Supplemental Information for

Activation of Rac1 by Src-dependent Phosphorylation of Dock180^{Y1811} Mediates PDGFRα-stimulated Glioma Tumorigenesis in Mice and Humans

Haizhong Feng, Bo Hu, Kun-Wei Liu, Yanxin Li, Xinghua Lu, Tao Cheng, Jia-Jean Yiin, Songjian Lu, Susan Keezer, Tim Fenton, Frank B. Furnari, Ronald L. Hamilton, Kristiina Vuori, Jann N. Sarkaria, Motoo Nagane, Ryo Nishikawa, Webster K. Cavenee and Shi-Yuan Cheng

This file includes:

Supplementary Data:

Supplementary Figure 3. Alignment of amino acid sequences surrounding Y1811 of Dock180 protein in members of the Dock family in humans.

Supplementary Figure 4. Corresponding to Figure 4B, p-Y of Dock180 at Y1811 is required for PDGFRα-promoted glioma cell survival and migration.

Supplementary Figure 5. Corresponding to Figure 4C-4K. Re-expression of shRNA-resistant $Dock180^{WT*}$ but not $Dock180^{Y1811F*}$ restored PDGFRα-promoted tumorigenesis of LN444/PDGF-A/shRNA gliomas in the brain. Figure S5D to S5L, enlarged images of Figure 4C-4K.

Supplementary Figure 6. Re-expression of shRNA-resistant $Dock180^{WT*}$ but not $Dock180^{Y1811F*}$ increased PDGFRα-promoted growth of neovessels and microglia cells but did not affect macrophage infiltration in brain gliomas.

Supplementary Figure 7. Effect of a PI3K inhibitor LY294002 on PDGFRα-stimulated p-Y of Dock180, Rac1 activities and cell migration in glioma cells.

Supplementary Figure 8. Effect of phosphorylation of $Dock180^{Y1811}$ on Dock180 association with PDGFRα.

Supplementary Figure 9. Corresponding to Figure 9A-9F. Enlarged images of IHC staining of a clinical primary WHO grade IV glioma specimen.

Supplementary Figure 10. IHC staining of a clinical primary WHO grade III glioma specimen.

Supplementary Figure 11. IHC staining of p-Dock180^{Y1811} in a normal brain and a WHO grade I glioma specimen.

Supplementary Table 1. LN443 and LN444 glioma cells express a group of genes that are statistically similar to a subgroup of signature genes in the proneural subtype of clinical human GBMs.

Supplementary Table 2. Immunohistochemical staining of PDGFRα, p-Src^{Y418} and p-Dock180^{Y1811} in human clinical glioma specimens.

Supplementary Table 3. Corresponding to Supplementary Table 2, statistical analysis of expression level of PDGFRα and p-Dock180^{Y1811} in human clinical glioma specimens by IHC staining.

Supplementary Table 4. Corresponding to Supplementary Table 2, spearman's rank correlation analysis of expression level of PDGFRα and p-Dock180^{Y1811} in human clinical glioma specimens by IHC staining.

Supplemental Experimental Procedures:

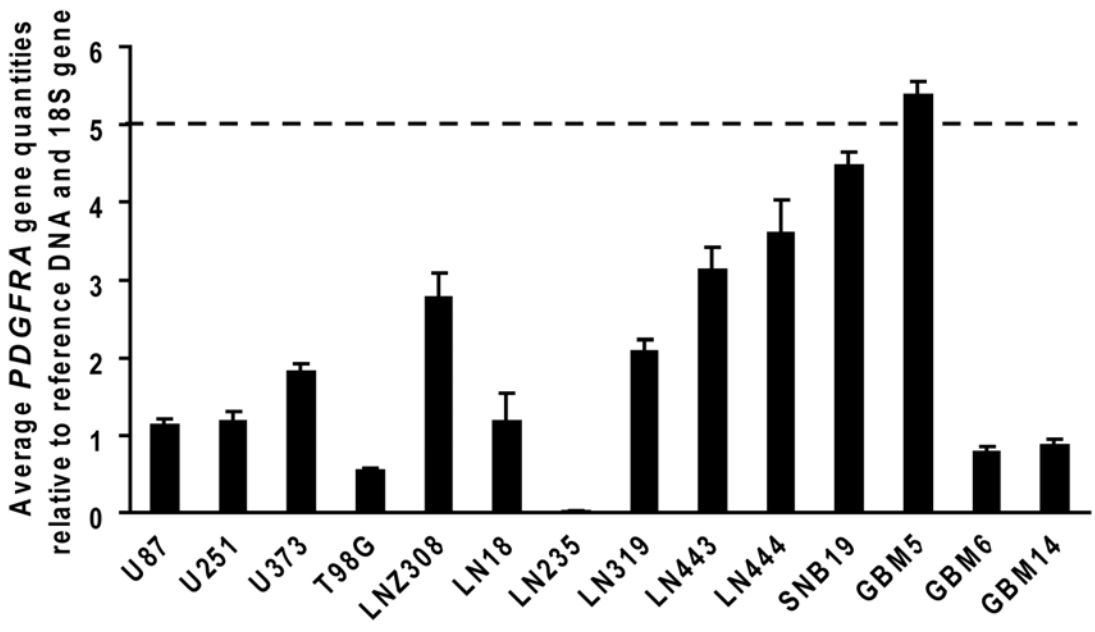

Figure S1. Corresponding to Figure 1A, *PDGFRA* gene quantitation in glioma cell lines and primary GBM cells. *PDGFRA* gene copy number was determined using q-PCR analysis as previously described (1, 2). The Y-axis indicates average *PDGFRA* gene quantities relative to reference sample (normal human astrocytes) and *18S* gene. Average values $\geq$ 5 were considered as gene amplification (dotted line). Bars, SD. *, $P<0.05$. Data represents three independent experiments with similar results.

