## Supplemental Methods

**Endotoxin Quantification** The UCS1 *E. coli* phage cocktail was serially diluted 3 logs ($10^{-3}$) in endotoxin-free water so that the endotoxin concentration fell within the range of the Endosafe Cartridge (PTS201, 0.1-1 EU/mL), then submitted to the Cell and Gene Therapy Center at Baylor College of Medicine for analysis. A Limulus Amoebocyte Assay (LAL) was performed on the diluted cocktail using a PTS201 Endosafe cartridge. The analysis certificate is in **Figure S4**.

**Sterility Testing** 100µL of UCS1 phage cocktail from each aliquot was sent to NOVA biologicals (Conroe, TX) for USP 71 sterility testing, with 50µL each inoculated into Fluid Thioglycollate medium and Tryptic Soy Broth. 100µL of the cocktail was also sent to NOVA for Acid Fast Bacteria (mycobacteria) testing. The analysis certificates are in **Figure S4**.

**Genomic DNA preparation and sequencing**

Phage genomic DNA was extracted and sequenced as described in Green et al 2017 [17]. Paired-end libraries were constructed from purified DNA. Amplicons were visualized via gel electrophoresis and purified to remove primer-dimers and non-specific amplicons (ChargeSwitch PCR Clean-up Kit, Invitrogen). Quantification utilized the Qubit® Fluorometer resulting in pooled samples at DNA mass of 100 ng per sample. The amplicon pool was sequenced on the Illumina MiSeq platform using reagent kit v2 (2 × 250 bp) paired-end protocol yielding paired-end reads that overlap almost completely.

Bacterial genomic DNA was purified using Omega E.Z.N.A. Bacterial DNA Kit (Cat#D3350) according to manufacturer's protocol, and sequencing performed by Novogene Corporation Inc.

