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Long-term antibiotic exposure promotes mortality after systemic fungal infection by driving lymphocyte dysfunction and systemic escape of commensal bacteria

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Fig 1

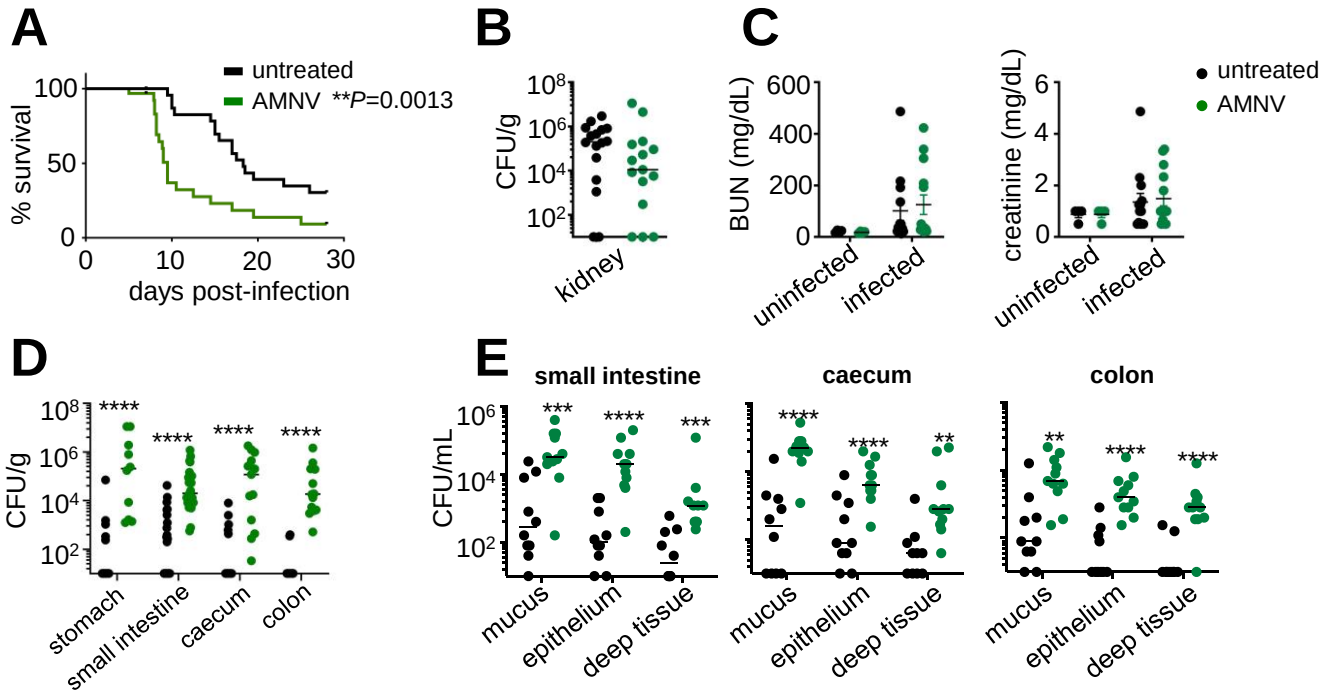

A
untreated
AMNV **P=0.0013
% survival
100
50
0
0
10
20
30
days post-infection
B
CFU/g
10^8
10^6
10^4
10^2
kidney
C
BUN (mg/dL)
600
400
200
0
uninfected
infected
creatinine (mg/dL)
6
4
2
0
uninfected
infected
untreated
AMNV
D
CFU/g
10^8
10^6
10^4
10^2
****
****
****
****
stomach
small intestine
caecum
colon
E
small intestine
caecum
colon
CFU/mL
10^6
10^4
10^2
***
****
***
****
****
**
**
****
****
mucus
epithelium
deep tissue
mucus
epithelium
deep tissue
mucus
epithelium
deep tissue

