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# 1 Interplay between ionizing radiation effects and aging in *C. elegans*

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## 11 Abstract

12 Living species are chronically exposed to environmental ionizing radiations from sources that can be  
13 overexpressed by nuclear accidents. In invertebrates, reproduction is the most radiosensitive studied  
14 endpoint, likely to be connected with aging. Surprisingly, aging is a sparsely investigated endpoint  
15 after chronic ionizing radiation, whereas understanding it is of fundamental interest in biology and  
16 medicine. Indeed, aging and aging-related diseases (e.g., cancer and degenerative diseases) cause  
17 about 90 % of deaths in developed countries. Therefore, *glp-1* sterile *Caenorhabditis elegans*  
18 nematode was used to assess the impact of chronic gamma irradiation on the lifespan. Analyses were  
19 performed, at the individual level, on aging and, in order to delve deeper into the mechanisms, at the  
20 molecular level, on oxidative damage (carbonylation), biomolecules (lipids, proteins and nucleic  
21 acids) and their colocalization. We observed that ionizing radiation accelerates aging (whatever the  
22 duration (3 to 19 days)/dose (0.5 to 24 Gy)/dose rate (7 and 52 mGy.h<sup>-1</sup>) tested) leading to a longevity  
23 value equivalent to that of *wt* nematode (~25-30 days). Moreover, the level of protein oxidative  
24 damage (carbonylation) turned out to be good cellular biomarker of aging, since it increases with age.  
25 Conversely, chronic radiation treatments reduced carbonylation levels and induced neutral lipid  
26 catabolism whatever the dose rate and the final delivered dose. Finally, under some conditions a lipid-  
27 protein colocalization without any carbonyl was observed; this could be linked to yolk accumulation in  
28 *glp-1* nematodes. To conclude, we noticed through this study a link between chronic gamma exposure,  
29 lifespan shortening and lipid level decrease associated with a decrease in the overall carbonylation.

30

## 31 **1. Introduction**

32 As nature provided environmental chronic ionizing radiations that can be overexpressed by nuclear  
33 accidents, e.g., Fukushima [1], environmental species are chronically exposed to ionizing radiation.  
34 Reported effects on gamma ray exposure are heterogeneous, depending on the mode of irradiation, and  
35 suffer from a lack of knowledge regarding the impact of chronic gamma irradiation. For many  
36 invertebrates, reproduction is the most radiosensitive studied parameter, after both acute and chronic  
37 irradiation [2-4]. This also includes roundworm *Caenorhabditis elegans* (*C. elegans*), where reduction  
38 of the progeny number is observed after both acute and chronic irradiation, whereas the hatching  
39 success decreases after acute irradiation [5-8]. While germline ablation has been shown to impact  
40 lifespan, a reproduction impairment could also influence the aging process and, finally, the longevity.  
41 Aging is considered to be a complex process involving both several molecular pathways and stochastic  
42 events for its modulation [9]. Energy resources are of crucial importance for biomolecular fitness,  
43 which slows down the aging process. There is a constant trade-off between investment in somatic  
44 maintenance and reproduction. Indeed, in organisms such as *C. elegans*, the reproduction has been  
45 proven to be an energetically expensive process with profound metabolic effect on the lifespan [10].  
46 Moreover, germline ablated nematodes showed a significantly prolonged lifespan of 60 %, and some  
47 of these conditional mutants are well suited to study the biochemistry of aging [11]. *C. elegans* is one  
48 of the premier metazoan models extensively used to investigate the molecular mechanisms of aging  
49 [12]. Despite the apparent complexity of aging, experiments performed on the *C. elegans* model have  
50 helped to outline the few key mechanisms that affect lifespan [13]: germline ablation [14], mTOR  
51 pathway [15] and IIS pathway [16]. Some of the transcription factors that are involved in lifespan  
52 extension by germline elimination are DAF-2, DAF-12 and DAF-16-, which are also involved in the  
53 IIS pathway [17]. Moreover, another gene-block is known to regulate worm development, phenotype  
54 and lifespan, e.g., clock genes (*clk-1*, *clk-2*, *clk-3* and *gro-1*) [18]. Numerous mechanisms, like  
55 autophagy and proteostasis control [19], modulate aging and influence these genes. Finally, various  
56 genetic factors, e.g., the mammalian tumour suppressor p53 ortholog CEP-1, were reported to regulate  
57 aging and induce an adaptive response in *C. elegans* [20].

58 The most popular theory of aging, called the “free-radical theory of aging” (FRTA), is based on  
59 free-radical induced oxidative damage. Reactive Oxygen Species (ROS) appear to be an important  
60 signalling intermediate, whereas mitochondria are crucial organelles for life and aging by hosting  
61 numerous metabolic processes, particularly electron transport and ATP-production [21]. Various  
62 biomolecular targets have been recognized to be involved in the molecular processes that regulate  
63 aging. Recently, it was shown that epigenetic drift, mediated by various PTMs such as DNA  
64 methylation and histone modification, impacts gene expressions and biomolecular interactions, which  
65 in turn finally affects aging [22]. Moreover, as the aging proceeds, massive metabolome and proteome  
66 changes that include decreased abundance of proteins acting for transcription termination, mRNA

67 degradation, mRNA stability, protein synthesis, and proteasome function [23] occur. Furthermore, a  
68 decrease in protein turnover has been identified as one of the biomarkers of aging with no relation to  
69 the protein abundance over time [24]. In the course of aging, there is an exponential increase of  
70 oxidative damage in proteins of human fibroblasts between the ages of 17 and 80 [25], while lipid  
71 membrane modifications have been proposed to play a major role in the process of aging [26].  
72 Elevation of lipid droplets (highly conserved neutral lipid storage), can cause various metabolic  
73 syndromes, including accelerated aging. One of the most studied oxidative damages to proteins is  
74 carbonylation [27]. Protein carbonylation is an irreversible post-translational modification, and is also  
75 an aging and oxidative stress biomarker [28]. Protein carbonylation is not an enzymatic process and  
76 can be induced via four chemical mechanisms [29].

77 Since the FRTA theory of aging and gamma ionizing radiation are mainly mediated through ROS  
78 generation, they can share some similar mechanisms. An old theory [30] recalled recently [31], stated  
79 that ionizing radiation accelerates the process of aging by sharing ROS intermediates and similar  
80 mechanisms [32]. Given that aging is a poorly investigated endpoint after chronic exposure to ionizing  
81 radiations, a conditional sterile *glp-1 C. elegans* mutant (a dedicated model for lifespan studies) was  
82 used to assess the impact of chronic gamma irradiation on its lifespan without any influence of either  
83 reproduction or progeny. Glp-1 regulates both somatic and germline growth and also differentiation in  
84 *C. elegans*, whereas the *glp-1* sterile mutant facilitates the study of radiation effects on a *C. elegans*  
85 cohort that does not reproduce. To test the impact of the irradiation period, the dose, and the dose rate,  
86 chronic gamma radiation exposure was performed at 2 different dose rates, i.e., 7 and 52 mGy.h<sup>-1</sup>,  
87 during 2 different periods, i.e., from eggs to young adult stage (64 h), and from eggs to old stage  
88 (19 days), with recovery conditions. This was accompanied by non-irradiated control, in order to  
89 follow the normal aging process. The lifespan, the levels of carbonyls, i.e., carbonylation of proteins,  
90 and of biomolecules like proteins, lipids and nucleic acids, were monitored under all of these  
91 conditions. Conversely, despite already known radioinduced apoptosis [33, 34], DNA damage was not  
92 analyzed here mainly because DNA repair, which is a rapid process, and apoptosis are expected to be  
93 low in germline-less *glp-1 C. elegans*.

