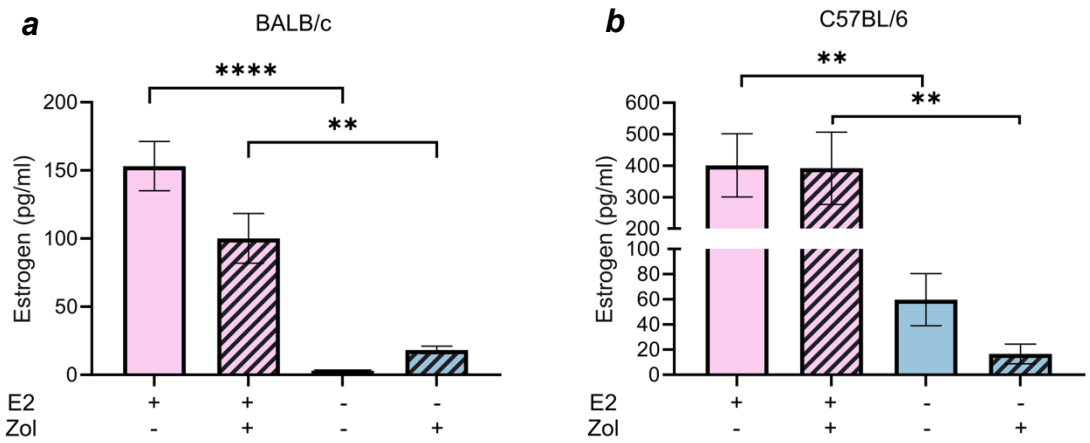
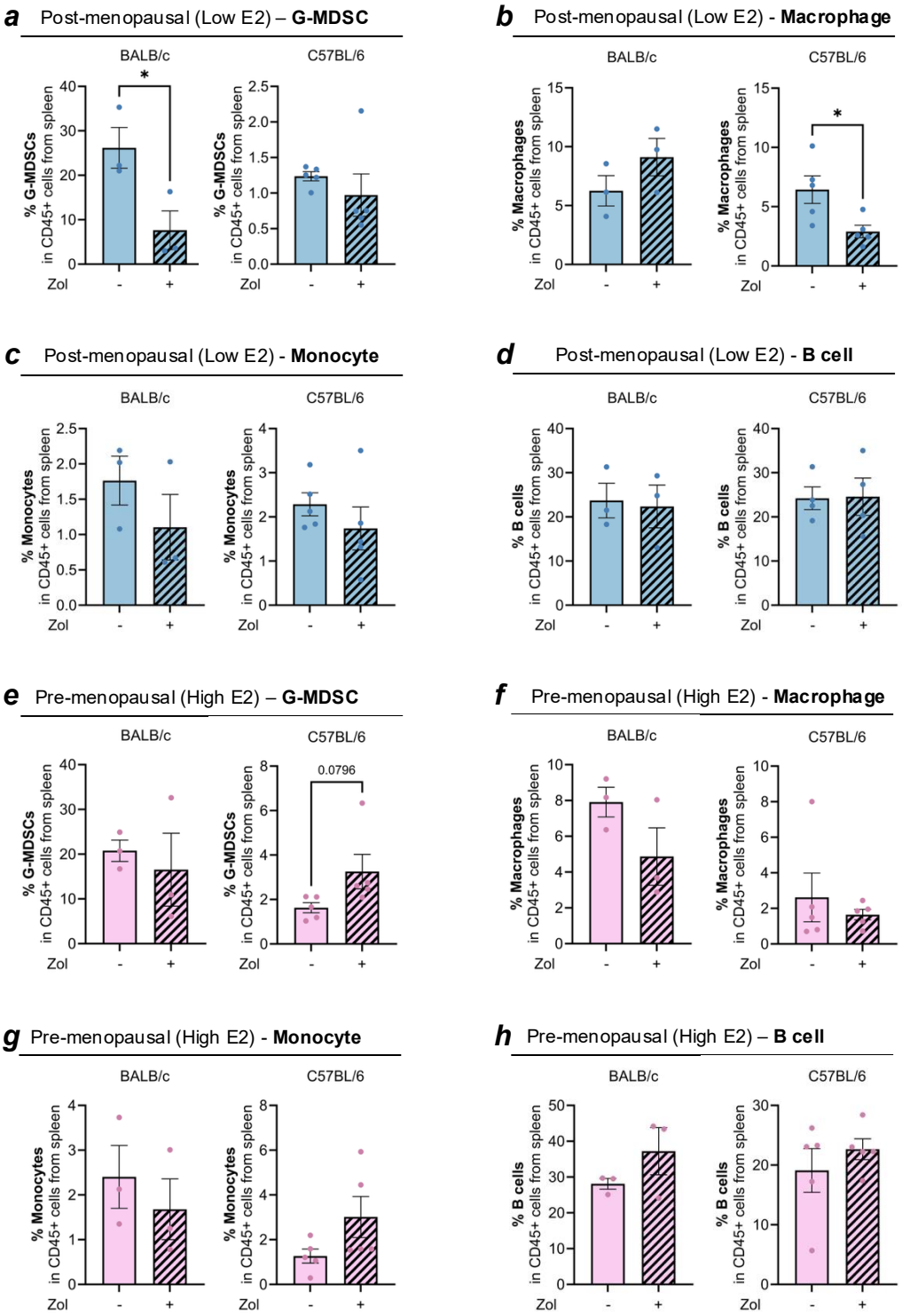


