IRSN
INSTITUT
DE RADIOPROTECTION
ET DE SÛRETÉ NUCLÉAIRE

Enhancing nuclear safety

Effect of chronic low doses of ionizing radiations on central nervous system development of zebrafish

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EUROPEAN TECHNICAL SAFETY ORGANISATIONS NETWORK

Introduction

Industrial, military and medical sources

Chernobyl and Fukushima

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Chernobyl and Fukushima

Controlled rejects ↓

↓ Uncontrolled rejects

What are the effects of chronic low dose exposures on the environment?

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Objectives

- Determining biomarkers of effects
- Establishing links between molecular and phenotypic effects during the development of the central nervous system

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- Establishing links between molecular and phenotypic effects during the development of the central nervous system

The central nervous system is sensitive to ionizing radiations

- Reduction of brain size on:
  - Birds at Chernobyl (Moller *et al*. 2011)
  - Macaques at Fukushima (Hayama *et al.* 2017)
  - Mice (Vereet *et al*. 2016)

- Behavioral effects on mice (Vereet *et al*. 2016)

Material and methods

Biological model : zebrafish

- Transparent embryos and quick development
- Sequenced genome

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Dose rates studied

- Irradiation by a ^{137}Cs source
- 0.5mGy/h
- 5mGy/h
- 50mGy/h

Low dose rate < 6mGy/h
(UNSCEAR. 2012)

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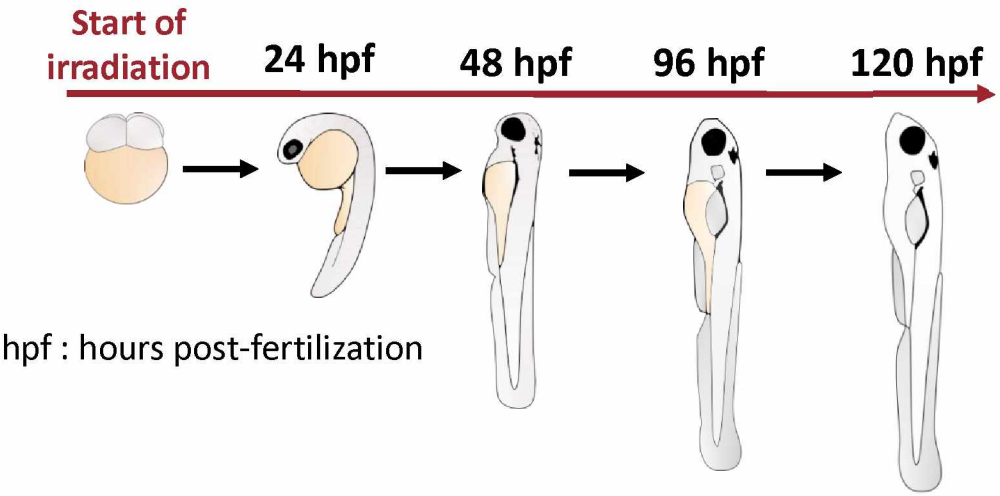

hpf : hours post-fertilization

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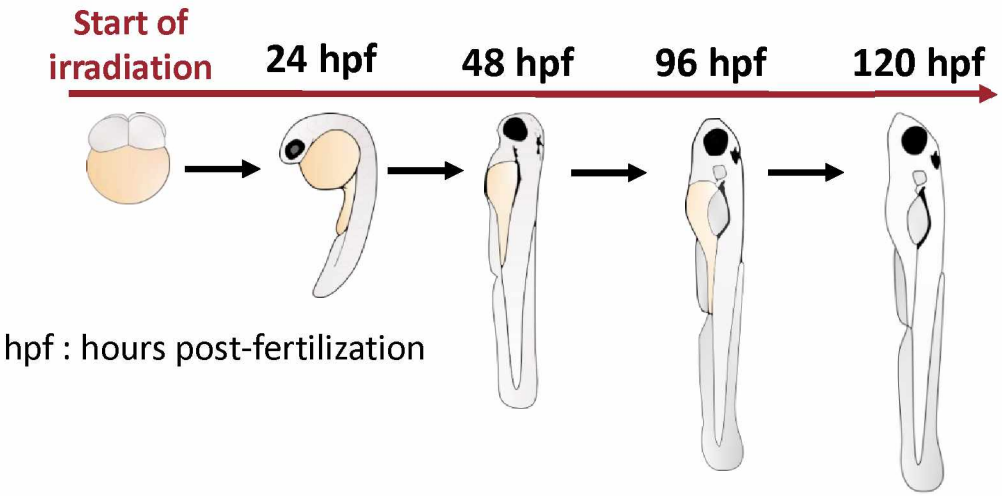

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Molecular scale

- Transcriptomics (RNA-seq)
- RT-qPCR
- Hybridization *in-situ*
- Proteomics
- Detection of foci γ-H2AX

Phenotypical scale

- Mortality
- Hatching
- Morphology
- Heart rate
- Behavioral tests
- Transmission electron microscopy

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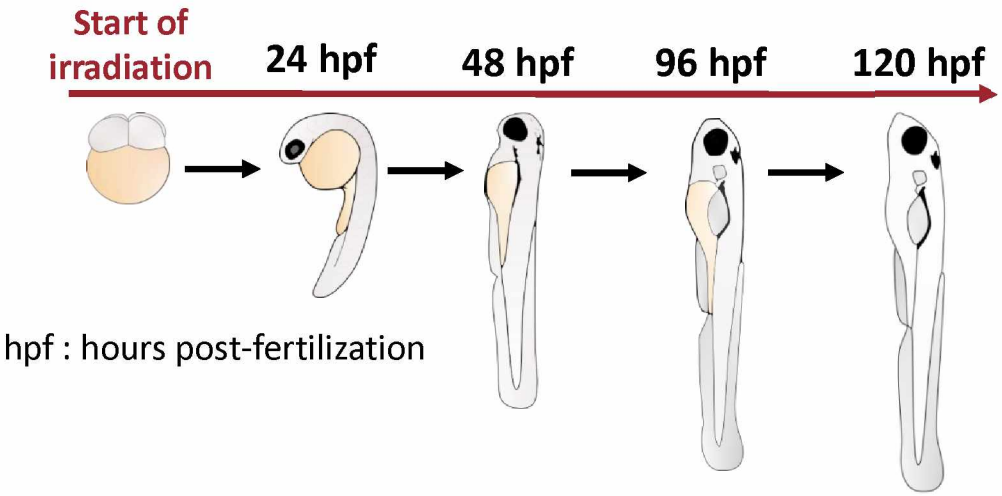

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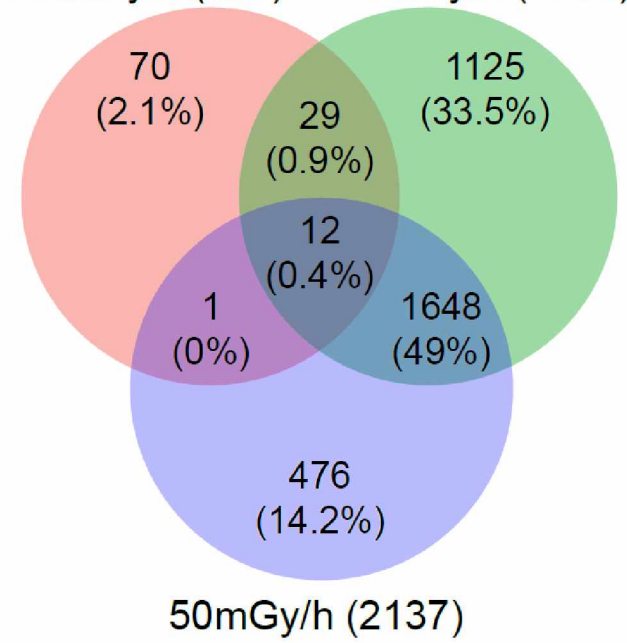
Results - Transcriptomics

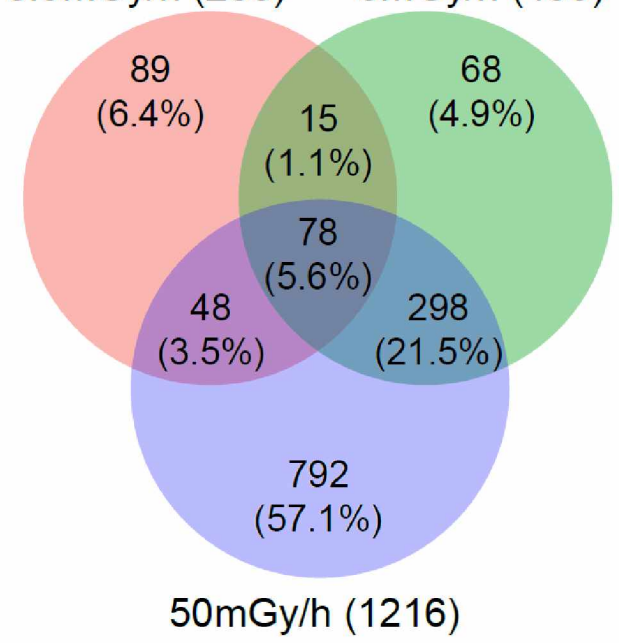
Number of **differentially expressed genes** between dose rates

Criteria for differentially expressed genes
Fold-change ≥ 1.5
Adjusted p-value ≤ 0.01

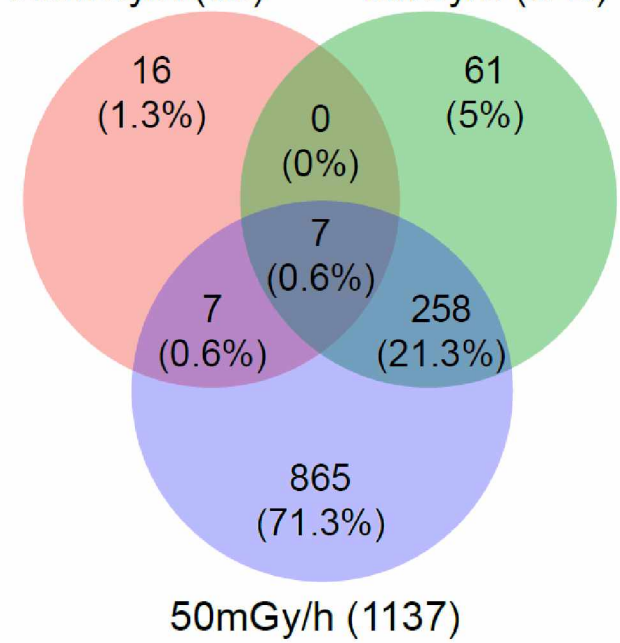
24hpf



48hpf



96hpf



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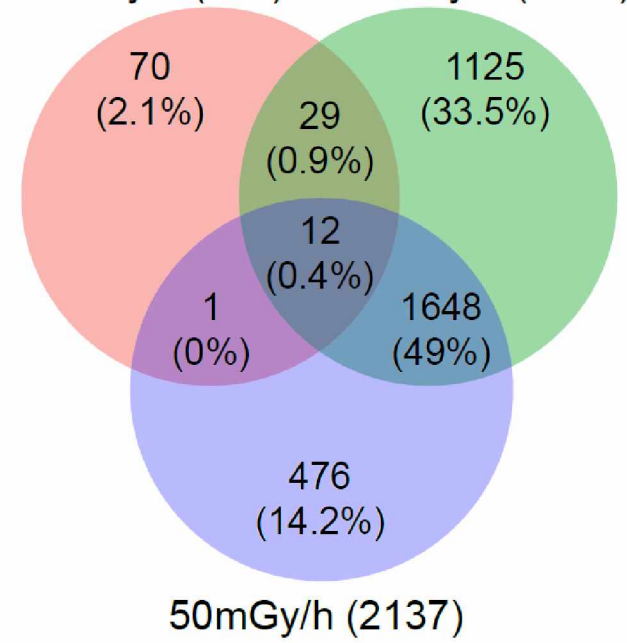

