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Supporting Information for

IL-33 controls IL-22-dependent antibacterial defense by modulating the microbiota

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This PDF file includes:

Figures S1 to S8

Other supporting materials for this manuscript include the following:

Raw sequencing data for Figures 2, 5, S4 and S8 are uploaded on NCBI GEO repository: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE236344

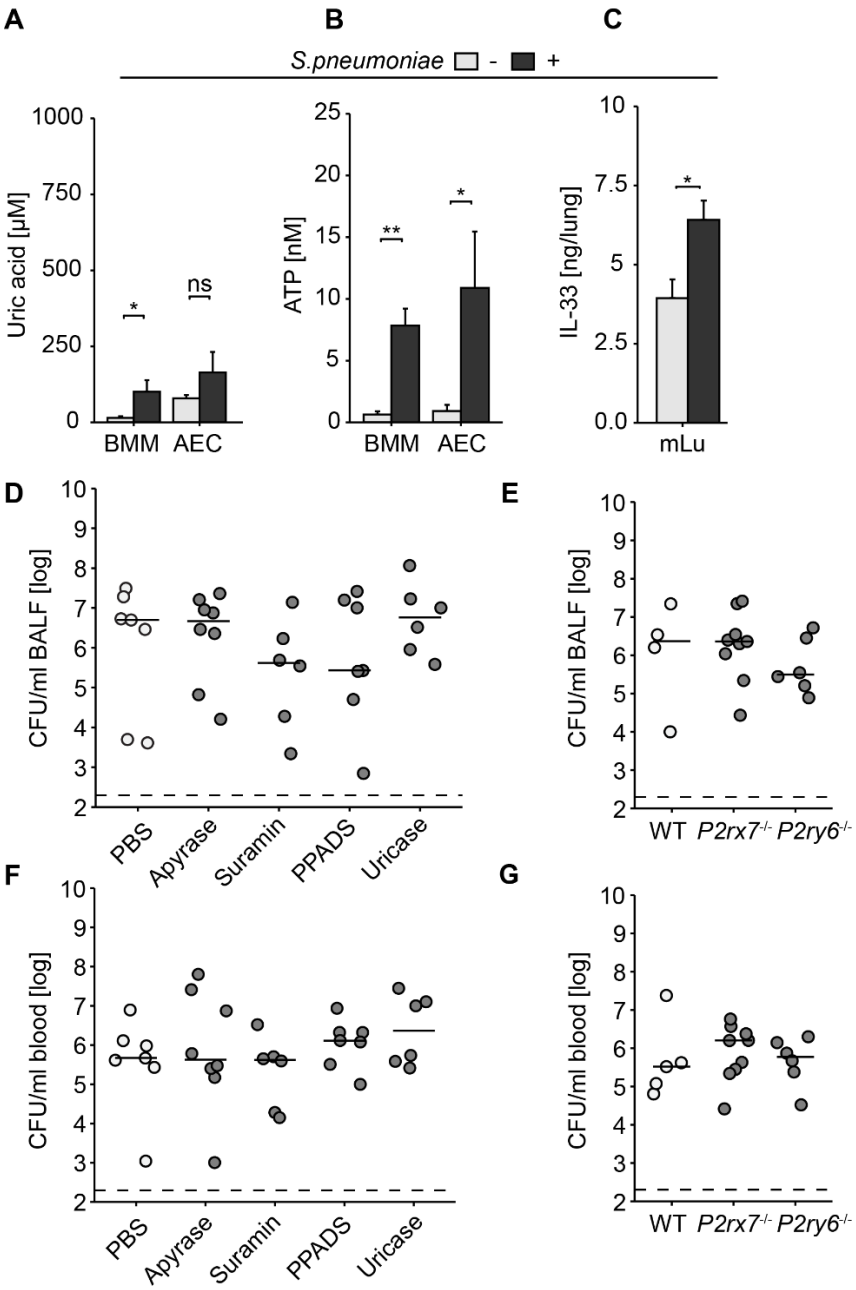

Figure S1. Role of alarmins in *S. pneumoniae* infection. (**A**-**C**) Murine bone marrow-derived macrophages (BMM), alveolar epithelial cells (AEC) and murine lung tissue (mLu) were left untreated or infected with 10^6 CFU of *S. pneumoniae* for 16-24 h. Uric acid (**A**), ATP (**B**) and IL-33 (**C**) were measured. Data are shown as mean + SEM (n = 8-14 per group); Wilcoxon rank sum test; ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$. (**D-G)**

