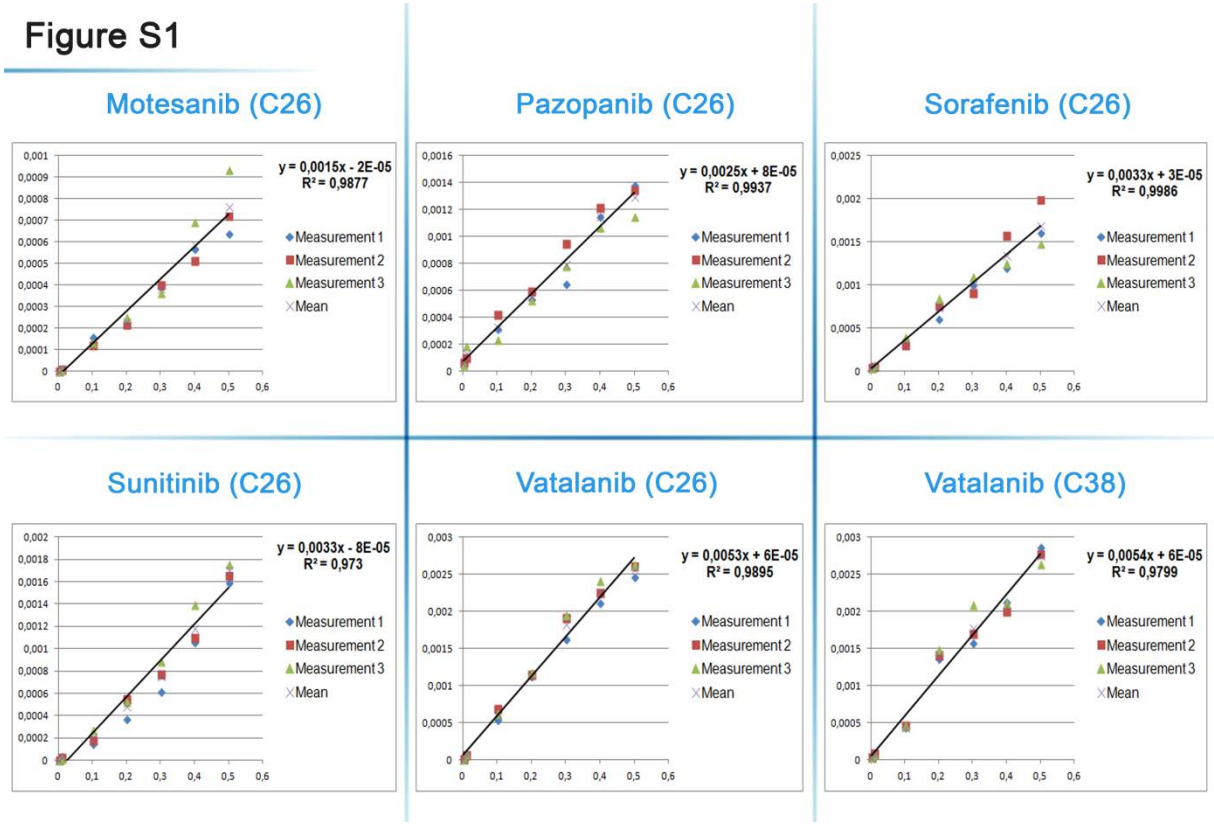

Figure S1. Calibration curves of antiangiogenic RTKIs. Drugs were dissolved and diluted in 50% methanol in the concentration range of 0.001–0.5 µmol·mL−1. 0.5 µL of the compound solution was applied on the control tumor tissue surfaces. Spraying and detection conditions were the same as those during the analysis of in vivo-treated tumors. Average signal intensities of the applied concentrations were measured and normalized to Total Ion Current (TIC) by using Xcalibur v 2.0.7. and ImageQuest™ softwares.

