

Supplementary materials

Punnanitinont et al., 2020, on effect of radiation on cancer cell-lines.

1. Figure S1. Effect of radiation on cell viability after four days.
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Figure S1. *Effect of radiation on cell viability.* Sub-confluent adherent cultures of indicated cell-lines that had been grown in parallel to same cell density were treated with one dose of 0, 7.5, or 15 Gy X-rays. Viability of adherent cells after four days was assessed from 7-amino-actinomycin D staining in fluorescence flow cytometry. Mean and its standard error for viability measurements in three independent experiments are plotted.

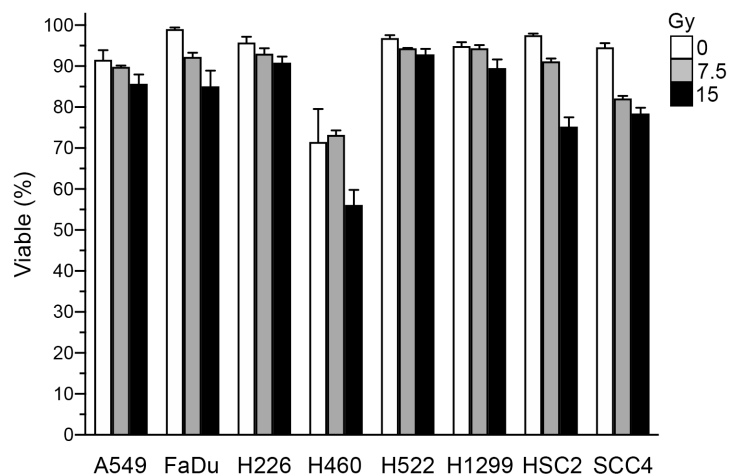


Figure S2. *Effect of radiation on cell surface protein measurement by flow cytometry.* Sub-confluent adherent cultures of indicated cell-lines that had been grown in parallel to same cell density were treated with one dose of 0, 7.5, or 15 Gy X-rays. After four days, adherent cells were collected by scraping and examined by direct fluorescence flow cytometry for cell surface expression of CD23, calreticulin (*CALR*), and mucin 1 (*MUC1*) proteins based on the binding of fluorophore-conjugated mouse IgG monoclonal antibodies (mAb) against them. Binding of IgG1 fraction of normal mouse serum (*mIgG1*) and of an anti-NY-ESO-1 mIgG1 mAb was indirectly assessed with a fluorophore-conjugated rat anti-mIgG1 mAb. Geometric mean fluorescence intensity (GMFI) values of viable cells based on 7-amino-actinomycin D staining was calculated from flow cytometry data. Mean and its standard error for GMFI determinations in three independent experiments are plotted.

