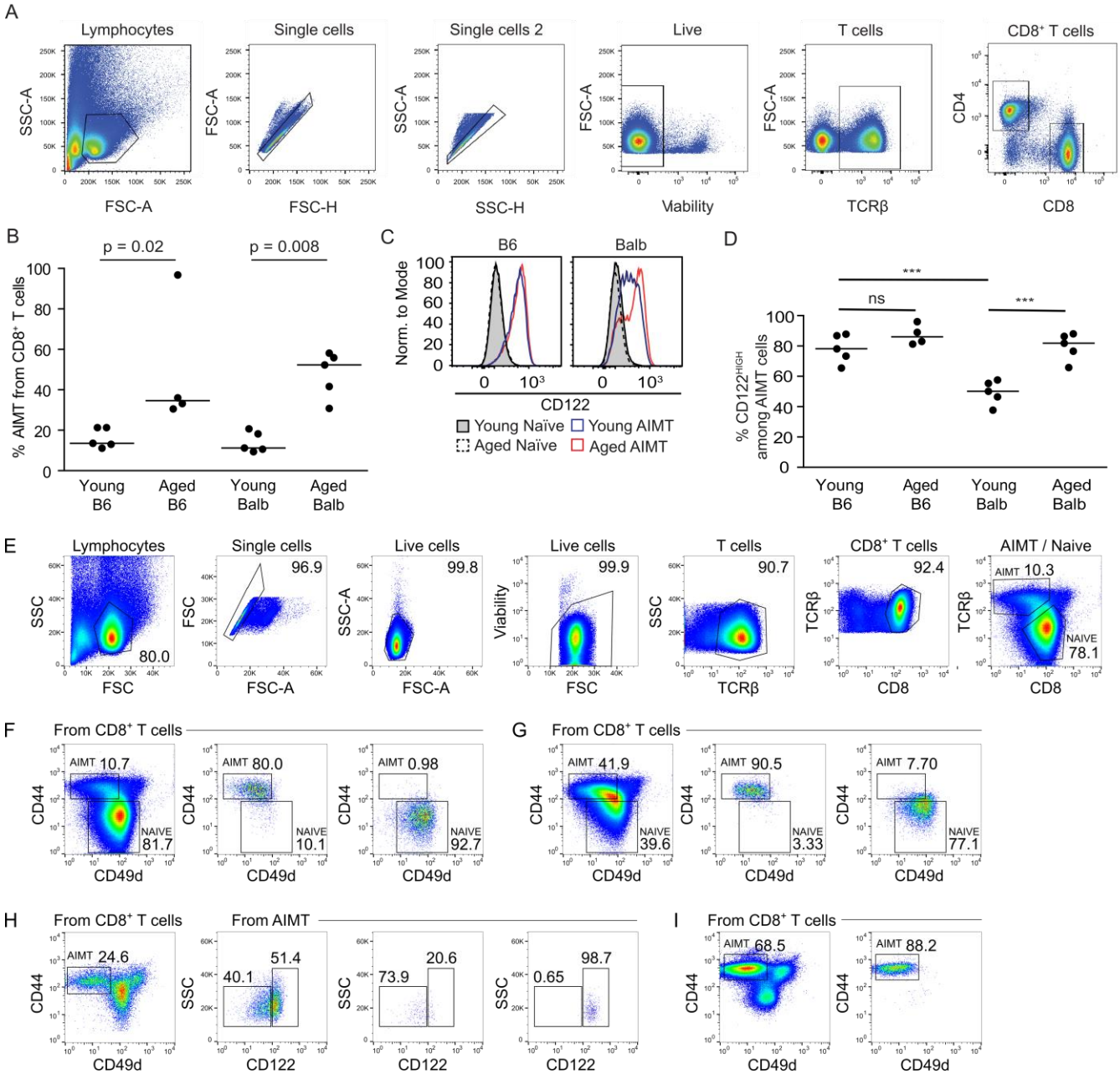
Figure S1


A
Lymphocytes
Single cells
Single cells 2
Live
T cells
CD8⁺ T cells
SSC-A
FSC-A
FSC-H
SSC-H
Viability
TCRβ
CD4
CD8
B
p = 0.02
p = 0.008
% AIMT from CD8⁺ T cells
Young B6
Aged B6
Young Balb
Aged Balb
C
B6
Balb
Norm. to Mode
CD122
Young Naïve
Young AIMT
Aged Naïve
Aged AIMT
D
ns
***
% CD122HIGH among AIMT cells
E
Lymphocytes
Single cells
Live cells
Live cells
T cells
CD8⁺ T cells
AIMT / Naive
80.0
96.9
99.8
99.9
90.7
92.4
AIMT 10.3
NAIVE 78.1
SSC
FSC
FSC-A
SSC-A
Viability
TCRβ
CD8
F
From CD8⁺ T cells
AIMT 10.7
NAIVE 81.7
AIMT 80.0
NAIVE 10.1
AIMT 0.98
NAIVE 92.7
CD44
CD49d
G
From CD8⁺ T cells
AIMT 41.9
NAIVE 39.6
AIMT 90.5
NAIVE 3.33
AIMT 7.70
NAIVE 77.1
H
From CD8⁺ T cells
From AIMT
AIMT 24.6
40.1
51.4
73.9
20.6
0.65
98.7
SSC
CD122
I
From CD8⁺ T cells
AIMT 68.5
AIMT 88.2

Supplemental Figure 1.

(A) Gating strategy for gating of $CD8^+$ T cells in the flow cytometry experiments shown in this study.

