UNIVERSITY OF LIVERPOOL

The radiobiology of proton beam therapy and impact on DNA damage repair

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nwcr NORTH WEST CANCER RESEARCH

Group Research Topics

- **Regulation of base excision repair proteins by ubiquitylation.**

Williams *et al* (2018) *Mol Cell Biol*

Edmonds *et al* (2017) *Nucleic Acids Res*

Carter and Parsons (2016) *Mol Cell Biol*

Parsons *et al* (2008, 2011) *Mol Cell*; Parsons *et al* (2009) *EMBO J*

- **Radiobiology of head and neck squamous cell carcinoma, colorectal cancer and uveal melanoma.**

Bowden *at al* (2018) *J. Proteomics*

Nickson *et al* (2017) *Oncotarget*

- **The radiobiology of proton beam therapy in the context of DNA damage and repair.**

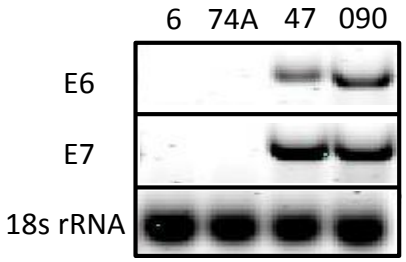
Carter *et al* (2018) *Int J Rad Oncol Biol Phys*

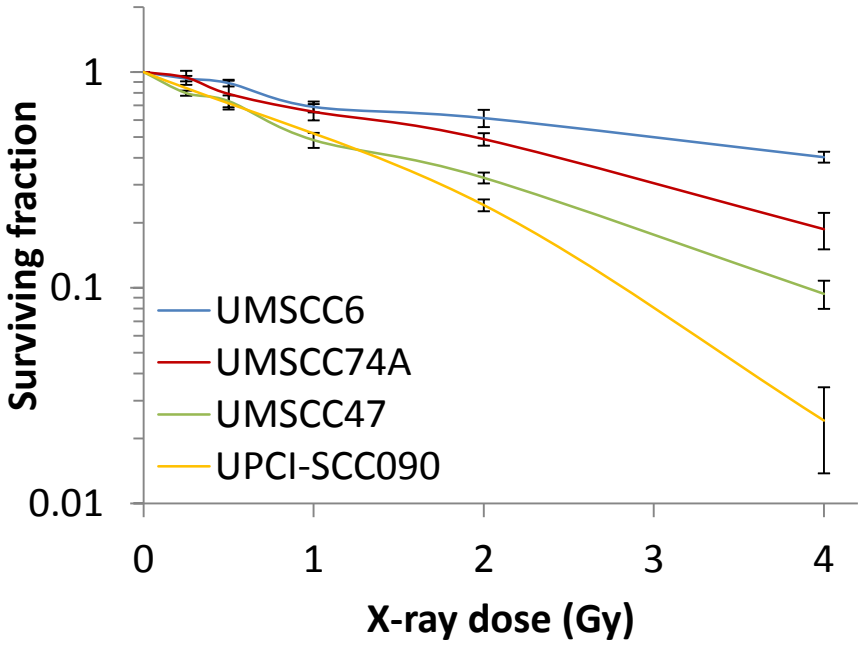
Carter *et al* (2019) *Int J Rad Oncol Biol Phys*

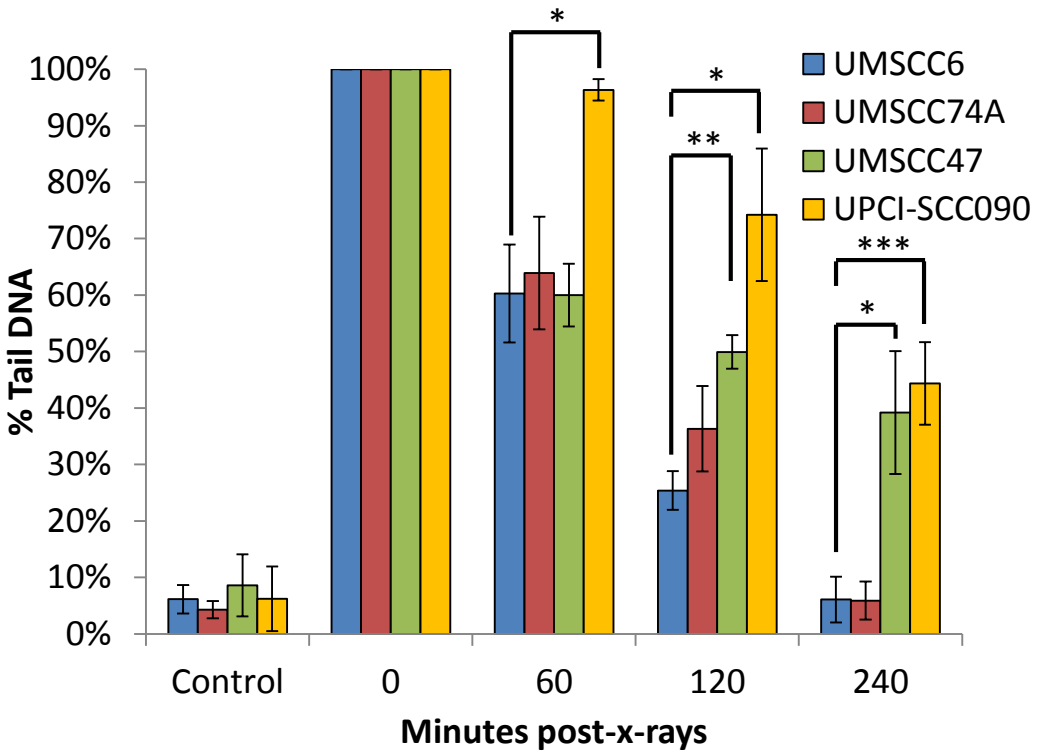
Head and neck squamous cell carcinoma (HNSCC)

- 6th most common cancer worldwide.
- Major contributory factors are smoking and drinking.
- Rapid rise in incidence of human papilloma virus (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.
- Underlying cellular mechanisms responsible for this effect are unclear.

Cells derived from HPV-positive OPSCC are more radiosensitive than HPV-negative cells due to defective DSB repair



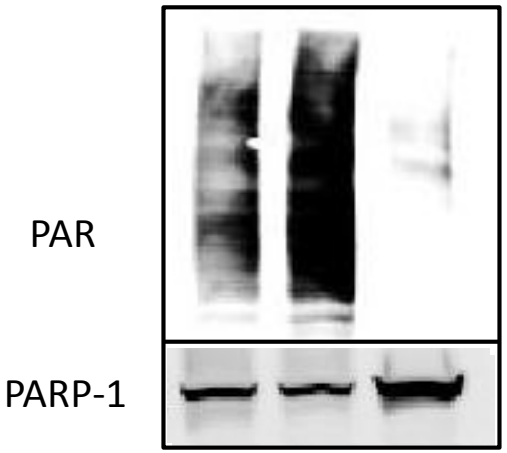




Nickson et al., (2017) Oncotarget

Radiosensitivity of HPV-negative OPSCC cells can be increased using PARP inhibitor

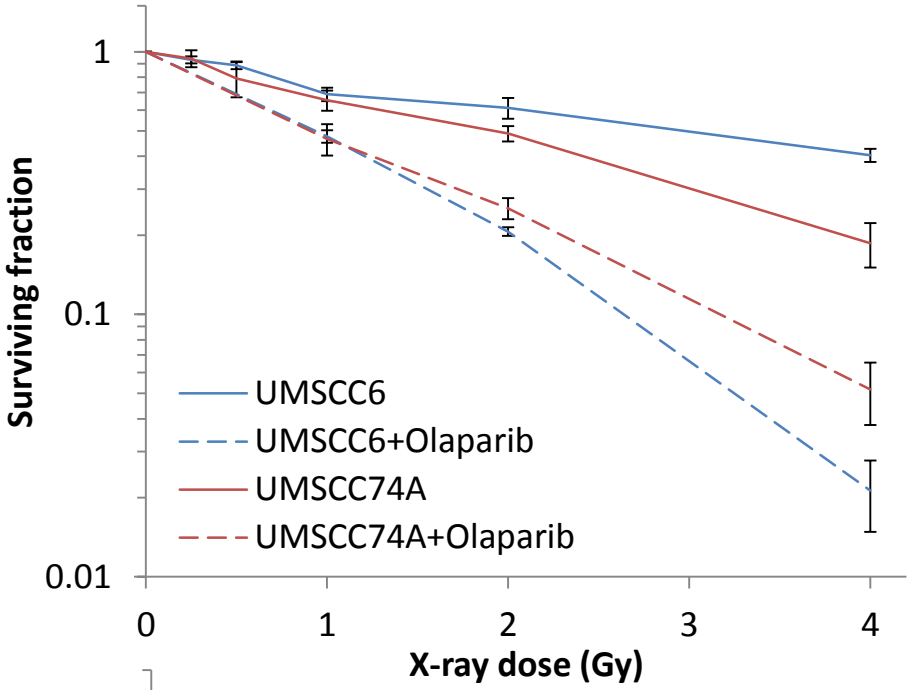
X-ray IR (4 Gy)	-	+	+
Olaparib (0.1 µM)	-	-	+

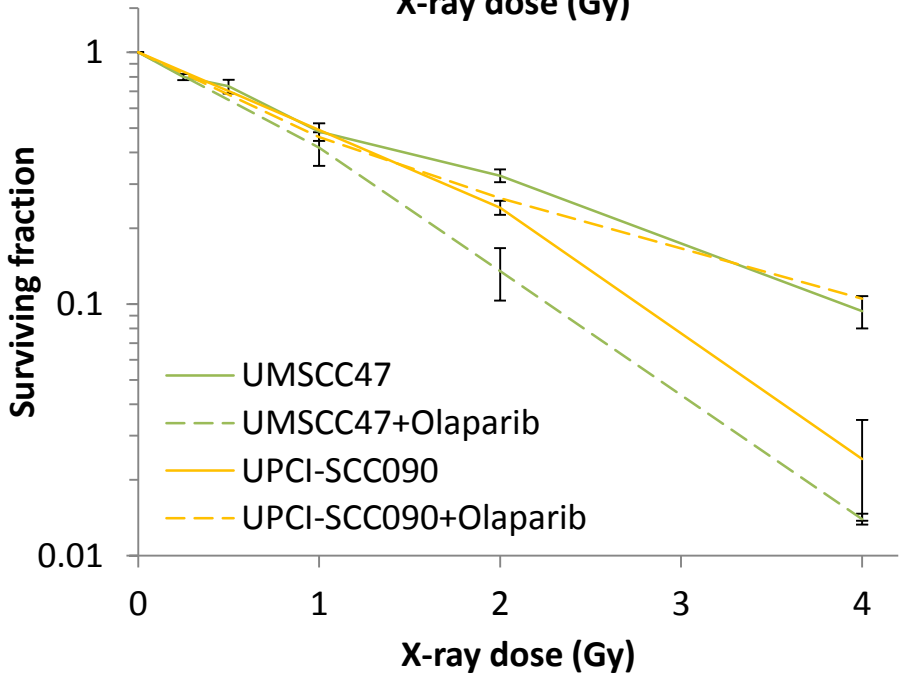


UPCI-SCC090

DER – 3.34 (UMSCC6), 1.76 (UMSCC74A), 1.51 (UMSCC47)