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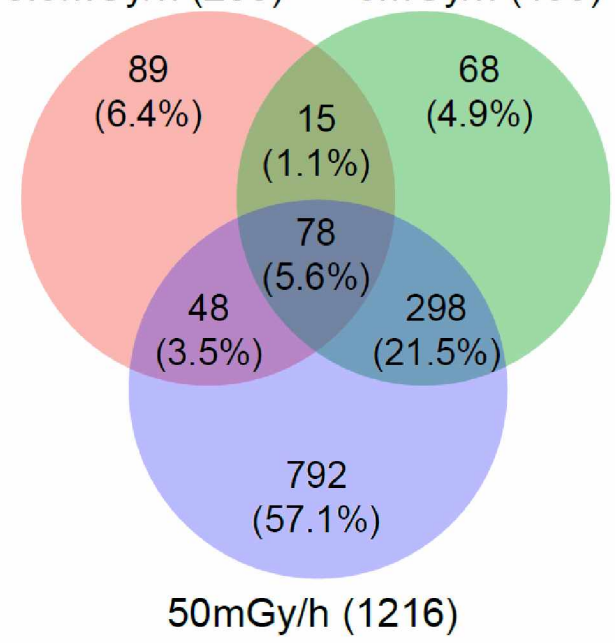

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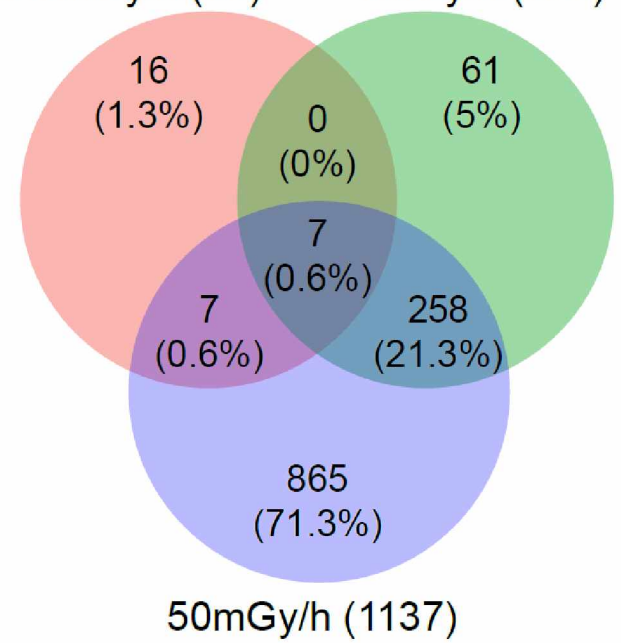
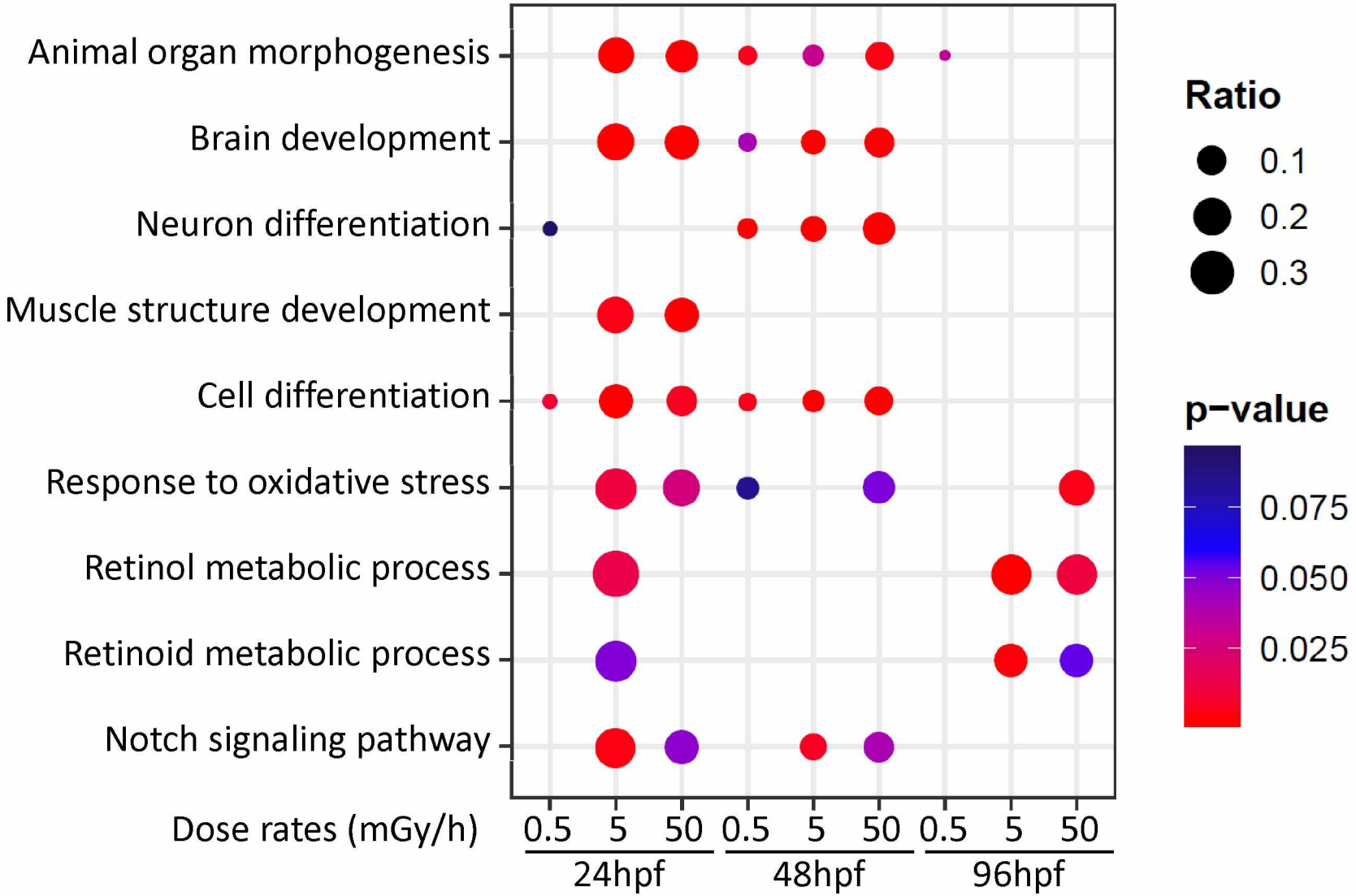


- Fewer differentially expressed genes at **0.5mGy/h**
- 20% to 50% of **common genes** between **5mGy/h and 50mGy/h**

Results - Transcriptomic - GO Term analysis



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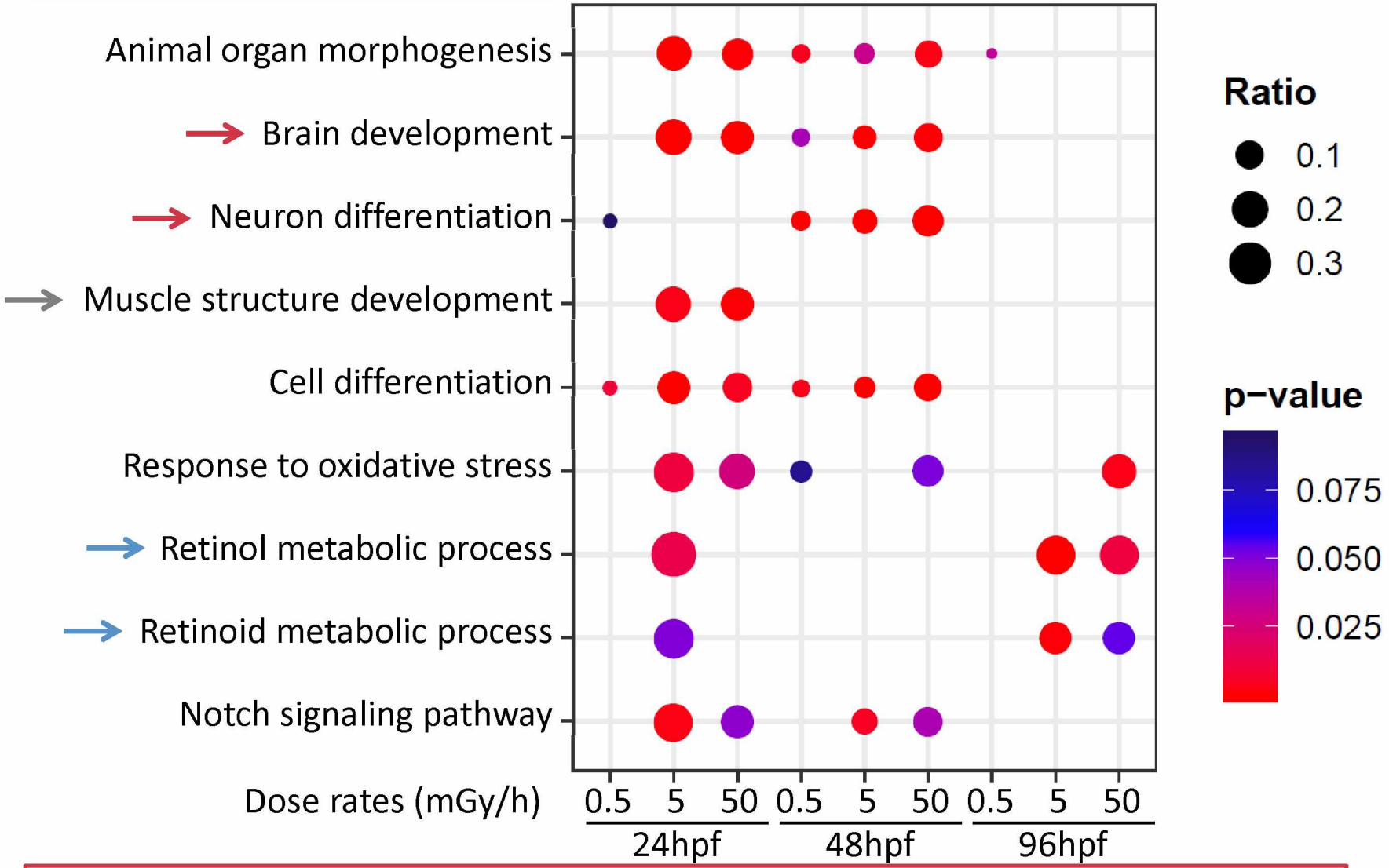
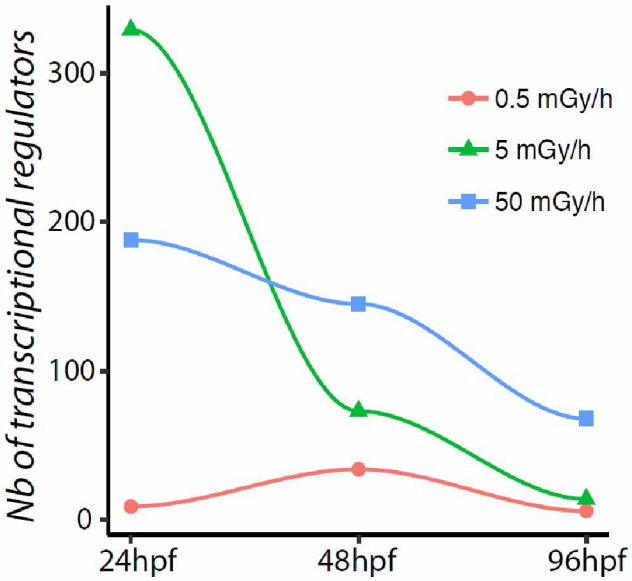


