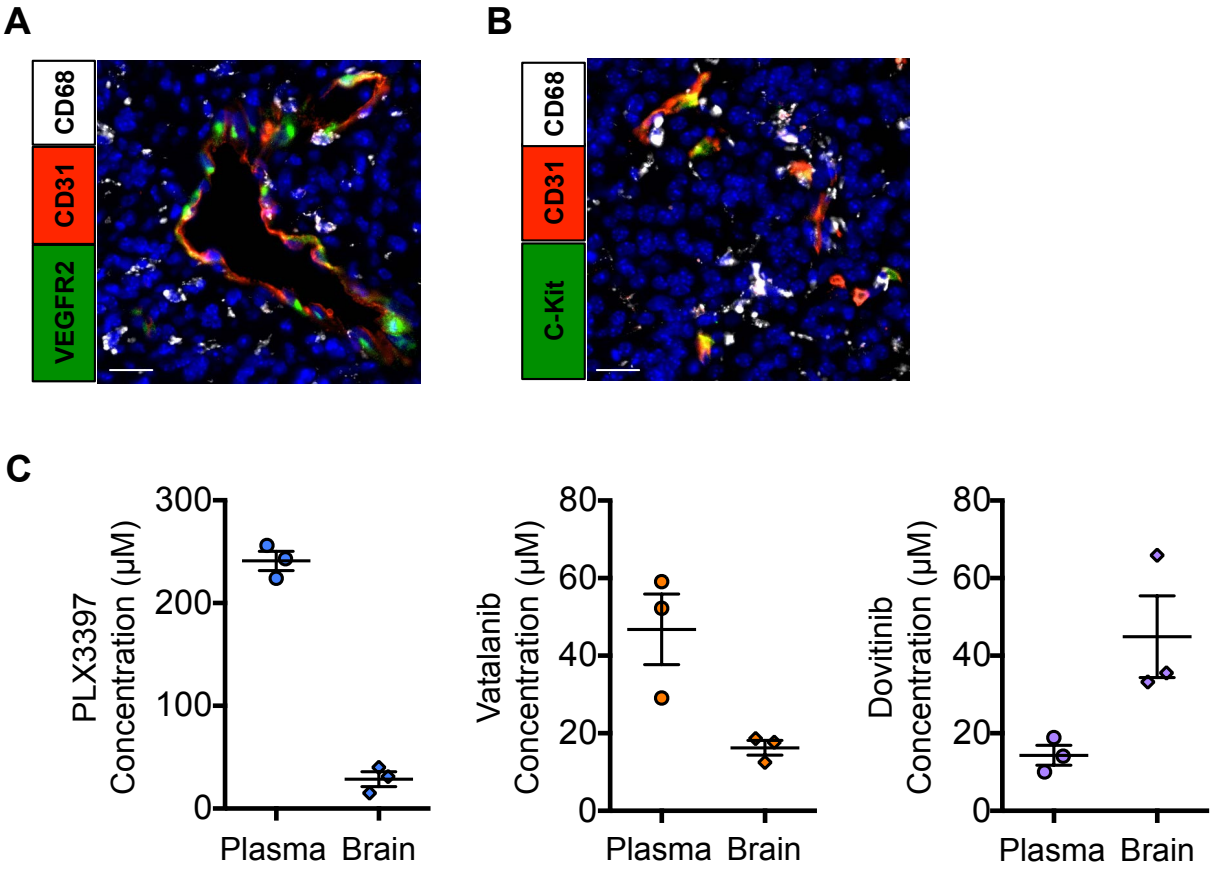
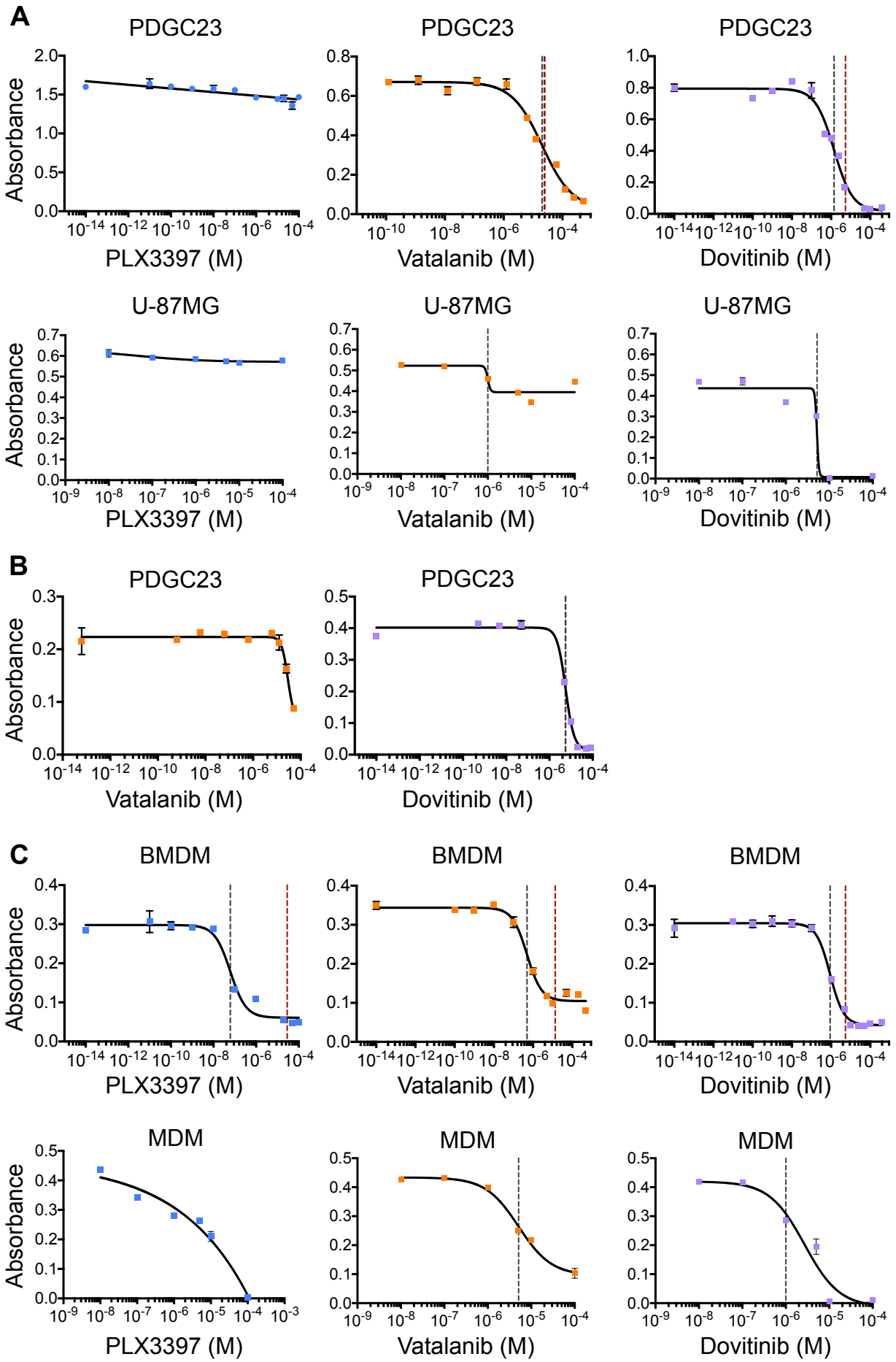
