Supplemental Table 1

	p_val	avg_logFC	p_val_adj
Ccl5	9.48E-15	1.64375	2.95E-10
Slpi	4.58E-12	1.32228	1.42E-07
Cxcl9	6.57E-13	1.29667	2.04E-08
C3	6.84E-22	1.27328	2.12E-17
Dusp1	9.80E-13	1.26357	3.04E-08
AA467197	3.52E-11	1.1813	1.09E-06
Ass1	7.62E-18	1.1622	2.37E-13
Ly6i	6.51E-24	1.06324	2.02E-19
Clec4d	8.96E-18	1.05614	2.78E-13
Neat1	1.30E-13	1.02051	4.05E-09
AW112010	7.45E-14	0.98972	2.31E-09
Fth1	3.87E-25	0.96366	1.20E-20
H2-Q7	8.31E-15	0.94834	2.58E-10
Ly6a	1.77E-19	0.91886	5.49E-15
Acod1	4.99E-14	0.84767	1.55E-09
Txn1	6.17E-15	0.80481	1.92E-10
Fosb	2.33E-13	0.72134	7.23E-09
Bst1	1.25E-17	0.70764	3.90E-13
B2m	2.21E-13	0.66535	6.87E-09
C1qc	8.51E-12	-0.42674	2.64E-07
mt-Nd1	2.22E-11	-0.42989	6.89E-07
C1qa	7.49E-13	-0.43631	2.33E-08
mt-Co2	1.47E-13	-0.44139	4.56E-09
mt-Cytb	1.89E-13	-0.44456	5.86E-09
mt-Nd4	1.88E-11	-0.44992	5.84E-07
mt-Co3	1.27E-15	-0.47723	3.94E-11
Tpm3	1.19E-13	-0.50229	3.69E-09
Ms4a7	7.37E-12	-0.64787	2.29E-07
mt-Nd3	1.73E-13	-0.65141	5.36E-09
mt-Atp6	1.02E-22	-0.66727	3.17E-18
Dab2	4.80E-15	-0.69147	1.49E-10
Fcrls	5.70E-12	-0.69249	1.77E-07
Tmem176a	1.45E-13	-0.70649	4.49E-09
Tmem176b	1.99E-12	-0.72074	6.18E-08
Mgl2	1.84E-14	-0.73344	5.71E-10
Lars2	7.17E-20	-0.78017	2.23E-15
Cd81	9.48E-17	-0.78229	2.94E-12
Apoe	5.75E-22	-0.86455	1.79E-17
Selenop	1.24E-17	-0.91699	3.86E-13
AY036118	1.25E-71	-2.99932	3.89E-67