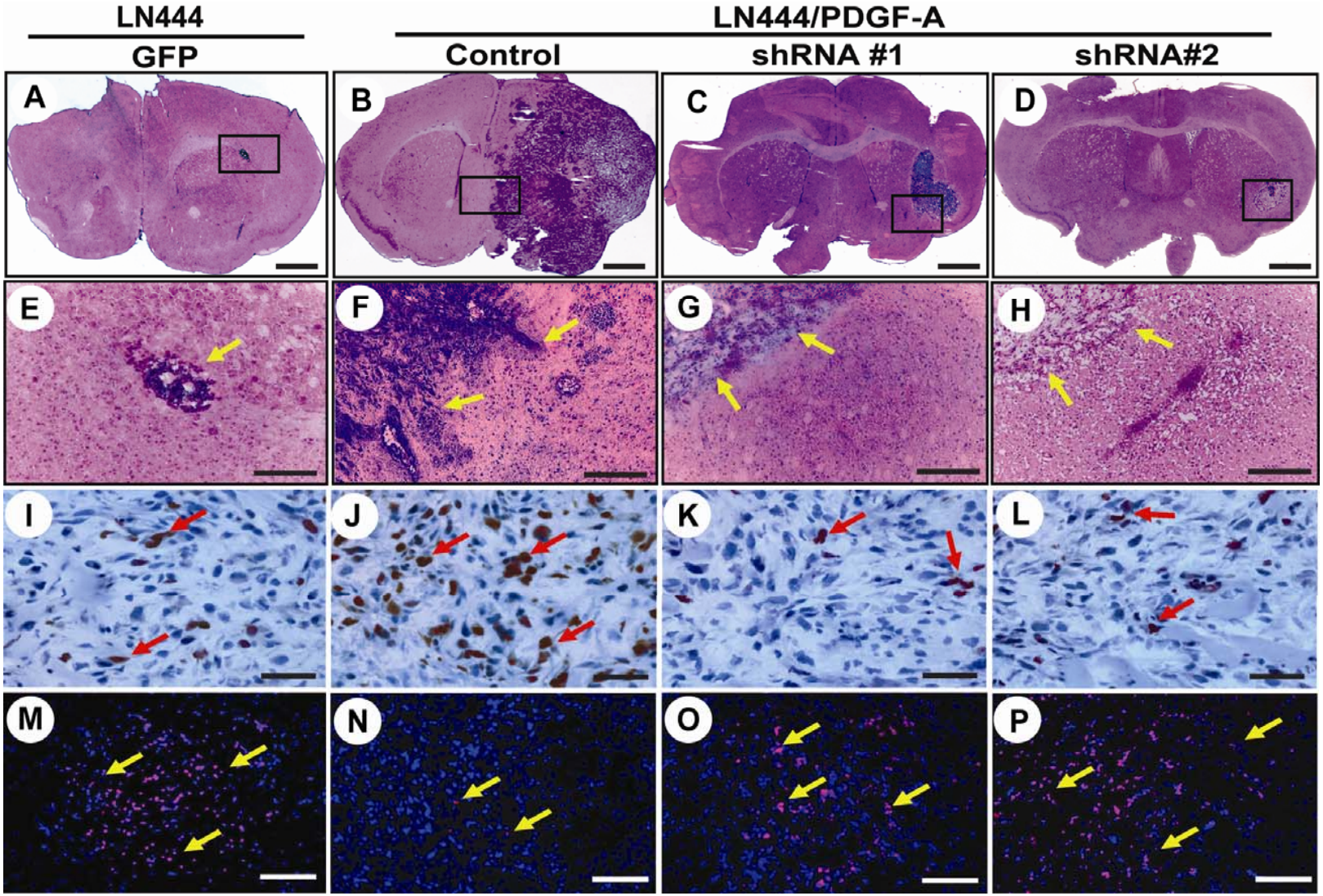

Figure S2. Corresponding to Figure 2D-O, depletion of Dock180 inhibits PDGFRα-promoted LN444 glioma growth and invasion in the brain. **A** to **D**, H&E staining of brain sections with indicated LN444 gliomas (scale bars, 1 mm). **E** to **H**, enlarged areas in A to D marked with squares (scale bars, 200 μm). Arrows indicate invasive tumor cells (F) or non-invasive tumor borders (E, G and H). **I** to **L**, Ki-67 staining (scale bars, 50 μm). **M** to **P**, TUNEL staining (scale bars, 100 μm). Data represents results of 5 mice per group from three independent experiments with similar results.

```
Dock180   1808 --VADYGNLME---NQDLLGSPTPPPPPH 1832
Dock2     1787 -----FLQLSD---GDKKTLTR-------- 1800
Dock3     1927 DLEPPYLPVHYSLSESAVLDSIKAQPCRSH 1956
Dock4     1860 SETSGFENQVN--EQSAPLPVPVPVPVPSY 1887
Dock5     1827 -----YEGSQR---NSTELAPP-------- 1840
Dock6     1996 --QKEYHRELERNYCRLREALQPLLTQRLP 2023
Dock7     1937 --QKEYQRELERNYHRLKEALQPLINRKIP 1964
Dock8     1971 --QREYQQELKKNYNKLKENLRPMIERKIP 1998
Dock9     2019 --QLEYQEEMKANYREMAKELSEIMHEQLG 2046
Dock10    2123 --QLEYQEELRSHYKDMLSELSTVMNEQIT 2150
Dock11    2010 --QVEYHEGLKSNFRDMVKELSDIIHEQIL 2037
```

Figure S3. Alignment of amino acid sequences surrounding Y1811 of Dock180 protein in members of the Dock family in humans.

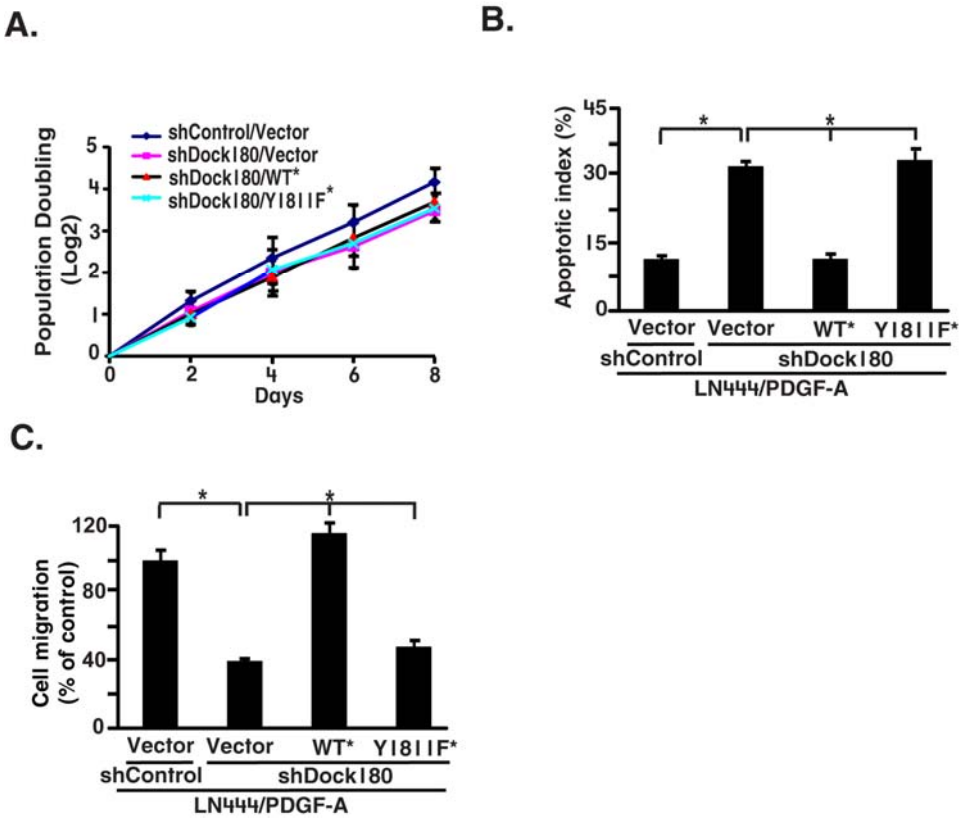

Figure S4. p-Y of Dock180 at Y1811 is required for PDGFRα-promoted glioma cell survival and migration. (**A**) In vitro cell proliferation assays. (**B**) In vitro cell viability assays. (**C**) In vitro cell migration assays were performed and analyzed as in Figure 1C. Bars, SD. *, $P < 0.05$. Data represents three independent experiments with similar results.