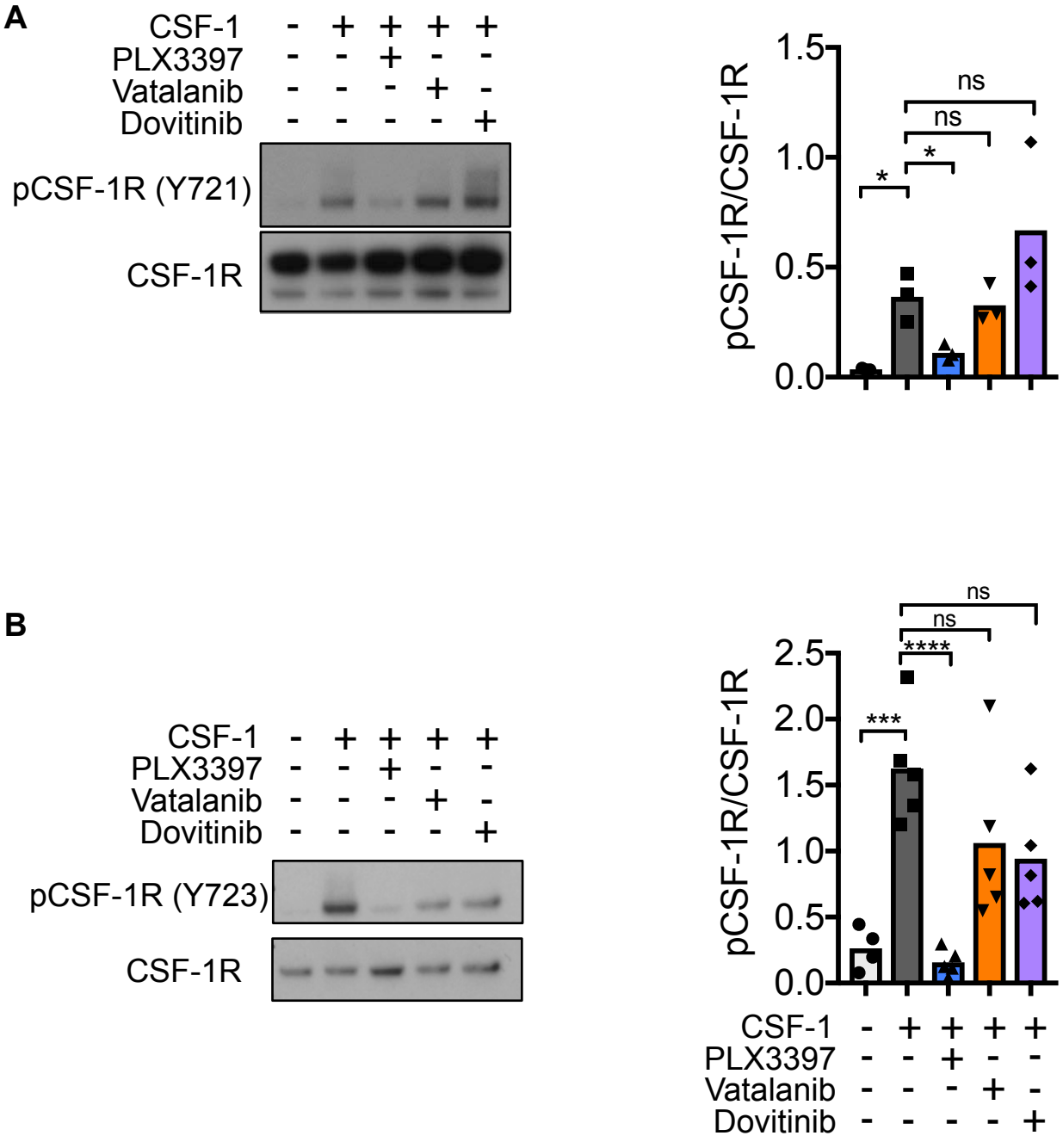

