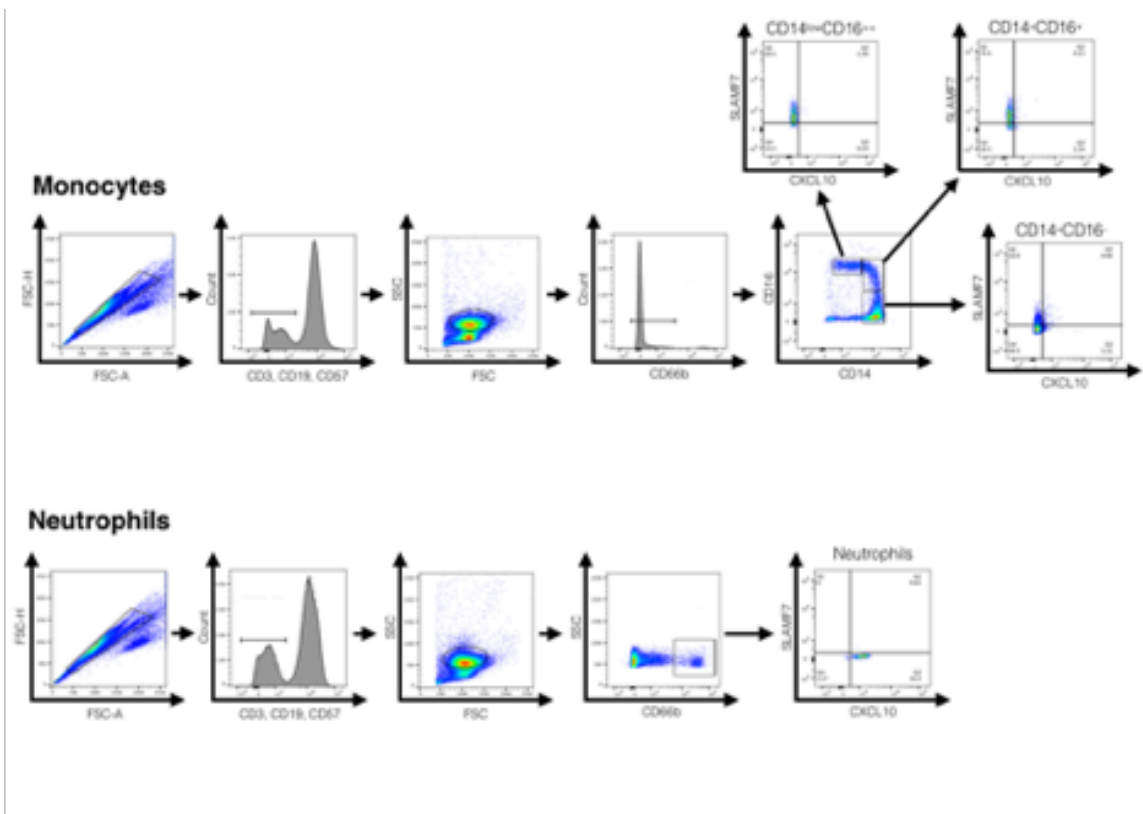
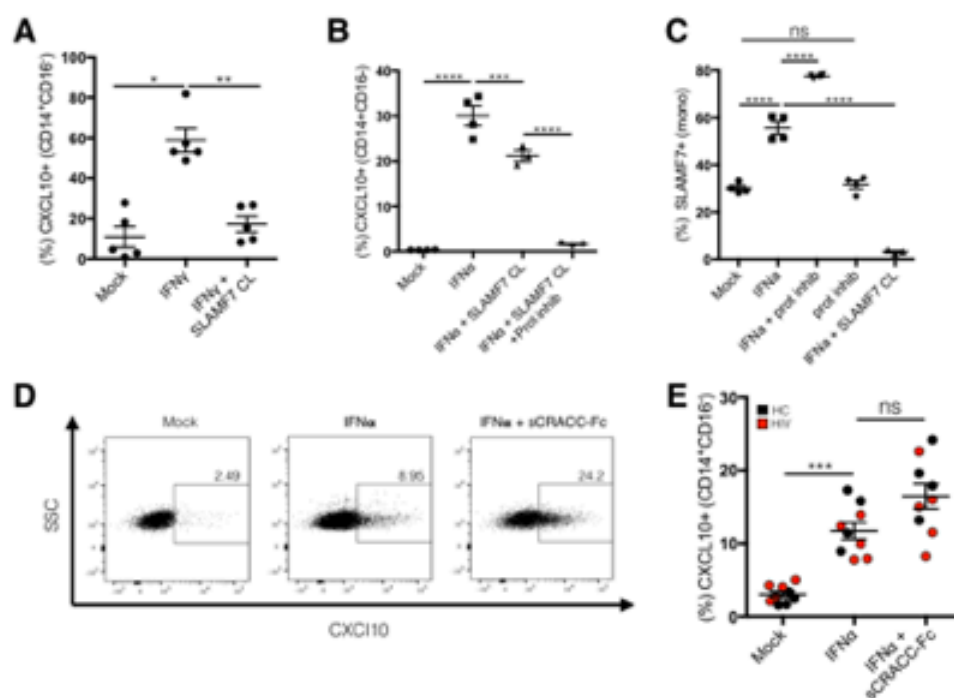


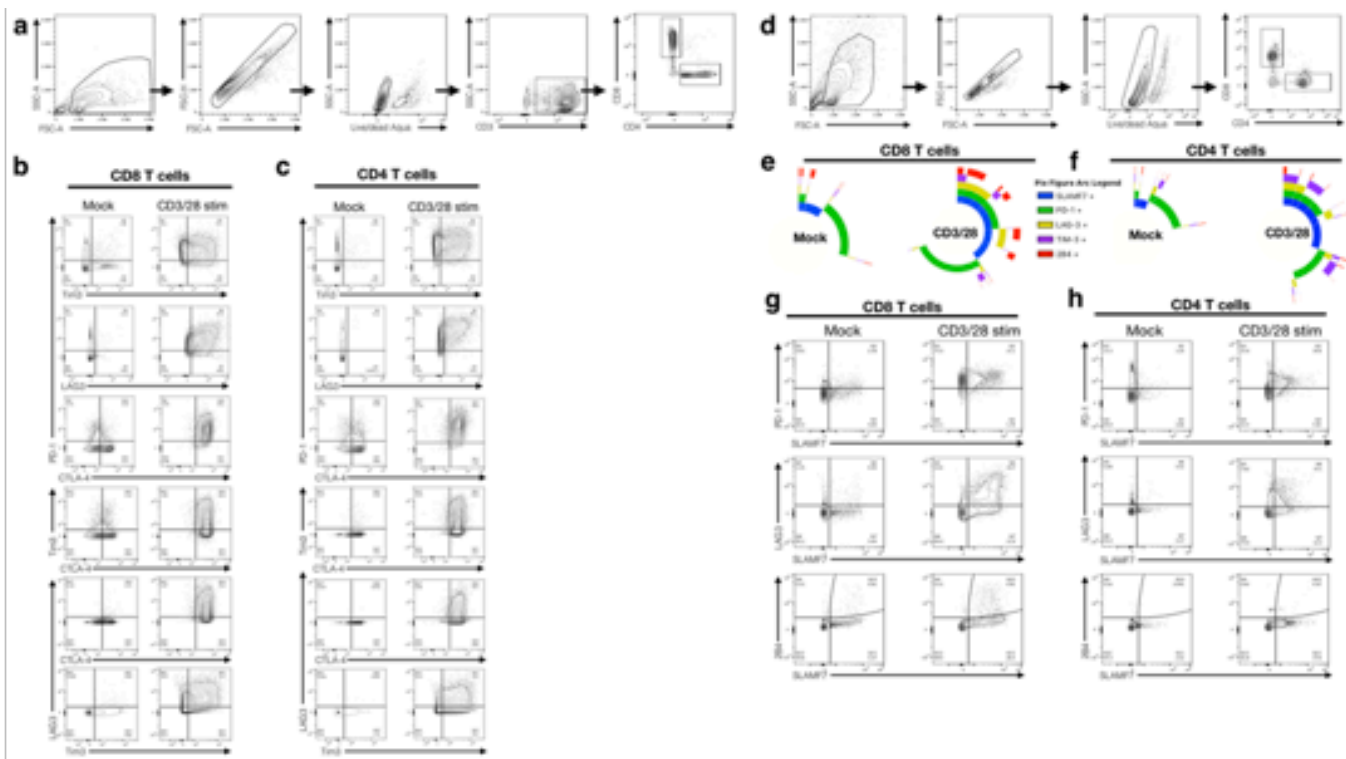
## **SUPPLEMENTAL FILES**



**Supplemental Figure 1.** Gating scheme used to identify monocyte subtypes and neutrophils.



**Supplemental Figure 2.** SLAMF7 activation inhibits IFN $\gamma$ -mediated CXCL10 production from monocytes and CXCL10 inhibition is not proteasome-mediated. (A) The same experiment carried out in (Fig. 2C and D) was performed with 100 IU/mL IFN $\gamma$  used in place of IFN $\alpha$ . The proteasome inhibitor Bortezomib was added where indicated at 100nM and CXCL10 expression was measured by flow cytometry (B). SLAMF7 expression on Bortezomib stimulated monocytes was also assessed by flow cytometry (C). (D and E) Soluble SLAMF7-Fc (sSLAMF7-Fc) was added to the supernatant of IFN $\alpha$  stimulated monocytes and CXCL10 expression was determined as in (Fig. 2C and D). Groups compared using a 1-way ANOVA with Tukey's multiple comparison test. Data presented as mean  $\pm$  SEM and are representative of 2 independent experiments (A) or 1 experiment (B, C, and E). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001



### Supplemental Fig. 3

(A) Gating scheme used for assessing T cells in Fig. 14.

(B) Co-expression of all combinations of exhaustion markers on CD8+ human T cells.

(C) Co-expression of all combinations of exhaustion markers on CD4+ human T cells.

