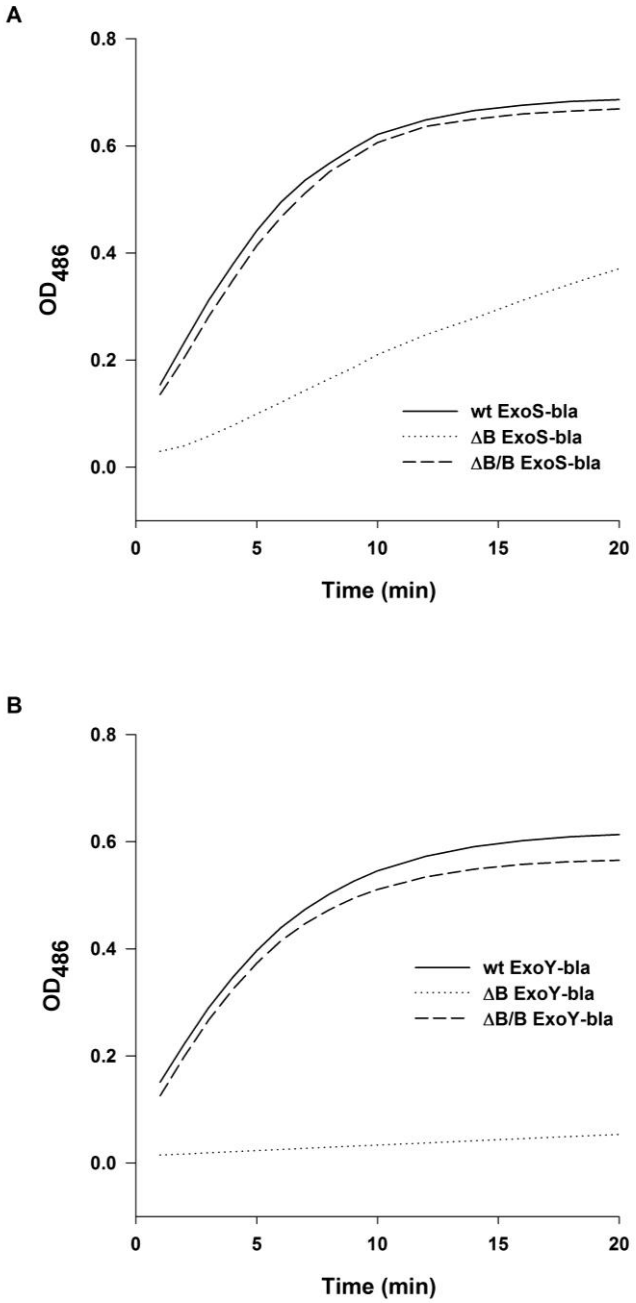
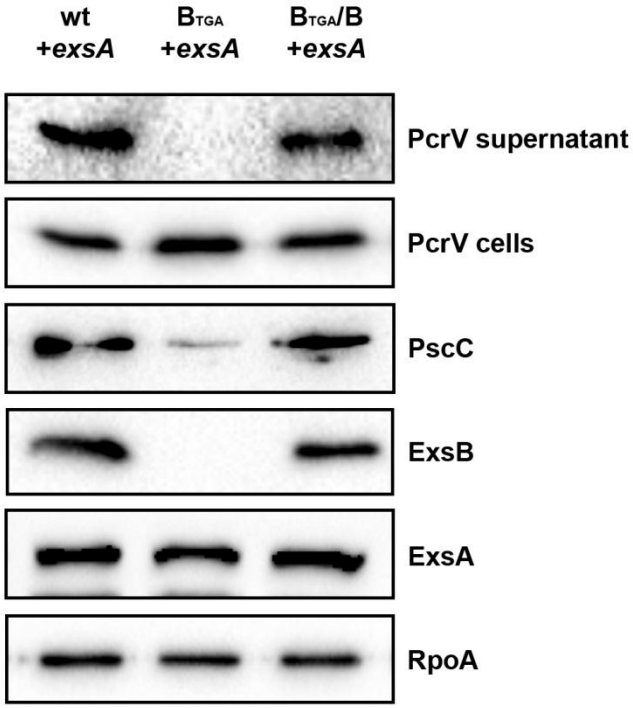
Supplementary Figure S1



Supplementary Fig S1: Quantification of secreted ß-lactamase chimeric Exotoxins.

