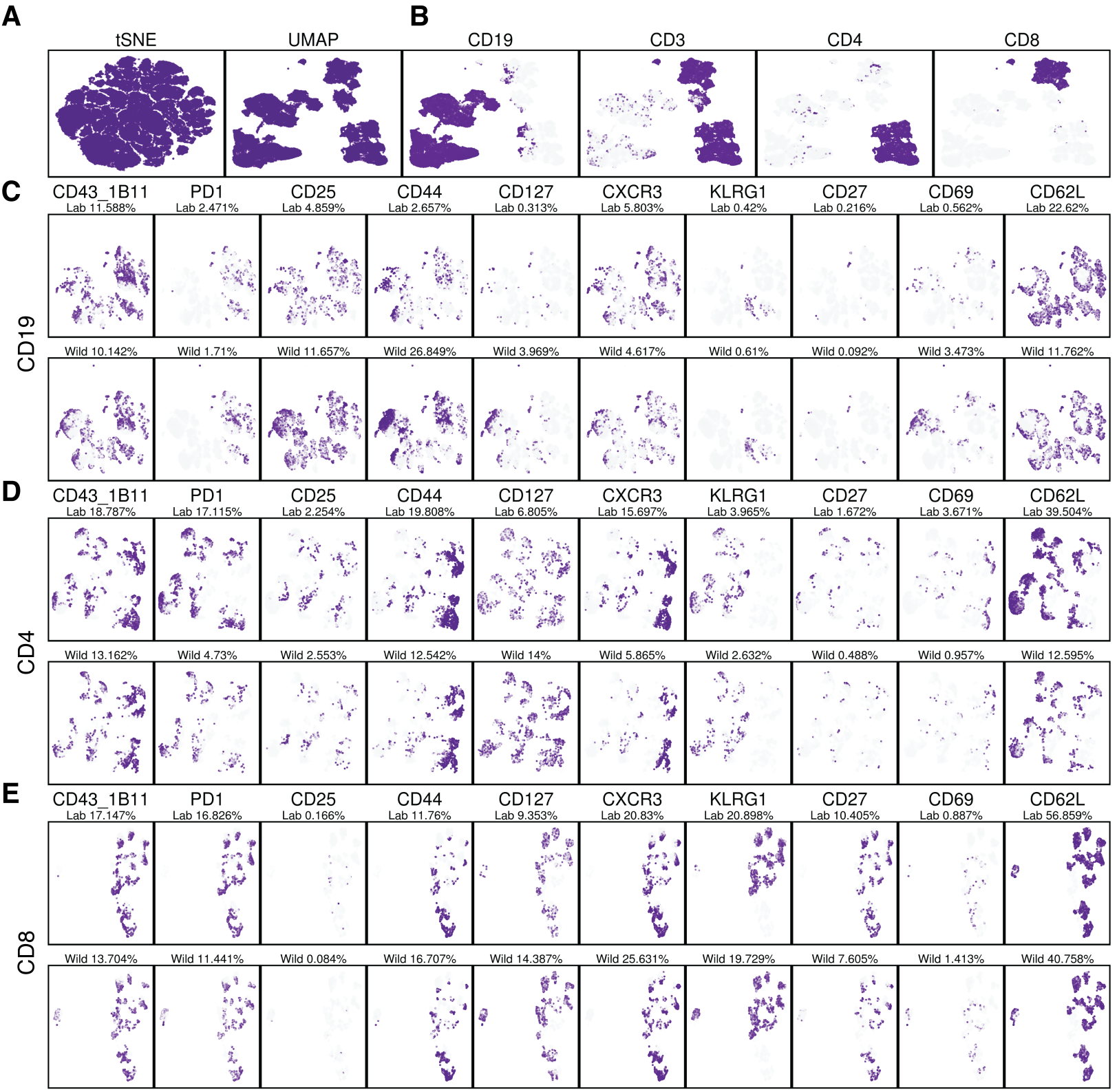
A
tSNE
UMAP
B
CD19
CD3
CD4
CD8
C
CD19
CD43_1B11
Lab 11.588%
Wild 10.142%
PD1
Lab 2.471%
Wild 1.71%
CD25
Lab 4.859%
Wild 11.657%
CD44
Lab 2.657%
Wild 26.849%
CD127
Lab 0.313%
Wild 3.969%
CXCR3
Lab 5.803%
Wild 4.617%
KLRG1
Lab 0.42%
Wild 0.61%
CD27
Lab 0.216%
Wild 0.092%
CD69
Lab 0.562%
Wild 3.473%
CD62L
Lab 22.62%
Wild 11.762%
D
CD4
CD43_1B11
Lab 18.787%
Wild 13.162%
PD1
Lab 17.115%
Wild 4.73%
CD25
Lab 2.254%
Wild 2.553%
CD44
Lab 19.808%
Wild 12.542%
CD127
Lab 6.805%
Wild 14%
CXCR3
Lab 15.697%
Wild 5.865%
KLRG1
Lab 3.965%
Wild 2.632%
CD27
Lab 1.672%
Wild 0.488%
CD69
Lab 3.671%
Wild 0.957%
CD62L
Lab 39.504%
Wild 12.595%
E
CD8
CD43_1B11
Lab 17.147%
Wild 13.704%
PD1
Lab 16.826%
Wild 11.441%
CD25
Lab 0.166%
Wild 0.084%
CD44
Lab 11.76%
Wild 16.707%
CD127
Lab 9.353%
Wild 14.387%
CXCR3
Lab 20.83%
Wild 25.631%
KLRG1
Lab 20.898%
Wild 19.729%
CD27
Lab 10.405%
Wild 7.605%
CD69
Lab 0.887%
Wild 1.413%
CD62L
Lab 56.859%
Wild 40.758%

