

High-parameter multi-panel mass cytometry reveals both B and T cell compartment remodeling in the tumor draining lymph nodes during anti-PD1 therapy

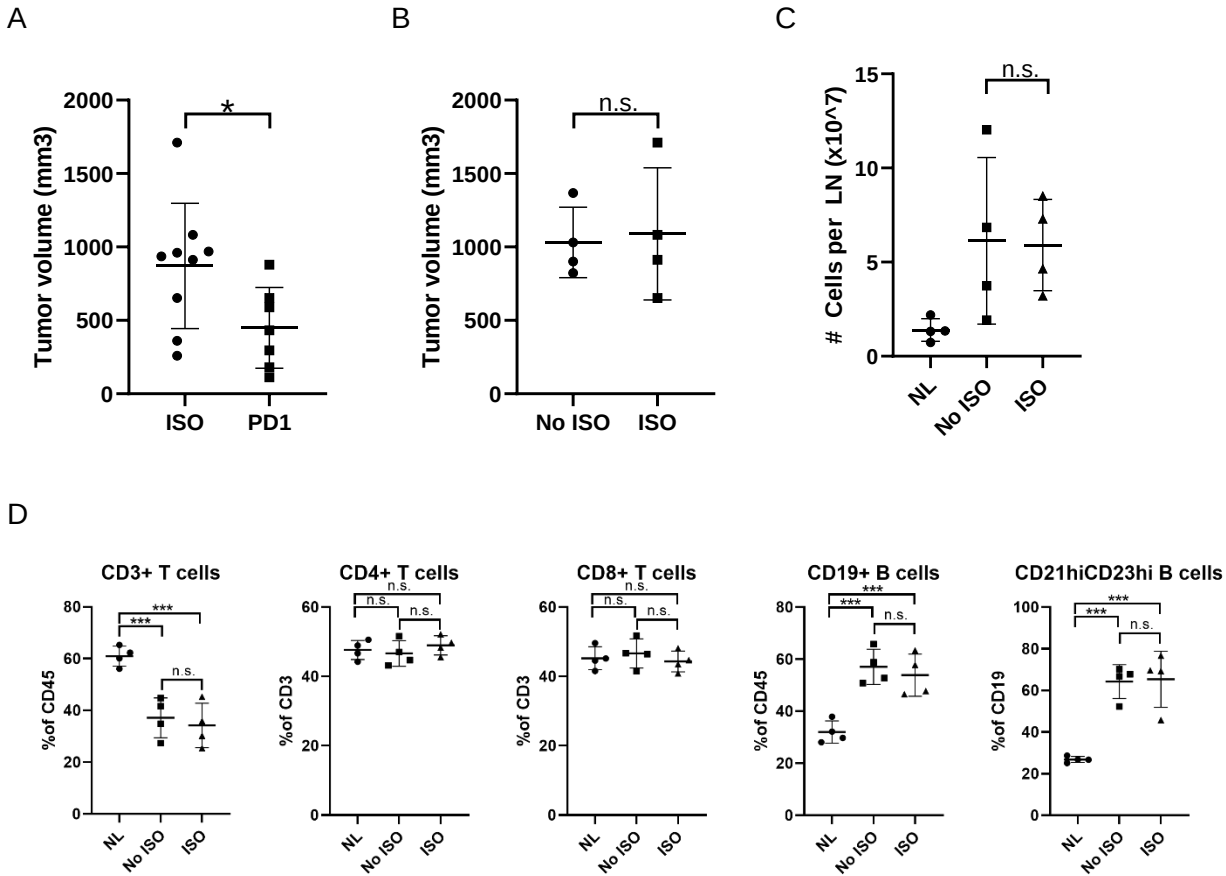
Won Jin Ho, Mark Yarchoan, Soren Charmsaz, Rebecca Munday, Ludmila Danilova, Marcelo B. Sztein, Elana J. Fertig, Elizabeth M. Jaffee

SUPPLEMENTARY MATERIALS

Supplementary Figures 1-29

Supplementary Tables 1-5

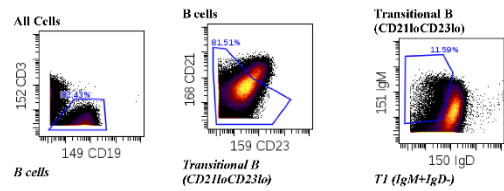
Supplementary Figure 1.



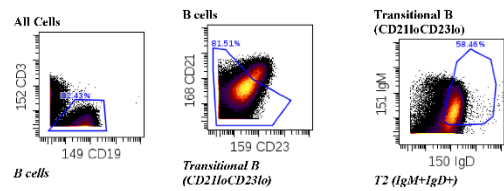
(A) Scatter plots showing MC38 tumor volumes on day 21 comparing mice treated with two injections on days 14 and 18 of anti-PD1 therapy ("PD1") to those treated with isotype antibody ("ISO"). Bars represent means $\pm$ SD (n=7-9) (B) MC38 tumor volumes in mice that are treated or not treated with the isotype antibody ("No ISO" vs. "ISO"). Bars represent Means $\pm$ SD (n=4). (C) Number of cells per lymph node comparing right inguinal lymph nodes from non-tumor bearing normal mice ("NL"), mice without isotype antibody treatment, and mice treated with isotype antibody. Bars represent means $\pm$ SD (n=4). (D) Immune cell subsets quantified as proportion of CD45+ cells, CD3+ cells, or CD19+ cells in the lymph nodes comparing the three groups. Bars represent means $\pm$ SD (n=4). P value not significant (n.s.) >0.05 , ≤ 0.05 , ≤ 0.01 , ≤ 0.005 .

Supplementary Figure 2.

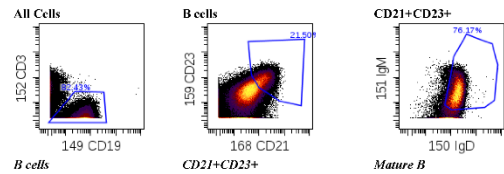
T1 (IgM+IgD-)



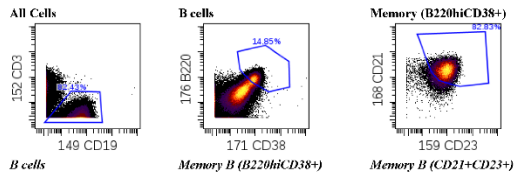
T2 (IgM+IgD+)



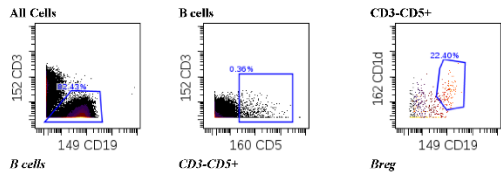
Mature B



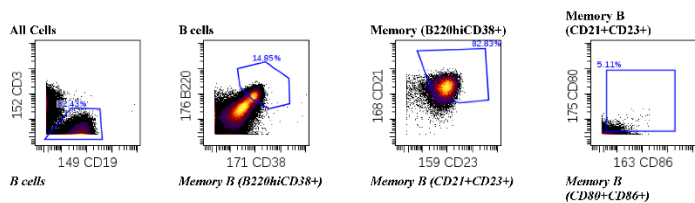
Memory B (CD21+CD23+)



Breg



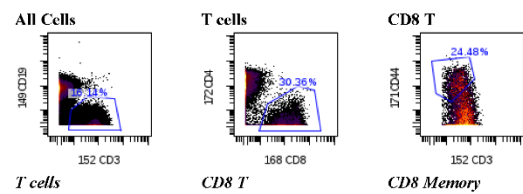
Memory B (CD80+CD86+)



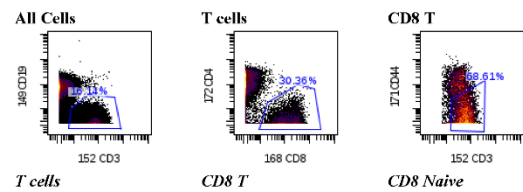
Gating hierarchy for B cell subtypes for supervised analysis

Supplementary Figure 3

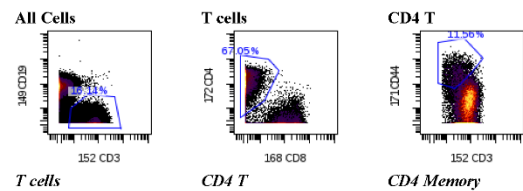
CD8 Memory



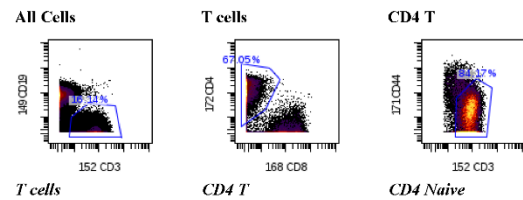
CD8 Naive



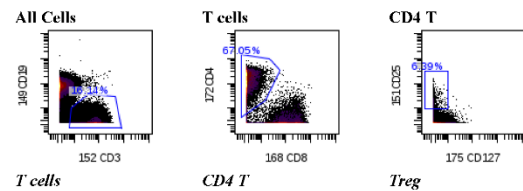
CD4 Memory



CD4 Naive

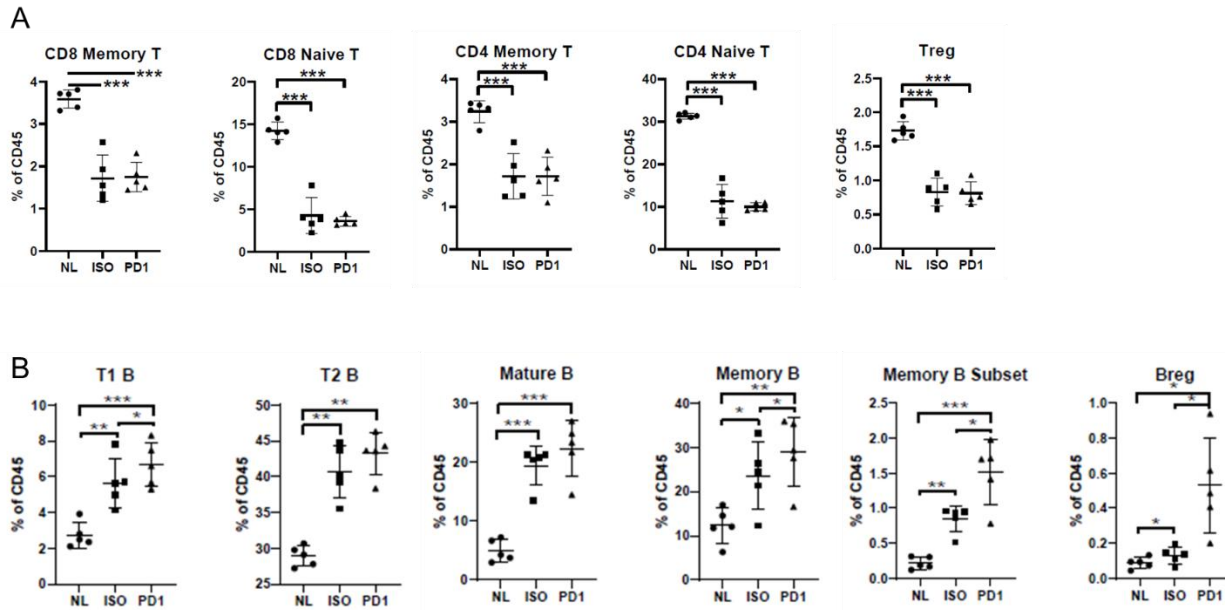


Treg



Gating hierarchy for T cell subtypes for supervised analysis

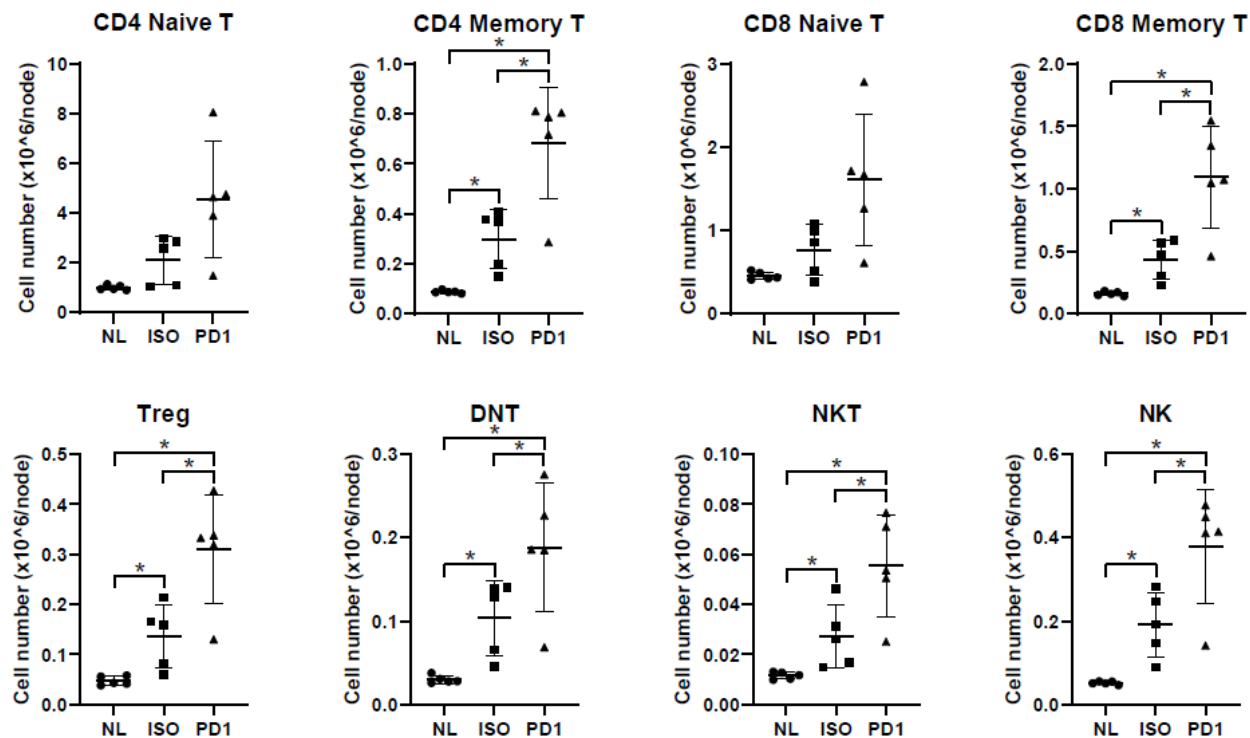
Supplementary Figure 4.



