SUPPLEMENTARY FIGURES & TABLE

Supplementary Figure 1. DOP treatment inhibits S1P lyase activity. A, The S1P enzymatic pathway. B, Chemical structure of DOP. C, SPL activity in ileum of TNFΔARE mice administered DOP or vehicle in drinking water for 2 weeks. D, mRNA expression of SPL in ileum of TNFΔARE mice receiving DOP or vehicle for 2 weeks (Data are expressed as mean ± SEM; n=4 mice/group; **P*<0.05 by two-tailed *t*-test.)

Supplementary Figure 2. Pyridoxine (Vitamin-B6)-deficient diet did not induce peripheral lymphopenia nor affected the severity of ileitis. A, Gating strategy for live LP CD4+T, CD8+T and B cells. B, Percentage of indicated lymphocytes in peripheral blood of TNFΔARE mice treated with standard diet or pyridoxine-deficient diet for 2 weeks. C, Total inflammatory index of TNFΔARE ileum receiving standard or pyridoxine-deficient diet fed mice. (Data are expressed as mean ± SEM*; n*≥10 mice/group).

Supplementary Figure 3. DOP induced peripheral lymphopenia in mice with chronic ileitis and down regulated S1PR1 expression on circulating T and B cells. A, B, Percentage and absolute counts of CD4 and CD8+ (total and subsets) in peripheral blood of S1P-eGFP reporter TNFΔARE mice treated with DOP or vehicle for 2 weeks. C, D, S1PR1 expression (median fluorescent intensity, MFI) of CD4+, CD8+T cells, and B220+ B cells from blood of TNFΔARE mice treated with DOP or vehicle control. (Data are expressed as mean ± SEM; *n*≥7 mice/group; **P*<0.05, ***P*<0.01, ****P*<0.001 by two-tailed *t*-test).

