Falcone et. al.,
Supplementary Information

Figure S1

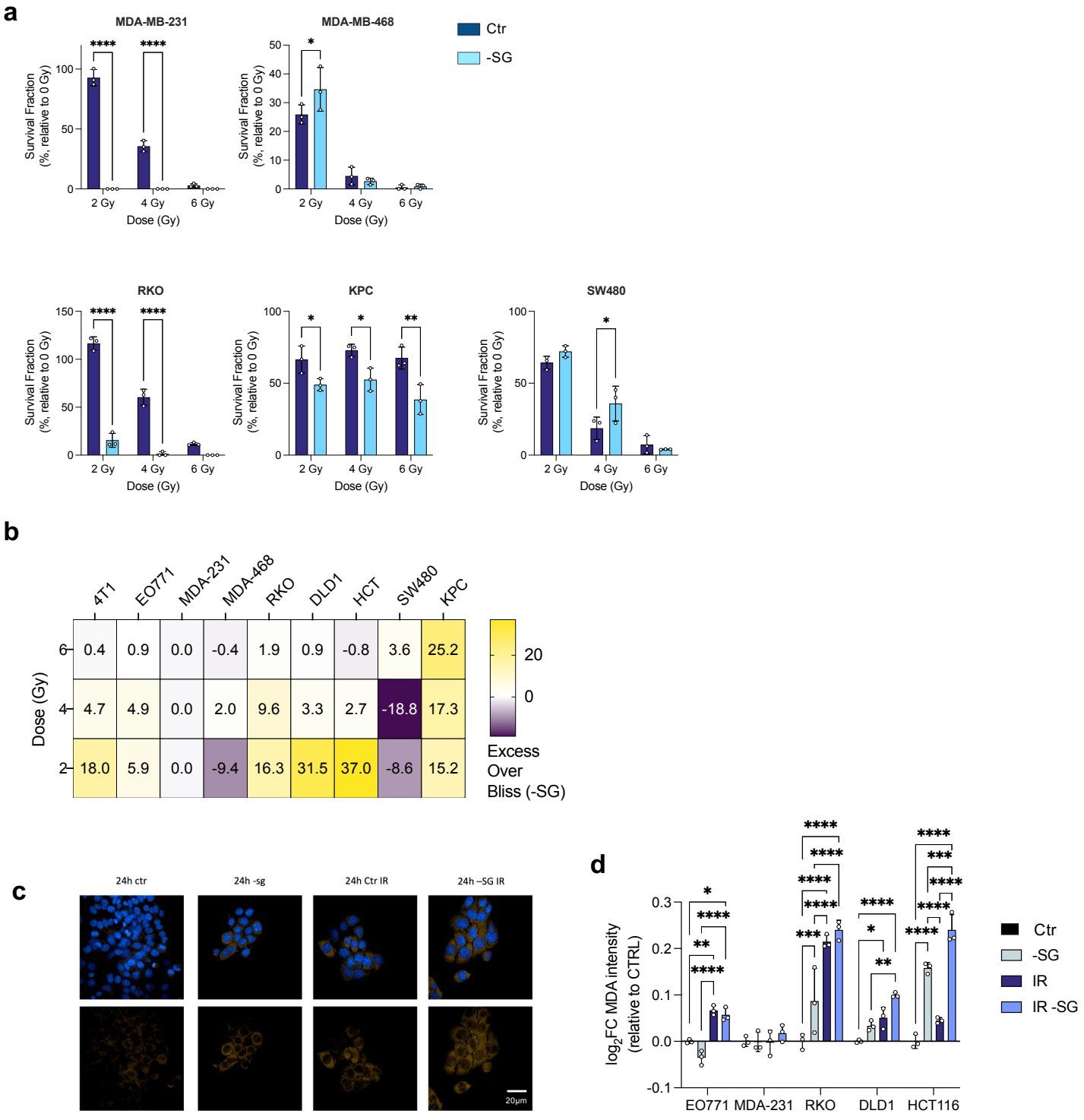


Figure S1. Serine and glycine starvation sensitises a range of cancer cells to radiation *in vitro* (linked to Figure 1)

a) Quantification of clonogenic assays for the displayed cell lines cultured with media containing serine and glycine (+SG) or not (-SG) and irradiated with increasing dose of radiation (0-6 Gy); surviving fraction relative to non-irradiated controls (mean +/-SD, one experiment in biological triplicate, Two-way ANOVA with Šídák's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005). For MDA-MB-231 cells there was complete cell death in the –SG condition for all doses of radiation.

b) Excess over Bliss score based on clonogenic assays of displayed lines cultured with media containing serine and glycine or not and irradiated with increasing dose of radiation (0-6 Gy) (mean, n = 4 experiments in biological triplicate for 4T1 and one experiment in biological triplicate for other cell lines).

c) Representative images of malondialdehyde (MDA) adduct detected using immunofluorescence staining 24h after treatment. 4T1 cells were cultured with media containing serine and glycine (Ctr) or not (-SG) and irradiated with a final dose of 4Gy X-ray radiation (IR) (scale bar 20um).

d) Quantification of malondialdehyde (MDA) adduct intensity relative to Ctr measured using immunofluorescence staining 72h after treatment. Cells were cultured with media containing serine and glycine (+SG) or not (-SG) and irradiated with a final dose of radiations of 4Gy (IR). (mean +/-SD, one experiment in biological triplicate, Two-way ANOVA with Tukey's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005).

