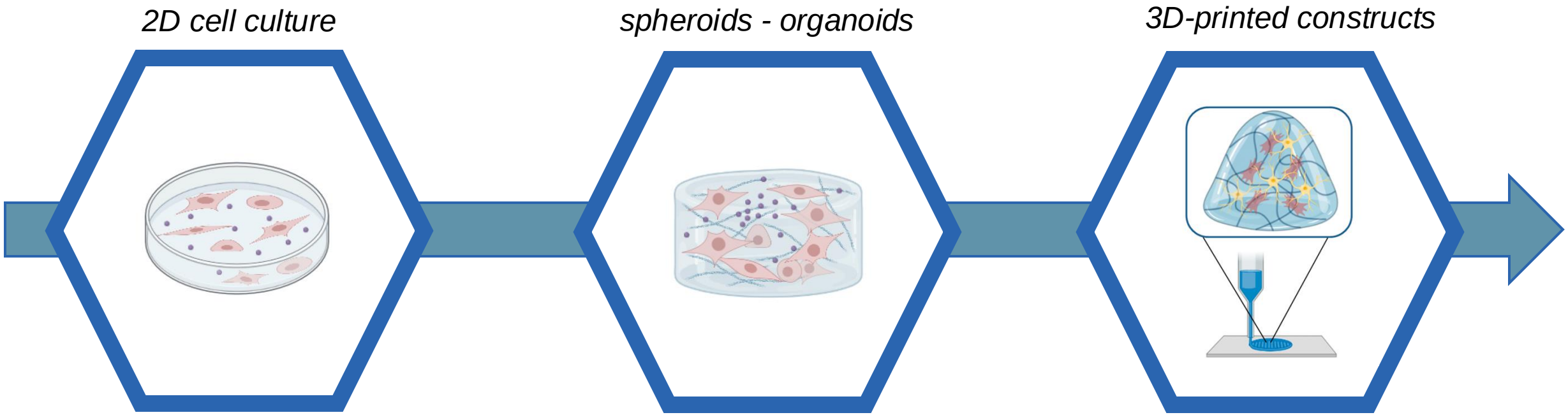
Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA
THE Tuscany Health Ecosystem

An *in vitro* platform to investigate the FLASH effects on melanoma and normal skin cells

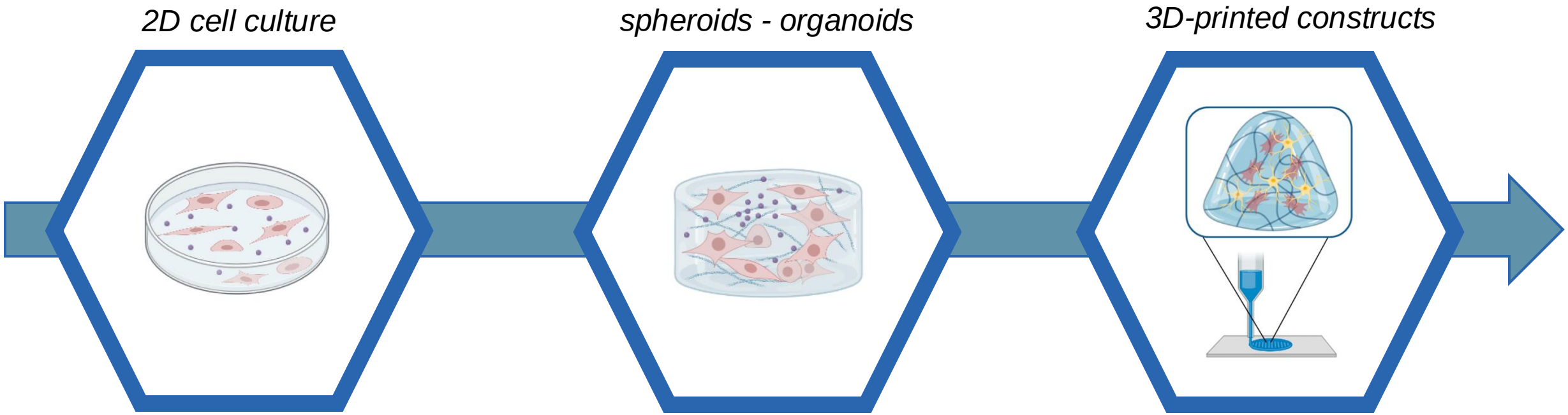
Alice Usai, PhD
THE Fellow

Institute of Clinical Physiology
Italian National Research Council

In vitro workflow: scaling up from 2D monolayer to complex 3D cell culture



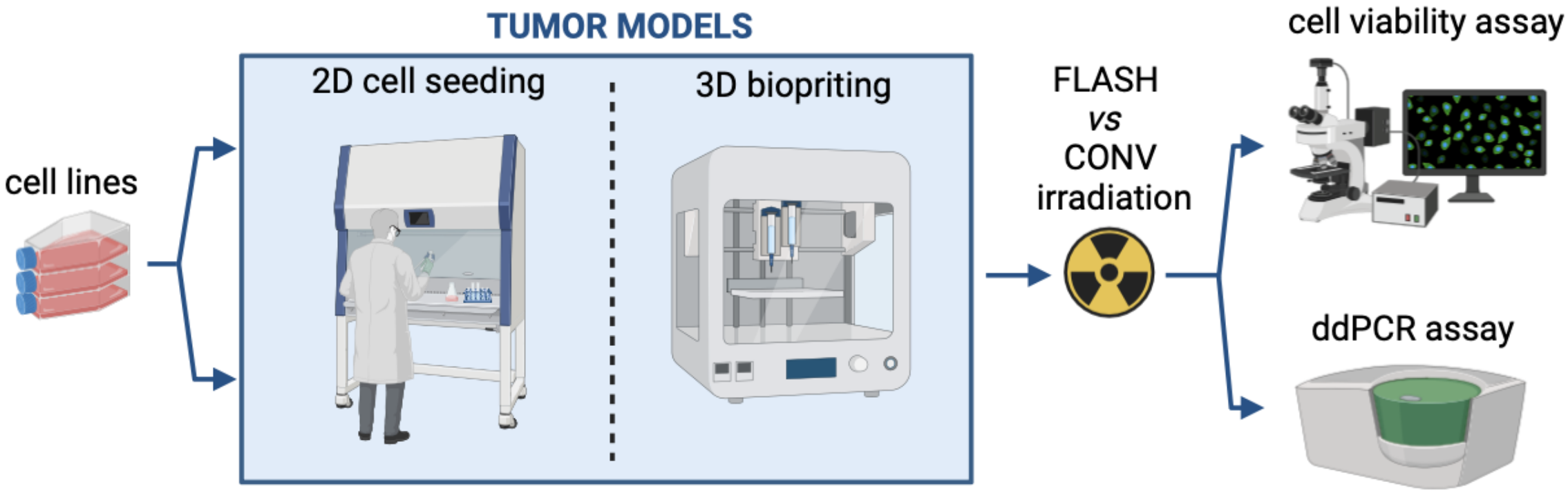
In vitro workflow: scaling up from 2D monolayer to complex 3D cell culture



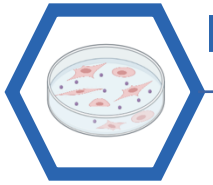
Goal

FLASH-RT minimizes the toxic effects on healthy cells
while preserving the cancer-fighting benefits of CONV-RT

Experimental plan



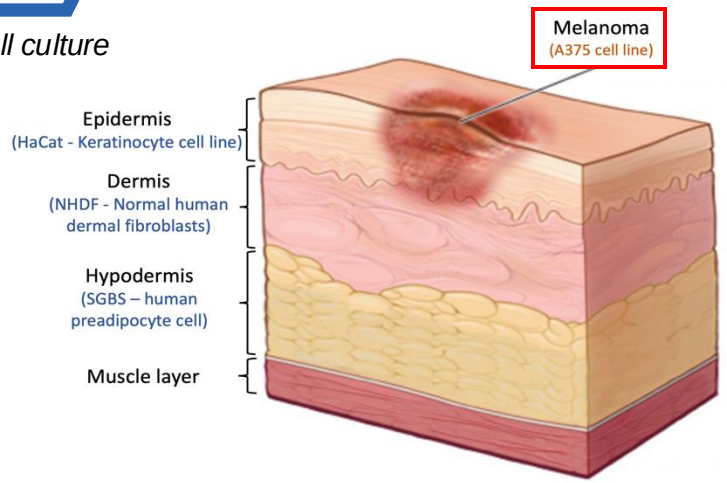
Evaluation of ionizing radiation effects at the molecular and cellular levels using 2D models



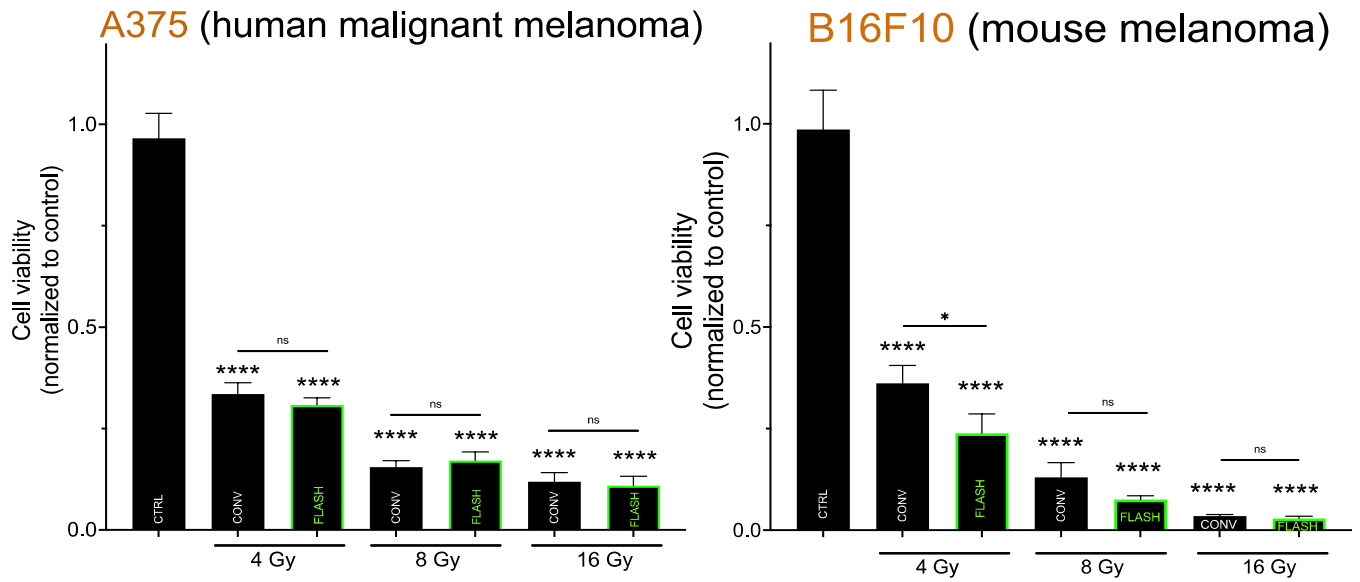
2D cell culture

Evaluation of ionizing radiation effects at the molecular and cellular levels using 2D models

2D cell culture

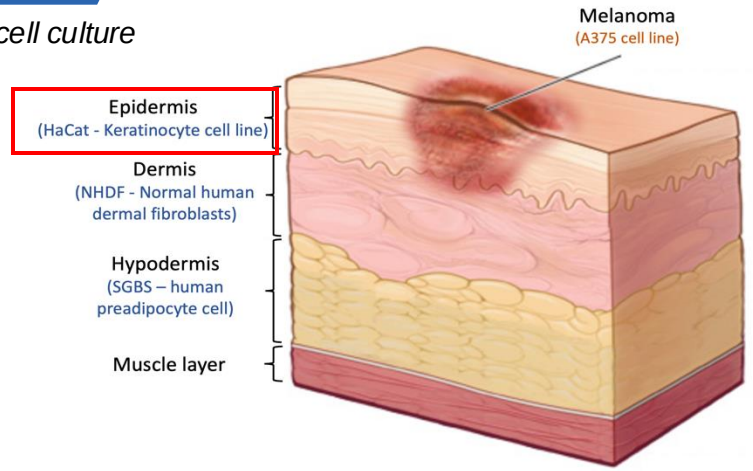


Tumor cells

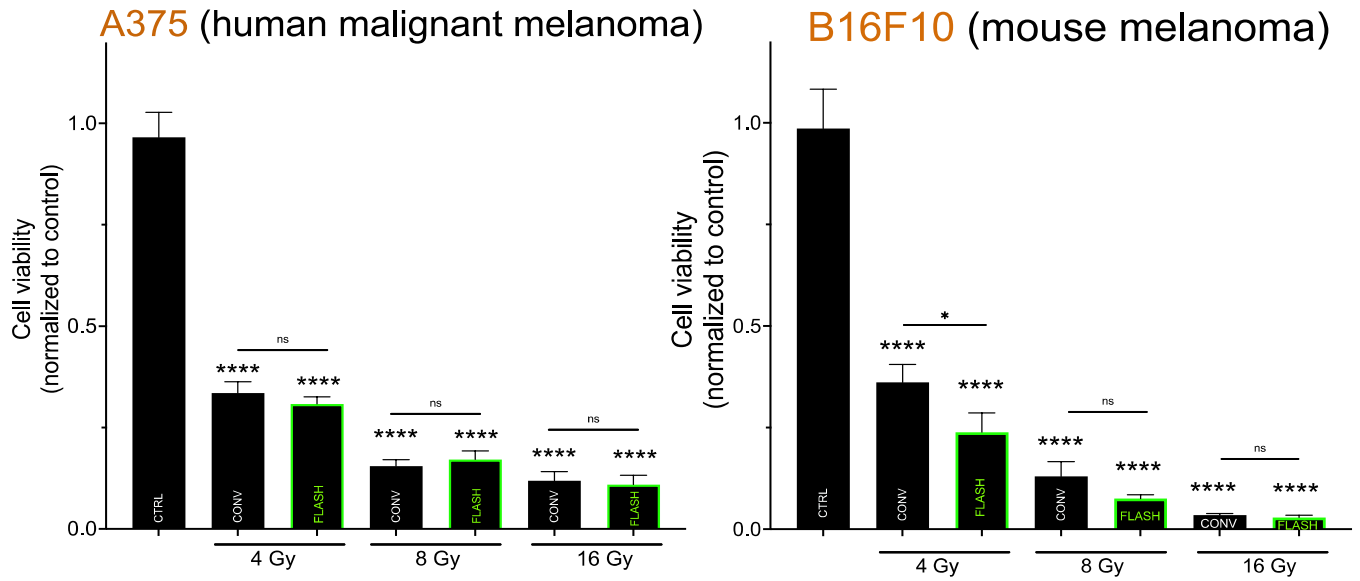


Evaluation of ionizing radiation effects at the molecular and cellular levels using 2D models

