

Characterization of pulmonary immune responses to hyperoxia
by high-dimensional mass cytometry analyses

Hanidziar D,^{1,5*} Nakahori Y,^{2,5} Cahill LA,² Gallo D,³ Keegan J,² Nguyen JP,²

Otterbein LE,³ Lederer JA,^{2,6} & Robson SC^{4,6}

¹Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts
General Hospital, Boston, MA 02114

²Department of Surgery, Brigham and Women's Hospital, Boston, MA 02115

³Department of Surgery, Beth Israel Deaconess Medical Center, Boston, MA 02115

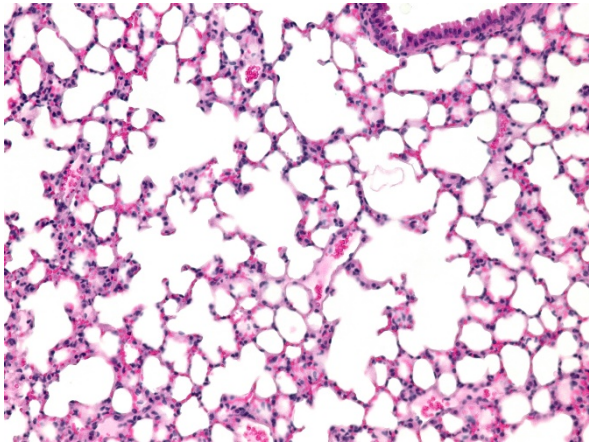
⁴Departments of Anesthesia and Medicine, Beth Israel Deaconess Medical Center,
Boston, MA 02115

⁵these authors contributed equally

⁶senior authors

Figure S1

NORMOXIA



HYPEROXIA

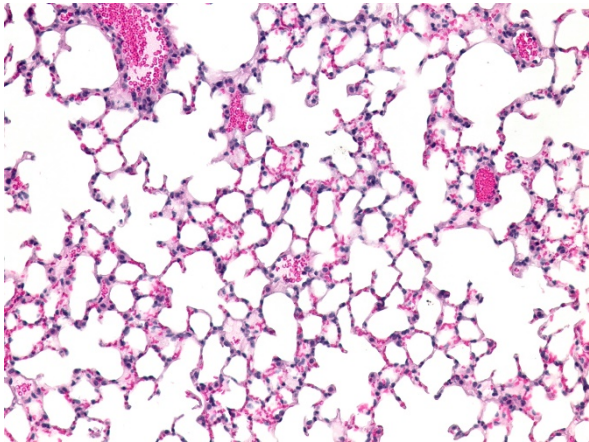


Figure S2

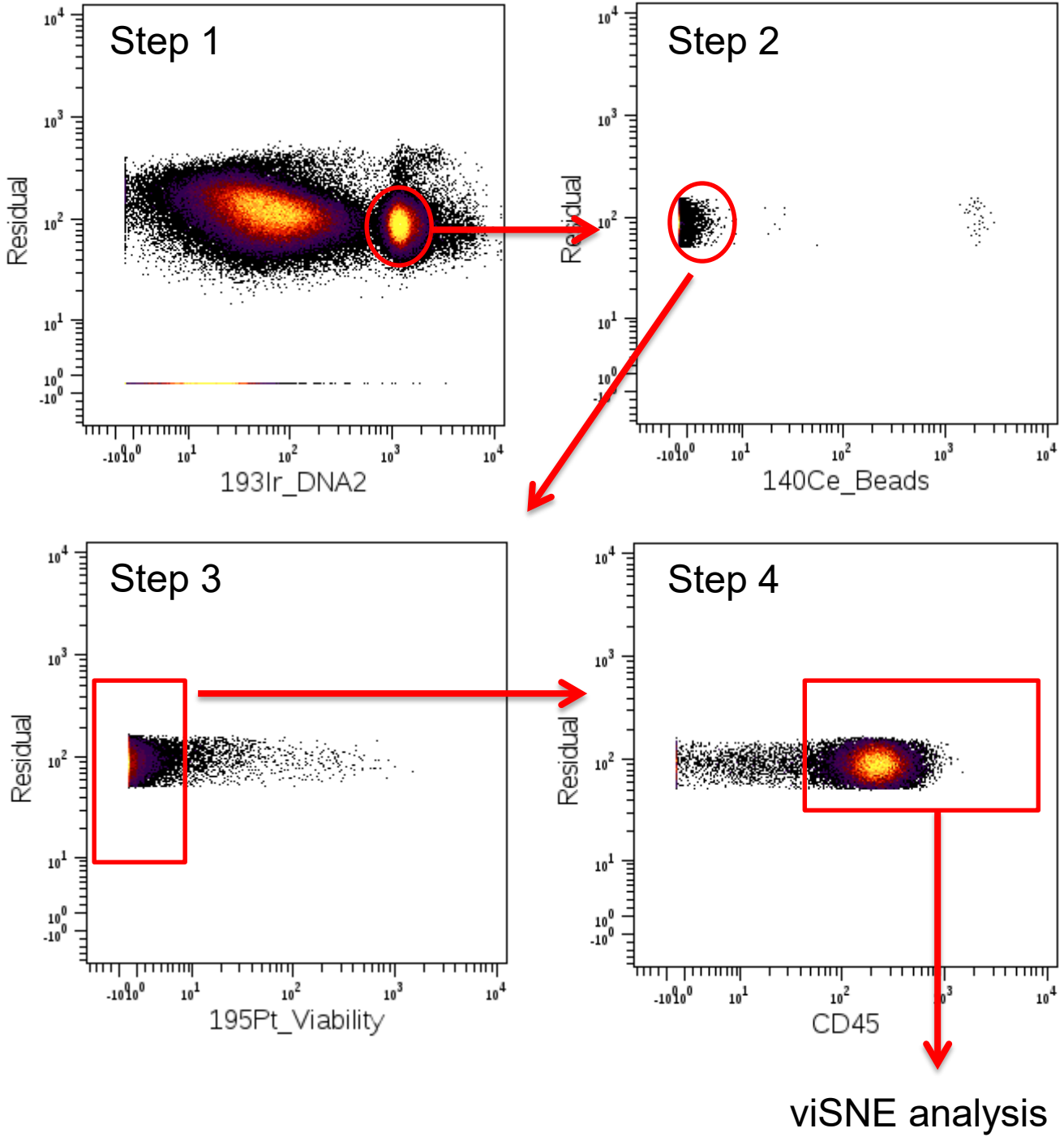
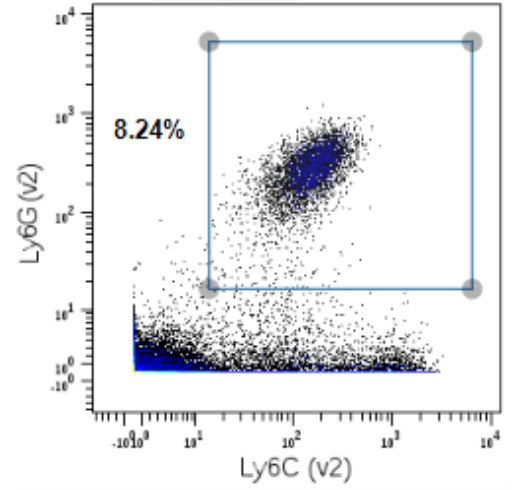
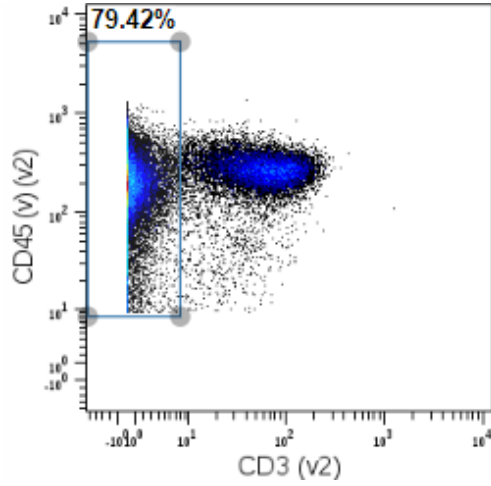