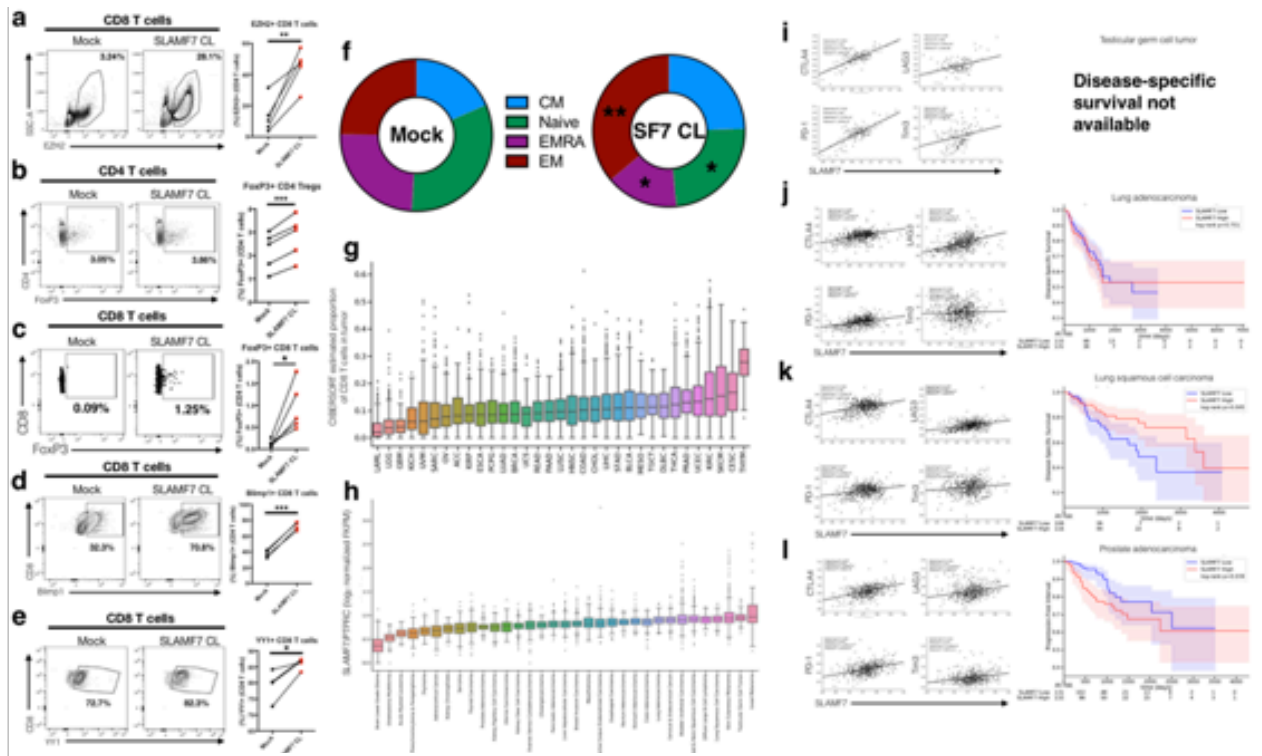
(D) Gating scheme used for assessing murine T cells.

(E) SPICE plots of mouse CD8+ T cells Mock or CD3/28 stimulated in vitro for 6 days. Plots are pooled results from (n=2) mice.

(F) SPICE plots of mouse CD4+ T cells Mock or CD3/28 stimulated in vitro for 3 days. Plots are pooled results from (n=2) mice.

(G, H) Representative biaxial plots showing SLAMF7 co-expression with exhaustion markers on CD8+ (G) and CD4+ (H) T cells.

Results in (D-H) are from a single experiment.



#### Supplemental Fig. 4

(A) Changes in EZH2 expression in Mock and SLAMF7 activated primary human CD8<sup>+</sup> T cells following 6 days of *in vitro* stimulation.

(B) Changes in FoxP3 expression in CD4<sup>+</sup> primary human T cells following 6 days of *in vitro* stimulation.

(C) Changes in FoxP3 expression in Mock and SLAMF7 activated primary human CD8<sup>+</sup> T cells following 6 days of *in vitro* stimulation.

(D) Changes in Blimp1 expression in Mock and SLAMF7 activated primary human CD8<sup>+</sup> T cells following 6 days of *in vitro* stimulation.

(E) Changes in YY1 expression in Mock and SLAMF7 activated primary human CD8<sup>+</sup> T cells following 6 days of *in vitro* stimulation.

(F) Changes in primary human CD8<sup>+</sup> T cell subsets following 6 days of SLAMF7 activation. Results shown are the averages of three individual donors run in duplicate. Asterisks in the SF7 CL donut graph indicate significant changes from Mock.

(G) CIBERSORT-generated estimations of CD8<sup>+</sup> T cell compositions from bulk RNA-seq data from TCGA participants was acquired from the Genomic Data Commons (GDC). TCGA abbreviations are available at: <https://gdc.cancer.gov/resources-tcga-users/tcga-code-tables/tcga-study-abbreviations>.

(H) SLAMF7 log<sub>2</sub> FKPM mRNA expression was divided by PTPRC log<sub>2</sub> FKPM mRNA expression to normalize for total immune cell contributions, and results were plotted for all TCGA participants by cancer type.

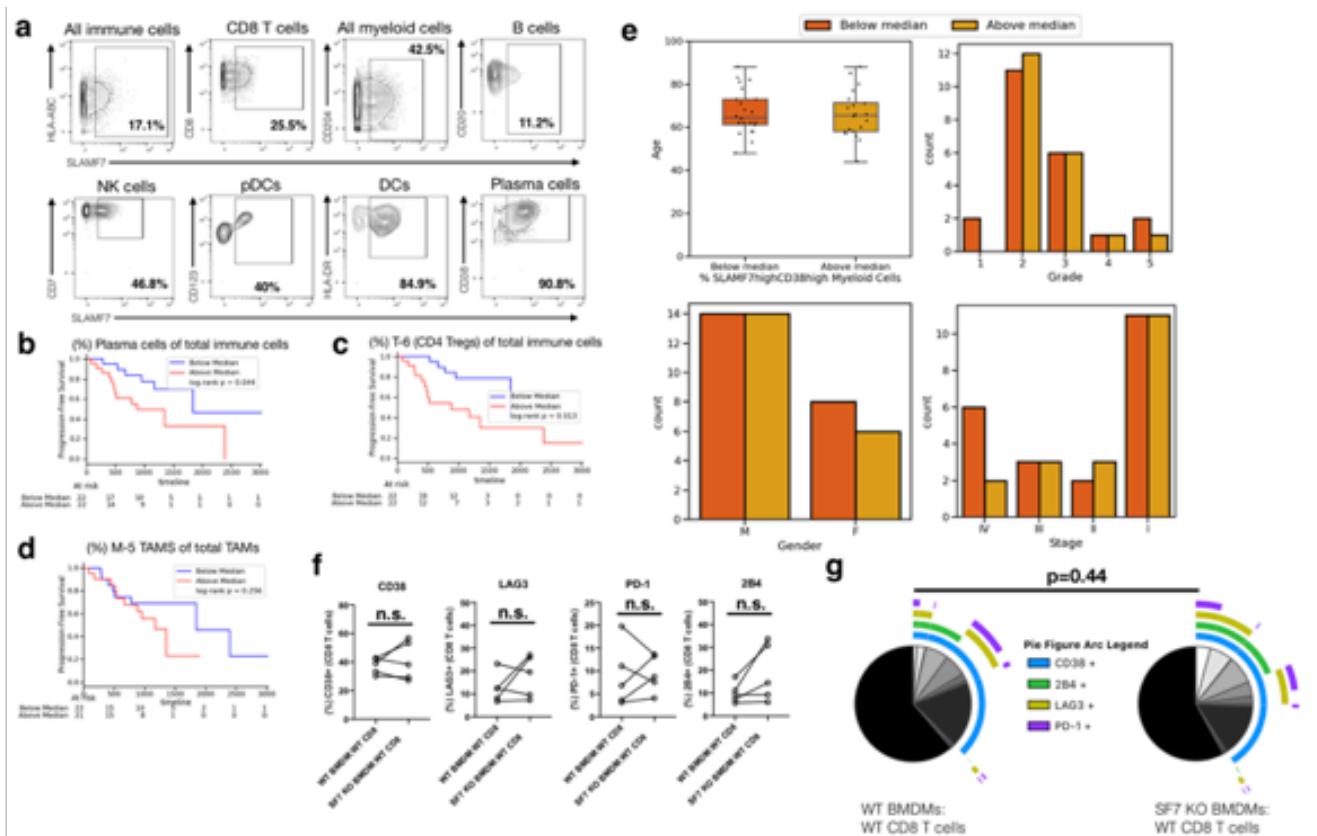
[i] Left, co-expression of SLAMF7 mRNA with various exhaustion marker mRNA from Testicular Germ Cell Tumor (n=139) (I), Lung Adenocarcinoma (n=539) (J), Lung Squamous Cell Carcinoma (n=490) (K), and Prostate Adenocarcinoma (n=550) (L), displayed as in **Figure 18**. Right, Kaplan-Meier curves showing disease specific survival for the top and bottom SLAMF7 expressing quartiles in Lung Adenocarcinoma (J) and Lung Squamous Cell Carcinoma (K). Prostate Adenocarcinoma (L) is shown as progression free survival. Accurate, disease specific survival data not available for Testicular Germ Cell Tumor (I).

Log-rank test is used to compare groups in [i] and shaded regions represent 95% confidence interval. Center line in box plots represents the median, the entire box represents the first and fourth quartiles, and the whiskers show the min and max, excluding outliers.

Results in (A-E) are from a single experiment, representative of 5 independent experiments (A, B, D, E) or 3 independent experiments (C). Groups in (A-E) compared using a two-way paired student's t-test.

Groups in (F) compared using a two-way ANOVA with Sidak's multiple comparison test. \*p<0.05;

\*\*p<0.01; \*\*\*p<0.001.



### Supplemental Fig. 5

(A) Representative gates of SLAMF7<sup>+</sup> cells from various immune cell types from Chevrier et al., 2017 CyTOF dataset.

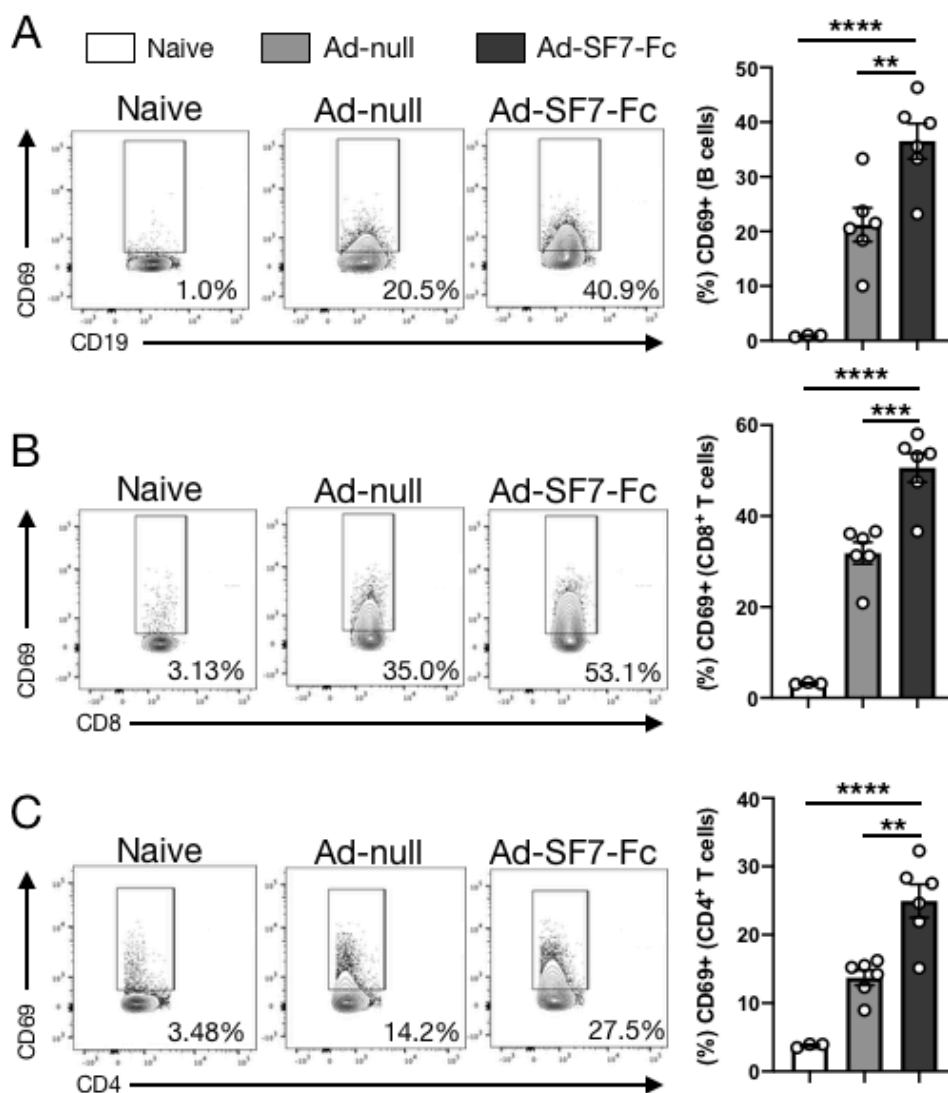
(B-D) Kaplan-Meier progression free survival curves with ccRCC patients stratified by percent of plasma cells from total immune cells (B), percent total CD4<sup>+</sup> Tregs from total immune cells (C), and percent total M-5 TAMs per total TAMs (D). Log-rank test is used to compare groups in (B-D).

