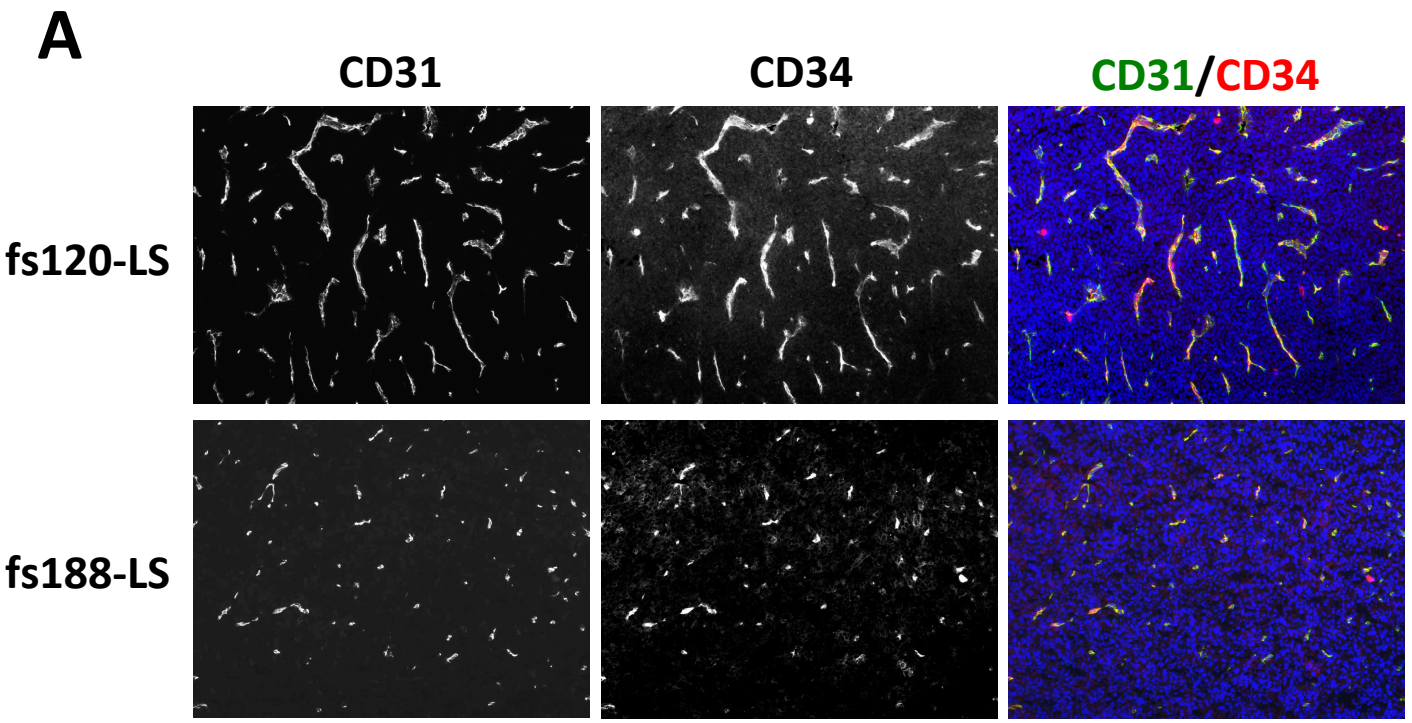
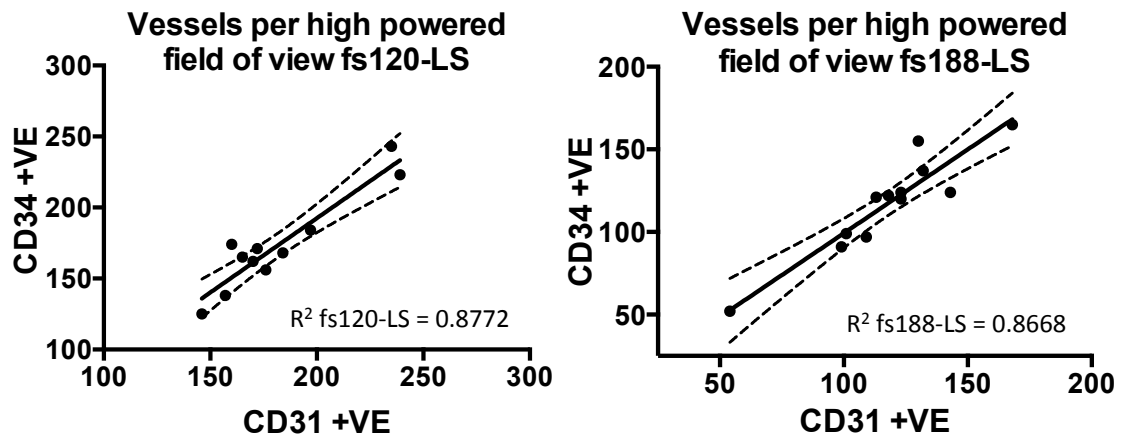
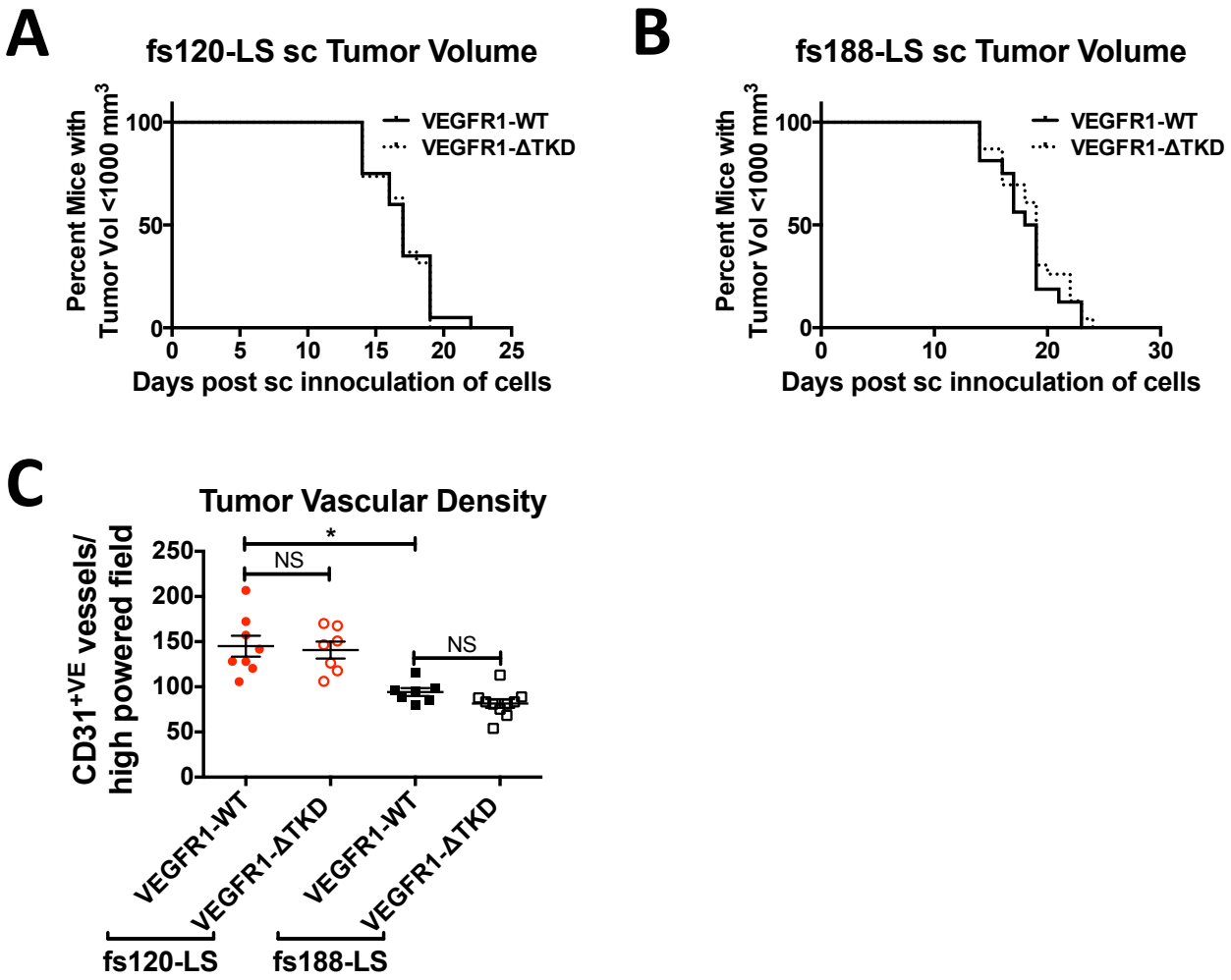
Supplementary Figure S1