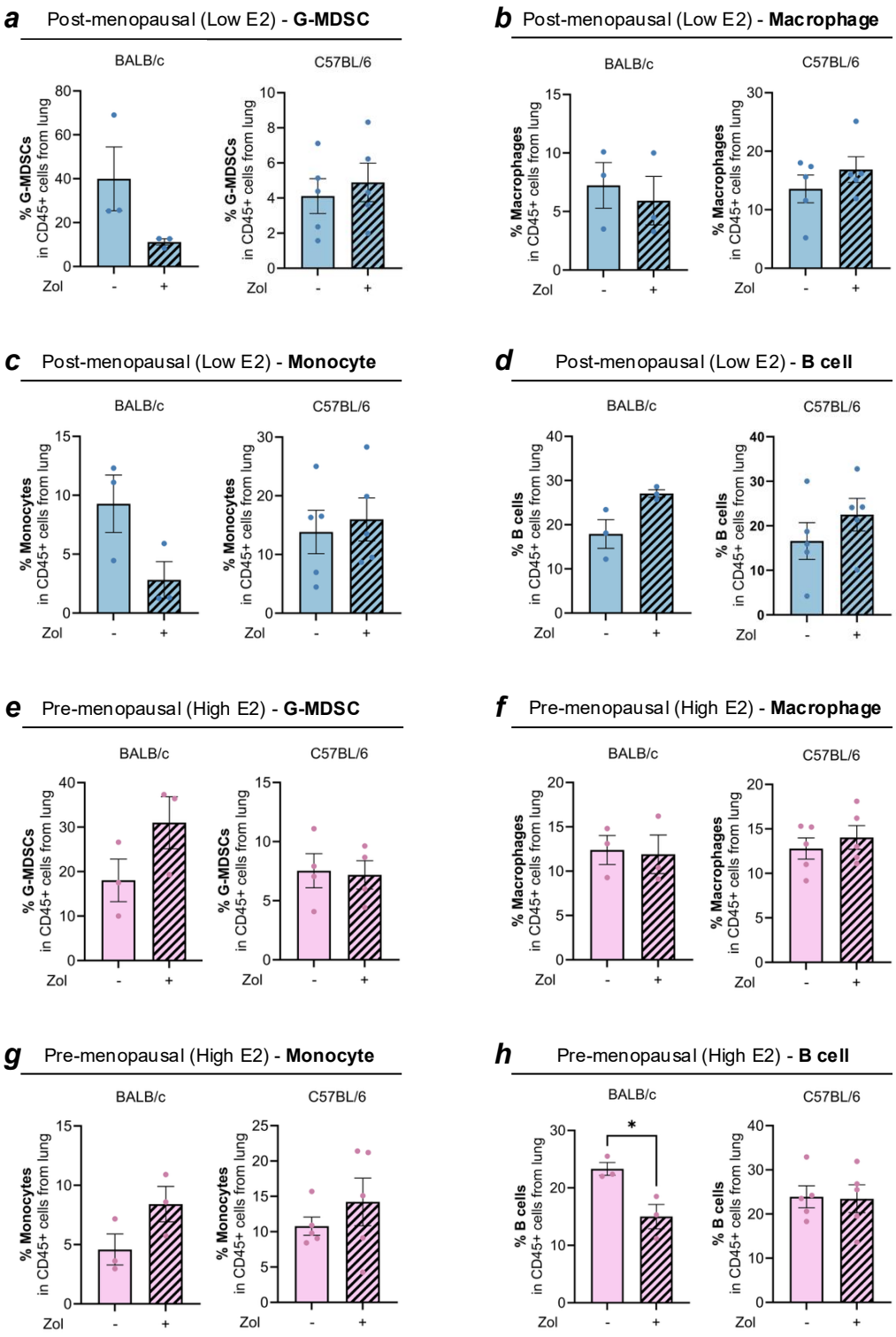
Supplementary figure. 1: Effects of ovariectomy and Zol +/- E2 supplementation on serum E2 concentrations in BALB/c and C57BL/6 mice.

12 - 14-week-old female C57BL/6 or BALB/c mice were ovariectomised and supplementing with 0 or 12.5mg/L 17ß-estradiol (E2) via drinking water plus addition of 40ug/kg/day goserelin. Effects on serum concentration of E2 are shown for BALB/c and C57BL/6 mice in **a)** and **b)** respectively. Data shown are mean +/− SEM, by one way ANOVA and Tucky's post hoc test. *P < 0.05; **P < 0.01, ****P<0.001.



