## FIGURE S1

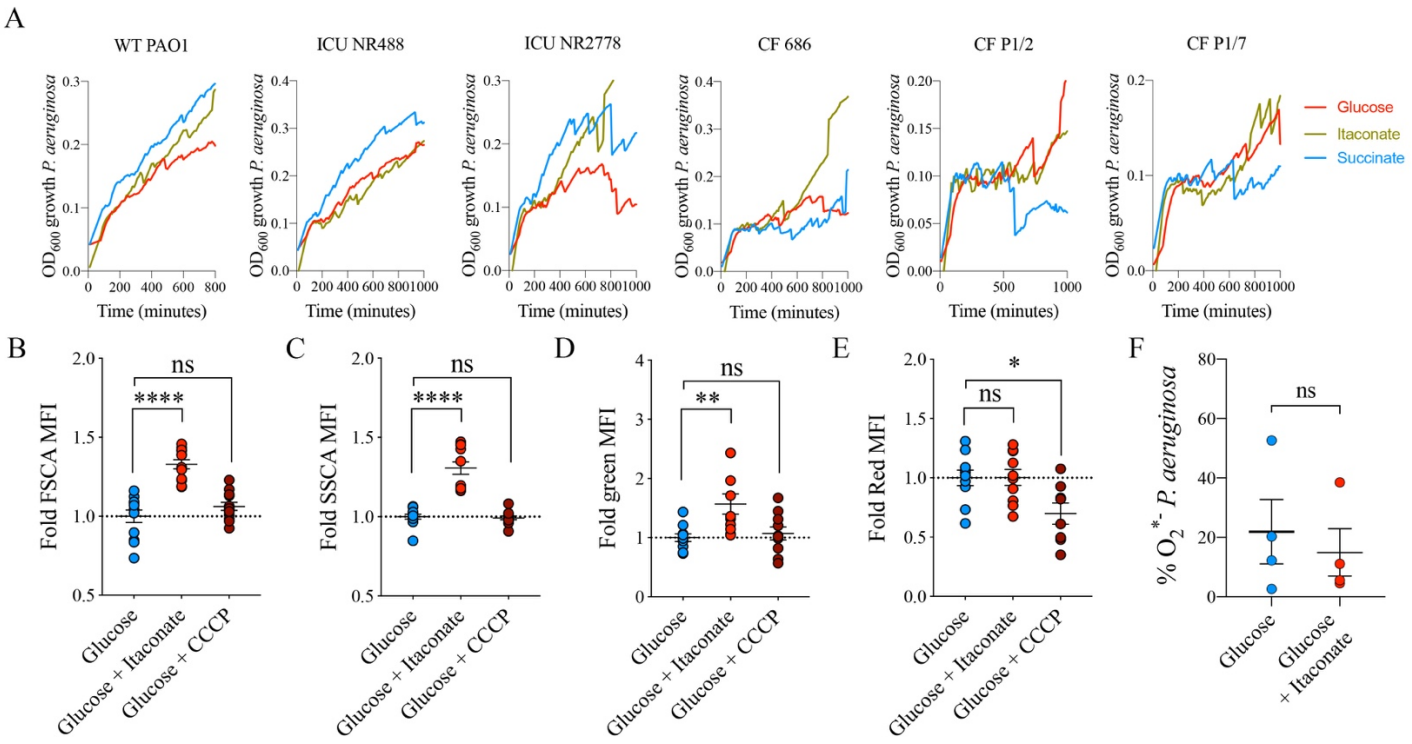



**FIGURE S1, related to FIGURE 1. Itaconate metabolism is associated with *in vivo* host adaptation and membrane deregulations in *P. aeruginosa*.**

**A**) *P. aeruginosa* growth curves in M9 media supplemented either with itaconate, succinate or glucose for PAO1, two ICU isolates (NR488, NR2778) and 3 CF clinical isolates (CF 686, CF P 1/2, CF P1/7).

**B-F**) Bacterial size (Forward FSCA) (B), bacterial surface granularity (Scatter SSCA) (C), $DiOC_2(3)$ green emission (D) and $DiOC_2(3)$ red emission (E) in *P. aeruginosa* PAO1 incubated overnight in either glucose or glucose + itaconate. CCCP, membrane potential uncoupler, was used as a positive control to determine $DiOC_2(3)$ specificity.

**F**) Anion superoxide ($O_2^{*-}$) production by flow cytometry using Mitosox in *P. aeruginosa* PAO1 incubated either in glucose or glucose + itaconate.

**A** are representative curves from n=2. **B-F**, from n=3. In **B-F**, data are shown as average +/- SEM. **B-E**: One-Way ANOVA; **F**: t-Student. ****: $P < 0.0001$; **: $P < 0.01$; *: $P < 0.05$; ns: non-significant.