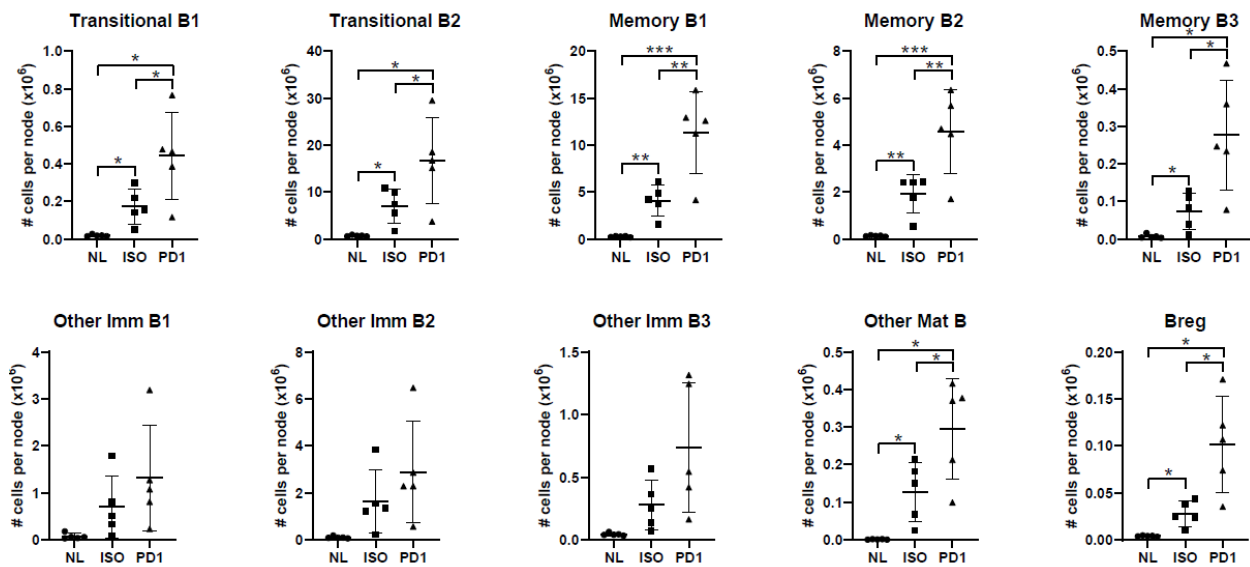
Hierarchical gating identified and quantified proportions of (A) T cell and (B) B cell subtypes within the lymph nodes. Proportions of each type as percentage of CD45⁺ were compared across the three groups: non-tumor bearing normal mice (“NL”), isotype-treated tumor-bearing mice (“ISO”) and anti-PD1-treated tumor-bearing mice (“PD1”). Results are shown as scatter plots of individual values and bars representing means $\pm$ SD (n=5). P value * <0.05 , ** <0.01 , *** <0.005 .

Supplementary Figure 5.

A

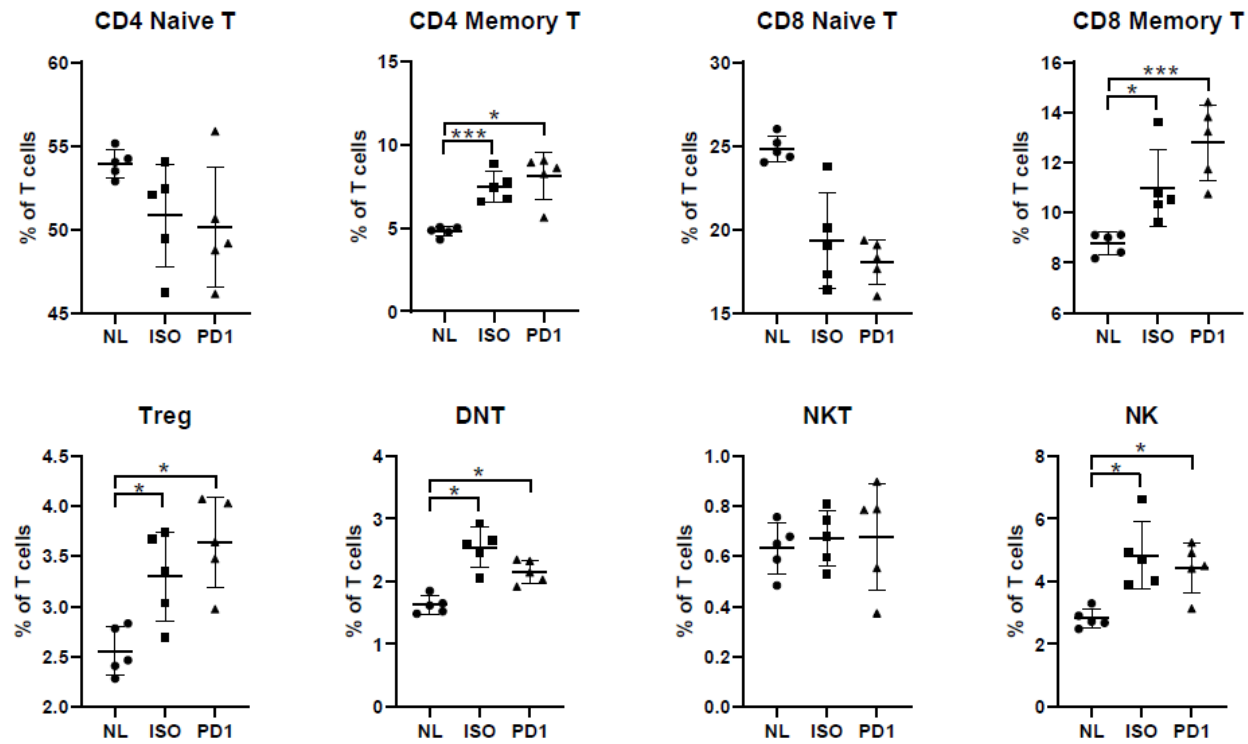


B



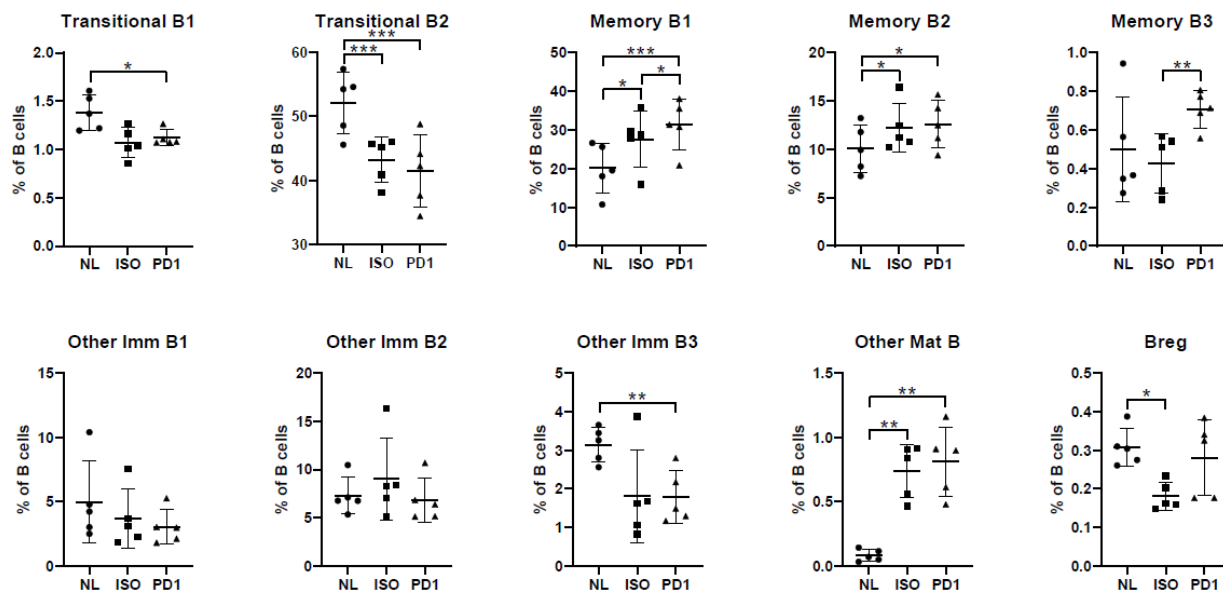
FlowSOM algorithm identified and quantified numbers of (A) T cell and (B) B cell subtypes within the lymph nodes. Numbers of each type were compared across the three groups: non-tumor bearing normal mice ("NL"), isotype-treated tumor-bearing mice ("ISO") and anti-PD1-treated tumor-bearing mice ("PD1"). Results are shown as scatter plots of individual values and bars representing means $\pm$ SD (n=5). P value * <0.05 , ** <0.01 , *** <0.005 .

Supplementary Figure 6



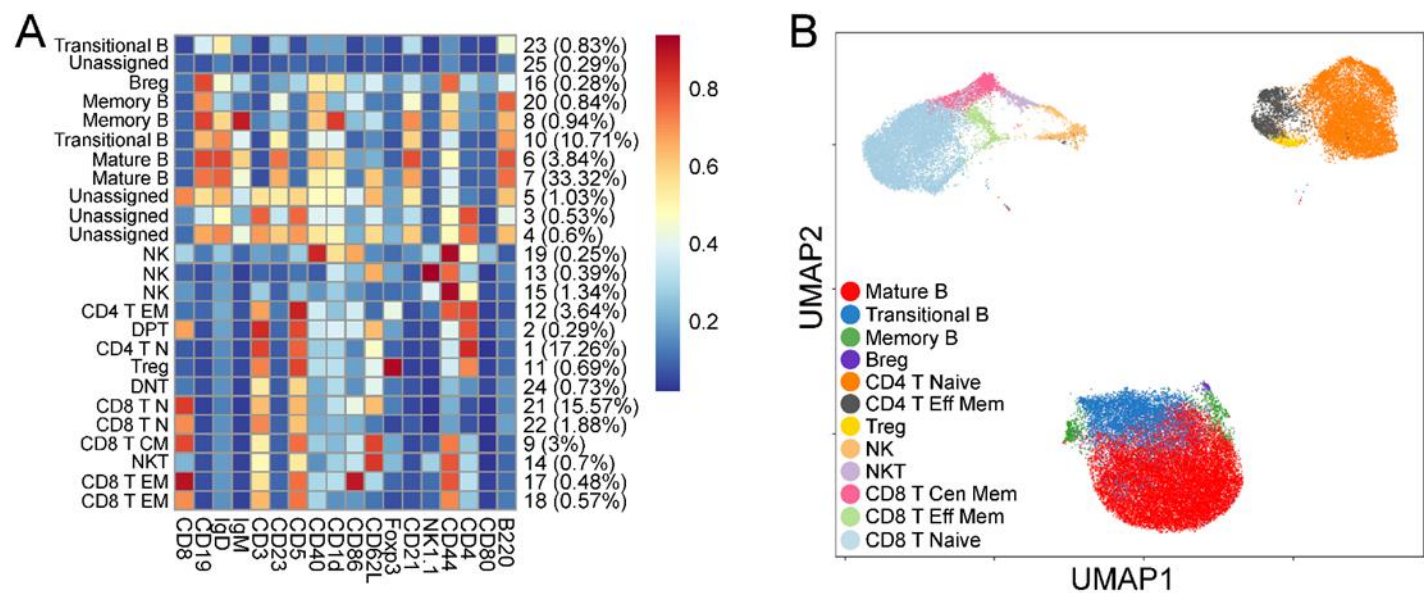
Each T cell subtype determined by FlowSOM algorithm are displayed in scatter plots as proportion of total T cells within the lymph nodes as individual values and means $\pm$ SD (n=5). P value * $\leq$ 0.05, ** $\leq$ 0.01, *** $\leq$ 0.005.

Supplementary Figure 7



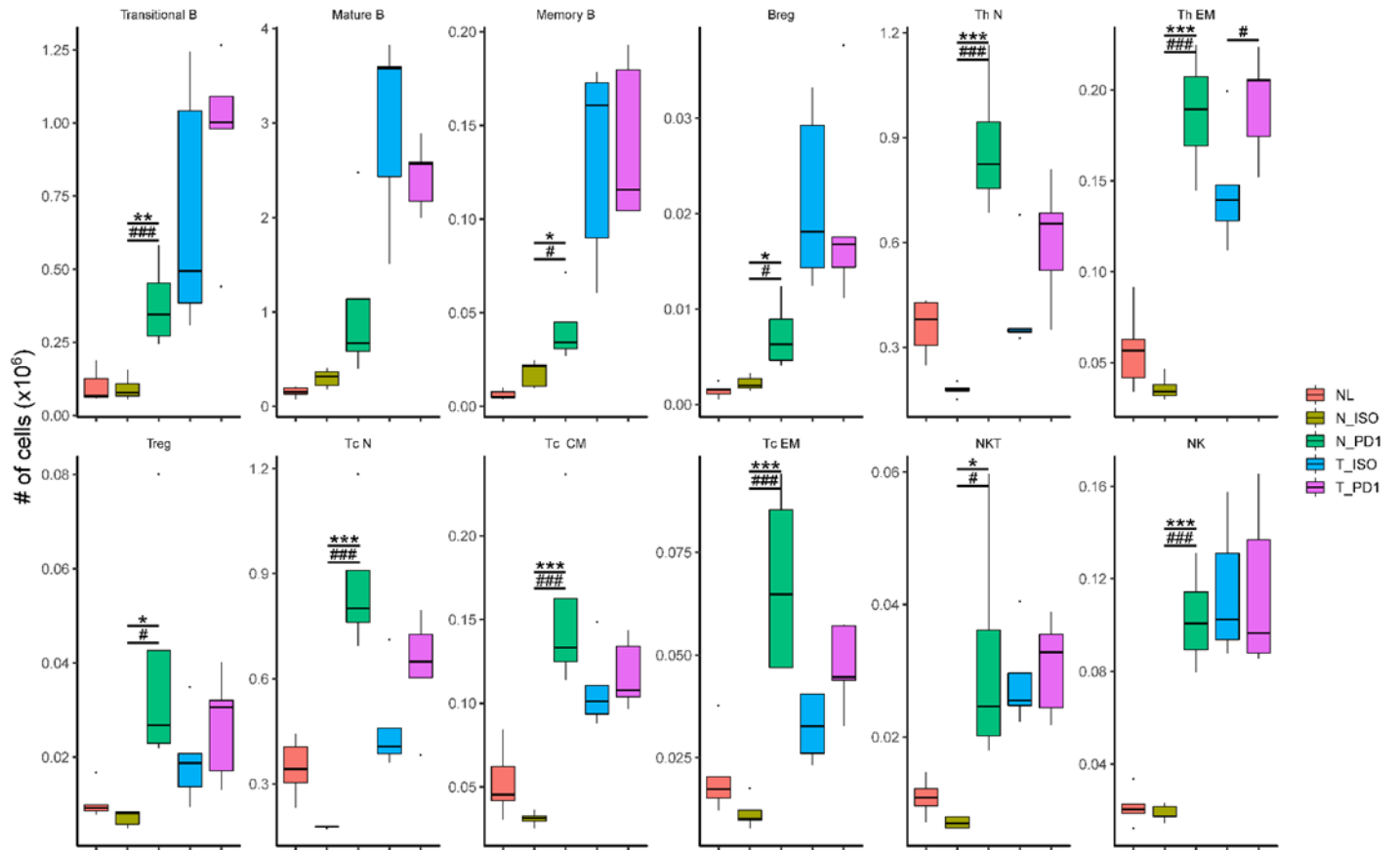
Each B cell subtype determined by FlowSOM algorithm are displayed in scatter plots as proportion of total B cells within the lymph nodes as individual values and means $\pm$ SD (n=5). P value * $\leq$ 0.05, ** $\leq$ 0.01, *** $\leq$ 0.005.

