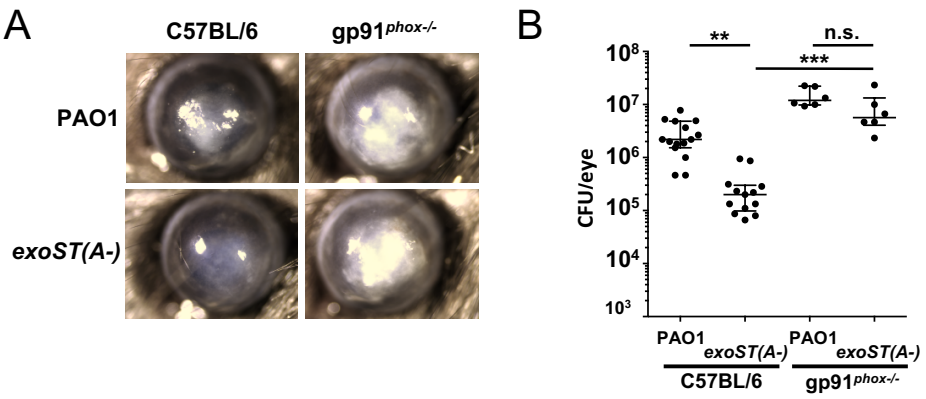

Figure S1. NADPH oxidase mediates ROS production by neutrophils facilitate clearance of *exoST(A-)* strain during bacterial keratitis. Related to Figure 1.

A) Representative images of corneal opacification 24 h post-infection of C57BL/6 and gp91^{phox} -/- (CGD) mice infected with 1x10^5 CFU PAO1 (WT) or with the *exoST(A-)* (APDRT of ExoS and ExoT are inactivated) mutant strain. (B) Colony forming units (CFU) recovered from infected corneas 24 h post-infection.

B) Data points represent individual corneas (n = 6-13 mice). Median and interquartile range are indicated. Statistical significance was calculated by Kruskal-Wallis test with Dunn's multiple comparison correction, **p<0.01, ***p<0.001, n.s. not significant.

