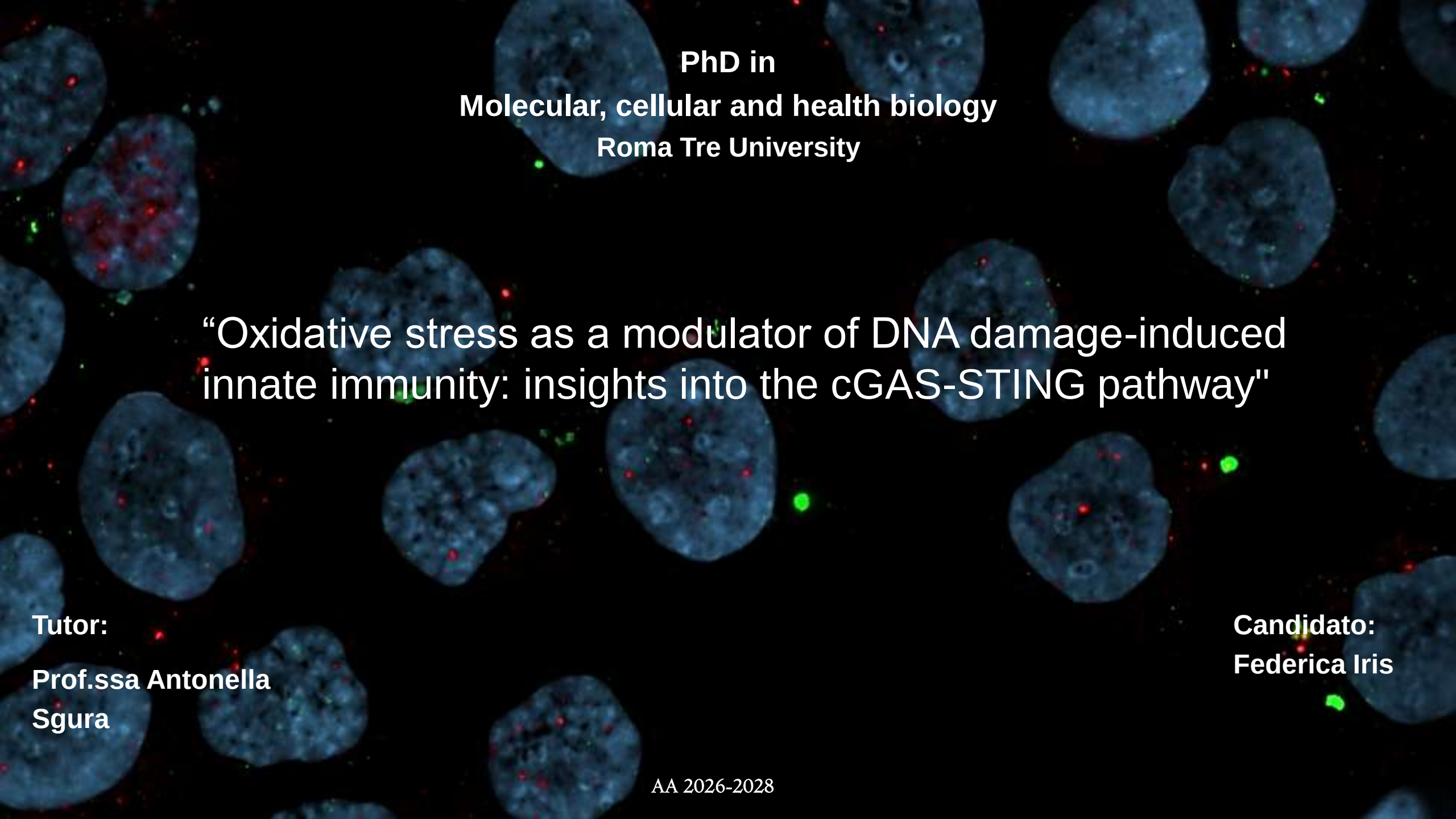




PhD in
Molecular, cellular and health biology
Roma Tre University

“Oxidative stress as a modulator of DNA damage-induced
innate immunity: insights into the cGAS-STING pathway”

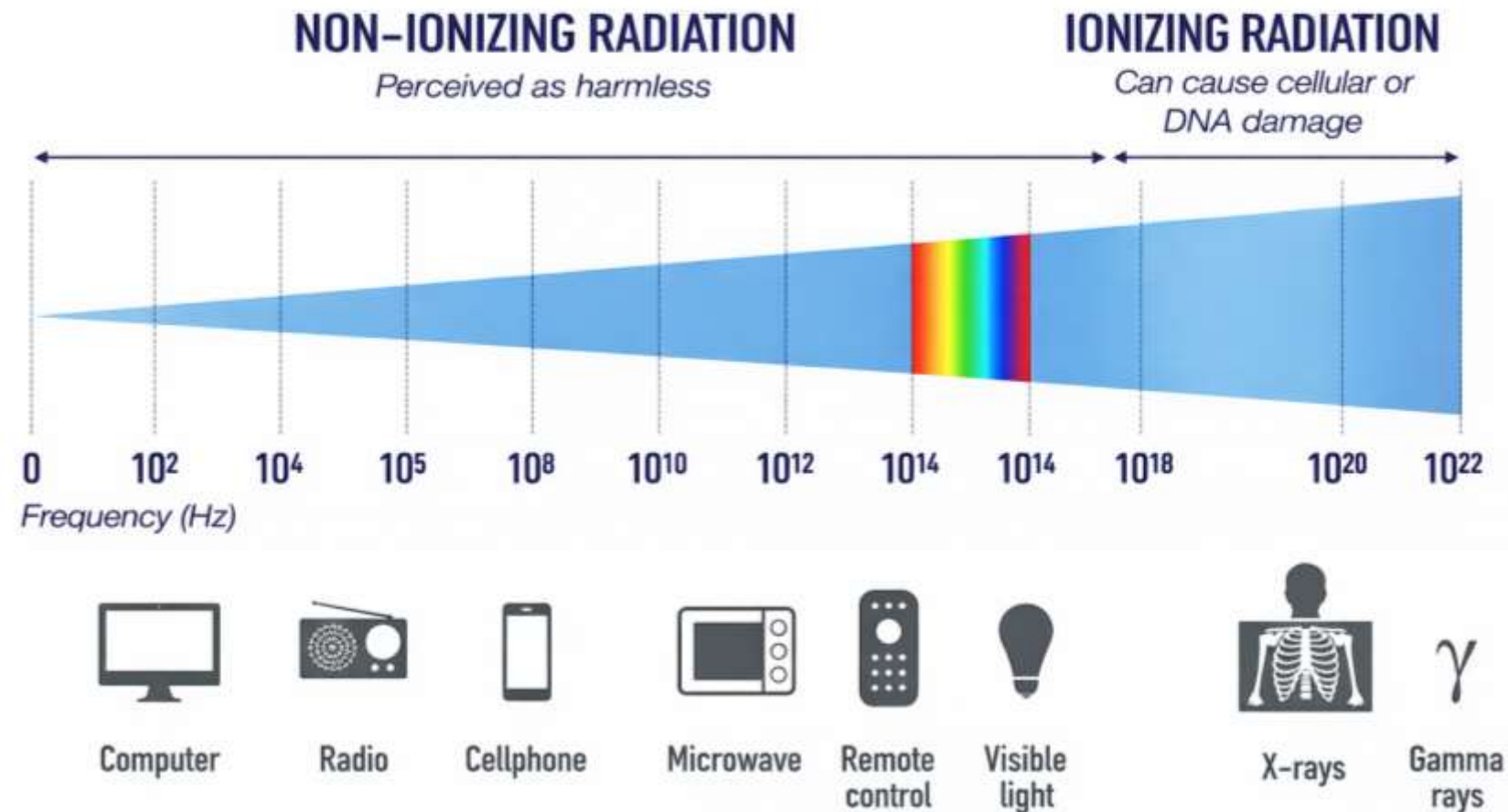
Tutor:
Prof.ssa Antonella
Sgura

Candidato:
Federica Iris

SCIENTIFIC BACKGROUND

Human exposure to radiation

- As frequency increases, the energy associated with radiation increases.
- **Ionizing radiation:** can remove electrons from atoms and is potentially harmful to cells.
- **Non-ionizing radiation:** does not ionize atoms but can produce thermal effects.

