Studies of the radiosensitization effect of gold NPs for hadrontherapy

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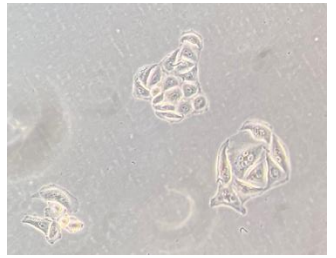
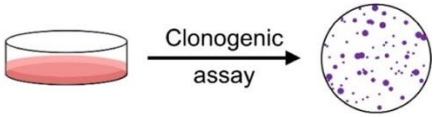
Radiobiology experiments: summary

❑ **Radiobiological experiments at the CNA cyclotron external beam line**

- ✓ Three measurement campaigns in October 2022 and 2023 and September 2024
- ✓ Hela cells and gold NPs of 50 and 20 nm (only in 2023) ⌀



- ✓ Effect of the radiation has been primary studied by **clonogenic assays**
  - The toxicity is evaluated by studying the ability of a single cell to form a colony
  - Samples are incubated for ~11 days and then the colonies are stained and counted

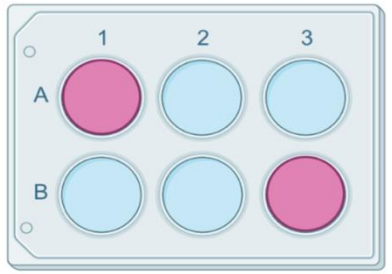


Year	Part .	E [MeV]	I_{beam} [pA]	Dose rate [Gy/min]	N	Treat. [h]	NP ⌀ size [nm]	Doses [Gy]
2022	p, γ	13.2	110-140	~4	50	~4	50	2,4,6,8
2023	p	13.2	110-140	~4	140	~4	50, 20	1,2,3,4,5,6
2024	p, γ	13.2	140-200	~4	43	4-6h	50	1,2,3,4,5,6

Radiobiology experiments: procedure

CABIMER

16-24h before irradiation:
cell monolayer seeding

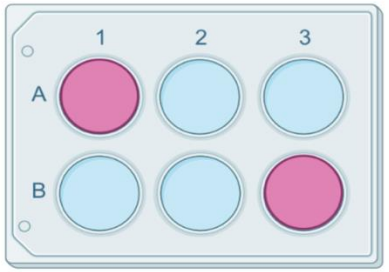


16-24h

Au NPs treatment

4h

AuNPs



Cleaning and
medium change +
adding a mylar film

CNA-cyclotron-~13 MeV p

Moving to
CNA
~5 min walk

Moving back
to CABIMER
~5 min walk

45min-1h out of the incubator

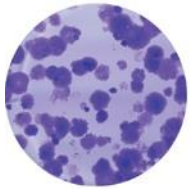
IFIC/UV

Data analysis

CABIMER

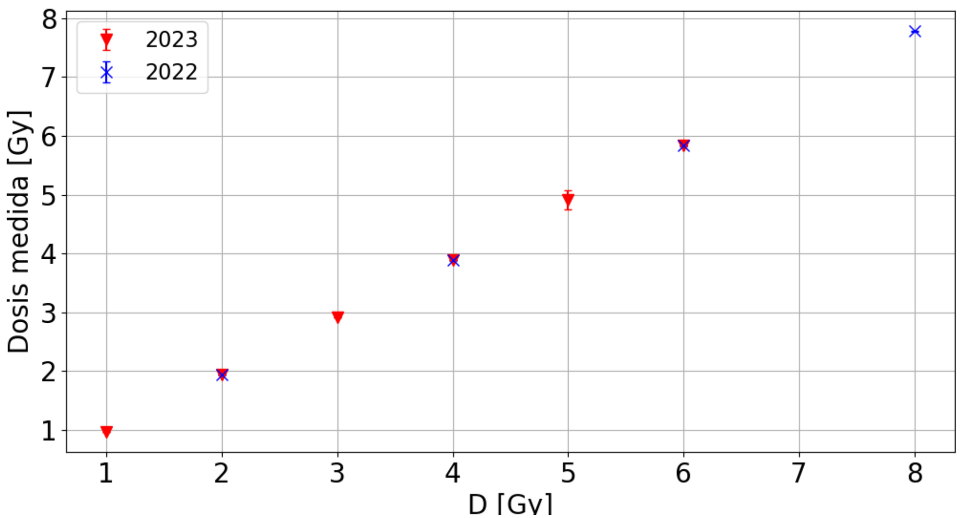
Remove mylar film and
change the medium

For clonogenic assays
place into the incubator
for 11-13 days

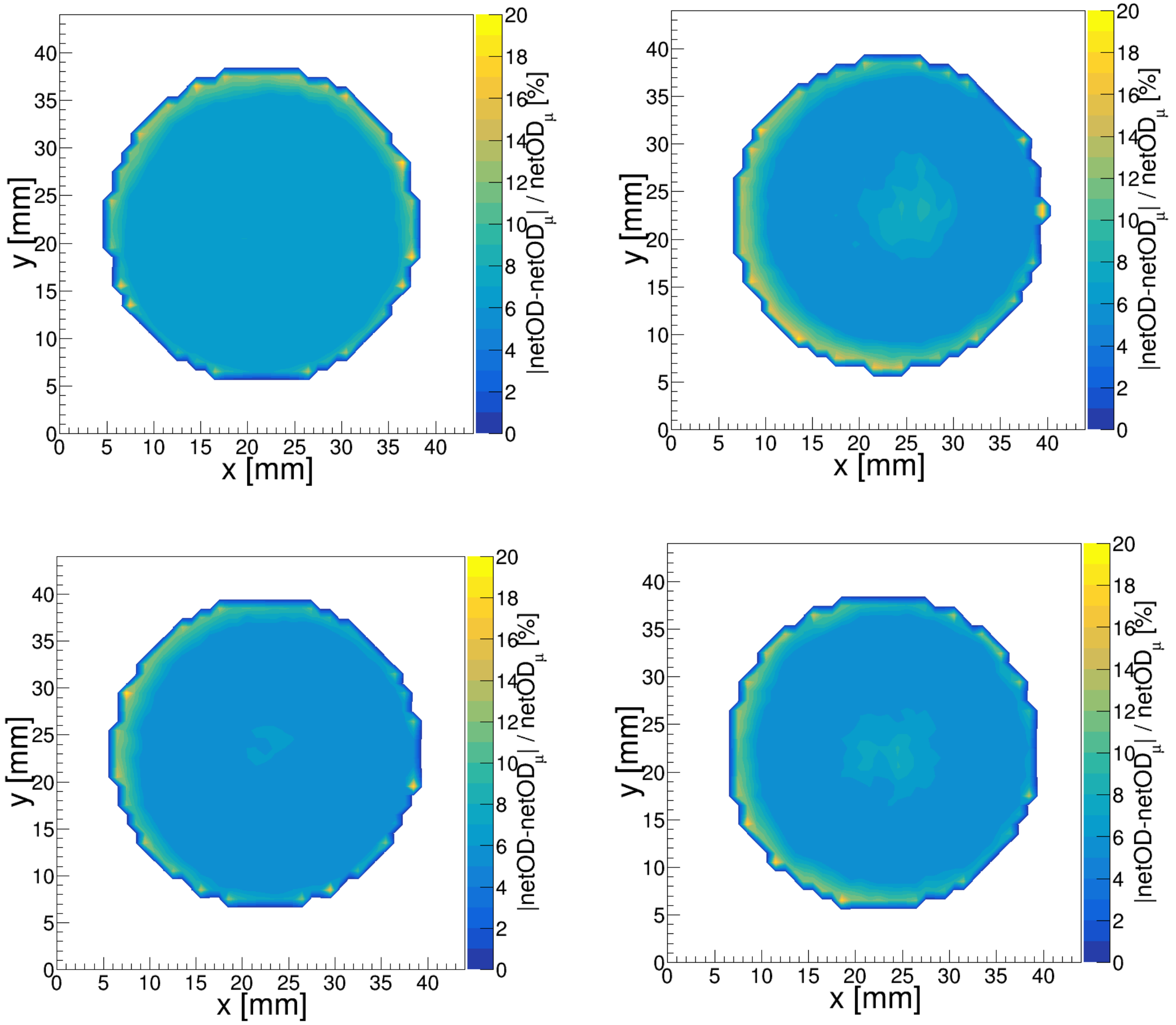


Radiobiology experiments: CNA beamline

Ionization chamber

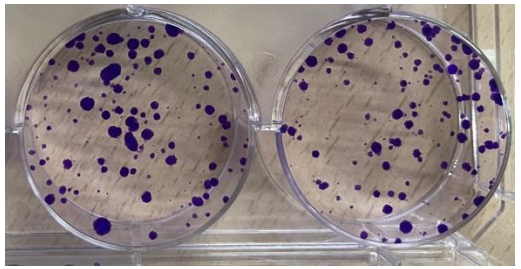


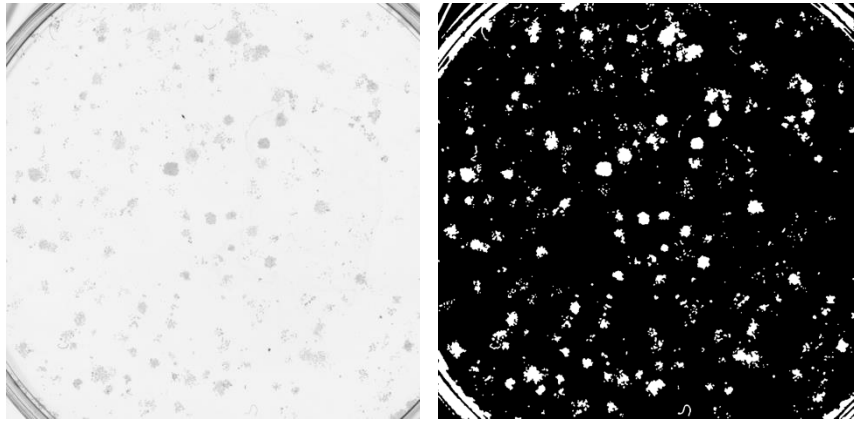
- Medidas de homogeneidad > Desviación media~4-5%



Radiobiology experiments: analysis

- The plates are scanned, and the colonies counted using **ImageJ**

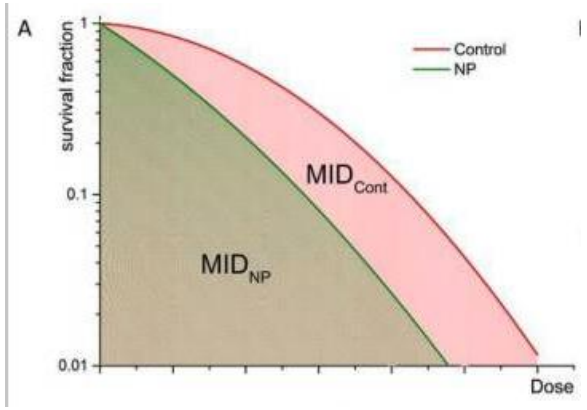




- To quantify the radiation effect, the **survival curves** are computed:

