

Supplementary information for “Enrichment of the Lung Microbiome with Gut Bacteria in Sepsis and the Acute Respiratory Distress Syndrome”

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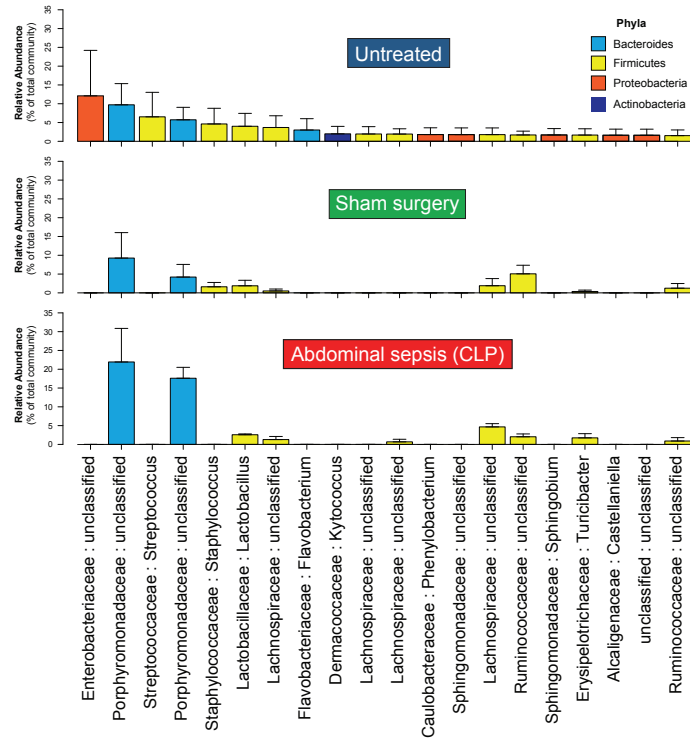
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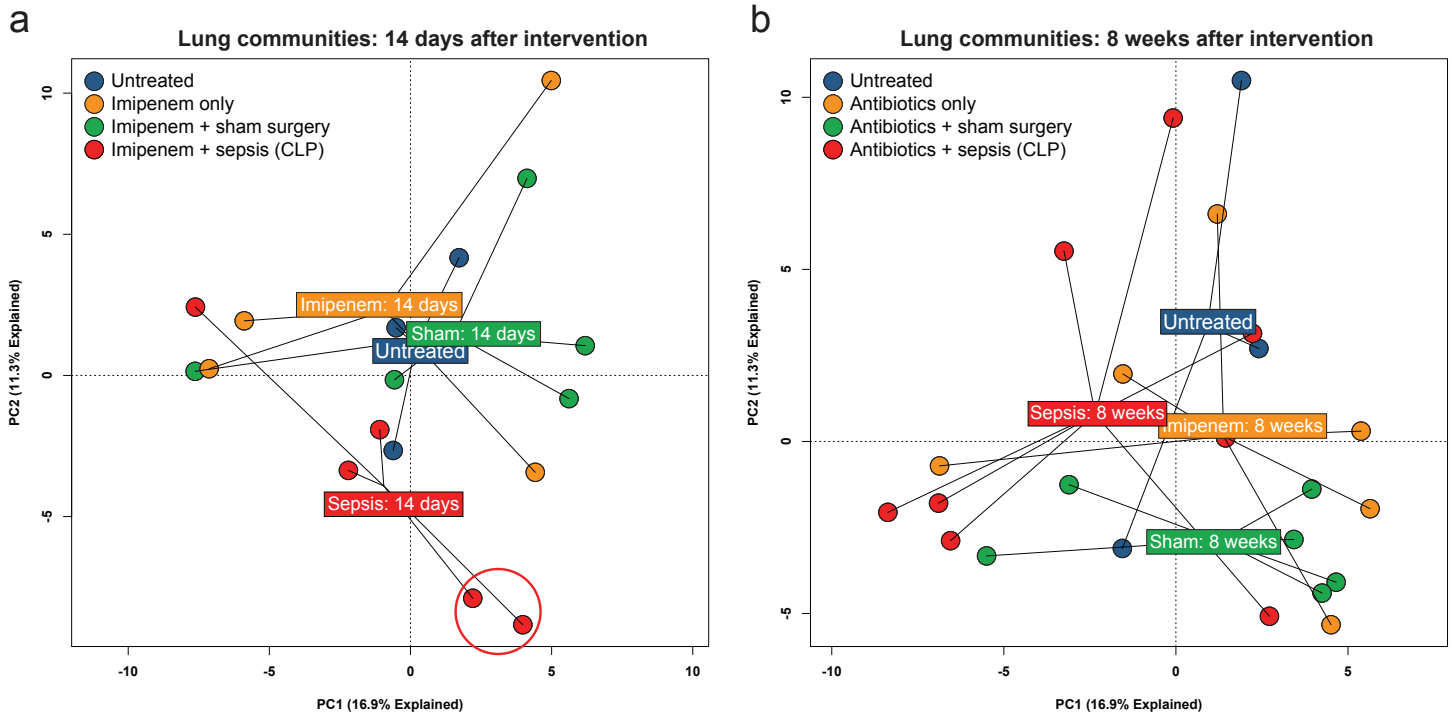
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Supplemental Table 1. Human subject characteristics.