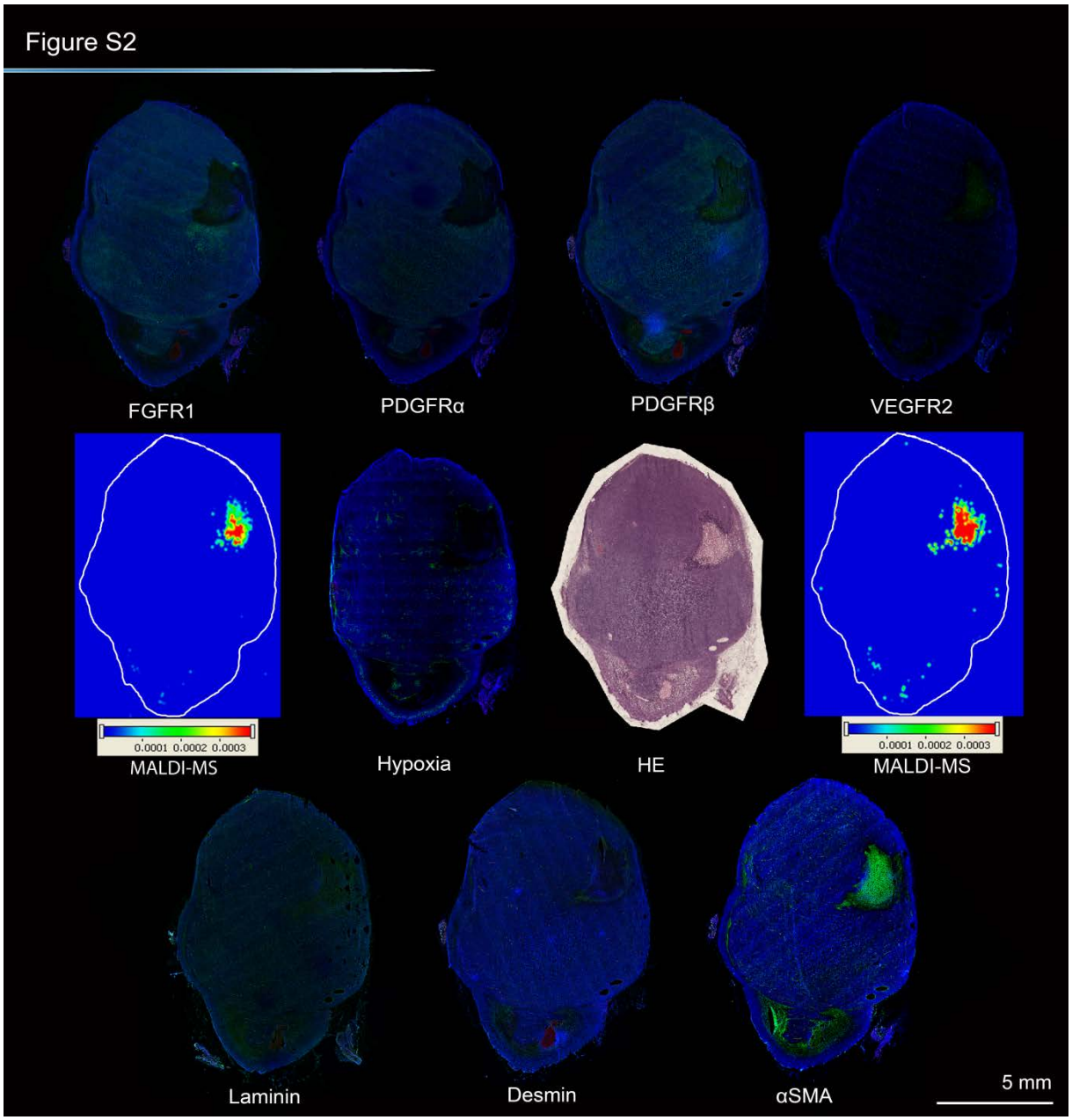

Figure S2. Overview of serial frozen tumor sections cut for MALDI-MS imaging, HE staining and immunofluorescence microscopy. Sections #1-4 were immunolabeled with antibodies directed against FGFR1, PDGFRα, PDGFRβ or VEGFR2. MALDI-MS imaging and subsequent HE staining were performed in sections #5 and #7. Section #6 was used for pimonidazole staining (hypoxia detection). Immunostainings for endothelial basement membrane (anti-laminin antibody) and pericytes (anti-desmin and anti-α-smooth muscle actin antibodies) were performed in sections #8, #9 and #10, respectively. Note that areas of necrotic tissue can also appear green due to non-specific binding of the secondary antibody in immunostained sections.