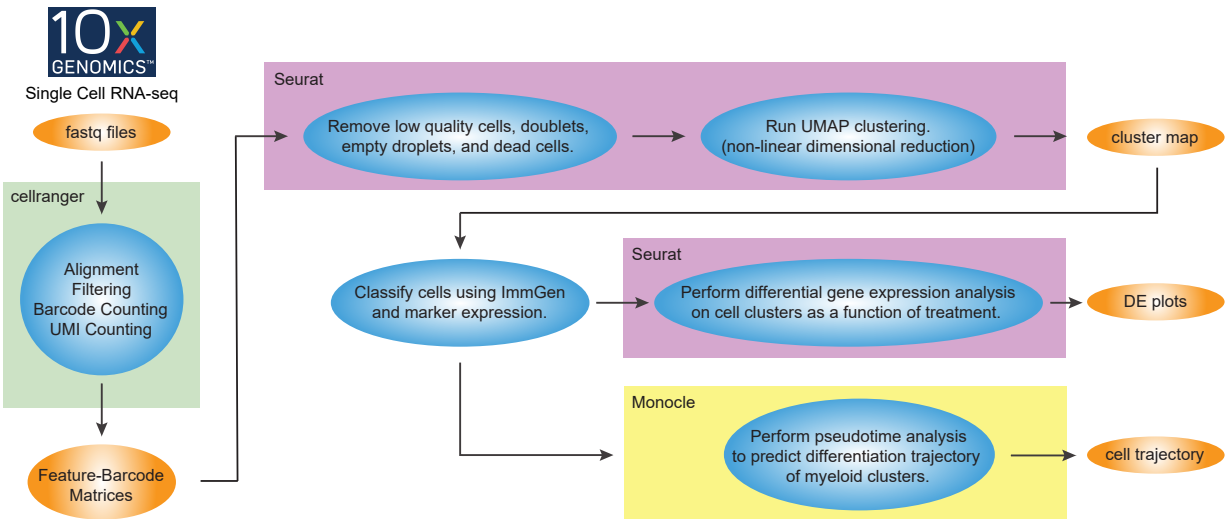
Supplemental Table 2

Target	Clone	Fluorophore	Source
F4/80	BM8	FITC	BioLegend
CD11b	M1/70	PerCP-Cy5.5	BD
CCL5	2E9/CCL5	PE	BioLegend
CD64	X54-5/7.1	PE-CF594	BioLegend
FoxP3	FJK-16s	PE-Cy5	Invitrogen
CD8a	53-6.7	PE-Cy7	BD
CD11c	HL3	APC	BD
CD45	30-F11	AF700	BioLegend
NK1.1	PK136	APC-Cy7	BioLegend
CD103	2E7	BV421	BioLegend
Viability	n/a	LD Aqua	Invitrogen
Ly6C	HK1.4	BV605	BioLegend
CD3	17A2	BV650	BioLegend
CD19	6D5	BV711	BioLegend
B220	RA3-6B2	BV711	BioLegend
I-A/I-E	M5/114.15.2	BV785	BioLegend
CD4	GK1.5	BUV395	BD
Ly6G	1A8	BUV805	BD
Tim3	RMT3-23	FITC	Invitrogen
LAG-3	C9B7W	PerCP-Cy5.5	BioLegend
CTLA-4	UC10-4B9	PE-CF594	BioLegend
CD62L	MEL-14	PE-Cy5	BioLegend
CD39	Duha59	AF647	BioLegend
FoxP3	MF-14	BV421	BioLegend
PD-1	29F.1A12	BV605	BioLegend
NK1.1	PK136	BV711	BioLegend
CD44	IM7	BV785	BioLegend
CD8a	53-6.7	BUV805	BD

A

Treatment Group	Sample Number	Estimated Number of Cells	Mean Reads per Cell	Median Genes per Cell
 Untreated	1	1,067	79,663	595
	2	712	66,662	730
	3	1,325	57,646	865
	4	1,816	55,261	1,019
 CD40 Agonist	1	1,774	60,080	1,173
	2	927	84,328	596
	3	252	51,345	1,036
	4	2,902	31,436	1,618
 ICB (αCTLA4+αPD-1)	1	2,670	43,033	889
	2	1,263	80,614	758
	3	1,516	74,173	1,514
	4	1,341	76,166	1,036
 CD40 + ICB	1	2,830	14,729	986
	2	5,324	7,225	833
	3	2,251	19,814	1,144
	4	3,738	12,005	1,057

