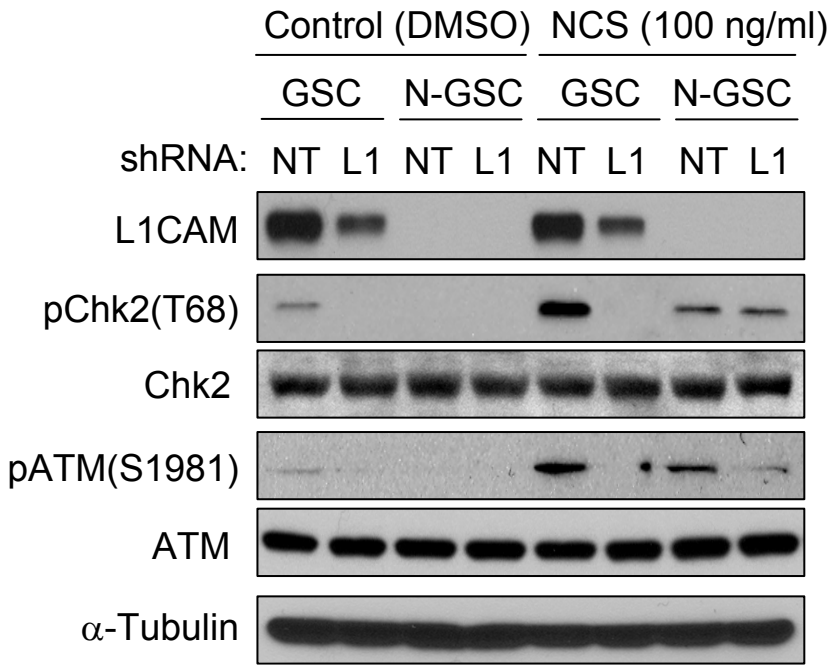
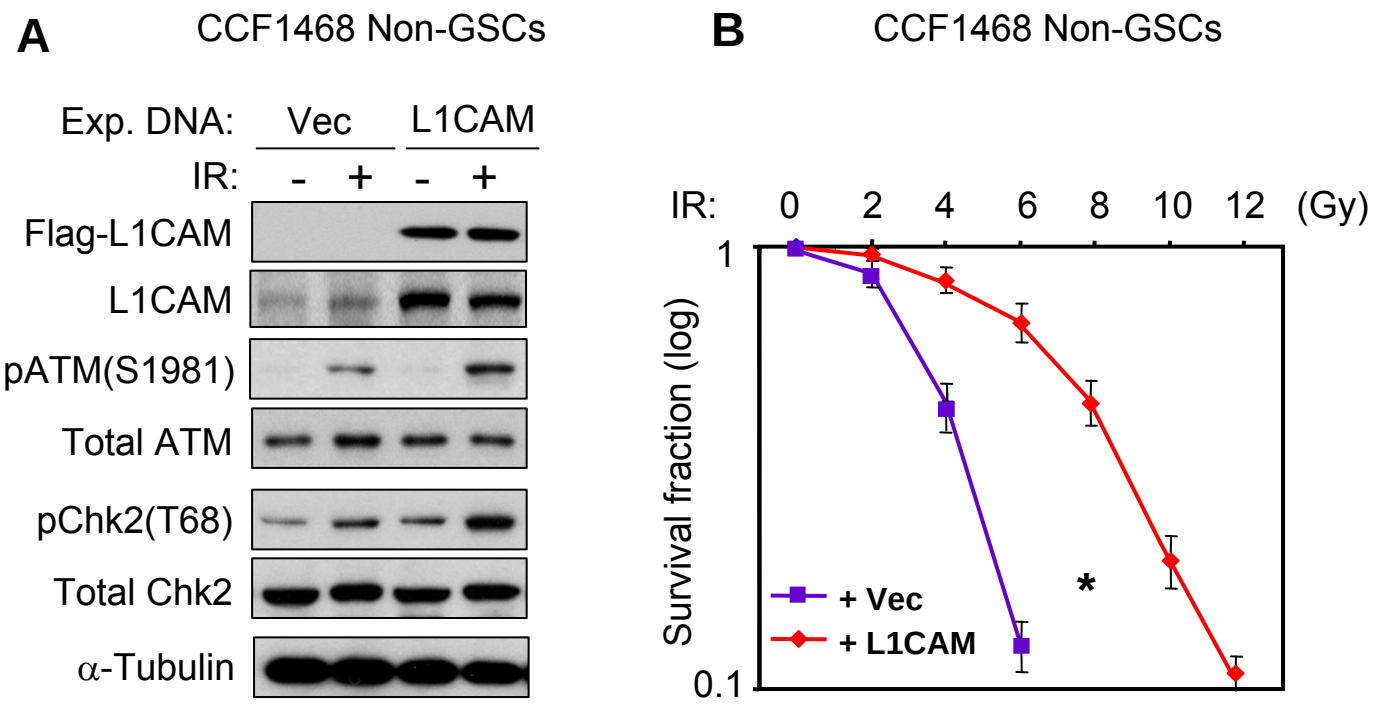

