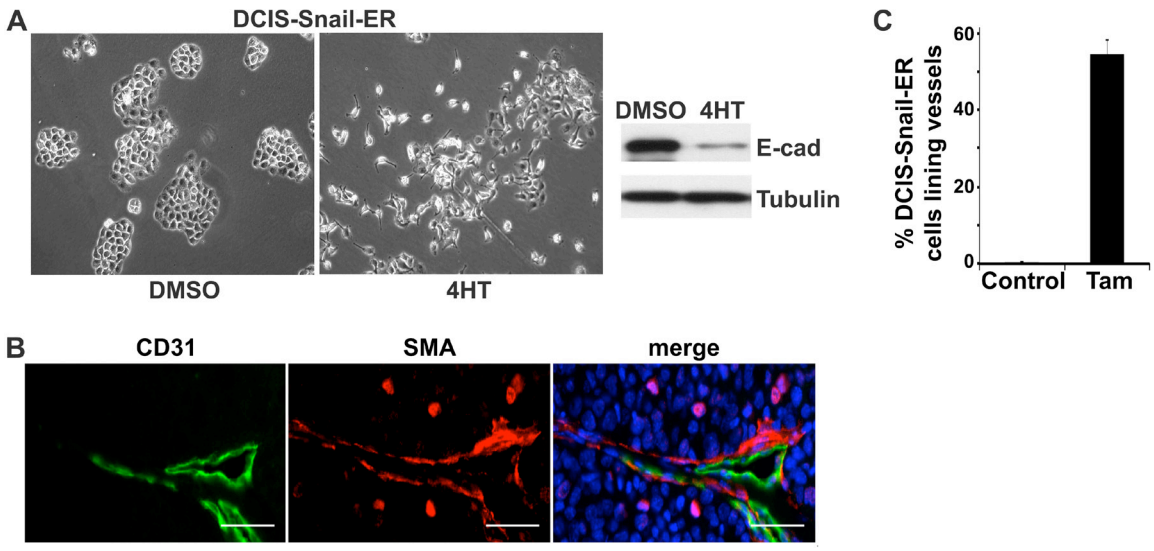
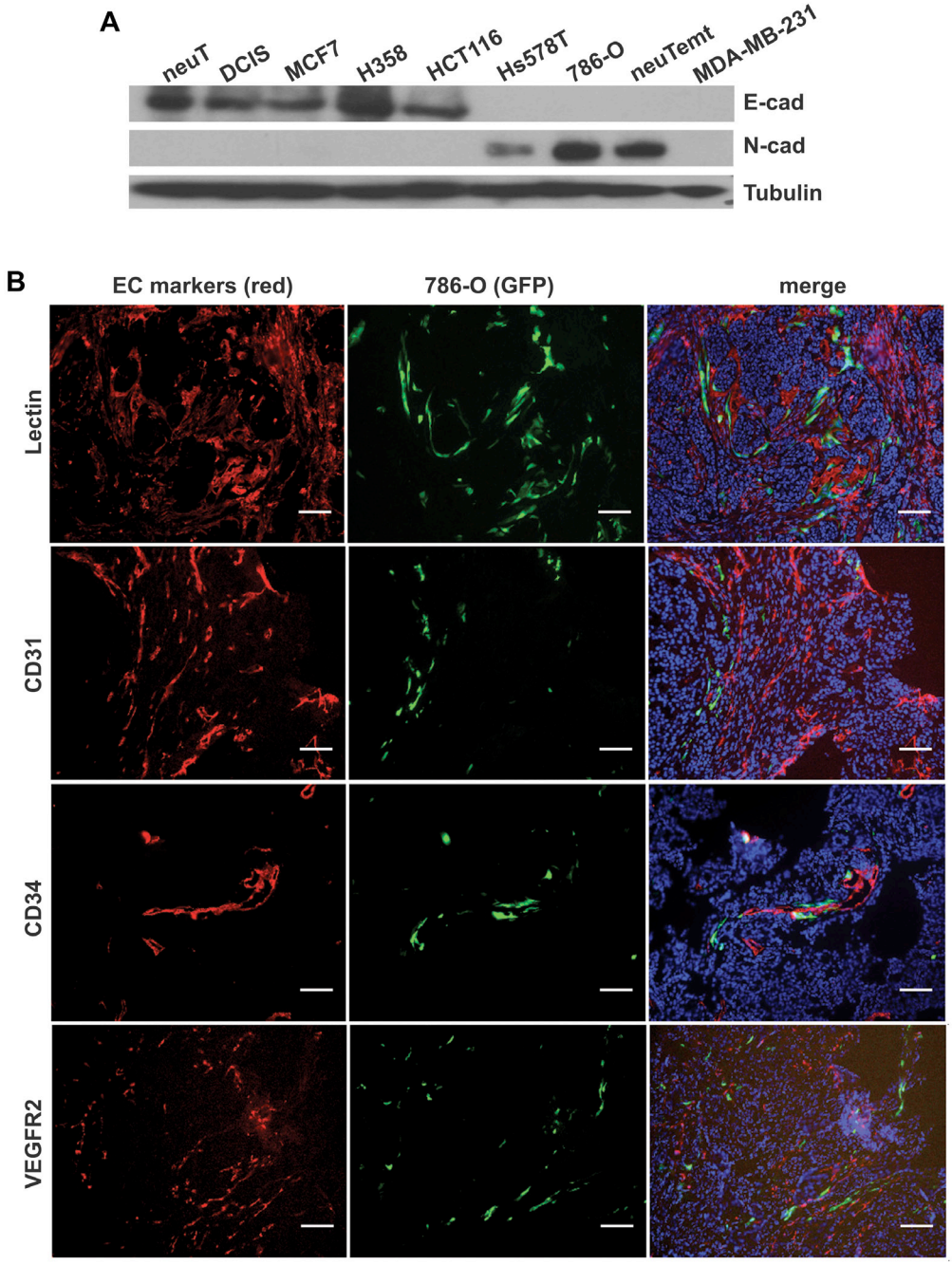
Supplementary information



