1   **PPAR-γ in macrophages limits pulmonary inflammation and promotes host recovery following**

2   **respiratory viral infection**

3

4   Su Huang[a, b], Bibo Zhu[a, b], In Su Cheon[a, b], Nick P. Goplen[a], Li Jiang[a, b], Ruixuan Zhang[a], R. Stokes

5   Peebles Jr.[c], Matthias Mack[d], Mark H. Kaplan[b], Andrew H. Limper[a] and Jie Sun[a, b]#

6

7   [a]Thoracic Diseases Research Unit, Division of Pulmonary and Critical Care Medicine, Department of

8   Medicine, Department of Immunology, Mayo Clinic College of Medicine and Science

9   [b]HB Wells Pediatric Research Center, Department of Pediatrics, Indiana University School of Medicine

10 [c]Department of Medicine, Vanderbilt University Medical Center

11 [d]Department of Nephrology, University Hospital Regensburg, Germany

12

13 Running title: PPAR-γ regulates influenza diseases and recovery

14

15 #Corresponding author: Jie Sun, Sun.Jie@mayo.edu.

16 S Huang and B Zhu contributed equally to this work

17

18

19

20

21

22

23

24

25

26

ABSTRACT

Alveolar macrophages (AM) play pivotal roles in modulating host defense, pulmonary inflammation and tissue injury following respiratory viral infections. However, the transcriptional regulation of AM function during respiratory viral infections is still largely undefined. Here we have screened the expression of 84 transcription factors in AM in response to influenza A virus (IAV) infection. We found that the transcription factor PPAR-γ was downregulated following IAV infection in AM through type I interferon (IFN)-dependent signaling. PPAR-γ expression in AM was critical for the suppression of exaggerated antiviral and inflammatory responses of AM following IAV and respiratory syncytial virus (RSV) infection. Myeloid PPAR-γ deficiency resulted in enhanced host morbidity and increased pulmonary inflammation following both IAV and RSV infections, suggesting that macrophage PPAR-γ is vital for restricting severe host disease development. Using approaches to selectively deplete recruiting monocytes, we demonstrated that PPAR-γ expression in resident AM was likely important in regulating host disease development. Furthermore, we showed that PPAR-γ was critical for the expression of wound healing genes in AM. As such, myeloid PPAR-γ deficiency resulted in impaired inflammation resolution and defective tissue repair following IAV infection. Our data have suggested a critical role of PPAR-γ expression in lung macrophages in modulating pulmonary inflammation, the development of acute host diseases and the proper restoration of tissue homeostasis following respiratory viral infections.

IMPORTANCE: Respiratory viral infections, like IAV and respiratory syncytial virus (RSV) infections, impose great challenges to the public health. Alveolar macrophages (AM) are lung resident immune cells that play important roles in protecting the host against IAV and RSV infections. However, the underlying molecular mechanisms by which AM modulating host inflammation, disease development and tissue recovery are not very well understood. Here we identify that PPAR-γ expression in AM is crucial to suppress pulmonary inflammation and diseases, and to promote fast host recovery from IAV and RSV infections. Our data suggest that targeting macrophage PPAR-γ may be a promising therapeutic option in the future to suppress acute inflammation and simultaneously promote recovery from severe diseases associated with respiratory viral infections.

INTRODUCTION

Acute respiratory viral infections, such as influenza A virus (IAV) and respiratory syncytial virus (RSV) infections, cause severe morbidity and mortality, and are among leading causes of death in children and the elderly (1, 2). Particularly, IAV virus infection kills ~500,000 people globally and up to 50,000 people in the United States each year (3). In addition to seasonal outbreaks, pandemic IAV viruses occasionally emerge and can cause catastrophic illness and widespread death. Current strategies for IAV prevention and treatment include yearly vaccination and anti-viral drugs. However, frequent changes in the surface antigens of IAV virus due to antigenic shift and drift can allow IAV to escape antibody-mediated immunity following vaccination (4, 5). Anti-viral treatment is generally only effective during a very short time period early after IAV infection. Furthermore, many circulating IAV strains have developed resistance to the current antiviral drugs (6). Thus, there is urgent need to better understand the pathophysiology and the protective immune responses to IAV infection for the development of future preventive and therapeutic means.

The disease pathogenesis associated with IAV infection results from a combination of the deleterious effects of virus replication and the host innate and adaptive immune response associated with control and ultimately clearance of virus (7, 8). The major contribution of the host response to lung injury during IAV infection is exemplified by the immune-mediated lung inflammation and injury associated with infections with the 1918 pandemic IAV or the highly pathogenic H5N1 avian IAV. The inability to control the host responses in these infections results in excessive inflammatory cell infiltration into the lungs and overproduction of pro-inflammatory mediators ((9, 10).

As important components of innate immunity, tissue macrophages and monocyte populations are heterogeneous multifunctional immune sentinel cells important in modulating tissue homeostasis, inflammation, injury and repair (11-15). The main macrophage population in the respiratory tract is alveolar macrophages (AM) that play important roles in lung homeostasis and pulmonary anti-microbial defense (16, 17). Compared to other tissue macrophages, monocytes and monocyte-derived cells, AM have distinct functions and phenotypes that include high autofluorescence, low CD11b expression, and high expression of CD11c and Siglec-F (16, 18). AM precursors develop mainly from fetal monocytes, which seed the lung prior to birth, and massively expand and develop into mature macrophages in response to GM-CSF and TGF-β after birth (18-20). A number of factors including PPAR-γ, mTORC1, phosphoinositide kinase PIKfyve and L-plastin were also recently shown to be important in AM development and function (19, 21-24). Interestingly, AM appear to be essential for the protection against

93   IAV and other respiratory viral infections (25-31) . To this end, AM were identified as a major cellular

94   source of the antiviral cytokines, type I interferons (IFNs) (29). Furthermore, AM can phagocytize virus

95   and virus infected cells, clear cellular debris and exudates, and protect alveolar type I cells (ATI) from

96   infection, thereby suppressing the development of lethal inflammation and injury during IAV infection

97   (25-31). AM, particularly AM undergoing alternative polarization (M2), have also been implicated in the

98   repair of damaged tissues following IAV infection (32). However, the underlying molecular mechanisms

99   regulating the protective function of AM against respiratory viral infections remain to be fully elucidated.

100

101   PPAR-γ is a nuclear transcription factor, usually forming heterodimer with RXR (retinoid X receptor)

102   which recruit different co-activators or co-repressors to form a complex binding to PPAR-responsive

103   regulatory elements in the genome to modulate the expression of genes involved in adipogenesis, lipid

104   metabolism and inflammation (33). PPAR-γ has been shown to be vital for M2 polarization and the

105   restriction of excessive production of inflammatory factors (34, 35), although the roles of PPAR-γ in

106   regulating macrophage inflammatory responses against viral infections have not been explored. AM

107   constitutively express high levels of PPAR-γ (19). Mice with *lox*P-flanked alleles encoding PPAR-γ

108   (*Pparg*fl/fl) and with CD11c-driven expression of Cre recombinase (Cd11c–cre) that is efficiently

109   expressed in fetal monocytes, exhibit severe defects in the AM compartment, suggesting that PPAR-γ is

110   essential for AM development from fetal monocytes (19). Interestingly, prophylactic or therapeutic

111   treatment of mice with natural or synthetic ligands which activate PPAR-γ resulted in diminished host

112   morbidity and mortality during IAV infection (36-40) . However, the cellular and molecular mechanisms

113   by which PPAR-γ agonists promote host protection against IAV infection have not been defined. In

114   addition, the physiological and cell type-specific function of PPAR-γ in response to endogenous ligands

115   during IAV infection are currently unknown.

116

117   In this report, we demonstrated that PPAR-γ was down-regulated in AM via IFNs following IAV

118   infection. PPAR-γ repressed macrophage pro-inflammatory responses and promoted the expression of

119   wound healing gene programs independent of M2 polarization, thereby modulating lung inflammation,

120   host morbidity and tissue repair. We further showed that PPAR-γ expression and function in AM were

121   likely important in dictating host diseases and recovery from respiratory viral infection.

122

123

124

125   **RESULTS**

126 **IAV infection downregulates PPAR-γ expression in macrophages through IFNs**

127 AM are important in regulating antiviral immunity and injury. However, the molecular mechanisms

128 regulating AM responses to viral infection are still not well understood. To explore the transcriptional

129 regulation of AM responding to viral infection, we infected WT AM with IAV PR8 (IAV, 10 MOI) *in*

130 *vitro* and then determined the expression of 84 transcription factors (TFs) following overnight culture

131 using Qiagen RT^2-PCR array. We found that a numbers of TFs involved in antiviral innate immunity were

132 upregulated, while several TFs including *Pparg* were downregulated in AM following IAV infection

133 (Figure 1 A and B). Quantitative PCR results also showed that *Pparg* was downregulated in AM

134 following IAV infection (Figure 1 C). Western blot analysis confirmed decreased PPAR-γ at the protein

135 level in IAV infected AM (Figure 1 D). To determine whether IAV infection downregulates *Pparg* in AM

136 *in vivo*, we sorted AM (CD11c$^+$/Siglec F$^+$) from the lungs of uninfected (day 0) or IAV-infected mice (4,

137 6, 10 or 15 days post infection (d.p.i.)) and examined *Pparg* expression by realtime RT-PCR (Figure 1 E).

138 We found that IAV infection diminished *Pparg* expression in AM, particularly at 6 d.p.i. (Figure 1 E).

139 Western blot analysis confirmed that AM isolated from IAV-infected mice (6 d.p.i.) exhibited decreased

140 PPAR-γ protein levels compared to AM isolated from uninfected mice (Figure 1 F). IAV infection

141 triggers the production of anti-viral cytokines type I IFNs by AM (6). We next examined whether type I

142 IFNs were involved in the regulation of PPAR-γ expression in AM. We found that IFN-α treatment

143 suppressed PPAR-γ expression in AM (Figure 1 G). Next, we infected AM with IAV and then blocked

144 type I IFN signaling with the inclusion of IFNAR1 blocking antibody (α-IFNAR1) in culture. We found

145 that α-IFNAR1 treatment abolished IAV-induced suppression of *Pparg* expression in AM (Figure 1H).