Fig 2

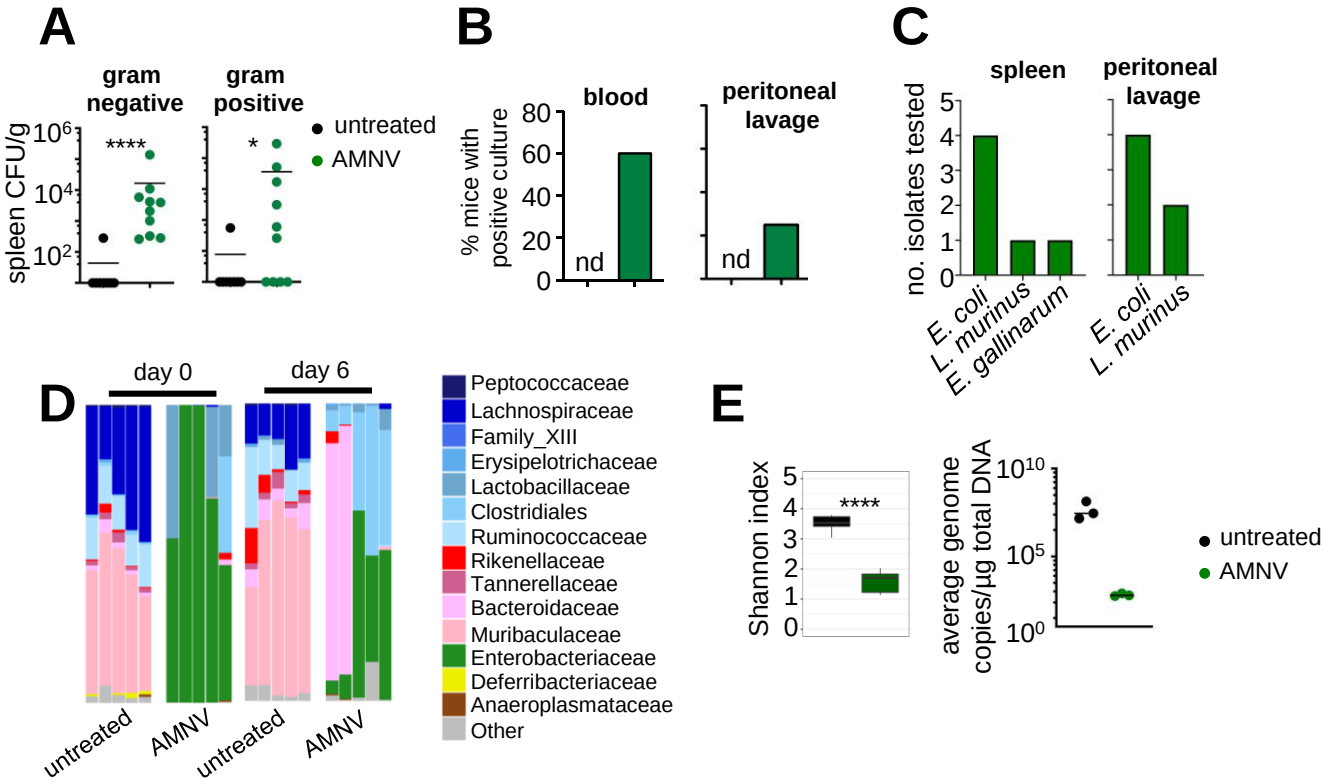

A
gram negative
gram positive
spleen CFU/g
10^6
10^4
10^2
****
*
untreated
AMNV
B
blood
peritoneal lavage
% mice with positive culture
80
60
40
20
0
nd
nd
C
spleen
peritoneal lavage
no. isolates tested
5
4
3
2
1
0
E. coli
L. murinus
E. gallinarum
E. coli
L. murinus
D
day 0
day 6
untreated
AMNV
untreated
AMNV
Peptococcaceae
Lachnospiraceae
Family_XIII
Erysipelotrichaceae
Lactobacillaceae
Clostridiales
Ruminococcaceae
Rikenellaceae
Tannerellaceae
Bacteroidaceae
Muribaculaceae
Enterobacteriaceae
Deferribacteriaceae
Anaeroplasmataceae
Other
E
Shannon index
5
4
3
2
1
0
****
average genome copies/µg total DNA
10^{10}
10^5
10^0
untreated
AMNV