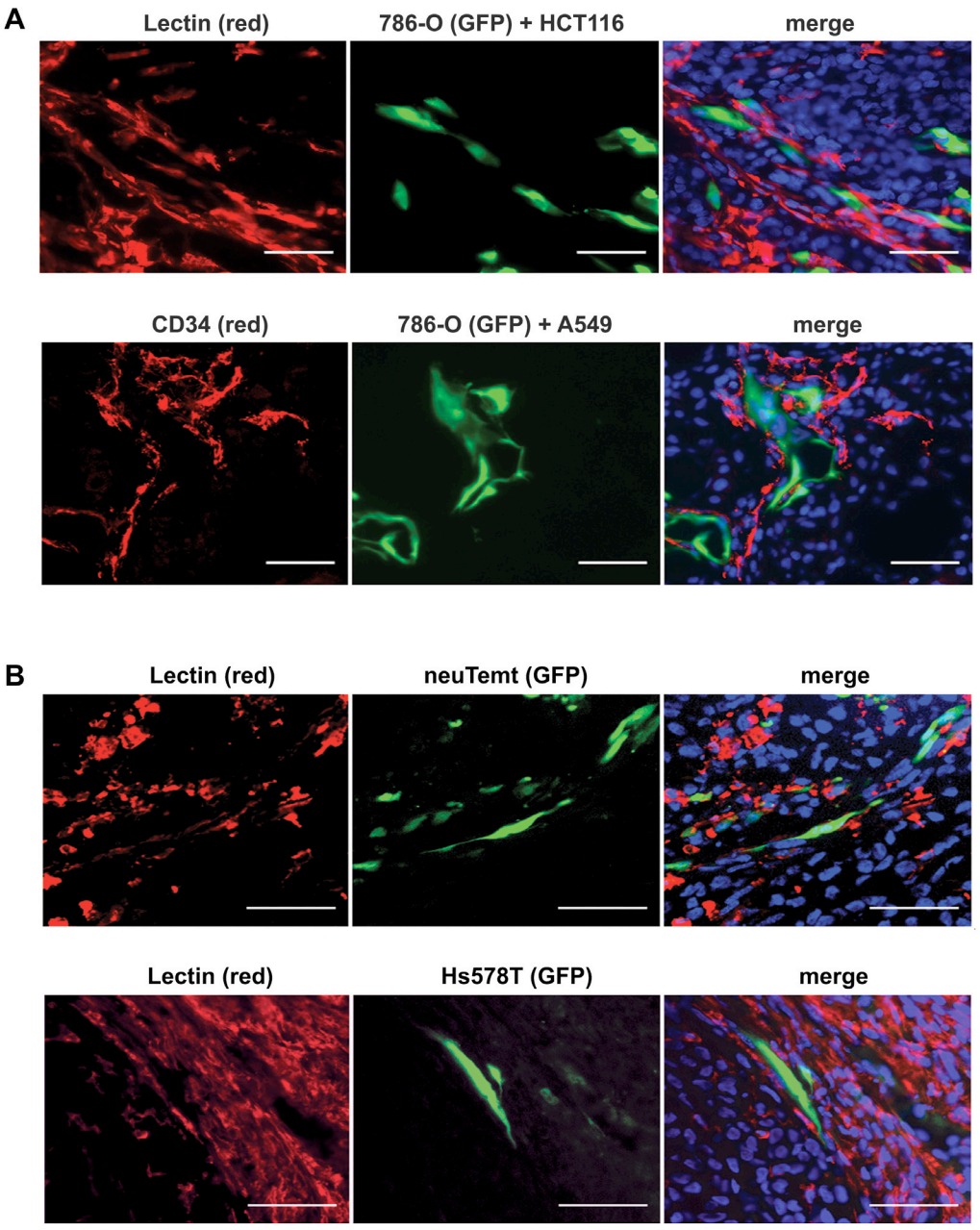
Supplementary Figure 1. An inducible EMT model in DCIS cells.
(A) DCIS cells transduced with lentiviral Snail-ER were treated with vehicle (DMSO) or 4HT for 2 days. 4HT transformed their cobblestone morphology to a fibroblastic shape. Downregulation of E-cadherin (E-cad) was confirmed by immunoblotting of cell lysates with an anti-E-cadherin antibody.
(B) Validation of antibodies recognizing vascular markers. Mouse neuT mammary epithelial tumor cells (1) were used to generate xenografts in mice. Tumor sections were stained with anti-CD31 antibody to label endothelial cells (ECs) (green) and anti-α-smooth muscle actin (SMA) antibody to mark mural cells (red). DNA was stained blue.
(C) Histogram shows the percentage of DCIS-Snail-ER cells that lined blood vessels in tumor xenografts in mice treated with vehicle (control) or tamoxifen (Tam) (corresponding to Figure 1). Error bars represent S.D. (n=5). $p<0.0001$. Statistical differences were determined by Student's t-test (2-tailed).



Supplementary Figure 2. Mesenchymal 786-O cancer cells are closely associated with blood vessels in tumor xenografts.

(A) Indicated epithelial and mesenchymal cancer cells were subjected to immunoblotting for E-cadherin (E-cad) and N-cadherin (N-cad) expression.

