Spermidine/spermine N1-acetyltransferase 1 (*SAT1*) – A Gene Target for Selective Sensitization of GB Cells using an Ionizable Lipid Nanoparticle to Deliver siRNA.

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SUPPORTING INFORMATION

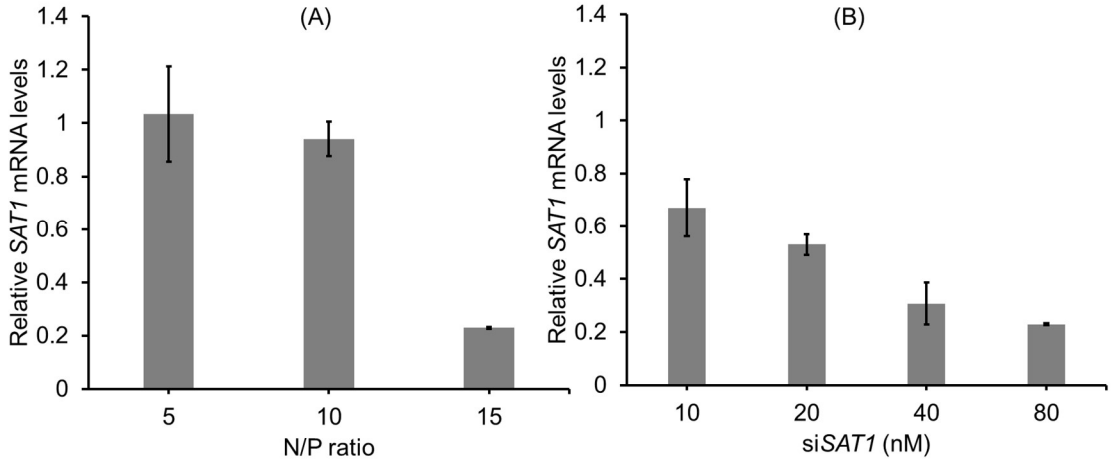


Figure S1. *SAT1* knockdown efficiency of LNP-si*SAT1* (si*SAT1* 80 nM with 1 μg/mL APOE) with different N/P (amine groups in DODAP/phosphate groups in si*SAT1*) ratio (A) and SAT1 knockdown efficiency of LNP-si*SAT1* (N/P = 15) at different concentration (B) in U251 cells compared to control cells that received LNP-siSCR. For all the preparations, the U251 cells cultured in 12 well plate (20,000/cm^2) was transfected in the presence of 1 μg/mL APOE. The *SAT1* mRNA levels were determined 48-hours after transfection (n = 3).

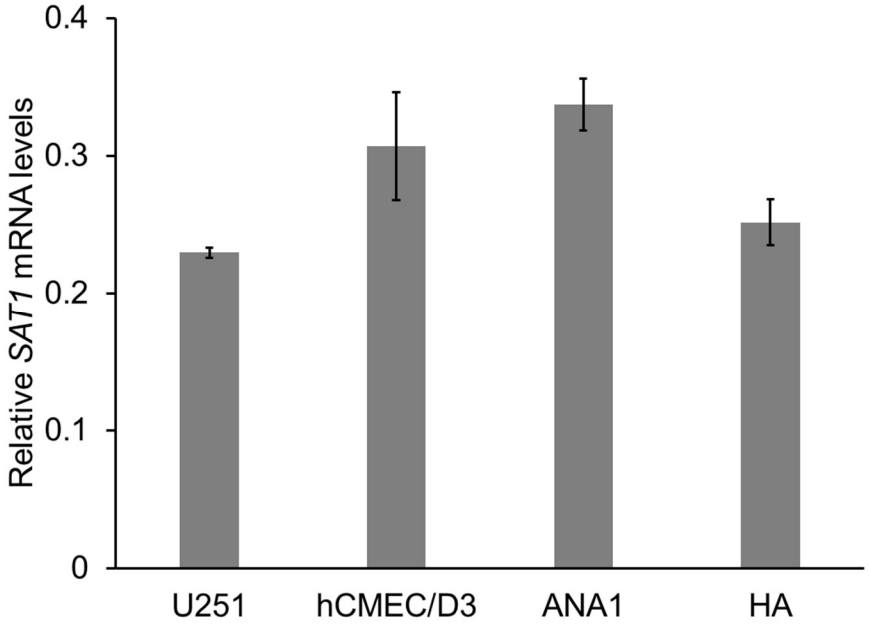
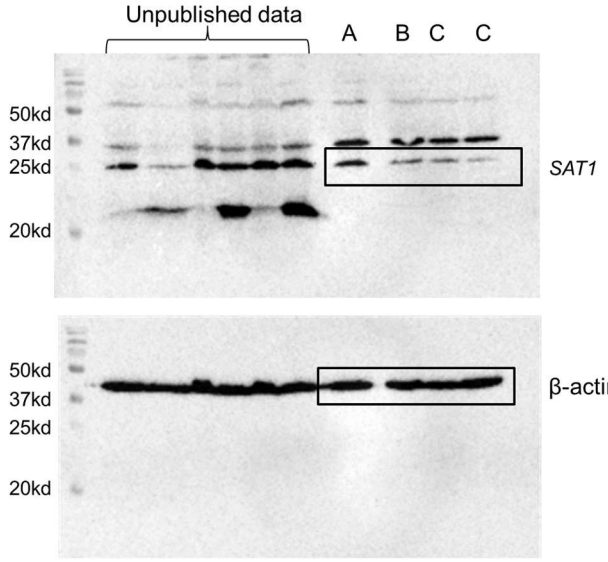


