

PhD PROJECT

Candidate: Daniele Di Chio

Roma, 4-07-24

To the examining committee for the Doctoral competition in MOLECULAR, CELLULAR AND ENVIRONMENTAL BIOLOGY, XL cycle, Roma Tre University

TITLE:

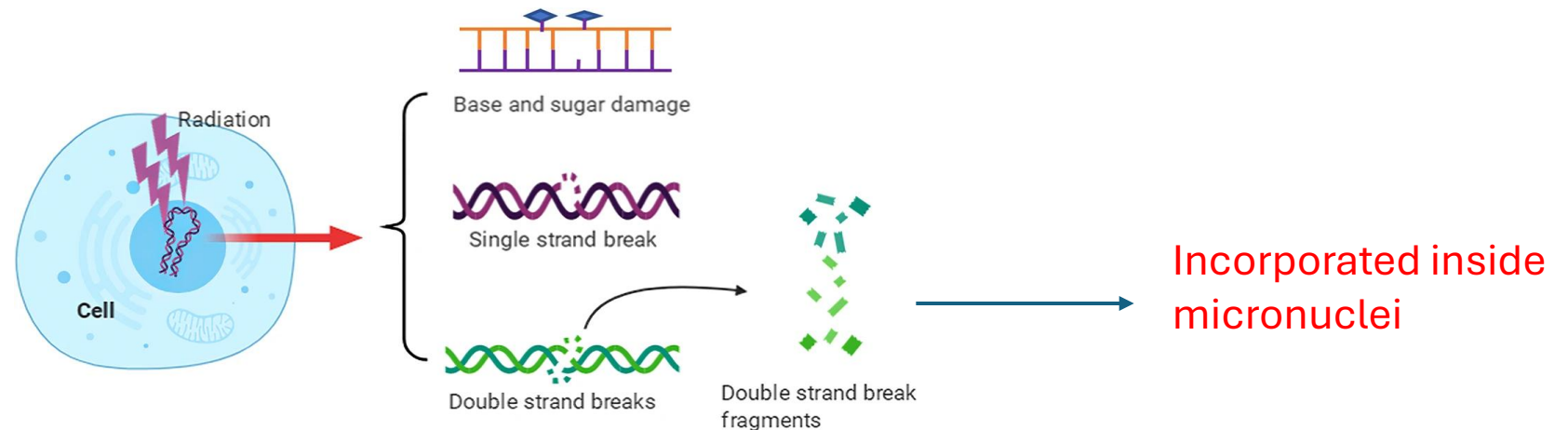
Evaluating cGAS Export Dynamics and Innate Immune Activation under Chronic Low-Dose Radiation Exposure"

EFFECTS OF IONIZING RADIATION ON DNA

“Ionizing radiation is a type of high-energy radiation that is able to release electrons from atoms and molecules generating ions which can break covalent bonds.”

“Ionizing radiation directly affects DNA structure by inducing DNA breaks, generating abasic sites and single strand breaks (SSB)”

Borrego-Soto G, Ortiz-López R, Rojas-Martínez A. Ionizing radiation-induced DNA injury and damage detection in patients with breast cancer. *Genet Mol Biol.* 2015 Dec;38(4):420-32. doi: 10.1590/S1415-475738420150019. Epub 2015 Nov 24. PMID: 26692152; PMCID: PMC4763322.