Fig 3

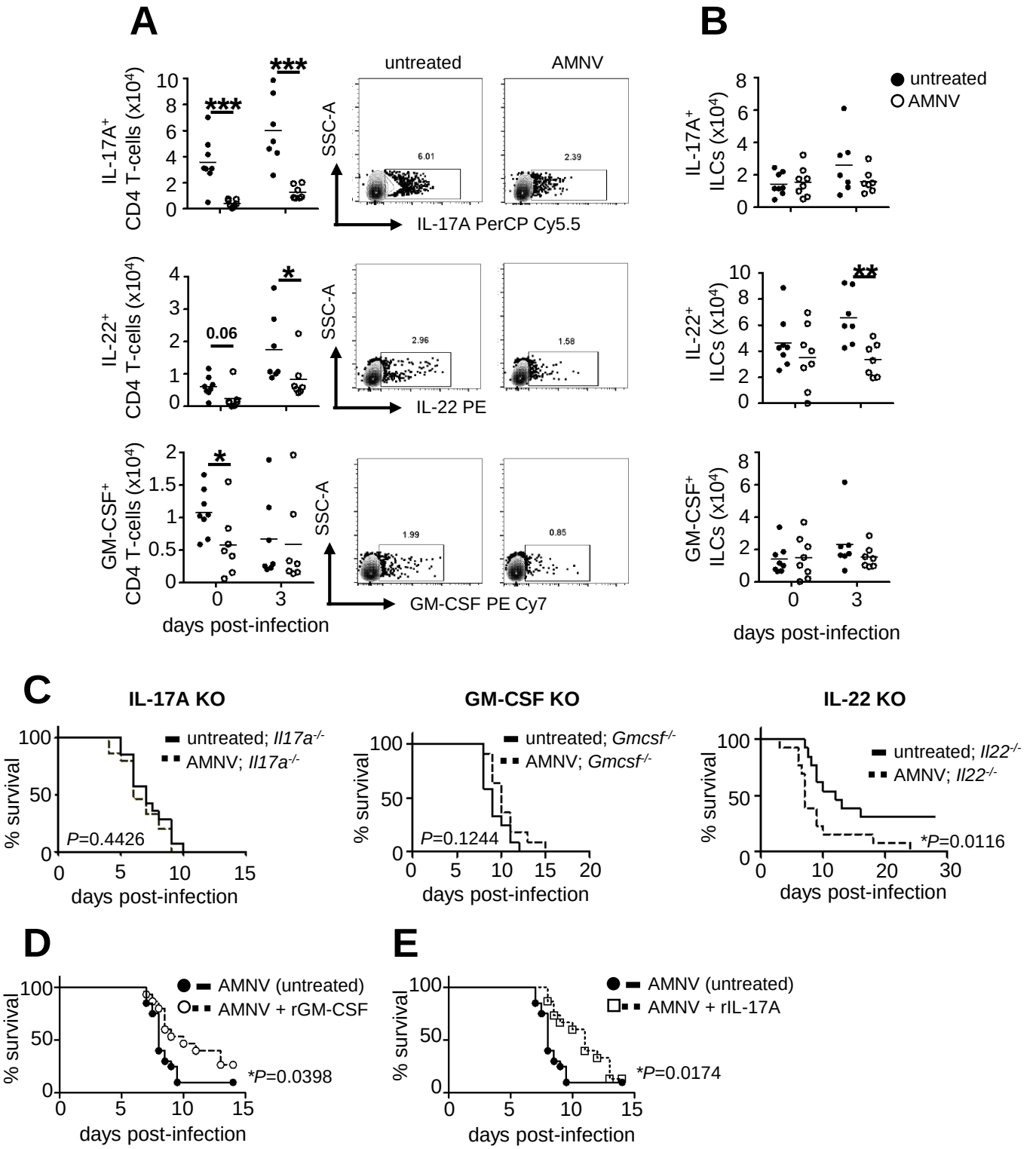

A
B
untreated
AMNV
IL-17A+ CD4 T-cells (x10^4)
IL-22+ CD4 T-cells (x10^4)
GM-CSF+ CD4 T-cells (x10^4)
SSC-A
IL-17A PerCP Cy5.5
IL-22 PE
GM-CSF PE Cy7
6.01
2.39
2.96
1.58
1.99
0.85
0.06
days post-infection
IL-17A+ ILCs (x10^4)
IL-22+ ILCs (x10^4)
GM-CSF+ ILCs (x10^4)
C
IL-17A KO
GM-CSF KO
IL-22 KO
% survival
untreated; Il17a-/-
AMNV; Il17a-/-
P=0.4426
untreated; Gmcsf-/-
AMNV; Gmcsf-/-
P=0.1244
untreated; Il22-/-
AMNV; Il22-/-
*P=0.0116
D
AMNV (untreated)
AMNV + rGM-CSF
*P=0.0398
E
AMNV + rIL-17A
*P=0.0174

Fig 4

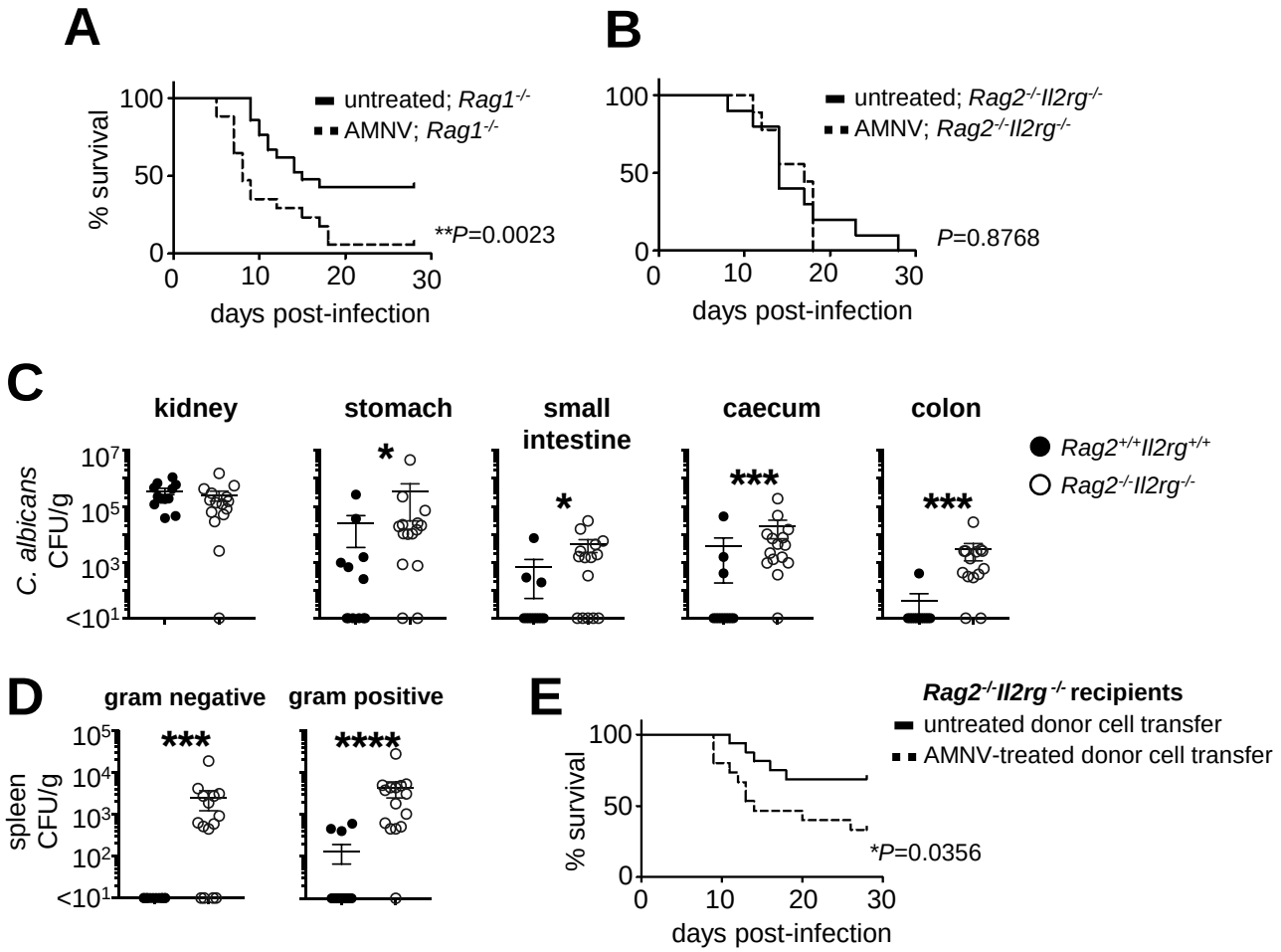

A
% survival
100
50
0
0
10
20
30
days post-infection
untreated; Rag1-/-
AMNV; Rag1-/-
**P=0.0023
B
100
50
0
0
10
20
30
days post-infection
untreated; Rag2-/-Il2rg-/-
AMNV; Rag2-/-Il2rg-/-
P=0.8768
C
kidney
stomach
small intestine
caecum
colon
C. albicans CFU/g
10^7
10^5
10^3
<10^1
*
*
***
***
Rag2+/+Il2rg+/+
Rag2-/-Il2rg-/-
D
gram negative
gram positive
spleen CFU/g
10^5
10^4
10^3
10^2
<10^1
***
****
E
Rag2-/-Il2rg-/- recipients
untreated donor cell transfer
AMNV-treated donor cell transfer
% survival
100
50
0
0
10
20
30
days post-infection
*P=0.0356