Supplementary Figure 8



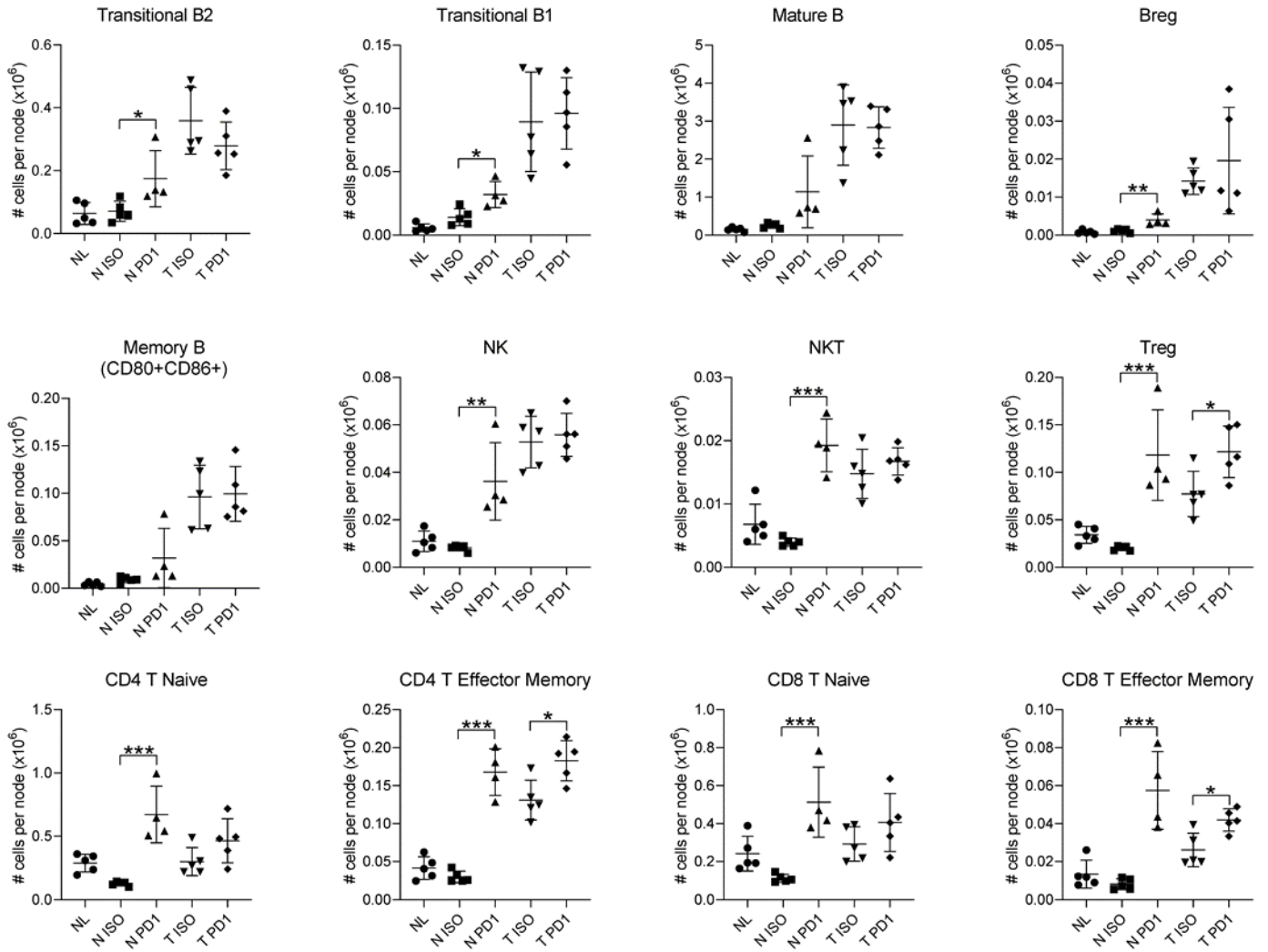
Unsupervised Analysis of Lymph Node Remodeling of a Repeat Run. (A) Based on the dataset from 18 canonical markers in a simplified, combined mass cytometry panel for profiling both T and B cell compartments, FlowSOM algorithm was used to yield 25 metaclusters annotated into 14 final clusters. The resulting phenograph is shown. (B) A representative UMAP plot for dimensionality reduction and visualization of all cell clusters is shown. Abbreviations: Breg, regulatory B cells; CM, central memory; DNT, double-negative T cells; DPT, double-positive T cells; EM, effector memory; N, naïve; NK, natural killer cells; NKT, natural killer T cells; Treg, regulatory T cells.

Supplementary Figure 9



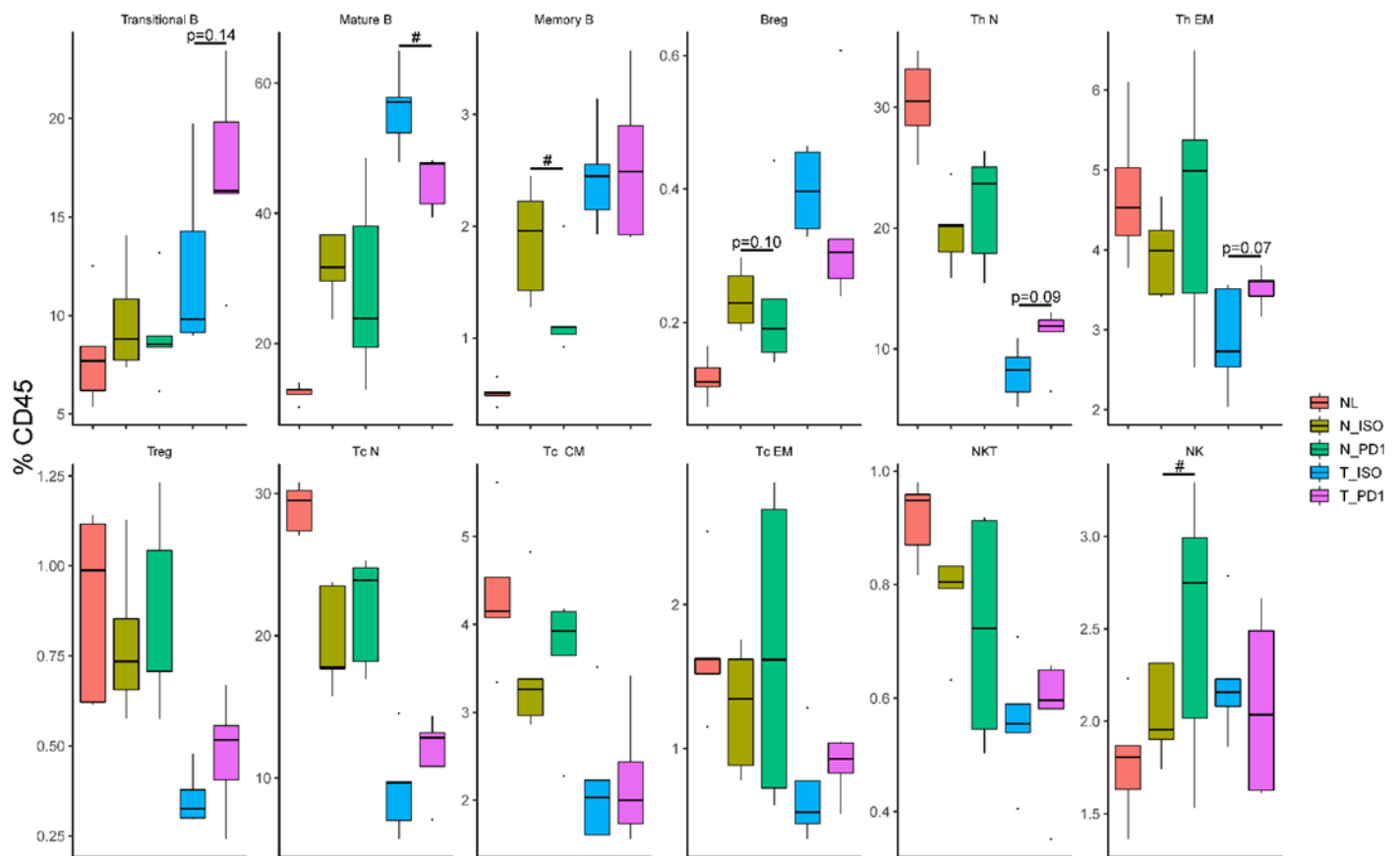
The number of cells per lymph node for each of the FlowSOM-defined cell type cluster from a repeat run is shown as boxplots with median bars and outlier dots for each of the five groups: non-tumor bearing normal (NL), non-tumor draining isotype-treated (N_ISO) or PD1-treated (N_PD1), and tumor draining isotype-treated (T_ISO) or PD1-treated (T_PD1) lymph nodes. Abbreviations: Breg, regulatory B cells; NK, natural killer cells; NKT, natural killer T cells; Tc N, naïve CD8 (cytotoxic) T cells; Tc CM, central memory CD8 (cytotoxic) T cells; Tc EM, effector memory CD8 (cytotoxic) T cells; Th N, naïve CD4 (helper) T cells; Th EM, CD4 (helper) T effector memory; Treg, regulatory T cells. Unadjusted p value # ≤ 0.05 , ### ≤ 0.005 . FDR-adjusted p value * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.005 .

Supplementary Figure 10



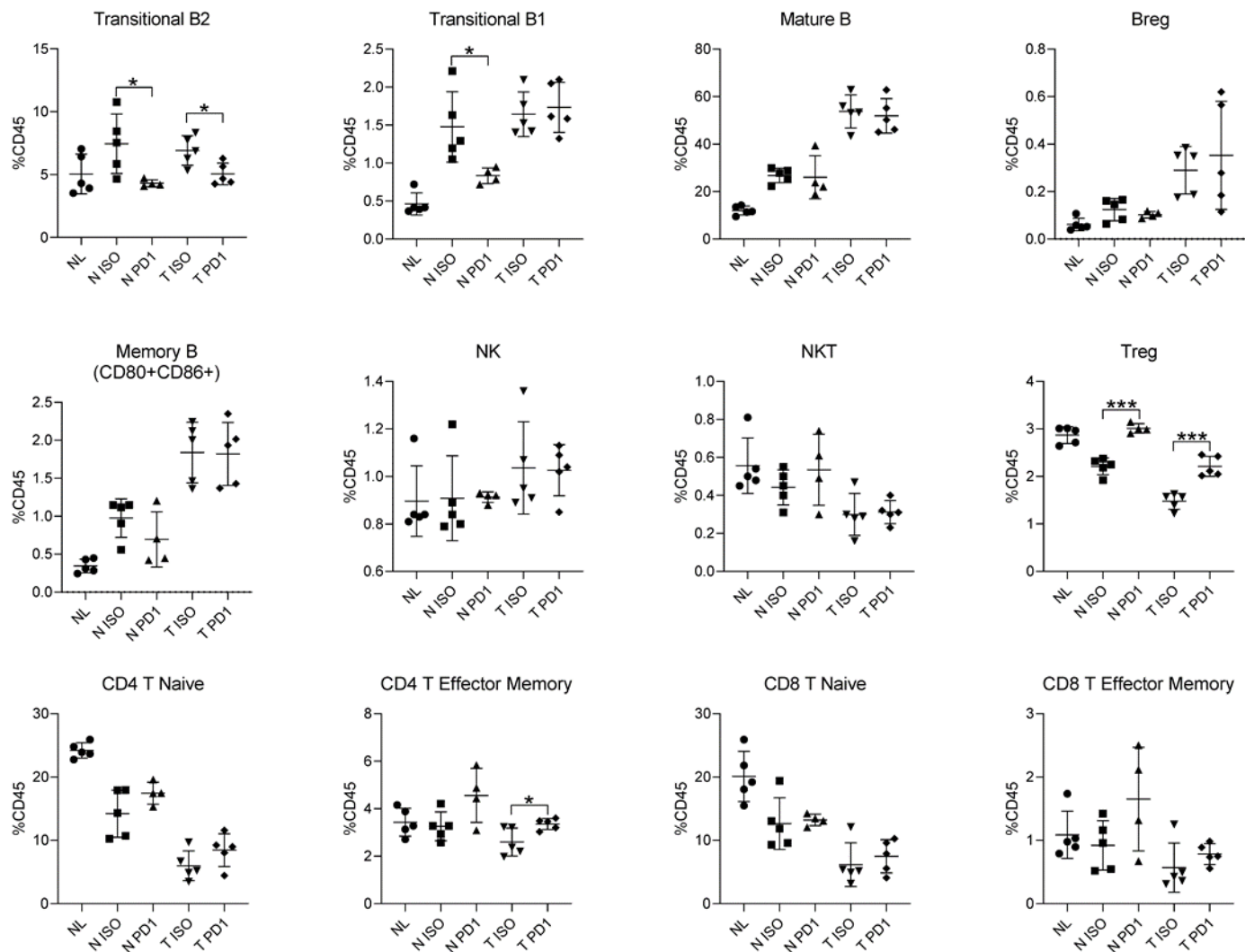
The number of cells per lymph node for each of the hierarchally gated cell type cluster from a repeat run is shown for each of the five groups: non-tumor bearing normal (NL), non-tumor draining isotype-treated (N ISO) or PD1-treated (N PD1), and tumor draining isotype-treated (T ISO) or PD1-treated (T PD1) lymph nodes. Abbreviations: Breg, regulatory B cells; NK, natural killer cells; NKT, natural killer T cells; Treg, regulatory T cells. Results for T-tests for N ISO vs. N PD1 and T ISO vs. T PD1 are shown as p value * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.005 .

Supplementary Figure 11



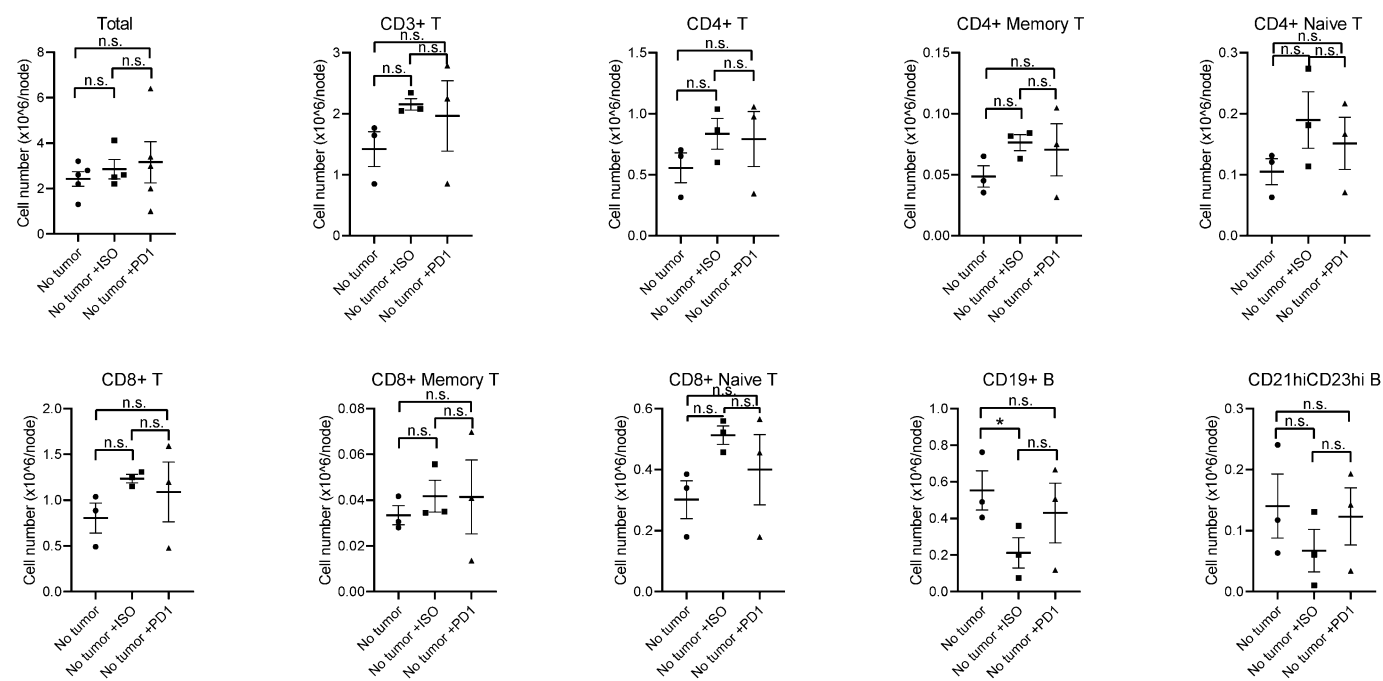
The proportions for each of the FlowSOM-defined cell type cluster out of the total CD45 cells from a repeat run are shown as boxplots with median bars and outlier dots for each of the five groups: non-tumor bearing normal (NL), non-tumor draining isotype-treated (N_ISO) or PD1-treated (N_PD1), and tumor draining isotype-treated (T_ISO) or PD1-treated (T_PD1) lymph nodes. Abbreviations: Breg, regulatory B cells; NK, natural killer cells; NKT, natural killer T cells; Tc N, naïve CD8 (cytotoxic) T cells; Tc CM, central memory CD8 (cytotoxic) T cells; Tc EM, effector memory CD8 (cytotoxic) T cells; Th N, naïve CD4 (helper) T cells; Th EM, CD4 (helper) T effector memory; Treg, regulatory T cells. Unadjusted p value $\# \leq 0.05$.

