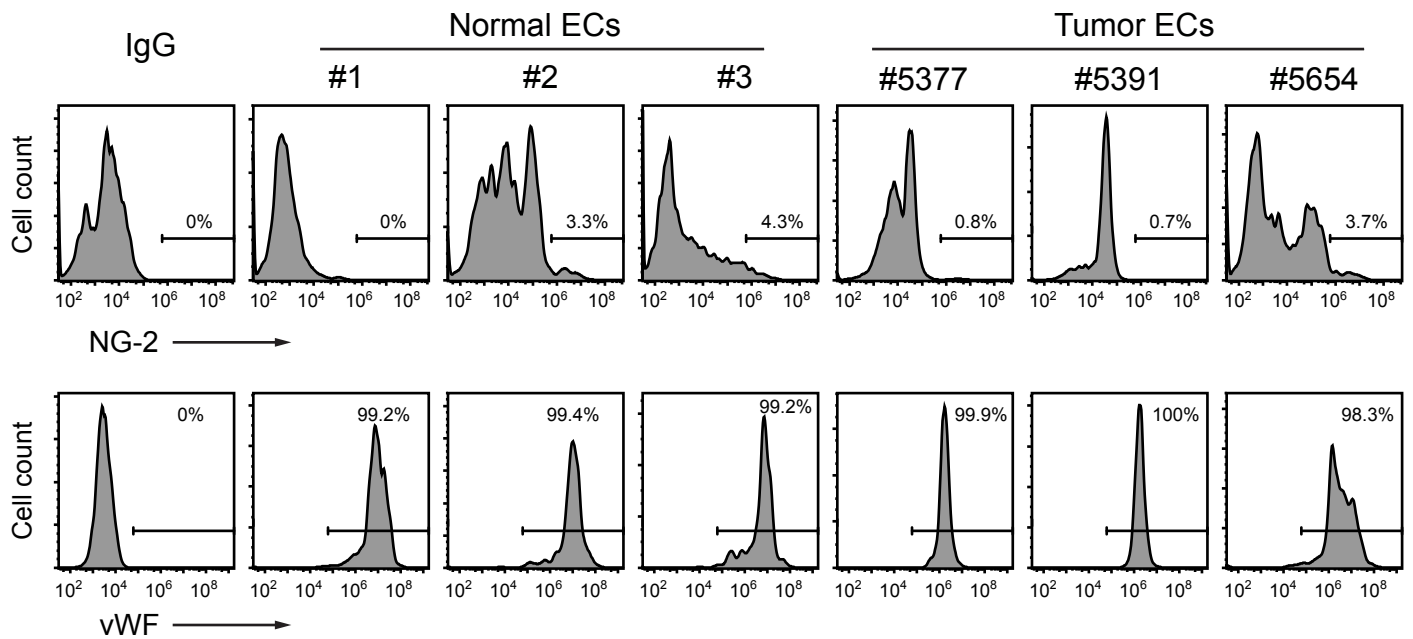


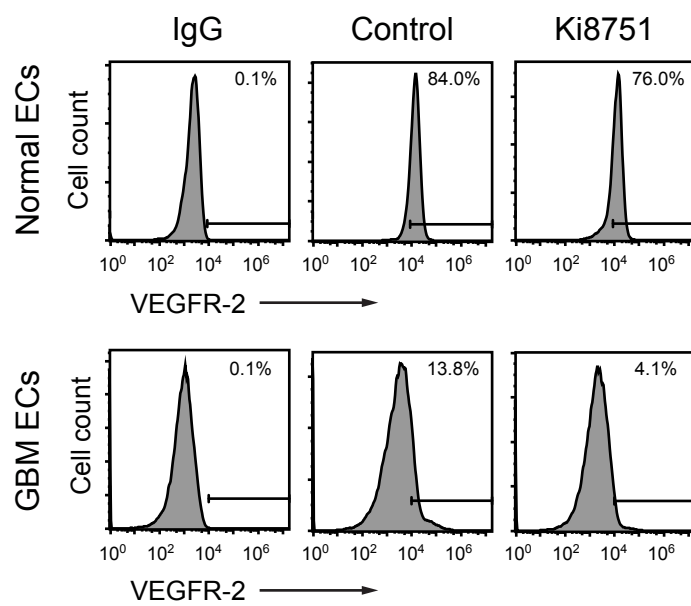
Supplementary Information

PDGF-mediated mesenchymal transformation renders endothelial resistance to anti-VEGF treatment in glioblastoma

Liu et al.

