1

2 ***Pseudomonas aeruginosa lasR* Mutants Resist Phagocytosis and Alter Inflammatory**

3 **Cytokine Production by Cystic Fibrosis Macrophages**

4

5

6 Daniel S. Aridgides[1], Diane L. Mellinger[1], Lorraine L. Gwilt[1], Thomas H. Hampton[2], Dallas L.

7 Mould[3], Deborah A. Hogan[2], and Alix Ashare[1,2*]

8 [1]Section of Pulmonary and Critical Care Medicine, Dartmouth-Hitchcock Medical Center,

9 Lebanon, NH

10 [2]Department of Microbiology and Immunology, Geisel School of Medicine, Hanover, NH

11 [3]Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT

12 * Correspondence: daniel.s.aridgides@hitchcock.org

13

Abstract

Cystic Fibrosis is characterized by chronic muco-obstructive lung disease and infection. People with CF (pwCF) are often colonized with *Pseudomonas aeruginosa* for years to decades, allowing for evolutionary adaptation. In chronic *P. aeruginosa* lung isolates from pwCF, the quorum sensing regulator LasR frequently is nonfunctional, however the factors enabling *lasR* loss-of-function (LOF) mutant selection are incompletely understood. We hypothesized that LOF mutations in *lasR* could allow *P. aeruginosa* to resist the selective pressure of phagocytosis. We found that in multiple strain backgrounds, LasR LOF decreased phagocytosis by both model THP-1 and primary monocyte-derived macrophages. While exogenous administration of the quorum-sensing autoinducer 3-oxo-C12-homoserine-lactone (3OC12HSL) that is made by an enzyme regulated by LasR activity inhibited phagocytosis and mitochondrial respiration, the phagocytosis resistance seen with *lasR* mutants appears to be bacterial cell intrinsic rather than due to secreted factors. Finally, we found that *lasR* LOF mutations altered the inflammatory profile upon infection of CF macrophages, with a shift from IL-1 family cytokine expression towards canonical inflammatory markers including IL-6 and TNFα. Collectively these data provide a potential explanation for both the prevalence of *lasR* mutants in the CF lung as well as their association with worse outcomes.

Importance

Cystic Fibrosis (CF) is a genetically inherited disease that leads to chronic lung infections. *Pseudomonas aeruginosa* is often implicated with worsening of lung disease, and it evolves in the lung over time to resist eradication. One of the most commonly disrupted gene targets identified in *Pseudomonas aeruginosa* isolates from chronically infected CF lungs is *lasR*, which encodes a transcription factor which regulates multiple virulence factors. What contributes to the apparent fitness of *lasR* mutants in the CF lung is not well known. Our study shows that *lasR* loss-of-function (LOF) mutants resist phagocytosis by macrophages, one of the fundamental

39 mechanisms of clearance by the immune system. We identify mechanisms promoting
40 resistance to phagocytosis, and explore the downstream consequences on inflammatory
41 responses. Understanding why *lasR* mutations arise could inform strategies to eradicate them
42 from the CF lung and improve outcomes.

43

44 **Introduction**

45 Cystic Fibrosis (CF) is a multisystem disorder caused by mutations in the Cystic Fibrosis
46 Transmembrane Conductance Regulator (*CFTR*) gene, encoding a chloride and bicarbonate
47 channel (1). In the lungs of people with CF (pwCF), CFTR mutations lead to dehydrated, sticky
48 mucus and subsequent colonization with bacteria often including *Pseudomonas aeruginosa* (2).
49 Novel highly effective modulator compounds which improve trafficking and function of the CFTR
50 protein have markedly improved outcomes for the subset of pwCF harboring CFTR mutations
51 that are responsive to them (3–12), however CFTR modulators cannot restore sterilizing
52 immunity to the lungs, particularly when there is pre-existing structural damage (13, 14). Given
53 their relatively recent availability, the long-term trajectory of lung function in pwCF remains
54 unknown, even for those who are clinically improved on modulators.

55 Lung function over time is impacted by bacterial colonization, with cycles of infection and
56 dysregulated inflammation leading to lung damage (1). The specific composition of the lung
57 microbiota as well as the host response to them play a role in determining whether lung function
58 worsens or remains stable. Low bacterial diversity, *P. aeruginosa* colonization, and detection of
59 *P. aeruginosa* with disruptions to the LasR/I quorum sensing system have all been shown to
60 correlate with accelerated lung function decline in pwCF (15–17).

61 LasR loss-of-function (LOF) mutants are one of the most commonly isolated morphotypes found
62 in CF lungs (18). LasR LOF mutants have also been isolated from environments such as pond
63 water, and will arise de novo when *P. aeruginosa* are cultured in vitro (18–21). Their common
64 emergence across various settings argues for a broad (or common) competitive advantage over
65 LasR intact (LasR+) comparators, however the reasons for selection *in vivo* are not entirely
66 clear. Social cheating whereby *lasR* LOF mutants can utilize secreted products from other *P.*
67 *aeruginosa* isolates without incurring the metabolic cost of producing them has been proposed
68 as a mechanism for *lasR* mutant selection. *lasR* mutation has also been shown increase

69 microoxic fitness and phosphate scavenging (22–24), providing additional possibilities for its
70 evolutionary advantage. Indeed *lasR* LOF mutants are selected for in vitro cultures due to a
71 growth advantage in post exponential phase (25–27) however these experimental systems lack
72 the host component. Interestingly, the phenotype of lack of protease activity has been shown to
73 increase in *P. aeruginosa* isolates that persist in pwCF on highly effective modulators,
74 suggesting *lasR* mutation may also be increased post modulator therapy (28).

75 In the CF lung *P. aeruginosa* must coexist with host immune cells which are attempting to
76 eradicate it, yet the effects of *lasR* LOF mutations on *P. aeruginosa* interactions with host
77 immune cells remain comparatively unstudied. Studies on interaction with airway epithelial cells
78 (29, 30), neutrophils (31, 32), and macrophages (33) have revealed variable effects on
79 inflammatory responses. Macrophages are responsible for orchestrating the host immune
80 response in the lung, phagocytosis of pathogens and debris, and have been shown to have
81 altered inflammatory and effector activity in CF (34). Although CFTR modulators have been
82 shown to improve CF macrophage function (35–37), an improved mechanistic understanding of
83 how modulators affect the host immune response could benefit the majority of pwCF who are
84 now eligible for highly effective modulator treatment and and lead to novel interventions that
85 might reduce bacterial persistence.

86 We hypothesized that loss of LasR function would alter interactions with host macrophages as a
87 mechanism of *lasR* mutant persistence within the CF lung, and that CFTR modulators would
88 further impact host response. We found that *lasR* LOF mutants indeed resist phagocytosis in
89 multiple *P. aeruginosa* backgrounds including laboratory and clinical isolates. Phagocytosis
90 resistance was maintained in mixed cultures with LasR+ relatives. *lasR* mutant resistance to
91 phagocytosis by primary CF monocyte derived macrophages (MDMs) was maintained despite
92 the presence of highly effective CFTR modulators. Finally, *lasR* mutation shifted the
93 inflammatory response of MDMs to *P. aeruginosa* infection away from IL-1 family cytokines

94 towards canonical proinflammatory cytokines IL-6 and TNFα. Collectively these results highlight
95 the importance of understanding *P. aeruginosa lasR* LOF in the context of immune cells and
96 provide potential explanations for its increased prevalence and virulence in CF. Some of these
97 results have previously been presented in the form of abstracts (38–42).

98 **Results**

99 ***lasR* loss-of-function mutations confer phagocytosis resistance independent of**
100 **background *P. aeruginosa* strain**

101 To test the hypothesis that loss-of-function mutations in *lasR* confer phagocytosis resistance we
102 utilized model THP-1 monocytes which can be differentiated into macrophage-like cells by the
103 addition of the protein kinase C activator phorbol 12-myristate 13-acetate (43). We performed
104 gentamicin-protection assays with three pairs of LasR+ and LasR- *P. aeruginosa* to compare
105 rates of bacterial uptake. We chose a 20 minute timepoint to study phagocytosis as this
106 provides an investigation of uptake alone, whereas longer timepoints may see a mixture of
107 phagocytosis and killing (36). In strain PA14Δ*lasR*-THP-1 co-cultures, fewer cells were
108 phagocytosed then the in the otherwise isogenic wild-type (WT) strain (**Figure 1A**). We then
109 utilized two closely related pairs of LasR+/LasR- clinical isolates for which *lasR* LOF evolved
110 over the course of infection to confirm this effect with *P. aeruginosa* derived from the CF lung.
111 LasR LOF continued to confer phagocytosis resistance in both clinical isolate backgrounds
112 (**Figure 1B,C**) demonstrating that this was not a strain-specific effect.

113 **LasR but not RhlR autoinducer homoserine lactone inhibits phagocytosis**

114 One of the primary signaling molecules regulated by LasR is the autoinducer N-(3-
115 oxododecanoyl)-L-homoserine lactone (3OC12HSL) that binds to the LasR regulator to induce a
116 positive feedback cycle. 3OC12HSL has been shown to have direct effects on eukaryotic
117 metabolism and signaling (44–55), as well as enhancing phagocytosis of yeast (56). We

118 therefore tested whether 3OC12HSL would increase phagocytosis of *P. aeruginosa* as an
119 explanation for the decreased *lasR* mutant phagocytosis. Contrary to this hypothesis, we found
120 that 3OC12HSL inhibited phagocytosis of both LasR+ and LasR- PA14 at concentrations of 50
121 μM or higher in a dose-dependent manner (**Figure 2A**). By comparison, the RhlR autoinducer
122 C4-homoserine lactone (C4HSL) had no appreciable effect (**Figure 2B**). Under all
123 concentrations of HSLs tested there continued to be a reproducible difference between LasR+
124 and LasR- phagocytosis. These results suggest that 3OC12HSL is not responsible for the
125 effects seen in **Figure 1**.

126 **Phagocytosis resistance of LasR- strains is maintained in mixed cultures**

127 We therefore sought to ascertain whether *lasR* mutation effects were due to secreted or cell
128 intrinsic factors. We performed phagocytosis assays with a mixture of LasR+/LasR- PA14
129 derivatives at varying percentages (0/100, 10/90, 30/70, 50/50, 70/30, 90/10 and 100/0). The
130 LasR- competitor in the PA14 background bore a *lacZ* reporter gene and plating onto X-gal
131 plates allowed for post-assay determination of strain identities when mixed with an untagged
132 wild type. While we found bacterial uptake was generally proportional to the percent input for
133 each strain (**Figure 3A**), there was a persistently lower amount of LasR- *P. aeruginosa*
134 phagocytosed. **Figure 3B** displays the same data as in **3A** graphed relative to percent input for
135 each strain such that the decreased phagocytosis of LasR- strains is evident. These data
136 suggest that the effects of *lasR* mutation on phagocytosis is due to cell intrinsic factors.