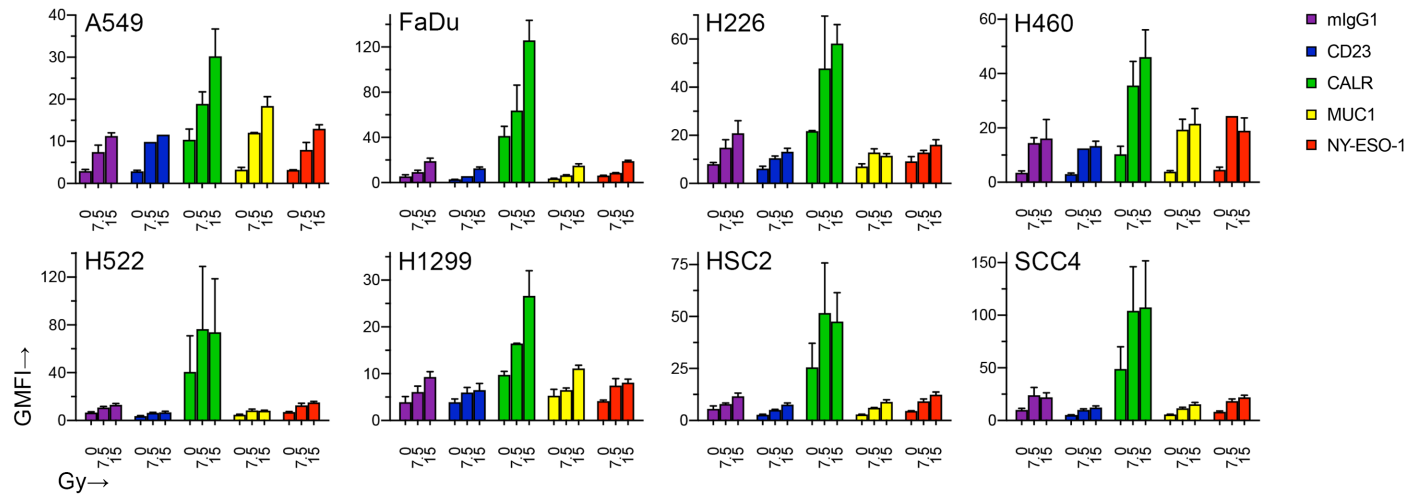


Figure S3. *Effect of radiation on cell size measurement by flow cytometry.* Sub-confluent adherent cultures of indicated cell-lines that had been grown in parallel to same cell density were treated with one dose of 0 or 15 Gy X-rays. After four days, adherent cells were collected by scraping and examined by flow cytometry. Representative histograms of forward scatter of viable cells identified by 7-amino-actinomycin D staining are shown.

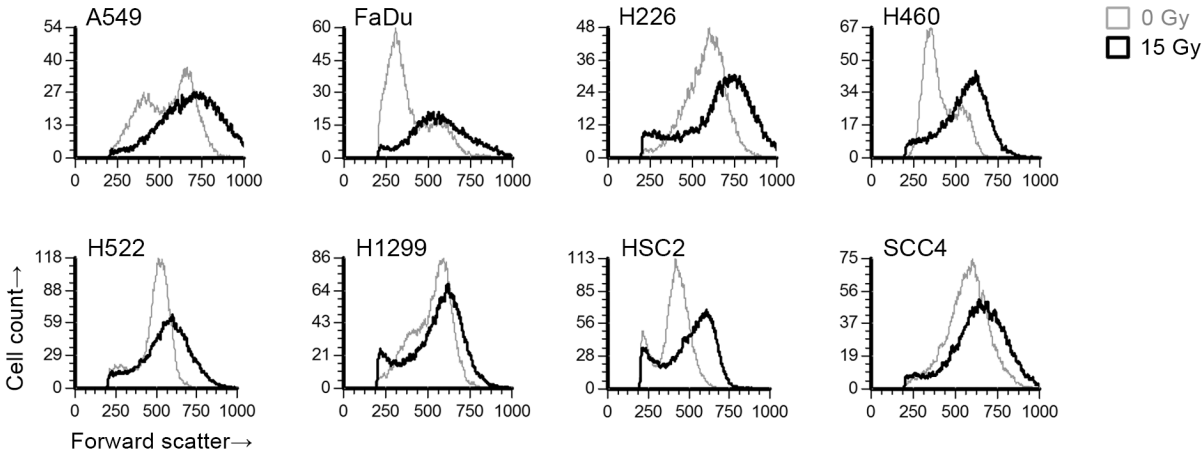


Figure S4. *Effect of radiation on expression of genes encoding cancer/testis antigens (CTAs).* Heatmap shows effect of 15 Gy radiation, calculated as \log_2 fold-change compared to untreated cells, on expression in indicated cell-lines of 105 CTA genes whose expression was detected in at least one cell-line. Genes for which there was no effect are in grey (nominal $P \geq 0.05$ in likelihood ratio test in edgeR). Those with $P < 0.05$ are colored as per the displayed scale. Genes whose expression was considered too low were not examined and are in white. Sub-confluent adherent cultures of cell-lines that had been grown in parallel to same cell density were treated with one dose of 0 or 15 Gy X-rays in three independent experiments. After four days, adherent cells were examined for gene expression by RNA sequencing.