Control (DMSO)
NCS (100 ng/ml)
GSC
N-GSC
GSC
N-GSC
shRNA: NT L1 NT L1 NT L1 NT L1
L1CAM
pChk2(T68)
Chk2
pATM(S1981)
ATM
α-Tubulin


A
CCF1468 Non-GSCs
Exp. DNA:
Vec
L1CAM
IR:
- + - +
Flag-L1CAM
L1CAM
pATM(S1981)
Total ATM
pChk2(T68)
Total Chk2
α-Tubulin
B
CCF1468 Non-GSCs
IR:
0 2 4 6 8 10 12 (Gy)
1
0.1
Survival fraction (log)
+ Vec
+ L1CAM
*

Supplemental Figure

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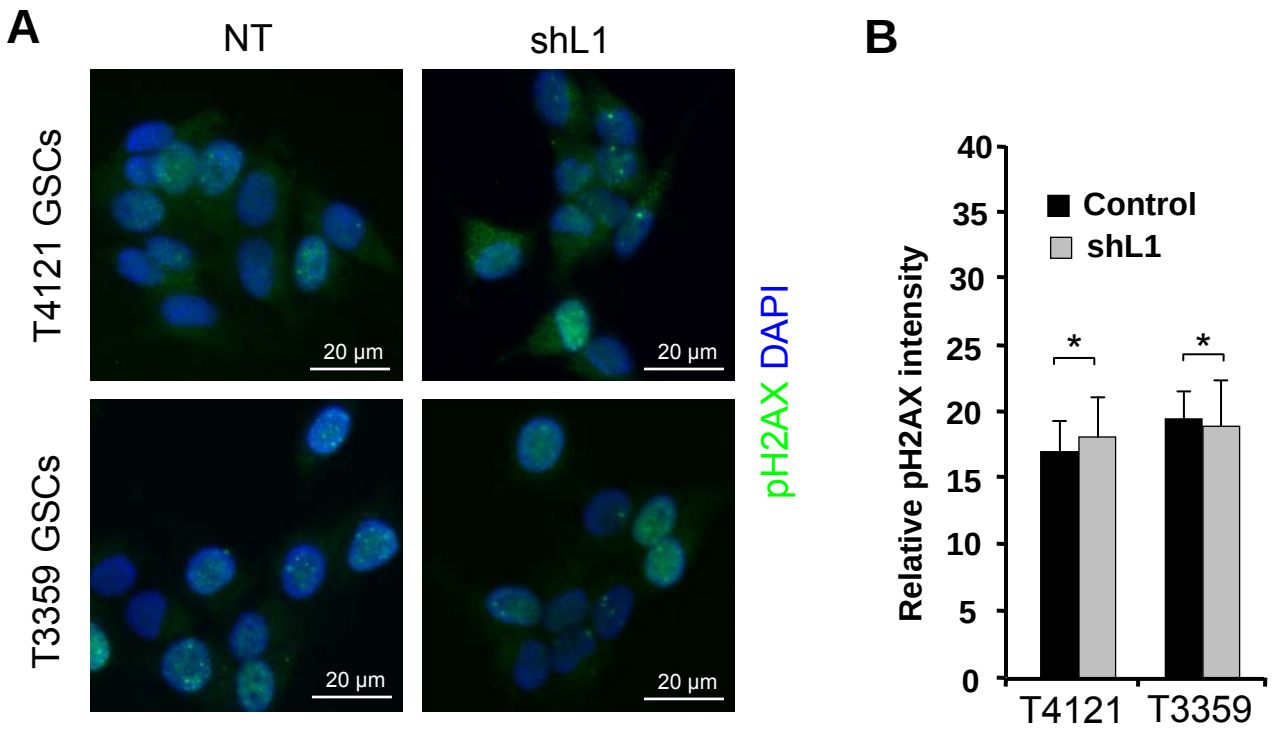
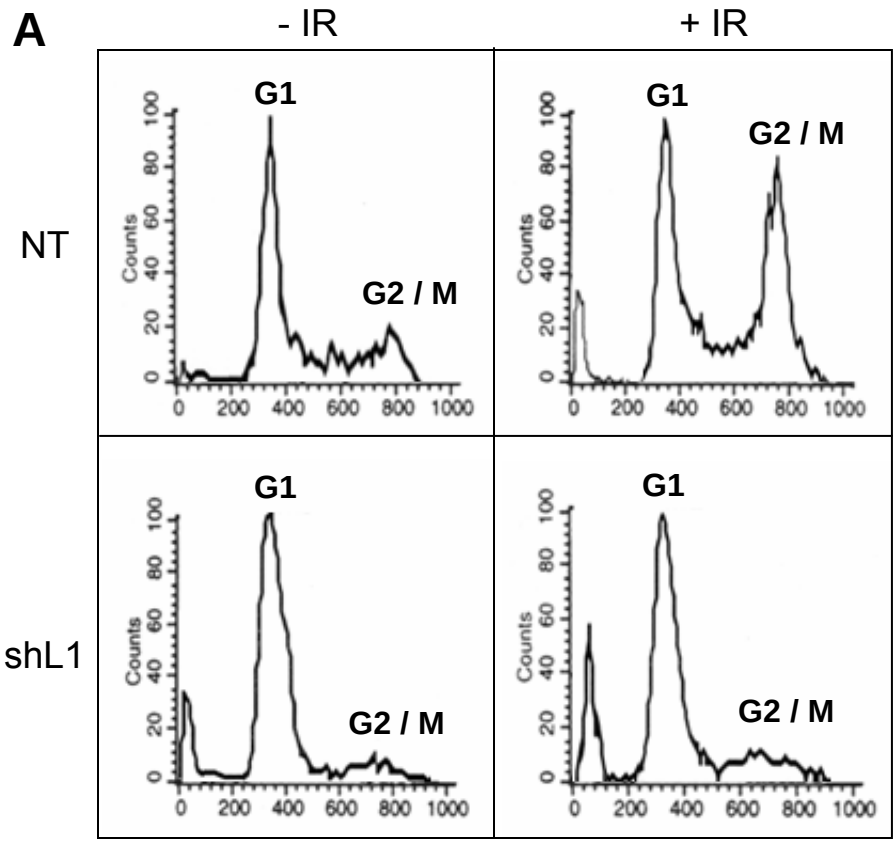
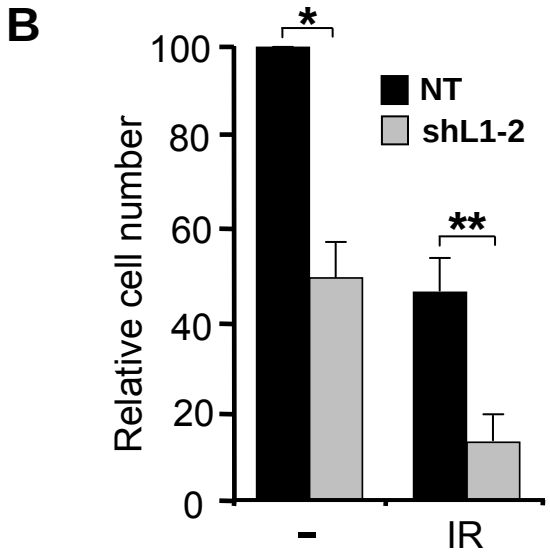
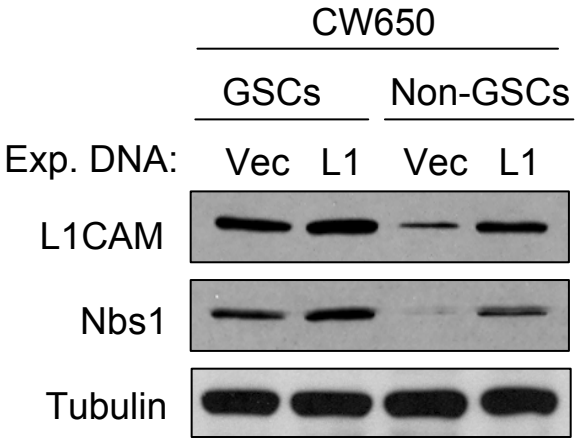
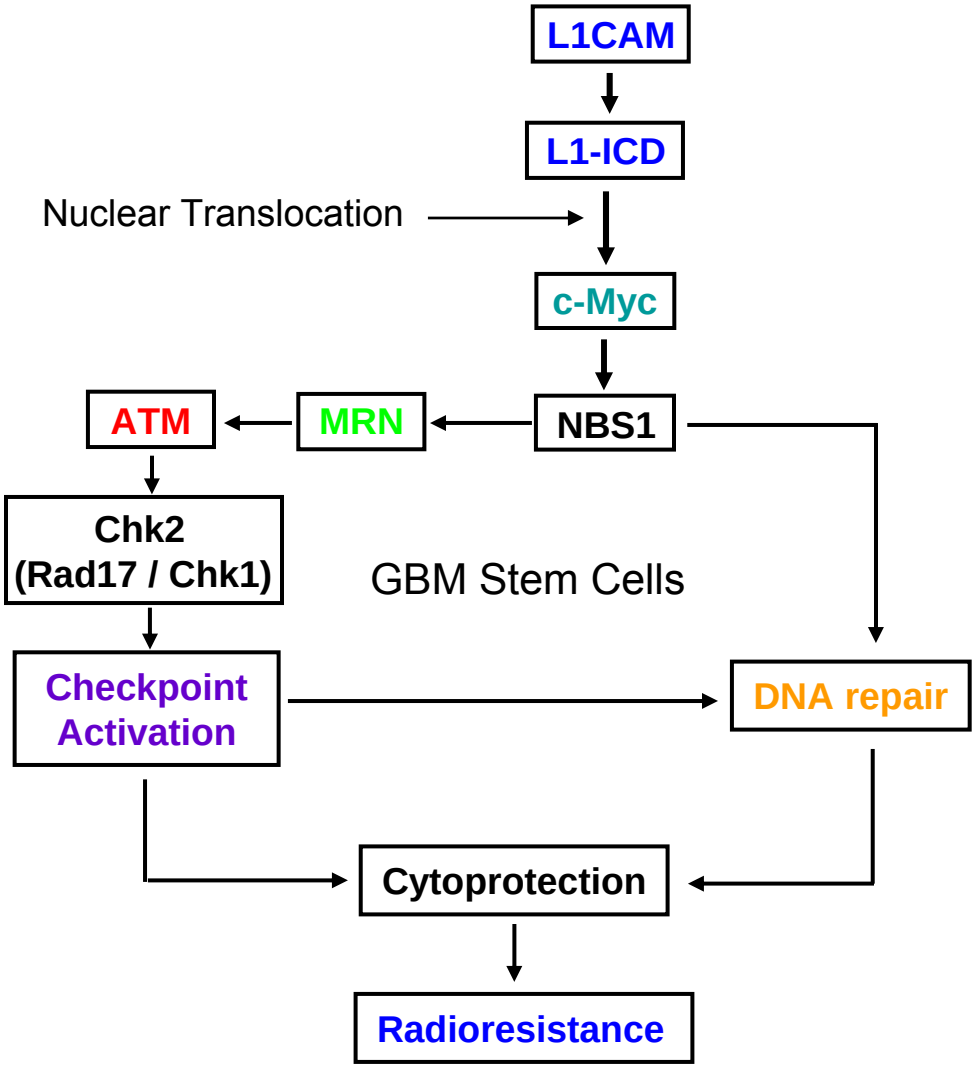



