SUPPLEMENTAL METHODS

IL6 Enzyme-Linked ImmunoSorbant Assay (ELISA)

GSCs and non-stem glioma cells were plated in 24-well plates in equal cell number ($2x10^5$ cells/well) with fresh neural basal medium. After 48 hours, conditioned media were collected and IL6 ELISA was performed with the Human IL6 ELISA Kit (R&D Systems) according to manufacturer's instruction.

Thymidine Incorperation

Cells were labeled for 4 hours with 4 μCi [^{3}H]thymidine, fixed in 10% trichloroacetic acid, and lysed in 0.2 N NaOH. [^{3}H]thymidine incorporation into DNA was measured with a scintillation counter.

SUPPLEMENTAL TABLES

Supplemental Table 1. Characteristics of Brain Tumor Patient Specimens. Patient and pathological information associated with brain tumor samples used for the isolation of cancer stem cells and non-stem cells is provided if known. The age and gender of patients as well as the tumor stage and histopathology are included. FISH (Fluorescence in situ hybridization) data determining Chromosome 7, Chromosome 10, EGFR, and PTEN status is included.

Supplemental Table 2. Detailed information of lentiviral shRNA clones (Sigma Mission RNAi) targeting IL6Rα and IL6. The shRNA sequences utilized for targeting IL6Rα and IL6 are displayed.

SUPPLEMENTAL FIGURES

Supplemental Figure 1. Co-localization of IL6Rα and CD133 in neurospheres and glioma patient specimens. (**A**) Co-staining of CD133 with IL6Rα and gp130 in neurospheres derived from GSCs isolated from a T3359 patient specimen passaged short-term in immunocompromised mice. (**B**) Immunofluorescent staining of IL6Rα and CD133 in the freshly frozen human glioma surgical biopsy specimens HP323 and HP591. Nuclei in all images were counterstained with Hoechst 33342.

Supplemental Figure 2. Analysis of IL6 and IL6 receptor mRNA in a primary patient specimen. Real-Time PCR was used to determine the relative mRNA levels of IL6Rα, gp130, IL6 and Olig2 in mRNA collected from the primary glioblastoma patient specimen CCF1863. The mRNA levels of IL6Rα and gp130 were higher in GSCs, whereas the mRNA level of IL6 was higher in non-stem glioma cells. Olig2 had higher mRNA levels in isolated GSC populations. *, $p < 0.05$ with comparison of non-stem glioma cells to matched GSCs.

Supplemental Figure 3. Non-stem glioma cells secreted more IL6 ligand than matched GSCs. ELISA was employed to determine the relative protein levels of IL6 ligand in GSCs and non-stem glioma cells isolated from T3359, T3832, T4105 and T4121 patient specimens passaged short term in immunocompromised mice. GSCs and non-stem glioma cells were plated in 24-well plates in equal cell number ($2x10^5$ cells/well) with fresh neural basal medium. After 48 hours, conditioned media were collected and IL6 ELISA was performed with the Human IL6 ELISA Kit. *, $p < 0.05$ with comparison of non-stem glioma cells to matched GSCs.

Supplemental Figure 4. Targeting IL6Rα or IL6 in a primary glioma specimen decreases GSC survival. (**A**) Targeting IL6Rα and IL6 via lentiviral shRNA significantly retarded the growth of CCF1863 GSCs as assessed with the Cell Titer Assay (Promega). (**B**) Targeting IL6Rα and IL6 respectively decreased the survival of

CCF1863 GSCs as demonstrated by Caspase 3/7 activity. (**C**) Targeting IL6Rα and IL6 expression respectively attenuated the efficiency of CCF1863 GSCs to form neurospheres. The percentage of wells with neurospheres is indicated when infected cells were plated with 50 cells per well in 24-well plates. (**D**) Representative images of neurospheres of non-target control. IL6Rα and IL6 knockdown cells did not form neurospheres. *, $p < 0.01$ with comparison to non-targeting shRNA.

Supplemental Figure 5. Targeting IL6Rα or IL6 expression reduced GSC proliferation. Targeting IL6Rα or IL6 via lentiviral shRNA reduced the proportion of S-phase T3359 (**A**) or D456MG (**B**) GSCs as determined by cell cycle analysis. Results were analyzed using Flowjo software. IL6Rα or IL6 knockdown decreased thymidine cooperation in T3359 (**B**) and D456MG (**D**) GSCs. *, $p < 0.05$ with comparison of shRNA targeted GCSs to matched non-targeting controls.

Supplemental Figure 6. Targeting IL6Rα or IL6 increased differentiation marker expression. **(A)** After IL6Rα knockdown, real-time PCR determined increased relative mRNA levels of the differentiation markers S100β (astrocyte lineage) and GalC (oligodendrocyte lineage). **(B)** Targeting IL6 also increased S100β and GalC expression.

Supplemental Figure 7. Elevated STAT3 phosphorylation in GSCs and induction by exogenous IL6. (**A**) STAT3 phosphorylation at Tyr 705 is elevated in D456MG and T4121 GSCs in comparison to matched non-stem glioma cells. (**B**) Western blotting demonstrated elevated Stat3 phosphorylation at Tyr 705 2 hours after addition of 40 ng/mL IL6 to the media of T3691 GSCs.

Supplemental Figure 8. Targeting STAT3 activity in GSCs with small molecule inhibitors decreased proliferation and increased cell death. (**A**) Treatment of D456MG GSCs with 5 μM Stattic or 5 μM JSI-124 for 4 hours have reduced STAT3 phosphorylation assessed via Western in comparison to vehicle treated control.