Supplementary Figure 12



The proportion of cells per lymph node for each of the hierarchically gated cell type cluster from a repeat run is shown for each of the five groups: non-tumor bearing normal (NL), non-tumor draining isotype-treated (N ISO) or PD1-treated (N PD1), and tumor draining isotype-treated (T ISO) or PD1-treated (T PD1) lymph nodes. Abbreviations: Breg, regulatory B cells; NK, natural killer cells; NKT, natural killer T cells; Treg, regulatory T cells. Results for T-tests for N ISO vs. N PD1 and T ISO vs. T PD1 are shown as p value ≤ 0.05 , $*** \leq 0.005$.

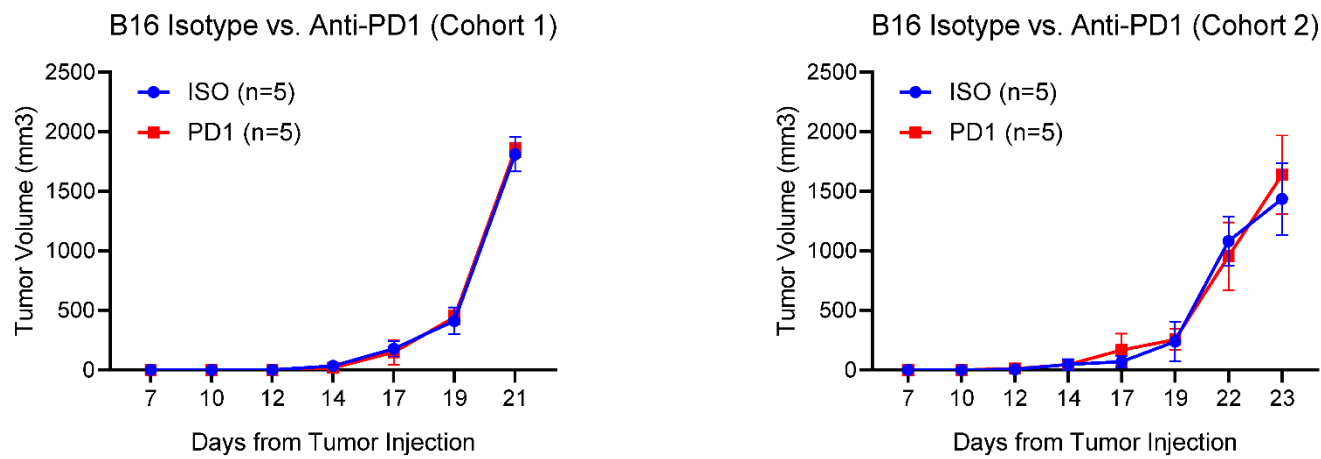
Supplementary Figure 13



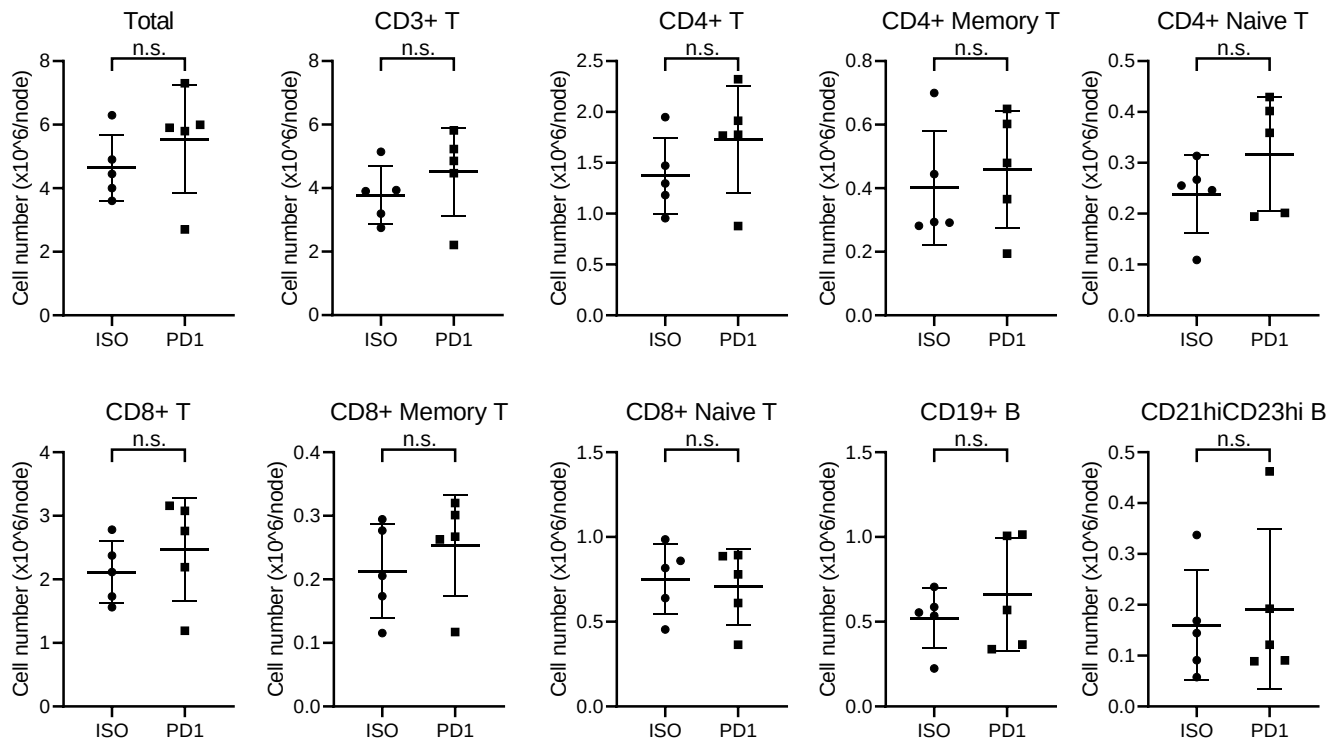
Comparison of T and B cell subtypes in non-tumor bearing mice (“No tumor”), isotype antibody-treated (“No tumor +ISO”), and anti-PD1 antibody treated (“No tumor +PD1”) by conventional flow cytometry reveal largely insignificant differences. Results are shown as scatter plots of individual values and bars representing means ± SD (n=5). Not significant (n.s.) represents p values >0.05.

Supplementary Figure 14

A

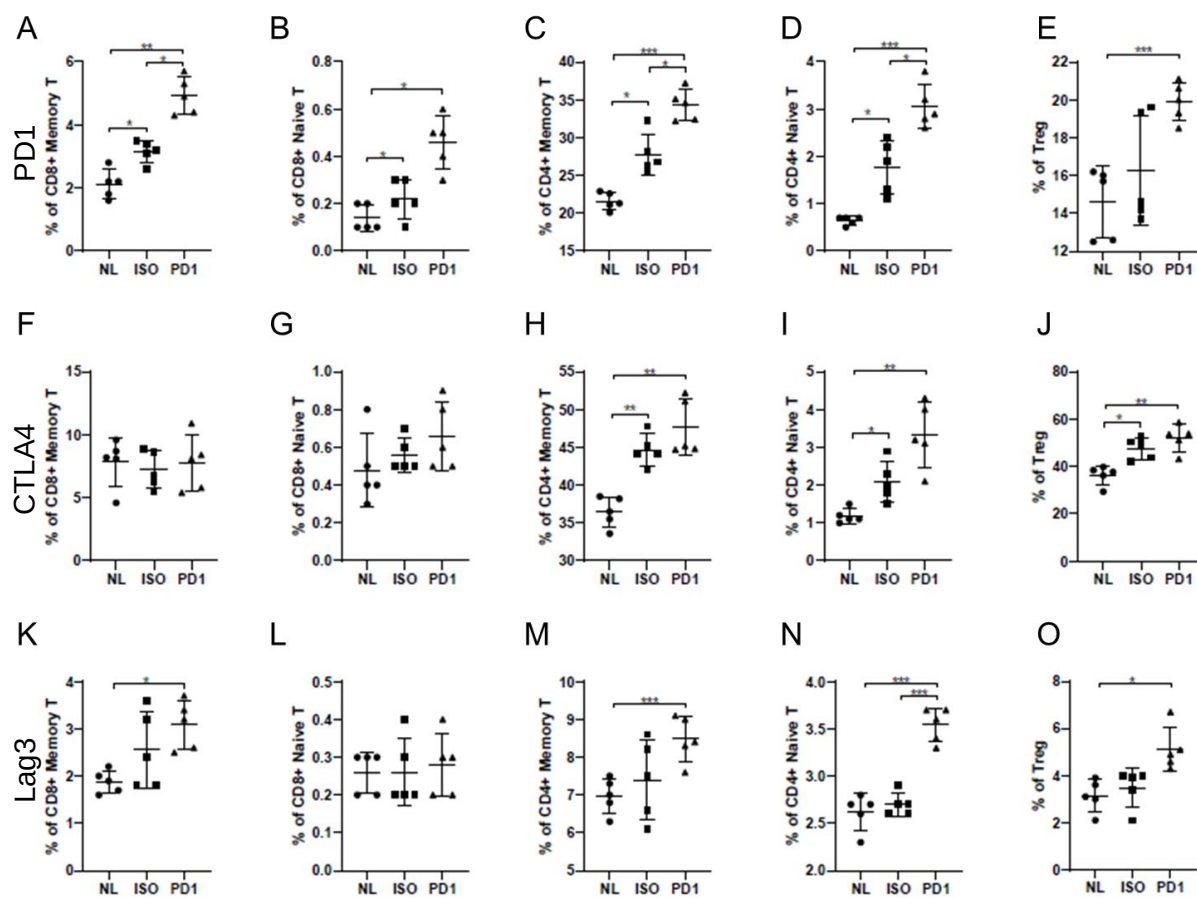


B



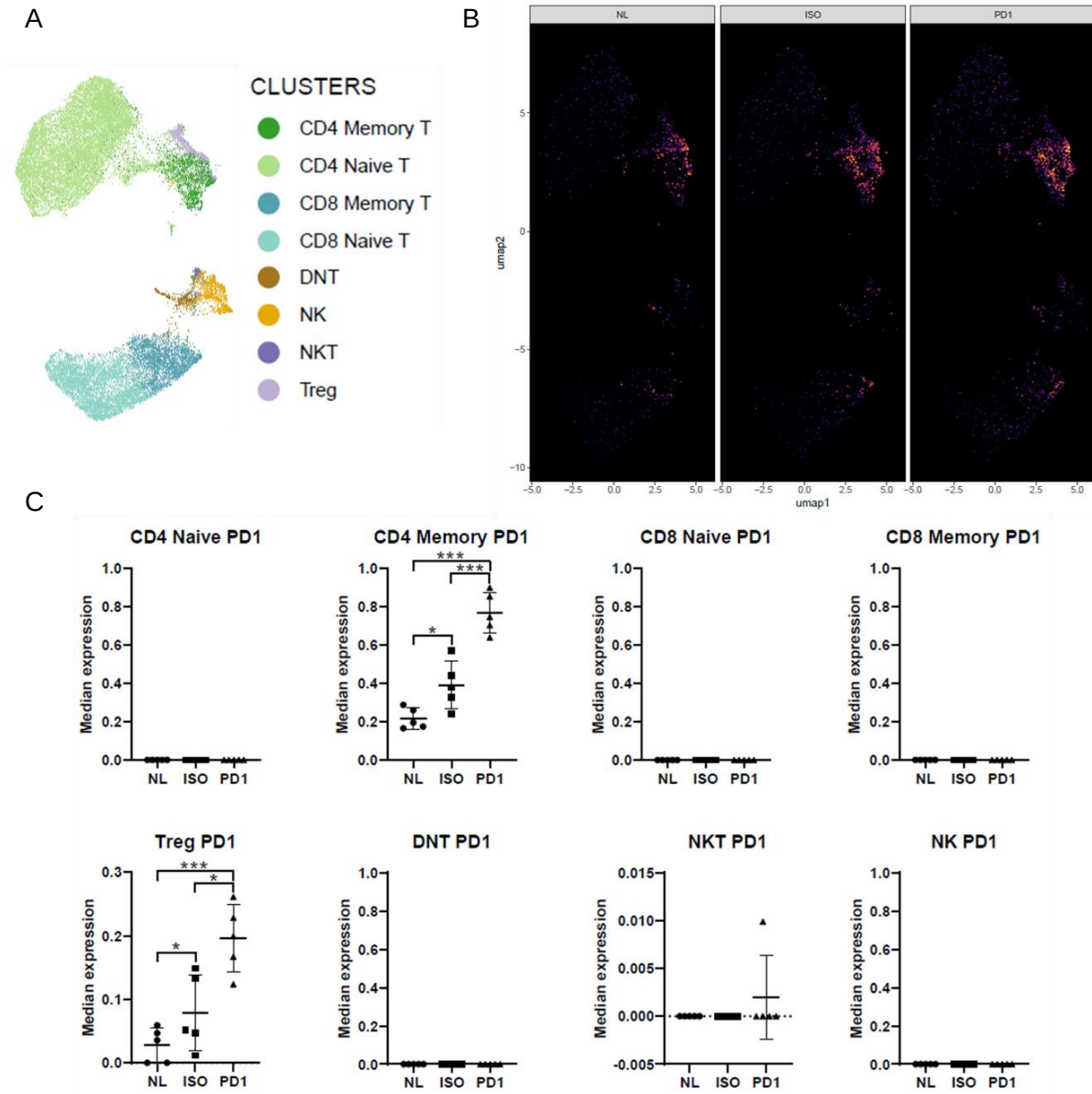
(A) Anti-PD1 treatment has no therapeutic effect on B16 tumors. Two replicate experiments are shown for tumor growth over time treated intraperitoneally with isotype (2A3) or PD1 (RMP1-14) 100ug twice weekly starting D3. (B) T and B cell subtypes in isotype antibody treated ("ISO") and anti-PD1 antibody treated ("PD1") B16-F10 tumor (PD1-resistant) draining lymph nodes are compared by conventional flow cytometry. Results are shown as scatter plots of individual values and bars representing means $\pm$ SD (n=5). P value <0.05 .