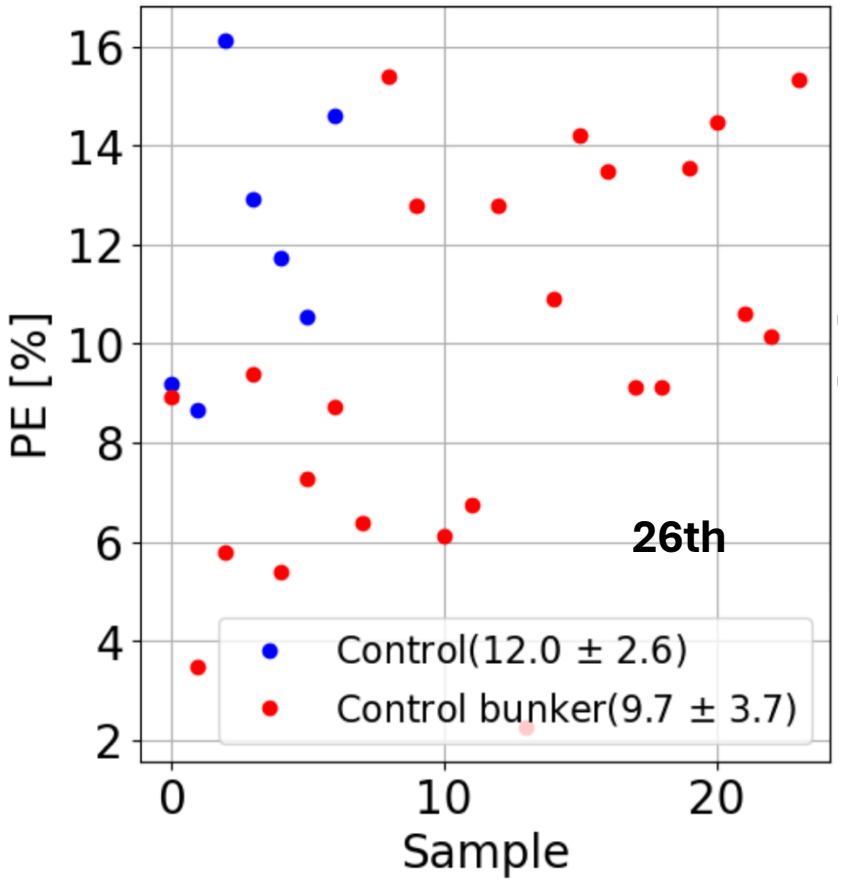
$$SF = \frac{colonies\ after\ irradiation\ at\ X\ Gy}{Seeded\ cells \times PE} \times 100 \qquad PE = \frac{colonies\ without\ irradiation}{Seeded\ cells} \times 100$$

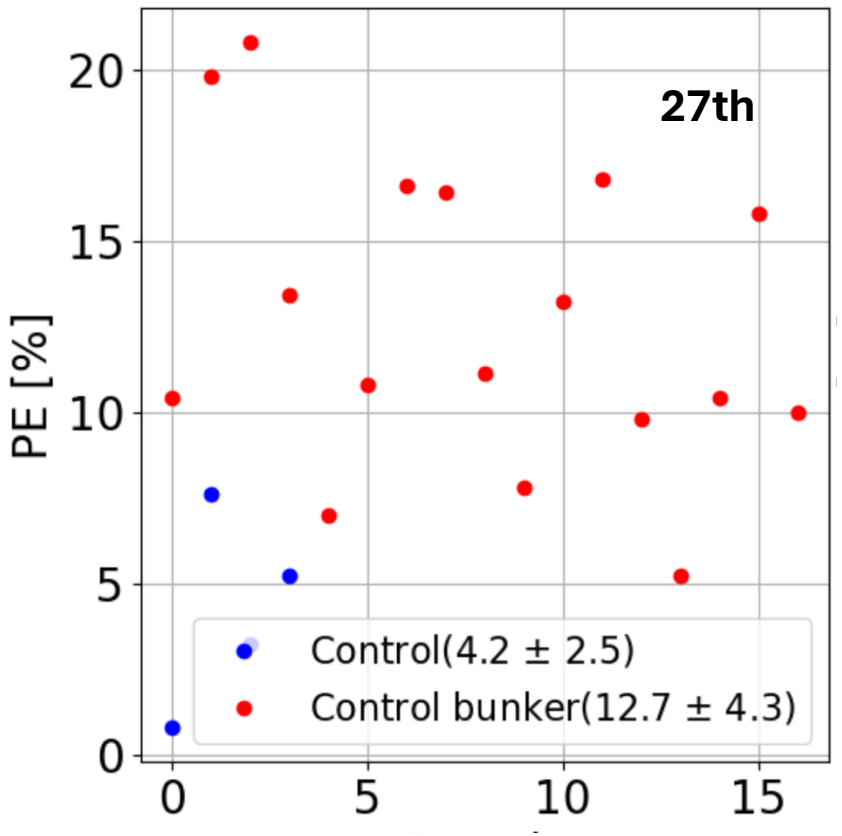
- Fit to LQ model $SF = \exp(-\alpha D - \beta D^2)$
- SER (Sensitization Enhancement Ratio) calculation $SER_{NP} = \frac{MID_{Cont}}{MID_{NP}}$
- ANOVA statistical test



ICRU report: Quantitative Concepts and Dosimetry in radiobiology. Measurements International Commission on RadiationUnits. Washington DC (1979). Establece que si las curvas no son proporcionales para hacer la comparación con y sin NPs hay que tener en cuenta la curva entera.

Clonogenic assays 2022



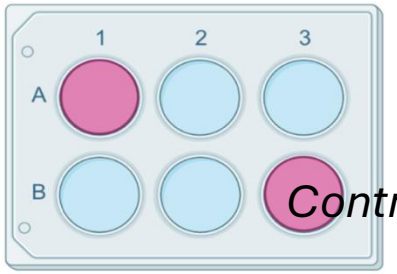


Dose (Gy)	Ncell 2022 26th/27th
0	250
1	-
2	250/750
3	-
4	500/1500
5	-
6	1000/3000
8	1000/3000

- Large PE dispersion observed
- Comparison between controls staying at CABIMER, controls travelling and controls inside the bunker during irradiation: preliminary conclusions no significant difference

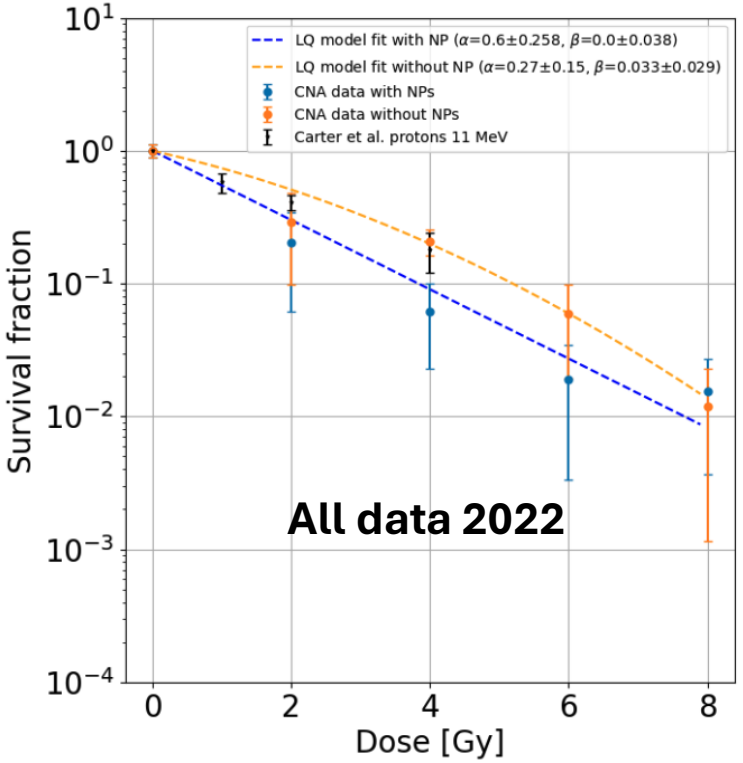
$$PE = \frac{colonies\ without\ irradiation}{Seeded\ cells} \times 100$$

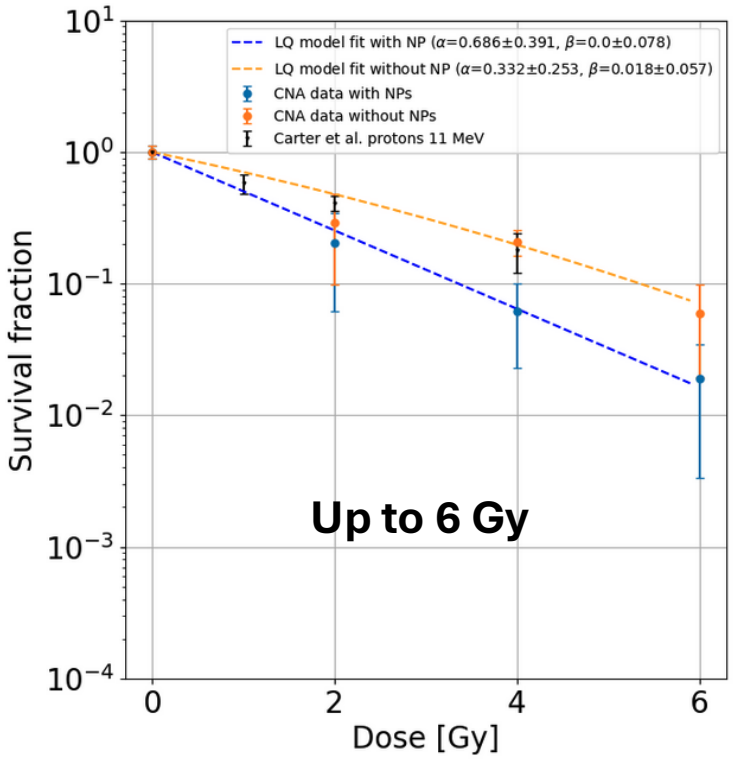
Irradiated

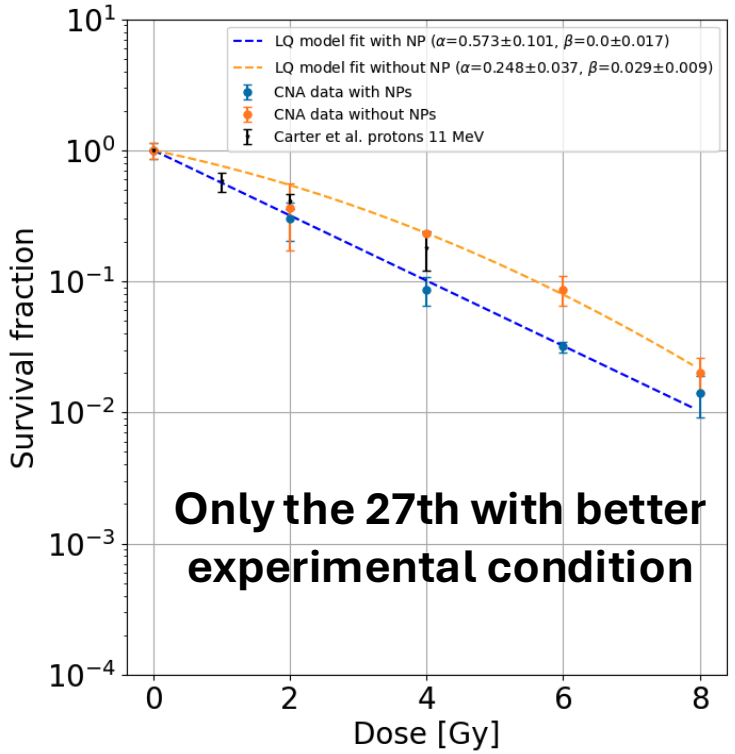


Control

Clonogenic assays 2022







	Treatment	α	β	R^2	SER
2,4,6,8 Gy	+	0.69 ± 0.39	0.00 ± 0.08	0.905	1.63
	-	0.33 ± 0.25	0.018 ± 0.057	0.990	
2,4,6 Gy	+	0.60 ± 0.25	0.00 ± 0.038	0.936	1.49
	-	0.27 ± 0.15	0.033 ± 0.029	0.997	
Only 27th	+	0.57 ± 0.10	0.00 ± 0.017	0.937	1.52
	-	0.25 ± 0.04	0.029 ± 0.009	0.999	

