1 **Host-derived oxidized phospholipids initiate effector-triggered immunity fostering lethality**

2 **upon microbial encounter.**

3

4 Marco Di Gioia[1], Valentina Poli[1], Piao J Tan[2], Roberto Spreafico[3], Anne Chu[1], Alex G Cuenca[4],

5 Philip LSM Gordts[5], Laura Pandolfi[6], Federica Meloni[6], Joseph L Witztum[7], Janet Chou[1], James

6 R Springstead[2], Ivan Zanoni[1]

7

8

9 [1]Harvard Medical School and Boston Children's Hospital, Division of Immunology and Division of

10 Gastroenterology, MA 02115, USA.

11 [2]Department of Chemical and Paper Engineering, Western Michigan University, Kalamazoo, MI,

12 USA.

13 [3]Institute for Quantitative and Computational Biosciences, University of California, Los Angeles,

14 Los Angeles, CA, USA.

15 [4]Department of Surgery, Boston Children's Hospital, Boston, MA 02115, USA; Center for

16 Transplantation Sciences, Department of Surgery, Massachusetts General Hospital, MA 02114,

17 USA.

18 [5]Division of Endocrinology and Metabolism, Department of Medicine, University of California, San

19 Diego, La Jolla, CA, USA; Glycobiology Research and Training Center, University of California,

20 San Diego, La Jolla, California, USA.

21 [6]Respiratory Disease Unit IRCCS San Matteo Hospital Foundation, and Department of Internal

22 Medicine and Pharmacology, University of Pavia, Pavia, 27100, Italy Pavia, 27100, Italy.

23 [7]Division of Endocrinology and Metabolism, Department of Medicine, University of California, San

24 Diego, La Jolla, CA, USA.

25 **Abstract**

26   Macrophages detect invading microorganisms via pattern recognition receptors that

27 recognize pathogen-associated molecular patterns, or via sensing the activity of virulence factors

28 that initiates effector-triggered immunity (ETI). Tissue damage that follows pathogen encounter

29 leads to the release of host-derived factors that participate to inflammation. How these *self*-

30 derived molecules are sensed by macrophages and their impact on immunity remain poorly

31 understood. Here we demonstrate that, in mice and humans, host-derived oxidized phospholipids

32 (oxPLs) are formed upon microbial encounter. oxPL blockade restricts inflammation and prevents

33 the death of the host, without affecting pathogen burden. Mechanistically, oxPLs bind and inhibit

34 AKT, a master regulator of immunity and metabolism. AKT inhibition potentiates the methionine

35 cycle, and epigenetically dampens *Il10*, a pluripotent anti-inflammatory cytokine. Overall, we

36 found that host-derived inflammatory cues act as "*self*" virulence factors that initiate ETI and that

37 their activity can be targeted to protect the host against excessive inflammation upon microbial

38 encounter.

Introduction

The immune system evolved to protect the host against infections and to maintain tissue and organismal homeostasis. This is accomplished through a delicate balance between immune resistance, tissue tolerance and damage, and repair. Phagocytes of the innate immune system initiate the immune response to pathogens by sensing pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors [1]. Phagocytes also detect the presence of host-derived damage-associated molecular patterns (DAMPs) that are released by the host under stress conditions. DAMPs are major drivers of sterile inflammatory diseases and, similarly to PAMPs, are widely believed to be recognized via PRRs [2]. Since the "infectious *non-self*, non-infectious *self*" theory [3] and the "danger model" [4] were proposed, much discussion raised about the distinct contribution of PAMPs and DAMPs to the immune response elicited against pathogens [5]. For example, inflammasome activation plays key anti-microbial roles, and it has been associated with the sensing of both *self* and non-*self* signals [6]. Nevertheless, so far, no consensus has been reached on whether the DAMPs that activate the inflammasome, such as ATP or uric acid crystals, are necessary to control pathogen growth.

Beside utilizing PRRs, phagocytes can also recognize the presence of invading microorganisms by sensing changes in physiological processes caused by virulence factors released by pathogens. The inhibition of critical immune pathways by the activity of virulence factors initiates an effector-triggered immunity (ETI) that does not rely on PRRs. While this mode of activating the immune response is well-described in plants and is known as the "guard model" [7], ETI has been reported also in eukaryotic cells [8-10]. For example, the ICP0 viral effector initiates ETI by degrading MORC3, a host repressor of viral replication. MORC3 degradation in turn unleashes the induction of anti-viral interferons, independently of PRR stimulation [11]. So far, no example of DAMPs inducing an ETI response has been described.

Our work centers on a set of DAMPs derived from non-enzymatic oxidation of phosphocholine-containing phospholipids (hereafter oxPLs). While oxPLs drive inflammatory

65   diseases such as atherosclerosis and nonalcoholic steatohepatitis [12, 13], their role during an

66   infection is controversial. oxPLs have been shown to increase inflammation during lung virus

67   infections via activating the PRR Toll-like receptor 4 (TLR4) [14, 15], while their role during

68   bacterial encounter remains debated [16]. Administering exogenous oxPLs before exposure to

69   bacteria reduces inflammation [17]. In contrast, when oxPLs are administered after exposure to

70   PAMPs, they increase the production of pro-interleukin (IL)-1β and trigger the inflammasome

71   activation [18-20]. Of note, the capacity of the host to produce oxPLs and their relevance in

72   shaping the immune response during microbial encounter remain unknown.

73   Here, we tested whether oxPLs are produced upon microbial encounter and whether they

74   contribute to the lethal inflammatory response that follows: i) a polymicrobial systemic infection;

75   ii) the encounter of methicillin-resistant *S. aureus* (MRSA); and iii) a viral mimic that causes lung

76   tissue damage.

77

78   **Results**

79   *oxPLs are induced and increase mortality and morbidity following microbial detection.*

80   oxPLs are DAMPs that react with a wide variety of innate PRRs [16] and antibodies,

81   including the innate natural IgM antibody E06 [21], which can be used to assess oxPLs levels *in*

82   *vivo* [12, 13]. We initially tested whether oxPLs are increased during the immune response

83   initiated by PRR stimulation. The levels of circulating oxPLs, as well as their accumulation in the

84   spleen, were tested in mice injected systemically with distinct classes of PAMPs. We found that

85   PRR stimulation induced the accumulation of oxPLs in the blood and in the white and red pulp of

86   the spleen (**Figure 1A, B,** and **Figure S1A**). We also found that among the different immune cells

87   that we tested (**Figure S1B**), macrophages showed the most significant accumulation of oxPLs

88   (**Figure 1C, Figure S1C**).

89   To test whether oxPLs affected the outcome of the immune response elicited by microbial

90   encounter, we performed cecal ligation and puncture (CLP) to induce a systemic polymicrobial

91   infection in wild-type (WT) mice, or in mice that express the single-chain fragment variable (scFv)

92   of the E06 antibody (E06-scFv mice), which efficiently and specifically neutralizes

93   phosphocholine-containing oxPLs *in vivo* [12, 13, 22]. We initially confirmed that oxPLs were

94   produced and efficiently neutralized in the spleen of E06-scFv, compared to WT, mice subjected

95   to CLP (**Figure S1D**). WT mice in which the activity of oxPLs was not neutralized showed

96   significant increase in mortality and morbidity (as measured by temperature loss), but a similar

97   microbial burden to E06-scFv mice (**Figure 1D-F**).

98   The CLP model is driven by a polymicrobial infection and takes >2 days to cause death.

99   To further explore the impact of neutralizing oxPLs in an even more inflammatory model, we

100   injected a lethal dose of MRSA sufficient to kill most mice within the first 24h. In keeping with the

101   capacity of oxPLs to increase lethality in the CLP model, mice in which oxPLs were not neutralized

102   showed a significant increased mortality compared to E06-scFv mice when injected with MRSA

103   (**Figure 1G**). When bacterial titers were measured, no significant difference was found in WT,

104   compared to E06-scFv, mice (**Figure 1H**).

105   Previous studies found increased levels of oxPLs during influenza A virus or severe acute

106   respiratory syndrome coronavirus (SARS-CoV) infections [14, 15]. We, thus, tested whether

107   oxPLs were also augmented in the bronchoalveolar lavages (BALs) of patients infected with

108   SARS-CoV-2, compared to individuals that were transplanted or had sarcoidosis. The samples

109   we utilized were previously characterized for the presence of interferons and inflammatory

110   cytokines, and showed a strong inflammatory response in SARS-CoV-2, but not transplanted or

111   sarcoidosis, patients [23]. We found that the BALs of patients with severe-to-critical coronavirus

112   disease 2019 (COVID-19) contained significantly increased amounts of oxPLs compared to the

113   controls (**Figure 1I**). We, next, utilized a mouse model based on repetitive injections of the

114   synthetic viral ligand polyinosinic-polycytidylic acid (poly(I:C)) to mimic a persistent lung virus

115   infection. This viral mimic was previously utilized to study severe COVID-19 [24] or post-acute

116   syndrome COVID-19 (PASC) [25]. We confirmed that, upon repetitive injections of poly(I:C), the

117   levels of oxPLs in the lungs of WT mice were significantly increased compared to mice that were

118   not injected with poly(I:C), or to E06-scFv mice that received poly(I:C) (**Figure 1J**). Morbidity

119   (measured as temperature or weight loss) and mortality were significantly increased in mice in

120   which the activity of oxPLs was not neutralized (**Figure 1K, L**). Increased mortality was associated

121   with augmented inflammation in the lungs of WT, compared to E06-scFv, mice, as determined by

122   histology (**Figure 1M**).

123   Overall, our data demonstrate that endogenous oxPLs cause the death of the host upon

124   encounter with pathogens or following chronic stimulation of PRRs. Also, we demonstrate that

125   oxPLs contribute to the immunopathology elicited upon microbial detection independently of the

126   load of the invading microorganisms.

127

128   *oxPLs worsen a polymicrobial infection by inhibiting IL-10.*

129   Next, we thought to measure global transcriptional changes in macrophages exposed to

130   a microbial ligand and/or to oxPLs to identify inflammatory programs governed by oxPLs that can

131   explain how blocking oxPLs *in vivo* significantly enhances host survival upon microbial encounter.

132   As a source of oxPLs, we utilized the oxidation products of the common phospholipid 1-palmitoyl-

133   2-arachidonyl-sn-glycero-3-phosphocholine (oxPAPC). Bone marrow-derived macrophages

134   (BMDMs) were treated with lipopolysaccharide (LPS) for 3 hours, followed by oxPAPC treatment

135   for 3 or 18 hours, and Illumina HiSeq RNA sequencing (RNAseq) was performed (**Figure 2A**). In

136   keeping with our previous findings [20], *Il1b* was upregulated late upon oxPAPC administration in

137   the presence of LPS. However, we also found a set of genes that were downregulated early, and

138   remained suppressed also at late time points. Among these genes, we focused on the key anti-

139   inflammatory cytokine IL-10 [26] and confirmed that oxPAPC inhibits the transcription of *Il10* in

140   macrophages activated with LPS (**Figure 2B**).

141   To account for the reduced pathology and increased survival in in E06-scFv, compared to

142   WT, mice in our three models of microbial-driven inflammation (**Figure 1D-M**), we hypothesized

143 that the inhibition of the activity of oxPLs in E06-scFv mice increased IL-10 levels. Indeed, we

144 found that levels of IL-10 were significantly increased in E06-scFv, compared to WT, mice in all

145 the three models utilized (**Figure S2A-C**). In contrast, levels of pro-inflammatory cytokines were

146 negatively regulated, or left unaltered, in E06-scFv, compared to WT, mice (**Figure S2A-C**).

147   These data support the hypothesis that oxPLs increase mortality upon microbial encounter

148 by inhibiting IL-10 production. To test whether inhibition of IL-10 by oxPLs is the cause of the

149 death of mice, we inhibited IL-10 signaling in E06-scFv mice. For this experiment, we focused on

150 the CLP polymicrobial infection model that allows to follow the mice over time and to detect not

151 only pro- and anti-inflammatory cytokines, but also to test pathogen control by assessing microbial

152 titers. We found that blockade of IL-10 signaling in E06-scFv mice reverted their phenotype,

153 increasing morbidity, mortality, and restoring levels of pro-inflammatory cytokines similar to WT

154 mice, without affecting bacterial count (**Figure 2C-E**).

155   Overall, these data demonstrate that naturally occurring oxPLs produced in response to

156 microbial encounters exacerbates immunopathology by reducing the levels of IL-10.

157

158 *oxPLs regulate IL-10 levels by inhibiting AKT.*

159   To determine the molecular mechanisms that leads to IL-10 inhibition upon oxPLs

160 encounter, we utilized oxPAPC on BMDMs. Since we previously found that oxPAPC alters the

161 metabolism of macrophages [20], we tested whether the rapid change in the transcription of *Il10*

162 that follows oxPAPC administration is driven by changes in the respiratory and/or glycolytic

163 capacity of macrophages. We found that oxPAPC, but not dipalmitoylphosphocholine (DPPC) (a

164 phospholipid that cannot be oxidized and is immunologically inactive [18-20]), rapidly inhibited

165 glycolysis, in the presence or absence of LPS (**Figure 3A, S3A**), while OXPHOS was only

166 marginally affected (**Figure 3B, S3B**). To test whether glycolysis inhibition drives the reduction of

167 IL-10, we inhibited the activity of hesokinase-2 or of lactate dehydrogenase in LPS-treated cells

168 exposed (or not) to oxPAPC. We found that inhibition of the glycolytic cascade did not cause a

169   change in the levels of IL-10 (or TNF, used as a control cytokine, which levels are not affected by

170   oxPAPC [27]) either in the presence or absence of oxPAPC stimulation (**Figure S3C, D**). In

171   keeping with these results, culturing macrophages in a low glucose medium did not affect the

172   levels of IL-10 in response to LPS and/or oxPAPC stimulation (**Figure S3E**).

173   We next tested whether oxPAPC alters the activation of AKT, which is the first enzyme that

174   controls the early wave of glycolysis downstream of TLR4 [28, 29], but it is also a master regulator

175   of multiple inflammatory processes [30]. oxPAPC, but not DPPC, administration, in the presence

176   or absence of LPS, inhibited AKT phosphorylation in a dose- and time-dependent manner (**Figure**

177   **3C-E**). Targets and downstream kinases of AKT were also inhibited by oxPAPC, confirming that

178   oxPAPC inhibits the activity of AKT (**Figure S3F**). Of note, administration of the AKT inhibitor VIII

179   (AKTi) mirrored the capacity of oxPAPC to inhibit IL-10 (but not TNF) production and glycolysis

180   (**Figure 3F, S3G, H**).

181   We next assessed whether oxPAPC maintained its capacity to inhibit AKT and dampen

182   IL-10 production in phagocytes of different origin. Mouse dendritic cells (DCs) were differentiated

183   *in vitro* using GM-CSF. Alternatively, primary mouse macrophages were isolated from the

184   peritoneum. The capacity of oxPAPC to inhibit AKT, glycolysis, and IL-10, but not TNF, production

185   was assessed. Our data demonstrate that oxPAPC maintains its activity on the different mouse

186   phagocytes (**Figure S3I-N**).

187   Finally, we tested the activity of oxPAPC when cells were activated with different PAMPs.

188   We utilized PAMPs of bacterial, viral, and fungal origin and found that, regardless of the PRR

189   stimulated, oxPAPC maintained its inhibitory activities (**Figure S4A-F**). Of note, in macrophages

190   stimulated with type I interferons, oxPAPC inhibited AKT and the induction of *Il10* (while other

191   interferon-stimulated genes were only marginally affected) (**Figure S4G, H**). AKT inhibition also

192   dampened the transcription of *Il10* in cells stimulated with type I interferons (**Figure S4I**).

193   Overall, these data demonstrate that oxPAPC leads to AKT inhibition, and consequently

194   blocks IL-10 production, in response to distinct PAMPs and in multiple mouse phagocytes.

195

196   *oxPLs exert their activities in human phagocytes and are inversely correlated with IL-10 levels in*

197   *patients with polymicrobial infections.*

198   We next tested whether oxPAPC also acts on human phagocytes. Human monocytes,

199   monocyte-derived-DCs or monocyte-derived macrophages were stimulated with LPS in the

200   presence or absence of oxPAPC. For all human cells, oxPAPC maintained the capacity to dampen

201   AKT activation, glycolysis induction, and the production of IL-10, but not of TNF (**Figure 4A-I**).

202   Based on these data, we investigated whether the presence of oxPLs in subjects with

203   polymicrobial infections was correlated with the levels of IL-10. We focused on pediatric

204   individuals sampled when they entered the intensive care unit (ICU) for evaluation of possible

205   sepsis. Hypotension requiring vasopressor medications and identification of infectious pathogens

206   occurred more frequently in patients meeting diagnostic criteria for sepsis [31] compared to those

207   without sepsis. Patient demographics, respiratory compromise, ventilatory support, and

208   glucocorticoid use were comparable between the two groups. Of note, oxPAPC levels were

209   inversely correlated with circulating IL-10 levels only in the cohort of critically infected patients

210   with a confirmed diagnosis of sepsis (**Figure 4J**).

211   Overall, these data demonstrate that the capacity of oxPLs to block AKT activation and

212   glycolysis, and to inhibit IL-10 production is maintained across multiple human phagocytes. Our

213   data also show that critical patients with polymicrobial infections present reduced levels of IL-10

214   when the blood concentration of oxPLs is increased.

215

216   *oxPLs directly bind and inhibit AKT.*

217   We next determined how oxPAPC inhibits AKT. We initially tested whether oxPAPC

218   administration affects the three major pathways that activate AKT, namely PI3K, TBK1/IKKε, and

219   PDK1 [28, 30] (**Figure S5A**). We found that none of these pathways was altered by oxPAPC

220   administration to macrophages treated, or not, with LPS (**Figure S5B**). Of note, we did not reveal

221 any major change in the activity of the complex 1 or 2 of mTOR (**Figure S5C**). We, thus, tested

222 whether any of the known receptors of oxPAPC [5, 17, 32-39] were involved in the inhibition of

223 AKT, glycolysis, and IL-10 induction. We excluded the involvement of TLR4 and TLR2 (**Figure**

224 **5A, B**), and of CD14 and CD36 (**Figure 5C, D**). Although we confirmed that oxPAPC activates

225 NRF2 in macrophages [38] (**Figure S5D**), we found that oxPAPC maintained the capacity to inhibit

226 AKT, glycolysis, and IL-10 in *Nfe2l2*$^{-/-}$ cells (**Figure 5E**). In keeping with the fact that NRF2 is not

227 involved in regulating AKT and IL-10, administration of the NRF2 activator 15-Deoxy-$\Delta^{12,14}$-

228 prostaglandin J2 (15d-PGJ$_2$) [40] did not affect AKT activation or IL-10 levels in LPS-stimulated

229 macrophages (**Figure S5E, F**).