Supplementary Figure 15



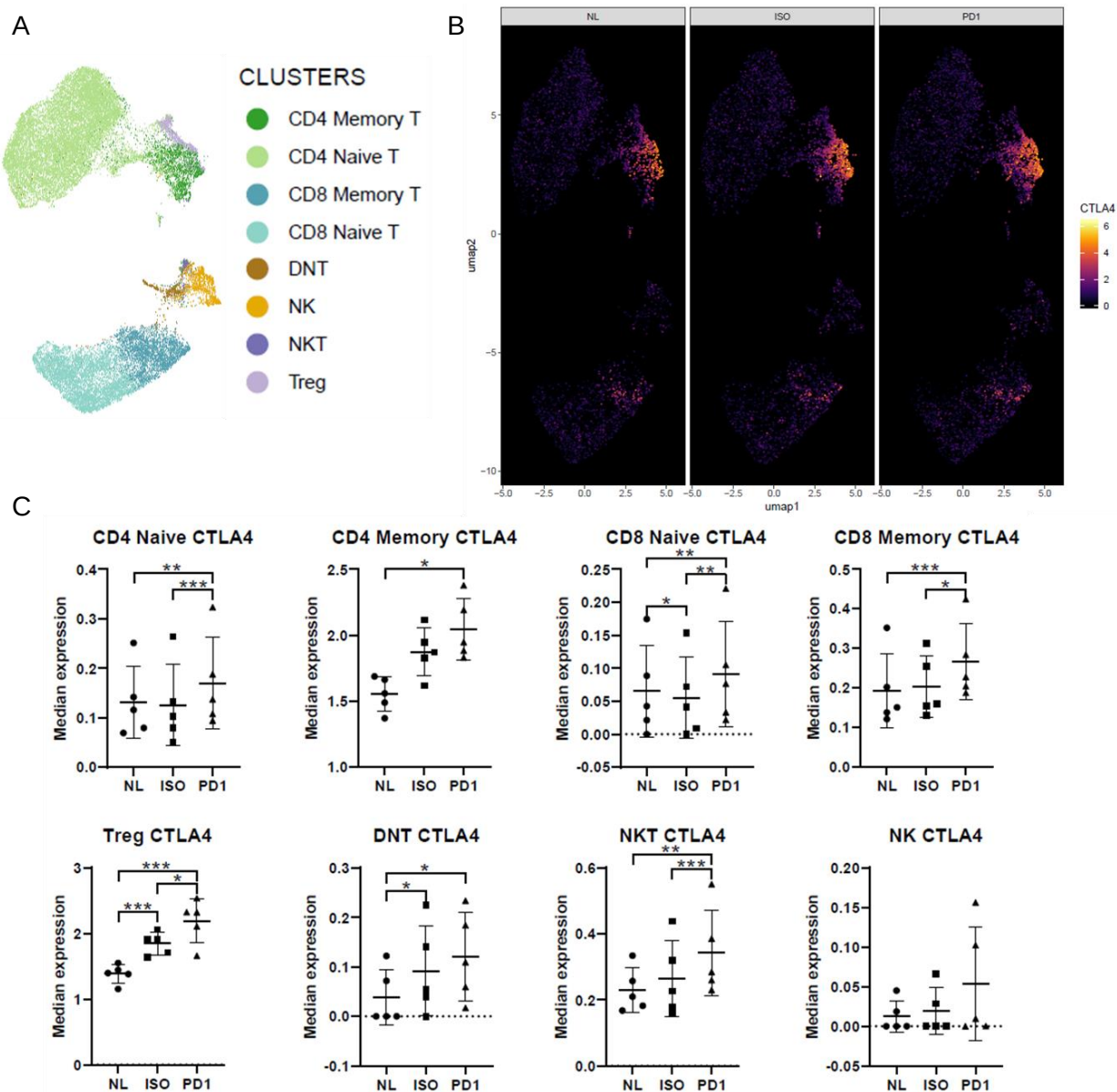
Percent of immune modulatory marker expression within each T cell type analyzed by supervised gating of cells positive for (A-E) PD1, (F-J) CTLA4, and (K-O) Lag3. Bars within the scatter plots represent means $\pm$ SD (n=5). P value * $\leq$ 0.05, ** $\leq$ 0.01, *** $\leq$ 0.005.

Supplementary Figure 16



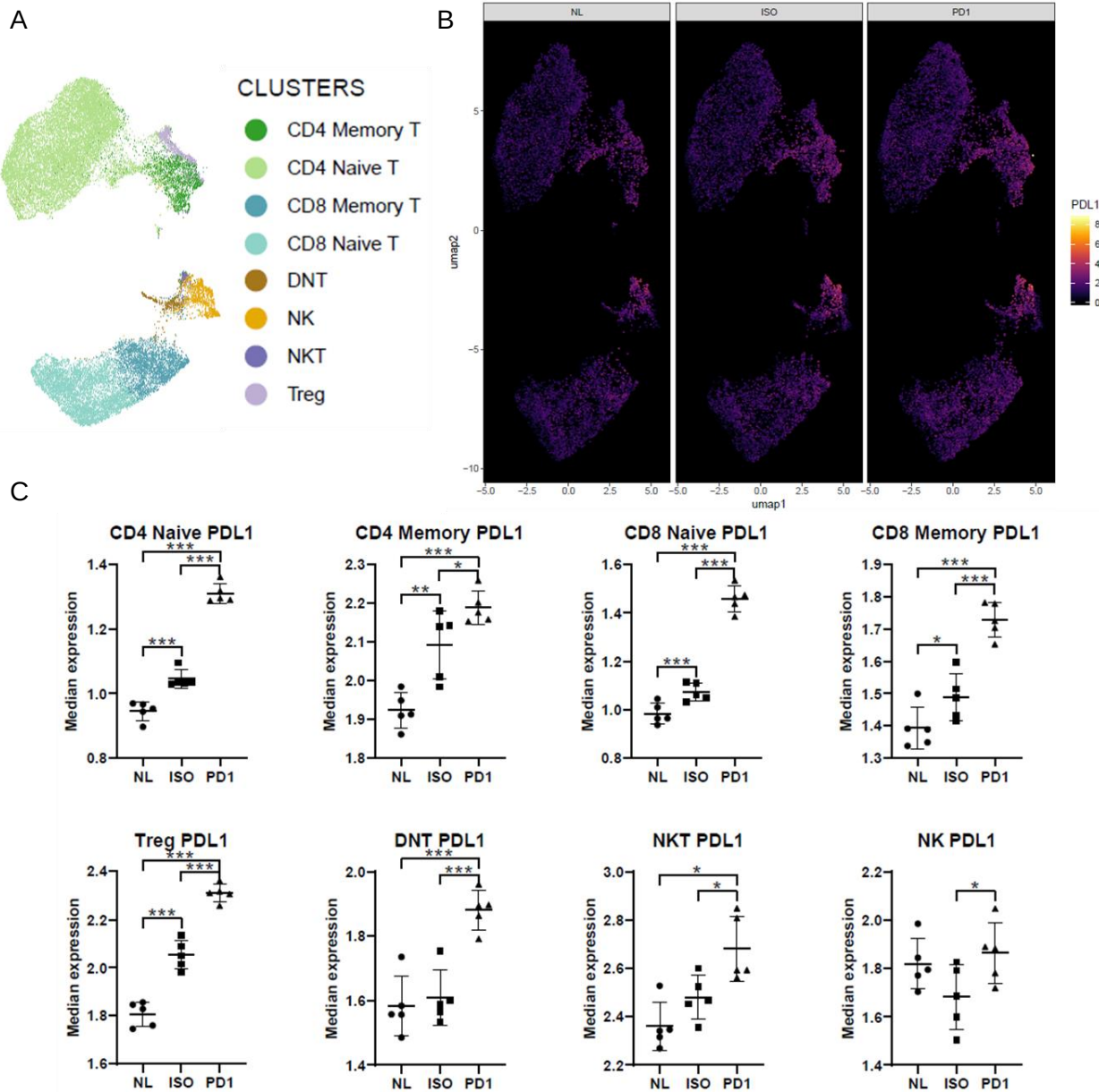
PD1 expression analysis by FlowSOM clustering followed by comparison of median expression within each T cell type. (A) Representative UMAP visualization of all T cell clusters in aggregate data of all samples as a reference for (B) PD1 expression heatmap superimposed comparing the lymph nodes from three groups, non-tumor bearing normal mice ("NL"), tumor bearing isotype-treated mice ("ISO"), and tumor bearing anti-PD1 treated mice ("PD1"). (C) Scatter plots of the median expression with bars representing means $\pm$ SD (n=5). P value $\ast \leq 0.05$, $\ast\ast \leq 0.01$, $\ast\ast\ast \leq 0.005$.

Supplementary Figure 17



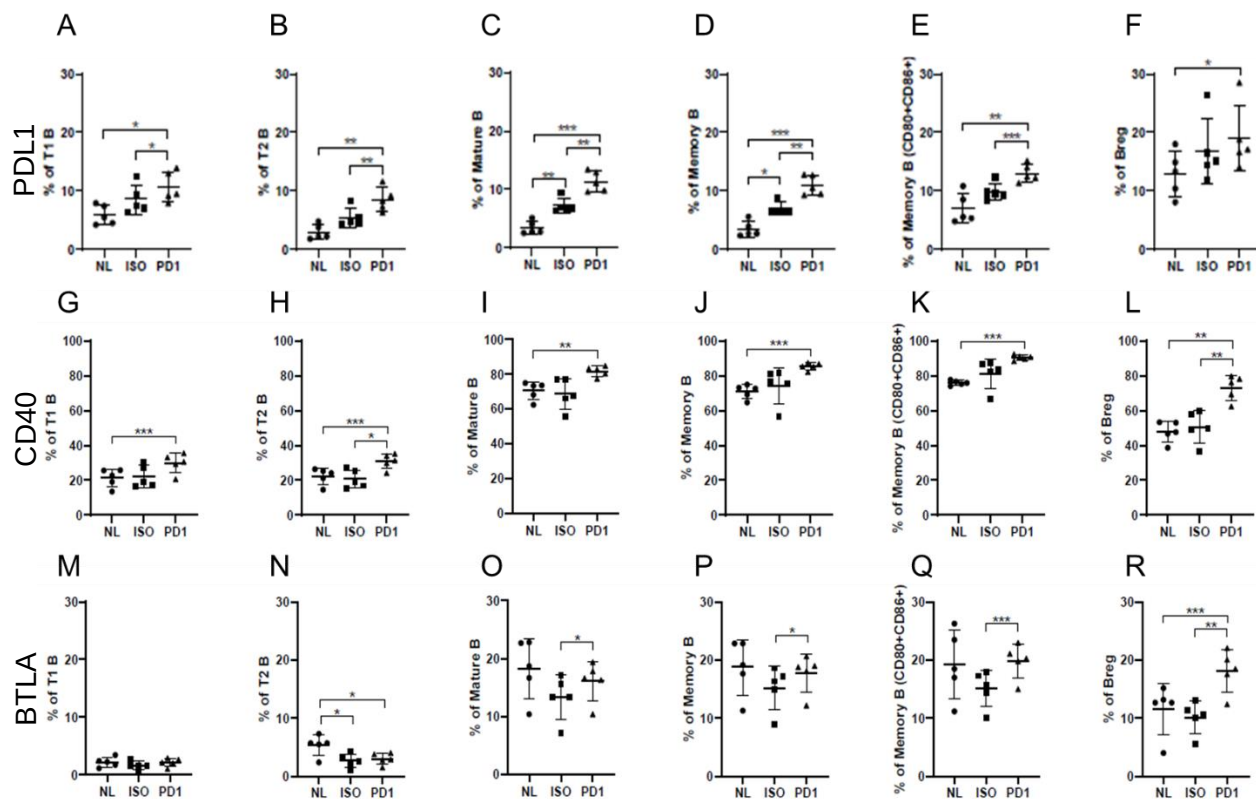
CTLA4 expression analysis by FlowSOM clustering followed by comparison of median expression within each T cell type. (A) Representative UMAP visualization of all T cell clusters in aggregate data of all samples as a reference for (B) PD1 expression heatmap superimposed comparing the lymph nodes from three groups, non-tumor bearing normal mice ("NL"), tumor bearing isotype-treated mice ("ISO"), and tumor bearing anti-PD1 treated mice ("PD1"). (C) Scatter plots of the median expression with bars representing means $\pm$ SD (n=5). P value $\ast \leq 0.05$, $\ast\ast \leq 0.01$, $\ast\ast\ast \leq 0.005$.

Supplementary Figure 18



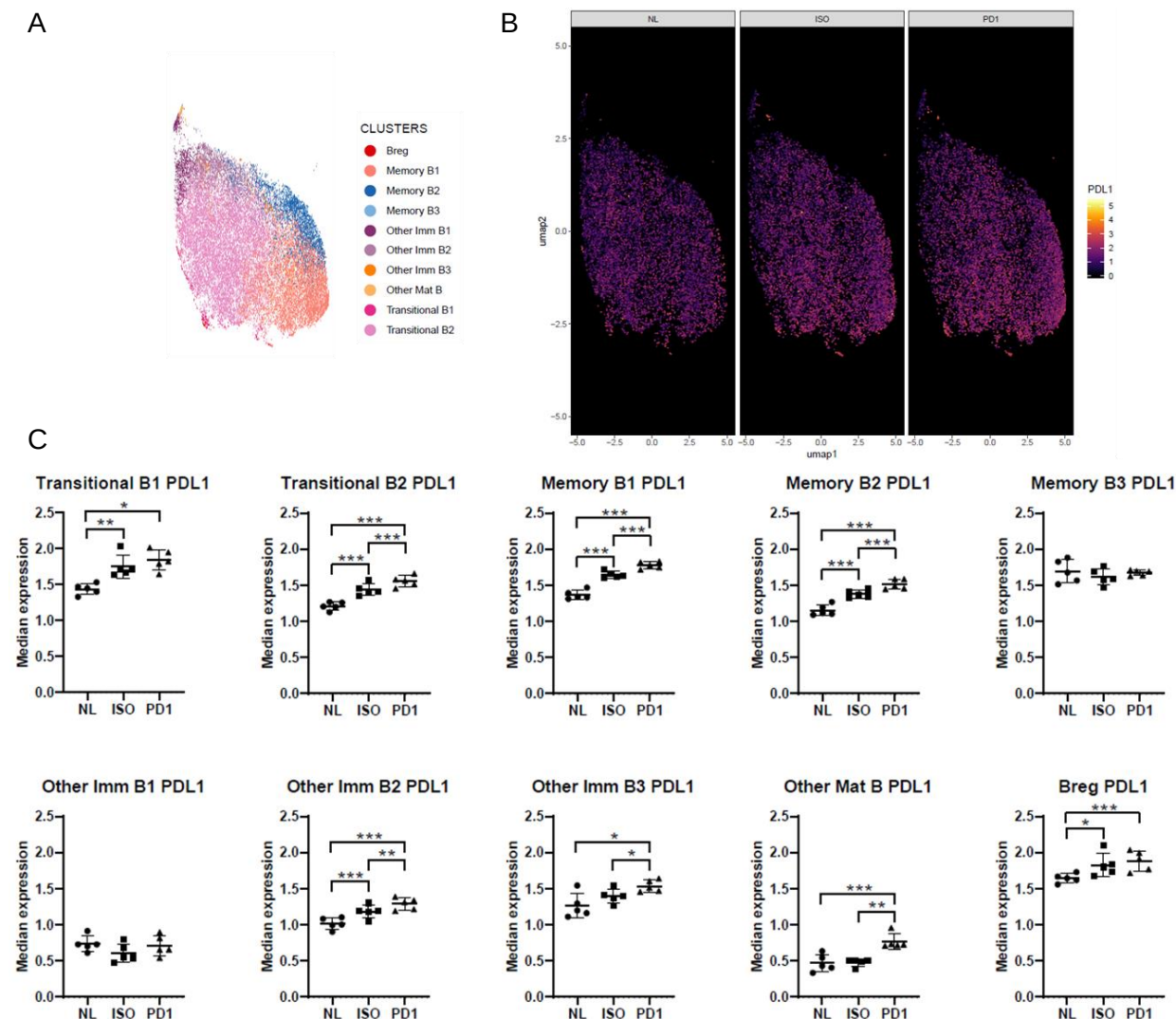
PDL1 expression analysis by FlowSOM clustering followed by comparison of median expression within each T cell type. (A) Representative UMAP visualization of all T cell clusters in aggregate data of all samples as a reference for (B) PDL1 expression heatmap superimposed comparing the lymph nodes from three groups, non-tumor bearing normal mice ("NL"), tumor bearing isotype-treated mice ("ISO"), and tumor bearing anti-PD1 treated mice ("PD1"). (C) Scatter plots of the median expression with bars representing means $\pm$ SD (n=5). P value $\ast \leq 0.05$, $\ast\ast \leq 0.01$, $\ast\ast\ast \leq 0.005$.

