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# Radiation induced DNA damage in human uveal melanoma cells

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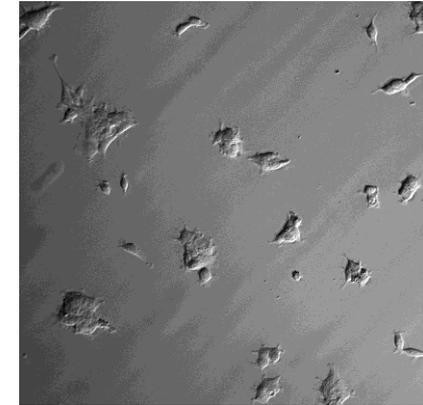
**ICTR-PHE**



**2016**



**Mel270** - human uveal melanoma cells, derived from primary tumor (Leiden University).



## Methods

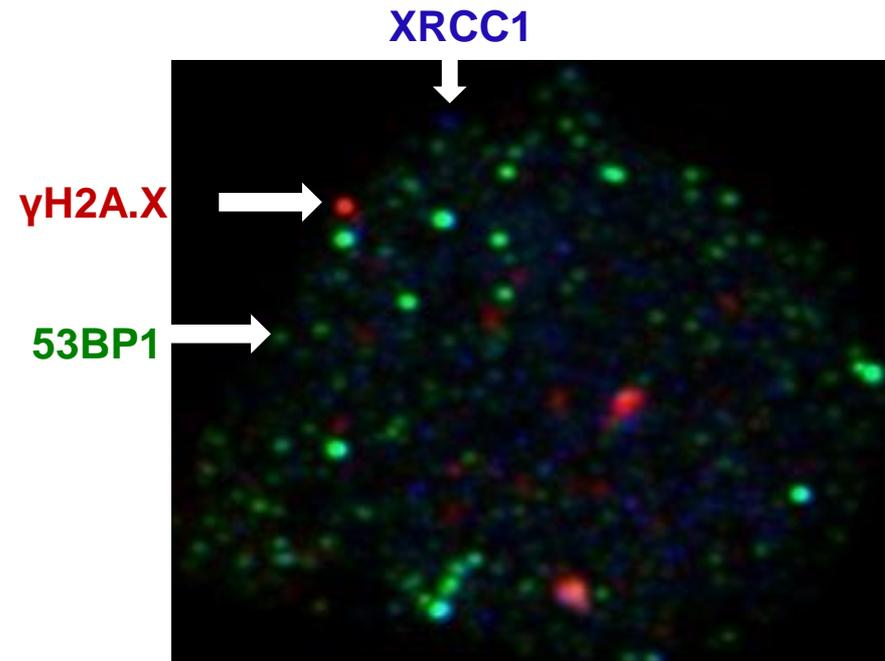
1. Two types of radiation:

- Proton beam radiation - 60 MeV from Proteus C-235 cyclotron; LET 2 keV/ $\mu\text{m}$
- X rays - 300 kVp Phillips, 1Gy/min.

2. Cells irradiated in suspension with doses: 1, 3 or 5 Gy.

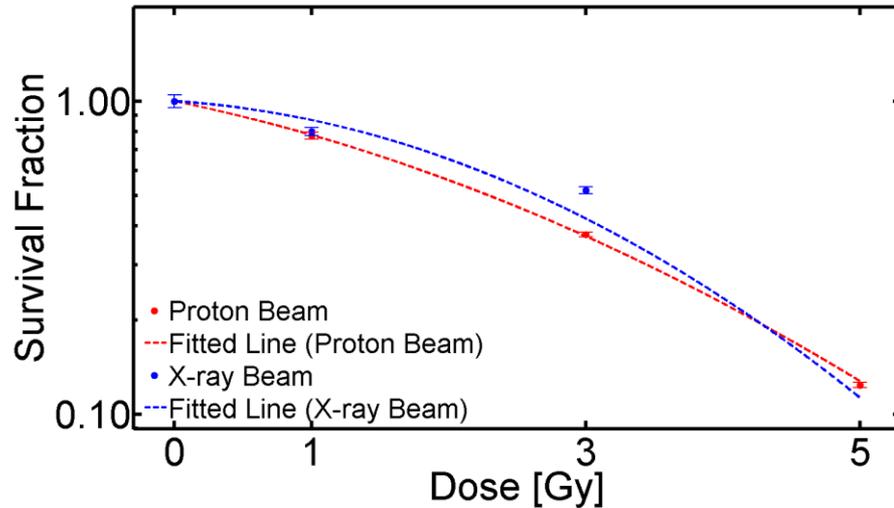
3. Clonogenic assay and proliferation study.

4. DNA damage was evaluated at **4 hours after irradiation (pilot experiment)** using immunofluorescence staining of  $\gamma$ -H2AX, 53BP1 (for double strand breaks) and XRCC1 (for single strand breaks). Detection with confocal microscopy.