B

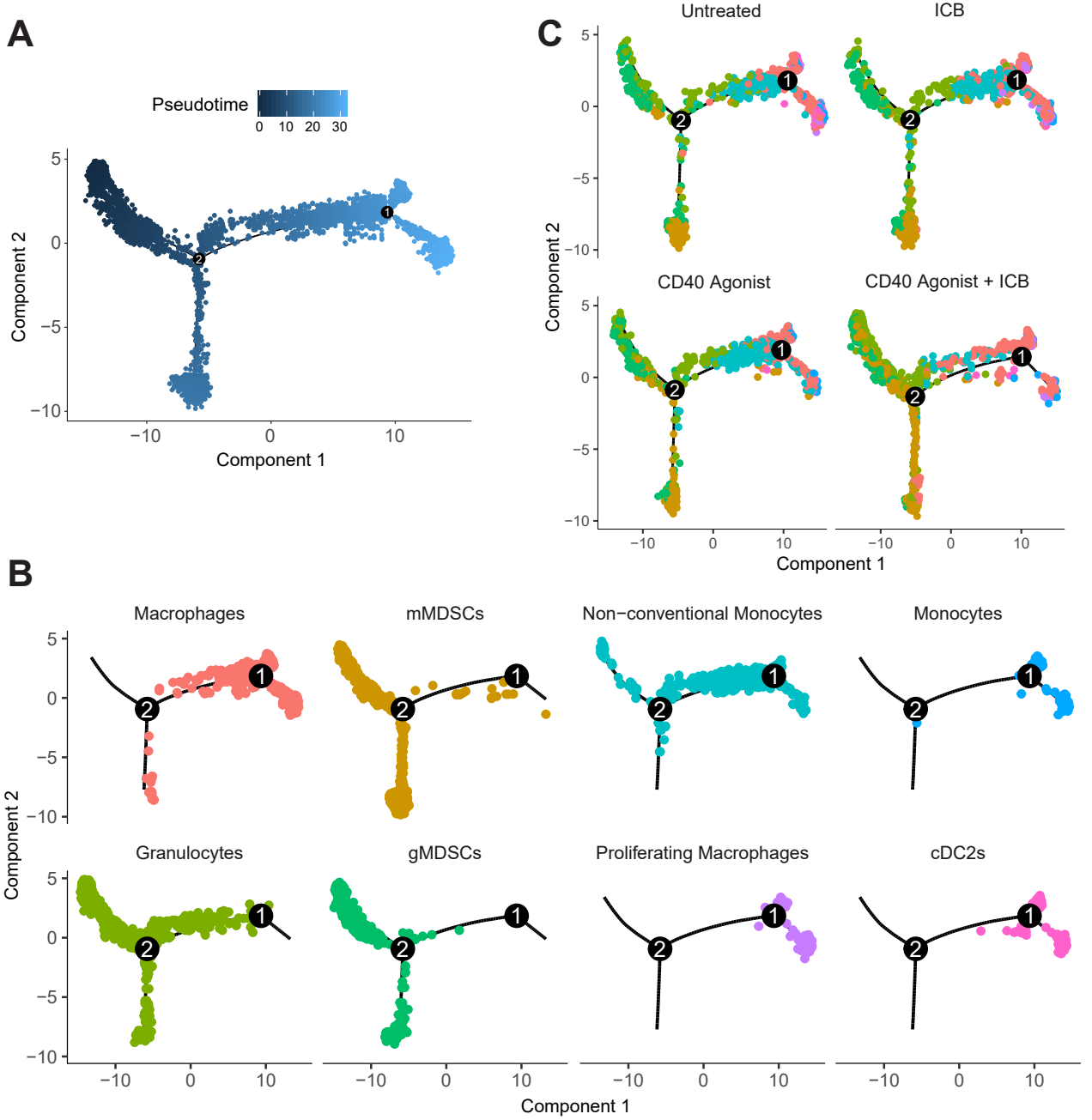


Supplemental Figure 1

Supplemental Figure 1 Single cell analysis pipeline and details.

(A) Cell and transcriptomic metrics from each single-cell library. Metrics were generated using the 10X Genomics CellRanger 3.0 software.

(B) Single-cell transcriptomic analysis pipeline following library sequencing. Software packages are color-coded.



Supplemental Figure 2

Supplemental Figure 2 Myeloid cell differentiation is unaffected by treatment with CD40 agonist and immune checkpoint blockade.

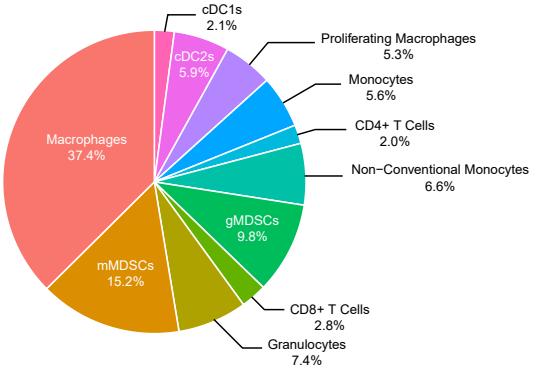
(A) Pseudotime trajectory of myeloid cell clusters across all treatment groups as calculated using Monocle2.

(B) Plots of each myeloid cell cluster along pseudotime trajectory.

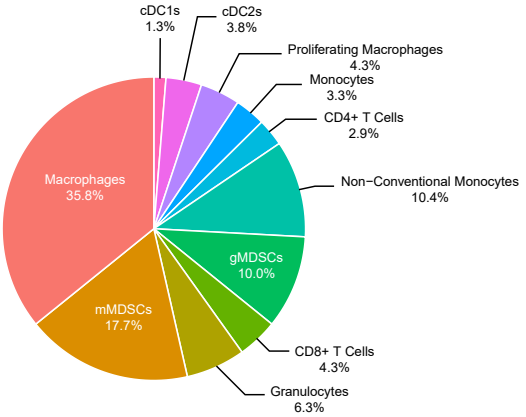
(C) Pseudotime trajectory of myeloid cell clusters split by treatment group.