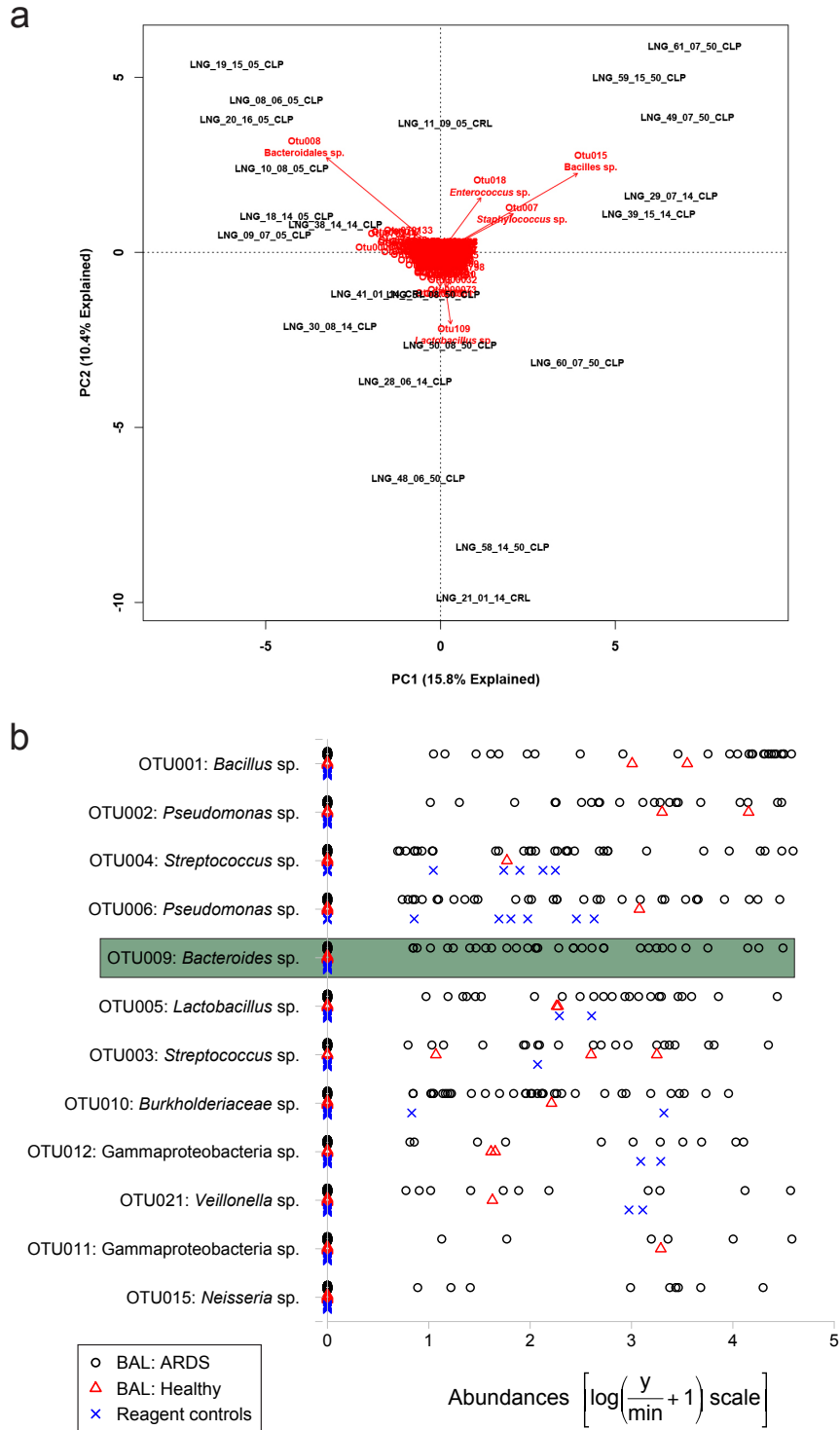
ARDS subjects (n=68)		
Patient demographics	Age	18-77 (47.4 ± 14.5)
	Male	46 (67%)
	Race: Caucasian	45 (66%)
	Race: African-American	19 (28.0%)
	Race: Other	4 (5.8%)
ARDS risk factor	Pneumonia	20 (29.4%)
	Aspiration	14 (20.6%)
	Sepsis	18 (26.4%)
	Other	16 (11.8%)
Illness severity at study enrollment	Oxygenation index	2.6 - 64.4 (16.0 ± 11.5)
	PaO ₂ :FiO ₂ Ratio	45 - 257 (117.6 ± 55.8)
	APACHE III index	24 - 128 (57.2 ± 17.1)
Outcomes	28-day mortality	14 (20.5%)
	Ventilator-free days	0 - 27 (8.7 ± 9.3)
Healthy subjects (n=7)		
Subject demographics	Age	47.4 ± 14.5
	Male	5 (71%)
	Smoking history (Active/Former/Never)	0/0/7



