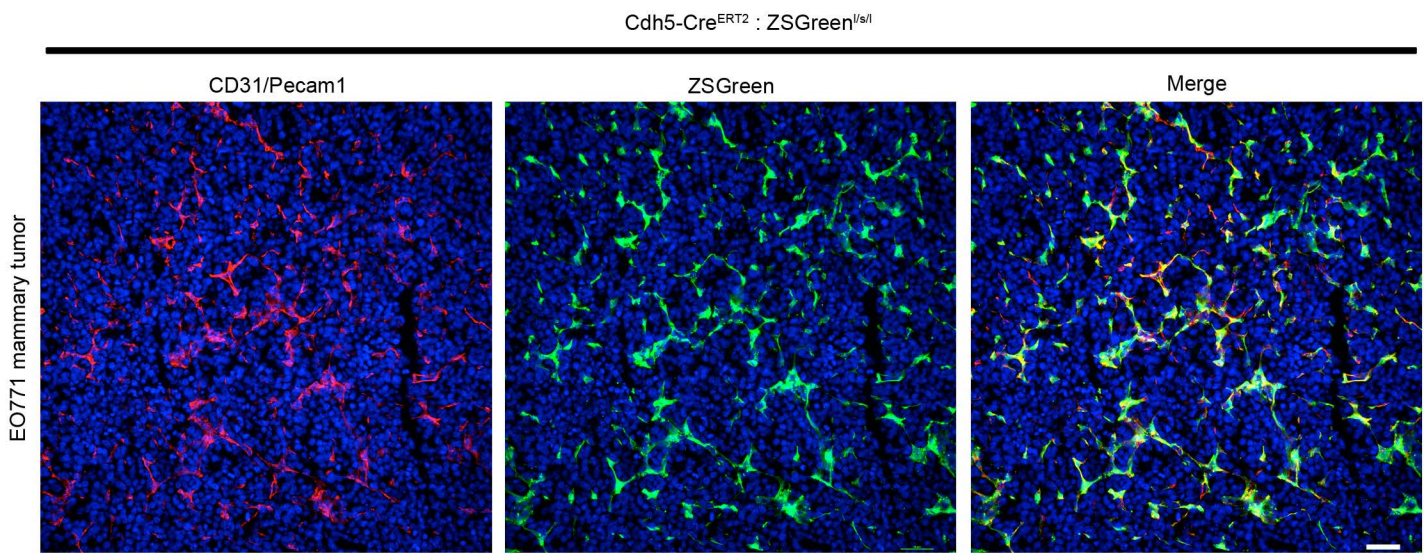
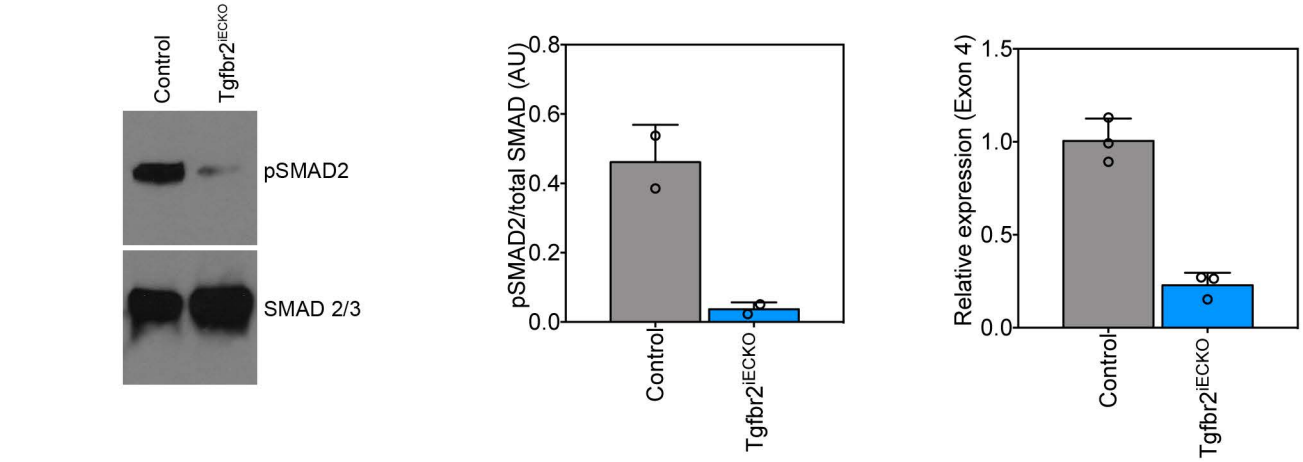
A


Cdh5-Cre^ERT2 : ZSGreen^l/s/l
CD31/Pecam1
ZSGreen
Merge
EO771 mammary tumor

B

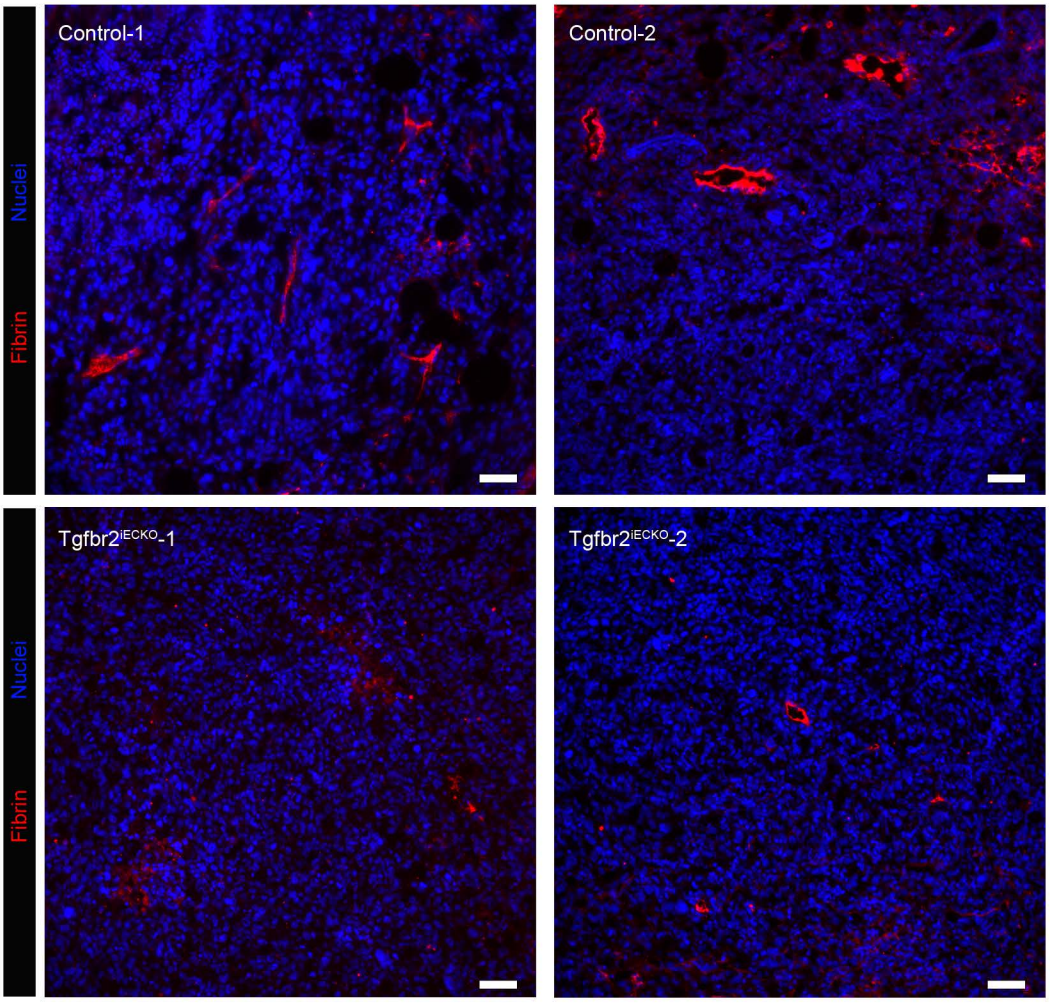

FACS-isolated ZSGreen+ ECs (EO771 mammary tumor)
Control
Tgfbr2iECKO
pSMAD2
SMAD 2/3
pSMAD2/total SMAD (AU)
0.0
0.2
0.4
0.6
0.8
Control
Tgfbr2iECKO
Relative expression (Exon 4)
0.0
0.5
1.0
1.5
Control
Tgfbr2iECKO

Supplemental figure legends

Fig S1) Confirmation of Cre activity in ECs from Cdh5-Cre^{ERT2} mice.

(A) CD31/Pecam staining and ZSGreen expression in E0771 mammary tumors orthotopically injected in Cdh5-Cre^{ERT2}:ZSGreen$^{l/s/l}$ mice. Scale bar = 50 um. (B) pSMAD2 and SMAD 2/3 western blot of FACS-sorted ZSGreen^{+} ECs from EO771 tumors in Cdh5-Cre^{ERT2}:ZSGreen$^{l/s/l}$ (control) and Tgfbr2^{iECKO} mice (n=2 independent pooled tumors). Western blot quantification of pSMAD2/total SMAD 2/3 from ZSGreen^{+} ECs. qPCR for Cre-excised exon (exon 4) from the same ZSGreen^{+} ECs.