## FIGURE S2

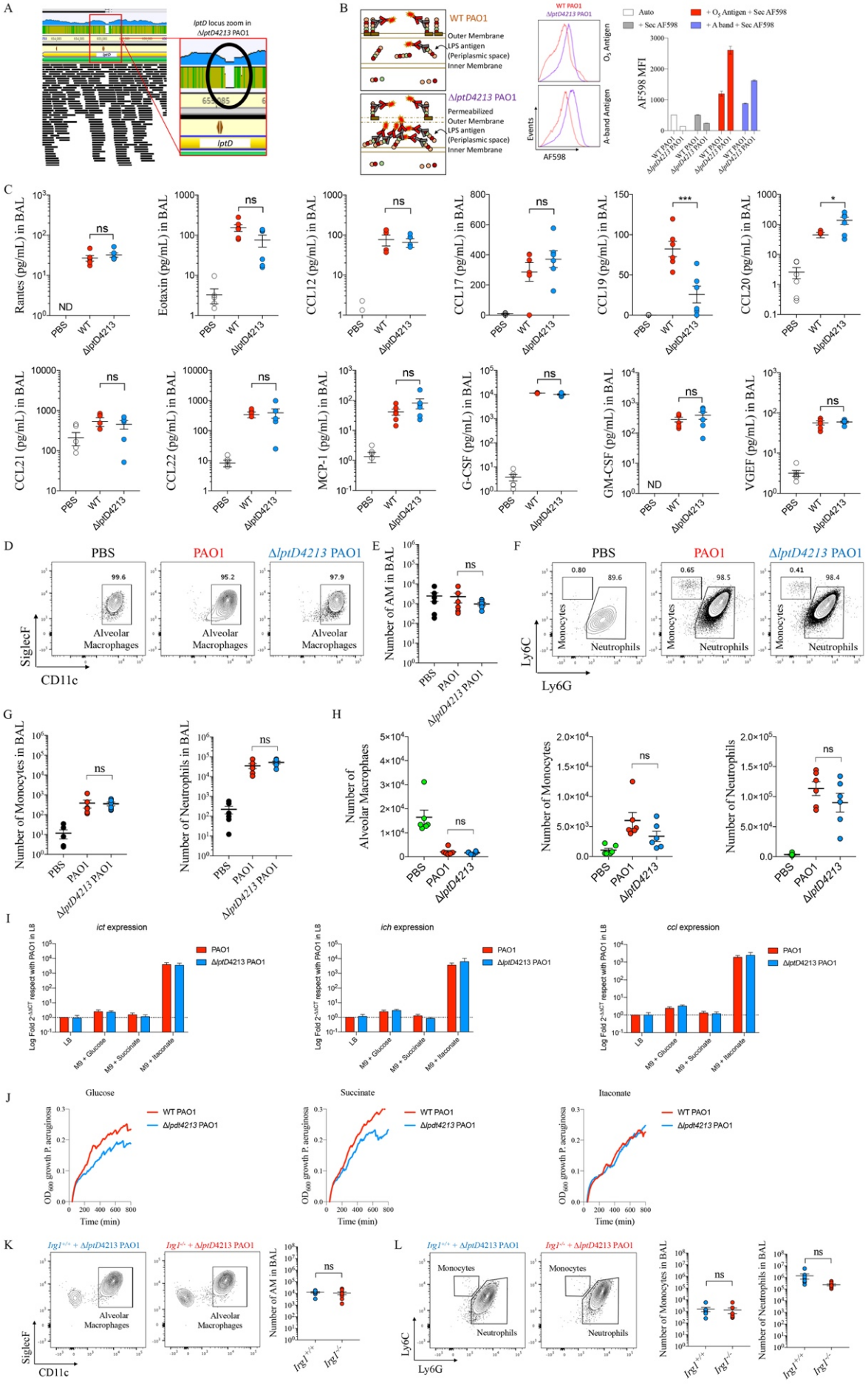

A
lptD locus zoom in ΔlptD4213 PAO1
lptD
B
WT PAO1
Outer Membrane
LPS antigen
(Periplasmic space)
Inner Membrane
ΔlptD4213 PAO1
Permeabilized
Outer Membrane
LPS antigen
(Periplasmic space)
Inner Membrane
WT PAO1
ΔlptD4213 PAO1
O$_x$ Antigen
A-band Antigen
Events
AF598
Auto
+ Sec AF598
+ O$_x$ Antigen + Sec AF598
+ A band + Sec AF598
AF598 MFI
C
Rantes (pg/mL) in BAL
Eotaxin (pg/mL) in BAL
CCL12 (pg/mL) in BAL
CCL17 (pg/mL) in BAL
CCL19 (pg/mL) in BAL
CCL20 (pg/mL) in BAL
CCL21 (pg/mL) in BAL
CCL22 (pg/mL) in BAL
MCP-1 (pg/mL) in BAL
G-CSF (pg/mL) in BAL
GM-CSF (pg/mL) in BAL
VGEF (pg/mL) in BAL
ND
ns
PBS
WT
ΔlptD4213
D
PBS
PAO1
ΔlptD4213 PAO1
99.6
95.2
97.9
Alveolar Macrophages
SiglecF
CD11c
E
Number of AM in BAL
F
0.80
89.6
0.65
98.5
0.41
98.4
Monocytes
Neutrophils
Ly6C
Ly6G
G
Number of Monocytes in BAL
Number of Neutrophils in BAL
H
Number of Alveolar Macrophaes
I
ict expression
ich expression
ccl expression
Log Fold 2-ΔΔCT respect with PAO1 in LB
LB
M9 + Glucose
M9 + Succinate
M9 + Itaconate
J
Glucose
Succinate
Itaconate
OD600 growth P. aeruginosa
Time (min)
WT PAO1
Δlpdt4213 PAO1
K
Irg1+/+ + ΔlptD4213 PAO1
Irg1-/- + ΔlptD4213 PAO1
Irg1+/+
Irg1-/-
L

**FIGURE S2, related to FIGURE 2. *lptD* deregulation conserves itaconate metabolism by PAO1**.

**A**) In respect to WT PAO1, Δ*lptD*4213 PAO1 exhibits a 69 nucleotide deletion (black circle) in the *lptD* gene that produces a truncated LptD protein lacking 23 amino acids.

