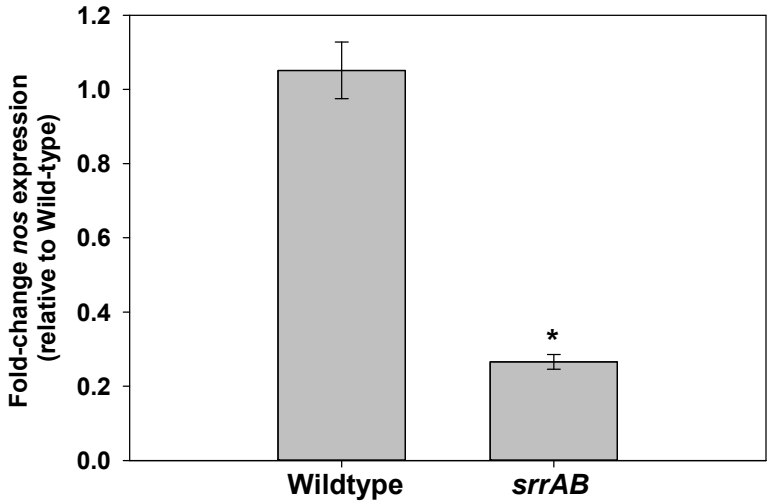

Figure S1: Real-time PCR of *nos* expression in wildtype and *srrAB* mutant low-oxygen cultures. Wild-type and *srrAB* mutant low-oxygen TSB+G cultures were grown for 6 hours, and RNA was extracted from cell pellets. Expression of *nos* was measured in cDNA synthesized from each RNA sample by real-time PCR. Relative fold expression was calculated using the Livak method ($2^{-\Delta\Delta Ct}$) and normalized to the reference housekeeping gene *sigA*. Data represent the average from n=3 biological samples, error bars = SEM. *P<0.001 Mann-Whitney Rank Sum Test.