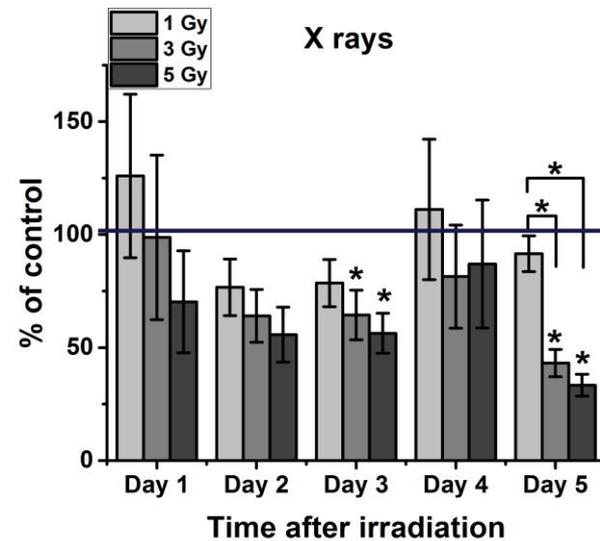
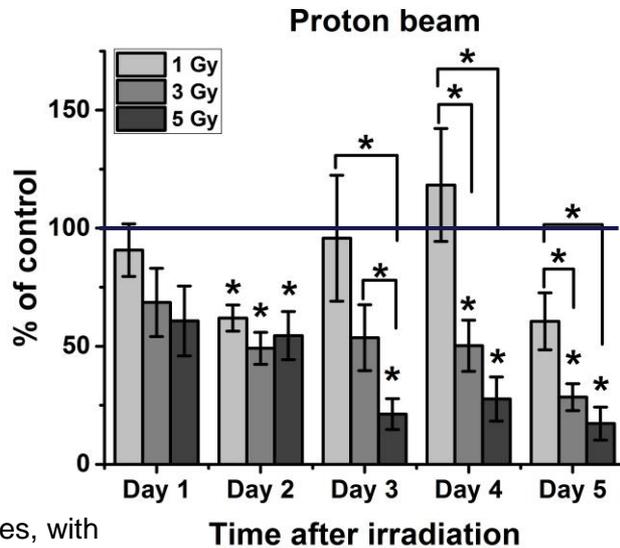


Merge of 3 channels (XRCC1,  $\gamma$ H2A.X, 53BP1)

