Supplementary Material

Innate immune interactions between *Bacillus anthracis* and host neutrophils

Janet Z. Liu¹, Syed Raza Ali¹, Ethan Bier², Victor Nizet^{1,3*}

¹Department of Pediatrics, Division of Host-Microbe Systems and Therapeutics, UC San Diego, La Jolla, CA, USA

²Section of Cell and Developmental Biology, Division of Biological Sciences, UC San Diego, La Jolla, CA, USA

³Skaggs School of Pharmacy and Pharmaceutical Sciences, UC San Diego, La Jolla, CA, USA

*Corresponding author Victor Nizet: email <u>vnizet@ucsd.edu</u>



Supplementary Figure S1 | Total number of neutrophil, macrophage and monocytes from murine spleen. Number of neutrophil and macrophage/monocytes recovered from the spleen of uninfected and wild-type *Bacillus anthracis* Sterne infected mice, treated with control antibody (Ab) or with neutrophil depleting 1A8 antibody. * P < 0.05, *** P < 0.001

LL-37					α -Defensin		
Donor:	1	2	3	Donor:	1	2	3
WT	0	,		WT	۲	۲	0
∆EF	۲	۲	0	∆EF	0	٢	۲
∆LF	۲	۲	۲	Δ LF	۲	0	0
ΔΡΑ	۲	۲	۲	∆ PA	0	۲	•
Uninfected			٢	Uninfected	0		0



Supplementary Figure S2 | Release of neutrophil granular proteins. (A) Dot-blots of LL-37 and α -defensin released by neutrophils infected the indicted *Bacillus anthracis* Sterne strain. Supernatant from infected neutrophils from three donors were collected 1 h post-infection and dotted on nitrocellulose membrane, then incubated with antibody for the indicated antimicrobial proteins. (B) Elastase assay as a read out of neutrophil elastase release during anthrax infection. Supernatant from infected neutrophils were collected at 1 h post-infection and mixed with p-nitroanilide. The resulting signal for elastase activity was measured using a spectrophotometer.



Supplementary Figure S3 | **Percent of dead neutrophils associated with bacteria.** Neutrophils were infected with the indicated strain of GFP-expressing *Bacillus anthracis* Sterne and stained 15 min post-infection with propidium iodide. As a control, neutrophils were incubated for 20 min before infection with cytochalasin D to inhibit phagocytosis.



Supplementary Figure S4 | Growth of wild-type anthrax in the presence of neutrophil function inhibitors. Wild-type *Bacillus anthracis* Sterne was incubated for 15 min in the presence of the indicated neutrophil function inhibitors. The percent survival was determined by plating on BHI agar and normalized to untreated anthrax.