Supplemental Fig. S1.

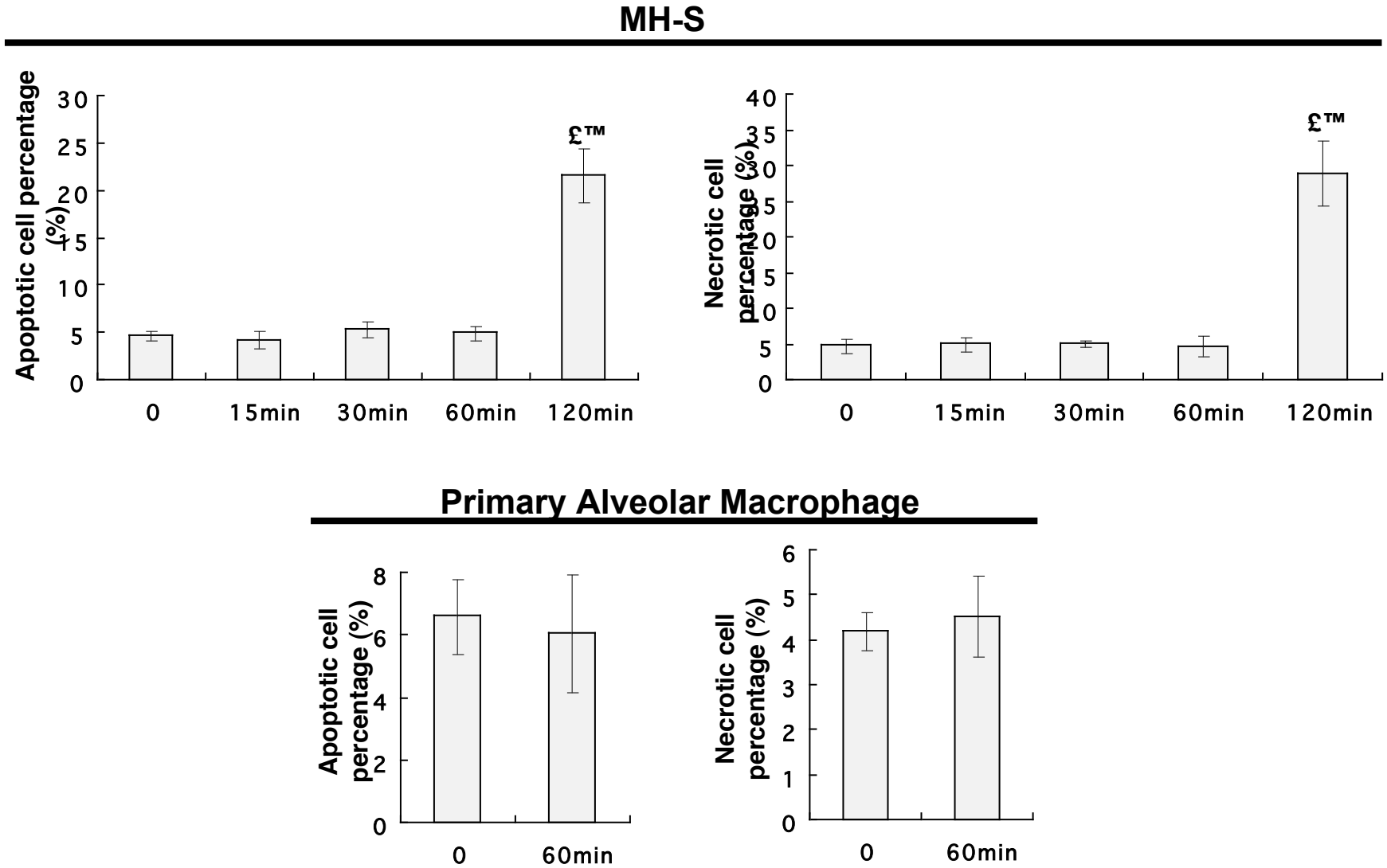

Fig. S1. Analysis of cell death with MH-S and primary AM following PA infection. The cells were infected with PAO1 at the indicated times and cell death (apoptosis and necrosis) was measured using Vybrant assay (Invitrogen). Apoptosis and necrosis were calculated according to the intensity of YO-PRO1 (green, apoptosis) and propidium iodide (red, necrosis) (*$p<0.05$).

