Supporting Information

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SI Materials and Methods

Neurosphere Formation by Glioblastoma (GBM) Center and Periphery Tissue Specimens. Paired GBM biopsy samples (center and periphery) were received from the operating room 30–60 min after resection and minced under sterile conditions. Cell aliquots were plated (10^4 cells/cm^2) into nonadherent 6-well culture dishes (Corning) in PROL media supplemented with 20 ng/mL epidermal growth factor/basic fibroblast growth factor (EGF/bFGF) (R&D Systems) and 10 ng/mL EGF/bFGF was added every other day thereafter. Neurosphere forming capacity of cells derived from periphery and center biopsies were compared beginning at culture passage 5. Briefly, a single-cell suspension of 10^4 cells/cm^2 was diluted in 1% methylcellulose-containing PROL media supplemented with 20 ng/mL EGF/bFGF, and inoculated into nonadhesive culture dishes. A total of 10 ng/mL EGF/bFGF was added every other day thereafter. At 21 ± 4 d in culture, neurospheres were quantified and single-cell suspensions were replated under identical conditions for analysis of secondary or higher degree neurosphere formation.

Primers Used.

CD133:
Forward, 5′-ctggggctgctgtttattattctg-3′
Reverse, 5′-acgccttgtccttggtagtgttg-3′

Nestin:
Forward, 5′-aggatgtggaggtagtgaga-3′
Reverse, 5′-ggagatctcagtggctctt-3′

Musashi-1:
Forward, 5′-gagactgacgcgccccagcc-3′
Reverse, 5′-cgcctggtccatgaaagtgacg-3′

Sox2:
Forward, 5′-accggcggcaaccagaagaacag-3′
Reverse, 5′-gcgccgcggccggtatttat-3′

Nanog:
Forward, 5′-ctaagaggtggcagaaaaaca-3′
Reverse, 5′-ctggtggtaggaagagtaaagg-3′

Oct4:
Forward, 5′-actgcagcagatgacggagatcg-3′
Reverse, 5′-atcctctcgttgtgcatagtcgc-3′

c-Myc:
Forward, 5′-tcaagaggcgaacacacaac-3′
Reverse, 5′-ggccttttcattgttttcca-3′

Klf4:
Forward, 5′-ccccgtgtgtttacggtagt-3′
Reverse, 5′-gagttcccatctcaaggcac-3′

Tuj1:
Forward, 5′-caacagcacggccatccagg-3′
Reverse, 5′-cttggggccctgggcctccga-3′

GFAP:
Forward, 5′-ggcaaaagcaccaaagacgg-3′
Reverse, 5′-ggcggcgttccatttacaat-3′

c-Met:
Forward, 5′-tgggaatctgcctgcgaa-3′
Reverse, 5′-ccagaggacgacgccaaa-3′

18S:
Forward, 5′-acaggattgacagattgatagctc-3′
Reverse, 5′-caaatcgctccaccaactaagaa-3′

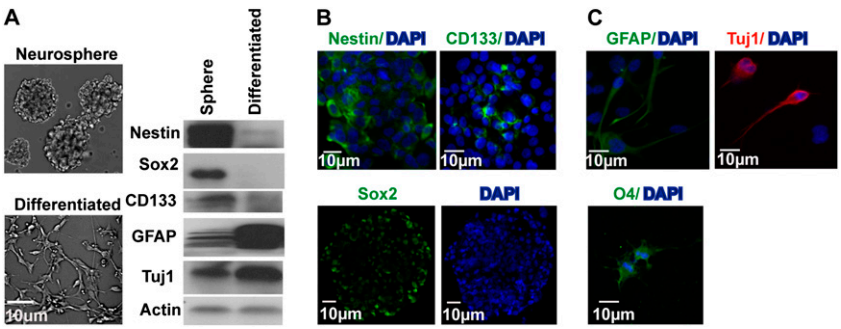

Fig. S1. Characteristics of primary neurospheres derived from Mayo39 human glioblastoma xenografts. (*A*) Cells dissociated from xenografts form nonadherent neurospheres when cultured in serum-free neurosphere medium containing EGF/FGF (*Upper Left*). Sphere-derived cells become adherent and process bearing when cultured under forced differentiation conditions (1% serum, Matrigel) (*Lower Left*). Immunoblot shows decreased expression of stem/progenitor markers (Sox2, Nestin, and CD133) and increased expression of lineage-specific markers (GFAP and Tuj1) following forced differentiation (*Right*). (*B*) Immunofluorescence showing stem/progenitor marker expression by neurospheres. (*C*) Immunofluorescence showing lineage-specific marker expression following the forced differentiation of neurosphere cells.