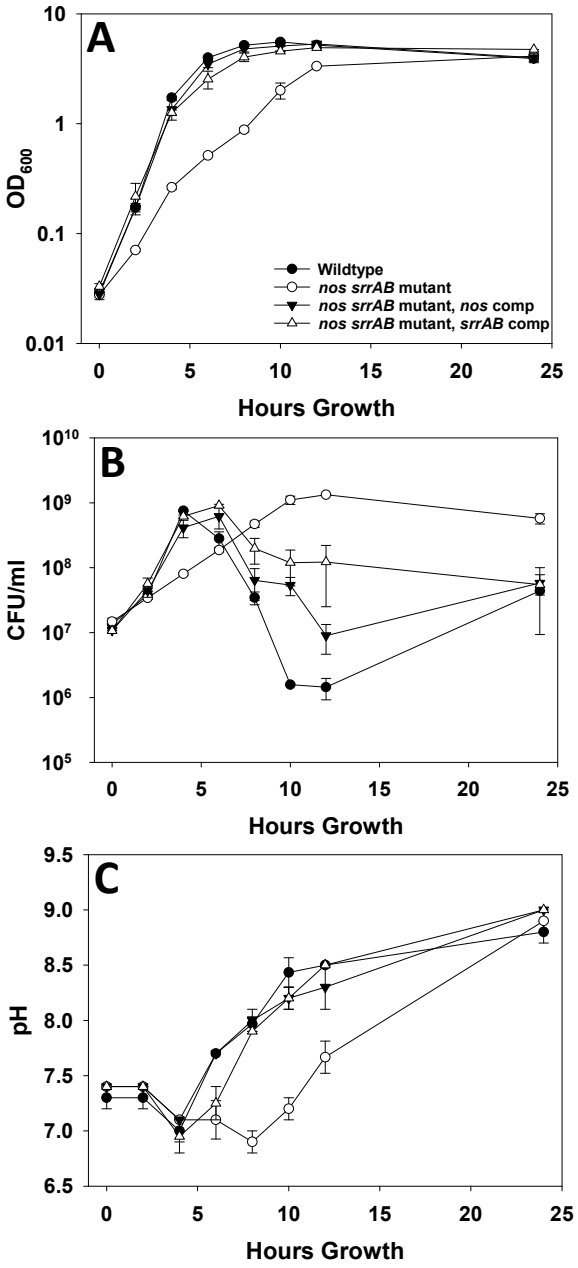

Figure S2: Growth analysis of UAMS-1 (wildtype), *nos srrAB* mutant, and *nos srrAB* complement strains. Aerobic TSB-G cultures were inoculated to an OD_{600} = 0.025 in TSB-G media and grown at 37°C with aeration (250 RPM; 1:12.5 volume to flask ratio). Growth over a 24-hour period was monitored by tracking OD_{600} measurements (A), CFU/ml by serial dilution plating (B), and pH measurement (C). Data points represent the average of 3 independent experiments and error bars = SEM.

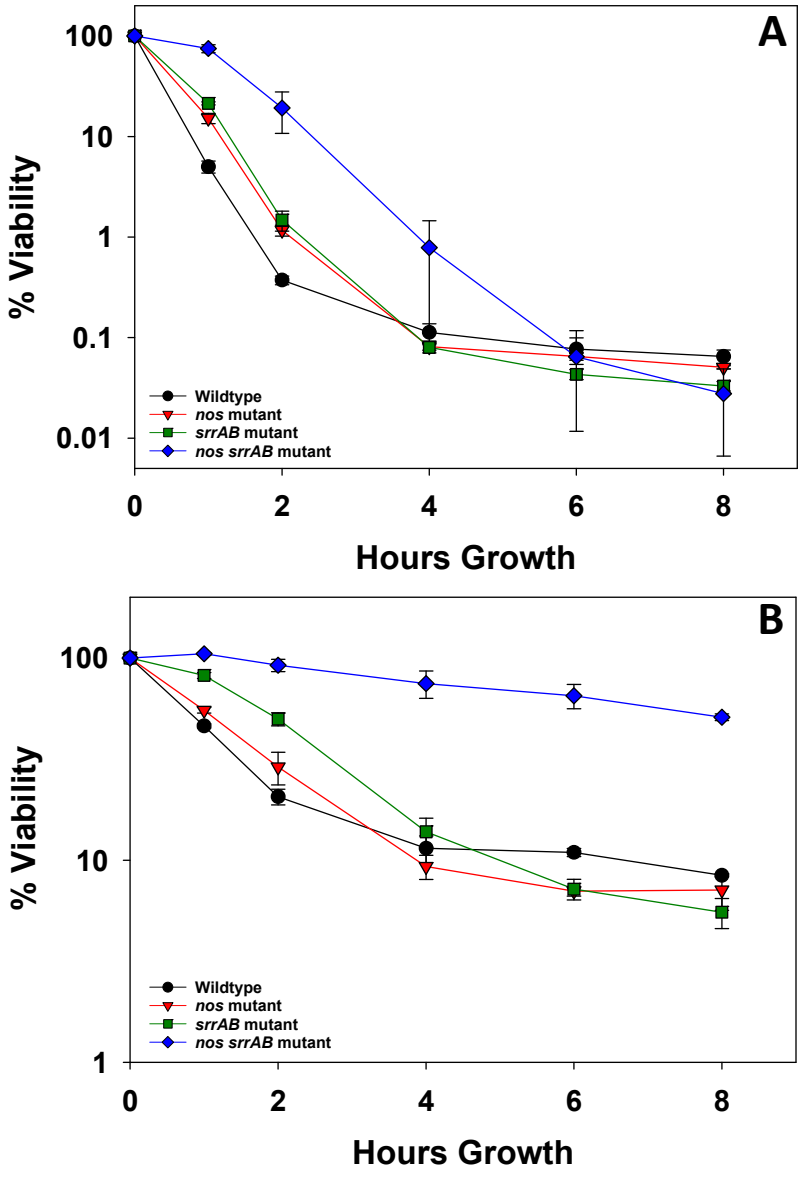

Figure S3: Contributions of saNOS and SrrAB to antibiotic killing. Early-exponential phase aerobic TSB-G cultures of UAMS-1 (wildtype) and isogenic *nos*, *srrAB*, and *nos srrAB* mutants were treated with final concentrations of either 30 µg/ml ciprofloxacin (A) or 40 µg/ml vancomycin (B), and CFU/ml were monitored at t=0 (just prior to antibiotic treatment), 1, 2, 4, 6, 8 hours post-treatment, as described in Materials and Methods. All data represent the average of n=3 independent experiments, error bars = SEM.