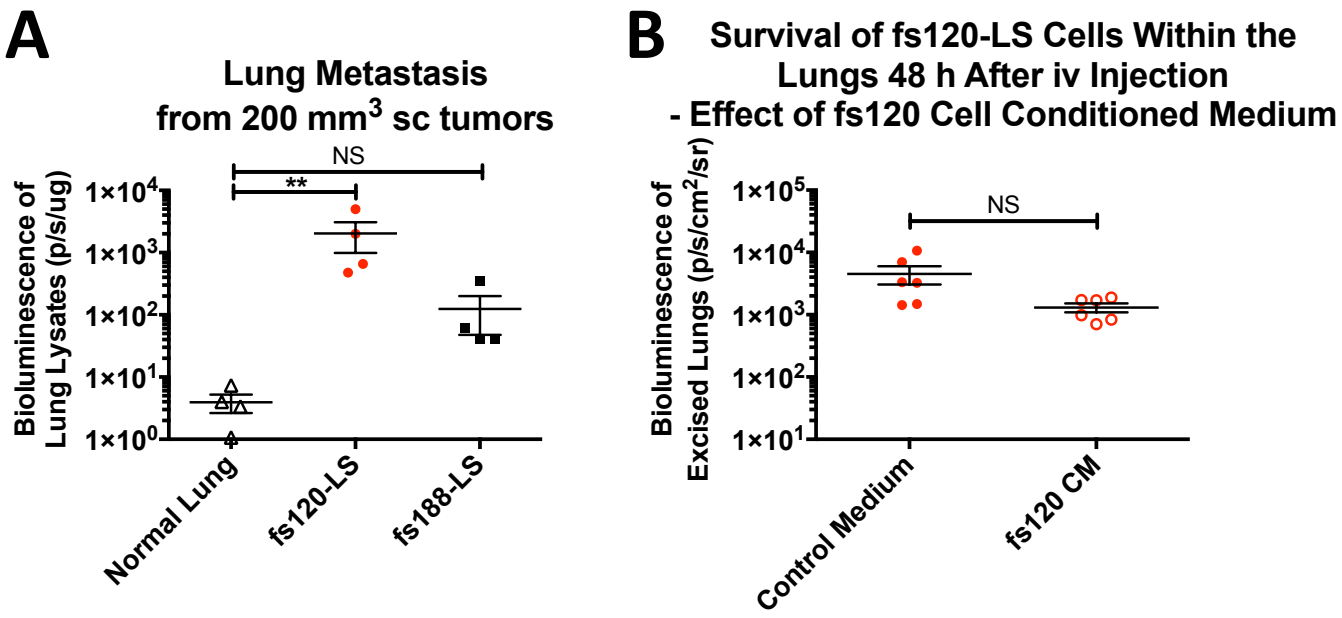
Supplementary Figure S1. CD31 and CD34 co-localize to the vasculature in fs120-LS and fs188-LS subcutaneous tumors. **A.** fs120-LS and fs188-LS tumors of between 800-1000 mm^3 were frozen and embedded in OCT before sectioning and staining with anti-CD31 (Alexa-488, green) and anti-CD34 (Alexa-555, red). Representative images of CD31 and CD34 staining for each tumor type are shown in black and white and as a color image merging CD31 (green), CD34 (red) and nuclei (DAPI, blue). **B.** Plots of CD31 and CD34 positive vessels ($CD31^{+VE}$ & $CD34^{+VE}$ respectively) per high-powered field of view from 3 separate areas and from 4 tumors. The solid line is the linear regression and the dotted lines are the 95% confidence intervals. The R^2 value is also shown.

