Supplemental Information
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RNA-Seq-Based Monitoring of Infection-Linked Changes in *Vibrio cholerae* Gene Expression

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Figure S1, related to Figure 1

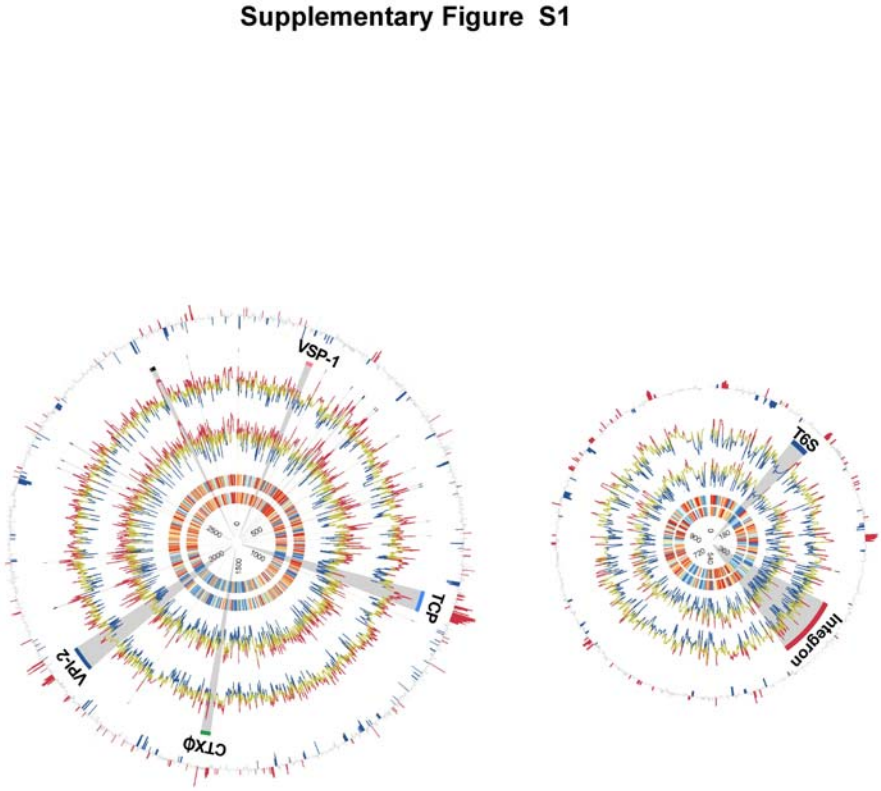


Figure S2, related to Figure 2

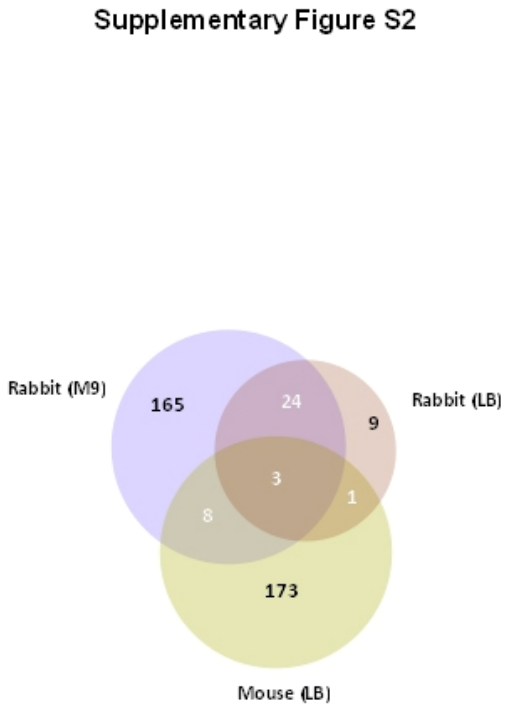
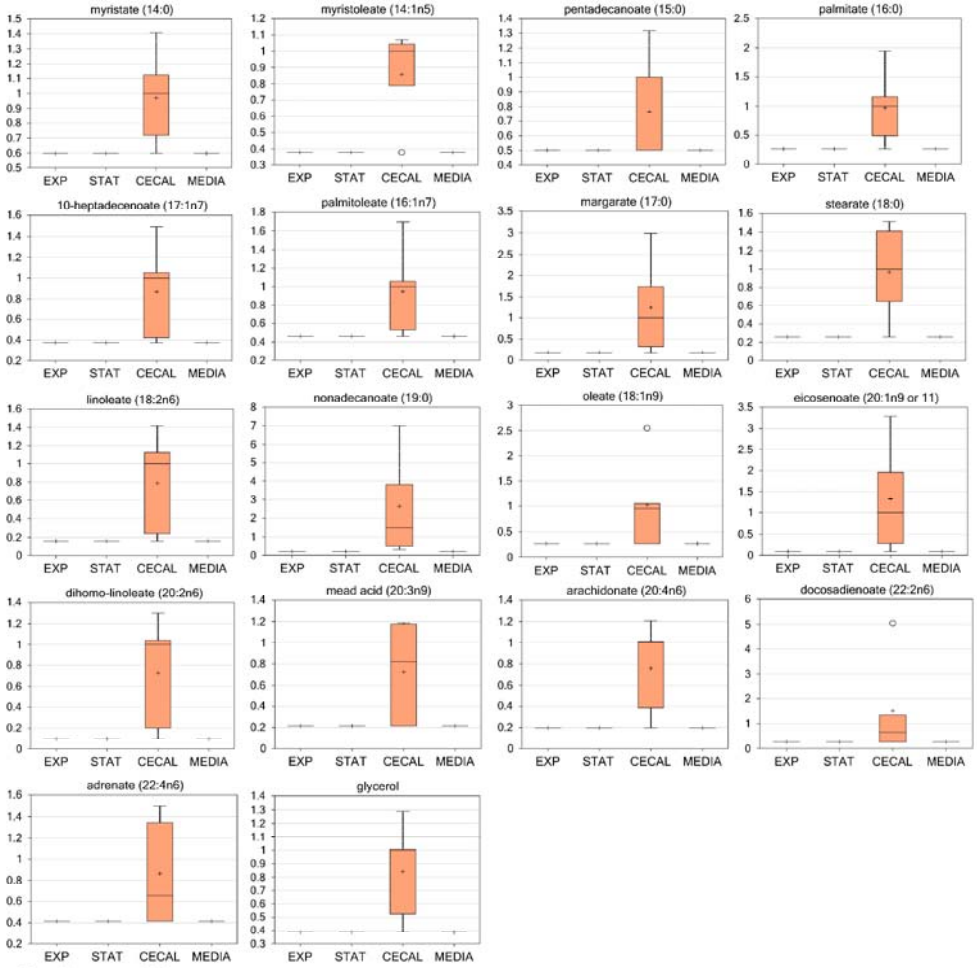


