

Supplementary data

Table S1. List of qPCR primers with respective forward (fw) and reverse (rev) primer sequence.

Target	Primer	Sequence 5'-3'
<i>glyceraldehyde-3-phosphate dehydrogenase (GAPDH)</i>	fw	CCT-GCA-CCA-CCA-ACT-GCT-TAG-C
	rev	GCC-AGT-GAG-CTT-CCC-GTT-CAG-C
<i>hypoxanthine phosphoribosyltransferase 1(HPRT1)</i>	fw	GCG-AAA-GTG-GAA-AAG-CCA-AGT
	rev	GCC-ACA-TCA-ACA-GGA-CTC-TTG-TAG
<i>collagen type II alpha 1 chain (Col2α1)</i>	fw	GGCCAGGATGCCCGAAAATTA
	rev	GTCACCTCTGGGTCCTTGTTT
<i>collagen type X alpha 1 chain (ColX)</i>	fw	CCTCTGAGCACCAGAATCCA
	rev	CGTCAAGGACACTAGCAGCA
<i>Bone morphogenetic protein 2 (BMP-2)</i>	fw	AAGCCAAACACAAACAGCGG
	rev	GCCACGATCCAGTCATTCCA
<i>runt-related transcription factor 2 (Runx2)</i>	fw	GCGCATTCCTCATCCCAGTA
	rev	GGTGGGGAGGATTGTGTCTG
<i>Osteoprotegerin (OPG)</i>	fw	CTCACTTGGCCTCCTGCTAA
	rev	TCGCACAGGGTGACATCTAT
<i>Osteopontin (SPP1)</i>	fw	CCAGCCAAGGACCAACTACA
	rev	AGTGTTTGCTGTAATGCGCC
<i>Alkaline Phosphatase (ALPL)</i>	fw	CGGTTGGTAGCCTCCTTCTG
	rev	GACGTTCCGATCCTGAGTGG

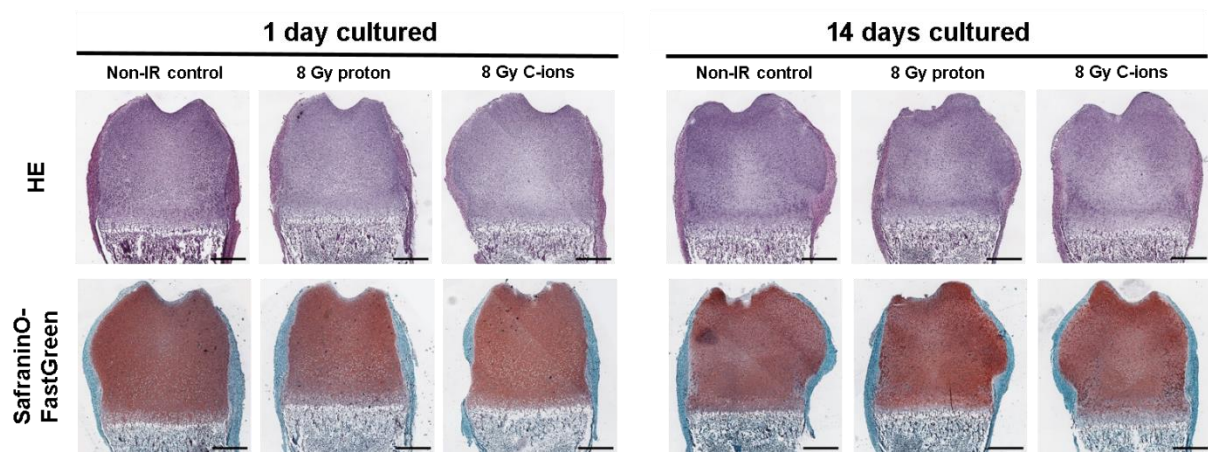


Figure S1. Overview of distal end of the femur histologically stained. Representative images of Haematoxylin&Eosin (H&E) and SafraninO-Fast Green staining of *ex vivo* bone cultures exposed to either 8 Gy proton or C-ions radiation with their respective controls followed by subsequent 1- and 14- days *in vitro* (DIV) cultivation. Scale bar – 500 μm .