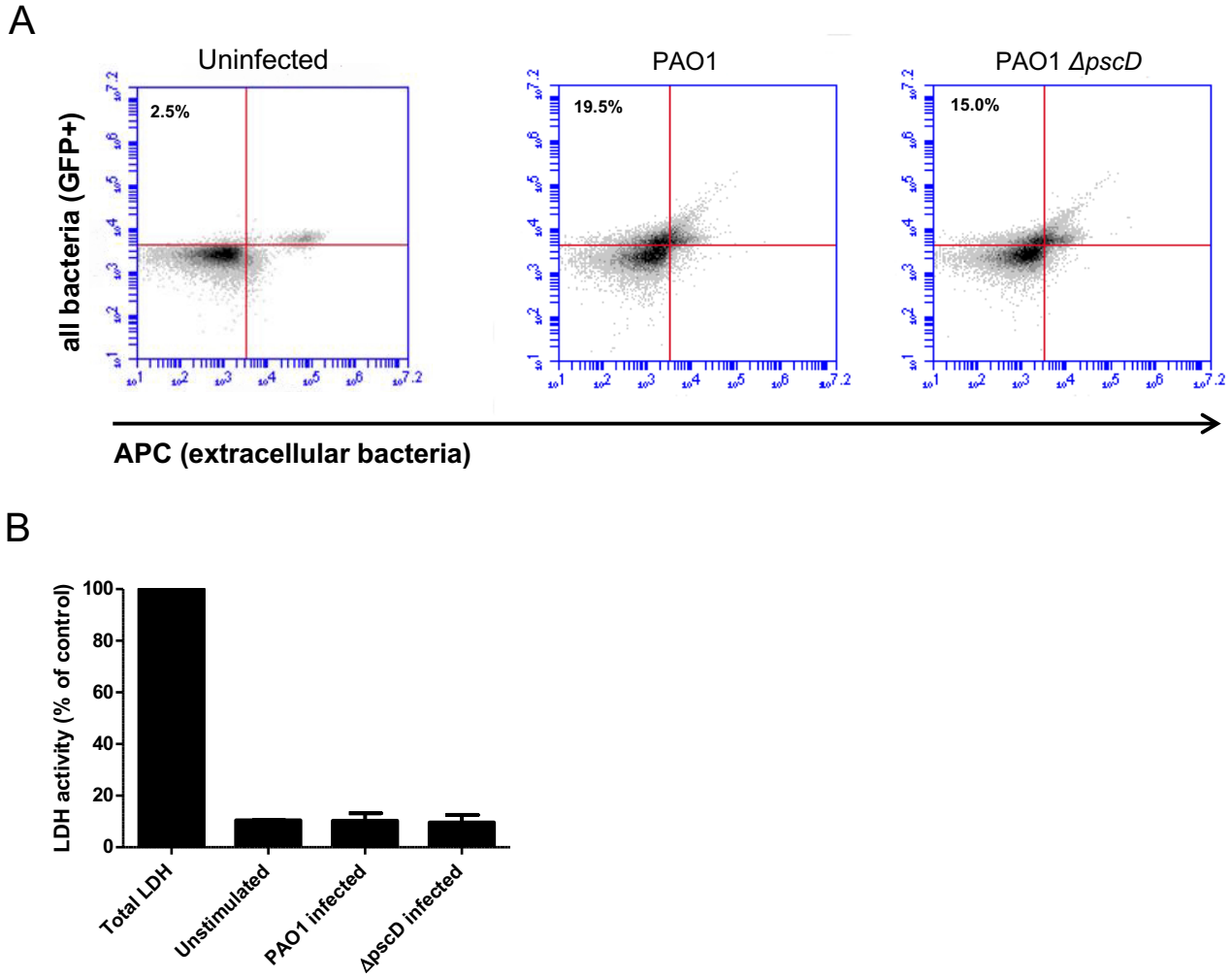

Figure S2. The type-III secretion system does not affect initial neutrophil phagocytosis or cell death. Related to Figures 2 and 4.

A) Human neutrophils were infected with biotin labelled GFP+-*P. aeruginosa* for 15 minutes. After washing and fixing with 4% paraformaldehyde, extracellular *P. aeruginosa* were labelled with streptavidin-APC. Gates were set to exclude mock infected neutrophils that had been subjected to the same fixing/staining protocol. The percentage of GFP+/APC- cells (top left quadrant), representing intracellular bacteria, is indicated. The experiments were repeated 2 times with similar results.

B) Human neutrophils were infected with *P. aeruginosa,* and lactate dehydrogenase (LDH) release was measured after 2 hours. The strains used in these experiments were wild type (PAO1) and a T3SS null mutant (*ΔpscD).* Total LDH represents LDH release after freezing and thawing neutrophils. The experiments were repeated 2 times with similar results.

