

Figure S1. Confirmation of attenuation of autophagy and its effects on inflammasome activation. **(A)** Western blot of cell lysates infected as shown

and immunostained for LC3. The unmodified (LC3-I) and lipidated isoform (LC3-II) are shown in 3 separate experiments. TUBB5 is shown as a loading control. **(B)** fluorescent imaging of BMDMs cells infected as indicated; LC3 is shown green, nuclei in blue (DAPI stain). Scale bar = 10 μ m. **(C)** quantification of LC3 puncta as shown in **(B)**. Columns are the mean number of puncta per cell, infected as shown; error bars are sem. *** significant difference between WT animals and *Vav-atg7^{-/-}* mice, $p < 0.001$. **(D)** as **(A)**, but in cells treated with Control siRNA or siRNA specific for *Atg5*. Cells were infected for 4 h as shown. Infected lysates are from 4 independent experiments. **(E)** immunoblot of lysates of cells infected and treated as shown. TUBB5 is shown as a loading control. Graphs show IL1B and TNF secretion from cells treated as shown. Columns are means of triplicate independent determinations; error bars are sem. ** indicates significant difference from infected cells without 3-MA, $P < 0.01$. Data are representative of 3 independent experiments. **(F)** as **(E)** but in cells infected with the PA01 strain. * and ** indicate significant difference from infected but untreated cells, $P < 0.05$ and 0.001 respectively.

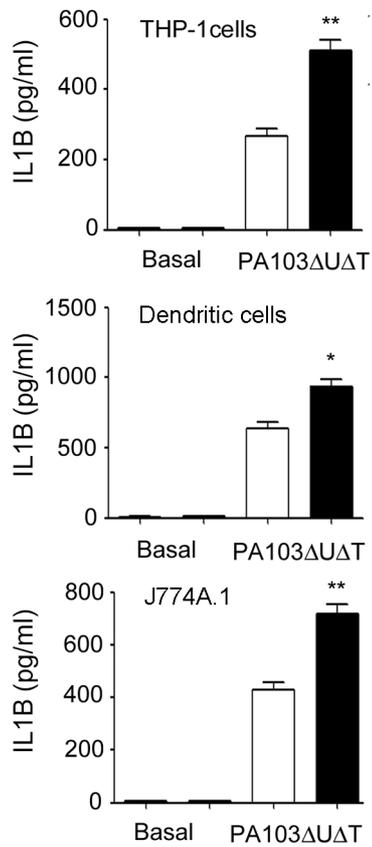


Figure S2. Effect of 3-MA inhibition of autophagy on inflammasome activation. Levels of secreted IL1B from cells as shown in the absence (open bars) or presence (filled bars) of 3-MA. Columns are means of triplicate independent determinations; error bars are sem. * indicate significant differences between the levels in the presence and absence of 3-MA, $P < 0.05$, and ** $P < 0.01$.