146 Similarly, α-IFNAR1 treatment abolished Poly IC induced suppression of *Pparg* expression in AM

147 (Figure 1H). Together, these data suggest that IAV infection inhibited PPAR-γ expression in AM

148 through IFN signaling. Consistent with the notion, we found that STAT1 could bind to *Pparg* locus

149 following IFN-α treatment, suggesting that STAT1 activation following IFN signaling may directly

150 modulate *Pparg* transcription in AM (Figure 1I).

151

152 **PPAR-γ suppresses antiviral inflammation, but does not regulate M2 genes following infection**

153 PPAR-γ is required for AM development because the deletion of PPAR-γ in CD11c$^+$ cells (*Pparg^{ΔCD11c}*)

154 resulted in impaired AM generation (Figure 2A and (19)). However, compared to CD11c-cre, Lyz2-cre

155 expression in fetal monocytes is incomplete (19). As the result, Lyz2-cre driven PPAR-γ deficiency

156 (*Pparg^{ΔLyz2}*) in AM resulted in relatively normal AM development (Figure 2A and (19). In comparison to

157 the severe defects of AM development and maturation (evidenced by dramatic increase of CD11b (19))

158 observed in *Pparg^{ΔCD11c}* mice, *Pparg^{ΔLyz2}* mice had comparable percentages of AM and only slightly

159   increased CD11b expression compared to those of control mice, suggesting that AM development and

160   maturation were relatively normal in *Pparg^{ΔLyz2}* mice (Figure 2A). Nevertheless, Lyz2-cre is able to

161   mediate gene recombination in adult AM compartments and AM from adult *Pparg^{ΔLyz2}* mice exhibited

162   impaired PPAR-γ expression (Figure 2 B and (30)). We therefore used AM from littermate control

163   (*Pparg^{fl/fl}*, WT) or *Pparg^{ΔLyz2}* mice for our further analysis on the roles of PPAR-γ in regulating AM

164   function during respiratory viral infections. We first isolated AM from uninfected control or *Pparg^{ΔLyz2}*

165   mice, and infected the AM with IAV *in vitro* as in Figure 1. Following infection, WT and *Pparg^{ΔLyz2}* AM

166   showed relatively comparable levels of viability (data not shown). We then examined the expression of

167   type I IFNs, inflammatory cytokines and M2 genes in control or PPAR-γ-deficient AM following IAV

168   infection. We found that PPAR-γ deficiency enhanced the expression of *Ifna4*, *Ifnb1*, *Tnf*, *Il1b* and *Ccl2*

169   expression, but did not affect the expression of *Retnla* (encoding RELM-α protein) and *Arg1* (encoding

170   Arginase 1 protein) (Figure 2C). These data suggest that PPAR-γ suppressed AM antiviral and

171   inflammatory responses, but did not change macrophage polarization following IAV infection. We next

172   infected control or *Pparg^{ΔLyz2}* mice with IAV and then sorted AM from the lungs of infected mice at 1 or

173   3 d.p.i. We found that PPAR-γ deficient AM exhibited enhanced type I IFN and inflammatory gene

174   expression, but showed similar levels of *Retnla* and *Arg1* expression compared to those of control AM at

175   3 d.p.i. (Figure 2D). These data suggest PPAR-γ functioned to inhibit antiviral and inflammatory

176   responses, but did not regulate M2 polarization following IAV infection.

177

178   **Myeloid PPAR-γ suppresses lung inflammation, host morbidity and mortality**

179   To explore PPAR-γ expression in macrophages in regulating host antiviral responses and disease

180   development following IAV infection, we infected control or *Pparg^{ΔLyz2}* mice with IAV and examined

181   host mortality, morbidity, viral replication and inflammatory responses at different days post infection.

182   Compared to control mice, *Pparg^{ΔLyz2}* mice had enhanced host mortality and morbidity, and delayed

183   weight recovery following IAV infection (Figure 3 A, B). We examined the kinetics of IAV replication in

184   the respiratory tract using plaque forming unit (pfu) assay and found that *Pparg^{ΔLyz2}* mice exhibited

185   significant increased virus titers early days following IAV infection (4 d.p.i.) compared to control mice

186   (Figure 3C). However, *Pparg^{ΔLyz2}* mice had comparable viral titers at 7 d.p.i. and most of the mice cleared

187   their infectious virus around 10 d.p.i. (3 out of 11 mice exhibited detectable viruses in control or

188   *Pparg^{ΔLyz2}* bronchoalveolar lavage fluid (BAL)) (Figure 3 C). Thus, *Pparg^{ΔLyz2}* mice showed similar viral

189   clearance kinetics as control mice and suggest that the enhanced morbidity and mortality observed in

190   *Pparg^{ΔLyz2}* mice was not merely due to the failure of viral clearance. Consistent with the viral clearance

191 data, we found that $Pparg^{\Delta Lyz2}$ mice exhibited comparable levels of IAV-specific CD8$^+$ T cell responses

192 (both H2d^b NP$_{366-374}$ tetramer$^+$ and H2d^b PA$_{224-233}$ tetramer$^+$) at 7, 10 and 15 d.p.i. (Figure 3D).

193

194 Next, we measured lung inflammatory cytokine (CCL2 and TNF-α) levels in the BAL at different days

195 following IAV infection to determine whether $Pparg$ expression in myeloid cells regulates pulmonary

196 inflammation. We found that $Pparg^{\Delta Lyz2}$ mice had significant higher CCL2 and TNF-α levels at early days

197 post IAV infection (i.e. 1 or 3 d.p.i.) (Figure 3E). Notably, although the differences did not reach

198 statistical significance, $Pparg^{\Delta Lyz2}$ mice showed trend of increased CCL2 protein levels in the BAL at 7,

199 10 or 15 d.p.i., indicating that $Pparg^{\Delta Lyz2}$ mice may have modest increased pulmonary inflammation at

200 later days post infection. To this end, we used a more sensitive approach to examine inflammatory gene

201 expression in the lungs of control or $Pparg^{\Delta Lyz2}$ mice by Qiagen RT^2-PCR array. We found that lungs of

202 $Pparg^{\Delta Lyz2}$ mice exhibited altered expression of inflammation-related genes including higher expression of

203 a number of pro-inflammatory genes (such as *Il6, Cxcl1* and *Fos*) at day 10 d.p.i. (Figure 3 F). Taken

204 together, these data suggest that myeloid PPAR-γ deficiency leaded to enhanced early viral replication,

205 exuberant inflammatory reaction and increased severity of host sickness.

206

207 Myeloid PPAR-γ inhibits inflammation and morbidity during RSV infection

208 To examine whether PPAR-γ controls AM inflammatory responses to other virus infection, we infected

209 isolated AM from control or $Pparg^{\Delta Lyz2}$ mice with RSV, a virus that affects millions of children. Similar

210 to what we have observed following IAV infection (Figure 2C), we found that PPAR-γ deficiency

211 enhanced *Ifna4, Ifnb1, Tnf, Il1b* and *Ccl2* expression following RSV (line 19, 10 MOI) infection *in vitro*,

212 suggesting that PPAR-γ also controls antiviral and inflammatory responses against RSV infection (Figure

213 4A). We then infected control or $Pparg^{\Delta Lyz2}$ mice with RSV (line 19) and examined host morbidity and

214 lung inflammatory responses. We found that myeloid PPAR-γ deficiency increased weight loss following

215 RSV infection (Figure 4 B). We also found that $Pparg^{\Delta Lyz2}$ mice had enhanced inflammatory innate

216 immune cells (neutrophils and monocytes) present in the lungs at 4 d.p.i. (Figure 4C), suggesting that

217 $Pparg^{\Delta Lyz2}$ mice had higher pulmonary inflammation compared to control mice. Consistently, BAL of

218 $Pparg^{\Delta Lyz2}$ mice had higher TNF-α and IL-1β levels compared to those of control mice at 4 d.p.i. (Figure

219 4D). Thus, myeloid PPAR-γ was required for the suppression of exuberant host inflammation and

220 exaggerated morbidity following RSV infection. These data suggest that macrophage PPAR-γ may

221 restrict host disease development in a broad spectrum of respiratory viral infections.

222 PPAR-γ expression in resident AM is likely required for controlling host disease development

223 Lysozymes are widely expressed in myeloid cells including neutrophils, monocytes and macrophages. We

224 crossed Lyz2-cre mouse with a cre reporter strain R26R-eYFP mouse to examine Cre deletion in the

225 myeloid compartment. In agreement with previous report (35), we observed Lyz2-cre activity in majority

226 of alveolar macrophages and neutrophils, partially in CD11b$^+$ monocytes/macrophages (Figure 5A).

