Supplementary Information for

Albumin-binding nanobody cancer vaccines coordinate lymphatic T cell priming and inflammatory remodeling of the tumor microenvironment

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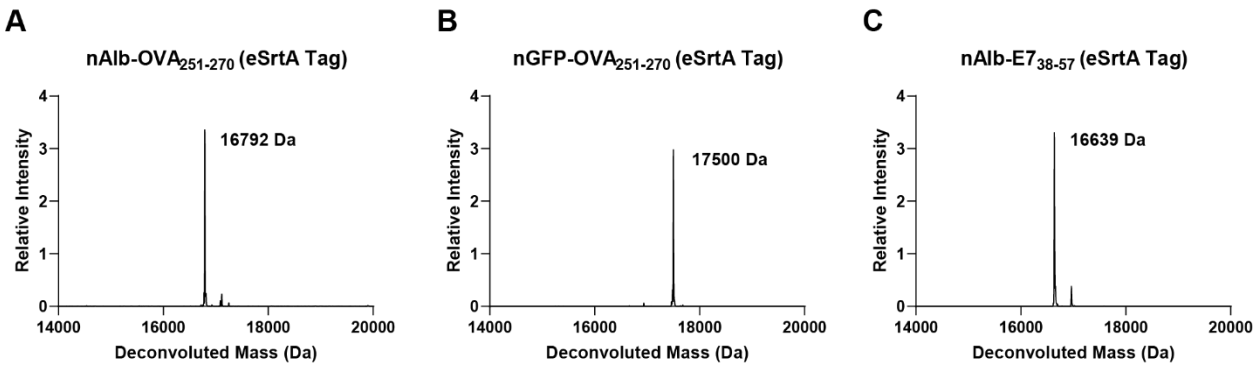


Fig. S1. ESI-MS of nanobody-antigen fusions. Deconvoluted mass spectra of **(A)** nAlb-$OVA_{251-270}$, **(B)** nGFP-$OVA_{251-270}$, and **(C)** nAlb-$E7_{38-57}$.

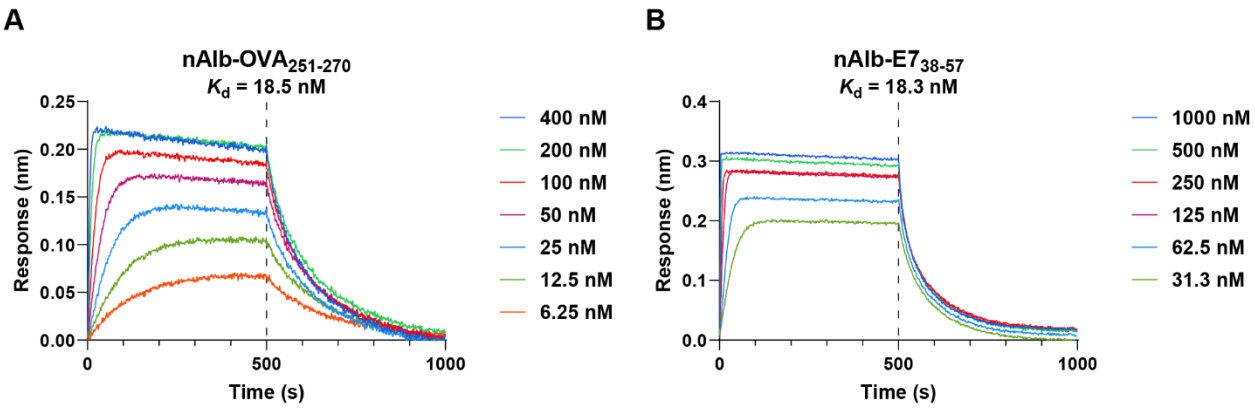


Fig. S2. BLI of nAlb-antigen fusions binding mouse serum albumin (MSA). (A) BLI binding curves of nAlb-OVA$_{251\text{-}270}$ binding MSA. **(B)** BLI binding curves of nAlb-E7$_{38\text{-}57}$ binding MSA.

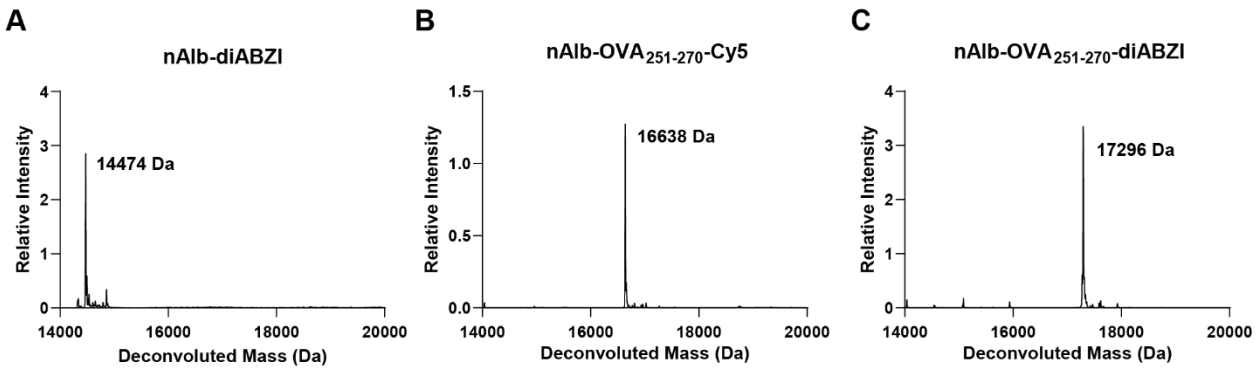


Fig. S3. ESI-MS of nanobody-drug conjugates (NDCs). Deconvoluted mass spectra of **(A)** nAlb-diABZI, **(B)** nAlb-OVA$_{251-270}$-Cy5, and **(C)** nAlb-OVA$_{251-270}$-diABZI.

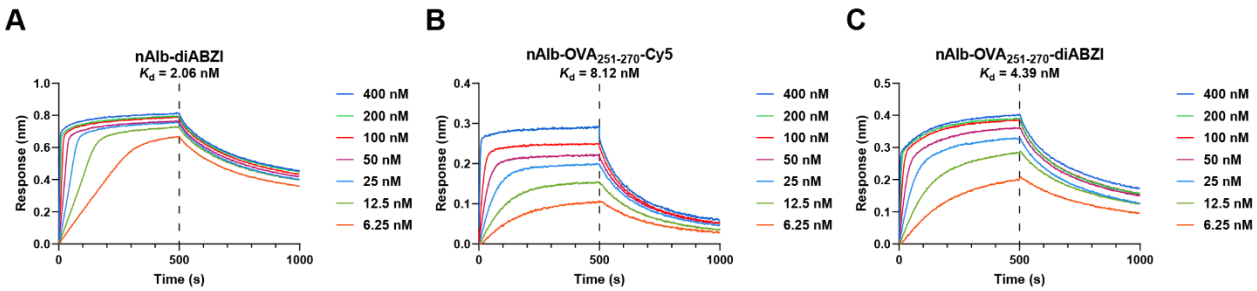


Fig. S4. BLI of nAlb- and nAlb-antigen-drug conjugates binding mouse serum albumin (MSA). (A) BLI binding curves of nAlb-diABZI binding MSA. **(B)** BLI binding curves of nAlb-OVA$_{251-270}$-Cy5 binding MSA. **(C)** BLI binding curves of nAlb-OVA$_{251-270}$-diABZI binding MSA.

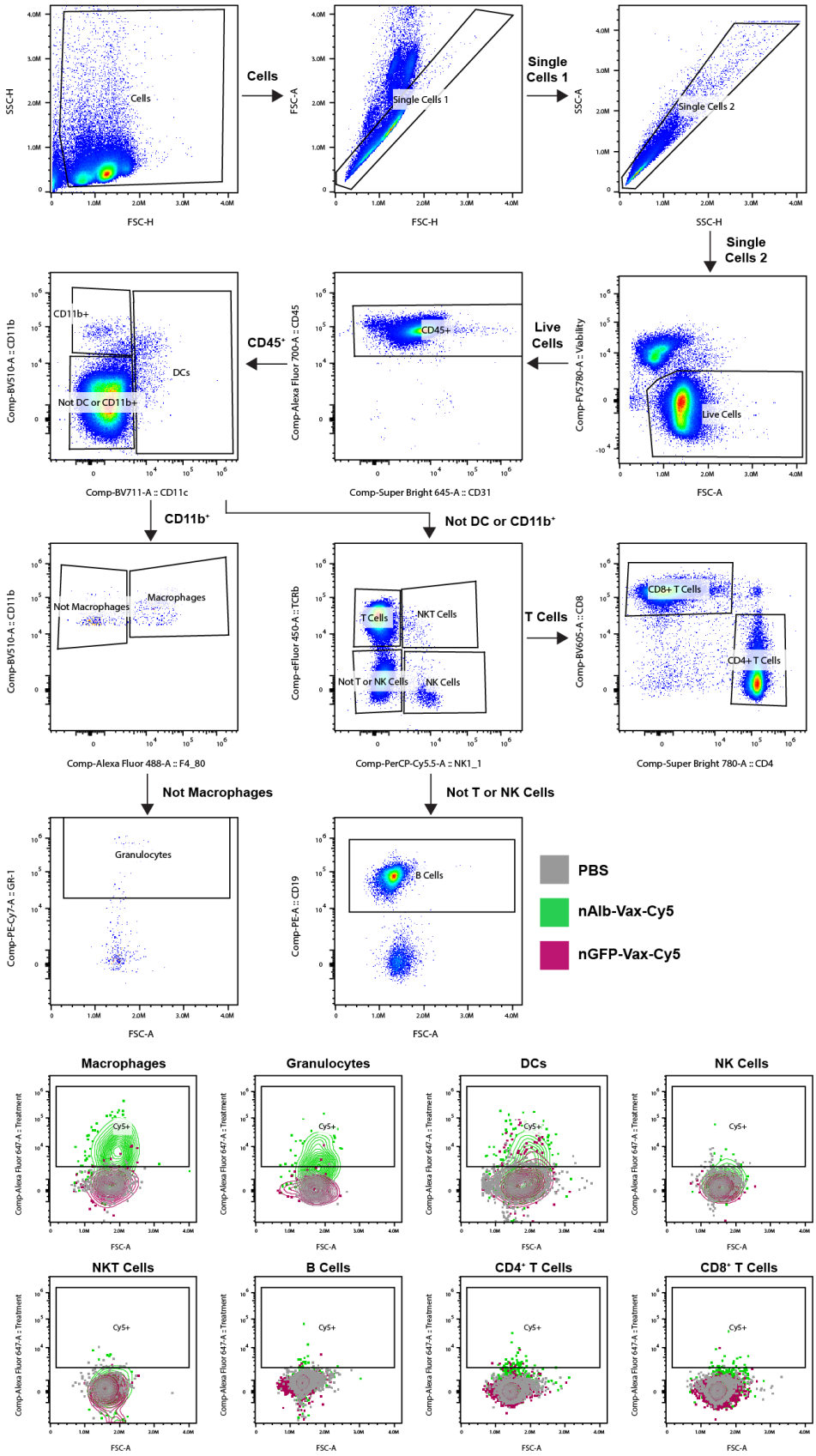


Fig. S5. Gating strategy and representative flow cytometry dot plots for Fig. 1J-K.

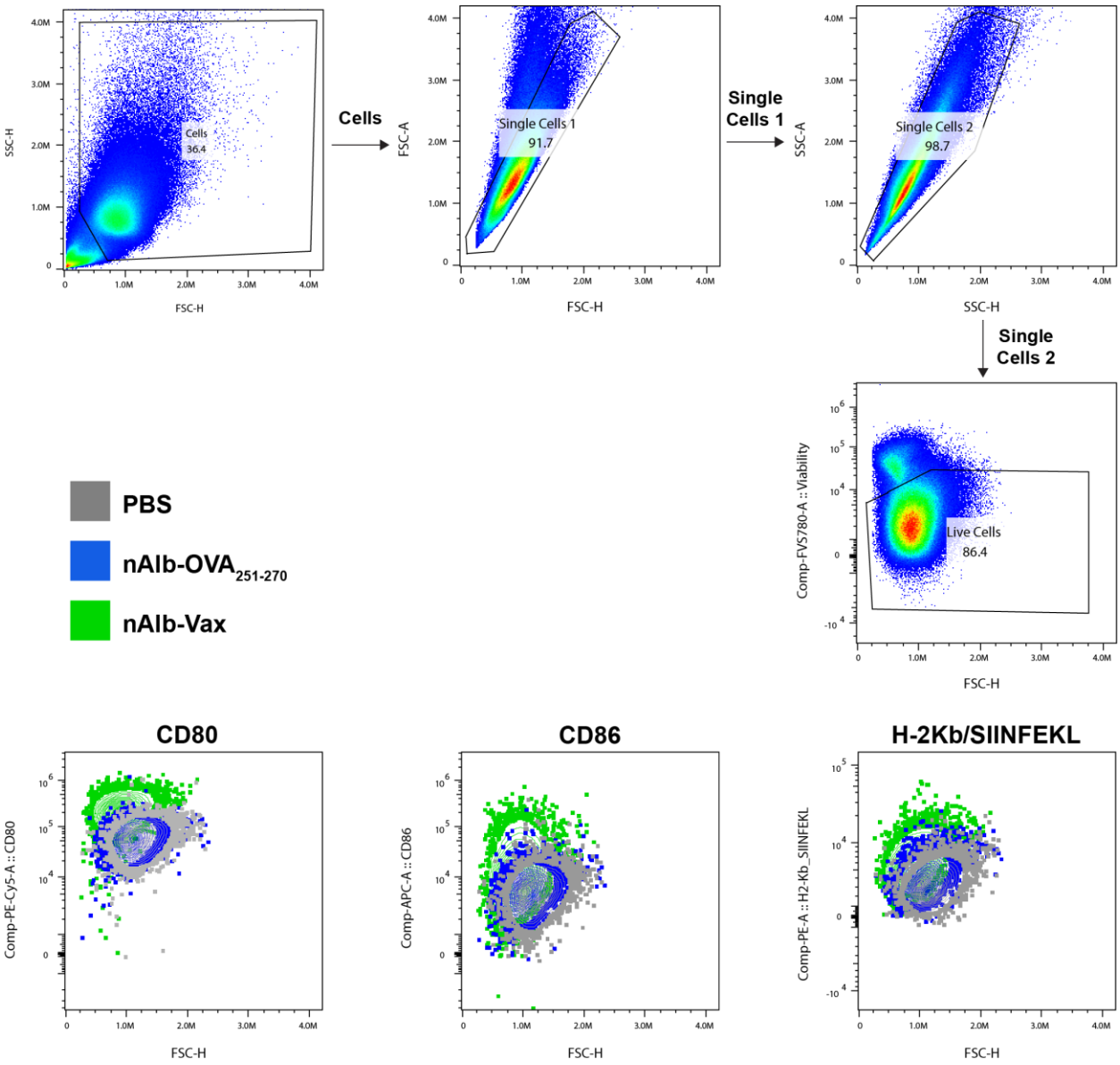


Fig. S6. Gating strategy and representative flow cytometry dot plots for Fig. 2C-E, K.

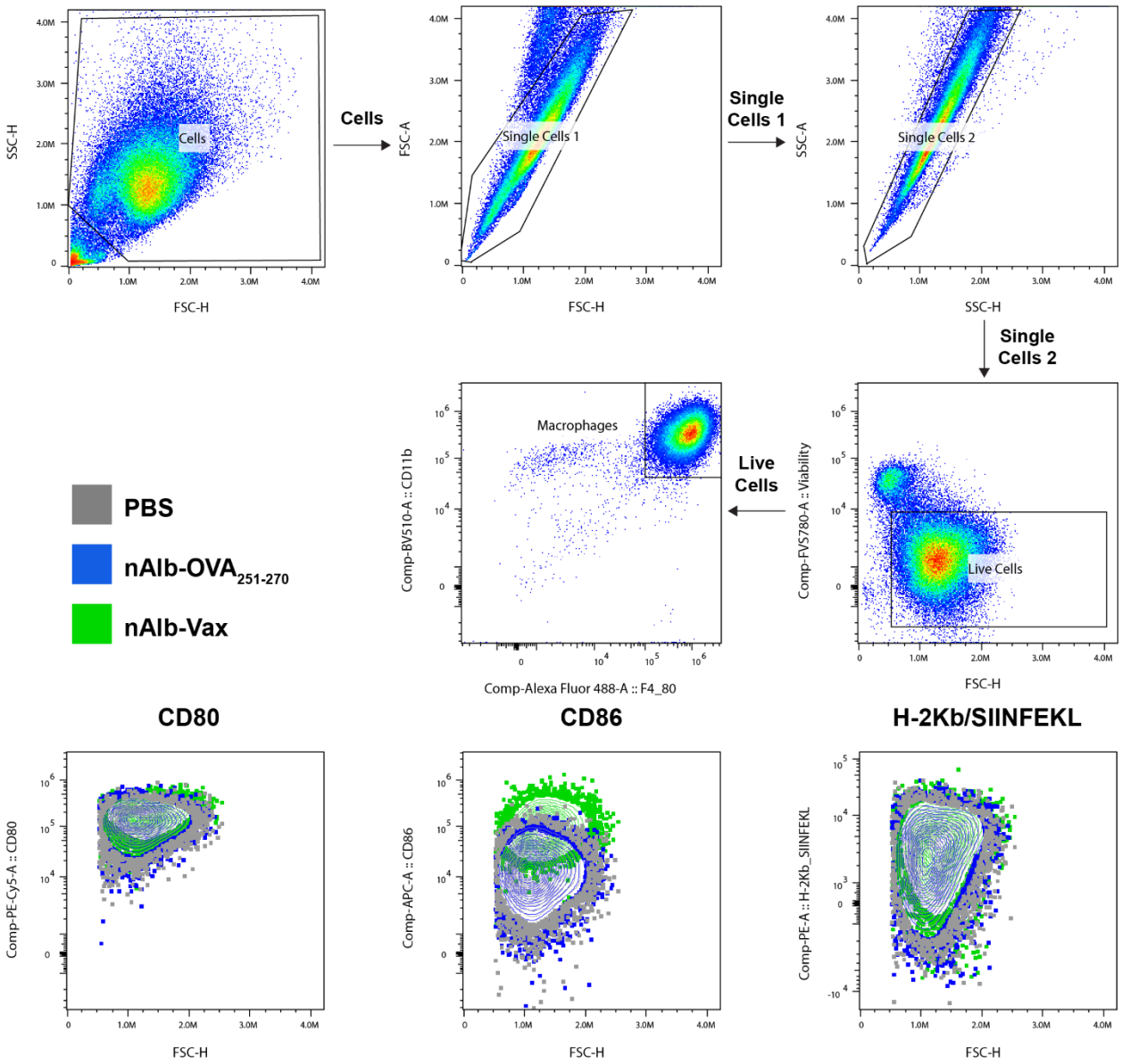


Fig. S7. Gating strategy and representative flow cytometry dot plots for Fig. 2H-J, L.

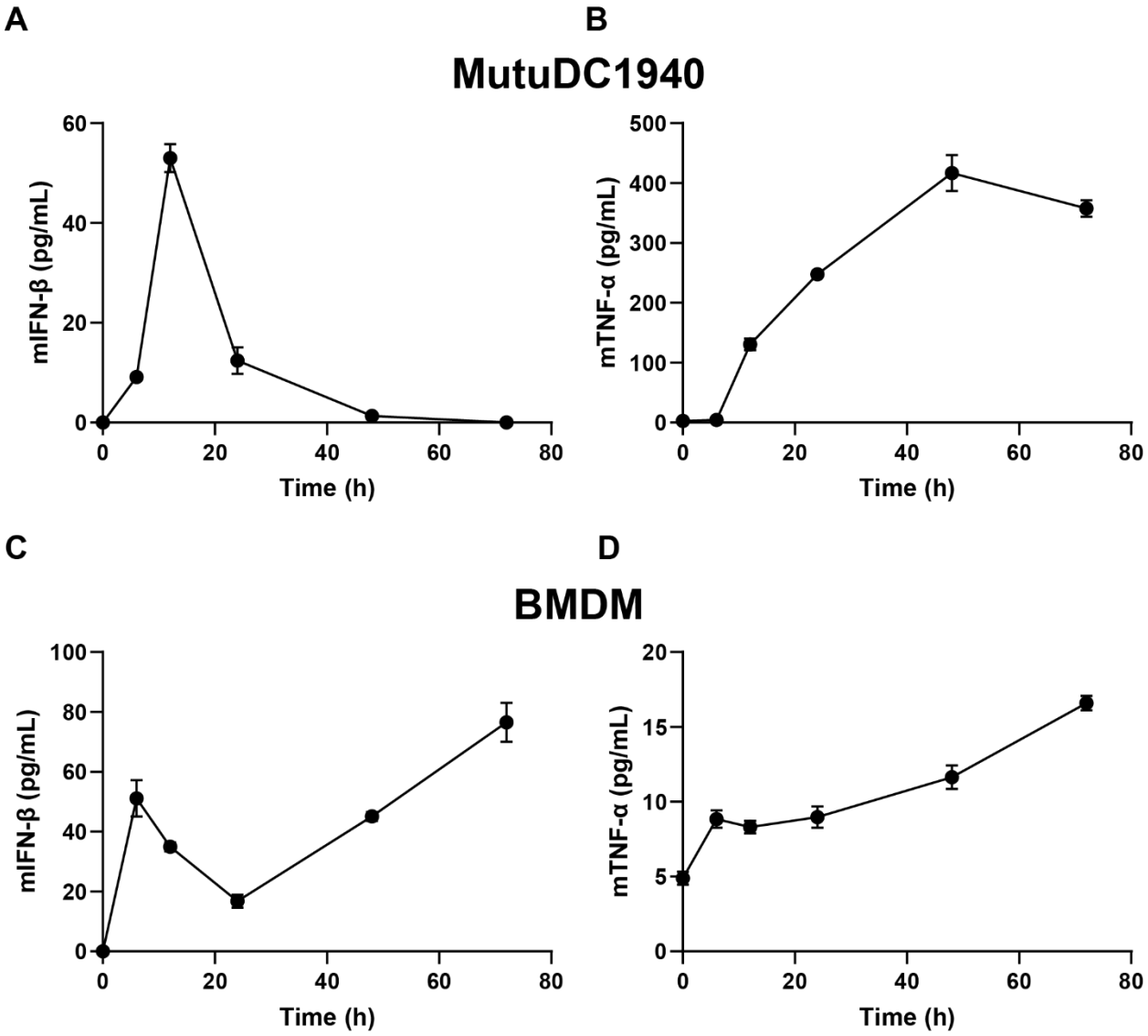


Fig. S8. ELISA quantification of IFN-β and TNF-α secretion by MutuDC1940 cells and BMDMs over time following treatment with nAlb-Vax. Quantification of **(A)** IFN-β and **(B)** TNF-α secretion by MutuDC1940 cells and **(C)** IFN-β and **(D)** TNF-α secretion by BMDMs following treatment with nAlb-Vax as a function of time (mean ± SEM, $n = 3$).

