Supplemental Material

Endothelial Tie1 regulates tumor angiogenesis, vascular abnormalization and metastatic dissemination

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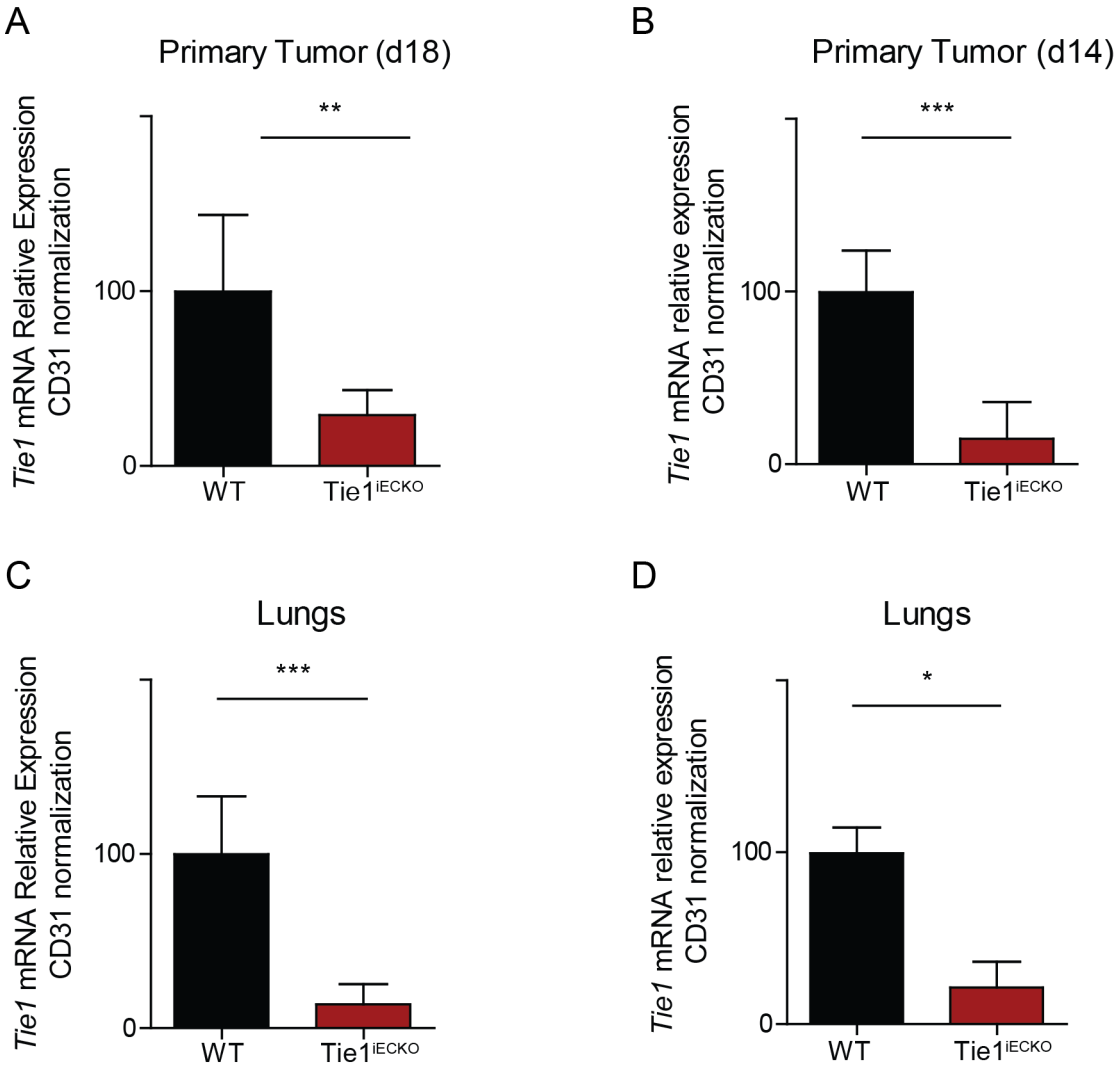

Figure S1. Tie1 deletion efficiency in primary tumor and lung vasculature.

