# Supplementary Information for

## Exploiting evolutionary trade-offs for post-treatment management of drug-resistant populations.


**Authors**

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**Figures**



**Supplementary Data** (available online: 10.6084/m9.figshare.11886288)

Supplementary Data 1 | Sanger sequencing of 120 colonies from the evolved tavaborole-resistant populations of *E. coli* (evolution in the presence of tavaborole only)

Supplementary Data 2 | Time-resolved genome sequencing of the evolving populations A and B. (the file contains links to the sequencing databases)

Supplementary Data 3 | The LeuRS-coding plasmid *leuS*-pBAD28.

Supplementary Data 4 | The integration plasmid to insert sfGFP-coding gene into the *E. coli* genome.

Supplementary Data 5 | Sanger sequencing of 90 colonies from the evolved tavaborole-resistant populations of *E. coli* (evolution in the presence of tavaborole and norvaline)

| Population A | Population B | Population C | Population D | Population E | Population F |
|---|---|---|---|---|---|
| R344S | R344C | G225D | M336MAV | V338D | G331C |
| R344S | R344C | R344S | M336MAV | V338D | G229V G331C |
| R344S | R344S | G225D | M336MAV | V338D | G225D G331R |
| G225D | R344S | R344S | M336MAV | R344S | G229V |
| G225D | R344S | R344S | M336MAV | V338D | G331C |
| G225D | R344C | G225D | M336MAV | V338D | G331C |
| R344S | R344S | G225D | M336MAV | V338D | G225D G331C |
| G225D | Y330F | G225D | M336MAV | V338D | G225D |
| R344S | R344S | R344S | Q269P | R344S | G331C |
| G225D | Y330F | A334E | M336MAV | R344S | G225D G331C |
| G225D | R344C | G229V | M336MAV | R344S | G225D |
| R344S | R344S | R344S | M336MAV | V338D | G331C |
| R344S | L354R | A334E | Q269P | R344S | G331C |
| G225D | R344S | R344S | M336MAV | V338D | G225D |
| G229V | G229T | M336I | Q269P | V338D | G225D |
| G229V | R344C | G225D | M336MAV | R344S | G331C |
| G225D | R344S | G225D | M336MAV | V338D | G331C |
| G225D | R344S | R344S | M336MAV | V338D | G229V |
| G225D | R344S | R344S | M336MAV | V338D | G331C |
| G225D | V335VMA | R344S | M336MAV | V338D | G225D |

| Population co-A | Population co-B | Population co-C | Population co-D | Population co-E | Population co-F |
|---|---|---|---|---|---|
| - | - | - | - | - | - |
| - | - | - | - | - | - |
| - | - | - | - | - | - |
| - | - | - | - | - | - |
| - | - | - | - | - | - |
| R344S | - | - | - | - | - |
| - | - | - | - | - | - |
| - | - | - | - | - | - |
| R344S | - | - | - | - | M336MAV |
| - | - | - | - | - | - |
| - | - | - | - | - | - |
| R344S | - | G229V | - | - | - |
| - | - | - | - | - | - |
| - | - | - | - | - | - |
| R344S | - | - | - | - | - |

**Table S1. Mutations observed in the tavaborole-resistant *E. coli* colonies.** The table summarizes mutations that were observed in *leuS* gene in 120 tavaborole-resistant colonies of the *E. coli* that were evolved in the presence of tavaborole (populations A to D) or tavaborole and norvaline (populations co-A to co-D). The corresponding Sanger sequencing data are deposited as (**Supplementary Data 1**, for the populations A to D) and (**Supplementary Data 5**, for the populations co-A to co-D).