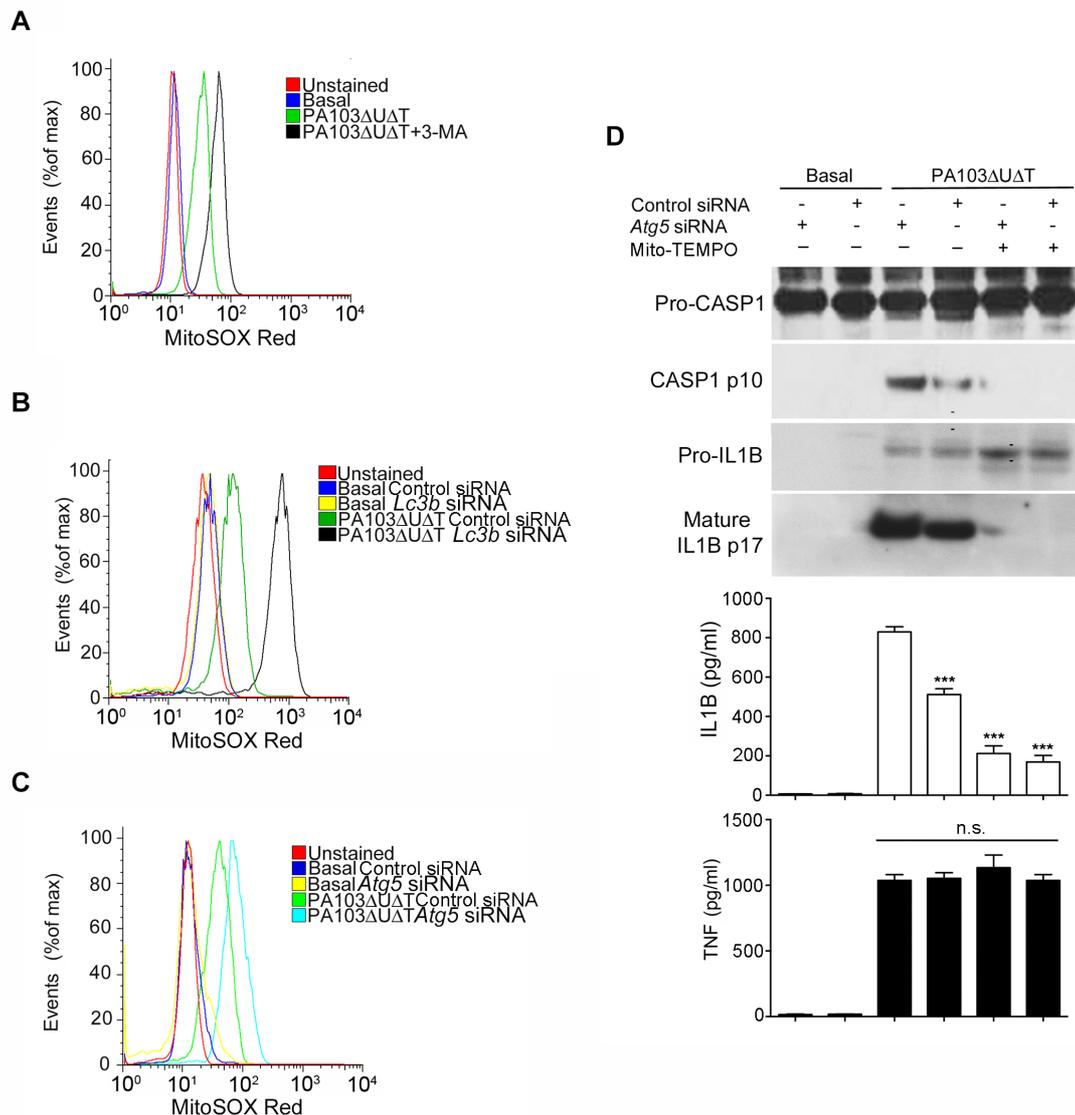


Figure S3. Inhibiting autophagy increases production of reactive mitochondrial oxygen following infection with *P. aeruginosa*. (**A to C**) Flow cytometry of BMDMs left uninfected (basal) or infected with PA103ΔUΔT (MOI 25) for 4 hours and stained with MitoSox. Cells were treated as indicated with 3-MA (**A**), control siRNA or siRNA for *Map1lc3b* (**B**), or control siRNA or siRNA for *Atg5* (**C**). (**D**) shows results using BMDMs treated with control siRNA or siRNA specific for *Atg5* and with the indicated infection and treatments. All experiments were repeated 2 or 3 times.

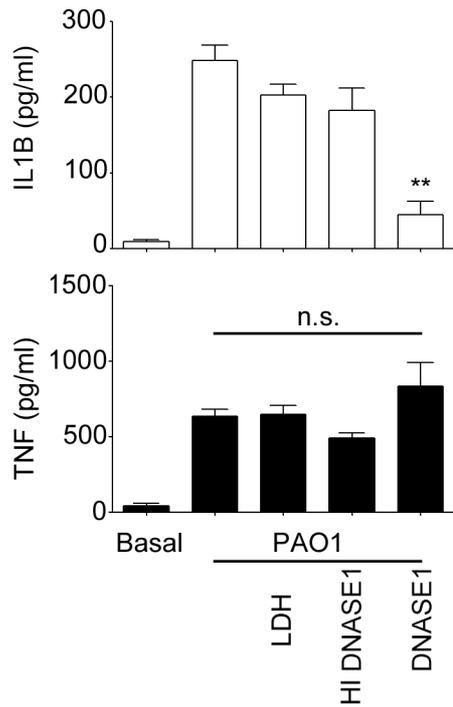


Figure S4. DNase I reduces secreted IL1B production in PAO1 infection. BMDMs were left uninfected (basal) or infected with PAO1 with the indicated proteins transfected into the cells as in **Fig. 5A**.