Figure S1. Identification of immune cell populations that differ between lab and rewilded mice with UMAP. Related to Figure 1. (**A**) Projection of ~180,000 CD45+ cells visualized by tSNE vs UMAP. (**B**) Identification of the major lymphocyte populations on UMAP based on CD19, CD3, CD4 and CD8 expression. (**C** to **E**) Expression of cell surface molecules on CD19+ cells (C), CD4+ cells (D) and CD8+ cells (E). In (C), (D) and (E), expression of 1B11, PD1, CD25, CD44, CD127, CXCR3, KLRG1, CD27, CD69, and CD62L are shown as high (purple) or low (grey) from cut-off values based on overall distribution of fluorescence.

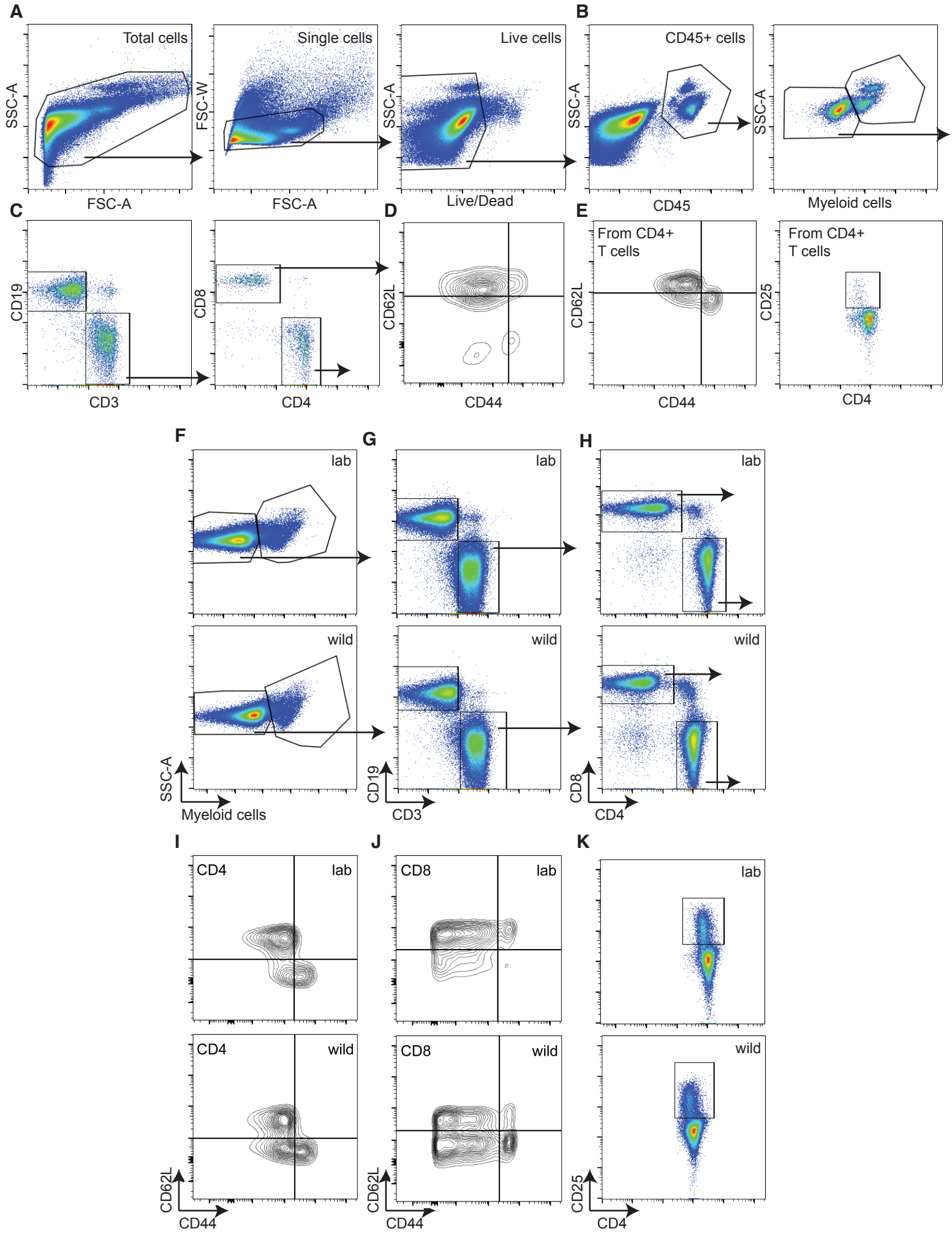
A
Total cells
SSC-A
FSC-A
Single cells
FSC-W
FSC-A
Live cells
SSC-A
Live/Dead
B
CD45+ cells
SSC-A
CD45
SSC-A
Myeloid cells
C
CD19
CD3
CD8
CD4
D
CD62L
CD44
E
From CD4+
T cells
CD62L
CD44
From CD4+
T cells
CD25
CD4
F
lab
wild
SSC-A
Myeloid cells
G
lab
wild
CD19
CD3
H
lab
wild
CD8
CD4
I
CD4
lab
CD4
wild
CD62L
CD44
J
CD8
lab
CD8
wild
CD62L
CD44
K
lab
wild
CD25
CD4