A


Control-1
Control-2
Tgfbr2iECKO-1
Tgfbr2iECKO-2
Nuclei
Fibrin

B

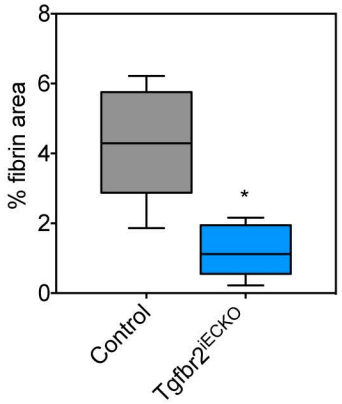
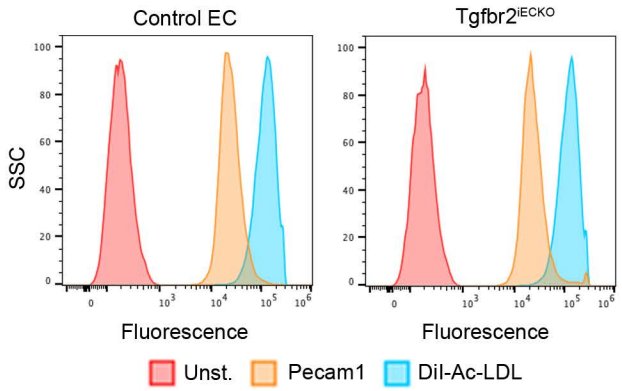

% fibrin area
8
6
4
2
0
*
Control
Tgfbr2iECKO

Fig S2) Mammary tumors in Tgfbr2^{iECKO} mice show reduced intratumoral fibrin compared to controls.

(A) Representative fibrin staining in E0771 mammary tumors harvested from control versus Tgfbr2^{iECKO} mice. Cryosections were stained as described in the methods section. Scale bar =100 um. (B) Quantification of fibrin staining using automated image analysis software (n=5 tumors per group). Data are means +/- SEM. *p=<0.05 using Student's t-test.

A


Control EC
Tgfbr2iECKO
SSC
Fluorescence
Fluorescence
Unst.
Pecam1
DiI-Ac-LDL

B

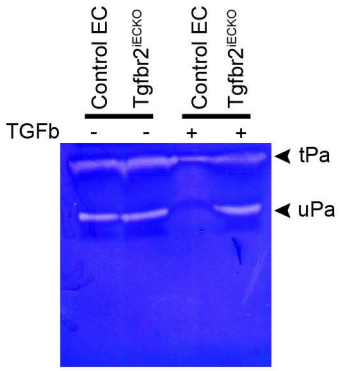
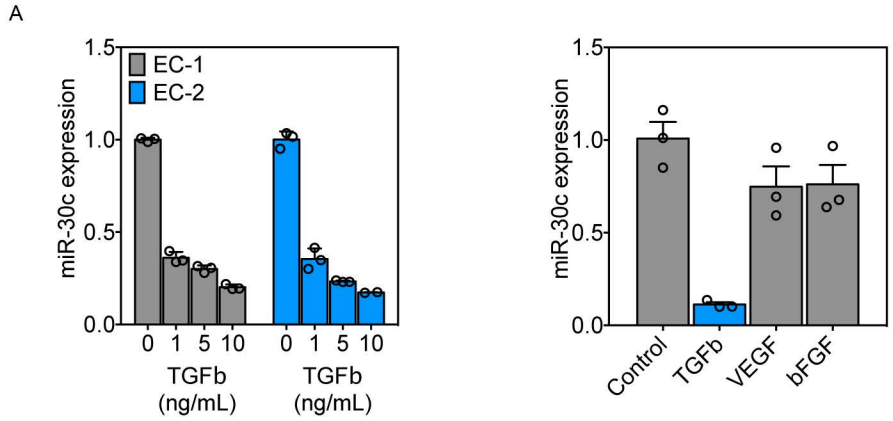
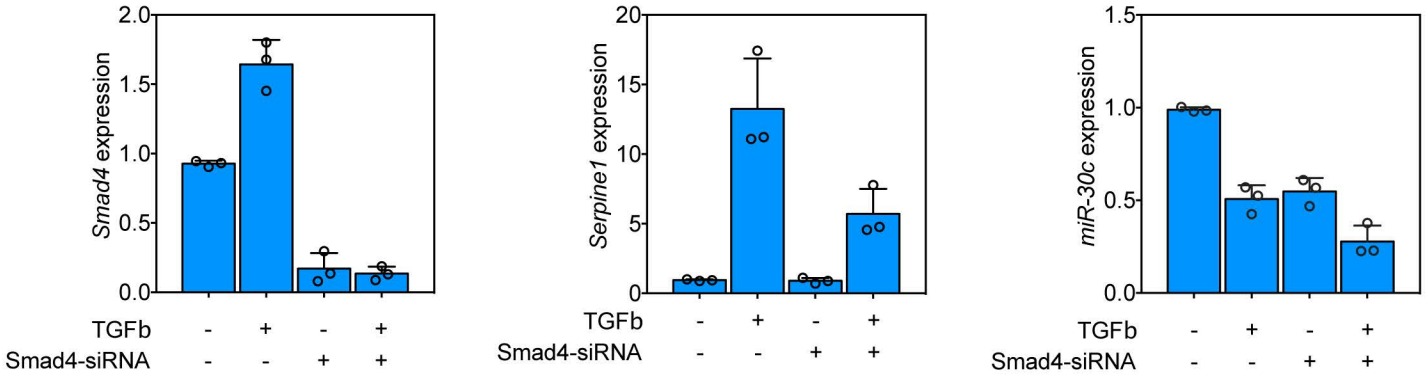

Control EC
Tgfbr2IECKO
Control EC
Tgfbr2IECKO
TGFb
- - + +
tPa
uPa

Fig S3) Confirmation of EC purity via FACS and fibrin zymography gel using EC conditioned media.

(A) EC purity confirmed using FACS where ZSGreen^{+} ECs were incubated with DiI-Ac-LDL and simultaneously stained with PECAM antibody**.** (B) Fibrin zymography gel from ECs treated with 10 ng/mL TGFb for 48 hours. Arrows indicate expected size of the plasminogen activators tPA (~ 75 kDa) and uPa (~ 55 kDa).


A
EC-1
EC-2
miR-30c expression
1.5
1.0
0.5
0.0
0 1 5 10
TGFb (ng/mL)
0 1 5 10
TGFb (ng/mL)
miR-30c expression
Control
TGFb
VEGF
bFGF


B
Smad4 expression
TGFb - + - +
Smad4-siRNA - - + +
Serpine1 expression
TGFb - + - +
Smad4-siRNA - - + +
miR-30c expression
TGFb - + - +
Smad4-siRNA - - + +

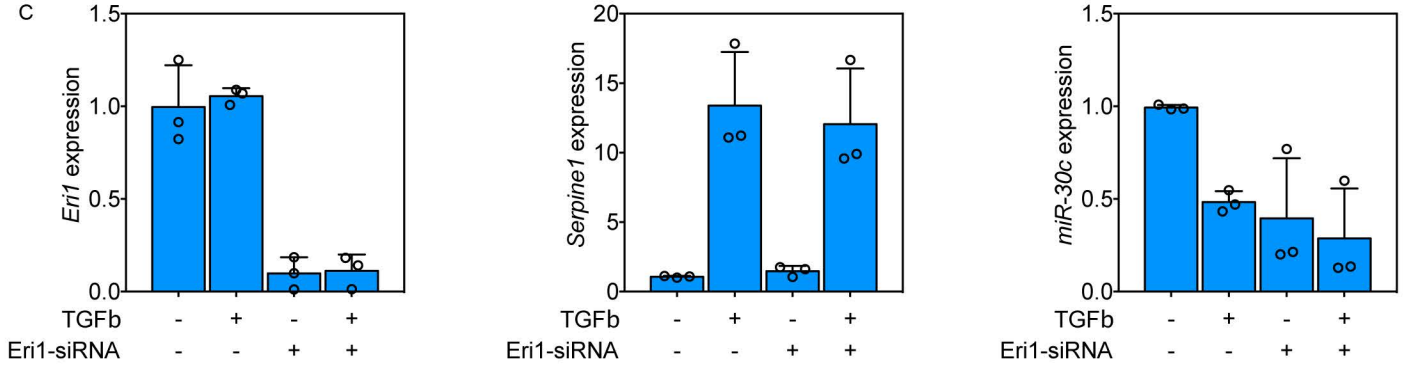

C
Eri1 expression
TGFb - + - +
Eri1-siRNA - - + +
Serpine1 expression
TGFb - + - +
Eri1-siRNA - - + +
miR-30c expression
TGFb - + - +
Eri1-siRNA - - + +

Fig S4) Additional studies characterizing the regulation of miR-30c by TGFb.

(A) qPCR analysis of miR-30c expression in ECs (two independent isolates) challenged with TGFb at the indicated dose for 48 hours. qPCR analysis of miR-30c expression in ECs challenged with TGFb (10 ng/ml), VEGF (10 ng/ml) or bFGF (5 ng/mL) for 48 hours (n=3). (B) qPCR analysis of *SMAD4*, *Serpine1* and miR-30c expression in control versus SMAD4 siRNA (20 nM) transfected ECs. Where indicated, cells were challenged with 10 ng/mL TGFb for 48 hours (n=3). (C) qPCR analysis of *Eri1*, *Serpine1* and miR-30c expression in control versus Eri1 siRNA (20 nM) transfected ECs. Where indicated, cells were challenged with 10 ng/mL TGFb for 48 hours (n=3).

