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Supplemental information

SerpinB3 drives cancer stem cell survival in glioblastoma

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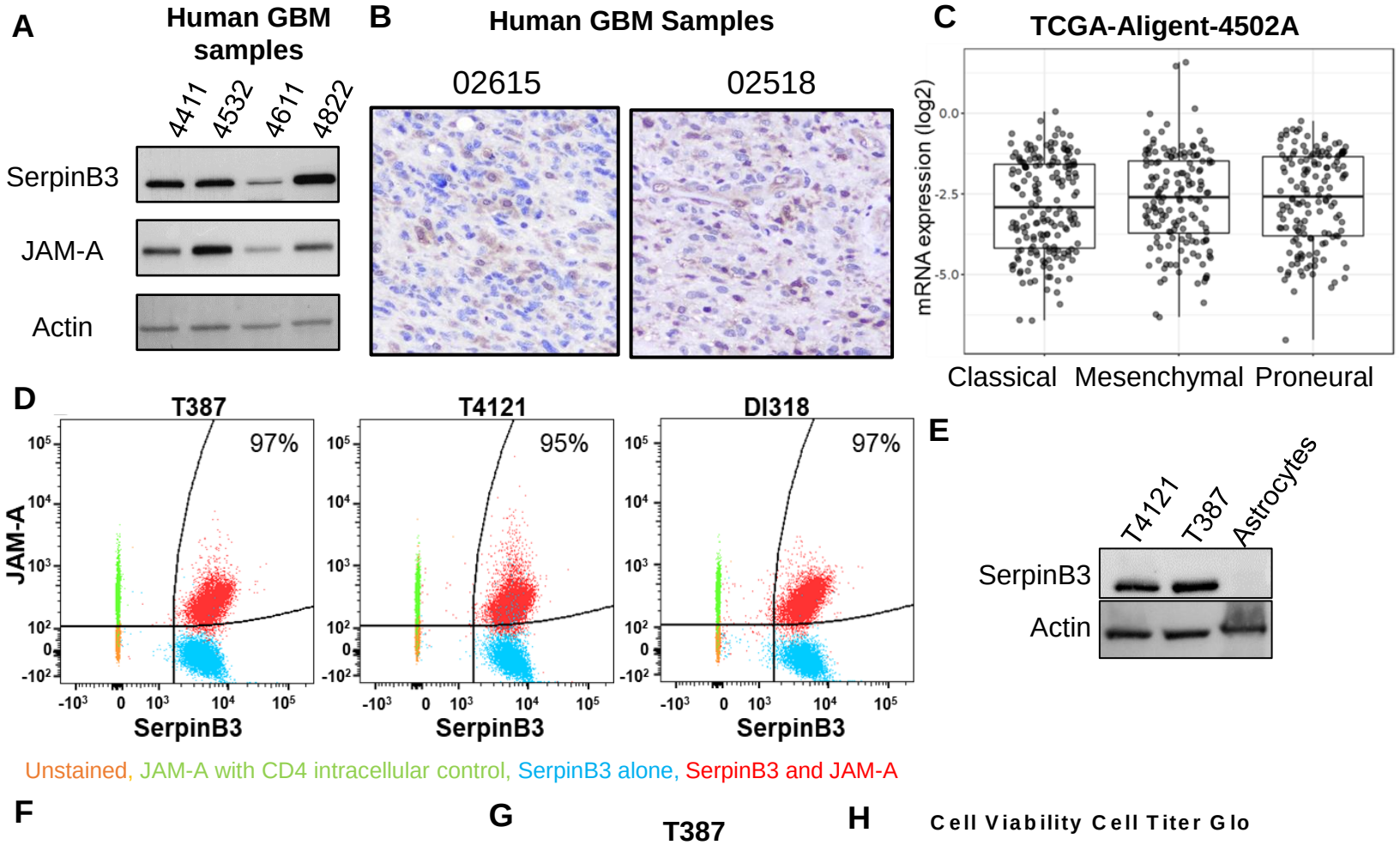
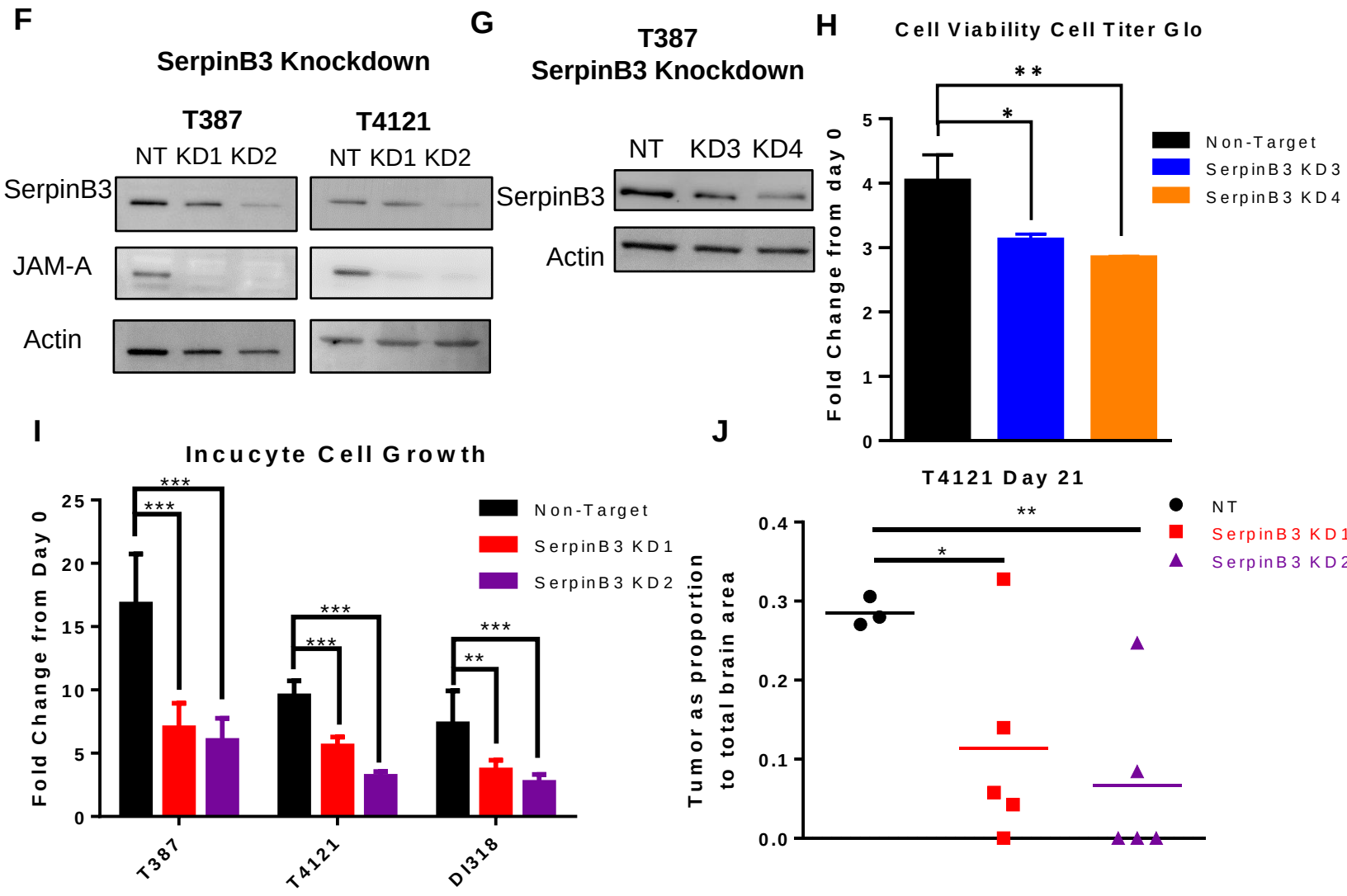
Supplemental Figure 1, SerpinB3 is required for GBM tumor growth, related to Figure 1



