Peripheral blood mononuclear cell populations correlate with outcome in patients with metastatic breast cancer

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SUPPLEMENTAL TABLES

Supplemental Table S1. Characteristics of the included patients. [a] De novo MBC denotes MBC at initial breast cancer diagnosis. [b] Distant recurrent MBC denotes MBC diagnosis after >0 years after the primary diagnosis. [c] Visceral metastasis is defined as involvement of lung, liver, peritoneal and/or pleura. [d] Progression is defined as progressive disease at three months evaluation, using modified RECIST 1.1 criteria. *Abbreviations:* ECOG; Eastern Cooperative Oncology Group, NHG; Nottingham Histological Grade, PT; primary tumor, ER; estrogen receptor, PR; progesterone-receptor, HER2; human epidermal growth factor receptor 2, MET; metastasis.

Variable	*Total N=32*	%
Age		
< 65 years	17	53.1
≥65 years	15	46.9
Baseline ECOG		
0	22	68.8
1	6	18.8
2	4	12.5
Unknown	0	0.0
PT Tumor type		
Ductal	21	65.5
Lobular	6	18.8
Other	4	12.5
Unknown	1	3.1
PT NHG		
I	2	6.3
II	13	40.6
III	7	21.9
Unknown	10	31.3
PT ER status		
ER-negative	8	25.0
ER-positive	20	62.5
ER-unknown	4	12.5
PT PR status		
PR-negative	12	37.5
PR-positive	15	46.9
PR-unknown	5	15.6
PT HER2 status		
HER2-negative	16	50.0
HER2-positive	5	15.6
HER2-unknown	11	34.4
Type of MBC		
De novo [a]	5	15.6
Distant recurrent [b]	27	84.4
Metastatic sites, number		
< 3 metastatic sites	19	59.4
≥ 3 metastatic sites	13	40.6
Metastatic sites, localization		
Lymph nodes No / Yes	20 / 12	62.5 / 37.5
Lung No / Yes	20 / 12	62.5 / 37.5

Liver No / Yes	23 / 9	71.9 / 28.1
Bone No / Yes	7 / 25	21.9 / 78.1
Visceral [c] No / Yes	13 / 19	40.6 / 59.4
Bone only No / Yes	23 / 9	71.9 / 28.1
MET ER status		
ER-negative	1	3.1
ER-positive	27	84.4
ER-unknown	4	12.5
MET PR status		
PR-negative	16	50.0
PR-positive	11	34.4
PR-unknown	5	15.6
MET HER2 status		
HER2-negative	23	71.9
HER2-positive	3	9.4
HER2-unknown	6	18.8
***Progression at 3 mo evaluation*[d]**		
Non-progression	25	78.1
Progression	3	9.4
Unknown	4	12.5

Supplemental Table S2. Antibodies used for flow cytometry with clone and dilution indicated.
All from BD Biosciences unless otherwise stated. [a] from Miltenyi Blood dendritic cell enumeration kit that also includes CD14 and CD19 to exclude monocytes and B lymphocytes, respectively.

Antibody	Clone	Dilution
CD14-FITC	M5E2	1:10
CD16-PE	3G8	1:20
HLA-DR-APC	G46-6	1:50
CD3-FITC	HIT3a	1:25
CD3-APC	HIT3a	1:20
CD4-PE	RPA-T4	1:25
CD8-PE	HIT8a	1:25
CD25-FITC	2A3	1:10
CD127-biotin / SA-APC	HIL-7R-M21	1:10 / 1:10
CD56-APC	B159	1:10
CD19-FITC	HIB19	1:20
CD33-APC	WM53	1:10
BDCA-1 (CD1c)[a]	NA[a]	1:10
BDCA-2 (CD303)[a]	NA[a]	1:10
BDCA-3 (CD141)[a]	NA[a]	1:10

Supplemental Table S3. Summary of the analyzed immune cell populations. Circulating immune cell populations were analyzed by flow cytometry (see Supplementary Figure 1 for gating strategies). Values represent percentage of immune cell population of PBMCs or, where indicated, of all CD4^{+} T cells. [a.] Ratio calculated as percentage of CD4^{+} cells of all lymphocytes / CD8^{+} cells of all lymphocytes. [b.] Ratio calculated as percentage of CD14^{+}CD16^{+} cells / CD14^{+}CD16^{-} cells. Median values with SEM and number of patients analyzed shown.