**B**) Dysfunctional LptD produces membrane permeability and accumulation of LPS-associated sugars in the periplasmic space of *P. aeruginosa*. Anti-LPS antibodies penetrate the outer membrane and detect accumulated endotoxin-associated carbohydrates (middle histograms and right graphs, by flow cytometry). Anti-LPS carbohydrates antibodies used were anti $O_5$ antigen and anti A-band sugars.

**C-H**) BAL chemokines by ELISA (C) and myeloid cell numbers in BAL (D-G) and lungs (H) by flow cytometry from WT mice treated with PBS or infected for 16h either with WT PAO1 or Δ*lptD*4213 PAO1.

**I**) Expression of *ict*, *ich* and *ccl* by qRT-PCR in WT PAO1 and Δ*lptD*4213 PAO1 grown in different media.

**J**) Growth curves for WT PAO1 and Δ*lptD*4213 PAO1 in M9 media supplemented either with succinate, glucose or itaconate.

**K-L**) Alveolar macrophages (K) and monocytes/neutrophils (L) by flow cytometry in *Irg1*$^{+/+}$ and *Irg1*$^{-/-}$ BAL from mice 16h-infected with Δ*lptD*4213 PAO1.

Data are shown as average +/- SEM. **C-H** and **K-L** are from n=2 with a total of 6 mice. **C-H**: One-Way ANOVA; **K-L**: t-Student test. ****: P* < 0.001*; *: P* < 0.05*;* ns: non-significant.

## Figure S3

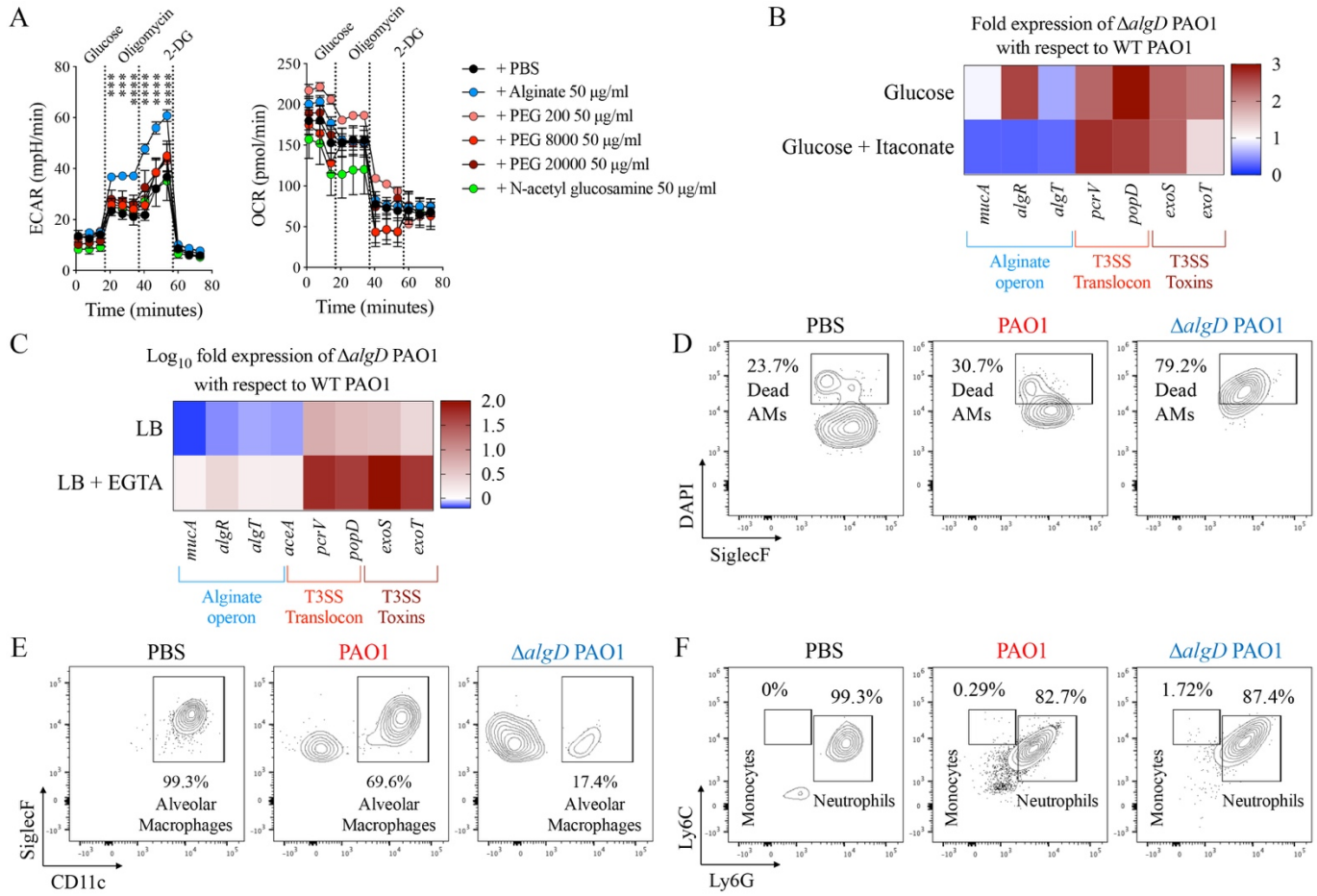



**FIGURE S3, related to FIGURE 3, 4. Increased alginate production promotes myeloid cell activation and accumulation in the murine airway.**