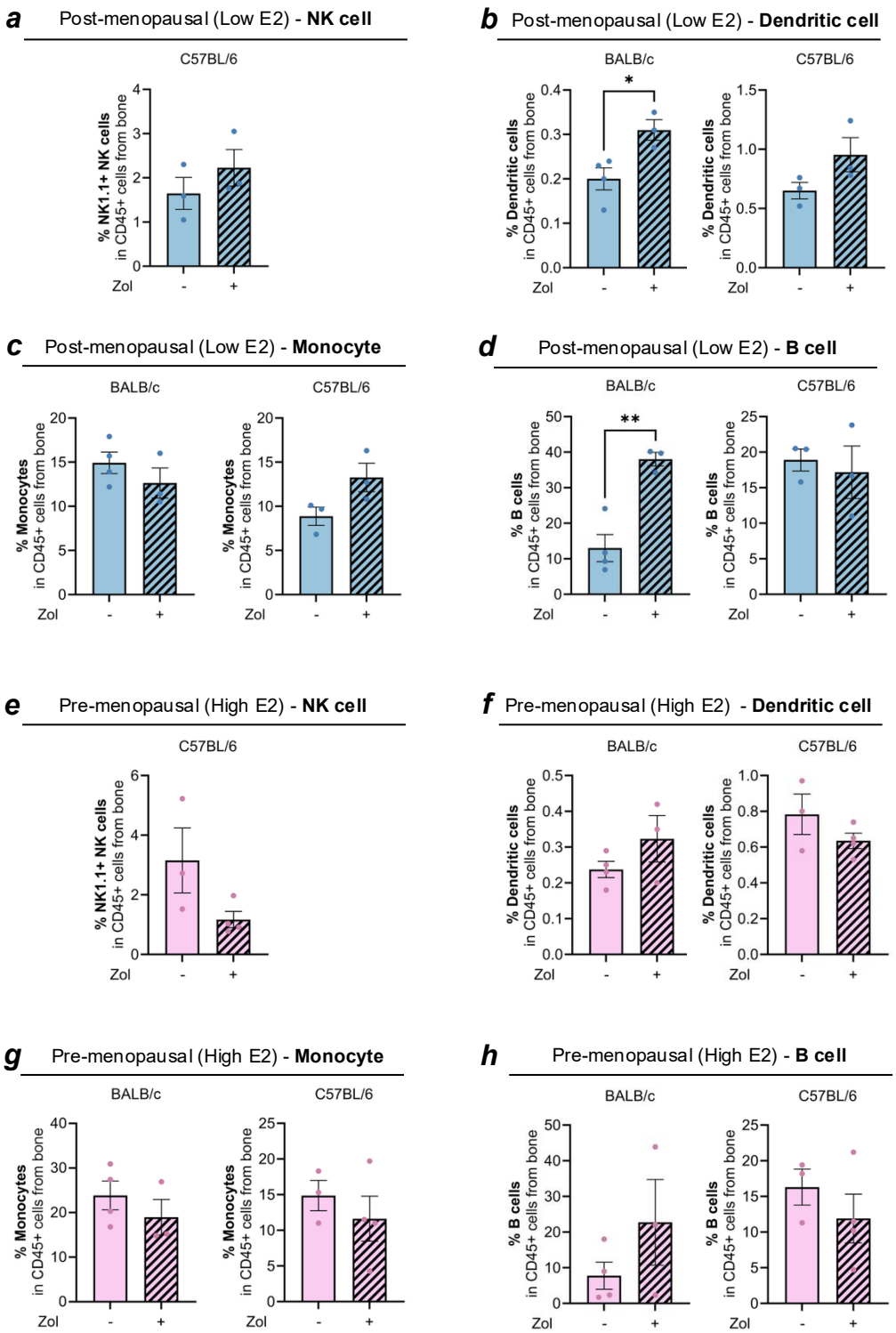
Supplementary figure. 2: Effects of Zol +/- E2 on splenic macrophage, B cell, G-MDSC and monocyte populations in BALB/c and C57BL/6 mice.

BALB/c and C57BL/6 mice were ovariectomized followed by administration of E2/diluent in the drinking water to produce serum E2 concentrations indicative of those found in pre- and postmenopausal women. 4T1-luc2 or E0771-luc2 cells were administered via intracardiac injection 7-days post ovariectomy and mice treated with either PBS control or 100 µg/kg/week Zol 3-days later. Spleens were dissected 9 and 11 days after injection of 4T1-luc2 or E0771-luc2 cells, respectively, and analyzed for relative quantities of immune cell populations. Effects of Zol on **a)** G-MCSC cells, **b)** macrophages, **c)** monocytes and **d)** B cells are shown for low E2 conditions (post-menopausal). Effects of Zol on **e)** G-MCSC, **f)** macrophage, **g)** monocyte and **h)** B cell populations are shown for high E2 conditions (pre-menopausal). Data are mean +/− SEM, t test. *P < 0.05.