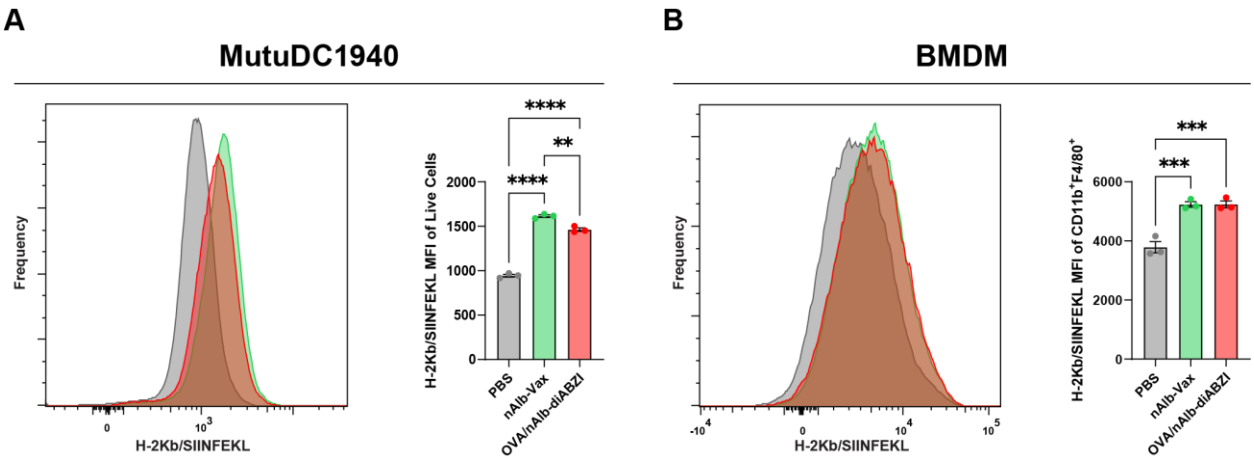


Fig. S9. Comparison of antigen cross presentation by nAlb-OVA$_{251-270}$ and full-length OVA. **(A)** Representative histograms and MFIs of H-2Kb/SIINFEKL expression by MutuDC1940 cells 24 h after treatment with indicated constructs (mean ± SEM, n = 3). Analyzed via one-way ANOVA with Tukey's *post-hoc*. **(B)** Representative histograms and MFIs of H-2Kb/SIINFEKL expression by BMDMs 24 h after treatment with indicated constructs (mean ± SEM, n = 3). Analyzed via one-way ANOVA with Tukey's *post-hoc*. ****$P < 0.0001$. ***$P < 0.001$. **$P < 0.01$. *$P < 0.05$.

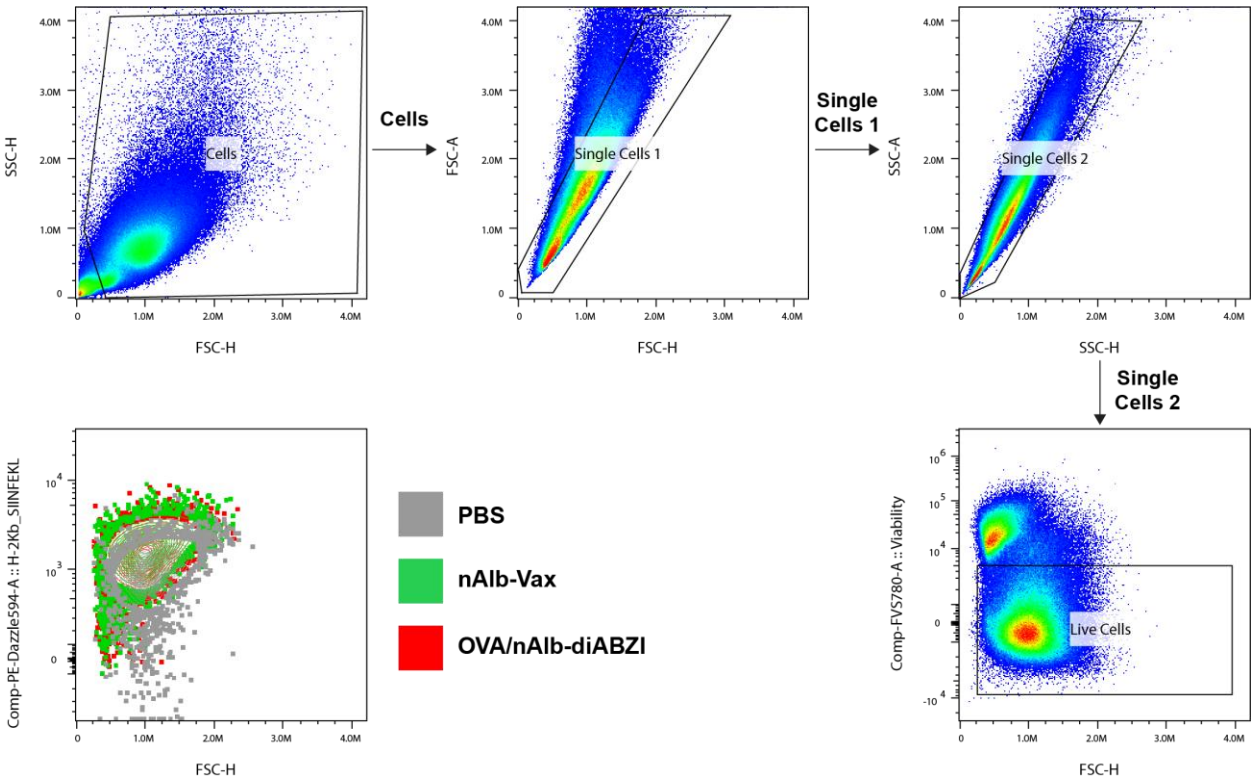


Fig. S10. Gating strategy and representative flow cytometry dot plots for Fig. S9A.

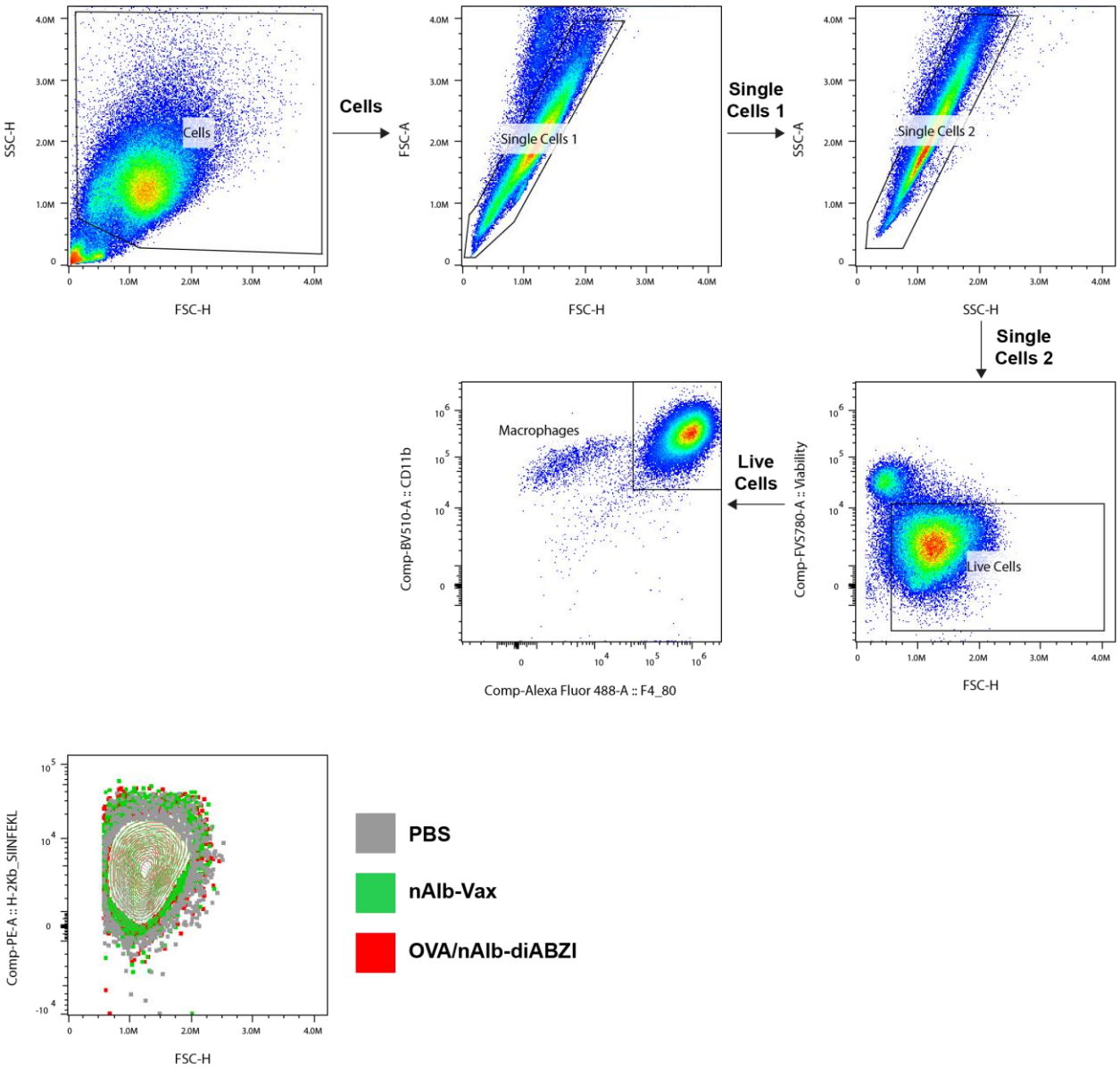


Fig. S11. Gating strategy and representative flow cytometry dot plots for Fig. S9B.

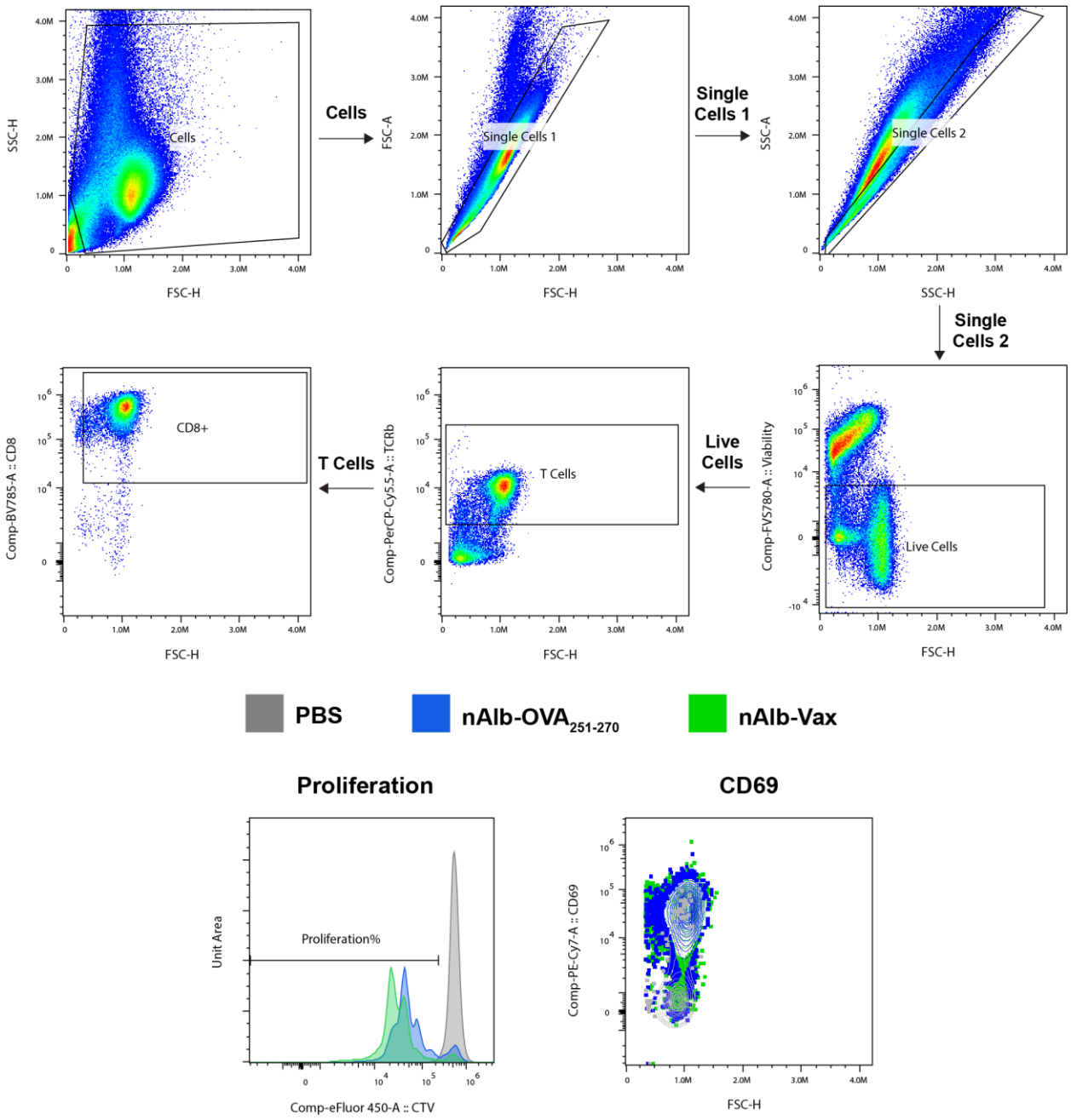


Fig. S12. Gating strategy and representative flow cytometry histograms and dot plots for Fig. 2M-O.

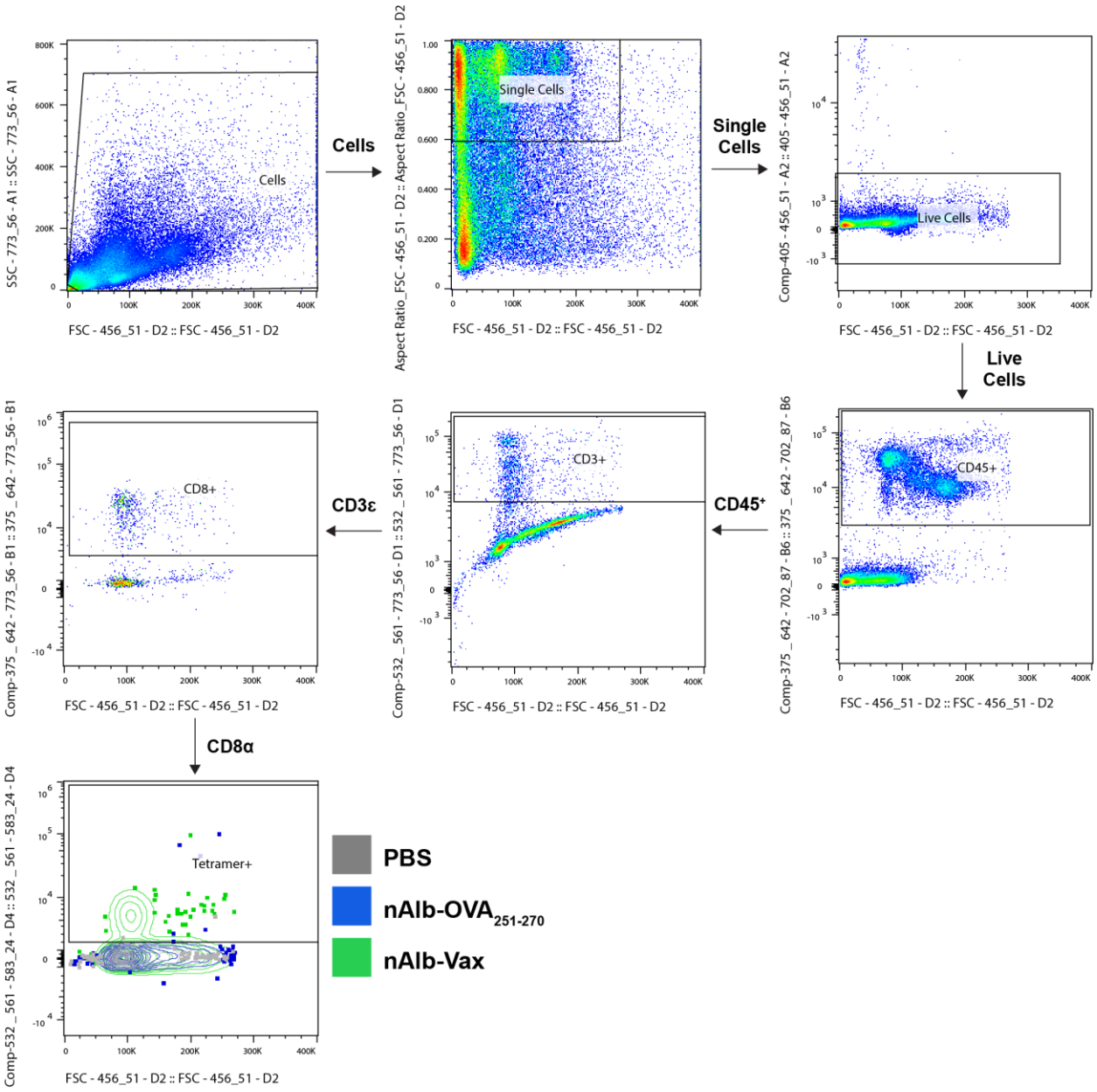


Fig. S13. Gating strategy and representative flow cytometry dot plots for Fig. 3A.

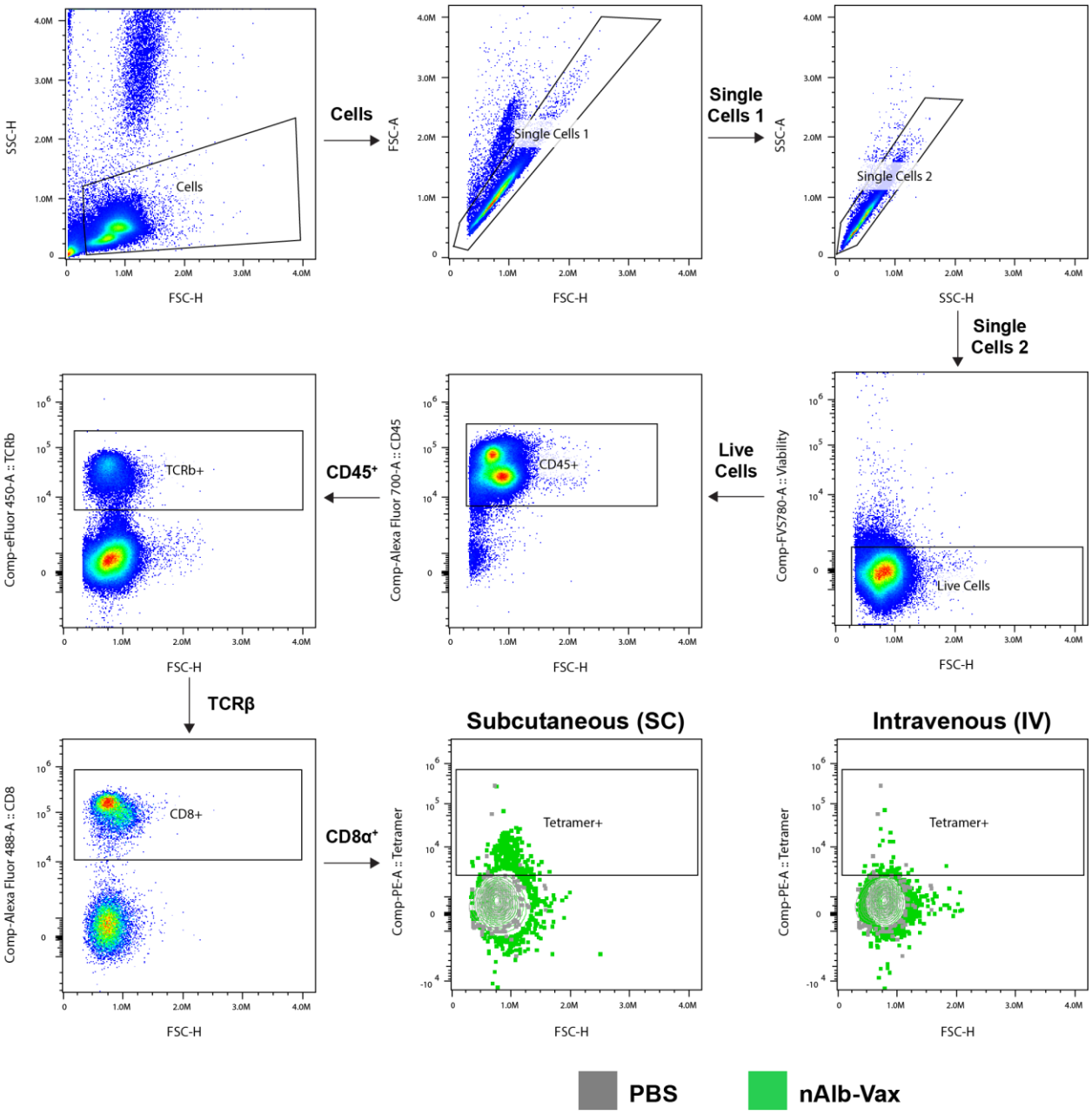


Fig. S14. Gating strategy and representative flow cytometry dot plots for Fig. 3B.

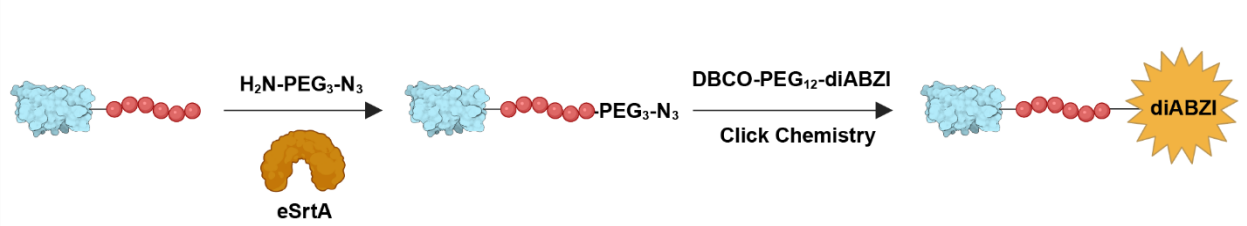


Fig. S15. Schematic depicting series of sortase and strain-promoted azide-alkyne cycloaddition (SPAAC) reactions used to label nAlb-OVA$_{251-270}$ with DBCO-PEG$_{12}$-diABZI. Created with Biorender.com.

