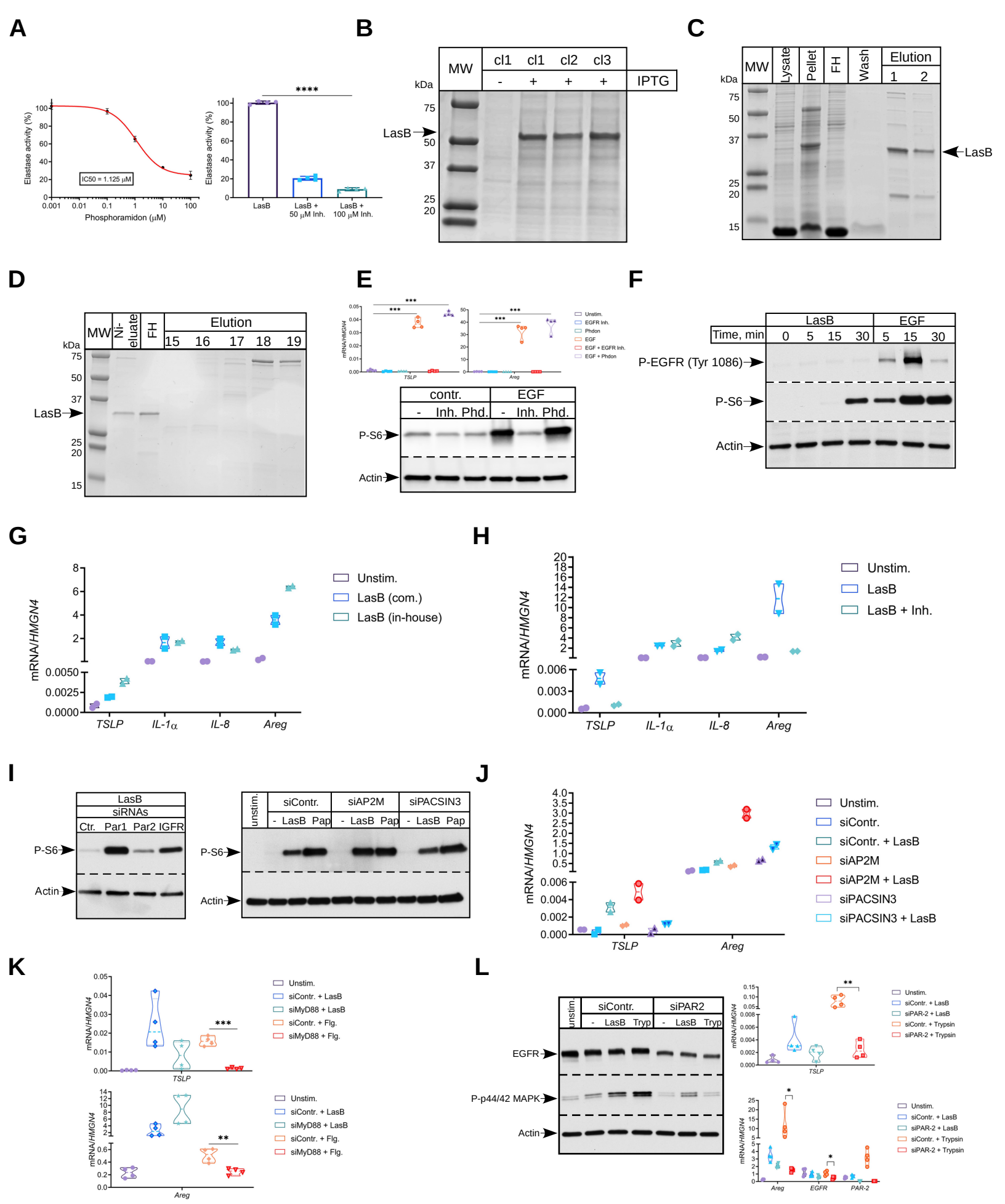
A
Elastase activity (%)
IC50 = 1.125 μM
Phosphoramidon (μM)
****
LasB
LasB + 50 μM Inh.
LasB + 100 μM Inh.
B
MW
cl1 cl1 cl2 cl3
- + + +
IPTG
kDa
LasB
C
MW
Lysate
Pellet
FH
Wash
Elution
1 2
kDa
LasB
D
MW
Ni-eluate
FH
Elution
15 16 17 18 19
kDa
LasB
E
***
TSLP
Areg
Unstim.
EGFR Inh.
Phdon
EGF
EGF + EGFR Inh.
EGF + Phdon
contr.
EGF
- Inh. Phd. - Inh. Phd.
P-S6
Actin
F
LasB
EGF
Time, min
0 5 15 30 5 15 30
P-EGFR (Tyr 1086)
P-S6
Actin
G
mRNA/HMGN4
Unstim.
LasB (com.)
LasB (in-house)
TSLP
IL-1α
IL-8
Areg
H
mRNA/HMGN4
Unstim.
LasB
LasB + Inh.
TSLP
IL-1α
IL-8
Areg
I
LasB
siRNAs
Ctr. Par1 Par2 IGFR
P-S6
Actin
unstim.
siContr.
siAP2M
siPACSIN3
- LasB Pap - LasB Pap - LasB Pap
P-S6
Actin
J
mRNA/HMGN4
Unstim.
siContr.
siContr. + LasB
siAP2M
siAP2M + LasB
siPACSIN3
siPACSIN3 + LasB
TSLP
Areg
K
mRNA/HMGN4
***
**
TSLP
Areg
Unstim.
siContr. + LasB
siMyD88 + LasB
siContr. + Flg.
siMyD88 + Flg.
L
unstim.
siContr.
siPAR2
- LasB Tryp - LasB Tryp
EGFR
P-p44/42 MAPK
Actin
mRNA/HMGN4
**
*
TSLP
Areg
EGFR
PAR-2
Unstim.
siContr. + LasB
siPAR-2 + LasB
siContr. + Trypsin
siPAR-2 + Trypsin

Figure S1

Figure S1. *P. aeruginosa* toxin engages EGFR pathway, independent of receptor endocytosis or MyD88, to stimulate epithelial cells, Related to Figure 1

(A) (Left) Inhibition of LasB activity with phosphoramidon using *in vitro* elastin cleavage assay. IC50 is shown in inset box (n=5). (Right) Elastase activity of purified recombinant LasB with increasing concentrations of LasB inhibitor, phosphoramidon (n=3).

(B) Expression of recombinant *P. aeruginosa* (PA01) 6XHis-tagged LasB in E. coli. LasB expression was induced by addition of 0.4 mM IPTG for 2 hr at 37 ºC (n=3).

(C) First-step chromatography of recombinant LasB on Ni-NTA column. LasB expression was induced by addition of 0.2 mM IPTG into 200 ml of E. coli cell culture for 4 hrs. FH – flow through fraction.

(D) Second step chromatography of recombinant LasB on ion-exchange MonoQ column.

