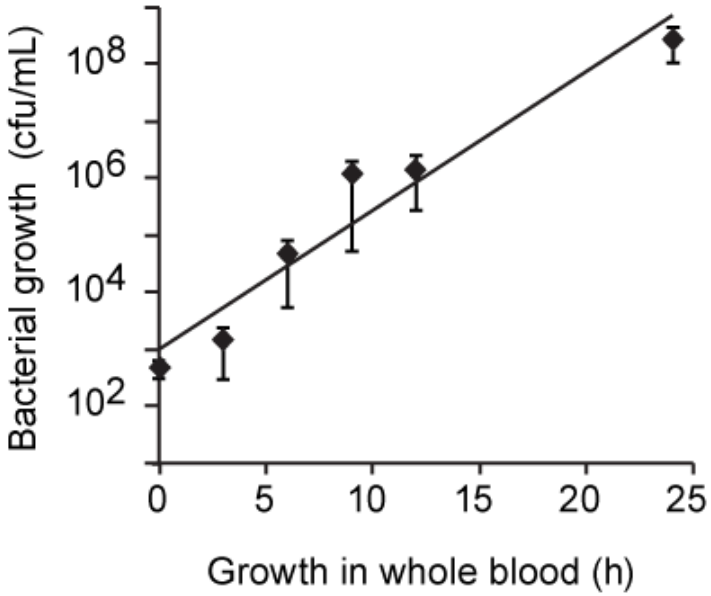
Supplementary Material to Franks, Campbell et al. "Methicillin-resistant *Staphylococcus aureus*-induced thrombo-inflammatory response is reduced with timely antibiotic administration" (Thromb Haemost 2013; 109.4)

Table 1: Early antibiotic administration inhibits MSSA induced thrombin generation.

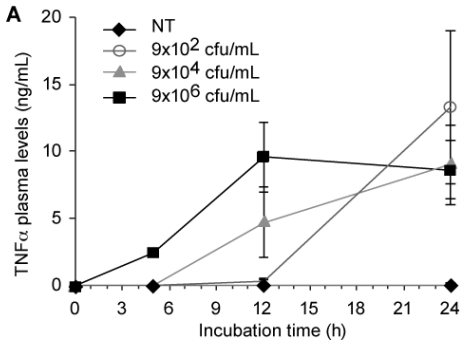
Time (hrs)	**-**		**0**		**6**		**12**	
Antibiotic	**-**		**VANC**	**LZ**	**VANC**	**LZ**	**VANC**	**LZ**
MSSA	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
Lag (min)	18.5 ± 2.5	6.7 ± 1.2*	16.0 ± 1**	15.5 ± 1.9**	15.3 ± 0.9**	15.7 ± 0.9**	12.7 ± 2.4	11.7 ± 1.3
Peak (nM)	212.1 ± 44.5	325.2 ± 43.9	246.1 ± 30	247.9 ± 49.1	220.8 ± 36.1	224.8 ± 39	236.5 ± 47.8	269.2 ± 22.2
Time to Peak (min)	29.3 ± 4.7	10.7 ± 1.8*	25.0 ± 2	24.3 ± 4	24.2 ± 2.1	24.3 ± 2.2	20.7 ± 3.8	18.7 ± 1.7
Rate (nM/min)	27.0 ± 13.1	89.0 ± 22.5	29.3 ± 7.2	37.4 ± 18.8	27.4 ± 8.3	30.4 ± 9.5	34.9 ± 14.3	39.1 ± 3.3
AUC (nM*min)	2570.6 ± 434.4	3165.6 ± 446.7	2733.0 ± 411.7	2624.6 ± 405.6	2765.8 ± 448.6	2637.3 ± 452.1	2866.6 ± 466.9	3112.4 ± 419.2

LZ: linezolid, VANC: vancomycin. *p<0.05 vs. no *S. aureus.* **p<0.05 vs. *S. aureus* alone without antibiotics.



