SUPPLEMENTAL DATA:

SUPPLEMTARY FIGURE LEGENDS

FIGURES S1-S6

TABLES S1-S4

Heterogeneity of perivascular coverage in breast cancer is coordinated by Angiopoietin-2 and impacts metastasis and response to chemotherapy

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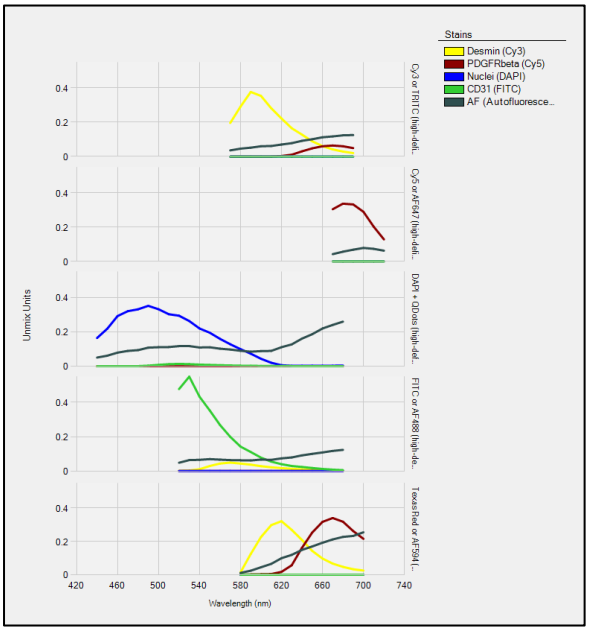
Supplementary Figure 1

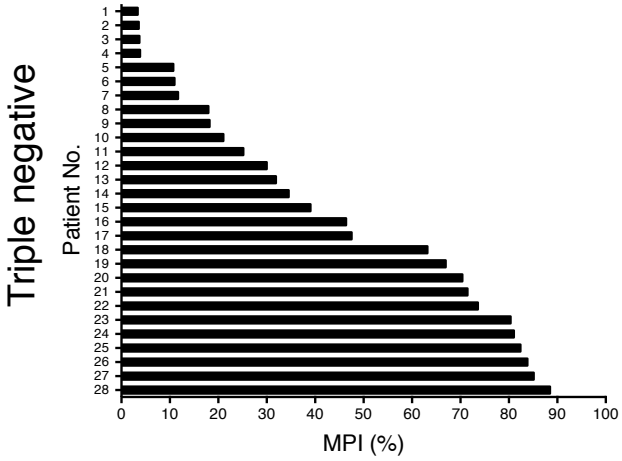
A



B

	Phenotype	Markers	Description
●	Blood vessels	CD31⁺	Endothelial tubular shape
●	Pericyte (P⁺D⁻)	PDGFRβ⁺Desmin⁻	Perivascular mesenchymal cells
●	Pericyte (P⁻D⁺)	PDGFRβ⁻Desmin⁺	Perivascular mesenchymal cells
●	Pericyte (P⁺D⁺)	PDGFRβ⁺Desmin⁺	Perivascular mesenchymal cells
●	Other	CD31-PDGFRβ⁻ Desmin⁻	Negative for all vascular and perivascular markers

C



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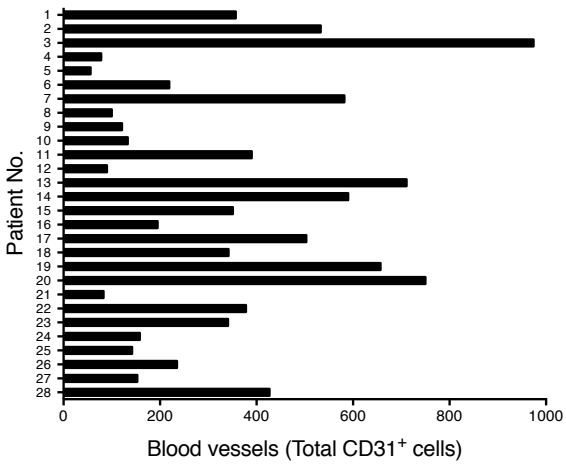

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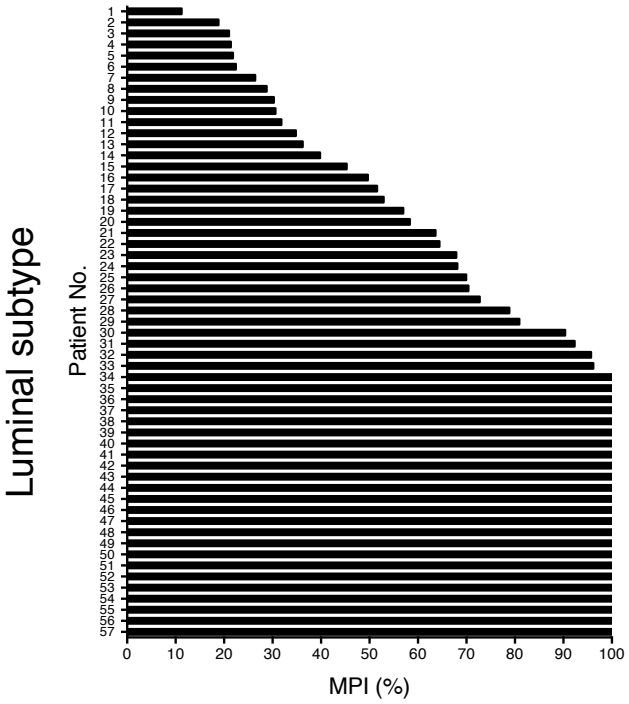