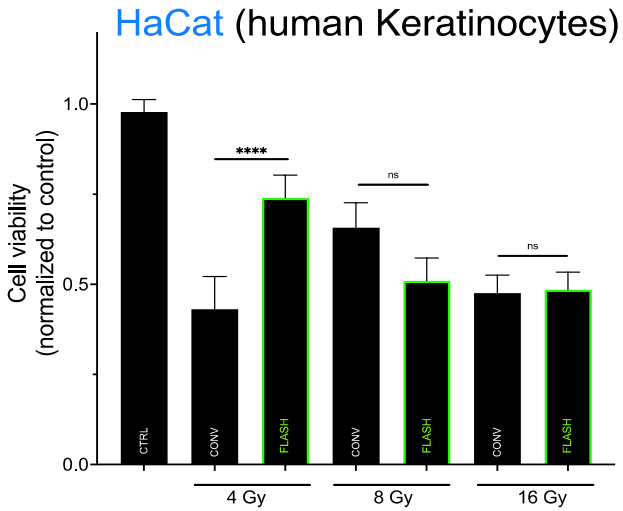
2D cell culture



Tumor cells

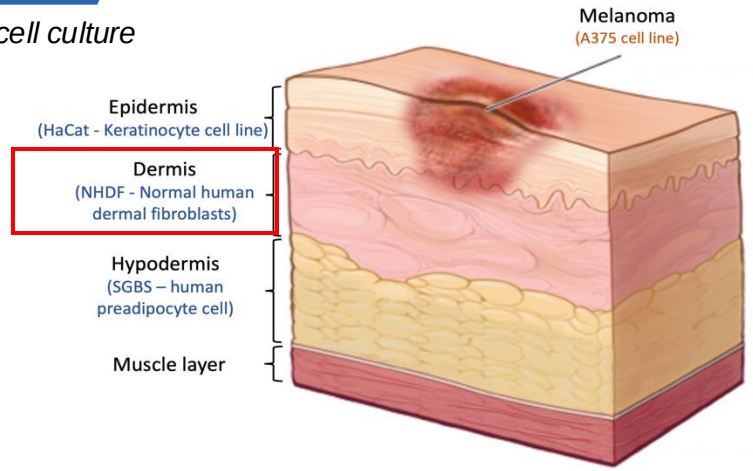


Normal cells

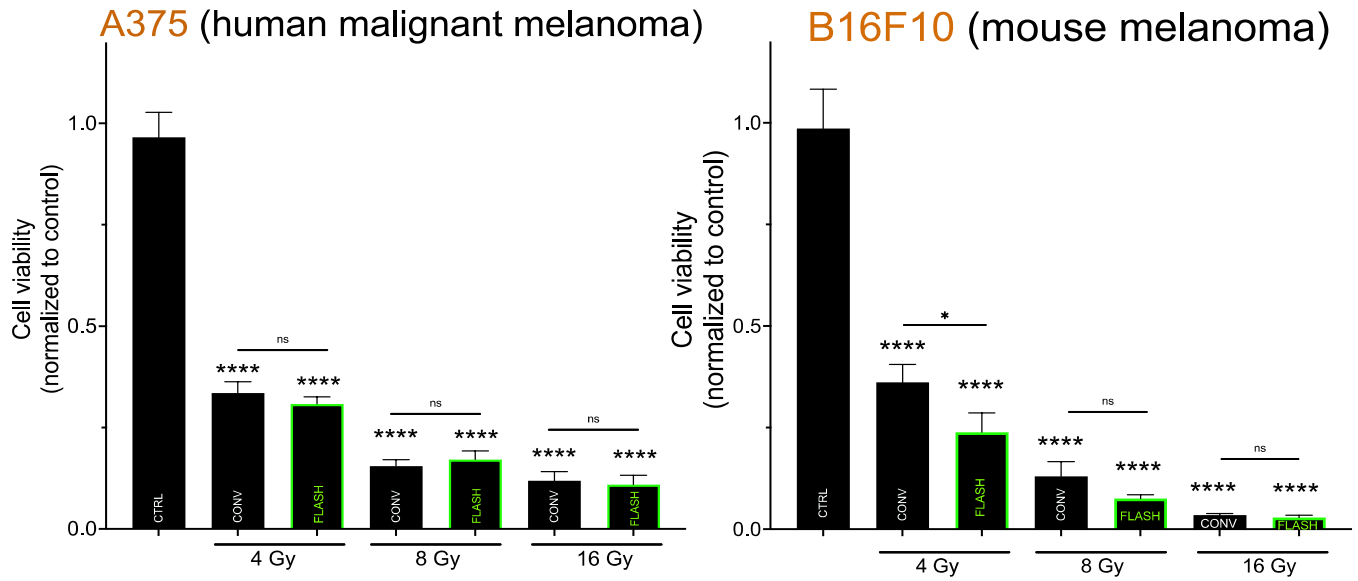


Evaluation of ionizing radiation effects at the molecular and cellular levels using 2D models

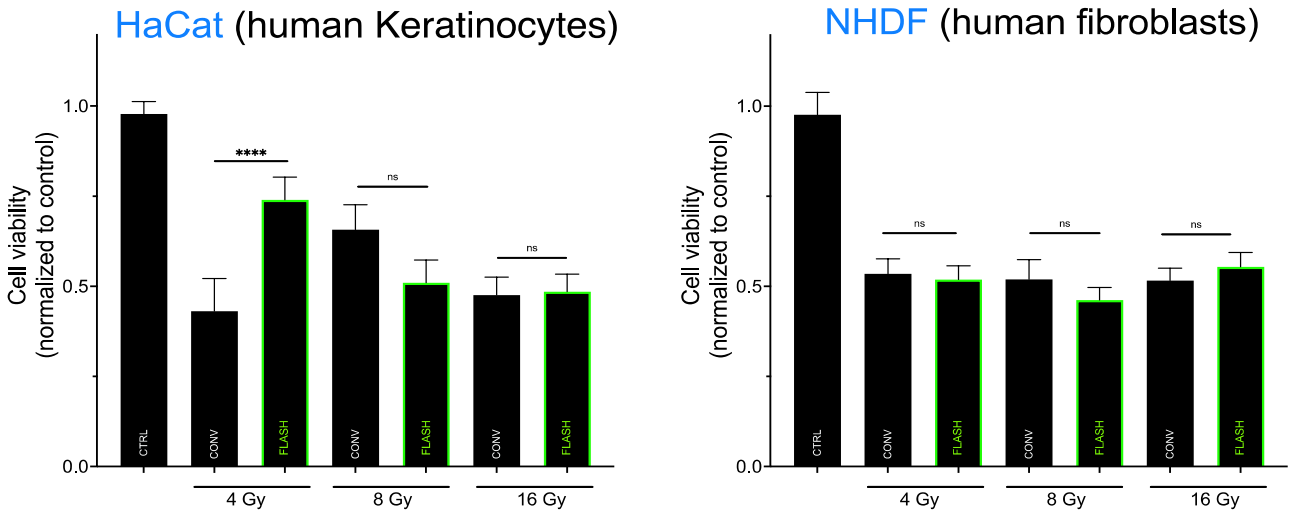
2D cell culture



Tumor cells



Normal cells



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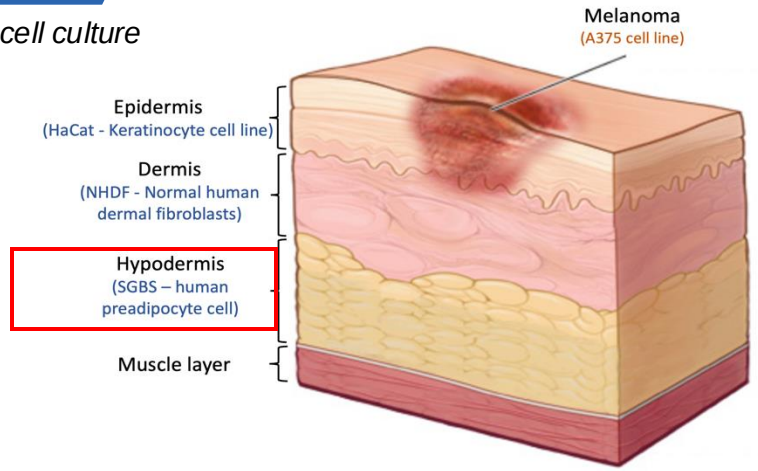
2D cell culture



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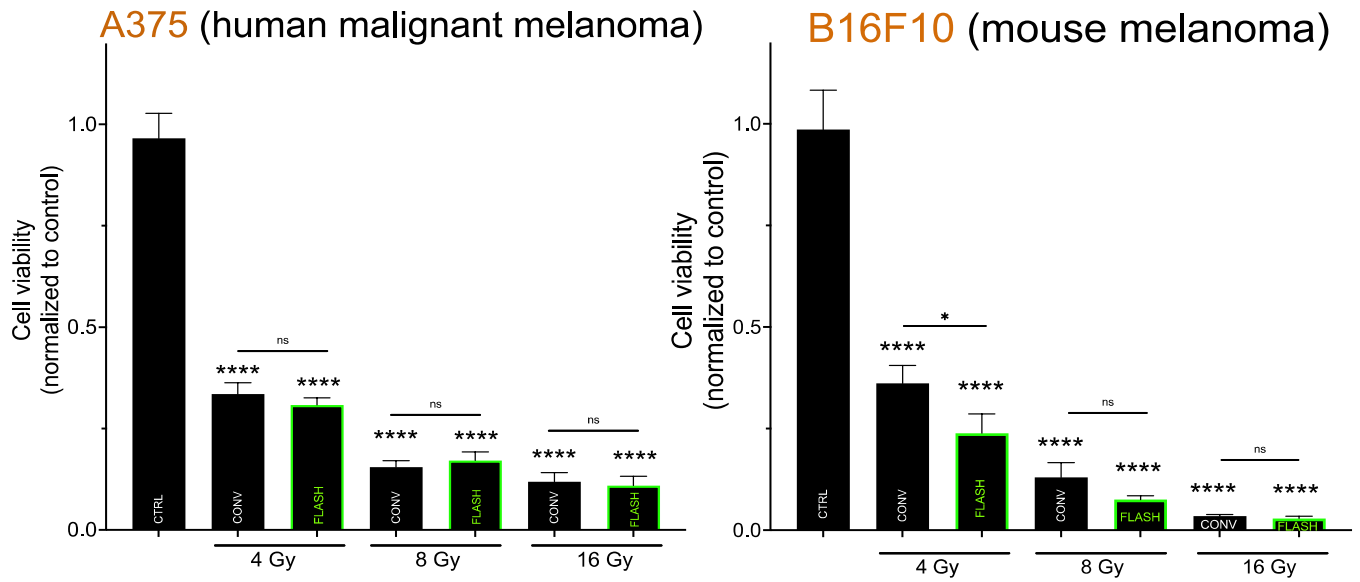

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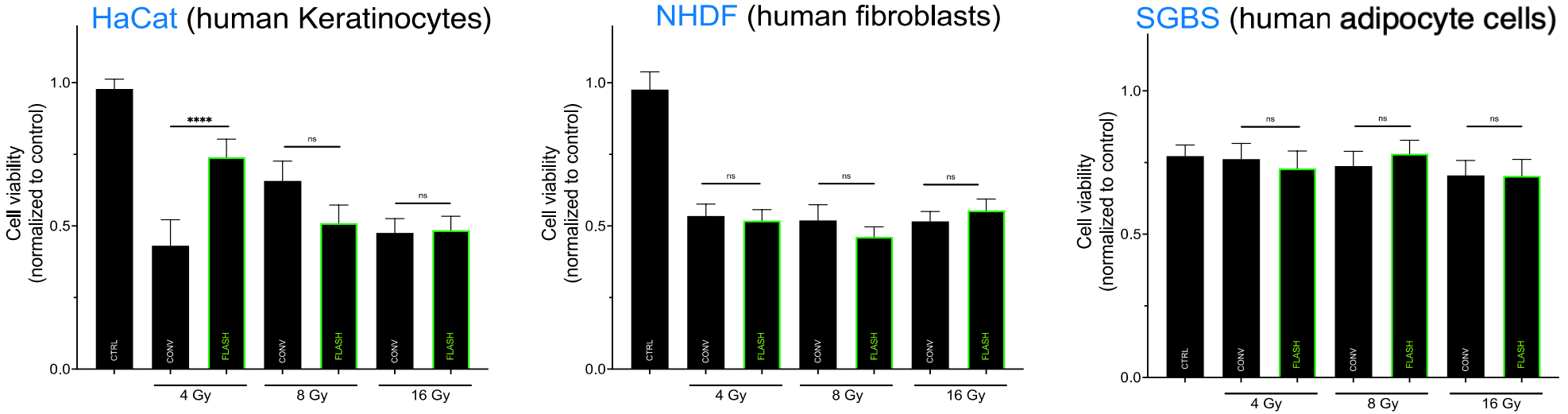