(B-D) Analysis of lymph nodes from the same mice as in the experiments in Figure 1A-D. Median.

(B) Quantification of the percentage of $CD44^+$ $CD49d^-$ AIMT cells among CD8 T cells. n=4 (Balb) or 5 (B6) mice from 2-5 independent experiments. The statistical significance was tested using 2-tailed Mann-Whitney test.

(C) Histograms of CD122 expression in $CD44^-$ naïve and $CD44^+$ $CD49d^-$ AIMT cells from indicated mice. A representative experiment out of two in total.

(D) Quantification of $CD122^{HIGH}$ cells among $CD8^+$ AIMT cells. Median. The statistical significance was tested using 1-way ANOVA ($p < 0.0001$) with Bonferroni's Multiple Comparison (post)Tests. *** $p \leq 0.001$.

(E-I) Gating strategies and re-analyses for cell sorting for the mRNA sequencing experiment shown in Fig 3. Representative samples per condition out of 3 in total are shown.

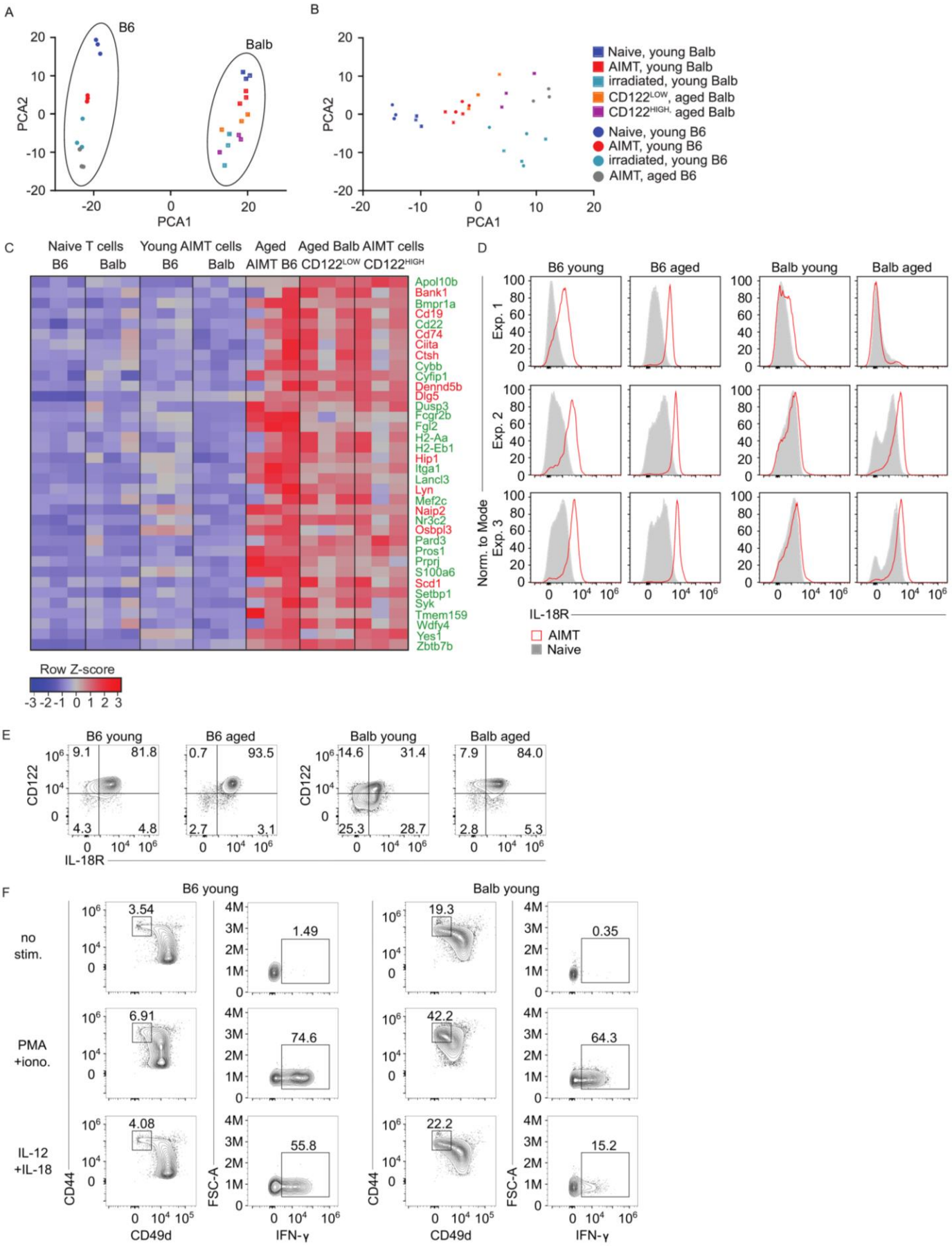
(E) General gating strategy for gating AIMT/Naïve $CD8^+$ T cells for sorting.

