Supplements

Table S1

Antigen	Clone	Isotype	Label	Dilution	Manufacturer
CD3	SK7	mouse IgG1	APC-H7	1:50	BD Pharmingen™
CD3 (LIN)	HIT3a	mouse IgG2a	FITC	1:100	BD Pharmingen™
CD4	RPA-T4	mouse IgG1	Pacific Blue™	1:50	BD Pharmingen™
CD8	RPA-T8	mouse IgG1	V500	1:50	BD Horizon™
CD11b	ICRF11	mouse IgG1	APC-Cy7	1:50	BD Pharmingen™
CD14	MφP9	mouse IgG2b	PE-Cy7	1:50	BD Pharmingen™
CD15	HI98	Mouse IgM	APC	1:100	BD Pharmingen™
CD19 (LIN)	HIB19	mouse IgG1	FITC	1:100	BD Pharmingen™
CD25	M-A251	mouse IgG1	PE-Cy7	1:50	BD Pharmingen™
CD33	P67.6	mouse IgG1	PE	1:100	BioLegend®
CD45	HI30	mouse IgG1	AF700	1:50	BD Pharmingen™
CDD45	2D1	mouse IgG1	V450	1:50	BD Horizon™
CD56 (LIN)	B159	mouse IgG1	FITC	1:100	BD Pharmingen™
CD127	HIL-7R-M21	mouse IgG1	FITC	1:50	BD Pharmingen™
Foxp3	259D/C7	mouse IgG1	PE	1:25	BD Pharmingen™
HLA-DR	G46-6	mouse IgG2a	V500	1:50	BD Horizon™
PD-1	MIH4	mouse IgG1	FITC	1:10	BD Pharmingen™
PD-L1	MIH1	mouse IgG1	BV605	1:50	BD OptiBuild™

Abbreviations: AF: Alexa Fluor; APC: allophycocyanin, BV: brilliant violett, CD: cluster of differentiation, FITC: flourescein isothiocyanate, Foxp3: forkhead box p3; HLA: human leukocyte antigen; LIN: linage; PD: programmed death; PD-L: programmed death ligand; PE: phycoerythrin; Ig: immunoglobulin