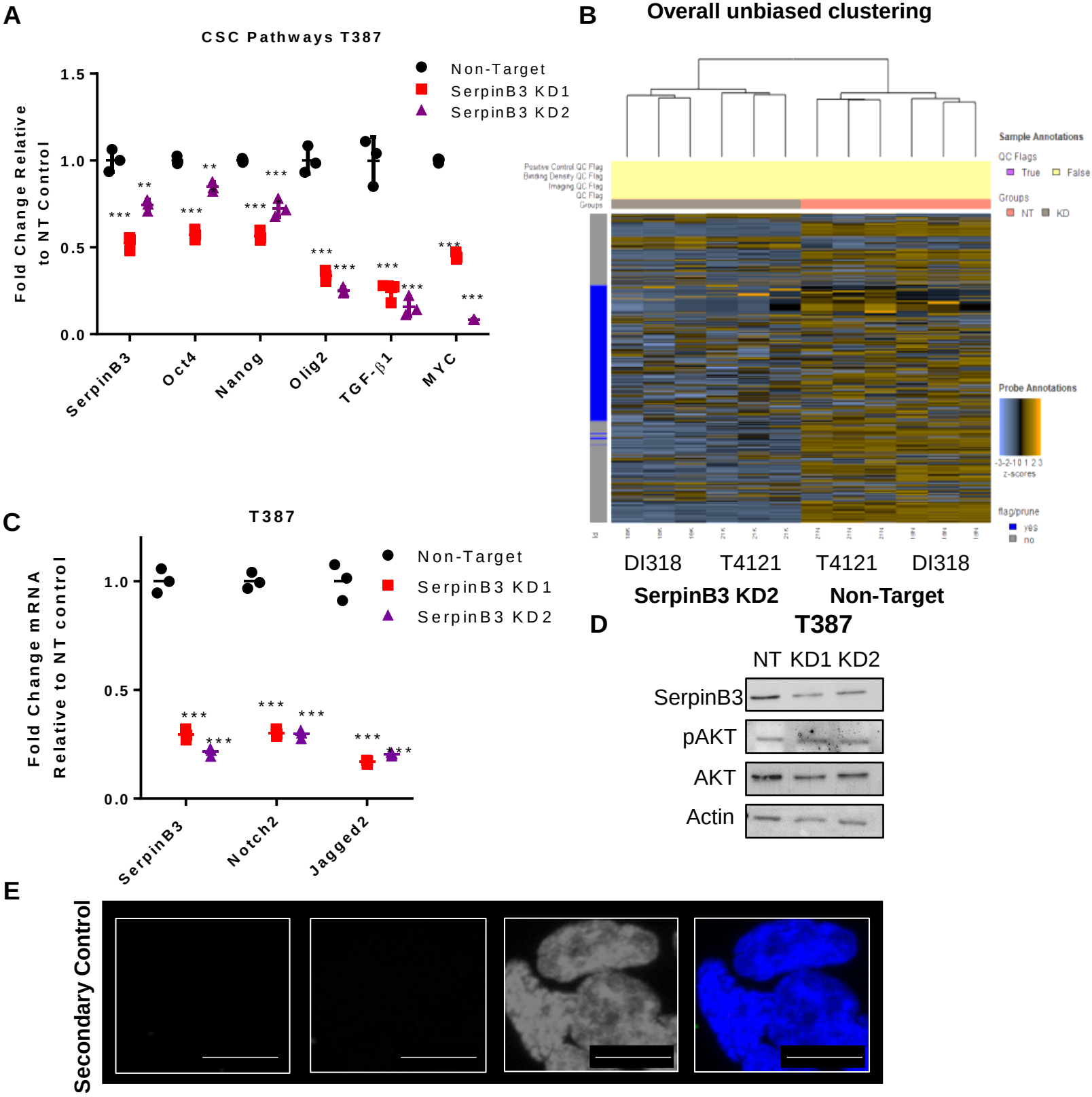
Unstained, JAM-A with CD4 intracellular control, SerpinB3 alone, SerpinB3 and JAM-A