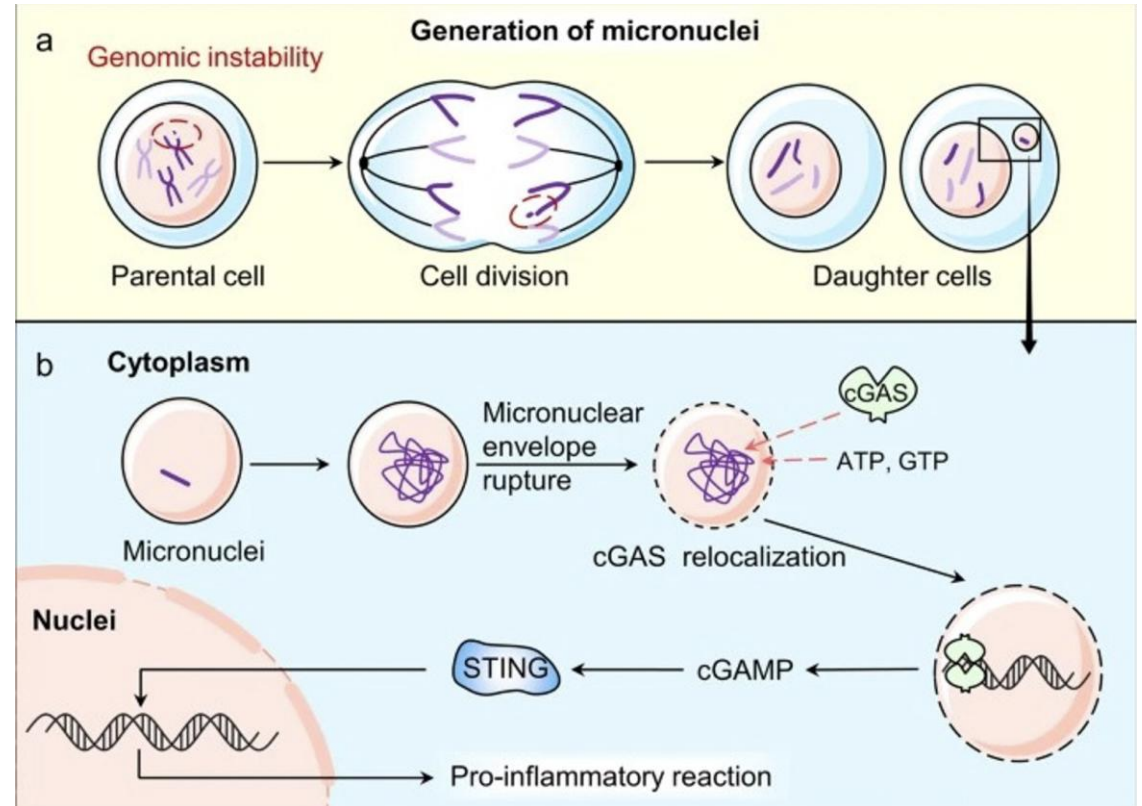


MICRONUCLEI

Causes:

- lagging chromosomes or acentric chromosome fragments that do not incorporate into daughter nuclei.
- Acentric chromosome fragments are produced by unrepaired DNA double strand breaks