**A**) Glycolysis (ECAR) and oxygen consumption rates (OCR) by Seahorse technology of mouse BMDMs stimulated either with PBS, alginate, polyethylene glycol (200, 8000, 20000 MW) or N-acetyl glucosamine.

**B**) Respect with WT PAO1, fold expression of *mucA, algT*, *algR*, *pcrV*, *popD*, *exoS* and *exoT* by qRT-PCR for Δ*algD* PAO1 incubated in M9 + glucose or M9 + glucose + itaconate.

**C**) Respect with WT PAO1, $Log_{10}$ fold expression of *mucA, algT*, *algR*, *pcrV*, *popD*, *exoS* and *exoT* by qRT-PCR for Δ*algD* PAO1 incubated in LB treated or not with EGTA, which activates type 3 secretion system function.

**D**) $DAPI^{+}$ staining (dead cells) for BAL alveolar macrophages from mice treated with PBS or infected either with WT and Δ*algD* PAO1.

**E-F**) Representative density plots for alveolar macrophages (E) and $Ly6C^{high}Ly6G^{low/-}$ monocytes and $Ly6C^{low/neg}Ly6G^{+}$ neutrophils (F) from BAL of WT mice treated with PBS or infected with PAO1 WT or Δ*algD* PAO1. Data are shown as average +/- SEM. A is from n=2. **A**: Two-Way ANOVA. **D-F**: In vivo data are from n=2 (7-8 mice total). ****: $P < 0.0001$; ***: $P < 0.001$; **; ns: non-significant.

## FIGURE S4

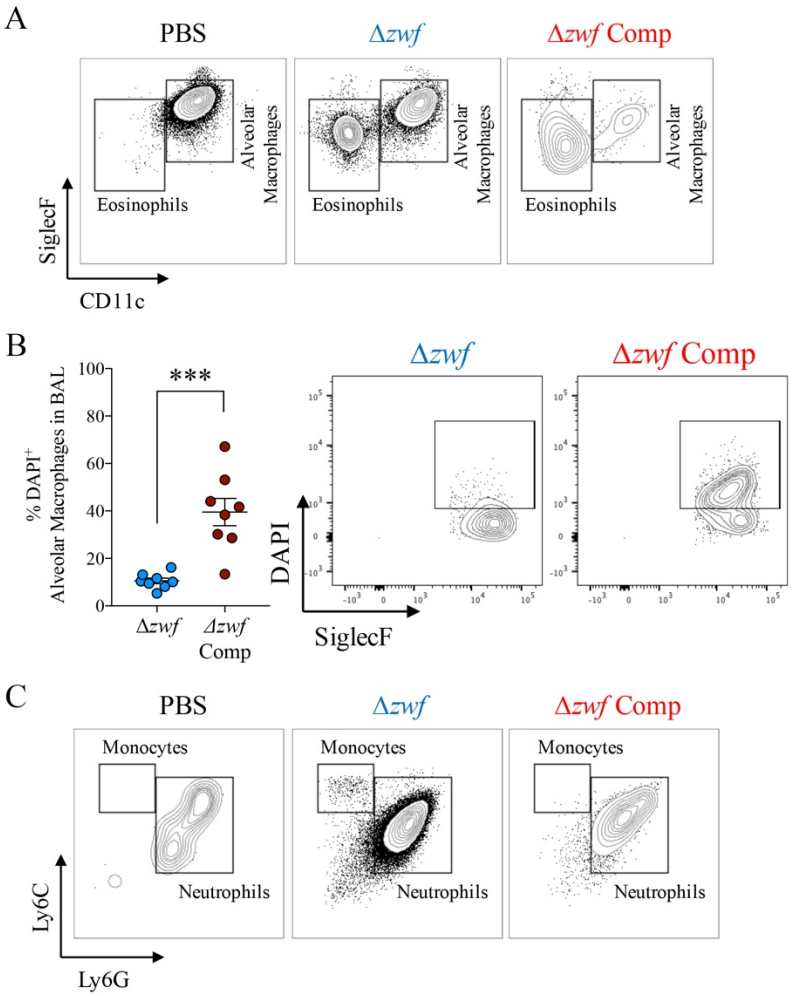



**FIGURE S4, related to 5. *P. aeruginosa* G6PDH activity regulates myeloid cells death.**

**A-C**) Representative density plots for live ($DAPI^-$) alveolar macrophages (A), $DAPI^+$ (dead cells) alveolar macrophages (B) and $Ly6C^{high}Ly6G^{low/-}$ monocytes and $Ly6C^{low/neg}Ly6G^+$ neutrophils (C) in BAL of mice uninfected (PBS) or 16h infected with Δ*zwf* and Δ*zwf* complemented PAO1. Data are shown as average +/- SEM. **A-C** are representative from n=2 (7-8 mice total). **B**: t-Student test. ***: $P < 0.001$; ns: non-significant.