Supplementary Figure S2



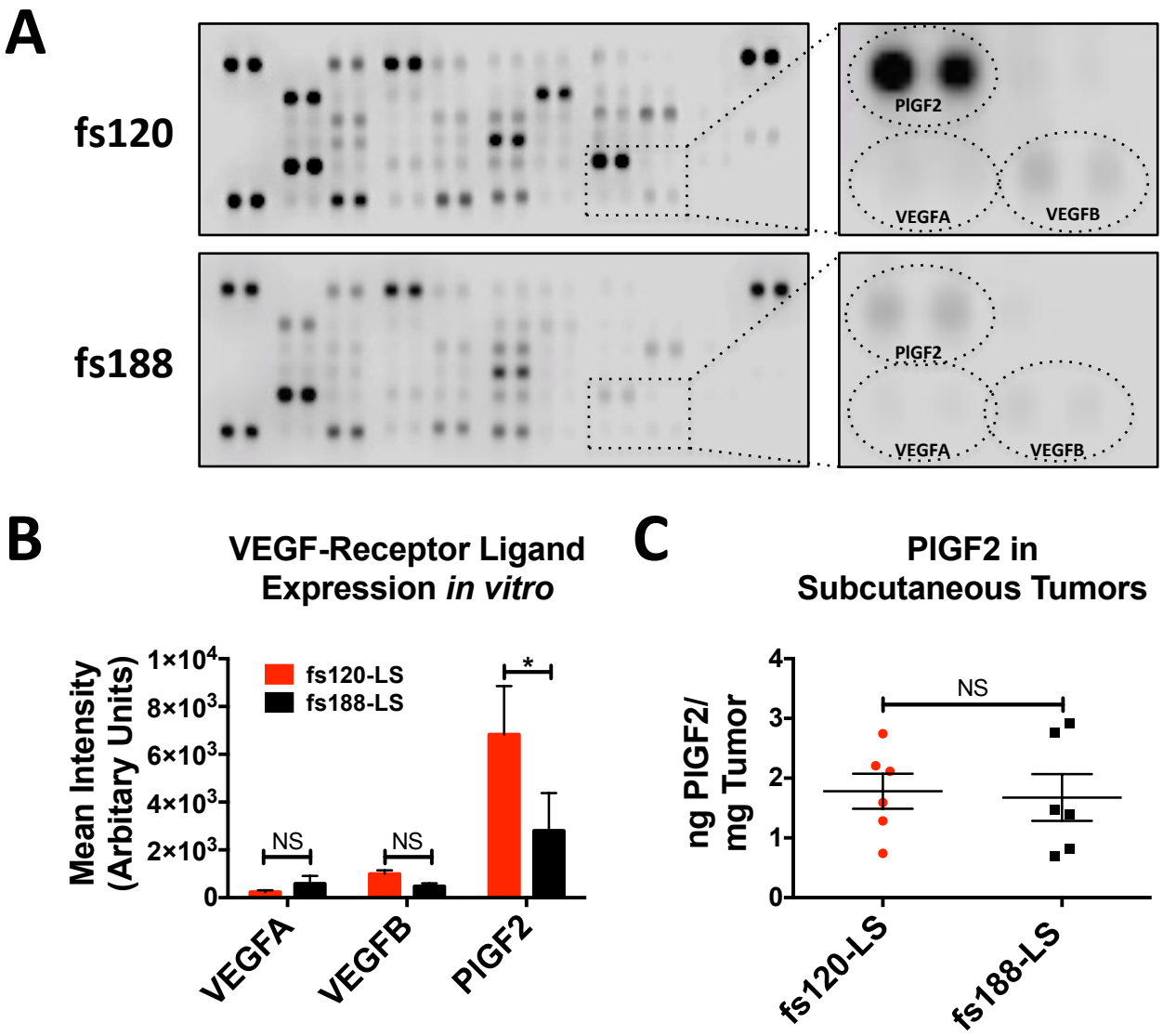
Supplementary Figure S2. VEGFR1 activity has no effect on tumor growth or vascular density of subcutaneous fs120-LS or fs188-LS tumors. **A – B.** C57Bl6/SCID mice with VEGFR1-WT or VEGFR1-ΔTKD were subcutaneously injected with 1 x 10^6 fs120-LS **(A)** or fs188-LS cells **(B)** and tumors grown to 1000 mm^3 (end-point size). Kaplan-Meier plots for end-point analysis of tumor volume < 1000 mm^3 are shown. There was no significant difference between VEGFR1-WT or VEGFR1-ΔTKD mice for the two cell lines (n = 16-23 in each of the 4 groups). **C.** FFPE tumors from C57Bl6/SCID VEGFR1-WT or VEGFR1-ΔTKD mice injected with fs120-LS or fs188-LS cells were sectioned and stained for CD31 to detect blood vessels. Mean vascular density per high-powered field of view was calculated by counting vessels within viable regions using a 20 X objective. Each symbol represents the mean value for an individual tumor. Error bars are ± SEM. $P < 0.05$ = *, NS = Not Significant.