137 **Primary human macrophages augment phagocytosis with CFTR modulators, but LasR**
138 **phagocytosis resistance is maintained**

139 To determine if the differences between phagocytosis of LasR+ and LasR- cells seen in THP-1
140 cells were also observed when using primary human cells, we isolated peripheral blood derived
141 mononuclear cells (PBMCs) from volunteers and differentiated them into monocyte-derived

142 macrophages (MDMs) in vitro for seven days. We first tested cells from CF volunteers. We and
143 others have demonstrated that CFTR modulators have a positive effect on macrophage and
144 monocyte phagocytosis (36, 37, 57–63), and as a majority of pwCF are now eligible for CFTR
145 modulators it is important to understand whether they may play an additional role in modifying
146 the response to *lasR* mutants. We tested one pair of laboratory isolates and one pair of clinical
147 isolates (DH1137/6) in MDMs that were treated for 48 hours with CFTR modulators or DMSO as
148 a control. The PA14Δ*lasR* (LasR-) strain was less efficiently phagocytosed by primary CF MDMs
149 relative to its LasR+ progenitor (**Figure 4A**). Tezacaftor/ivacaftor (TI) and
150 elexacaftor/tezacaftor/ivacaftor (ETI) improved phagocytosis of both strains in the PA14
151 background (**Figure 4A**) however there was no statistically significant interaction with *lasR*
152 status. We found similar results using paired clinical *P. aeruginosa* isolates, where *lasR* mutants
153 were less efficiently phagocytosed and CFTR modulators improved phagocytosis of both strains
154 with no difference in response to modulators between isolates (**Figure 4B**). We have previously
155 demonstrated that nonCF MDMs respond similarly to CFTR modulators when compared with
156 CF MDMs (37), therefore we tested whether any of the effects on *lasR* mutant phagocytosis
157 might be modified by *CFTR* genotype. NonCF MDMs displayed a similar pattern, with
158 decreased phagocytosis of *lasR* mutants, improved phagocytosis in the presence of CFTR
159 modulators, and no statistically significant interaction between modulators and *lasR* mutation
160 (**Figure 4C**). With the LasR+/LasR- clinical isolates, there continued to be an effect of *lasR*
161 mutation (**Figure 4D**) however double CFTR modulator treatment only displayed a non-
162 significant trend towards enhanced phagocytosis, with triple modulators still proving effective
163 (**Figure 4D**). Collectively these results confirm that *lasR* mutants exhibit reduced phagocytosis
164 by primary human CF and nonCF macrophages, and that while CFTR modulator treatment is
165 still beneficial, it fails to restore phagocytosis to the level of LasR+ strains.

166 ***lasR* mutation abrogates the inhibitory effect of *P. aeruginosa* conditioned medium on**
167 **macrophage mitochondrial respiration**

168 Macrophage metabolism impacts their inflammatory predilection, with activated macrophages
169 relying on glycolysis for ATP generation whereas pro-resolution utilized aerobic respiration (64,
170 65). We have previously demonstrated that secreted products from *P. aeruginosa* inhibit aerobic
171 respiration in MDMs (37), and we hypothesized that *lasR* mutation may impact this effect. In
172 addition, we saw that ETI inhibits aerobic respiration without significantly affecting glycolysis.
173 We therefore performed Seahorse metabolic flux analysis on CF MDMs pre-treated with CFTR
174 modulators and then then conditioned media from either LasR+ or LasR- PA14 strains was
175 added acutely. Oxygen consumption rate (OCR) was measured as a proxy for mitochondrial
176 respiration and extracellular acidification rate (ECAR) as a proxy for lactate
177 generation/glycolysis. **Figure 5A-C** represent example readouts of OCR from a single CF
178 subject pre-treated with DMSO, TI or ETI, respectively and **Figure 5D-F** are the corresponding
179 readouts for ECAR. Addition of oligomycin, FCCP and rotenone/antimycin A allow for
180 measurement of additional parameters related to mitochondrial respiration. In CF MDMs, ETI
181 decreased basal respiration similarly to prior experiments whereas TI had no effect (**Figure 5G**).
182 Upon acute injection of LasR+ conditioned medium, there was a decrease in mitochondrial
183 respiration (**Figure 5H**), however LasR- conditioned medium completely lost this effect. In the
184 setting of low basal mitochondrial respiration with ETI, the effect of *P. aeruginosa* conditioned
185 medium (either LasR+ or LasR-) was lost (**Figure 5H**). Corresponding decreases in ATP-linked
186 respiration were seen with LasR+ conditioned medium and ETI pre-treatment (**Figure 5G**).
187 Basal glycolysis was not affected by CFTR modulators or different prior to injection (**Figure 5J**)
188 however LasR+ but not LasR- conditioned medium caused an acute rise in glycolysis (**Figure**
189 **5K**). This effect was lost upon ETI pre-treatment (**Figure 5K**). OCR/ECAR ratio is a way of
190 gauging relative contributions of each pathway to ATP-generation, and in line with the effects

191 seen on ATP-linked respiration and glycolysis, OCR/ECAR was decreased with LasR+ but not
192 LasR- conditioned media, as well as by ETI (**Figure 5L**). Measurements of maximal respiration,
193 spare respiratory capacity, non-mitochondrial respiration and proton leak are shown in
194 **Supplemental Figure 1**. These results collectively demonstrate that *lasR* mutation eliminates
195 the mitochondrial effects of *P. aeruginosa* exoproducts. Additionally, CFTR modulators inhibit
196 basal mitochondrial activity and there is no additive effect of *P. aeruginosa* exoproducts. Both of
197 these observations have potential downstream implications for induction of inflammation.

198 We next examined nonCF MDMs to determine whether there may be any differences in how
199 they respond to *lasR* mutants. Similarly to CF MDMs, we found the ETI inhibits basal respiration
200 (**Figure 5M**) with no effect of TI, in agreement with our prior published work (37). LasR+ but not
201 LasR- conditioned medium inhibited mitochondrial respiration (**Figure 5N**) and decreased ATP-
202 linked respiration (**Figure 5O**). These effects were decreased or eliminated by ETI-pretreatment.
203 Modulators had no effect on basal glycolysis (**Figure 5P**), with increased glycolysis secondary
204 to LasR+ but not LasR- conditioned medium (**Figure 5Q**). In contrast to CF MDMs, differences
205 in OCR/ECAR were not statistically significant (**Figure 5R**). Measurements of maximal
206 respiration, spare respiratory capacity, non-mitochondrial respiration and proton leak are shown
207 in **Supplemental Figure 2**.

208 **LasR autoinducer homoserine lactone inhibits macrophage mitochondrial respiration**
209 **and induces glycolysis**

210 The divergence between LasR+ and LasR- conditioned medium effects on MDM metabolism led
211 us to hypothesize that 3OC12HSL might underlie these effects. To test this, we returned to THP-
212 1 derived model macrophages and performed Seahorse metabolic analysis with acute injection
213 of 3OC12HSL, with C4HSL as a control. After first confirming no differences in basal respiration
214 prior to injection (**Figure 6A**), we found a dose responsive effect of 3OC12HSL injection (**Figure**
215 **6B**) causing inhibition of mitochondrial respiration whereas C4-HSL had no effect. This was

216 partially reflected in differences in ATP-linked respiration (**Figure 6C**) although the effects were
217 not statistically significant. Simultaneous measurement of glycolytic rates confirmed no basal
218 differences in glycolysis (**Figure 6D**), with a dose-dependent increase in glycolysis upon
219 3OC12HSL injection (**Figure 6E**) and no effect of C4HSL. The shift in metabolic profile from
220 mitochondrial respiration to glycolysis by 3OC12HSL was confirmed with decreased OCR/ECAR
221 (**Figure 6F**). Measurements of maximal respiration, spare respiratory capacity, non-
222 mitochondrial respiration and proton leak are shown in **Supplemental Figure 3**. These results
223 suggest that 3OC12HSL may underlie the effects of LasR+ *P. aeruginosa* exoproducts on
224 mitochondrial respiration.

225 ***lasR* mutation shifts inflammatory cytokine profiles of primary human macrophages**
226 **away from IL-1 family cytokines towards IL-6 and TNFα irrespective of CFTR modulators**
227 **and *CFTR* mutation status**

228 Finally, we investigated whether *lasR* mutation impacted inflammatory profiles of MDMs, and the
229 interplay with CFTR modulators. We pretreated MDMs with CFTR modulators for 48 hours, then
230 infected MDMs for two hours with LasR+ or LasR- clinical isolates of *P. aeruginosa* and
231 performed cytokine multiplex analysis on the supernatants. Unsurprisingly we found broad
232 induction of multiple inflammatory cytokines upon *P. aeruginosa* infection of CF MDMs (**Figure**
233 **7A**). There was little appreciable effect of CFTR modulators, consistent with our prior work and
234 others' (37, 66). NonCF MDMs had a similar inflammatory response to CF MDMs, with no
235 statistically significant differences between the groups (**Figure 7B**). We then combined results
236 and tested specifically for differences between LasR+ and LasR- infection. IL-1 family cytokines
237 IL-1α, IL-1β and IL-18 were higher with LasR+ infection, whereas IL-6, IL-10, MCP-1, MIP-1α,
238 and MIP-1β were higher with LasR- infection (**Figure 7C**), demonstrating a specific alteration in
239 the inflammatory profile after macrophage response to *lasR* mutants.

240 **Discussion**

241 Loss-of-function mutations in *lasR* have been demonstrated to be important for *P. aeruginosa*
242 persistence and virulence within the CF lung (15, 18). How this impacts interactions with host
243 immune cells and specifically in the presence of highly effective CFTR modulators is
244 incompletely understood (29–33, 67). We have identified for the first time a robust and
245 reproducible resistance to phagocytosis by macrophages that is hypothesized to contribute to
246 persistence in the CF lung. While the difference was modest (0.3 to 0.5 log depending on the
247 assay), this represents a relative difference of 2-3 fold uptake in 20 minutes, therefore over
248 years this could represent a significant evolutionary pressure. The phagocytosis resistance was
249 present in multiple background strains including a common laboratory strain (PA14) and two
250 pairs of clinical isolates from the lungs of pwCF (**Figure 1**), increasing the generalizability of our
251 findings.

252 We also identified potential factors by which LasR mutants resist phagocytosis. The LasR
253 autoinducer 3OC12HSL inhibited *P. aeruginosa* phagocytosis (**Figure 2**), in contrast to prior
254 studies of yeast phagocytosis (56). If 3OC12HSL were responsible for differences in
255 phagocytosis however, it would be expected that LasR mutants would exhibit *higher*
256 phagocytosis rates, which was the opposite of what was seen. Differences in phagocytosis rates
257 were also maintained between LasR+ and LasR- strains when they were mixed in different
258 ratios (**Figure 3**), arguing that the responsible factors are cell intrinsic rather than secreted.

259 We also explored the interplay between CFTR modulators and *lasR* mutation with phagocytosis
260 and inflammation in primary MDMs. While CFTR modulators were able to enhance
261 phagocytosis of all strains tested consistent with our prior work (37), the decrement with *lasR*
262 mutation was maintained (**Figure 4**). Given the known persistence of *P. aeruginosa* in the lungs
263 of pwCF despite highly effective modulator therapy (14), this is relevant to understanding *P.*
264 *aeruginosa* colonization. We previously saw that *P. aeruginosa* secreted products inhibited
265 mitochondrial respiration in MDMs (37), as did the triple modulator combination ETI. Intriguingly

266 we found that *lasR* mutation essentially eliminated this effect (**Figure 5**). In combination with the
267 effects of exogenous 3OC12HSL (**Figure 6**), this suggests that the acyl-homoserine lactone is
268 the responsible factor, in line with previous studies of epithelial cells and fibroblasts (45, 46, 48–
269 51, 68).

270 Unsurprisingly given the variable rates of phagocytosis and differences in effects on
271 mitochondrial function in MDMs, we found that *lasR* mutation impacted inflammatory cytokine
272 profiles upon infection of CF and nonCF MDMs (**Figure 7**). While infection generally induced a
273 broad inflammatory cytokine profile, there was a shift from IL-1 family cytokines IL-1α, IL-1β, IL-
274 1RA and IL-18 towards more classical inflammatory markers IL-6 and TNFα, as well as
275 macrophage inflammatory protein 1α, 1β and macrophage chemoattractant protein 1. There
276 was a trend towards increased IL-8 for *lasR* mutant infection that was not statistically significant
277 after adjustment for multiple comparisons (**Figure 7C**). A shift towards IL-1 family cytokines may
278 reflect increased inflammasome activation versus direct proteolytic activation of IL-1 family
279 precursor proteins. The LasR-regulated elastase LasB has previously been demonstrated to
280 directly activate pro-IL-1β in mice and human macrophages (69, 70), consistent with our data
281 showing less mature IL-1β for infections with *lasR* loss-of-function mutants (**Figure 7C**). Early
282 CF lung *P. aeruginosa* isolates have been shown to be more potent inflammasome activators
283 when compared to later isolates (71), however LasR functional status was not tested in the
284 study by Phuong et al. Decreased protease expression has been seen in persistent *P.*
285 *aeruginosa* isolates in those on ETI, also suggesting *lasR* mutation may be increased post
286 modulators (28). It is important to note that in our system significant LasR quorum sensing
287 activation would not necessarily be expected, as bacteria are sub-cultured at low density prior to
288 addition to cells, and cells were infected for 2 hours. In contrast to its effects increasing
289 processed IL-1β, LasB can decrease IL-8 levels upon infection of airway epithelial cells via
290 direct digestion of IL-8 (72). Future experiments will help to elucidate the exact mechanism

291 whereby *lasR* mutation alters the inflammatory cytokine milieu in the shorter term. We are also
292 currently conducting transcriptomic studies on macrophages infected in parallel with LasR+ or
293 LasR- isolates which should shed light on whether the effects are transcriptional versus post-
294 transcriptional.