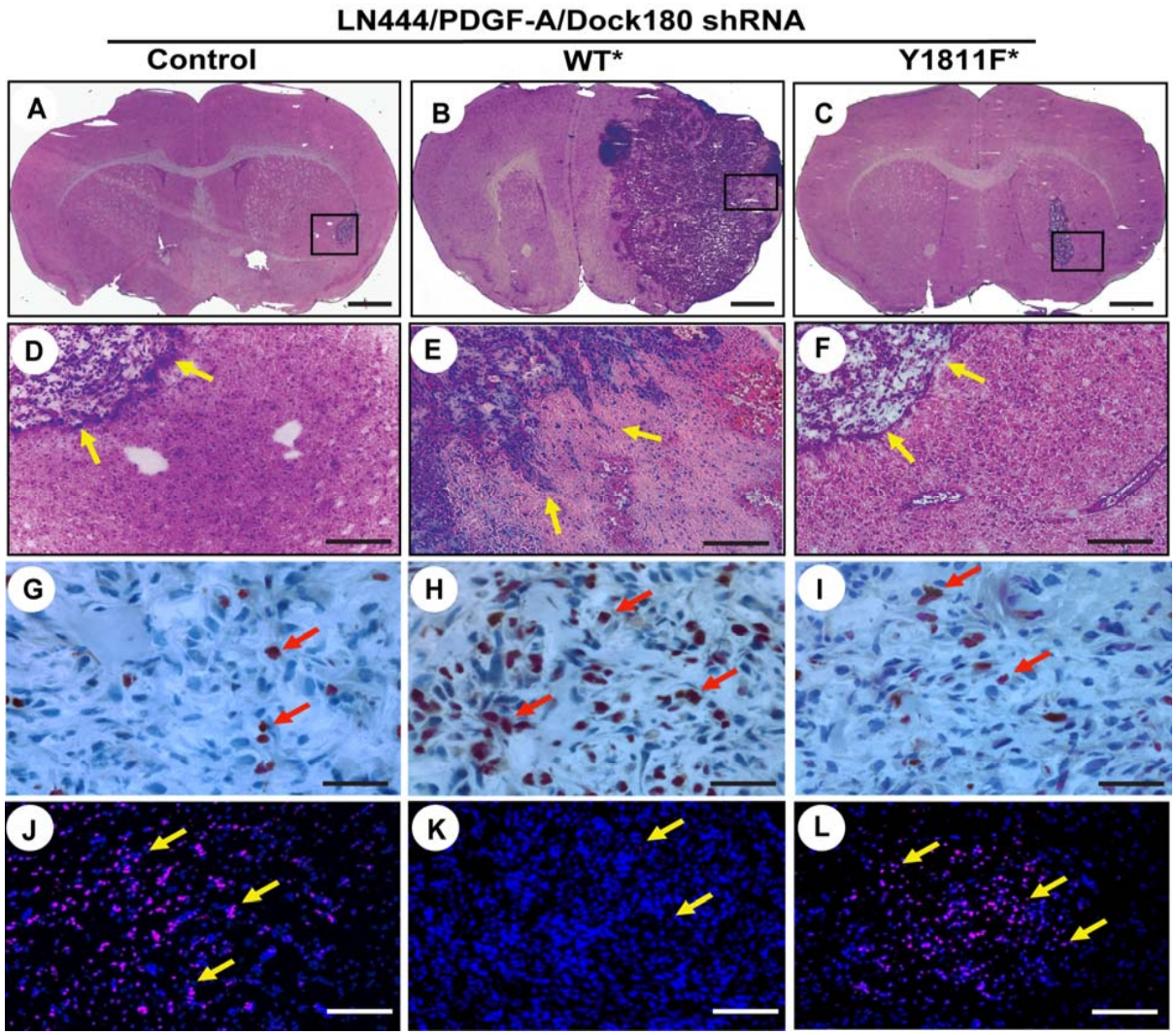

Figure S5. Corresponding to **Figure 4C**-**4K**, re-expression of shRNA-resistant Dock180^{WT*} but not Dock180^{Y1811F*} restored PDGFRα-promoted tumorigenesis of LN444/PDGF-A/shDock180 gliomas in the brain. **A** to **C**, H&E staining (scale bars, 1 mm). **D** to **F**, enlarged areas in **A** to **C** marked with squares (scale bars, 200 μm). Arrows indicate invasive tumor cells (**E**) or non-invasive tumor borders (**D** and **F**). **G** to **I**, Ki-67 staining (scale bars, 50 μm). **J** to **L**, TUNEL staining (scale bars, 100 μm). Arrows in **G** to **L** indicate positively stained tumor cells. Images represent results of 5 mice per group from three independent experiments with similar results.