| Primer | Sequence | Description |
|---|---|---|
| 1 | CATCCGCCAAAACAGCTTAGCCAACGACCAGATTGAGGAGTTT | *leuS* amplification |
| 2 | AGCAGCGGCCAAGAGCAATACCGC | *leuS* amplification |
| 3 | TGGATAAACTGGATCACTGGCCAGAC | editing domain segment amplification |
| 4 | TTACCACATCTTCCGGCAGGATCAC | editing domain segment amplification |
| 5 | GCTGTTTTGGCGGATGAGAGAAG | pBAD28 amplification for *leuS* cloning |
| 6 | GCTAGCCCAAAAAAACGGGTATGG | pBAD28 amplification for *leuS* cloning |
| 7 | GGTGATCCTGCCGGAAGATGTGGTAA | pBAD28 amplification for ed-domain cloning |
| 8 | GGTGATCCTGCCGGAAGATGTGGTAA | pBAD28 amplification for ed-domain cloning |
| 9 | TTCTGTTTTATCAGACCGCTTCTGCG | pBAD28 insert analysis |
| 10 | ATAGCATTTTTATCCATAAGATTAGCGGATCC | pBAD28 insert analysis |
| 11 | CCTAATACGACTCACTATAGCCGAAGTGGCGAAATCGGTAGA | DNA template for $tRNA^{Leu}$ synthesis |
| 12 | GCCGAAGTGGCGAAATCGGTAGACGCAGTTGATTCAAAATCAACCGTAGAAATACGTGC | DNA template for $tRNA^{Leu}$ synthesis |
| 13 | TGGTGCCGAAGGCCGGACTCGAACCGGCACGTATTTCTACGGTTGATTTTGAATCAAC | DNA template for $tRNA^{Leu}$ synthesis |
| 14 | ACCGTTTACGTTGTCCGCCCGGACACCTTTATGGG | *leuS* mutagenesis: T247V, T248V |
| 15 | GGCGGACAACGTAAACGGTCAGCGTGTTGTCATAGTC | *leuS* mutagenesis: T247V, T248V |

**Table S2. Primers used in the study.**

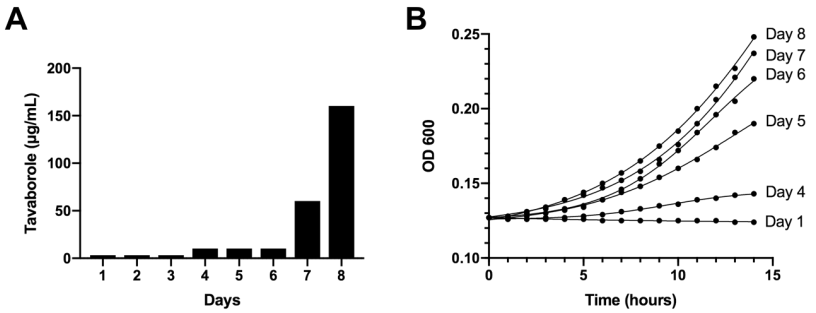


**Fig. S1 | Tavaborole-resistance evolution in *E. coli*. A.** The diagram shows tavaborole concentrations that were used to evolve resistance in 10 populations of *E. coli* that were grown in parallel. Tavaborole was added to the growth media to 2.5 ug/ml concentration. As the populations showed the sign of tavaborole-resistance, tavaborole concentrations were gradually increased to the final concentration of 160 ug/ml. **B.** Growth curves of one of the evolving E. coli culture (lineage 1) collected at days 1-8 of the experiment and regrown simultaneously in the presence of tavaborole (16ug/ml). As the diagram shows, the initial cell population (collected at Day 1) cannot grow in the presence of tavaborole (16ug/ml), however in the course of the evolution experiment the population acquires the ascendingly rapid growth in the presence of the drug.

