Impact of Tumor Vascularity on Responsiveness to Anti-angiogenesis in a Prostate Cancer Stem Cell-derived Tumor Model

Supplemental Table

Supplemental Figures 1-7

Kexiong Zhang and David J. Waxman

Division of Cell and Molecular Biology, Department of Biology

Boston University, 5 Cummington Street, Boston, MA 02215

Supplemental Table 1. Primer sequences used for quantitative real-time PCR (qPCR) analysis

Gene	Oligo Name	Primer sequence
hm-VEGFA	ON-3610	Forward, 5'-CGGGCCTCCGAAACCATGAAC -3'
	ON-3611	Reverse, 5'- ATGGGTGCAGCCTGGGACCA -3'
h-CCL2	ON-3860	Forward, 5'- AGCAAGTGTCCCAAAGAAGCTG-3'
	ON-3861	Reverse, 5'-CTCCTTGGCCACAATGGTCT-3'
hm-VEGFR2	ON-4154	Forward, 5'-GTCATCATCCTACGGACCGTTAAG-3'
	ON-4155	Reverse, 5'-GATGGACAAGTAGCCTGTCTTCAG-3'
h-VEGFR2	ON-5685	Forward, 5'-AACTGACTTGGCCTCGGTCA
	ON-5886	Reverse, 5'-CACTAACAGAAGCAATAAATGGAGATCT
m-VEGFR2	ON-5687	Forward, 5'-ACGTCGACATAGCCTCCACTG
	ON-5688	Reverse, 5'-GGCGATGAATGGTGATCTGTAAT
m-VE-cadherin	ON-3790	Forward, 5'-GTGGCCAAAGACCCTGACAA-3'
	ON-3791	Reverse, 5'-TCACTGGTCTTGCGGATGGA-3'
hm-VEGFC	ON-4156	Forward, 5'- TTCCATTATTAGACGTTCCCTGC-3'
	ON-4157	Reverse, 5'- GGTCTTGTTCGCTGCCTGAC-3'
h-IL8	ON-3078	Forward, 5'- CTGGGTGCAGAGGGTTGTGGAGA-3'
	ON-3079	Reverse, 5'- TGGCAACCCTACAACAGACCCACA-3'

hm-: the primer can amplify both human and mouse genes; h-: human specific primer; m-: mouse specific primer.

Supplemental Figure 1. Immunohistochemical staining of PC3/2G7 and PC3 tumors using anti-human-specific CD31 antibody. No immuostaining was seen in either tumor model, indicating that the large increase in tumor blood vessels in the PC3 stem-like cell-derived PC3/2G7 tumors was not due to differentiation of the human stem-like cells into tumor blood vessels. **A**, anti-human CD31 staining of human tonsil tissue, which served as a positive control (magnification, 20x). **B, C**. PC3/2G7 and PC3 tumor sections (magnification, 20x). Sections were counterstained with hematoxylin (blue) to visualized cell nuclei.

Supplemental Figure 2. Staining of paraffin-embedded PC3/2G7 and PC3 tumor sections, with and without 12 days axitinib treatment, as shown in Fig. 2. Show are representative stained images for CD31 (**A**), hematoxylin (**B**), PCNA (**C**) and TUNEL assay (**D**), as indicated (magnification, 20x). Quantitative data based on a large number of such images are shown in Fig. 2B-2E.

Supplemental Figure 3. Cell growth inhibition assay. Cultured PC3/2G7, PC3, and 9L gliosarcoma cells (positive control) were treated with axitinib at the indicated concentrations for 4 days continuously and the effect on final cell numbers, indicative of relative growth rate, was determined by crystal violet staining. Results show that axitinib does not directly inhibit PC3 or PC3/2G7 cell growth in culture, in contrast to its inhibitory effects on 9L gliosarcoma cell growth, which served as a positive control. Data shown are mean ± SD values, for n = 6 replicate samples per data point.