230 Based on these data, we tested the hypothesis that oxPAPC can directly bind and inhibit

231 AKT. Initially, we utilized a biotinylated analog of oxPAPC (oxPAPE-N-biotin) to pull down a HA-

232 tagged AKT. The experiment was performed in the presence or absence of oxPAPC to compete

233 for the binding to AKT. Our data demonstrated that oxPAPC and AKT interact with each other

234 (**Figure 5F**). Next, to assess whether oxPAPC directly binds AKT, we used MicroScale

235 thermophoresis (MST) [41]. Based on the Immgen database, tissue resident macrophages mostly

236 express *Akt1* and *Akt2*, except for peritoneal macrophages that also express *Akt3* (**Figure S5G**).

237 We confirmed that bone-marrow derived macrophages express all three isoforms of AKT (**Figure**

238 **S5H**). We, thus, tested the capacity of oxPAPC to bind each of the three AKT isoforms and we

239 found that oxPAPC, but not DPPC, directly binds AKT1-3 with a K$_d$ that varies between 22.7μM

240 and 65.3μM, depending on the AKT isoform (**Figure 5G, S5I, J**). To identify the domain of AKT

241 bound by oxPAPC, we expressed the following HA-tagged AKT1 proteins: i) full length; ii) amino

242 acid (AA)1-149 (PH domain); iii) AA120-433 (KD domain); and iv) AA1-408 (PH and KD domains)

243 (**Figure 5H**). These proteins were then tested for their ability to interact with oxPAPE-N-biotin. We

244 found that oxPAPC binds the catalytic domain of AKT (**Figure 5H**).

245   Since oxPAPC binds to the catalytic domain of AKT, we assessed whether oxPAPC directly

246   inhibits AKT activity. The enzymatic activity of AKT1, 2 and 3 was tested in the presence of

247   oxPAPC (or DPPC) in a cell-free assay. oxPAPC, but not DPPC, inhibited the activity of AKT1-3,

248   but not of the related kinase PDK1, in a dose-dependent manner (**Figure 5I, S5K, L**).

249   Overall, these data demonstrate that oxPAPC directly binds and inhibits all three isoforms

250   of AKT.

251

252   *oxPLs-dependent AKT inhibition reprograms the landscape of metabolites in macrophages.*

253   We next investigated how oxPAPC-dependent AKT inhibition affects IL-10 production.

254   Cells were stimulated with LPS, and the main transcriptional pathways activated downstream of

255   the Myddosome were assessed (**Figure S6A**). We found that canonical NF-kB activation was not

256   altered in LPS-stimulated cells exposed to oxPAPC (**Figure S6B, C**). Similarly, the ERK, JNK,

257   and p38 pathways were not altered by oxPAPC (**Figure S6D**). Previous reports implicated CREB

258   in AKT-dependent regulation of IL-10 [42, 43], but we did not detect changes in CREB activation

259   in the presence of oxPAPC in LPS-stimulated cells (**Figure S6D**). Similar to Myddosome

260   dependent signaling, oxPAPC did not alter TRIF- and IRF3-dependent responses (**Figure S6E-**

261   **G**). These results are in agreement with our previous data showing that oxPAPC activity happens

262   across multiple stimuli and independently of distinct PRRs.

263   To identify the pathway that regulates IL-10 production upon oxPAPC-dependent inhibition

264   of AKT, we interrogated the landscape of metabolites in cells activated, or not, with LPS, and

265   exposed, or not, to oxPAPC, or to AKTi. A sparse partial least squares discriminant analysis

266   (sPLS-DA) revealed that the various treatments induced a unique landscape of metabolites

267   (**Figure 6A, B**). Also, a pathway analysis revealed the regulation of several metabolic pathways,

268   some of which were shared by cells exposed to oxPAPC or AKTi (**Figure 6C**). Several of the

269   common metabolic pathways, such as "Purine" or "Serine/glycine" metabolism, converge on the

270   methionine cycle (**Figure 6D**). S-adenosyl-homocysteine (SAH) and/or homocysteine, which are

271 intermediates of the methionine cycle, were among the metabolites most upregulated in cells

272 exposed to LPS and oxPAPC, or AKTi (**Figure 6E**). Notably, the conversion of S-adenosyl-

273 methionine (SAM) into SAH/homocysteine liberates methyl groups that can be used for epigenetic

274 modifications such as H3K27me2me3, a central modulator of gene silencing in mammals [44, 45].

275 Given that AKT suppresses the activity of the methyltransferase EZH2 (which methylates H3K27

276 [46]), we hypothesized that oxPAPC-dependent AKT inhibition enhances EZH2 activity, and thereby

277 suppresses *Il10* transcription.

278   oxPAPC administration, as well as AKTi treatment, reduced the inhibitory phosphorylation

279 of EZH2 on S21 [46] (**Figure 6F**). Furthermore, our RNAseq analysis showed that *Id3*, a gene

280 activated by EZH2 [47], was upregulated in oxPAPC-treated cells.

281   Overall, our data demonstrate that oxPAPC rewires the metabolic landscape of LPS-

282 stimulated macrophages, suggesting that epigenetic changes can be the cause of the inhibition in

283 the induction of *Il10*.

284

285 *oxPLs-dependent AKT inhibition epigenetically reprograms Il10 transcription.*

286   To directly test the capacity of oxPAPC to alter H3K27me2me3 in the *Il10* locus, we

287 performed chromatin immune precipitation (ChIP) followed by qPCR analysis of several conserved

288 non-coding sequences (CNS) (**Figure 7A**). H3K27me2me3 was highly enriched in *Hoxc10* and

289 poorly present in *Gadph*, utilized as positive and negative controls, respectively, independently of

290 cell stimulation. We found that oxPAPC treatment significantly restored the levels of

291 H3K27me2me3 in multiple CNS of cells treated with LPS and exposed to oxPAPC, compared to

292 cells treated with LPS alone (**Figure 7A**). Of note, inhibition of the activity of EZH2 with two different

293 highly selective methyltransferase inhibitors [48, 49], as well as with an EED degrader [50],

294 restored IL-10 production in cells treated with LPS and oxPAPC, only minimally impacting TNF

295 levels (**Figure 7B, S7A-C**). In keeping with the *in vitro* activity of oxPAPC on EZH2, when EZH2

296 was inhibited *in vivo*, mice were protected against the polymicrobial infection that follows CLP

297 (**Figure 7C, D**) and IL-10 levels were increased, while, as a consequence of IL-10 activity, pro-

298 inflammatory cytokines levels were dampened (**Figure 7E**). As shown before, bacterial levels

299 were not significantly affected (**Figure 7F**), demonstrating that targeting oxPL-dependent

300 activation of EZH2 ameliorates disease development, without altering the anti-microbial activity

301 of the immune system.

302 Overall, our data demonstrate that via the inhibition of AKT, oxPAPC rewires the

303 methionine cycle and boosts the activity of EZH2, which in turns increases the trimethylation of

304 H3K27, thus dampening IL-10 production (**Figure 7G**). Also, that targeting EZH2 *in vivo*

305 counterbalances the activity of oxPLs, ameliorating the immunopathology driven by excessive

306 inflammation that follows microorganism encounter.

307

308 **Discussion**

309 oxPLs are host-derived inflammatory cues that are generated in the context of sterile

310 inflammation and inflammatory diseases [16]. In our work, we demonstrate that host-derived

311 oxPLs are formed as a consequence of the encounter with distinct PAMPs, and that oxPLs are

312 key determinants of the outcome of the immune response. In particular, we found that oxPLs

313 reduce the production of IL-10, thus exaggerating the inflammatory response that is secondary to

314 a disseminated polymicrobial infection, to MRSA encounter, and to persistent sensing of a viral

315 mimic in the lung. oxPLs exert this function by inhibiting AKT and rewiring the methionine cycle,

316 which epigenetically switches off the *Il10* locus via the activity of the epigenetic writer EZH2.

317 By using the E06-scFv transgenic mouse model, whose secreted sc-Fv targets the

318 phosphocholine moieties of oxPLs, we show that oxPLs blockade significantly increased the

319 survival of mice in multiple mouse models of microbial sensing. Our data demonstrate that oxPLs

320 do not compromise immune resistance and, instead, are fundamental to determine the extent of

321 the inflammation elicited in response to microbial sensing. In addition, our findings that targeting

322 oxPLs in distinct infectious models was effective in preventing mortality open to the development

323   of a novel approach against microbial infections, switching the focus of therapeutic intervention

324   from the pathogen to the host. This is particularly relevant in light of our findings that septic

325   patients present an inverse correlation between the levels of oxPLs and circulating IL-10. Under

326   these circumstances, the use of drugs that target the metabolic and epigenetic adaptions initiated

327   by oxPLs during sepsis may become a new valuable therapeutic tool.

328   From a physiological point of view, our data reveal that oxPLs serve as sensors of tissue

329   damage and alert the immune system to potentiate its response against invading microorganisms.

330   The primary function of the immune system is to prevent pathogen invasion and spread. It is,

331   thus, necessary that the cells of the immune system recognize the presence of host damage

332   caused by the growth of pathogens that are not properly controlled. In this context, the immune

333   system reacts by increasing the potency and extent of the immune response. The "collateral"

334   damage to the tissues of the host caused by the potentiated immune response would be

335   evolutionary justified by the necessity to control the spread of the pathogen. In our *in vivo*

336   experimental models, we mimicked inflammatory conditions that are extreme, as represented by

337   systemic polymicrobial or Gram-positive-driven infections, or a persistent stimulation of the

338   immune response by a viral mimic, similarly to what has been described in the context of severe

339   COVID-19 or PASC [24, 25]. Under these conditions, the excessive inflammation driven by oxPL-

340   dependent inhibition of IL-10 causes the death of the host. In contrast, under less potent

341   inflammatory conditions, the production of oxPLs may tip the balance of the immune response in

342   a way that will help to alert the immune system to eradicate the invading microorganisms, without

343   causing excessive inflammation and lethality.

344   Our data also showed that none of the known receptors for oxPLs are involved in

345   regulating IL-10 levels. In contrast, the reduction in the production of IL-10 is driven by the block

346   of the "physiological" activity of AKT and by the consequent activation of the epigenetic writer

347   EZH2. Under our experimental conditions, oxPAPC acts as a "*self*" virulence factor that initiates

348   ETI by directly binding and inhibiting AKT. AKT is a key inflammatory and metabolic checkpoint

349 that regulates the immune response [30]. Thus, it is important for the host to "guard" the activity

350 of AKT, and, in case of alteration of its activation, to instruct the immune system to respond

351 adequately. In the case of oxPAPC-dependent inhibition of AKT, the phagocytes of the host

352 respond by decreasing IL-10 production. The reduced activity of IL-10 *in vivo* on bystander cells

353 in turns leads to increased pro-inflammatory cytokine induction. Intriguingly, AKT is the target of

354 multiple virus and bacterial effectors that can either inhibit or boost its activity. For example, AKT

355 activation is blocked by *Yersinia* YopJ, *Pseudomonas* ExoT, *Vibrio cholerae* MakA, and

356 uropathogenic *Escherichia coli* alpha-hemolysin [51-53]. In these contexts, it will be important in

357 future studies to assess whether the host elicits a response similar to the one initiated in response

358 to oxPLs to reduce IL-10. In contrast to virulence factors that block AKT, several bacterial or viral

359 pathogens such as *Salmonella*, *Shigella*, influenza viruses, or herpesviruses exploit the activation

360 of the AKT pathway to prevent cell death and favor proliferation [51, 54, 55]. Under these

361 circumstances, oxPL production may exert anti-microbial roles, and may be evolutionary

362 advantageous. Therefore, it will be important to contextualize the activity of oxPLs *in vivo* based

363 on the pathogen encountered and according to the extent of inflammation elicited.

364 Overall, our data revealed that the activity of oxPLs can elicit an ETI response, expanding

365 our understanding of the functioning of the immune system and of the contribution of external-

366 and self-derived inflammatory cues to controlling the extent of the inflammatory response.

367

374

375   **Author contributions:**

376   MDG performed *in vitro* and *in vivo* experiments, conceived the experiments, and helped

377   to write the manuscript; VP performed CLP experiments and the analyses related to *in vivo*

378   experiments; PJT prepared oxPAPC; RS performed the bioinformatics analyses; AC identified

379   and isolated samples from septic patients; AGC performed CLP experiments; PLSMG performed

380   the MRSA experiments; LP and FM analyzed oxPL levels in the BALs; JLW provided transgenic

381   mice and gave advice; JC helped to perform and analyze the experiments with human samples;

382   JRS provided oxPAPC and gave advices; IZ conceived and supervised the experiments, and

383   wrote the paper.

384

385   **Main Figure Legends**

386   **Figure 1: A**) oxPLs quantification in serum of mice treated with vehicle (saline) or LPS (2 mg Kg^{-1}

387   ) for 8h. *n* = 20 mice per group. Graph shows means ± SD. Statistical significance was calculated

388   using two-tailed *t* test. **B**) Spleens of mice treated with vehicle (saline) or LPS (2 mg Kg^{-1}) for 24h

389   were immunostained with E06 antibody (brown). Representative photomicrographs are shown.

390   Scale bar = 200 μm. **C**) Representative histograms (*left*) and quantification (*right*) of oxPLs

391   accumulation (E06-PE MFI) in the indicated cell populations of the spleens of mice treated with

392   vehicle (saline) or LPS (2 mg Kg^{-1}) for 24h. *n* = 6 mice (vehicle), *n* = 10 mice (LPS). Graphs show

393   means ± SD. Statistical significance was calculated using two-tailed *t* test. **D-F**) WT (*n* = 11) and

394   E06-scFv (*n* = 11) mice were subjected to CLP. Survival was followed over time. Kaplan–Meier

395   curves with log-rank (Mantel-Cox) test are shown (**D**). Body temperature loss was measured 8h

396   after surgery (**E**). Bacteria loads in serum were analyzed 24h after CLP (**F**). Graphs show means

397   ± SD. Statistical significance was calculated using two-tailed *t* test. **G-H**) WT (*n* = 53) and E06-

398   scFv (*n* = 52) mice were injected with MRSA (1.8 x 10^{8} CFU/mouse) and monitored for lethality

399   over 120 hours. Kaplan–Meier curves with log-rank (Mantel-Cox) test are shown (**G**). Bacteria

400   loads in serum (*left*) and spleen (*right*) were analyzed 24h after MRSA injection (**H**). Graphs show

401   means ± SD. Statistical significance was calculated using two-tailed t test. **I**) oxPLs levels were

402   measured in the BAL of COVID-19 (n = 27) and non-microbially infected (subjects affected by

403   sarcoidosis, n = 10 and transplant, n = 10) patients. Graphs show mean ± SD. Statistical

404   significance was calculated using two-tailed t test. **J-M**) WT (n = 9) and E06-scFv (n = 8) mice

405   were intratracheally administered with poly(I:C) (2.5 mg Kg^{-1}) or saline (WT, n = 4) daily for 6 days.

406   oxPLs were quantify from lung homogenates. Graph shows means ± SD. Statistical multiple

407   comparisons were calculated by one-way ANOVA and Tukey's test (**J**). Body temperature (*left*)

408   and weight (*right*) loss were measured at the indicated time points. Graphs show means ± 95%

409   CL. Statistical comparisons were calculated by two-way ANOVA and Sidak's test (**K**). Survival

410   was followed over time. Kaplan–Meier curves with log-rank (Mantel-Cox) test are shown (**L**).

411   Representative H&E images of control (saline) and poly(I:C) WT and E06-scFv treated mice.

412   Scale bar = 1 mm. (*left*). Histological inflammation score (*right*). Graph shows means ± SD.

413   Statistical significance was calculated using two-tailed t test (**M**).

414

415   **Figure 2: A**) Scatter plot of transcript fold-change expression with s-value $<$ 0.01 (RNA-seq) in

416   LPS-primed BMDMs treated, or not, with oxPAPC (100 µg ml^{-1}) for 3h (x-axis) or 18h (y-axis).

417   Points correspond to genes. **B**) BMDMs were primed with LPS (1 µg ml^{-1}) and then treated, or

418   not, with oxPAPC (100 µg ml^{-1}). *Il10* mRNA levels were measured at the indicated time points by

419   qPCR. n = 3, statistical significance was calculated using two-way ANOVA and Sidak's multiple

420   comparisons test. **C-E**) WT (n =16) and E06-scFv mice (n =10, isotype control n = 10, anti-IL10R)

421   undergoing CLP were treated with anti-IL-10R antibody or isotype control, as indicated. Survival

422   was followed over time. Kaplan–Meier curves with log-rank (Mantel-Cox) test are shown (**C**). (**D**)

423   Body temperature loss was measured 8h after surgery (left) and bacteria loads in serum were

424   analyzed 24h after CLP (right). Statistical significance was calculated using two-tailed t test. (**E**)

425   WT and E06-scFv mice undergoing CLP were treated with anti-IL-10R antibody or isotype control,

426   as indicated, and serum levels of IL-10, TNF, IL-1β and IL-6 were analyzed 8h after CLP. n =10

427   mice per group. Graph show means ± SD. Statistical significance was calculated using two-tailed

428   *t* test.

429

430   **Figure 3: A**) Real-time changes in ECAR (*left*), basal ECAR and maximal glycolytic capacity

431   (MGC) quantifications (*right*) of BMDMs untreated or treated with LPS (1 µg ml^{-1}) and then

432   challenged with different doses of oxPAPC, as indicated. **B**) Real-time changes in OCR (*left*),

433   basal OCR and maximal respiratory capacity (MRC) quantifications (*right*) of BMDMs treated as

434   in **A**. *n* = 6, graphs are representative of three independent experiments and show means ± SEM.

435   Statistical significance was calculated using two-way ANOVA and Dunnett's multiple comparisons

436   test. **C**) BMDMs were primed, or not, with LPS and then stimulated with oxPAPC (100, 50 or

437   25 µg ml^{-1}). AKT phosphorylation was analyzed after 1h by immunoblotting. **D**) BMDMs were

438   primed, or not, with LPS and then stimulated with oxPAPC (100 µg ml^{-1}). AKT phosphorylation

439   was analyzed at the indicate time points by immunoblotting. **E**) BMDMs were primed, or not, with

440   LPS and then treated with DPPC (100, 50 or 25 µM). AKT phosphorylation was analyzed after 1h

441   by immunoblotting. Images are representative of three independent experiments. **F**) BMDMs were

442   primed with LPS and then stimulated with oxPAPC (100, 50 or 25 µg ml^{-1}) (*n* = 4) (*left*) or AKTi

443   (10, 5, 25 µM) (*n* = 9) (*right*). IL-10 and TNF production was quantified by ELISA 18h later. Graphs

444   are representative of four independent experiments and show means ± SEM. Statistical multiple

445   comparisons were calculated by two-way ANOVA and Dunnett's test.