## 94 2. Results

### 95 2.1. Impact of low chronic gamma irradiation on the lifespan

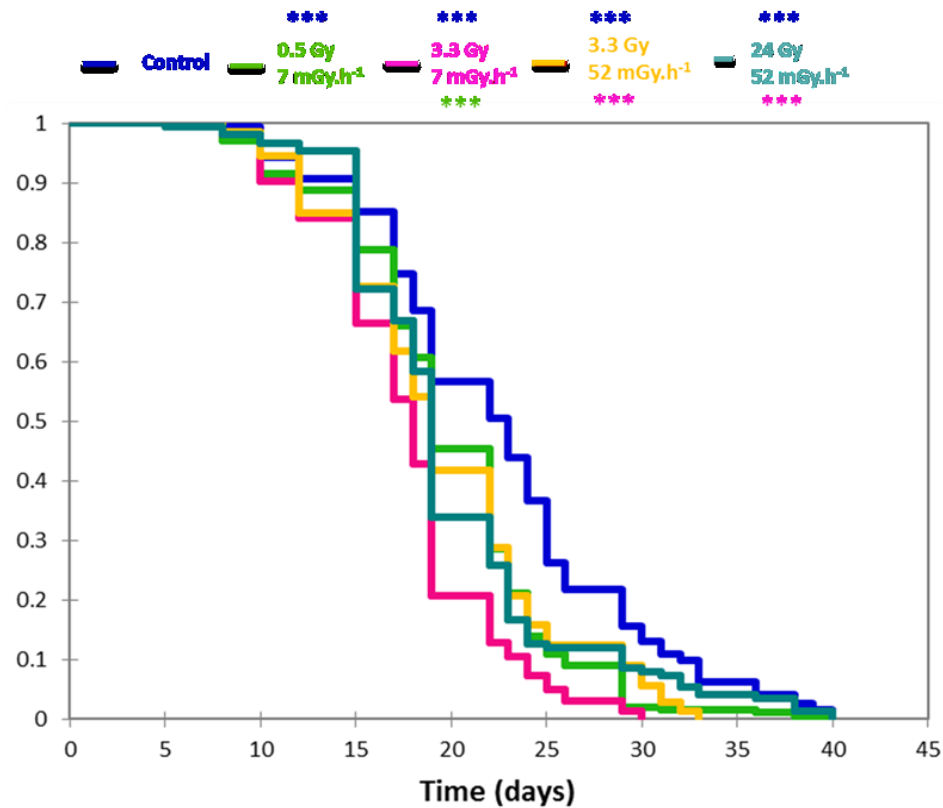
96 We used the *glp-1* mutant strain of *C. elegans*, which are sterile at a restrictive temperature (25°C).  
97 Together with control experiments, we performed chronic gamma ray irradiations (as described in the  
98 Materials and Methods section, summary in **Figure S1**) with two different dose rates of 7 and 52  
99 mGy.h<sup>-1</sup> and during two different periods of life, as follows:

- 100 - Gamma irradiation from eggs to young adult stage (i.e., 64 hours of development time,  
101 equivalent to the 1<sup>st</sup> day of adulthood);
- 102 - Gamma irradiation from eggs to old stage (i.e., 19 days, which is equivalent to the 17<sup>th</sup> day of  
103 adulthood).

104 The aim of the study was, firstly, to compare the effects induced by these 2 irradiation dose rates  
105 (given that chronic exposure conditions are being used) for a same period of exposure (eggs to L4,  
106 given 2 different doses) and also for a same dose, i.e., 3.3Gy (at the old stage, with one exposure  
107 ending earlier, yielding recovery groups). As shown in **Figure 1**, we noticed a highly significant  
108 lifespan shortening (Log-Rank  $p < 0.0001$ ) upon low chronic gamma irradiation in comparison with  
109 non-irradiated controls, which showed an expected lifespan as previously reported [11]. Moreover, we  
110 also noticed that the highly significant lifespan shortening was observed whatever the dose rate, the  
111 final dose or the duration of irradiation (**Fig. 1**). More precisely, the irradiation condition of 7 mGy.h<sup>-1</sup>  
112 differed significantly from all of the other irradiation conditions assessed (**Fig. 1**) and showed a  
113 maximum lifespan shortening effect. This result indicates that the “long term irradiation” treatment  
114 (which spans 19 days) with a dose of 7 mGy.h<sup>-1</sup> may induce the maximal shortening effect on the  
115 animal’s lifespan expectancy and differs significantly from the other irradiation treatments.  
116 Furthermore, we observed that a higher dose did not result in a worsening effect on the lifespan  
117 reduction. Accordingly, as shown in **Fig. 1a and b**, a “long-term irradiation” with a higher dose rate  
118 like 52 mGy.h<sup>-1</sup> did not result in a greater shortening of the lifespan expectancy. To complete the  
119 irradiation effect on the worm population, we performed a “recovery treatment”, which involves  
120 gamma ray irradiation from eggs to young adult stage (over a development period spanning 64 h)  
121 followed by a recovery period, until animal death. We then observed that the animals of the “recovery  
122 treatment” groups, irradiated with either 7 or 52 mGy.h<sup>-1</sup>, were not significantly different from each  
123 other (**Fig. 1b**). Altogether, these results could indicate that gamma ray irradiation can induce a  
124 phenomenon with a binary response without any dose response relationship, i.e., as soon as *C. elegans*  
125 have been irradiated at the egg stage, the lifespan has been shortened and nearly equals that of N2 wild  
126 type *C. elegans* (**Figure S2**). To compare, the lifespan of N2 *C. elegans* exposed to chronic gamma  
127 irradiation from eggs to L4 stage with the dose rate of 52 mGy.h<sup>-1</sup> did not show a significant difference  
128 relative to the unirradiated control ones (Kaplan-Meier analysis, P-value = 0.075) (**Fig. S2**). To

129 investigate the underlying mechanisms in order to explain our results, we monitored the assay at the  
130 molecular levels.

(a)



(b)

Dose	0.5 Gy	3.3 Gy	3.3 Gy	24 Gy
Dose rate	7 mGy.h <sup>-1</sup>	7 mGy.h <sup>-1</sup>	52 mGy.h <sup>-1</sup>	52 mGy.h <sup>-1</sup>
Condition	Recovery	Radiation	Recovery	Radiation
0				
0	<0.0001	<0.0001	<0.0001	<0.0001
Control				
0.5 Gy				
7 mGy.h <sup>-1</sup>	/	<0.0001	0.984	0.9
Recovery				
3.3 Gy				
7 mGy.h <sup>-1</sup>	<0.0001	/	0.000	0.000
Radiation				
3.3 Gy				
52 mGy.h <sup>-1</sup>	0.984	0.000	/	0.484
Recovery				

131

132 **Figure 1 – (a).** Kaplan-Meier estimation of the *glp-1* mutant lifespan after gamma irradiation. Various conditions were considered as individual statistical groups: Control (blue line)- *C. elegans*  
 133 without irradiation treatment; 0.5 Gy (green line)- Gamma irradiation from eggs to L4 stage with 7 mGy.h<sup>-1</sup> and recovery until death occurs; 3.3Gy (pink line)- Gamma irradiation from eggs to  
 134 old stage with 7 mGy.h<sup>-1</sup> and recovery until death occurs; 3.3 Gy (yellow line)- Gamma irradiation from eggs to L4 stage with 52 mGy.h<sup>-1</sup> and recovery until death occurs; 24 Gy (turquoise  
 135 line)- Gamma irradiation from eggs to old stage with 52 mGy.h<sup>-1</sup> and recovery until death occurs. The experiment was performed in triplicate. Treatments with stars are significantly different  
 136 (Log-Rank,  $\alpha = 0.01$ ,  $p < 0.0001$ \*\*\*). Blue stars depict a significant difference between control and all treatments; green stars depict a significant difference between 0.5 Gy and 3.3 Gy  
 137 irradiations with a same dose rate (7 mGy.h<sup>-1</sup>); pink stars depict a significant difference between 3.3 Gy cumulative irradiation (with 7 mGy.h<sup>-1</sup>) and treatments with 52 mGy.h<sup>-1</sup> (with cumulative  
 138 doses of 3.3 and 24 Gy). **(b)** Statistical analysis summary (XLSTAT software, Addinsoft, New York, NY, USA) of the results plotted in Figure 1-(a), showing significant degree differences  
 139 between survivals calculated with the Log-Rank test with 99% confidence.

## 140 2.2. Biomolecular characterization of aging, radiation and recovery processes

141 *In situ* visualization of *C. elegans* carbonylation and biomolecular colocalization were performed by a  
142 recently published method [35]. **Figure 2a** gives an example of carbonyl and biomolecule  
143 visualization under our conditions. As shown in **Figure 2**, we were able to uncover the evolution of  
144 both the carbonylation and biomolecule levels in the course of the normal aging and as a response to  
145 contrasted ionizing radiation conditions. **Figures 2b and f** (carbonyl panel) reveal a significant  
146 carbonylation increase, 2.5-3 fold, between 1 (young adult worm group) and 17 days (elderly worm  
147 group) of adulthood. Interestingly, we also noticed that irradiated groups did show a comparable  
148 increase in carbonylation. Conversely, a chronic gamma ray irradiation induced a detectable reduction  
149 of the carbonyl staining (**Figs. 2b and f, carbonyl panel**) in both young (2-3 fold change) and old  
150 worm (1.5-5 fold change) groups. We also observed that this carbonylation decrease did not depend on  
151 the dose rate, the final dose or the duration of the gamma irradiation (**Figs. 2b and f**). On the basis of  
152 the results presented above, we can conclude that a chronic gamma irradiation decreases the level of  
153 carbonylation, whereas aging can increase the carbonylation levels in both unirradiated and irradiated  
154 nematodes. All of these results have been confirmed by protein carbonylation measurement *in vitro* on  
155 extracted proteins using new methodology recently developed [36] (data not shown).

156 Interestingly, lipid level trends after irradiation are similar to carbonylation ones. Namely, irradiation  
157 decreases lipid levels at the young (3.75-7.5 fold) and old stages (3-13 fold) (**Figs. 2c and g, lipid**  
158 **panel**). However, no significant changes in the lipid levels have been observed with aging. These  
159 results have been confirmed by lipid extraction and thin layer chromatography (HPTLC) measurement  
160 (data not shown) to avoid erroneous conclusions due to a methodology bias; e.g., loss of labelling  
161 signal due to the disruption of fat storage organelles [37]. In addition, the lipid distribution along the  
162 nematode body appears to be impacted by aging and irradiation. Those observations are simplified in  
163 **Scheme S1**. However, only a few worms were observed per condition and need to be verified through  
164 visualization of lipids and their distribution; e.g., with electron microscopy.