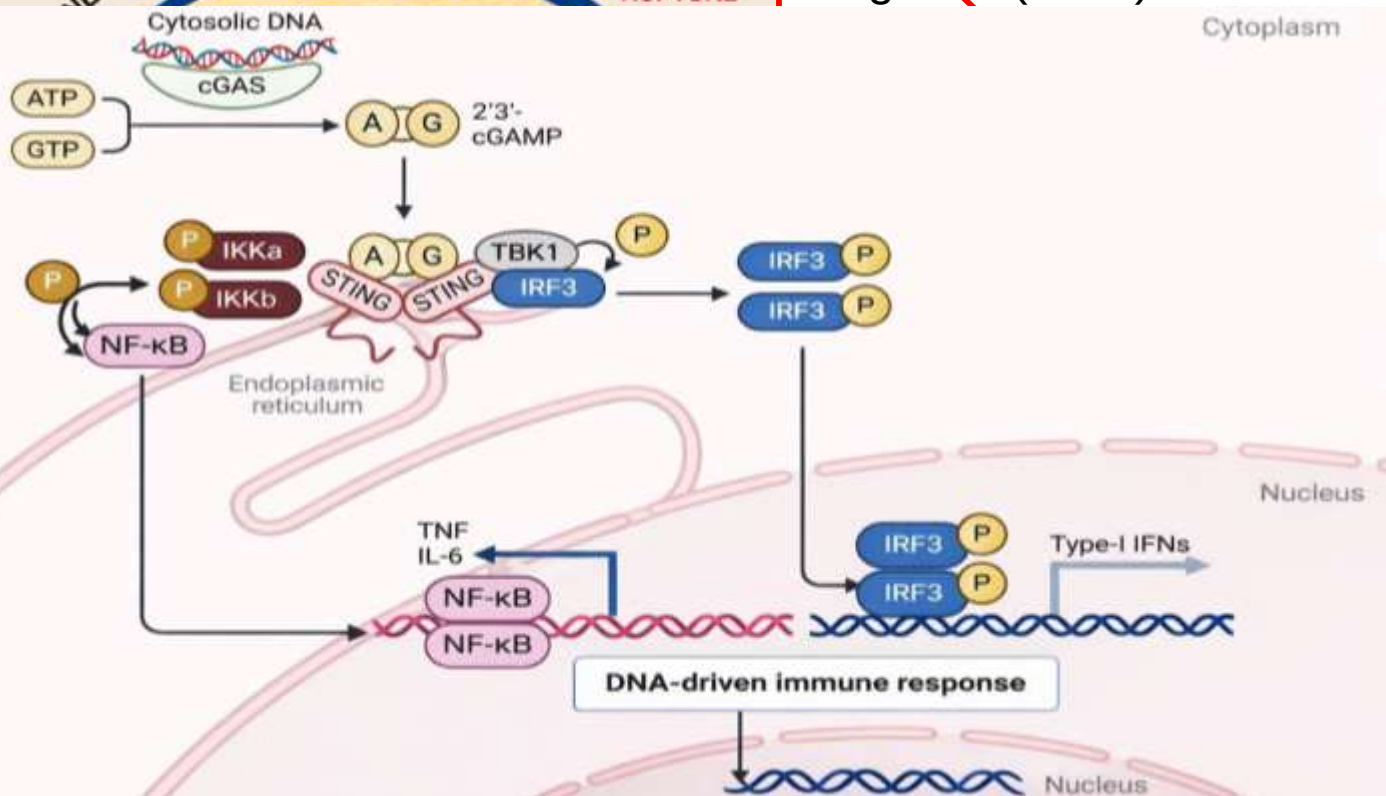
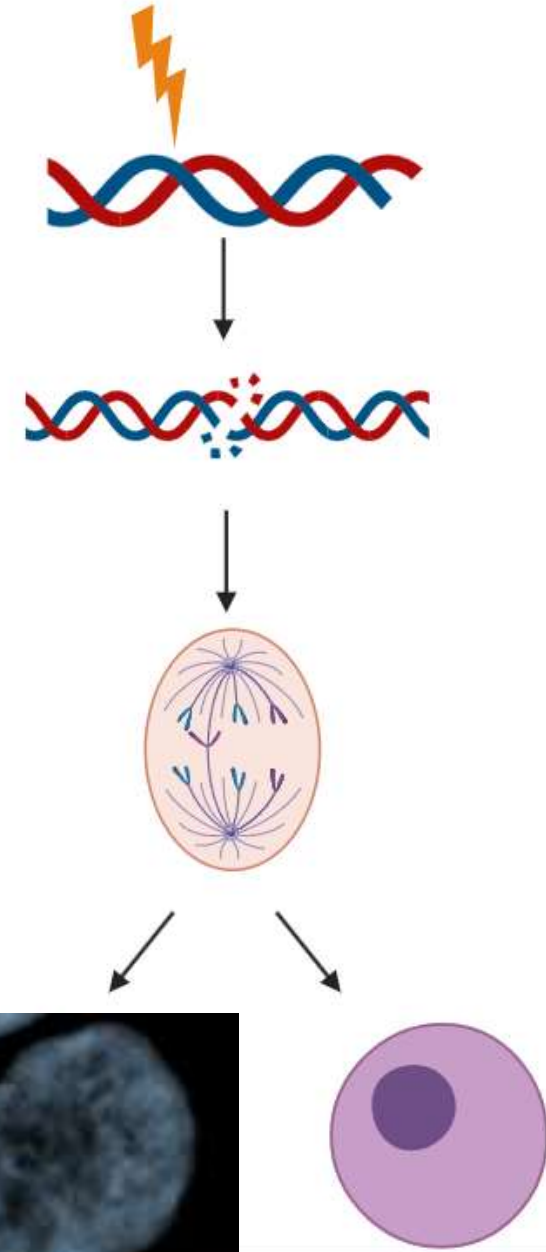


SCIENTIFIC BACKGROUND

Ionizing radiation (IR) induces DNA damage and oxidative stress (ROS).

Micronuclei (MNI), formed from double strand breaks (DSBs), can rupture and release DNA into the cytosol.

leading to the production of type I interferons (ISGs).