227 Western-blot analysis on sorted myeloid cell populations isolated from the lungs revealed that AM

228 expressed high levels of PPAR-γ and lung CD11b$^+$ monocyte/macrophage population expressed

229 comparatively lower levels of PPAR-γ (Figure 5B), while neutrophils did not express detectable PPAR-γ,

230 which is consistent with previous reports (35) (Figure 5B). To explore the potential roles of PPAR-γ in

231 regulating inflammation of AM, monocytes/monocyte-derived macrophages and/or epithelial cells, we

232 sorted AM, CD11b$^+$ monocytes/macrophages and CD45$^-$ (mainly epithelial cells) from IAV-infected

233 lungs of control or $Pparg^{\Delta Lyz2}$ mice at 1 and 3 d.p.i. and examined inflammatory cytokine expression. We

234 found that elevated Tnf and $Ccl2$ expression was mainly observed in AM, but not in CD11b$^+$

235 monocytes/macrophages nor in CD45$^-$ cells (Figure 5C). We next explored the relative contributions of

236 PPAR-γ in AM and monocytes/monocyte-derived macrophages in controlling host disease development

237 during IAV infection. To this end, we crossed $Pparg^{\Delta Lyz2}$ mice to $Ccr2^{-/-}$ mice to block monocyte traffic to

238 the infected lungs (40-43). We found that, compared to $Ccr2^{-/-}/Pparg^{fl/fl}$ mice, $Ccr2^{-/-}/Pparg^{\Delta Lyz2}$ mice lost

239 more weight and exhibited delayed recovery (Figure 5D), suggesting that enhanced disease development

240 in $Pparg^{\Delta Lyz2}$ mice is independent of monocytes or monocyte-derived cells. We next assessed whether

241 treatment of anti-CCR2 (MC21 mAb), which selectively depletes recruiting monocytes (44) could affect

242 host morbidity in $Pparg^{\Delta Lyz2}$ mice. As reported (43), MC21 treatment greatly decreased monocyte

243 infiltration to the lung (Figure 5E). However, MC21 treatment did not significantly alter host weight loss

244 in neither control nor $Pparg^{\Delta Lyz2}$ mice (Figure 5F), again suggesting that monocytes are dispensable for

245 phenotypes observed in $Pparg^{\Delta Lyz2}$ mice following IAV infection. Taken together, these data suggest that

246 PPAR-γ expression in AM, rather than in monocytes or monocyte-derived cells, is probably responsible

247 for the restriction of exaggerated pulmonary inflammation and the suppression of the development of

248 severe diseases following respiratory viral infection.

249

250 **Macrophage PPAR-γ promotes tissue repair**

251 Following the clearance of IAV, the inflammatory responses in the lung resolve and the damaged tissue

252 undergoing repair process to restore normal tissue homeostasis. AM are thought to be involved in the

253 tissue repair process following lung injury (32). We therefore examined whether PPAR-γ affects AM

254 tissue repair function. To this end, we isolated control or PPAR-γ-deficient AM from WT or $Pparg^{\Delta Lyz2}$

255 mice and performed Qiagen RT^2-PCR array to determine wound healing gene expression. We found that

256   PPAR-γ deficiency resulted in impaired expression of a large numbers of wound healing-related genes

257   including epithelial and endothelial growth factors such as *Vegf*, *Egf* and *Fgf7* (Figure 6 A, B). A numbers

258   of factors involved in tissue remodeling including *Mmp7*, *Mmp9* and *Timp1* were also decreased in

259   PPAR-γ-deficient AM (Figure 6 A, B). These data suggested that PPAR-γ expression is important in

260   regulating wound healing and tissue repair function of AM.

261

262   Therefore, we examined whether *Pparg^{ΔLyz2}* mice had impaired tissue recovery *in vivo* following viral

263   clearance. To this end, we examined lung histopathology with Hematoxylin and Eosin (H&E) staining of

264   lung sections at 15 d.p.i., when infectious virus has been cleared from IAV infection (Figure 3C). We

265   found that *Pparg^{ΔLyz2}* mice still had significant higher proportions of the inflamed and/or damaged areas

266   that were not properly repaired at day 15 p.i., when mice already recovered most of their lost weight

267   (Figure 3B and 6 C). To further explore the roles of myeloid PPAR-γ in regulating lung inflammation

268   resolution and tissue repair, we first examined airway inflammatory cell content (monocytes and

269   neutrophils, reflection of lung inflammatory resolution). We found that *Pparg^{ΔLyz2}* mice exhibited higher

270   neutrophil numbers at 15 d.p.i., suggesting that *Pparg^{ΔLyz2}* mice had impaired pulmonary inflammation

271   resolution (Figure 6 D). We also measured total protein concentrations in the BAL (reflection of

272   endothelial/epithelial leakage) at different days following IAV infection and observed that *Pparg^{ΔLyz2}*

273   mice had drastically higher protein levels in the BAL compared to those of control mice at 15 d.p.i

274   (Figure 6 E). These data indicate that *Pparg^{ΔLyz2}* mice had impaired inflammation resolution and

275   decreased damage repair. In further support of this view, we examined alveolar type II (AT II) epithelial

276   gene expression in the lungs of control and *Pparg^{ΔLyz2}* mice as a surrogate of tissue recovery at 8, 10 or 15

277   d.p.i. We found that ATII specific genes, *Sftpb* and *Abca3,* were comparable between control and

278   *Pparg^{ΔLyz2}* lungs at 8 d.p.i. However, lungs of *Pparg^{ΔLyz2}* mice exhibited lower *Sftpb* and *Abca3*

279   expression compared to those of control mice at 10 or 15 d.p.i. (Figure 6 F), indicating that lungs of

280   infected *Pparg^{ΔLyz2}* mice had diminished ATII cell regeneration and lung recovery during viral clearance.

281   Taken together, these data suggest that PPAR-γ promoted AM tissue repair function and myeloid-

282   deficiency of PPAR-γ resulted in diminished inflammation resolution and impaired tissue recovery

283   following IAV infection.

284

285

286

287   **DISCUSSION**

288   The transcriptional regulation of lung macrophage responses against respiratory viral infections is largely

289   undefined. Here we identify that PPAR-γ expression in AM is vital for their proper responses during both

290   IAV and RSV infection. PPAR-γ is an anti-inflammatory transcription factor able to antagonize NF-κb-

291   mediated cytokine production constitutively and in response to TLR ligand stimulation (45). Consistent

292   with the notion, we showed that PPAR-γ-deficient AM produced increased levels of both antiviral and

293   pro-inflammatory cytokines in response to IAV and RSV infection. Notably, AM constitutively express

294   high levels of PPAR-γ, which may help to maintain a tolerogenic environment in the lung during

295   homeostasis. However, AM can also rapidly produce inflammatory cytokines following microbial

296   challenge (17, 46). The down-regulation of PPAR-γ in AM may help the AM to rapidly respond to certain

297   microbial challenges and provide beneficial functions under certain conditions. Nevertheless, the

298   complete loss of PPAR-γ in macrophages caused exaggerated release of inflammatory mediators and

299   enhanced disease development *in vivo* following IAV and RSV infections. These data suggest that PPAR-

300   γ counter-regulates the pathogenic inflammatory responses *in vivo*, and acts to ensure the proper function

301   of lung macrophages during respiratory viral infections.

302

303   The differential functions of AM and recruited monocyte/macrophage populations during homeostasis

304   and disease conditions have only begun to be appreciated. During respiratory viral infections, circulating

305   monocytes infiltrate the lungs in a CCR2-dependent manner and can give rise to exudate or inflammatory

306   macrophages at the site of infection (42). These CCR2-dependent inflammatory monocytes and

307   monocyte-derived cells have been associated with the development of immunopathology, although these

308   cells also contribute to the normal antiviral responses as the blockage of their migration to the lung due to

309   CCR2 inhibition or deficiency impaired and/or delayed host viral clearance during RSV and IAV

310   infections (31, 40-42, 44). Notably, CCR2 deficiency or CCR2 blockade did not significantly change

311   overall host morbidity in PPAR-γ sufficient or deficient background in our experimental system than what

312   was reported before(31, 41), in which CCR2 deficiency significantly diminished host morbidity and

313   mortality. Variations in infection schemes, virus stocks and/or microbiota (46) may contribute to the

314   different results observed. However, our results are supported by the findings of Aldridge et al (40), in

315   which *Ccr2$^{-/-}$* deficient mice exhibited similar morbidity and mortality as WT mice following IAV

316   infection.

317

318   In contrast, lung resident AM are often beneficial to the host during respiratory viral infections as AM

319   depletion impairs host antiviral responses with concomitant development of severe lung injury during

320   respiratory viral infections (25-31). However, AM do release inflammatory mediators following viral

321   infections and thus may contribute to the development respiratory inflammation and/or injury if their

322   responses are not tightly regulated. Multiple lines of evidence present in this study suggest that PPAR-γ

323   expression in AM rather than in monocytes and/or monocyte-derived cells is important in controlling host

324   inflammation and subsequent disease development. First, AM expressed high levels of PPAR-γ compared

325   to monocytes and monocyte-derived cells. Furthermore, sorted AM rather than monocytes or monocyte-

326   derived cells exhibited increased inflammatory responses. Finally, disruption of monocyte recruitment

327   into the lungs by using anti-CCR2 or genetic CCR2 deletion did not majorly impact the outcome of IAV

328   infection in WT and myeloid PPAR-γ deficient mice, suggesting that PPAR-γ expression in monocytes

329   may be dispensable for the regulation of the development of severe diseases following respiratory viral

330   infection. Interestingly, PPAR-γ expression also regulated the wound healing function of AM and tissue

331   recovery through the promotion of various growth factors and tissue remodeling factors. Notably, PPAR-γ

332   deficiency did not result in decreased M2 gene expression in AM with or without IAV or RSV infection,

333   suggesting that PPAR-γ may regulate AM repair function independent of M2 polarization. Thus, PPAR-γ

334   is vital for the proper function of AM during respiratory viral infection by restricting their inflammatory

335   features and simultaneously promoting their repair roles.

336

337   Type I IFNs are widely recognized as host-beneficial, anti-viral cytokines. They lead to the transcription

338   of IFN-stimulated genes that aim to eliminate the virus and prevent its spread by promoting anti-viral

339   state in nearby cells (47). However, type I IFNs are also the key initiators of pulmonary inflammatory

340   responses during respiratory viral infections and thus their actions must also be finely balanced to

341   maximize viral clearance while inflicting minimal damage to the tissue (48). Indeed, the exaggerated

342   production of type I IFNs have been implicated in the development of exuberant pulmonary inflammation,

343   severe host morbidity and mortality following respiratory viral infections (44, 49, 50). In this report, the

344   enhanced type I IFN production was observed in PPAR-γ deficient AM, but the absence of PPAR-γ in

345   AM resulted in significantly increased viral titers at four days following IAV infection, suggesting that

346   the enhanced production of type I IFNs by PPAR-γ-deficient AM was not sufficient to diminish viral

347   replication in the lungs. The exact reasons underlying the phenomenon warrant further investigation.

