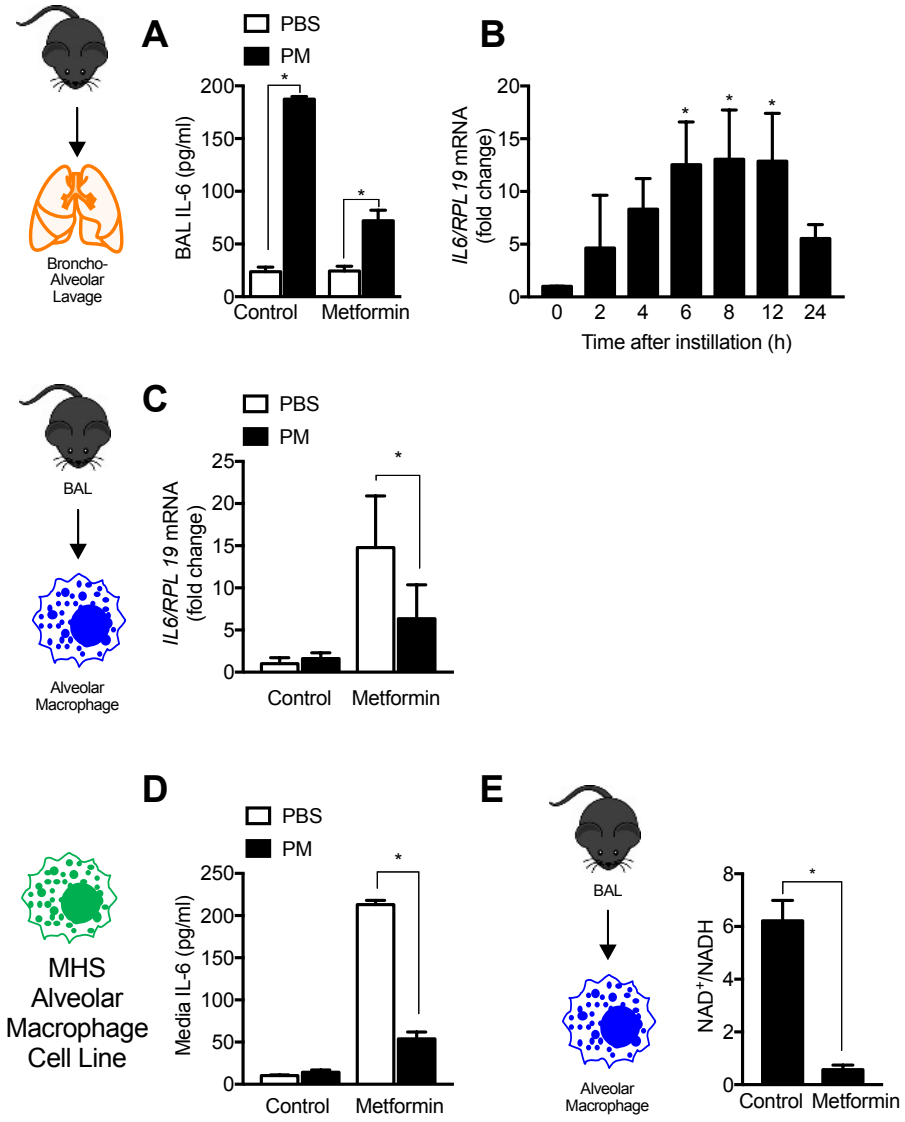
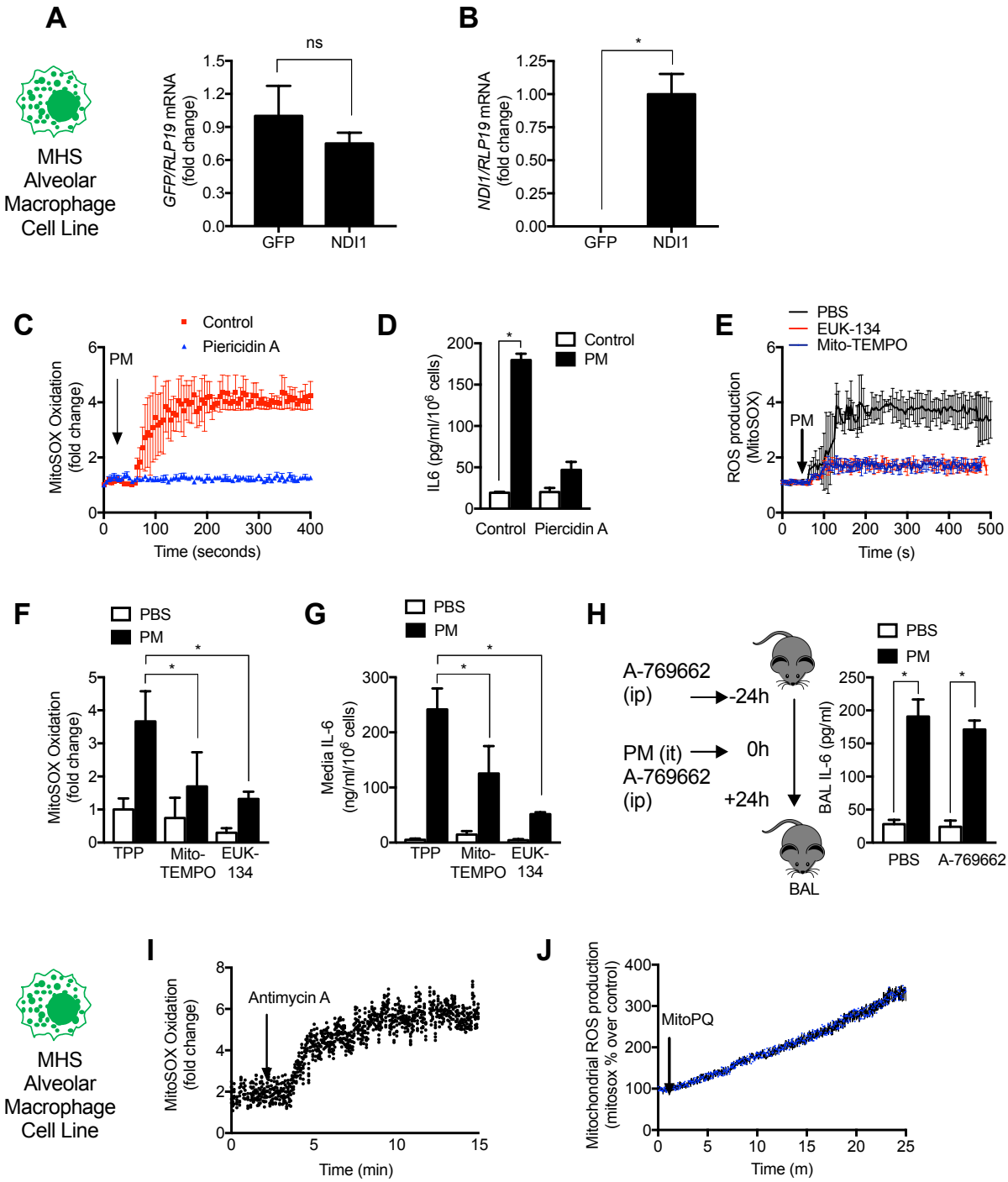
Supplemental Information



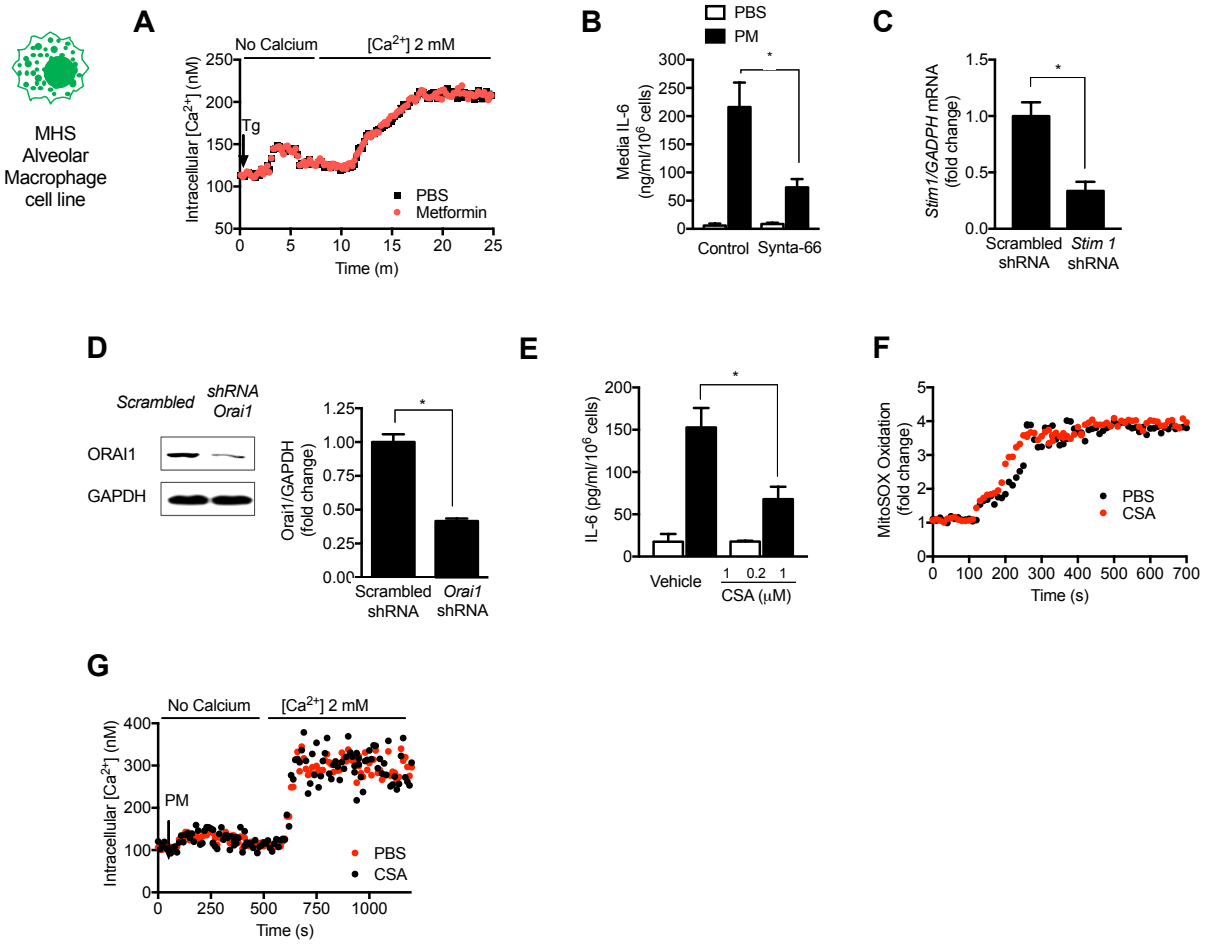
Supplemental Figure 1. Refers to Figure 1. Mice were treated with metformin in their drinking water beginning 24 hours before the intratracheal administration of PM (National Institute of Standards and Technology (NIST), 10 µg/animal in 50 µL PBS) and (**A**) the levels of IL-6 were measured in the bronchoalveolar lavage fluid 24 hours later (n=3–6 per condition, * $p < 0.05$). (**B**) Wild-type C57Bl/6 mice were treated with PM (10 µg/mouse) at time 0 and alveolar macrophages were obtained from BAL fluid at the indicated times after the instillation of PM and the levels of *Il6* mRNA were measured using RT-qPCR (n=3 animal per condition, $p < 0.05$ for comparison with control). (**C**) Mice were administered metformin in their drinking water for 24 hours before the intratracheal administration of PM and the levels of *Il6* mRNA in alveolar macrophages isolated from BAL were measured 6 hours later (n=4 animals per condition, * $p < 0.05$). (**D**) MHS cells were treated with PM (10 µg/cm^3) in the presence or absence of metformin (1 mM) and the levels of IL-6 were measured (n=3, * $p<0.05$). (**E**) Mice were treated with metformin in the drinking water for 24 hours and the levels of oxidized and reduced

nicotinamide adenine dinucleotide (NAD^+/NADH) were measured in BAL fluid macrophages using a colorimetric assay (n=6 animals per condition, * $p<0.05$).