Supplementary Figure S3



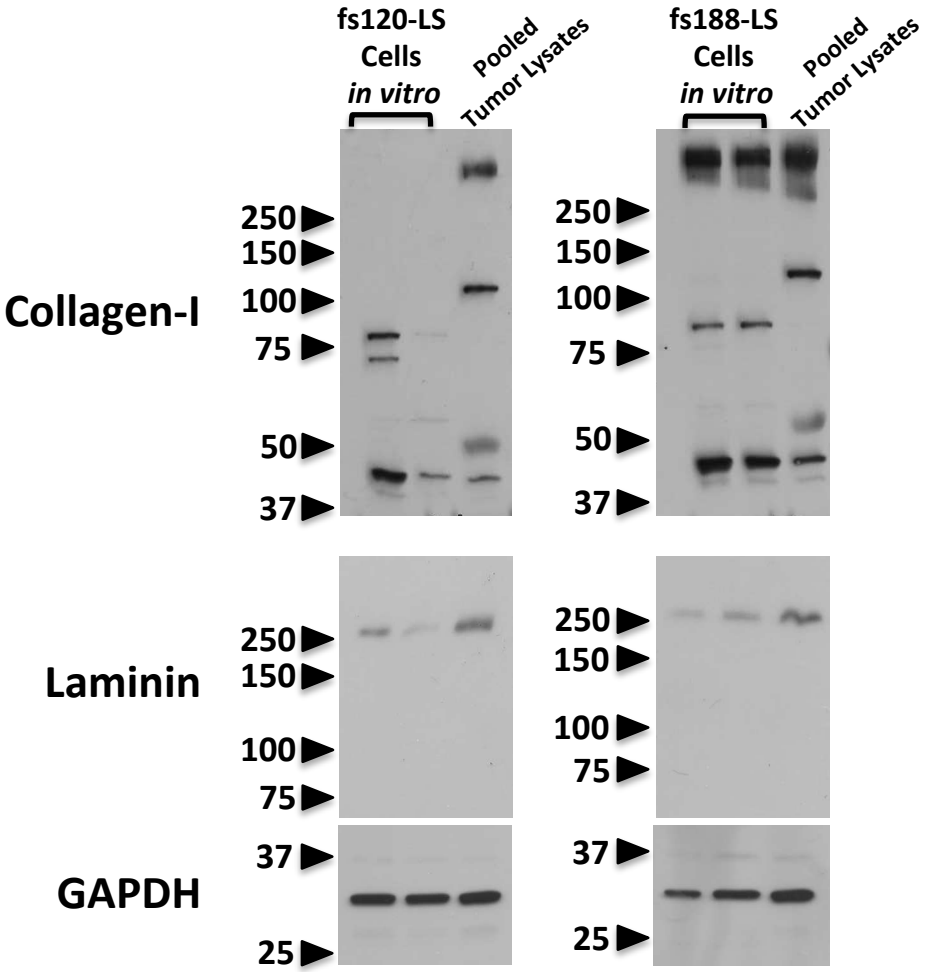
Supplementary Figure S3. fs120-LS and fs188-LS cells metastasize to the lung early during subcutaneous tumor growth. **A.** Mice were injected with 1 x 10^6 fs120-LS or fs188-LS cells subcutaneously and tumors grown to 200-300 mm^3 before lungs were excised. Lungs were homogenized and luciferase activity measured as described in the Materials and Methods section (n = 4 per group). Background bioluminescence of lungs from normal mice is shown as 'normal lung'. Error bars are ± SEM. P < 0.01 = **, NS = Not Significant. **B.** fs120 cells were grown in either atmospheric or 1% O_2 and conditioned medium (fs120 CM) harvested, combined and injected ip daily over 5 days before mice were injected iv with 5 x 10^4 fs120-LS cells and bioluminescence of lung lobes was measured on necropsy (n = 6). Untreated medium (Control Medium) was used as a vehicle control (n = 6).

