Supplementary Methods

Mice lines

C57BL/6, Rosa Cre-ER^{T2} mice, *PDGFRα* $^{loxP/loxP}$ mice, NG2-DsRed mice, and *UBC-GFP* mice were obtained from Jackson Laboratories. Rosa Cre-ER^{T2} mice, *PDGFRα* $^{loxP/loxP}$ mice, NG2-DsRed mice, and *UBC-GFP* mice were originally in C57BL/6 background, and PDGF-C deficient mice were backcrossed to C57BL/6 background for 8 generations. Littermate controls were used in all animal experiments. RosaCre-ER^{T2} mice were crossed with *PDGFRa* $^{loxP/loxP}$ mice to generate RosaCre ER^{T2} x*PDGFRa* $^{fl/fl}$ mice. The littermate RosaCre-ER^{T2} x *PDGFRa* $^{+/+}$ served as controls. All animal experiments were performed under the guidelines set by the Institutional Animal Care and Use Committee.

Genotyping

For genotyping methods, primer sequences, and PCR conditions, see below.

Rosa Cre-ER^{T2} :

Wild type forward, AAA GTC GCT CTG AGT TGT TAT

Wild type reverse, GGA GCG GGA GAA ATG GAT ATG

Mutant reverse, CCT GAT CCT GGC AAT TTC G

94x30s, 65x30s, 72x60s, 35 cycles. Wild Type band 650 bp, Mutant band 825.

PDGFRa loxP/loxP:

Wild type forward, CCC TTG TGG TCA TGC CAA AC

Wild type reverse, GCT TTT GCC TCC ATT ACA CTG G

Mutant reverse, ACG AAG TTA TTA GGT CCC TCG AC

94x30s, 65x60s, 72x60s, 35 cycles. Wild Type band 450 bp, Mutant band 250.

PDGF-C:

Wild type forward: AGC TGA CAT TTG ATG AGA GAT

Wild type reverse: AGT AGG TGA AAT AAG AGG TGA ACA

Mutant forward: CTG ATG TTC TCG TGA CTC TGA

Mutant reverse: TAG CTA GTC GAT ACC GTC GA

95x30s, 55x30s, 72x30s, 35 cycles. Wild Type band 200 bp, Knock Out band 350.

Western blot.

Western blot and immunofluorescence analyses were carried out by standard methods as previously described (1). Anti-bodies used for western blotting include: PDGFRα, pPDGFRα (Cell Signaling, Cat. 3164, 4547, Dilution 1:1000); GFP (Abcam, Cat. Ab290, Dilution 1:2000); GAPDH (Santa Cruz, Cat. FL335, Dilution 1:2000).

Intracranial injections and tumor imaging

The intracranial injections have been described previously (1). Briefly, 2ul of $1x10^5$ Luciferase labeled Gl261 cells were stereotactically inoculated into the right hemisphere of 8 week-old mouse brains (1.5 mm posterior to the bregma and 1.5 mm to the right of the sagittal suture to a depth of 2.5 mm below the surface of the skull using a 10 ul Hamilton syringe). Mice were euthanized when they presented with neurological symptoms or a significant loss of weight; mice were examined and weighed daily. Bioluminesence and magnetic resonance imaging (MRI) were performed to monitor the progression of tumor. Bioluminesence was perfomed under the IVIS-100 (xenogen)

system, and images were acquired and analyzed by Living image (Caliper). High-resolution MRI images were acquired on a 7.0-T animal scanner system (Bruker Biospin). 200 ul of 40 mM Gd-DTPA were injected by tail vein as to measure brain tumor contrast enhancement. During imaging, animals were under isoflurane anesthesia and monitored for respiration and heart rate; the temperature was kept at 37°C. Images were analyzed by OsiriX and ImageJ. The area of tumor in each slice was manually outlined. The respective region of interest (ROI) of each slice was added to the ROI manager. The area of each ROI was calculated and then multiplied by the slice thickness. Finally, all slice volumes were added up to calculate the volume of each three-dimensional structure.

Tomoxifen administration

To induce PDGFRα gene ablation 3 days post-injection, mice with intracranially implanted tumor cells were treated with tamoxifen (100 mg/kg intraperitoneally) for 6 days, interrupted for 3 days after the third dose. After 3 days of respite, the fourth dose was reinstituted for an additional 3 days, resulting in RosaCre-ER^{T2} x *PDGFRa* $^{fl/fl}$ (*PDGFRa* $^{fl/fl}$) mice that were deficient in PDGFRa at both alleles. The control animals were RosaCre-ER^{T2}x *PDGFRa* $^{+/+}$ mice.

Cell count of GFP^{+} HCMEC/D3

The number of HCMEC/D3 cells was quantified based on GFP-cell counting. Six 100X fields were randomly chosen to count for each well. The means of GFP^{+} cells were used

to represent cell count in each well. The mean value from three experimental wells was used to represent one biological sample. "N" of biological replicates was six.

Immunohistochemistry & Immunostaining

Tissue was harvested and preserved in 4% PFA for paraffin embedding and subsequent IHC and H&E staining or preserved in 1.6% PFA/20% sucrose solution for OCT embedding and subsequent immunofluoresence.

Antibodies utilized for IHC and immunostainning included: anti-human PDGFRα (Goat IgG R&D systems, Rabbit IgG Cell Signaling); anti-murine PDGFRα (Goat IgG R&D systems, Rabbit IgG Cell Signaling; Anti-Collagen IV (Abcam); anti-Nestin (Abcam); anti-GFAP (Abcam); anti-CD31 (BD Biosciences); anti-NG2 (Millipore); anti-von willebrand factor (Millipore); anti-Ki67 (Vector Laboratories); IDH1-R132H (Dianova, Clone H09, Dilution 1:50). Secondary antibodies utilized included: FITC and Cy3 conjugated Donkey anti-goat/anti-rabbit/anti-rat (Jackson ImmunoResearch); Alexa 488 and Alexa 555 conjugated Donkey anti-Mouse (Invitrogen); Biotinylated Goat anti-rabbit (Vector).

Cell Preparation and Flow Cytometry

Brain tumor stromal cells were obtained from tumor specimens by initially washing in ice-cold HBSS, and then manual mincing, followed by digestion utilizing PAPAIN in PIPES buffer with DNase. 30 minutes later, reactions were quenched by adding 1ml of FCS. Dissociated cells were then twice washed by complete medium and filtered through 70 um filters. Red blood cells were lysed using ACK buffer (Gibco, Invitrogen). Bone

marrow cells flushed from femurs were counted and one million cells in each sample were subjected to flow analysis on FACS-Calibur (BD). All cells were stained at 4° F in PBS with 5% FCS. The following antibodies were used for staining: APC-anti-mouse PDGFRα, APC-Rat IgG isotype (eBioscience); Biotin Mouse Lineage Panel (BD Pharmingen)/FITC-Streptavidin (eBiosciece); PE-anti-C-kit (eBiosciece)

Microarray analysis

GFP^+ bone marrow derived cells were sorted by FACS (BD) from Gl261 orthotopic model-derived and normal brains, which were processed as described in "Cell preparation". Then, $2x10^5$ $F4/80^+$ and GFP^+ cells in each group were sorted by MACS (miltenyibiotec) based on manufacture instructions, and total RNA was prepared with RNeasy micro kit (Qiagen) for subsequent cDNA amplification and microarray. 3 individual samples from normal brain, and 4 individual samples from Gl261 tumors were subjected to microarray analysis (Mouse genome 430.20, Affymetrix). Gene expression data was analyzed using limma package in R/Bioconductor software, using p values of less than 0.01 and fold change greater than 2, 881 genes were identified as significantly changed. By unsupervised analysis, these samples can be clustered in two separate groups: genes that are either up- or down-regulated. Z score was used in the cluster analysis. Microarray data can be accessed from Gene Expression Omnibus (GEO), under accession NO. GSE38283.

TCGA Kaplan-Meier Analysis.

PDGF-C expression data were retrieved from the MSKCC cBio Cancer Genomics Portal. Patients with GBM were subdivided into high PDGF-C expression (threshold of Z-score = 2.0), and the rest were considered as PDGF-C low expression for purposes of the analysis.