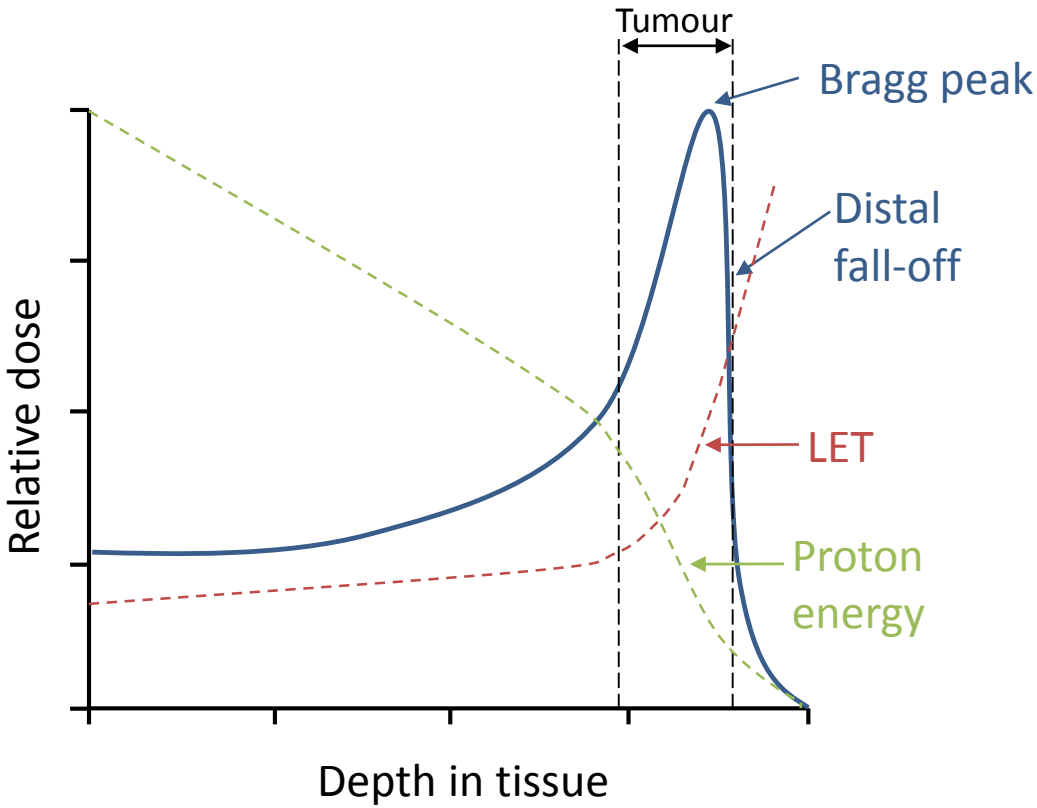
Nickson et al., (2017) Oncotarget





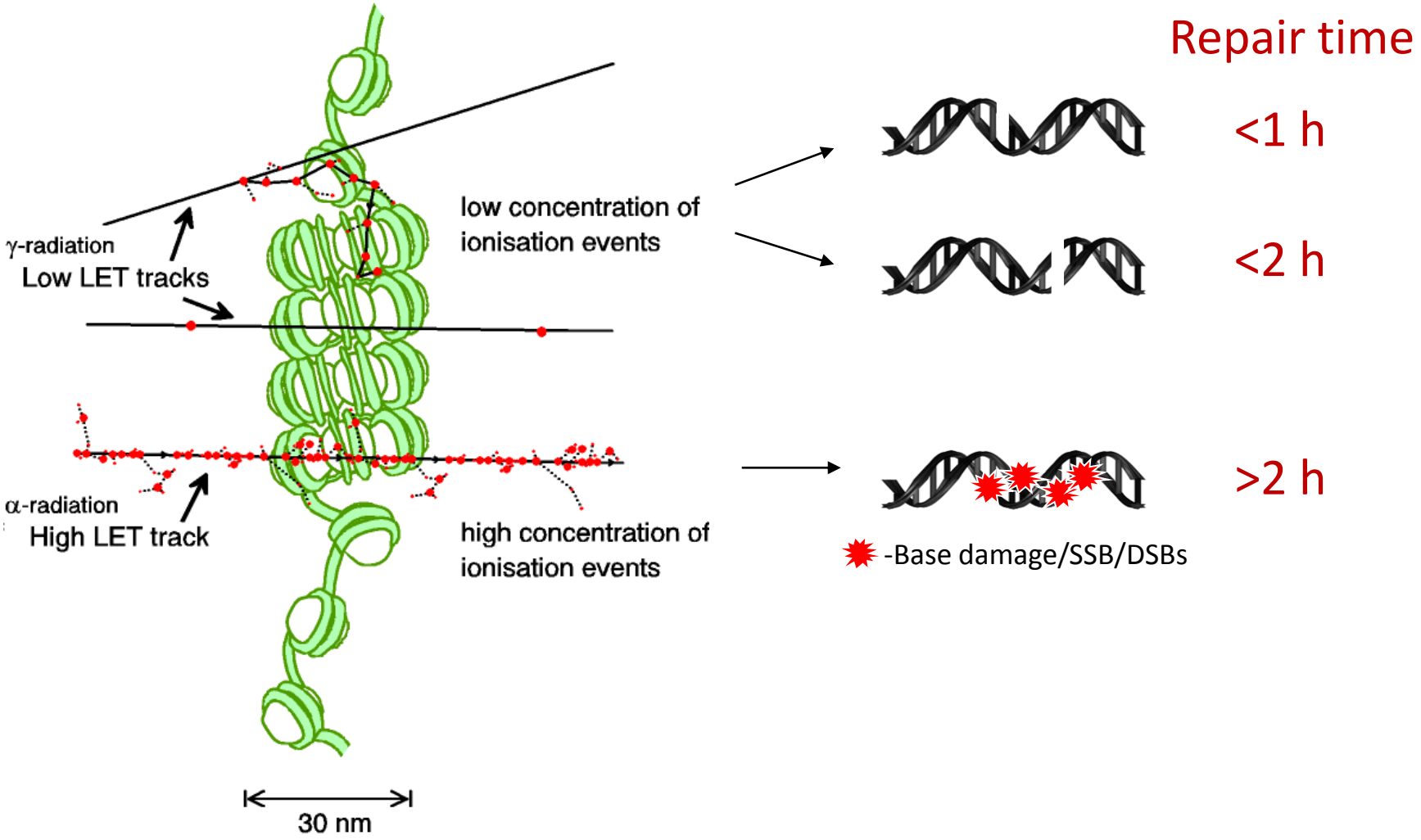
Advantages of proton beam therapy

- In contrast to conventional x-rays, protons can deliver energy within a finite region (termed the Bragg peak) which can directly target cancer cells.
- This limits radiation dose to proximal normal, healthy tissues.



- Surprisingly, the radiobiological effects of proton irradiation (in comparison to x-ray irradiation) remain poorly understood.

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



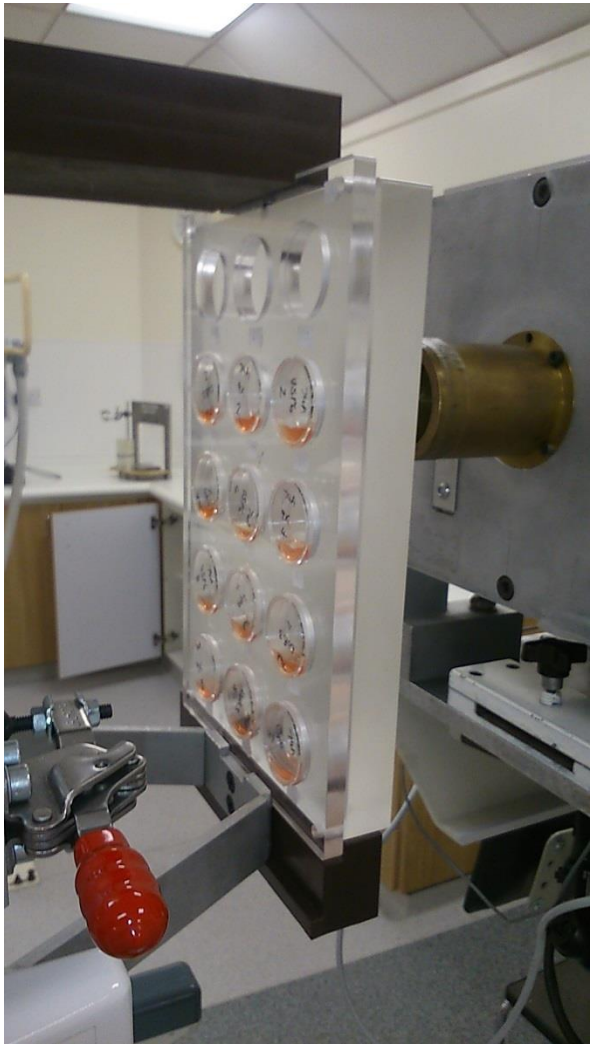
Complexity of DNA damage induced by IR

Particle	No break (%)	SSB (%)	SSB⁺ (%)	2SSB (%)	DSB (%)	DSB⁺ (%)	DSB⁺⁺ (%)	SSB_c (%)	SSB_{cb} (%)	DSB_c (%)	DSB_{cb} (%)	SSB_I (%)	SSB_D (%)	DSB_I (%)	DSB_D (%)	Base damage without strand break (%)
Electrons																
0.3 keV[b]	66.4	26.5	3.3	0.4	2.4	0.9	0.09	12	52	28	68	34	66	29	71	—
1.5 keV[b]	70.5	24.3	2.4	0.4	1.7	0.6	0.07	10	41	29	60	39	62	27	73	—
4.5 keV[b]	71.4	24.1	2.3	0.3	1.5	0.5	0.04	9	37	29	62	34	66	23	76	—
Protons																
0.3 MeV	55.2	26.5	7.1	1.5	4.8	3.6	1.3	24	70	51	90	40	60	33	67	28
0.5 MeV[b]	60.4	26.3	5.4	1.1	3.9	2.3	0.6	20	70	46	86	39	61	33	67	29
0.75 MeV	63.5	25.8	4.6	1.0	3.3	1.6	0.3	18	68	37	82	38	62	34	66	32
1.0 MeV[b]	66.6	24.8	3.7	0.7	2.7	1.1	0.3	15	59	37	80	40	60	38	63	33
2.0 MeV	70.6	23.5	2.6	0.5	2.0	0.7	0.1	12	52	28	76	39	61	33	67	38
4.0 MeV[b]	73.6	21.7	2.1	0.5	1.6	0.5	0.1	10	45	26	66	40	60	39	61	42
α particles																
2.0 MeV[b]	51.3	23.0	7.0	2.0	4.8	6.2	5.7	28	75	73	96	46	54	31	69	32
3.0 MeV	51.4	24.4	7.6	2.0	5.3	5.7	3.7	28	78	64	95	44	57	30	70	31
4.0 MeV	56.7	24.9	6.5	1.5	4.3	4.1	2.2	24	73	60	92	41	59	31	69	29
6.0 MeV[b]	58.5	21.5	5.1	1.2	3.5	2.7	1.3	23	70	56	90	40	60	29	71	28
8.0 MeV	61.8	25.1	5.1	1.0	3.9	2.4	0.7	20	65	47	84	39	61	31	69	25
10.0 MeV[b]	64.3	24.8	4.4	1.0	3.2	1.9	0.4	18	65	45	84	42	59	35	65	26

Nikjoo *et al* (2001) *Rad Res*

- <30 % complex DNA damage is induced by low-LET IR but this is >90 % with high-LET IR.
- Proton beam therapy, particularly at low energies, can induce complex DNA damage formation.

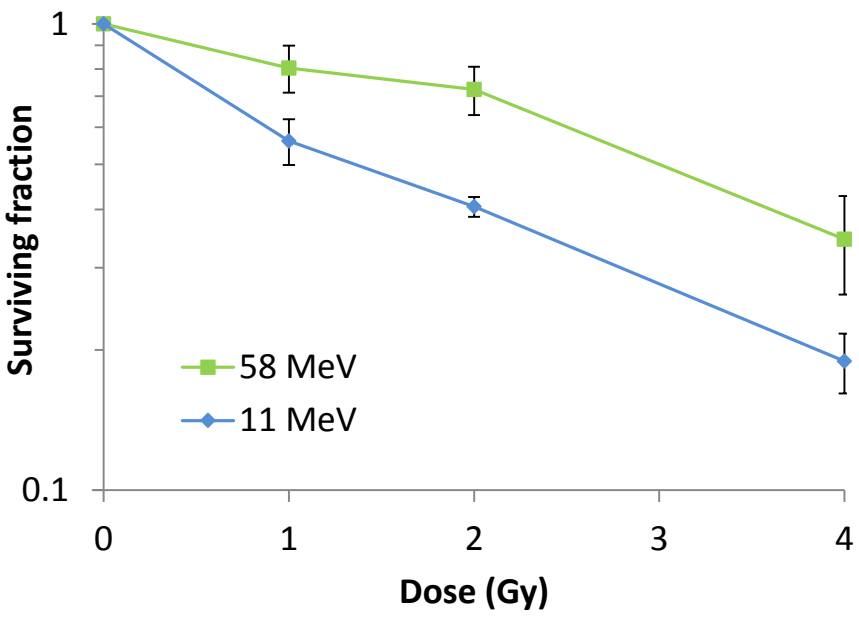
Proton beam and radiobiology facilities at the Clatterbridge Cancer Centre (CCC)



Collaboration with Andrzej Kacperek

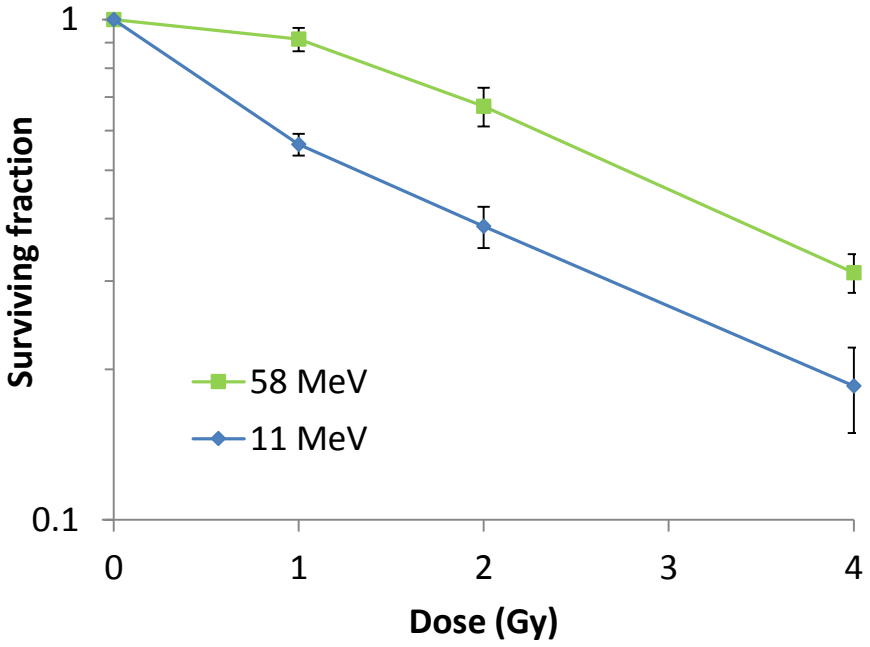
Low energy protons cause a decrease in cell survival compared to high energy protons

HeLa



SF2, p<0.02

UMSCC6



SF2, p<0.01

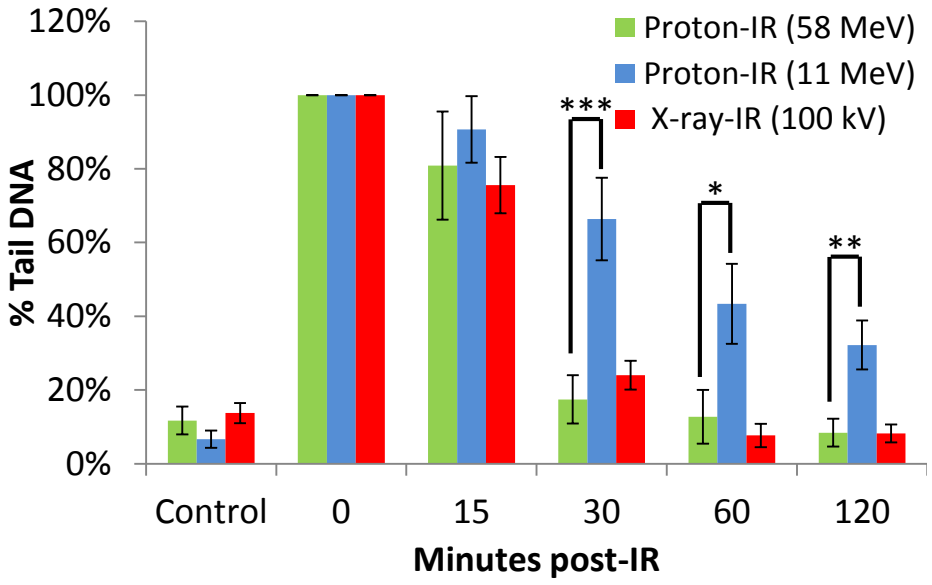
58 MeV (1 keV/µm); 11 MeV (12 keV/µm)

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

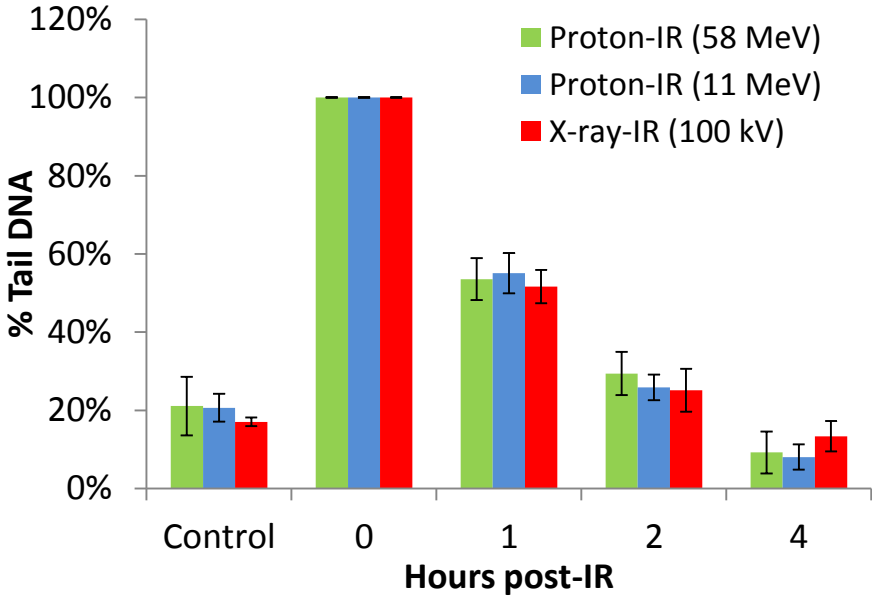
The repair of DNA single strand breaks/alkali labile sites is slower in cells following low energy protons

HeLa

Alkaline (SSB)