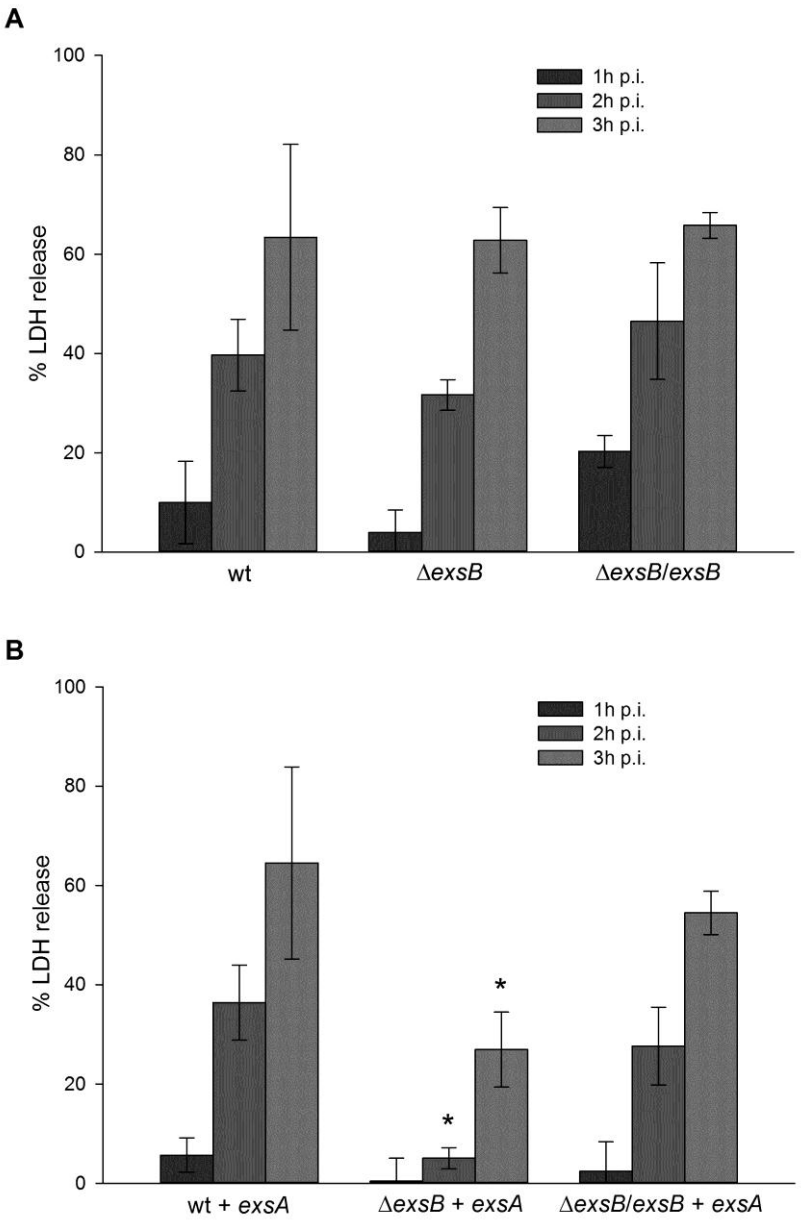
Wild-type (wt), Δ*exsB* (ΔB) and Δ*exsB/exsB* (ΔB/B) strains harboring plasmids enabling the production of ExoS (A) or ExoY (B) toxins fused to the ß-lactamase (Verove *et al*, 2012) were grown to OD=1 under T3SS induction conditions (calcium depletion). Supernatants were then incubated with nitrocefin, a substrate of ß-lactamase. Nitrocefin hydrolysis by the chimeric exotoxins was monitored during 20 min by measuring the absorbance at 486nm.