# Similar survival after both types of radiation



$$RBE_{37} = 1,075$$



Mean values, with SEM, \* $p < 0,05$

**Control**

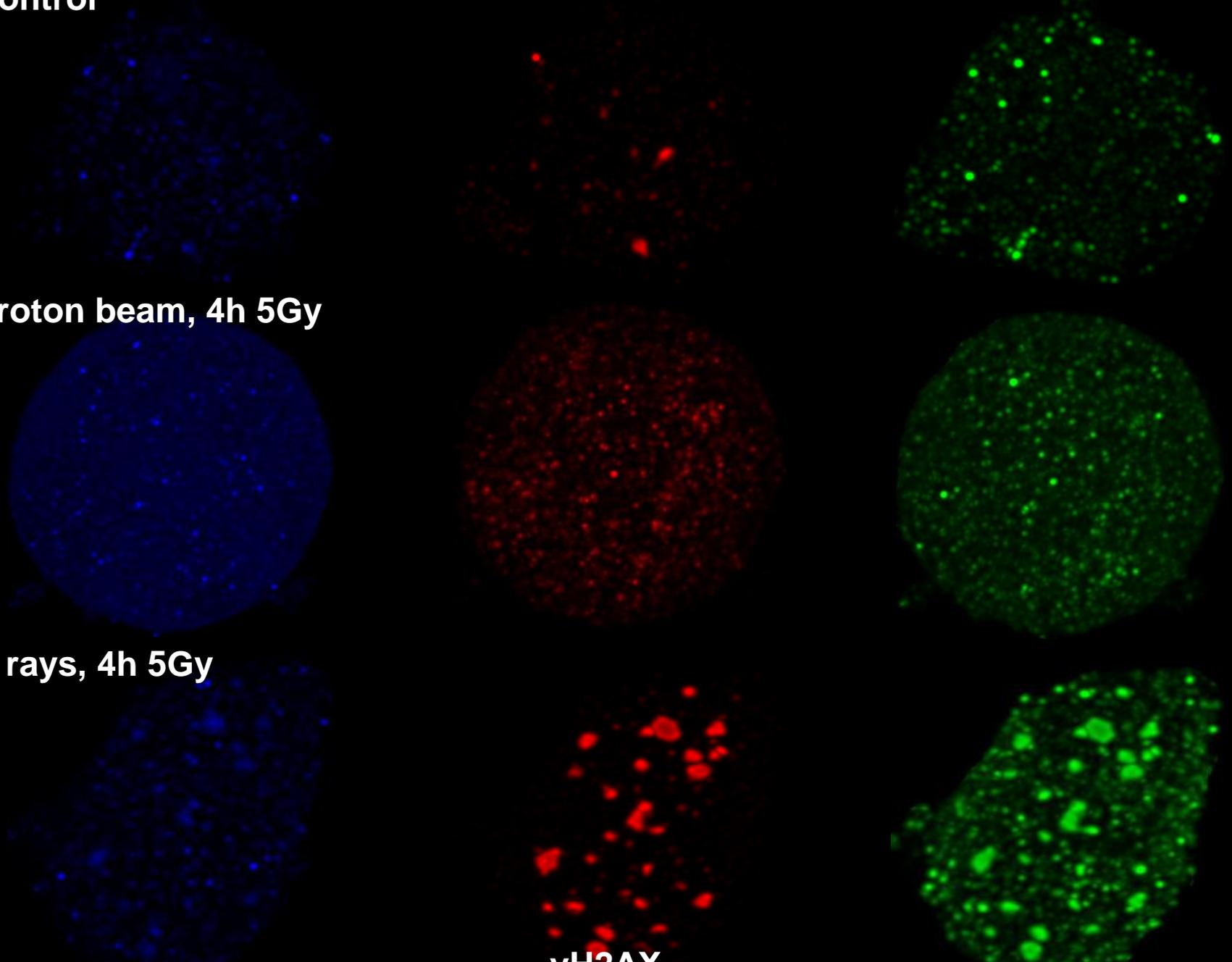
**Proton beam, 4h 5Gy**

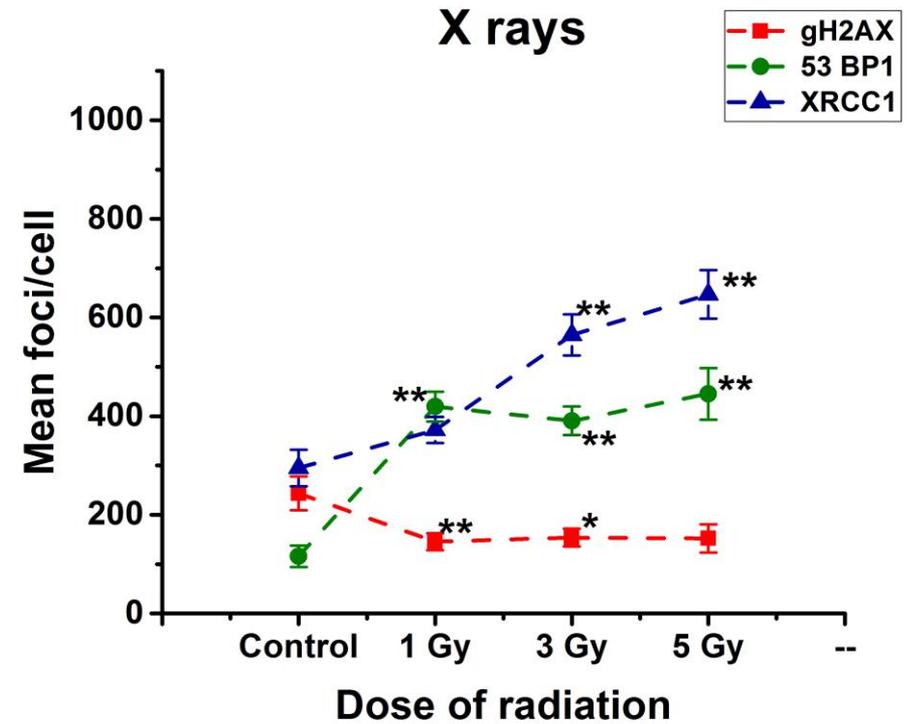
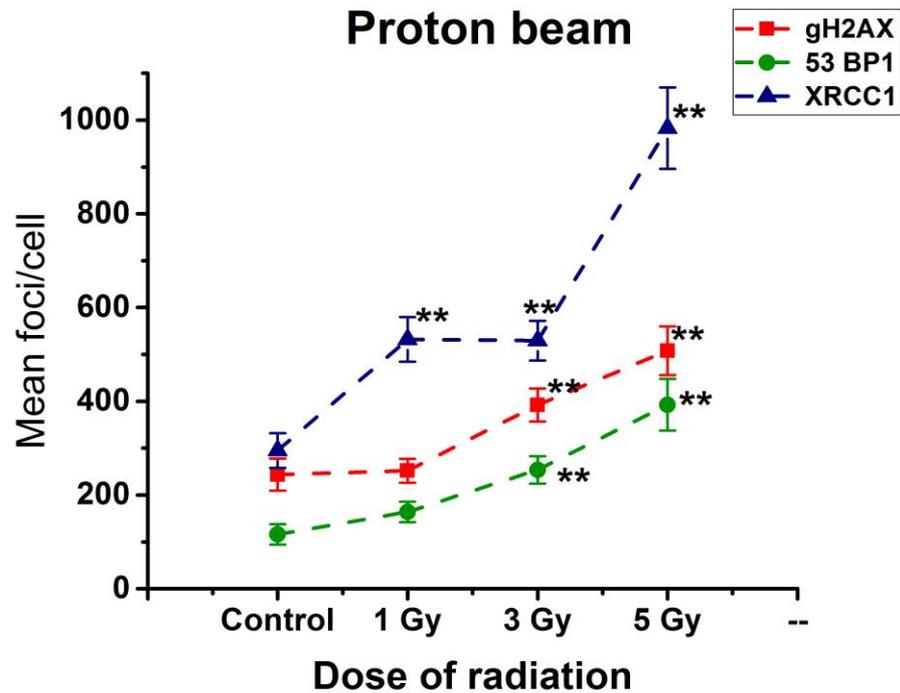
**X rays, 4h 5Gy**

