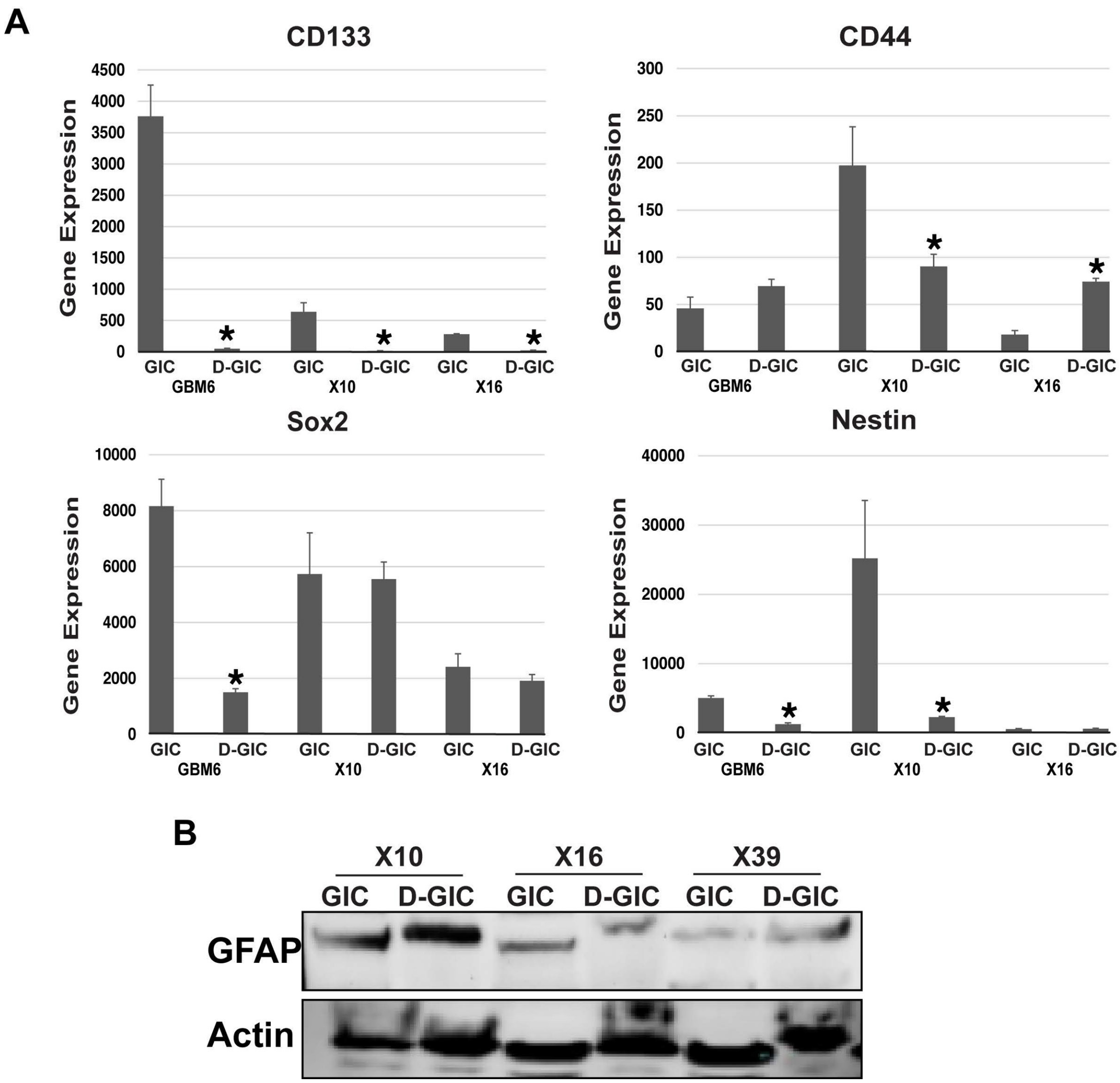
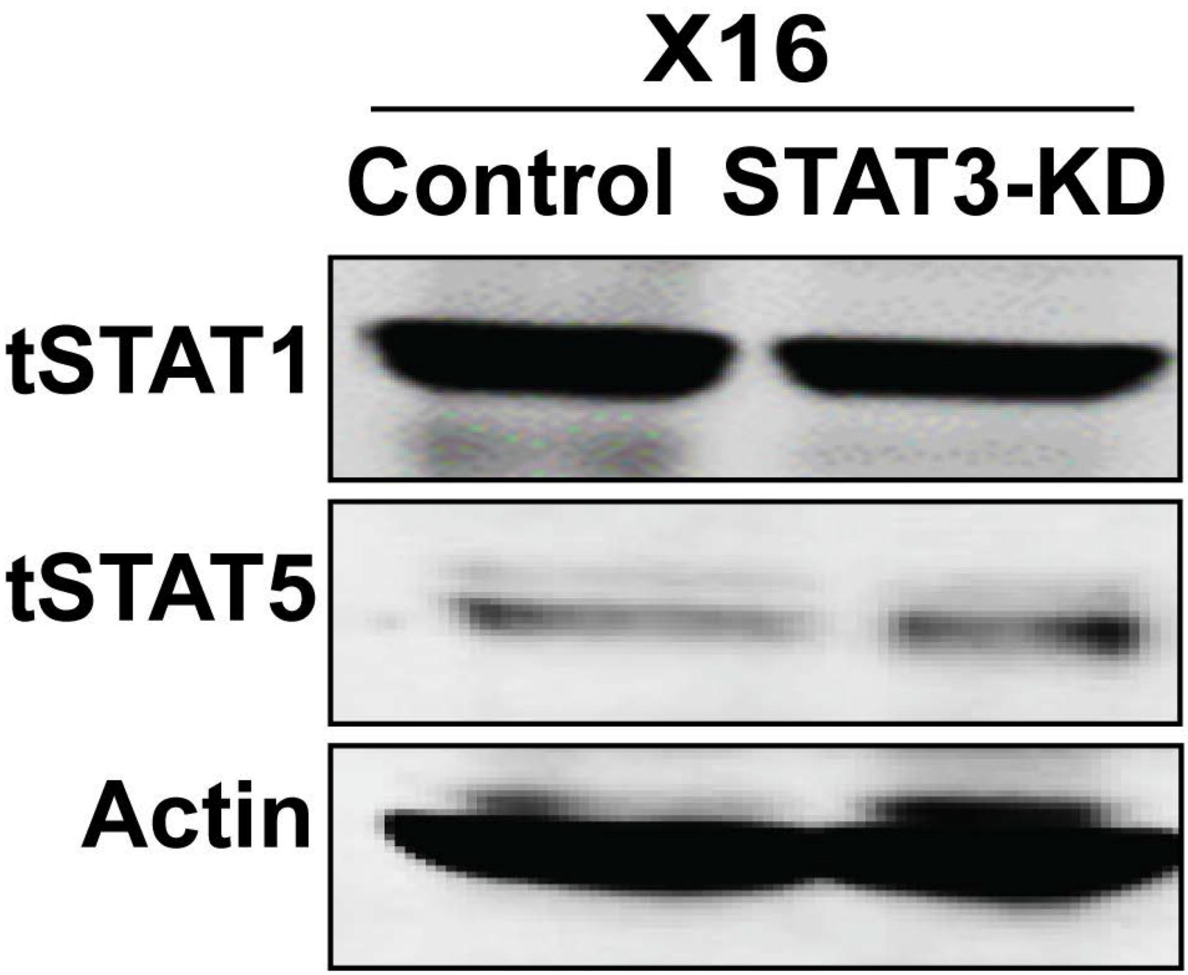
The critical role that STAT3 plays in glioma-initiating cells: STAT3 addiction in glioma

