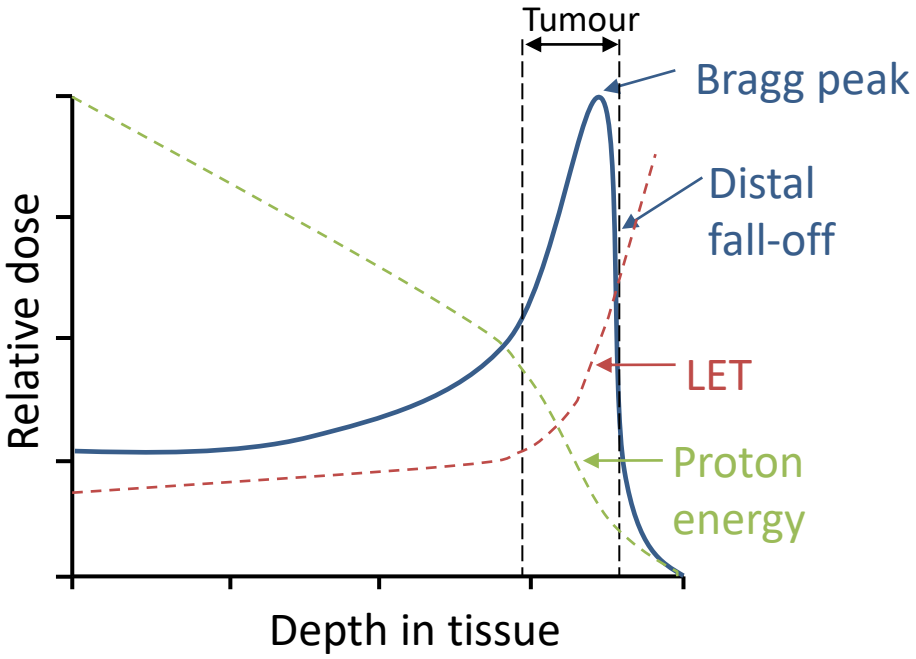
Summary of radiobiology research at Clatterbridge/Liverpool: The cellular response to complex DNA damage induced by proton beam therapy

Dr Jason Parsons
Cancer Research Centre
Department of Molecular and Clinical Cancer Medicine

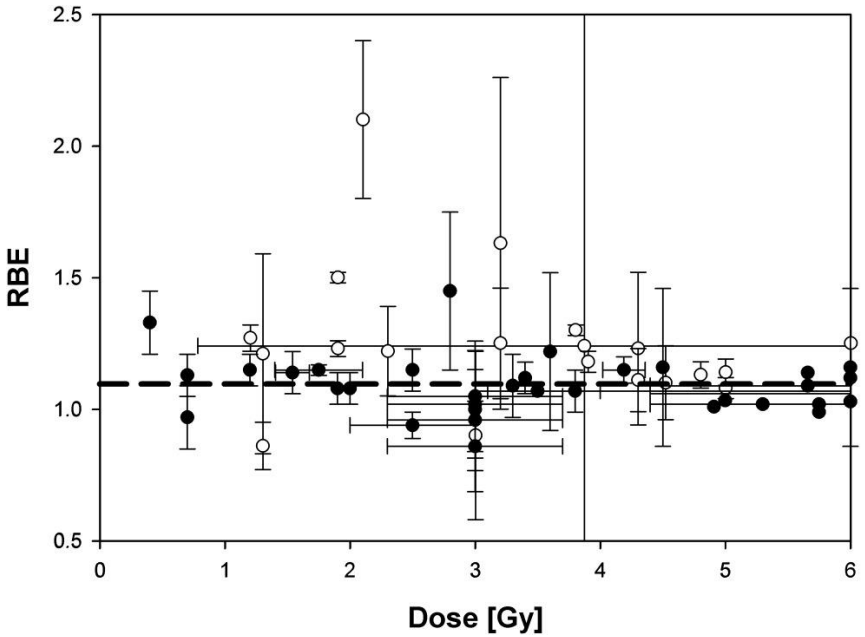
Head and neck squamous cell carcinoma (HNSCC)

- **6th most common cancer worldwide.**
- **Major contributory factors are smoking and drinking.**
- **Rapid rise in incidence of human papillomavirus (HPV-16) associated cancers of the oropharynx (OPSCC).**
- **HPV-positive tumours are more sensitive to radiotherapy and chemotherapy, thus improved prognosis, than HPV-negative tumours.**
- **HPV-positive OPSCC display defects in the repair of DNA double strand breaks (*Nickson et al., 2017, Oncotarget*).**

The radiobiology of protons



Vitti and Parsons (2019) Cancers



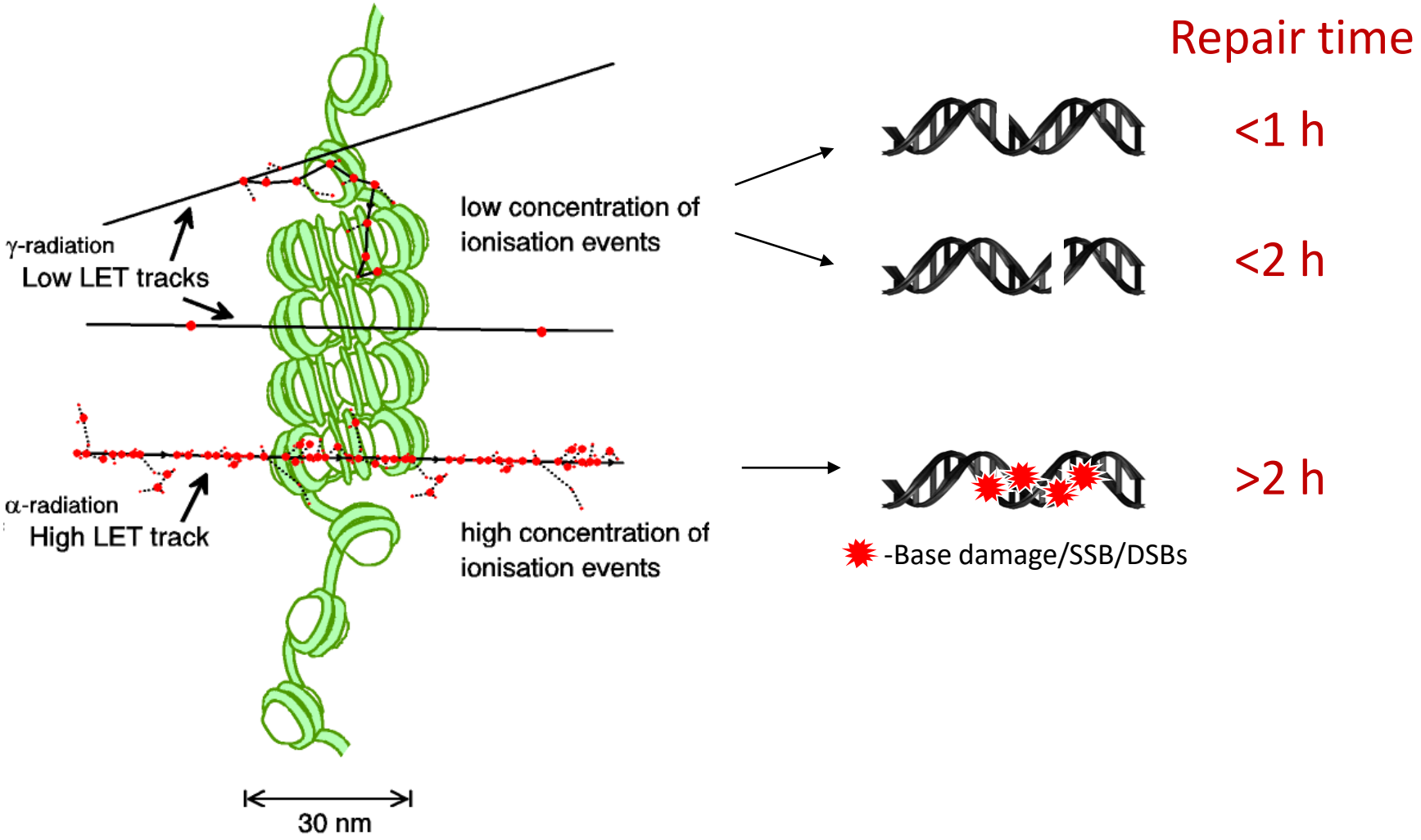
Taken from Paganetti and van Luijk (2013) Sem Rad Oncol

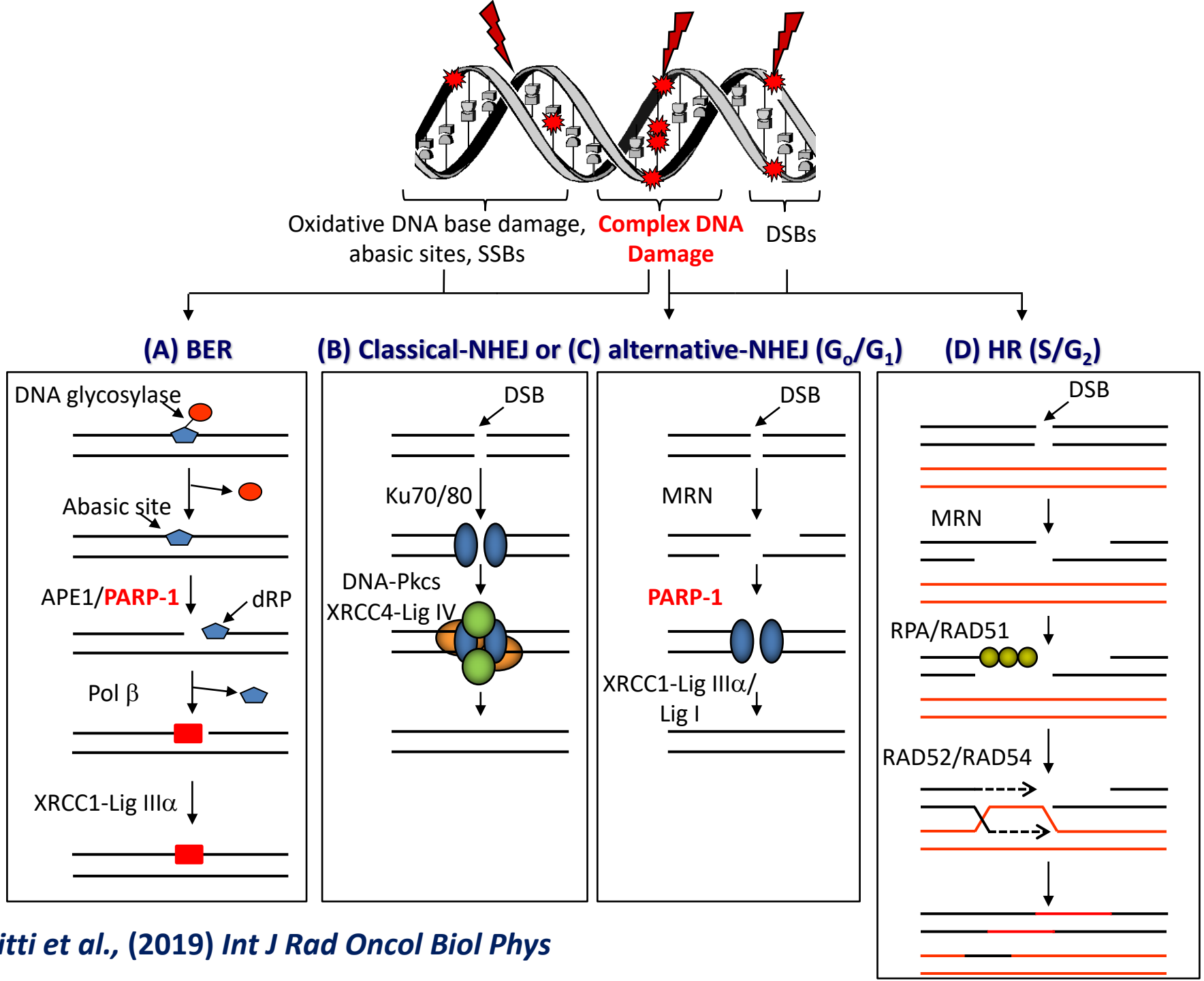
A constant relative biological effectiveness (RBE) value of 1.1 is used in clinical practice. However, there is a large uncertainty with using this approach as RBE is variable and dependent on many factors, including:-

- Proton energy (therefore linear energy transfer, LET) and dose.
- Radiosensitivity/radiobiology of the tissue.
- Biological end-point examined (e.g. clonogenic survival, tumour growth delay).

