Table S1. Log2FC of each guide RNA in the random library computed for strain MG1655

Table S2. Statistics of the log2FC of guide RNAs grouped by their 5nt seed sequence for strain MG1655.

Table S3. Statistics of the log2FC of guide RNAs grouped by their 4nt seed sequence for strain MG1655.

Table S4. Statistics of the log2FC of guide RNAs grouped by their 6nt seed sequence for strain MG1655.

Table S5. Strains used in this study.

Table S6. Pasmids used in this study.

Table S7. Guide RNAs used in this study.

Table S8. Oligonucleotides used in plasmid construction.

Table S9. Oligonucleotides used in RT-qPCR.

Table S10. Oligonucleotides used to introduce mutations in the chromosome of *E. coli* through recombineering. Bases marked with a star symbol are phosphorothioated.

Table S11. Oligonucleotides used for Illumina sequencing.

Table S12. Read counts obtained from the screen of 12 Enterobacteriaceae. The first column is the guide sequence. Following columns are named as follow: species name, or strain name in the case of E. coli species, followed by REF (reference sample) or IND (induced sample), and finally 1 or 2 indicate the replicate number.

Table S13. Log2FC of guide RNAs computed for each of the 12 Enterobacteria strains.

Table S14. List of the top toxic seed sequences in strain LC-E18 and candidate off-target sites.