Figure S3

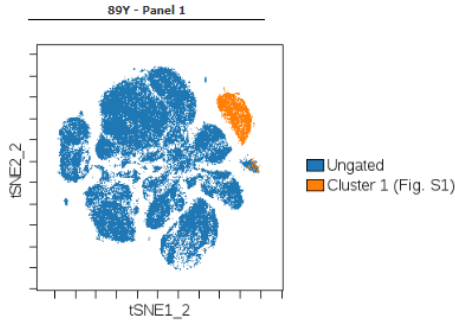
Cluster 1
Neutrophil

Manual gating

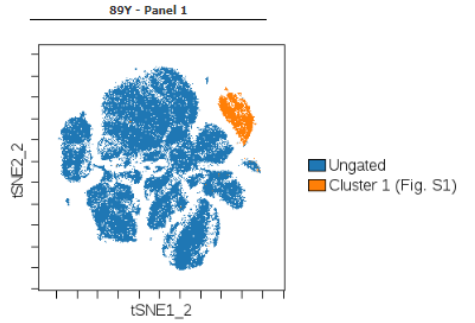


Overlay on viSNE

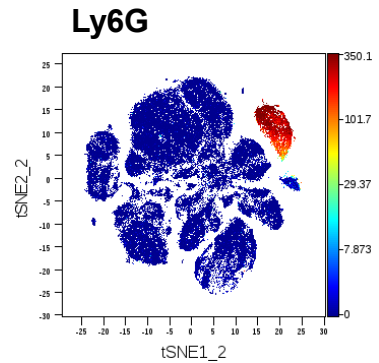
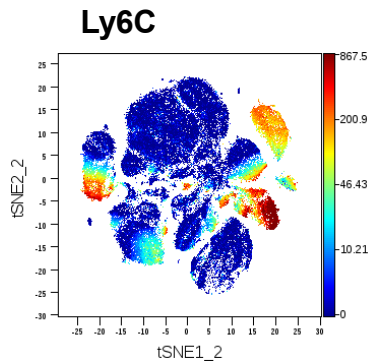
Sham_Ungated_visNE.fcs - Sham_Ungated_visNE.fcs