Supplementary Figure S4



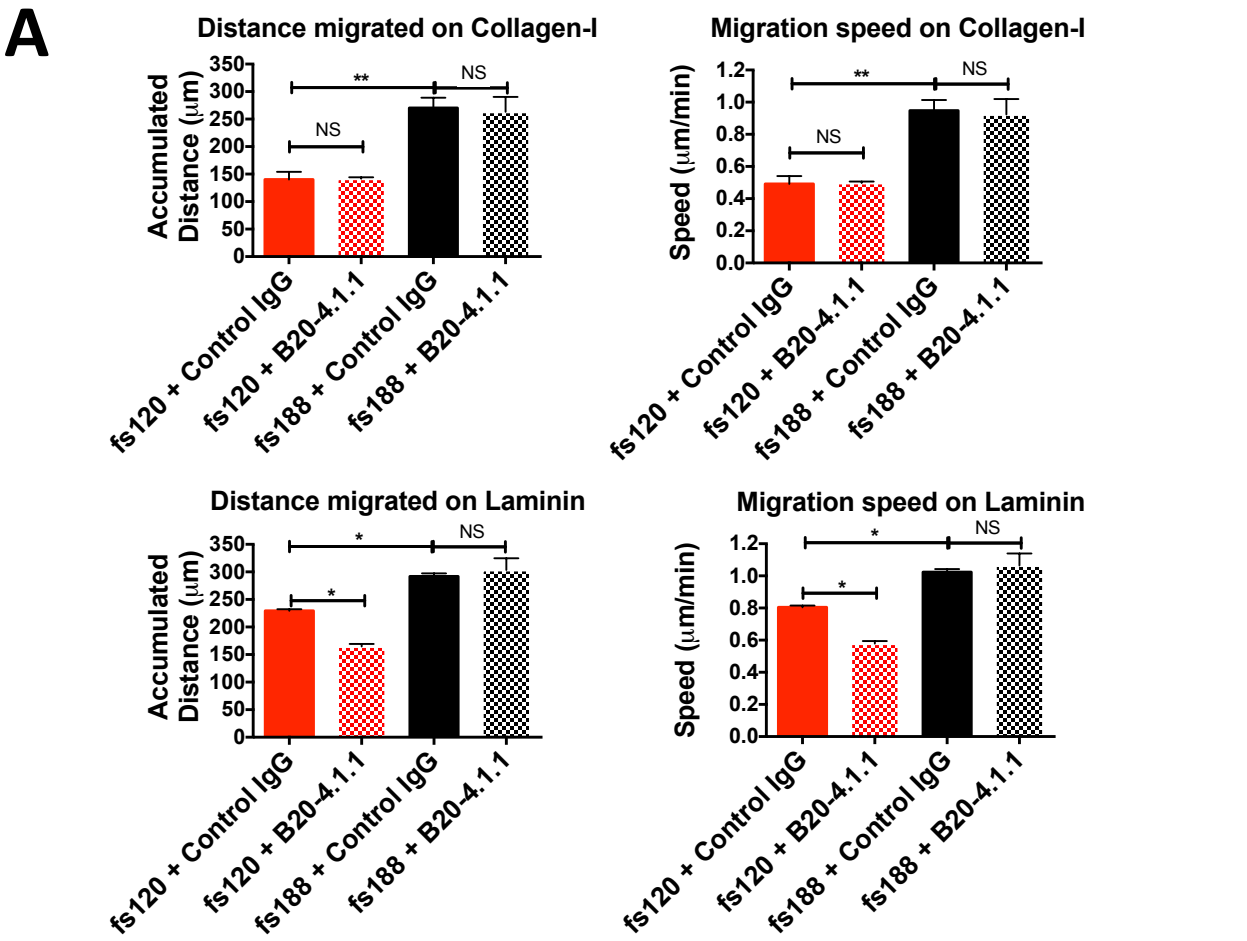
Supplementary Figure S4. fs120-LS cells express higher levels of PlGF2 than fs188-LS cells in vitro, with no difference in levels of PLGF2 detected in subcutaneous tumours. **A and B.** Lysates from 3D multicellular spheroids were prepared as described in the Materials and Methods and analyzed on protein profiler angiogenesis arrays. An example array is shown in A, with the spots detecting VEGFA, VEGFB and PlGF2 highlighted within the enlargements with dashed ovals. Quantification of mean spot intensity from 4 independent experiments is shown in B, normalized to the mean intensity of the control spots provided on each array. Error bars are ± SEM. **C.** Mice were injected with 1 x 10^6 fs120-LS or fs188-LS cells and tumors grown to 1000 mm^3. PlGF2 was measured in tumor lysates by ELISA and corrected for total protein extracted (n = 6, ± SEM). * = $P < 0.05$. NS = Not Significant.