Supplemental Figure 1, SerpinB3 is required for GBM tumor growth, related to Figure 1

A) SerpinB3 and JAM-A expression in four fresh human GBM tumor samples. B) Immunohistochemistry of SerpinB3 in two IDH-wild-type glioblastoma patients. C) SerpinB3 expression across GBM subtypes in the TCGA-Aligent-4502A dataset. D) Three separate GBM PDX CSC models were stained with either SerpinB3 alone (blue), JAM-A plus CD4 intracellular IgG control (green), JAM-A and SerpinB3 (red), or left unstained (orange). Percentage double-positive cells is indicated. E) SerpinB3 expression in PDX CSCs was compared to normal human astrocytes. F) SerpinB3 was knocked down in T387 and T4121 tumor cells, and JAM-A expression was measured via western blot. G) Western blot of SerpinB3 knockdown 3 (KD3) and knockdown 4 (KD4). H) Cell viability was measured with CellTiter Glo at day 7 in multiple GBM PDX models with KD3 and KD4 shRNA constructs (five technical replicates per condition, per tumor model). I) IncuCyte growth data, with cell count after 7 days, graphed as fold change relative to time 0. J) T4121 CSCs were intracranially injected into NSG mice. On day 21, mice were euthanized (two non-target mice reached endpoint before day 21 and could not be analyzed). Sections were cut and subjected to hematoxylin and eosin staining, and the largest tumor cross-section for each brain was identified. ImageJ was utilized to quantify the area of the entire brain and area of the tumor. The tumor proportion of cross-section was calculated. $p<0.05$ was considered statistically significant. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ as determined by one-way ANOVA with Dunnett's multiple comparisons or Student's t-Test (J). Error bars represent standard deviation.