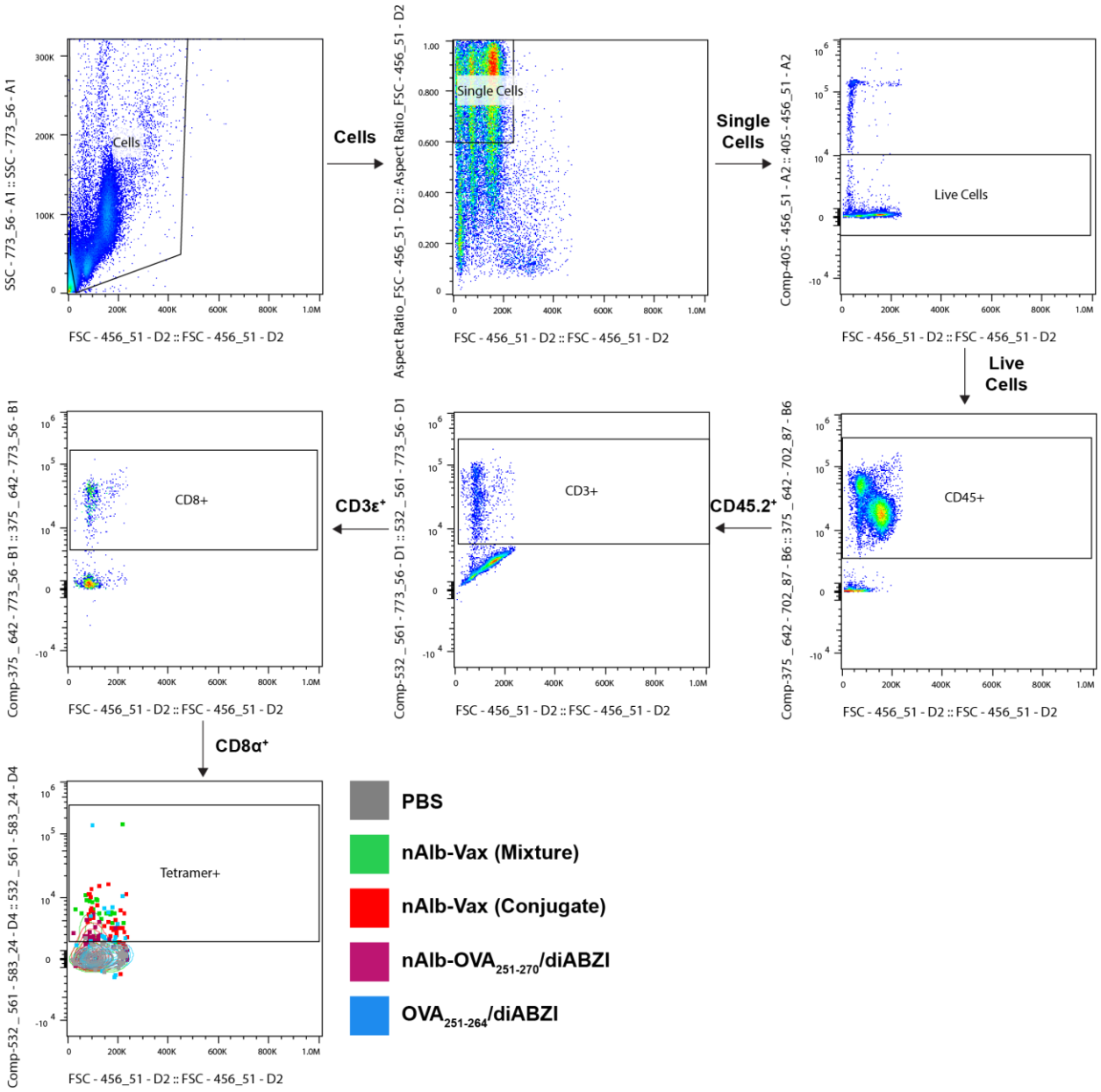


Fig. S16. Gating strategy and representative flow cytometry dot plots for Fig. 3D.

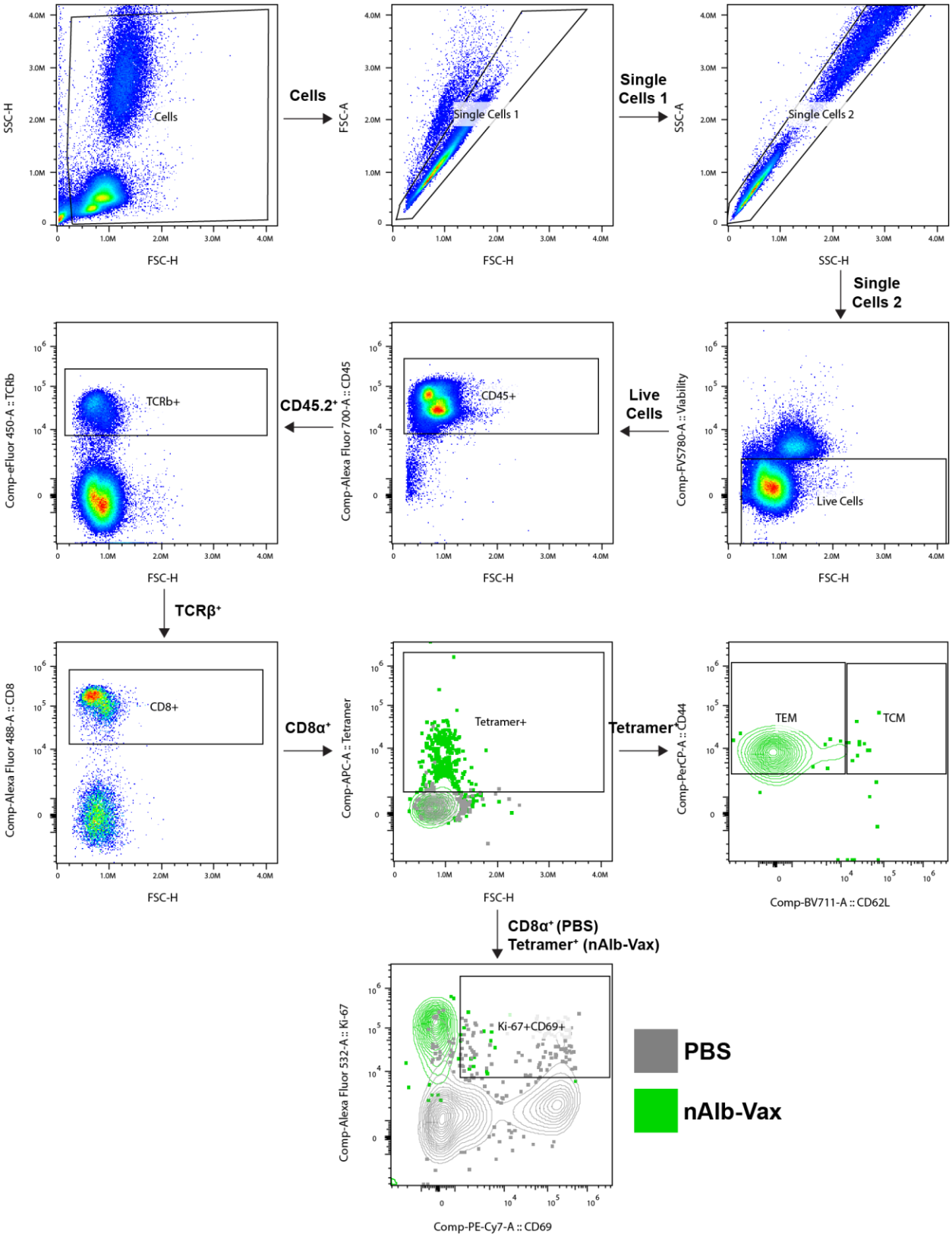


Fig. S17. Gating strategy and representative flow cytometry dot plots for Fig. 3F-H.

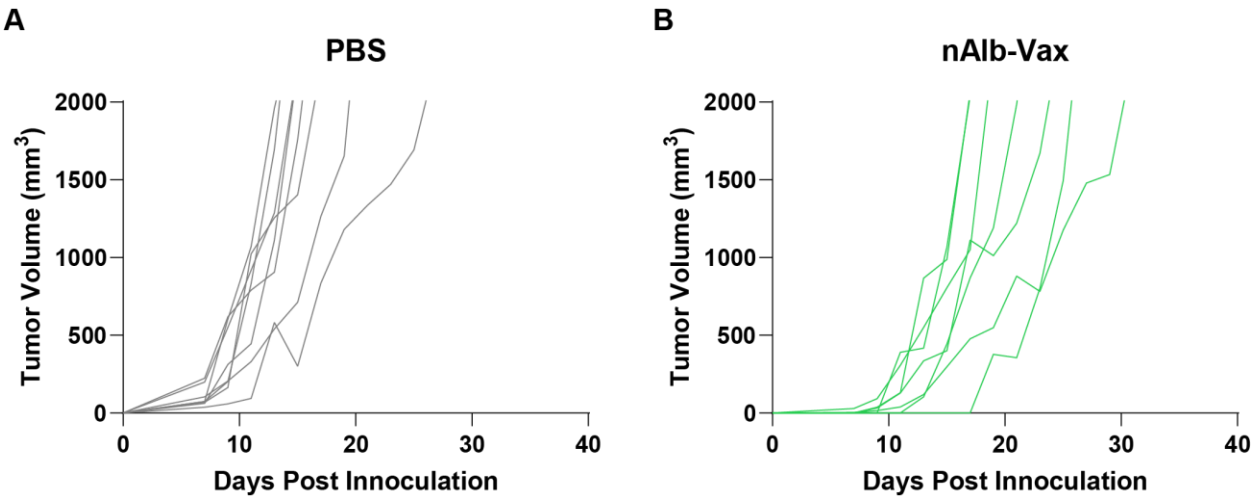


Fig. S18. Individual tumor growth curves for Fig. 3I. Individual tumor growth curves for Fig. 3I following treatment with **(A)** PBS or **(B)** nAlb-Vax.

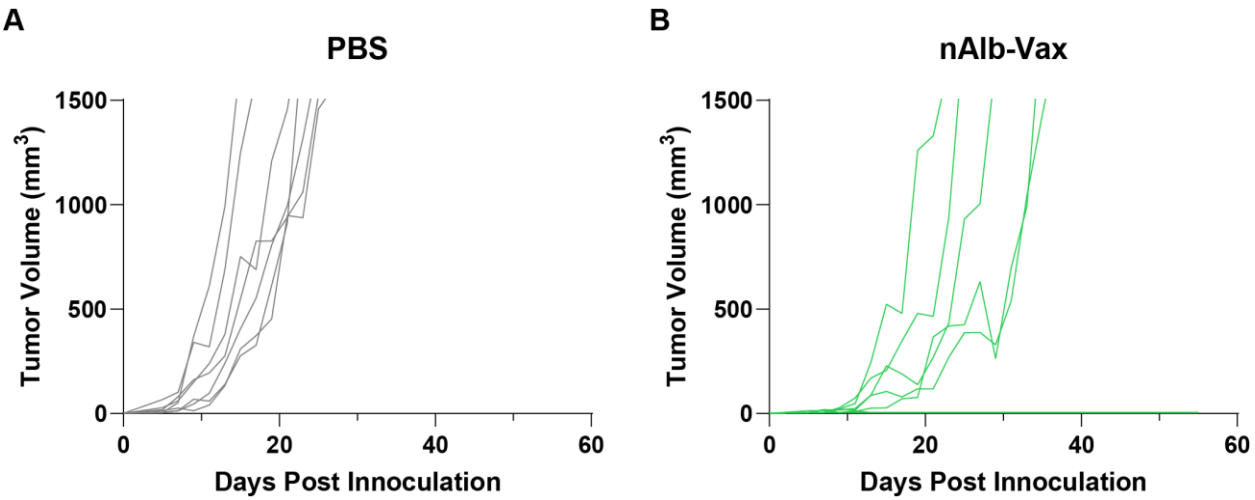


Fig. S19. Individual tumor growth curves for Fig. 3L. Individual tumor growth curves for Fig. 3L following treatment with **(A)** PBS or **(B)** nAlb-Vax.

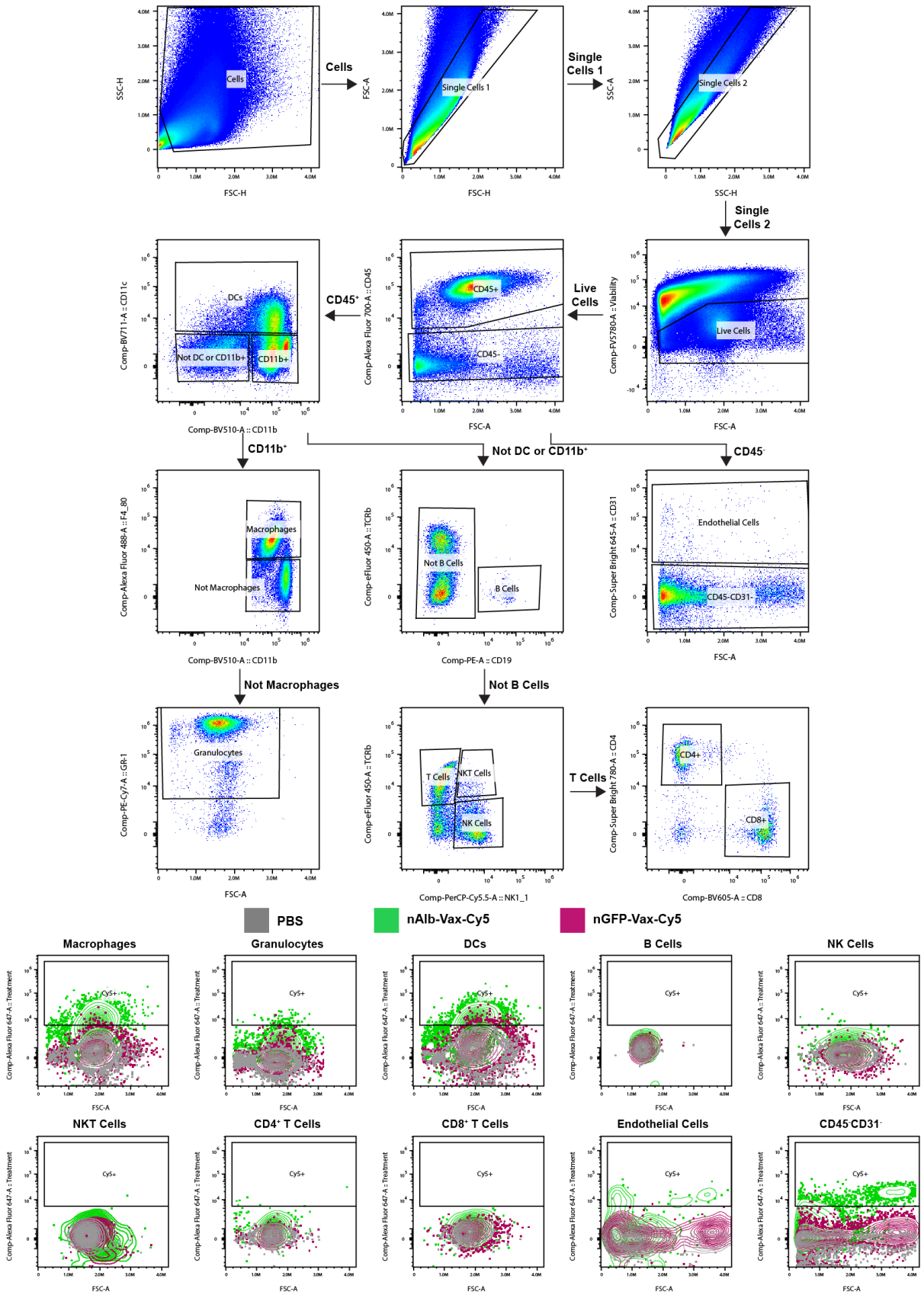


Fig. S20. Gating strategy and representative flow cytometry dot plots for Fig. 4E-F.

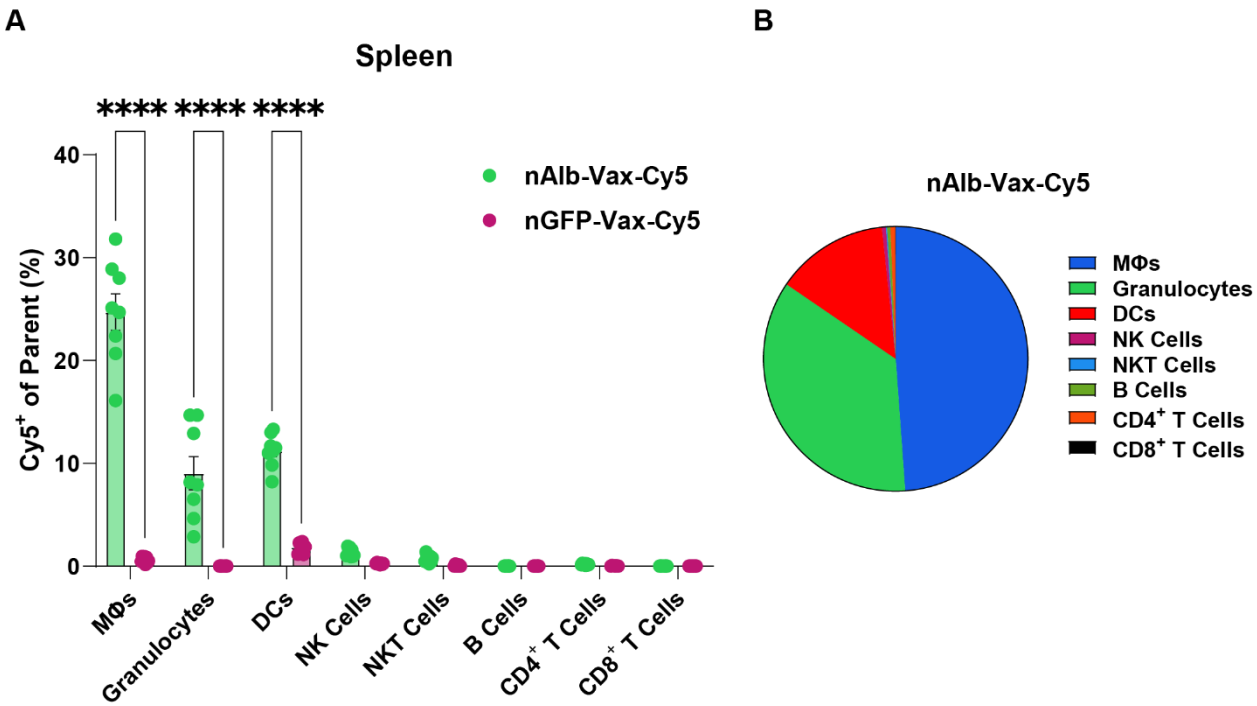


Fig. S21. Quantification of Cy5 uptake by major immune cell populations in the spleen. (A) Quantification of Cy5 uptake by major immune cell populations in the spleen 24 h after SC administration of indicated formulations (mean ± SEM, n = 6-8). Analyzed via two-way ANOVA with Tukey's *post-hoc*. **(B)** Pie charts depicting Cy5 uptake by major immune cell populations in the spleen as a percentage of all $Cy5^+$ cells following treatment with nAlb-Vax-Cy5. ****$P < 0.0001$. ***$P < 0.001$. **$P < 0.01$. *$P < 0.05$.

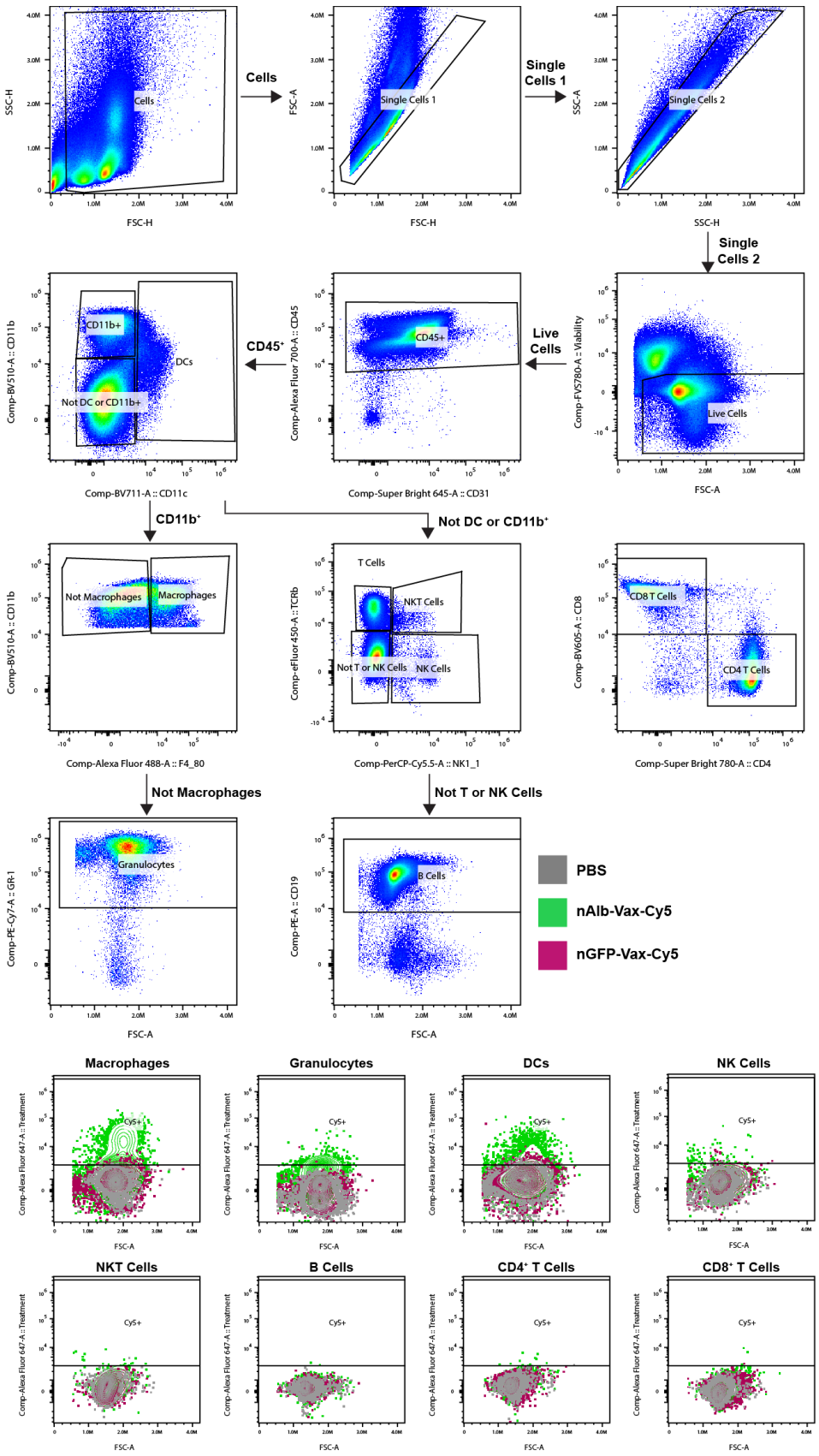


Fig. S22. Gating strategy and representative flow cytometry dot plots for Fig. S21A-B.