(A) Relative *Tie1* expression analyzed 18 days post-injection from total tumor RNA of WT and Tie1^{iECKO} LLC tumor bearing mice (n=5; two-tailed Mann Whitney test, **, p<0.01). **(B)** Relative *Tie1* expression analyzed at the time of tumor resection (d14) from total tumor RNA of WT and Tie1^{iECKO} LLC tumor bearing mice treated with tamoxifen during primary tumor growth (n=8 [WT] and 10 [Tie1^{iECKO}]; two-tailed Mann Whitney test, ***, p<0.001). **(C)** Relative *Tie1* expression from total lung RNA in the experimental metastasis protocol, two weeks after B16F10 injection (n=4 [WT] and 5 [Tie1^{iECKO}]; two-tailed Mann Whitney test, ***, p<0.001). **(D)** Relative *Tie1* expression analyzed at the time of sacrifice (d35) from total lung RNA of WT and Tie1^{iECKO} mice. Tamoxifen was administrated after surgery (n=8; two-tailed Mann Whitney test, ***, p<0.001).

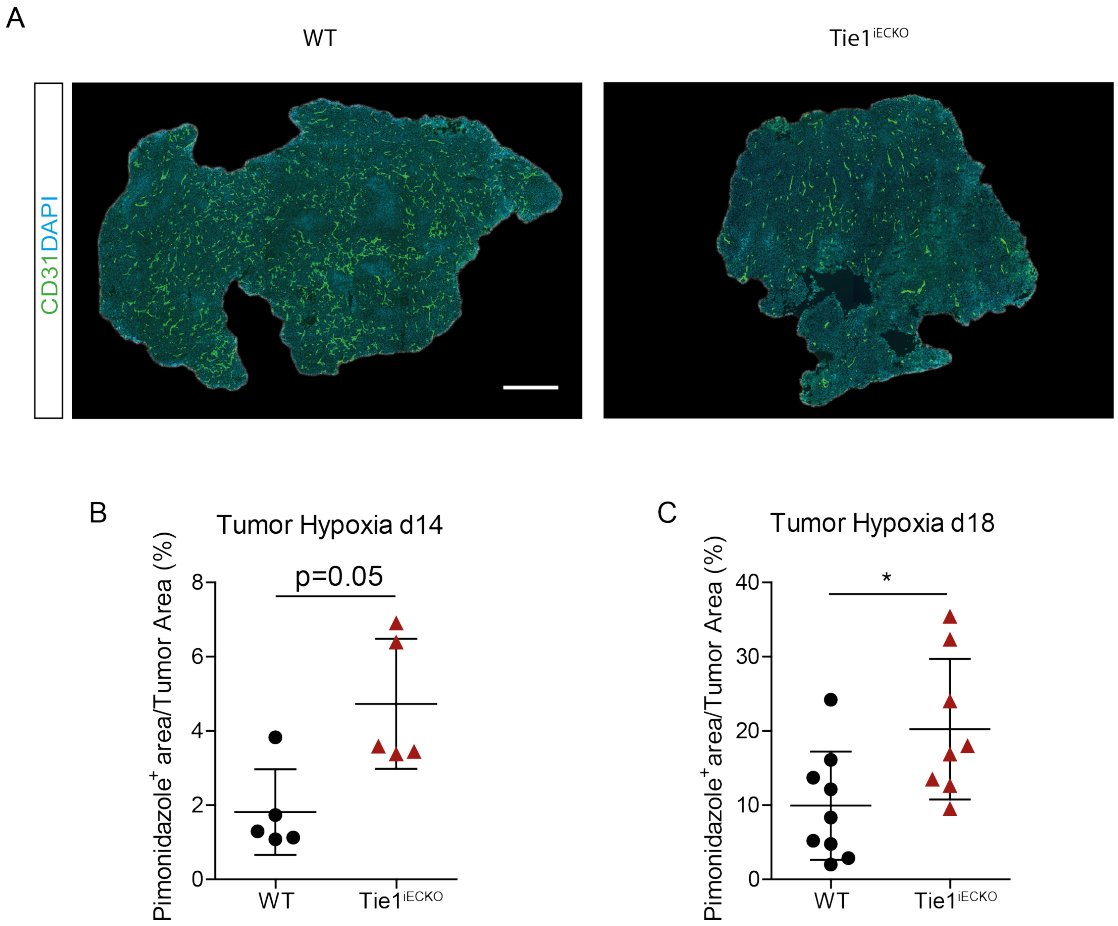

Figure S2. Tie1 endothelial deletion leads to reduced microvessel area and density.
(A) Representative microscopy pictures of LLC tumor grown in WT and Tie1^{iECKO} 14 days after tumor cells inoculation. Blood vessels are stained with the pan-endothelial marker CD31 and nuclei with DAPI. Scale bar: 500 µm. **(B)** Quantification of hypoxic area, stained with pimonidazole, 14 days post LLC-inoculation (n=5; two-tailed Mann Whitney test). **(C)** Quantification of hypoxic area, stained with pimonidazole, 18 days post LLC-inoculation (n=9 [WT] and 8 [Tie1^{iECKO}]; two-tailed Mann Whitney test).

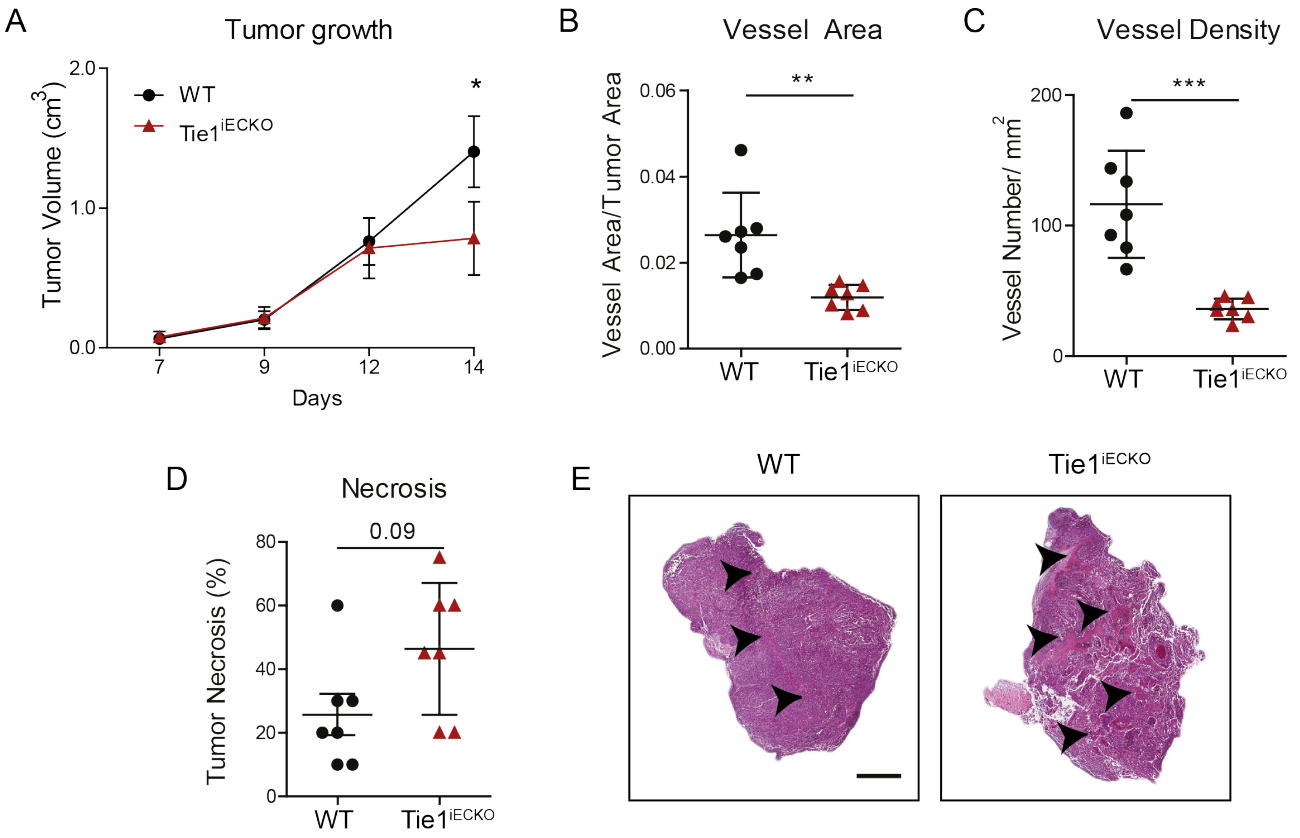

Figure S3. Tie1^{iECKO} delays primary tumor growth and leads to reduced tumor angiogenesis and increased necrosis