NUCLEAR ENVELOPE rupture is always irreversible and can lead to exposure of MN DNA to the cytoplasm



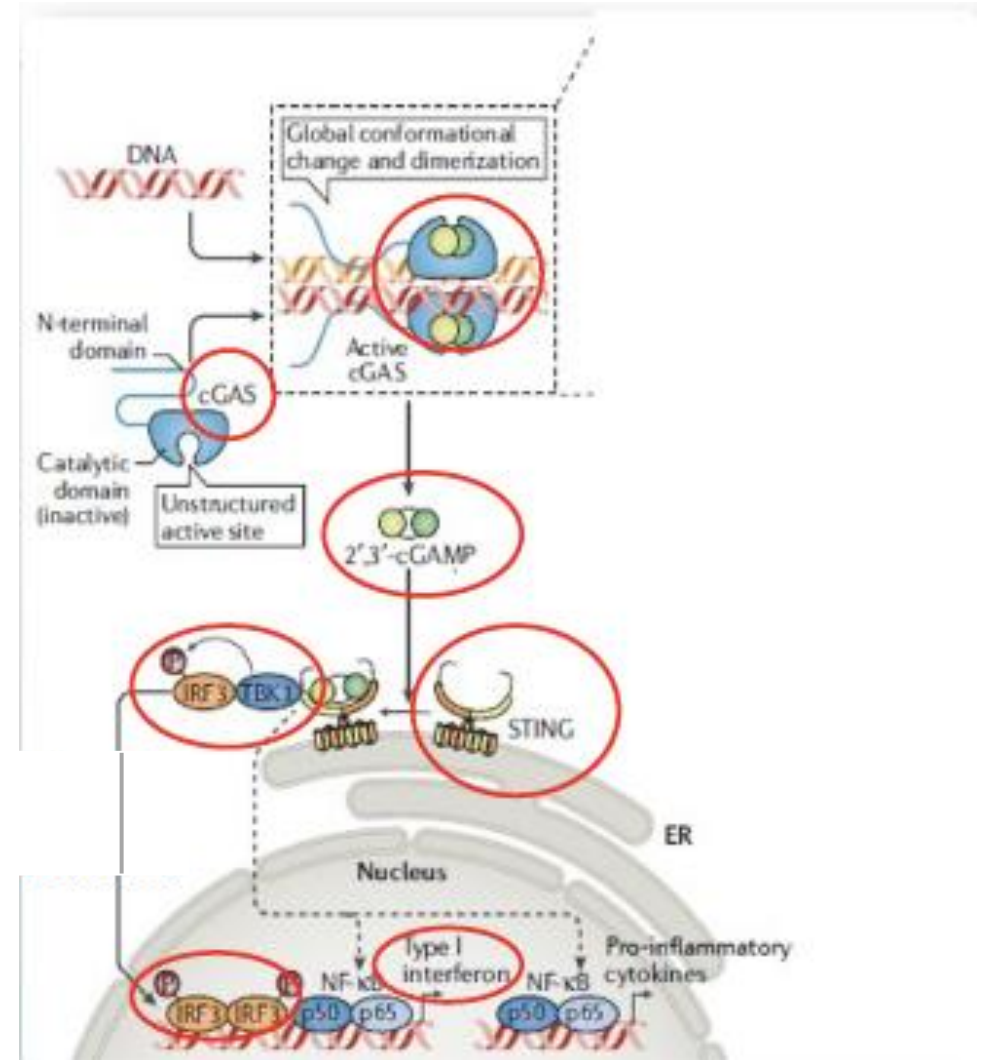
cGAS-STING pathway in literature

cGAS is able to recognize DNA fragments derived from:

- bacteria and viruses (Decout A et al. 2021)
- DNA damage, induced for example by radiation.