(A): n=6,510 cells from myeloid cell clusters across all treatment groups as determined in Seurat were used as input for Monocle pseudotime analysis. (B): n=1,764 macrophages, n=1,301 mMDSCs, n=1218 granulocytes, n=802 gMDSCs, n=710 non-conventional monocytes, n=252 monocytes, n=241 proliferating macrophages, and n=222 cDC2s shown. (C): n=1,448 untreated, n=1,635 ICB, n=1,284 CD40 agonist, n=2,143 CD40/ICB cells shown.

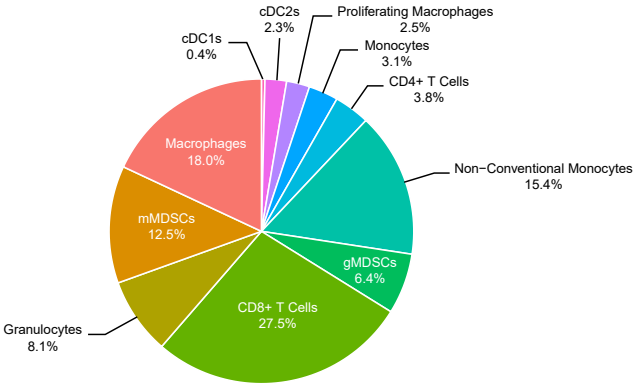
Untreated



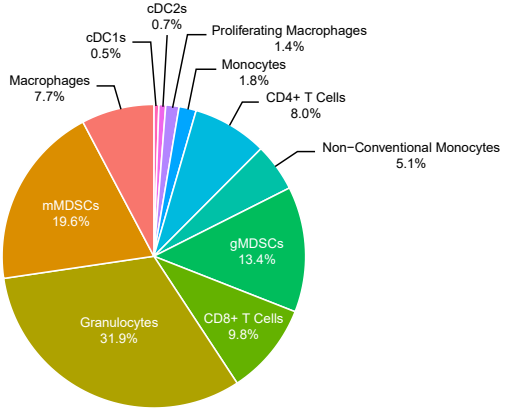
ICB



CD40 Agonist



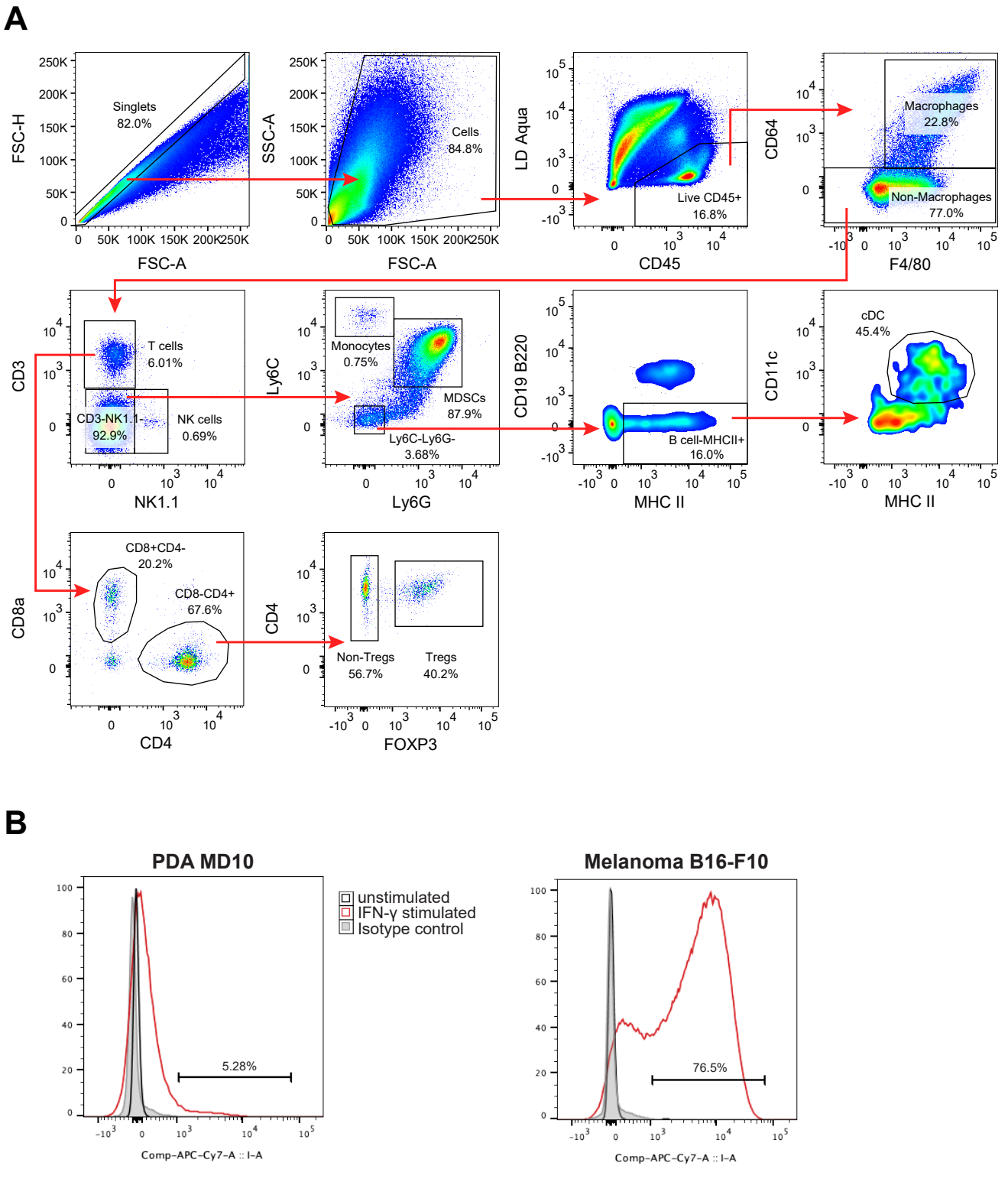
CD40 Agonist + ICB



Supplemental Figure 3

Supplemental Figure 3

Proportion of each immune cell cluster split by treatment group.

