SUPPLEMENTAL FIGURES LEGENDS

Supplementary Figure S1. The L1CAM+ glioma cell subpopulation correlates with the CD133+ glioma cell population. *A*, FACS analysis indicated that the fraction of CD133+ cells was similar to that of L1CAM+ cells in primary glioblastomas T3837 and T3852. Unsorted tumor cells from primary glioma samples were labeled with anti-CD133-APC, or anti-L1CAM-PE, and then subjected to FACS analysis for CD133+ population or L1CAM+ population. The fractions of CD133+ cells and L1CAM+ cells in total unsorted tumor cells were very similar in each case. *B*, FACS analyses of the L1CAM+ cell fraction in CD133+ and CD133- subpopulations isolated from the glioblastoma surgical specimens T3691, T3832, and T3946 are displayed. Sorted CD133+ and CD133- populations were cultured short term and then labeled as in (*B*). 78-93% of CD133+ cells also expressed L1CAM, whereas CD133- glioma cells rarely expressed L1CAM (*, $p < 0.001$).

Supplementary Figure S2. Knockdown of L1CAM in CD133+ glioma cells reduces self-renewal due to increased apoptosis. *A*, Targeting L1CAM expression disrupted neurosphere formation of CD133+ brain tumor cells derived from a T3691 glioblastoma patient specimen or a D456MG pediatric glioblastoma xenograft. CD133+ cells infected with lentivirus expressing non-targeting shRNA (NT) formed neurospheres, whereas infection with lentivirus expressing L1CAM shRNA attenuated neurosphere formation. *B*, Knockdown of L1CAM expression via lentiviral shRNA significantly reduced neurosphere formation of CD133+ brain tumor cells isolated from the primary

glioblastomas T3852 and T3946. *, p <0.001. *C*, FACS analysis of apoptosis with Annexin V-FITC staining confirmed that L1CAM knockdown induces apoptotic cell death of CD133+ brain tumor cells from both the glioblastoma surgical specimen T3691 and the pediatric glioblastoma xenograft D456MG. *, $p < 0.001$ with comparison to non-targeting shRNA.

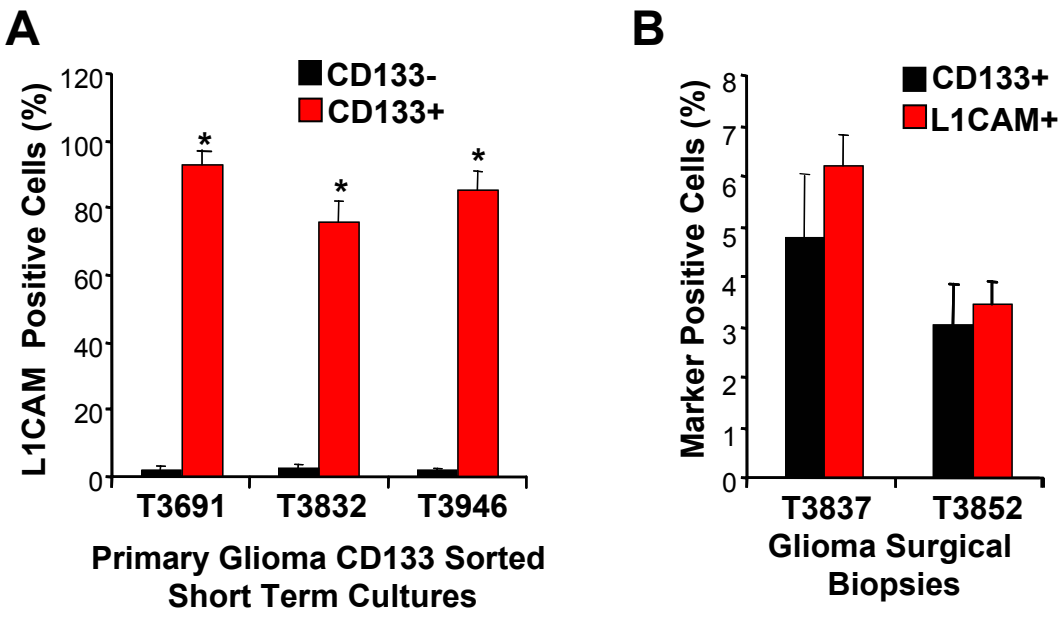
Supplementary Figure S3. Knockdown of L1CAM expression preferentially suppresses the growth of CD133+ glioblastoma cells. Identical numbers of CD133+ brain tumor cells (3 x 10^5) (*A*), matched CD133- cells (*B*), and CD133+ normal neural progenitor cells (*C*) were infected with lentivirus expressing non-targeting control shRNA (NT) or L1CAM shRNA. CD133+ and CD133- cells were isolated from a glioblastoma T4105 patient specimen. Live cells were quantified on days 3, 5 and 7 after infection using trypan blue staining. All cells were cultured under identical conditions in neurobasal media with B27 and EGF/FGF growth factors (20 ng/ml for each). *, $p < 0.01$, ** $p < 0.001$ with comparison to non-targeting control cells on the same day.

