Supplementary Data

The neural stem-cell marker *CD24* is specifically upregulated in IDH-mutant glioma

Patricia D. B. Tiburcio[a,b,1], Mary C. Locke[c], Srividya Bhaskara[b,c], Mahesh B. Chandrasekharan[c], L. Eric Huang[a,b,*]

[a]Department of Neurosurgery, Clinical Neurosciences Center, University of Utah, Salt Lake City, Utah, USA

[b]Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah, USA

[c]Department of Radiation Oncology, Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah, USA

*Address all correspondence to: L. Eric Huang, Department of Neurosurgery, Clinical Neurosciences Center, 175 North Medical Drive East, Salt Lake City, UT 84132, USA.

E-mail: eric.huang@hsc.utah.edu

[1]Current address: Children's Medical Center Research Institute at UT Southwestern.

Supplementary Table 1. Primer sequences of reverse transcription–quantitative PCR

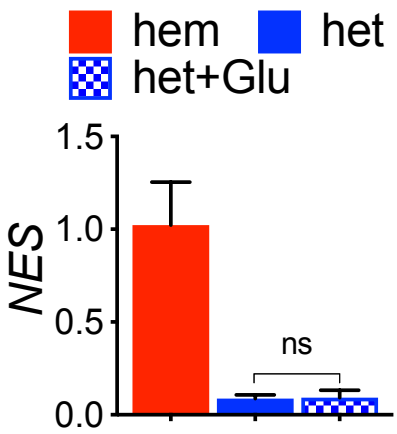
Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
CD24	ATGGGCAGAGCAATGGUG	GGAATAAATCTGCGTGGGTAGG
CD44	CGCCAAACACCCAAAGAA	GTGTTGTCCTTCCTTGCATT
NES	GAGAACTCCCGGCTGCAAA	TTGGGGTCCTGAAAGCTGAG
PROM1	AGATTTGGATGGCCTGGT	GTCGTGGTTTGGCGTTGT
RPL30	AGTCTTTCCTTTCTCGTTCCCC	GCCACCATCTTCCTGCCTTAG
UBC	GGTCGCAGTTCTTGTTTGTGG	ACCAGTCAGAGTCTTCACGAA
YWHAZ	CATCTTGGAGGGTCGTCT	GCTCCGTCTCAATTTTCTCTCT

Supplementary Table 2. Primer sequences of bisulfite DNA sequencing

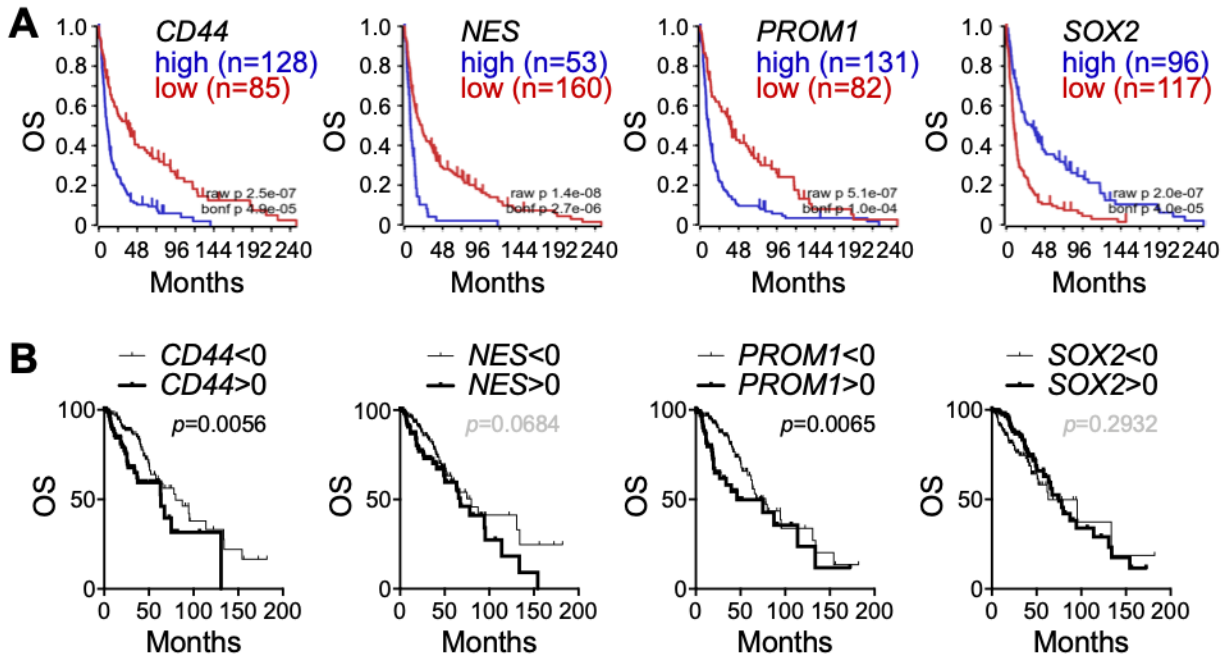
Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
CD24	GTTAGGGTTTTTTAGGTTTAGTTTT	AAAATCCCCATATTATTTTAACCCA
NES	GTATTTTGGGGAAGTAGGAATAGAG	TCTAACCCACTAAAAATAAACAAAC