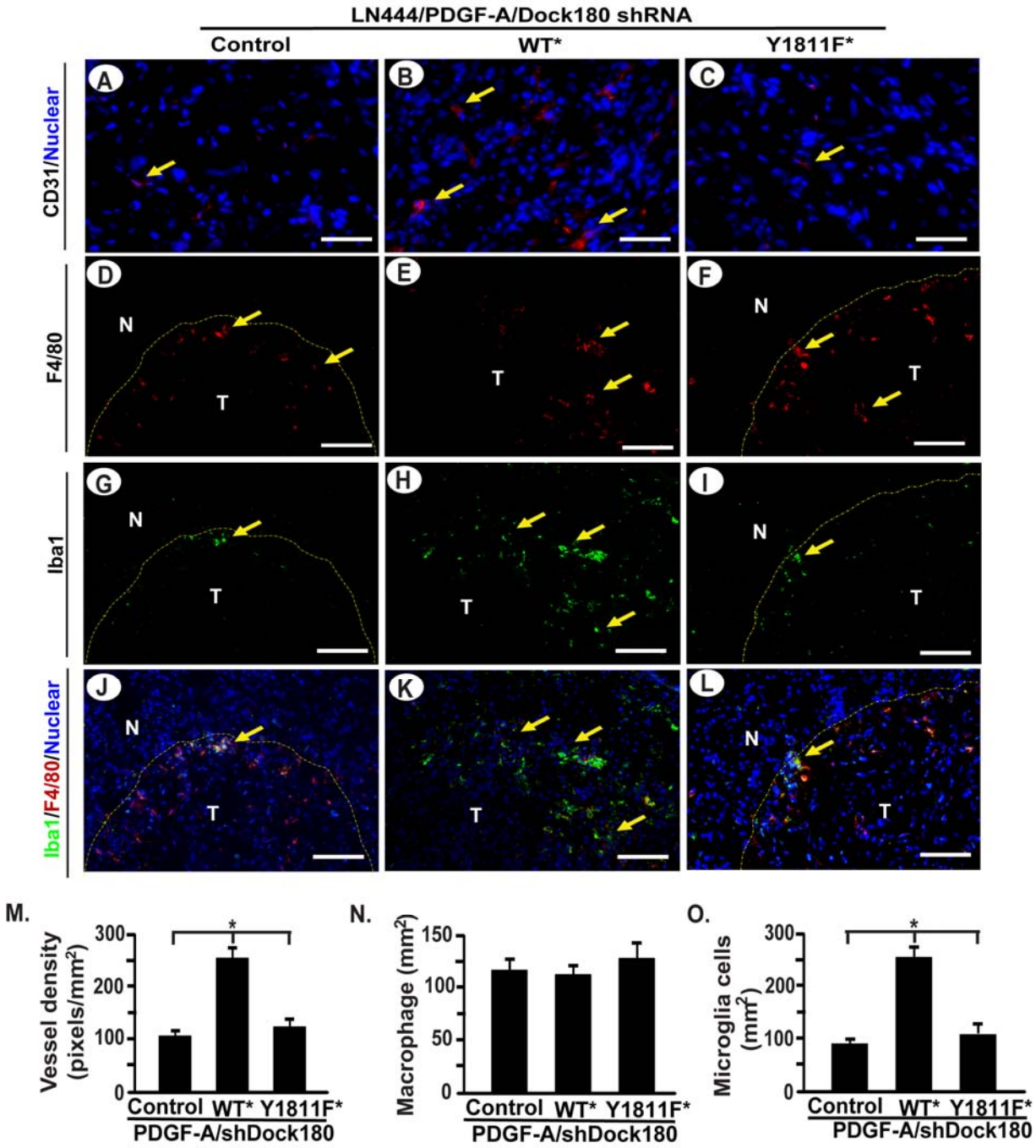

Figure S6. Re-expression of shRNA-resistant Dock180^{WT*} but not Dock180Y^{1811F*} increased growth of tumor neo-vessel and microglia but did not affect macrophage infiltration. **A** to **C**, CD31 and Hoechst 33342 nuclear staining (scale bars, 50 μm). **D** to **F**, F4/80 staining (scale bars, 100 μm). **G** to **I**, Iba1 staining (scale bars, 100 μm). **J**, merge of D, G and nuclear. **K**, merge of E, H and nuclear. **L**, merge of **F**, **I** and nuclear. Images represent results of 5 mice per group with similar results. Arrows indicate positively stained cells. N, normal tissues. T, tumors. **M** to **O** Quantifications of vessel density (CD31 staining), macrophages (F4/80 staining) or microglia cells (Iba1 staining). Bars, SD. *, $P < 0.05$. Data represents three independent experiments with similar results. Dot lines in **D, F, G, T, J** and **L,** tumor borders.

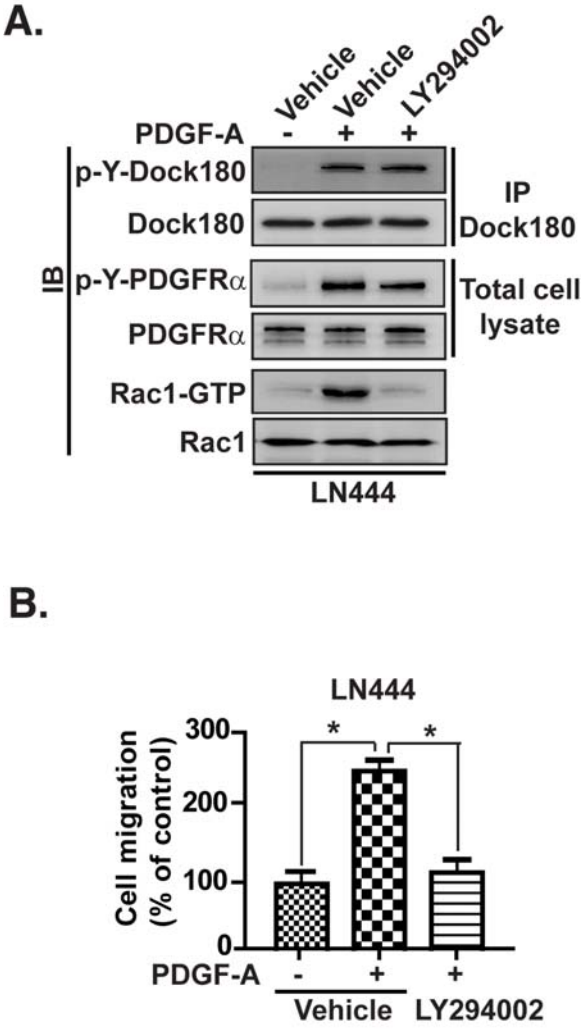

Figure S7. Effect of a PI3K inhibitor LY294002 on PDGFRα-stimulated p-Y of Dock180, Rac1 activities and cell migration in glioma cells. (**A**) Serum-starved LN444 cells were pretreated with or without LY294002 (10 μM) for 1 hr, and then stimulated with or without PDGF-A (50 ng/ml) for 5 min. p-Y-Dock180 is detected with a pan-phospho-tyrosine antibody (4G10). (**B**) In vitro cell migration assays were performed and analyzed as in Figure 1C. *, $P < 0.05$. Data represents two independent experiments with similar results.

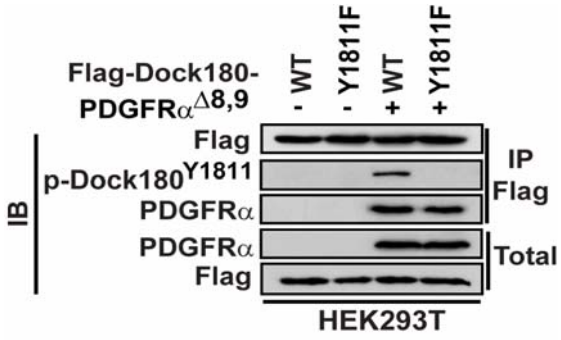

Figure S8. Effect of phosphorylation of Dock180^{Y1811} on Dock180 association with PDGFRα. Dock180^{WT} or Dock180^{Y1811F} was separately co-transfected with or without PDGFR$\alpha^{\Delta 8,9}$ into HEK293T cells. The impact on PDGFRα-induced association of Dock180 with PDGFRα was examined by IP and IB analysis. p-Dock180^{Y1811} is detected using an anti-p-Dock180^{Y1811} antibody. Expression of PDGFRα and Flag-tagged Dock180 were used as loading controls. Data represents two independent experiments with similar results.

