Figures and figure supplements

The bacterial quorum sensing signal 2′-aminoacetophenone rewires immune cell bioenergetics through the Ppargc1a/Esrra axis to mediate tolerance to infection

Arijit Chakraborty and Arunava Bandyopadhaya *et al*.

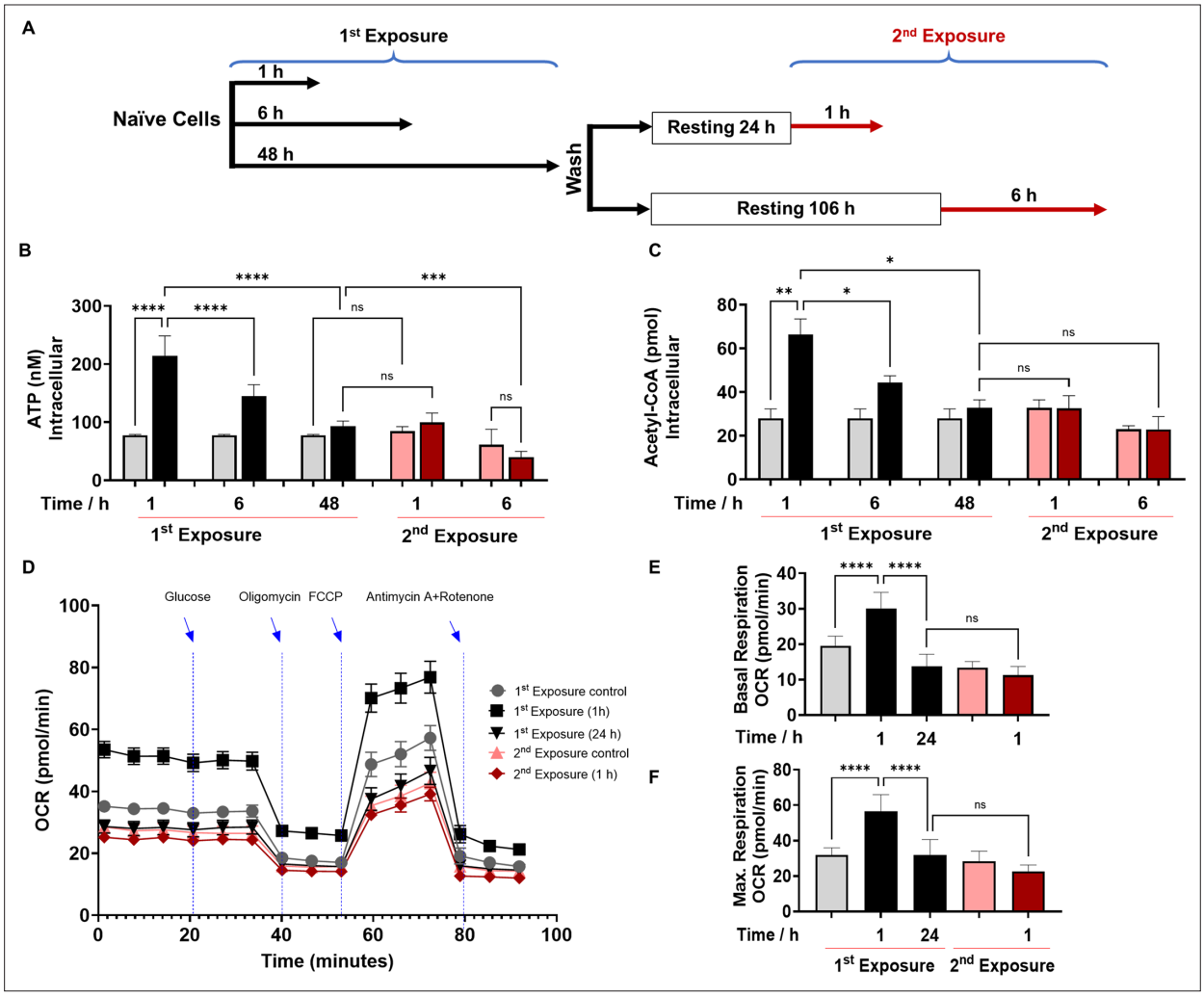

Figure 1. 2′-Aminoacetophenone (2-AA) tolerization decreases crucial metabolites of cellular energy and affects mitochondrial respiration in mouse BMDM. (**A**) Schematic representation showing experimental design: naïve cells were exposed to 2-AA for 1, 6, or 48 hr (black). Cells exposed to 2-AA (400 µM) for 48 hr were washed, rested for 24 or 106 hr, and re-exposed (200 µM) for 1 or 6 hr (red), respectively. The same color code, black cells after first exposure and red cells after second exposure, was kept throughout the manuscript with corresponding controls in gray and pink, respectively. The levels of (**B**) adenosine triphosphate (ATP) and (**C**) acetyl-CoA in BMDM cells after first and second 2-AA exposure. (**D**) Real-time oxygen consumption rate (OCR) traces were recorded using a Seahorse XF analyzer and normalized to protein content. Cells were exposed to 2-AA for 1 or 24 hr (black), washed and rested for 24 hr, and re-exposed for 1 hr (red). Mitochondrial respiratory parameters, (**E**) basal respiration, and (**F**) maximal respiration. Data are presented as mean ± SD, n≥4, *p<0.05, **p<0.01, ***p<0.001, and ns indicates no significant difference. One-way ANOVA followed by Tukey's post hoc test was applied.