Fig 5

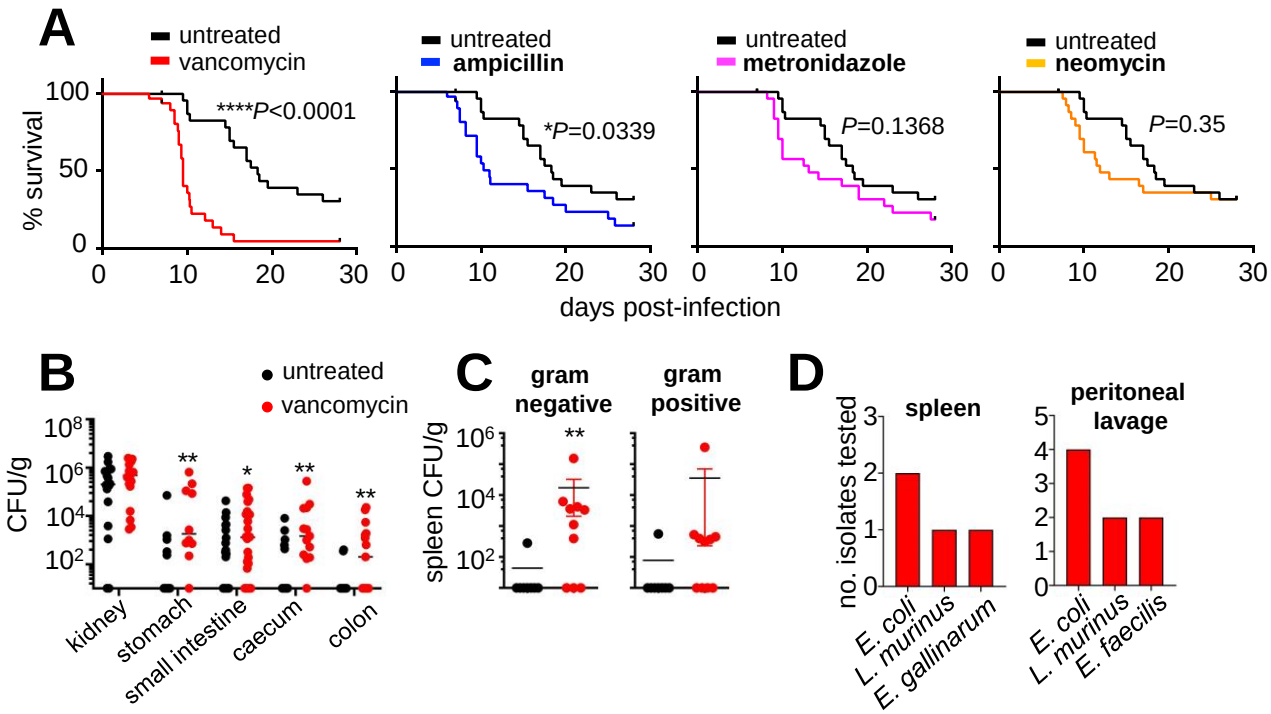

A
untreated
vancomycin
****P<0.0001
% survival
100
50
0
0
10
20
30
untreated
ampicillin
*P=0.0339
untreated
metronidazole
P=0.1368
untreated
neomycin
P=0.35
days post-infection
B
untreated
vancomycin
CFU/g
10^8
10^6
10^4
10^2
**
*
**
**
kidney
stomach
small intestine
caecum
colon
C
gram negative
gram positive
spleen CFU/g
10^6
10^4
10^2
**
D
spleen
peritoneal lavage
no. isolates tested
E. coli
L. murinus
E. gallinarum
E. faecilis