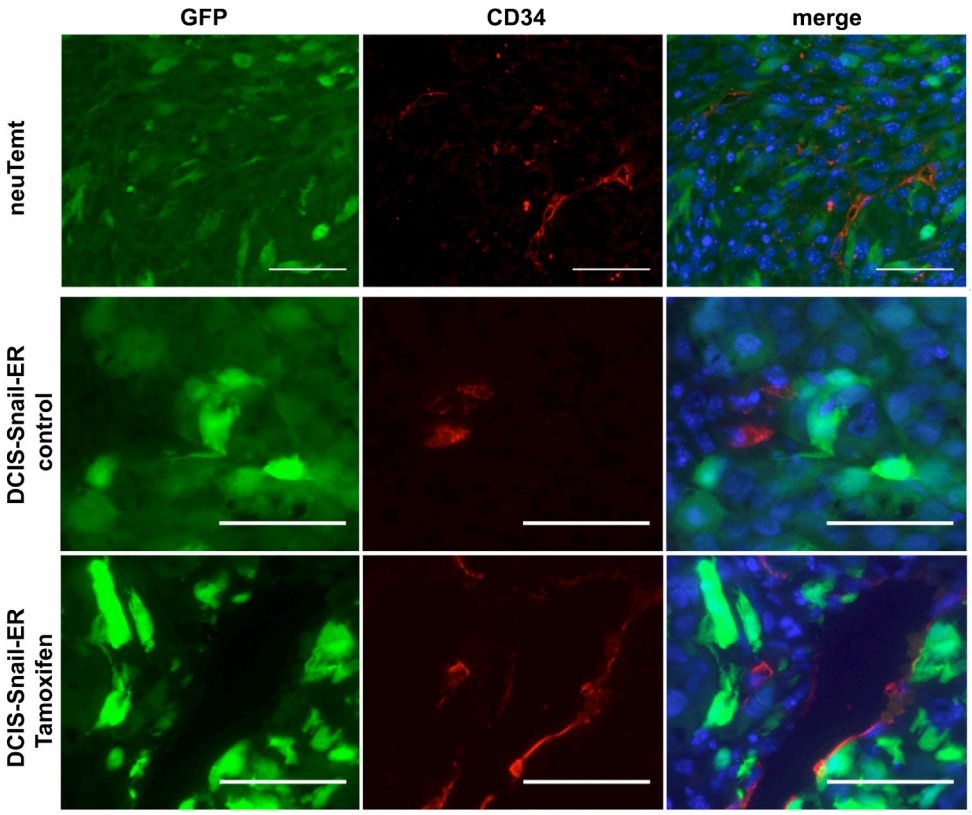
(B) Lower magnification images showing association of mesenchymal 786-O cells with endothelium in tumors (corresponding to Figure 2A). GFP-tagged 786-O cells were mixed in a 1:4 ratio with unlabeled DCIS cells to generate tumor xenografts. Tumor vasculature was immunostained red with lectin or antibodies for indicated endothelial markers. DNA was stained blue. Scale bar: 100μm.



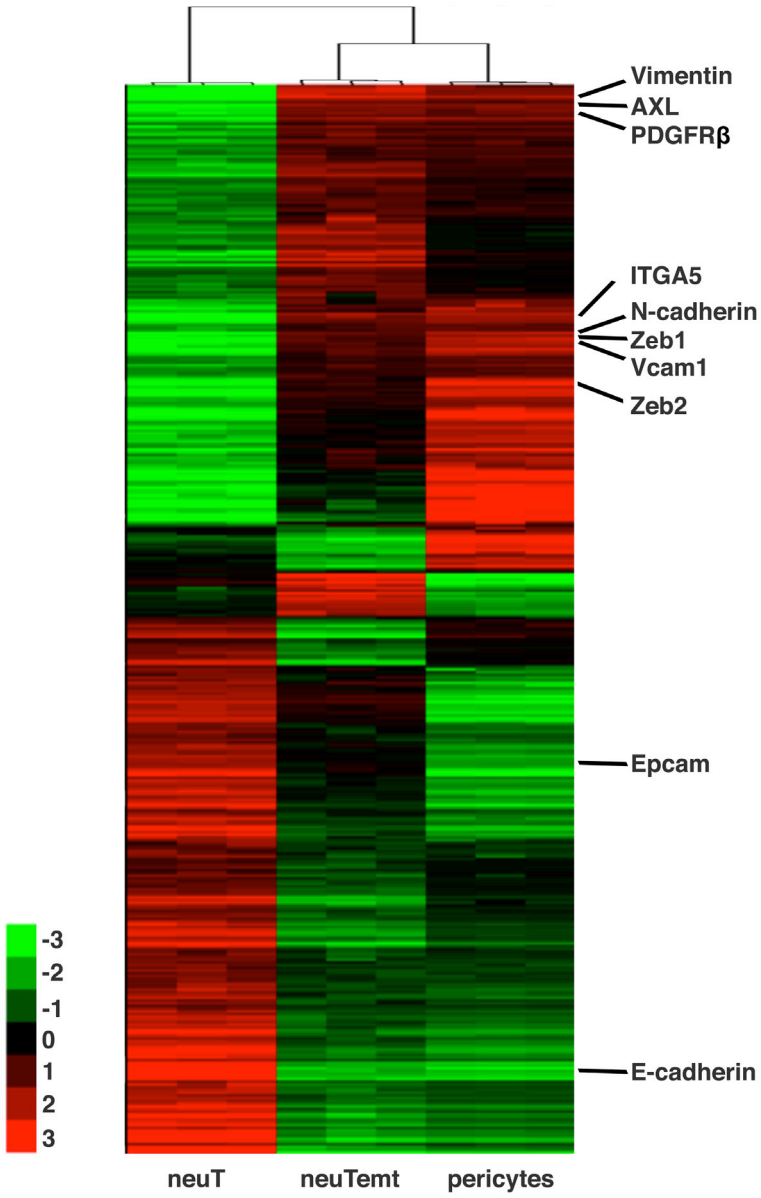
Supplementary Figure 3. Mesenchymal cancer cells exhibit vascular association in tumor xenografts.

(A) GFP-tagged 786-O cells (green) were mixed in a 1:4 ratio with unlabeled HCT116 or A549 epithelial cancer cells for tumor transplantation. Tumor vasculature was marked red by lectin or anti-CD34 antibody staining. Scale bar: 50μm.