O2 injury_Ungated_visNE.fcs - O2 injury_Ungated_visNE.fcs

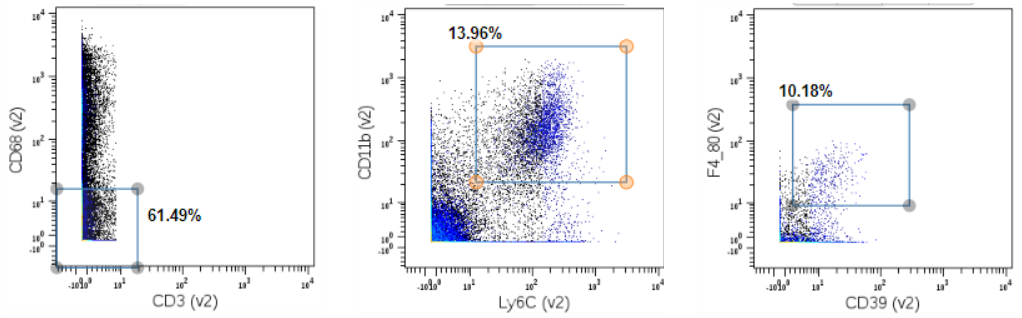


Expression of lineage markers

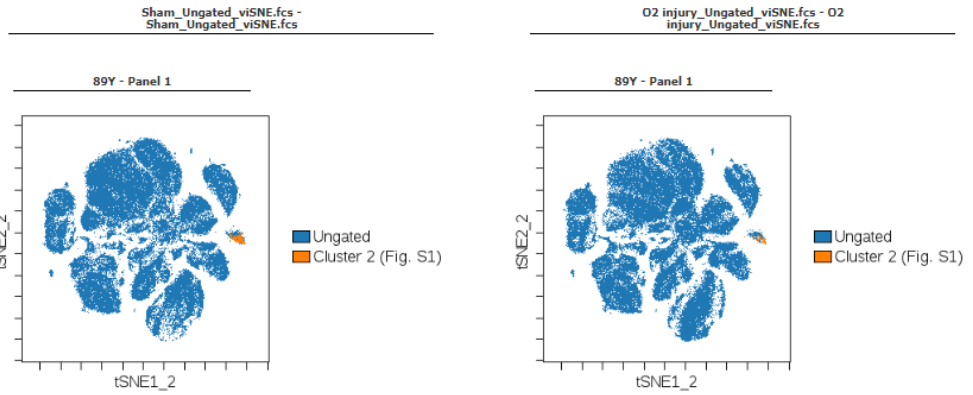


Cluster 2
Myeloid regulatory cell

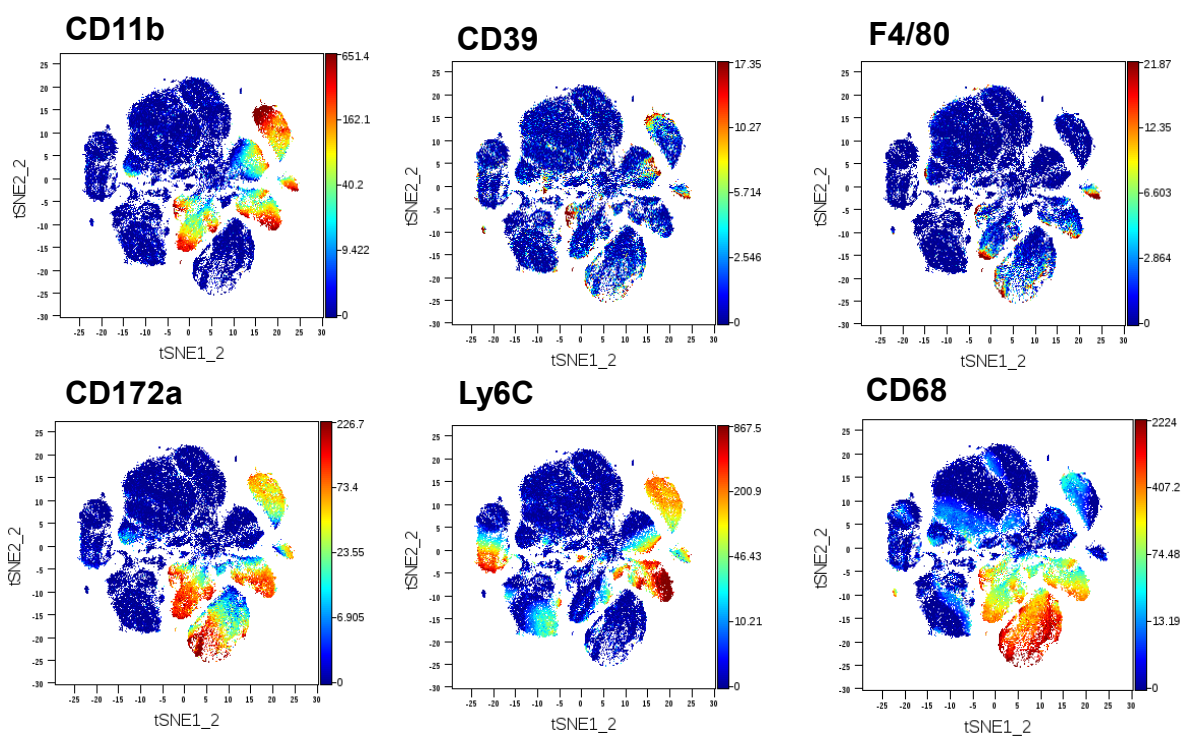
Manual gating



Overlay on viSNE

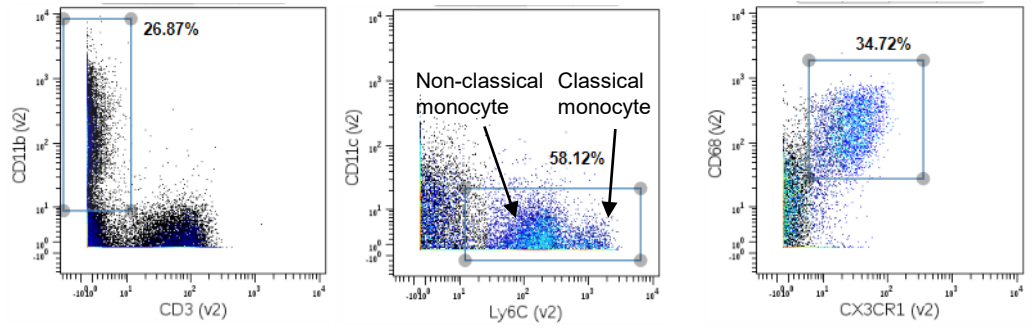


Expression of lineage markers

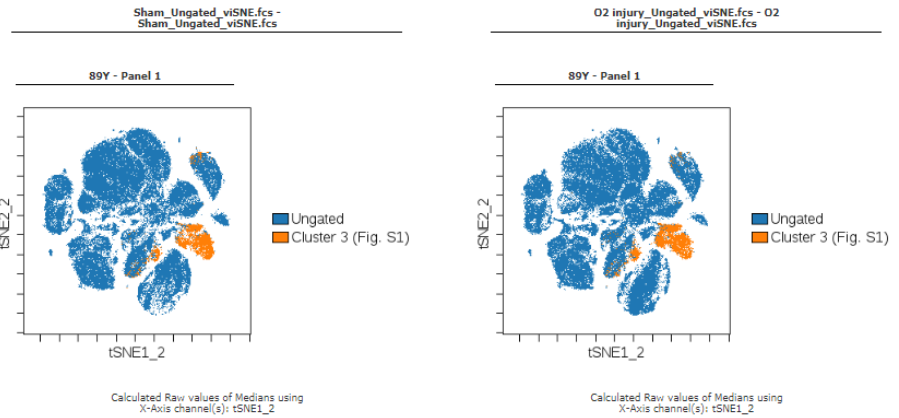


Cluster 3 Monocyte

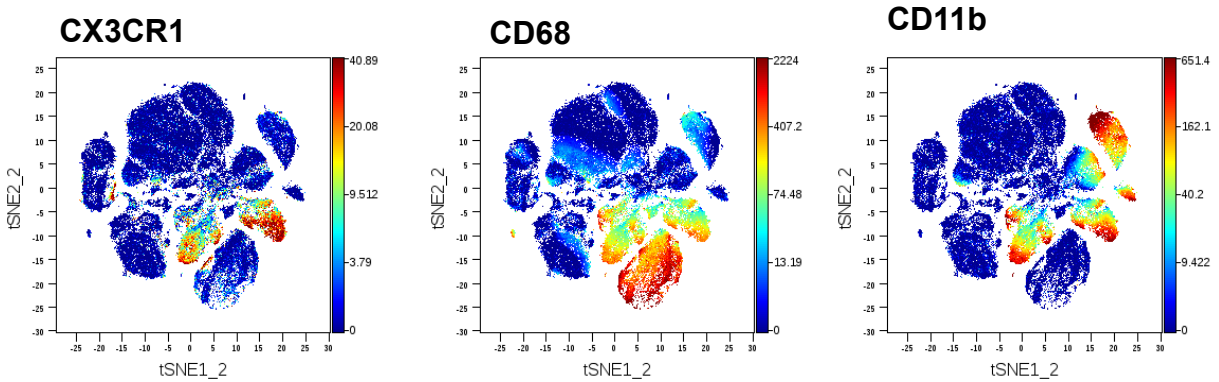
Manual gating



Overlay on viSNE

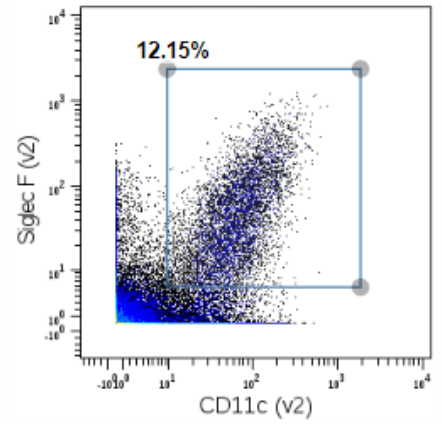
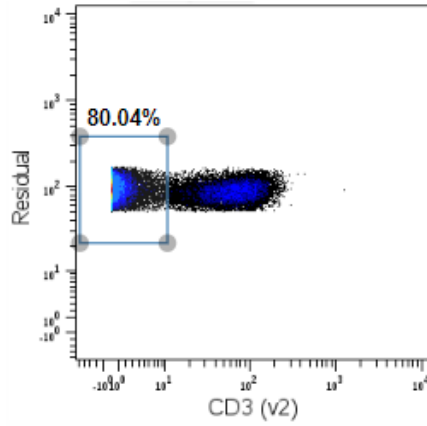


Expression of lineage markers

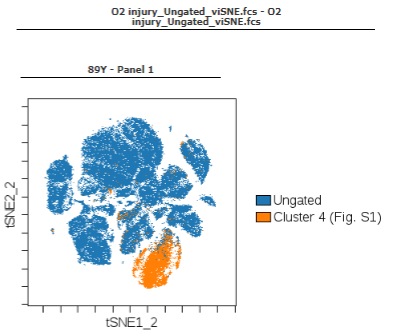
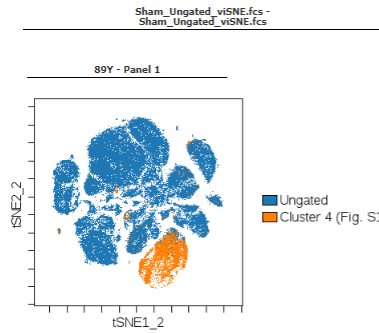


Cluster 4
Alveolar macrophage

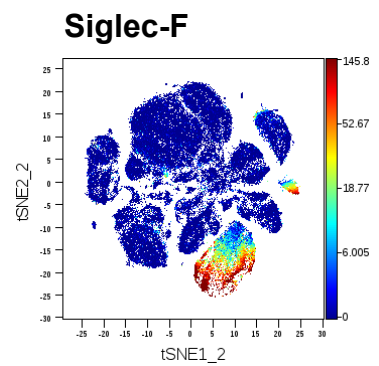
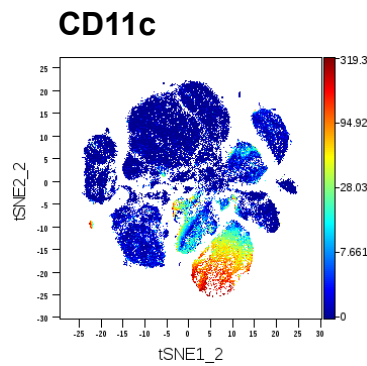
Manual gating