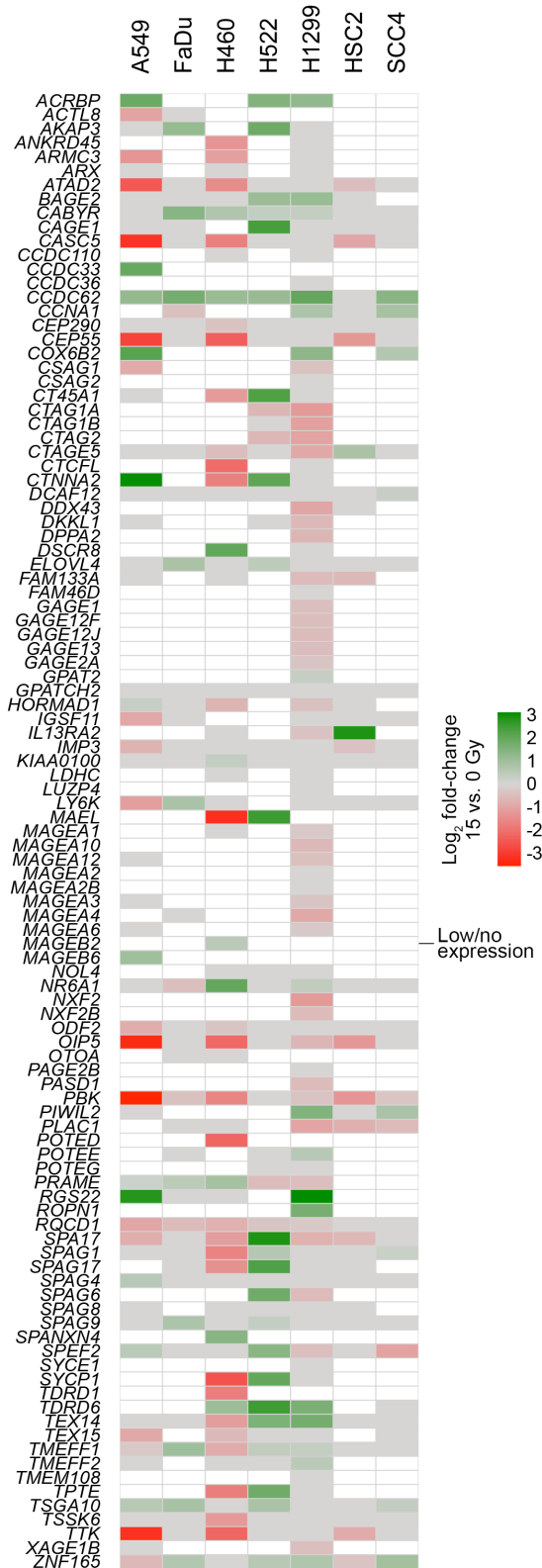


Table S1. Results of differential gene expression analyses applied to identify effect of radiation.

File *Table S1.xlsx* (Microsoft® Excel™ document, 9.4 MB).

Table S2. Genes whose expression was down-regulated by radiation in all cell-lines^a.