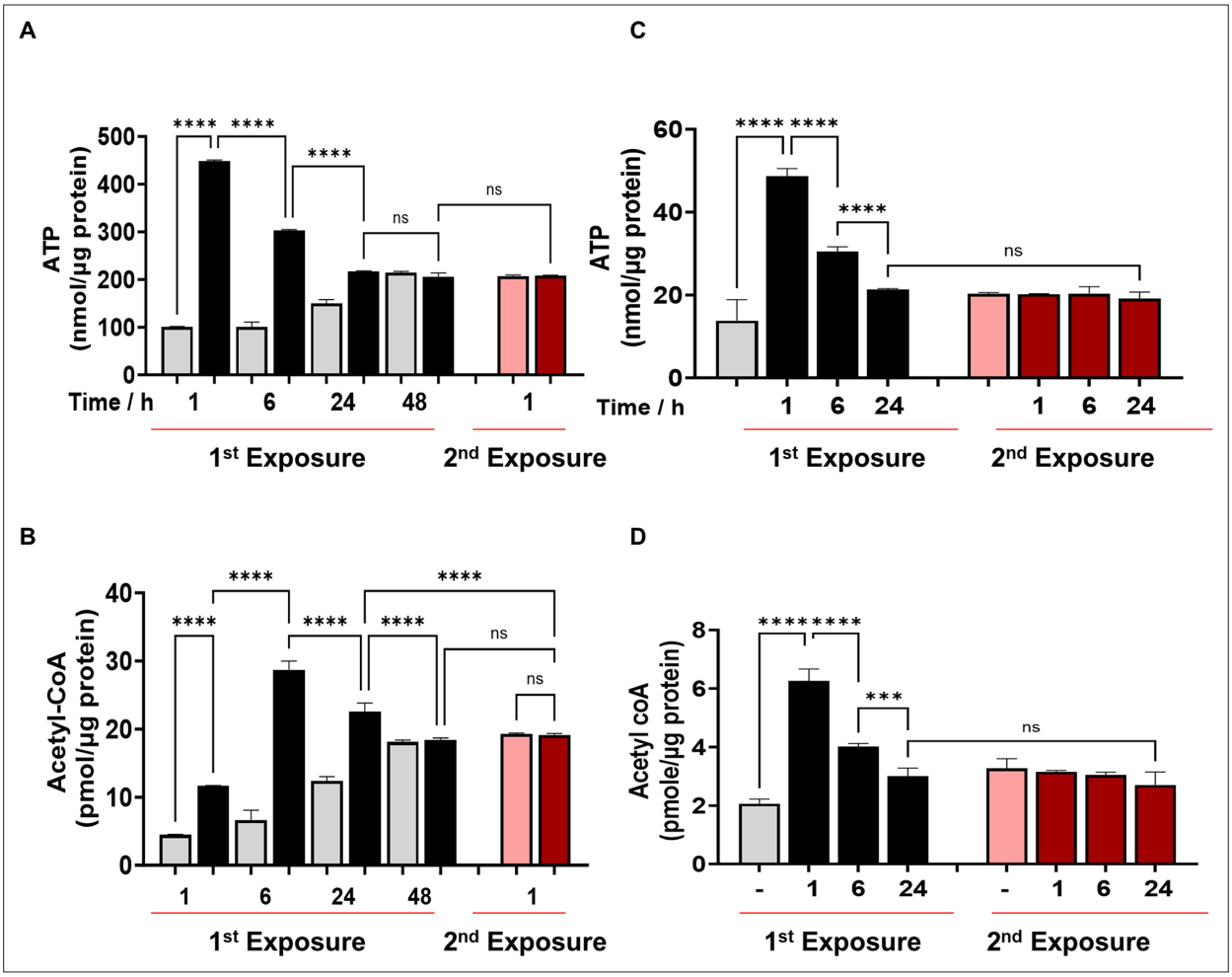

Figure 1—figure supplement 1. 2′-Aminoacetophenone (2-AA) tolerization decreases metabolites in murine RAW 264.7 (**A–B**) and human THP-1 (**C–D**) cells. (**A and C**) Adenosine triphosphate (ATP), (**B and D**) acetyl-CoA, and (**E**) cytosolic and (**F**) mitochondrial pyruvate levels were quantified in 2-AA (400 µM) exposed and re-exposed cells for the hours indicated using the same condition as shown in ***Figure 1A***. Mean ± SD is shown (n=3); ***p<0.001, and ns indicates no significant difference. One-way ANOVA followed by Tukey's post hoc test was applied. The same color code, black cells after first exposure and red cells after second exposure, was kept throughout the main manuscript, with corresponding controls shown in gray and pink, respectively.