Isolation of oligodendritic progenitors, astrocytes and endothelial cells

The isolation of oligodendritic progenitors and astrocytes was described previously with minor modifications (2, 3). Forebrains, stripped of meninges and dissected from diencephalon, were minced and then incubated at 37°C with PAPAIN in PIPES buffer, resuspending after 20 min. After quenching the digestion with FCS, the suspension was again disrupted with a pipette and passed through 100um cell filters. Cells were collected immediately by centrifugation and seeded onto poly L-lysine-coated petri-dishes with complete medium containing 10% FCS in DMEM overnight. The dishes were shaken horizontally clockwise 10 times and counter-clockwise 10 times every other hour for 12 hours. The cells that were disrupted and were in suspension were removed and spun down to isolate oligodendritic progenitors. The remaining attached cells were primarily astrocytes, maintained in DMEM with 10% FCS, and passaged at least 3 times. Detached cells from the previous step were subjected to MACS with anti-PDGFRα (eBiosciece) and anti-rat magnetic beads (Miltenyi Biotech). Sorted cells were maintained in DMEM/F12 supplemented with B27 and bFGF (invitrogen). Brain endothelial cells were isolated based on CD31 by MCAS from dissociated forebrain as described above and maintained in EBM-2 (Lonza) supplemented with 5% FCS, ECGF

and bFGF. The purity of astrocytes and endothelial cells were determined by GFAP (Abcam) and VE-cadherin (BD Bioscience) staining.

RT-PCR

RNA from the sorted or cultured cells was extracted using the RNeasy kit (Qiagen), and reverse transcribed using Superscript III reverse transcriptase (Invitrogen). QPCR was performed on a 7500 Fast Real Time PCR System (AppliedBiosystems).

TaqMan primers and probes for amplification of PDGF-C were designed from sequences deposited in the GenBank database under "Mm00480205_m1"; Sox10 was designed from "Hs00366918_m1"; Olig2 was designed from "Hs00300164_s1"; GFAP was designed from "Hs00909233_m1"; VE-cadherin was designed from "Hs00901463_m1" (Applied Biosystems). As an internal control, an rRNA fragment was amplified using the TaqMan Ribosomal RNA Control Reagents (Applied Biosystems).

In vitro blood brain barrier assay

This assay was based on previous work (4, 5) with minor modifications. Briefly, 1x 10^4 HCMEC/D3 cells were seeded inside transwells, pre-coated with gelatin in EGM-2 medium with 5% FCS. After an endothelial monolayer formed (checked with VE-cadherin staining), 1x 10^4 human primary astrocytes (ScienCell), OPCs or both, were seeded on opposite sides of transwells for 12 hours by placing transwells upside down. U251 conditional medium was then used to replace the medium for a subsequent 12 hours. Purified luciferase was added to the upper chamber to a final concentration of

1ug/ml. 12 hours later, medium from the lower chamber was taken out for bioluminescence assays.

Endothelial sprouting assay

This assay was based on previous work (6) with some modifications. GFP labeled HCMEC/D3 cells ($1x10^3$) were seeded in a 50ul collagen matrix (with 5% FCS and bFGF) and placed upside down for 48 hours in humidified chamber to form endothelial spheroids. The endothelial spheroids were moved to 3D Matrigel (BD biosciences), co-cultured with different cells including astroyctes and OPCs in U251 conditional medium for another 48 hours. For antibody neutralization, PDGFRα antibody (R&D) or control IgG, were added into co-cultured systems to a final concentration 20 ug/ml. Spatial organization and sprout configuration were assessed after 2 days using in situ fluorescence imaging. Cumulative sprout lengths of spheroids represented by the area of the GFP^+ field were quantitatively analyzed using ImageJ and please refer to "Quantification" section for more details.

Endothelial tubule formation

GFP labeled HCMEC/D3 (5×10^4/well) cells were seeded on 24-well plates coated with matrigel containing growth factors (200 μl/well: BD Biosciences) and incubated for 6 hrs followed by co-culture with human astrocytes and OPCs in U251 conditional medium. The length of endothelial tubule in each field was quantified by ImageJ, and please refer to "Quantification" section for more details. For antibody neutralization, PDGFRα

antibody (R&D) or control IgG were applied into both lower and upper chambers with final concentrations of 20 ug/ml.

Quantification

All data are given as mean ± SD or ± SEM. "N" was used to show the number of biological replicates within figure legends. 1) Quantification of IHC: "N" stands for number of biological samples within each group. Firstly, we randomly selected 5 slides from each sample/mouse. 4~6 fields from each slide were counted and an average number per field was used to represent each slide. Then, the mean of values from 5 slides was used to represent each sample/mouse, the graph were generated based on "N" independent samples/ mice. Two independent observers blindly measured or counted to determine intra- and inter- observer variability. 2) "N" in animal experiments represents the number of mice in each group. 3) "N" in RT-PCR and cell count experiments represents number of independent wells of treatment per condition, each of which represented the mean value from three experimental wells. In "Endothelial sprouting assay", "Tubule formation assay" and "Proliferation assay", the value of each well was the mean of cell counts of six 100X fields randomly chosen within this well, and "N" is the number of independent wells of treatment per condition. Differences between the mean parameter values derived from two groups were compared using Student's t-tests, One-way ANOVA, followed by post-hoc test for comparison of multiple groups. Significance was inferred at P value less than 0.05.

Supplementary References

1. Kawakami K, Kioi M, Liu Q, Kawakami M, Puri RK. Evidence that IL-13R alpha-2 chain in human glioma cells is responsible for the antitumor activity mediated by receptor-directed cytotoxin therapy. Journal of Immunotherapy 2005; 28: 193-202.
2. Chen Y, Balasubramaniyan V, Peng J, Hurlock EC, Tallquist M, Li J, et al. Isolation and culture of rat and mouse oligodendrocyte precursor cells. Nature Protocols 2007; 2: 1044-51.
3. Szuchet S, Yim SH. Characterization of a Subset of Oligodendrocytes Separated on the Basis of Selective Adherence Properties. Journal of Neuroscience Research 1984; 11: 131-44.
4. Bos PD, Zhang XHF, Nadal C, Shu WP, Gomis RR, Nguyen DX, et al. Genes that mediate breast cancer metastasis to the brain. Nature 2009; 459: 1005-U137.
5. Eugenin EA, Berman JW. Chemokine-dependent mechanisms of leukocyte trafficking across a model of the blood-brain barrier. Methods 2003; 29: 351-61.
6. Korff T, Augustin HG. Tensional forces in fibrillar extracellular matrices control directional capillary sprouting. Journal of Cell Science 1999; 112: 3249-58.