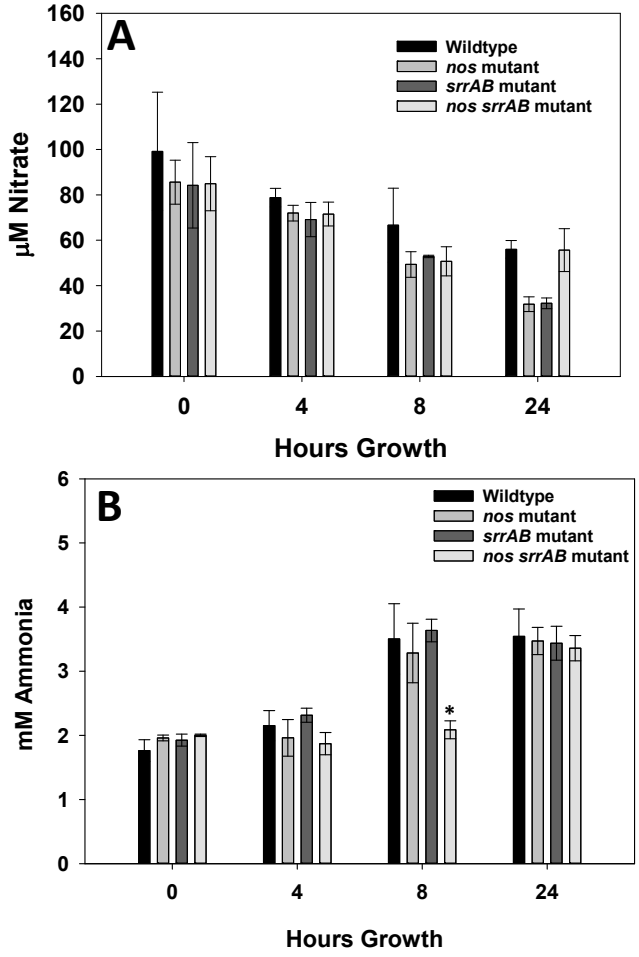

Figure S4: Nitrate and ammonia concentrations in supernatants. Extracellular nitrate and ammonia was measured from supernatants of wild-type, *nos* mutant, *srrAB* mutant, and *nos srrAB* mutant aerobic growth curves in TSB-G media. A. Nitrate measurements B. Ammonia measurements. For both A and B: Samples were collected at T = 0, 4, 8, 24 hours after inoculation. Measurements were performed using R-Biopharm kits in 96-well cell culture plates and each supernatant had an n=3 technical replicates and for each condition N=3 biological replicates, error bars = SEM). *P = 0.03 T-test (relative to wildtype).