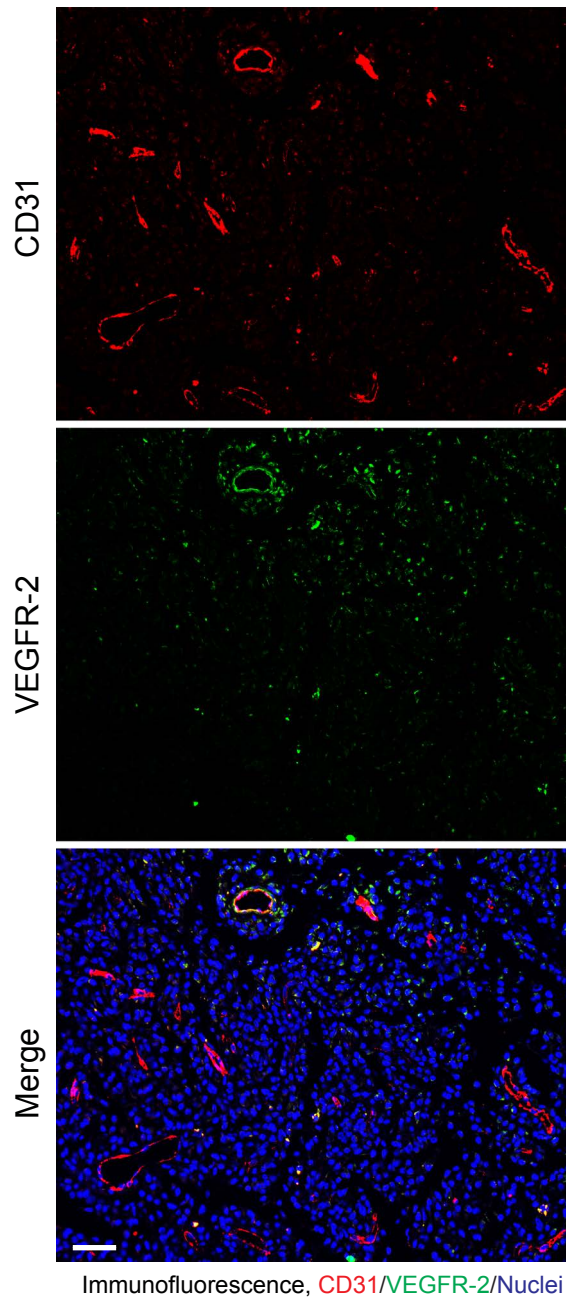


Supplementary Figure 1. Identification of microvascular ECs isolated from normal human brains and GBM tumors. ECs derived from normal human brains (#1 and #2 from adult brains and #3 from fetal brain) and from human GBM tumors (patients #5377, #5391, and #5654) were immunostained with anti-NG-2 or anti-vWF antibody or isotype IgG, followed by flow cytometry analysis. Representative sortings are shown.



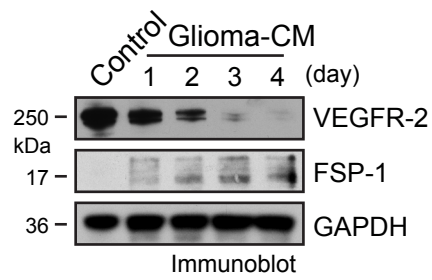
Supplementary Figure 2. VEGFR-2 expression after Ki8751 treatment in ECs.

ECs derived from normal human brain and from human GBM tumor (patients #5377) were treated with 1 μ M Ki8751 or control medium. Single cell suspensions were immunostained with anti-VEGFR-2 antibody or isotype IgG, followed by flow cytometry analysis. Representative sortings are shown.



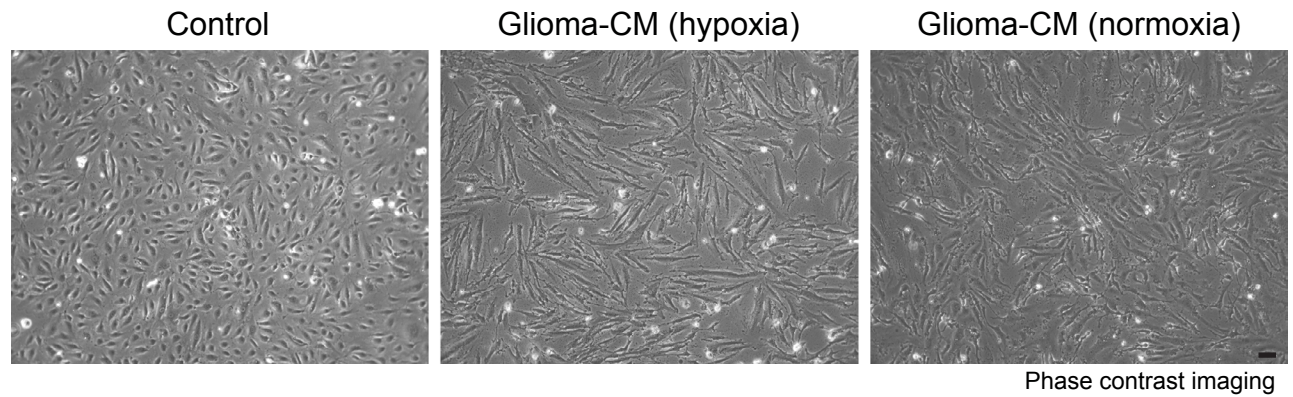
Supplementary Figure 3. Diminished expression of VEGFR-2 in GBM-associated ECs.

Tissue sections from surgical specimens of human patients with GBM (n = 3) were stained with anti-CD31 and anti-VEGFR-2 antibodies, followed by immunofluorescence analysis. Representative images are shown. Scale bar: 100 μ m.



Supplementary Figure 4. Effects of glioma-CM on Endo-MT in ECs.

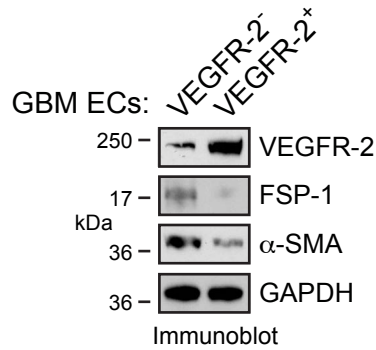
Glioma-CM was harvested from the medium of U251 human glioma cells cultured under normoxia. Human brain ECs were treated with glioma-CM or control normal medium. EC lysates were immunoblotted.