(F-G) Naïve and AIMT cell gates for pre-sorted cells (left) and re-analyses of the sorted samples (AIMT – center, Naïve – right) from young B6 (F) or Balb (G) mice.

(H) AIMT cell gate for pre-sorted cells in aged Balb mice (1^{st} panel from the left). Sorting gate (2^{nd} panel) and re-analyses of the sorted samples from aged Balb mice (3^{rd} panel – $CD122^{LOW}$ AIMT, 4^{th} panel –$CD122^{HIGH}$ AIMT).

(I) AIMT cell gate for pre-sorted cells (left) and re-analysis (right) of the sorted sample from aged B6 mice.

Figure S2


A
B6
Balb
PCA2
PCA1
B
Naive, young Balb
AIMT, young Balb
irradiated, young Balb
CD122LOW, aged Balb
CD122HIGH, aged Balb
Naive, young B6
AIMT, young B6
irradiated, young B6
AIMT, aged B6
C
Naive T cells
Young AIMT cells
Aged AIMT B6
Aged Balb AIMT cells
B6
Balb
CD122LOW
CD122HIGH
Apol10b
Bank1
Bmpr1a
Cd19
Cd22
Cd74
Ciita
Ctsh
Cybb
Cyfip1
Dennd5b
Dlg5
Dusp3
Fcgr2b
Fgl2
H2-Aa
H2-Eb1
Hip1
Itga1
Lancl3
Lyn
Mef2c
Naip2
Nr3c2
Osbpl3
Pard3
Pros1
Prprj
S100a6
Scd1
Setbp1
Syk
Tmem159
Wdfy4
Yes1
Zbtb7b
Row Z-score
D
B6 young
B6 aged
Balb young
Balb aged
Exp. 1
Exp. 2
Exp. 3
Norm. to Mode
IL-18R
AIMT
Naive
E
B6 young
B6 aged
Balb young
Balb aged
CD122
IL-18R
9.1
81.8
4.3
4.8
0.7
93.5
2.7
3.1
14.6
31.4
25.3
28.7
7.9
84.0
2.8
5.3
F
B6 young
Balb young
no stim.
PMA +iono.
IL-12 +IL-18
CD44
CD49d
FSC-A
IFN-γ
3.54
1.49
19.3
0.35
6.91
74.6
42.2
64.3
4.08
55.8
22.2
15.2

Supplemental Figure 2.

(A-B) PCA analysis (top 500 variable genes) of the gene expression profiles of the individual samples (Fig. S1E-I, Fig. 3) prior to the normalization between strains (A) and after removing genes differentially expressed between strains (B).