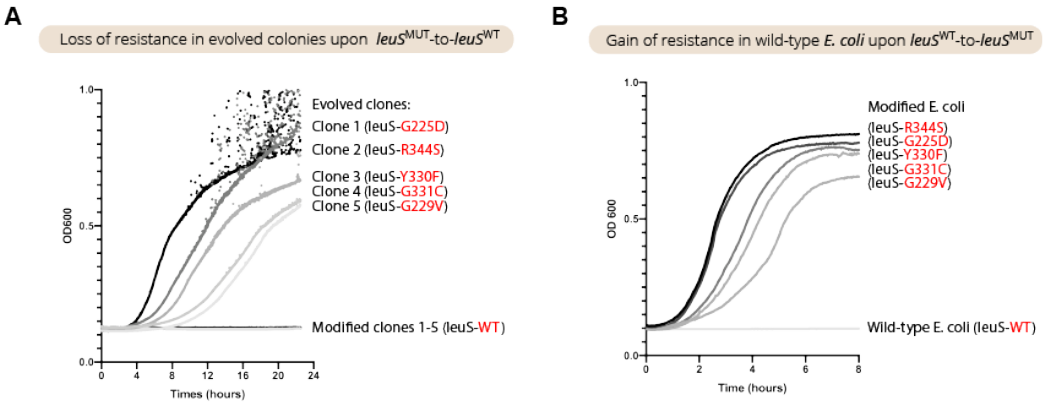


**Fig. S2 | Mutations in the *leuS* gene confer tavaborole-resistance.** The panels show growth rate assays of the evolved and genetically engineered strains of *E. coli* to test if mutations in the editing domain of LeuRS indeed confer tavaborole resistance. **A.** Growth curves comparing two sets of *E. coli* cells: the evolved *E. coli* clones (clones 1–5), in which *leuS* contained one of the five most frequently observed mutations, and the same clones after their *leuS* gene had been replaced with the wild-type *leuS* gene. When the evolved clones were modified and their *leuS* sequence was reverted to the wild-type, they lost their tavaborole resistance, indicating that the tavaborole-resistant phenotype is determined by mutations in the *leuS* gene. **B.** Growth curves comparing wild-type *E. coli* with the derived clones in which the *leuS* gene was modified to introduce one of the five most frequently observed mutations in the *leuS* gene. When *E. coli* acquire one mutation in *leuS* in their genomic DNA, they become resistant to tavaborole.

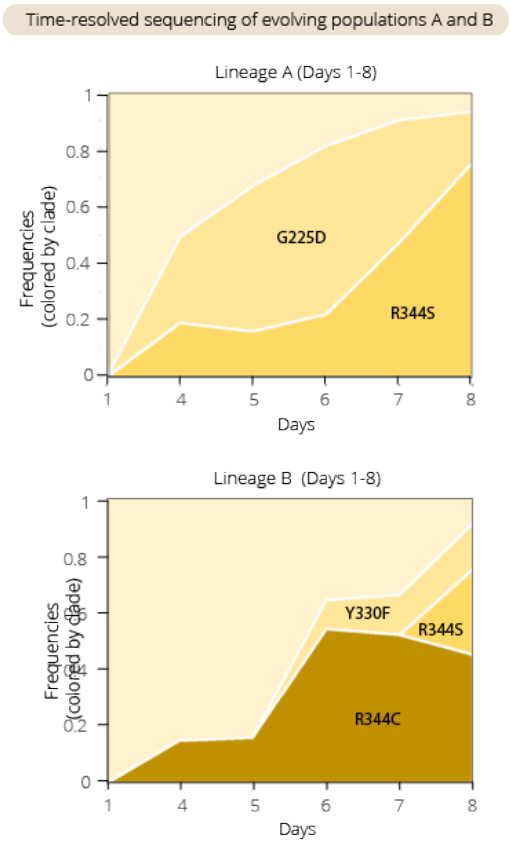


**Fig. S3 | Time-resolved whole-population genome sequencing illustrates rapid propagation of *leuS* mutations in the evolving *E. coli*.** Time-resolved whole-population DNA sequencing for two independent populations of *E. coli* (populations A and B) growing in the presence of tavaborole. The panels show that mutations in the *leuS* gene accumulated in a time-dependent manner and were eventually present in the majority of the evolving cells, illustrating that the majority of tavaborole-resistant *E. coli* have mutated editing domain in LeuRS.