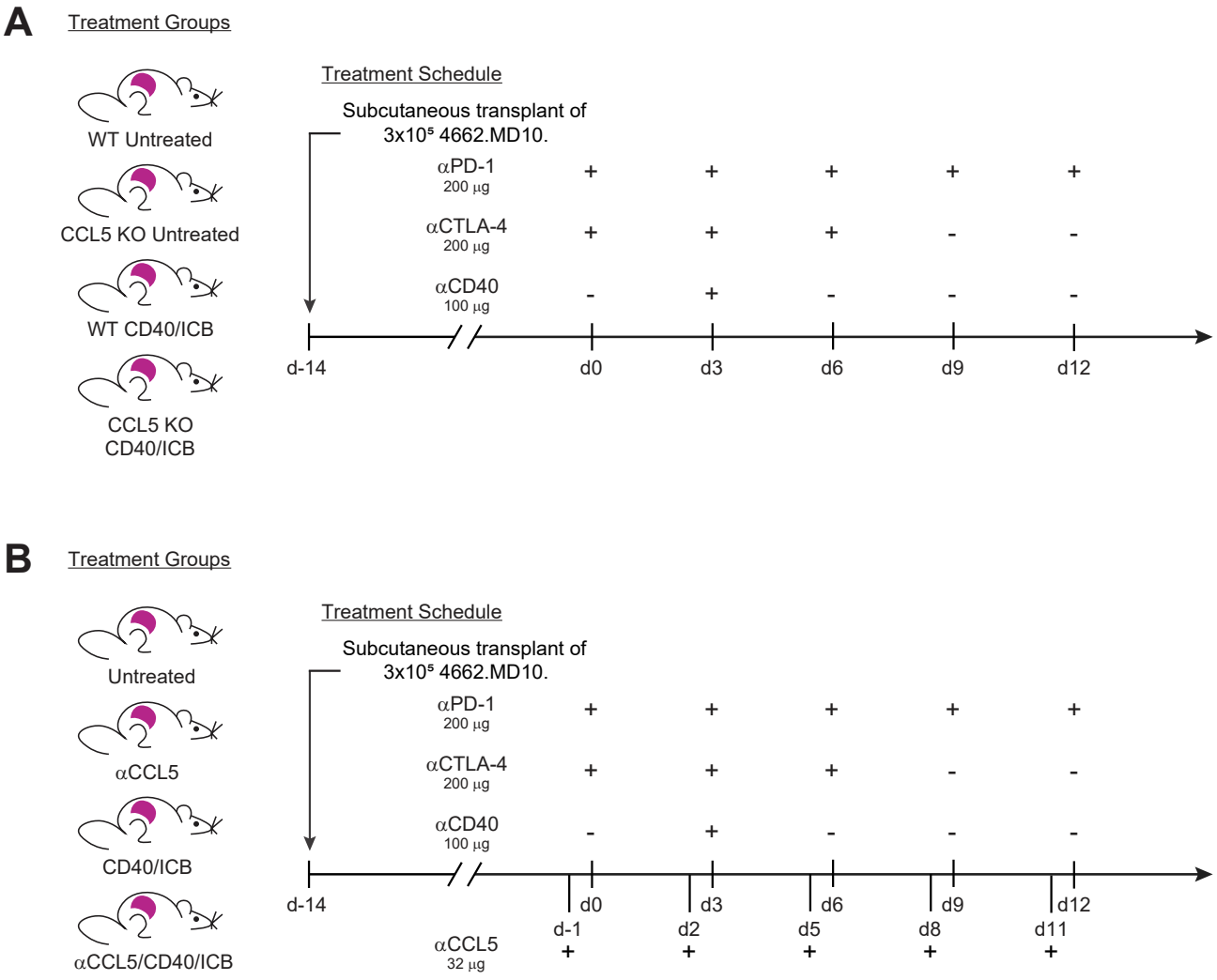


Supplemental Figure 4

Supplemental Figure 4

(A) Gating scheme for flow cytometric identification of immune populations in a representative subcutaneously implanted KPC tumor.

(B) Flow cytometry comparison of 4662.MD10 and B16-F10 MHCII expression.

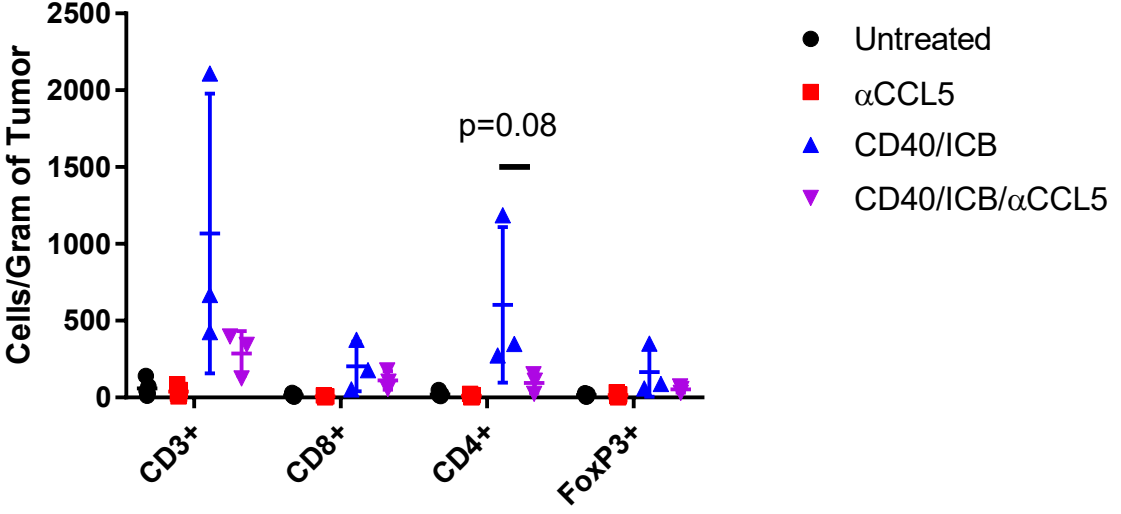


Supplemental Figure 5

Supplemental Figure 5

(A) Treatment schema of C57BL/6J and CCL5 KO mice subcutaneously implanted with clonal KPC cell line *4662.MD10* with combination CD40/ICB.

(B) Treatment schema of C57BL/6J mice subcutaneously implanted with clonal KPC cell line *4662.MD10* with combination CD40/ICB +/- anti-CCL5.


Cells/Gram of Tumor
2500
2000
1500
1000
500
0
p=0.08
CD3+
CD8+
CD4+
FoxP3+
Untreated
αCCL5
CD40/ICB
CD40/ICB/αCCL5

Supplemental Figure 6

Enumeration of T-cell populations per gram of tumor in mice treated with combination CD40/ICB +/- αCCL5 day 12 post-implantation. Corresponds to Figure 5B.