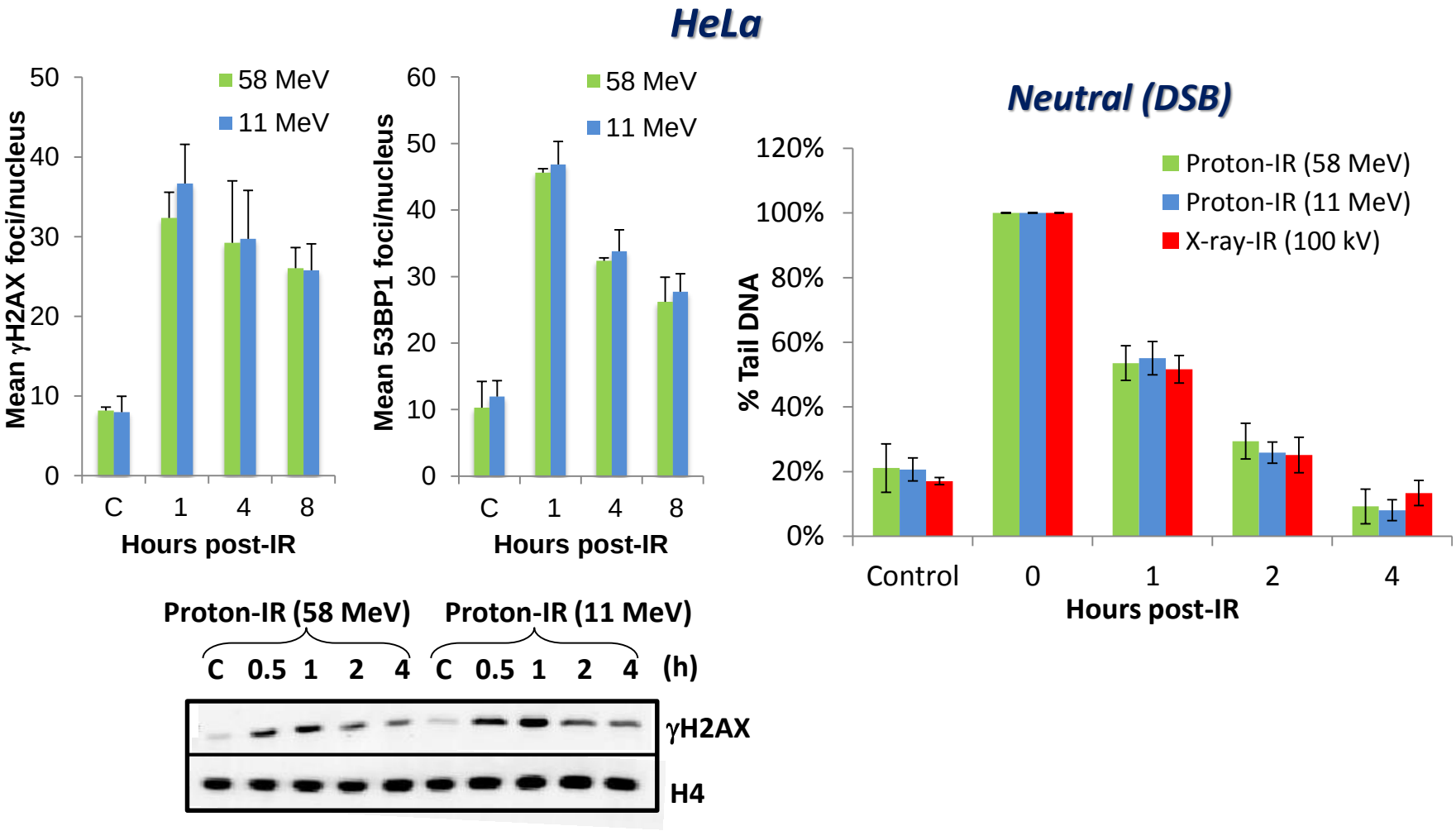
Neutral (DSB)



*p<0.005, **p<0.002, ***p<0.001

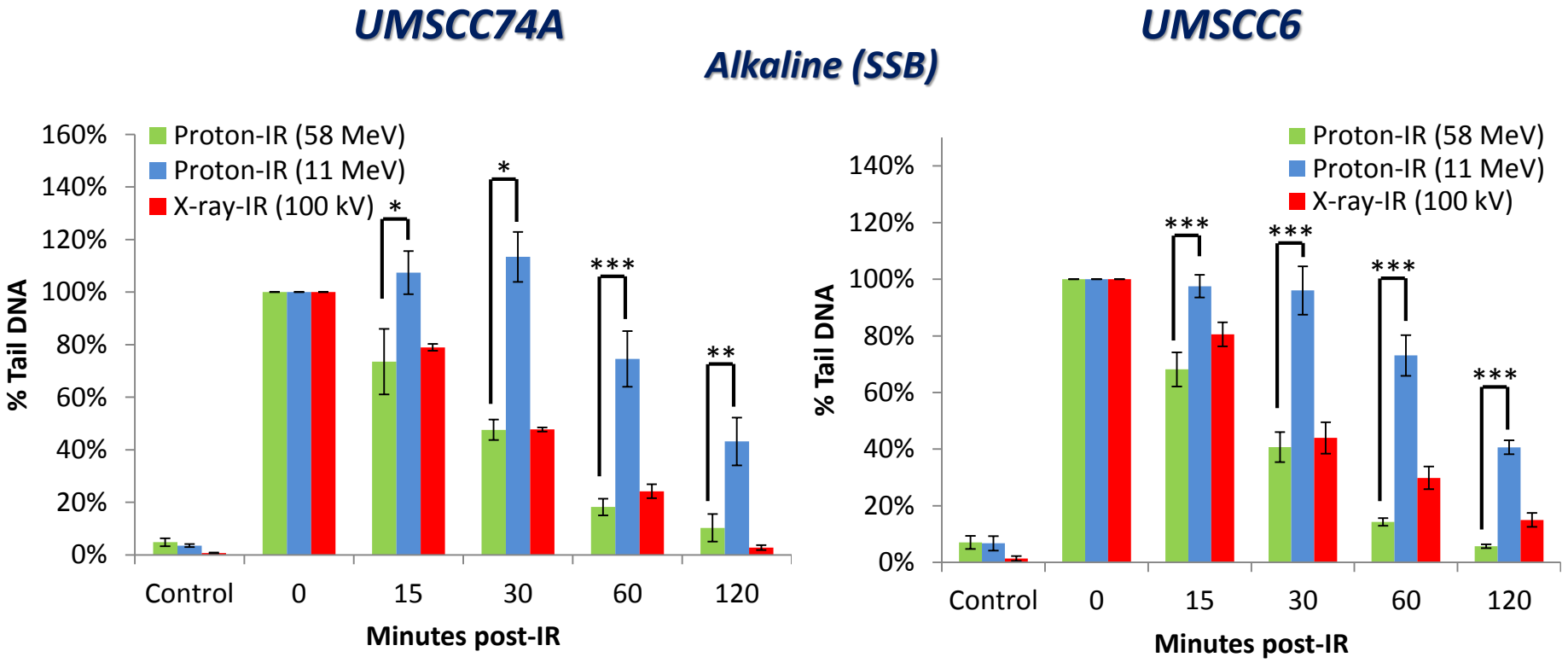
Carter et al., (2018) Int J Rad Oncol Biol Phys

The repair of DNA single strand breaks/alkali labile sites is slower in cells following low energy protons



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

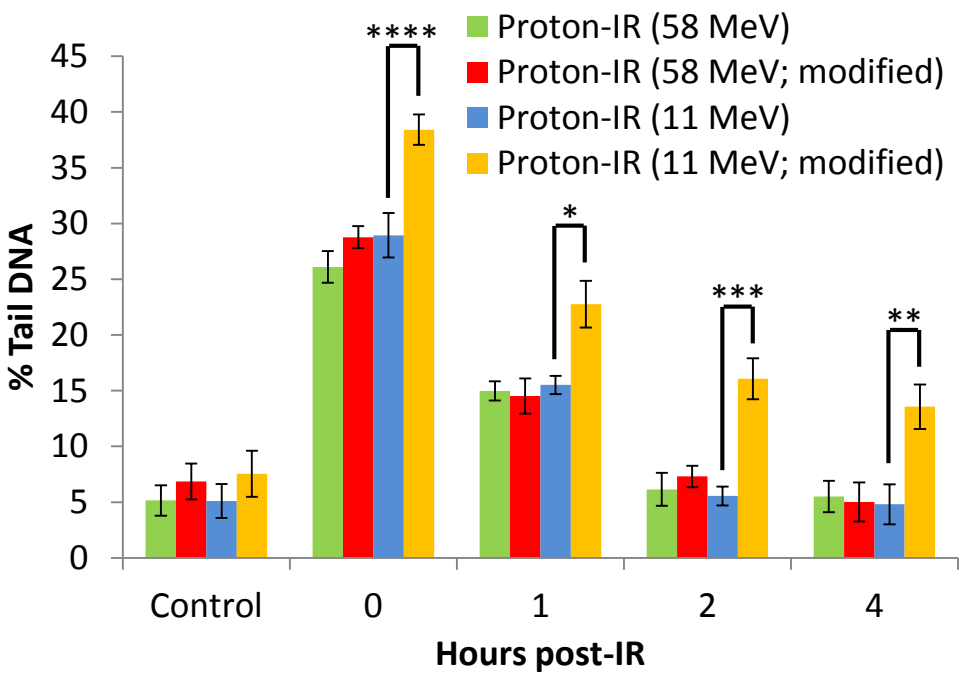
The repair of DNA single strand breaks/alkali labile sites is slower in OPSCC cells following low energy protons



*p<0.005, **p<0.002, ***p<0.001

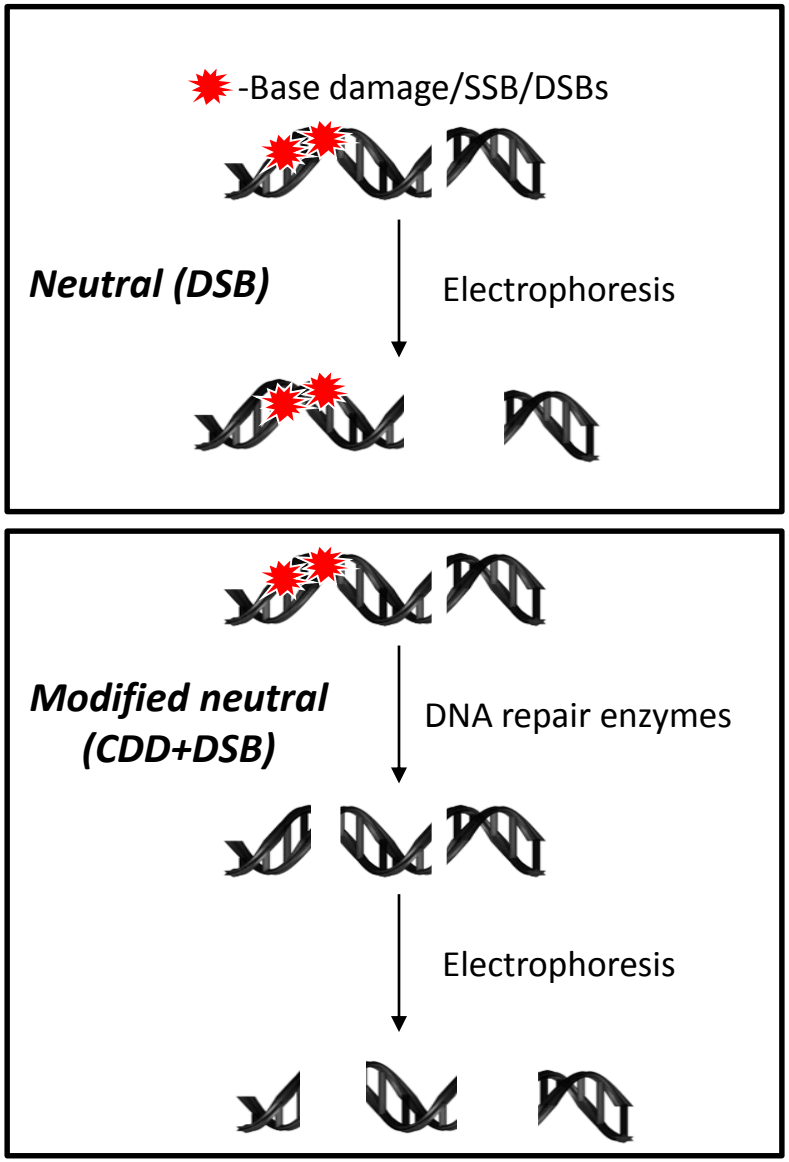
Carter et al., (2018) Int J Rad Oncol Biol Phys

Low energy protons induce the formation of CDD



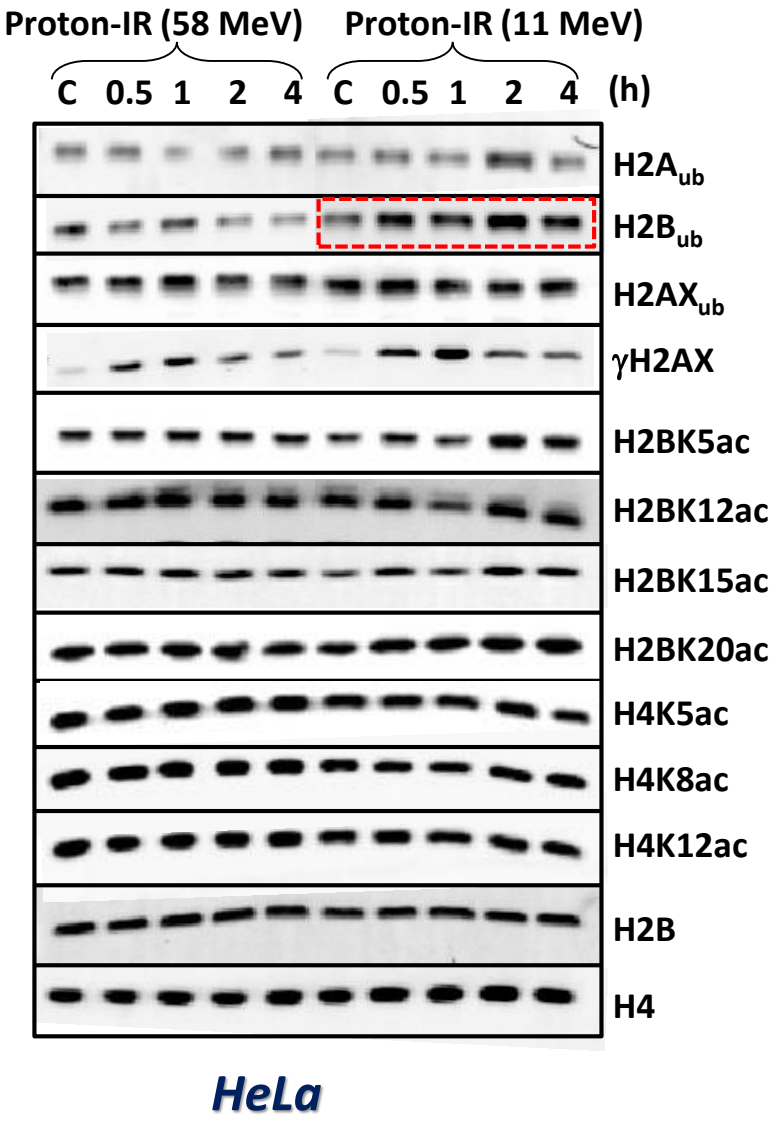
*p<0.02, **p<0.01, ***p<0.005, ****p<0.001