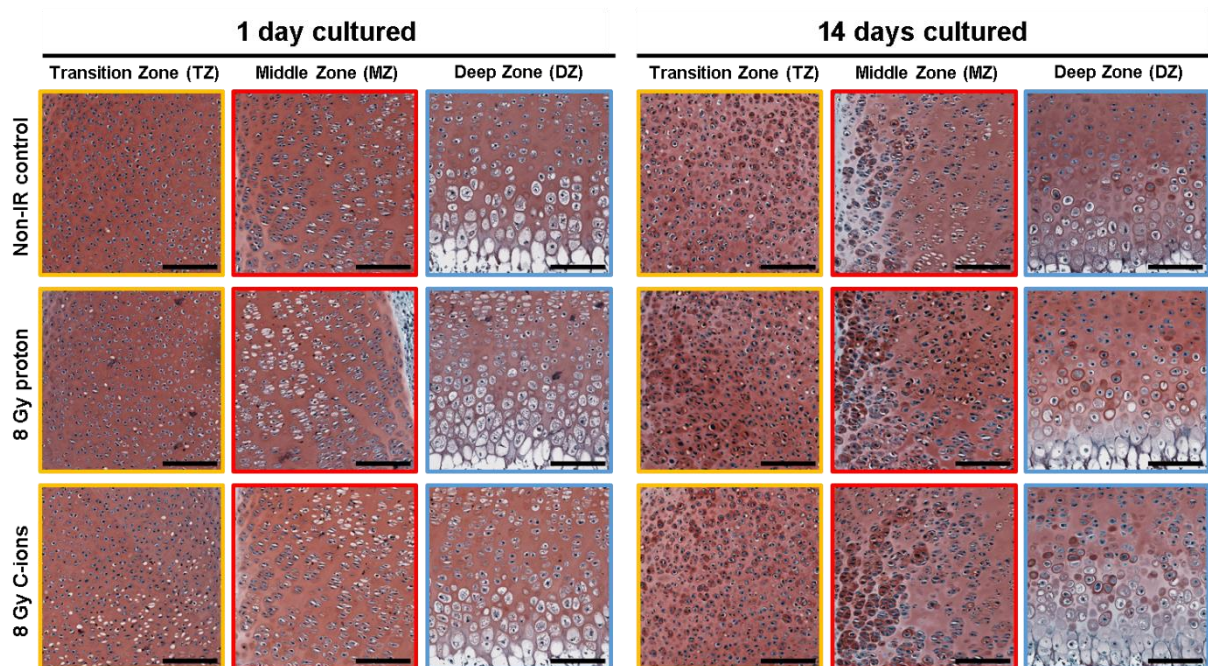


Figure S2. Increased proteoglycan production due to radiation. Representative images of Safranin O-Fast Green staining of *ex vivo* bone cultures exposed to either 8 Gy proton or C-ions radiation with their respective controls followed by subsequent 1- and 14- days *in vitro* (DIV) cultivation. Coloured squares represent transition zone (TZ, yellow), middle zone (MZ, red) and deep zone (DZ, blue). Scale bar - TZ/MZ/DZ = 150 μm .