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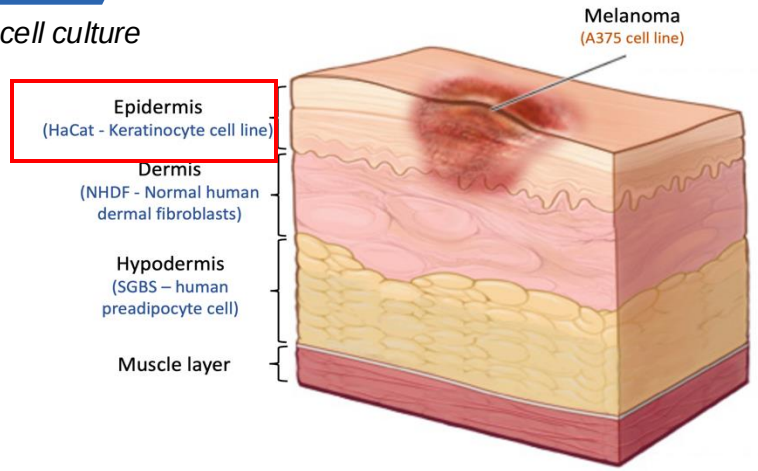
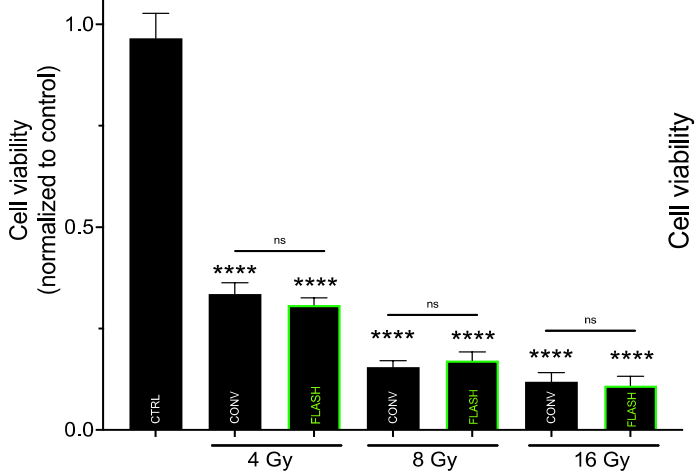
2D cell culture

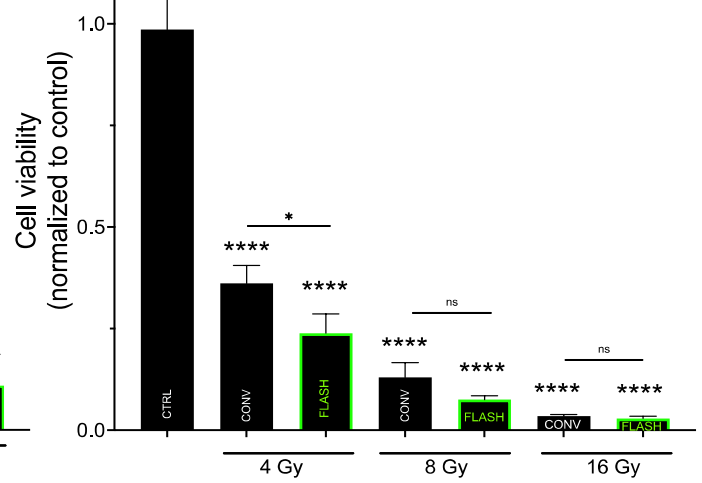


Tumor cells

A375 (human malignant melanoma)

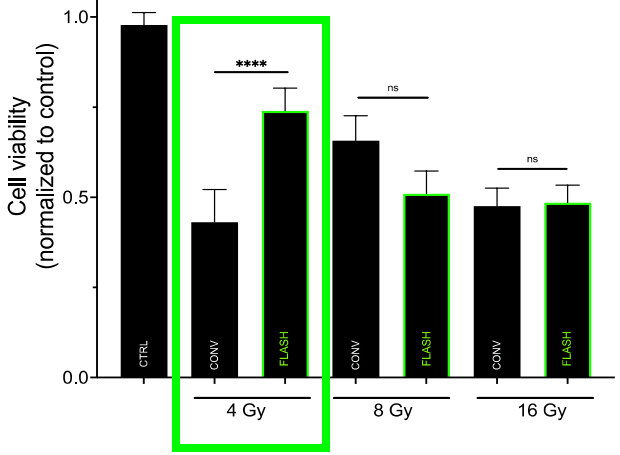


B16F10 (mouse melanoma)

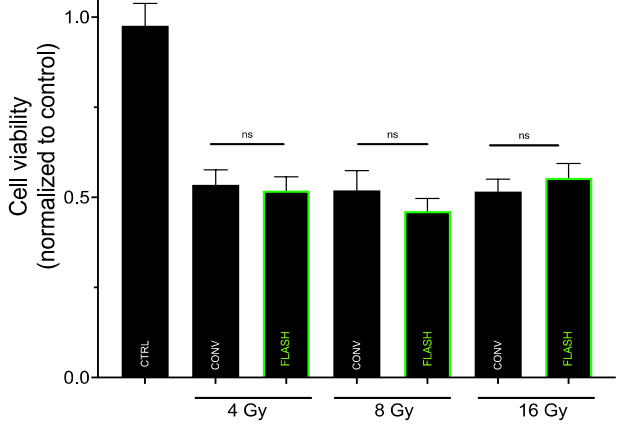


Normal cells

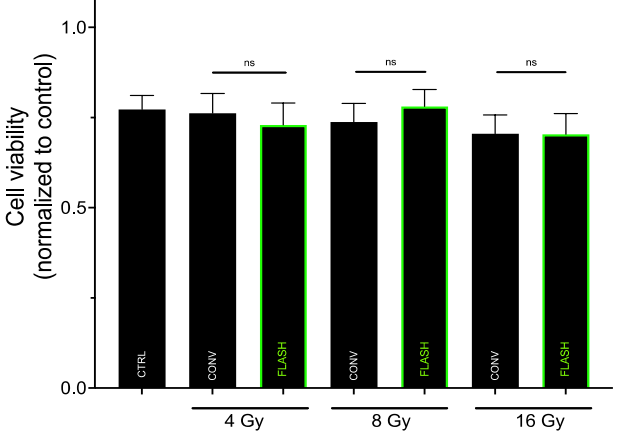
HaCat (human Keratinocytes)



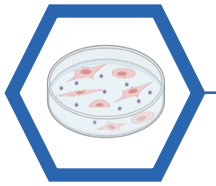
NHDF (human fibroblasts)



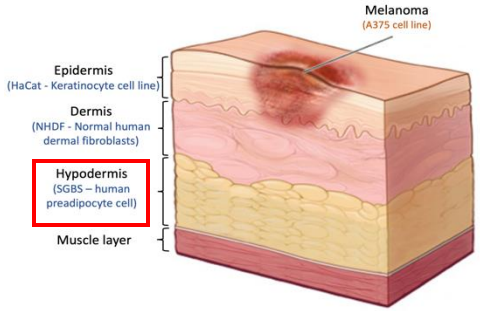
SGBS (human adipocyte cells)

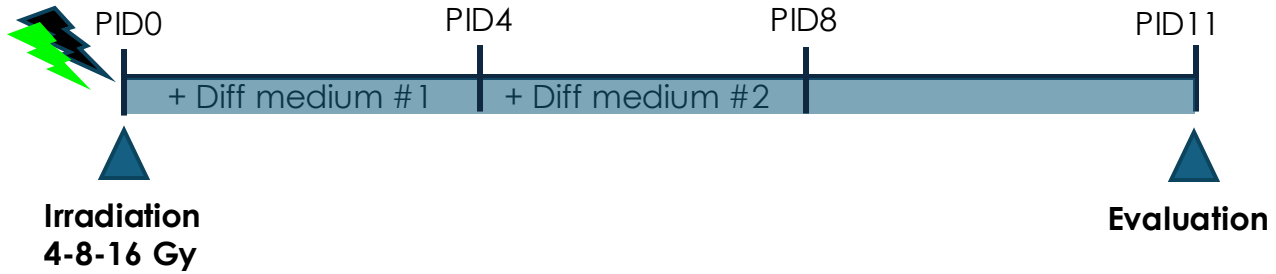


CONV-RT *vs* FLASH-RT on SGBS adipogenesis

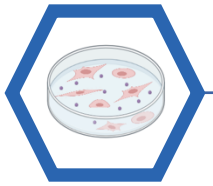


2D cell culture

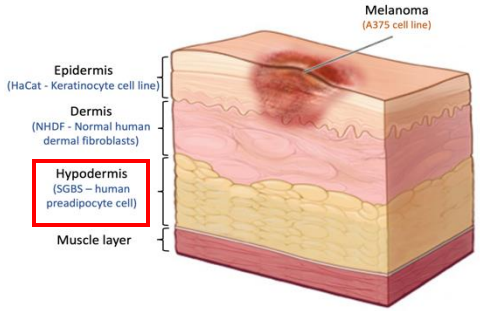


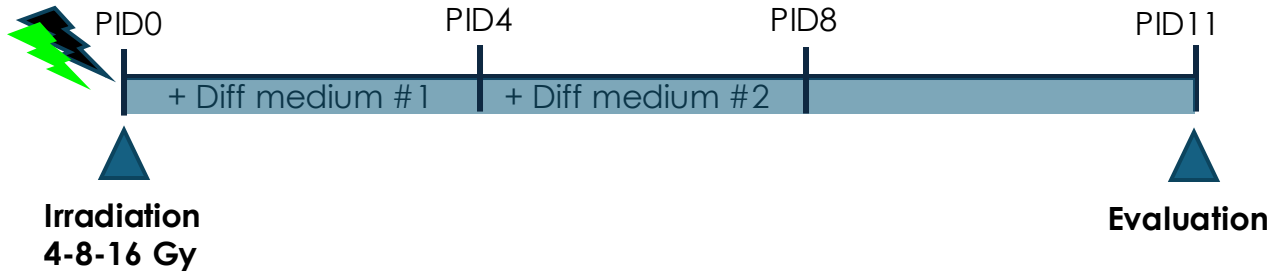


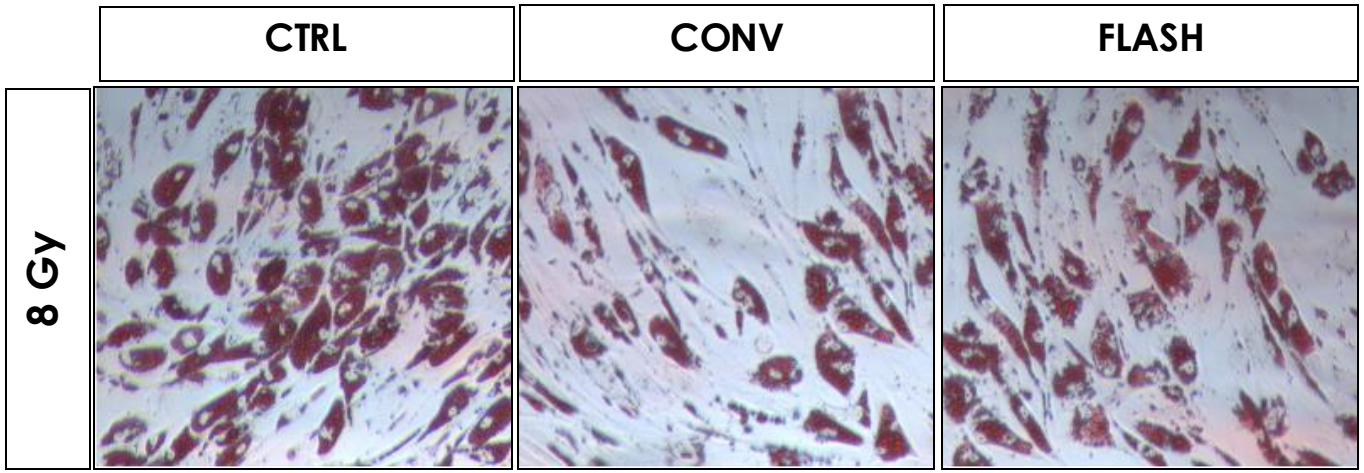
CONV-RT *vs* FLASH-RT on SGBS adipogenesis

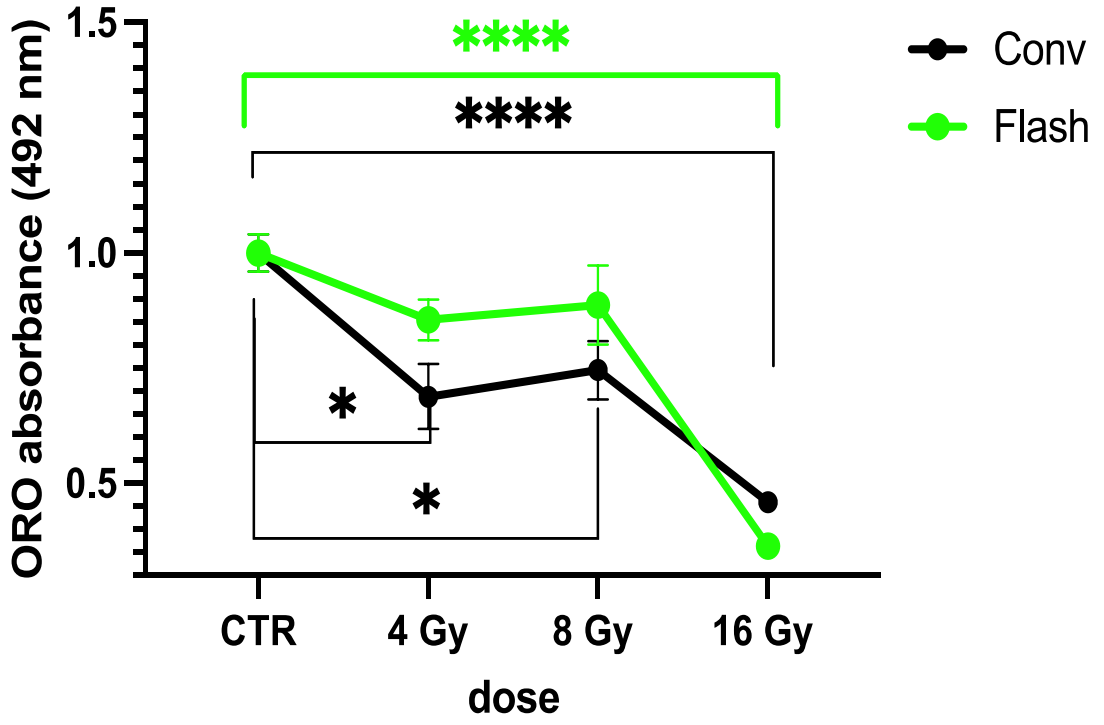


2D cell culture



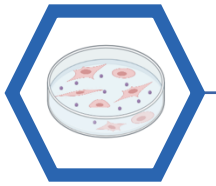




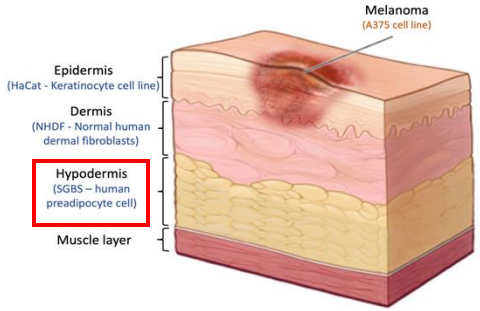


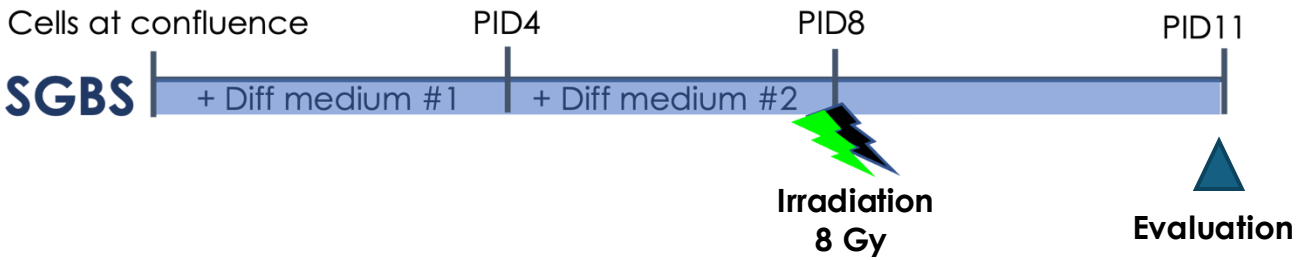
- Adipogenesis is compromised by RT in a dose response manner.
- A modest sparing effect is observed under **FLASH** mode.