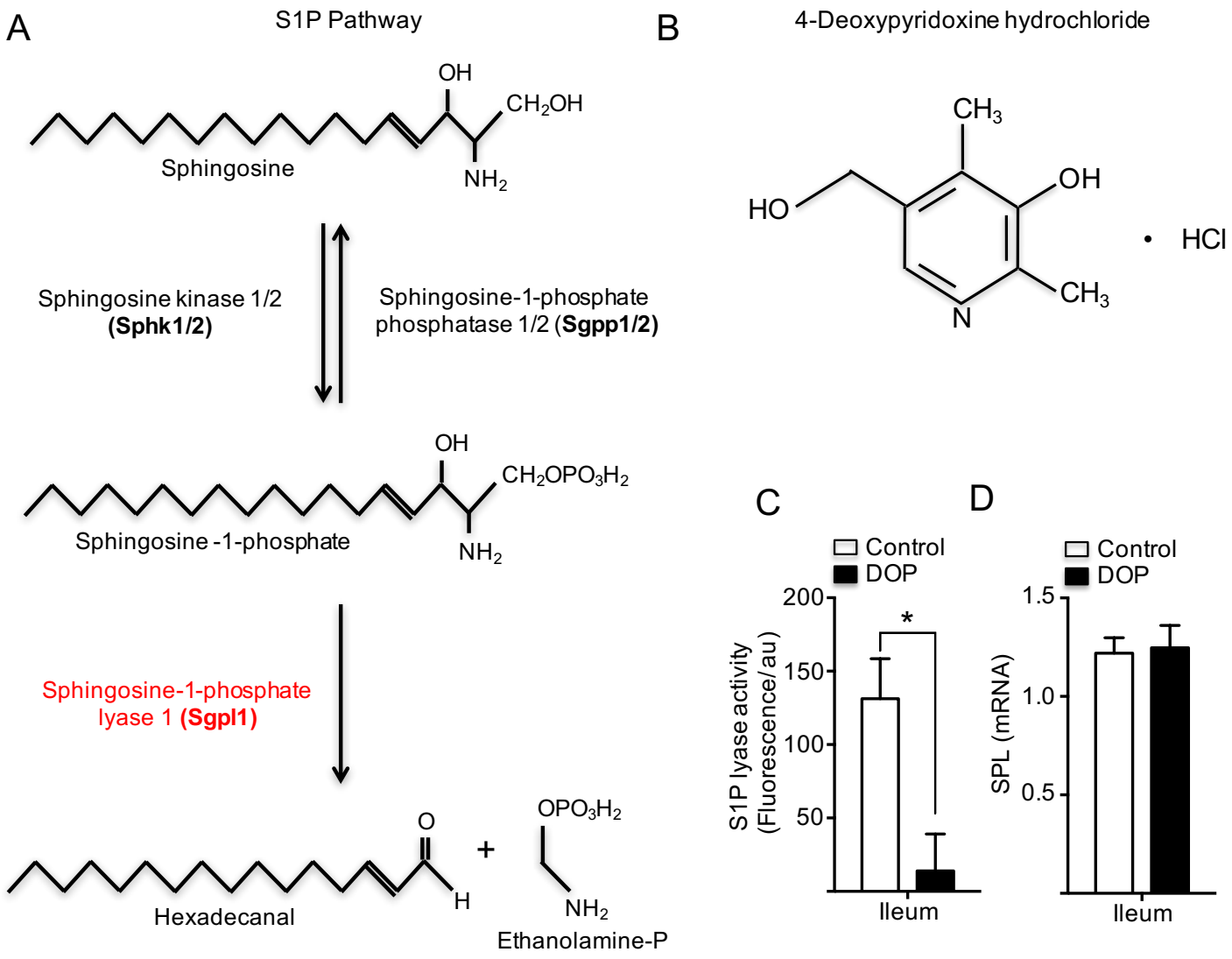
Supplementary Figure 4. DOP treatment for 1 week induced lymphopenia but did not affect the severity of ileitis in TNFΔARE mice. A, Percentages and absolute counts of indicated lymphocyte subsets in peripheral blood of TNFΔARE mice treated with DOP or vehicle control. B, Inflammatory histopathological indices of ileitis severity of TNFΔARE of DOP- and vehicle-treated control mice. C, Representative micrographs. (Data are expressed as mean ± SEM; *n*=5 mice/group, ***P*<0.01 by two-tailed *t*-test).

Supplementary Figure 5. Mass cytometry analyses of the effects of SPL inhibition on leukocyte distribution within distinct immune compartments of mice with chronic ileitis. A-E, Blood, MLN and ileum LP were harvested from control or DOP treated TNFΔARE mice, with cells from each tissue stained with a panel of 34 metal-conjugated antibodies and acquired on a Helios mass cytometer. Data depict PhenoGraph-defined cellular distribution and clustering within peripheral blood (panels A-E), F,J, MLN or K-O, ileum LP. Panels A, F and K show PhenoGraph-defined cellular distribution and clustering of all viable singlets