(E) Demographic and clinical parameter comparison between ccRCC patients in the "above median" and "below median" groups of patients stratified by SLAMF7<sup>high</sup>CD38<sup>high</sup> TAMs shown in **Figure 19e**.

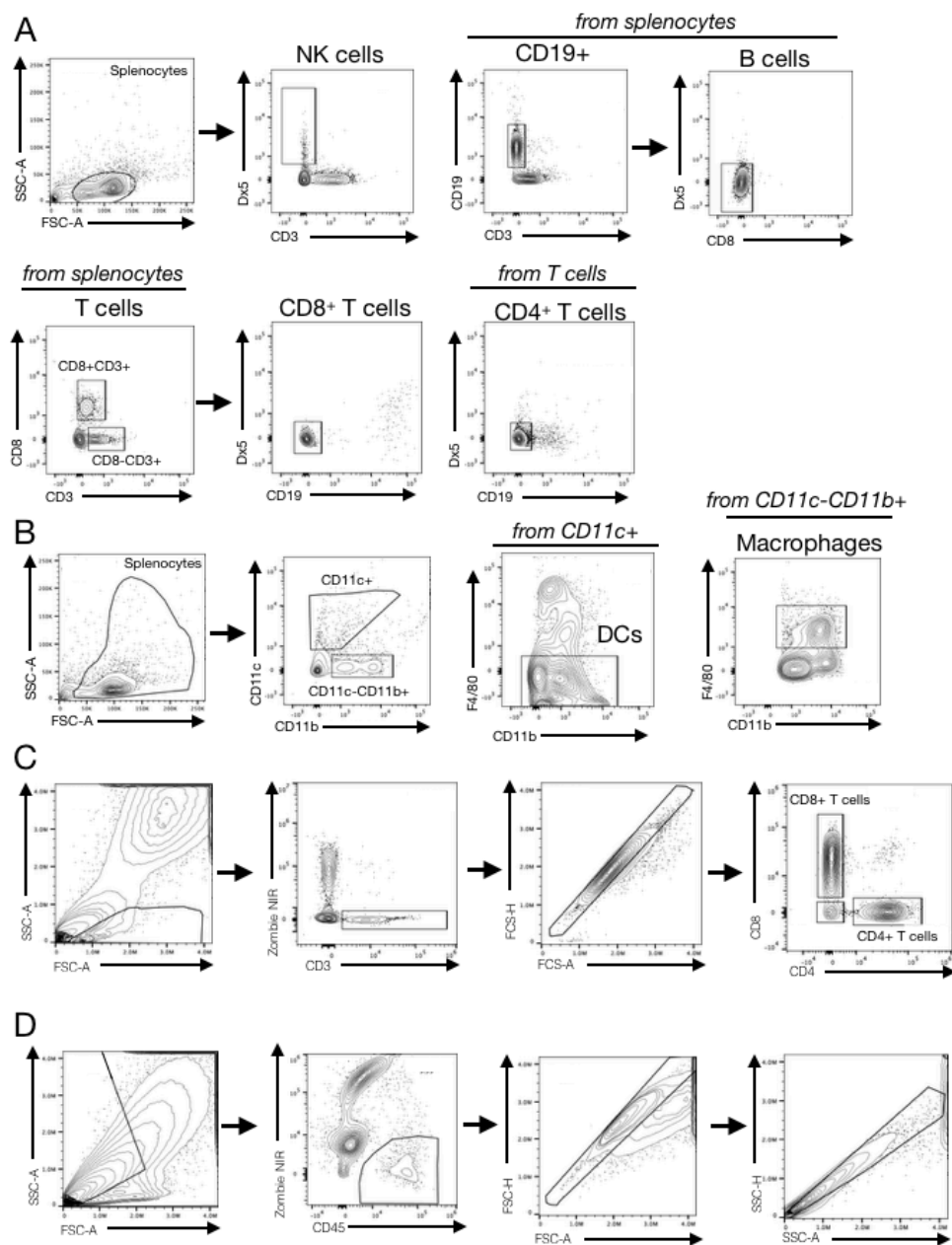
(F) Changes in expression of exhaustion markers on CD8<sup>+</sup> T cells from co-cultured with bone marrow-derived macrophages (BMDMs) from WT or SLAMF7<sup>-/-</sup> mice (n=5). Groups compared using a paired student's t-test. (G) SPICE plots of data from (F) compared using Permutation test.

Rank	Population 1	Population 2	Spearman Coefficient	Pearson Coefficient	P-value
1	T-0	SF7 <sup>high</sup> CD38 <sup>high</sup> TAMs	0.71	0.78	1.02E-15
2	T-0	SF7+ Myeloid cells	0.67	0.77	2.03E-15
3	T-0	SF7+ M-2 TAMs	0.56	0.69	3.65E-11
4	T-0	SF7+ M-1 TAMs	0.59	0.67	1.5E-09
5	T-0	SF7+ M-0 TAMs	0.53	0.64	2.91E-09
6	T-0	SF7+ all immune cells	0.63	0.63	1.49E-09
7	T-1	SF7+ Myeloid cells	0.57	0.58	6.29E-08
8	T-0	SF7+ M-3 TAMs	0.52	0.55	9.46E-07
9	T-1	SF7 <sup>high</sup> CD38 <sup>high</sup> TAMs	0.59	0.52	2.49E-06
10	T-1	SF7+ M-0 TAMs	0.47	0.47	3.96E-05
1	T-4	SF7+ NK cells	-0.61	-0.69	1.03E-11
2	T-4	SF7+ DN T cells	-0.54	-0.62	3.7E-09
3	T-4	SF7+ CD8 T cells	-0.49	-0.59	2.93E-08
4	T-3	SF7+ M-2 TAMs	-0.58	-0.57	2.08E-07
5	T-4	SF7+ all immune cells	-0.55	-0.56	1.84E-07
6	T-3	SF7+ Myeloid cells	-0.52	-0.54	8E-07
7	T-3	SF7+ M-8 TAMs	-0.44	-0.49	3.42E-05
8	T-3	SF7+ all immune cells	-0.45	-0.47	2.11E-05
9	T-3	SF7+ M-10 TAMs	-0.42	-0.47	3.61E-05
10	T-3	SF7 <sup>high</sup> CD38 <sup>high</sup> TAMs	-0.49	-0.47	3.78E-05

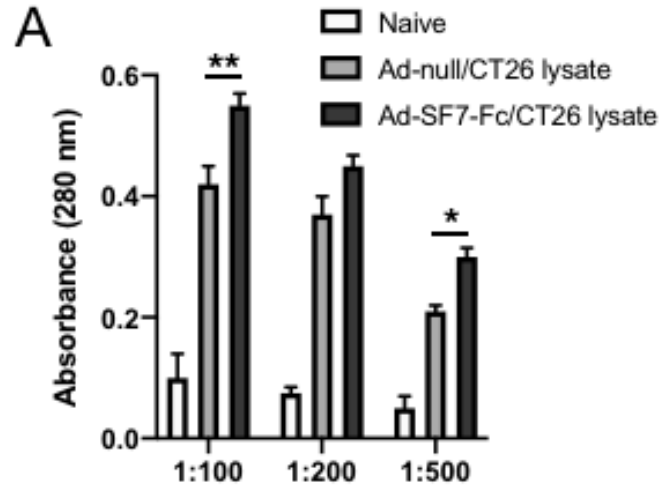
**Supplementary Table 1. Top 10 positive and negative correlations between SLAMF7<sup>+</sup> cell populations and T cell subsets ranked by Pearson coefficient. Related to Figure 19c.**



**Supplemental Figure 6 (Related to Figure 22). 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 + SEM and representative of a single experiment. Groups compared with one-way ANOVA with Tukey's multiple comparison test. \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ .

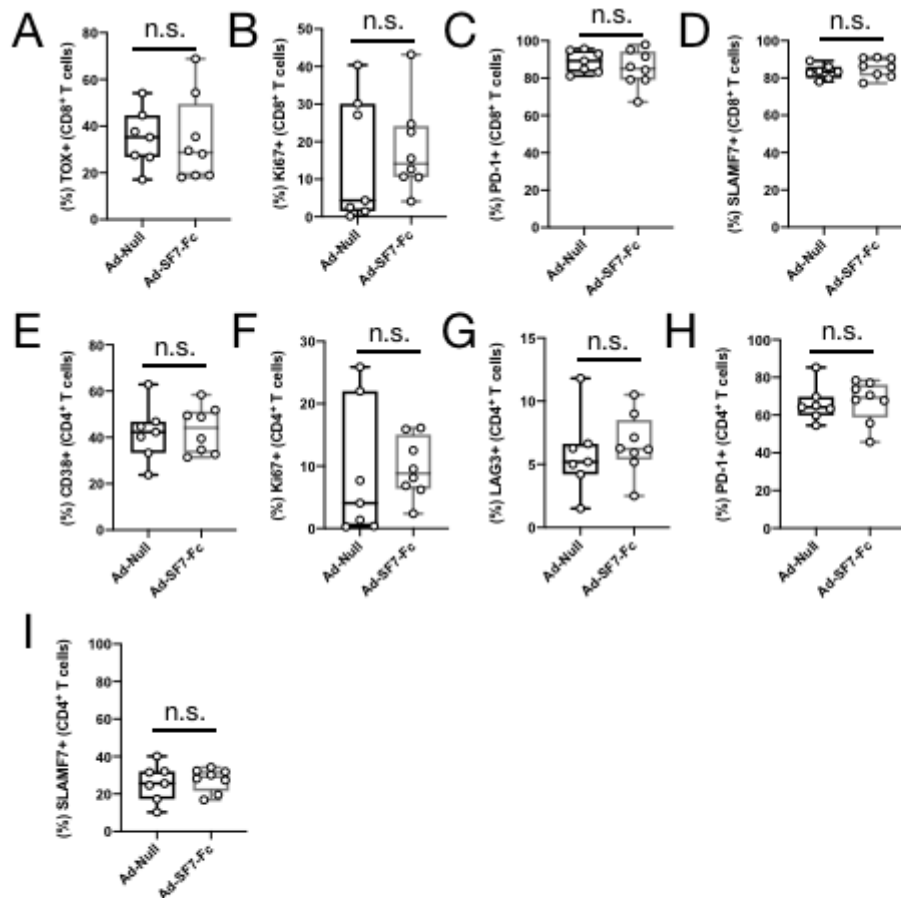


**Supplemental Figure 7. Flow cytometry and spectral cytometry gating strategies.** (A) Flow cytometry gating approach used for lymphocytes in Fig. 22 and Supplemental Fig. 6. (B) Flow cytometry gating approach used for myeloid cells in Fig. 22. (C) Spectral cytometry gating approach used for B16 TIL T cell analysis in Fig. 25C-G and Supplemental Fig. 9. (D) Spectral cytometry gating approach used for B16 high dimensional TME profiling in Fig. 25H-J, Fig. 26, Fig. 28, Supplemental Fig. 10, and Supplemental Fig. 11. We utilized a broad-based strategy so as to capture all tumor-infiltrating immune cells, and cleaned up data with an additional singlet gate.