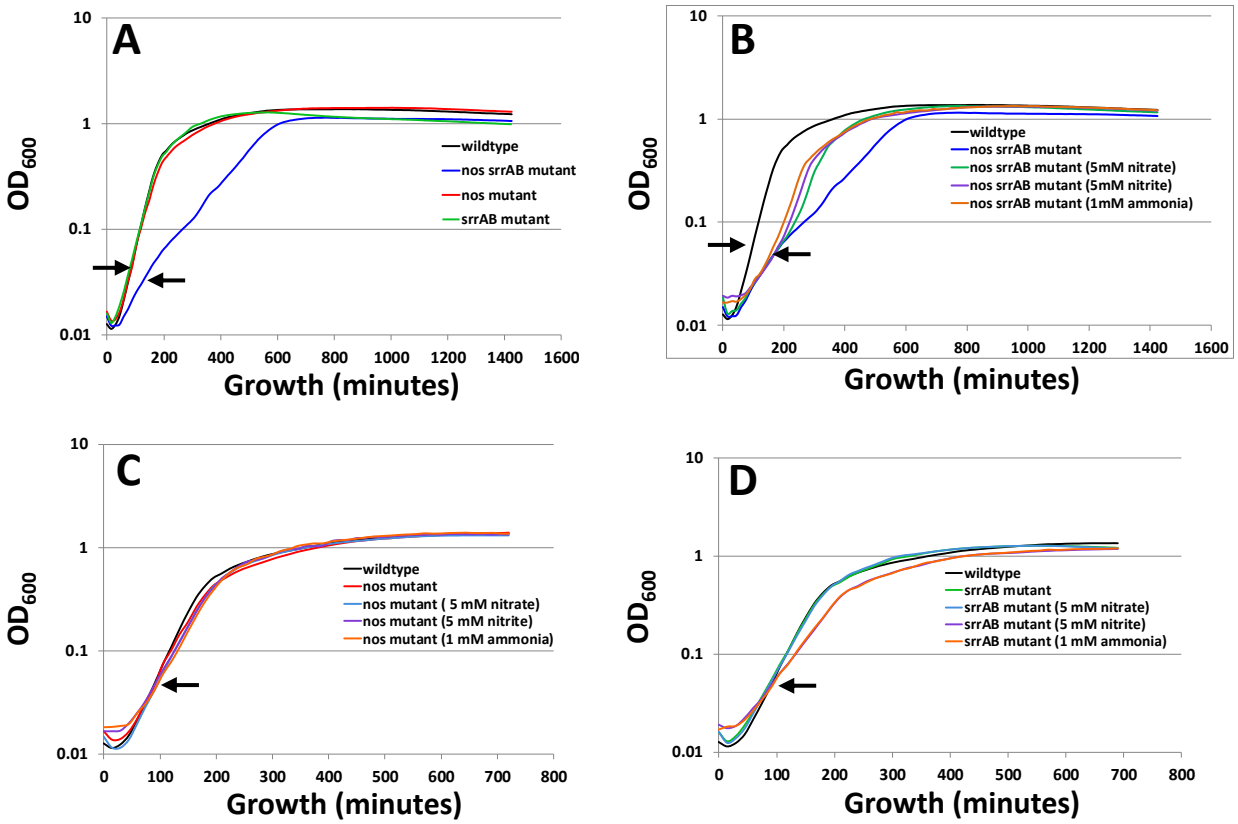

Figure S5: Bioscreen growth curves. Wildtype, *nos* mutant, *srrAB* mutant, and *nos srrAB* mutant were inoculated to a final OD_{600} = 0.025 in TSB-G and were grown untreated or supplemented with either 5 mM nitrate, 1 mM nitrite, or 1 mM ammonia. 200 µl aliquots were dispensed into quadruplicate wells of a Bioscreen C plate. Cultures were incubated in a Bioscreen C plate reader with maximum shaking at 37°C and growth was monitored by measuring the OD_{600} every 15 minutes for 24 hours. Graphs are representative of n=3 biological replicates. Black arrows represent the linear region of the graph in which maximum specific growth rates were calculated for Figure 6.