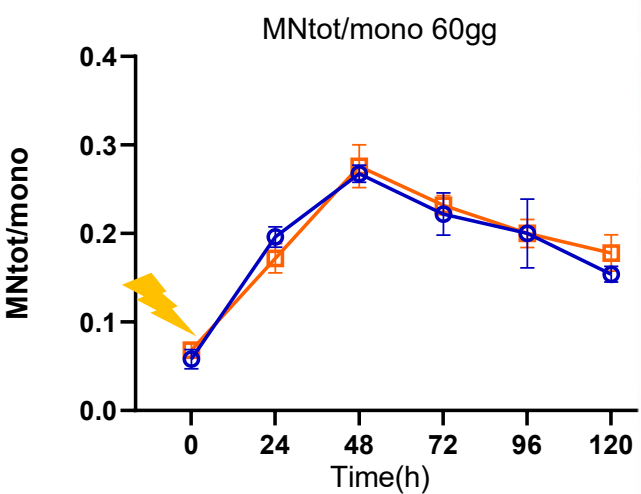
A major target of cGAS are the MNs because of the presence of large amount of DNA inside it.

When it is fragmented in smaller molecules, the interaction between cGAS and the DNA is allowed.

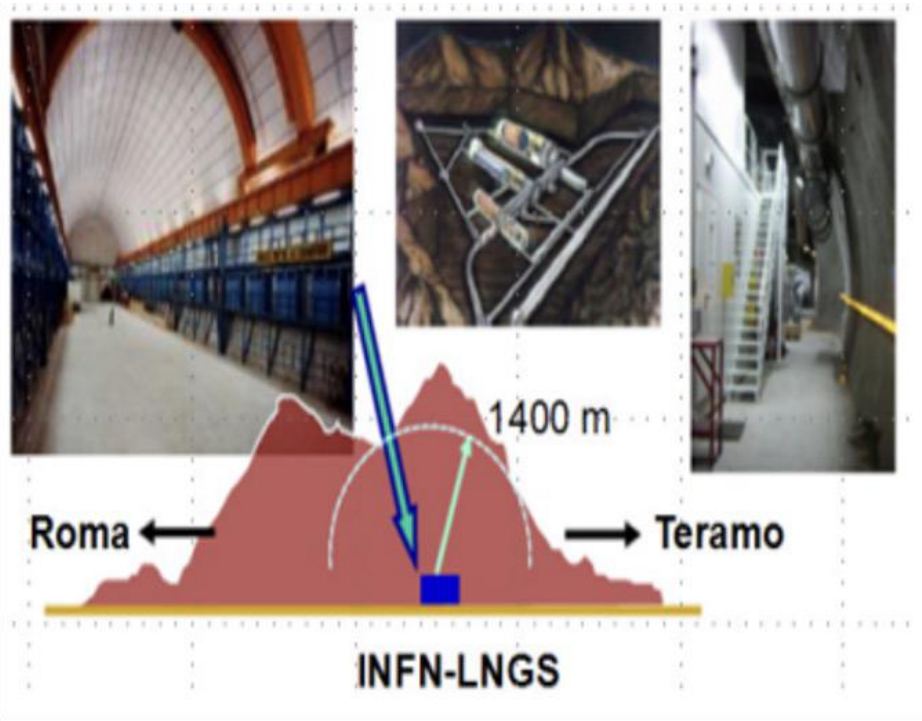


Data gathered during my master's degree thesis

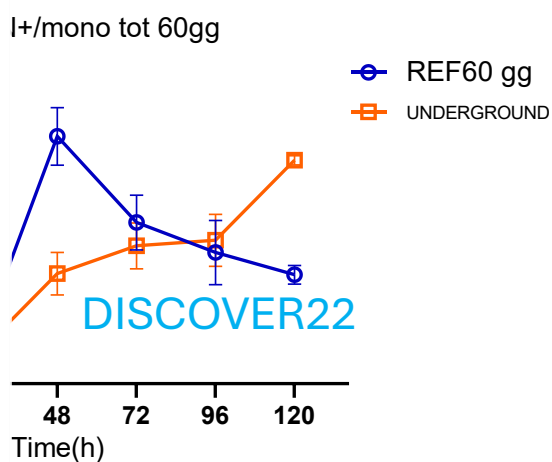
Micronuclei fraction after 1Gy



LNGS laboratory

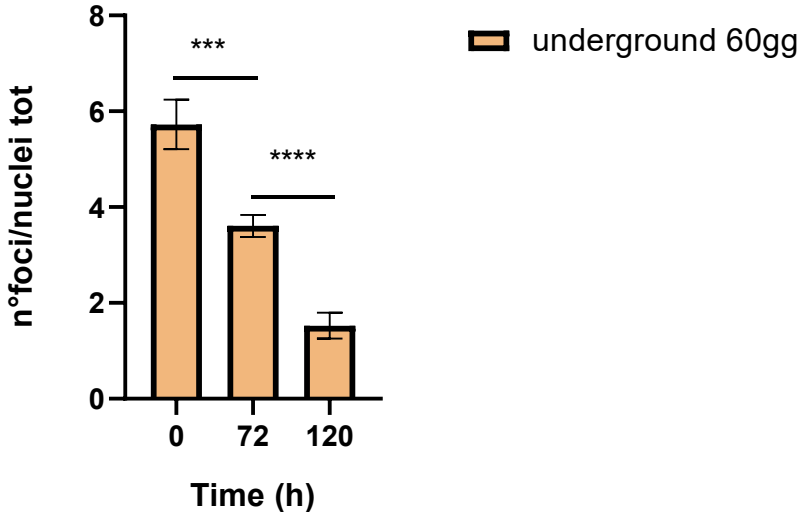
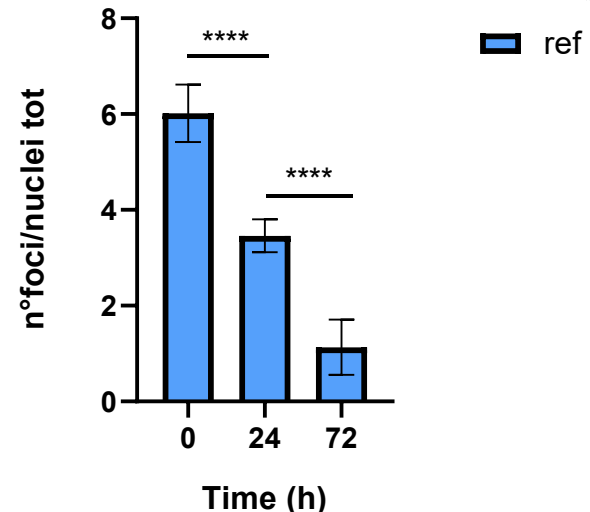