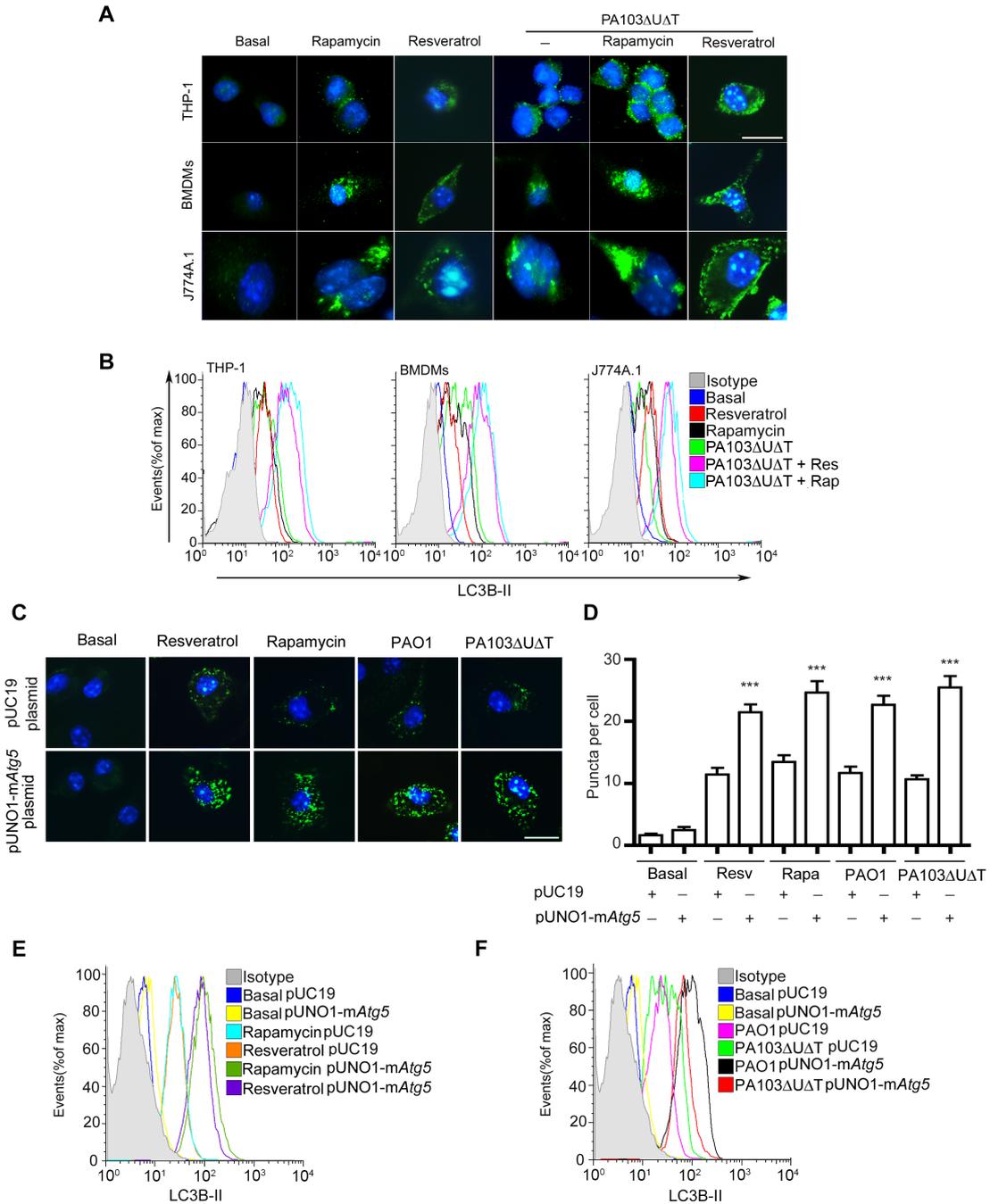


Figure S5. Induction of autophagy. **(A)** representative immunofluorescence images of LC3 in THP-1, BMDMs, and J774A.1 cells. Cells left uninfected (Basal), treated with rapamycin (50 μ g/ml) or resveratrol (50 μ g/ml) for 4 h, or infected with PA103 Δ U Δ T or PA103 Δ U Δ T+rapamycin or reseveratrol for 4 h at a MOI of 25. Cells were stained with DAPI to visualize nuclei (blue), and LC3 staining is shown as green. Scale bar = 10 μ m (representative of 3

independent experiments). **(B)** Flow cytometric analysis of LC3B-II protein following infection with PA103 Δ U Δ T (MOI 25 for 4 h), in the presence and absence of rapamycin or resveratrol. **(C)** immunofluorescence images for LC3B (green) and nuclei (blue) of BMDMs transfected with control plasmid or expression plasmid for murine *Atg5* (pUNO-*mAtg5*) and treated or infected (4 h, MOI 25) as shown. **(D)** quantification of the puncta formed as shown in **(C)**. Bars are means of determinations for at least 50 cells; error bars are sem. ***, indicates significant difference from control transfected cells, $P < 0.001$. **(E** and **F)**, as in **(B)**, with cells treated as shown.