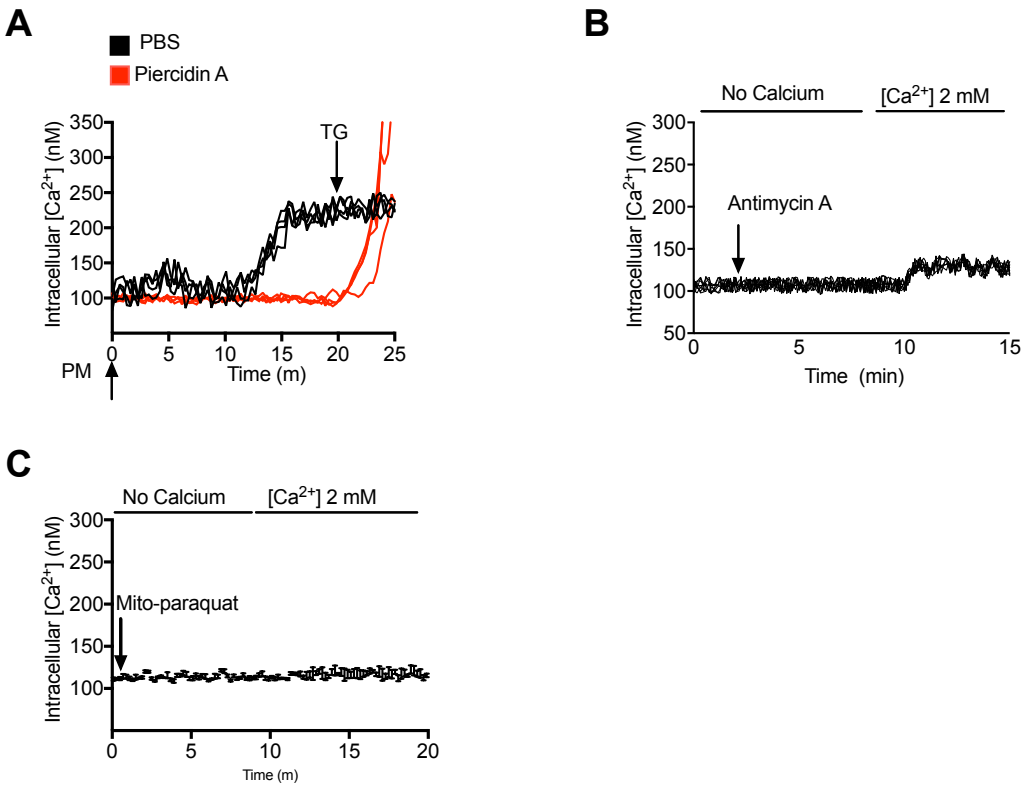


Supplemental Figure 2. Refers to Figure 2. (**A,B**) MHS cells were stably transfected with a lentivirus encoding GFP (control) or GFP-NDI1, a yeast protein capable of transferring electrons from NADH to complex II/III but incapable of ROS generation, and mRNA encoding GFP and NDI1 were measured by RT-qPCR (n=3, * $p < 0.05$). (**C,D**) MHS cells were treated with the complex I inhibitor piercidin A (1μM) and mitochondrial ROS generation was estimated from the oxidation of a mitochondrially localized oxidant sensitive fluorescent dye (MitoSOX) on the

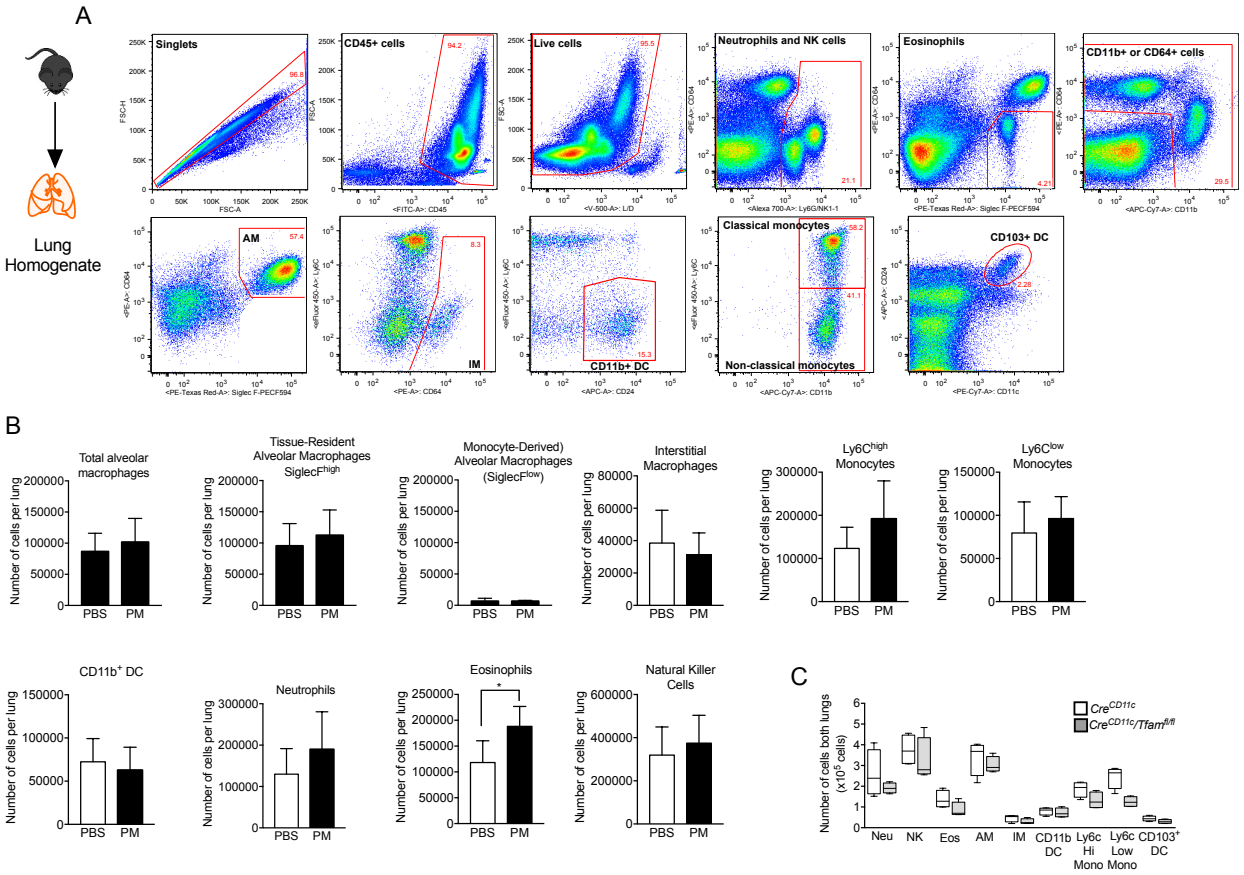
stage of an epifluorescent microscope and IL-6 release into the media was measured 24 hours later (* $P < 0.05$ n=3). (**E-F**) MHS cells were treated with the mitochondrially targeted antioxidant Mito-TEMPO or the control cation (TPP), both 5 μM, or the non-selective superoxide dismutase/catalase mimetic EUK-134 (20 μM) before treatment with PM and mitochondrial ROS generation was estimated from the oxidation of a mitochondrially localized oxidant sensitive fluorescent dye (MitoSOX) on the stage of an epifluorescent microscope (n=3-8 per condition, * $P<0.05$). (**G**) MHS cells were pretreated with Mito-TEMPO or TPP (both 5 μM) or the combined superoxide dismutase/catalase mimetic EUK-134 (20 μM) and IL-6 was measured in the media 24 hours later (n=3-8 per condition, * $P<0.05$). (**H**) Mice were treated with A-769662 (30 mg/kg, i.p or vehicle) 24 hours before and on the day of treatment with PM (10 μg/mouse, i.t.) and BAL fluid levels of IL-6 were measured 24 hours later (n=5 animals per group, differences not significant). (**I,J**) MHS cells were treated with Antimycin A (10 μM) or mitochondrially targeted paraquat (5 μM) and mitochondrial ROS generation was estimated from the oxidation of a mitochondrially localized oxidant sensitive fluorescent dye (MitoSOX) on the stage of an epifluorescent microscope (3 areas from one representative replicate are shown).