Supplementary Figure 19



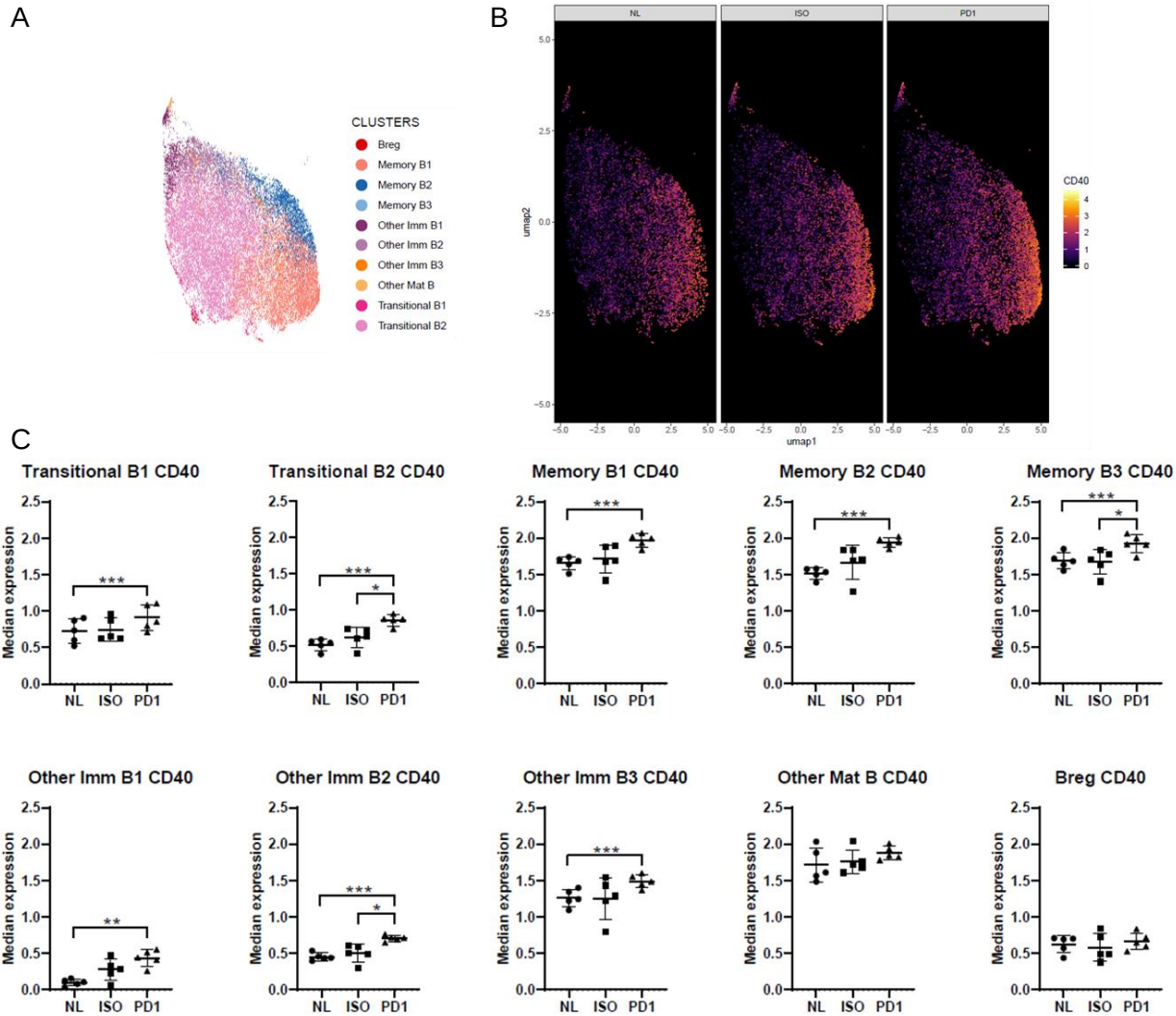
Percent of immune modulatory marker expression within each B cell type analyzed by supervised gating of cells positive for (A-F) PDL1, (G-L) CD40, and (M-R) BTLA. Bars within the scatter plots represent means $\pm$ SD (n=5). P value ≤ 0.05 , ≤ 0.01 , ≤ 0.005 .

Supplementary Figure 20



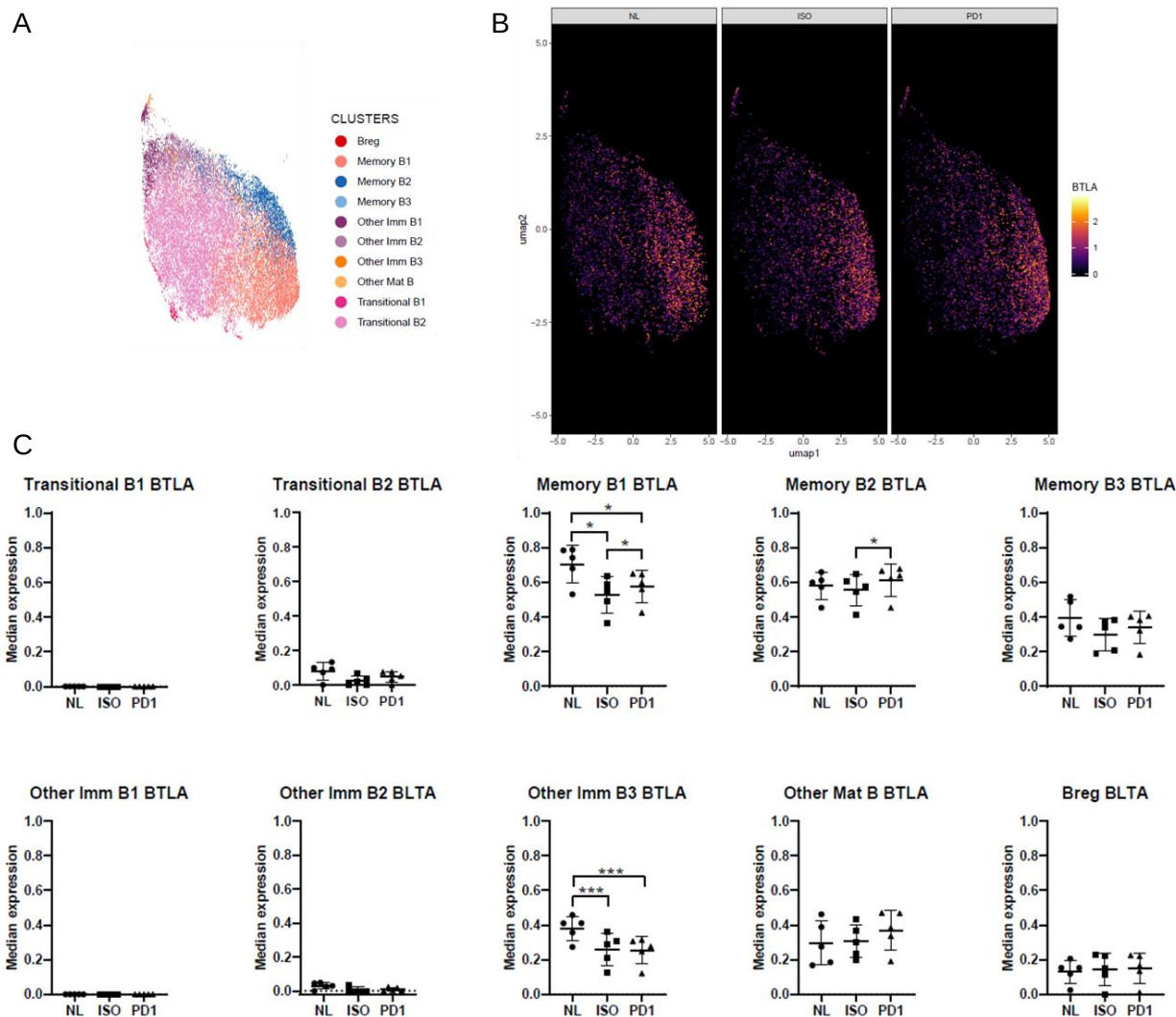
PDL1 expression analysis by FlowSOM clustering followed by comparison of median expression within each B cell type. (A) Representative UMAP visualization of all B cell clusters in aggregate data of all samples as a reference for (B) PDL1 expression heatmap superimposed comparing the lymph nodes from three groups, non-tumor bearing normal mice ("NL"), tumor bearing isotype-treated mice ("ISO"), and tumor bearing anti-PD1 treated mice ("PD1"). (C) Scatter plots of the median expression with bars representing means $\pm$ SD (n=5). P value $\ast \leq 0.05$, $\ast\ast \leq 0.01$, $\ast\ast\ast \leq 0.005$.

Supplementary Figure 21



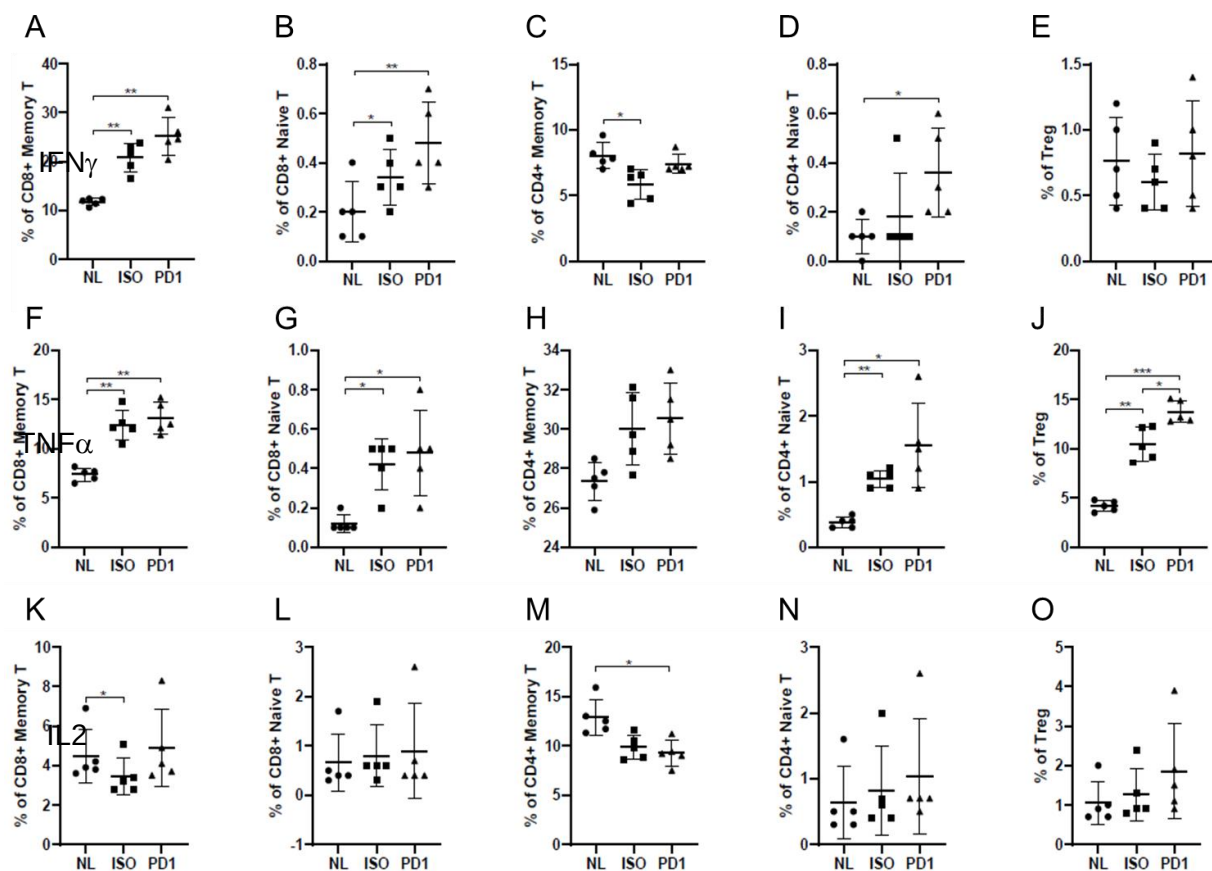
CD40 expression analysis by FlowSOM clustering followed by comparison of median expression within each B cell type. (A) Representative UMAP visualization of all B cell clusters in aggregate data of all samples as a reference for (B) CD40 expression heatmap superimposed comparing the lymph nodes from three groups, non-tumor bearing normal mice ("NL"), tumor bearing isotype-treated mice ("ISO"), and tumor bearing anti-PD1 treated mice ("PD1"). (C) Scatter plots of the median expression with bars representing means $\pm$ SD (n=5). P value $\ast \leq 0.05$, $\ast\ast \leq 0.01$, $\ast\ast\ast \leq 0.005$.