(A) B16F10 tumor growth curves in WT and Tie1^{iECKO} mice (n=8; two-way ANOVA test, *, p<0.05. Data are expressed as mean ± SEM). Quantifications of **(B)** vessel area and **(C)** vessel density in B16F10 primary tumors after 14 days from tumor cells injection (n=7; two-tailed Mann Whitney test, **, p<0.01, ***, p<0.001). **(D)** Primary tumor necrosis quantification (n=7; two-tailed Mann Whitney test) and **(E)** representative pictures. Scale bar: 1 mm.

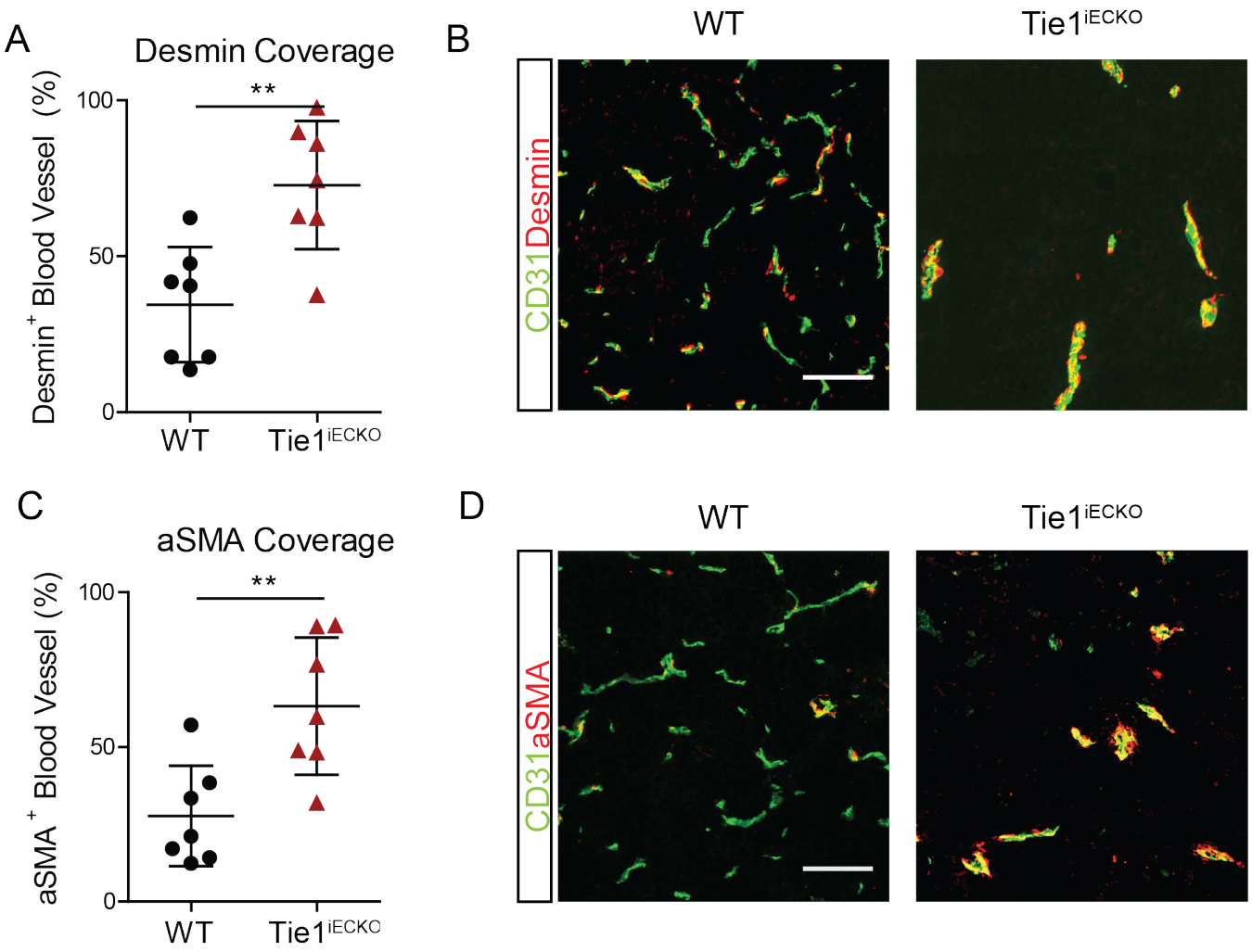

Figure S4. Tie1^{iECKO} improves perivascular coverage in B16F10 tumors.
(A) Quantification of desmin positive area colocalized with CD31 (n=7; two-tailed Mann Whitney test, **, p<0.01), and **(B)** representative pictures. Scale bar: 100 µm. **(C)** Quantification of aSMA positive area colocalized with CD31 (n=7; Mann Whitney test, **, p<0.01), and **(D)** representative pictures. Scale bar: 100 µm.