(**B**) Treatment of D456MG GSCs with 1 μM Stattic or 1 μM JSI-124 for 48 hours demonstrated an increased percentage of apoptotic cells as measured by FACS analysis with Annexin V-FITC and PI staining compared to DMSO control. (**C**) Treatment of D456MG GSCs with 1 μM Stattic or 1 μM JSI-124 for 24 hours demonstrated increased activity in the caspase 3/7 assay (Promega) compared with DMSO control. **(D)** T3359 GSCs treated with 5 μM Stattic or 5 μM JSI-124 for 24 hours demonstrated decreased DNA synthesis compared with DMSO control. *, $p < 0.05$ with ANOVA comparison to vehicle treated control.

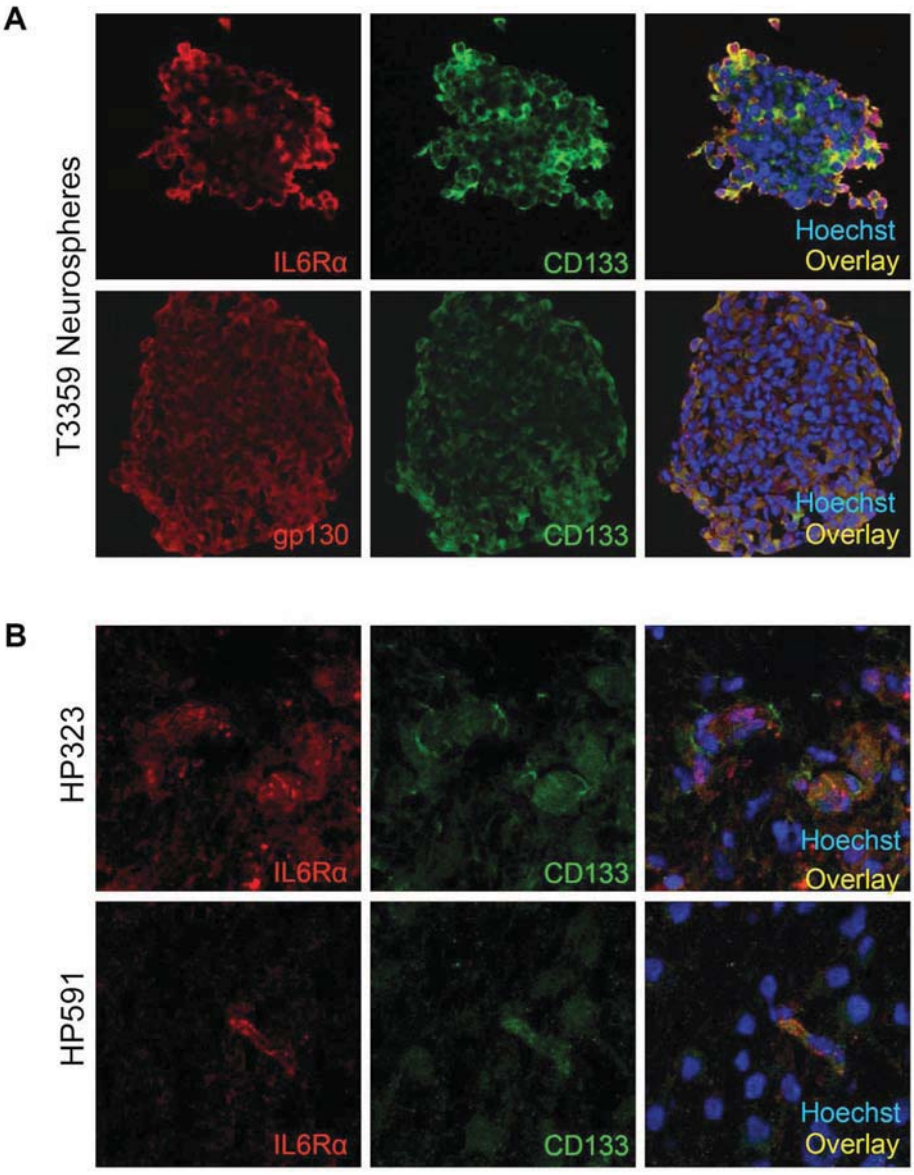
Supplemental Figure 9. Representative images of GSC-initiated human brain tumor xenografts. (**A**) Hematoxylin and eosin stains of sections of brains of immunocompromised mice injected with T3359 GSCs infected with non-targeting control shRNA or shRNAs derived against IL6Rα. (**B**) Hematoxylin and eosin stains of sections of brains of immunocompromised mice injected with D456MG GSCs infected with non-targeting control shRNA or shRNAs derived against IL6. For these experiments, all animals were sacrificed when any one animal developed neurological signs.

Supplemental Figure 10. Higher gp130 and LIF mRNA expression is correlated with poorer patient survival but higher CNTF mRNA expression is not. **(A)** Clinical data from the National Cancer Institute's Repository for Molecular Brain Neoplasia Data (REMBRANDT) database indicates two fold or greater upregulation of gp130 correlates with poor survival. *, $p = 0.0281$ with comparison of survival probabilities for patients with up-regulated gp130 expression ($n = 4$) to those with intermediate expression ($n = 198$). **(B)** REMBRANDT data indicates three fold or greater upregulation of LIF correlates with poor survival. *, $p = 0.0031$ with comparison of survival probabilities for patients with up-regulated ($n = 65$) LIF expression to those with intermediate expression ($n = 124$). **(C)** REMBRANDT data indicates two fold or greater upregulation of CNTF does not correlate with poor survival. $p = 0.2578$ with comparison of survival probabilities for patients with up-regulated ($n = 28$) CNTF expression to those with intermediate expression ($n = 156$).

