

Supplementary Figures and Tables:

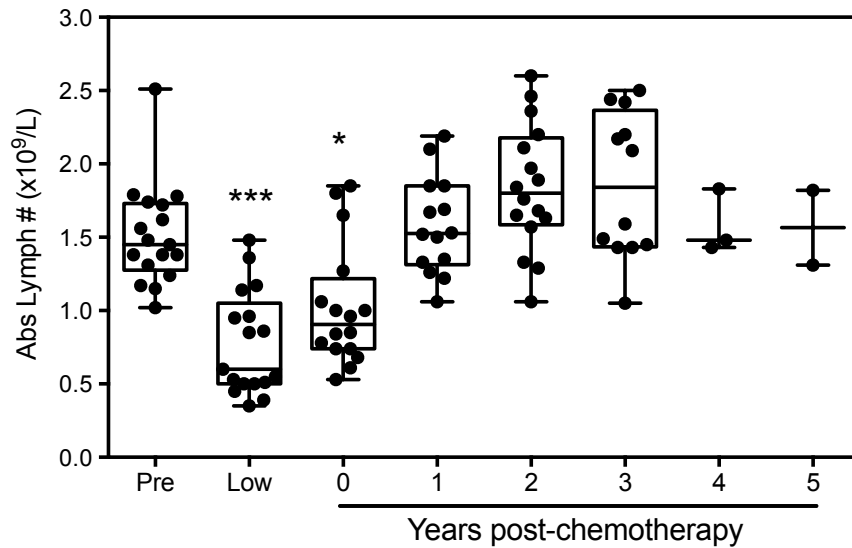


Figure S1. Longitudinal absolute lymphocyte counts in breast cancer patients. Absolute lymphocyte counts in peripheral blood in our cohort of patients (n=15) before chemotherapy treatment (“Pre”), during treatment (“Low”), at the completion of treatment (“0”) and 1-5 years after the end of treatment. P-values are compared to pre-treatment levels using Kruskal-Wallis with Dunn’s comparison. *P<0.05, *** P<0.001