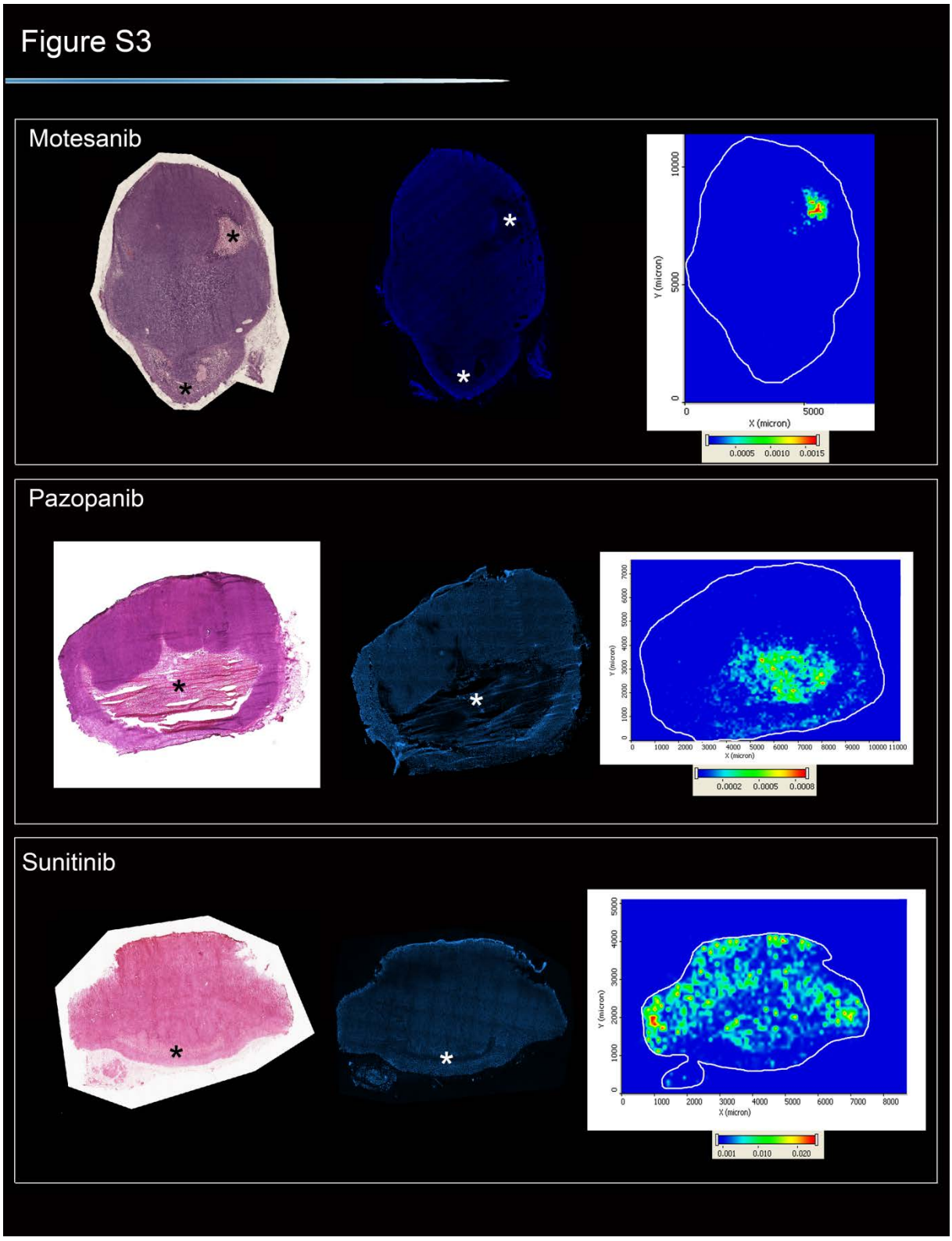

Figure S3. Co-localization of drug compounds (as visualized by MALDI-MSI) and tumor necrosis in C26 tumors treated with motesanib, pazopanib and sunitinib. Asterisks mark intratumoral areas of necrotic tissue in H&E sections (left). In Hoechst nuclear-counterstained sections (middle) these areas (asterisks) appear black due to lack of nuclear counterstain (Hoechst33342, blue). Tumor boundaries are delineated with white line in MALDI-MS images (right).