Overlay on viSNE

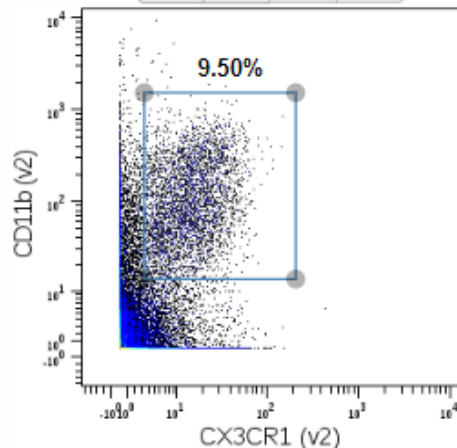
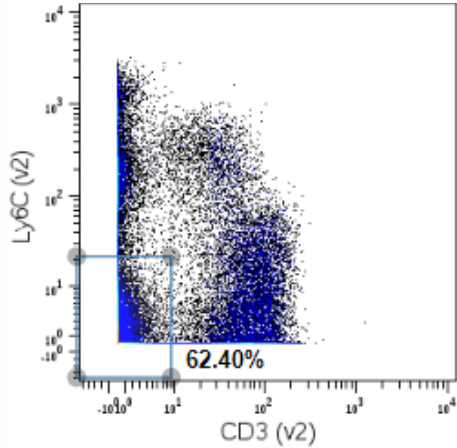


Expression of lineage markers

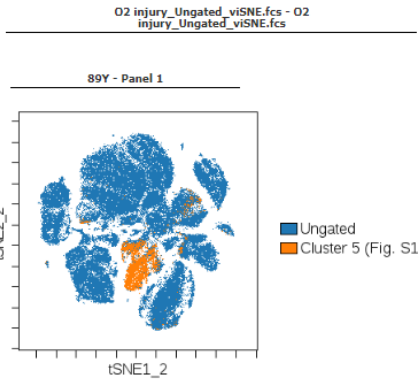
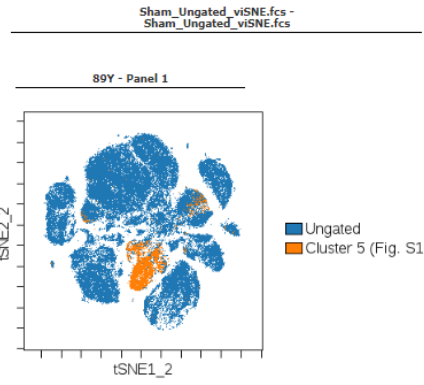


Cluster 5
Interstitial macrophage

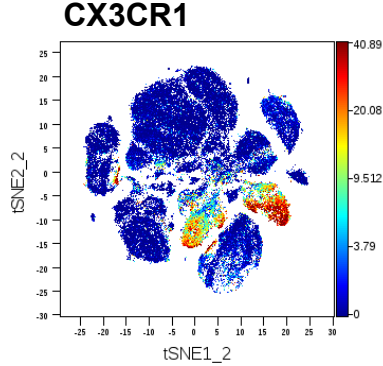
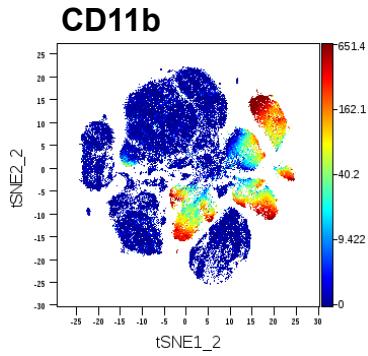
Manual gating