C57BL/6J WT mice were left untreated or treated with different alarmin inhibitors (n = 7-8 per group) and WT (n = 5), *P2rx7*$^{-/-}$ (n = 9) and *P2rx6*$^{-/-}$ mice (n = 6) were infected with *S. pneumoniae* and bacterial loads in BALF (**D, E**) and blood (**F, G**) were measured. Data are shown as individual points, lines represent median and dashed lines the lower detection limit.

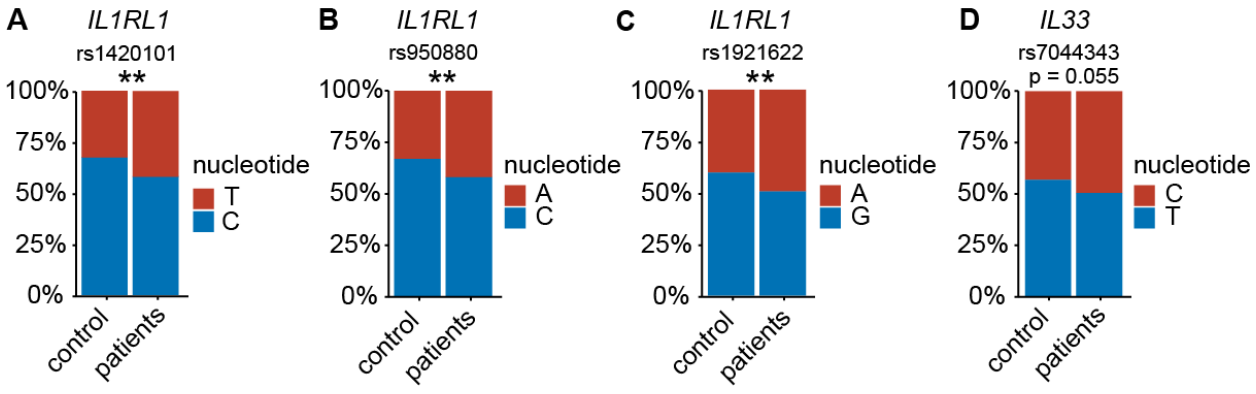

Figure S2. SNPs in *IL33* and *IL1RL1* are associated with pneumococcal pneumonia. (**A-D**) Frequencies of SNP alleles of the *IL33* and *IL1RL1* genes were assessed in 238 patients with community-acquired pneumococcal pneumonia and 238 age- and sex- matched controls. Allele frequencies are visualized; Fisher's exact test, ** = $p < 0.01$.