Figure S2. Flow cytometry gating strategy for the quantification of immune populations in the blood and MLN of lab and rewilded mice. Related to STAR Methods. (**A** to **E**) Representative flow cytometry plots illustrating gating strategy (A) of lymphoid populations in the blood including the total CD45+ cells, total myeloid cells (B), total B and T cells, total CD4 and CD8 T cells (C), $CD44^{lo}CD62L^{hi}$, $CD44^{hi}CD62L^{hi}$, $CD44^{hi}CD62L^{lo}$, $CD44^{lo}CD62L^{lo}$ CD8 T cells (D), $CD44^{lo}CD62L^{hi}$, $CD44^{hi}CD62L^{hi}$, $CD44^{hi}CD62L^{lo}$, $CD44^{lo}CD62L^{lo}$ CD4 T cells, and CD25+CD4+ T cells (E). (**F** to **K**) Representative flow cytometry plots for immune cell populations after gating for CD45+ cells in the MLNs of lab or rewilded C57BL/6 mice; for the total abundance of myeloid cells (F), B and T cells (G), CD4 and CD8 T cells (H), $CD44^{lo}CD62L^{hi}$, $CD44^{hi}CD62L^{hi}$, $CD44^{hi}CD62L^{lo}$, $CD44^{lo}CD62L^{lo}$ CD4 T cells (I), $CD44^{lo}CD62L^{hi}$, $CD44^{hi}CD62L^{hi}$, $CD44^{hi}CD62L^{lo}$, $CD44^{lo}CD62L^{lo}$ CD8 T cells (J), and CD25+CD4+ T cells (K).