(E) Relative mRNA expression of TSLP and Areg (top) and immunoblot analysis of P-S6 in H292 cells stimulated with EGF (50 ng/ml) +/- EGFR inhibitor (EGFR Inh., 20 μM), LasB inhibitor (Phdon, 50 μM) for 3 hr (n=4).

(F) Immunoblot analysis of P-EGFR Tyr1068 and P-S6 in H292 cells stimulated with LasB (5 μg/ml) and EGF (10 ng/ml) for indicated times (n=3).

(G) Relative mRNA expression of pro-inflammatory (IL-1α, IL-8) and tissue repair (TSLP, Areg) genes in H292 cells treated with commercial and recombinant in-house LasB (2 μg/ml) for 3 hr (n=2).

(H) Relative mRNA expression of pro-inflammatory (IL-1α, IL-8) and tissue repair (TSLP, Areg) cytokine genes in H292 cells treated with recombinant in-house LasB (2 μg/ml) +/- inhibitor (50 μM) for 3 hr (n=2).

(I and J) Immunoblot analysis of P-S6 (I) and relative mRNA expression of TSLP and Areg (J) in H292 cells treated with control, AP2M or PACSIN3 siRNA and stimulated with LasB (5 μg/ml) and papain (8 μg/ml) for 3hr (n=2). I left – immunoblot analysis of P-S6 in H292 cells treated with “control” (targeting EGFR), Par1, Par2 or IGFR siRNA.

(K) Relative mRNA expression of TSLP and Areg in H292 cells treated with control or MyD88 siRNA and stimulated with LasB (5 μg/ml) or flagellin (10 μg/ml) for 3hr (n=4).

(L) Immunoblot analysis of P-p44/42 MAPK (left) and relative mRNA expression of TSLP, Areg, EGFR and PAR-2 (right) in H292 cells treated with control and PAR-2 siRNA and stimulated with LasB (5 μg/ml) or Trypsin (25 nM) (n=4).

mRNA expression was measured relative to HMGN4. ß-actin was used as loading control. n = independent experiments. Data are mean ± SD and were analyzed by one-way ANOVA with Tukey’s multiple comparisons test (A, E) or two-tailed unpaired Student’s t test (K, L). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

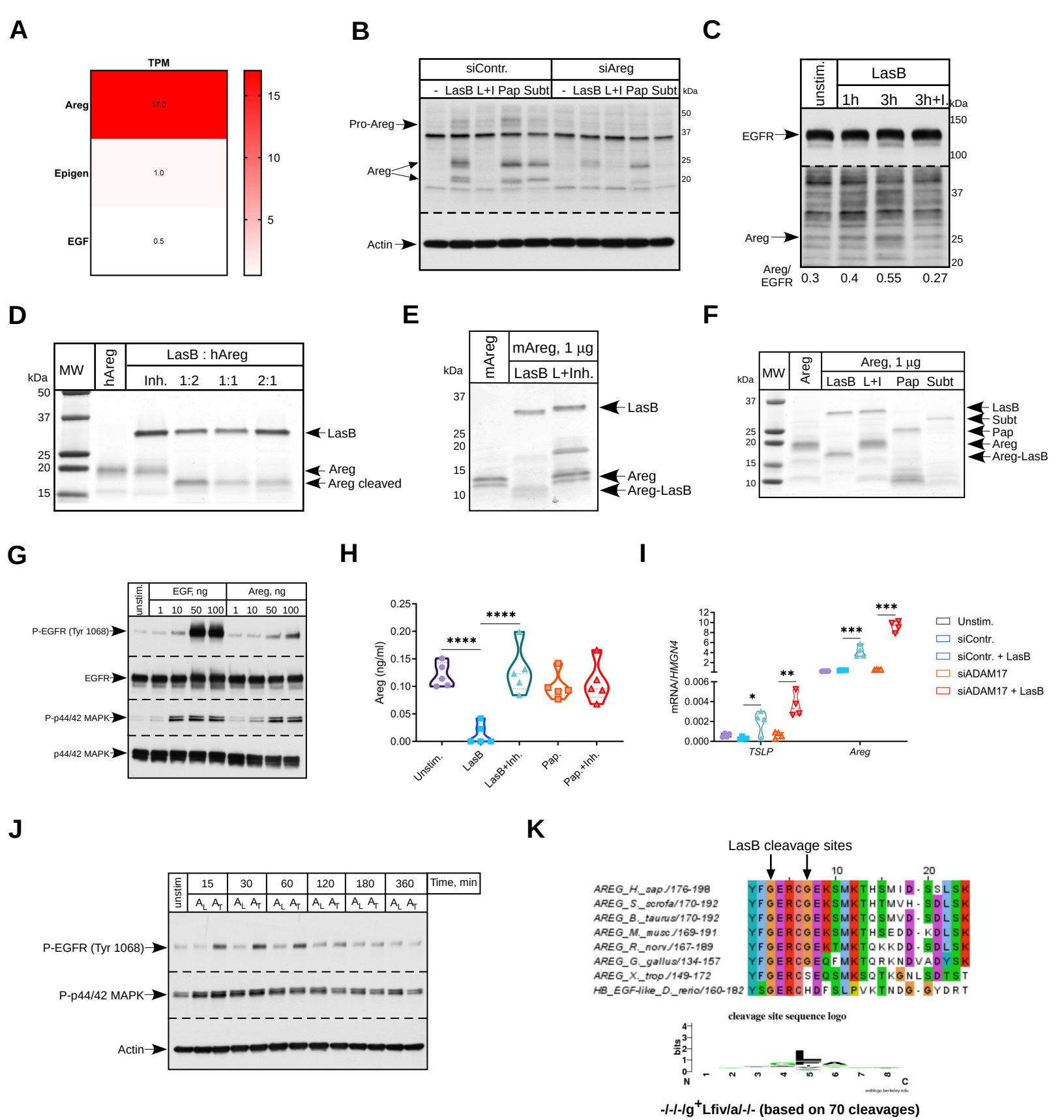
A
TPM
Areg
Epigen
EGF
17.0
1.0
0.5
15
10
5
B
siContr.
siAreg
- LasB L+I Pap Subt
- LasB L+I Pap Subt
kDa
Pro-Areg
Areg
Actin
50
37
25
20
C
unstim.
LasB
1h
3h
3h+I.
kDa
EGFR
Areg
Areg/
EGFR
0.3
0.4
0.55
0.27
150
100
37
25
20
D
kDa
MW
hAreg
LasB : hAreg
Inh.
1:2
1:1
2:1
50
37
25
20
15
LasB
Areg
Areg cleaved
E
kDa
mAreg
mAreg, 1 µg
LasB L+Inh.
37
25
20
15
10
LasB
Areg
Areg-LasB
F
kDa
MW
Areg
Areg, 1 µg
LasB
L+I
Pap
Subt
37
25
20
15
10
LasB
Subt
Pap
Areg
Areg-LasB
G
unstim.
EGF, ng
Areg, ng
1
10
50
100
1
10
50
100
P-EGFR (Tyr 1068)
EGFR
P-p44/42 MAPK
p44/42 MAPK
H
Areg (ng/ml)
0.25
0.20
0.15
0.10
0.05
0.00
****
****
Unstim.
LasB
LasB+Inh.
Pap.
Pap.+Inh.
I
mRNA/HMGN4
12
10
8
6
4
2
0.006
0.004
0.002
0.000
*
**
***
***
TSLP
Areg
Unstim.
siContr.
siContr. + LasB
siADAM17
siADAM17 + LasB
J
unstim
15
30
60
120
180
360
Time, min
A_L A_T
P-EGFR (Tyr 1068)
P-p44/42 MAPK
Actin
K
LasB cleavage sites
10
20
AREG_H._sap./176-198
AREG_S._scrofa/170-192
AREG_B._taurus/170-192
AREG_M._musc./169-191
AREG_R._norv./167-189
AREG_G._gallus/134-157
AREG_X._trop./149-172
HB_EGF-like_D._rerio/160-182
cleavage site sequence logo
bits
4
3
2
1
0
N
C
-/-/-/g+Lfiv/a/-/- (based on 70 cleavages)
L
101
SVRVEQVVKPPQNKTESENTSDKPKRKKKGGKNGKNRRNRKKKNPCNAEF
QNFCIHGECKYIEHLEAVTCKCQQEYFGERCGEKSMKTHSMIDSSLSK
198