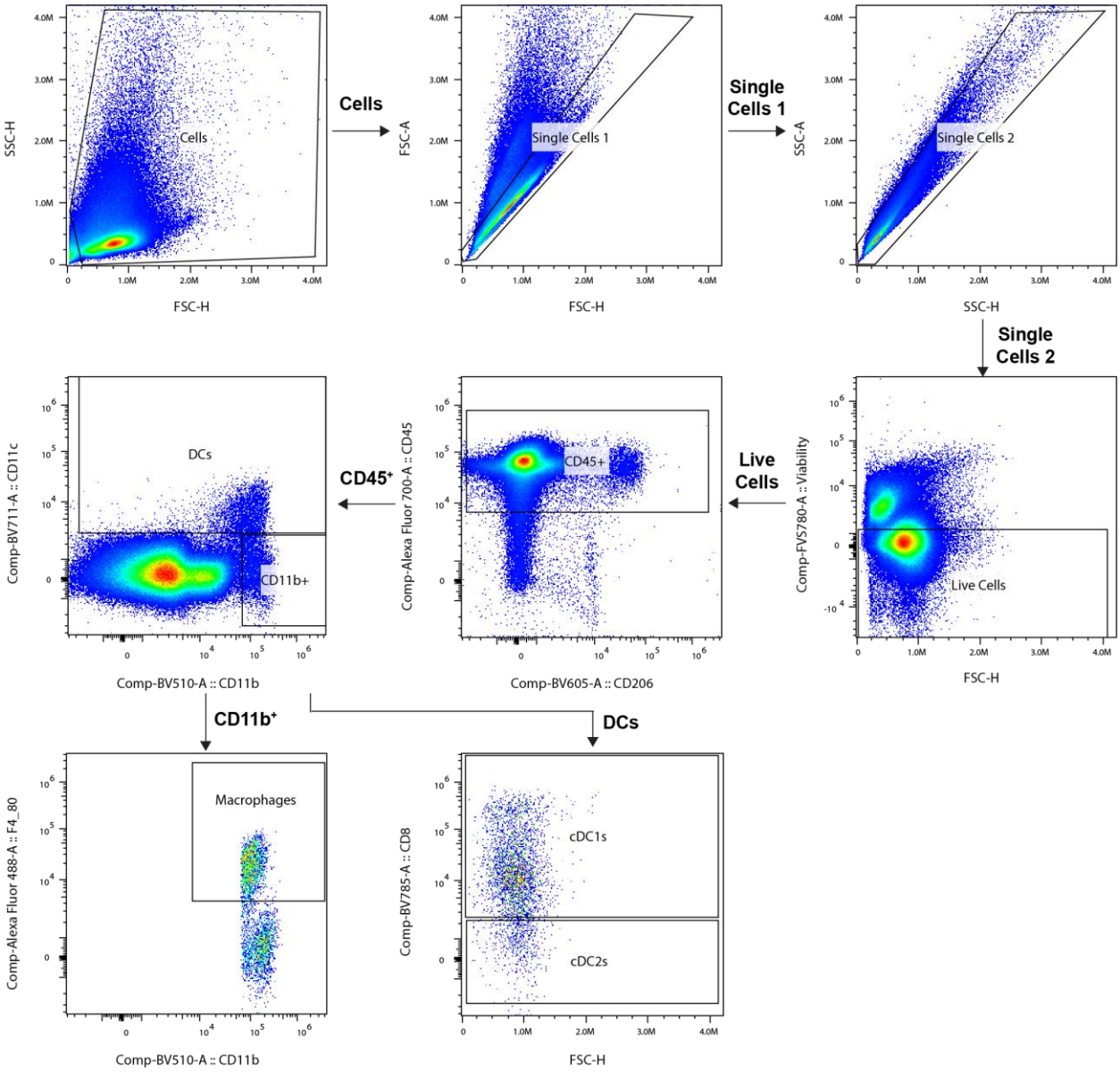


Fig. S23. Gating strategy for Fig. 5B (Injection Draining LN), Fig. 5C-D, Fig. 5G-H, Fig. 5K-L, Fig. S29 (Injection Draining LN), and Fig. S30 (Injection Draining LN).

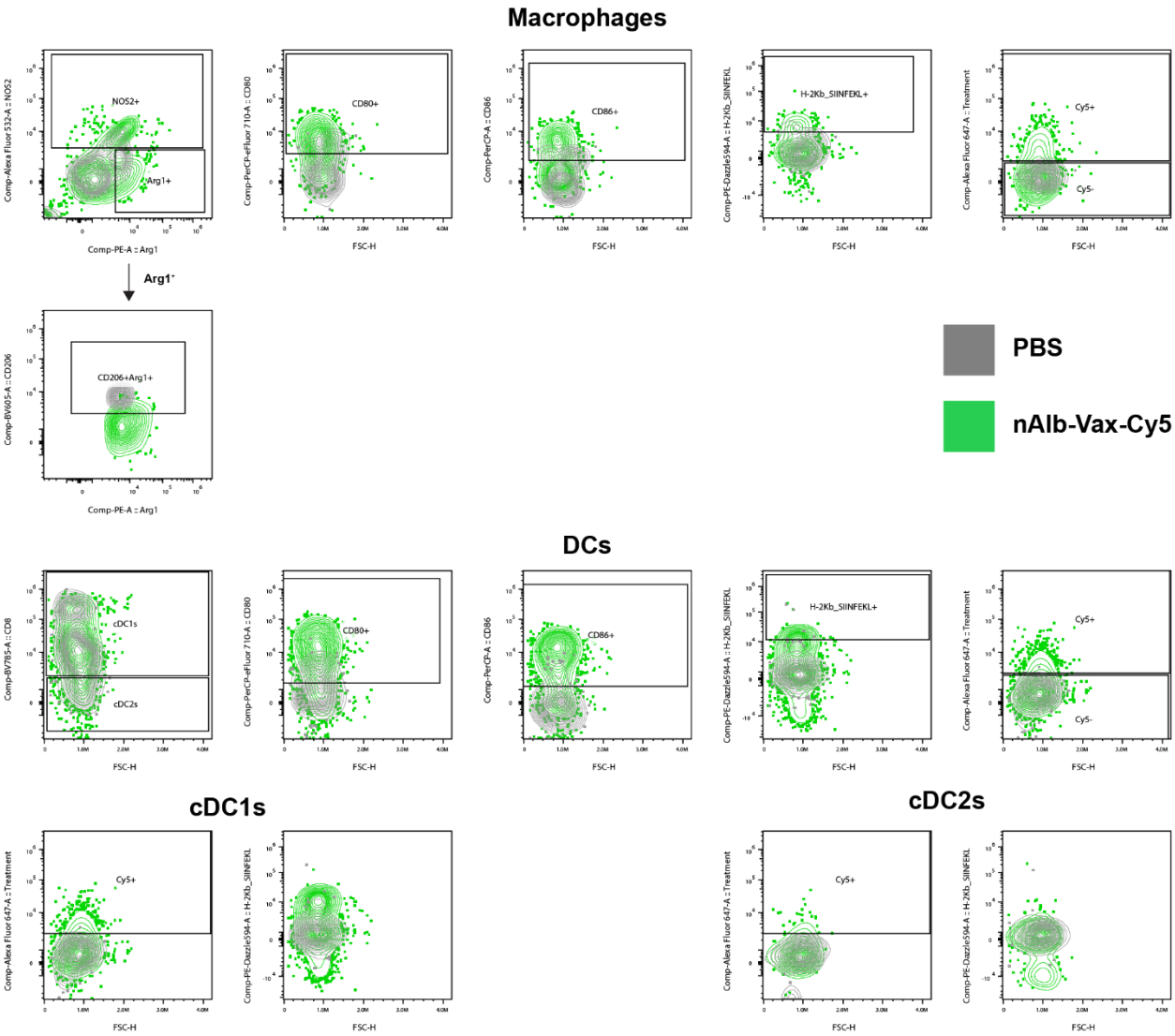


Fig. S24. Representative flow cytometry dot plots for Fig. 5B (Injection Draining LN), Fig. 5C-D, Fig. 5G-H, Fig. 5K-L, Fig. S29 (Injection Draining LN), and Fig. S30 (Injection Draining LN).

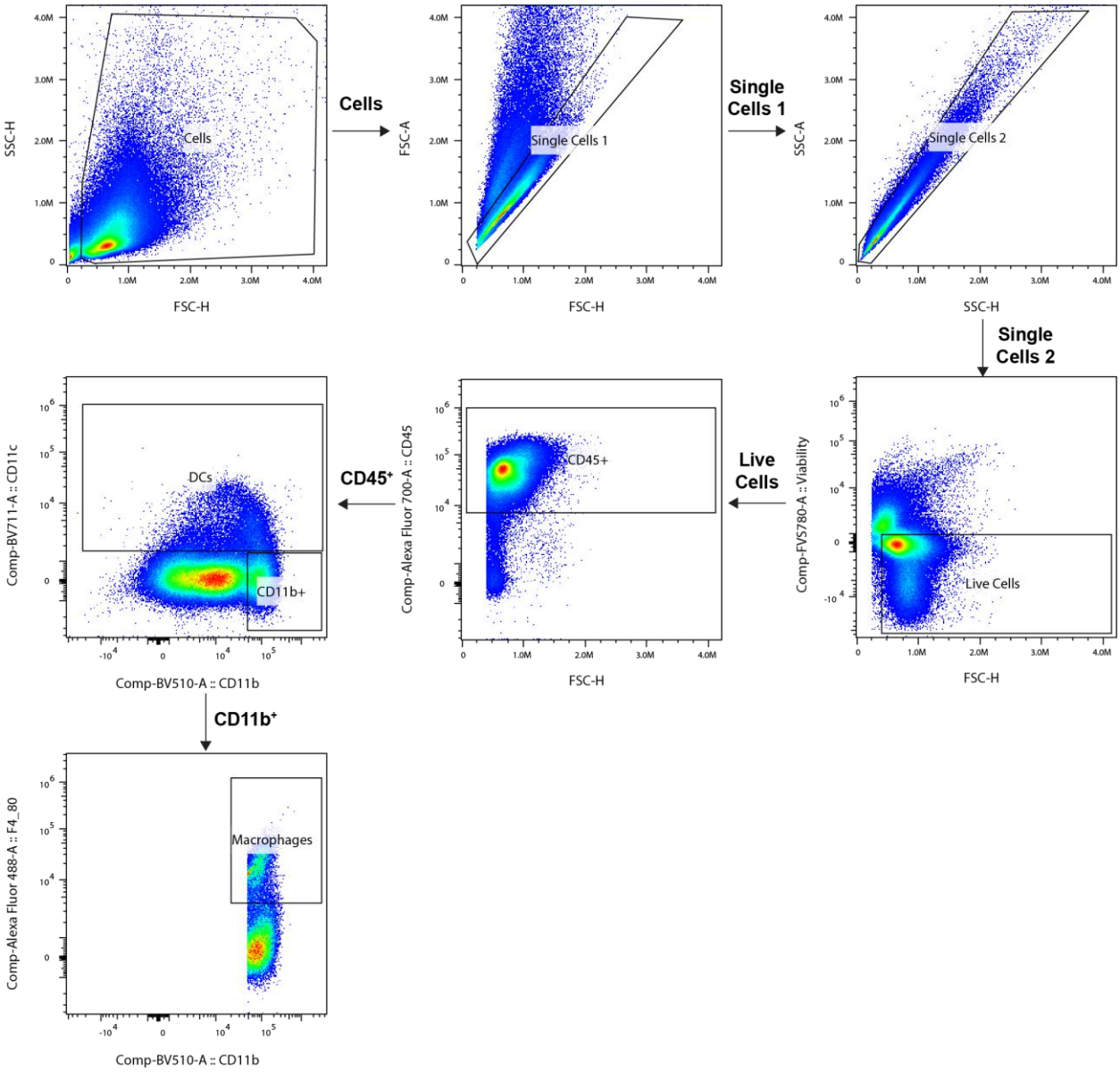


Fig. S25. Gating strategy for Fig. 5B (Spleen), Fig. S29 (Spleen), and Fig. S30 (Spleen).

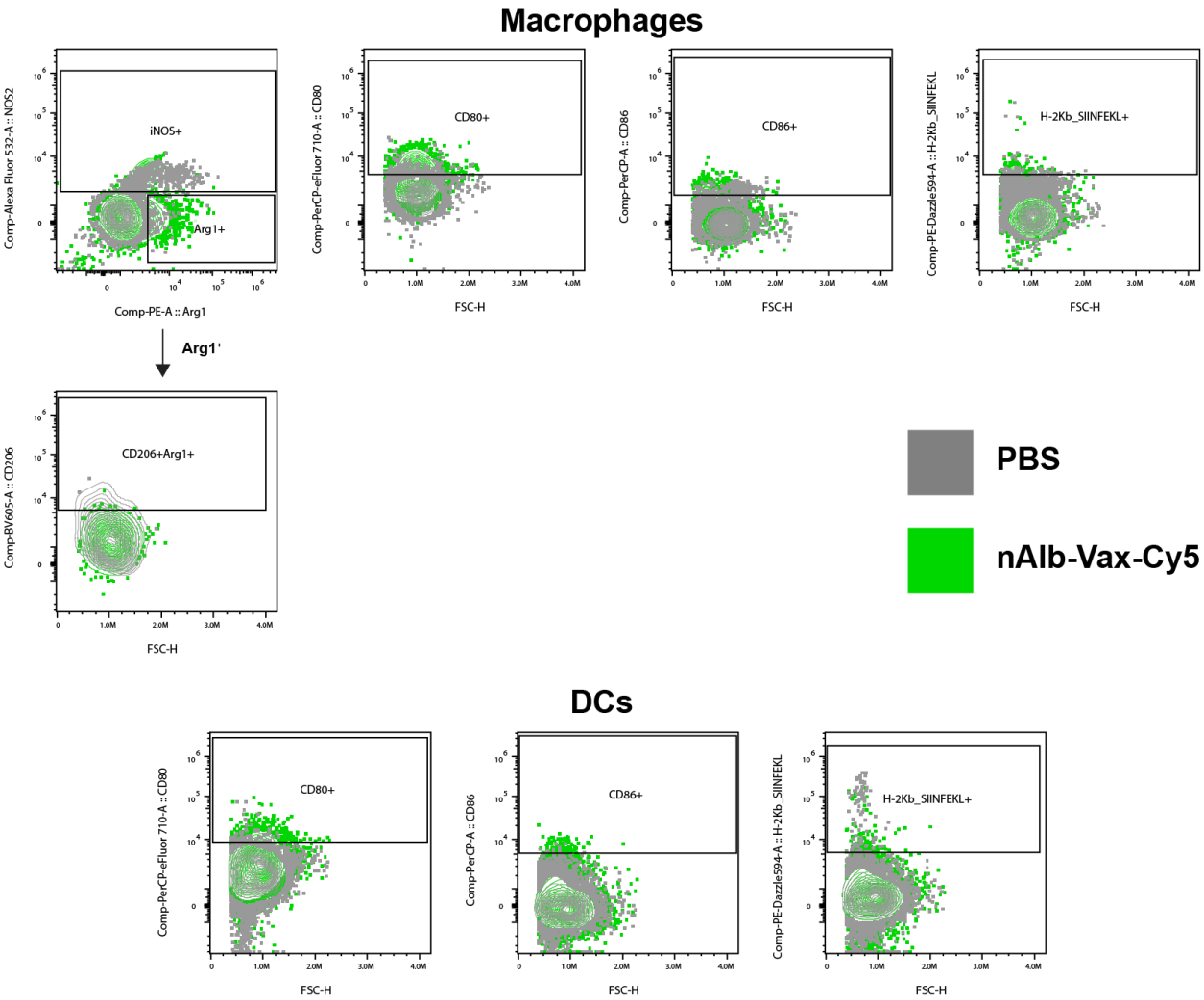


Fig. S26. Representative flow cytometry dot plots for Fig. 5B (Spleen), Fig. S29 (Spleen), and Fig. S30 (Spleen).

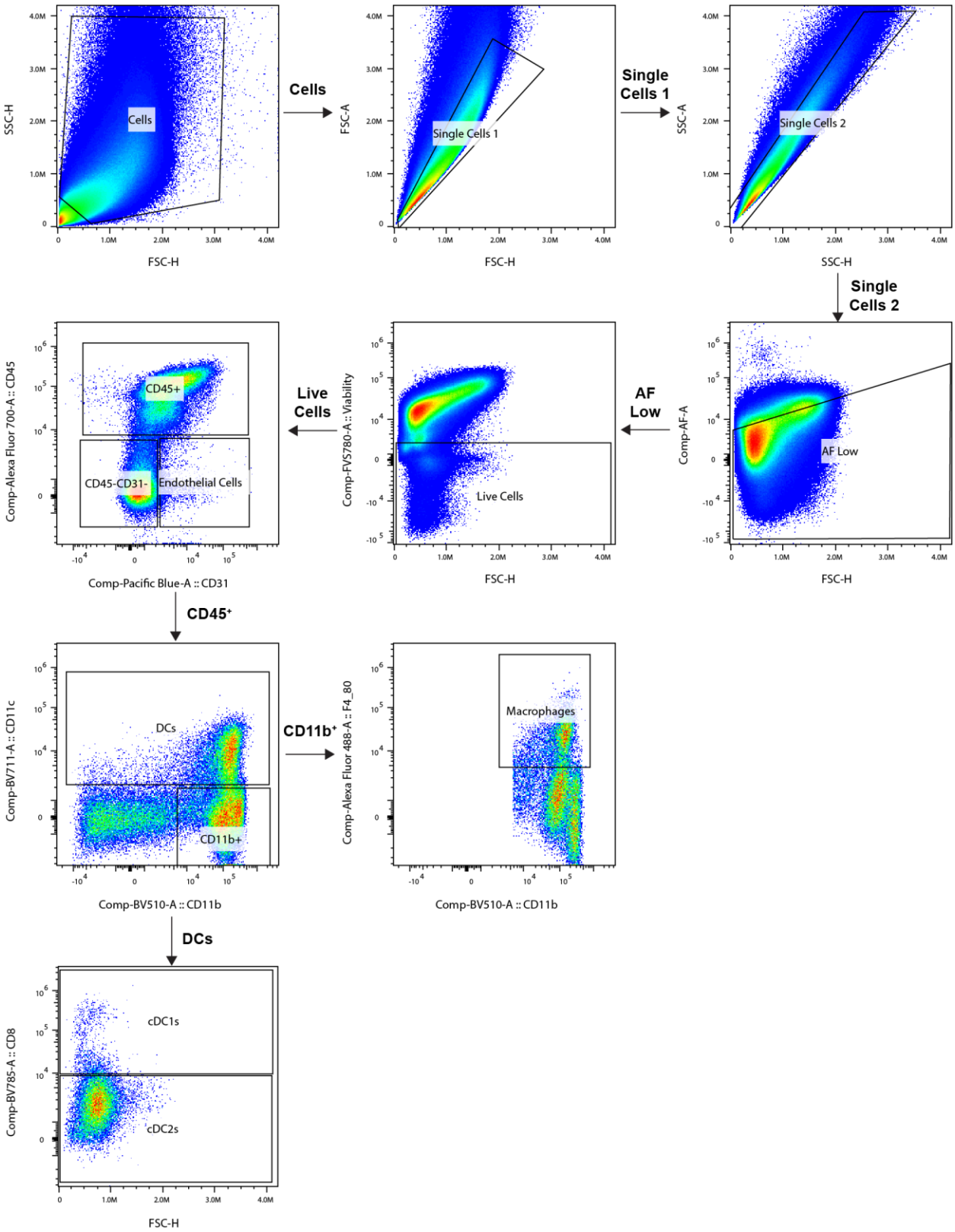


Fig. S27. Gating strategy for Fig. 5B (Tumor), Fig. 5E-F, Fig. 5I-J, Fig. 5M-N, Fig. S29 (Tumor), and Fig. S30 (Tumor).

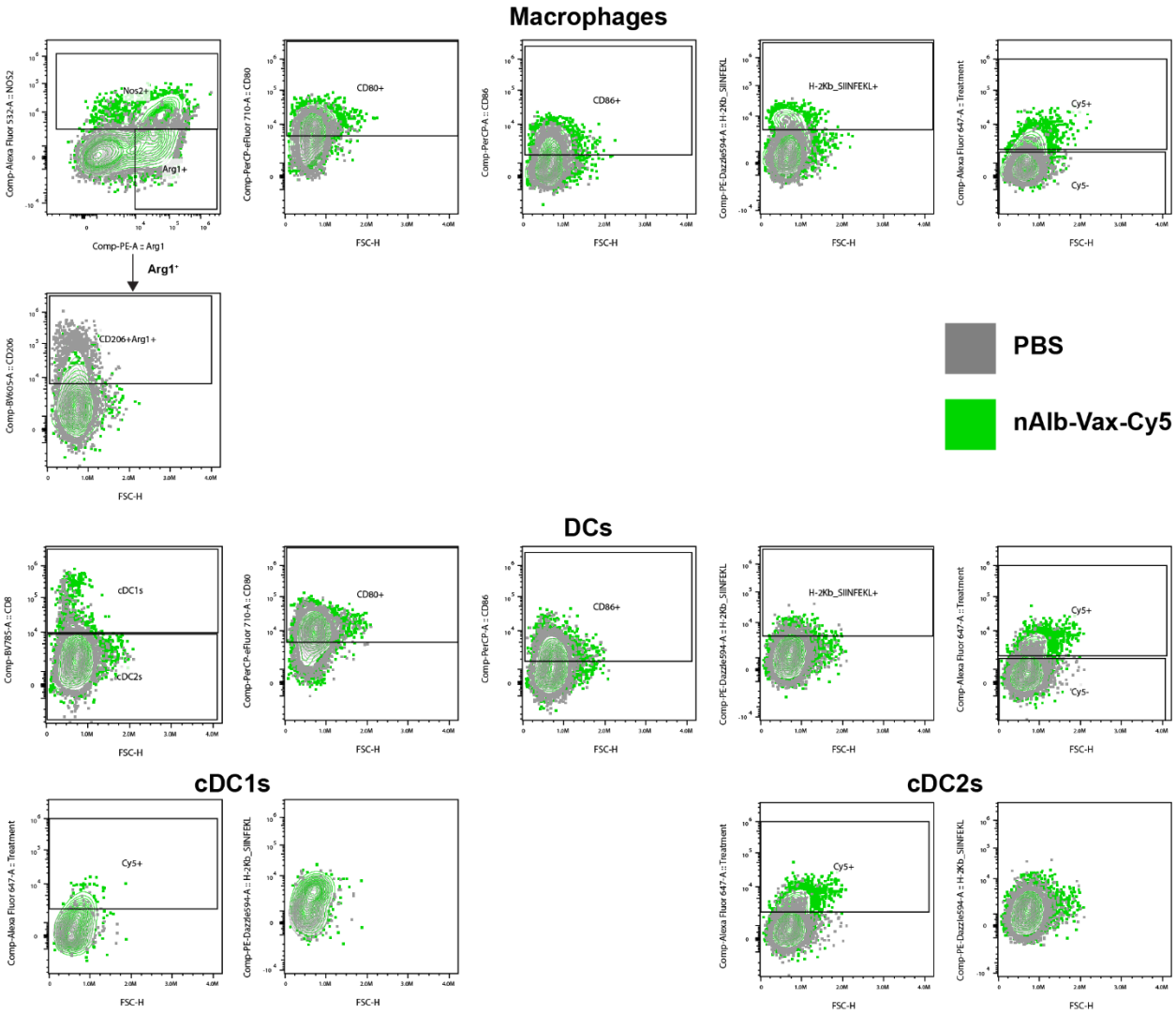


Fig. S28. Representative flow cytometry dot plots for Fig. 5B (Tumor), Fig. 5E-F, Fig. 5I-J, Fig. 5M-N, Fig. S29 (Tumor), and Fig. S30 (Tumor).

