

**Figure S1.** Flow-diagram illustrating the study design.

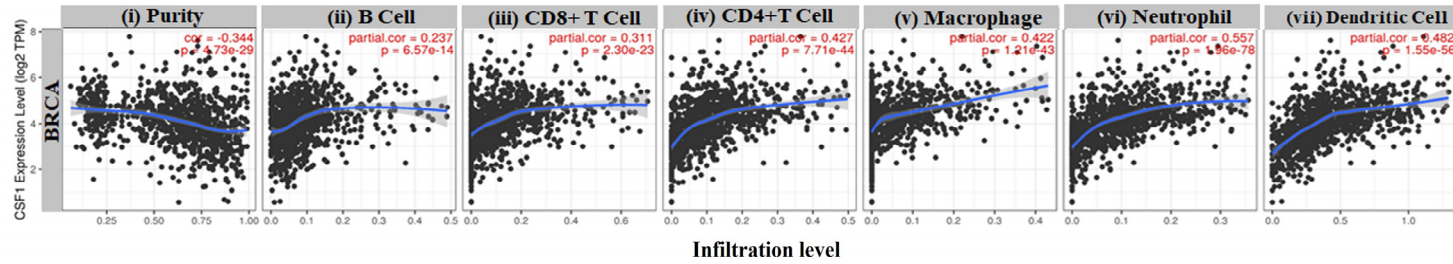
**Table S1.** Correlation of *CSF1R* mRNA with 37-gene tumor associated macrophage signature [1].

TAM signature genes	Purity-adjusted partial spearman's rho value	p-value
<i>IRF8</i>	0.686	3.75E-139
<i>CCL2</i>	0.396	1.06E-38
<i>C1QC</i>	0.746	1.97E-177
<i>GBP5</i>	0.486	2.73E-55
<i>HCST</i>	0.545	4.36E-78
<i>LILRB4</i>	0.719	6.06E-159
<i>AIF1</i>	0.790	4.25E-213
<i>PSMB9</i>	0.343	6.93E-29
<i>GBP4</i>	0.414	1.86E-42
<i>GBP1</i>	0.379	3.20E-35
<i>HLA-DOA</i>	0.725	5.10E-163
<i>C1QA</i>	0.664	1.50E-127
<i>CCL4</i>	0.526	7.25E-72
<i>NCF1C</i>	0.586	1.03E-92
<i>LAP3</i>	0.394	2.64E-38
<i>TNFAIP3</i>	0.501	2.21E-64
<i>ITGB2</i>	0.783	1.17E-206
<i>LAIR1</i>	0.792	8.87E-215

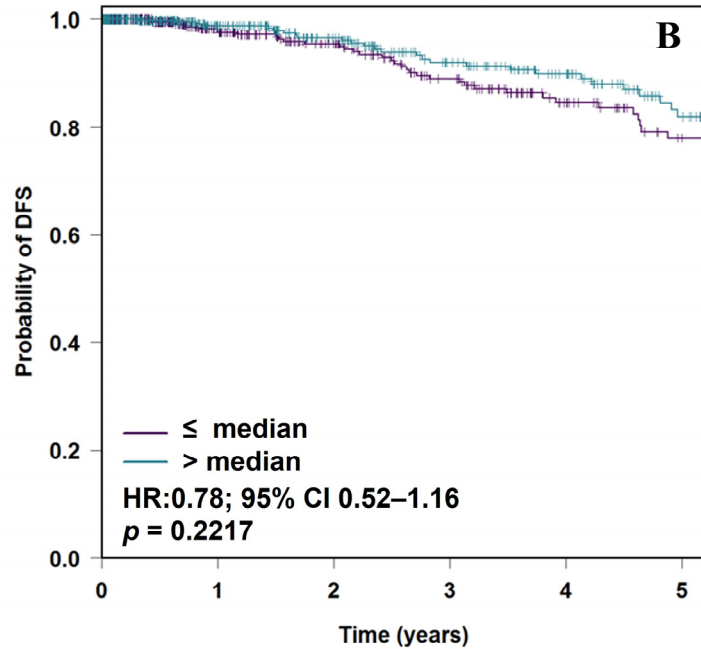
<b>TAM signature genes</b>	<b>Purity-adjusted partial spearman's rho value</b>	<b><i>p</i>-value</b>
<i>FOLR2</i>	0.528	2.31E-72
<i>CD83</i>	0.551	4.59E-80
<i>SIGLEC1</i>	0.603	1.45E-99
<i>TCN2</i>	0.450	1.33E-50
<i>PLTP</i>	0.466	8.80E-55
<i>C1QB</i>	0.703	7.61E-149
<i>DOK2</i>	0.608	1.99E-104
<i>GIMAP6</i>	0.581	6.40E-91
<i>CD40</i>	0.531	2.18E-73
<i>CCL3</i>	0.549	6.20E-53
<i>CCL8</i>	0.350	5.64E-30
<i>FCN1</i>	0.434	7.03E-47
<i>CD4</i>	0.838	8.07E-263
<i>VAV1</i>	0.642	2.00E-116
<i>TLR7</i>	0.750	1.78E-180
<i>FGD2</i>	0.742	1.29E-174
<i>LST1</i>	0.667	5.58E-129
<i>VSIG4</i>	0.730	1.79E-166
<i>CLEC7A</i>	0.620	1.72E-106

