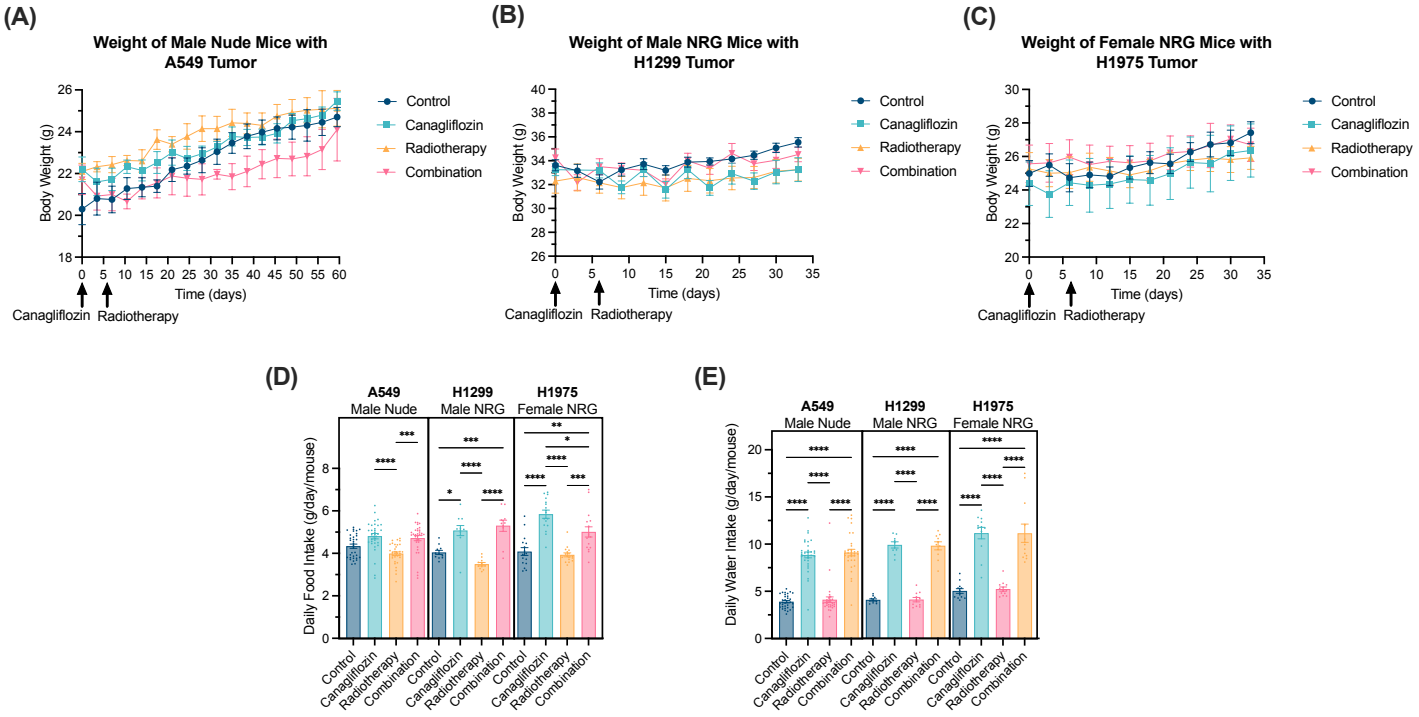
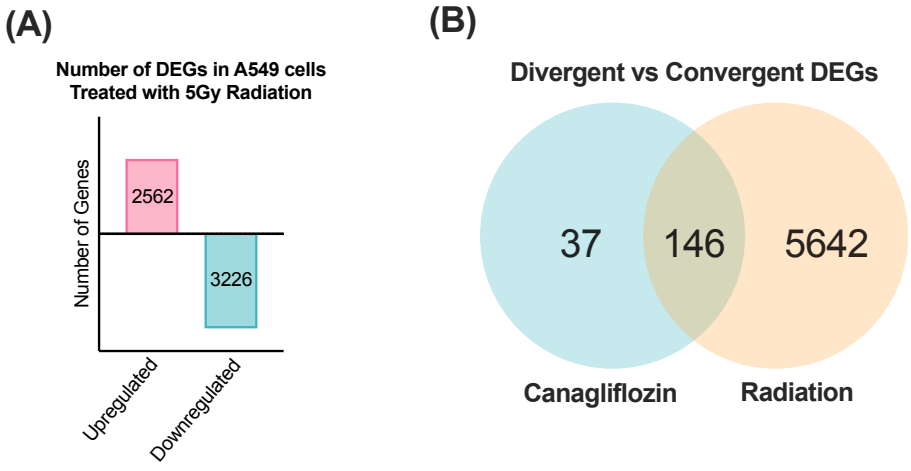
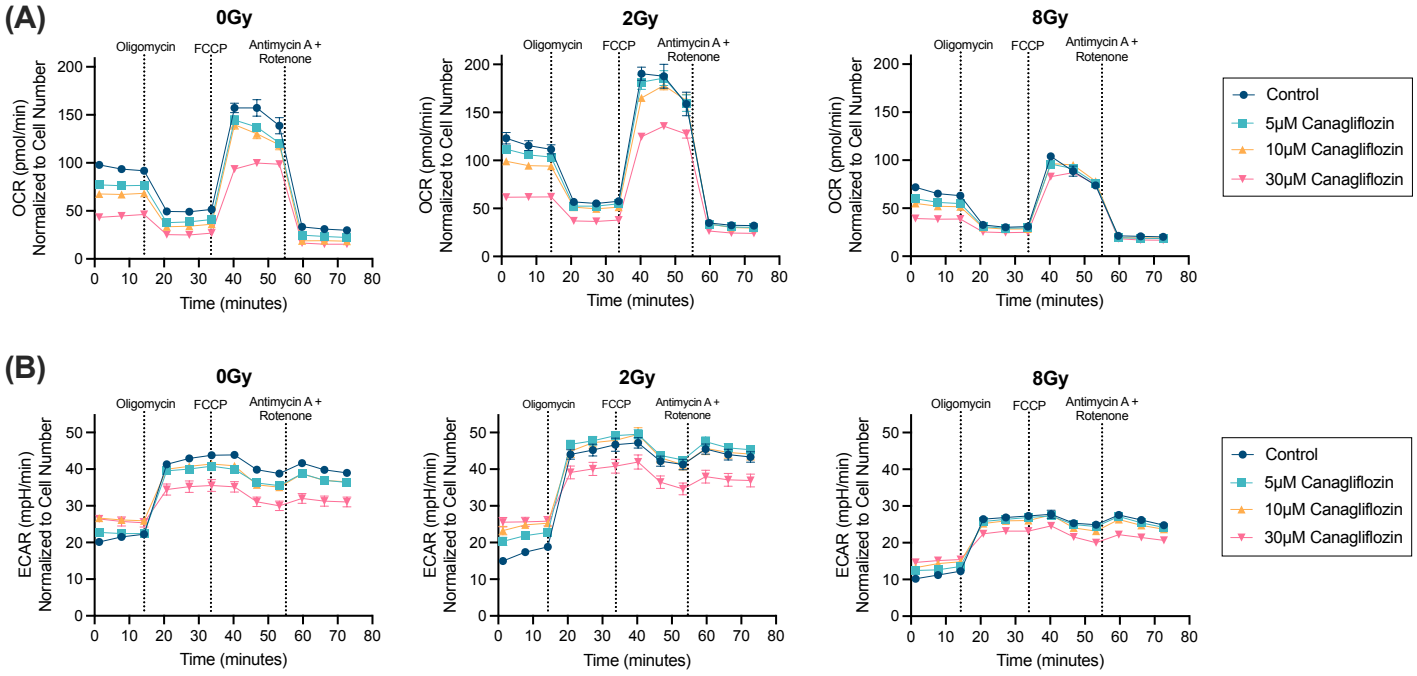