446

447   **Figure 4. A**) Human monocytes were primed, or not, with LPS and treated or not with oxPAPC

448   (50, 12 or 12.5 µg ml^{-1}) for 1h. AKT phosphorylation was assessed by immunoblot. Data are

449   representative of three independent experiments. **B**) ECAR of human monocytes, treated as

450   indicated, was measured using a Seahorse analyzer. oxPAPC was used at 50 µg ml^{-1}. Data are

451   representative of three independent experiments. **C**) Human monocytes were treated as in **A** and

452   TNF and IL-10 release was quantified by ELISA after 18h. *n* = 8, from 4 different donors. Graphs

453   show means ± SEM. Statistical multiple comparisons were calculated by two-way ANOVA and

454   Dunnett's test. **D**) Human mo-DCs were primed, or not, with LPS and treated or not with oxPAPC

455   (50, 12 or 12.5 μg ml^{-1}) for 1h. AKT phosphorylation was assessed by immunoblot. Data are

456   representative of three independent experiments. **E**) ECAR of human mo-DCs, treated as

457   indicated, was measured using a Seahorse analyzer. oxPAPC was used at 50 μg ml^{-1}. Data are

458   representative of three independent experiments. **F**) Human mo-DCs were treated as in **D** and

459   TNF and IL-10 release was quantified by ELISA after 18h. n = 10, from 5 different donors. Graphs

460   show means ± SEM. Statistical multiple comparisons were calculated by two-way ANOVA and

461   Dunnett's test. **G**) Human macrophages were primed, or not, with LPS and treated or not with

462   oxPAPC (50, 12 or 12.5 μg ml^{-1}) for 1h. AKT phosphorylation was assessed by immunoblot. Data

463   are representative of three independent experiments. **H**) ECAR of human macrophages was

464   measured using a Seahorse analyzer. oxPAPC was used at 50 μg ml^{-1}. Data are representative

465   of three independent experiments. **I**) Human macrophages were treated as in **G** and TNF and IL-

466   10 release was quantified by ELISA after 18h. n = 6, from 3 different donors. Graphs show means

467   ± SEM. Statistical multiple comparisons were calculated by two-way ANOVA and Dunnett's test.

468   **J**) Scatter plot of oxPLs and IL-10 levels in plasma of patients with organism-defined sepsis (*left*,

469   n = 13) and critically ill patients without sepsis (*right*, n = 14), obtained within 48 hours of admission

470   to the intensive care unit. Plots show results of Spearman's rank correlation tests and a linear

471   regression line with 95% confidence intervals.

472

473   **Figure 5: A-E**) WT and *Tlr4*$^{-/-}$ (**A**), *Tlr2*$^{-/-}$ (**B**), *Cd14*$^{-/-}$ (**C**), *Cd36*$^{-/-}$ (**D**) *or Nfe2l2*$^{-/-}$ (**E**) BMDMs were

474   primed, or not, with R848 (1 μg ml^{-1}) (**A, C**) or LPS (1 μg ml^{-1}) (**B-E**) and treated or not with oxPAPC

475   (100 μg ml^{-1}) for 1h (*left, center*) or 24h (*right*). AKT phosphorylation (**A-E**) or NRF2 accumulation

476   (**E**) were assessed by immunoblot (*left*). Data are representative of three independent

477   experiments. ECAR (*center*) was measured using a Seahorse analyzer. n = 6 (**A-C, E**), n = 5 (**D**),

478   data are representative of three independent experiments and show means ± SEM. Statistical

479   multiple comparisons were calculated by two-way ANOVA and Tukey's test. IL-10 and TNF

480   release (*right*) was quantified by ELISA. *n* = 3, graphs are representative of three independent

481   experiments and show means ± SEM. Statistical multiple comparisons were calculated by two-

482   way ANOVA and Tukey's test. **F**) oxPAPC binding capacity of AKT was determined by pull down

483   assay. Cellular lysate of 293T cells expressing HA-tagged human AKT1 was incubated with

484   oxPAPE-N-Biotin and the indicated doses of oxPAPC. AKT associated with biotinylated lipids was

485   captured by streptavidin beads and revealed by immunoblotting using anti-HA antibody. β-actin

486   was used as a negative control. Data are representative of three independent experiments. **G**)

487   Microscale thermophoresis (MST) analysis of oxPAPC and AKT interactions. Traces of

488   fluorescently labeled human recombinant AKT1 incubated with oxPAPC (1000, 500, 250,125,

489   62.5, 31.25, 15.62, 7.8, 3.9, 1.9, 0.97, 0.488, 0.24, 0.12, 0.0061 and 0.030 μM) or DPPC (500,

490   250,125, 62.5, 31.25, 15.62, 7.8, 3.9, 1.9, 0.97, 0.488, 0.24, 0.12, 0.0061 and 0.030 μM) (*left and*

491   *central*). oxPAPC-AKT binding curve was derived from the quantification of normalized

492   fluorescence changes (*right*). *n* = 3, graph shows means ± SD. Images are representative of three

493   independent experiments. **H**) Cellular lysate of 293T cells expressing the indicated HA-tagged

494   AKT1 truncated forms were incubated with oxPAPE-N-Biotin. HA-proteins associated with

495   biotinylated lipid were captured by streptavidin beads and revealed by immunoblotting. Data

496   shown are representative of three independent experiments. **I**) Active human recombinant AKT1

497   or PDK1 were incubated with oxPAPC or DPPC (1000, 500, 250,125, 62.5, 31.25, 15.62, 7.8, 3.9,

498   and 1.9 μM) and kinase-specific FRET-peptide substrates (Z'-LYTE). Kinase inhibition was

499   measured as the ability of lipid to block substrate phosphorylation. *n* = 4, graph shows means ±

500   SD. Data are representative of three independent experiments.

501

502   **Figure 6. A-C**) BMDMs were primed, or not, with LPS (1 μg ml^{-1}) treated or not with oxPAPC (100

503   μg ml^{-1}) or AKTi (10 μM). Mass-spectrometry polar metabolomics analysis was performed 3h later.

504   sPLS-DA plot showing the effect on variance during oxPAPC (**A**) or AKTi (**B**) treatments. Each

505   point represents an independent biological sample. KEGG pathway enrichment analysis of the

506   indicated treatments (**C**). **D**) Schematic showing the interconnection between methionine cycle

507   and histone methylation processes. **E**) BMDMs were primed with LPS and then stimulated with

508   oxPAPC (100 µg ml^{-1}) (n = 3) (left) or AKTi (10 µM) (n = 3) (right). Volcano plots show the change

509   of polar metabolites after 3h. **F**) BMDMs were treated as in **E** and EZH2 phosphorylation was

510   measured by flow cytometry after 1h. *Left*: cytofluorimetry histograms. *Right*: bar graphs. Bars

511   represent the MFI of p-EZH2. n = 3, graph shows means ± SEM. Statistical multiple comparisons

512   were calculated by two-way ANOVA and Tukey's test. Data are representative of three

513   independent experiments.

514

515   **Figure 7: A**) BMDMs were primed, or not, with LPS and then stimulated with oxPAPC (100 µg ml^{-}

516   ^{1}). Chromatin immunoprecipitation and quantitative PCR (ChIP–qPCR) of H3K27me2me3 at CNS

517   regions related to the *Il10* locus (indicated in the scheme), was performed 3h later. *Hoxc10* and

518   *Gapdh* were used as positive and negative controls, respectively. n = 3 independent

519   immunoprecipitations, graphs show means ± SEM. Statistical significance was calculated using

520   two-way ANOVA. **B**) BMDMs were incubated with GSK343 (10 µM) for 30 minutes, primed with

521   LPS and then stimulated with oxPAPC (100 µg ml^{-1}). IL-10 and TNF production was quantified by

522   ELISA after 18h. n = 6, graphs are representative of four independent experiments and show

523   means ± SEM. Statistical multiple comparisons were calculated by two-way ANOVA and Tukey's

524   test. **C-F**) WT mice (n = 10) were treated with GSK343 and subjected to CLP. Survival was

525   followed over time. Kaplan–Meier curves with log-rank (Mantel-Cox) test are shown (**C**). Body

526   temperature loss was measured 8h later after surgery (**D**). Serum levels of IL-10, TNF, IL-1β and

527   IL-6 were analyzed 8h after CLP (**E**). Bacteria loads in serum were analyzed 24h after CLP (**F**).

528   Statistical significance was calculated using two-tailed t test. **G**) Schematic showing how oxPLs

529   control IL-10 production by guarding the activity of AKT.

Supplementary Figure Legends

Supplementary Figure 1. A) Mice were i.p. injected with vehicle (saline), CpG (15 mg Kg^{-1}), R848 (10 mg Kg^{-1}) or WGP (15 mg Kg^{-1}). oxPLs were quantified in serum after 8h (*left*) or in spleen after 24h (*right*). n = 10 mice per group. Statistical significance was calculated using two-tailed t test. Spleens were immunostained with E06 antibody. Representative photomicrographs are shown. Scale bar = 200 μm. **B, C**) Mice were treated with vehicle (saline) or LPS (2 mg Kg^{-1}) for 24h and splenic cell populations were analyzed for oxPLs accumulation using flow cytometry (**B**, gate strategy) or (**C**) fluorescent immunohistochemistry. Representative photomicrographs are shown. Scale bar = 200 μm. **D**) Mice were subjected to CLP. oxPLs were quantified in serum after 8h (*left*) or in spleen after 24h (*right*). n = 8 (sham control) n = 10 (CLP) mice per group. Statistical significance was calculated using two-tailed t test. Spleens were immunostained with E06 antibody. Representative photomicrographs are shown. Scale bar = 200 μm.

Supplementary Figure 2. A) WT and E06-scFv mice (n = 9) were subjected to CLP. The indicated cytokines were quantified after 8h from serum. Graph shows means ± SD. Statistical significance was calculated using two-tailed t test. **B**) WT and E06-scFv mice (n = 10) were injected with MRSA (1 x 10^8 CFU/mouse) and the indicated cytokines were measured from serum 24h post-infection. Graph shows means ± SD. Statistical significance was calculated using Mann-Whitney Test. **C**) WT (n = 9) and E06-scFv (n = 8) mice were intratracheally daily administered with poly(I:C) (2.5 mg Kg^{-1}). At day 6, the indicated cytokines were measured from lung homogenates. Graph shows means ± SD. Statistical significance was calculated using two-tailed t test.

Supplementary Figure 3. A, B) Real-time changes in ECAR (**A**) and OCR (**B**) of BMDMs untreated or treated with LPS (1 µg ml^{-1}) and then challenged with different doses of DPPC, as indicated. n = 6, graphs are representative of three independent experiments and show means ±

555   SEM. **C-E**) BMDMs were incubated with TAT-peptide (10 µM), TAT-HK2-peptide (10 µM) (**C**),

556   oxamate (30 mM) (**D**) or seed in high glucose (25 mM) and low glucose (1 mM) medium (**E**) for

557   30 minutes. The cells were primed with LPS and then stimulated with oxPAPC (100 µg ml^{-1}). IL-

558   10 and TNF production was quantified by ELISA after 18h. n = 3, graphs are representative of

559   three independent experiments and show means ± SEM. Statistical multiple comparisons were

560   calculated by two-way ANOVA and Sidak's multiple comparisons test. **F**) BMDMs were primed, or

561   not, with LPS and then stimulated with oxPAPC (100, 50 or 25 µg ml^{-1}). The indicated AKT targets

562   and downstream kinases were analyzed after 1h by immunoblotting. Data are representative of

563   three independent experiments. **G**) BMDMs were primed, or not, with LPS and then treated with

564   AKTi (10 µM). AKT phosphorylation was analyzed after 1h by immunoblotting. Data are

565   representative of three independent experiments. **H**) Real-time changes in ECAR (*left*) and OCR

566   (*right*) of BMDMs untreated or treated with LPS (1 µg ml^{-1}) and then challenged with AKTi (10 µM),

567   as indicated. n = 6, graphs are representative of three independent experiments and show means

568   ± SEM. **I**) Murine GM-CSF-derived DCs (BMDCs) were primed, or not, with LPS and treated or

569   not with oxPAPC (100, 50 or 25 µg ml^{-1}) for 1h. AKT phosphorylation was assessed by

570   immunoblot. Data are representative of three independent experiments **J**) ECAR of BMDCs,

571   treated as indicated, was measured using a Seahorse analyzer. oxPAPC was used at 100 µg ml^{-}

572   ^{1}. Data are representative of three independent experiments. **K**) BMDCs were treated as in **I** and

573   TNF and IL-10 release was quantified by ELISA after 18h. n = 3, graphs are representative of

574   three independent experiments and show means ± SEM. Statistical multiple comparisons were

575   calculated by two-way ANOVA and Dunnett's test. **L**) Murine peritoneal macrophages were

576   primed, or not, with LPS and treated or not with oxPAPC (50, 12 or 12.5 µg ml^{-1}) for 1h. AKT

577   phosphorylation was assessed by immunoblot. Data are representative of three independent

578   experiments. **M**) ECAR murine peritoneal macrophages, treated as indicated, was measured

579   using a Seahorse analyzer. oxPAPC was used at 50 µg ml^{-1}. Data are representative of three

580   independent experiments. **N**) Murine peritoneal macrophages were treated as in **L** and TNF and

581   IL-10 release was quantified by ELISA after 18h. *n* = 6, graphs are representative of three

582   independent experiments and show means ± SEM. Statistical multiple comparisons were

583   calculated by two-way ANOVA and Dunnett's test.

584

585   **Supplementary Figure 4. A**) BMDMs were primed, or not, with LPS (1 µg ml⁻¹), P3C (1 µg ml⁻¹),

586   CpG (5 µM) or R848 (1 µg ml⁻¹) and then stimulated with oxPAPC (100, 50 or 25 µg ml⁻¹). AKT

587   phosphorylation was analyzed after 1h by immunoblotting. Data are representative of three

588   independent experiments. **B**) ECAR of BMDMs, treated as indicated, was measured using a

589   Seahorse analyzer. oxPAPC was used at 100 µg ml⁻¹. *n* = 6, data are representative of three

590   independent experiments and show means ± SEM. Statistical significance was calculated using

591   two-way ANOVA and Sidak's multiple comparisons test. **C**) BMDMs were treated as in (**A**) and IL-

592   10 and TNF release was quantified by ELISA after 18h. *n* = 4, graphs are representative of three

593   independent experiments and show means ± SEM. Statistical multiple comparisons were

594   calculated by two-way ANOVA and Dunnett's test. **D**) BMDMs were primed, or not, with poly(I:C)

595   (10 µg ml⁻¹) or transfected with 3p-hpRNA/LyoVec complexes (1 µg ml⁻¹) and then stimulated with

596   oxPAPC (50 µg ml⁻¹). AKT phosphorylation was analyzed after 1h by immunoblotting. Data are

597   representative of three independent experiments. **E**) BMDMs were primed with poly(I:C) (*n* = 7)

598   or 3p-hpRNA/LyoVec (*n* = 3) as in (**D**) and then stimulated with oxPAPC (100, 50 or 25 µg ml⁻¹).

599   IL-10 and TNF release was quantified by ELISA after 18h. Graphs are representative of three

600   independent experiments and show means ± SEM. Statistical multiple comparisons were

601   calculated by two-way ANOVA and Dunnett's test. **F**) BMDMs were pre-treated with GM-CSF.

602   After 16h, cells were primed, or not, with WGP (1 µg ml⁻¹) and then stimulated with oxPAPC (100,

603   50 or 25 µg ml⁻¹). AKT phosphorylation was analyzed after 1h by immunoblotting. Data are

604   representative of three independent experiments. **G**) BMDMs were treated with oxPAPC (100, 50

605   or 25 µg ml⁻¹) for 30 minutes and then stimulated, or not, with IFNβ (10 ng ml⁻¹) for 1h. AKT and

606   STAT1 phosphorylation were analyzed after 1h by immunoblotting. Data are representative of

607   three independent experiments. **H-I**) BMDMs were treated with vehicle or oxPAPC (100 µg ml^{-1})

608   (**H**) or AKTi (10 µM) (**I**) for 30 minutes and then stimulated with IFNβ (10 ng ml^{-1}). The indicated

609   transcripts were analyzed by qPCR at the indicated time points. *n* = 3, data are representative of

610   three independent experiments and show means ± SEM. Statistical significance was calculated

611   using two-way ANOVA and Sidak's multiple comparisons test.

612

613   **Supplementary Figure 5. A**) Schematic shows upstream and downstream kinases in the AKT

614   signaling network. **B**) BMDMs were primed, or not, with LPS and then stimulated with oxPAPC

615   (100, 50 or 25 µg ml^{-1}). Phosphorylation of upstream AKT regulators (TBK1, IKKε, PDK1, PTEN

616   and PI3K) was analyzed by immunoblot. Images are representative of three independent

617   experiments. **C**) BMDMs were primed, or not, with LPS (1 µg ml^{-1}) and then stimulated with

618   oxPAPC (100, 50 or 25 µg ml^{-1}). The phosphorylation of the indicated mTORC1/2-associated

619   proteins was analyzed after 1h by immunoblotting. Data are representative of three independent

620   experiments. **D**) WT and *Nfe2l2*$^{-/-}$ BMDMs were primed, or not, with LPS (1 µg ml^{-1}) and treated

621   or not with oxPAPC (100 µg ml^{-1}) for 1h. The indicated transcripts were analyzed by qPCR. *n* = 3,

622   data are representative of three independent experiments and show means ± SEM. Statistical

623   significance was calculated using two-way ANOVA and Sidak's multiple comparisons test. **E-F**)

624   BMDMs were primed, or not, with LPS (1 µg ml^{-1}) and treated or not with 15d-PGJ$_2$ (20, 10 or

625   1 µM in **E** and 10 µM in **F**) for 1h (**E**) or 24h (**F**). AKT phosphorylation and NRF2 accumulation

626   were assessed by immunoblot (**E**). Data are representative of three independent experiments. IL-

627   10 and TNF release was quantified by ELISA (**F**). *n* = 6, graphs are representative of three

628   independent experiments and show means ± SEM. Statistical multiple comparisons were

629   calculated by two-way ANOVA and Sidak's test. **G**) Expression of *Akt* isoforms (RNA-seq,

630   Immgen.org database) in the indicated murine macrophage populations. **H**) Normalized

631   expression of *Akt1*, *Akt2* and *Akt3* in BMDMs, analyzed by qPCR. *n* = 3, data are representative

632   of three independent experiments and show means ± SEM. **I, J**) Microscale thermophoresis

633   (MST) analysis of oxPAPC interactions with AKT2 (**I**) and AKT3 (**J**). Traces of fluorescently labeled

634   human recombinant AKT incubated with oxPAPC (1000, 500, 250,125, 62.5, 31.25, 15.62, 7.8,

635   3.9, 1.9, 0.97, 0.488, 0.24, 0.12, 0.0061 and 0.030 µM) or DPPC (500, 250,125, 62.5, 31.25,

636   15.62, 7.8, 3.9, 1.9, 0.97, 0.488, 0.24, 0.12, 0.0061 and 0.030 µM). oxPAPC-AKT binding curve

637   was derived from the quantification of normalized fluorescence changes. $n = 3$, graph shows

638   means ± SD. Images are representative of three independent experiments. **K, L**) Active human

639   recombinant AKT2 (**K**) or AKT3 (**L**) were incubated with oxPAPC and a kinase-specific FRET-

640   peptide substrate (Z-'LYTE). Kinase inhibition was measured as the ability of lipid to block

641   substrate phosphorylation. $n = 4$, graph shows means ± SD. Data are representative of three

642   independent experiments.