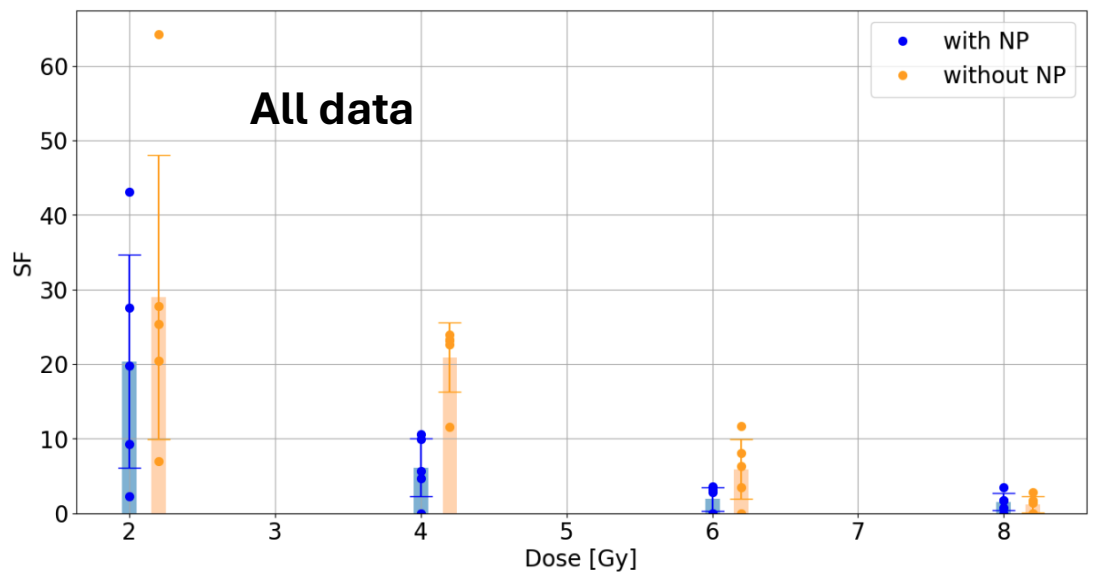
Clonogenic assays 2022

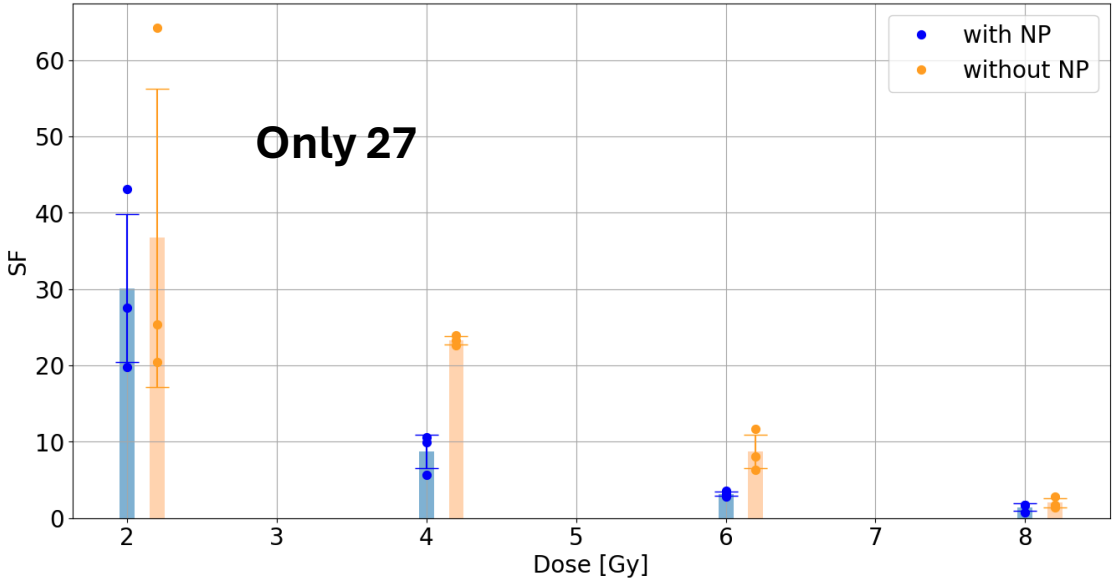
The one-way ANOVA tests the hypothesis that two or more groups have the same population mean. The test is applied to samples from two or more groups, possibly with differing sizes.

Dose (Gy)	p_value All data	p_value Only 27
2	0,49	0,69
4	0,0012	0,0008
6	0,097	0,024
8	0,68	0,34

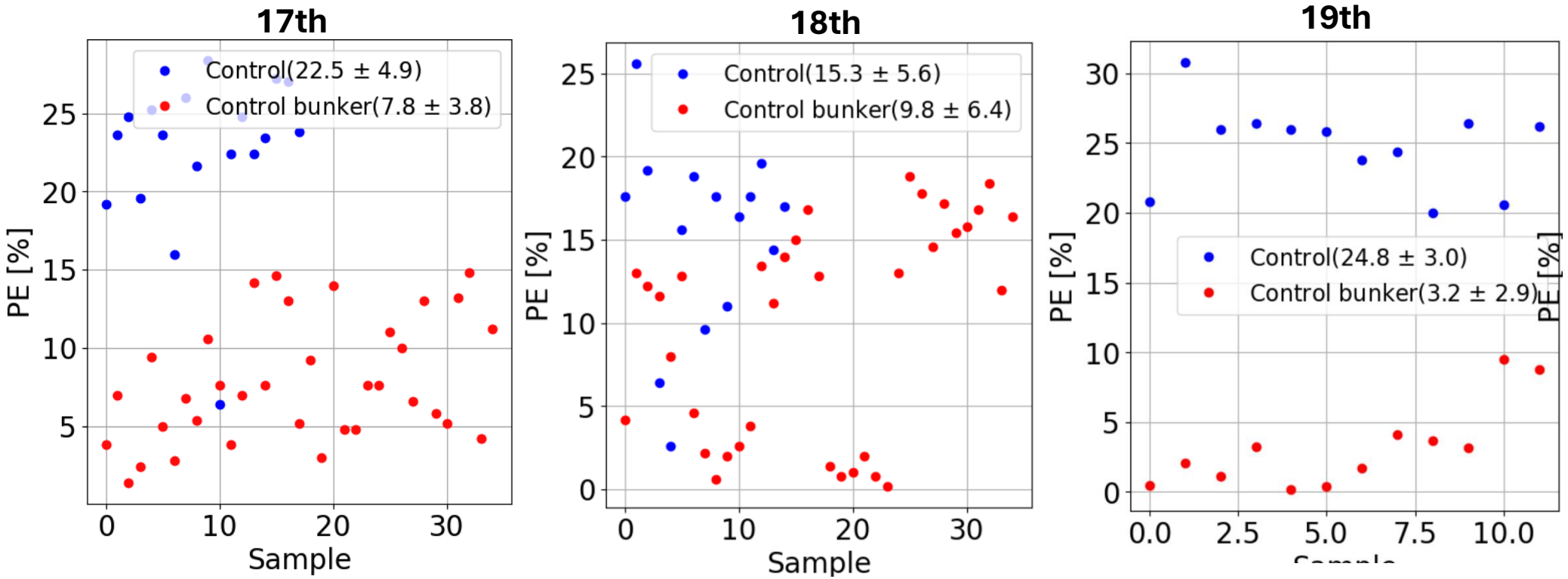
Low colony statistics

$p < 0.05$ indicates that at least one group has a mean value different statistically significant





Clonogenic assays 2023 - 50 nm



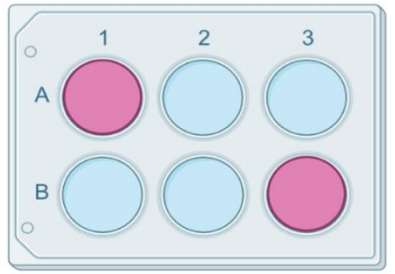
Dose (Gy)	Ncell 2023
0/1	250/500
2	1000
3/4	1500
5/6	3000

- Large difference between controls outside and inside the bunker during irradiation

Sources:

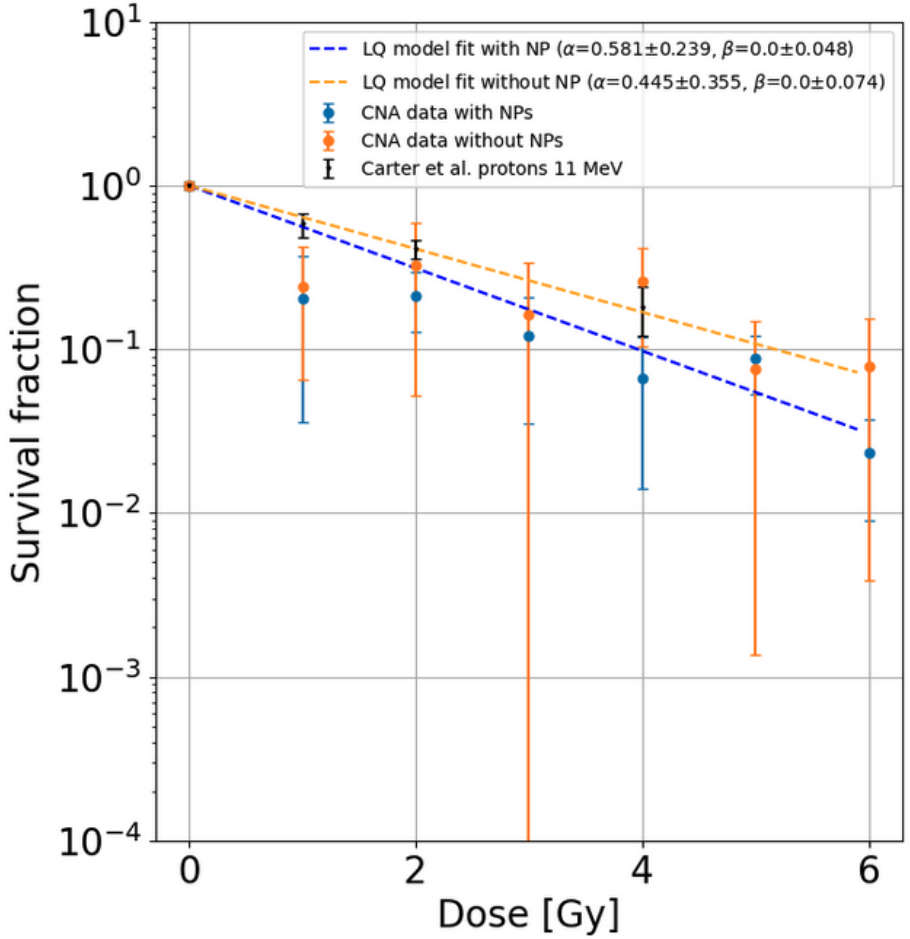
- Some problems found with the mylar tape and loss of liquid in some samples
- ???

