

Supplemental Figures

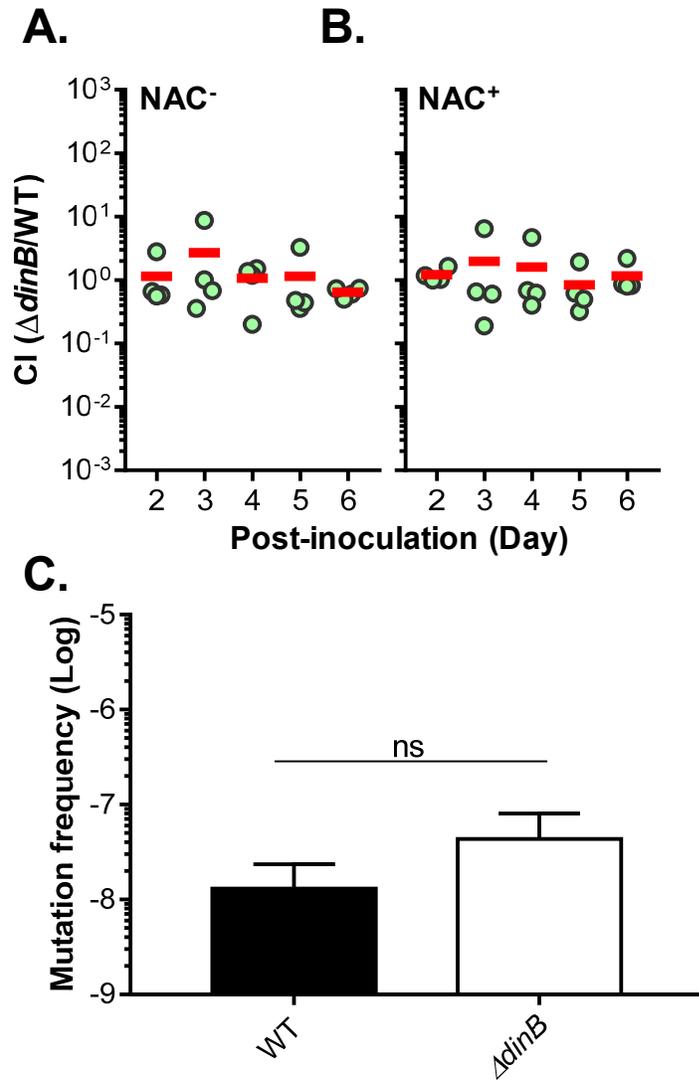


Fig. A. The effect of *dinB* on colonization and mutation rate. **A&B.** Colonization of in-frame *dinB* deletion mutants. 10^8 cells of wildtype and $\Delta dinB$ mutants were mixed in a 1:1 ratio and intragastrically administered to NAC⁻ (**A**) and NAC⁺. (**B**) mice. Fecal pellets were collected from each mouse at the indicated time points and plated onto selective plates. The competitive index (CI) was calculated as the ratio of mutant to wildtype colonies normalized to the input ratio. Horizontal line: mean CI of 4 mice. **B.** Mutation frequency. Cultures of wildtype and $\Delta dinB$ strains were grown in LB until saturation and then plated onto LB agar and LB agar + 50 μ g/ml rifampicin. After overnight growth at 37°C, rifampicin resistant colonies were scored. Error bars represent means and SDs from three independent assays. ns: Student t-test no significance.

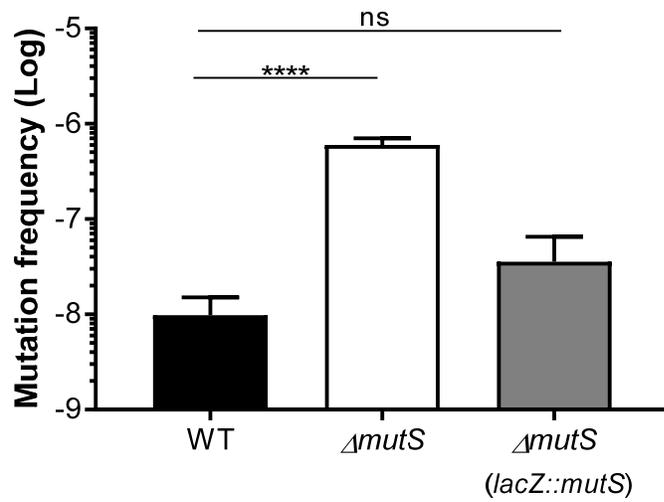


Fig. B. Chromosomal complementation of *mutS*. Cultures of wildtype, $\Delta mutS$, and chromosomally inserted (in *lacZ* locus) *mutS* in $\Delta mutS$ were grown in LB until saturated and then plated on LB agar and LB agar + 50 $\mu\text{g/ml}$ rifampicin. After overnight growth at 37°C, rifampicin resistant colonies were scored. ****: One-way ANOVA $P < 0.0001$. ns: no significance.