348   Nevertheless, given the potential inflammatory function of type I IFNs, it is possible that the altered

349   production of type I IFNs along with the dysregulated inflammatory cytokine production in PPAR-γ

350   deficient AM contribute to the severe outcome of IAV infection in the myeloid PPAR-γ deficient mice.

351

352   In summary, our findings have uncovered critical roles of PPAR-γ in regulating inflammatory responses

353   of AM, the development of acute host disease and the proper restoration of tissue homeostasis following

354   respiratory viral infections. Further studies are warranted to examine the therapeutic potential of

355   modalities that can specifically modulate the expression of PPAR-γ in AM for the treatment of severe

356   respiratory viral infections and their associated pathologies.

357

358

359

360

361

362

363

364

365

366

367

368

369

370

371

372

373

374

375

376

377

378

379

380

381

382

383

384

385

386

387

388

389

390

391   **MATERIALS AND METHODS**

392

393 **Mouse and infection.** WT C57/BL6 mice were purchased from the Jackson Laboratory. Lyz2-cre,

394 CD11c-cre, *Pparg^{fl/fl}*, R26R-eYFP, *Ccr2^{-/-}* were purchased from the Jackson Laboratory and bred in house.

395 *Pparg^{ΔLyz2}* mice were generated by crossing *Pparg^{fl/fl}* mice with Lyz2-cre mice. Pparg^{ΔCD11c} were

396 generated by crossing *Pparg^{fl/fl}* mice with CD11c-cre mice. *Ccr2^{-/-} Pparg^{fl/fl}* and *Ccr2^{-/-} Pparg^{ΔLyz2}* mice

397 were generated by crossing *Pparg^{fl/fl}* or Pparg^{ΔLyz2} mice with *Ccr2^{-/-}* mice. Lyz2-cre R26R-eYFP reporter

398 mice were generated by crossing R26R-eYFP mice with Lyz2-cre mice. All mice housed in a specific

399 pathogen-free environment. For IAV infection, influenza A/PR8/34 strain (~200 pfu/mouse) was diluted

400 in FBS-free DMEM media (Corning) on ice and inoculated in anesthetized mice through intranasal route

401 as described before (51). Host mortality was determined based on humane endpoint (more than 30%

402 weight loss or moribund) or deaths before humanely sacrifice. For RSV infection, RSV (strain line 19,

403 ~$5×10^6$ pfu/mouse) was diluted in FBS-free DMEM media (Corning) on ice and inoculated in

404 anesthetized mice through intranasal route as described (52).

405

406 **AM culture and infection *in vitro*.** AM were obtained from BAL. Briefly, alveolar lavages were pooled

407 from BAL washes from 3-5 mice (PBS with 2 mM EDTA) and stored on ice. Red blood cell lysis was

408 then performed in ACK lysis buffer (0.15 M NH_4Cl, 1 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.2) at room

409 temperature for 2 min. Freshly isolated cells were rested in complete medium (RPMI-1640, 10% FBS, 1%

410 Pen/Strep) for 4 h at 37℃ and 5% CO_2. The non-adherent cells were discarded, and the plates were rinsed

411 with warm PBS. For AM infection *in vitro*, seeded cells were infected with or without 10 MOI of IAV

412 PR8 virus or RSV line 19 as indicated in the text for 1 hour and then cultured for overnight. For AM IFN

413 treatment *in vitro*, 10^5 AM were plated in 12-well plate and treated with 50 ng/ml IFN-α (BioLegend) or

414 vehicle overnight in the presence of recombinant GM-CSF to keep AM alive (Biolegend, 10ng/ml). Cell

415 lysates were analyzed by quantitative RT-PCR or western blot.

416

417 **Quantitative RT-PCR.** mRNA from cultured AM (pooled from multiple mice), *in vivo* sorted AM

418 (pooled from multiple mice) or homogenates from individual lungs as indicated in the text was isolated

419 with Total RNA purification kit (Sigma) and treated with DNase I (Invitrogen). Random primers

420 (Invitrogen) and MMLV reverse transcriptase (Invitrogen) were used to synthesize first-strand cDNAs

421 from equivalent amounts of RNA from each sample. RT-PCR was performed with Fast SYBR Green

422 PCR Master Mix (Applied Biosystems). qPCR was conducted in duplicates in QuantStudio3 (Applied

423 Bioscience). Data were generated with the comparative threshold cycle (Delta CT) method by

424 normalizing to hypoxanthine phosphoribosyltransferase (HPRT). Sequences of primers used in the studies

425 are provided as follows. *Abca3:* TTCTGGTTCTCCGCTCTGTT, GTACATGAGGGGGATGATGG.

426   *Arg1:* CAATGAAGAGCTGGCTGGTG, TGAGCATCCACCCAAATGAC.

427   *Ccl2:* GTCACCAAGCTCAAGAGAGAGGTC, CCTACAGAAGTGCTTGAGGTGGTT.

428   *Hprt:* CTCCGCCGGCTTCCTCCTCA, ACCTGGTTCATCATCGCTAATC.

429   *Ifna4:* TCCATCAGCAGCTCAATGAC, AGGAAGAGAGGGCTCTCCAG.

430   *Ifnb1:* TCCACCAGCAGACAGTGTT, CTTTGCACCCTCCAGTAATAGC.

431   *Il1b:* GGGCCTCAAAGGAAAGAATC, TACCAGTTGGGGAACTCTGC.

432   *Pparg:* TGCCAGTTTCGATCCGTAGA, ATGAATCCTTGGCCCTCTGA.

433   *Retnla:* TGCCCTGCTGGGATGACTGCT, GGACAGTTGGCAGCAGCGGG.

434   *Sftpb:* CTGTGCCAAGAGTGTGAGGA, TTGGGGTTAATCTGGCTCTG.

435   *Tnf:* CATGCGTCCAGCTGACTAAA, TCCCCTTCATCTTCCTCCTT.

436

437   **RT^2 Profiler PCR Array.** Total RNA from lung tissue or AM was extracted as described above. Equal

438   amount of total RNA was used for the synthesis of first strand cDNA with kit from Qiagen. First strand

439   cDNA was mixed with 2xFast SYBR Green Master Mix (Applied Bioscience) and water in a formula

440   directed in the manual. 25 μl of the mixture was added into each well of the 96 well plate provided by

441   manufacture. The wells in the plate include different primers in each well to detect 84 target genes,

442   housekeeping genes, negative and positive control genes. qPCR was conducted in QuantStudio3 (Applied

443   Bioscience). Obtained raw data was analyzed in software provided by Qiagen (accessible online on the

444   website of Qiagen). Following the instruction step by step, upload Excel file, designating control group,

445   select housekeeping gene to normalize result and calculate the relative expression quantity.

446

447   **Cell depletion.** For monocyte depletion, mice were treated intraperitoneally (i.p.) with anti-CCR2

448   antibody (clone: MC21, 25 μg/mouse in 200 μl of PBS)(53) or control IgG daily from day 0 to day 6.

449

450   **Lung histopathology.** Following euthanasia, mice were perfused with PBS (10 mL) via the right

451   ventricle. 10% paraformaldehyde (PF) was then gently instilled into the lung and left inflated for 1 minute

452   before excising and moving lobe to 10% PF for 48 hours followed by transfer to ethanol (70%). Samples

453   were shipped to Mayo Clinic Histology Core Lab (Scottsdale, AZ) where they were embedded in paraffin

454   and 5 um sections were cut for Hematoxylin and eosin stain. To quantify percent of inflamed or disrupted

455   alveolar area, H&E slides were scanned through the Aperio whole slide scanning system and exported to

456   image files. Computer-based image analysis was performed using the Image J software (NIG, Bethesda,

457   MD, USA). We first determined the total lung area by converting the image into gray scale followed with

458   red highlighting through the adjustment of the Threshold. For determination of the inflamed and disrupted

459   area, color images were split into single channels. We then used the green channel, highlighted the

460　inflamed areas in red by adjusting the Threshold and measured the areas based on pixel. The percentages

461　of disrupted and inflamed lung areas were calculated based on the ratio of highlighted disrupted areas to

462　the total lung area in each lung section.

463

464　**Western Blot analysis.** Same numbers of cultured or FACS-sorted AM were lysed in lysis buffer

465　(62.5mM Tris-HCL (pH 6.8), 2% SDS and 10% glycerol) with a protease inhibitor cocktail (Roche). The

466　lysates were then separated by SDS-PAGE and transferred to Immuno-Blot Nitrocellulose Membrane

467　(Bio-Rad,). The membranes were blocked with 5% non-fat milk in 20 mM Tris (pH 7.5), 0.5 M NaCl and

468　0.05% Tween 20 (TBST) for 1h at room temperature (RT), followed by incubation with primary Ab

469　against PPAR-γ (1:1000, Cell Signaling Technology) or β-actin (1:5000, Santa Cruz Biotechnology)

470　overnight at 4°C. After washing with TBST buffer, membranes were incubated with goat anti-rabbit or

471　anti-mouse secondary Ab (Promega). Peroxidase activity was detected with enhanced

472　chemiluminesecence (ECL).