<i>Gene</i>	<i>Description</i>	<i>Log₂ fold-change</i>	<i>P</i>
<i>ABCB10</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 10	-1.1–-0.4	3.0E-15–1.5E-02
<i>AK2</i>	Adenylate kinase 2	-0.8–-0.2	4.6E-23–4.6E-02
<i>AL662801.1</i>	C6orf47 antisense RNA 1	-0.7–-0.3	4.3E-12–9.2E-03
<i>ALYREF</i>	Aly/REF export factor	-1.8–-0.2	1.9E-74–3.5E-02
<i>ATP6V1G2-DDX39B</i>	ATP6V1G2-DDX39B readthrough (NMD candidate)	-0.7–-0.3	1.1E-12–4.1E-02
<i>BCL7C</i>	B-cell CLL/lymphoma 7C	-0.9–-0.3	6.5E-11–4.7E-02
<i>BTF3</i>	Basic transcription factor 3	-0.6–-0.2	1.7E-08–2.4E-02
<i>C12orf10</i>	Chromosome 12 open reading frame 10	-0.8–-0.4	5.1E-07–3.2E-02
<i>C17orf75</i>	Chromosome 17 open reading frame 75	-0.7–-0.2	1.9E-05–2.2E-02
<i>C8orf82</i>	Chromosome 8 open reading frame 82	-1.0–-0.4	1.7E-09–4.9E-02
<i>CDCA7</i>	Cell division cycle associated 7	-1.5–-0.3	2.4E-60–3.9E-02
<i>CDKN2C</i>	Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	-1.8–-0.5	1.3E-37–2.3E-02
<i>COX20</i>	COX20 cytochrome c oxidase assembly factor	-1.1–-0.3	1.1E-17–2.1E-02
<i>CYC1</i>	Cytochrome c-1	-1.0–-0.3	1.7E-30–4.0E-02
<i>DCPS</i>	Decapping enzyme, scavenger	-1.5–-0.5	1.0E-21–2.5E-02
<i>DHPS</i>	Deoxyhypusine synthase	-0.9–-0.3	6.6E-19–2.3E-02
<i>ECI2</i>	Enoyl-CoA delta isomerase 2	-1.0–-0.4	7.1E-08–7.9E-03
<i>EEF2KMT</i>	Eukaryotic elongation factor 2 lysine methyltransferase	-1.3–-0.4	1.0E-16–4.2E-02
<i>ENO1</i>	Enolase 1, (alpha)	-0.9–-0.2	3.1E-09–8.2E-03
<i>ESD</i>	Esterase D	-0.9–-0.4	2.0E-09–4.0E-02
<i>FAM20B</i>	Family with sequence similarity 20, member B	-0.9–-0.3	6.2E-22–7.3E-03
<i>FH</i>	Fumarate hydratase	-0.8–-0.4	4.2E-23–2.0E-02
<i>H2AFV</i>	H2A histone family, member V	-1.2–-0.2	9.4E-35–9.0E-03
<i>H2BFS</i>	H2B histone family, member S (pseudogene)	-2.2–-0.8	2.2E-13–1.6E-02
<i>H3F3B</i>	H3 histone, family 3B (H3.3B)	-0.9–-0.3	5.5E-13–2.8E-02
<i>HIST1H1B</i>	Histone cluster 1, H1b	-4.2–-0.9	1.7E-219–4.8E-09
<i>HIST1H1C</i>	Histone cluster 1, H1c	-2.3–-1.3	4.6E-75–9.3E-12
<i>HIST1H1D</i>	Histone cluster 1, H1d	-3.8–-1.1	5.7E-142–1.1E-05
<i>HIST1H1E</i>	Histone cluster 1, H1e	-2.7–-1.1	2.6E-74–5.1E-06
<i>HIST1H2AB</i>	Histone cluster 1, H2ab	-3.9–-0.8	1.1E-140–2.3E-04
<i>HIST1H2AC</i>	Histone cluster 1, H2ac	-1.6–-0.2	6.8E-40–4.0E-02
<i>HIST1H2AH</i>	Histone cluster 1, H2ah	-4.0–-1.0	7.4E-166–3.9E-09
<i>HIST1H2AI</i>	Histone cluster 1, H2ai	-3.9–-1.1	3.8E-119–4.1E-07
<i>HIST1H2AK</i>	Histone cluster 1, H2ak	-3.2–-0.9	4.5E-82–3.8E-05
<i>HIST1H2AL</i>	Histone cluster 1, H2al	-4.2–-1.1	2.1E-86–1.7E-04
<i>HIST1H2AM</i>	Histone cluster 1, H2am	-3.5–-1.0	1.1E-133–2.3E-05
<i>HIST1H2BB</i>	Histone cluster 1, H2bb	-3.1–-1.0	9.2E-151–1.4E-08
<i>HIST1H2BC</i>	Histone cluster 1, H2bc	-2.6–-0.8	2.4E-118–4.2E-04
<i>HIST1H2BD</i>	Histone cluster 1, H2bd	-2.7–-0.7	2.0E-121–1.0E-06
<i>HIST1H2BJ</i>	Histone cluster 1, H2bj	-3.5–-1.1	4.9E-109–6.1E-08
<i>HIST1H2BK</i>	Histone cluster 1, H2bk	-2.6–-0.7	1.8E-72–5.1E-08
<i>HIST1H2BL</i>	Histone cluster 1, H2bl	-3.9–-1.0	1.3E-154–5.4E-05
<i>HIST1H2BN</i>	Histone cluster 1, H2bn	-3.2–-0.7	5.0E-145–7.0E-07
<i>HIST1H2BO</i>	Histone cluster 1, H2bo	-3.9–-0.8	1.3E-140–3.5E-05
<i>HIST1H3A</i>	Histone cluster 1, H3a	-3.1–-1.2	1.3E-103–4.7E-05
<i>HIST1H3B</i>	Histone cluster 1, H3b	-4.0–-1.4	1.8E-166–1.9E-13
<i>HIST1H3H</i>	Histone cluster 1, H3h	-3.9–-1.0	5.5E-212–2.0E-08
<i>HIST1H3J</i>	Histone cluster 1, H3j	-3.8–-0.9	1.4E-142–5.3E-06
<i>HIST1H4A</i>	Histone cluster 1, H4a	-3.7–-1.1	1.7E-104–4.5E-05