Supplement Figure 1

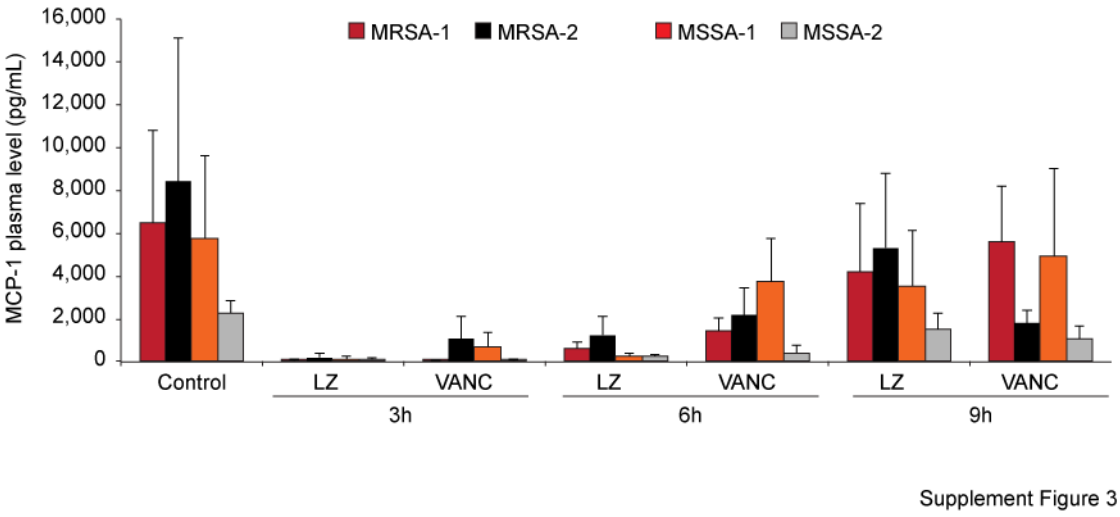
Suppl. Figure 1: Bacterial growth in whole blood occurs in logarithmic phase. Bacteria (9 x 10^2 cfu/mL, initial) were grown in whole blood for indicated amount of time and plated on blood agar and incubated overnight at 37ºC. Bacterial cfu were counted. MRSA bacterial growth is represented by $\log_{10}$ units on the vertical axis. The data reflect the mean (±SEM) of 3-5 experiments.



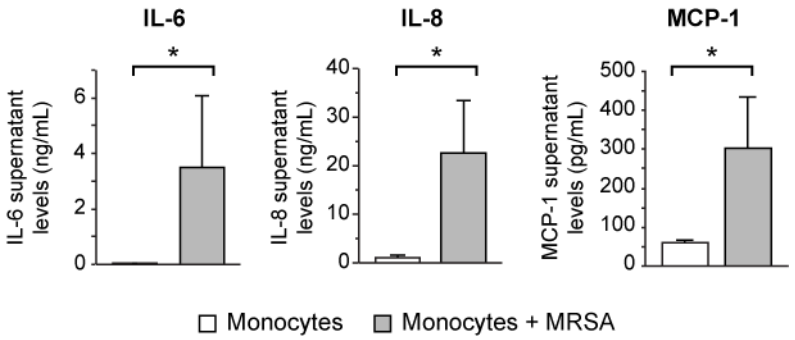
Supplement Figure 2

Suppl. Figure 2: MRSA induces the release of TNF-α and IL-1β in whole blood. MRSA was grown overnight and then inoculated in two mL of whole blood at a starting concentration of $9x10^2$, $9x10^4$, $9x10^6$ cfu/mL, or not treated (NT). Whole blood inoculated with MRSA was incubated at 37ºC for 24