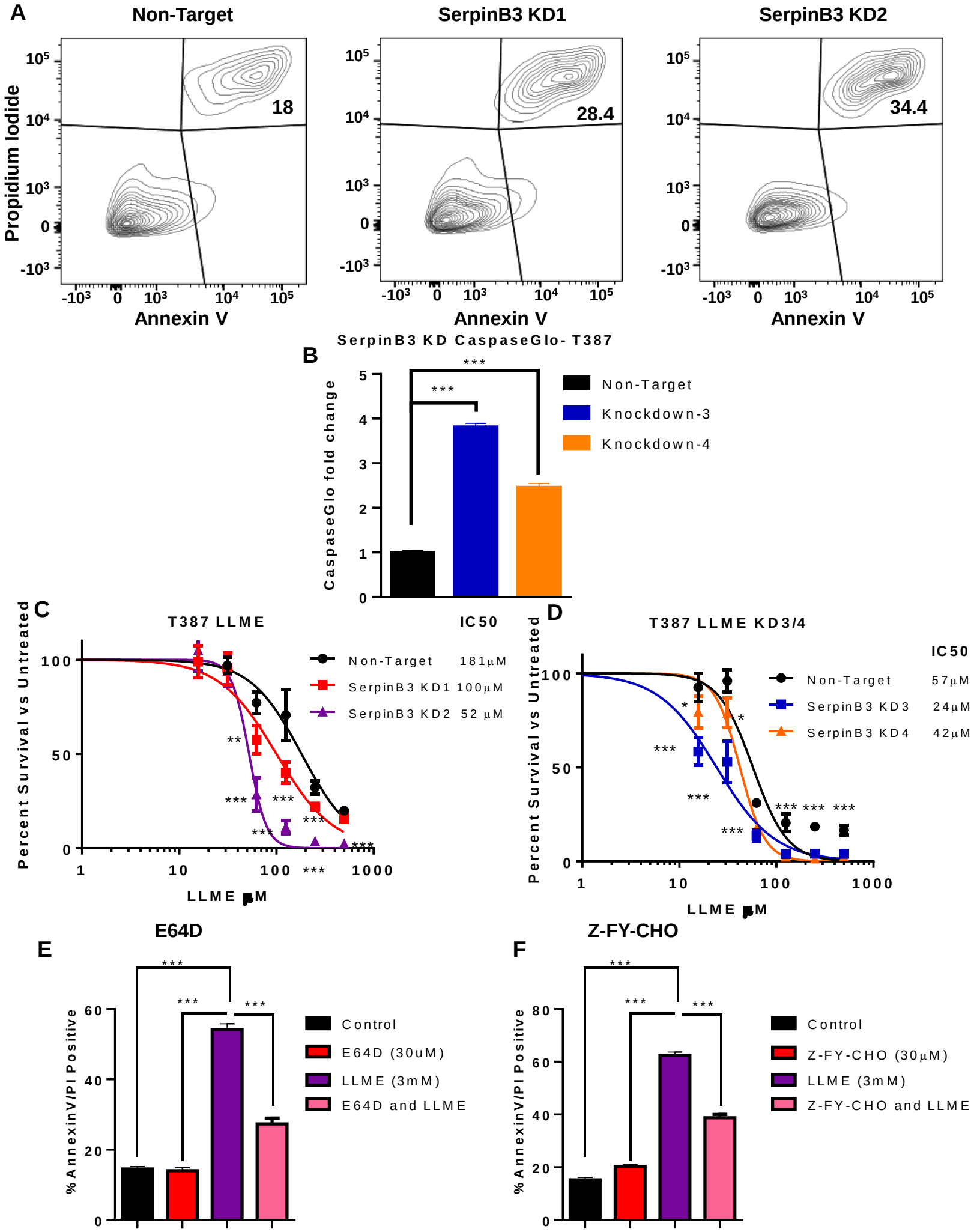
Supplemental Figure 2, Loss of SerpinB3 disrupts CSC pathways, related to Figure 2



Supplemental Figure 2, Loss of SerpinB3 disrupts CSC pathways, related to Figure 2

A) RNA was isolated after SerpinB3 knockdown in T387 cells, and qPCR was performed for SERPINB3, OCT4, NANOG, OLIG2, MYC, and TGF-β1 and normalized to Actin (three technical replicates). B) Overall unbiased clustering from the NanoString platform analysis of DI318 and T4121 PDX models with non-target and SerpinB3 KD2 shRNA. C) mRNA expression of Notch2 and Jagged2 after SerpinB3 knockdown in T387 CSCs, normalized to GAPDH. D) SerpinB3 was knocked down in T387 cells and phosphoAKT (pAKT) and AKT were measured. E) Secondary control for SerpinB3 and SOX2 staining. Scale bar represents 10μM *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ as determined by Student's t-Test. Error bars represent standard deviation.