Figure S2

Figure S2. *P. aeruginosa* toxin utilizes amphiregulin to activate epithelial cells, Related to Figure 2

(A) TPM of EGF-family ligands from RNA-seq of 675 commonly used human cancer cell lines (Klijn et al., 2015).

(B) Immunoblot analysis of Areg in H292 cells treated with control or Areg siRNA and stimulated with LasB (5 µg/ml) +/- inhibitor (50 µM), papain (8 µg/ml) or subtilisin (1 µg/ml) for 3hr (n=3).

(C) Immunoblot analysis of Areg in input samples before immunoprecipitation with anti-EGFR antibodies in H292 cells stimulated with LasB (3 µg/ml) +/- inhibitor (50 µM) (n=3).

(D) *In vitro* cleavage of recombinant human Areg by LasB. Ratios (in µg) of recombinant human Areg to LasB (1:2, 1:1, 2:1) +/- inhibitor (50 µM) were used (n=7).

(E and F) *In vitro* cleavage of recombinant mouse (E) or human (F) Areg (1 µg) by LasB (0.5 µg) +/- inhibitor (50 µM), papain and subtilisin (both 0.5 µg) (n=3-5).

(G) Immunoblot analysis of P-EGFR Tyr1068 and P-p44/42 MAPK in H292 cells treated with indicated amounts of EGF or Areg for 1hr (n=3).

(H) ELISA of Areg amounts in H292 cell supernatants pre-treated with LasB or papain inhibitor (both 50 µM) for 10-15 min and stimulated with LasB (2 µg/ml) or papain (8 µg/ml) for 3hr (n=5).

(I) Relative mRNA expression of TSLP and Areg in H292 treated with control or ADAM17 or TACE siRNA and stimulated with LasB (2 µg/ml) for 3hr (n=4).

(J) Immunoblot analysis of P-EGFR Tyr 1068 and P-p44/42 MAPK in H292 cells stimulated with A_L or A_T (50 ng/ml) (n=3).

(K) Sequence alignment of Areg from different species (top) and cleavage site sequence for LasB based on MEROPS database (bottom) (Rawlings et al., 2018). EGF-like domain containing LasB cleavage sites was used for alignment. LasB cleavages sites are shown by arrows.

(L) Sequence of recombinant human Areg with depicted cleavage sites for ADAM17 or TACE (K184 and K187 in orange) and LasB (G178 and G182 in blue). EGF-like domain is highlighted in red. Areg peptide likely cleaved by LasB is highlighted in yellow box.

mRNA expression was measured relative to HMGN4. ß-actin, total EGFR and p44/42 MAPK were used as loading controls. n = independent experiments. Data were analyzed by one-way ANOVA with Tukey's multiple comparisons test (H, I). *p < 0.05; **p < 0.01; ***p < 0.001, ****p < 0.0001.

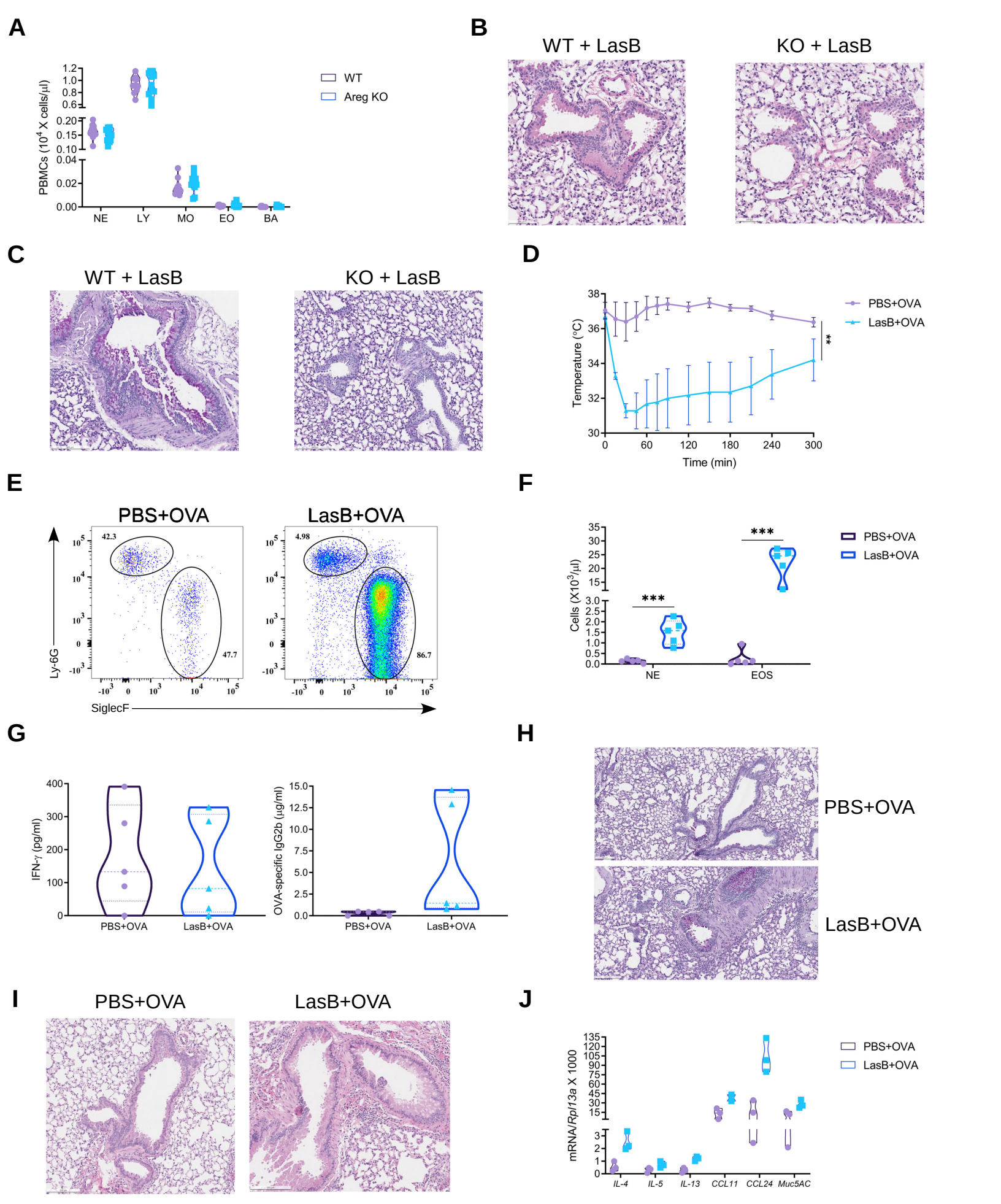
A
PBMCs (10^4 X cells/μl)
1.2
1.0
0.8
0.6
0.20
0.15
0.10
0.04
0.02
0.00
NE
LY
MO
EO
BA
WT
Areg KO
B
WT + LasB
KO + LasB
C
WT + LasB
KO + LasB
D
Temperature (°C)
38
36
34
32
30
0
60
120
180
240
300
Time (min)
**
PBS+OVA
LasB+OVA
E
PBS+OVA
LasB+OVA
42.3
47.7
4.98
86.7
Ly-6G
SiglecF
F
Cells (X10^3/μl)
35
30
25
20
15
3.0
2.5
2.0
1.5
1.0
0.5
0.0
***
***
NE
EOS
PBS+OVA
LasB+OVA
G
IFN-γ (pg/ml)
400
300
200
100
0
PBS+OVA
LasB+OVA
OVA-specific IgG2b (μg/ml)
15.0
12.5
10.0
7.5
5.0
2.5
0.0
PBS+OVA
LasB+OVA
H
PBS+OVA
LasB+OVA
I
PBS+OVA
LasB+OVA
J
mRNA/*Rpl13a* X 1000
135
120
105
90
75
60
45
30
15
3
2
1
0
IL-4
IL-5
IL-13
CCL11
CCL24
Muc5AC
PBS+OVA
LasB+OVA