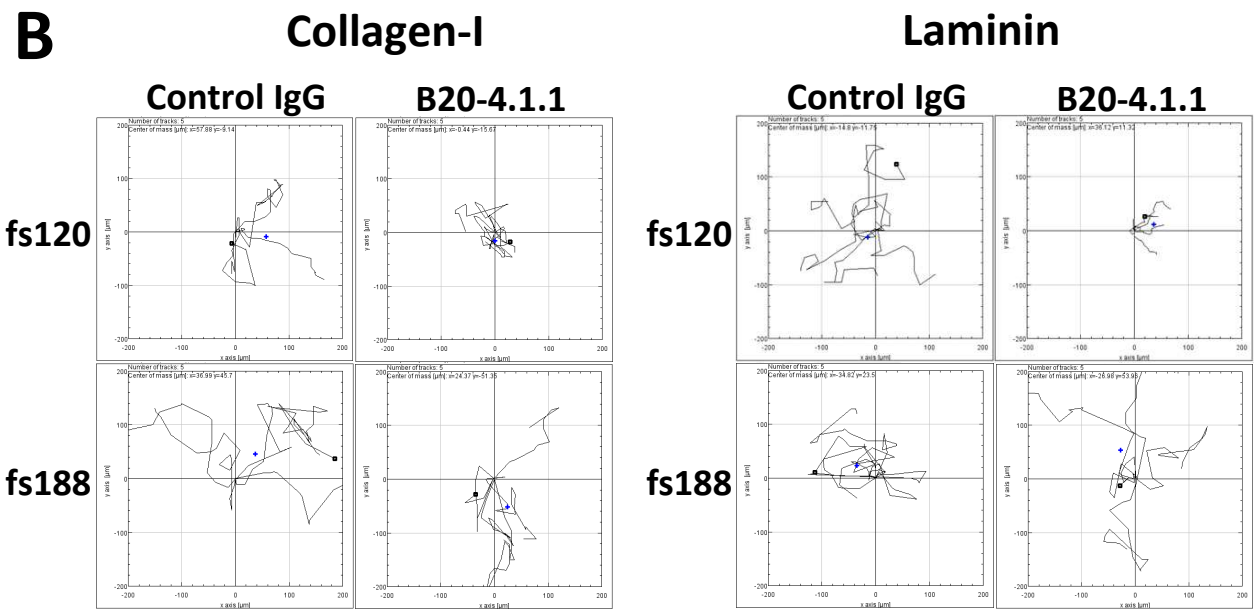
Supplementary Figure S5



Supplementary Figure S5. *Expression of laminin and collagen-I in lysates of fs120-LS and fs188-LS subcutaneous tumors.* Western blots of cell lysates from fs120-LS and fs188-LS cells from two independent *in vitro* cultures are shown. For comparison, a sample from the same pooled lysate of subcutaneous fs120-LS and fs188-LS tumors was run in each case. The upper blots are from non-reducing SDS-PAGE gels probed for collagen-I. Tumor lysates contain high molecular weight aggregates (upper bands) and 139 kDa monomers. High molecular weight aggregates are present in fs188 cell lysates and absent from fs120-LS cell lysates. Laminin was detected in both cell types on western blotting of reducing SDS-PAGE. Molecular weight standards are indicated after tracing over the positions of pre-stained markers on the nitrocellulose membrane. GAPDH was used as a control to demonstrate equal loading of cell and tumor lysates and for normalization of samples.