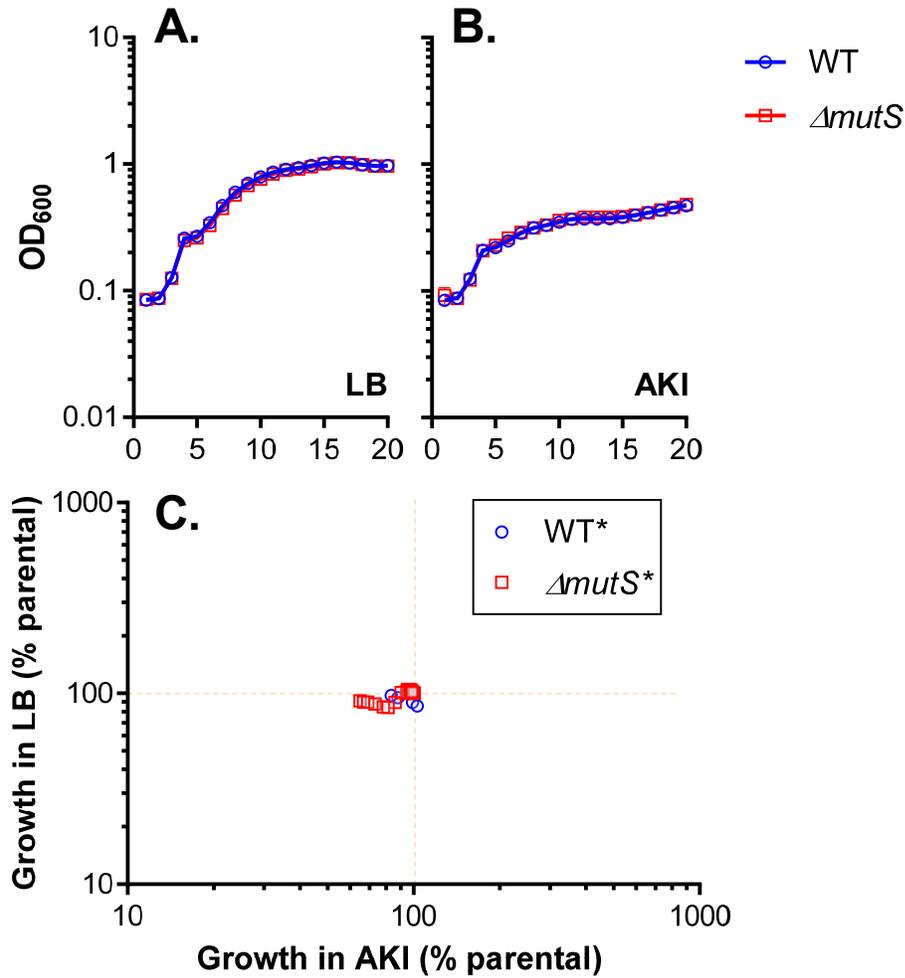


Fig. C. The effect of *mutS* on *V. cholerae* growth. Wildtype and $\Delta mutS$ growth in LB (shaking)(**A**) and AKI medium (standing)(**B**). OD₆₀₀ was measured. **C.** Growth of WT* and $\Delta mutS^*$ in LB and AKI to mid-log phase. OD₆₀₀ was measured and compared with their parental strains.