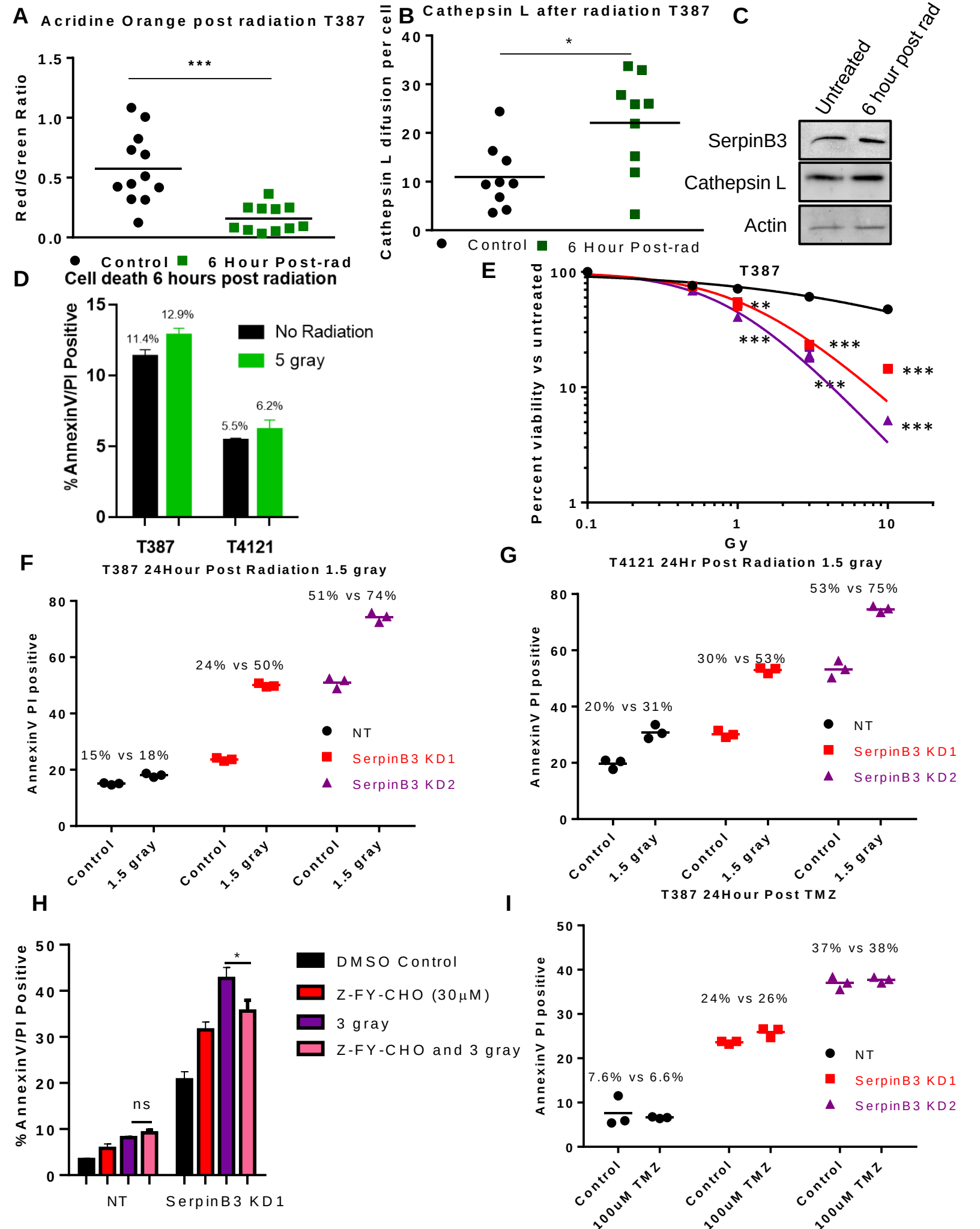
Supplemental Figure 3, SerpinB3 inhibits cathepsin L, related to Figure 3



Supplemental Figure 3, SerpinB3 inhibits cathepsin L, related to Figure 3

A) Annexin V- and propidium iodide-positive sample flow plots (T4121). B) Caspase 3/7 activity in T387 cells depleted for SerpinB3 with shRNA KD3 and KD4. Normalized to Cell-Titer Glo (five technical replicates per condition, per tumor model). C-D) T387 cells were treated with LLME at varying concentrations for 7 days. On day 7, cell viability was quantified via Cell Titer Glo and compared to untreated controls for each condition (NT, KD1, and KD2). The IC50 was determined (five replicates per condition). E-F) T4121 cells were treated with 3mM LLME for 6 hours with or without E) E64D or F) Z-FY-CHO. After 6 hours, annexin V/PI-positive percentage was quantified for each condition. $p<0.05$ was considered statistically significant *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ as determined by one-way ANOVA with Dunnett's multiple comparisons or Students t-test (E-F). Error bars represent standard deviation.