**FIGURE S5**

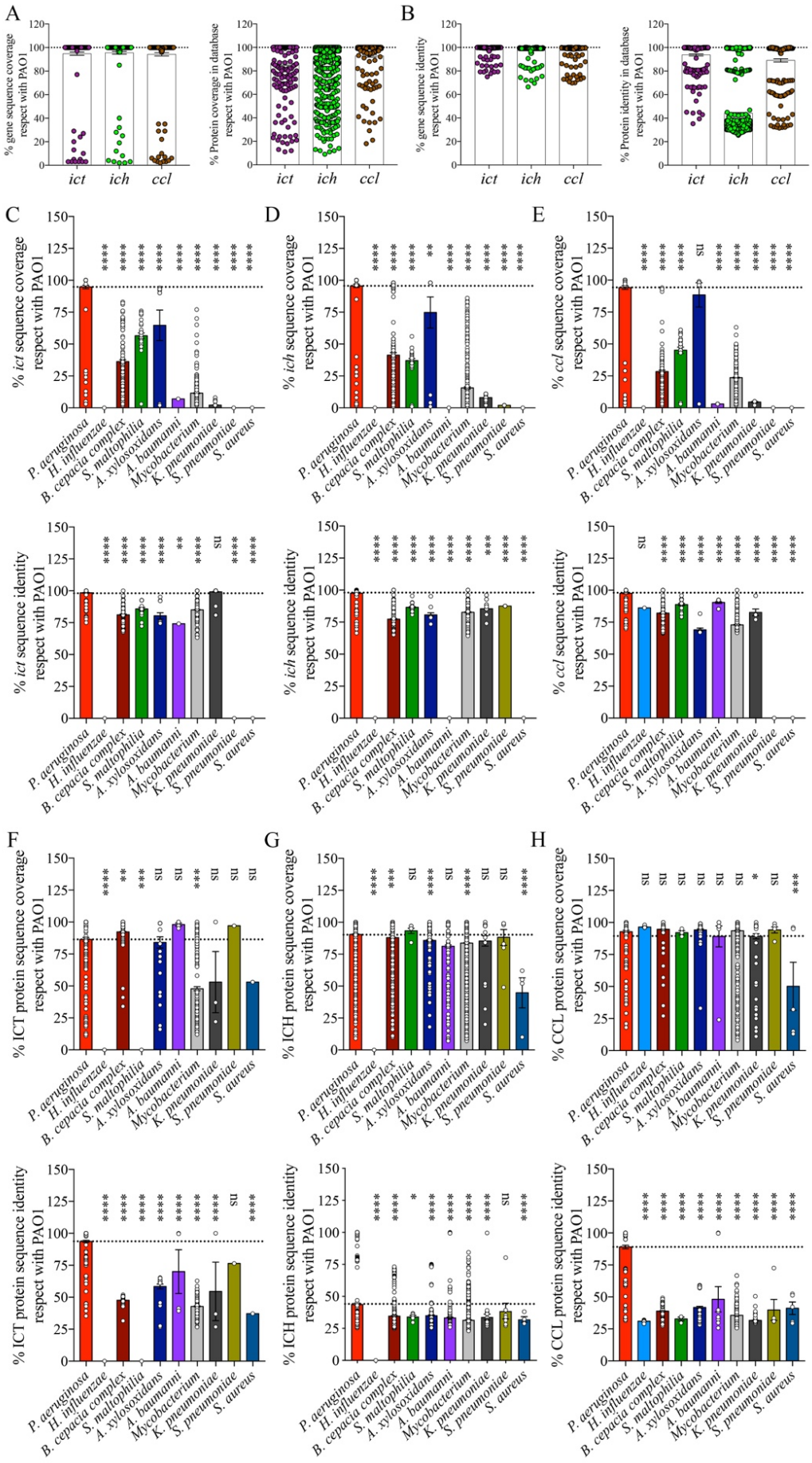

A
B
% gene sequence coverage respect with PAO1
% Protein coverage in database respect with PAO1
% gene sequence identity respect with PAO1
% Protein identity in database respect with PAO1
ict
ich
ccl
C
D
E
% ict sequence coverage respect with PAO1
% ich sequence coverage respect with PAO1
% ccl sequence coverage respect with PAO1
% ict sequence identity respect with PAO1
% ich sequence identity respect with PAO1
% ccl sequence identity respect with PAO1
F
G
H
% ICT protein sequence coverage respect with PAO1
% ICH protein sequence coverage respect with PAO1
% CCL protein sequence coverage respect with PAO1
% ICT protein sequence identity respect with PAO1
% ICH protein sequence identity respect with PAO1
% CCL protein sequence identity respect with PAO1
P. aeruginosa
H. influenzae
B. cepacia complex
S. maltophilia
A. xylosoxidans
A. baumanni
Mycobacterium
K. pneumoniae
S. pneumoniae
S. aureus

**FIGURE S5, related to FIGURE 6. Genes/proteins that regulate itaconate assimilation are conserved in *P. aeruginosa* strains and are found less often in genomes of other respiratory pathogens**.