Figure S2

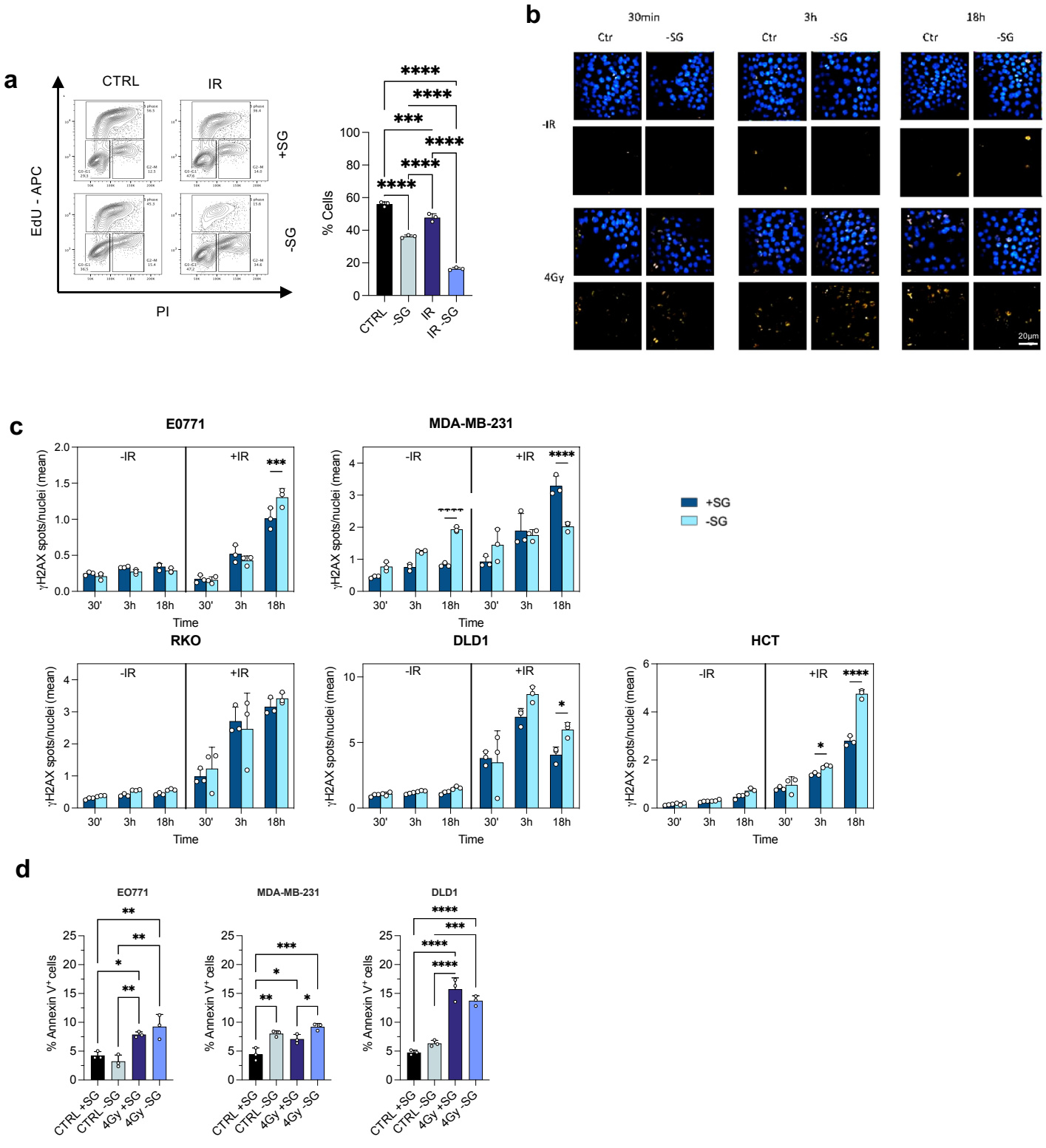


Figure S2. Serine and glycine starvation increases damage from radiation *in vitro* (linked to Figure 1)

a) EdU incorporation measured using flow cytometry 48h after treatment. Representative FACS plots of EdU fluorescence vs. Propidium Iodide (PI) of HCT116 cells cultured with media containing serine and glycine (+SG; top row) or not (-SG; bottom row) and irradiated with a final dose of radiations of 4Gy (IR). Numbers indicate percentage of EdU positive cells in each gate, with quantification (mean +/-SD, one experiment in biological triplicate, One-way ANOVA with Tukey's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005).

b) Representative images of γ-H2AX foci at different time points using immunofluorescence staining in 4T1 cells cultured with media containing serine and glycine (Ctr) or not (-SG) and irradiated or not with a final dose of 4Gy X-ray radiation (+/-IR) (scale bar 20um).

c) Quantification of γ-H2AX foci at different time points in cells cultured with media containing serine and glycine (+SG) or not (-SG) and irradiated with a final dose of radiations of 4Gy (IR) (mean +/-SD, one experiment in biological triplicate, Two-way ANOVA with Tukey's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005).

d) Quantification of Annexin V positive cells using flow cytometry 24h after treatment. Cells cultured with media containing serine and glycine (+SG; top row) or not (-SG; bottom row) and irradiated with a final dose of radiations of 4Gy (IR). Numbers indicate percentage of positive cells (mean +/-SD, one experiment in biological triplicate, One-way ANOVA with Tukey's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005).