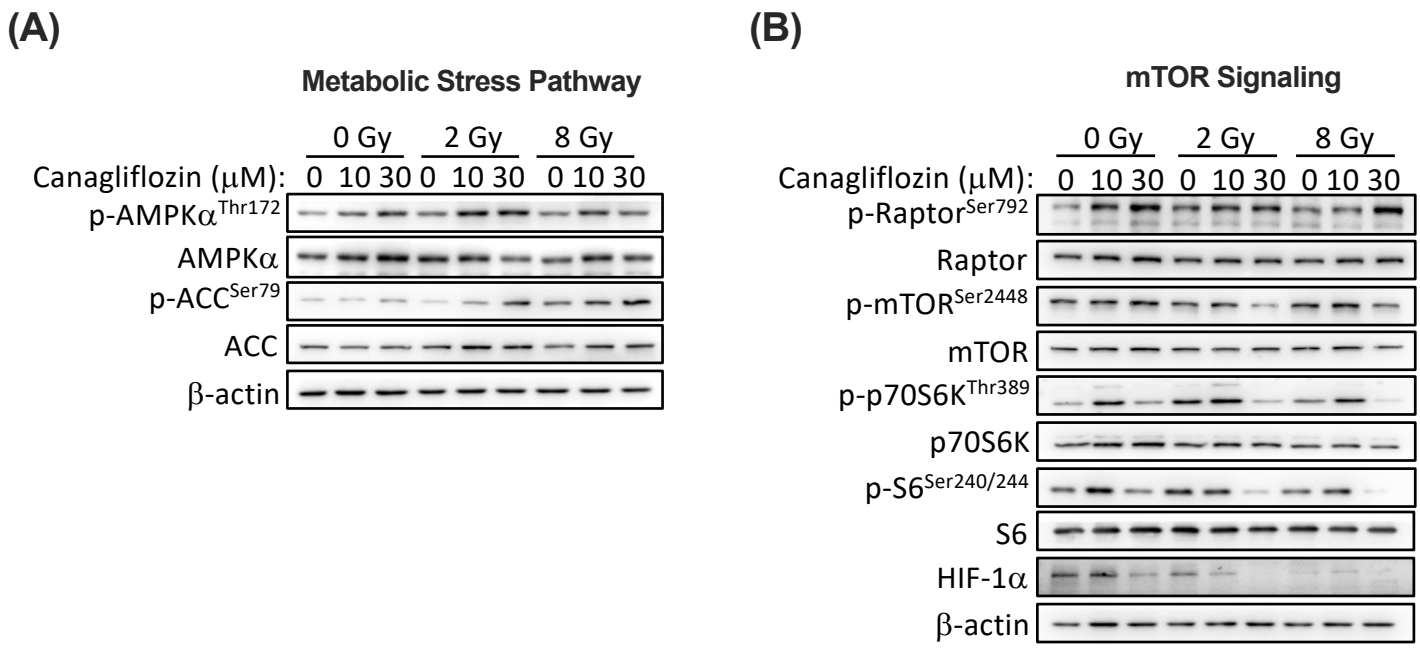
Supplementary Figure 1. Body weight and food and water intake of mice bearing non-small cell lung cancer (NSCLC) tumors. Total body weight of **(A)** male BALB/c nude mice with A549 tumors (*n*=4–5/group), **(B)** male NRG mice with H1299 tumors (*n*=5/group) and **(C)** female NRG mice with H1975 tumors (*n*=5/group) treated with vehicle control, canagliflozin, RT (5Gy) or a combination treatment. **(D)** Diet and **(E)** water were weighed 2–3 times per week to estimate the daily consumption by the animals. Data are presented as mean ± SEM. **(A–C)** Two-way repeated measures ANOVA or **(D, E)** ordinary one-way ANOVA followed by Tukey's multiple comparisons test was used. **p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001.