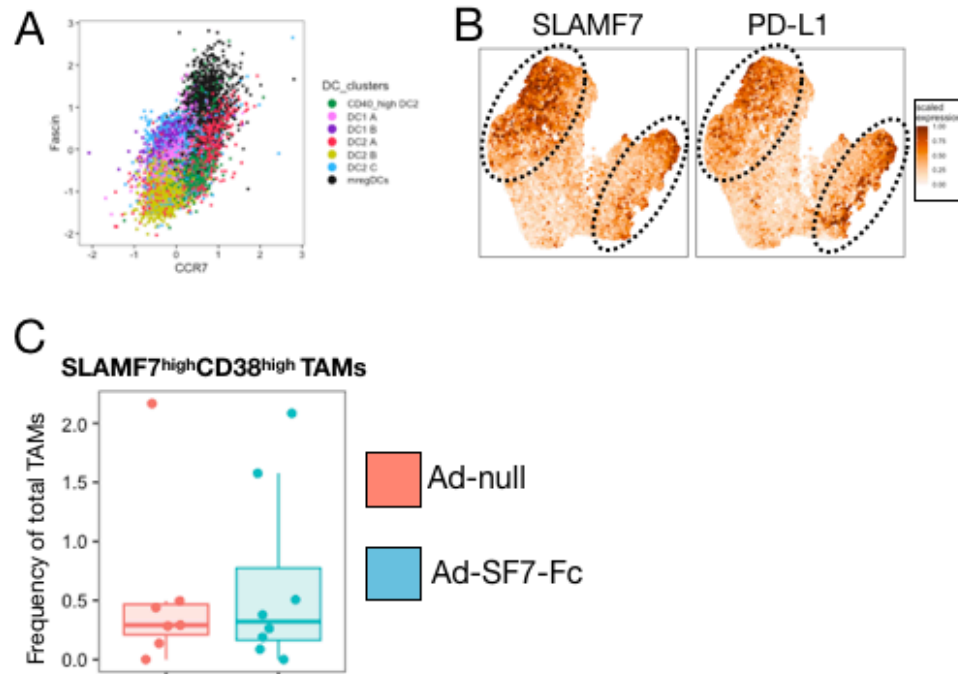
**Supplemental Figure 8 (Related to Figure 24). CT26-specific IgG responses following adenovirus vaccination.**

(A) 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  $\pm$  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.



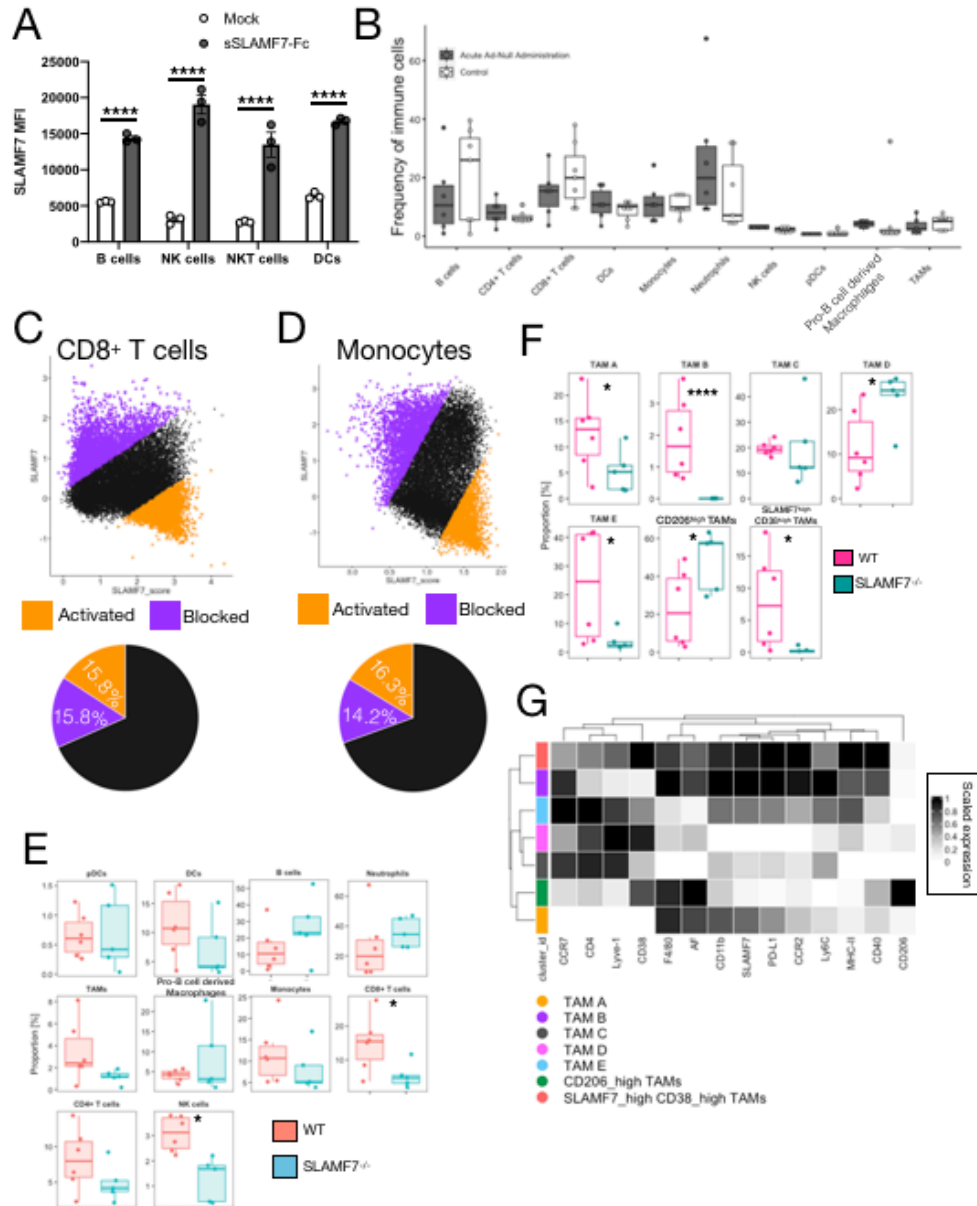
**Supplemental Figure 9 (Related to Figure 25). CD4<sup>+</sup> and CD8<sup>+</sup> B16 TIL phenotypes from adenovirus treated mice.**

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



**Supplemental Figure 10 (Related to Figure 26). 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. (B) Expression of SLAMF7 and PD-L1 overlaid onto UMAP plot of TAM subsets as depicted in Figure 26G. (C) Frequency of SLAMF7<sup>high</sup>CD38<sup>high</sup> TAMs between Ad-null and Ad-SF7-Fc treated B16 tumors.



**Supplemental Figure 11 (Related to Figure 28). Supporting data for SF7-Fc predictions and TME changes in B16 tumors of SLAMF7<sup>-/-</sup> mice.**

(A) Expression of SLAMF7 on splenic immune cell subsets from WT mice, in vitro stimulated with 10  $\mu$ g/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<sup>+</sup> T cells (C) and Monocytes (D) depicted as in Fig. 28B-E. (E) Frequency of tumor-infiltrating immune cell subsets from B16 tumors of WT (Ad-null) and SLAMF7<sup>-/-</sup> (Ad-null) mice assessed by spectral cytometry similarly to Fig. 25J. (F) Frequency of TAM subsets from B16 tumors between WT (Ad-null) and SLAMF7<sup>-/-</sup> (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.

<b><u>Feature</u></b>	<b><u>SF7 Fc_priors</u></b>
IL_1a_lysate	1
IL_1b_lysate	1
IL_2_lysate	1
IL_3_lysate	1
IL_4_lysate	1
IL_5_lysate	1
IL_6_lysate	1
IL_9_lysate	1
IL_10_lysate	1
IL_12p40_lysate	1
IL_12p70_lysate	1
IL_13_lysate	1
IL_17_lysate	1
Eotaxin_lysate	1
G_CSF_lysate	1
GM_CSF_lysate	1
IFNg_lysate	1
CXCL1_lysate	1
MCP_1_lysate	1
MIP_1a_lysate	1
MIP_1b_lysate	1
CCL5_lysate	1
TNF_a_lysate	1
IL_1a_plasma_day1	0.9
IL_1b_plasma_day1	0.9
IL_3_plasma_day1	0.2
IL_4_plasma_day1	0.2
IL_5_plasma_day1	0.2
IL_6_plasma_day1	1
IL_10_plasma_day1	0.5
IL_12p40_plasma_day1	1
IL_12p70_plasma_day1	1
IL_17_plasma_day1	0.2
Eotaxin_plasma_day1	1
G_CSF_plasma_day1	0.4
GM_CSF_plasma_day1	0.9
IFNg_plasma_day1	0.4
CXCL1_plasma_day1	1
MCP_1_plasma_day1	0.8
MIP_1a_plasma_day1	0.5
MIP_1b_plasma_day1	0.5
CCL5_plasma_day1	0.6

TNF_a_plasma_day1	1
IL_1a_plasma_day0	1
IL_1b_plasma_day0	1
IL_3_plasma_day0	1
IL_4_plasma_day0	1
IL_5_plasma_day0	1
IL_6_plasma_day0	1
IL_10_plasma_day0	1
IL_12p40_plasma_day0	1
IL_12p70_plasma_day0	1
IL_17_plasma_day0	1
Eotaxin_plasma_day0	1
G_CSF_plasma_day0	1
GM_CSF_plasma_day0	1
IFNg_plasma_day0	1
CXCL1_plasma_day0	1
MCP_1_plasma_day0	1
MIP_1a_plasma_day0	1
MIP_1b_plasma_day0	1
CCL5_plasma_day0	1
TNF_a_plasma_day0	1
2B4+_CD8	1
CD38+_CD4	0.5
CD38+_CD8	1
Ki67+_CD4	0.1
Ki67+_CD8	0.2
LAG3+_CD4	0.5
LAG3+_CD8	0.9
PD1_high_CD8	0.8
PD1+_CD4	0.3
SLAMF7+_CD4	1
SLAMF7+_CD8	1
Tex_int	1
Tex_prog1	1
Tex_prog2	1
Tex_term	1
TOX_high_PD1_high_CD8	1
TOX_PD1_dp_CD8	1
TOX+_CD8	1
B cells	1
CD4+ T cells	1
CD8+ T cells	1
DCs	1