473

474　**Chromatin Immunoprecipitation (ChIP).** AM were obtained from the lung of naïve WT C57BL6 mice,

475　using anti-CD169 magnetic beads, as recommended by the manufacturer (Miltenyi Biotec). AM were

476　cultured in complete medium supplemented with 10 ng/ml GM-CSF in the presence of 50 ng/ml IFN-α

477　(BioLegend) or vehicle overnight. Then the cells were subjected to ChIP assay as previously described

478　(54). In brief, $8x10^6$ AM were crosslinked for 10 min at 37℃ by the addition of 1% freshly made

479　formaldehyde. Fixed cells were pelleted at 4℃ and washed with ice-cold PBS. The cells were lysed with

480　SDS lysis buffer (1% SDS, 10mM EDTA, 50mM Tris, pH 8.1) containing protease inhibitors (Roche) on

481　ice for 10 min and sonicated to an average size of 200-500bp. After sonication, samples were centrifuged

482　at 13,000 rpm for 10 min at 4℃ and 5% of sonicated cell extracts were saved as input. The resulting

483　whole-cell extract was incubated with Protein A/G Agarose (Santa Cruz) for 1h at 4℃. Precleared extracts

484　were then incubated with 60 ul of Protein A/G Agarose (Santa Cruz) for ChIP with 5μg of the appropriate

485　antibody overnight at 4℃. STAT1 ChIP antibody (clone D1K9Y) was from Cell Signaling. After

486　overnight incubation, beads were washed once with low salt immune complex wash buffer (0.1% SDS, 1%

487　Triton X-100, 2 mM EDTA, 20 mM Tris-HCL pH 8.1, 150 mM NaCl), once with high salt immune

488　complex wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCL pH 8.1, 500 mM

489　NaCl), once with LiCl wash buffer (10 mM Tris-HCl pH 8.1, 1 mM EDTA, 250 mM LiCl, 1% NP-40),

490　and twice with TE wash buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). DNA was eluted in freshly

491　prepared elution buffer (1% SDS, 0.1M NaHCO3). Cross-links were reversed by overnight incubation

492　with 5 M NaCl at 65℃. RNA and protein were digested using RNase A and proteinase K (Roche),

493   respectively and DNA was purified by Qiagen MinElute PCR Purification kit according to the

494   manufacturer's instructions. The immunoprecipitated DNA was analyzed by quantitative real-time PCR

495   and normalized relative to input DNA amount. Primers were designed to a segment that was centered on

496   the PPAR-γ coverage regions. Primers used in this study are listed in as follows. Realtime PCR data is

497   represented as fold levels over control. Primers sequence are as following. *Pparg* -4.3k:

498   TGGAATGAAAGAATCCTCCAA, GTTGGTGCCACATGGATTTT. *Pparg* -16.8k:

499   GCAGATTTGTGCCAAGAACA, TGCAGCCGCTGAATAAATAC.

500

501   **ELISA analysis of BAL cytokines.** 50 µl of each BAL sample was analyzed with the ELISA using

502   commercially available kits for mouse IL-1β, CCL2 and TNF-α (Biolegend) following the manufacturer's

503   protocol. The VERSAmax microplate reader (Molecular Devices) was used for colorimetric

504   quantification and analysis at 450nM wavelength.

505

506   **BCA protein assay.** BCA protein assay kit was obtained from Thermo Scientific. 2µl of each BALF

507   sample was used. VERSAmax microplate reader (Molecular Devices) was used for colorimetric

508   quantification and analysis at 570nm wavelength.

509

510   **Plaque Assay.** IAV plaque assays were performed as described before (55). Briefly, MDCK cells were

511   grown in 6-well plates and incubated with series dilution of BALF for 1 h. The plates were then overlaid

512   with low melting temperature agarose (0.6 %) in MEM with BSA and trypsin and cultured for 3 days in

513   37°C incubator. Plates were then fixed with formaldehyde and virus plaques were visualized with the

514   staining of neutral red.

515

516   **FACS analysis.** Fluorescence-conjugated FACS Abs were purchased from Biolegend, BD Biosciences or

517   eBioscience. Ab clones are provided. We defined cell populations based on following cell surface markers:

518   AM (CD11c$^+$ Siglec F$^+$ CD11b^{low}), Neutrophils (CD11b$^+$ Ly6G$^+$), total CD11b$^+$ Monocyte/Macrophage

519   population (Ly6G$^-$ Siglec F$^-$ CD11b$^+$), Monocytes (Ly6G$^-$ Siglec F$^-$ CD11b$^+$ Ly6C$^+$), NP$_{366}$ tetramer$^+$ cells

520   (CD8$^+$ NP$_{366}$-tet$^+$), PA$_{224}$ tetramer$^+$ cells (CD8$^+$ PA$_{224}$-tet$^+$). Samples were collected on FACS Attune or

521   FACS Attune NXT flow cytometer (Life technologies) and analyzed using Flow Jo software (Tree Star).

522

523   **Statistical analysis.** Data are mean ± SEM of values from individual mice (*in vivo* experiments).

524   Unpaired two-tailed Student's t-test (two group comparison), Multiple t-tests (weight loss) or Logrank

525 test (survival study) were used to determine statistical significance by GraphPad Prism software. We

526 consider P values < 0.05 as significant.

527

ACKNOWLEDGMENTS

535

536

537

538

539

540

541

542

543

544

545

546

547

548

549

550

551

552

553

554

REFERENCES

556

557 1.   **Openshaw PJM, Chiu C, Culley FJ, Johansson C.** 2017. Protective and Harmful Immunity to RSV Infection.
558   Annu Rev Immunol **35:**501-532.

559   2.   **Zhou H, Thompson WW, Viboud CG, Ringholz CM, Cheng PY, Steiner C, Abedi GR, Anderson LJ, Brammer**
560   **L, Shay DK.** 2012. Hospitalizations associated with influenza and respiratory syncytial virus in the United
561   States, 1993-2008. Clin Infect Dis **54:**1427-1436.
562   3.   **Molinari NA, Ortega-Sanchez IR, Messonnier ML, Thompson WW, Wortley PM, Weintraub E, Bridges CB.**
563   2007. The annual impact of seasonal influenza in the US: measuring disease burden and costs. Vaccine
564   **25:**5086-5096.
565   4.   **Doherty PC, Turner SJ, Webby RG, Thomas PG.** 2006. Influenza and the challenge for immunology. Nat
566   Immunol **7:**449-455.
567   5.   **Thomas PG, Keating R, Hulse-Post DJ, Doherty PC.** 2006. Cell-mediated protection in influenza infection.
568   Emerg Infect Dis **12:**48-54.
569   6.   **Hussain M, Galvin HD, Haw TY, Nutsford AN, Husain M.** 2017. Drug resistance in influenza A virus: the
570   epidemiology and management. Infect Drug Resist **10:**121-134.
571   7.   **Braciale TJ, Sun J, Kim TS.** 2012. Regulating the adaptive immune response to respiratory virus infection.
572   Nat Rev Immunol **12:**295-305.
573   8.   **Sun J, Braciale TJ.** 2013. Role of T cell immunity in recovery from influenza virus infection. Curr Opin Virol
574   **3:**425-429.
575   9.   **de Jong MD, Simmons CP, Thanh TT, Hien VM, Smith GJ, Chau TN, Hoang DM, Chau NV, Khanh TH, Dong**
576   **VC, Qui PT, Cam BV, Ha do Q, Guan Y, Peiris JS, Chinh NT, Hien TT, Farrar J.** 2006. Fatal outcome of
577   human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat Med **12:**1203-
578   1207.
579   10. **Kobasa D, Jones SM, Shinya K, Kash JC, Copps J, Ebihara H, Hatta Y, Kim JH, Halfmann P, Hatta M,**
580   **Feldmann F, Alimonti JB, Fernando L, Li Y, Katze MG, Feldmann H, Kawaoka Y.** 2007. Aberrant innate
581   immune response in lethal infection of macaques with the 1918 influenza virus. Nature **445:**319-323.
582   11. **Lavin Y, Mortha A, Rahman A, Merad M.** 2015. Regulation of macrophage development and function in
583   peripheral tissues. Nat Rev Immunol **15:**731-744.
584   12. **Geissmann F, Mass E.** 2015. A stratified myeloid system, the challenge of understanding macrophage
585   diversity. Semin Immunol **27:**353-356.
586   13. **Geissmann F, Gordon S, Hume DA, Mowat AM, Randolph GJ.** 2010. Unravelling mononuclear phagocyte
587   heterogeneity. Nat Rev Immunol **10:**453-460.
588   14. **Ginhoux F, Guilliams M.** 2016. Tissue-Resident Macrophage Ontogeny and Homeostasis. Immunity
589   **44:**439-449.
590   15. **Perdiguero EG, Geissmann F.** 2016. The development and maintenance of resident macrophages. Nat
591   Immunol **17:**2-8.
592   16. **Hussell T, Bell TJ.** 2014. Alveolar macrophages: plasticity in a tissue-specific context. Nat Rev Immunol
593   **14:**81-93.
594   17. **Kopf M, Schneider C, Nobs SP.** 2015. The development and function of lung-resident macrophages and
595   dendritic cells. Nat Immunol **16:**36-44.
596   18. **Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, De Prijck S, Deswarte K, Malissen B, Hammad H,**
597   **Lambrecht BN.** 2013. Alveolar macrophages develop from fetal monocytes that differentiate into long-
598   lived cells in the first week of life via GM-CSF. J Exp Med **210:**1977-1992.
599   19. **Schneider C, Nobs SP, Kurrer M, Rehrauer H, Thiele C, Kopf M.** 2014. Induction of the nuclear receptor
600   PPAR-gamma by the cytokine GM-CSF is critical for the differentiation of fetal monocytes into alveolar
601   macrophages. Nat Immunol **15:**1026-1037.
602   20. **Yu X, Buttgereit A, Lelios I, Utz SG, Cansever D, Becher B, Greter M.** 2017. The Cytokine TGF-beta
603   Promotes the Development and Homeostasis of Alveolar Macrophages. Immunity **47:**903-912 e904.
604   21. **Schneider C, Nobs SP, Heer AK, Hirsch E, Penninger J, Siggs OM, Kopf M.** 2017. Frontline Science:
605   Coincidental null mutation of Csf2ralpha in a colony of PI3Kgamma-/- mice causes alveolar macrophage
606   deficiency and fatal respiratory viral infection. J Leukoc Biol **101:**367-376.
607   22. **Deng W, Yang J, Lin X, Shin J, Gao J, Zhong XP.** 2017. Essential Role of mTORC1 in Self-Renewal of Murine
608   Alveolar Macrophages. J Immunol **198:**492-504.