A
CD68
CD31
VEGFR2
B
CD68
CD31
C-Kit
C
PLX3397 Concentration (μM)
300
200
100
0
Plasma Brain
Vatalanib Concentration (μM)
80
60
40
20
0
Plasma Brain
Dovitinib Concentration (μM)
80
60
40
20
0
Plasma Brain
D
Vehicle PLX3397 Vatalanib Dovitinib
pCSF-1R (Y721)
CSF-1R
pCSF1R normalized to total CSF1R (r.u.)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
***
Vehicle
PLX3397
Vatalanib
Dovitinib
Vehicle PLX3397 Vatalanib Dovitinib
pPDGFR-α (Y762)
PDGFR-α (short exposure)
PDGFR-α (long exposure)
pPDGFR-α normalized to total PDGFR-α (r.u.)
1.6
1.2
0.8
0.4
0.0
****
****
Vehicle
Vatalanib
Dovitinib

**Supplementary Figure 1. Intratumoral expression of receptor tyrosine kinases and on-target inhibition *in vivo*.** **(A)** Co-localization of VEGFR2 and $CD31^+$ endothelial cells in PDG tumors. **(B)** Expression pattern of c-Kit staining showing overlap with $CD31^+$ endothelial cells and $CD31^-$ $CD68^-$ tumor cells. Scale bars in panels A and B: 20 μm. **(C)** Pharmacokinetic analysis of PLX3397, vatalanib and dovitinib. Mouse plasma and brain samples (from non-tumor-bearing mice) were analyzed to determine compound concentration 2 hours after dosing, n=3 mice. **(D)** Representative immunoblots of phosphorylated CSF-1R, phosphorylated PDGFR-α and respective total receptors using fractionated whole tumor lysates from animals treated with indicated inhibitors. Densitometry analysis in the right panels shows inhibition of CSF-1R by PLX3397 and inhibition of PDGFR-α by vatalanib and dovitinib, n=3 tumors for each group. The effect of PLX3397 on PDGFR-α phosphorylation could not be assessed due to very low numbers of PDGFR-α-positive tumor cells in the efficiently-debulked gliomas of this treatment group. One-way ANOVA with Sidak's multiple comparisons test was used to calculate statistical significance. * $P<0.05$; *** $P<0.001$; **** $P<0.0001$.