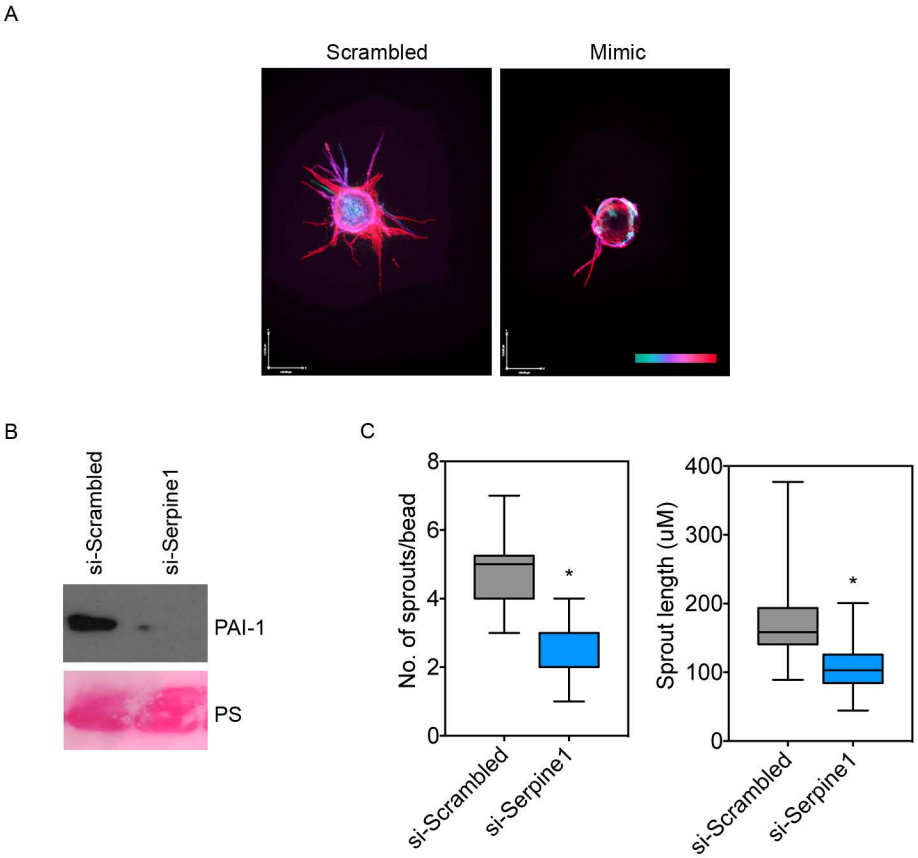
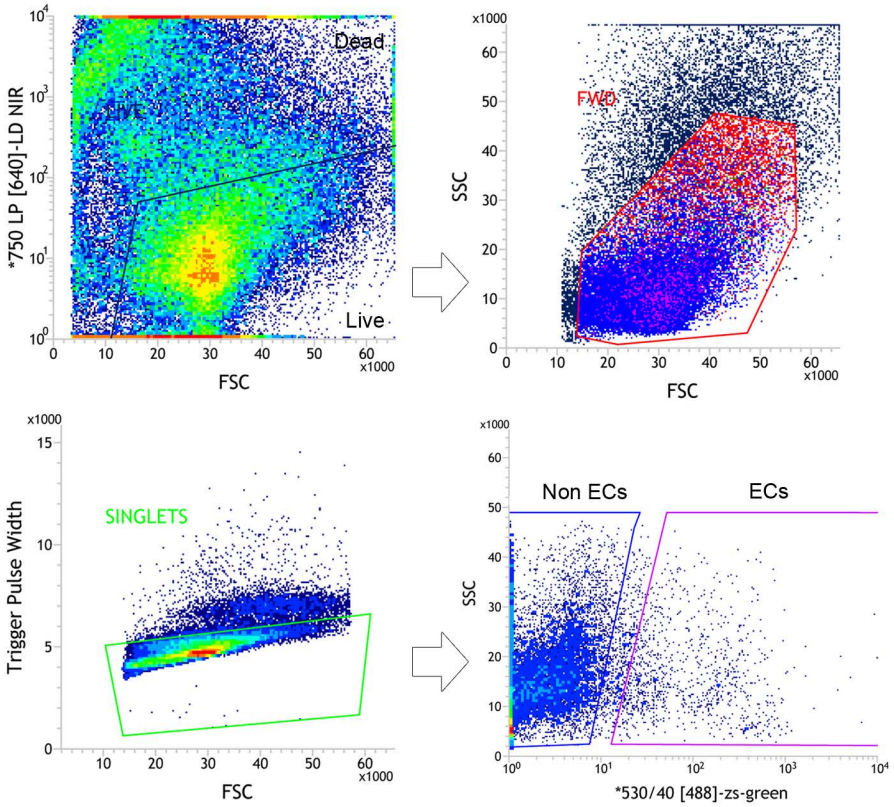

A
Scrambled
Mimic
B
si-Scrambled
si-Serpine1
PAI-1
PS
C
No. of sprouts/bead
Sprout length (uM)
si-Scrambled
si-Serpine1
si-Scrambled
si-Serpine1

Fig S5) Characterization of sprouting in fibrin in ECs treated with miR-30c mimics or *Serpine1* siRNA.

(A) Phalloidin-stained ECs in the fibrin bead sprouting assay treated with 40 nM scrambled control or 40 nM miR-30c mimic for 72 hours. The scale at bottom right indicates sprout depth in the 3D fibrin matrix. Scale bar =40 um (x) X 54 um (y). (B) Western blot of secreted PAI-1 in ECs treated with 20 nM *Serpine1* siRNA. (C) Number and length of sprouts in ECs treated with *Serpine1* siRNA for 72 hours (n=30 beads). Results were analyzed using ANOVA and statistical significance is indicated with an asterisk.

A


Dead
Live
*750 LP [640]-LD NIR
FSC
x1000
FWD
SSC
SINGLETS
Trigger Pulse Width
Non ECs
ECs
*530/40 [488]-zs-green

B

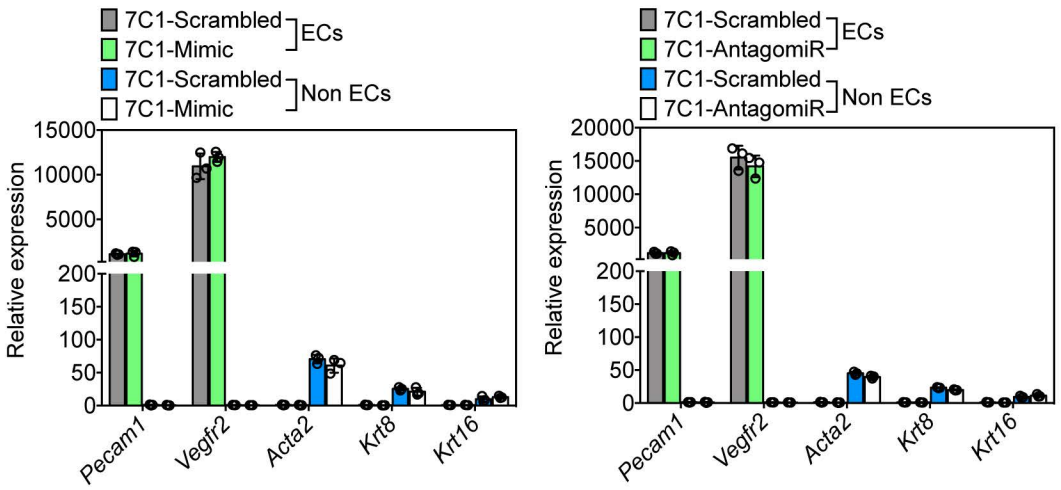

7C1-Scrambled
7C1-Mimic
ECs
7C1-Scrambled
7C1-Mimic
Non ECs
Relative expression
Pecam1
Vegfr2
Acta2
Krt8
Krt16
7C1-Scrambled
7C1-AntagomiR
ECs
7C1-Scrambled
7C1-AntagomiR
Non ECs

Fig S6) FACS gating strategies and characterization of ZSGreen^{+} ECs and ZSGreen^{-} non-ECs.

(A) Representative flow plots for sorting ECs from Cdh5-Cre^{ERT2}:ZSGreen$^{l/s/l}$ mice. (B) qPCR validation showing sorting efficiency of ECs from Cdh5-Cre^{ERT2}:ZSGreen$^{l/s/l}$ mice**.** *Pecam1 and Vegfr2* were used as markers for ECs, *Acta2* was used as a marker for fibroblasts and *Krt8* and *Krt16* were used as markers for tumor cells. Samples were assayed in triplicate (n=2 independent pooled tumors).