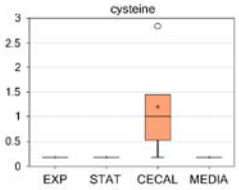
Figure S3, related to Figure 3

Supplemental Figure 3

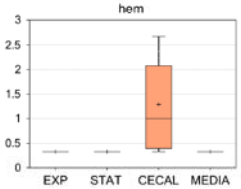
Long chain fatty acids and glycerol



Amino acids



Heme



Symbol legend

+	Mean value
-	Median value
o	Extreme data point

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Profiles of *V. cholerae* gene expression in rabbits and minimal media
Profiles of *V. cholerae* gene expression in culture and during infection. Plots for chromosome I are on the left and for chromosome II are on the right and are based on data from two biological replicates for each condition. From inside to outside, the 6 circles in each plot correspond to the following: 1-2) heatmap of ranked coverage in 5 kb windows in vitro and in vivo, respectively 3-4) log2 of RPKMO for each gene in vitro and in vivo, respectively. In circles 1-4 red, yellow, and blue correspond to windows/genes with high, middle, and low expression, respectively. 5) Regions encoding ribosomal proteins (black) or corresponding to indicated genomic islands. 6) Log2 of fold expression in vivo vs. in vitro. Genes whose fold expression is statistically significant and > 4 fold higher or lower in vivo are highlighted in red and blue, respectively; the height of the bars corresponds to log2 of the differential abundance *in vivo* vs LB.

Figure S2. Venn diagram of genes under-expressed in mice and rabbits
Genes were considered under-expressed if their differential abundance between in vivo and in vitro samples was $\leq$ 0.25-fold and had a P value < $1x10^{-5}$.

Figure S3. Comparison of the abundance of selected metabolites in cecal fluid and culture supernatants
Culture supernatants of *V. cholerae* grown in minimal media to exponential (exp) (4 samples) or stationary phase (stat) (4 samples), of spun cecal fluid specimens from 5 infected rabbits, and media control were subjected to biochemical profiling by mass spectrometry. In the box plots, the y-axis is the scaled intensity for each metabolite tested. All of the biochemicals listed were only detected in cecal fluid samples; the values shown for the culture supernatants and media samples represent imputed minimum values on a per biochemical basis. The levels of each of these metabolites were significantly elevated ($p \leq 0.05$) in the cecal fluid compared to other conditions based on Welch's two-sample *t*-tests.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Rabbit infection:

Infant rabbits were infected with *V. cholerae* largely as described (Ritchie et al., 2010). Two three-day-old infant rabbits were treated with cimetidine (50-mg/kg via intraperitoneal injection) 3 h prior to orogastric inoculation with *V. cholerae* with a size 5 French catheter (Arrow International, Reading, PA). In most experiments, rabbits were inoculated with ~1 × 10^9 CFU of *V. cholerae*. To prepare the inocula, overnight cultures of *V. cholerae* grown at 30°C were harvested by centrifugation, and the cell pellets were resuspended at a final concentration of ~2 × 10^9 CFU/ml in a sodium bicarbonate solution (2.5 g in 100ml; pH 9). Rabbits were euthanized at 22 h postinfection. Necropsy was done at 37°C and cecal fluid was harvested in an eppendorf tube.

