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Supplemental information

Adenoviral delivery of an immunomodulatory protein to the tumor microenvironment controls tumor growth

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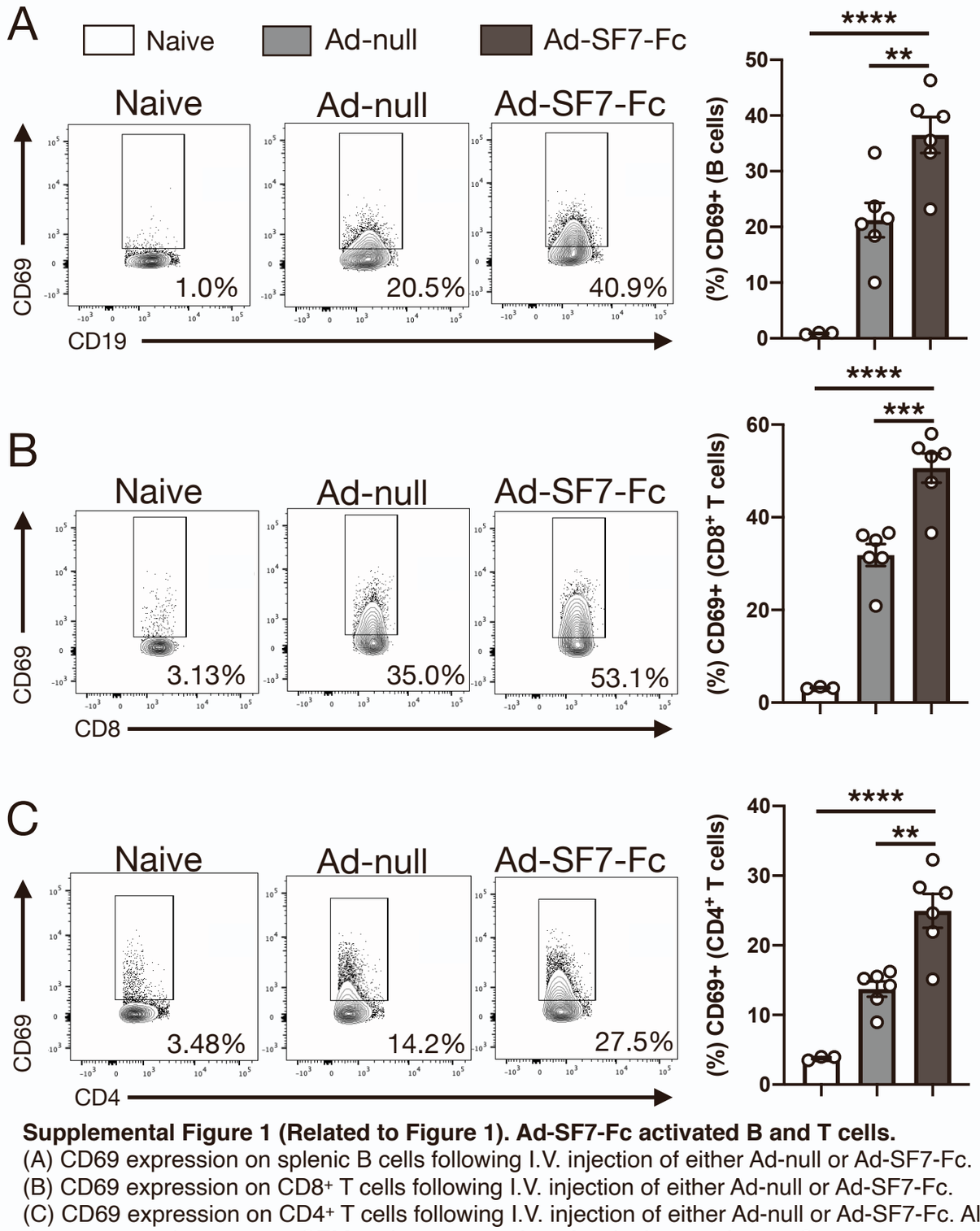
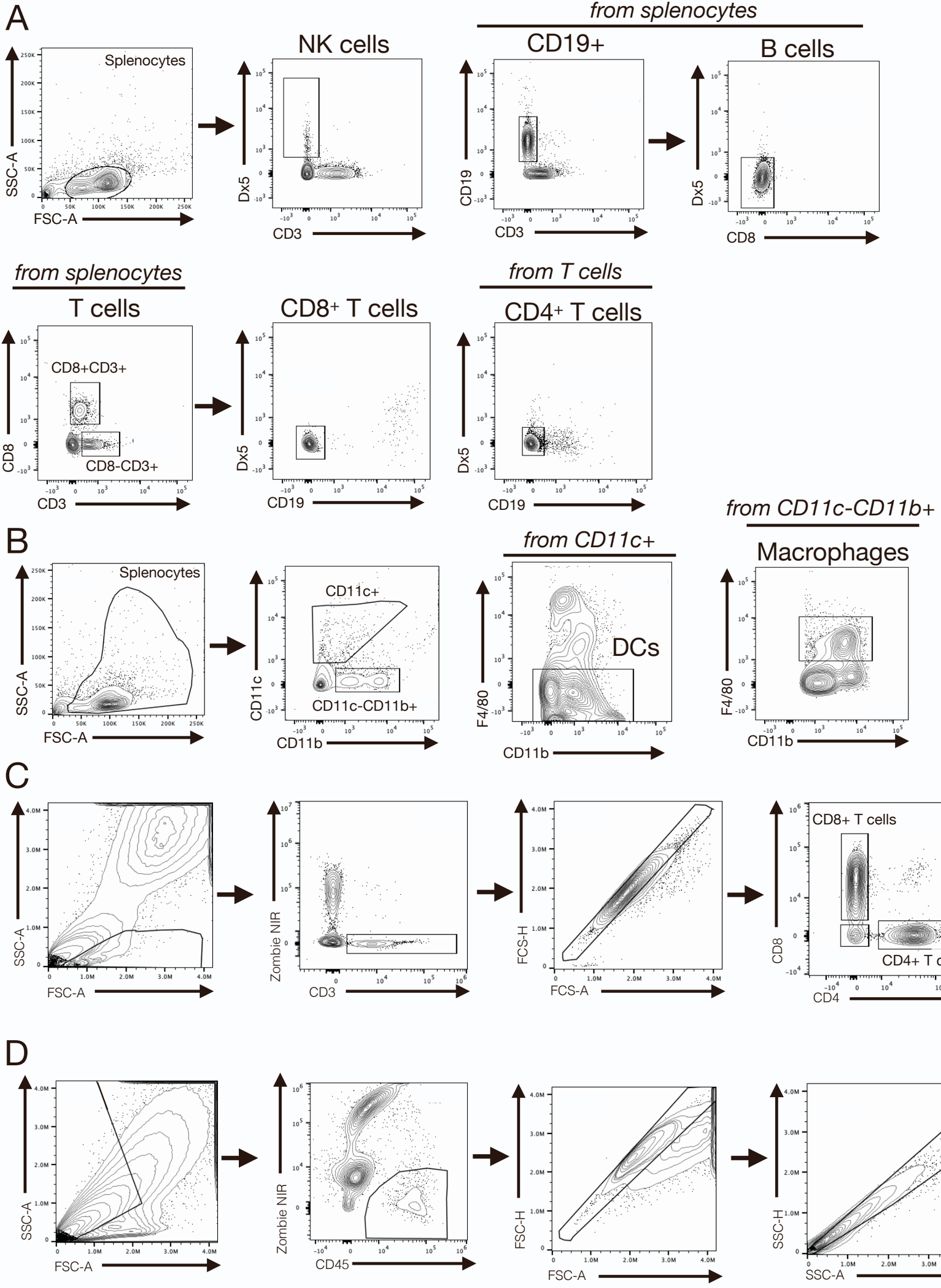
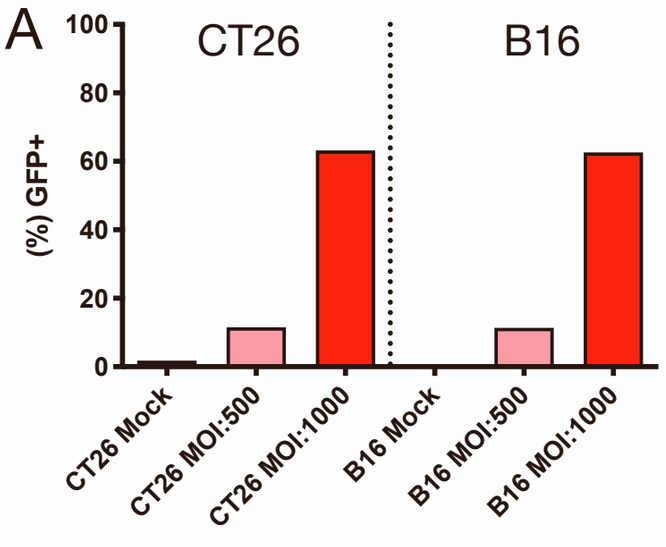
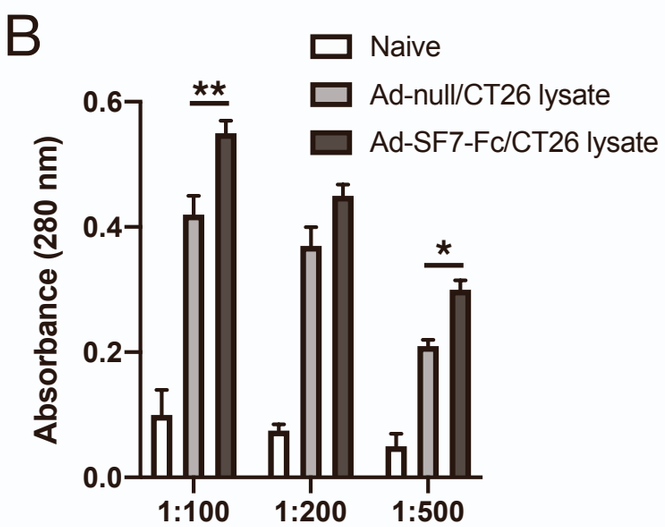