- Potential impact on **neurogenesis** **at all dose rates**
- At **5 and 50mGy/h** potential impact on **retinoic acid pathway** and **myogenesis**
- **Hypothesis**: perturbation of retinoic acid pathway may disrupt neurogenesis

Results - Transcriptomic - Transcription factors analysis

Number of differentially expressed transcription factors



- **Decrease** of number of **transcriptional factors** misregulated during development

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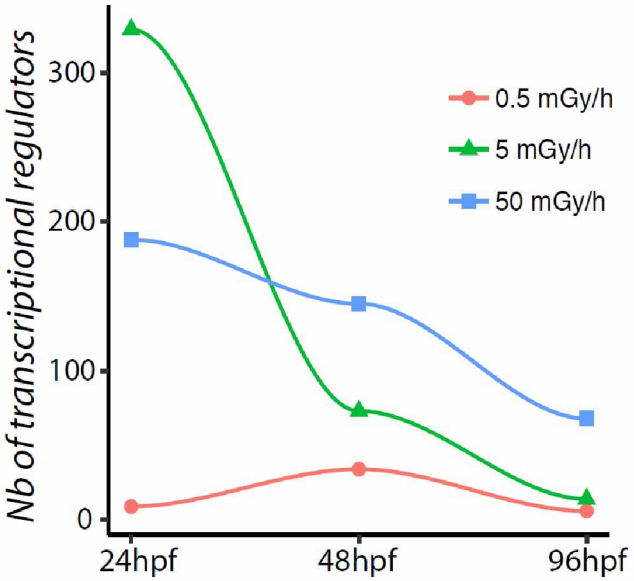
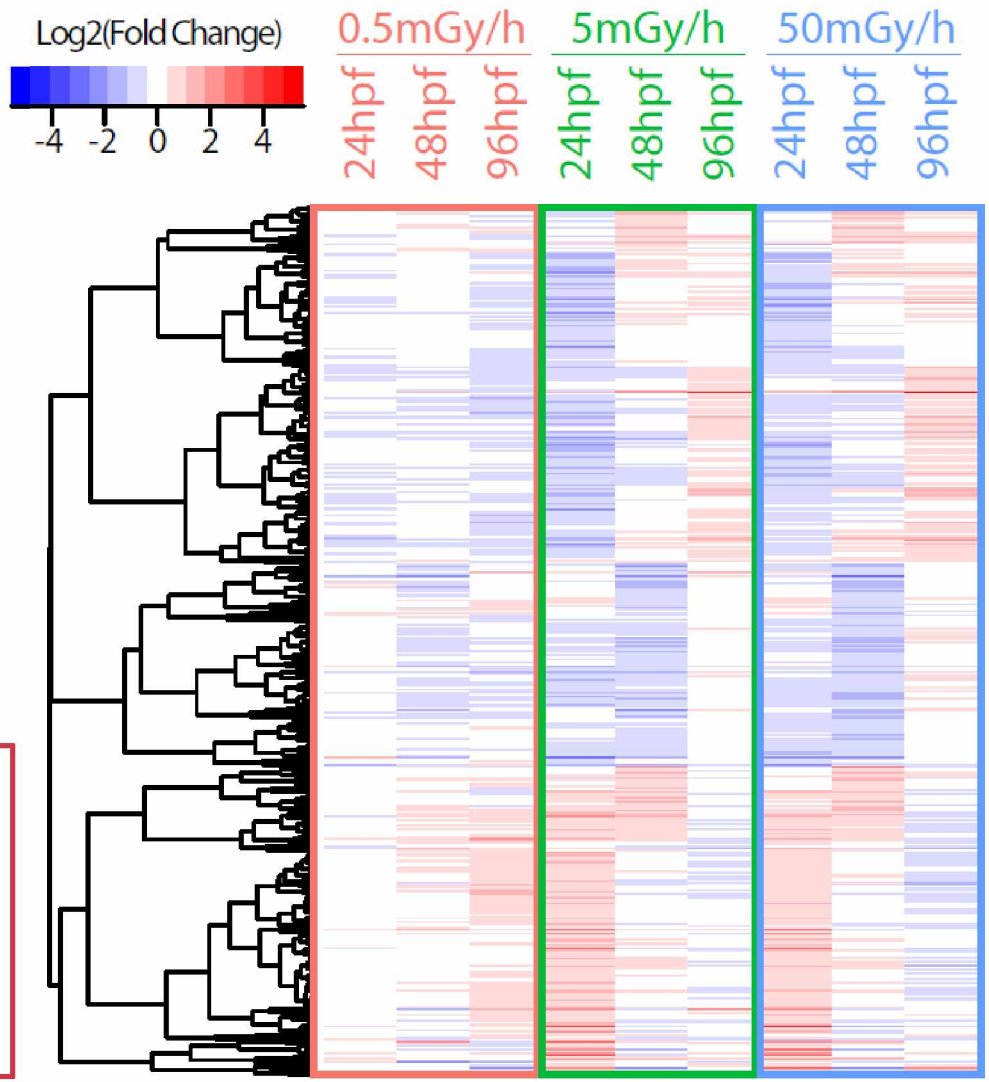


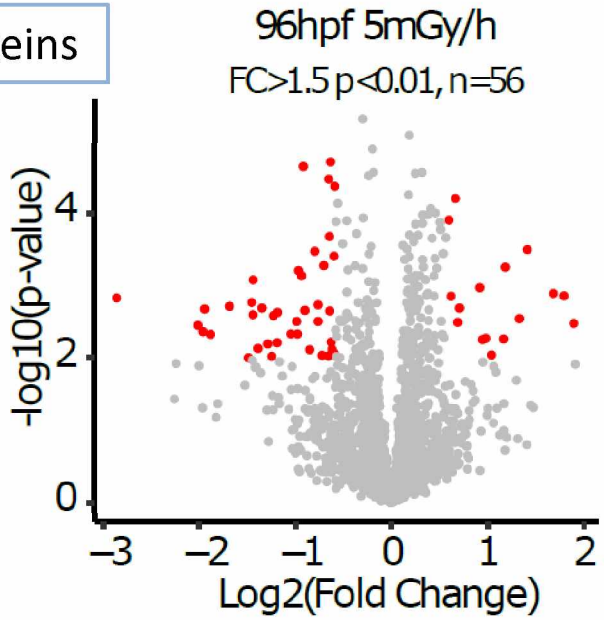
Clusterization of transcription factors

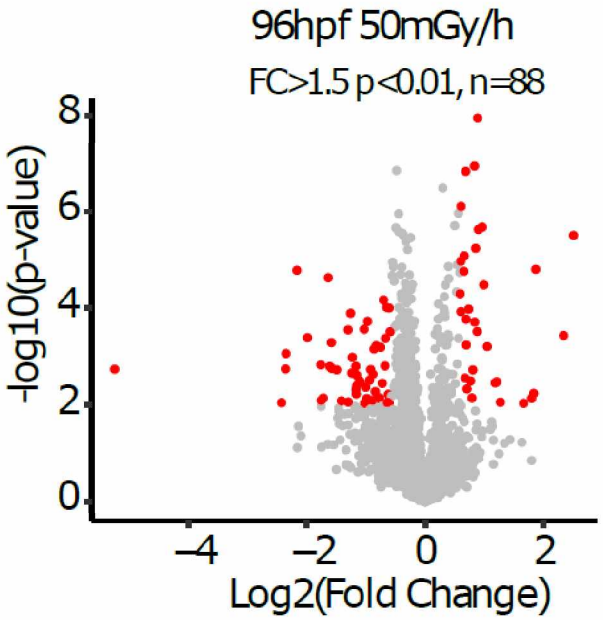


- **Decrease** of number of **transcriptional factors** misregulated during development
- **Similar** expression profile between **5 and 50mGy/h**

Results - Proteomics at 96hpf

Differentially expressed proteins





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Results - Proteomics at 96hpf

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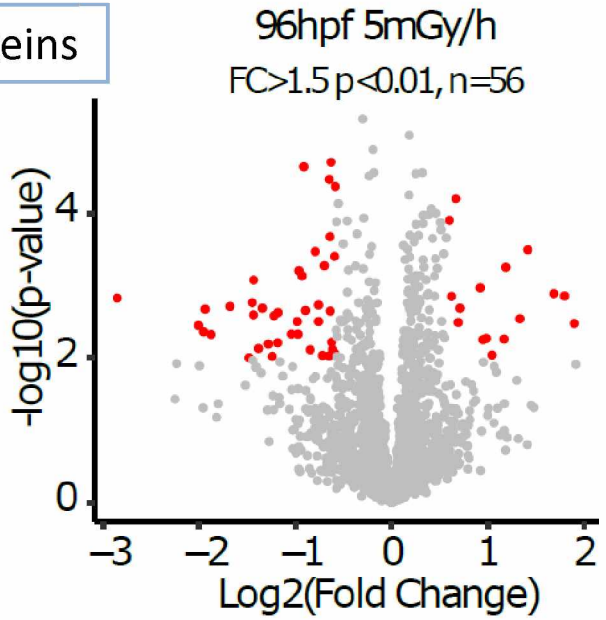
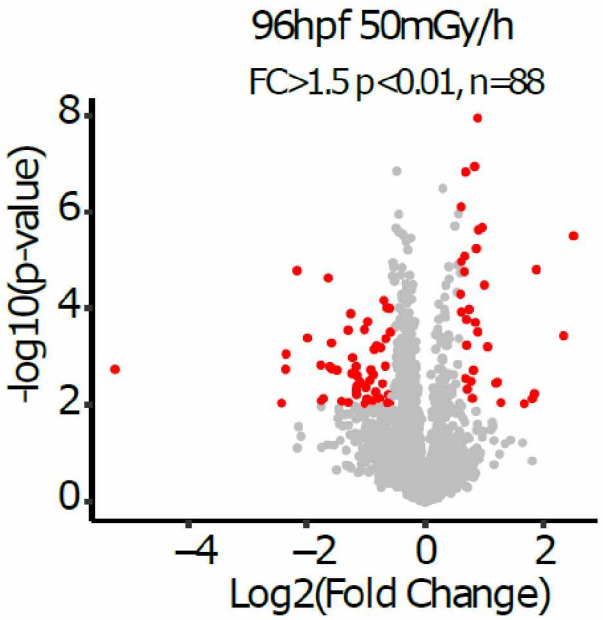
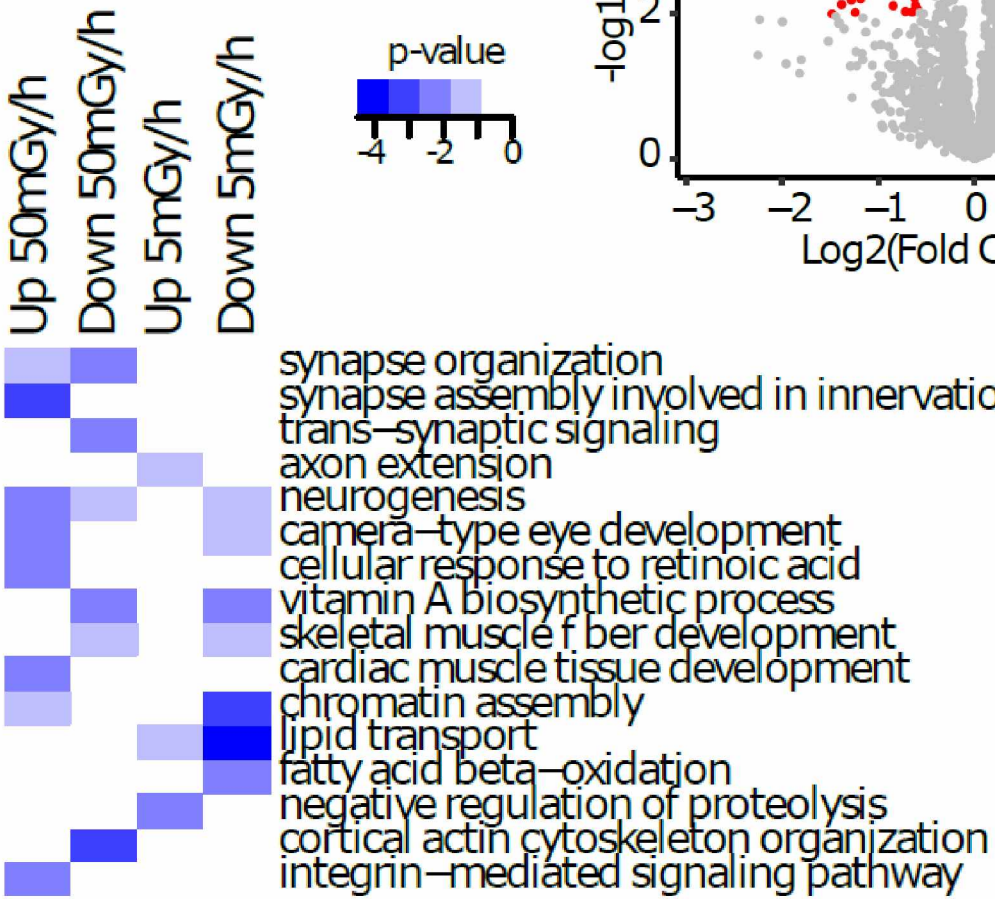