165 The protein level is not significantly changed either by aging or by irradiation (**Figs. 2e and h, protein**  
166 **panel**). Beyond this result, the stable level of protein constituted a labelling control, showing that old  
167 worms are not easier to permeabilize than young ones are. A complementary assay for protein  
168 measurement has been done by extraction and BCA assay measurement. Protein concentrations vary  
169 between 1 and 1.52 mg.ml<sup>-1</sup> over all conditions (young and old worms, irradiated and unirradiated  
170 controls); protein concentrations are not significantly different between conditions (Kruskal-Wallis  
171 test, P-value = 0.1257) (**Supplementary data, Figure S3**).

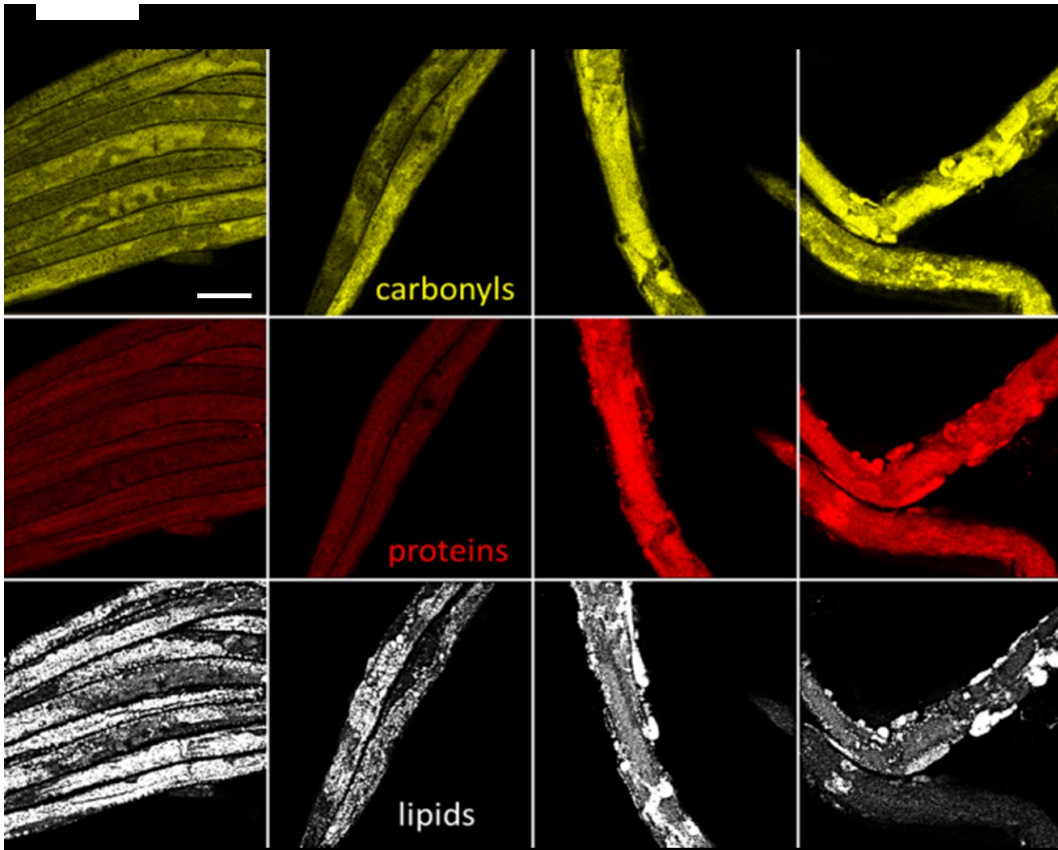
172 On the other hand, chronic exposure of N2 *C. elegans* to gamma rays (with 3 different dose rates: 50,  
173 75 and 100 mGy.h<sup>-1</sup>) did not significantly alter the levels of carbonylation and biomolecules (protein,



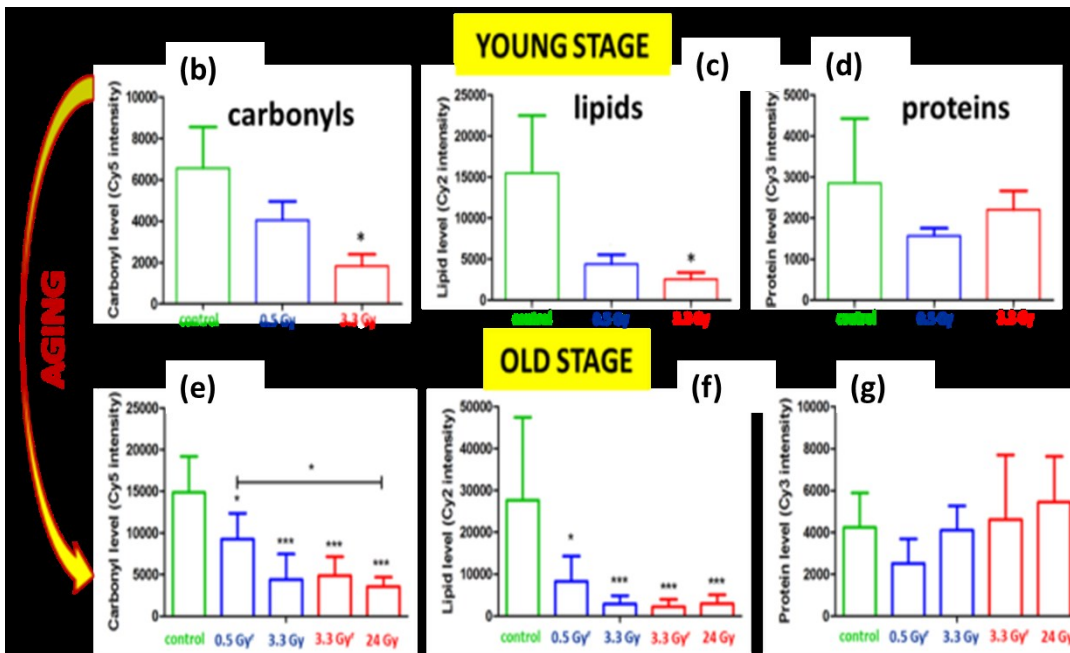
174 lipid and DNA) [8] compare to the controls, except at 6.8 Gy where the lipid levels decreased  
175 dramatically (**Figure S4**).

176

(a)