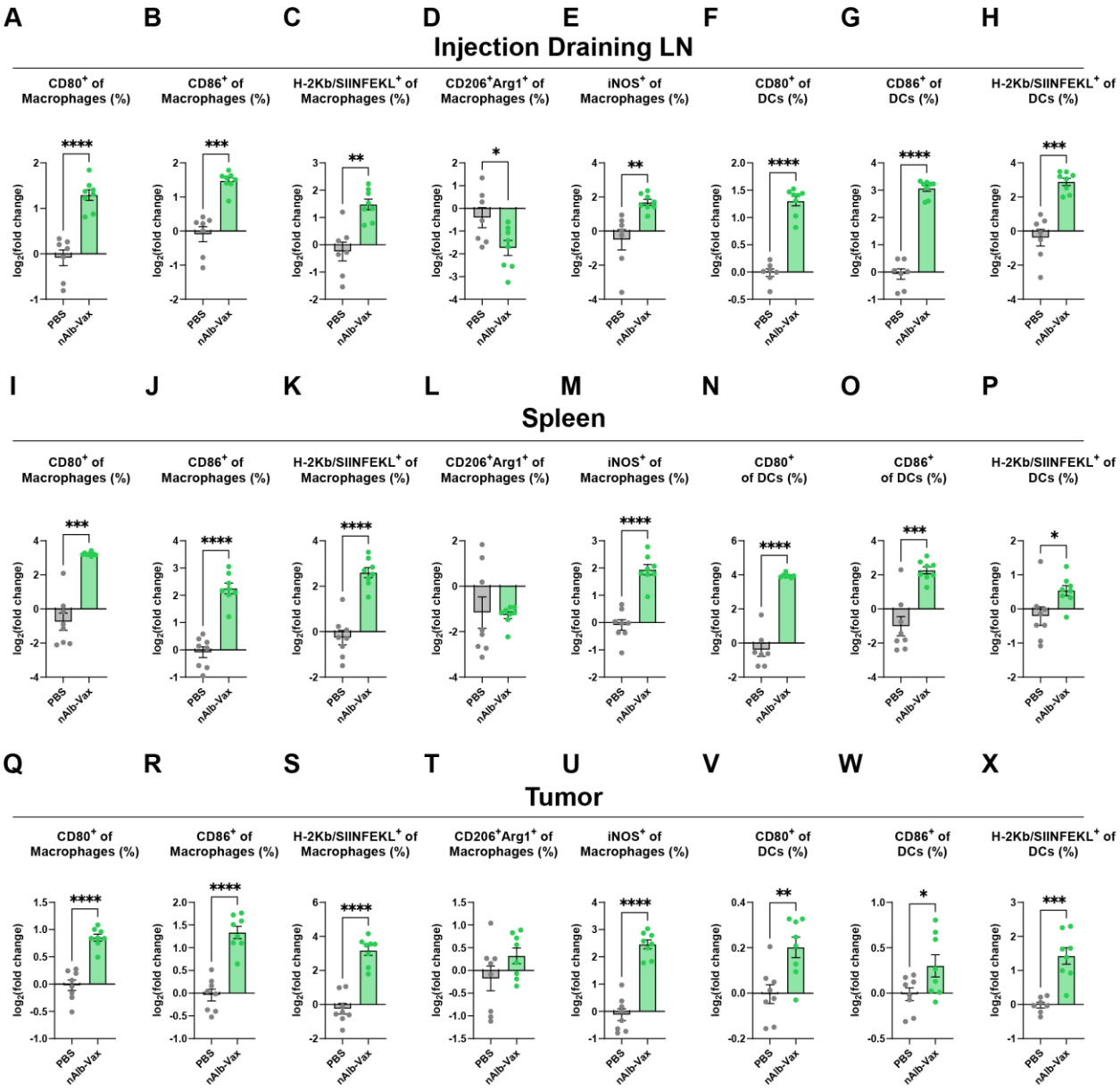


Fig. S29. Bar graphs of the log$_2$(fold change) in the percentage of indicated cell populations relative to PBS control in indicated tissues presented in Fig. 5B. Bar graphs of the log$_2$(fold change) in the percentage of **(A)** CD80$^+$, **(B)** CD86$^+$, **(C)** H-2Kb/SIINFEKL$^+$, **(D)** CD206$^+$Arg1$^+$, and **(E)** iNOS$^+$ of macrophages and **(F)** CD80$^+$, **(G)** CD86$^+$, and **(H)** H-2Kb/SIINFEKL$^+$ of DCs in the injection draining LN relative to PBS control (mean ± SEM, n = 6-8). Bar graphs of the log$_2$(fold change) in the percentage of **(I)** CD80$^+$, **(J)** CD86$^+$, **(K)** H-2Kb/SIINFEKL$^+$, **(L)** CD206$^+$Arg1$^+$, and **(M)** iNOS$^+$ of macrophages and **(N)** CD80$^+$, **(O)** CD86$^+$, and **(P)** H-2Kb/SIINFEKL$^+$ of DCs in the spleen relative to PBS control (mean ± SEM, n = 6-8). Bar graphs of the log$_2$(fold change) in the percentage of **(Q)** CD80$^+$, **(R)** CD86$^+$, **(S)** H-2Kb/SIINFEKL$^+$, **(T)** CD206$^+$Arg1$^+$, and **(U)** iNOS$^+$ of macrophages and **(V)** CD80$^+$, **(W)** CD86$^+$, and **(X)** H-2Kb/SIINFEKL$^+$ of DCs in the tumor relative to PBS control (mean ± SEM, n = 6-8). Analyzed via Welch's two-tailed t test. ****$P < 0.0001$. ***$P < 0.001$. **$P < 0.01$. *$P < 0.05$.

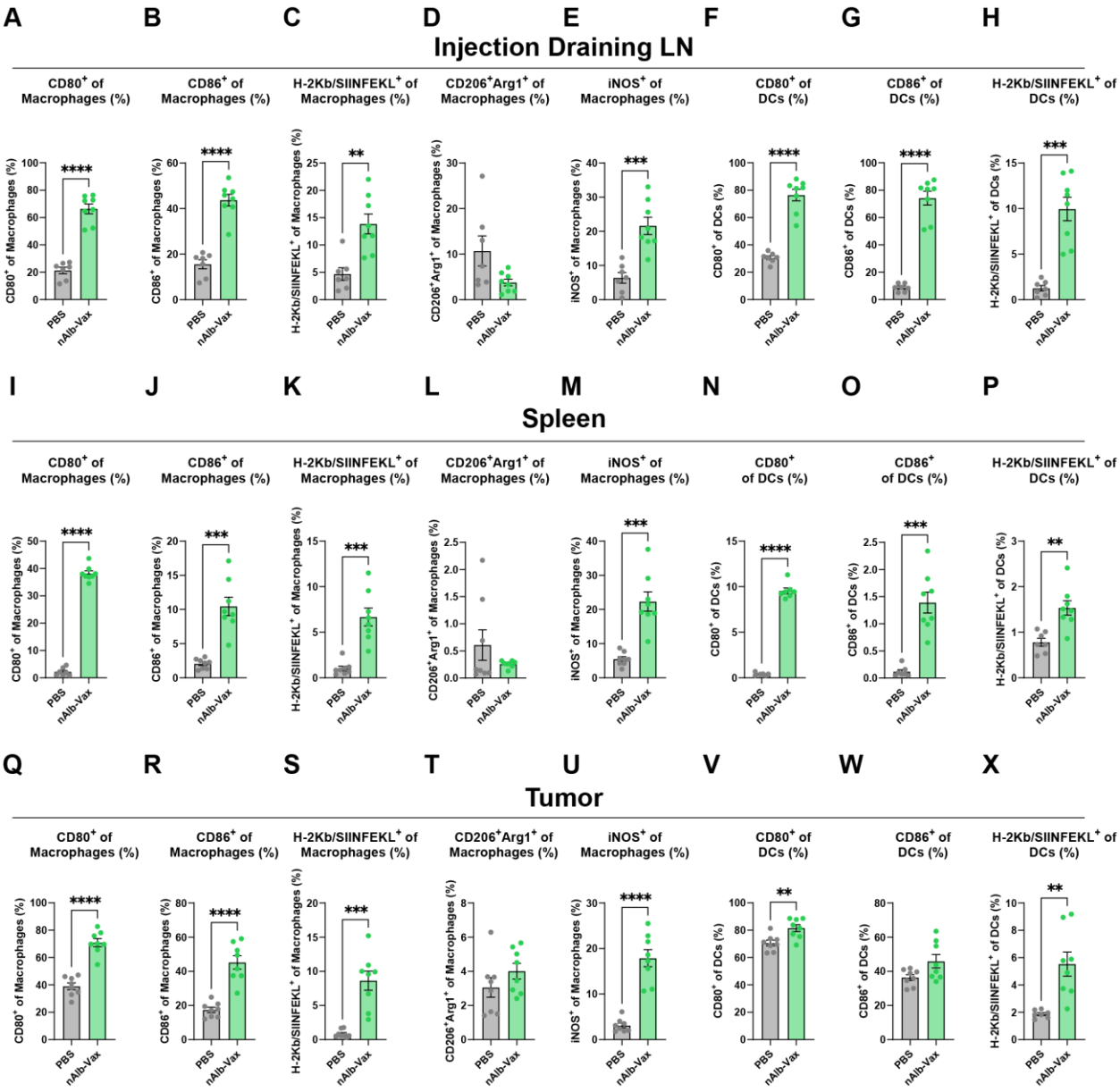


Fig. S30. Bar graphs depicting the frequency of indicated cell populations following treatment with indicated constructs prior to data transformation presented in Fig. 5B and Fig. S29. Bar graphs of the frequency of **(A)** $CD80^+$, **(B)** $CD86^+$, **(C)** H-$2Kb/SIINFEKL^+$, **(D)** $CD206^+Arg1^+$, and **(E)** $iNOS^+$ of macrophages and **(F)** $CD80^+$, **(G)** $CD86^+$, and **(H)** H-$2Kb/SIINFEKL^+$ of DCs in the injection draining LN following treatment with indicated constructs (mean ± SEM, n = 7-8). Bar graphs of the frequency of **(I)** $CD80^+$, **(J)** $CD86^+$, **(K)** H-$2Kb/SIINFEKL^+$, **(L)** $CD206^+Arg1^+$, and **(M)** $iNOS^+$ of macrophages and **(N)** $CD80^+$, **(O)** $CD86^+$, and **(P)** H-$2Kb/SIINFEKL^+$ of DCs in the spleen following treatment with indicated constructs (mean ± SEM, n = 7-8). Bar graphs of the frequency of **(Q)** $CD80^+$, **(R)** $CD86^+$, **(S)** H-$2Kb/SIINFEKL^+$, **(T)** $CD206^+Arg1^+$, and **(U)** $iNOS^+$ of macrophages and **(V)** $CD80^+$, **(W)** $CD86^+$, and **(X)** H-$2Kb/SIINFEKL^+$ of DCs in the tumor following treatment with indicated constructs (mean ± SEM, n = 6-8). Analyzed via Welch's two-tailed t test. $^{****}P < 0.0001$. $^{***}P < 0.001$. $^{**}P < 0.01$. $^{*}P < 0.05$.

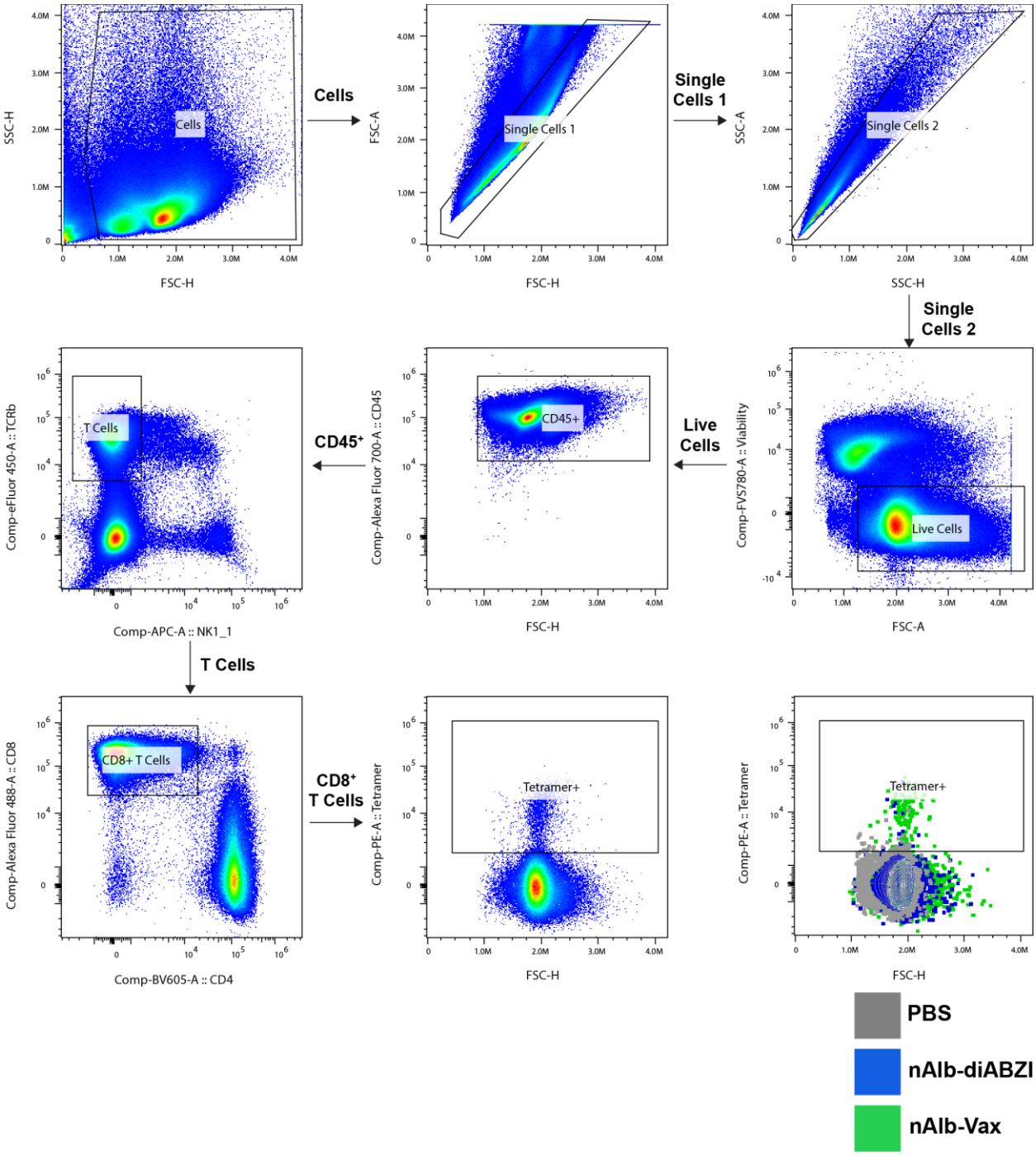


Fig. S31. Gating strategy and representative flow cytometry dot plots for Fig. 6B.

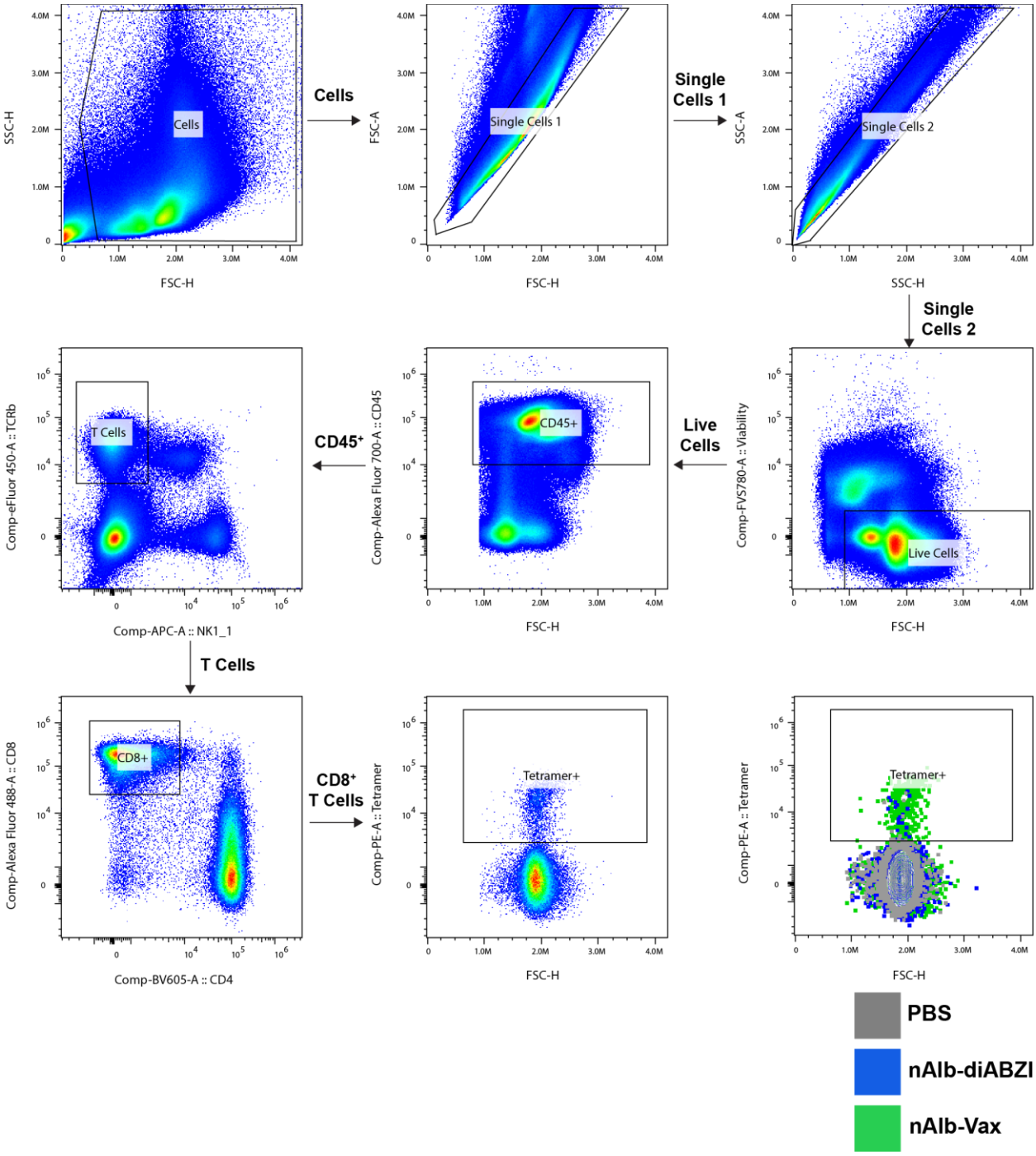


Fig. S32. Gating strategy and representative flow cytometry dot plots for Fig. 6C.

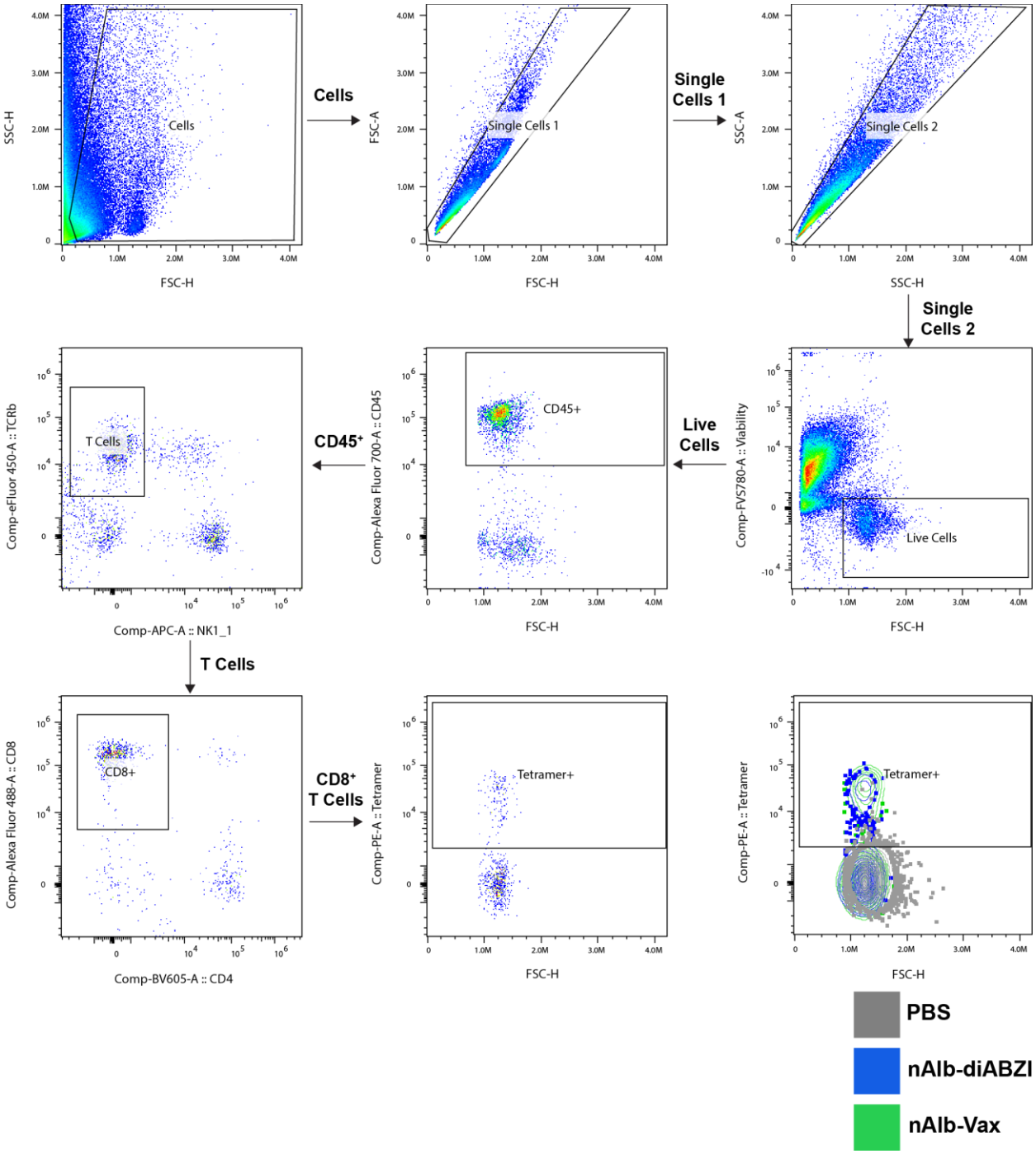


Fig. S33. Gating strategy and representative flow cytometry dot plots for Fig. 6D.

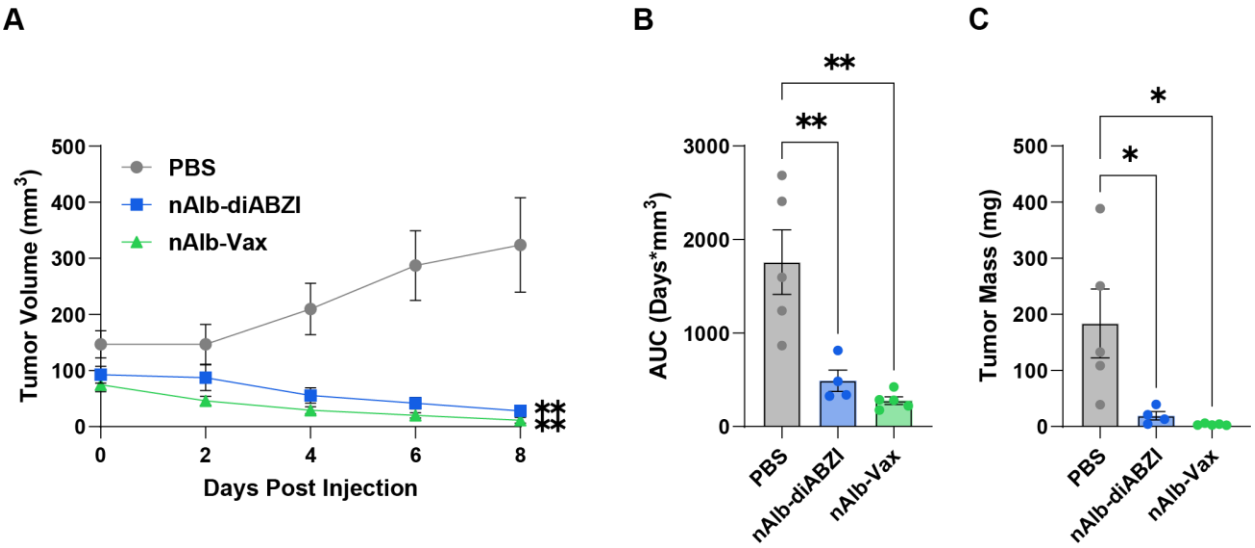


Fig. S34. MC38-OVA is highly responsive to therapeutic treatment with nAlb-diABZI and nAlb-Vax. (A) Tumor growth curves, (B) AUC of individual tumor growth curves, and (C) *ex vivo* tumor mass at endpoint following treatment with indicated formulation (mean ± SEM, $n = 4$-8). Analyzed via one-way ANOVA with Tukey's *post-hoc*. ****$P < 0.0001$. ***$P < 0.001$. **$P < 0.01$. *$P < 0.05$.