GO term analysis



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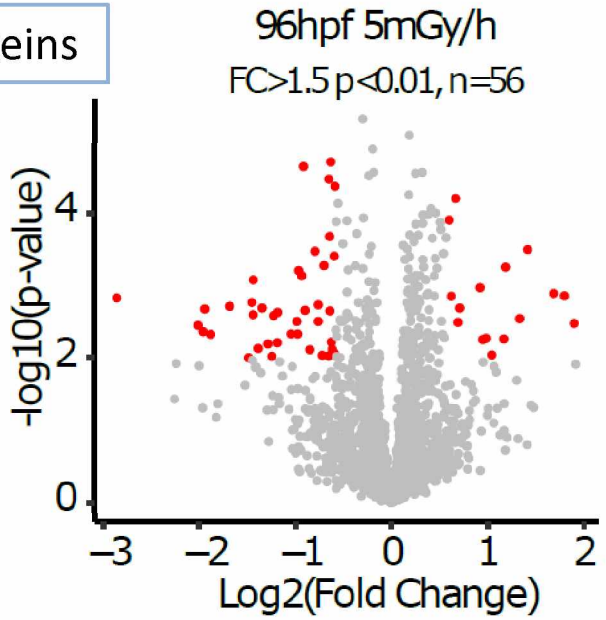

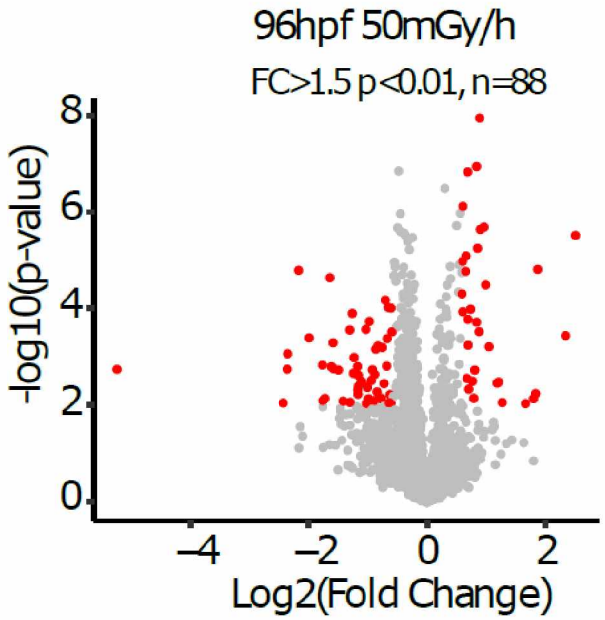

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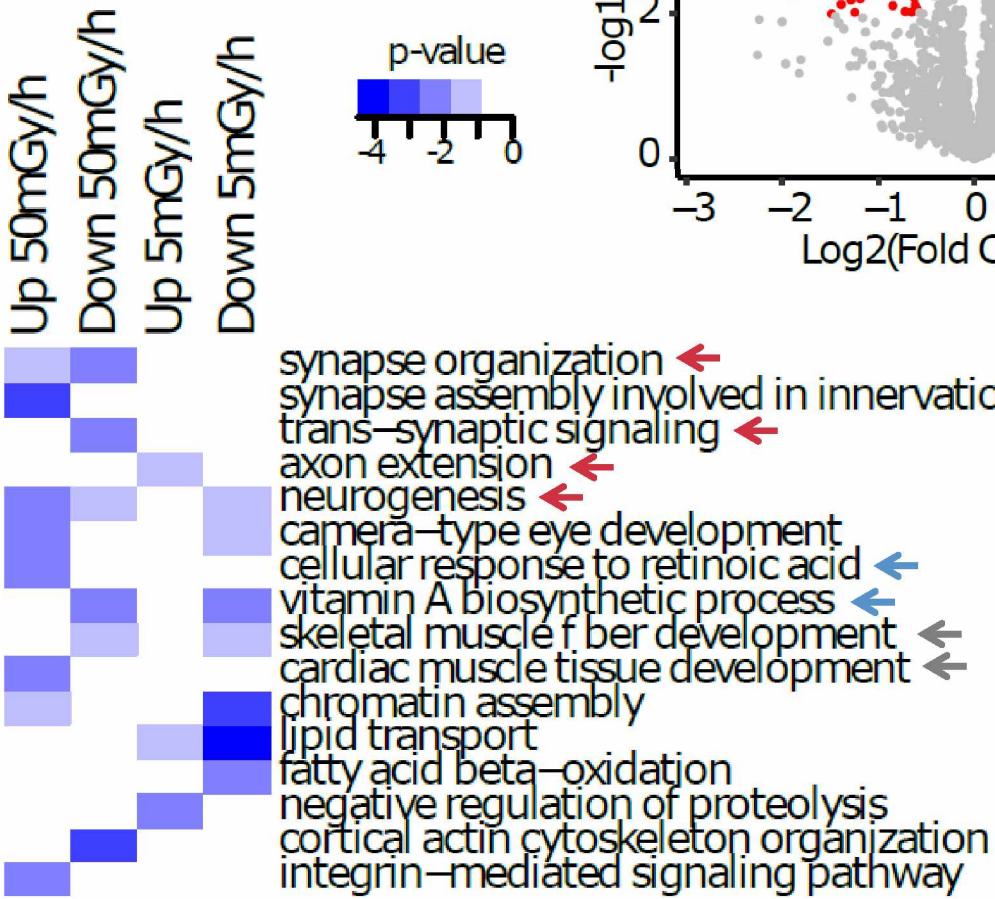

- Potential impact on **neurogenesis** and myogenesis
- Disruption of **retinoic acid pathway**
- **Concordance of results with RNA-seq**

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Results - Behavioral tests

Integrative parameters of locomotor functions development

Brustein *et al*. 2003
Goulding. 2009
Kiehn. 2016

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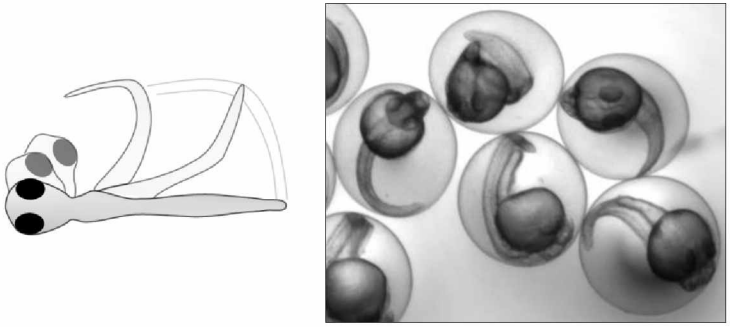
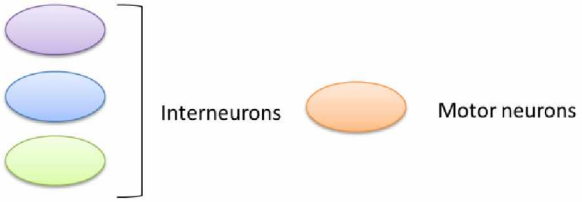
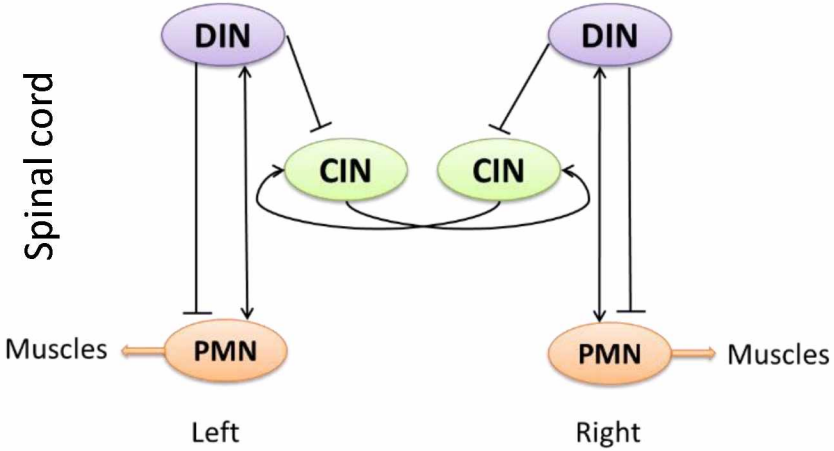
Test n°1 : Tail coiling (24hpf)