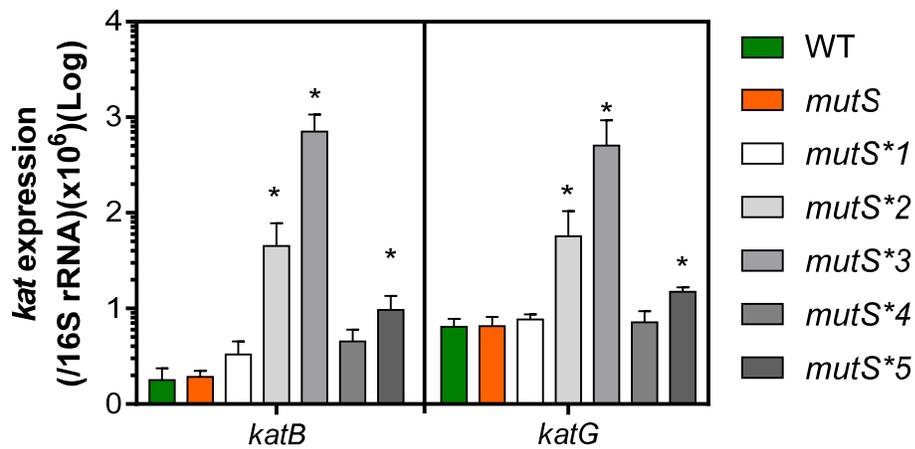


Fig. D. Expression of catalase genes in $\Delta mutS^*$ isolates. Mid-log cultures of wildtype, $\Delta mutS$, and selected $\Delta mutS^*$ were induced with 500 μM H_2O_2 for 1 hr. Total RNA was extracted and cDNA was synthesized. Reverse transcription-quantitative PCR (qRT-PCR) was carried out and normalized against 16S rRNA as the internal standard. Error bars represent means and SDs from three independent assays. *: One-way ANOVA $P < 0.05$ (compared to wildtype).

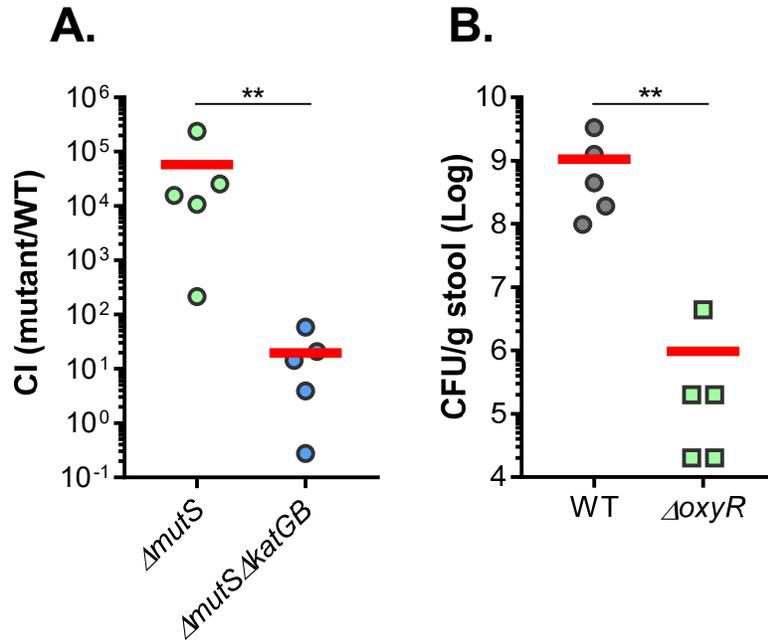


Fig. E. Colonization of ROS-sensitive mutants in NAC^- mice. **A.** $\Delta katGB$, $\Delta mutS$ or $\Delta mutS \Delta katGB$ mutants were mixed with wildtype at 1:1 ratio and intragastrically administered to NAC^- mice. Fecal pellets were collected from each mouse at 4-day PI and plated onto X-gal plates with appropriate antibiotics. The competitive index (CI) was calculated as the ratio of mutants to wildtype normalized to the input ratio. Horizontal line: mean CI of 5 mice. **: Mann-Whitney test P value < 0.01 . **B.** $\Delta oxyR$. $\Delta oxyR$ mutants were mixed with wildtype in a 1:1 ratio and intragastrically administered to NAC^- mice. Fecal pellets were collected from each mouse at 4-day PI and plated onto X-gal plates with 10 $\mu g/ml$ catalase and appropriate antibiotics. The competitive index (CI) was calculated as the ratio of mutants to wildtype normalized to the input ratio. Horizontal line: mean CI of 5 mice. **: Mann-Whitney test P value < 0.01 .

