

Content of Supplementary Tables

TS1 describes the characteristics of the cell lines used in this study, including hormone receptor status and type of breast cancer.

TS2 lists the antibodies used and the method by which immunohistochemistry stained breast cancer specimens were scored.

TS3 describes the levels of IL8, Jak2, P-Ja2, STAT3, P-STAT3 and monocyte macrophages (CD68, CD163) in normal breast tissue and IBC specimens.

TS4 lists the expression levels of 80 cytokines, chemokines and growth factors in the conditioned media from SUM149 cells. Data were obtained from at least three independent studies.

TS5 lists the sequences of siRNAs that were used for gene silencing in this study.

TS6 lists the primer sequences and the genes targeted for qPCR studies carried out.

Supplementary Table 1. Source, clinical, and pathological features of breast cancer cell lines used

Cell line	Subtype	ER	PR	HER2	Source	Tumor Type
MCF-7	Luminal	+	+	-	PE	Met AC
T47D	Luminal	+	+	-	PE	IDC
MDA-MB-231	Basal	-	-	-	PE	Met AC
BT549	Basal	-	-	++ ^M	P.Br	IDC, papillary
SUM149	Basal	-	-	-	PE	Inf
IBC-80	Basal	-	-	-	P.Br	Inf

Met AC=Metastatic adenocarcinoma; Inf= Inflammatory carcinoma; IDC= Invasive ductal carcinoma; PE= Pleural effusion; P.Br= Primary breast

Supplementary Table 2. IHC Antibodies and Scoring Methods

Antibody	Origin/Reference	Scoring		Ordinal or Continuous Variable
		Negative	Positive	
IL-8	BD Biosciences/554717	0	1+, 2+,3+	Ordinal
pJAK2	Abcam/ab32101	0	1+, 2+, 3+	Ordinal
pSTAT3	Cell Signaling/4113	0	1+, 2+	Ordinal
CD68	Dako/M0876	Scored as 0-150 cells/HPF		Continuous
CD163	NCL-CD163	Scored as 0-99 cells/HPF		Continuous

/HPF: per high power field 63X objective

Supplementary Table 3: Expression Levels in Tumor Tissue Compared to Matched Normal Breast Tissue

		IBC Cases (N=45)	Companion Normal Breast Controls (N=45)	P-Value
IL-8	0	8 (19.0%)	12 (33.3%)	<0.0001 [^]
	1+	8 (19.0%)	10 (27.8%)	
	2+	4 (9.5%)	8 (22.2%)	
	3+	22 (52.4%)	6 (16.7%)	
	No normal tissue		6	
	Analysis N/A	3	3	
pJAK2	0	2 (4.8%)	20 (60.6%)	<0.0001 [^]
	1+	21 (50.0%)	10 (30.3%)	
	2+	19 (45.2%)	2 (6.1%)	
	3+	0	1 (3.0%)	
	No normal tissue		9	
	Analysis N/A	3	3	
pSTAT3	0	18 (45.0%)	20 (66.7%)	0.0002 [^]
	1+	19 (47.5%)	4 (13.3%)	
	2+	3 (7.5%)	6 (20.0%)	
	No normal tissue		10	
	Analysis N/A	5	5	
CD68	Median (range)	40 (4-100)	15 (0-150)	<0.0001 ^{^^}
	No tumor available	5		
	No normal tissue		5	
CD163	Median (range)	28 (3-80)	12 (2-40)	0.0048 ^{^^}
	No Tumor Available	4		
	No normal tissue		4	

[^]Fisher's Exact Test

^{^^} Kruskal-Wallis non parametric analysis of variance

Supplementary Table 4. Relative expression of cytokines, chemokines and growth factors in serum-free SUM149 CM (from highest to lowest)

