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Supplementary Information for

Blocking IL-1β reverses the immunosuppression in mouse breast cancer and synergizes with anti-PD-1 for tumor abrogation

Irena Kaplanov, Yaron Carmi, Rachel Kornetsky, Avishai Shemesh, Galina V. Shurin, Michael R. Shurin, Charles A. Dinarello, Elena Voronov and Ron N. Apte
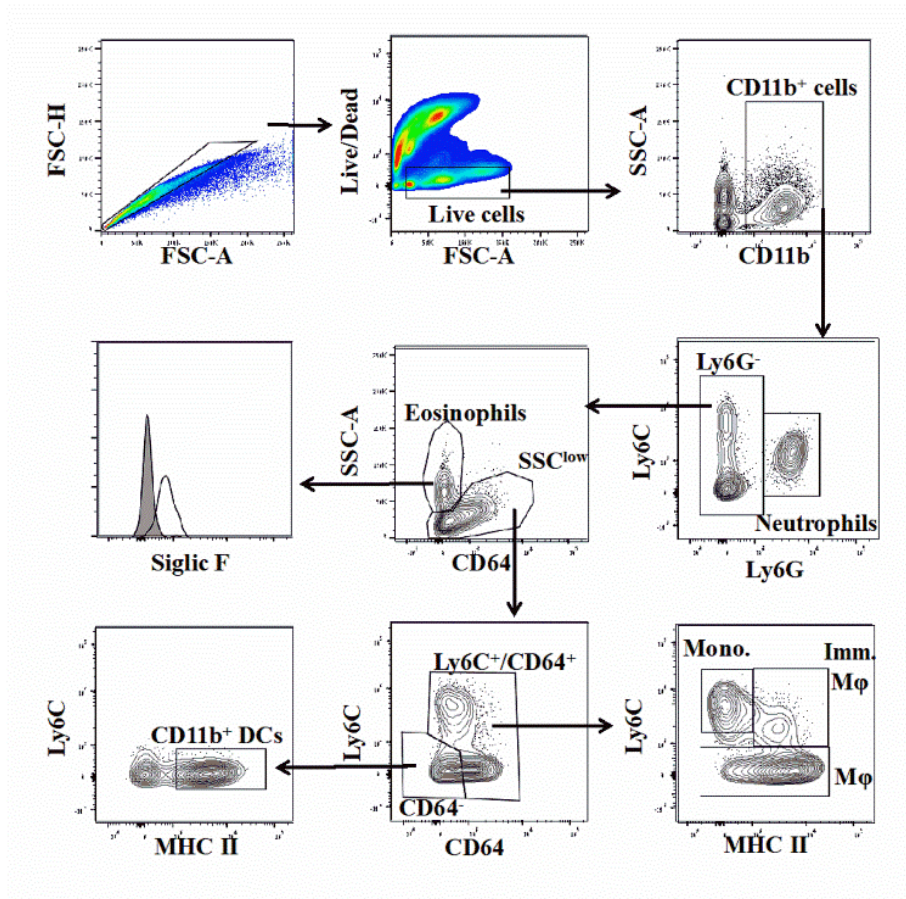
Corresponding author: Ron N. Apte
Email: rapte@bgu.ac.il
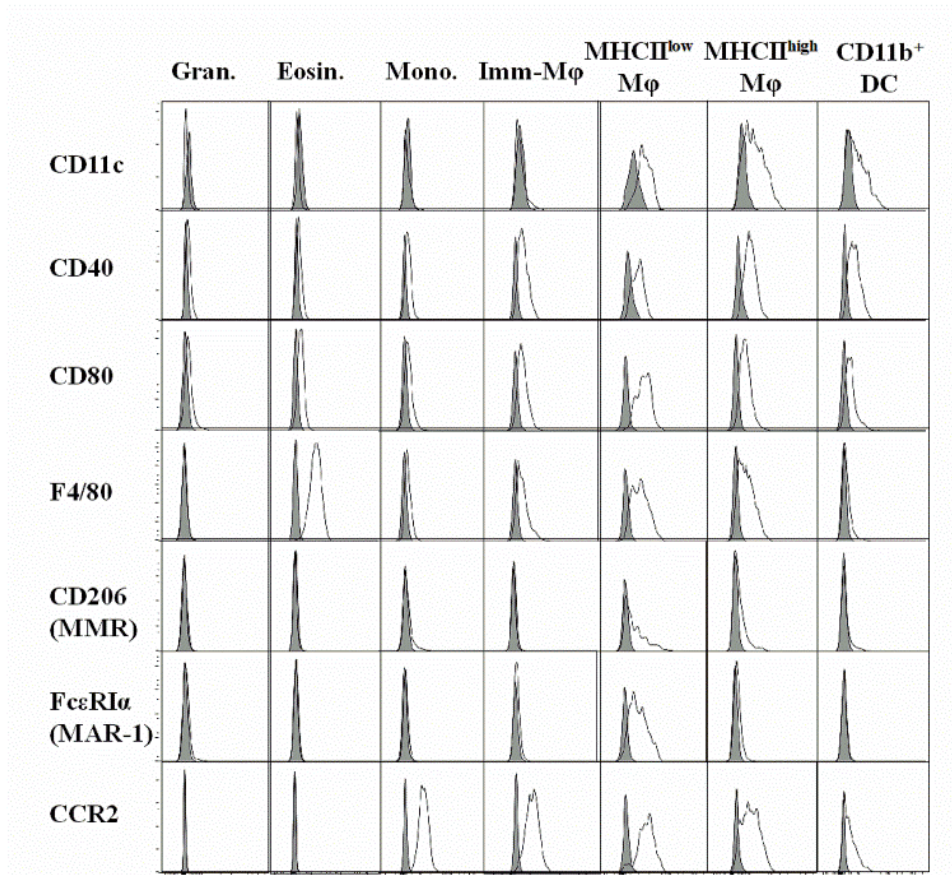
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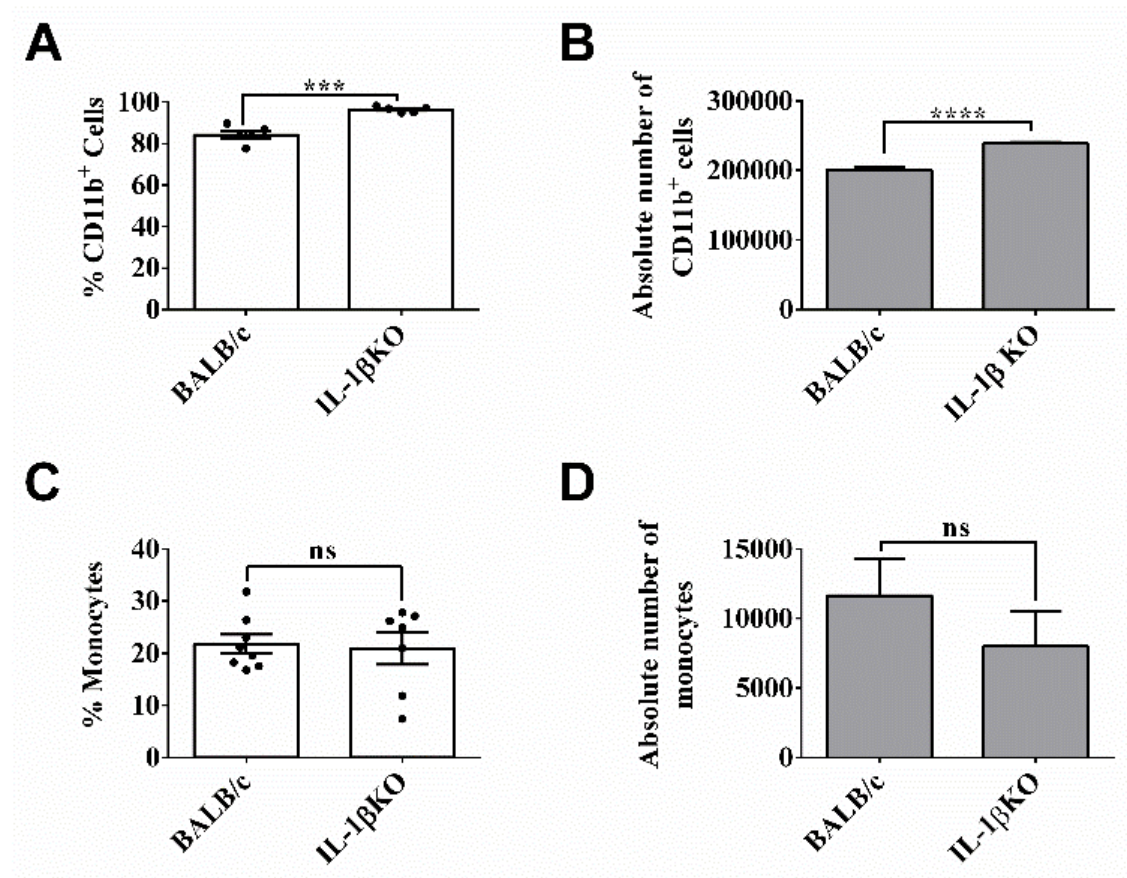**Supplementary Information Text**

