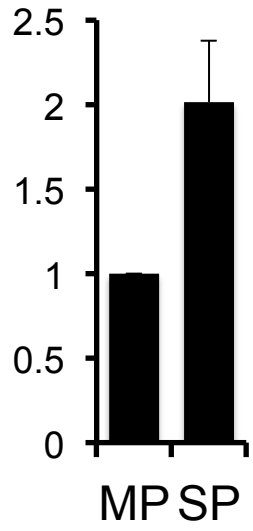
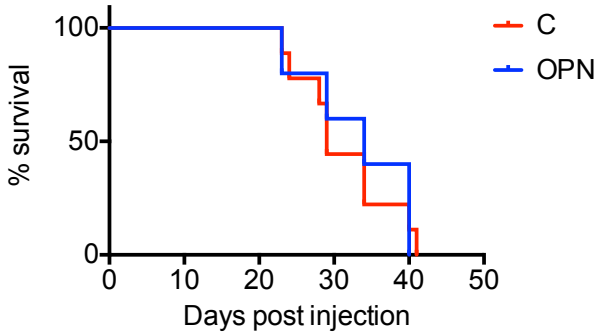
**Figure S1**

**A**


2.5
2
1.5
1
0.5
0
MP SP

**B**


100
50
0
% survival
0
10
20
30
40
50
Days post injection
C
OPN

## Figure S2

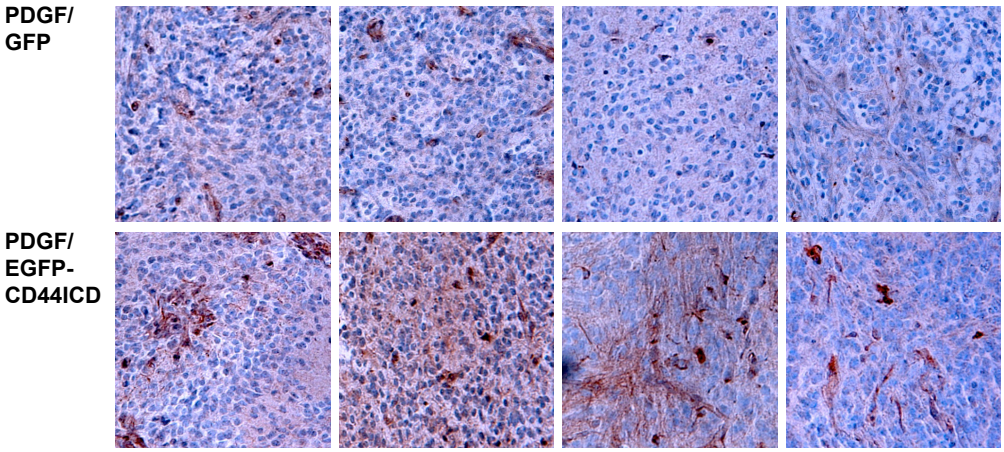


**Figure S3**

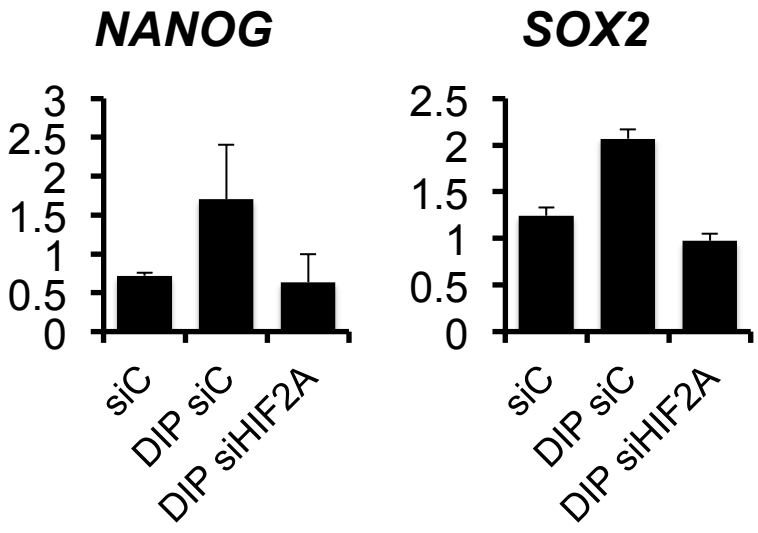

NANOG
SOX2
3
2.5
2
1.5
1
0.5
0
2.5
2
1.5
1
0.5
0
siC
DIP siC
DIP siHIF2A
siC
DIP siC
DIP siHIF2A