cGAS+ micronuclei fraction

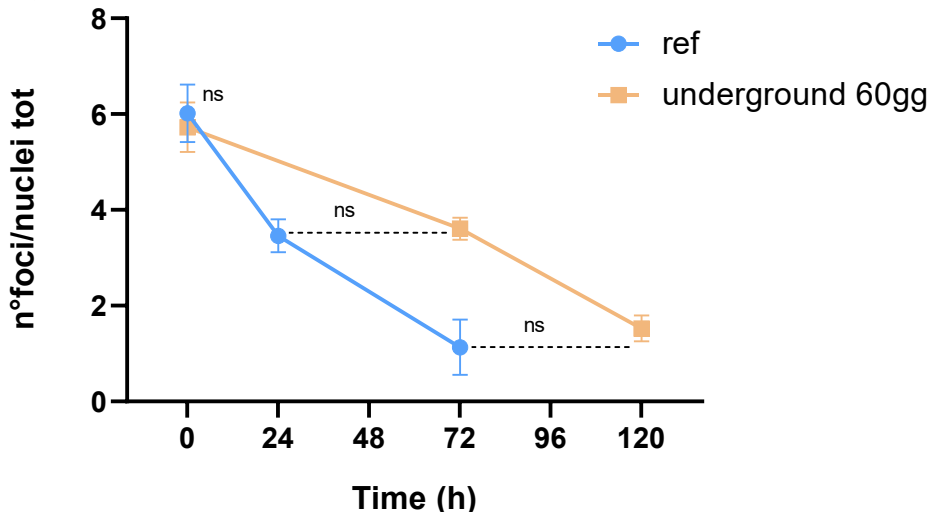


Analysis of cGAS presence after irradiation

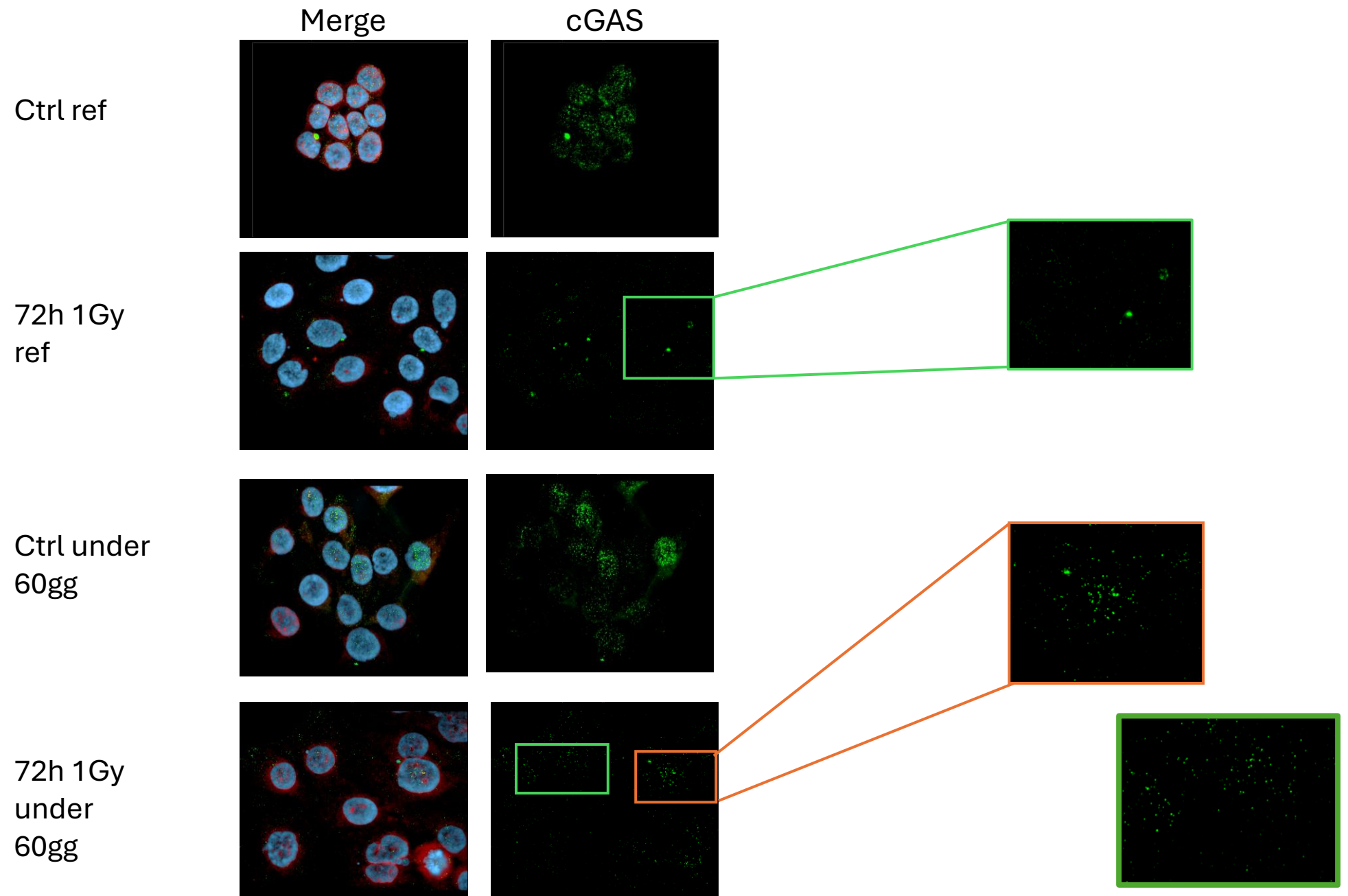
ref



ref vs under 2.0



HaCat (60gg)



PhD schedule

In order to better understand the interaction between cGAS export from the nucleus and the recognition of cytosolic DNA:

AIMS

1) Create a cell line that has an increased basal level of DNA damage due to low dose radiation, acclimatizing them to a different radiation background

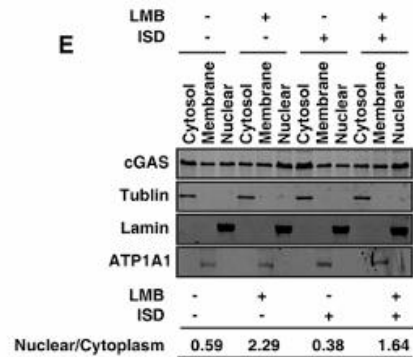
2) The first simple but fundamental investigation is on the transcription and translation of cGAS at different time points after 1Gy of X-rays (challenging dose) in both the cell lines.

EXPERIMENTS AND METHODS

- • Irradiate the cells with a sub-chronic very low dose in a large amount of time
- • This analysis will be made using RT-qPCR and Western blot.