TAM, tumor associated macrophages. 37 genes represent the tumor associated macrophage signature described by Cassetta et al. (Reference # 24). (Spearman's rho: 0, no correlation; 0.1–0.3, weak; 0.4–0.6, moderate; 0.7–0.9, strong; 1, perfect; *p*-value < 0.05).

**A**

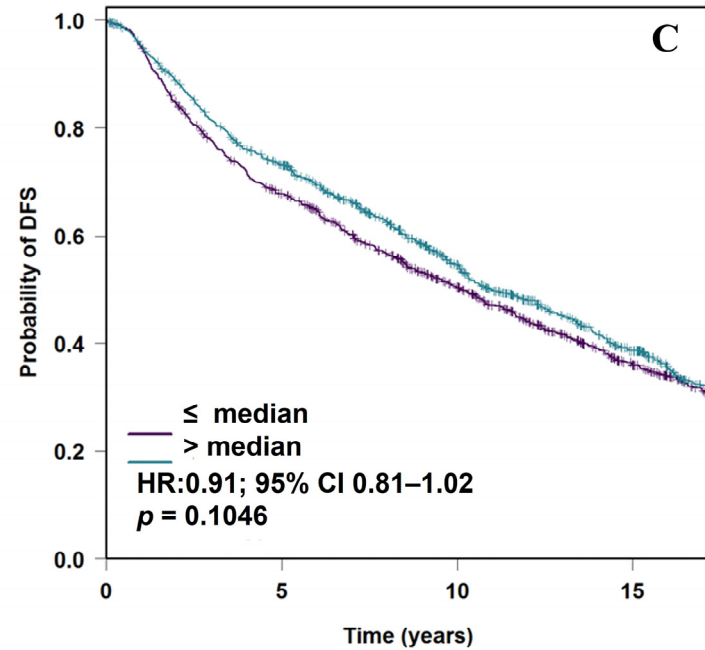


Kaplan-Meier survival estimates of *CSF1* expression  
(node all; ER all; PR all)  
(TCGA data)



Patients at risk:						(Events)
—	517	278	198	149	94	66 (57)
—	516	269	194	143	105	66 (42)

Kaplan-Meier survival estimates of *CSF1* expression  
(node all; ER all; PR all)  
(METABRIC data)



Patients at risk:				(Events)
—	990	651	417	220 (654)
—	990	686	408	201 (581)

**Figure S2.** Correlation of *CSF1* mRNA expression with immune cell infiltrates and prognosis in breast cancer. TIMER2.0 web server was used to evaluate the correlation between *CSF1* mRNA expression and immune cell infiltrates. the left-most panel (i) shows *CSF1* mRNA expression against tumor purity (proportion of cancer cells in the sample), showing a significantly negative correlation. scatter plots (ii-vii) are showing the purity-adjusted partial spearman's rho value and statistical significance for positive correlation of *CSF1* mRNA with immune cell types (spearman's rho: 0, no correlation; 0.1-0.3, weak; 0.4-0.6, moderate; 0.7-0.9, strong; 1, perfect; p-value <0.05). five year Kaplan Meier curves showing an insignificant association of *CSF1* mRNA with disease free survival (DFS) in the TCGA (b) and METABRIC (c) invasive breast cancer cohorts. *p*-value and hazard ratios (HR) with corresponding 95% confidence intervals were estimated by the log-rank test. The survival curves for TCGA and METABRIC cohorts were generated using bc-GenExMiner v4.7.

