the slide. Last but not the least, perform the freeze-cracking when the slide is still frozen. Defrosted slides will not provide the strength to open worm cuticles when freeze-cracking. In extreme scenarios, attempting to freeze-crack a defrosted slide might lead to immense loss of worms.

Problem 3

Optimized selection of blocking buffer (step 17)

Potential solution

For the general practice of blocking, normal serum ranging from 5% to 10% v/v from the same host species as the fluorophore-labeled secondary antibody should work effectively. Taking example of this protocol, we used 10% donkey serum (diluted with PBS) as blocking buffer because all secondary

antibodies utilized therein are raised in donkey. Therefore, the IgG in donkey serum can occupy sticky sites on the sample, preventing any non-specific binding of labeled secondary antibodies. In contrast, do not use the normal serum from the same host species as the primary antibody for blocking as it leads to higher background when sticky points being recognized by secondary antibodies throughout the sample.

Problem 4

Strong background after immunostaining (Imaging and expected outcome)

Potential solution

Remaining bacteria due to incomplete wash will glow under fluorescent microscopy, rendering unfavorable background noise when imaging. Throughout the whole procedure, do not let the specimen dry out; otherwise the specimen might be auto-fluorescent when imaging. It is highly recommended to block the slides with 10% donkey serum (diluted with PBS) for at least 30 min at 20°C–22°C before incubating with primary antibodies. The selection of secondary antibodies may also influence the overall background. If applicable, perform a pilot experiment for optimizing the antibody combinations beforehand.

Problem 5

Quantification and normalization

Potential solution

In order to precisely quantify the PGC signals in different channels, use the polygon selection tool in ImageJ and carefully select the PGC, each PGC at one time in one channel. Once selected, click Analyze > Measure to obtain the mean intensity or integrated density of the selected area in the indicated channel. To ensure the exact same area is selected across channels, copy the selected area and paste it in a different channel where ImageJ will automatically copy the background together with the selection frame into the next channel. Click the window of the next channel and by clicking Edit > Undo the selection frame will remain while the carry-over background being eliminated. Now the exact same area can be utilized for measuring the fluorescent intensity of the desired PGC across different channels.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Prof. Dr. Björn Schumacher (bjoern.schumacher@uni-koeln.de).

Materials availability

Requests for materials mentioned in this study should be directed to and will be fulfilled by the lead contact, Prof. Dr. Björn Schumacher (bjoern.schumacher@uni-koeln.de).

Data and code availability

Requests for raw data mentioned in this study should be directed to and will be fulfilled by the lead contact, Prof. Dr. Björn Schumacher (bjoern.schumacher@uni-koeln.de) or the technical contact, Dr. Hui-Ling Ou (hlo30@cam.ac.uk).

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AUTHOR CONTRIBUTIONS

Scientific conceptualization, H.-L.O. and B.S.; experimental design, H.-L.O.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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