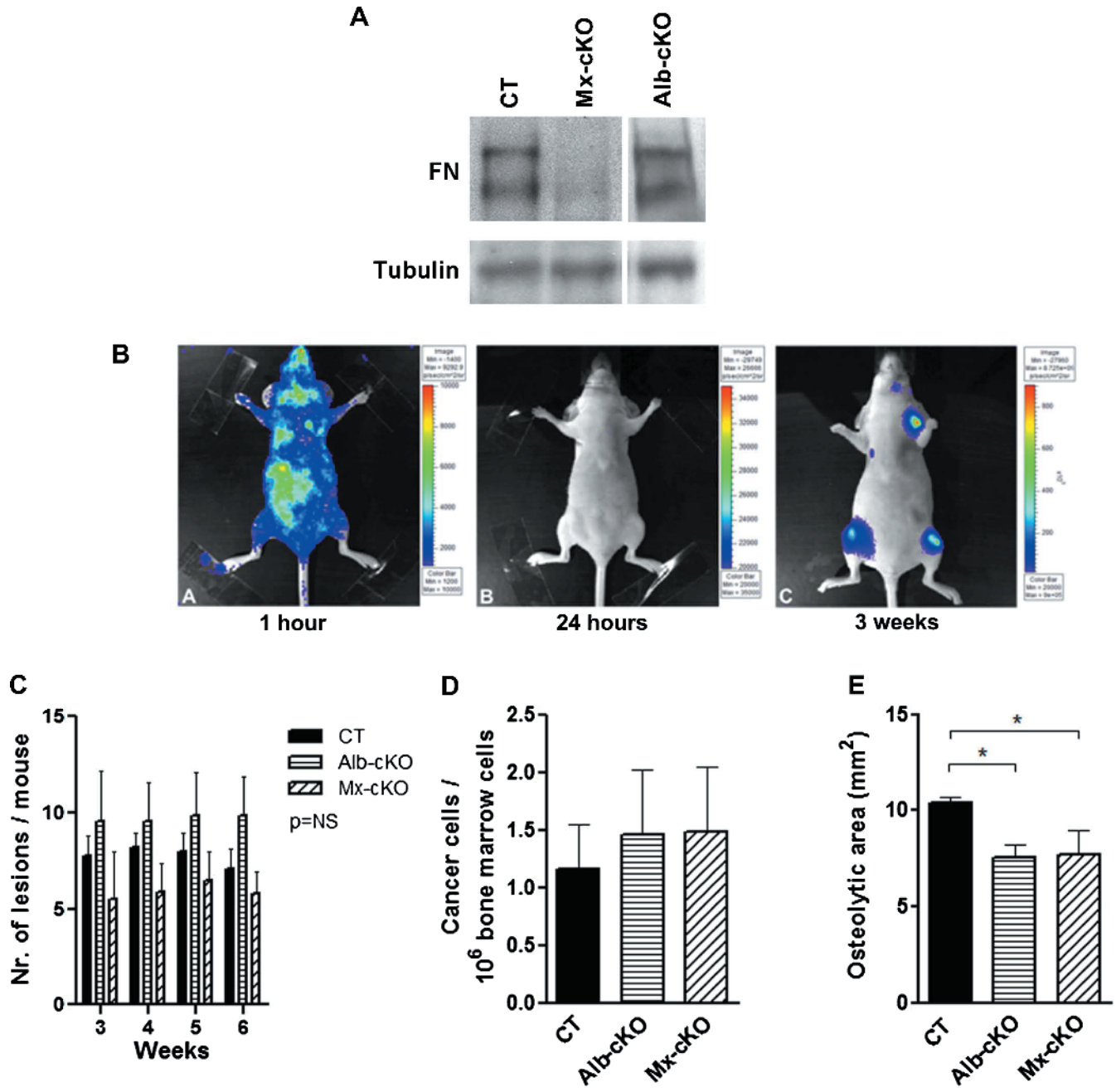

Figure W1. (A) Western blot of bone marrow lysate confirms decreased fibronectin content (FN) in Mx-cKO and normal content in Alb-cKO. Bone marrow was flushed with 100 μl of protein lysis buffer/femur. (B) Bioluminescence signal after intracardiac injection of MDA-MB-231/B+: 1 hour after intracardiac injection, a very weak signal can be detected (embedded A), which is gone by 24 hours after injection (embedded B). Lesions are detected by 3 weeks after injection (embedded C). To measure the bioluminescence signal, mice were injected with 150 mg/kg luciferin exactly 5 minutes before starting measurements. (C) Intracardiac injection of MDA-MB-231 in Alb- and Mx-cKO mice results in a similar number of lesions per mouse as determined by BLI. The *x*-axis represents weeks since intracardiac injection (N = 11-12/group). (D) Homing of cancer cells to the bone marrow was not affected by the deletion of circulating fibronectin