Supplementary Figure S1

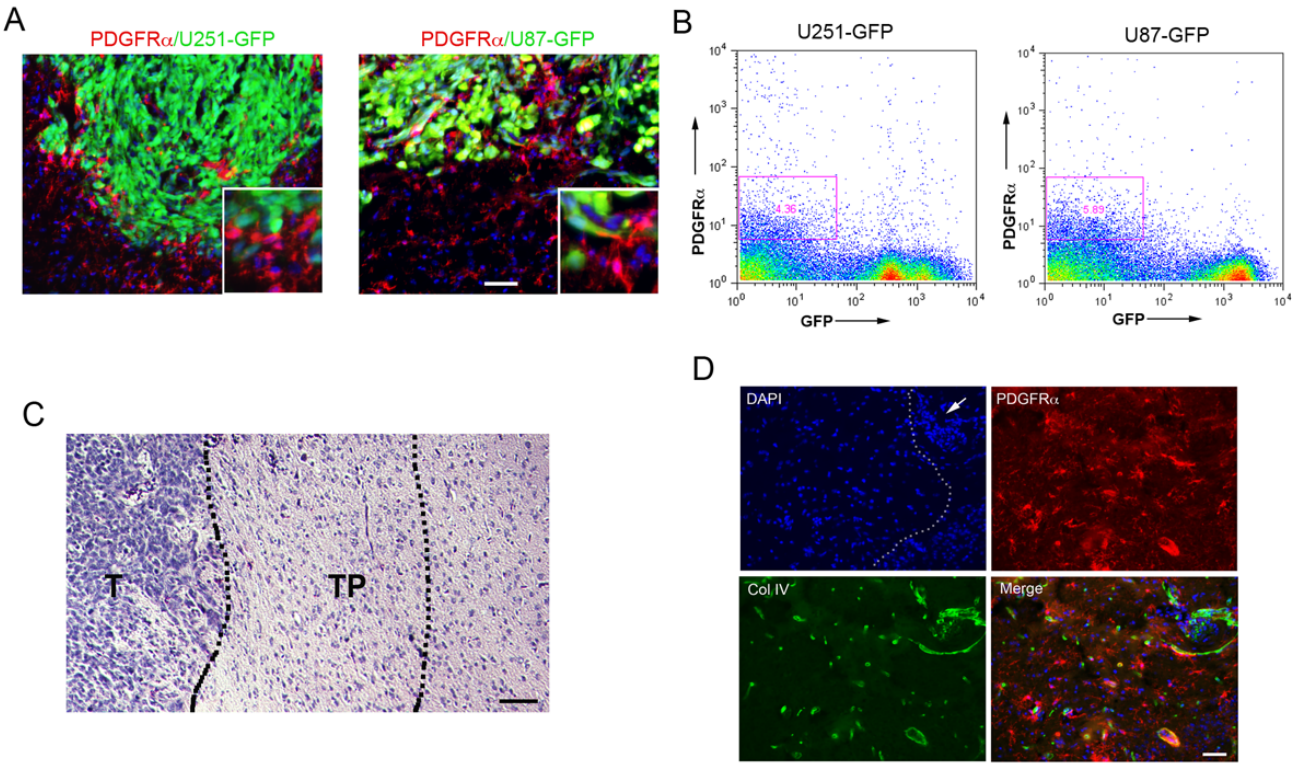

Figure S1 Stromal cells of glioma express PDGFRα. (A) U87-MG and U251 tumors have PDGFRα+ stromal cells. GFP labeled U87-MG and U251 cells were intracranially injected into nude mice. At D28, PDGFRα was stained (red) on U251(left) and U87-MG (right) tumors. Scale bar, 50 μm. (B) Non-GFP population expresses PDGFRα. Flow cytometry was performed on GFP-U251 (left) and GFP-U87 MG (right) tumors with PDGFRa antibody. (C) H&E staining of invading front Gl261 tumor. “T” represents tumor inside and “TP” represents tumor periphery. OPC counts in this study were performed within the tumor periphery with approximately 30 cell-diameters. Scale bar, 50 μm. (D) Invading front of KR158 tumor was stained with PDGFRα and Collagen IV (ColIV) for OPCs and blood vessels. Nuclei were counterstained with DAPI. The dashed line in DAPI-panel shows tumor rim, and arrow shows tumor nodule on the right of tumor rim. Scale bar, 50 μm.

Supplementary Figure S2

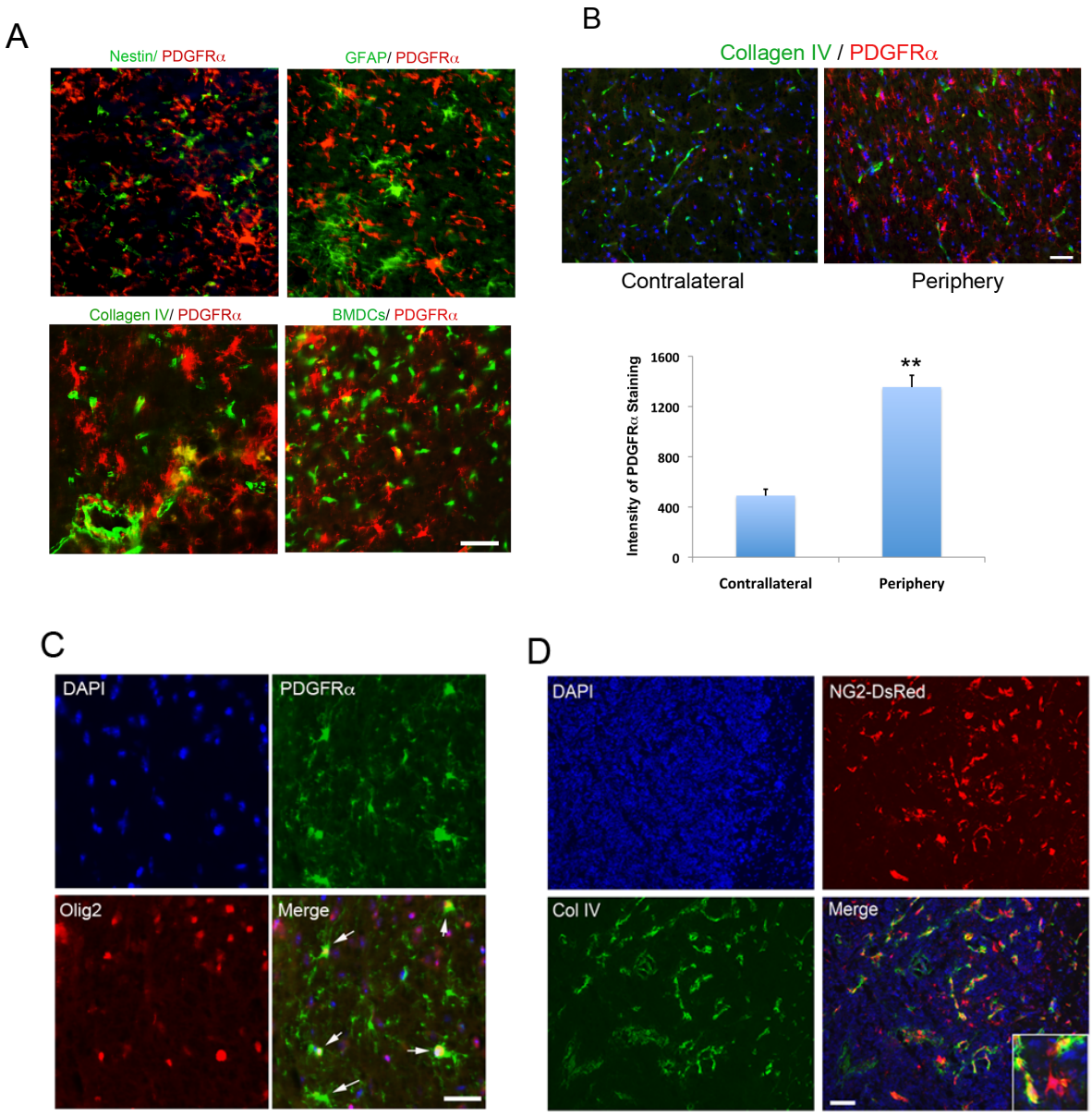

Figure S2 PDGFRα+ stromal cells are cells in the oligodendritic lineage.
(A) In the Gl261 tumor periphery, PDGFRα+ cells (red) did not co-stain with Nestin, GFAP or Collagen IV (green). PDGFRα was stained in mice with adoptive GFP-bone marrow transplantation. Scale bar, 25 μm. (B) PDGFRα (Red) and Collagen IV (Green) staining in periphery of Gl261tumor and contralateral hemisphere. Scale bar, 50 μm. Quantification of intensity of PDGFRα staining is provided to represent its expression level. Means +/-SD, n=5 mice, T-test, **P< 0.001. (C) PDGFRα (Green) was co-stained with Olig2 (Red) in tumor periphery. Scale bar, 25 μm. (D) Intra-tumoral area of Gl261 tumor, which were implanted in NG2-DsRed mice, was counter-stained with Collagen IV (Col IV) and DAPI. NG2-DsRed is shown in red. Scale bar, 50 μm.