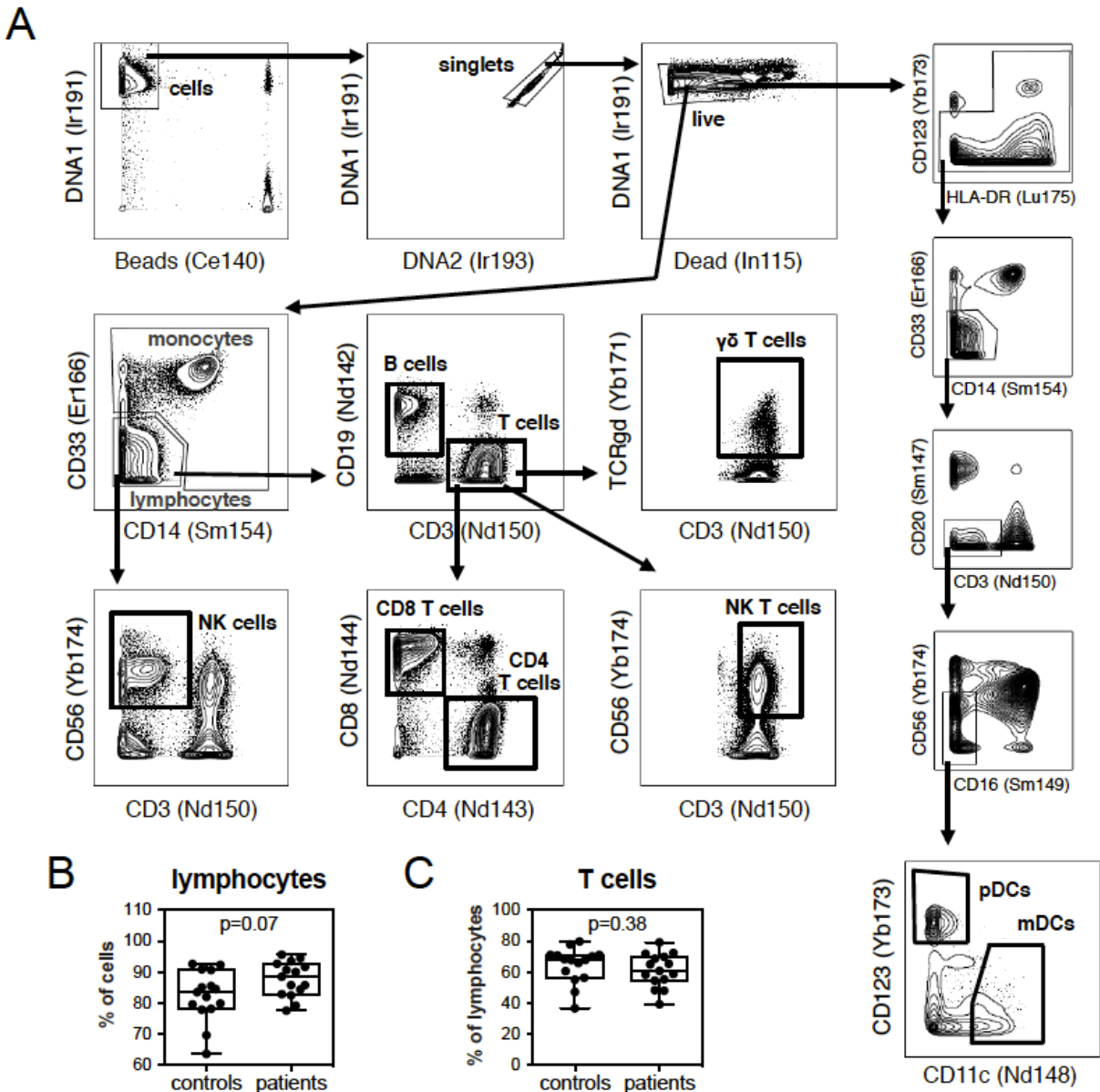


Figure S2. Determining immune cell subsets in breast cancer patients via mass cytometry. (A) The gating strategy for immune cell subsets in peripheral blood mononuclear cells. (B) The frequency of lymphocytes ($CD14^{neg}CD33^{neg}$) within live cells in patients > 1 year post-chemotherapy (n=15) and controls (n=15). (C) The frequency of T cells ($CD3^{+}CD19^{neg}$) within lymphocytes in patients (n=15) and controls (n=15). P-values were determined using Mann-Whitney tests.