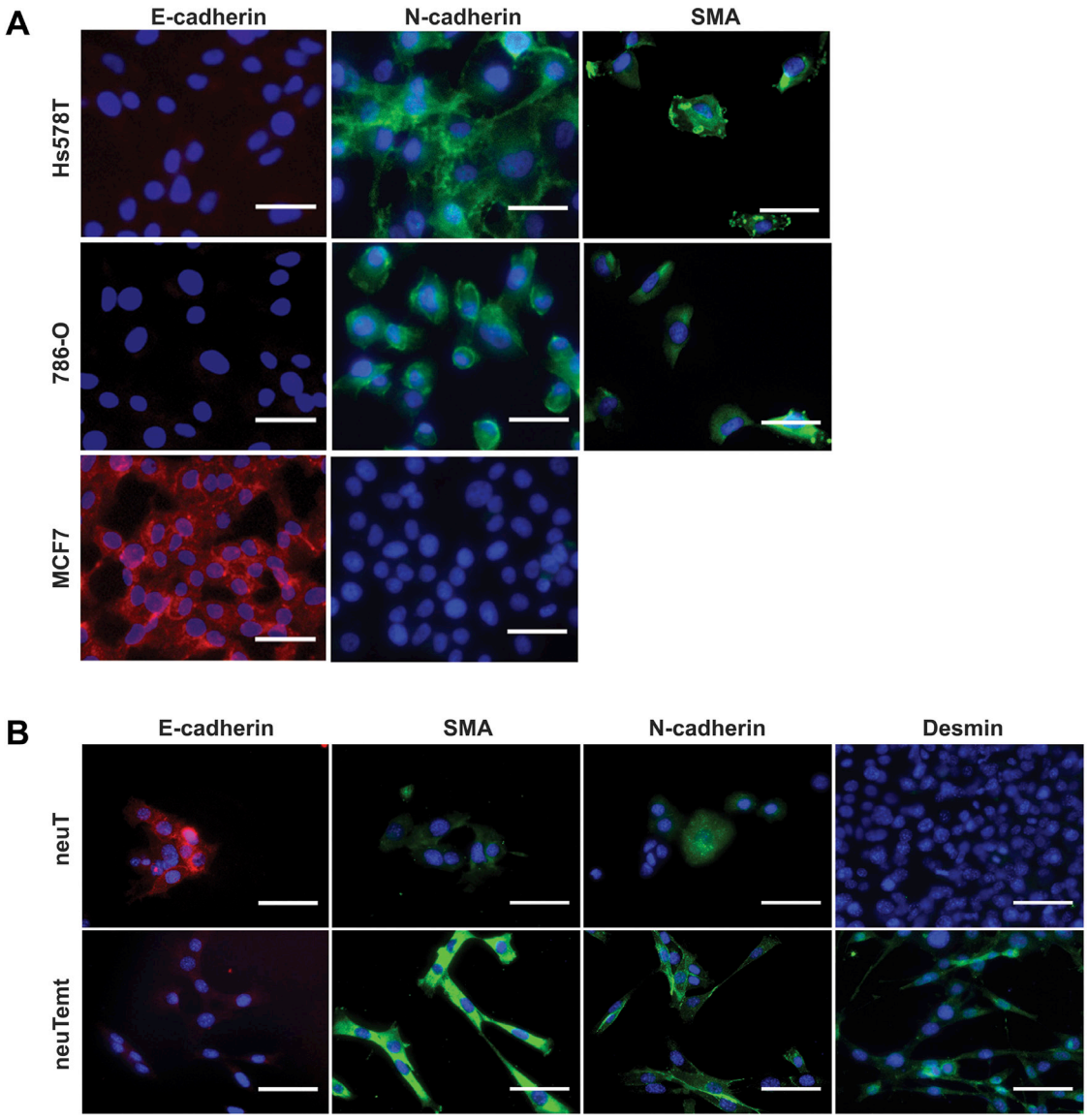
(B) GFP-tagged mesenchymal neuTemt and Hs578T cells (green) were mixed with unlabeled DCIS cells (1:4 ratio) to generate tumor xenografts. Tumor vasculature was stained with lectin (red). Scale bar: 50μm.



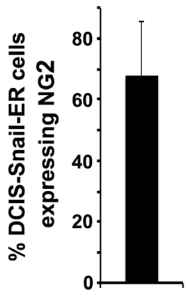
Supplementary Figure 4. Association of mesenchymal or EMT cancer cells with vasculature in mesenchymal tumors.
GFP-labeled neuTemt mesenchymal or DCIS-Snail-ER tumor cells were injected into mouse mammary fat pad. Mice bearing DCIS-Snail-ER tumors were treated with vehicle or tamoxifen. Tumor sections were stained with anti-CD34 antibody to mark endothelial cells (red). DNA was stained with blue. Scale bar: 50μm.



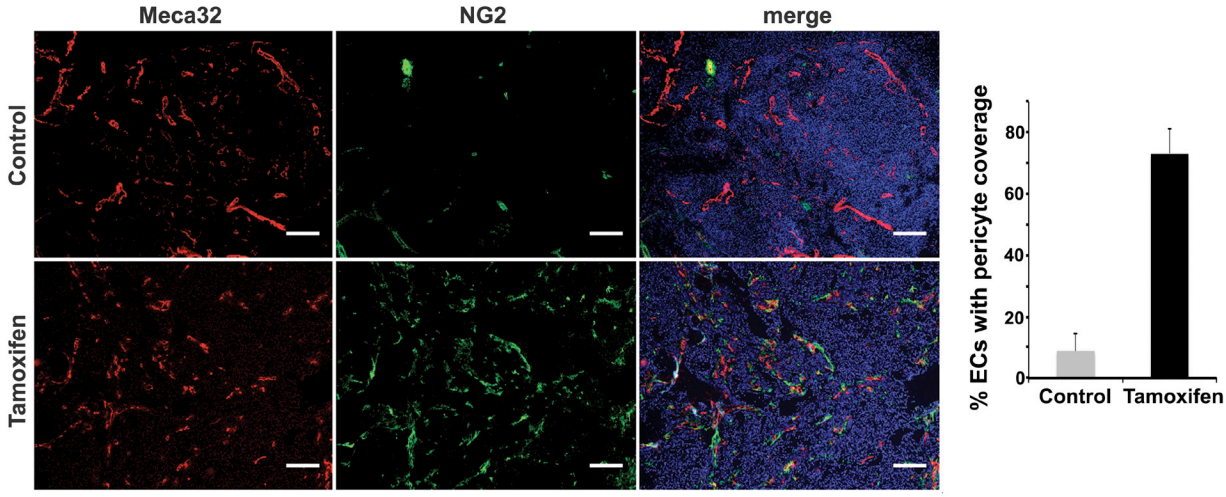
Supplementary Figure 5. EMT cancer cells resemble pericytes.
A heatmap shows gene expression levels in RNA samples isolated from neuT epithelial tumor cells, neuTemt mesenchymal tumor cells, and mouse primary pericytes. These cells are clustered based on the selected genes that are differentially expressed between neuT and neuTemt cells. A few EMT and/or pericyte markers are indicated. Mouse pericyte microarray dataset is from GSE46564 (2), and microarray data of neuT and neuTemt cells were previously described (1).