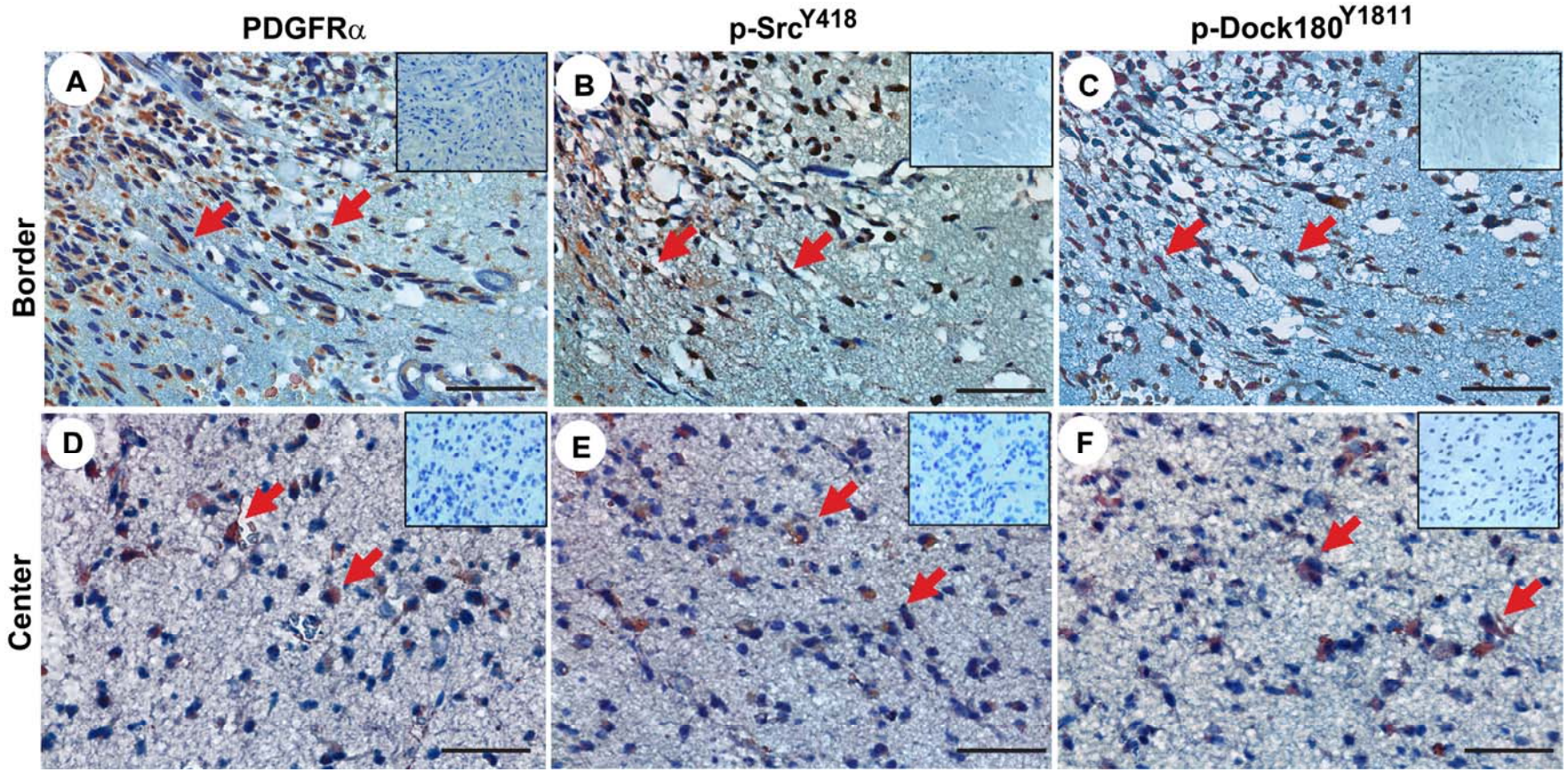

Figure S9. Enlarged images of Figure 9A-9F, IHC staining of a clinical primary WHO grade IV glioma specimen.

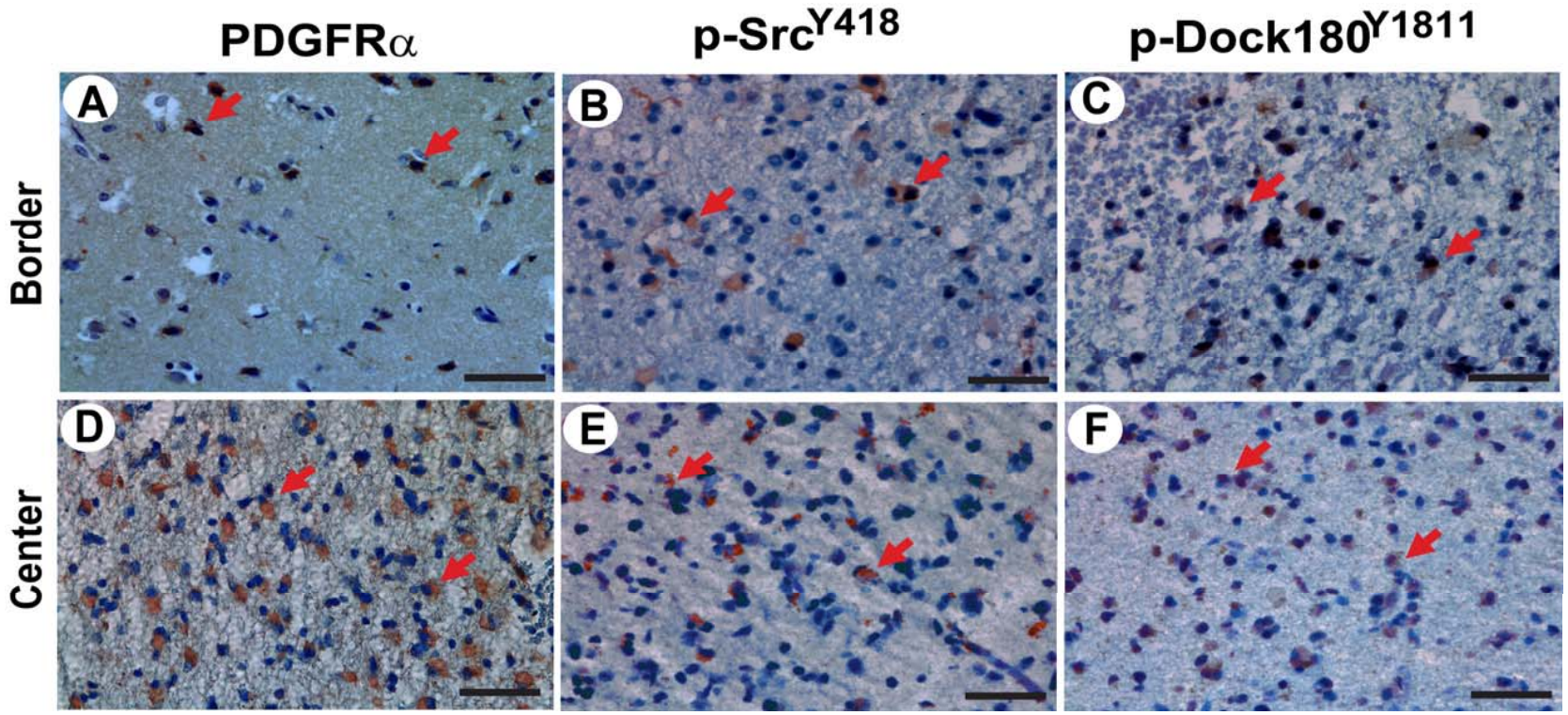

Figure S10. IHC staining of a clinical primary WHO grade III glioma specimen. Representative images of serial sections of a WHO grade III glioma tissue using anti-PDGFRα (**A** and **D**), anti-p-Src^{Y418} (**B** and **E**), and anti-p-Dock180^{Y1811} (**C** and **F**) antibodies are shown. **A** to **C**, invasive area; **D** to **F**, central region. Arrows, positive staining. Scale bars, 50 μm.

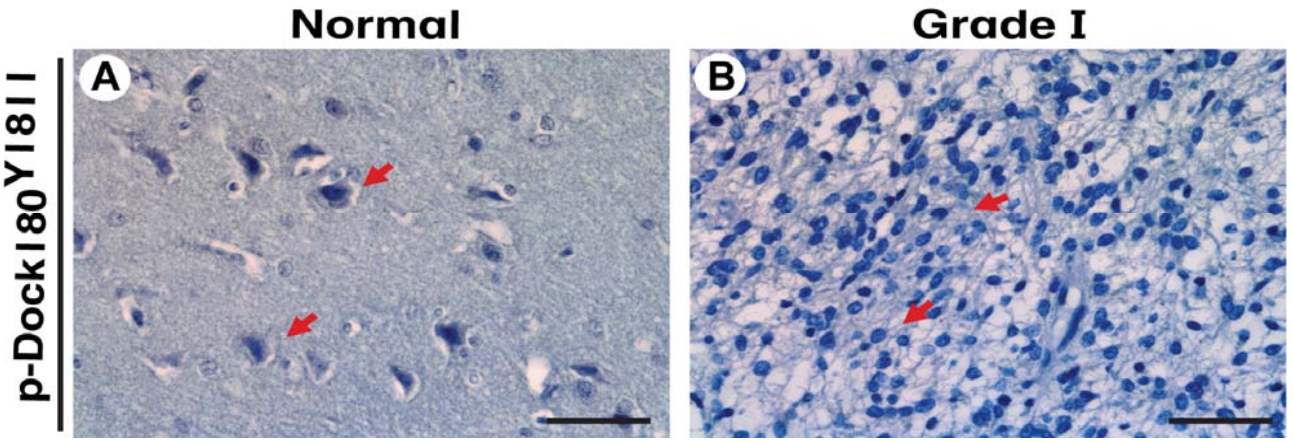

Figure S11. IHC staining of p-Dock180^{Y1811} in a normal brain and a clinical WHO grade I glioma specimen using the specific anti-p-Dock180^{Y1811} antibody. **A**, a normal brain specimen. **B**, a pilocytic astrocytoma (WHO grade I) specimen. Arrows, negative stained cells of p-Dock180^{Y1811}. Scale bars: 50 μm. Data represents two independent IHC analyses on 4 normal brain and 6 WHO grade I glioma specimens with similar results.