**XRCC1**

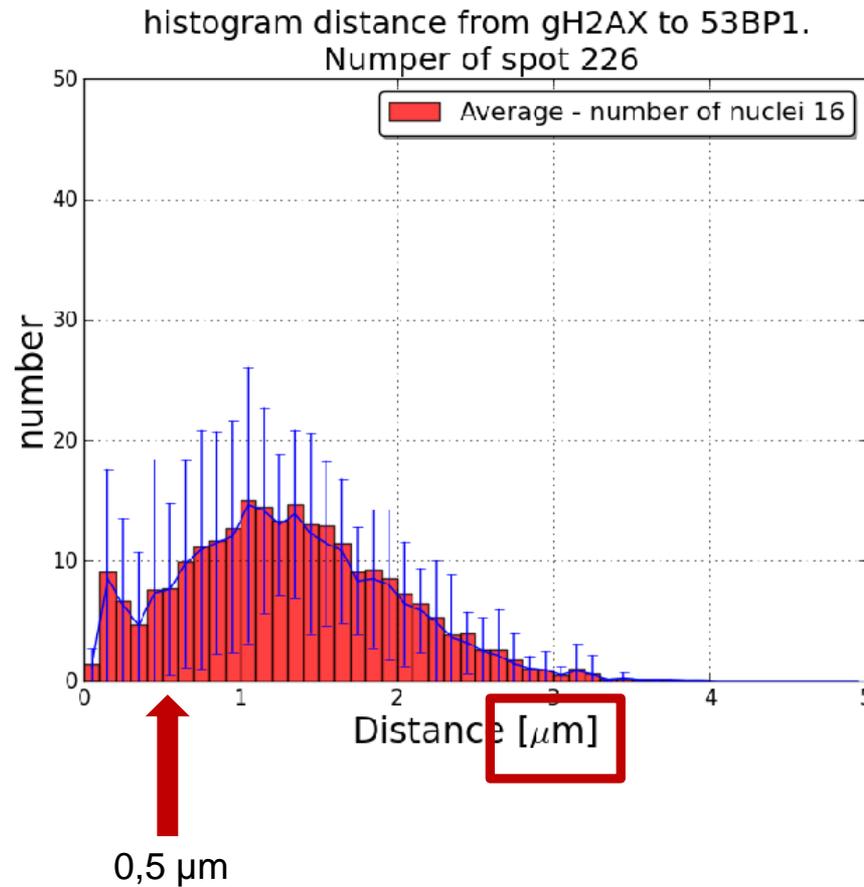
**$\gamma$ H2AX**

**53BP1**





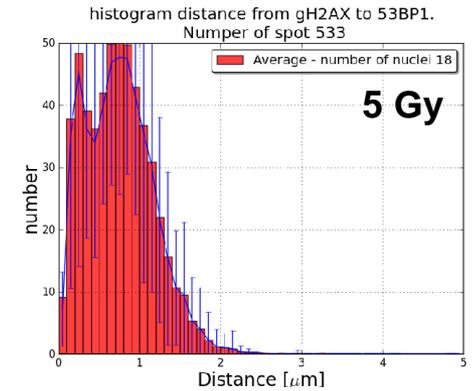
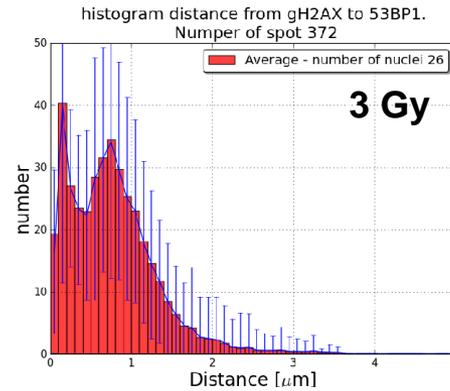
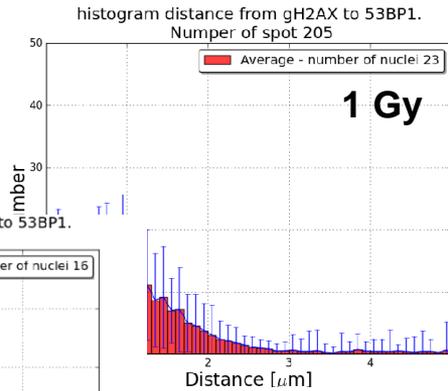
Mean values, with SEM, \* $p < 0,05$ ; \*\* $p < 0,01$



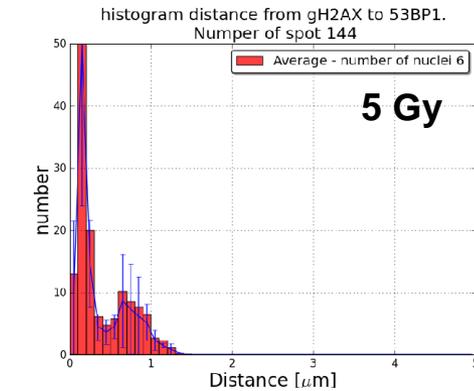
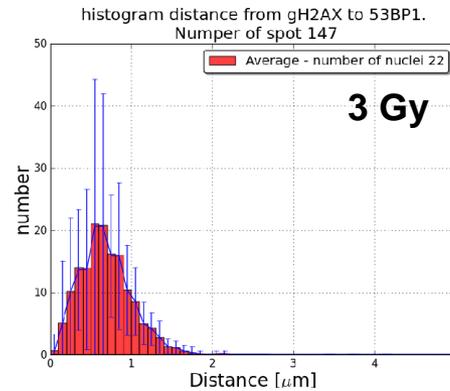
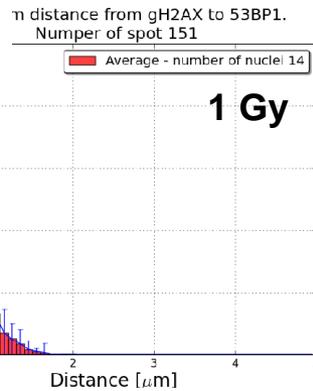
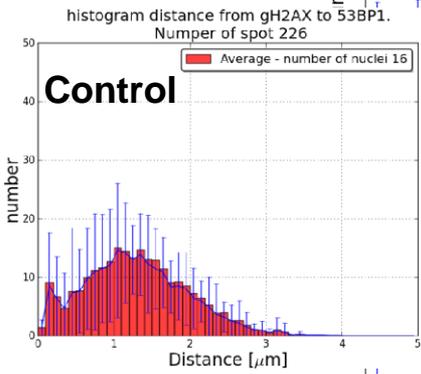
## 2 groups:

- 1) with high colocalization preference, below 0,5  $\mu\text{m}$  between foci;
- 2) random distribution, above 0,5  $\mu\text{m}$  between foci.