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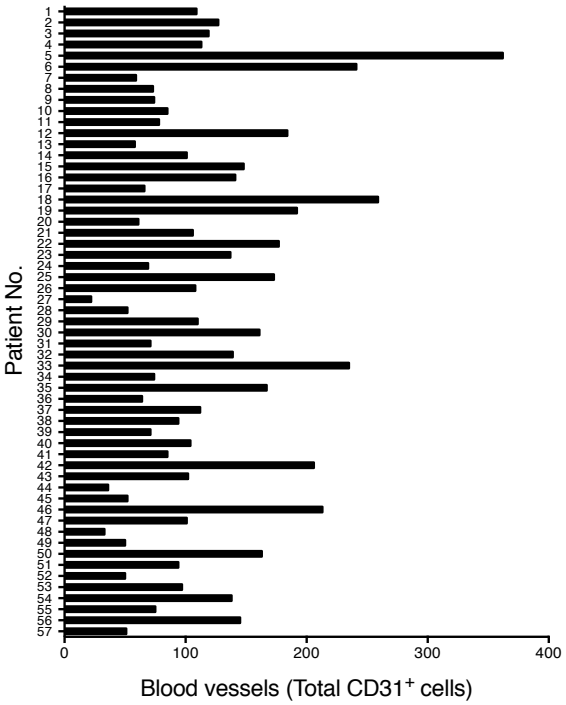
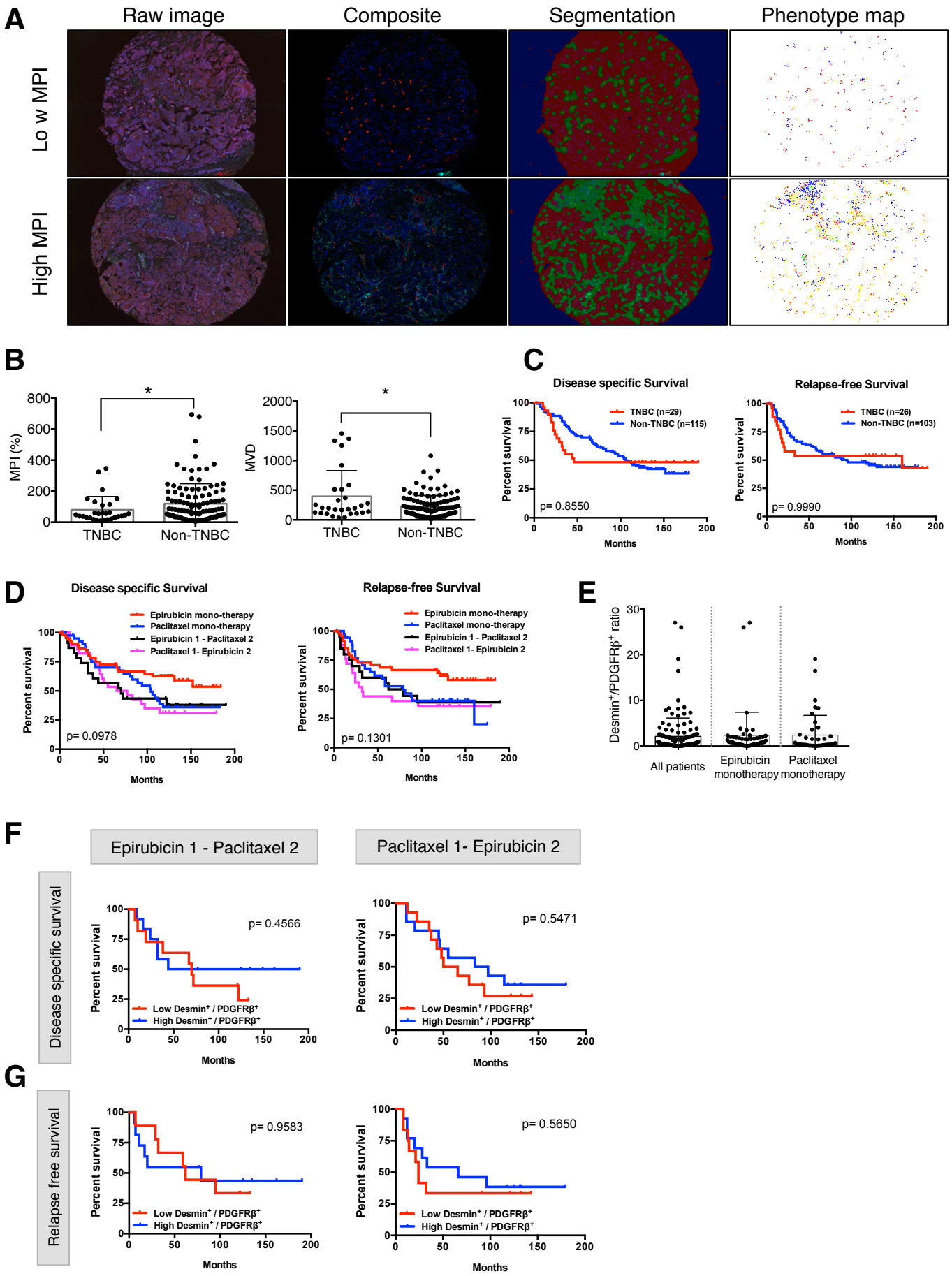


Supplementary Figure Legends:

Supplementary Figure 1. Overview of the spectral unmixing and phenotyping analysis of multiplex stained tissues and individual patient distribution of pericyte coverage across different breast cancer subtypes in patient cohort 1

A. Emission spectrum of all 5 markers obtained using the different filter cubes within the Vectra imaging system and used for spectral unmixing of multispectral images. **B**. Phenotype table identifying all cell phenotypes of interest based on the individual expression of CD31, Desmin and/or PDGFRβ. **C-F.** MPI (microvessels pericyte coverage index, %) (**C** and **E**) and total $CD31^+$ cells (**D** and **F**) for individual patient with either TNBC (**C** and **D**) or Luminal breast cancer (**E** and **F**). TNBC, n=28; Luminal, n=57.