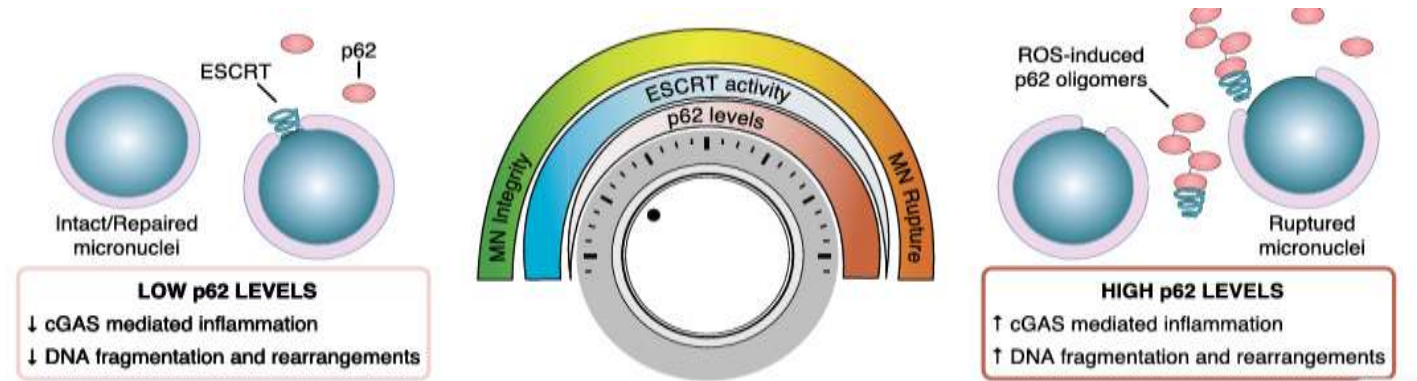
ROLE OF p62 – AN AUTOPHAGY RECEPTOR

ROS influence the localization of p62

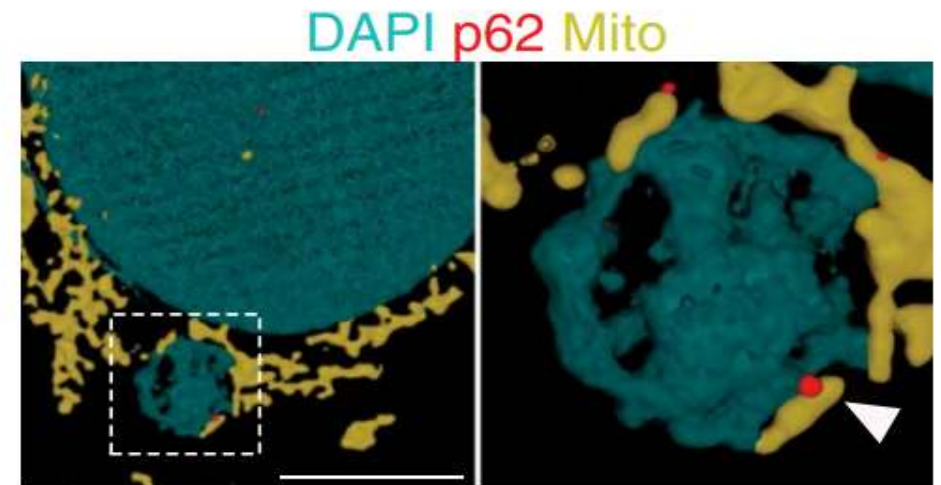
ROS-induced homo-oligomerization of p62 affected nuclear and micronuclear integrity by inhibiting the activity of ESCRT-III (endosomal sorting complex)

The presence of p62 has been correlated with damage and rupture of membranes

Proximity between mitochondria and MNi exposes MNi to elevated ROS levels



Martin S et al. (2024)



Martin S et al. (2024)

Laboratori Nazionali del Gran Sasso (LNGS)

NBR: Natural Background Radiation

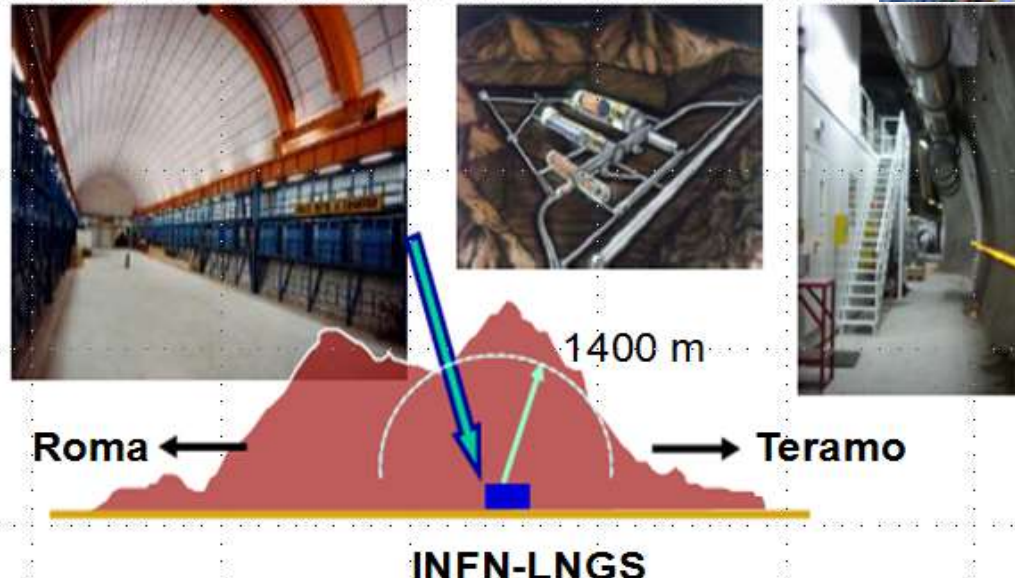


HaCaT cell line

BBR: Below Background Radiation



Radiation	NBR Lab
Muons	47 nSv/h
γ -rays	31 nSv/h
Neutrons	1.4 nSv/h
Radon	~ 20 Bq/m ³

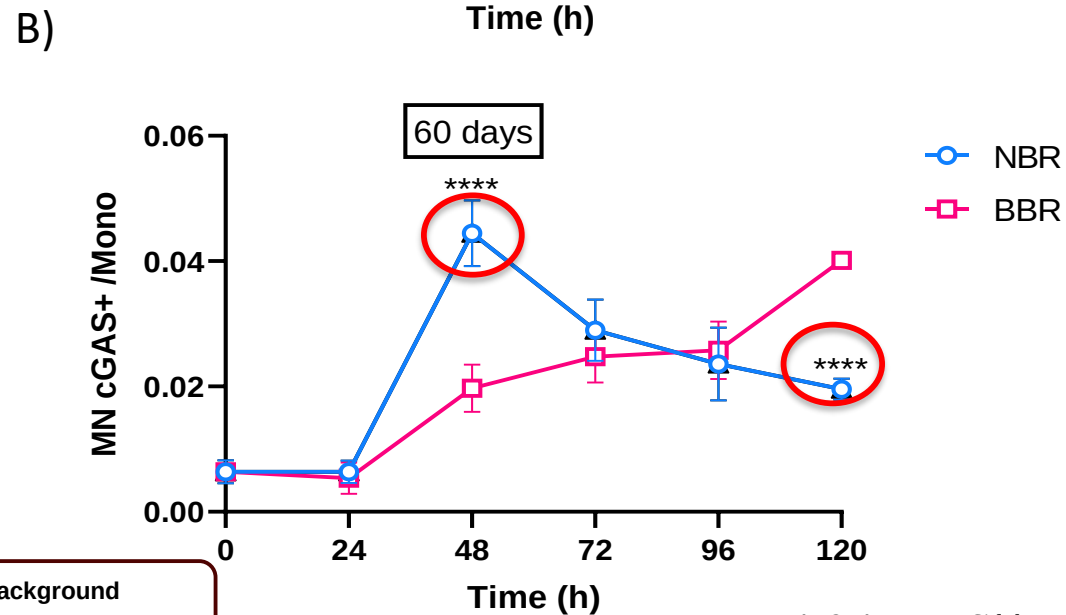
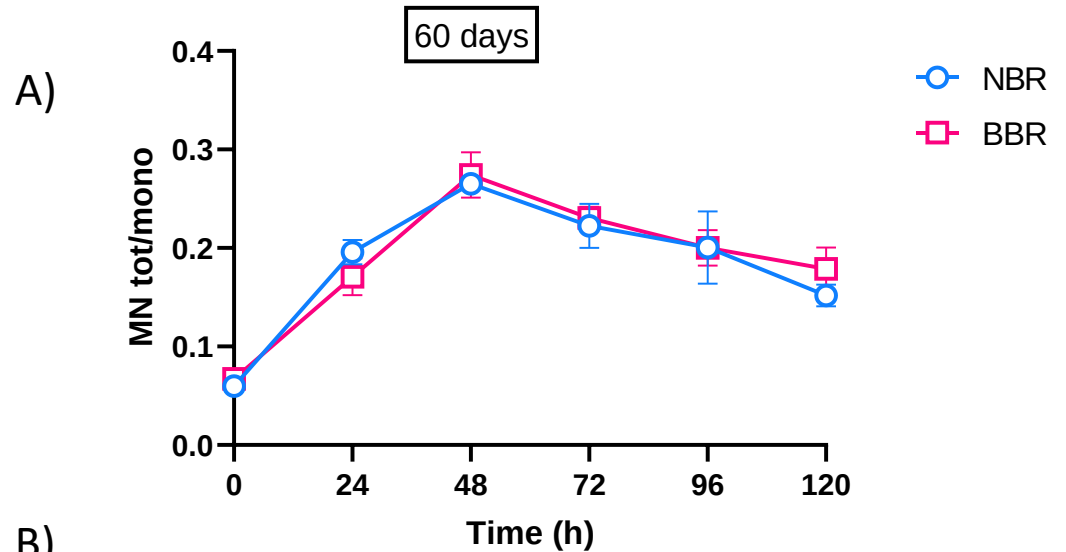