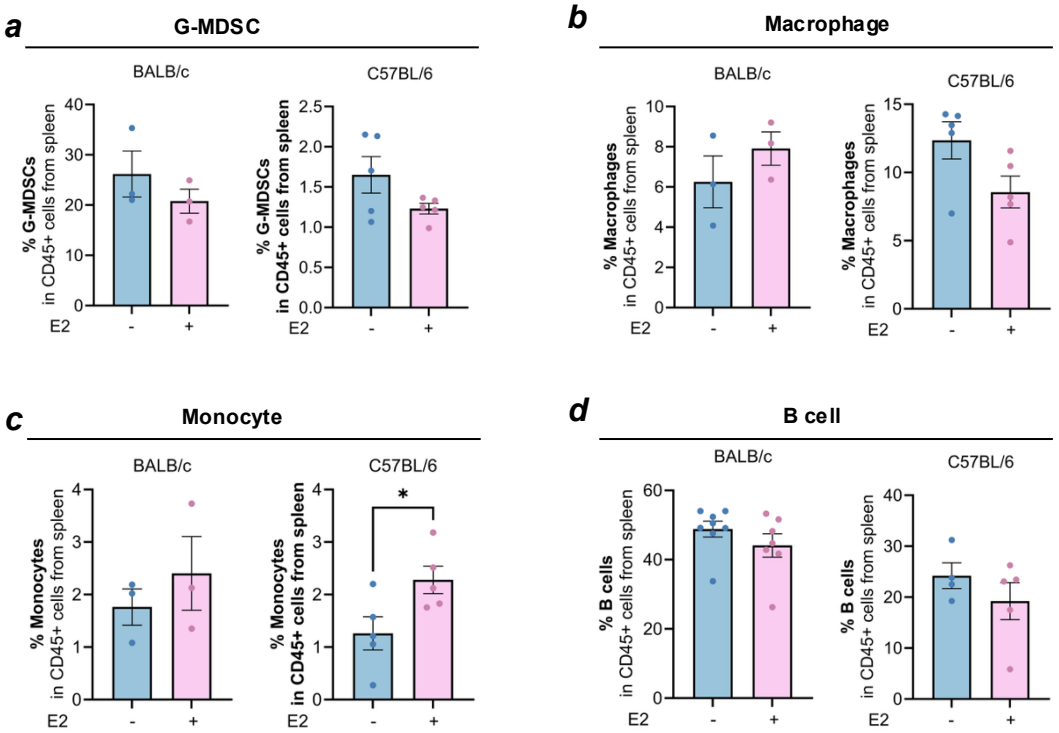
Supplementary figure 3: Effects of Zol +/- E2 on macrophage, B cell, G-MDSC and monocyte populations in tumor bearing lungs from BALB/c and C57BL/6 mice.

BALB/c and C57BL/6 mice were ovariectomized followed by administration of E2/diluent to produce serum E2 concentrations indicative of those found in pre- and postmenopausal women. 4T1-luc2 or E0771-luc2 cells were administered via intracardiac injection 7-days post ovariectomy and mice treated with either PBS control or 100µg/kg/week Zol 3-days later. Tumor bearing lungs were dissected 9 and 11 days after injection of 4T1-luc2 or E0771-luc2 cells, respectively, and analyzed for relative quantities of immune cell populations. Effects of Zol on **a)** G-MCSC cells, **b)** macrophages, **c)** monocytes and **d)** B cells are shown for low E2 conditions (post-menopausal). Effects of Zol on **e)** G-MCSC cells, **f)** macrophages, **g)** monocytes and **h)** B cells are shown for high E2 conditions (pre-menopausal). Data are mean +/− SEM, t test. *$P < 0.05$.



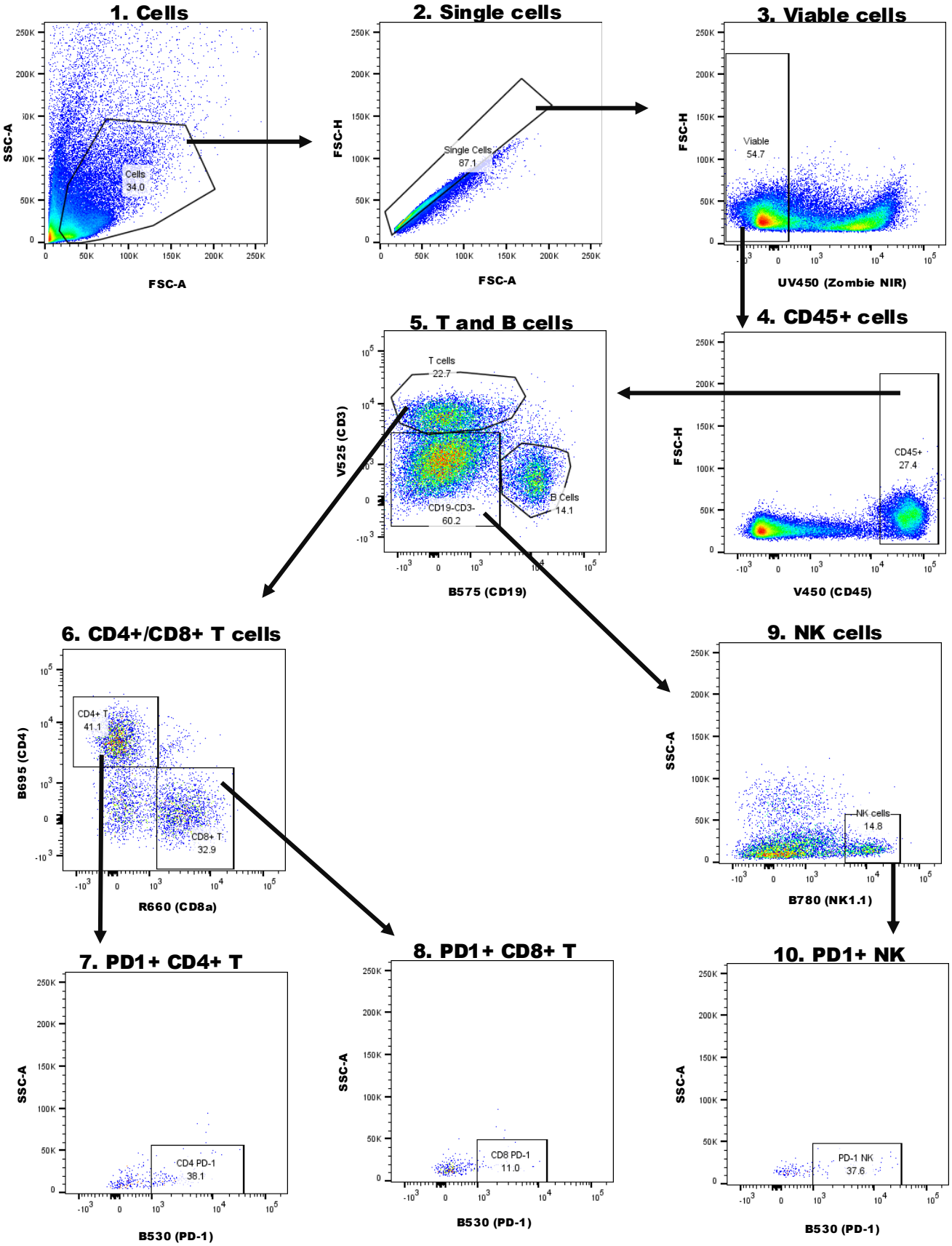
Supplementary figure 4: Effects of Zol +/- E2 on macrophage, monocyte and B cell populations in tumor bearing bones from BALB/c and C57BL/6 mice.