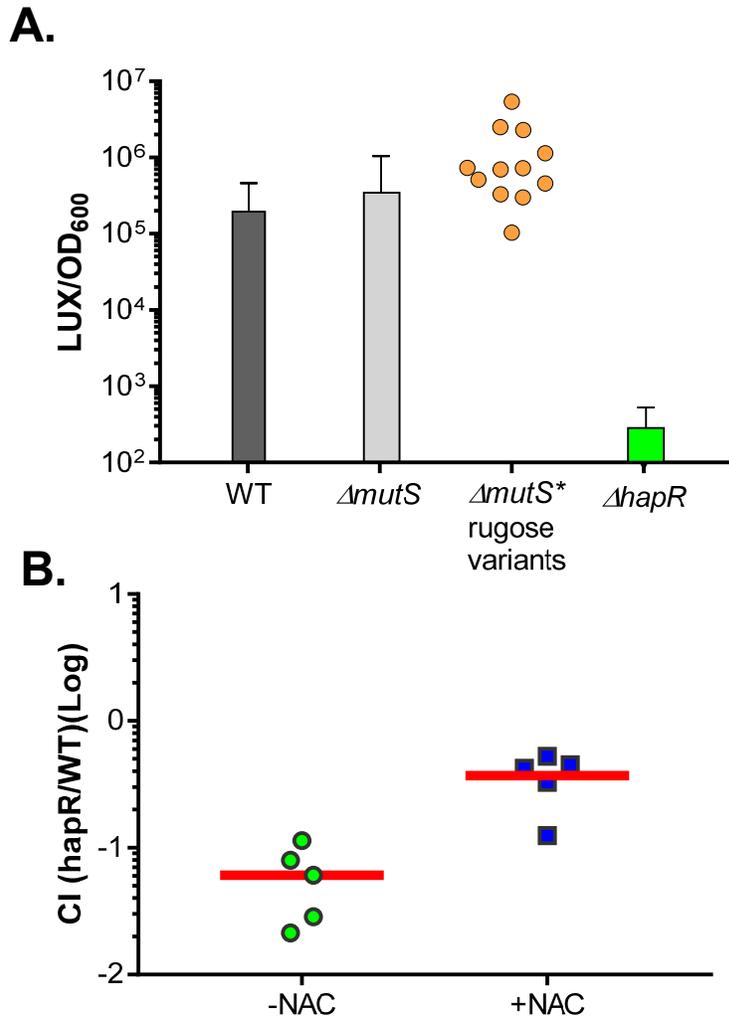


Fig. F. The relationship between quorum sensing regulator HapR and rugose variants of $\Delta mutS^*$. **A.** pBB1 expression in $\Delta mutS^*$. Wildtype, $\Delta mutS$, and $\Delta mutS^*$ rugose variants containing a HapR-regulated *luxCDABE* (pBB1) [68] were grown in LB with appropriate antibiotics at 30°C overnight, diluted to a concentration of 1:100 in fresh LB and transferred to white opaque 96 well plates and incubated while shaking at 30°C. Luminescence was read at $OD_{600} = 1$. **B.** Colonization. Wildtype and $\Delta hapR$ were co-inoculated into 6-week-old CD-1 mice with or without NAC treatment. Fecal pellets were collected after 5 days and plated onto selective plates. The competitive index was calculated as the ratio of mutant to wildtype colonies normalized to the input ratio.

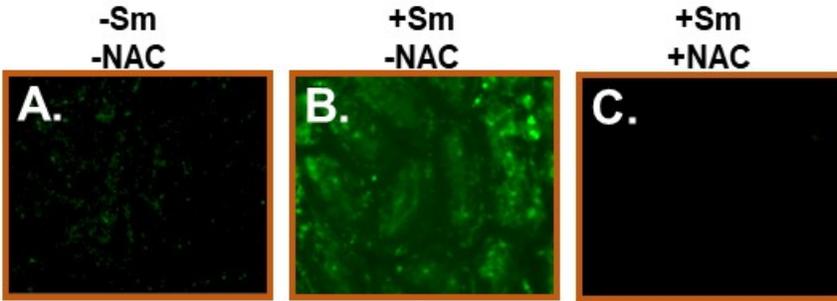


Fig. G. ROS production in adult mouse intestinal tissues. Small intestinal frozen tissue sections from mice with no treatment (**A**, -Sm, -NAC), treated with streptomycin (**B**, +Sm, -NAC), and with streptomycin and N-acetyl cysteine (**C**, +Sm, +NAC) were stained with CM-H₂DCFDA (Invitrogen) for 60 min at 37 °C. Images were taken using a fluorescence microscope (IX81; Olympus). Five randomly selected areas were photographed with the same exposure time. The images were processed using the same fixed threshold in all samples by Slidebook 5.0, and cropped using Adobe Photoshop. Representative images are shown.

