

Supplementary Materials

Title

Inhibition of macrophage inflammasome assembly and pyroptosis with GC-1 ameliorates acute lung injury

Authors

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This file includes: Figure S1-4

Supplementary figures

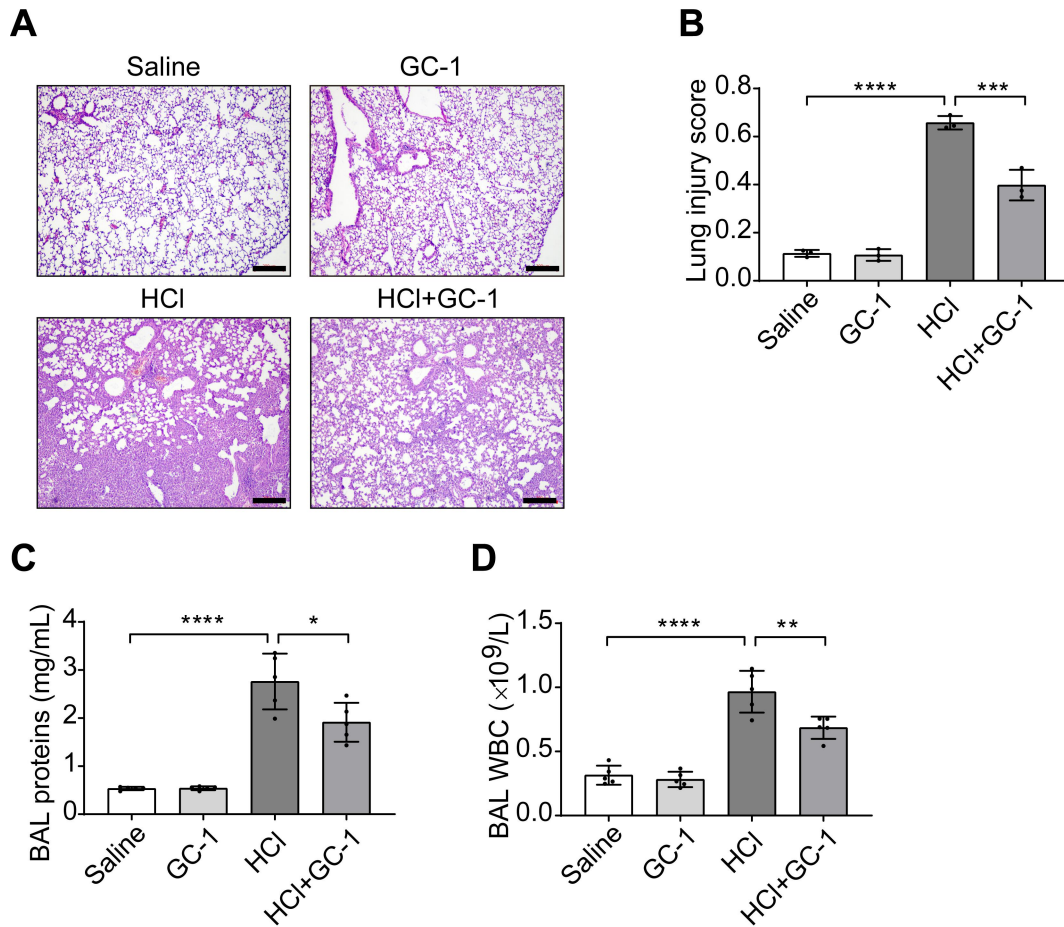


Figure S1. GC-1 alleviated lung injury induced by HCl in mice. The treatment of C57BL/6 mice was the same as in Figure 1. (A) Representative paraffin sections from HCl-induced mice ($n = 3$), H&E staining, Scale bars, 200 μm . (B) Lung injury score of HCl-induced ALI according to the ATS lung injury scoring system ($n = 3$). The levels of (C) BAL protein content and (D) BAL WBC concentration were measured in HCl-induced mice ($n = 5$). BAL: bronchoalveolar lavage. The data represent one of at least three independent experiments. The values are shown as mean \pm SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

