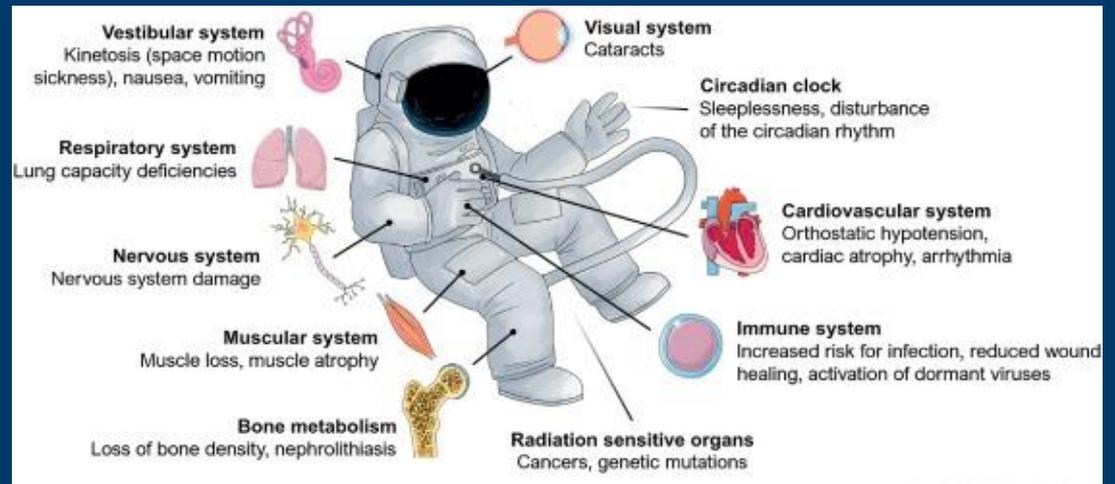


# IMPIEGO DI MODELLI MURINI IN BIOMEDICINA PER LA RADIOBIOLOGIA SPAZIALE



Simonetta Pazzaglia (ENEA)

Moroni et al. *Trends in biotechnology* 2022, vol. 40, 398-411

15-17 March 2023, Simposio ASI “Biomedicina Spaziale per le Future Missioni di Esplorazione Umana dello Spazio: a Call to Action”





### Team

Personale esperto in istopatologia, specializzato in processazione tissutale e tecniche molecolari per la valutazione di alterazioni patologiche e genetiche negli animali da esperimento.

Personale medico/veterinario per la cura degli animali



### Progetti UE

DoReMi, ProCardio, CEREBRAD, Dark-Risk e OPERRA (6/7° PQ), LD-LensRad e SEPARATE (Horizon 2020)  
Horizon Europe è partner di PIANAFORTE  
Piattaforme europee: EURADOS e MELODI e NERIS

### Obiettivi

**Focus:** aspetti meccanicistici delle alterazioni molecolari/cellulari responsabili dell'induzione di patologie da radiazioni a breve e lungo termine

Valutazione dei rischi da radiazioni

- Insorgenza di tumori
- Effetti «non-cancer» (cataratta, effetti neurologici, effetti metabolici, sull'intestino e microbioma)

### Priorità in ambito aerospaziale

Effetti dell'esposizione a radiazioni cosmiche a livello sistemico e tessuto specifico, in particolare sulle ripercussioni a carico del sistema circolatorio e nervoso

Conoscenza approfondita dei meccanismi patogenetici delle radiazioni

Rischio da radiazioni sulla salute



**CONTROMISURE**

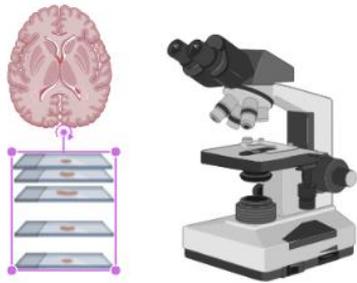


# ENEA

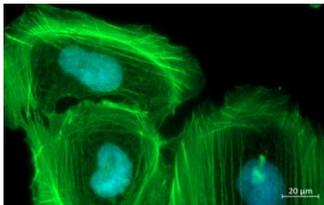
## LABORATORIO DI TECNOLOGIE BIOMEDICHE



Modelli animali



Istologia



Microscopia

Raggi X



Raggi  $\gamma$



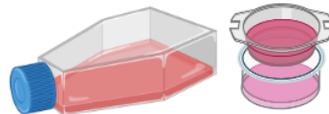
Protoni  
( $<70$  MeV)



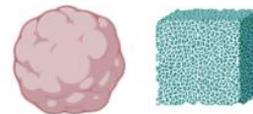
Neutroni



2D

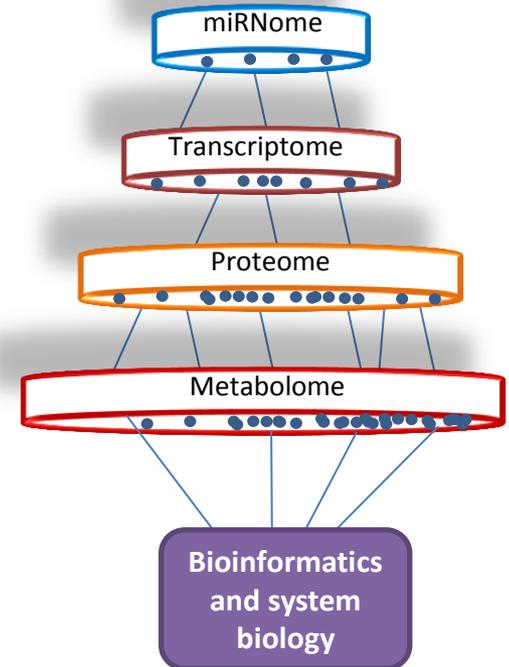


3D



Modelli *in vitro*

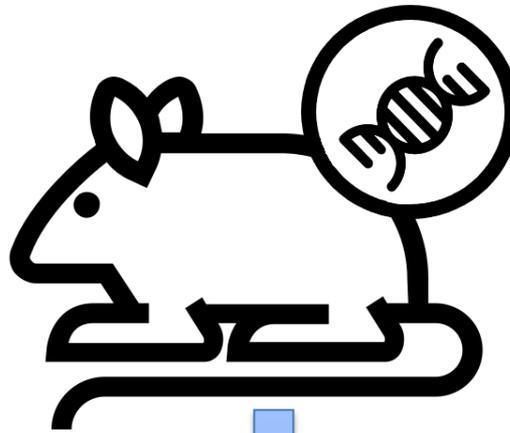
### Analisi «omiche»



# MODELLI MURINI di interesse in ambito radioprotezionistico



Stabulario



KO mice per geni  
del riparo al DNA,  
• DNA-PK<sup>-/-</sup>  
• Rad54<sup>-/-</sup>  
• PARP1<sup>-/-</sup>