**Figure S4**

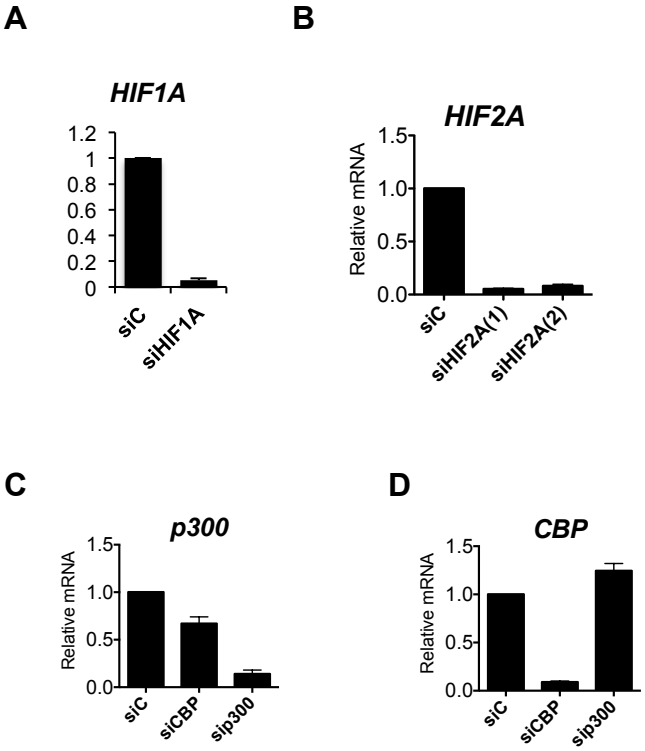

A
HIF1A
1.2
1
0.8
0.6
0.4
0.2
0
siC
siHIF1A
B
HIF2A
Relative mRNA
1.5
1.0
0.5
0.0
siC
siHIF2A(1)
siHIF2A(2)
C
p300
Relative mRNA
1.5
1.0
0.5
0.0
siC
siCBP
sip300
D
CBP
Relative mRNA
1.5
1.0
0.5
0.0
siC
siCBP
sip300