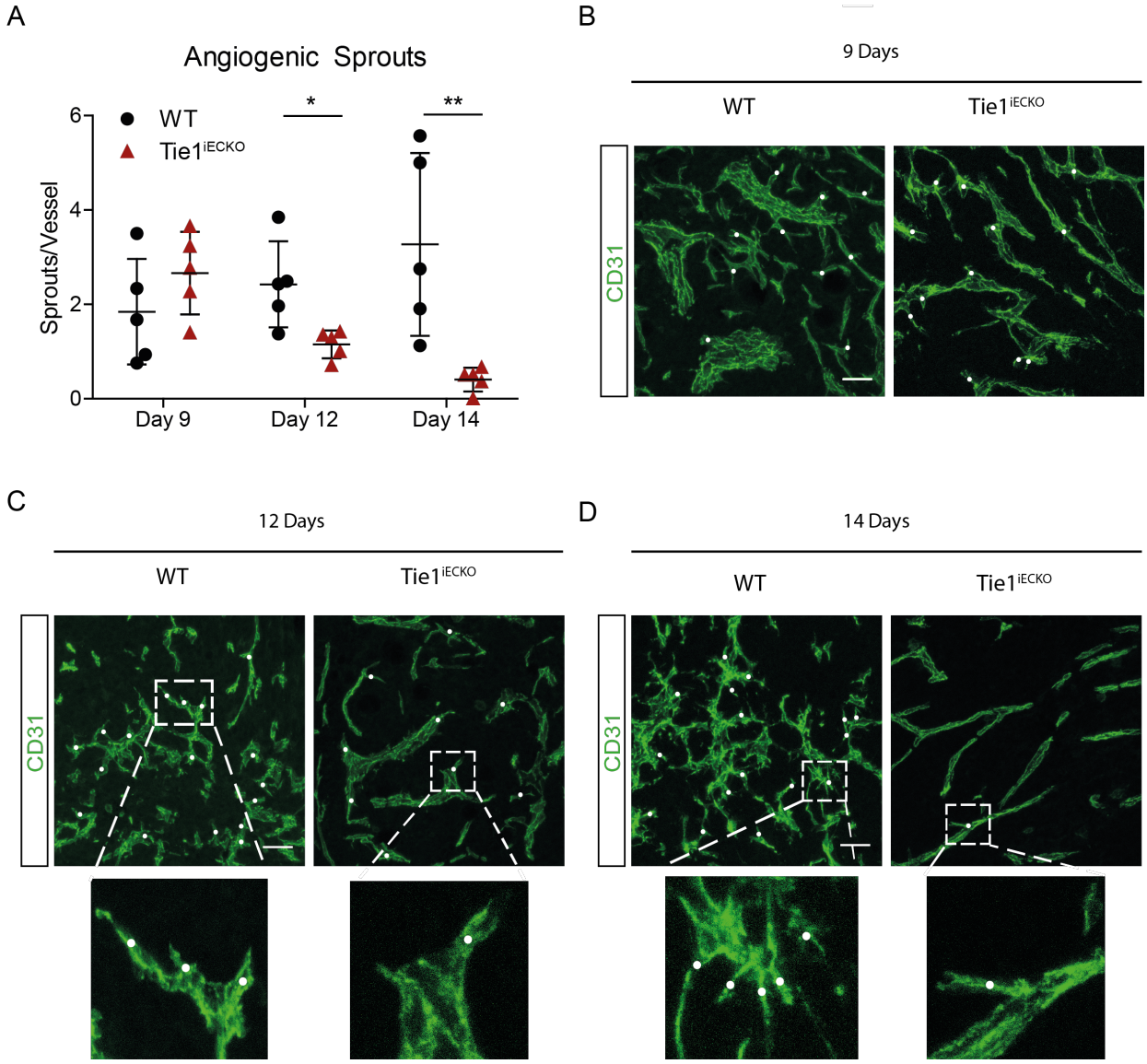

Figure S5. Tie1^{iECKO} reduces tip cells overtime.

Quantification of vessel sprouting 9, 12 and 14 days after LLC-inoculation (n=5; two-tailed Mann Whitney test, *, p<0.05, **, p<0.01) **(A).** Representative microscope pictures of CD31 stained blood vessels after 9 days **(B)**, 12 days **(C)** and 14 days post-LLC inoculation. White dots indicate angiogenic sprouts. Scale bar: 50 μm.