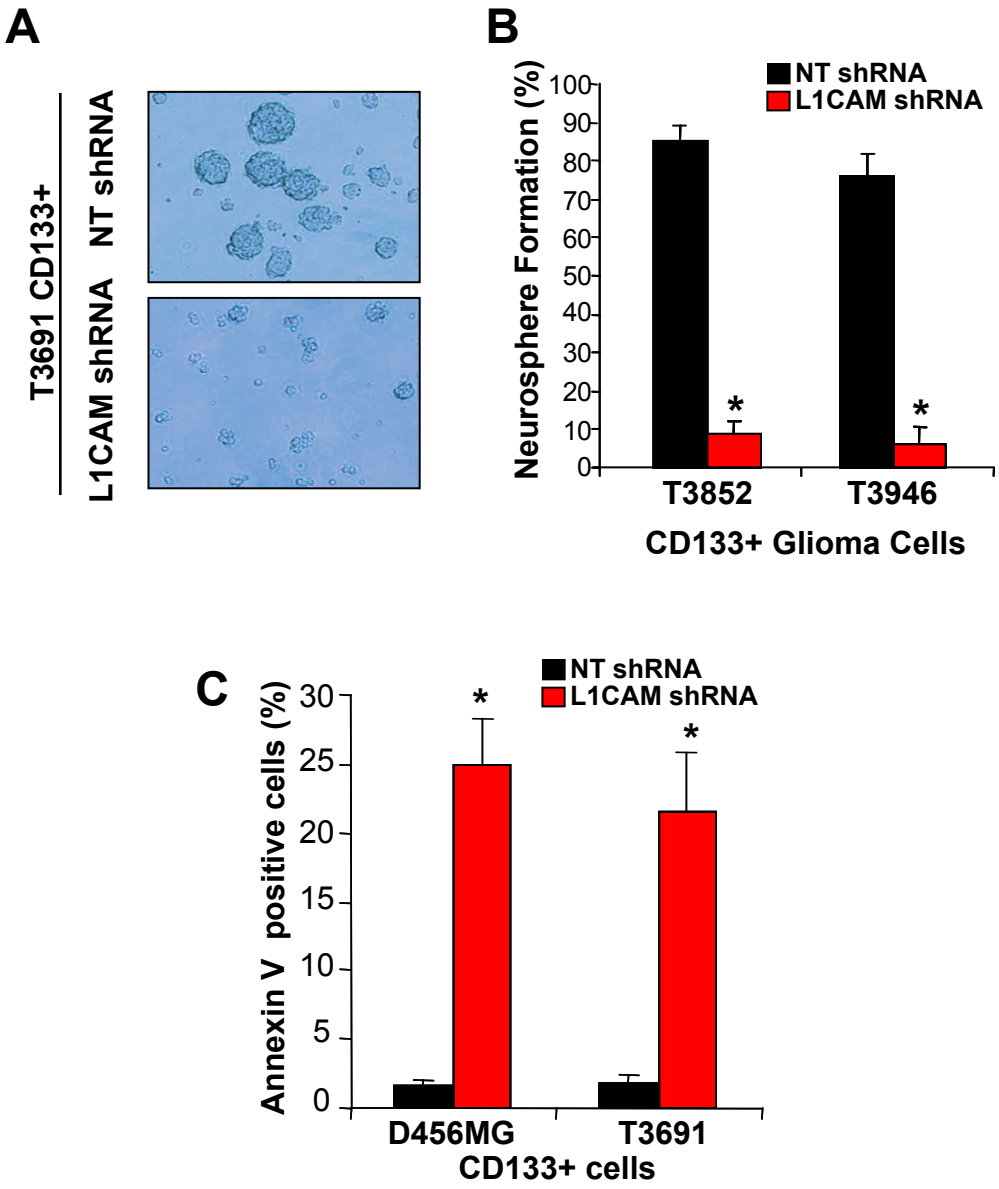
Supplementary Figure S4. Knockdown of L1CAM decreases tumor formation by targeting CD133+ glioma cells. *A*, Representative images of brains of mice injected with CD133+ cells isolated from the anaplastic astrocytoma T3565 patient specimen and infected with lentivirus expressing non-targeting shRNA (NT) or L1CAM shRNA are displayed. Identical numbers of viable cells (10^5 cells/mouse) 48 hours after infection were implanted and brains examined on day 17 after injection. *B*, Knockdown of L1CAM in CD133+ cells isolated from the anaplastic astrocytomas patient specimen