Figure S3

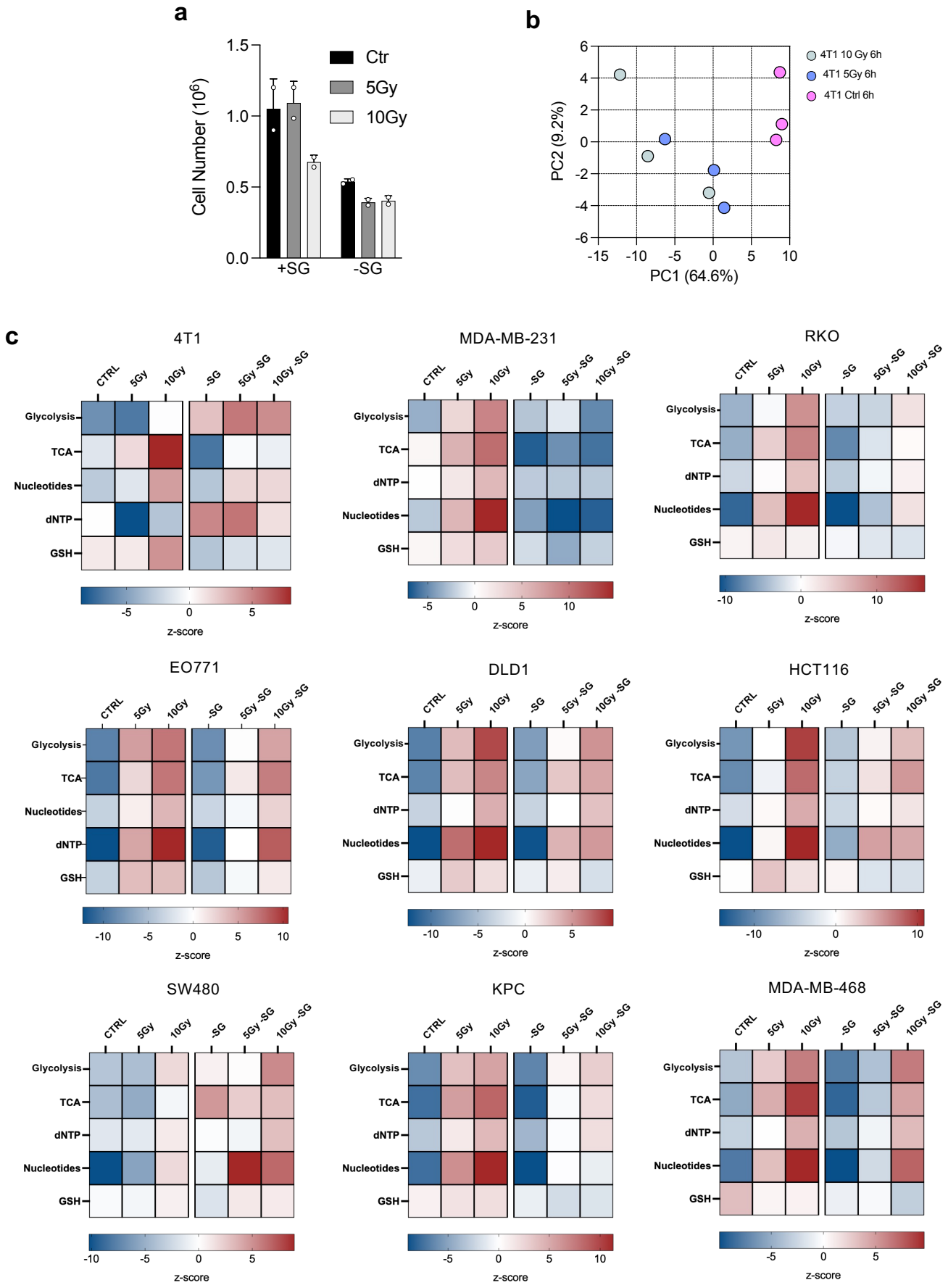


Figure S3. Metabolomic impact of radiation and SG starvation (linked to Figure 2)

a) Absolute number of 4T1 cells used for metabolomics experiment (shown in Figure 3) cultured with media containing serine and glycine (Ctr) or not (-SG) and irradiated with increasing doses of X-ray radiation (0, 5 and 10 Gy) for 24h (mean +/-SD, counts done in a single experiment in biological duplicate).

b) Spatial division displayed as PCA plot based on the metabolomic profile of 4T1 cells cultured in complete medium irradiated with increasing dose of X-ray radiation (0/Ctr, 5 and 10 Gy) and harvested at 6h post-radiation (one experiment in biological triplicate).

c) Summarized pathway activity for the displayed cell lines cultured with media containing serine and glycine (CTRL) or not (-SG) and irradiated with increasing dose of radiations (0, 5 and 10 Gy) for 24h (one experiment in biological triplicate for each cell line).