Supplemental Figure 1 Altered bacteria in the lung microbiome 24 hours after sepsis and sham surgery. The 20 most abundant operational taxonomic units detected in the lungs of untreated mice are shown across experimental arms. OTUs are ranked in descending order of mean abundance in the lungs of untreated mice. Values presented as means \pm standard error of the mean.



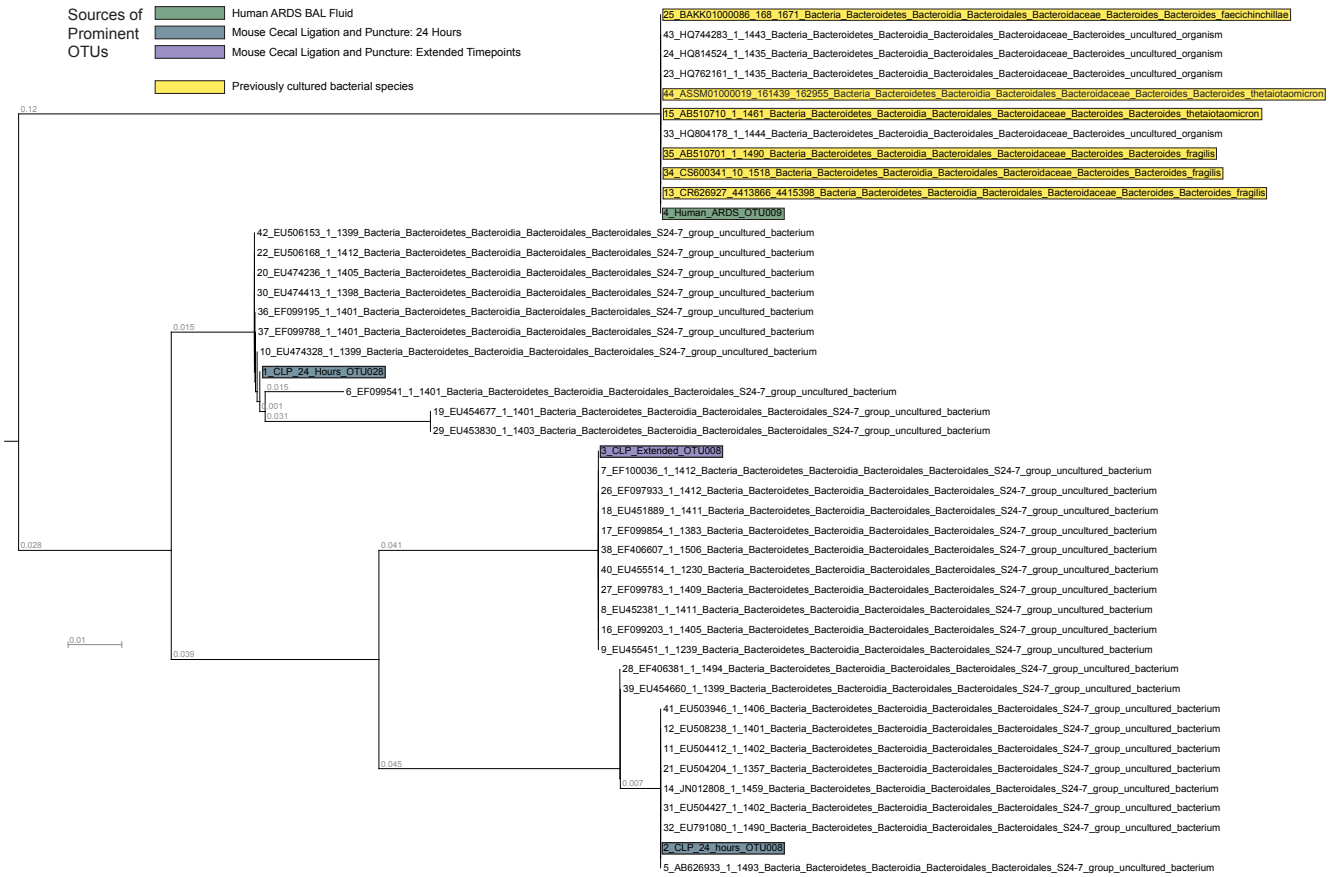
Supplemental Figure 2 Bacterial communities in the lungs of mice at extended timepoints. Mice were exposed to abdominal sepsis (cecal ligation and puncture and imipenem) and compared at multiple timepoints to three experimental control groups: untreated, imipenem only, and imipenem with sham surgery. Lung communities are shown at 14 days following exposure (a) and 8 weeks following exposure (b). Post-sepsis lungs were not collectively distinct from the lungs of control mice at either timepoint ($P > 0.05$). Post-sepsis lung communities within the red circle were enriched with four OTUs classified at the family level as *Lachnospiraceae*, comprising 37.2% of sequences. These same OTUs comprised only 5.7% of sequences in the remaining 14-day CLP lungs, and 6.9% of sequences in all other 14-day lungs.