SGBS: RT effects on adipocytes, 8 Gy



2D cell culture





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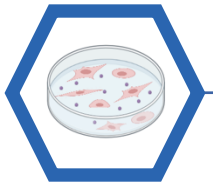
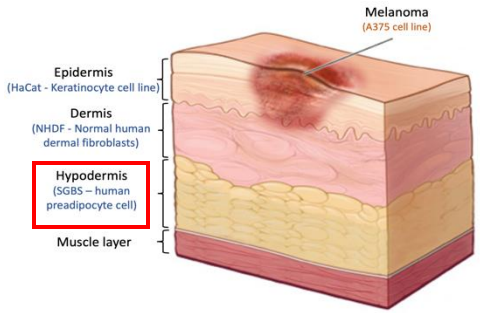
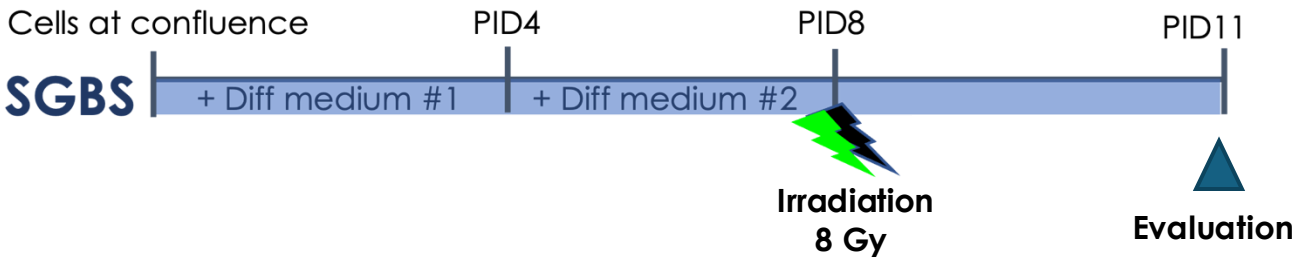
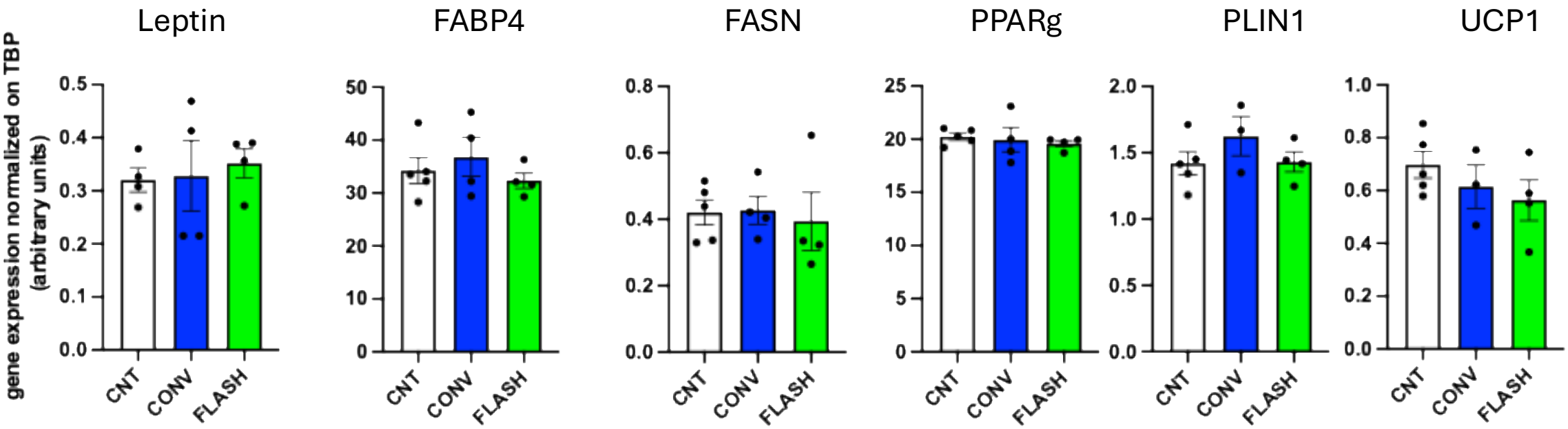


2D cell culture



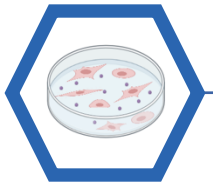


Gene expression studies – markers of terminal differentiation

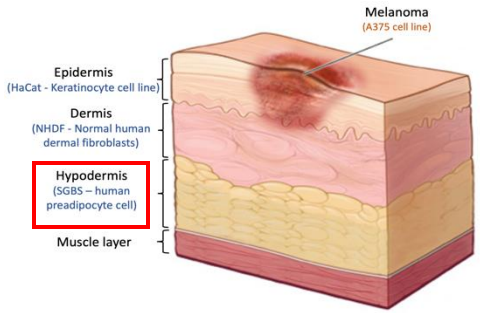


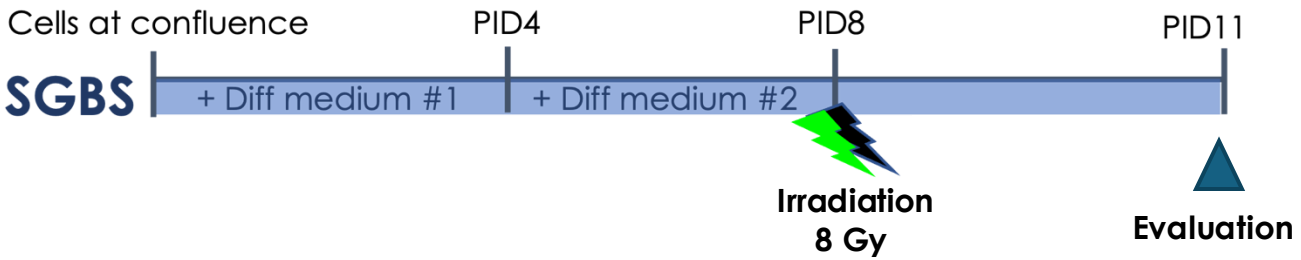
Adipocyte function is not compromised

SGBS: RT effects on adipocytes, 8 Gy

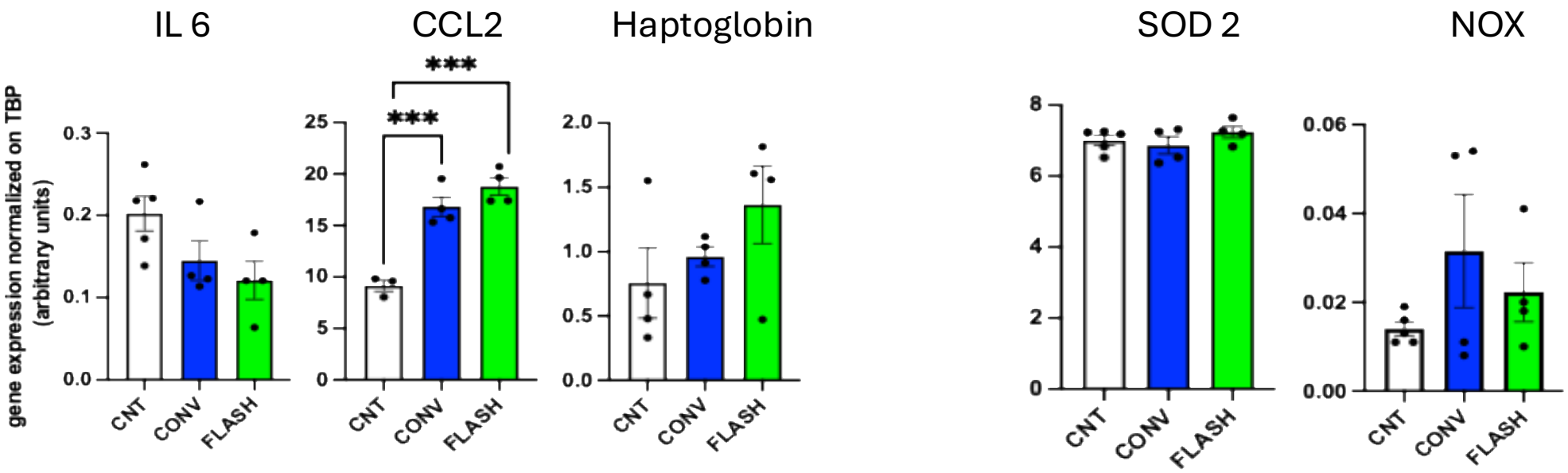


2D cell culture

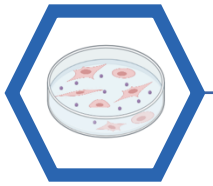




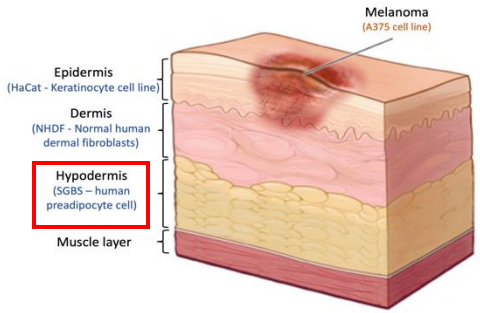
Gene expression studies – Inflammation and OS response