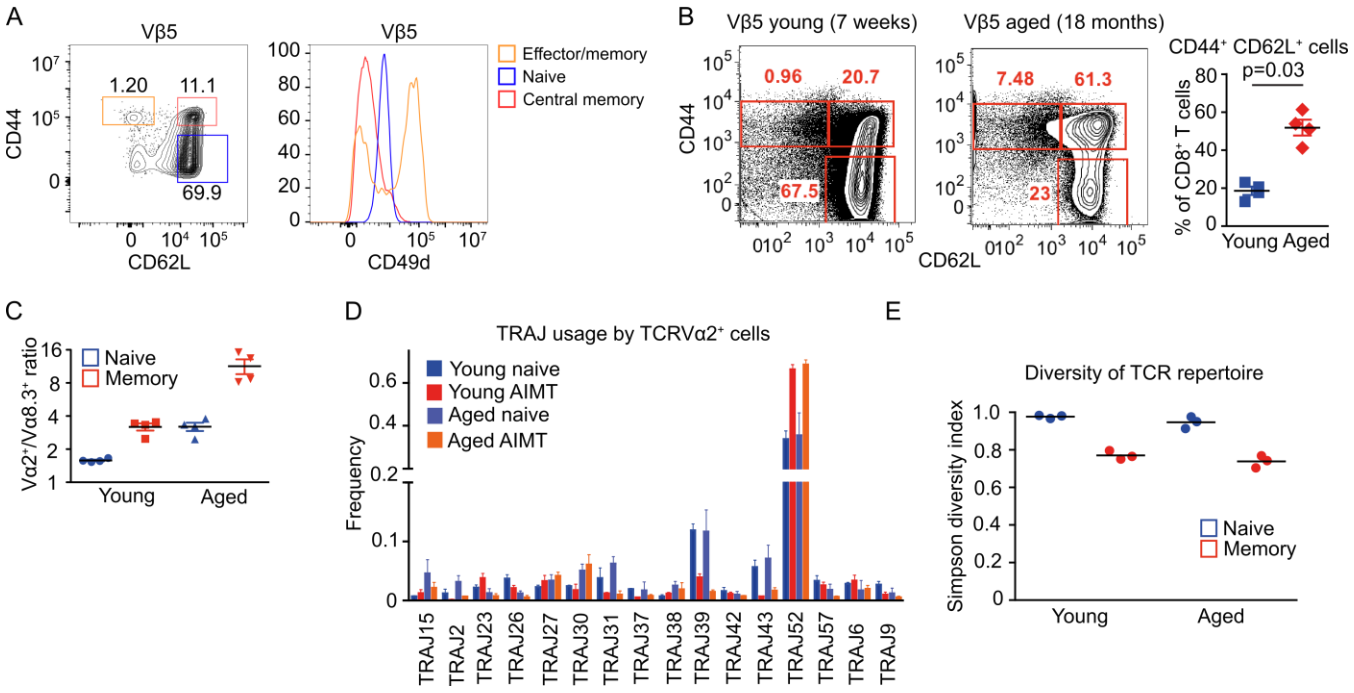
(C) Heatmap showing relative expression of 36 genes showing significant upregulation in AIMT cells from aged mice in comparison to AIMT cells in young mice in B6 and Balb strains (both in $CD122^{HIGH}$ and $CD122^{LOW}$ AIMT cells from aged Balb mice). Names of genes upregulated in AIMT cells from aged B6 mice in comparison to young B6 mice in the previously published dataset [36] are in green.

(D) Surface levels of IL-18R in naïve and AIMT $CD8^+$ T cells from young and aged Balb and B6 mice measured by flow cytometry. Supplemental histograms for the experiment shown in Fig. 3G. Three independent experiments are shown.

(E) Surface levels of IL-18R and CD122 in AIMT $CD8^+$ T cells from young and aged Balb and B6 mice measured by flow cytometry. A representative experiment out of three in total (same experiments as shown in Figure S2D).

(F) Production of IFN-γ by AIMT cells (gated as $CD8^+$ $CD44^+$ $CD49d^-$) isolated from young B6 or Balb mice measured by flow cytometry. Supplemental representative contour plots for the experiment shown in Fig. 3H. A representative experiment out of three in total (same experiments as shown in Figure S2D).

Figure S3



Supplemental Figure 3.

