

Supplementary Data

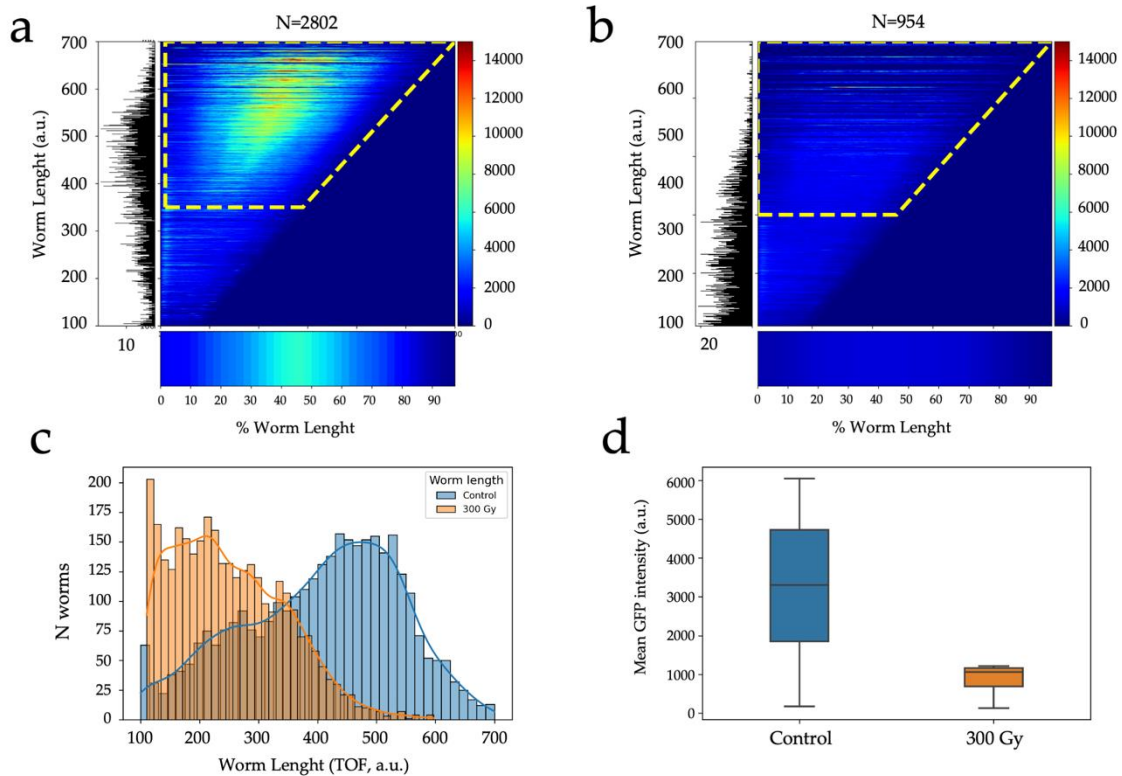
Proton Microbeam targeted Irradiation of the Gonad Primordium Region Induces Developmental Alterations Associated with Heat Shock Responses and Cuticle Defense in *Caenorhabditis elegans*

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Supplementary Figure S1: Radiation-induced developmental alterations in *C. elegans* following unselective irradiation analyzed by COPAS. (a) Longitudinal fluorescence profiles (GFP) in control population as a function of development (worm length, TOF). The appearance of gonads at the L3/L4 stage and above (TOF>250-300, dotted yellow region of interest) is converted into a bar where the calculated mean GFP longitudinal intensity profile is indicated with the length corresponds to the animal's size and the color codes for the fluorescence intensity (bottom figure). The corresponding distribution of worm length is shown on left panel. (b) Longitudinal profiles of GFP fluorescence in the population micro-irradiated at 300 Gy. The appearance of gonads at the L3/L4 stage and above (TOF>250-300, dotted yellow region of interest) is visible in calculated mean GFP longitudinal profile (bottom figure). (c) Length distribution of worms in control population (blue) and irradiated population (orange). (d) Average fluorescence intensity in worms above L3/L4 stage (control, N=2802; Gy, N=954).

Supplementary Table S1: Radiation-induced differentially expressed genes in *C. elegans* following selective irradiation (300 Gy) analyzed by Long Read Sequencing (Oxford Nanopore technologies).

