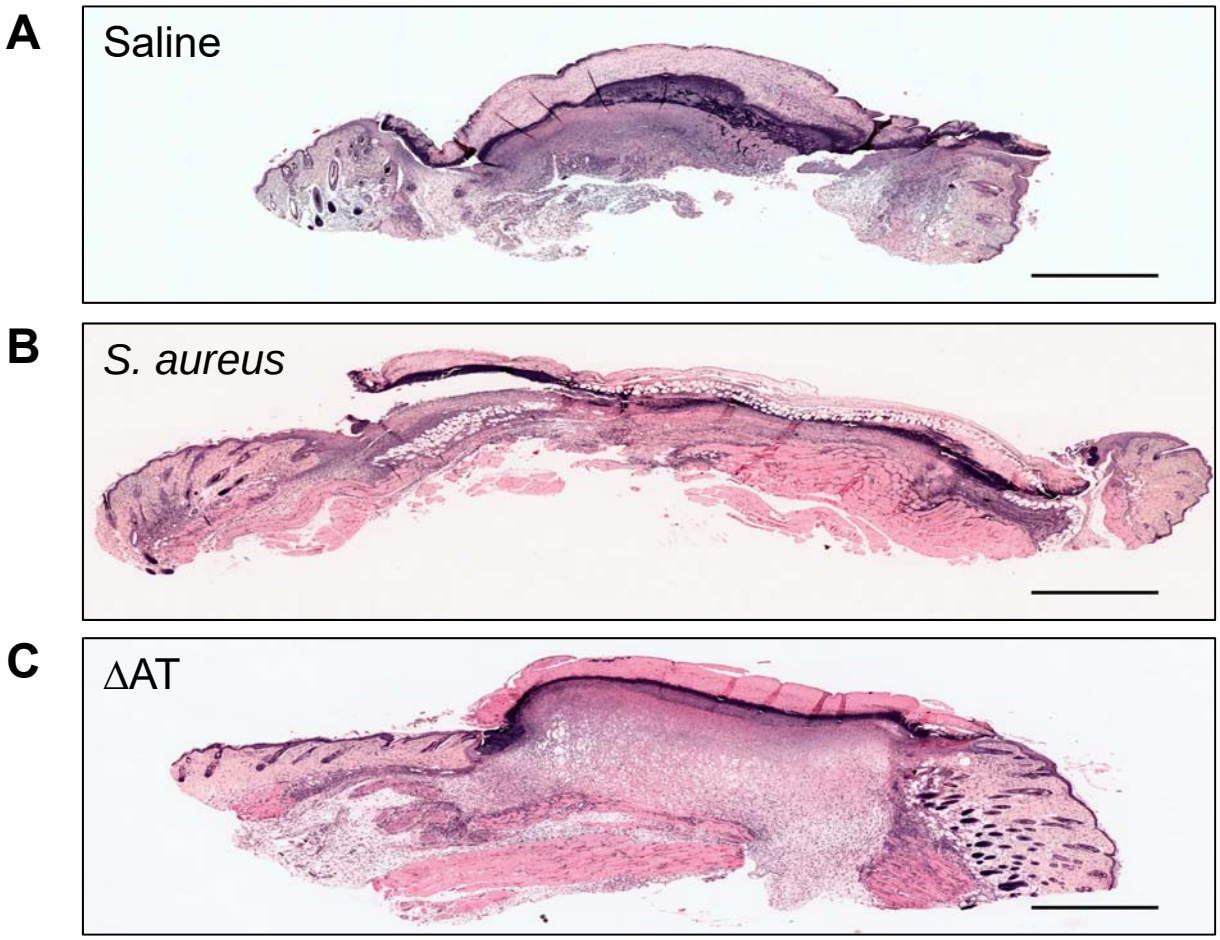
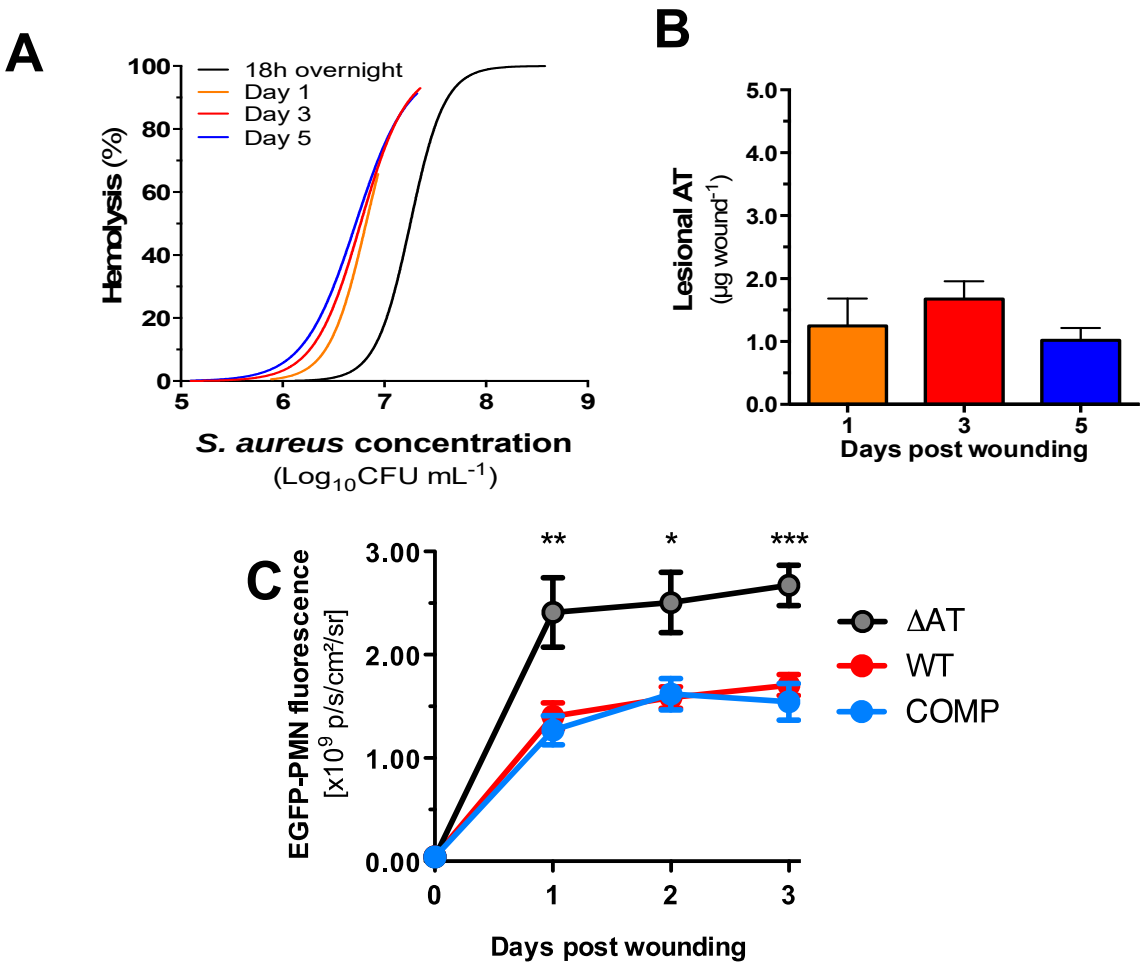
Alpha-toxin regulates local granulocyte expansion from hematopoietic stem and progenitor cells in *Staphylococcus aureus*-infected wounds

Patrick C. Falahee,[1]* Leif S. Anderson,[1]* Mack B. Reynolds,* Mauricio Pirir,* Bridget E. McLaughlin,† Carly A. Dillen, § Ambrose L. Cheung, ‡ Lloyd S. Miller, § and Scott I. Simon*

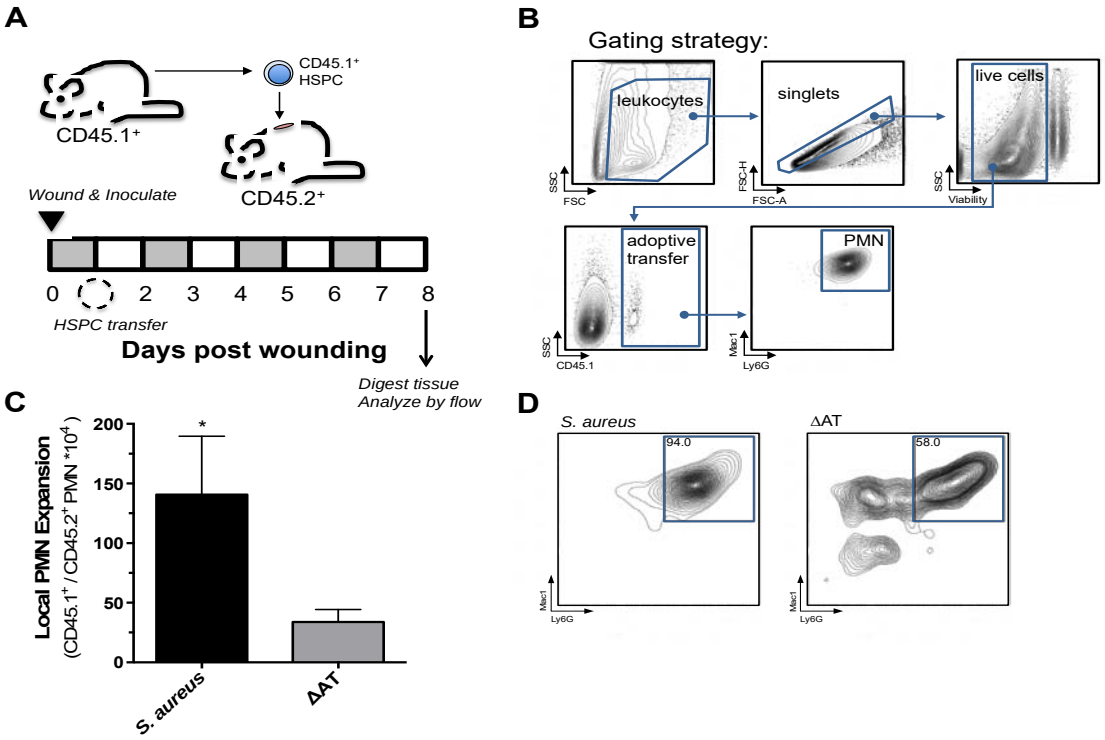
Supplementary Material



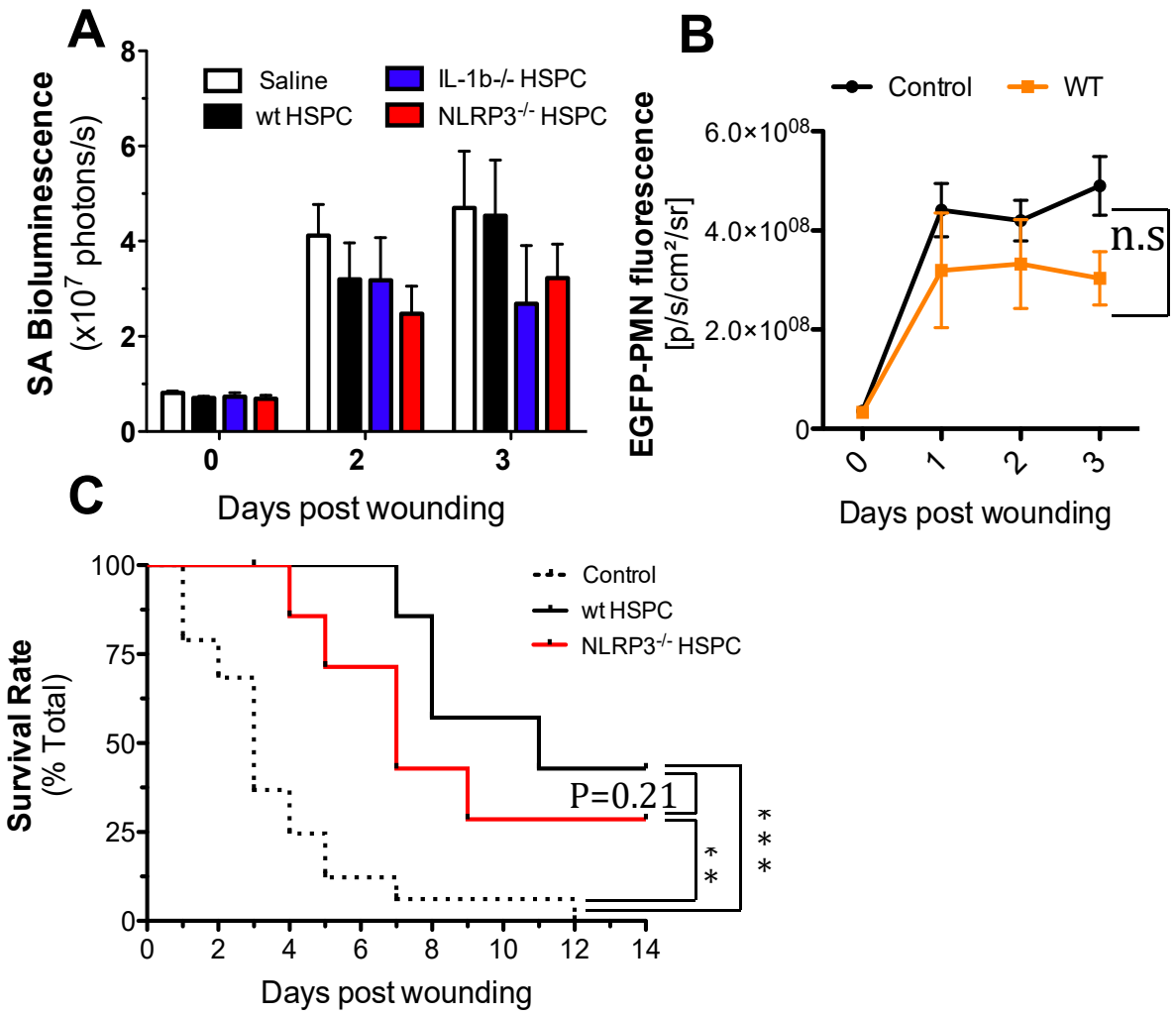
Supplemental Figure 1. Histological comparison of wound healing between mice inoculated with saline or *S. aureus* versus ΔAT. C57BL/6 mice were wounded and inoculated with either sterile saline or 1e7 CFU of *S. aureus* versus ΔAT. At day 3 post inoculation, mice were euthanized and wound tissue biopsied. Samples were immediately embedded in paraffin and sectioned for H&E staining. Representative photomicrographs of sections from mice of each treatment group are shown. Scale bars = 200 μm.