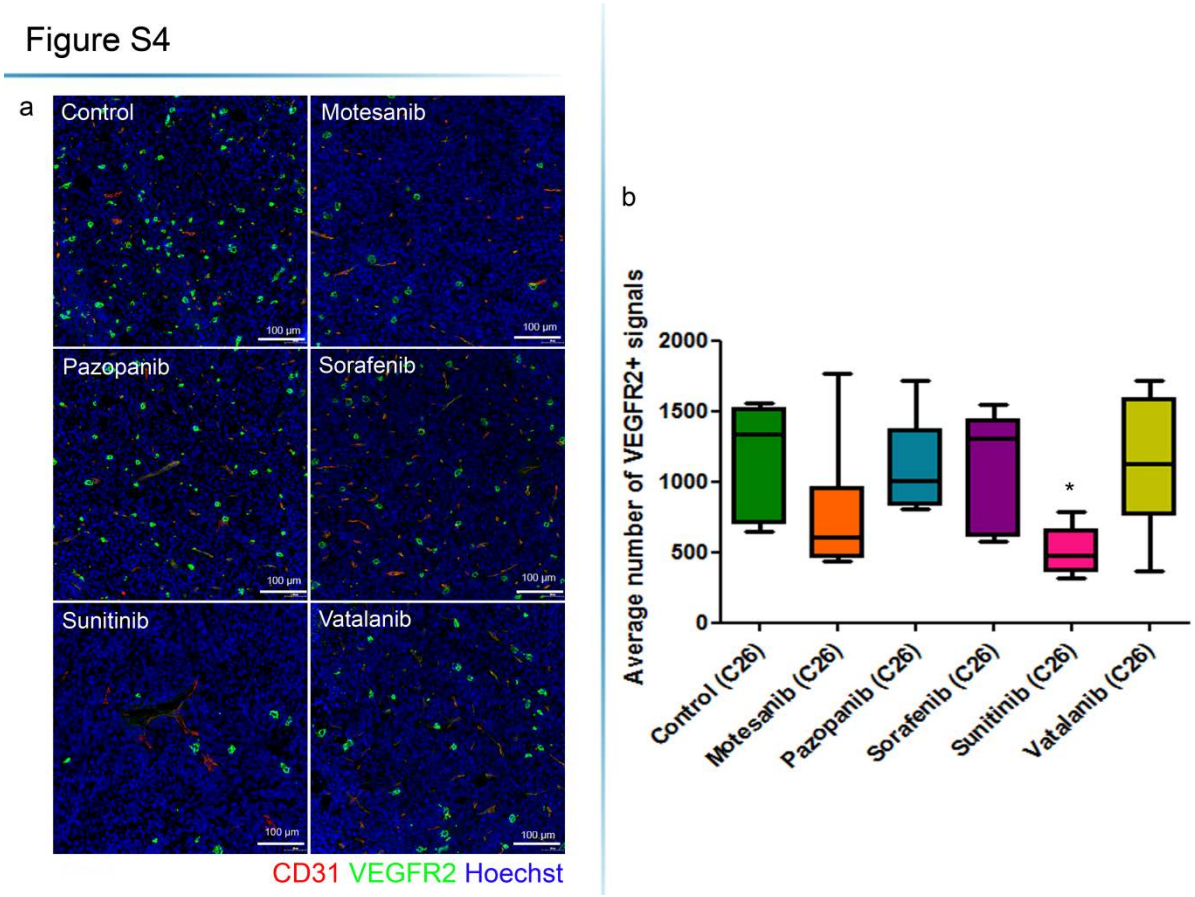

Figure S4. Intratumoral expression and distribution patterns of VEGFR2 in antiangiogenic RTKI-treated and control C26 tumors as visualized by immunofluorescent labeling. Tumor sections are immunolabeled for VEGFR2 (green) and CD31 (red). Nuclei are counterstained with Hoechst33342 (blue) (***a***). Note the focal VEGFR2 expression pattern of the tumor cells in both the control and treated animals. In (***b***), data are shown as box (first and third quartiles) and whisker (maximum to minimum) plots with the mean (horizontal bar) from 6 animals per group. *P<0.05