Supplemental Figure 11. Comparison of mRNA expression of LIF and CNTF ligand and receptor in GSCs and matched non-stem glioma cells. Real-Time PCR was used to determine the relative mRNA levels of LIF **(A)**, LIF receptor **(B)**, CNTF **(C)** and CNTF receptor **(D)** in GSCs and non-stem glioma cells from the long term human glioma xenograft D456MG, T3359 and T3832 patient specimens passaged short-term in immunocompromised mice, as well as the primary glioblastoma specimen CCF1863. **(E)** Olig2, a reported marker for GSCs, had consistently higher mRNA levels in isolated GSC populations. *, $p < 0.05$ with comparison of non-stem glioma cells to matched GSCs.

Supplemental Figure 12. Systemic treatment with IL6 antibody inhibited the growth of human glioma xenografts *in vivo*. (**A**) Subcutaneous tumor volume was significantly decreased with IL6 antibody treatment. Animals were subcutaneously injected with GSCs isolated from a D456MG xenograft. 24 hours after tumor cell injection, animals were injected intravenously with IL6 antibody at 100 mg every two days or PBS as a control. (**B**) Total tumor burden was reduced with IL6 antibody treatment. Tumor weights were measured at the completion of the experiment and demonstrated that IL6 antibody treated xenografts were significantly smaller than controls. #, $p < 0.05$ with comparison to non-targeting control. (**C**) Images of xenografts measured in B.

Supplemental Figure 13. Histological analysis of T3359 xenografts in Fig. 7. **(A)** Representative images of control and IL6 antibody treated T3359 xenografts stained with hematoxylin and eosin. (**B**) Representative images of Nestin staining of controls and IL6 antibody treated T3359 xenografts.



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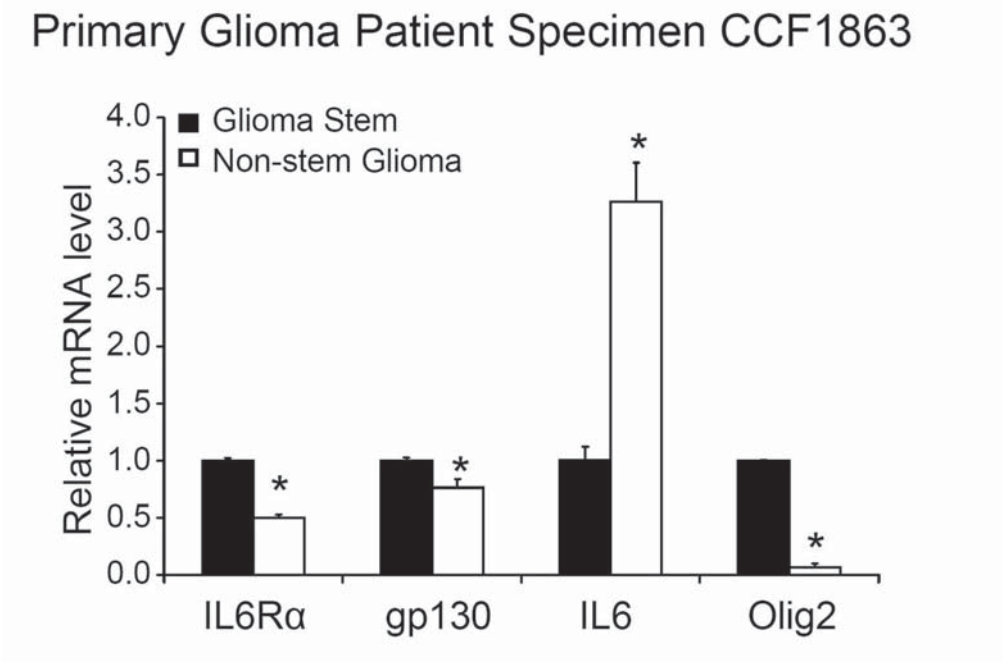

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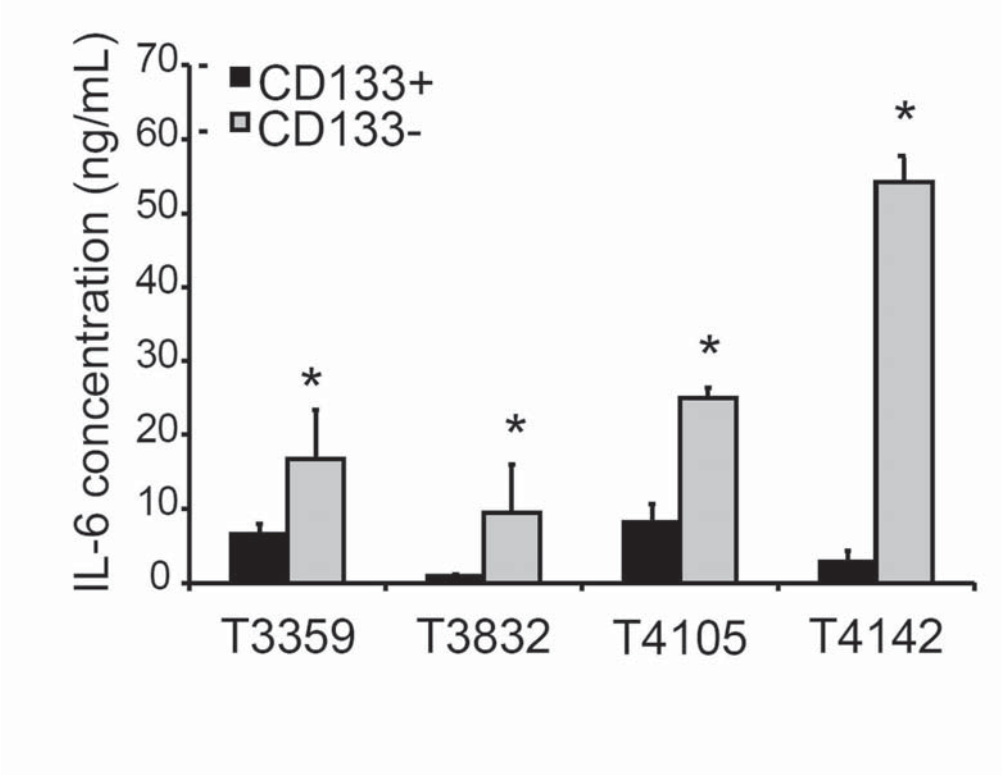