Supplemental Figure 1 (Related to Figure 1). Ad-SF7-Fc activated B and T cells.
(A) CD69 expression on splenic B cells following I.V. injection of either Ad-null or Ad-SF7-Fc. (B) CD69 expression on $CD8^+$ T cells following I.V. injection of either Ad-null or Ad-SF7-Fc. (C) CD69 expression on $CD4^+$ T cells following I.V. injection of either Ad-null or Ad-SF7-Fc. All data presented as mean $\pm$ SEM and representative of a single experiment. Groups compared with one-way ANOVA with Tukey's multiple comparison test. **p<0.01; ***p<0.001; ****p<0.0001.

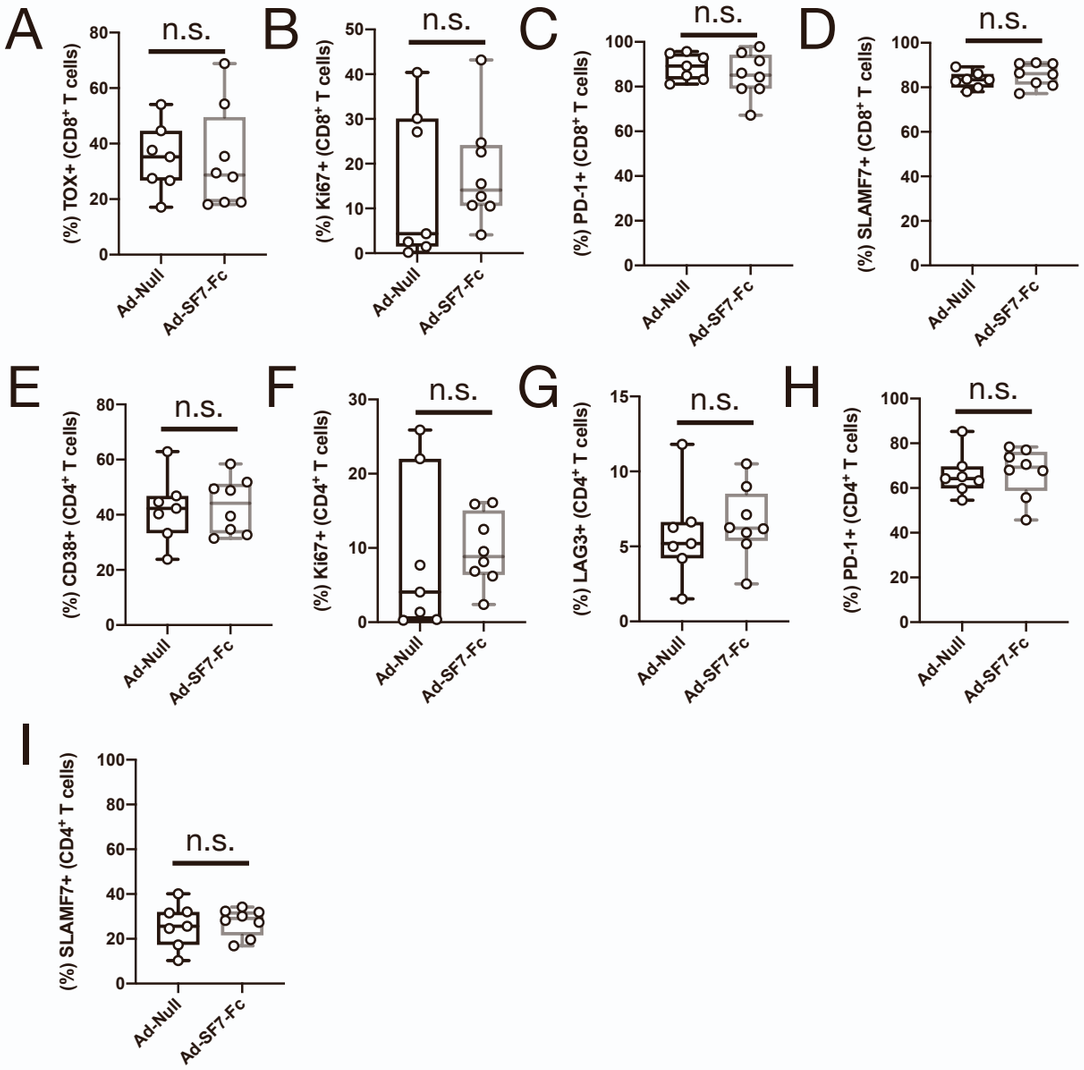


Supplemental Figure 2. Flow cytometry and spectral cytometry gating strategies.
(A) Flow cytometry gating approach used for lymphocytes in Fig. 1 and Supplemental Fig. 1. (B) Flow cytometry gating approach used for myeloid cells in Fig. 1. (C) Spectral cytometry gating approach used for B16 TIL T cell analysis in Fig. 4C-G and Supplemental Fig. 4. (D) Spectral cytometry gating approach