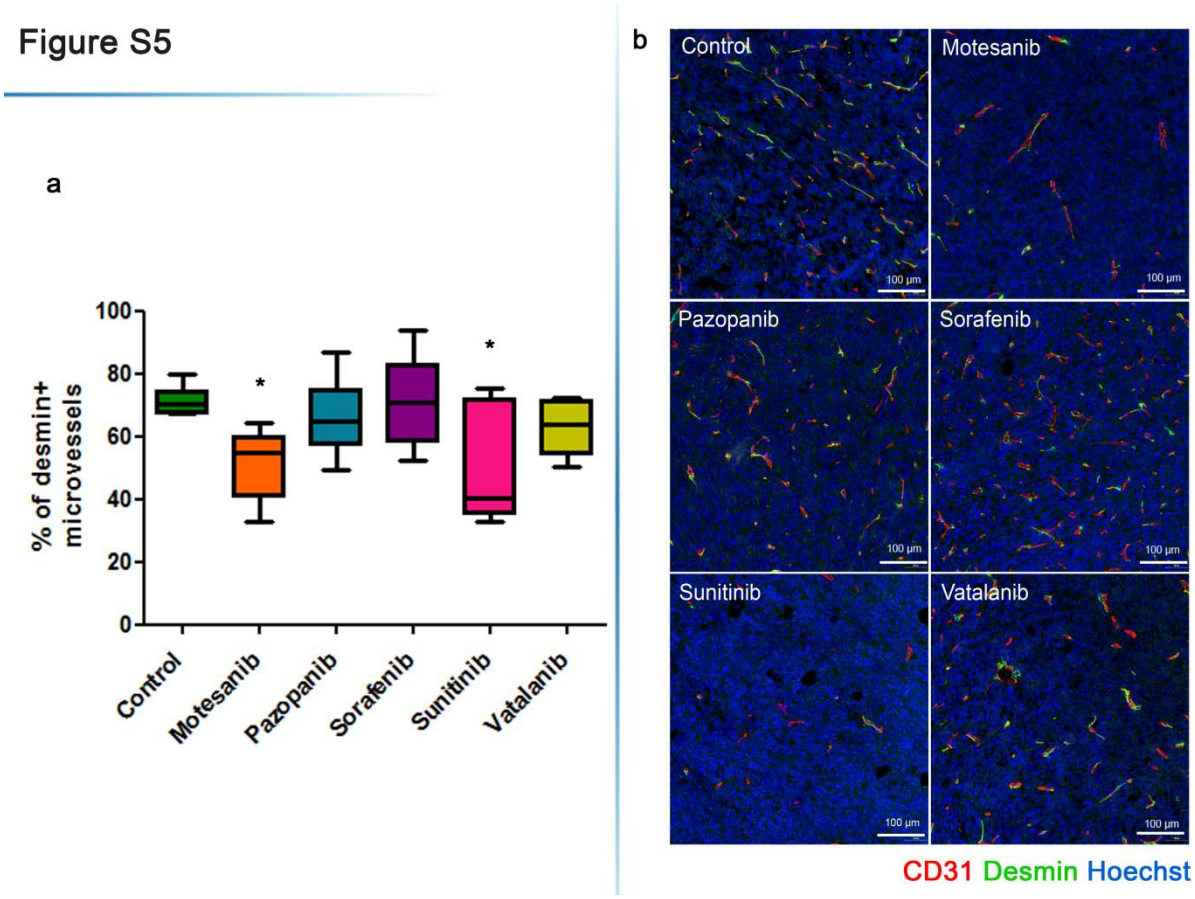

Figure S5. Desmin expression in antiangiogenic RTKI-treated and control C26 tumors as visualized by immunofluorescent labeling. In (***a***), data are shown as box (first and third quartiles) and whisker (maximum to minimum) plots with the mean (horizontal bar) from 6 animals per group (*P<0.05). Tumor sections are immunolabeled for pericyte desmin (green) and CD31 (red). Nuclei are counterstained with Hoechst 33342 (blue) (***b***).