Therefore, further research exploiting the biological impact of protons is vital for investigating RBE, and thus improving clinical use of proton beam therapy.

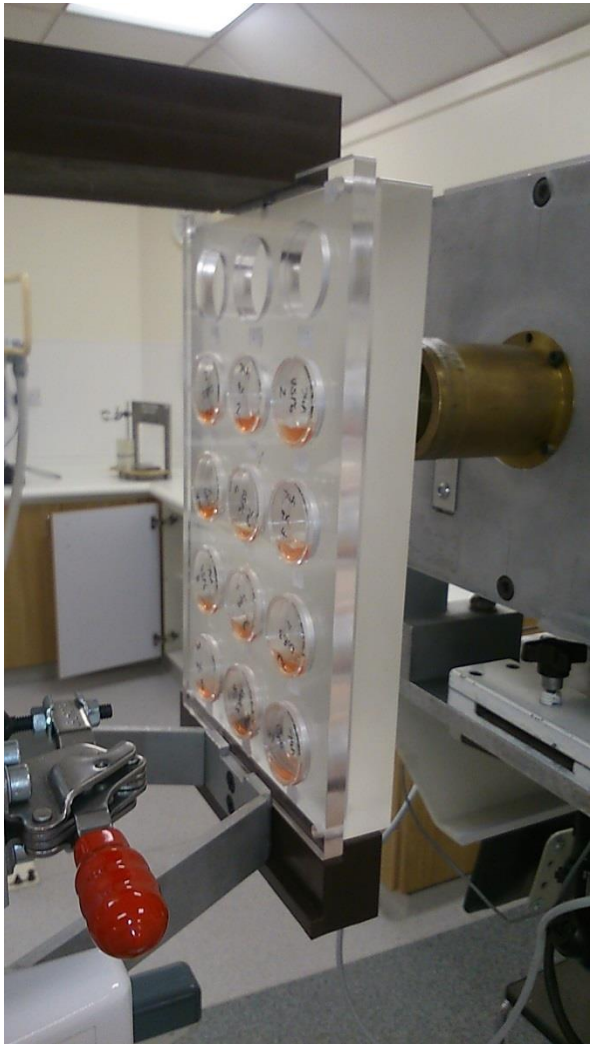
The critical cellular target for IR is DNA and damage complexity is dependant on ionisation density





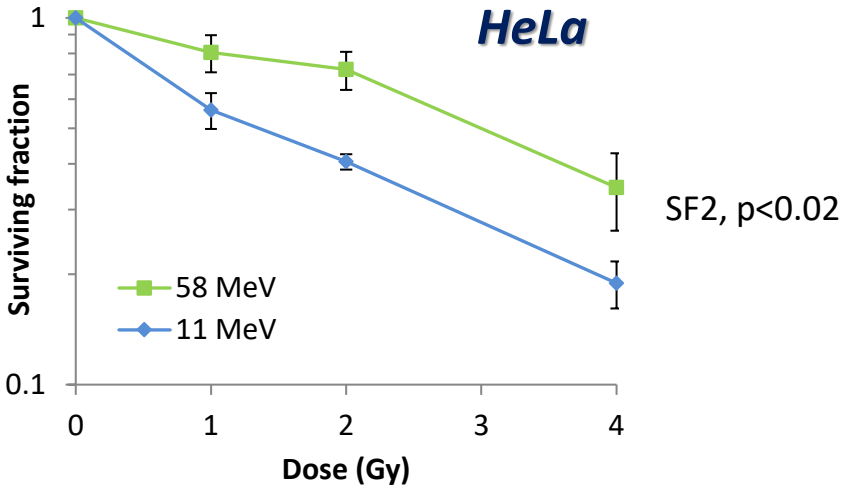
Vitti et al., (2019) *Int J Rad Oncol Biol Phys*

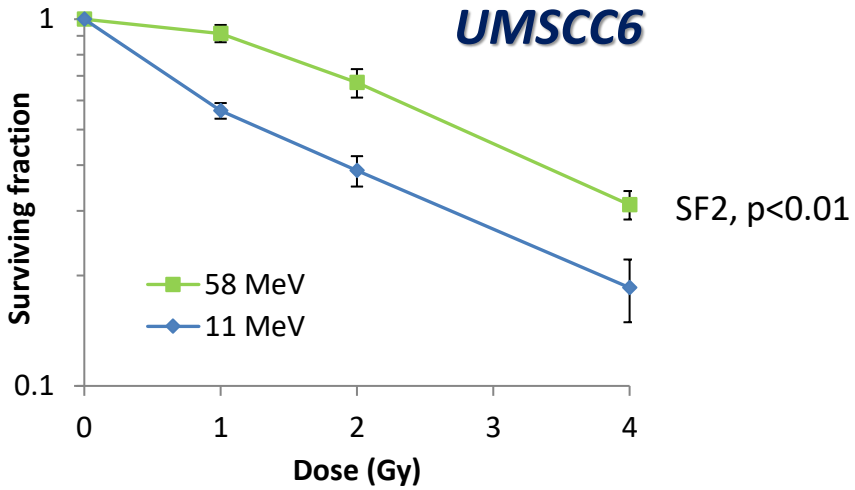
Proton beam and radiobiology facilities at the Clatterbridge Cancer Centre (CCC)