**Proton beam**

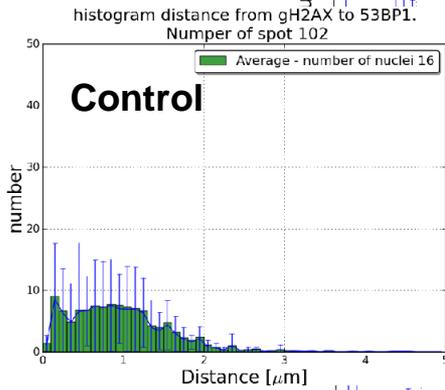


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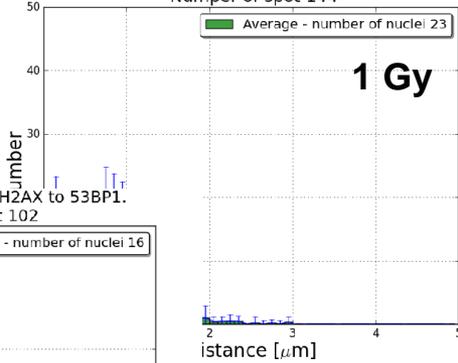


**X rays**

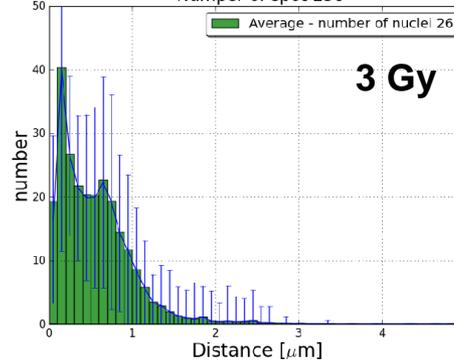
**Proton beam**



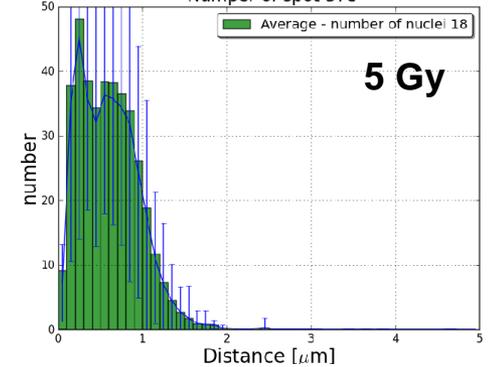
histogram distance from  $\gamma$ H2AX to 53BP1.  
Number of spot 144



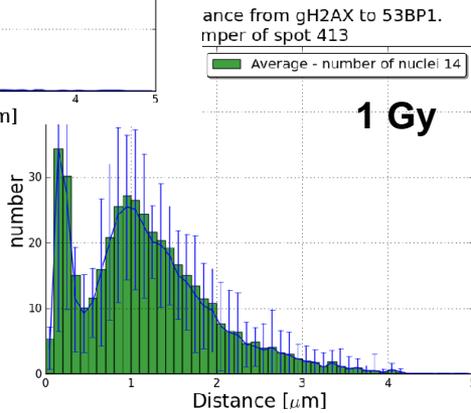
histogram distance from  $\gamma$ H2AX to 53BP1.  
Number of spot 230



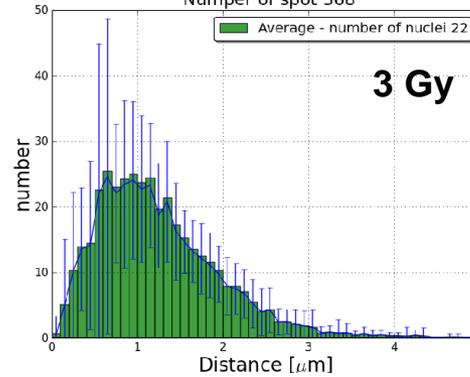
histogram distance from  $\gamma$ H2AX to 53BP1.  
Number of spot 378