**Training cohort: correlation CSF-1R+ carcinoma cells with clinicopathological features, immune biomarkers and prognosis**

The optimal scoring, and positivity thresholds were first finalized on the training cohort (n=1183). Of these, 23.6% demonstrated high expression ( $\geq 10\%$ ) with a significant positive association with age <50 years, high proliferation index (Ki67  $\geq 14\%$ ), negative expression of ER and progesterone receptor, positive expression of HER2 and non-luminal breast cancer subtypes (supplementary Table S2). In addition, CSF-1R+ carcinoma cells show a significant correlation with intraepithelial lymphocytes expressing PD-1, TIM3, LAG3, with PD-L1+ carcinoma cells and CSF-1R+ M2 macrophages (supplementary Table 3). Cases exhibiting high expression of CSF-1R by carcinoma cells are associated with significantly adverse breast cancer specific survival in the full training set (supplementary Figure 2A) and in ER positive cases (supplementary Figure 2C-D). In contrast, CSF-1R+ macrophages did not show any significant prognostic associations in the training cohort (supplementary Figure 2B). Cases with high CSF-1R+ carcinoma cells were associated with significantly greater hazard of breast cancer specific death in multivariate analysis, independent of the standard clinicopathological features (supplementary Table S4).

**Table S2.** BC Cancer series training set: correlation of CSF-1R+ carcinoma cells with clinicopathological features.

Clinicopathological variables	CSF-1R expression on carcinoma cells		<i>p</i> -value
	Low (<10%) 904 (76.4)	High (>10%) 279 (23.6)	
<b>Age at diagnosis</b>			
< 50	259 (28.7)	105 (37.6)	0.004*
$\geq 50$	645 (71.3)	174 (62.4)	
<b>Tumor size (cm)</b>			
$\leq 2$	482 (53.6)	131 (47.3)	0.07
> 2	418 (46.4)	146 (52.7)	
<b>Tumor grade</b>			
1 & 2	53 (6.2)	9 (3.3)	0.08
3	808 (93.8)	261 (96.7)	
<b>Axillary lymph node status</b>			
Negative	510 (56.5)	150 (54)	0.45
Positive	392 (43.5)	128 (46)	
<b>Lymphovascular invasion</b>			
Negative	475 (55.2)	143 (53.4)	0.60
Positive	386 (44.8)	125 (46.6)	
<b>ER expression</b>			
Negative	196 (21.7)	131 (47)	<0.001*
Positive	706 (78.3)	148 (53)	
<b>Progesterone receptor expression</b>			
Negative	345 (40.5)	176 (64.7)	<0.001*
Positive	506 (59.5)	96 (35.3)	
<b>HER2 overexpression/amplification</b>			
Negative			<0.001*

Positive	802 (90.9)	202 (73.5)	
	80 (9.1)	73 (26.5)	
<b>Ki-67 proliferation index</b>			
<14%	486 (58.4)	108 (40.3)	<0.001*
≥14%	346 (41.6)	160 (59.7)	
<b>Breast cancer subtypes (IHC)</b>			
Luminal NOS	42 (4.6)	3 (1.1)	
Luminal A	416 (46)	68 (24.4)	
Luminal B/HER2-/Ki67+	224 (24.8)	54 (19.4)	
Luminal / HER2+	47 (5.2)	26 (9.3)	<0.001*
HER2+	32 (3.5)	45 (16.1)	
Basal	69 (7.6)	47 (16.8)	
Additional basal by TNP	43 (4.8)	30 (10.8)	
Unassignable	31 (3.4)	6 (2.2)	