Supplementary Figure S3

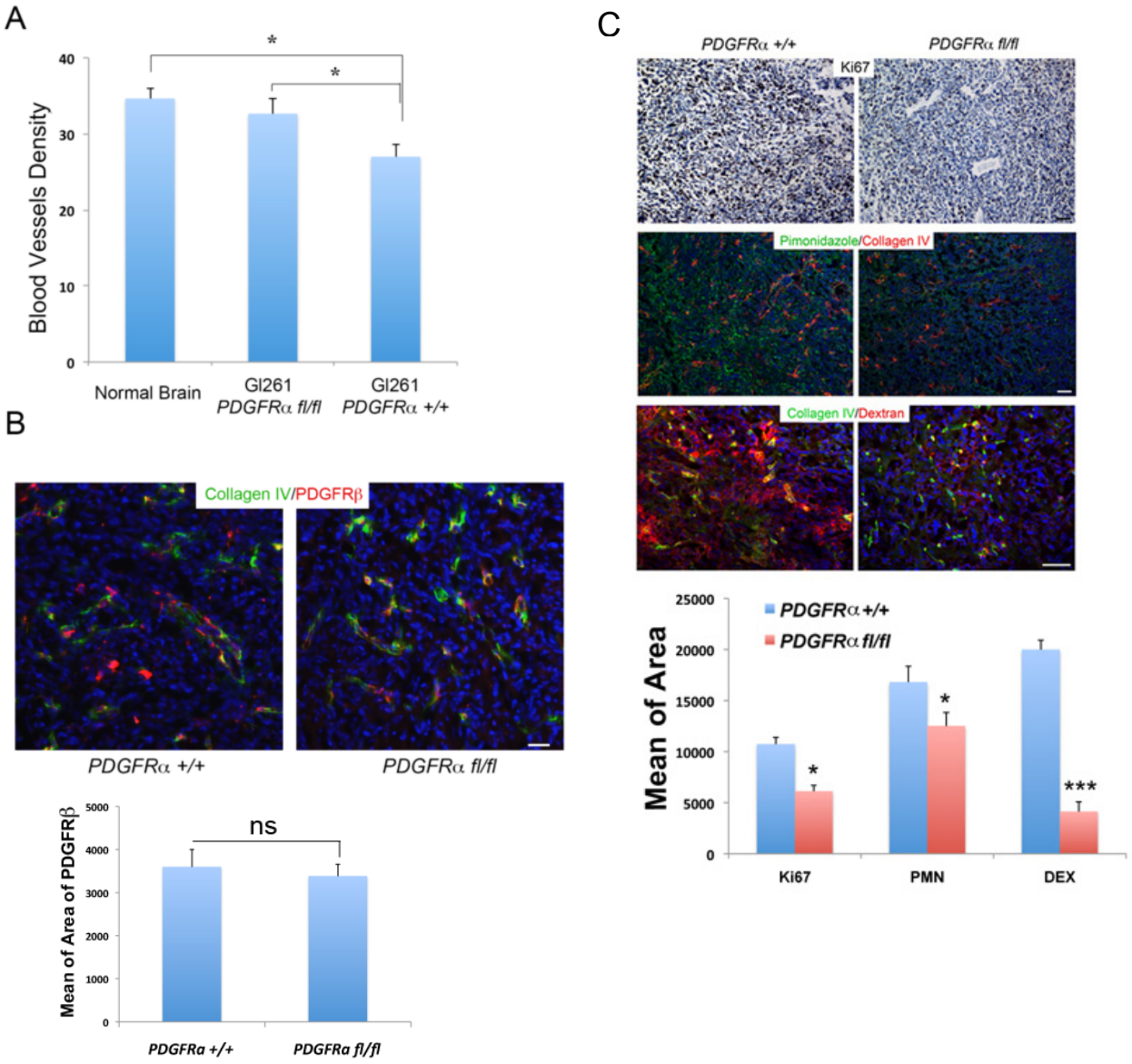

Figure S3 Tumor blood vessels are less dense but maintain comparable amount of pericytes. (A) Blood vessel density was slightly greater in PDGFRα $^{fl/fl}$ group. Quantification of the number of blood vessels per field, in Gl261 tumor from PDGFRα $^{fl/fl}$ mice and PDGFRα $^{+/+}$ mice as well as normal brain tissue. Means +/- SD, n=5 mice, One way-ANOVA *P< 0.05. (B) Blood vessels maintain comparable pericyte coverage. Co-staining PDGFRβ (pericytes) and Collagen IV (endothelium) in the tumors from PDGFRα$^{fl/fl}$ mice and PDGFRα$^{+/+}$ mice demonstrated the existence and distribution of pericytes. Scale bar, 50 μm. Quantification of the area of PDGFRβ staining is provided. Means +/- SD, n=5 mice, T-test no-significance. (C) Proliferation (Ki67), hypoxia (pimonidazole), and vascular leakiness (Dextran) was measured in GL261 tumors implanted in PDGFRα$^{fl/fl}$ and PDGFRα$^{+/+}$ groups. Scale bar, 100 μm. Intensity of positive staining was quantified based on means of area. Means +/- SD, “n” of biological samples =6 mice, T-test *P<0.05 ***P<0.001.

Supplementary Figure S4

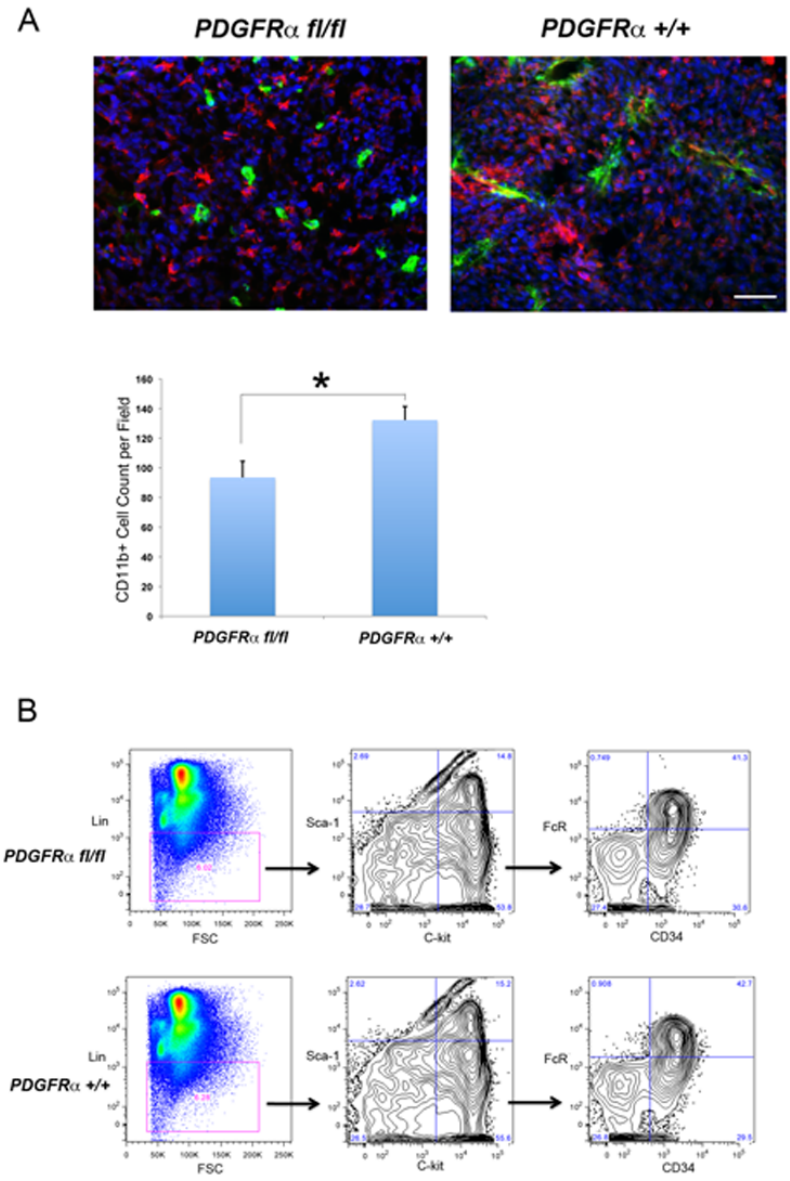

Figure S4 Knocking out PDGFRα suppresses infiltration of BMDCs without affecting hematopoiesis. (A) Co-stain of CD11b (Red) and Collagen IV (Green) was performed on GI261 tumor in PDGFRα $^{fl/fl}$ mice and in PDGFRα$^{+/+}$mice. The number of CD11b+ cells were quantified. Error bars are +/- SD, n=6 mice, T-test *P <0.05. (B) Hematopoiesis in PDGFRα$^{fl/fl}$ mice was not affected. The bone marrow cells from PDGFRα $^{fl/fl}$ mice and PDGFRα $^{+/+}$ mice were stained by lineage markers (Lin), hematopoietic progenitor markers (C-kit+/Sca-1+) and myeloid progenitor markers (CD34+/FcR+/-).