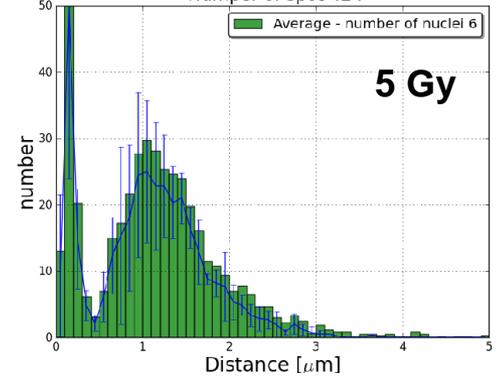
**X rays**



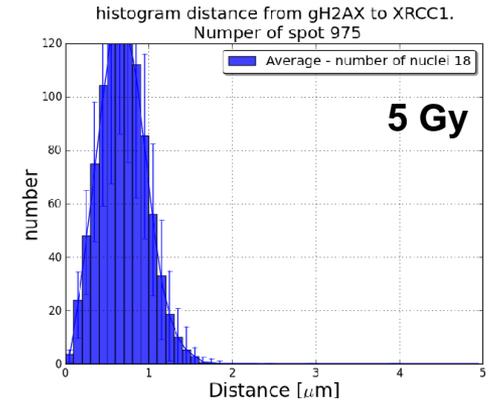
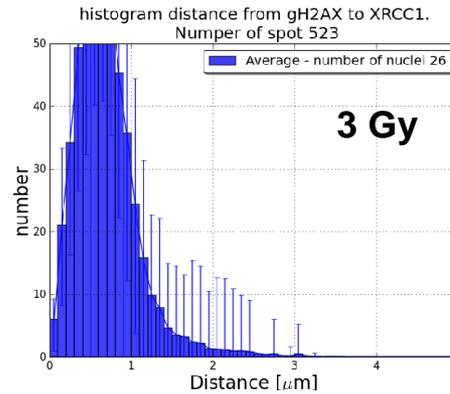
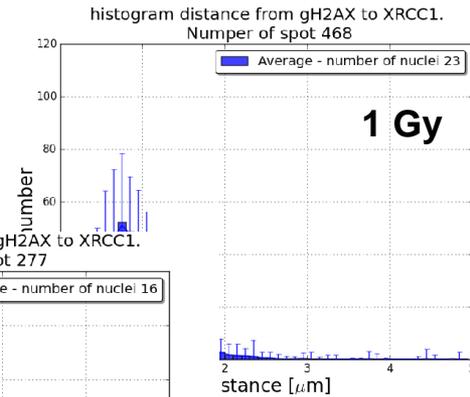
histogram distance from  $\gamma$ H2AX to 53BP1.  
Number of spot 368



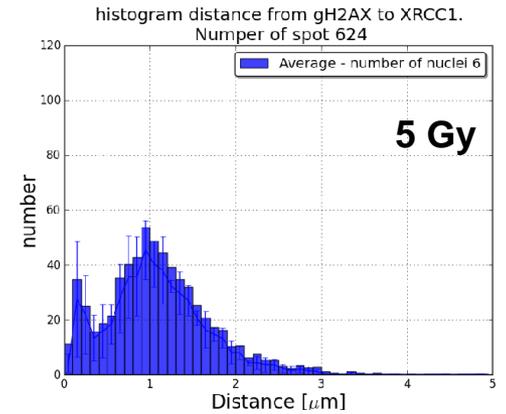
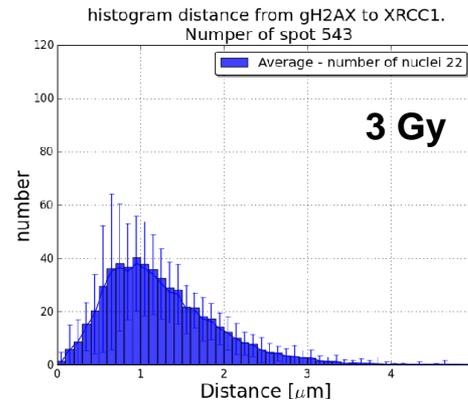
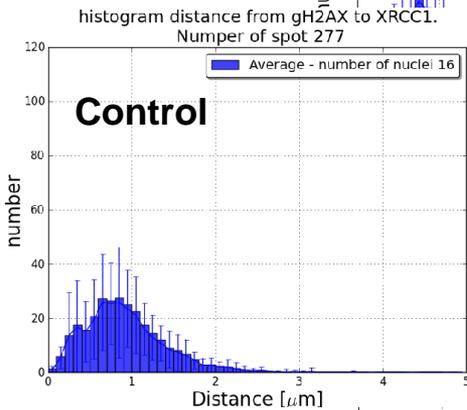
histogram distance from  $\gamma$ H2AX to 53BP1.  
Number of spot 424



**Proton beam**

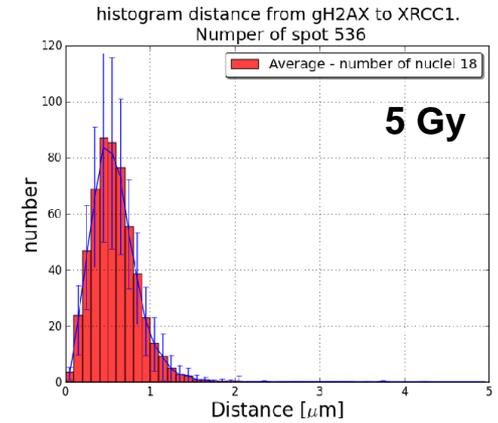
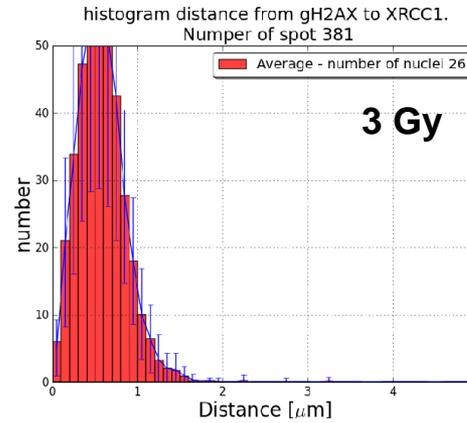
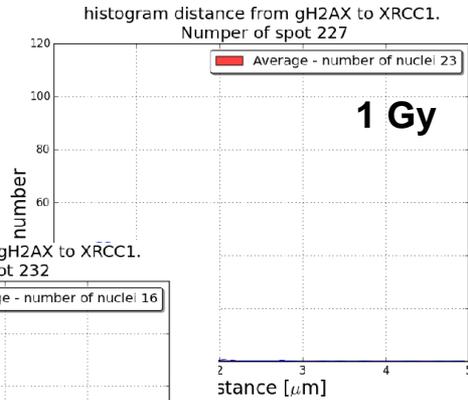