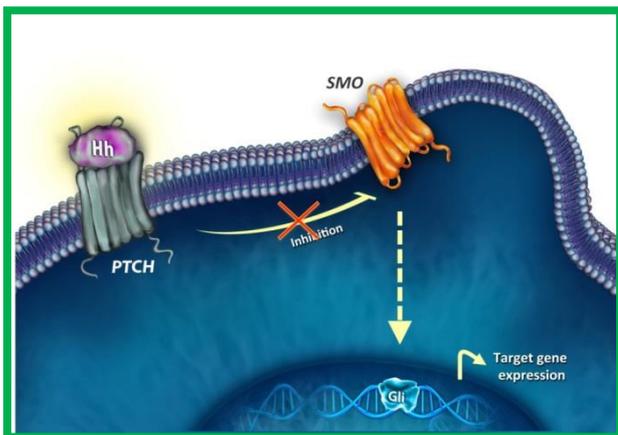
Topi ApoE<sup>-/-</sup>

Topi wild type

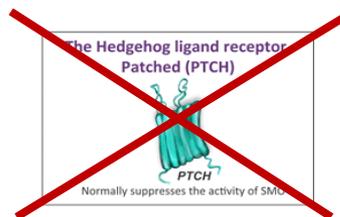
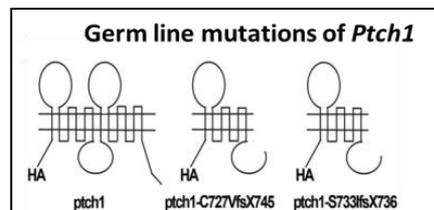
Topi *Patched*<sup>+/-</sup>

# MODELLO MURINO *PATCHED1* (*Ptch1*<sup>+/-</sup>)

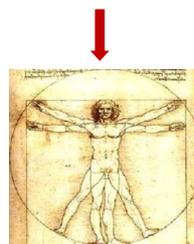
## The Shh signaling pathway



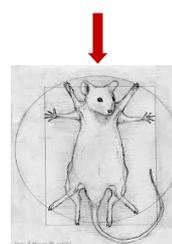
The Shh signaling pathway is an important regulator of cell growth and differentiation in a wide array of tissues during normal embryonic development



## *Ptch1* mutations in humans and mice



Gorlin syndrome



*Ptch1*<sup>+/-</sup> mice model of the Gorlin syndrome

### Common features

#### Developmental defects

Generalized overgrowth

Polydactily

Radiation hypersensitivity

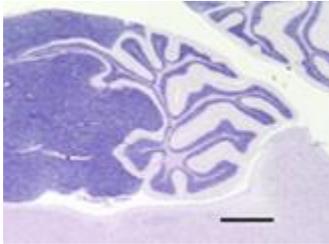
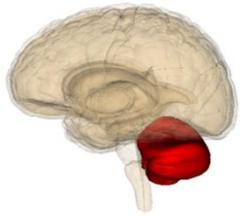
Tumor predisposition

# Topi *Ptch1*<sup>+/-</sup> per lo studio di patologie radioindotte

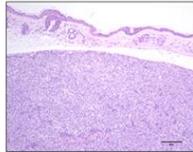
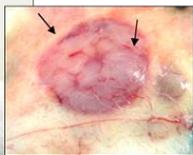
CANCER



NON-CANCER



Induzione di medulloblastoma



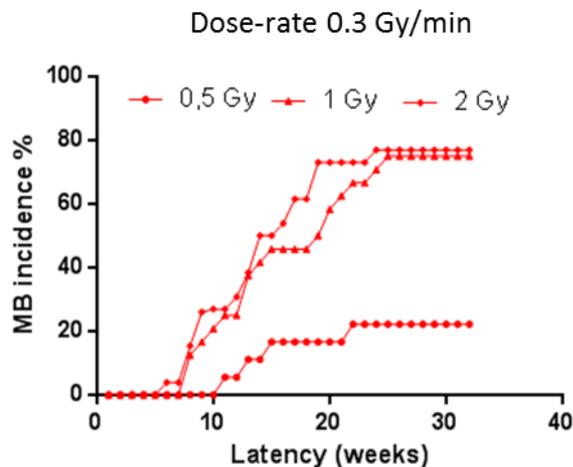
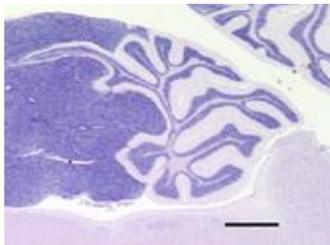
Induzione di BCC



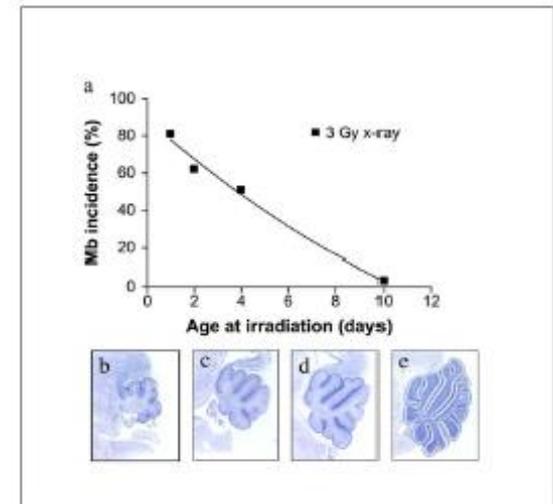
Induzione di cataratta

# Topi *Ptch1*<sup>+/-</sup>: INDUZIONE DI MEDULLOBLASTOMA (Raggi- $\gamma$ )

- ✓ Medulloblastoma (MB) è il tumore cerebrale pediatrico più maligno
- ✓ Circa il 30% dei MB sporadici mostra attivazione del pathway di Shh
- ✓ 7% dei topi *Ptch1*<sup>+/-</sup> sviluppa spontaneamente MB