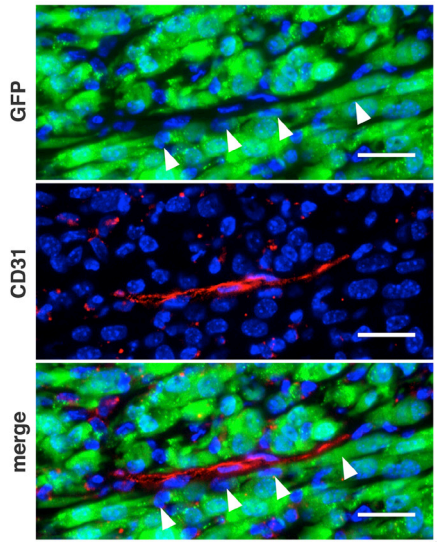
Supplementary Figure 6. Expression of EMT and pericyte markers in epithelial and mesenchymal cancer cells.
(A) Human Hs578T and 786-O mesenchymal, and MCF7 epithelial cancer cells as well as **(B)** mouse neuT epithelial and neuTemt mesenchymal tumor cells were immunostained with indicated antibodies. Scale bar: 50μm.



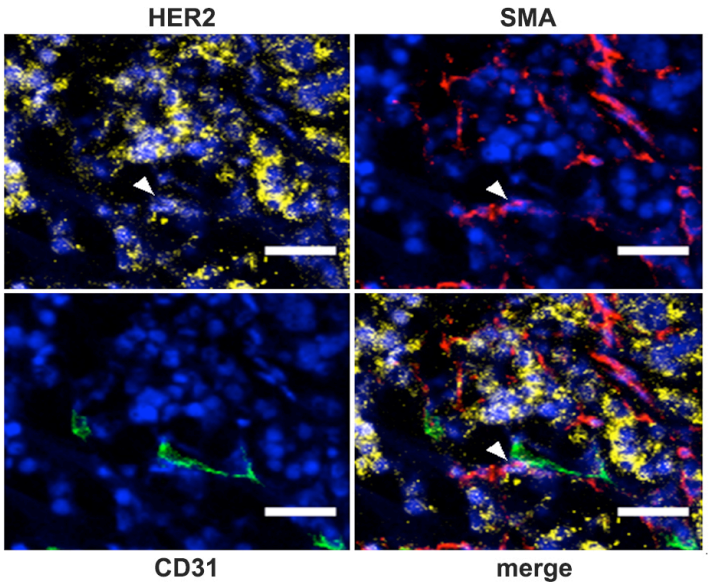
Supplementary Figure 7. DCIS-Snail-ER cells express NG2 in vivo.
Histogram shows the percentage of DCIS-Snail-ER cells that express NG2 in tumor xenografts in mice treated with tamoxifen (corresponding to Figure 3C). Error bars represent S.D. (n=5).



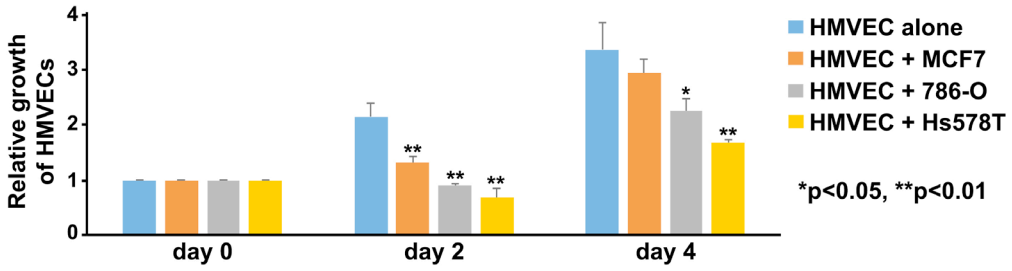
Supplementary Figure 8. Inducible EMT cancer cells increase pericyte coverage in tumor xenografts.
Mice bearing tumor xenografts resulting from mixed DCIS-Snail-ER and DCIS cells (in a 1:4 ratio) were treated with vehicle (control) or tamoxifen. Tumor sections were fixed with acetone, followed by immunostaining of pericytes with anti-NG2 antibody and ECs with anti-Meca32 antibody. Scale bar: 200μm. (right) Histogram shows the percentage of ECs covered by NG2-positive pericytes. Error bars represent S.D. (n=5). $p<0.0001$. Statistical differences were determined by Student's t-test (2-tailed).



Supplementary Figure 9. Spontaneous EMT cancer cells are associated with vasculature.
Mouse neuT mammary epithelial tumor cells were stably labeled with GFP through lentiviral transduction, and orthotopically implanted into mice. Tumor sections were stained with anti-CD31 antibody to mark endothelial cells (red). Arrowheads indicate elongated EMT tumor cells that are adjacent to a blood vessel. Scale bar: 25μm.