Overlay on viSNE

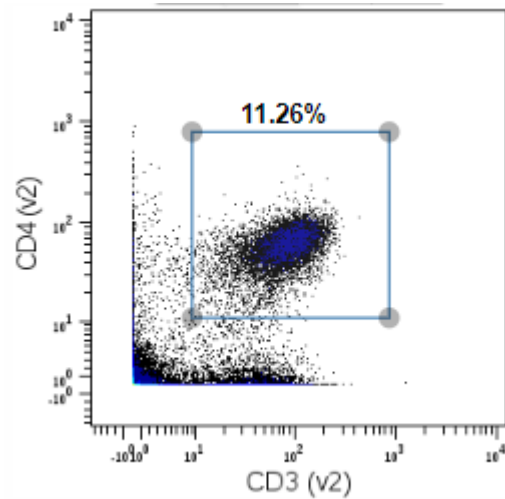


Expression of lineage markers

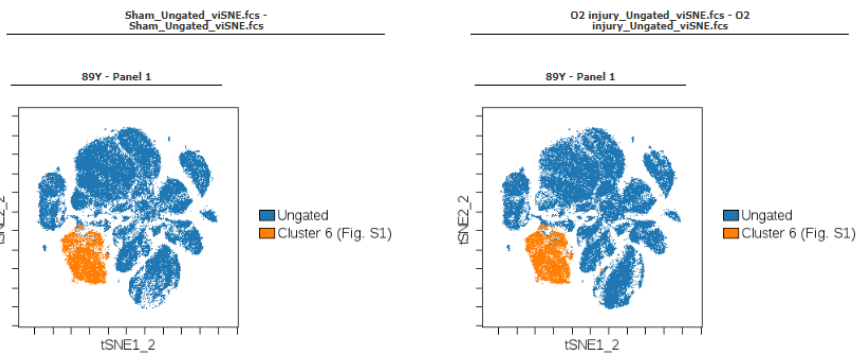


Cluster 6
CD4+ T cell

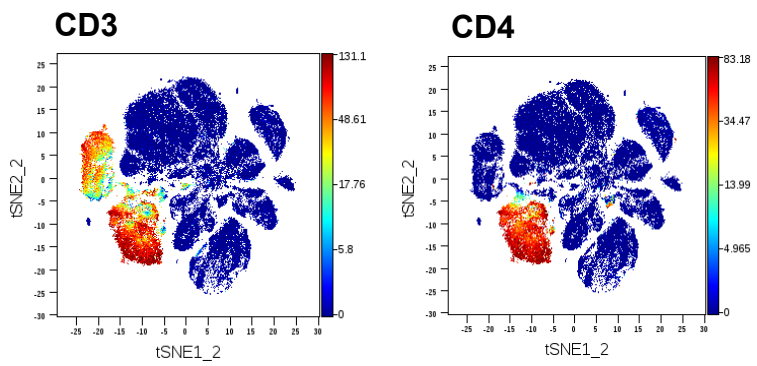
Manual gating



Overlay on viSNE

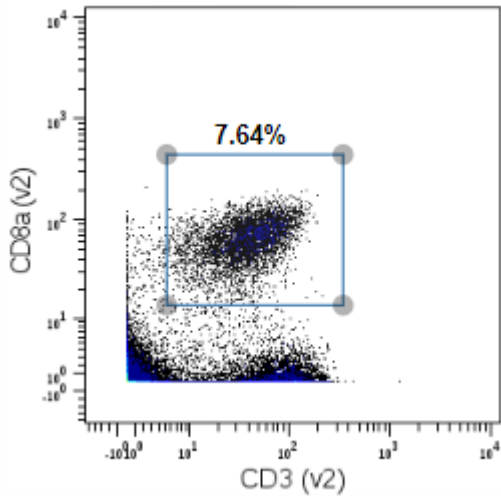


Expression of lineage markers

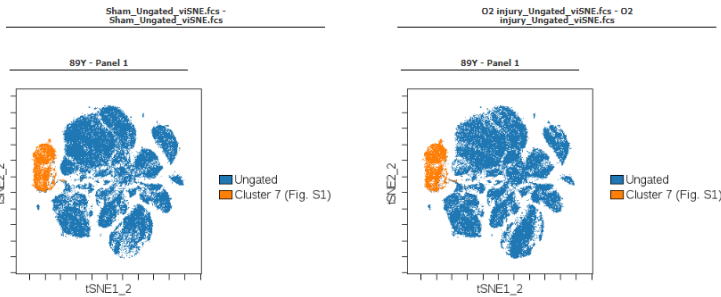


Cluster 7
CD8+ T cell

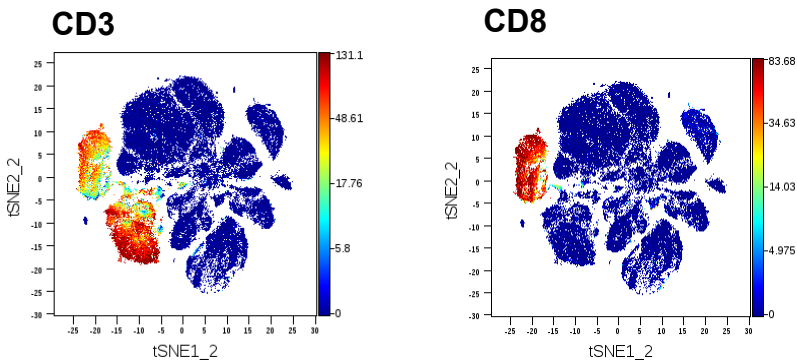
Manual gating



Overlay on viSNE

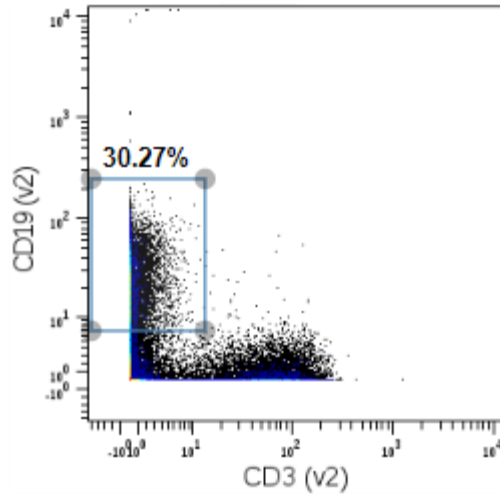


Expression of lineage markers

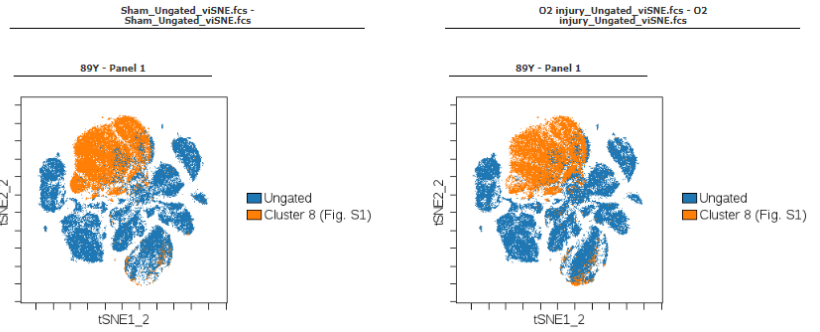


Cluster 8
B cell

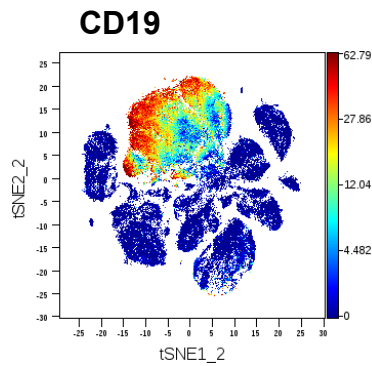
Manual gating



Overlay on viSNE

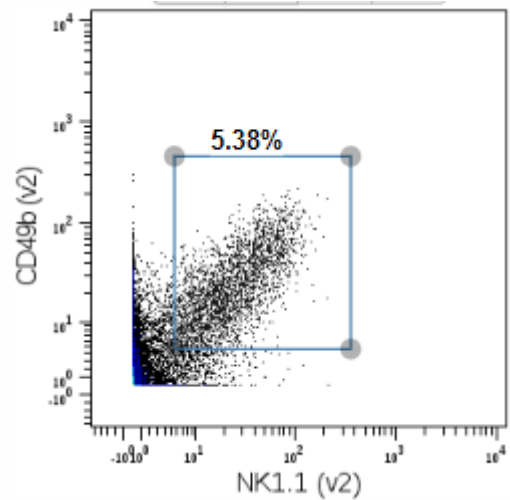
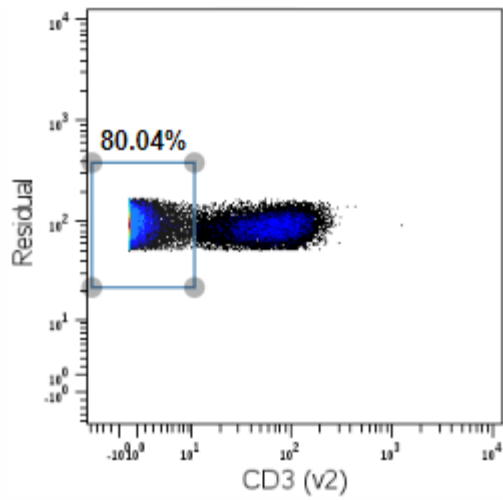


Expression of lineage markers

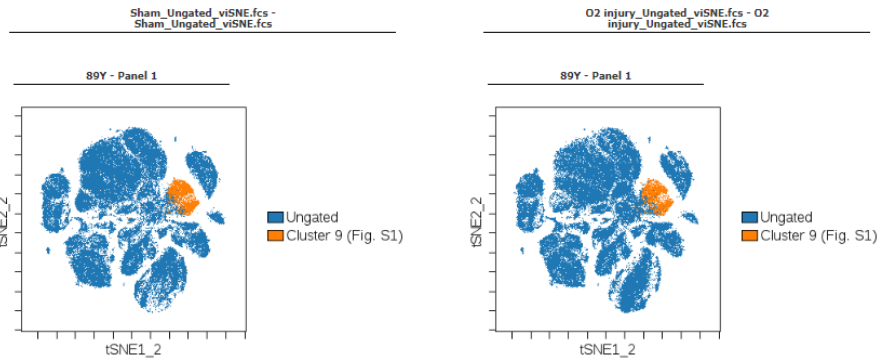


**Cluster 9
NK cell**

Manual gating



Overlay on viSNE



Expression of lineage markers

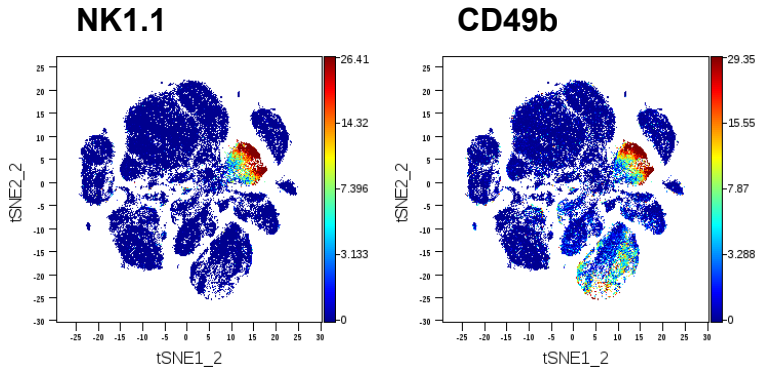
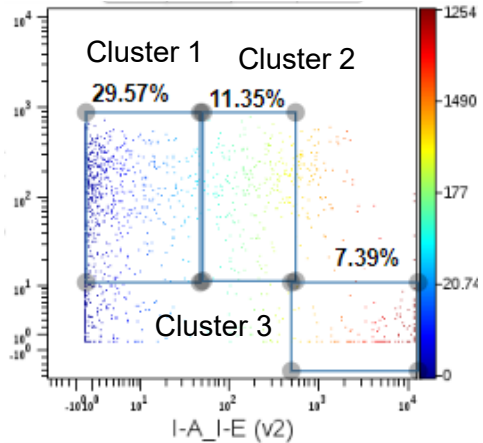
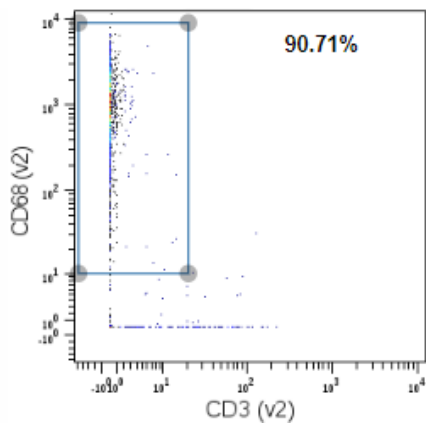