Figure S3

Figure S3. LasB is an adjuvant for allergic response, Related to Figures 3 and 4

(A) Analysis of PBMCs counts in WT and *Areg*$^{-/-}$ (KO) mice at basal state (combined of 2 experiments yielding 10-11 mice per group).

(B and C) H&E (B, scale bar = 200 μm) and PAS (C, scale bar = 500 μm) staining in the lungs of LasB-challenged WT and *Areg*$^{-/-}$ (KO) mice in innate airway inflammation model (representative of 3 experiments, 3-5 mice per group).

(D) Rectal temperature measurements of mice immunized with OVA +/- LasB in airway allergic inflammation model (combined of 2 experiments yielding 6 mice per group).

(E and F) Representative FACS plots (n=2) (E) and graphs showing cell numbers (F) of eosinophils (CD45+MHCII–CD11b+Siglec-F+) and neutrophils (CD45+MHCII–CD11b+Ly6G+) out of CD45+ cells in the BAL of mice immunized as in (D) (combined of 2 experiments yielding 5 mice per group).

(G) ELISA of IFN-g in the BAL (left) and OVA-IgG2b in the serum (right) of mice immunized with OVA +/- LasB (combined of 2 experiments yielding 5 mice per group).

(H and I) PAS (H) and H&E (I) staining in the lungs of mice immunized as in (D) (representative of 3 experiments, 3 mice per group, scale bar = 500 μm).

(J) Relative mRNA expression of type 2 cytokines (IL-4, 5, 13), eotaxins (Ccl11, 24) and Muc5AC in the lungs of mice immunized as in (D) (representative of 3 experiments, 3 mice per group).

Data are mean ± SD and were analyzed by two-tailed unpaired Student's t test or one-way ANOVA (D). **p < 0.01; ***p < 0.001.

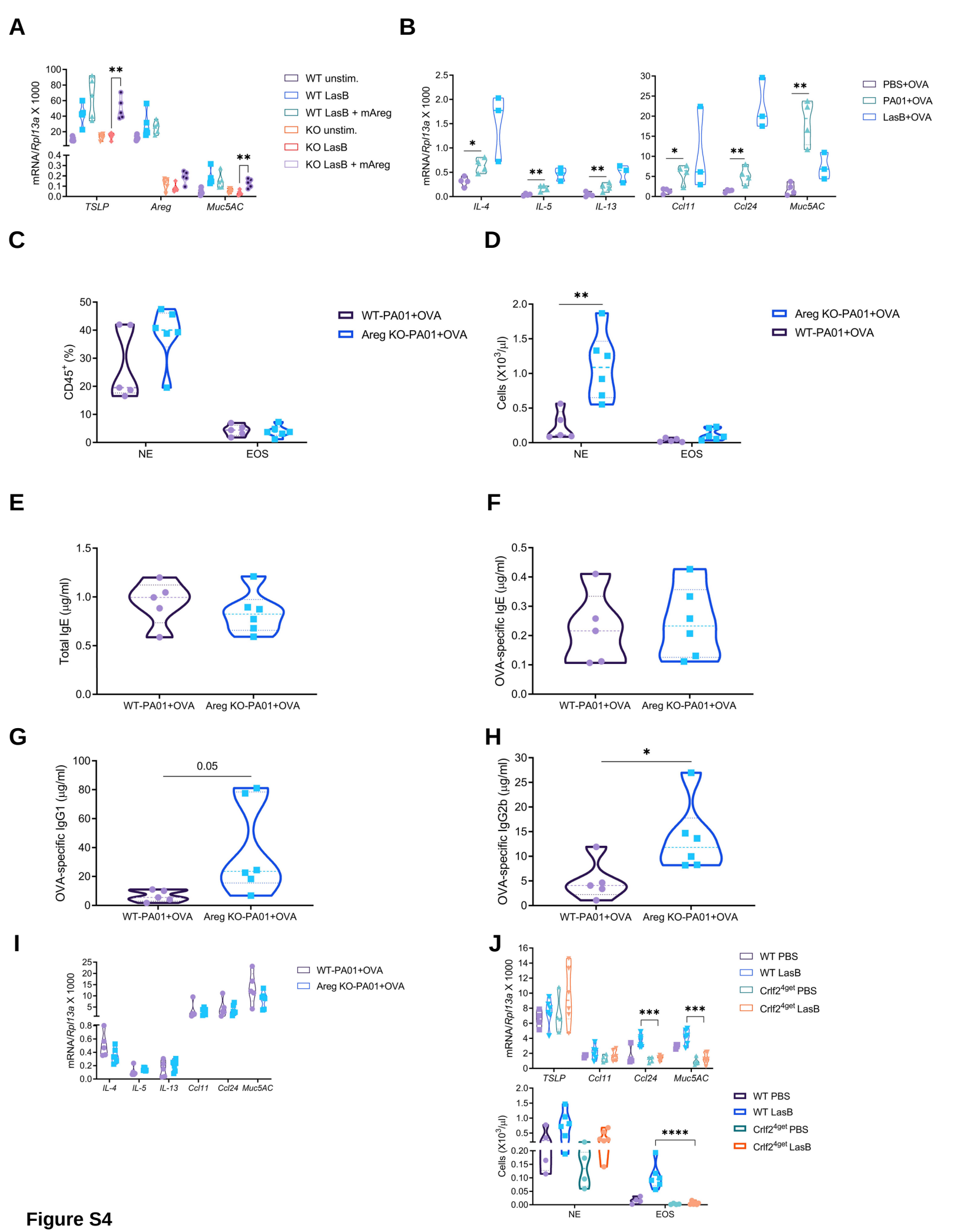
A
mRNA/Rpl13a X 1000
TSLP
Areg
Muc5AC
WT unstim.
WT LasB
WT LasB + mAreg
KO unstim.
KO LasB
KO LasB + mAreg
B
mRNA/Rpl13a X 1000
IL-4
IL-5
IL-13
Ccl11
Ccl24
Muc5AC
PBS+OVA
PA01+OVA
LasB+OVA
C
CD45+ (%)
NE
EOS
WT-PA01+OVA
Areg KO-PA01+OVA
D
Cells (X10³/µl)
NE
EOS
Areg KO-PA01+OVA
WT-PA01+OVA
E
Total IgE (µg/ml)
WT-PA01+OVA
Areg KO-PA01+OVA
F
OVA-specific IgE (µg/ml)
WT-PA01+OVA
Areg KO-PA01+OVA
G
OVA-specific IgG1 (µg/ml)
0.05
WT-PA01+OVA
Areg KO-PA01+OVA
H
OVA-specific IgG2b (µg/ml)
WT-PA01+OVA
Areg KO-PA01+OVA
I
mRNA/Rpl13a X 1000
IL-4
IL-5
IL-13
Ccl11
Ccl24
Muc5AC
WT-PA01+OVA
Areg KO-PA01+OVA
J
mRNA/Rpl13a X 1000
TSLP
Ccl11
Ccl24
Muc5AC
WT PBS
WT LasB
Crlf2^4get PBS
Crlf2^4get LasB
Cells (X10³/µl)
NE
EOS
WT PBS
WT LasB
Crlf2^4get PBS
Crlf2^4get LasB