295 Strengths of our work include analysis of *lasR* mutations in multiple backgrounds including a
296 common laboratory strain as well as naturally occurring clinical isolates to improve the
297 generalizability. We began to investigate the mechanisms by which *lasR* loss-of-function
298 mutants may confer phagocytosis resistance although work remains to further elucidate the full
299 mechanism. We confirmed the results found in a human cell line (THP-1 cells) with primary
300 human macrophages from multiple subjects and investigated the interplay with CFTR
301 modulators and genotype (mutant vs wild-type CFTR). Studies performed in parallel on cells
302 from the same subject allowed for a close interrogation of the effects of CFTR modulators and
303 LasR mutation on phagocytosis, metabolism and inflammation.

304 Our work also carries several limitations. MDMs, while they are a commonly used surrogate for
305 primary lung macrophages due to their ease of procurement, do not behave identically to lung
306 macrophages (73). We plan to expand our work with primary lung macrophages isolated via
307 bronchoscopy (74), and compare them with classically generated MDMs as well as the novel
308 differentiation protocol proposed by Pahari et al. Additionally, while our ex vivo studies provided
309 the opportunity to directly compare effects of LasR status and modulators in paired samples
310 from the same subject, they are necessarily reductive and cannot replicate in vivo conditions.
311 Mouse models differ from human CF in chronicity and acuity of lung infection, however there are
312 data suggesting *lasR* mutants are more virulent in mice as well (31, 72). Finally, we found no
313 direct effect of CFTR modulators including ETI on inflammatory responses by MDMs, in contrast
314 to human studies pre- and post-modulator which demonstrate decreased inflammation (14, 60,

315 60, 75–78). In vivo studies however cannot separate direct effects on immune cells from
316 decreased mucus and pathogen burden leading to a systemic decrease in inflammation.

317 In summary, *lasR* mutation confers reproducible resistance to phagocytosis, altered metabolic
318 effects on macrophages, and a change in inflammatory cytokine repertoire upon infection.
319 These effects were independent of background strain of *P. aeruginosa*, as well as CFTR
320 genotype and CFTR modulator therapy. Our work adds to the body of literature on how and why
321 *lasR* mutants are problematic in the lungs of pwCF and will likely continue to be so in the highly
322 effective modulator era.

323

324 **Materials and methods**

325 **Source of key chemicals and bacterial strains**

	Source of materials	**Catalog number**
M-CSF	Miltenyi	130-096-491
Pan monocyte isolation kit	Miltenyi	130-096-537
3-oxo-C12-homoserinelactone (3OC12HSL)	Sigma	O9139
C4-homoserinelactone (C4HSL)	Chemodex	B0267
Ivacaftor	Selleckchem	S1144
Tezacaftor	Selleckchem	S7059
Elexacaftor	Selleckchem	S8851
Seahorse mitostress kit	Agilent	
THP-1 cells	ATCC	TIB-202
PMA	Sigma	P1585

PA14 WT	(79)	Hogan Lab (DH122)
PA14 Δ*lasR*	(80)	Hogan Lab (DH164)
PA14 Δ*lasR* att::P*rhlI-lacZ*	(81)	Hogan Lab
DH1137 (LasR+)	(82)	Hogan Lab (DH1137)
DH1136 (LasR-)	(82)	Hogan Lab (DH1136)
DH2417 (LasR+), NC-AMT0101-1-2 (parent of NC-AMT0101-1)	(82)	Hogan Lab (DH2417)
DH2415 (LasR-), NC-AMT0101-1-1, related to DH2417 with LasR LOF (frameshift) allele	(82)	Hogan Lab (DH2415)

326

327 **THP-1 cell culture**

328 THP-1 cells were cultured in RPMI with 10% FBS, 50 μM β-mercaptoethanol, and 50 μg/mL
329 gentamicin in a 37°C humidity-controlled incubator with 5% CO_2. The initial vial was expanded
330 and frozen down in aliquots which were stored in liquid nitrogen with DMSO. Cultures from
331 individual aliquots were maintained in vitro for ~2 months prior to being discarded. THP-1
332 monocytes were differentiated into macrophages by addition of 50 nM Phorbol 12-myristate 13-
333 acetate (PMA) for 48 hours prior to experiments.

334 **Gentamicin protection phagocytosis assays with THP-1 derived macrophages**

335 THP-1 cells were differentiated at 5 x 10^5/mL in 24-well plates. They were then washed with
336 PBS and medium was replaced with identical medium without antibiotics. Overnight LB cultures
337 of bacteria of indicated strains were sub-cultured 1:10 into LB for 60 minutes, centrifuged at
338 8000 x g for 2 minutes, washed twice with antibiotic-free THP-1 medium, then resuspended and
339 bacterial density was determined by spectrophotometry at 600 nm. OD600 of 1.0 was

340 empirically determined to represent 10^9 bacteria/mL media. After dilution, bacteria were added to
341 THP-1 cells at a multiplicity of infection (MOI) of 10 bacteria per macrophage. Plates were
342 incubated at 37°C for 20 minutes, then cells were washed once with PBS, then THP-1 medium
343 with 10x gentamicin (500 µg/mL) was added for 15 minutes. Cells were then washed twice with
344 PBS at 4°C followed by lysis with 250 µL 0.1% Triton X-100. Lysates were diluted 1:3 in PBS
345 and then 10 µL was then plated overnight onto LB agar plates for colony forming unit (CFU)
346 determination. Colonies were counted the next day and bacteria per 10^6 macrophages was
347 calculated. MOI was also determined empirically from the stock bacterial solutions for each
348 assay as an internal control. Average of at least three technical replicates per assay was then
349 log-transformed and results were combined from at least 5 assays performed on separate days
350 (n for each experiment listed in figure legends).

351 **Phagocytosis competition assay**

352 PA14 Δ*lasR* with a *lacZ* reporter gene integrated at a neutral site on the chromosome was used
353 along with PA14 wild type (WT). Assay was identical to above except fixed percentages of PA14
354 WT and the Δ*lasR* strain were used at 100/0, 90/10, 70/30, 50/50, 30/70, 10/90, and 0/100 such
355 that the total MOI of both strains together was constant at 10. Lysates were plated onto LB agar
356 plates with 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) for blue-white colony
357 discrimination. 100/0 samples were entirely white and 0/100 samples were entirely blue,
358 confirming the fidelity of the reporter. Total PA14 WT and Δ*lasR* colonies were counted for each
359 well. Each experiment was performed in triplicate and the means of technical replicates for each
360 assay were log-transformed and combined.

361 **Human subjects**

362 Our blood draw protocol has been approved by the Dartmouth Health Institutional Review
363 Board. Subjects were recruited from our CF clinic population if they were 18-65 years old,

364 F508del homozygous or heterozygous, at clinical baseline without symptoms of exacerbation,
365 and had normal hemoglobin levels. NonCF control subjects were recruited by IRB-approved
366 flyers; smokers or those with respiratory disease, immunologic disorders, or pulmonary
367 medication use were excluded. Phlebotomy was performed (100 mL whole blood isolated) for
368 monocyte isolation by our research nurse and taken directly to the laboratory for downstream
369 processing.

370 **Monocyte derived macrophage (MDM) generation and phagocytosis**

371 MDMs were generated according to previously published protocols (37). Briefly, monocytes
372 were isolated from peripheral blood by Ficoll gradient separation. They were quantified and
373 plated directly onto plates as per intended assay to avoid the need for detachment and re-
374 plating. MDMs were generated by treatment with 100 ng/mL M-CSF for seven days in
375 RPMI/10% FCS with 50 µg/mL gentamicin. Medium was exchanged every 2-3 days. After seven
376 days of differentiation, MDMs were cultured in RPMI/10% FCS + gentamicin, and CFTR
377 modulators were added for 48 hours prior to the experiment. CFTR modulators were replaced
378 with all media exchanges. Gentamicin protection assay was performed as above. Three to four
379 technical replicates per subject were averaged and log-transformed. Data on paired *lasR*
380 mutants were combined with previously published data on *lasR*-WT strains (37).

381 **Seahorse extracellular flux assay**

382 Monocytes were plated initially onto a Seahorse 96-well plate at 50,000 per well and
383 differentiated into MDM directly in the wells for seven days as above. At that point, differentiation
384 medium was removed and CFTR modulators were added for 48 hours. Pa-conditioned medium
385 was prepared in advance by culturing bacteria overnight in Seahorse mitostress assay medium,
386 normalizing cultures to an OD600 of 0.500, centrifuging to pellet bacteria then passing

387 supernatant through a 0.22 µm filter. This was defined as 100% conditioned medium. Aliquots
388 were then frozen at -20° C until use.

389 On the day of assay, cell culture medium was exchanged for mitostress assay medium as per
390 manufacturer's instructions. A mitostress assay with acute injection was run on an Agilent
391 Seahorse XFe96 platform, with 20 µL conditioned medium in injection port A (final concentration
392 10%), plain mitostress medium was included for control wells. Oligomycin, FCCP, and
393 Rotenone/Antimycin A were placed into ports B-D respectively. Four to eight technical replicates
394 per condition were run. Parameters including response to injection, maximal respiration, and
395 others were calculated as per manufacturer's instructions. Data were combined with previously
396 published results with a *lasR*-WT strain only (37).

397 **THP-1 Seahorse extracellular flux assay**

398 THP-1 macrophages were differentiated directly on a Seahorse 96-well plate with 50 nM PMA at
399 50,000 cells/well for 48 h. Seahorse extracellular flux assay was then run with varying
400 concentrations of 3OC12HSL or C4HSL in injection port A. Five to six technical replicates were
401 run per condition for each assay and averaged. Five independent experiments were run.

402 **Cytokine analysis**

403 MDMs were differentiated for seven days as above then treated for 48 hours with CFTR
404 modulators or DMSO. Bacteria were prepared in an identical manner to the phagocytosis
405 assays. After exchange for antibiotic-free media, MDMs were infected with indicated strains at
406 an MOI of 10 for 2 hours, then washed and placed in media with gentamicin for an additional 2
407 hours. Supernatants were then collected, centrifuged at 14,000 x g at 4°C for 10 minutes,
408 transferred to a new tube, and frozen at -20°C until needed. Samples were batched and
409 cytokine multiplex analysis was run by the Dartmouth Immunology and Flow Cytometry Core.

410 One replicate per condition was run. Samples with levels below the limit of the detection of the
411 assay were set to a concentration of 0, and data were log-transformed prior to analysis.

412 **Figure generation and statistical analysis**

413 Figures were generated with GraphPad Prism 10. Statistical analyses were performed
414 separately in R, with mixed model linear analysis using nlme package version 3.1. Models
415 include all variables of interest as fixed variables and assay date (for THP-1 experiments) or
416 subject (for MDM experiments) as random variables.

417 **Acknowledgments**

418 Funding for DSA provided by the CF Foundation (ARIDGI19B0, ARIDGI20L0, STANTO19R0).
419 THH is supported by DartCF (NIH grant P30-DK117469) and Cystic Fibrosis Foundation
420 Research Development Program (BOMBER24G0). AA is supported by the NIH (R56HL158923,
421 R01HL174700) and CFF (ASHARE21G0). Dartmouth Immunology and Flow Cytometry Core is
422 supported by NIH 5P30CA023108. The authors declare no competing interests and the funders
423 had no direct role in the design or conduct of these studies.

424

425 1. Elborn JS. 2016. Cystic fibrosis. Lancet 388:2519–2531.