Holt and Georgakilas, 2007

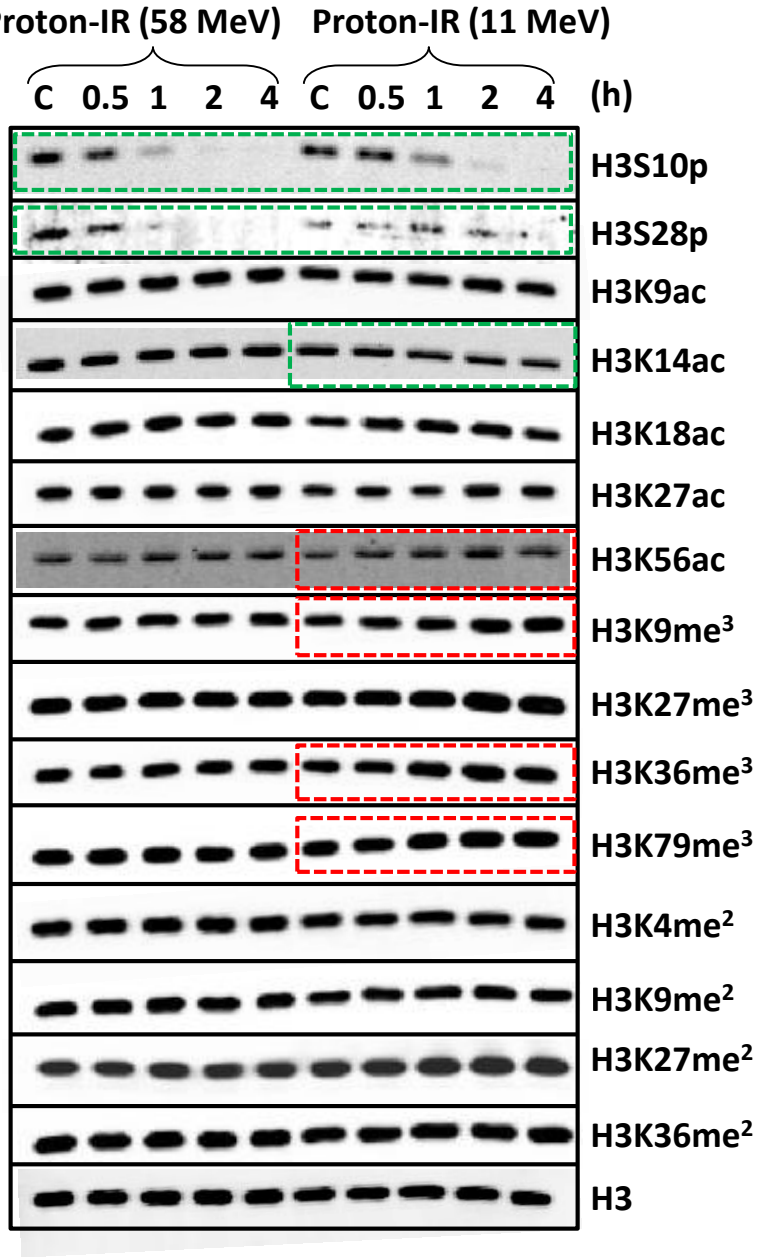


Carter et al., (2018) *Int J Rad Oncol Biol Phys*

Histone modifications following proton irradiation



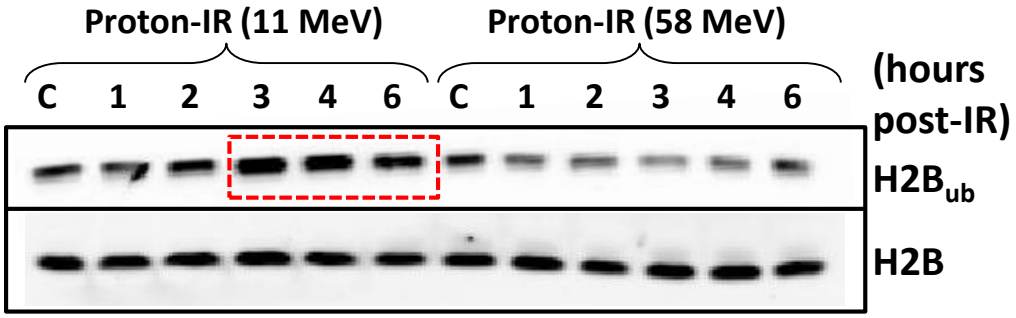
HeLa

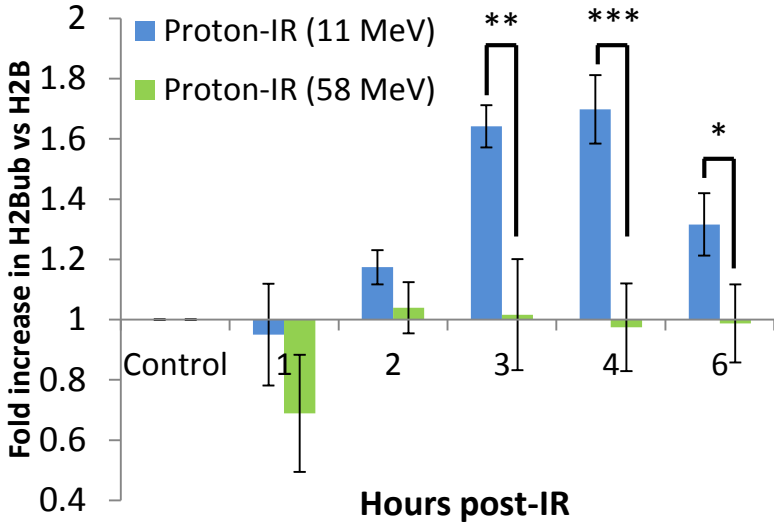


Carter et al., (2018) Int J Rad Oncol Biol Phys

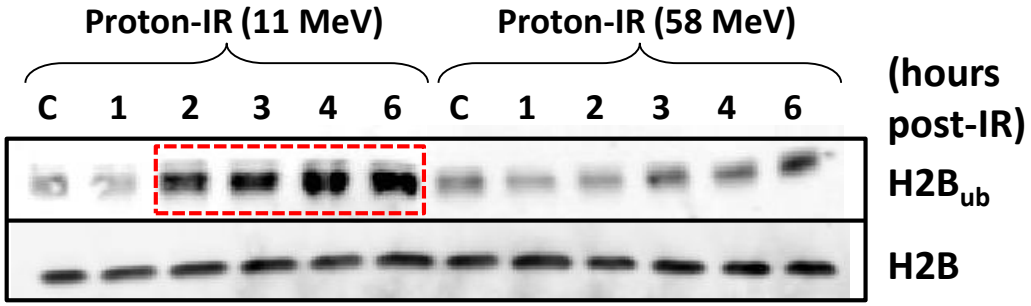
Histone H2B ubiquitylation (K120) is induced in HeLa cells in response to CDD by low energy protons

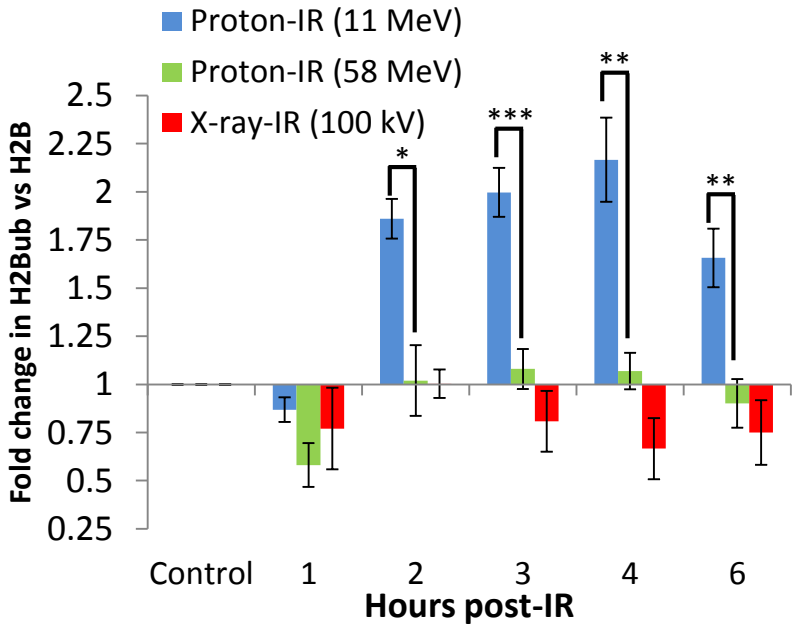
HeLa





UMSCC74A

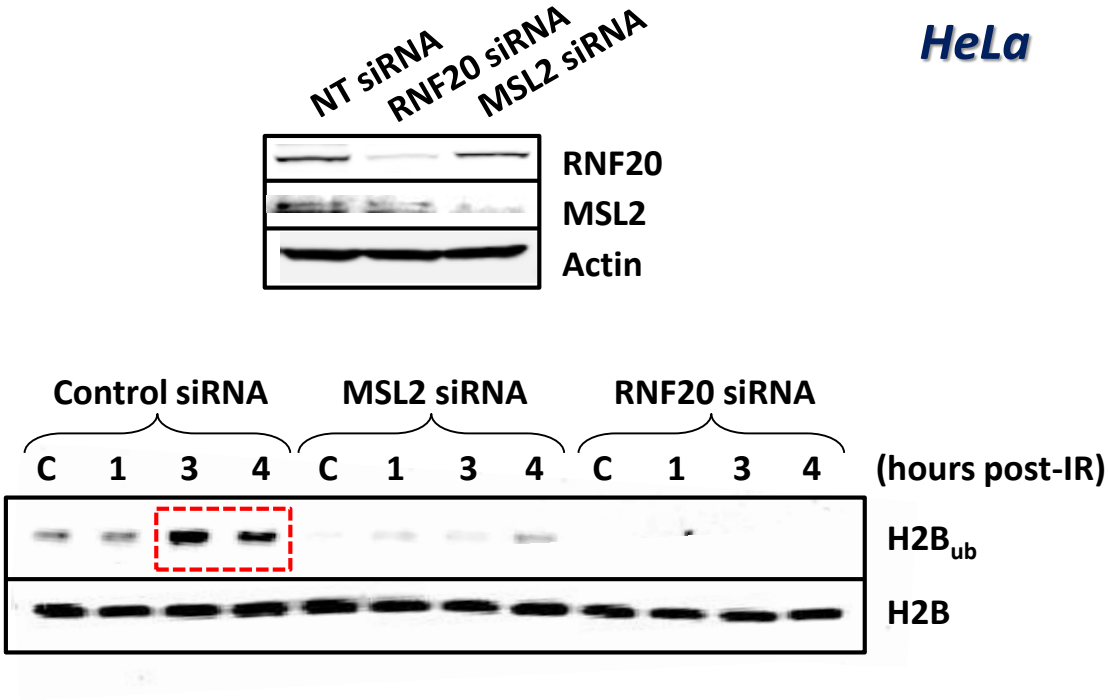


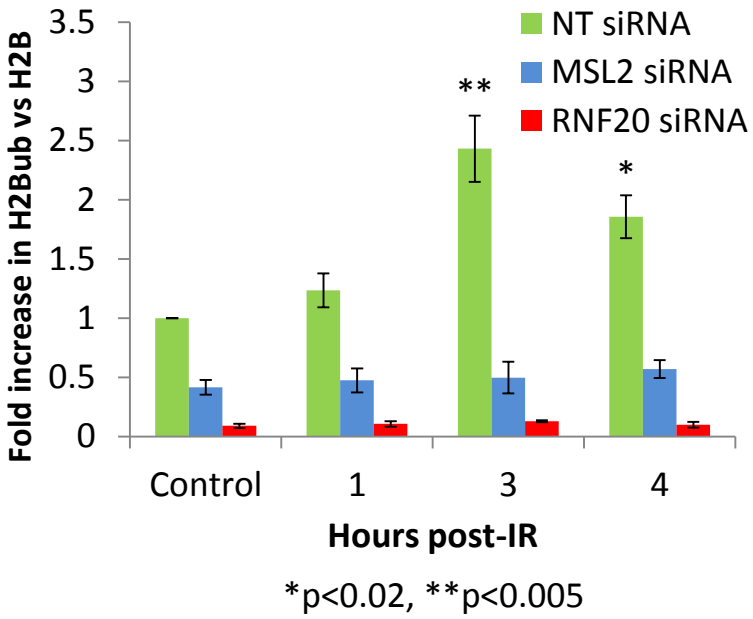


*p<0.05, **p<0.005, ***p<0.002

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

Histone H2B ubiquitylation (K120) is driven by MSL2 and RNF20/40 E3 ubiquitin ligases

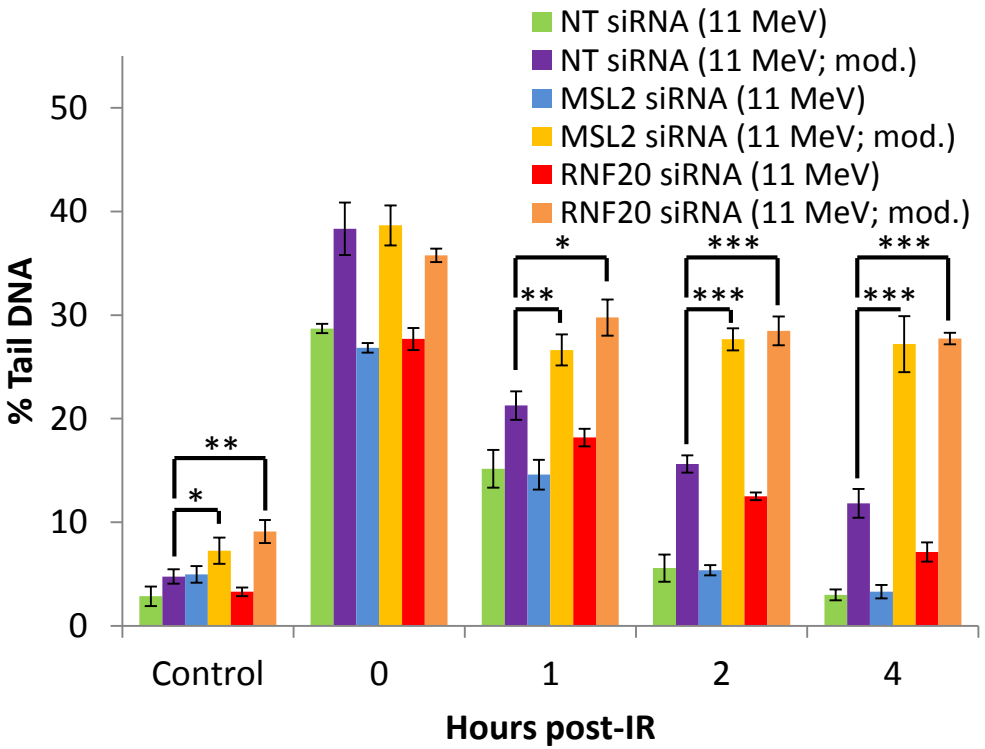




*p<0.02, **p<0.005

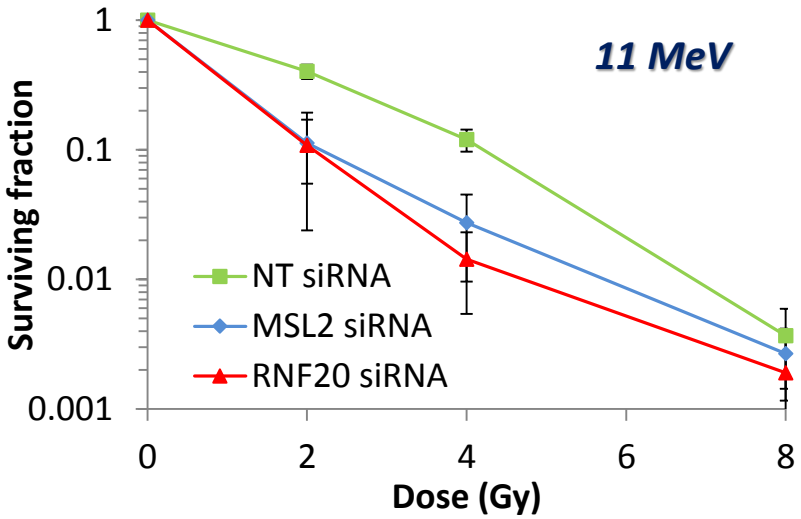
Carter et al., (2018) Int J Rad Oncol Biol Phys