Figure S4

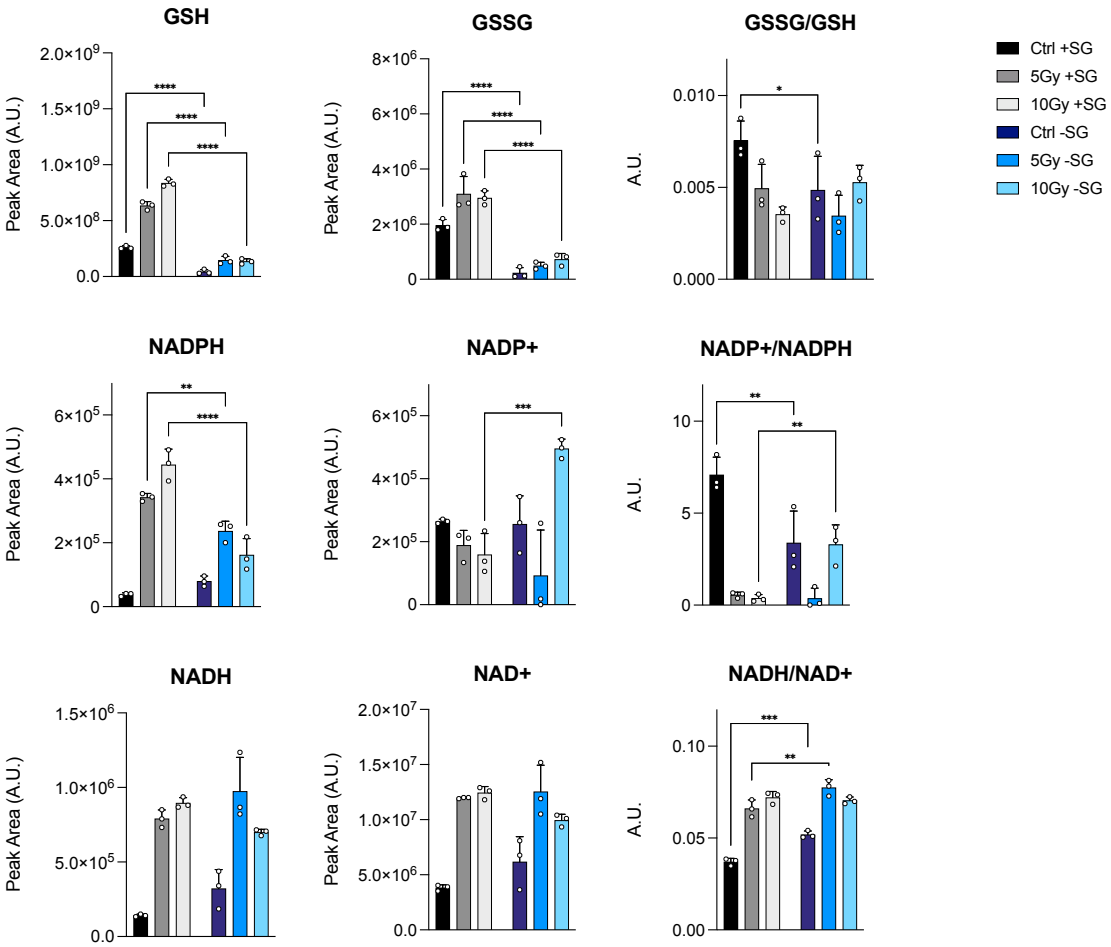
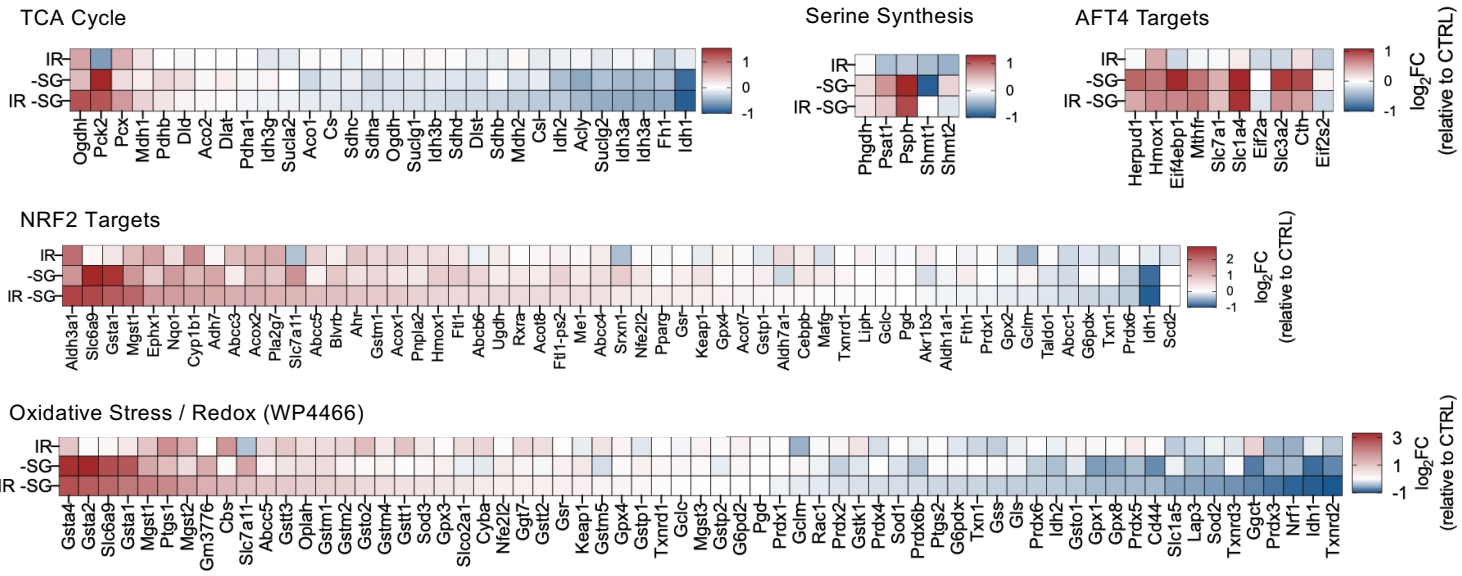