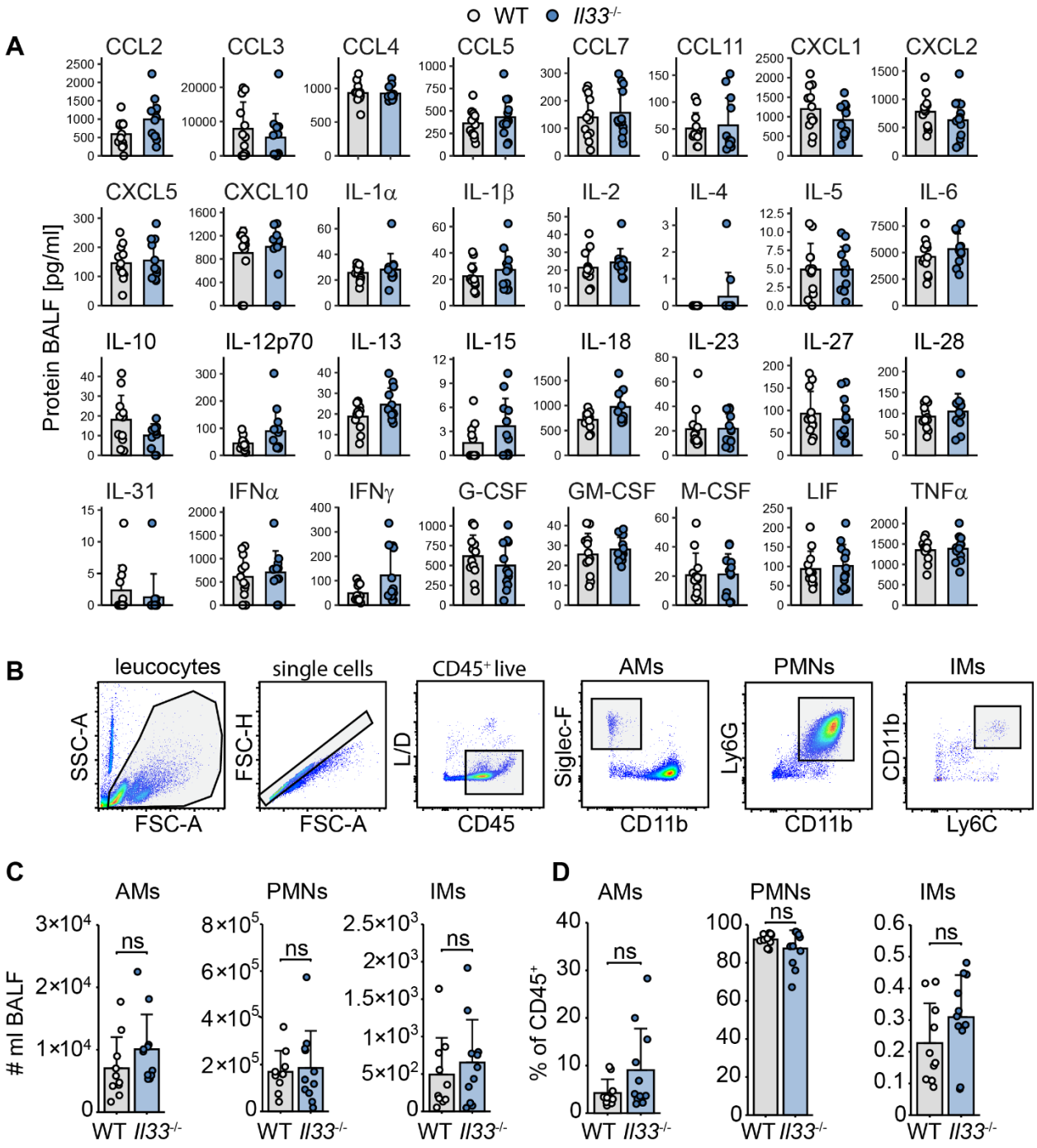

Figure S3. IL-33 deficiency does not influence production of several inflammatory cytokines or number or proportion of major innate leukocytes. (**A**) WT and Il33$^{-/-}$ mice were intranasally infected with *S. pneumoniae*. After 18 h, cytokine and chemokine levels in BALF were quantified by multiplex ELISA (n = 16 per group). (**B**) Representative gating strategy to analyze macrophages,

PMNs and inflammatory monocytes (IMs) by flow cytometry. (**C, D**) WT (n = 10) and *Il33*$^{-/-}$ mice (n = 11) were infected, sacrificed after 18 h, and absolute numbers and frequencies of leucocytes were measured in BALF by flow cytometry. Bars represent mean + SD, Wilcoxon rank sum test; ns = $p > 0.05$.