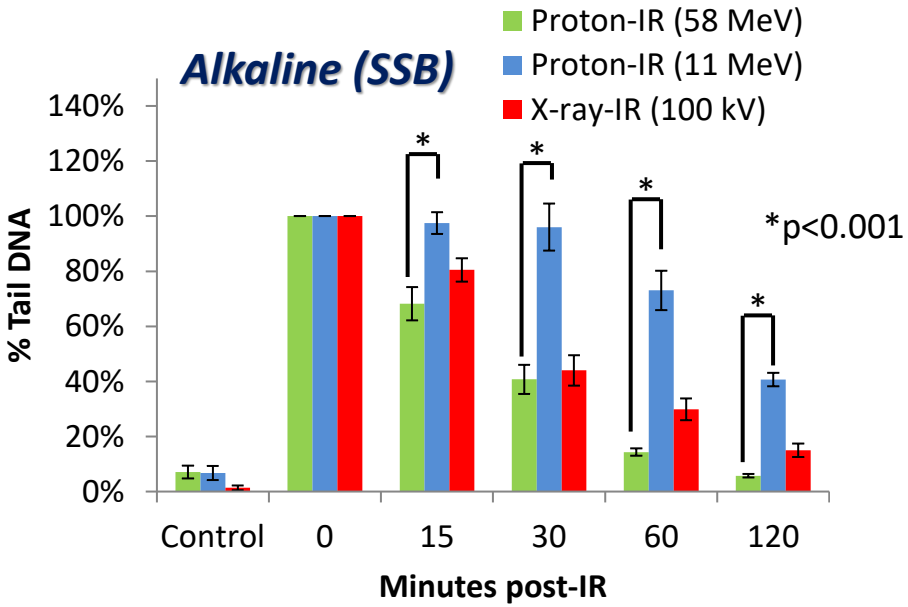


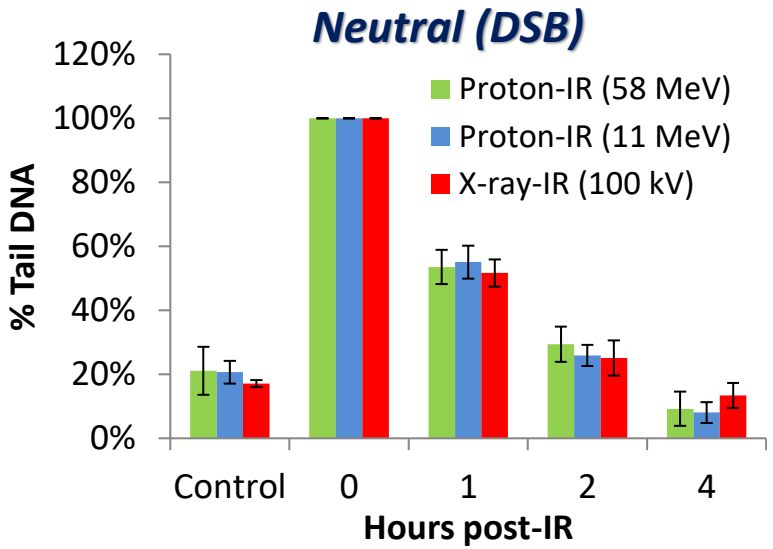
Collaboration with Andrzej Kacperek

Low energy protons cause a decrease in cell survival through delayed SSB repair





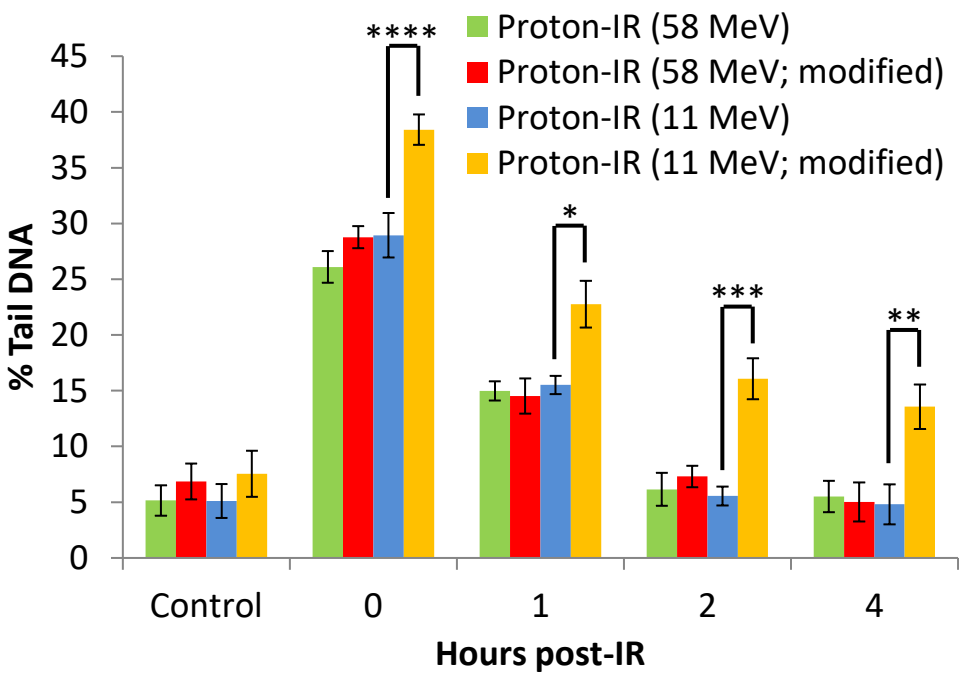




Carter et al., (2018) *Int J Rad Oncol Biol Phys*

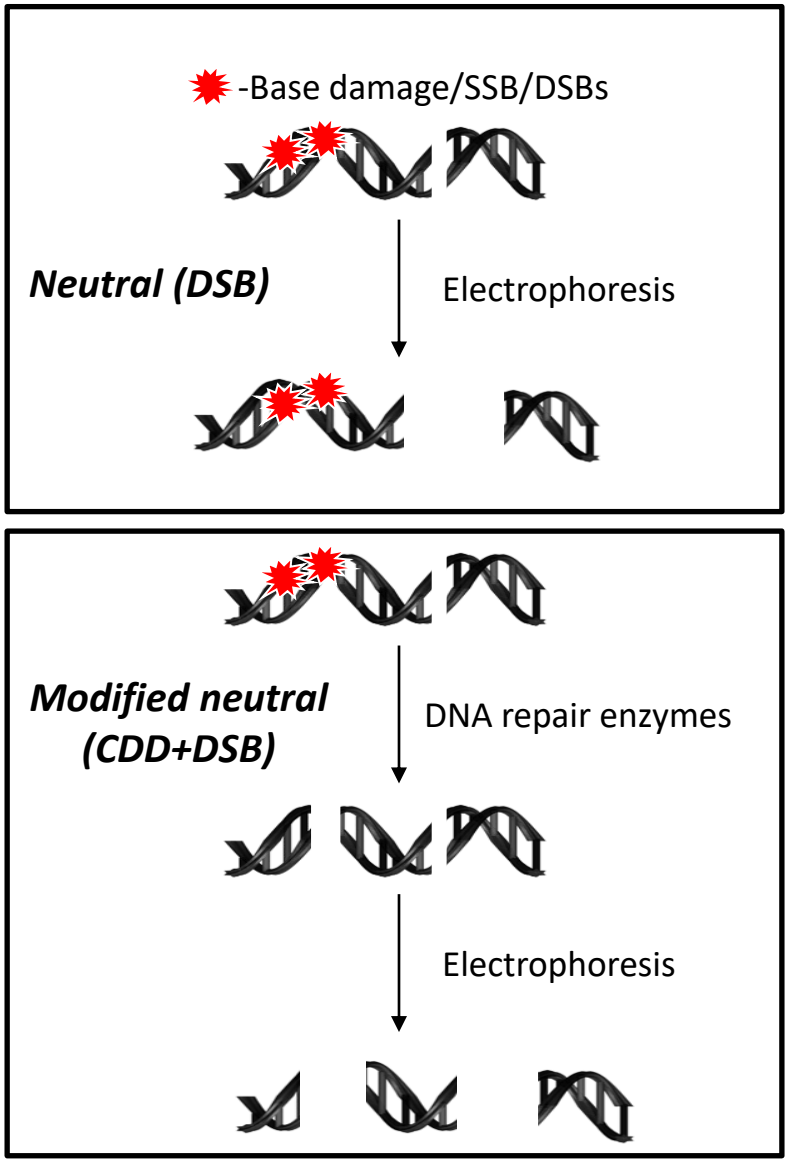
58 MeV (1 keV/µm); 11 MeV (12 keV/µm)

Low energy protons induce the formation of CDD



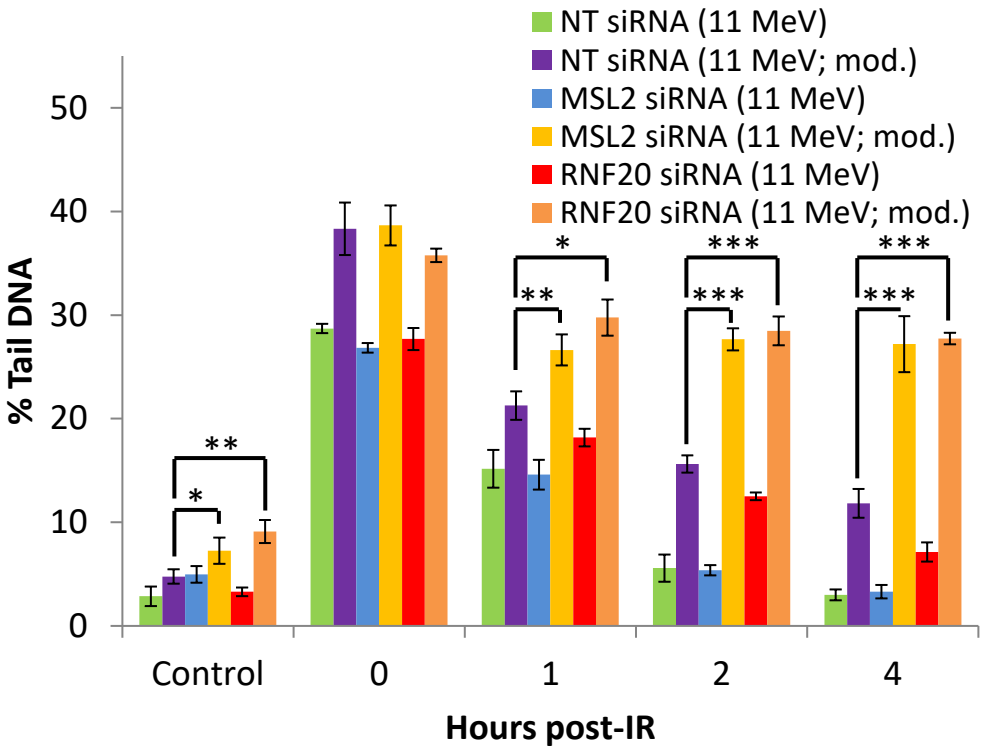
*p<0.02, **p<0.01, ***p<0.005, ****p<0.001

Holt and Georgakilas, 2007



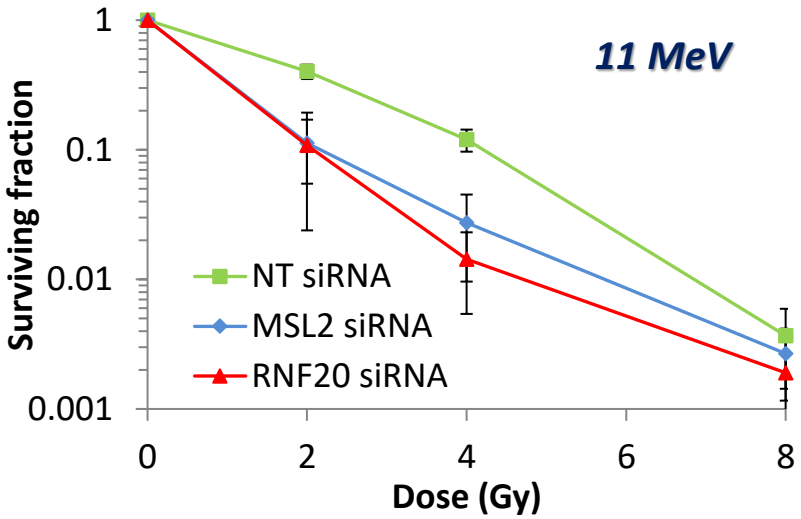
Carter et al., (2018) *Int J Rad Oncol Biol Phys*

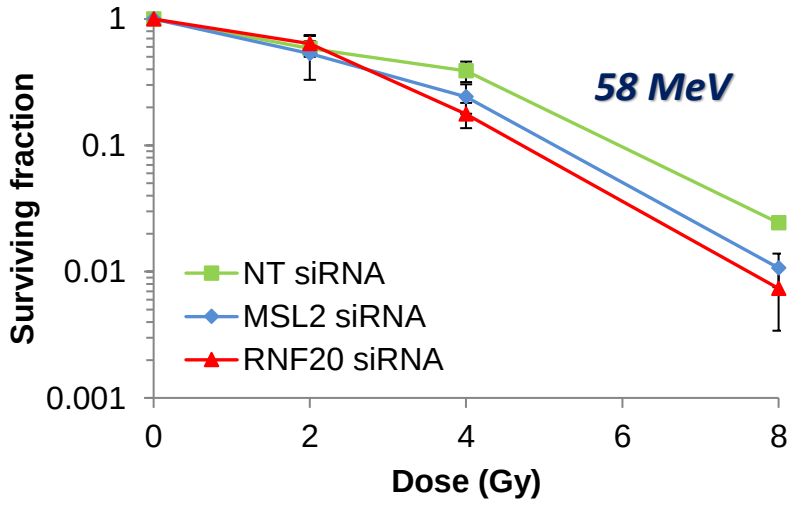
MSL2 and RNF20/40 regulate the repair of CDD and radiosensitivity following low energy protons



*p<0.02, **p<0.005, ***p<0.001

SF2, p<0.01 (MSL2), p<0.05 (RNF20)





HeLa

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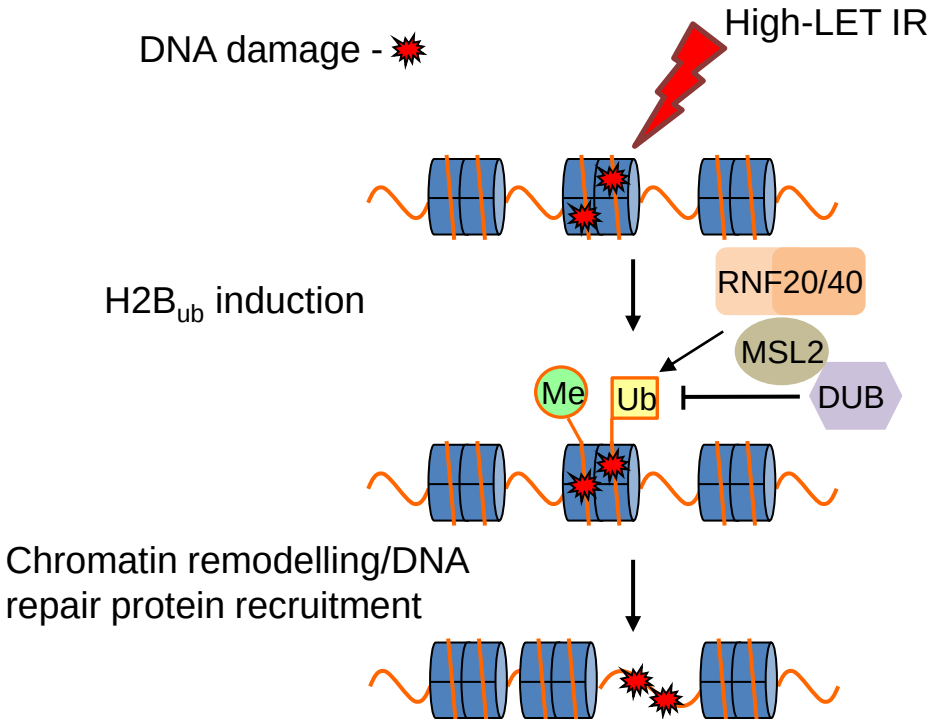
Model for the recognition and repair of CDD in chromatin