643

644   **Supplementary Figure 6. A**) Schematic shows MyD88-dependent TLR4 downstream signaling.

645   **B-D**) BMDMs were primed, or not, with LPS (1 µg ml^{-1}) and treated or not with oxPAPC (100 µg ml^{-}

646   ^{1} in **B**, **D** and 100, 50 or 25 µg ml^{-1} in **B, D** and 100 µg ml^{-1} in **C**) for 1h. NF-κB (**B**) and MAPK

647   pathway activation (**D**) were assessed by immunoblot. Images are representative of three

648   independent experiments. NF-κB nuclear translocation was analyzed by immunofluorescence

649   (**C**). Scale bar 50 µm. Data are representative of three independent experiments. **E**) Schematic

650   shows TRIF-dependent TLR4 downstream signaling. **F, G**) BMDMs were primed, or not, with LPS

651   (1 µg ml^{-1}) and treated or not with oxPAPC (100, 50 or 25 µg ml^{-1}). IFN-β production (**F**) was

652   quantified by ELISA after 24h. $n = 4$, data are representative of three independent experiments

653   and show means ± SEM. Statistical multiple comparisons were calculated by two-way ANOVA

654   and Tukey's test. Type I IFN pathway activation was assessed by immunoblot (**G**). Data are

655   representative of three independent experiments.

656

657   **Supplementary Figure 7. A**) BMDMs were incubated with UNC1999 (5 µM) for 30 minutes,

658   primed with LPS and then stimulated with oxPAPC (100 µg ml^{-1}). IL-10 and TNF production was

659   quantified by ELISA after 18h. n = 4, graphs are representative of three independent experiments

660   and show means ± SEM. Statistical multiple comparisons were calculated by two-way ANOVA

661   and Tukey's test. **B**) BMDMs were treated with UNC6852 (50, 25, 12.5, 6.25, 3 and 1.5 µM) for

662   24h and the indicated components of PRC2 complex were quantified by immunoblot. Image is

663   representative of three independent experiments. **C**) BMDMs were incubated with UNC6852 (50

664   µM) for 24h, primed with LPS and then stimulated with oxPAPC (100 µg ml^{-1}). IL-10 and TNF

665   production was quantified by ELISA after 18h. n = 4, graphs are representative of three

666   independent experiments and show means ± SEM. Statistical multiple comparisons were

667   calculated by two-way ANOVA and Tukey's test.

668 **Methods**

669 **Mouse strains and murine cell cultures**

670 C57BL/6J (Jax 000664), $Tlr4^{-/-}$ (Jax 029015), $Tlr2^{-/-}$ (Jax 004650), $Cd14^{-/-}$ (Jax 003726), $Cd36^{-/-}$

671 (Jax 019006), $Nfe2l2^{-/-}$ (Jax 017009) mice were acquired from The Jackson Laboratory. E06-scFv

672 animals on C57BL/6J background were provided by J.L. Witztum (University of California San

673 Diego). Macrophages were differentiated from bone marrow using Dulbecco's modified Eagle

674 medium (DMEM, Thermo Fisher Scientific, Cat# 11965092), with 30% L929-M-CSF (BMDMs) or

675 10% B16-GM-CSF supernatant (GM-derived phagocytes), 10% fetal bovine serum (FBS), 2mM

676 L-glutamine (Thermo Fisher Scientific, Cat# 25030081) and 100U/100μg ml^{-1}

677 penicillin/streptomycin (Thermo Fisher Scientific, Cat# 15140122). Primary peritoneal

678 macrophages were obtained by peritoneal lavage of mice treated intraperitoneally with 4%

679 thioglycolate (Sigma-Aldrich, Cat# T9032) for 4 days. Prior to stimulations, cultured cells were

680 rinsed with phosphate-buffered saline (PBS) and re-plated in DMEM supplemented with 10%

681 FBS, 2mM L-glutamine and 100U/100μg ml^{-1} penicillin/streptomycin at a concentration of 1×10^6

682 cells ml^{-1}. In all experiments requiring LPS priming, LPS was used at the concentration of 1 μg

683 ml^{-1} for 3 hours, unless otherwise stated.

684

685 **Human phagocytes cell cultures**

686 Human phagocytes were isolated or differentiated from collars of blood received from Boston

687 Children's Hospital healthy donors. Briefly, blood was diluted 1:2 in PBS and peripheral blood

688 mononuclear cells (PBMCs) were isolated using Histopaque (Sigma, Cat# 1077-1) gradient.

689 Monocytes were positively selected from PBMCs with CD14 MicroBeads (Miltenyi Biotec, Cat#

690 130-050-201). Mo-DCs and macrophages were differentiated from monocytes in Roswell Park

691 Memorial Institute (RPMI) 1640 medium (Thermo Fisher Scientific , 61870127) supplemented

692 with 10% FBS in presence of GM-CSF (20 ng ml^{-1}, BioLegend Cat# 572903) and IL-4 (20 ng ml^{-}

693   [1], BioLegend Cat# 574004) (Mo-DCs) or M-CSF (50 ng ml^{-1}, BioLegend Cat# 574804) and IL-4

694   (20 ng ml^{-1}) (macrophages) for 7 days. Prior to stimulations, cultured cells were rinsed with PBS

695   and re-plated in RPMI 1640 supplemented with 10% FBS, 2mM L-glutamine and 100U/100μg ml^{-}

696   ^{1} penicillin/streptomycin at a concentration of 1×10^6 cells ml^{-1}. In all experiments with human cells

697   requiring LPS priming, LPS was used at the concentration of 0.1 μg ml^{-1} for 3 hours, unless

698   otherwise stated.

699

700   **Sepsis patient cohort and clinical characteristics**

701   The Third International Consensus Definitions for Sepsis and Septic Shock were used to identify

702   patients with sepsis [31]. Patients with sepsis had an identified infection with > 2 point increase in

703   the Pediatric Sequential Organ Failure Assessment score at the time of study enrollment

704   compared to their pre-admission score. All patients were admitted to the critical care unit, with

705   blood obtained within 48 hours of admission. This study was approved by the institutional review

706   board of Boston Children's Hospital.

707

708   **Clinical samples for oxPLs measurement in BAL**

709   BALs were obtained from COVID-19 and control patients previously described in[56]. Briefly,

710   BALs from SARS-CoV-2 positive patients (27) hospitalized in the Intensive Care Unit (ICU) were

711   collected from September to November 2020 at Luigi Sacco Hospital (Milan, Italy). Control

712   samples, sarcoidosis patients (10) and lung transplant patients (10), were collected by IRCCS

713   Policlinico San Matteo Foundation (Pavia, Italy). None of the control patients were diagnosed a

714   respiratory infection.

715

716   **Stimuli and reagents**

717   oxPAPC and oxPAPE-N-Biotin were custom made starting from PAPC (Avanti Polar Lipids, Cat#

718   850459) and PAPE (Avanti Polar Lipids, Cat# 850759). DPPC (Cat# 850355) was purchased from

719   Avanti Polar Lipids. 15d-PGJ$_2$ (Cat# 18570) and 2-deoxy-D-Glucose (2-DG, Cat# 14325) were

720   purchased from Cayman Chemicals. *Escherichia coli* LPS (Serotype O555:B5, TLRgrade, Cat#

721   ALX-581-013-L002) was purchased from Enzo Life Sciences. Pam3CSK4 (Cat# tlrl-pms), CpG

722   ODN 1826 (Cat# tlrl-1826), R848 (Cat# tlrl-r848), poly(I:C) (Cat# tlrl-pic-5), 5' triphosphate hairpin

723   RNA (3p-hpRNA, Cat# tlrl-hprna), LyoVec (Cat# lyec-1) and dispersible whole glucan particles

724   (WGP, Cat# tlrl-wgp) were purchased from InvivoGen. Recombinant murine IFN-β1 (Cat#

725   581304) was purchased from BioLegend. AKT inhibitor VIII (Cat# A6730), sodium oxamate (Cat#

726   O2751), FCCP (Cat# C2920), oligomycin A (OM, Cat# 75351), rotenone (Cat# R8875) and

727   Antimycin A (Cat# A8674) were purchased from Sigma-Aldrich. GSK343 (Cat# T6059), UNC6852

728   (Cat# T13954) and UN1999 (Cat# T3057) were purchased from TargetMol Chemicals. Peptides

729   TAT-HKII (MIASHMIACLFTELN(β-Ala)GYGRKKRRQRRG-amide) and TAT (GYGRKKRRQRRG-

730   amide) were custom-made by GenScript.

731

732   **oxPAPE-N-Biotin synthesis.**

733   A solution of PAPE in dry dichloromethane was added dropwise to a magnetically stirred solution

734   of biotin, N,N'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in dry

735   dichloromethane under argon at room temperature. The solution was mixed with a magnetic

736   stirrer under argon for 12-24h at room temperature. oxPAPE-N-Biotin was directly purified with a

737   syringe filter (PES 0.45 um) and SPE kit.

738

739   **Lipid oxidation**

740   PAPC and PAPE-N-Biotin were oxidized using a method similar to that previously reported [57].

741   Briefly, phospholipids were transferred to clean borosilicate tubes in 0.5 mg aliquots in chloroform,

742   dried, and oxidized for 24–72h, while monitoring oxidation by flow injection on an electrospray

743   ionization (ESI) instrument (LCQ Fleet, Thermo Fisher Scientific). Lipid concentrations were

744   analyzed by phosphorus assay.

745

Seahorse metabolic analysis

The ECAR and OCR were measured with a Seahorse XFe96 Extracellular Flux Analyzer. Phagocytes (8×10^4 per well) were seeded in a Seahorse 96-well plate in DMEM complete medium (murine cells) or RPMI complete medium (human cells). After 4h, cells were washed and incubated in the Seahorse Assay Medium (Agilent Technologies, Seahorse XF DMEM medium, Cat# 103575-100, or Seahorse XF RPMI medium, Cat# 103576-100) supplemented with 10 mM glucose and 2 mM glutamine at 37 °C for 45 min. The OCR and ECAR were measured under basal conditions and after injection of LPS (1 or 0.1 µg ml^{-1}), oxPAPC, DPPC, FCCP (1.5 µM) and rotenone (0.5 µM) plus antimycin A (0.5 µM) plus 2-DG (50 mM) (rot. + AA + 2-DG), as indicated in the figures.

756

Gene expression analysis

RNA was isolated from cell cultures using Direct-zol RNA Miniprep Kit (Zymo Research, Cat# R2053). Purified RNA was analyzed for gene expression on a CFX384 real-time cycler (Bio-Rad Laboratories) using Power SYBR Green RNA-to-CT 1-Step Kit (Thermo Fisher Scientific, Cat# 4389986) with predesigned primers (Sigma-Aldrich, KiCqStart SYBR Green Primers) specific for murine *Il10*, *Cxcl10*, *Ifit2*, *Gbp2*, *Hmox1*, *Nqo1*, *Txnrd1* and *Rpl13a* (housekeeping). In order to analyze *Akt* isoforms expression, TaqMan RNA-to-CT 1-Step Kit (Thermo Fisher Scientific, Cat# 4392938) was used in association with the following probes: *Akt1* (Thermo Fisher Scientific, Cat# Mm01331626_m1), *Akt2* (Thermo Fisher Scientific, Cat# Mm05804787_gH), *Akt3* (Thermo Fisher Scientific, Cat# Mm00442194_m1) and *Rpl13a* (Thermo Fisher Scientific, Cat# Mm05910660_g1).

768

Measurement of cytokines by ELISA

770   Cytokine production was quantified from cell supernatants or serum using the following ELISA

771   kits: ELISA MAX Deluxe Set Mouse IL-10 (BioLegend, Cat# 431414), ELISA MAX Deluxe Set

772   Human IL-10 (BioLegend, Cat# 430604), ELISA MAX Deluxe Set Mouse TNF-α (BioLegend, Cat#

773   430915), ELISA MAX Deluxe Set Human TNF-α (BioLegend, Cat# 430204), ELISA MAX Deluxe

774   Set Mouse IL-6 (BioLegend, Cat# 431315) and ELISA MAX Deluxe Set Mouse IL-1β (BioLegend,

775   Cat# 432604).

776

777   **Immunoblotting**

778   For western blotting, phagocytes (1×10^6) were treated as indicated and subsequently lysed in

779   100 μl RIPA buffer (Boston BioProducts, Cat# BP-116TX) added with protease and phosphatase

780   inhibitors (Thermo Fisher Scientific, Halt Protease and Phosphatase Inhibitor Cocktail, Cat#

781   78440). Total protein concentrations were determined using BCA protein assay kit (Genesee

782   Scientific, Cat# 18-440). Proteins were separated using SDS-PAGE electrophoresis and then

783   transferred to polyvinylidene difluoride (PVDF) membranes. After 1h incubation with blocking

784   buffer (5% milk or BSA in Tris-buffered saline (TBS) with 0.05% Tween-20), membranes were

785   probed with the following primary antibodies: phospho-AKT (Ser473) (1:1000, Cell Signaling

786   Technology, Cat# 4060), phospho-AKT (Thr308) (1:1000, Cell Signaling Technology, Cat# 13038),

787   AKT (pan) (1:1000, Cell Signaling Technology, Cat# 4691), β-actin (1:3000, Sigma–Aldrich, Cat#

788   A5441), phospho-SIN1 (Thr86) (1:1000, Cell Signaling Technology, Cat# 14716), SIN1

789   (Proteintech Group, Cat# 15463-1-AP), phospho-NDRG1 (Thr346) (1:1000, Cell Signaling

790   Technology, Cat# 5482), NDRG1 (1:1000, Abcam, Cat# ab37897), phospho-PRAS40 (Thr246)

791   (1:1000, Cell Signaling Technology, Cat# #13175), PRAS40 (Cell Signaling Technology, Cat#

792   #2610), phospho-GSK3β (Ser9) (1:1000, Cell Signaling Technology, Cat# #5558), GSK3β

793   (Proteintech Group, Cat# 22104-1-AP), phospho-TBK1 (Ser172) (1:1000, Cell Signaling

794   Technology, Cat# 5483), TBK1 (1:1000, Cell Signaling Technology, Cat# 38066), phospho-IKKε

795   (Ser172) (1:1000, Sigma-Aldrich, Cat# 06-1340), IKKε (1:1000, Cell Signaling Technology, Cat#

796   3416), phospho-PDK1 (Ser241) (1:1000, Cell Signaling Technology, Cat# #3438), PDK1 (1:1000,

797   Cell Signaling Technology, Cat# 3062), phospho-PTEN (Ser380) (1:1000, Cell Signaling

798   Technology, Cat# 9551), PTEN (1:1000, Cell Signaling Technology, Cat# 9559), phospho-PI3K

799   p85 (Tyr458)/p55 (Tyr199) (1:1000, Cell Signaling Technology, Cat# 4228), PI3K p85 (1:1000,

800   Cell Signaling Technology, Cat# 4257), phospho-SYK (Tyr525/526) (1:1000, Cell Signaling

801   Technology, Cat# 2710), SYK (1:1000, Cell Signaling Technology, Cat# 13198), phospho-mTOR

802   (Ser2448) (1:1000, Cell Signaling Technology, Cat# 5536), mTOR (1:1000, Cell Signaling

803   Technology, Cat# 2983), phospho-RICTOR (Thr1135) (1:1000, Cell Signaling Technology, Cat#

804   3806), RICTOR (1:1000, Cell Signaling Technology, Cat# 2114), phospho-PKCα/β$_{II}$ (Thr638/641)

805   (1:1000, Cell Signaling Technology, Cat# 9375), PKCα (Proteintech Group, Cat# 21991-1-AP),

806   phospho-p70S6K (Thr389) (1:1000, Cell Signaling Technology, Cat# 9234), p70S6K (1:1000, Cell

807   Signaling Technology, Cat# 34475), phospho-S6 (Ser235/Ser236) (1:500, BioLegend, Cat#

808   608602), S6 (1:500, BioLegend, Cat# 691802), phospho-STAT1 (Tyr701) (1:1000, Becton,

809   Dickinson and Company, Cat# 612133), STAT1 (1:1000, Cell Signaling Technology, Cat# 14994),

810   RSAD2 (1:1000, BioLegend, custom made 91736), NRF2 (1:1000, Cell Signaling Technology,

811   Cat# 12721), phospho-ERK1/2 (Thr202/Tyr204) (1:1000, Cell Signaling Technology, Cat# 9106),

812   ERK1/2 (1:1000, Cell Signaling Technology, Cat# 9102), phospho-p38 (Thr180/Tyr182) (1:1000,

813   Cell Signaling Technology, Cat# 4511), p38 (1:500, BioLegend, Cat# 622403), phospho-

814   SAPK/JNK (Thr183/Tyr185) (1:1000, Cell Signaling Technology, Cat# 4668) , SAPK/JNK (1:1000,

815   Cell Signaling Technology, Cat# 9252), phospho-CREB (Ser133) (1:1000, Cell Signaling

816   Technology, Cat# 9198), CREB1 (1:2000, Proteintech Group, Cat# 12208-1-AP), phospho-NF-κB

817   (Ser536) (1:1000, Cell Signaling Technology, Cat# 3033), NFκB p65 (1:1000, Cell Signaling

818   Technology, Cat# 6956), EED (1:1000, Cell Signaling Technology, Cat# 85322), EZH2 (1:1000,

819   Cell Signaling Technology, Cat# 5246) and SUZ12 (1:1000, Cell Signaling Technology, Cat#

820   3737).

