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Supplemental information

Engineering cGAS-agonistic oligonucleotides as therapeutics for cancer immunotherapy

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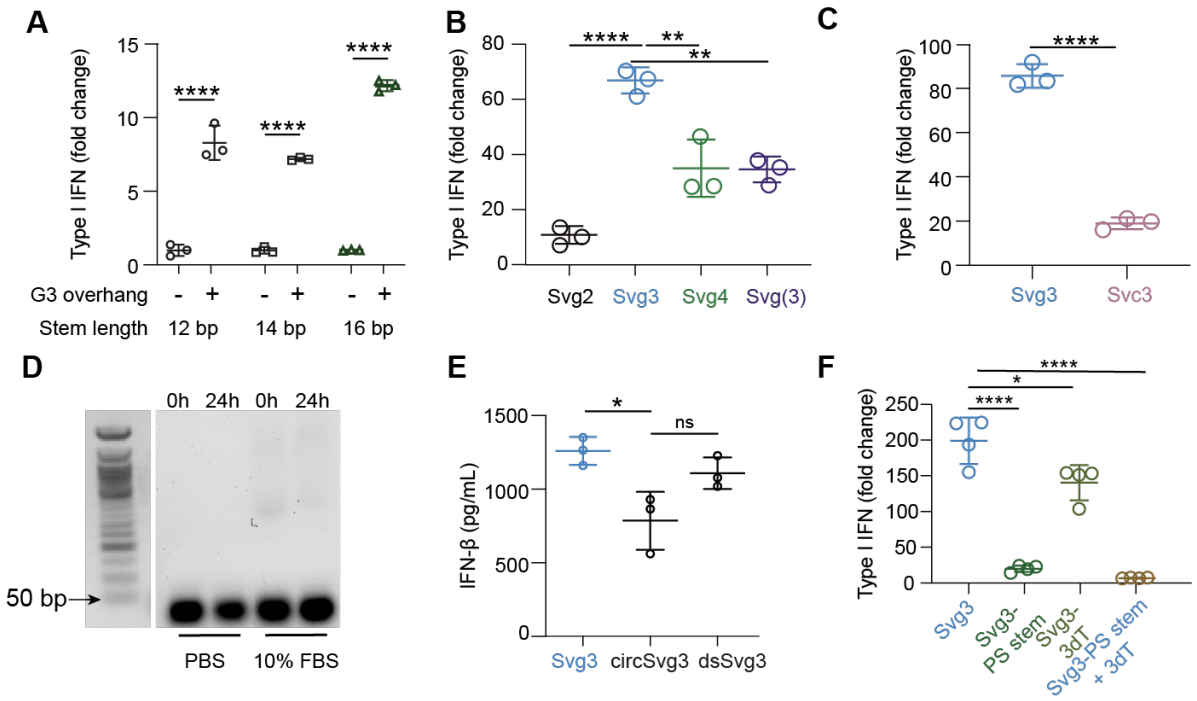

Figure S1. **Screening for and characterization of Svg3 as a cGAS agonist.** A) IFN-I response in Raw-ISG cells after 24 h transfection with short hairpin DNAs with or without consecutive guanosine in the overhangs. B) IFN-I response in Raw-ISG cells after 24 h transfection with different G content in the overhangs of short hairpin DNAs. C) Comparison of IFN-I response in Raw-ISG cells of hairpin DNA containing consecutive guanosine or cytosine in the overhangs. D) Integrity of Svg3 after incubation in PBS or cell culture medium containing 10% FBS for 24 h verified by 2% agarose gel electrophoresis. E) IFN-β expression in Raw 264.7 cells after transfection of Svg3, circularized Svg3 or double-stranded Svg3 (with two open overhangs) respectively, for 24 h. F) IFN-I response in Raw-ISG cells after 24 h transfection of chemically modified Svg3.