Biology Contribution

Complex DNA Damage Induced by High Linear Energy Transfer Alpha-Particles and Protons Triggers a Specific Cellular DNA Damage Response

Rachel J. Carter, PhD,* Catherine M. Nickson, PhD,* James M. Thompson, PhD,† Andrzej Kacperek, PhD,‡ Mark A. Hill, PhD,† and Jason L. Parsons, PhD *

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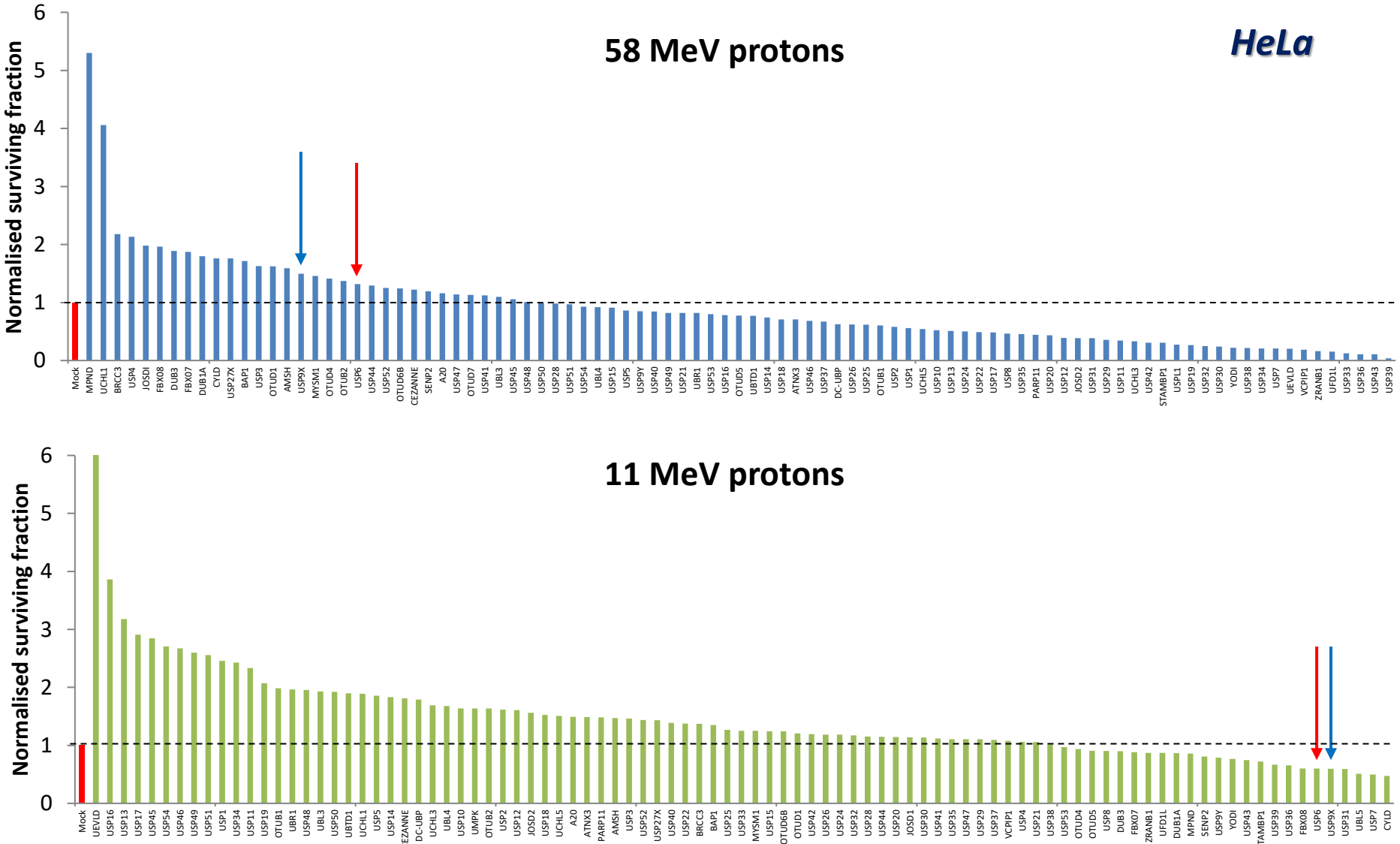
Modulation of proton-induced cellular sensitivity following DUB siRNA knockdown



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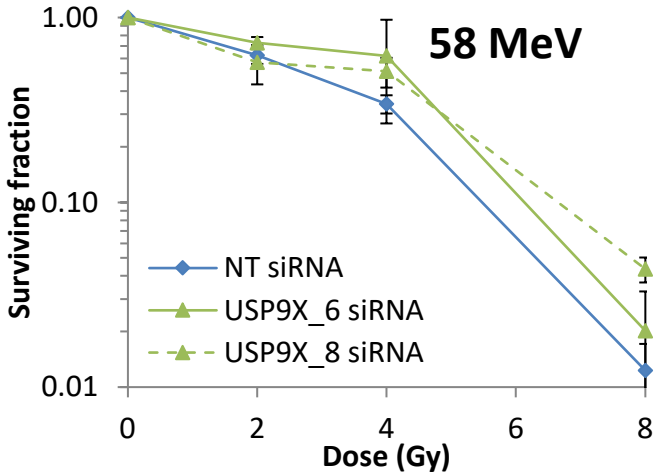
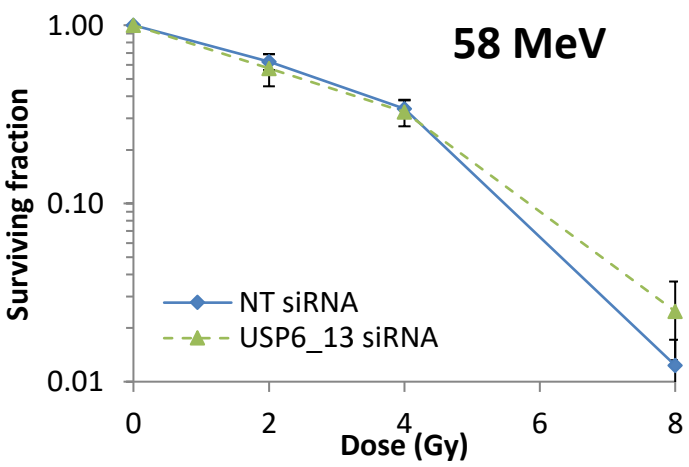
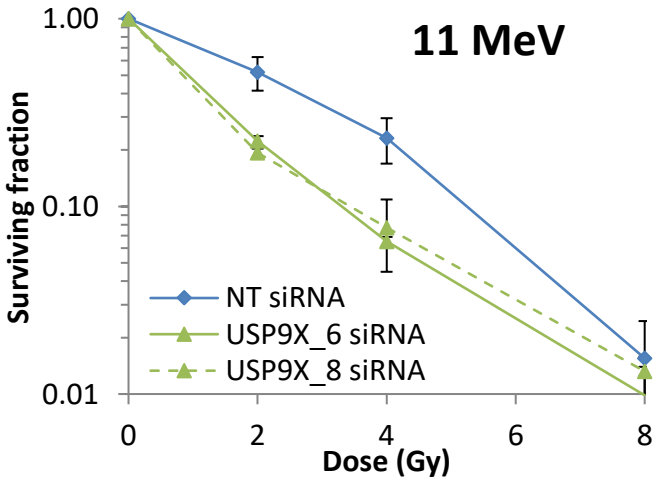
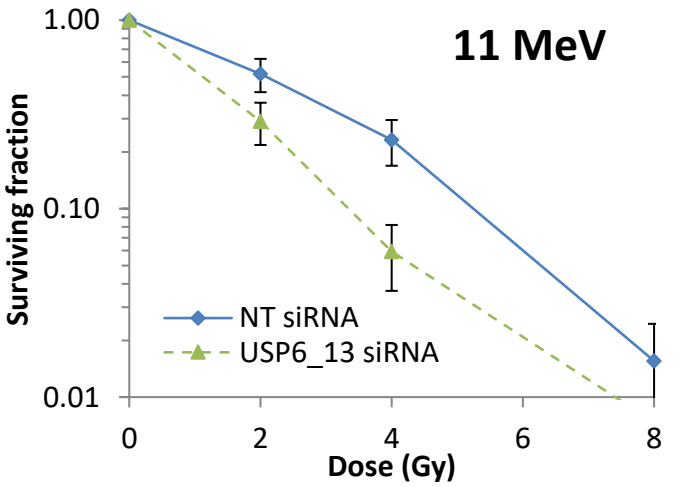
USP9X and USP6 control in the cellular response to low energy protons