Figure S4

Figure S4. *P. aeruginosa* can serve as an adjuvant for an allergic response, Related to Figure 3 and 5

(A) Relative mRNA expression of TSLP, Areg and Muc5AC in WT and *Areg*$^{-/-}$ (KO) mouse lung organ cultures stimulated with LasB (5 µg/ml) +/- mAreg (10 ng/ml) for 8 hr (n=5).

(B) Relative mRNA expression of type 2 cytokines (IL-4, 5, 13), eotaxins (Ccl11, 24) and Muc5AC (from left to right) in the lungs of mice immunized with OVA +/- WT PA01 or LasB (representative of 3 experiments, 3-4 mice per group).

(C and D) Cell frequency (B) and numbers (C) of eosinophils (CD45+MHCII–CD11b+CD64-Siglec-F+) and neutrophils (CD45+MHCII–CD11b+CD64-Ly6G+) out of CD45+ cells in the BAL of WT and *Areg*$^{-/-}$ (KO) mice immunized with OVA +/- WT PA01.

(E-H) ELISA of total IgE (E), OVA-specific IgE (F), OVA-specific IgG1 (G) and OVA-specific IgG2b (H) in the serum of WT and *Areg*$^{-/-}$ (KO) mice immunized as in (C and D).

(I) Relative mRNA expression of type 2 cytokines (IL-4, 5, 13), eotaxins (Ccl11, 24) and Muc5AC in the lungs of mice immunized as in (C and D).

(J) (Top) Relative mRNA expression of Tslp, Ccl11, Ccl24 and Muc5AC in the lungs of LasB-treated WT and *Crlf2*^{4get} mice and (bottom) graphs showing cell numbers of eosinophils (CD45+MHCII–CD11b+Siglec-F+) and neutrophils (CD45+MHCII–CD11b+Ly6G+) out of CD45+ cells in the BAL of LasB-treated WT and *Crlf2*^{4get} mice (combined of 2 experiments yielding 4-6 mice per group).

For (C-I) representative of 2 experiments, 5-6 mice per group. mRNA expression was measured relative to Rpl13a. n = independent experiments. Data were analyzed by two-tailed unpaired Student's t test and one-way ANOVA with Tukey's multiple comparisons test (J). *p < 0.05; **p < 0.01, ***p < 0.01, ****p < 0.001.