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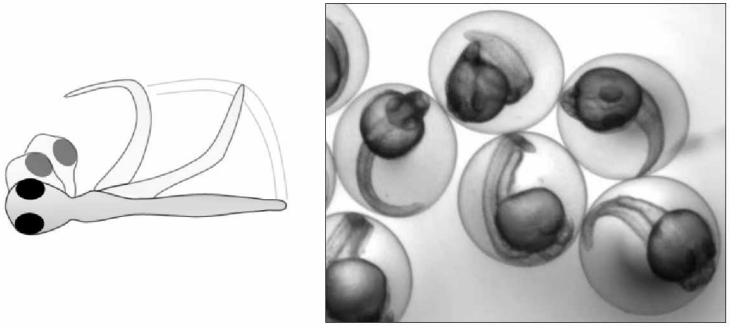
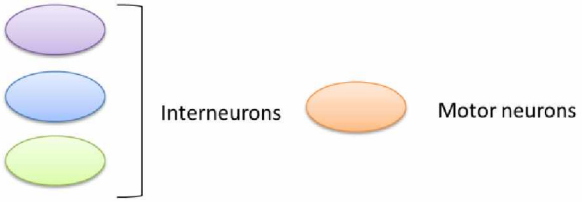
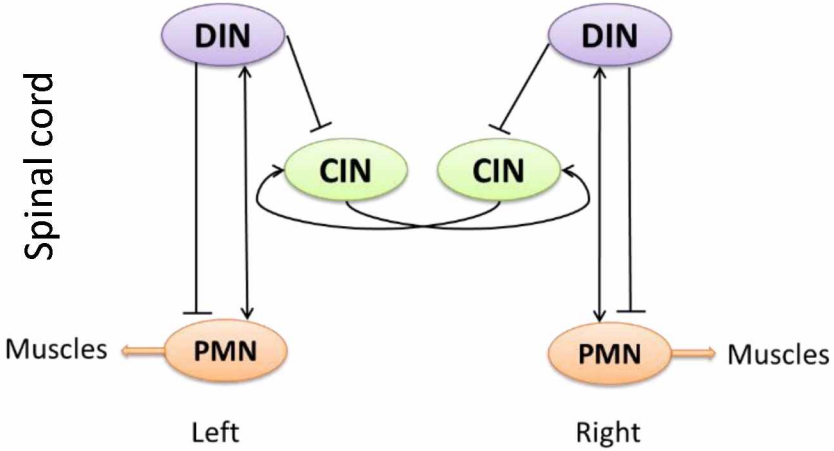
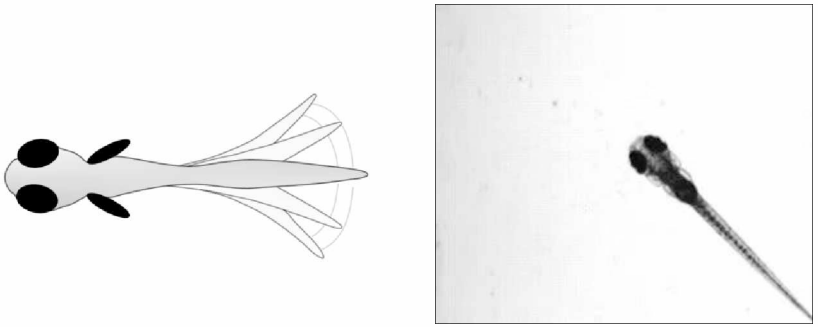
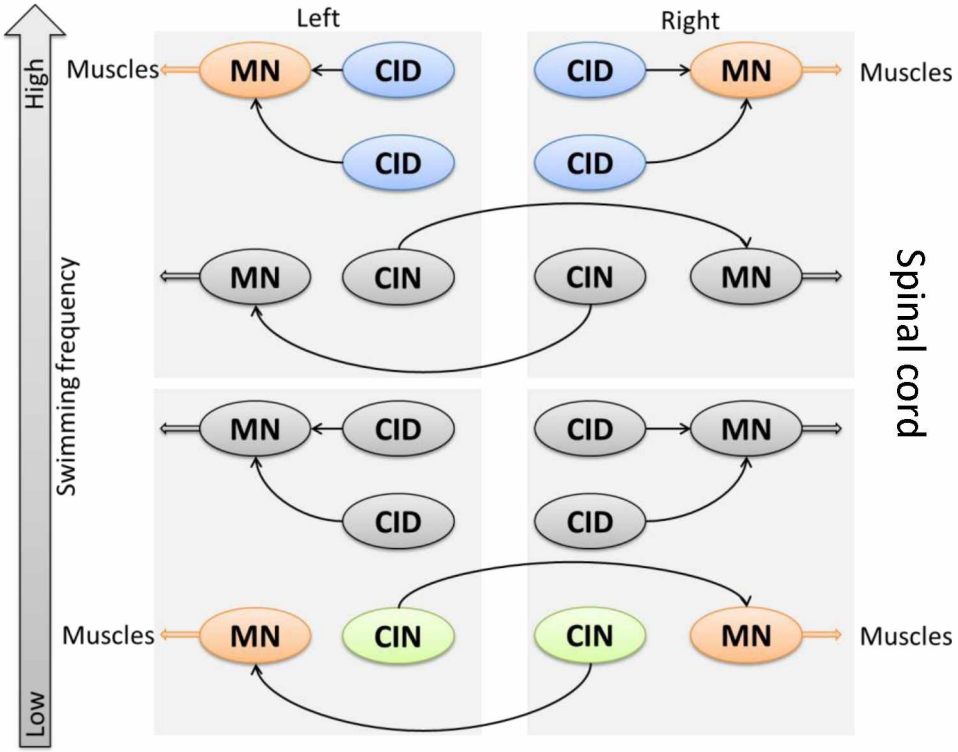




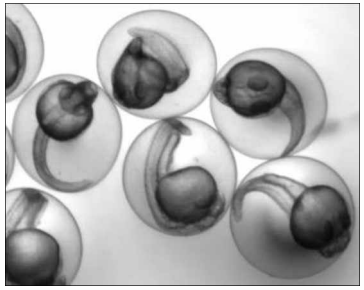


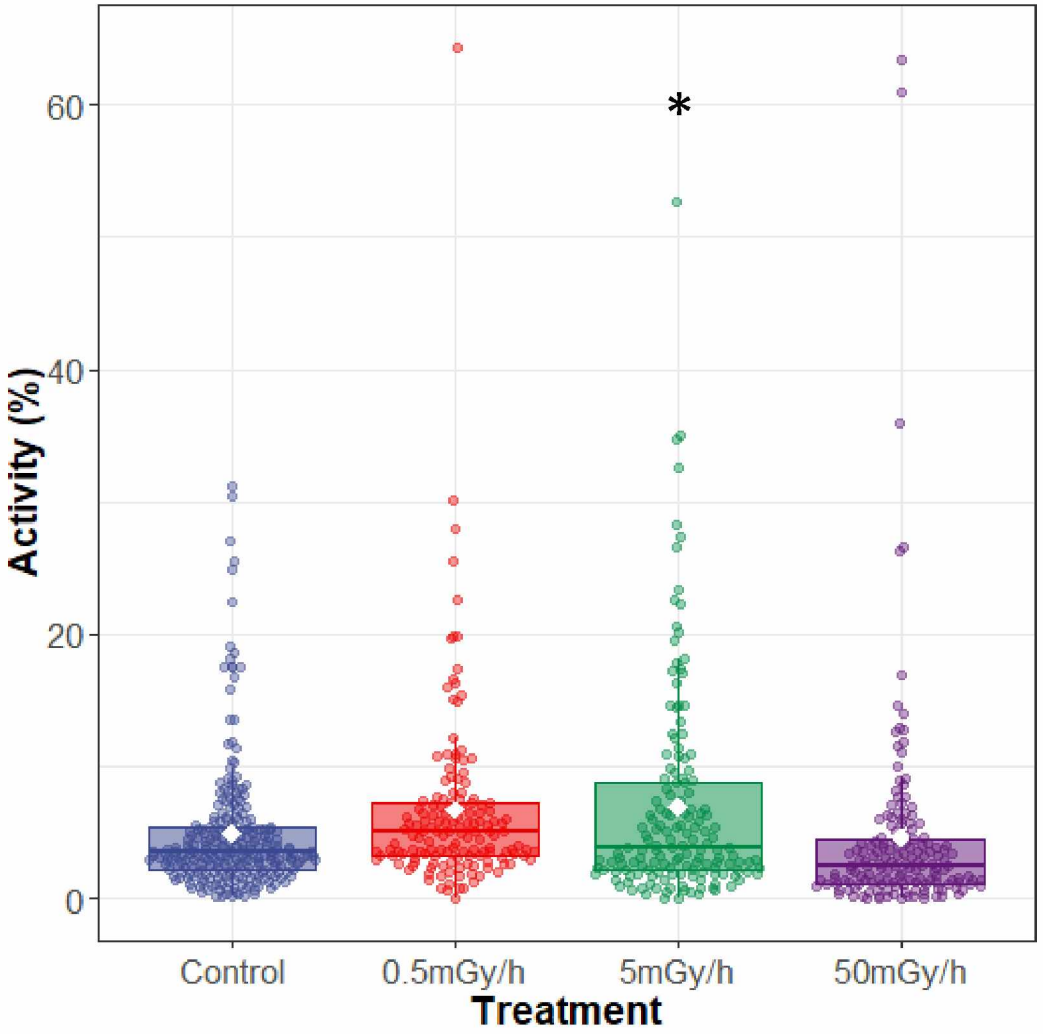
Test n°2 : Visual motor response (120hpf)





Results - Tail coiling at 24hpf





Permutation test
p-value < 0,05
n = 150

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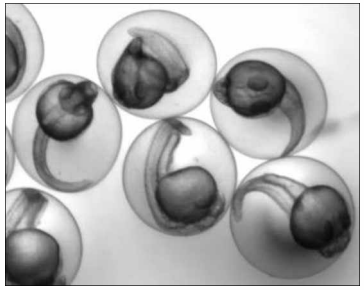

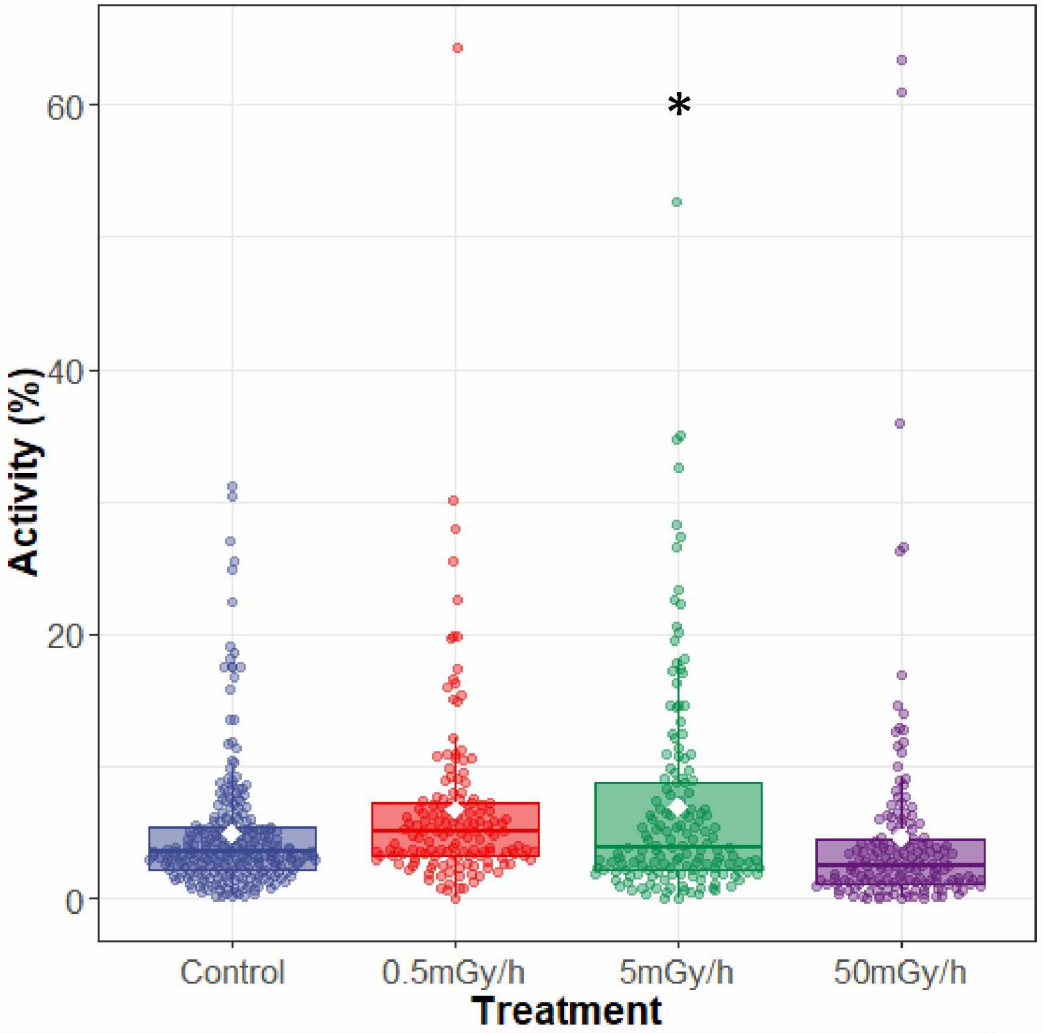

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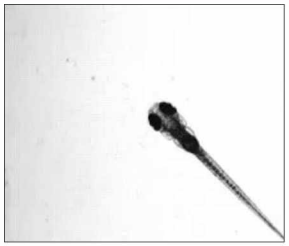
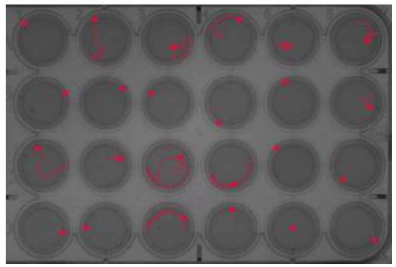
n = 150