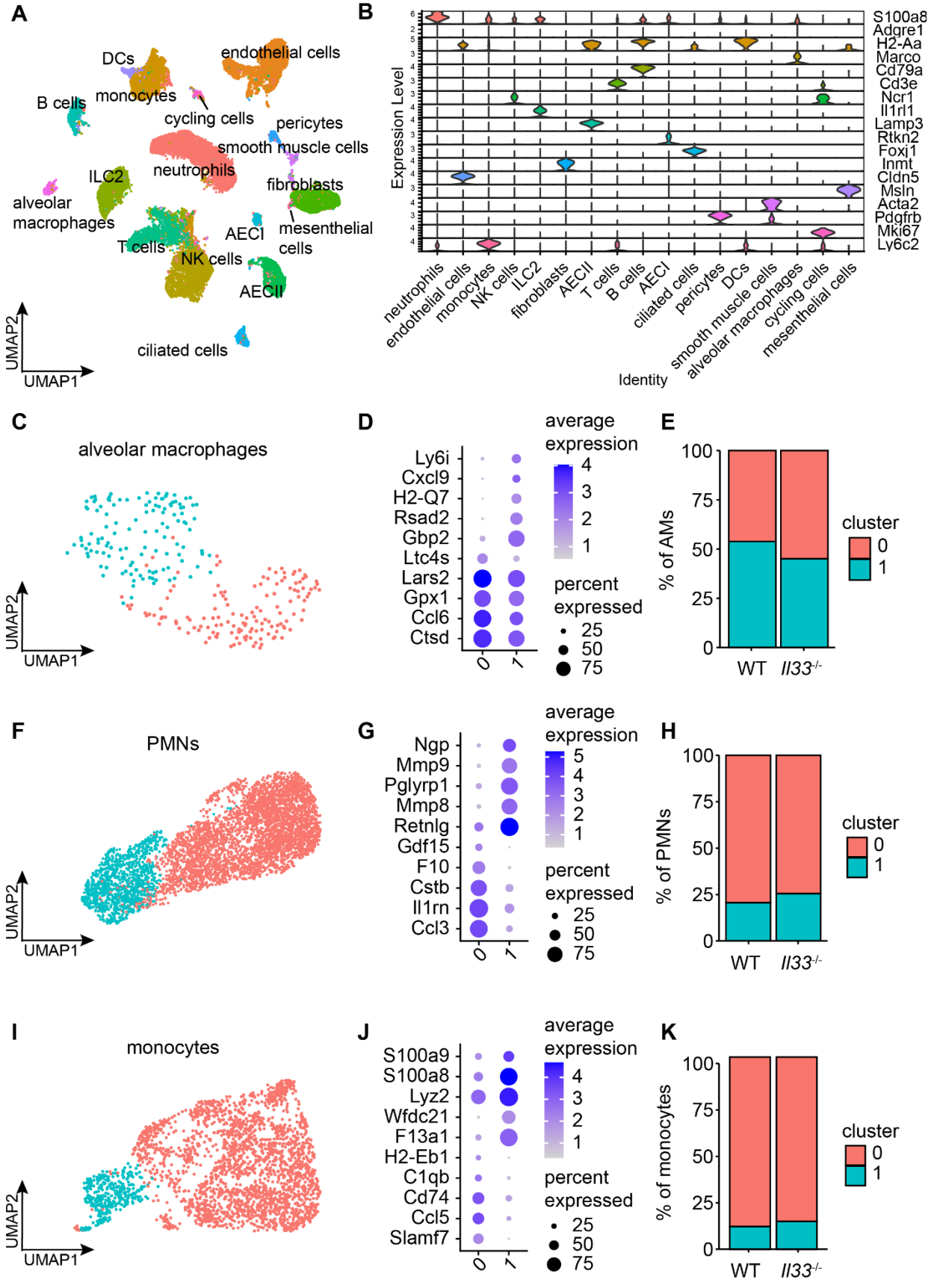
A
DCs
endothelial cells
B cells
monocytes
cycling cells
pericytes
smooth muscle cells
neutrophils
ILC2
fibroblasts
alveolar macrophages
AECI
mesenthelial cells
T cells
NK cells
AECII
ciliated cells
UMAP2
UMAP1
B
Expression Level
S100a8
Adgre1
H2-Aa
Marco
Cd79a
Cd3e
Ncr1
Il1rl1
Lamp3
Rtkn2
Foxj1
Inmt
Cldn5
Msln
Acta2
Pdgfrb
Mki67
Ly6c2
neutrophils
endothelial cells
monocytes
NK cells
ILC2
fibroblasts
AECII
T cells
B cells
AECI
ciliated cells
pericytes
DCs
smooth muscle cells
alveolar macrophages
cycling cells
mesenthelial cells
Identity
C
alveolar macrophages
UMAP2
UMAP1
D
Ly6i
Cxcl9
H2-Q7
Rsad2
Gbp2
Ltc4s
Lars2
Gpx1
Ccl6
Ctsd
0
1
average expression
4
3
2
1
percent expressed
25
50
75
E
% of AMs
100
75
50
25
0
WT
Il33-/-
cluster
0
1
F
PMNs
UMAP2
UMAP1
G
Ngp
Mmp9
Pglyrp1
Mmp8
Retnlg
Gdf15
F10
Cstb
Il1rn
Ccl3
0
1
average expression
5
4
3
2
1
percent expressed
25
50
75
H
% of PMNs
100
75
50
25
0
WT
Il33-/-
cluster
0
1
I
monocytes
UMAP2
UMAP1
J
S100a9
S100a8
Lyz2
Wfdc21
F13a1
H2-Eb1
C1qb
Cd74
Ccl5
Slamf7
0
1
average expression
4
3
2
1
percent expressed
25
50
75
K
% of monocytes
100
75
50
25
0
WT
Il33-/-
cluster
0
1