<i>HIST1H4B</i>	Histone cluster 1, H4b	-3.0—1.4	3.4E-89–3.7E-09
<i>HIST1H4C</i>	Histone cluster 1, H4c	-2.7—1.1	1.4E-67–3.7E-15
<i>HIST1H4D</i>	Histone cluster 1, H4d	-3.4—0.7	2.0E-78–6.5E-05
<i>HIST1H4E</i>	Histone cluster 1, H4e	-2.0—1.1	1.1E-59–2.4E-11
<i>HIST1H4H</i>	Histone cluster 1, H4h	-2.9—1.2	3.6E-96–4.6E-11
<i>HIST1H4J</i>	Histone cluster 1, H4j	-3.2—0.8	1.0E-42–7.2E-04
<i>HIST1H4K</i>	Histone cluster 1, H4k	-2.5—0.7	1.9E-20–3.8E-04
<i>HIST2H2AA3</i>	Histone cluster 2, H2aa3	-3.0—0.8	1.7E-39–1.6E-02
<i>HIST2H2AA4</i>	Histone cluster 2, H2aa4	-2.7—1.1	5.3E-56–2.3E-05
<i>HIST2H2AB</i>	Histone cluster 2, H2ab	-3.8—1.0	1.2E-163–2.7E-07
<i>HIST2H2AC</i>	Histone cluster 2, H2ac	-2.4—0.9	2.3E-67–2.1E-03
<i>HIST2H2BD</i>	Histone cluster 2, H2bd (pseudogene)	-3.3—0.8	2.1E-68–2.3E-03
<i>HIST2H2BE</i>	Histone cluster 2, H2be	-2.4—0.8	2.2E-78–1.3E-05
<i>HIST2H2BF</i>	Histone cluster 2, H2bf	-3.3—0.5	1.9E-161–4.7E-02
<i>HIST2H3A</i>	Histone cluster 2, H3a	-4.0—0.9	3.3E-106–1.4E-03
<i>HIST2H3C</i>	Histone cluster 2, H3c	-4.4—0.8	3.2E-78–6.7E-04
<i>HIST4H4</i>	Histone cluster 4, H4	-1.6—0.8	5.8E-37–7.5E-06
<i>HMGB1</i>	High mobility group box 1	-1.7—0.4	1.4E-63–3.5E-03
<i>HMGB1P5</i>	High mobility group box 1 pseudogene 5	-2.0—0.5	5.7E-66–3.2E-03
<i>HMG2</i>	High mobility group nucleosomal binding domain 2	-1.7—0.4	1.3E-61–6.6E-03
<i>HMG2P3</i>	HMG2 pseudogene 3	-1.8—0.5	2.2E-34–3.5E-02
<i>LBR</i>	Lamin B receptor	-1.8—0.4	1.9E-98–8.2E-03
<i>LMNB1</i>	Lamin B1	-2.8—0.3	7.9E-143–1.4E-02
<i>LSM2</i>	LSM2 homolog, U6 small nuclear RNA associated	-1.5—0.4	1.2E-29–5.4E-03
<i>MCRS1</i>	Microspherule protein 1	-0.8—0.3	2.8E-19–8.6E-03
<i>MRPL3</i>	Mitochondrial ribosomal protein L3	-1.1—0.3	2.1E-23–5.0E-02
<i>MTF1</i>	Mitochondrial fission process 1	-1.4—0.5	3.5E-31–5.1E-03
<i>NDC1</i>	NDC1 transmembrane nucleoporin	-1.8—0.3	2.3E-92–3.3E-02
<i>NOL7</i>	Nucleolar protein 7, 27kDa	-0.9—0.3	3.0E-17–2.8E-02
<i>NPM3</i>	Nucleophosmin/nucleoplasmin 3	-1.5—0.3	2.5E-38–3.9E-03
<i>NUCKS1</i>	Nuclear casein kinase and cyclin-dependent kinase substrate 1	-1.1—0.4	2.0E-20–1.5E-03