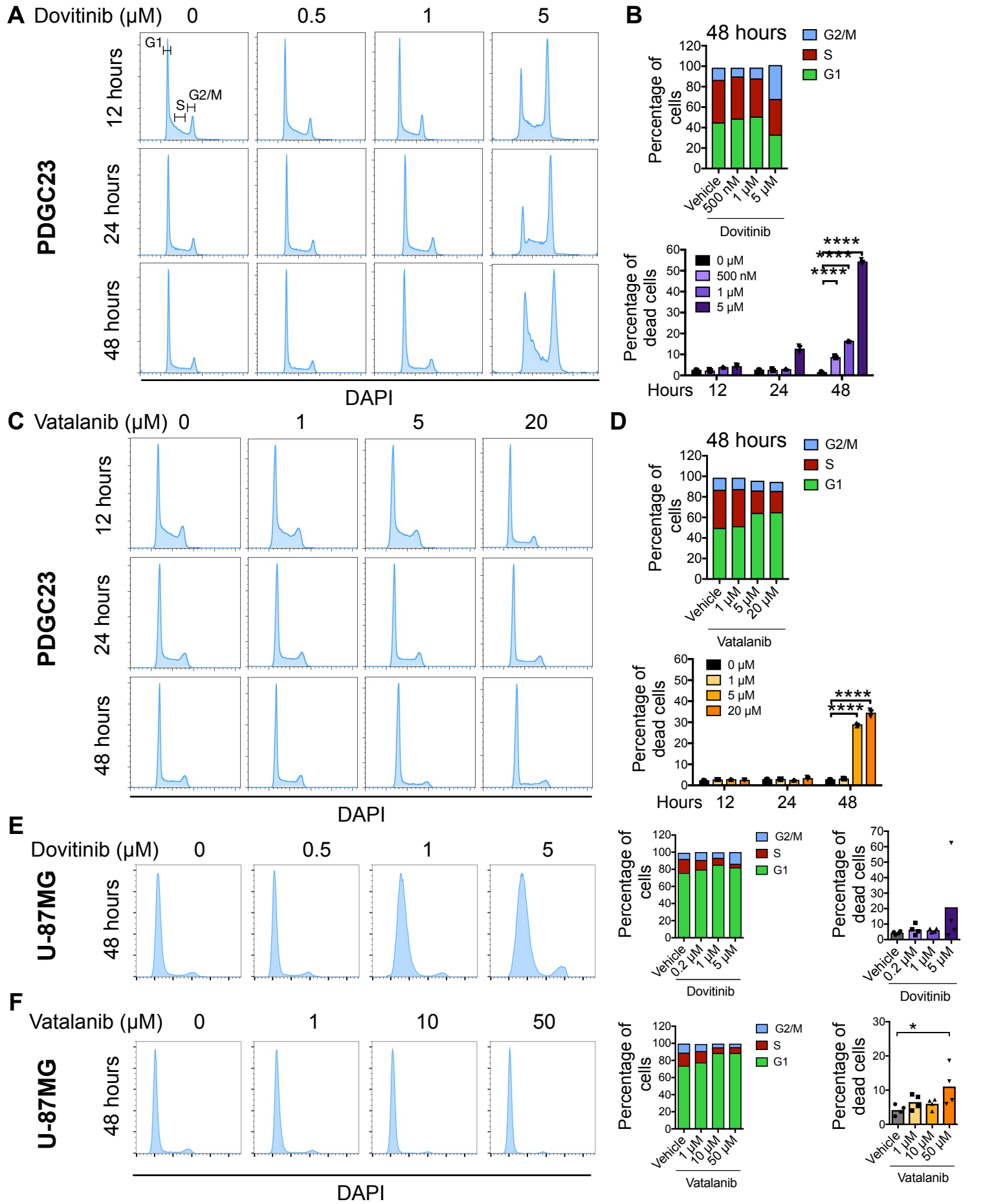

A
PDGC23
PDGC23
PDGC23
Absorbance
PLX3397 (M)
Vatalanib (M)
Dovitinib (M)
U-87MG
U-87MG
U-87MG
Absorbance
PLX3397 (M)
Vatalanib (M)
Dovitinib (M)
B
PDGC23
PDGC23
Absorbance
Vatalanib (M)
Dovitinib (M)
C
BMDM
BMDM
BMDM
Absorbance
PLX3397 (M)
Vatalanib (M)
Dovitinib (M)
MDM
MDM
MDM
Absorbance
PLX3397 (M)
Vatalanib (M)
Dovitinib (M)

**Supplementary Figure 2. On-target inhibition of receptor tyrosine kinases *in vitro*. (A)** Dose-response curves of murine PDGC23 cells (upper part) and human U-87MG cells (lower part) treated with PLX3397, vatalanib and dovitinib, in serum-free media as assessed using MTT assays. Gray dashed line indicates cellular $IC_{50}$ and red dashed line denotes physiological compound concentration in the brain parenchyma determined by pharmacokinetic analysis. Graphs depict a representative experiment from a minimum of 4 independent assays. **(B)** MTT assay showing the dose-response relationship between vatalanib or dovitinib and PDGC23 viability, in the presence of PDGF-BB (1 ng/mL). **(C)** Dose-response curves of murine bone marrow-derived macrophages (BMDMs) and human monocyte-derived macrophages (MDMs) treated with PLX3397, vatalanib or dovitinib in the presence of CSF-1 (10 ng/mL), with the gray dashed line indicating cellular $IC_{50}$ and the red dashed line denoting physiological compound concentration in the brain parenchyma. Graphs depict a representative experiment from 6 independent experiments for both cell types.


A
CSF-1 - + + + +
PLX3397 - - + - -
Vatalanib - - - + -
Dovitinib - - - - +
pCSF-1R (Y721)
CSF-1R
pCSF-1R/CSF-1R
1.5
1.0
0.5
0.0
ns
ns
*
*
B
CSF-1 - + + + +
PLX3397 - - + - -
Vatalanib - - - + -
Dovitinib - - - - +
pCSF-1R (Y723)
CSF-1R
pCSF-1R/CSF-1R
2.5
2.0
1.5
1.0
0.5
0.0
ns
ns
****
***