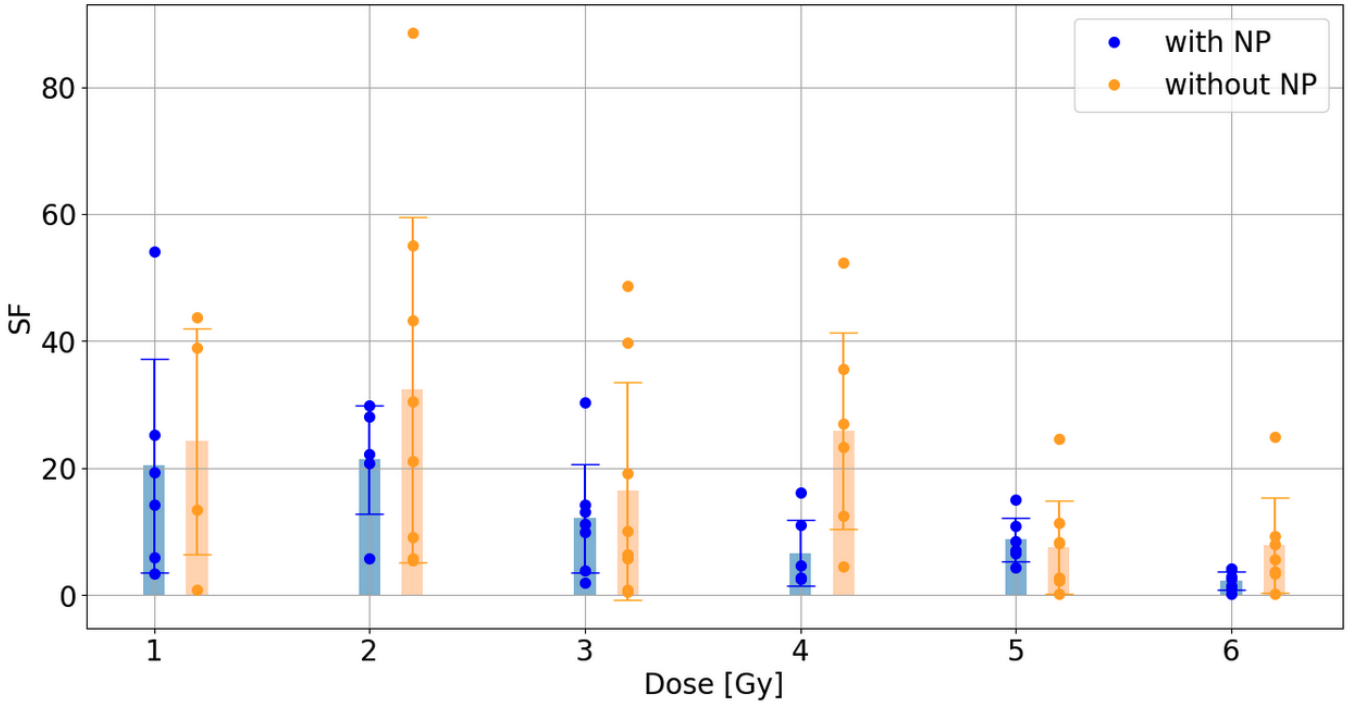
$$PE = \frac{colonies\ without\ irradiation}{Seeded\ cells} \times \frac{}{100}$$