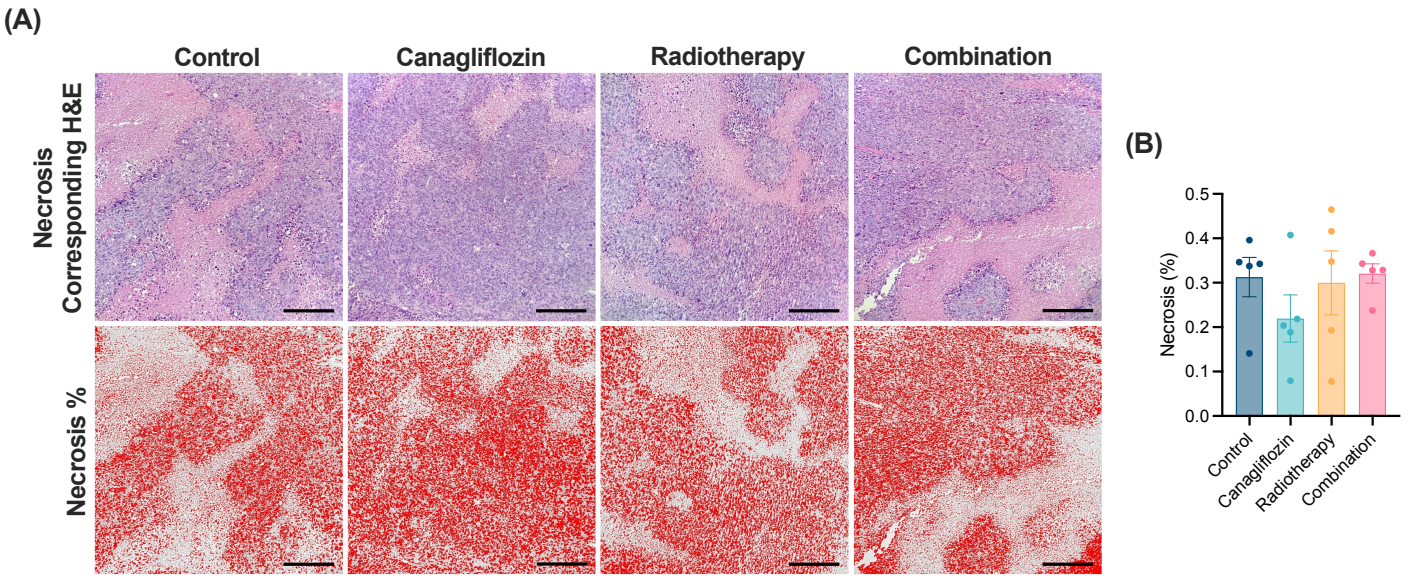
Supplementary Figure 2. Bioinformatics analysis of differentially expressed genes (DEGs) in A549 non-small cell lung cancer (NSCLC) cells treated with canagliflozin or radiotherapy (RT). RNA-sequencing (seq) was used to provide insight into the transcriptome of A549 cells treated with 10μM canagliflozin or 5Gy for 24h *(n*=3). **(A)** The number of significantly upregulated and downregulated DEGs in A549 cells following RT (5Gy) with a False Discovery Rate (FDR) *q*-value <0.05. **(B)** A Venn diagram illustrating the number of divergent and convergent DEGs by canagliflozin (10μM) vs. RT (5Gy) (FDR *q*-value <0.05).