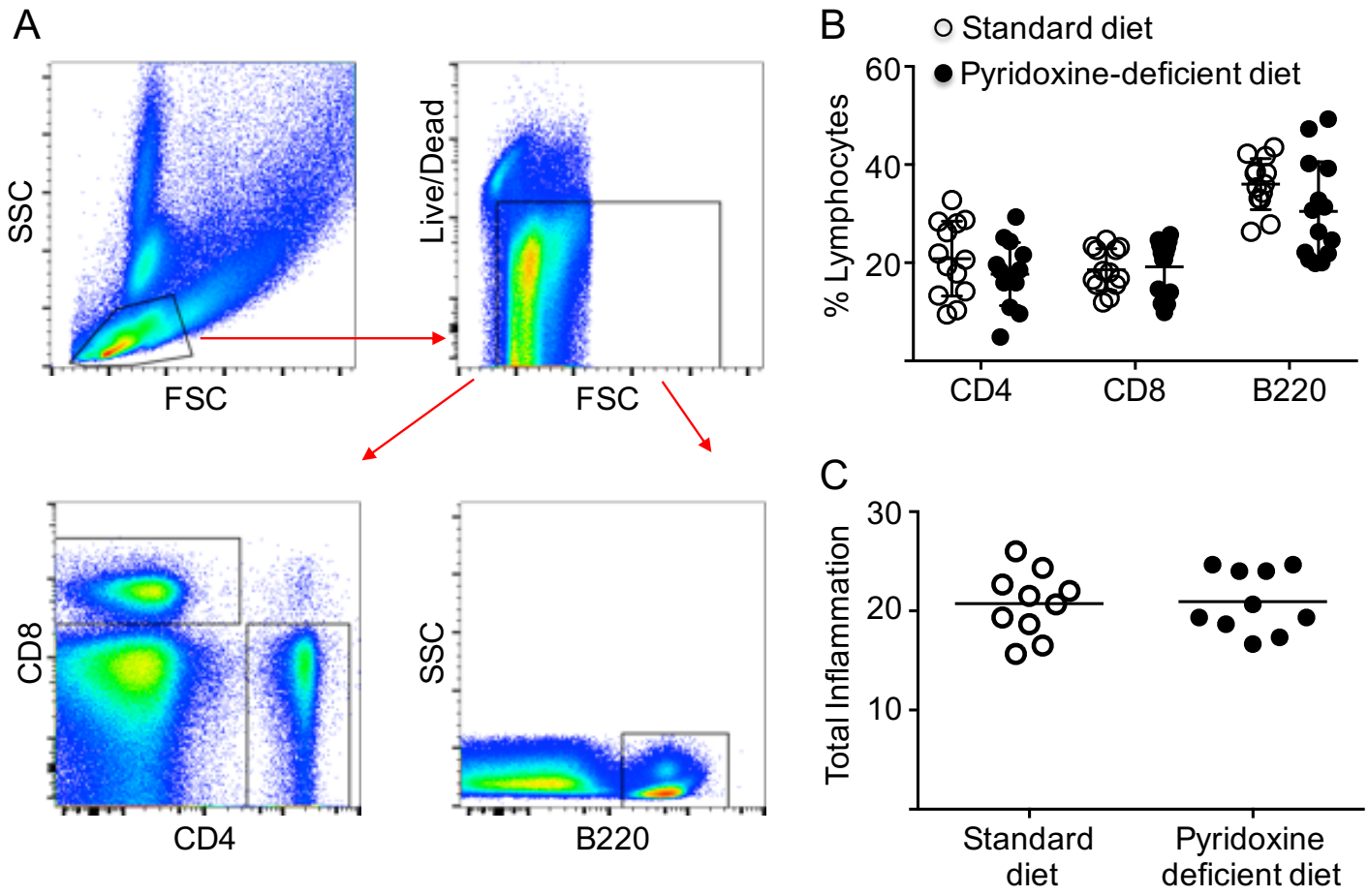
(defined as 191Ir+ 193Ir+ 195Pt-), plotted according to tSNE1 and tSNE2, and colored by phenotype designation as noted. Panels B, G, and L quantify the relative frequencies of CD4+, CD8+, CD11b+, and CD19+ cells among viable singlets. Panels C, H, and M show all viable T cells (defined as 191Ir+ 193Ir+ 195Pt- CD3+ MHCII-) subjected to the PhenoGraph algorithm, plotted according to tSNE1 and tSNE2, with panels according to protein expression for CD4, CD8, CD44, and CD62L. Panels D, I, and N show PhenoGraph-defined cellular phenotypes among T cells, plotted according to tSNE1 and tSNE2 and colored by the designated phenotype. Panels E, J, and O quantify the relative frequencies of T cell subsets. Statistical analysis done using an unpaired t test between control and DOP treated conditions, subjected to multiple testing correction, with statistical significance identified as follows: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. No designation, not significant.