and/or fibronectin in the bone marrow, as determined by qPCR. Cancer cells (10^5) were injected intracardially, and 24 hours later, DNA was extracted and examined by qPCR for human alu. Results are adjusted to murine bone marrow (N = 6/group). (E) Intratibial injection is associated with a decrease in growth in both Alb- and Mx-cKO. The results of bioluminescence signal measurements are shown in Figure 1*F*. The decrease in the area of osteolysis at the end of the experiment at day 40 after intratibial injection is shown here (N = 9-11/group). *$P < .05$.

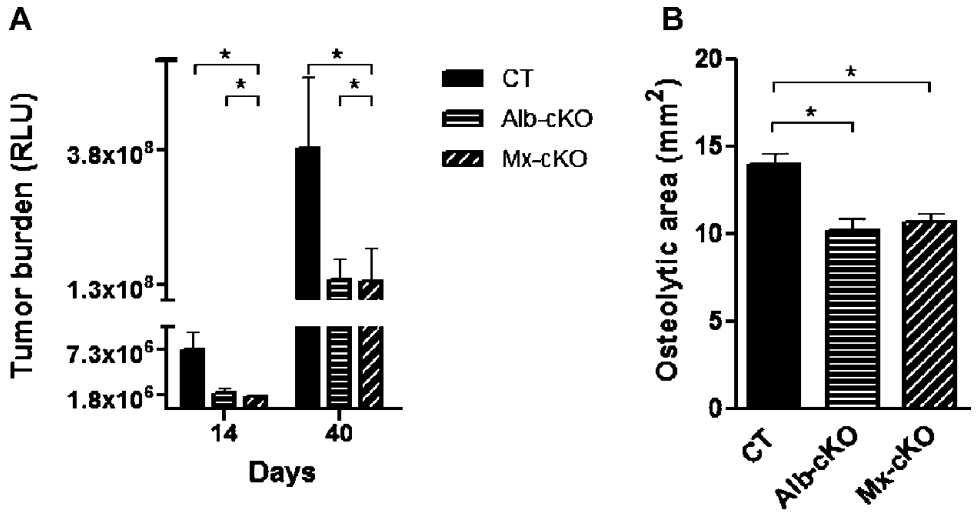

Figure W2. Findings in a model of prostate cancer. Tumor cells of the line PC3Mpro4/luc+ were injected intratibially in male mice, and bioluminescence signal was examined at the time points shown on A. (A) Decreased growth by bioluminescence signal ($N = 12$/group). (B) Area of osteolysis was diminished at the time of death 40 days after intratibial injection.