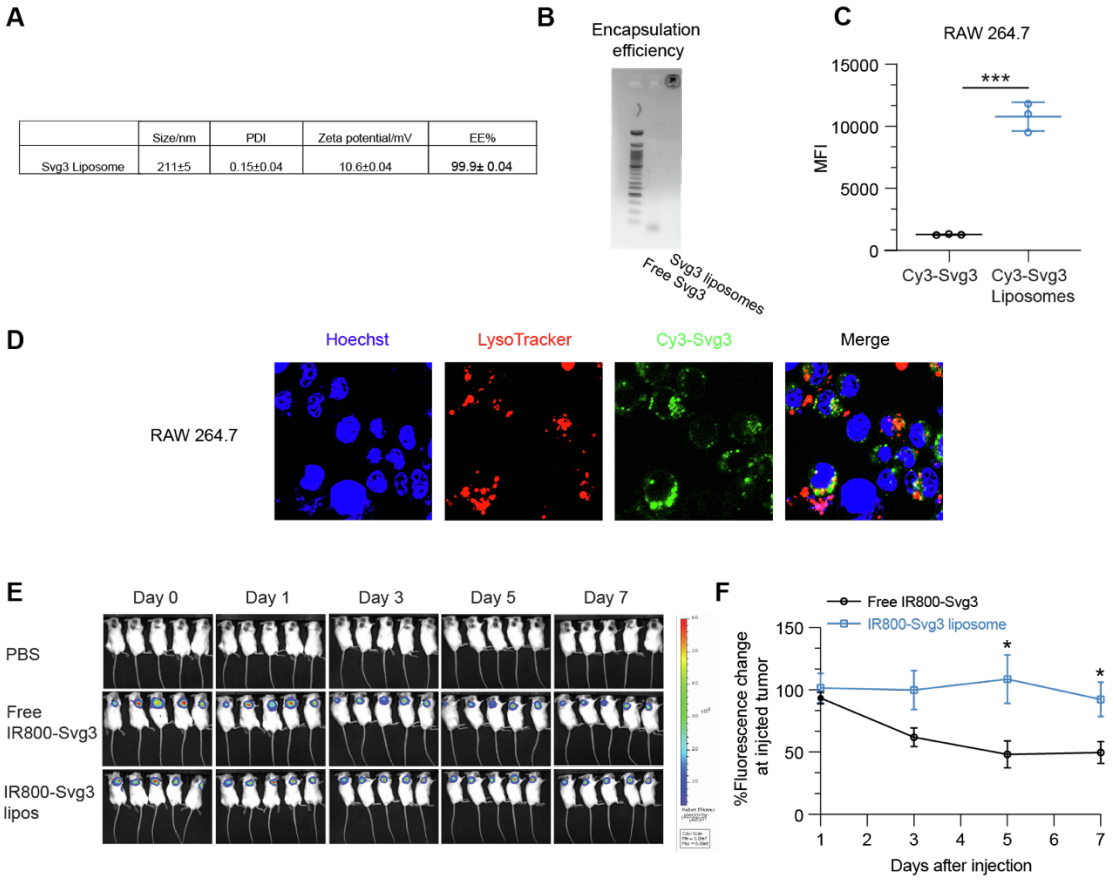

Figure S2. Liposomes readily loaded Svg3 for efficient delivery and retention of Svg3 in tissues and cells. A) Characterization of the size, polydispersity index (PDI), zeta potential and encapsulation efficiency (EE%) of nanoparticles used in the study. B) Encapsulation efficiency of Svg3 liposomes evaluated by 2% agarose gel electrophoresis. C) Median Cy3 fluorescence intensity from Cy3-Svg3 loaded in liposomes. D) Confocal images of RAW 264.7 cells after incubated with liposomal Cy3-Svg3 for 5 h. E) IVIS imaging of 4T1 tumors after i.t. injection of liposomal IR800-Svg3. F) Fluorescence intensities of 4T1 tumors quantified from IVIS imaging results.