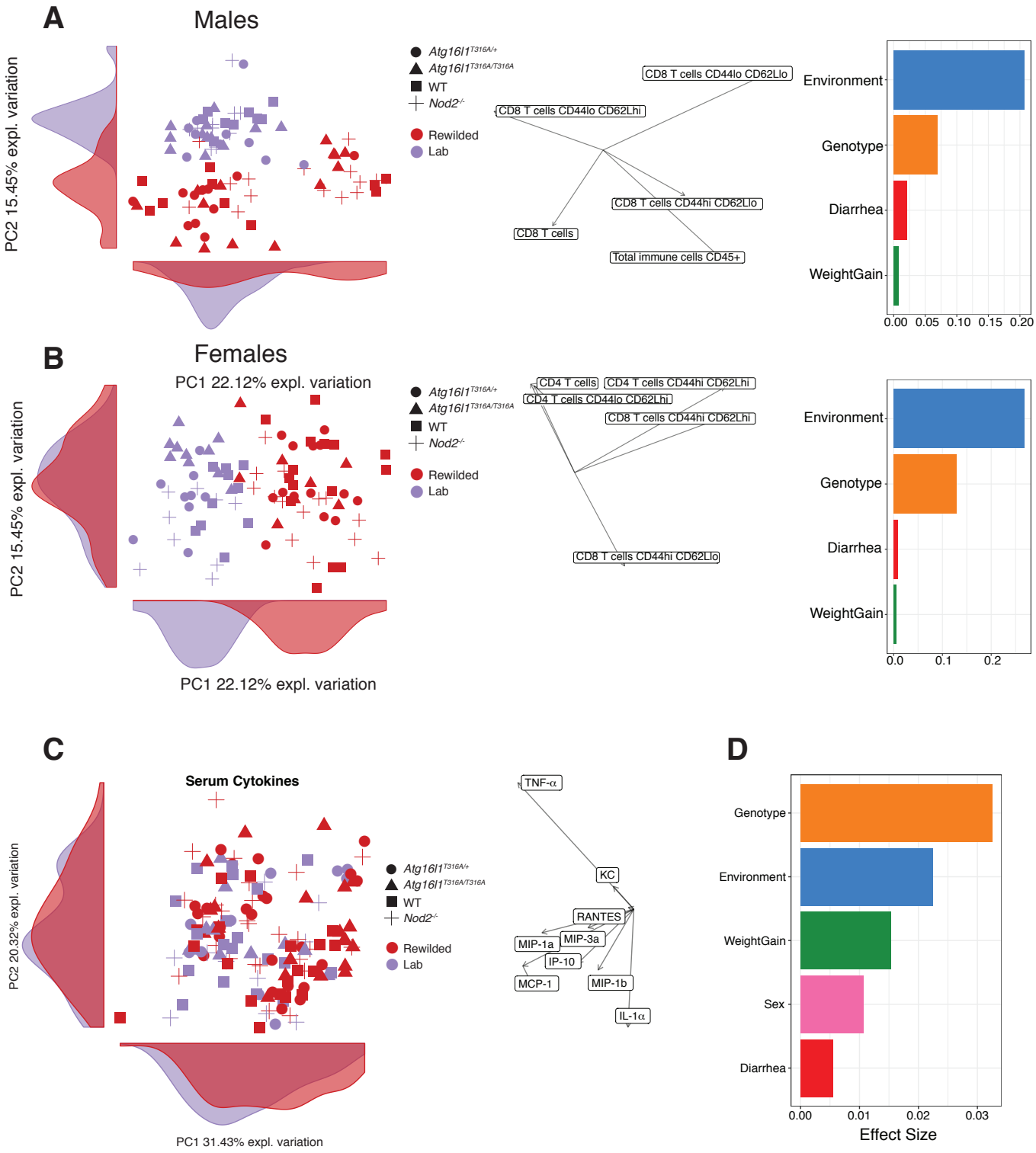
A
Males
PC2 15.45% expl. variation
Atg16l1T316A/+
Atg16l1T316A/T316A
WT
Nod2-/-
Rewilded
Lab
CD8 T cells CD44lo CD62Llo
CD8 T cells CD44lo CD62Lhi
CD8 T cells CD44hi CD62Llo
CD8 T cells
Total immune cells CD45+
Environment
Genotype
Diarrhea
WeightGain
0.00 0.05 0.10 0.15 0.20
B
Females
PC1 22.12% expl. variation
PC2 15.45% expl. variation
CD4 T cells
CD4 T cells CD44hi CD62Lhi
CD4 T cells CD44lo CD62Lhi
CD8 T cells CD44hi CD62Lhi
CD8 T cells CD44hi CD62Llo
0.0 0.1 0.2
C
Serum Cytokines
PC2 20.32% expl. variation
PC1 31.43% expl. variation
TNF-α
KC
RANTES
MIP-1a
MIP-3a
IP-10
MCP-1
MIP-1b
IL-1α
D
Genotype
Environment
WeightGain
Sex
Diarrhea
0.00 0.01 0.02 0.03
Effect Size

Figure S3. The effects of environment and genotype on immune cell compositions and plasma cytokine levels for lab and rewilded mice. Related to Figure 1. (**A** and **B**) Principal component analysis (PCA) of gated immune cell populations in the blood and the density of each population along the principal components (PC) for male (A) and female (B) mice separately. Middle panel indicate biplots of the gated immune cell populations that are projected onto PC1 and PC2 that have the biggest effect sizes. Right panels are Bar plots showing the pseudo R^2 measure of effect size on the entire distance matrix used to calculate the PCA of immune cell populations in the blood. (**C** and **D**) Levels of RANTES, MIP-3a, KC, TNF-α, MCP-1, IP-10, MIP-1a, MIP-1b, IL-1α, IL-6 cytokines were measured from the plasma of lab and rewilded mice at the time of sacrifice with bead-based immunoassays (LEGENDplex). (C) PCA of plasma cytokine levels in the blood of individual mice and the density of each population along the principal components (PC). Right panel indicate biplots of the plasma cytokines are projected onto PC1 and PC2. (D) Bar plot showing the pseudo R^2 measure of the effect size of different variables on the variance of plasma cytokine levels.