Monocytes	1
Neutrophils	1
NK cells	1
pDCs	1
Pro-B cell derived Macrophages	1
TAMs	1
CD45_B cells	1
CD45_CD4+ T cells	1
CD45_CD8+ T cells	1
CD45_DCs	1
CD45_Monocytes	1
CD45_Neutrophils	1
CD45_NK cells	1
CD45_pDCs	1
CD45_Pro-B cell derived Macrophages	1
CD45_TAMs	1
CD11b_B cells	0.1
CD11b_CD4+ T cells	0.2
CD11b_CD8+ T cells	0.2
CD11b_DCs	0.8
CD11b_Monocytes	0.8
CD11b_Neutrophils	1
CD11b_NK cells	0.5
CD11b_pDCs	0.3
CD11b_Pro-B cell derived Macrophages	1
CD11b_TAMs	1
CD40_B cells	0.4
CD40_CD4+ T cells	0
CD40_CD8+ T cells	0
CD40_DCs	0.8
CD40_Monocytes	0.1
CD40_Neutrophils	0
CD40_NK cells	0
CD40_pDCs	0
CD40_Pro-B cell derived Macrophages	0
CD40_TAMs	0.7
CD4_B cells	0
CD4_CD4+ T cells	1
CD4_CD8+ T cells	0
CD4_DCs	0.2
CD4_Monocytes	0.2
CD4_Neutrophils	0

CD4_NK cells	0
CD4_pDCs	0
CD4_Pro-B cell derived Macrophages	0.3
CD4_TAMs	0.3
CD8_B cells	0
CD8_CD4+ T cells	0
CD8_CD8+ T cells	1
CD8_DCs	0.3
CD8_Monocytes	0
CD8_Neutrophils	0.2
CD8_NK cells	0.2
CD8_pDCs	0
CD8_Pro-B cell derived Macrophages	0
CD8_TAMs	0
NK1.1_B cells	0
NK1.1_CD4+ T cells	0
NK1.1_CD8+ T cells	0
NK1.1_DCs	0
NK1.1_Monocytes	0
NK1.1_Neutrophils	0
NK1.1_NK cells	1
NK1.1_pDCs	0
NK1.1_Pro-B cell derived Macrophages	0
NK1.1_TAMs	0
CD19_B cells	1
CD19_CD4+ T cells	0
CD19_CD8+ T cells	0
CD19_DCs	0
CD19_Monocytes	0
CD19_Neutrophils	0
CD19_NK cells	0
CD19_pDCs	0
CD19_Pro-B cell derived Macrophages	0
CD19_TAMs	0
SLAMF7_B cells	1
SLAMF7_CD4+ T cells	0.5
SLAMF7_CD8+ T cells	1
SLAMF7_DCs	1
SLAMF7_Monocytes	1
SLAMF7_Neutrophils	0
SLAMF7_NK cells	1
SLAMF7_pDCs	1

SLAMF7_Pro-B cell derived	
Macrophages	1
SLAMF7_TAMs	1
F4/80_B cells	0
F4/80_CD4+ T cells	0
F4/80_CD8+ T cells	0
F4/80_DCs	0.3
F4/80_Monocytes	0.4
F4/80_Neutrophils	0.2
F4/80_NK cells	0
F4/80_pDCs	0
F4/80_Pro-B cell derived Macrophages	0.5
F4/80_TAMs	1
Ly6C_B cells	1
Ly6C_CD4+ T cells	1
Ly6C_CD8+ T cells	1
Ly6C_DCs	1
Ly6C_Monocytes	1
Ly6C_Neutrophils	1
Ly6C_NK cells	1
Ly6C_pDCs	1
Ly6C_Pro-B cell derived Macrophages	1
Ly6C_TAMs	1
Ly6G_B cells	0
Ly6G_CD4+ T cells	0
Ly6G_CD8+ T cells	0
Ly6G_DCs	0
Ly6G_Monocytes	0
Ly6G_Neutrophils	1
Ly6G_NK cells	0
Ly6G_pDCs	0
Ly6G_Pro-B cell derived Macrophages	0
Ly6G_TAMs	0
MHC-II_B cells	1
MHC-II_CD4+ T cells	0
MHC-II_CD8+ T cells	0
MHC-II_DCs	1
MHC-II_Monocytes	1
MHC-II_Neutrophils	0
MHC-II_NK cells	0.5
MHC-II_pDCs	0.4
MHC-II_Pro-B cell derived	
Macrophages	0.3

MHC-II_TAMs	1
PD-L1_B cells	0.2
PD-L1_CD4+ T cells	0
PD-L1_CD8+ T cells	0
PD-L1_DCs	1
PD-L1_Monocytes	1
PD-L1_Neutrophils	0.2
PD-L1_NK cells	0.5
PD-L1_pDCs	0
PD-L1_Pro-B cell derived Macrophages	1
PD-L1_TAMs	1
CD11c_B cells	0.2
CD11c_CD4+ T cells	0
CD11c_CD8+ T cells	0
CD11c_DCs	1
CD11c_Monocytes	0.6
CD11c_Neutrophils	0
CD11c_NK cells	1
CD11c_pDCs	1
CD11c_Pro-B cell derived Macrophages	1
CD11c_TAMs	1
B220_B cells	1
B220_CD4+ T cells	0.3
B220_CD8+ T cells	0.3
B220_DCs	0
B220_Monocytes	0
B220_Neutrophils	0
B220_NK cells	0
B220_pDCs	1
B220_Pro-B cell derived Macrophages	1
B220_TAMs	0
PD-1_B cells	0
PD-1_CD4+ T cells	1
PD-1_CD8+ T cells	1
PD-1_DCs	0.5
PD-1_Monocytes	0.5
PD-1_Neutrophils	0
PD-1_NK cells	1
PD-1_pDCs	0.5
PD-1_Pro-B cell derived Macrophages	0.4
PD-1_TAMs	0.5
IgD_B cells	1
IgD_CD4+ T cells	0

IgD_CD8+ T cells	0
IgD_DCs	0
IgD_Monocytes	0
IgD_Neutrophils	0
IgD_NK cells	0
IgD_pDCs	0
IgD_Pro-B cell derived Macrophages	0
IgD_TAMs	0
CCR2_B cells	0
CCR2_CD4+ T cells	0
CCR2_CD8+ T cells	0
CCR2_DCs	1
CCR2_Monocytes	1
CCR2_Neutrophils	1
CCR2_NK cells	0
CCR2_pDCs	0
CCR2_Pro-B cell derived Macrophages	1
CCR2_TAMs	1
CD38_B cells	1
CD38_CD4+ T cells	1
CD38_CD8+ T cells	1
CD38_DCs	1
CD38_Monocytes	1
CD38_Neutrophils	0
CD38_NK cells	1
CD38_pDCs	1
CD38_Pro-B cell derived Macrophages	1
CD38_TAMs	1
CD206_B cells	0
CD206_CD4+ T cells	0
CD206_CD8+ T cells	0
CD206_DCs	0.5
CD206_Monocytes	0.5
CD206_Neutrophils	0
CD206_NK cells	0
CD206_pDCs	0
CD206_Pro-B cell derived Macrophages	0.5
CD206_TAMs	1
AF_B cells	1
AF_CD4+ T cells	1
AF_CD8+ T cells	1
AF_DCs	1

AF_Monocytes	1
AF_Neutrophils	1
AF_NK cells	1
AF_pDCs	1
AF_Pro-B cell derived Macrophages	1
AF_TAMs	1
CD3_B cells	0
CD3_CD4+ T cells	1
CD3_CD8+ T cells	1
CD3_DCs	0
CD3_Monocytes	0
CD3_Neutrophils	0
CD3_NK cells	0.2
CD3_pDCs	0
CD3_Pro-B cell derived Macrophages	0
CD3_TAMs	0
Fascin_B cells	1
Fascin_CD4+ T cells	0
Fascin_CD8+ T cells	0
Fascin_DCs	1
Fascin_Monocytes	1
Fascin_Neutrophils	1
Fascin_NK cells	1
Fascin_pDCs	1
Fascin_Pro-B cell derived Macrophages	1
Fascin_TAMs	1
Lyve-1_B cells	0
Lyve-1_CD4+ T cells	0
Lyve-1_CD8+ T cells	0
Lyve-1_DCs	1
Lyve-1_Monocytes	1
Lyve-1_Neutrophils	1
Lyve-1_NK cells	0
Lyve-1_pDCs	0
Lyve-1_Pro-B cell derived Macrophages	1
Lyve-1_TAMs	1
CD90_B cells	0.3
CD90_CD4+ T cells	1
CD90_CD8+ T cells	1
CD90_DCs	0
CD90_Monocytes	0
CD90_Neutrophils	0

CD90_NK cells	1
CD90_pDCs	0
CD90_Pro-B cell derived Macrophages	0.3
CD90_TAMs	0
CCR7_B cells	1
CCR7_CD4+ T cells	1
CCR7_CD8+ T cells	1
CCR7_DCs	1
CCR7_Monocytes	1
CCR7_Neutrophils	1
CCR7_NK cells	1
CCR7_pDCs	1
CCR7_Pro-B cell derived Macrophages	1
CCR7_TAMs	1
Monocytes_activated	1
Monocytes_blocked	1
pDCs_activated	1
pDCs_blocked	1
DCs_activated	1
DCs_blocked	1
TAMs_activated	1
TAMs_blocked	1
CD8_T_cells_activated	1
CD8_T_cells_blocked	1
NK_cells_activated	1
NK_cells_blocked	1

**Supplementary table 2. Priors used for iEN machine learning.**

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_lystate	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_lystate	0.181000311	Inc. in responders
IFNg_lystate	0.177540472	Inc. in responders
TAMs_activated	0.160726842	Inc. in responders
MIP_1a_lystate	0.154900386	Inc. in responders
MCP_1_lystate	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_lystate	0.114914635	Inc. in responders
SLAMF7_DCcs	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_lystate	0.0425075	Inc. in responders
Eotaxin_lystate	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_lystate	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_lystate	0.009106385	Inc. in responders
G-CSF_lystate	0.008431265	Inc. in responders
TOX_high_PD1_high_CD8	0.007180879	Inc. in responders

CCR2_DCs	0.006887445	Inc. in responders
IL_4_lystate	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_lystate	-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_lystate	-0.38754388	Dec. in responders