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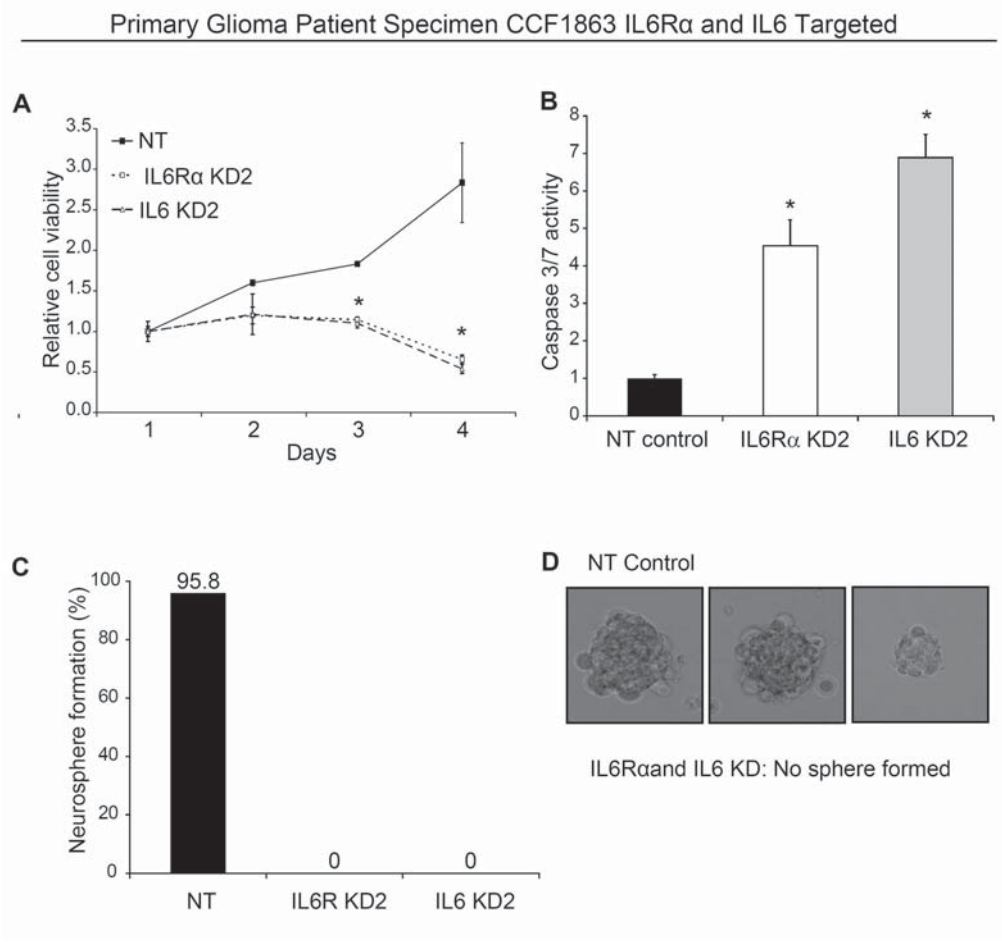

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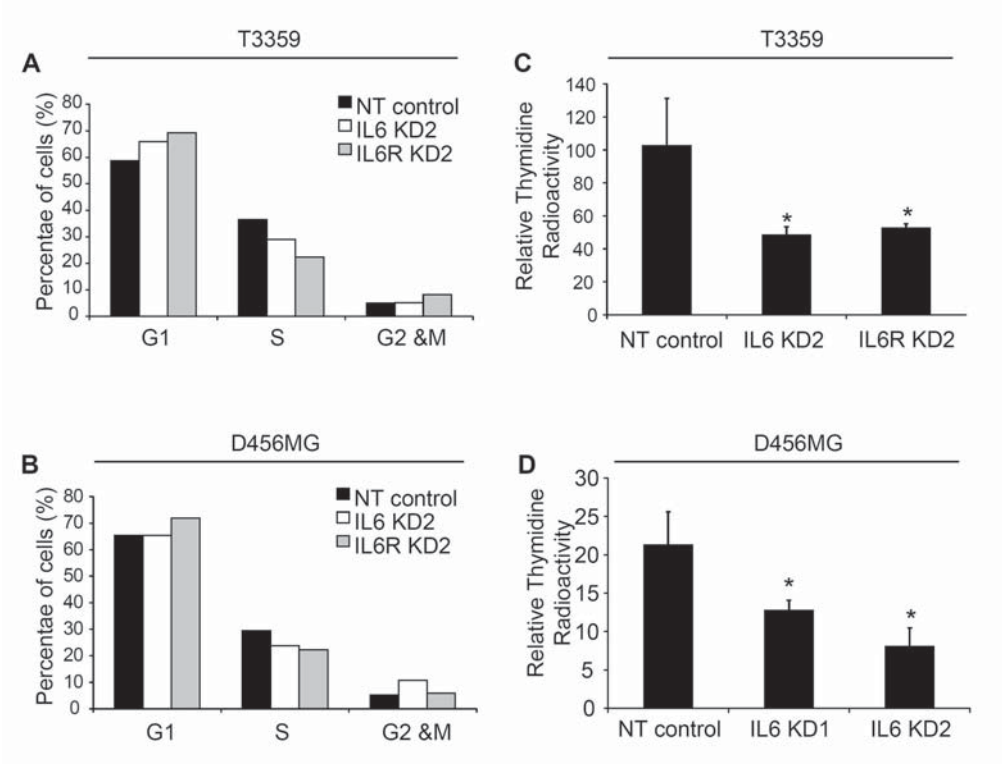