Figure S4. scRNAseq analysis of pulmonary cells from WT and *Il33*$^{-/-}$ mice. Mice were infected, sacrificed after 36 h (n = 3-4 per group) and lungs were subjected to scRNAseq. (**A**) Two-dimensional embedding computed by UMAP on 24612 computationally identified cells. (**B**) Stacked violin plot depicting representative marker genes for each cell type. (**C - K**) Dataset was first subsetted on alveolar macrophages, PMNs and monocytes and separated in two clusters by unbiased clustering (**C, F, I**). Dotplots of cluster specific marker genes (**D, G, J**) and frequencies of subclusters in WT and *Il33*$^{-/-}$ are represented in a barplot (**E, H, K**).

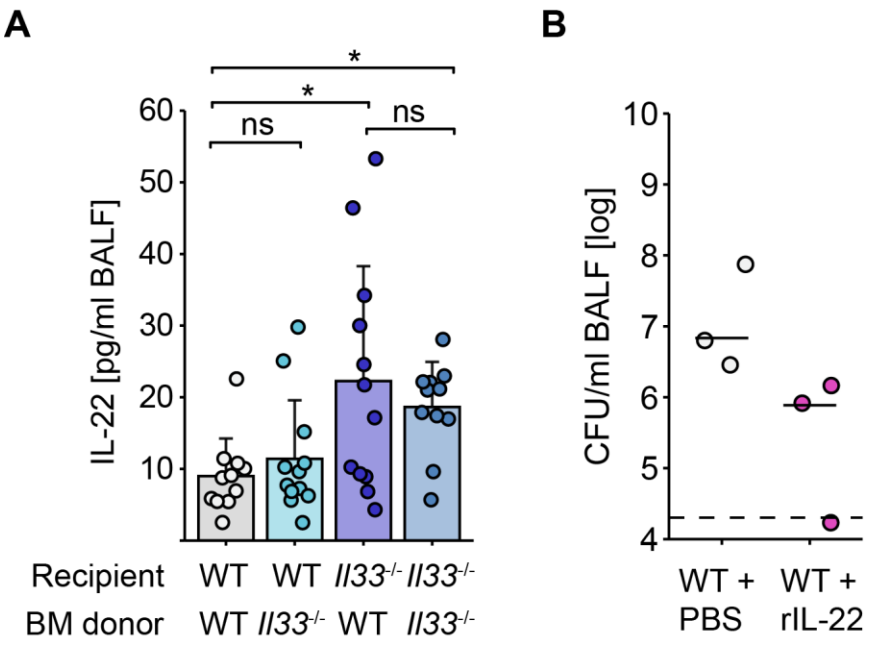

Figure S5. Non-hematopoietic IL-33 influences IL-22 levels in the lung, and administration of recombinant IL-22 improves antibacterial defense against *S. pneumoniae*. (**A**) Bone marrow chimera mice were infected and IL-22 levels in BALF were determined 48 h post infection, (n = 12 per group). Bars represent median + SD. Kruskal-Wallis followed by Dunn's posthoc test; * = $p < 0.05$, ns = $p > 0.05$. (**B**) WT mice were treated intranasally and intraperitoneally with 1 µg rIL-22 or PBS as control. CFU were measured 48 hours after infection (n = 3 per group). Data are shown as individual data points, lines represent median and dashed line lower detection limit.