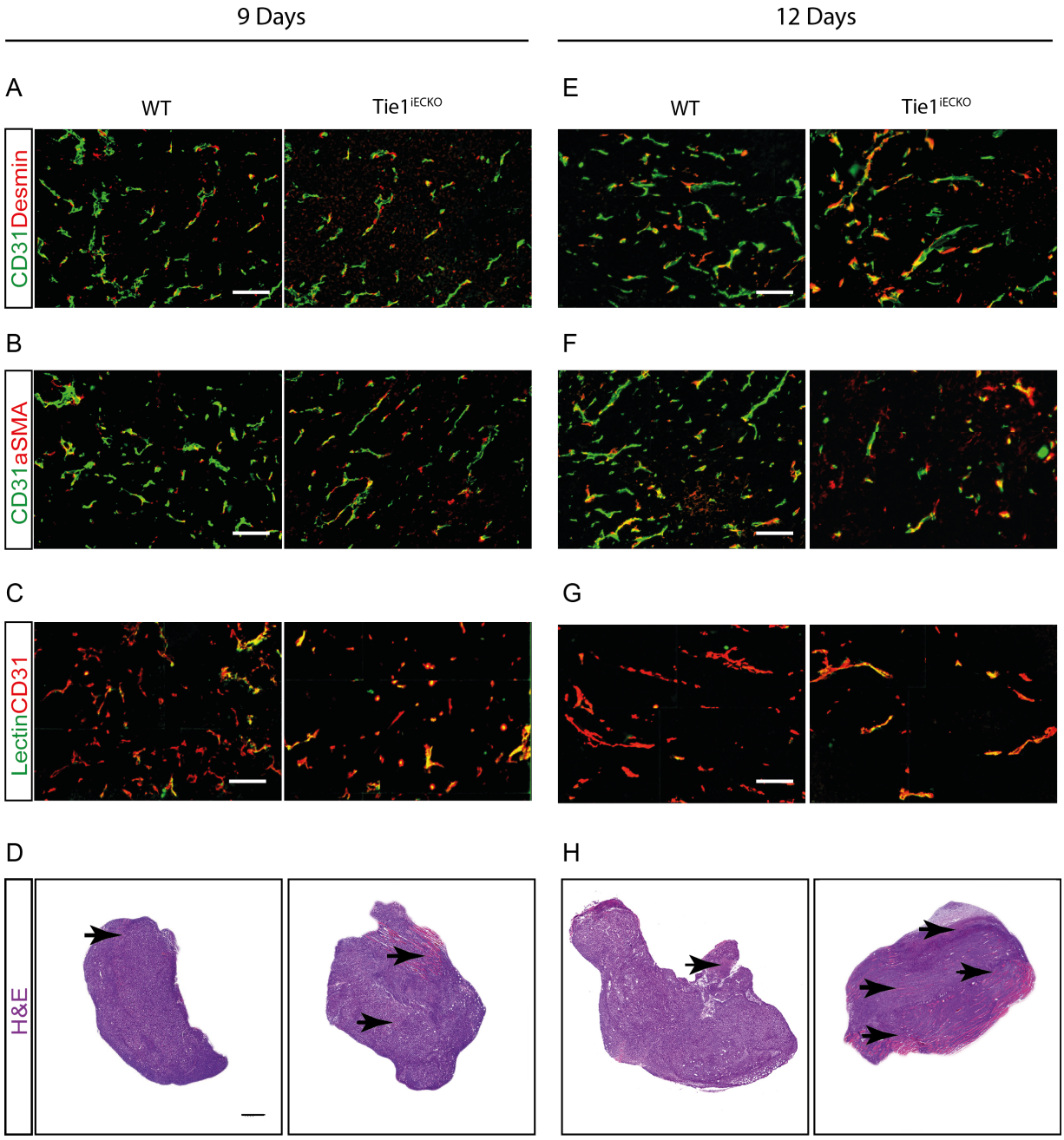

Figure S6. Tie1^{iECKO} induces vascular changes at later stages.

Representative microscope images of **(A)** Desmin^{+}, **(B)** aSMA^{+} and **(C)** lectin^{+} blood vessels 9 days post LLC-inoculation. **(D)** H&E staining from 9 days LLC primary tumors.

Images of primary tumor blood vessels positive for **(E)** Desmin **(F)** aSMA and **(G)** lectin 12 days post-injection. **(H)** H&E staining from 12 days primary tumors.