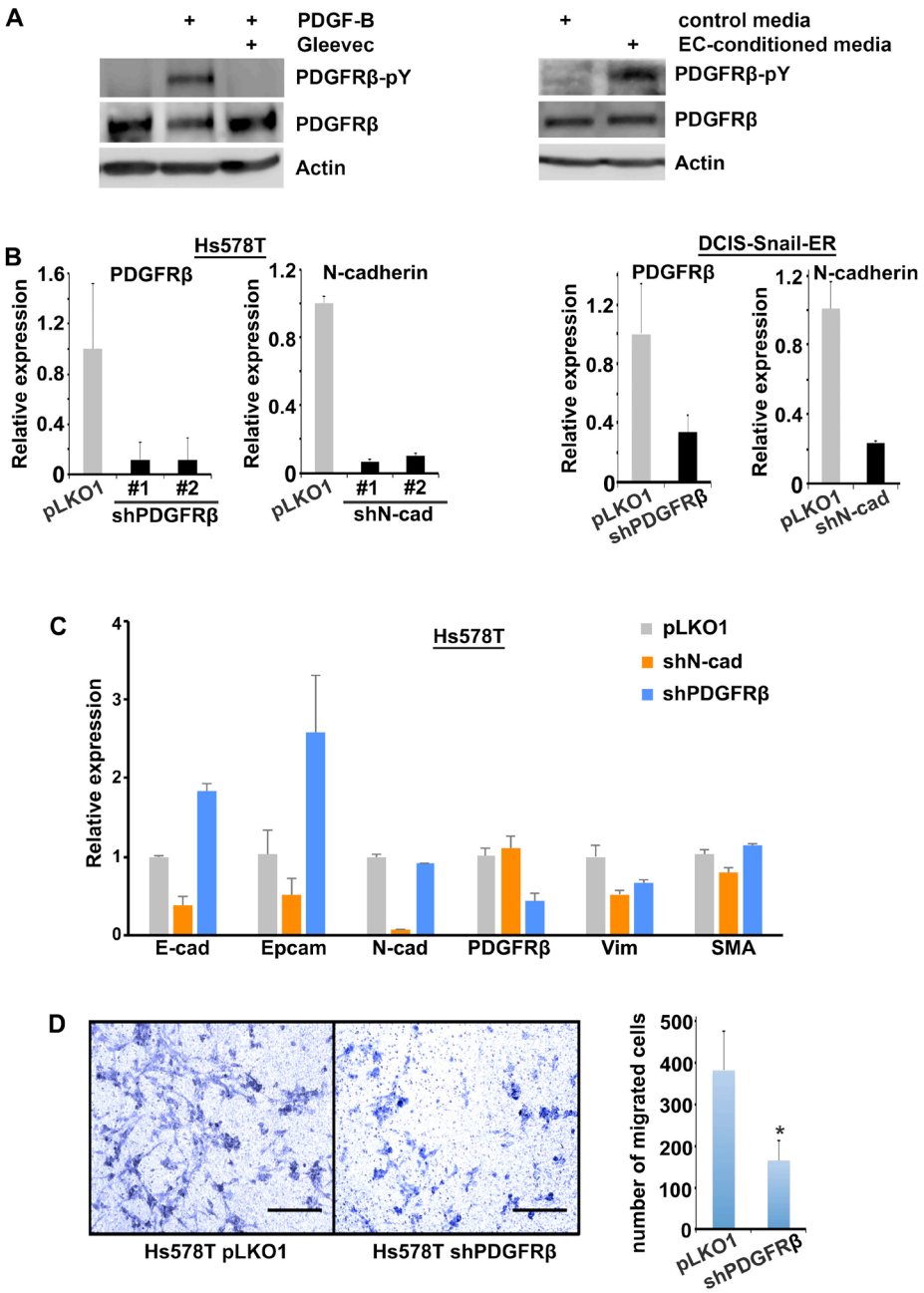


Supplementary Figure 10. A spontaneous EMT cancer cell in human breast tumor is associated with endothelium.
A HER2-positive human breast tumor section was stained simultaneously with rabbit monoclonal anti-HER2 antibody for cancer cells, rat monoclonal anti-CD31 antibody for ECs, and mouse monoclonal Cy3-conjugated anti-SMA antibody (red) for pericytes, followed by Alexa Fluor 647-conjugated anti-rabbit (far-red, pseudo-colored yellow) and Fluor 488-conjugated anti-rat (green) secondary antibodies. Arrowhead indicates a cell that expresses HER2 and SMA, and attaches to EC. Scale bar: 50μm.



Supplementary Figure 11. Mesenchymal cancer cells inhibit EC proliferation in vitro.

HMVEC cells were stably labeled with GFP, mixed with equal number of indicated cancer cells, and cultured for indicated periods. The number of EC cells were counted under a fluorescence microscope. Error bars represent S.D. (n=3). Statistical differences were determined by Student's t-test (2-tailed).



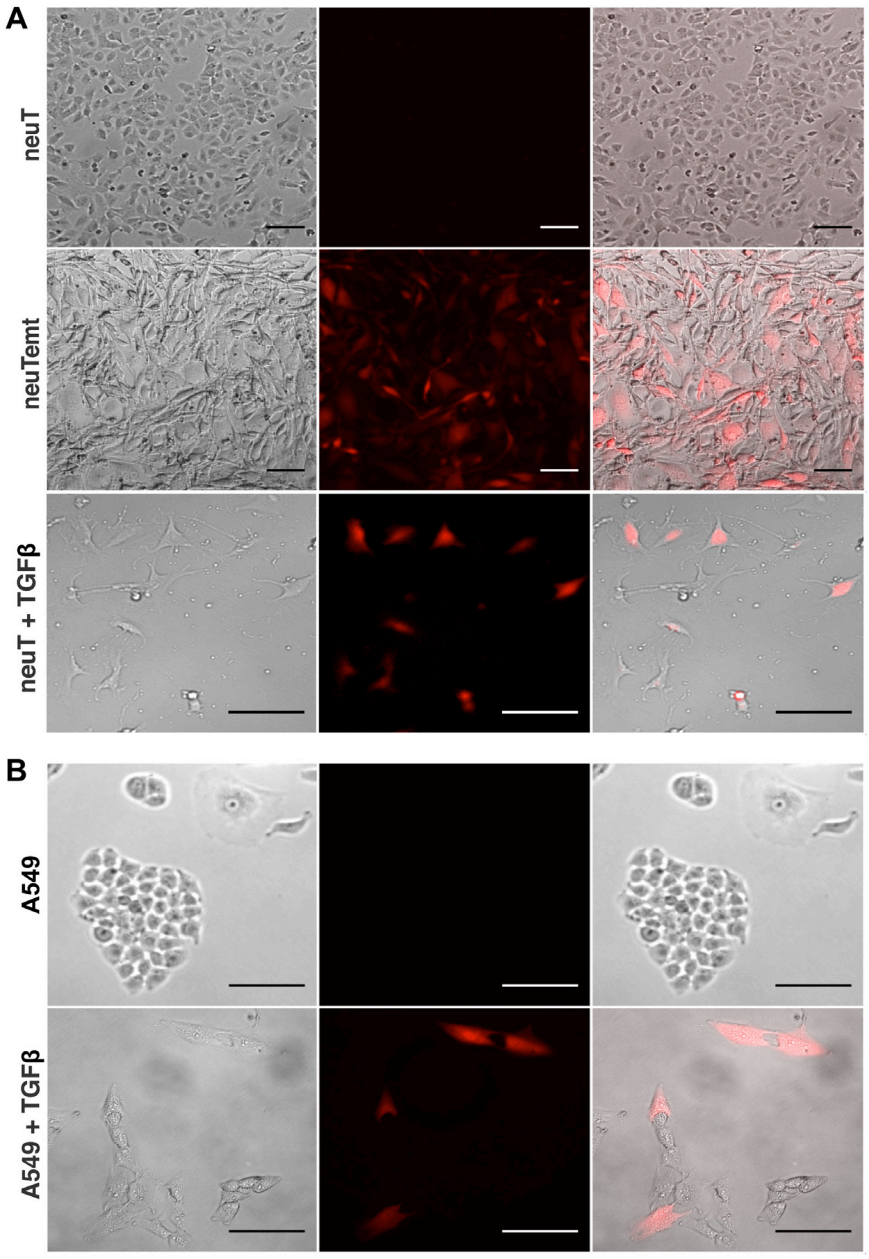
Supplementary Figure 12. Functional characterization of PDGFRβ and N-cadherin in mesenchymal or EMT cancer cells.