Figure S4

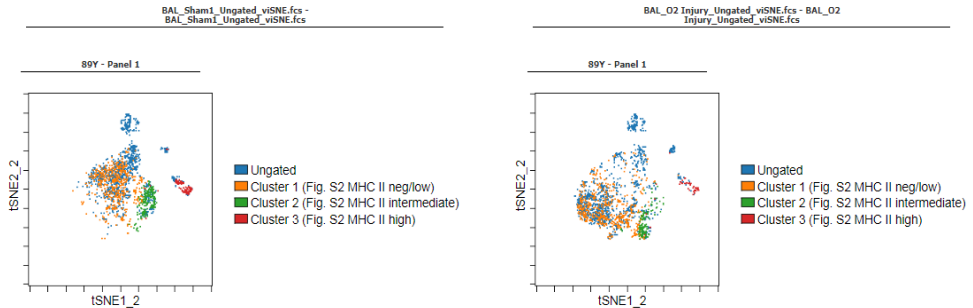
Cluster 1
 Alveolar macrophage
 CD68+ Siglec-F+
 MHC low

Cluster 2
 Alveolar macrophage
 CD68+ Siglec-F+
 MHC intermediate

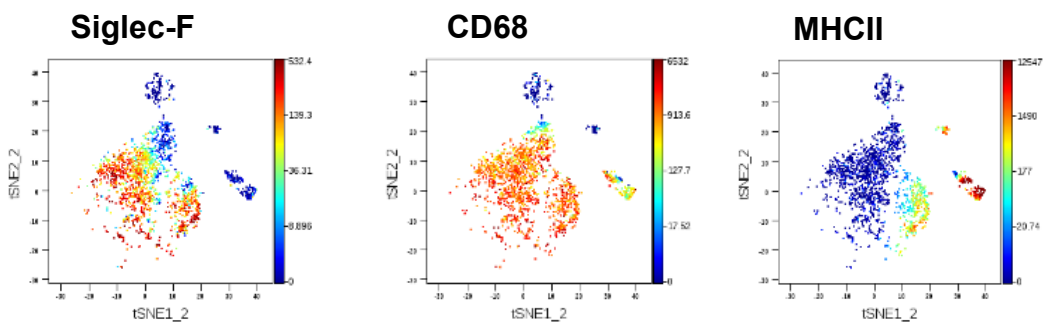
Cluster 3
 Myeloid cell
 CD68+ Siglec-F-
 MHC high



Overlay on viSNE



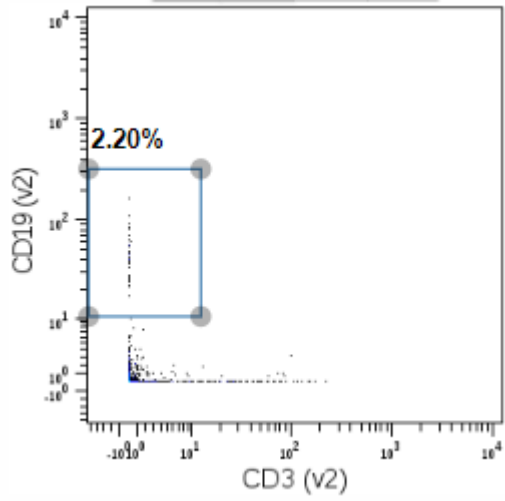
Expression of lineage markers



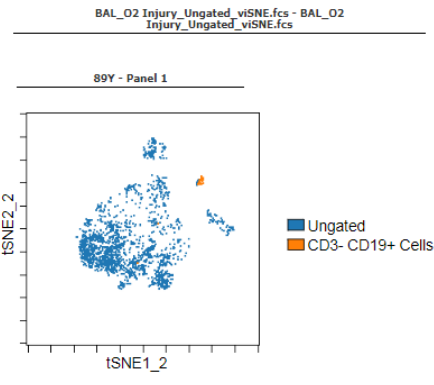
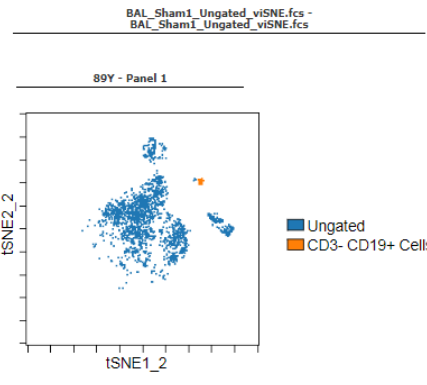
Manual gating

Cluster 4
B cell
CD19+

Cluster 5
T cell
CD3+



Overlay on viSNE



Expression of lineage markers

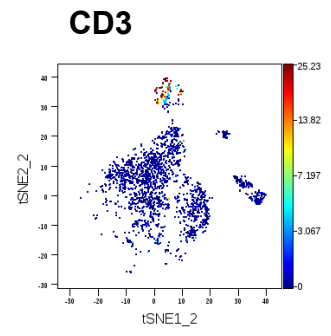
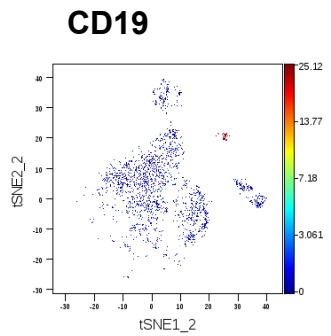
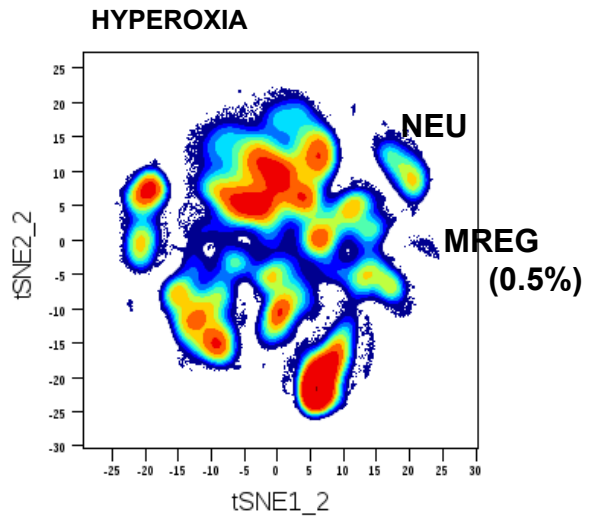
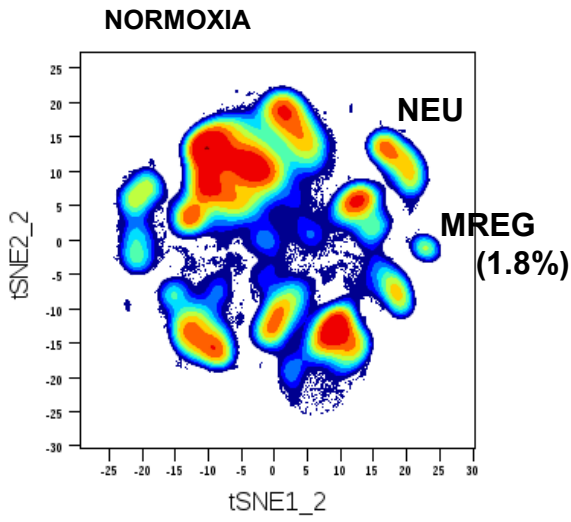