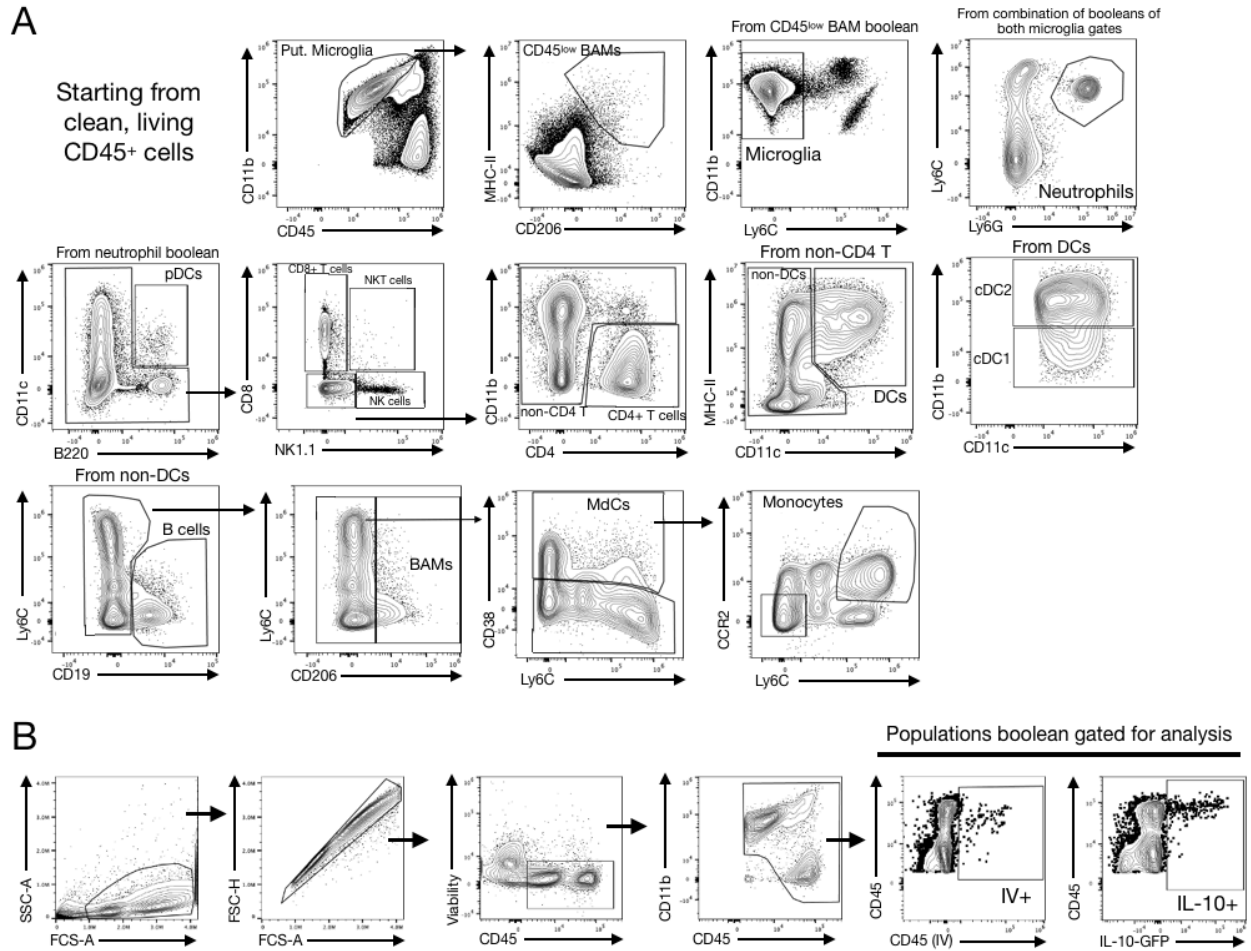
**Supplementary table 3. iEN model coefficients.** Only non-0 coefficients included.

<b>Antibody</b>	<b>Conjugate</b>	<b>Source</b>
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

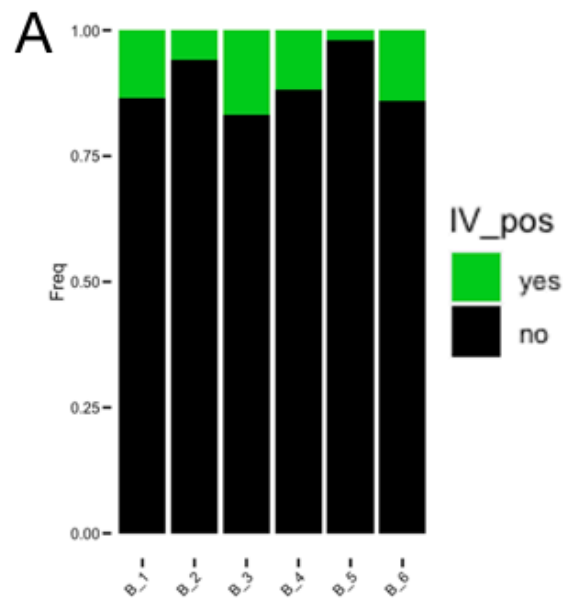
**Supplementary table 4. List of all antibodies used.**

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 Polymer™ - 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 Probe™ - 10 minutes  ProMark Rat on Mouse HRP Polymer™ - 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 Polymer™ - 20 minute  AEC Chromogen – 5 minutes CATHE Hematoxylin 1:10 – 1 minute

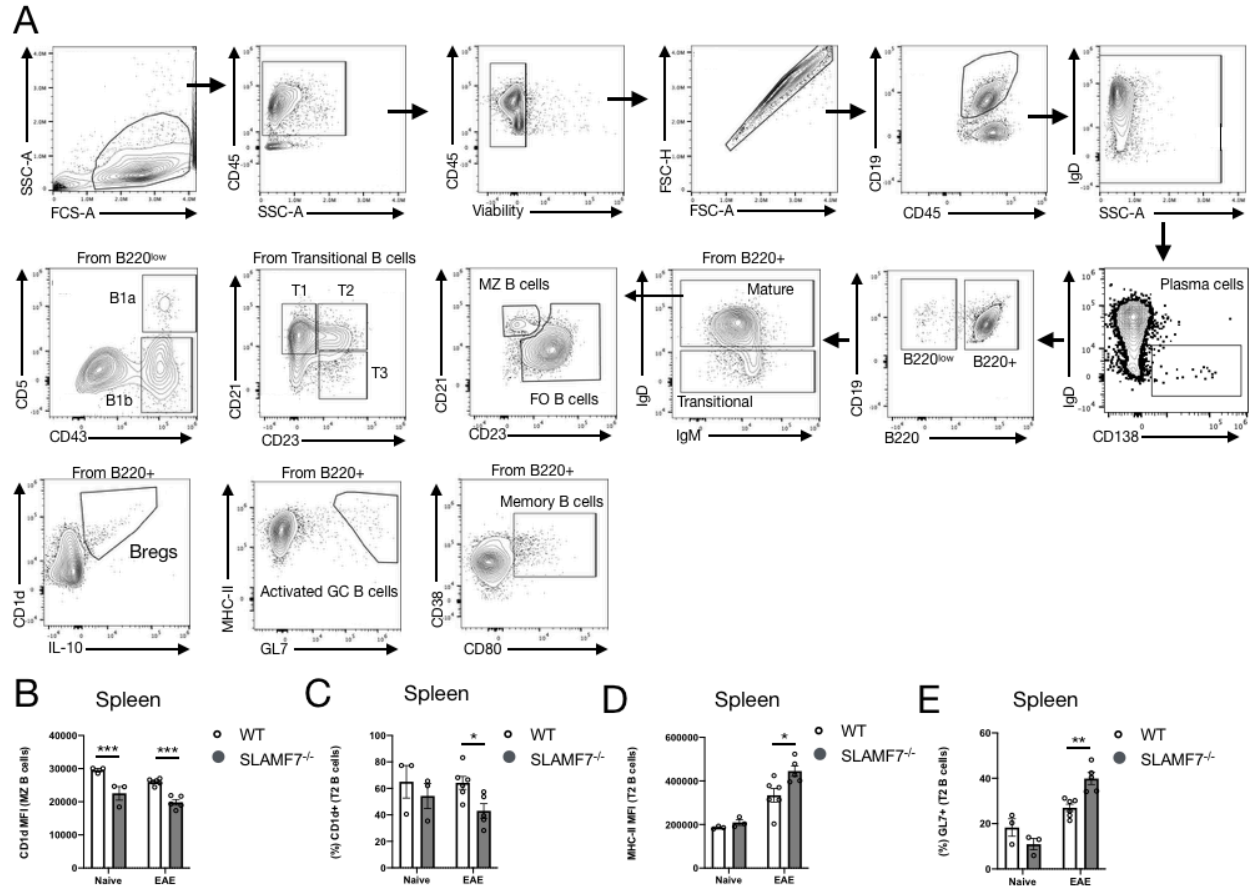
**Supplementary table 5. IHC antibodies and staining specifications.**



**Supplemental figure 12. Gating schemes.** (A) Gating scheme used to manually annotate nearly all CNS immune cell subsets (used in [Fig. 30G] and [Fig. 31A, B, D]). This gating scheme is able to identify approximately 98% of all CD45<sup>+</sup> CNS immune cells. (B) Gating scheme to clean up datasets for high dimensional single-cell analysis in R for experiments assessing IL-10 production from CNS resident immune cells (used in [Fig. 30C-F], [Fig. 31C], and [Fig. 32]).



**Supplemental figure 13. Resident frequency of CNS B cell subsets (related to Fig. 33).** (A) Frequency of each CNS B cell subset from (Fig. 33D-F) that is CNS resident [IV<sup>-</sup>] versus circulating (IV<sup>+</sup>).