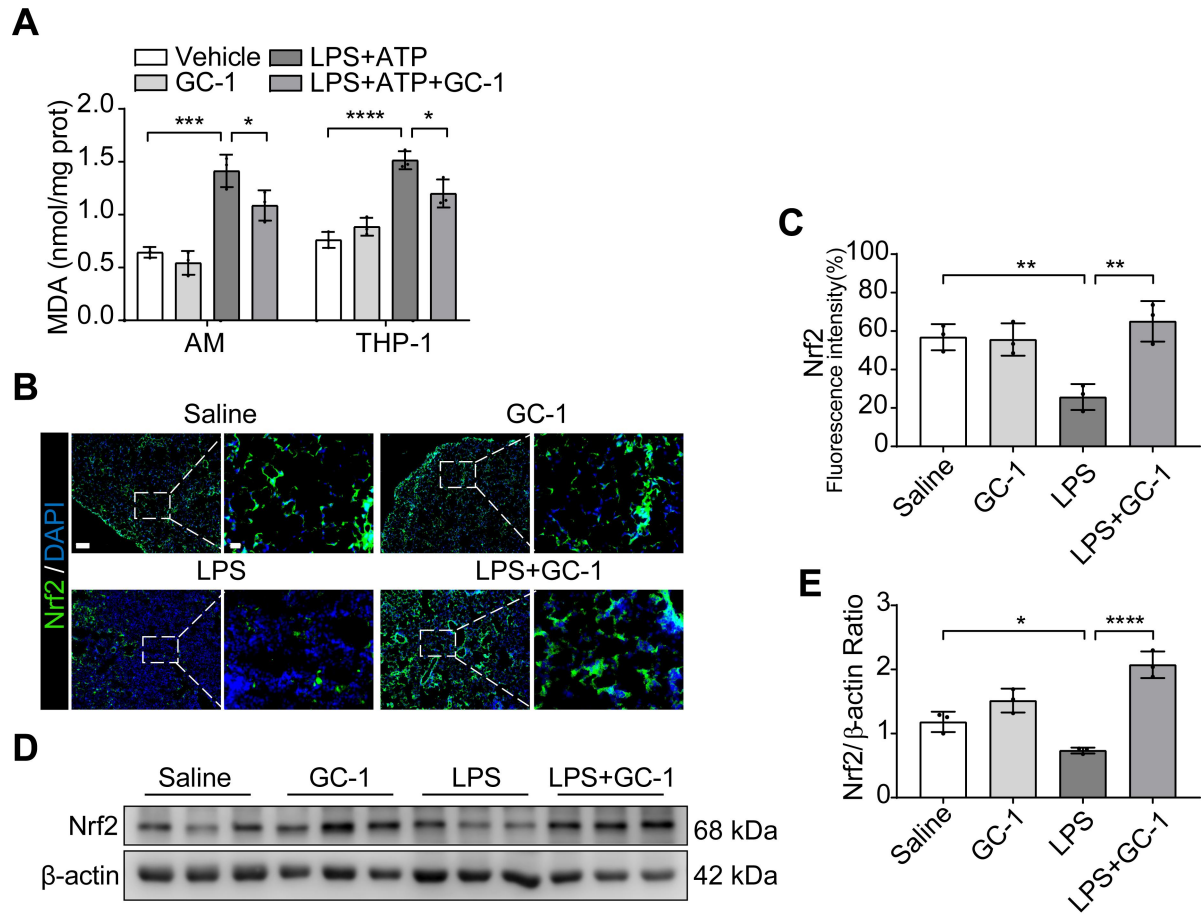


Figure S2. GC-1 mitigated oxidative damage in vivo and in vitro. (A) Detection of MDA concentrations in AM and THP-1 (n = 3). (B) Representative IF images in mice (n = 3), Scale bars, 100 μ m and 20 μ m (insets). (C) Quantitative statistical results of fluorescence intensity from Nrf2 with DAPI as reference (n = 3). (D) Western blot analysis of Nrf2 expression in lung tissue (n = 3). (E) Quantification of immunoblot grayscale values (n = 3). The data represent one of at least three independent experiments. MDA: malondialdehyde; Nrf2: nuclear factor erythroid 2-related factor 2. The values are shown as mean \pm SD. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; ns = not significant.