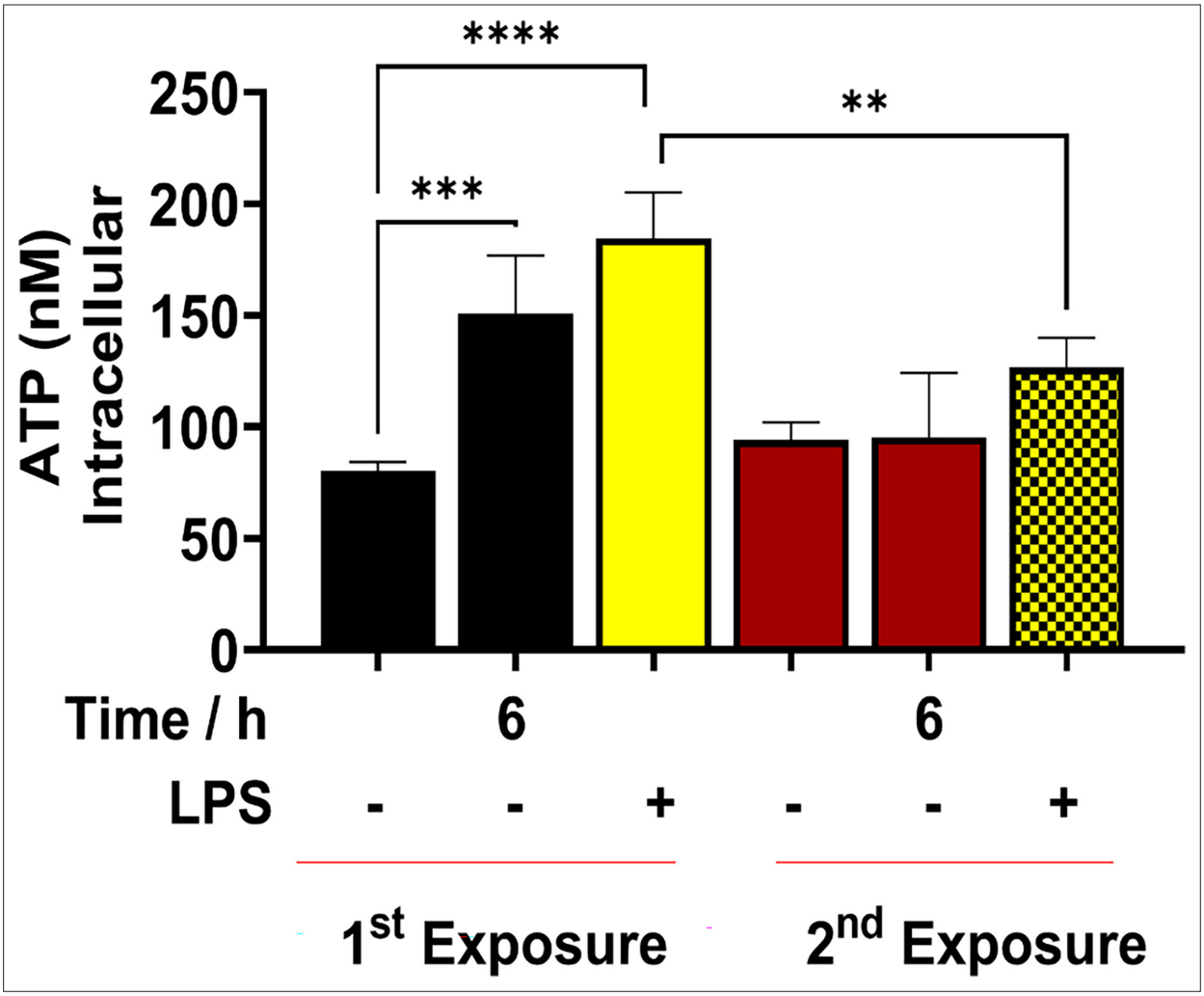

Figure 1—figure supplement 2. Adenosine triphosphate (ATP) levels in murine BMDM (**A**) cells with and without 2′-aminoacetophenone (2-AA) (400 µM) or lipopolysaccharide (LPS) stimulation (100 µg/mL). Each dot represents one experimental replicate of four independent experiments (n=4). Data are presented as mean ± SD, **p<0.01, ***p<0.001, and ns indicates no significant difference. One-way ANOVA followed by Tukey's post hoc test was applied.

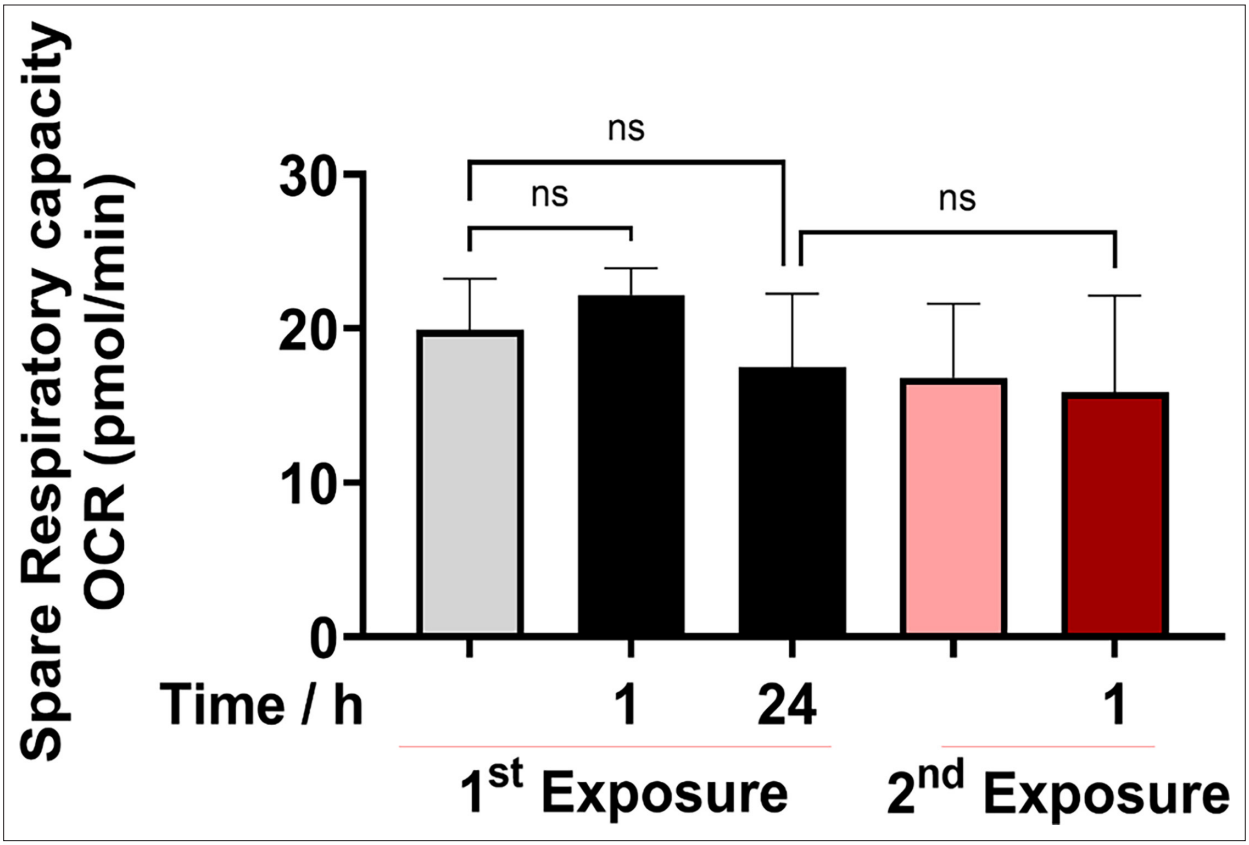

Figure 1—figure supplement 3. Effect of 2′-aminoacetophenone (2-AA) on mitochondrial spare respiratory capacity in BMDM non-tolerized (black) and tolerized (red) macrophages. Controls are shown in gray and pink, respectively. Mitochondrial spare respiratory capacity data extrapolated from the oxygen consumption rate (OCR) profiles shown in ***Figure 1D***. Unstimulated BMDM macrophages were used as a control (**c**). Means ± SDs are shown, n=6, *p<0.05, ***p<0.001, and ns indicates no significant difference. One-way ANOVA followed by Tukey's post hoc test was applied.