Figure S2. Relative *SAT1* mRNA expression level in LNP-si*SAT1* transfected GBM (U251), brain endothelial (hCMEC/D3), microglia (ANA-1) and astrocyte (HA) cells compared to control cells which received LNP-siSCR. Expression was determined 48-hours after treatment and represent the mean ± SEM ($n = 3$). .



Bands	Densitometry readings			
	A	B	C	C
SAT1	168.26	93.56	90.04	53.50
β-actin	226.30	230.27	218.68	227.69

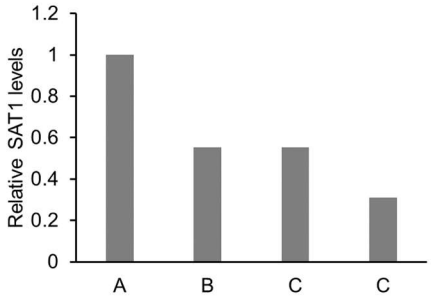


Figure S3. Left panel: The whole blot for the bands (highlighted) shown in Figure 2C of the manuscript. The top and bottom images show the immunoblotting for *SAT1* and β-actin from the same membrane; A – Control, B and C – LNP-si*SAT1* 40 and 80 nM, respectively. Right panel: The densitometry readings and relative *SAT1* levels calculated from the highlighted bands on the blots from the left panel.

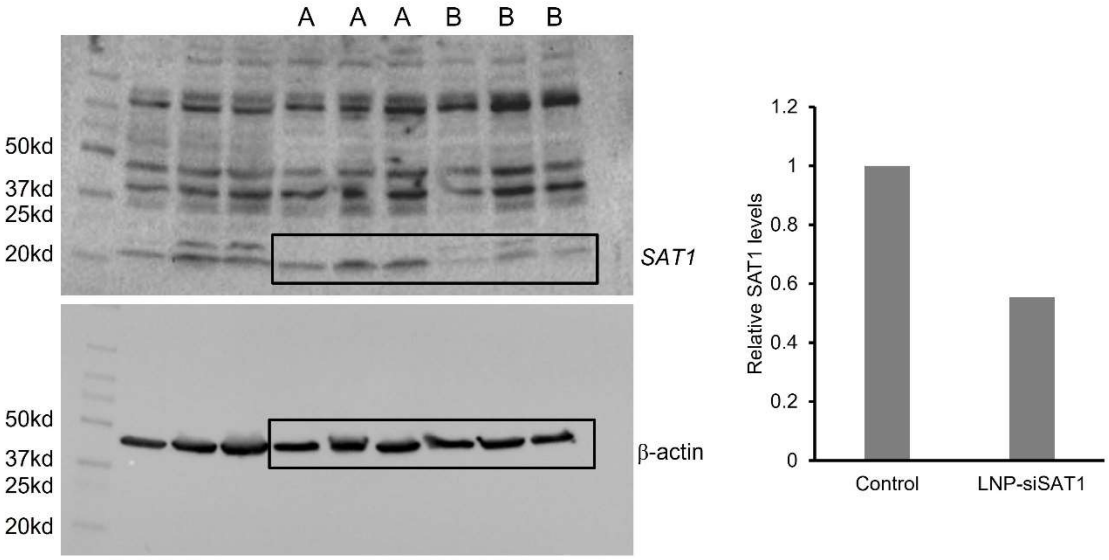


Figure S4. Left panel: The whole blot for the bands (highlighted) shown in Figure 2F of the manuscript. The top and bottom images show the immunoblotting for *SAT1* and β-actin from the same membrane; A – Control and B – LNP-si*SAT1* (80 nM). Right panel: The relative *SAT1* levels calculated from the highlighted bands on the blots from the left panel.

