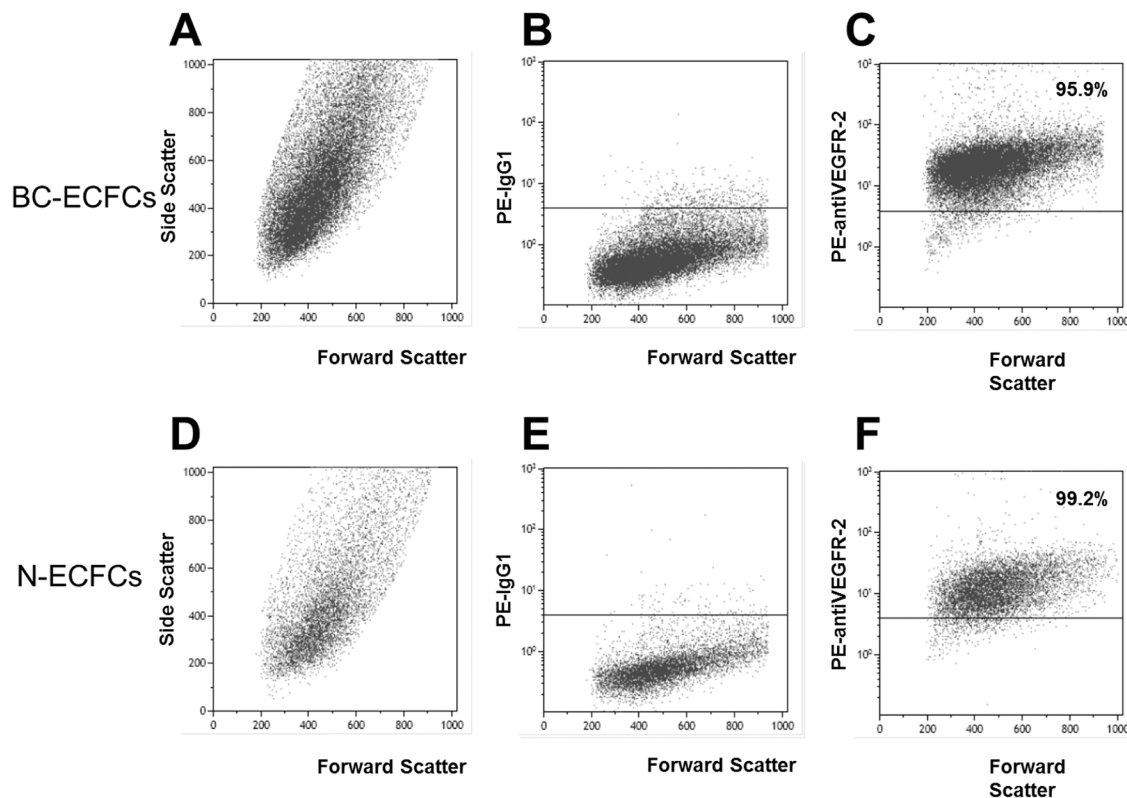
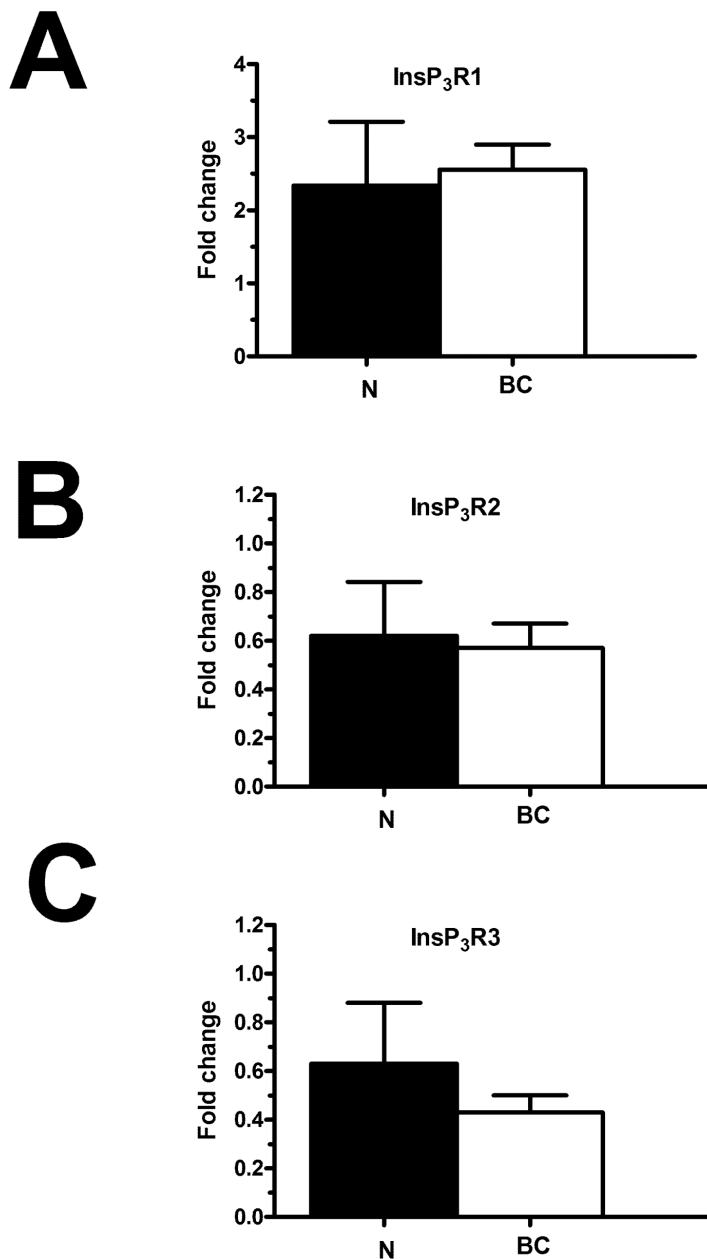