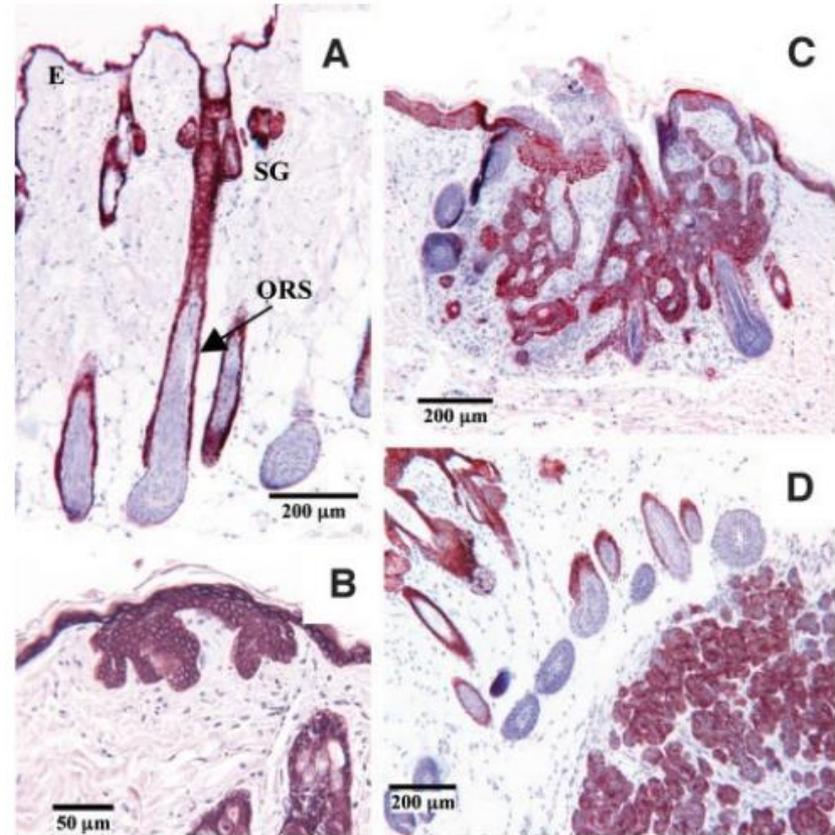
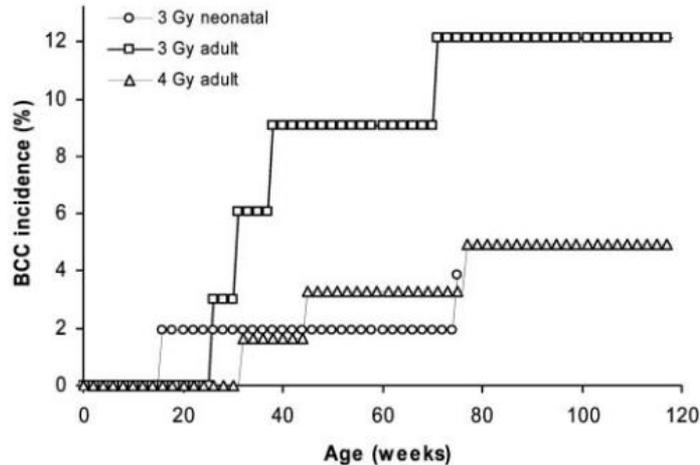
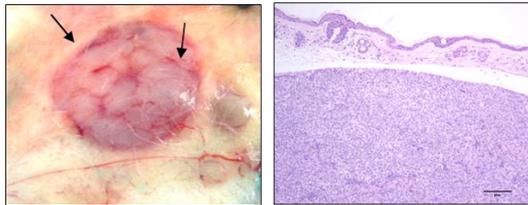


Anche dopo irraggiamento con 0.5 Gy, topi *Ptch1*<sup>+/-</sup> mostrano un aumento significativo dell'induzione di MB ( $P = 0.018$ )



La possibilità di indurre MB dopo irraggiamento è limitata all'età neonatale

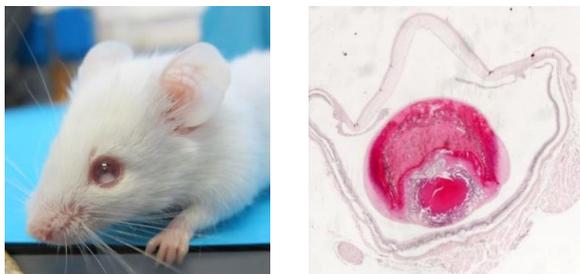
# Topi *Ptch1*<sup>+/-</sup>: INDUZIONE DI BCC DA RADIAZIONI



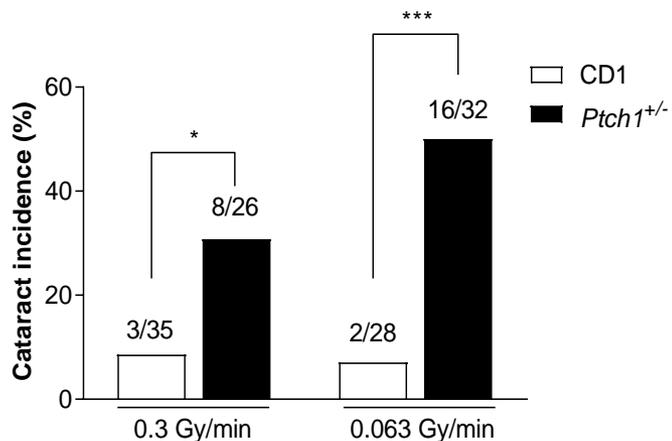
Anti-CK14-staining of skin lesions

# Topi *Ptch1*<sup>+/-</sup>: induzione di cataratta (Raggi- $\gamma$ )

## Role of genetic background in controlling the induction of lens opacity in *Ptch1*<sup>+/-</sup> and WT mice



2 Gy



Incidence of macroscopic cataract in 2 Gy irradiated *Ptch1*<sup>+/-</sup>/CD1 and CD1 WT mice

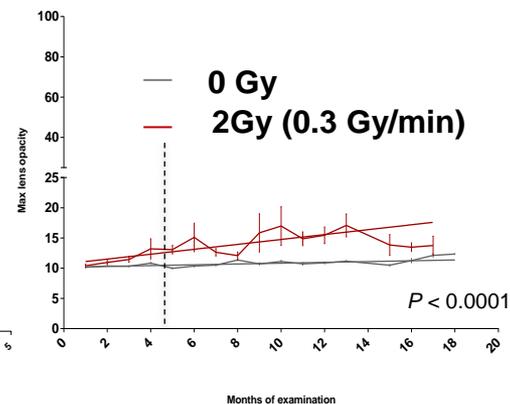
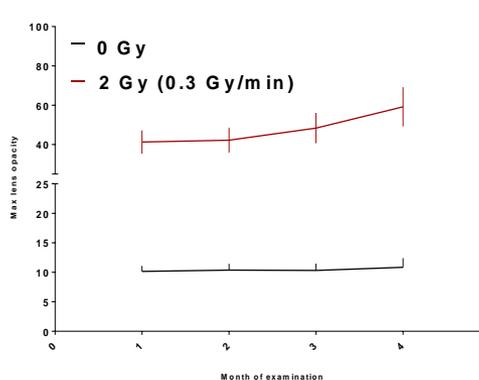
## Age-dependency for radiation-induced cataract in *Ptch1*<sup>+/-</sup>/CD1 mice



Neonatal irradiation (P2)



Adult irradiation (10W)



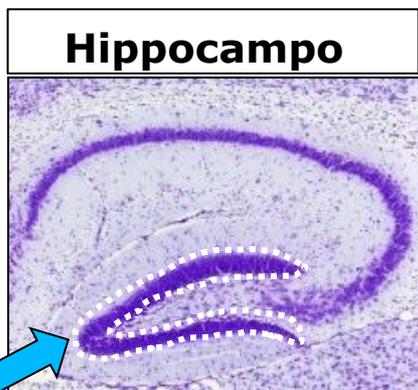
Strong decrease in sensitivity to radiation-induced lens opacity with increasing of mouse age at irradiation