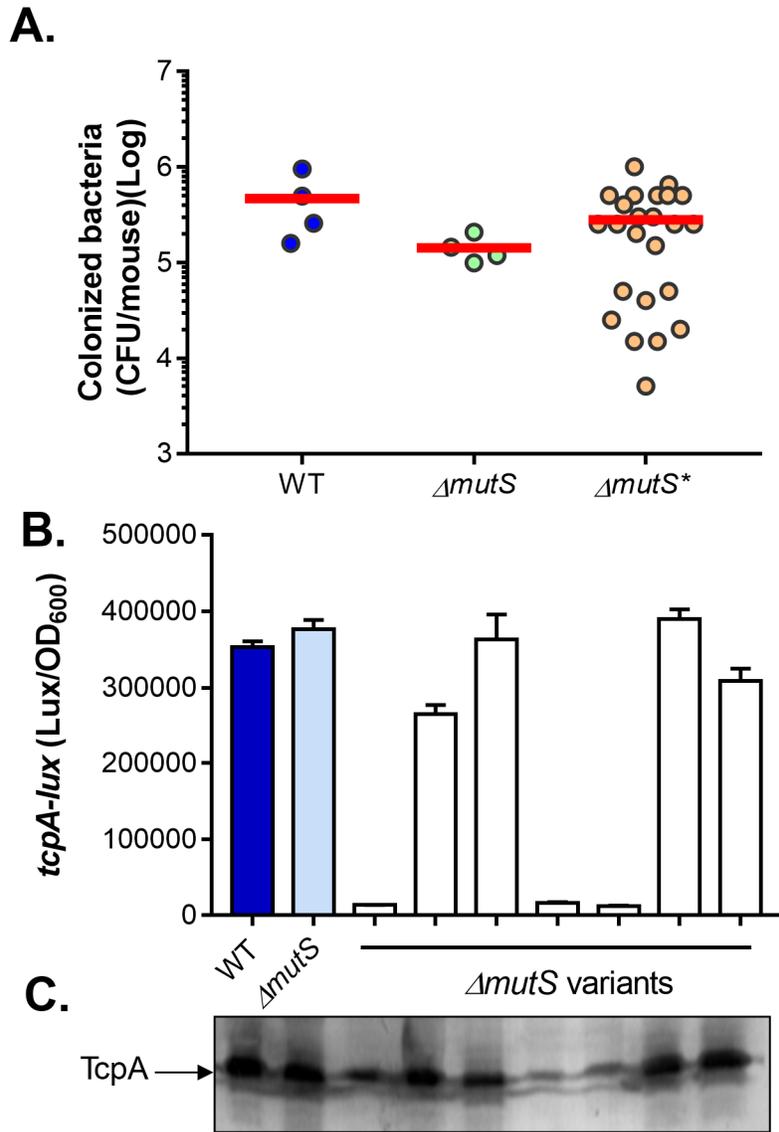


Fig. H. The effects of *mutS* on virulence factor production and infant mouse colonization. **A.** The infant mouse colonization assays. Mid-log phase cultures of WT (*lacZ*⁺) and mutants (*lacZ*⁻) were mixed in a 1:1 ratio and approximately 10⁵ cells were intragastrically inoculated into 5-day-old CD-1 suckling mice. After a 20-hr period of incubation, mice were sacrificed. Small intestines were harvested and homogenized, the ratio of mutants to WT bacteria was determined by plating onto LB agar containing antibiotics and X-Gal. **B.&C.** Overnight cultures of wildtype, $\Delta mutS$ and $\Delta mutS^*$ containing P_{*tcpA-luxCDABE*} transcriptional fusion plasmids were inoculated 1:10000 into AKI medium [37] and incubated without shaking at 37°C for 4 hrs, followed by shaking at 37°C for an additional 3 hrs. Luminescence was then measured at the indicated time points and normalized to OD₆₀₀ (**B**). At the final time point, 10⁹ cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting using anti-TcpA antiserum (**C**).