used for B16 high dimensional TME profiling in Fig. 4H-J, Fig. 5, Fig. 7, Supplemental Fig. 5, and Supplemental Fig. 6. We utilized a broad-based strategy so as to capture all tumor-infiltrating immune cells, and cleaned up data with an additional singlet gate.





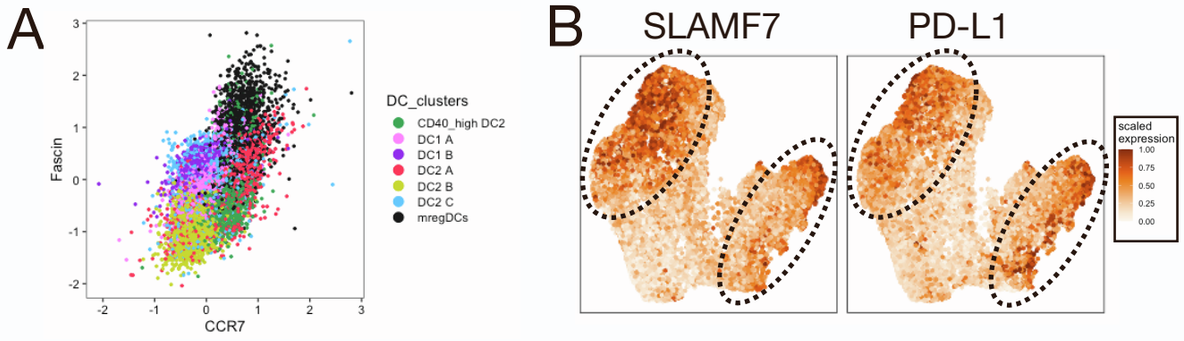
Supplemental Figure 3 (Related to Figure 3). CT26-specific IgG responses following adenovirus vaccination and Ad infection testing of CT26 and B16. (A) CT26 and B16 tumor cells were infected in vitro for 18 hrs with varying MOI's of Ad-GFP to determine optimal dose for tumor cell transduction and treatment. (B) Relative abundance of tumor specific IgG antibodies in plasma of non-vaccinated (naive) (n=2), Ad-null/CT26 lysate (n=6), and Ad-SF7-Fc/CT26 lysate (n=7) vaccinated mice using different dilutions. All data presented as mean ± SEM and representative of a single experiment. Groups compared with one-way ANOVA with Tukey's multiple comparison test. *p<0.05; **p<0.01.