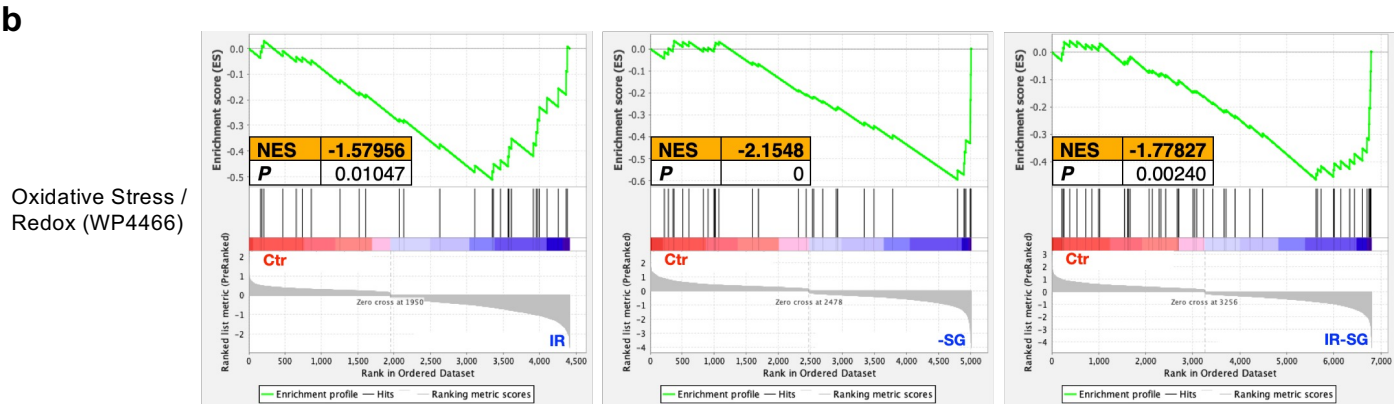
Figure S4. Impact of radiotherapy on redox related metabolites (linked to Figure 3)
Metabolite levels analysed by LCMS in 4T1 cells cultured with media containing serine and glycine (+SG) or not (-SG) and irradiated with increasing doses of X-ray radiation (0, 5 and 10 Gy) for 24h (mean +/-SD, one experiment in biological triplicate, Two-way ANOVA with Šídák's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005).

Figure S5

a



b



c

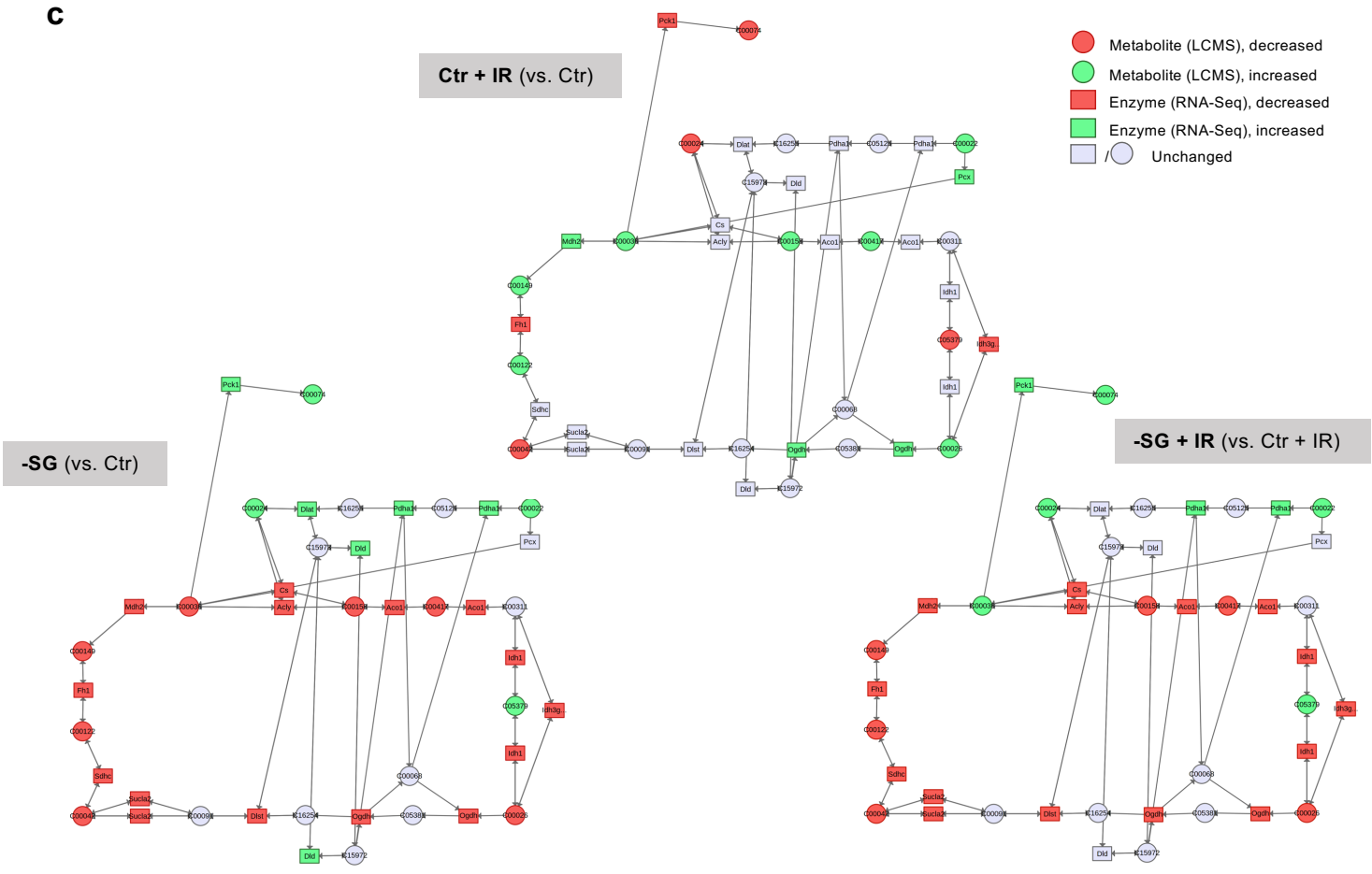


Figure S5

Figure S5. TCA cycle ans stress responses impact of radiation and SG starvation (linked to Figure 4)

a) Heat map representation of gene expression (as log_2FC relative to Ctr condition) of members of the displayed pathways based on RNA-Seq profile of 4T1 cells cultured with media containing either serine and glycine (+SG) or not (-SG) and subsequently irradiated with a final dose of radiations 5 Gy (IR) for 24h.

b) GSEA of the IR, -SG, IR-SG vs Ctr respectively using the WP4466 (Oxidative stress / Redox) gene sets (NES, normalized enrichment score).

c) TCA cycle KEGG pathway overview displaying both enzyme (rectangles) and metabolite (circles) ratios between the highlighted conditions (one experiment in biological triplicate for both metabolomics profiling and RNA-Sequencing).