(A) Activation of PDGFRβ in neuTemt cells by PDGF or EC-conditioned media. NeuTemt cells were incubated with recombinant PDGF-B or HMVEC-conditioned media. Cells were lysed and immunoblotted with indicated antibodies.

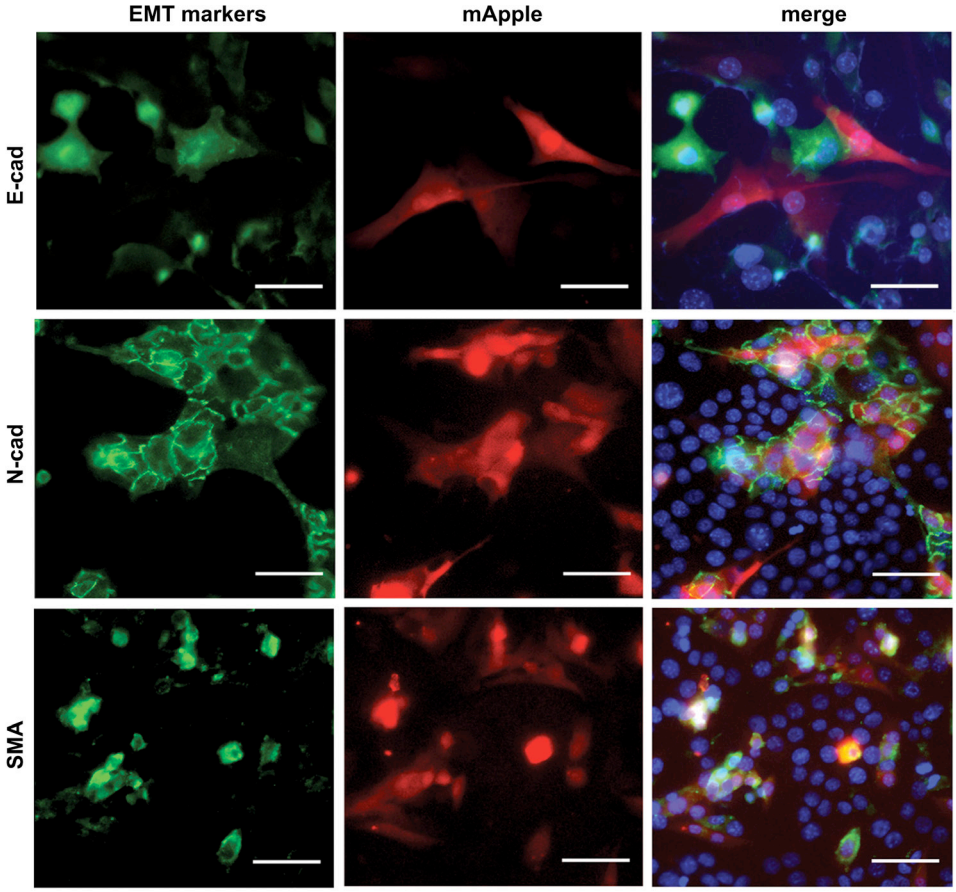
(B) Depletion of PDGFRβ and N-cadherin in Hs578T and DCIS-Snail-ER cells with lentiviral shRNAs. Knockdown efficiency was verified by quantitative RT-PCR. Error bars represent S.D. (n=3). Hs578T cells with two independent shRNAs were combined.

(C) Expression of indicated EMT and pericyte markers in Hs578T cells depleted of PDGFRβ or N-cadherin. Error bars represent S.D. (n=3).

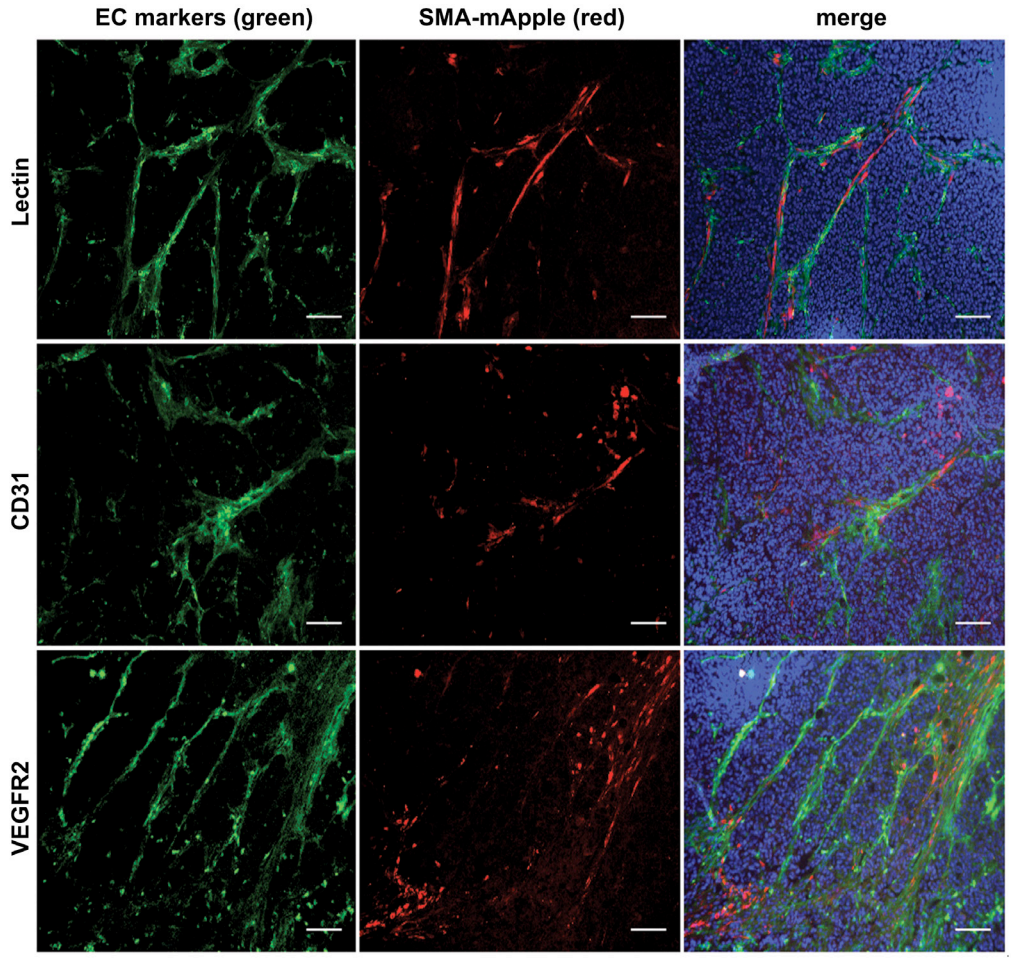
(D) PDGFRβ is required for chemotaxis of mesenchymal cancer cells to ECs. In the Transwell, HMVECs were plated on the bottom chamber, whereas control and PDGFRβ-depleted Hs578T cells were added to the upper chamber. After 48 hrs, migrated Hs578T cells were stained and counted. Error bars represent S.D. (n=4). Statistical differences were determined by student t-test (2-tailed).