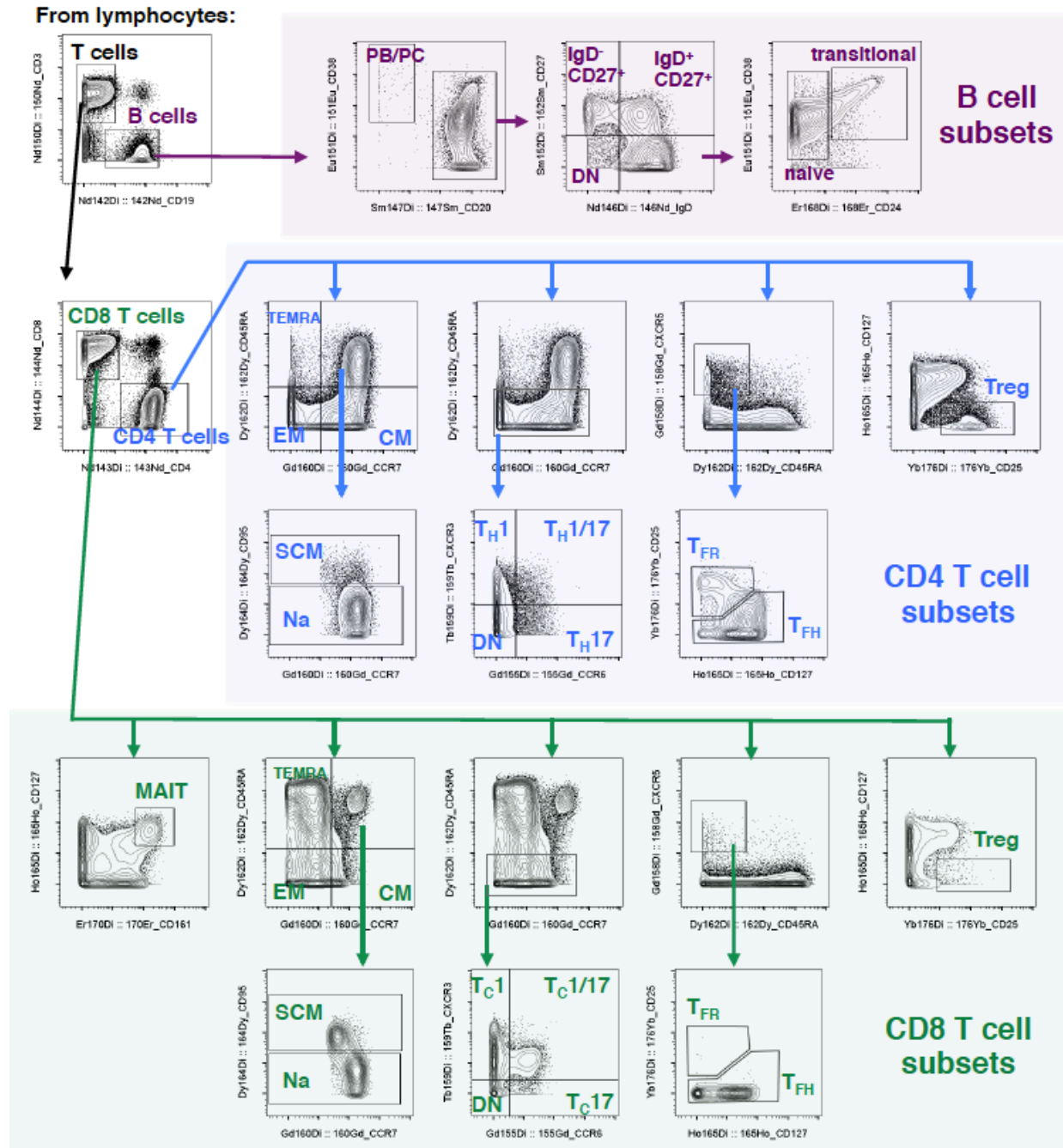


Figure S3. Gating strategy for B cell, CD4 T cell and CD8 T cell subsets for scaffold analysis. Example gating strategy for B cell and T cell subsets, pre-gated on live, single lymphocytes.

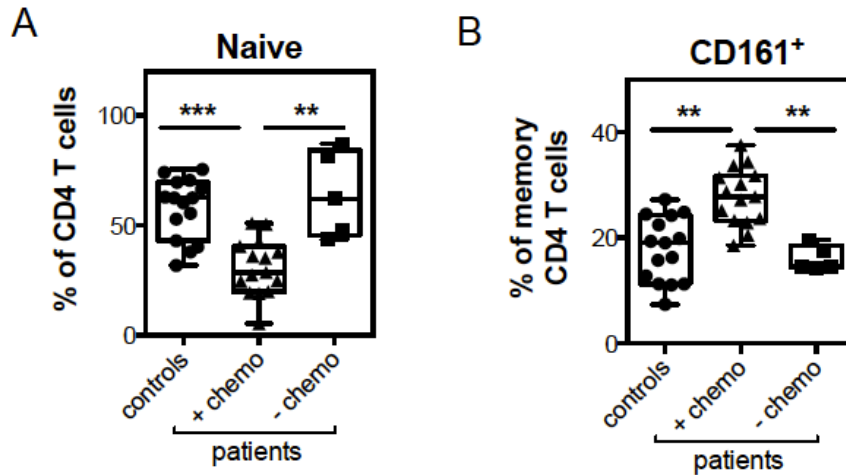


Figure S4. Comparison of CD4 T cell populations in breast cancer patients who received or did not receive chemotherapy treatment. The frequencies of (A) naïve (CCR7⁺CD45RA⁺) within CD4 T cells and (B) CD161⁺ cells within memory CD4 T cells in breast cancer patients with (+ chemo; n=15) and without (- chemo; n=5) chemotherapy treatment compared to chemotherapy-naïve healthy controls (n=15). P-values determined by Kruskal-Wallis with Dunn's comparison. **P<0.01, *** P<0.001