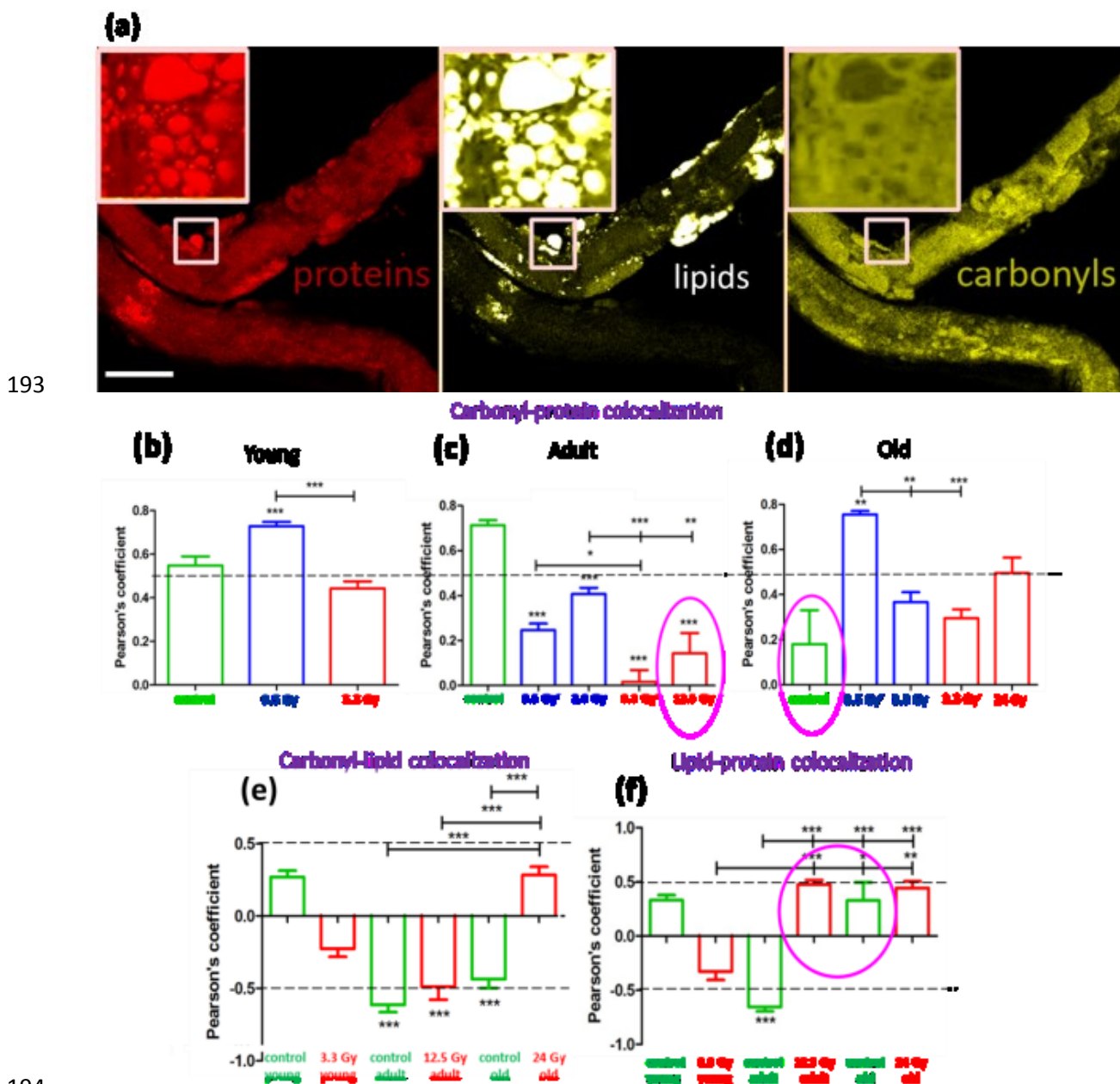
177



178

179 **Figure 2 – (a).** Impact of aging, radiation and recovery after different radiation treatments on biomolecule levels of *C.*  
180 *elegans*, at young (3<sup>rd</sup> day of nematode life) and old (19<sup>th</sup> day of nematode life) stages. Biomolecules were visualized *in situ*  
181 with confocal microscope. Mean  $\pm$  SE, n=15. Scale bar is 10  $\mu$ m. Confocal images were visualized by using a colour-blind  
182 friendly palette. Those images are examples of young and old worms (control and irradiated) with the labeling of carbonyls  
183 (yellow), proteins (red) and lipids (white). Irradiated worms represented here had been subjected to a dose rate of 52 mGy.h<sup>-1</sup>  
184 cumulating the dose of 3.3 Gy and 24 Gy at the young and old stages, respectively. Biomolecular levels of carbonyls **(b, e)**,  
185 of lipids **(c, f)**, and of proteins **(d, g)** were measured *in situ* and quantified with ImageQuantTL at two stages; i.e., the young  
186 **(b, c, d)** and old stages **(e, f, g)**. Mean  $\pm$  SE, n=3, 3, 6 (young: control, 0.5 and 3.3 Gy), n=9, 6, 6, 9, 6 (old: control, 0.5 Gy,  
187 3.3 Gy, 3.3 Gy recovery and 24 Gy). Treatments with stars are significantly different (Tukey and Kruskal-Wallis tests; \* <  
188 0.05; \*\*<0.01; \*\*\*<0.001.

189 To highlight which biomolecule holds carbonyls, the biomolecule colocalizations were assessed from  
 190 the young stage (3<sup>rd</sup> day of nematode life) up until the old stage (19<sup>th</sup> day of nematode life) using a  
 191 dedicated software [38], and following a methodology as depicted in **Scheme S1**. Therefore,  
 192 biomolecule colocalization under all conditions during adulthood was compared (**Figure 3**).



194 **Figure 3 – (a)**. Biomolecules were visualized *in situ* with a confocal microscope. This confocal image is an example of old  
 195 worms that display the colocalization between lipids and proteins with partial exclusion of carbonyls, with the higher  
 196 magnification in the white zoom box. Confocal images were visualized by using a colour-blind-friendly palette. Mean  $\pm$  SE,  
 197  $n=15$ . Scale bar is 10  $\mu\text{m}$ . **(b-f)** Impact of aging, radiation and recovery after irradiation on colocalization between  
 198 biomolecules in young (3<sup>rd</sup> day of nematode life), adult (10<sup>th</sup> day of adulthood) and old (19<sup>th</sup> day of nematode life)  
 199 *C. elegans*. Colocalization was measured via Pearson's coefficient in ImageJ using the JACoP plugin. The colour green  
 200 represents the controls in different stages, blue represents irradiation with a dose rate of 7 mGy.h<sup>-1</sup>, and red represents  
 201 irradiation with 52 mGy.h<sup>-1</sup>. 'r' stands for recovery after irradiation that took place during the development from eggs to young  
 202 stage. Carbonyl-protein colocalization was calculated at the young, adult and old stage in Graphs b, c and d, respectively.  
 203 Carbonyl-lipid colocalization is calculated in Graph e, while protein-lipid colocalization is depicted in Graph f. Pink circles  
 204 represent the samples that were finally analyzed for lipid and protein colocalization. Treatments with stars are significantly  
 205 different (Tukey and Kruskal-Wallis tests; \* < 0.05; \*\* < 0.01; \*\*\* < 0.001). The dashed line indicates limits of Pc coefficient-  
 206 significance.  
 207

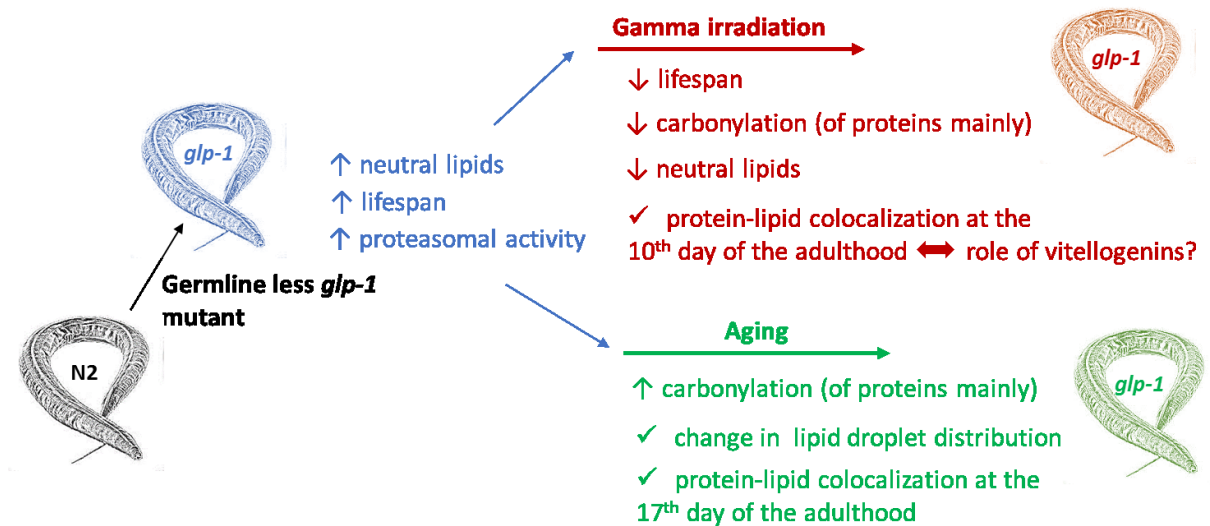
208 Positive and negative colocalizations are considered to be significant if the value of Pearson's  
209 coefficient (Pc) ranges between 0.5 and 1 and between -0.5 and -1, respectively. Values in between -  
210 0.5 and 0.5 were then considered as non-significant and values close to zero mean that there is no  
211 correlation. Results are shown in **Figures 3b-f**. Significant differences between Pc, illustrating  
212 colocalization between two macromolecules, were observed at the young, adult and old stage; i.e., 1<sup>st</sup>  
213 day, 10<sup>th</sup> day and 17<sup>th</sup> day of adulthood. Colocalization of carbonyls and proteins was firstly analyzed  
214 (**Figures 3b-d, first row**). Under most conditions, carbonyls colocalize mainly with proteins.  
215 However, at the young stages, the high dose rate (52 mGy.h<sup>-1</sup>) irradiation tends to display a lower  
216 colocalization between carbonyls and proteins than the low dose rate (7 mGy.h<sup>-1</sup>) irradiation (**Figure**  
217 **3b**); it is the same at the 10<sup>th</sup> day of nematode adulthood, under all tested irradiations compared to the  
218 control group (**Figure 3c**). However, at the same adulthood stage (10<sup>th</sup> day), high dose rate (52 mGy.h<sup>-1</sup>  
219 <sup>1</sup>, in recovery and long-radiation groups) irradiated worms manifested something never observed yet;  
220 namely, no colocalization between carbonyls and proteins (**Figure 3c**). This phenomenon is also  
221 observed in control old nematodes (19 days), at the 17<sup>th</sup> day of adulthood; that is, with a delay of 7  
222 days compared to the adult stage irradiated group (52 mGy.h<sup>-1</sup>) (**Figure 3d**).