Supplementary Figure 6. Effect of S1P lyase inhibition on thymic size. Representative images of thymi from TNFΔARE mice treated with DOP (30 mg/L) or vehicle at indicated time points.

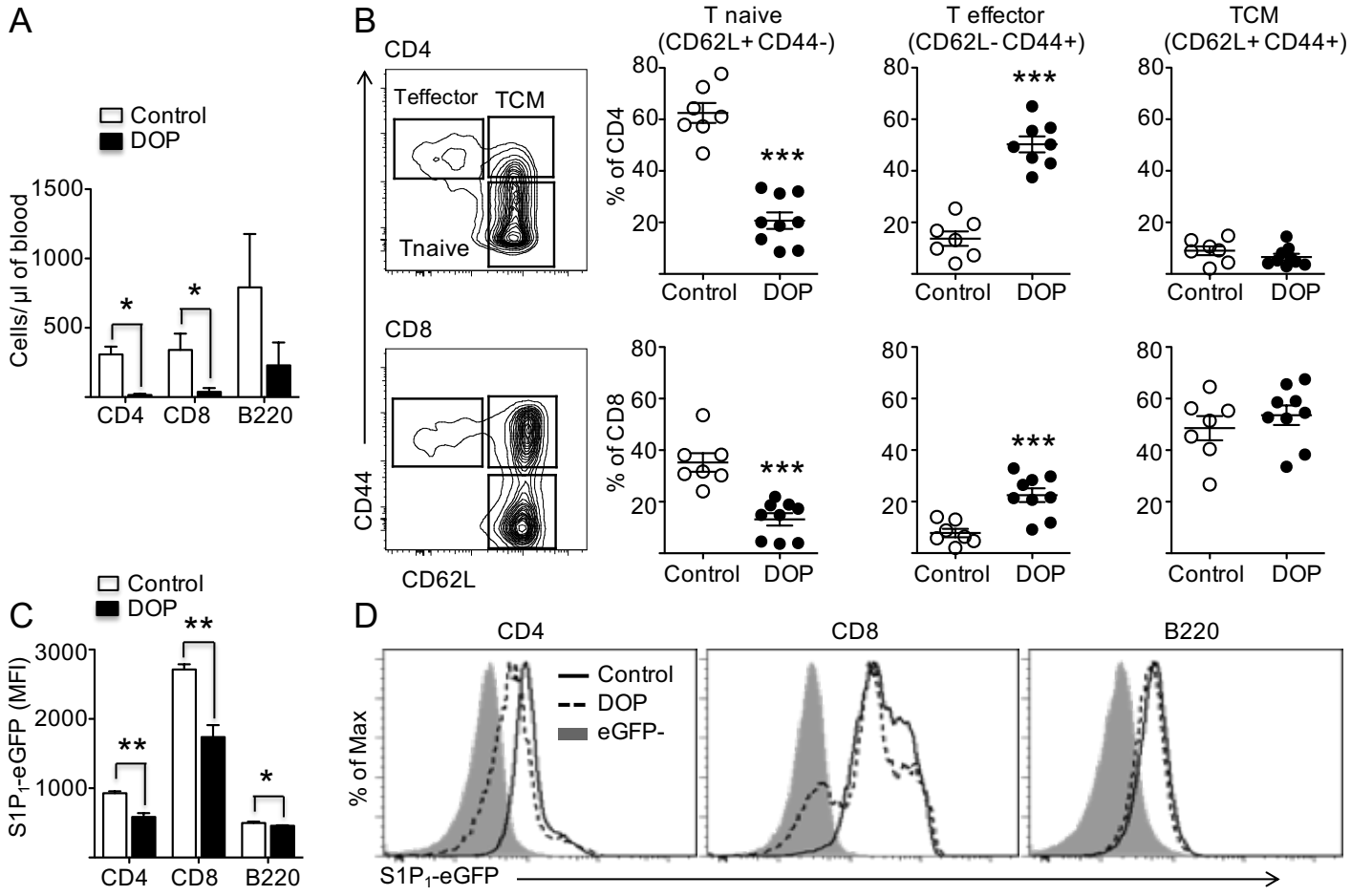
Supplementary Table 1. CyTOF antibody staining panel epitopes, clones, suppliers, and isotope label. All Fluidigm antibodies were purchased pre-conjugated. All other supplier antibodies were purchased in a carrier-free format and conjugated with the respective metal isotope using the MaxPar-X8 Conjugation Kit (Fluidigm).