BALB/c and C57BL/6 mice were ovariectomized followed by administration of E2/diluent to produce serum E2 concentrations indicative of those found in pre- and postmenopausal women. 4T1-luc2 or E0771-luc2 cells were administered via intracardiac injection 7-days post ovariectomy and mice treated with either PBS control or 100µg/kg/week Zol 3-days later. Tumor bearing bones were dissected 9 and 11 days after injection of 4T1-luc2 or E0771-luc2 cells, respectively, and analyzed for relative quantities of immune cell populations. Effects of Zol on **a)** NK, **b)** dendritic, **c)** monocyte and **d)** B cells are shown for low E2 conditions (post-menopausal). Effects of Zol on **e)** NK, **f)** dendritic, **g)** monocyte and **h)** B cells are shown for high E2 conditions (pre-menopausal). Data are mean +/− SEM, t test. *P < 0.05, **P < 0.01, ***P < 0.005.



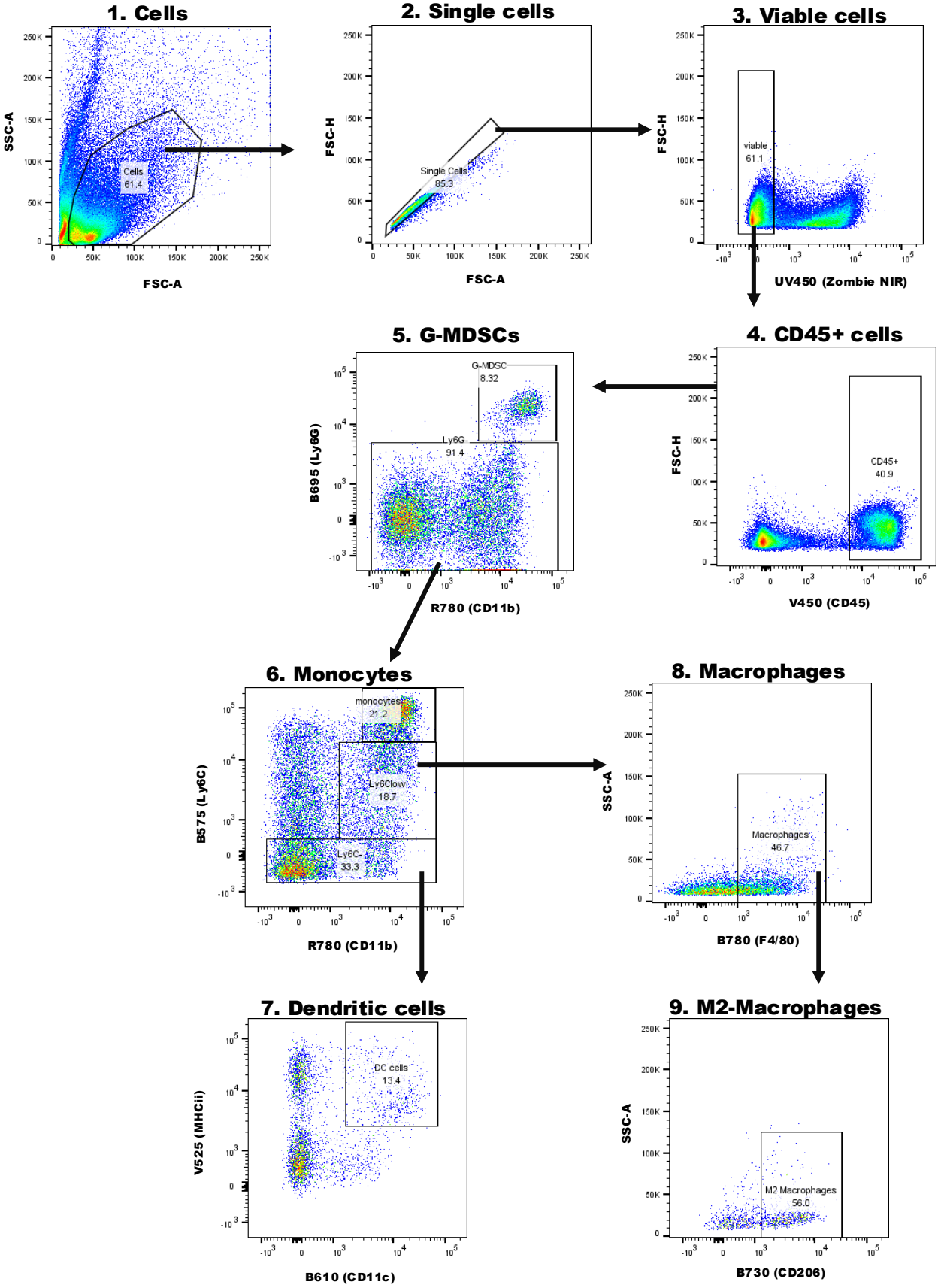
Supplementary figure 5: Effects of Estrogen on systemic G-MDSC cells, macrophages monocytes and B cells.