Table S1. LN443 and LN444 glioma cells express a group of genes that are statistically similar to a subgroup of signature genes in the proneural subtype of clinical human GBMs.

Comparison	PN[a] similarity score	SD
LN444 vs TCGA_GBMs[b]	0.6613	0.1498
LN444/PDGF-A vs TCGA_GBMs	0.7482[c]	0.1275
LN443 vs TCGA_GBMs	0.6994	0.1298
LN443/PDGF-A vs TCGA_GBMs	0.6632	0.1359

[a]PN, proneural. *SOX3, ALCAM, CKB, DPYSL4, FXYD6, GABRA3, GADD45G, HRASLS, MAPT, MAST1, NRXN2, PODXL2, SLC1A1* and *GALNT13* proneural signature genes in (3) were used as the gene set.
[b]TCGA_GBMs, 54 GBM tumor samples that were classified into proneural group (3).
[c]LN444/PDGF-A and LN444 are significantly different, $P = 0.0024$.

Supplemental Table 2. Immunohistochemical staining of PDGFRα, p-Src^{Y418} and p-Dock180^{Y1811} in human glioma specimens

Case	Histology (WHO Grade)	PDGFRα			p-Src^{Y418}			p-Dock180^{Y1811}		
		Border	Center	Invasion	Border	Center	Invasion	Border	Center	Invasion
J7	GBM(IV)	2+	3+	3+	N/A	N/A	N/A	N/A	N/A	N/A
J10	A.A.(III)	1+	2+	3+	N/A	N/A	N/A	N/A	N/A	N/A
J16	A.O.(III)	2+	3+	3+	3+	2+	2+	2+	1+	2+
J44	D.A.(II)	2+	3+	2+	2+	1+	3+	3+	2+	3+
J57	D.A.(II)	2+	2+	2+	3+	3+	3+	2+	1+	2+
J69	GBM(IV)	3+	3+	2+	2+	1+	2+	3+	2+	3+
J72	GBM(IV)	1+	2+	1+	-	-	-	-	-	-
J80	GBM(IV)	2+	2+	3+	2+	-	3+	2+	-	2+
J94	O.D.(II)	2+	-	2+	3+	-	3+	3+	1+	3+
J152	GBM(IV)	2+	1+	3+	3+	±	3+	3+	2+	3+
J156	GBM(IV)	1+	1+	3+	3+	3+	3+	2+	1+	2+
J158	GBM(IV)	3+	3+	3+	1+	1+	1+	2+	1+	1+
J159	GBM(IV)	1+	3+	2+	-	-	-	2+	±	2+
J161	GBM(IV)	1+	2+	1+	3+	3+	3+	3+	2+	2+
J163	GBM(IV)	3+	2+	3+	2+	2+	2+	2+	2+	1+
J165	A.A.(III)	1+	2+	2+	1+	3+	2+	2+	3+	3+
J171	A.O.D.(III)	2+	3+	3+	1+	±	2+	-	-	-
J186	O.D.(II)	-	-	-	1+	2+	1+	±	-	±
J191	O.D.(II)	-	-	-	2+	3+	2+	1+	1+	±
J194	O.D.(II)	-	-	-	2+	2+	2+	1+	±	1+
J196	A.A.(III)	N/A	2+	N/A	2+	1+	2+	2+	2+	1+
J206	GBM(IV)	-	-	-	±	1+	±	±	±	±
J208	GBM(IV)	-	-	-	1+	2+	1+	2+	2+	2+
J210	GBM(IV)	-	-	-	2+	2+	1+	1+	2+	1+
J211	GBM(IV)	±	2+	±	1+	2+	2+	2+	1+	±
J212	GBM(IV)	1+	1+	3+	2+	1+	1+	3+	2+	1+
J214	GBM(IV)	-	-	-	±	1+	±	±	-	±
J216	GBM(IV)	1+	3+	1+	2+	1+	2+	1+	-	1+
J223	GBM(IV)	-	-	-	2+	1+	2+	±	-	±
J228	GBM(IV)	-	-	-	1+	-	1+	±	-	±
J229	GBM(IV)	-	-	-	N/A	1+	N/A	N/A	±	N/A
J233	GBM(IV)	N/A	1+	N/A	2+	±	1+	2+	1+	1+
J235	GBM(IV)	2+	1+	3+	1+	-	2+	2+	-	2+
JKU01	GBM(IV)	1+	1+	2+	2+	-	2+	2+	-	2+
JKU02	GBM(IV)	2+	±	3+	2+	1+	2+	2+	2+	1+
JKU03	GBM(IV)	1+	2+	3+	1+	-	2+	1+	1+	2+
JKU04	GBM(IV)	2+	2+	2+	2+	3+	3+	1+	-	1+
JKU05	A.A.(III)	1+	2+	3+	1+	-	1+	2+	2+	3+
JKU06	GBM(IV)	2+	2+	3+	3+	3+	3+	3+	3+	3+
JKU07	GBM(IV)	1+	2+	2+	3+	3+	3+	2+	1+	3+
JKU08	GBM(IV)	3+	3+	3+	3+	2+	3+	3+	1+	3+
JKU09	GBM(IV)	2+	2+	1+	3+	2+	3+	2+	2+	3+
JKU10	GBM(IV)	2+	±	2+	1+	3+	1+	2+	2+	2+

D.A., diffuse astrocytoma; O.A., oligoastrocytoma; O.D., oligodendroglioma; A.A.; anaplastic astrocytoma; A.O.D., anaplastic oligodendroglioma; A.O.A., anaplastic oligoastrocytoma.

3+, signals in most tumor cells (~50%); 2+, signals in some tumor cells (<25%); 1+, signals in few tumor cells (<5%); ±, low or no signals in few tumor cells (<1%); - No detectable signals in all tumor cells (0%).

N/A, not available.

Table S3. Statistical analysis of expression level of PDGFRα and p-Dock180^{Y1811} in human clinical glioma specimens by IHC staining

IHC Score	PDGFRα			p-Dock180^{Y1811}		
	Border	Center	Invasion	Border	Center	Invasion
3+	4	14	16	7	5	10
2+	12	15	11	19	15	14
1+	15	6	4	11	11	9
±	1	2	1	6	4	9
-	99	97	99	84	90	84
N/A*	3	0	3	7	9	8

* N/A, not available.

Table S4. Spearman's rank correlation analysis of expression level of PDGFRα and p-Dock180^{Y1811} in human clinical glioma specimens by IHC staining

PDGFRα vs p-Dock180^{Y1811}	r	*P*
Border vs Border	0.9000*	0.0417
Center vs Center	0.9000*	0.0417
Invasion vs Invasion	0.8721*	0.0417

*. Correlation is significant at the 0.05 level (1-tailed).