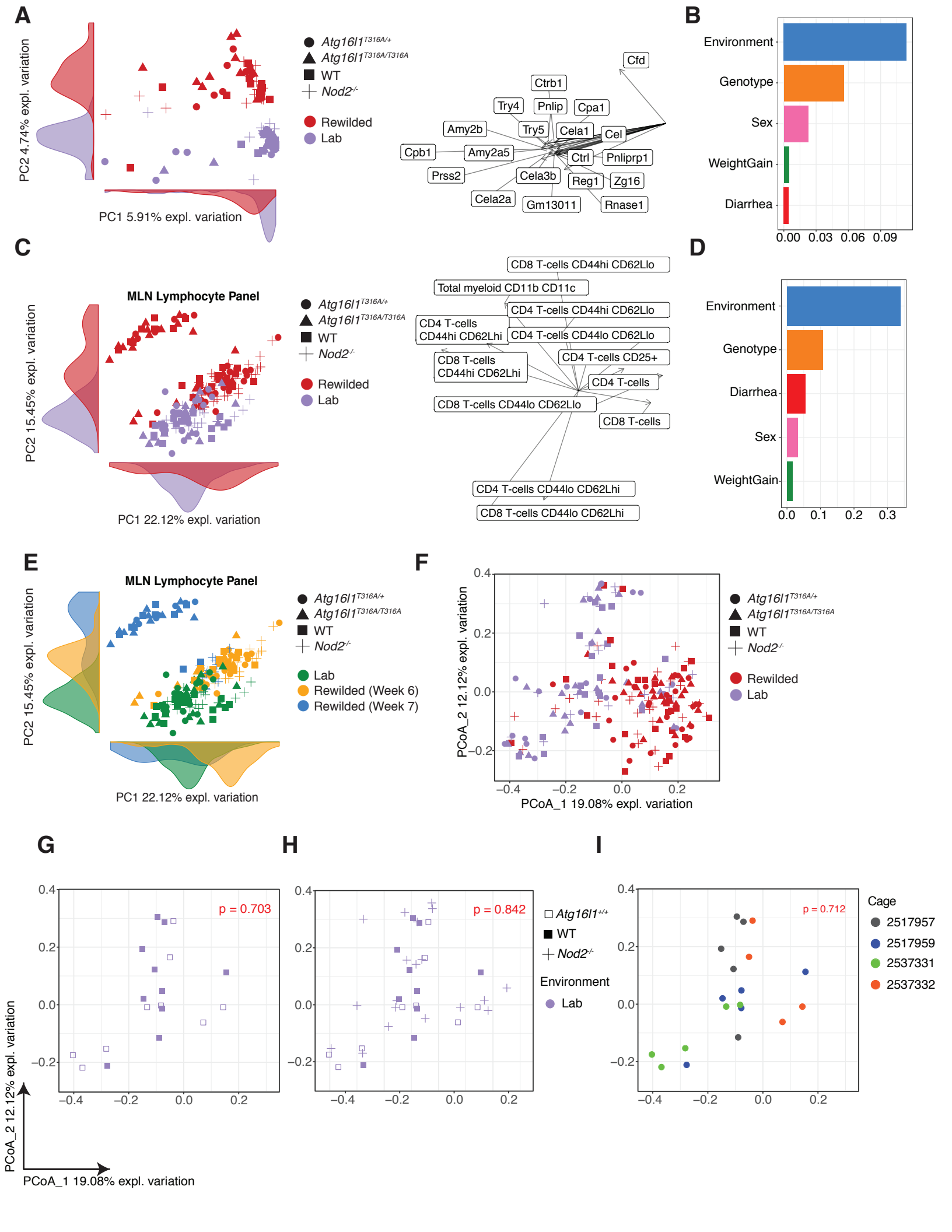
A
PC2 4.74% expl. variation
PC1 5.91% expl. variation
Atg16l1T316A/+
Atg16l1T316A/T316A
WT
Nod2-/-
Rewilded
Lab
Cfd
Ctrb1
Try4
Pnlip
Cpa1
Amy2b
Try5
Cela1
Cel
Cpb1
Amy2a5
Ctrl
Pnliprp1
Prss2
Cela3b
Reg1
Zg16
Cela2a
Gm13011
Rnase1
B
Environment
Genotype
Sex
WeightGain
Diarrhea
0.00 0.03 0.06 0.09
C
MLN Lymphocyte Panel
PC2 15.45% expl. variation
PC1 22.12% expl. variation
CD8 T-cells CD44hi CD62Llo
Total myeloid CD11b CD11c
CD4 T-cells CD44hi CD62Llo
CD4 T-cells CD44hi CD62Lhi
CD4 T-cells CD44lo CD62Llo
CD8 T-cells CD44hi CD62Lhi
CD4 T-cells CD25+
CD4 T-cells
CD8 T-cells CD44lo CD62Llo
CD8 T-cells
CD4 T-cells CD44lo CD62Lhi
CD8 T-cells CD44lo CD62Lhi
D
Environment
Genotype
Diarrhea
Sex
WeightGain
0.0 0.1 0.2 0.3
E
MLN Lymphocyte Panel
PC2 15.45% expl. variation
PC1 22.12% expl. variation
Lab
Rewilded (Week 6)
Rewilded (Week 7)
F
PCoA_2 12.12% expl. variation
PCoA_1 19.08% expl. variation
G
H
I
p = 0.703
p = 0.842
p = 0.712
Atg16l1+/+
Environment
Cage
2517957
2517959
2537331
2537332
PCoA_2 12.12% expl. variation
PCoA_1 19.08% expl. variation

Figure S4: Environment has the greatest effect on variation of transcriptional profiles, lymphoid populations, and microbial composition. Related to Figure 4. (**A** to **B**) Transcriptional profiles of MLNs were determined by Celseq. (**A**) PCA based on gene profiles in the MLN and the density of each population along the principal components (PC). Right panel indicates biplots of the genes projected onto PC1 and PC2. (**B**) Bar plot showing the pseudo R^2 measure of effect size on the entire distance matrix used to calculate the PCA of gene expression levels. (**C** to **E**) PCA based on lymphoid populations in the MLN and the density of each population along the principal components (PC). In plot (C), dot color coded by rewilded (red) and