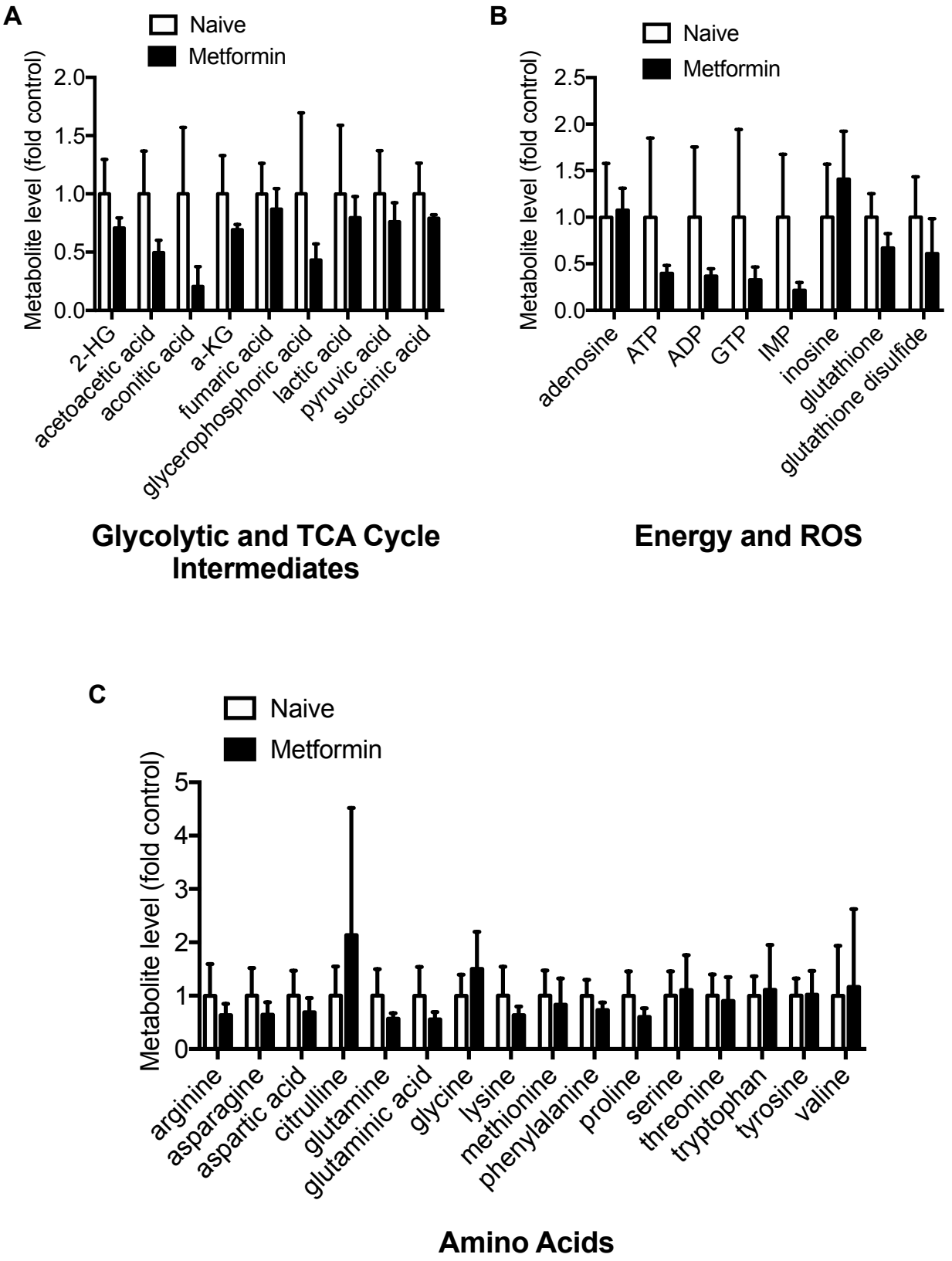
Supplemental Figure 3. Refers to Figure 3. (**A**) MHS cells were loaded with Fura-2 on the stage of an epifluorescent microscope and then treated with thapsigargin (25 nM) in the presence or absence of metformin (1 mM) and intracellular calcium concentrations were measured. (**B**) MHS cells were treated with PM (10 μg/cm^2) in the presence or absence of Synta-66 (10 μM) and the level of IL-6 in the media was measured 24 hours later. (**C**) MHS cells were stably transfected with a lentivirus encoding Stim1 mRNA and the levels of *Stim1* mRNA were measured. **(D)** MHS cells were stably transfected with a lentivirus encoding a shRNA against *Orai1* or a standard scrambled lentivirus and the levels of ORAI1 protein were assessed by immunoblotting. (**E**) MHS cells were treated with cyclosporine A (CSA) (200nM) prior to exposure to PM (10 μg/cm^2) and the levels of IL-6 in the media were measured 24 hours later (n=3, * P<0.05). (**F**) MHS cells were loaded with MitoSox on the stage of an epifluorescent microscope and treated with PM in the presence or absence of CSA (200 nM). (**G**) MHS cells were loaded with Fura-2 on the stage of an epifluorescent microscope and treated with PM in the presence or absence of CSA (200 nM) and intracellular calcium was measured (representative experiment from three replicates shown).



Supplemental Figure 4. Refers to Figure 4. (**A**) MHS cells were loaded with Fura-2 on the stage of an epifluorescent microscope and intracellular calcium was measured after the addition PM (10 μg/cm^3) in the presence or absence of Piercidin A (1 μM). (**B,C**) MHS cells were loaded with Fura-2 on the stage of an epifluorescent microscope and intracellular calcium was measured after the addition of Antimycin A (10 μM) or mito-paraquat (5 μM) (n=3, * P<0.05).



