Expanded View Figures

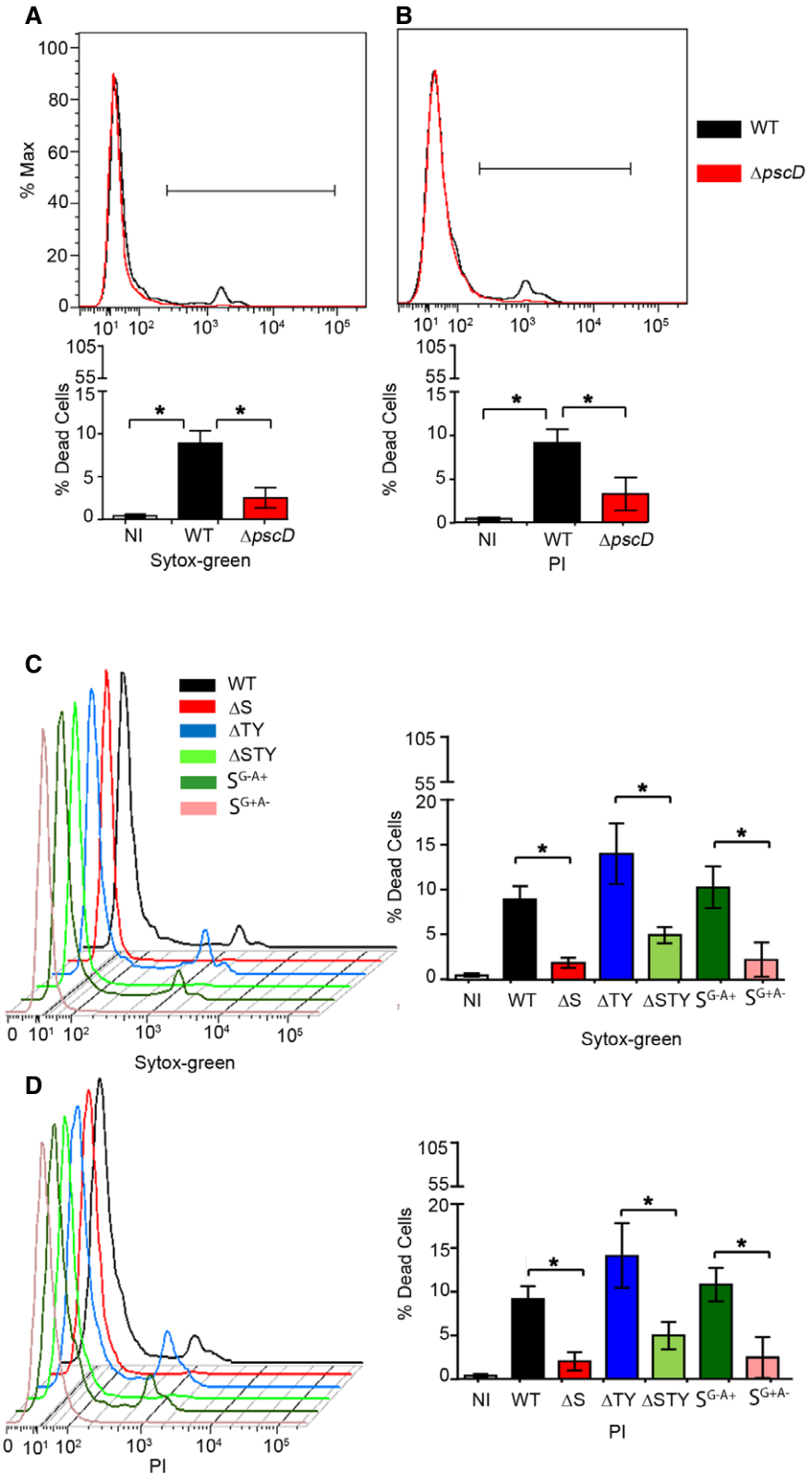

Figure EV1. Evaluation of cell death after *P. aeruginosa* infection.

A–D A549 cells were either left uninfected (NI) or infected for 4 h with WT and different *P. aeruginosa* mutants. Cells were then stained with Sytox-green (A, C) or propidium iodide (PI) (B, D) and the percentage of dead cells was analyzed by flow cytometry. Data are mean +/− SD from three independent experiments; one-way ANOVA analysis followed Dunn's multiple-comparison posttest *t*, $*P \leq 0.05$.