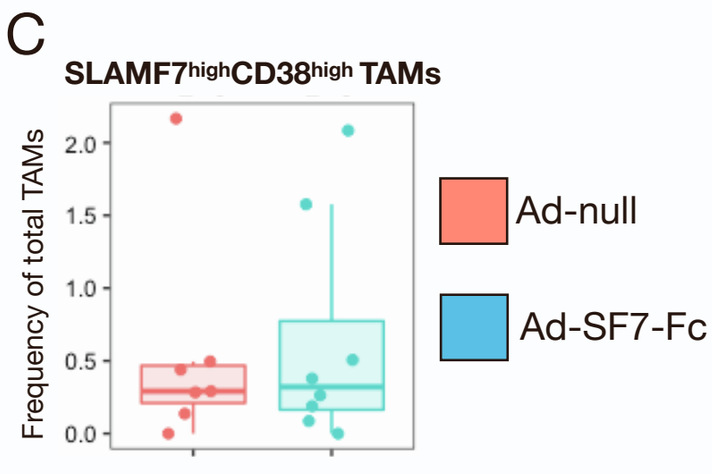


Supplemental Figure 4 (Related to Figure 4). CD4⁺ and CD8⁺ B16 TIL phenotypes from adenovirus treated mice.

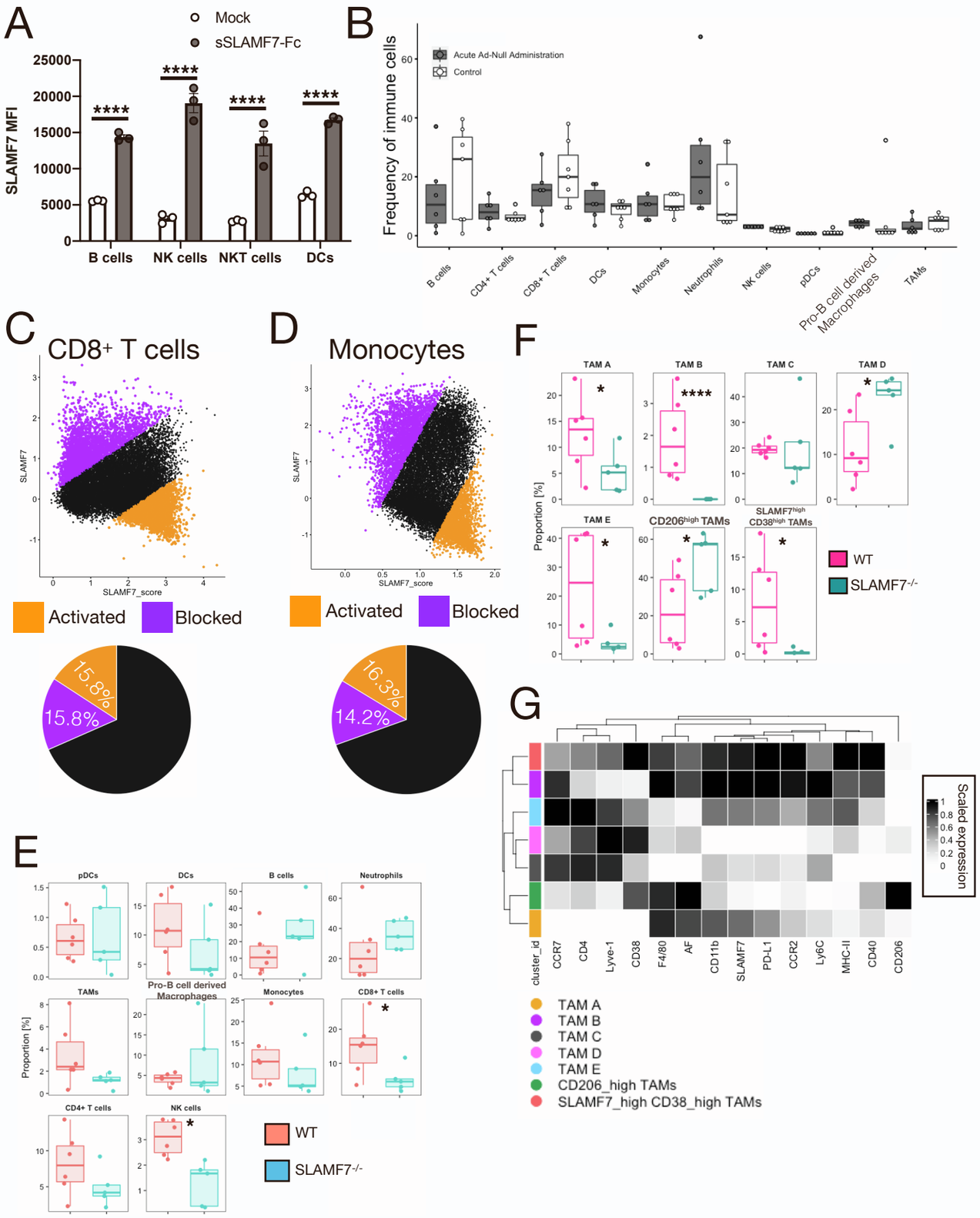
(A) Frequency of TOX⁺ CD8⁺ TILs between Ad-null and Ad-SF7-Fc treated B16 tumors. (B) Frequency of Ki67⁺ CD8⁺ TILs between Ad-null and Ad-SF7-Fc treated B16 tumors.