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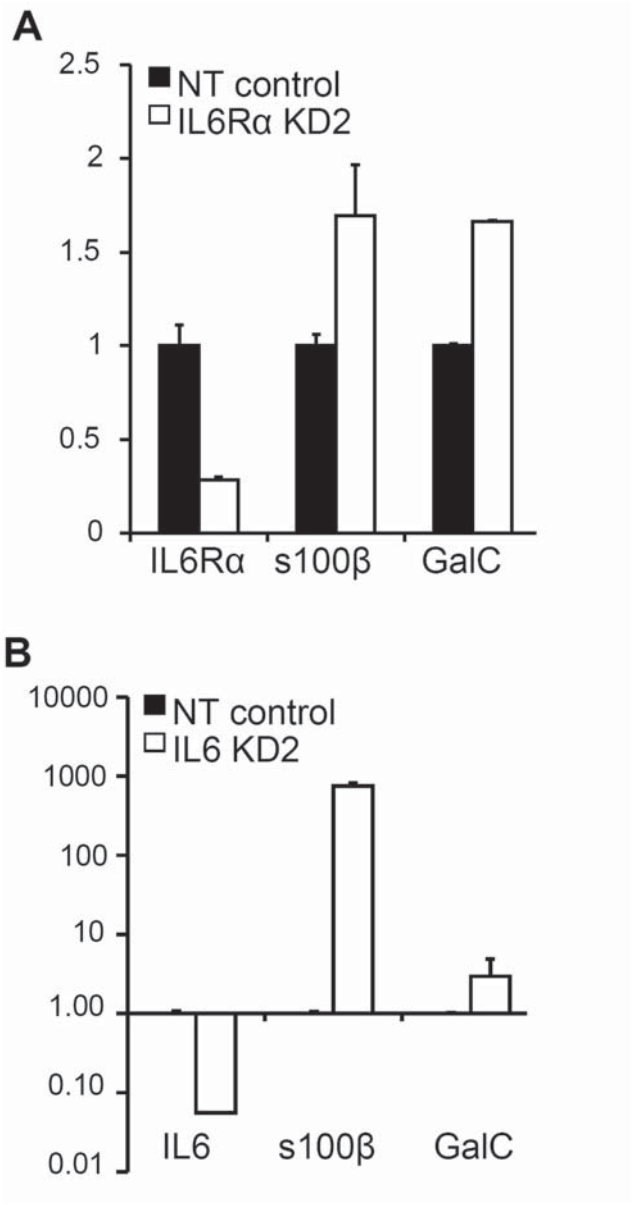

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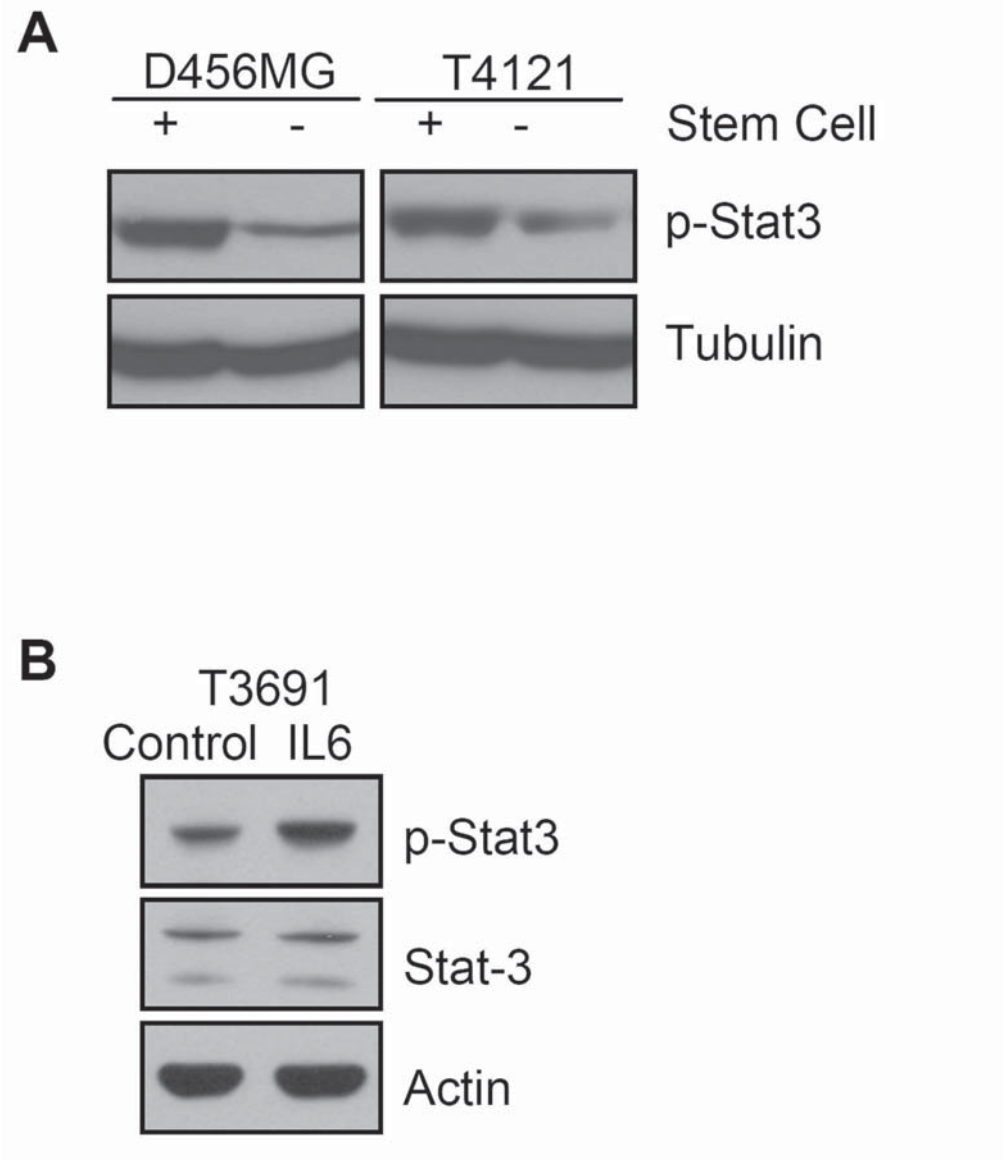