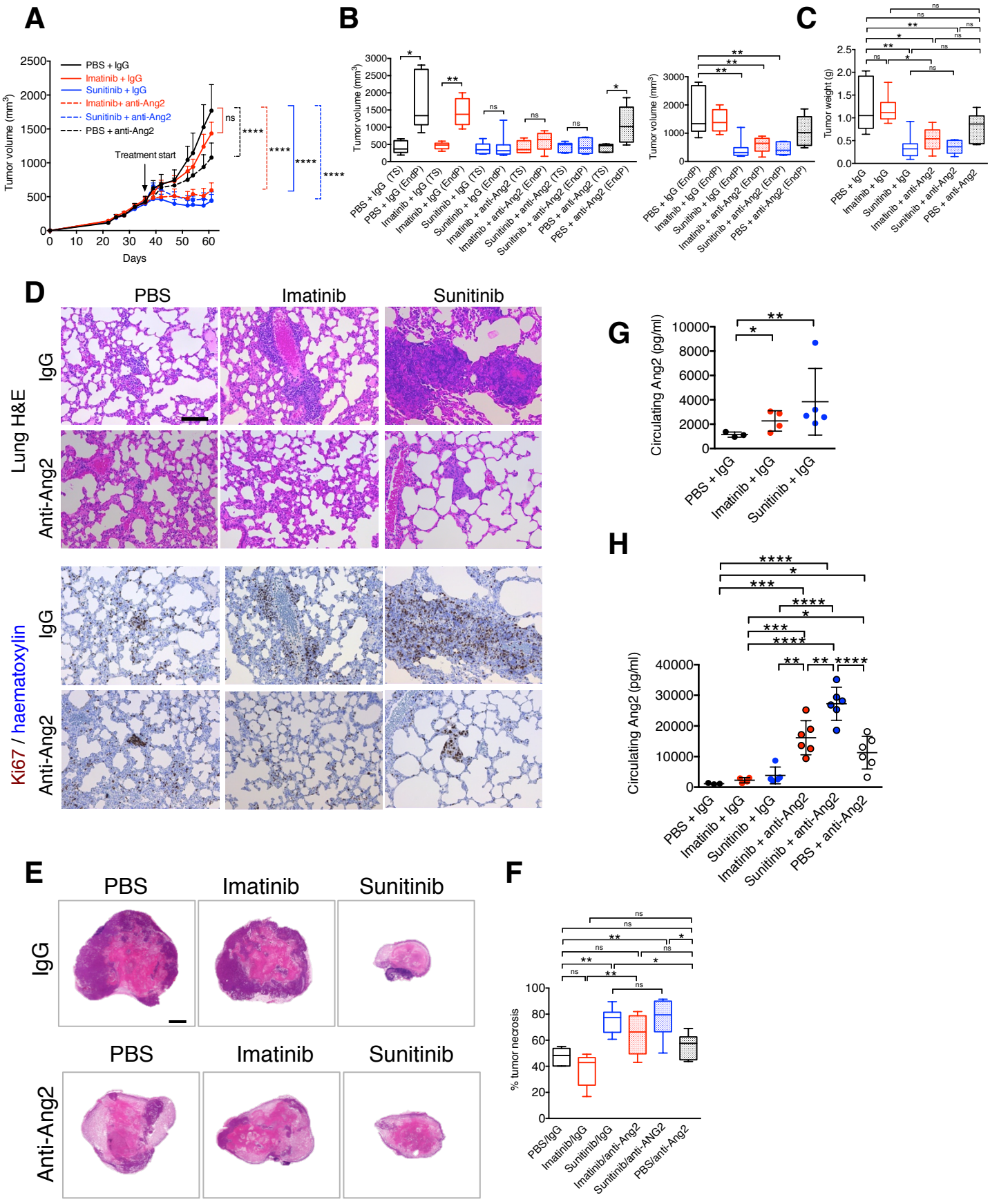
Supplementary Figure 2



Supplementary Figure 2. Differential microvascular and pericyte distribution and survival analyses of subgroups of patient in cohort 2

A. Stitched high power (200x) images showing representative TMA tumor cores with differential pericytes coverage. Images displayed are raw, composite, perivascular segmentation and the cellular phenotype map displaying all previously defined cell populations ($CD31^+$ endothelial cells - red, $PDGFR\beta^-$ $Desmin^+$ pericytes - green, $PDGFR\beta^+$ $Desmin^-$ pericytes - yellow, $PDGFR\beta^+$ $Desmin^+$ pericytes - orange, and other; blue). **B.** Quantification of MPI and MVD in indicated patient groups. TNBC, n=29; non-TNBC, n=115. Data is represented as the mean +/- S.D. Unpaired two-tailed t-test was used to determine statistical significance. * $p < 0.05$. **C.** Disease-specific survival and relapse-free survival analysis of all patients based on breast cancer type (TNBC and non-TNBC). **D.** Disease-specific survival and relapse-free survival analysis based on different neoadjuvant treatment groups. **E.** $PDGFR\beta^-$ $Desmin^+$ / $PDGFR\beta^+$ $Desmin^-$ ratio distribution for all patient samples assessed and epirubicin and paclitaxel monotherapy groups (Table S3). **F-G.** Disease-specific survival (F) and relapse-free survival (G) analysis of patients in the epirubicin1-paclitaxel2 and paclitaxel1-epirubicin2 groups based on $PDGFR\beta^-$ $Desmin^+$ / $PDGFR\beta^+$ $Desmin^-$ pericytes ratio. High and low values were defined based on the median ratio values. Disease-specific survival analysis, epirubicin 1 - paclitaxel 2, n=23; paclitaxel 1- epirubicin 2, n=28. For relapse-free survival analysis, epirubicin 1 - paclitaxel 2, n=20; paclitaxel 1- epirubicin 2, n=25. Unless otherwise indicated, Log-rank test was used to determine statistical significance.