(C) Frequency of PD-1⁺ CD8⁺ TILs between Ad-null and Ad-SF7-Fc treated B16 tumors. (D) Frequency of SLAMF7⁺ CD8⁺ TILs between Ad-null and Ad-SF7-Fc treated B16 tumors. (E) Frequency of CD38⁺ CD4⁺ TILs between Ad-null and Ad-SF7-Fc treated B16 tumors. (F) Frequency of Ki67⁺ CD4⁺ TILs between Ad-null and Ad-SF7-Fc treated B16 tumors. (G) Frequency of LAG3⁺ CD4⁺ TILs between Ad-null and Ad-SF7-Fc treated B16 tumors. (H) Frequency of PD-1⁺ CD4⁺ TILs between Ad-null and Ad-SF7-Fc treated B16 tumors. (I) Frequency of SLAMF7⁺ CD4⁺ TILs between Ad-null and Ad-SF7-Fc treated B16 tumors.





Supplemental Figure 5 (Related to Figure 5). mregDC identification and SLAMF7/PD-L1 co-expression on TAMs.
(A) Dot plot of Fascin and CCR7 co-expression on DC subsets from adenovirus treated tumors. Points are colored by DC subset. mregDCs are defined by high co-expression of both of these markers.18 (B) Expression of SLAMF7 and PD-L1 overlaid onto UMAP plot of TAM subsets as depicted in Figure 5G. (C) Frequency of SLAMF7^{high}CD38^{high} TAMs between Ad-null and Ad-SF7-Fc treated B16 tumors.



Supplemental Figure 6 (Related to Figure 7). Supporting data for SF7-Fc predictions and TME changes in B16 tumors of SLAMF7$^{-/-}$ mice.
(A) Expression of SLAMF7 on splenic immune cell subsets from WT mice, in vitro stimulated with 10 μg/mL soluble SLAMF7-Fc protein (sSLAMF7-Fc) for two days. (B) Frequency of TME immune cell subsets between mice who received a single injection of Ad-null at day 7 post B16 tumor inoculation (Control) and mice who also received a second injection 24 hrs before sacrifice (Acute Ad Null Administration). Activating versus blocking predictions of SF7-Fc in CD8^{+} T cells (C) and Monocytes (D) depicted as in Fig. 7B-E. (E) Frequency of tumor-infiltrating immune cell subsets from B16 tumors of WT (Ad-null) and SLAMF7$^{-/-}$ (Ad-null) mice assessed by spectral cytometry similarly to Fig. 4J. (F) Frequency of TAM subsets from B16 tumors between WT (Ad-null) and SLAMF7$^{-/-}$ (Ad-null) mice. (G) Marker expression on TAM subsets from (F). Data in (A, F-G) representative of a single experiment. Data in (B) aggregated from multiple experiments. Data in (C and D) representative of two independent experiments showing similar results. Data in (A) presented as mean $\pm$ SEM and groups compared with two-way ANOVA with Sidak's multiple comparison test. Groups in (E and F) compared with a GLMM. *p<0.05; ****p<0.0001. AF; autofluorescence.