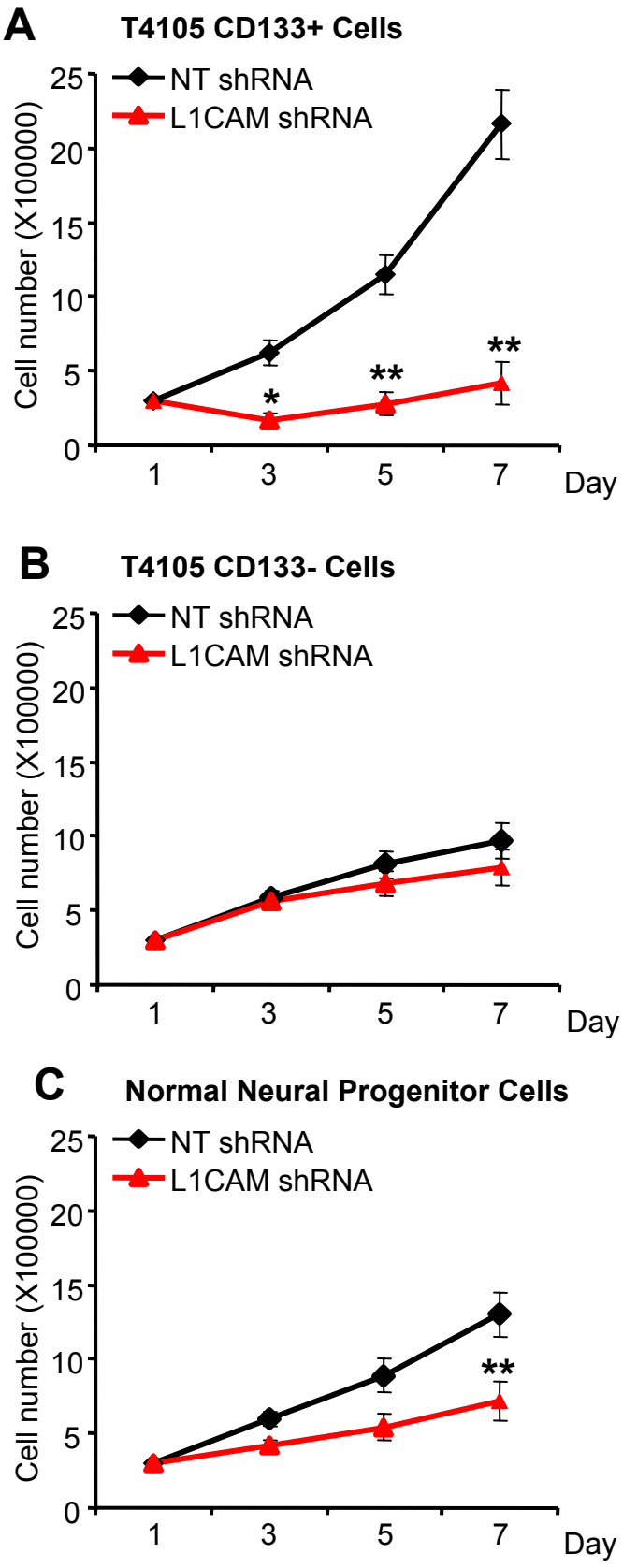
T3565 prior to intracranial implantation increased survival. $p < 0.001$ with comparison to non-targeting control. CD133+ cells isolated from brain tumor patient specimens and infected with non-targeting shRNA (NT) or L1CAM shRNA prior to injection were implanted into the brains of immunocompromised mice as in (*A).* *C*, Targeting L1CAM in CD133+ cells isolated from the glioblastoma patient specimen T4105 decreased their tumorigenic potential *in vivo*. Decreasing numbers of CD133+ cells (10^6 to 100) infected with non-targeting shRNA (NT) or L1CAM shRNA were implanted into the brains of immunocompromised mice with five mice per condition and animals were permitted to survive until the development of neurologic signs. The incidence of tumor formation and the average number of days of survival is indicated. *D*, Targeting L1CAM in CD133+ cells isolated from the anaplastic astrocytoma patient specimen T3565 decreases their tumorigenic potential *in vivo*. Decreasing numbers of CD133+ cells (10^6 to 500) infected with non-targeting shRNA (NT) or L1CAM shRNA were implanted into the brains of immunocompromised mice with five mice per condition and animals were permitted to survive until the development of neurologic signs. The incidence of tumor formation and the average number of days of survival is indicated.