| Immune cell population or ratio	*N*	Median ± SEM
% All T lymphocytes (CD3^{+} cells)	32	23.23 % ± 2.05
% Cytotoxic T lymphocytes (CD8^{+} cells)	32	6.59 % ± 0.96
% T helper cells (CD4^{+} cells)	32	14.63 % ± 1.31
% Tregs (CD4^{++}CD25^{+}CD127^{low/-} cells of all CD4^{++} cells)	31	3.68 % ±0.42
% CD8^{+} cells of all CD3^{+} T lymphocytes	32	34.34 % ± 2.02
% CD4^{+} cells of all CD3^{+} T lymphocytes	32	64.54 % ± 2.66
Ratio CD4^{+} T cells / CD8^{+} T cells[a]	32	1.86 ±0.32
% B lymphocytes (CD19^{+} cells)	26	4.34 % ± 0.86
% NK cells (CD56^{+}CD3^{-} cells)	32	2.82 % ± 0.49
% NKT cells (CD56^{+}CD3^{+} cells)	32	0.53 % ± 0.28
% Classical monocytes (CD14^{++}CD16^{-} cells)	27	7.36 % ±0.91
% Intermediate monocytes (CD14^{++}CD16[+/++] cells)	27	2.59 % ± 0.46
% Non-classical monocytes (CD14^{+}CD16^{++} cells)	27	0.27 % ± 0.06
Ratio CD16^{+} / CD16^{-} monocytes[b]	27	0.23 ± 0.05
% MDC1 (BDCA-1^{+} cells)	31	0.31 % ± 0.05
% MDC2 (BDCA-3^{+} cells)	31	0.02 % ± 0.01
% PDC (BDCA-2^{+} cells)	31	0.58 % ±0.12
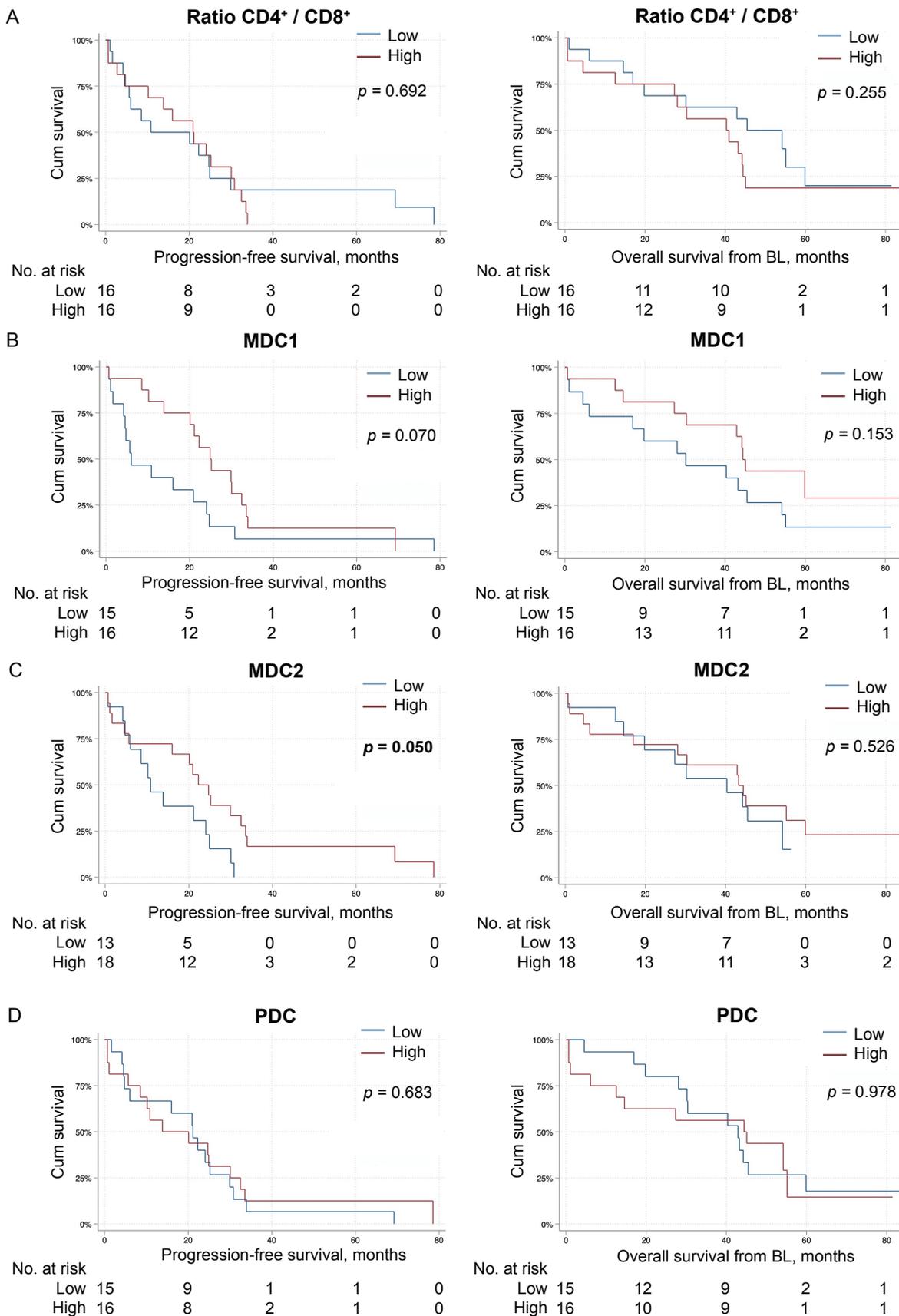
Supplemental Figure S1. High levels of systemic MDC1 associate with improved progression-free survival. Kaplan-Meier curves of progression-free survival (PFS; left panels) and overall survival (OS) from baseline (right panels) according to the levels (percentages) of indicated immune cell population in MBC patients. **A**, Ratio of % CD4^{++} T-helper cells / % CD8$^+$ CTLs. **B**, BDCA-1$^+$ MDC1. **C**, BDCA-3$^+$ MDC2. **D**, BDCA-2$^+$ PDC. *N*=31 for all populations except for CD4$^+$/CD8$^+$ ratio (*N*=32). Statistics by Log-rank test, *p*-val-ues < 0.05 highlighted in bold.