Supplementary Figure 3

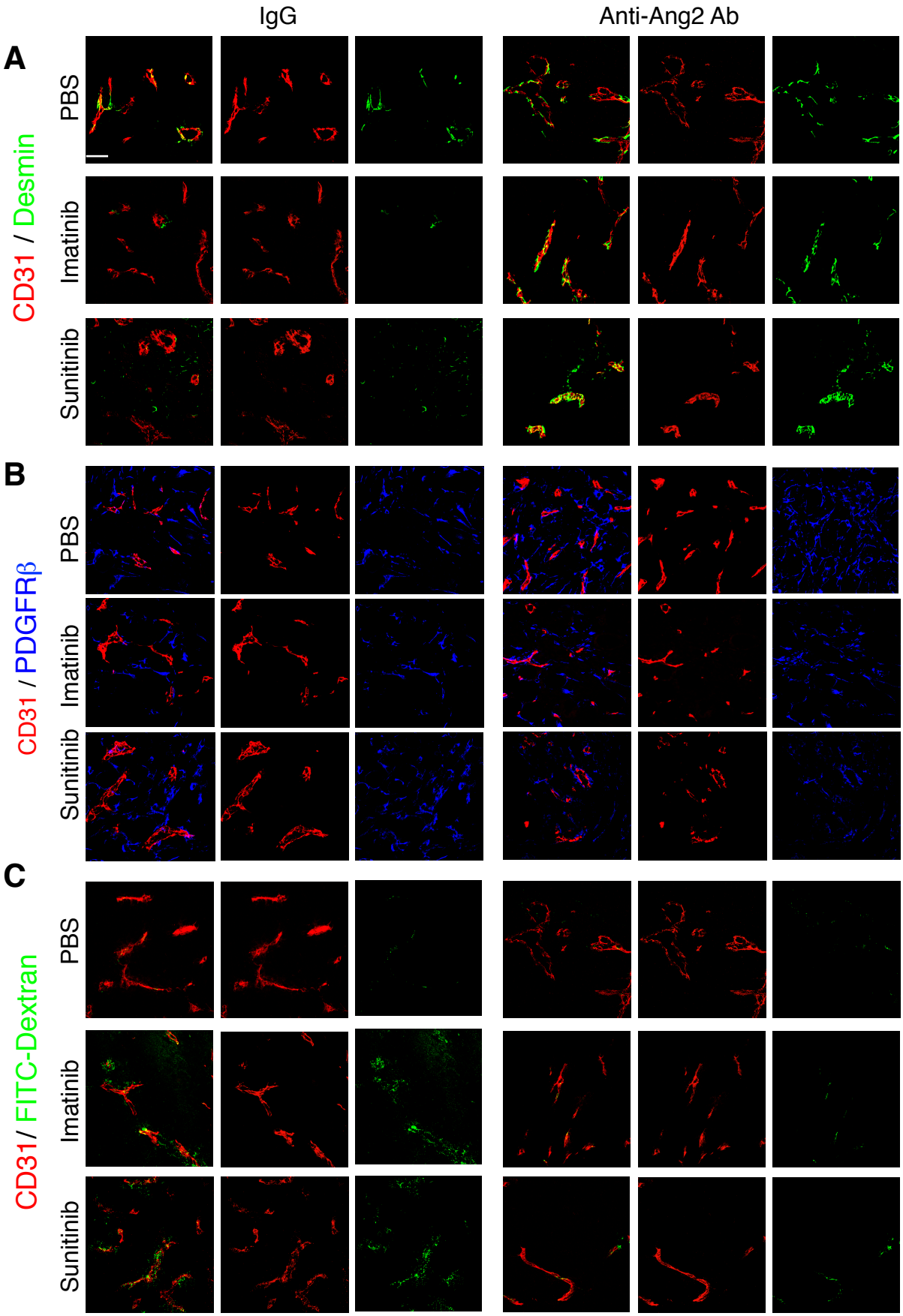


Supplementary Figure 3. Combinatorial treatment of imatinib / sunitinib and Anti-Ang2 antibody synergistically suppresses tumor growth and lung metastasis

A. Tumor volume measurements of orthotopically implanted MDA-MB-231 tumors over time in experimental groups (IgG: isotype control for anti-Ang2 Ab treatment, PBS: control for imatinib or sunitinib). Treatments started when average tumor burden reached 500 mm^3. Statistical significance was determined by two-way ANOVA with Sidak's multiple comparison test. **B.** Tumor volumes at treatments start (TS) and experimental end point (EndP) within indicated experimental groups. Statistical significance was determined by unpaired two-tailed t-test. IgG + PBS, n=5; IgG + imatinib, n=6; anti-ANG2 + imatinib, n=6; IgG + sunitinib, n=7; anti-ANG2 + sunitinib, n=6; anti-ANG2 + PBS, n=6. **C.** Quantification of tumor weights at experimental endpoint in the indicated experimental groups. IgG + PBS, n=5; IgG + imatinib, n=6; anti-ANG2 + imatinib, n=6; anti-ANG2 + PBS, n=6; IgG + sunitinib, n=7; anti-ANG2 + sunitinib, n=6. **D.** H&E staining and Ki67 immunostaining on consecutive FFPE sections of lungs from the indicated experimental groups to show lung metastasis. Scale bar: 100 μm. **E.** Representative images of total tumors from the indicated groups stained with H&E, depicting tumor necrosis. **F**. Quantification of the percentage of tumor necrotic area. IgG + PBS, n=5; IgG + imatinib, n=5; anti-ANG2 + imatinib, n=6; anti-ANG2 + PBS, n=6; IgG + sunitinib, n=7; anti-ANG2 + sunitinib, n=6. **G.** Quantification of plasma circulating Ang2 protein levels in the indicated experimental groups. IgG + PBS, n=3; IgG + imatinib, n=4; IgG + sunitinib, n=5. Unpaired two-tailed t-test was used to determine statistical significance. **H.** Quantification of circulating plasma Ang2 protein levels in the indicated experimental groups. IgG + PBS, n=3; IgG + imatinib, n=4; anti-

ANG2 + imatinib, n=6; anti-ANG2 + PBS, n=6; IgG + sunitinib, n=5; anti-ANG2 + sunitinib, n=6. Data is represented as the mean +/- S.D. Unless otherwise indicated, One-way ANOVA with Tukey's multiple comparison test was used to determine statistical significance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: not significant.