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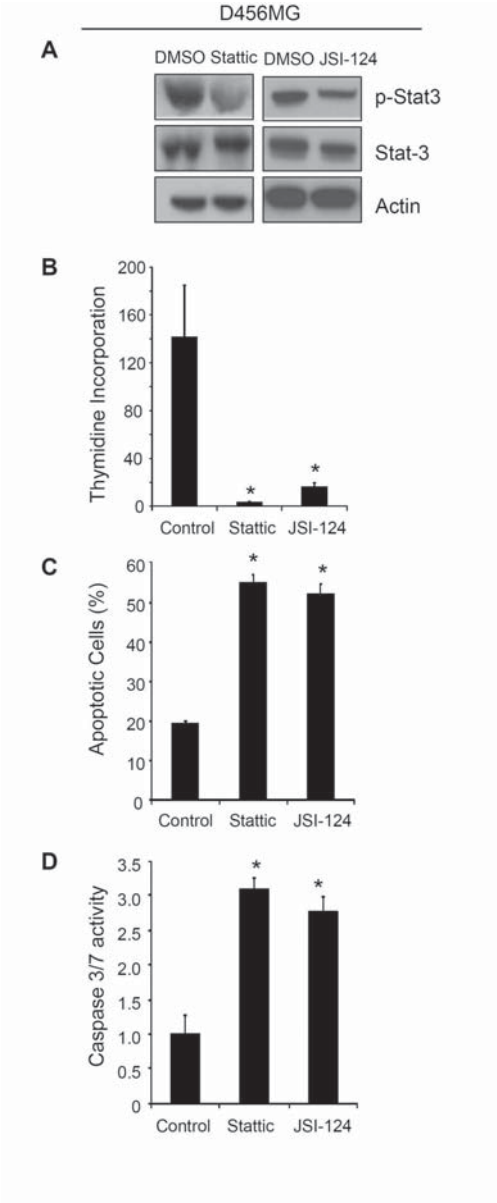

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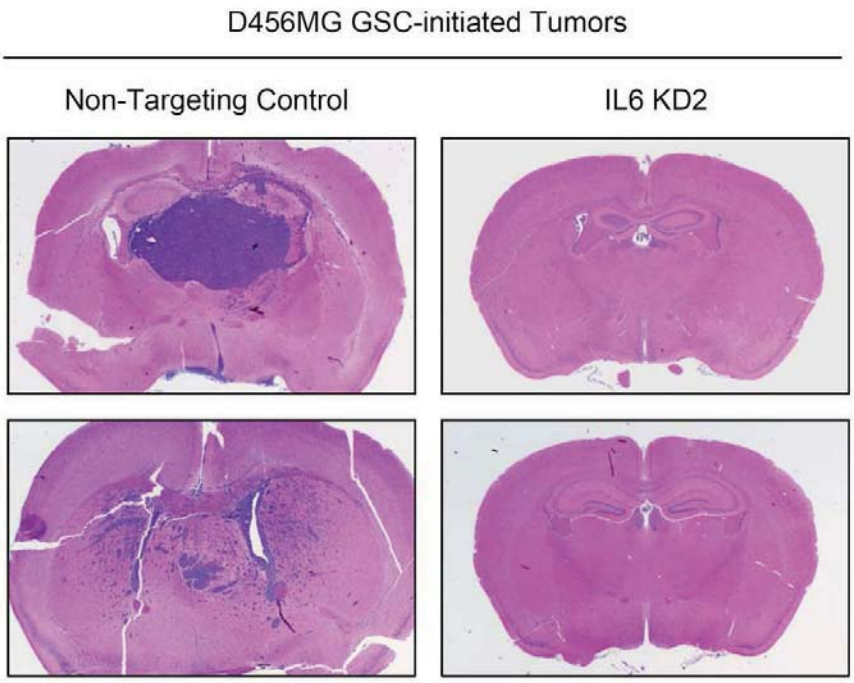

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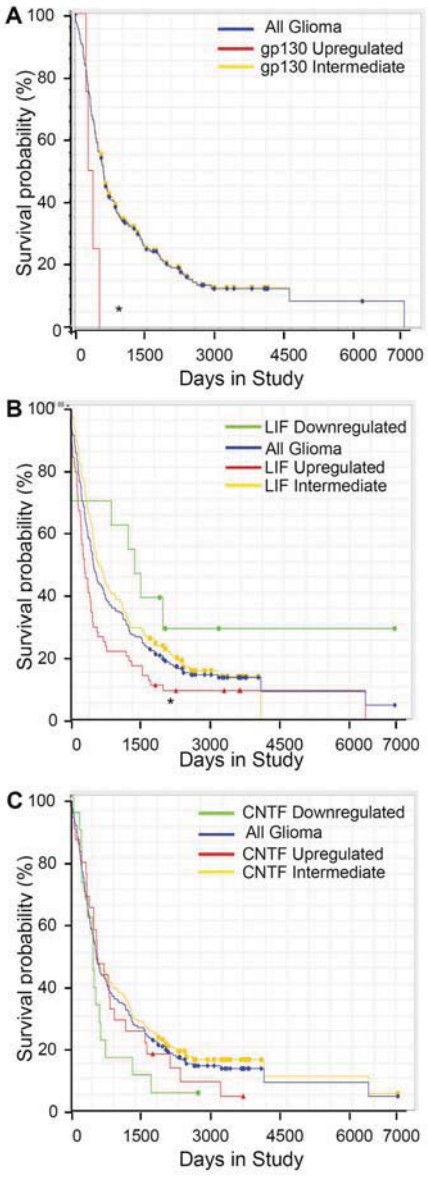