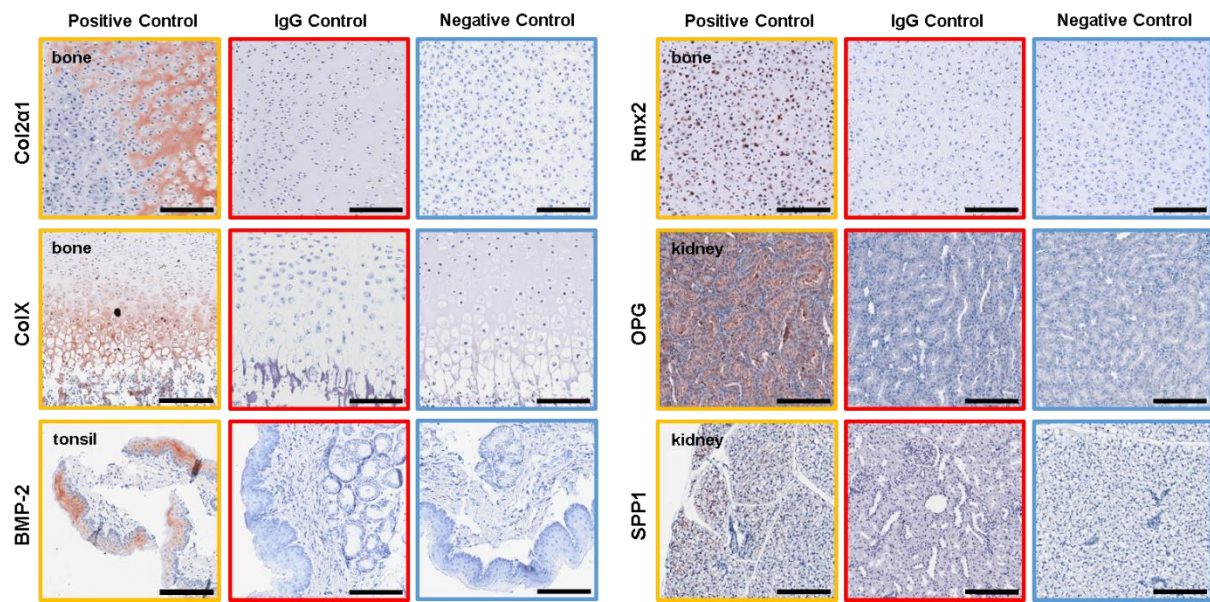


Figure S3. Immunohistochemistry controls. Positive control, IgG control – IgG antibody with respective concentration of primary antibody and negative control – only antibody dilution of appropriate tissue for Col2αa, ColX, BMP-2, Runx2, OPG, and SPP1 are presented. Scale bar = 150 μm.