223 Although carbonyls do not colocalize with proteins, they might colocalize with other biomolecules,  
224 such as nucleic acids or lipids. Namely, carbonyls colocalize positively with nucleic acids only in  
225 young stage irradiated worms (0.5 Gy), under the same condition in which nucleic acids also display a  
226 colocalization with proteins (data not shown). On the other hand, carbonyls and lipids do not  
227 colocalize in young-stage nematodes for control (Pc <0.5) (**Figure 3e**). Conversely, 52 mGy.h<sup>-1</sup>  
228 radiation seems to induce a negative correlation (still weak as <-0.5) between carbonyls and lipids,  
229 except for old-stage worms, in which a non-significant positive correlation is observed. This negative  
230 correlation is enhanced and becomes significant at the adult stage (in both control and irradiated  
231 worms) and at the old stage (in control worms) (**Fig. 3e**).

232 Finally, the Pc between lipids and proteins has been calculated (**Figure 3f**). We observed a similar  
233 pattern to that found for carbonyls and lipids at the young stage (both control and irradiated), for adult  
234 controls (showing a negative correlation) and for irradiated old worms (**Fig. 3e**). Conversely, at the  
235 adult stage (10<sup>th</sup> day of adulthood) after chronic irradiation at 52 mGy.h<sup>-1</sup> and for the old control, the  
236 Pc pattern is totally opposite to that between lipids and carbonyls, as it shows a slight positive  
237 colocalization between lipids and proteins (**Fig. 3f**). Positive colocalization between proteins and  
238 lipids that partially exclude carbonyl groups can also be visualized from the confocal image  
239 (**Figure 3a**).

240 To conclude with regard to these biomolecular colocalization results, proteins and carbonyls mostly  
241 colocalize at the young stage of *C. elegans*, as published before [35], while there is only a weak  
242 colocalization between proteins and lipids. This can be due to the level change of carbonyls, which

243 makes possible the colocalization measurement with more or less accuracy. Conversely, we observed  
 244 that adult irradiated nematodes and old control worms show negative colocalization between carbonyls  
 245 and proteins associated with a positive lipid and protein colocalization. A scheme has been included to  
 246 sum up the results (**Scheme 1**).



247 **Scheme 1.** Illustrations of summarized conclusions are displayed here upon aging and chronic ionizing radiation of sterile  
 248 *C. elegans*.  
 249

250

### 251 3. Discussion

252 Understanding aging is of fundamental interest in biology and medicine [17], since aging and  
253 aging-related diseases (e.g., cancer and degenerative diseases) cause about 90% of deaths in developed  
254 countries. However, questions remain about i) the specificity of aging-related mechanisms, and ii) the  
255 influence of organism environment on aging.

256 A low-dose gamma irradiation can modulate the lifespan of various organisms [39]. With regard to  
257 *C. elegans*, only acute gamma irradiations above 1000 Gy have already been observed to reduce the  
258 lifespan [40], but no results have been found after chronic irradiations. We showed herein that the  
259 *C. elegans* sterile *glp-1* mutant had a significantly shortened lifespan after all irradiation treatments.  
260 Furthermore, different radiation treatments, i.e., different dose, dose rate and duration of exposure, did  
261 not result in a significant difference between treated groups. It is possible that our experimental  
262 conditions resulted in an increased protective mechanism with a threshold reached by the first  
263 delivered dose to organisms, independently of the duration and dose rate, and then led to a similar  
264 lifespan after irradiation. As some developmental stages are more sensitive to radiations than others  
265 [40], shorter radiation exposure but with the same dose rates, over specific developmental stages, e.g.,  
266 diakinesis, would be interesting to perform in order to test for modulation effects on the lifespan. Our  
267 work has been done on a conditional sterile mutant that has prolonged lifespan due to germline  
268 mutation. Therefore, noted changes in longevity cannot be simply associated to metabolic trade-offs  
269 between reproduction and longevity, but rather are instead consequences of distinct, pro- and anti-  
270 longevity signals originating from various regions of the gonad [41]. In addition, irradiation effects on  
271 the lifespan can then be more emphasized in this mutant as the period of observation is more extended  
272 than for wild worms.

273 A biochemical study of radiation-induced accelerated aging was performed with emphasis on protein  
274 damage monitoring; i.e., protein carbonylation, a molecular modification that has been previously  
275 known to be irreversible and impacted by aging and ionizing radiation [42, 43]. In fact, carbonylation  
276 turned out to be a good biomarker of aging, since old nematodes display higher carbonylation than  
277 young ones. These results are in accordance with previous reports on aging [25, 44]. Moreover, a  
278 decrease in protein turnover has been identified as one of the biomarkers of aging with no relation to  
279 protein abundance over time [24]. Such a phenomenon perhaps explains the higher susceptibility to  
280 carbonylation and aggregation of proteins in old worms. Otherwise, radiation treatment gave a rather  
281 unexpected result regarding carbonylation level detection compared to its control counterpart; i.e.,  
282 radiation reduces carbonylation regardless of the dose or duration of the irradiation. The decrease in  
283 carbonylation could be partially explained by chronic irradiation induction of chymotrypsin-like  
284 proteasomal activity [8], whereas proteasome activity of germline ablated *glp-1* worm mutants has  
285 already highly increased caspase, trypsin, and chymotrypsin-like proteasome activities in comparison

286 to wild worms [45]. In addition, 26S proteasome regulators, like *rpn-6.1*, that reduce proteotoxic stress  
287 have already been reported to be upregulated following a constant mild-heat stress, such as 25°C [45].  
288 Our experiments also involved a temperature of 25°C under all our working conditions, including the  
289 control ones. In *wt* nematodes, the protein carbonylation level has been found to be quite stable after  
290 chronic irradiation, with an increasing proteasomal activity [8]. As some hypotheses stated that  
291 carbonylation partially takes place in mitochondria [46], the difference between both models could be  
292 due to the mitochondria number, which is higher in N2 than in *Glp-1* mutants (without germ cells).

293 With regard to lipids, their level in *glp-1* is higher than in N2 *wt* nematodes, which is in agreement  
294 with a previous report [14]. Moreover, our experimentation showed that radiation also induced neutral  
295 lipid catabolism whatever the dose or the dose rate. Our results indicate that lipid catabolism induced  
296 by radiation correlates with a reduced longevity, which is finally equivalent to the longevity value of  
297 *wt* nematodes (~25-30 days). Lipids are very important signalling molecules that constitute the cell  
298 membrane and form comprehensive energy storage. Lipid homeostasis is in charge of endocrine  
299 signalling of longevity. However, regulating mechanisms of lipids in *C. elegans* are not fully  
300 understood and are very complex [47]. It was already reported that germline ablation in worms alters  
301 lipid metabolism and affects longevity. With our methodology, the neutral lipids were mainly labelled  
302 in this study. In *C. elegans*, lipids are stored in 2 types of organelles, lipid droplets and yolk. Lipids  
303 were observed at the young stage in a small dotted pattern, which is consistent with previous research  
304 where LDs appear as bead organelles highly enriched in Tri Acyl Glycerol (TAGs) [48, 49]. One of  
305 the assumptions for lipid catabolism after irradiation can be that irradiated worms tend to have a lower  
306 bacterial food intake after irradiation, resulting in a reduced uptake of lipids, which is not the case in  
307 the normal aging process [24]. Another possibility can be that a higher rate of TAG molecule  
308 degradation would increase the release of free-fatty acids and the production of energy [47].

309 With regard to biomolecular colocalization during radiation and aging, especially between lipids,  
310 proteins and carbonyls, explanations are unclear at this time, particularly for the lipid-protein  
311 colocalization partially excluding carbonyls. One category of protein that could correspond to this  
312 “lipoprotein” description of positive colocalization between lipids and proteins is vitellogenin. Indeed,  
313 vitellogenin is the most abundant lipoprotein of the yolk, which is highly accumulated in the aged  
314 *glp-1* sterile mutant nematodes [50] and overexpressed after gamma chronic irradiation of *wt C.*  
315 *elegans* (Dubois et al, Submitted 2018). In addition, this protein is not very prone to carbonylation  
316 due to its constitutive amino-acids [51]. To confirm this hypothesis, additional experiments need to be  
317 performed, such as triplex labelling between lipids, proteins and vitellogenin. This phenomenon could  
318 constitute a marker of both irradiation and aging, but requires further investigations to be fully  
319 elucidated.