426 2. Patient Registry | Cystic Fibrosis Foundation. https://www.cff.org/medical-
427 professionals/patient-registry. Retrieved 1 June 2022.

428 3. Barry PJ, Mall MA, Álvarez A, Colombo C, de Winter-de Groot KM, Fajac I, McBennett
429 KA, McKone EF, Ramsey BW, Sutharsan S, Taylor-Cousar JL, Tullis E, Ahluwalia N, Jun
430 LS, Moskowitz SM, Prieto-Centurion V, Tian S, Waltz D, Xuan F, Zhang Y, Rowe SM,
431 Polineni D, VX18-445-104 Study Group. 2021. Triple Therapy for Cystic Fibrosis
432 Phe508del-Gating and -Residual Function Genotypes. N Engl J Med 385:815–825.

433 4. Bower JK, Volkova N, Ahluwalia N, Sahota G, Xuan F, Chin A, Weinstock TG, Ostrenga
434 J, Elbert A. 2023. Real-world safety and effectiveness of elexacaftor/tezacaftor/ivacaftor in
435 people with cystic fibrosis: Interim results of a long-term registry-based study. J Cyst
436 Fibros S1569-1993(23)00066–8.

437 5. Bass R, Stalvey M, Solomon G, Rowe S, Nichols D, Schwarzenberg SJ, Freedman S,
438 Walega R, Kelly A. 2025. Cholesterol and triglyceride concentrations following 12-
439 18 months of clinically prescribed elexacaftor-tezacaftor-ivacaftor-PROMISE sub-study. J
440 Clin Transl Endocrinol 40:100391.

441 6. Burgel P-R, Durieu I, Chiron R, Ramel S, Danner-Boucher I, Prevotat A, Grenet D,
442 Marguet C, Reynaud-Gaubert M, Macey J, Mely L, Fanton A, Quetant S, Lemonnier L,
443 Paillasseur J-L, Da Silva J, Martin C, Andrejak C, Becourt A, Mounard J, Poulet C, Rames
444 C, Talleux M, Chevalier M-C, Darviot E, Jouvenot M, Marien C, Paris A, Pelatan C,
445 Person C, Priou P, Troussier F, Urban T, Dalphin M-L, Dalphin J-C, Ladaurade A, Pernet

D, Richaud-Thiriez B, Roux-Claude P, Blanc N, Boisserie-Lacroix V, Bui S, Collet C, Debelleix S, Macey J, Bergot E, Brouard J, Campbell K, Laurans M, Ribault V, Borderon C, Heraud M-C, Labbe G, Montcouquiol S, Petit I, Ruivard M, Delestrain C, Douvry B, Epaud R, Maitre B, Remus N, Beltramo G, Fanton A, Houzel A, Huet F, Perez S, Boldron-Ghaddar A, Scalbert M, Bouzioukh R, Mely L, Simon C, Camara B, Hamidfar R, Llerena C, Pin I, Quetant S, Deschildre A, Gicquello A, Le Rouzic O, Leroy C, Paris N, Perez T, Prevotat A, Thumerelle C, Turck D, Wizla N, Dupuy-Grasset M, Languepin J, Masson-Rouchaud A, Menetrey C, Durieu I, Durupt S, L'Excellent S, Nove-Josserand R, Ohlmann C, Reix P, Reynaud Q, Werck-Gallois M-C, Baravalle M, Coltey B, Desmazes-Dufeu N, Dubus J-C, Gautier C, Rey J-B, Reynaud-Gaubert M, Stremler N, Caimmi D, Chiron R, Devrait M, Moreau J, Billon Y, Blondé A, Guillaumot A, Kiefer S, Peretti L, Tatopoulos A, Tiotiu A, Benhamida M, Bihouee T, Danner-Boucher I, Eschapasse E, Tissot A, Giannantonio M, Leroy S, Piccini-Bailly C, Pradelli J, Messika J, Boussaud V, Burgel P-R, Carlier N, Honoré I, Hubert D, Kanaan R, Martin C, Bailly-Botuha C, Chedevergne F, De Blic J, Delacourt C, Drummond D, Fauroux B, Karila C, Le Bourgeois M, Sermet I, Delaisi B, Gerardin M, Houdouin V, Leclainche L, Aubertin G, Berdah L, Clement A, Corvol H, Nathan N, Prevost B, Richard N, Tamalet A, Taytard J, Thouvenin G, Tourniaire B, Abely M, Bessaci-Kabouya K, Dury S, Ravoninjatovo B, Dabadie A, Dagorne M, Deneuville E, Jamin M, Ribault M, Vigier C, Belleguic C, Brinchault G, Desrues B, Barzic A, Dirou-Prigent A, Le Bihan J, Ramel S, Revert K, Ropars T, Couderc L, Dominique S, Marguet C, Morisse-Pradier H, Pramil S, Thiberville L, Allou N, Enaud L, Gachelin E, Huchot E, Payet A, Perisson C, Piyaraly S, Valois S, Herzog A, Kessler R, Porzio M, Weiss L, Beaumont L, Brugiere O, Colin S, Verdiere de, Cuquemelle E, De Miranda S, Grenet D, Monem Hamid

469 A, Parquin F, Picard C, Roux A, Roy C, Bremont F, Didier A, Dupuis M, Faviez G,
470 Labouret G, Mittaine M, Murris-Espin M, Roditis L, Cosson L, Diot P, Flament T, Giraut
471 C, Mankikian J, Arnouat B, Mousset G, Storni V, Vigneron P, Coirier-Duet E, Gabsi A.
472 2021. Rapid Improvement after Starting Elexacaftor–Tezacaftor–Ivacaftor in Patients with
473 Cystic Fibrosis and Advanced Pulmonary Disease. Am J Respir Crit Care Med 204:64–73.

474 7. Daines CL, Polineni D, Tullis E, Costa S, Linnemann RW, Mall MA, McKone EF, Quon
475 BS, Ringshausen FC, Selvadurai H, Taylor-Cousar JL, Withers NJ, Sawicki GS, Lee T,
476 Ahluwalia N, Morlando Geiger J, Jennings M, Tan YV, Waltz D, Ramsey B, Griese M,
477 VX17-445-105 Study Group. 2025. Long-Term Safety and Efficacy of
478 Elexacaftor/Tezacaftor/Ivacaftor in Adults and Adolescents with Cystic Fibrosis and at
479 Least One F508del Allele: A Phase 3, Open-Label Extension Study. Am J Respir Crit Care
480 Med https://doi.org/10.1164/rccm.202411-2231OC.

481 8. Daines CL, Tullis E, Costa S, Linnemann RW, Mall MA, McKone EF, Polineni D, Quon
482 BS, Ringshausen FC, Rowe SM, Selvadurai H, Taylor-Cousar JL, Withers NJ, Ahluwalia
483 N, Moskowitz SM, Prieto-Centurion V, Tan YV, Tian S, Weinstock T, Xuan F, Zhang Y,
484 Ramsey B, Griese M, VX17-445-105 Study Group. 2023. Long-term safety and efficacy of
485 elexacaftor/tezacaftor/ivacaftor in people with cystic fibrosis and at least one F508del
486 allele: 144-week interim results from a 192-week open-label extension study. Eur Respir J
487 62:2202029.

488 9. Heijerman HGM, McKone EF, Downey DG, Van Braeckel E, Rowe SM, Tullis E, Mall
489 MA, Welter JJ, Ramsey BW, McKee CM, Marigowda G, Moskowitz SM, Waltz D, Sosnay
490 PR, Simard C, Ahluwalia N, Xuan F, Zhang Y, Taylor-Cousar JL, McCoy KS, VX17-445-

491 103 Trial Group. 2019. Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor
492 combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a
493 double-blind, randomised, phase 3 trial. Lancet 394:1940–1948.

494 10. McNally P, Lester K, Stone G, Elnazir B, Williamson M, Cox D, Linnane B, Kirwan L,
495 Rea D, O'Regan P, Semple T, Saunders C, Tiddens HAWM, McKone E, Davies JC,
496 RECOVER Study Group. 2023. Improvement in Lung Clearance Index and Chest
497 Computed Tomography Scores with Elexacaftor/Tezacaftor/Ivacaftor Treatment in People
498 with Cystic Fibrosis Aged 12 Years and Older - The RECOVER Trial. Am J Respir Crit
499 Care Med 208:917–929.

500 11. Middleton PG, Mall MA, Dřevínek P, Lands LC, McKone EF, Polineni D, Ramsey BW,
501 Taylor-Cousar JL, Tullis E, Vermeulen F, Marigowda G, McKee CM, Moskowitz SM, Nair
502 N, Savage J, Simard C, Tian S, Waltz D, Xuan F, Rowe SM, Jain R, VX17-445-102 Study
503 Group. 2019. Elexacaftor-Tezacaftor-Ivacaftor for Cystic Fibrosis with a Single Phe508del
504 Allele. N Engl J Med 381:1809–1819.

505 12. Nichols DP, Paynter AC, Heltshe SL, Donaldson SH, Frederick CA, Freedman SD,
506 Gelfond D, Hoffman LR, Kelly A, Narkewicz MR, Pittman JE, Ratjen F, Rosenfeld M,
507 Sagel SD, Schwarzenberg SJ, Singh PK, Solomon GM, Stalvey MS, Clancy JP, Kirby S,
508 Van Dalfsen JM, Kloster MH, Rowe SM, PROMISE Study Group. 2021. Clinical
509 Effectiveness of Elexacaftor/Tezacftor/Ivacaftor in People with Cystic Fibrosis. Am J
510 Respir Crit Care Med https://doi.org/10.1164/rccm.202108-1986OC.

511 13. Heltshe SL, Mayer-Hamblett N, Burns JL, Khan U, Baines A, Ramsey BW, Rowe SM,
512 GOAL (the G551D Observation-AL) Investigators of the Cystic Fibrosis Foundation

513 Therapeutics Development Network. 2015. Pseudomonas aeruginosa in cystic fibrosis
514 patients with G551D-CFTR treated with ivacaftor. Clin Infect Dis 60:703–712.

515 14. Nichols DP, Morgan SJ, Skalland M, Vo AT, Van Dalfsen JM, Singh SB, Ni W, Hoffman
516 LR, McGeer K, Heltshe SL, Clancy JP, Rowe SM, Jorth P, Singh PK, PROMISE-Micro
517 Study Group. 2023. Pharmacologic improvement of CFTR function rapidly decreases
518 sputum pathogen density, but lung infections generally persist. J Clin Invest 133:e167957.

519 15. Hoffman LR, Kulasekara HD, Emerson J, Houston LS, Burns JL, Ramsey BW, Miller SI.
520 2009. PSEUDOMONAS AERUGINOSA lasR MUTANTS ARE ASSOCIATED WITH
521 CYSTIC FIBROSIS LUNG DISEASE PROGRESSION. J Cyst Fibros 8:66–70.

522 16. Delhaes L, Monchy S, Fréalle E, Hubans C, Salleron J, Leroy S, Prevotat A, Wallet F,
523 Wallaert B, Dei-Cas E, Sime-Ngando T, Chabé M, Viscogliosi E. 2012. The Airway
524 Microbiota in Cystic Fibrosis: A Complex Fungal and Bacterial Community—Implications
525 for Therapeutic Management. PLOS ONE 7:e36313.

526 17. Zhao K, Yang X, Zeng Q, Zhang Y, Li H, Yan C, Li JS, Liu H, Du L, Wu Y, Huang G,
527 Huang T, Zhang Y, Zhou H, Wang X, Chu Y, Zhou X. 2023. Evolution of lasR mutants in
528 polymorphic Pseudomonas aeruginosa populations facilitates chronic infection of the lung.
529 Nat Commun 14:5976.

530 18. Feltner JB, Wolter DJ, Pope CE, Groleau M-C, Smalley NE, Greenberg EP, Mayer-
531 Hamblett N, Burns J, Déziel E, Hoffman LR, Dandekar AA. 2016. LasR Variant Cystic
532 Fibrosis Isolates Reveal an Adaptable Quorum-Sensing Hierarchy in Pseudomonas
533 aeruginosa. MBio 7.