Supplemental Experimental Procedures

Antibodies and reagents

The following antibodies were used in our studies: anti-Dock180 (H-4 and N-19), anti-PDGFRα (C-20), anti-PDGF-A (N-30), anti–β-actin (I-19), anti-Met (C-12), anti-phospho-PDGFRα (Tyr754) and anti-Src (SRC 2) antibodies (Santa Cruz Biotechnology); an anti-pan-phosphotyrosine antibody (4G10)(Millipore-Upstate); anti-Rac1, anti-Crk and anti-$p130^{Cas}$ antibodies (BD Transduction Laboratories); a monoclonal anti-Flag M2 antibody (Sigma-Aldrich); anti-phospho-p44/42 MAP Kinase (Thr202/Tyr204, #9101), anti-p44/42 MAP Kinase (#9102), anti-phospho-Akt (Ser473, #4051), anti-Akt (#9272), anti-phospho-PAK1(Thr423)/PAK2(Thr402) and anti-phospho-EGFR (Tyr1045) antibodies (Cell Signaling Technology); an anti-Ki67 antigen (NCL-Ki67p) antibody (Leica Microsystems Inc.); an anti-c-Met ($pYpY^{1230/1234/1235}$) antibody (BioSource International, Inc.), and a Rac1 activation assay kit (Millipore-Upstate). The secondary antibodies were from Vector Laboratories or Jackson ImmunoResearch Laboratories. Peroxidase blocking reagent was from DAKO; AquaBlock was from East Coast Biologics, Inc. Cell culture media and other reagents were from Hyclone, Invitrogen, Sigma-Aldrich, and Fisher Scientific.

Knockdown of Dock180 and Src by siRNA and shRNA

Endogenous Dock180 in various glioma cells was knocked down using siRNA as previously described (4). Briefly, a pool containing three separate Dock180 siRNAs (Santa Cruz Biotechnology) was transiently transfected into LN443, LN444 and GBM5 cells. To stably knock down endogenous Dock180 in LN444/PDGF-A cells, two separate pG-SUPER-Dock180 shRNAs were constructed as previously described (5). pG-SUPER-

Dock180 shRNAs and pG-SUPER-GFP were separately co-transfected with a pcDNA3 vector at a ratio of 10:1 into LN444/PDFG-A cells with Effectene reagent. G418-resistant cell populations were selected and resorted by GFP expression via FACS. Efficiencies of Dock180 knockdown in these cells was determined by IB analysis. To stably deplete endogenous Src in glioma cells, LN444/PDGF-A cells that re-expressed vector control, shRNA-resistant Dock180^{WT} or Dock180^{Y1811F} were separately infected by lentivirus-mediated shRNA #4 and #5 for Src obtained from the Broad Institute of Harvard and MIT. The puromycin-resistant cell populations were expanded and examined for efficiency of Src knockdown by IB analysis.

Plasmids

The pcDNA3-Flag-Dock180 plasmid was derived from a pCXN2-Flag-Dock180 (a gift from Dr. M. Matsuda) (6). Briefly, a 596-bp DNA fragment of pCXN2-Dock180 amplified by PCR was cloned into a pcDNA3 vector (Invitrogen) with XhoI and XbaI to generate a pcDNA3-Flag-Dock180-XbaI. Then a 5.314-kb DNA fragment released from a pCXN2-Dock180 by XbaI was ligated to a pcDNA3-Flag-Dock180-XbaI to generate a pcDNA3-Flag-Dock180. The pcDNA3-(His)$_6$-Dock180 plasmid was derived from a pcDNA3-Flag-Dock180.

pcDNA3-PDGFR$\alpha^{\Delta 8,9}$ was derived from a pUC119-PDGFR$\alpha^{\Delta 8,9}$ (a gift from Dr. I. Clarke) (7). pLVX-Flag-Dock180 plasmid was constructed from a pcDNA3-Flag-Dock180 (Clontech). pMXI-PDGFRα and pMXI-PDGFA were generated from a pcDNA3-PDGFRα and a pLNCX2-PDGFA (a gift from Dr. C.-H. Heldin), respectively. pEBB-CrkII wild type, pSGT-Src-wild type, Src-kinase dead and Src-Y527F were the gifts from Drs. R. Birge and

S. Courtneidge, respectively. A pcDNA3-EGFP-Rac1-WT (Addgene plasmid 12980) was purchased from Addgene Inc. (Cambridge, MA) (8).

Deletion Mutants and Mutagenesis

To construct pcDNA3-Flag-Dock180-ΔDHR1 or -ΔDHR2, the fragment including -ΔDHR1 or -ΔDHR2 released from pcDNA3.1-Dock180-ΔDHR1 or -ΔDHR2 by XbaI was cloned to a pcDNA3-Flag-Dock180-XbaI (9). pcDNA3-Flag-Dock180-ApaI, -ΔDHR1-ApaI, -ΔDHR1-ApaI-StuI, -ΔDHR1- ApaI-PvuI, and -ΔDHR1- ApaI–BglII was derived from pcDNA3-Flag-Dock180 or -ΔDHR1. A pcDNA3-Flag-Dock180-Δ160 was constructed by inserting a 53-bp fragment into pcDNA3-Flag-Dock180 at XhoI and XbaI sites. The fragment was synthesized with an oligo pair: forward, 5' TCGAG ATGGACTACAAAGACGATGACGATAAAGACTACAAAGACGATGACGATAAAATT 3' and reverse, 5' CTAGAATTTTATCGTCATCGTCTTTGTAGTC TTTATCGTCATCGTCTTTGTAGTCCATC 3'.

Mutations of Dock180^{Y1811F}, siRNA-resistant Dock180^{WT*} and Dock180^{Y1811F*} were performed with the QuikChange multisite-directed mutagenesis kit (Stratagene), according to the manufacturer's instructions, by using the following oligonucleotide: 5' GGCAGCGTGGCAGATTTCGGGAATTTGATGGAAAAC CAGG 3' and 5' GGAACAGCAAACATCAAGAAATCTTTGAGAAAGAATTTAAACCC 3', respectively. Mutant constructs were verified by automated DNA sequencing.

Cell Proliferation and Viability Assays

In vitro cell proliferation analyses of various glioma cell lines were performed as we recently described (10). Briefly, 50,000 cells were seeded in 10% FBS/ DMEM, split, counted, and re-seeded every 2 days in a 6-well plate. Population doubling was calculated by dividing the total cell number by the cells seeded (50,000 cells), using log2 versus the days in culture to determine the proliferation rate of each type of cells.

To determine cell survival and apoptosis, various cells were plated on 8-well chamber slides. After overnight incubation, the medium was replaced with fresh DMEM plus 0.5% FBS. After 48 hr, slides were fixed by 4% formaldehyde in ice-cold PBS and permeabilized with 0.2% Triton X-100 solution. After equilibration, slides were immersed in TUNEL reaction buffer at 37°C for 60 min inside the humidified chamber. Reaction was stopped by 2X saline sodium citrate (SSC) and slides were counterstained with Hoechst 33258 and mounted in VECTASHIELD (Vector Lab). One thousand cells of each slide were randomly examined and numbers of TUNEL-positive cells were counted under a microscope equipped with a SPOT digital camera. The data were analyzed using GraphPad Software.

In vitro Cell Migration and Rac1 Activation Assays

In vitro cell migration assays were performed as we previously described (5). Briefly, various cells were serum-starved for 24 hr, washed with PBS and resuspended in DMEM plus 0.1% FBS. 50 µl of cells (5 x 10^5/ml) was placed into the top compartment of a Boyden chamber and the bottom chamber was added with or without 50 ng/ml PDGF-A and indicated inhibitors. The cells were allowed to migrate through an 8-µm pore size membrane precoated with fibronectin (10 µg/ml) for 10 or 16 hr at 37°C. Afterwards, the

membrane was fixed, stained and analyzed. Rac1 activation assays were preformed using a Rac1 activation assay kit (Millipore-Upstate) as we previously described (4).