Supplemental Fig. S2

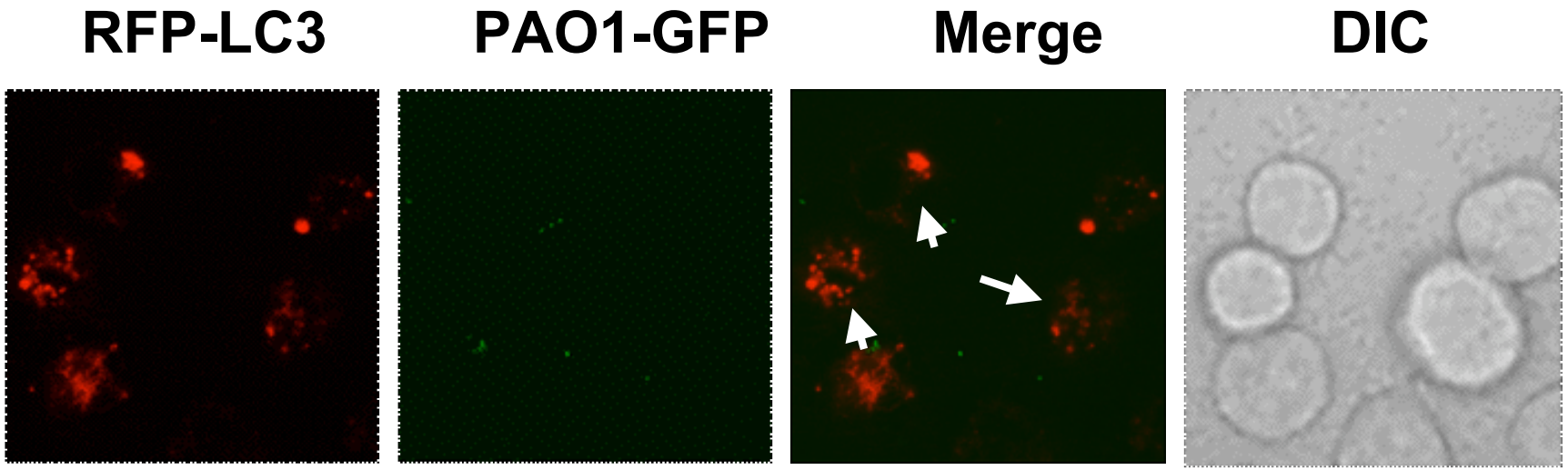

Fig. S2. PA induced autophagy in primary mouse AM cells. The cells were transiently transfected with RFP-LC3 plasmid and infected with PAO1-GFP (arrows showing the puncta that are colocalized with PAO1). Non-infection and vector only control did not show puncta staining (not shown). To account for the experimental errors, cells were also pre-treated with either rapamycin (3 mM, 12 h, positive control for induction of autophagy) or 3-MA (3 mM, 3 h, for blockade of autophagy) before infection (data not shown).