Supplementary Figure 22



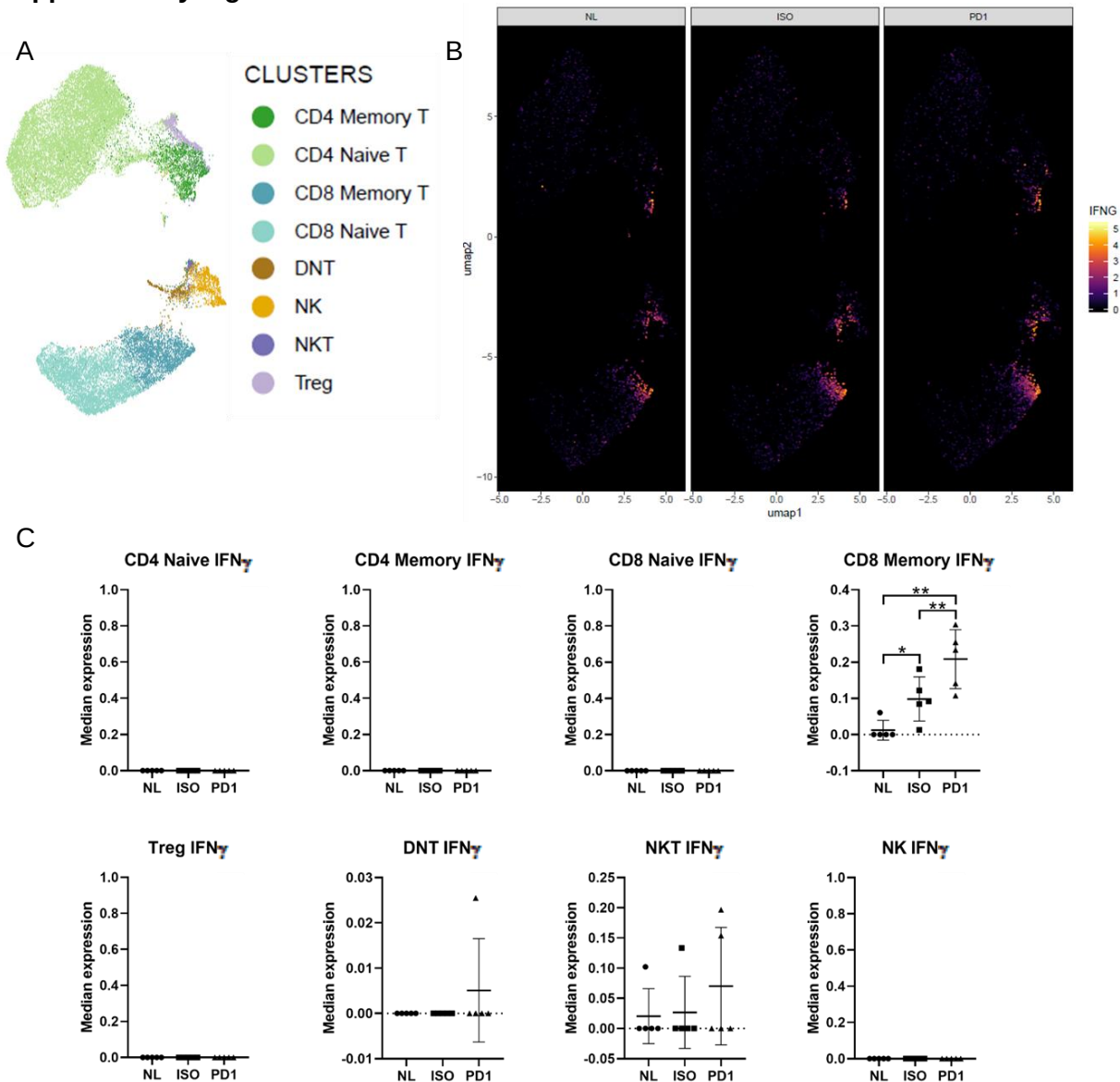
BTLA expression analysis by FlowSOM clustering followed by comparison of median expression within each B cell type. (A) Representative UMAP visualization of all B cell clusters in aggregate data of all samples as a reference for (B) BTLA expression heatmap superimposed comparing the lymph nodes from three groups, non-tumor bearing normal mice ("NL"), tumor bearing isotype-treated mice ("ISO"), and tumor bearing anti-PD1 treated mice ("PD1"). (C) Scatter plots of the median expression with bars representing means $\pm$ SD (n=5). P value $\ast \leq 0.05$, $\ast\ast \leq 0.01$, $\ast\ast\ast \leq 0.005$.

Supplementary Figure 23



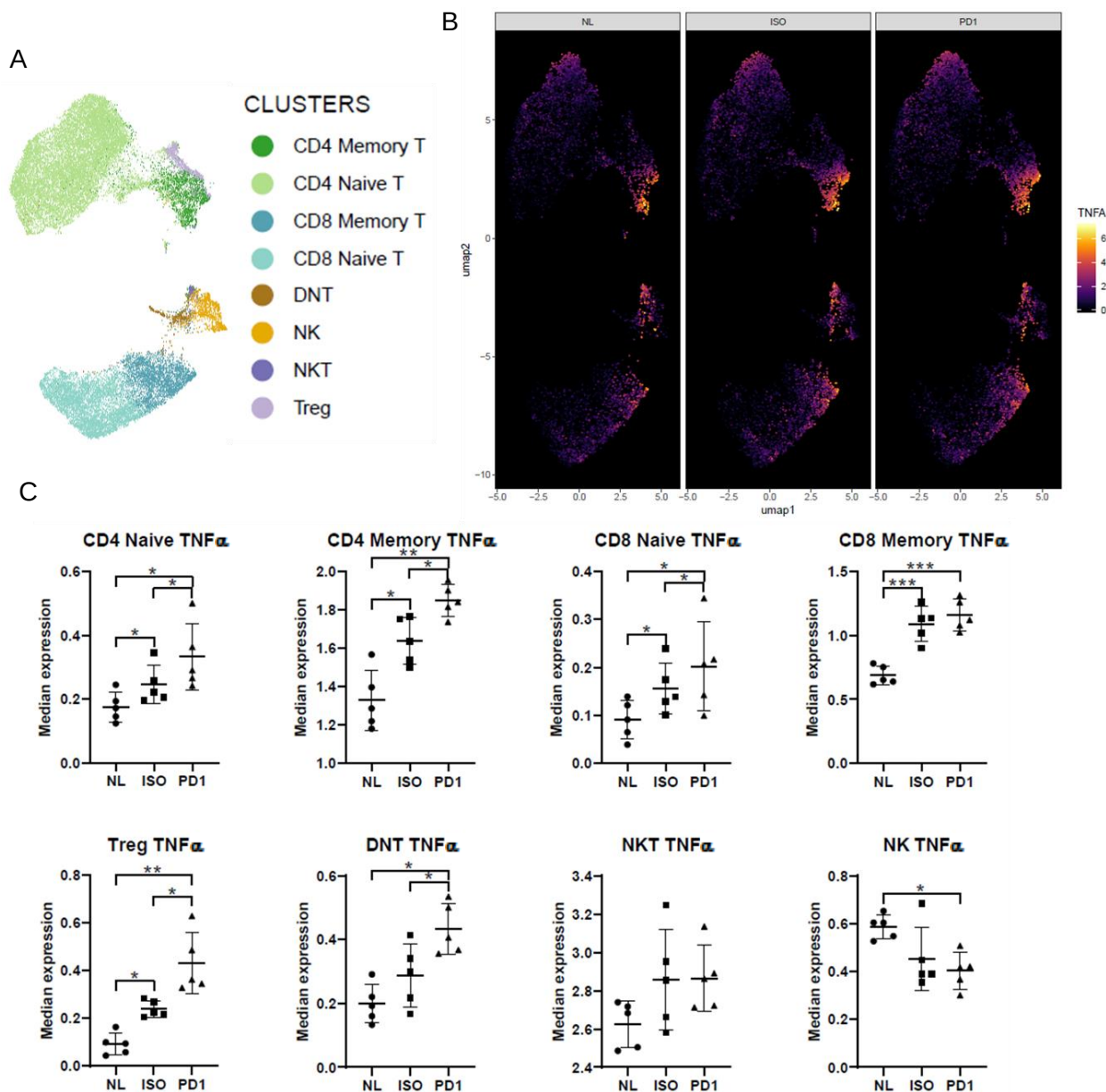
Percent of cytokine expression within each T cell type analyzed by supervised gating of cells positive for (A-E) IFN γ , (F-J) TNF α , and (K-O) IL2. Bars within the scatter plots represent means $\pm$ SD (n=5). P value * $\leq$ 0.05, ** $\leq$ 0.01, *** $\leq$ 0.005.

Supplementary Figure 24



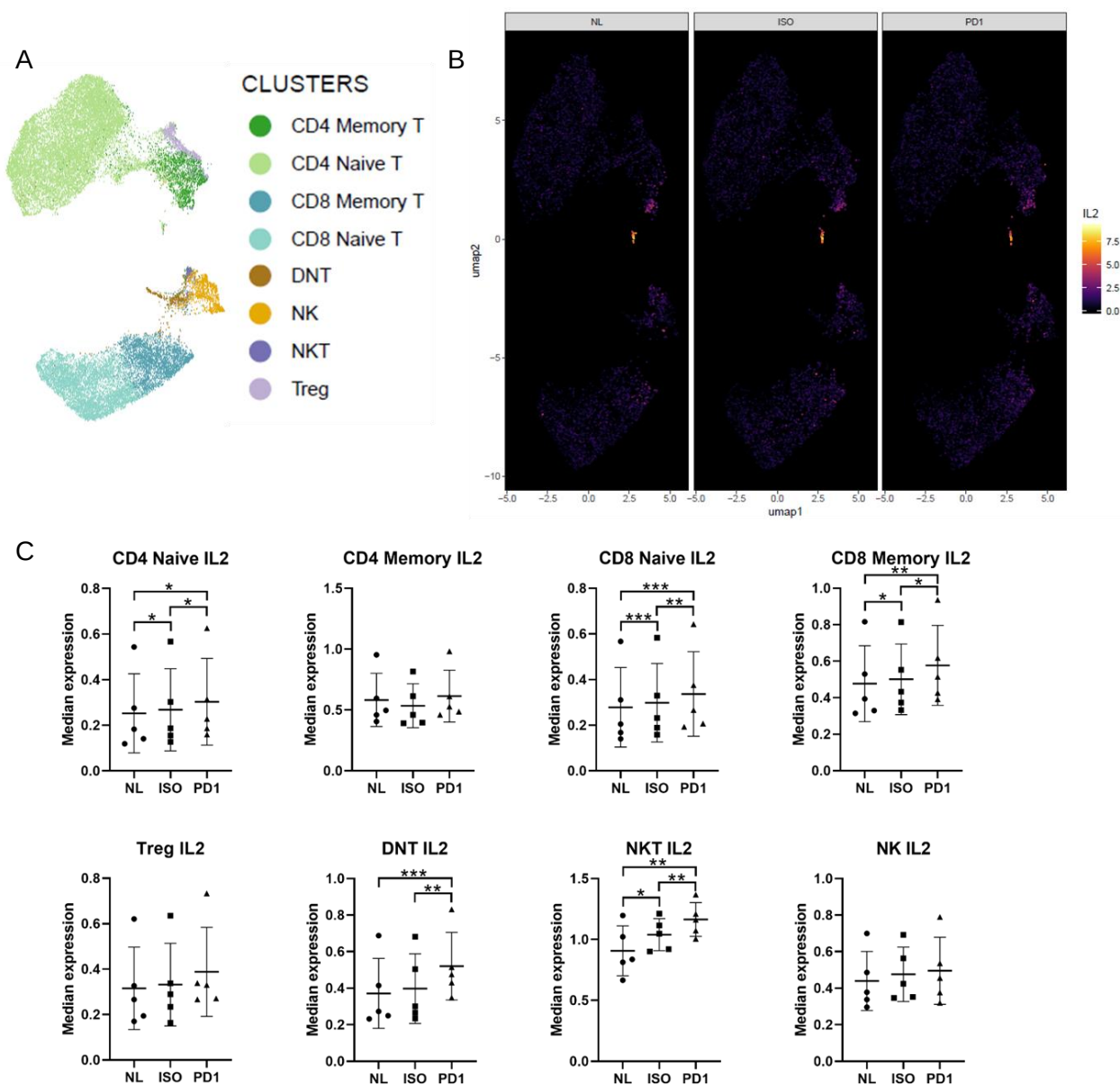
IFN γ expression analysis by FlowSOM clustering followed by comparison of median expression within each T cell type. (A) Representative UMAP visualization of all T cell clusters as a in aggregate data of all samples reference for (B) IFN γ expression heatmap superimposed comparing the lymph nodes from three groups, non-tumor bearing normal mice ("NL"), tumor bearing isotype-treated mice ("ISO"), and tumor bearing anti-PD1 treated mice ("PD1"). (C) Scatter plots of the median expression with bars representing means $\pm$ SD (n=5). P value * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.005 .

Supplementary Figure 25



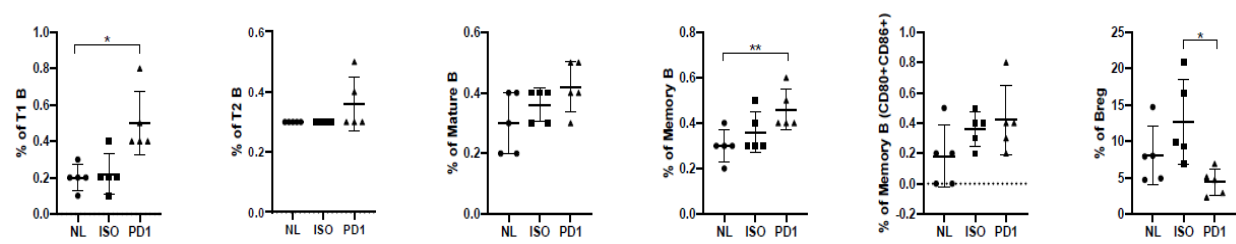
TNF α expression analysis by FlowSOM clustering followed by comparison of median expression within each T cell type. (A) Representative UMAP visualization of all T cell clusters in aggregate data of all samples as a reference for (B) TNF α expression heatmap superimposed comparing the lymph nodes from three groups, non-tumor bearing normal mice ("NL"), tumor bearing isotype-treated mice ("ISO"), and tumor bearing anti-PD1 treated mice ("PD1"). (C) Scatter plots of the median expression with bars representing means $\pm$ SD (n=5). P value * $\leq$ 0.05, ** $\leq$ 0.01, *** $\leq$ 0.005.