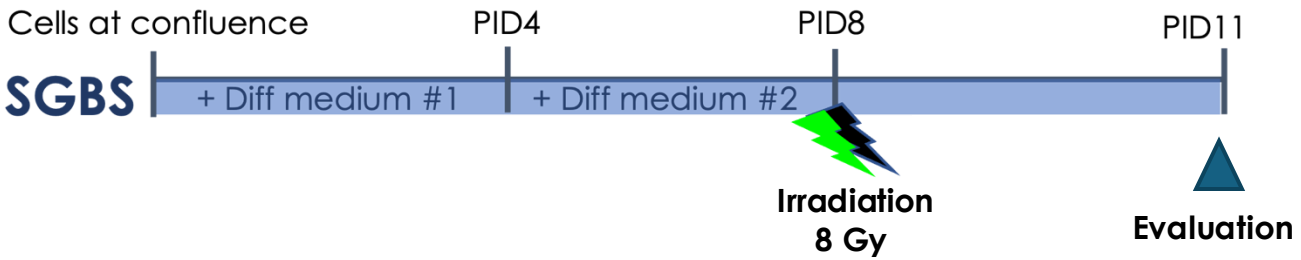


SGBS: RT effects on adipocytes, 8 Gy

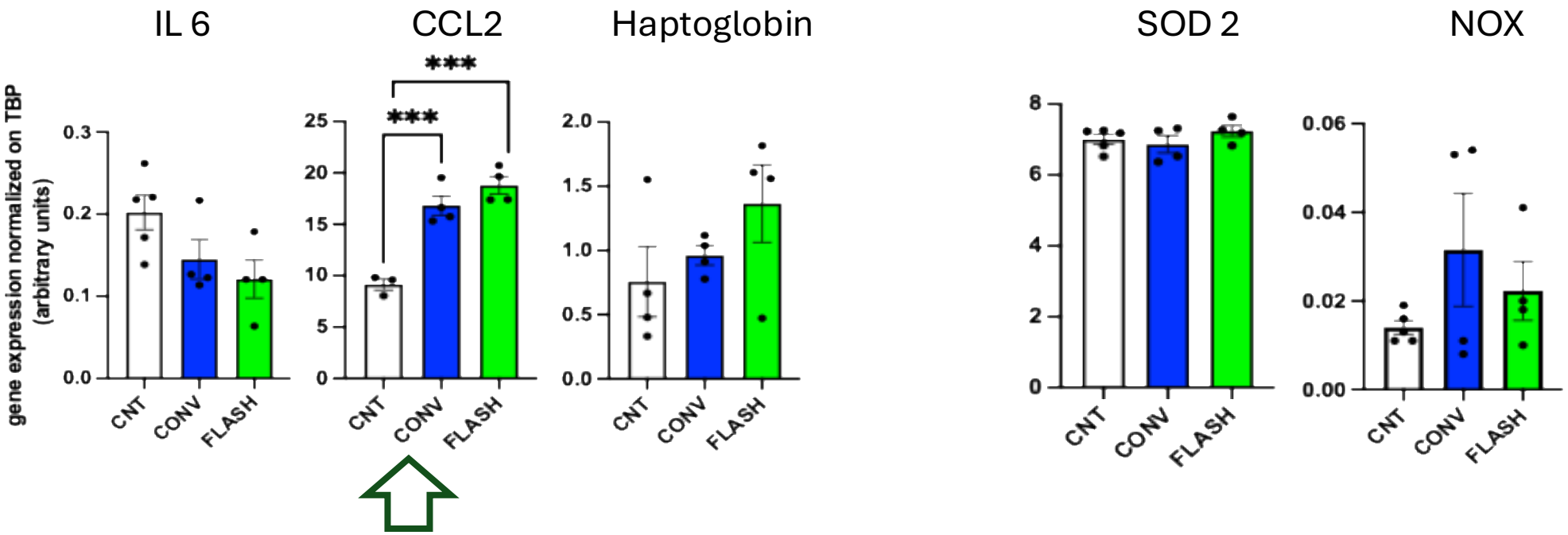


2D cell culture



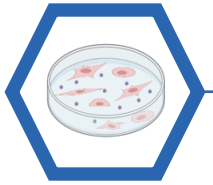


Gene expression studies – Inflammation and OS response

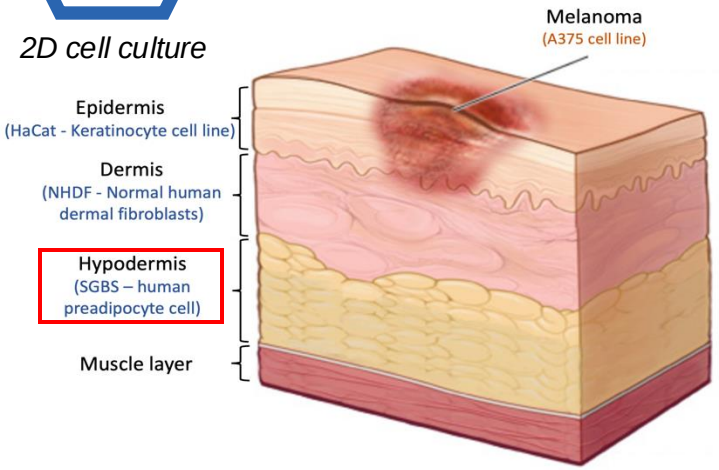


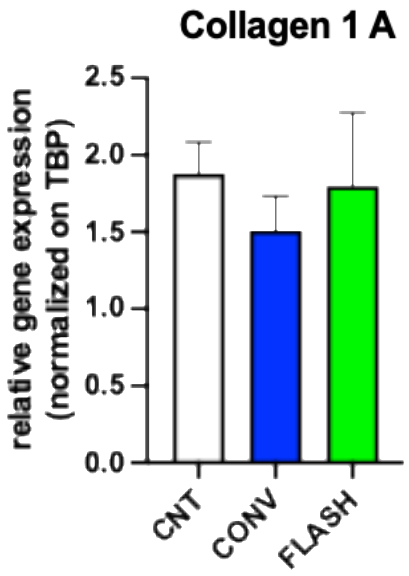
Inflammation: NO difference **FLASH-RT** *vs* **CONV-RT**

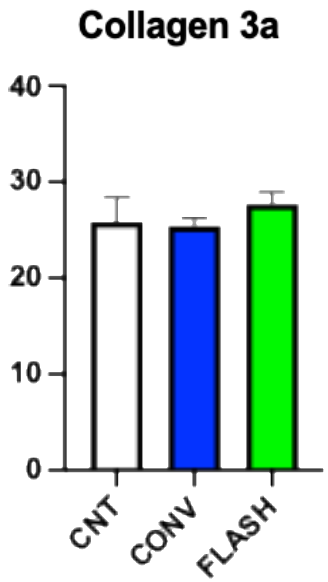
RT effects on fibrosis - 8 Gy

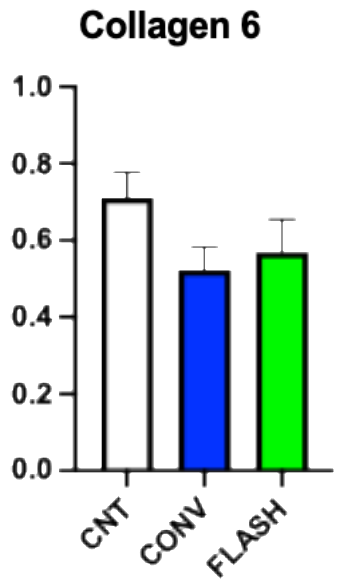


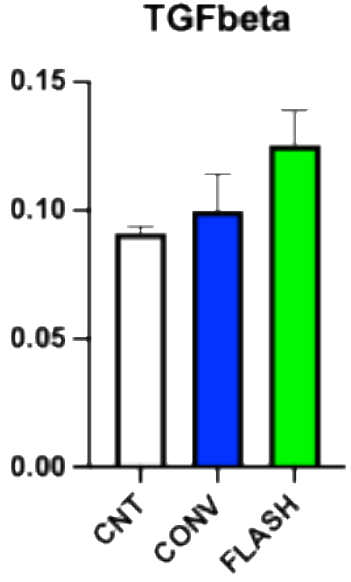
2D cell culture



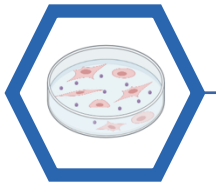




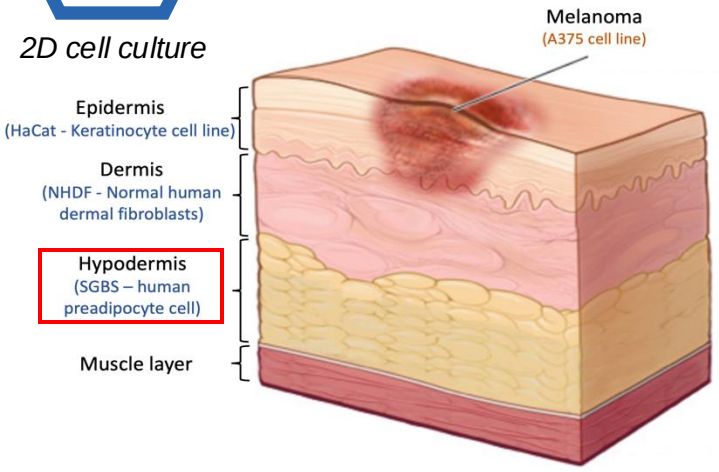


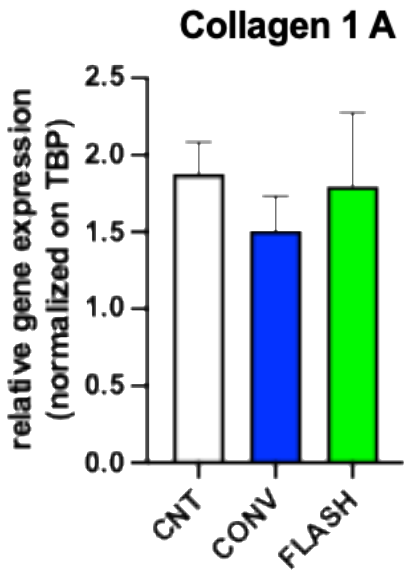


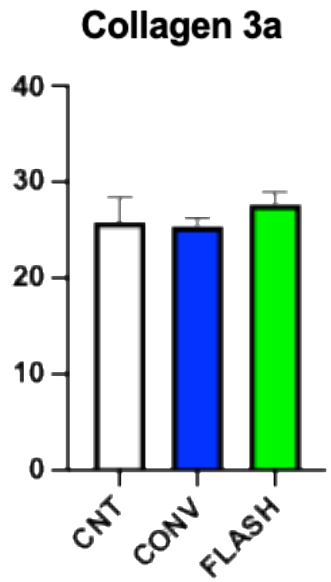
RT effects on fibrosis - 8 Gy

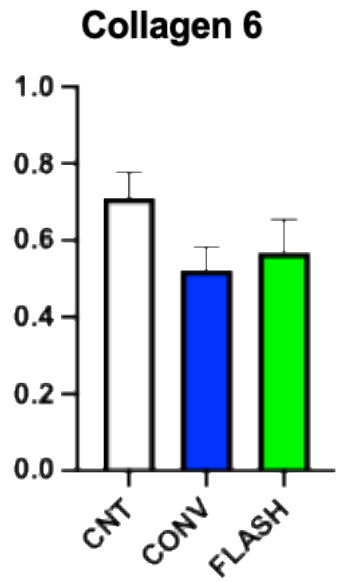


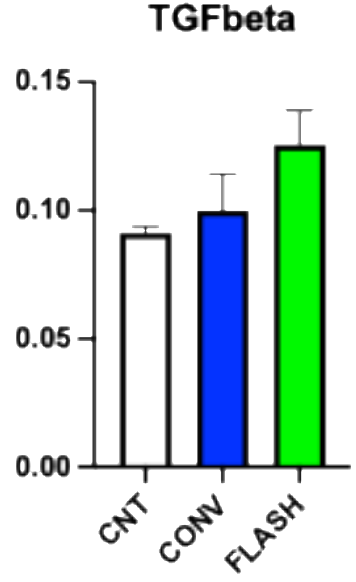
2D cell culture

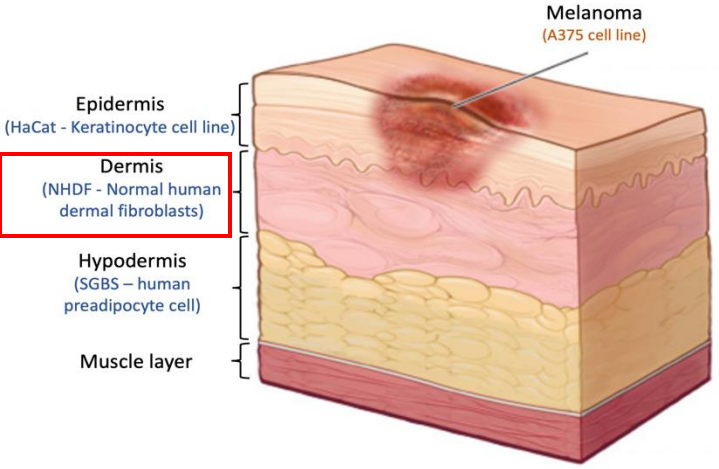


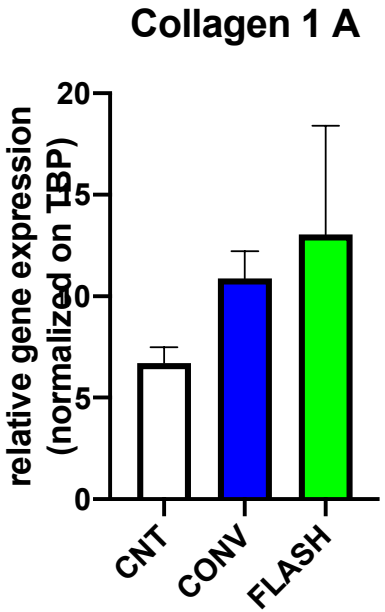


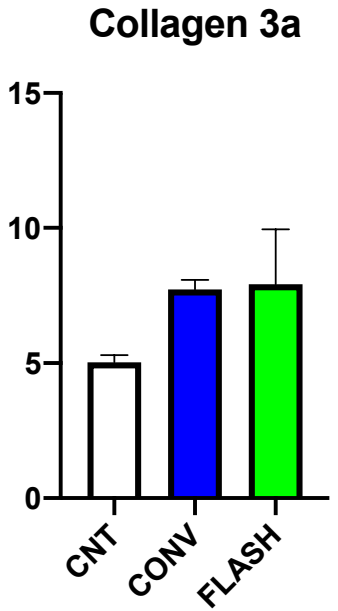


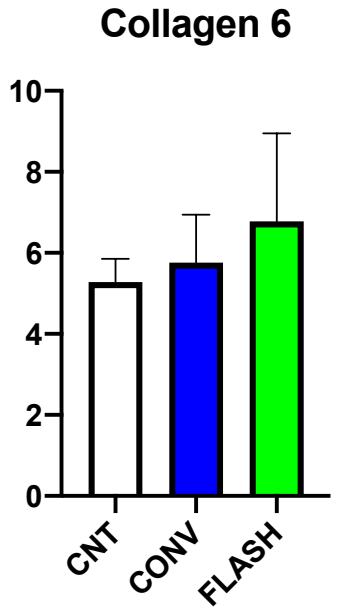


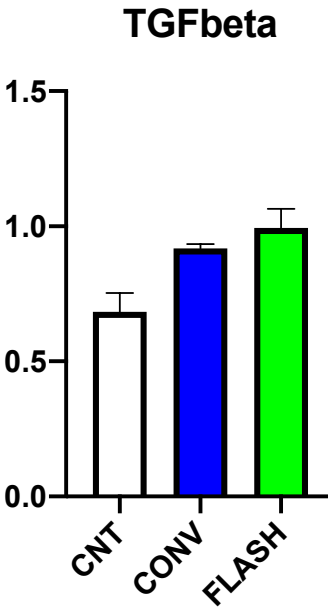








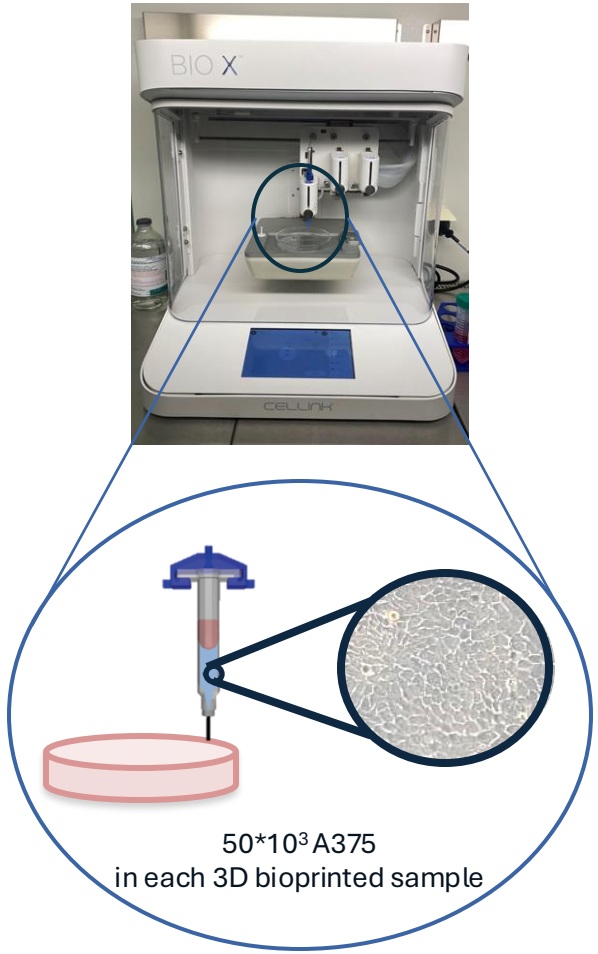




3D bioprinting of A375-GFP laden bioink

spheroids

@ Laboratorio di Medicina Rigenerativa, Biomateriali e Terapie Avanzate



Cavallo A, et al. Fibrinogen-Based Bioink for Application in Skin Equivalent 3D Bioprinting. J Funct Biomater. 2023 5;14(9):459. doi: 10.3390/jfb14090459.