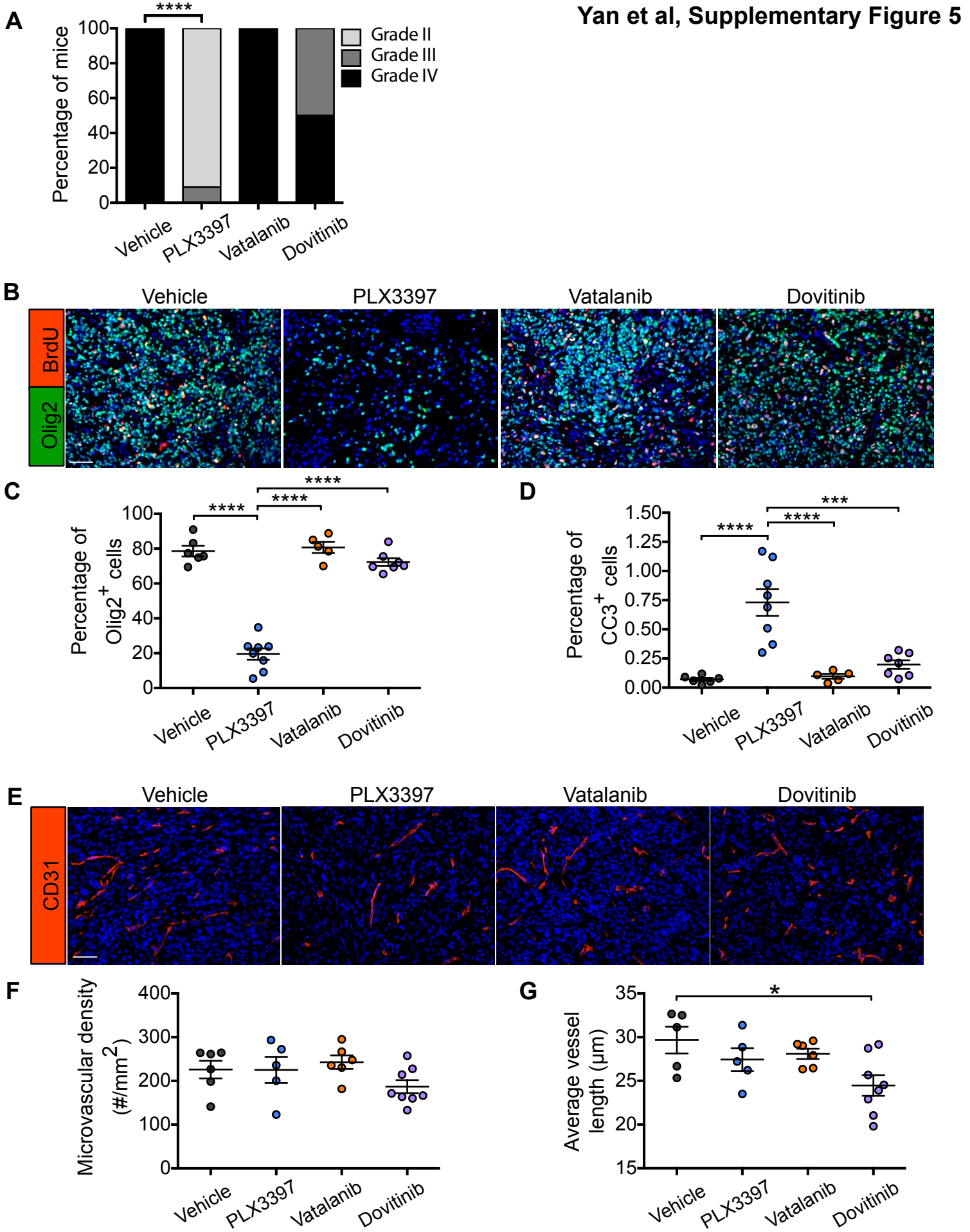
**Supplementary Figure 3. Analysis of CSF-1R phosphorylation in the presence of receptor tyrosine kinase inhibitors.** Representative immunoblot of phosphorylated CSF-1R and total CSF-1R using lysates from (A) murine BMDMs and (B) human MDMs. Cells were cultured in the absence of CSF-1 for 12 hours and pre-treated with inhibitors: PLX3397 (70 nM or 500 nM, respectively), vatalanib (500 nM) and dovitinib (900 nM or 500 nM, respectively) for 1 hour, and then stimulated with CSF-1 (10 ng/mL) for 5 minutes. Graphs depict the quantification of 3 (BMDMs) and 5 (MDMs) independent experiments. For (A), paired t-test was used to assess statistical significance and for (B) one-way ANOVA with Dunnett's multiple comparison test was used to calculate statistical significance. *P<0.05; *** *P*<0.001; **** *P*<0.0001.

# Yan et al, Supplementary Figure 4



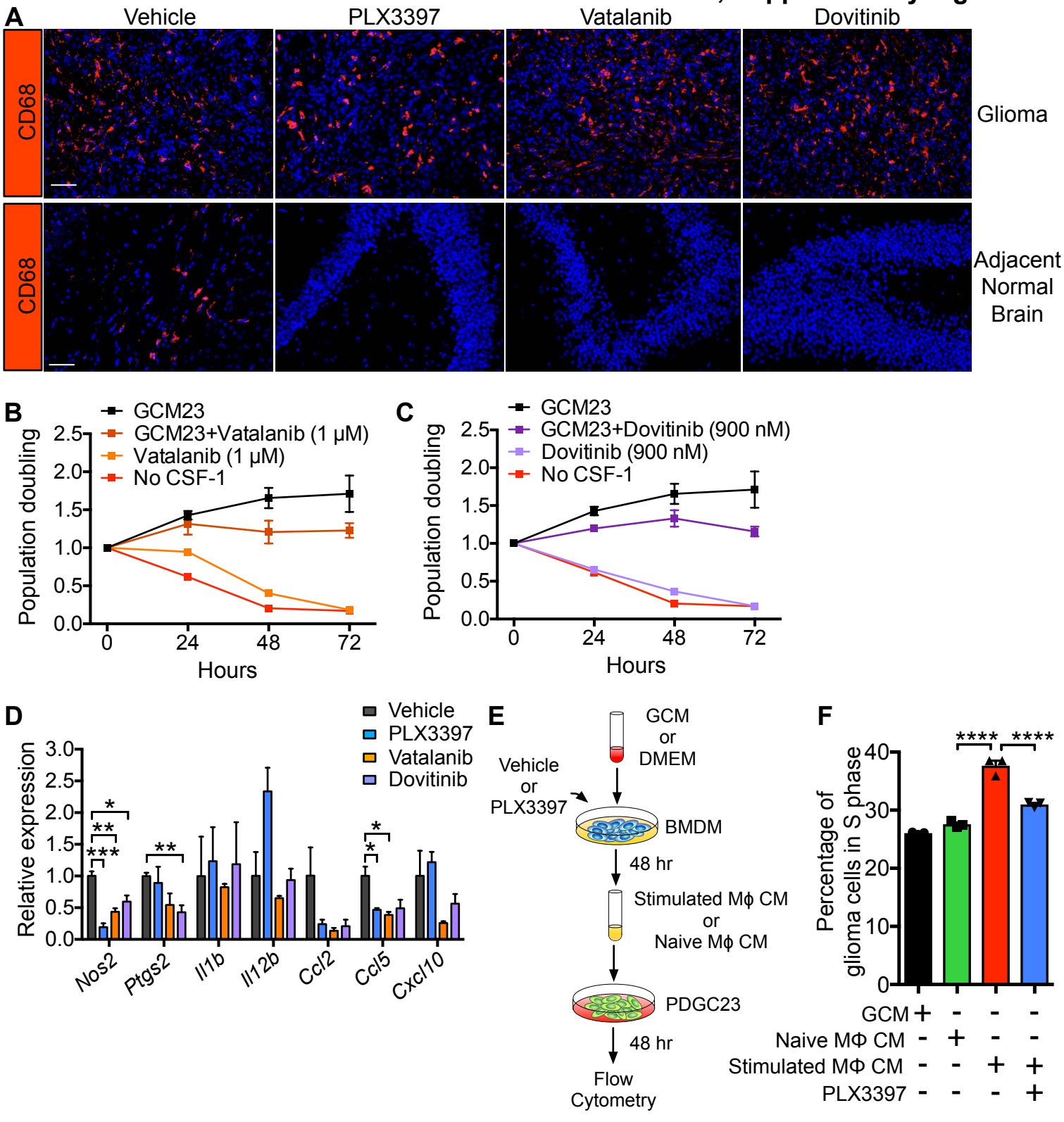