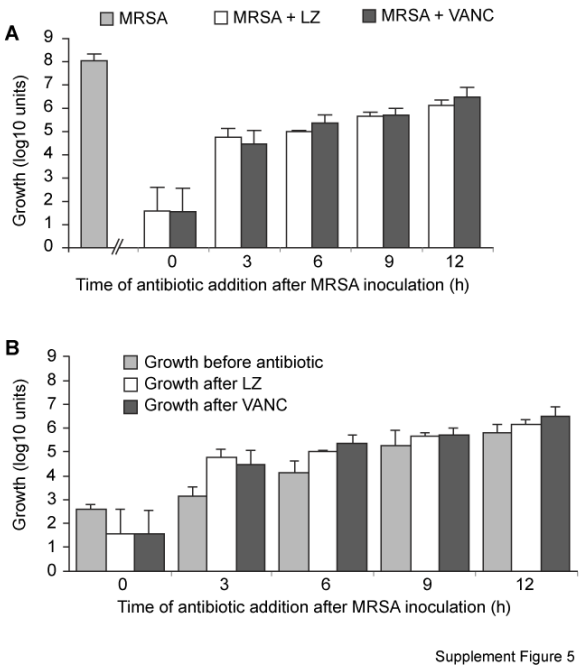
hours and plasma levels of each cytokine were measured over time by ELISA. Each point reflects the mean (±SEM) of 3-5 experiments.



Suppl. Figure 3: MRSA and MSSA strains induce similar cytokine responses in whole blood . Two unique MRSA and two unique MSSA strains were grown overnight and then inoculated in 2 mL of whole blood at a starting concentration of $9x10^2$ cfu/mL. Bacteria were incubated at 37ºC in 5% CO_2 for 24 hours. Plasma levels of IL-6, IL-8, and MCP-1 were measured at 24 hours. The left bars reflect the amount of MCP-1 produced during 24 hours of inoculation with MRSA and MSSA strains in the absence of any antibiotics. In additional experiments, linezolid (LZ, 5 μg/mL final concentration) or vancomycin (VANC, 5 μg/mL final concentration) were added 3, 6, and 9 hours after the inoculation was begun and cytokine synthesis was measured. Notice each strain produces a highly variable response. However, early antibiotic treatment reduces cytokine response compared to late administration regardless of strain of bacteria. Each point reflects the mean (±SEM) of 3 experiments.

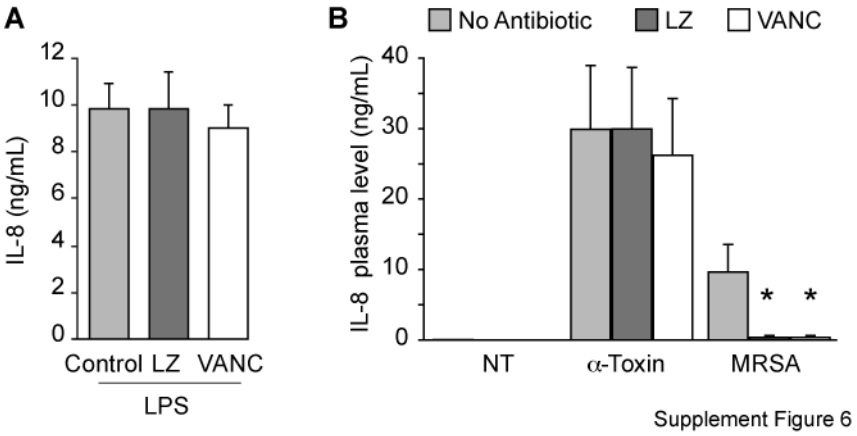


Suppl. Figure 4: MRSA induces cytokine synthesis in isolated monocytes. MRSA was grown overnight and then inoculated with freshly isolated monocytes (2 x 10^6/mL) at a concentration of 9x10^2 colony cfu/mL. MRSA-induced IL-6, IL-8, and MCP-1 synthesis by isolated monocytes was measured using ELISA. These data are representative of ≥3 individual experiments. (*$p<0.05$ compared to NT.)