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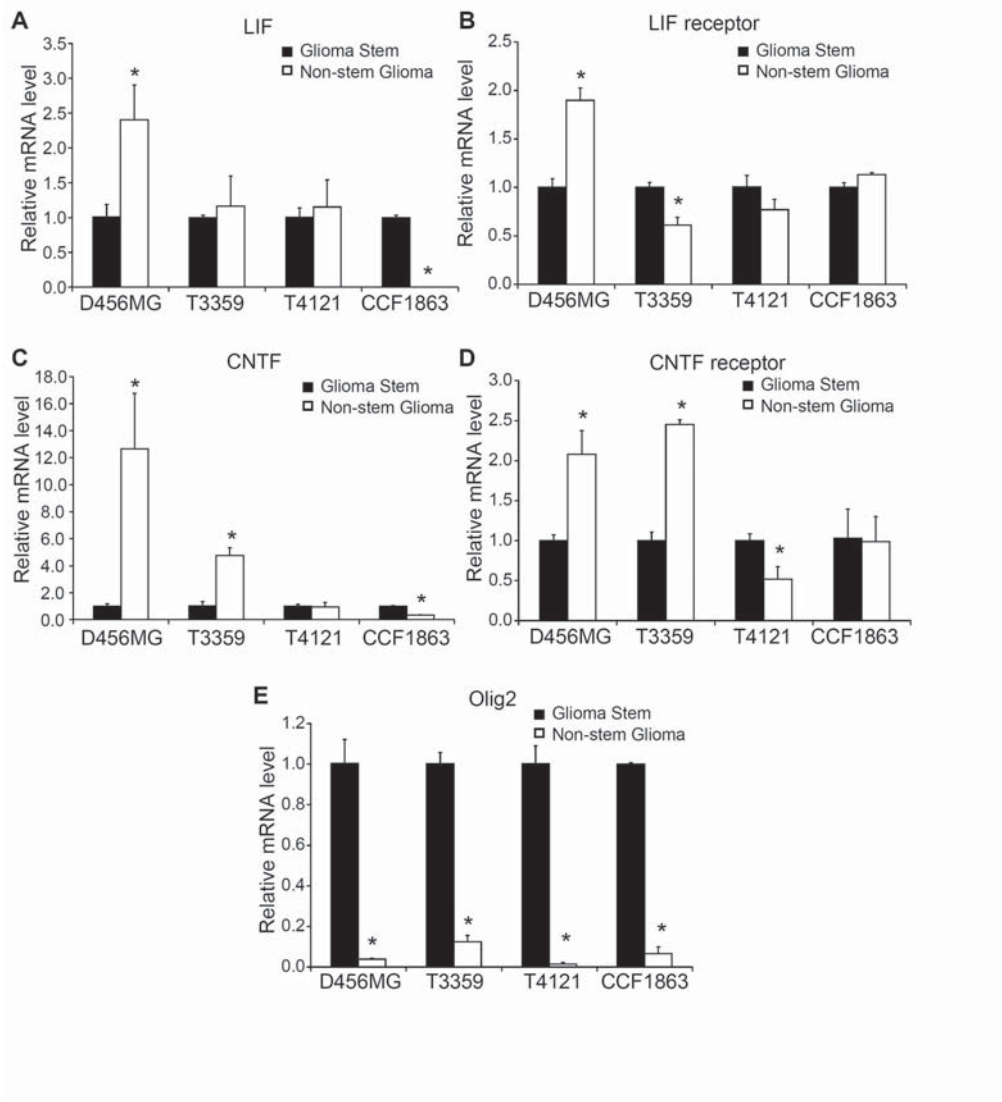

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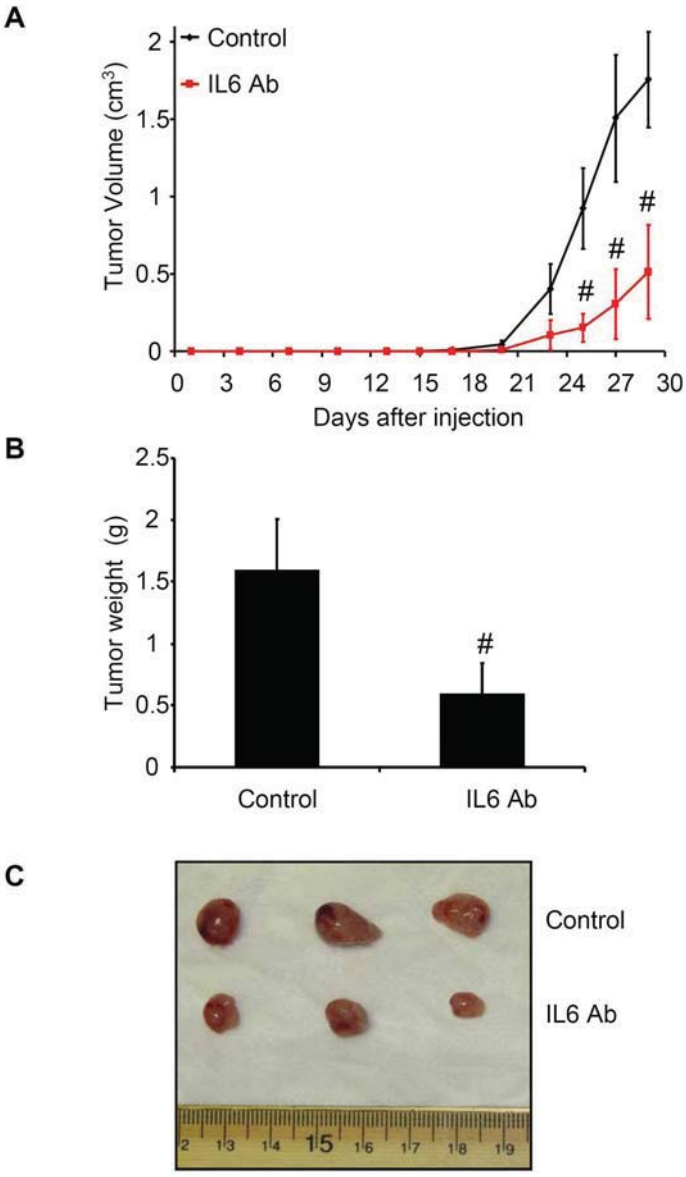