Clonogenic assays 2023

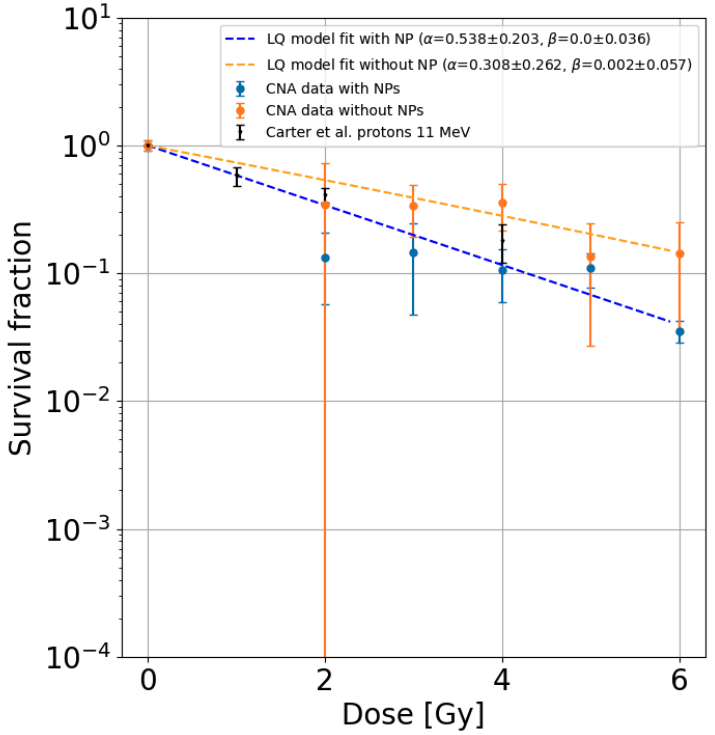
All data

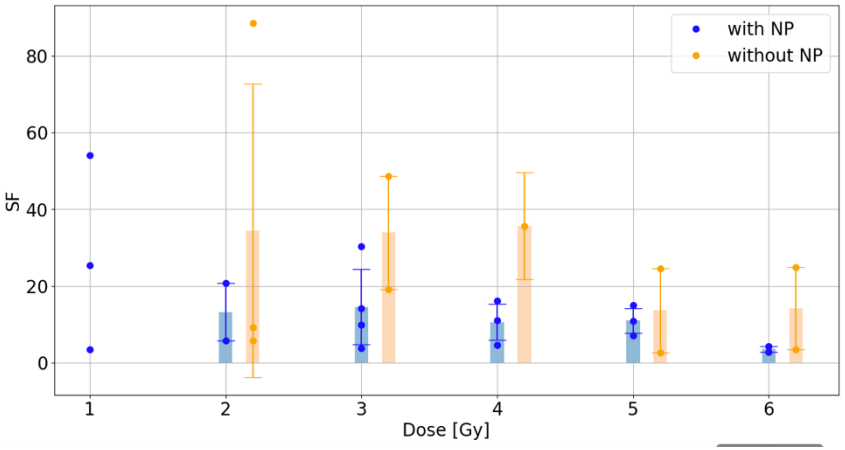




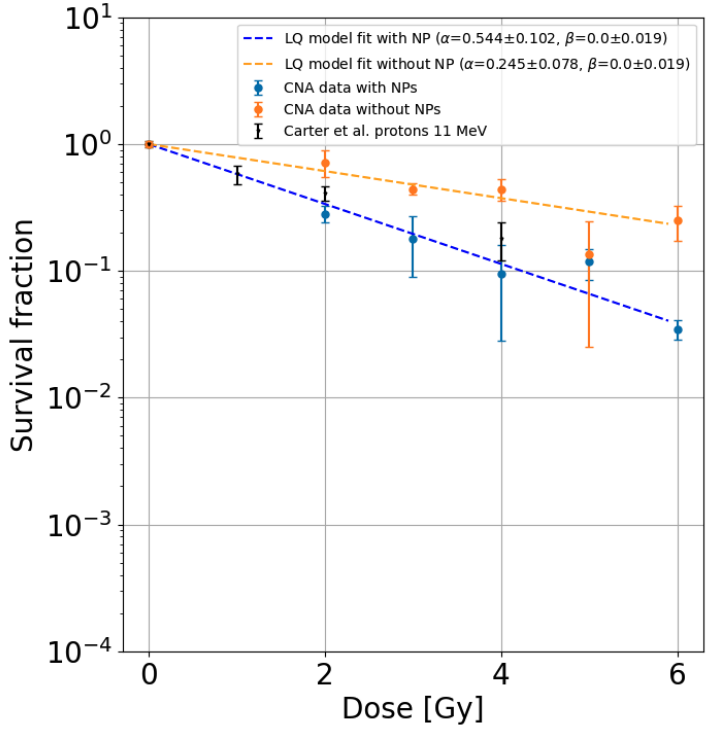
Clonogenic assays 2023

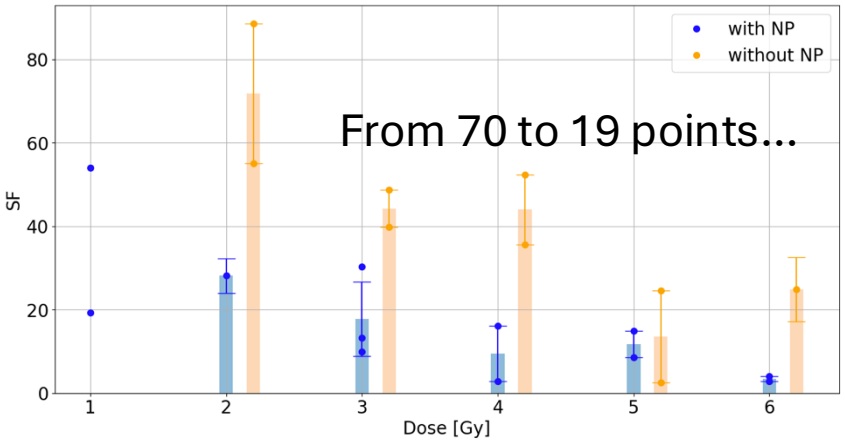
Only data from 18th with better PE



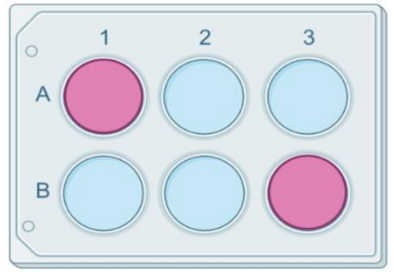


Filtered data





Irradiated



Control

Removeing dose having control-irradiated sample with PE< 3%

Measurements without NPs 1 or 2 points, strictly not possible to apply ANOVA

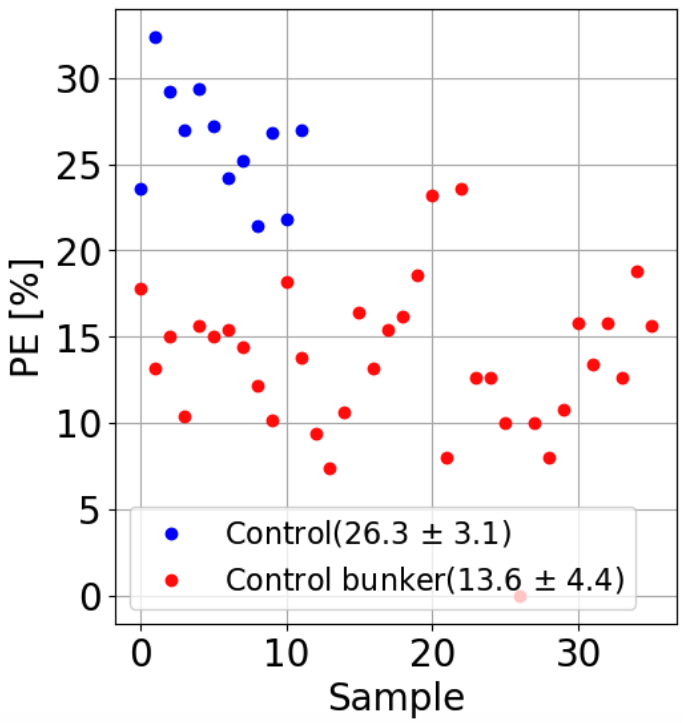
Clonogenic assays 2023

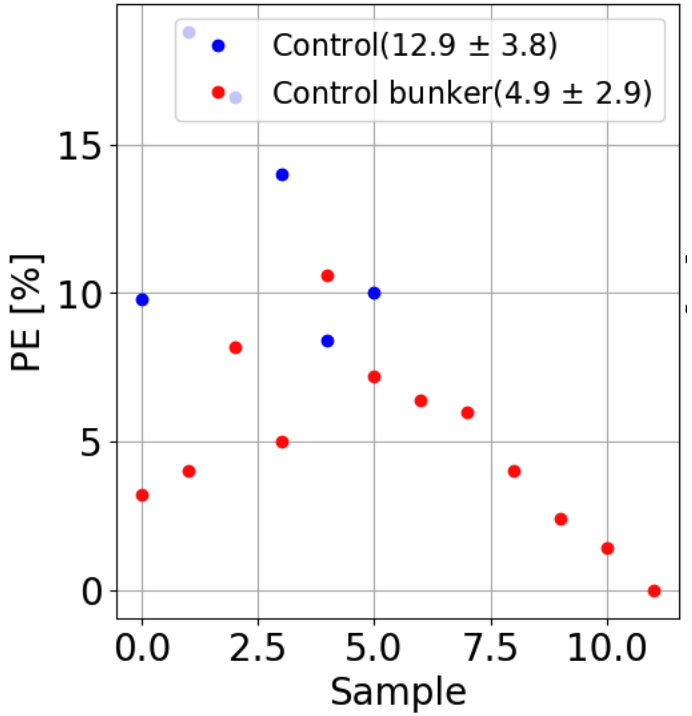
Dose (Gy)	p_value All data	p_value Only 27 2022	p_value 2023 All data	Only 28th
1			0.38	
2	0,49	0,69	0.43	0.59
3			0.24	0.19
4	0,0012	0,0007	0.02	0.06
5			0.95	0.78
6	0,097	0,024	0.12	0.42
8	0,68	0,34		

With filters the number of points are reduced, and ANOVA can not be applied

	Treatment	α	β	R^2	SER	Comment
All data	+	0.58 ± 0.24	0.00 ± 0.05	0.81	1.27	Compatible with 2022. Large errors. Lower SER
	-	0.44 ± 0.35	0.00 ± 0.07	0.86		
Only 18th	+	0.54 ± 0.20	0.00 ± 0.04	0.86	1.62	Compatible with 2022. Large errors. Same SER
	-	0.31 ± 0.26	0.002 ± 0.006	0.94		
Filtering PE>3%	+	0.55 ± 0.10	0.00 ± 0.019	0.91	1.89	Compatible with 2022. Smaller errors. HIgher SER. Low statistics
	-	0.25 ± 0.08	0.000 ± 0.019	0.97		

Clonogenic assays 2023- 20 nm

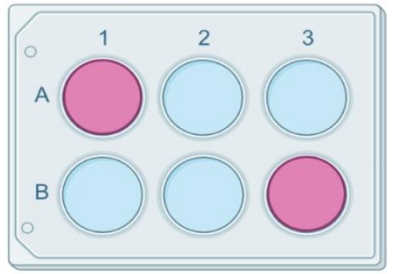




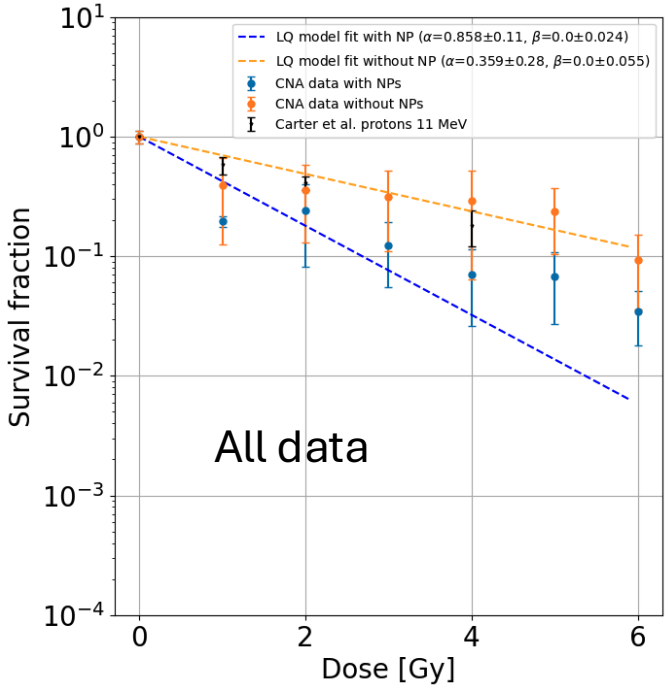
Dose (Gy)	Ncell 2023
0/1	250/500
2	1000
3	1500
3/4	1500
5/6	3000

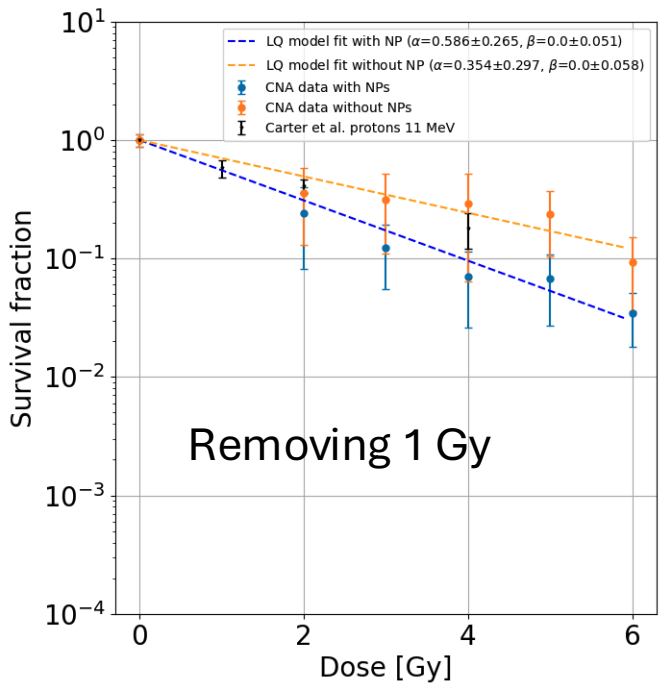
- Large difference between controls outside and inside the bunker during irradiation
- Some problems found with the mylar tape

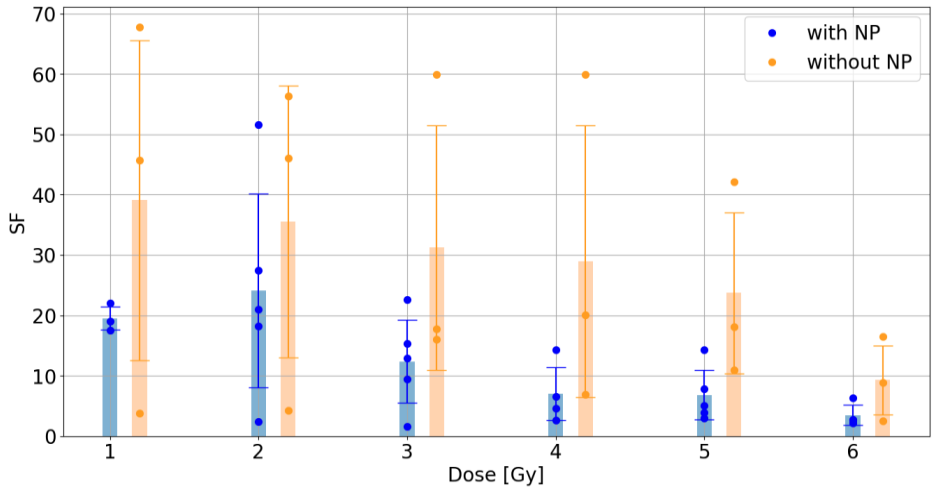
$$PE = \frac{colonies\ without\ irradiation}{Seeded\ cells} \times \frac{}{100}$$