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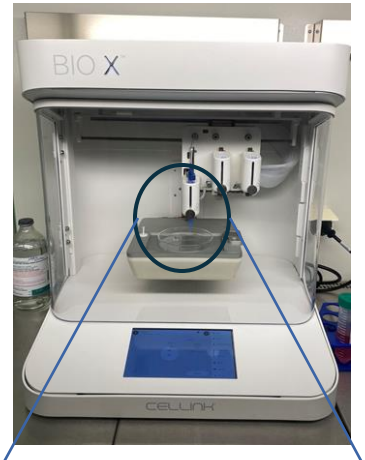
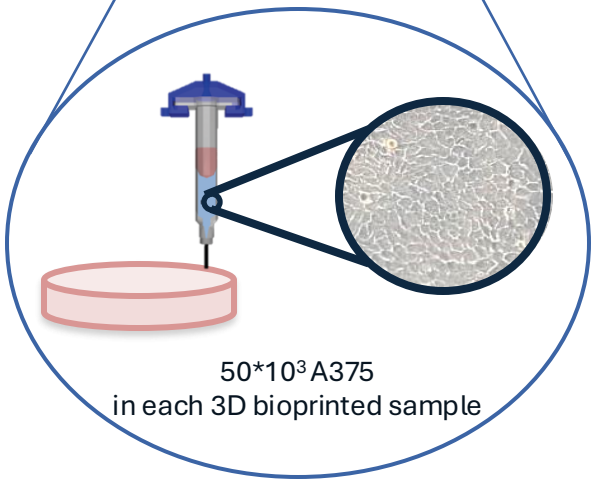
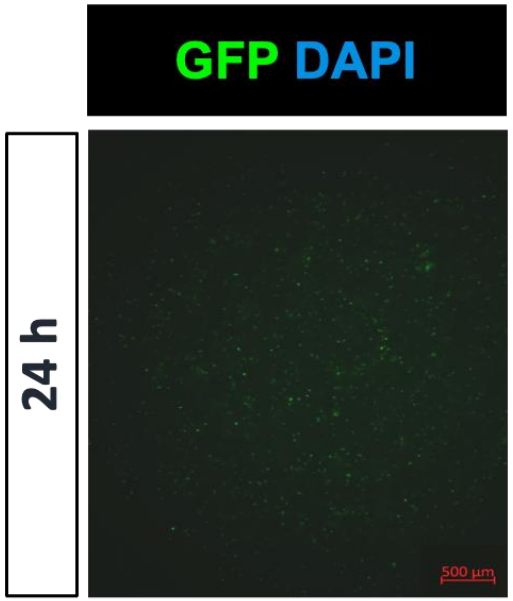
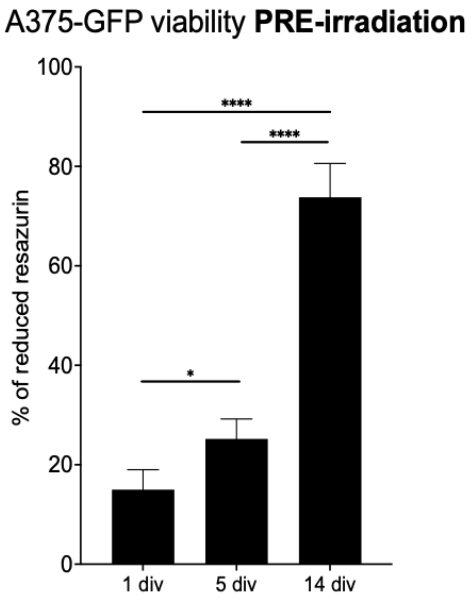
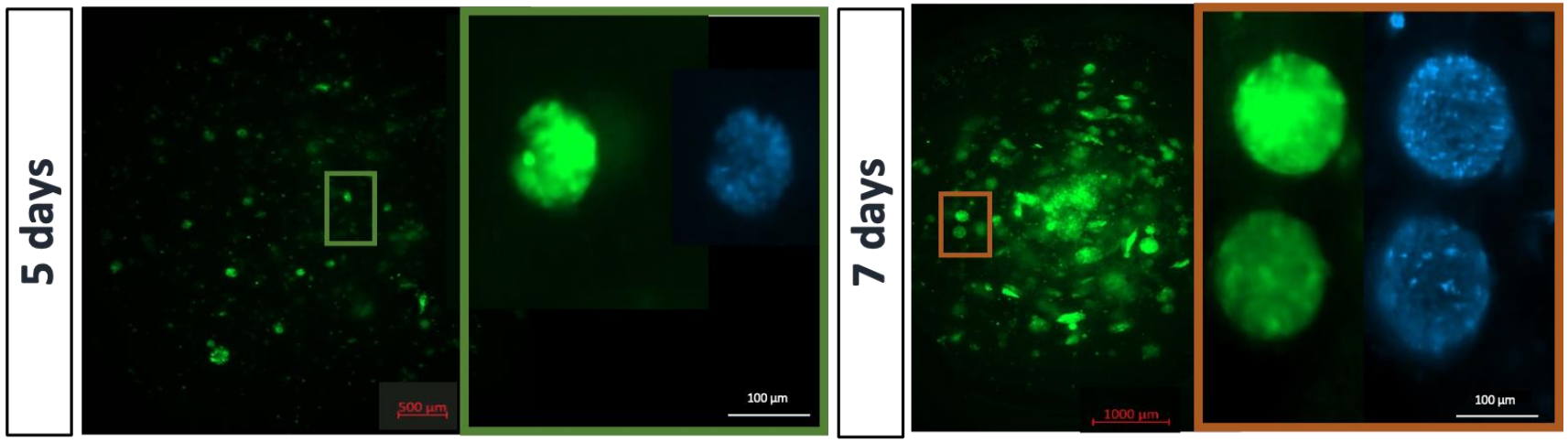




50*10^3 A375
in each 3D bioprinted sample







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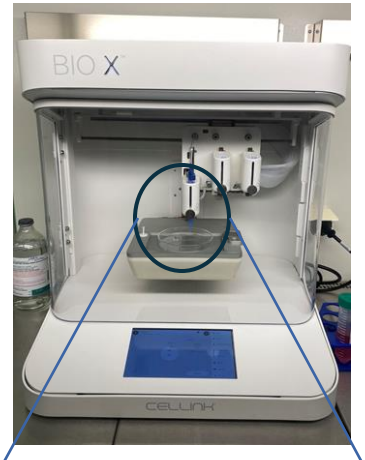
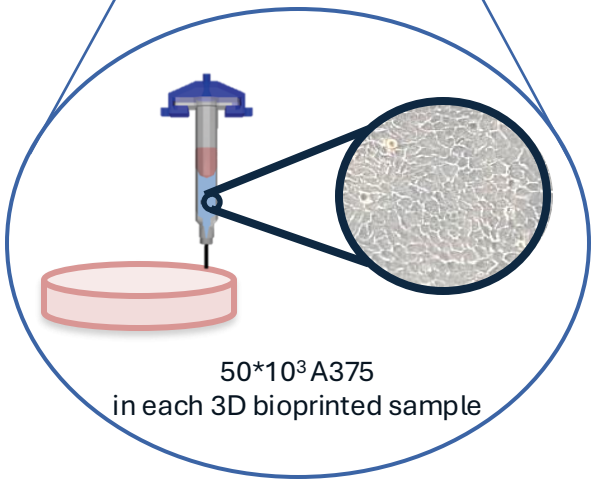
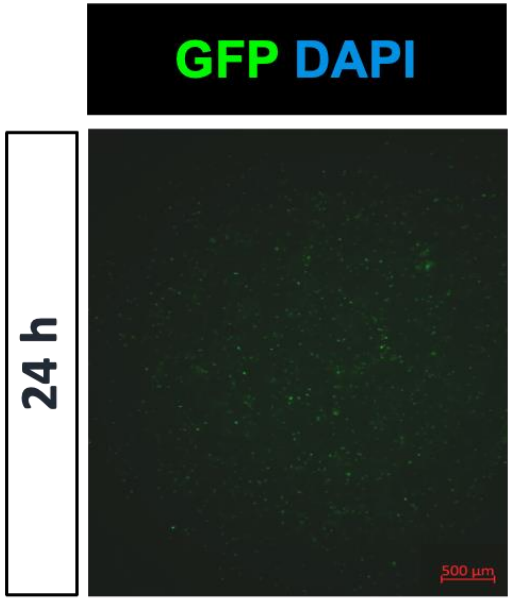
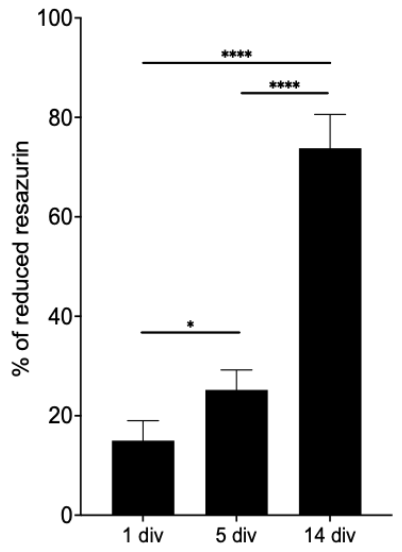
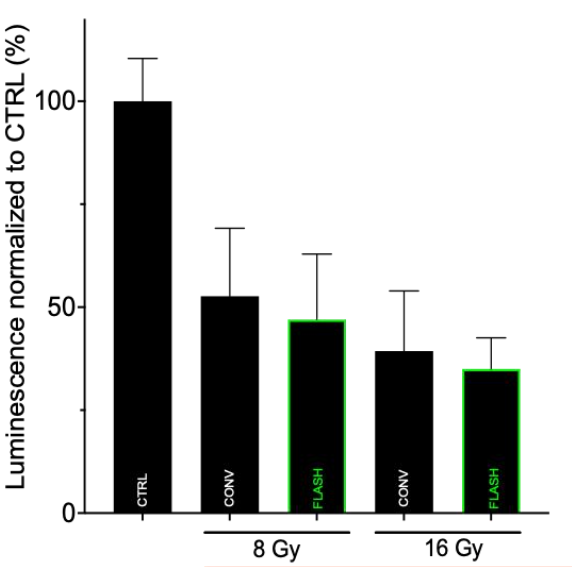


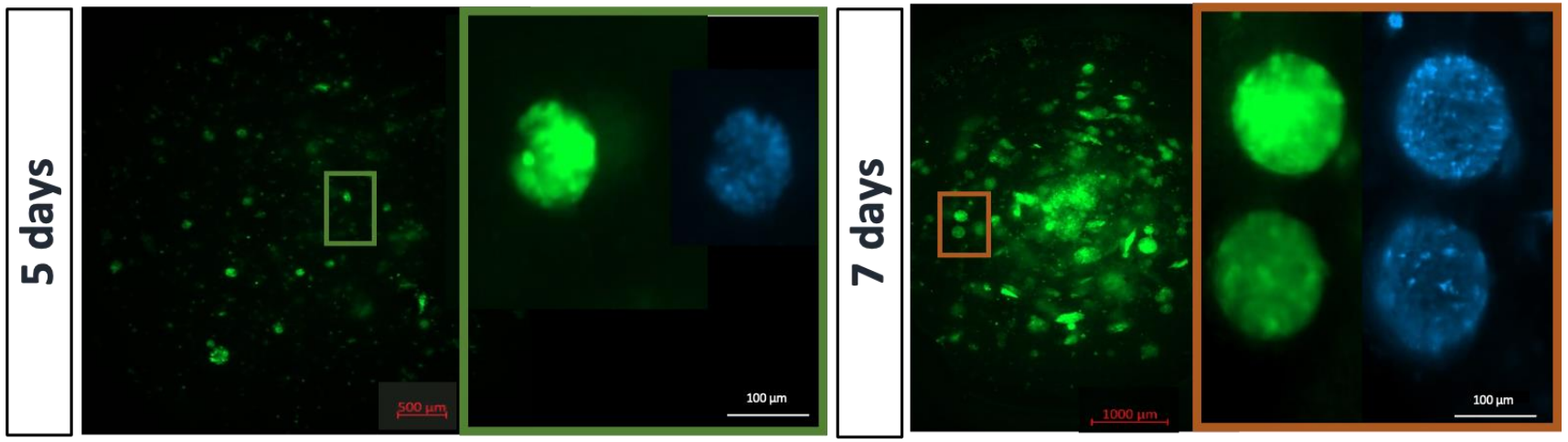


50*10³ A375
in each 3D bioprinted sample









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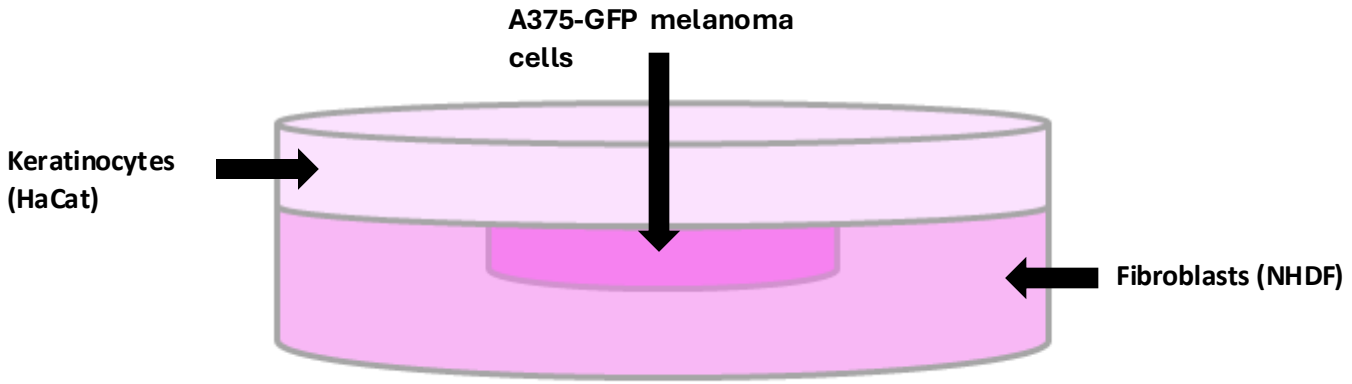
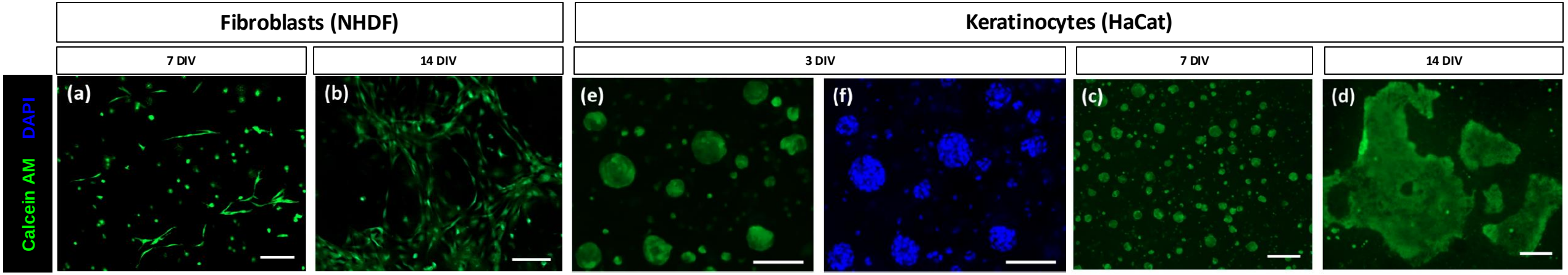
Biofabrication of a tri-layered 3D-bioprinted malignant melanoma model