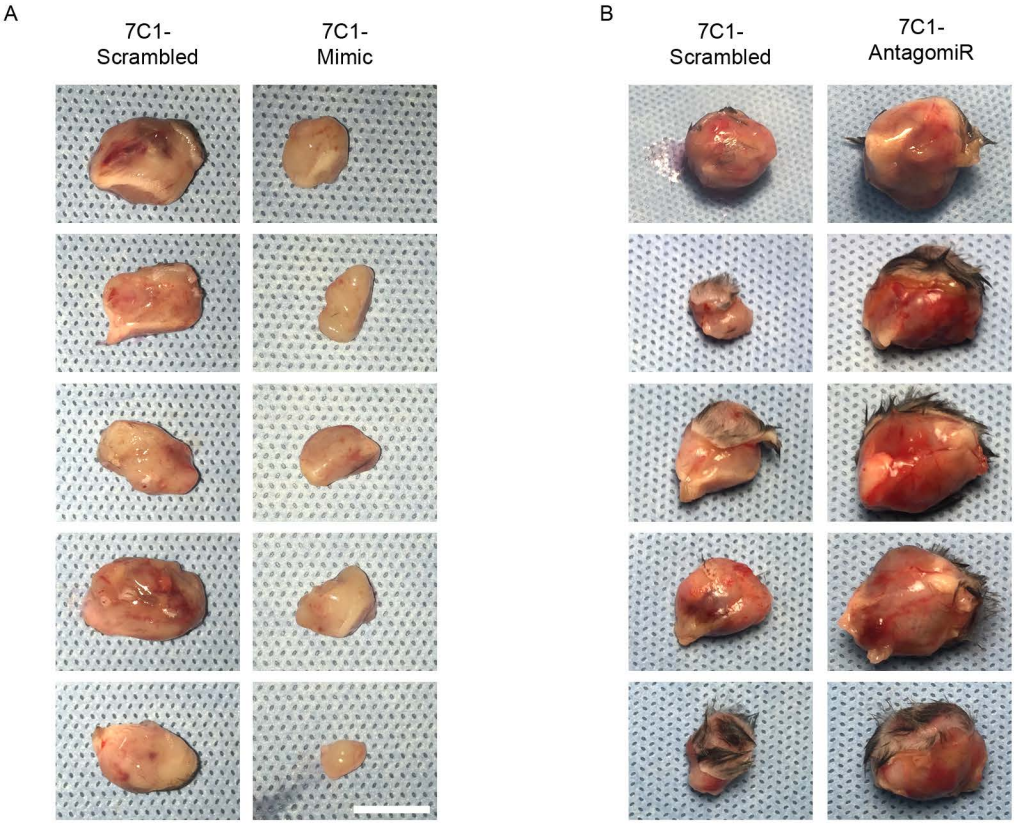

A
7C1-
Scrambled
7C1-
Mimic
B
7C1-
Scrambled
7C1-
AntagomiR

Fig S7) E0771 mammary tumors from miR-30c mimic or miR-30c antagomiR-treated mice.

(A) Representative photographs of orthotopic E0771 mammary tumors from mice treated with 7C1-mimic. (B) Representative photographs of orthotopic E0771 mammary tumors from mice treated with 7C1-antagomiR.

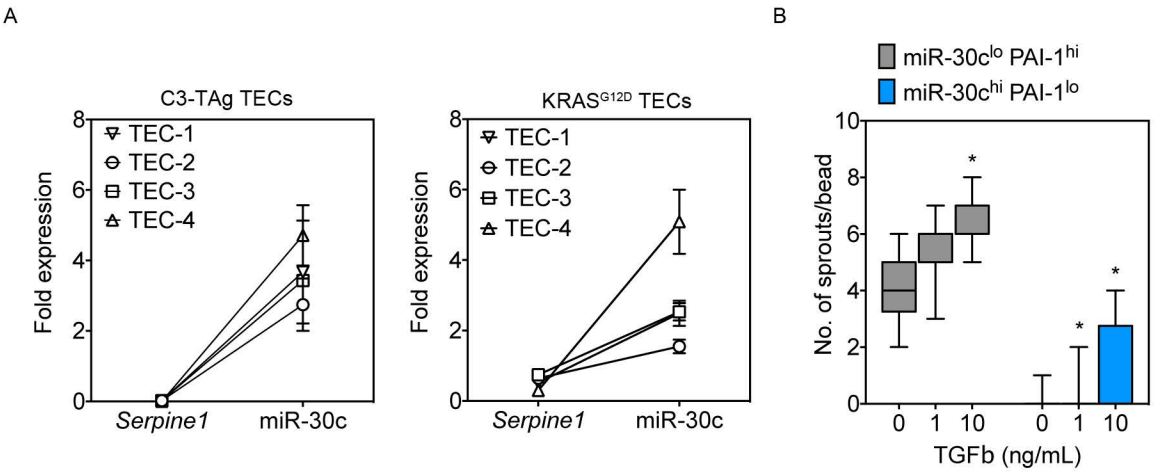

A
C3-TAg TECs
KRAS^{G12D} TECs
TEC-1
TEC-2
TEC-3
TEC-4
Fold expression
Serpine1
miR-30c
B
miR-30c^{lo} PAI-1^{hi}
miR-30c^{hi} PAI-1^{lo}
No. of sprouts/bead
TGFb (ng/mL)

Fig S8) Pearson correlation analysis of miR-30c and *Serpine1* in TECs isolated from C3-TAg mammary tumors and KRAS^{G12D} lung tumors.

(A) qPCR analysis showing the relative association between *Serpine1* and miR-30c expression in individual isolates of C3-TAg TECs or KRAS^{G12D} TECs. Samples were assayed in triplicate. $R^2 = 0.58$ and $p = 0.0277$ using qPCR analysis from all TECs combined. (B) Length of sprouts per bead in miR-30c^{lo}/PAI-1^{hi} ECs and miR-30c^{hi}/PAI-1^{lo} ECs. Cells were treated with the indicated concentration of TGFb for 48 hours. Results were analyzed using ANOVA.

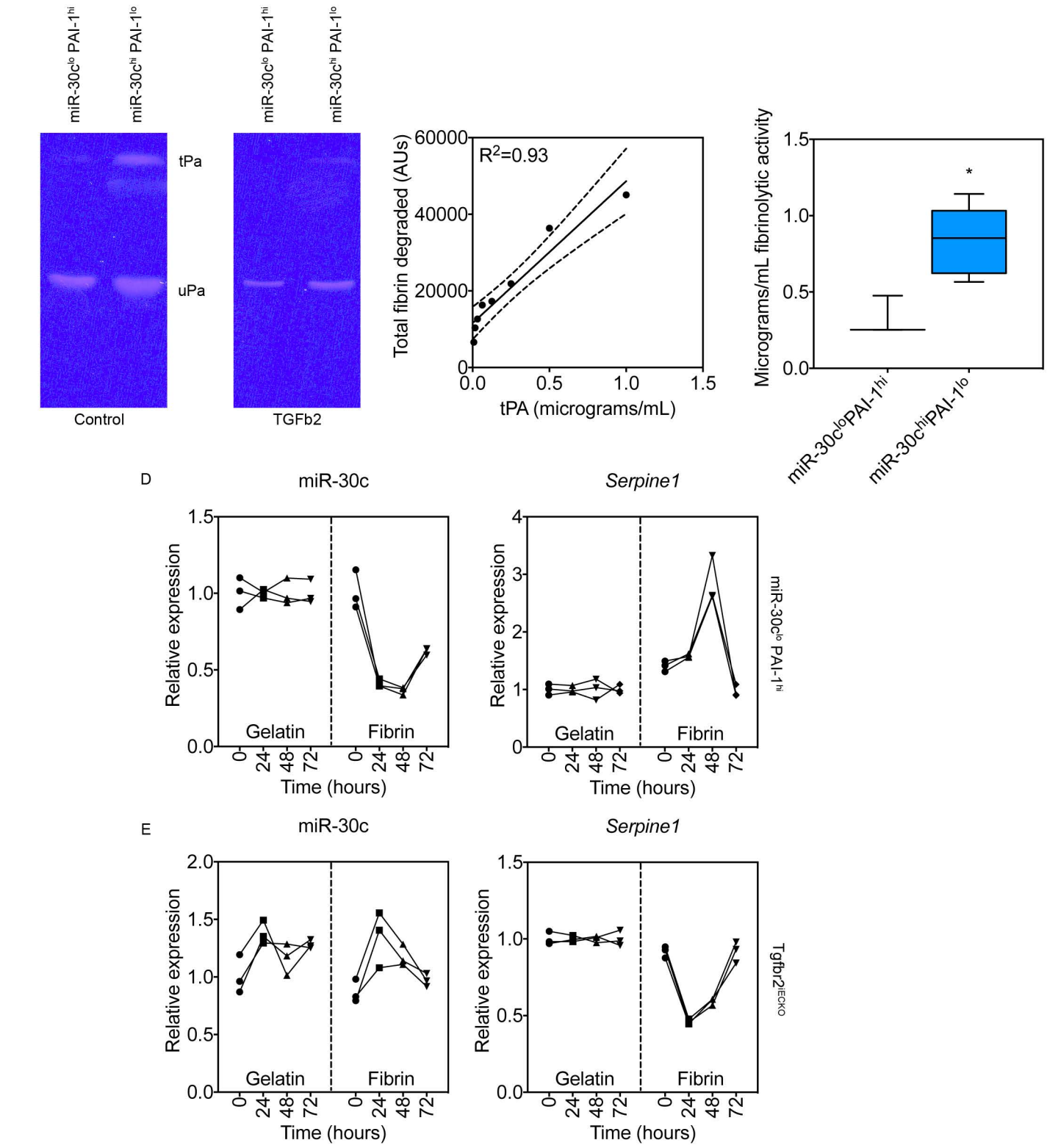
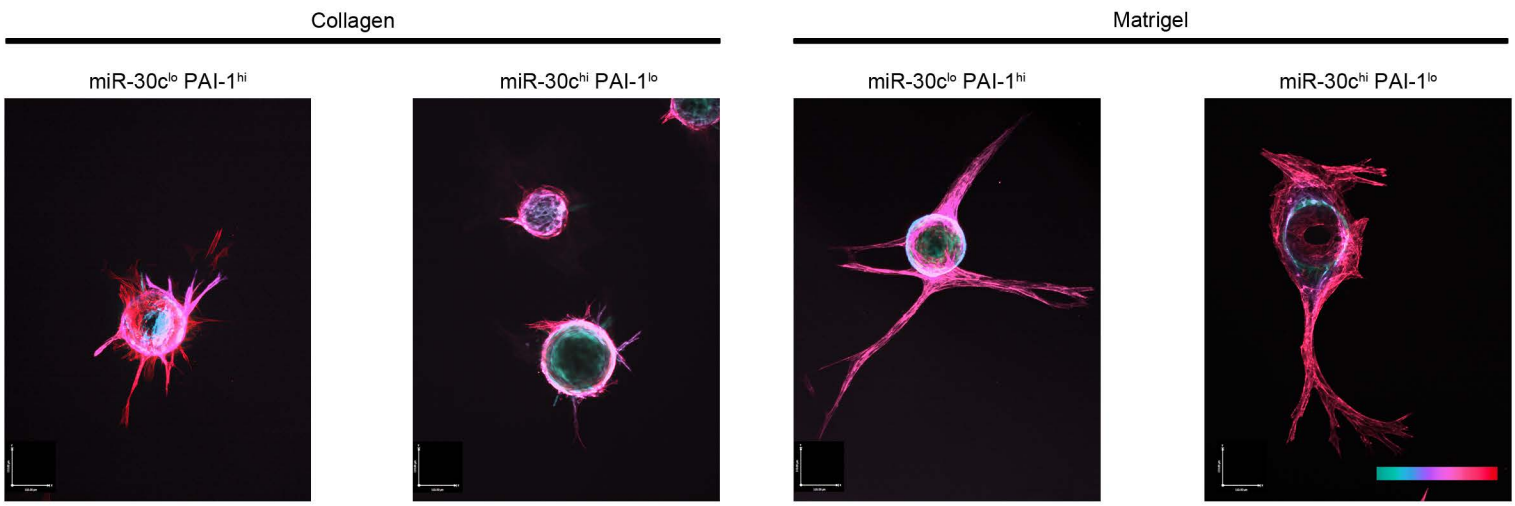