609 23. **Kawasaki T, Ito K, Miyata H, Akira S, Kawai T.** 2017. Deletion of PIKfyve alters alveolar macrophage
610   populations and exacerbates allergic inflammation in mice. EMBO J **36:**1707-1718.
611 24. **Todd EM, Zhou JY, Szasz TP, Deady LE, D'Angelo JA, Cheung MD, Kim AH, Morley SC.** 2016. Alveolar
612   macrophage development in mice requires L-plastin for cellular localization in alveoli. Blood **128:**2785-
613   2796.
614 25. **Laidlaw BJ, Decman V, Ali MA, Abt MC, Wolf AI, Monticelli LA, Mozdzanowska K, Angelosanto JM, Artis
615   D, Erikson J, Wherry EJ.** 2013. Cooperativity between CD8+ T cells, non-neutralizing antibodies, and
616   alveolar macrophages is important for heterosubtypic influenza virus immunity. PLoS Pathog **9:**e1003207.
617 26. **Schneider C, Nobs SP, Heer AK, Kurrer M, Klinke G, van Rooijen N, Vogel J, Kopf M.** 2014. Alveolar
618   macrophages are essential for protection from respiratory failure and associated morbidity following
619   influenza virus infection. PLoS Pathog **10:**e1004053.
620 27. **Kim HM, Lee YW, Lee KJ, Kim HS, Cho SW, van Rooijen N, Guan Y, Seo SH.** 2008. Alveolar macrophages
621   are indispensable for controlling influenza viruses in lungs of pigs. J Virol **82:**4265-4274.
622 28. **Purnama C, Ng SL, Tetlak P, Setiagani YA, Kandasamy M, Baalasubramanian S, Karjalainen K, Ruedl C.**
623   2014. Transient ablation of alveolar macrophages leads to massive pathology of influenza infection
624   without affecting cellular adaptive immunity. Eur J Immunol **44:**2003-2012.
625 29. **Kumagai Y, Takeuchi O, Kato H, Kumar H, Matsui K, Morii E, Aozasa K, Kawai T, Akira S.** 2007. Alveolar
626   macrophages are the primary interferon-alpha producer in pulmonary infection with RNA viruses.
627   Immunity **27:**240-252.
628 30. **Cardani A, Boulton A, Kim TS, Braciale TJ.** 2017. Alveolar Macrophages Prevent Lethal Influenza
629   Pneumonia By Inhibiting Infection Of Type-1 Alveolar Epithelial Cells. PLoS Pathog **13:**e1006140.
630 31.   **Goritzka M, Makris S, Kausar F, Durant LR, Pereira C, Kumagai Y, Culley FJ, Mack M, Akira S, Johansson C.**
631   2015. Alveolar macrophage-derived type I interferons orchestrate innate immunity to RSV through
632   recruitment of antiviral monocytes. J Exp Med **212:**699-714.
633 32. **Gorski SA, Hufford MM, Braciale TJ.** 2012. Recent insights into pulmonary repair following virus-induced
634   inflammation of the respiratory tract. Curr Opin Virol **2:**233-241.
635 33. **Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, Evans RM.** 2013. PPARgamma signaling
636   and metabolism: the good, the bad and the future. Nat Med **19:**557-566.
637 34. **Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, Red Eagle A,
638   Vats D, Brombacher F, Ferrante AW, Chawla A.** 2007. Macrophage-specific PPARgamma controls
639   alternative activation and improves insulin resistance. Nature **447:**1116-1120.
640 35. **Chawla A.** 2010. Control of macrophage activation and function by PPARs. Circ Res **106:**1559-1569.
641 36. **Moseley CE, Webster RG, Aldridge JR.** 2010. Peroxisome proliferator-activated receptor and AMP-
642   activated protein kinase agonists protect against lethal influenza virus challenge in mice. Influenza Other
643   Respir Viruses **4:**307-311.
644 37. **Darwish I, Mubareka S, Liles WC.** 2011. Immunomodulatory therapy for severe influenza. Expert Rev Anti
645   Infect Ther **9:**807-822.
646 38. **Cloutier A, Marois I, Cloutier D, Verreault C, Cantin AM, Richter MV.** 2012. The prostanoid 15-deoxy-
647   Delta12,14-prostaglandin-j2 reduces lung inflammation and protects mice against lethal influenza
648   infection. J Infect Dis **205:**621-630.
649 39. **Fedson DS.** 2013. Treating influenza with statins and other immunomodulatory agents. Antiviral Res
650   **99:**417-435.
651 40. **Aldridge JR, Jr., Moseley CE, Boltz DA, Negovetich NJ, Reynolds C, Franks J, Brown SA, Doherty PC,
652   Webster RG, Thomas PG.** 2009. TNF/iNOS-producing dendritic cells are the necessary evil of lethal
653   influenza virus infection. Proc Natl Acad Sci U S A **106:**5306-5311.
654 41. **Herold S, Steinmueller M, von Wulffen W, Cakarova L, Pinto R, Pleschka S, Mack M, Kuziel WA, Corazza
655   N, Brunner T, Seeger W, Lohmeyer J.** 2008. Lung epithelial apoptosis in influenza virus pneumonia: the
656   role of macrophage-expressed TNF-related apoptosis-inducing ligand. J Exp Med **205:**3065-3077.
657 42. **Lin KL, Suzuki Y, Nakano H, Ramsburg E, Gunn MD.** 2008. CCR2+ monocyte-derived dendritic cells and
658   exudate macrophages produce influenza-induced pulmonary immune pathology and mortality. J Immunol
659   **180:**2562-2572.

660   43.   **Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, Becker CD, See P, Price J, Lucas D,**
661   **Greter M, Mortha A, Boyer SW, Forsberg EC, Tanaka M, van Rooijen N, Garcia-Sastre A, Stanley ER,**
662   **Ginhoux F, Frenette PS, Merad M.** 2013. Tissue-resident macrophages self-maintain locally throughout
663   adult life with minimal contribution from circulating monocytes. Immunity **38:**792-804.
664   44.   **Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK, Perlman S.** 2016. Dysregulated
665   Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-
666   CoV-Infected Mice. Cell Host Microbe **19:**181-193.
667   45.   **Gautier EL, Chow A, Spanbroek R, Marcelin G, Greter M, Jakubzick C, Bogunovic M, Leboeuf M, van**
668   **Rooijen N, Habenicht AJ, Merad M, Randolph GJ.** 2012. Systemic analysis of PPARgamma in mouse
669   macrophage populations reveals marked diversity in expression with critical roles in resolution of
670   inflammation and airway immunity. J Immunol **189:**2614-2624.
671   46.   **Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, Iwasaki A.** 2011. Microbiota regulates
672   immune defense against respiratory tract influenza A virus infection. Proc Natl Acad Sci U S A **108:**5354-
673   5359.
674   47.   **Garcia-Sastre A, Biron CA.** 2006. Type 1 interferons and the virus-host relationship: a lesson in detente.
675   Science **312:**879-882.
676   48.   **Teijaro JR, Walsh KB, Cahalan S, Fremgen DM, Roberts E, Scott F, Martinborough E, Peach R, Oldstone**
677   **MB, Rosen H.** 2011. Endothelial cells are central orchestrators of cytokine amplification during influenza
678   virus infection. Cell **146:**980-991.
679   49.   **Teijaro JR.** 2015. The role of cytokine responses during influenza virus pathogenesis and potential
680   therapeutic options. Curr Top Microbiol Immunol **386:**3-22.
681   50.   **Davidson S, Crotta S, McCabe TM, Wack A.** 2014. Pathogenic potential of interferon alphabeta in acute
682   influenza infection. Nat Commun **5:**3864.
683   51.   **Sun J, Madan R, Karp CL, Braciale TJ.** 2009. Effector T cells control lung inflammation during acute
684   influenza virus infection by producing IL-10. Nat Med **15:**277-284.
685   52.   **Yao S, Jiang L, Moser EK, Jewett LB, Wright J, Du J, Zhou B, Davis SD, Krupp NL, Braciale TJ, Sun J.** 2015.
686   Control of pathogenic effector T-cell activities in situ by PD-L1 expression on respiratory inflammatory
687   dendritic cells during respiratory syncytial virus infection. Mucosal Immunol **8:**746-759.
688   53.   **Mack M, Cihak J, Simonis C, Luckow B, Proudfoot AE, Plachy J, Bruhl H, Frink M, Anders HJ, Vielhauer V,**
689   **Pfirstinger J, Stangassinger M, Schlondorff D.** 2001. Expression and characterization of the chemokine
690   receptors CCR2 and CCR5 in mice. J Immunol **166:**4697-4704.
691   54.   **Yao S, Buzo BF, Pham D, Jiang L, Taparowsky EJ, Kaplan MH, Sun J.** 2013. Interferon regulatory factor 4
692   sustains CD8(+) T cell expansion and effector differentiation. Immunity **39:**833-845.
693   55.   **Huprikar J, Rabinowitz S.** 1980. A simplified plaque assay for influenza viruses in Madin-Darby kidney
694   (MDCK) cells. J Virol Methods **1:**117-120.
695
696
697
698
699
700
701
702
703