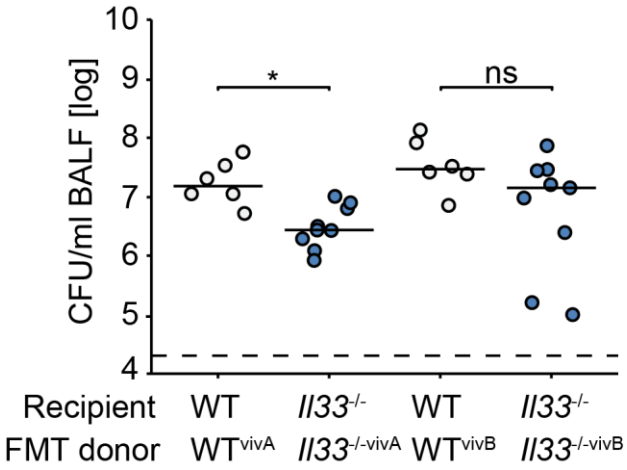

Figure S6. Antibacterial resistance of *Il33*$^{-/-}$ mice depends on the microbiota. WT and *Il33*$^{-/-}$ mice were treated orally with an antibiotic cocktail to deplete their own microbiota. Afterwards, mice were transplanted with fecal samples derived from WT or *Il33*$^{-/-}$ animals, each originating from mice of two different vivaria (for vivA n = 6-9 per group and for vivB n = 6-9 per group). After a reconstitution time of approximately 8 days, mice were intranasally infected with *S. pneumoniae* and bacterial loads in BALF were assessed. Kruskal-Wallis test followed by Dunn's posthoc test; * = $p < 0.05$, ns = $p > 0.05$.

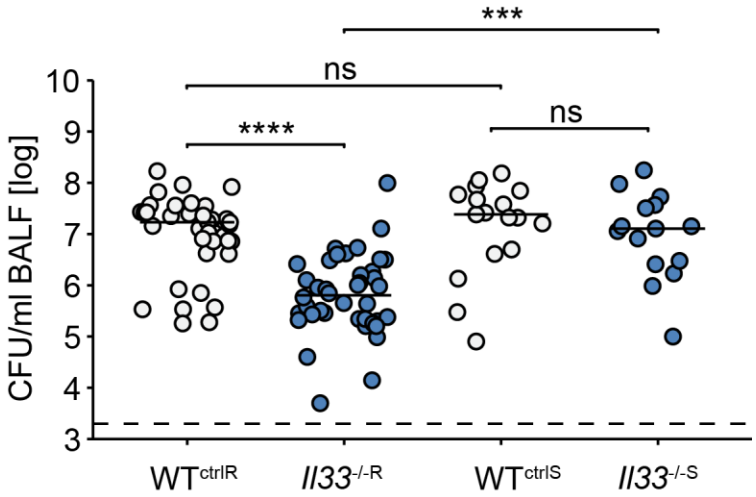

Figure S7. The antibacterial resistance of *Il33*$^{-/-}$ but not WT animals seems to be influenced by the housing environment. Accumulated CFU data from Fig. 4 classified by genotype and phenotype ('resistant' *Il33*$^{-/-}$ = *Il33*$^{-/-R}$ (n = 40), corresponding WT = WT^{ctrlR} (n = 39), 'susceptible' *Il33*$^{-/-}$ = *Il33*$^{-/-S}$ (n = 15), corresponding WT = WT^{ctrlS} (n = 17)). Data are shown as individual data points, lines represent median and dashed line lower detection limit, Kruskal-Wallis followed by Dunn's posthoc test; ns = $p > 0.05$, *** = $p < 0.001$, **** = $p < 0.0001$.