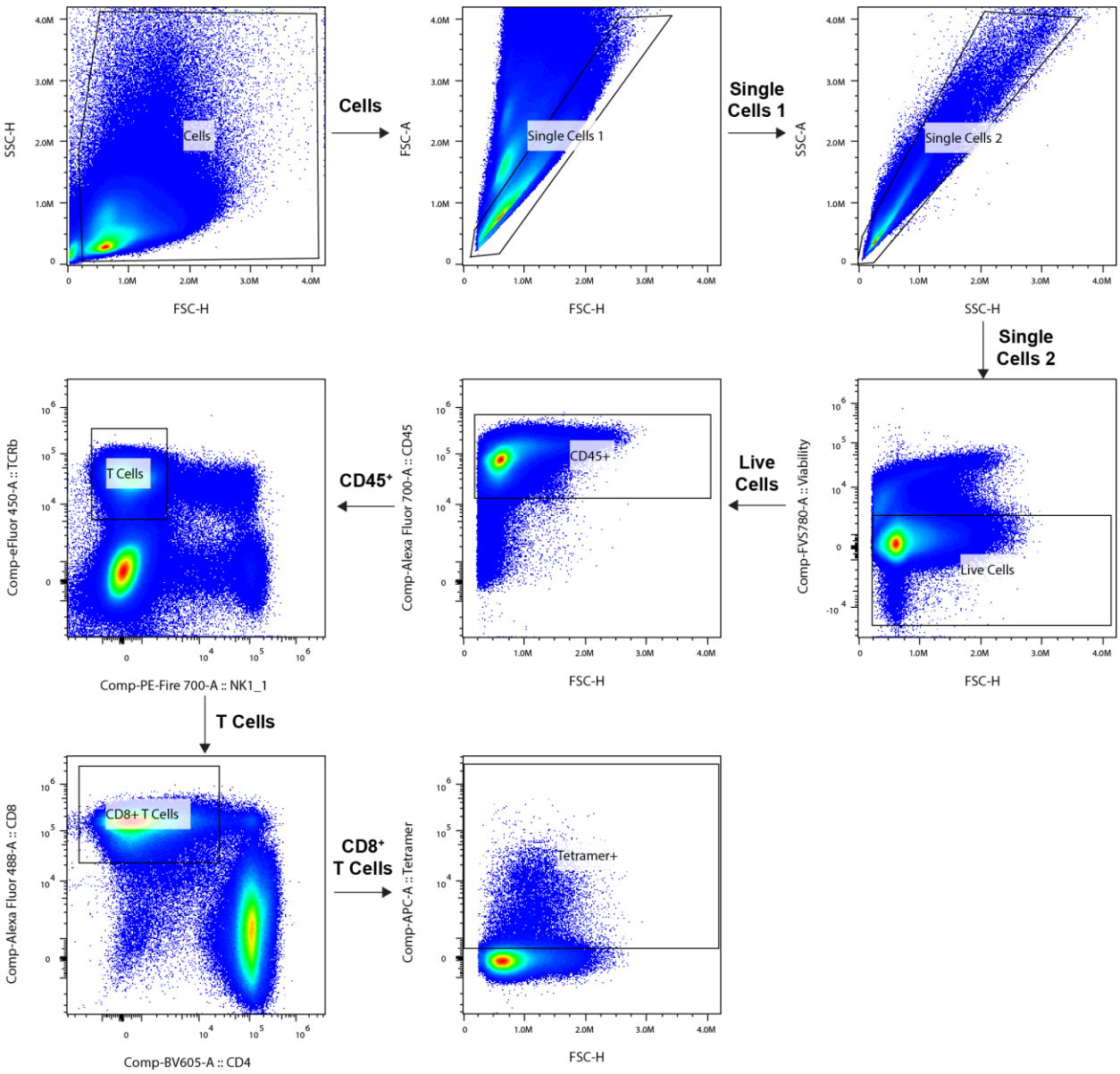


Fig. S35. Gating strategy for Fig. 6F, Fig. 6I (Injection Draining LN), Fig. S41 (Injection Draining LN), and Fig. S42 (Injection Draining LN).

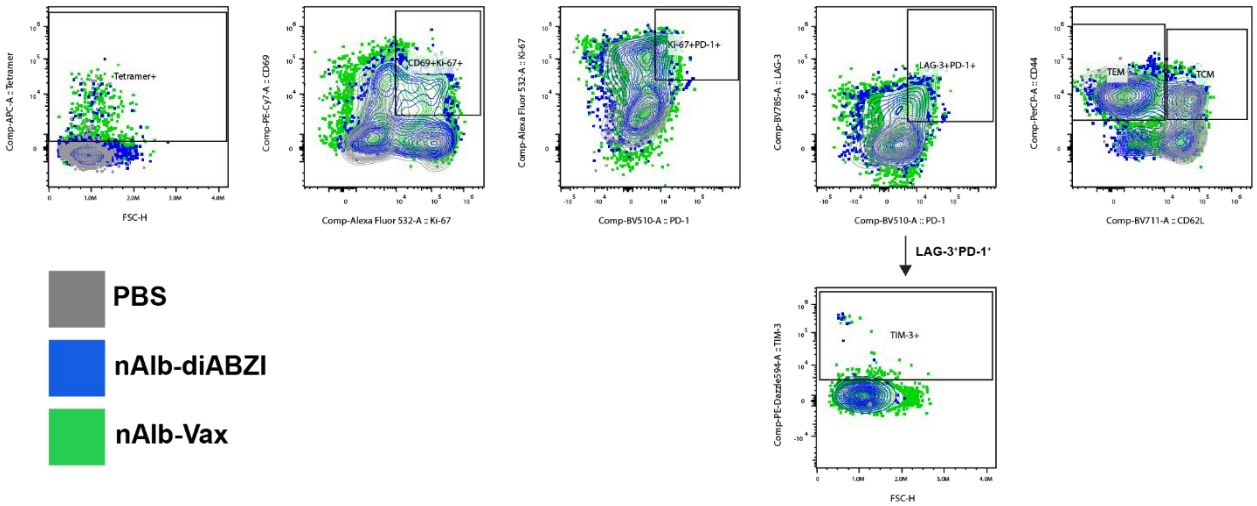


Fig. S36. Representative flow cytometry dot plots for Fig. 6F, Fig. 6I (Injection Draining LN), Fig. S41 (Injection Draining LN), and Fig. S42 (Injection Draining LN).

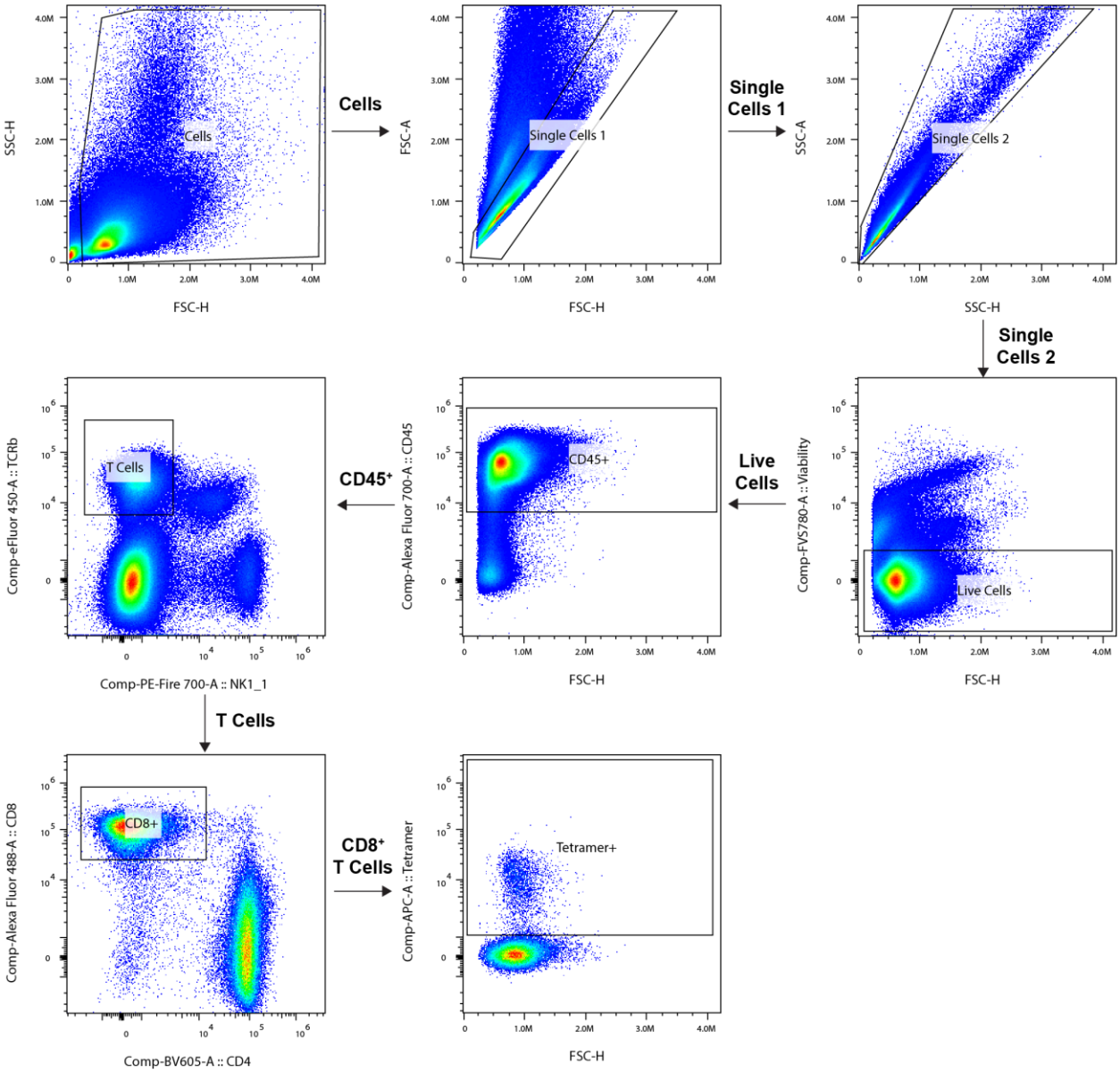


Fig. S37. Gating strategy for Fig. 6G, Fig. 6I (Spleen), Fig. S41 (Spleen), and Fig. S42 (Spleen).

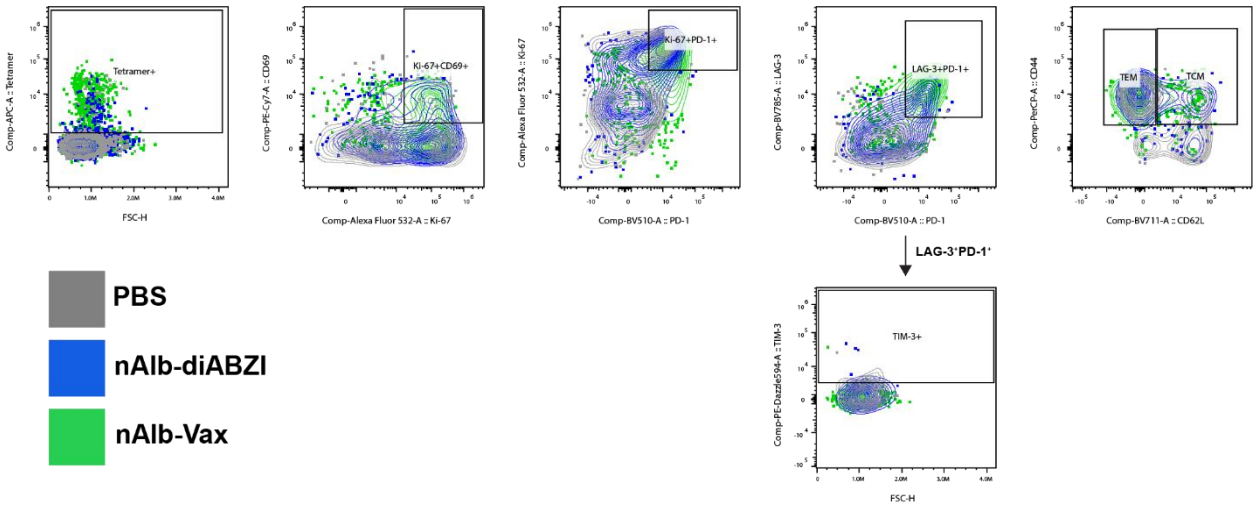


Fig. S38. Representative flow cytometry dot plots for Fig. 6G, Fig. 6I (Spleen), Fig. S41 (Spleen), and Fig. S42 (Spleen).

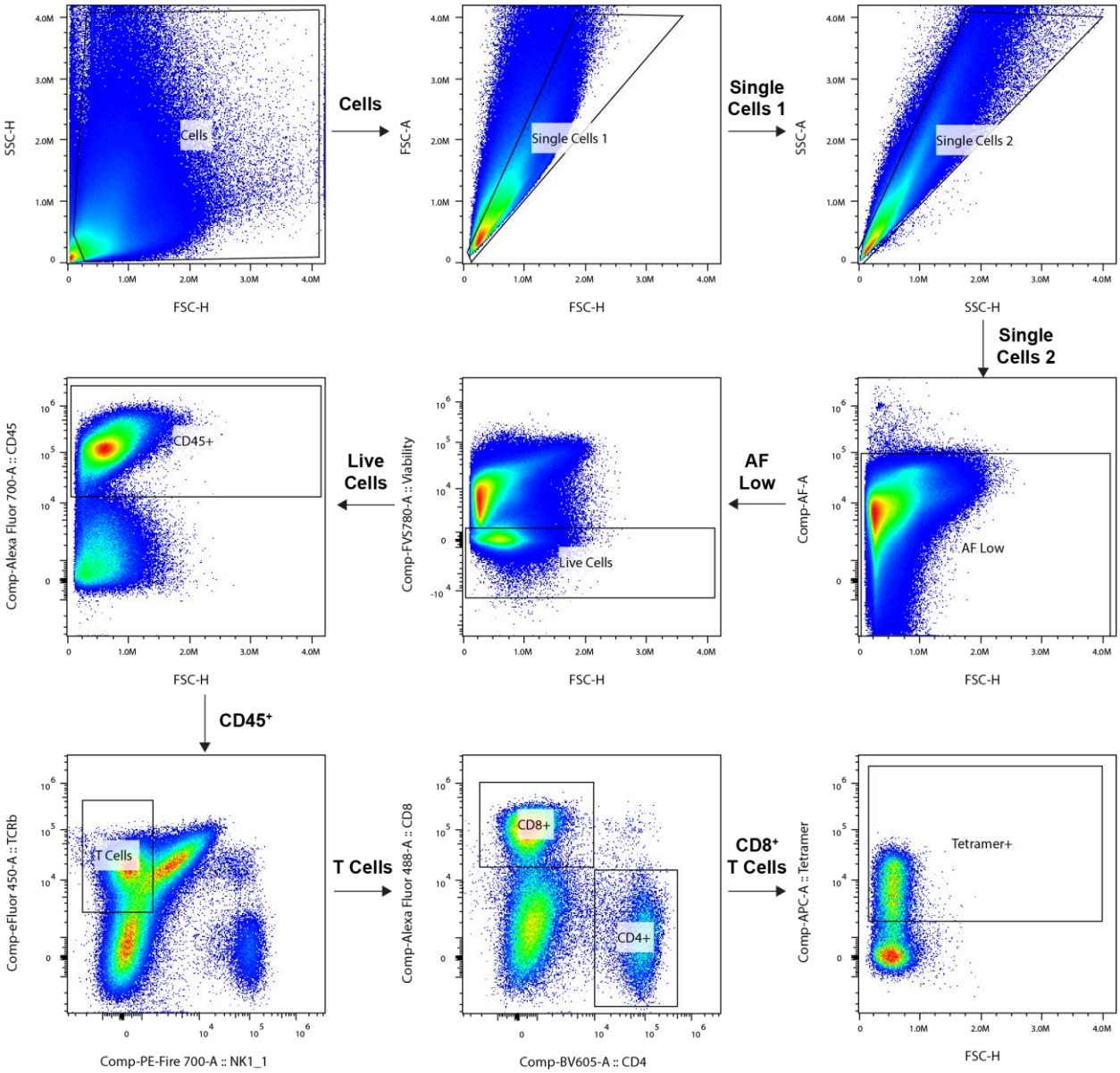


Fig. S39. Gating strategy for Fig. 6H, Fig. 6I (Tumor), Fig. 6J, Fig. 6K, Fig. S41 (Tumor), and Fig. S42 (Tumor).

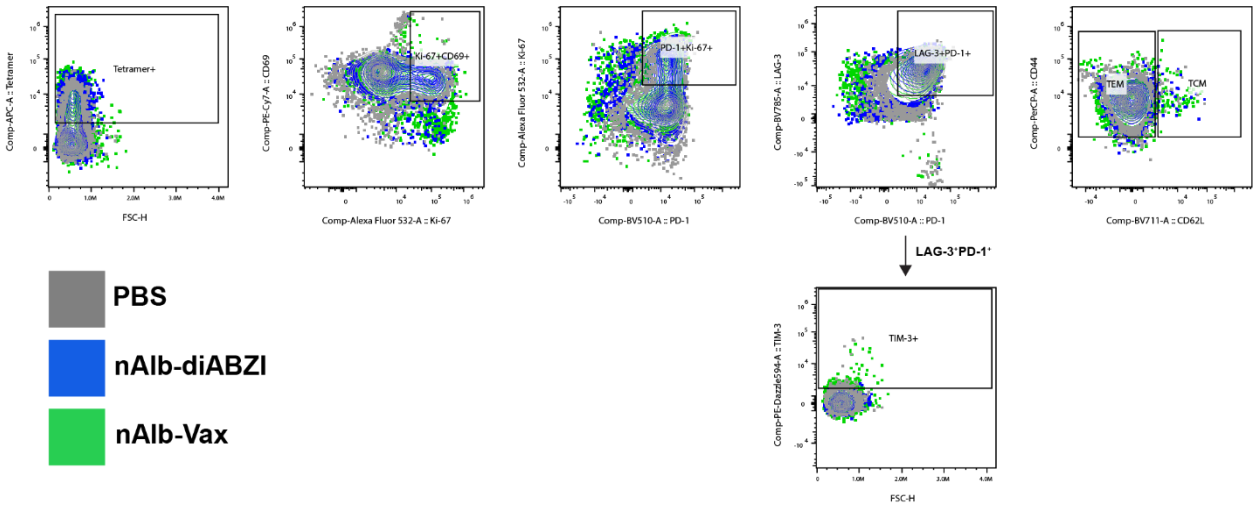


Fig. S40. Representative flow cytometry dot plots for Fig. 6H, Fig. 6I (Tumor), Fig. S41 (Tumor), and Fig. S42 (Tumor).

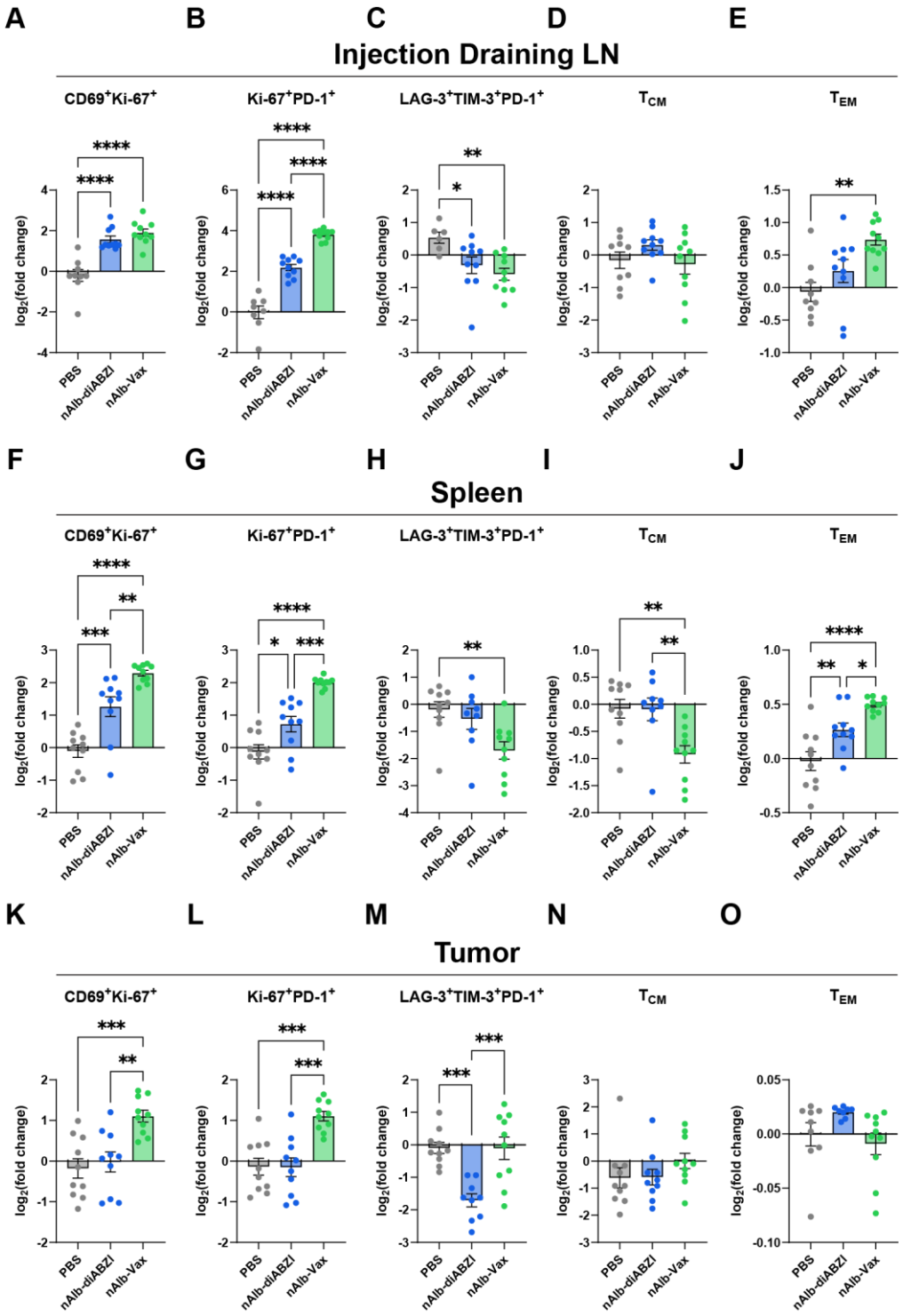


Fig. S41. Bar graphs of the log$_2$(fold change) in the percentage of indicated cell populations relative to PBS control in indicated tissues presented in Fig. 6I. Bar graphs of the log$_2$(fold change) in the percentage of **(A)** activated, proliferating (CD69$^+$Ki-67$^+$), **(B)** ICB-responsive (Ki-67$^+$PD-1$^+$), **(C)** exhausted (LAG-3$^+$TIM-3$^+$PD-1$^+$), **(D)** T_{CM} (CD44$^+$CD62L$^+$), and **(E)** T_{EM} (CD44$^+$CD62L$^-$) of H-2Kb/SIINFEKL tetramer$^+$ T cells following treatment with indicated formulations in the injection draining LN relative to PBS control (mean ± SEM, n = 6-10). Bar

graphs of the log_2(fold change) in the percentage of **(F)** activated, proliferating ($CD69^+Ki\text{-}67^+$), **(G)** ICB-responsive ($Ki\text{-}67^+PD\text{-}1^+$), **(H)** exhausted ($LAG\text{-}3^+TIM\text{-}3^+PD\text{-}1^+$), **(I)** T_{CM} ($CD44^+CD62L^+$), and **(J)** T_{EM} ($CD44^+CD62L^-$) of H-2Kb/SIINFEKL $tetramer^+$ T cells following treatment with indicated formulations in the spleen relative to PBS control (mean ± SEM, n = 6-10). Bar graphs of the log_2(fold change) in the percentage of **(K)** activated, proliferating ($CD69^+Ki\text{-}67^+$), **(L)** ICB-responsive ($Ki\text{-}67^+PD\text{-}1^+$), **(M)** exhausted ($LAG\text{-}3^+TIM\text{-}3^+PD\text{-}1^+$), **(N)** T_{CM} ($CD44^+CD62L^+$), and **(O)** T_{EM} ($CD44^+CD62L^-$) of H-2Kb/SIINFEKL $tetramer^+$ T cells following treatment with indicated formulations in the tumor relative to PBS control (mean ± SEM, n = 6-10). Analyzed via one-way ANOVA with Tukey's *post-hoc*. $^{****}P < 0.0001$. $^{***}P < 0.001$. $^{**}P < 0.01$. $^{*}P < 0.05$.