Figure S1

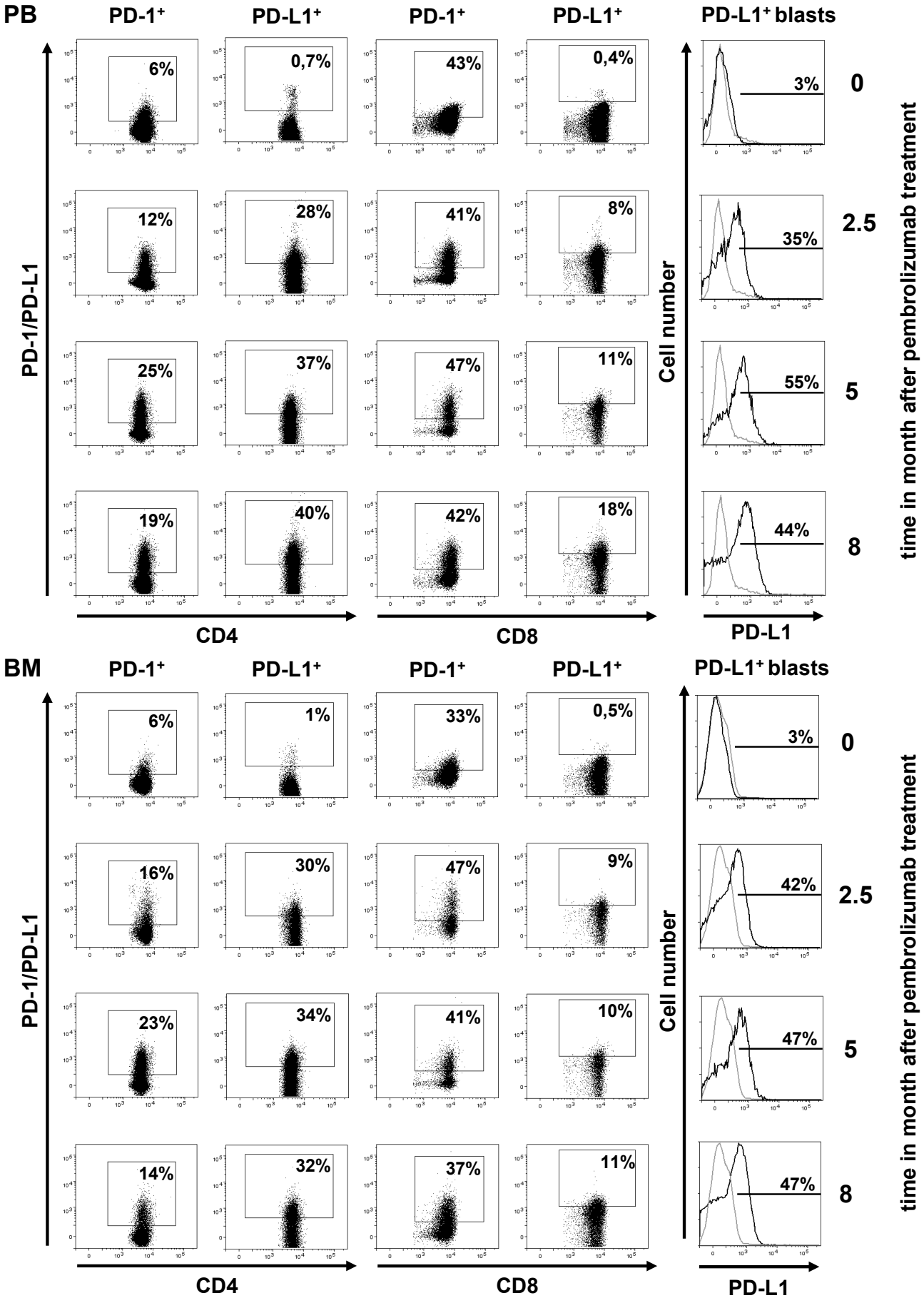
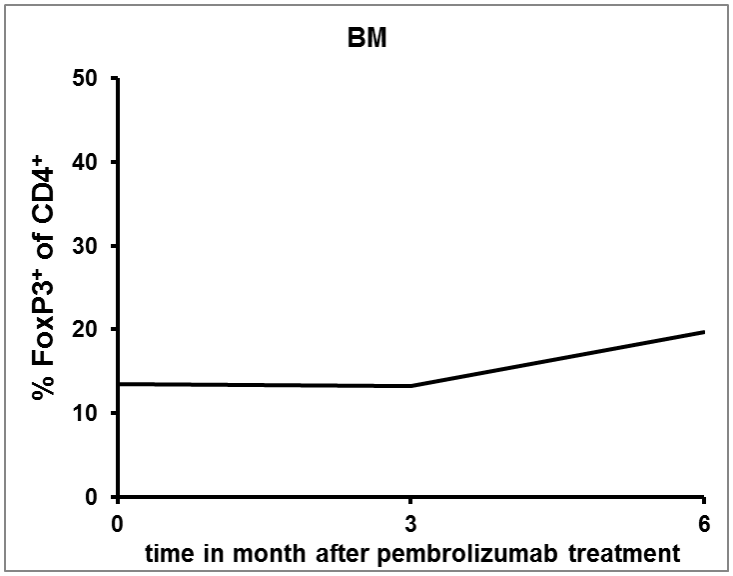
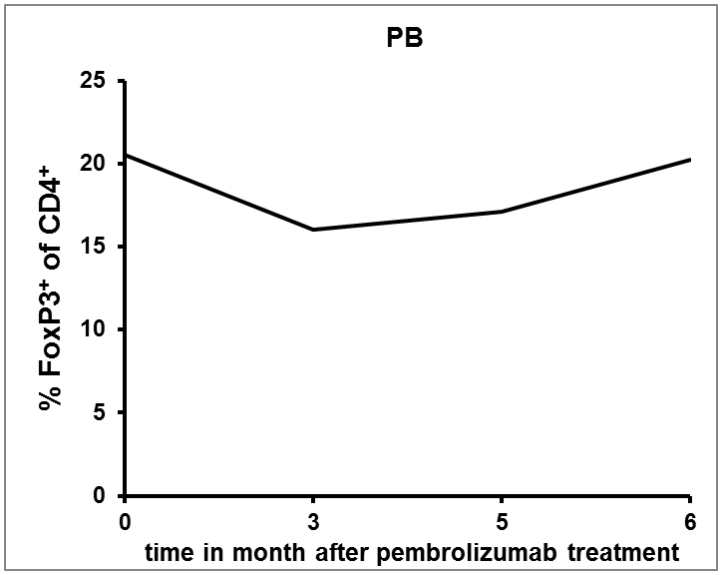

