SUPPLEMENTARY INFORMATION

SUPPLEMENTARY RESULTS AND DISCUSSION

Human Studies

Patient selection - Of the 1153 patients screened (**Supplementary Table 1**), 796 (69%) were disqualified because of immediate prior antibiotic usage. Another 100 were disqualified because their diarrhea began more than 24 h before enrollment, while 132 were excluded due to lack of a permanent address for follow-up. Of the 11 adults (all males) who entered the study, four could not be contacted by staff after discharge from the hospital; their samples were not included in our analysis, leaving a total of seven individuals (A-G; see **Supplementary Table 2** for clinical metadata). The seven patients included in the study experienced diarrhea for 8.0±2.6 hours (mean±SD) prior to hospital admission and for 41.5±16.7 hours during their hospital stay. They had an average of 1.5±1.0 diarrheal stools per hour (**Supplementary Table 2**).

For ethical reasons, we could not withhold treatment with azithromycin (or for that matter oral rehydration therapy). Therefore, our study design did not allow us to isolate the nature and effect sizes of various elements of the treatment protocol on the temporal patterns of change in the gut microbiota during the acute and recovery phases of infection, versus those produced by the diarrheal disease *per se*.

Identifying community-wide changes in representation of 97%-identity OTU and species during diarrhea and recovery phases - Across all seven individuals, 58.6% of 97%-identity OTUs with a species-level taxonomic assignment associated with recovery (see below) were also detected during both diarrhea and recovery phases (**Extended Data Figs. 1,2**). For individuals C and E, where higher time-resolution analysis was performed, 41.1% and 29.9% of species-level taxa were identified in both diarrhea and recovery samples respectively, with 6.9% and 11.1% of the identified species detected only in recovery phase samples (**Supplementary Table 4c**).

Phylogenetic diversity (PD) of the cholera fecal microbiota decreased markedly during D-Ph2/D-Ph3, approaching the PD of healthy Bangladeshi children, before rising during R-Ph1-R-Ph3 in a temporal pattern that paralleled the UniFrac measurements of similarity to reference healthy adult controls (**Extended Data Fig. 5e**).

Indicator species analysis was performed on the set of 236 fecal specimens selected from the diarrheal and recovery phases of subjects A-G. The statistical significance of associations was defined using permutation tests in which permutations were constrained within subjects: a bacterial species was considered significantly associated if it had a FDR-adjusted $P<0.05$. This approach identified 260 bacterial species consistently associated with either the diarrheal or recovery phases. 219 of these 260 species also had a significant correlation to community UniFrac distance to healthy control microbiota (FDR-adjusted $P<0.05$; **Supplementary Tables 5,6, Extended Data Fig. 2d**). For species with positive correlations, higher relative abundances in a given microbiota state correlated to an increased difference to healthy fecal microbiota. Interpersonal differences in the distribution of 97%-identity OTUs comprising these species were also evident (**Extended Data Fig. 2c**, **3).**

Of the 31 age-discriminatory species-level bacterial taxa in the developing gut microbiota of healthy Bangladeshi children, 24 including *R. obeum* and *F. prausnitzii,* also had a significant Spearman rank correlation value between their relative abundance in each fecal sample and the mean weighted UniFrac distance between that sample and all healthy adult Bangladeshi microbiota (**Supplementary Tables 5,6,** see **Extended Data Fig. 2-4** for 97%-identity OTU analysis). In addition, Spearman rank correlations revealed that (i) 23 of the 27 species had relative abundances that significantly correlated with chronologic age in healthy children *and* with time following onset of acute diarrhea, and (ii) the direction of change

(increase or decrease) was concordant between the two datasets for all 23 species (**Supplementary Table 4d**).

Changes in relative abundance of genes encoding ECs in fecal microbiomes sampled during diarrhea and recovery - Shotgun sequencing of DNA prepared from diarrhea and recovery phase human fecal samples, followed by binning reads into assignable KEGG Enzyme Commission numbers (ECs), revealed that genes encoding enzymes involved in carbohydrate metabolism comprised the largest category of ECs that changed in relative abundance within the fecal microbiome during the course of cholera (see **Extended Data Fig. 6** and **Supplementary Table 8** for EC-based abundance analysis, including Spearman rank correlations of EC abundance as a function of time across D-Ph1 through R-Ph3). These results led us to include *Bacteroides cellulosilyticus, Bacteroides thetaiotaiotaomicron*, and *Clostridium scindens* in the artificial community of human gut symbionts, even though they did not satisfy the criteria described above as recovery phase-indicative or significantly correlated to normal maturation of the Bangladeshi infant microbiota.