clonogenic assays 2023 – 20 nm



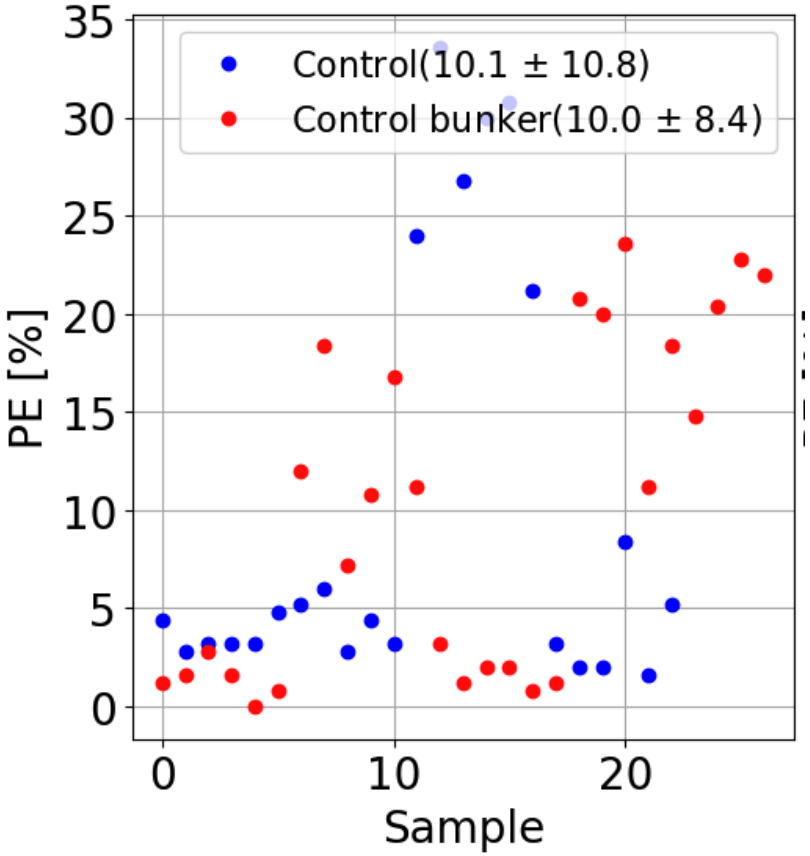


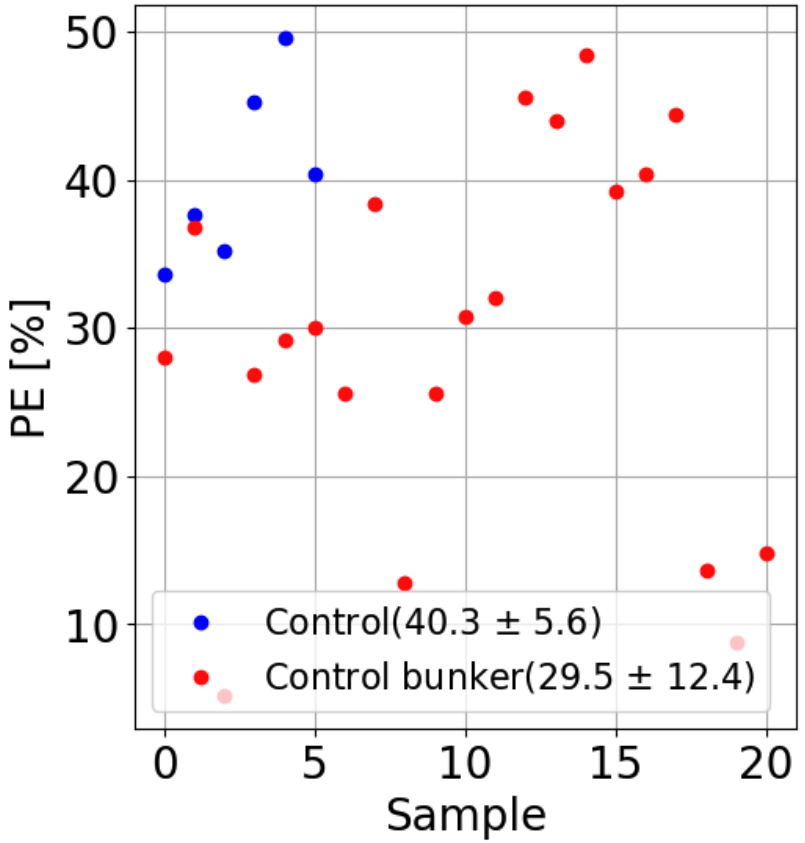


		α	β	R^2	SER	Comment
All data	+	0.93 ± 0.11	0.00 ± 0.05	0.77	2.46	Higher effect. Large errors.
	-	0.36 ± 0.28	0.00 ± 0.06	0.93		
Removing 1 Gy	+	0.60 ± 0.29	0.00 ± 0.06	0.95	1.62	Compatible with 50 nm. Large errors. Same SER
	-	0.35 ± 0.30	0.00 ± 0.06	0.99		

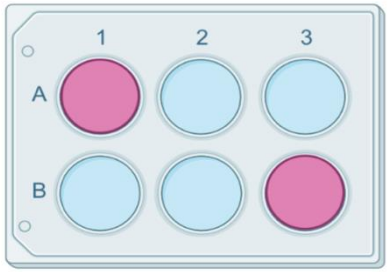
Dose (Gy)	p_value 2023 All data
1	0.35
2	0.49
3	0.15
4	0.17
5	0.06
6	0.16

Clonogenic assays 2024





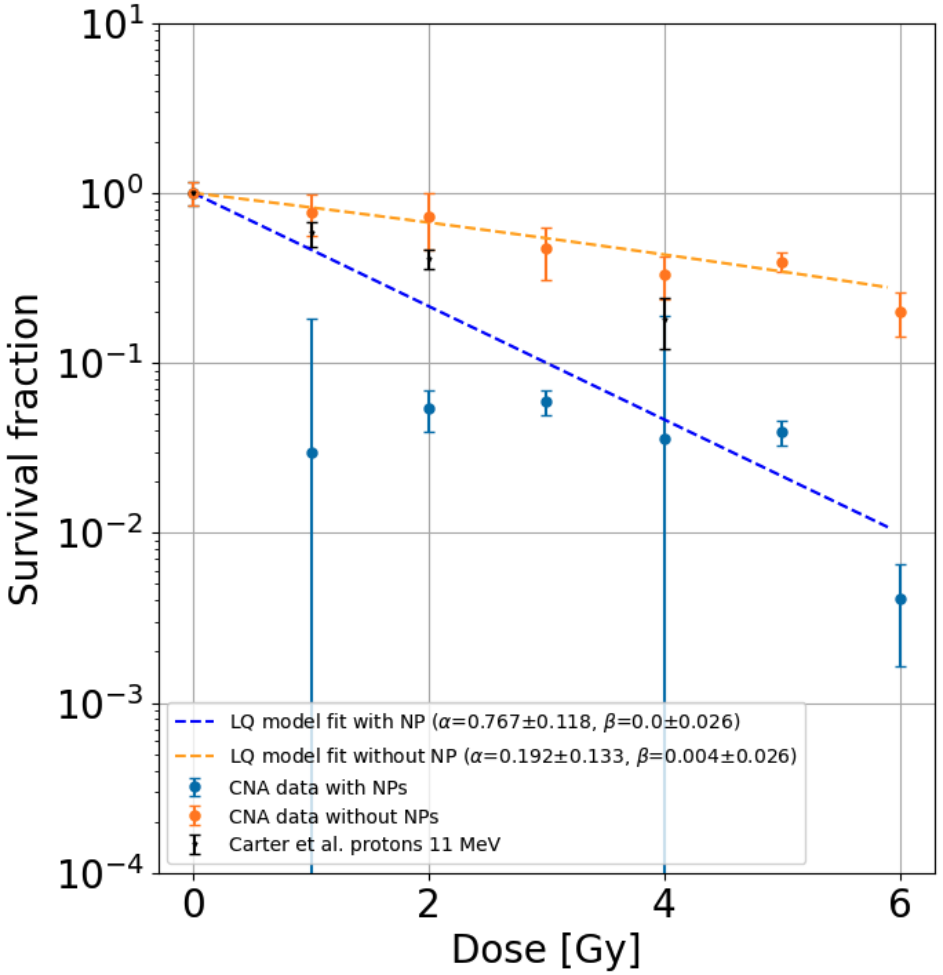
Dose (Gy)	Ncell 2023
0/1	250/500
2	1000
3/4	1500
5/6	3000



- First day many samples with low PE and second day better situation
- Not ideal conditions, due to problems with cyclotron larger waiting times and time outside the incubator

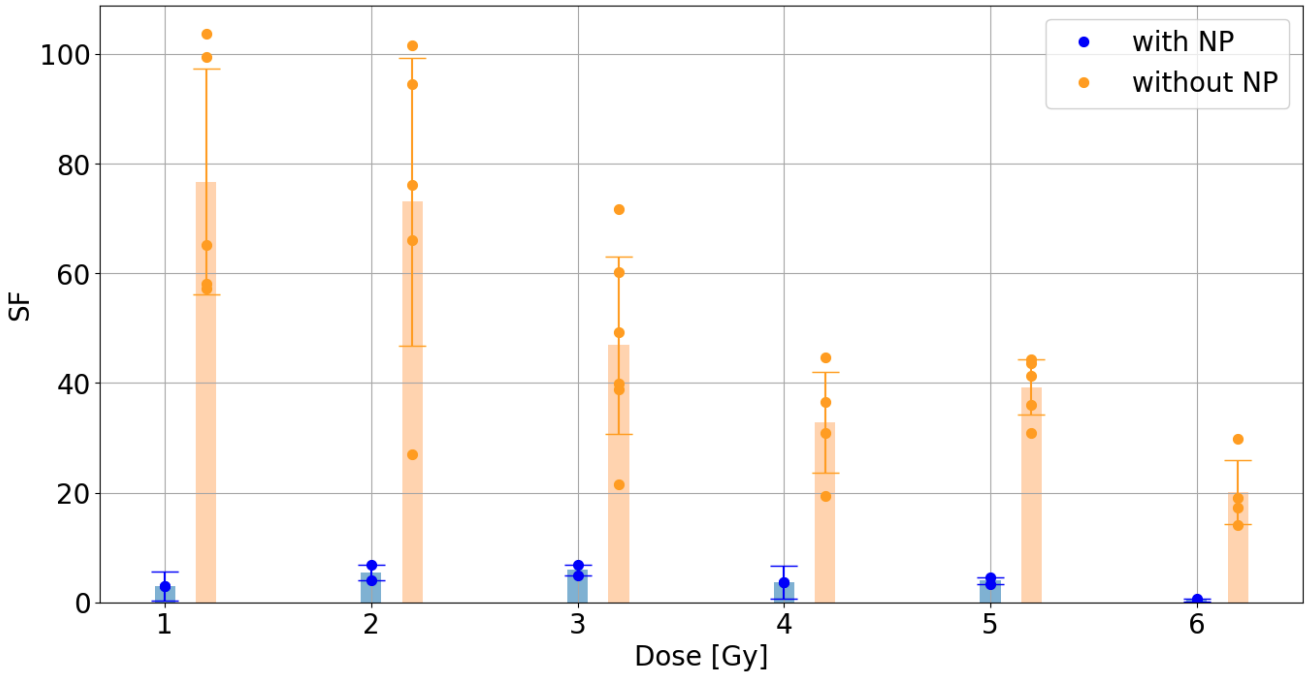
$$PE = \frac{colonies\ without\ irradiation}{Seeded\ cells} \times \frac{}{100}$$