lab (purple) and in plot (E), dot color coded by lab (green), rewilded week 6 (yellow), rewilded week 7 (blue). Plot (C) also shows the biplots of the MLN lymphocyte populations are projected onto PC1 and PC2. (**D**) Bar plot showing the pseudo R^2 measure of effect size on the entire distance matrix used to calculate the PCA of immune populations. (**F** to **I**) PCoA based on Bray Curtis distances of 16s rRNA sequencing profiles in all lab and rewilded mice across all genotypes (F), lab wild type (WT) and *Atg16l*$^{+/+}$ only (G), lab wild type (WT), *Atg16l*$^{+/+}$ and *Nod2*$^{-/-}$ (H), and lab wild type (WT), *Atg16l*$^{+/+}$ and *Nod2*$^{-/-}$ by respective cages (I). P-values were determined by an Adonis test from Bray Curtis distances.

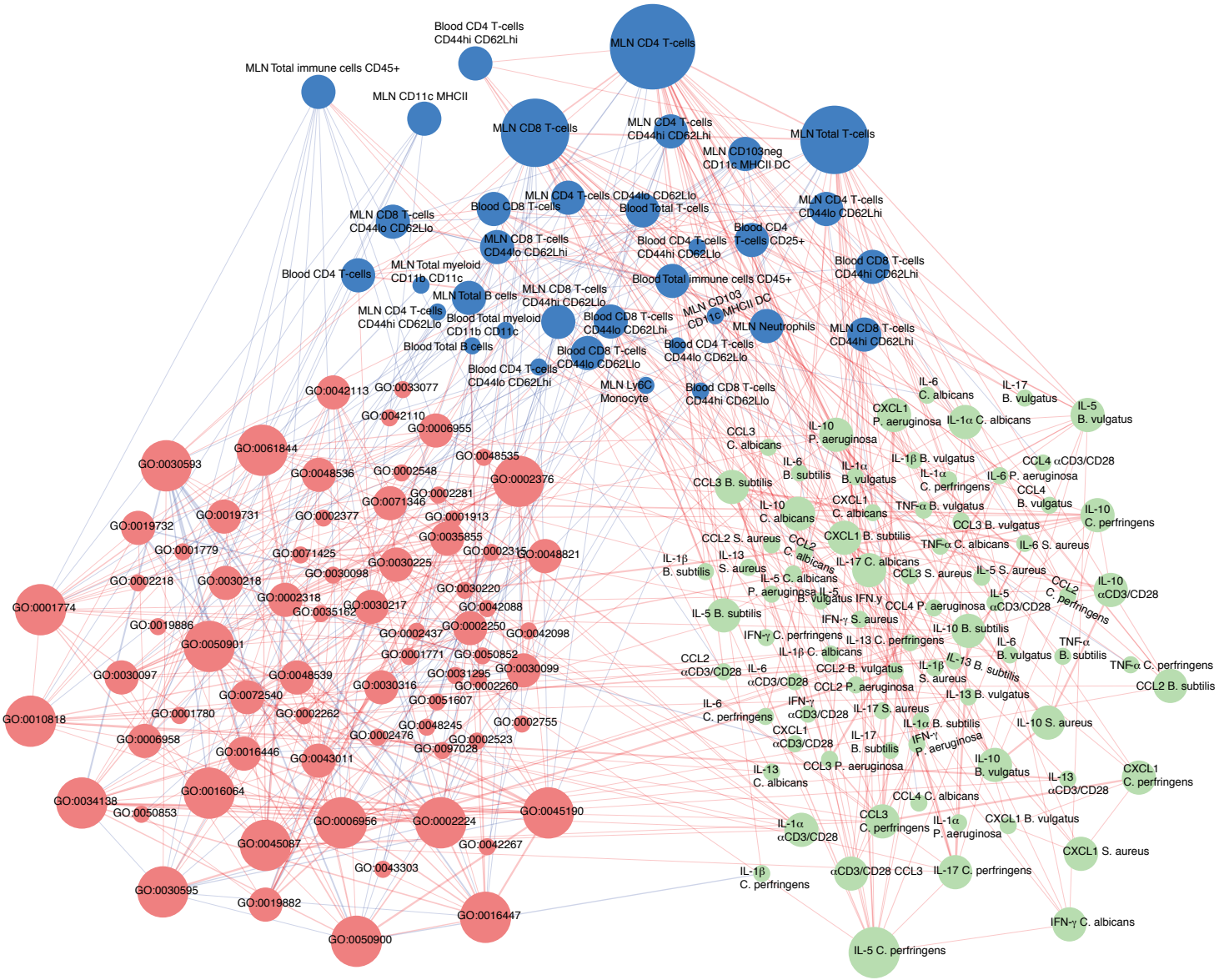
Blood CD4 T-cells CD44hi CD62Lhi
MLN CD4 T-cells
MLN Total immune cells CD45+
MLN CD11c MHCII
MLN CD8 T-cells
MLN CD4 T-cells CD44hi CD62Lhi
MLN Total T-cells
MLN CD103neg CD11c MHCII DC
MLN CD4 T-cells CD44lo CD62Lo
Blood CD8 T-cells
Blood Total T-cells
MLN CD4 T-cells CD44lo CD62Lhi
MLN CD8 T-cells CD44lo CD62Lo
Blood CD4 T-cells CD25+
MLN CD8 T-cells CD44lo CD62Lhi
Blood CD4 T-cells CD44hi CD62Lo
Blood CD8 T-cells CD44hi CD62Lhi
Blood CD4 T-cells
MLN Total myeloid CD11b CD11c
Blood Total immune cells CD45+
MLN Total B cells
MLN CD8 T-cells CD44hi CD62Lo
MLN CD4 T-cells CD44hi CD62Lo
Blood Total myeloid CD11b CD11c
Blood CD8 T-cells CD44lo CD62Lhi
MLN CD103 CD11c MHCII DC
MLN Neutrophils
MLN CD8 T-cells CD44hi CD62Lhi
Blood Total B cells
Blood CD8 T-cells CD44lo CD62Lo
Blood CD4 T-cells CD44lo CD62Lo
Blood CD4 T-cells CD44lo CD62Lhi
MLN Ly6C Monocyte
Blood CD8 T-cells CD44hi CD62Llo
GO:0042113
GO:0033077
GO:0042110
GO:0006955
GO:0061844
GO:0048535
GO:0030593
GO:0048536
GO:0002548
GO:0002376
GO:0002281
GO:0071346
GO:0019731
GO:0002377