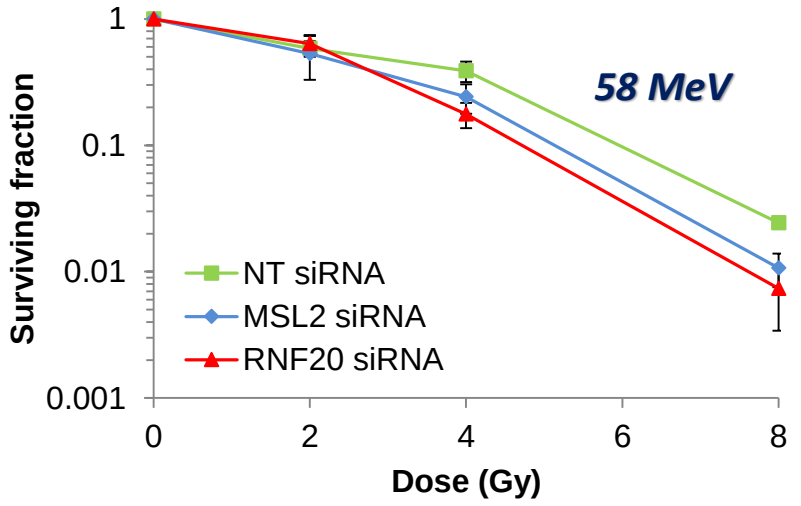
MSL2 and RNF20/40 regulate the repair of CDD and radiosensitivity following low energy protons



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

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

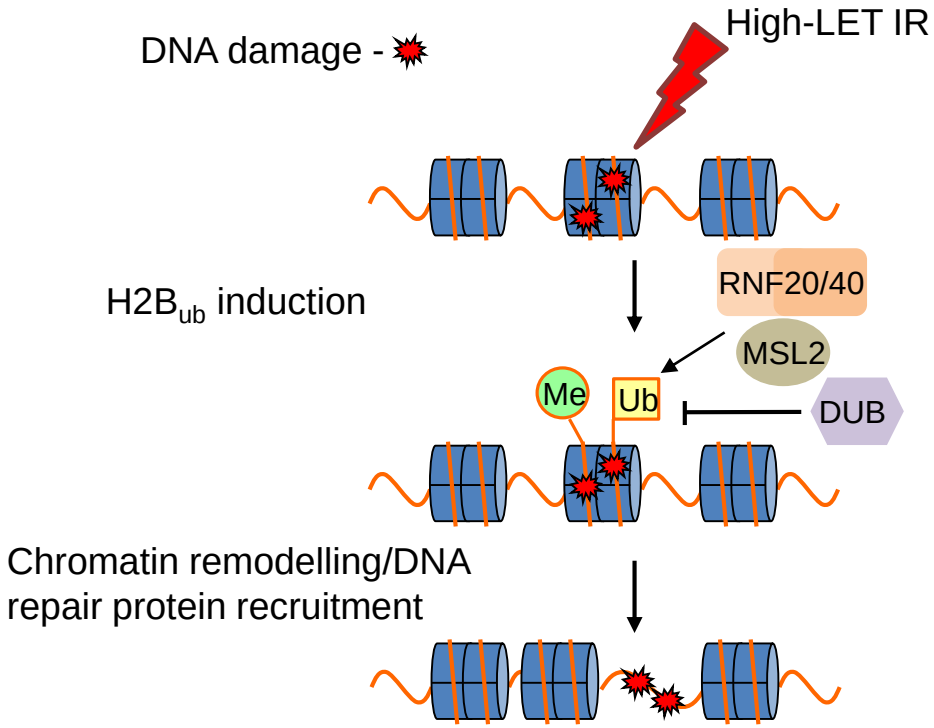




HeLa

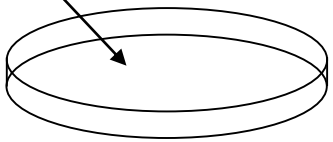
Carter et al., (2018) Int J Rad Oncol Biol Phys

Model for the recognition and repair of CDD in chromatin

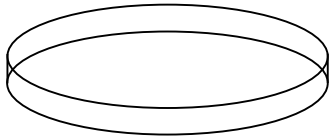


Identification of DUBs involved in the response to complex DNA damage induced by IR

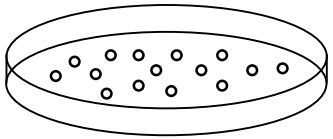
HeLa cells as a monolayer



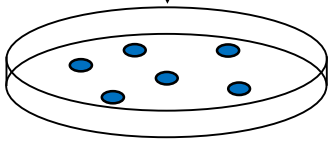
Transfect cells with DUB plasmids/siRNA

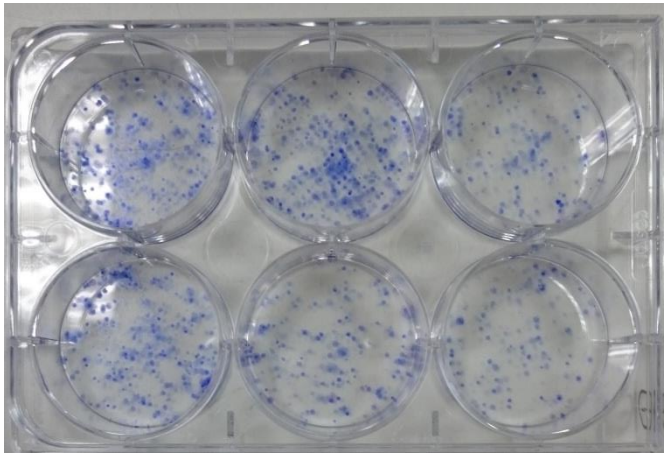


Irradiate (x-rays, α-particles or protons), trypsinise, count and seed into individual cells



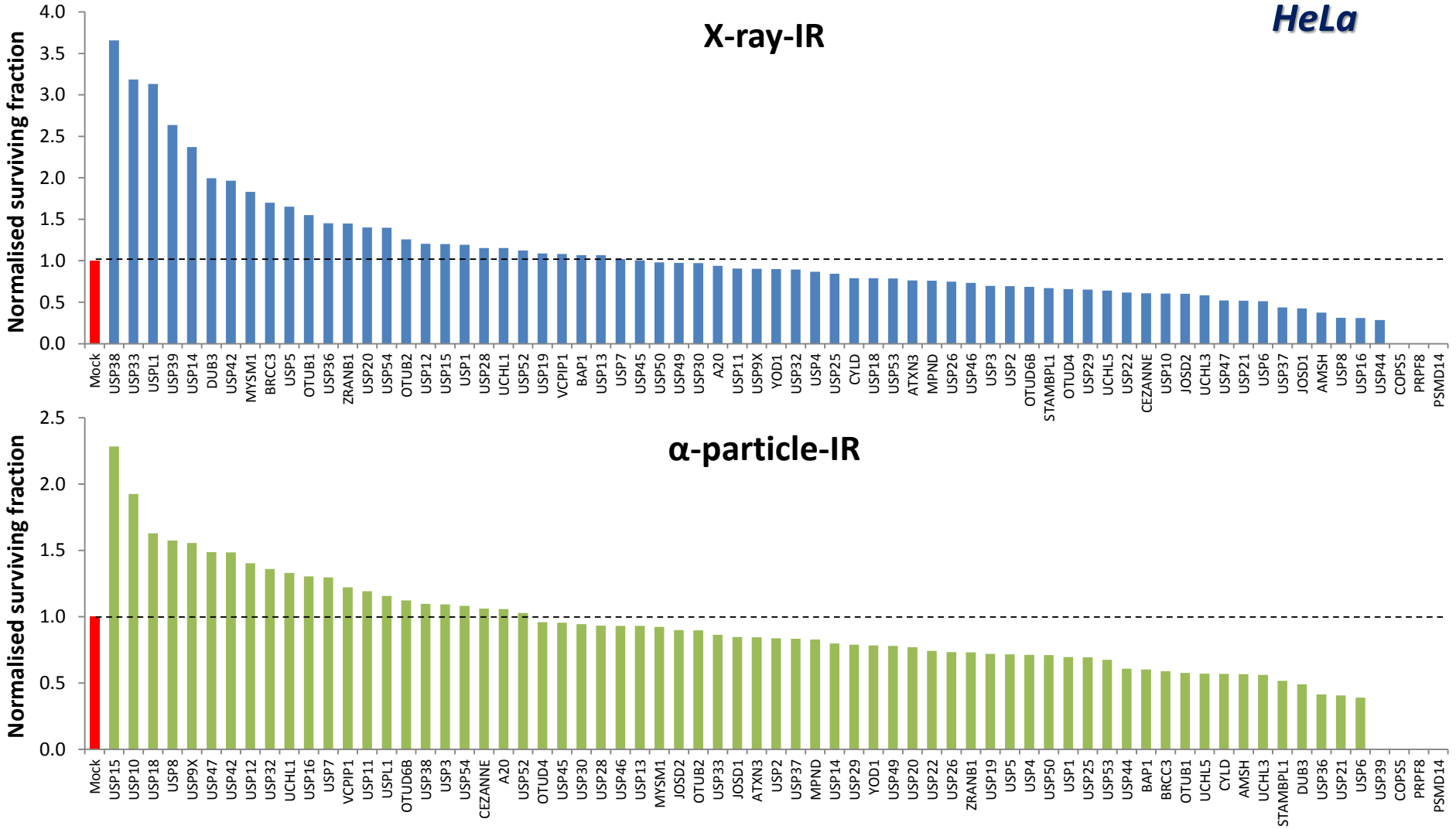
Allow colonies to grow for ~7 days, fix, stain and count





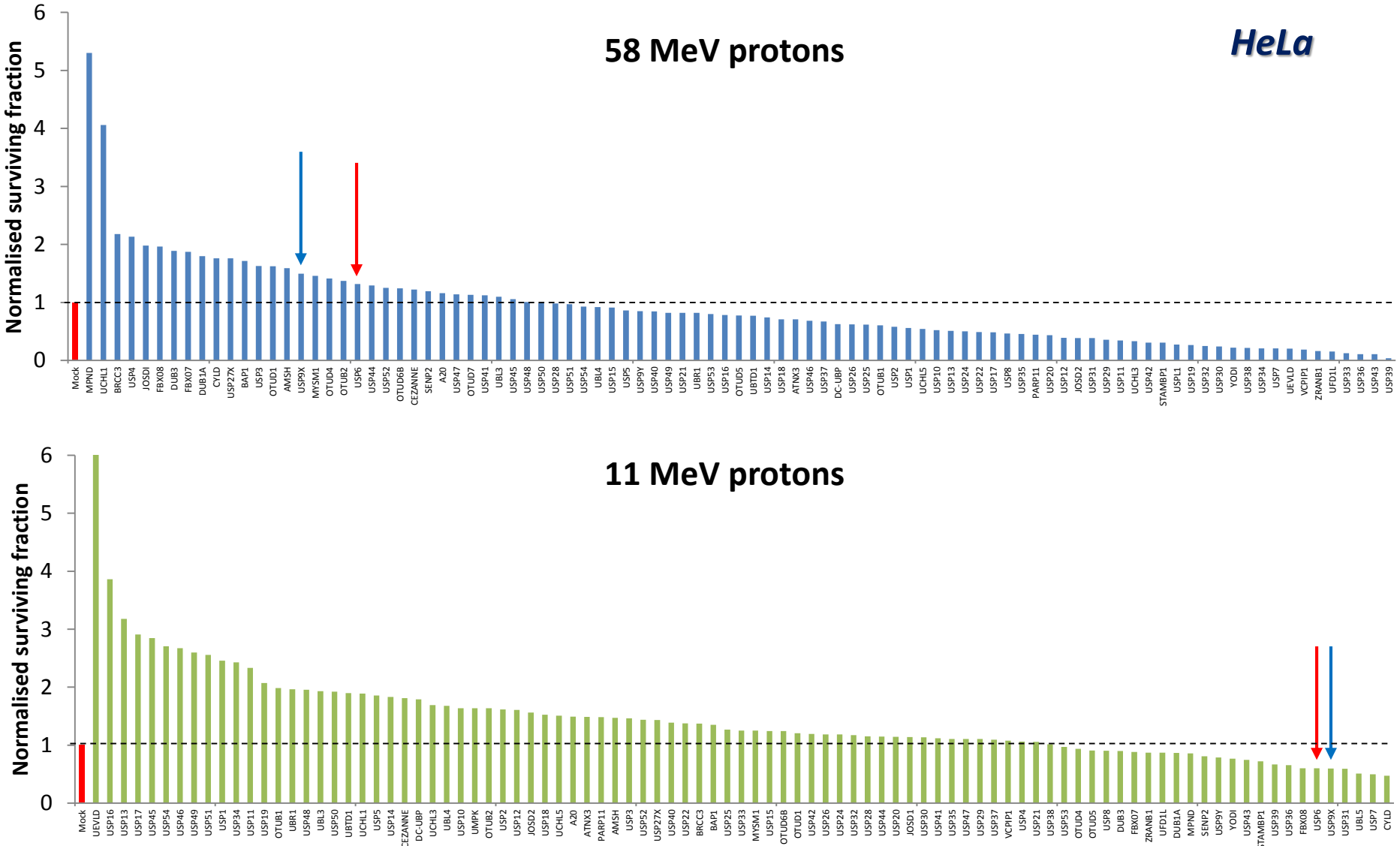
In collaboration with Mike Clague and Sylvie Urbe