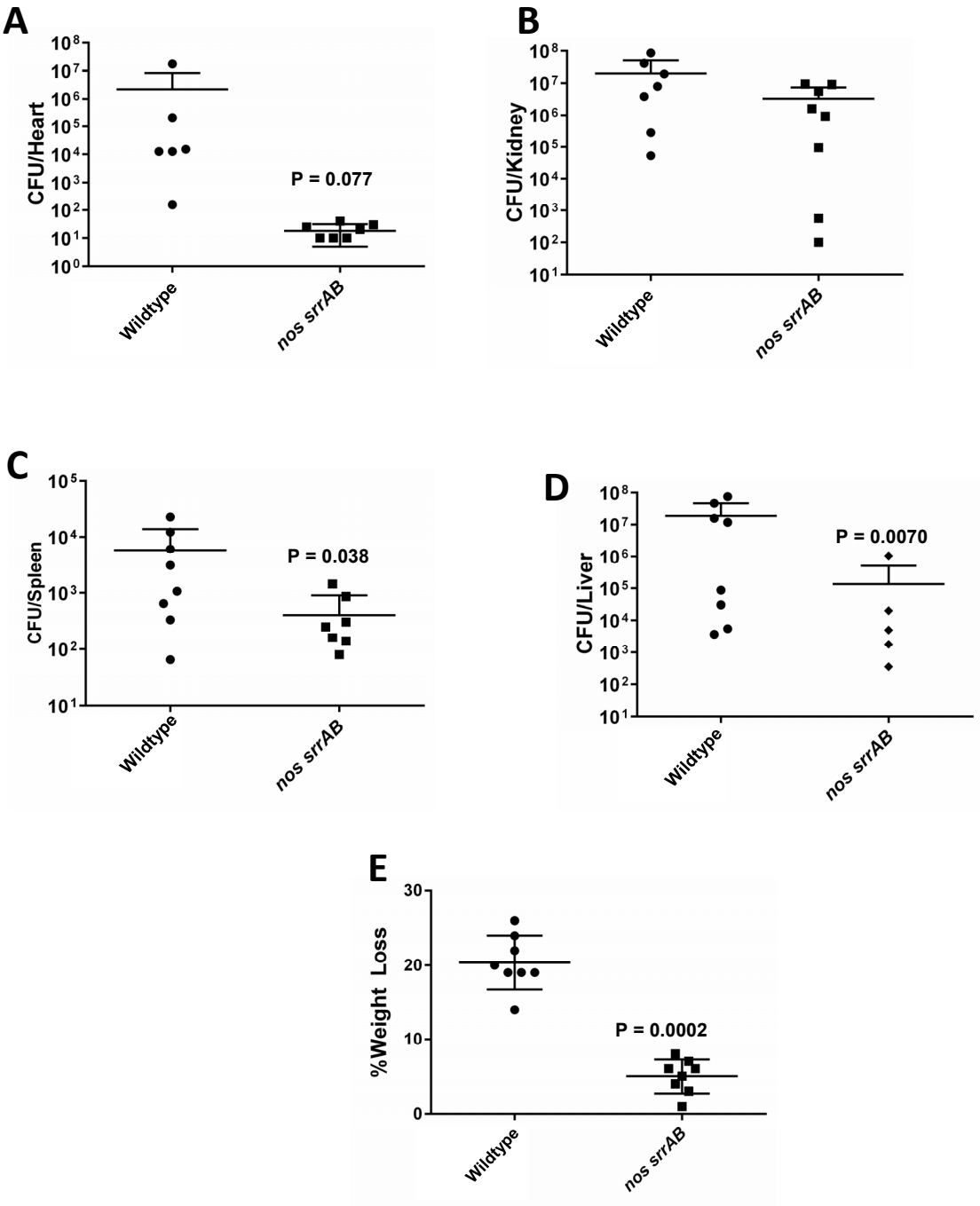

Figure S6: 4-day sepsis infection. UAMS-1 (wildtype; 3.95 x 10^7 CFU) and isogenic *nos srrAB* (3.9 x 10^7 CFU) mutant cultures were each retro-orbitally injected into 8-week-old C57BL/6 female mice (n=8 mice per group). Mice were euthanized 4-days post-infection, and organs were processed for CFU/organ determination as described in Materials and Methods. Graphs depict the calculated bacterial burdens for heart (A), kidney (B), spleen (C) and liver (D). The percent weight loss at 4-days post infection of each animal group is also reported (E). For A–E, data are graphed as scatter plots with mean (solid line) and standard deviation (error bars). Two-tailed P-values are reported for all significantly different comparisons to wild-type using a Mann-Whitney Test.