BALB/c and C57BL/6 mice were ovariectomized followed by administration of E2/diluent to produce serum E2 concentrations indicative of those found in pre- and postmenopausal women. 4T1-luc2 or E0771-luc2 cells were administered via intracardiac injection 7-days post ovariectomy. Effects of E2 on splenic **a)** G-MDSC cells, **b)** macrophages, **c)** monocytes and **d)** B cell populations are shown as mean +/- SEM.



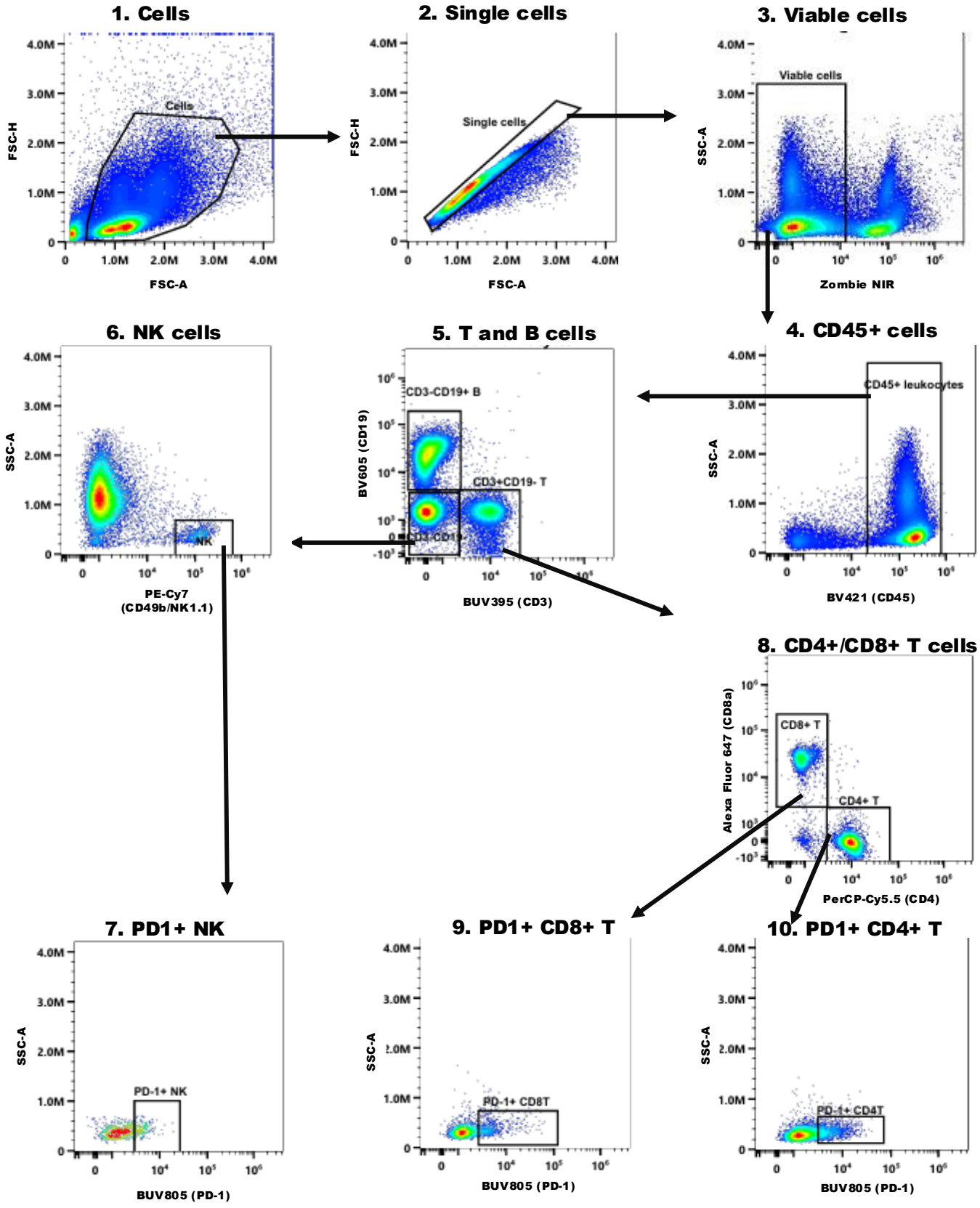
B cell: CD45+ CD3- CD19+
T cell :CD45+ CD3+ CD19-
CD4+ T cell :CD45+ CD3+ CD19- CD4+
CD8+ T cell :CD45+ CD3+ CD19- CD8+
NK cell :CD45+ CD3- CD19- NK1.1+

Supplementary figure. 6: Gating strategy for flow cytometric analysis of lymphoid cell populations using BD LSR II