Fig 6

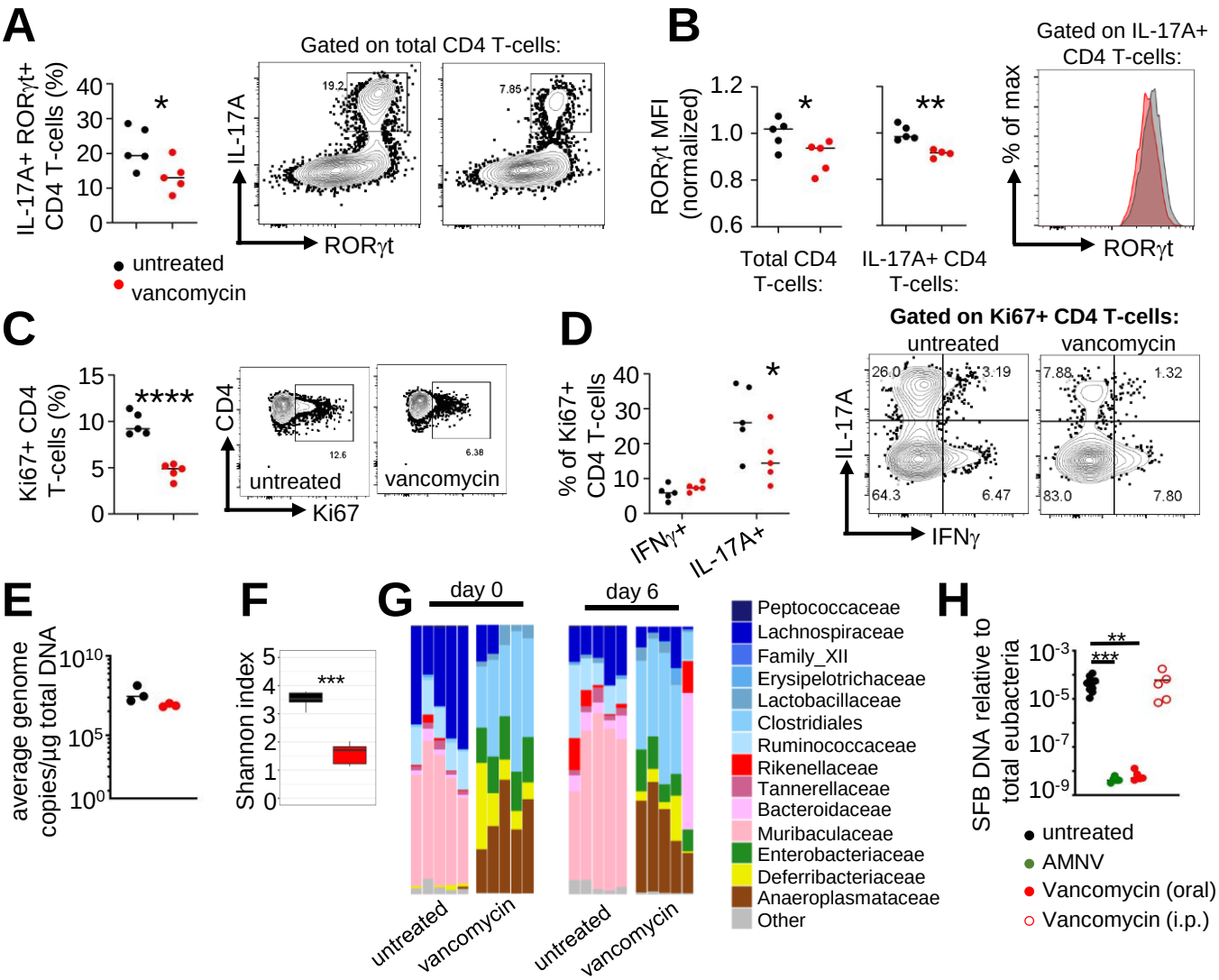

A
IL-17A+ RORγt+ CD4 T-cells (%)
Gated on total CD4 T-cells:
IL-17A
RORγt
19.2
7.85
untreated
vancomycin
B
RORγt MFI (normalized)
Total CD4 T-cells:
IL-17A+ CD4 T-cells:
Gated on IL-17A+ CD4 T-cells:
% of max
RORγt
C
Ki67+ CD4 T-cells (%)
CD4
Ki67
untreated
vancomycin
12.6
6.38
D
% of Ki67+ CD4 T-cells
IFNγ+
IL-17A+
Gated on Ki67+ CD4 T-cells:
untreated
vancomycin
IL-17A
IFNγ
26.0
3.19
64.3
6.47
7.88
1.32
83.0
7.80
E
average genome copies/µg total DNA
F
Shannon index
G
day 0
day 6
untreated
vancomycin
Peptococcaceae
Lachnospiraceae
Family_XII
Erysipelotrichaceae
Lactobacillaceae
Clostridiales
Ruminococcaceae
Rikenellaceae
Tannerellaceae
Bacteroidaceae
Muribaculaceae
Enterobacteriaceae
Deferribacteriaceae
Anaeroplasmataceae
Other
H
SFB DNA relative to total eubacteria
untreated
AMNV
Vancomycin (oral)
Vancomycin (i.p.)