Figure S6

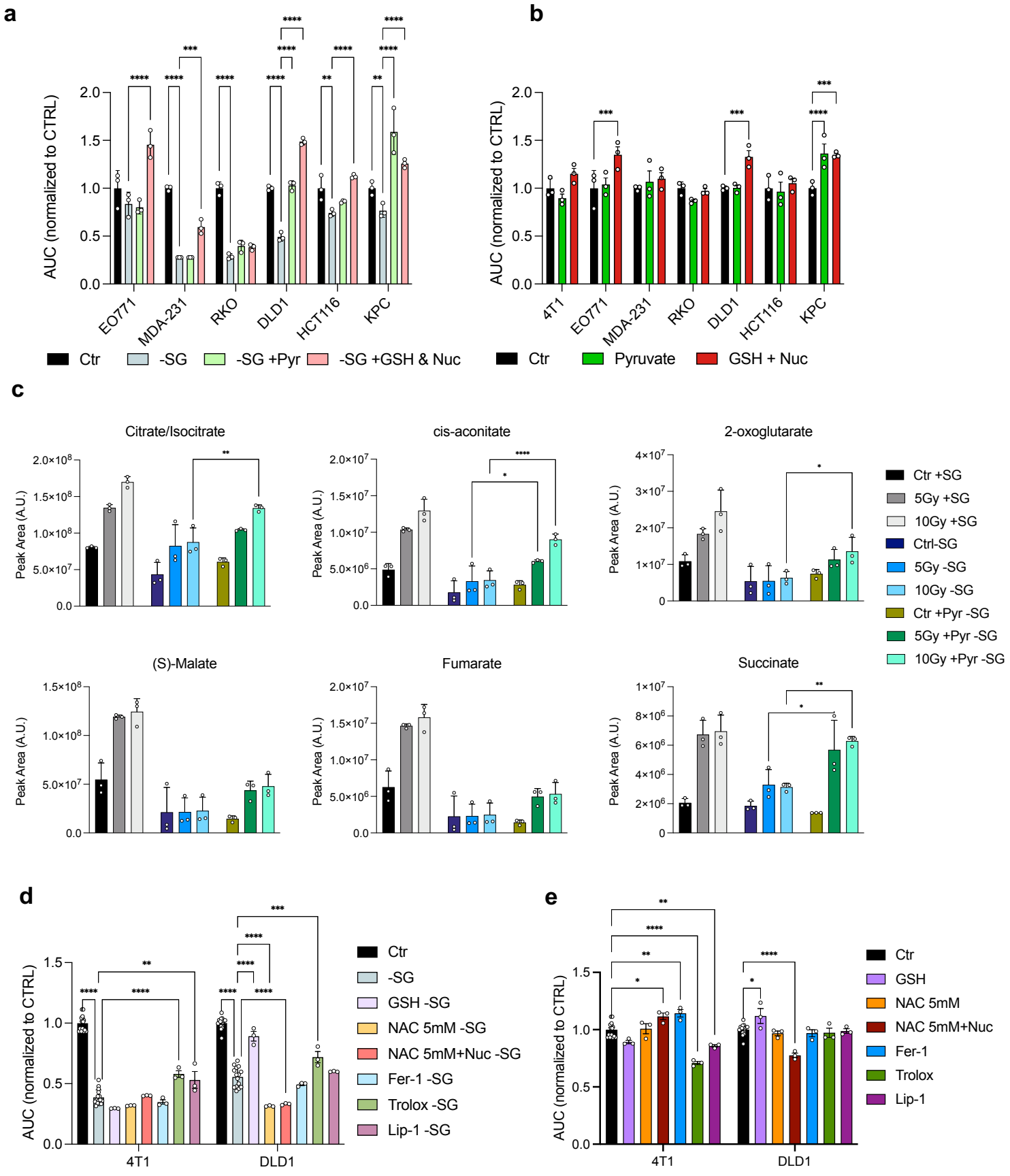

a
AUC (normalized to CTRL)
EO771
MDA-231
RKO
DLD1
HCT116
KPC
Ctr
-SG
-SG +Pyr
-SG +GSH & Nuc
b
AUC (normalized to CTRL)
4T1
EO771
MDA-231
RKO
DLD1
HCT116
KPC
Ctr
Pyruvate
GSH + Nuc
c
Citrate/Isocitrate
cis-aconitate
2-oxoglutarate
(S)-Malate
Fumarate
Succinate
Peak Area (A.U.)
Ctr +SG
5Gy +SG
10Gy +SG
Ctrl-SG
5Gy -SG
10Gy -SG
Ctr +Pyr -SG
5Gy +Pyr -SG
10Gy +Pyr -SG
d
AUC (normalized to CTRL)
4T1
DLD1
Ctr
-SG
GSH -SG
NAC 5mM -SG
NAC 5mM+Nuc -SG
Fer-1 -SG
Trolox -SG
Lip-1 -SG
e
AUC (normalized to CTRL)
4T1
DLD1
Ctr
GSH
NAC 5mM
NAC 5mM+Nuc
Fer-1
Trolox
Lip-1

Figure S6

Figure S6. Metabolite rescue (linked to Figure 4)

a) Clonogenic assay shown as normalized area under the curve (AUC) of the displayed cells lines cultured with media containing serine and glycine (Ctr) or not (-SG), irradiated with increasing dose of X-ray radiation (0-6 Gy) and with or without supplementation with 2mM Pyruvate or 2.5mM reduced glutathione & nucleosides mix (mean +/-SD, one experiment in biological triplicate, Two-way ANOVA with Dunnet's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005).