Table S1. List of bacterial strains and plasmids used in this study

Strain or plasmid name	Description	Reference or source
E. coli DH5α	Host strain for construction of recombinant plasmids	(1)
Staphylococcus aureus		
RN4220	Easily transformable restriction deficient strain	(2)
UAMS-1	Osteomyelitis clinical isolate	(3)
KR1010	UAMS-1 *nos*::*erm* insertion mutant	(4)
KB6004	UAMS-1 Δ*srrAB* deletion mutant	(5)
KR1014	UAMS-1 *nos*::*erm* Δ*srrAB* mutant	This study
pTR27	*nos::erm* allele-replacement plasmid in pBT2; Erm^R/Cm^R	(4)
pBT2	Temperature-sensitive shuttle vector; Cm^R/Amp^R	(6)
pMK*nos*	*nos* complementation plasmid; Cm^R	(4)
pMK4	Shuttle vector; Cm^R/Amp^R	(7)
pIHW58	*srrAB* complementation plasmid in pJB94; Tet^R	(8)

Table S2. PCR primers used in this study

Primer	Purpose	Sequence (5′-3′)	Reference or Source
sigA-F sigA-R	Real-time PCR	F - CAAGCAATCACTCGTGCAAT R - GGTGCTGGATCTCGACCTAA	(4)
sar0233 (*hmp*)	Real-time PCR	F - AGAGGCATGCAATCTTCAGC R - AGTGCGCAGTGTTTATATGC	(9)
sar0256 (*scdA*)	Real-time PCR	F - TGCGGCGGACAAGTAAGTAT R - GCGAACCTGGTGTATTCGTT	(9)
sar0218 (*pflB*)	Real-time PCR	F - GCTGTTAAAGCAGCCTACCG R - AGAAGCATATGCCCCTTCAC	(9)
sar1032 (*qoxC*)	Real-time PCR	F - ACGCATGGTTGTCACGTATC R - TGTCTAATCCGCGTCGTTG	(9)
sar2680 (*ldh2*)	Real-time PCR	F - CTTGCAGTTTGGTCACAAGC R - TTCCGCTTTAGCTTCGCTAC	(9)
sar0234 (*ldh1*)	Real-time PCR	F - GGTGTTGCAATGGGATTAGC R - TGTGCGAACTTGCTTTGTTC	This Paper
sar2486 (*narG*)	Real-time PCR	F - CGGCAAGAGCAGTTATTTCG R - GACCCAGGCGTTTGAATATG	(9)
sar2489 (*nirB*)	Real-time PCR	F - TGCAGAACATAACGGCAAAG R - TCCCTTGTATCCGTTCGTTT	This Paper
sar2493 (*narT*)	Real-time PCR	F - TATGGGGACGACTGGGTAAA R - GCGGTAAATCTGGTTCGTGT	This Paper
sar2714 (*arcA*)	Real-time PCR	F - AGCACGACGACGAGAATCAA R - AAACAAGTTCATCGCCGCCT	This Paper
sar2713 (*arcB*)	Real-time PCR	F - TGGTGTACCGGTGTGGAATG R - ACGTCCATCTCCAACGTAAG	This Paper
sar0184 (*argJ*)	Real-time PCR	F - GGTGCGTTTAACGAGGCAAT R - TCCTTTAGCACTGCCACCAA	This Paper
sar0923 (*argG*)	Real-time PCR	F - GCATTCGCACCTGTACGTGA R - TCATTCGCTCTGCCCCATAG	This Paper
sar1142 (*otc*)	Real-time PCR	F - AGGTGCTGCCATTATGGGTA R - GTTGCCACCATTTTCAGCGG	This Paper
sar2255 (*rocF*)	Real-time PCR	F - CGCCAGGCACAGGTACTAGA R - CCGATTGTTCAGCAGTATGGT	This Paper
sar2007 (*nos*)	Real-time PCR	F - TATGGTGCTAAAATGGCTTG R - ACGATGCTTCGTCAGTAACA	(4)

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