3D-printed constructs





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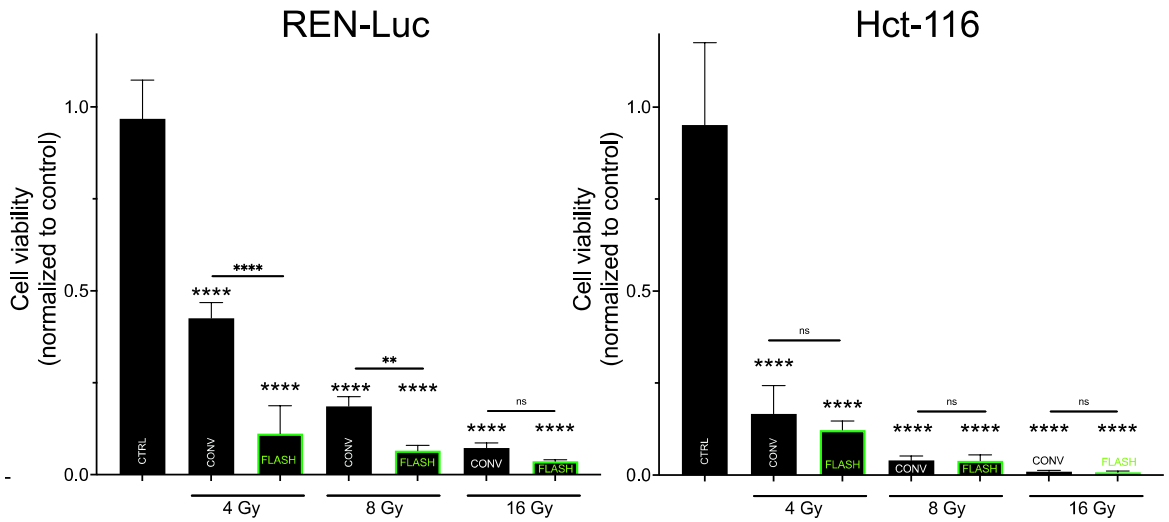
Conclusions

- Simple, low-cost, and accurate pre-clinical models are needed to screen novel radiotherapy approaches;
- Our work achieves a cross fertilization effort between the experimentation of new technologies and the implementation of the state-of-the art of FLASH-RT;
- The administration of FLASH-RT would lead to therapeutic effects on the tumor, but limits the damage to healthy tissues.

Future perspective



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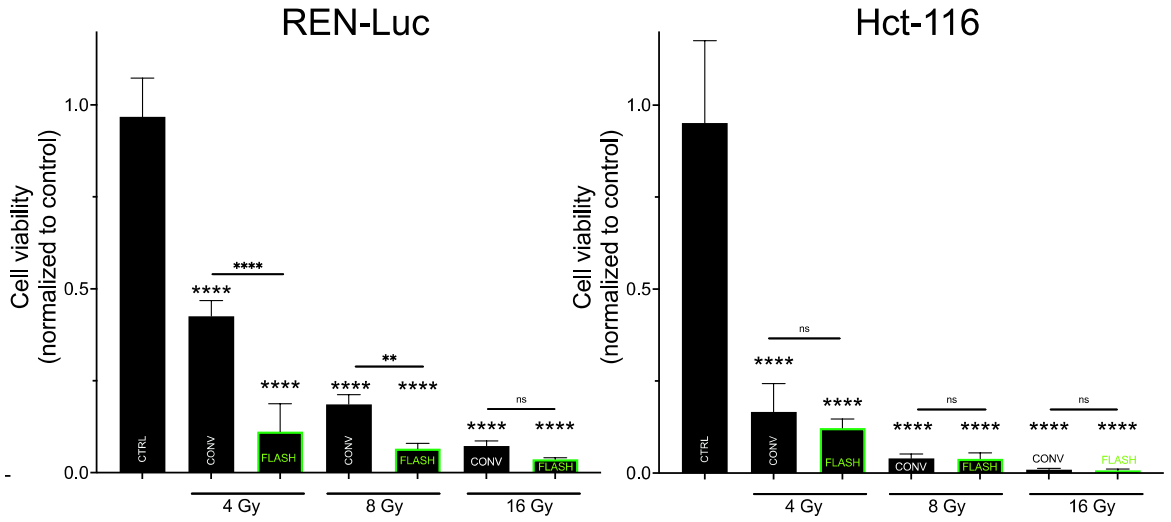

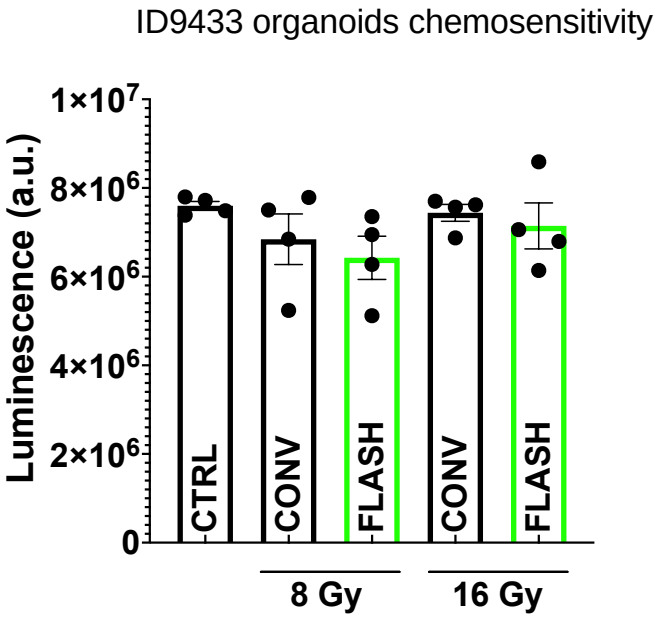

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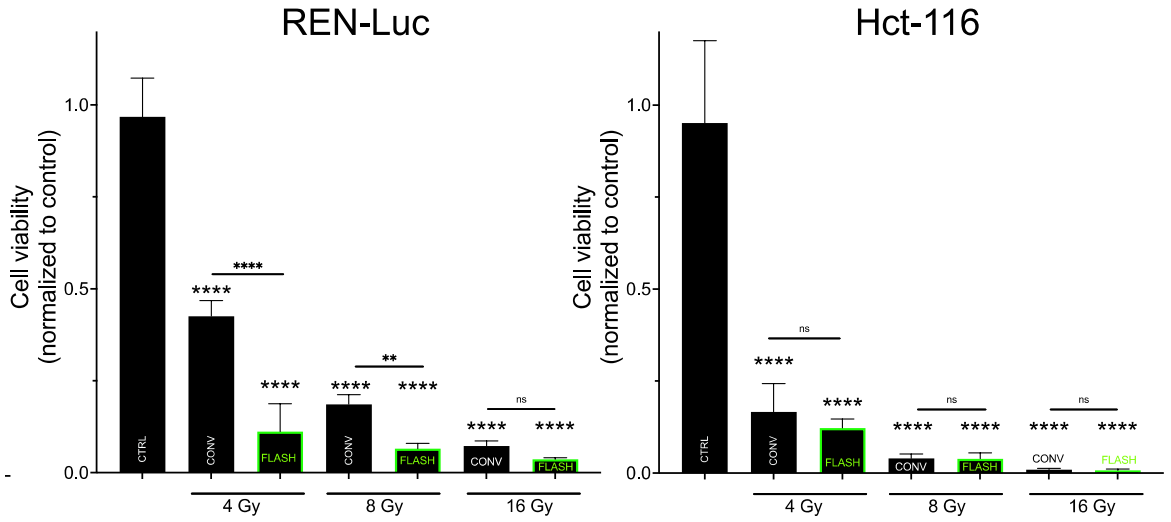

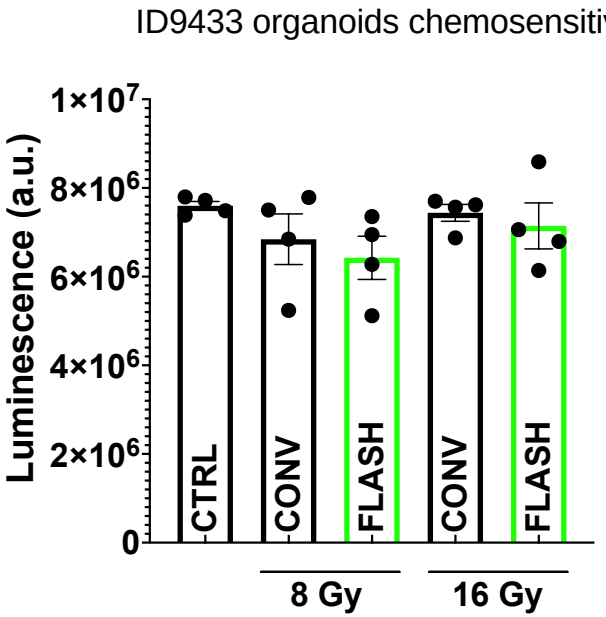

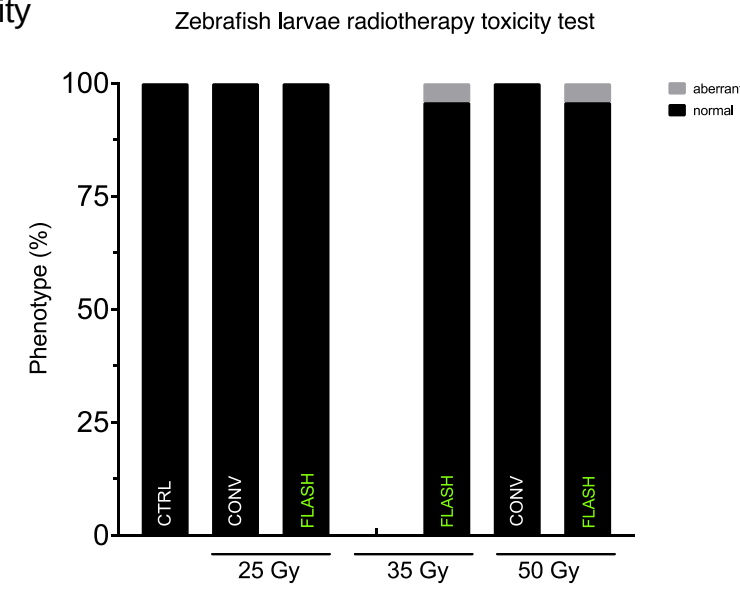

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Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA

THE Tuscany Health Ecosystem

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CISUP Center for Instrument Sharing UNIVERSITY OF PISA

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Alice Usai
aliceusai@cnr.it

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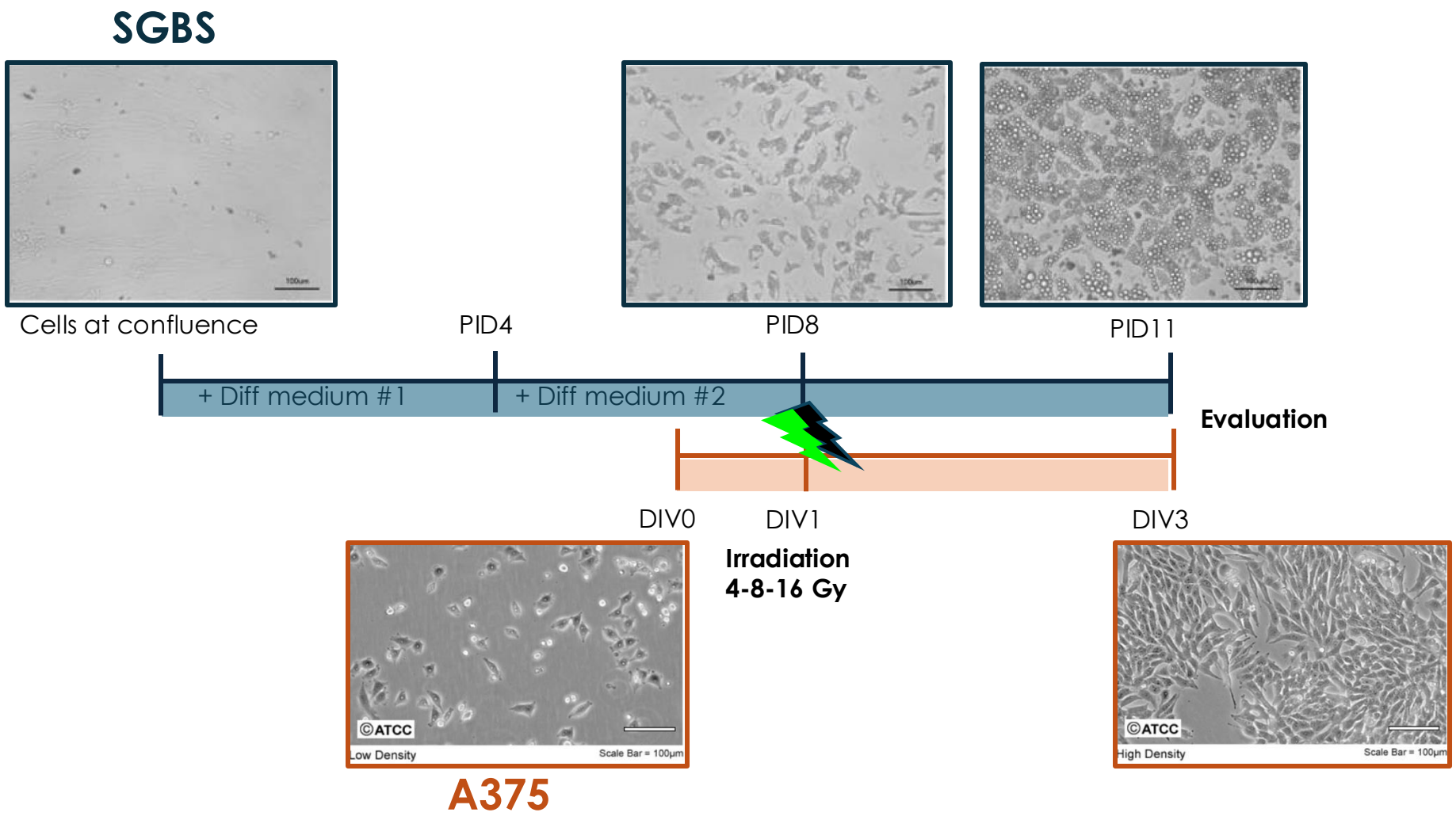
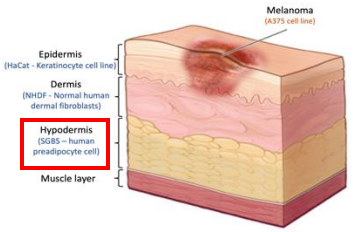
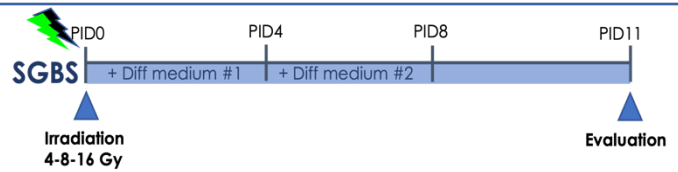
Experimental plan