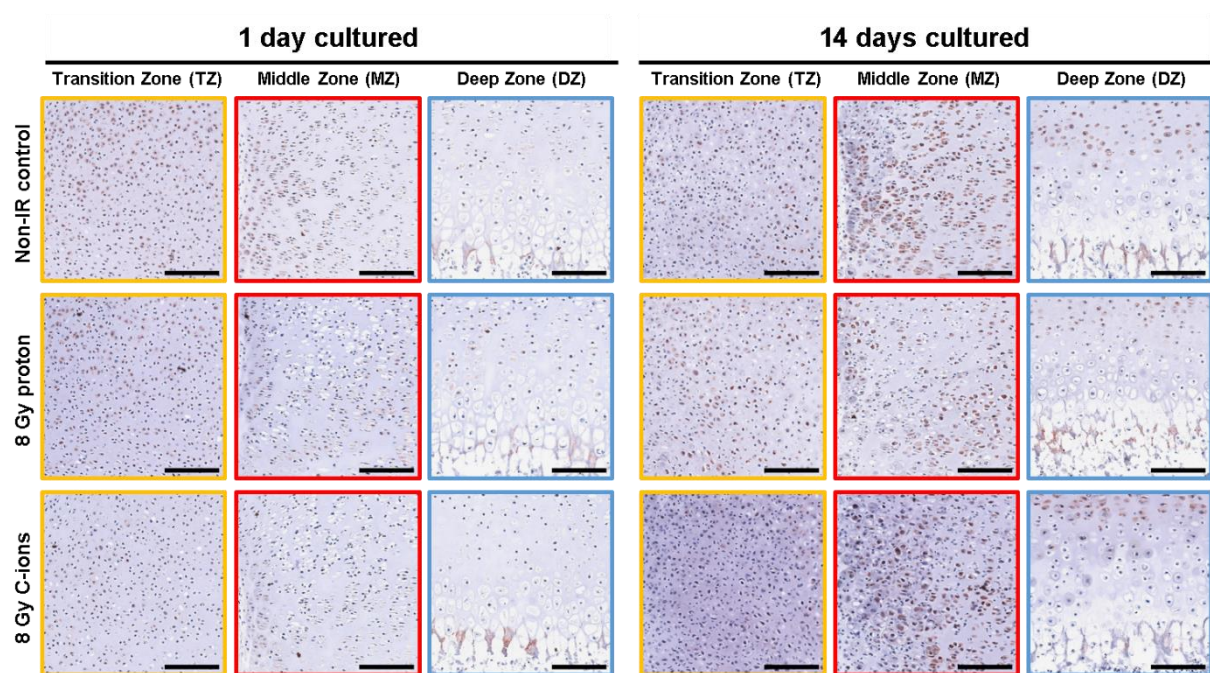


Figure S4. Immunohistochemistry of Bone morphogenetic factor 2 (BMP-2). Representative images of BMP-2 IHC of *ex vivo* bone cultures exposed to either 8 Gy proton or C-ions radiation with their respective controls followed by subsequent 1- and 14- days *in vitro* (DIV) cultivation. Coloured squares represent transition zone (TZ, yellow), middle zone (MZ, red) and deep zone (DZ, blue). Scale bar - TZ/MZ/DZ = 150 μm.