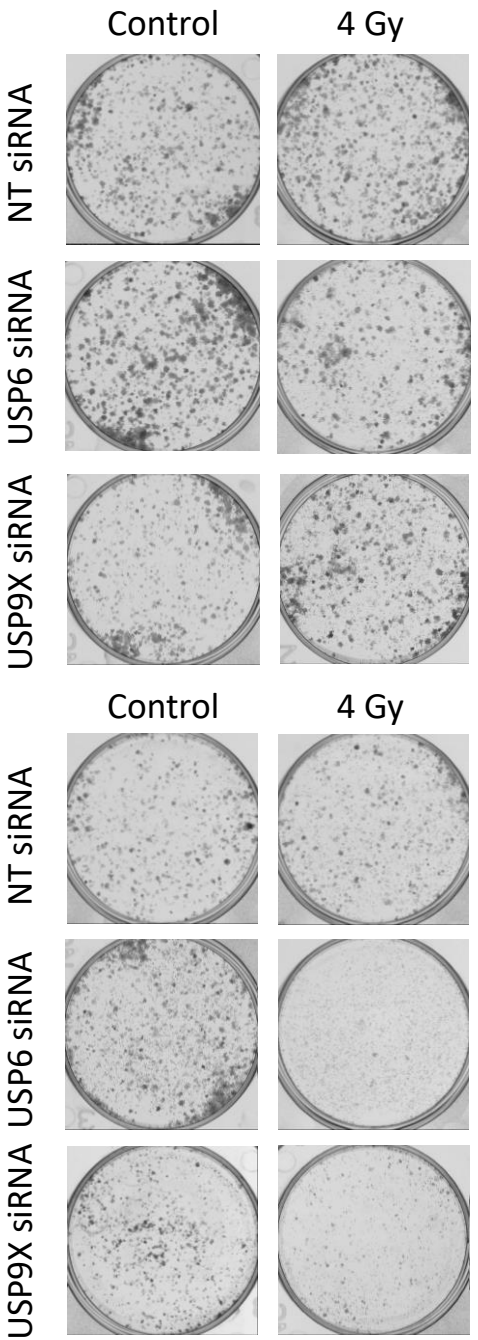
UMSCC74A











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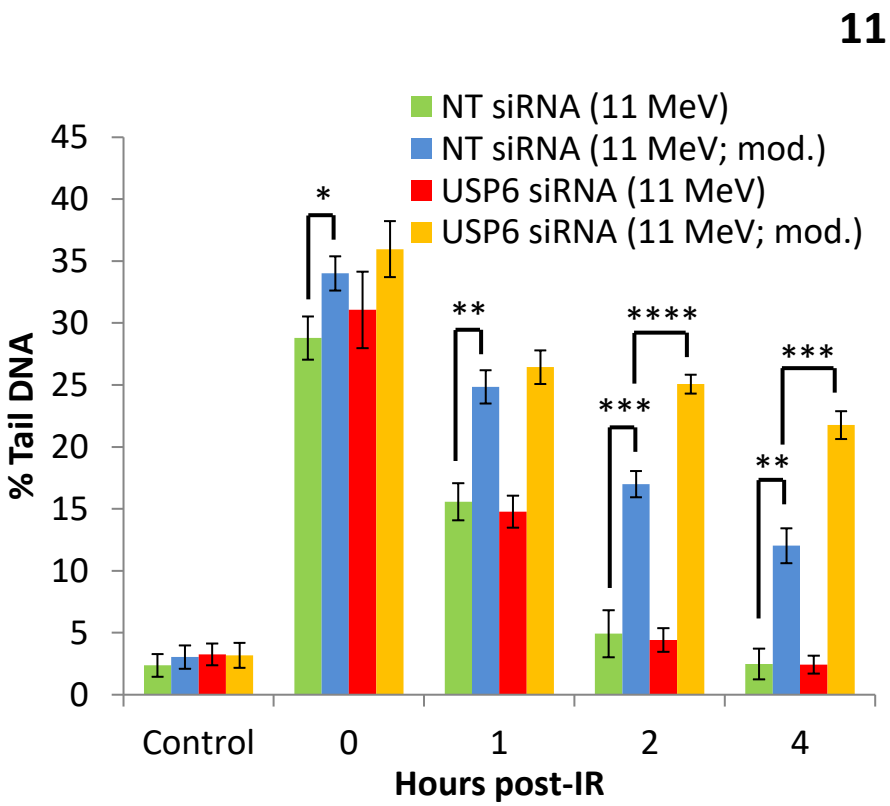
Knockdown of USP6 causes persistence of complex DNA damage, G2/M arrest and PARP-1 protein instability in response to low energy protons



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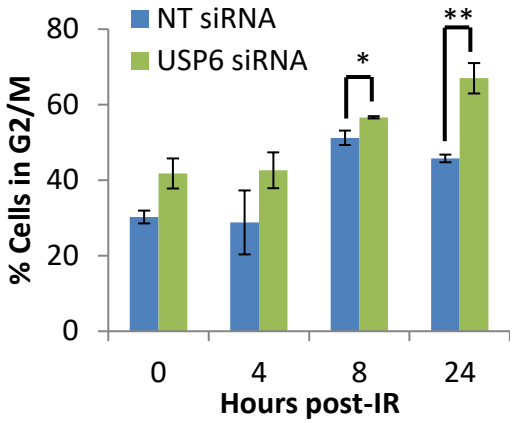
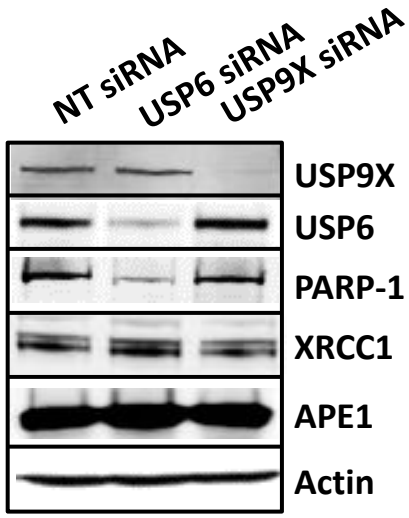


*p<0.05
**p<0.01,



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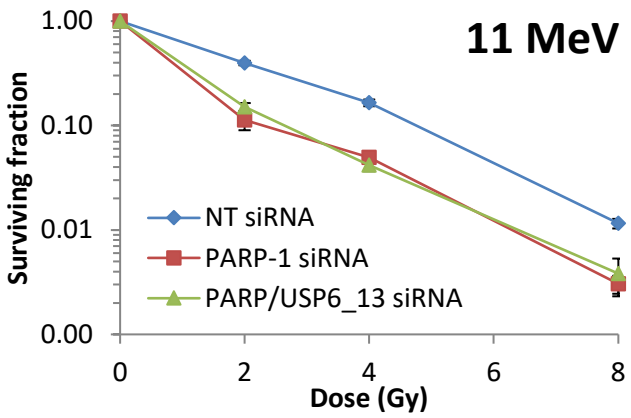
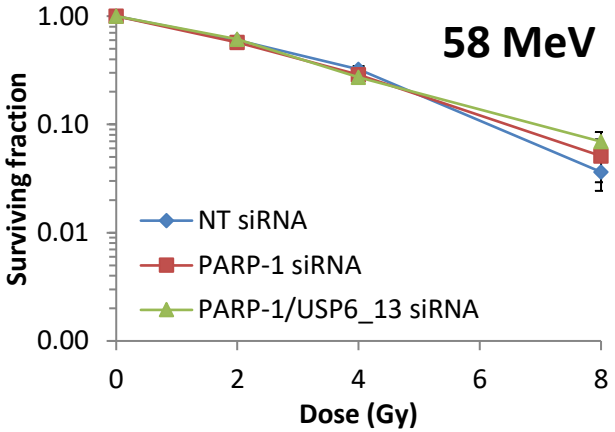
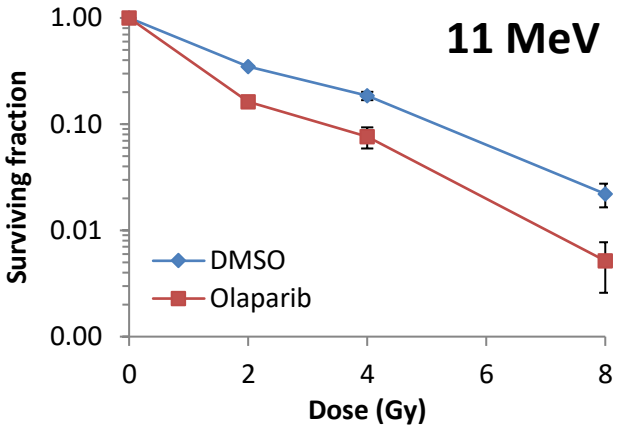
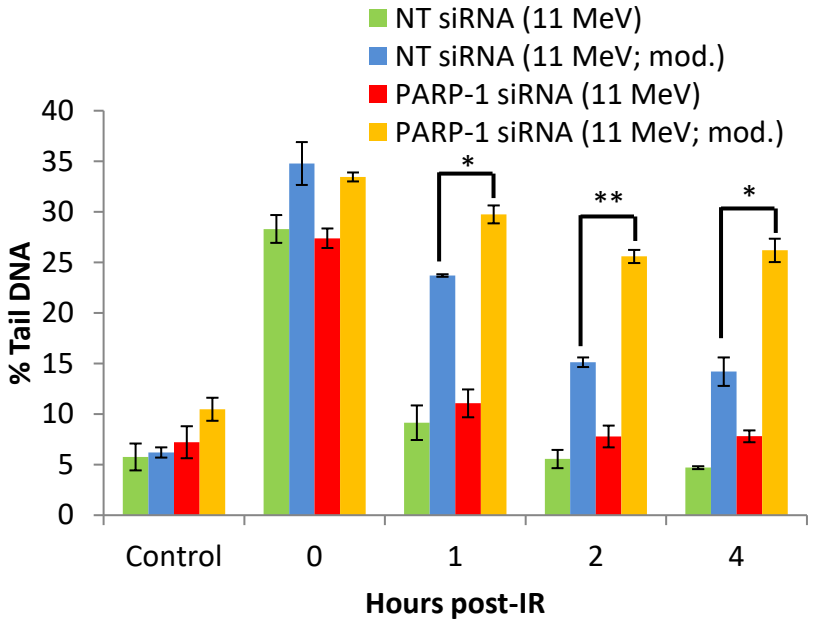
Knockdown/inhibition of PARP-1 sensitises cells to low energy protons through deficiencies in CDD repair









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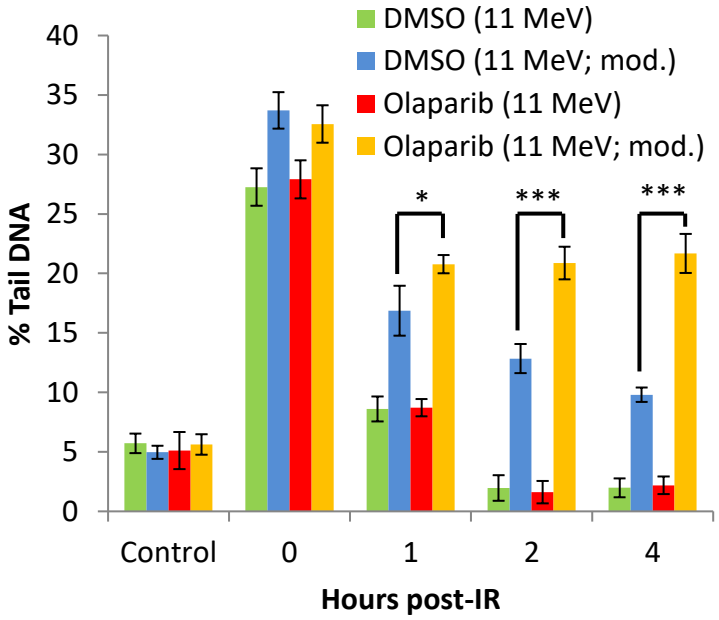
**p<0.005
***p<0.001