Radiation	BBR Lab
Muons	$\ll 1$ nSv/h
γ -rays	27 nSv/h
Neutrons	$\ll 1$ pSv/h
Radon	~ 20 Bq/m ³

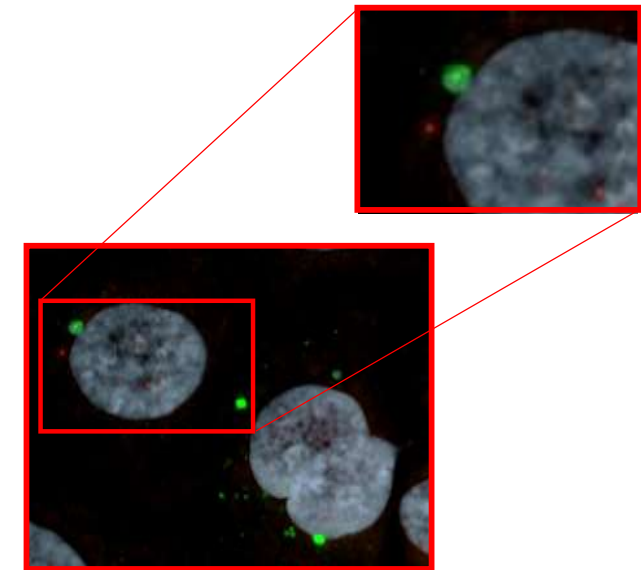
PRELIMINARY RESULTS

Preliminary data have shown a temporally distinct recruitment of cGAS to MNi after IR in NBR and BBR.

Immortalized human keratinocytes
(HaCaT cell line)
IR: 1Gy of X- rays



Two-way ANOVA, post test Sidak;
**** $p < 0,0001$

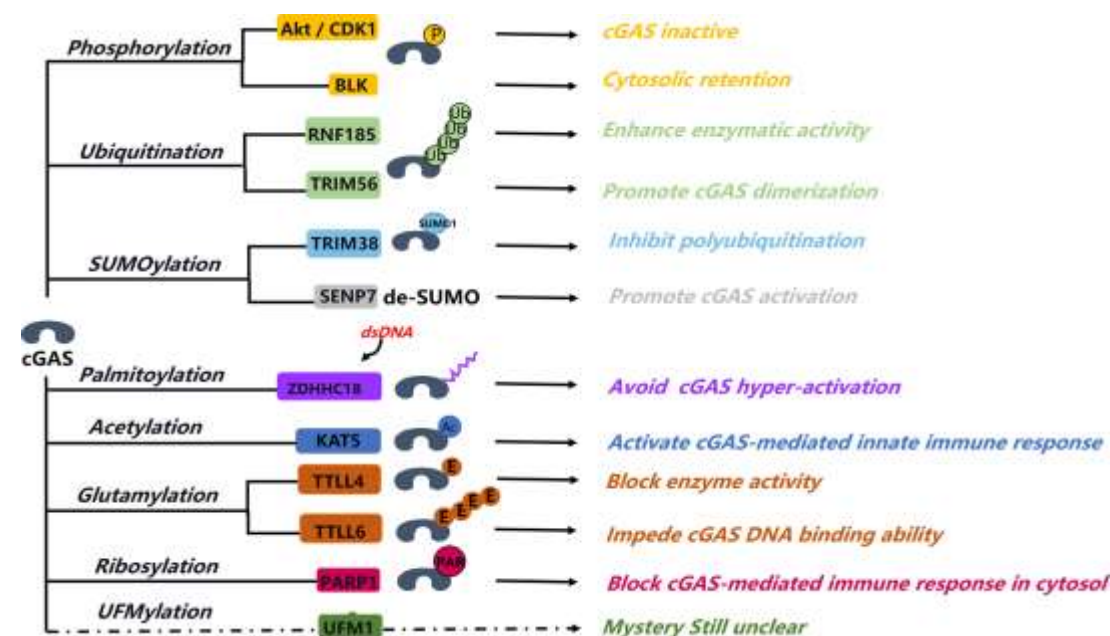
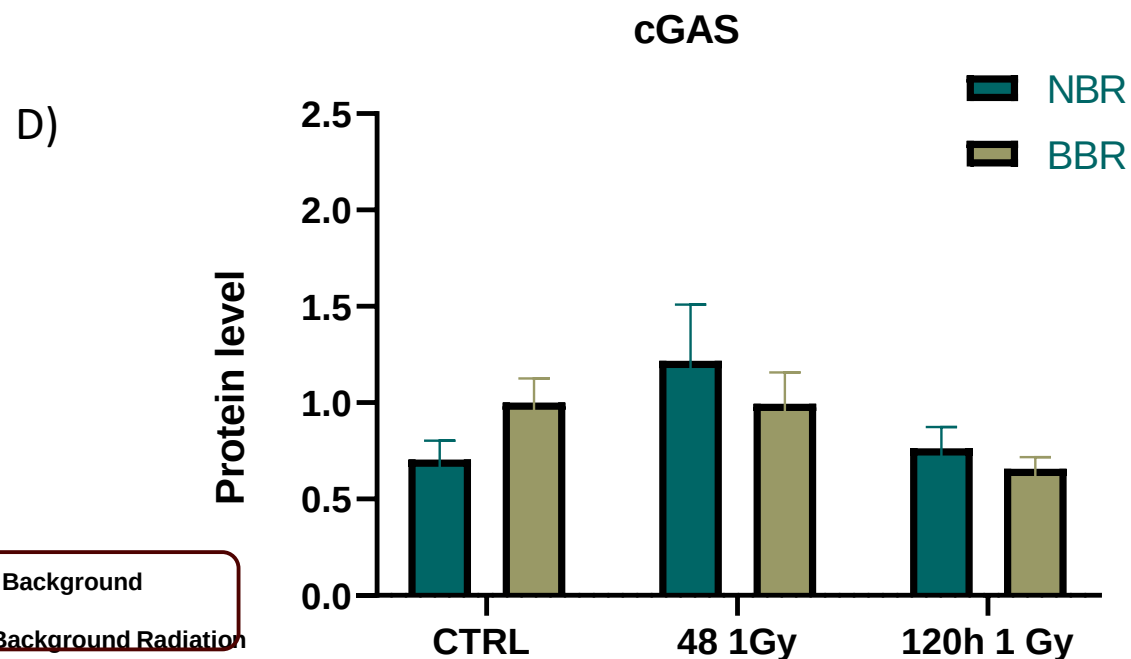
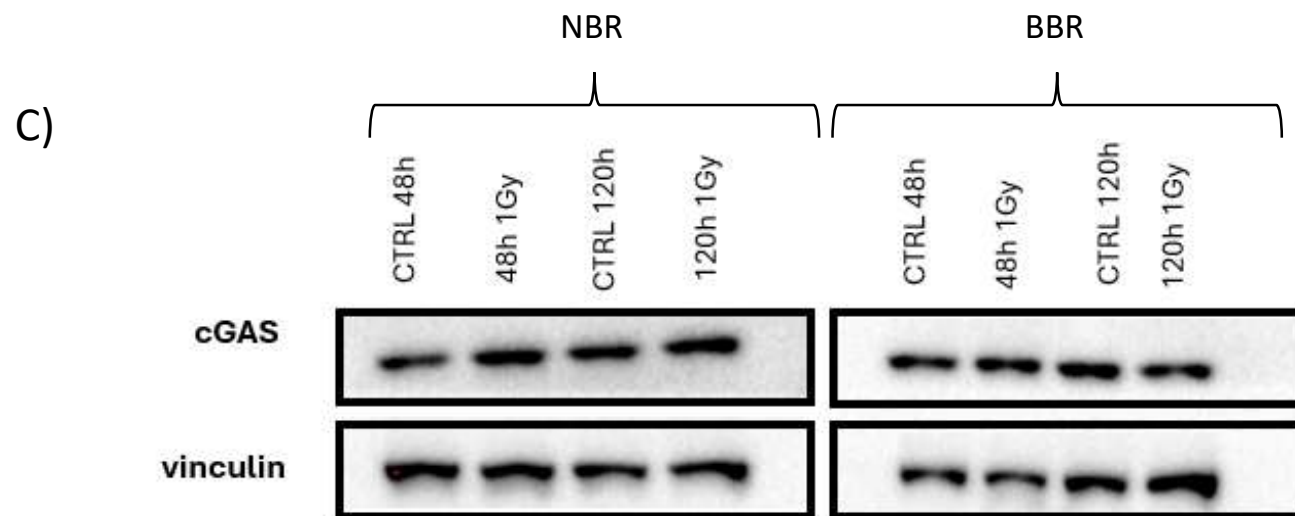