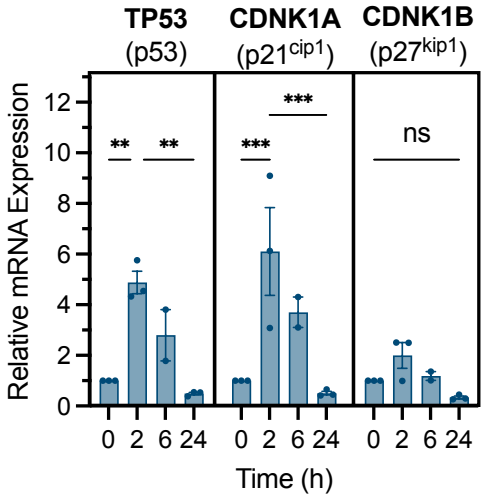
Supplementary Figure 3. Effects of canagliflozin and radiotherapy (RT) on mitochondrial respiration in A549 non-small cell lung cancer (NSCLC) cells. A549 cells were pre-treated for 5h with the indicated canagliflozin (5–30μM) doses prior to RT (single fraction of 2 or 8Gy) and **(A)** oxygen consumption rate (OCR) and **(B)** extracellular acidification rate (ECAR) were measured with the Seahorse Mito Stress Test 48h later.



Supplementary Figure 4. Regulation of metabolic pathways by canagliflozin and radiotherapy (RT) in H1299 non-small cell lung cancer (NSCLC) cells. Representative protein immunoblots of H1299 cells pre-treated with canagliflozin (10 or 30μM) for 5h followed by RT (2 or 8Gy) and probed for markers in the **(A)** metabolic stress and **(B)** mTOR signaling pathways 48h post-RT.