Fig. S1.

Myeloid cells gaiting strategy. Gating strategy for identification of myeloid cell populations. Starting from the upper left, arrows indicate directionality of sub-gates. Markers are indicated to the left and bottom of each polychromatic dot plot. Identified populations are marked in black text.

Fig. S2.

Myeloid cells surface antigens. 4T1 tumor-derived myeloid cells were analyzed by Flow cytometry for different membrane antigens. Markers are indicated to the left and cell population type above the histograms. Representative histograms of different myeloid populations stained with specific antibodies (white) and isotype control antibodies (gray).

Fig. S3.

Myeloid cells and monocytes in tumor microenvironment. On day 12 after 4T1 cell injection, BALB/c and IL-1β KO mice were sacrificed and single-cell suspension from the primary tumors was prepared as described (methods). (A) Percentage of CD11b$^+$ cells from CD45$^+$ cells. (B) The absolute number of CD11b$^+$ cells from 250,000 CD45$^+$. (C) Percentage of monocytes from CD11b$^+$Ly6G$^-$SSC^{low} cells. (D) The absolute number of monocytes cells from 250,000 CD45$^+$ cells. Graphs show mean ± SEM, n=4-8, ns, not significant, ***, p<0.001, ****, p<0.0001.

Table S1. List of Primers for qPCR

Gene	Forward	Reverse
CCL2	AGGTCCCTGTCATGCTTCTG	TCTGGACCCATTCCTTCTTG
CSF1	CTTCATGCCAGATTGCCTTT	TCATGGAAAGTTCGGACACA
CD8A	TCAGTTCTGTCGTGCCAGTC	ATCACAGGCGAAGTCCAATC
GZMB	GGGGAGATCATCGGGGGACA	GCCCCCACATATCGCCTCAG
ITGAM	AGTGCTGGGAGACGTGAATG	CACTCAGAGACTGCCCGAAG
IL10	GGGAAGACAATAACTGCACCCA	TGTTTGAAAGAAAGTCTTCACCT
IL12A	TCTTTGATGATGACCCTGTGC	TCTCCCACAGGAGGTTTCTG
IL12B	CACACTGGACCAAAGGGACT	CAGCACCAGCTTCTTCATCA
ACTB	GCATTGTTACCAACTGGGAC	GGTCTCAAACATGATGTGGG

Table S2. List of Flow cytometry antibodies

Antigen	Clone	Source
CD45	30-F11	Biogems, eBioscience
CD11b	M1/70	Biogems, eBioscience
Ly6C	HK1.4	eBioscience
Gr1	RB6-8C5	eBioscience
Ly6G	1A8	Biolegend
CD64	X54-5/7.1	Biolegend
IA/IE	M5/114.15.2	eBioscience
CD206 (MMR)	C068C2	Biolegend
FcεRIα	MAR1	Biolegend
CD11c	N418	eBioscience
IL-12/IL-23 p40	C17.8	eBioscience
CD40	3/23	BD Bioscience
IL-10	JES5 16E3	eBioscience
CCR2	475301	R&D systems
CD3	145-2C11	eBioscience
CD4	GK1.5	eBioscience
CD8	53-6.7	eBioscience
F4/80	BM8	eBioscience
TNFα	MP6-XT22	eBioscience
IFNγ	XMG1.2	eBioscience
SiglecF (CD170)	1RNM44N	eBioscience
CD80	16-10A1	eBioscience
Rat IgG2a	eBR2a	eBioscience
Rat IgG2b, κ	eB149/10H5	eBioscience
Rat IgG1	BRG1	eBioscience
Armenian hamster IgG	eBio299Arm	eBioscience
Mouse IgG1, κ	MOPC-21	Biolegend