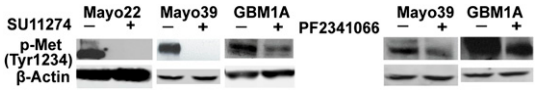

Fig. S2. c-Met inhibitor SU11274 and PF2341066 inhibited c-Met phosphorylation in GBM neurosphere cells. Mayo22, Mayo39, and GBM1A neurosphere cells were treated with 500 nM SU11274 or 300 nM PF2341066 for 1 h; c-Met phosphorylation was examined by immunoblotting.

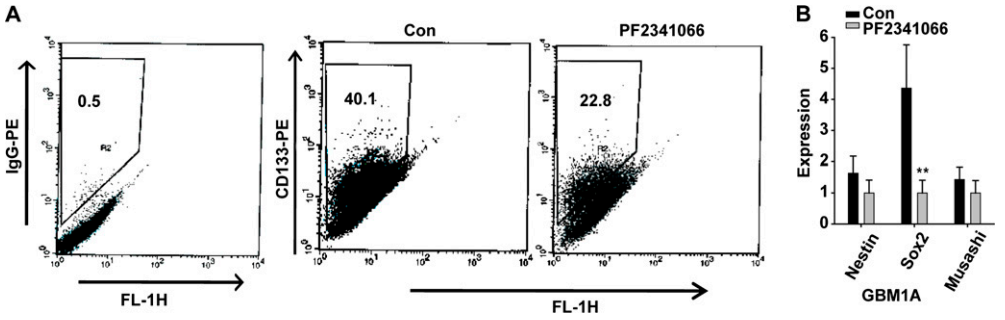

Fig. S3. c-Met inhibitor PF2341066 significantly inhibited expression of stem cell markers CD133 and Sox2. GBM1A cells were treated with PF2341066 for 7 d. (*A*) The cells were labeled with phycoerythrin (PE)-conjugated anti-CD133 antibody according to manufacturer's instructions. The cells were analyzed by flow cytometry. (*B*) Whole cell RNA was isolated from control and PF2341066-treated cells and subjected to quantitative RT-PCR for Sox2, Nestin, and Musashi. $*P < 0.05$, $**P < 0.01$.

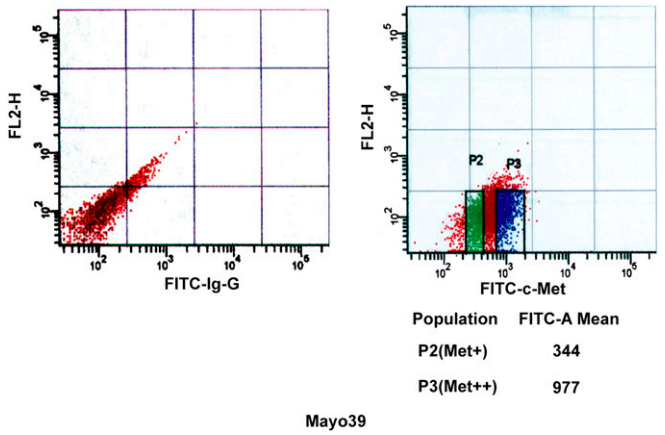

Fig. S4. Separation of low c-Met–expressing (Met^+) and high c-Met–expressing (Met^{++}) GBM-derived neurosphere cells. Primary Mayo39 neurospheres were dissociated, labeled with control IgG FITC (*Left*) or with anti–c-Met FITC (*Right*) and then sorted using the FACS Vantage SE flow cytometer (BD). (*Right*) Windows used to separate c-Met^+ cells (P2) from c-Met^{++} cells (P3).