A
miR-30c^lo PAI-1^hi
miR-30c^hi PAI-1^lo
miR-30c^lo PAI-1^hi
miR-30c^hi PAI-1^lo
tPa
uPa
Control
TGFb2
B
R²=0.93
Total fibrin degraded (AUs)
tPA (micrograms/mL)
C
Micrograms/mL fibrinolytic activity
*
miR-30c^loPAI-1^hi
miR-30c^hiPAI-1^lo
D
miR-30c
Serpine1
Relative expression
Gelatin
Fibrin
Time (hours)
miR-30c^lo PAI-1^hi
E
miR-30c
Serpine1
Relative expression
Gelatin
Fibrin
Time (hours)
Tgfbr2^iECKO

Fig S9) Fibrin degradation in EC subtypes.

(A) Fibrin zymogram in EC subtypes as indicated treated with 10 ng/mL TGFb for 48 hours. The arrows point to the respective migratory positions of tPA and uPA. (B) Standard curve prepared by titrating increasing amounts of recombinant tPA in a fibrin zymogram containing fibrinogen and thrombin (see methods section for details). Total fibrin degraded was quantified by measuring the band area in ImageJ and is presented as arbitrary units (AUs). (C) Quantification of fibrinolytic activity in EC subtypes as indicated. Results were generated by extrapolating from the tPA dose response standard curve generated. Results were analyzed using Student's t-test and statistical significance is indicated with an asterisk. (D) qPCR analysis of miR-30c and *Serpine1* in miR-30c^{lo}PAI1^{hi} ECs plated on gelatin or fibrin for the indicated time points. Samples were assayed in triplicate (n=3). (E) qPCR analysis of miR-30c and *Serpine1* in Tgfbr2^{iECKO} ECs plated on gelatin or fibrin for the indicated time points. Samples were assayed in triplicate (n=3).

A


Collagen
Matrigel
miR-30c^{lo} PAI-1^{hi}
miR-30c^{hi} PAI-1^{lo}
miR-30c^{lo} PAI-1^{hi}
miR-30c^{hi} PAI-1^{lo}

B

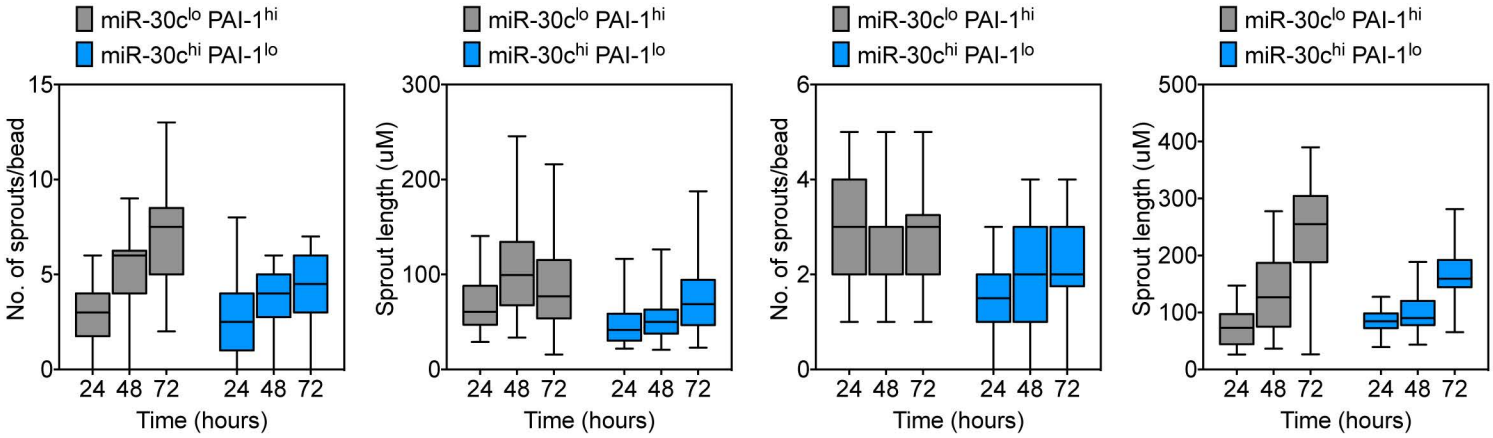

miR-30c^{lo} PAI-1^{hi}
miR-30c^{hi} PAI-1^{lo}
No. of sprouts/bead
Sprout length (uM)
Time (hours)
24 48 72 24 48 72

Fig S10) Characterization of in vitro sprouting using miR-30c^{lo}/PAI-1^{hi} and miR-30c^{hi}/PAI-1^{lo} ECs in different matrices.

(A) Representative images of vascular structures using miR-30c^{lo}/PAI-1^{hi} and miR-30c^{hi}/PAI-1^{lo} ECs in collagen or matrigel matrices as indicated. Images were captured after 48 hours. The scale at bottom right indicates sprout depth in the 3D matrix. Scale bar =40 um (x) X 54 um (y). (B) Number of sprouts per bead and average sprout length of miR-30c^{lo}/ PAI-1^{hi} and miR-30c^{hi}/PAI-1^{lo} ECs. Sprouts were counted at the indicated time, n=30 beads per time point.