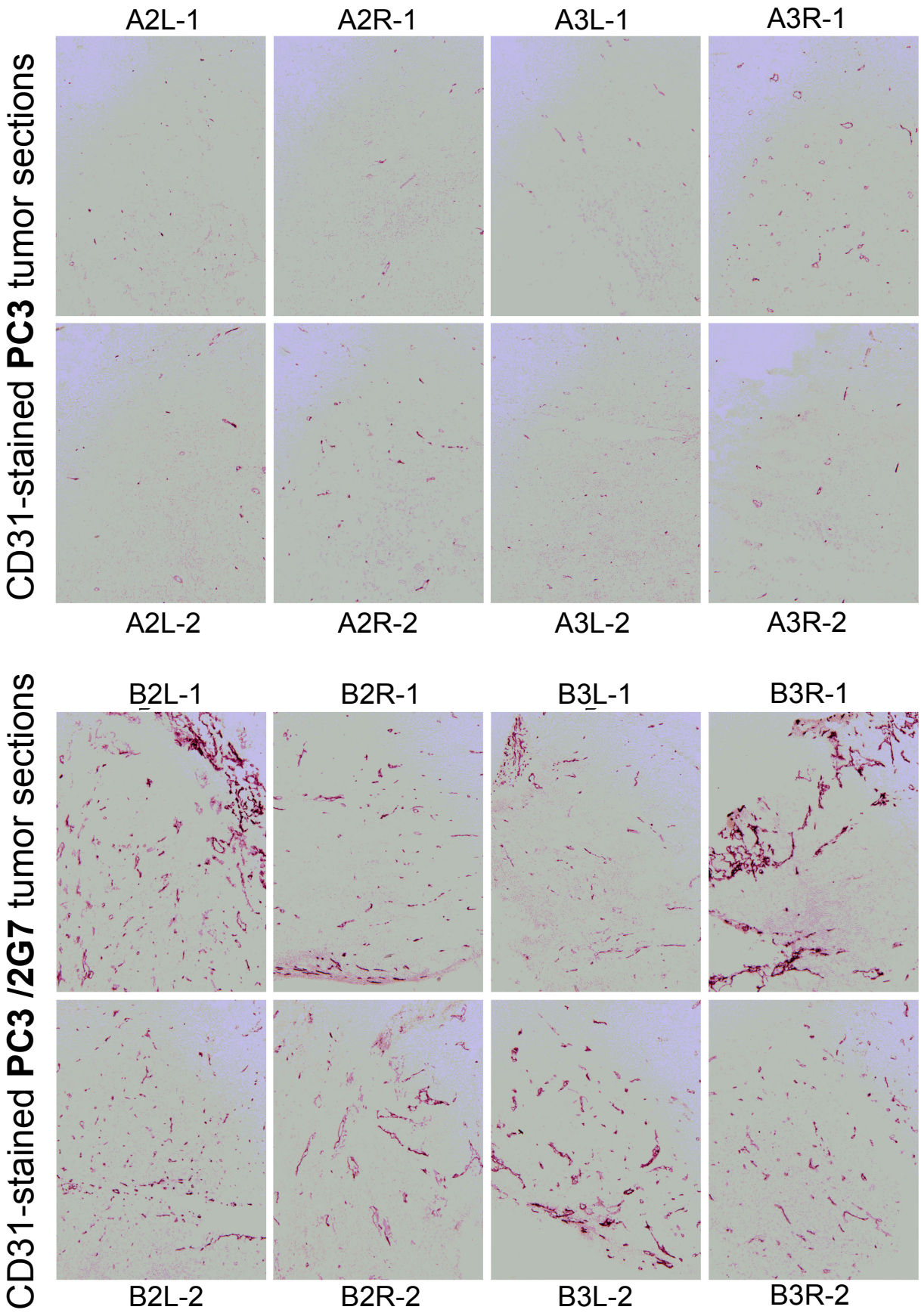
Supplemental Figure 4. Effects of daily sorafenib treatment on growth of PC3 and PC3/2G7 tumors. Tumor cells (4×10^6 cells) were implanted bilaterally by s.c. injection into each flank of male scid mice. Tumor sizes were measured every 3 days using digital calipers. Sorafenib was administered to the tumor-bearing mice daily for 21 days (first injection on day 0) by i.p. injection at 25 mg/kg body weight. Body weight measurements was taken every 3 days during treatment as a monitor of host toxicity (*bottom panels*).

Supplemental Figure 5. Impact of DC101 treatment on growth of PC3 and PC3/2G7 tumors. Tumor cells (4×10^6 cells) were implanted bilaterally by s.c. injection into each flank of male scid mice. Tumor sizes were measured every 3 days using digital calipers. DC101 was administered to the tumor-bearing mice every 3 days by i.p. injection at 28.6 mg/kg body weight. A total of 10 cycles DC101 were administered, beginning on day 0 and concluding on day 27. Body weight measurements were taken every 3 days during treatment as a monitor of host toxicity (*bottom panels*).