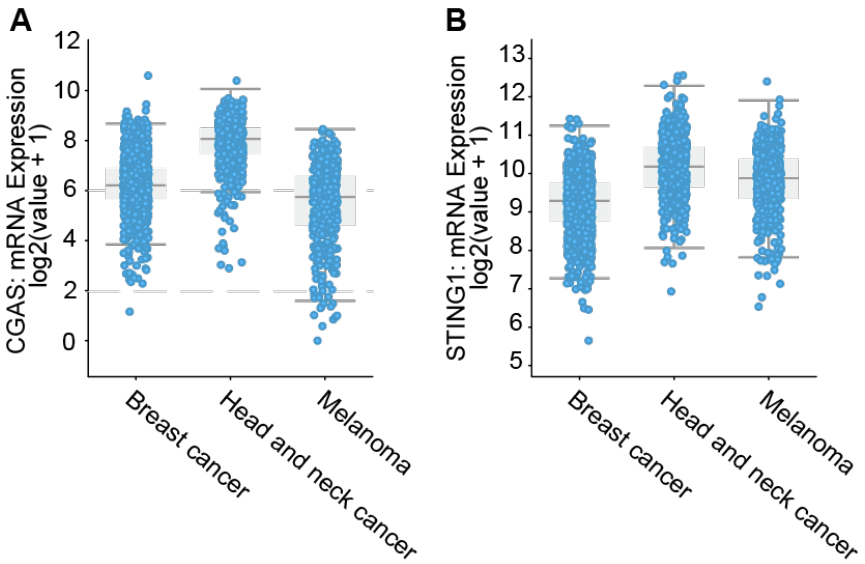

Figure S3. The transcript levels of *cGas* (A) and *Sting* (B) in the indicated three types of human cancers. Human transcript data were downloaded from cBioPortal.

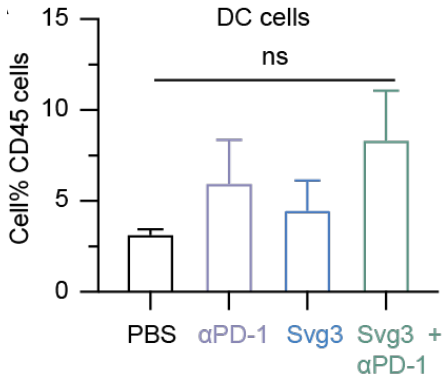

Figure S4. Percentages of DC cells among $CD45^{+}$ cells in as-treated 4T1 tumors.

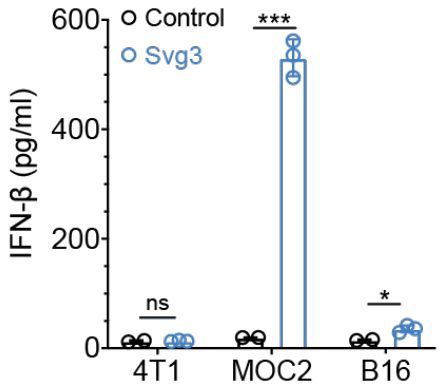

Figure S5. Svg3 elicited different levels of IFN-I responses in three types of murine tumor cells, as shown by the IFN-β levels in the culture medium after Svg3 transfection (500 nM, 24 h).

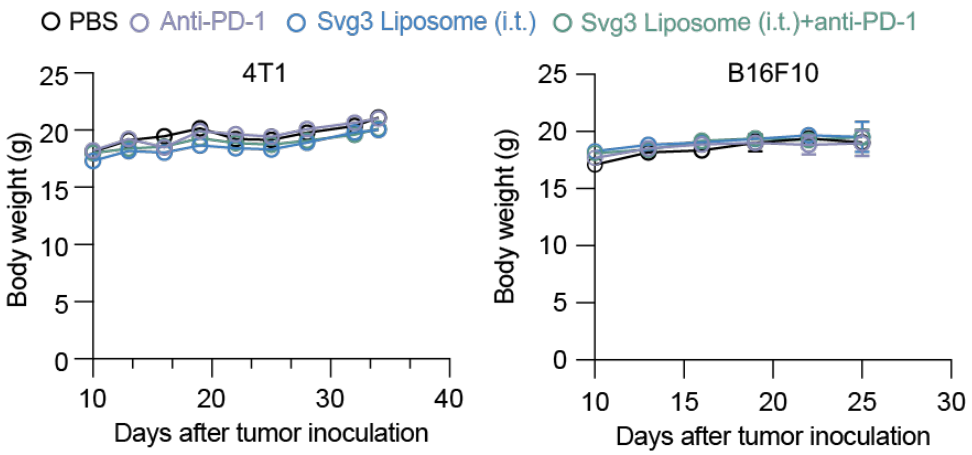

Figure S6. Mouse body weights during the course of tumor therapy using the indicated treatments.