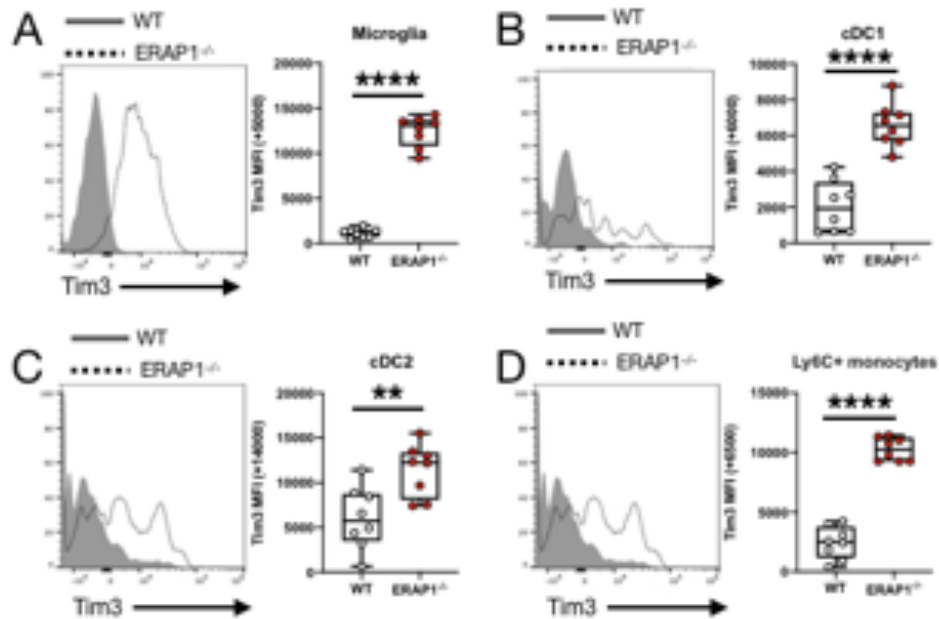
**Supplemental figure 14. Gating and additional analyses of B cell deep phenotyping (related to Fig. 34).** (A) Gating scheme used to clean up datasets and manually annotate all CNS and splenic B cell subsets from high dimensional B cell profiling experiments. (B) CD1d expression on MZ B cells. (C) CD1d expression on T2 B cells. (D) MHC-II expression on T2 B cells. (E) GL7 expression on T2 B cells. Groups in (B-E) compared with a two-way ANOVA with Sidak's test for multiple comparisons. FO, follicular; MZ, marginal zone; Bregs, regulatory B cells. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Antibody	Conjugate	Source
<b>Confocal antibodies</b>		
Anti-goat Iba1	n/a	Novus
Anti-rabbit SLAMF7 (bs-2544R)	n/a	Bioss
<b>Neuroimmune phenotyping panel (IL-10)</b>		
CD45 (30-F11)	Alexa Fluor 532	ThermoFischer
Cd11b (M1/70)	BV570	BioLegend
Tim3 (B8.2C12)	APC/Fire 750	BioLegend
CD4 (RM4-5)	eFluor 450	ThermoFischer
CD8a (53-6.7)	A700	eBioscience
NK1.1 (PK136)	PE-Cy7	eBioscience
CD19 (1D3)	PerCP-Cy5.5	Fischer Scientific
SLAMF7 (4G2)	APC	R&D Systems
NKG2D (CX5)	PE	ThermoFischer
Ly-6C (HK1.4)	BV421	BioLegend
Live/Dead	Zombie NIR	BioLegend
Ly-6G (1A8)	BV711	BioLegend
MHCII (M5/114.15.2)	BV785	BioLegend
CD3 (17A2)	BUV737	BD Biosciences
Siglec-H (440c)	BV650	BD Biosciences
CD11c (HL3)	PE-CF594	BD Biosciences
B220 (RA3-6B2)	BV480	Fischer Scientific
CD49b (DX5)	PerCP-eFluor 710	ThermoFischer
IgD (IA6-2)	Super Bright 436	eBioscience
CD206 (C068C2)	Alexa Fluor 647	BioLegend
CD38 (90/CD38)	BV510	BD Biosciences
CCR2 (4575301)	BV750	BD Biosciences
LYVE1 (ALY7)	eFluor 615	ThermoFischer
CD90 (53-2.1)	BUV395	BD Biosciences
Lag-3 (C9B7W)	BUV496	BD Biosciences
<b>uMT immunophenotyping panel</b>		
CD45 (30-F11)	Alexa Fluor 488	BioLegend
CD19 (1D3)	PerCP-Cy5.5	Fischer Scientific
CD3 (145-2C11)	APC-Cy7	BioLegend
CD4 (RM4-5)	eFluor450	ThermoFisher
CD8a (53-6.7)	A700	eBioscience
Live/Dead	Zombie NIR	BioLegend
T-bet (4B10)	PE Dazzle 594	BioLegend
GATA3 (TWAJ)	eFluor 660	ThermoFischer
IFN gamma (XMG1.2)	APC	eBiosciences
TNF alpha (MP6-XT22)	PE-Cy7	eBiosciences
ROR gamma(t) (B2D)	PE	ThermoFischer
IL-17 (TC11-18H10.1)	BV785	BioLegend
IgD (IA6-2)	SB436	ThermoFischer

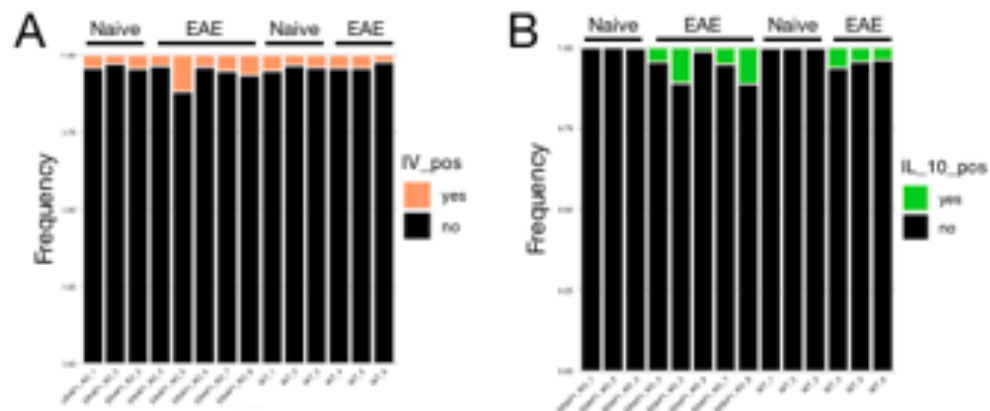
B220 (RA3-6B2)	VioletFluor450	Fischer Scientific
<b>Neuroimmune phenotyping panel (SF9)</b>		
CD45 (30-F11)	Alexa Fluor 488	ThermoFischer
Cd11b (M1/70)	BV570	BioLegend
Tim3 (B8.2C12)	APC/Fire 750	BioLegend
CD4 (RM4-5)	eFluor 450	ThermoFischer
CD8a (53-6.7)	A700	eBioscience
NK1.1 (PK136)	PE-Cy7	eBioscience
CD19 (1D3)	PerCP-Cy5.5	Fischer Scientific
CD80 (16-10A1)	PE	eBioscience
SLAMF7 (4G2)	APC	R&D Systems
Ly-6C (HK1.4)	BV421	BioLegend
Live/Dead	Live/dead Aqua	ThermoFisher
Ly-6G (1A8)	BV711	BioLegend
SLAMF9	Secondary stain w/ Alexa555	Biorbyt
MHCII (M5/114.15.2)	BV785	BioLegend
Siglec-H (440c)	BV650	BD Biosciences
CD11c (HL3)	PE-CF594	BD Biosciences
B220 (RA3-6B2)	BV480	Fischer Scientific
PD-1 (RMP1-30)	PerCP-eFluor 710	eBiosciences
CXCR5 (L138D7)	BV605	BioLegend

**Supplemental table 6. List of antibodies used.**

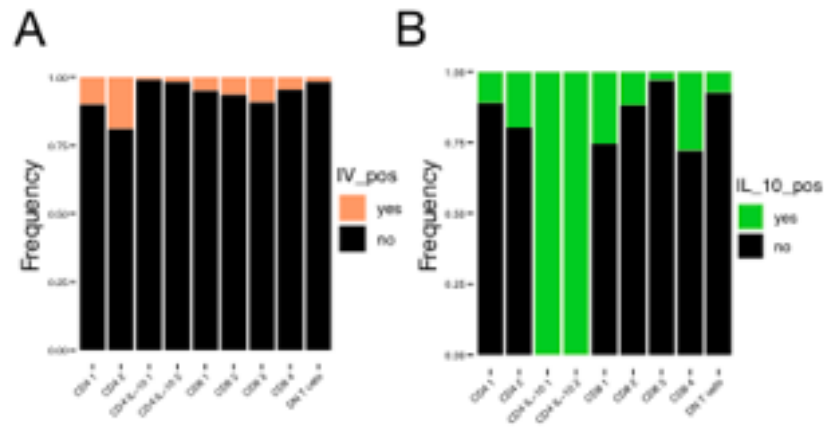




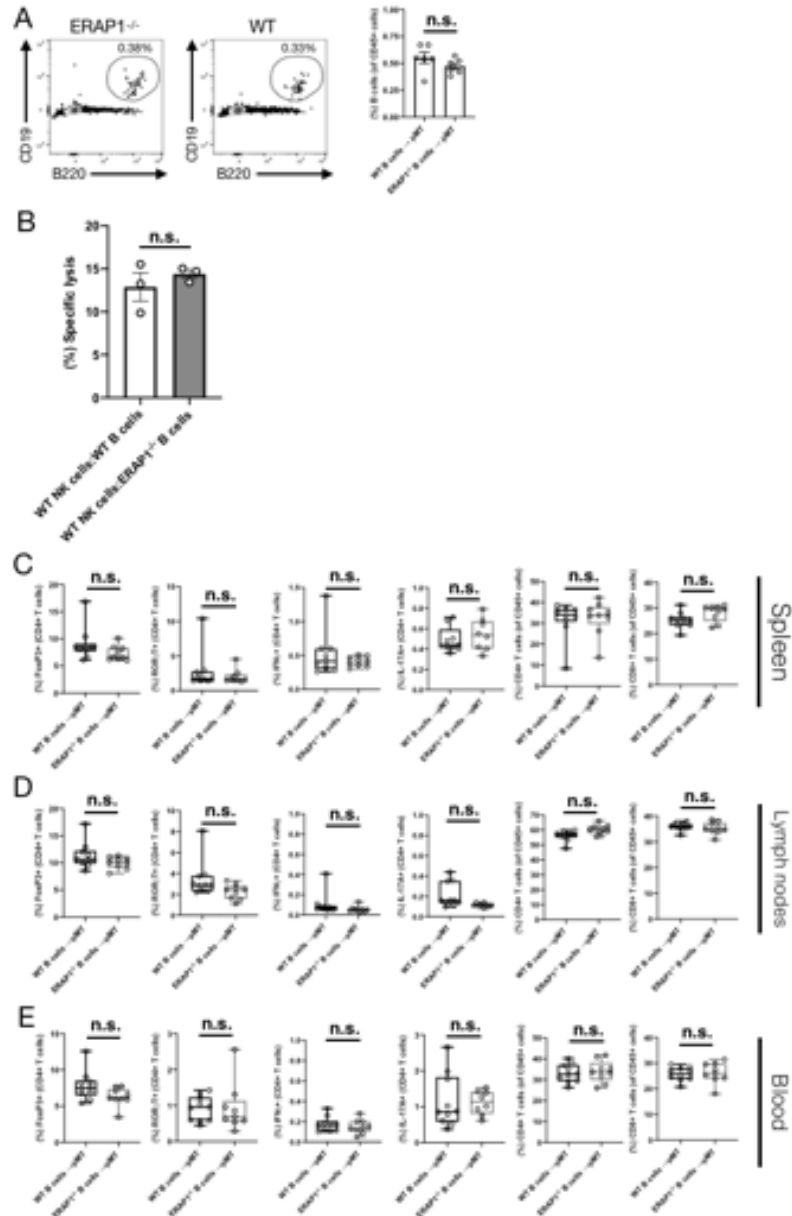
**Supplemental figure 16. Tim3 expression on CNS myeloid cells during EAE (related to Fig. 35).** (A) Tim3 expression on microglia from WT and ERAP1<sup>-/-</sup> mice subjected to EAE with rmMOG<sub>1-125</sub>. (B) Tim3 expression on cDC1 cells from WT and ERAP1<sup>-/-</sup> mice subjected to EAE with rmMOG<sub>1-125</sub>. (C) Tim3 expression on cDC2 cells from WT and ERAP1<sup>-/-</sup> mice subjected to EAE with rmMOG<sub>1-125</sub>. (D) Tim3 expression on Ly6C<sup>+</sup> monocytes from WT and ERAP1<sup>-/-</sup> mice subjected to EAE with rmMOG<sub>1-125</sub>. Representative of two independent experiments showing similar results. Groups compared with unpaired two-way t-test. \*\*p<0.01, \*\*\*p<0.0001.