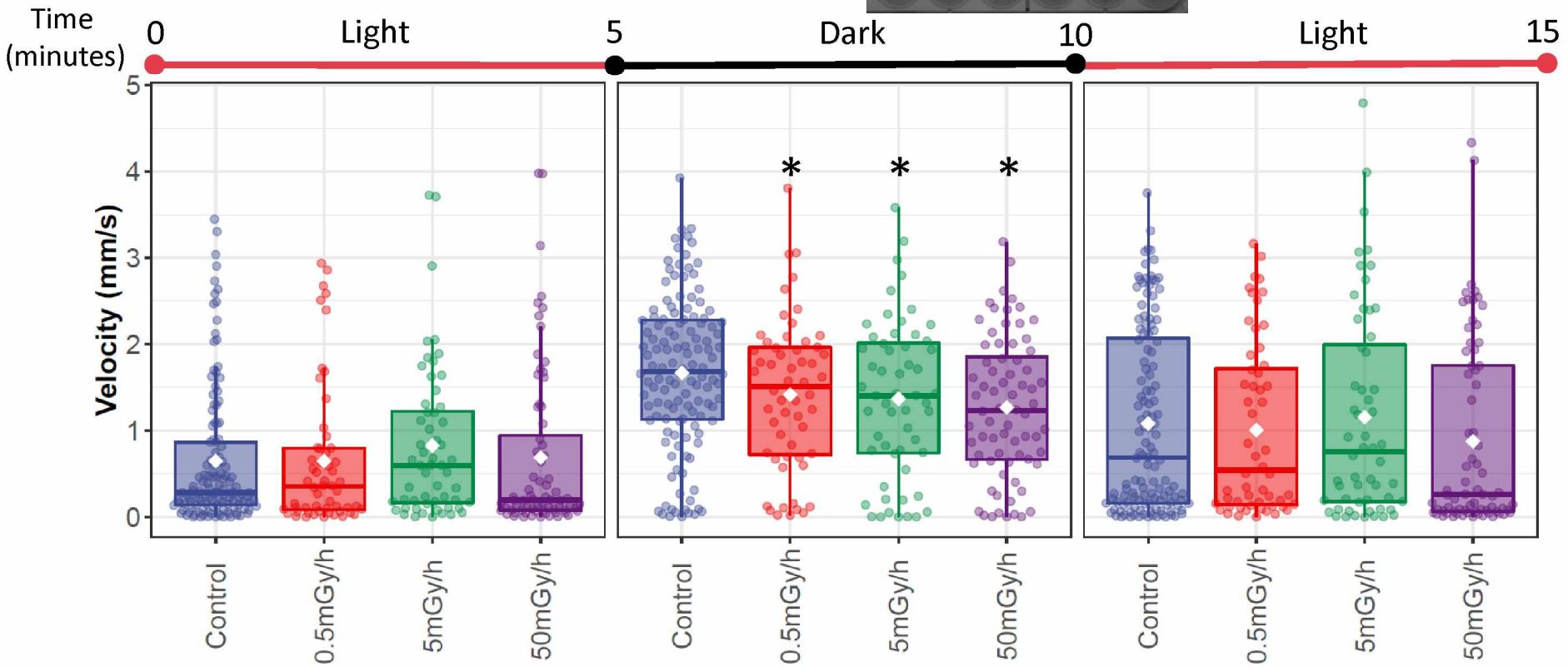
Increase of embryo activity at 5mGy/h

- **Neuromuscular effects at 5mGy/h?**
- **Hypothesis:** Effect may be linked to a high number of misregulated transcription factors at 24hpf at 5mGy/h

Results - Visual motor response at 120hpf







Permutation test
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Kiehn. 2016

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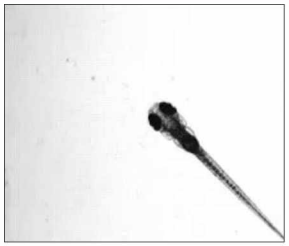

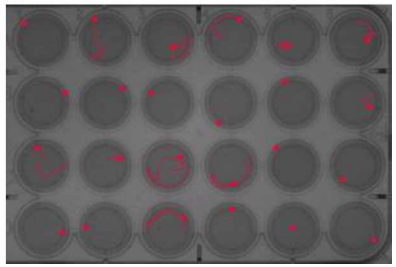

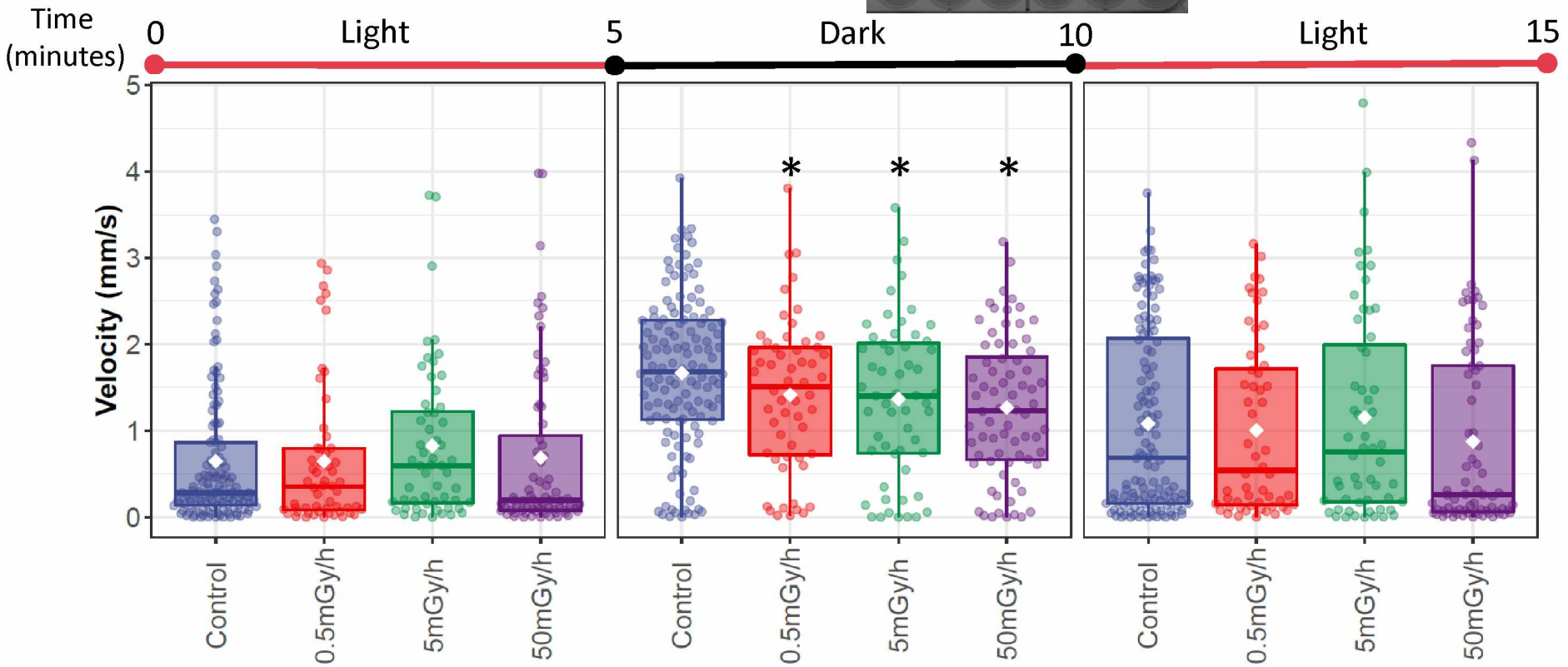

Permutation test
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Decrease of velocity in dark phase at all dose rates

- **Impact on neuronal network controlling fast swim?**
- **Stress response may also be involved**