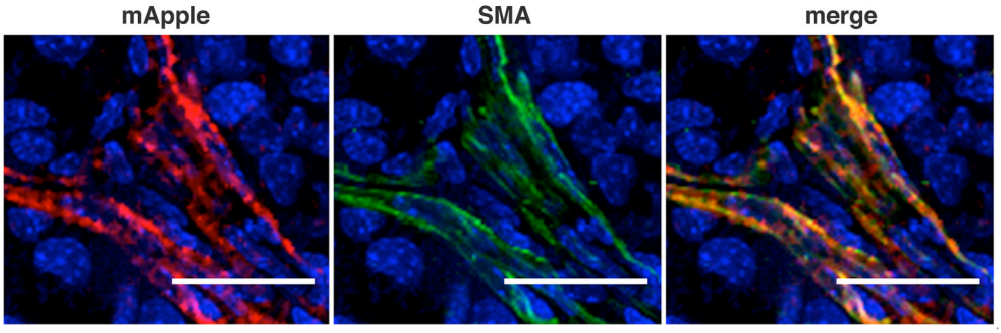
Supplementary Figure 13. The SMA-mApple reporter can detect EMT cells.
(A) Epithelial neuT and mesenchymal neuTemt tumor cells were infected with high-titer lentiviruses carrying the SMA-mApple reporter. Some infected neuT cells were subsequently treated with TGFβ for 5 days to induce EMT. Expression of mApple (red) was monitored by fluorescence microscopy.
(B) A549 epithelial cancer cells were transduced with lentiviral SMA-mApple and treated with or without TGFβ for 4 days.



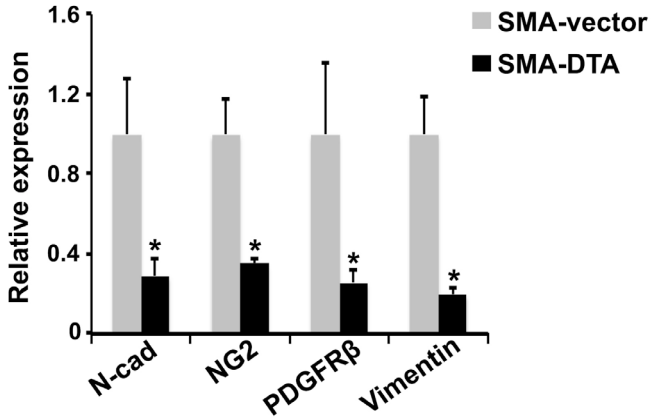
Supplementary Figure 14. The SMA-mApple reporter marks neuT tumor cells that undergo EMT.
NeuT epithelial tumor cells were infected with the lentiviral SMA-mApple reporter. Following prolonged culture, a subset of infected cells expressed mApple (red). Cells were immunostained with anti-E-cadherin (top), anti-N-cadherin (mid), and anti-SMA (bottom) antibodies. mApple-expressing cells are negative for E-cadherin and positive for N-cadherin and SMA. Scale bar: 50μm.



Supplementary Figure 15. Spontaneous EMT cancer cells are closely associated with blood vessels in tumor xenografts.
Lower magnification images (corresponding to Figure 6A) showing tight association of mApple-expressing neuT cells (red) with vascular endothelium (green). Endothelial cells were stained with anti-CD31 and anti-VEGFR2 antibodies. For lectin staining, tumor-bearing mice with intravenously injected with fluorescent lectin before tumors were harvested. Scale bar: 100μm.



Supplementary Figure 16. Spontaneous EMT cancer cells in tumor xenografts express pericyte marker SMA.
Higher magnification confocal images (corresponding to Figure 6D) showing overlapping of mApple and SMA. Sections of tumor xenografts derived from SMA-mApple neuT cells were stained anti-SMA antibody to mark pericytes (green). Scale bar: 25μm.



Supplementary Figure 17. Elimination of spontaneous EMT cancer cells reduces the amount of pericytes in tumor xenografts.
Quantitative RT-PCR analysis showing expression of indicated pericyte markers in RNA samples extracted from tumor xenografts generated by control or SMA-DTA neuT cells (corresponding to Figure 7A). Error bars represent S.D. (n=4). *p < 0.05. Statistical differences were determined by student t-test (2-tailed).

References:

1. Jahn SC et al. An in vivo model of epithelial to mesenchymal transition reveals a mitogenic switch.. *Cancer letters* 2012;326(2):183–90.
2. Hosaka K et al. Tumour PDGF-BB expression levels determine dual effects of anti-PDGF drugs on vascular remodelling and metastasis.. *Nature communications* 2013;4:2129.