Supplementary Figure S2



Supplementary Fig S2: Impaired T3SS secretion in the ExsB-TGA mutant overproducing ExsA.
The T3SS proteins PcrV, PscC, ExsB and ExsA were monitored by western blot in bacterial extracts. In addition, secreted PcrV was detected in culture supernatants. The wild-type strain (wt), the mutant harboring the *exsB* gene disrupted by a TGA stop-codon (B_{TGA}) and the complemented strain (B_{TGA}/B) were transformed with the pDD2 plasmid (+*exsA*). All these strains moderately overproduce the T3SS activator ExsA to the same level, as detected by western blot. RpoA was used as a loading control.

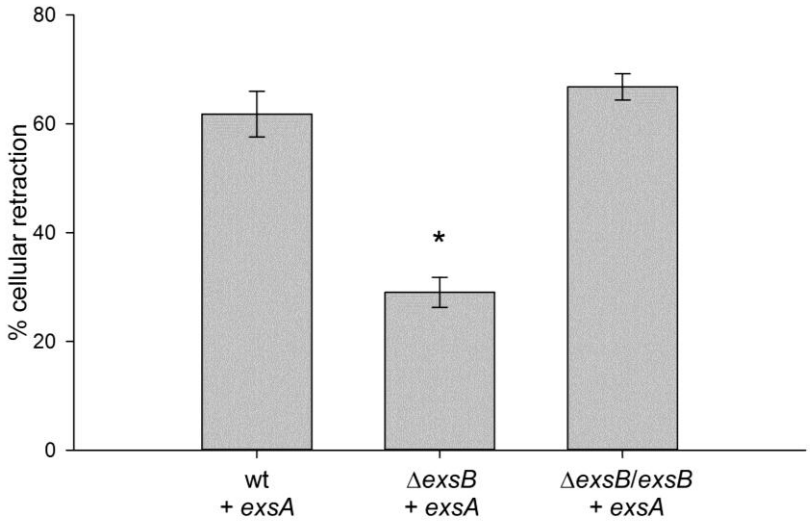
Supplementary Figure S3



Supplementary Fig 3: Cytotoxicity assay on J774 mouse macrophages.

The percentage of intracellular lactate dehydrogenase (LDH) release by the infected cells was measured at 1, 2 and 3 hours post-infection at a MOI of 1. The cytotoxicity assay was first performed with the wild-type (wt), mutant lacking *exsB* (Δ*exsB*) and complemented (Δ*exsB/exsB*) strains of *P. aeruginosa* (A). In these conditions, no difference was observed between all the strains. The assay was then performed with the same strains overproducing the T3SS activator ExsA (B). One-way ANOVA was performed (overall *P* value < 0.02). The stars indicate a statistically significant difference of cytotoxicity caused by Δ*exsB+exsA* compared to wt+*exsA* and Δ*exsB/exsB+exsA* at 2h and 3h post-infection *(*Tukey post-hoc test p < 0.05)

Supplementary Figure S4



Supplementary Fig 4: Cytotoxicity assay on HUVEC with ExsA overproducing strains.

The percentage of cellular retraction was measured after 3 hours of infection by the wild-type (wt), mutant (Δ*exsB*) and complemented (Δ*exsB*/*exsB*) strains overproducing ExsA from the pDD2 plasmid. One-way ANOVA was performed (overall P value = 0.001). The star indicates a statistically significant difference of cytotoxicity caused by Δ*exsB* compared to wt and Δ*exsB*/*exsB* (Tukey post-hoc test $p < 0.001$).