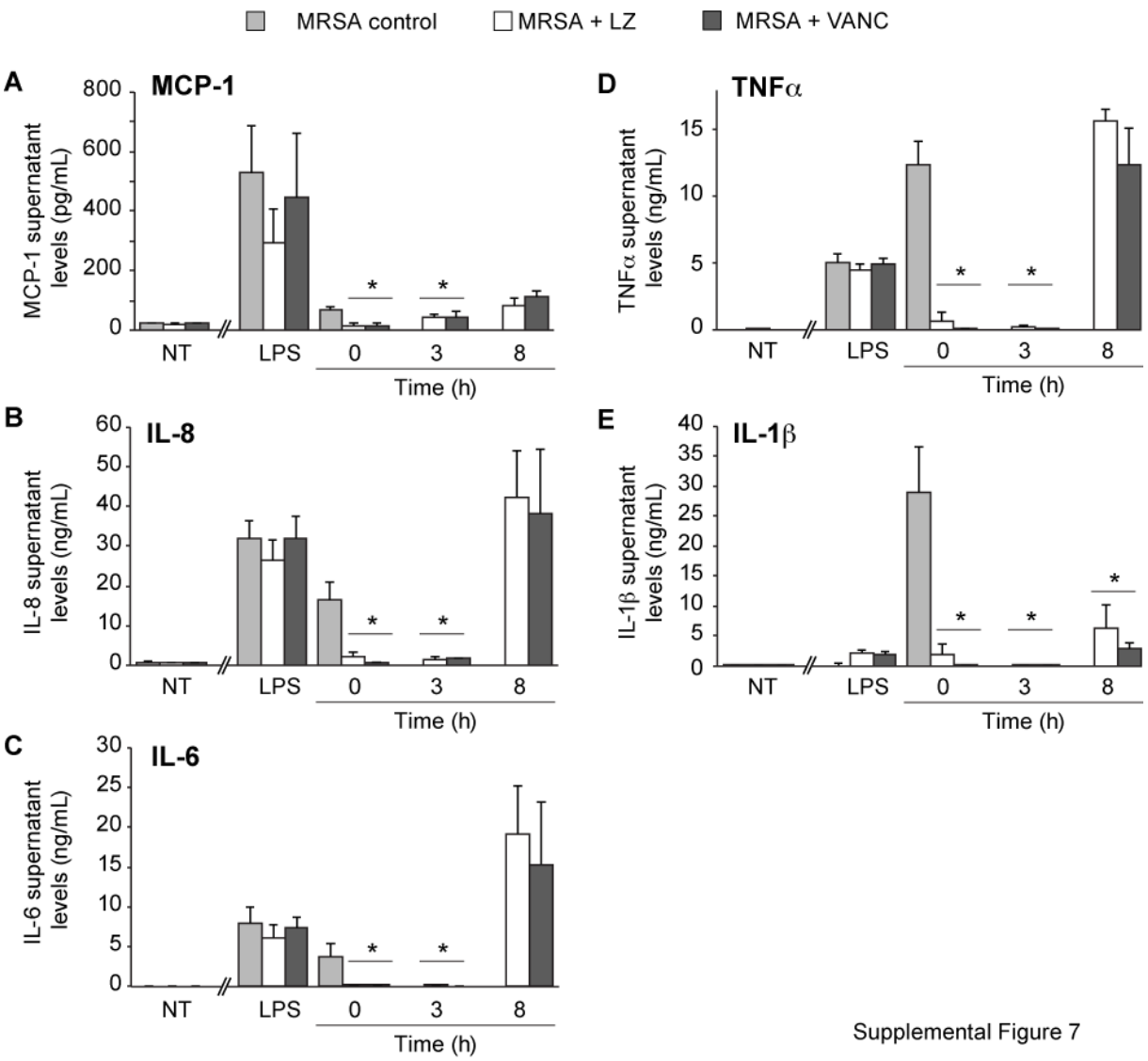


Suppl. Figure 5: Antibiotics concentrations were bacteriostatic in whole blood. Bacteria were grown in whole blood for indicated amount of time and plated on blood agar and incubated overnight at 37ºC. Bacterial cfu were counted. Bacterial growth is represented by $\log_{10}$ units on the vertical axis (A). In some experiments, linezolid (LZ, 5 μg/mL) or vancomycin (VANC, 5 μg/mL) were added at indicated times to determine the effect on growth. The far left bar (MRSA Control) reflects