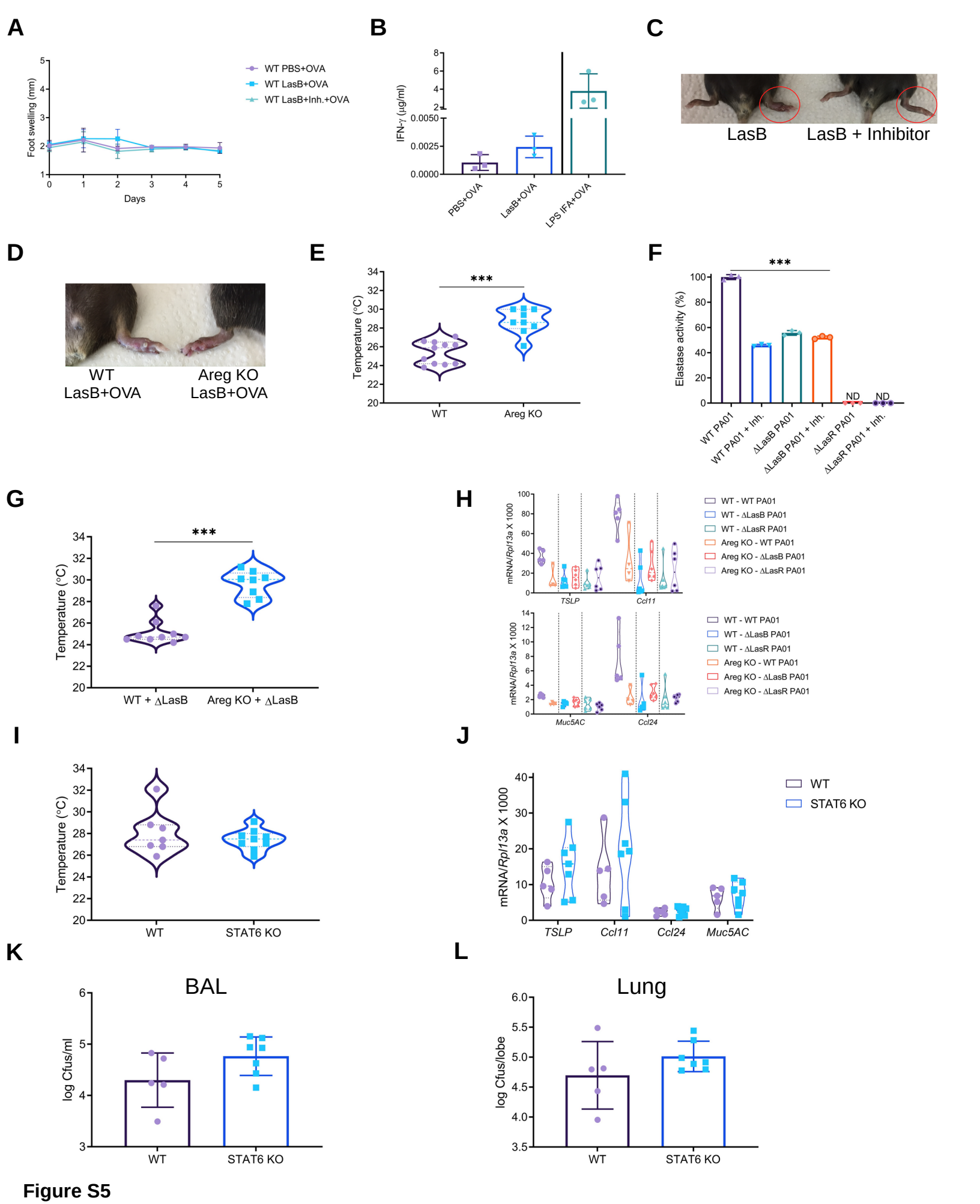
A
Foot swelling (mm)
Days
WT PBS+OVA
WT LasB+OVA
WT LasB+Inh.+OVA
B
IFN-γ (μg/ml)
PBS+OVA
LasB+OVA
LPS IFA+OVA
C
LasB
LasB + Inhibitor
D
WT
LasB+OVA
Areg KO
LasB+OVA
E
Temperature (°C)
***
WT
Areg KO
F
Elastase activity (%)
***
ND
ND
WT PA01
WT PA01 + Inh.
ΔLasB PA01
ΔLasB PA01 + Inh.
ΔLasR PA01
ΔLasR PA01 + Inh.
G
Temperature (°C)
***
WT + ΔLasB
Areg KO + ΔLasB
H
mRNA/Rpl13a X 1000
TSLP
Ccl11
Muc5AC
Ccl24
WT - WT PA01
WT - ΔLasB PA01
WT - ΔLasR PA01
Areg KO - WT PA01
Areg KO - ΔLasB PA01
Areg KO - ΔLasR PA01
I
Temperature (°C)
WT
STAT6 KO
J
mRNA/Rpl13a X 1000
TSLP
Ccl11
Ccl24
Muc5AC
WT
STAT6 KO
K
BAL
log Cfus/ml
WT
STAT6 KO
L
Lung
log Cfus/lobe
WT
STAT6 KO

Figure S5

Figure S5. Amphiregulin is required for successful *P. aeruginosa* infection, Related to Figures 4 and 6

(A) Kinetics of swelling in non-injected right footpad of mice immunized with LasB+OVA +/- inhibitor in skin allergic inflammation model (combined of 2 experiments yielding 3-6 mice per group).

(B) IFNγ production after *ex vivo* stimulation of total lymph node cells from mice immunized as in (A) or with LPS and IFA and OVA (representative of 2 experiments, 3 mice per group).

(C and D) Photograph of left hind footpads of WT mice (C) or WT and *Areg*$^{-/-}$ (KO) mice (D) immunized with LasB+OVA +/- inhibitor.

(E) Temperature measurements of WT and *Areg*$^{-/-}$ (KO) mice 12 hr after WT PA01 infection (combined of 2 experiments yielding 9-11 mice per group).

(F) Elastase activity measurements in supernatants collected from WT, ΔLasB and ΔLasR PA01 cell cultures (n=3).

(G) Temperature measurements of WT and *Areg*$^{-/-}$ (KO) mice 12 hr after infection with ΔLasB PA01 (combined of 2 experiments yielding 8-9 mice per group).

(H) Relative mRNA expression of TSLP, eotaxins (Ccl11, 24) and Muc5AC in the lungs of WT and *Areg*$^{-/-}$ (KO) mice infected with WT, ΔLasB or ΔLasR PA01 for 12 hr (representative of 3 experiments, 4-6 mice per group).

(I) Temperature measurements of WT and *STAT6*$^{-/-}$ (KO) mice 12 hr after WT PA01 infection (combined of 2 experiments yielding 7-9 mice per group).

(J) Relative mRNA expression of TSLP, eotaxins (Ccl11, 24) and Muc5AC in the lungs of WT and *STAT6*$^{-/-}$ (KO) mice infected with WT PA01 for 12 hr.

(K and L) BAL (K) and lung (L) bacterial load of WT PA01 in WT and *STAT6*$^{-/-}$ (KO) mice 12 hr after infection. J-L - representative of 3 experiments, 5-7 mice per group. mRNA expression was measured relative to Rpl13a. Data are mean ± SD and were analyzed by two-tailed unpaired Student's t test or one-way ANOVA with Tukey's multiple comparisons test (F). ***$p < 0.001$.