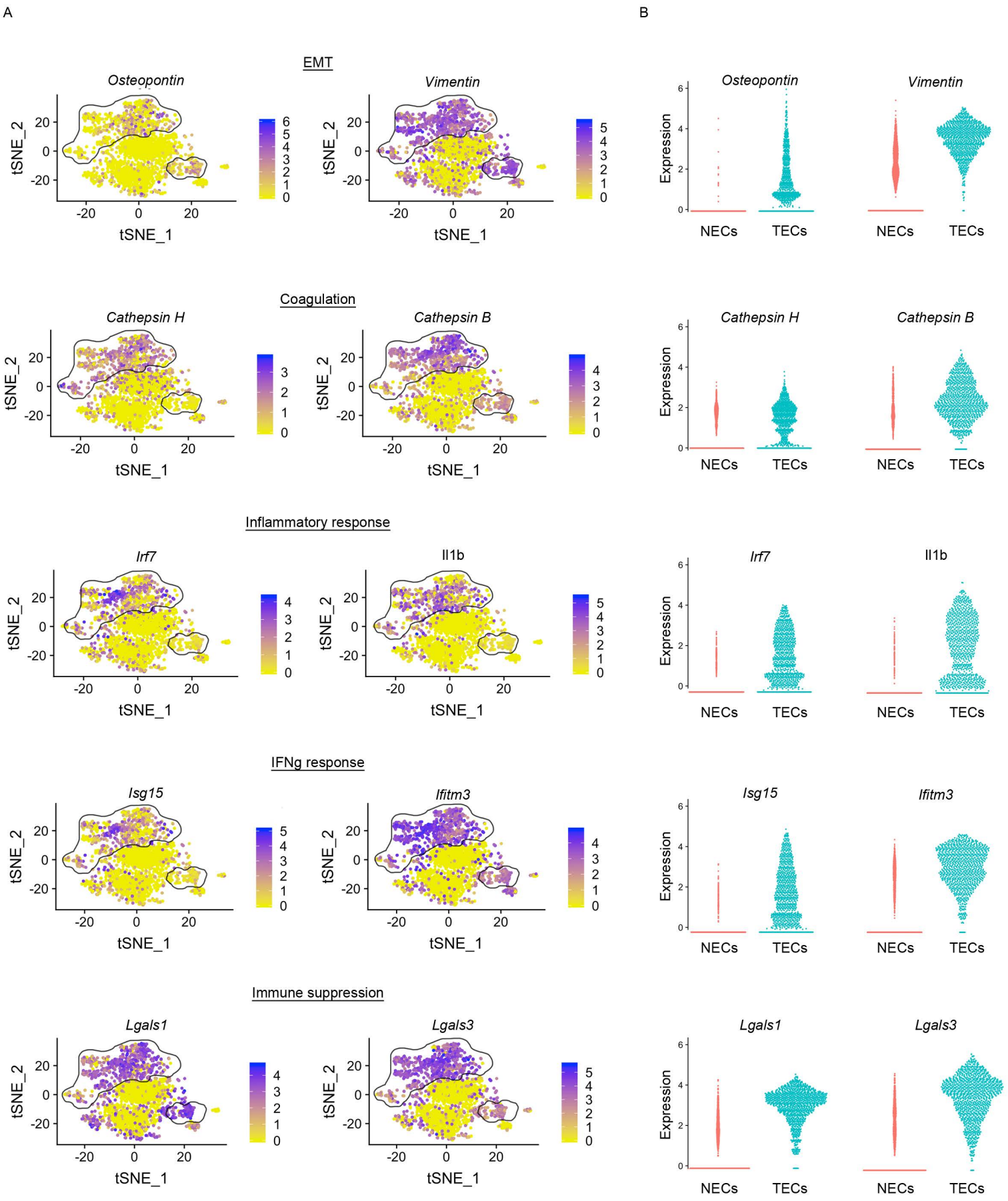

A
B
EMT
Osteopontin
Vimentin
Coagulation
Cathepsin H
Cathepsin B
Inflammatory response
Irf7
Il1b
IFNg response
Isg15
Ifitm3
Immune suppression
Lgals1
Lgals3
tSNE_1
tSNE_2
Expression
NECs
TECs

Fig S11) Selected candidate genes from the GSEA analysis that are important during EMT, coagulation, inflammatory responses, IFNg responses, and immune suppression.

(A) tSNE plots of NEC versus TEC populations showing enrichment for selected genes from the indicated GSEA pathway. Two of the major TEC subpopulations are circled for reference. (B) Bee swarm plots showing distribution in expression of the selected genes from the GSEA analysis.

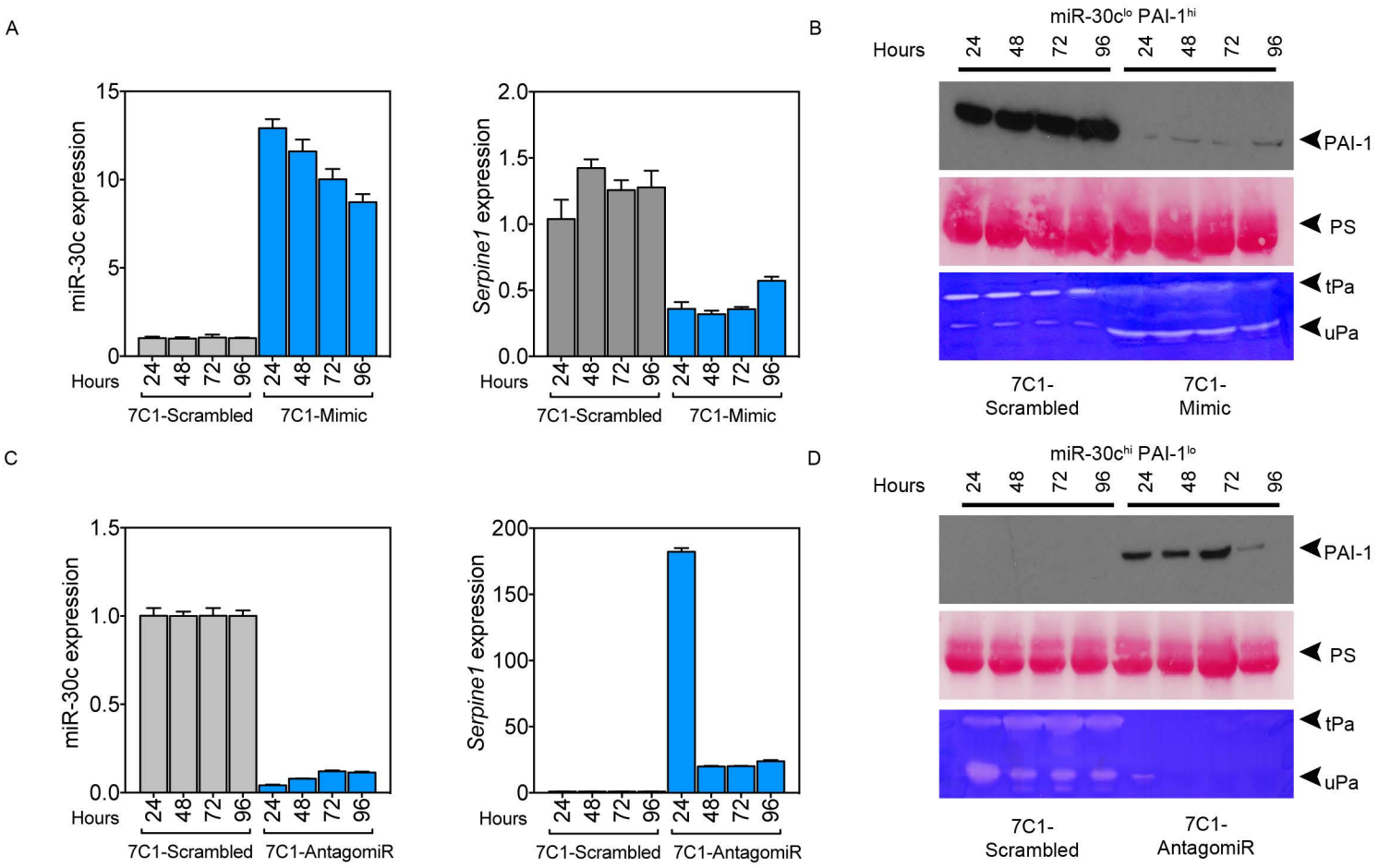
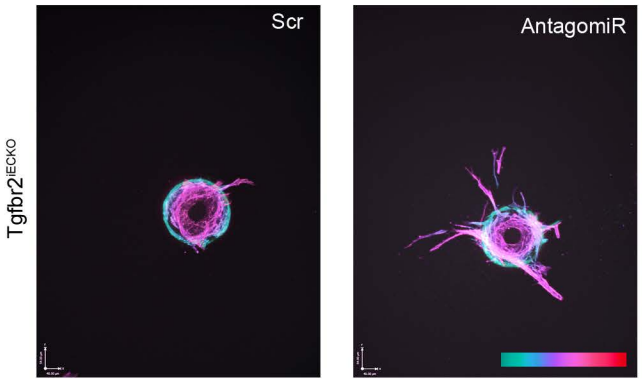

A
miR-30c expression
15
10
5
0
Hours
24
48
72
96
7C1-Scrambled
7C1-Mimic
Serpine1 expression
2.0
1.5
1.0
0.5
0.0
B
miR-30clo PAI-1hi
PAI-1
PS
tPa
uPa
7C1-Scrambled
7C1-Mimic
C
1.5
1.0
0.5
0.0
200
150
100
50
0
7C1-AntagomiR
D
miR-30chi PAI-1lo

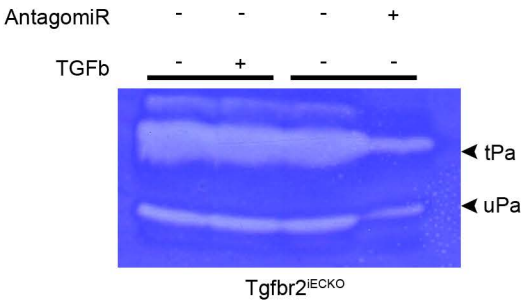
Fig S12) Characterization of activity using 7C1-mimics and 7C1-antagomiRs in EC subtypes.