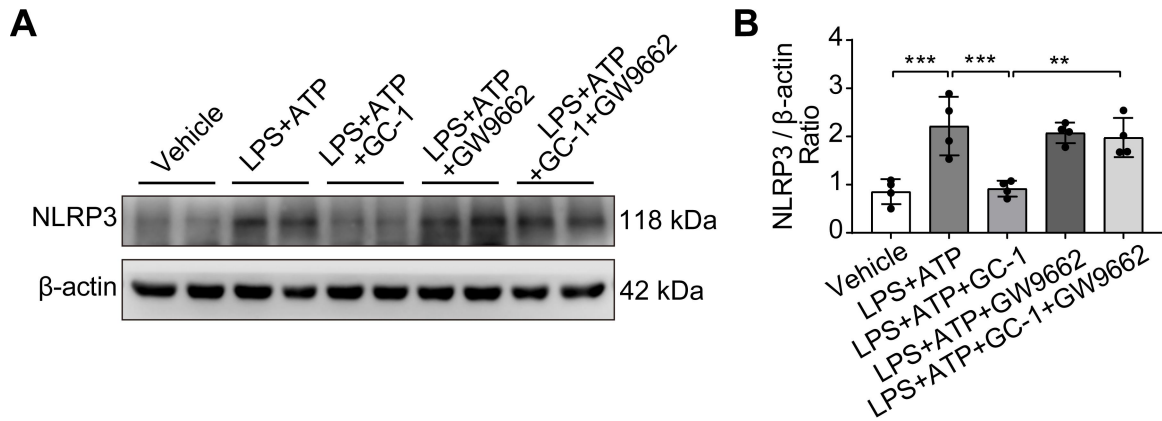


Figure S3. Inhibition of PPAR γ reversed the effect of GC-1 on NLRP3. THP-1 cells were pretreated with GC-1 (100 nM) and/or the PPAR γ inhibitor GW9662 (5 μ M) for 12 h, followed by treatment with LPS and ATP. **(A)** Representative images of Western blot for NLRP3 in THP-1 cells. **(B)** Quantitative analysis of immunoblot grayscale values (n = 4). NLRP3: nlr family pyrin domain-containing 3. The data represent one of at least three independent experiments. The values are shown as mean \pm SD. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

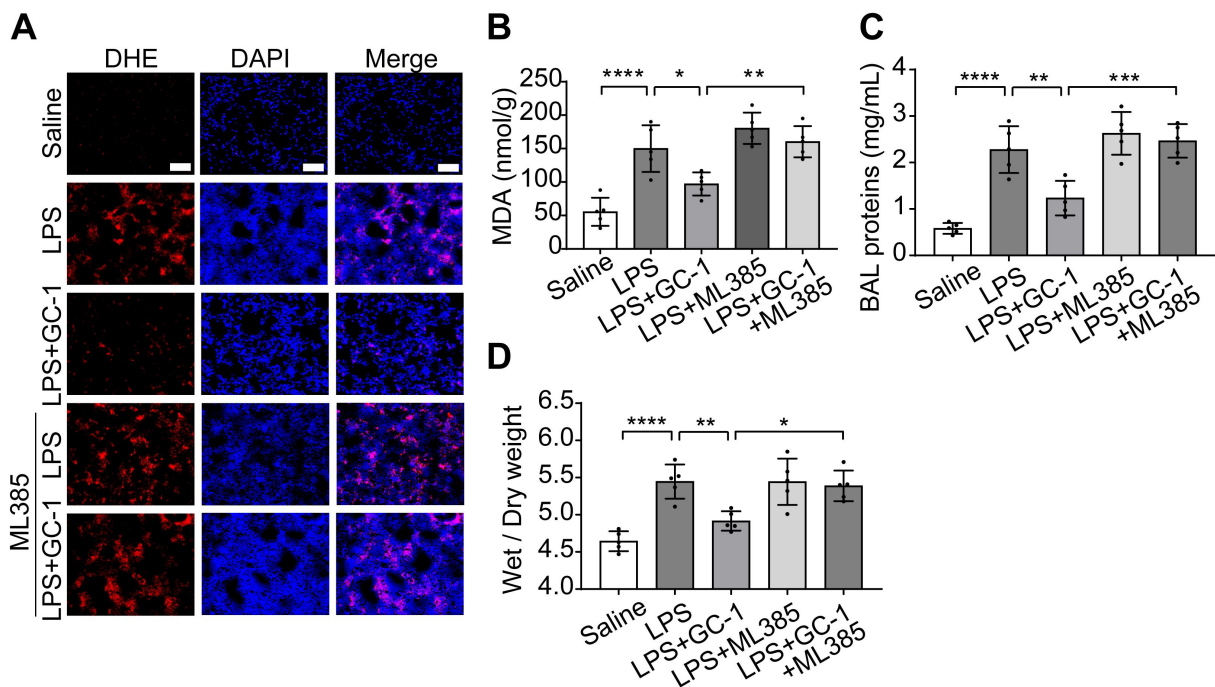


Figure S4. Inhibition of Nrf2 exacerbated oxidative damage and lung injury in ALI mice.

(A) Representative frozen sections of lung tissue (n = 3), stained with DHE, Scale bars, 100 μ m. (B) Measurement of MDA concentrations in lung tissue (n = 5). (C) BAL protein content and (D) lung wet/dry weight ratio were measured in LPS-induced mice (n = 5). The data represent one of at least three independent experiments. BAL: bronchoalveolar lavage; DHE: Dihydroethidium; MDA: malondialdehyde. The values are shown as mean \pm SD. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.