AIMS

3) After treatment with a challenging dose , will be done experiments to measure cytosolic DNA and its interaction with cGAS



Civril, F., Deimling, T., de Oliveira Mann, C. C., Ablasser, A., Moldt, M., Witte, G., & Hopfner, K. P. (2013). A Nuclear Export Signal Is Required for cGAS to Sense Cytosolic DNA. *Nature*, 505(7485), 394–398. <https://doi.org/10.1038/nature12889>

4) To demonstrate different export kinetics from nucleus to cytosol in normal environmental radiation background cells and sub-chronic low-dose irradiated cells after challenging dose of X-rays

EXPERIMENTS AND METHODS

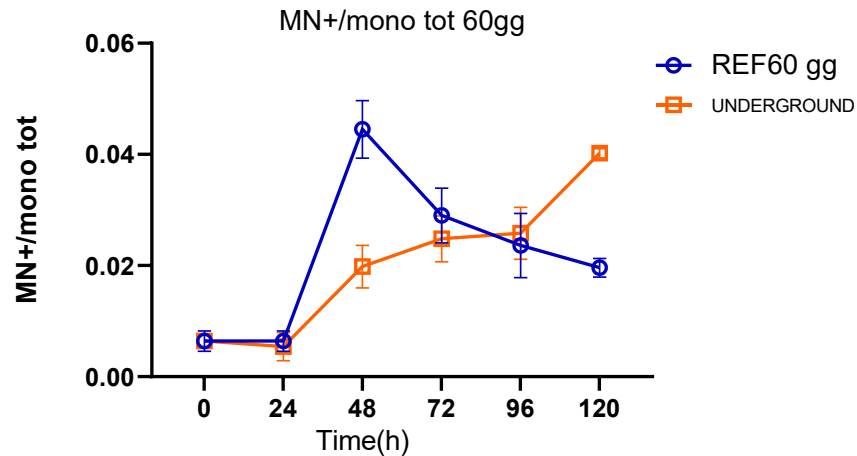
- Extraction and quantification of cytosolic DNA to demonstrate the induction in the sub-chronic line
- Immunoprecipitation will be performed to verify the formation of cGAS and cytosolic DNA complexes.
- WB of cGAS protein following cytosolic and nuclear extraction, to measure the different levels of protein in both cell lines, at different time post-irradiation

- Immunofluorescence of cGAS in the nucleus at different time points after challenging dose

HYPOTESIS?...

AIMS

It would be interesting to define a threshold value of free cytosolic DNA that activates the pathway.



EXPERIMENTS AND METHODS

- Quantification of cytosolic DNA at peak of cGAS activation in both cell lines using a fluorescence probe (picogreen)

Khatoor, M., Yang, J., Gee, K. R., & Gasser, S. (2019). Evaluation of PicoGreen variants for use in microscopy and flow cytometry. *bioRxiv*. <https://doi.org/10.1101/584037>

To verify the correlation between cGAS-STING activation and innate immune system response

- Studies on the production of IFN-1 and ISG15 paired to the previous experiment, to verify the activation of the immune response.

SO FAR SO GOOD

During my first months of PhD:

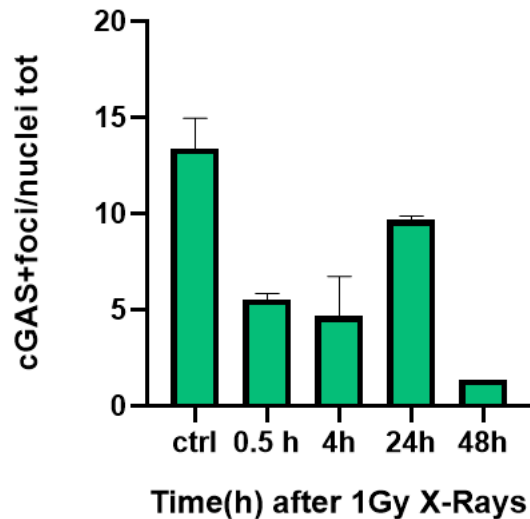
Last year of DISCOVER22

Roma Tre

Istituto Superiore di Sanità

More experiments about the cGAS export dynamics and correlation with DNA damage

protocol development:
Immunofluorescence of cGAS and γ H2AX



Characterize the effect of the radiation background on cell differentiation

Study of the CDs of HL60, which can be differentiated in
MACROPHAGES or
NEUTROPHILES

Protocol development to understand if these cells have the same efficiency upon activation

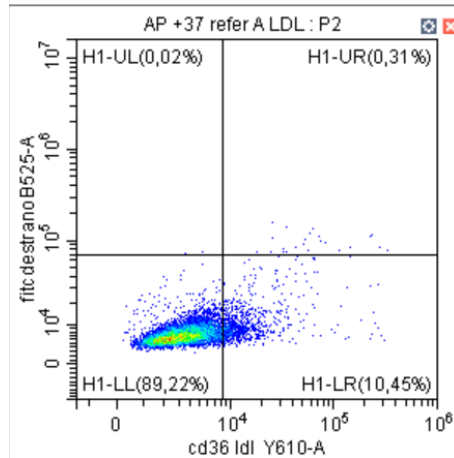
EFFICIENCY UPON DIFFERENTIATION

MACROPHAGES(M ϕ)