b) Clonogenic assay shown as normalized area under the curve (AUC) of the displayed cells lines cultured with media containing serine and glycine, irradiated with increasing dose of radiations (0-6 Gy) and supplemented with either 2mM Pyruvate or with 2.5mM reduced glutathione & nucleosides mix (mean +/-SD, one experiment in biological triplicate, Two-way ANOVA with Dunnet's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005).

c) Metabolite levels analysed by LCMS in 4T1 cells cultured with media containing serine and glycine (Ctr) or not (-SG) and with or without 2mM Pyruvate, subsequently irradiated with increasing doses of X-ray radiation (0, 5 and 10 Gy) for 24h (mean +/-SD, one experiment in biological triplicate Two-way ANOVA with Tukey's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005).

d) Clonogenic assay shown as normalized area under the curve (AUC) of 4T1 and DLD1 lines cultured with media containing serine and glycine (Ctr) or not (-SG), subsequently irradiated with increasing dose of radiations (0-6 Gy) and supplemented with 2.5mM reduced glutathione (GSH), 5mM N-acetylcysteine (NAC), 5mM NAC+nucleosides mix (NAC+Nuc), 2uM Ferrostatin-1 (Fer-1), 50uM Trolox or 100nM Liproxstatin-1 (Lip-1) (mean +/-SD, one experiment in biological triplicate, Two-way ANOVA with Dunnet's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005).

e) Clonogenic assay shown as normalized area under the curve (AUC) of 4T1 and DLD1 lines cultured with media containing serine and glycine, subsequently irradiated with increasing dose of radiations (0-6 Gy) and supplemented with 2.5mM reduced glutathione (GSH), 5mM N-acetylcysteine (NAC), 5mM NAC+nucleosides mix (NAC+Nuc), 2uM Ferrostatin-1 (Fer-1), 50uM Trolox or 100nM Liproxstatin-1 (Lip-1) (mean +/-SD, one experiment in biological triplicate, Two-way ANOVA with Dunnet's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005).

Figure S7

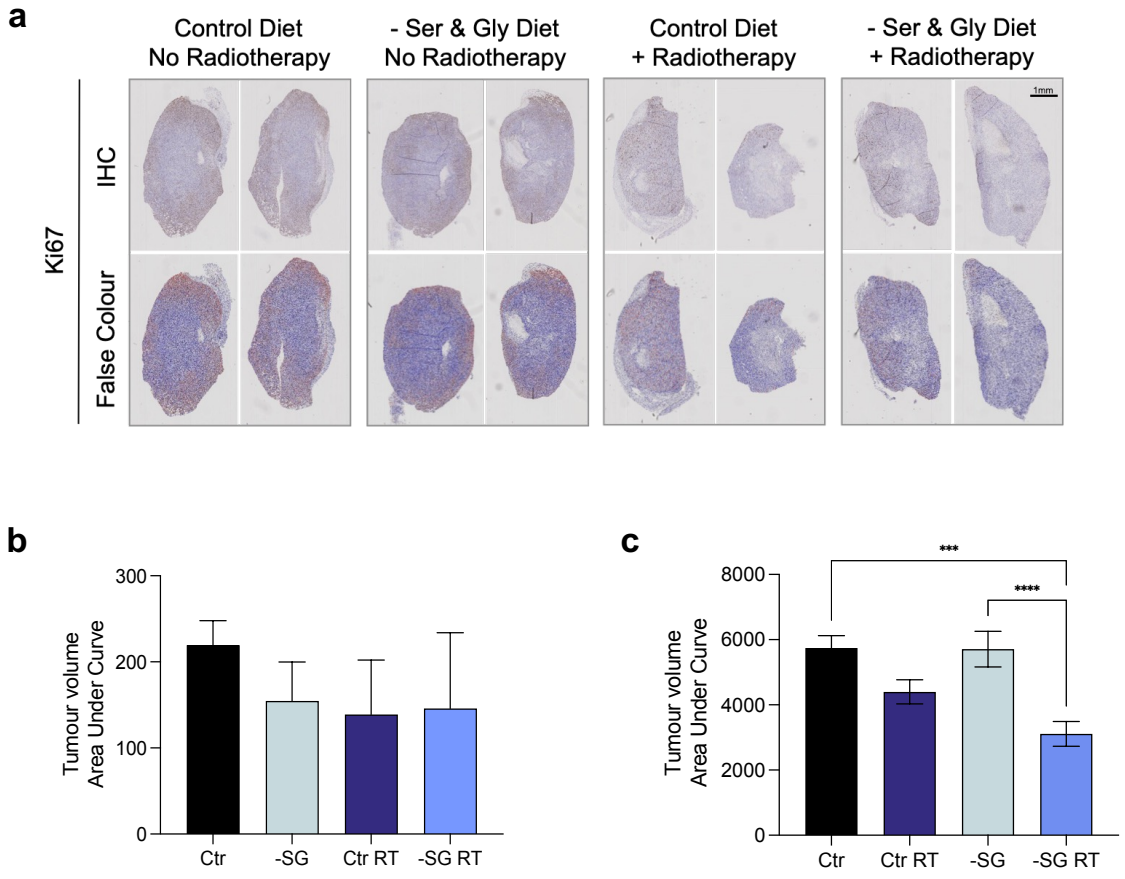


Figure S7. Tumour growth area under curve (linked to figure 5)

a) Representative formalin fixed paraffin embedded tissue sections from KPC cell syngeneic subcutaneous isografts were stained for Ki67. Once measurable tumours had formed, mice were transferred to a diet regime consisting of either a control diet containing serine and glycine (Control diet) or a matched diet lacking serine and glycine (-SG diet); after three days on diet, tumours were treated with 20 Gy targeted X-ray radiation. None of the tumours reached size endpoint due to skin ulceration occurring in all experimental groups, so all tumours were harvested for IHC analysis within 7 days of radiation.