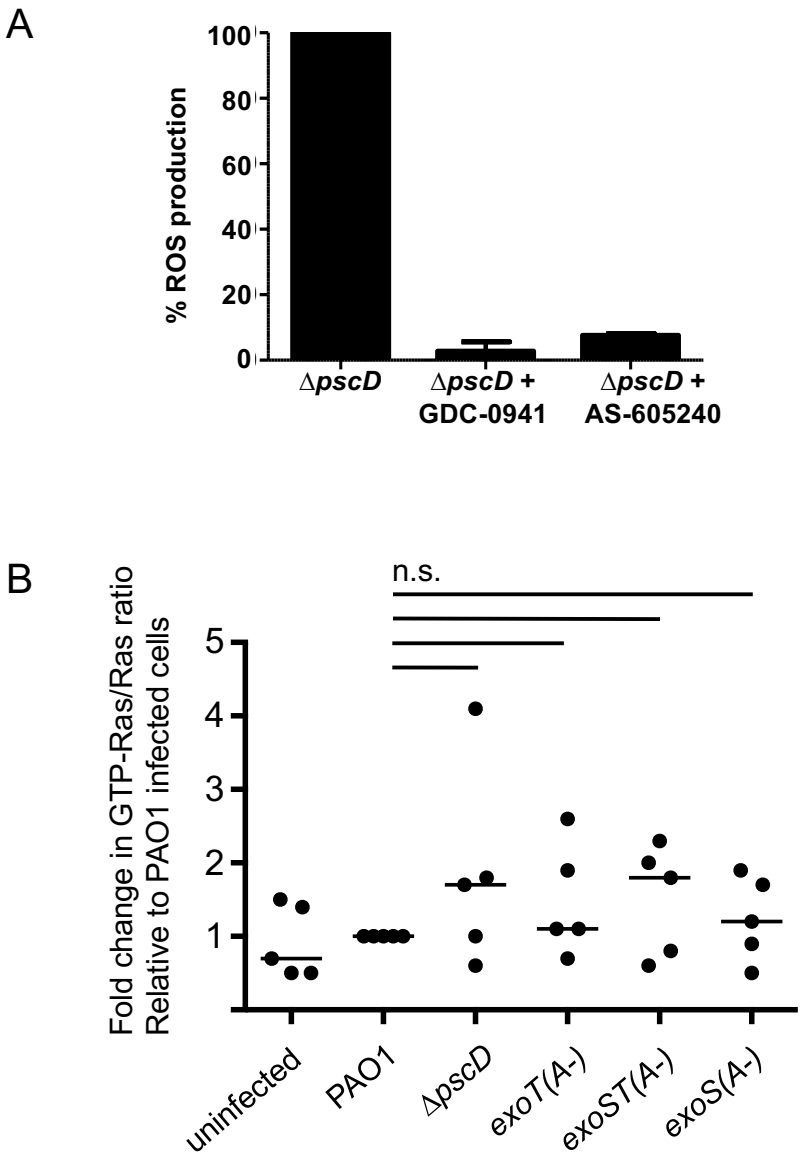

Figure S3. ROS production in neutrophils infected by the Δ*pscD* mutant strain requires PI3-kinase, but ExoS ADP-ribosylation does not affect the GTP-Ras/Ras ratio in infected neutrophils. Related to Figures 3.

A) ROS production was assayed in human neutrophils infected with a T3SS null mutant strain (Δ*pscD*) incubated with PI3K inhibitors GDC-0941 or AS-605240 (both 1μM), or vehicle (DMSO, column 1). Activity was determined by integrating the area under the curve of the luminescence plots, and is expressed as a percentage of ROS production by infected neutrophils in the absence of inhibitors. The data represent an average of 2 independent experiments (n = 2 donors).

B) The ratio of GTP-bound Ras to total Ras was determined in 5 independent experiments and is plotted as fold change relative to the GTP-Ras/Ras ratio in PAO1-infected neutrophils. GTP-bound Ras was isolated by Raf1-mediated affinity chromatography, and was detected by Western blot. Total Ras was detected in the corresponding cellular lysate. Densitometry values were normalized relative to the Ras-band of PAO1-infected neutrophils of each blot to allow comparison between experiments. The ratio of GTP-bound/total Ras was plotted for each experiment and infection condition. None of the GTP-Ras/Ras ratios changed significantly compared to PAO1-infected neutrophils (n.s. = not significant by one-way ANOVA, with Bonferroni multiple comparison test).