821 After incubation with the specific HRP-conjugated antibodies anti-rabbit (1:10000, Thermo Fisher

822 Scientific, Cat# G-21234), anti-mouse (1:10000, Jackson ImmunoResearch, Cat# 715-035-151)

823 or anti-goat (1:10000, Santa Cruz Biotechnology, Cat# sc-2354), the immunoreactive bands were

824 detected by iBright FL100 Imaging System (Thermo Fisher Scientific).

825

826 **Measurement of total oxPLs by ELISA**

827 The concentration of total immunodetectable oxPLs in mouse and human samples (serum, BAL

828 or lung homogenate) was measured using a modification of a competitive ELISA technique, as

829 previously described [13]. Briefly, white immunoplates (Thermo Fisher Scientific, Cat# 436110)

830 were coated with an IgG form of the E06 antibody (Absolute Antibody, Cat# Ab02746-23.0) and

831 left overnight at 4°C. Plates were blocked with 1% fatty acid free BSA for 1 hour and then diluted

832 serum samples were added in triplicate to the wells. Next, Phosphorylcholine Keyhole Limpet

833 Hemocyanin (PC-KLH, LGC Biosearch Technologies, Cat# PC-1013-5) was applied at a

834 concentration of 100 ng per well. After 1h incubation, alkaline phosphatase conjugated anti-KLH

835 antibody (Rockland Inc, Cat# 600–405-466) was added to detect the bound PC-KLH, using Lumi-

836 Phos 530 (Lumigen, Cat# P-501). The luminescence emitted was measured using a SpectraMax

837 i3x plate reader (Molecular Devices). In order to calculate the concentration of oxPLs in the

838 samples, each plate included a standard curve of oxPAPC.

839

840 **Flow Cytometry**

841 Spleens collected from the indicated mice were mechanically dissociated and filtered to obtain a

842 cell suspension. After red blood cell lysis, dead cells were stained with Zombie Violet (BioLegend,

843 Cat# 423114) and cellular surface markers were stained with the following fluorophore-conjugated

844 anti-mouse antibodies: Alexa Fluor 488 CD3 (BioLegend, Cat# 100210), Alexa Fluor 488 CD19

845 (BioLegend, Cat# 115521), Alexa Fluor 488 NK1.1 (BioLegend, Cat# 108718), BV605 CD11b

846 (BioLegend, Cat# 101257), BV510 Ly6G (BioLegend, Cat# 127633), APC F4/80 (BioLegend,

847   Cat# 123116). Cells were fixed (Fixation buffer, BioLeged, Cat# 420801), permeabilized

848   (Intracellular Staining Permeabilization Wash Buffer, BioLegend, Cat# 421002) and incubated for

849   30 minutes with TruStain FcX™ PLUS (anti-mouse CD16/32) antibody (BioLegend, Cat#

850   156604). Immunodetectable oxPLs were analyzed using custom-made PE-conjugated E06 rabbit

851   antibody (Absolute antibody) and PE-conjugated isotype control (rabbit IgG, Cell Signaling

852   Technology, Cat# 5742S).

853   To analyze the phosphorylation status of EZH2, BMDMs were treated as indicated, fixed and

854   permeabilized using True-Phos Perm Buffer (BioLegend, Cat# 425401). Cells were incubated

855   with phospho-EZH2 (S21) (Affinity Biosciences, Cat# AF3822) or rabbit IgG isotype control

856   (BioLegend, Cat# 910801) followed by PE-conjugated anti-rabbit antibody (BioLegend, Cat#

857   406421) staining.

858   Samples were acquired with LSRFortessa Cell Analyzer (Becton, Dickinson and Company) and

859   analyzed by FlowJo v10.9 (Becton, Dickinson and Company).

860

861   **Microscopy, histology and immunostaining**

862   Immunofluorescent analysis of BMDMs were performed seeding 1.5×10^4 cells on sterile 12mm

863   coverslips (Neuvitro Corporation, GG-12-1.5-Fibronectin). Cells were treated as indicated and

864   then fixed with 4% paraformaldehyde. After permeabilization using 0.1% Triton X-100 0.2% BSA-

865   PBS, cells were blocked in 2% BSA-PBS and incubated with NF-κB p65 (1:100, Cell Signaling

866   Technology, Cat# 6956S) followed by Alexa Fluor 488 conjugated chicken anti-mouse IgG (1:300,

867   Thermo Fisher Scientific, Cat# A-21200). Nuclei were stained with DAPI (1:10000, Thermo Fisher

868   Scientific, Cat# D3571).

869   Histological analysis on collected spleens and lungs were performed at Histology core facility and

870   Confocal imaging and Immunohistochemistry (IHC) core facility at Beth Israel Deaconess Medical

871   Center. The IHC-DAB staining was performed on paraffin embedded mouse spleen sections.

872   Slides were cut, deparaffinized and hydrated. Antigen retrieval was performed by boiling the slides

873 in 10 mM sodium citrate (pH 6) in a pressure cooker. Endogenous peroxidase activity was blocked

874 with 3% hydrogen peroxide and sections were blocked with 2% BSA (Jackson ImmunoResearch

875 Lab, Cat# 001-000-162) for 1h at room temperature. After serum blocking, the slides were

876 incubated with rabbit anti-oxidized phospholipid E06 IgG (1:100, Absolute Antibody, Cat#

877 Ab02746-23.0) primary antibody overnight at 4°C. After wash, the slides were incubated with goat

878 anti-rabbit IgG conjugated with HRP polymer (Abcam, Cat#: ab214880) for 90 minutes at room

879 temperature. The slides were washed before developing in DAB (diaminobenzidine) metal

880 enhanced kit (Vector lab, Cat# SK4105) and counter stained with hematoxylin (Thermo Fisher

881 Scientific, Cat# 411160250). Lastly, the IHC slides were processed through dehydration steps

882 before mounted in Permount (Fisher Scientific, Cat# SP15-100). The triple immunofluorescence

883 antigen labeling was performed on paraffin-embedded mouse spleen sections. The paraffin

884 sections were deparaffinized and rehydrated, followed by antigen retrieval using Tris-EDTA buffer

885 (pH 9). After three washes, the sections were incubated with 2% BSA for 1 h at room temperature.

886 Slides were then incubated with rabbit anti-oxidized phospholipid E06 IgG (1:100, Absolute

887 antibody, Cat#: Ab02746-23.0), mouse anti-CD68 (1:100, Santa Cruz Biotechnology, Cat# sc-

888 20060) and rat anti-Ly6G/Ly6C (1:50, Santa Cruz Biotechnology, Cat#: dc-59338) overnight at

889 4°C. The slides were washed three times and incubated with Alexa Fluor 488 conjugated donkey

890 anti-rabbit (1:300, Jackson ImmunoResearch Lab, Cat# 711-545-152), Cy3 conjugated donkey

891 anti-mouse secondary antibody (1:300, Jackson ImmunoResearch Lab, Cat# 715-165-151) and

892 Alexa Fluor 647 conjugated donkey anti-rat secondary antibody (1:300, Jackson

893 ImmunoResearch Lab, Cat# 712-605-153) for 90 minutes at room temperature. Samples were

894 counterstained with Hoechst 33342 (1:10000, Life Technology, Cat#: H21492), washed and then

895 mounted in Prolong Gold anti-fade mounting media (Thermo Fisher Scientific, Cat#: P36930).

896 To analyze tissue inflammation and damage in lungs, the organs were collected, fixed in 10%

897 formalin and processed for paraffin inclusion. 5 μm sections were stained with hematoxylin and

898   eosin and perivascular and peribronchial inflammation were evaluated by severity-based ordinal

899   scoring, based on cellular infiltration: 0 = none, 1 = solitary infiltration of cells, 2 = a ring of

900   inflammatory cells 1 cell layer deep, 3 = a ring of inflammatory cells 2–4 cells deep and 4 = a ring

901   of inflammatory cells >4 cells deep. Interstitial inflammation score was evaluated as follow: 0 =

902   none, 1 = presence of few cells in septa, 2 = increased alveolar thickness and moderate cellular

903   infiltration of septa, 3 = severe cellular infiltration in thickened septa and in air-space lumens.

904   Each score was normalized for the surface of affected lung. The final inflammation score of each

905   lung (0-11) was obtained by the sum of the normalized scores of perivascular, peribronchial and

906   interstitial inflammation.

907   All the images were acquired using EVOS M7000 Imaging System (Thermo Fisher Scientific,

908   Cat# AMF7000) and analyzed using ImageJ (NIH).

909

910   *In vivo* **models**

911   The animals were housed in the same vivarium room at the Boston Children's Hospital animal

912   facility, up to five animals per cage, under controlled temperature, 12:12 light-dark cycle, and

913   free access to normal food and water. All animal experiments were performed under the Guide

914   of Care and Use of Laboratory Animals of the National Institute of Health. All animal

915   experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of

916   Boston Children's Hospital.

917   Indicated mice (females, 8-10 weeks old) were kept under specific-pathogen-free conditions and

918   were co-housed for at least 4 weeks before any experiment.

919   For PRR challenge, PRR agonists (LPS, CpG, R848 or WPG) were i.p. injected, at the indicated

920   concentrations, and blood and spleens were collected at 8h and 24h.

921   In CLP experiments, the day before the procedure, mice were implanted with temperature

922   transponders (Bio Medic Data Systems, Cat# IPTT-300) and the abdomen was shaved. For acute

923   IL-10 signaling inhibition, the following antibodies were i.p. injected at 0.5 mg per mouse: rat anti-

924   mouse CD210 (IL-10R, clone 1B1.3a, BioLegend, Cat# 112712, Lot# B360179,) and rat IgG1,

925   isotype control (BioLegend, Cat# 400458, Lot# B302875). For EZH2 inhibition, GSK343 (10 mg

926   Kg^{-1}), resuspended in 10% DMSO, 10% PEG-400, 10% Tween-80, 70% saline, was i.p. injected

927   48h and 12h before the experiment. For CLP procedure, mice were anesthetized, and cecum was

928   exteriorized. The cecum was tightly suture ligated at about 1 cm to the distal end and was

929   perforated by single through-and-through puncture midway between the ligation and the tip of the

930   cecum in a mesenteric-to-antimesenteric direction with a 20G needle. After removing the needle,

931   the cecum was then gently squeezed to extrude a small amount of feces. The cecum was

932   relocated into the abdominal cavity, the peritoneum was closed by applying simple running

933   sutures and skin was closed using metallic clips. In sham group, mice were operated following

934   the same protocol without CLP procedure. After operation, mice were resuscitated by

935   subcutaneously injecting pre-warmed saline and the analgesic buprenorphine extended-release

936   (Fidelis Animal Health, Ethiqa XR) was administered subcutaneously at 3.25 mg Kg^{-1}. Animals

937   were monitored for 4 days for body temperature drop (Bio Medic Data Systems, Bluetooth

938   Wireless Reader, Cat# DAS-8027-BLU System), weight loss and mortality comparison. Blood and

939   spleens were collected at the indicated time points. Blood was left undisturbed at room

940   temperature for 15 minutes and centrifugated at 4°C 2000 x g for 10 minutes to collect serum.

941   Serum was immediately used for the measurement of cytokines and oxPLs levels. Alternatively,

942   serum was immediately stored at -80°C.

943   The MRSA experiments were conducted at the University of California San Diego (UCSD) and

944   protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of UCSD.

945   Male and female mice were injected with MRSA via tail vein with a lethal dose (1.8 x 10⁸

946   CFU/mouse) and monitored for mortality over 120 hours. Blood was collected at 24 hours for

947   measurement of bacterial load. In a separate experiment, male mice were injected with a

948   sublethal dose (1.0 x 108 CFU/mouse) and monitored for 120 hours for mortality. Serum was

949   collected for cytokine quantification at 24 hours.

950   To mimic persistent or post-acute viral infection, 2.5 mg Kg^{-1} of poly(I:C) or saline were daily

951   administrated in mice via intratracheal instillation for the indicated time points. Body temperature,

952   recorded using subcutaneous transponders (Bio Medic Data Systems, Cat# IPTT-300), and body

953   weight were monitored every 24 hours. Survival was followed overtime, using humane endpoint

954   at weight loss >20%. After 6 days, mice were euthanized by CO_2 inhalation. Mice were

955   transcardially perfused with 10 ml of PBS or until liver and lungs were clear. Lungs were harvested

956   and processed for histology inspection or lysed in RIPA buffer, added with protease and

957   phosphatase inhibitors, using a Bead Mill 24 Homogenizer (Fisher, Cat# 15-340-163). After

958   centrifugation at 17000 x g for 10 minutes at 4°C, the resulted clear tissue homogenates were

959   immediately used for the quantification of cytokines and oxPLs.

960

Bacterial Counts

962   Collected whole blood was serially diluted in sterile PBS and cultured overnight at 37°C on 5%

963   blood agar plates (Hardy Diagnostics, Cat# A10BX). Bacteria colonies were counted, and the

964   results were expressed as log of colony-forming units (CFU) per ml.

965

Metabolomics analysis

967   Metabolomics profiling was performed at the Beth Israel Deaconess Medical Center Mass

968   Spectrometry Facility. Briefly, polar metabolites were collected by methanol-based extraction, as

969   previously described [58]. Samples were resuspended using HPLC-grade water for mass

970   spectrometry and analyzed using a 5500 QTRAP hybrid triple quadrupole mass spectrometer (AB

971   Sciex) coupled to a Prominence UFLC HPLC system. A total of 284 endogenous water-soluble

972 metabolites were analyzed). Statistical analysis was performed using MetaboAnalyst

973 (http://www.metaboanalyst.ca, free online software).

974

Microscale thermophoresis (MST)

976 Microscale thermophoresis experiments were performed at the Harvard Center for

977 Macromolecular Interactions (CMI). His-tagged recombinant human AKT1 (BPS Bioscience, Cat#

978 40003), AKT2 (BPS Bioscience, Cat# 40011) and AKT3 (BPS Bioscience, Cat# 40012) were

979 fluorescently labeled using His-Tag Labeling Kit RED-Tris-NTA 2nd Generation (NanoTemper, Cat#

980 MO-L018). The proteins (at fixed concentration) were incubated with oxPAPC or DPPC, at the

981 indicated concentrations, and samples were loaded in Monolith capillaries (NanoTemper, Cat#

982 MO-K022). MST was run in Monolith NT.115 Instrument (NanoTemper) and analyzed with MO

983 Affinity Analysis software, Version 2.3 (NanoTemper).

984

Kinase activity

986 The inhibition of kinase activity mediated by phospholipids was determined using the following Z'-

987 LYTE Kinase Assay Kits: Z'-LYTE Kinase Assay Kit - Ser/Thr 6 Peptide (Thermo Fisher Scientific,

988 Cat# PV3179) used to measure AKT activity and the Z'-LYTE Kinase Assay Kit - Ser/Thr 7 Peptide

989 (Thermo Fisher Scientific, Cat# PV3180) used to measure PDK1 activity. All assays were run in

990 accordance with the manufacturer's instructions. Briefly, recombinant human AKT1 (BPS

991 Bioscience, Cat# 40003), AKT2 (BPS Bioscience, Cat# 40011) and AKT3 (BPS Bioscience, Cat#

992 40012) were used at 7.5 ng per reaction and recombinant human PDK1 (BPS Bioscience, Cat#

993 40080) was used at 50 ng per reaction. Phospholipids were prepared by 2-fold serial dilution in

994 kinase buffer and incubated with the target protein for at least 10 minutes at room temperature.

995 Specific Fluorescence Resonance Energy Transfer (FRET)-peptides were added at 2 μM and the

996 solution was supplemented with ATP (100 μM for AKT or 50 μM for PDK1) to initiate the reaction.

997　ATP was omitted in control reactions to obtain a complete kinase activity inhibition (100%

998　inhibition). After 1h, development reagent, that selectively cleaves non-phosphorylated peptides

999　disrupting FRET between the donor (coumarin) and acceptor (fluorescein) fluorophores on the

1000　FRET-peptides, was added and incubated for 1 hour at room temperature. After stopping the

1001　reaction with stop reagent, coumarin and FRET based fluorescein emission was measured on a

1002　SpectraMax i3x plate reader (Molecular Devices).

1003

1004　**Immunoprecipitation**

1005　293T cells were transiently transfected using Lipofectamine 3000 (Thermo Fisher Scientific, Cat#

1006　L3000001) with the following plasmids: pCDNA3-HA-Akt1 (Addgene plasmid # 73408,

1007　RRID:Addgene_73408), pCDNA3-HA-Akt1-aa1-149 (Addgene plasmid # 73410,

1008　RRID:Addgene_73410), pCDNA3-HA-Akt1-aa1-408 (Addgene plasmid # 73412,

1009　RRID:Addgene_73412) and pCDNA3-HA-Akt1-aa120-433 (Addgene plasmid # 73411,

1010　RRID:Addgene_73411). These plasmids were a gift from Jie Chen (University of Illinois at Urbana-

1011　Champaign) [59]. Cell lysates were incubated with oxPAPE-N-Biotin (10 μg) with or without

1012　competing oxPAPC doses at 4°C on a nutator for either 6h or overnight. Lipid-protein complexes

1013　were captured using High Capacity Streptavidin Agarose beads (Thermo Fisher Scientific, Cat#

1014　20357) and then heated at 98°C in Laemmli Sample Buffer (Bio-rad, Cat# 1610747). Samples

1015　were run in a SDS-PAGE gel and lipid-interacting proteins were detected by immunoblotting using

1016　HA-tag antibody (Proteintech Group, Cat# 51064-2-AP).

1017

1018　**RNA-Seq**

1019　BMDMs (1×10^6) were treated as indicated and RNA was purified using Direct-zol RNA Miniprep

1020　(Zymo Research Corporation, Cat# R2053). Samples were submitted to Azenta Life Sciences for

1021　standard RNA-seq processing. RNA was quantified using Qubit 2.0 Fluorometer (Thermo Fisher

1022 Scientific) and RNA integrity was checked with RNA Screen Tape on Agilent 2200 TapeStation

1023 (Agilent Technologies). After library preparation (Illumina, PolyA selection), samples were

1024 sequenced using Illumina, 2x150 bp, ~350M PE reads (~105GB), single index.