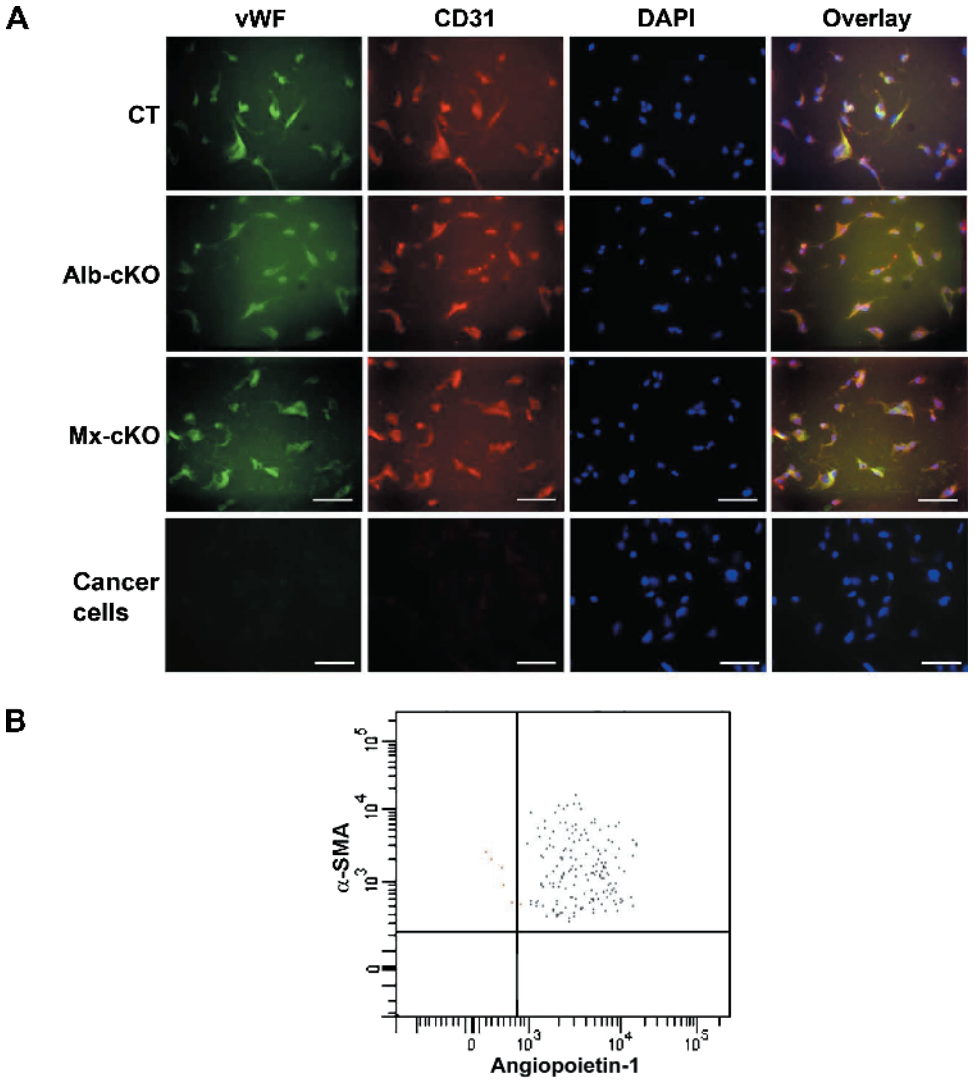

Figure W3. Confirmation of identity of isolated endothelial cells and pericytes. (A) Endothelial cells isolated using CD31-coated Dynabeads stained positive for both vWF and CD31. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). Cancer cells did not show any staining with either endothelial cell markers. Bars represent 50 μm. (B) Pericytes isolated by sorting stained positive for both angiopoietin-1 and α-SMA by flow cytometry.

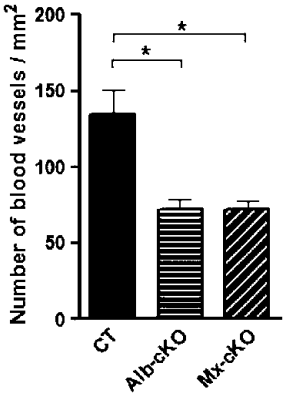

Figure W4. Angiogenesis in a prostate cancer model. Tumor cells of the line PC3Mpro4/luc+ were injected intratibially in male mice. Forty days after injection, mice were killed and tumors were examined using CD31 staining. There was a significant decrease in blood vessel numbers in Alb-cKO and Mx-cKO tumors, but no difference between Alb- and Mx-cKO tumors ($N = 4$/group). *$P < .05$.