320 In *C. elegans*, it is believed that IIS (insulin/IGF1 signalling) and germline originating signals can  
321 modify DAF-16 transcription factor activity, which can, in turn, modulate the lifespan expectancy  
322 [16]. By recruiting different signalling pathways (DAF-16, SKN-1/Nrf, oleic acid, lipase, etc.),  
323 germline ablation can enhance immune defence, proteostasis and stress resistance. Notably, fat  
324 metabolism, i.e., fatty acid desaturation and thus fat composition, can be profoundly modified [52].  
325 Indeed, through the mutation of the *glp-1* gene, *C. elegans* overexpress triglyceride lipase Lip1-4 and  
326 fatty acid desaturases, which induces autophagy, lipophagy and desaturation into higher but healthier  
327 lipid status [53]. Lipids can be beneficial but also detrimental for longevity [14]. After gamma  
328 radiation exposure, *Glp-1* individuals seem to lose their properties; it seems that the decrease of lipids  
329 is associated to a reduction of the lifespan. To fight against chronic irradiation, N2 individuals  
330 stimulate the 20S proteasome complex [8] and conversely seem to maintain their lipid content at the  
331 same level for the reproductive function, despite an overexpression of lipid transporters (**Dubois et al,**  
332 **Submitted 2018**). However, a decrease occurs at 6.8 Gy. Two different compensatory mechanisms  
333 could occur during gamma ray exposure: one increasing the level of lipids (with an increase of the  
334 lipid transporter expression), and one decreasing the level of lipids. Recently, a nuclear protein,  
335 SKN-1, was reported to drive two opposite responses in *C. elegans*, thus promoting longevity by a  
336 mechanism other than that set for protection against oxidative damage [54]. In addition, SKN-1 plays  
337 a direct role in maintaining lipid homeostasis [14]. Irradiation yields oxidative stress, e.g., protein  
338 oxidation and lipid peroxidation, and then triggers protection against oxidative damage. SKN-1, in this  
339 context, could then be implied in the response to radiation and trigger lipid catabolism. However, lipid  
340 catabolism can also be induced by several other pathways and the question of the link between lipids,  
341 reproduction and lifespan is far from elucidated [41, 47].

342 To conclude, this study contributes to assert that fat catabolism is not necessarily beneficial, as we  
343 showed that the lifespan reduction after chronic gamma exposure is associated with a lipid level  
344 decrease. In addition, and by the same way, a decrease in carbonyl content cannot be considered as a  
345 reliable marker of health because it appears to be linked with a lifespan decrease after chronic gamma  
346 irradiation. A proteolysis increase induced by a commitment in fighting oxidative stress induced by  
347 irradiation can probably yield energy consumptions that are of negative impact for organism health.  
348 Finally, whereas some phenomena, i.e., colocalization of lipids and proteins in some developmental  
349 stages, seem shared by aging and irradiation processes, other mechanisms, such as lipid metabolism  
350 and oxidative protein level, seem different as organisms react in opposite ways. These results enable to  
351 conclude that aging and irradiation processes undergo different mechanisms under our conditions.  
352 Numerous cascades of events can yield this result. Some specific tests on SKN-1 and downstream  
353 proteins would be necessary to address this question and give evidence of the specific mechanisms  
354 that take place during irradiation exposure and aging.



## 355 **4. Materials and methods**

### 356 **4.1. Strain and maintenance**

357 The *glp-1* sterile *C. elegans* strain (Bristol N2) used in this study was provided by the Caenorhabditis  
358 Genetics Center (MN, USA). Nematode growth medium (NGM) and M9 medium were prepared by  
359 standard protocols [55]. The nematodes were cultured at 15 °C, 70 % relative humidity in darkness, on  
360 NGM agar seeded with *Escherichia coli* OP50 strain, according to the standard method [55]. Bacteria  
361 were prepared following the standard protocol [56]. Seeded plates were exposed to UVC (20 min,  
362 Bio-LinkCrosslinker; 254 nm, 200  $\mu\text{Wm}^2$ ) to suppress bacterial activity [57].

### 363 **4.2. Double-synchronized population for ionizing radiation**

364 Samples for ionizing radiation were prepared in 3 steps. All those steps were developed at 15 °C and  
365 70 % relative humidity in darkness, which was constantly controlled by HOBO data logger [58]. All  
366 NGM plates that were used for this experiment were seeded with 10 x concentrated bacteria (OP 50) to  
367 prevent starvation and metabolic changes. The first step was to transfer 100 gravid worms with a  
368 worm picker onto 10 NGM large plates (9-cm diameter plates) followed by 5 days incubation at 15 °C  
369 to stimulate reproduction. The second step represents 1<sup>st</sup> synchronization on the fifth day, in which  
370 those plates were rinsed with M9 buffer and the worms were cleansed 3 times in M9 buffer in glass  
371 tubes with a centrifuge speed of 400 g for 2 minutes. Cleaned samples were subjected to bleaching (20  
372 % NaOH, 10 % hypochlorite and 70 % H<sub>2</sub>O) for 5 minutes in order to release the eggs from the  
373 worms' uterus. After this, fast centrifuge is necessary to prevent egg degeneration (800 g for 1 minute  
374 followed by the removal of the maximum amount possible of supernatant). Cleaning of the samples  
375 was the same as above (3 times). The number of eggs was counted under the binocular in 1  $\mu\text{l}$  of M9.  
376 From there, the volume that contained 5000 eggs was transferred onto 20 NGM large plates followed  
377 by 5 days of incubation, as mentioned above. The last and third day involved synchronizing these  
378 plates. The rinsing and washing were performed as in the second step. Samples were passed through  
379 the saccharose gradient (3 and 7 %), in order to separate only gravid worms whose eggs from the  
380 uterus should be synchronized over 3 hours (internal communication). Bleaching was performed on  
381 those gravid worms and the eggs were counted as in the second step. This time, the volume  
382 corresponding to 3000 eggs was transferred onto 50 6-cm NGM plates because their volume fitted the  
383 <sup>137</sup>Cs source. Furthermore, 50 eggs were transferred to 15 6-cm NGM plates to follow the life span.

### 384 **4.3. Ionizing radiation**

385 A Monte Carlo simulation of the dose was performed before irradiation to determine by modelling the  
386 correct position of the petri dish. The same homogeneous <sup>137</sup>Cs source was used for both irradiations,  
387 varying the distance only to yield 7 or 52 mGy.h<sup>-1</sup>. Plates were arranged perpendicularly to the <sup>137</sup>Cs  
388 source, with 1 to 3 RPL (Radio photo luminescent) dosimeters placed on the back of the container to

389 perform a posterior metrology analysis of dosimeters and check the stability of the dose rate during the  
390 experiment. Irradiation was conducted at 25 °C and 70 % relative humidity in darkness. IR began from  
391 eggs at different dose rates in one incubator, where the temperature and humidity were controlled by  
392 data logger [58]. Supplementary data, **Figure S1a** shows the experimental device, as well as a  
393 summary of these results **S1b**. Homogeneous data have been obtained for all of the experiments.

394 There were four irradiation schemes with two different time exposures and two different dose rates:  
395 the first irradiation groups were from eggs to L4 stage (during 64 hours) with 7 and 52 mGy.h<sup>-1</sup>  
396 reaching 0.5 and 3.3 Gy, respectively; the second irradiation groups were from eggs to “old” stage  
397 (during 19 days) with the same dose rates reaching 3.3 Gy and 24 Gy, respectively. Due to the  
398 limitation of the maximum dose rate (52 mGy.h<sup>-1</sup>) from the <sup>137</sup>Cs gamma irradiation source (**Sup data,**  
399 **Fig S1a**), there was no possibility of obtaining a cumulative dose of 24 Gy at the young stage (L4, 64  
400 h of irradiation). After each completed treatment, samples were washed 3 times with M9 and quickly  
401 frozen in a grinder tube with the minimal volume. Samples were stored at -80 °C until use.

#### 402 **4.4. Experimental design for following biomolecular levels during radiation and aging**

403 Although the study was focused on the young and old stage of worms during radiation and aging, the  
404 interest was also to follow their biomolecule levels in between the young and old stage. The  
405 biomolecules of interest were: carbonyls, proteins, lipids and nucleic acids. Various effects were  
406 monitored every second or third day, when also HOBO data were undertaken. On those days plates  
407 were washed 3 times with M9 as mentioned above. After washing the worms, they were transferred  
408 onto fresh 6-cm NGM plates with 10 times concentrated bacteria to avoid starvation. The samples for  
409 biomolecular labelling were collected from all replicates; i.e., 10 worms from 10 replicates were taken  
410 and quickly frozen in 100 µl of M9. Aging, radiation and recovery effects were followed from the  
411 young stage to the old stage, whereas dose and dose rate effects were observed within the same stage.  
412 The aging effect represents a normal control without any radiation treatment. The radiation effect  
413 represents long-term radiation (from eggs to old stage) with either of the chosen dose rates, 7 and 52  
414 mGy.h<sup>-1</sup>. However, the recovery effect represents short-term radiation, where nematodes were  
415 irradiated during the developmental period from eggs to the young stage with both dose rates (7 and  
416 52 mGy.h<sup>-1</sup>), and after letting them recover up until the old stage. The dose rate effect compares two  
417 different dose rates but the same final dose; i.e., 7 and 52 mGy.h<sup>-1</sup> at the old stage. Finally, the dose  
418 effect represents all of the different doses at the same stage, including control which represents 0 dose.