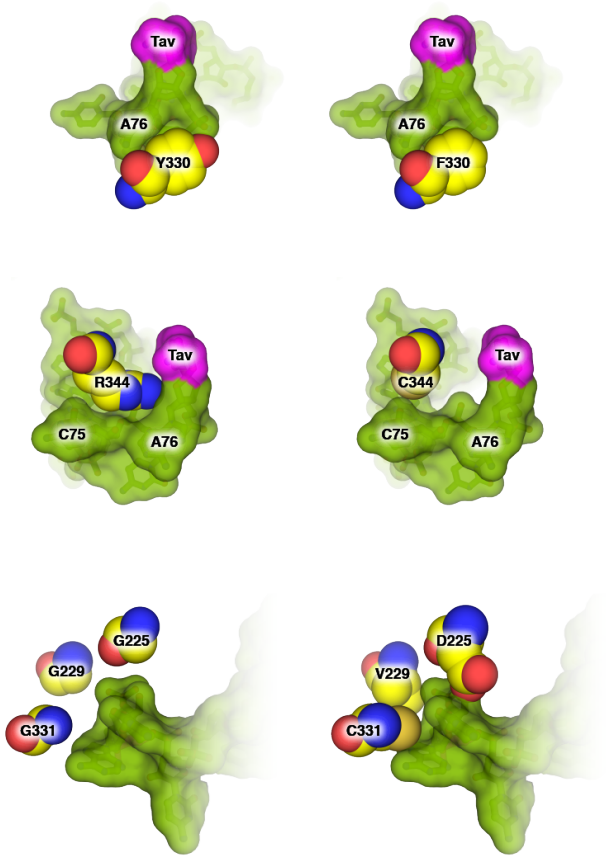


**Fig. S4 | Mutations in LeuRS editing site appear to compromise tRNA binding to the editing domain.** The panels show fragments of the crystal structure of bacterial LeuRS/tRNA/tavaborole complex (left panels, pdb id **2v0g**) and the hypothetic structures of LeuRS mutants (right panels) to illustrate an apparent mechanism by which LeuRS mutations prevent tRNA accommodation in the editing site. The structure suggests that several mutations confer tavaborole-resistance by disrupting tRNA contacts with LeuRS editing domain (such as the hydrogen bonds between Y330 residue and the 3'-terminal phosphate in $tRNA^{Leu}$ , or the salt bridging-contact between R344 and the 3'-terminal phosphate in $tRNA^{Leu}$). Other mutations, including G225D, G229V, and G331C, appear to create a steric clash between $tRNA^{Leu}$ and LeuRS, thereby preventing $tRNA^{Leu}$ binding to the editing site.