1025

1026 **ChIP-qPCR**

1027 BMDMs (5×10^6) were treated as indicated and fixed with 1% formaldehyde for 10 minutes at

1028 room temperature. After quenching with 650 mM glycine for 5 minutes, cells were washed in

1029 chilled PBS and collected by scraping. Subsequent sample processing was performed using

1030 Chromatrap Enzymatic kit (Chromatrap Porvair Sciences, Cat# 500191). Briefly, cells were lysed,

1031 and chromatin was sheared by enzymatic digestion to obtain 150-500 bp fragments. Next,

1032 chromatin was incubated with H3K27me2me3 antibody (2μg, Active Motif, Cat# 39435) or mouse

1033 IgG isotype antibody (2μg, Santa Cruz Biotechnology, Cat# sc-2025) for 1h at 4°C and antibody-

1034 chromatin complexes were selectively collected by protein A spin columns. Samples were reverse

1035 cross-linked, and proteins digested with proteinase K. DNA fragments were purified using ChIP

1036 DNA Clean & Concentrator columns (Zymo Research Corporation, Cat# D5205).

1037 Specific primer pairs were used to amplified conserved noncoding sequences (CNS) related to

1038 murine *Il10* locus. The following primers were obtained from Neumann et al. 2014 [60]: CNS -4.5

1039 (Fwd: GCCACGATTCTCAGGACATT, Rev: GTATCCAACCCCACTTGCAC) and CNS -0.5 (Fwd:

1040 CTCTCCTCTGACCAACTGCC, Rev: TGGGTTGAACGTCCGATATT). The following primers

1041 were obtained from Lee et al. 2012 [61]: CNS -0.12 (Fwd: TCTGTACATAGAACAGCTGTC, Rev:

1042 CTGGTCGGAATGAACTTCTG), CNS +1.65 (Fwd: GTCTCTTGCTCATCTGTCTC, Rev:

1043 GCTAATAACCCACAATGACTC), CNS +2.98 (Fwd: ACTAGGTGTTGAGGAGAGTG, Rev:

1044 GAATTCTGCTTTCTGCTCGT), CNS +6.45 (Fwd: GTGGTCATTTTTTCAGTAAGACC, Rev:

1045 CCTAACCTTTCATCTCACAG). Control primers were purchased from Active Motif: *Hoxc10* (Cat#

1046 71019) and *Gapdh* (Cat# 71014).

1047

1048   **Statistical analysis**

1049   The statistical tests used and P values for all the experiments are specified in figure captions.

1050   Statistical significance for the experiments with more than two groups and two factors was tested

1051   with two-way analysis of variance (ANOVA), and Sidak's, Dunnet's or Tukey's multiple-

1052   comparisons tests were performed according to the nature of the comparison tested. When

1053   comparisons between only two groups were made, an unpaired two-tailed t-test was used to

1054   assess statistical significance. For survival experiments, P values were determined by long-rank

1055   test. To establish the appropriate test, normal distribution and variance similarity were assessed

1056   with the D'Agostino–Pearson omnibus normality test. All statistical analyses were performed

1057   using Prism 9 (GraphPad Software, version 9.4.0).

References

1. Brubaker, S.W., et al., *Innate Immune Pattern Recognition: A Cell Biological Perspective.* Annu Rev Immunol, 2015.

2. Gong, T., et al., *DAMP-sensing receptors in sterile inflammation and inflammatory diseases.* Nat Rev Immunol, 2020. **20**(2): p. 95-112.

3. Janeway, C.A., Jr., *Approaching the asymptote? Evolution and revolution in immunology.* Cold Spring Harb Symp Quant Biol, 1989. **54 Pt 1**: p. 1-13.

4. Matzinger, P., *Tolerance, danger, and the extended family.* Annu Rev Immunol, 1994. **12**: p. 991-1045.

5. Medzhitov, R., *Approaching the asymptote: 20 years later.* Immunity, 2009. **30**(6): p. 766-75.

6. Fu, J. and H. Wu, *Structural Mechanisms of NLRP3 Inflammasome Assembly and Activation.* Annu Rev Immunol, 2023. **41**: p. 301-316.

7. Dangl, J.L. and J.D. Jones, *Plant pathogens and integrated defence responses to infection.* Nature, 2001. **411**(6839): p. 826-33.

8. Remick, B.C., M.M. Gaidt, and R.E. Vance, *Effector-Triggered Immunity.* Annu Rev Immunol, 2023. **41**: p. 453-481.

9. Lopes Fischer, N., et al., *Effector-triggered immunity and pathogen sensing in metazoans.* Nat Microbiol, 2020. **5**(1): p. 14-26.

10. Orzalli, M.H. and P. Parameswaran, *Effector-triggered immunity in mammalian antiviral defense.* Trends Immunol, 2022. **43**(12): p. 1006-1017.

11. Gaidt, M.M., et al., *Self-guarding of MORC3 enables virulence factor-triggered immunity.* Nature, 2021. **600**(7887): p. 138-142.

12. Que, X., et al., *Oxidized phospholipids are proinflammatory and proatherogenic in hypercholesterolaemic mice.* Nature, 2018. **558**(7709): p. 301-306.

13. Sun, X., et al., *Neutralization of Oxidized Phospholipids Ameliorates Non-alcoholic Steatohepatitis.* Cell Metab, 2020. **31**(1): p. 189-206 e8.

14. Imai, Y., et al., *Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury.* Cell, 2008. **133**(2): p. 235-49.

15. Shirey, K.A., et al., *The TLR4 antagonist Eritoran protects mice from lethal influenza infection.* Nature, 2013. **497**(7450): p. 498-502.

16. Di Gioia, M. and I. Zanoni, *Dooming Phagocyte Responses: Inflammatory Effects of Endogenous Oxidized Phospholipids.* Front Endocrinol (Lausanne), 2021. **12**: p. 626842.

17. Bochkov, V.N., et al., *Protective role of phospholipid oxidation products in endotoxin-induced tissue damage.* Nature, 2002. **419**(6902): p. 77-81.

18. Zanoni, I., et al., *By Capturing Inflammatory Lipids Released from Dying Cells, the Receptor CD14 Induces Inflammasome-Dependent Phagocyte Hyperactivation.* Immunity, 2017. **47**(4): p. 697-709 e3.

19. Zanoni, I., et al., *An endogenous caspase-11 ligand elicits interleukin-1 release from living dendritic cells.* Science, 2016. **352**(6290): p. 1232-6.

20. Di Gioia, M., et al., *Endogenous oxidized phospholipids reprogram cellular metabolism and boost hyperinflammation.* Nat Immunol, 2020. **21**(1): p. 42-53.

21. Binder, C.J., N. Papac-Milicevic, and J.L. Witztum, *Innate sensing of oxidation-specific epitopes in health and disease.* Nat Rev Immunol, 2016. **16**(8): p. 485-97.

22. Upchurch, C.M., et al., *Targeting oxidized phospholipids by AAV-based gene therapy in mice with established hepatic steatosis prevents progression to fibrosis.* Sci Adv, 2022. **8**(28): p. eabn0050.

23. Sposito, B., et al., *The interferon landscape along the respiratory tract impacts the severity of COVID-19.* Cell, 2021. **184**(19): p. 4953-4968 e16.

1107   24.   Broggi, A., et al., *Type III interferons disrupt the lung epithelial barrier upon viral*
1108   *recognition.* Science, 2020. **369**(6504): p. 706-712.

1109   25.   Wong, A.C., et al., *Serotonin reduction in post-acute sequelae of viral infection.* Cell, 2023.

1110   26.   Moore, K.W., et al., *Interleukin-10 and the interleukin-10 receptor.* Annu Rev Immunol,
1111   2001. **19**: p. 683-765.

1112   27.   Tannahill, G.M., et al., *Succinate is an inflammatory signal that induces IL-1beta through*
1113   *HIF-1alpha.* Nature, 2013. **496**(7444): p. 238-42.

1114   28.   Everts, B., et al., *TLR-driven early glycolytic reprogramming via the kinases TBK1-*
1115   *IKKvarepsilon supports the anabolic demands of dendritic cell activation.* Nat Immunol,
1116   2014. **15**(4): p. 323-32.

1117   29.   O'Neill, L.A. and E.J. Pearce, *Immunometabolism governs dendritic cell and macrophage*
1118   *function.* J Exp Med, 2016. **213**(1): p. 15-23.

1119   30.   Manning, B.D. and A. Toker, *AKT/PKB Signaling: Navigating the Network.* Cell, 2017.
1120   **169**(3): p. 381-405.

1121   31.   Singer, M., et al., *The Third International Consensus Definitions for Sepsis and Septic*
1122   *Shock (Sepsis-3).* JAMA, 2016. **315**(8): p. 801-10.

1123   32.   Nathan, C., *Points of control in inflammation.* Nature, 2002. **420**(6917): p. 846-52.

1124   33.   Erridge, C., et al., *Oxidized phospholipid inhibition of toll-like receptor (TLR) signaling is*
1125   *restricted to TLR2 and TLR4: roles for CD14, LPS-binding protein, and MD2 as targets for*
1126   *specificity of inhibition.* J Biol Chem, 2008. **283**(36): p. 24748-59.

1127   34.   Bretscher, P., et al., *Phospholipid oxidation generates potent anti-inflammatory lipid*
1128   *mediators that mimic structurally related pro-resolving eicosanoids by activating Nrf2.*
1129   EMBO Mol Med, 2015. **7**(5): p. 593-607.

1130   35.   Kerur, N., et al., *cGAS drives noncanonical-inflammasome activation in age-related*
1131   *macular degeneration.* Nat Med, 2017.

1132   36.   Kadl, A., et al., *Oxidized phospholipid-induced inflammation is mediated by Toll-like*
1133   *receptor 2.* Free Radic Biol Med, 2011. **51**(10): p. 1903-9.

1134   37.   Serbulea, V., et al., *Macrophages sensing oxidized DAMPs reprogram their metabolism*
1135   *to support redox homeostasis and inflammation through a TLR2-Syk-ceramide dependent*
1136   *mechanism.* Mol Metab, 2018. **7**: p. 23-34.

1137   38.   Kadl, A., et al., *Identification of a novel macrophage phenotype that develops in response*
1138   *to atherogenic phospholipids via Nrf2.* Circ Res, 2010. **107**(6): p. 737-46.

1139   39.   Serbulea, V., et al., *Macrophage phenotype and bioenergetics are controlled by oxidized*
1140   *phospholipids identified in lean and obese adipose tissue.* Proc Natl Acad Sci U S A, 2018.
1141   **115**(27): p. E6254-E6263.

1142   40.   Itoh, K., et al., *Transcription factor Nrf2 regulates inflammation by mediating the effect of*
1143   *15-deoxy-Delta(12,14)-prostaglandin j(2).* Mol Cell Biol, 2004. **24**(1): p. 36-45.

1144   41.   Jerabek-Willemsen, M., et al., *Molecular interaction studies using microscale*
1145   *thermophoresis.* Assay Drug Dev Technol, 2011. **9**(4): p. 342-53.

1146   42.   Martin, M., et al., *Toll-like receptor-mediated cytokine production is differentially regulated*
1147   *by glycogen synthase kinase 3.* Nat Immunol, 2005. **6**(8): p. 777-84.

1148   43.   Hu, X., et al., *IFN-gamma suppresses IL-10 production and synergizes with TLR2 by*
1149   *regulating GSK3 and CREB/AP-1 proteins.* Immunity, 2006. **24**(5): p. 563-74.

1150   44.   Sankar, A., et al., *Histone editing elucidates the functional roles of H3K27 methylation and*
1151   *acetylation in mammals.* Nat Genet, 2022.

1152   45.   Scacchetti, A. and R. Bonasio, *Histone gene editing probes functions of H3K27*
1153   *modifications in mammals.* Nat Genet, 2022.

1154   46.   Cha, T.L., et al., *Akt-mediated phosphorylation of EZH2 suppresses methylation of lysine*
1155   *27 in histone H3.* Science, 2005. **310**(5746): p. 306-10.

1156   47.   He, S., et al., *Ezh2 phosphorylation state determines its capacity to maintain CD8(+) T*
1157   *memory precursors for antitumor immunity.* Nat Commun, 2017. **8**(1): p. 2125.