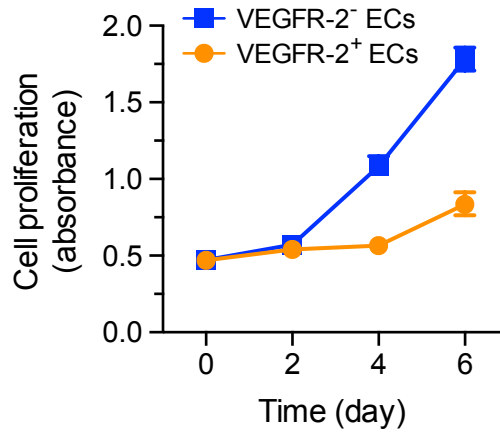
Supplementary Figure 5. Effects of glioma-CM on EC morphology.

Human brain ECs were treated with control normal medium or glioma-CM that was isolated from U251 human glioma cells cultured under hypoxia or normoxia. After 48 h incubation, cells were imaged. Scale bar: 100 μ m.

a



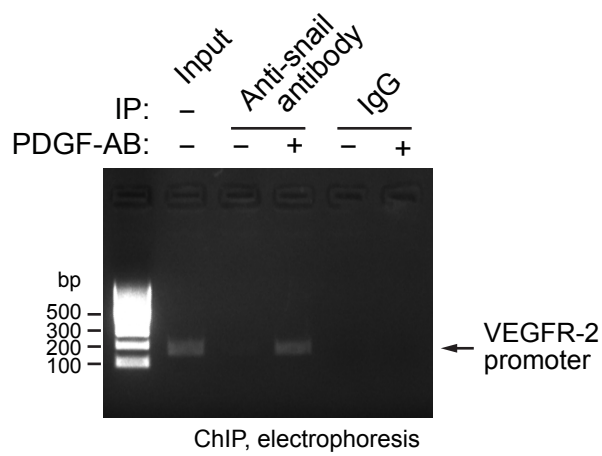
b



Supplementary Figure 6. VEGFR2⁺ and VEGFR2⁻ GBM ECs.

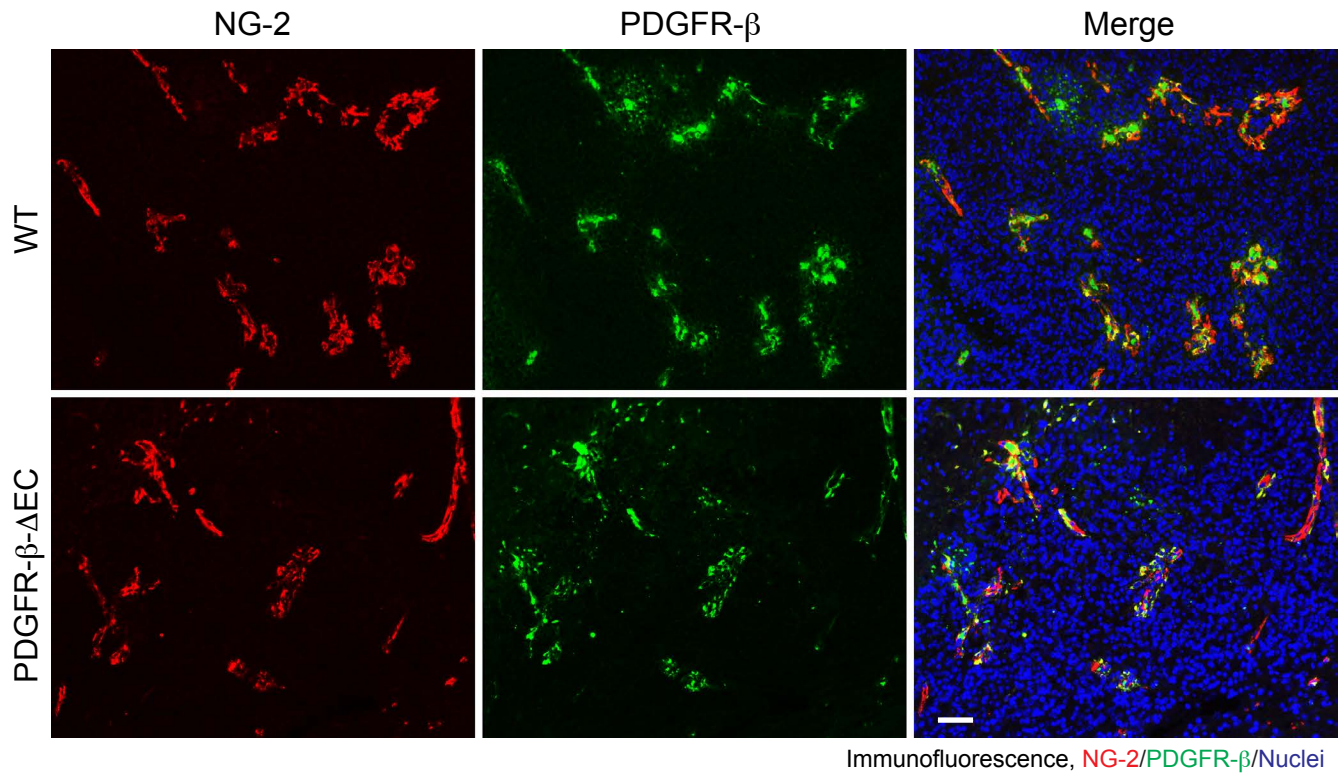
ECs were isolated from the GBM tumor of human patient #5377. Cells were incubated with anti-VEGFR-2 antibody-conjugated magnetic beads, followed by magnetic-activating cell sorting (MACS). VEGFR-2⁺ and VEGFR-2⁻ cells were isolated and cultured.