Supplementary Figure S5

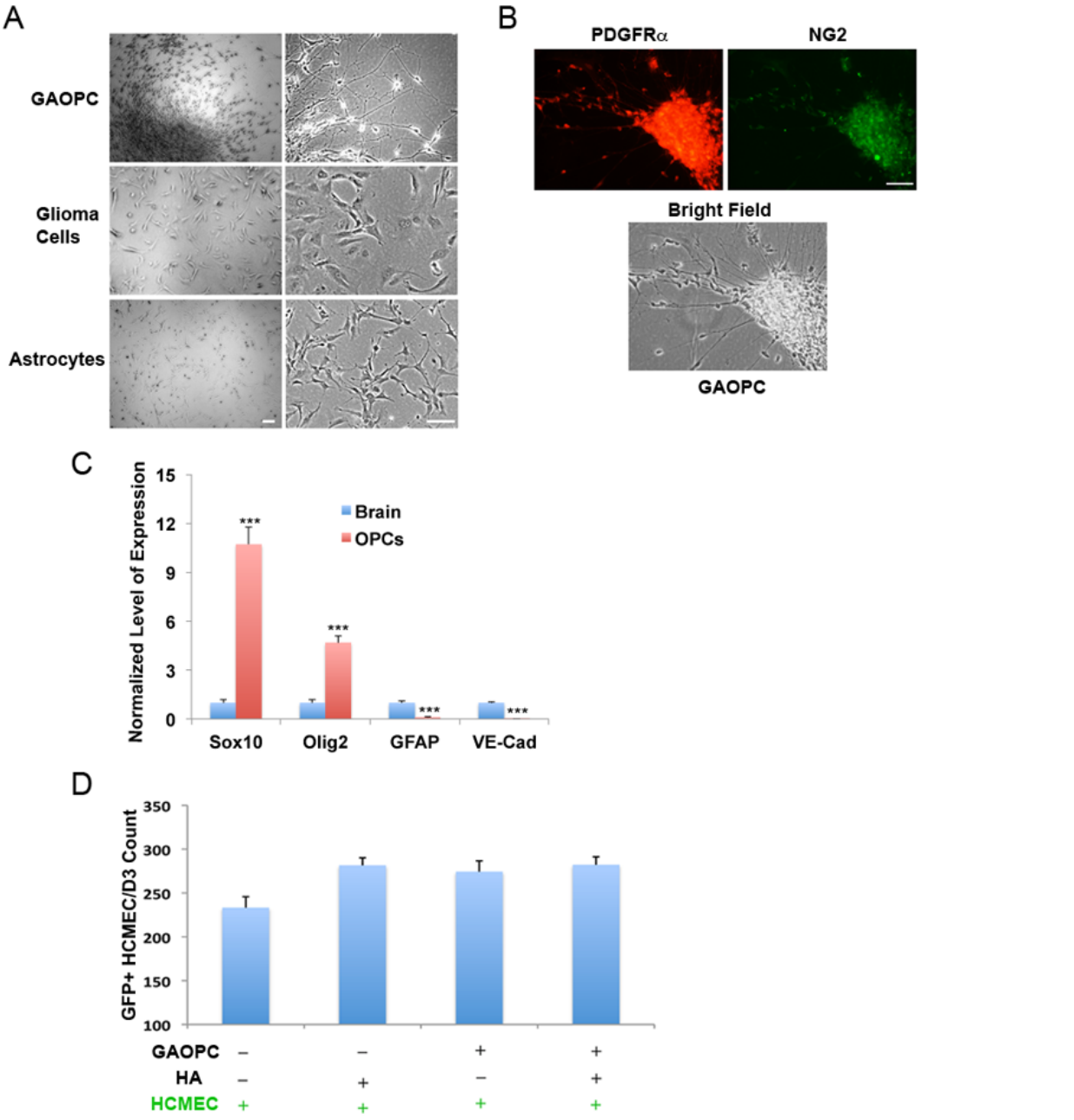

Figure S5 Isolation of OPCs. (A) Morphology of human derived OPCs. OPCs were isolated from a low-grade astrocytoma, and maintained in serum-free neural stem cell medium. Glioma-associated OPCs, counterpart tumor cells, and normal astrocytes are shown in bright field. Scale bar, 50 um. (B) Progenitor markers of OPCs remained. OPCs were stained by PDGFRα (red) and NG2 (green). Scale bar, 50 μm. (C) RT-PCR was performed on brain tissue and cultured OPCs with primers of Sox10, Olig2, GFAP, and VE-Cad. Means +/-SEM, n=6, T-test, ***P<0.001. (D) The number of GFP^+ HCMEC/D3 was measured, after co-culture with GAOPC, HA or both, respectively. Error bars are +/- SEM, n=6, One-way ANOVA, no-significance. “N” is the number of independent wells.

Supplementary Figure S6

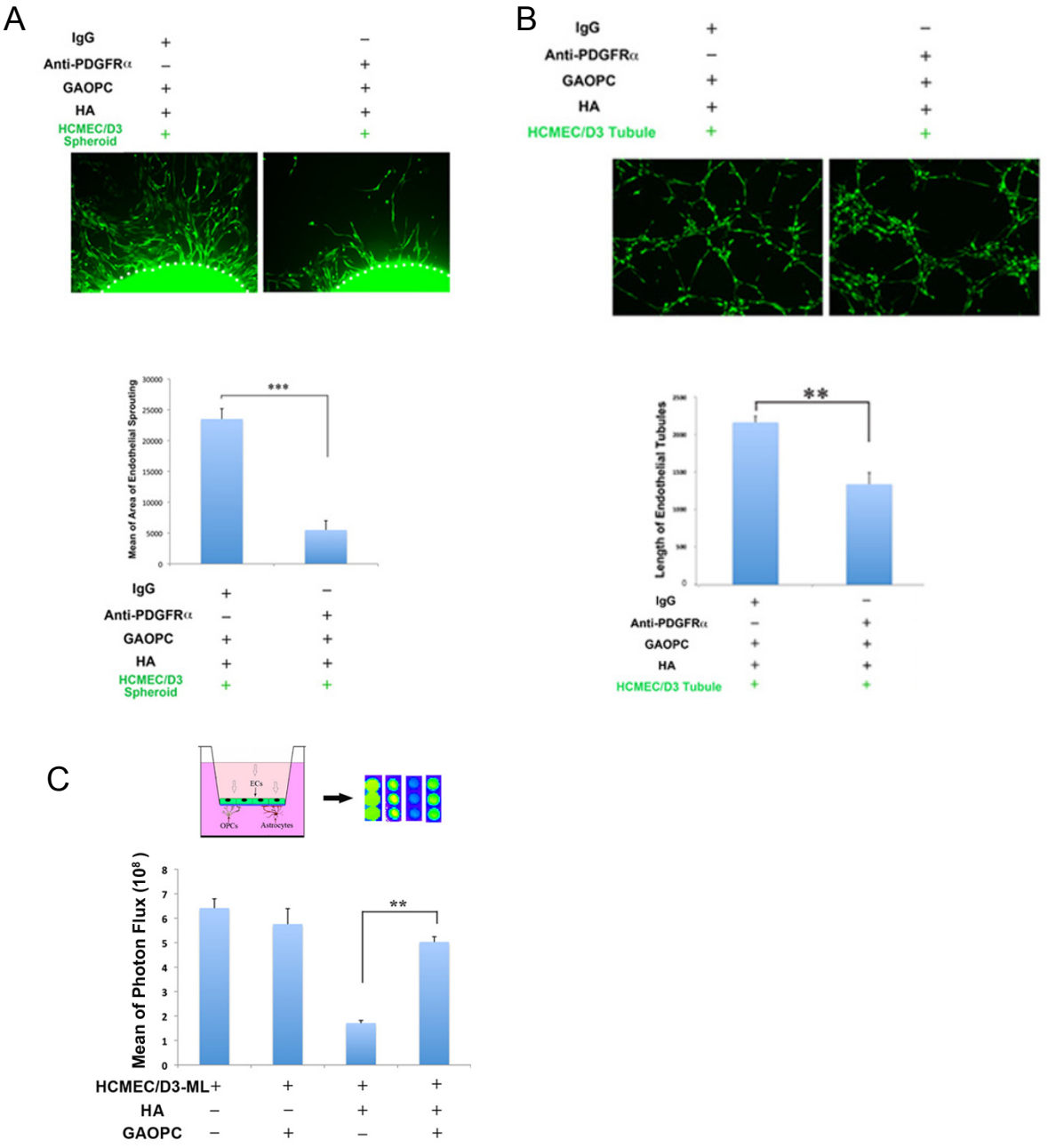

Figure S6 GA-OPCs abrogate the inhibitory functions of astrocytes on endothelial tubule formation and endothelial sprouting. (A) The pro-sprouting effect is dependent on PDGFRα. Neutralizing PDGFRα antibody or control IgG was applied to the co-culture of GAOPC, HA and HCMEC/D3 spheroids. Error bars are +/- SEM, n=6, T-test ***P< 0.001. (B) The effect of pro-tubule formation is dependent on PDGFRα. In the endothelial tubule formation assay, neutralizing PDGFRα or isotype IgG was applied to the co-culture of GAOPC, HA and HCMEC (endothelial tubules). Error bars are +/- SEM, n=6, T-test ***P<0.001. (C) Tumor modified blood-brain barrier model demonstrates increased permeability of endothelial junctions as measured by bioluminescence of diffused luciferase. Endothelial monolayer permeability (HCMEC/D3-ML) in different

culture condition was measured. Means+/-SEM, n=5, One way ANOVA **P<0.01. “N” is the number of independent wells.