1158 48. Zhou, J., et al., *Targeting EZH2 histone methyltransferase activity alleviates experimental*
1159 *intestinal inflammation.* Nat Commun, 2019. **10**(1): p. 2427.
1160 49. Konze, K.D., et al., *An orally bioavailable chemical probe of the Lysine Methyltransferases*
1161 *EZH2 and EZH1.* ACS Chem Biol, 2013. **8**(6): p. 1324-34.
1162 50. Potjewyd, F., et al., *Degradation of Polycomb Repressive Complex 2 with an EED-*
1163 *Targeted Bivalent Chemical Degrader.* Cell Chem Biol, 2020. **27**(1): p. 47-56 e15.
1164 51. Krachler, A.M., A.R. Woolery, and K. Orth, *Manipulation of kinase signaling by bacterial*
1165 *pathogens.* J Cell Biol, 2011. **195**(7): p. 1083-92.
1166 52. Wiles, T.J., et al., *Inactivation of host Akt/protein kinase B signaling by bacterial pore-*
1167 *forming toxins.* Mol Biol Cell, 2008. **19**(4): p. 1427-38.
1168 53. Toh, E., et al., *Bacterial protein MakA causes suppression of tumour cell proliferation via*
1169 *inhibition of PIP5K1alpha/Akt signalling.* Cell Death Dis, 2022. **13**(12): p. 1024.
1170 54. Knodler, L.A., B.B. Finlay, and O. Steele-Mortimer, *The Salmonella effector protein SopB*
1171 *protects epithelial cells from apoptosis by sustained activation of Akt.* J Biol Chem, 2005.
1172 **280**(10): p. 9058-64.
1173 55. Liu, X. and J.I. Cohen, *The role of PI3K/Akt in human herpesvirus infection: From the*
1174 *bench to the bedside.* Virology, 2015. **479-480**: p. 568-77.
1175 56. Sposito, B., et al., *Severity of SARS-CoV-2 infection as a function of the interferon*
1176 *landscape across the respiratory tract of COVID-19 patients.* bioRxiv, 2021: p.
1177 2021.03.30.437173.
1178 57. Springstead, J.R., et al., *Evidence for the importance of OxPAPC interaction with*
1179 *cysteines in regulating endothelial cell function.* J Lipid Res, 2012. **53**(7): p. 1304-15.
1180 58. Yuan, M., et al., *A positive/negative ion-switching, targeted mass spectrometry-based*
1181 *metabolomics platform for bodily fluids, cells, and fresh and fixed tissue.* Nat Protoc, 2012.
1182 **7**(5): p. 872-81.
1183 59. Zeng, M., W.A. van der Donk, and J. Chen, *Lanthionine synthetase C-like protein 2*
1184 *(LanCL2) is a novel regulator of Akt.* Mol Biol Cell, 2014. **25**(24): p. 3954-61.
1185 60. Neumann, C., et al., *Role of Blimp-1 in programing Th effector cells into IL-10 producers.*
1186 J Exp Med, 2014. **211**(9): p. 1807-19.
1187 61. Lee, C.G., et al., *Interaction of Ets-1 with HDAC1 represses IL-10 expression in Th1 cells.*
1188 J Immunol, 2012. **188**(5): p. 2244-53.
1189
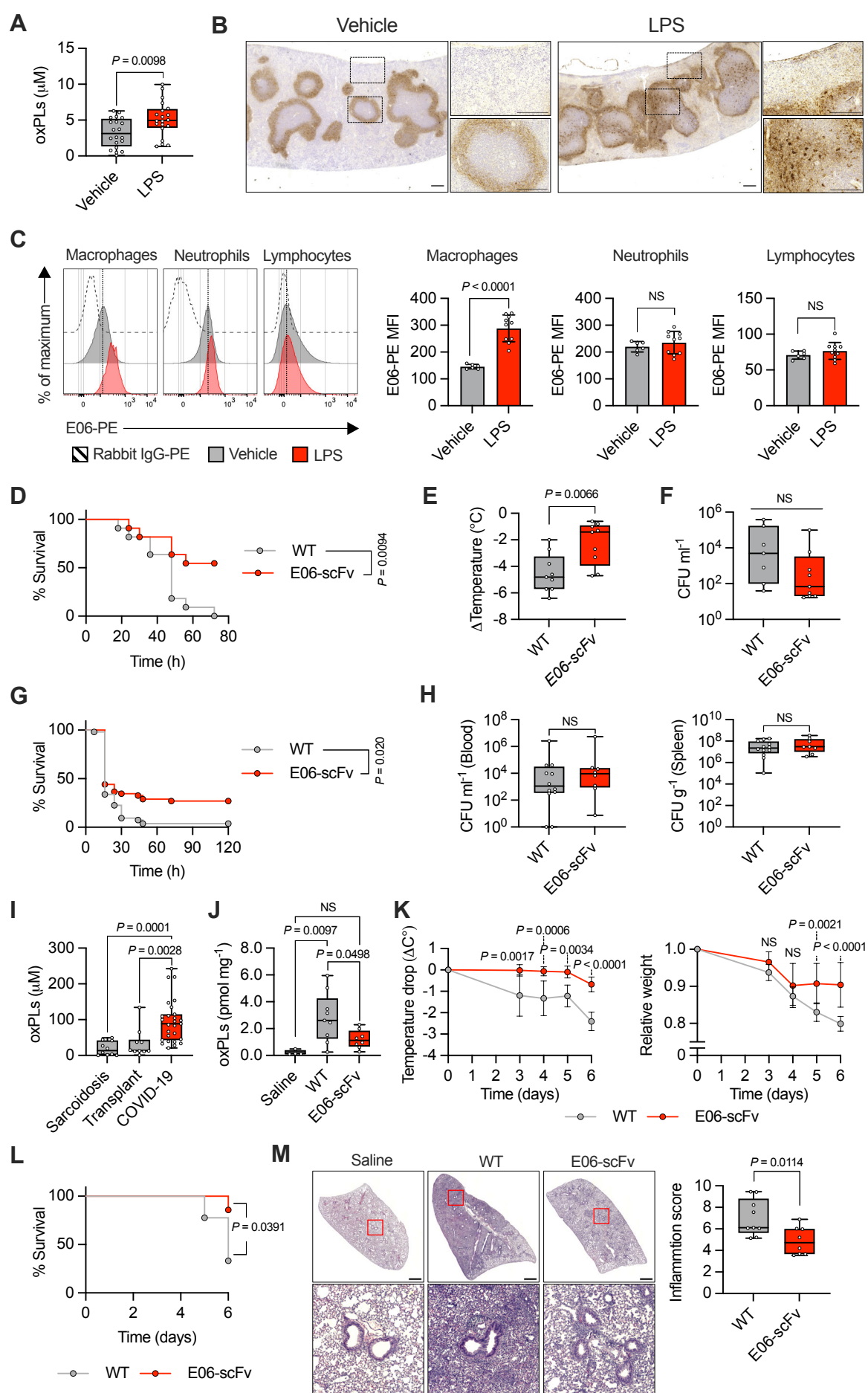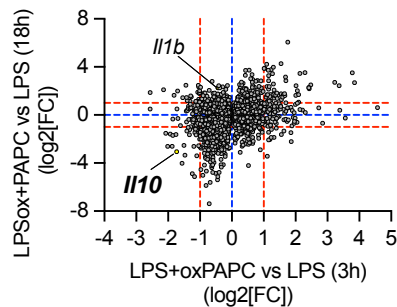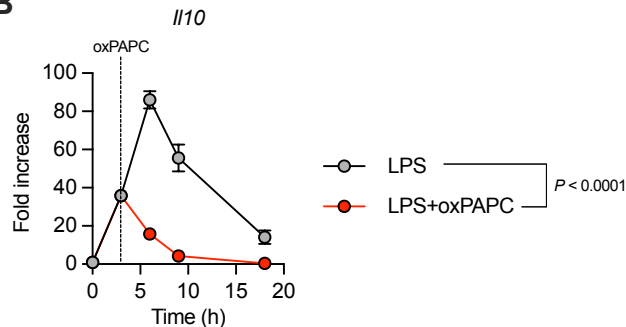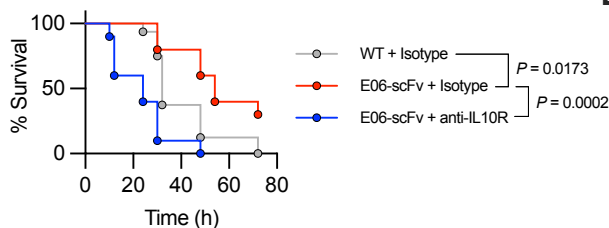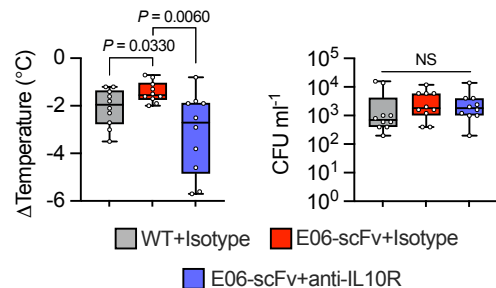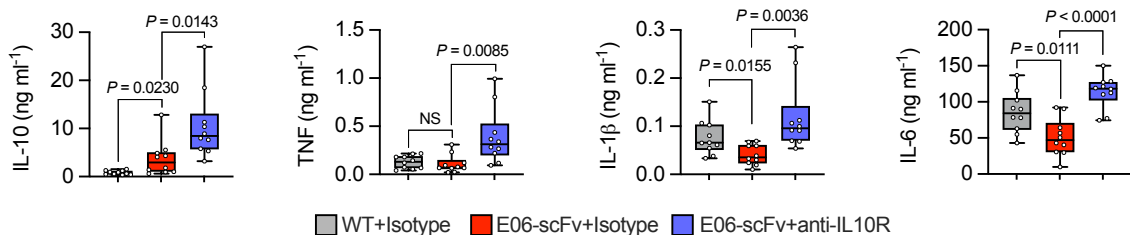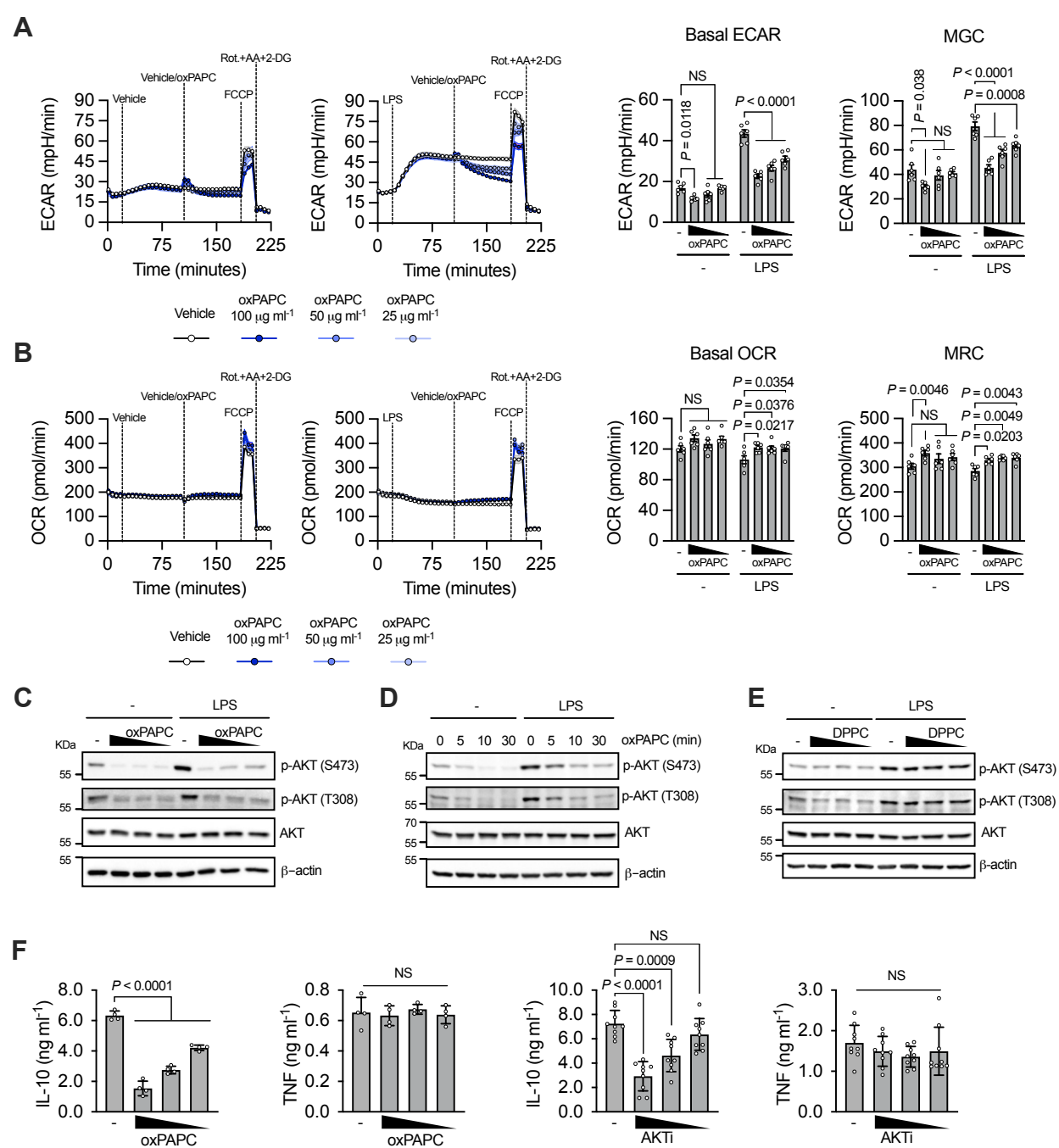
Figure 1
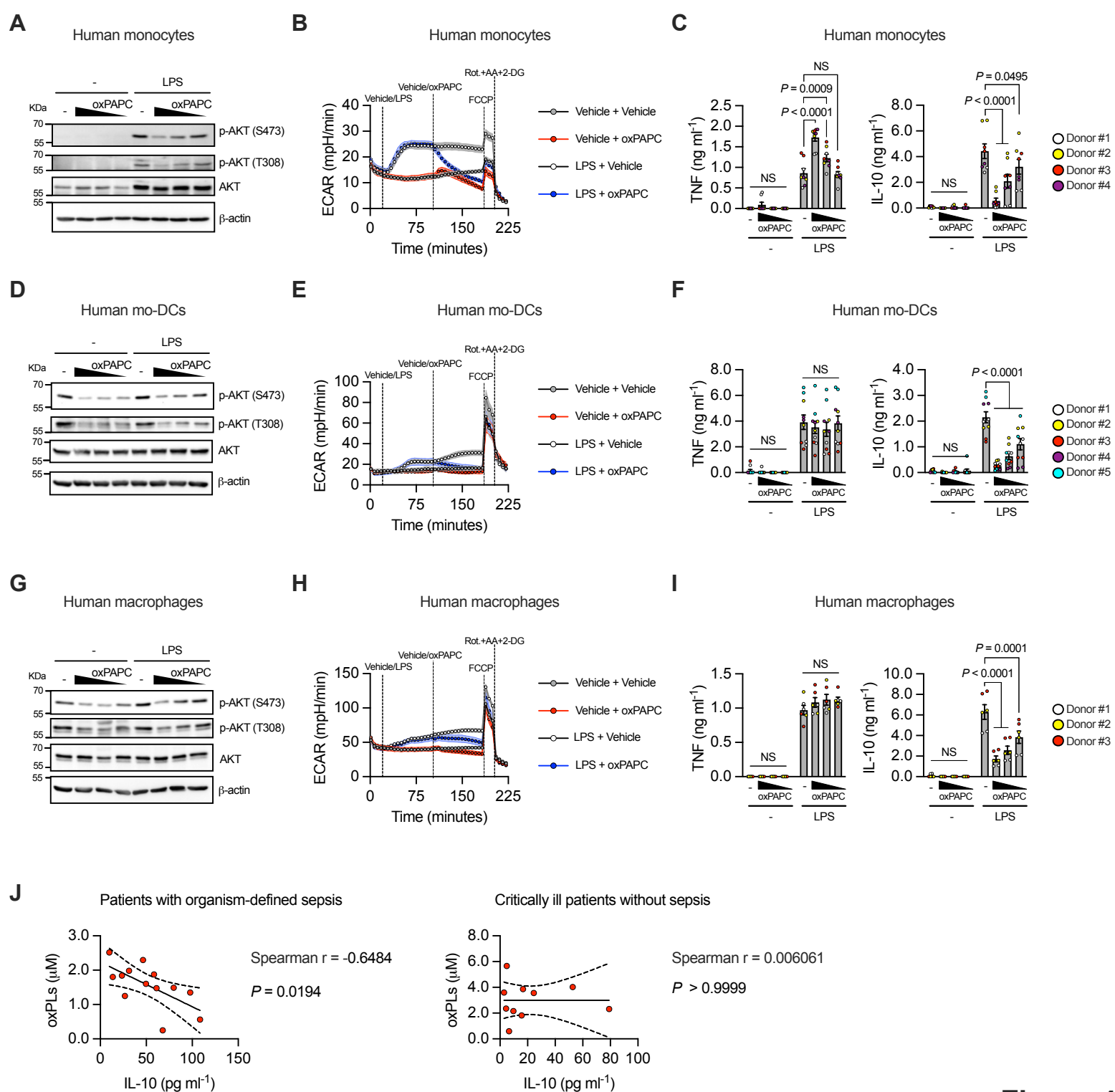
**Figure 2**