(a) Cell lysates were analyzed by immunoblot. (b) Cells were subjected to proliferation analysis (n = 3, mean \pm SD).



Supplementary Figure 7. PDGF-AB induces Snail binding to VEGFR-2 promoter in ECs.

Human brain ECs were treated with PDGF-AB or control medium for 24 h. Nuclear extracts were immunoprecipitated with anti-Snail antibody or IgG, and subjected to ChIP analysis. DNA was resolved by agarose electrophoresis, and imaged. The arrow indicates the amplified DNA in VEGFR-2 promoter.



Supplementary Figure 8. Pericyte and PDGFR- β expression in mouse GBM tumors.

The primary GBM was induced in *Ntv-a;Ink4a-Arf^{-/-};Pten^{-/-};LSL-Luc* donor mice by RCAS-mediated somatic gene transfer. Single-cell tumor suspension was implanted into *Pdgfrb^{fl/fl}* (WT) or *Tie2-Cre;Pdgfrb^{fl/fl}* (PDGFR- β - Δ EC) recipient mice. Tumor sections were stained with anti-NG-2 and anti-PDGFR- β antibodies, followed by immunofluorescence analysis. Representative images are shown (n = 4 mice). Scale bar: 100 μ m.

Fig. 1c

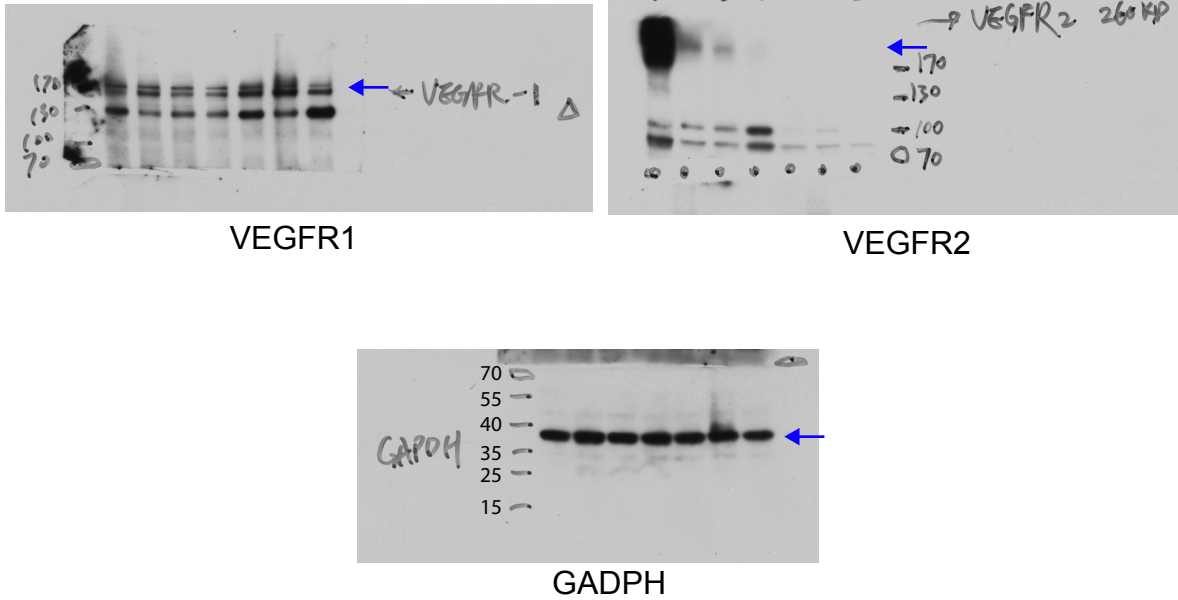


Fig. 2c

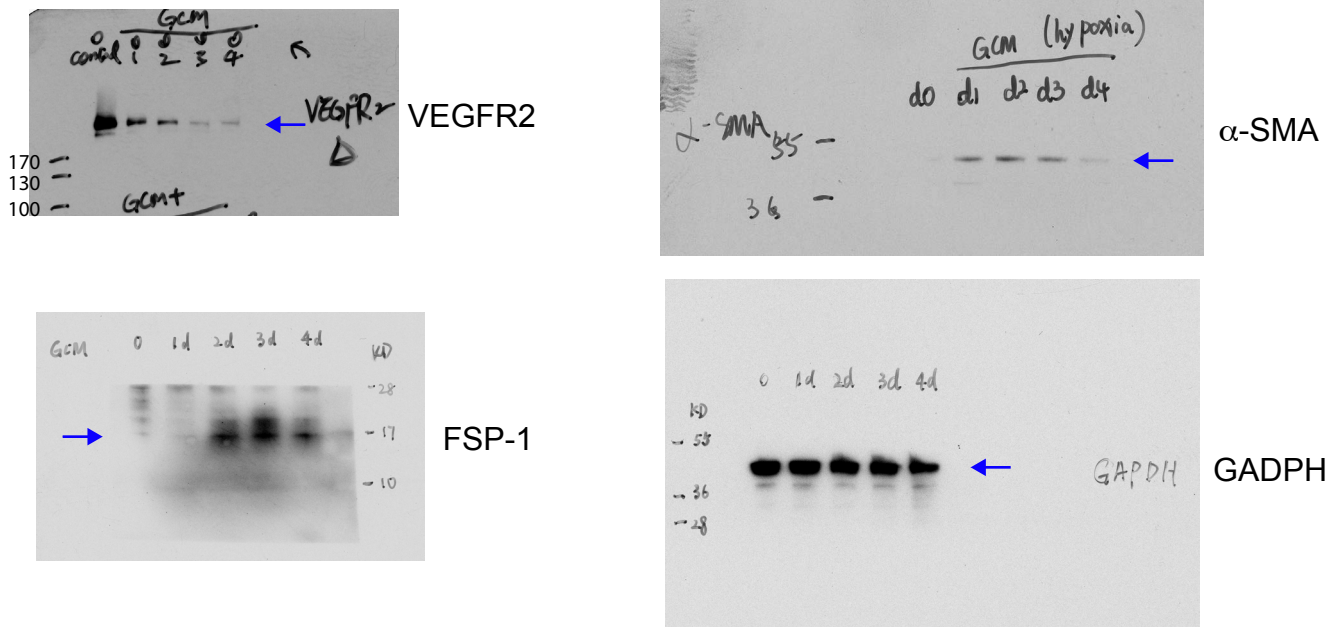
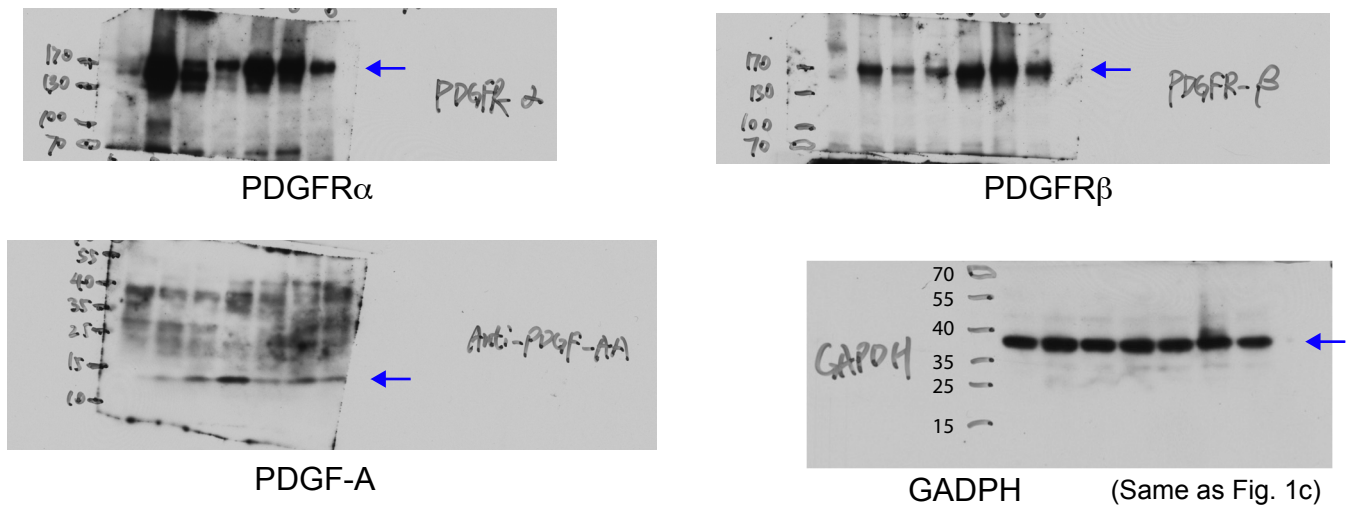
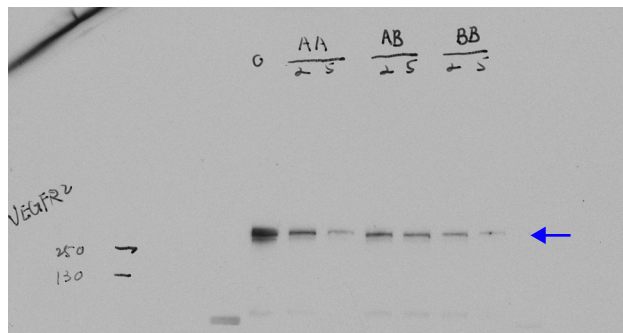
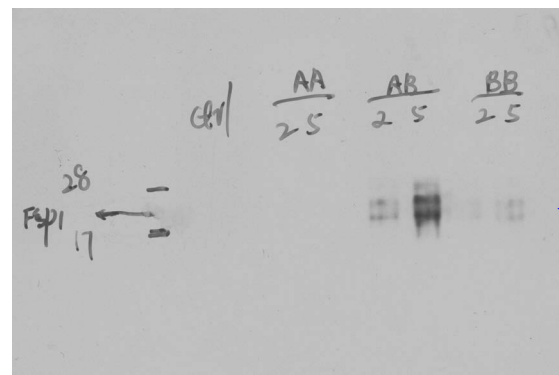