NBR: Natural Background
Radiation
BBR: Below Background Radiation

PRELIMINARY RESULTS

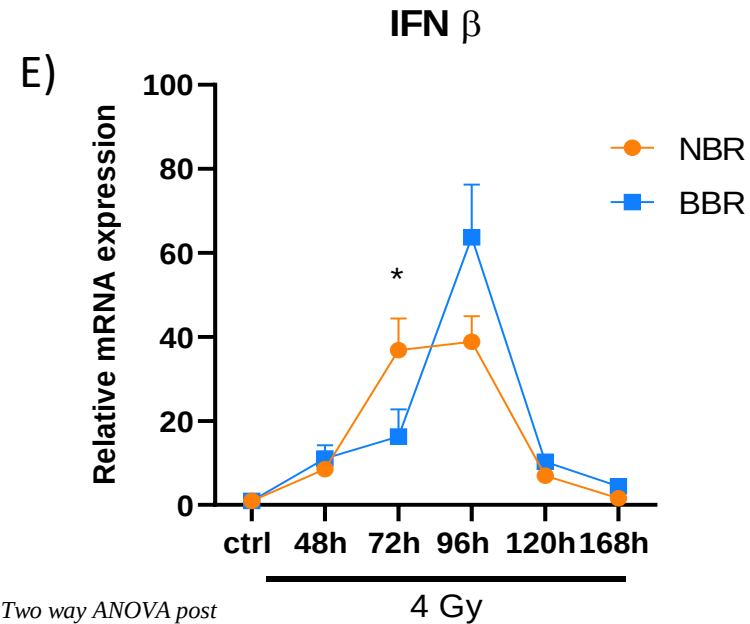
Total protein level of cGAS doesn't change upon after IR in NBR and BBR.

Immortalized human keratinocytes
(HaCaT cell line)
IR: 1Gy of X- rays

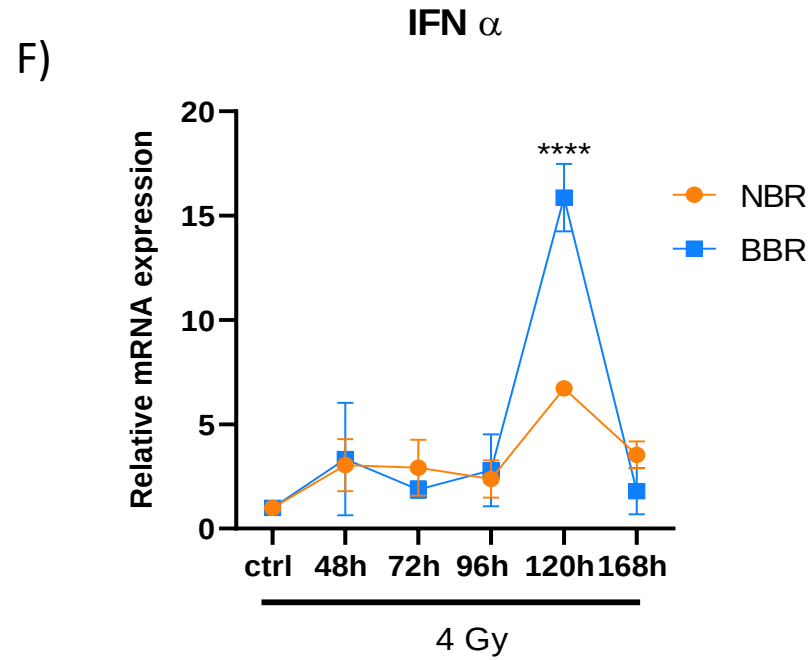


PRELIMINARY RESULTS

IFN- α and IFN- β show different mRNA level after IR in NBR and BBR. ISG15 show different protein level after IR in NBR and BBR.