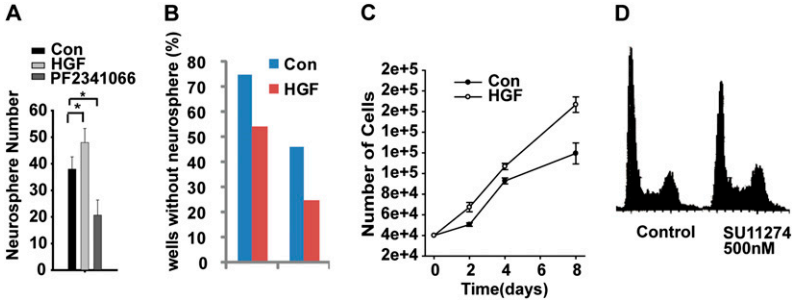

Fig. S5. Effects of c-Met activity on neurosphere formation and neurosphere cell proliferation. (*A*) Dissociated viable GBM1A cells (3.5×10^4 cells per well in six-well plates) were cultured overnight in neurosphere medium lacking EGF/FGF before treatment ± hepatocyte growth factor (HGF) or c-Met inhibitor PF2341066 for 7 d. Neurospheres were fixed in neurosphere medium with 1% agarose. Neurospheres >100 μm diameter were counted by computer-assisted image analysis. (*B*) Mayo39 neurospheres were dissociated and subjected to forced differentiation (1% serum, Matrigel) for 7 d and transferred to serum-free neurosphere medium ± HGF for an additional 7 d. Cells were then cultured for 14 d in neurosphere medium containing EGF/FGF. Limited dilution assay results are similar to those obtained with GBM1A as shown in Fig. 3*B*. (*C*) Growth curve of GBM1A neurosphere cells cultured in neurosphere medium ± HGF for 8 d. (*D*) GBM1A neurosphere cells were cultured in neurosphere medium containing EGF/FGF ± the c-Met inhibitor SU11274 for 24 h, labeled with propidium iodide, and subjected to cell cycle analysis by flow cytometry. $*P < 0.05$, $**P < 0.01$.

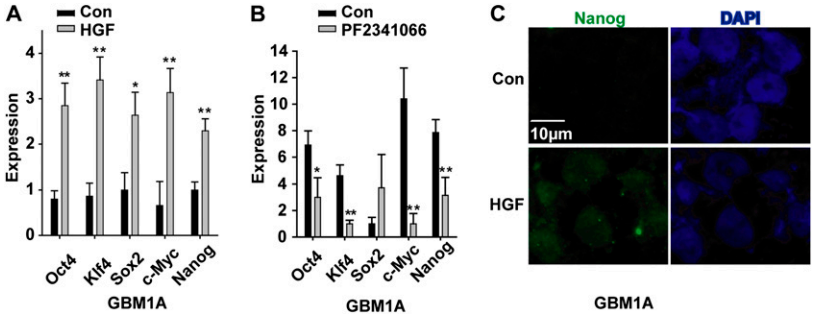

Fig. S6. HGF induces the expression of reprogramming transcription factors in GBM-derived neurospheres. (*A*) GBM1A neurosphere cells were treated ± HGF for 7 h. Expression levels of reprogramming factors normalized to 18S RNA were then quantified by qRT-PCR. Results are similar to those using primary Mayo39 neurosphere cells as shown in Fig. 4*A*. (*B*) GBM1A neurospheres were cultured in neurosphere medium and treated ± PF2341066 for 1 h. Basal reprogramming factor expression is inhibited by PF2341066. (*C*) GBM1A neurospheres were treated ± HGF as in *A*. Immunofluorescence staining of neurosphere cytospin shows that HGF induces nuclear Nanog. $*P < 0.05$, $**P < 0.01$.

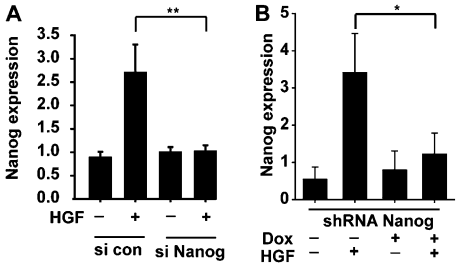

Fig. S7. Inhibition of Nanog induction by siRNA and doxycycline-inducible shRNA. (*A*) Dissociated GBM1A neurosphere cells were treated with either control or Nanog siRNA followed by treatment ± HGF for 7 h. Total RNA was isolated and Nanog expression relative to 18S RNA quantified by qRT-PCR. (*B*) GBM1A neurosphere cells transfected with Dox-shRNA-Nanog were treated ± doxycycline for 48 h followed by treatment ± HGF for 7 h. Total RNA was isolated and Nanog expression relative to 18S RNA quantified by qRT-PCR. Both siRNA and shRNA potently inhibited Nanog induction by HGF. $*P < 0.05$, $**P < 0.01$.

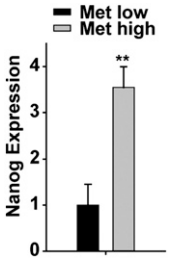

Fig. S8. Neurosphere cells expressing high c-Met show higher levels of Nanog relative to cells expressing low c-Met. Primary Mayo39 neurosphere cells expressing high and low levels of c-Met (Met^{++}, Met^{+}, respectively) were separated by flow cytometry as in Fig. S4. Quantitative RT-PCR analysis shows that c-Met^{++} cells are enriched for Nanog expression. $*P < 0.05$, $**P < 0.01$.