(A) qPCR analysis for miR-30c and *Serpine1* expression in miR-30c^{lo}PAI-1^{hi} ECs treated with 20 nM 7C1-Scrambled or 20 nM 7C1-mimic for the indicated time. Samples were assayed in triplicate (n=3). (B) Western blot for secreted PAI-1 (top) and fibrin zymogram (bottom) in miR-30c^{lo}PAI-1^{hi} ECs treated with 20 nM 7C1-Scrambled or 20 nM 7C1-mimic for the indicated time. Ponceau stain (PS) was used to show equal loading. (C) qPCR analysis for miR-30c and *Serpine1* expression in miR-30c^{hi}PAI-1^{lo} ECs treated with 20 nM 7C1-Scrambled or 20 nM 7C1-antagomiR for the indicated time. Samples were assayed in triplicate (n=3). (D) Western blot for secreted PAI-1 (top) and fibrin zymogram (bottom) in miR-30c^{hi}PAI-1^{lo} ECs treated with 20 nM 7C1-Scrambled or 20 nM 7C1-antagomiR for the indicated time. Ponceau stain (PS) was used to show equal loading.

A


Scr
AntagomiR
Tgfbr2iECKO

C


AntagomiR - - - +
TGFb - + - -
tPa
uPa
Tgfbr2iECKO

B

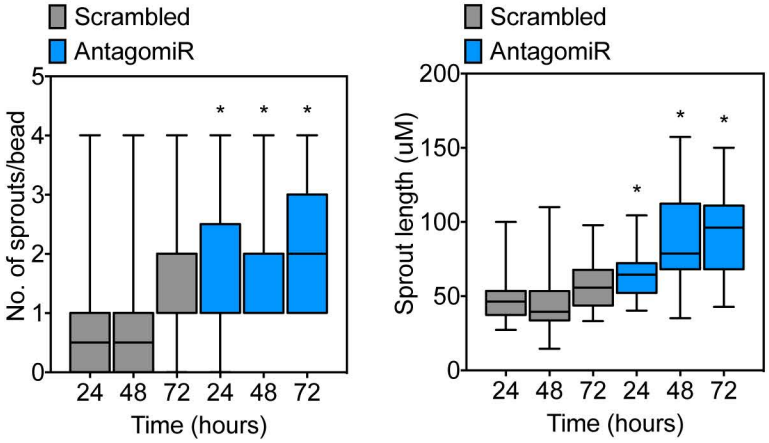

Scrambled
AntagomiR
No. of sprouts/bead
0
1
2
3
4
5
24 48 72 24 48 72
Time (hours)
Scrambled
AntagomiR
Sprout length (uM)
0
50
100
150
200
24 48 72 24 48 72
Time (hours)

Fig S13) miR-30c antagomiRs partially rescue in vitro sprouting in Tgfbr2^{iECKO} ECs.

(A) Representative images of phalloidin-stained Tgfbr2^{iECKO} ECs treated with 20 nM scrambled control or 20 nM miR-30c antagomiR for 72 hours. The scale at bottom right indicates sprout depth in the 3D fibrin matrix. Scale bar =40 um (x) X 54 um (y). (B) Number and length of sprouts per bead in Tgfbr2^{iECKO} ECs treated 20 nM Scrambled control or 20 nM miR-30c antagomiR for the indicated time. Results were analyzed using Student's t-test and statistical significance is indicated with an asterisk. (C) Fibrin zymogram using conditioned medium from Tgfbr2^{iECKO} ECs treated with miR-30c antagomiR or with 10 ng/mL TGFb for 48 hours. The antagomiR reduces fibrin degradation indicated by the arrow heads.

Supplemental Methods

7C1 synthesis and miRNA conjugation: To formulate nanoparticles, 7C1 was mixed with $C_{14}PEG_{200}$ and mixed with miRNA in a microfluidic device. Particles were then dialyzed against 1X PBS before sterile filtration through a 0.22 um filter. miRNA concentration was determined using Quant-iT^{TM} RiboGreen (Invitrogen) using HPLC absorbance against a known standard.

In vitro nanoparticle treatment: NECs and TECs were treated with 20-40 nM of nanoparticles in vitro by growing cells in LG-DMEM with 20% FBS and then adding nanoparticles to cells for the indicated time points. RNA and conditioned media were harvested from the cells at the indicated times in the figure.

a-amanitin treatment: NECs were treated with a-amanitin at defined time points in the figure in LG-DMEM with 20% FBS. For TGFb and a-amanitin treatment, NECs were pretreated with a-amanitin for 2 hours before TGFb was added.

Human EC isolation: Resected tissue (lung TECs or healthy lung tissue for NECs) was washed with 5-10 mL of LG-DMEM on ice. Tissue was then placed in a 10 cm plate with 2.2 mL of digestion buffer (see above) and mechanically dissociated using a sterile scalpel blade. The mechanically digested tissue was then transferred to a 15 mL conical tube and incubated on a shaker at 37°C for 60 minutes. The digested solution was then filtered through 40 um cell strainer and the strainer was washed once with an additional ten mL of MACS buffer to collect any residual cells adhered to the filter. The cell suspension was then spun at 1,200 rpm for five minutes. The supernatant was removed and the resulting cell pellets were resuspended in two mL of ACK lysis buffer and incubated for two minutes at room temperature. Then, three mL of MACS buffer was added, and the solution was spun at 1,200 rpm for five minutes. The supernatant was then removed and the resulting cell pellet was resuspended in 100 uL of MACS buffer. The staining antibodies were then added to the cell solution: FITC mouse anti-human EpCAM (BD Bioscience Cat#347197), PerCP-Cy5.5 mouse anti-human CD45 (BD Bioscience Cat#A0080), AlexaFluor 647 mouse anti-human CD31 (BD Bioscience Cat#561654), PE rabbit anti-human LYVE-1 (Novus Biologics Cat#NB100-725PE) and Biolegend Zombie Aqua viability dye (Biolegend Cat#423101). Cells were stained for one hour in the dark, on ice, with agitation every 15-20 minutes. Cells were then washed with MACS buffer and resuspended in a final volume of 300 uL MACS buffer and filtered through a cell strainer. The samples were sorted on a Becton Dickinson FACSAria II and were sorted directly into RNA lysis buffer. Blood endothelial cells were identified as $CD45^-/APC^-/CD31^+/LYVE\text{-}1^-$. ArC (Amine Reactive Compensation) bead Kit was used for compensation control for the viability dye and onecomp eBeads were used for single color gating controls.

Vessel branching: To determine vessel branching number and length, cryosections stained with CD31 antibodies were imported into FIJI. After converting to an 8-bit image and adjusting the threshold, images were converted to binary and then “skeletonized” using the skeletonize plugin included with the software. To quantify branching, the “analyze skeleton” feature was used. Values including number of branches and branch lengths were finally plotted using Prism.

Immunofluorescence: Tumor tissue was resected from mammary fat pads and placed in 4% PFA in PBS for 24 hours at 4°C. Tumors were then transferred to 30% sucrose in PBS for cryoprotection. After sucrose, tumors were embedded in OCT and cut in seven um sections. Slides were fixed in ice-cold methanol for 20 minutes at -20°C and then rinsed three times with PBS. Blocking buffer (PBS + 5% BSA + 5% goat serum) was added for 30 minutes at room temperature. The primary antibody was then diluted in blocking buffer and added to slides. Primary antibodies used: 1:100 rabbit anti mouse fibrin (Dako), rat anti-mouse CD31 clone MEC 13.3 (BD Bioscience Cat#550274). Slides were then incubated overnight at 4°C and then washed three times with PBS. The secondary antibody was diluted in blocking buffer and incubated at room temperature for one hr. Secondary antibodies used: goat anti-rat 488 (Thermo Fisher Cat#A-11006), and goat anti-rabbit 488 or 594 (Thermo Fisher Cat#A-11034 and Cat#A-11037). The slides were washed three times with PBS and then stained with DAPI. Coverslips were then mounted to the side using VectaShield Mounting Media Hard Set. CD31 and fibrin primary antibodies were used at 1:100 dilutions in blocking buffer. Secondary antibodies were used at 1:200.

Western Blot Analysis: Cells were lysed in RIPA buffer containing phosphatase and protease inhibitors. Protein concentrations were determined by Bradford assays and approximately 25 ug per sample of cell lysate or CM was used for western blots. All membranes were blocked and antibodies added in 5% milk in TBS with 0.1% Tween 20 (TBS-T) except pSMAD2 which was done in 5% BSA. Membranes were incubated with primary antibodies at 4°C overnight and then secondary antibodies at room temperature for one hour. Primary antibodies: 1:1,000 rabbit anti-mouse PAI-1 (Novus Biologics Cat#NBP1-19773), 1:1,000 phospho-Smad2 (Ser465/467) (Cell Signaling Cat#18338T), 1:1,000 rabbit anti-SMAD2/3 (Cell Signaling Cat#8685), and 1:2,500. Secondary HRP conjugated antibodies: 1:10,000 goat anti-rabbit or anti-mouse HRP (Vector PI-100 and PI-2000). Ponceau S Solution was used to stain CM western blots for total protein concentrations. In short, western membranes were incubated 5-10 minutes with shaking at room temperature in Ponceau S stain and then imaged for total protein detection.