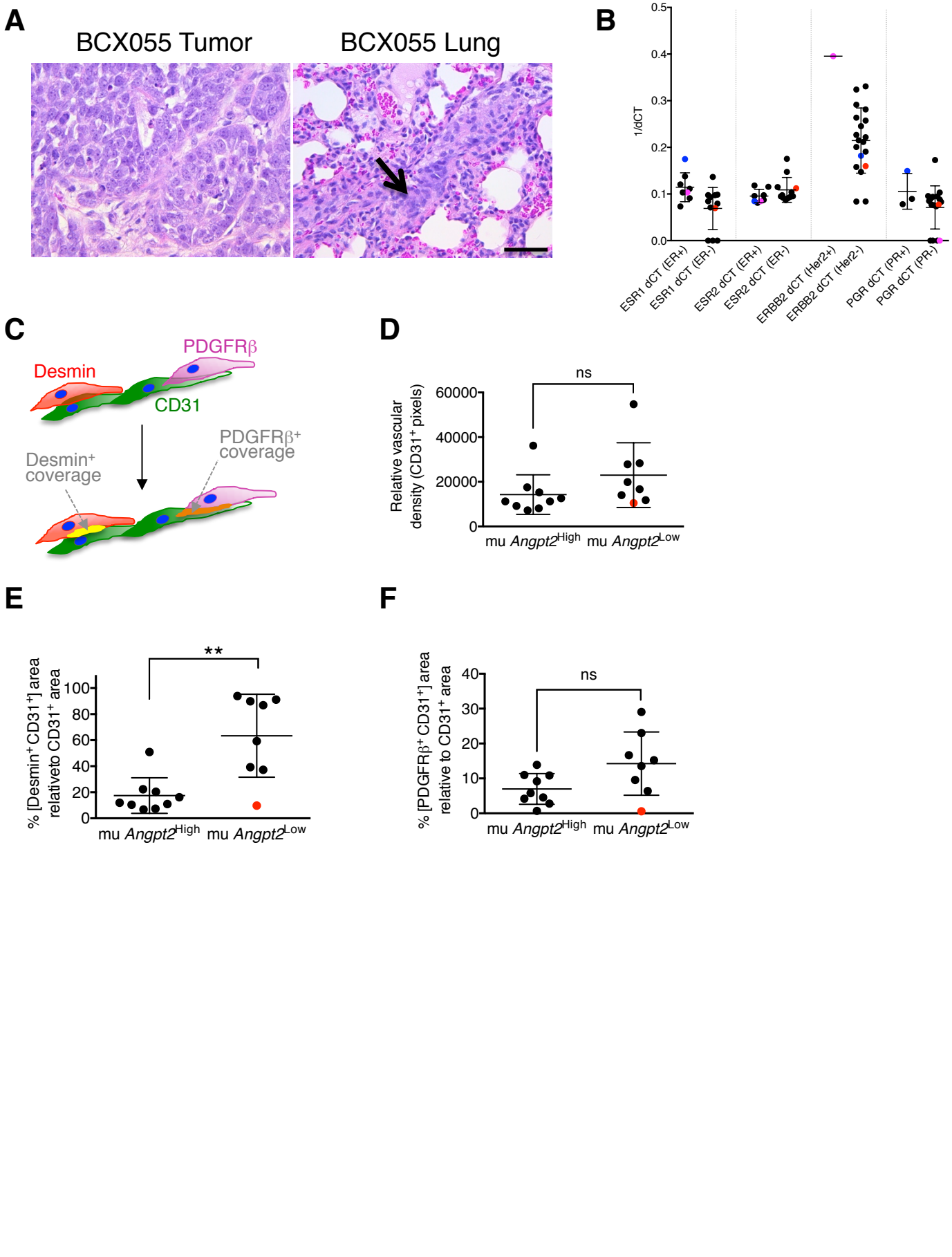
Supplementary Figure 4



Supplementary Figure 4. Representative images of individual channels for differential pericyte coverage and vascular leakage levels in MDA-MB-231 tumor xenografts

A-C. Immunostaining of CD31/Desmin (A) and CD31/PDGFRβ (B) as well as visualization of perfused 2,000 kDa FITC-dextran (C) associated to CD31 immunostaining on MDA-MB-231 FFPE tumor sections in the indicated experimental groups. Separate channel images are provided. Scale bar: 50μm.