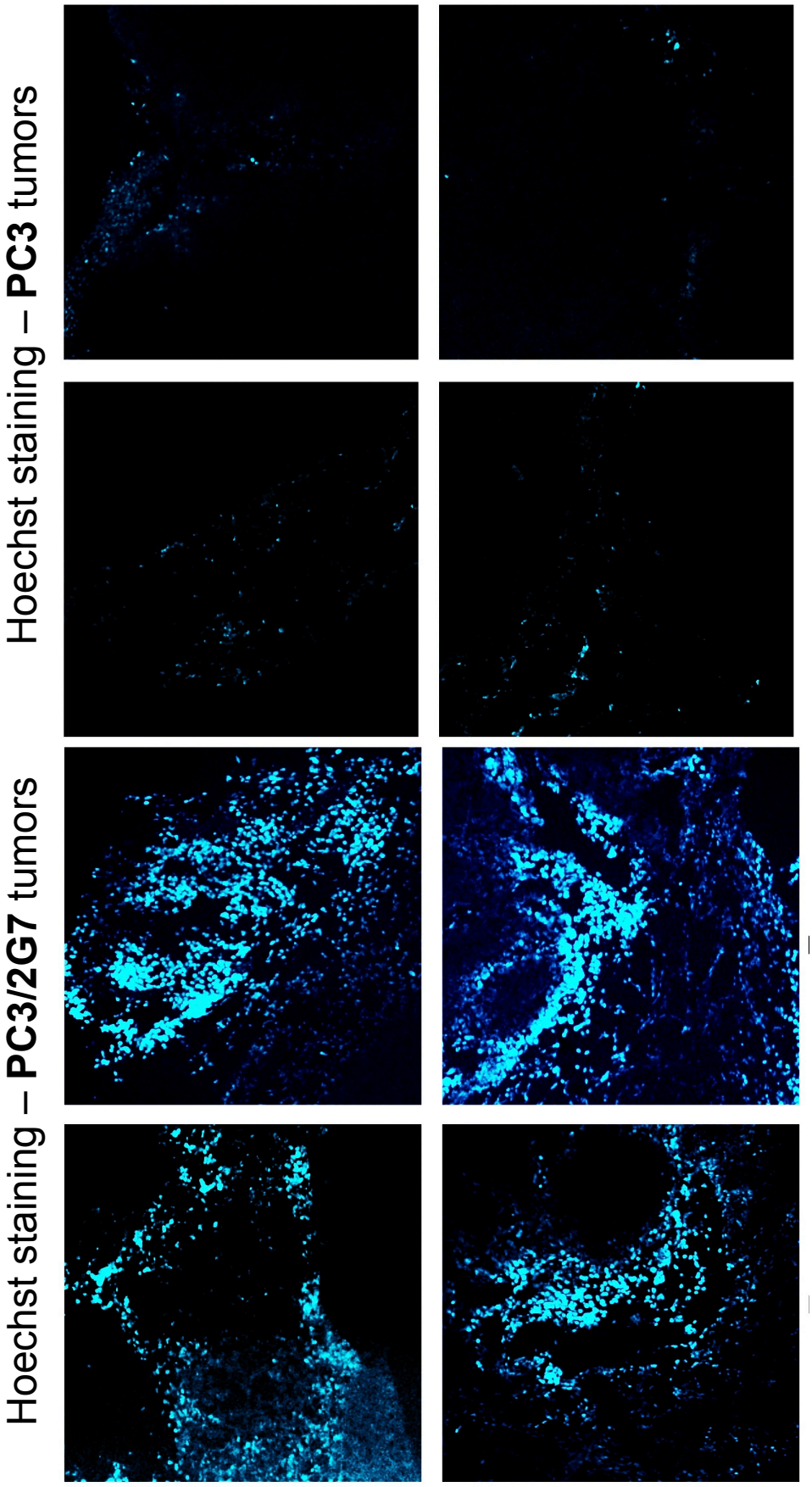
Supplemental Figure 6. Expression of VEGFC (**A**) and interleukin-8 (IL8) (**B**) in PC3/2G7 and PC3 tumors, determined by qPCR. VEGFC were not significantly changed, and IL8 levels were significantly decreased in PC3/2G7 tumors compared to PC3 tumors. Data presented are from two independent studies. qPCR Ct numbers are shown above select bars at the left.

Supplemental Figure 7. Images showing increased CD31 immunostained microvessel density in PC3/2G7 tumors after 58 days axitinib treatment, as in Fig. 6D, right. Neovascularization near the tumor periphery was apparent (magnification, 4.2x), indicating escape of PC3/2G7 tumors from anti-angiogenesis.