b) Quantification of KPC cell syngeneic subcutaneous isografts shown in Fig. 5a as Area under the curve (AUC) for tumour volume (mean +/-SD, Control diet No RT n = 2 mice, Control diet +RT *n* =4 mice, -SG diet No RT *n* = 6 mice, -SG diet +RT *n* = 6 mice).

c) Quantification of Fig. 5c. 4T1 cell orthotopic isografts as Area under the curve (AUC) for tumour volume (mean +/-SD, Control diet No RT n = 13 mice, Control diet +RT *n* =12 mice, -SG diet No RT *n* = 15 mice, -SG diet +RT *n* = 12 mice, Two-way ANOVA with Tukey's multiple comparisons test, *p<0.05, **p<0.005, ***p<0.0005, ****p<0.00005).

	Ctr vs 5Gy	Ctr vs 10Gy	Ctr vs -SG	Ctr vs 5Gy -SG	Ctr vs 10Gy -SG	5Gy vs 10Gy	5Gy vs -SG	5Gy vs 5Gy -SG	5Gy vs 10Gy -SG	10Gy vs -SG	10Gy vs 5Gy -SG	10Gy vs 10Gy -SG	5Gy -SG vs 10Gy -SG	-SG vs 5Gy -SG	-SG vc 10Gy -SG
D-Glucose	0.4603	0.9802	0.0264	0.1608	0.0223	0.8346	0.4654	0.9685	0.4139	0.0833	0.4191	0.0707	0.8299	0.8729	>0.9999
Fructose 1,6-bisphosphate	0.2032	0.6333	0.0017	0.7586	0.2835	0.9341	<0.0001	0.0228	0.0050	0.0002	0.1054	0.0231	0.9360	0.0152	0.0707
Glyceraldhyde 3-phosphate	0.0074	0.3066	0.0082	<0.0001	0.0001	0.2654	<0.0001	<0.0001	<0.0001	0.0003	<0.0001	<0.0001	0.7110	0.0116	0.1256
1,3-bisphosphoglycerate	0.9957	0.0027	0.0021	0.0002	0.0004	0.0012	0.0010	<0.0001	0.0002	>0.9999	0.5154	0.7905	0.9955	0.6019	0.8613
3-phosphoglycerate	0.9957	0.0997	0.1862	0.0099	0.2070	0.2130	0.3695	0.0225	0.4038	0.9983	0.7378	0.9964	0.4735	0.5121	>0.9999
PEP	>0.9999	0.0393	0.0047	0.0013	0.0199	0.0392	0.0047	0.0013	0.0198	0.7941	0.3621	0.9982	0.5760	0.9625	0.9474
Pyruvate	0.9118	0.0499	0.9855	0.0315	0.0032	0.2411	0.9991	0.1600	0.0167	0.1415	0.9997	0.5887	0.7437	0.0912	0.0092
Acetyl-CoA	0.8776	>0.9999	<0.0001	<0.0001	<0.0001	0.9090	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	>0.9999	0.0034	0.0045
Citrate/Isocitrate	0.7060	0.0027	0.4233	0.8940	0.9628	0.0294	0.0481	0.9984	0.9853	0.0001	0.0151	0.0100	0.9998	0.0923	0.1372
cis-Aconitate	0.2264	0.0005	0.5821	0.9915	>0.9999	0.0216	0.0152	0.4761	0.2107	<0.0001	0.0011	0.0004	0.9877	0.2961	0.6104
2-Oxoglutarate	0.5011	0.0345	0.2279	0.9778	0.9363	0.5105	0.0120	0.1978	0.1442	0.0007	0.0105	0.0075	>0.9999	0.5549	0.6733
Succinate	0.9955	>0.9999	0.6000	0.9997	0.6695	0.9787	0.8596	>0.9999	0.9061	0.4846	0.9956	0.5522	0.8215	0.7607	>0.9999
Fumarate	0.6708	0.0005	0.5466	0.9895	0.9803	0.0047	0.0634	0.9378	0.9591	<0.0001	0.0011	0.0013	>0.9999	0.2612	0.2293
Malate	0.6828	0.0009	0.4274	0.9896	0.9582	0.0093	0.0455	0.9427	0.9833	<0.0001	0.0022	0.0031	0.9999	0.1901	0.1339
Oxaloacetate	0.9982	0.5834	0.1714	0.3098	0.8540	0.3671	0.0900	0.1720	0.6418	0.9267	0.9934	0.9948	0.8938	0.9981	0.6973
dATP	0.0724	<0.0001	0.1864	0.0269	0.0306	<0.0001	0.0011	0.9897	0.9945	<0.0001	0.0001	<0.0001	>0.9999	0.0005	0.0005
dGTP	0.0028	0.0023	0.1810	0.1069	0.8815	>0.9999	<0.0001	0.3048	0.0005	<0.0001	0.2561	0.0004	0.0177	0.0016	0.6796
dCTP	0.9569	0.0002	0.9997	0.0006	0.0009	0.0007	0.9931	0.0021	0.0033	0.0003	0.9836	0.9258	0.9996	0.0009	0.0014
dTTP	0.2222	<0.0001	0.8165	<0.0001	<0.0001	<0.0001	0.8300	0.0002	0.0001	<0.0001	0.3835	0.5215	0.9998	<0.0001	<0.0001
Serine	0.8875	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	>0.9999	>0.9999	>0.9999
Glycine	<0.0001	0.0078	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.9798	0.9915	0.8021

Table S1. P-values from two-way ANOVA with Tukey's multiple comparison tests ($p < 0.05$ are shaded grey) for the LCMS data shown in Figure 3.