Supplementary Figure S7

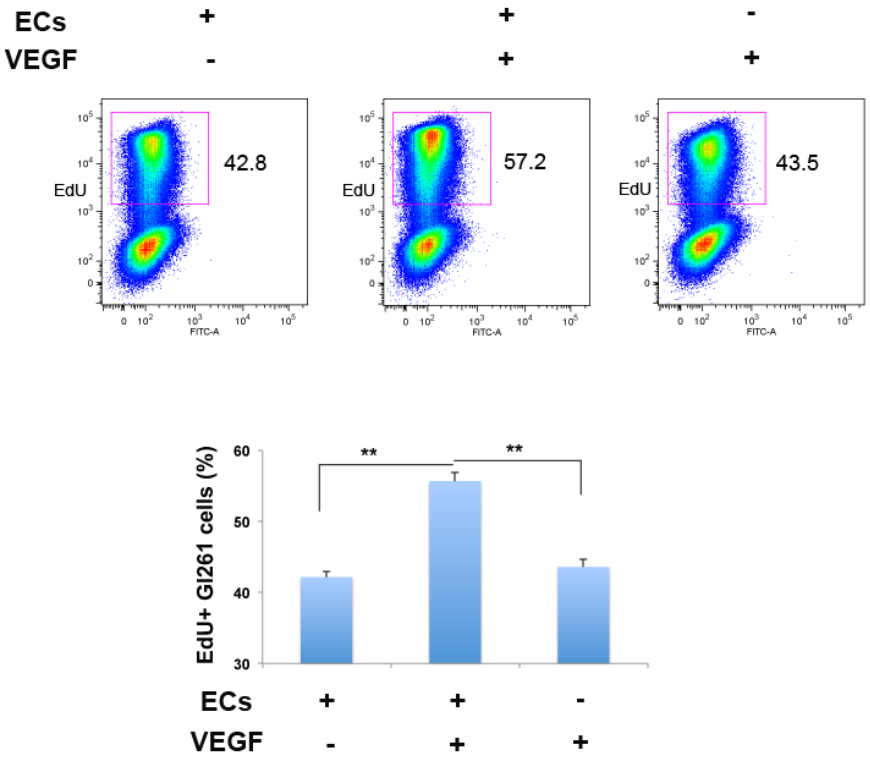

Figure S7 Activated endothelial cells enhance the mitosis of glioma cells.
Co-culture of GI261 cells with GFP-HCMEC/D3 treated with or without VEGF, or with VEGF alone. GI261 cells were sorted and proliferation was demonstrated by flow cytometry detecting incorporation of EdU. Statistical chart of EdU^+ GI261 cells versus total GI261 cells is shown in the lower panel. Means ± SD, n = 5, One-way ANOVA, **p < 0.01. “N” is the number of independent wells.

Supplementary Figure S8

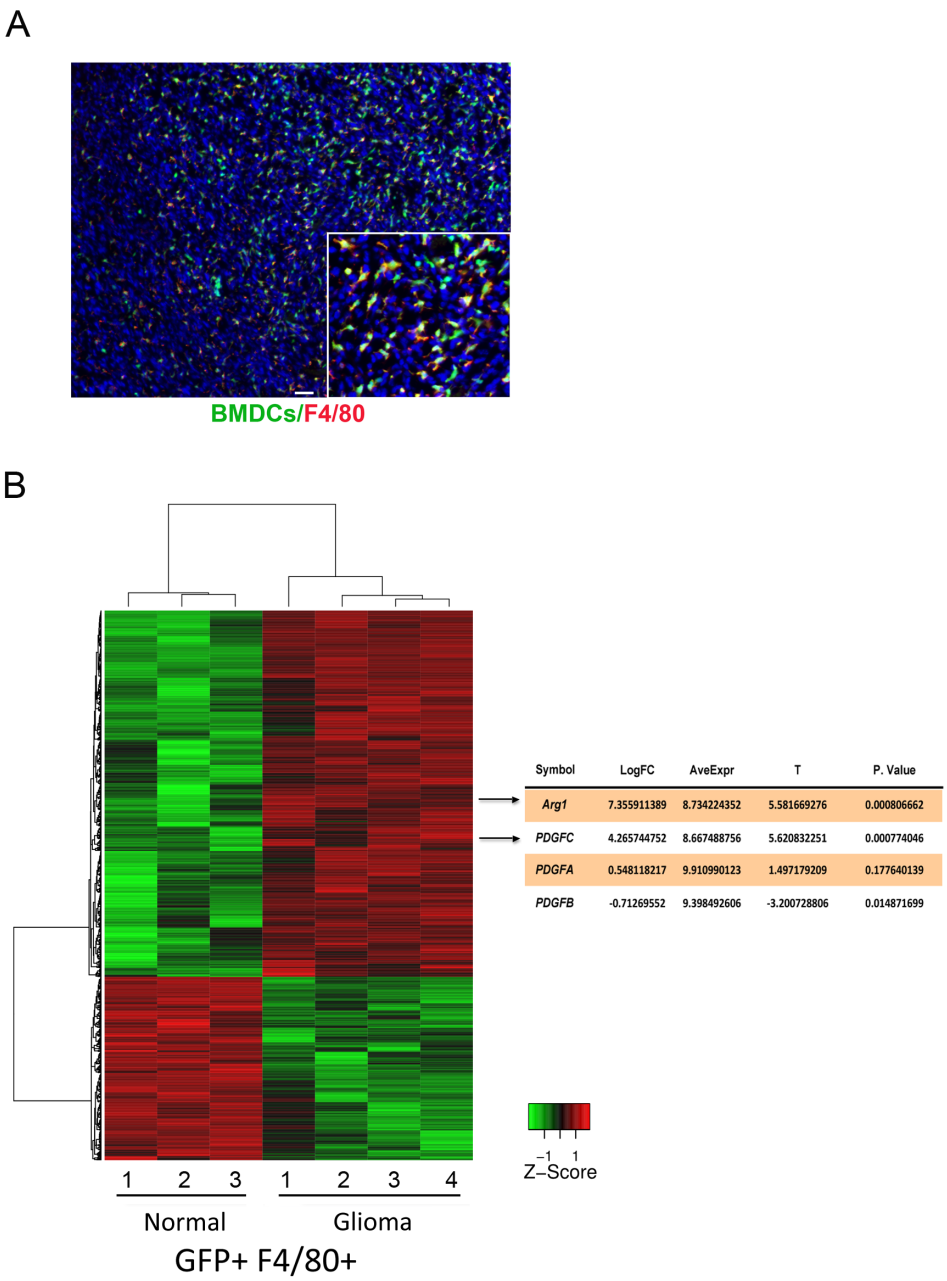

Figure S8 Microarray analysis on GFP+/F4/80+ BMDCs. (A) Majority of BMDCs in Gl261 tumors are F4/80 positive. F4/80 (red) was counterstained in Gl261 tumors from mice with adoptively transplanted. GFP+ bone marrow. Inset is an enlarged and representative field. Scale= 50 µm. (B) Microarray analysis on GFP+/F4/80+ cells. Microarray was performed on the mRNA, isolated from GFP+ /F4/80+ cells from Gl261 tumors and normal brain. Those genes, which were significantly up or down regulated ($p<0.01$) and altered in expression level (fold change > 2), were clustered and shown in a heatmap. The change of PDGF-A, PDGF-B, PDGF-C and Arg1 (arginase 1) are presented.