Sprouting assay: ECs were mixed with hydrated cytodex beads at $1x10^6$ cells/2,500 beads in 1.5 mL of culture media. Cells and beads were combined in a FACs tube and placed in a tissue culture incubator for four hours. Cells and beads were dispersed into suspension every ten minutes by removing tubes from the incubator and flicking several times. The suspension was then moved to a 60 mm dish and cells were allowed to grow overnight. The next day, the beads now coated with cells were checked under light microscopy. Fibrinogen solution (2mg/mL in PBS) was incubated at 37°C with shaking for ~ 15 minutes to allow fibrinogen to dissolve. The solution was then sterile filtered with a 0.2 um filter and aprotinin was added at a final concentration of 0.15 U/mL. The suspension was then moved to a 15 mL conical tube and the beads were allowed to settle with gravity. The beads and cells were washed once with PBS and then resuspended in five mL of fibrinogen solution. Five hundred uL of bead and cell suspension was then combined with 0.625 U/mL of thrombin in a 24-well plate. The plate was then left on the bench for five minutes (to allow fibrin to form) and then moved to the incubator for 10-15 minutes to allow further polymerization. Once the fibrin gel formed, one mL of LG-DMEM with 20% FBS was added to each well. Finally, mouse fibroblasts were seeded on top of the fibrin gel at a concentration of 20,000 cells/well. Beads were then imaged every 24 hours and media was changed every 48 hours. Transfection of ECs during sprouting assays took place before cells and beads were embedded in fibrin. In short, after checking beads for cell coating, media was removed from the plate and the transfection was performed as outlined above. The transfection was allowed to take place for four hours before cells and beads were embedded in the fibrin matrix. rPAI (EMD Millipore) was added to fibrinogen/bead mixture at defined concentrations just prior to the addition of thrombin. The fibrinogen/bead/thrombin solution was mixed in each well of a 24-well plate and sprouts were imaged as defined above. For Matrigel (Corning) and collagen (Ibidi) sprouting assays, cytodex beads were coated at the same bead to cell ratio as outlined above. Both were carried out in 96-well plates in 100 uL of matrix with 75 beads per well. Matrix composition for the Matrigel sprouting assay was 50% Matrigel: 50% LG-DMEM with 20% FBS. The collagen sprouting assay was carried out at a final concentration of 1.5 mg/ml collagen.

Phalloiden staining: After 72 hours, the sprouting assay media was removed and 2.5X trypsin was used to remove fibroblasts (while watching under microscope to confirm fibroblast detachment). Trypsin was neutralized with 20% FBS in PBS and then removed. Each well was fixed with 4% PFA for 20 minutes at room temperature and the PFA was removed and wells were washed four times with PBS. Cell membranes were permeabilized with 0.5% Triton-X 100 in PBS for two hours at room temperature. Wells were blocked overnight in blocking solution (5% goat serum, 1% BSA, 0.3% Triton-X 100 in PBS) at 4°C. Phalloidin was diluted 1:50 in blocking solution and the blocking solution was removed from the plate and replaced with 500 uL of phalloidin in blocking solution. The plate was placed at 4°C overnight with shaking and protected from light. Finally, the phalloidin solution was removed and the wells were washed three times with PBS before imaging.

Media concentration: ECs were grown in LG-DMEM supplemented with 1% FBS and mammary tumor cells were grown in HG-DMEM with 1% FBS. Media was collected from cells grown for indicated time points and then spun at 1,200 rpm to pellet any detached cells. Supernatant was then transferred to a Microsep Advance Centrifugal Filter (with three kDa cut off). Samples were then spun at 20,000 rpm for ~30 minutes. Samples were concentrated ~10-fold from starting volume (e.g. two mL of media concentrated to 200 uL). Protein concentration was determined using a Bradford assay.

Fibrin Zymography: First, a resolving gel was poured with the following recipe: 10 mL of PBS containing 36 mg of dissolved fibrinogen, 12 mL of 30% acrylamide, 7.5 mL of 1.5 M Tris, 150 uL of 20% SDS, 300 uL of 10%

APS, 60 uL of thrombin dilution (1 uL of 50U/mL thrombin in 999 uL of water) and 12 uL of TEMED. The resolving gel was allowed to polymerize and then the stacking gel portion was poured containing 2.5 mL of 30% acrylamide, 3.8 mL of 0.5 M Tris-HCL, 8.5 mL of PBS, 75 uL of 20% SDS, 150 uL of 10% APS, and 15 uL of TEMED. Gels were poured in 1.0 mm glass plates with 10-well combs. Once the gel was poured, 25 ug of protein from conditioned media samples was mixed with non-reducing buffer and loaded onto the gel. The gel was run at 150 V for 1.5 hours and then the gel was removed from the glass plates and washed three times with 2.5% triton and then three times with water. After washing, the gel was submerged in incubation Tris-HCL buffer and placed at 37°C with shaking for ~ 16-18 hours. Parafilm was placed over the gel to reduce evaporation. Next, incubation buffer was removed and gels were stained with coomassie blue and then scanned. Recombinant tissue plasminogen activator was used to create a standard curve to quantify fibrinolytic activity. In short, known amounts of rTPA (0.0007 pg/mL - 1 ug/mL) were run on a fibrin zymography gel as described above and then stained. The gel was then scanned and analyzed in ImageJ using the measure area function. The total area of fibrin degradation was plotted against known amounts of rTPA to create standard curves. This standard curve was then used to extrapolate fibrinolytic activity in unknown samples by plotting total fibrin degradation area on the standard curve.

Fibrin Plug Assay: $0.8x10^6$ wildtype ECs or $Tgfbr2^{iECKO}$ ECs were re-suspended in 200 uL of fibrinogen solution (3mg/mL). 50 uL of thrombin solution (10 U/mL) was then injected subcutaneously in the upper dorsal region using a 26-guage needle. Next, 200 uL of fibrinogen/cell suspension was injected at the same site. On day seven, 50 ug of isolectin GS-IB4-alexaFluor 594 (Molecular Probes) was injected intravenously to visualize perfusion of vessels.

Polymerase chain reaction and real-time quantitative PCR (qPCR): mRNA primers were designed using NCBI-Primer Blast. miRNA TaqMan primers were ordered from Life Technologies (specific miRNA primer info below). Total RNA was isolated using the Quick-RNA miniprep kit and cDNA synthesis generated using iScript cDNA Synthesis Kit (250 ng of RNA/reaction). cDNA for miRNA was generated using the TaqMan MicroRNA Reverse Transcription kit (10 ng of RNA/reaction) and specific TaqMan miRNA primers. mRNA qPCR was run in triplicate with Maxima SYBR Green on an Applied Biosystems Quant Studio 6 instrument. miRNA qPCR was run in triplicate with TaqMan Universal Master Mix II on an Applied Biosystems Quant Studio 6 instrument. miRNA taqman primers used (all from Thermo) were: miR-30a, miR-30b, miR-30c, miR-30d, miR-30e, and snoRNA234.

Nanostring Array: RNA was isolated as described above from control cells or cells treated with 10 ng/mL of TGFb for 48 hours. After following the manufacturer's instructions included with the kit, samples were submitted to the Nanostring array core at UNC Chapel Hill. Analysis of the Nanostring array was done using nCounter software. Background subtraction was performed to account for false positives. Positive controls and code-set content (house keeping gene) normalization was performed using the nCounter on all samples.

Target site blockers: Target site blockers (TSB) were designed by Exiqon, Inc. using *Serpine1* accession number NM_008871.2 and miR-30c MIMAT0000514. TSB was transfected into ECs using the same RNAiMAX protocol as described above.

TGFb treatment: Cells were treated with 10 ng/mL of TGFb in LG-DMEM with 20% FBS (ECs) or HG-DMEM with 20% FBS (mammary tumor cells) unless indicated otherwise for the time points indicated. For conditioned media (CM) experiments, cells were treated with the same dose but grown in LG-DMEM supplemented with 1% FBS (ECs) or in HG-DMEM with 1% FBS (mammary tumor cells).

miR-30c mimic, AntagomiR, and siRNA transfection: ECs were transfected with miR-30c mimic, miR-30c AntagomiR, SMAD4 siRNA, Eri1 siRNA, or scrambled control following RNAiMax manufacturer's protocol. See figures for specific siRNA or miRNA concentrations and duration of the treatment. All transfections were done in either LG-DMEM with 20% FBS (ECs) or HG-DMEM with 20% FBS (mammary tumor cells) except for CM experiments which were carried out in 1% FBS as described above.

Flow Cytometry: Flow cytometry was performed on a FACS caliber with data analyzed post hoc using Flowjo (V10.5). Antibodies: PE rat anti-mouse CD31 (BD Bioscience Cat#553373) and live/dead fixable far red dead cell stain kit (Invitrogen) were used. Cells were sorted on an Influx cell sorter based on ZSGreen expression.

PAI-1 ELISA: Circulating PAI-1 levels were measured by obtaining ~ one mL of blood from each mouse via cardiac puncture. The syringe and collection tubes were washed with 3.8% sodium citrate to prevent clotting. Samples were then spun at 6,500 rpm at 4°C for 15 minutes. Platelet free plasma was then removed, with care taken not to disturb the buffy coat and RBC pellet. PAI-1 levels were then measured using Molecular Innovations mouse PAI-1 total antigen assay ELISA kit. Following the manufacturer's protocol, PAI-1 concentrations were determined by plotting on standard curves using rPAI-1.