534 19. Hammond JH, Hebert WP, Naimie A, Ray K, Van Gelder RD, DiGiandomenico A, Lalitha
535 P, Srinivasan M, Acharya NR, Lietman T, Hogan DA, Zegans ME. 2016. Environmentally
536 Endemic Pseudomonas aeruginosa Strains with Mutations in lasR Are Associated with
537 Increased Disease Severity in Corneal Ulcers. mSphere 1.

538 20. Scribner MR, Stephens AC, Huong JL, Richardson AR, Cooper VS. 2022. The nutritional
539 environment is sufficient to select coexisting biofilm and quorum-sensing mutants of
540 Pseudomonas aeruginosa. J Bacteriol JB0044421.

541 21. Groleau M-C, Taillefer H, Vincent AT, Constant P, Déziel E. 2021. Pseudomonas
542 aeruginosa isolates defective in function of the LasR quorum sensing regulator are frequent
543 in diverse environmental niches. Environ Microbiol https://doi.org/10.1111/1462-
544 2920.15745.

545 22. Mould DL, Stevanovic M, Ashare A, Schultz D, Hogan DA. 2022. Metabolic basis for the
546 evolution of a common pathogenic Pseudomonas aeruginosa variant. Elife 11:e76555.

547 23. Mould DL, Finger CE, Conaway A, Botelho N, Stuut SE, Hogan DA. 2024. Citrate cross-
548 feeding by Pseudomonas aeruginosa supports lasR mutant fitness. mBio 15:e0127823.

549 24. Clay ME, Hammond JH, Zhong F, Chen X, Kowalski CH, Lee AJ, Porter MS, Hampton
550 TH, Greene CS, Pletneva EV, Hogan DA. 2020. Pseudomonas aeruginosa lasR mutant
551 fitness in microoxia is supported by an Anr-regulated oxygen-binding hemerythrin. PNAS
552 https://doi.org/10.1073/pnas.1917576117.

553 25. Feng X, Kostylev M, Dandekar AA, Greenberg EP. 2019. Dynamics of cheater invasion in
554 a cooperating population of Pseudomonas aeruginosa. Sci Rep 9:10190.

555 26. Chen R, Déziel E, Groleau M-C, Schaefer AL, Greenberg EP. 2019. Social cheating in a
556 Pseudomonas aeruginosa quorum-sensing variant. PNAS 201819801.

557 27. D'Argenio DA, Wu M, Hoffman LR, Kulasekara HD, Déziel E, Smith EE, Nguyen H,
558 Ernst RK, Larson Freeman TJ, Spencer DH, Brittnacher M, Hayden HS, Selgrade S,
559 Klausen M, Goodlett DR, Burns JL, Ramsey BW, Miller SI. 2007. Growth phenotypes of
560 Pseudomonas aeruginosa lasR mutants adapted to the airways of cystic fibrosis patients.
561 Mol Microbiol 64:512–533.

562 28. Durfey SL, Kapnadak SG, Pena T, Willmering MM, Godwin JD, Teresi ME, Heltshe SL,
563 Vo AT, Villacreses RA, Aliukonyte I, Boyken L, Stroik MR, Morgan SJ, Wang GM, Betts
564 HL, Zhang S, Goss CH, Clancy JP, Aitken ML, Steele C, Feder AF, Esther CR, Tiddens
565 HAWM, Woods JC, Stoltz DA, Singh PK. 2025. Pseudomonas infections persisting after
566 CFTR modulators are widespread throughout the lungs and drive lung inflammation. Cell
567 Host Microbe 33:1428-1445.e4.

568 29. Pseudomonas aeruginosa Modulates the Antiviral Response of Bronchial Epithelial Cells -
569 PubMed. https://pubmed.ncbi.nlm.nih.gov/32117250/. Retrieved 15 June 2022.

570 30. Dulon S, Leduc D, Cottrell GS, D'Alayer J, Hansen KK, Bunnett NW, Hollenberg MD,
571 Pidard D, Chignard M. 2005. Pseudomonas aeruginosa elastase disables proteinase-
572 activated receptor 2 in respiratory epithelial cells. Am J Respir Cell Mol Biol 32:411–419.

573 31. Hennemann LC, LaFayette SL, Malet JK, Bortolotti P, Yang T, McKay GA, Houle D,
574 Radzioch D, Rousseau S, Nguyen D. 2021. LasR-deficient Pseudomonas aeruginosa

575 variants increase airway epithelial mICAM-1 expression and enhance neutrophilic lung
576 inflammation. PLoS Pathog 17:e1009375.

577 32. Skopelja-Gardner S, Theprungsirikul J, Lewis KA, Hammond JH, Carlson KM, Hazlett HF,
578 Nymon A, Nguyen D, Berwin BL, Hogan DA, Rigby WFC. 2019. Regulation of
579 Pseudomonas aeruginosa-Mediated Neutrophil Extracellular Traps. Front Immunol
580 10:1670.

581 33. Bastaert F, Kheir S, Saint-Criq V, Villeret B, Dang PM-C, El-Benna J, Sirard J-C,
582 Voulhoux R, Sallenave J-M. 2018. Pseudomonas aeruginosa LasB Subverts Alveolar
583 Macrophage Activity by Interfering With Bacterial Killing Through Downregulation of
584 Innate Immune Defense, Reactive Oxygen Species Generation, and Complement
585 Activation. Front Immunol 9.

586 34. Bruscia EM, Bonfield TL. 2016. Cystic Fibrosis Lung Immunity: The Role of the
587 Macrophage. J Innate Immun 8:550–563.

588 35. Hazlett HF, Hampton TH, Aridgides DS, Armstrong DA, Dessaint JA, Mellinger DL,
589 Nymon AB, Ashare A. 2020. Altered iron metabolism in cystic fibrosis macrophages: the
590 impact of CFTR modulators and implications for Pseudomonas aeruginosa survival. Sci
591 Rep 10:10935.

592 36. Barnaby R, Koeppen K, Nymon A, Hampton TH, Berwin B, Ashare A, Stanton B. 2017.
593 Lumacaftor (VX-809) restores the ability of CF macrophages to phagocytose and kill
594 Pseudomonas aeruginosa. Am J Physiol Lung Cell Mol Physiol ajplung004612017.

595 37. Aridgides DS, Mellinger DL, Gwilt LL, Hampton TH, Mould DL, Hogan DA, Ashare A.
596 2023. Comparative effects of CFTR modulators on phagocytic, metabolic and inflammatory
597 profiles of CF and nonCF macrophages. Sci Rep 13:11995.

598 38. 2018. The 32nd Annual North American Cystic Fibrosis Conference, Colorado Convention
599 Center, Denver, Colorado October 18-20, 2018. Pediatric Pulmonology 53:S148–S456.

600 39. 2019. The 33rd Annual North American Cystic Fibrosis Conference, October 31-
601 November 2, 2019. Pediatric Pulmonology 54:S155–S480.

602 40. 2020. The 34th Annual North American Cystic Fibrosis Conference, October 7-23, 2020.
603 Pediatr Pulmonol 55 Suppl 2:S38–S361.

604 41. Aridgides D, Mellinger D, Ashare A. 2022. 553 Determinants of LasR mutant
605 Pseudomonas aeruginosa phagocytosis resistance. Journal of Cystic Fibrosis 21:S307–
606 S308.

607 42. Aridgides D, Mellinger D, Dessaint J, Hogan D, Ashare A. 2021. 432: Pseudomonas
608 aeruginosa LasR loss-of-function mutants evade phagocytosis by CF macrophages despite
609 CFTR triple modulator therapy. Journal of Cystic Fibrosis 20:S203.

610 43. Lund ME, To J, O'Brien BA, Donnelly S. 2016. The choice of phorbol 12-myristate 13-
611 acetate differentiation protocol influences the response of THP-1 macrophages to a pro-
612 inflammatory stimulus. J Immunol Methods 430:64–70.

613 44. Cruz RL, Asfahl KL, Van den Bossche S, Coenye T, Crabbé A, Dandekar AA. 2020. RhlR-
614 Regulated Acyl-Homoserine Lactone Quorum Sensing in a Cystic Fibrosis Isolate of
615 Pseudomonas aeruginosa. mBio 11:e00532-20.

616 45. Jacobi CA, Schiffner F, Henkel M, Waibel M, Stork B, Daubrawa M, Eberl L, Gregor M,
617 Wesselborg S. 2009. Effects of bacterial N-acyl homoserine lactones on human Jurkat T
618 lymphocytes-OdDHL induces apoptosis via the mitochondrial pathway. International
619 Journal of Medical Microbiology 299:509–519.

620 46. Josephson H, Ntzouni M, Skoglund C, Linder S, Turkina MV, Vikström E. 2020.
621 Pseudomonas aeruginosa N-3-Oxo-Dodecanoyl-Homoserine Lactone Impacts
622 Mitochondrial Networks Morphology, Energetics, and Proteome in Host Cells. Front
623 Microbiol 11:1069.

624 47. Kravchenko VV, Kaufmann GF, Mathison JC, Scott DA, Katz AZ, Wood MR, Brogan AP,
625 Lehmann M, Mee JM, Iwata K, Pan Q, Fearns C, Knaus UG, Meijler MM, Janda KD,
626 Ulevitch RJ. 2006. N-(3-oxo-acyl)homoserine lactones signal cell activation through a
627 mechanism distinct from the canonical pathogen-associated molecular pattern recognition
628 receptor pathways. J Biol Chem 281:28822–28830.

629 48. Neely AM, Zhao G, Schwarzer C, Stivers NS, Whitt AG, Meng S, Burlison JA, Machen
630 TE, Li C. N-(3-Oxo-acyl)-homoserine lactone induces apoptosis primarily through a
631 mitochondrial pathway in fibroblasts. Cellular Microbiology 20:e12787.

632 49. Schwarzer C, Fu Z, Shuai S, Babbar S, Zhao G, Li C, Machen TE. 2014. Pseudomonas
633 aeruginosa Homoserine Lactone Triggers Apoptosis and Bak/Bax-Independent Release of
634 Mitochondrial Cytochrome C in Fibroblasts. Cell Microbiol 16:1094–1104.

635 50. Singh PK, Yadav VK, Kalia M, Sharma D, Pandey D, Agarwal V. 2019. Pseudomonas
636 aeruginosa quorum-sensing molecule N-(3-oxo-dodecanoyl)-L-homoserine lactone triggers
637 mitochondrial dysfunction and apoptosis in neutrophils through calcium signaling. Med
638 Microbiol Immunol 208:855–868.

639 51. Tao S, Niu L, Cai L, Geng Y, Hua C, Ni Y, Zhao R. 2018. N-(3-oxododecanoyl)-l-
640 homoserine lactone modulates mitochondrial function and suppresses proliferation in
641 intestinal goblet cells. Life Sciences 201:81–88.

642 52. Tateda K, Ishii Y, Horikawa M, Matsumoto T, Miyairi S, Pechere JC, Standiford TJ,
643 Ishiguro M, Yamaguchi K. 2003. The Pseudomonas aeruginosa Autoinducer N-3-
644 Oxododecanoyl Homoserine Lactone Accelerates Apoptosis in Macrophages and
645 Neutrophils. Infection and Immunity 71:5785–5793.

646 53. Telford G, Wheeler D, Williams P, Tomkins PT, Appleby P, Sewell H, Stewart GSAB,
647 Bycroft BW, Pritchard DI. 1998. The Pseudomonas aeruginosaQuorum-Sensing Signal
648 MoleculeN-(3-Oxododecanoyl)-l-Homoserine Lactone Has Immunomodulatory Activity.
649 Infection and Immunity 66:36–42.

650 54. Yadav VK, Singh PK, Sharma D, Singh SK, Agarwal V. 2019. Mechanism underlying N-
651 (3-oxo-dodecanoyl)-L-homoserine lactone mediated intracellular calcium mobilization in
652 human platelets. Blood Cells Mol Dis 79:102340.

653 55. Wagner C, Zimmermann S, Brenner-Weiss G, Hug F, Prior B, Obst U, Hänsch GM. 2007.
654 The quorum-sensing molecule N-3-oxododecanoyl homoserine lactone (3OC12-HSL)
655 enhances the host defence by activating human polymorphonuclear neutrophils (PMN).
656 Anal Bioanal Chem 387:481–487.