**Supplementary Figure 4. Perturbations of cell cycle and cell viability by receptor tyrosine kinase inhibitors.** **(A)** Cell cycle analysis of dovitinib-treated murine PDGC23 cells at 12-hour, 24-hour and 48-hour time points. **(B)** Quantification of the percentage of cells in each of phase of cell cycle at 48h (upper panel) and dead glioma cells (APC-Cy7$^{+}$) after 12-hour, 24-hour and 48-hour incubation with dovitinib (500 nM, 1 μM, 5 μM) (bottom panel), n=3 independent experiments. **(C)** Cell cycle analysis of vatalanib-treated PDGC23 cells at 12-hour, 24-hour and 48-hour time points. **(D)** Quantification of the percentage of cells in each of phase of cell cycle at 48h (upper panel) and dead glioma cells (APC-Cy7$^{+}$) (bottom panel) after 12-hour, 24-hour and 48-hour incubation with vatalanib (1 μM, 5 μM, 20 μM), n=3 independent experiments. **(E)** Cell cycle analysis of dovitinib-treated U-87MG cells at the 48-hour time point, **(F)** Cell cycle analysis of vatalanib-treated U-87MG cells at the 48-hour time point. For B, D, E and F: individual points represent independent experiments. For B, D, E, F: One-way ANOVA with Dunnett's multiple comparisons test was used to calculate statistical significance, **** $P$<0.0001.