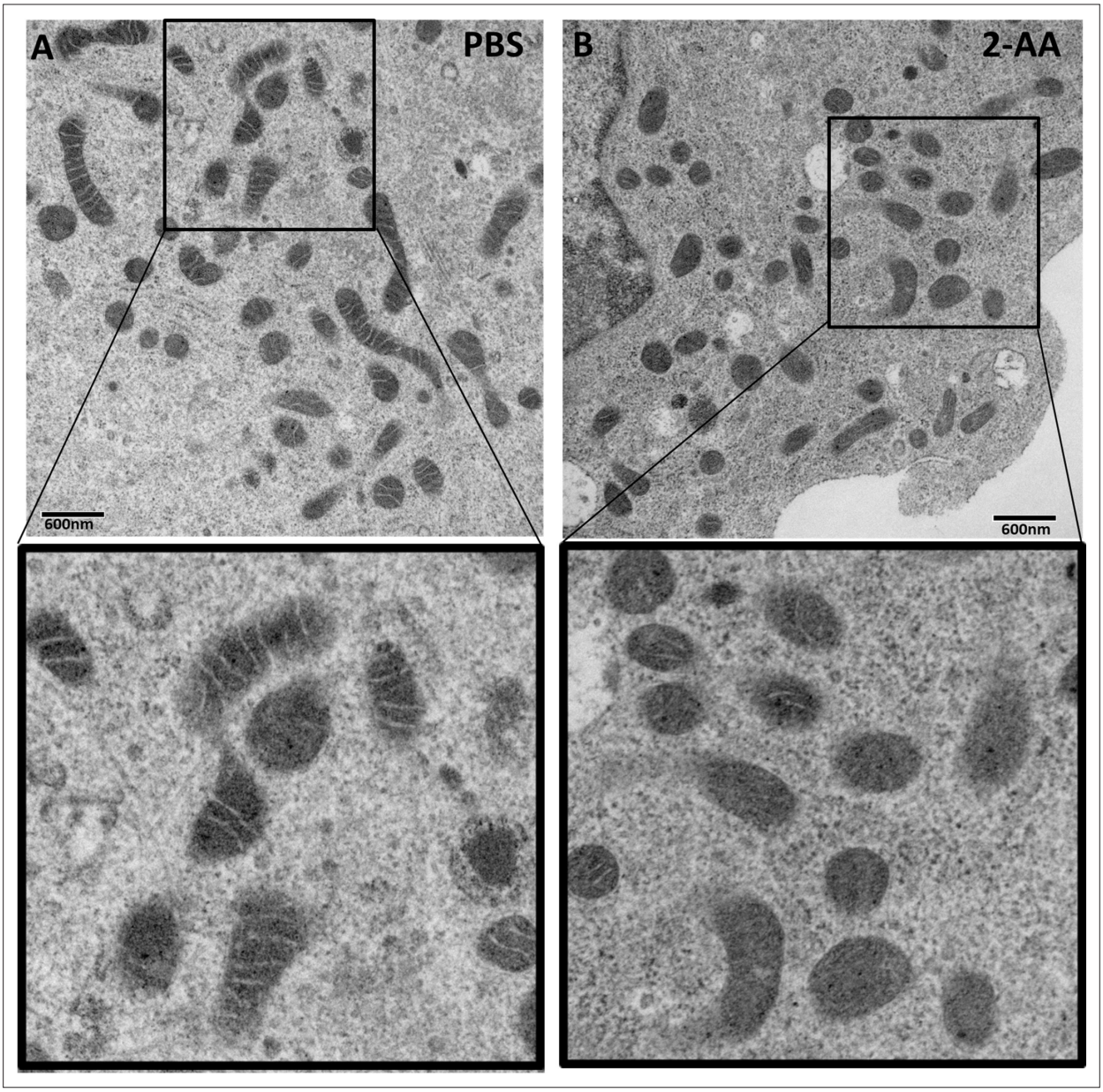

Figure 1—figure supplement 4. Transmission electron microscopy (TEM) images showing structural alterations of mitochondria in 2′-aminoacetophenone (2-AA) exposed macrophages for 48 hr. High-magnification (×30,000) TEM images showing mitochondrial abundance and structural changes in 2-AA exposed macrophages as compared to the non-exposed macrophages.

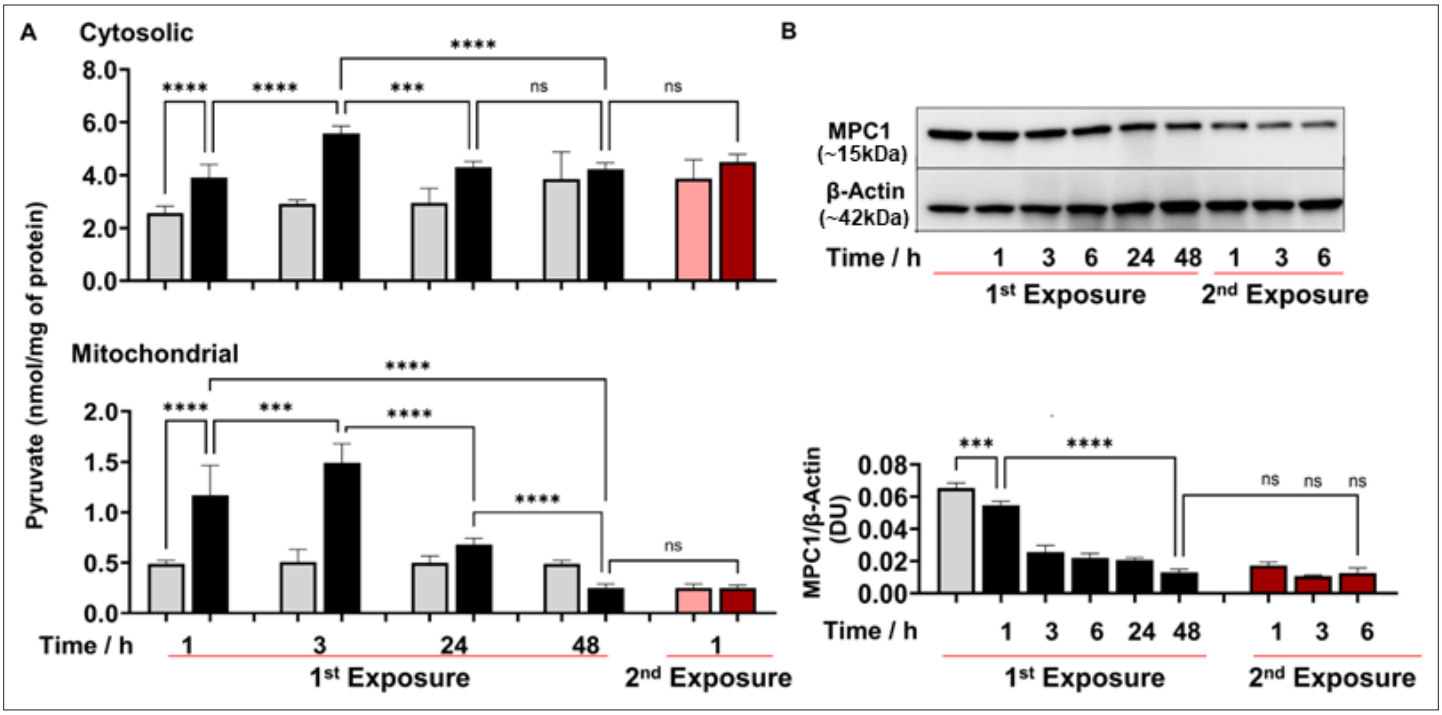

Figure 2. 2′-Aminoacetophenone (2-AA) perturbs the mitochondrial Mpc1-mediated import and metabolism of pyruvate. (**A**) Cytosolic and mitochondrial pyruvate levels following 2-AA exposure (black) or re-exposure (red) and corresponding controls in gray and pink, respectively, for indicated time points. (**B**) Representative western blot and results of densitometric analysis of Mpc1 protein levels following 2-AA exposure or re-exposure for indicated time points. β-Actin was used as a control. Corresponding controls are shown in gray or pink, respectively. Mean ± SD is shown, n=3, ***p<0.001, ****p<0.0001, and ns indicates no significant difference. One-way ANOVA followed by Tukey's post hoc test was applied.