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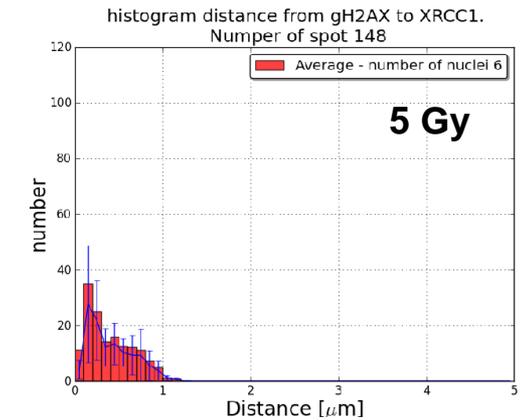
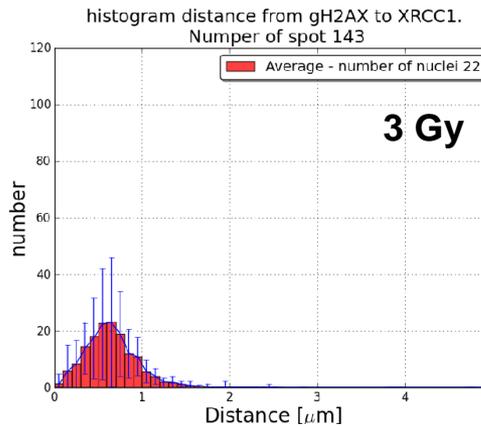
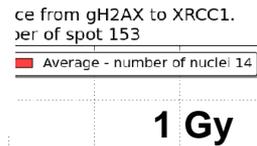
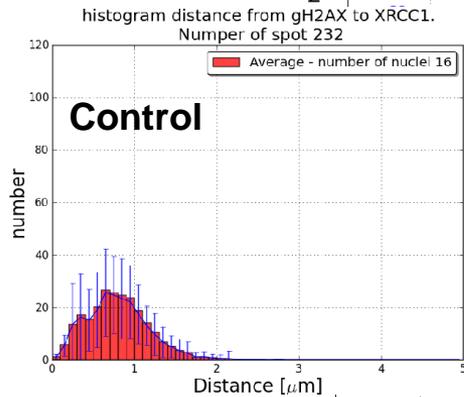


**X rays**

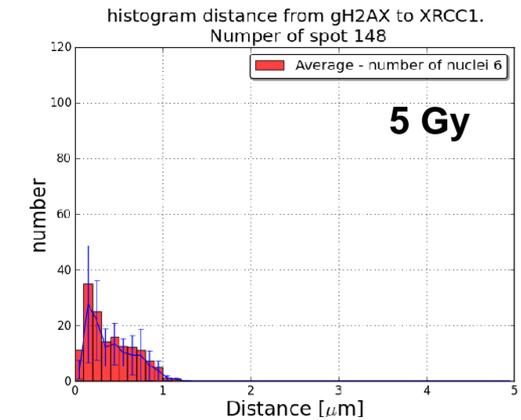
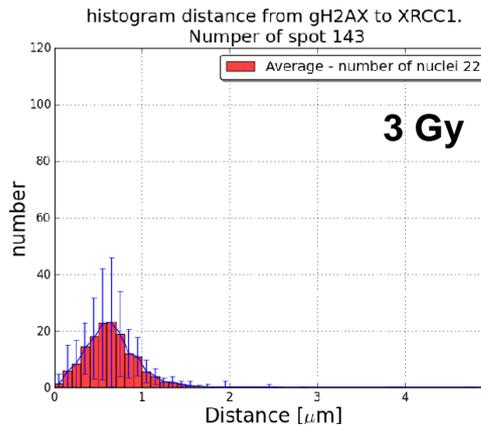
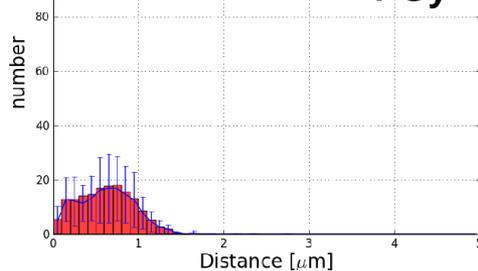
**Proton beam**



