**Supplementary Methods**

*Genome resequencing of small colony variants*

Genomic DNA was purified from A8-S and A4-S SCV isolates or wild type *E. coli* using the following protocol. Bacterial cell pellets from 2-3 mL saturated cultures were suspended in 500 µL TE buffer (10 mM Tris-HCl pH 7.5, 10 mM EDTA) and lysed by adding SDS (final concentration 0.5 %). The lysate was then treated with 200 µg/mL RNAse A (Sigma-Aldrich/Merck, USA) for 2 hours at 37 °C and then with 200 µg/mL Proteinase K (Sigma-Aldrich/Merck, USA) overnight at 50-55 °C. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was then added. Genomic DNA was precipitated from the aqueous phase with 0.1 volume 3 M sodium acetate solution and 2.5 volumes ethanol for 1 hour at -20 °C. Obtained precipitate was washed with 70 % ethanol, dried and dissolved in 50-100 µL of TE buffer. Genomic DNA was cleaned up using DNA binding spin columns (Qiagen, USA) as per the manufacturer’s instructions. DNA was quantitated spectrophotometrically and its integrity was verified by 0.8 % agarose gel electrophoresis.

Genome sequencing services were provided by Genotypic Technology (India). Paired-end whole-genome sequencing was performed using Next-Generation Sequencing on a MiSeq system (Illumina, USA) with read lengths of 150 bps. Processed reads were aligned to the reference genome *E. coli* K-12 MG1655 (NC\_000913) using bowtie2. A cut-off of a minimum of 20x coverage was employed, which made >97% of the reads for each of the samples permissible for analysis. Variant calling and prediction of new junctions in the genome to identify large structural mutations such as insertions and deletions was done using Breseq using default settings (Barrick *et al.* 2014; Deatherage and Barrick 2014; Deatherage *et al.* 2014). All variants and new junctions that were present in the wild type ancestor were discarded.

*Protein content and RpoB expression level*

Saturated cultures of wild type or SCV strains were diluted 1:100 in 6 mL fresh LB medium and allowed to grow for 90 minutes at 37 °C with shaking at 180 rpm. Cultures were centrifuged and bacterial pellets were lysed in 1 mL TrisHCl-buffered saline by sonication. Protein content was determined using Bradford’s assay and normalised to optical density of the cultures to calculate per capita protein content.

To evaluate the expression level of RpoB in wild type or SCV E. coli strains, immunoblotting was performed. Briefly, 2 µg of total lysate protein was subjected to SDS-PAGE followed by electroblotting onto a PVDF membrane. The blot was blocked with 5 % delipidated milk for 1 hour at room temperature, treated with primary antibody (anti-RNA Pol β monoclonal 8RB13, Invitrogen/Thermo Fisher, USA) overnight at 4 °C and then with HRP-linked Anti-mouse IgG F(ab’)2 fragment secondary antibody (GE Healthcare, USA) for 1 hour at room temperature. Bound antibody was determined by chemiluminescence.

*Bacterial cell length*

For determining bacterial cell length mid-log phase cultures (O.D. at 600 nm between 0.6 and 1) of appropriate strains were normalised for cell density and 10 µL of each culture was smeared onto a grease-free glass slide using a nichrome loop. The bacterial smear was heat fixed, stained with 0.1% safranin (Himedia Laboratories, India) and visualised initially at 10x magnification to visualise pellicle formation and then under 100x using an oil immersion lens on a light microscope. Multiple fields were photographed and bacterial cell length was measured using ImageJ. At least 2 independent cultures were used for each test bacterial strain.

*Sedimentation efficiency of bacterial cultures*

To measure the sedimentation efficiency of *E. coli*, late-log phase cultures of appropriate strains were normalised for cell density (based on optical density at 600 nm) and 1 mL of each culture was centrifuged at 3500 rpm (822 g) for 1 min in a 1.5 mL conical microfuge tube. The optical density of the supernatant was measured and reduction in OD was used to calculate sedimented fraction for comparison across strains.

*Amplification and sequence determination of rifampicin resistance determining regions (RRDR) of rifampicin-resistant isolates*

The rifampicin resistance determining region (RRDR) was amplified from purified genomic DNA of appropriate strains by polymerase chain reaction using Taq DNA polymerase (MP Biomedicals, India or New England Biolabs, U.S.A.) and RRDR\_RpoB\_fwd (5’-CGTCGTATCCGTTCCGTTGGC-3’) and RRDR\_RpoB\_rev (5’-CCTGGTCGCGGCTGAACAAGC-3’) primers or RRDR\_ClusterI\_fwd (5’– GGAATGTCAAATCCGTGGCGT-3’) and RRDR\_ClusterI\_rev (5’- CGACCAACCGCAGACAAGTCATA-3’) primers (Sigma-Aldrich/Merck, USA). RRDR\_RpoB fwd or RRDR\_ClusterI\_fwd were used as sequencing primers. Sequencing services were provided by First Base Laboratories (Malaysia). All sequence comparisons were done using Ape plasmid editor (<http://biologylabs.utah.edu/jorgensen/wayned/ape/>). The sequence of wild type *rpoB* was obtained from NCBI (http://www.ncbi.nlm.nih.gov/). The RRDR of two ancestral strains used for our selections was sequenced to ensure that no mutations were present at this locus to begin with.

*Isolation of spontaneous rifampicin-resistant mutants*

Spontaneous rifampicin-resistant mutants were isolated in the wild type or high fitness SCV (A8-S) background using a fluctuation test. Briefly, ~109 bacteria each from 3 stationary phase cultures of the appropriate strain were harvested and spread onto LB agar supplemented with rifampicin (50 µg/mL). Plates were incubated at 37 °C for 18 hours and 10 random colonies from each strain were used for further characterization.

*Genome engineering*

The His526Gln (C1578A) or Ile572Leu (A1714C) mutation was engineered in the genomic copy of the *rpoB* gene in the appropriate strain of *E. coli* using a modified Lambda Red recombineering protocol (Datsenko and Wanner 2000). Briefly, 100-200 ng of purified PCR amplicon harbouring the *rpoB* His526Gln (C1578A)/ Ile572Leu (A1714C) mutation was electroporated into a recipient strain containing the pKD46 plasmid (Datsenko and Wanner 2000). Recombinants were selected on media supplemented with rifampicin (50 µg/mL) at 37°C. The pKD46 plasmid was cured by repeated passages at 37 °C in the absence of ampicillin. Recombinant strains were confirmed by sequencing of the RRDR locus.