Figure S6

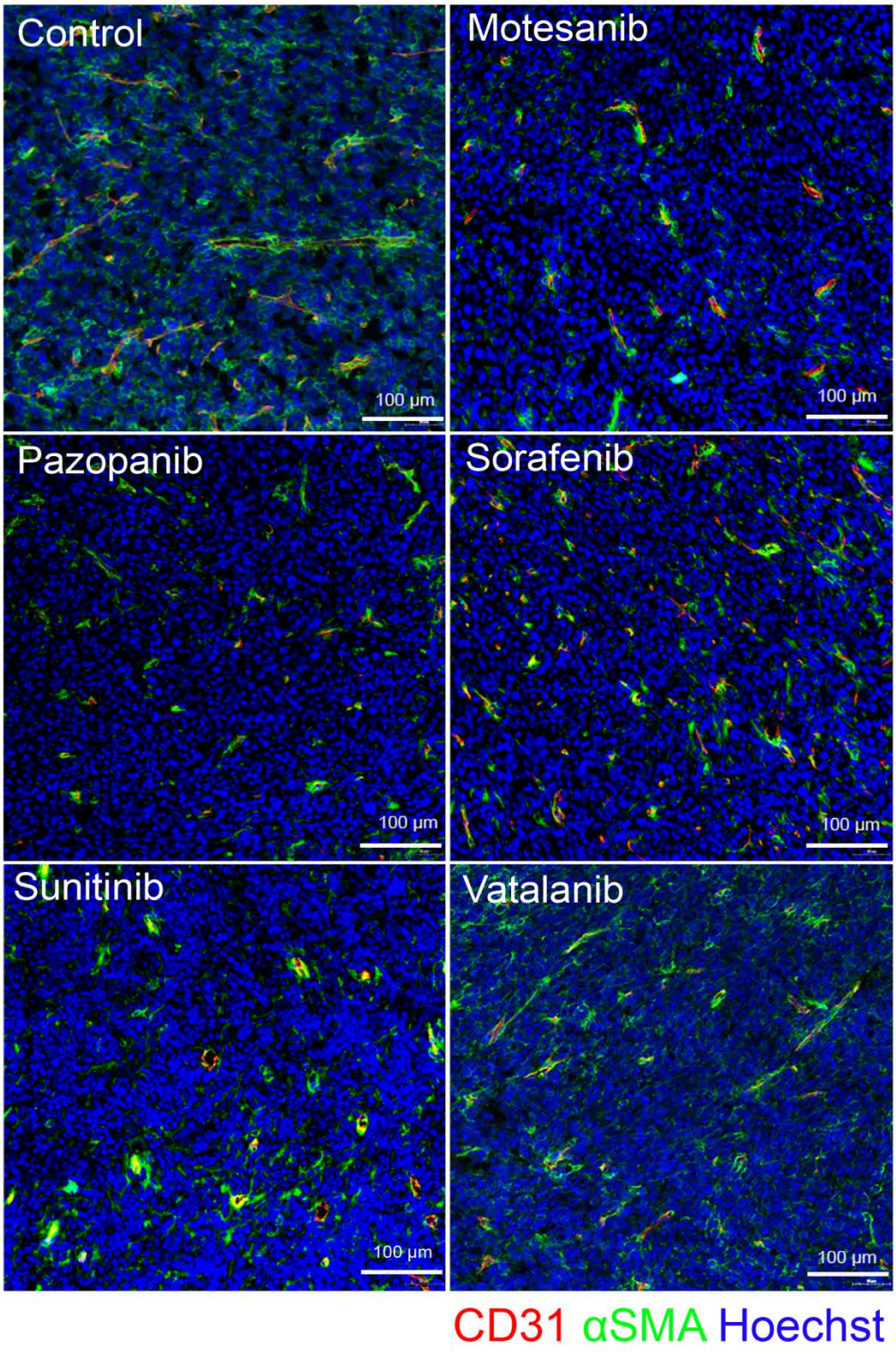

Figure S6. Low power views of antiangiogenic RTKI-treated and control C26 tumor sections stained for pericyte αSMA (green) and CD31 (red). Nuclei are stained with Hoechst33342 (blue).

Figure S7

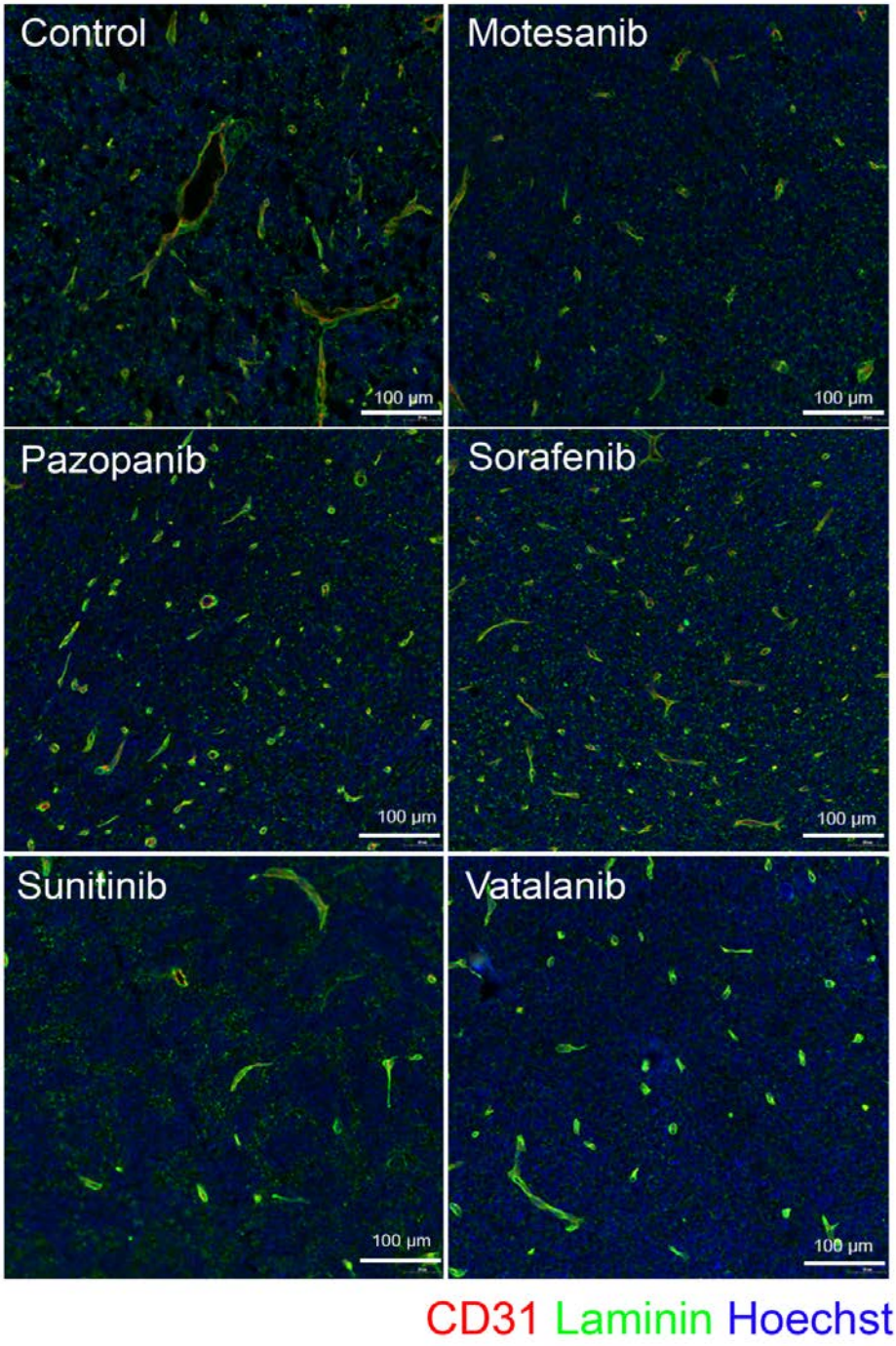

Figure S7. Low power views of antiangiogenic RTKI-treated and control C26 tumor sections stained for the capillary basement membrane component laminin (green) and CD31 (red). Nuclei are stained with Hoechst33342 (blue). Note that tumor cells are also weakly positive for laminin.