Supplementary Figure S5. *A*, Representative images of subcutaneous tumors treated with lentivirus expressing non-targeting shRNA (NT) or L1CAM directed shRNA are shown. Identical numbers of CD133+ glioma cells (10^5 cells/mouse) isolated from the glioblastoma T3832 patient specimen were implanted into nude mice to establish small subcutaneous tumors. After tumor establishment, lentivirus expressing non-targeting control shRNA (NT) or L1CAM shRNA was delivered to the subcutaneous tumors

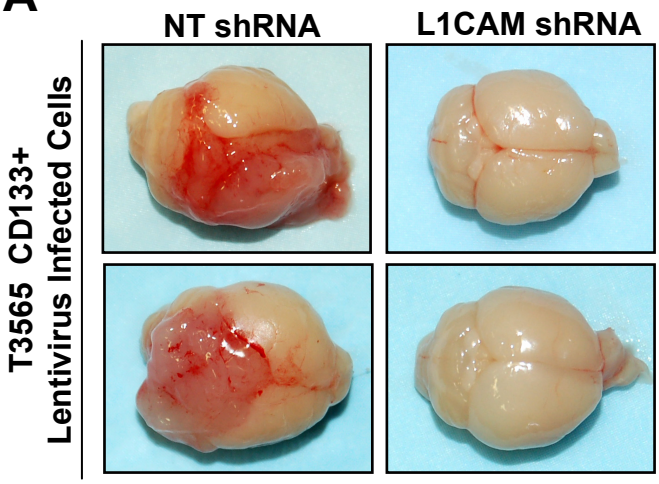
through direct injection to the tumor site for 21 days (once every other day). *B*, Growth of established subcutaneous tumors was reduced by treatment with lentivirus expressing L1CAM shRNA in comparison to non-targeting shRNA (NT) as demonstrated by significantly reduced average tumor weight (*, $p < 0.01$). *C*, Western blot confirms that injection of lentivirus expressing L1CAM shRNA (L1) to the tumor site reduces the expression of L1CAM protein in glioma xenografts compared to treatment with lentivirus expressing non-targeting shRNA (NT). *D*, FACS analysis further confirmed that the CD133+ glioma cell population was reduced in tumors treated with L1CAM shRNA targeting lentivirus relative to that treated with non-targeting control lentivirus.


A
CD133-
CD133+
L1CAM Positive Cells (%)
120
100
80
60
40
20
0
*
*
*
T3691
T3832
T3946
Primary Glioma CD133 Sorted
Short Term Cultures
B
CD133+
L1CAM+
Marker Positive Cells (%)
8
7
6
5
4
3
2
1
0
T3837
T3852
Glioma Surgical
Biopsies