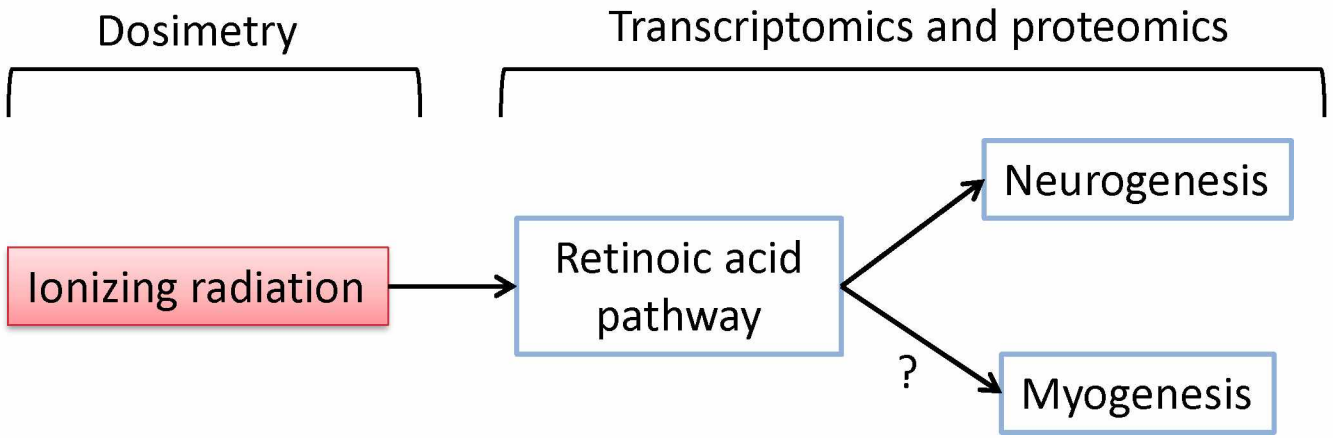
Kiehn. 2016

Conclusions and Perspectives

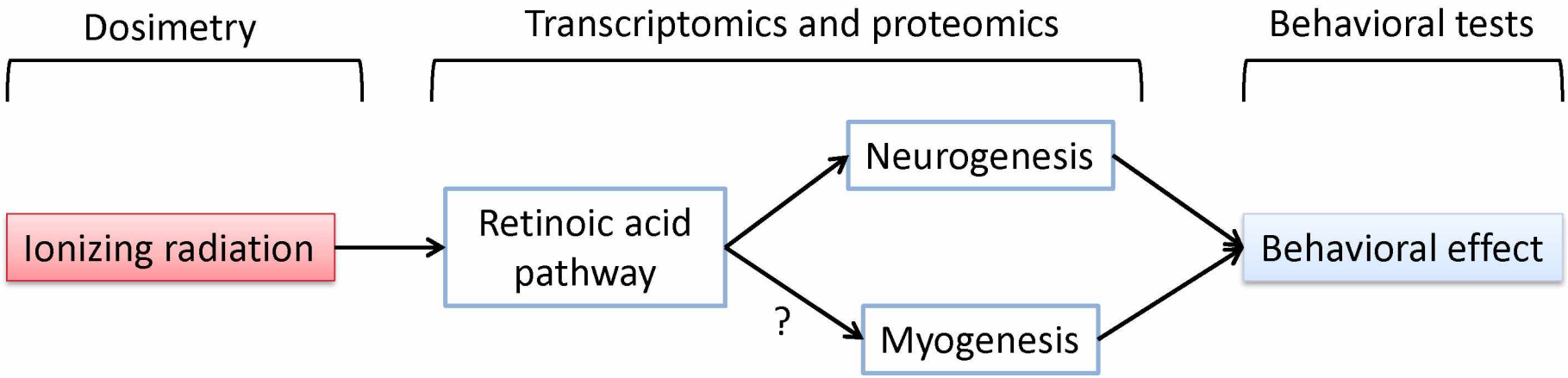
Dosimetry

Ionizing radiation

Conclusions and Perspectives



Conclusions and Perspectives



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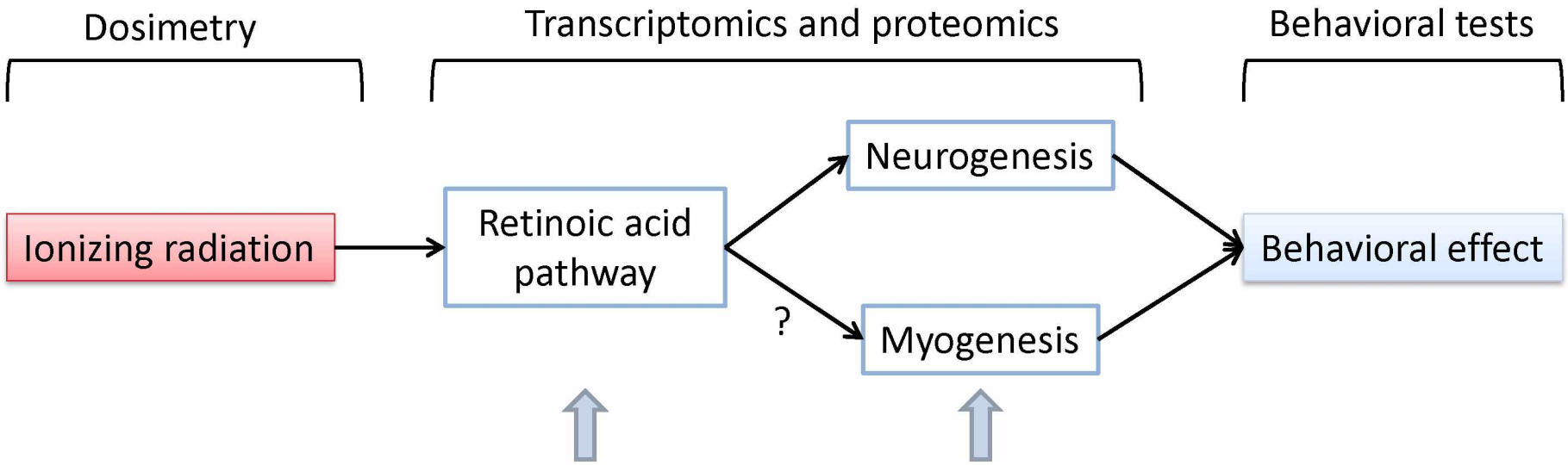


Biomarkers of effects?

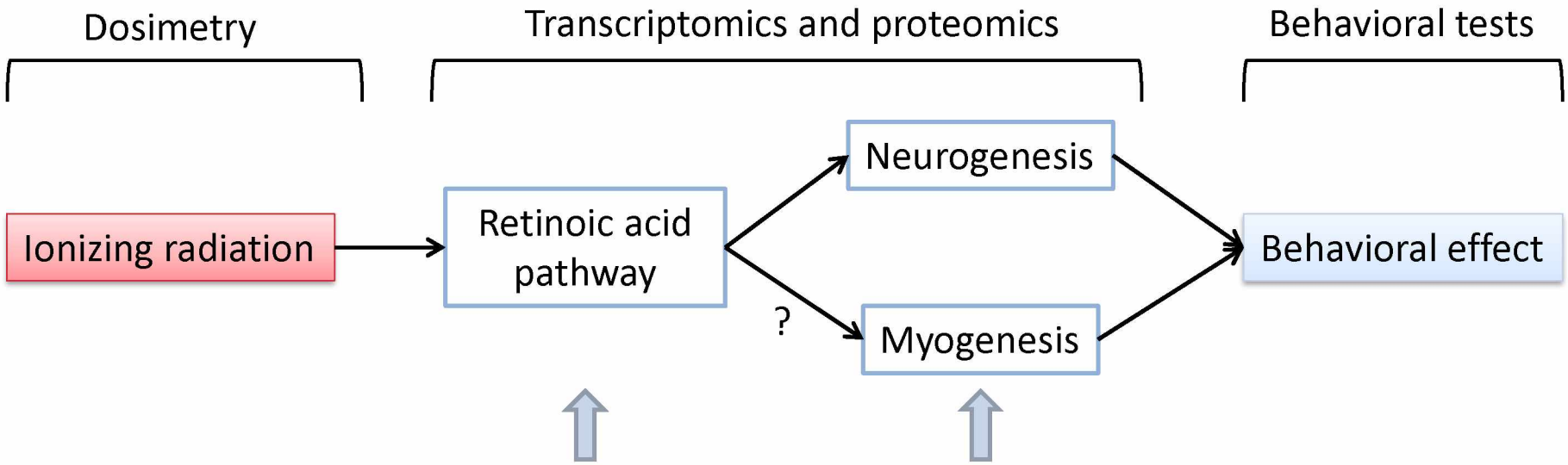
Potential biomarkers:

Retinoic acid : rdh5, rdh10b

Neurogenesis: dbx1, olig1

Myogenesis: myog, myf5

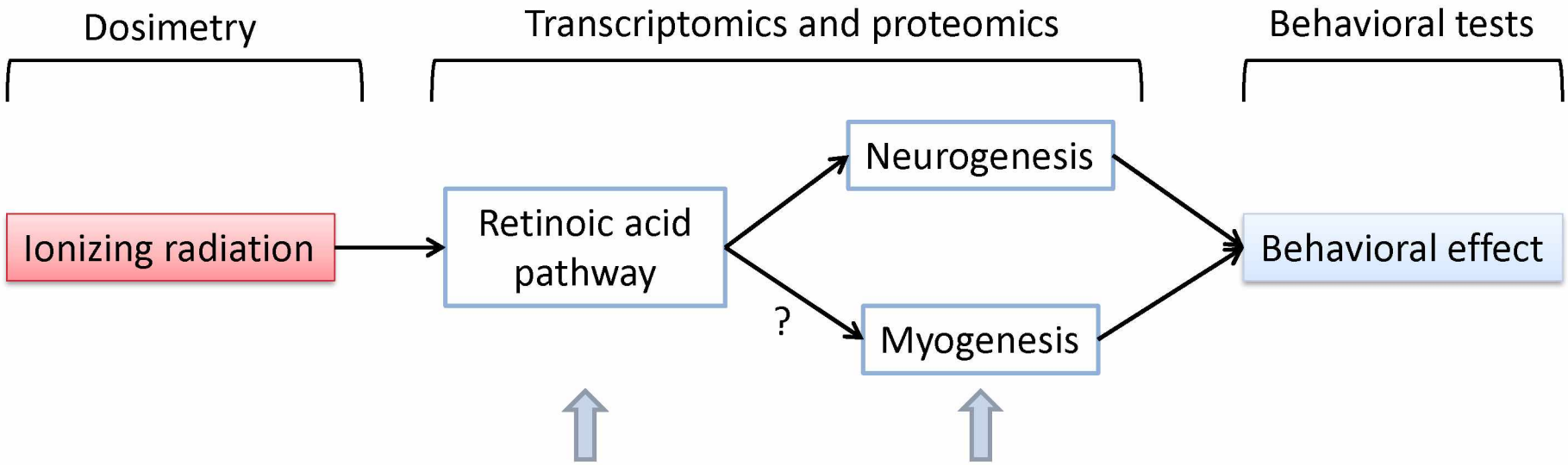
Conclusions and Perspectives



Ongoing analyses

- Muscles observation with transcription electron microscopy
- Transcription factors pattern expression analysis with hybridization *in-situ*
- Methylome analysis at shield stage

Conclusions and Perspectives



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Myogenesis: myog, myf5

Ongoing analyses

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Perspectives

- Investigate the difference in response to lower (0.5mGy/h) and higher (5 and 50mGy/h) dose rates
- Explore the mode of action of ionizing radiations on retinoic acid pathway and neurogenesis
- Confirm potential biomarkers of effects

Acknowledgment

IRSN
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NEEDS nucléaire énergie • environnement • déchets • société

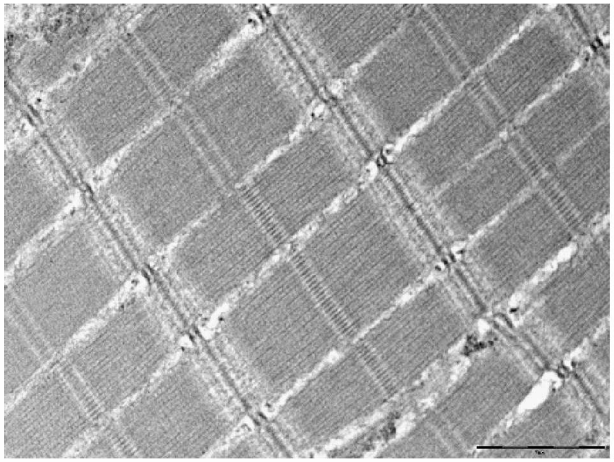
DE LA RECHERCHE À L'INDUSTRIE cea

Funding:
NEEDS Environment
CEA / IRSN project

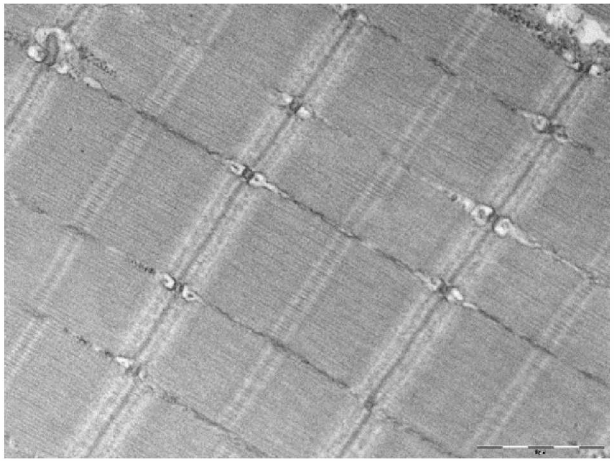
Supplementary results - Transmission Electron Microscopy