Supplemental Fig. 1A

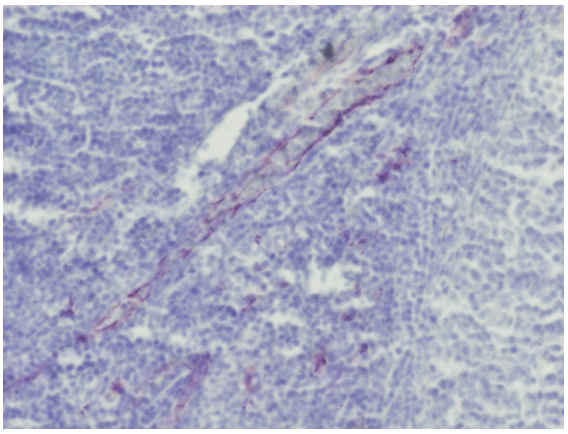


Supplemental Fig. 1B

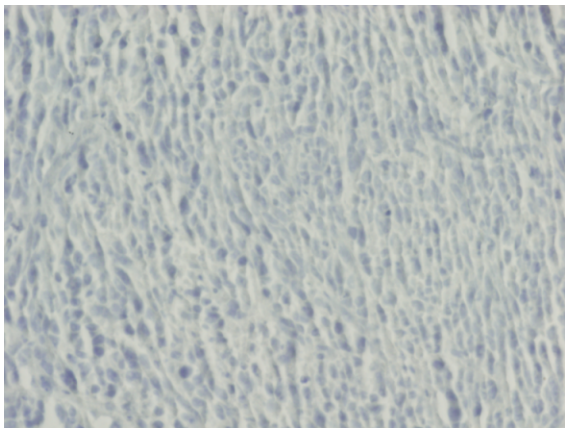


Supplemental Fig. 1C

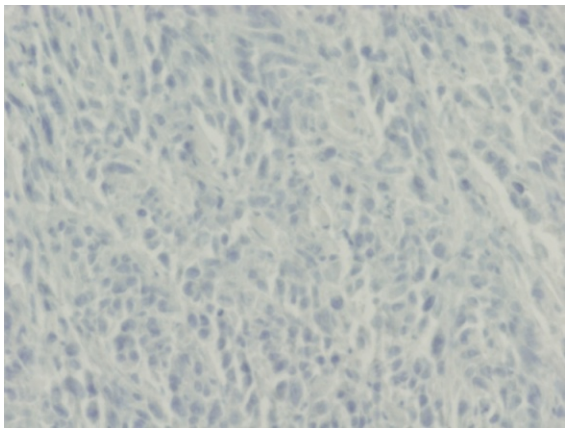
Human CD31-stained tissue sections



Human tonsil