Gene Symbol	Gene Name	DE Status	Fold Changes	p-value	Identified Pathways
Heat shock stress					
T27E4.8	hsp-16.1	Up	1.439653399	4.09E-15	Heat shock stress
Y46H3A.3	hsp-16.2	Up	1.599351631	7.04E-05	Heat shock stress
T27E4.3	hsp-16.48	Up	1.293580584	3.71E-13	Heat shock stress
T27E4.9	hsp-16.49	Up	1.567863911	2.41E-10	Heat shock stress
F44E5.4		Up	1.231622987	6.94E-06	Heat shock stress, Hsp70 family
F44E5.5		Up	1.252968616	5.41E-12	Heat shock stress, Hsp70 family
F57A8.1		Up	1.651892213	7.01E-08	Heat shock stress
F59C12.4		Up	1.306556184	8.43E-18	Heat shock stress
R11A5.3		Up	2.308296289	1.26E-15	Heat shock stress
Fatty Acid Elongase activity					
F11E6.5	elo-2	Down	-1.114049169	2.19E-06	Fatty acid elongase activity. Involved in determination of adult lifespan; lipid metabolic process; regulation of gene expression.
F41H10.7	elo-5	Down	-1.139646916	2.77E-08	Fatty acid elongase activity. Involved in determination of adult lifespan; lipid metabolic process; regulation of gene expression.
F41H10.8	elo-6	Down	-1.041064083	3.87E-06	Fatty acid elongase activity. Involved in determination of adult lifespan; lipid metabolic process; regulation of gene expression.
UDG glucuronosyl transferases family					
C10H11.6	ugt-26	Down	-1.755353953	3.09E-09	Metabolism stress response, UDG glucuronosyl transferases family, involved in chemical detoxification
Y49C4A.8	ugt-29	Down	-1.90468465	6.01E-11	Metabolism stress response, UDG glucuronosyl transferases family, involved in chemical detoxification
Y39G10AR.6	ugt-31	Down	-1.522264659	6.26E-07	Metabolism stress response, UDG glucuronosyl transferases family, involved in chemical detoxification
Innate immune activity and signaling pathway					
K08D8.5		Down	-1.257810805	5.11E-07	Innate immune activity and signaling pathway (p38 MAPK)
F49E11.10	scl-2	Down	-1.391884051	1.16E-05	Cuticle, Innate immune activity and signaling pathway (p38 MAPK)
R08B4.5	nlp-66	Down	-1.231782492	2.47E-06	Cuticle, Innate immune activity and signaling pathway (p38 MAPK)
Related to gonadal/vulval and epithelial development					
F23A7.4		Up	0.704692227	1.59E-07	Vulval lineage transcriptome
F23A7.8		Up	1.17361793	1.01E-10	Germ-line specific transcriptome. affected by chemicals exposure
C52B9.7	paf-2	Up	1.344196824	4.35E-06	epithelial morphogenesis
Pathways related to heat and metal induced stress					
K11G9.6	mtl-1	Up	1.816797026	4.51E-08	Heat response, Response to metal induced stress
C25F9.2		Up	2.521799072	7.94E-05	Heat stress
Y38E10A.13	nspe-1	Down	-1.163088168	2.25E-07	Heat shock stress, arsenic response
Others pathways					
ZK973.6	anc-1	Up	0.63505832	6.28E-05	Nucleophagy, nucleolar homeostasis (tumor suppressor)
T22B11.4	kin-36	Up	1.368273355	1.15E-08	uncharacterized
R08D7.3	eif-3.D	Down	-0.799341075	1.12E-06	mRNA translation
C15C8.3	hrp-7	Down	-1.551418033	1.42E-08	Intestinal heme homeostasis (protease)
F08F3.7	cyp-14A5	Down	-1.01822292	5.90E-05	Response to Ionizing Radiation, Phase I detoxification enzyme
K09E4.4		Down	-1.282228823	9.55E-06	uncharacterized
C55A1.6		Down	-0.98767716	8.51E-06	uncharacterized
E04F6.6		Down	-0.952725622	1.77E-08	uncharacterized
C36H8.3	flp-9	Down	-0.837940796	9.96E-05	SKN-1 stress pathway