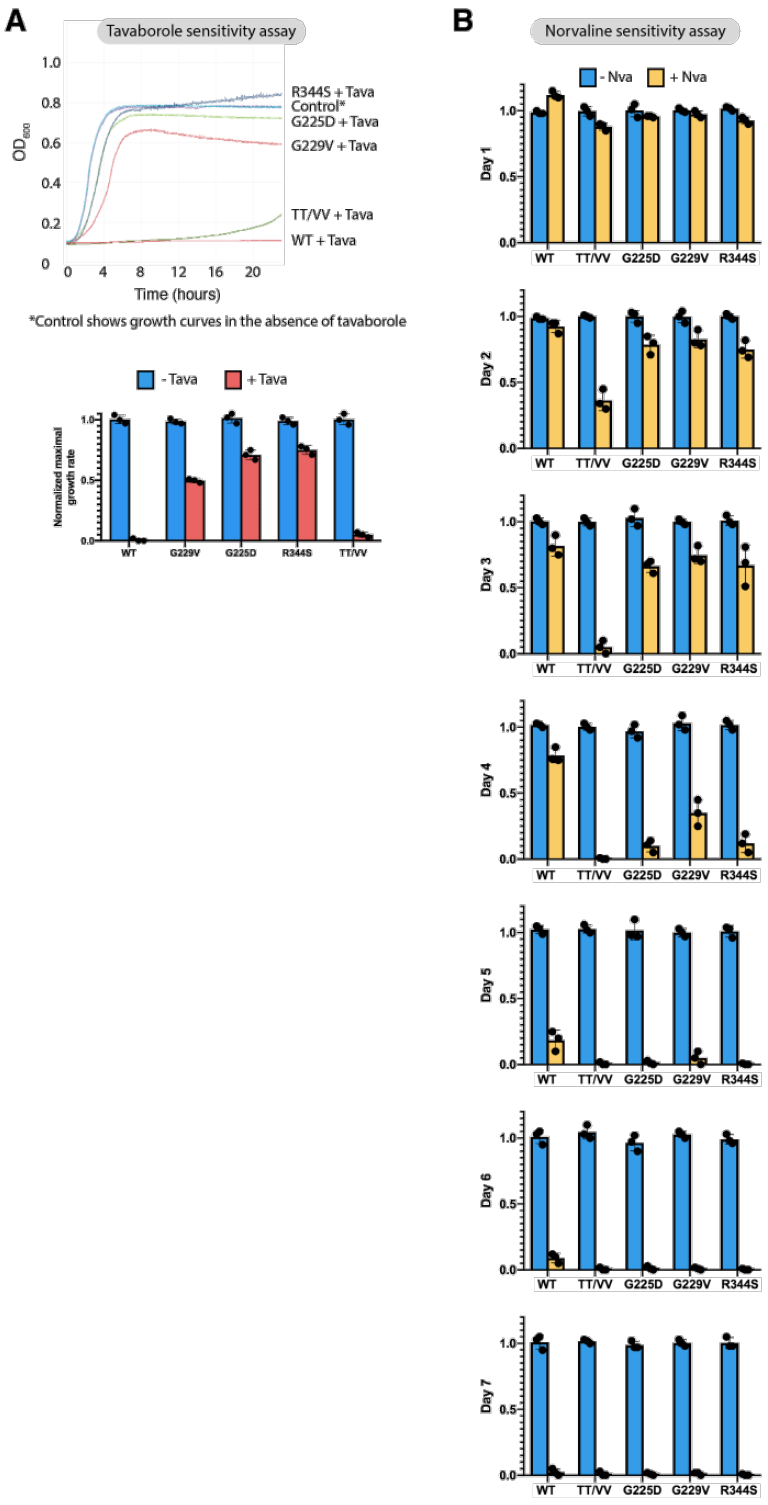


**Figure S5. Tavaborole-resistant *E. coli* colonies are hypersensitive to norvaline toxicity.** The panels compare growth rates of the parental wild-type *E. coli* and the experimentally evolved tavaborole-resistant *E. coli* strains with mutations G225D, G229V, and R344S in *leuS* gene. As a

positive control, we engineered the *E. coli* strain with the double-mutation T247V/T248V in LeuRS (**Materials and Methods**). Previously, Martinis laboratory showed that T247V, T248V mutations inactivate the editing activity of LeuRS, rendering *E. coli* hypersensitive to norvaline toxicity (Ref. 43).

**A.** First, we retested tavaborole sensitivity in these strains by measuring growth rates of these strains in media LB in the presence or absence of tavaborole (10 μg/ml). We found that, in the evolved *E. coli* mutants grew rapidly in the presence of tavaborole, with R344S showing the fastest growth, consistent with the highest frequency of R344S mutation in the experimentally evolved populations A-D. By contrast, the wild type *E. coli* and the *leuS*$^{T247V,\ T248V}$ mutant were effectively inhibited by tavaborole. This experiment illustrated that loss of LeuRS editing activity does not necessarily correlate with loss of tavaborole resistance because some mutations can inactivate the editing without altering the drug binding. However, in the evolved *E. coli* strains, loss of the editing activity did correlate with the mutation frequency, with the most frequent mutant (R344S) being the most norvaline-hypersensitive.

The experimental conditions: before the experiment, all strains were grown overnight in LB media at 37°C and then diluted with LB media to $OD_{600}$ 0.1, transferred into 24 well plates (Cornig), and incubated in Synergy|HTX multi-mode plate reader (BioTek) at 37°C, measuring the $OD_{600}$ once a minute.

**B.** We then measured growth rates of these strains in the presence or absence of norvaline (1 mM). For this purpose, we observed these strains for 8 days in a serial transfer experiment. Cells were grown in the chemically-defined NMM20 media at 37°C in 24 well plates (Cornig) using Synergy|HTX multi-mode plate reader (BioTek), measuring the $OD_{600}$ once a minute. Every 24 hours, each culture was diluted to to OD 0.1 with NMM20 media and transferred to a new plate with the total volume of 1 ml per well.

We observed that norvaline addition to wild-type *E. coli* subtly accelerated growth rate on day 1, and then caused gradual decrease in growth rate, leading to growth arrest on day 7. This observation was consistent with previous studies showing that even editing-competent *E. coli* cannot fully prevent norvaline incorporation in cellular proteins when a growth media is supplemented with high levels of norvaline (10 mM)[61]. By contrast, the *leuS*$^{T247V,T248V}$ *E. coli* mutant was rapidly inhibited by norvaline, with the growth arrest observed on day 3. For the evolved tavaborole-resistant clones – “G225D”, “G229V”, and “R344S” – we observed growth arrest on day 5, 6, and 6 respectively. Overall, this experiment showed that, compared to wild-type *E. coli*, the tavaborole-resistant strains are more sensitive to norvaline toxicity, with stronger tavaborole-resistant mutants showing higher norvaline sensitivity.