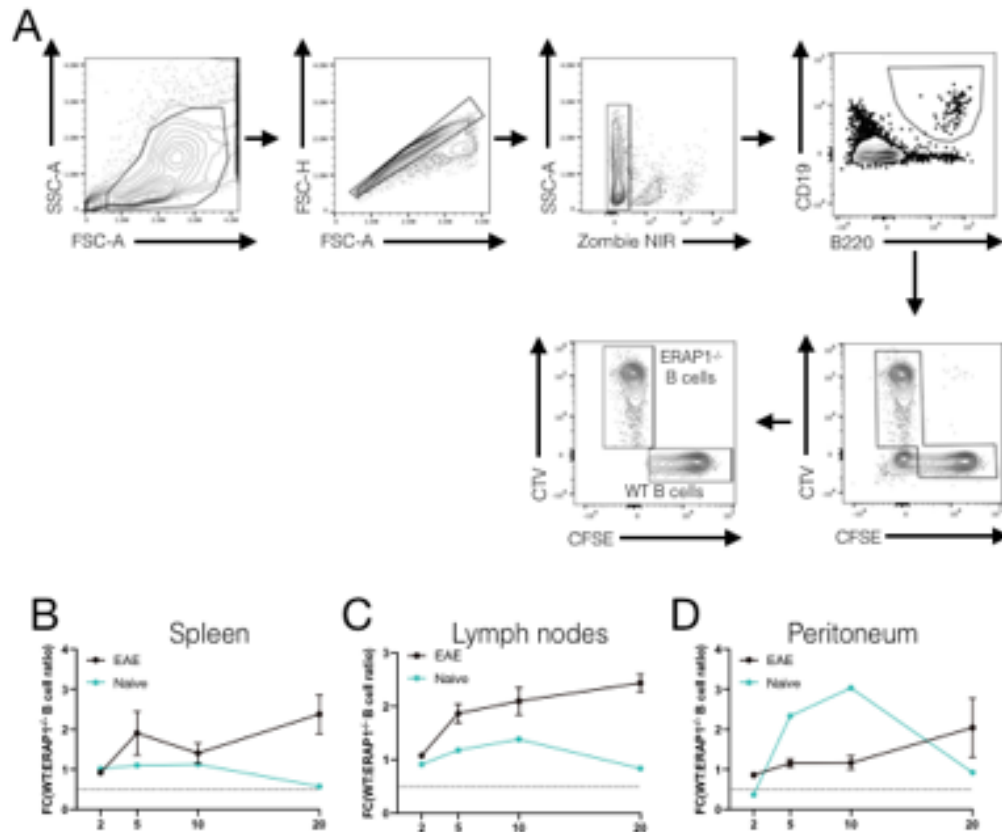
**Supplemental figure 17. IV labeling and IL-10 expression on CNS immune cells by sample (related to Fig. 36).** (A) Frequency of IV<sup>+</sup> CNS immune cells from naive and EAE mice. Equivalent low-level IV labeling across all samples indicating tissue processing consistency and effectiveness of trans-cardiac perfusion. (B) Frequency of IL-10-GFP<sup>+</sup> immune cells in the CNS of naive and EAE mice. Substantial numbers of IL-10<sup>+</sup> cells are only detectable in mice subjected to EAE as expected.



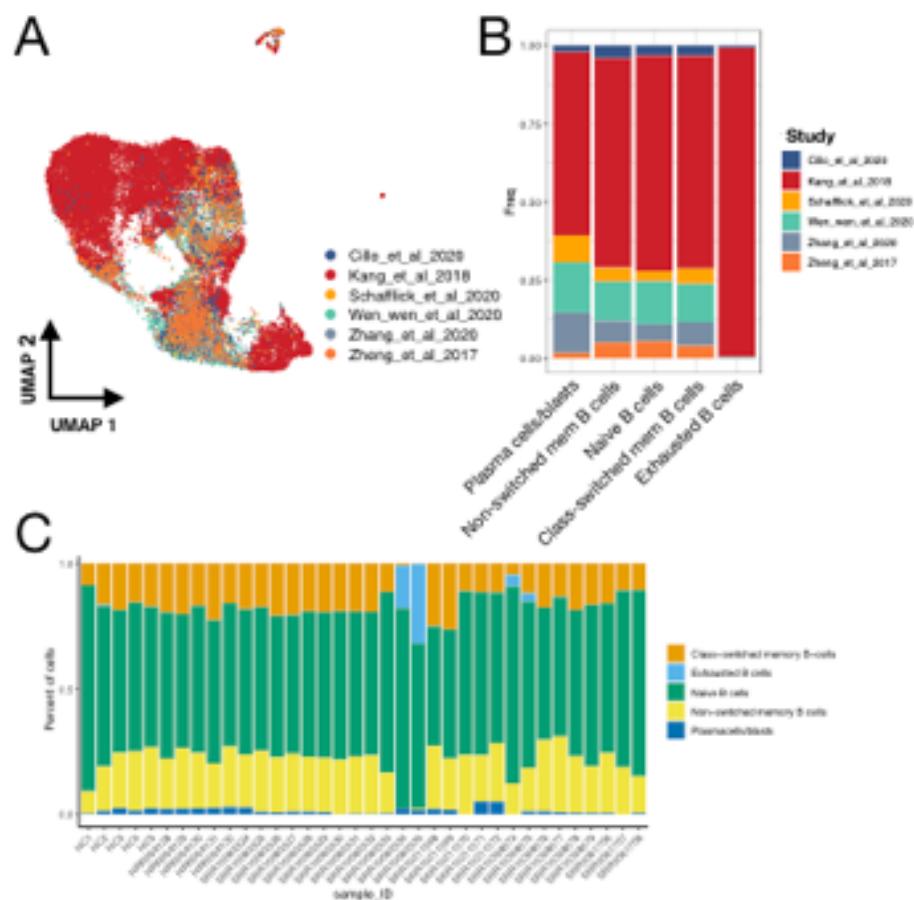
**Supplemental figure 18. IV labeling and IL-10 expression on CNS T cell subsets (related to Fig. 37).** (A) Frequency of IV<sup>+</sup> CNS T cells in WT and ERAP1<sup>-/-</sup> mice during EAE. (B) Frequency of IL-10-GFP<sup>+</sup> CNS resident T cells in WT and ERAP1<sup>-/-</sup> mice during EAE. Both CD4<sup>+</sup> clusters labeled as “IL-10” show 100% of cells being IL-10<sup>+</sup>.



**Supplemental figure 19. Adoptive B cell transfer validation and T cell phenotypes (related to Fig. 38).** (A) Validation of the transfer of equivalent numbers of WT and ERAP1<sup>-/-</sup> B cells into uMT mice via FACS of peripheral blood collected three days post-transfer. (B) Splenic T cell ratios, polarization-defining transcription factor expression, and cytokine expression from uMT mice following adoptive transfer of B cells and EAE induction. (C) Same measurements as (B), but from inguinal lymph nodes. (D) Same measurements from (B), but from peripheral blood. All data is representative of two independent experiments showing equivalent results. Data in all figures compared with a two-way unpaired t-test. n.s. not significant.



**Supplemental figure 20. Gating scheme and comparison to naive mice for B cell co-transfer experiment (related to Fig. 40).** (A) Gating scheme employed for all lymphoid organs assessed. (B-D) Fold change in the ratio of WT to ERAP1<sup>-/-</sup> B cells transferred into the same uMT mouse over time in mice subjected to EAE or left untreated. Data in (B-D) representative of a single experiment with three mice analyzed per timepoint in the EAE group and one mouse per timepoint in the naive group.



**Supplemental figure 21. Study- and sample-level assessment of human scRNA-seq B cell analysis (related to Fig. 41).** (A) UMAP of all B cells/plasma cells colored by study. (B) Bar plot of study contribution to each B cell subset. (C) Bar plot of cluster breakdown per each individual.

Antibody	Conjugate	Source
<b>Immunophenotyping panel 1</b>		
PD1 (J43)	PerCP-eFluor710	ThermoFischer
CD4 (RM4-5)	eFluor 450	ThermoFischer
CD8 (53-6.7)	A700	eBioscience
Lag-3 (C9B7W)	BV785	ThermoFischer
Tim-3 (RMT3-23)	PE-Cy7	Biolegend
CD3 (145-2C11)	APC	ThermoFischer
SLAMF7 (4G2)	BV421	eBioscience
CD19 (1D3)	PerCP-Cy5.5	Fischer Scientific
B220 (RA3-6B2)	VioletFluor450	Fischer Scientific
IgD (11-26c)	Super Bright 436	eBioscience
Live/Dead	Zombie NIR	BioLegend
<b>Immunophenotyping panel 2</b>		
CD45 (30-F11)	Alexa Fluor 532	ThermoFischer
Cd11b (M1/70)	BV570	BioLegend
Tim3 (B8.2C12)	APC/Fire 750	BioLegend
CD4 (RM4-5)	eFluor 450	ThermoFischer
CD8a (53-6.7)	A700	eBioscience
NK1.1 (PK136)	PE-Cy7	eBioscience
CD19 (1D3)	PerCP-Cy5.5	Fischer Scientific
SLAMF7 (4G2)	APC	R&D Systems
NKG2D (CX5)	PE	ThermoFischer
Ly-6C (HK1.4)	BV421	BioLegend
Live/Dead	Zombie NIR	BioLegend
Ly-6G (1A8)	BV711	BioLegend
MHCII (M5/114.15.2)	BV785	BioLegend
CD3 (17A2)	BUV737	BD Biosciences
Siglec-H (440c)	BV650	BD Biosciences
CD11c (HL3)	PE-CF594	BD Biosciences
B220 (RA3-6B2)	BV480	Fischer Scientific
CD49b (DX5)	PerCP-eFluor 710	ThermoFischer
IgD (IA6-2)	Super Bright 436	eBioscience
CD206 (C068C2)	Alexa Fluor 647	BioLegend
CD38 (90/CD38)	BV510	BD Biosciences
CCR2 (4575301)	BV750	BD Biosciences
LYVE1 (ALY7)	eFluor 615	ThermoFischer
CD90 (53-2.1)	BUV395	BD Biosciences
Lag-3 (C9B7W)	BUV496	BD Biosciences
<b>uMT immunophenotyping panel</b>		
CD45 (30-F11)	Alexa Fluor 488	BioLegend
CD19 (1D3)	PerCP-Cy5.5	Fischer Scientific
CD3 (145-2C11)	APC-Cy7	BioLegend
CD4 (RM4-5)	BV510	BioLegend
CD8a (53-6.7)	A700	eBioscience

Live/Dead	Zombie NIR	BioLegend
CD20 (SA275A11)	APC	BioLegend
CD80 (16-10A1)	PE	eBioscience
T-bet (4B10)	PE Dazzle 594	BioLegend
GATA3 (TWAJ)	eFluor 660	ThermoFischer
FOXP3 (FJK-16s)	eFluor 450	ThermoFischer
IFN gamma (XMG1.2)	APC	eBiosciences
TNF alpha (MP6-XT22)	PE-Cy7	eBiosciences
ROR gamma(t) (B2D)	PE	ThermoFischer
IL-17 (TC11-18H10.1)	BV785	BioLegend
IgD (IA6-2)	SB436	ThermoFischer
B220 (RA3-6B2)	VioletFluor450	Fischer Scientific
WT stain	CFSE	Sigma
ERAP1 KO stain	CellTrace Violet	ThermoFischer
CD43 (S11)	PE-Dazzle 594	BioLegend

**Supplementary table 7. List of antibodies used.**