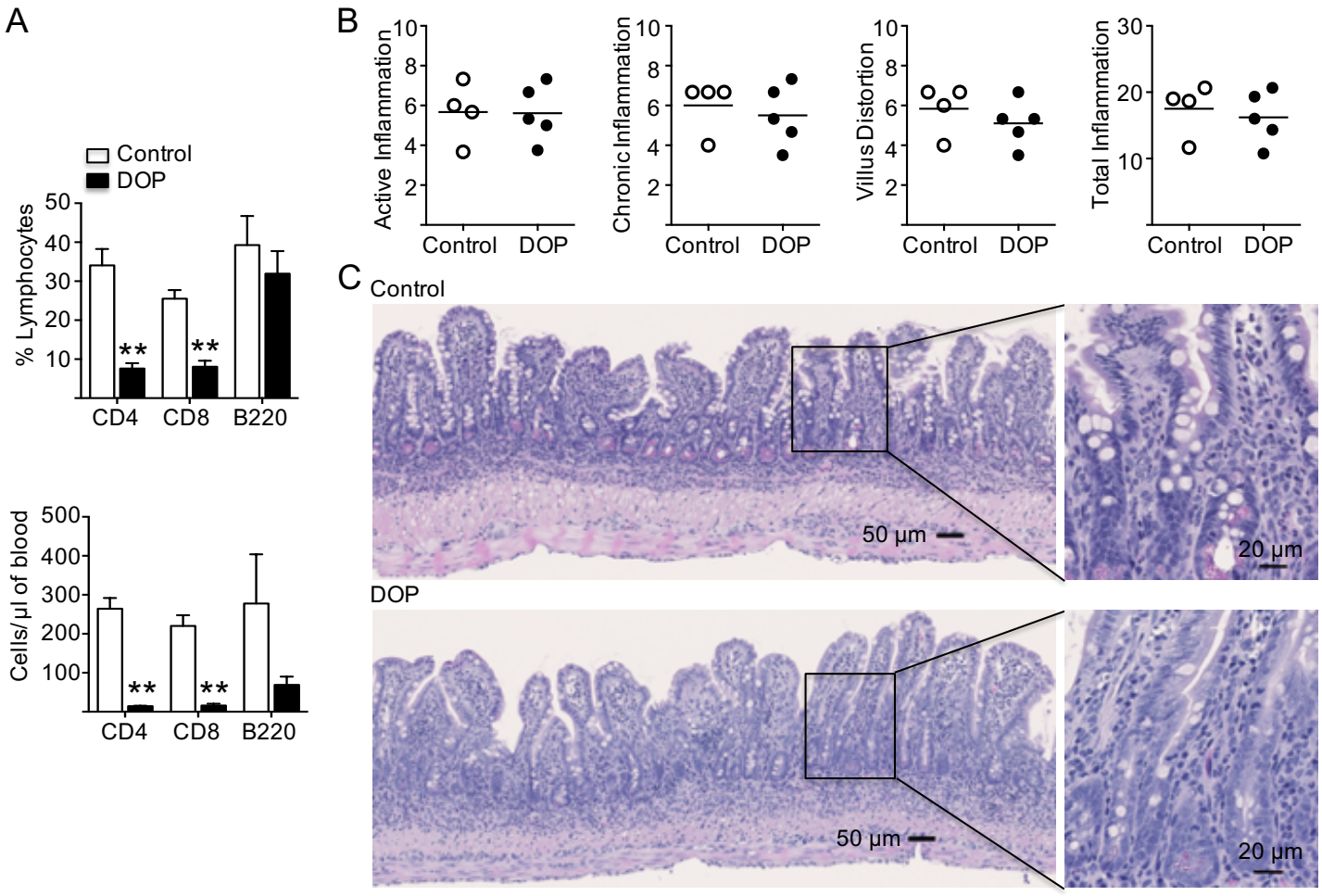
Supplementary Figure 1



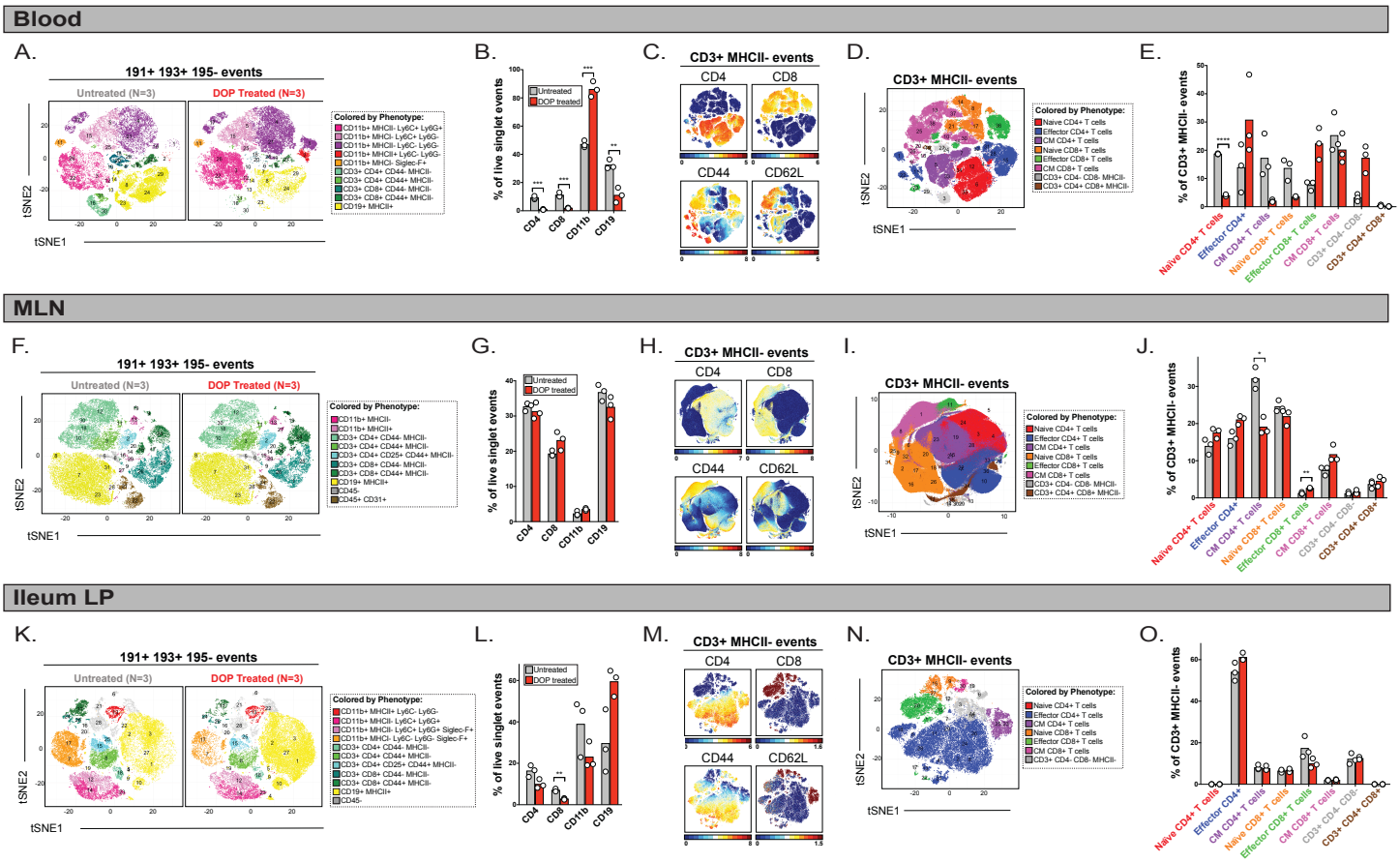
Supplementary Figure 2



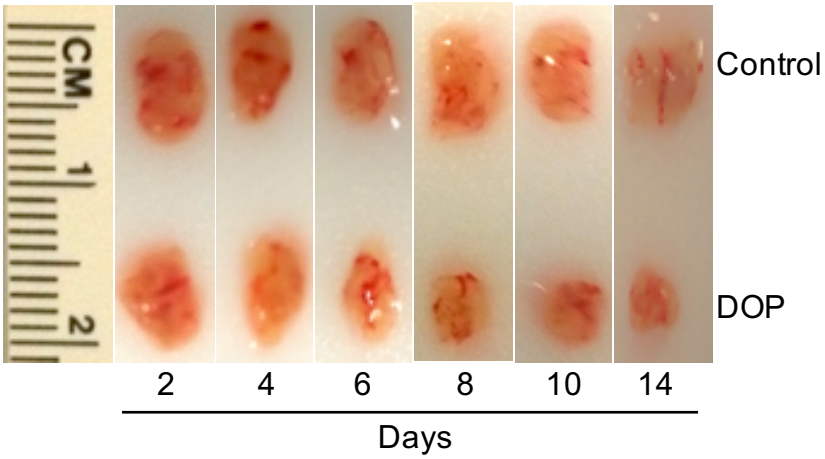
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6

Target epitope	Clone	Supplier	Isotopic label
CD31 (PECAM-1)	390	Fluidigm	165Ho
CD14	Sa14-2	Fluidigm	156Gd
F4/80	BM8	Fluidigm	146Nd
CD11b (Mac-1)	M1/70	Fluidigm	143Nd
DEC205	NLDC-145	BioLegend	173Yb