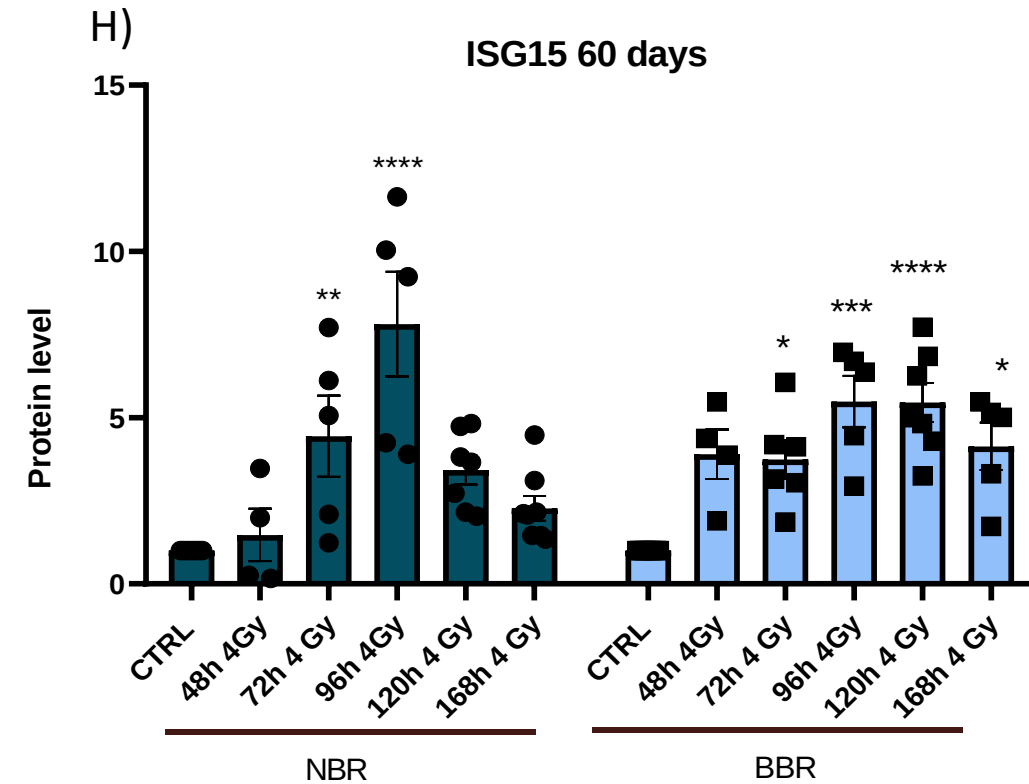
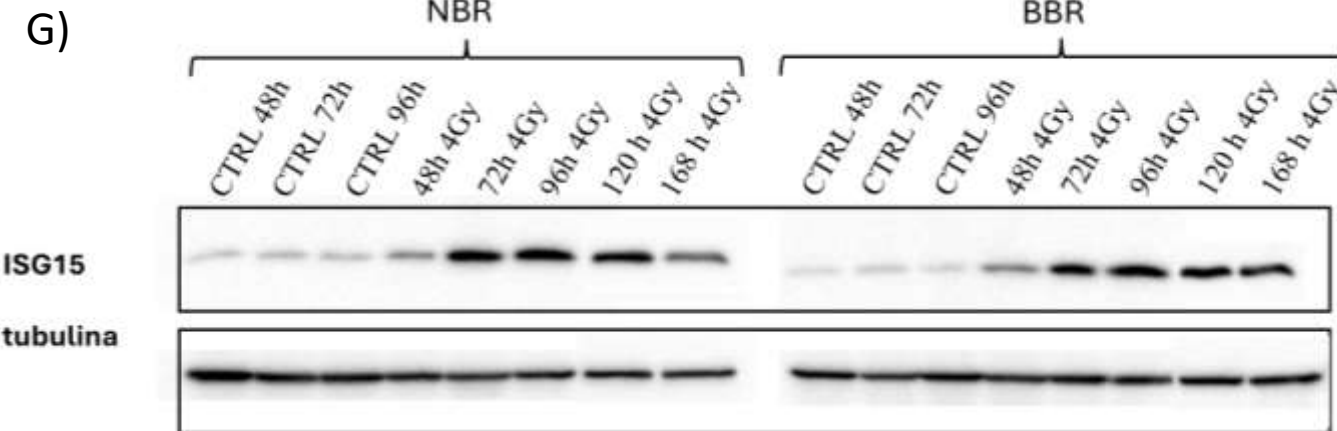


Two way ANOVA post test Tukey (* $p < 0.05$, **** $p < 0.0001$)



Immortalized human keratinocytes
(HaCaT cell line)
IR: 4Gy of X- rays

Two way ANOVA post test Tukey (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).



AIM



The role of oxidative stress as a modulator of DNA damage-induced innate immunity in **NBR** and **BBR**.



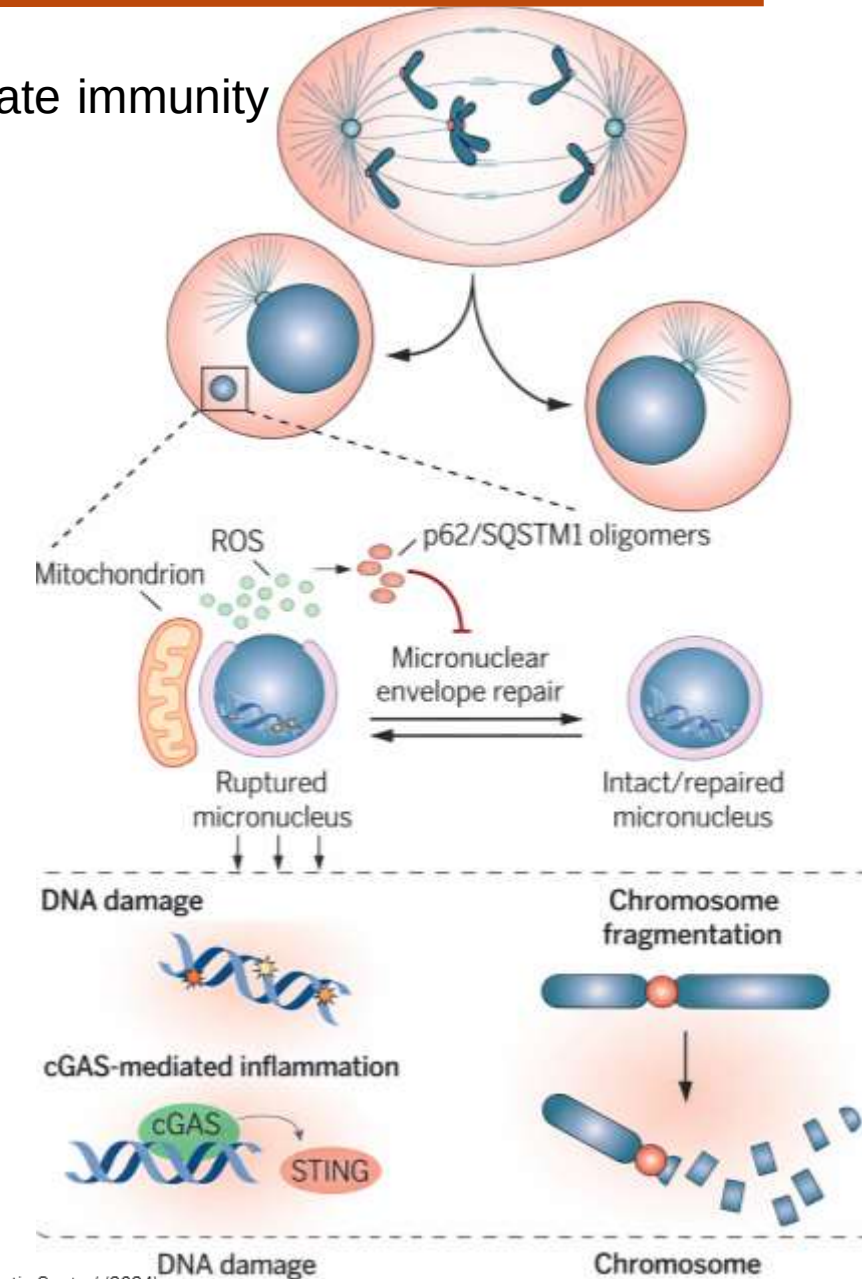
Investigate how oxidative stress influences the recruitment of cGAS following DNA damage

Determine whether ROS levels modulate activation of the cGAS–STING pathway

Analyze the impact of oxidative stress on the immune response

Evaluate post-translational modifications of cGAS

Dissect the mitochondrial metabolic shift (glycolysis and OXPHOS) under different conditions (NBR and BBR) and study the contribution of mitochondria in the activation of the pathway following IR



FIRST-YEAR OBJECTIVES

Treatment : 1 Gy of X-rays at different time-points: 24h, 48h, 72h, 96h in HaCaT cell line kept in **NBR** and **BBR** conditions.

cGAS

Co-immunofluorescence:
cGAS+ MNi and p62+ MNi.