GO:0001913
GO:0019732
GO:0035855
GO:0001779
GO:0071425
GO:0002315
GO:0048821
GO:0030225
GO:0002218
GO:0030218
GO:0030098
GO:0030220
GO:0002318
GO:0030217
GO:0042088
GO:0001774
GO:0035162
GO:0019886
GO:0002437
GO:0002250
GO:0042098
GO:0050901
GO:0001771
GO:0050852
GO:0030097
GO:0048539
GO:0031295
GO:0030099
GO:0030316
GO:0002260
GO:0072540
GO:0051607
GO:0010818
GO:0001780
GO:0002262
GO:0002755
GO:0048245
GO:0006958
GO:0002476
GO:0002523
GO:0097028
GO:0016446
GO:0043011
GO:0034138
GO:0016064
GO:0050853
GO:0045190
GO:0006956
GO:0002224
GO:0045087
GO:0042267
GO:0043303
GO:0030595
GO:0019882
GO:0016447
GO:0050900
IL-6 C. albicans
IL-17 B. vulgatus
CXCL1 P. aeruginosa
IL-1α C. albicans
IL-5 B. vulgatus
IL-10 P. aeruginosa
CCL3 C. albicans
IL-6 B. subtilis
IL-1α B. vulgatus
IL-1β B. vulgatus
CCL4 αCD3/CD28
CCL3 B. subtilis
IL-1α C. perfringens
IL-6 P. aeruginosa
CCL4 B. vulgatus
IL-10 C. albicans
CXCL1 C. albicans
TNF-α B. vulgatus
IL-10 C. perfringens
CCL3 B. vulgatus
CCL2 S. aureus
CCL2 C. albicans
CXCL1 B. subtilis
TNF-α C. albicans
IL-6 S. aureus
IL-1β B. subtilis
IL-13 S. aureus
IL-17 C. albicans
CCL3 S. aureus
IL-5 S. aureus
IL-5 C. albicans
CCL2 C. perfringens
P. aeruginosa IL-5 B. vulgatus IFN.γ
IL-10 αCD3/CD28
CCL4 P. aeruginosa
IL-5 αCD3/CD28
IL-5 B. subtilis
IFN-γ S. aureus
IL-10 B. subtilis
IFN-γ C. perfringens
IL-13 C. perfringens
IL-6 B. vulgatus
TNF-α B. subtilis
IL-1β C. albicans
TNF-α C. perfringens
CCL2 αCD3/CD28
IL-6 αCD3/CD28
CCL2 B. vulgatus
IL-1β S. aureus
IL-13 B. subtilis
CCL2 B. subtilis
CCL2 P. aeruginosa
IL-13 B. vulgatus
IL-6 C. perfringens
IFN-γ αCD3/CD28
IL-17 S. aureus
IL-1α B. subtilis
IL-10 S. aureus
CXCL1 αCD3/CD28
IL-17 B. subtilis
IFN-γ P. aeruginosa
CCL3 P. aeruginosa
IL-10 B. vulgatus
IL-13 C. albicans
IL-13 αCD3/CD28
CXCL1 C. perfringens
CCL4 C. albicans
CCL3 C. perfringens
IL-1α P. aeruginosa