# Effetti indotti dalle radiazioni ionizzanti in vari tessuti di topi WT

Topi wild type

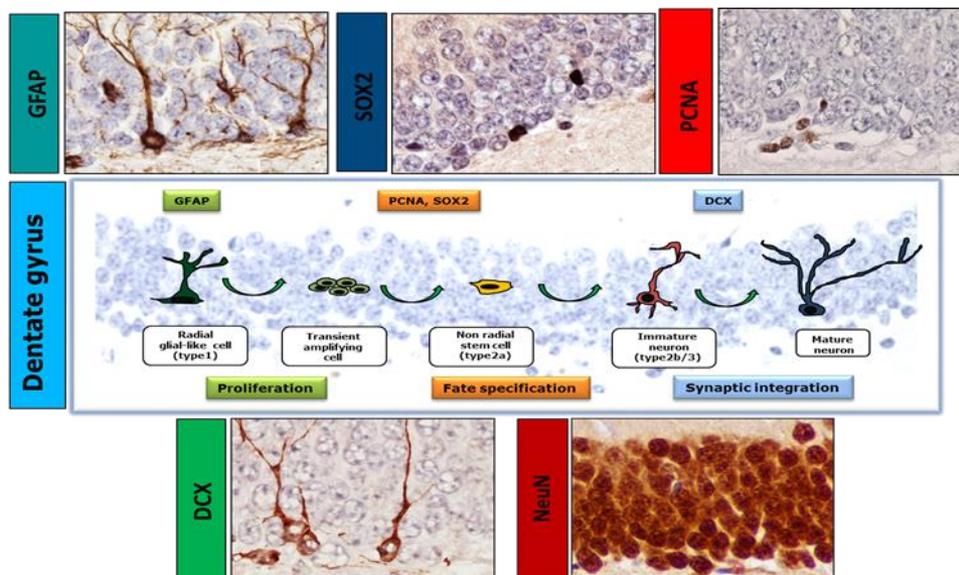


# Neurogenesi dell'ippocampo

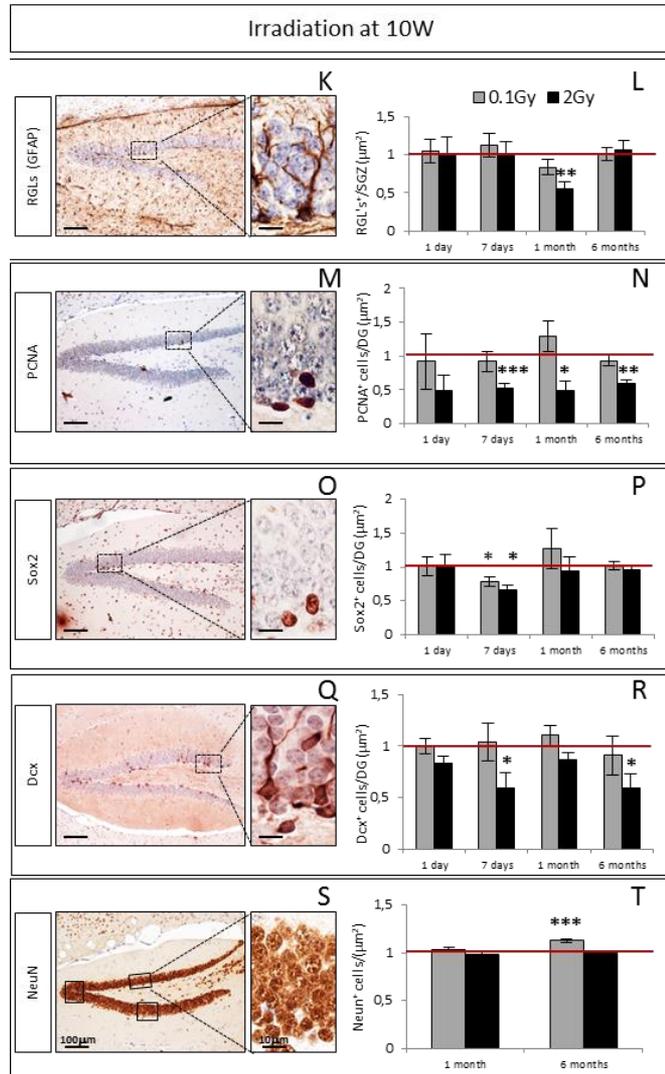


Il giro dentato (DG) dell'ippocampo è una delle strutture del sistema nervoso centrale dove la neurogenesi è osservata per tutta la vita e la maggioranza dei neuroni è generata postnatalmente.

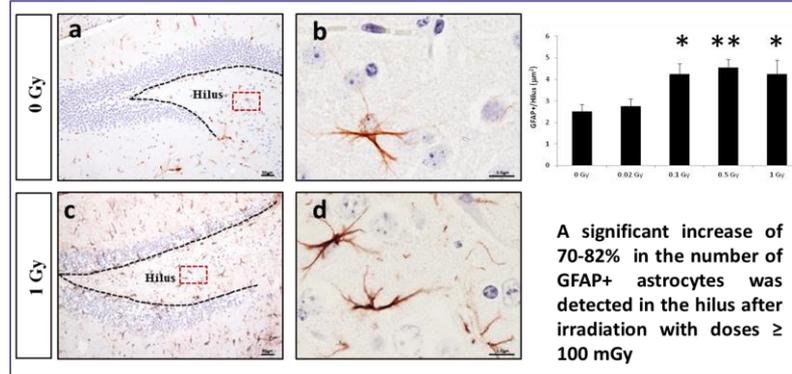
L'esposizione alla radiazioni ionizzanti è nota rallentare la neurogenesi dell'ippocampo.



# Effetti delle radiazioni sulla neurogenesi dell'ippocampo di topi wt



## Microenvironment is another critical player in hippocampal neurogenesis



Chronic inflammation may alter hippocampal neurogenesis causing defects in cognitive function

[www.impactjournals.com/oncotarget/](http://www.impactjournals.com/oncotarget/)

Oncotarget, Vol. 7, No. 19

### Age-related effects of X-ray irradiation on mouse hippocampus

Arianna Casciati<sup>1</sup>, Katalin Dobos<sup>2</sup>, Francesca Antonelli<sup>1</sup>, Anett Benedek<sup>2</sup>, Stefan J. Kempf<sup>3,8</sup>, Montserrat Bellés<sup>3</sup>, Andrea Balogh<sup>2</sup>, Mirella Tanori<sup>1</sup>, Luis Heredia<sup>2</sup>, Michael J. Atkinson<sup>4,5</sup>, Christine von Toerne<sup>6</sup>, Omid Azimzadeh<sup>4</sup>, Anna Saran<sup>1</sup>, Geza Sáfrány<sup>2</sup>, Mohammed A. Benotmane<sup>7</sup>, M. Victoria Linares-Vidal<sup>3</sup>, Soile Tapio<sup>4</sup>, Katalin Lumniczky<sup>2</sup>, Simonetta Pazzaglia<sup>1</sup>