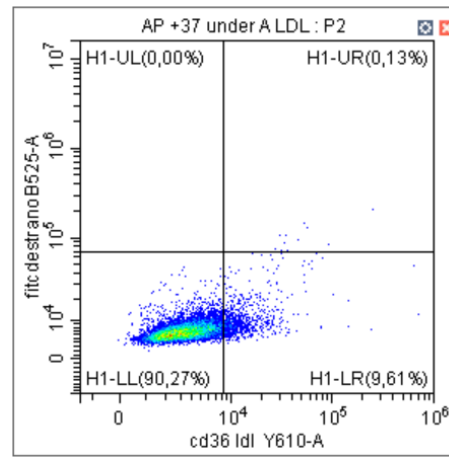
NEUTROPHILS

HL60

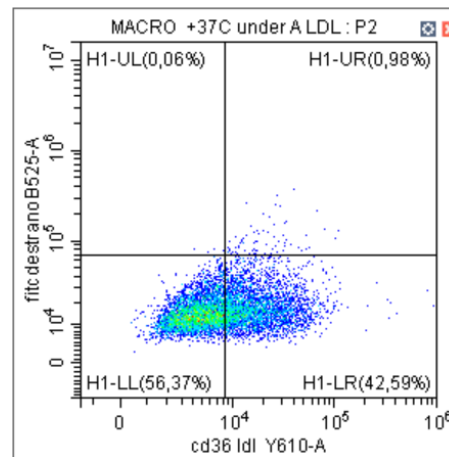
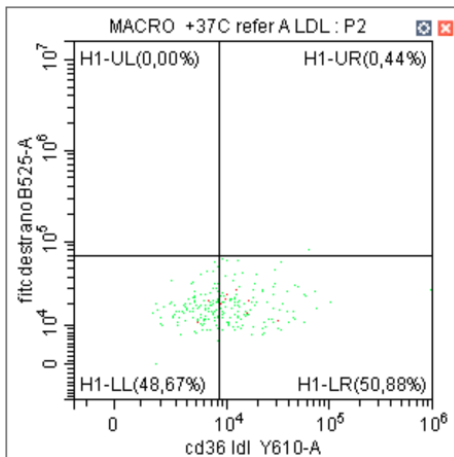
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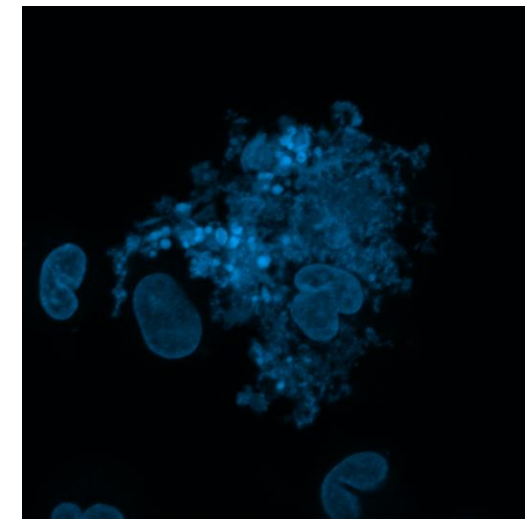
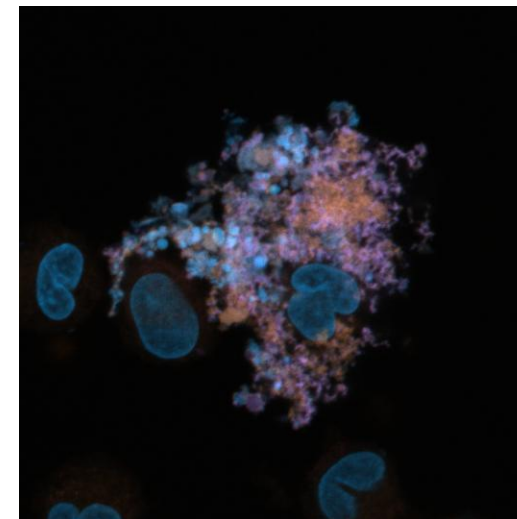
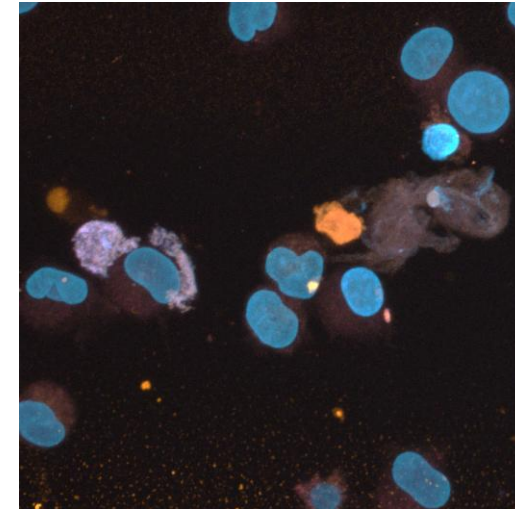
UNDERGROUND