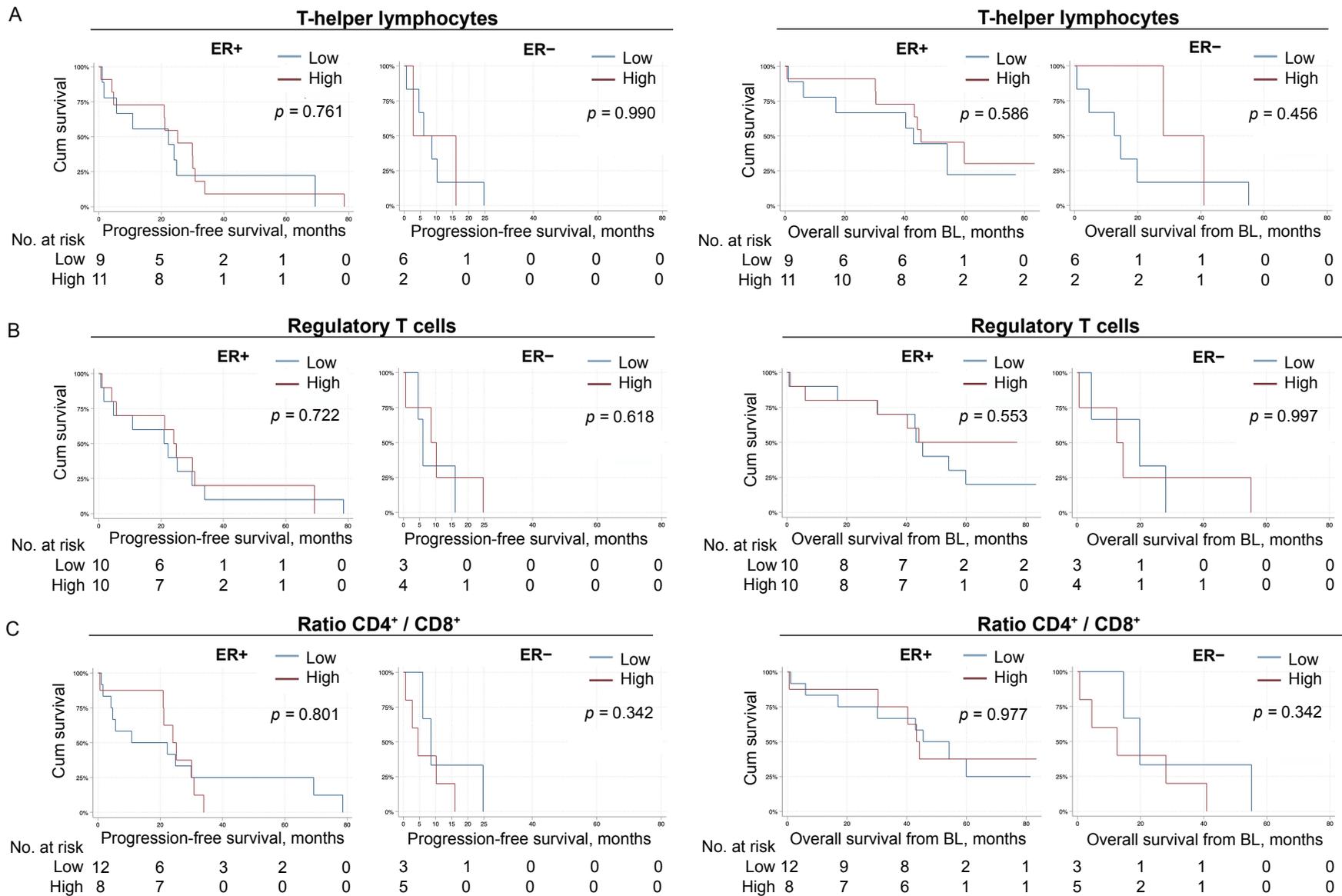
Supplemental Figure S2. The levels of T-helper or T$_{regs}$ do not associate with survival in patients with ER-positive or ER-negative primary tumors. Kaplan-Meier curves of progression-free survival (PFS; left panels) and overall survival (OS, right panels) according to the levels of indicated immune cell populations in patients with MBC stratified for primary tumor ER status. **A**, CD4$^+$ T-helper cells. **B**, CD4$^+$CD25$^+$CD127$^{low/-}$ cells of CD4$^+$ cells. **C**, ratio of % CD4$^+$ T-helper cells / % CD8$^+$ CTLs. N=20 ER-positive and N=8 ER-negative for all populations except for T$_{regs}$ (N=20 and N=7, respectively). Statistics by Log-rank test, p-values < 0.05 highlighted in bold.