## Supplementary Figures

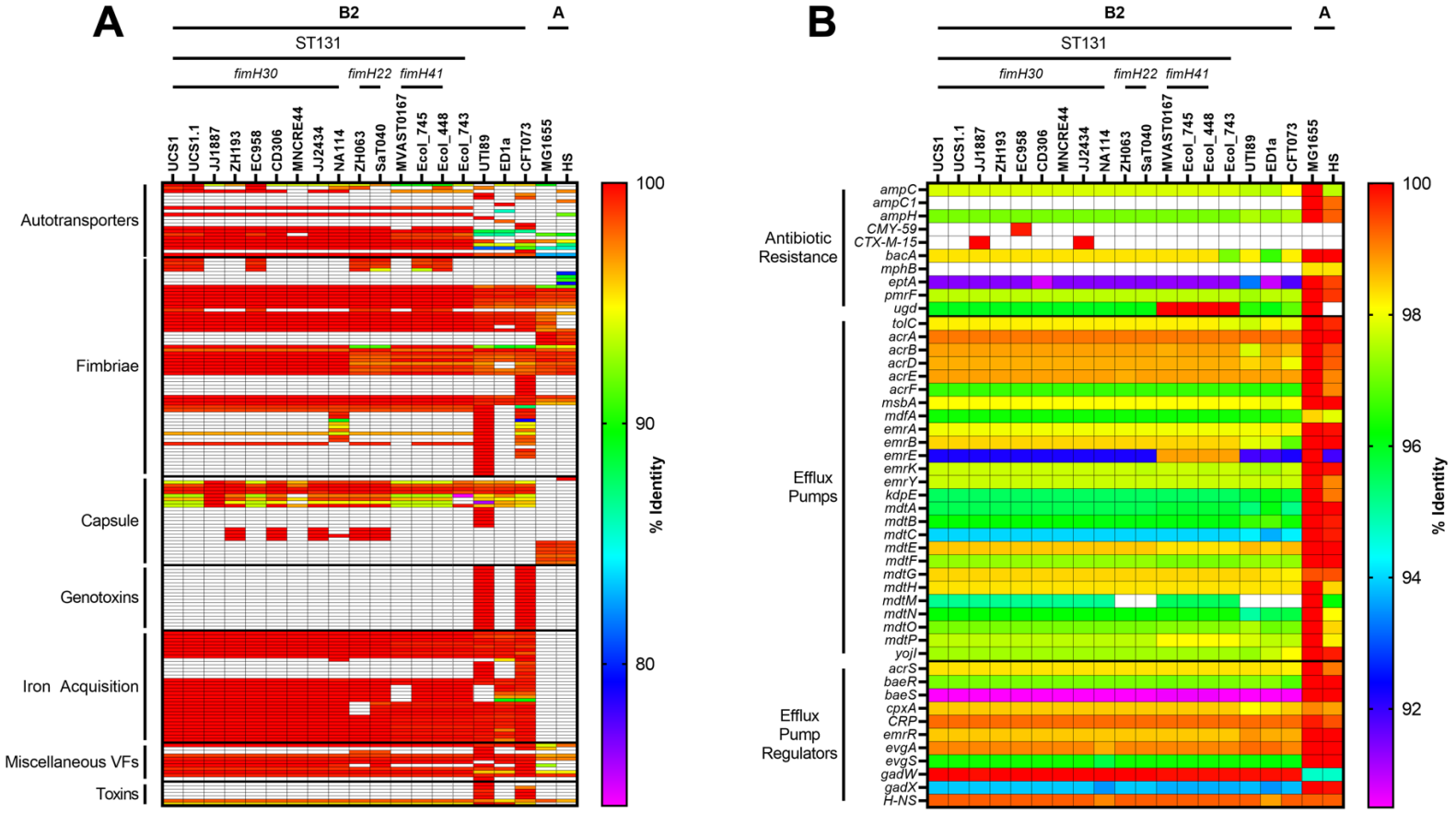


**Figure S1.** Comparative pathogenomics and resistomics of UCS1 and UCS1.1 with other *E. coli* species**.**

Several ST131 strains, including archetypal members of well-known ExPEC-associated ST95 (UTI89) and ST73 (CFT073) sequence types, and 3 non-pathogenic strains (ED1a, HS, and K-12 MG1655) were used as queries against either a custom *E. coli* virulance factor (VF) database (**A**) [37] or against the Comprehensive Antibiotic Resistance Database (CARD) (**B**). Heatmaps show the percent identity relative to the reference gene in the database. To generate the heatmaps with Graphpad Prism 9.2.0, megaBLAST aligned VFs or antibiotic resistance genes against each strain. The percent identity of High-scoring Segment Pairs (HSPs) that covered >90% of the reference gene was extracted and mapped.