**Figure S5**

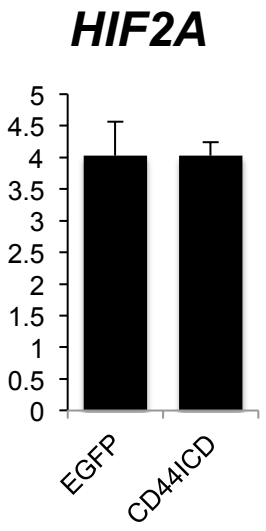


**Figure S6**

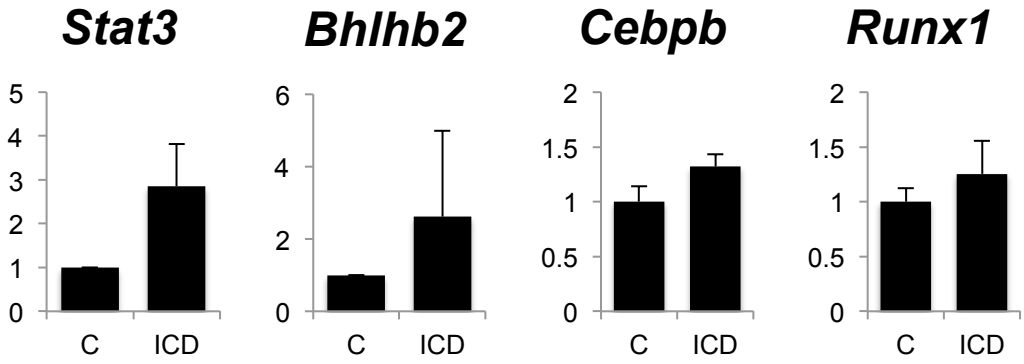
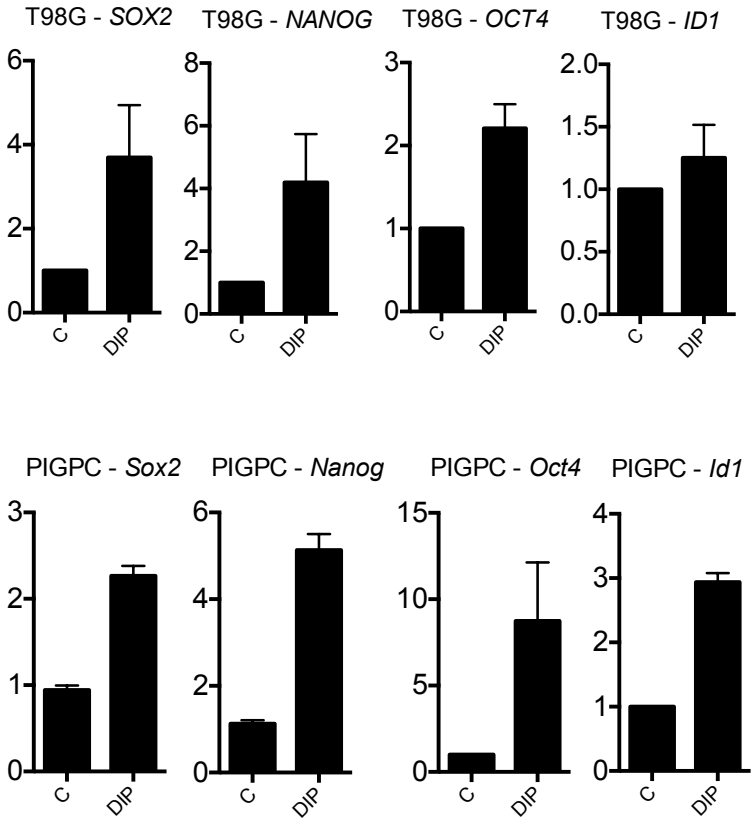

Stat3
5
4
3
2
1
0
C
ICD
Bhlhb2
6
4
2
0
C
ICD
Cebpb
2
1.5
1
0.5
0
C
ICD
Runx1
2
1.5
1
0.5
0
C
ICD

**Figure S7**


T98G - SOX2
T98G - NANOG
T98G - OCT4
T98G - ID1
PIGPC - Sox2
PIGPC - Nanog
PIGPC - Oct4
PIGPC - Id1
C
DIP

## Supplemental Figure Legends

Figure S1. A. *Cd44* mRNA levels as measured by qPCR in sorted MP and SP PIGPCs as indicated. Error bars represent SEM. B. Kaplan-Meier plots showing survival of mice injected with PIGPCs pre-treated or not with OPN as indicated. Related to Fig. 2.

Figure S2. Examples of CD44 c-terminal IHC stains from PDGF/GFP and PDGF/CD44ICD tumors as indicated. Related to Fig. 3.

Figure S3. *NANOG* and *SOX2* mRNA levels as measured by qPCR in U251 cells treated as indicated with or without DIP and transfected with control siRNA or siRNA targeting *HIF2A*. Error bars represent SEM. Related to Fig. 5.

Figure S4. qPCR validation of siRNA knockdown efficiencies. Error bars represent SEM. Related to Fig. 5.

Figure S5. *HIF2A* mRNA levels as measured by qPCR in U251 cells expressing EGFP or CD44ICD as indicated. Error bars represent SEM. Related to Fig. 6.

Figure S6. Relative mRNA levels of mesenchymal GBM subtype markers as measured by qPCR in PIGPCs expressing EGFP or CD44ICD as indicated. Error bars represent SEM. Related to Fig. 7.

Figure S7. Relative mRNA levels of indicated stem cell marker genes in T98G cells and PIGPCs as indicated, treated or not with DIP to stabilize HIFs, as measured by qPCR. Error bars represent SEM. Related to Fig. 2.