Supplemental Figure 5. Refers to Figure 4 and 5. (**A**) Flow cytometry gating strategy used to identify myeloid subpopulations in the mouse lung. (**B**) Intratracheal administration of PM (10 µg per mouse intratracheally) does not evoke recruitment of monocyte-derived alveolar macrophages. Mice were treated with intratracheally administered PM (10 µg/animal) and the lungs were processed for flow cytometric analysis 24 hours later. (**C**) *Cre*^{CD11c}*Tfam*$^{flox/flox}$ mice do not exhibit changes in myeloid populations in the lung in the steady state.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Naive
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1.5
1.0
0.5
0.0
2-HG
acetoacetic acid
aconitic acid
a-KG
fumaric acid
glycerophosphoric acid
lactic acid
pyruvic acid
succinic acid
Glycolytic and TCA Cycle Intermediates
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1.5
1.0
0.5
0.0
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ATP
ADP
GTP
IMP
inosine
glutathione
glutathione disulfide
Energy and ROS
C
Naive
Metformin
Metabolite level (fold control)
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4
3
2
1
0
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asparagine
aspartic acid
citrulline
glutamine
glutaminic acid
glycine
lysine
methionine
phenylalanine
proline
serine
threonine
tryptophan
tyrosine
valine
Amino Acids

Supplemental Figure 6. Related to Figure 7. Mice were administered metformin in the drinking water (150 mg/kg/day) or received standard drinking water beginning two days before the intratracheal administration of PM (10 μg/mouse) or no treatment, and continuing until harvest 24 hours after the administration of PM. BAL macrophages were isolated from the mice as rapidly as possible, washed, pelleted and counted for normalization. 100,000 BAL macrophages in 75 μL PBS were snap frozen for mass spectroscopy based measures of cellular metabolites (See STAR Methods: Metabolomic analysis). ANOVA of 154 detected metabolites revealed significant differences between macrophages from naïve and metformin treated animals ($P<0.001$) but individual metabolites did not meet criteria for discovery (FDR q value of 0.1) using two stage linear step up procedure of Benjamini, Krieger Benjamini, Krieger and Yekutieli. (**A**) Metabolites involved in glycolysis or TCA cycle metabolism, (**B**) Metabolites involved in energy homeostasis and redox maintenance, (**C**) Amino Acids.