A
- IR
+ IR
NT
shL1
G1
G2 / M
Counts
0
20
40
60
80
100
0
200
400
600
800
1000


B
Relative cell number
100
80
60
40
20
0
*
**
NT
shL1-2
-
IR


CW650
GSCs
Non-GSCs
Exp. DNA:
Vec
L1
Vec
L1
L1CAM
Nbs1
Tubulin


L1CAM
L1-ICD
Nuclear Translocation
c-Myc
ATM
MRN
NBS1
Chk2
(Rad17 / Chk1)
GBM Stem Cells
Checkpoint
Activation
DNA repair
Cytoprotection
Radioresistance

Legends to Supplementary Figures

Supplementary Figure S1. L1CAM knockdown in non-stem glioma cells showed little or no effect on the activation of checkpoint proteins in response to DNA damage. Matched GSCs and non-stem tumor cells (N-GSCs) derived from CW654 GBM tumor were transduced with L1CAM shRNA (L1) or non-targeting (NT) shRNA through lentiviral infection, treated with or without NCS, and then subjected for immunoblot analysis with specific antibodies against pATM(S1981) and pChk2(T68) phosphorylated checkpoint proteins and the total checkpoint proteins. L1CAM knockdown reduced the activating phosphorylation of Chk2 and ATM in GSCs, but showed minor or no effects on the phosphorylation of these checkpoint proteins in non-stem tumor cells that display little L1CAM expression and less checkpoint activation in response to DNA damage.