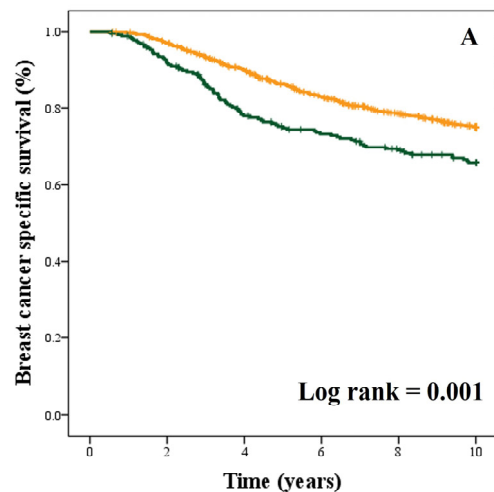
\* Denotes differences between low and high CSF-1R groups that are significant at the Bonferroni-corrected p-value of <0.005 (0.05/10); ER, estrogen receptor; HER2, human epidermal growth factor receptor; TNP, triple negative phenotype.

**Table S3.** BC Cancer series training set: correlation of CSF-1R+ carcinoma cells with immune biomarkers.

Variables	CSF-1R+ carcinoma expression		p-value
	Low (<10%) 904 (76.4)	High (≥10%) 279 (23.6)	
<b>H&amp;E sTIL count (%)</b>			
< 10	728 (86)	197 (74.1)	<0.001*
≥ 10	119 (14)	69 (25.9)	
<b>CD8 iTIL count</b>			
< 1	580 (67.2)	162 (60.4)	0.042
≥ 1	283 (32.8)	106 (39.6)	
<b>PD-1 iTIL count</b>			
< 1	803 (93)	230 (84.6)	<0.001*
≥ 1	60 (7.0)	42 (15.4)	
<b>PD-L1+ carcinoma cells (%)</b>			
0	789 (92.5)	223 (83.5)	<0.001*
≥ 1	64 (7.5)	44 (16.5)	
<b>FOXP3 iTIL count</b>			
< 2	577 (66.9)	157 (58.4)	0.010
≥ 2	285 (33.1)	112 (41.6)	
<b>TIM3 iTIL count</b>			
< 1	796 (91.1)	225 (82.1)	<0.001*
≥ 1	78 (8.9)	49 (17.9)	
<b>LAG3 iTIL count</b>			
< 1	786 (90.6)	225 (81.6)	<0.001*
≥ 1	82 (9.4)	50 (18.2)	
<b>CSF-1R macrophages</b>			
<2	544 (63.6)	117 (42.4)	<0.001*
≥2	312 (36.4)	159 (57.6)	
<b>CD163+ M2 macrophages</b>			
<2	332 (41.7)	75 (28)	<0.001*
≥2	465 (58.3)	193 (72)	

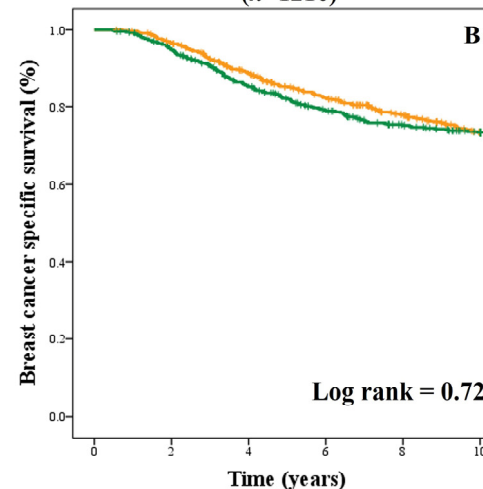
H&E, hematoxylin and eosin stained; iTILs, intraepithelial tumor infiltrating lymphocytes; sTILs, stromal tumor infiltrating lymphocytes; PD-1/L1, programmed cell death protein-1 /ligand 1; FOXP3, forkhead box P3; TIM3, T-cell immunoglobulin domain and mucin domain 3; LAG3, lymphocyte activation gene 3 protein; CSF-1R, colony stimulating factor-1 receptor.