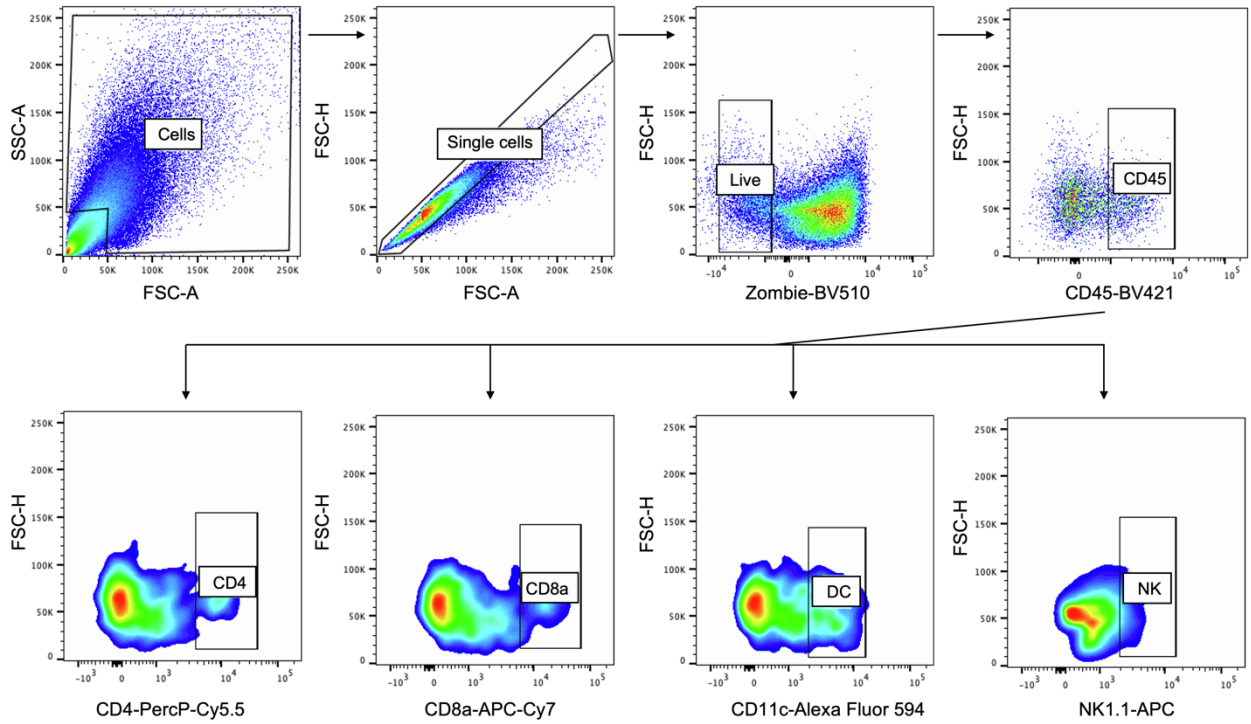

Figure S7. Flow cytometry gating trees for the analysis of $CD4^+$ T cells, $CD8^+$ T cells, DCs, and NK cells in 4T1 tumor microenvironment.