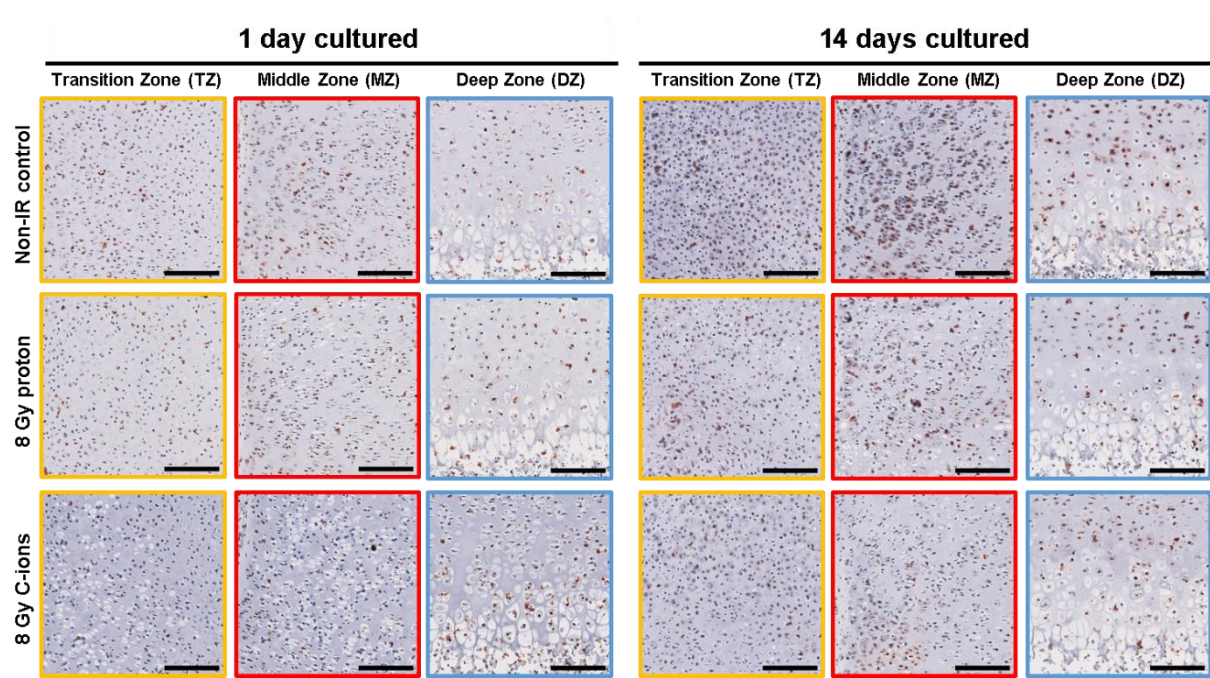


Figure S5. Immunohistochemistry of runt related factor 2 (Runx2). Representative images of Runx2 IHC of *ex vivo* bone cultures exposed to either 8 Gy proton or C-ions radiation with their respective controls followed by subsequent 1- and 14- days *in vitro* (DIV) cultivation. Coloured squares represent transition zone (TZ, yellow), middle zone (MZ, red) and deep zone (DZ, blue). Scale bar - TZ/MZ/DZ = 150 μ m.

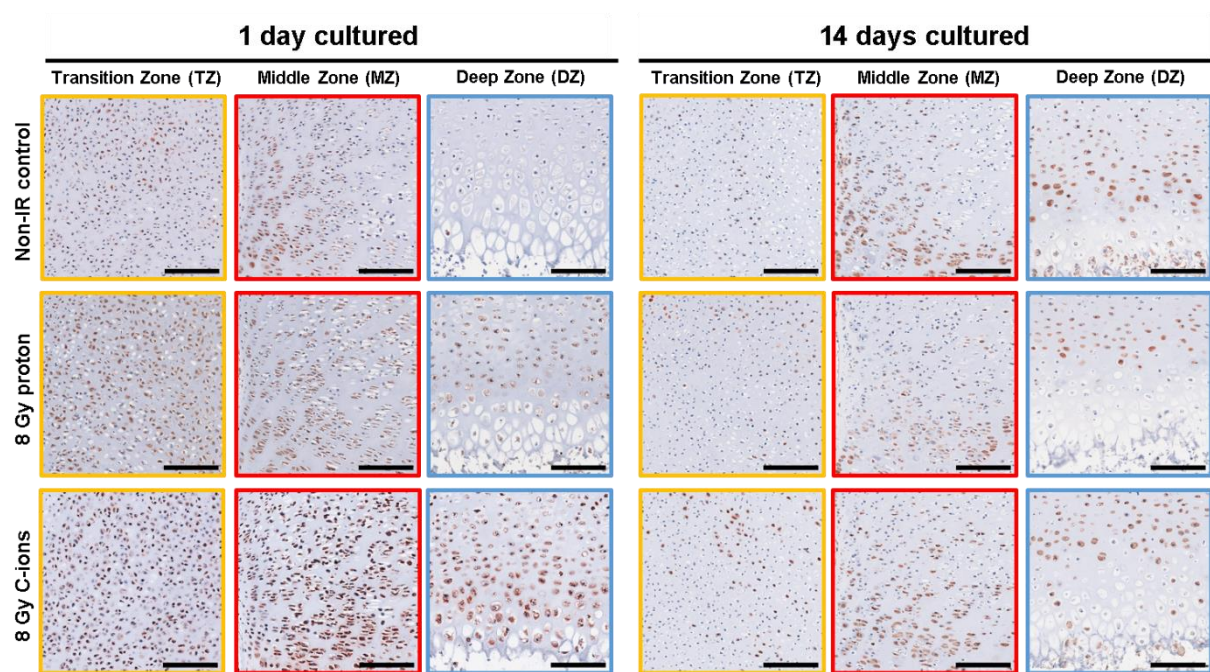


Figure S6. Immunohistochemistry of Osteopontin (OPG). Representative images of OPG IHC of *ex vivo* bone cultures exposed to either 8 Gy proton or C-ions radiation with their respective controls followed by subsequent 1- and 14- days *in vitro* (DIV) cultivation. Coloured squares represent transition zone (TZ, yellow), middle zone (MZ, red) and deep zone (DZ, blue). Scale bar - TZ/MZ/DZ = 150 μ m.