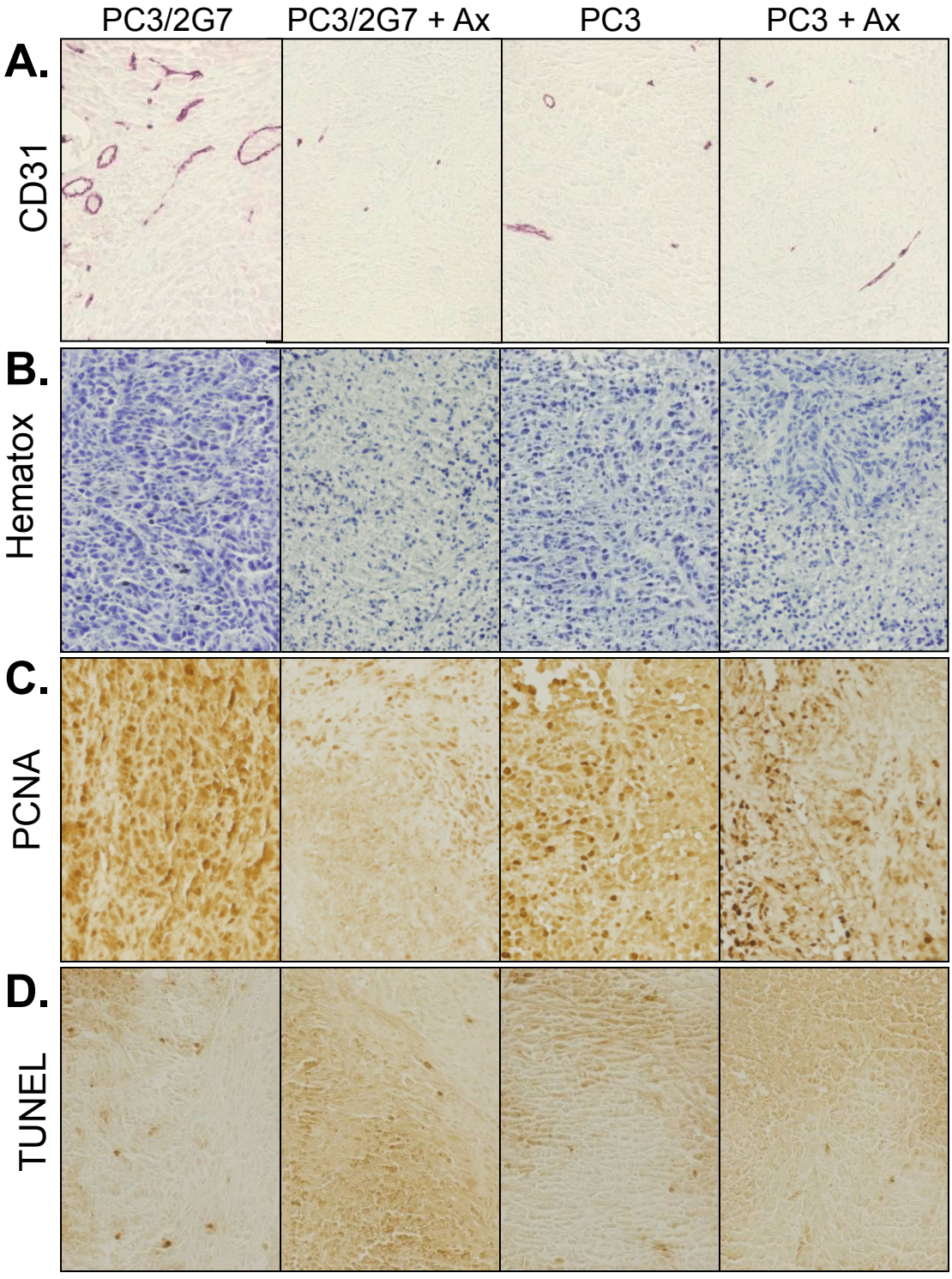


PC3/2G7 tumor

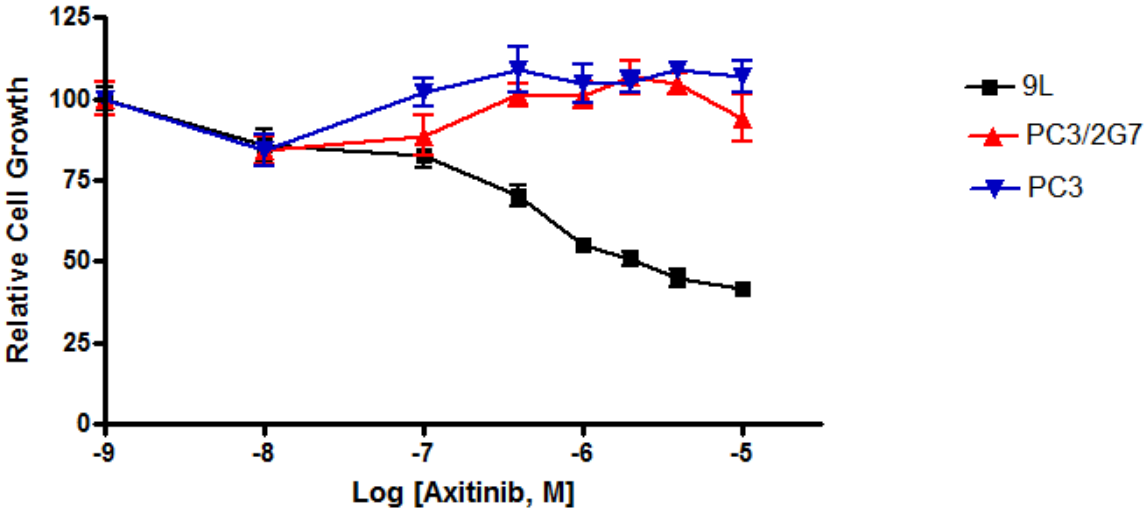


PC3 tumor

Supplemental Fig. 2

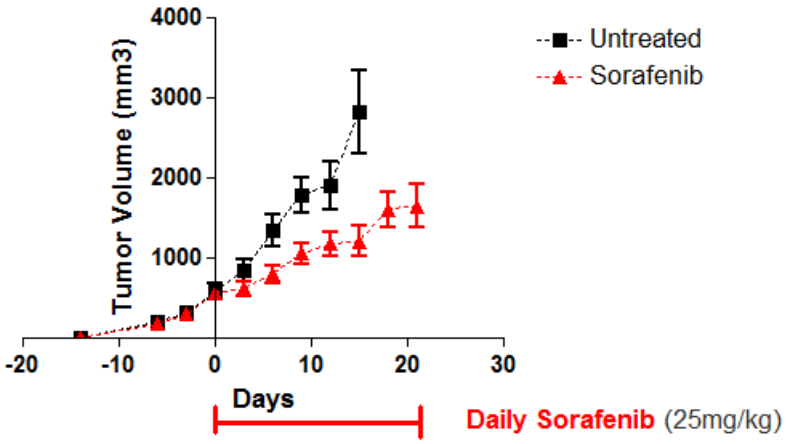


Supplemental Fig. 3

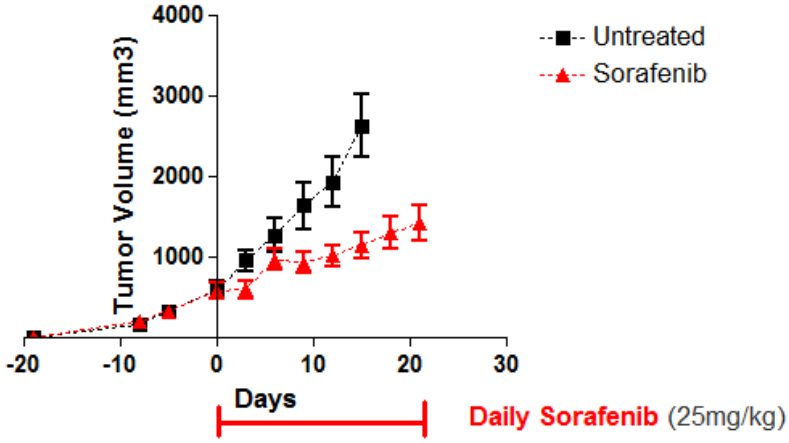


Supplemental Fig. 4