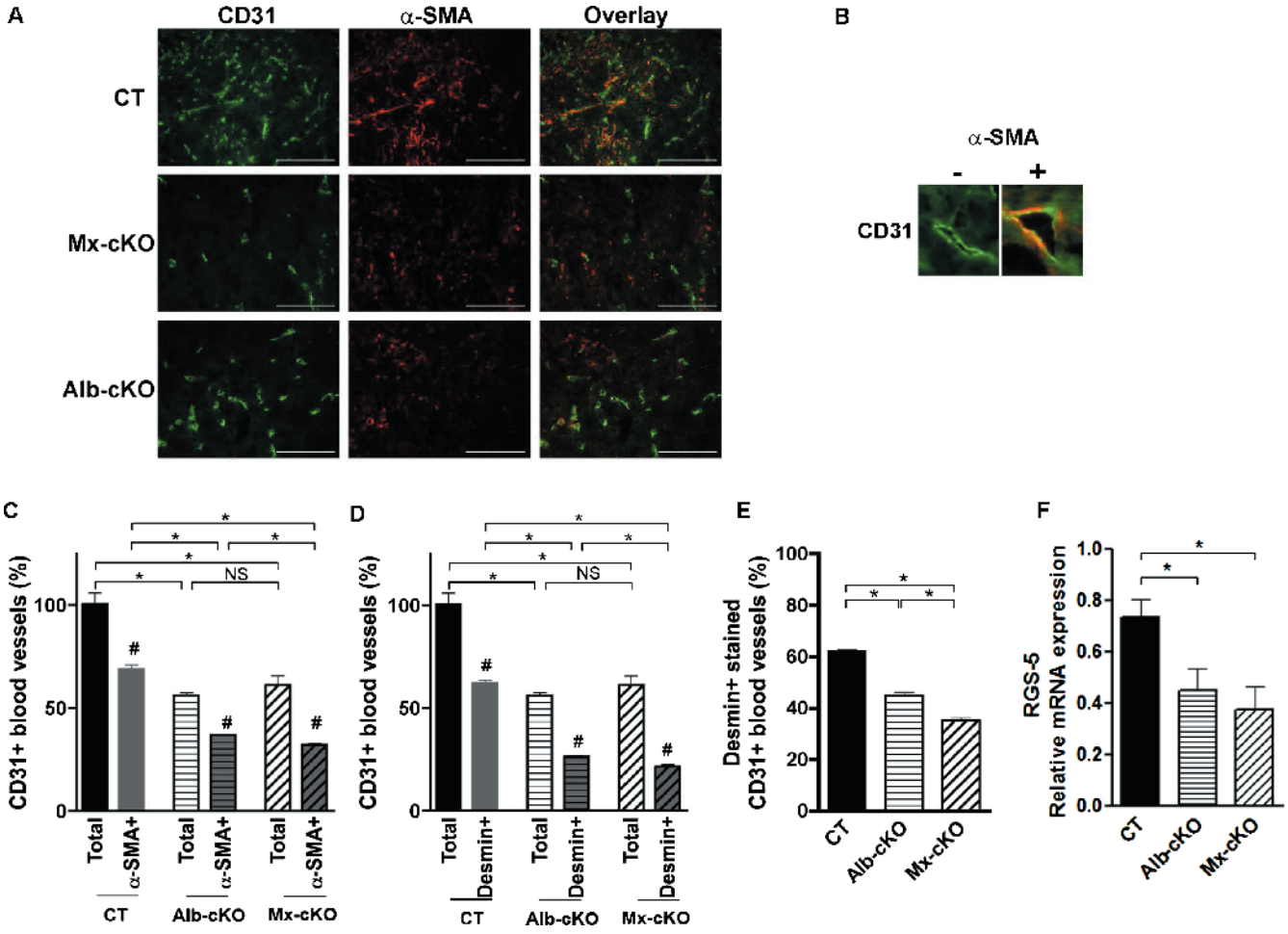

Figure W5. Effects of fibronectin on vessel maturation. (A) CD31+ blood vessels were additionally stained with α-SMA. Bars represent 200 μm. (B) A detailed view of a blood vessel co-stained with CD31 and α-SMA. The quantification is shown in Figure 3*E* and in C. (C) CD31+ blood vessel number was decreased in both cKO models to almost the same degree compared to CT (total). In addition, there was a decrease in double positive CD31+ α-SMA+ blood vessels in both cKOs, but their percentage was higher in Alb-cKO compared to Mx-cKO. The number of blood vessels corrected to the area in CT tumors was set at 100% to allow for comparison of relative changes between the groups. $^{\#}P < .05$ for α-SMA+ *versus* total in each group ($N = 3, 3, 6$ mice, four sections/mouse). (D) This graph is similar to C except that it shows the data for desmin staining instead of α-SMA. The number of blood vessels corrected to the area in CT tumors was set at 100% to allow for comparison of relative changes between the groups. $^{\#}P < .05$ for desmin+ *versus* total in each group. N as in C. (E) The decrease in the percentage of desmin+ blood vessels was more pronounced in Mx- than in Alb-cKO tumors. For the purpose of this graph, CT, Alb-cKO, and Mx-cKO CD31+ vessels were set at 100%. This graph is similar to Figure 3*E* except that it shows the data for desmin staining instead of α-SMA. N as in C. (F) Relative mRNA expression of the pericyte marker RGS5 was diminished in both cKOs ($N = 7$ CT, 4 Alb-cKO, and 5 Mx-cKO). $^{*}P < .05$, $^{**}P < .01$.