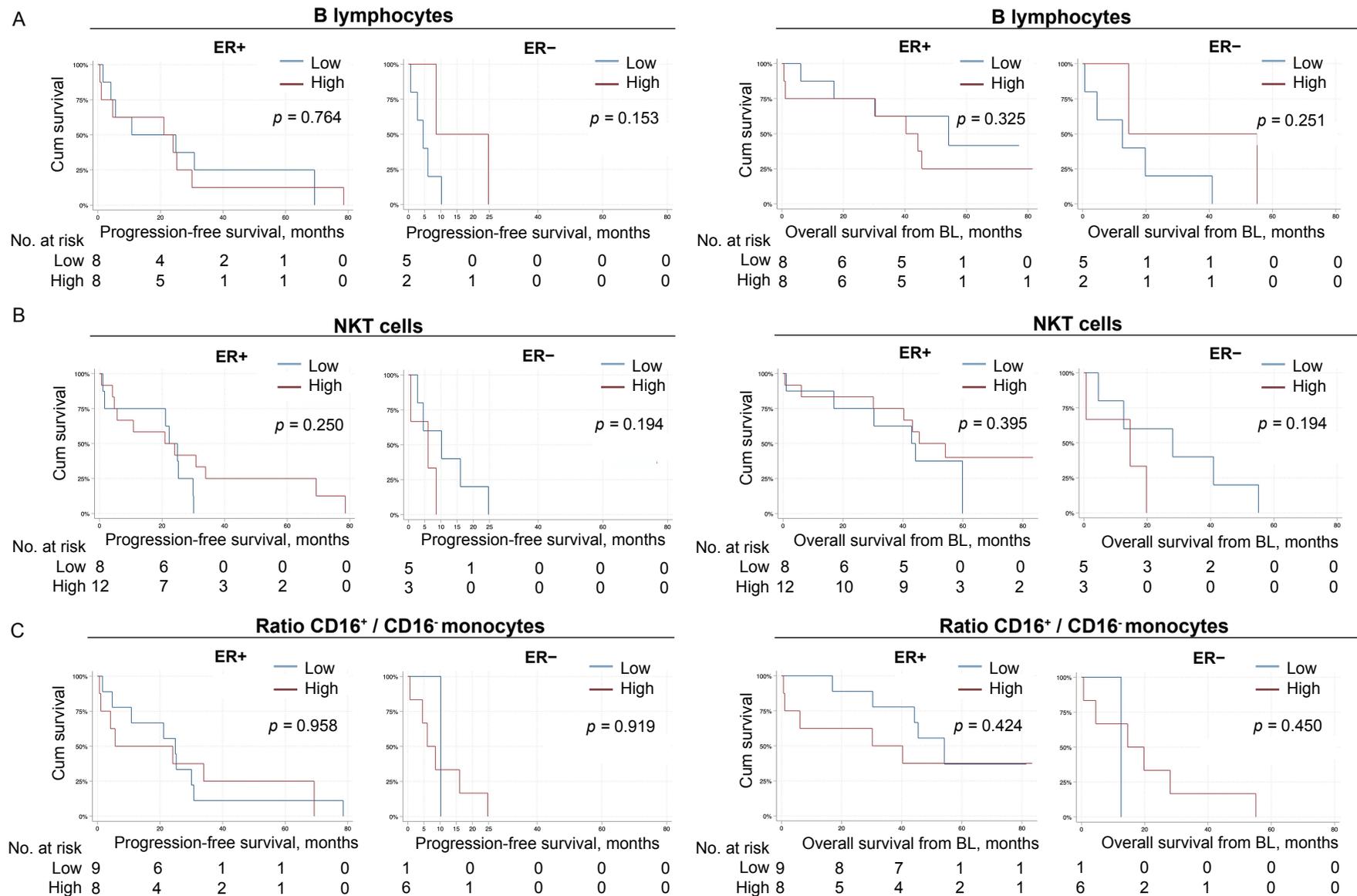
Supplemental Figure S3. The levels of B lymphocytes or NKT cells do not associate with survival in patients with ER-positive or ER-negative primary tumors. Kaplan Meier curves of progression-free survival (PFS; left panels) or overall survival (OS) from baseline (right panels) according to the levels of indicated immune cell populations in patients with MBC stratified for primary tumor ER status. **A**, CD19+ B lymphocytes, *N*=16 ER-positive and *N*=7 ER-negative. **B**, CD56+CD3+ NKT cells, *N*=20 ER-positive and *N*=8 ER-negative. **C**, ratio of % CD16+ monocytes / % CD16− monocytes, *N*=17 ER-positive and *N*=7 ER-negative. Statistics by Log-rank test, with *p*-values indicated.