Kempf et al. *Molecular Neurodegeneration* 2014, 9:57  
<http://www.molecularneurodegeneration.com/content/9/1/57>



RESEARCH ARTICLE

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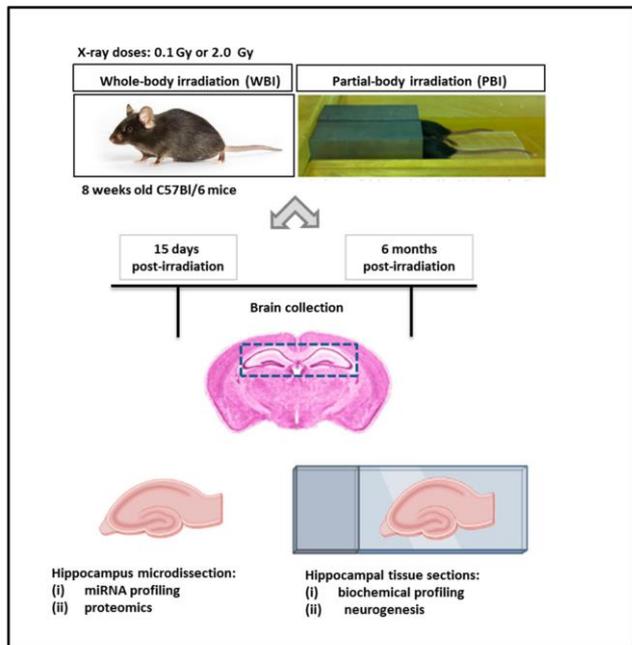
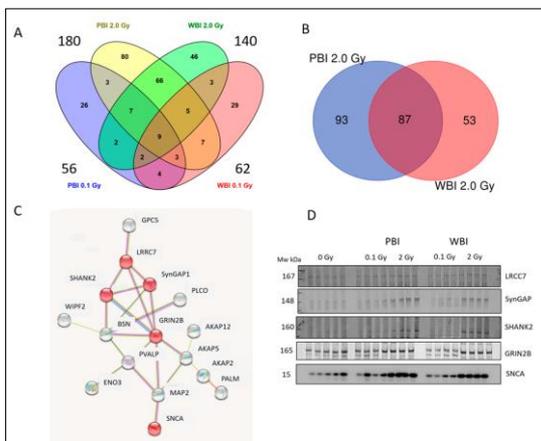
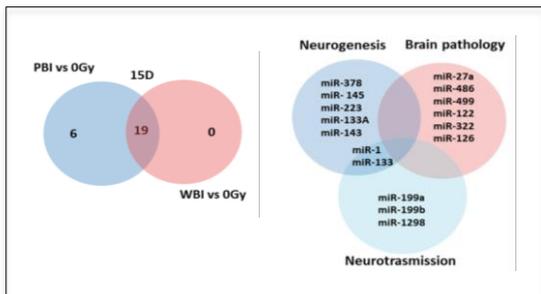
The cognitive defects of neonatally irradiated mice are accompanied by changed synaptic plasticity, adult neurogenesis and neuroinflammation

Stefan J Kempf<sup>1</sup>, Arianna Casciati<sup>2†</sup>, Sonja Buratovic<sup>3†</sup>, Dirk Janik<sup>4</sup>, Christine von Toerne<sup>5</sup>, Marius Ueffing<sup>5</sup>, Frauke Neff<sup>6</sup>, Simone Moertl<sup>1</sup>, Bo Stenerlöw<sup>6</sup>, Anna Saran<sup>7</sup>, Michael J Atkinson<sup>1,2</sup>, Per Eriksson<sup>3</sup>, Simonetta Pazzaglia<sup>3</sup> and Soile Tapio<sup>1\*</sup>

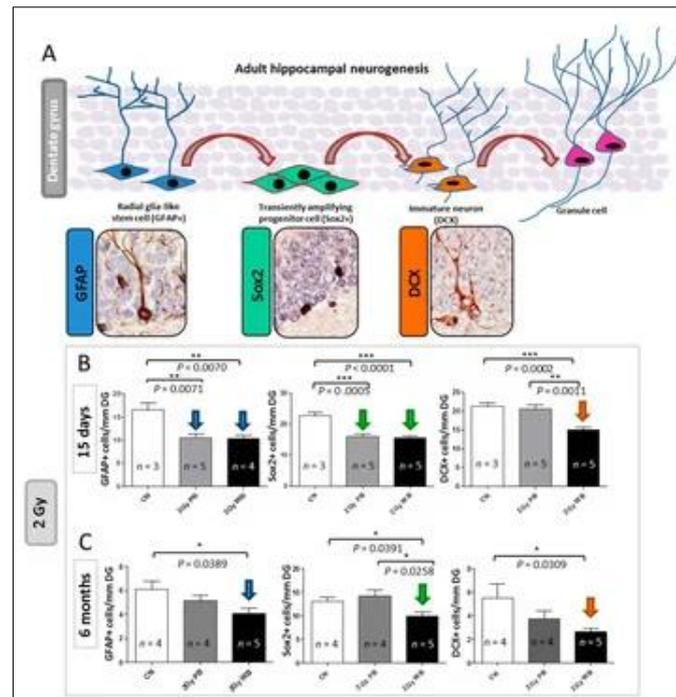
Article  
**Long-Term Effects of Ionizing Radiation on the Hippocampus: Linking Effects of the Sonic Hedgehog Pathway Activation with Radiation Response**  
 Francesca Antonelli<sup>1\*</sup>, Arianna Casciati<sup>1</sup>, Montserrat Bellés<sup>2</sup>, Noemi Serra<sup>2</sup>, Maria Victoria Linares-Vidal<sup>2</sup>, Carmela Marino<sup>1</sup>, Mariateresa Mancuso<sup>1</sup> and Simonetta Pazzaglia<sup>1\*</sup>