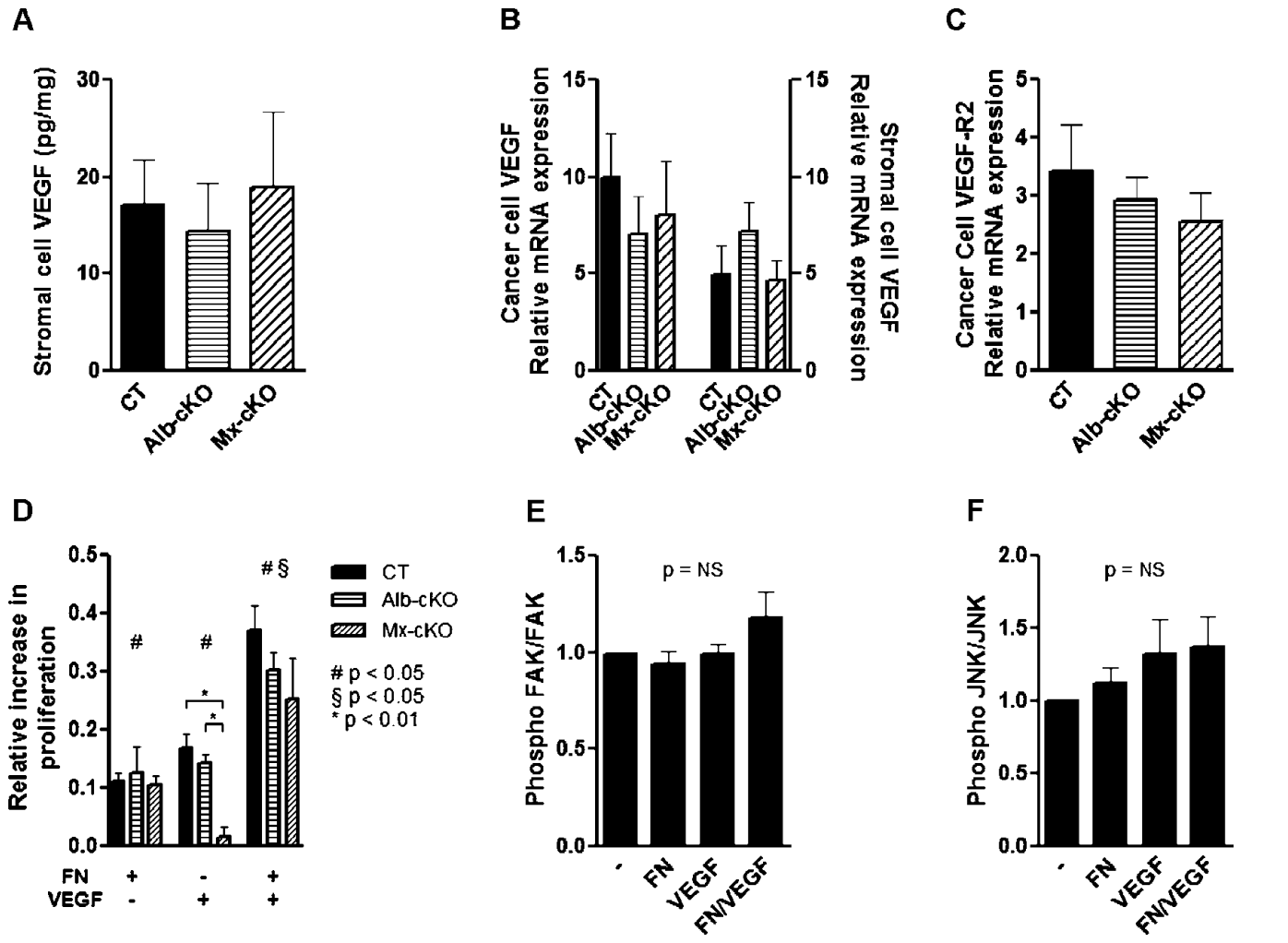

Figure W6. Changes in VEGF, VEGFR-2, and signaling *in vivo* and *in vitro*. (A) Murine VEGF originating from stromal cells was not affected in cKO tumors (measured using murine-specific ELISA), while cancer cell (human) VEGF was (shown in Figure 4*A*; $N = 4$-6/group). (B) Neither cancer cell nor stromal VEGF mRNA differed between CT and cKO tumors ($N = 4$/group). (C) Cancer cell (human) VEGFR-2 mRNA expression was not affected in cKO tumors, but murine VEGFR-2 (shown in Figure 4*D*) was ($N = 4$/group). (D) Cell proliferation was significantly increased when endothelial cells were treated with either fibronectin or VEGF compared to untreated cells, except for Mx-cKO endothelial cells, which failed to proliferate in response to VEGF alone. The addition of both simultaneously resulted in a more pronounced increase in proliferation in the three groups; # denotes significant change compared to baseline proliferation; § denotes significant increase compared to either fibronectin (FN) or VEGF alone ($N = 3$ experiments with three to five replicates/condition per experiment. (E and F) Fibronectin, VEGF, or the combination of both does not affect FAK (E) or JNK (F) phosphorylation in tumor endothelial cells. The data are the average of four experiments with three to five replicates/condition per experiment. Equal amounts of protein as measured by BCA were loaded. The Western blot is shown in Figure 4*G*.