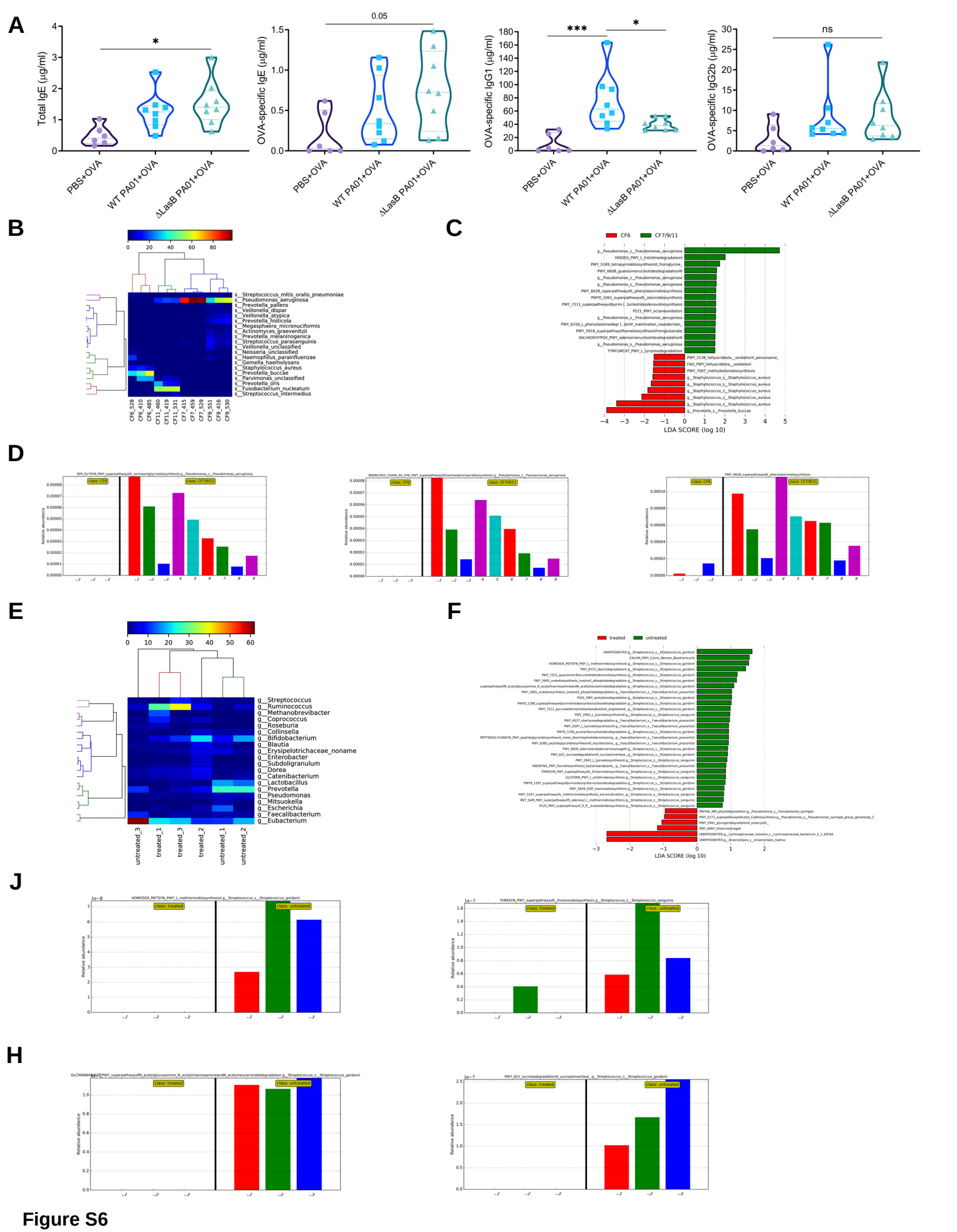
A
Total IgE (μg/ml)
OVA-specific IgE (μg/ml)
OVA-specific IgG1 (μg/ml)
OVA-specific IgG2b (μg/ml)
PBS+OVA
WT PA01+OVA
ΔLasB PA01+OVA
*
0.05
***
ns
B
C
CF6
CF7/9/11
LDA SCORE (log 10)
D
E
F
treated
untreated
J
H
Relative abundance
class: treated
class: untreated

Figure S6

Figure S6. Human lung and gut microbiomes use known mucin utilization pathways under conditions mimicking type-2 response, Related to Figure 7

(A) ELISA of total IgE, OVA-specific IgE, IgG1, IgG2b (from left to right) in the serum of mice immunized with OVA +/- WT or ΔLasB PA01 (combined of 2 experiments yielding 6-8 mice per group, analyzed by one-way ANOVA with Tukey's multiple comparisons test, *$p < 0.05$; ***$p < 0.001$).

(B) Taxonomic classification of the lung microbiome of cystic fibrosis patients at different stages of disease.

(C) Histogram of the linear discriminant analysis (LDA) scores computed for differentially abundant microbiome metabolic pathways (based on HUMAnN2) of cystic fibrosis patients colonized (CF7/9/11) vs non-colonized (CF6) with *P. aeruginosa*.

(D) Histogram of the relative abundances of the metabolic pathways (from left to right – serine and glycine, branched chain amino acids and phenylalanine biosynthesis) in the lung microbiome of cystic fibrosis patients colonized (CF7/9/11) vs non-colonized (CF6) with *P. aeruginosa*. Classes (patients) are shown in the top of the histograms. Subclasses (individual patient samples at different stages of disease) are differentially colored, and the relative abundances of the pathways are indicated.

(E) Taxonomic classification of the fecal microbiome of worm-infected patients treated vs non-treated with anthelminthics.

(F) Histogram of the LDA scores computed for differentially abundant microbiome metabolic pathways (based on HUMAnN2) between worm-infected patients treated vs non-treated with anthelminthic.

(G and H) Histogram of the relative abundances of the amino acid biosynthesis (G) and polysaccharide degradation (H) pathways in the fecal microbiome of worm-infected patients treated vs non-treated with anthelmintic. Classes (patients) are shown in the top of the histograms. Subclasses (individual patient samples) are differentially colored, and the relative abundances of the pathways are indicated.