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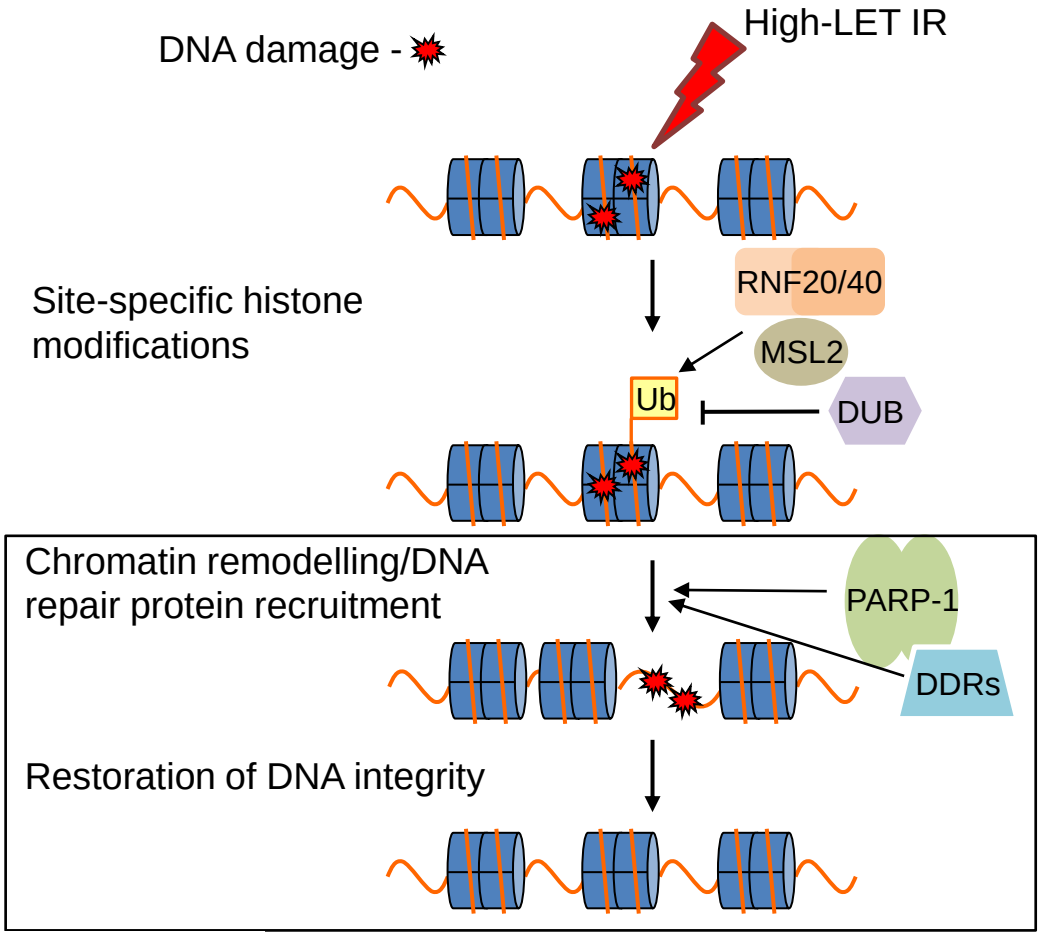
Model for the recognition and repair of CDD in chromatin



Biology Contribution

Characterisation of Deubiquitylating Enzymes in the Cellular Response to High-LET Ionizing Radiation and Complex DNA Damage

Rachel J. Carter, PhD,* Catherine M. Nickson, PhD,* James M. Thompson, PhD,† Andrzej Kacperek, PhD,† Mark A. Hill, PhD,† and Jason L. Parsons, PhD*

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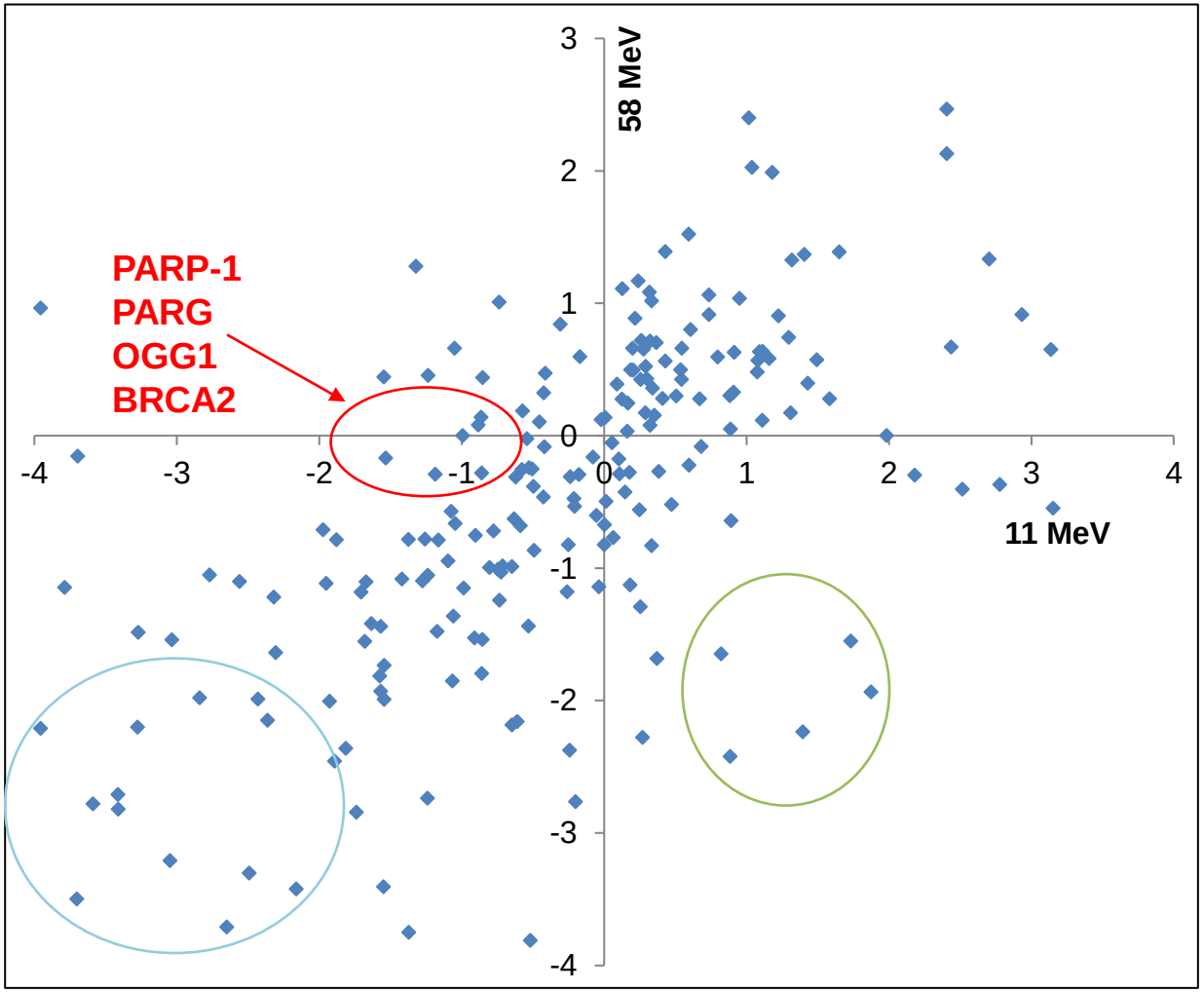
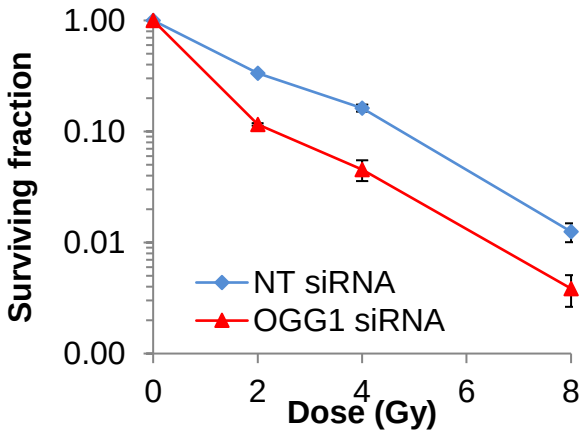
Modulation of proton-induced cellular sensitivity following DDR siRNA knockdown

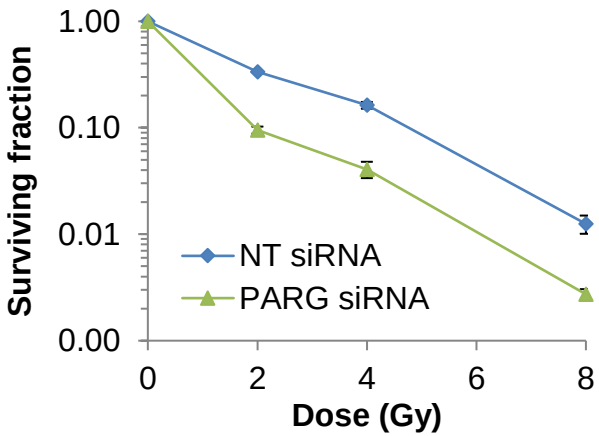


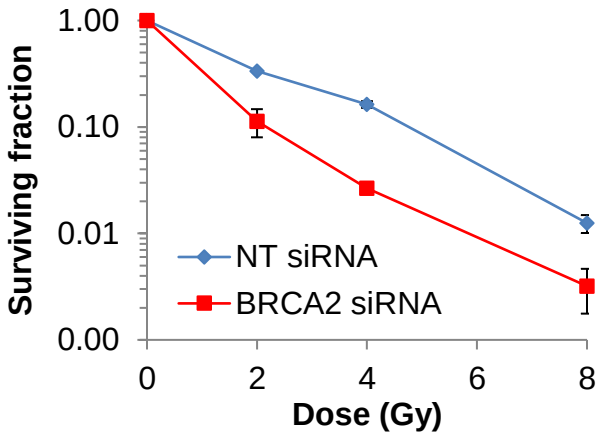
Nickson, Fabbrizi et al., unpublished

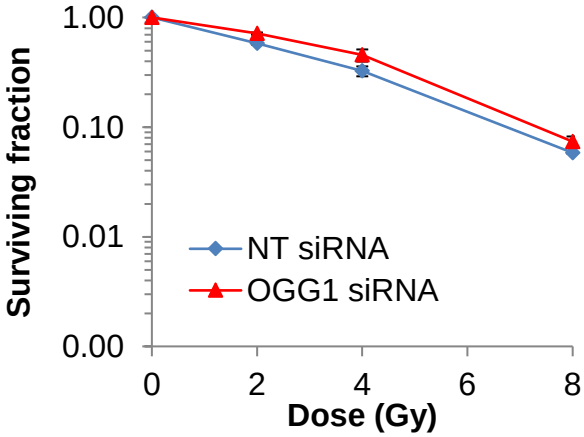
Knockdown of OGG1, PARG and BRCA2 sensitises cells to low energy protons