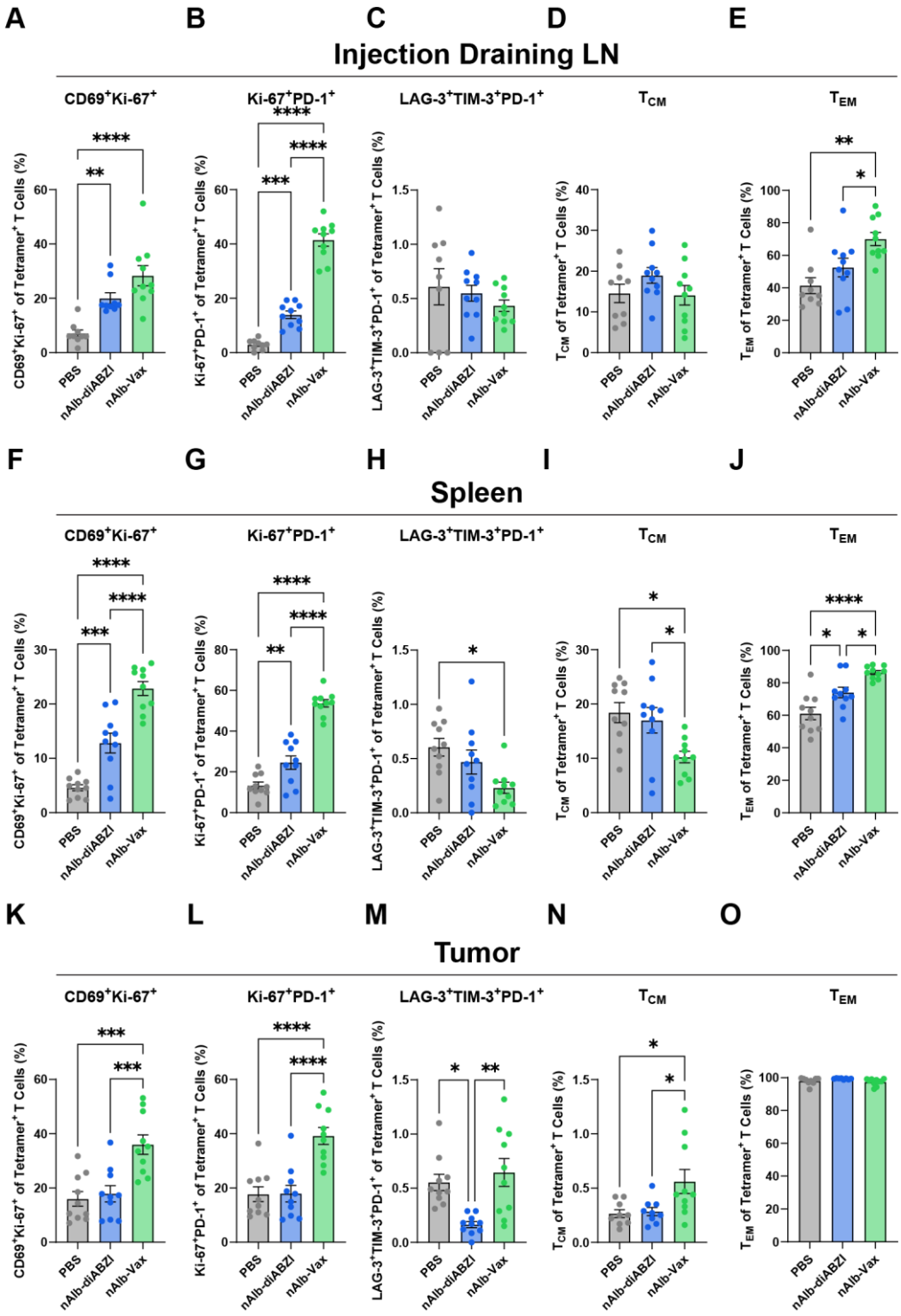


Fig. S42. Bar graphs depicting the frequency of indicated cell populations following treatment with indicated constructs prior to data transformation presented in Fig. 6I and Fig. S41. Bar graphs of the frequency of **(A)** activated, proliferating ($CD69^+Ki\text{-}67^+$), **(B)** ICB-responsive ($Ki\text{-}67^+PD\text{-}1^+$), **(C)** exhausted ($LAG\text{-}3^+TIM\text{-}3^+PD\text{-}1^+$), **(D)** T_{CM} ($CD44^+CD62L^+$), and **(E)** T_{EM} ($CD44^+CD62L^-$) of H-2Kb/SIINFEKL $tetramer^+$ T cells following treatment with indicated formulations in the injection draining LN (mean ± SEM, n = 9-10). Bar graphs of the

frequency of **(F)** activated, proliferating ($CD69^+Ki\text{-}67^+$), **(G)** ICB-responsive ($Ki\text{-}67^+PD\text{-}1^+$), **(H)** exhausted ($LAG\text{-}3^+TIM\text{-}3^+PD\text{-}1^+$), **(I)** T_{CM} ($CD44^+CD62L^+$), and **(J)** T_{EM} ($CD44^+CD62L^-$) of H-2Kb/SIINFEKL $tetramer^+$ T cells following treatment with indicated formulations in the spleen (mean ± SEM, $n = 10$). Bar graphs of the frequency of **(K)** activated, proliferating ($CD69^+Ki\text{-}67^+$), **(L)** ICB-responsive ($Ki\text{-}67^+PD\text{-}1^+$), **(M)** exhausted ($LAG\text{-}3^+TIM\text{-}3^+PD\text{-}1^+$), **(N)** T_{CM} ($CD44^+CD62L^+$), and **(O)** T_{EM} ($CD44^+CD62L^-$) of H-2Kb/SIINFEKL $tetramer^+$ T cells following treatment with indicated formulations in the tumor (mean ± SEM, $n = 8\text{-}10$). Analyzed via one-way ANOVA with Tukey's *post-hoc*. $^{****}P < 0.0001$. $^{***}P < 0.001$. $^{**}P < 0.01$. $^{*}P < 0.05$.

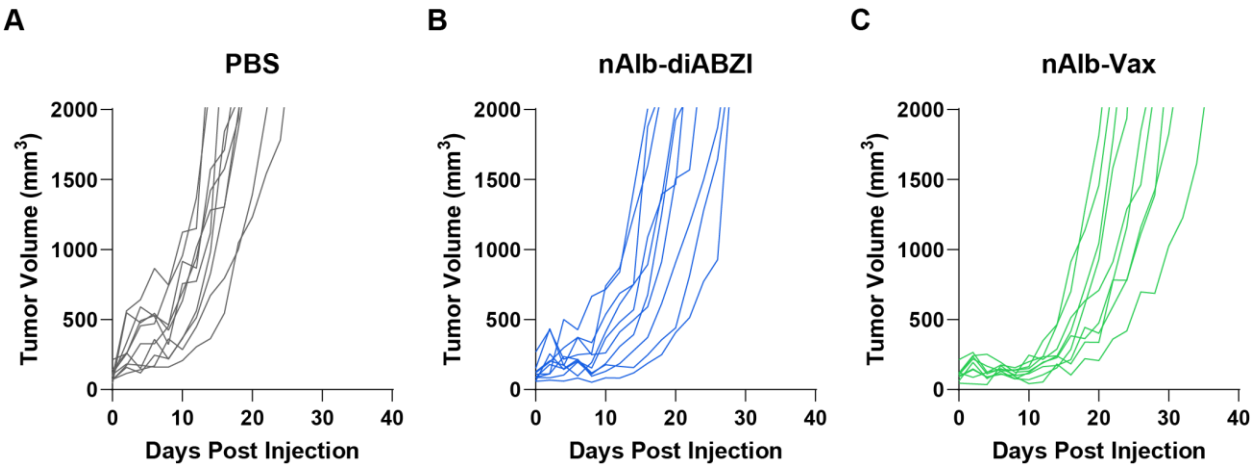


Fig. S43. Individual tumor growth curves for Fig. 6M. Individual tumor growth curves for Fig. 6M following treatment with **(A)** PBS, **(B)** nAlb-diABZI, or **(C)** nAlb-Vax.

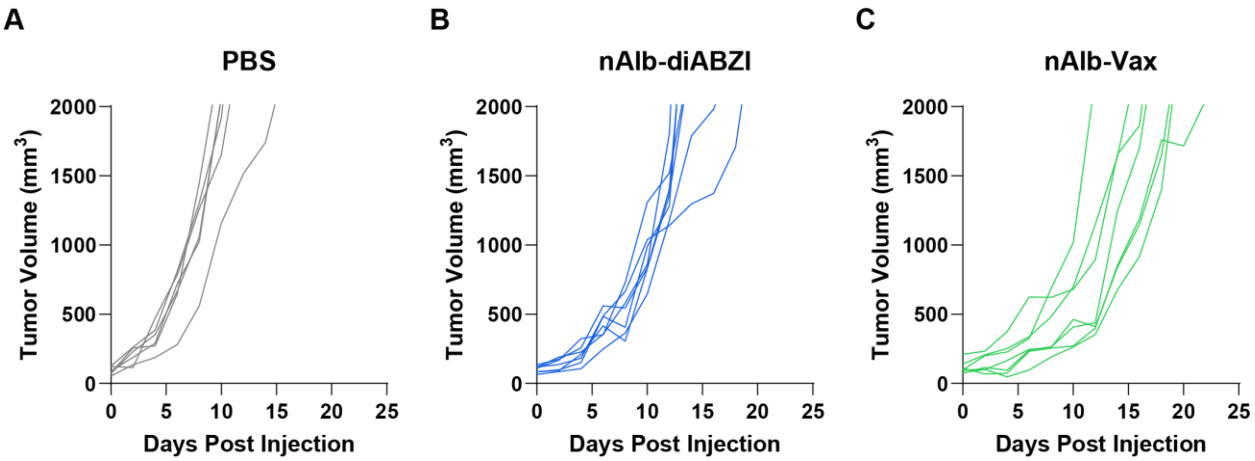


Fig. S44. Individual tumor growth curves for Fig. 7B. Individual tumor growth curves for Fig. 7B following treatment with **(A)** PBS, **(B)** nAlb-diABZI, or **(C)** nAlb-Vax.

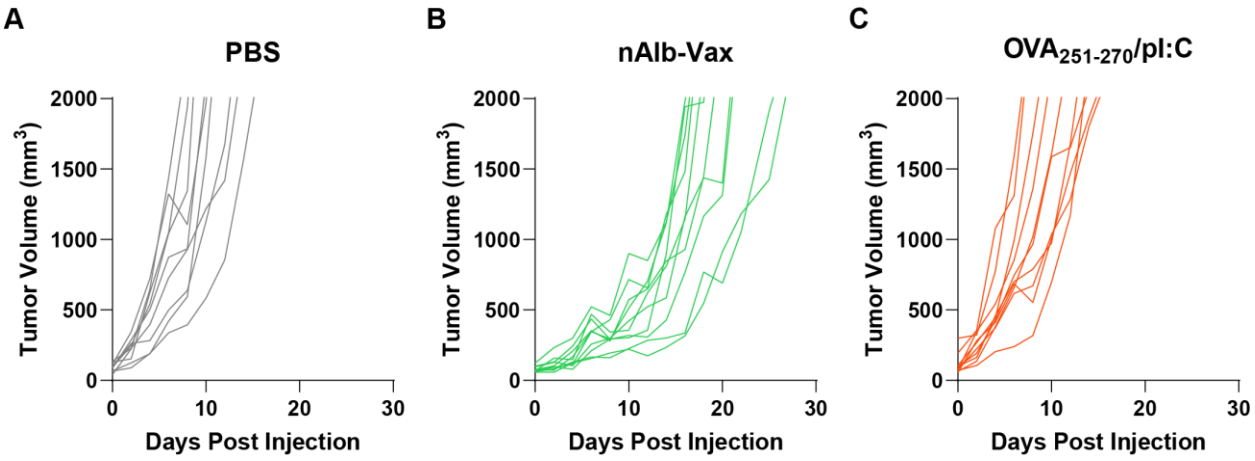


Fig. S45. Individual tumor growth curves for Fig. 7F. Individual tumor growth curves for Fig. 7F following treatment with **(A)** PBS, **(B)** nAlb-Vax, or **(C)** $OVA_{251-270}$/pI:C.

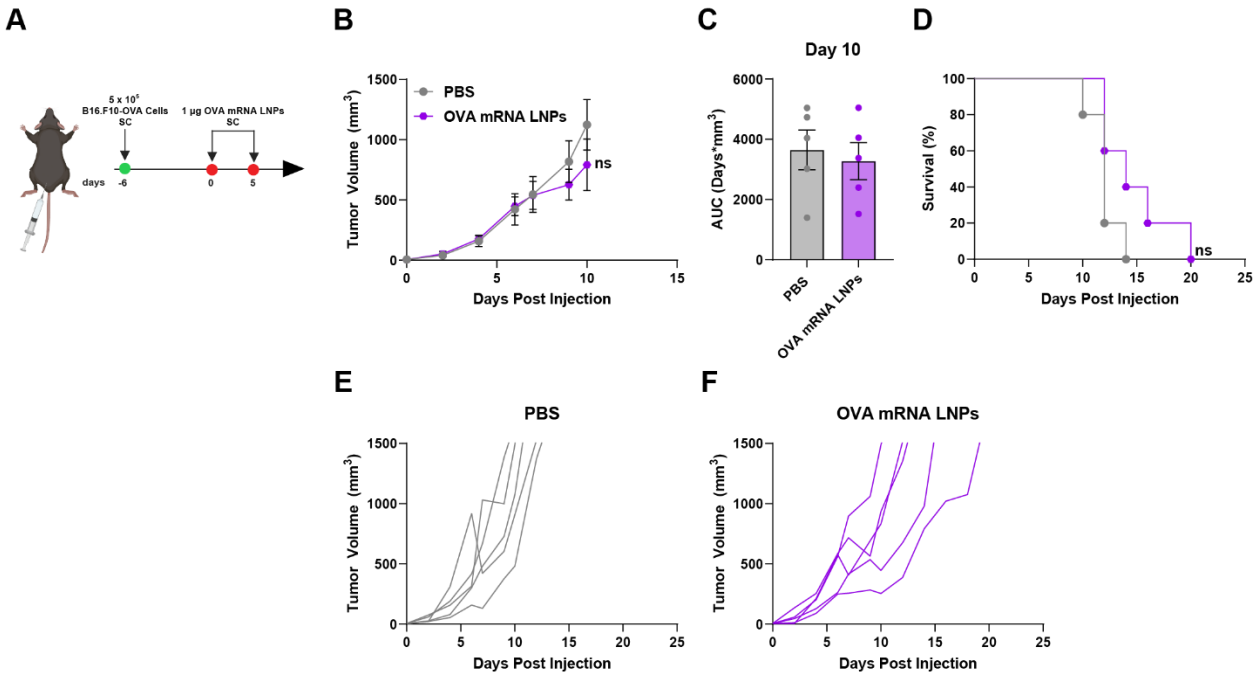


Fig. S46. Therapeutic vaccination with OVA mRNA lipid nanoparticles (LNPs) exhibits minimal antitumor efficacy. (A) Schematic of treatment timeline. Created with Biorender.com. **(B)** B16.F10-OVA tumor volumes, **(C)** AUC of the individual tumor growth curves, and **(D)** Kaplan-Meier survival plots following treatment with PBS or OVA mRNA LNPs (mean ± SEM, $n = 5$). Analyzed via Welch's two-tailed t test or Mantel-Cox log-rank test as appropriate. Individual tumor growth curves following treatment with **(E)** PBS or **(F)** OVA mRNA LNPs. $^{****}P < 0.0001$. $^{***}P < 0.001$. $^{**}P < 0.01$. $^{*}P < 0.05$.

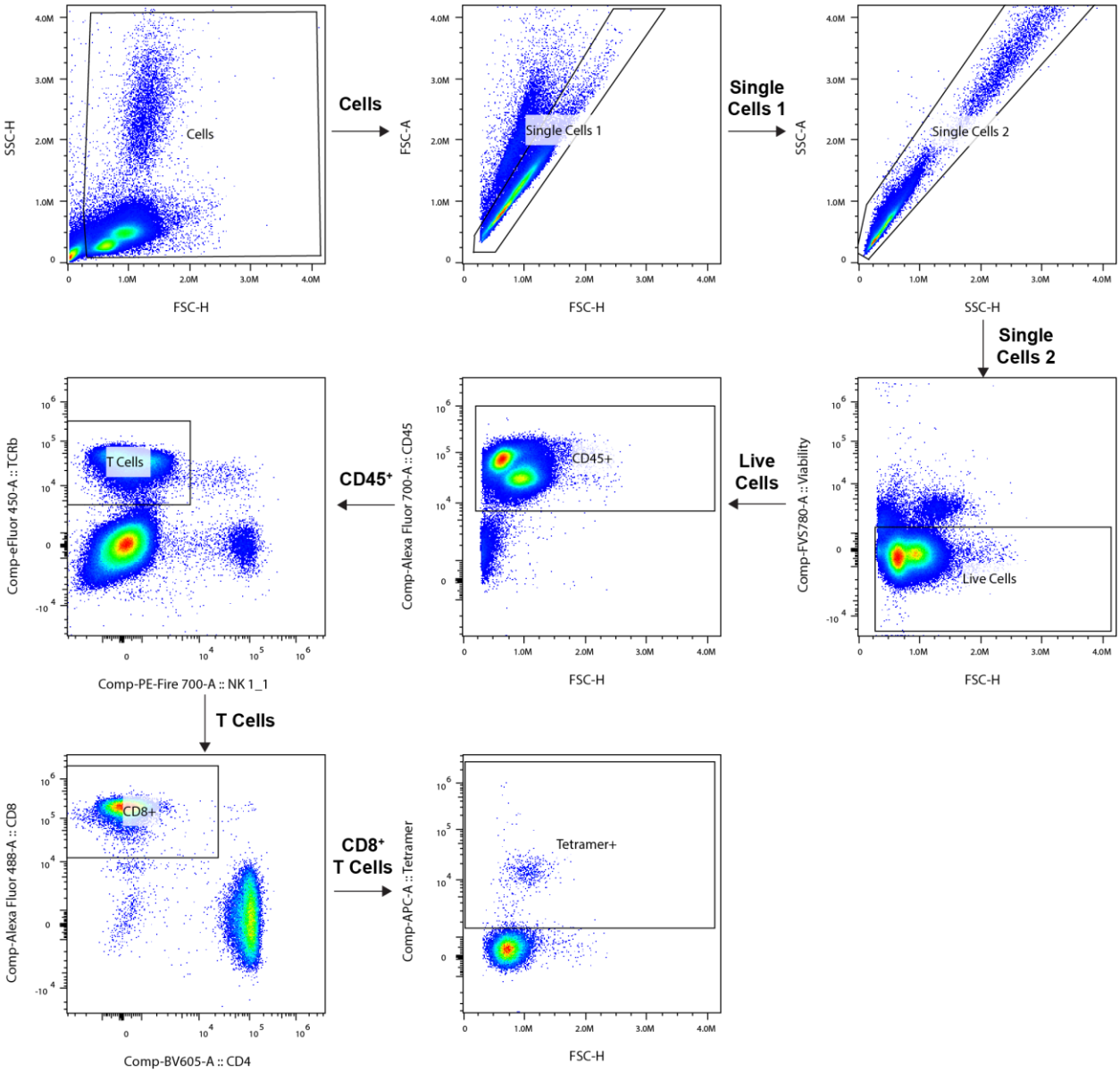


Fig. S47. Gating strategy for Fig. 7J-M.

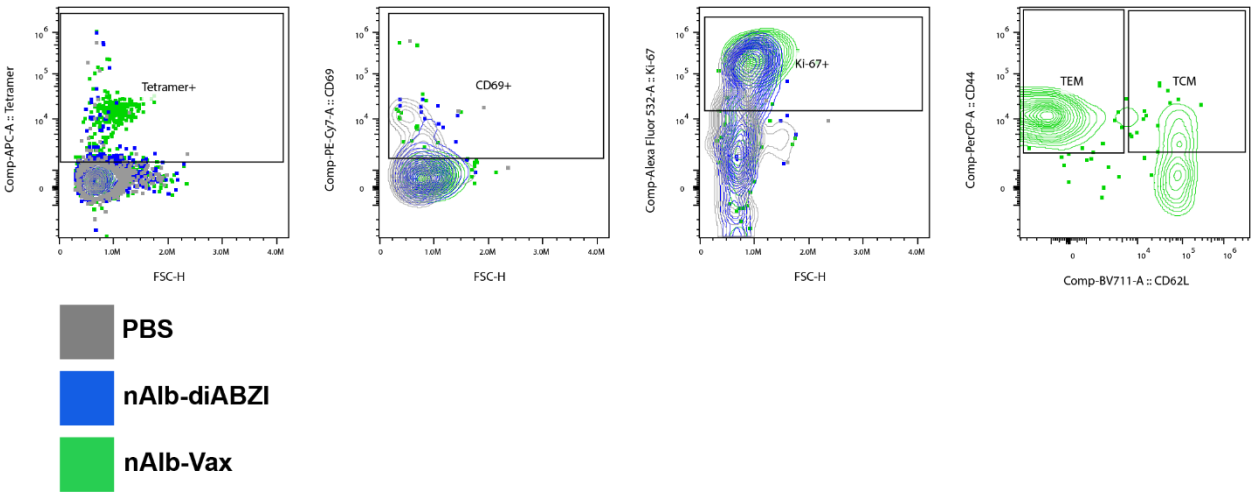


Fig. S48. Representative flow cytometry dot plots for Fig. 7J-M.