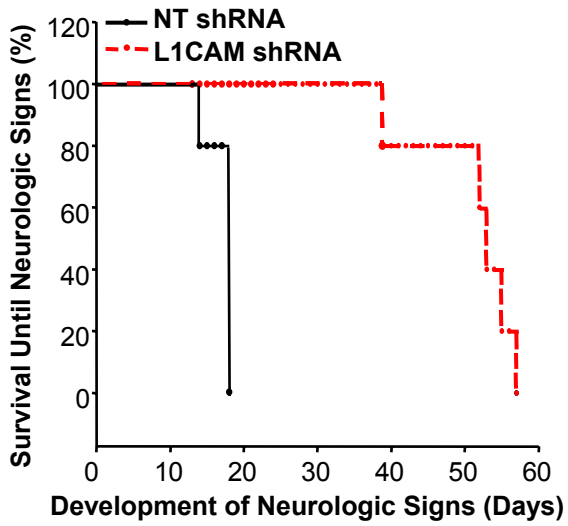

A
T3691 CD133+
NT shRNA
L1CAM shRNA
B
NT shRNA
L1CAM shRNA
Neurosphere Formation (%)
100
90
80
70
60
50
40
30
20
10
0
*
*
T3852
T3946
CD133+ Glioma Cells
C
NT shRNA
L1CAM shRNA
Annexin V positive cells (%)
30
25
20
15
10
5
0
*
*
D456MG
T3691
CD133+ cells


A
T4105 CD133+ Cells
NT shRNA
L1CAM shRNA
Cell number (X100000)
25
20
15
10
5
0
1
3
5
7
Day
*
**
**
B
T4105 CD133- Cells
NT shRNA
L1CAM shRNA
Cell number (X100000)
25
20
15
10
5
0
1
3
5
7
Day
C
Normal Neural Progenitor Cells
NT shRNA
L1CAM shRNA
Cell number (X100000)
25
20
15
10
5
0
1
3
5
7
Day
**