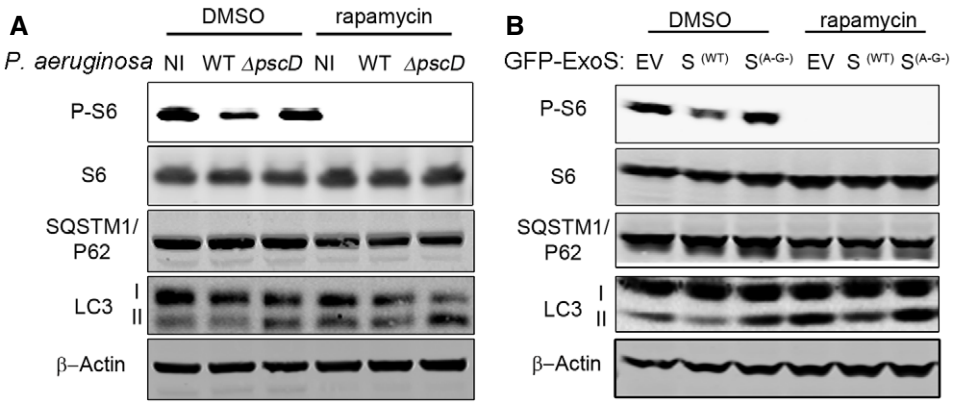

Figure EV2. Induction of autophagy was attenuated by *P. aeruginosa* T3SS ExoS.

A A549 cells were treated for 16 h with DMSO or rapamycin. Cells were then left uninfected (NI) or infected for 4 h with WT or *ΔpscD P. aeruginosa* and cell lysates were evaluated by immunoblotting.

B A549 cells were transfected, for 24 h, with a plasmid containing an empty vector (EV), GFP-ExoS or an inactive mutant (GFP-ExoS^{G-A-}). Cells were then treated for 16 h with DMSO or rapamycin and cell lysates were evaluated by immunoblotting.

Source data are available online for this figure.

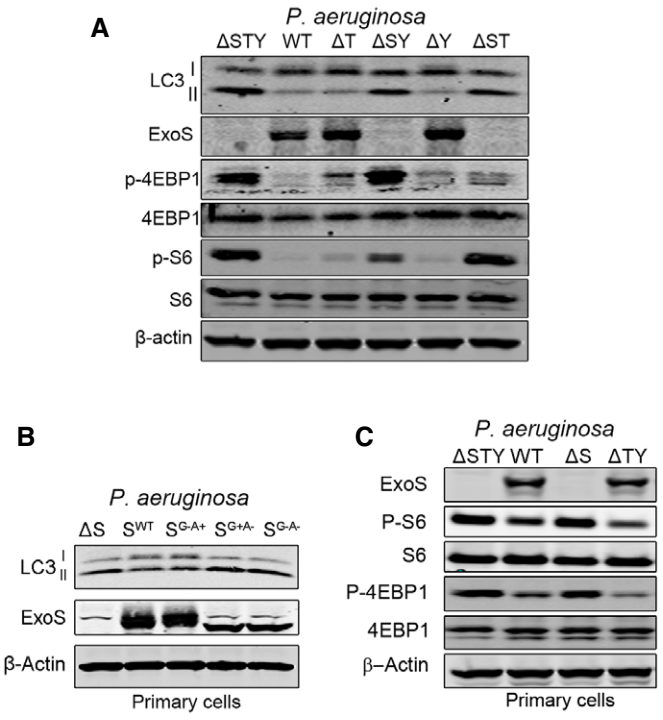

Figure EV3. ExoS ADP-ribosyltransferase has the same effect on autophagy in primary epithelial cells; Single cytotoxin ExoT or ExoY does not affect mTOR or autophagy.