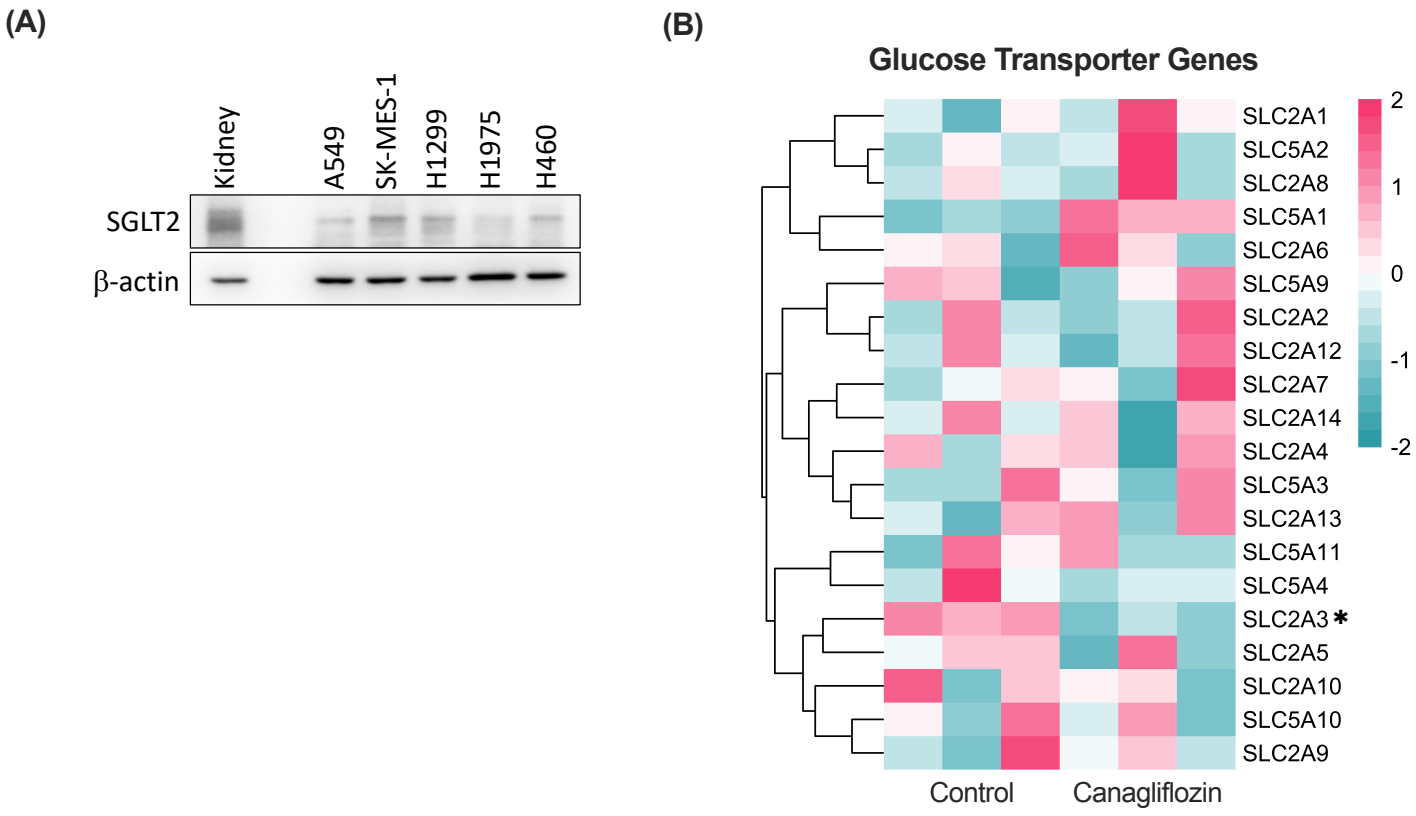


Supplementary Figure 5. Assessment of necrosis in H1299 non-small cell lung cancer (NSCLC) tumors. (A) Representative H&E images (top row) of H1299 tumors (*n*=5/group) that were treated with vehicle control, canagliflozin, radiotherapy or combination therapy and the necrosis percentage images (second row) were generated using ImageJ (scale bar=400μm). Red indicates the viable areas, while the pale color represents necrotic areas. **(B)** Quantification of the whole tumor section was determined following the ImageJ User Guide for tissue quantification. Necrotic Area = Total Area – Viable Tissue Area; Necrotic Tissue Percentage = Necrotic Area/Total Area. Data are presented as mean ± SEM. Ordinary one-way ANOVA **(B)** was used to detect statistical significance.