NAC treatment (antioxidant, every 24h) → reduce ROS, test effect on MNi rupture & cGAS recruitment.

ROS quantification: DCFH-DA assay with flow cytometry.

LIBIS system (Istituto Superiore di Sanità): chronic low-dose γ -rays as **preconditioning** and evaluate the innate immune response after IR (1 Gy of X-rays). Also compare the obtained results with past results.

- IFN-I (α and β)
- ISG15



SECOND-YEAR OBJECTIVES

Experimental Conditions

- ⑩ Standard culture
- ⑩ 1 Gy X-rays
- ⑩ 1 Gy X-rays + NAC treatment

This analysis will help determine whether oxidative stress influence different **cGAS** recruitment in response to radiation in BBR conditions .

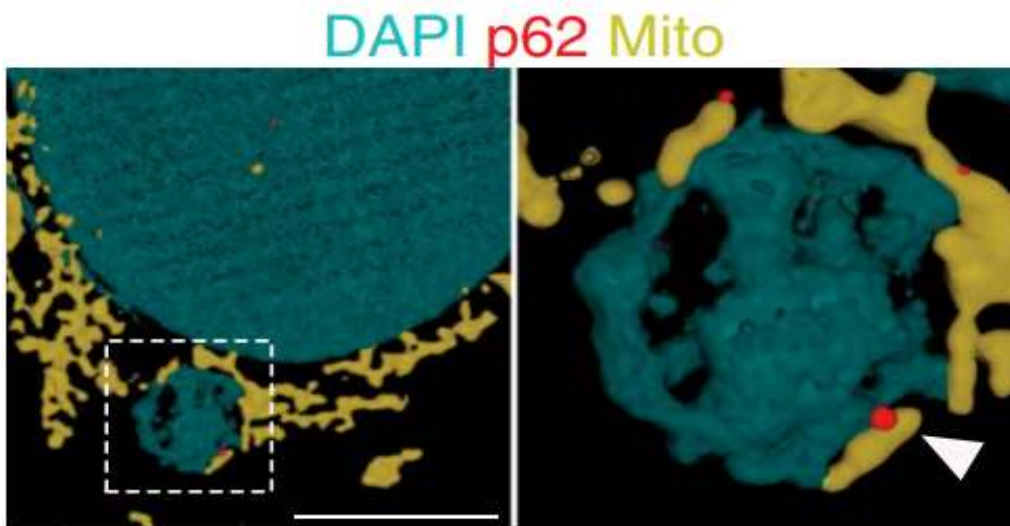


Methods

Seahorse XF Analyzer

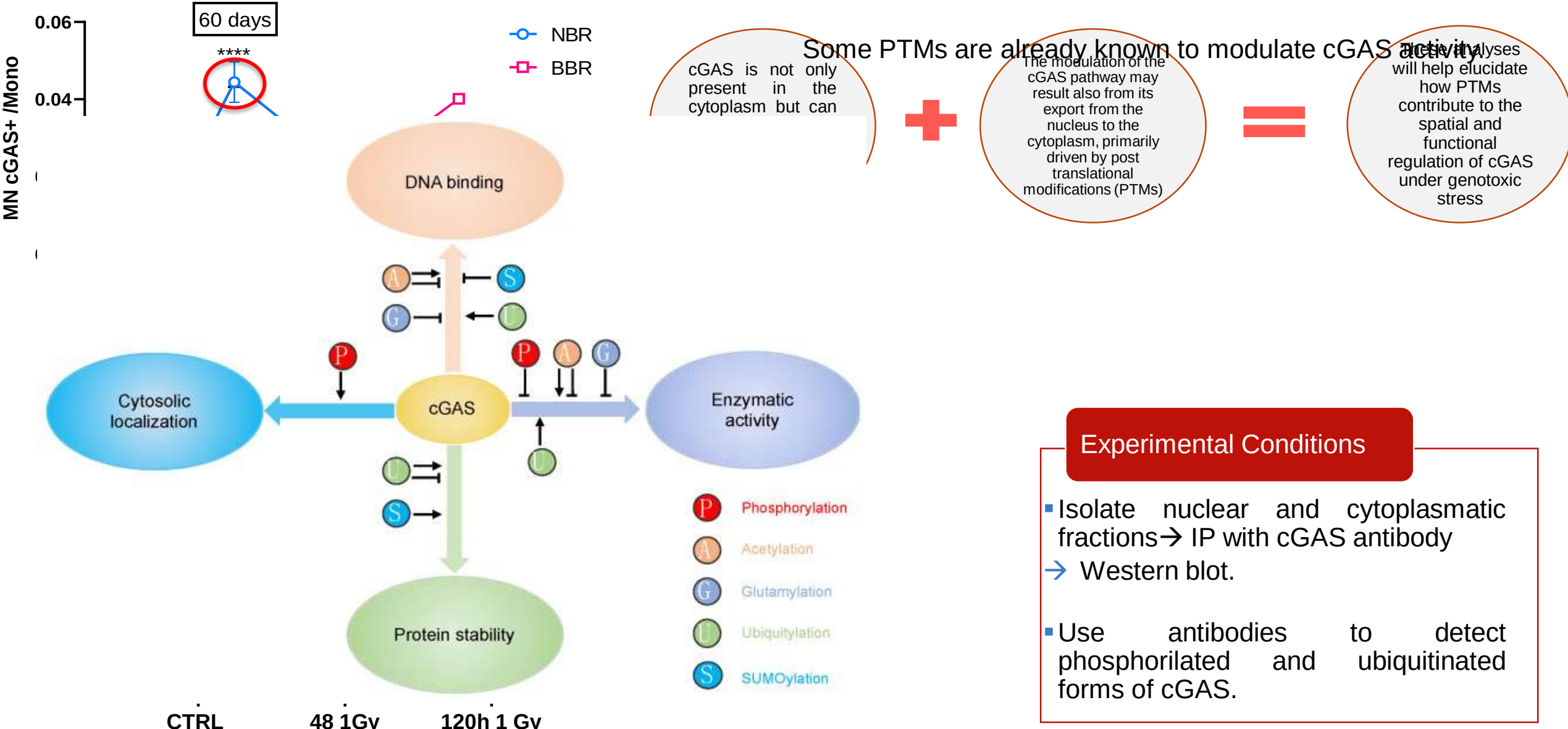
- Oxygen consumption rate (OCR) → oxidative phosphorylation
- Extracellular acidification rate (ECAR) → glycolysis

Electron microscopy → mitochondrial ultrastructure & health



THIRD-YEAR OBJECTIVES

How cGAS is spatially and functionally controlled under genotoxic stress in NBR and BBR?



GOALS AND FUTURE IMPLICATIONS

Understanding how cGAS is modulated.

Characterize cGAS and p62 kinetics



Define the role of p62 in the activation of cGAS after micronuclear rupture.

Clarify PTM regulation of cGAS



Investigate how PTMs fine-tune cGAS activity and localization.

Test effects of pre-conditioning



Determine if chronic exposure to low dose of IR can tune immune responses to DNA damage.

Define mitochondrial contribution to cGAS signaling



Explore how mitochondria act in NBR vs BBR.

Provide insights relevant to radioimmunotherapy and cancer biology.

A microscopic image showing numerous cells, likely lymphocytes, with prominent nuclei and some visible nucleoli, set against a dark background.