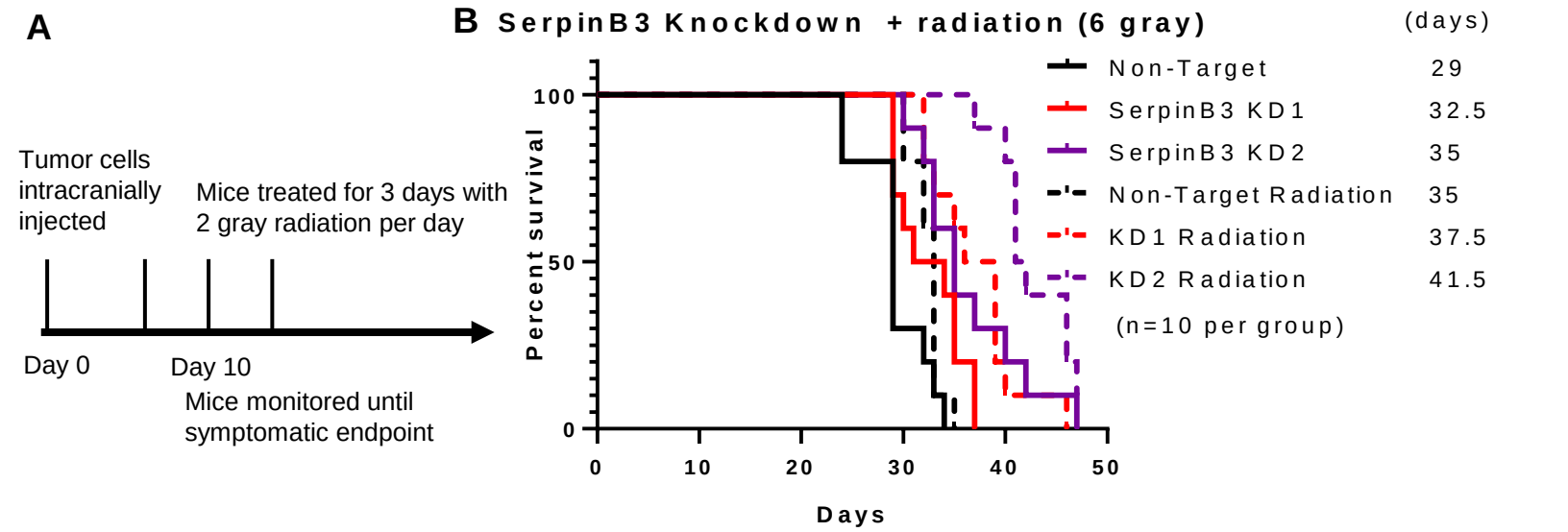
Supplemental Figure 4, Radiation cause cathepsin L mediated cell death, related to Figure 4