Figure S8

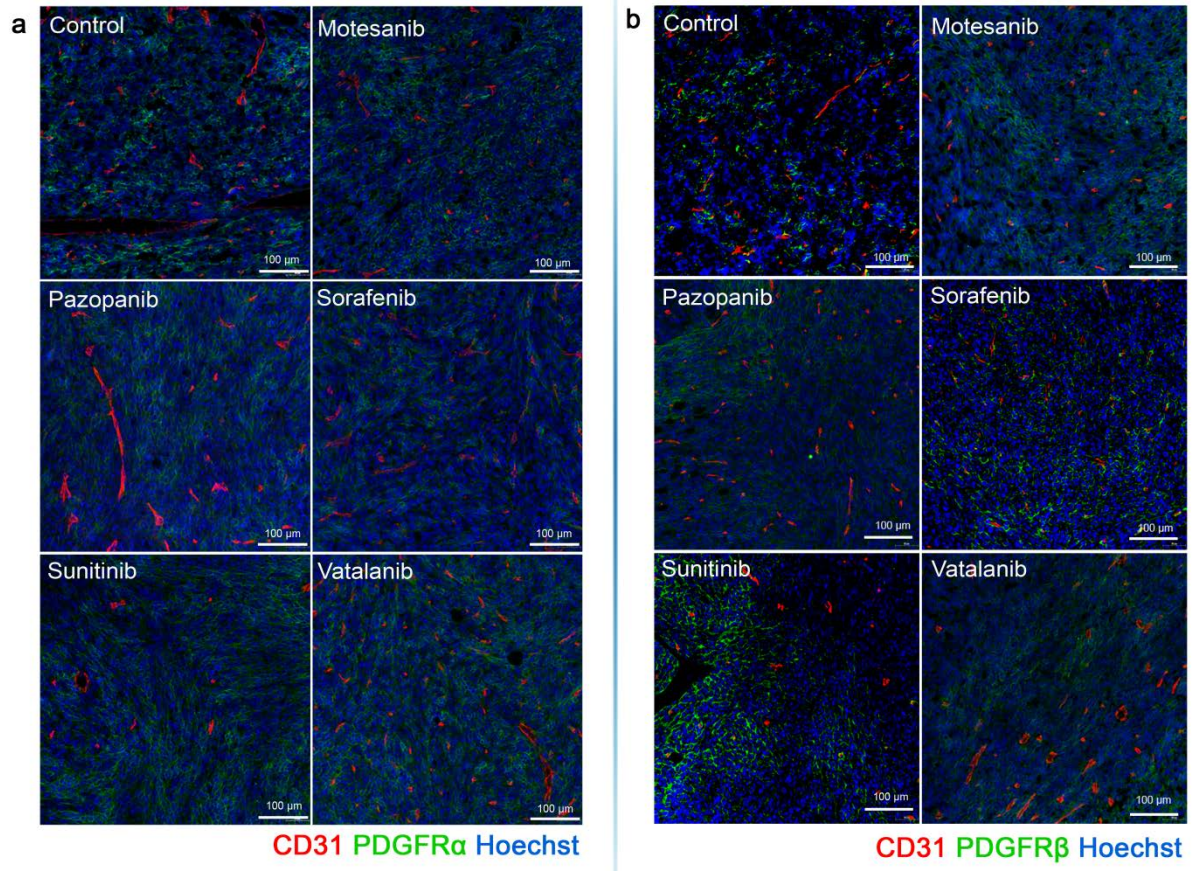

Figure S8. Low power views of antiangiogenic RTKI-treated and control C26 tumor sections stained for PDGFRα (green, left panel) and PDGFRβ (green, right panel). Microvessels are labeled with anti-CD31 (red). Nuclei are stained with Hoechst33342 (blue). Note the focal PDGFRα and PDGFRβ expression patterns of the tumor cells in both the control and treated animals.