Fig S1

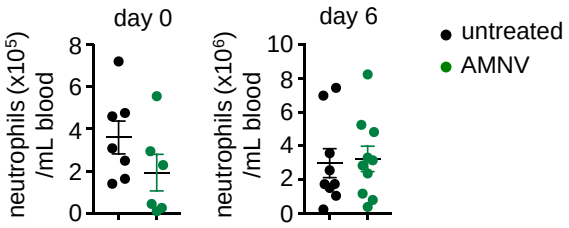


Fig S2

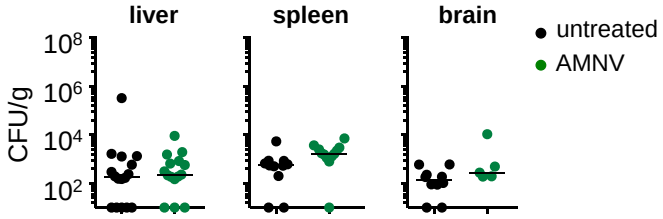


Fig S3

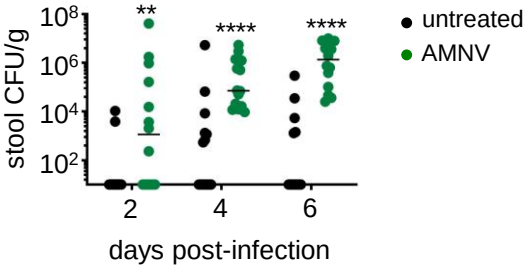


Fig S4

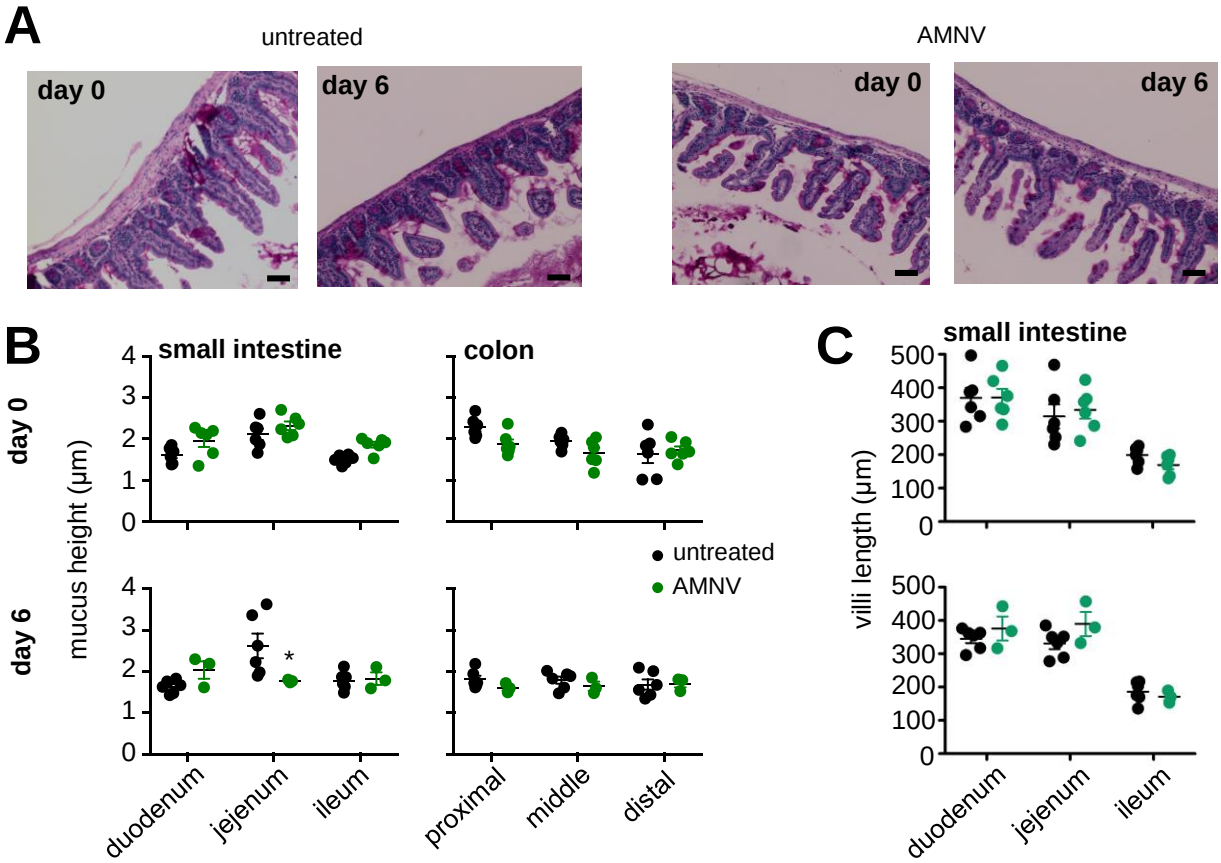
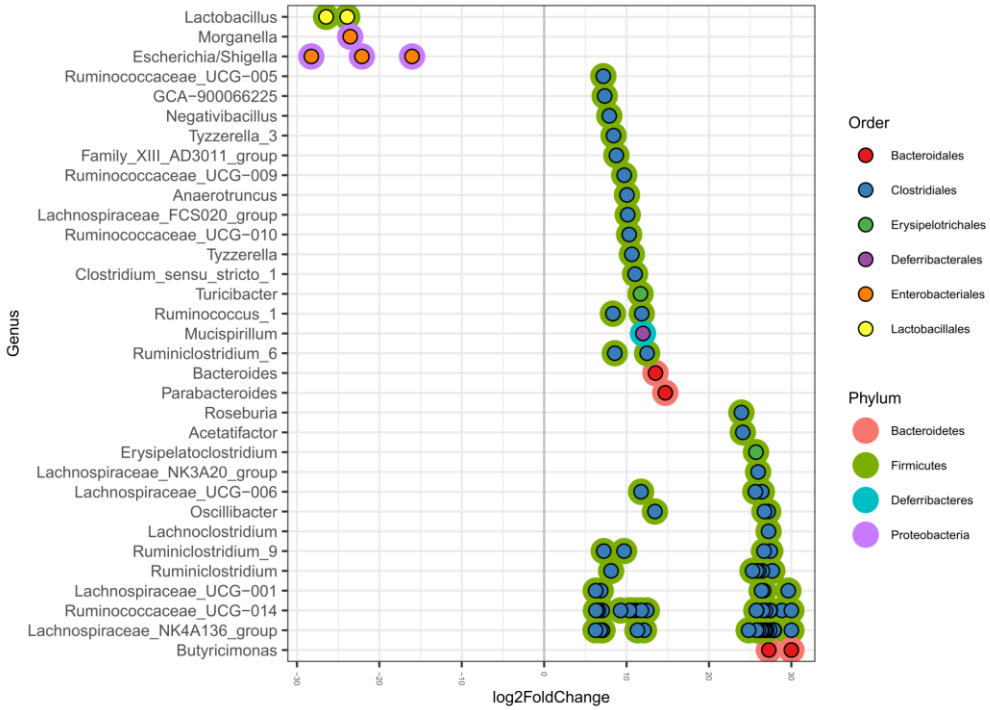

A
untreated
AMNV
day 0
day 6
day 0
day 6
B
small intestine
colon
day 0
day 6
mucus height (µm)
untreated
AMNV
*
duodenum
jejenum
ileum
proximal
middle
distal
C
small intestine
villi length (µm)
duodenum
jejenum
ileum

Fig S5

A


Lactobacillus
Morganella
Escherichia/Shigella
Ruminococcaceae_UCG−005
GCA−900066225
Negativibacillus
Tyzzerella_3
Family_XIII_AD3011_group
Ruminococcaceae_UCG−009
Anaerotruncus
Lachnospiraceae_FCS020_group
Ruminococcaceae_UCG−010
Tyzzerella
Clostridium_sensu_stricto_1
Turicibacter
Ruminococcus_1
Mucispirillum
Ruminiclostridium_6
Bacteroides
Parabacteroides
Roseburia
Acetatifactor
Erysipelatoclostridium
Lachnospiraceae_NK3A20_group
Lachnospiraceae_UCG−006
Oscillibacter
Lachnoclostridium
Ruminiclostridium_9
Ruminiclostridium
Lachnospiraceae_UCG−001
Ruminococcaceae_UCG−014
Lachnospiraceae_NK4A136_group
Butyricimonas
Genus
log2FoldChange
Order
Bacteroidales
Clostridiales
Erysipelotrichales
Deferribacterales
Enterobacteriales
Lactobacillales
Phylum
Bacteroidetes
Firmicutes
Deferribacteres
Proteobacteria