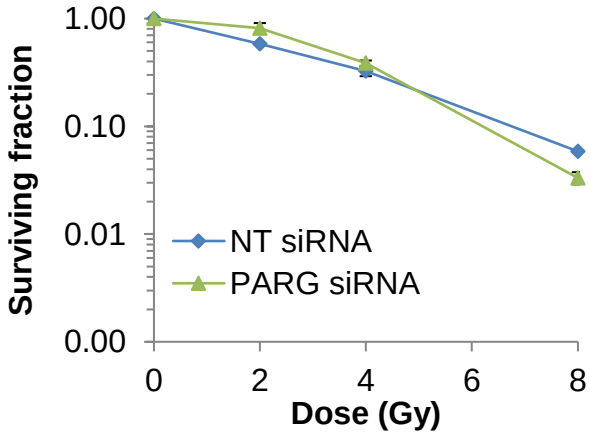
11 MeV

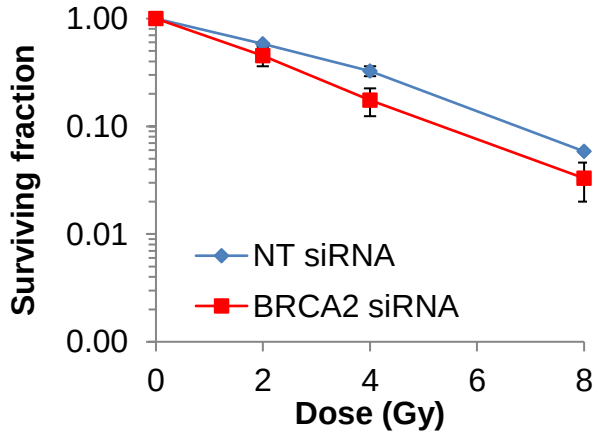






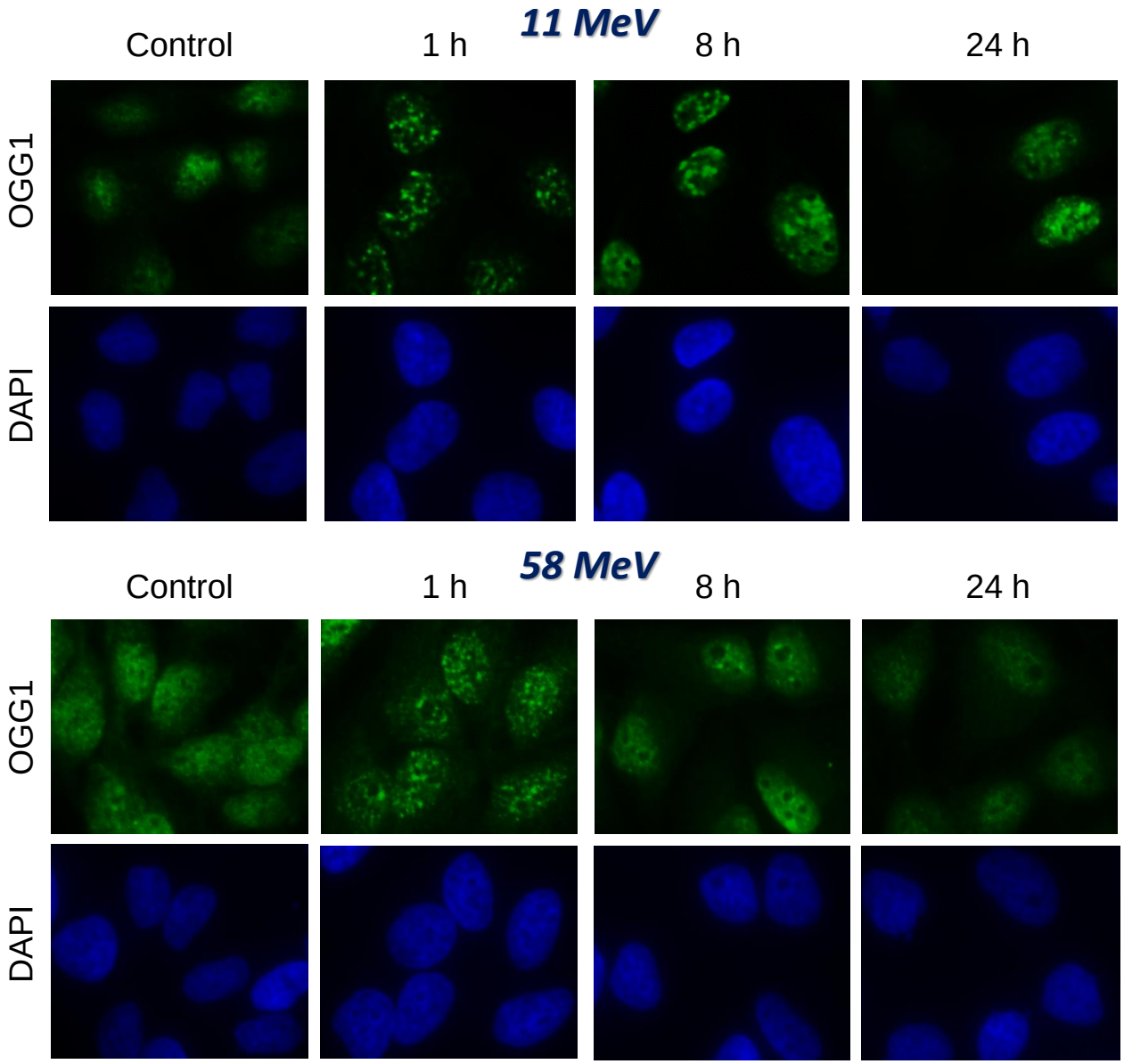
58 MeV

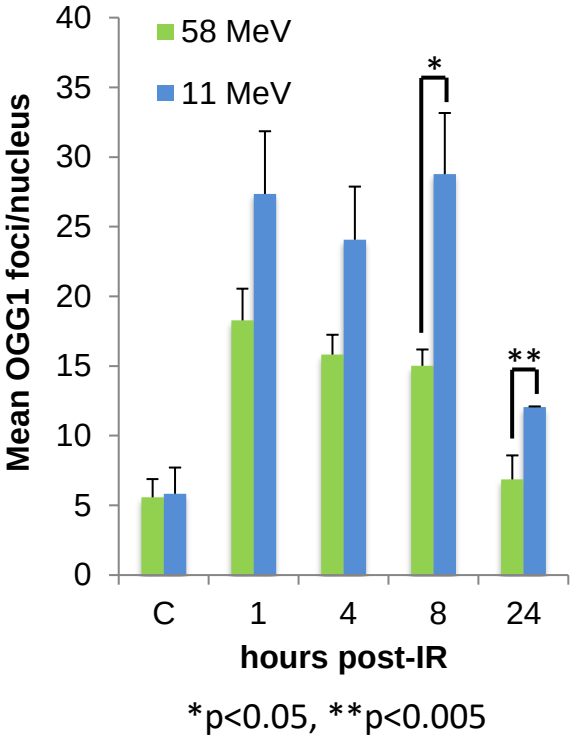




***Nickson, Fabbrizi et al.*, unpublished**

OGG1 localises to DNA repair foci that persist following high-LET protons

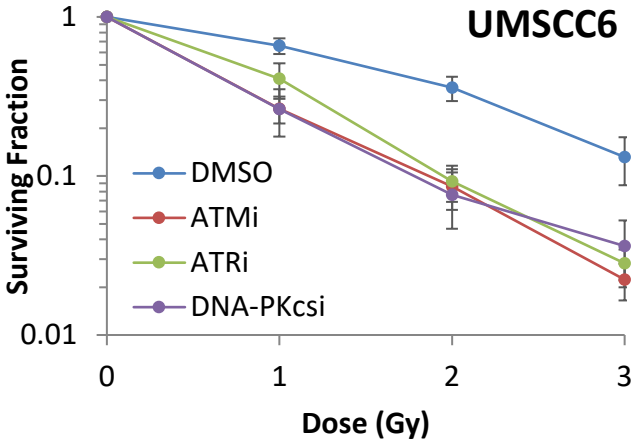


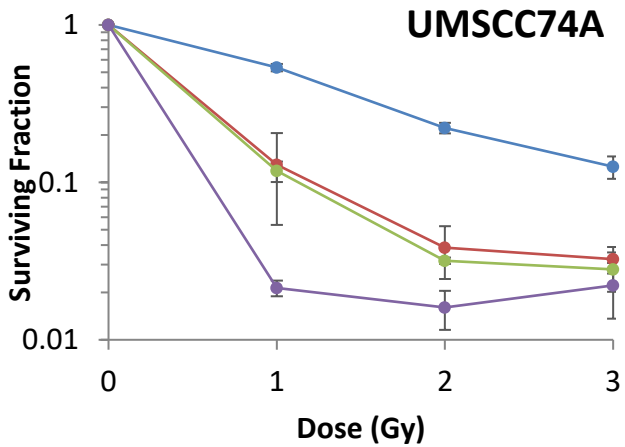


*p<0.05, **p<0.005

Targeting DNA double strand break repair in HNSCC with photons and protons

X-rays

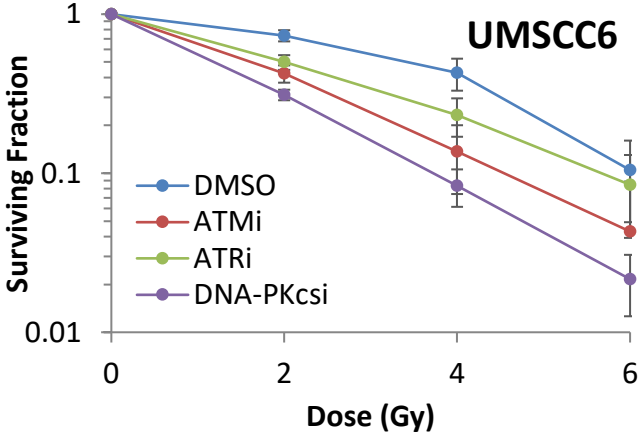


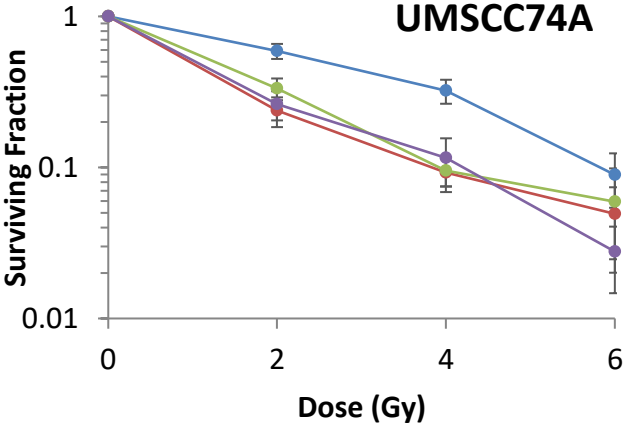


Dose enhancement ratios (DER)

Inhibitor	6	74A	47	090
ATM	2.06	1.91	1.38	1.15
ATR	1.91	2.01	1.36	1.02
DNA-Pkcs	1.93	2.39	1.69	1.36