<i>PIR</i>	Pirin (iron-binding nuclear protein)	-1.2—0.4	5.5E-13–1.1E-02
<i>POLD2</i>	Polymerase (DNA directed), delta 2, accessory subunit	-1.2—0.2	1.7E-36–3.1E-02
<i>PP1L1</i>	Peptidylprolyl isomerase (cyclophilin)-like 1	-1.0—0.3	4.9E-24–3.4E-02
<i>PPP1CA</i>	Protein phosphatase 1, catalytic subunit, alpha isozyme	-0.8—0.2	3.8E-18–3.5E-02
<i>PRMT1</i>	Protein arginine methyltransferase 1	-1.2—0.2	2.4E-41–4.3E-02
<i>PSMA1</i>	Proteasome (prosome, macropain) subunit, alpha type, 1	-0.6—0.3	8.1E-08–3.5E-02
<i>PTMA</i>	Prothymosin, alpha	-1.5—0.5	3.3E-45–8.4E-03
<i>RBMX</i>	RNA binding motif protein, X-linked	-0.9—0.4	4.3E-22–1.3E-02
<i>RCC1</i>	Regulator of chromosome condensation 1	-0.8—0.2	2.5E-19–2.3E-02
<i>SET</i>	SET nuclear proto-oncogene	-1.2—0.2	1.2E-27–3.8E-02
<i>SLC25A15</i>	Solute carrier family 25, member 15	-1.0—0.4	4.3E-16–3.7E-02
<i>SLC25A5</i>	Solute carrier family 25, member 5	-1.1—0.4	1.3E-22–1.3E-02
<i>SRSF2</i>	Serine/arginine-rich splicing factor 2	-1.1—0.3	2.6E-37–2.7E-02
<i>SSRP1</i>	Structure specific recognition protein 1	-1.6—0.4	3.7E-60–6.7E-03
<i>STAG1</i>	Stromal antigen 1	-1.0—0.4	2.5E-20–2.2E-02
<i>SUMO3</i>	Small ubiquitin-like modifier 3	-0.8—0.3	3.6E-12–6.1E-03
<i>TAF9B</i>	TAF9B RNA polymerase II, TBP-associated factor, 31kDa	-1.2—0.6	9.8E-25–1.3E-04
<i>TRMT112</i>	TRNA methyltransferase 11-2 homolog (<i>S. cerevisiae</i>)	-1.0—0.3	1.8E-27–3.1E-02
<i>UQCRC3</i>	Ubiquinol-cytochrome c reductase complex assembly factor 3	-1.2—0.4	1.1E-27–4.1E-02
<i>WDR34</i>	WD repeat domain 34	-1.1—0.5	1.2E-34–9.1E-03
<i>XRCC6</i>	X-ray repair complementing defective repair in Chinese hamster cells 6	-1.0—0.3	5.9E-24–1.1E-02
<i>ZNRD1</i>	Zinc ribbon domain containing 1	-0.8—0.4	2.0E-08–4.5E-02

^aGene expression differences between three pairs of 15 Gy-irradiated and non-irradiated cells of seven cell-lines was analyzed with the likelihood ratio test in edgeR software. Genes down-regulated by radiation with nominal $P \leq 0.05$ in all cell-lines are listed along with ranges of \log_2 fold-change (15 Gy vs. 0 Gy) and P values among the cell-lines.