# EFFETTO BYSTANDER INDOTTO DALLE RADIAZIONI NELL'IPPOCAMPO

## Analisi «omiche»



## Istologia

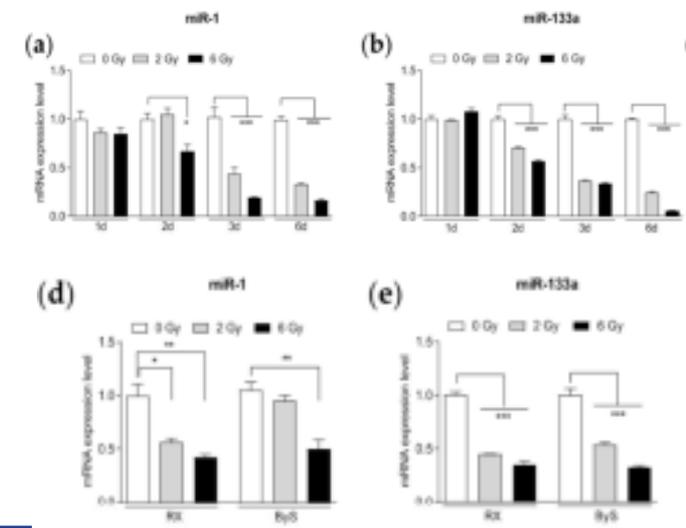
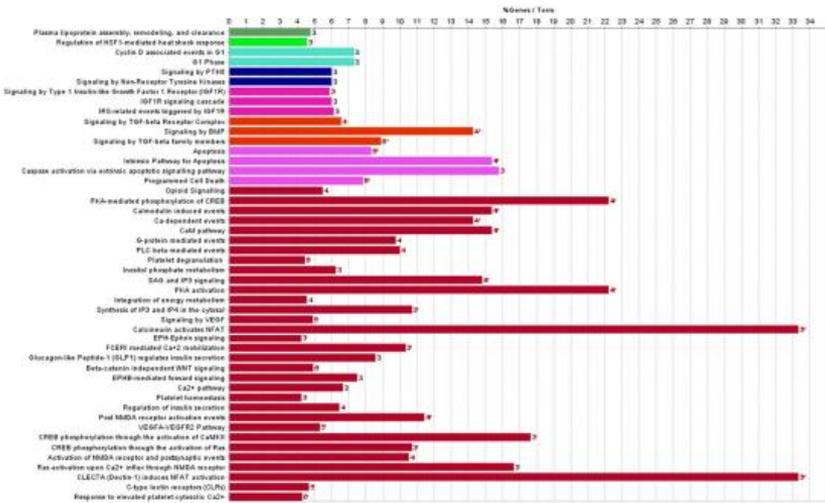
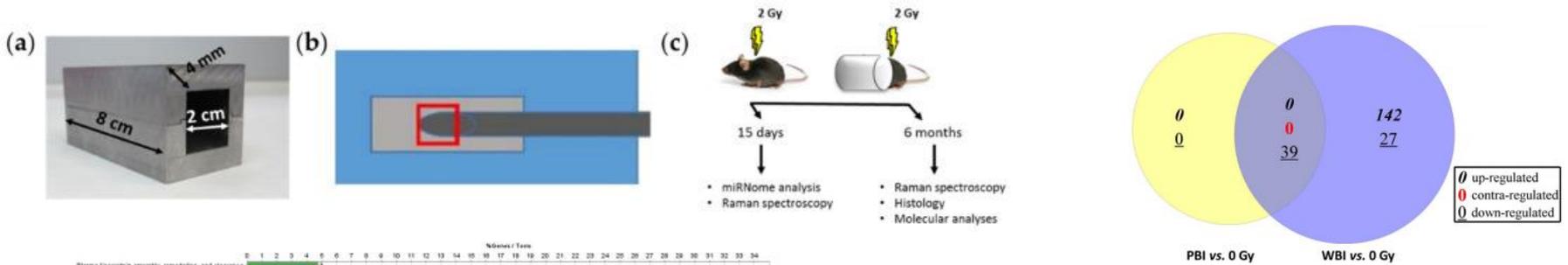


### Article

## Out-of-Field Hippocampus from Partial-Body Irradiated Mice Displays Changes in Multi-Omics Profile and Defects in Neurogenesis

Simonetta Pazzaglia<sup>1,\*</sup>, Barbara Tanno<sup>1</sup>, Francesca Antonelli<sup>1</sup>, Paola Giardullo<sup>1</sup>, Gabriele Babini<sup>2,3,†</sup>, Prabal Subedi<sup>4,5</sup>, Omid Azimzadeh<sup>4,5</sup>, Zohaib N. Khan<sup>4</sup>, Kateryna Oleksenko<sup>4</sup>, Fabian Metzger<sup>6,†</sup>, Christine von Toerne<sup>6</sup>, Damien Traynor<sup>7</sup>, Dinesh Medipally<sup>7</sup>, Aidan D. Meade<sup>7</sup>, Munira Kadhim<sup>8</sup>, Fiona M. Lyng<sup>7</sup>, Soile Tapio<sup>4</sup>, Anna Saran<sup>1</sup> and Mariateresa Mancuso<sup>1,†</sup>

# I MiRNA MEDIANO L'EFFETTO BYSTANDER INDOTTO DALLE RADIAZIONI NEL CUORE



✓ Mir-1 and miR-133a, membri della famiglia dei myomiR, erano altamente deregolati dopo analisi NGS eseguita a 15 days postirradiation nei cuori WBI e PBI



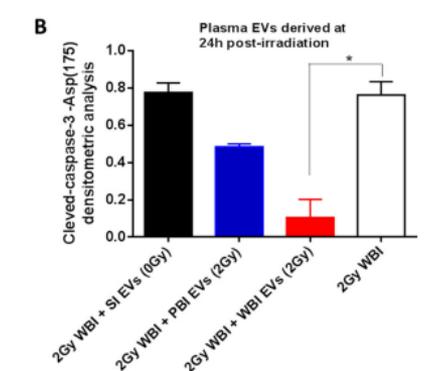
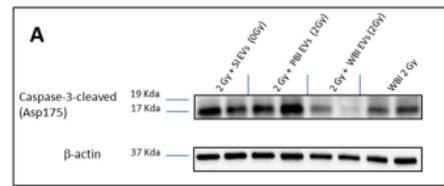
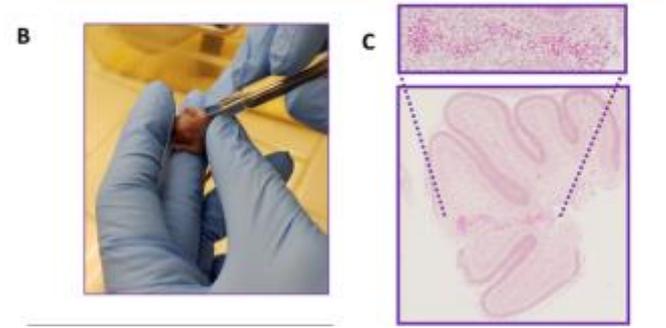
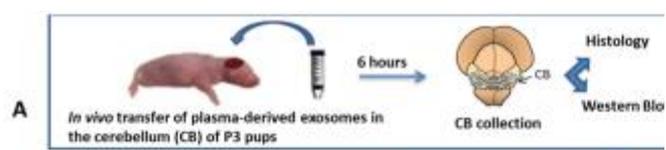
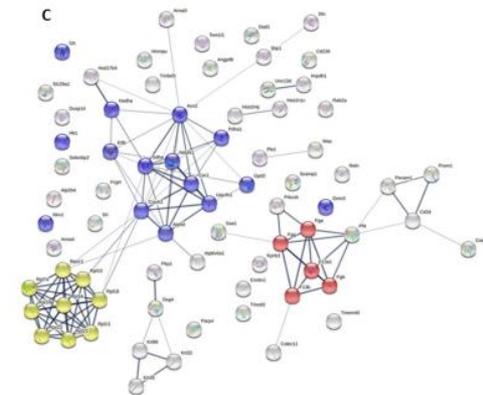
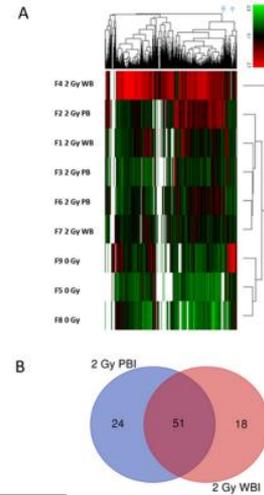
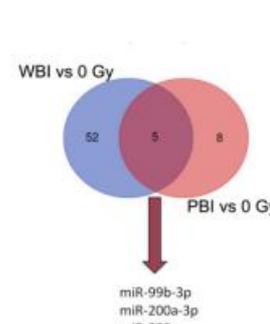
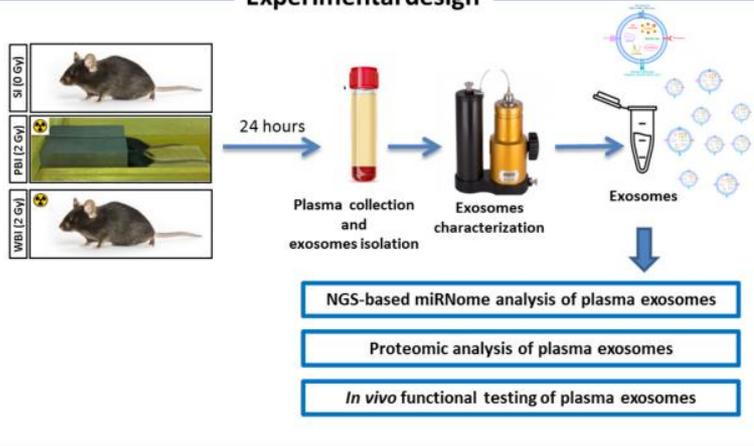
## Article MiRNA-Mediated Fibrosis in the Out-of-Target Heart following Partial-Body Irradiation