Figure S9

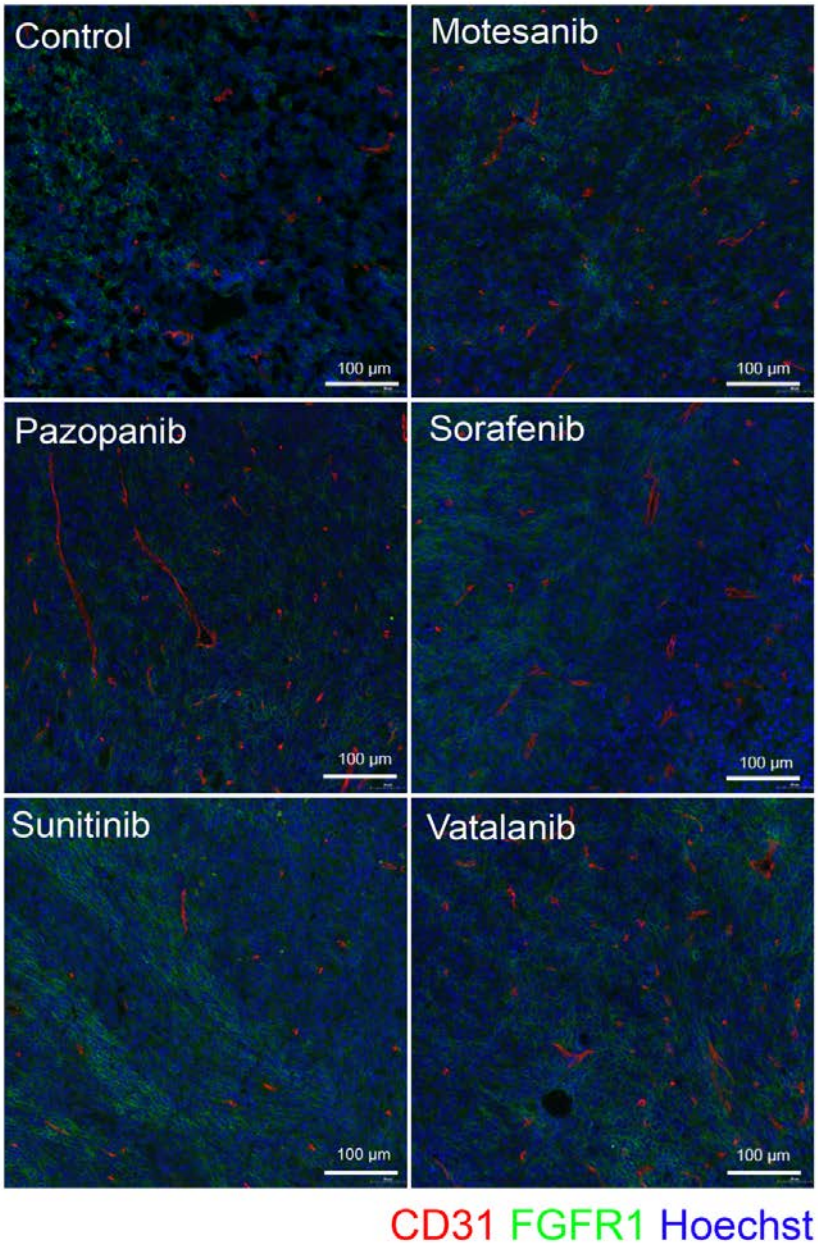

Figure S9. Low power views of antiangiogenic RTKI-treated and control C26 tumor sections stained for FGFR1 (green). Microvessels are labeled with anti-CD31 (red). Nuclei are stained with Hoechst33342 (blue). Note the focal FGFR1 expression pattern of the tumor cells in both the control and treated animals.

Table S1. List of antibodies used in the study

Antibody	Species	Manufacturer	Catalog No.	Dilution
Primary				
Anti-CD31	rat monoclonal	BD Pharmingen, BD Biosciences, FranklinLakes, NJ, USA	550275	1:50
Hypoxyprobe-1 Plus Kit (FITC-Mab1)	mouse monoclonal	Hypoxyprobe Inc., MiddlesexTurnpike Burlin, MA USA	HP1-100Kit	1:100
Anti-Laminin	rabbit polyclonal	DAKO, Glostrup Denmark	Z0097	1:200
Anti-Desmin	rabbit polyclonal	Abcam, Cambridge, UK	Ab32362	1:200
Anti-α-SMA	mouse monoclonal	DAKO, Glostrup Denmark	M0851	1:200
Anti-FGFR1	rabbit polyclonal	Cell Signaling Technology, Danvers, MA, USA	9740	1:50
Anti-PDGFRα	rabbit polyclonal	Cell Signaling Technology, Danvers, MA, USA	3174	1:50
Anti-PDGFRβ	rabbit polyclonal	Cell Signaling Technology, Danvers, MA, USA	4564	1:50
Anti-VEGFR2	rabbit polyclonal	Cell Signaling Technology, Danvers, MA, USA	2479	1:50
Secondary				
Alexa 488	Anti-mouse goat $F(ab')_2$	Cell Signaling Technology, Danvers, MA, USA	4408	1:1000
Alexa 488	Anti-rabbit goat $F(ab')_2$	Cell Signaling Technology, Danvers, MA, USA	4412	1:1000
Alexa 555	Anti-rat goat $F(ab')_2$	Cell Signaling Technology, Danvers, MA, USA	4413	1:1000
Nucleic acid stain				
Hoechst 33342		Molecular probes, Eugene, OR, USA	H3570	1:10000