Group	Patient ID	Gender	Race	Stage	chemotherapy regimen	At blood draw		Experiments		
						Age	Years since end of treatment	CytoF	TCRseq	TRECS
Early (1-2.5 yrs)	1	F	W	IIA	A-C-T	49	1	Y	Y	Y
	2	F	B	IB	A-C-T	54	1.1	Y	Y	Y
	3	F	A	IA	T-C	47	1.2	Y	Y	Y
	4	F	W	IIA	T-Ca-Ab + Ta-Ab	55	1.2	Y	Y	Y
	5	F	H	IIA	T-Ca-Ab	58	1.3	Y	Y	Y
	6	F	W	IIB	T-Ab-O	51	1.4	Y	Y	Y
	7	F	A	IA	T-Ca-Ab	50	1.9	Y	Y	Y
	8	F	W	IIA	A-C-T	52	2			Y
	9	F	W	IIB	A-C-T	49	2.1	Y		
	10	F	A	IIA	A-C-T-O	47	2.1	Y		
	11	F	W	IIB	A-C-T	54	2.2	Y		
	12	F	W	IIIA	T-C	50	2.3	Y		
Late (3+ yrs)	13	F	W	IIA	A-C-T-Ab	57	3.5	Y		
	14	F	W	IIB	G-Ca-O	63	3.6	Y		
	15	F	A	IIA	T-C	58	4.4	Y		
	16	F	W	IIIA	T-C	50	4.4	Y		
	17	F	W	IIIA	G-Ca-O	54	4.9	Y		

Table S1. Cohort demographics. All patients were female (F). Race: White (W), Black (B), Asian (A) and Hispanic (H). Chemotherapy regimens include taxotere (T), Cytoxan (C), Carboplatin (Ca), Herceptin, Pertuzamab &/or Trastuzumab (Ab), Gemcitabine (G), Adriamycin (A) and other (O). In Experiments, Y indicates that the patient was included.

	Frequencies (median[range])		Mann-Whitney p-value	adjusted p-value
	Controls (n=15)	Patients (n=15)		
of CD4 T cells				
Naïve	62.4 (31.7-75.4)	28.5 (5.27-50.9)	< 0.0001	0.0009
SCM	1.89 (0.52-3.78)	2.78 (0.8-24.3)	0.0675	0.2700
CM	29.3 (15.8-46.7)	48 (26.2-79)	0.0001	0.0009
EM	7.58 (1.95-25.5)	13 (6.96-27.1)	0.0023	0.0161
TEMRA	0.6 (0.092-9.93)	1.03 (0.13-6.16)	0.4426	0.4674
TH1	8.66 (2.52-25.3)	19 (6.7-31.5)	0.0019	0.0152
TH17	2.14 (0.92-6.7)	7.69 (2.9-13.7)	< 0.0001	0.0009
TH1/17	1.34 (0.24-5.14)	4.2 (1.53-12.9)	< 0.0001	0.0009
DN	21.4 (15.8-40.6)	30 (17.6-49.4)	0.042	0.2100
TFH	4.22 (0.63-18.9)	9.18 (4.12-24.1)	0.0051	0.0306
TFR	0.62 (0.032-2.38)	0.82 (0.05-3.27)	0.4674	0.4674
Treg	3.8 (1.74-9.31)	5.65 (1.96-9.6)	0.2717	0.4674
	Frequencies (median(range))		Mann-Whitney p-value	adjusted p-value
	Controls (n=15)	Patients (n=15)		
of CD8 T cells				
Naïve	56.6 (6.29-78.9)	38.9 (18-62.4)	0.0613	0.6743
SCM	3.55 (2.01-6.86)	6.15 (1.87-14.9)	0.0037	0.0481
CM	4.74 (1.44-10.6)	8.51 (1.72-30.9)	0.0358	0.4296
EM	13 (2.3-27)	16.6 (2.72-40.3)	0.4185	0.7112
TEMRA	20 (5.51-55.9)	20.9 (4.82-40.9)	0.7112	0.7112
Tc1	13.2 (2.56-24.9)	13.6 (2.63-41.3)	0.2368	0.7112
Tc17	0.57 (0.048-2.36)	0.94 (0.066(4.03)	0.2854	0.7112
Tc1/17	1.66 (0.18-8.32)	2.97 (0.28-7.36)	0.2806	0.7112
DN	4.12 (0.8-10.9)	5.46 (1.47-17.2)	0.3093	0.7112
TFH	0.57 (0.067-5.77)	0.79 (0.12-2.44)	0.1837	0.7112
TFR	0.015 (0-.14)	0.021 (0.003-0.078)	0.1637	0.7112
Treg	0.44 (0.12-1.52)	0.57 (0.26-1.12)	0.0911	0.7112
MAIT	4.61 (1.03-14.9)	2.73 (0.72-17)	0.2017	0.7112

Table S2. Frequencies of hand-gated CD4 and CD8 T cell subsets. Adjusted p-values were calculated using Hochberg multiple comparison.