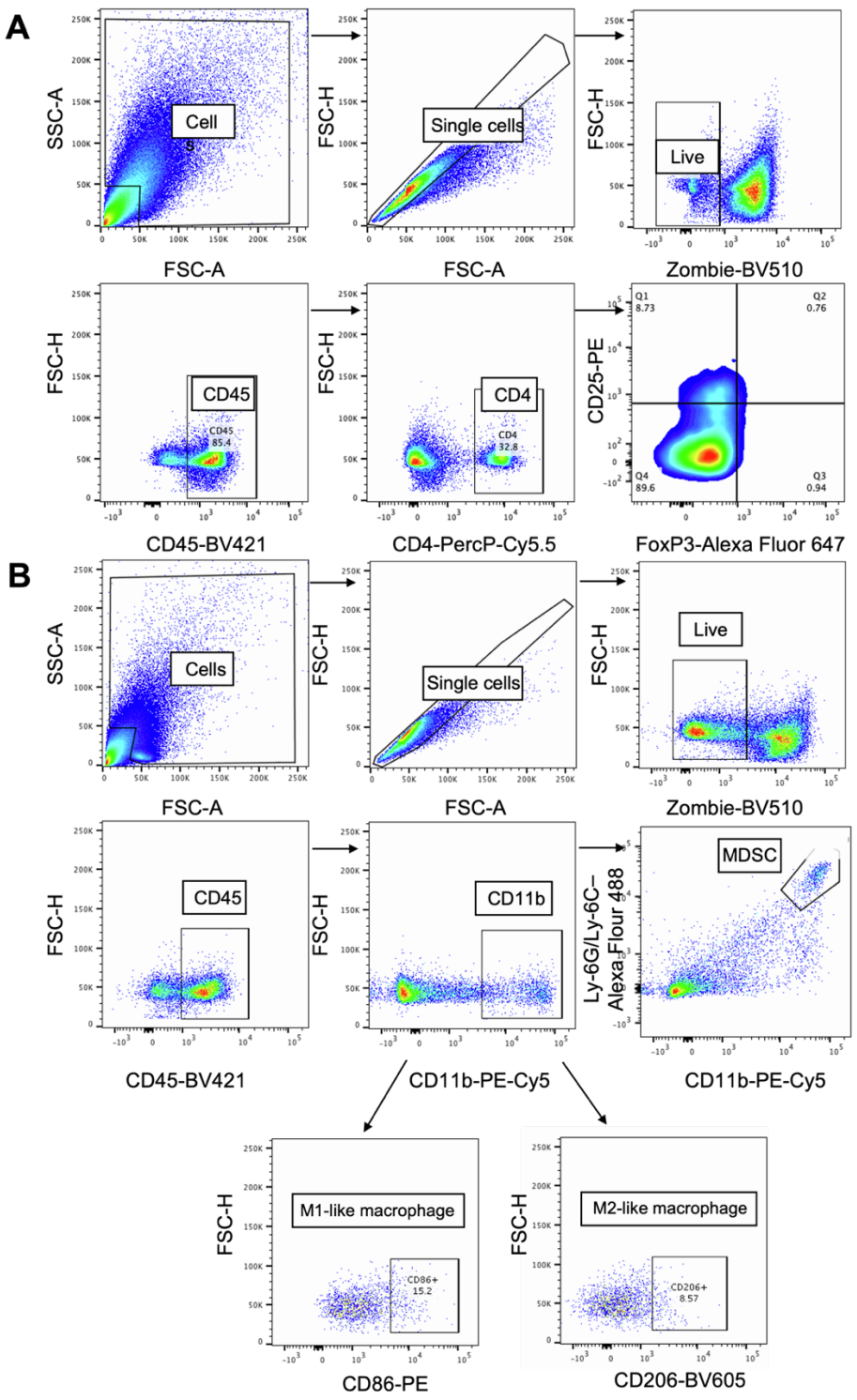

Figure S8. A) Flow cytometry gating strategy for regulatory T cells among $CD4^+$ T cells in 4T1 tumor microenvironment analysis. **B)** Flow cytometry gating strategy for myeloid derived suppressor cells, M1-like macrophage and M2-like macrophage in 4T1 tumor microenvironment analysis.

Table S1. DNA sequences used for oligonucleotide engineering and screening.