## Supplemental Experimental Procedures

### *Patient material and histology*

Paraffin-embedded human glioma specimens from the proneural, mesenchymal and classical subtype were obtained from the MSKCC brain tumor bank. Tissue was routinely fixed in 10% formalin, embedded in paraffin and stained for H/E. For DAB staining, a Ventana DAB-MAP system was used with the following antibodies: CD44 C-terminal (Abcam ab65829), CD44 (BD Biosciences), Osteopontin (Abcam ab8448).

### *Cell culture and reagents*

PIGPCs were isolated from murine brains using mechanical dissociation and/or papain (Worthington), then cultured in DMEM (ATCC) containing 10% FBS, 1% PenStrep. Recombinant mouse and human OPN were obtained from R&D systems, doxycycline from Clontech, 2,2-dipyridyl from Sigma-Aldrich, MK-003 was a kind gift from Merck, and IM7 anti-CD44 and IgG blocking antibodies were obtained from BD. Cells were transfected using Fugene 6 (Roche) for plasmids and HiPerFect (Qiagen) for siRNA according to manufacturers' recommendations. ON-TARGETplus siRNAs targeting *HIF2A* or non-targeting control were obtained from Thermo Scientific and transfected at a final concentration of 20 nM.

### *Western blot*

Cells were lysed in RIPA buffer, and lysates were run on Novex NuPage Bis-Tris precast gels (Invitrogen). The following antibodies were used: Myc-tag (Sigma 9E10), CD44 (Cell Signaling 8E2), HIF-2α (Abcam ab199), Actin (Santa Cruz).

### *Primers sequences for qPCR.*

| Gene | Forward primer | Reverse primer |
|---|---|---|
| *CBP* (human) | 5'-CAACCCCAAAAGAGCCAAACT-3' | 5'-CCTCGTAGAAGCTCCGACAGT-3' |
| *ID1* (human) | Purchased from Qiagen (QT00230650) | Purchased from Qiagen (QT00230650) |
| *Id1* (mouse) | Purchased from Qiagen (QT01743756) | Purchased from Qiagen (QT01743756) |
| *HIF1A* (human) | 5'-CTGCCACCACTGATGAATTA-3' | 5'-GTATGTGGGTAGGAGATGGA-3' |
| *HIF2A* (human) | 5'-GTGCTCCCACGGCCTGTA-3' | 5'-TTGTCACACCTATGGCATATCACA-3' |
| *NANOG* (human) | 5'-TCTGGACACTGGCTGAATCCT-3' | 5'-CGCTGATTAGGCTCCAACCAT-3' |
| *Nanog* (mouse) | 5'-CCTCAGCCTCCAGCAGATGC-3' | 5'-CCGCTTGCACTTCACCCTTTG-3' |
| *OCT4* (human) | 5'-GCTCGAGAAGGATGTGGTCC-3' | 5'-CGTTGTGCATAGTCGCTGCT-3' |
| *Oct4* (mouse) | 5'-GTTGGAGAAGGTGGAACCAA-3' | 5'-CTCCTTCTGCAGGGCTTTC-3' |
| *P300* (human) | 5'-AGCCAAGCGGCCTAAACTC-3' | 5'-TCACCACCATTGGTTAGTCCC-3' |
| *SDHA* (human) | 5'-TGGGAACAAGAGGGCATCTG-3' | 5'-CCACCACTGCATCAAATTCATG-3' |
| *Sdha* (mouse) | 5'-CTTGAATGAGGCTGACTGTG-3' | 5'-ATCACATAAGCTGGTCCTGT-3' |
| *SOX2* (human) | 5'-GCCTGGGCGCCGAGTGGA-3' | 5'-GGGCGAGCCGTTCATGTAGGTCTG-3' |
| *Sox2* (mouse) | 5'-TCCAAAAACTAATCACAACAATCG-3' | 5'-GAAGTGCAATTGGGATGAAAA-3' |
| *UBC* (human) | 5'-ATTTGGGTCGCGGTTCTTG-3' | 5'-TGCCTTGACATTCTCGATGGT-3' |
| *Ubc* (mouse) | 5'-AGCCCAGTGTTACCACCAAG-3' | 5'-ACCCAAGAACAAGCACAAGG-3' |
| *VEGF* (human) | 5'-AGGAGGAGGGCAGAATCATCA-3' | 5'-CTCGATTGGATGGCAGTAGCT-3' |