PBMC phenotyping panel			
Metal label	Specificity	Clone/Company	Source
113In or Cd Qdot	CD57	HCD57, BioLegend	In house
115In	live/dead		
142Nd	CD19	SJ25C1, Southern BioTech	In house
143Nd	CD4	SK3, BioLegend	In house
144Nd	CD8	SK1, BioLegend	In house
146Nd	IgD	IA6-2, BioLegend	In house
147Sm	CD20	2H7, Fluidigm	purchased
148Nd	CD11c	Bu15, BioLegend	In house
149Sm	CD16	3G8, BioLegend	In house
150Nd	CD3	UCHT1, BD	In house
151Eu	CD38	HB-7, BD	In house
152Sm	CD27	L128, BD	In house
153Eu	CD11b	ICRF44, BioLegend	In house
154Sm	CD14	M5E2, BioLegend	In house
155Gd	CCR6	11A9, BD or G034E3, BioLegend	In house
156Gd	CD94	HP-3D9, BD	In house
157Gd	CD86	IT2.2, BioLegend	In house
158Gd	CXCR5	RF8B2, BD	In house
159Tb	CXCR3	G025H7, BioLegend	In house
160Gd	CCR7	150503, R&D Systems	In house
162Dy	CD45RA	HI100, BioLegend	In house
164Dy	CD95	DX2, Fluidigm	purchased
165Ho	CD127	A019D5, BioLegend	In house
166Er	CD33	P67.8, BD	In house
167Er	CD28	L293, BD	In house
168Er	CD24	ML5, BioLegend	In house
169Tm	ICOS	DX29, BD	In house
170Er	CD161	DX12, BD	In house
171Yb	TCRgd	B1, BioLegend	In house
172Yb	PD-1	EH12.1, BD	In house
173Yb	CD123	9F5, BD	In house
174Yb	CD56	NCAM16.2, BD	In house
175Lu	HLA-DR	G46-6, BD	In house
176Yb	CD25	M-A251, BD	In house

Table S3. Mass cytometry antibody information.

	Live Single cells	CD4 T cells			CD8 T cells			B cells	
Figure	1	2	7 (early)	7 (late)	2	7 (early)	7 (late)	7 (early)	7 (late)
Numbers of controls/patients	15/15	15/15	10/10	5/5	15/15	10/10	5/5	10/10	5/5
Cluster number	200	100			50			100	
Pre-gated markers	none	CD33 (neg) CD14 (neg) CD3 (pos) CD19 (neg) CD4 (pos) CD8 (neg)			CD33 (neg) CD14 (neg) CD3 (pos) CD19 (neg) CD4 (neg) CD8 (pos)			CD33 (neg) CD14 (neg) CD3 (neg) CD19 (pos)	
Markers used in analysis	CCR6 CCR7 CD11b CD11c CD123 CD127 CD14 CD16 CD161 CD19 CD20 CD24 CD25 CD27 CD28 CD38 CD45RA CD56 CD57 CD8 CD86 CD94 CD95 CXCR5 HLA-DR ICOS IgD PD-1 TCRgd	CCR6 CCR7 CD127 CD161 CD25 CD27 CD28 CD38 CD45RA CD56 CD57 CD95 CXCR3 CXCR5 HLADR ICOS PD-1			CCR6 CCR7 CD127 CD161 CD25 CD27 CD28 CD38 CD45RA CD56 CD57 CD95 CXCR3 CXCR5 HLADR ICOS PD-1			CCR6 CCR7 CD11b CD11c CD20 CD24 CD28 CD38 CD45RA CD86 CD95 CXCR5 HLADR IgD	

Table S4. Cell surface markers used in Statistical Scaffold analyzes. neg = negative, pos = positive