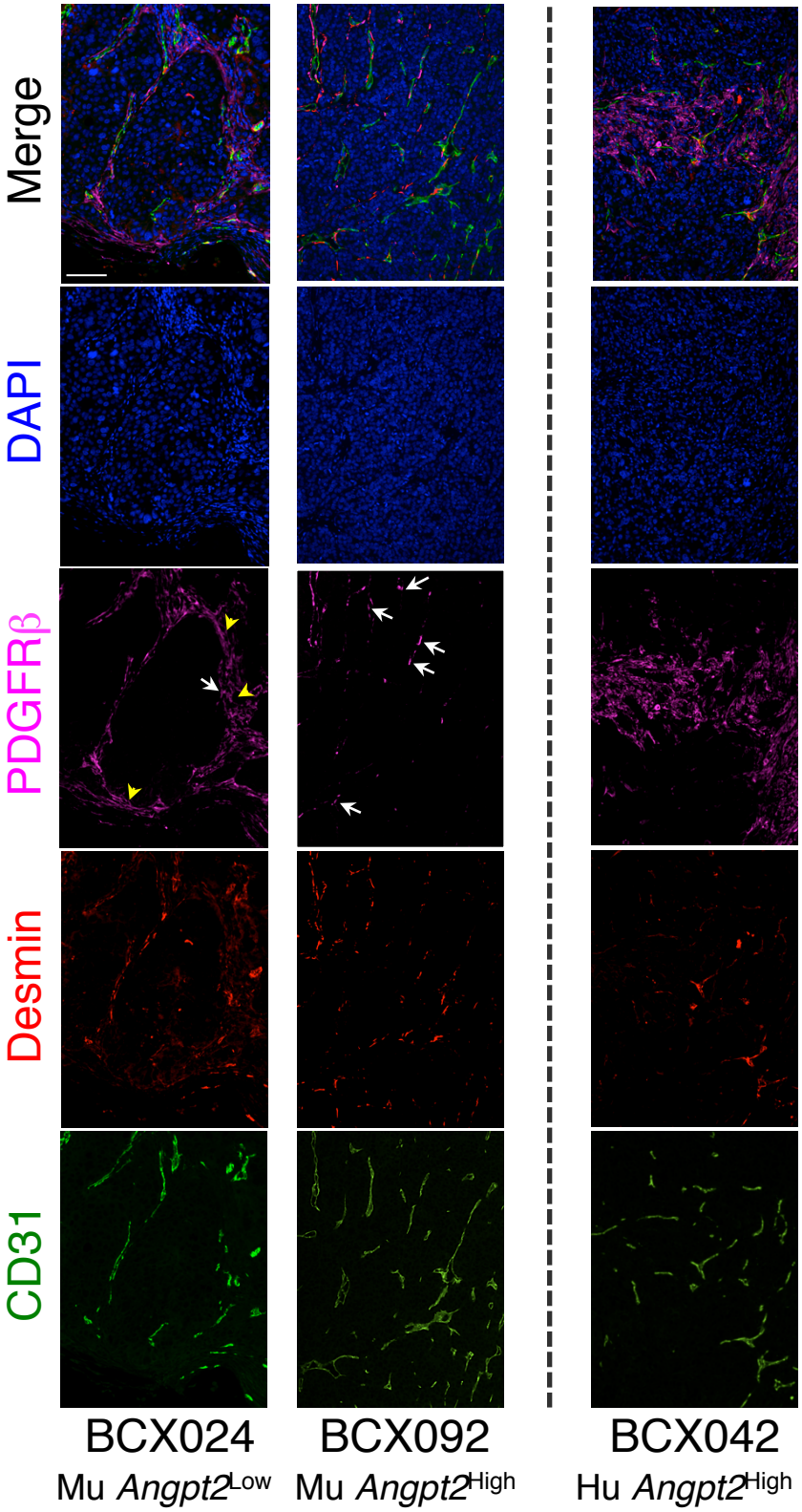
Supplementary Figure 5



Supplementary Figure 5. Characterization of patient-derived xenograft (PDX) models of breast cancer

A. Representative images of H&E stained PDX tumor and lung sections. Note lung section showing micrometastasis. Scale bar: 50 μm. **B.** Analysis of transcript levels of ESR1, ESR2, ERBB2 and PGR in each PDX model. Data is represented as 1/dCT. For each gene, values are split based on different primary tumor receptor status (ER^+ or ER^-; $Her2^+$ or $Her2^-$; PGR^+ or PGR^-). Transcripts from MCF7 (Blue dot; $ESR1^+$; $ESR2^-$; $ERBB2^-$; PGR^+), SKBR3 (Pink dot; $ESR1^-$; $ESR2^-$; $ERBB2^+$; PGR^-) and MDA-MB-231 (Red dot; $ESR1^-$; $ESR2^-$; $ERBB2^-$; PGR^-) cells are used as controls to determine positivity for each gene. **C.** Illustration of pericyte covered tumor vessels depicting co-localization of CD31 (endothelial cells) and pericytes markers (Desmin and PDGFRβ). **D.** Quantification of relative vascular density ($CD31^+$ area) in different BCX models according to *Angpt2* expression levels. **E.** Quantification of relative percentage of $Desmin^+$ pericytes associated with $CD31^+$ vessels in different BCX models according to *Angpt2* expression levels. **F.** Quantification of relative percentage of $PDGFR\beta^+$ pericytes associated with $CD31^+$ vessels in different BCX models according to *Angpt2* expression levels. The red dot in **D-F** identifies BCX042. Experimental n numbers were as follows, Mu $Angpt2^{High}$, n=9; Mu $Angpt2^{Low}$, n=8. Data is represented as the mean +/- S.D. Unpaired two-tailed t-test was used to determine statistical significance. ** $p < 0.01$. ns : not significant, ns: not significant.

Supplementary Figure 6



Supplementary Figure 6. Representative images of differential pericyte and vascular coverage levels in different PDX breast cancer models

Representative images of tumors immunolabeled for CD31, Desmin and PDGFRβ in indicated PDX models. Separate channel images are provided. White arrows indicate $PDGFR\beta^{+}Desmin^{-}$ pericytes. Yellow arrowheads indicate $PDGFR\beta^{+}$ non-perivascular cells that were excluded from quantification. Note that BCX042 tumor expresses exceptionally high levels of cancer derived Hu *Angpt2*. Scale bar: 100 μm.

Table S1. Baseline demographics and clinical characteristics of cohort 1

	TNBC	Luminal
	n=28	n=57
Median age at diagnosis		
	48 (range 32-73)	54 (range 31-88)
Tumor size		
< 2.0	12	28
2.0 to < 5.0	10	24
> or = 5.0	4	4
N/A	2	1
Stage		
I	0	21
II	0	11
III	0	2
IA	10	0
IIA	8	10
IIB	4	3
IIIA	1	1
IIIB	5	2
N/A	0	7
Lymph node		
N0	20	32
N1	6	18
N2	2	1
N/A	0	6
Tumor size		
T1	10	30
T2	11	19
T3	2	3
T4	5	2
N/A	0	3
Distant metastasis		
M0	28	49
M1	0	1
N/A	0	7
Histological grade		
1	1	7
2	5	32
3	20	17
N/A	2	1
Relapse		
0	21	48
1	7	9
Disease specific death		
0	25	52
1	2	5
N/A	1	0
Neoadjuvant chemotherapy		
0	23	46
1	5	1
N/A	0	10
Adjuvant chemotherapy		
0	7	21
1	21	35
N/A	0	1
Radiation therapy		
0	8	23
1	20	33
N/A	0	1

Table S1. Baseline demographics and clinical characteristics of cohort 1

Patient demographic and clinical characteristics, TNBC: triple negative breast cancer. Histological grade: 1, low grade/well differentiated; 2, intermediate/moderately differentiated; 3, high grade/poorly differentiated. Relapse, Disease specific death, neoadjuvant chemotherapy, Adjuvant chemotherapy, Radiation therapy: 0, no; 1, yes; N/A: not applicable/unknown.