Observation of muscle and neuro-muscular junctions

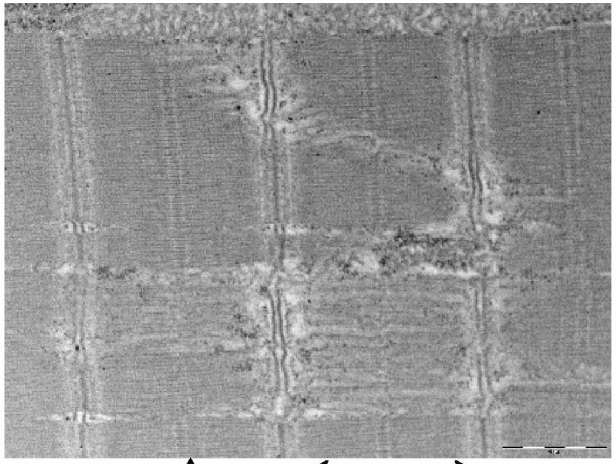
Control



5 mGy/h

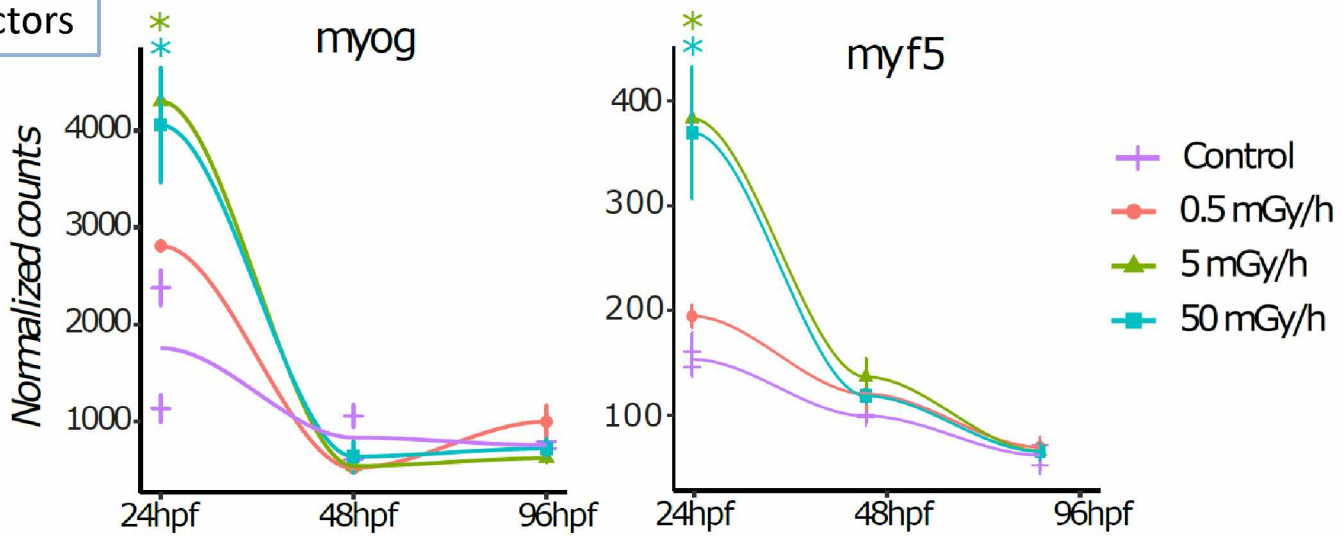


50 mGy/h

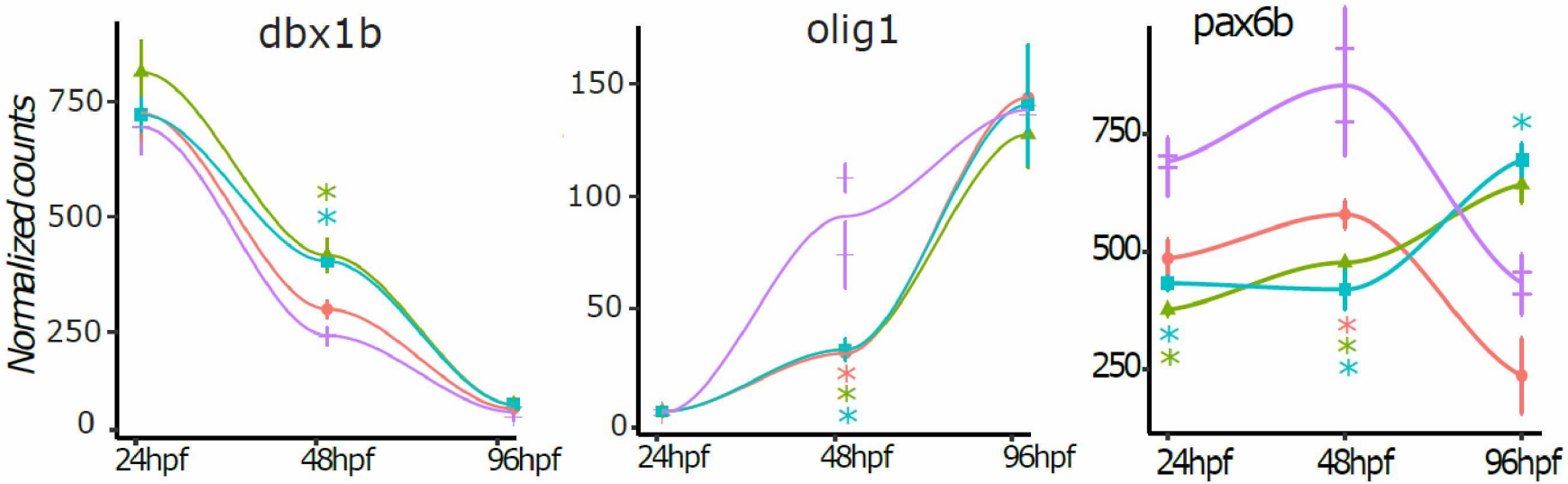


Supplementary results - Transcriptomic - Transcription factors analysis

Myogenesis transcription factors

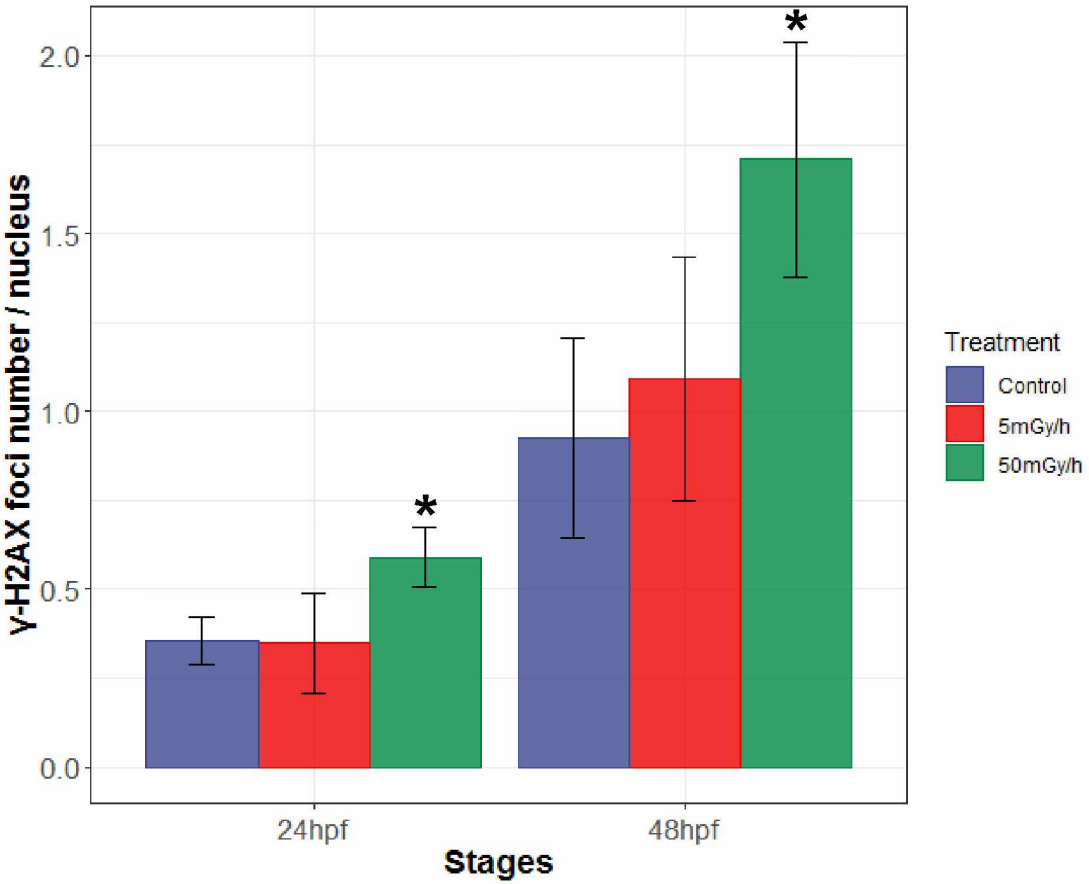


Neurogenesis transcription factors



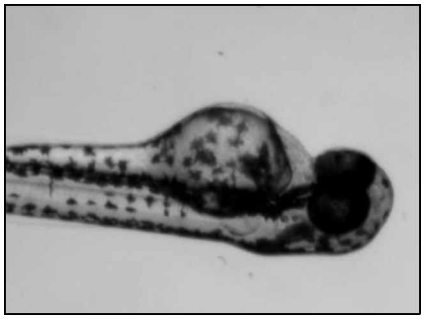
Supplementary results - Immunostaining

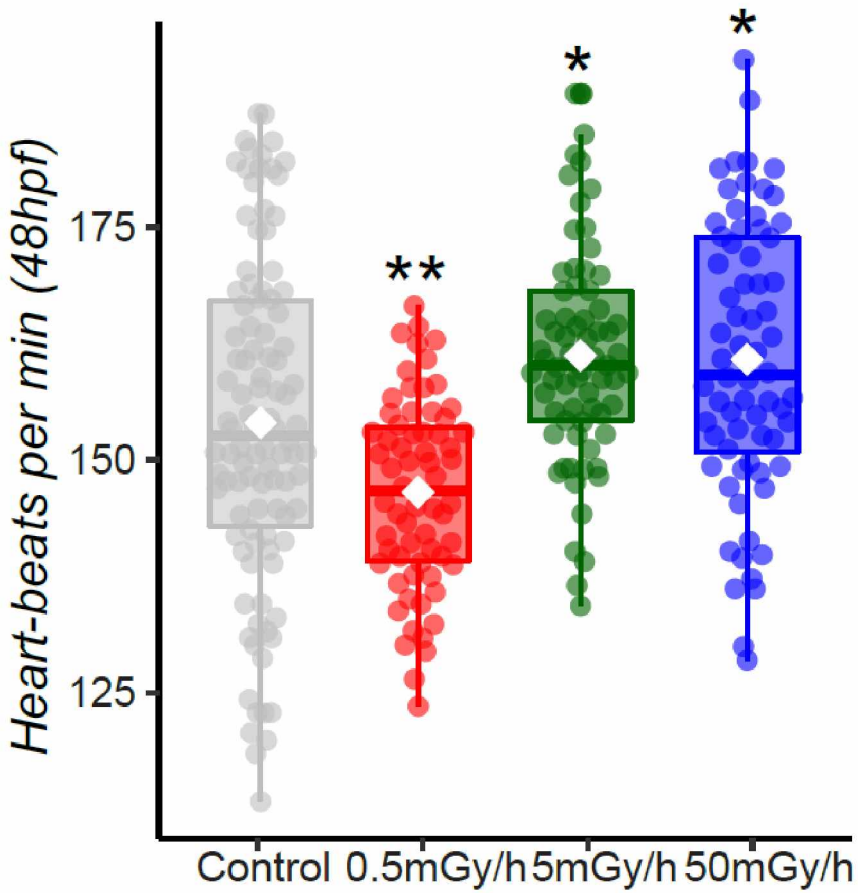
Detection of gamma-H2AX foci



T-test
p-value < 0,05
n = 400
Error bar: Confidence interval at 95%

Supplementary results - Heart rate at 48hpf





T-test
* p-value < 0,05
** p-value < 0,001
n = 70

Supplementary data - Cumulated doses (mGy)

MICADO installation

	0.5mGy/h	5mGy/h	50mGy/h
24hpf	12	120	1200
48hpf	24	240	2400
96hpf	48	480	4800
120hpf	60	600	6000