M ϕ



Moreover we are in contact with Max Planck institute and Dr Zychlinsky to establish a collaboration and use their 3D9 antibody, which is specific for NETosis, allowing us to also use cytofluorimetry



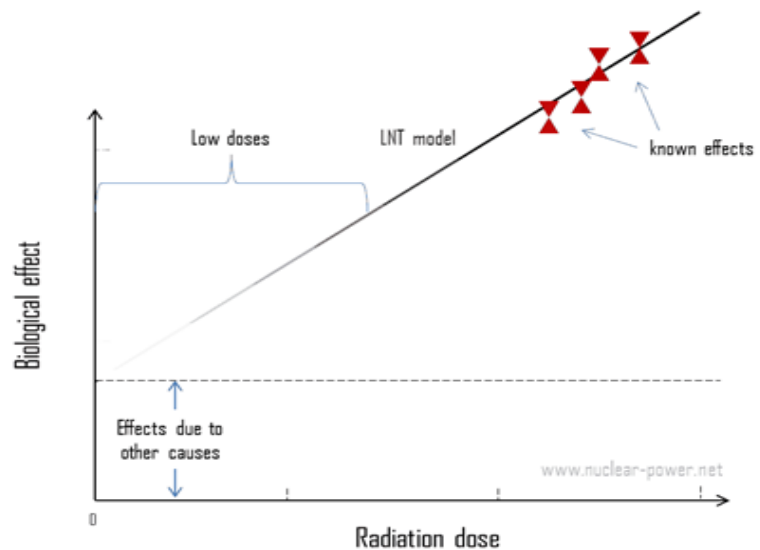
HORMESIS

“In contrast to high-dose radiation (HDR), **LDR is able to promote growth and development ,suppress the aging process, enhance immune functions, and delay cancer progression.** This interesting phenomenon of the beneficial effects of LDR is often called ‘radiation hormesis’”

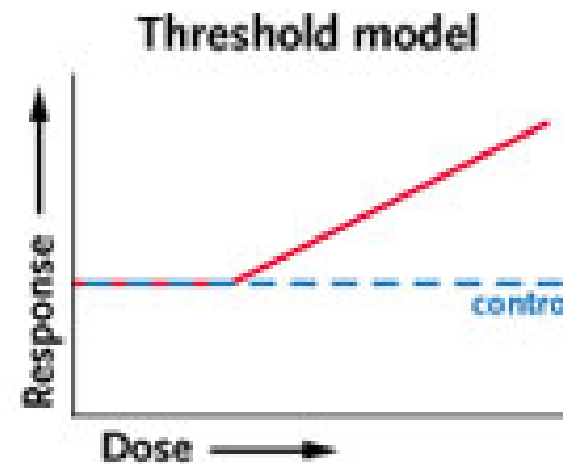
Several recent investigations found that LDR was more effective in cancer treatment than conventional radiotherapy, as LDR can stimulate the immune system, whereas high radiation doses usually suppress it.

For example, in a well-recognized model of thymic lymphoma induced by HDR, an LDR treatment (0.05 to 0.2 Gy) 6–24 h preceding each fractionated HDR could significantly reduce the lymphoma incidence

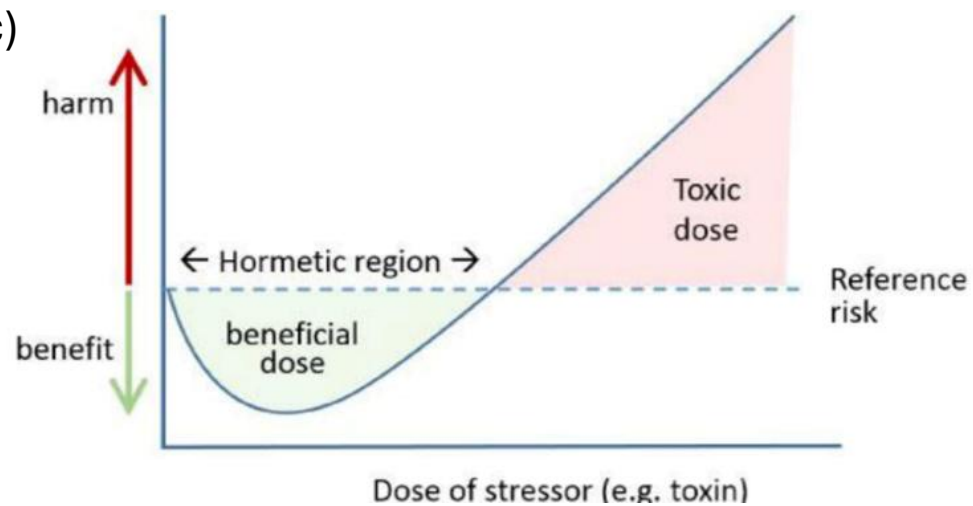
a)



b)



c)



d)