Barbara Tanno <sup>1,\*</sup>, Flavia Novelli <sup>1</sup>, Simona Leonardi <sup>1</sup>, Caterina Merla <sup>1</sup>, Gabriele Babini <sup>2,†</sup>, Paola Giardullo <sup>1</sup>, Munira Kadhim <sup>3</sup>, Damien Traynor <sup>4</sup>, Dinesh K. R. Medipally <sup>1</sup>, Aidan D. Meade <sup>4</sup>, Fiona M. Lyng <sup>4</sup>, Soile Tapio <sup>5</sup>, Luca Marchetti <sup>1,6</sup>, Anna Saran <sup>1,7</sup>, Simonetta Pazzaglia <sup>1</sup> and Mariateresa Mancuso <sup>1,\*</sup>



# Micro-RNA and Proteomic Profiles of Plasma-Derived Exosomes from Irradiated Mice Reveal Molecular Changes Preventing Apoptosis in Neonatal Cerebellum

## Experimental design



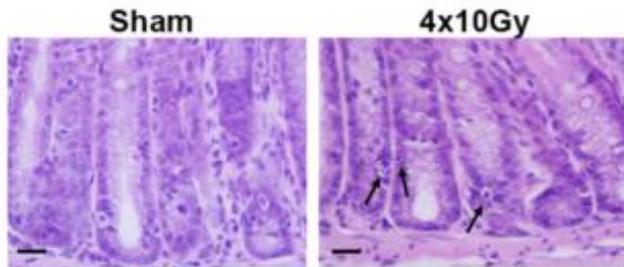
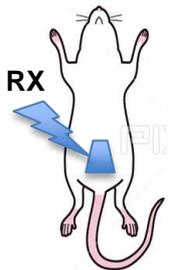
International Journal of Molecular Sciences



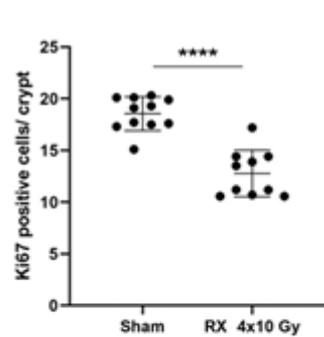
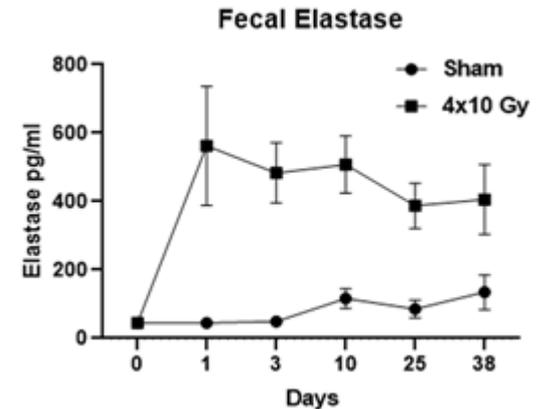
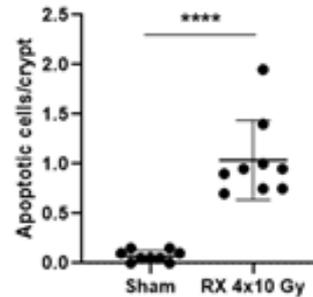
## Micro-RNA and Proteomic Profiles of Plasma-Derived Exosomes from Irradiated Mice Reveal Molecular Changes Preventing Apoptosis in Neonatal Cerebellum

Simonetta Pazzaglia <sup>1,\*</sup>, Barbara Tanno <sup>1</sup>, Ilaria De Stefano <sup>1</sup>, Paola Giardullo <sup>1</sup>, Simona Leonardi <sup>1</sup>, Caterina Merla <sup>1</sup>, Gabriele Babini <sup>2,†</sup>, Seda Tuncay Cagatay <sup>3</sup>, Ammar Mayah <sup>3</sup>, Munira Kadhim <sup>3</sup>, Fiona M. Lyng <sup>4</sup>, Christine von Toerne <sup>5</sup>, Zohaib N. Khan <sup>5</sup>, Prabal Subedi <sup>5</sup>, Soile Tapio <sup>5</sup>, Anna Saran <sup>1</sup> and Mariateresa Mancuso <sup>1,\*</sup>