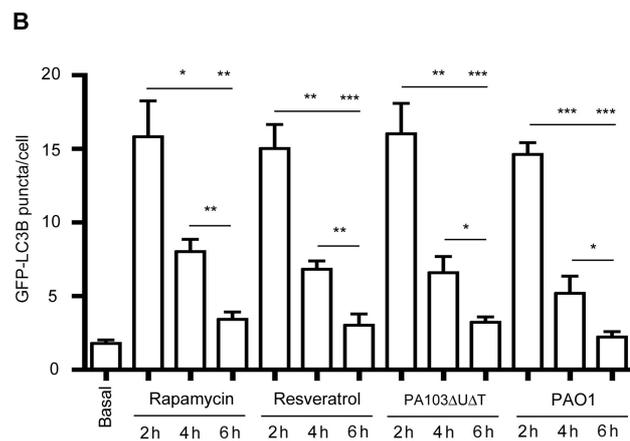
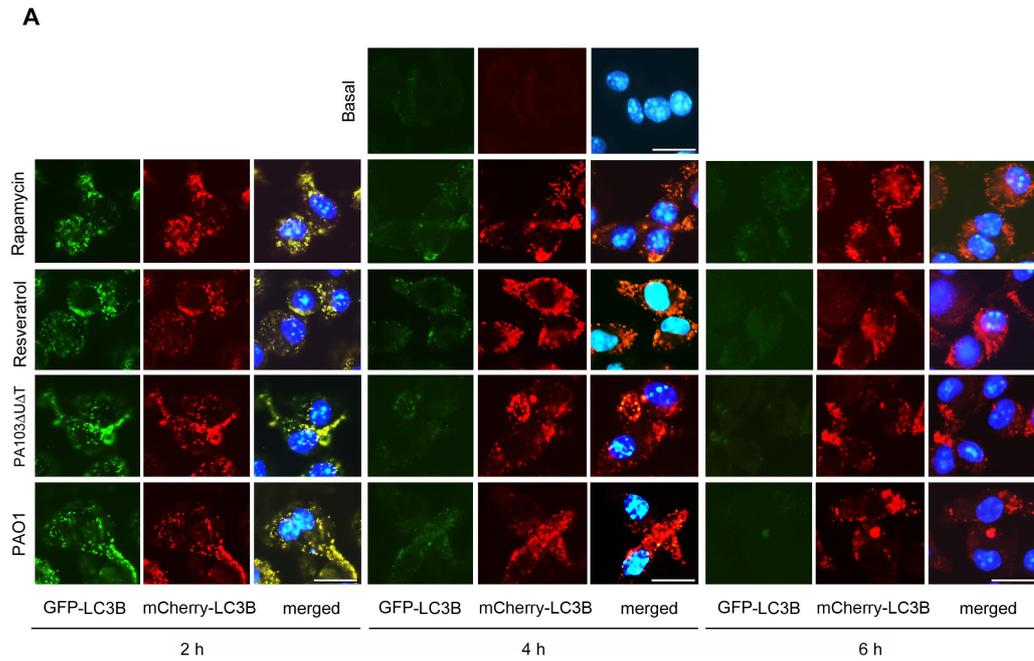


Figure S6. Flux through autophagic pathway following infection. **(A)** immunofluorescent images of BMDMs transfected with the expression plasmid for tandem GFP-mCherry-LC3B and treated or infected (MOI 25) as shown for the indicated times. Panels show signal from GFP-LC3B (green), mCherry-LC3B (red), and merged (overlapping signal yellow, nuclei, blue). **(B)** Quantification of data from **(A)**. Bars show mean numbers of GFP-LC3B puncta per cell for at least 50 cells; bars are SEM. Treatments and infections as shown. Asterixes show significant differences over time, *, **, and *** with $P < 0.05$, 0.01 and 0.001 respectively.