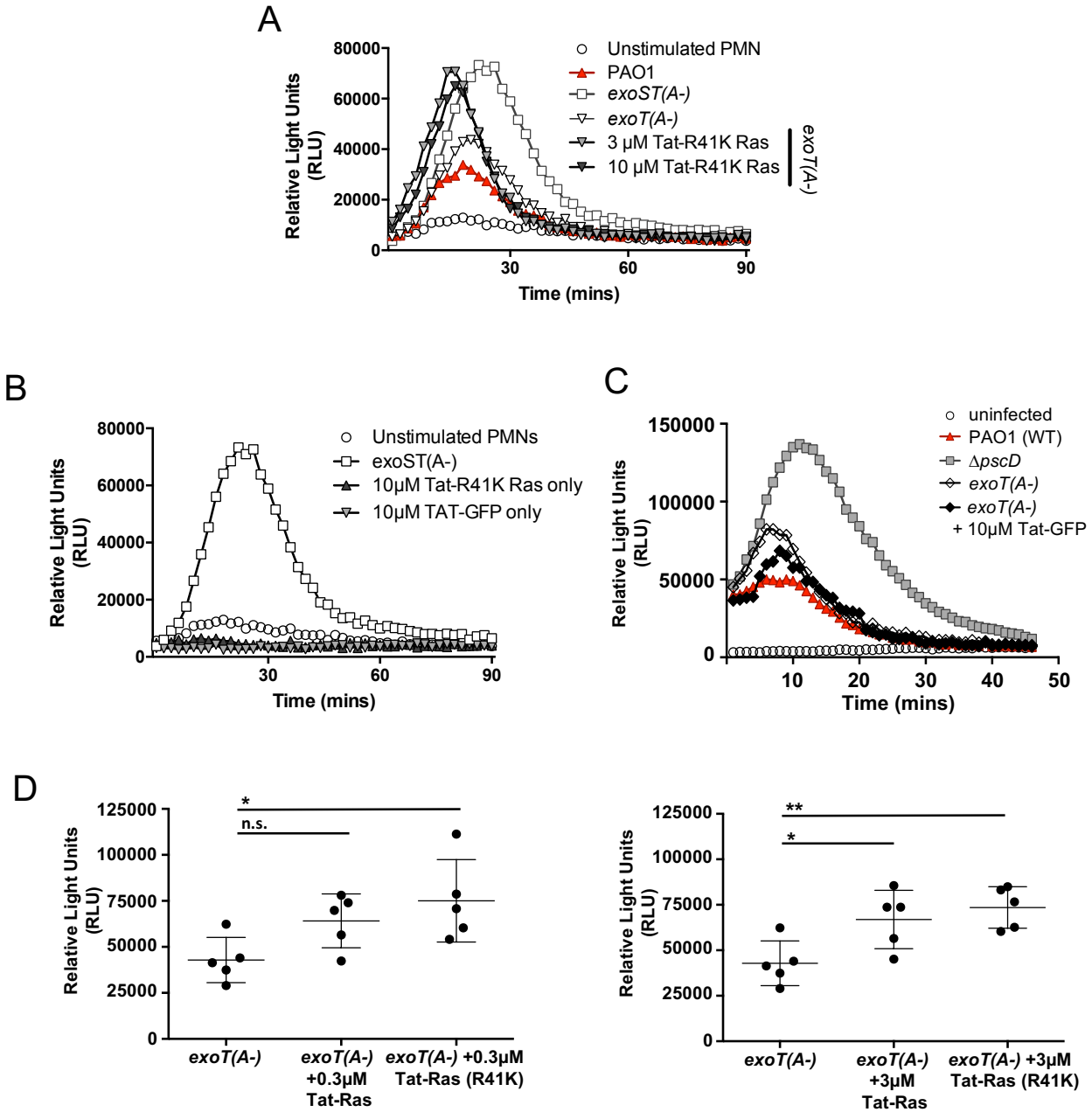

Figure S4. Tat-Ras(R41K) and Tat-GFP do not induce ROS production in human neutrophils, nor does Tat-GFP restore ROS production in infected neutrophils. Related to Figure 4.

A) ROS production by human neutrophils infected with PAO1, *exoT*(A-), or *exoST*(A-) *P. aeruginosa* was measured by chemiluminescence. Where indicated, cells were pre-incubated with Tat-Ras(R41K) thirty minutes prior to infection. The plot is representative of three independent experiments.

B) ROS production by human neutrophils infected with *exoST*(A-) *P. aeruginosa*, or incubated only with Tat-GFP or Tat-Ras(R41K), no infection. The plot is representative of three independent experiments.

C) ROS production by human neutrophils infected with PAO1, *exoT*(A-), or *exoST*(A-) *P. aeruginosa* . Where indicated, cells were pre-incubated with Tat-GFP thirty minutes prior to infection. The plot is representative of two independent experiments.

D) Area under the curve values for ROS production (relative light units) by neutrophils from five volunteers infected with PAO1 *exoT(A-)*. Neutrophils were either left untreated, or pretreated with 0.3μM (left panel) or 3μM (right panel) Tat-Ras or Tat-Ras (R41K), as indicated. Mean values, and standard deviation are indicated. Samples were compared by 1-way ANOVA with Bonferroni correction. ** $p<0.01$,* $p<0.05$, n.s. not significant.