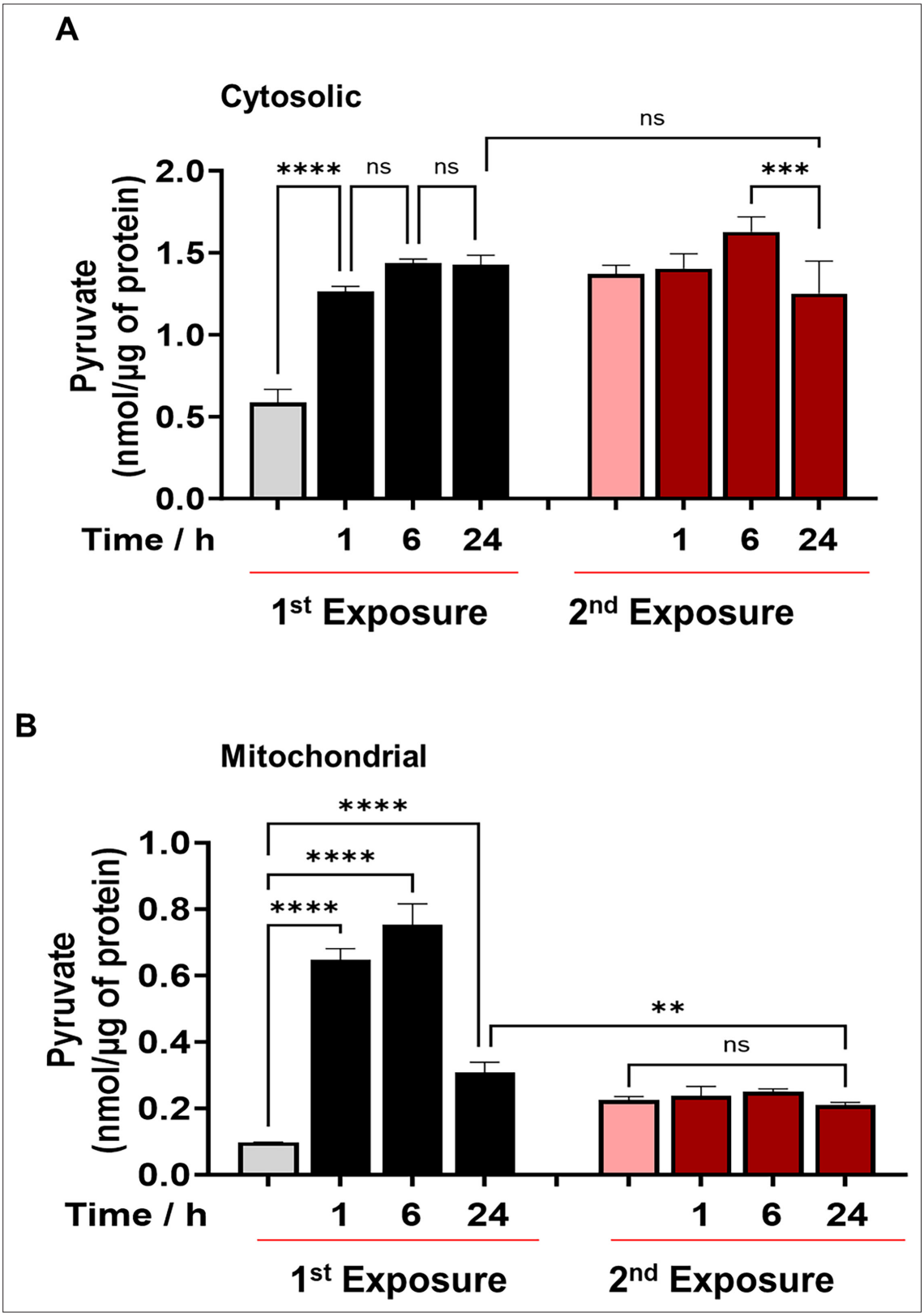

Figure 2—figure supplement 1. 2′-Aminoacetophenone (2-AA) tolerization decreases pyruvate levels in human THP-1 cells. Cytosolic (**A**) and mitochondrial (**B**) pyruvate were quantified in 2-AA (400 µM) exposed and re-exposed cells for the hours indicated using the same condition as shown in ***Figure 1A***. Mean ± SD is shown (n=3); ***p<0.001, and ns indicates no significant difference. One-way ANOVA followed by Tukey's post hoc test was applied. The same color code, black cells after first exposure and red cells after second exposure, was kept throughout the main manuscript, with corresponding controls shown in gray and pink, respectively.