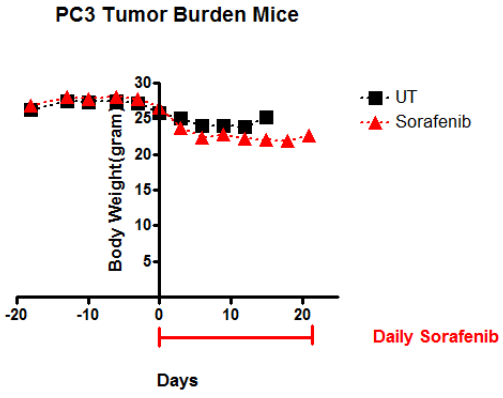
PC3 Tumors

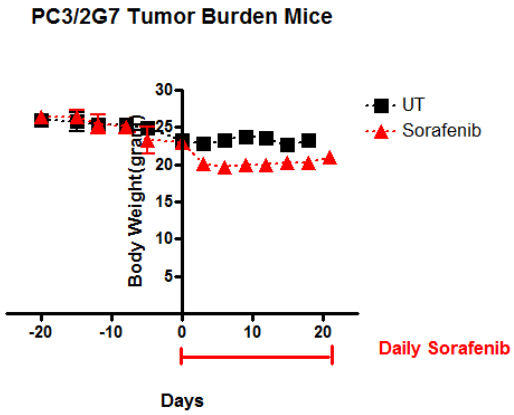


PC3/2G7 Tumors

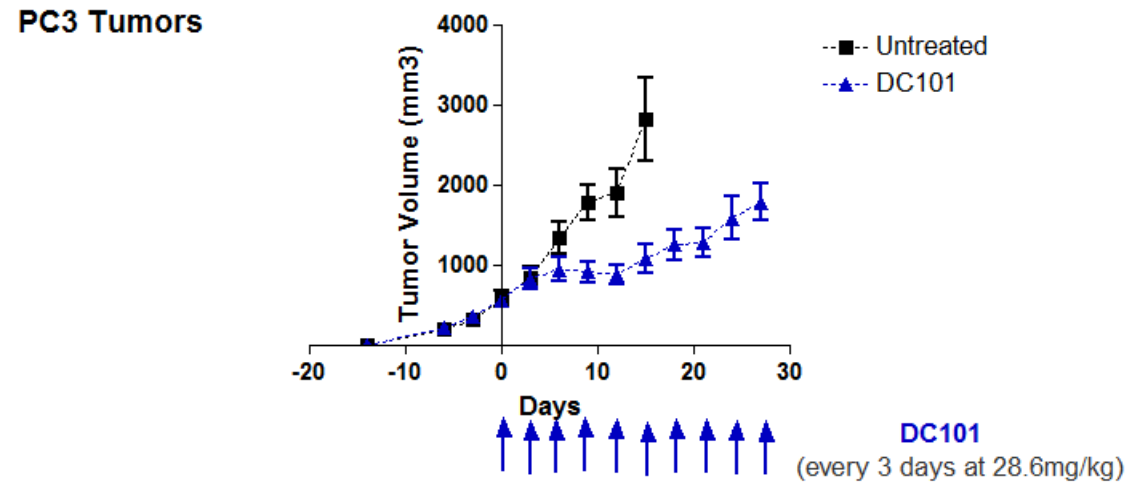


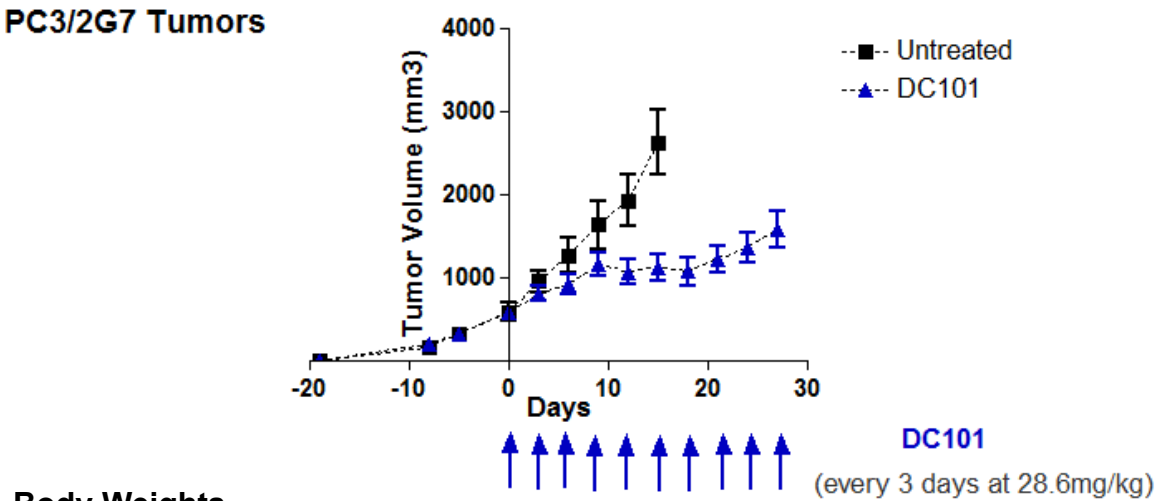
Body Weights



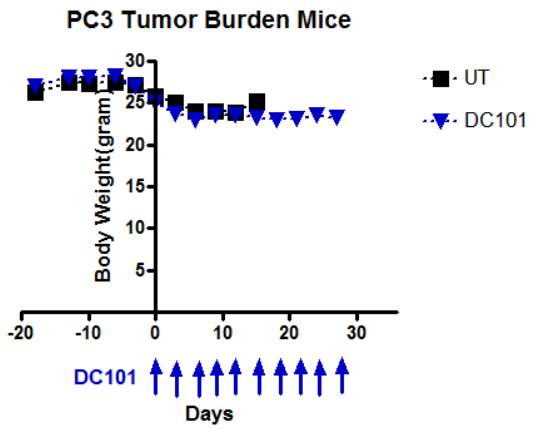