Protons





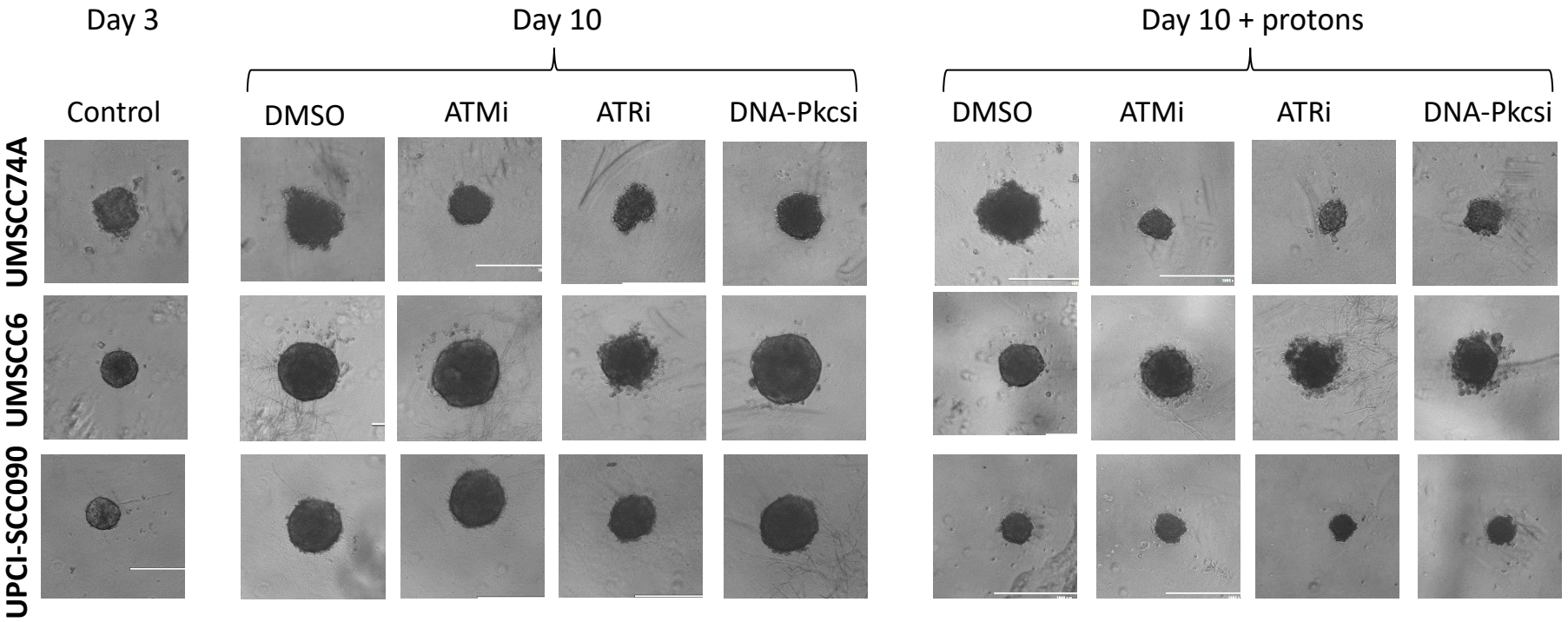
Dose enhancement ratios (DER)

Inhibitor	6	74A	47	090
ATM	1.62	1.52	1.49	1.24
ATR	1.25	1.42	1.28	1.30
DNA-Pkcs	2.01	1.64	1.38	1.32

Vitti and Parsons, unpublished

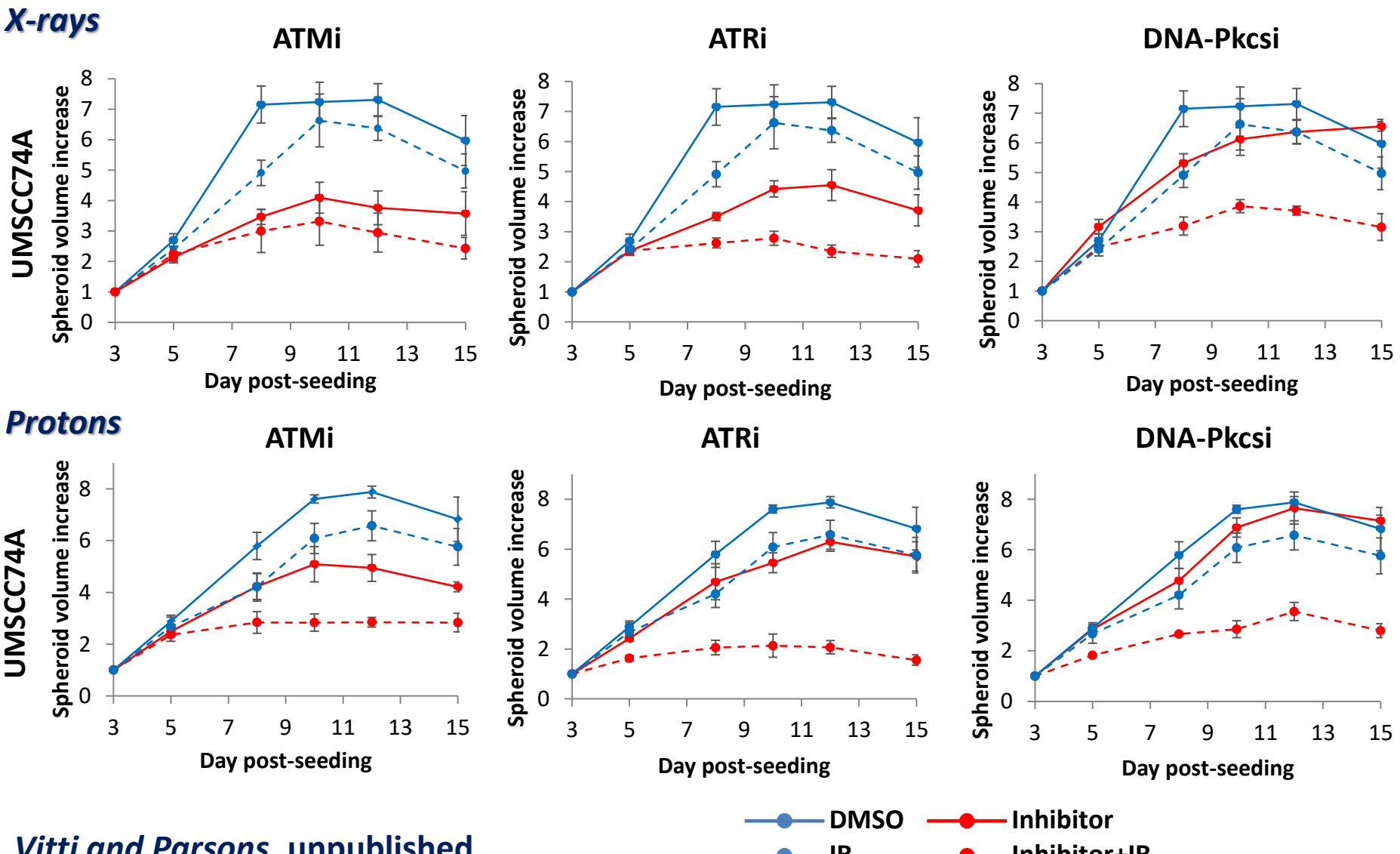
KU-55933 (ATM); VE-821 (ATR); KU-57788 (DNA-Pk)

Targeting DNA double strand break repair in HNSCC with photons and protons



Vitti and Parsons, **unpublished**

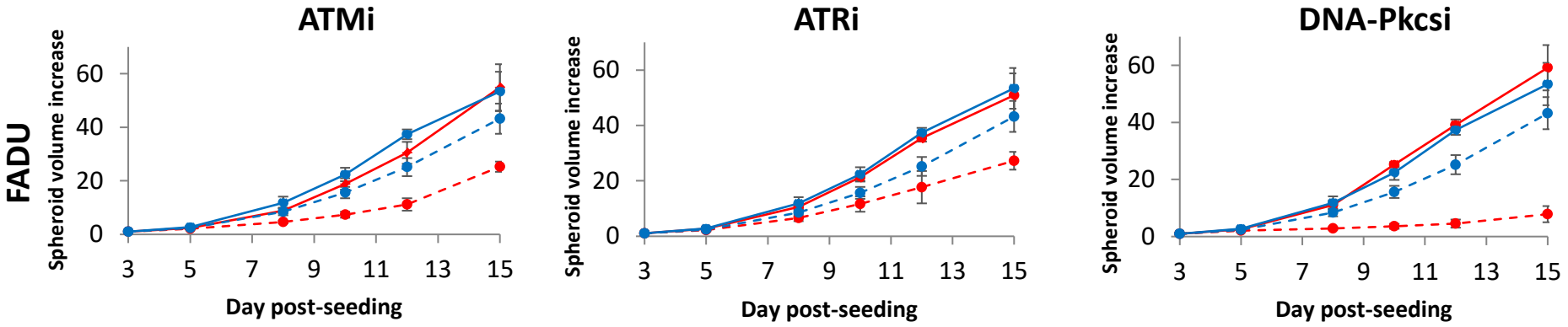
Targeting DNA double strand break repair in HNSCC with photons and protons



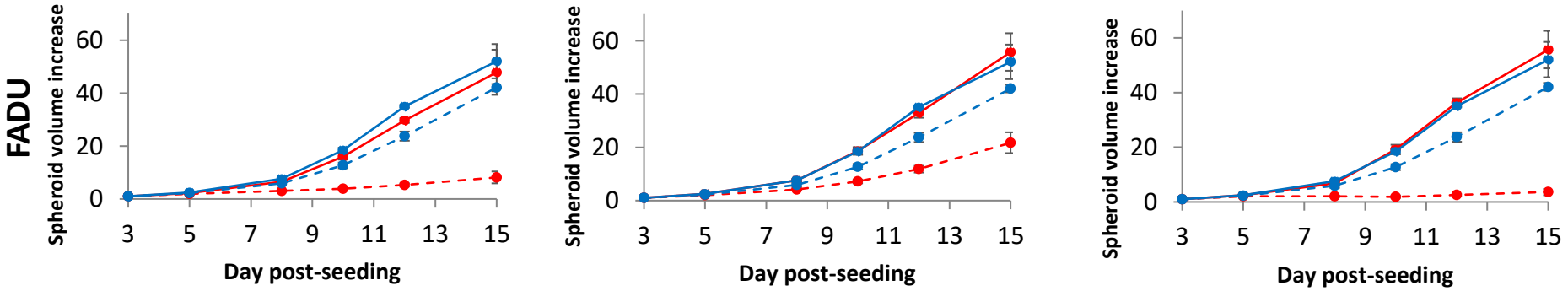
Vitti and Parsons, unpublished

Targeting DNA double strand break repair in HNSCC with photons and protons

X-rays



Protons



DMSO — Inhibitor

IR — Inhibitor+IR

Vitti and Parsons, **unpublished**

Summary

- **High-LET protons (at Bragg peak distal end), can generate complex DNA complex that increases cellular radiosensitivity.**
- **Repair of complex DNA damage induced by high-LET protons is promoted by histone H2B K120 ubiquitylation mediated by RNF20/40 and MSL2.**
- **A subset of DUBs (USP9X and USP6) control the cellular response to complex DNA damage induced by high-LET protons.**
- **Synergy between PARP inhibition/loss and complex DNA damage induction in promoting cancer cell killing.**
- **Specific DDR proteins and pathways co-ordinate the repair of complex DNA damage generated by high-LET protons.**

Acknowledgements

Rachel Carter
Katie Nickson
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Terpsi Vitti
Eleanor Madders
Chumin Zhou
Jonathan Hughes
Sifaddin Konis
Rachael Clifford
Hayley Fowler
Rumana Hussain

Institute of Translational Medicine
Mike Clague, Sylvie Urbe

Clatterbridge Cancer Centre
Andrzej Kacperek

Oxford Institute for Radiation Oncology
Mark Hill, James Thompson

Imperial College London
Ken Long, Ajit Kurup

University of Birmingham
Stuart Green, Ben Phoenix