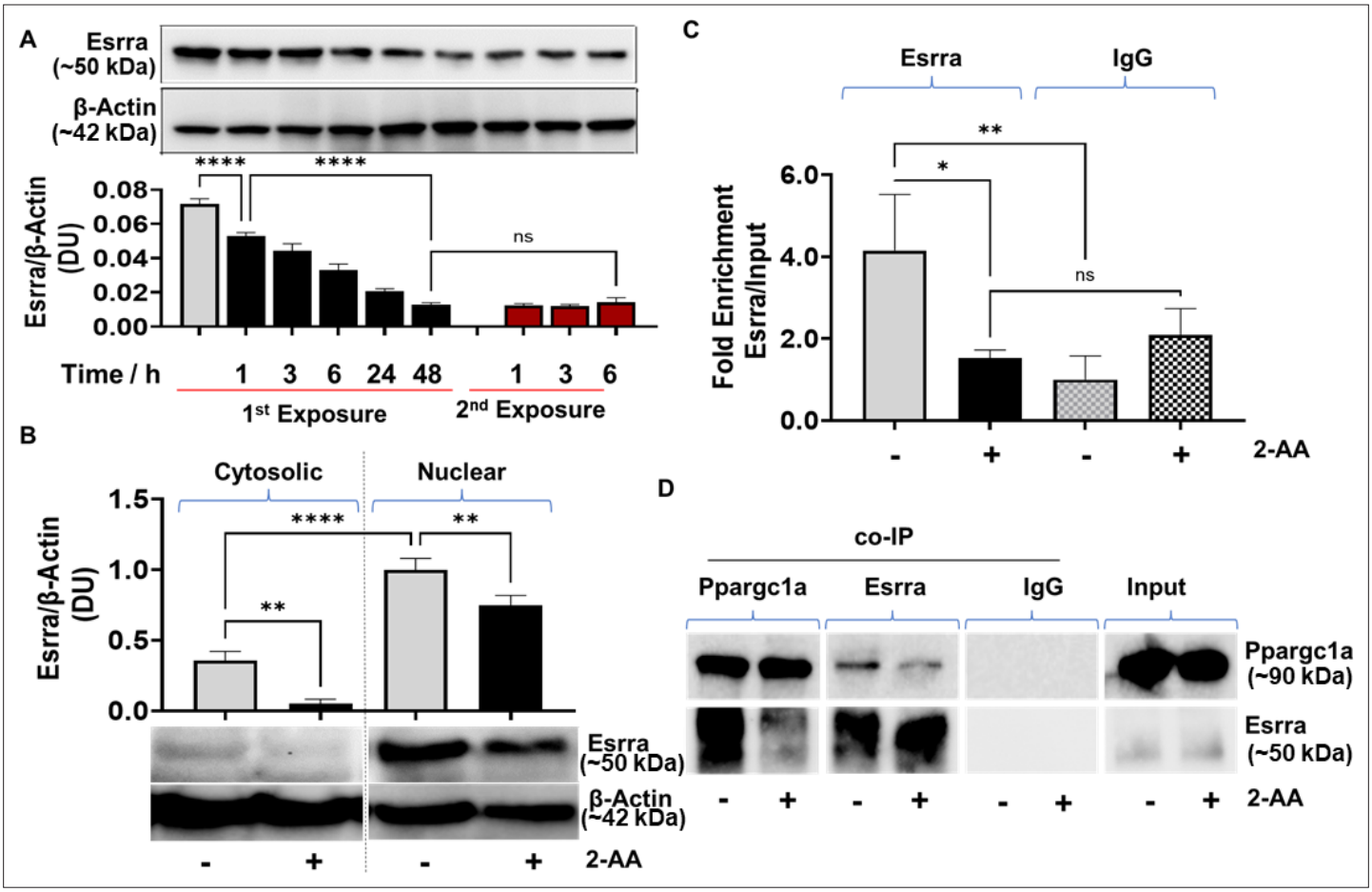

Figure 3. 2′-Aminoacetophenone (2-AA)-mediated macrophage tolerization deranges Ppargc1a/Esrra-dependent metabolic programming. (**A**) Representative western blot and results of densitometric analysis of Esrra protein levels following 2-AA exposure or re-exposure for indicated time points. β-Actin was used as a control. (**B**) Western blots and its corresponding densitometric analysis of Esrra in cytoplasmic or nuclear lysates of tolerized macrophages exposed to 2-AA for 24 hr or not exposed to 2-AA. (**C**) Chromatin immunoprecipitation (ChIP)-qPCR assay of Esrra binding at the *Mpc1* promoter in RAW 264.7 tolerized macrophages exposed to 2-AA (200 μM) for 24 hr (black) compared to untreated control macrophages (gray). IgG served as a negative control. (**D**) Representative western blot of co-immunoprecipitation (co-IP) studies of Esrra and Ppargc1a in nuclear extracts of 2-AA-tolerized (24 hr) and control RAW 264.7 cells. Pull-down with IgG served as a negative control. 2-AA-tolerized macrophages are shown in black, and untreated control macrophages in gray. Mean ± SD is shown, n ≥3, *p<0.05, **p<0.01, ****p<0.0001, and ns indicates no significant difference. One-way ANOVA followed by Tukey's post hoc test was applied.