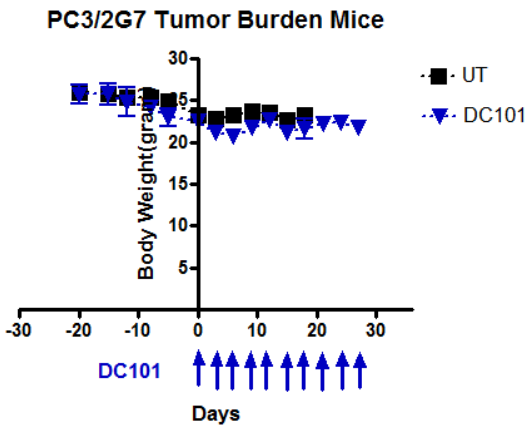
Supplemental Fig. 5



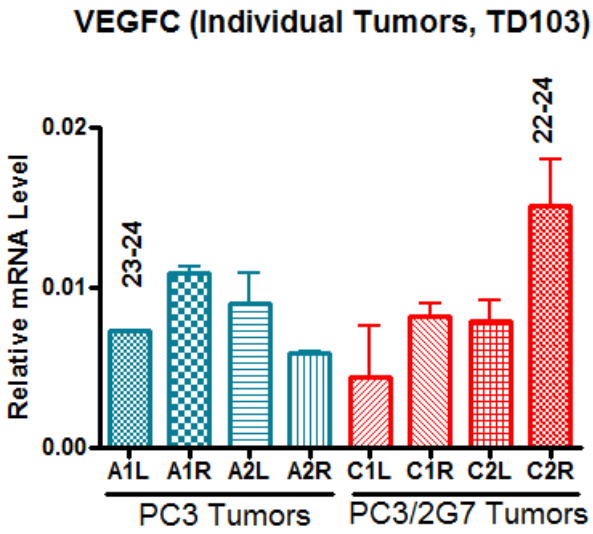


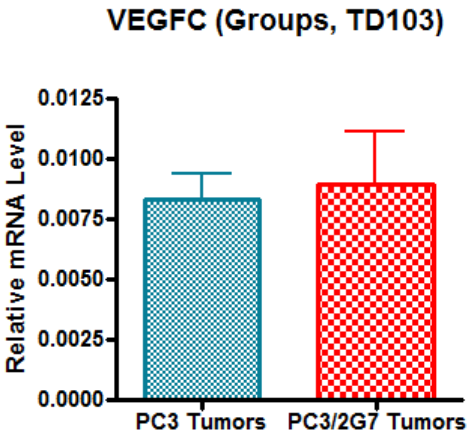
Body Weights

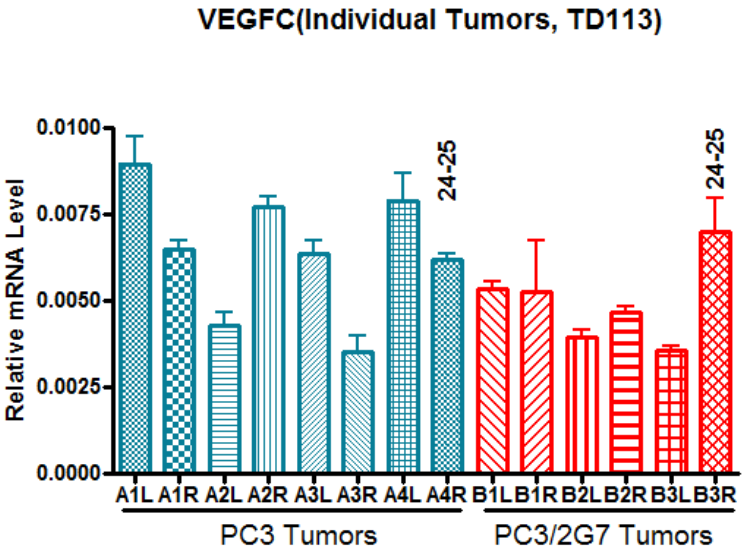


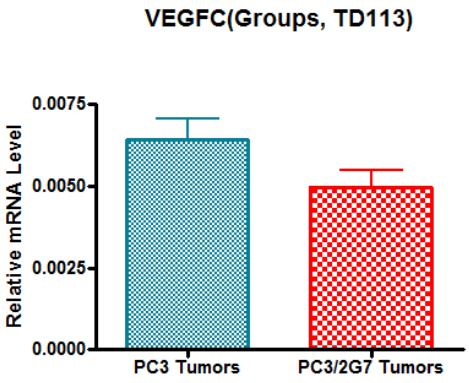