Supplemental Figure 2. Alpha toxin activity correlates closely with bacterial abundance. (A) Rabbit erythrocytes were co-incubated with various concentrations of 0.2 µm-filtered overnight culture supernatants (black line) or homogenized wound tissue collected at either 1, 3 or 5 days post wounding and inoculation with *S. aureus*. Hemolytic activity was calculated based on the absorbance at 450 nm. (B) Alpha toxin protein expression from homogenized wound tissue collected 1, 3, and 5 day post wounding and inoculation with WT S. aureus as measured by ELISA. (C) LysM-EGFP mice were wounded and inoculated with $1x10^7$ CFU of WT, ΔAT or ΔAT-comp S. aureus, and EGFP-PMN fluorescence was measured at wound sites daily over 3 days. Data are derived from 5-8 mice per group and are expressed as mean±SEM. * $p < .05$, ** $p < .01$, *** $p < .001$, S. aureus and ΔAT-comp versus ΔAT .



Supplemental Figure 3. Flow cytometric analysis of wounds following expansion of HSPC. (A) C57BL/6 mice ($CD45.2^+$) were wounded and inoculated with 1e7 CFU of *either S. aureus* or ΔAT. At 24 hours post wounding, $2x10^6$ HSPC from congenic $CD45.1^+$ donor mice were adoptively transferred directly into wound beds as shown. (B) After 7 days, mice were euthanized and wounds biopsied. Digested tissue was stained and analyzed by flow cytometry to determine the phenotype of locally expanded $CD45.1^+$ progeny according to the gating scheme depicted. (C) Relative frequency of locally expanded $CD45.1^+$ $Ly6G^{hi}$ $Mac1^{hi}$ from mice infected with *S. aureus* versus ΔAT is shown. (D) Representative plots showing the prevalence of $CD45.1^+$ PMN from each group are depicted. Data are expressed as mean±SEM and represent 4-5 mice per group. * $p < .05$.



Supplemental Figure 4. Adoptive transfer of HSPC confers protection from lethal *S. aureus* infection in MyD88$^{-/-}$ mice—additional characterization. MyD88$^{-/-}$ or MyD88$^{-/-}$ x LysM-EGFP mice were wounded and inoculated with 2e7 CFU of *S. aureus* lux. At 6 hours post inoculation, mice received a local injection of vehicle control or 1x10^{5} HSPC derived from wild-type (wt), IL-1β$^{-/-}$ or NLRP3$^{-/-}$ donors. (A) Bacterial bioluminescence in MyD88$^{-/-}$ mice was monitored daily. (B) Endogenous PMN kinetics were measured in MyD88$^{-/-}$ x LysM-EGFP over 3 days following wounding and infection. (C) Kaplan-Meier survival plots including NLRP3$^{-/-}$ HSPC group. Data represent 2-12 animals per group and are expressed as mean±SEM. ** $p <$ .01, *** p < .001