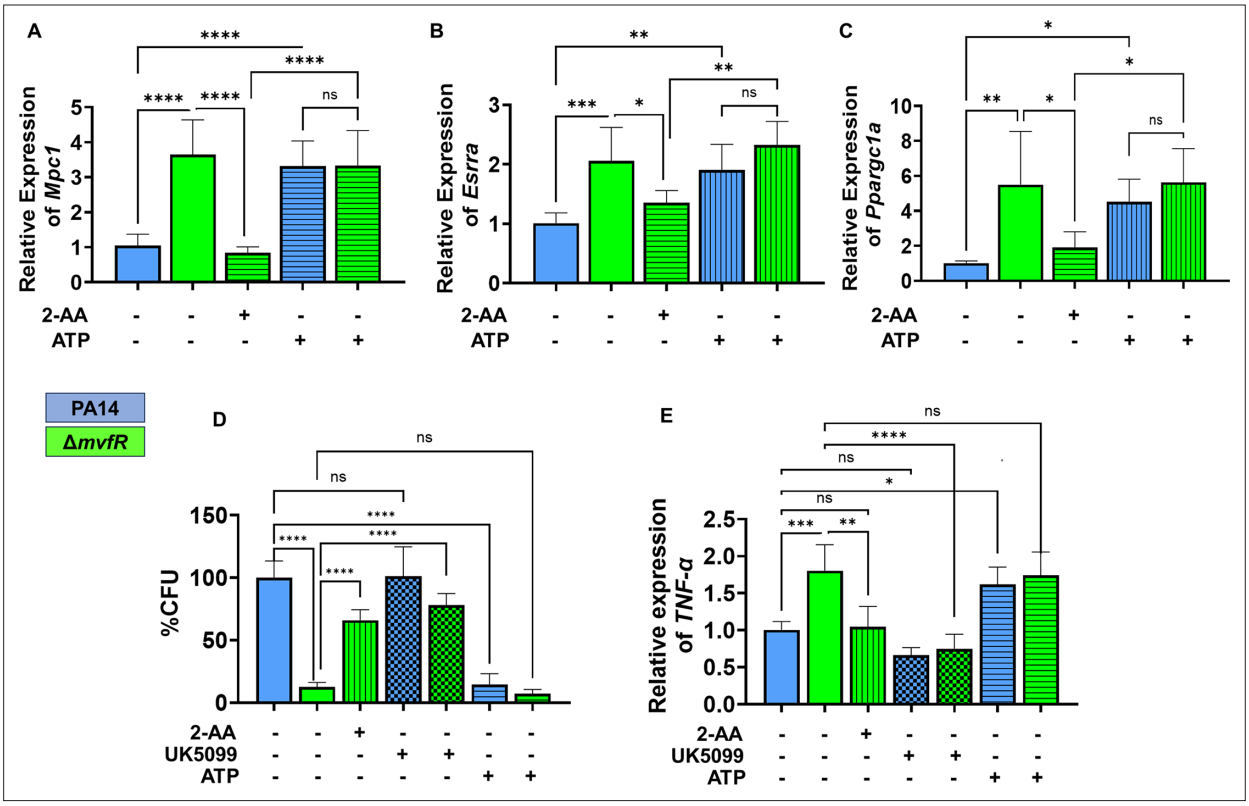

Figure 4. Increased intracellular burden in macrophages is associated with decreased expression of *Mpc1, Esrra,* and *TNF-α genes.* Real-time PCR analysis of *Mpc1* (**A**), *Esrra* (**B**), and *Ppargc1a* (**C**) expression in RAW 246.7 macrophages infected with PA14 or *ΔmvfR* in the presence or absence of exogenous addition of 2′-aminoacetophenone (2-AA) or adenosine triphosphate (ATP) for 6 hr as indicated. Transcript levels were normalized to 18S-rRNA. PA14-infected cells served as controls. (**D**) The intracellular burden of PA14 or *ΔmvfR* of infected macrophages in the presence or absence of exogenous addition of 2-AA, UK5099, or ATP. Untreated cells infected with PA14 were set as 100%. (**E**) Real-time PCR analysis of TNF-α expression in RAW 246.7 macrophages infected with PA14 or *ΔmvfR* in the presence or absence of exogenous addition of 2-AA, ATP, or UK5099. Transcript levels were normalized to 18S-rRNA. PA14-infected cells served as controls. The compound concentration used for UK5099 was 10 μM and ATP 20 μM. Mean ± SD is shown, n=3, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, and ns indicates no significant difference. One-way ANOVA followed by Tukey's post hoc test was applied.

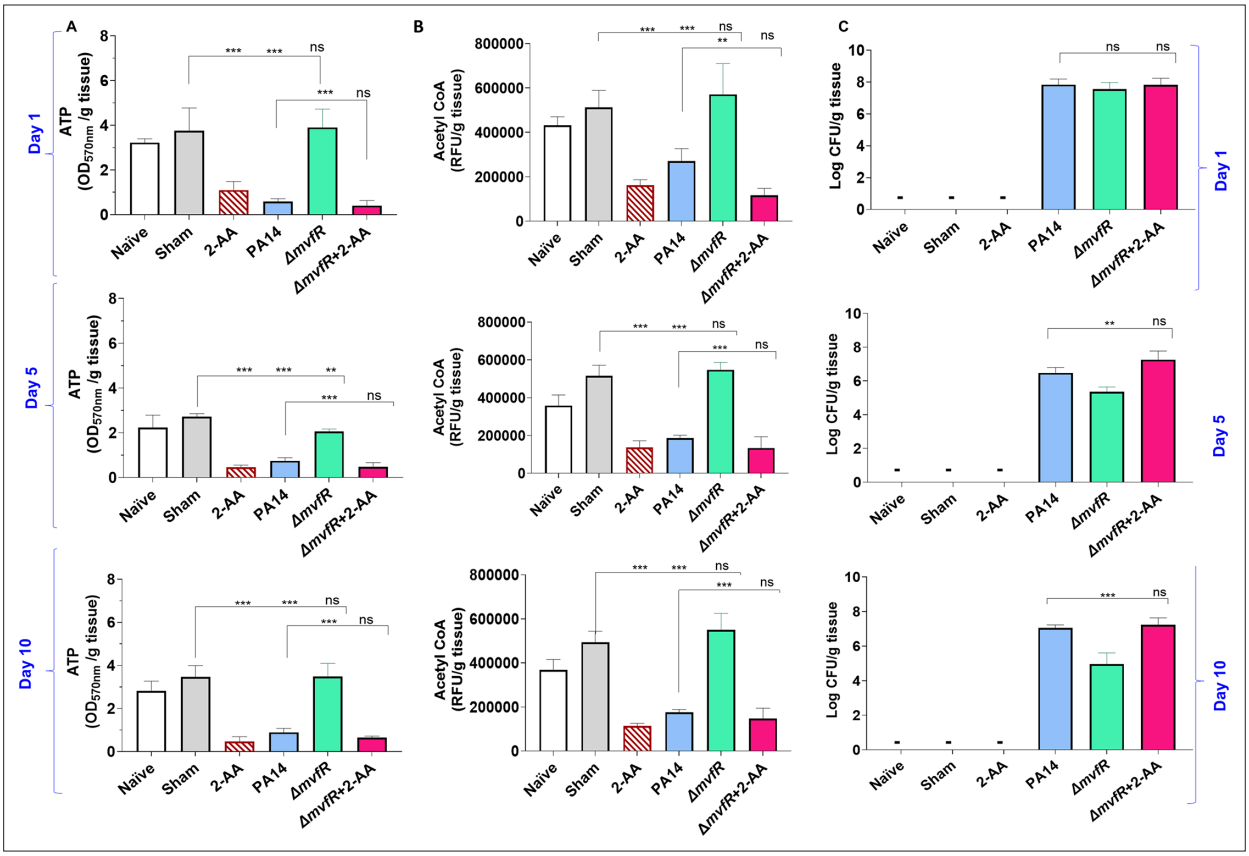

Figure 5. 2′-Aminoacetophenone (2-AA) promotes a long-lasting decrease in adenosine triphosphate (ATP), acetyl-CoA levels, and bacterial persistence in *P. aeruginosa* (*PA*)-infected mice. (**A**) ATP and (**B**) acetyl-CoA concentrations in the spleens of mice infected with *PA* wild-type (PA14), the isogenic mutant Δ*mvfR*, Δ*mvfR* injected with 2-AA at the time of infection (ΔmvfR + 2-AA), or non-infected but injected with 2-AA (6.75 mg/kg). (**C**) Bacterial burden in muscles expressed as colony-forming unit (CFU) count was analyzed using the Kruskal-Wallis non-parametric test with Dunn's post-test; ***p<0.001, and ns indicates no significant difference. Control mice groups: naïve were not given 2-AA; mice receiving 2-AA were given a single intraperitoneal injection of 2-AA; sham represents a burn/PBS group since the burn and infection model was used. Results of three independent replicates with four mice per group for 1, 5, and 10 days are shown. Means ± SD are shown, *p<0.05, **p<0.01, ***p<0.001, and ns indicate no significant difference. One-way ANOVA followed by Tukey's post hoc test was applied.