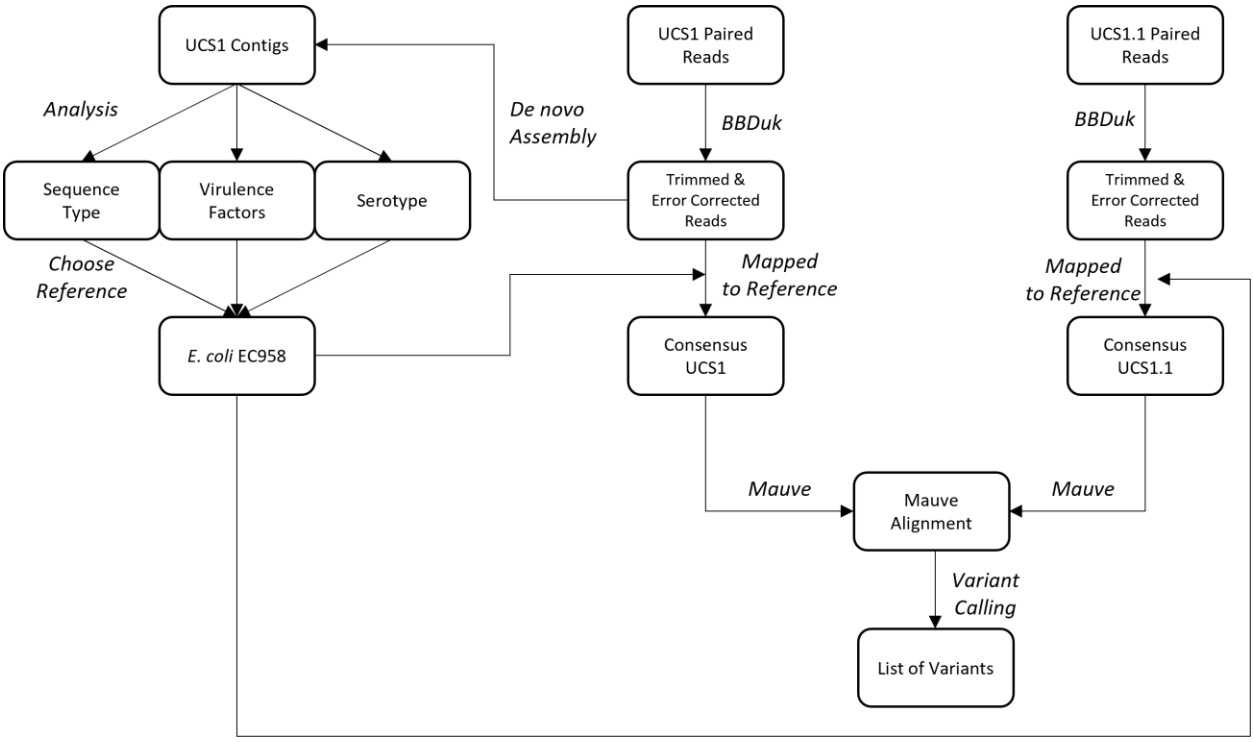


**Figure S2.** Bioinformatic pipeline for genomic comparison between UCS1 and UCS1.1

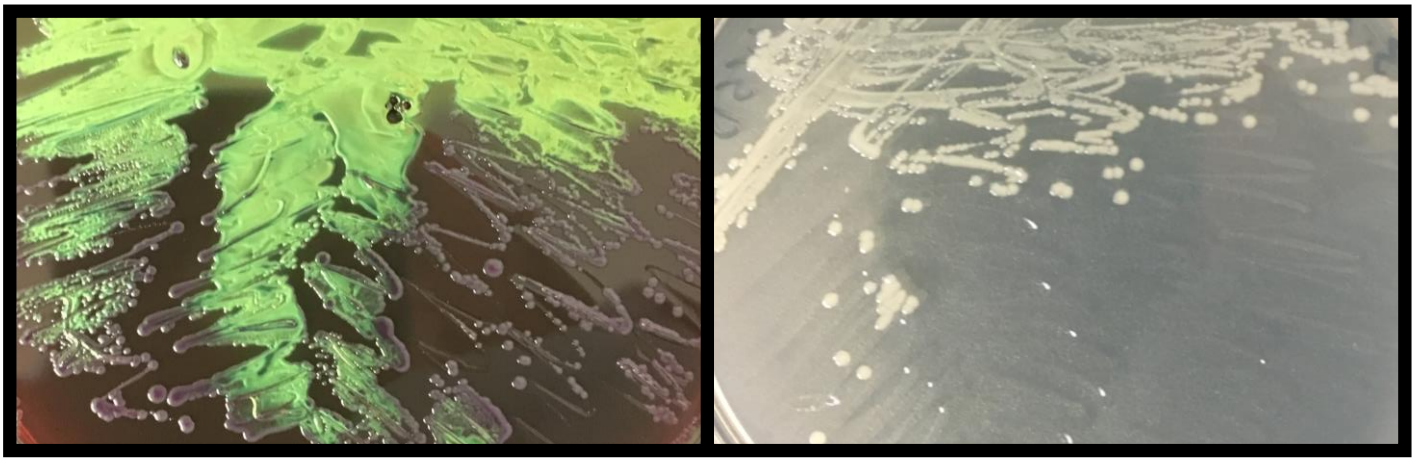

**Figure S3**. Colony Morphology of UCS1 *E. coli*. Clonal UCS1 colonies were streaked onto EMB agar (left) and LB agar (right) and incubated overnight at 37C.

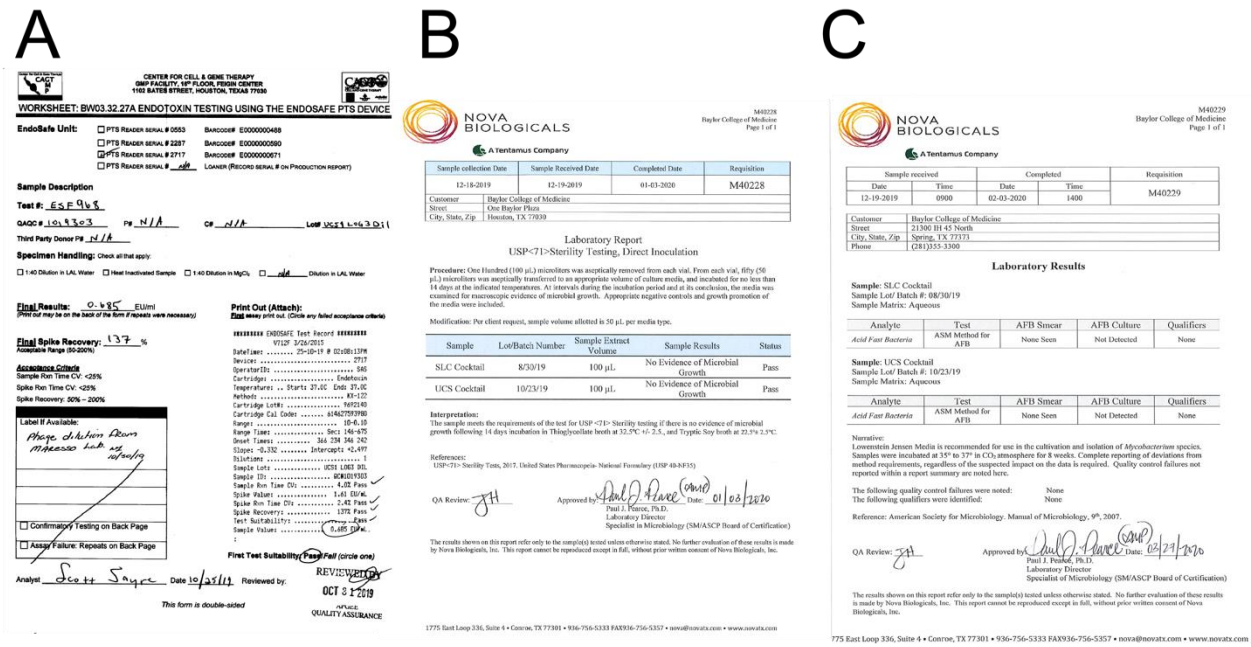


**Figure S4. Characterization of UCS1 cocktail by certified laboratories**. **A.** UCS1 phage cocktail was subjected to USP 85 endotoxin testing by limulus amoebocyte assay (Endosafe cartridges), **B.** USP 71 sterility testing by direct inoculation in rich media (Tryptic Soy Broth, Thioglycollate Broth), and **C.** Acid Fast Bacteria testing (Lowenstein Jensen Media)