Fig. 2e

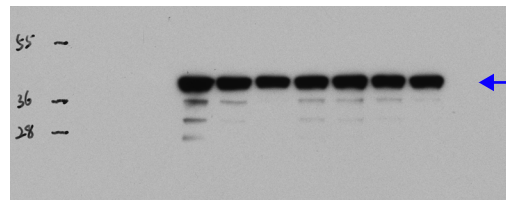




VEGFR2

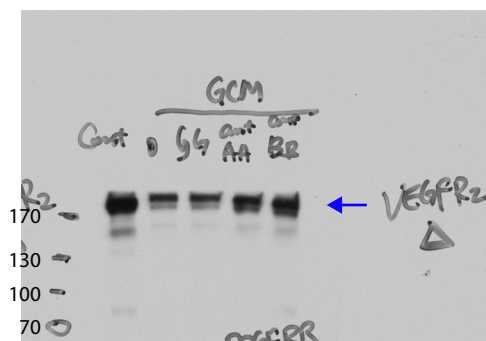


FSP-1

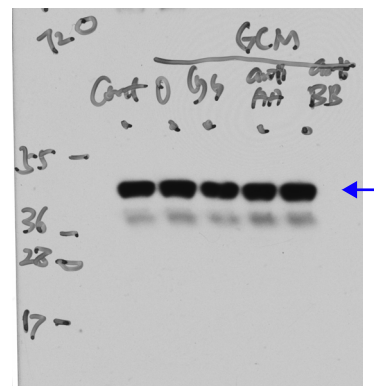


GADPH

Fig. 2f

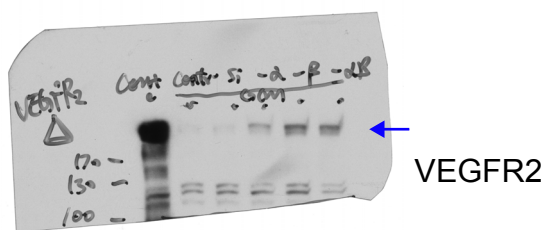


VEGFR2

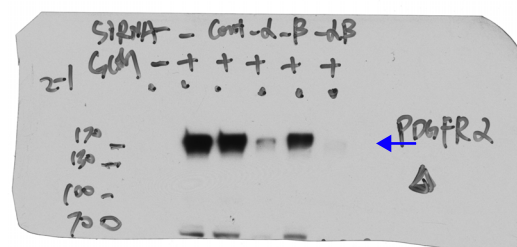


GADPH

Fig. 3a

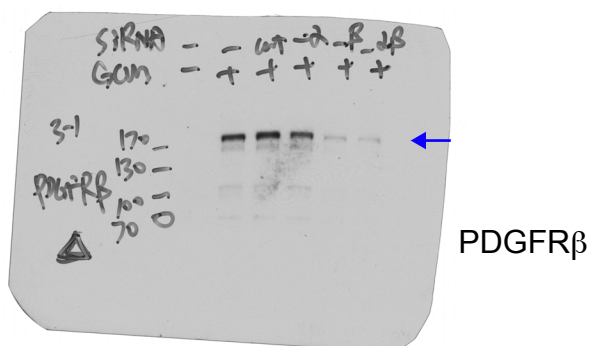