B

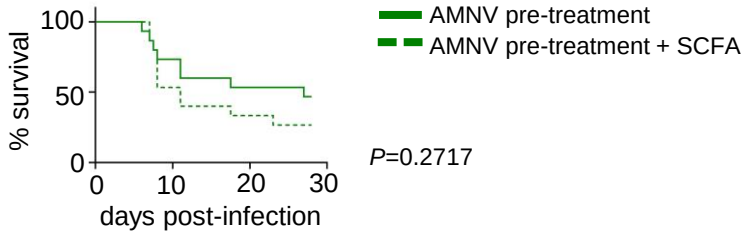

100
50
0
% survival
0
10
20
30
days post-infection
AMNV pre-treatment
AMNV pre-treatment + SCFA
P=0.2717

Fig S6

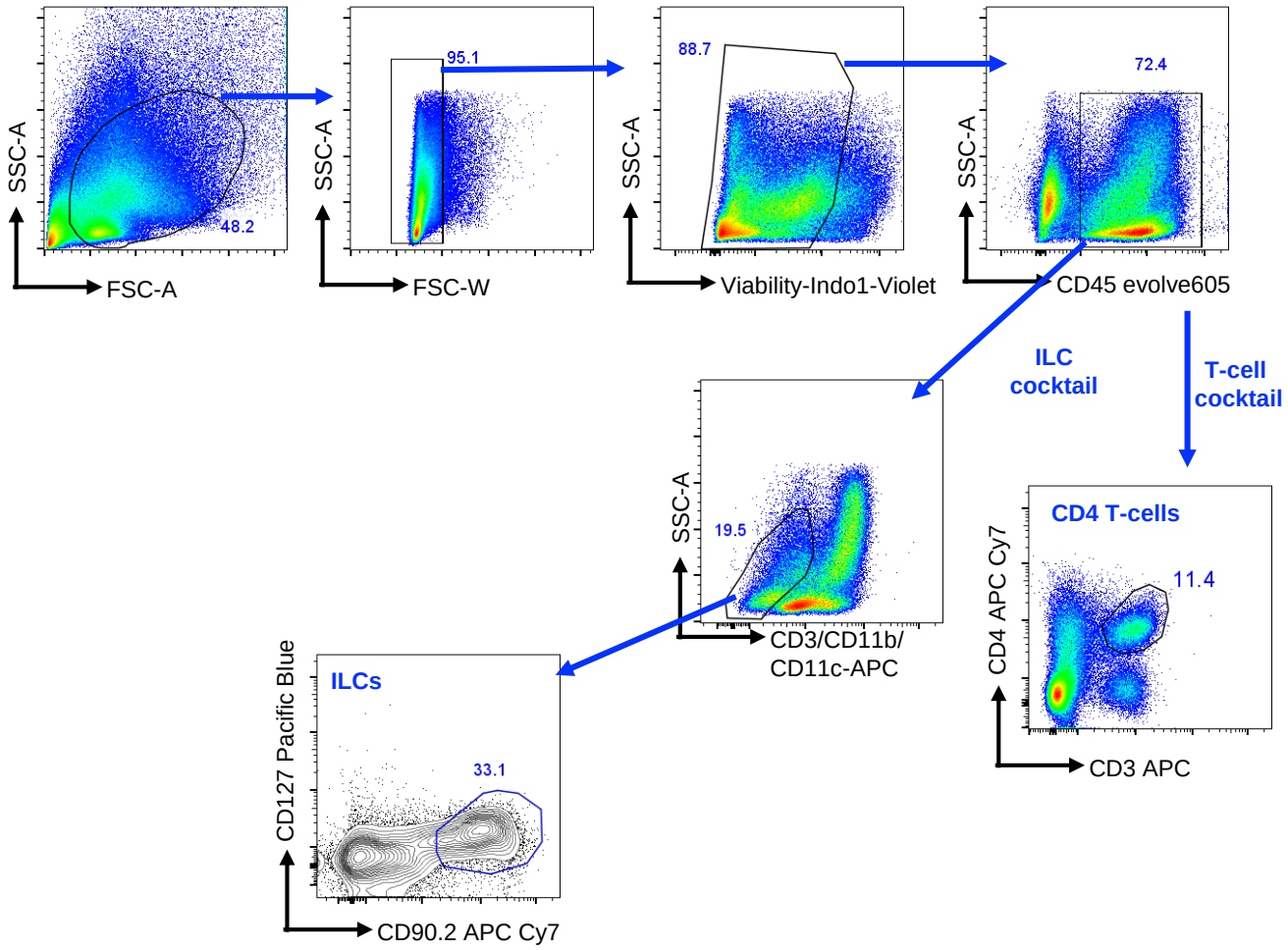

SSC-A
FSC-A
48.2
SSC-A
FSC-W
95.1
SSC-A
Viability-Indo1-Violet
88.7
SSC-A
CD45 evolve605
72.4
ILC cocktail
T-cell cocktail
SSC-A
CD3/CD11b/ CD11c-APC
19.5
CD4 T-cells
CD4 APC Cy7
CD3 APC
11.4
ILCs
CD127 Pacific Blue
CD90.2 APC Cy7
33.1