Modulation of IR-induced cellular sensitivity following DUB siRNA knockdown



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

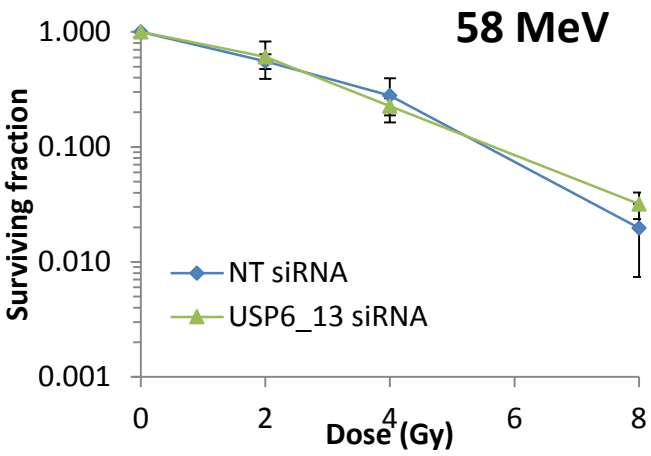
Modulation of proton-induced cellular sensitivity following DUB siRNA knockdown

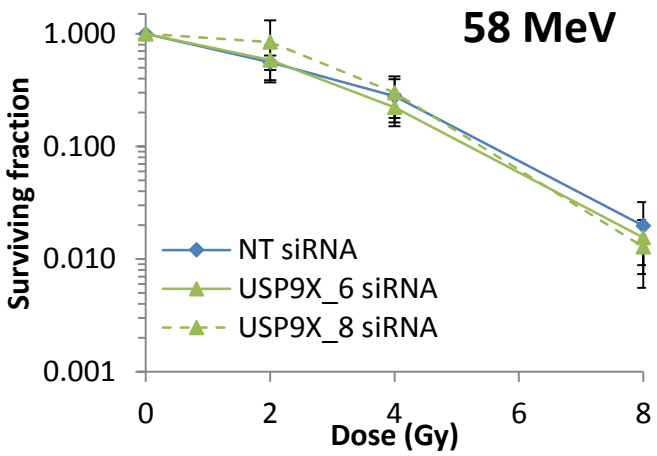


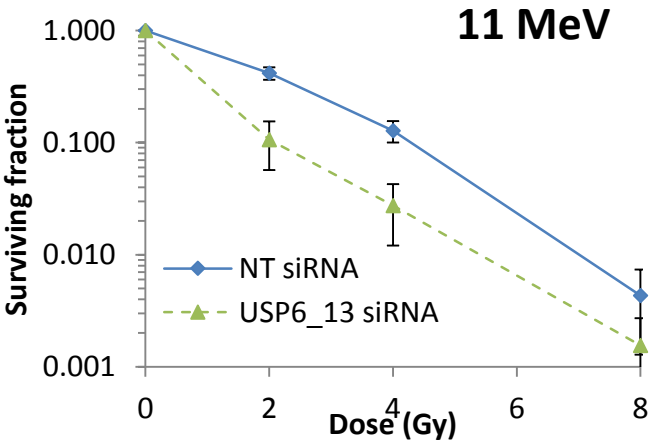
Carter et al., (2019) *Int J Rad Oncol Biol Phys*

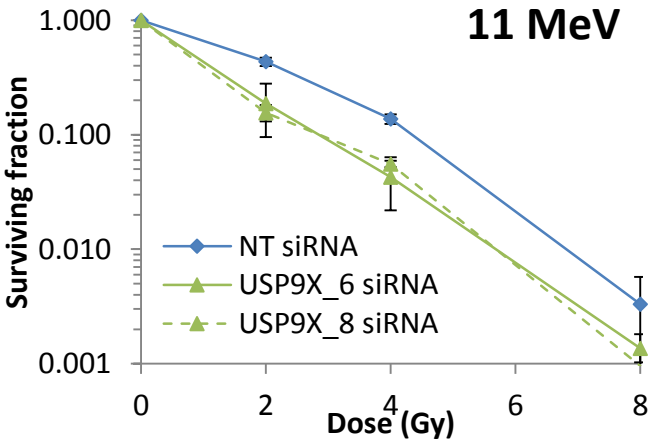
USP9X and USP6 control the cellular response to low energy protons

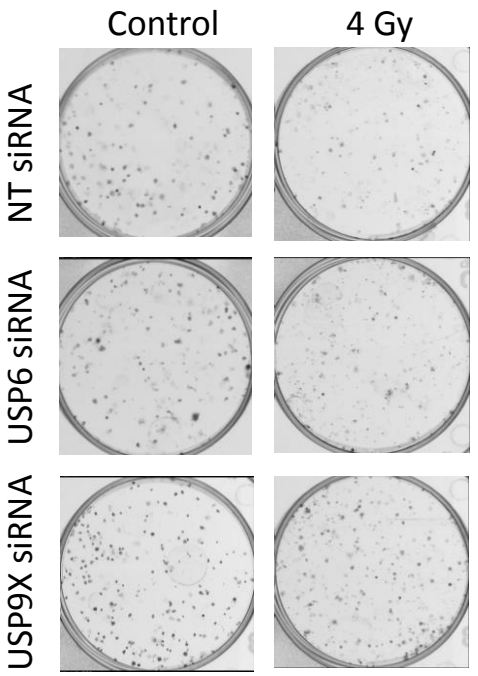
HeLa

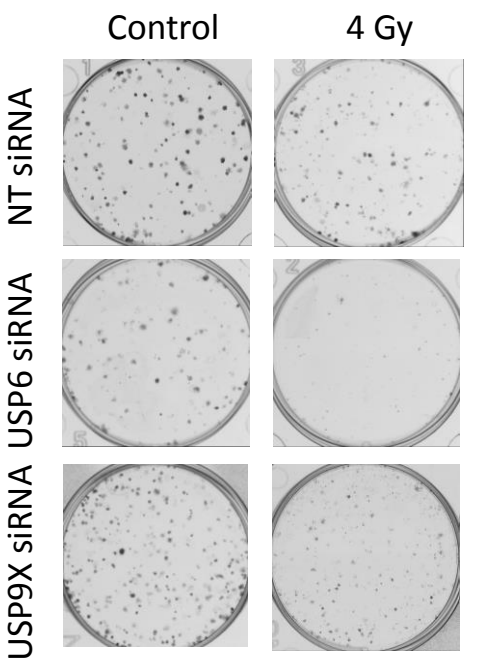








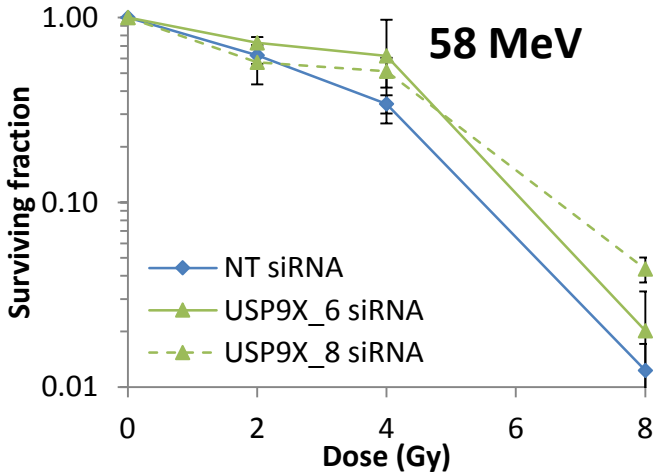


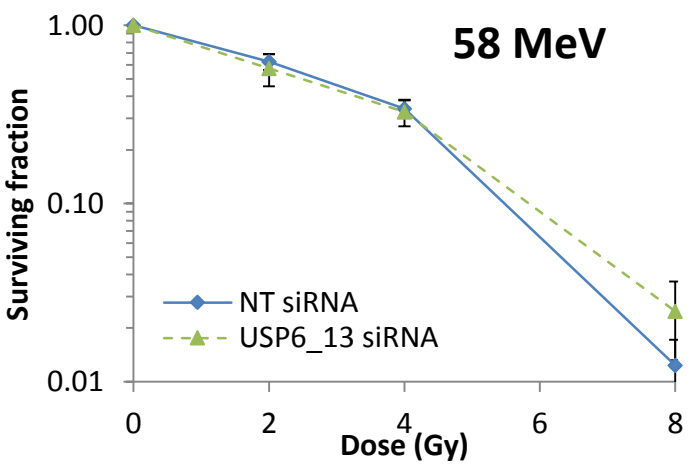


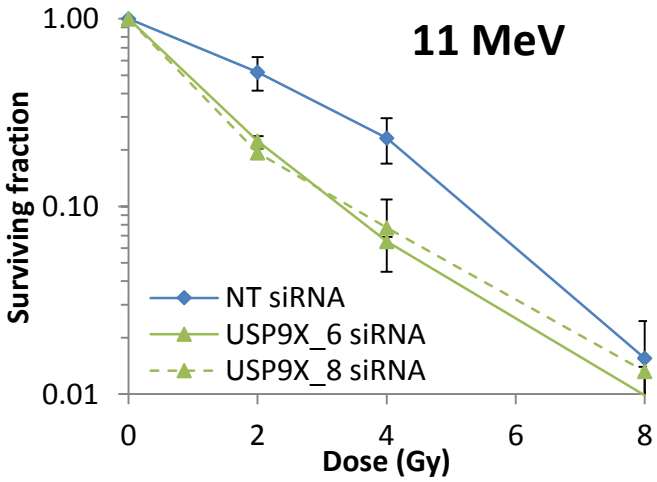
Carter et al., (2019) Int J Rad Oncol Biol Phys

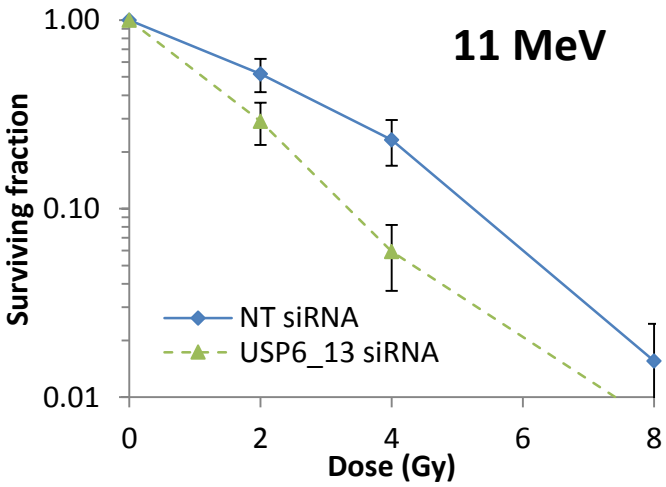
USP9X and USP6 control in the cellular response to low energy protons

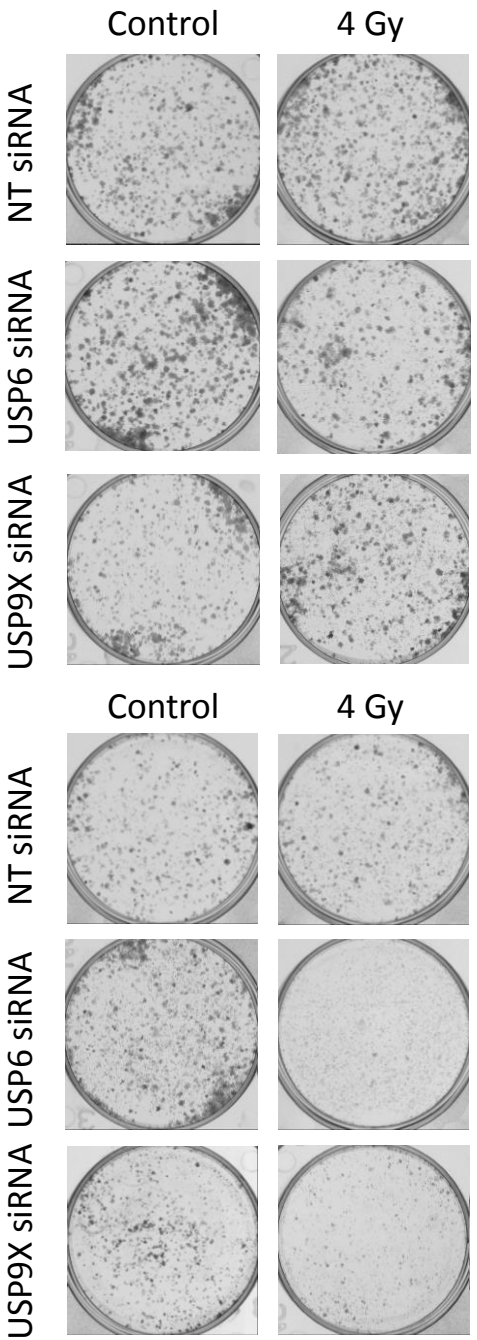
UMSCC74A











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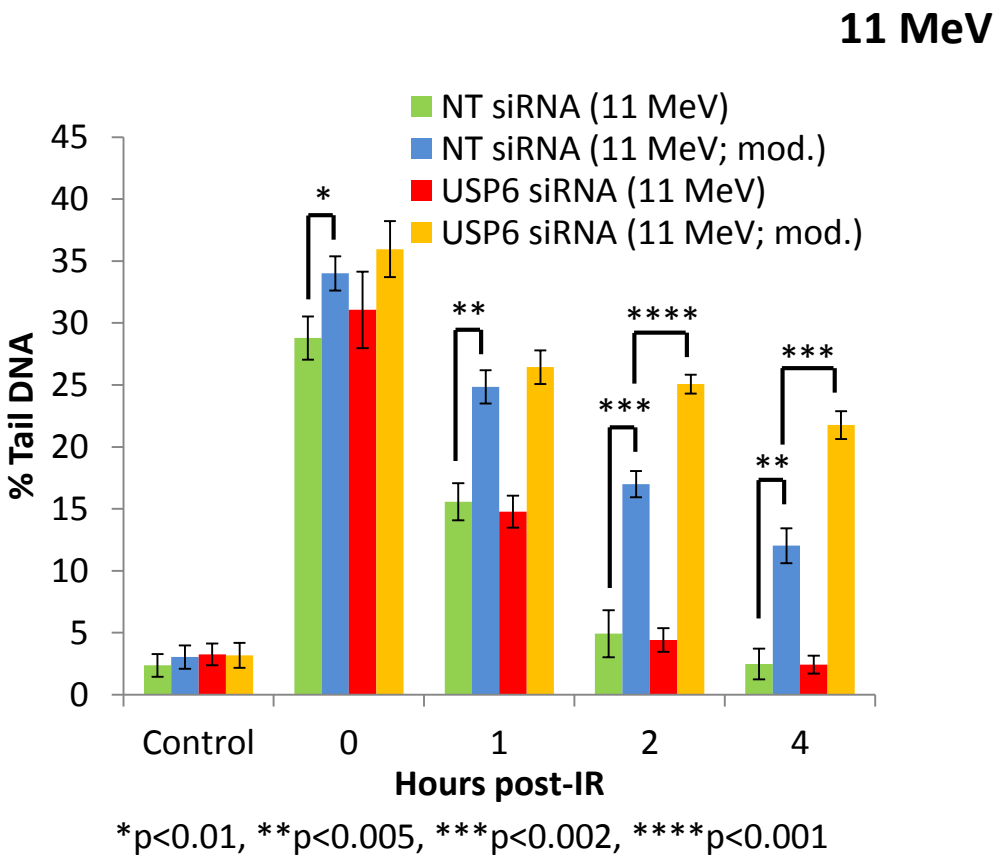
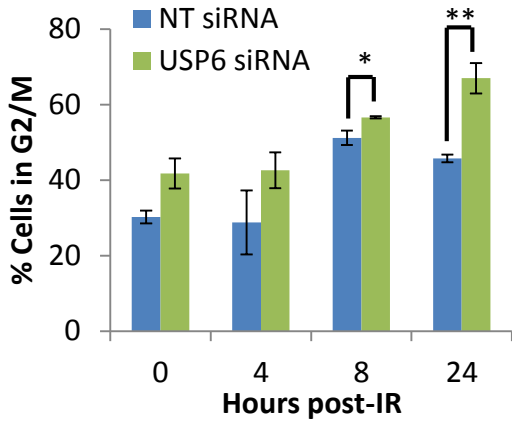
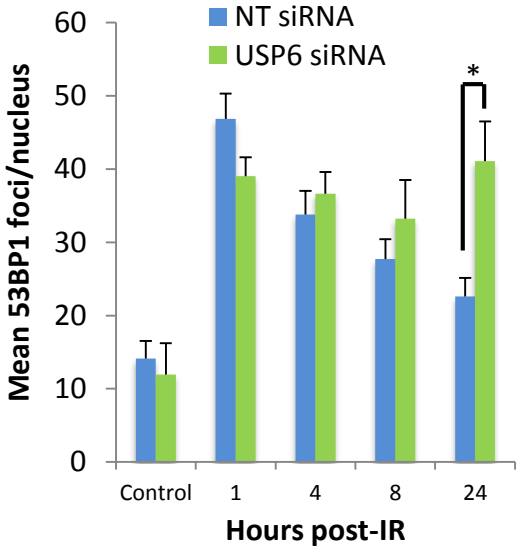
Knockdown of USP6 causes persistence of complex DNA damage and G2/M arrest in response to low energy protons