**Yan et al, Supplementary Figure 5**

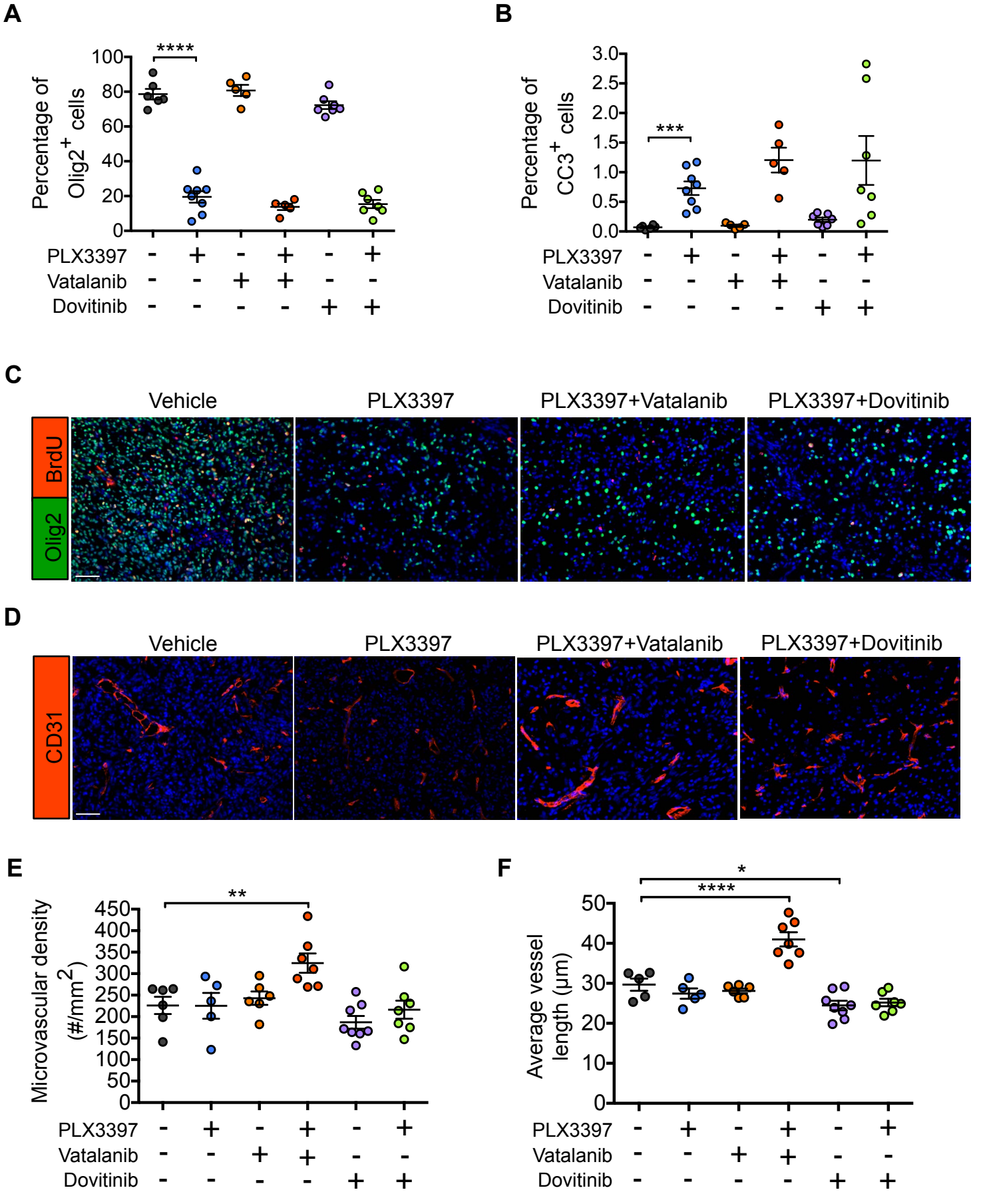


**Supplementary Figure 5. Analysis of alterations in tumorigenic processes within treated gliomas.** **(A)** Composition of tumor grades in all treatment arms, based on the 2007 WHO criteria. Chi-square test was used for statistical comparisons. **(B)** Representative immunofluorescence images of glioma cell proliferation, with glioma cells labeled by Olig2 positivity and proliferation indicated by BrdU positivity. **(C)** Quantification of percentages of $Olig2^+$ tumor cells. **(D)** Quantification of percentages of $CC3^+$ apoptotic cells within gliomas. **(E)** Representative immunofluorescence images of intratumoral $CD31^+$ vasculature. **(F)** Quantification of microvascular density within gliomas. **(G)** Quantification of blood vessel length within gliomas. One-way ANOVA with Sidak's multiple comparisons test and Dunnett's multiple comparisons test were used to assess statistical significance in (C-D) and (F-G) respectively. * $P<0.05$; **** $P<0.0001$. Scale bars in panels B and E: 50 μm. For C, D, F and G each point represents an independent tumor.