Table S2. Baseline demographics and clinical characteristics of cohort 2

	Epirubicin mono therapy	Paclitaxel mono therapy	Epirubicin 1 - Paclitaxel 2	Paclitaxel 1- Epirubicin 2	All patients
	n=51	n=42	n=23	n=28	n=144
Median age at diagnosis(range)					
	53 (range 31-68)	52 (range 28-70)	51(range 29-68)	n=47 (range 31-66)	51 (range 28-70)
Response to first treatment					
CR	3	4	0	0	7
PR	36	31	0	0	67
SD	12	7	15	20	54
PD	0	0	8	8	16
Tumor size					
T1	0	0	1	0	1
T2	1	1	0	0	2
T3	45	30	17	23	115
T4	5	11	5	5	26
Lymph node involvement					
N0	20	16	8	9	53
N1	24	19	9	16	68
N2	6	7	5	3	21
N3	1	0	0	0	1
N/A	0	0	1	0	1
Distant metastasis					
M0	48	36	20	25	129
M1	3	6	3	3	15
Estrogen receptor					
Positive	24	27	11	17	79
Negative	27	15	12	10	64
N/A	0	0	0	1	1
Progesterone receptor					
Positive	24	23	10	10	67
Negative	27	18	13	17	75
N/A	0	1	0	1	2
HER2					
Positive	13	10	7	9	39
Negative	28	25	12	10	75
N/A	10	7	4	9	30
TNBC(ER/PGR/HER2 negative)					
	12	7	6	4	29
TP53 mutations					
Mutant	12	9	8	6	35
Wild-type	39	33	15	22	109

Table S2. Baseline demographics and clinical characteristics of cohort 2

Patient demographic and clinical characteristics. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. N/A: not applicable/unknown.

Table S3. Baseline demographics and clinical characteristics of sub-groups within cohort 2

Neoadjuvant therapy	Epirubicin monotherapy		Paclitaxel monotherapy		Epirubicin 1 - Paclitaxel 2		Paclitaxel 1 - Epirubicin 2		All patients	
Desmin+/PDGFRβ+ ratio	Low	High	Low	High	Low	High	Low	High	Low	High
	n=25	n=26	n=21	n=21	n=11	n=12	n=14	n=14	n=72	n=72
Median age at diagnosis(range)										
	52(38-68)	55(31-68)	49(28-67)	53(38-70)	54(31-67)	49(44-68)	48(33-66)	46(31-57)	51(28-70)	52(31-70)
Response to first treatment										
CR	0	3	1	3	0	0	0	0	1	6
PR	17	19	18	13	0	0	0	0	37	30
SD	8	4	2	5	7	8	10	10	26	28
PD	0	0	0	0	4	4	4	4	8	8
Tumor size										
T1	0	0	0	0	0	1	0	0	0	1
T2	0	1	0	1	0	0	0	0	0	2
T3	23	22	16	14	8	9	11	12	59	56
T4	2	3	5	6	3	2	3	2	13	13
Lymph node involvement										
N0	11	9	8	8	4	4	3	6	29	24
N1	12	12	12	7	6	3	9	7	36	32
N2	1	5	1	6	1	4	2	1	6	15
N3	1	0	0	0	0	0	0	0	1	0
N/A	0	0	0	0	0	1	0	0	0	1
Distant metastasis										
M0	23	25	18	18	9	11	12	13	63	66
M1	2	1	3	3	2	1	2	1	9	6
Estrogen receptor										
Positive	10	14	14	13	6	5	7	10	39	40
Negative	15	12	7	8	5	7	6	4	32	32
N/A	0	0	0	0	0	0	1	0	1	0
Progesterone receptor										
Positive	10	14	12	11	5	5	4	6	31	36
Negative	15	12	8	10	6	7	9	8	39	36
N/A	0	0	1	0	0	0	1	0	2	0
HER2										
Positive	6	7	5	5	3	4	6	3	21	18
Negative	13	15	13	12	7	5	5	5	36	39
N/A	6	4	3	4	1	3	3	6	15	15
TNBC(ER/PGR/HER2 negative)										
	6	6	2	5	2	4	2	2	11	18
TP53 mutations										
Mutant	5	7	2	7	5	3	3	3	16	19
Wild-type	20	19	19	14	6	9	11	11	56	53

Table S3. Baseline demographics and clinical characteristics of sub-groups within cohort 2

Patient demographic and clinical characteristics. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. N/A: not applicable/unknown. Patients were segregated into the following sub-groups: epirubicin monotherapy (n=51), paclitaxel monotherapy (n=42), epirubicin 1 - paclitaxel 2 (epirubicin, followed by paclitaxel; n=23), paclitaxel 1- epirubicin 2 (paclitaxel, followed by epirubicin; n=28).

Table S4. Clinical characteristics of patients (left) and histophathological analysis of patient derived xenografts (right).