A–C A549 cells (A) and primary NHBE cells (B, C) were infected for 4 h, with WT or *P. aeruginosa* mutants (ΔSTY, ΔT, ΔSY, ΔY, ΔST S^{WT}, S^{G-A-}, S^{G-A+}, and S^{G+A-}), and cell lysate were evaluated by immunoblotting using indicated antibodies. *P. aeruginosa mutant* strains legend: S^{WT} (*P. aeruginosa* contains only active ExoS), S^{G-A+} (*P. aeruginosa* contains only ExoS with loss-of-function mutations in the GTPase-activating domain), S^{G+A-} (*P. aeruginosa* contains ExoS with loss-of-function in the ADP ribosylation domain), S^{G-A-} (*P. aeruginosa* contains a nonfunctional ExoS);ΔSTY (mutant deficient for the three cytotoxins), ΔT (deficient for ExoT but still express ExoS and ExoY cytotoxins), ΔSY (deficient for ExoS and ExoY only express ExoT), ΔY (deficient for ExoY but still express ExoS and ExoT cytotoxins), ΔST (deficient for ExoS and ExoT only express ExoY).

Source data are available online for this figure.

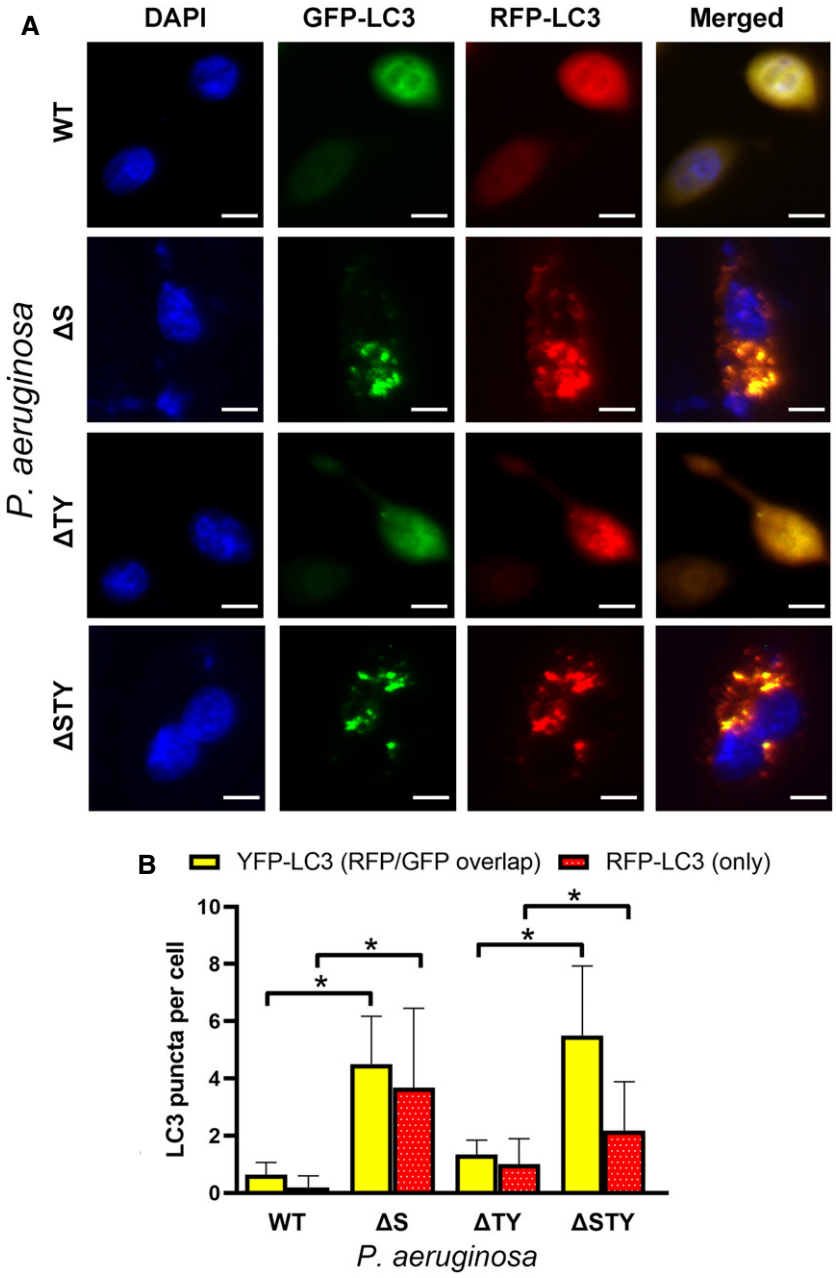

Figure EV4. ExoS reduced autophagosome and autolysosome formation.