(A) Expression of the CD49d marker on naïve ($CD44^-$ $CD62L^+$), central memory phenotype ($CD44^+$ $CD62L^+$), and effector/memory ($CD44^+$ $CD62L^+$) cells in the lymph nodes of young Vβ5 mice. A representative sample is shown out of 5 independent experiments.

(B-C) Analysis of the same young and aged Vβ5 mice as the experiment in Fig. 4A.

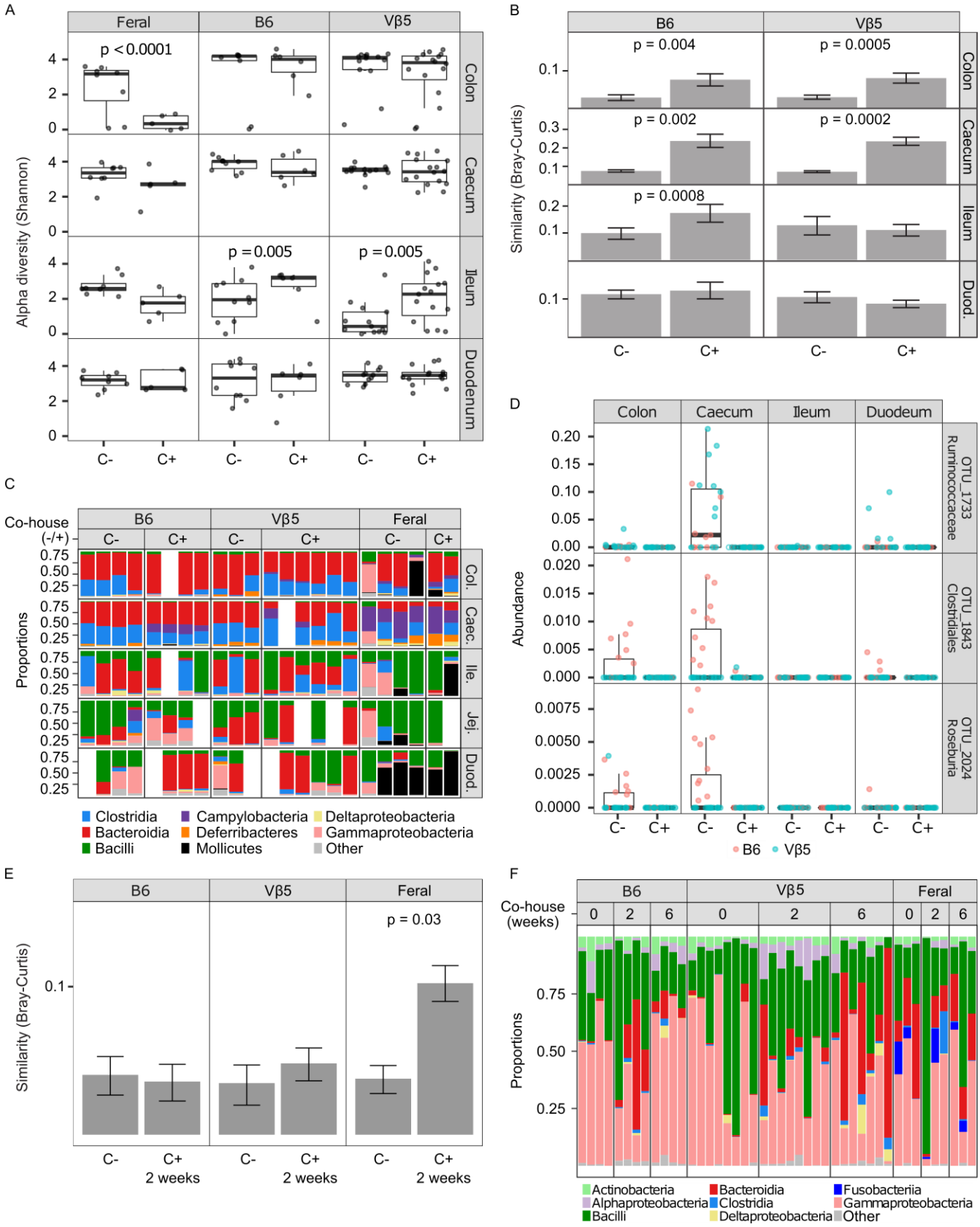
(B) Percentage of naïve ($CD44^-$ $CD62L^+$), AIMT ($CD44^+$ $CD62L^+$), and effector/memory ($CD44^+$ $CD62L^-$) cells in the lymph nodes of young and aged mice. Mean ± SEM is shown. A representative experiment and the quantification of 4 mice per group are shown. Statistical significance was calculated using Mann-Whitney test.