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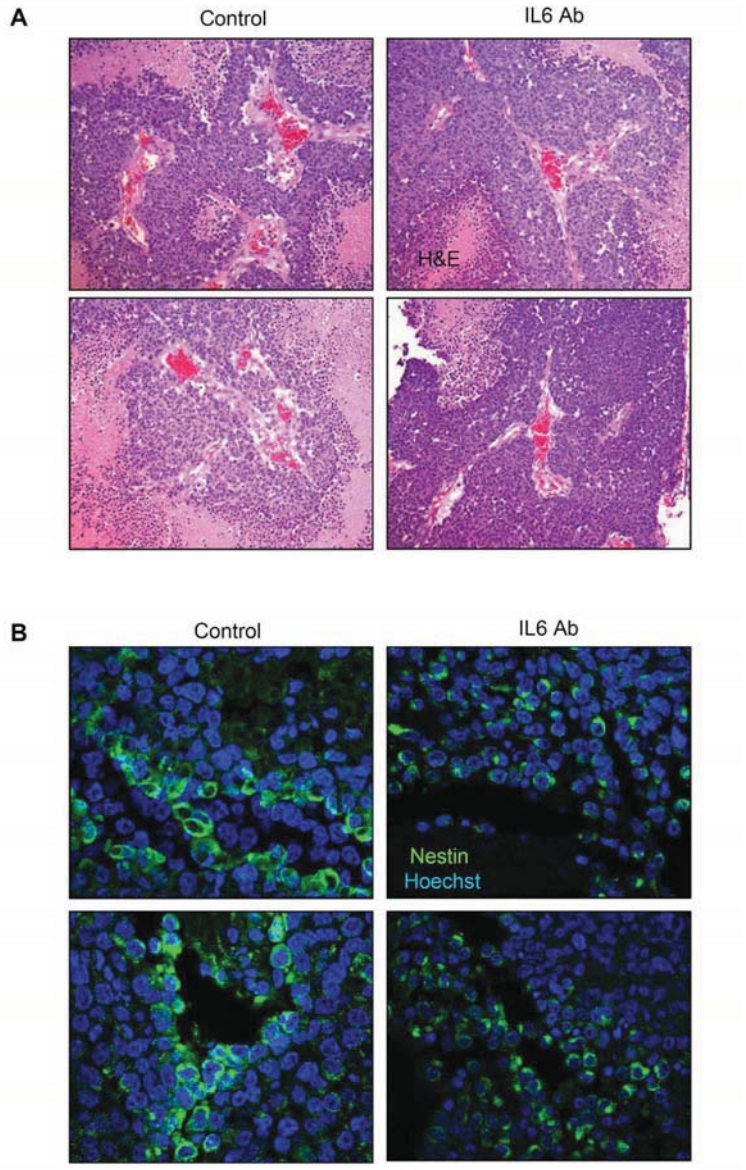

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Specimen	Age	Gender	Tumor State	Histopathology	FISH			
					Chromosome 7	EGFR	Chromosome 10	PTEN
T3359	31	M	New Diagnosis	GBM	Polysomy 62%	Polysomy 70%	Polysomy 80%	Polysomy 57%
T3691	59	F	New Diagnosis	GBM	Polysomy 77%	Polysomy 83%	Loss 54%	Loss 49%
T3832	75	F	New Diagnosis	GBM	Polysomy 82%	Polysomy 97%	Polysomy 70%	Polysomy 73%
T4105	75	M	New Diagnosis	GBM				
T4121	26	M	Recurrent	GBM	Polysomy 78%	Polysomy 79%	Intact	Loss 48%
CCF1863	69	M	New Diagnosis	Gliosarcoma	Aneusomic	Not Amplified		

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Name of shRNA constructs	TCR number	Clone ID	Sequence
IL6Rα KD1	TRCN0000058781	NM_000565.2-1800s1c1	CCGGAGCCCTTATGACATCAGCAATCTCGAGATTGCTGATGTCATAAGGGCTTTTTTG
IL6Rα KD2	TRCN0000058778	NM_000565.2-1430s1c1	CCGGGCAGGCACTTACTACTAATAACTCGAGTTATTAGTAGTAAGTGCCTGCTTTTTG
IL6 KD1	TRCN0000059205	NM_000600.1-636s1c1	CCGGCATCTCATTCTGCGCAGCTTTCTCGAGAAAGCTGCGCAGAATGAGATGTTTTTG
IL6 KD2	TRCN0000059207	NM_000600.1-211s1c1	CCGGCAGAACGAATTGACAAACAAACTCGAGTTTGTTTGTCAATTCGTTCTGTTTTTG

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