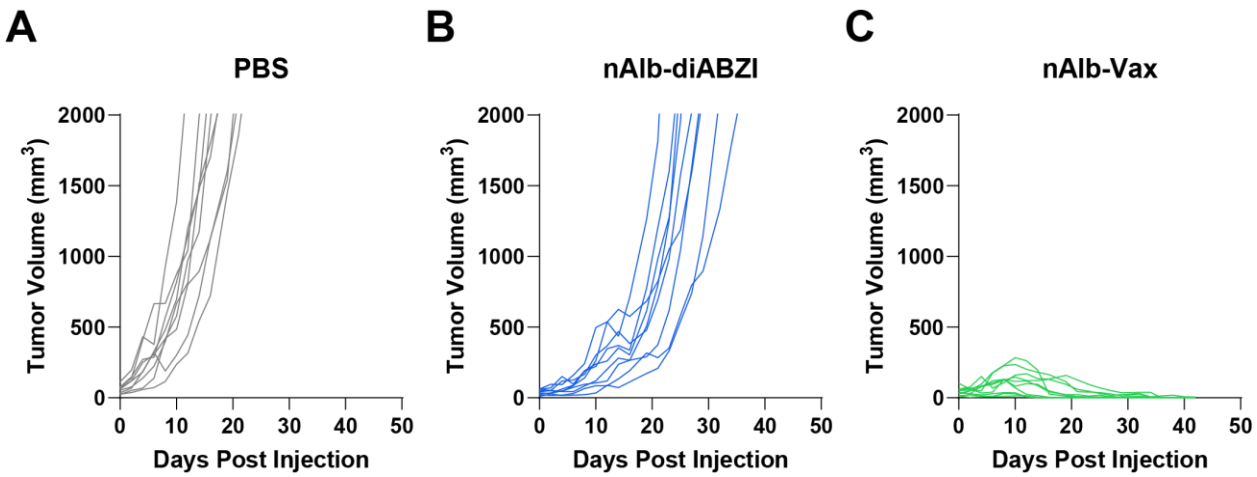


Fig. S49. Individual tumor growth curves for Fig. 7N. Individual tumor growth curves for Fig. 7N following treatment with **(A)** PBS, **(B)** nAlb-diABZI, or **(C)** nAlb-Vax.

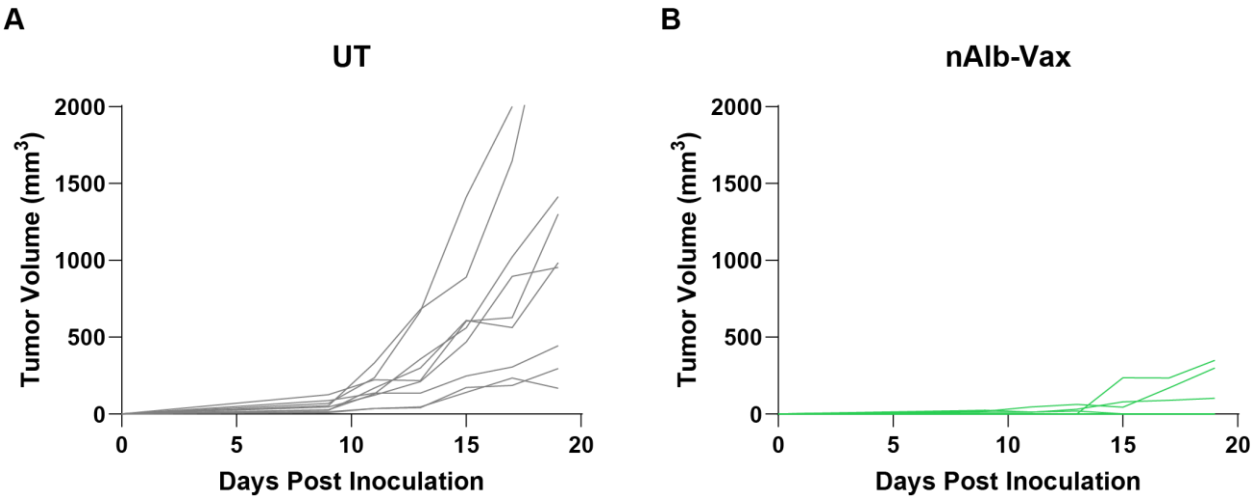


Fig. S50. Individual tumor growth curves for Fig. 7R. Individual tumor growth curves for Fig. 7R for mice that were previously **(A)** untreated (UT) or **(B)** treated with nAlb-Vax.

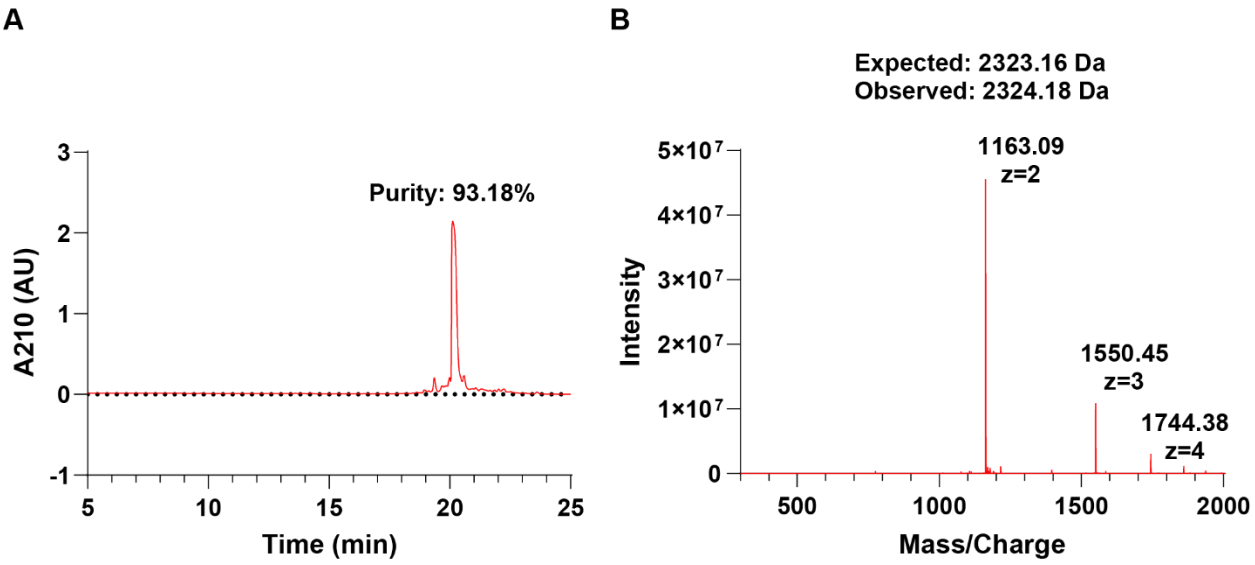


Fig. S51. Characterization of OVA$_{251-270}$ peptide. (A) HPLC chromatogram of purified OVA$_{251-270}$ indicating that the compound is > 90% pure. **(B)** Mass spectra of purified OVA$_{251-270}$ indicating that the peptide exhibits the appropriate molecular weight.

Table S1. Antibodies used for immunofluorescent microscopy in Fig. 1I.

Marker (Species)	Cell Type	Fluorophore	Dilution	Vendor (Catalog)
F4/80	Macrophages	BV421	1:100	BioLegend (123132)
CD11c	Dendritic Cells	FITC	1:200	BioLegend (117306)
CD3ε (Hamster)	T Cells	N/A	1:200	BD (553238)
Anti-Hamster Secondary Ab	N/A	Alexa Fluor 568	1:1000	Invitrogen (A-21112)
Cy5	Treatment	Cy5	N/A	N/A

Table S2. Antibodies/stains used for flow cytometry in Fig. 1J-K.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
CD4	$CD4^+$ T Cells	Super Bright 780	1:80	Invitrogen (78-0042-82)
CD8α	$CD8^+$ T Cells	BV605	1:40	BioLegend (100744)
CD11b	Macrophages, Granulocytes	BV510	1:50	BioLegend (101263)
CD11c	DCs	BV711	1:1000	BioLegend (117349)
Gr-1	Granulocytes	PE-Cy7	1:333	BioLegend (108416)
F4/80	Macrophages	AlexaFluor 488	1:50	BioLegend (123120)
CD19	B Cells	PE	1:100	BioLegend (115508)
NK1.1	NK Cells	PerCp-Cy5.5	1:40	BioLegend (108728)
CD31	Endothelial Cells	Super Bright 645	1:80	Invitrogen (64-0311-82)
Cy5	Treatment	Alexa Fluor 647	N/A	N/A

Table S3. Antibodies/stains used for flow cytometry in Fig. 2C-E, K.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD80	Activation	PE-Cy5	1:100	BioLegend (104712)
CD86	Activation	APC	1:80	BioLegend (105012)
H-2Kb/SIINFEKL	Antigen Presentation	PE	1:80	BioLegend (141604)

Table S4. Antibodies/stains used for flow cytometry in Fig. 2H-J, L.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD11b	Macrophages	BV510	1:40	BioLegend (101263)
F4/80	Macrophages	AlexaFluor 488	1:50	BioLegend (123120)
CD80	Activation	PE-Cy5	1:100	BioLegend (104712)
CD86	Activation	APC	1:80	BioLegend (105012)
H-2Kb/SIINFEKL	Antigen Presentation	PE	1:80	BioLegend (141604)

Table S5. Antibodies/stains used for flow cytometry in Fig. S9A.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
H-2Kb/SIINFEKL	Antigen Presentation	PE	1:80	BioLegend (141604)

Table S6. Antibodies/stains used for flow cytometry in Fig. S9B.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD11b	Macrophages	BV510	1:40	BioLegend (101263)
F4/80	Macrophages	AlexaFluor 488	1:50	BioLegend (123120)
H-2Kb/SIINFEKL	Antigen Presentation	PE	1:80	BioLegend (141604)

Table S7. Antibodies/stains used for flow cytometry in Fig. 2M-O.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CellTrace Violet	Proliferation	eFluor 450	N/A	Invitrogen (C34557)
CD69	Activation	PE-Cy7	1:100	BioLegend (104512)
TCRβ	T Cells	PerCp-Cy5.5	1:80	BioLegend (109228)
CD8α	$CD8^+$ T Cells	BV785	1:40	BioLegend (100750)

Table S8. Antibodies/stains used for flow cytometry in Fig. 3A.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	DAPI	N/A	Invitrogen (D3571)
CD45.2	Immune Cells	APC	1:80	BioLegend (109814)
CD3ε	T Cells	PE-Cy7	1:80	BioLegend (100220)
CD8α	$CD8^+$ T Cells	APC-Cy7	1:20	BioLegend (100714)
Tetramer	Ag-Specific T Cells	PE	1:33	N/A

Table S9. Antibodies/stains used for flow cytometry in Fig. 3B.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
CD8α	$CD8^+$ T Cells	AlexaFluor 488	1:100	BioLegend (100723)
Tetramer	Ag-Specific T Cells	PE	1:33	N/A

Table S10. Antibodies/stains used for flow cytometry in Fig. 3D.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	DAPI	N/A	Invitrogen (D3571)
CD45.2	Immune Cells	APC	1:80	BioLegend (109814)
CD3ε	T Cells	PE-Cy7	1:80	BioLegend (100220)
CD8α	$CD8^+$ T Cells	APC-Cy7	1:20	BioLegend (100714)
Tetramer	Ag-Specific T Cells	PE	1:33	N/A

Table S11. Antibodies/stains used for flow cytometry in Fig. 3F-H.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
CD8α	$CD8^+$ T Cells	AlexaFluor 488	1:100	BioLegend (100723)
CD69	Activation	PE-Cy7	1:100	BioLegend (104512)
CD44	Memory	PerCP	1:80	BioLegend (103036)
CD62L	Memory	BV711	1:100	BioLegend (104445)
Ki-67	Proliferation	AlexaFluor 532	1:80	Invitrogen (58-5698-82)
Tetramer	Ag-Specific T Cells	PE	1:33	N/A

Table S12. Antibodies/stains used for flow cytometry in Fig. 4E-F.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
CD4	$CD4^+$ T Cells	Super Bright 780	1:80	Invitrogen (78-0042-82)
CD8α	$CD8^+$ T Cells	BV605	1:40	BioLegend (100744)
CD11b	Macrophages, Granulocytes	BV510	1:50	BioLegend (101263)
CD11c	DCs	BV711	1:1000	BioLegend (117349)
Gr-1	Granulocytes	PE-Cy7	1:333	BioLegend (108416)
F4/80	Macrophages	AlexaFluor 488	1:50	BioLegend (123120)
CD19	B Cells	PE	1:100	BioLegend (115508)
NK1.1	NK Cells	PerCp-Cy5.5	1:40	BioLegend (108728)
CD31	Endothelial Cells	Super Bright 645	1:80	Invitrogen (64-0311-82)
Cy5	Treatment	Alexa Fluor 647	N/A	N/A

Table S13. Antibodies/stains used for flow cytometry in Fig. S21A-B.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
CD4	$CD4^+$ T Cells	Super Bright 780	1:80	Invitrogen (78-0042-82)
CD8α	$CD8^+$ T Cells	BV605	1:40	BioLegend (100744)
CD11b	Macrophages, Granulocytes	BV510	1:50	BioLegend (101263)
CD11c	DCs	BV711	1:1000	BioLegend (117349)
Gr-1	Granulocytes	PE-Cy7	1:333	BioLegend (108416)
F4/80	Macrophages	AlexaFluor 488	1:50	BioLegend (123120)
CD19	B Cells	PE	1:100	BioLegend (115508)
NK1.1	NK Cells	PerCp-Cy5.5	1:40	BioLegend (108728)
CD31	Endothelial Cells	Super Bright 645	1:80	Invitrogen (64-0311-82)
Cy5	Treatment	Alexa Fluor 647	N/A	N/A

Table S14. Antibodies/stains used for flow cytometry in Fig. 5B (Injection Draining LN), Fig. 5C-D, Fig. 5G-H, Fig. 5K-L, Fig. S29 (Injection Draining LN), and Fig. S30 (Injection Draining LN).

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
CD11b	Macrophages	BV510	1:50	BioLegend (101263)
CD11c	DCs	BV711	1:1000	BioLegend (117349)
F4/80	Macrophages	AlexaFluor 488	1:50	BioLegend (123120)
CD80	Activation	PerCp-eFluor 710	1:160	Invitrogen (46-0801-82)
CD86	Activation	PerCp	1:80	BioLegend (105026)
H-2Kb/SIINFEKL	Antigen Presentation	PE-Dazzle 594	1:40	BioLegend (141612)
CD8α	cDC1s	BV785	1:40	BioLegend (100750)
CD206	M2 Marker	BV605	1:40	BioLegend (141721)
Arg1	M2 Marker	PE	1:40	Invitrogen (12-3697-82)
iNOS	M1 Marker	AlexaFluor 532	1:160	Invitrogen (58-5920-82)
Cy5	Treatment	Alexa Fluor 647	N/A	N/A

Table S15. Antibodies/stains used for flow cytometry in Fig. 5B (Spleen), Fig. S29 (Spleen), and Fig. S30 (Spleen).

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
CD11b	Macrophages	BV510	1:50	BioLegend (101263)
CD11c	DCs	BV711	1:1000	BioLegend (117349)
F4/80	Macrophages	AlexaFluor 488	1:50	BioLegend (123120)
CD80	Activation	PerCp-eFluor 710	1:160	Invitrogen (46-0801-82)
CD86	Activation	PerCp	1:80	BioLegend (105026)
H-2Kb/SIINFEKL	Antigen Presentation	PE-Dazzle 594	1:40	BioLegend (141612)
CD206	M2 Marker	BV605	1:40	BioLegend (141721)
Arg1	M2 Marker	PE	1:40	Invitrogen (12-3697-82)
iNOS	M1 Marker	AlexaFluor 532	1:160	Invitrogen (58-5920-82)

Table S16. Antibodies/stains used for flow cytometry in Fig. 5B (Tumor), Fig. 5E-F, Fig. 5I-J, Fig. 5M-N, Fig. S29 (Tumor), and Fig. S30 (Tumor).

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
CD31	Endothelial Cells	Pacific Blue	1:40	Invitrogen (RM5228)
CD11b	Macrophages	BV510	1:50	BioLegend (101263)
CD11c	DCs	BV711	1:1000	BioLegend (117349)
F4/80	Macrophages	AlexaFluor 488	1:50	BioLegend (123120)
CD80	Activation	PerCp-eFluor 710	1:160	Invitrogen (46-0801-82)
CD86	Activation	PerCp	1:80	BioLegend (105026)
H-2Kb/SIINFEKL	Antigen Presentation	PE-Dazzle 594	1:40	BioLegend (141612)
CD8α	cDC1s	BV785	1:40	BioLegend (100750)
CD206	M2 Marker	BV605	1:40	BioLegend (141721)
Arg1	M2 Marker	PE	1:40	Invitrogen (12-3697-82)
iNOS	M1 Marker	AlexaFluor 532	1:160	Invitrogen (58-5920-82)
Cy5	Treatment	Alexa Fluor 647	N/A	N/A

Table S17. Antibodies/stains used for flow cytometry in Fig. 6B.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
CD8α	$CD8^+$ T Cells	AlexaFluor 488	1:100	BioLegend (100723)
CD4	$CD4^+$ T Cells	BV605	1:160	BioLegend (100548)
NK1.1	NK Cells	APC	1:40	BioLegend (156506)
Tetramer	Ag-Specific T Cells	PE	1:33	N/A

Table S18. Antibodies/stains used for flow cytometry in Fig. 6C.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
CD8α	$CD8^+$ T Cells	AlexaFluor 488	1:100	BioLegend (100723)
CD4	$CD4^+$ T Cells	BV605	1:160	BioLegend (100548)
NK1.1	NK Cells	APC	1:40	BioLegend (156506)
Tetramer	Ag-Specific T Cells	PE	1:33	N/A

Table S19. Antibodies/stains used for flow cytometry in Fig. 6D.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
CD8α	$CD8^+$ T Cells	AlexaFluor 488	1:100	BioLegend (100723)
CD4	$CD4^+$ T Cells	BV605	1:160	BioLegend (100548)
NK1.1	NK Cells	APC	1:40	BioLegend (156506)
Tetramer	Ag-Specific T Cells	PE	1:33	N/A

Table S20. Antibodies/stains used for flow cytometry in Fig. 6F, Fig. 6I (Injection Draining LN), Fig. S41 (Injection Draining LN), and Fig. S42 (Injection Draining LN).

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
CD8α	$CD8^+$ T Cells	AlexaFluor 488	1:100	BioLegend (100723)
CD4	$CD4^+$ T Cells	BV605	1:160	BioLegend (100548)
NK1.1	NK Cells	PE-Fire 700	1:80	BioLegend (108774)
Tetramer	Ag-Specific T Cells	APC	1:33	N/A
CD69	Activation	PE-Cy7	1:100	BioLegend (104512)
Ki-67	Proliferation	AlexaFluor 532	1:80	Invitrogen (58-5698-82)
PD-1	Activation/ Checkpoint	BV510	1:160	BioLegend (135241)
LAG-3	Exhaustion	BV785	1:26.67	BioLegend (125219)
TIM-3	Exhaustion	PE-Dazzle 594	1:50	BioLegend (134014)
CD44	Memory	PerCP	1:80	BioLegend (103036)
CD62L	Central Memory	BV711	1:100	BioLegend (104445)

Table S21. Antibodies/stains used for flow cytometry in Fig. 6G, Fig. 6I (Spleen), Fig. S41 (Spleen), and Fig. S42 (Spleen).

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
CD8α	$CD8^+$ T Cells	AlexaFluor 488	1:100	BioLegend (100723)
CD4	$CD4^+$ T Cells	BV605	1:160	BioLegend (100548)
NK1.1	NK Cells	PE-Fire 700	1:80	BioLegend (108774)
Tetramer	Ag-Specific T Cells	APC	1:33	N/A
CD69	Activation	PE-Cy7	1:100	BioLegend (104512)
Ki-67	Proliferation	AlexaFluor 532	1:80	Invitrogen (58-5698-82)
PD-1	Activation/ Checkpoint	BV510	1:160	BioLegend (135241)
LAG-3	Exhaustion	BV785	1:26.67	BioLegend (125219)
TIM-3	Exhaustion	PE-Dazzle 594	1:50	BioLegend (134014)
CD44	Memory	PerCP	1:80	BioLegend (103036)
CD62L	Central Memory	BV711	1:100	BioLegend (104445)

Table S22. Antibodies/stains used for flow cytometry in Fig. 6H, Fig. 6I (Tumor), Fig. 6J, Fig. 6K, Fig. S41 (Tumor), and Fig. S42 (Tumor).

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
CD8α	$CD8^+$ T Cells	AlexaFluor 488	1:100	BioLegend (100723)
CD4	$CD4^+$ T Cells	BV605	1:160	BioLegend (100548)
NK1.1	NK Cells	PE-Fire 700	1:80	BioLegend (108774)
Tetramer	Ag-Specific T Cells	APC	1:33	N/A
CD69	Activation	PE-Cy7	1:100	BioLegend (104512)
Ki-67	Proliferation	AlexaFluor 532	1:80	Invitrogen (58-5698-82)
PD-1	Activation/ Checkpoint	BV510	1:160	BioLegend (135241)
LAG-3	Exhaustion	BV785	1:26.67	BioLegend (125219)
TIM-3	Exhaustion	PE-Dazzle 594	1:50	BioLegend (134014)
CD44	Memory	PerCP	1:80	BioLegend (103036)
CD62L	Central Memory	BV711	1:100	BioLegend (104445)

Table S23. Antibodies/stains used for flow cytometry in Fig. 7J-M.

Marker	Purpose	Fluorophore	Dilution	Vendor (Catalog)
Viability	Live Cells	eFluor 780	1:16000	Invitrogen (65-0865-18)
CD45.2	Immune Cells	AlexaFluor 700	1:50	BioLegend (109822)
TCRβ	T Cells	eFluor 450	1:80	Invitrogen (48-5961-82)
NK1.1	NK Cells	PE-Fire 700	1:80	BioLegend (108774)
CD8α	$CD8^+$ T Cells	AlexaFluor 488	1:100	BioLegend (100723)
CD4	$CD4^+$ T Cells	BV605	1:160	BioLegend (100548)
Tetramer	Ag-Specific T Cells	APC	1:200	Baylor College of Medicine (H-2Db/HPV16.E7.RAH APC)
CD69	Activation	PE-Cy7	1:100	BioLegend (104512)
Ki-67	Proliferation	AlexaFluor 532	1:80	Invitrogen (58-5698-82)
CD44	Memory	PerCP	1:80	BioLegend (103036)
CD62L	Central Memory	BV711	1:100	BioLegend (104445)