**A**) *P. aeruginosa* PAO1 *ict*, *ich* and *ccl* genes were compared against all published *P. aeruginosa* genomes (taxid 136841). Upper panel: gene coverage respect with PAO1; lower panel: sequence identity for the coverage matched in the upper panel.

**B**) *P. aeruginosa* PAO1 ICT, ICH and CCL protein sequences were blasted against all published *P. aeruginosa* genomes (taxid 136841). Upper panel: protein coverage respect with PAO1; lower panel: sequence identity for the protein coverage matched in the upper panel.

**C-E**) *P. aeruginosa* PAO1 *ic*t, *ich* and *ccl* genes were compared against all published genomes for different respiratory pathogens. Upper panel: gene coverage respect to PAO1; lower panel: sequence identity for the coverage matched in the upper panel.

**F-H**) *P. aeruginosa* PAO1 ICT, ICH and CCL protein sequences were compared against all published genomes for different respiratory pathogens. Upper panel: protein coverage respect to PAO1; lower panel: sequence identity for the protein coverage matched in the upper panel. Blasts were performed at https://blast.ncbi.nlm.nih.gov/Blast.cgi. *P. aeruginosa* taxid 136841; *H. influenzae* taxid 727; *B. cepacia complex* taxid 87882; *S. maltophilia* taxid 995085; *A. xylodoxidans* 85698; *A. baumanni* taxid 470; *Mycobacteriu*m taxid 85007; *K. pneu*moniae taxid 573; *S. pneumoniae* taxid 1313; *S. aureus* taxid 1280.

Data are shown as average +/- SEM. Data was analyzed by One-Way ANOVA. ****: $P < 0.0001$; ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: non-significant.