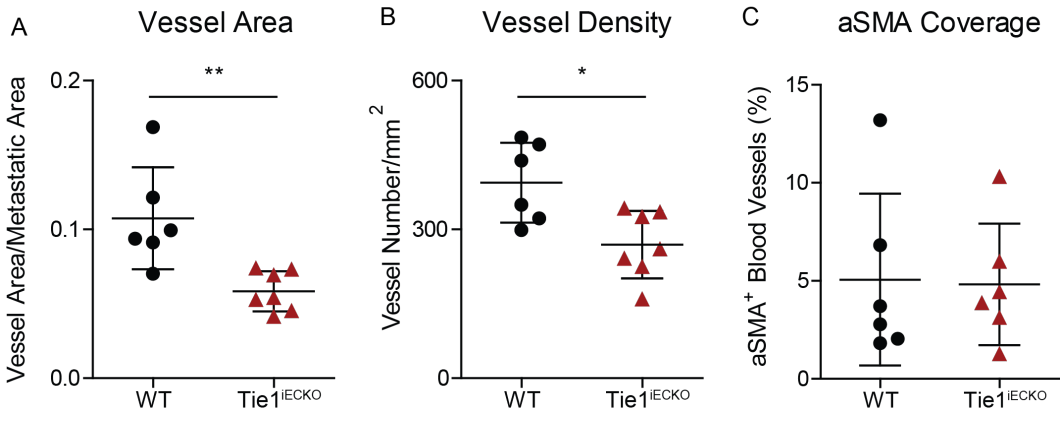

Figure S7. Tie1 deletion after primary tumor removal reduces microvessel area and density in the lung metastatic vasculature.

Quantification of microvessel area **(A)**, microvessel density **(B)** and **(C)** aSMA positive blood vessel in lung metastasis (n=6 [WT] and 6-7 [Tie1^{iECKO}]; two-tailed Mann Whitney test, *, p<0.05, **, p<0.01). Tamoxifen was administrated after primary tumor removal.