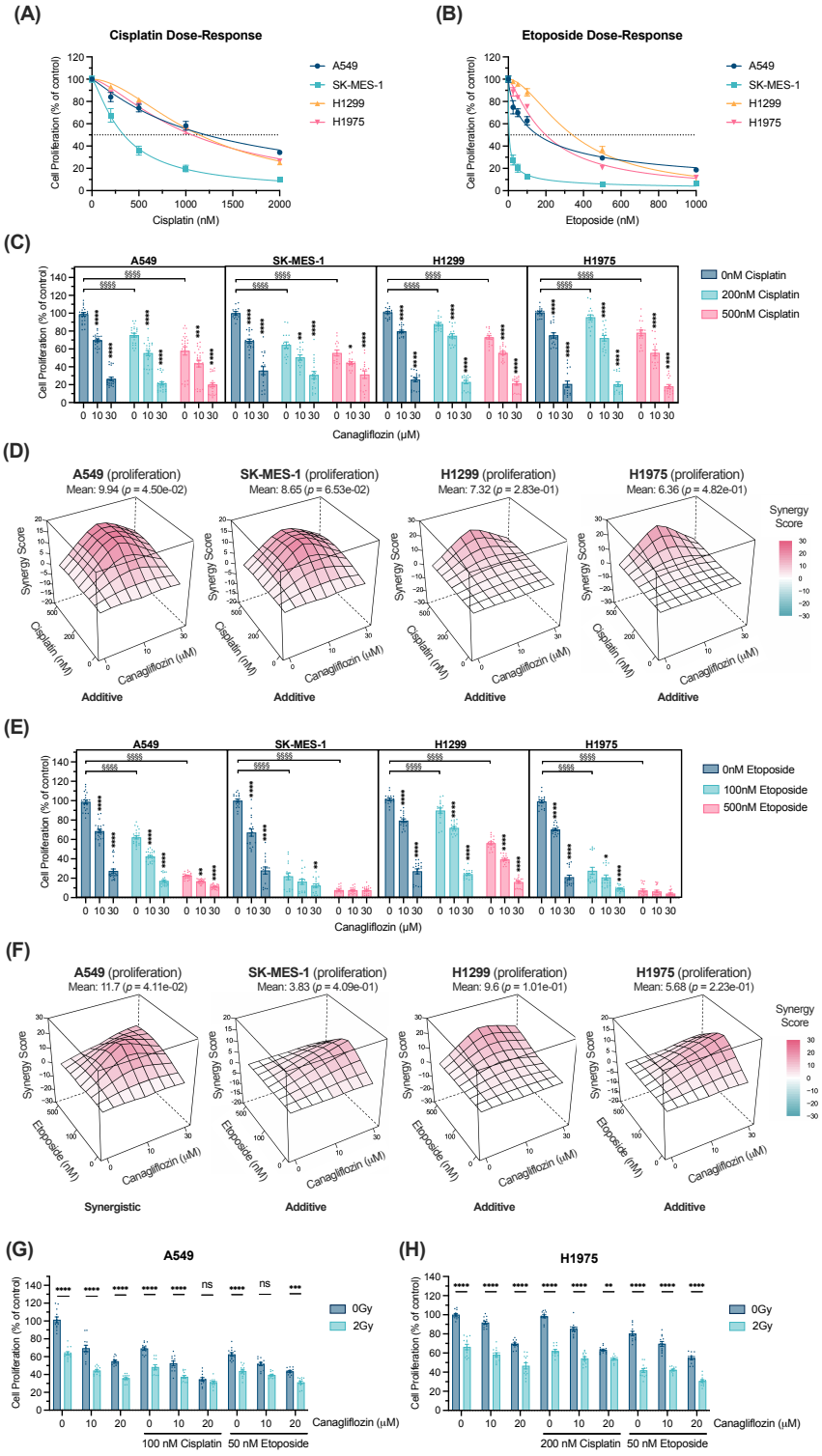


Supplementary Figure 6. Analysis of cell cycle regulation genes in A549 non-small cell lung cancer (NSCLC) cells treated with canagliflozin. Relative mRNA expression, determined with RT-qPCR, of cell cycle regulation genes in A549 cells treated with canagliflozin (30μM) for 0, 2, 6 and 24h. Data were obtained from three independent experiments and presented as mean ± SEM. Ordinary one-way ANOVA followed by Tukey's multiple comparisons test was used to detect statistical significance. ns: non-significant, ***p*<0.01, ****p*<0.001.