Patient	BCX #	Race	Histology	Stage at Dx	ER	PR	HER2	HER2 FISH	KI67	BCX #	Passage #	Tumor Weight (g)	Lung metastasis (Y/N/S)	Hypoxyprobe	Desmin+ coverage	PDGFRβ+ coverage	Vascular Density	mo Angpt2 (dCt)	Hu Angpt2 (dCt)
Patient 1	6	Black	Ductal	IIIB	0	0	0	NA	NA	BCX006 (1)	P36	NR	N						
										BCX006 (2)	P36	1.50	N						
										BCX006 (3)	P36	1.10	N	35.52726477	11.97%	4.54%	7875.59375	0.2663460	10.1075096
Patient 2	10	White	Metaplastic Spindle	IV	0	0	0	1.1	80	BCX010 (2)	P55	1.90	N	18.54694249	7.39%	5.82%	11204.06	0.9074020	11.4350452
										BCX010 (3)	P55	1.20	N	16.81299819	41.26%	2.53%		-0.1210938	11.7031002
Patient 3	11	Black	Metaplastic	IIIA	0	0	0	NA	35	BCX011	P40	0.80	N	26.86286634	46.62%	6.77%	7724.978723	-0.8455544	13.7869034
										BCX011	P40	0.80	N	28.39817693	65.97%	20.99%	5408	-0.0963135	14.2656021
Patient 4	17	White	Ductal	IIB	25	0	1	4.12	50	BCX017	P20	0.59	Y	2.381922017	29.30%	6.79%	13246	0.2345772	11.4958553
										BCX017	P20	1.10	N	7.267274984	91.16%	39.21%	28348.61	0.8058739	10.9765911
Patient 5	22	Black	Cystic papillary Carcinoma	IIA	2	0	0	NA	75	BCX022	P20	1.70	Y		54.88%	8.94%	10555.18605	-0.0952530	11.2142353
										BCX022	P21	0.30	Y	23.61642402	16.22%	9.47%	4985.304348	-0.4286366	11.0269032
Patient 6	24	Asian	Ductal	IA	10	0	0	1.07	NA	BCX024 (1)	P17	0.90	N	6.456143959	95.13%	36.11%	32797.66667	0.3666611	UD
										BCX024 (2)	P17	0.90	N	7.213879033	92.83%	22.01%	23650.94444	0.5243073	11.5877323
Patient 7	42	White	Ductal	IIA	10	3	0	NA	50	BCX042	P9	NR	N	5.909260243	9.77%	0.58%	10482.625	1.4875526	1.1710567
Patient 8	51	Black	Ductal	IIA	0	0	0	NA	80	BCX051	P14	1.40	S	3.493418752	20.41%	10.97%	15273.5	-2.4764862	7.8169918
Patient 9	55	white	Ductal	IIA	0	0	0	NA	80	BCX055	P12	0.90	Y	3.943870996	3.50%	0.31%	3237.061224	-0.6089668	UD
										BCX055	P12	0.60	Y	2.868395478	17.29%	1.07%	14601.55556	-0.3597717	UD
Patient 10	70	BLack	Ductal	IIA	0	0	0	NA	90	BCX070	P11	NR	N	33.3934411	53.63%	12.16%	232849		
										BCX070	P11	1.00	N	35.8844175	48.44%	14.44%	9105.377778	0.4515724	12.2367744
										BCX070	P11	1.00	N	33.92165799	21.97%	12.27%	12123.94444	0.7607002	12.3241940
										BCX070	P12	1.80	N	7.582641619	24.90%	21.98%	8562.266667	0.3712311	11.8959141
Patient 11	80	Black	Ductal	IIB	0	0	1	NA	70	BCX080	P7	2.50	N	0.017294979	59.28%	13.56%	31897.36842	1.2849083	10.6739445
Patient 12	84	White	Ductal	IIA	10	0	1	NA	80	BCX084	P8	NR	N	20.25933386	11.23%	6.14%	4650.962243		
										BCX084 (1)	P8	2.50	N	11.73451036	10.58%	5.60%	5249.608696	-0.9630890	UD
Patient 13	92	White	Ductal	IA	2	0	1	NA	50	BCX092	P3	1.50	N	14.88966123					
										BCX092	P3	2.50	N	8.452650088	6.77%	2.79%	36167.55556	0.3638458	13.3749065
Patient 14	94	Black	DUctal	IIA	0	0	0	NA	70	BCX094	P7	NR	N	1.086136169	39.30%	16.64%	19888.68182	0.8276196	UD
Patient 15	100	Hispanic	Ductal (spindloid)	IIIB	20	1	0	NA	70	BCX100	P4	NR	N	7.187716284				0.4773979	9.5969734
										BCX100 (1)	P4	1.00	N	12.40126213	43.74%	5.48%	25745.13514	0.1979465	7.6808128
										BCX100 (2)	P4	0.40	N	5.917346433	89.92%	7.26%	14014.25	1.0477448	7.3940868
Patient 16	102	Black	Ductal	IIA	0	0	2+	1.09		BCX102	P3	0.80	N	7.885162865	86.83%	10.96%	11763	1.4707050	UD
										BCX102	P3	NR	S	8.669022915	63.82%	8.16%	6525.266667	1.0398598	12.6946354
Patient 17	105	White	Ductal	IIA	40	40	0	NA	80	BCX105	P2	0.80	N	23.7782772	38.08%	10.85%	29399.14642	-1.2432938	10.6454391

Y Yes
N No
NA Not applicable

mo Angpt2 > median dCt
mo Angpt2 < or = median dCt

Y Yes
N No
S Suspected
NR Not recorded

Table S4. Clinical characteristics of patients (left) and histophathological analysis of patient derived xenografts (right).

33 BCX from 17 patients were analyzed for murine (Mu) and human (Hu) *Angpt2* transcripts. The pink shaded cells indicates BCX with high mouse *Angpt2* transcript (Mu *Angpt2*^{High}) and the unshaded cells indicates BCX with low mouse *Angpt2* transcript (Mu *Angpt2*^{Low}) using the median dCt as a cutoff. The Ki67 column is expressed as percent positive tumor cells among the total number of tumor cells assessed. In the BCX # column, the numbers within parentheses reflect BCX from the same patients but harvested from distinct mice. Dx, diagnosis; NA, not applicable; UD, undetectable; Y, yes; N, no; S, suspected; NR, not recorded; empty cells, the analyses were not performed.