Supplementary Table 3. Primer sequences of chromatin immunoprecipitation–quantitative PCR

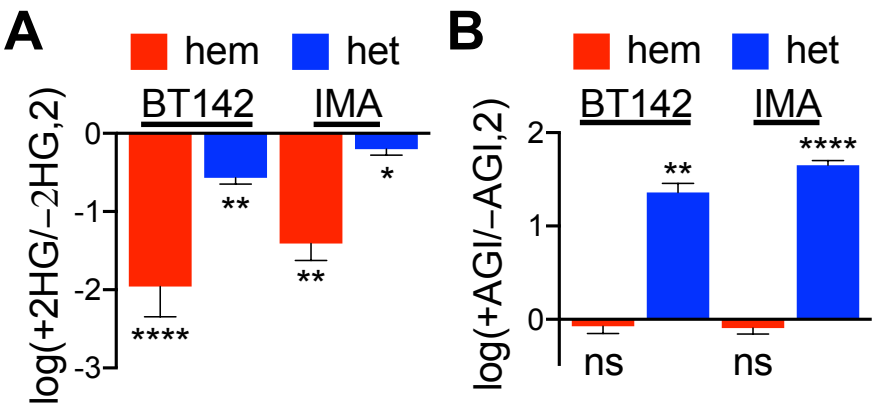
Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
CD24	GGACCGGGAGAGAATCTTG	AGGGAATGGAAAAATGGGG
NES	CGTTGGAACAGAGGTTGGA	ACTTTTCAGTAGCCCGCA