Figure 3

Figure 4
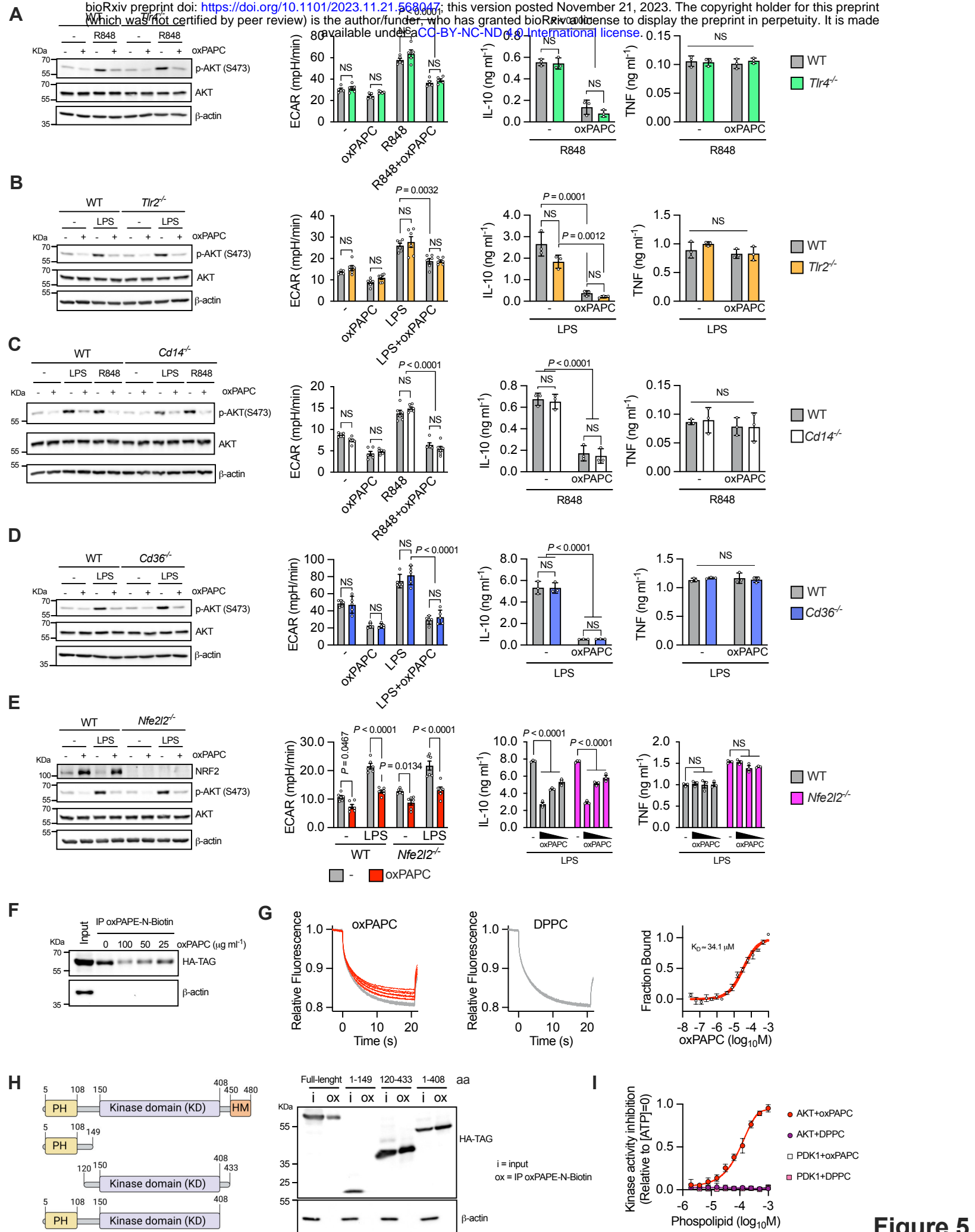
Figure 5
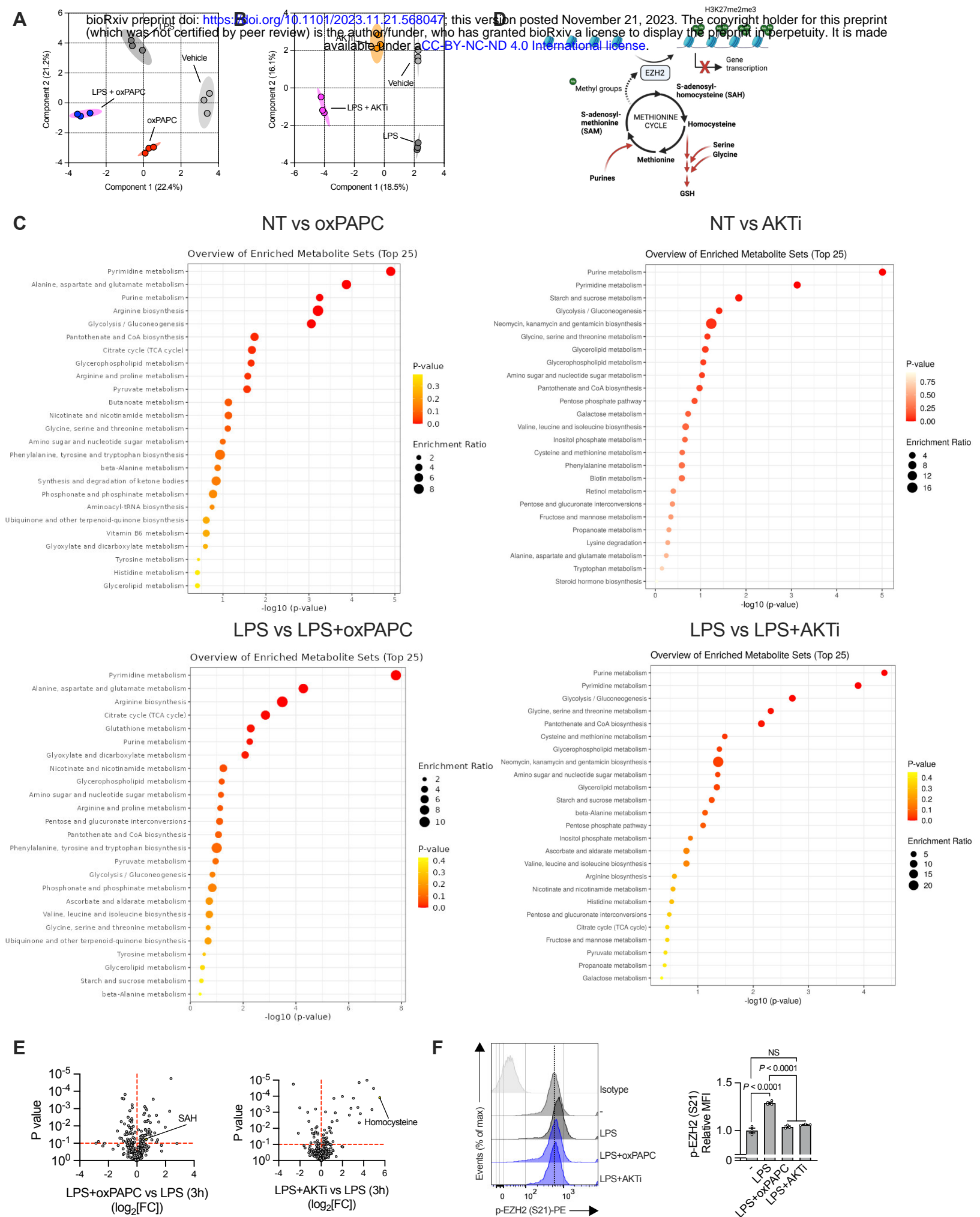
Figure 6
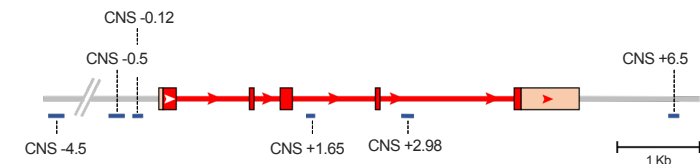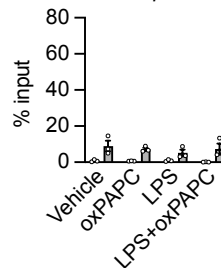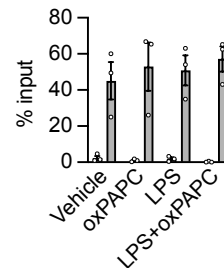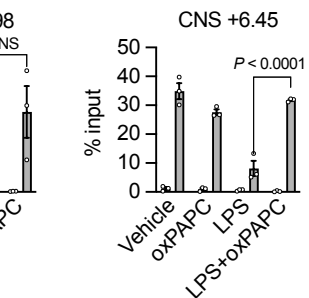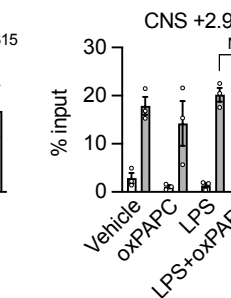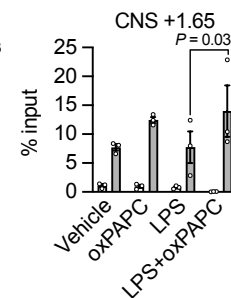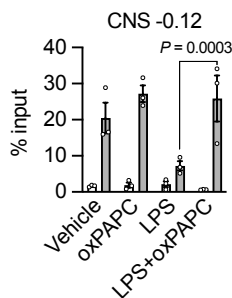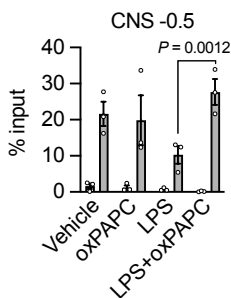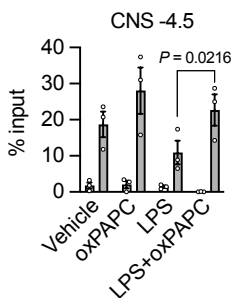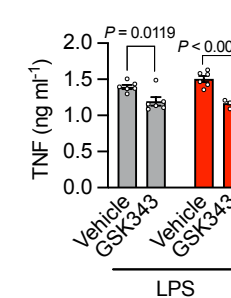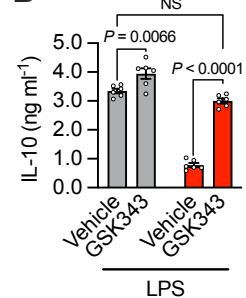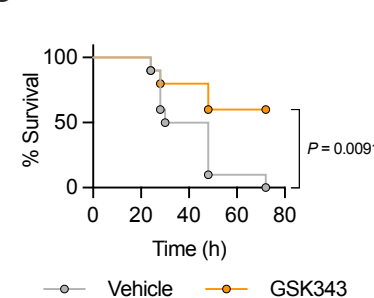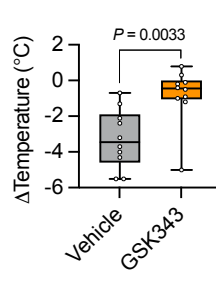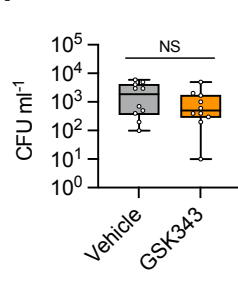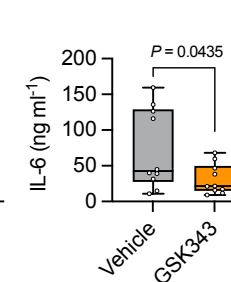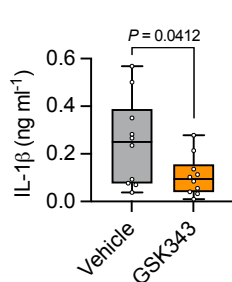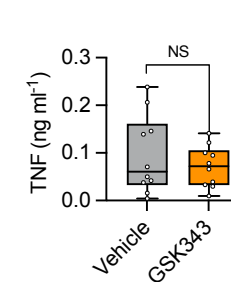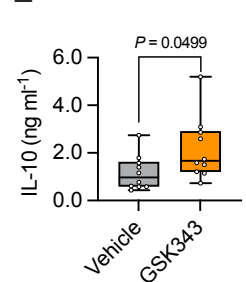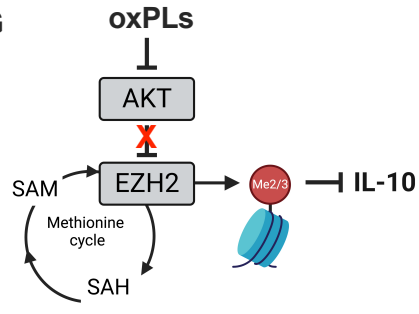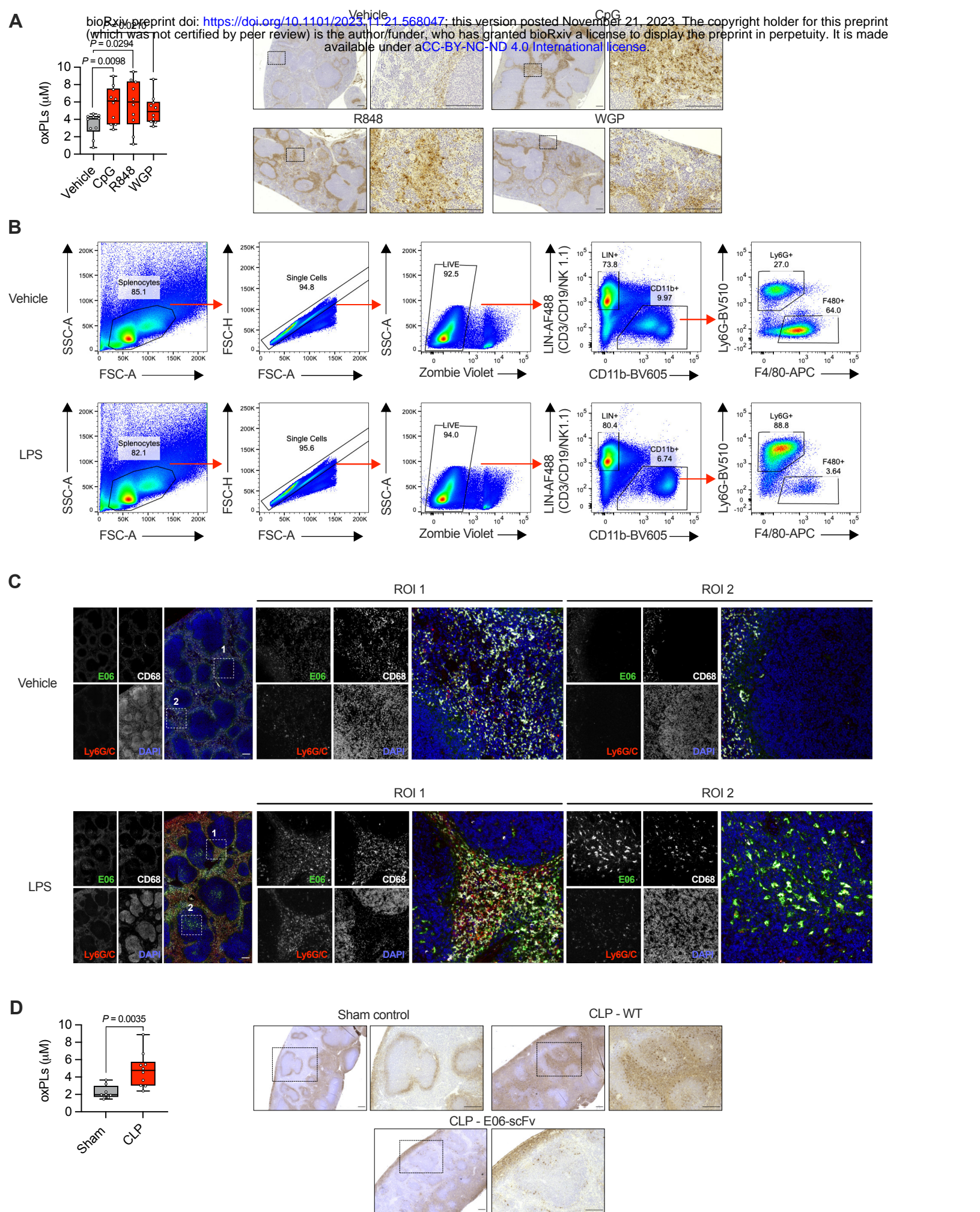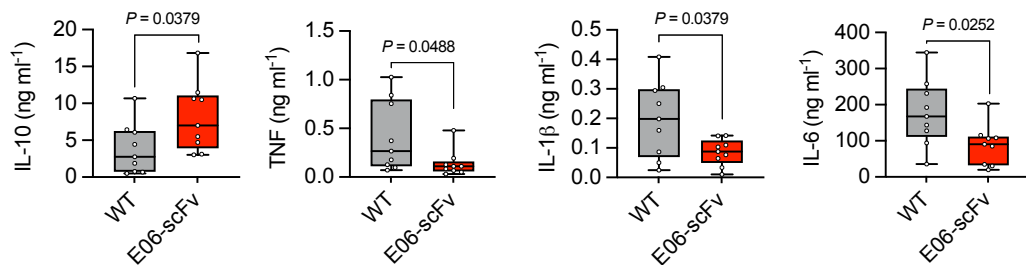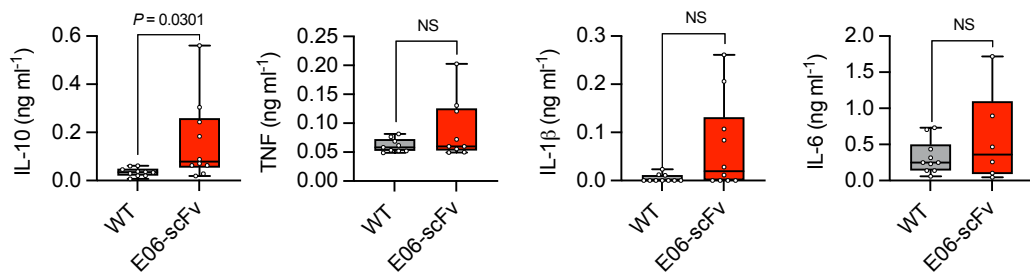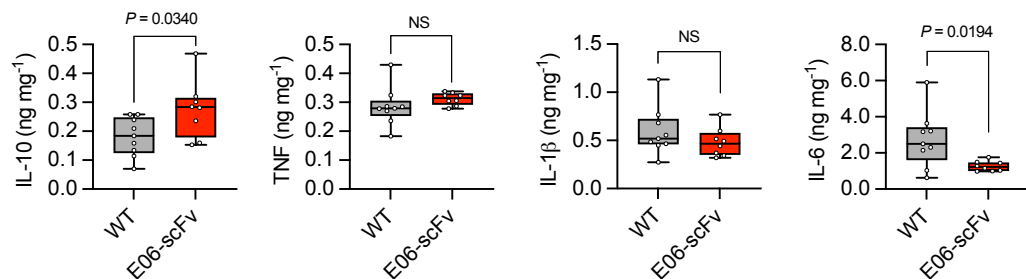
Figure 7
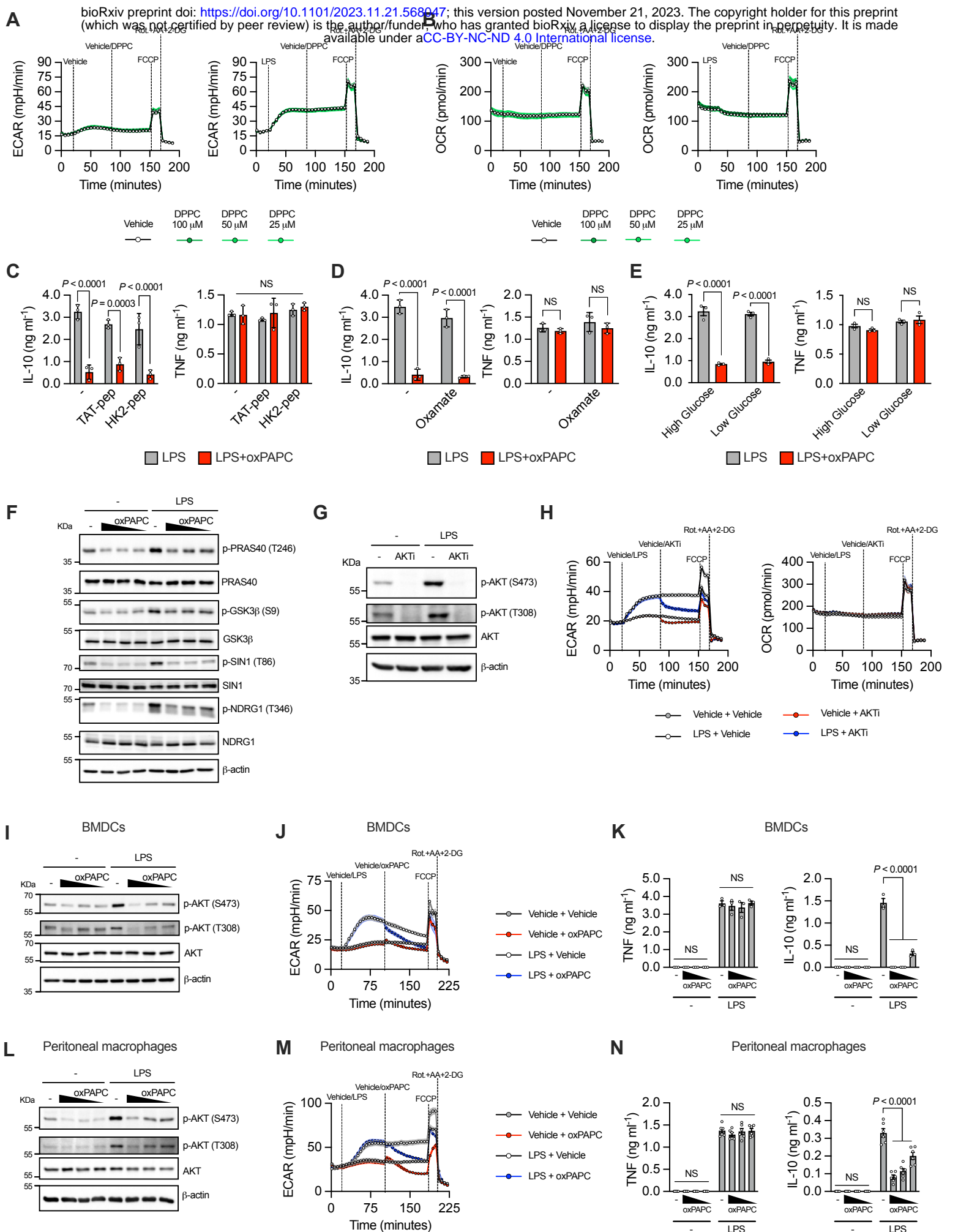
Figure S1
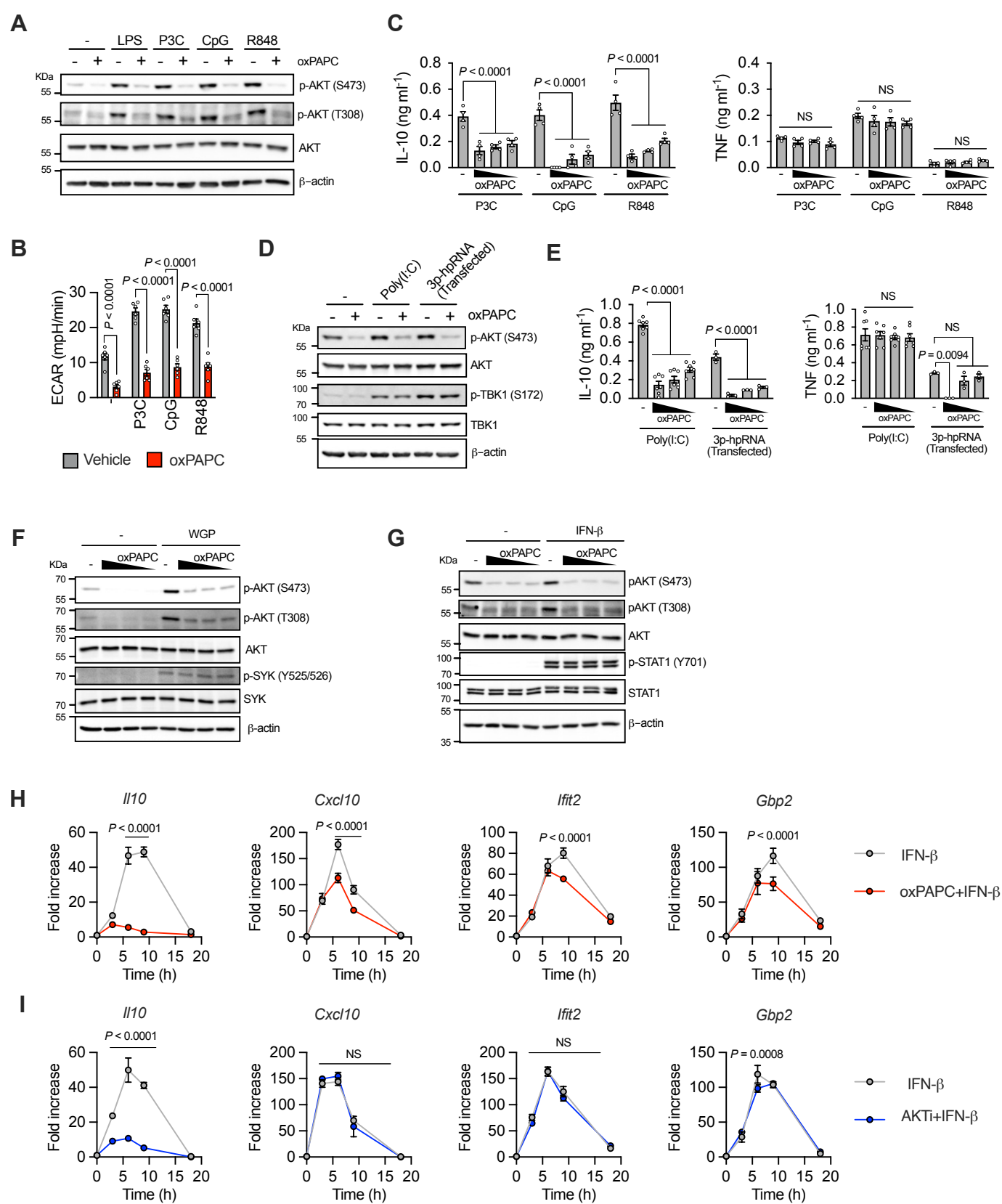
Figure S2

Figure S3

Figure S4

Figure S5

Figure S6

Figure S7