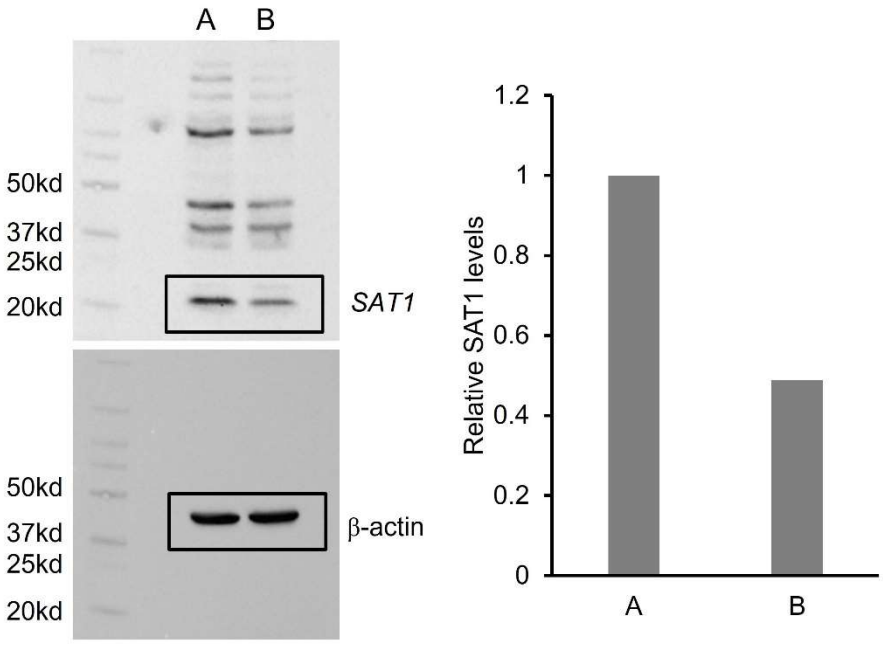


Figure S5. Left panel: The whole blot for the bands (highlighted) shown in Figure 2G of the manuscript. The top and bottom images show the immunoblotting for *SAT1* and β-actin from the same membrane; A – Control and B – LNP-si*SAT1* (80 nM). Right panel: The relative *SAT1* levels calculated from the highlighted bands on the blots from the left panel.

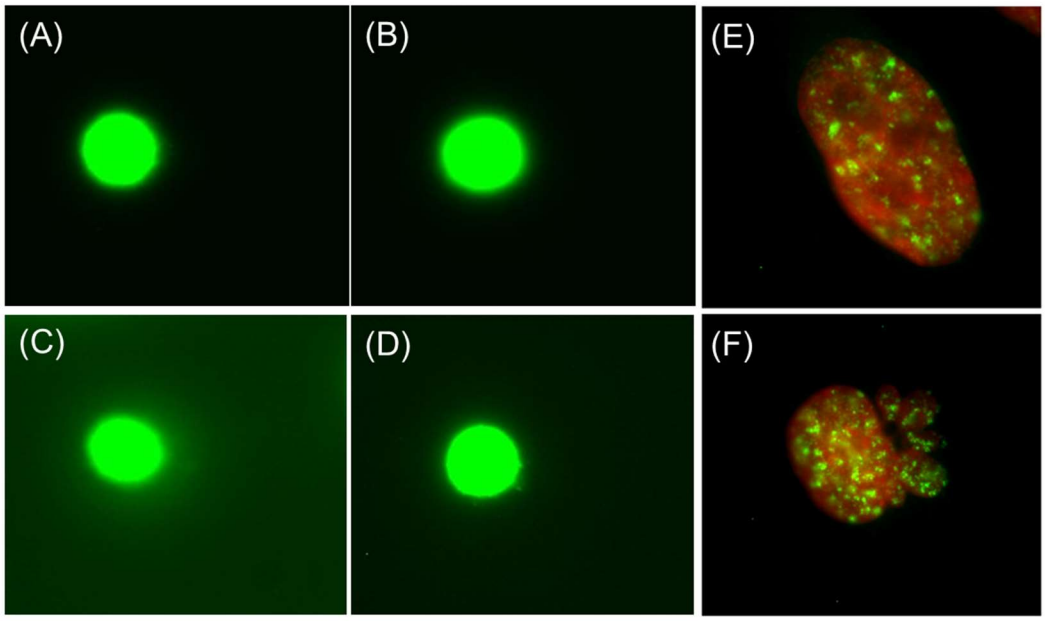


Figure S6. Single cell gel electrophoresis (comet assay) of U251 cells treated with LNP-siSCR (A) and LNP-si*SAT1*(B) that were not exposed to radiation. Single-cell gel electrophoresis (comet assay) on U251 cells treated with LNP-siSCR (C) and LNP-si*SAT1* (D) 24-hours after 10 Gy radiation. The reduction in the observable tail moment as compared to 6-hours could be attributed to the completion of the DNA damage repair. γ-H2AX (green) immunofluorescence and DAPI overlay of LNP-siSCR (E) and LNP-si*SAT1* (F) cells 6-hrs following exposure to 10 Gy radiation. A considerably higher amount of γ-H2AX foci compared to 1 Gy radiation was observed, indicating that the DNA DSB is radiation dose-dependent.