704   **FIGURE LEGENDS**

705   **Figure 1. IAV down-regulates PPAR-γ expression in AM.**

706   A. Comparison of the expression of 84 transcription factors in AM (isolated and pooled from at least
707   3 mice) with or without IAV (IAV) infection for overnight *in vitro* by RT^2 Profiler PCR array.
708   Dotted line: fold cutoff of gene expression (1.5 fold). Red dots, genes up-regulated following IAV
709   infection. Green dots, genes down-regulated following IAV infection.
710   B. List of up- or down-regulated transcription factors in AM (isolated and pooled from at least 3
711   mice) following IAV infection *in vitro* for overnight by RT^2 Profiler PCR array.
712   C. Relative expression of *Pparg* in AM (isolated and pooled from at least 3 mice) with or without
713   IAV infection for overnight *in vitro* by qRT-PCR.
714   D. Western blot analysis of PPAR-γ levels in AM (isolated and pooled from at least 3 mice) with or
715   without IAV infection for overnight. Bar graph represents relative density of PPAR-γ band pooled
716   from three independent experiments.
717   E. Relative expression of *Pparg* in sorted AM isolated from non-infected (day 0) or IAV-infected
718   mice at 4, 6, 10 or15 p.i
719   F. Western blot analysis of PPAR-γ expression *ex vivo* in AM (isolated and pooled from at least 3
720   mice) isolated from non-infected (day 0) or IAV-infected lungs (6 d.p.i.). Bar graphs represent
721   relative density of PPAR-γ band pooled from three independent experiments.
722   G. Western blot analysis of PPAR-γ expression in AM (isolated and pooled from at least 3 mice) with
723   or without IFN-α treatment for overnight. Bar graph represent relative density of PPAR-γ band
724   pooled from three independent experiments.
725   H. Relative expression of *Pparg* in AM (isolated and pooled from at least 3 mice) with or without
726   IAV infection in the presence or absence of α-IFNAR1 for overnight *in vitro* by qRT-PCR.
727   I. STAT1 binding to *Pparg* locus in AM following overnight IFN-α treatment *in vitro* was
728   determined through ChIP (pooled from n>20 mice). Numbers in Red are distances of the binding
729   sites to start codon.
730   Data are representative of two to three independent experiments. *, P < 0.05.
731
732 **Figure 2. PPAR-γ suppresses antiviral inflammation, but not regulates M2 genes following infection**
733   A. Airway AM percentages and CD11b expression on AM from control (*Pparg$^{fl/fl}$*) or *Pparg^{ΔCD11c}*
734   mice, and control or *Pparg^{ΔLyz2}* mice.
735   B. Western blot of PPAR-γ expression in sorted AM (isolated and pooled from 2-3 mice) from
736   control (*Pparg$^{fl/fl}$*) or *Pparg^{ΔLyz2}* mice at 0 and 3 d.p.i.

737   C. qRT-PCR analysis of *Ifna4, Ifnb1, Il1b, Tnf, Ccl2, Retnla* and *Arg1* expression in AM (isolated

738   and pooled from 3 mice) from control ($Pparg^{fl/fl}$) or $Pparg^{\Delta Lyz2}$ mice following IAV infection *in*

739   *vitro* for overnight.

740   D. Control or $Pparg^{\Delta Lyz2}$ mice were infected with IAV. *Ifna4, Ifnb1, Tnf, Ccl2, Retnla* and *Arg1* gene

741   expression in AM (isolated and pooled from 2-3 mice) of control or $Pparg^{\Delta Lyz2}$ mice at day 1 and 3

742   p.i.

743 Data are representative of at least two independent experiments. *, $P < 0.05$.

744

745 **Figure 3. Myeloid PPAR-γ suppresses host mortality, morbidity and pulmonary inflammation.**

746 Control or $Pparg^{\Delta Lyz2}$ mice were infected with IAV.

747   A. Host mortality (% survival) was monitored.

748   B. Host morbidity (% initial weight) was monitored.

749   C. Airway IAV titers (pfu assay) were determined at day 4, 7, 10 or 15 p.i..

750   D. Lung IAV-specific PA_{224} and NP_{366} tetramer$^+$ CD8$^+$ T cells at day 7, 10 and 15 p.i.

751   E. CCL2 and TNF-α levels in the BAL were quantified by ELISA at day 1, 3, 7, 10 or 15 p.i.

752   F. Comparison of the expression of 84 inflammation-related genes in lungs from control or

753     $Pparg^{\Delta Lyz2}$ mice at day 10 p.i. by RT^2 Profiler PCR array. Dotted line: 1.5 fold difference cutoff.

754   Red dots, genes up-regulated in the lungs of $Pparg^{\Delta Lyz2}$ mice. Green dots, genes down-regulated in

755   the lungs of $Pparg^{\Delta Lyz2}$ mice.

756 Data are representative of at least two independent experiments (n=3-6 mice per group) except A, B,

757 C (pooled data from 2-6 experiments). *, $P < 0.05$.

758

759 **Figure 4. Myeloid PPAR-γ suppresses pulmonary inflammation during RSV infection.**

760   A. qRT-PCR analysis of *Ifna4, Ifnb1, Il1b, Tnf, Ccl2, Retnla* and *Arg1* expression in AM (isolated

761   and pooled from at least 3 mice) from control ($Pparg^{fl/fl}$) or $Pparg^{\Delta Lyz2}$ mice following RSV

762   infection (10 MOI) *in vitro* for overnight.

763   B-D. Control ($Pparg^{fl/fl}$) or $Pparg^{\Delta Lyz2}$ mice were infected with RSV.

764   B. Host morbidity (% initial weight) was monitored daily.

765   C. Numbers of lung neutrophils or monocytes at 4 d.p.i.

766   D. BAL TNF and IL-1β concentrations were determined through ELISA at 4 d.p.i.

767 Data are representative of at least two independent experiments (n=3-4 mice per group) except B

768 (pooled data from 2 experiments). *, $P < 0.05$.

769

770 **Figure 5. PPAR-γ expression in resident alveolar macrophages is likely required for the suppression**
771 **of host morbidity**

    A. Lyz2-cre gene recombination in AM, neutrophils and CD11b$^+$ monocytes/macrophages is reported
773   by % eYFP expression following crossing with R26R-eYFP reporter mice.

    B. Western blot analysis of PPAR-**γ** protein expression in sorted AM, CD11b$^+$
775   monocytes/macrophages and neutrophils in the lungs from naïve WT mice (pooled from 3 mice).

    C. *Tnf* and *Ccl2* expression in indicated cell populations in the lungs of control (*Pparg$^{fl/fl}$*) or
777   *Pparg^{ΔLyz2}* mice at day 1 and 3 p.i. (pooled from 2-3 mice per group).

    D. *Ccr2$^{-/-}$ Pparg$^{fl/fl}$* and *Ccr2$^{-/-}$ Pparg^{ΔLyz2}* mice were infected with IAV. Host morbidity (% initial
779   weight) was monitored.

    E. WT mice were infected with IAV and treated with control IgG or MC21 mAb. % lung AM (upper
781   panel) and monocytes (lower panel) in CD45$^+$ Ly6G$^-$ cells are depicted at 5 d.p.i.

    F. Control (*Pparg$^{fl/fl}$*) and *Pparg^{ΔLyz2}* mice were infected with IAV and treated with control IgG or
783   MC21 mAb. Host morbidity (% initial weight) was monitored.

784 Data are representative of at least two to three independent experiments except C (pooled data from 3
785 experiments) and D. *, $P < 0.05$.

787 **Figure 6. Macrophage PPAR-γ modulates inflammation resolution and tissue repair.**

    A. Comparison of the expression of 84 wound healing genes of AM isolated (pooled from 3 mice)
789   from uninfected control or *Pparg^{ΔLyz2}* mice *in vitro*. Dotted line: fold cutoff of gene expression
790   (1.5 fold). Red dots, genes up-regulated in PPAR-γ-deficient AM. Green dots, genes down-
791   regulated in PPAR-γ-deficient AM.

    B. List of up- or down-regulated wound healing genes in AM (pooled from 3 mice) from control or
793   *Pparg^{ΔLyz2}* mice by RT^2 Profiler PCR array.

794 C-F. Control (*Pparg$^{fl/fl}$*) or *Pparg^{ΔLyz2}* mice were infected with IAV (n=3-4).

    C. H&E staining of lung sections of control or *Pparg^{ΔLyz2}* mice at day 15 p.i. Left panel,
796   representative images. Right panel, quantification of percentages of inflamed and disrupted
797   alveolar area in the lungs of control (*Pparg$^{fl/fl}$*) and *Pparg^{ΔLyz2}* mice.