VEGFR2

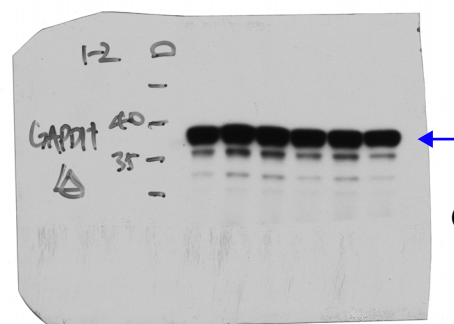


PDGFR α

Fig. 3c



PDGFR β



GADPH

Fig. 4b



Fig. 4d

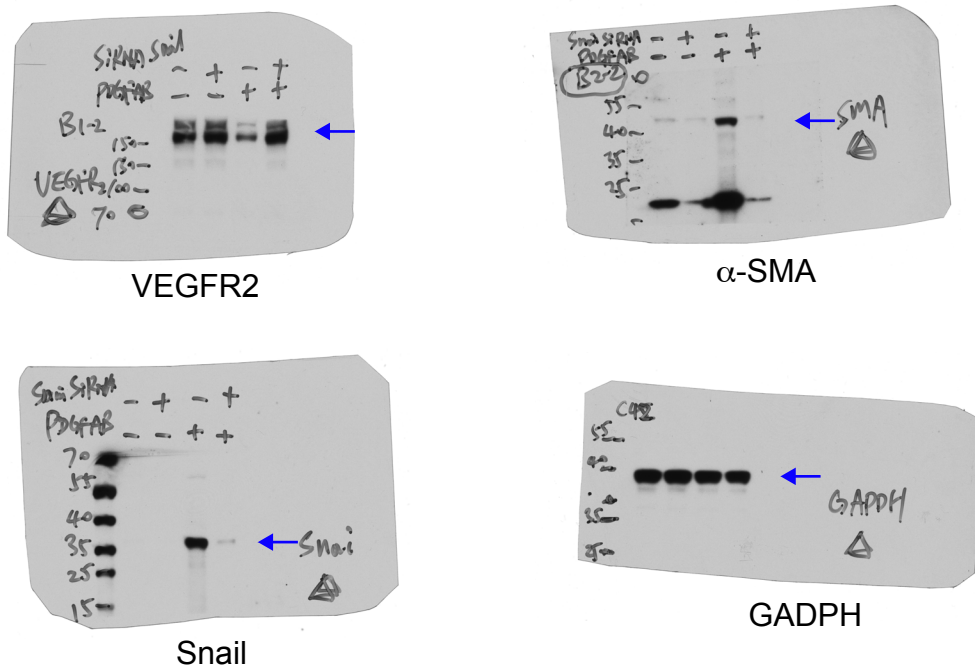


Fig. 4e

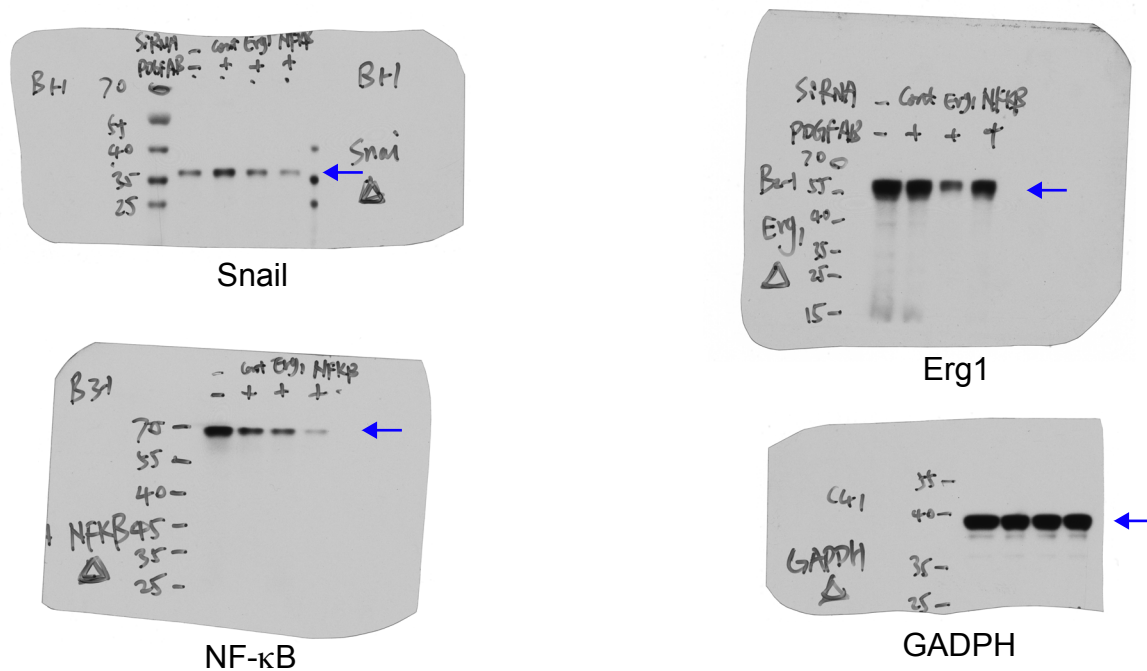
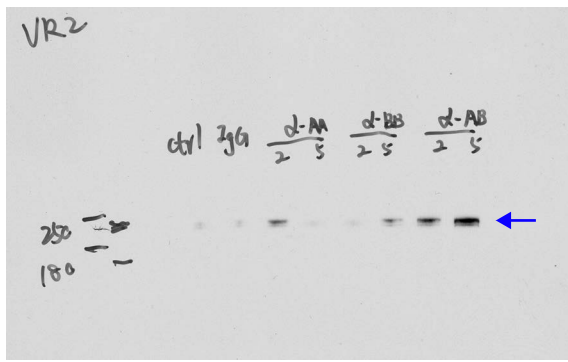
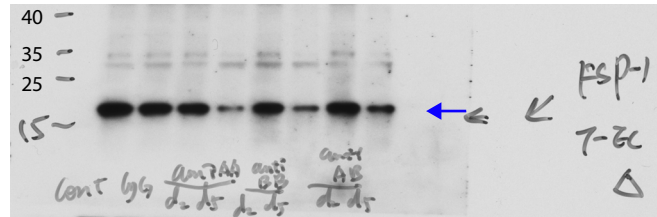