PB
PD-1+
PD-L1+
PD-1+
PD-L1+
PD-L1+ blasts
6%
0,7%
43%
0,4%
3%
0
12%
28%
41%
8%
35%
2.5
25%
37%
47%
11%
55%
5
19%
40%
42%
18%
44%
8
PD-1/PD-L1
Cell number
CD4
CD8
PD-L1
time in month after pembrolizumab treatment
BM
PD-1+
PD-L1+
PD-1+
PD-L1+
PD-L1+ blasts
6%
1%
33%
0,5%
3%
0
16%
30%
47%
9%
42%
2.5
23%
34%
41%
10%
47%
5
14%
32%
37%
11%
47%
8
PD-1/PD-L1
Cell number
CD4
CD8
PD-L1
time in month after pembrolizumab treatment

Figure S2

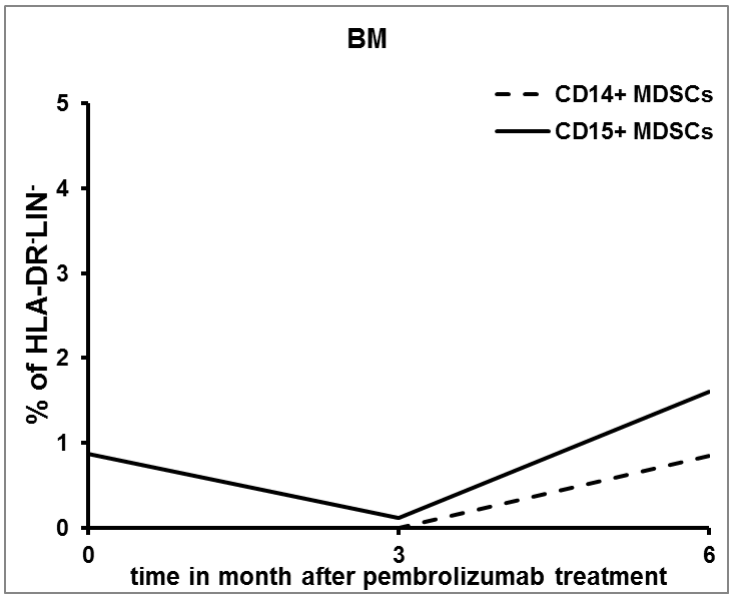
A



B



C



D

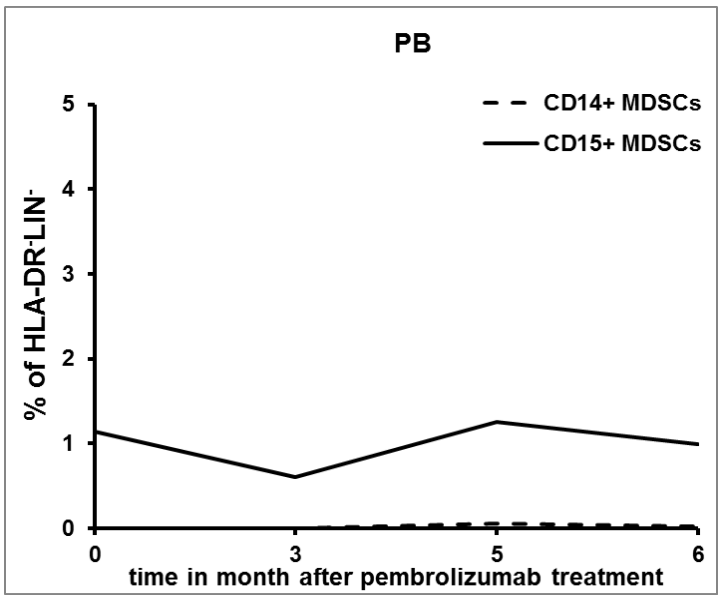
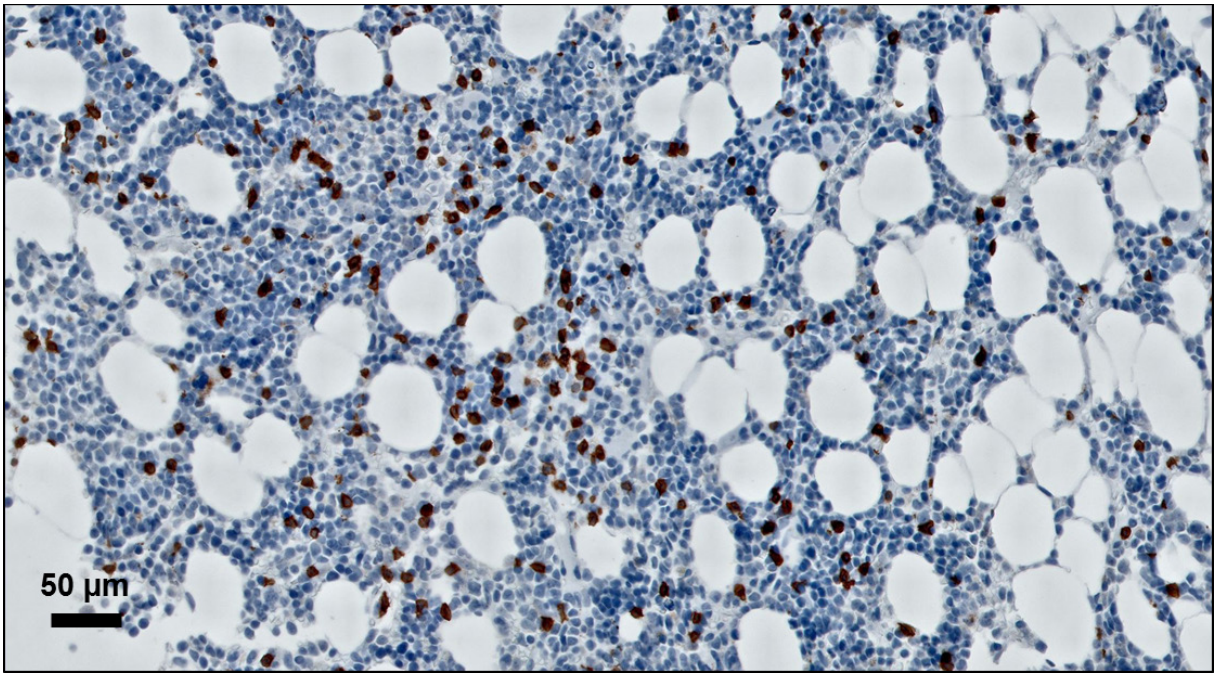
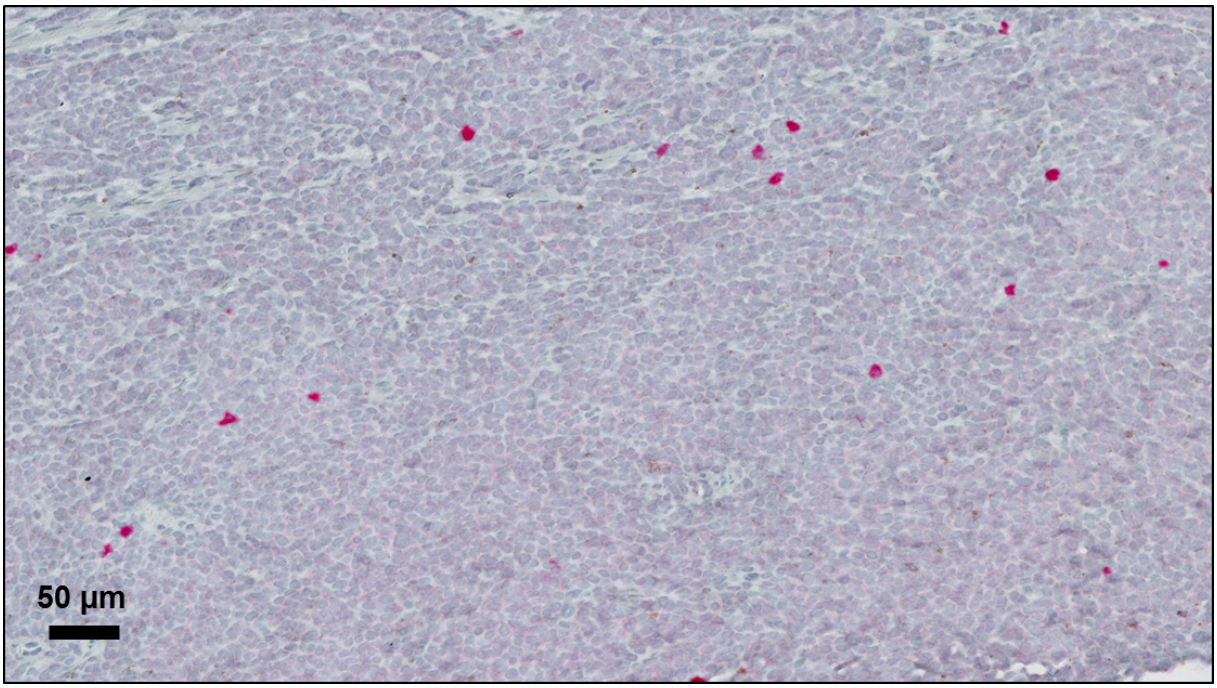