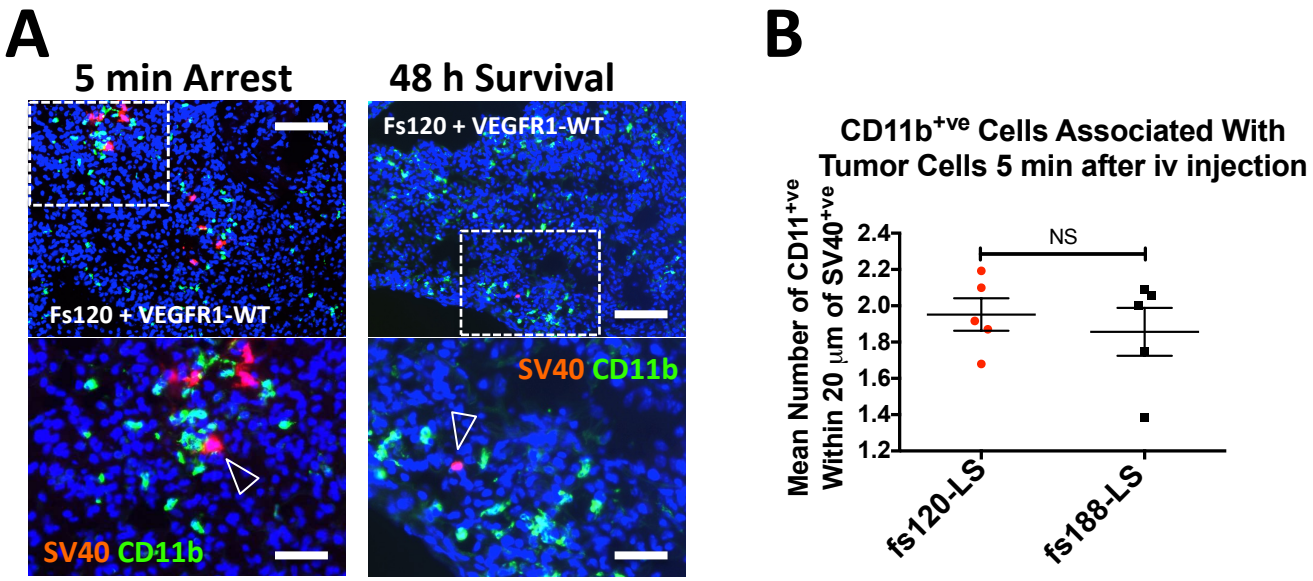
Supplementary Figure S6





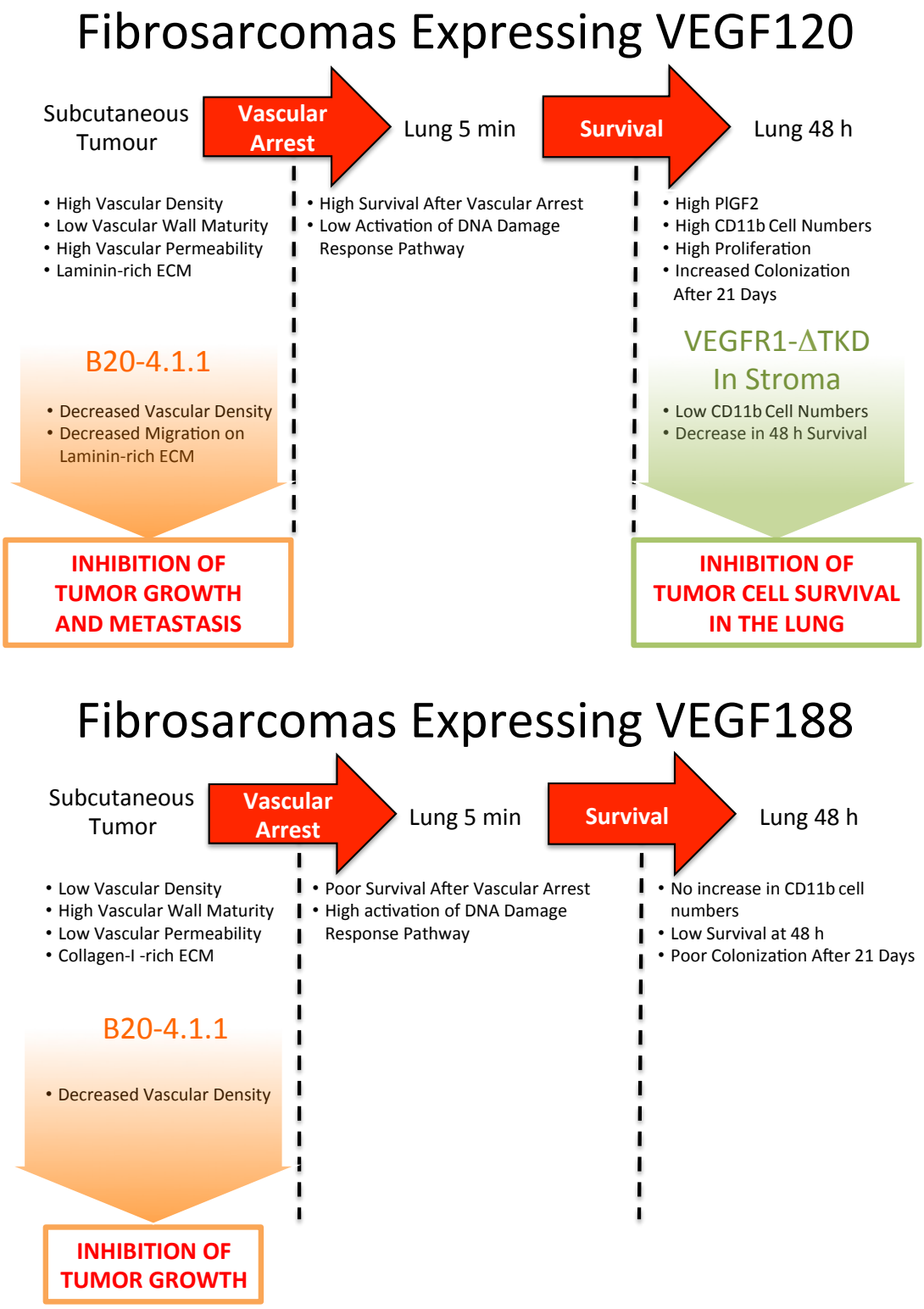
Supplementary Figure S6. Quantification of single cell migration using live video microscopy. **A.** Quantification of live video single cell migration of fs120 and fs188 cells on collagen-I or laminin coated plastic. Data shown is the mean value from three independent experiments each tracking 30 cells. Error bars are ± SEM. $P < 0.05$ = *, $P < 0.01$ = **, NS = Not Significant. **B.** Example migration tracks from 5 cells from data shown in A, centered with a common origin. Axes are ± 250 μm.