BC Cancer cohort: training set  
(*n*=1183)



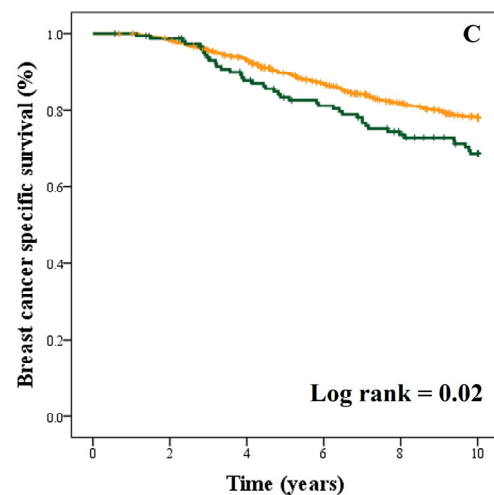
— CSF-1R+ carcinoma cells < 10% (reference)  
— CSF-1R+ carcinoma cells  $\geq 10\%$  (HR 1.53, 95% CI 1.20–1.96)

BC Cancer cohort: training set  
(*n*=1216)



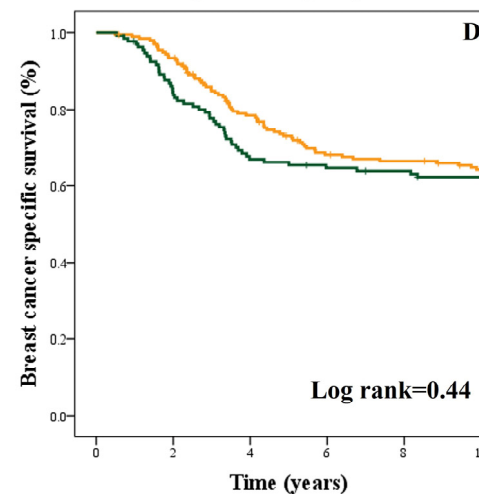
— CSF-1R+ tumor associated macrophages < 2 (reference)  
— CSF-1R+ tumor associated macrophages (HR 1.04, 95% CI 0.83–1.31)

BC Cancer cohort: training set  
ER positive subgroup (*n*=854)



— CSF-1R+ carcinoma cells < 10% (reference)  
— CSF-1R+ carcinoma cells  $\geq 10\%$  (HR 1.51, 95% CI 1.07–2.13)

BC Cancer cohort: training set  
ER negative subgroup (*n*=327)



— CSF-1R+ carcinoma cells < 10% (reference)  
— CSF-1R+ carcinoma cells  $\geq 10\%$  (HR 1.16, 95% CI 0.80–1.67)

**Figure S3.** Kaplan Meier survival curves showing breast cancer specific survival in the training set of BC Cancer series stratified by CSF-1R+ carcinoma cells (A) and CSF-1R+ tumor associated macrophages (B). Prespecified subgroup analysis for CSF-1R expressing carcinoma cells in ER positive cases (C) and ER negative cases (D). .

**Table S4.** Multivariate analysis for CSF-1R+ carcinoma cells in the training set of BC Cancer cohort.

Co-variates in the model	Breast Cancer Specific Survival	
	Adjusted HR (95% CI)	p-value
<b>Age at diagnosis (years)</b>		
<50	1	0.53
≥50	0.92 (0.72–1.83)	
<b>Tumor size (cm)</b>		
≤2	1	<0.001
>2	1.85 (1.43–2.4)	
<b>Tumor grade</b>		
1 & 2	1	<0.001
3	2.13-1.64–2.77)	
<b>Axillary LN status</b>		
Negative	1	<0.001
Positive	2.47 (1.86–3.29)	
<b>LVI</b>		
Negative	1	0.11
Positive	1.26 (0.95–1.66)	
<b>CSF-1R+ carcinoma cells</b>		
Low (<10%)	1	0.02
High (≥10%)	1.38 (1.07–1.79)	

LN, lymph node; LVI, lymphovascular invasion; CSF-1R, colony stimulating factor-1 receptor.

## References

1. Cassetta L, Fragkogianni S, Sims AH, et al. Human Tumor-Associated Macrophage and Monocyte Transcriptional Landscapes Reveal Cancer-Specific Reprogramming, Biomarkers, and Therapeutic Targets. *Cancer Cell*. 2019;35(4):588-602 e510.