Clonogenic assays 2024



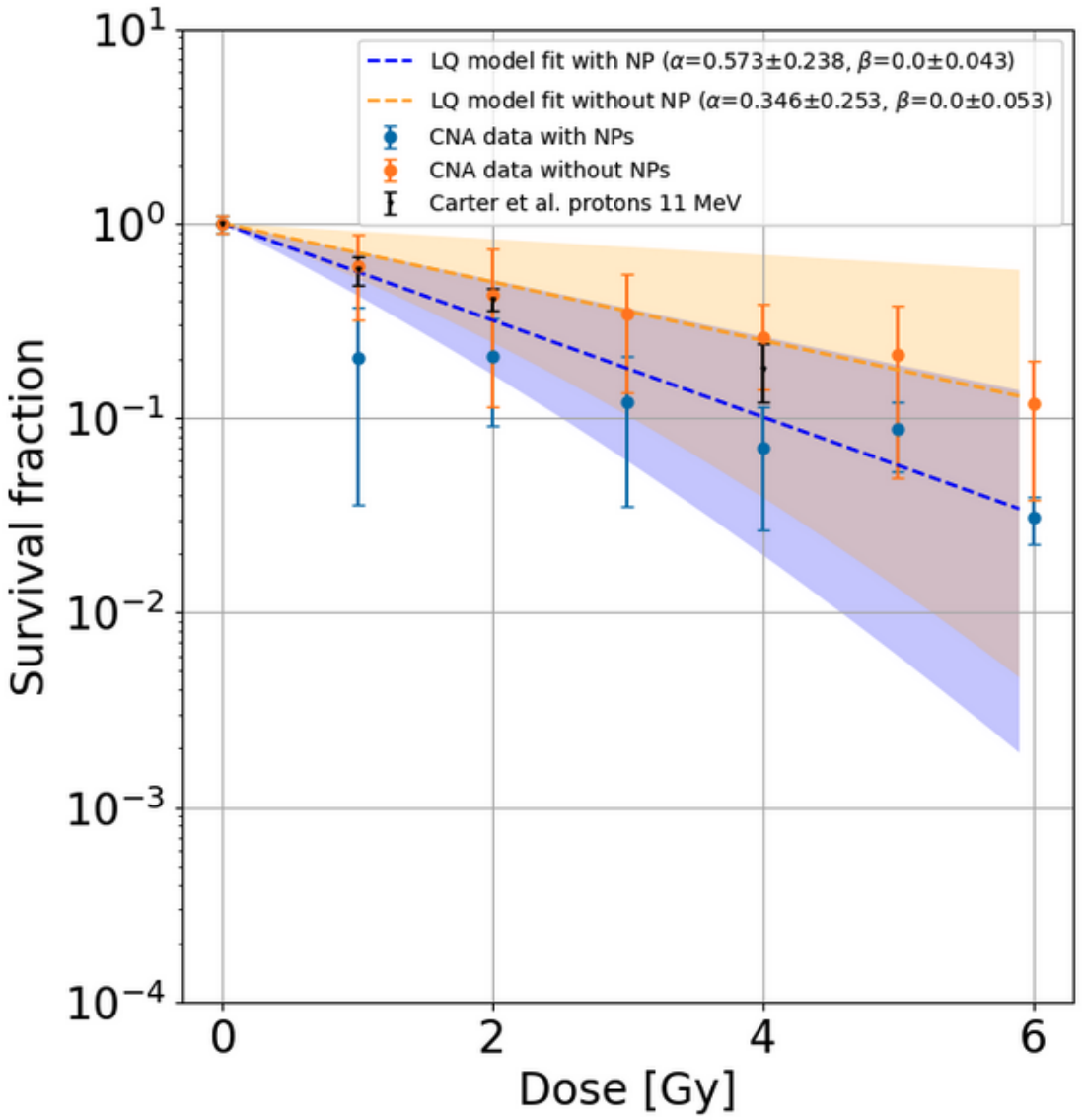
2024

- Problems with cyclotron vacuum
- 5 sets of measurements without NPs and 2 with NPs
- Unfortunately, the sets with NPs are not okay