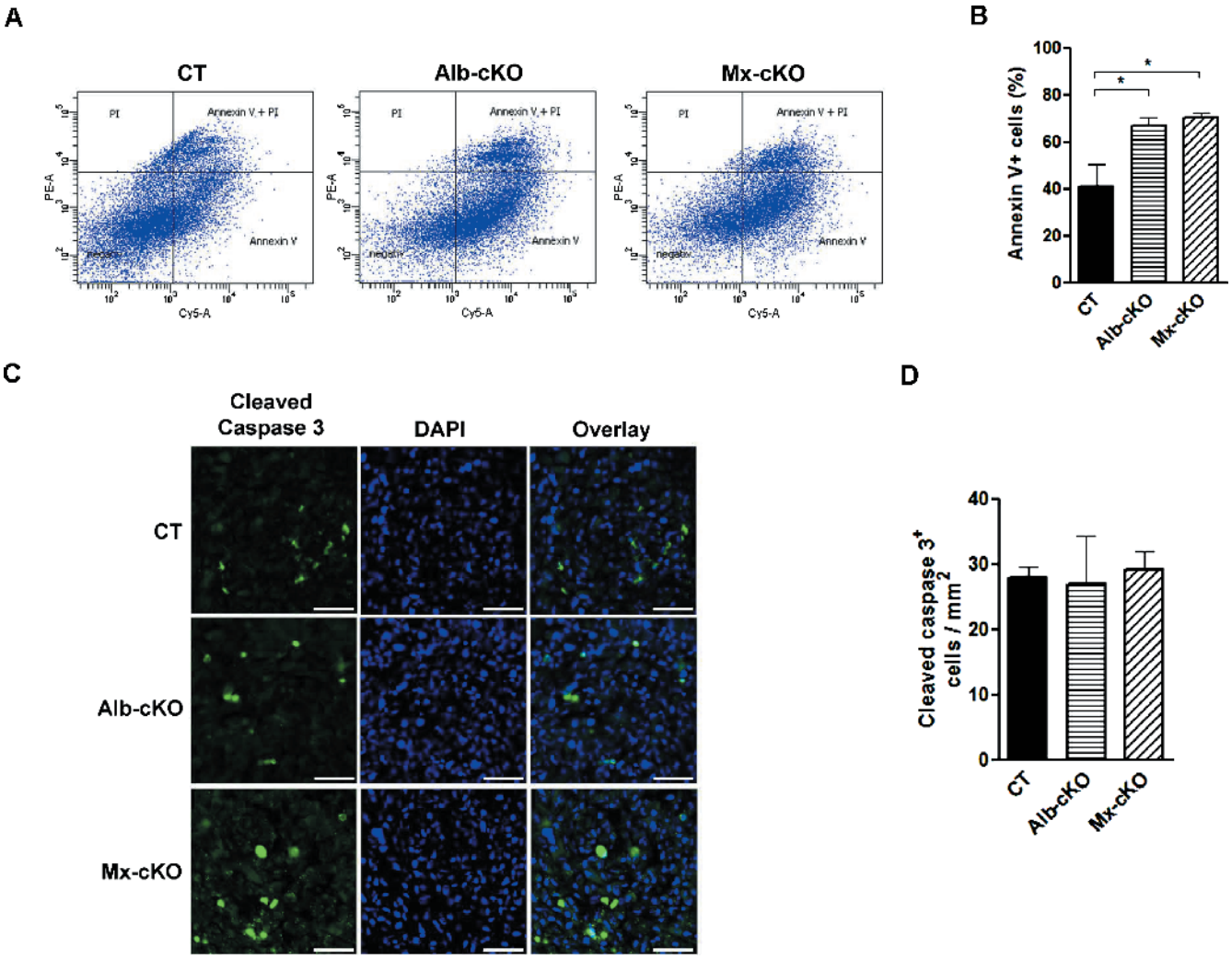

Figure W7. Effects of fibronectin on apoptosis. (A) Apoptosis in tumor was determined by digesting tumor tissue followed by staining for AV and PI. (B) The number of tumor cells stained positive with AV (AV+/PI+ and AV+/PI−) is increased in Mx-cKO and Alb-cKO tumors (N = 3/group). (C) Cleaved caspase-3 was not affected in Mx- and Alb-cKO tumors compared to CT. Bars represent 100 μm. (D) Quantification confirms absence of a difference (N = 6 CT, 3 Alb-cKO, and 3 Mx-cKO).