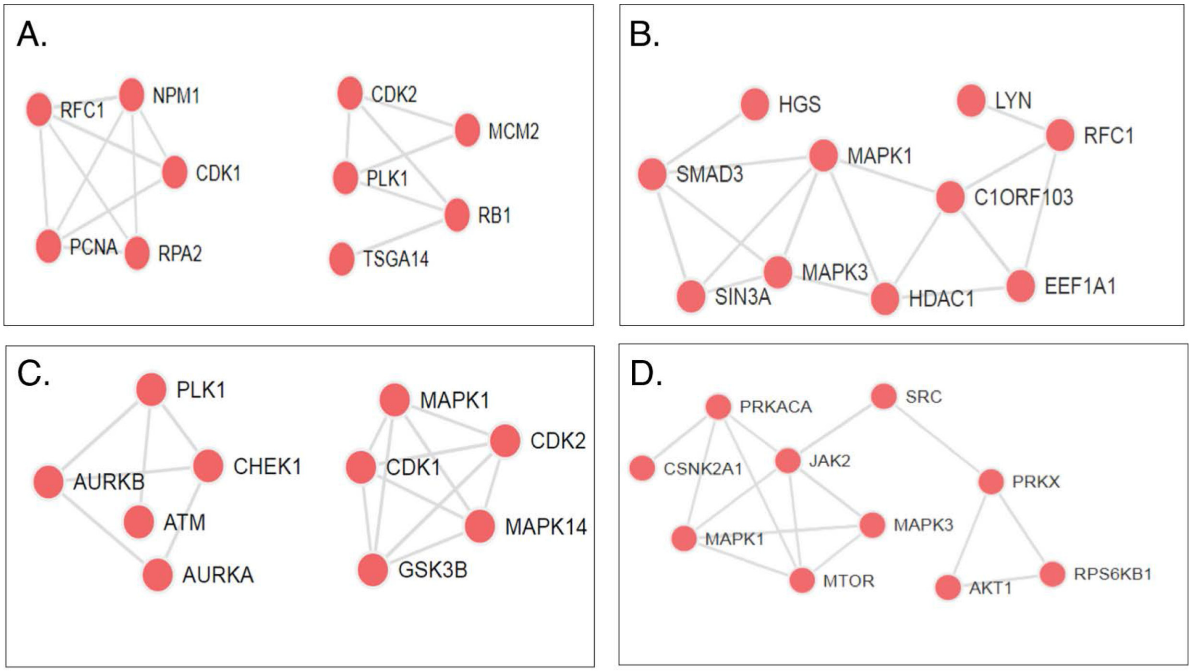
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Stem cell marker expression in the GICs and GICs induced to differentiate. GICs were grown under stem cell conditions or induced to differentiate for several passages in serum-containing medium (D-GICs). **(A)** qPCR was performed on RNA for *CD133*, *CD44*, *SOX2*, *NESTIN* and *Polr2A*, and gene expression normalized to Polr2A expression. **(B)** Protein lysates were immunoblotted for the astrocyte differentiation marker, GFAP, in the GBMX10, GBMX16 and GBX39 GICs and corresponding D-GICs.