#### 419 **4.5. Life span**

420 There were approximately 50 worms on a 6-cm plate to follow their life span. Life span was  
421 monitored in biological triplicate for every condition. Furthermore, 4 different radiation treatments  
422 were performed at least for 64 hours in radiation incubators. All of the effects mentioned above were

423 followed: normal aging (control), cumulative radiation (from eggs to old stage with 7 and 52 mGy.h<sup>-1</sup>)  
424 and recovery effect (radiation from eggs to the young stage and recovery). The time points for  
425 following the life span under the binocular were the same as in the previous section; i.e., every second  
426 or third day during radiation and after every day except on weekends. The important strategy to notice  
427 here is that, when radiations were finished and plates were transferred to control incubators without  
428 radiation, they were separated from the control plates by lead and shell to prevent a bystander effect.  
429 The radiation-induced bystander effect is the phenomenon occurring in non-irradiated cells displaying  
430 damaged phenotype received from nearby irradiated cells [59].

#### 431 **4.6. Multiplex *in situ* labelling**

##### 432 *4.6.1. Labelling of carbonyls, proteins and lipids*

433 Chemoporation and labelling were performed in 40 % isopropanol as in the previous protocol [35];  
434 i.e., carbonyls with 4 µg.ml<sup>-1</sup> Cy5 Hz (Cy<sup>TM</sup>5 Mono Hydrazide, GE Healthcare Life Sciences,  
435 Buckinghamshire, UK); proteins with 0.5 µg.ml<sup>-1</sup> Cy3 NHS ester (Cyanine3 NHS ester minimal dye,  
436 Interchim, Montluçon, France) and lipids with 2 µg.ml<sup>-1</sup> Bodipy 505/515 (Bodipy 505/ 515, Molecular  
437 Probes ThermoFisher Scientific, Eugene, Oregon, USA).

##### 438 *4.6.2. Labelling of carbonyls, proteins and nucleic acids*

439 The protocol was the same as above, but the last labelling was for nucleic acids with 5X Sybr Green II  
440 in 70 % ethanol (Sybr® Green II Nucleic Acid Stain, Lonza, Basel, Switzerland) for 15 minutes.  
441 Sybr green II labels DNA and RNA *in situ* [35]. There is significantly higher quantity of RNA  
442 compared to DNA generally, but particularly here because there is less DNA in sterile nematodes  
443 compared to wild ones, since the gonad is empty (no cells nor embryos). To conclude, most of the  
444 signal obtained here is from RNA.

#### 445 **4.7. Slide preparation, confocal microscopy imaging and fluorescence quantification**

446 Images were collected with an LSM 780 confocal microscope (Carl Zeiss, France) by using a 10× dry  
447 N.A. 0.45 and 20× dry (N.A. 0.80) or 63× oil (N.A. 1.4) oil immersion objective, with a pinhole  
448 setting of 1.31 A.U. and 8 or 12-bit images. Imaging of multiplex labelled samples and fluorescence  
449 quantification was performed according to the previous protocol [35].

#### 450 **4.8. Colocalization**

451 Biomolecular colocalization was measured across the whole body of *C. elegans* with Pearson's  
452 coefficient, as depicted with a blue square in **Scheme S1**. Namely, we sampled 15 different points  
453 across the body of *C. elegans* (**Scheme S1**, yellow numeric points in a blue square) and tested them on  
454 different colocalizations: protein-carbonyl, carbonyl-lipid and protein-lipid colocalization. These 15

455 different points were quite homogeneous in terms of the colocalization parameter and they were  
456 averaged in the end.

#### 457 **4.9. Statistical analyses**

458 Statistical analysis significance was calculated in GraphPad Prism. Before each analysis, the normality  
459 (Shapiro-Wilk test) and homogeneity of data variance (Levene's test) were tested. When these  
460 assumptions were met, then parametric tests were used; i.e., one-way ANOVA with post-hoc Tukey  
461 test. Otherwise, nonparametric tests were used; i.e., Kruskal-Wallis with Dunn's multiple comparison  
462 test. The lifespan statistical analyses were done with XLSTAT software (Addinsoft, New York, NY,  
463 USA). Survival data by the Kaplan-Meier method and differences between survivals were calculated  
464 using the Log-Rank test with 99% confidence. Three statistical parameters are presented: Log-rank,  
465 Wilcoxon and Tarone-Ware.

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474 **Authors' contribution**

475 Mira Kuzmic participated in the experimental design, performed data acquisition, analysis and  
476 interpretation and most of the writing of the article.

477 Simon Galas performed statistical analysis of the lifespan, critical analysis, associated figures and  
478 captions, and contributed to the writing of the article.

479 Catherine Lecomte contributed to setting up the global experimental design, and to the breeding and  
480 maintenance of the nematodes with her expertise in *C. elegans*, as well as to writing the article.

481 Cecile Dubois participated in carbonylation quantification in the Oxy-DIGE method.

482 Nicolas Dubourg contributed to the ionizing radiation experiment and the dosimetry calculation.

483 Sandrine Frelon contributed to the global experimental design throughout the entire project, and she  
484 also performed signal integration, data acquisition, analysis and interpretation, and participated in the  
485 writing of the article.

486 **Conflict of interest**

487 No benefit of any kind will be received from this paper either directly or indirectly by any of the  
488 authors.

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496 **5. References**

- 497 1. Yasunari, T.J., et al., *Cesium-137 deposition and contamination of Japanese soils due to the*  
498 *Fukushima nuclear accident*. Proc Natl Acad Sci U S A, 2011. **108**(49): p. 19530-4.
- 499 2. Harrison, F.L. and S.L. Anderson, *Effects of acute irradiation on reproductive success of the*  
500 *polychaete worm, Neanthes arenaceodentata*. Radiat Res, 1994. **137**(1): p. 59-66.
- 501 3. Knowles, J.F. and L.N. Greenwood, *The effects of chronic irradiation on the reproductive*  
502 *performance of Ophryotrocha diadema (polychaeta, dorvilleidae)*. Marine Environmental  
503 Research, 1994. **38**(3): p. 207-224.
- 504 4. Hertel-Aas, T., et al., *Effects of chronic gamma irradiation on reproduction in the earthworm*  
505 *Eisenia fetida (Oligochaeta)*. Radiat Res, 2007. **168**(5): p. 515-26.
- 506 5. Takanami, T., et al., *Hyper-resistance of meiotic cells to radiation due to a strong expression*  
507 *of a single recA-like gene in Caenorhabditis elegans*. Nucleic Acids Research, 2000. **28**(21): p.  
508 4232-4236.
- 509 6. Bailly, A.P., et al., *The Caenorhabditis elegans homolog of Gen1/Yen1 resolvases links DNA*  
510 *damage signaling to DNA double-strand break repair*. PLoS Genet, 2010. **6**(7): p. e1001025.
- 511 7. Buisset-Goussen, A., et al., *Effects of chronic gamma irradiation: a multigenerational study*  
512 *using Caenorhabditis elegans*. J Environ Radioact, 2014. **137**: p. 190-197.
- 513 8. Dubois, C., et al., *Precocce and opposite response of proteasome activity after acute or chronic*  
514 *exposure of C. elegans to gamma-radiation*. Sci Rep, 2018. **8**(1): p. 11349.
- 515 9. Herndon, L.A., et al., *Stochastic and genetic factors influence tissue-specific decline in ageing*  
516 *C. elegans*. Nature, 2002. **419**(6909): p. 808-14.
- 517 10. Hansen, M., T. Flatt, and H. Aguilaniu, *Reproduction, Fat Metabolism, and Lifespan – What Is*  
518 *the Connection?* Cell metabolism, 2013. **17**(1): p. 10-19.
- 519 11. Berman, J.R. and C. Kenyon, *Germ-Cell Loss Extends C. elegans Life Span through Regulation*  
520 *of DAF-16 by kri-1 and Lipophilic-Hormone Signaling*. Cell, 2006. **124**(5): p. 1055-1068.
- 521 12. Tissenbaum, H.A., *Using C. elegans for aging research*. Invertebrate reproduction &  
522 development, 2015. **59**(sup1): p. 59-63.
- 523 13. Hekimi, S., et al., *Genetics of lifespan in C. elegans: molecular diversity, physiological*  
524 *complexity, mechanistic simplicity*. Trends Genet, 2001. **17**(12): p. 712-8.
- 525 14. Steinbaugh, M.J., et al., *Lipid-mediated regulation of SKN-1/Nrf in response to germ cell*  
526 *absence*. Elife, 2015. **4**.
- 527 15. Robida-Stubbs, S., et al., *TOR signaling and rapamycin influence longevity by regulating SKN-*  
528 *1/Nrf and DAF-16/FoxO*. Cell metabolism, 2012. **15**(5): p. 713-724.
- 529 16. Uno, M. and E. Nishida, *Lifespan-regulating genes in C. elegans*. NPJ Aging Mech Dis, 2016. **2**:  
530 p. 16010.
- 531 17. Hsin, H. and C. Kenyon, *Signals from the reproductive system regulate the lifespan of C.*  
532 *elegans*. Nature, 1999. **399**(6734): p. 362-6.
- 533 18. Lakowski, B. and S. Hekimi, *Determination of life-span in Caenorhabditis elegans by four clock*  
534 *genes*. Science, 1996. **272**(5264): p. 1010-3.
- 535 19. Taylor, R.C. and A. Dillin, *Aging as an event of proteostasis collapse*. Cold Spring Harb  
536 Perspect Biol, 2011. **3**(5).
- 537 20. Yanase, S., et al., *Impaired p53/CEP-1 is associated with lifespan extension through an age-*  
538 *related imbalance in the energy metabolism of C. elegans*. Genes Cells, 2017. **22**(12): p. 1004-  
539 1010.
- 540 21. Baruah, A., et al., *CEP-1, the Caenorhabditis elegans p53 homolog, mediates opposing*  
541 *longevity outcomes in mitochondrial electron transport chain mutants*. PLoS Genet, 2014.  
542 **10**(2): p. e1004097.
- 543 22. Holliday, R., *Aging and the decline in health*. Health, 2010. **2**(6): p. 5.
- 544 23. Copes, N., et al., *Metabolome and proteome changes with aging in Caenorhabditis elegans*.  
545 Exp Gerontol, 2015. **72**: p. 67-84.