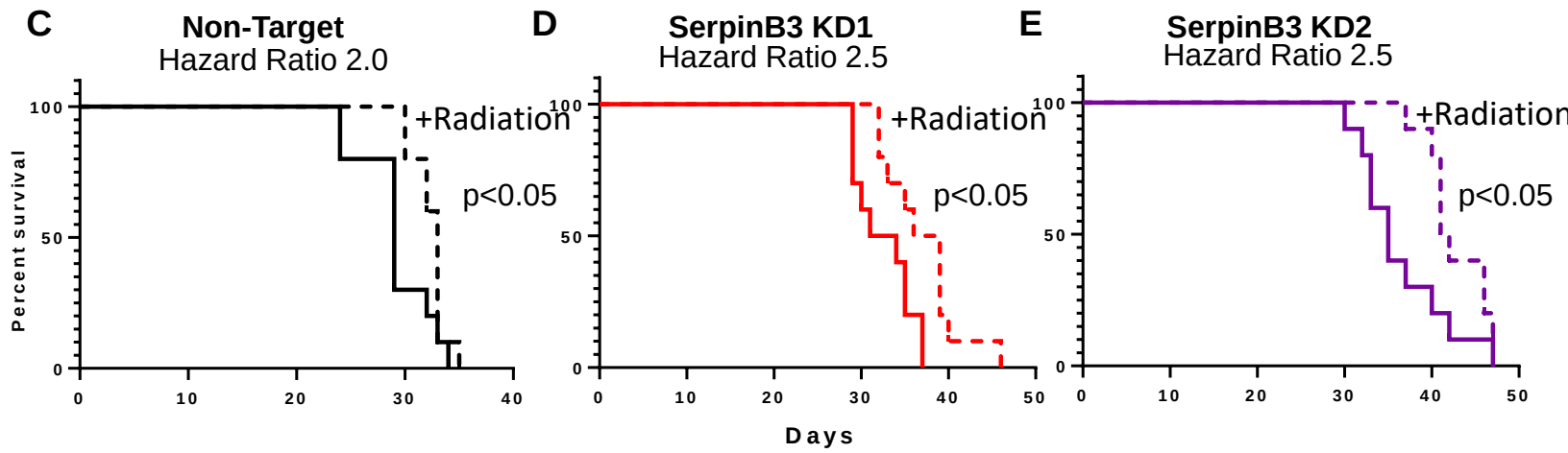


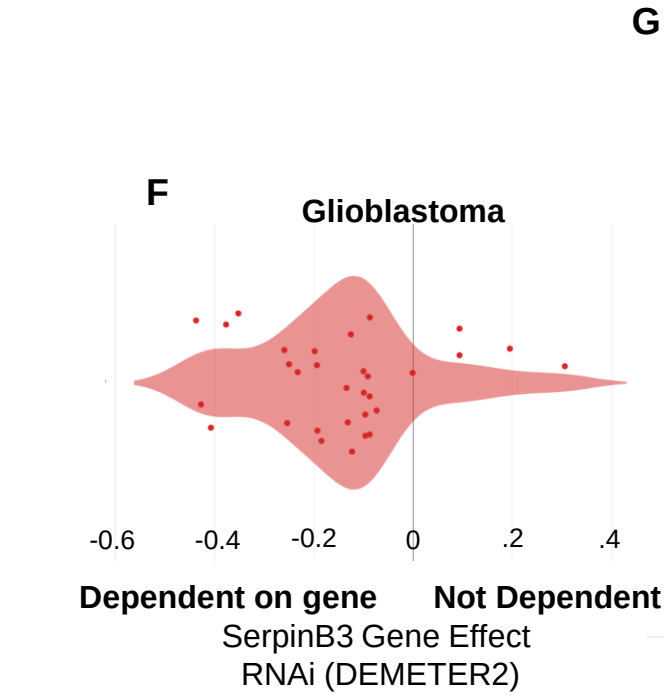
Supplemental Figure 4, Radiation cause cathepsin L mediated cell death, related to Figure 4

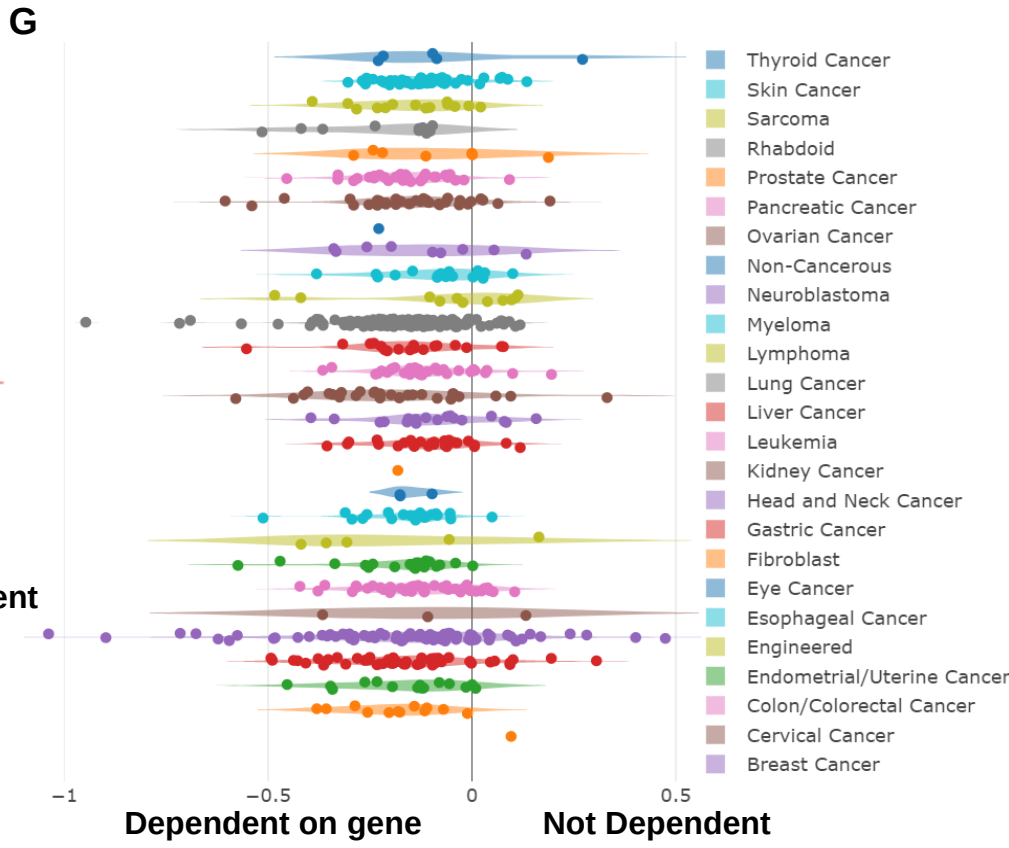
A) Acridine orange was added to T387 cells 6 hours after irradiation with 5 Gy, and images from 12 random visual fields were taken. The red/green ratio per image was calculated comparing control to irradiated conditions. B) Six hours post irradiation, T387 cells were fixed with paraformaldehyde and stained for cathepsin L. Images from 9 random fields were taken for each group. The integrated density of cathepsin L per cell was determined and compared between the irradiated and control groups. C) Western blot demonstrating total expression of SerpinB3 and cathepsin L 6 hours after irradiation. D) T387 and T4121 tumor cells were irradiated at 5 Gy. After 6 hours, the annexin V/PI-positive percentage was quantified and compared with untreated cells. E) Cell viability was measured after two days of varying doses of radiation, and the percent of viable cells is shown compared to each group's untreated control at each dose of radiation (three technical replicates per condition). F-G) T387 and T4121 CSCs expressing SerpinB3 shRNA or NT control were irradiated with 1.5 Gy. After 24 hours, percent of annexin V/PI-positive cells was quantified and compared to cells that did not received radiation. H) T4121 tumor cells were treated with 3 Gy radiation with or without 30μM Z-FY-CHO. Twenty-four hours post radiation, annexin V/PI-positive cells were quantified. I) T387 tumor cells were treated with 100μM temozolomide (TMZ) and annexin V/PI-positive cells were quantified after 24 hours. $p<0.05$ was considered statistically significant *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ as determined by one-way ANOVA with E) Dunnett's multiple comparisons or Students t-test (A,B,H). Error bars represent standard deviation.