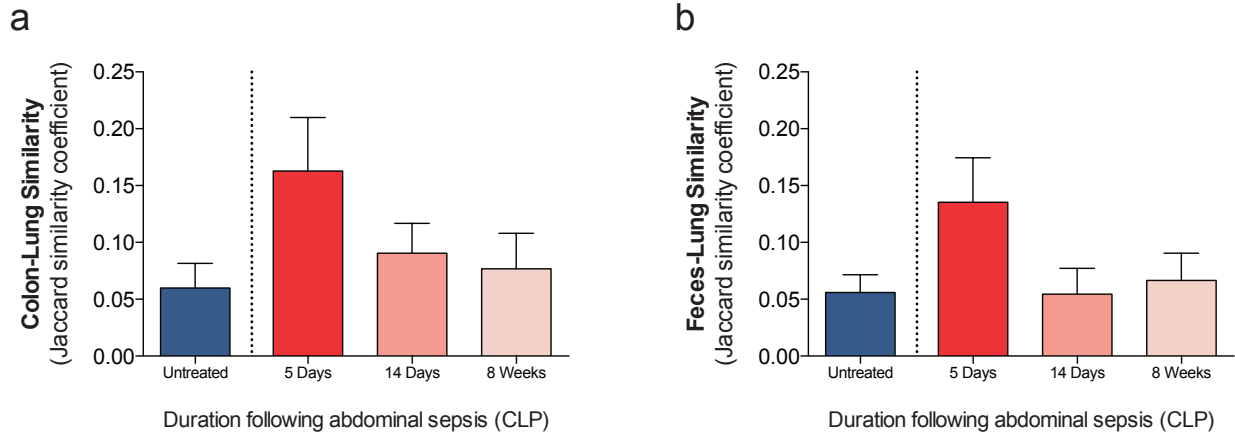
Supplemental Figure 3 Enriched lung bacteria in experimental sepsis and human ARDS. (a) Biplot analysis of lungs of bacterial communities detected in lungs of untreated mice and at various timepoints following sepsis. A single taxonomic group (OTU008, *Bacteroidales* sp.) drove the community difference between post-sepsis lungs (5 days) and all other intervention arms. (b) Model-based analysis of multivariate abundance data (mvabund package in R¹⁵) of taxonomic groups relatively enriched in the BAL fluid of patients with ARDS compared to that of healthy subjects. Identified taxa corresponded to bacterial genera found in the lower intestinal tract (e.g. *Bacillus*, *Bacteroidetes*, *Lactobacillus*), the upper respiratory tract (*Streptococcus*, *Burkholderiaceae*, *Neisseria*) and both (*Pseudomonas*, *Gammaproteobacteria* spp.). OTU009 (*Bacteroides* sp., highlighted in green) was selected for further study given its frequency and abundance in ARDS BAL specimens, its absence from healthy human BAL specimens and the previously reported microbiota of the upper respiratory tract, and its absence from reagent control specimens.

Sources of
 Prominent
 OTUs

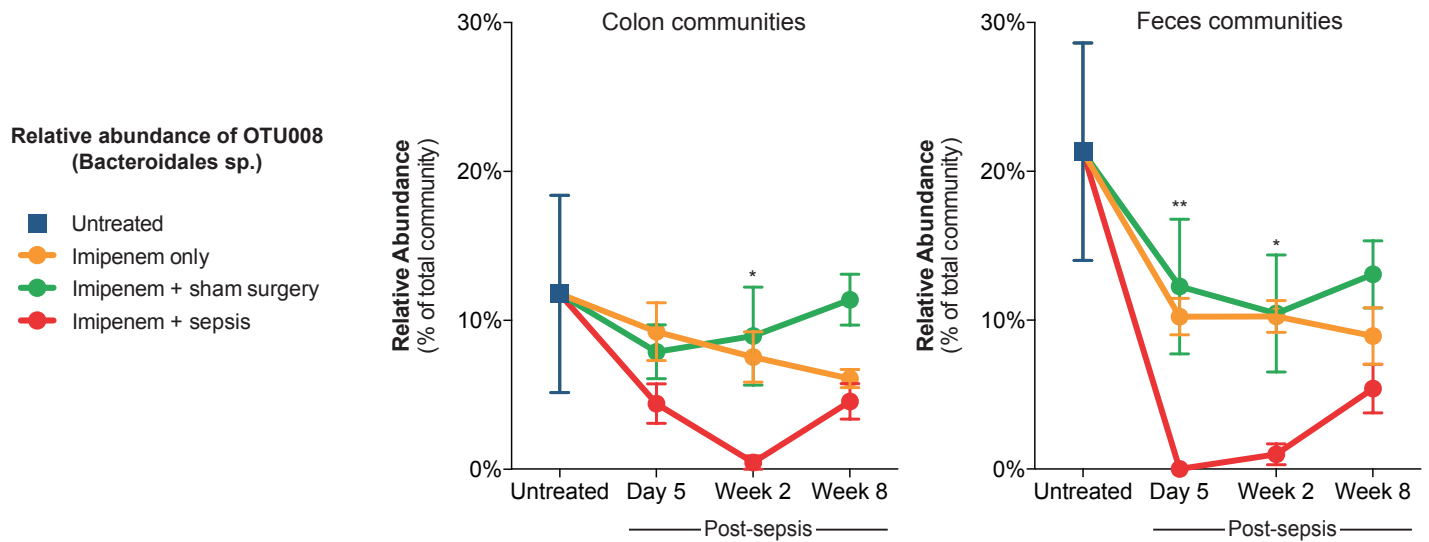
- Human ARDS BAL Fluid
- Mouse Cecal Ligation and Puncture: 24 Hours
- Mouse Cecal Ligation and Puncture: Extended Timepoints
- Previously cultured bacterial species