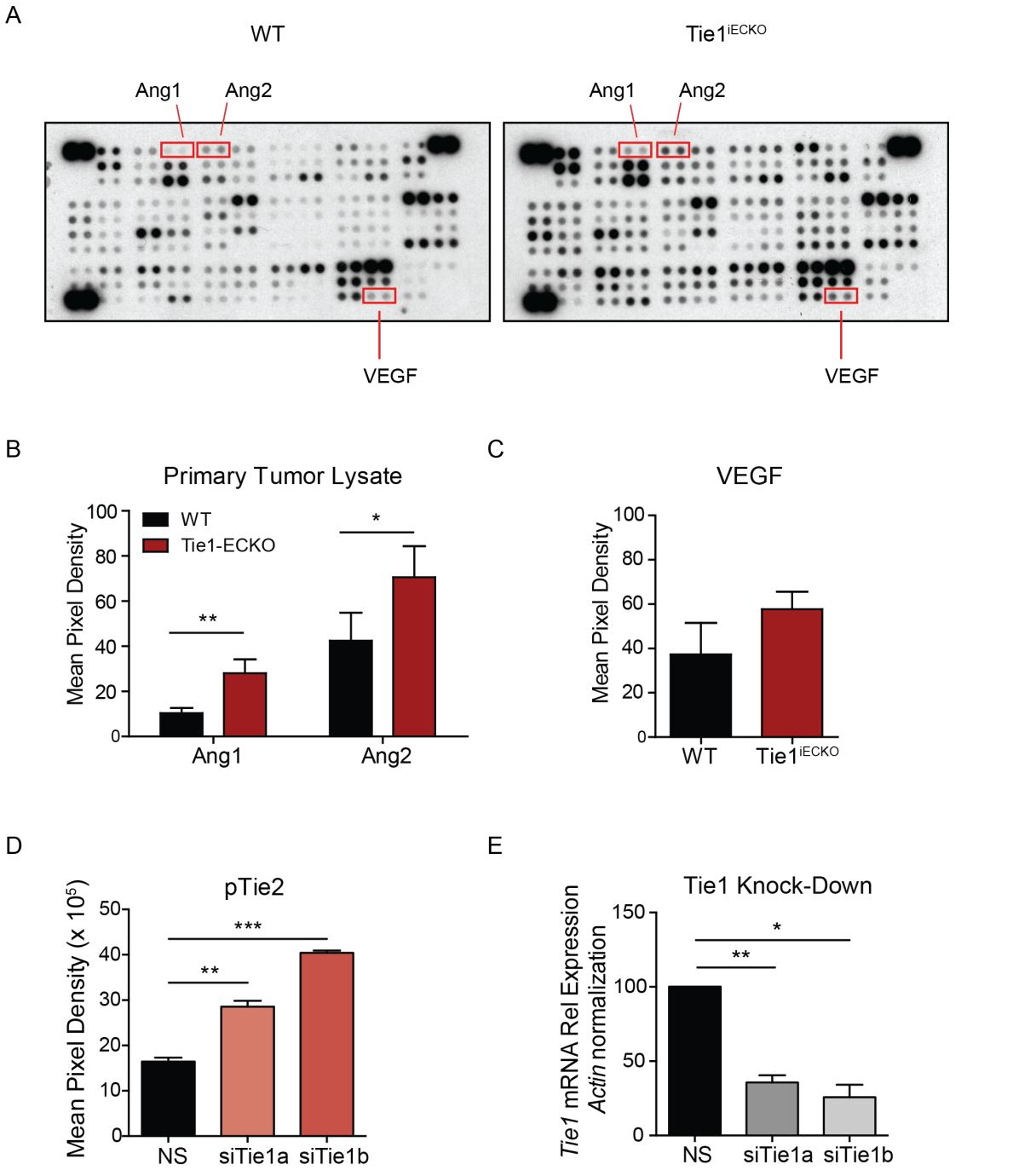

Figure S8. Cytokine array made on tumor lysate.

(A) Representative immunoblotting images of proteome profiler. Mice were injected with LLC and after 14 days, primary tumors were used for the cytokine array and quantified. **(B)** Densitometric quantification of Ang1 and Ang2 in WT and Tie1^{iECKO} primary tumor (n=4; two-tailed Mann Whitney test, *, p<0.05). **(C)** Densitometric quantification of VEGF in WT and Tie1^{iECKO} primary tumor. **(D)** Human phospho-receptor tyrosine kinase array. Densitometric quantification of phospho-Tie2 in control HUVEC (NS) and siTie1-KD HUVEC (siTie1a, siTie1b) stimulated contemporary with Ang1 and Ang2. (n= 4 independent experiments; Lysates of the same conditions were pooled. Two-tailed unpaired T-test; **, p<0.01, ***, p<0.001). **(E)** Relative *Tie1* expression from HUVEC RNA lysate 24 h after siRNA transfection. Values are expressed as the average of 4 independent experiments. Two-tailed unpaired T-test; *, p<0.05, **, p<0.01).