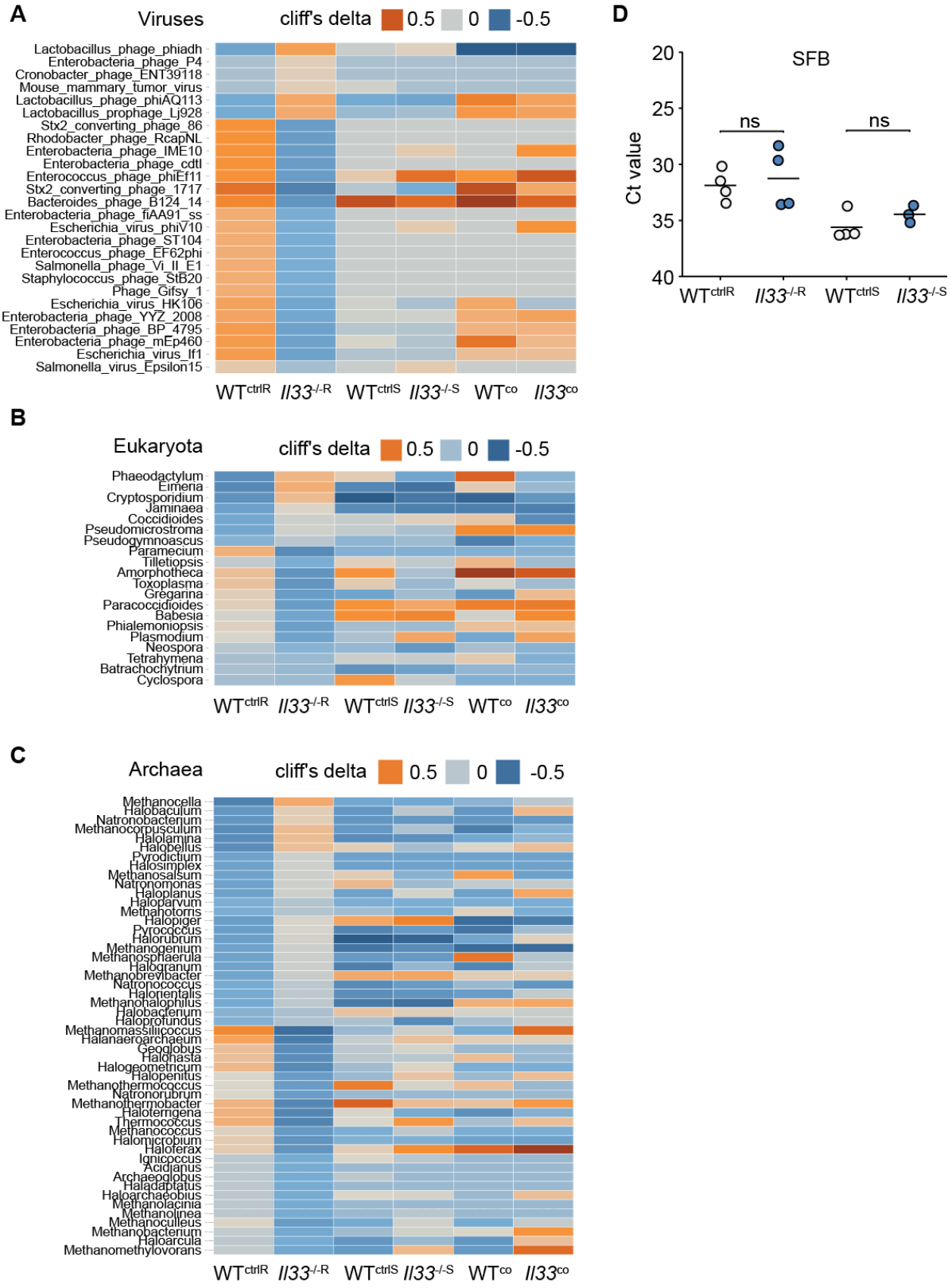
A
Viruses
cliff's delta 0.5 0 -0.5
Lactobacillus_phage_phiadh
Enterobacteria_phage_P4
Cronobacter_phage_ENT39118
Mouse_mammary_tumor_virus
Lactobacillus_phage_phiAQ113
Lactobacillus_prophage_Lj928
Stx2_converting_phage_86
Rhodobacter_phage_RcapNL
Enterobacteria_phage_IME10
Enterobacteria_phage_cdtI
Enterococcus_phage_phiEf11
Stx2_converting_phage_1717
Bacteroides_phage_B124_14
Enterobacteria_phage_fiAA91_ss
Escherichia_virus_phiV10
Enterobacteria_phage_ST104
Enterococcus_phage_EF62phi
Salmonella_phage_Vi_II_E1
Staphylococcus_phage_StB20
Phage_Gifsy_1
Escherichia_virus_HK106
Enterobacteria_phage_YYZ_2008
Enterobacteria_phage_BP_4795
Enterobacteria_phage_mEp460
Escherichia_virus_If1
Salmonella_virus_Epsilon15
WTctrlR Il33-/-R WTctrlS Il33-/-S WTco Il33co
B
Eukaryota
cliff's delta 0.5 0 -0.5
Phaeodactylum
Eimeria
Cryptosporidium
Jaminaea
Coccidioides
Pseudomicrostroma
Pseudogymnoascus
Paramecium
Tilletiopsis
Amorphotheca
Toxoplasma
Gregarina
Paracoccidioides
Babesia
Phialemoniopsis
Plasmodium
Neospora
Tetrahymena
Batrachochytrium
Cyclospora
WTctrlR Il33-/-R WTctrlS Il33-/-S WTco Il33co
C
Archaea
cliff's delta 0.5 0 -0.5
Methanocella
Halobaculum
Natronobacterium
Methanocorpusculum
Halolamina
Halobellus
Pyrodictium
Halosimplex
Methanosalsum
Natronomonas
Haloplanus
Haloparvum
Methanotorris
Halopiger
Pyrococcus
Halorubrum
Methanogenium
Methanosphaerula
Halogranum
Methanobrevibacter