Supplemental Fig. S3

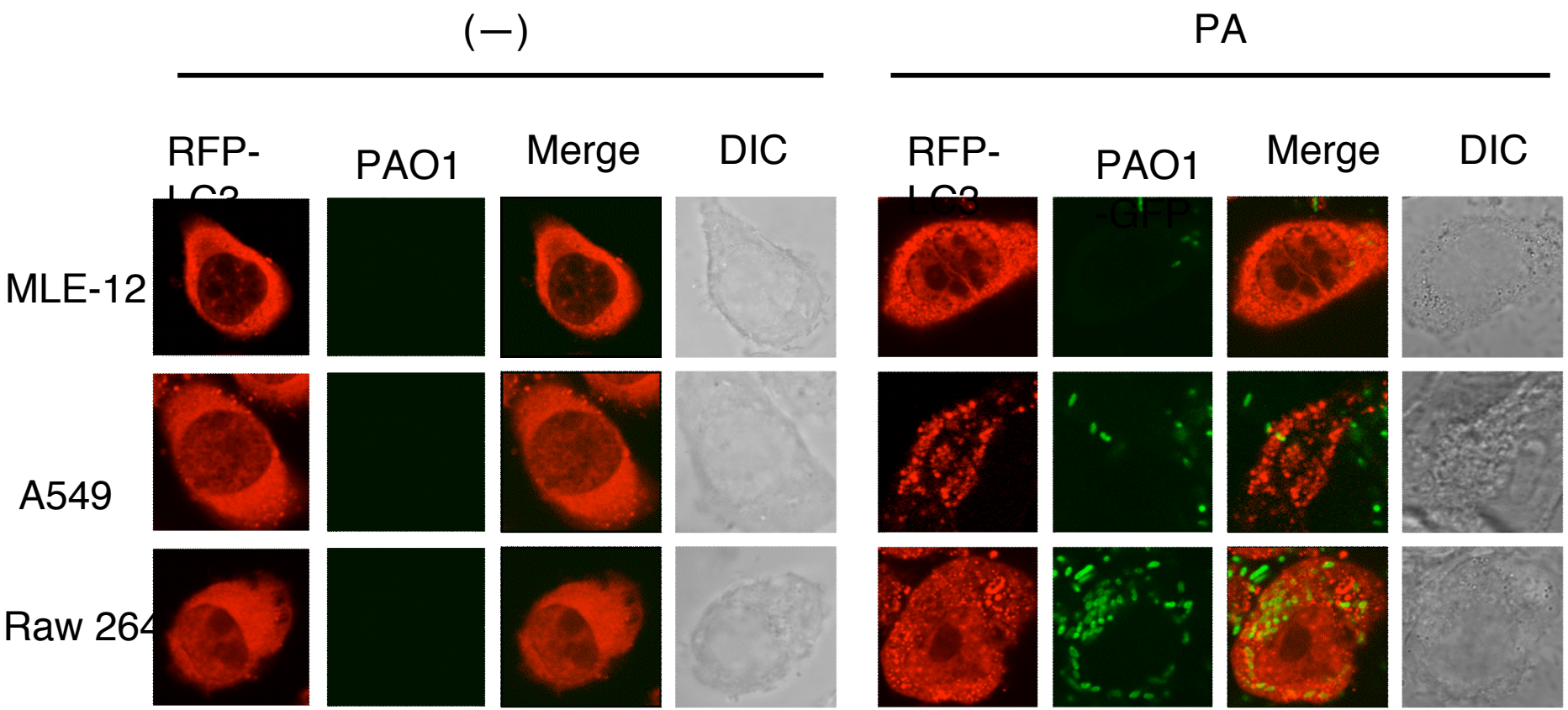

Fig. S3. PA infection induces autophagy in MLE-12, A549, and RAW264.7 cells. The cells were transfected with RFP-LC3 plasmid for 24 h and then infected with PAO1-GFP for 1 h (MOI=10:1). To account for the experimental errors, cells were also pre-treated with either rapamycin (3 mM, 12 h, positive control for induction of autophagy) or 3-MA (3 mM, 3 h, for blockade of autophagy) before infection (data not shown).

Supplemental Fig. S4

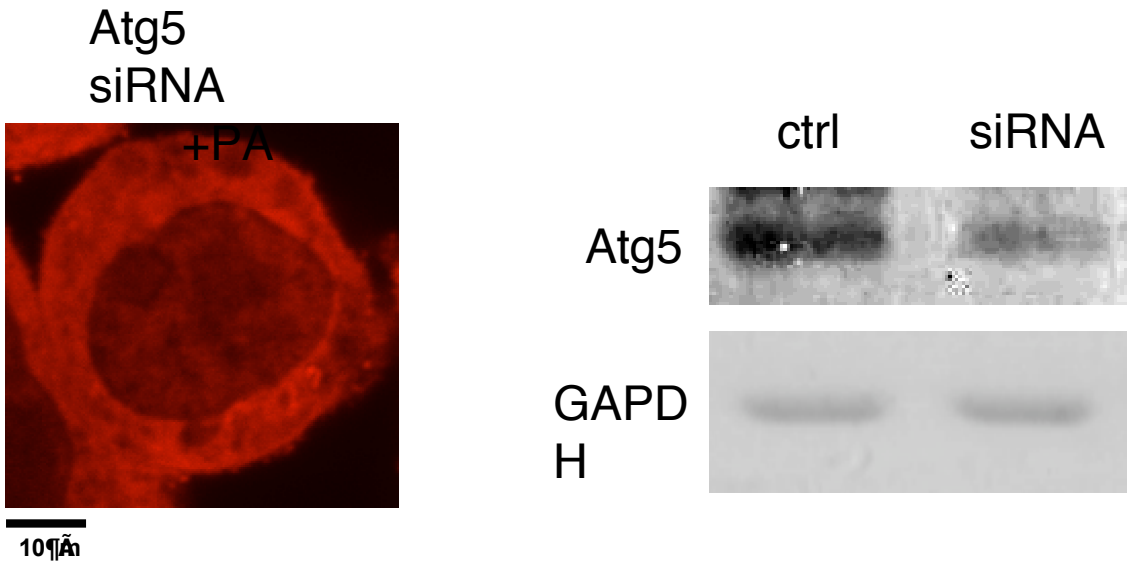

Fig. S4. PA induced-autophagy is dependent on Atg5. After transfection with a LC-3-RFP plasmid, the cells were also treated with negative control siRNA or Atg5 siRNA. Confocal images were presented to show LC3 puncta induction. Western blotting of Atg5 was performed to show the successful reduction in Atg5. GAPDH was probed as a loading control.