Supplementary Figure S9

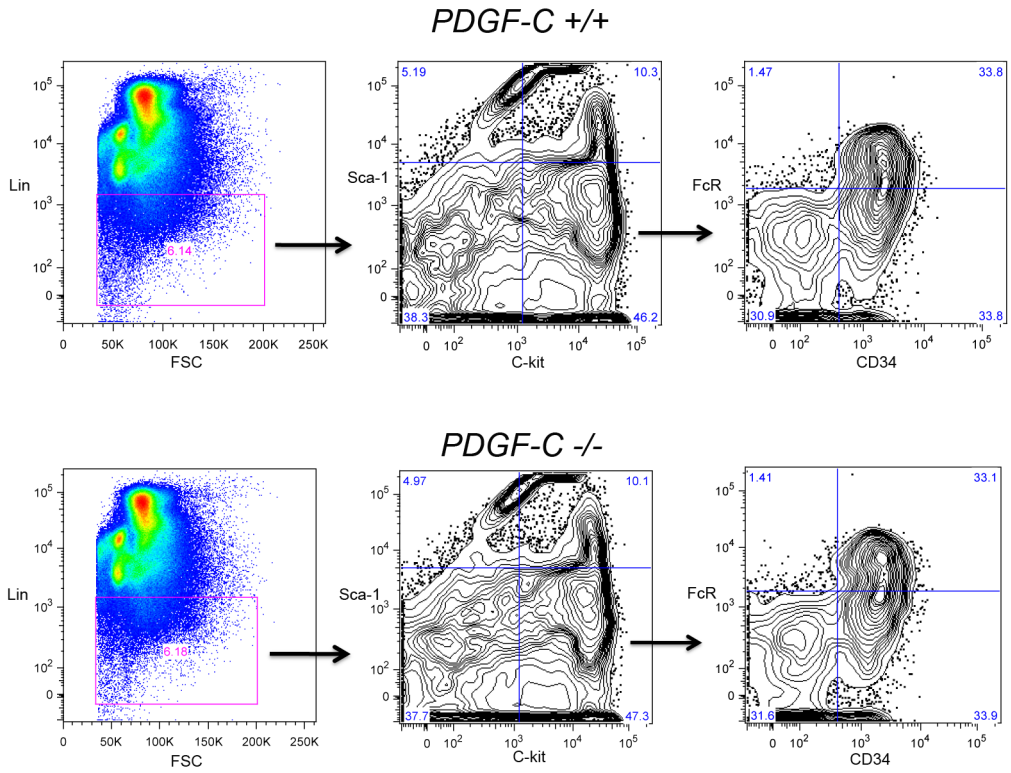

Figure S9 Hematopoiesis in PDGF-C $^{-/-}$ mice was not affected. The bone marrow cells from PDGF-C $^{-/-}$ mice and PDGF-C$^{+/+}$ mice were analyzed utilizing a lineage marker (Lin). Hematopoietic stem cells (C-kit+/Sca-1+) and myeloid progenitor cells (CD34+/FcR+/-) did not show significant differences.

Supplementary Figure S10

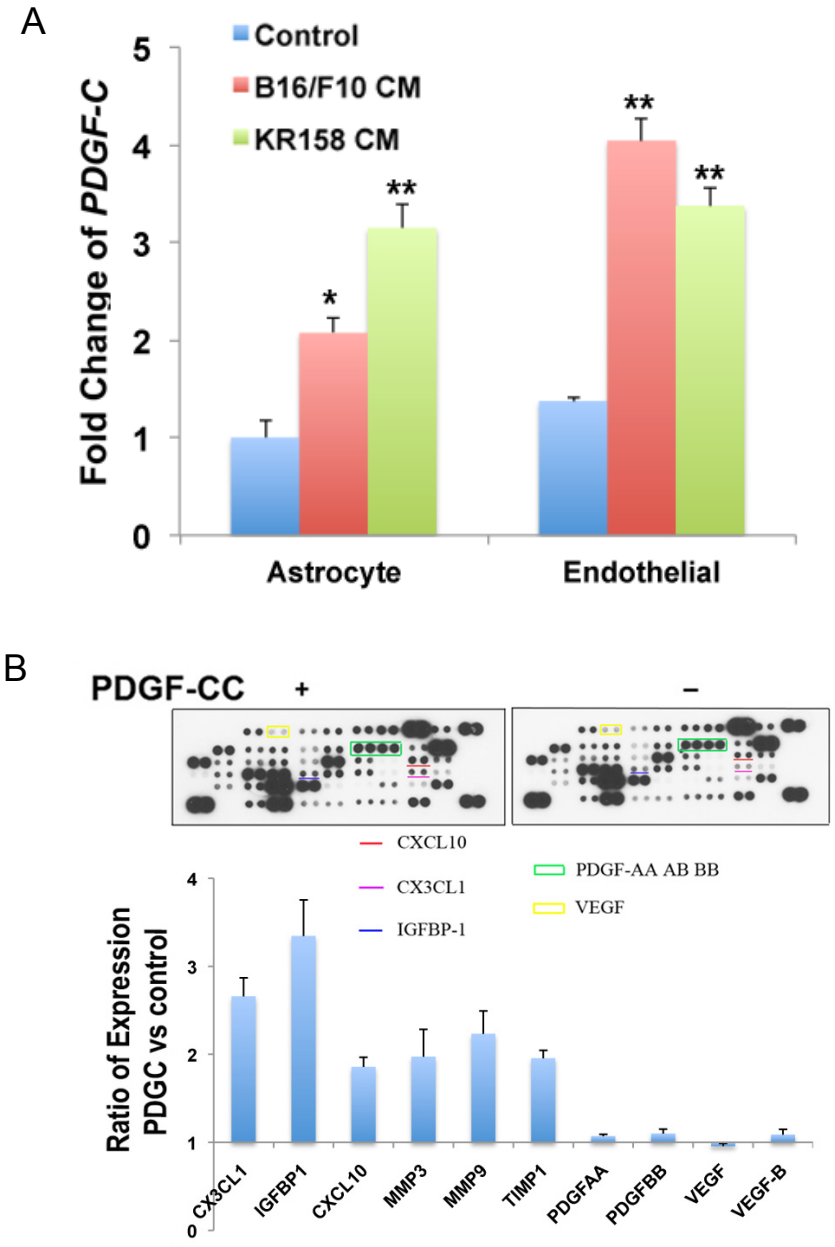

Figure S10 Astrocytes and endothelial cells express PDGF-C, which induces pro-angiogenesis factors in OPCs.
(A) Endothelial cells and astrocytes isolated from cortical tissue of C57BL/6 mice were treated with B16/F10 conditioned medium (B16/F10 CM), KR158 conditioned medium (KR158 CM) or control medium (Control). Quantitative RT-PCR of PDGF-C was performed showing the up-regulation of PDGF-C in both endothelial and astrocytic cells in response to two different types of tumor cells. Means +/- SEM, n=6, One way ANOVA, vs control *P< 0.05, **P<0.01. (B) Mice OPCs treated in conditioned medium derived from cells with or without PDGF-CC. Cell lysates were blotted with an antibody arrays. Expression levels were quantified by pixel density. The pro-angiogenic factors (PDGF-CC treated vs non-treated; fold change> 1.8) shown with representative unchanged targets. CXCl10, CX3Cl1, IGBFP1 had significant upregulation. Means +/-SD, n=3, T-test. P<0.05. “N” is the number of independent wells.

Supplementary Figure S11

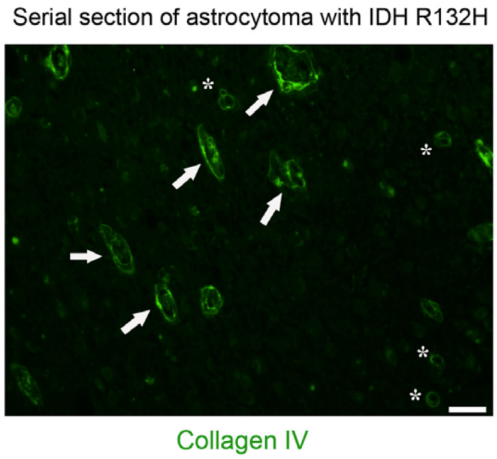

Figure S11 Patients' samples show transition areas of enlarging blood vessels. Serial sections from a Grade III astrocytoma shown in Figure 7A, were stained with anti-Collagen IV to detect blood vessels. Arrows indicat blood vessels with enlarged lumens, and stars indicat normal brain blood vessels. Scale bar, 50 μ m.

Supplementary Figure S12

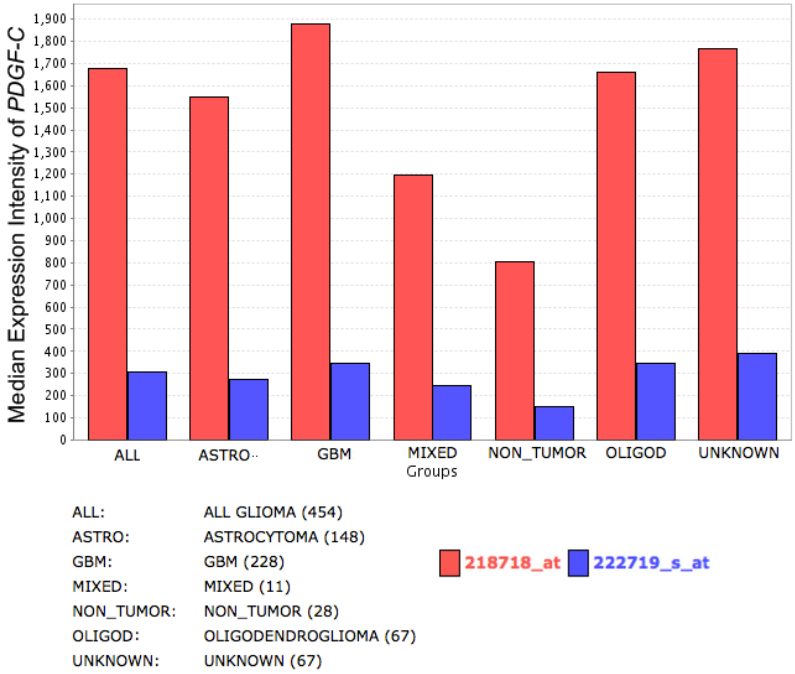

Figure S12 Expression of *PDGF-C* was analyzed in **REMBRANDT** database. The level of PDGF-C was determined by two probe sets within multiple subgroups of gliomas as indicated including astrocytoma, oligodendroglioma, mixed, GBM, and non-tumor.