Supplementary Figure S2. Ectopic expression of L1CAM enhanced checkpoint activation and cell survival in no-stem glioma cells (Non-GSCs) in response to IR. (**A**) Ectopic expression of L1CAM in Non-GSCs increased the activating phosphorylation of ATM and Chk2 checkpoint proteins after DNA damage induced by IR. No-GSCs isolated from CCF1468 GBM tumor were transduced with Flag-L1CAM or vector control for 36 hours through lentiviral infection, and treated with irradiation (3 Gy) followed by a 3 hour recovery, and then harvested for immunoblot analysis with specific antibodies against pATM(S1981) and pChk2(T68) phosphorylated checkpoint proteins and the total checkpoint proteins. (**B**) Dose

response survival curve of non-stem glioma cells (Non-GSCs) without or with L1CAM ectopic expression in response to a range of IR treatment. Ectopic expression of L1CAM in non-stem tumor cells derived from CCF1468 GBM increased cell survival and resistance to radiation. *, $p < 0.002$.

Supplementary Figure S3. In the condition without induction of DNA damage L1CAM knockdown in GSCs showed little effect on endogenous DNA damage as demonstrated by imunofluorescent staining of pH2AX. (**A**) GSCs derived from T3359 and T4121 GBM xenografts were targeted with L1CAM shRNA (shL1-2) or NT shRNA for 48 hours, and then immuno-stainned with the specific antibody against pH2AX (green) and counterstained with DAPI (blue). (**B**) The pH2AX staining intensity from (**A**) was quantified and statistically analyzed. GSCs populations expressing shL1 and NT shRNA did not show a significant difference in pH2AX staining intensity when cells were cultured under normal condition without induction of DNA damage. *, $p > 0.5$.

Supplementary Figure S4. L1CAM knockdown affected cell cycle profiles in GSCs and reduced cell viability under non-irradiated and irradiated conditions. (**A**) Cell cycle profiling showed that L1CAM knockdown in GSCs reduced G2 arrest in response to irradiation and increased cell death under non-irradiated and irradiated conditions. GSCs derived from CW702 GBM xenograft were targeted with L1CAM shRNA (shL1) or NT shRNA for 48 hours, untreated or treated with IR (5 Gy), and

then harvested for FACS at 22 hours after IR treatment. GSCs expressing NT shRNA displayed G2/M arrest after IR, while the GSCs expressing shL1CAM showed reduced G2/M arrest and increased cell death. (**B**) Cell viability analysis showed that L1CAM knockdown in GSCs reduced survival of non-irradiated cells and further decreased survival of irradiated cells. GSCs derived from CW619 GBM were targeted with L1CAM shRNA (shL1-2) or NT shRNA through lentiviral infection for 48 hours, and treated without or with IR (5 Gy). Cell viability was analyzed 72 hours after IR by trypan blue staining and a TC10 automatic cell counter. *, $p < 0.002$; **, $p < 0.001$.

Supplementary Figure S5. Ectopic expression of L1CAM increased NBS1 expression in both GSCs and non-stem tumor cells (Non-GSCs) as demonstrated by immunoblotting analysis. GSCs or non-stem tumor cells derived from CW650 GBM surgical specimen were transduced with Flag-L1CAM (L1) or vector control (Vec). L1CAM and NBS1 protein levels were analyzed at 36 hrs after transfection.

Supplementary Figure S6. A schematic illustration shows that L1CAM up-regulates NBS1 of the MRN complex through c-Myc to enhance DNA damage checkpoint activation and DNA repair that promote cytoprotection and radioresistance of GSCs. As L1CAM is preferentially expressed in GSCs, L1CAM up-regulates expression of c-Myc and NBS1 that activates MRN-ATM-Chk2 signaling to augment checkpoint activation and DNA repair, while elevated NBS1 itself also promotes DNA repair

process in response to the radiation-induced DNA damage. Both effects may contribute to the enhanced cytoprotection and the increased radioresistance of GSCs.