    D. BAL neutrophil or monocyte numbers were enumerated at 15 d.p.i.

    E. BAL total protein concentrations were determined at 1, 3, 7, 10 or 15 d.p.i.

    F. *Sftpb* and *Abca3* gene expression in lungs from control or *Pparg^{ΔLyz2}* mice at 8, 10 or 15 d.p.i.

801 Data are representative of at least two independent experiments, *, $P < 0.05$.
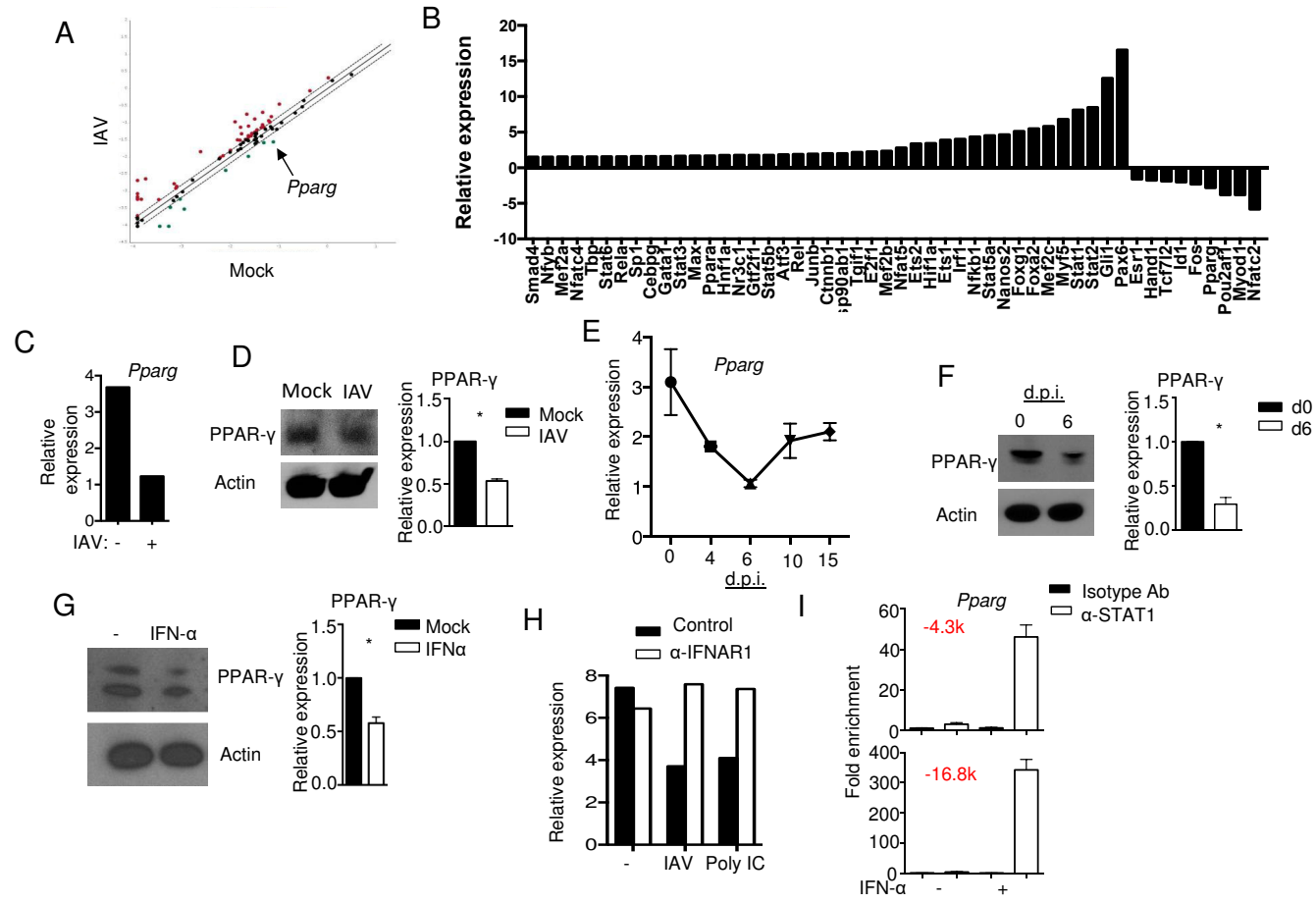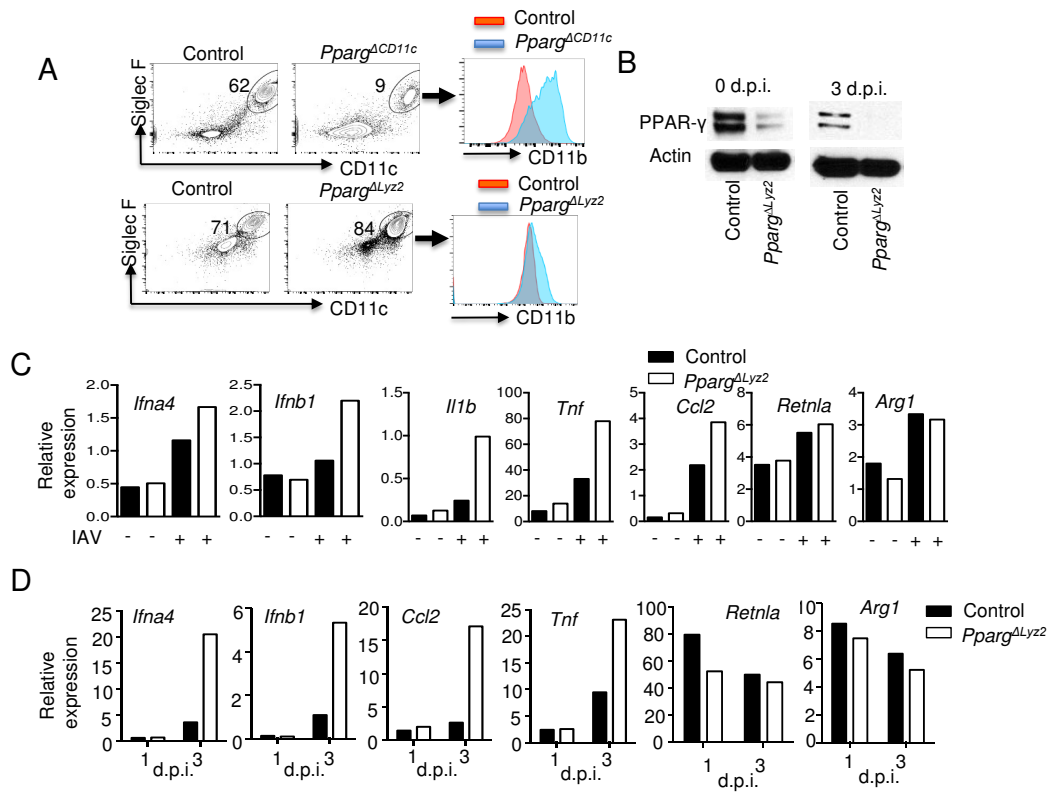
803

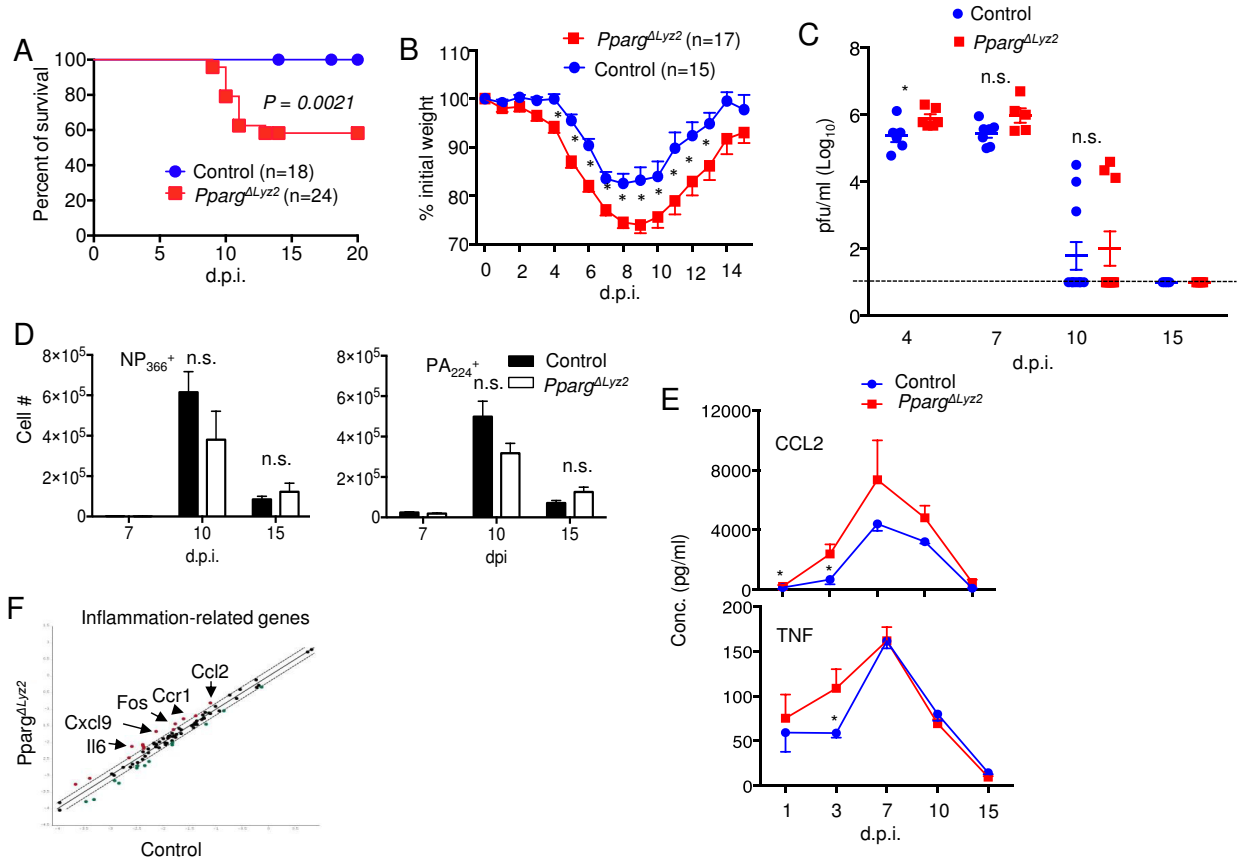Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

A

Rosa26-eYFP

Lyz2-cre
R26R-eYFP

AM   Neutrophils   CD11b+
                            Mono/MΦ

Siglec F   Ly6G   CD11b

<1   <1   <1

83   74   24

eYFP

B

PPAR-γ

Actin

AM CD11b+ Neutrophils
    Mono/MΦ

C

Control
PpargΔLyz2

AM   CD45-   CD11b+
                          Mono/MΦ

Tnf

Ccl2

Relative expression

1   3   1   3   1   3
         d.p.i.

D

Ccr2-/- Ppargfl/fl (n=12)
Ccr2-/- PpargΔLyz2 (n=7)

% initial weight

0 2 4 6 8 10 12 14 16 18
d.p.i.

E

5 d.p.i.

Gated on CD45+ Ly6G- cells
IgG   MC21

Siglec F   8.5   10

Ly6C   41   6

                          CD11c

                          CD11b

F

Control + IgG (n=5)
PpargΔLyz2 +IgG (n=5)
Control + MC21 (n=5)
PpargΔLyz2 + MC21 (n=5)

% initial weight

0 2 4 6 8 10 12 14

Figure 6

A — Wound healing PCR array; PPAR-γ-deficient AM vs Control AM

B — Relative expression

C — 15 d.p.i.; Control; Pparg^ΔLyz2; % disrupted area

D — Neutrophils; Monocytes; Cell #

E — BAL protein; Conc. (pg/ml); d.p.i.

F — 8 d.p.i.; 10 d.p.i.; 15 d.p.i.; Sftpb; Abca3; Relative expression