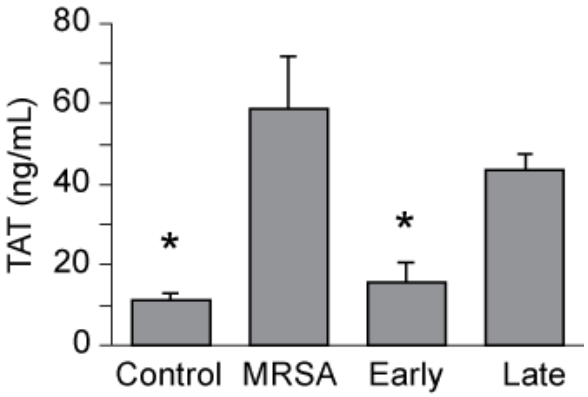
bacterial growth after 24 hours of inoculation with MRSA in the absence of any antibiotics. These data reflect the mean (±SEM) of 3-5 experiments. In some experiments, as described above and at indicated times, bacteria were counted and antibiotics were added to determine if further growth occurred after addition of antibiotics (B).



Suppl. Figure 6: Antibiotics do not inhibit LPS or alpha-toxin induced synthesis of IL-8. MRSA (9 x 10^2 cfu/mL bacteria) and LPS (100 ng/mL final concentration) (A) or alpha toxin (2 μg/mL final concentration) (B) were incubated in 2 mL of whole blood for 24 hours in the presence of linezolid (LZ, 5 μg/mL final concentration) or vancomycin (VANC, 5 μg/mL final concentration). Plasma levels of IL-8 were measured using ELISA. Each point reflects the mean (±SEM) of 3-5 experiments (*$p<0.05$ versus MRSA without antibiotics).



Suppl. Figure 7: Antibiotics reduce cytokine synthesis in MRSA-treated purified monocytes. Monocytes were purified as described in material and methods and incubated with LPS (100 ng/mL) or MRSA (9 x 10^2 cfu/mL) for 24 hours. At indicated time after treatment of LPS or MRSA, linezolid (LZ, 5 μg/mL) or vancomycin (VANC, 5 μg/mL) were added. Early addition of antibiotics reduces MCP-1 (A), IL-8 (B), IL-6 (C), TNF-α (D), and IL-1β (E), while later administration has little effect on cytokine production. Antibiotics had no effect on LPS-induced cytokine synthesis. The data reflect the mean (±SEM) of 3 experiments (*$p<0.05$ versus MRSA without antibiotics).



Supplement Figure 8

Suppl. Figure 8: Early antibiotic administration reduces TAT levels after MRSA infection in mice. C57BL/6 mice (weighing 20–25 g) were randomly assigned to one of four groups: (1) control intravenous (i.v.) saline injection (PBS 100 μL) (2) MRSA i.v. injection (3.3 x 10^6 total bacteria) 3) MRSA i.v. injection (3.3 x 10^6 total bacteria) followed by i.v. vancomycin injections (60 mg/kg) at three hours post infection (4) MRSA i.v. injection (3.3 x 10^6 total bacteria) followed by vancomycin i.v. injections (60 mg/kg) at nine hours post infection. Twenty-four hours after infection, mice were anesthetized with avertin and blood drawn into ACD through the carotid artery. TAT measurements were performed using an ELISA. Early vancomycin administration significantly reduced TAT levels compared to late administration. The data reflect the mean (±SEM) of 3-4 mice per group (*$p<0.05$ compared to control)