VEGF-induced intracellular Ca^{2+} oscillations are down-regulated and do not stimulate angiogenesis in breast cancer-derived endothelial colony forming cells

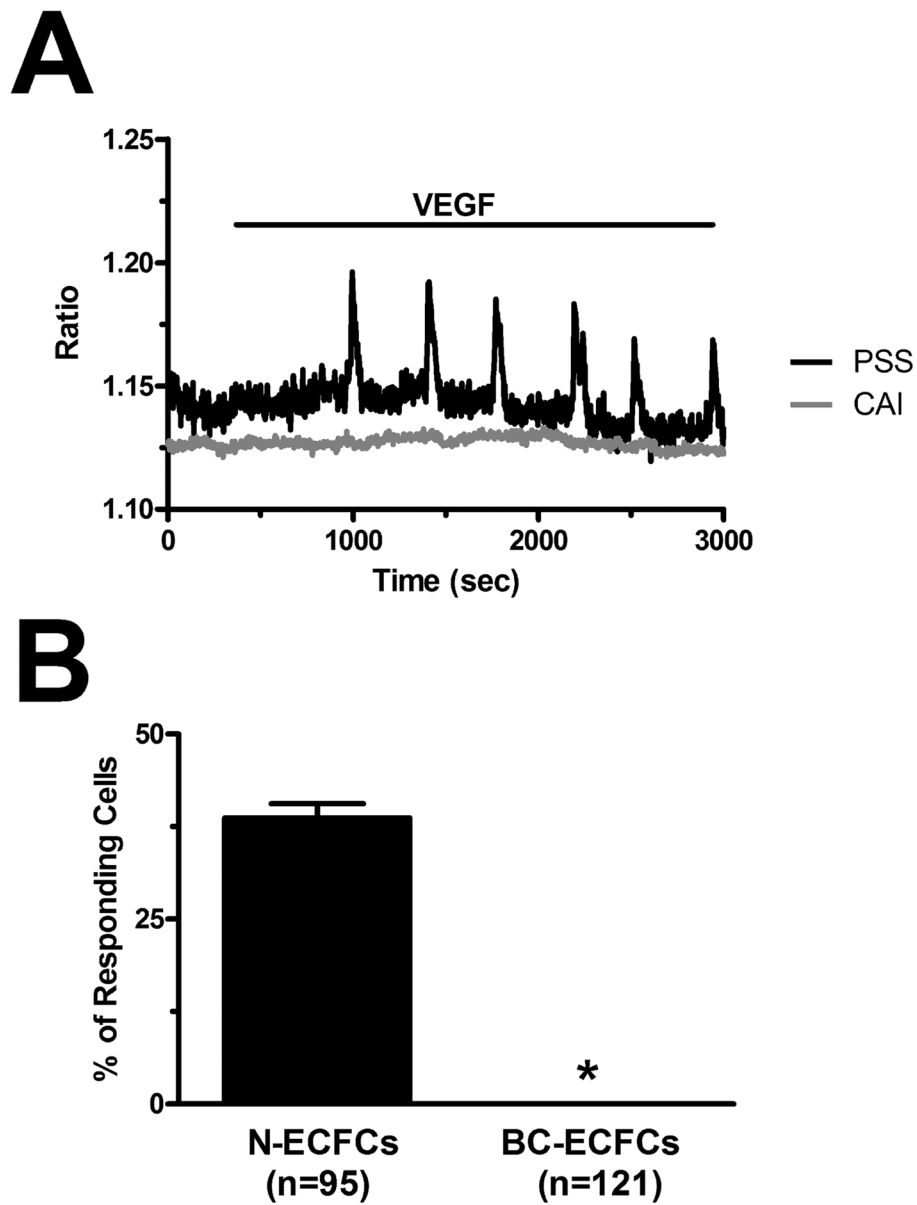
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Comparable expression of VEGFR-2 on normal and breast cancer-derived endothelial colony forming cells. Viable cultured ECFCs, as shown by the typical picture in the forward scatter/side scatter plots (A and D), stained with the PE-IgG1 isotype control (B and E) and with the Pe-anti-VEGFR-2 antibody (C and F).