## FIGURE S6

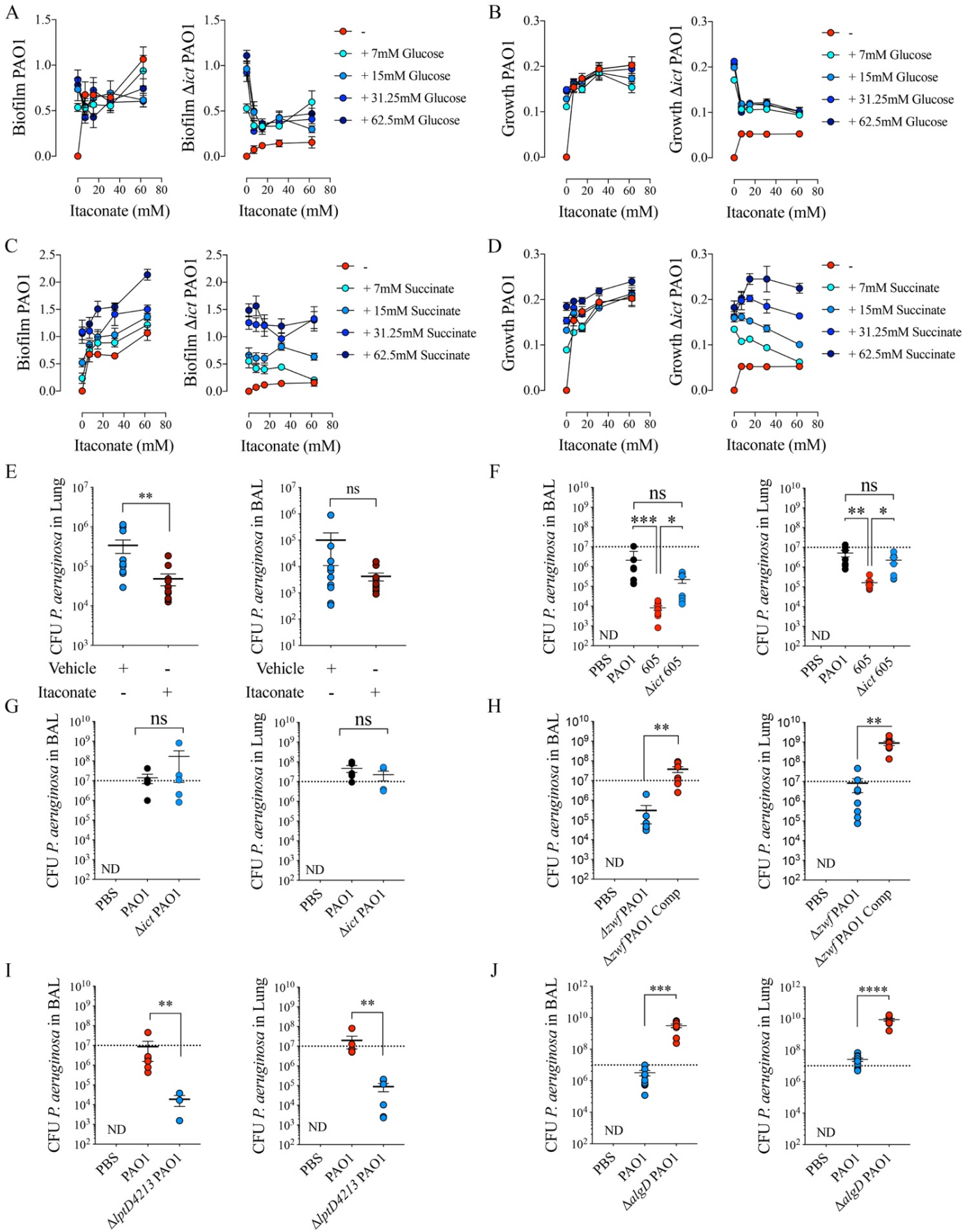

A
Biofilm PAO1
Biofilm Δict PAO1
Itaconate (mM)
-
+ 7mM Glucose
+ 15mM Glucose
+ 31.25mM Glucose
+ 62.5mM Glucose
B
Growth PAO1
Growth Δict PAO1
Itaconate (mM)
C
Biofilm PAO1
Biofilm Δict PAO1
Itaconate (mM)
+ 7mM Succinate
+ 15mM Succinate
+ 31.25mM Succinate
+ 62.5mM Succinate
D
Growth PAO1
Growth Δict PAO1
Itaconate (mM)
E
CFU P. aeruginosa in Lung
CFU P. aeruginosa in BAL
Vehicle
Itaconate
ns
F
CFU P. aeruginosa in BAL
CFU P. aeruginosa in Lung
ND
PBS
PAO1
605
Δict 605
G
CFU P. aeruginosa in BAL
CFU P. aeruginosa in Lung
ND
PBS
PAO1
Δict PAO1
H
CFU P. aeruginosa in BAL
CFU P. aeruginosa in Lung
ND
PBS
Δzwf PAO1
Δzwf PAO1 Comp
I
CFU P. aeruginosa in BAL
CFU P. aeruginosa in Lung
ND
PBS
PAO1
ΔlptD4213 PAO1
J
CFU P. aeruginosa in BAL
CFU P. aeruginosa in Lung
ND
PBS
PAO1
ΔalgD PAO1

**FIGURE S6, related to FIGURE 6. Long-term adaptation to itaconate stress modifies the infective properties of *P. aeruginosa*.**

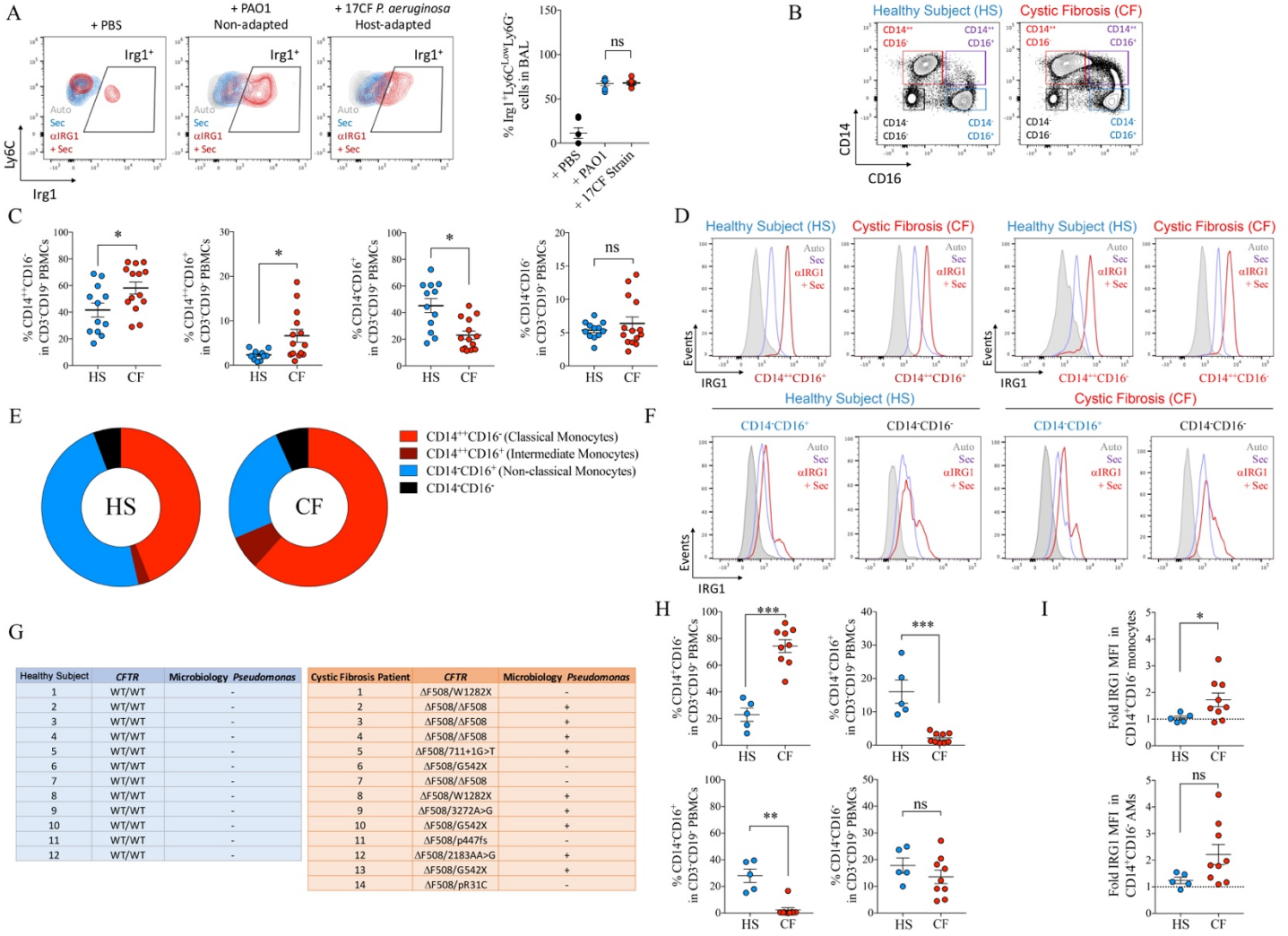
**A-D**) Itaconate-based biofilm and growth for PAO1 and its isogenic Δ*ict* PAO1 mutant cultured overnight in glucose (A-B) or succinate (C-D).