Gene expression analysis

Total RNA extracted from glioma cell lines was used as template for the generation of labeled cRNA that was hybridized to Agilent 4 x 4 4k whole human genome microarrays and scanned with an Agilent scanner and Feature Extraction Software (Agilent Technologies, Santa Clara, CA) as recommended. The complete data set has been submitted and is currently available in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/; accession number: GSE28748).

Comparison of gene expression profiles of glioma cell lines with TCGA data

Fourteen proneural signature genes were selected based on published TCGA data (3): *SOX3, ALCAM, CKB, DPYSL4, FXYD6, GABRA3, GADD45G, HRASLS, MAPT, MAST1, NRXN2, PODXL2, SLC1A1* and *GALNT13*. The overall expression profiles of the cell lines in this study were determined using Agilent GE array. For each cell line, the gene expression profile of above genes was represented as a 14-dimensional vector containing the expression values of the genes; the vectors were further normalized as unit vectors. The gene expression data for 54 GBM tumor samples classified into proneural group (3) were downloaded from the TCGA data portal; expression values of these 14 genes in these samples were extracted. Expression profile of above signature genes in a sample was also represented as a 14-dimensional unit vector. The similarity of two gene expression profiles was measured using a linear algebra metric referred to as "dot product" of two unit vectors, which reflects the cosine of the angle between two vectors in a vector

space. The range of a dot product is (0, 1); the larger a dot product, the smaller the angle between the two vectors. For each cell line, we calculated the dot products of the gene expression vector with each of the 54 proneural vectors. The dot products (similarity scores) between the parent cell line and proneural tumors were compared to those derived between the experimental cell lines and the proneural tumors. A Welch two sample t-test was applied to compare the dot products from parental against those from an experiment cell line.

Analysis of *PDGFRA* gene copy number status

As previously described (1), *PDGFRA* gene status was determined by q-PCR analysis in glioma cells: U87, U251, U373, T98G, LNZ308, LN18, LN235, LN319, LN443, LN444, SNB19, and primary GBM cells: GBM5, GBM6, GBM14. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) and quantified. Reference DNA was obtained from normal human astrocytes. q-PCR was performed in triplicate using gene-specific primer sets for *PDGFRA* or 18S, respectively. Reagents and protocols of a 7900 HT Fast Real-Time PCR System were from Applied Biosystems. The PCR amplification was performed in a 10-µl reaction volume under the following conditions: 2X Power SYBR Green PCR Master Mix (Applied Biosystems), 0.5 µM primers, and 20 ng DNA. The $\Delta\Delta$Ct method was used to calculate *PDGFRA* gene copy number in the tumor DNA sample normalized to the reference gene (*18S*) and calibrated to normal DNA. Fold increase of PDGFRA gene was calculated by $2^{(\Delta\Delta Ct)}$. Values $\geq$5 were considered as gene amplification and values between 2 and 5 were considered as increased gene copy numbers (1, 2).

Immunoprecipitation (IP) and Immunoblotting (IB)

IB and IP analyses were performed as we previously described (5). Briefly, various cells that were treated with or without 50 ng/ml PDGF-A or indicated inhibitors were lysed in an IP buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 2 mM Na_3VO_4, 5 mM NaF, 1%Triton X-100 and protease inhibitor cocktail) at 4°C for 30 min. The lysates were centrifuged for 20 min at 12,000 x g to remove debris. Protein concentrations were determined with a BCA protein assay kit. Equal amounts of cell lysates were immunoprecipitated with specific antibodies and protein G-agarose beads (Invitrogen). Immunoprecipitates were washed five times with IP buffer, resolved in a 2X SDS lysis buffer and analyzed by IB (5, 10).

Mouse glioma xenografts

All animal experiments were approved by Institutional Animal Care and Use Committee (IACUC) of University of Pittsburgh. Various human glioma cells (5 $X10^5$ in 5 µl PBS) were stereotactically implanted into the brain of individual mice with 5 mice per group. The glioma-bearing mice were sacrificed 8 weeks post-implantation. The brains were removed, processed, and analyzed as previously described (10).

References

1. Martinho, O, Longatto-Filho, A, Lambros, MB, Martins, A, Pinheiro, C, Silva, A, Pardal, F, Amorim, J, Mackay, A, Milanezi, F, et al. Expression, mutation and copy number analysis of platelet-derived growth factor receptor A (PDGFRA) and its ligand PDGFA in gliomas. *Br J Cancer.* 2009;101(6):973-982.

2. Ozawa, T, Brennan, CW, Wang, L, Squatrito, M, Sasayama, T, Nakada, M, Huse, JT, Pedraza, A, Utsuki, S, Yasui, Y, et al. PDGFRA gene rearrangements are frequent genetic events in PDGFRA-amplified glioblastomas. *Genes Dev.* 2010;24(19):2205-2218.

3. Verhaak, RG, Hoadley, KA, Purdom, E, Wang, V, Qi, Y, Wilkerson, MD, Miller, CR, Ding, L, Golub, T, Mesirov, JP, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell.* 2010;17(1):98-110.

4. Jarzynka, MJ, Hu, B, Hui, KM, Bar-Joseph, I, Gu, W, Hirose, T, Haney, LB, Ravichandran, KS, Nishikawa, R, and Cheng, SY. ELMO1 and Dock180, a bipartite Rac1 guanine nucleotide exchange factor, promote human glioma cell invasion. *Cancer Res.* 2007;67(15):7203-7211.

5. Hu, B, Shi, B, Jarzynka, MJ, Yiin, JJ, D'Souza-Schorey, C, and Cheng, SY. ADP-ribosylation factor 6 regulates glioma cell invasion through the IQ-domain GTPase-activating protein 1-Rac1-mediated pathway. *Cancer Res.* 2009;69(3):794-801.

6. Kiyokawa, E, Hashimoto, Y, Kurata, T, Sugimura, H, and Matsuda, M. Evidence that DOCK180 up-regulates signals from the CrkII-p130(Cas) complex. *J Biol Chem.* 1998;273(38):24479-24484.

7. Clarke, ID, and Dirks, PB. A human brain tumor-derived PDGFR-alpha deletion mutant is transforming. *Oncogene.* 2003;22(5):722-733.

8. Subauste, MC, Von Herrath, M, Benard, V, Chamberlain, CE, Chuang, TH, Chu, K, Bokoch, GM, and Hahn, KM. Rho family proteins modulate rapid apoptosis induced by cytotoxic T lymphocytes and Fas. *J Biol Chem.* 2000;275(13):9725-9733.

9. Cote, JF, Motoyama, AB, Bush, JA, and Vuori, K. A novel and evolutionarily conserved PtdIns(3,4,5)P3-binding domain is necessary for DOCK180 signalling. *Nat Cell Biol.* 2005;7(8):797-807.

10. Liu, KW, Feng, H, Bachoo, R, Kazlauskas, A, Smith, EM, Symes, K, Hamilton, RL, Nagane, M, Nishikawa, R, Hu, B, et al. SHP-2/PTPN11 mediates gliomagenesis driven by PDGFRA and INK4A/ARF aberrations in mice and humans. *J Clin Invest.* 2011;121(3):905-917.