Supplementary Figure 2: InsP₃ receptors are normally expressed in breast cancer-derived endothelial colony forming cells. There is no difference in the expression of InsP₃R1 (A), InsP₃R2 (B) and InsP₃R3 (C) between normal and breast cancer-derived ECFCs. mRNA levels were measured by RT-PCR relative to the β -actin internal standard (see Materials and Methods) and the values obtained were reported as Δ Ct. Bars represent the mean \pm SE of at least 4 different experiments each from different RNA extracts. *P<0.05 versus InsP₃R1 (1-way ANOVA followed by Newman-Keuls's Q test). The PCR products were of the expected size: InsP₃R1, 180 bp; InsP₃R2, 158 bp; InsP₃R3, 173 bp. The specific primers described in Supplementary Table 2 have been utilized to examine the expression levels of InsP₃R transcripts.



Supplementary Figure 3: CAI inhibits VEGF-induced intracellular Ca^{2+} oscillations in breast cancer-derived endothelial colony forming cells. (A), CAI (10 μM , 20 min) prevents VEGF-induced intracellular Ca^{2+} spikes in BC-ECFCs. (B), mean \pm SE of the percentage of VEGF-responding cells in the absence and the presence of CAI. The asterisk indicates $p < 0.05$.

Supplementary Table 1: Primer sequences used for real time reverse transcription/polymerase chain reaction of InsP₃ receptors

Gene	Primer sequences	Size (bp)	Accession number	
InsP ₃ R1	Forward	5'- TCAACAAACTGCACCACGCT -3'	180	ENSG00000150995
	Reverse	5'- CTCTCATGGCATTCTTCTCC -3'		
InsP ₃ R2	Forward	5'- ACCTTGGG GTTAGTGGATGA -3'	158	ENSG00000123104
	Reverse	5'- CCTTGTTTGGCTTGCTTTGC -3'		
InsP ₃ R3	Forward	5'- TGGCTTCATCAGCACTTTGG -3'	173	ENSG00000096433
	Reverse	5'- TGTCCTGCTTAGTCTGCTTG -3'		
β-actin	Hs_ACTB_1_SG, QuantiTect Primer Assay QT00095431, Qiagen		146	NM_001101

Supplementary Table 2: Primer sequences used for real time reverse transcription/polymerase chain reaction of TRPC1-7, Stim1-2 and Orai1-3

Gene	Primer sequences	Size (bp)	Accession number	
TRPC1	Forward	5'-ATCCTACACTGGTGGCAGAA-3'	307	NM_003304.4
	Reverse	5'-AACAAAGCAAAGCAGGTGCC-3'		
TRPC3	Forward	5'-GGAGATCTGGAATCAGCAGA-3'	336	NM_001130698.1 variant 1
	Reverse	5'-AAGCAGACCCAGGAAGATGA-3'		NM_003305.2 variant 2
TRPC4	Forward	5'-ACCTGGGACCTCTGCAAATA-3'	300	NM_016179.2 variant alpha
	Reverse	5'-ACATGGTGGCACCAACAAAC-3'		NM_001135955.1 variant beta NM_001135956.1 variant gamma NM_001135957.1 variant delta NM_003306.1 variant epsilon NM_001135958.1 variant zeta
TRPC5	Forward	5'-GAGATGACCACAGTGAAGAG-3'	221	NM_012471.2
	Reverse	5'-AGACAGCATGGGAAACAGGA-3'		
TRPC6	Forward	5'-AGCTGTTCCAGGGCCATAAA-3'	341	NM_004621.5
	Reverse	5'-AAGGAGTTCATAGCGGAGAC-3'		
TRPC7	Forward	5'-CACTTGTGGAACCTGCTAGA-3'	387	NM_020389.1
	Reverse	5'-CATCCCAATCATGAAGGCCA-3'		
Orai1	Forward	5'-AGTTACTCCGAGGTGATGAG-3'	257	NM_032790.3
	Reverse	5'-ATGCAGGTGCTGATCATGAG-3'		
Orai2	Forward	5'-CCATAAGGGCATGGATTACC-3'	334	NM_001126340.1 variant 1
	Reverse	5'-CAGGTTGTGGATGTTGCTCA-3'		NM_032831.2 variant 2
Orai3	Forward	5'-CCAAGCTCAAAGCTTCCAGCC-3'	159	NM_152288.2
	Reverse	5'-CAAAGAGGTGCACAGCCACCA-3'		
Stim1	Forward	5'-CCTCAGTATGAGGAGACCTT-3'	347	NM_003156.3
	Reverse	5'-TCCTGAAGGTCATGCAGACT-3'		
Stim2	Forward	5'-AAACACAGCCATCTGCACAG-3'	186	NM_020860.2
	Reverse	5'-GGGAAGTGTTCGTTCCCTTTGA-3'		
β -actin		Hs_ACTB_1_SG, QuantiTect Primer Assay QT00095431, Qiagen	146	NM_001101