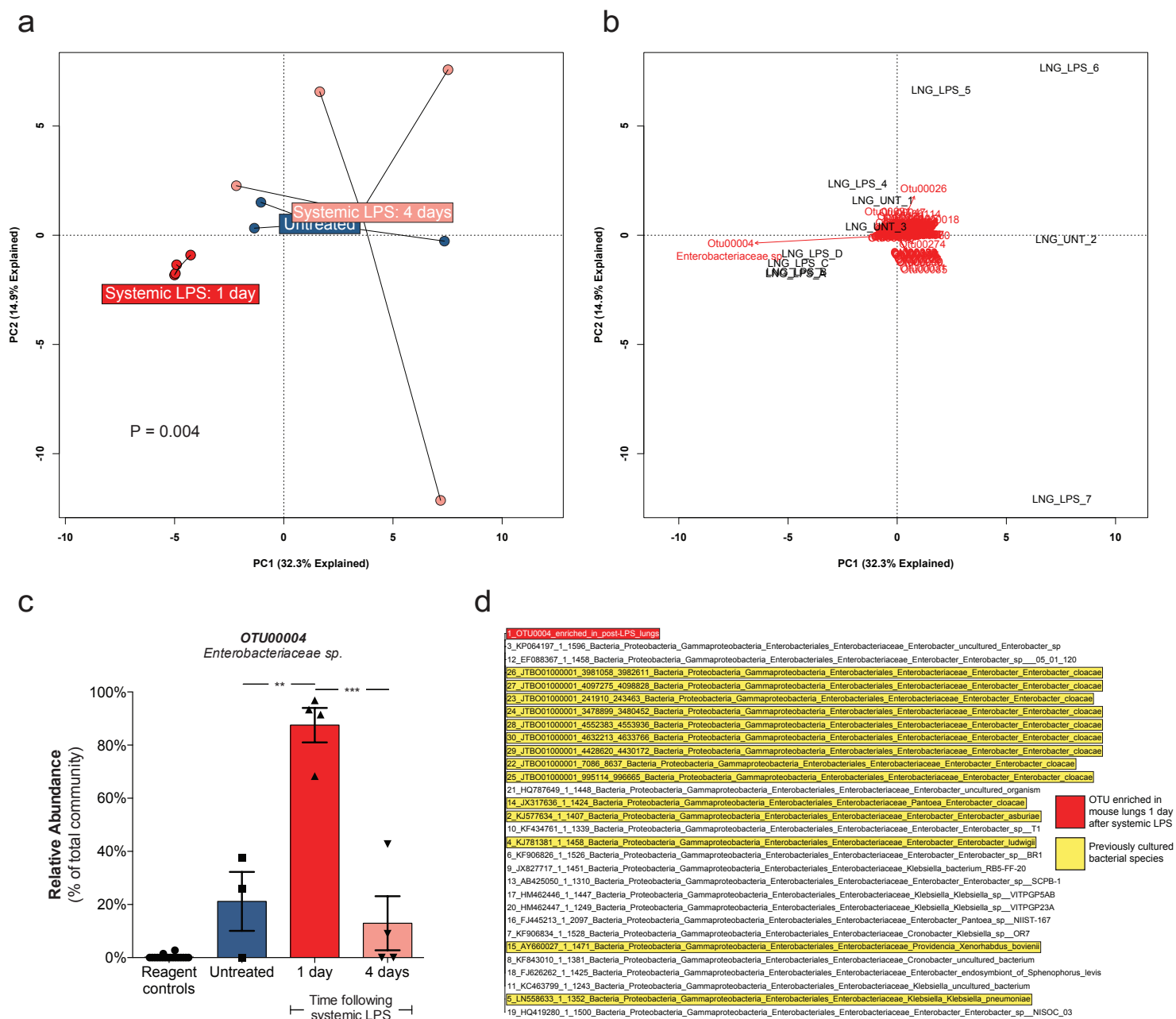
Supplemental Figure 4 Phylogenetic tree of prominent OTUs and closely-aligned sequences from a reference database. Prominent OTUs detected the BAL fluid of humans with ARDS (green) and the lungs of mice following cecal ligation and puncture at 24 hours (blue) and extended timepoints (purple) were compared with 40 closely-aligned sequences obtained from the SILVA database (<http://www.arb-silva.de/>). Sequences obtained from cultivated organisms are highlighted in yellow. All related sequences were classified as belonging to the Bacteroidales order within the Bacteroidetes phylum. Tree generated with MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/>).