Natrococcus
Halorientalis
Methanohalophilus
Halobacterium
Haloprofundus
Methanomassiliicoccus
Halanaeroarchaeum
Geoglobus
Halohasta
Halogeometricum
Halopenitus
Methanothermococcus
Natronorubrum
Methanothermobacter
Halotermigena
Thermococcus
Methanococcus
Halomicrobium
Haloferax
Ignicoccus
Acidianus
Archaeoglobus
Haladaptatus
Haloarchaeobius
Methanolacinia
Methanolinea
Methanoculleus
Methanobacterium
Haloarcula
Methanomethylovorans
WTctrlR Il33-/-R WTctrlS Il33-/-S WTco Il33co
D
SFB
Ct value
20
25
30
35
40
ns
ns
WTctrlR Il33-/-R WTctrlS Il33-/-S

Figure S8. IL-33 influences the gut virome and eukaryotic microbial communities. (**A - C**) Heatmap of shotgun-sequenced microbiota derived from resistant, susceptible and co-housed mice ('resistant' *Il33*$^{-/-}$ = *Il33*$^{-/-R}$ (n = 15), corresponding WT = WT^{ctrlR} (n = 15), 'susceptible' *Il33*$^{-/-}$ = *Il33*$^{-/-S}$ (n = 13), corresponding WT = WT^{ctrlS} (n = 12), cohoused WT = WT^{co} (n = 7), cohoused *Il33*$^{-/-}$ (n = 7)). Cliff's delta was applied to quantify effect sizes of viruses (**A**), eukaryota (genus level) (**B**) and archaea (genus level) (**C**) between *Il33*$^{-/-R}$ and WT^{ctrlR} mice. (**D**) SFB was quantified in fecal samples from WT^{ctrlR}, *Il33*$^{-/-R}$, WT^{ctrlS} and *Il33*$^{-/-S}$ mice (for each group n = 4) by qPCR. Lines represent median, Wilcoxon rank sum test; ns = $p > 0.05$.

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