- 546 24. Dhondt, I., et al., *Changes of Protein Turnover in Aging Caenorhabditis elegans*. Mol Cell  
547 Proteomics, 2017. **16**(9): p. 1621-1633.
- 548 25. Stadtman, E.R., *Protein oxidation and aging*. Free Radic Res, 2006. **40**(12): p. 1250-8.
- 549 26. Rikans, L.E. and K.R. Hornbrook, *Lipid peroxidation, antioxidant protection and aging*.  
550 Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1997. **1362**(2-3): p. 116-  
551 127.
- 552 27. Stadtman, E.R. and B.S. Berlett, *Reactive oxygen-mediated protein oxidation in aging and*  
553 *disease*. Chem Res Toxicol, 1997. **10**(5): p. 485-94.
- 554 28. Dalle-Donne, I., et al., *Protein carbonyl groups as biomarkers of oxidative stress*. Clin Chim  
555 Acta, 2003. **329**(1-2): p. 23-38.
- 556 29. Fedorova, M., R.C. Bollineni, and R. Hoffmann, *Protein carbonylation as a major hallmark of*  
557 *oxidative damage: Update of analytical strategies*. Mass Spectrometry Reviews, 2014. **33**(2):  
558 p. 79-97.
- 559 30. Upton, A.C., et al., *Some Delayed Effects of Atom-Bomb Radiations in Mice*. Cancer Res, 1960.  
560 **20**(8 Part 2): p. 1-60.
- 561 31. Candeias, S.M., et al., *Low-dose radiation accelerates aging of the T-cell receptor repertoire in*  
562 *CBA/Ca mice*. Cell Mol Life Sci, 2017.
- 563 32. Johnson, H.A., *Age and Sensitivity to Radiation Life Shortening*. Radiat Res, 1964. **23**: p. 19-25.
- 564 33. Bailly, A. and A. Gartner, *Germ cell apoptosis and DNA damage responses*. Adv Exp Med Biol,  
565 2013. **757**: p. 249-76.
- 566 34. Buisset-Goussen, A., et al., *Effects of chronic gamma irradiation: a multigenerational study*  
567 *using Caenorhabditis elegans*. J Environ Radioact, 2014. **137**: p. 190-7.
- 568 35. Kuzmic, M., et al., *In situ visualization of carbonylation and its co-localization with proteins,*  
569 *lipids, DNA and RNA in Caenorhabditis elegans*. Free Radic Biol Med, 2016. **101**: p. 465-474.
- 570 36. Pyr Dit Ruys, S., J.M. Bonzom, and S. Frelon, *Benchmarking of protein carbonylation analysis*  
571 *in Caenorhabditis elegans: specific considerations and general advice*. Free Radic Biol Med,  
572 2016. **99**: p. 364-373.
- 573 37. Mullaney, B.C. and K. Ashrafi, *C. elegans fat storage and metabolic regulation*. Biochim  
574 Biophys Acta, 2009. **1791**(6): p. 474-8.
- 575 38. Bolte, S. and F.P. Cordelieres, *A guided tour into subcellular colocalization analysis in light*  
576 *microscopy*. J Microsc, 2006. **224**(Pt 3): p. 213-32.
- 577 39. Zhikrevetskaya, S., et al., *Effect of Low Doses (5-40 cGy) of Gamma-irradiation on Lifespan*  
578 *and Stress-related Genes Expression Profile in Drosophila melanogaster*. PLoS One, 2015.  
579 **10**(8): p. e0133840.
- 580 40. Johnson, T.E. and P.S. Hartman, *Radiation effects on life span in Caenorhabditis elegans*. J  
581 Gerontol, 1988. **43**(5): p. B137-41.
- 582 41. Lemieux, G.A. and K. Ashrafi, *Investigating Connections between Metabolism, Longevity, and*  
583 *Behavior in Caenorhabditis elegans*. Trends Endocrinol Metab, 2016. **27**(8): p. 586-596.
- 584 42. Stadtman, E.R., *Protein oxidation and aging*. Science, 1992. **257**(5074): p. 1220-4.
- 585 43. Krisko, A. and M. Radman, *Protein damage and death by radiation in Escherichia coli and*  
586 *Deinococcus radiodurans*. Proc Natl Acad Sci U S A, 2010. **107**(32): p. 14373-7.
- 587 44. Adachi, H., Y. Fujiwara, and N. Ishii, *Effects of oxygen on protein carbonyl and aging in*  
588 *Caenorhabditis elegans mutants with long (age-1) and short (mev-1) life spans*. J Gerontol A  
589 Biol Sci Med Sci, 1998. **53**(4): p. B240-4.
- 590 45. Vilchez, D., et al., *RPN-6 determines C. elegans longevity under proteotoxic stress conditions*.  
591 Nature, 2012. **489**(7415): p. 263-8.
- 592 46. Aguilaniu, H., et al., *Asymmetric inheritance of oxidatively damaged proteins during*  
593 *cytokinesis*. Science, 2003. **299**(5613): p. 1751-3.
- 594 47. Watts, J.L. and M. Ristow, *Lipid and Carbohydrate Metabolism in Caenorhabditis elegans*.  
595 Genetics, 2017. **207**(2): p. 413-446.
- 596 48. Zhang, P., et al., *Proteomic study and marker protein identification of Caenorhabditis elegans*  
597 *lipid droplets*. Mol Cell Proteomics, 2012. **11**(8): p. 317-28.

- 598 49. Vrablik, T.L., et al., *Lipidomic and proteomic analysis of Caenorhabditis elegans lipid droplets*  
599 *and identification of ACS-4 as a lipid droplet-associated protein*. *Biochim Biophys Acta*, 2015.  
600 **1851**(10): p. 1337-45.
- 601 50. Lemieux, G.A. and K. Ashrafi, *Stressing about misplaced fat is a key to longevity*. *Elife*, 2015.  
602 **4**.
- 603 51. Chen, J.S., T.W. Sappington, and A.S. Raikhel, *Extensive sequence conservation among insect,*  
604 *nematode, and vertebrate vitellogenins reveals ancient common ancestry*. *J Mol Evol*, 1997.  
605 **44**(4): p. 440-51.
- 606 52. Goudeau, J., et al., *Fatty acid desaturation links germ cell loss to longevity through NHR-*  
607 *80/HNF4 in C. elegans*. *PLoS Biol*, 2011. **9**(3): p. e1000599.
- 608 53. Ackerman, D. and D. Gems, *The mystery of C. elegans aging: an emerging role for fat. Distant*  
609 *parallels between C. elegans aging and metabolic syndrome?* *Bioessays*, 2012. **34**(6): p. 466-  
610 71.
- 611 54. Tullet, J.M.A., et al., *The SKN-1/Nrf2 transcription factor can protect against oxidative stress*  
612 *and increase lifespan in C. elegans by distinct mechanisms*. *Aging Cell*, 2017. **16**(5): p. 1191-  
613 1194.
- 614 55. Stiernagle, T., *Maintenance of C. elegans*. *WormBook*, 2006: p. 1-11.
- 615 56. Margerit, A., et al., *Nested interactions in the combined toxicity of uranium and cadmium to*  
616 *the nematode Caenorhabditis elegans*. *Ecotoxicol Environ Saf*, 2015. **118**: p. 139-48.
- 617 57. Goussen, B., et al., *Consequences of a multi-generation exposure to uranium on*  
618 *Caenorhabditis elegans life parameters and sensitivity*. *Ecotoxicology*, 2013. **22**(5): p. 869-78.
- 619 58. Bernstein, J.A., et al., *A pilot study to investigate the effects of combined dehumidification*  
620 *and HEPA filtration on dew point and airborne mold spore counts in day care centers*. *Indoor*  
621 *Air*, 2005. **15**(6): p. 402-7.
- 622 59. Najafi, M., et al., *The mechanisms of radiation-induced bystander effect*. *J Biomed Phys Eng*,  
623 2014. **4**(4): p. 163-72.
- 624
- 625