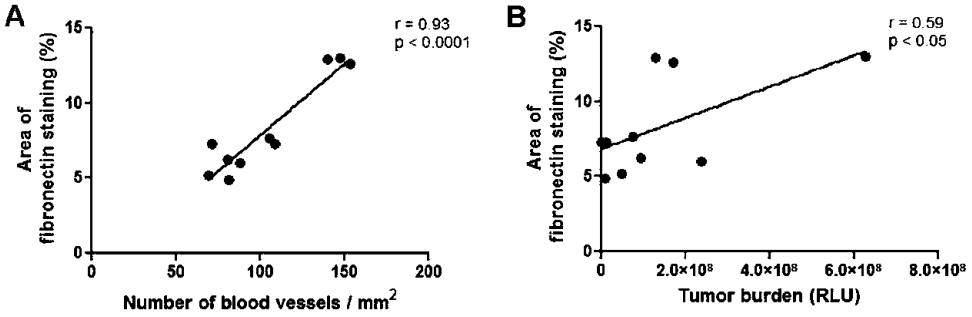

Figure W8. Correlations between fibronectin staining in intratibial MDA-MB-231b/luc+ tumors and blood vessel numbers or bioluminescence signal. (A) Area of fibronectin staining correlates with blood vessel numbers (N = 4 CT, 3 Alb-cKO, and 3 Mx-cKO). (B) Area of fibronectin staining correlates with bioluminescence signal in the same groups as A.