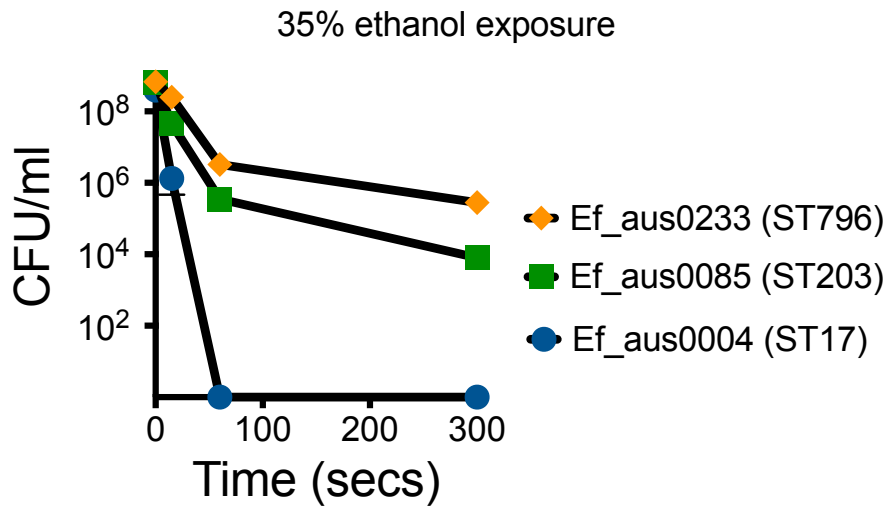
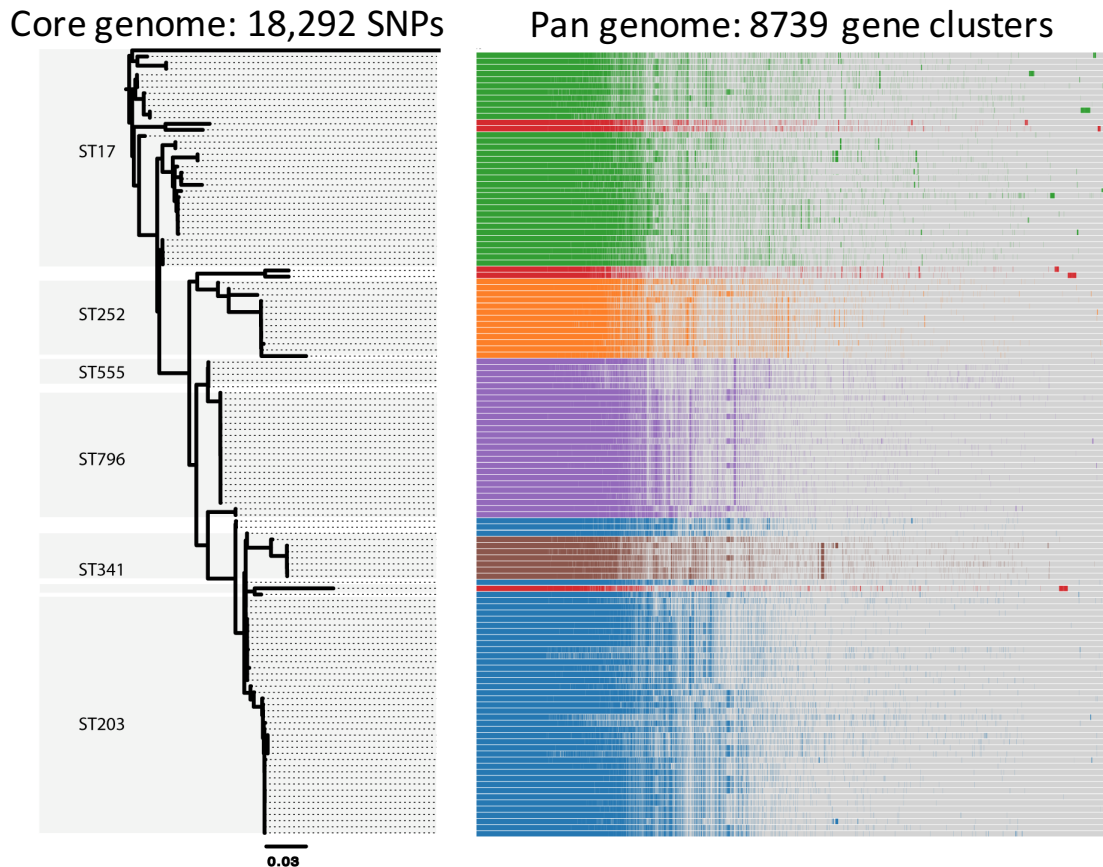