A A549 cells, transfected with RFP-GFP-LC3 vectors for 24 h, were infected with different *P. aeruginosa* mutant strains. Representative images form fluorescence microscopy assays were shown. Scale bars: 10 μm.

B Quantitative analysis of yellow puncta generated from overlapping GFP and RFP puncta (represent autophagosome) and RFP-LC3 puncta (represent autolysosome). The puncta from more than 100 cells were counted and the ratios of these puncta per cell are shown. Data are mean +/− SD from three independent experiments. The significance of differences between treatments was determined using two-tailed Student's *t*-test with Welch's correction, $*P \leq 0.05$.

Source data are available online for this figure.

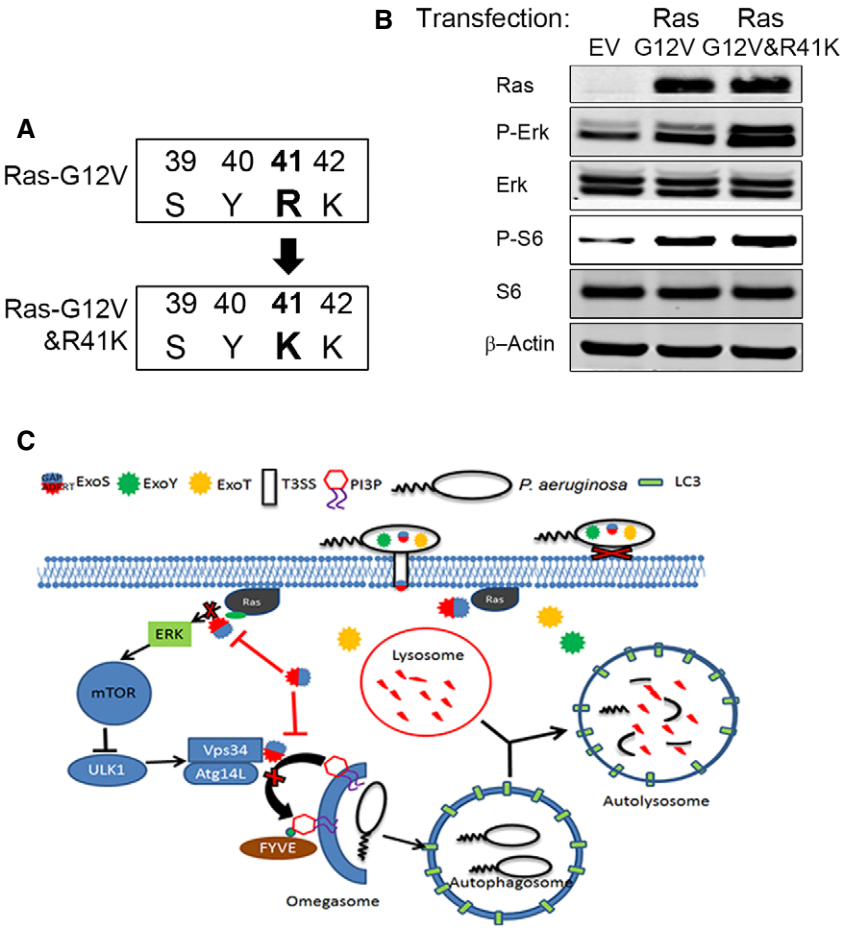

Figure EV5. Evaluation of cellular activity of Ras mutants and Working model of ExoS facilitates intracellular survival of *P. aeruginosa* by inhibiting autophagy and mTOR.

A Schematic of the generation of Ras-G12V&R41K from Ras-G12V.

B A549 cells were transfected, for 24 h, with plasmids containing empty vector (EV), Ras-G12V or Ras-G12V&R41K and cell lysates were evaluated by immunoblotting.

C Working model of *P. aeruginosa* ExoS role in inhibiting host cells autophagy and mTOR to facilitate intracellular bacterial survival. *P. aeruginosa* injects toxins including ExoS, ExoY, and ExoT through the T3SS. ExoS-mediated ADP ribosylation of RAS and leads to inhibition of mTOR. In addition, ExoS's ADP ribosylation activity suppresses autophagy initiation complex Atg14L-Vps34 kinase activity preventing autophagosome formation. The inhibition of autophagy by ExoS ADP ribosylation protects bacteria from elimination by autolysosome digestion.

Source data are available online for this figure.