**E**) CFUs recovered from BAL and lung of WT mice 16h-infected with a collection of 17 CF host-adapted *P. aeruginosa* isolates and treated intranasally with 60mM itaconate. Itaconate treatment was 24h previous CFUs counts.

**F-J**) CFUs recovered from BAL and lung of mice 16h-infected either with 605 control, Δ*ict* 605 or WT PAO1 (F), Δ*ict* PAO1 or WT PAO1 (G), Δ*zwf* PAO1 or Δ*zwf* complelemented PAO1 (H), Δ*lptD*4213 PAO1 or WT PAO1 (I) or Δ*algD* PAO1 or WT PAO1 (J).

Data are shown as average +/- SEM. All data is from n=2 (in vivo, 5-9 mice in total). **E**: t-Student test; **F-J**: One-Way ANOVA. ****: $P < 0.0001$; ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: non-significant.

## FIGURE S7




| Healthy Subject | *CFTR* | Microbiology *Pseudomonas* |
|---|---|---|
| 1 | WT/WT | - |
| 2 | WT/WT | - |
| 3 | WT/WT | - |
| 4 | WT/WT | - |
| 5 | WT/WT | - |
| 6 | WT/WT | - |
| 7 | WT/WT | - |
| 8 | WT/WT | - |
| 9 | WT/WT | - |
| 10 | WT/WT | - |
| 11 | WT/WT | - |
| 12 | WT/WT | - |

| Cystic Fibrosis Patient | *CFTR* | Microbiology *Pseudomonas* |
|---|---|---|
| 1 | ΔF508/W1282X | - |
| 2 | ΔF508/ΔF508 | + |
| 3 | ΔF508/ΔF508 | + |
| 4 | ΔF508/ΔF508 | + |
| 5 | ΔF508/711+1G>T | + |
| 6 | ΔF508/G542X | - |
| 7 | ΔF508/ΔF508 | - |
| 8 | ΔF508/W1282X | + |
| 9 | ΔF508/3272A>G | + |
| 10 | ΔF508/G542X | + |
| 11 | ΔF508/p447fs | - |
| 12 | ΔF508/2183AA>G | + |
| 13 | ΔF508/G542X | + |
| 14 | ΔF508/pR31C | - |

**FIGURE S7, related to FIGURE 7. Local and peripheral itaconate-producing cells are associated with *P. aeruginosa* lung infection.**

**A**) Representative density plots (left panel) and percentage of Irg1-expressing Ly6C$^{low}$Ly6G$^{-}$ monocytes (right graph) from mice treated either with PBS or infected with PAO1 or a collection of 17 CF-host adapted *P. aeruginosa* isolates.

**B**) Human PBMCs were sorted (DAPI$^{-}$CD45$^{+}$CD3$^{-}$CD19$^{-}$) and analyzed for monocytes using CD14 and CD16 markers by flow cytometry.

**C**) Dot plots for frequency quantification of the four populations found in B in healthy versus subjects with CF.

**D**) Intracellular IRG1 levels by flow cytometry in the CD14$^{++}$CD16$^{-}$ and CD14$^{++}$CD16$^{+}$ blood monocyte cells.

**E**) Frequency quantification of the four populations found in B in healthy subjects versus individuals with CF.

**F**) Intracellular IRG1 levels by flow cytometry in the CD14$^{-}$CD16$^{-}$ and CD14$^{-}$CD16$^{+}$ blood monocyte cells.

**G**) List of human samples analyzed depicting *CFTR* mutation involved and whether patients were actively colonized by *P. aeruginosa* (+) or not (-).

**H**) Percentage of CD14$^{+}$CD16$^{-}$, CD14$^{+}$CD16$^{+}$ (alveolar macrophages), CD14$^{-}$CD16$^{+}$ and CD14$^{-}$CD16$^{-}$ cells in DAPI$^{-}$ CD45$^{+}$CD3$^{-}$CD19$^{-}$ sputum cells from healthy subjects and individuals with CF.

**I**) Fold IRG1 MFI over secondary antibody staining for CD14$^{+}$CD16$^{-}$ and CD14$^{+}$CD16$^{+}$ (alveolar macrophages) sputum cells.

Data are shown as average +/- SEM. A is representative from n=2 with a total of 5 mice. Sputum from 9 individuals with CF and 5 healthy subjects were analyzed. In **C, H** and **I**, each dot represents a single subject. In **A**, **C**, **H** and **I** data were analyzed by t-Student test. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: non-significant.