Supplemental Fig. 6A

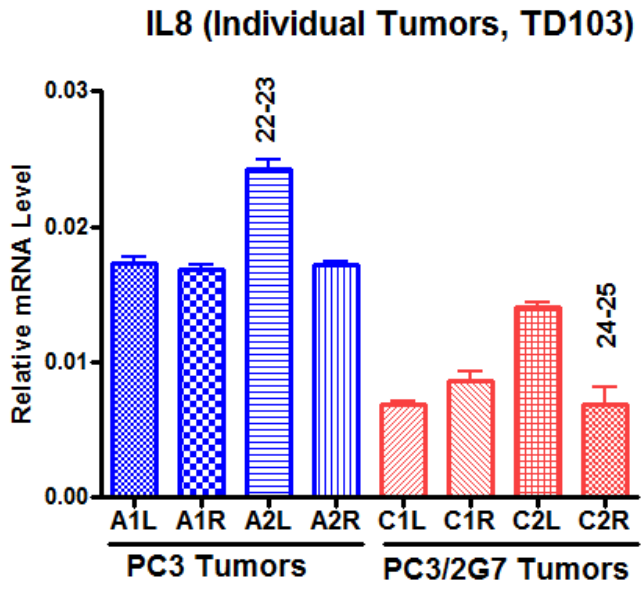


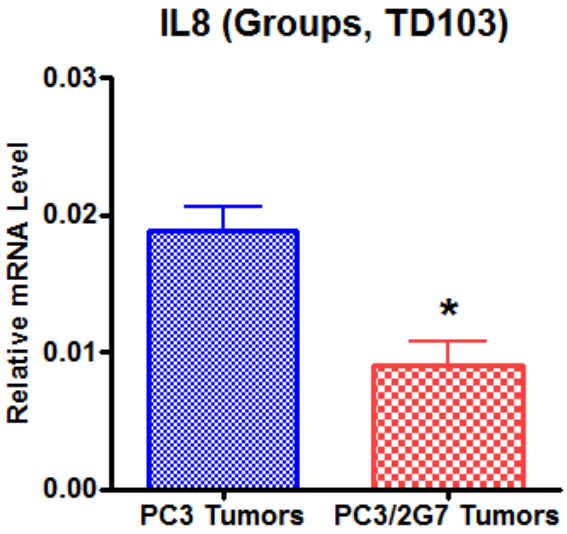


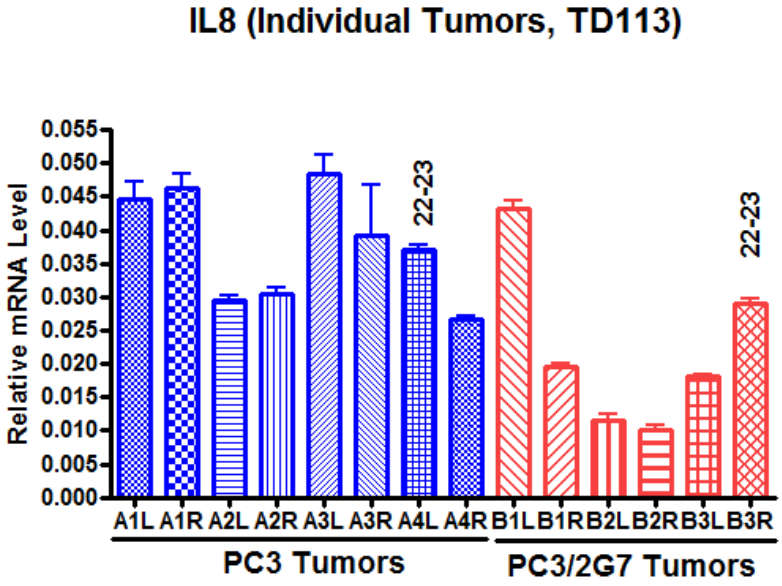


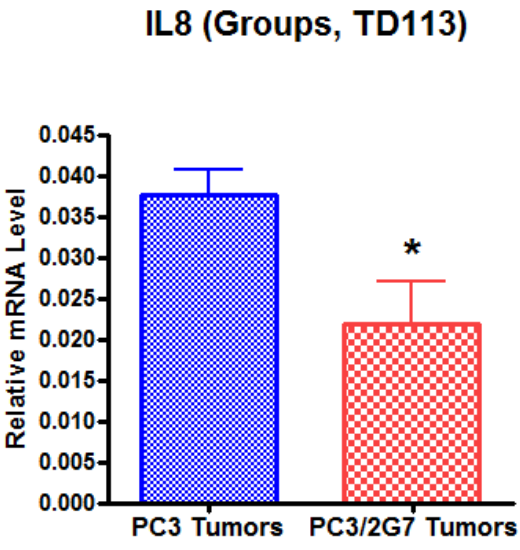


Supplemental Fig. 6B









Supplemental Fig. 7