Supplemental Figure 5, SerpinB3 contributes to radiation resistance *in vivo*, related to Figure 5









Supplemental Figure 5, SerpinB3 contributes to radiation resistance in vivo, related to Figure 5

A) Schematic of in vivo radiation experiment with 10 Gy total. B-G) A total of 20,000 tumor cells per condition were intracranially injected into mice. Ten days after irradiation, mice heads received 2 Gy of radiation every other day for 3 days (total of 6 Gy) to the head. B) All treatment groups plotted with median survival values. The groups were subsequently divided into: C) Non-target with or without radiation, D) SerpinB3 knockdown 1 with or without radiation, E) SerpinB3 knockdown 2 with or without radiation. G) SerpinB3 dependency of 31 GBM cells lines by RNAi screen.F) SerpinB3 dependency across three large RNAi screens on tumor cell lines from a variety of tumors. $p<0.05$ was considered statistically significant. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ as determined by log-rank test.

Supplemental Table 1, Results from JAM-A pulldown LC/MS, Related to Figure 1

Protein (gene)	Control His	JAM-A His	Ratio
Junctional adhesion molecule A (F11R)	3	126	42
SerpinB3 (SERPINB3)	**0**	**37**	**JAM-A only**
Myosin-14 (MYH14)	0	24	JAM-A only
hydroxysteroid (17-beta) dehydrogenase 4 (HSD17B4)	0	13	JAM-A only
Calmodulin like protein 3 (CALML3)	0	12	JAM-A only
Involucrin (IVL)	0	10	JAM-A only
Calmodulin like protein 5 (CALML5)	1	24	24
Protein POF1B (POF-1B)	2	34	17
transglutaminase 1 (TGM1)	1	13	13
Truncated profilaggrin (FLG)	5	46	9.2
Protein-glutamine gamma-glutamyltransferase E (TGM3)	5	42	8.4
Caspase-14 (CASP14)	10	49	4.9
Probable fibrosin-1 (FBRS)	3	12	4
Suprabasin (SBSN)	6	21	3.5
Purine nucleoside phosphorylase (NP)	4	13	3.3
Bleomycin hydrolase (BLMH)	6	19	3.2
Probable tRNA N6-adenosinethreonylcarbamoyltransferase (OSGEP)	3	10	3
Protein S100-A9 (S100-A9)	5	15	3

JAM-A only

Ratio

Supplemental Table 2, CSC PCR primers, related to Figure 2

Gene	Forward	Reverse
SERPINB3	CGCGGTCTCGTGCTATCTGG	AGAAGAGGATGCTGTTGGTC
OCT4	TGAGTCAGTGAACAGGGAATG	AATCTCCCCTTTCCATTCGG
NANOG	GAAATACCTCAGCCTCCAGC	GCGTCACACCATTGCTATTC
OLIG 2	AGCTCCTCAAATCGCATCC	AAAAGGTCATCGGGCTCTG
c-MYC	TTCGGGTAGTGGAAAACCAG	AGTAGAAATACGGCTGCACC
TGF-β1	AAGTGGACATCAAGGGTTC	GTCCTTGCGGAAGTCAATGT
GAPDH	ACATCGCTCAGACACCATG	TGTAGTTGAGGTCAATGAAGGG
NOTCH2	GTGCCTATGTCCATCTGGATGG	AGACACCTGAGTGCTGGCACAA
JAGGED2	GCTGCTACGACCTGGTCAATGA	AGGTGTAGGCATCGCACTGGAA