Thanks for your attention

ACKNOWLEDGMENTS

Prof.ssa Sgura Antonella
Prof. Berardinelli Francesco
Dott.ssa Barbato Federica
Laboratori Nazionali del Gran Sasso
Laboratorio di Genetica Roma Tre

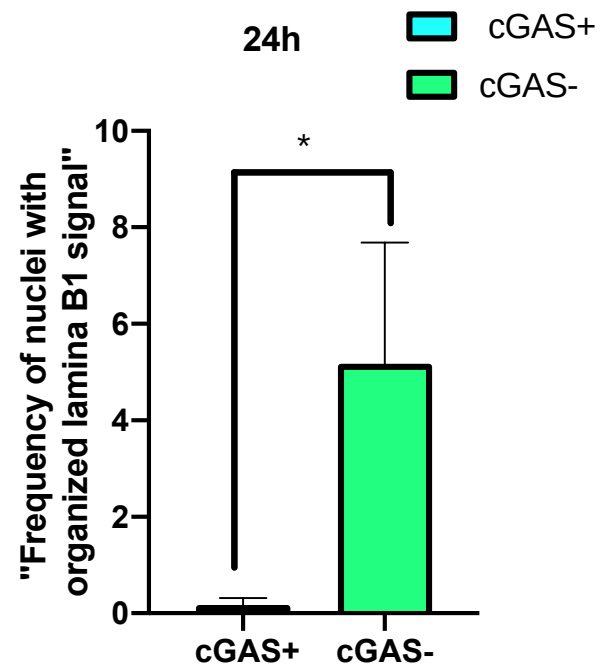
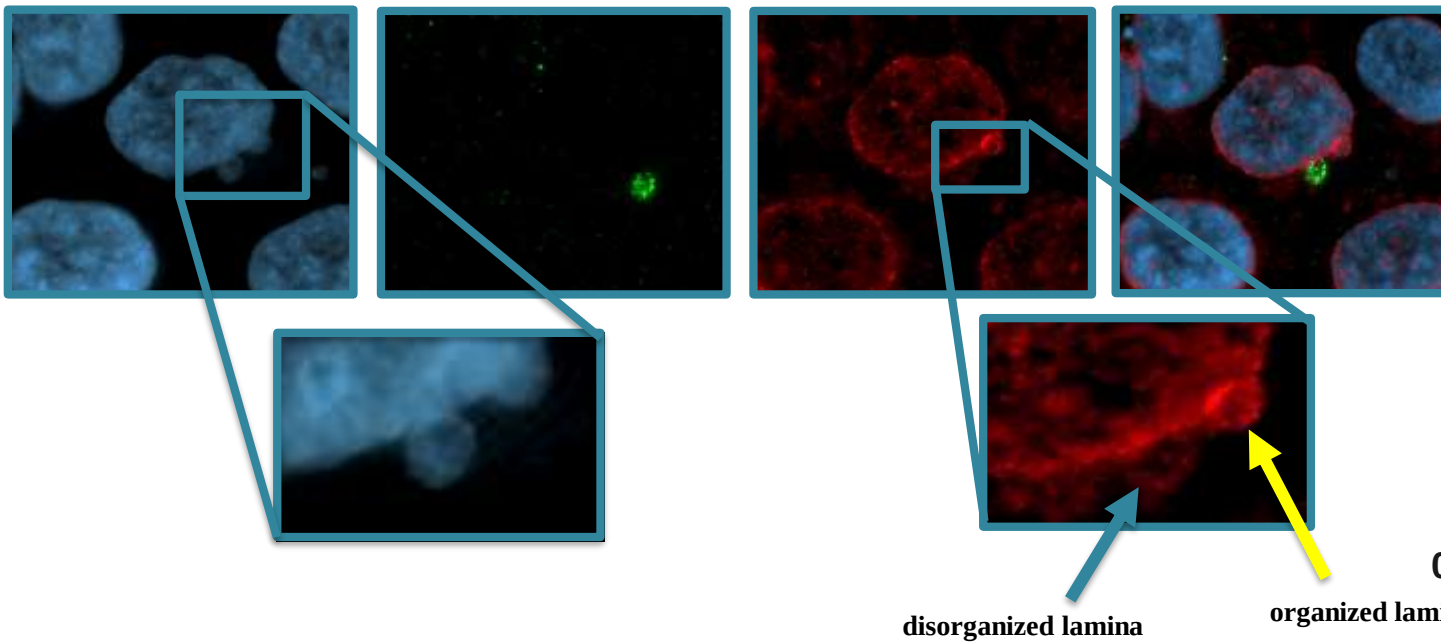


DAPI

cGAS

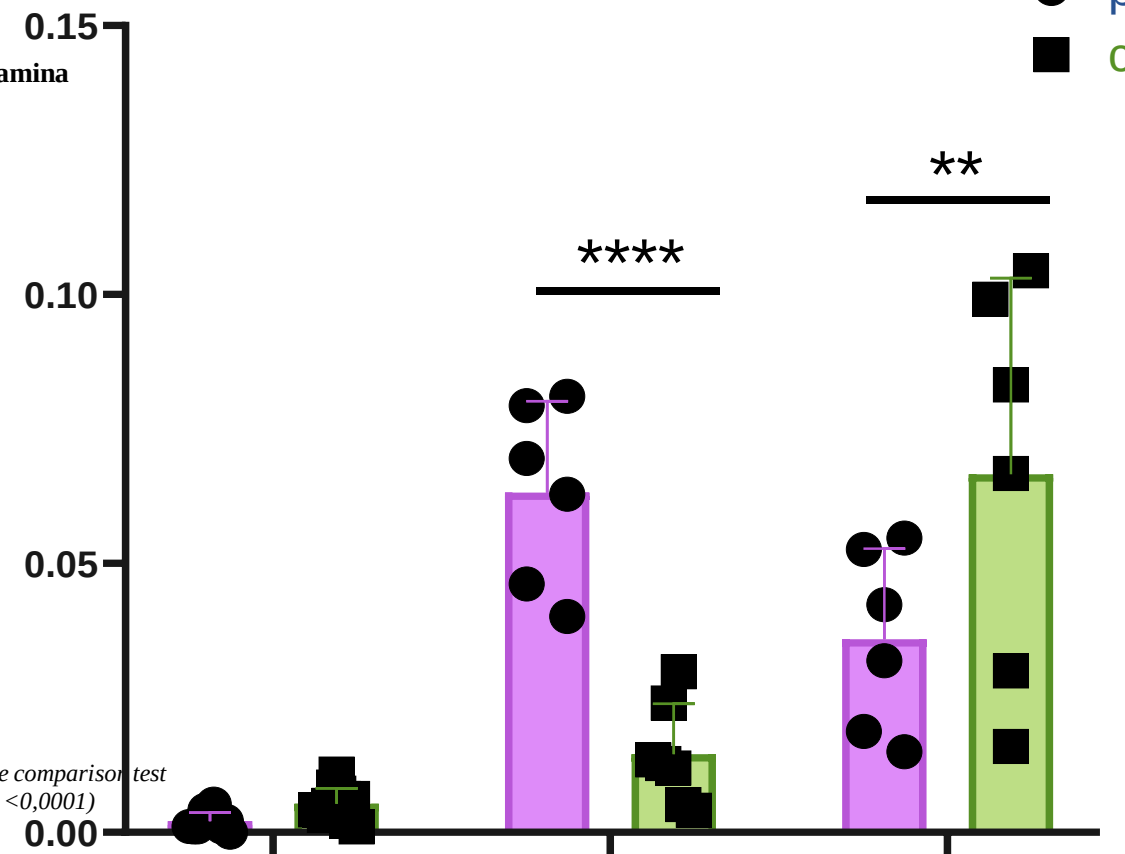
LAMIN B1

MERGE



Two-way ANOVA, multiple comparison test
Sidak (** $p < 0.02$, **** $p < 0.0001$)

MNI+/ mono cells



FIRST-YEAR OBJECTIVES

Treatment : 1 Gy of X-rays at different time-points: 24h, 48h, 72h, 96h in HaCaT cell line.

cGAS