Gnotobiotic mouse experiments

Genome-wide analysis of how V. cholerae influences the R. obeum transcriptome in vivo - Mice were first mono-colonized with *R. obeum* or *V. cholerae* for 7 days, followed by introduction of the other organism (**Extended Data Fig. 1c**). RNA-Seq was performed using fecal samples collected from both groups of mice on the day prior to and 2 days after co-colonization (**Supplementary Table 12)**. An analysis of changes across the entire *R. obeum* transcriptome 2d after introduction of *V. cholerae* revealed few (n=7) functionally annotated transcripts with significant differences in expression (P<0.05 after multiple-hypothesis testing in DESeq[40]; see **Supplementary Table 10c**). LuxS serves a dual role: production of the precursor AI-2 molecule [(*S*)-4,5-dihydroxyl-2,3-pentanedione] and participation in the pathway that re-generates

homocysteine for use in the activated methyl cycle; in the absence of *luxS*, homocysteine is produced via oxaloacetate involving aspartate and glutamate as intermediates[41,42] Consistent with this, three *R. obeum* genes encoding ECs involved in glutamate biosynthesis were significantly down-regulated after introduction of *V. cholerae*.

Genome-wide analysis of how R. obeum influences the V. cholerae transcriptome in vivo - *R. obeum* increased the expression of several *V. cholerae* genes whose functions could impact its colonization, including five that encode products involved in iron acquisition and transport [*VC0365* (bacterioferritin), *VC0364* (bacterioferritin-associated ferredoxin), *VC0608* (iron(III) transporter), *VC0750* (*hesB* family protein)] plus five genes thought to be involved in cell wall modification (*VC0246, VC0247, VC0245, VC0259, VC0249* in **Supplementary Table 10b**; modifications in *V. cholerae* LPS have been reported to be important in colonization of mice[17]). Cholera toxin gene *(ctxA, ctxB*) expression was below the limits of reliable detection in each of the *V. cholerae* treatment groups, consistent with previous reports that it is not required for colonization of adult mice[11-12].

Bile acids and regulation of virulence genes - *V. cholerae* senses host signals, including bile acids, in order to coordinately up-regulate expression of colonization factor genes[13,43-47] and down-regulate expression of anti-colonization factors such as the mannose-sensitive hemagglutinin pilus[48]. The gut microbiota could, in principle, affect colonization by modulating levels of host-derived signals that impact *V. cholerae*, or the microbiota could produce signaling molecules that directly modulate *V. cholerae* pathogenesis. To explore these possibilities, we used ultra-performance liquid chromatography-mass spectroscopy (UPLC-MS) to characterize the representation of bile acid species in fecal samples collected from mice colonized with the 14-member community and this community minus *R. obeum*, (n=5-6 animals/group). We detected 10 bile acid species, and expressed each of their levels as a proportion of the aggregate

levels of all 10 bile acids. Comparing fecal samples collected from mice after colonization with the 14- and 13-member communities, we found that the presence or absence of *R. obeum* did not have a statistically significant effect on levels of the primary bile acids taurocholic acid, tauro-beta-muricholic acid, and beta-muricholic acid that together comprise >80% of the measured pool (P>0.05, unpaired Mann-Whitney U test). One primary bile acid, alpha muricholic acid, and a secondary bile acid, urosodeoxycholic acid, were affected, but in aggregate they represent minor constituents (<11%) of the measured pool (**Extended Data Fig. 8**). While we could not rule out that the effect of *R. obeum* on these minor bile acid species impacts *V. cholerae* colonization/virulence, given the observed lack of change in the predominant bile acid species, we turned our attention to classes of microbial factors known to affect virulence (see *Main Text*).

R. obeum AI-2 production is stimulated by V. cholerae in vitro and in co-colonized animals - In mice initially mono-colonized with *R. obeum* for 7d, *R. obeum luxS* expression increased more than 2-fold 2d following introduction of *V. cholerae* (P<0.01, unpaired Mann-Whitney U test; **Extended Data Fig. 7d**). Using the BB170 AI-2 assay[24], we measured fecal AI-2 levels from mice mono-colonized with *R. obeum* or co-colonized with *R. obeum* and Δ*luxS V. cholerae* (MM883)[14], and confirmed that AI-2 levels were modestly but significantly higher in the co-colonized group (**Extended Data Fig. 7e**). When these bacteria were co-cultured *in vitro* under anaerobic conditions, *R. obeum* AI-2 signal increased significantly [2.8±0.1-fold (mean±SEM), P<0.01, unpaired Mann-Whitney; **Extended Data Fig. 7f**]. Furthermore, we induced expression of cloned *R. obeum* and *V. cholerae luxS* genes using an arabinose-inducible P_{BAD} promoter in an AI-2-deficient *E. coli* strain (DH5 α)[25] and observed that supernatants from these strains were able to induce increased BB170 bioluminescence over vector controls [7.2±1.1 and 8.8±2.4-fold (mean±SEM), respectively].

SUPPLEMENTARY TABLES