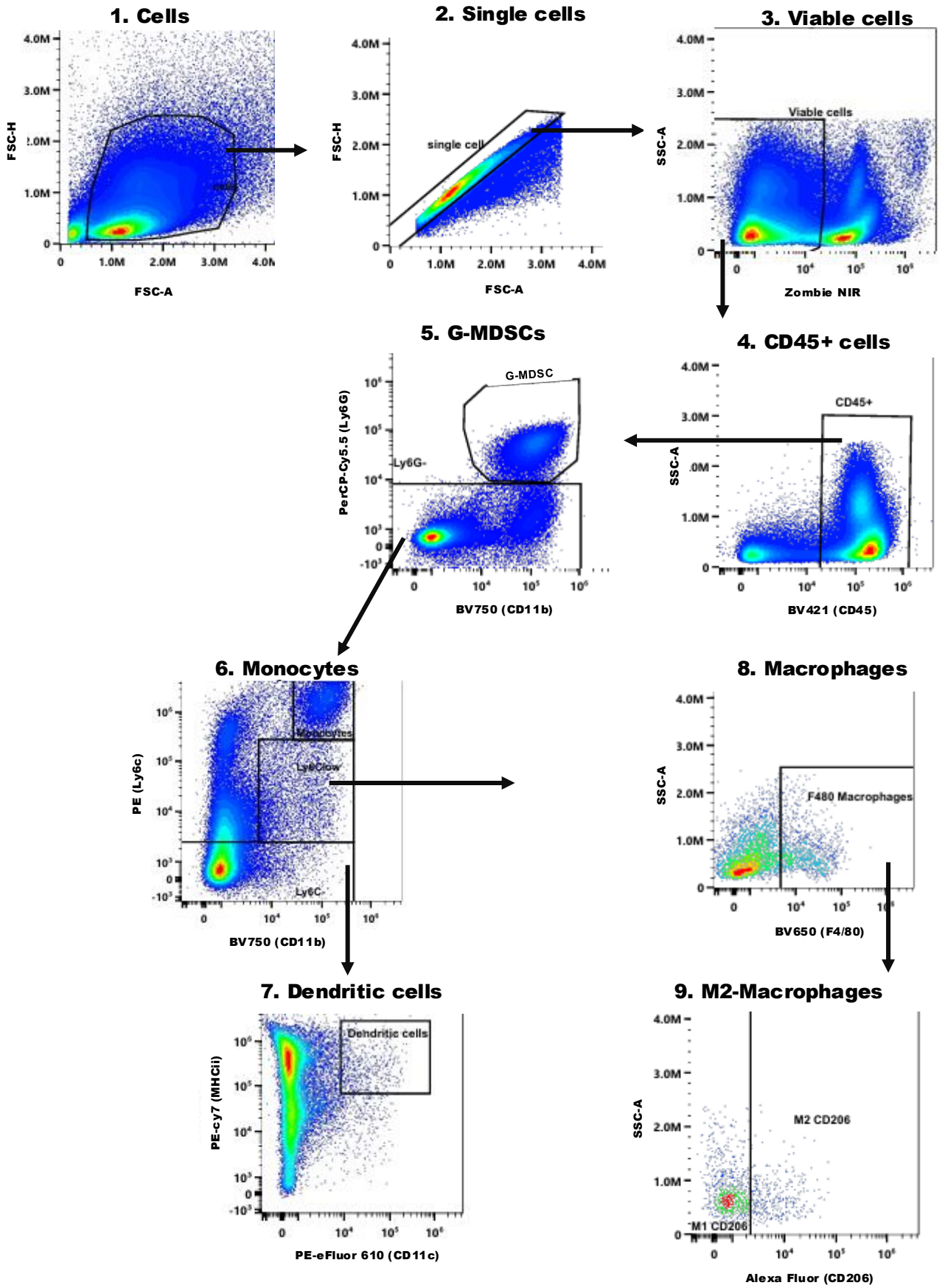
G-MDSC: CD45+ Ly6G+ CD11b+
Monocyte: CD45+ Ly6G- CD11b+ Ly6Chigh
Macrophage: CD45+ Ly6G- CD11b+ Ly6Clow F4/80+
Macrophage: CD45+ Ly6G- CD11b+ Ly6Clow F4/80+ CD206+
Dendritic cell: CD45+ Ly6G- Ly6C- MHC+ CD11c+

Supplementary figure 7: Gating strategy for flow cytometric analysis of myeloid cell populations using BD LSR II



B cell: CD45+ CD3- CD19+
T cell :CD45+ CD3+ CD19-
CD4+ T cell :CD45+ CD3+ CD19- CD4+
CD8+ T cell :CD45+ CD3+ CD19- CD8+
NK cell :CD45+ CD3- CD19- NK1.1+/CD49b+

Supplementary figure. 8: Gating strategy for flow cytometric analysis of lymphoid cell populations using Cytek Aurora 5



G-MDSC: CD45+ Ly6G+ CD11b+
Monocyte: CD45+ Ly6G- CD11b+ Ly6Chigh
Macrophage: CD45+ Ly6G- CD11b+ Ly6Clow F4/80+
Macrophage: CD45+ Ly6G- CD11b+ Ly6Clow F4/80+ CD206+
Dendritic cell: CD45+ Ly6G- Ly6C- MHC+ CD11c+

Supplementary figure 9: Gating strategy for flow cytometric analysis of myeloid cell populations using Cytek Aurora 5