Supplementary Figure 26



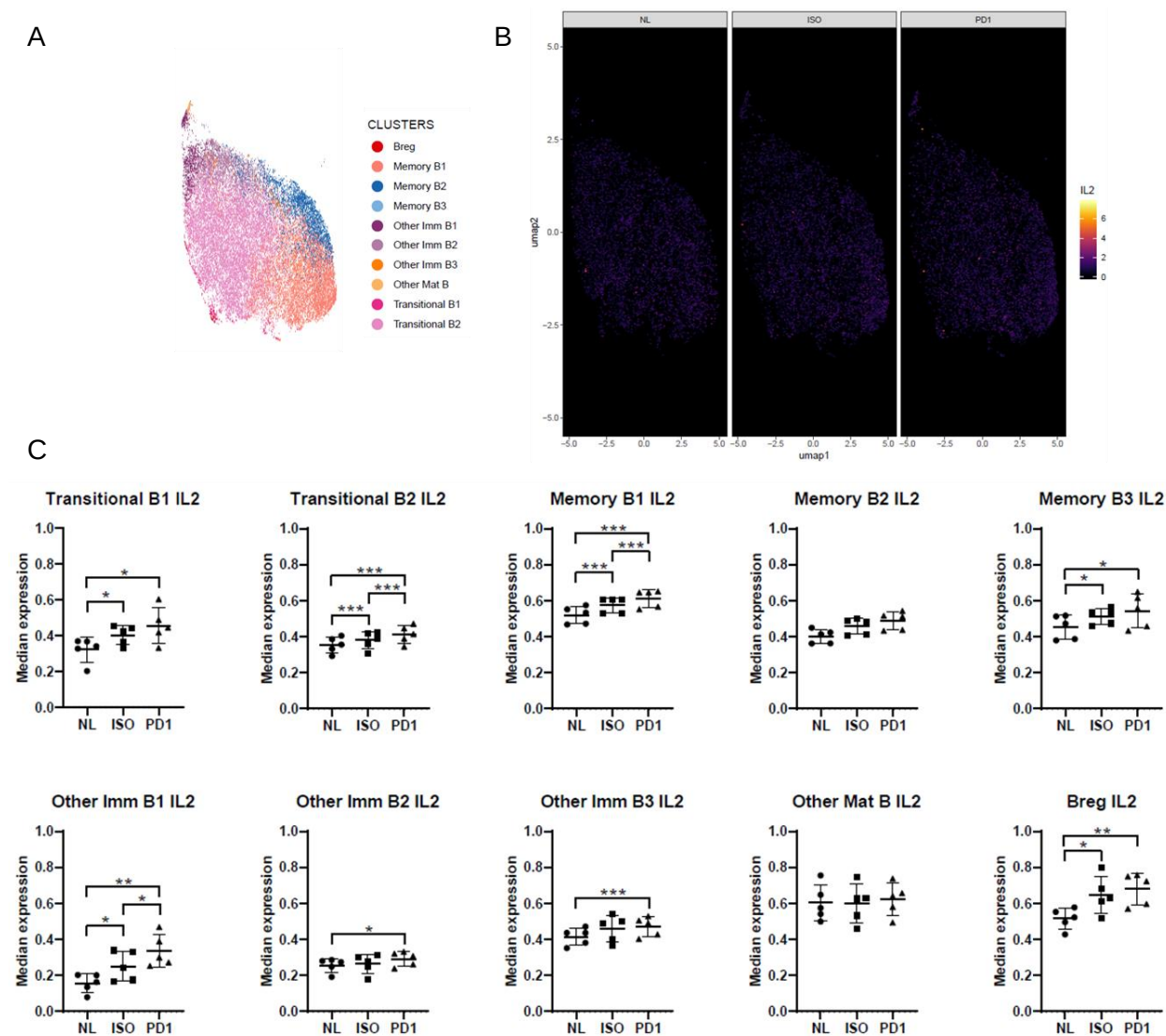
IL2 expression analysis by FlowSOM clustering followed by comparison of median expression within each T cell type. (A) Representative UMAP visualization of all T cell clusters in aggregate data of all samples as a reference for (B) IL2 expression heatmap superimposed comparing the lymph nodes from three groups, non-tumor bearing normal mice ("NL"), tumor bearing isotype-treated mice ("ISO"), and tumor bearing anti-PD1 treated mice ("PD1"). (C) Scatter plots of the median expression with bars representing means $\pm$ SD (n=5). P value $\ast \leq 0.05$, $\ast\ast \leq 0.01$, $\ast\ast\ast \leq 0.005$.

Supplementary Figure 27



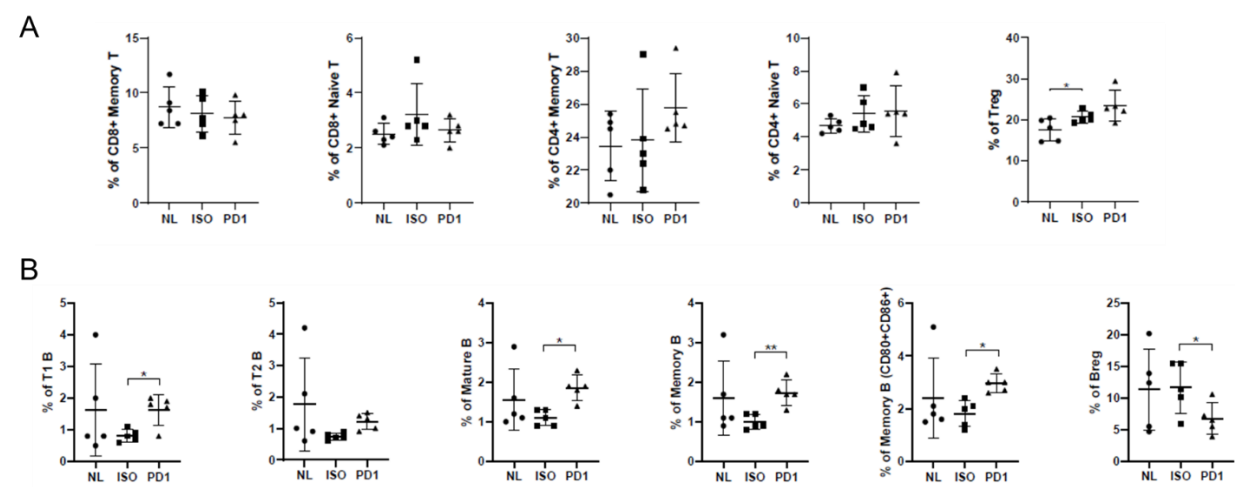
Percent of IL2 expression within each B cell type analyzed by supervised gating of IL2 positive cells. Bars within the scatter plots represent means $\pm$ SD (n=5). P value * $\leq$ 0.05, ** $\leq$ 0.01, *** $\leq$ 0.005.

Supplementary Figure 28



IL2 expression analysis by FlowSOM clustering followed by comparison of median expression within each B cell type. (A) Representative UMAP visualization of all B cell clusters in aggregate data of all samples as a reference for (B) IL2 expression heatmap superimposed comparing the lymph nodes from three groups, non-tumor bearing normal mice ("NL"), tumor bearing isotype-treated mice ("ISO"), and tumor bearing anti-PD1 treated mice ("PD1"). (C) Scatter plots of the median expression with bars representing means $\pm$ SD (n=5). P value $\ast \leq 0.05$, $\ast\ast \leq 0.01$, $\ast\ast\ast \leq 0.005$.

Supplementary Figure 29



Percent of CD69 expression within each (A) T cell type and (B) B cell type analyzed by supervised gating of CD69 positive cells. Bars within the scatter plots represent means $\pm$ SD (n=5). P value * $\leq$ 0.05, ** $\leq$ 0.01, *** $\leq$ 0.005.

Supplementary Table 1. T Cell Focused Mass Cytometry Panel

T Cell Subtyping Panel							
Metal	Isotope	Antigen	Clone	Source	Titration	Surface	Intracellular
In	113	CD45	30-F11	Biolegend (custom)	1:100	x	
In	115	CD45	30-F11	Biolegend (custom)	1:100	x	
Nd	144	IL2	JES6-5H4	Fluidigm	1:100		x
Nd	145	CD69	H1.2F3	Fluidigm	1:100	x	
Sm	149	CD19	6D5	Fluidigm	1:100	x	
Eu	151	CD25	3C7	Fluidigm	1:100	x	
Sm	152	CD3e	145-2C11	Fluidigm	1:75	x	
Eu	153	PDL1	10F.9G2	Fluidigm	1:100	x	
Sm	154	CTLA4	UC10-4B9	Fluidigm	1:100 and 1:200	x	x
Tb	159	PD1	29F.1A12	Fluidigm	1:100	x	
Gd	160	CD5	53-7.3	Fluidigm	1:200	x	
Dy	162	TNFA	MP6-XT22	Fluidigm	1:100		x
Ho	165	IFNG	XMG1.2	Fluidigm	1:100		x
Er	168	CD8a	53-6.7	Fluidigm	1:75	x	
Er	170	CD161	PK136	Fluidigm	1:50	x	
Yb	171	CD44	IM7	Fluidigm	1:100	x	
Yb	172	CD4	RM4-5	Fluidigm	1:100	x	
Yb	174	LAG3	C9B7W	Fluidigm	1:100	x	
Lu	175	CD127	A7R34	Fluidigm	1:100	x	
Pt	194	CD45	30-F11	Biolegend (custom)	1:100	x	
Rh	103	Cell-Id		Fluidigm	1:1000		
Pd	106/8	Viability		Sigma-Aldrich	500nM		

Supplementary Table 2. B Cell Focused Mass Cytometry Panel

B Cell Subtyping Panel							
Metal	Isotope	Antigen	Clone	Source	Titration	Surface	Intracellular
In	113	CD45	30-F11	Biolegend (custom)	1:100	x	
In	115	CD45	30-F11	Biolegend (custom)	1:100	x	
Nd	144	IL2	JES6-5H4	Fluidigm	1:100		x
Nd	145	CD69	H1.2F3	Fluidigm	1:100	x	
Sm	149	CD19	6D5	Fluidigm	1:100	x	
Nd	150	IgD	1126c.2a	Fluidigm	1:100	x	
Eu	151	IgM	RMM1	Fluidigm	1:100	x	
Sm	152	CD3e	145-2C11	Fluidigm	1:75	x	
Eu	153	PDL1	10F.9G2	Fluidigm	1:100	x	
Gd	156	BTLA	6F7	Fluidigm	1:100	x	
Tb	159	CD23	B3B4	Fluidigm	1:100	x	
Gd	160	CD5	53-7.3	Fluidigm	1:200	x	
Dy	161	CD40	HM40-3	Fluidigm	1:50	x	
Dy	162	CD1d	1B1	Fluidigm	1:200	x	
Dy	163	CD86	GL-1	Biolegend (custom)	1:100	x	
Ho	165	IFNG	XMG1.2	Fluidigm	1:100		x
Er	168	CD21	7G6	Fluidigm	1:400	x	
Yb	171	CD38	90	Fluidigm	1:200	x	
Lu	175	CD80	16-10A1	Biolegend (custom)	1:400	x	
Yb	176	B220	30-F11	Fluidigm	1:100	x	
Pt	194	CD45	30-F11	Biolegend (custom)	1:100	x	
Rh	103	Cell-Id		Fluidigm	1:1000		
Pd	106/8	Viability		Sigma-Aldrich	500nM		

Supplementary Table 3.

Subtype	CytoF Panel	Key Marker Combination to Define Subtype
B	T Cell	CD19+CD3-
Breg	B Cell	CD19+CD3- CD1d+CD5+CD38+
CD4 Memory T	T Cell	CD3+CD5+CD4+CD25-CD44+
CD4 Naïve T	T Cell	CD3+CD5+CD4+CD25-CD44-/loCD127-
CD8 Memory T	T Cell	CD3+CD5+CD8+CD44+
CD8 Naïve T	T Cell	CD3+CD5+CD8+CD44-
DNT	T Cell	CD3+CD5+CD4-CD8-
Memory B1	B Cell	CD19+B220hiCD3- CD21hiCD23hi IgMhiIgDhi CD38+CD86-
Memory B2	B Cell	CD19+B220hiCD3- CD21hiCD23hi IgM-IgDhi CD38+CD86-
Memory B3	B Cell	CD19+B220hiCD3- CD21hiCD21hi IgM-IgDhi CD38+CD86+
NK	T Cell	CD161+CD3-CD19-
NKT	T Cell	CD161+CD3+CD19-
Other Imm B1	B Cell	CD19+B220intCD3- CD21-CD23- IgM-IgDint CD38-CD86-
Other Imm B2	B Cell	CD19+B220intCD3- CD21hiCD23- IgM-IgDhi CD38intCD86-
Other Imm B3	B Cell	CD19+B220hiCD3- CD21-CD23- IgM-IgDhi CD38+CD86-
Other Mat B	B Cell	CD19+B220hiCD3- CD21intCD23hi IgM-IgD- CD38-CD86+
T	B Cell	CD19-CD3+
Transitional B1	B Cell	CD19+B220intCD3- CD21-CD23- IgMhiIgDlo CD38intCD86-
Transitional B2	B Cell	CD19+B220intCD3- CD21intCD23int IgMintIgDhi CD38intCD86-
Treg	T Cell	CD3+CD4+CD25+CD44+CD127-
Unassigned	Both	CD19+CD3+CD5+ or CD19-CD3-CD5-CD161-

Supplementary Table 4. Combined (Repeat Run) Mass Cytometry Panel

B Cell Subtyping Panel							
Metal	Isotope	Antigen	Clone	Source	Titration	Surface	Intracellular
Cd	112	CD45	30-F11	Biolegend (custom)	1:100	x	
In	113	CD45	30-F11	Biolegend (custom)	1:100	x	
Cd	114	CD45	30-F11	Biolegend (custom)	1:100	x	
In	115	CD45	30-F11	Biolegend (custom)	1:100	x	
Cd	116	CD45	30-F11	Biolegend (custom)	1:100	x	
Nd	144	CD8a	53-6.7	Biolegend (custom)	1:100	x	
Sm	149	CD19	6D5	Fluidigm	1:100	x	
Nd	150	IgD	1126c.2a	Fluidigm	1:100	x	
Eu	151	IgM	RMM1	Fluidigm	1:100	x	
Sm	152	CD3e	145-2C11	Fluidigm	1:75	x	
Tb	159	CD23	B3B4	Fluidigm	1:100	x	
Gd	160	CD5	53-7.3	Fluidigm	1:200	x	
Dy	161	CD40	HM40-3	Fluidigm	1:50	x	
Dy	162	CD1d	1B1	Fluidigm	1:200	x	
Dy	163	CD86	GL-1	Biolegend (custom)	1:400	x	
Dy	164	CD62L	MEL-14	Fluidigm	1:100	x	
Ho	165	Foxp3	FJK-16s	Fluidigm	1:100		x
Er	168	CD21	7G6	Fluidigm	1:400	x	
Er	170	CD161	NK1.1	Fluidigm	1:50	x	
Yb	171	CD44	IM7	Fluidigm	1:100	x	
Yb	172	CD4	RM4-5	Fluidigm	1:200	X	
Lu	175	CD80	16-10A1	Biolegend (custom)	1:400	x	
Yb	176	B220	30-F11	Fluidigm	1:100	x	
Pt	194	CD45	30-F11	Biolegend (custom)	1:100	x	
Rh	103	Cell-Id		Fluidigm	1:1000		
Pd	106/8	Viability		Sigma-Aldrich	500nM		

Supplementary Table 5.

Subtype	CyTOF Panel	Key Marker Combination to Define Subtype
Transitional B	Combined	CD19+B220loCD3-CD21loCD23loCD40lo
Mature B	Combined	CD19+B220hiCD3-CD21hiCD23hiCD40hi
Memory B	Combined	CD19+B220hiCD3-CD21hiCD23intCD40hiCD86+
Breg	Combined	CD19+B220+CD3-CD1d+CD5+
CD4 T Naïve	Combined	CD3+CD5+CD4+CD8-CD44-CD62L+
CD4 T Effector Memory	Combined	CD3+CD5+CD4+CD8-CD44+CD62L-
Treg	Combined	CD3+CD5+CD4+CD8-Foxp3+
CD8 T Naïve	Combined	CD3+CD5+CD4-CD8+CD44-CD62L+/-
CD8 T Central Memory	Combined	CD3+CD5+CD4-CD8+CD44+CD62L+
CD8 T Effector Memory	Combined	CD3+CD5+CD4-CD8+CD44+CD62L-
NK	Combined	CD161+CD3-CD19-
NKT	Combined	CD161+CD3+CD19-
DPT	Combined	CD3+CD5+CD19-CD4+CD8+
DNT	Combined	CD3+CD5+CD19-CD4-CD8-
Unassigned	Combined	CD3-CD5-CD19- or CD3+CD5+CD19+