Supplementary Figure S7



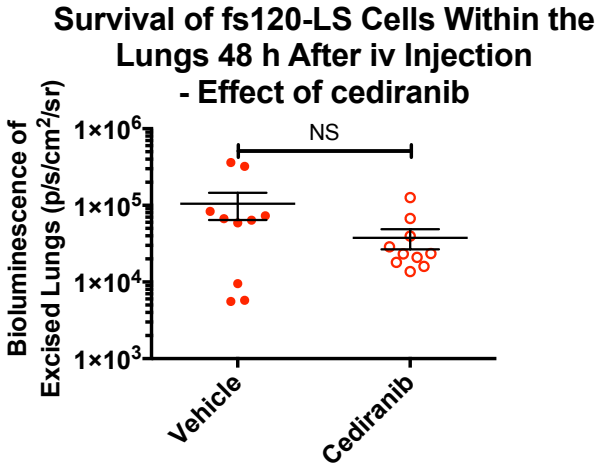
Supplementary Figure S7. Localization of CD11b cells with respect to intravenously injected fibrosarcoma cells within the lung. **A.** Representative images of SV40+VE fs120-LS fibrosarcoma cells (red), CD11b^{+VE} cells (green) and nuclei (DAPI, blue) within the lung of VEGFR1-WT mice at 5 min and 48 h after intravenous injection. The dotted region in the upper images is shown enlarged in the lower image. CD11b^{+VE} cells were observed to cluster around SV40^{+VE} cells at 5 min, but not at 48 h. Scale bar is 200 µm in the upper image and 70 µm in the lower image. **B.** Quantification of the number of CD11b^{+VE} cells within 20 µm of an SV40^{+VE} fibrosarcoma cell at 5 min. Each data point is the mean count of CD11b^{+VE} cells associated with each SV40^{+VE} fibrosarcoma cell (n = 7 – 25) from the lung of each mouse (n = 5 - 6).

Supplementary Figure S8



Supplementary Figure S8. Summary of results showing key differences between fibrosarcomas expressing VEGF120 and VEGF188 and the effects of B20-4.1.1 and stromal VEGFR1 activity. Red arrows show the processes that link the different areas of investigation (vascular arrest and retention/survival). The effects of anti-VEGFA therapy (B20-4.1.1) and stromal VEGFR1 activity determined using mice lacking the VEGFR1 tyrosine kinase domain (VEGFR1 ΔTKD) are shown within the orange and green downward arrows respectively.

Supplementary Figure S9



Supplementary Figure S9. Effect of cediranib on survival of fs120-LS cells in the lung 48 h after iv injection. Mice were dosed daily by gavage with 6 mg/kg of the VEGFR tyrosine kinase inhibitor cediranib or vehicle on days 1 – 4. On day 3, mice were injected intravenously with 5 x 10^4 fs120-LS cells. On day 5, bioluminescence was measured of intact lung lobes on necropsy (n = 10). Error bars are ± SEM. NS = Not Significant.