Supplemental table 2. iEN model coefficients. Only non-0 coefficients are included.

Feature	Beta	Interpretation
CD8+ T cells	0.636777322	Inc. in responders
PD-L1_TAMs	0.576284123	Inc. in responders
B220_B cells	0.574742634	Inc. in responders
IL_12p40_lysate	0.429617979	Inc. in responders
SLAMF7_TAMs	0.3491533	Inc. in responders
IgD_B cells	0.278947827	Inc. in responders
CD11c_TAMs	0.272528427	Inc. in responders
Tex_prog1	0.207656434	Inc. in responders
Ly6C_pDCs	0.195718603	Inc. in responders
Pro-B cell derived Macrophages	0.193926537	Inc. in responders
IL_10_lysate	0.181000311	Inc. in responders
IFNg_lysate	0.177540472	Inc. in responders
TAMs_activated	0.160726842	Inc. in responders
MIP_1a_lysate	0.154900386	Inc. in responders
MCP_1_lysate	0.136453361	Inc. in responders
CD45_TAMs	0.134739305	Inc. in responders
Ly6C_CD8+ T cells	0.134206359	Inc. in responders
TOX+_CD8	0.115547706	Inc. in responders
IL_6_lysate	0.114914635	Inc. in responders
SLAMF7_DCs	0.098996632	Inc. in responders
Tex_int	0.079320436	Inc. in responders
pDCs_activated	0.076432825	Inc. in responders
PD-L1_Pro-B cell derived Macrophages	0.059414611	Inc. in responders
MHC-II_TAMs	0.058278209	Inc. in responders
CCR2_TAMs	0.053394087	Inc. in responders
CD8_T_cells_activated	0.043799318	Inc. in responders
GM_CSF_lysate	0.0425075	Inc. in responders
Eotaxin_lysate	0.041849572	Inc. in responders
B220_Pro-B cell derived Macrophages	0.033258237	Inc. in responders
CD38_B cells	0.02633125	Inc. in responders
CD11b_TAMs	0.021632477	Inc. in responders

AF_B cells	0.021309016	Inc. in responders
Ly6G_Neutrophils	0.021267713	Inc. in responders
MIP_1b_lysate	0.021107824	Inc. in responders
PD-1_CD8+ T cells	0.015923065	Inc. in responders
CD90_CD8+ T cells	0.013698046	Inc. in responders
CD11c_NK cells	0.011434616	Inc. in responders
TNF_a_plasma_day1	0.010473118	Inc. in responders
CD90_CD4+ T cells	0.010203013	Inc. in responders
IL_3_lysate	0.009106385	Inc. in responders
G_CSF_lysate	0.008431265	Inc. in responders
TOX_high_PD1_high_CD8	0.007180879	Inc. in responders
CCR2_DCs	0.006887445	Inc. in responders
IL_4_lysate	0.004009159	Inc. in responders
MHC-II_DCs	0.003805041	Inc. in responders
CCR2_Monocytes	0.003246796	Inc. in responders
CD90_NK cells	0.000369813	Inc. in responders
CCL5_plasma_day0	-0.017050343	Dec. in responders
TAMs	-0.019593439	Dec. in responders
CD206_TAMs	-0.02316422	Dec. in responders
CD38_pDCs	-0.025184203	Dec. in responders
IL_4_plasma_day0	-0.034607044	Dec. in responders
Tex_prog2	-0.043115321	Dec. in responders
TNF_a_plasma_day0	-0.056736883	Dec. in responders
IL_1b_plasma_day0	-0.136661524	Dec. in responders
IL_12p70_lysate	-0.1835637	Dec. in responders
Neutrophils	-0.229220797	Dec. in responders
Lyve-1_Pro-B cell derived Macrophages	-0.236662769	Dec. in responders
IL_12p40_plasma_day0	-0.325532592	Dec. in responders
B220_pDCs	-0.335022487	Dec. in responders
IL_1a_lysate	-0.38754388	Dec. in responders

Supplemental table 3. List of all antibodies used.