Yan et al, Supplementary Figure 6

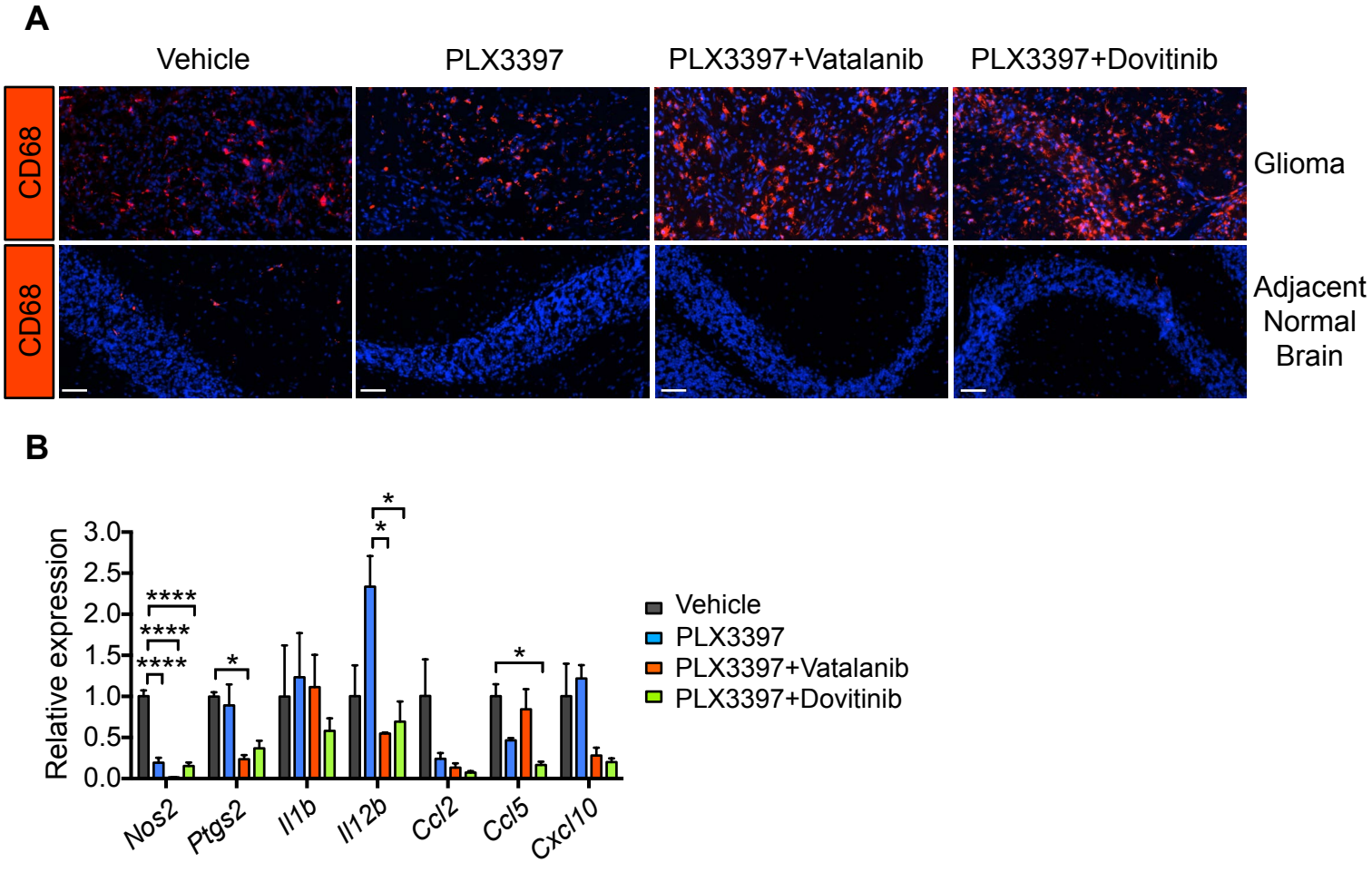


**Supplementary Figure 6. Macrophage protection by glioma cell-derived factors and PLX3397-mediated TAM gene expression changes.** **(A)** Representative immunofluorescence images of $CD68^+$ TAMs (upper row) and microglia in adjacent normal brain (lower row). Scale bars: 50 μm. **(B)** MTT assay showing cell viability of murine BMDMs treated with GCM, vatalanib (1 μM, based on 2-fold of estimated $IC_{50}$ from dose-response studies), or a combination of both, n=3 independent experiments. **(C)** MTT assay showing cell viability of BMDMs treated with GCM, dovitinib (900 nM, based on estimated $IC_{50}$ from dose-response studies), or a combination of both, n=3 independent experiments. **(D)** Quantification of mRNA expression of M1-like genes, including *Nos2*, *Ptgs2*, *Il1b*, *Il12b*, *Ccl2*, *Ccl5* and *Cxcl10*, in treated gliomas, n=3 tumors for each group. **(E)** Schematic of conditioned media cell culture assay. **(F)** Quantification of proportion of glioma cells in S phase, after incubation with naïve or stimulated MΦ CM generated from BMDMs treated with vehicle (DMSO) or PLX3397 (60 nM), n=3 independent experiments. One-way ANOVA with Sidak's multiple comparisons test and Tukey's multiple comparisons test were used to assess statistical significance in (D) and (E) respectively. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$.


A
Percentage of Olig2+ cells
100
80
60
40
20
0
****
PLX3397 - + - + - +
Vatalanib - - + + - -
Dovitinib - - - - + +
B
Percentage of CC3+ cells
3.0
2.5
2.0
1.5
1.0
0.5
0.0
***
PLX3397 - + - + - +
Vatalanib - - + + - -
Dovitinib - - - - + +
C
Vehicle
PLX3397
PLX3397+Vatalanib
PLX3397+Dovitinib
BrdU
Olig2
D
Vehicle
PLX3397
PLX3397+Vatalanib
PLX3397+Dovitinib
CD31
E
Microvascular density (#/mm2)
450
400
350
300
250
200
150
100
50
0
**
PLX3397 - + - + - +
Vatalanib - - + + - -
Dovitinib - - - - + +
F
Average vessel length (μm)
50
40
30
20
10
0
*
****
PLX3397 - + - + - +
Vatalanib - - + + - -
Dovitinib - - - - + +

**Supplementary Figure 7. Intratumoral changes following administration of combination therapies.** **(A)** Quantification of percentages of $Olig2^+$ tumor cells in the different treatment arms. **(B)** Quantification of percentages of $CC3^+$ apoptotic cells. Values in the single-agent groups are from Supplementary Fig. 4C-D for comparison purposes. **(C)** Representative immunofluorescence images of glioma cell proliferation. **(D)** Representative immunofluorescence images showing intratumoral vasculature. Scale bars in panels C and D: 50 μm. **(E)** Quantification of intratumoral microvascular density in the different treatment arms. **(F)** Quantification of intratumoral blood vessel length. Values in the single-agent groups are from Supplementary Fig. 4F-G for comparison purposes. One-way ANOVA with Sidak's multiple comparisons test and Dunnett's multiple comparisons test were used to assess statistical significance in (A-B) and (E-F) respectively. * $P<0.05$; ** $P<0.01$; **** $P<0.0001$; n.s. not significant. For A, B, E and F each dot represents an independent tumor.

**Yan et al, Supplementary Figure 8**



**Supplementary Figure 8. Gene expression changes in TAMs after combination therapies.** **(A)** Representative immunofluorescence images of $CD68^+$ TAMs in the different treatment arms. Scale bar: 50 μm. **(B)** Quantification of mRNA expression of M1-like genes, including *Nos2*, *Ptgs2*, *Il1b*, *Il12b*, *Ccl2*, *Ccl5* and *Cxcl10*, in treated gliomas, n=3 tumors for each group. One-way ANOVA with Sidak's multiple comparisons test was used to calculate statistical significance. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

**Supplementary Table 1:** List of antibodies used for immunofluorescence (IF) and Western blotting (WB)

| Antibody | Clone | Vendor | Dilution |
|---|---|---|---|
| BrdU | BU1/75(ICR1) | Serotec | IF 1:200 |
| c-Kit | D13A2 | Cell Signaling Technology | IF 1:400 |
| Cleaved caspase 3 (CC3) | - | Cell Signaling Technology | IF 1:500 |
| CD31 | - | R&D Systems | IF 1:100 |
| CD68 | FA-11 | Serotec | IF 1:1000 |
| CSF-1R | C-20 | Santa Cruz | WB 1:1000 |
| Phospho-CSF-1R (Y723) | 49C10 | Cell Signaling Technology | WB 1:1000 |
| Olig2 | - | Millipore | IF 1:200 |
| PDGFR alpha | D1E1E | Cell Signaling Technology | WB 1:1000 |
| Phospho-PDGFR alpha (Y762) | - | Cell Signaling Technology | WB 1:1000 |
| VEGFR2 | D5B1 | Cell Signaling Technology | IF 1:400 |