	Protein name	Relative expression		Protein name	Relative expression
1	GRO	2.926134559	41	IFN- γ	0.189073452
2	IL-8	1.865960201	42	LIGHT	0.182363621
3	GRO- α CXCL1	1.344020912	43	Ck β 8-1	0.178034375
4	TIMP-1	1.310561996	44	PARC	0.175683753
5	Angiogenin	0.984326088	45	BLC	0.172294406
6	NAP-2	0.951858411	46	EGF	0.169170522
7	Eotaxin-2	0.773934076	47	TGF-β3	0.165995298
8	IL-10	0.773139263	48	IL-6	0.155379211
9	TGF-β2	0.724478487	49	Osteoprotegerin	0.141164735
10	RANTES	0.665056538	50	MIP-1 δ	0.133031914
11	LIF	0.570961551	51	TGF-β1	0.132600163
12	GDNF	0.570961551	52	FGF-7	0.13180535
13	TIMP-2	0.567230518	53	FGF-4	0.128899094
14	IGFBP-2	0.537143427	54	Eotaxin-3	0.127146859
15	IGFBP-1	0.498748116	55	MCP-1	0.118313145
16	OSM	0.494346328	56	IGF-1	0.114881219
17	VEGF-A	0.451138681	57	NT-4	0.106573174
18	IP-10	0.429265889	58	PDGF-BB	0.105645717
19	MCSF	0.421927707	59	MIP-3 α	0.096709321
20	MIP-1 β	0.385985699	60	MDC	0.095957087
21	IL-3	0.379574975	61	IL-1 β	0.095534098
22	NT-3	0.370070329	62	SDF-1	0.088679181
23	MIF	0.364279195	63	SCF	0.088174713
24	HGF	0.344107297	64	ENA-78/CXCL5	0.074609966
25	IL-16	0.333747737	65	Fractalkine	0.072486082
26	PIGF	0.324388352	66	IGFBP-4	0.071208178
27	TARC	0.309041232	67	Flt-3-Ligand	0.059643431
28	BDNF	0.302733188	68	IL-5	0.059416867
29	GM-CSF	0.302177554	69	I-309	0.05754075
30	Eotaxin-1	0.298177378	70	IL-2	0.057168925
31	TNF- β	0.278727576	71	MIG	0.055963739
32	FGF-9	0.277078023	72	MCP-4	0.055036281
33	TNF- α	0.263884924	73	IL-12p40p70	0.054138962
34	IGFBP-3	0.250123752	74	Thrombopoietin	0.048954801
35	Leptin	0.241610345	75	MCP-3	0.044275109
36	IL-1 α	0.223263225	76	IL-4	0.043574215
37	FGF-6	0.210048924	77	IL-13	0.037368677
38	Osteopontin	0.209894903	78	IL-15	0.035265995
39	IL-7	0.209245349	79	MCP-2	0.034843006
40	GCP-2/CXCL6	0.207369232	80	GCSF	0.026184515

Cytokines, chemokines and growth factors known to play a role in EMT and or CSC-regulation are bolded

Supplementary Table 5. siRNA sequences

siRNA	sense	antisense
IL-8 siRNA-1	5'-AGAUGUCAGUGCAUAAAAGAtt-3'	5'-UCUUUAUGCACUGACAUCUaa-3'
IL-8 siRNA-2	5'-GAACUUAGAUGUCAGUGCAtt-3'	5'-UGCACUGACAUCUAAGUUCtt-3'
GRO- α siRNA-1	5'-CAGUCAUUAUGUJAAUJUtt-3'	5'-AAUJUUAACAUAAUGACUGga-3'
GRO- α siRNA-2	5'-AGUUCAAUCUGGAUUCAUAtt-3'	5'-UAUGAAUCCAGAUUGAACUaa-3'
GRO- β siRNA	5'-CAUCGCCCAUGGUUAAGAAtt-3'	5'-UUCUUAACCAUGGGCGAUGcg-3'
GRO- γ siRNA-1	5'-CCAACUGACAGGAGAGAAGUU -3'	5'-CUUCUCUCCUGU CAGUUGGUU -3'
GRO- γ siRNA-2	5'-CGAGGGUUCUACUUAUUUAtt-3'	5'-UAAAUAAGUAGAACCCUCGta-3'

Supplementary Table 6. qPCR primer sequences

Gene	Forward primer	Reverse primer
GRO- α	5'-AAGTGTGAACGTGAAGTCC-3'	5'-GGATTTGTCACTGTTTCAGCA-3'
GRO- β	5'-AAAGCTTGTCTCAACCCC-3'	5'-GGTCAGTTGGATTTGCCATTTT-3'
GRO- γ	5'-TCCCCCATGGTTCAGAAAATC-3'	5'-GGTGCTCCCCTTGTTTCAGTATCT-3'
IL-8	5'-ATGACTTCCAAGCTGGCCGTGGCT-3'	5'-TCTCAGCCCTCTTCAAAAATTCT-3'
IL-10	5'-TCAAACCTCACTCATGGCTTTGT-3'	5'-GCTGTCATCGATTCTTCCC-3'
IL-12p40	5'-CCCTGACATTCTGCGTTCA-3'	5'-AGGTCTTGTCCTGAAGACTCTA-3'
MMP2	5'-GTTGGCAGTGCAATACCTG-3'	5'-GGCAGTCCAAAGAACTTCTG-3'
MMP9	5'-GTACTCGACCTGTACCAGC-3'	5'-AACTGTATCCTTGGTCCGG-3'
TNF- α	5'-CCCAGGGACCTCTCTAATCA-3'	5'-AGCTGCCCTCAGCTTGAG-3'
VEGF-A	5'-CTACCTCCACCATGCCAAGT-3'	5'-CCATGAACCTCACCCTTCGT-3'
GAPDH	5'-TTAAAAGCAGCCCTGGTGAC-3'	5'-CTCTGCTCCTCCTGTTTCGAC-3'