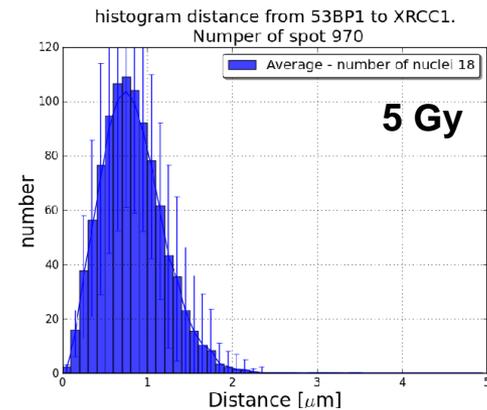
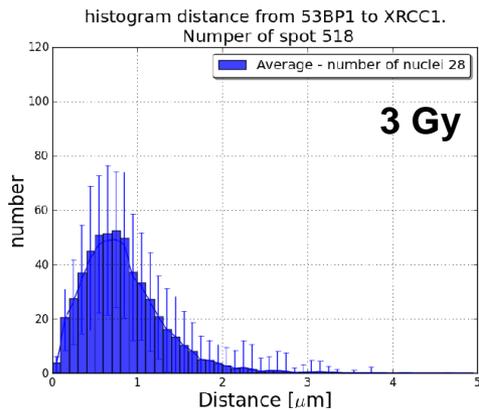
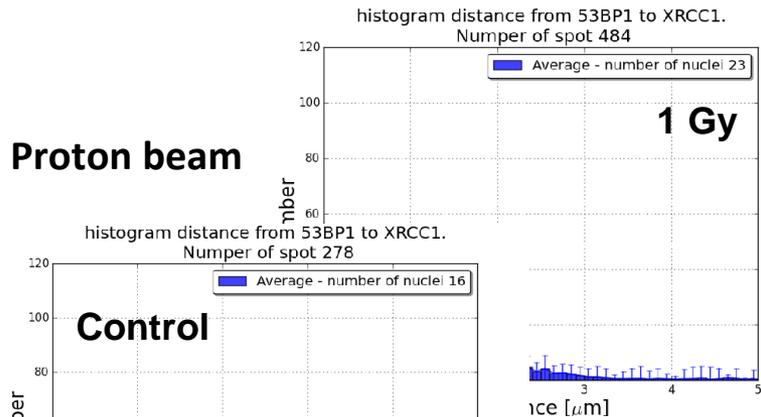
**Control**



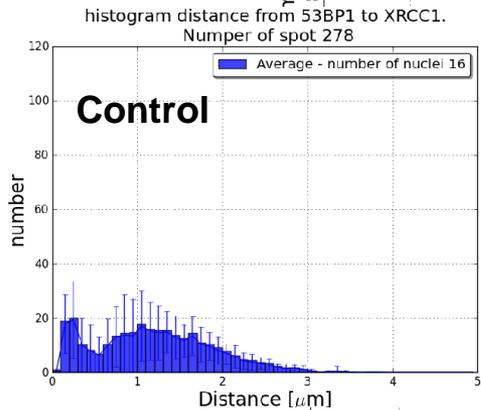
**X rays**



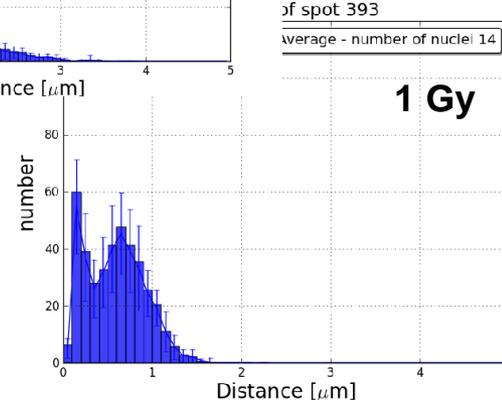
**Proton beam**



**Control**



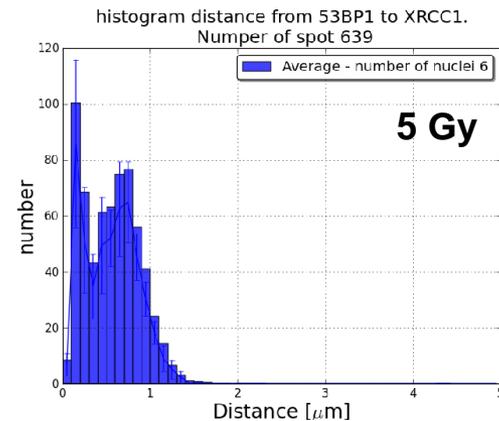
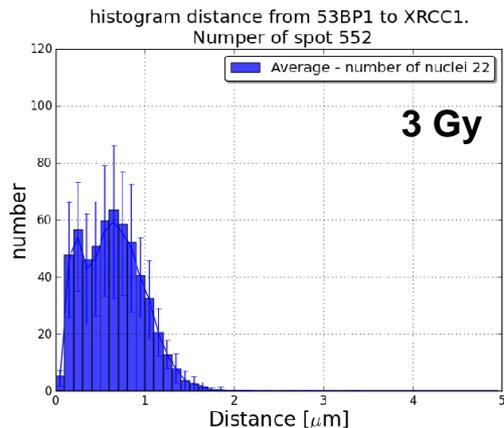
from 53BP1 to XRCC1.  
Number of spot 393



**1 Gy**

Average - number of nuclei 14

**X rays**



## Conclusions

- ✓ Survival of Mel270 cells did not differ after proton beam irradiation and X rays
- ✓ DNA damage after proton beam increased in dose dependent manner at 4h after irradiation
- ✓ High number of 53BP1 foci was seen after X rays
- ✓ There are two distinct groups of  $\gamma$ H2AX and 53BP1: colocalised and randomly localised
- ✓ There is no colocalisation of XRCC1 and  $\gamma$ H2AX or 53BP1 after proton beam radiation

## Acknowledgements

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A. Panek, PhD.

Please visit our poster, no 33 on „Proton beam irradiation inhibits cellular motility in vitro”