I-A/I-E (MHC class II)	M5/114.15.2	Fluidigm	174Yb
ROR gamma (t)	B2D	Fluidigm	159Tb
CD45	30-F11	Fluidigm	89Y
CD127 (IL-7Ra)	A7R34	Fluidigm	175Lu
CD161 (NK1.1)	PK136	Fluidigm	170Er
CD335 (NKp46)	29A1.4	Fluidigm	153Eu
CD40	HM40-3	Fluidigm	161Dy
CD86	GL1	Fluidigm	172Yb
CD11c	N418	TONBO	115In
CD19	6D5	Fluidigm	149Sm
CD45R (B220)	RA3-6B2	Fluidigm	176Yb
Tbet	4B10	Fluidigm	160Gd
Foxp3	FJK-16s	Fluidigm	158Gd
CD25 (IL-2R)	3C7	BioLegend	169Tm
CD49d (Integrin alpha 4)	R1-2	Fluidigm	151Eu
CD44	IM7	Fluidigm	171Yb
CD62L (L-selectin)	MEL-14	Fluidigm	164Dy
CD8a	53-6.7	Fluidigm	168Er
CD4	RM4-5	Fluidigm	145Nd
CD3e	145-2C11	Fluidigm	152Sm
CD29	HMβ1-1	BioLegend	166Er
CCR9	9B1	BioLegend	155Gd
CD103	2E7	BioLegend	163Dy
Integrin b7	FIB504	Fluidigm	162Dy
Gata3	TWAJ	Fluidigm	167Er
KLRG-1	2F1	TONBO	154Sm
Siglec F	E50-2440	BD	147Sm
Ly-6G	1A8	Fluidigm	141Pr
Ly-6C	HK1.4	Fluidigm	150Nd

Supplementary Table 1