Figure S3

A



B



Supplement Figure Legends

Figure S1

Single graphs to illustrate the expression of PD-L1 and PD-1 on AML blasts and T cells. PB- and BM-derived AML blasts and T cells were stained with fluorochrome-conjugated antibodies and detected by flow cytometry prior and at three time points during PEM treatment. T cells were characterized as $CD45^+$, $CD3^+$ and $CD4^+$ or $CD8^+$ populations. $CD8^+$ T and $CD4^+$ T cells were analyzed for their expression of PD-1 and PD-L1. Values in the dot plots show the percentages of cells stained positive for each molecule compared to unstained cells (not shown). Blasts were defined as $CD45^{low}$ cells and stained for PD-L1 expression. Values in the histogramms represent the percentages of cells stained positive for PD-L1 (black) compared to unstained (grey) cells.

Figure S2

The frequency of Treg cells and MDSCs. BM (A, C) and PB (B, D) samples were stained with fluorochrome-conjugated antibodies and analyzed by flow cytometry to detect Treg cells and MDSCs prior and during PEM treatment. (A, B) Treg cells were characterized as $CD45^+$, $CD3^+$, $CD4^+$, $CD25^+$, $CD127^-$ and $Foxp3^+$. (C, D) MDSCs were defined as $CD45^+$, LIN^-, $HLA\text{-}DR^-$, $CD33^+$ and $CD11b^+$ to identify a monocytic ($CD14^+$) and a granulocytic ($CD15^+$) subset. Values in the graphs represent the percentage of cells stained positive for each molecule at indicated different time points.

Figure S3

Lymphocyte infiltration into BM and melanoma tissue. (A, B) Immunohistochemical stainings on formalin-fixed, paraffin-embedded tissues were performed to detect $CD3^+$ T cells in BM tissue one month after start of PEM treatment (A) and in melanoma tissue prior to PEM therapy (B). $CD3^+$ T cells were visualized by 3,3´-Diaminobenzidine (A) or alkaline phospatase (B) staining. Sections were counterstained with Mayer´s hematoxilin.