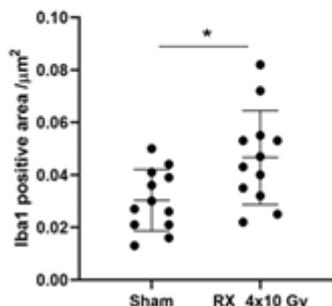
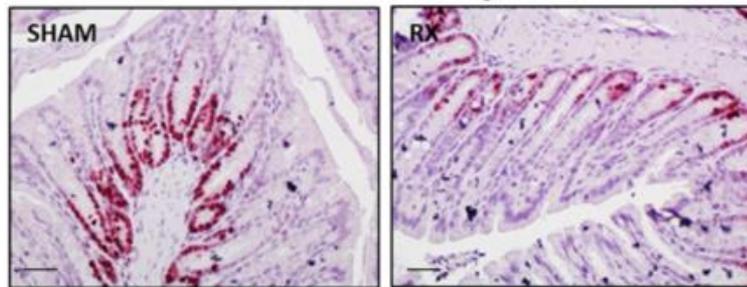
# Radioenterite da radiazioni



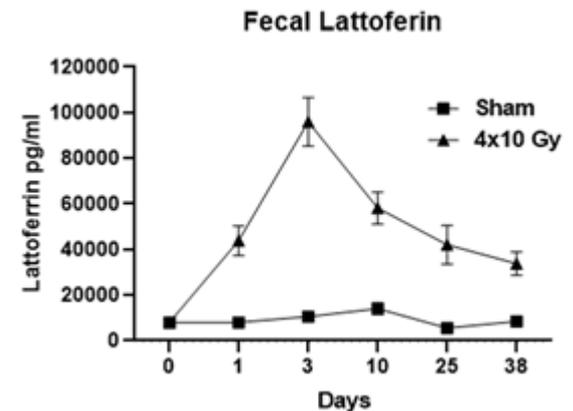
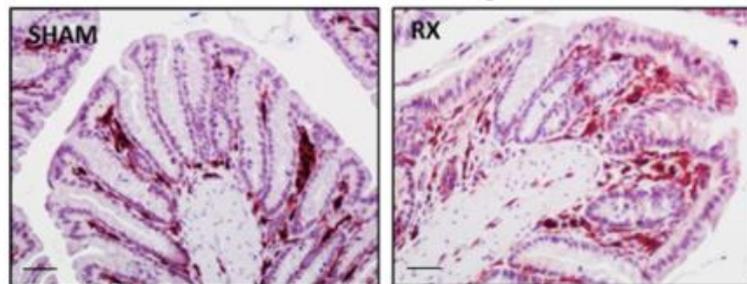
3 h post-irradiation



Ki67 immunostaining



Iba1 immunostaining



Quantitative analysis of intestinal inflammatory fecal markers Elastase and Lactoferrin

38 days post-irradiation

# CONCLUSIONI



- I nostri modelli murini, estremamente ben caratterizzati per la risposta alle radiazioni in molti tessuti rilevanti per la radiobiologia spaziale, permettono di valutare l'efficacia di contromisure *in vivo*, come ad esempio valutare se il rallentamento dello stato metabolico di un organismo può mitigare gli effetti dannosi delle radiazioni.

- L'identificazione dei meccanismi patogenetici delle radiazioni è cruciale per lo sviluppo di metodi per mitigare il rischio da radiazioni.

**LAB TECS**

M Mancuso  
A Casciati  
E Colantoni  
I De Stefano  
S Leonardi  
E Pasquali  
F Palone  
B Tanno  
M Tanori  
R Vitali



Simonetta Pazzaglia  
simonetta.pazzaglia@enea.it



Francesca Antonelli



1101 0110 1100  
0101 0010 1101  
0001 0110 1110  
1101 0010 1101  
1111 1010 0000



Emiliano Fratini



HORIZON EUROPE



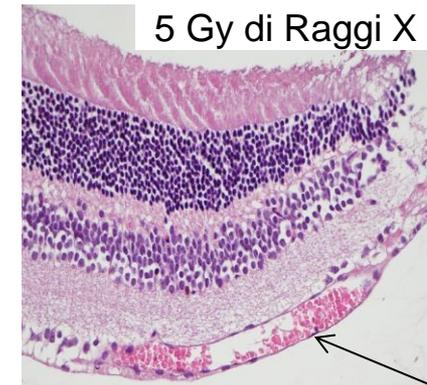
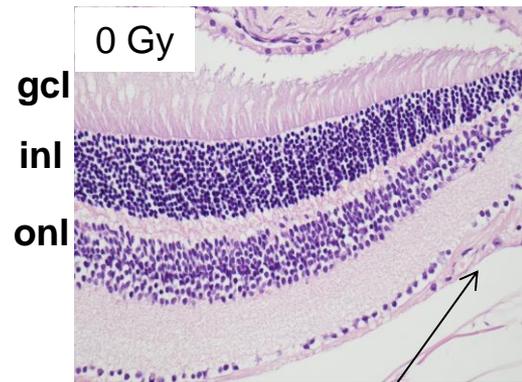
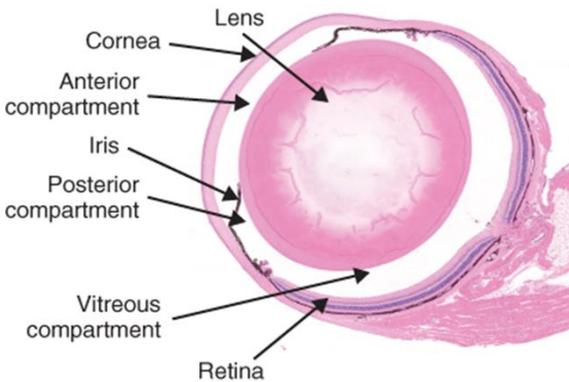
Daniela Giovannini

Grazie per l'attenzione



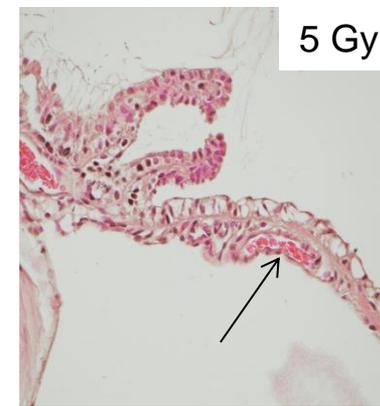
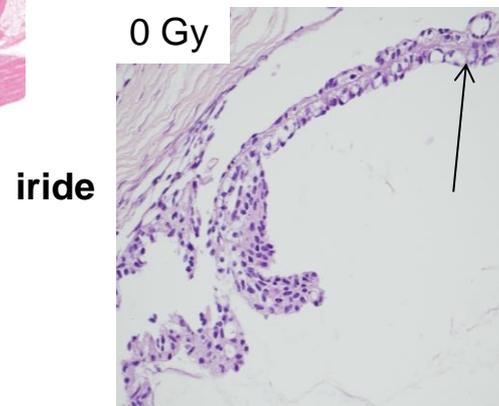
# Effetti dell'irraggiamento sulla retina wi topi WT

## Topi CD1 WT a 4 settimane post RX



Retiniti da radiazioni

- ✓ Aumento volume vasi superficiali periferici
- ✓ Diminuzione del numero di cellule dei gangli (RCG) nella retina sensoriale



Glucoma neovascolare

- ✓ Aumento numero e volume dei vasi nell'angolo anteriore dell'occhio-iride