Name	Sequence (5'-3')
Svg3	CAG GGG GGA CCA CTC TTA AGC CTC AAG GGA AGC TGG GTT GAG GCT TAA GAG TGG TCC CGG GT
Svg3-PS stem	CAG GGG* G*G*A* C*C*A* C*T*C* T*T*A* A*G*C* C*T*C* A*AG GGA AGC TGG G*T*T* G*A*G* G*C*T* T*A*A* G*A*G* T*G*G* T*C*C* CGG GT
Svg3-3dT	CAG GGG GGA CCA CTC TTA AGC CTC AAG GGA AGC TGG GTT GAG GCT TAA GAG TGG TCC CGG GT /3InvdT/
Svg3-PS stem-3dT	CAG GGG* G*G*A* C*C*A* C*T*C* T*T*A* A*G*C* C*T*C* A*AG GGA AGC TGG G*T*T* G*A*G* G*C*T* T*A*A* G*A*G* T*G*G* T*C*C* CGG GT/3InvdT/
Svc3	CAC CCG GGA CCA CTC TTA AGC CTC AAC CCA AGC TCC GTT GAG GCT TAA GAG TGG TCC CCC CT
Svg2	CGGGGACCACTCTTAAGCCTCAAGCTGTTGAGGCTTAAGAGTGGTCCC GT
Svg(3)	GGGACCACTCTTAAGCCTCAAGGGAAGCTGGGTTGAGGCTTAAGAGT GGTCCC
Svg4	CAGGGGGACCACTCTTAAGCCTCAAGGAAGCTGGTTGAGGCTTAAGA GTGGTCCCGGT
ISD (InvivoGen)	5'-TACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTACA-3'
	3'-ATGTCTAGATGATCACTAGATACTGACTAGACATGTACTAGATGT-5'
DNA_10 bp	TAC TCC AAG CAA GCC CGC TTG GAG TAG TCT TG
DNA_14 bp	GTT CTG TTG AGG ATT TAC GGA CAC AAC CGT AAA TCC TCA A
DNA_18 bp	GTT CTG TTG AGG ATT TAC GAG GTCAC ACA AGACC TCG TAA ATC CTC AA
DNA_20 bp	GTT CTG TTG AGG ATT TAC GAG GTCGTAC ACA AACGACC TCG TAA ATC CTC AA
DNA_21 bp	CAC CCC GGA CCA CTC TTA AGC CTC AAC CCA AGC TCC GTT GAG GCT TAA GAG TGG TCC CCC CT
DNA_22 bp	GTT CTG CTTTG AGG ATT TAC GAG GTCGTAC ACA AACGACC TCG TAA ATC CTC AAAG
DNA_24 bp	GTT CTG AGCTTTG AGG ATT TAC GAG GTCGTAC ACA AACGACC TCG TAA ATC CTC AAAGCT

Table S2. Primer sequences used for qPCR.

Human genes	5'-3'
***Gapdh*-Forward**	GGAGCGAGATCCCTCCAAAAT
***Gapdh*-Reverse**	GGCTGTTGTCATACTTCTCATGG
***Ifna2*-Forward**	GCTTGGGATGAGACCCTCCTA
***Ifna2*-Reverse**	CCCACCCCCTGTATCACAC
***Ifnb*-Forward**	ATGACCAACAAGTGTCTCCTCC
***Ifnb*-Reverse**	GGAATCCAAGCAAGTTGTAGCTC
***Il6*-Forward**	ACTCACCTCTTCAGAACGAATTG
***Il6*-Reverse**	CCATCTTTGGAAGGTTCAGGTTG
***Cxcl10*-Forward**	GTGGCATTCAAGGAGTACCTC
***Cxcl10*-Reverse**	TGATGGCCTTCGATTCTGGATT
Mouse genes	
***Gapdh*-Forward**	CTTTGTCAAGCTCATTTCCTGG
***Gapdh*-Reverse**	TCTTGCTCAGTGTCCTTGC
***Tnfa*-Forward**	GGTGCCTATGTCTCAGCCTCTT
***Tnfa*-Reverse**	GCCATAGAACTGATGAGAGGGAG
***Il6*-Forward**	GAGGATACCACTCCCAACAGACC
***Il6*-Reverse**	AAGTGCATCATCGTTGTTCATACA
***Cxcl10*-Forward**	ATCATCCCTGCGAGCCTATCCT
***Cxcl10*-Reverse**	GACCTTTTTTGGCTAAACGCTTTC

Table S3. Details of antibodies used in this study.

Targets	Fluorochrome	Clone	Vendor	Catalogue #
CD45	Brilliant Violet 421	30-F11	BioLegend	103133
CD11c	Alexa Fluor 594	N418	BioLegend	117346
CD11b	FITC	M1/70	BioLegend	101205
CD8a	APC/Cy7	53-6.7	BioLegend	100713
CD4	PerCP/Cy5.5	GK1.5	BioLegend	100433
CD103	Brilliant Violet 605	2.00E+07	BioLegend	121433
CD205	PE/Cy7	NLDC-145	BioLegend	138209
F4/80	APC/Cy7	BM8	BioLegend	123117
CD11b	PE/Cy5	M1/70	BioLegend	101209
NK1.1	APC	S17016D	BioLegend	156505
CD3	PerCP/Cy5.5	17A2	BioLegend	100217
CD44	PE/Cy5	IM7	BioLegend	103009
CD62L	FITC	MEL-14	BioLegend	104405
CD25	FITC	3C7	BioLegend	101907
FoxP3	Alexa Fluor 647	MF-14	BioLegend	126407
CD279 (PD-1)	N/A	RMP1-14-CP162	Bio X Cell	CP162