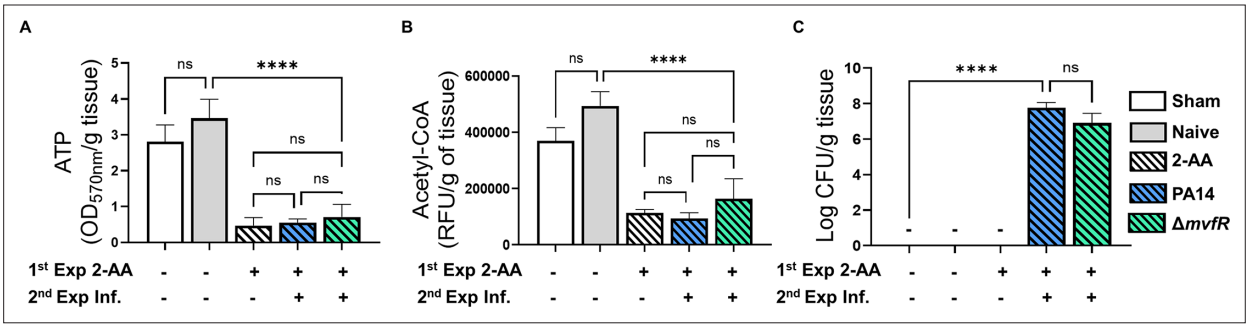

Figure 5—figure supplement 1. Exposure and re-exposure to 2′-aminoacetophenone (2-AA) promotes a long-lasting decrease in adenosine triphosphate (ATP) and acetyl-CoA levels and sustains bacterial presence in mice receiving first exposure to 2-AA by injecting 2-AA and second exposure through infection with PA14 or Δ*mvfR* 4 days post-2-AA injection. (**A**) ATP and (**B**) acetyl-CoA concentrations in the spleens of mice. (**C**) Bacterial burden in muscles expressed as colony-forming unit (CFU) counts was analyzed using the Kruskal-Wallis non-parametric test with Dunn's post-test; ***p<0.001, and ns indicates no significant difference. Control mice groups: naïve were not given 2-AA; mice receiving 2-AA were given a single intraperitoneal injection of 2-AA 4 days prior to infection; sham represents a burn/PBS group since the burn and infection model was used. N=4 mice per group, and 10 days post-infection is shown. Means ± SD are shown, *p<0.05, **p<0.01, ***p<0.001, and ns indicate no significant difference. One-way ANOVA followed by Tukey's post hoc test was applied.

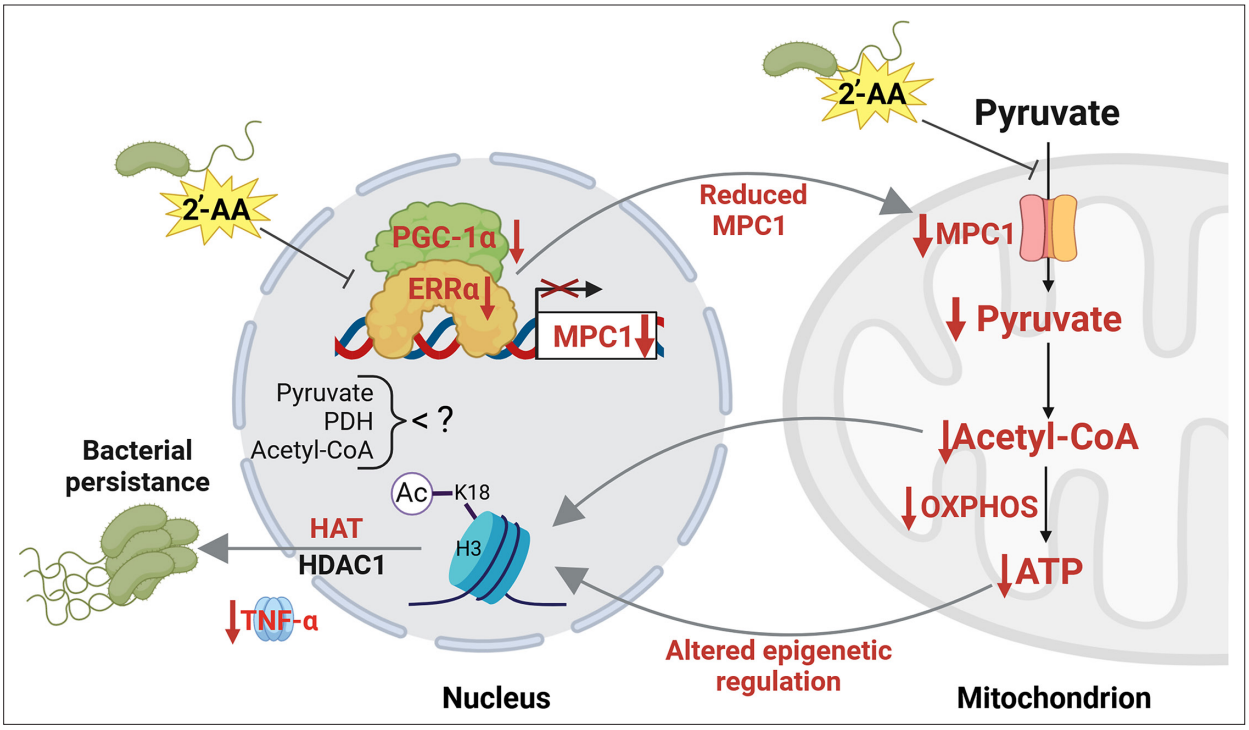

Figure 6. Proposed mechanism by which 2'-aminoacetophenone (2-AA) impairs bioenergetics through the inhibition of Mpc1-mediated pyruvate transport into mitochondria and its impact on the Ppargc1a/Esrra axis. 2-AA-tolerized macrophages exhibit diminished pyruvate levels in mitochondria due to the decreased expression of *Mpc1*, a consequence of the 2-AA impact on the interaction of Esrra with the transcriptional coactivator Ppargc1a for the effective transcription of *Esrra* since *Esrra* controls its own transcription and that of *Mpc1*. In the presence of 2-AA, the weakened interaction between Esrra and Ppargc1a results in reduced expression of *Mpc1* and *Esrra*. The reduction in mitochondrial pyruvate levels leads to decreased acetyl-CoA and adenosine triphosphate (ATP) levels, which modulate histone deacetylase 1 (HDAC1)- and histone acetyltransferase (HAT)-catalyzed remodeling of H3K18 acetylation. The diminished levels of this epigenetic mark have previously been associated with an increased intracellular presence of bacteria in macrophages, as demonstrated by our group (***Bandyopadhaya et al., 2016b***). Pathways, proteins, and metabolites that are negatively affected are indicated in red, while positively affected are denoted in black. Figure 6 was created with BioRender.com, and published using a CC BY-NC-ND license with permission.