Figure S5

EXPERIMENT 1 (N=7)



EXPERIMENT 2 (N=5)

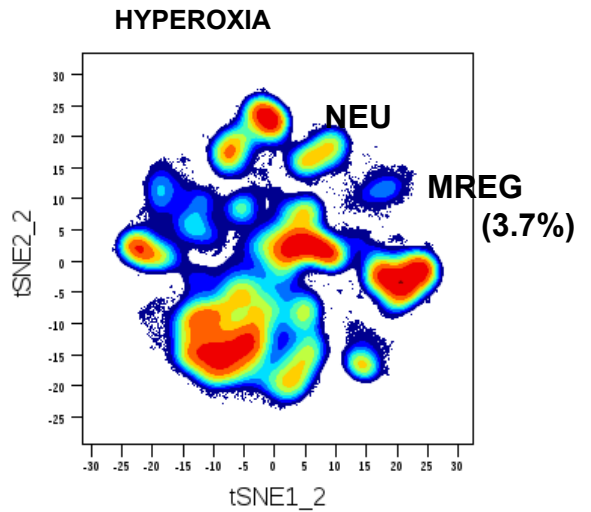
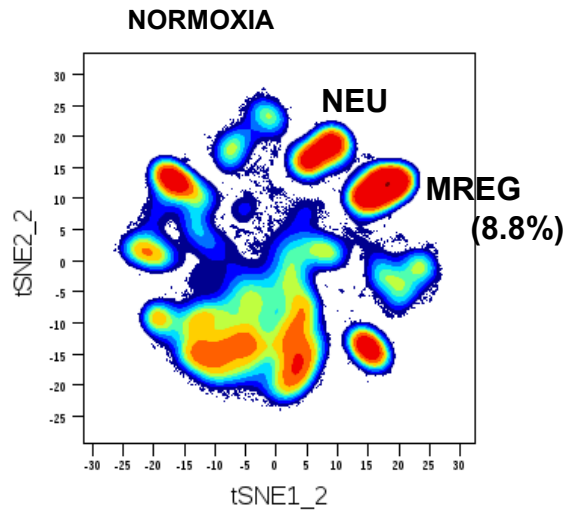
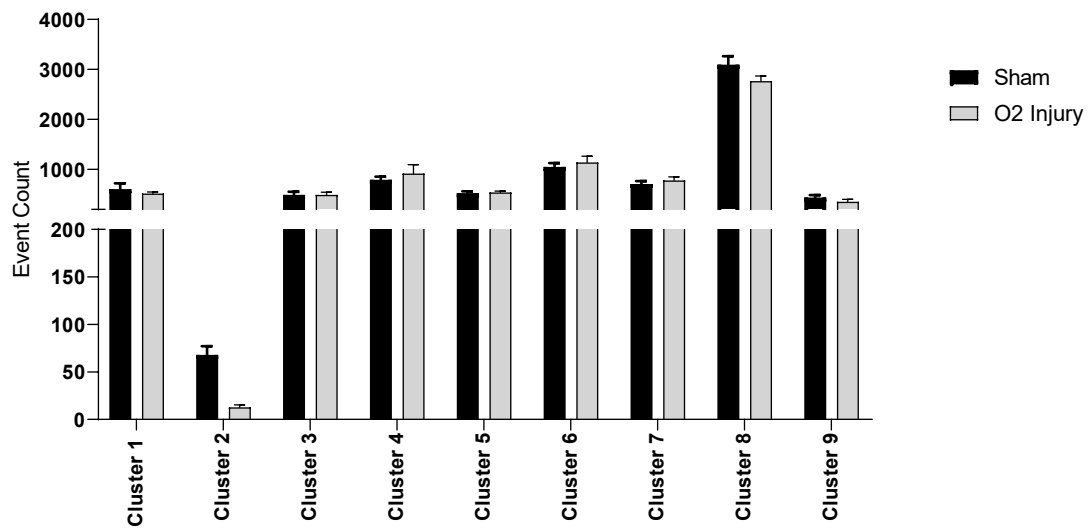


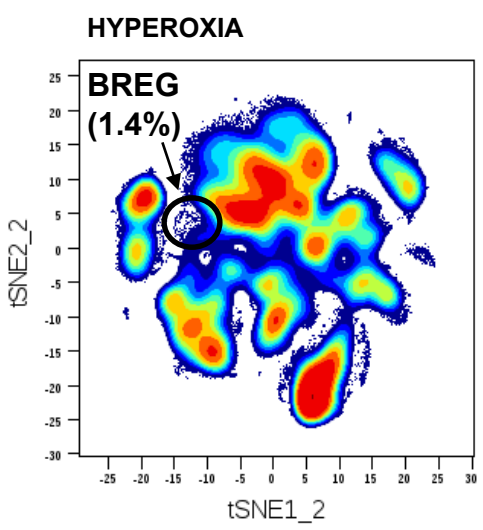
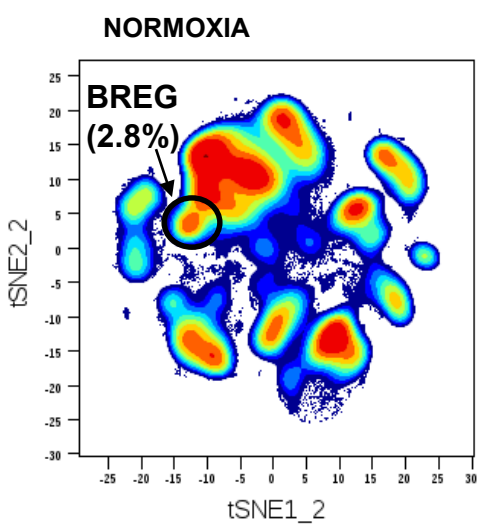
Figure S6



Cluster 1	neutrophils
Cluster 2	myeloid regulatory cells
Cluster 3	monocytes
Cluster 4	alveolar macrophages
Cluster 5	interstitial macrophages
Cluster 6	CD4+ T cells
Cluster 7	CD8+ T cells
Cluster 8	B cells
Cluster 9	NK cells

Figure S7

EXPERIMENT 1 (N=7)



EXPERIMENT 2 (N=5)

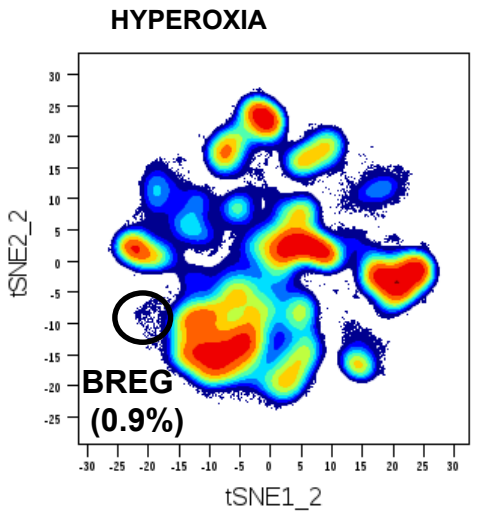
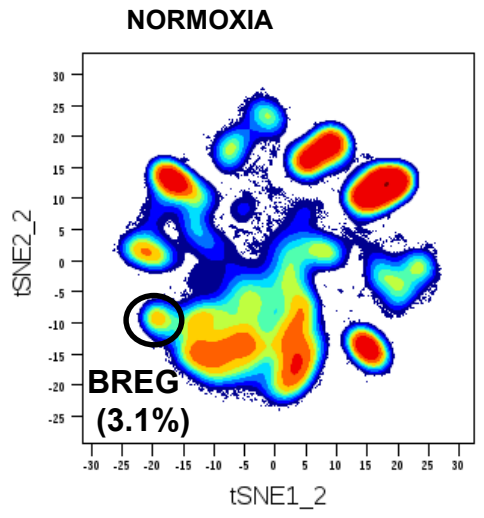