Antibody	Conjugate	Source
CD3 (clone 145-2C11)	APC	BD Biosciences
CD38 (clone 90/CD38)	BV510	BD Biosciences
CD8a (clone 53-6.7)	Alexa 700	Thermofisher
CD11c (clone HL3)	PE-Cy7	BD Biosciences
CD11b (clone M1/70)	APC-Cy7	BD Biosciences
F4/80 (Clone BMB)	PE	Thermofisher
CD86 (Clone GL1)	V450	BD Biosciences
CD3e (clone SK7)	APC-Cy7	BD Biosciences
CD19 (clone 1D3)	PerCp-Cy5.5	BD Biosciences
Dx5 (a.k.a. CD49b)	PE-Cy7	Thermofisher
CD69 (clone H1.2F3)	FITC	BD Biosciences
IFNg (clone XMG1.2)	Alexa 488	BD Biosciences
CD45 (clone 30-F11)	Alexa 532	Thermofisher
CD11b (clone M1/70)	BV570	BioLegend
CD4 (clone RM4-5)	eFluor450	Thermofisher
NK1.1 (clone PK136)	PE-Cy7	Thermofisher
SLAMF7 (clone 4G2)	APC	BioLegend
Ly6C (clone HK1.4)	BV421	BioLegend
Zombie NIR (viability)	N/A	BioLegend
Ly6G (clone 1A8)	BV711	BioLegend
MHC-II (clone M4/114.15.2)	BV785	BioLegend
CD3 (clone 17A2)	BUV737	BD Biosciences
CD11c (clone HL3)	PE-CF594	BD Biosciences
B220 (clone RA3-6B2)	BV480	BD Biosciences
IgD (clone 11-26c)	SuperBright 436	Thermofisher
CD206 (clone C06802)	Alexa 647	BioLegend
CCR2 (clone 747967)	BV750	BD Biosciences
Lyve1 (clone ALY7)	eFluor615	Thermofisher
CD90.2 (clone 53-2.1)	BUV396	BD Biosciences
PD-1 (clone RMP1-30)	PerCp-eFluor710	Thermofisher
LAG3 (clone C9B7W)	BV785	BioLegend

Ki67 (clone B56)	BV421	BD Biosciences
2B4 (clone m2B4 (B6)458.1)	FITC	BioLegend
TOX (clone TXRX10)	eFluor660	Thermofisher
SLAMF6 (clone 13B3)	BUV395	BD Biosciences
CD69 (clone H1.2F3)	PE-Cy7	BD Biosciences
CD40 (clone 3/23)	APC-Fire750	BioLegend
Fascin (clone 55K-2)	Alexa 488	Santa Cruz Biotech
CCR7 (clone 4B12)	BV605	BD Biosciences
PD-L1 (clone 10F.9G2)	BV650	BD Biosciences
EAT-2 (clone LS-C87204)	Alexa647	LSBio

Supplemental table 4. IHC antibodies and staining specifications.

Primary Ab	Vendor	Pretreatment	Primary	Staining system (BioCare Medical)
Rabbit anti – CD3 Polyclonal	Abcam #GR3194253-3 Cambridge, MA	Heat Retrieval – Citrate Buffer pH 6.0 – Pascal Pressure Cooker – 125°C for 15 sec, 80°C for 1 min, room temperature with lid off for 30 min	1:450 in NAD – 1 Hour	Rodent Block M – 20 minutes ProMark Rabbit on Rodent HRP PolymerTM - 35 minutes AEC Chromogen – 5 minutes CATHE Hematoxylin 1:10 – 1 minute
Rat anti – CD8 Monoclonal	Dianova *#DIA-808* Hamburg, Germany	Heat Retrieval – Citrate Buffer pH 6.0 – Steamer for 30 min, room temperature with lid off for 10 min	1:100 in NAD - 1 Hour	Rodent Block M – 10 minutes ProMark Rat on Mouse HRP ProbeTM - 10 minutes ProMark Rat on Mouse HRP PolymerTM - 10 minutes AEC Chromogen – 5 minutes CATHE Hematoxylin 1:10 – 1 minute
Rabbit anti – Integrin Alpha 2 (DX5) Monoclonal	Abcam #GR196223-25 Cambridge, MA	No Pretreatment	1:100 in NAD – 1 Hour	Rodent Block M – 20 minutes ProMark Rabbit on Rodent HRP PolymerTM - 20 minute AEC Chromogen – 5 minutes CATHE Hematoxylin 1:10 – 1 minute