657 56. Vikström E, Magnusson K-E, Pivoriūnas A. 2005. The Pseudomonas aeruginosa quorum-
658 sensing molecule N-(3-oxododecanoyl)-L-homoserine lactone stimulates phagocytic
659 activity in human macrophages through the p38 MAPK pathway. Microbes Infect 7:1512–
660 1518.

661 57. Cavinato L, Luly FR, Pastore V, Chiappetta D, Sangiorgi G, Ferrara E, Baiocchi P,
662 Mandarello G, Cimino G, Del Porto P, Ascenzioni F. 2022. Elexacaftor-Tezacaftor-
663 Ivacaftor corrects monocyte microbicidal deficiency in cystic fibrosis. Eur Respir J
664 2200725.

665 58. Assani K, Shrestha CL, Rinehardt H, Zhang S, Robledo-Avila F, Wellmerling J, Partida-
666 Sanchez S, Cormet-Boyaka E, Reynolds SD, Schlesinger LS, Kopp BT. 2018. AR-13
667 reduces antibiotic-resistant bacterial burden in cystic fibrosis phagocytes and improves
668 cystic fibrosis transmembrane conductance regulator function. J Cyst Fibros
669 https://doi.org/10.1016/j.jcf.2018.10.010.

670 59. Kopp BT, Nicholson L, Paul G, Tobias J, Ramanathan C, Hayes D. 2015. Geographic
671 variations in cystic fibrosis: An analysis of the U.S. CF Foundation Registry. Pediatr
672 Pulmonol 50:754–762.

673 60. Sheikh S, Britt RD, Ryan-Wenger NA, Khan AQ, Lewis BW, Gushue C, Ozuna H,
674 Jaganathan D, McCoy K, Kopp BT. 2023. Impact of elexacaftor-tezacaftor-ivacaftor on
675 bacterial colonization and inflammatory responses in cystic fibrosis. Pediatr Pulmonol
676 58:825–833.

677 61. Shrestha CL, Zhang S, Wisniewski B, Häfner S, Elie J, Meijer L, Kopp BT. 2020. (R)-
678 Roscovitine and CFTR modulators enhance killing of multi-drug resistant Burkholderia
679 cenocepacia by cystic fibrosis macrophages. Sci Rep 10:21700.

680 62. Bitto NJ, Baker PJ, Dowling JK, Wray-McCann G, De Paoli A, Tran LS, Leung PL, Stacey
681 KJ, Mansell A, Masters SL, Ferrero RL. 2018. Membrane vesicles from Pseudomonas
682 aeruginosa activate the noncanonical inflammasome through caspase-5 in human
683 monocytes. Immunology & Cell Biology 96:1120–1130.

684 63. Gabillard-Lefort C, Casey M, Glasgow AMA, Boland F, Kerr O, Marron E, Lyons A-M,
685 Gunaratnam C, McElvaney NG, Reeves EP. 2022. Trikafta Rescues CFTR and Lowers
686 Monocyte P2X7R-Induced Inflammasome Activation in Cystic Fibrosis. Am J Respir Crit
687 Care Med https://doi.org/10.1164/rccm.202106-1426OC.

688 64. Van den Bossche J, O'Neill LA, Menon D. 2017. Macrophage Immunometabolism: Where
689 Are We (Going)? Trends in Immunology 38:395–406.

690 65. Hamilton K, Krause K, Badr A, Daily K, Estfanous S, Eltobgy M, Khweek AA, Anne
691 MNK, Carafice C, Baetzhold D, Tonniges JR, Zhang X, Gavrilin MA, Parinandi NL, Amer
692 AO. 2020. Defective immunometabolism pathways in cystic fibrosis macrophages. Journal
693 of Cystic Fibrosis 0.

694 66. Zhang S, Shrestha CL, Robledo-Avila F, Jaganathan D, Wisniewski BL, Brown N, Pham
695 H, Carey K, Amer AO, Hall-Stoodley L, McCoy KS, Bai S, Partida-Sanchez S, Kopp BT.
696 2022. Cystic fibrosis macrophage function and clinical outcomes after
697 elexacaftor/tezacaftor/ivacaftor. Eur Respir J 2102861.

698 67. Hennemann LC, Nguyen D. 2021. LasR-regulated proteases in acute vs. chronic lung
699 infection: a double-edged sword. Microb Cell 8:161–163.

700 68. Maurice NM, Bedi B, Yuan Z, Goldberg JB, Koval M, Hart CM, Sadikot RT. 2019.
701 Pseudomonas aeruginosa Induced Host Epithelial Cell Mitochondrial Dysfunction. Sci Rep
702 9:11929.

703 69. Everett MJ, Davies DT, Leiris S, Sprynski N, Llanos A, Castandet JM, Lozano C, LaRock
704 CN, LaRock DL, Corsica G, Docquier J-D, Pallin TD, Cridland A, Blench T, Zalacain M,
705 Lemonnier M. 2023. Chemical Optimization of Selective Pseudomonas aeruginosa LasB
706 Elastase Inhibitors and Their Impact on LasB-Mediated Activation of IL-1β in Cellular and
707 Animal Infection Models. ACS Infect Dis 9:270–282.

708 70. Sun J, LaRock DL, Skowronski EA, Kimmey JM, Olson J, Jiang Z, O'Donoghue AJ, Nizet
709 V, LaRock CN. 2020. The Pseudomonas aeruginosa protease LasB directly activates IL-1β.
710 EBioMedicine 60:102984.

711 71. Phuong MS, Hernandez RE, Wolter DJ, Hoffman LR, Sad S. 2021. Impairment in
712 inflammasome signaling by the chronic Pseudomonas aeruginosa isolates from cystic
713 fibrosis patients results in an increase in inflammatory response. Cell Death Dis 12:241.

714 72. LaFayette SL, Houle D, Beaudoin T, Wojewodka G, Radzioch D, Hoffman LR, Burns JL,
715 Dandekar AA, Smalley NE, Chandler JR, Zlosnik JE, Speert DP, Bernier J, Matouk E,
716 Brochiero E, Rousseau S, Nguyen D. 2015. Cystic fibrosis-adapted Pseudomonas
717 aeruginosa quorum sensing lasR mutants cause hyperinflammatory responses. Sci Adv 1.

718 73. Pahari S, Arnett E, Simper J, Azad A, Guerrero-Arguero I, Ye C, Zhang H, Cai H, Wang Y,
719 Lai Z, Jarvis N, Lumbreras M, Maselli DJ, Peters J, Torrelles JB, Martinez-Sobrido L,
720 Schlesinger LS. 2023. A new tractable method for generating human alveolar macrophage-
721 like cells in vitro to study lung inflammatory processes and diseases. mBio 14:e0083423.

722 74. Aridgides D, Dessaint J, Atkins G, Carroll J, Ashare A. 2021. Safety of research
723 bronchoscopy with BAL in stable adult patients with cystic fibrosis. PLoS One
724 16:e0245696.

725 75. Jarosz-Griffiths HH, Scambler T, Wong CH, Lara-Reyna S, Holbrook J, Martinon F, Savic
726 S, Whitaker P, Etherington C, Spoletini G, Clifton I, Mehta A, McDermott MF, Peckham
727 D. 2020. Different CFTR modulator combinations downregulate inflammation differently
728 in cystic fibrosis. Elife 9:e54556.

729 76. Casey M, Gabillard-Lefort C, McElvaney OF, McElvaney OJ, Carroll T, Heeney RC,
730 Gunaratnam C, Reeves EP, Murphy MP, McElvaney NG. 2023. Effect of
731 elexacaftor/tezacaftor/ivacaftor on airway and systemic inflammation in cystic fibrosis.
732 Thorax 78:835–839.

733 77. Jarosz-Griffiths HH, Gillgrass L, Caley LR, Spoletini G, Clifton IJ, Etherington C, Savic S,
734 McDermott MF, Peckham D. 2024. Anti-inflammatory effects of

735 elexacaftor/tezacaftor/ivacaftor in adults with cystic fibrosis heterozygous for F508del.
736 PLoS One 19:e0304555.

737 78. De Vuyst RC, Bennard E, Kam CW, McKinzie CJ, Esther CR. 2023.
738 Elexacaftor/tezacaftor/ivacaftor treatment reduces airway inflammation in cystic fibrosis.
739 Pediatr Pulmonol 58:1592–1594.

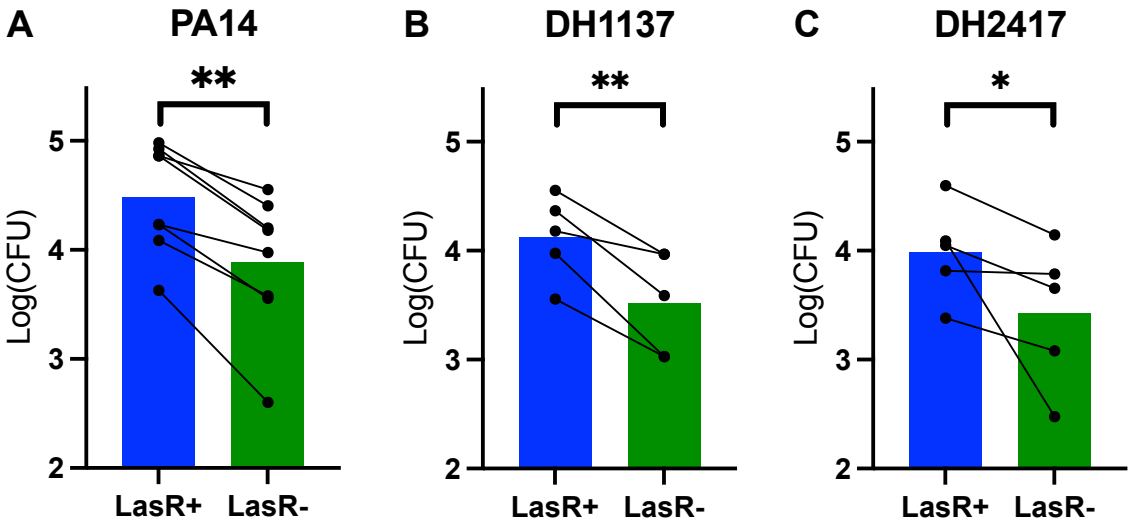
740 79. Rahme LG, Stevens EJ, Wolfort SF, Shao J, Tompkins RG, Ausubel FM. 1995. Common
741 virulence factors for bacterial pathogenicity in plants and animals. Science 268:1899–1902.

742 80. Hogan DA, Vik A, Kolter R. 2004. A Pseudomonas aeruginosa quorum-sensing molecule
743 influences Candida albicans morphology. Mol Microbiol 54:1212–1223.

744 81. Mould DL, Botelho NJ, Hogan DA. 2020. Intraspecies Signaling between Common
745 Variants of Pseudomonas aeruginosa Increases Production of Quorum-Sensing-Controlled
746 Virulence Factors. mBio 11:e01865-20.

747 82. Smith EE, Buckley DG, Wu Z, Saenphimmachak C, Hoffman LR, D'Argenio DA, Miller
748 SI, Ramsey BW, Speert DP, Moskowitz SM, Burns JL, Kaul R, Olson MV. 2006. Genetic
749 adaptation by Pseudomonas aeruginosa to the airways of cystic fibrosis patients. Proc Natl
750 Acad Sci U S A 103:8487–8492.