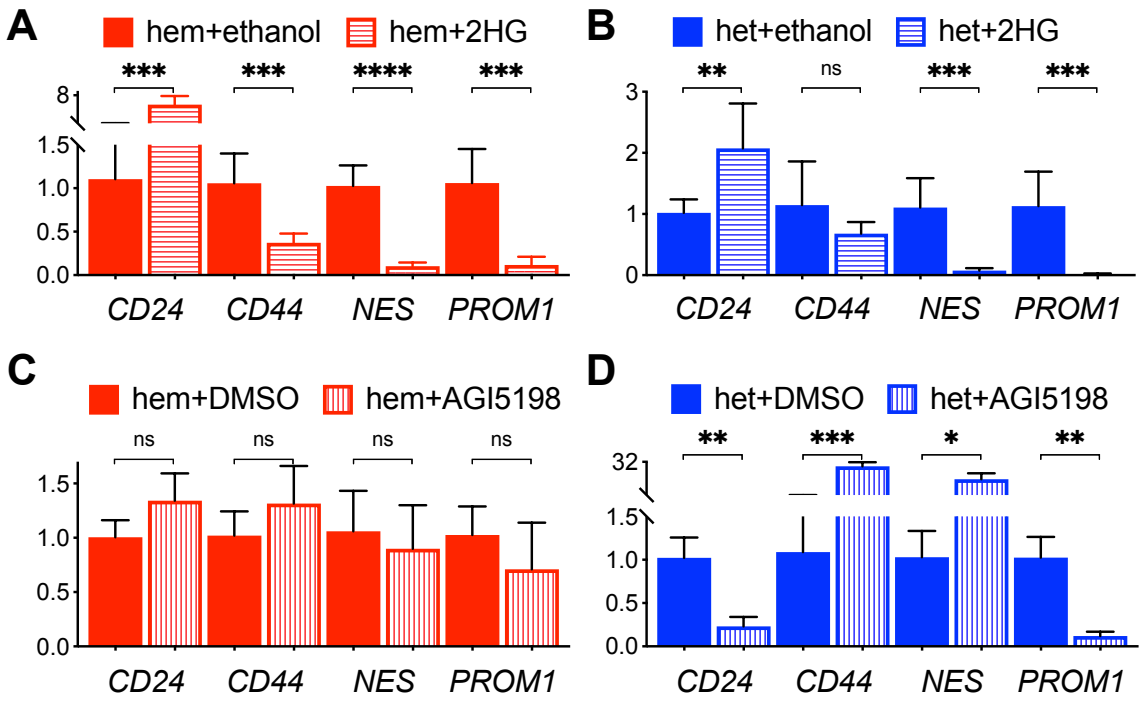
Supplementary Figure 1. Effect of glutamate on *NES* expression. The addition of glutamate to *IDH1*^{R132H}-heterozygous BT142 spheroid growth (het+Glu) showing no significant effects on *NES* expression at the mRNA level, as assayed by quantitative PCR (n=4). ns, not significant.



Supplementary Figure 2. Associations of glioma stem-cell marker genes with overall survival. **A,** Kaplan–Meier survival analysis of the GSE16011 data set showing negative associations with *CD44*, *NES*, and *PROM1* expression. Bonferroni-corrected *p*=4.9e-05 (*CD44*), 2.7e-06 (*NES*), and 1.0e-04 (*PROM1*). **B,** Log-rank tests of the TCGA-LGG data set confirming the negative associations with *CD44* and *PROM1*.



Supplementary Figure 3. D-2HG inhibits spheroid growth of glioma cells. **A**, octyl-(R)-2HG treatment resulting in marked decreases of *IDH1*^{R132H}-hemizygous (hem) spheroid growth in BT142 and IMA cells (n=3). Fold changes are expressed in log2 of treated (+2HG) versus untreated (−2HG). **B**, AGI-5198 treatment resulting in striking increases of *IDH1*^{R132H}-heterozygous (het) spheroid growth (n=3). Fold changes are expressed in log2 of treated (+AGI) versus untreated (−AGI). ns, not significant; *p<0.05; **p<0.01; and ****p<0.0001.



Supplementary Figure 4. Differential regulation of *CD24* and *NES* by D-2HG in IMA spheroid growth. **A** and **B**, Octyl-(R)-2HG treatment (+2HG) stimulating *CD24* expression in *IDH1*^{R132H}-hemizygous spheroids (***A***) but inhibiting *NES* expression in both *IDH1*^{R132H}-hemizygous and *IDH1*^{R132H}-heterozygous spheroids (***B***) in reference to vehicle treatment (+ethanol). **C** and **D**, In contrast to modest effects in *IDH1*^{R132H}-hemizygous spheroids (***C***), AGI-5198 treatment stimulating *NES* expression but inhibiting *CD24* expression in *IDH1*^{R132H}-heterozygous spheroids (***D***). Gene expression was assayed with quantitative PCR (n=4). ns, not significant; **p*<0.05; ***p*<0.01; ****p*<0.001; and *****p*<0.0001.