(C) Quantification of the ratio between the TCRVα2 and TCRVα8.3 T cells among AIMT or naïve $CD8^+$ T cells in Vβ5 young or aged mice. Mean ± SEM is shown.

(D) Quantification of the TRAJ usage by the indicated mice. Mean + SEM. Analysis of the same experiment as shown in Fig. 4B-F.

(E) Diversity of the CDR3 among TRAV14 TCRα in naïve and AIMT CD8+ T cells isolated from Vβ5 mice was estimated using Simpson diversity index. Mean is shown. The statistical significance was calculated using one-way ANOVA with Bonferroni's Multiple Comparison Posttest. ns; $p>0.05$. Analysis of the same experiment as shown in Fig. 4B-F.

Figure S4


A
Feral
B6
Vβ5
p < 0.0001
p = 0.005
p = 0.005
Colon
Caecum
Ileum
Duodenum
Alpha diversity (Shannon)
C-
C+
B
B6
Vβ5
p = 0.004
p = 0.0005
p = 0.002
p = 0.0002
p = 0.0008
Similarity (Bray-Curtis)
Colon
Caecum
Ileum
Duod.
C
Co-house (-/+)
B6
Vβ5
Feral
Proportions
Col.
Caec.
Ile.
Jej.
Duod.
Clostridia
Bacteroidia
Bacilli
Campylobacteria
Deferribacteres
Mollicutes
Deltaproteobacteria
Gammaproteobacteria
Other
D
Colon
Caecum
Ileum
Duodeum
Abundance
OTU_1733 Ruminococcaceae
OTU_1843 Clostridiales
OTU_2024 Roseburia
B6
Vβ5
E
B6
Vβ5
Feral
p = 0.03
Similarity (Bray-Curtis)
C-
C+
2 weeks
F
B6
Vβ5
Feral
Co-house (weeks)
0
2
6
Proportions
Actinobacteria
Alphaproteobacteria
Bacilli
Bacteroidia
Clostridia
Deltaproteobacteria
Fusobacteriia
Gammaproteobacteria
Other

Supplemental Figure 4.

(A-B) Analysis of the experiment shown in Figure 6A-C.

(A) Shannon-index based alpha diversity of the gut microbiota of feral and laboratory mice co-housed together (C+) or non-co-housed (C-). The statistical significance was tested using linear mixed effect model, while considering individual identity as a random effect and the effect of treatment level (co-housed vs non-co-housed), gut section, and their interactions as explanatory variables.

(B) Average Bray-Curtis similarity score of intestinal microbiota between laboratory B6 or Vβ5 mice co-housed (C+) or non-co-housed (C-) with feral mice and non-co-housed feral mice. Error bars correspond to 95% bootstrap confidence intervals, permutation-based p-values are shown for significant differences ($p < 0.05$). Duod. – Duodenum.

(C) Taxonomical composition of the intestinal microbiota of laboratory co-housed (C+) and non-co-housed (C-) B6, Vβ5, and feral mice (Experiment A, see Methods). Color bars represent proportions of dominant bacterial classes in each sample. Col. – Colon, Caec. – Caecum, Ile. – Ileum, Jej. – Jejunum, Duod. – Duodenum.

(D) Three most abundant operational taxonomic units with lower relative abundance in co-housed (C+) than non-co-housed (C-) B6 and Vβ5 laboratory mice. (Experiment B, see Methods).

(E) Average Bray-Curtis similarity of the salivary microbiota of co-housed (C+) or non-co-housed (C-) laboratory B6 or Vβ5 mice with feral mice to conventional feral mice (left, center), or co-housed or non-co-housed feral mice to laboratory B6 and Vβ5 controls (right) in the experiment shown in Figure 6D. Error bars correspond to 95% bootstrap confidence intervals. Permutation-based p-value is shown for significant differences ($p < 0.05$).

(F) Taxonomical composition of the salivary microbiota of laboratory B6 or Vβ5 mice co-housed vs. non-co-housed with feral mice (Experiment A, see Methods). Color bars represent proportions of dominant bacterial classes in each sample. Samples were collected from each mouse prior to the co-housing (0 weeks) and after 2 and 6 weeks during the co-housing experiment.