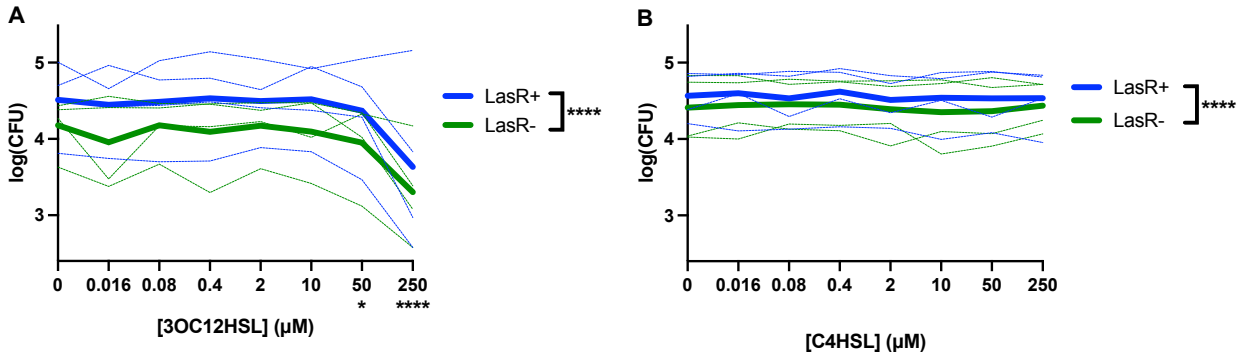
751



752

753 **Figure 1. *lasR* mutation confers phagocytosis resistance independent of parental strain.**
754 **A)** THP-1 cells were differentiated into macrophages by addition of 50 nM PMA for 48 hours.
755 Phagocytosis was then measured by a gentamicin protection assay at 20 minutes of bacterial
756 uptake. After removal of extracellular bacteria by washing and addition of gentamicin,
757 intracellular CFU were quantified by plating onto LB agar. Phagcoytosis of PA14 wild type and
758 PA14 Δ*lasR* (LasR-) was assessed in eight independent assays, log-transformed means of
759 three technical replicates per assay are displayed. ** $p<0.01$ for PA14 WT vs LasR- by mixed
760 model linear regression. **B)** Identical assays to **A** were run using naturally occurring paired
761 LasR+/- clinical isolates of *P. aeruginosa* derived from the same parental strain DH1137. **
762 $p<0.01$ for PA14WT vs LasR- by mixed model linear regression. **C)** Identical assays to **A** were
763 run using naturally occurring paired LasR+/- clinical isolates of *P. aeruginosa* derived from the
764 same parental strain DH2417. ** $p<0.01$ for PA14WT vs LasR- by mixed model linear
765 regression.

766



767

768 **Figure 2. LasR-regulated autoinducer inhibits phagocytosis in a dose-dependent manner.**

769 **A)** THP-1 macrophages were incubated with the indicated concentration of 3OC12HSL for 20

770 minutes prior to performing a gentamicin phagocytosis assay with PA14 WT and PA14Δ*lasR*

771 (LasR-) in parallel. Four separate experiments were performed with three technical replicates

772 each. The means of technical replicates from each individual experiment are shown in the light

773 lines, with darker lines at the means of the four experiments. *$p<0.05$, **** $p<0.0001$ for 50 and

774 250 μM 3OC12HSL relative to DMSO respectively, and LasR+ vs LasR- by mixed model linear

775 regression. **B)** Identical assays to **A** were performed with C4HSL in place of 3OC12HSL. ****

776 $p<0.0001$ by mixed model linear regression.

777

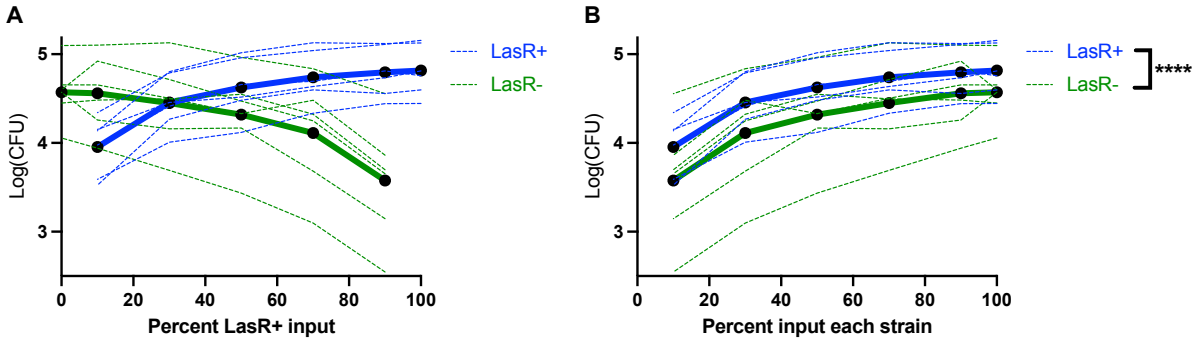


Figure 3. *lasR* mutation confers phagocytosis resistance in mixed cultures. A) A PA14Δ*lasR* strain engineered to express a LacZ reporter was mixed with PA14 wild type at 0%, 10%, 30%, 50%, 70%, 90% and 100% with a fixed total MOI of 10 for the two strains and a phagocytosis assay was performed as previously. After lysis, bacteria were plated onto X-gal plates to allow for differentiation of colony genotype. Means of three technical replicates from 6 independent experiments are graphed, with a dark line at the mean of the 6 experiments. **B)** The same data as in **A** are graphed relative to the percent of each strain input, rather than percent LasR- to better visualize differences. **** $p<0.0001$ by mixed model linear regression.

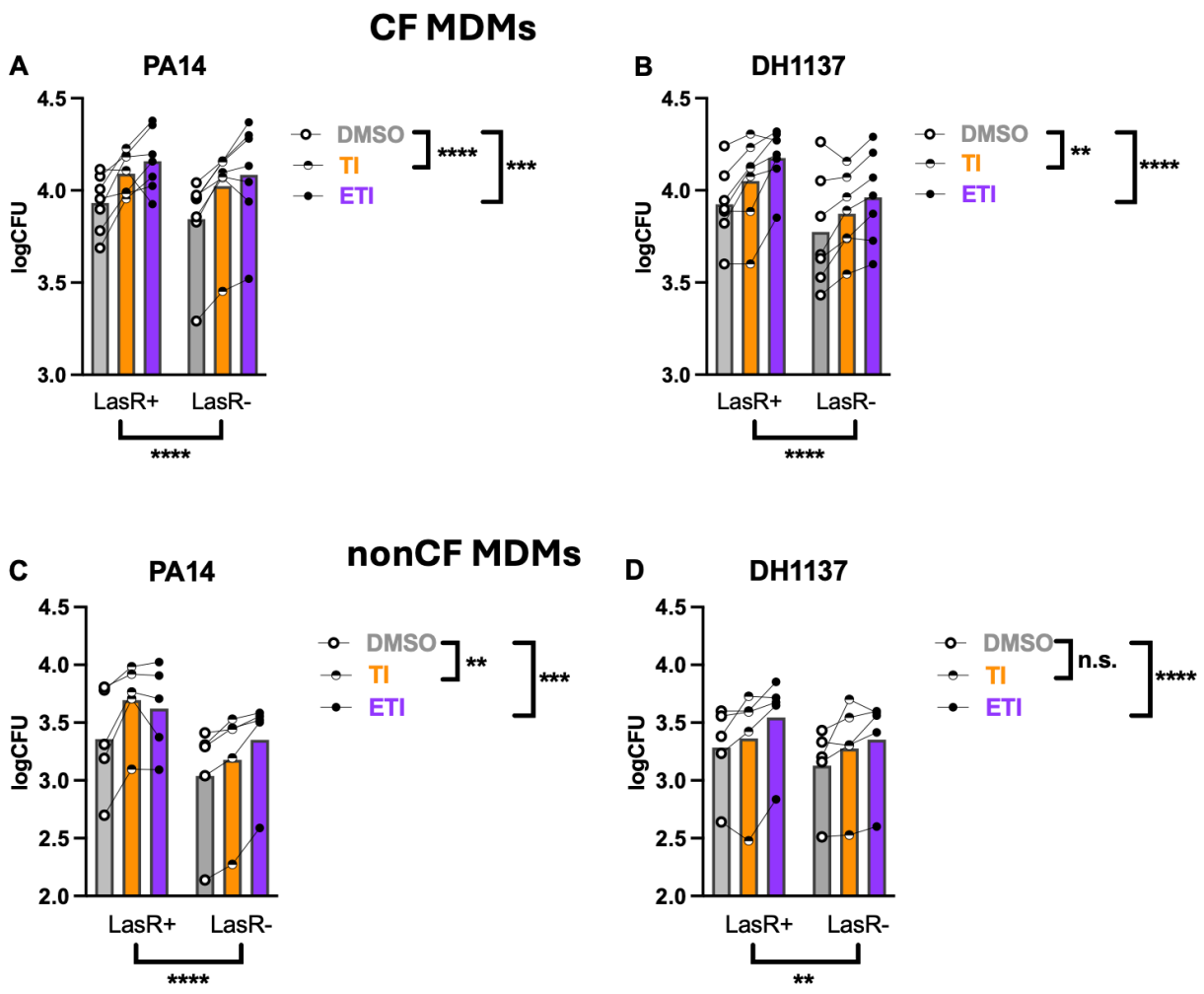
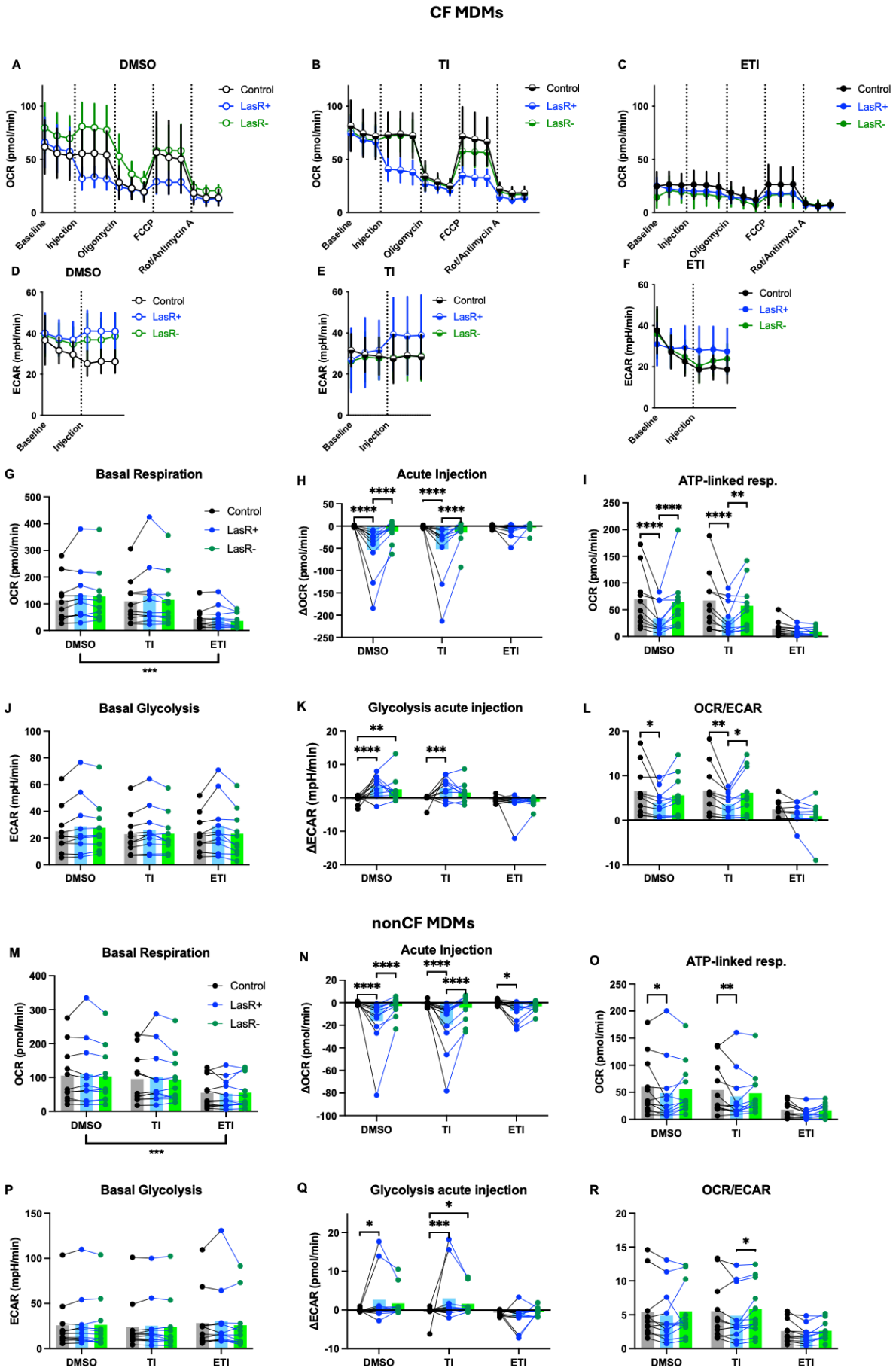


Figure 4. *lasR* mutants exhibit reduced phagocytosis by primary MDMs irrespective of CFTR genotype or modulators. A) CF MDMs were treated for 48 hours with CFTR modulators as indicated, then infected with PA14 WT or PA14 Δ*lasR* (LasR-). Lines connecting dots represent data from a single subject (n=7). **B)** As in **A** except the clinical isolate DH1137 and its derivative *lasR* mutant were used. **C)** NonCF MDMs (n=5) were infected with PA14 WT or LasR- derivative as in **A**. D) NonCF MDMs were infected with DH1137 or its *lasR* mutant derivative as in **B**. ** p<0.01, ***p<0.001, **** p<0.0001 for indicated comparisons by mixed model linear regression. Data were combined with previously published data on LasR+ strains only (37).