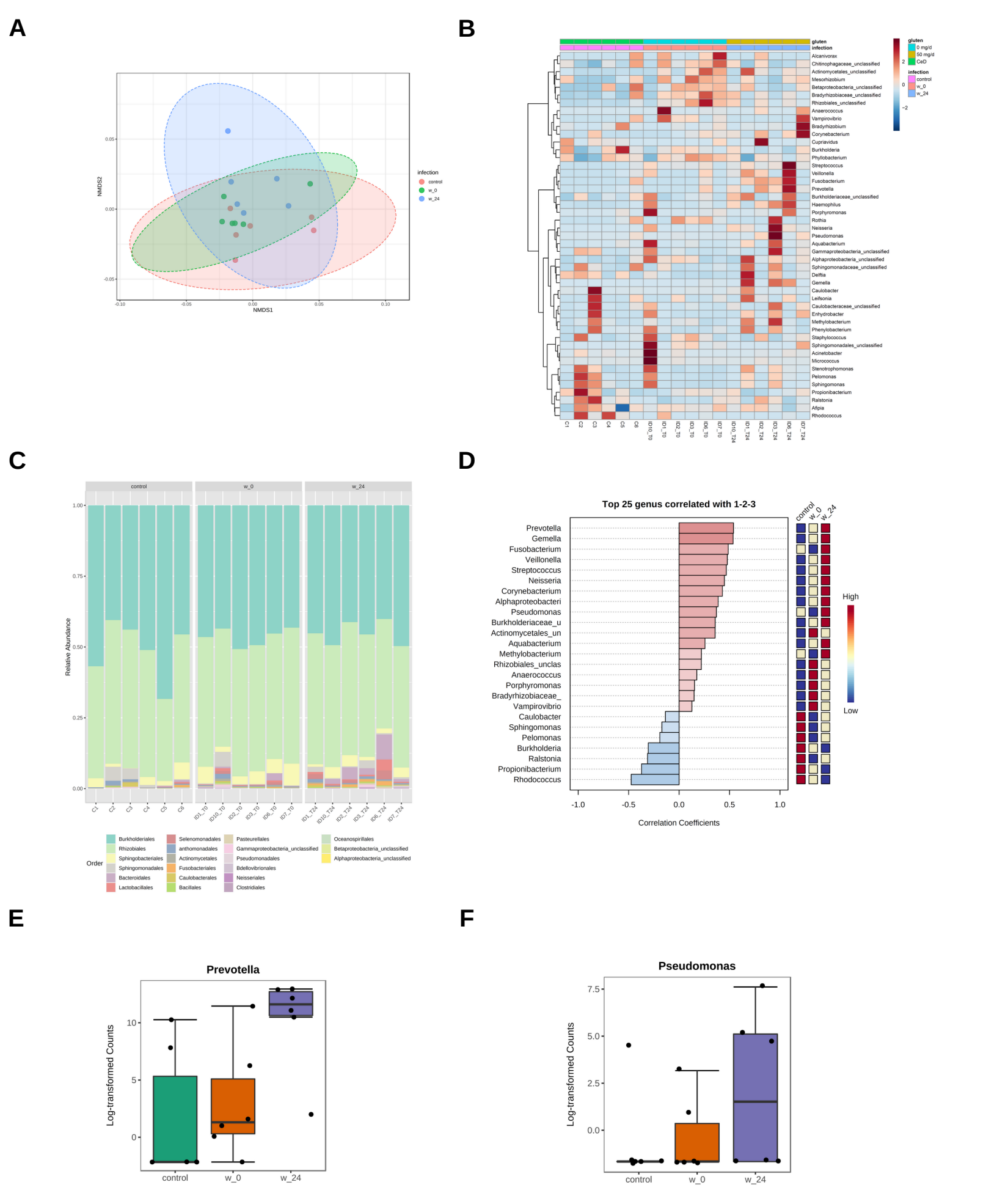
A
NMDS1
NMDS2
infection
control
w_0
w_24
B
gluten
infection
0 mg/d
50 mg/d
CeD
control
w_0
w_24
Alcanivorax
Chitinophagaceae_unclassified
Actinomycetales_unclassified
Mesorhizobium
Betaproteobacteria_unclassified
Bradyrhizobiaceae_unclassified
Rhizobiales_unclassified
Anaerococcus
Vampirovibrio
Bradyrhizobium
Corynebacterium
Cupriavidus
Burkholderia
Phyllobacterium
Streptococcus
Veillonella
Fusobacterium
Prevotella
Burkholderiaceae_unclassified
Haemophilus
Porphyromonas
Rothia
Neisseria
Pseudomonas
Aquabacterium
Gammaproteobacteria_unclassified
Alphaproteobacteria_unclassified
Sphingomonadaceae_unclassified
Delftia
Gemella
Caulobacter
Leifsonia
Caulobacteraceae_unclassified
Enhydrobacter
Methylobacterium
Phenylobacterium
Staphylococcus
Sphingomonadales_unclassified
Acinetobacter
Micrococcus
Stenotrophomonas
Pelomonas
Sphingomonas
Propionibacterium
Ralstonia
Afipia
Rhodococcus
C
control
w_0
w_24
Relative Abundance
Order
Burkholderiales
Rhizobiales
Sphingobacteriales
Sphingomonadales
Bacteroidales
Lactobacillales
Selenomonadales
anthomonadales
Actinomycetales
Fusobacteriales
Caulobacterales
Bacillales
Pasteurellales
Gammaproteobacteria_unclassified
Pseudomonadales
Bdellovibrionales
Neisseriales
Clostridiales
Oceanospirillales
Betaproteobacteria_unclassified
Alphaproteobacteria_unclassified
D
Top 25 genus correlated with 1-2-3
Prevotella
Gemella
Fusobacterium
Veillonella
Streptococcus
Neisseria
Corynebacterium
Alphaproteobacteri
Pseudomonas
Burkholderiaceae_u
Actinomycetales_un
Aquabacterium
Methylobacterium
Rhizobiales_unclas
Anaerococcus
Porphyromonas
Bradyrhizobiaceae_
Vampirovibrio
Caulobacter
Sphingomonas
Pelomonas
Burkholderia
Ralstonia
Propionibacterium
Rhodococcus
control
w_0
w_24
High
Low
Correlation Coefficients
E
Prevotella
Log-transformed Counts
control
w_0
w_24
F
Pseudomonas
Log-transformed Counts
control
w_0
w_24

Figure S7

Figure S7. *Prevotella* and *Pseudomonas* species are enriched in duodenum of worm-infected celiac patients, Related to Figure 7

(A) ß-diversity analysis of control, gluten-challenged uninfected or worm-infected celiac patients.

(B) Heatmap clustering of duodenal microbiome of celiac patients as in (A).

(C) Relative abundances of duodenal microbiome orders of celiac patients as in (A).

(D-F) Correlation analysis of duodenal microbiome at the level of different genera (D) and *Prevotella* (E – r(Pearson)=0.546, p=0.02) or *Pseudomonas* (F – r(Pearson)=0.37, p=0.13) genera of celiac patients as in (A).

Table S2. qPCR primers used in this study. Related to Figures 1-3, 6, 7 and S1-S5.

Gene name	**Forward primer**	**Reverse primer**
human *AREG*	TGATCCTCACAGCTGTTGCT	TCCATTCTCTTGTCGAAGTTTCT
human *EGFR*	GGAGAACTGCCAGAAACTGACC	GCCTGCAGCACTGGTTG
human *F2RL1* (*PAR-2*)	ATCTGGTTCCCCTTGAAGATTGC	GTTCACGACCCAATACCTC
human *IL-1a*	AAGCTGCAGCCAGAGAGGGA	AGCCTTCATGGAGTGGGCCATA
human *IL-8*	CCTGATTTCTGCAGCTCTGTGTGA	CACCCAGTTTTCCTTGGGGTCCA
human *HMGN4*	GTCACGGGCCTCAGCTGGGAT	AGCAGTGTTCGCAGGTGGCTT
human Muc5AC	CTATGTGCTGACCAAGCCCT	TCAGTGTCACGCTCTTCAGG
human *TSLP*	AAAGTACCGAGTTCAACAAC	GTAGCATTTATCTGAGTTTCCG
mouse *AREG*	ATCACAGTGCACCTTTGGAAAC	GTCCCGTTTTCTTGTCGAAGC
mouse *CCL11*	CTTCACCTCCCAGGTGCT	TTGTAGCTCTTCAGTAGTGTGTTGG
mouse *CCL24*	AGTGGTTAGCTACCAGTTGGC	CACTGCCTTGGCCCCTTTAG
mouse *IL-4*	CTGGATTCATCGATAAGCTG	TTTGCATGATGCTCTTTAGG
mouse *IL-5*	AGACTTCAGAGTCATGAGAAG	GCTGGTGATTTTTATGAGTAGG
mouse *IL-13*	CTTAAGGAGCTTATTGAGGAG	CATTGCAATTGGAGATGTTG
mouse Muc5AC	CCATGCAGAGTCCTCAGAACAA	TTACTGGAAAGGCCCAAGCA
mouse *RPL13a*	GAGGTCGGGTGGAAGTACCA	TGCATCTTGGCCTTTTCCTT
mouse *TSLP*	TCAATCCTATCCCTGGCTGC	GCCATTTCCTGAGTACCGTCA

Table S3. Patient demographics. Related to Figure 7 and STAR Methods Bacterial growth on Muc5AC and human sputum samples.

Patient#	Sex	Age	Race
1	M	60	Caucasian
2	M	20	African American
3	F	69	Caucasian
4	M	37	Caucasian