Supplementary Figure S13

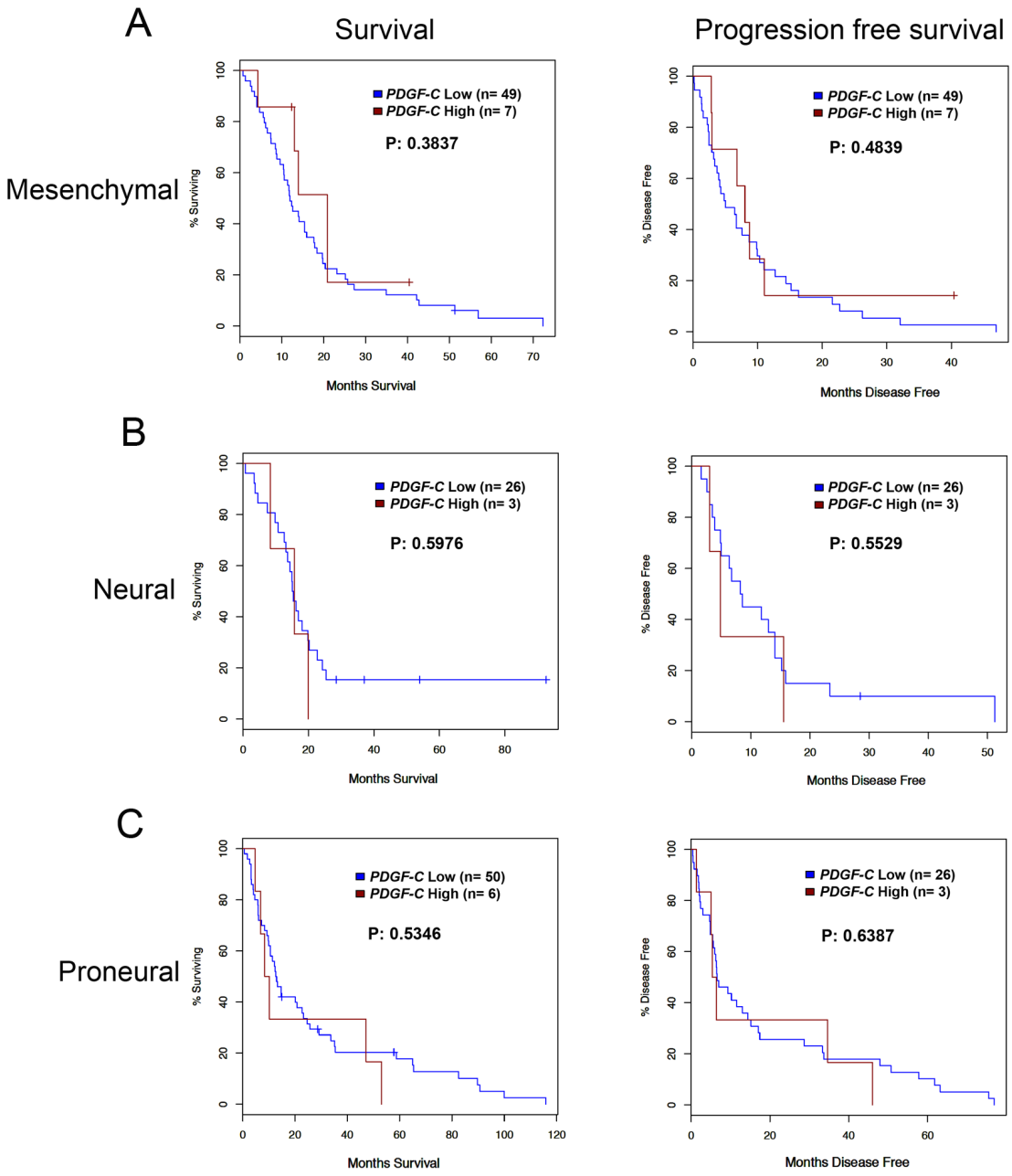

Figure S13 *PDGF-C* was not correlated with patient's survival in other subsets of GBM. Kaplan Meier survival and progression free survival analyses were done on subsets of mesenchymal, neural, proneural GBM samples based upon the expression of PDGF-C. Number of analyzed patients and P value are shown within each graph. P> 0.05 by log-rank test.

Supplementary Figure S14

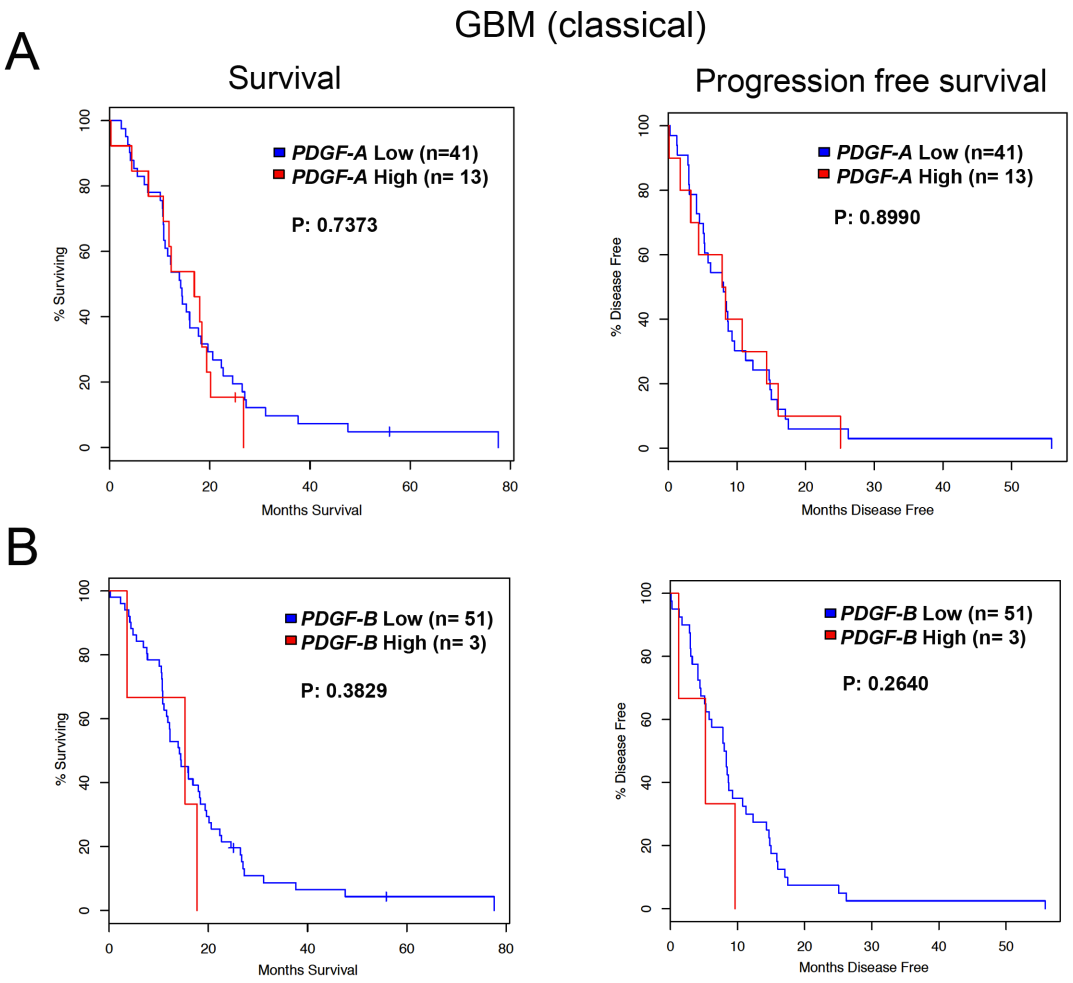

Figure S14 *PDGF-A, and PDGF-B* were not correlated with patient's survival within the classical subset of GBM. Kaplan Meier survival and progression free survival analyses were done on subsets of classical samples based on the expression of PDGF-A and PDGF-B. Number of analyzed patients and P value are shown within each graph. P> 0.05 by log-rank test.