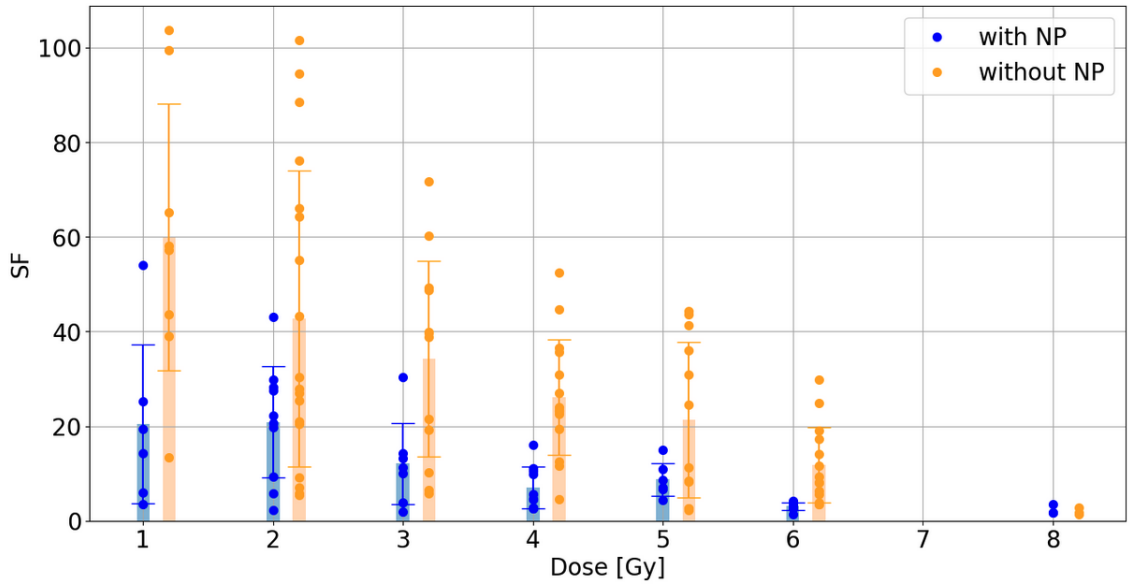


Combination 2022, 2023, 2024

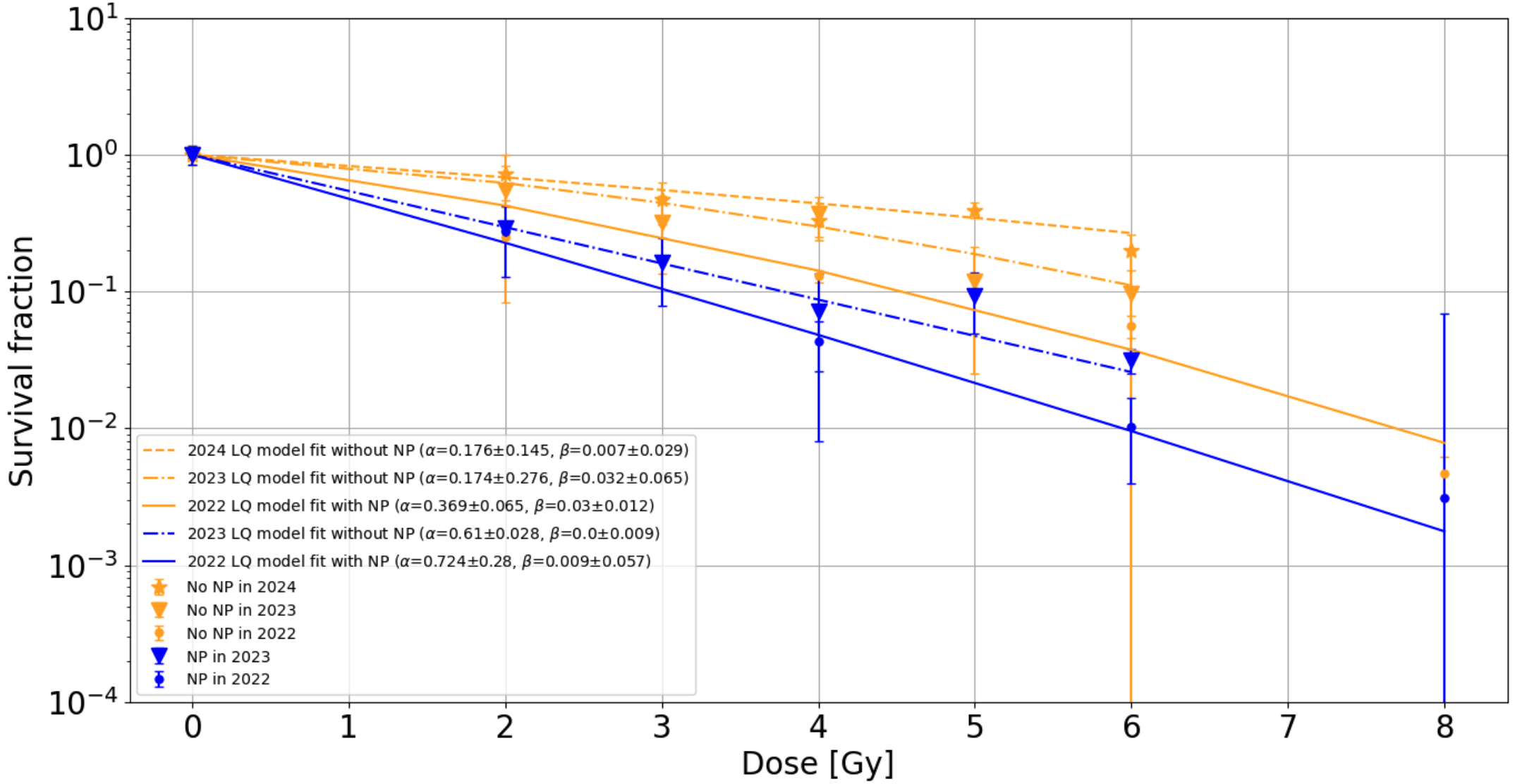
All data



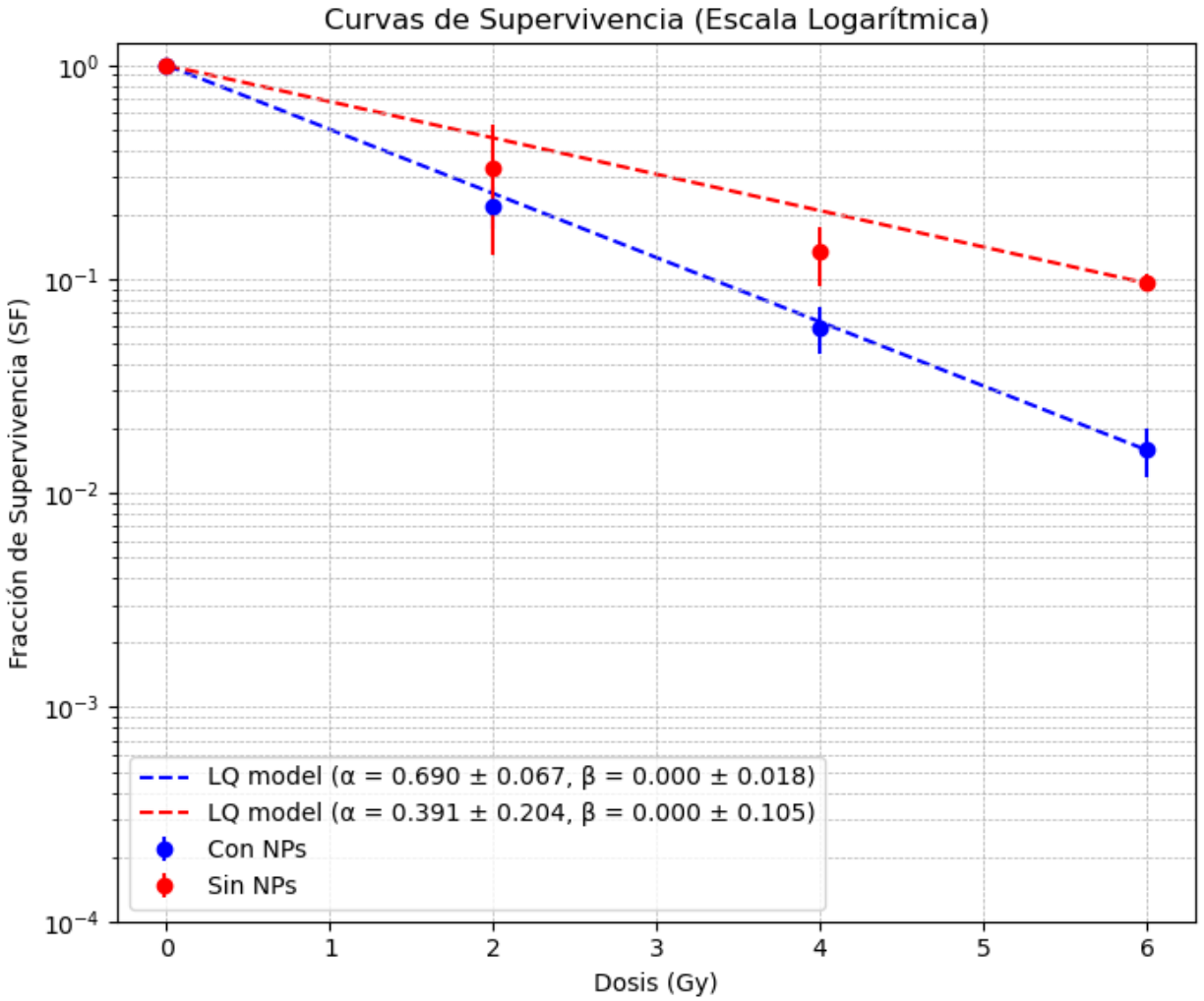
Dose (Gy)	p_value 2023 All data
1	0.015
2	0.05
3	0.02
4	0.00013
5	0.10
6	0.008



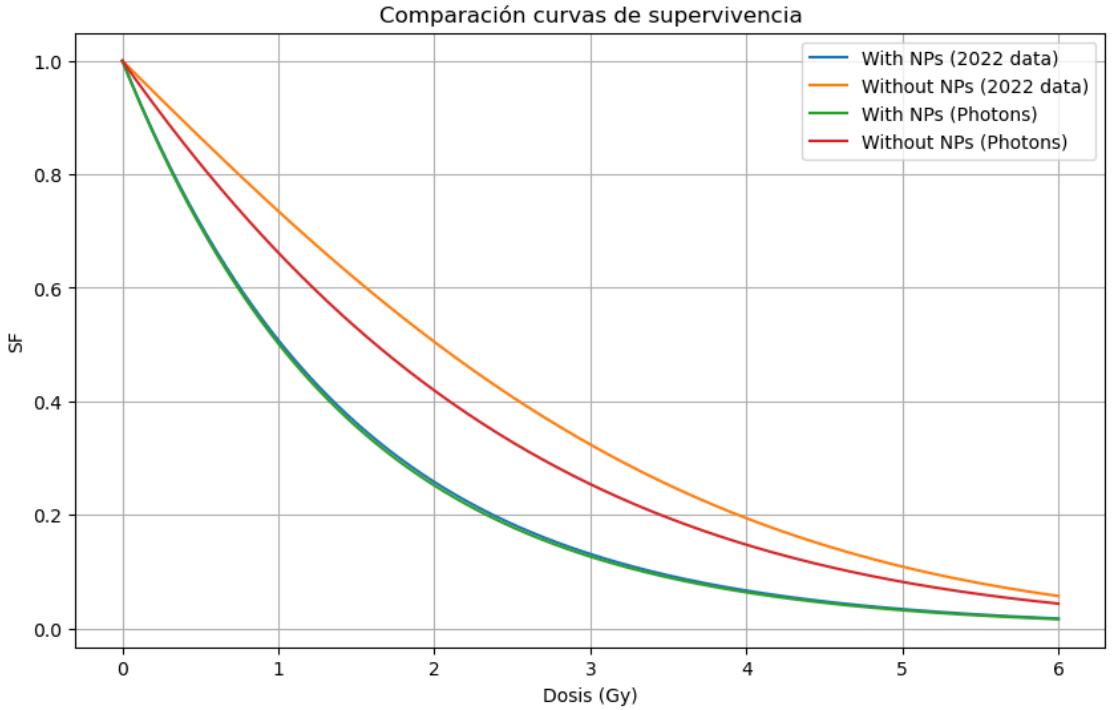
Comparison


Survival fraction
Dose [Gy]
2024 LQ model fit without NP (α=0.176±0.145, β=0.007±0.029)
2023 LQ model fit without NP (α=0.174±0.276, β=0.032±0.065)
2022 LQ model fit with NP (α=0.369±0.065, β=0.03±0.012)
2023 LQ model fit without NP (α=0.61±0.028, β=0.0±0.009)
2022 LQ model fit with NP (α=0.724±0.28, β=0.009±0.057)
No NP in 2024
No NP in 2023
No NP in 2022
NP in 2023
NP in 2022

Photons 2022 y 2024



Detail analysis in progress



Experiments and analysis on going to check that the irradiation dose at CNA and CABIMER are the same

Summary

Is it possible to reduce the biological variability observed?

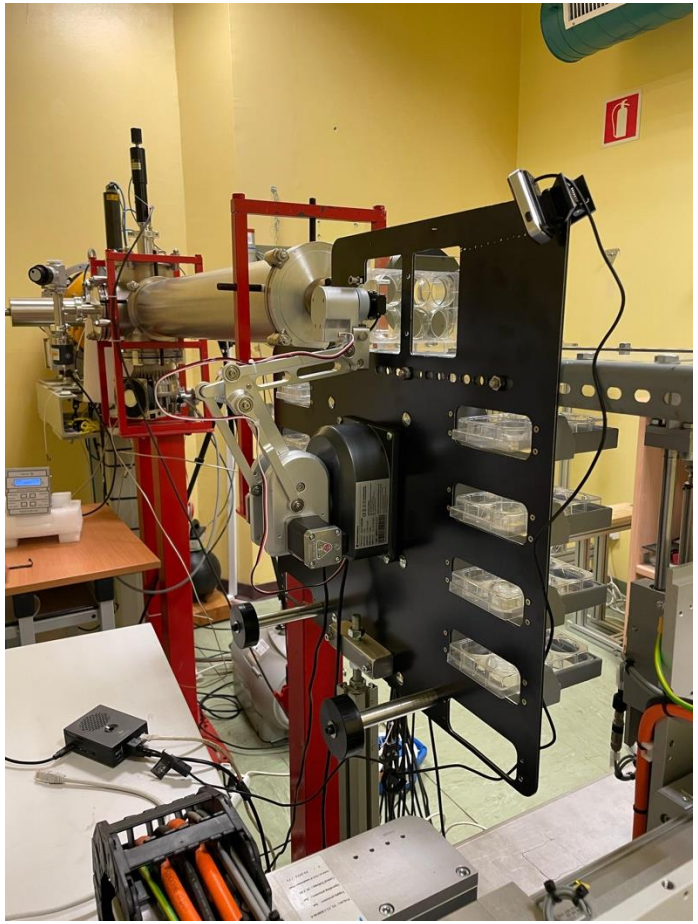
- Irradiation robot system
- Samples preparation and incubation next to the control room (incubator and minimum equipment required)

- The effect of AuNPs 50 nm diameter has been observed
  - SER ~1.5-1.8
  - ANOVA test significant from 3,4 and 6 Gy
  - 20 nm NPs similar effect observed
  - Comparison analysis photon vs proton on-going

- Priority in July finish the publication of the results obtained so far

- New collaboration with Silvia Pujals from Institute for Advanced Chemistry of Catalonia (IQAC-CSIC)
  - Expertise in the design, synthesis and characterization of conjugated-NPs

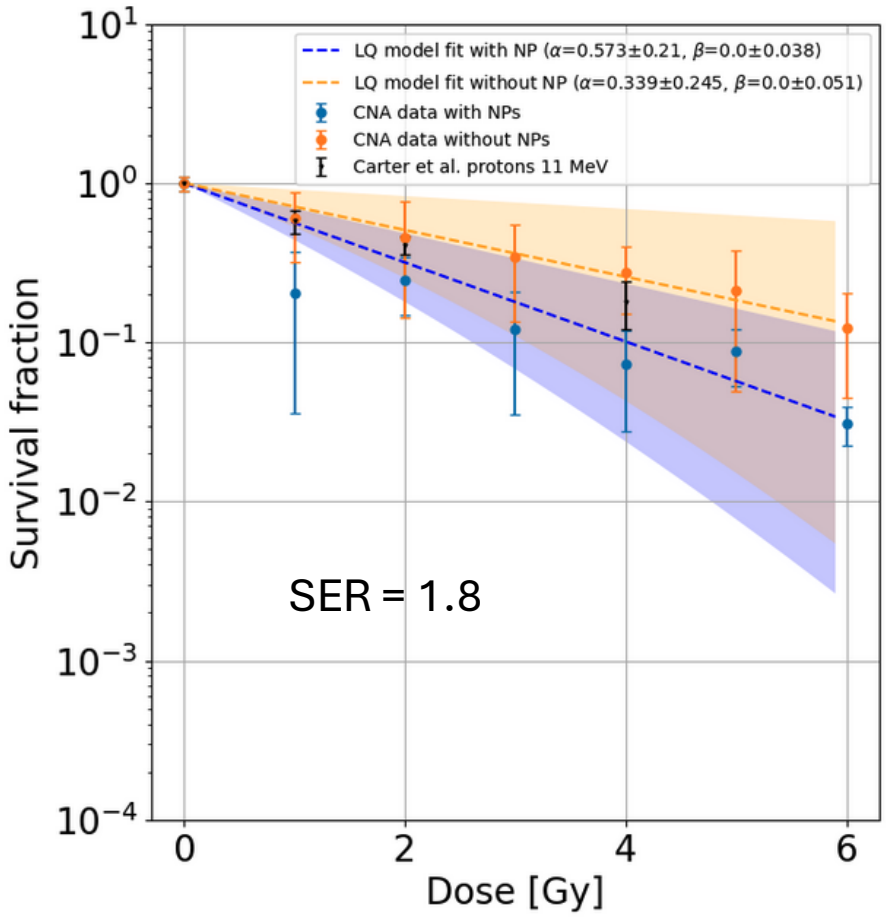
- Next experimental campaign (September-December 2025)
  - Validate the robot performance with old or new NPs?
  - Study the radiosensitization effect of new NPs with prostate cancer cells (detailed plan to be discussed)



Back-up

Combination 2022, 2023, 2024

27th from 2022
18th from 2023
All no NP from 2024



Dose (Gy)	p_value
1	0.015
2	0.09
3	0.02
4	0.0002
5	0.1
6	0.005