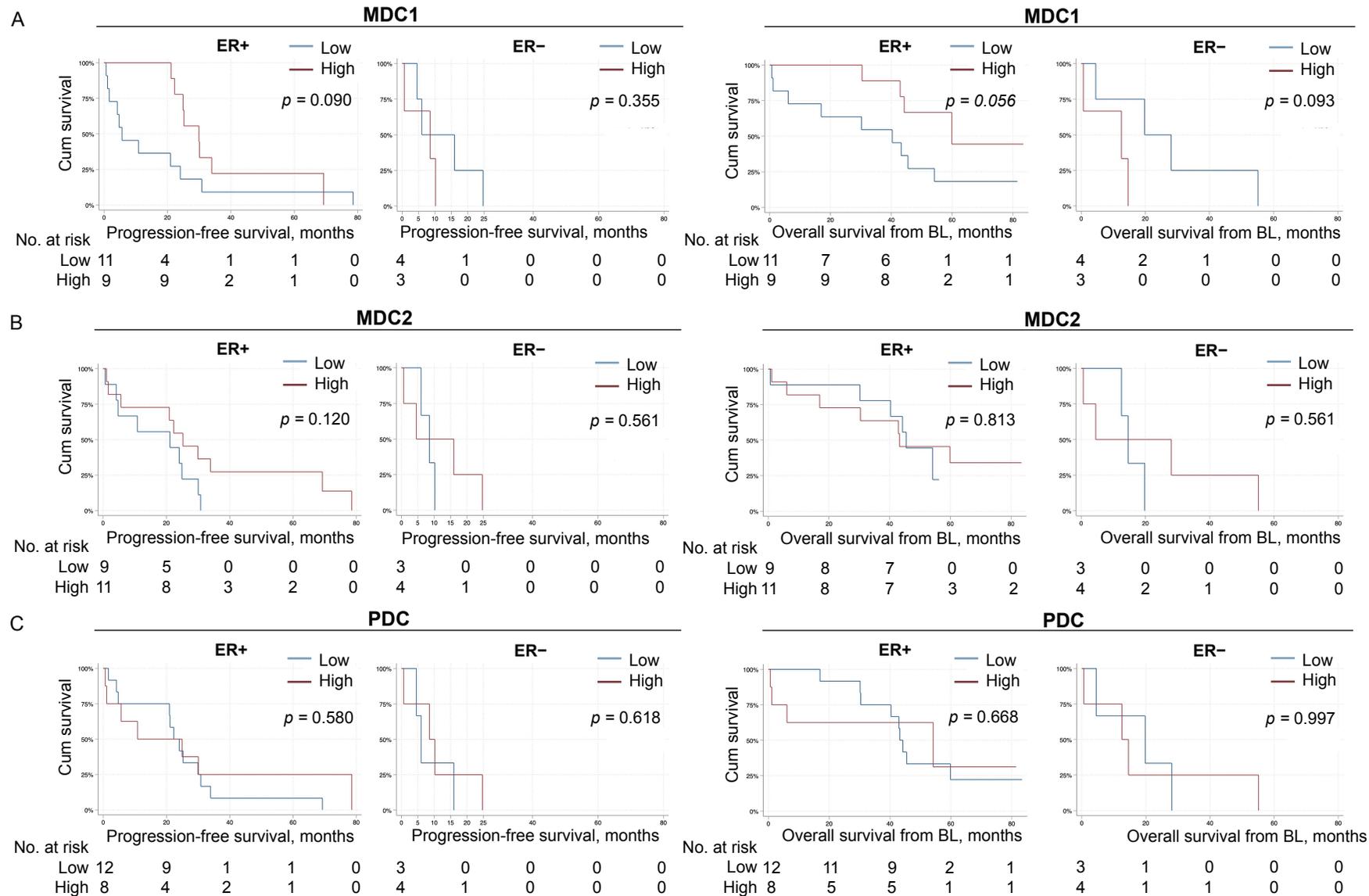
Supplemental Figure S4. The levels of MDC1 tend to associate with survival. Kaplan Meier curves of progression-free survival (PFS; left panels) or overall survival (OS) from baseline (right panels) according to the levels of indicated blood DC population in patients with MBC stratified for primary tumor ER status. **A**, BDCA-1^{+} MDC1. **B**, BDCA-3^{+} MDC2. **C**, BDCA-2^{+} PDC. *N*=20 ER-positive and *N*=7 ER-negative for all populations. Statistics by Log-rank test, with *p*-values indicated.