Fig S7

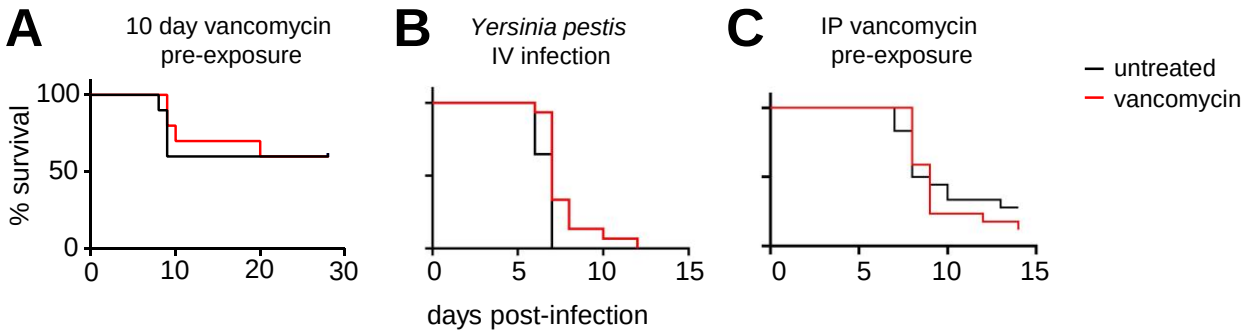

A
10 day vancomycin pre-exposure
% survival
100
50
0
0
10
20
30
B
Yersinia pestis IV infection
0
5
10
15
days post-infection
C
IP vancomycin pre-exposure
0
5
10
15
untreated
vancomycin

Fig S8

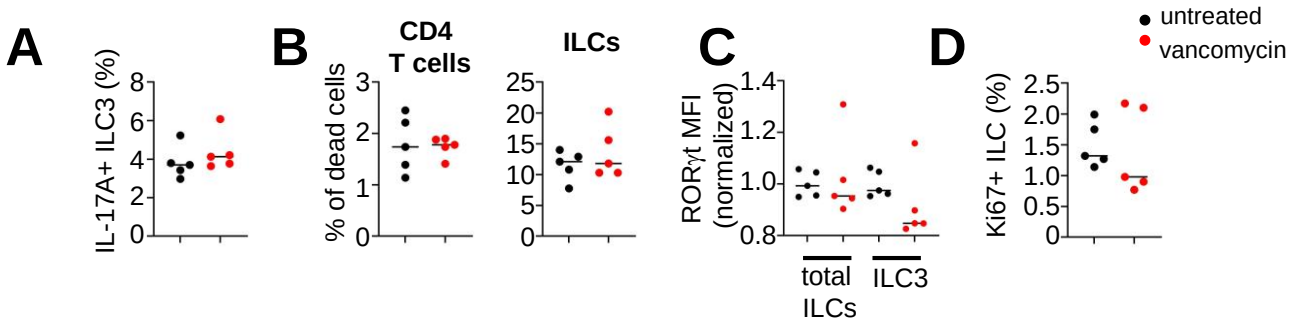
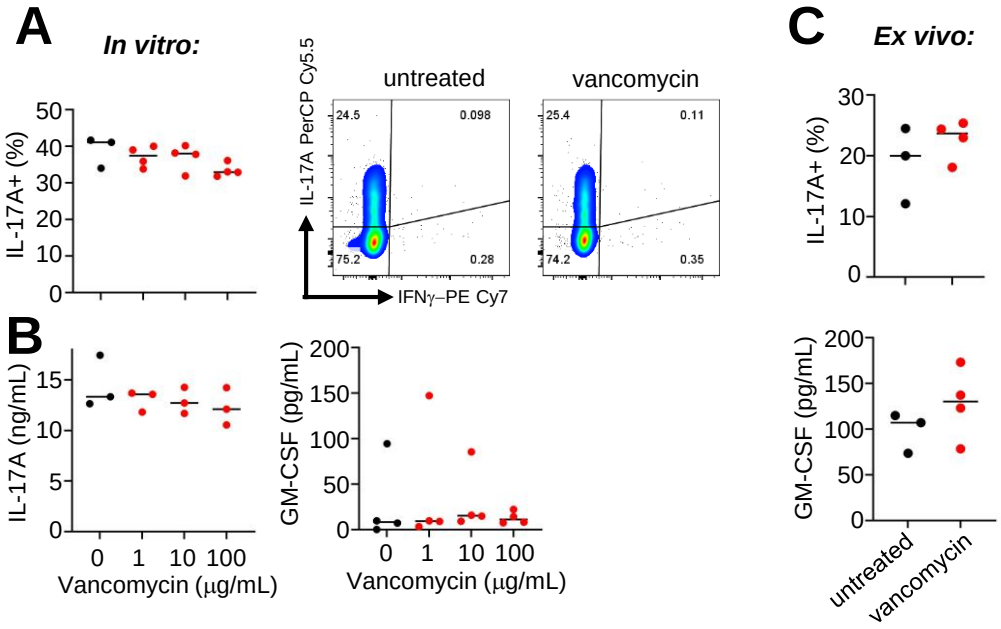

A
IL-17A+ ILC3 (%)
8
6
4
2
0
B
CD4 T cells
% of dead cells
3
2
1
0
ILCs
25
20
15
10
5
0
C
RORγt MFI (normalized)
1.4
1.2
1.0
0.8
total ILCs
ILC3
D
Ki67+ ILC (%)
2.5
2.0
1.5
1.0
0.5
0
untreated
vancomycin

Fig S9


A
In vitro:
IL-17A+ (%)
50
40
30
20
10
0
untreated
vancomycin
IL-17A PerCP Cy5.5
24.5
0.098
75.2
0.28
25.4
0.11
74.2
0.35
IFNγ–PE Cy7
B
IL-17A (ng/mL)
15
10
5
0
0 1 10 100
Vancomycin (μg/mL)
GM-CSF (pg/mL)
200
150
100
50
0
0 1 10 100
Vancomycin (μg/mL)
C
Ex vivo:
IL-17A+ (%)
30
20
10
0
GM-CSF (pg/mL)
200
150
100
50
0
untreated
vancomycin