Supplemental Figure 5 Time course of gut-lung community similarity following abdominal sepsis. For each mouse, community similarity was calculated for paired lung and colon communities (a) and paired lung and feces communities (b). Group means and standard errors of the mean are depicted.

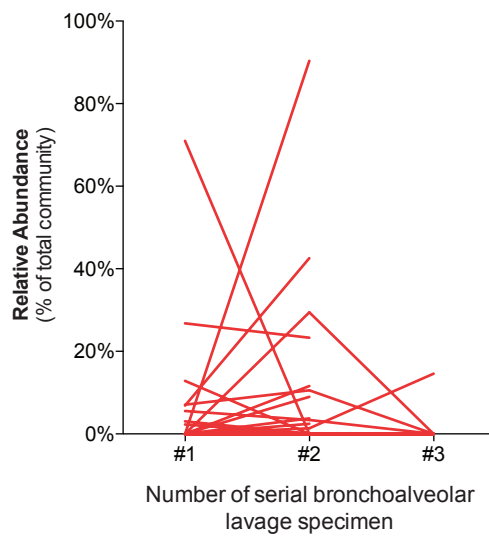


Supplemental Figure 6 Effect of experimental sepsis on the relative abundance of OTU008 Bacteroidales sp. in the murine gut. Mice were exposed to abdominal sepsis (cecal ligation and puncture and imipenem) and compared at multiple timepoints to three experimental control groups: untreated, imipenem only, and imipenem with sham surgery. The taxonomic group enriched in post-sepsis lungs (OTU008, Bacteroidales sp.) decreased in relative abundance in colon and fecal communities at day 5 and week 2, normalizing by week 8. Statistical significance was determined by Kruskal–Wallis one-way analysis of variance with Dunn’s multiple comparisons test. ** $P \leq 0.01$, * $P \leq 0.05$.



Supplemental Figure 7 Gut-associated bacteria in the lungs of mice following LPS-induced shock. Shock was induced via intraperitoneal injection of 5 mg/kg LPS, and lung bacterial communities were sequenced and analyzed after 1 day and 4 days. (a) One day after LPS-induced shock, lung communities were significantly distinct from those of untreated animals. This difference had resolved by 4 days. (b) Biplot analysis identified an Enterobacteriaceae-classified OTU (OTU00004) as the community member responsible for this difference. (c) This *Enterobacteriaceae*-classified OTU (OTU00004) had significantly increased relative abundance in the lung communities of LPS treated mice after 1 day; this difference had resolved by 4 days. (d) Phylogenetic tree of this OTU (OTU00004) compared with 30 neighboring sequences in the SILVA database (<http://www.arb-silva.de/>). Sequences obtained from cultivated organisms are highlighted in yellow. All sequences were genetically identical (distance = 0) to OTU00004, and belonged to the Enterobacteriaceae family within the Proteobacteria phylum. Tree generated with MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/>). ** $P \leq 0.01$, *** $P \leq 0.001$.

***Bacteroides* sp. (OTU009)**



Supplemental Figure 8 Stability of OTU009 (*Bacteroides* sp.) in serial BAL specimens from patients with ARDS. The relative abundance of OTU009 (*Bacteroides* sp.) is shown for BAL specimens collected from the 25 ARDS patients who underwent serial bronchoscopy.