*p<0.01, **p<0.005, ***p<0.002, ****p<0.001



*p<0.05
**p<0.01,



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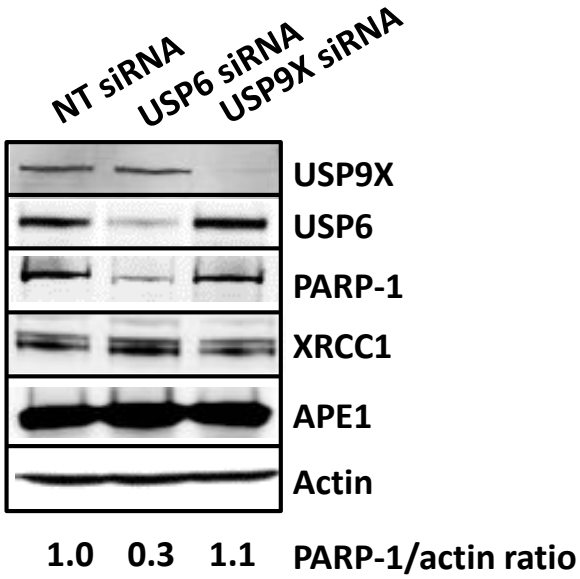
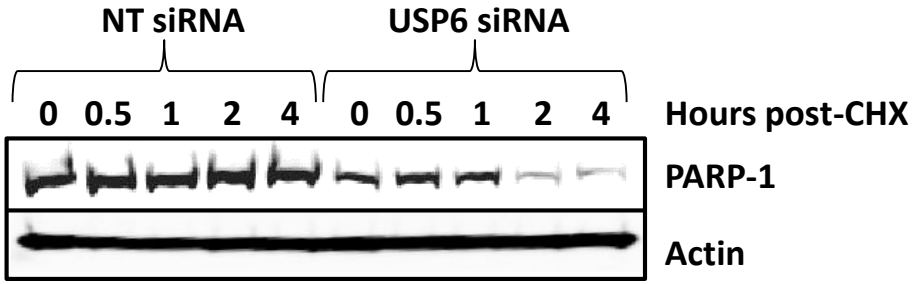
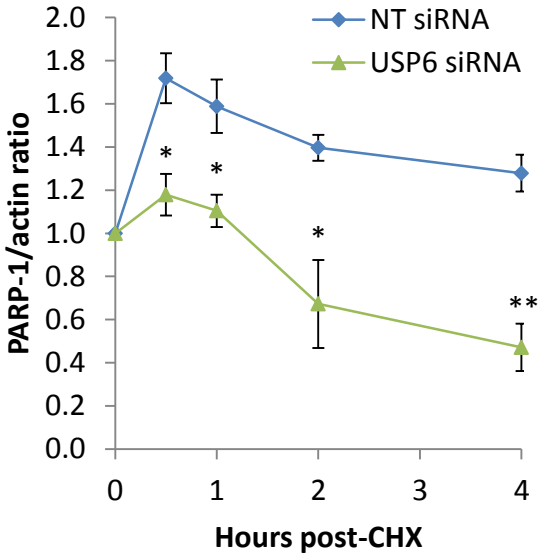
Knockdown of USP6 decreased PARP-1 stability







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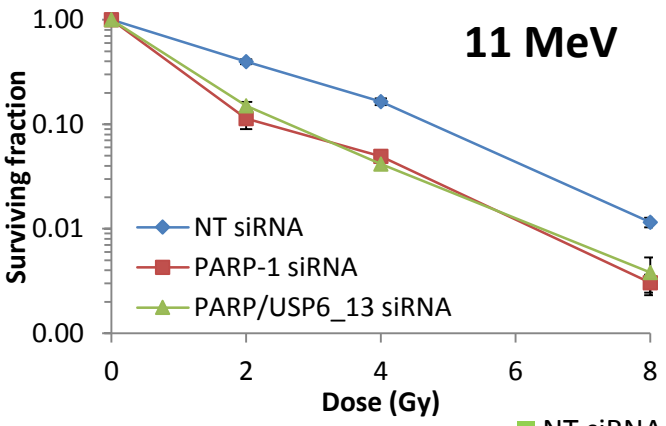
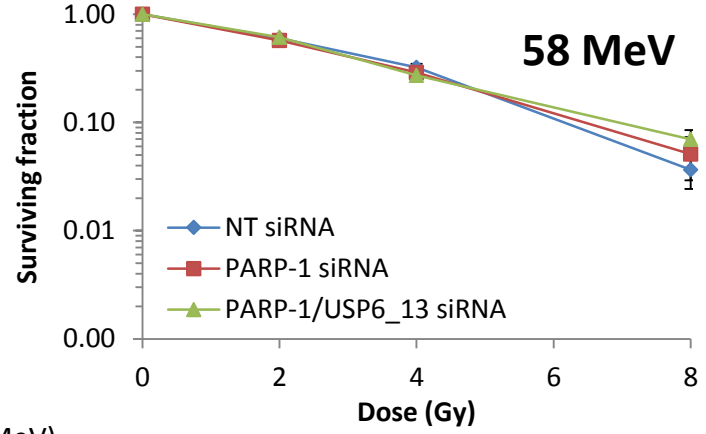
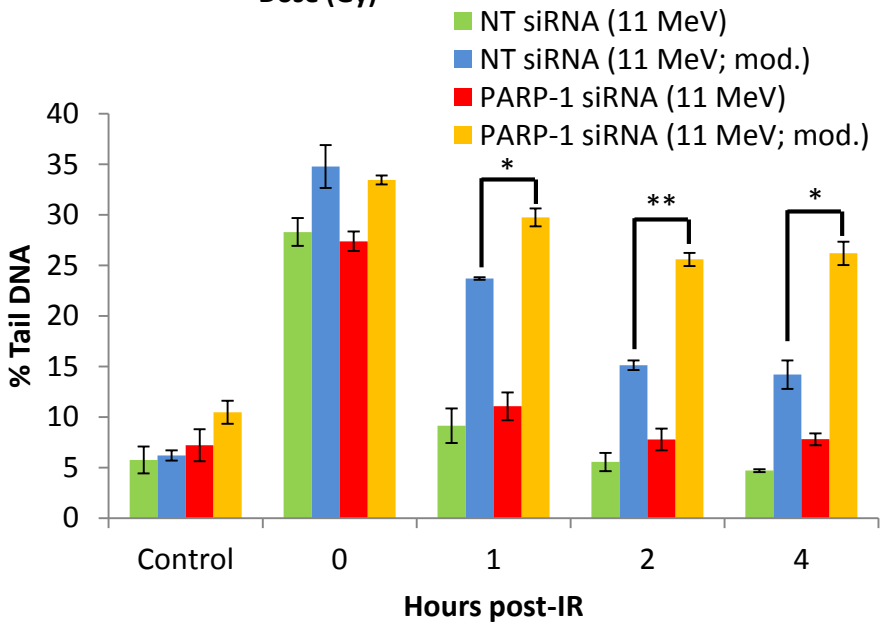
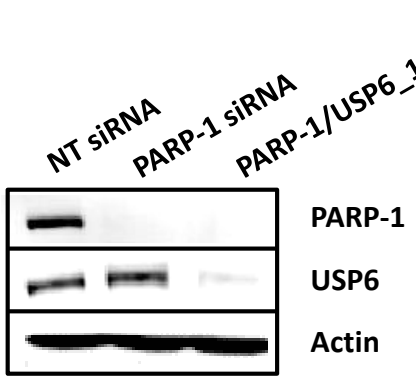
Knockdown of PARP-1 sensitises cells to low energy protons through deficiencies in CDD repair