A



B

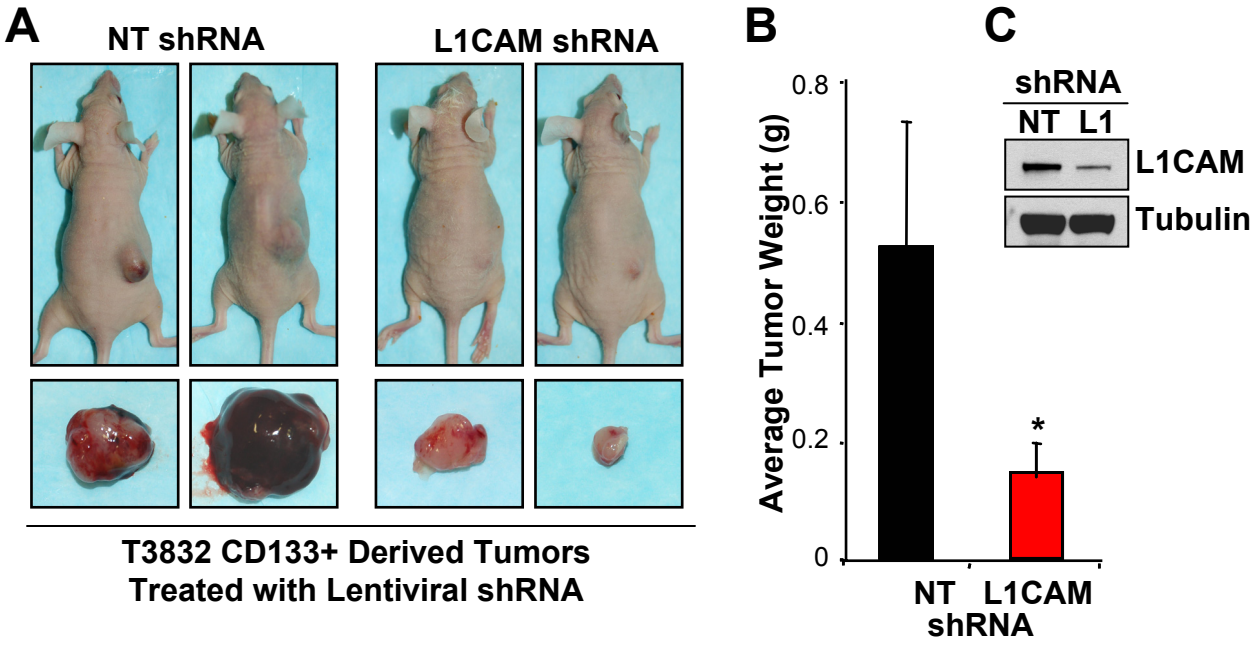


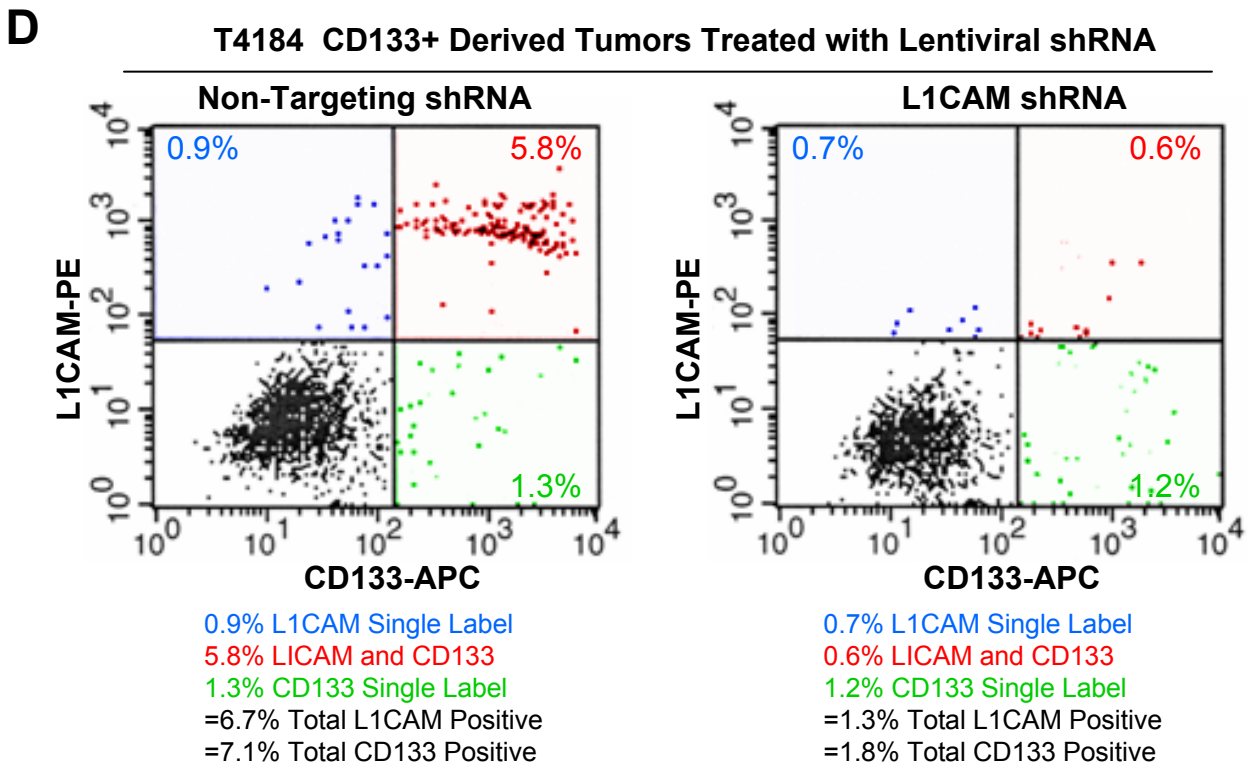
C

shRNA	T4105 CD133+ Lentivirus Infected Cells				
	Cell Number	1000000	100000	10000	1000
Non-Targeting	Incidence	5/5	5/5	5/5	5/5
	Median Survival	18	35	46	72
L1CAM	Incidence	5/5	5/5	4/5	0/5
	Median Survival	31	52	71	--

D

shRNA	T3565 CD133+ Lentivirus Infected Cells					
	Cell Number	1000000	100000	10000	1000	500
Non-Targeting	Incidence	5/5	5/5	5/5	5/5	4/5
	Median Survival	18	32	55	69	88
L1CAM	Incidence	5/5	5/5	0/5	0/5	0/5
	Median Survival	33	64	-	-	-


A
NT shRNA
L1CAM shRNA
T3832 CD133+ Derived Tumors
Treated with Lentiviral shRNA
B
C
shRNA
NT L1
L1CAM
Tubulin
Average Tumor Weight (g)
0.8
0.6
0.4
0.2
0
*
NT L1CAM
shRNA


D
T4184 CD133+ Derived Tumors Treated with Lentiviral shRNA
Non-Targeting shRNA
L1CAM shRNA
0.9%
5.8%
1.3%
0.7%
0.6%
1.2%
L1CAM-PE
CD133-APC
0.9% L1CAM Single Label
5.8% LICAM and CD133
1.3% CD133 Single Label
=6.7% Total L1CAM Positive
=7.1% Total CD133 Positive
0.7% L1CAM Single Label
0.6% LICAM and CD133
1.2% CD133 Single Label
=1.3% Total L1CAM Positive
=1.8% Total CD133 Positive