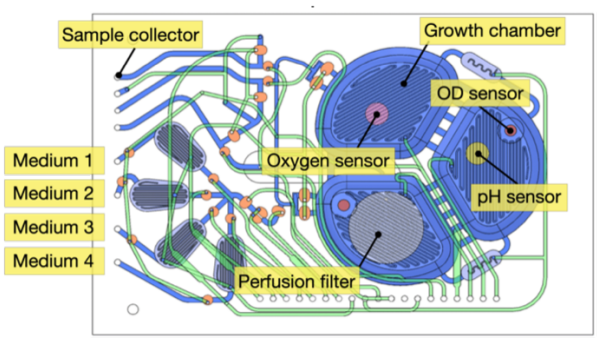


**Fig. S6 | The principal component of the microbioreactor used for the competition evolutionary experiment.** The scheme shows the milliliter-scale microbioreactors (as described in Ref. 65) used for the *E. coli* competition assay. Each reactor is a polycarbonate-PDMS membrane-polycarbonate sandwiched chip with active microfluidic circuits that are equipped for pneumatic routing of reagents, precise peristaltic injections, growth chamber mixing, and fluid extraction. Each chip has a total volume of 2 mL and allows continuous growth of a cell culture in a turbidostatic mode.

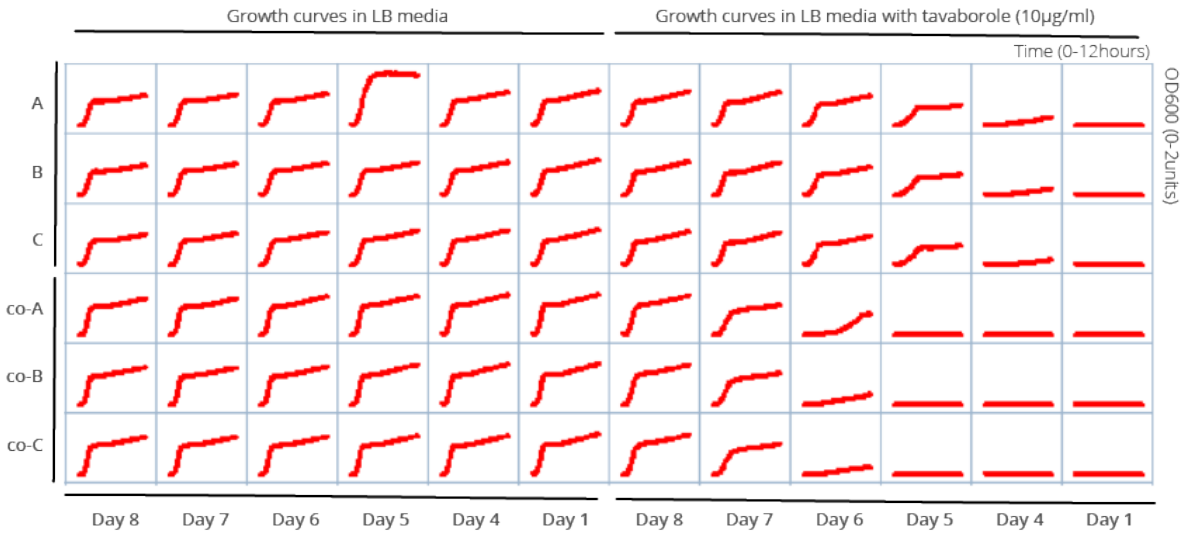


**Fig. S7 | Norvaline slows down tavaborole resistance evolution in *E. coli*.** The growth curves illustrate delayed evolution of tavaborole resistance in *E. coli* when tavaborole treatment is complemented with simultaneous treatment with norvaline (0.4 mM). For this experiment, aliquots of the evolving populations A-C (treated with tavaborole only) and co-A-co-C (cotreated with tavaborole and norvaline) were collected on Day 1-Day 8 of the evolutionary experiment, regrown to saturation (overnight at 37C in LB media), diluted to $OD_{600}$ 0.1 with LB media, and regrown in 24 well plates (Cornig) incubated in Synergy|HTX multi-mode plate reader (BioTek) at 37°C, measuring the $OD_{600}$ once a minute.