Supplementary Figure 7. Differentially expressed genes (DEGs) in A549 non-small cell lung cancer (NSCLC) cells treated with canagliflozin or radiotherapy (RT). Heatmaps of the **(A)** convergent DEGs regulated by canagliflozin and RT compared to the vehicle control, and **(B)** divergent DEGs regulated by only canagliflozin compared to the vehicle control *(n*=3; FDR *q*-value <0.05). The teal to pink scales represent downregulated to upregulated from -1.5 to 1.5, respectively. Data were obtained from the RNA-seq experiment.



Supplementary Figure 8. Expression of glucose transporters in non-small cell lung cancer (NSCLC) cells. (A) Representative protein immunoblot of the sodium-glucose cotransporter 2 (SGLT2) in mouse kidney (positive control) and human A549, SK-MES-1, H1299, H1975 and H460 NSCLC cells. **(B)** A heatmap of glucose transporter genes from the RNA-seq data in A549 cells (control vs. canagliflozin). A 24h treatment with 10μM canagliflozin significantly regulated one glucose transporter (*SLC2A3*) as indicated by an asterisk (*) (false discovery rate (FDR) *q*-value <0.05).



Supplementary Figure 9. Proliferation assay of human non-small cell lung cancer (NSCLC) cells treated with canagliflozin, chemotherapy and/or radiotherapy (RT). Proliferation assay of human NSCLC cells (A549, SK-MES-1, H1299 and H1975) treated with **(A)** cisplatin (200–2000nM), **(B)** etoposide (50–1000nM) or **(C, E)** a combination treatment concurrently with canagliflozin (10 or 30μM) for 5 days. **(D, F)** Proliferation assay results were subjected to synergy analysis using Highest Single Agent (HSA) modeling where a mean HSA synergy score of < -10 suggests antagonism, -10–10 suggests additivity and > 10 suggests synergism. Proliferation assay with **(G)** A549 and **(H)** H1975 cells treated with the indicated doses of canagliflozin (10 or 20μM), cisplatin (100 or 200nM), etoposide (50nM) and/or RT (single fraction of 2Gy). Drug treatments were administered concurrently 5h prior to RT. Results were derived from three independent experiments and presented as mean ± SEM. Data were calculated using two-way ANOVA **(C, E, G, H)** followed by Tukey's multiple comparisons test. ns: non-significant, *p<0.05, **p<0.01, ***/§§§p<0.001, ****/§§§§p<0.0001.

Supplementary Table 1. List of antibodies and PCR probes used. Antibodies were purchased from New England Biolabs (Ipswich, MA), Proteintech (Sankt Leon-Rot, Germany) or Vector Laboratories Inc (Burlingame, CA) and PCR probes were purchased from Life Technologies Inc (Carlsbad, CA).

Antibody or Probe	Catalog Number	Dilution (WB)	Dilution (IHC)	Manufacturer
AMPKα	2532	1:1000		NEB
p-AMPKα (Thr172)	2531	1:1000		NEB
ACC	3662	1:1000		NEB
p-ACC (Ser79)	11818	1:1000	1:200	NEB
Raptor	2280	1:1000		NEB
p-Raptor (Ser792)	2083	1:1000		NEB
mTOR	2983	1:1000		NEB
p-mTOR (Ser2448)	2971	1:1000		NEB
p70S6K	9202	1:1000		NEB
p-p70S6K (Thr389)	9205	1:1000		NEB
S6	2217	1:1000		NEB
p-S6 (Ser240/244)	2215	1:1000		NEB
4EBP1	9644	1:1000		NEB
p-4EBP1 (Ser65)	9451	1:1000		NEB
HIF-1α	36169	1:1000		NEB
H3	4499	1:1000		NEB
p-H3 (Ser10)	53348	1:1000		NEB
p21cip1	2947	1:1000		NEB
p27kip1	3688	1:1000		NEB
HDAC2	57156	1:1000		NEB
β-actin	5125	1:1000		NEB
SGLT2	24654-1-AP	1:1000		Proteintech
CC3 (Asp175)	9661		1:400	NEB
Anti-Rabbit IgG, HRP-linked secondary antibody	7074	1:10000		NEB
Goat anti-Rabbit IgG (H+L), Biotinylated	BA-1000		1:500	Vector Laboratories Inc
TP53 (p53)	4331182 (Hs01034249_m1)			Life Technologies Inc
CDKN1A (p21cip1)	4331182 (Hs00355782_m1)			Life Technologies Inc
CDKN1B (p27kip1)	4331182 (Hs00153277_m1)			Life Technologies Inc
GAPDH	4331182 (Hs02786624_g1)			Life Technologies Inc