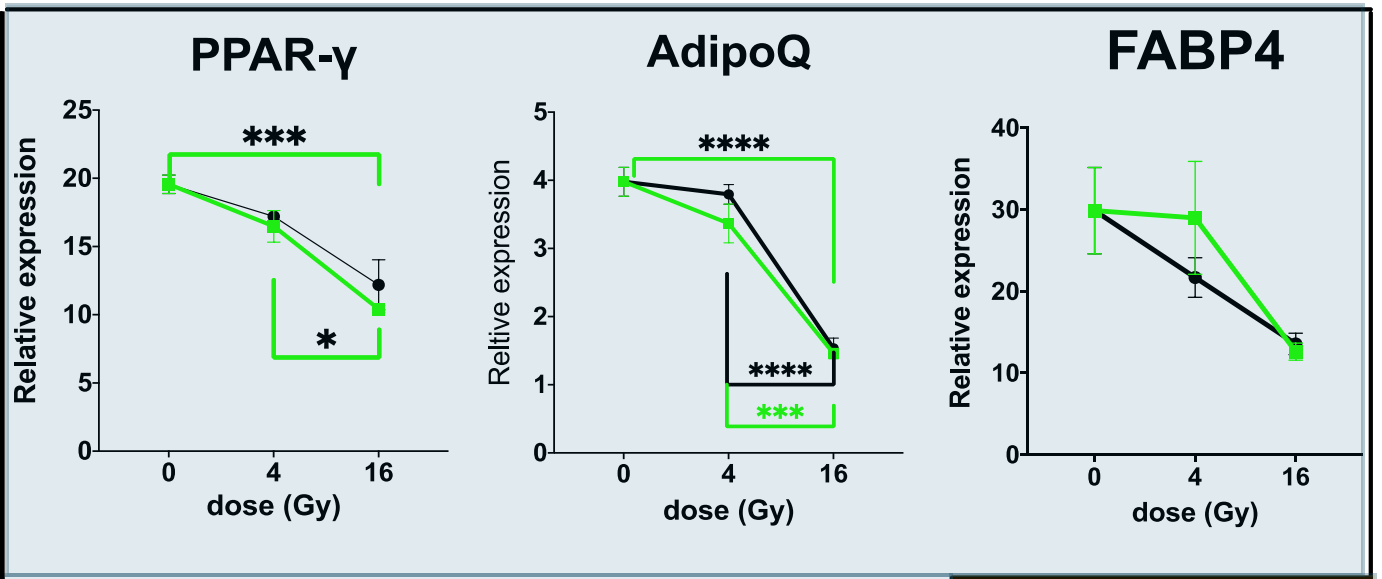


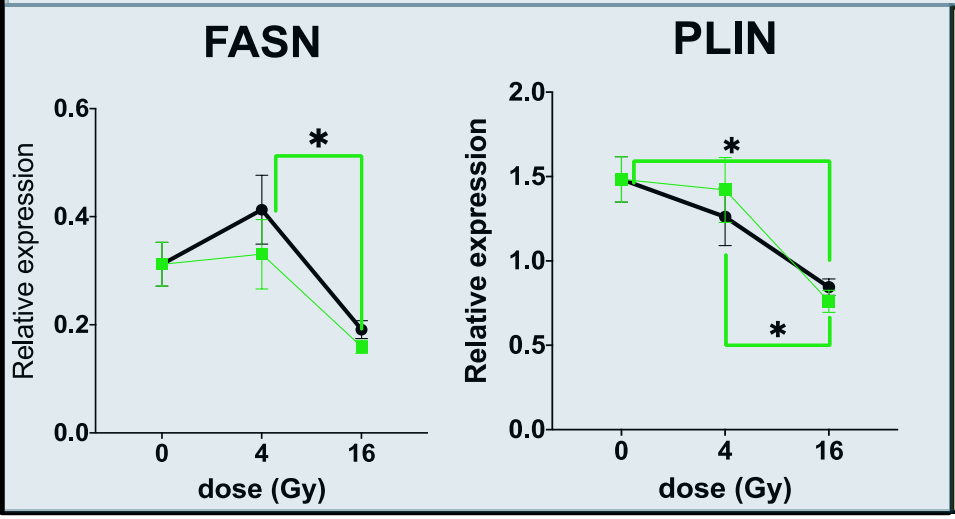
CONV-RT *vs* FLASH-RT on SGBS differentiation and senescence markers

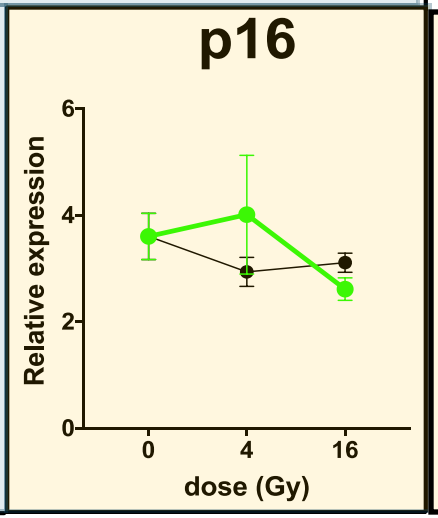




DIFFERENTIATION MARKERS

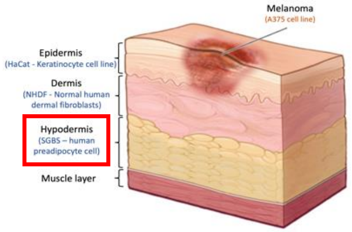


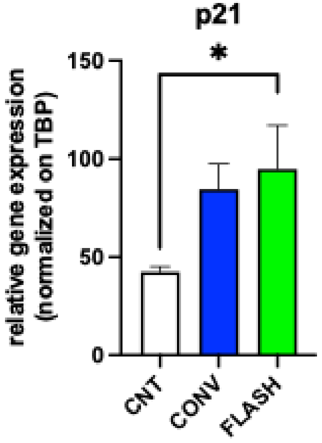


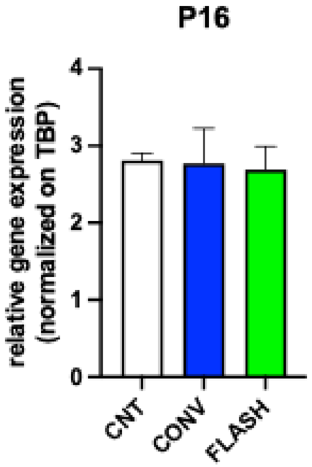


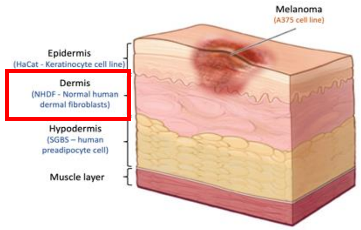
MARKER of SENESCENCE

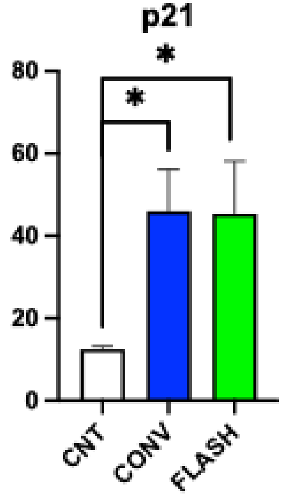
RT effects on senescence - 8 Gy

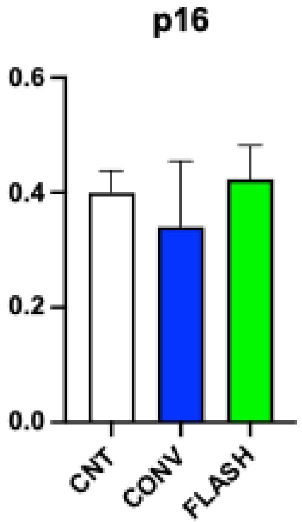


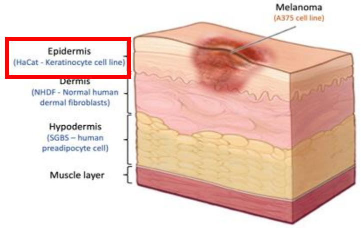


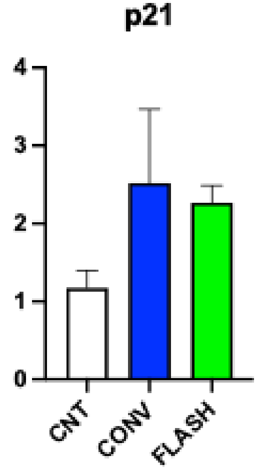


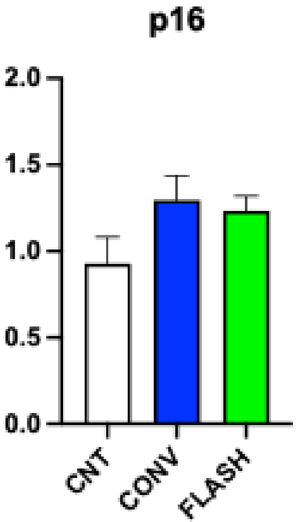


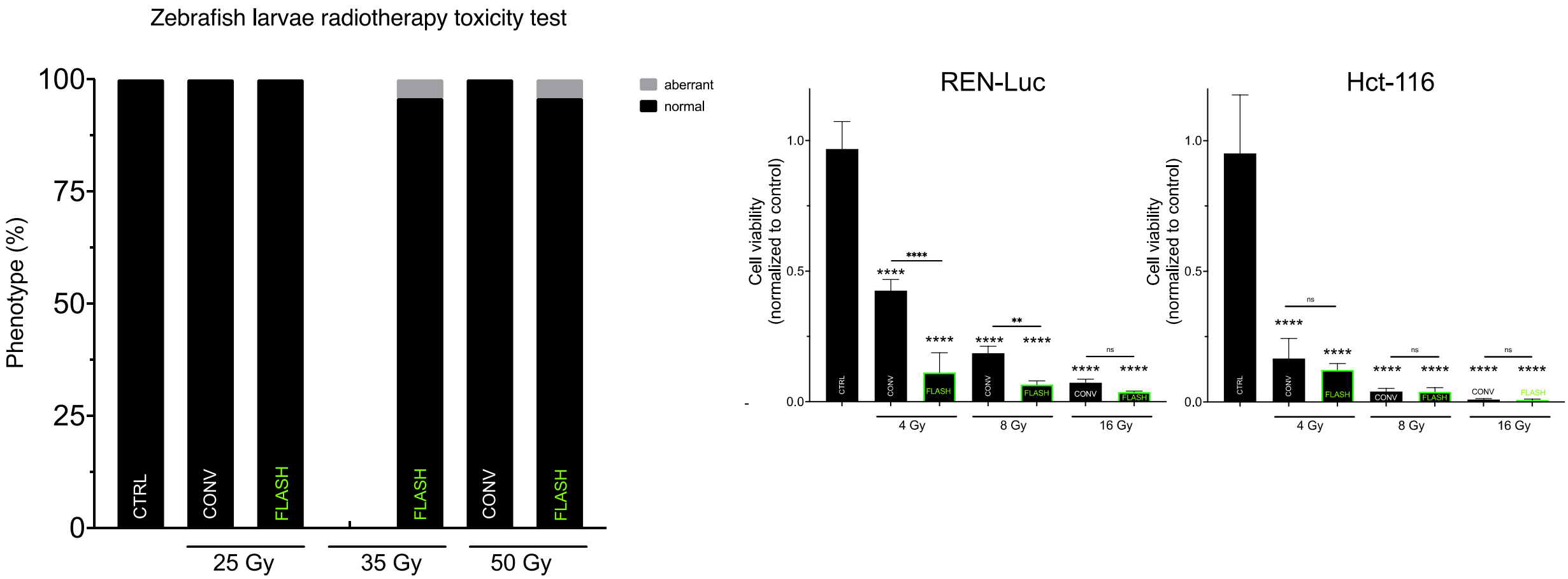











Zebrafish larvae radiotherapy toxicity test
Phenotype (%)
100
75
50
25
0
aberrant
normal
CTRL
CONV
FLASH
FLASH
CONV
FLASH
25 Gy
35 Gy
50 Gy
REN-Luc
Hct-116
Cell viability
(normalized to control)
1.0
0.5
0.0
****
**
ns
4 Gy
8 Gy
16 Gy