Mouse infection:

The protocol for infection of infant mice with *V.cholerae* was generally as described (Angelichio et al., 1999). In short, 5-day old mice were infected with *V. cholerae* from cultures that had been grown overnight at 37°C. Infant mice were separated from their mothers for 6 hours prior to oral inoculation with ~10^5 *V.cholerae* cells diluted into 50ul LB. Mice were sacrificed 18-20 hours post-infection and the small intestines were removed and suspended in 10ml of a 1:1 mixture of ice cold LB:RNALater (Ambion). Intestinal tissue was pulverized and the homogenate filtered by passing through a 30ml syringe packed with cheesecloth. *V. cholerae* were further culled from host cells by passage through a 5mm cellulose acetate membrane filter (Minisart). Filtered cells were concentrated by centrifugation at 5000 *g* for 12 minutes.

Bacterial RNA preparation:

A. RNA for Illumina sequencing: For the *in vitro* samples, RNA was extracted from 1ml of a mid-exponential phase culture using TRIzol (Invitrogen), and then treated with Turbo DNA-free (Ambion) and acid-phenol-chloroform (Ambion) to remove the residual DNA. For *V. cholerae* derived from infected rabbits, cecal fluid was centrifuged at 6000*g* for 2 minutes, and the cell pellet was then suspended in 1ml of TRIzol and treated, as were the *in vitro* samples.

B. RNA for Helicos sequencing: The concentrated cell pellet obtained from the mouse intestinal homogenate was resuspended in 500µl RNAlater and the RNA extraction and purification was completed using the mirVana PARIS (Ambion) protocol. For the in vitro samples, *V. cholerae* was grown in LB at 37°C. 5ml of culture was harvested during mid-log phase growth (O.D. 600~0.4) and then diluted 1:1 in RNAlater. Cells were concentrated by centrifugation at 5000 *g* for 12 minutes and resuspended in 500µl RNAlater for RNA extraction and purification using the mirVana PARIS (Ambion) protocol.

Sequencing:

For Illumina sequencing: The cDNA libraries were sequenced on an Illumina Genome Analyzer II at the Broad Genome Sequencing Platform. The libraries were quantified using quantitative PCR (KAPA biosystems) with specific probes for the ends of the adapters. Based on the qPCR quantification, libraries were normalized to 2nM and then denatured using 0.1 N NaOH. Cluster amplification of denatured templates occurred according to manufacturer's protocol (Illumina) using V4 Chemistry and V4 Flowcells. Sybr Green dye was added to all flowcell lanes to provide a quality control checkpoint after cluster amplification to ensure optimal cluster densities on the flowcells. Flowcells were

sequenced on Genome Analyzer IIs, using V4 Sequencing-by-Synthesis kits and analyzed with the Illumina RTA v1.8 pipeline. Standard quality control metrics including error rates, % passing filter reads, and total Gb produced were used to characterize process performance prior to downstream analysis.

For Helicos sequencing: 5-10 femtomoles of prepared product was loaded per lane on the Helicos flowcell resulting in 20-40 million reads. The average read length was 36 nt.

Mouse infection and competition studies:

Competition studies were performed as described previously (Angelichio et al., 1999). Overnight cultures of wild type C6706 *lacZ*$^{-}$ and various transposon insertion mutants (*lacZ*$^{+}$) were diluted 1:1000 and then mixed 1:1 to form the inocula. 3-5 day old CD-1 mice were orogastrically infected with the inocula. Mice were sacrificed at 22 h postinfection and their small intestines were removed, homogenized and plated on LB containing streptomycin and supplemented with X-Gal. CFU were counted the next day and competitive indices were calculated as the output ratio of mutant vs WT cells divided by the input ratio of mutant vs WT cells.

Biochemical profiling:

Culture supernatant from exponential (n=4) and stationary phase (n=4) cells grown in minimal media and cecal fluid supernatant (n=5) was sent for biochemical profiling at Metabolon, Inc. The samples were extracted and prepared as described (Evans et al., 2009). The extracted samples were split into equal parts for analysis on GC/MS and LC/MS/MS platforms. Following log transformation and imputation with minimum observed values for each compound, Welch's two-sample *t*-tests were used to identify biochemicals that differed significantly ($p \leq 0.05$) between groups.

SUPPLEMENTAL REFERENCES

Angelichio, M. J., Spector, J., Waldor, M. K., and Camilli, A. (1999). Vibrio cholerae intestinal population dynamics in the suckling mouse model of infection. Infect Immun *67*, 3733-3739.

Evans, A. M., DeHaven, C. D., Barrett, T., Mitchell, M., and Milgram, E. (2009). Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. Anal Chem *81*, 6656-6667.