Target	Fluorophore	Species / Reactivity	Isotype	Clone	Supplier	#Cat	Dilution	Filter
Lymphoid panel								
CD45	BV421	Rat anti-Mouse	IgG2b, κ	30-F11	BioLegend	103134	1:100	V450
CD3	BV510	Hamster anti-Mouse	IgG	145-2C11	BioLegend	100353	1:100	V525
CD19	BV605	Rat anti-Mouse	IgG2a, κ	6D5	BioLegend	115540	1:100	B575
CD4	PerCp-Cy5.5	Rat anti-Mouse	IgG2b, κ	GK1.5	BioLegend	100434	1:100	B695
NK1.1	PE-Cy7	Mouse anti-Mouse	IgG2a, κ	PK136	BioLegend	108714	1:100	B780
CD8a	AlexaFluor 647	Rat anti-Mouse	IgG2a, κ	53-6.7	BioLegend	100724	1:100	R660
PD-1	FITC	Rat anti Mouse	IgG2a, κ	J43	Invitrogen	11-9985-82	1:100	B530
Zombie	Zombie NIR	Amine reactive dye			BioLegend	423107	1:100	UV450
Myeloid panel								
CD45	BV421	Rat anti-Mouse	IgG2b, κ	30-F11	BioLegend	103134	1:100	V450
F4/80	PeCy7	Rat anti-Mouse	IgG2a, κ	BM8	BioLegend	123113	1:100	B780
CD11b	BV750	Rat anti-Mouse	IgG2b, κ	M1/70	BioLegend	101267	1:100	R780
Ly6C	PE	Rat anti-Mouse	IgG2c, κ	HK1.4	BioLegend	128008	1:100	B575
Ly6G	PerCP-Cy5.5	Rat anti-Mouse	IgG2a, κ	1A8	BioLegend	127610	1:100	B695
MHCII	PeCy7	Rat anti-Mouse	IgG2b, κ	M5/114.15.2	BioLegend	107630	1:100	V525
CD11c	PE-eFluor610	Hamster anti-Mouse/Human	IgG	N418	Invitrogen	61-0114-82	1:100	B610
CD206	AlexaFluor 700	Rat anti-Mouse	IgG2a, κ	C068C2	BioLegend	141734	1:100	R730
Zombie	Zombie NIR	Amine reactive dye			BioLegend	423107	1:100	UV450

Supplementary table 1: Antibodies for analyzing of immune cell populations on the BD LSR II

Target	Fluorophore	Species/ Reactivity	Isotype	Clone	Supplier	#Cat	Dilution	Filter
Lymphoid panel								
CD45	BV421	Rat anti-Mouse	IgG2b, κ	30-F11	BioLegend	103134	1:200	V1
CD3	BUV395	Hamster anti-Mouse	IgG1, κ	145-2C11	BDHorizon	563565	1:66	UV2
CD19	BV605	Rat anti-Mouse	IgG2a, κ	6D5	BioLegend	115540	1:100	V9
CD25	PE	Rat anti-Mouse	IgG1, λ	PC61	BioLegend	102008	1:200	YG1
CD4	PerCp-Cy5.5	Rat anti-Mouse	IgG2b, κ	GK1.5	BioLegend	100434	1:200	B9
NK1.1	PE-Cy7	Mouse anti-Mouse	IgG2a, κ	PK136	BioLegend	108714	1:200	YG9
CD49b	PE-Cy7	Hamster anti-Mouse	IgG	HMα2	BioLegend	103518	1:100	YG9
CD8a	AlexaFluor 647	Rat anti-Mouse	IgG2a, κ	53-6.7	BioLegend	100724	1:100	R1
CD69	AlexaFluor 488	Hamster anti-Mouse	IgG	H1.2F3	BioLegend	104516	1:100	B2
PD-1	BUV805	Rat anti-Mouse	IgG2a, κ	29F.1A12	BDHorizon	568609	1:100	UV16
Zombie	Zombie NIR	Amine reactive dye			BioLegend	423107	1:500	R5
Myeloid panel								
CD45	BV421	Rat anti-Mouse	IgG2b, κ	30-F11	BioLegend	103134	1:200	V1
F4/80	BV650	Rat anti-Mouse	IgG2a, κ	BM8	BioLegend	123149	1:66	V11
CD11b	BV750	Rat anti-Mouse	IgG2b, κ	M1/70	BioLegend	101267	1:100	V14
Ly6C	PE	Rat anti-Mouse	IgG2c, κ	HK1.4	BioLegend	128008	1:200	YG1
Ly6G	PerCP-Cy5.5	Rat anti-Mouse	IgG2a, κ	1A8	BioLegend	127610	1:100	B9
MHCII	PeCy7	Rat anti-Mouse	IgG2b, κ	M5/114.15.2	BioLegend	107630	1:100	YG9
CD11c	PE-eFluor610	Hamster anti-Mouse/Human	IgG	N418	Invitrogen	61-0114-82	1:66	YG2
CD163	APC	Rat anti Mouse	IgG2a, κ	S15049I	BioLegend	155306	1:200	R1
CD206	AlexaFluor 700	Rat anti Mouse	IgG2a, κ	C068C2	BioLegend	141734	1:100	R4
Zombie	Zombie NIR	Amine reactive dye			BioLegend	423107	1:500	R7

Supplementary table 2: Antibodies for analyzing of immune cell populations on the Cytek Aurora 5