CF MDMs
A
DMSO
B
TI
C
ETI
Control
LasR+
LasR-
OCR (pmol/min)
Baseline
Injection
Oligomycin
FCCP
Rot/Antimycin A
D
E
F
ECAR (mpH/min)
G
Basal Respiration
H
Acute Injection
I
ATP-linked resp.
ΔOCR (pmol/min)
J
Basal Glycolysis
K
Glycolysis acute injection
L
OCR/ECAR
ΔECAR (mpH/min)
nonCF MDMs
M
N
O
P
Q
R
ΔECAR (pmol/min)

799 **Figure 5. Effects of CFTR modulators and LasR+/- secreted products on macrophage**
800 **metabolism.** CF and nonCF MDM were differentiated with M-CSF then treated for 48 hours
801 with CFTR modulators. A Seahorse mitostress assay with acute injection of *P. aeruginosa*
802 conditioned medium was then performed. **A-C**) Representative oxygen consumption rate
803 tracings from a CF subject MDMs pretreated with DMSO, TI, or ETI. **D-F**) Representative
804 extracellular acidification rates from the same subject. **G-L)** Calculated parameters as indicated
805 based on the mitostress assay of CF MDMs (n=10). Each point with connecting lines represents
806 the mean of technical replicates from an individual subject. Bars represent means of all the
807 subjects within the group. **M-R**) Calculated parameters as indicated for nonCF MDMs (n=11).
808 *p<0.05, ** p<0.01, ***p<0.001, **** p<0.0001 for indicated comparisons by mixed model linear
809 regression. Data were combined with previously published data on LasR+ strains only (37).

810

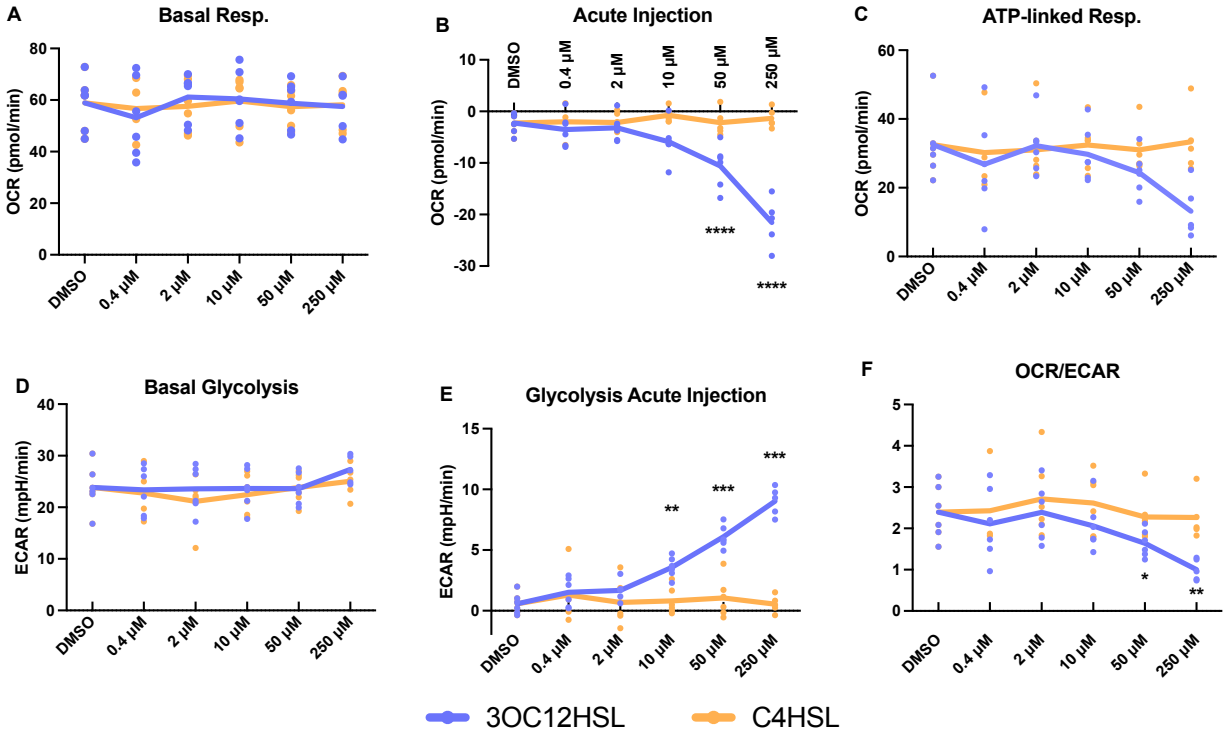
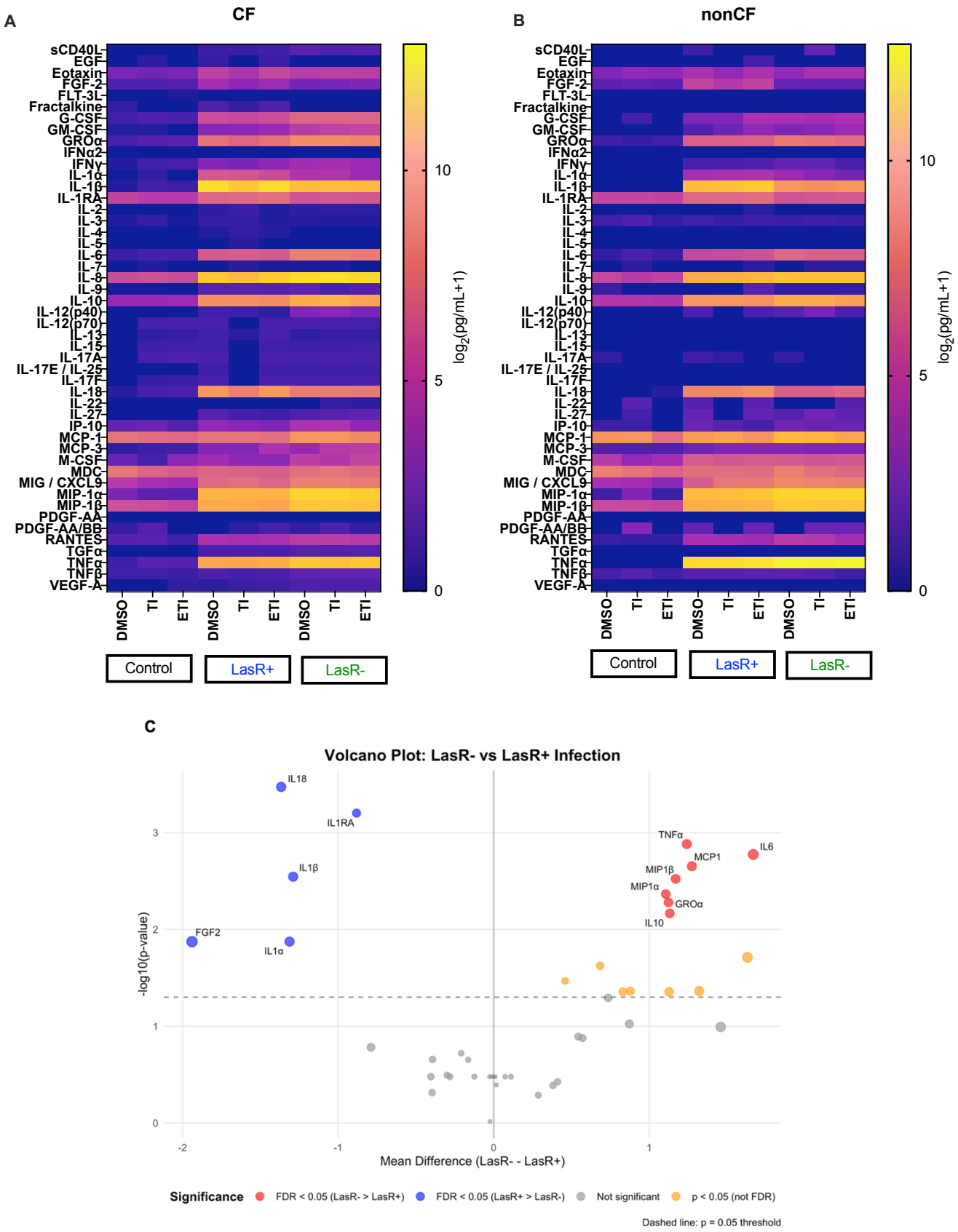


Figure 6. 3OC12HSL inhibits mitochondrial respiration in THP-1 macrophages. A-F) Seahorse mitostress assay was run with acute injection of various concentrations of 3OC12HSL or C4HSL. Parameters were calculated as per standard protocol. Points represent averages of 6-8 technical replicates from 6 individual experiments, with solid lines at the global means. *p<0.05, ** p<0.01, ***p<0.001, **** p<0.0001 for indicated comparisons relative to DMSO treated wells by mixed model linear regression.


A
CF
B
nonCF
sCD40L
EGF
Eotaxin
FGF-2
FLT-3L
Fractalkine
G-CSF
GM-CSF
GROα
IFNα2
IFNγ
IL-1α
IL-1β
IL-1RA
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
IL-9
IL-10
IL-12(p40)
IL-12(p70)
IL-13
IL-15
IL-17A
IL-17E / IL-25
IL-17F
IL-18
IL-22
IL-27
IP-10
MCP-1
MCP-3
M-CSF
MDC
MIG / CXCL9
MIP-1α
MIP-1β
PDGF-AA
PDGF-AA/BB
RANTES
TGFα
TNFα
TNFβ
VEGF-A
DMSO
TI
ETI
Control
LasR+
LasR-
log_2(pg/mL+1)
10
5
0
C
Volcano Plot: LasR- vs LasR+ Infection
IL18
IL1RA
IL1β
FGF2
IL1α
TNFα
IL6
MCP1
MIP1β
MIP1α
GROα
IL10
-log10(p-value)
Mean Difference (LasR- - LasR+)
Significance
FDR < 0.05 (LasR- > LasR+)
FDR < 0.05 (LasR+ > LasR-)
Not significant
p < 0.05 (not FDR)
Dashed line: p = 0.05 threshold

820

821 **Figure 7. *lasR* mutation alters inflammatory cytokine expression profile by CF and nonCF**
822 **MDMs. A)** CF MDMs (n=11 subjects) were pre-treated with CFTR modulators and then infected
823 with DH1137 (LasR+) or DH1136 (LasR-) for two hours, then cells were washed and media
824 replaced. Supernatants were collected 2 hours after washing and analyzed by cytokine
825 multiplex assay. Means of log-transformed data are graphed. **B)** nonCF MDMs (n=4) were
826 infected in the same way and cytokines analyzed by multiplex. **C)** Volcano plot of fold difference
827 between LasR+ and LasR- cytokine results, DMSO treated group only.

828

829

830

831