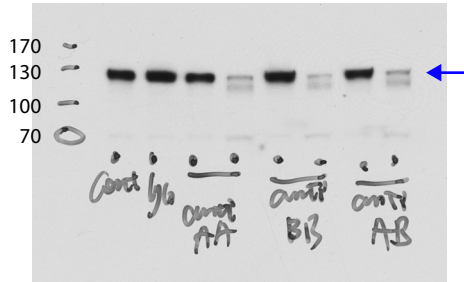
Fig. 5a



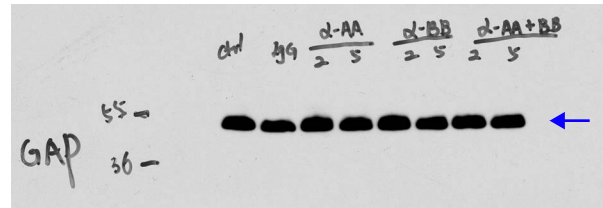
VEGFR2



FSP-1

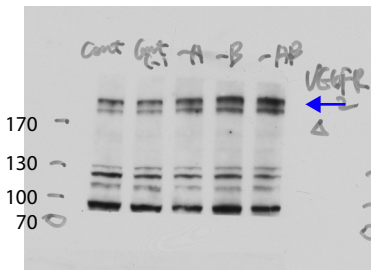


N-cadherin

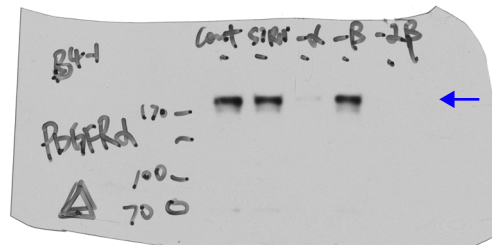


GAPDH

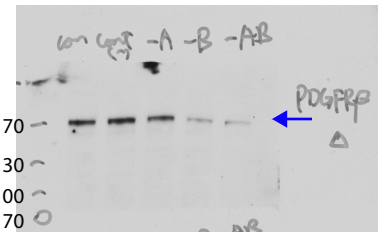
Fig. 5a



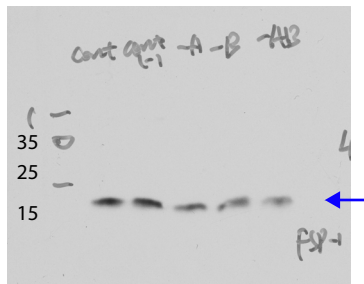
VEGFR2



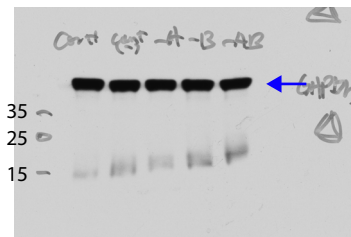
PDGFR α



PDGFR β

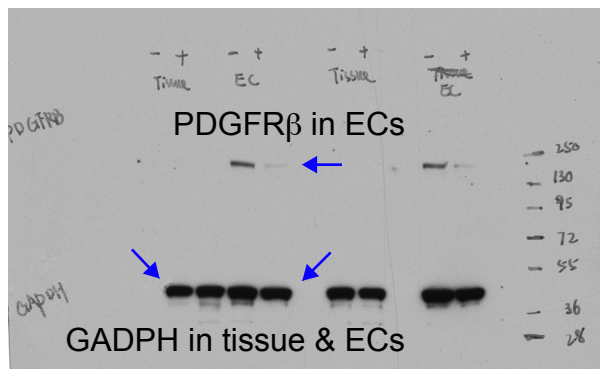


FSP-1

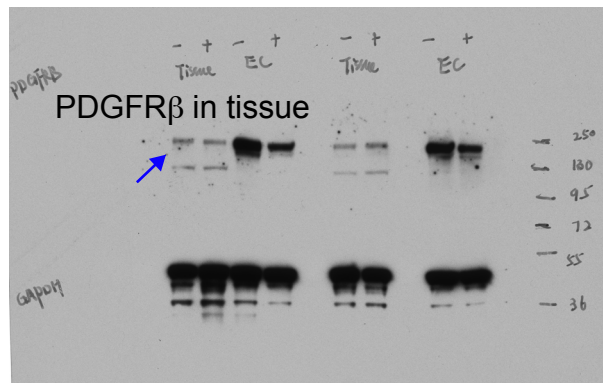


GAPDH

Fig. 7b

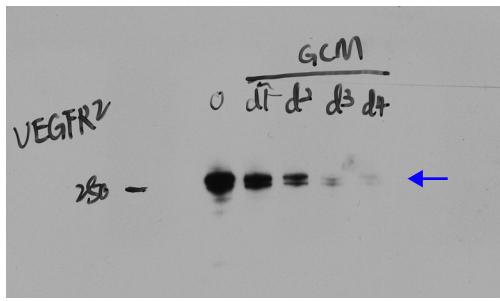


Light exposure

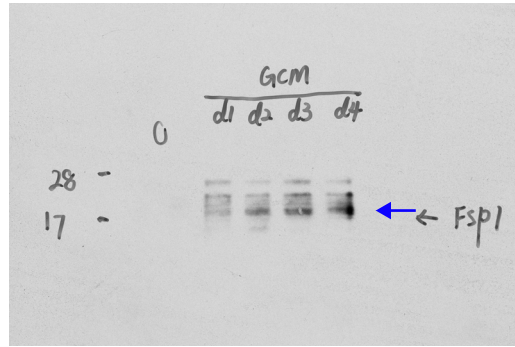


Heavy exposure

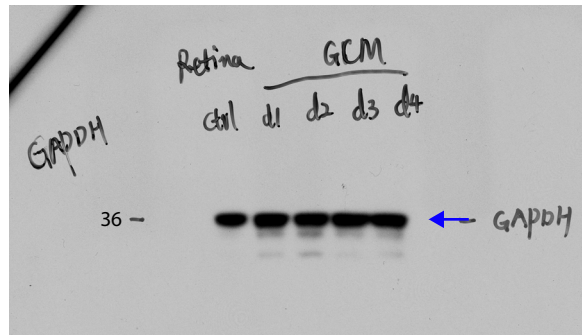
Fig. S4



VEGFR2

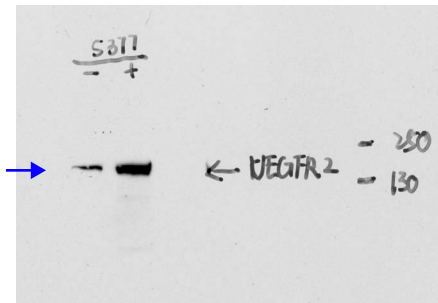


FSP-1

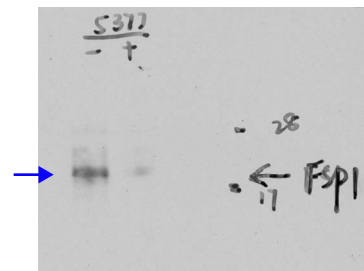


GAPDH

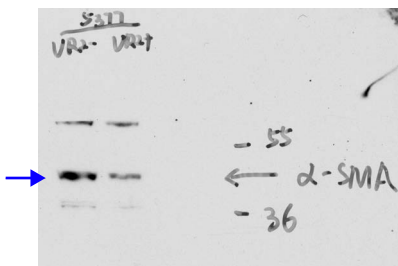
Fig. S6



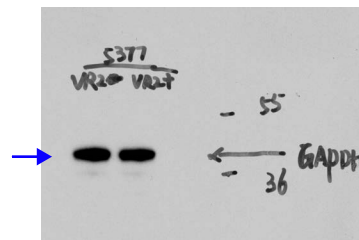
VEGFR2



FSP-1



α-SMA



GAPDH