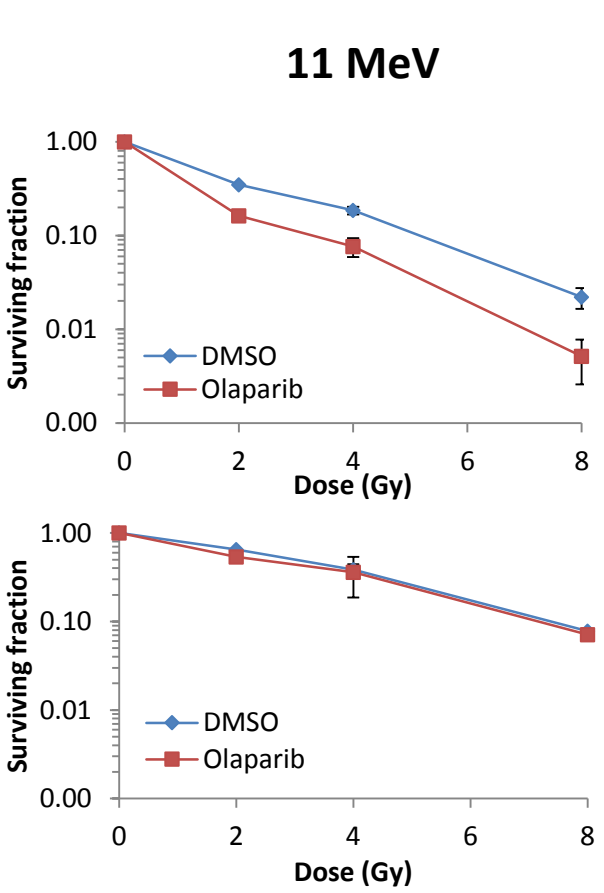


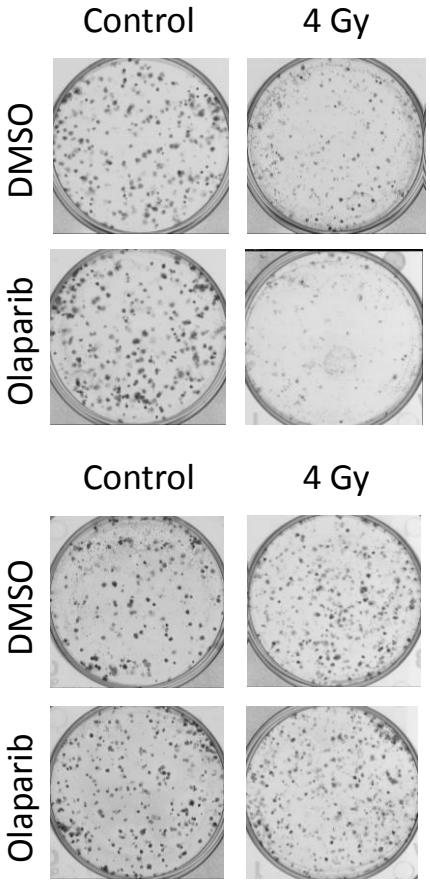
*p<0.01
**p<0.005

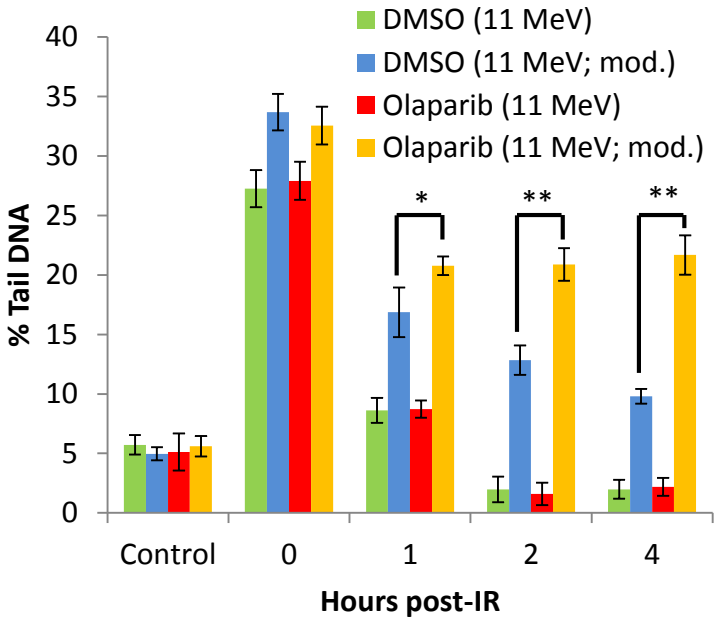


Carter et al., (2019) Int J Rad Oncol Biol Phys

Olaparib synergies with low energy protons in promoting cancer cell killing







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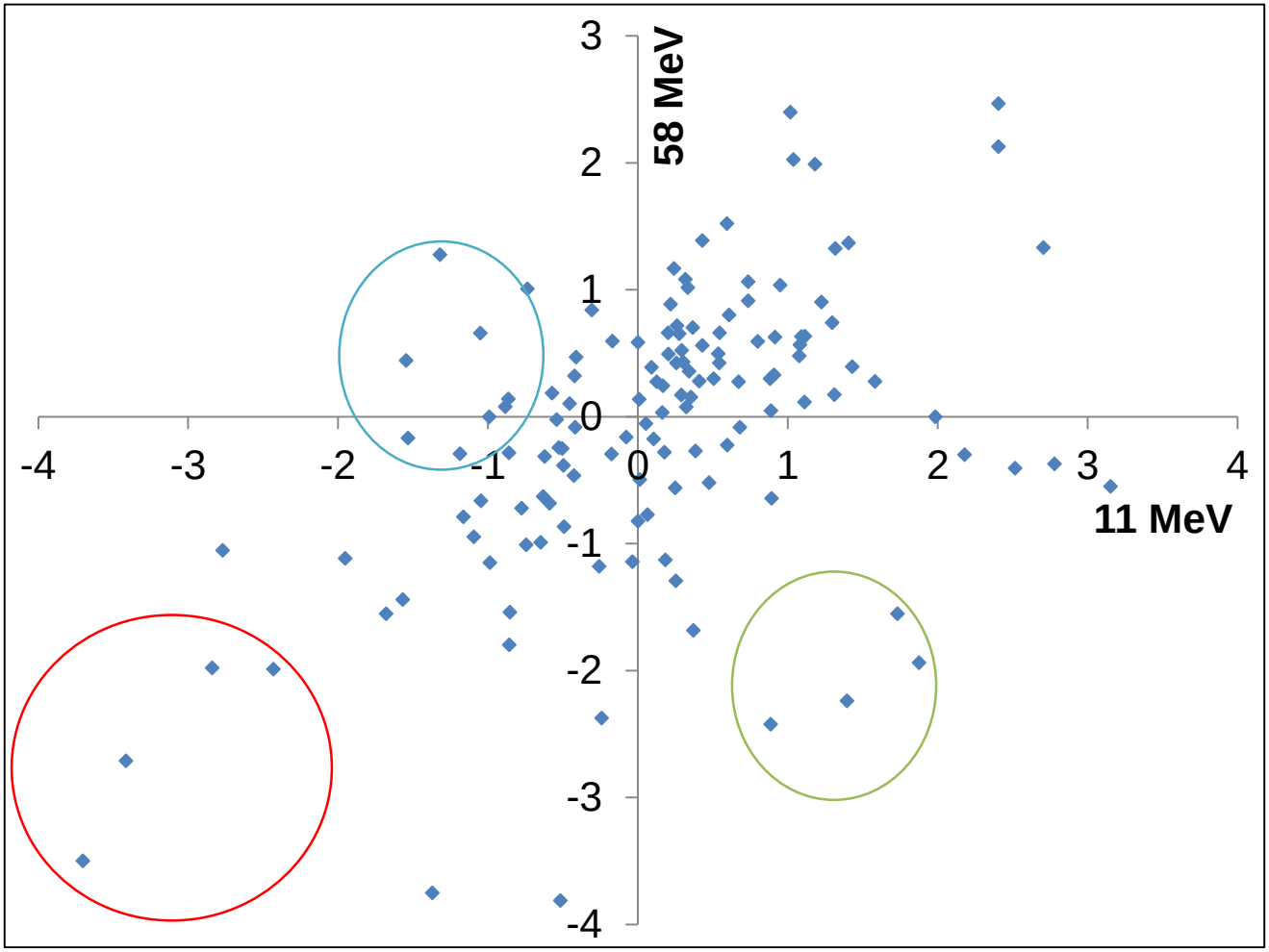
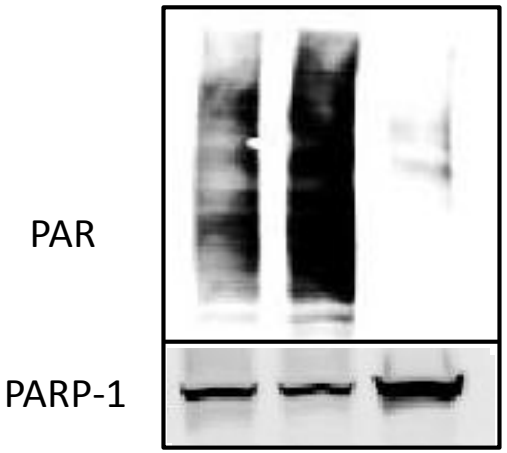
Modulation of proton-induced cellular sensitivity following DDR siRNA knockdown



Radiosensitivity of HPV-negative OPSCC cells can be increased using PARP inhibitor

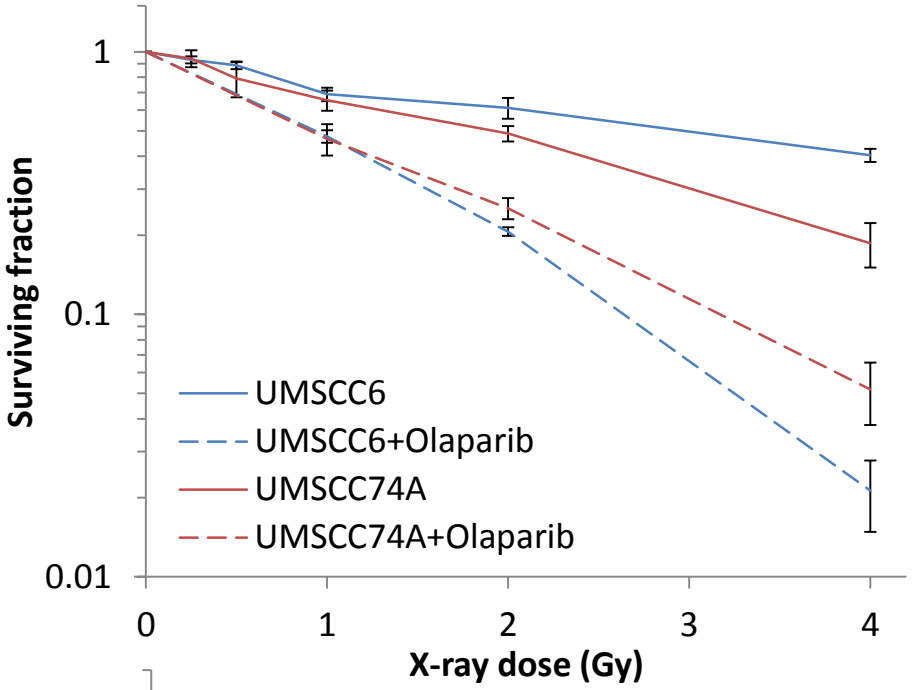
X-ray IR (4 Gy)	-	+	+
Olaparib (0.1 µM)	-	-	+

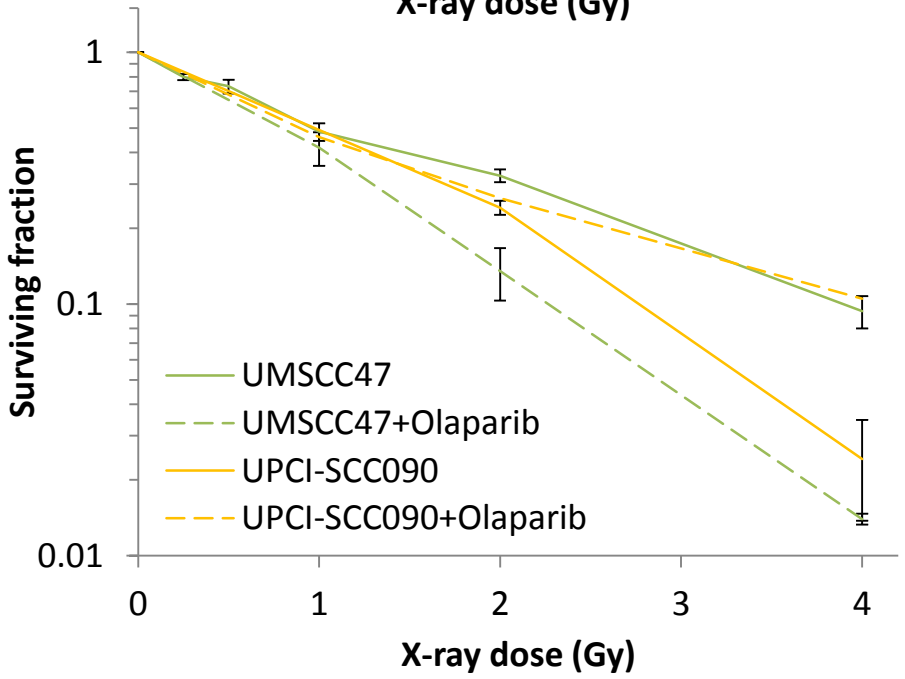


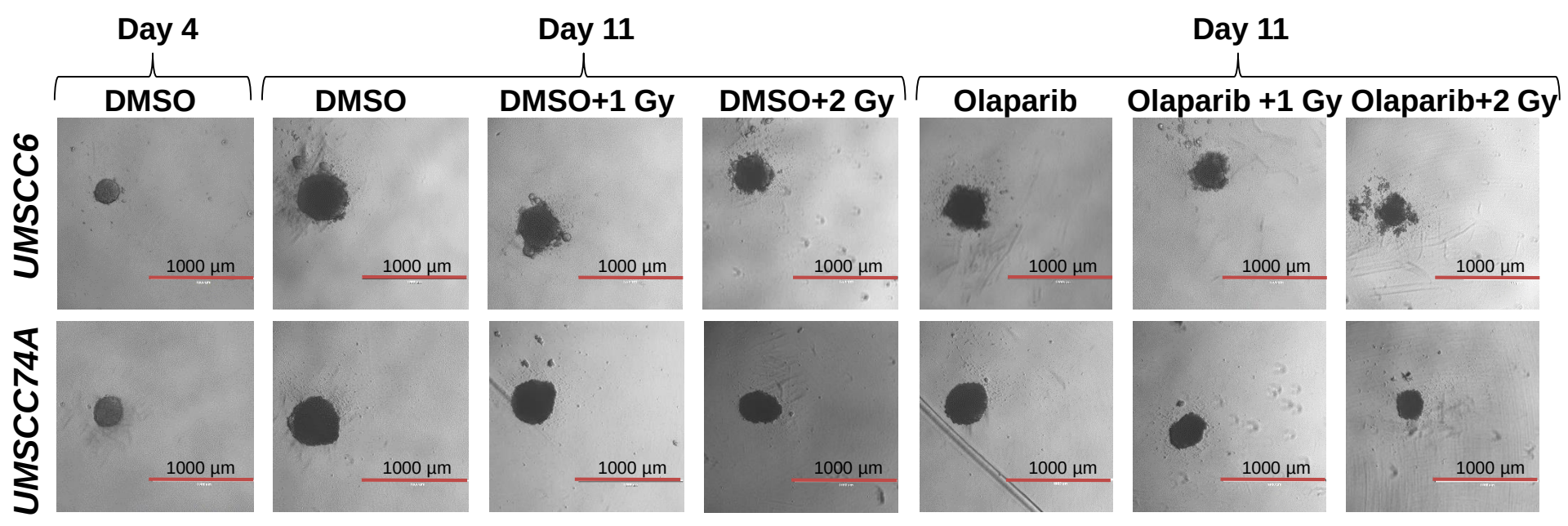
UPCI-SCC090

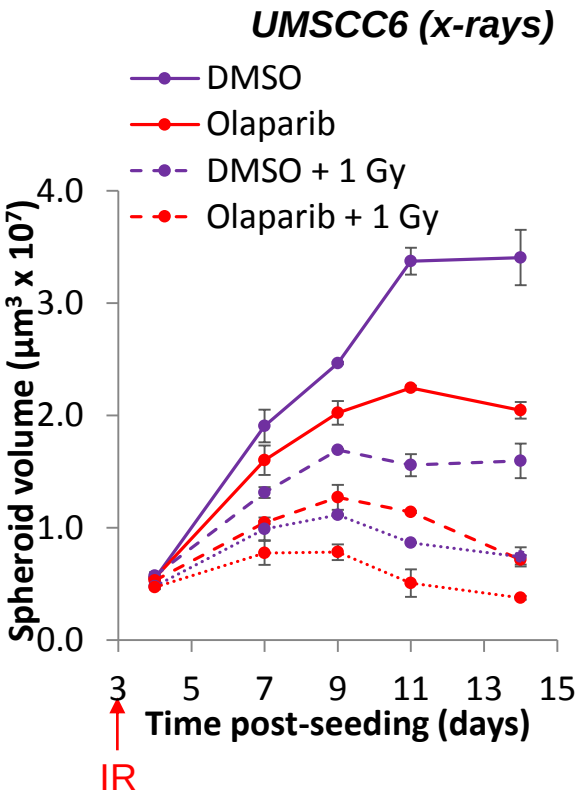
DER – 3.34 (UMSCC6), 1.76 (UMSCC74A), 1.51 (UMSCC47)

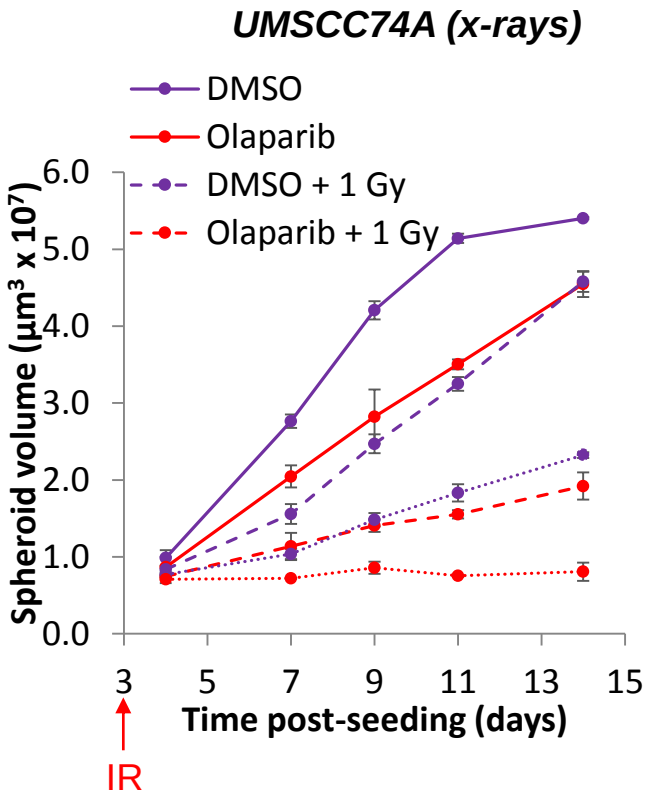
Nickson et al., (2017) Oncotarget






Day 4
Day 11
Day 11
DMSO
DMSO
DMSO+1 Gy
DMSO+2 Gy
Olaparib
Olaparib +1 Gy
Olaparib+2 Gy
UMSCC6
UMSCC74A
1000 µm


UMSCC6 (x-rays)
DMSO
Olaparib
DMSO + 1 Gy
Olaparib + 1 Gy
Spheroid volume (µm³ x 10⁷)
0.0
1.0
2.0
3.0
4.0
3
5
7
9
11
13
15
Time post-seeding (days)
IR


UMSCC74A (x-rays)
DMSO
Olaparib
DMSO + 1 Gy
Olaparib + 1 Gy
Spheroid volume (µm³ x 10⁷)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
3
5
7
9
11
13
15
Time post-seeding (days)
IR

Summary

- **Low energy (high-LET) protons, in contrast to high energy (low-LET) protons, can generate complex DNA complex that contributes to increased cellular radiosensitivity.**
- **Repair of complex DNA damage induced by low energy protons is promoted by histone H2B K120 ubiquitylation mediated by RNF20/40 and MSL2.**
- **A subset of DUBs, particularly USP9X and USP6, are involved in the cellular response to complex DNA damage induced by low energy protons.**
- **Synergy between PARP inhibition/loss and complex DNA damage induction in promoting cancer cell killing.**

Acknowledgements

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Mark Hill, James Thompson
Peter O'Neill

MRC Medical Research Council

nwcr NORTH WEST CANCER RESEARCH