Supplementary Figure 2: STAT3-KD has no effect on STAT1 and STAT5 protein expression in the X16 GICs. Immunoblots of protein lysates from iSTAT3-KD GBMX16 GICs treated with Dox using antibodies against STAT1 and STAT5.



Supplementary Figure 3: Protein-protein interaction and kinase substrate networks analysis of STAT3-regulated genes identified by RNA-seq. RNA-Seq analysis was performed on RNA prepared from GBMX16 GICs not treated with Dox, GBMX16 GICs treated with Dox (STAT3-KD GICs), and WT-STAT3 rescued STAT3-KD GICs. **(A)** Protein-protein interaction (PPI)-Hub proteins associated with STAT3 activated genes. **(B)** Kinase substrate networks associated with STAT3 activated genes. **(C)** PPI-Hub proteins associated with STAT3 suppressed genes. **(D)** Kinase substrate networks associated with STAT3 suppressed genes.

Supplementary Table 1: Sequences of primers used for qPCR

Target	Sequence (5'-3')
POLR2A	5'ATGTCTGTGACGGAGGGTGGCA 3'GGCCAGGACACTCTGTCATGTTT
STAT3	5'CAGCAGCTTGACACACGGTA 3'GCCCAATCTTGACTCTCAATCC
CD133	5'AGTCGGAAACTGGCAGATAGC 3'GGTAGTGTTGTACTGGGCCAAT
CD44	5'TTACAGCCTCAGCAGAGCAC 3'TGACCTAAGACGGAGGGAGG
SOX2	5'GCCGAGTGGAAACTTTTGTCG 3'GGCAGCGTGTACTTATCCTTCT
NESTIN	5'CTGCTACCCTTGAGACACCTG 3'GGGCTCTGATCTCTGCATCTAC
AMIGO	5'TTCCGTACCCCACCAGAACA 3'AGCTCTGGCCACCTCAAAAA
ARRB2	5'CCATGGGGGAGAAACCCG 3'GGTAGTCAGGGTCCACAAGC
ANG	5'TTTCCATTGTCCTGCCCGTT 3'TTGGCATCATAGTGCTGGGT
DRD2	5'GCTGCTGAAAACCATCTGGC 3'GGTCCTGGAAGGTGACTCGT
GATA2	5'ACTCATCAAGCCCAAGCGAA 3'CTTCATGGTCAGTGGCCTGT
CDS1	5'TGTTTGGATTCATTGCTGCCTAT 3'TGGATCTGGAAAGGGTACAAGC
CHD4	5'CCAATGCAGTCCTGCACAAA 3'CTCACAGCAACTGGGGGAAT
PBK	5'GCGGTGAGACTCTGGACTGA 3'CTGCATAAACGGAGAGGCCG
S100B	5'TGTAGACCCTAACCCGGAGG 3'TGCATGGATGAGGAACGCAT
THY1	5'GAAGACCCCAGTCCAGATCCA 3'TATTCTCATGGCGGCAGTCC
HMGB1	5'CAGAGCGGAGAGAGTGAGGA 3'TTGCCTCTCGGCTTCTTAGG
CDK1	5'CTTGGCTTCAAAGCTGGCTC 3'GGGTATGGTAGATCCCGGCT
HLA-DPB1	5'GTCGTGTCCACCAACCTGAT 3'ACTCCACGGTGACAGGACTA
CD200	5'CACCGTCTATGTACAGCCCA 3'CTGGTAACAGACGTGGTCCC
CCL2	5'ACCTGGACAAGCAAACCCAA 3'AGGGTGTCTGGGGAAAGCTA
BTG1	5'CGCAACACATGGACTACCCT 3'CAGTAGTCAGCACTGGGGTG
COL6A1	5'ATTGCCAAGGACTTCGTCGT 3'TCCACTGCAGGCTCTTGATG
PMP22	5'TGGAAGAAGGGGTTACGCTG 3'TCTGCCAGAGATCAGTTGCG
HIF1A F1	5'GAAAGCGCAAGTCTTCAAAG 3'TGGGTAGGAGATGGAGATGC