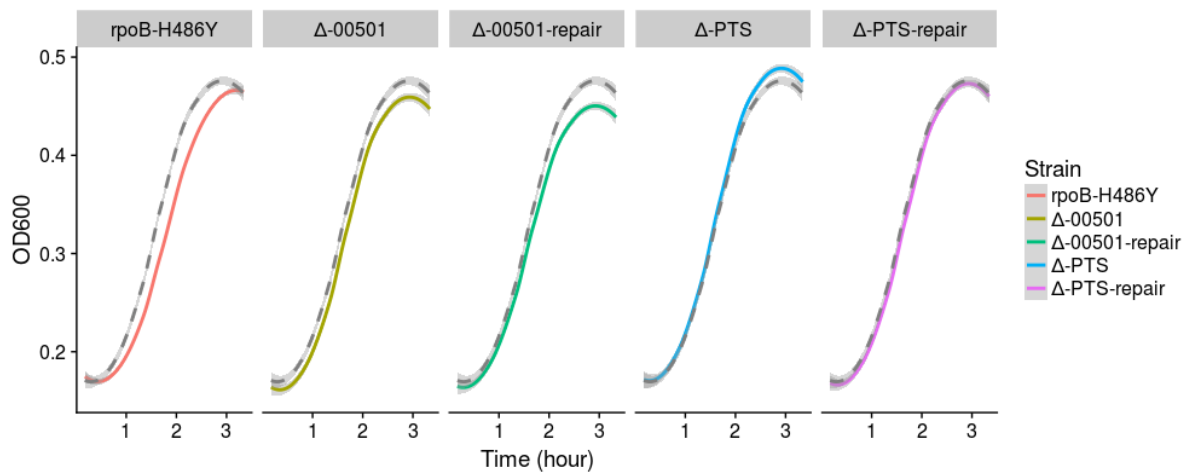
**SUPPLEMENTARY MATERIALS:**



**Fig. S1.** Tolerance of *E. faecium* to ethanol exposure. Time-kill assay showing tolerance of three *E. faecium* isolates to 35% v/v ethanol.



**Fig. S2.** Core and pan genome analysis of 129 *E. faecium* genomes. Core genome maximum likelihood phylogeny inferred from pairwise SNP alignments of 18,292 variable nucleotide positions against reference genome Ef\_aus0233, filtered to remove recombination. BAPS groups derived from the same core genome alignment are grey shaded blocks, with MLST indicated. Pan-genome analysis based on protein ortholog comparisons (Roary) and displayed using FriPan, showing shared and clone-specific gene content among the Efm population. Note the Ef\_aus0233 reference genome has 2,726 predicted CDS.



**Fig. S3.** Growth curves of mutants. Plots depict growth curves of wild type *E. faecium* Ef\_ aus0233 against the *rpoB* mutant and the two targeted mutants and their repaired versions, showing mutagenesis had no impact on the growth rate of the strains.

1 **Table S1:** Oligonucleotides used in this study

<b>Primer name</b>	<b>Sequence (5' – 3')</b>
IM585 501 OUT F	TTGTTTTTCCAAGTGTTCATCAAGG
IM586 501 OUT R	TCTGTCACTGTTTCAAATACTGATCC
IM587 501 AF	<b>CCTCACTAAAGGGAACAAAAGCTGGGTACCCATACGTTTACGATAGTTCGTAGC</b>
IM588 501 BR	CATAATCTGTATTCTCTCCTGTTC
IM589 501 CF	<i>TGAACAGGAGAGGAATACAGATTATGTAAGAAAGGATCTTGAAAATGAAGATTAC</i>
IM590 501 DR	<b>CGACTCACTATAGGGCGAATTGGAGCTCTGAATCGAACCAAATGCTGAGAAGC</b>
IM862 PTS AF	<b>CCTCACTAAAGGGAACAAAAGCTGGGTACCATCTCCAGTGATCATCATAGGAAGC</b>
IM863 PTS BR	CATCCTCAACACAAAAAACAGGCAG
IM864 PTS CF	<i>CTGCCTGTTTTTGTGTTGAGGATGTGAGCGCTCAGTAATACTATTGATGC</i>
IM865 PTS DR	<b>CGACTCACTATAGGGCGAATTGGAGCTCTTCGGACACCGTTACCTAAGGTAC</b>

2 **Notes:** Bold typeface indicates tails for SLiCE cloning into pIMAY-Z; italic typeface indicates complementary to B primer for SOE-PCR.

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