Figure S8

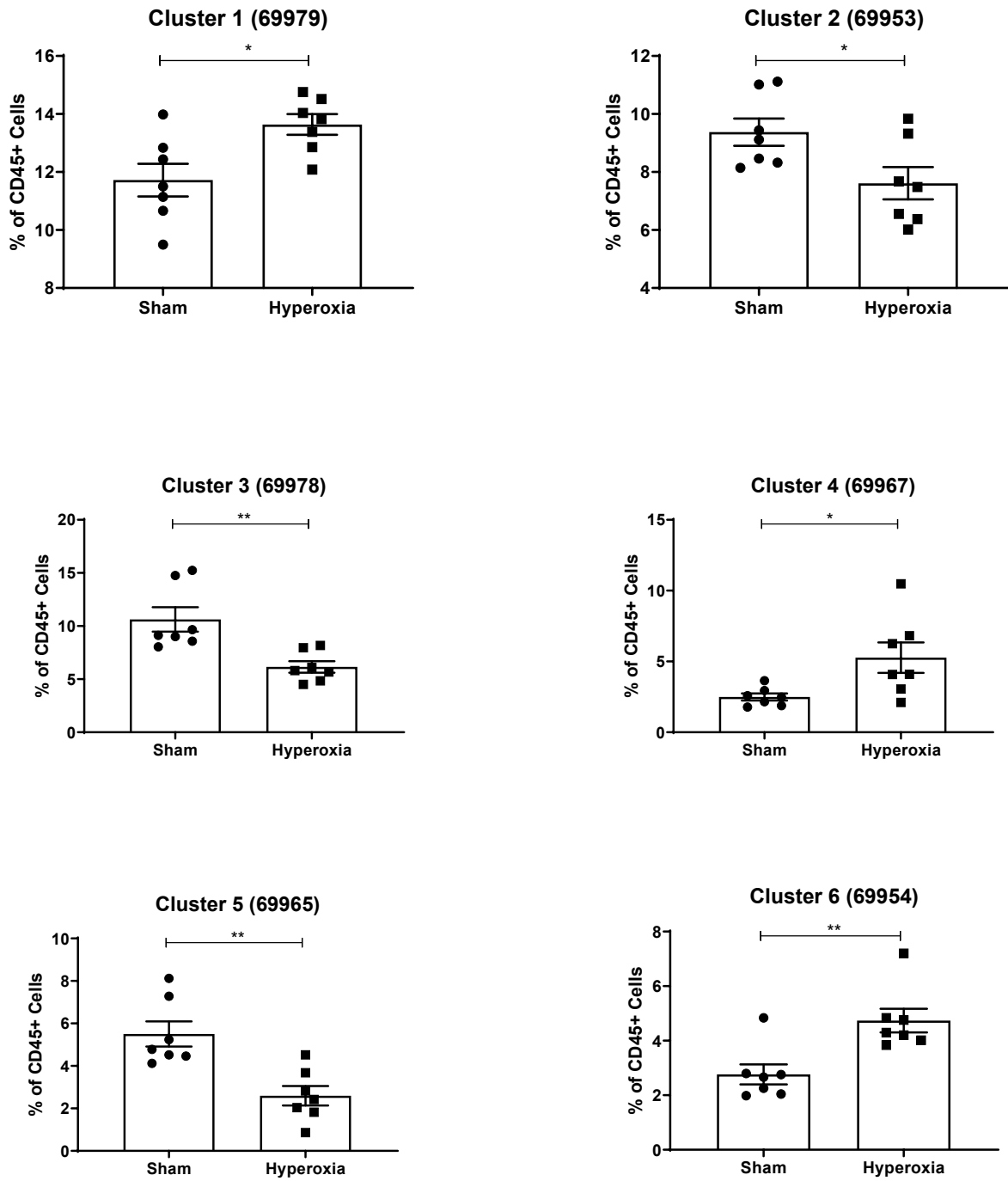


Table S1

Marker	Clone	Metal
EpCAM	G8.8	113In
CD44	IM7	115In
CD45	30-F11	141Pr
CD8a	53-6.7	142Nd
CD73	TY/11.8	143Nd
VEGF R1	141522	144Nd
CD4	RM4-5	145Nd
CD11c	N418	146Nd
Ly6G	1A8	148Nd
PD-L1	10F.9G2	149Sm
CX3CR1	SA011F11	150Nd
Ly6C	HK1.4	151Eu
CD3	145-2C11	152Sm
CD172a	P84	153Eu
CD103	2E7	154Sm
CD68	FA-11	155Gd
CD19	6D5	156Gd
CD205	NLDC-145	158Gd
C39	Duha59	159Tb
Sca-1	E13-161.7	160Gd
Arginase I	Poly	161Dy
Foxp3	FJK-16s	162Dy
NK1.1	PK136	163Dy
Ki67	16A8	164Dy
CD115	460615	165Ho
CD86	GL-1	166Er
Grz B	GB11	167Er
CD11b	M1/70	169Tm
Siglec-F	E50-2440	170Er
CD49b	DX5	171Yb
TCRgd	GL3	172Yb
CD69	H1.2F3	173Yb
I-A/I-E	M5/114.15.2	174Yb
F4/80	BM8	175Lu
TLR2	T2.5	176Yb
Autotaxin	Polyclonal	209Bi

Figure S1. Lung tissue during normoxia and hyperoxia. Lung parenchyma from WT mouse during normoxia and after 48 hours of hyperoxia. Representative sample, H&E staining, 400x magnified. No evidence of diffuse alveolar damage is seen at 48 hours.

Figure S2. Stepwise gating approach to identify live CD45+ immune cells for CyTOF analysis. Step 1: Single cells were gated based on DNA content (193Ir-DNA2+). Step 2: Elimination of normalization beads. Step 3: Gating on live cells. Step 4: Gating on CD45+ cells.

Figure S3. Identification of immune cell lineage of cell clusters in the whole lung based on the expression of surface and intracellular markers. Overlays of manually gated populations on tSNE plots.

Figure S4. Identification of immune cell lineage of cell clusters in BALF based on the expression of surface and intracellular markers. Overlays of manually gated populations on tSNE plots.

Figure S5. Myeloid regulatory cells during normoxia and hyperoxia. tSNE plots from 2 independent experiments (N=7 and N=5) show depletion of myeloid regulatory cells during hyperoxia.

Figure S6. Absolute cell numbers of 9 major immune clusters as acquired by CyTOF in the live gate before equal sampling (7 control mice and 7 mice in hyperoxia).

Figure S7. Regulatory B cells during normoxia and hyperoxia. tSNE plots from 2 independent experiments (N=7 and N= 5) show depletion of regulatory B cells during hyperoxia.

Figure S8. Comparison of frequencies of CITRUS-identified clusters during normoxia and hyperoxia with unpaired t-test . Each dot represents data from one lung. * indicated $p < 0.05$, ** indicated $p < 0.01$.

Table S1. Panel of CyTOF antibodies used in the study. 36 metal conjugated antibodies were used in the study.