CXCL1 B. vulgatus
IL-1α αCD3/CD28
CXCL1 S. aureus
IL-1β C. perfringens
αCD3/CD28 CCL3
IL-17 C. perfringens
IFN-γ C. albicans
IL-5 C. perfringens

Figure S5: An unsupervised PLS-regression multi-component network model for multi-omic integration. Related to Figure 5. A fully annotated multi-component network as shown in Figure 5A.

Table S1. Lymphocyte panel of flow cytometry. Related to Figure 1.

Immune population	**Gating strategy**
Total CD45+ cells	Total cells/Single cells/Live+/CD45+
Total myeloid cells	Total cells/Single cells/Live+/CD45+/CD11b+CD11c+
Total B cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3-CD19+
Total T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-
Total CD4 T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-/CD4+CD8-
Total CD8 T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-/CD4-CD8+
$CD44^{lo}CD62L^{hi}$ CD4 T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-/CD4+CD8-/ $CD44^{lo}CD62L^{hi}$
$CD44^{hi}CD62L^{hi}$ CD4 T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-/CD4+CD8-/ $CD44^{hi}CD62L^{hi}$
$CD44^{hi}CD62L^{lo}$ CD4 T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-/CD4+CD8-/ $CD44^{hi}CD62L^{lo}$
$CD44^{lo}CD62L^{lo}$ CD4 T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-/CD4+CD8-/ $CD44^{lo}CD62L^{lo}$
$CD44^{lo}CD62L^{hi}$ CD8 T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-/CD4-CD8+/ $CD44^{lo}CD62L^{hi}$
$CD44^{hi}CD62L^{hi}$ CD8 T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-/CD4-CD8+/ $CD44^{hi}CD62L^{hi}$
$CD44^{hi}CD62L^{lo}$ CD8 T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-/CD4-CD8+/ $CD44^{hi}CD62L^{lo}$
$CD44^{lo}CD62L^{lo}$ CD8 T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-/CD4-CD8+/ $CD44^{lo}CD62L^{lo}$
Total CD25+CD4+ T cells	Total cells/Single cells/Live+/CD45+/CD11b-CD11c-/ CD3+CD19-/CD4+CD8-/CD25+CD4+