Supplemental Fig. S5

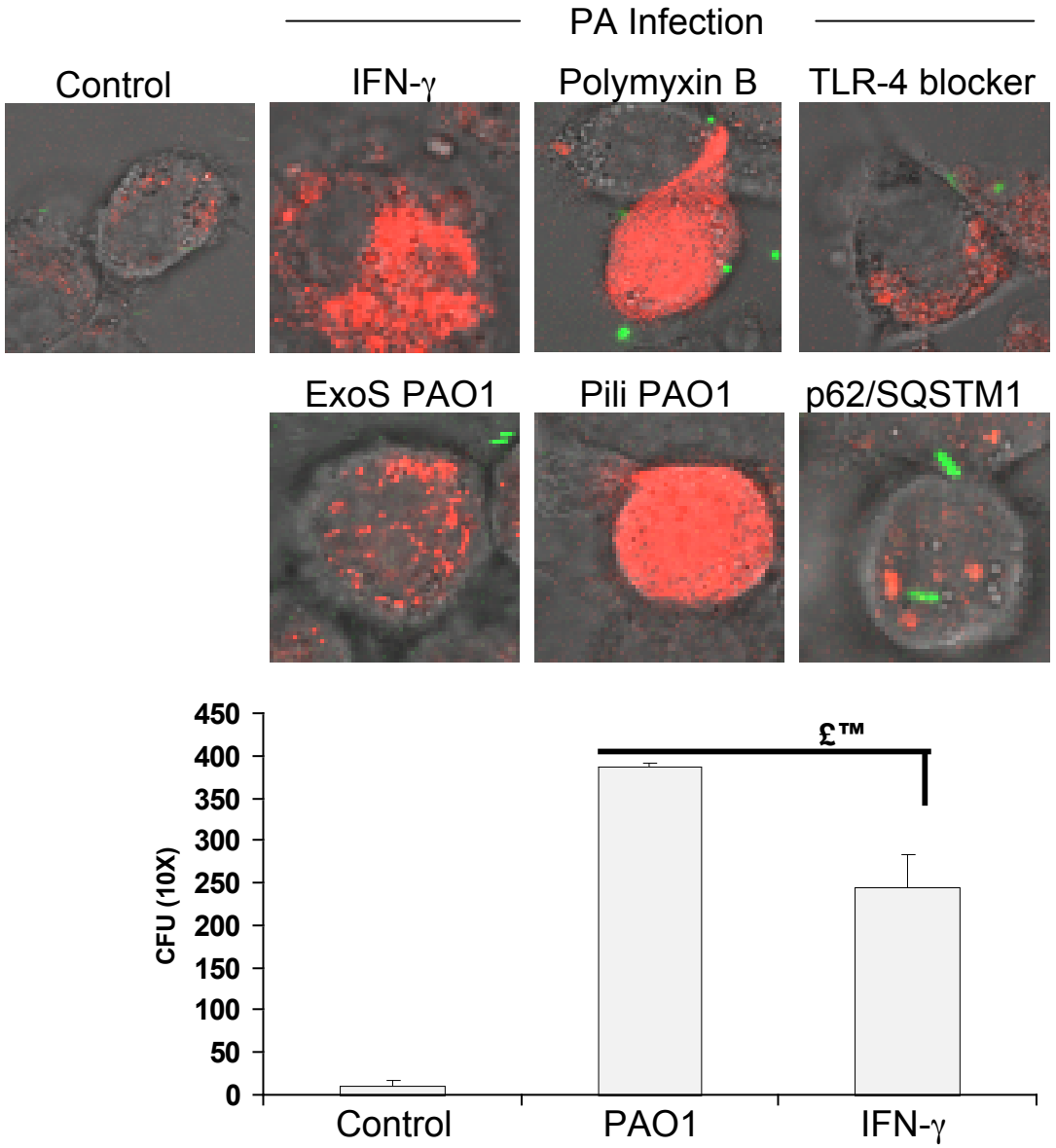

Fig. S5. PA induced-autophagy is dependent on its virulence and host factors (IFN-γ and TLR-4). MH-S cells were pretreated with IFN-γ for 12 h and infected with GFP-PAO1. We also evaluated a variety of host (TLR-4 and p62) and pathogen factors (exoenzyme S [ExoS PAO1] and pili [Pili PAO1] deficient strain) for their role in autophagy. Bacterial clearance was evaluated using CFU assay.