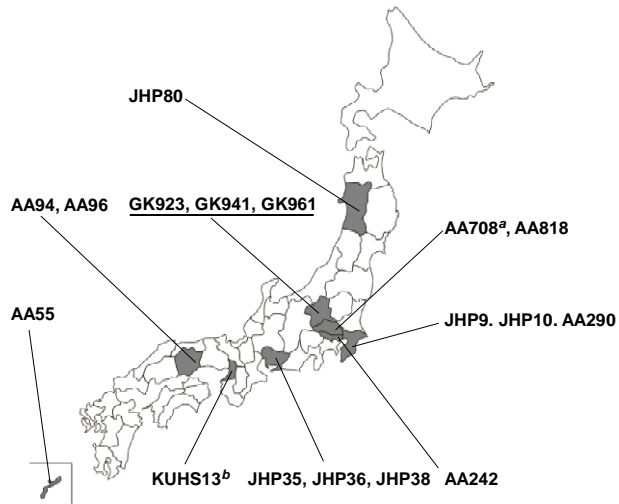
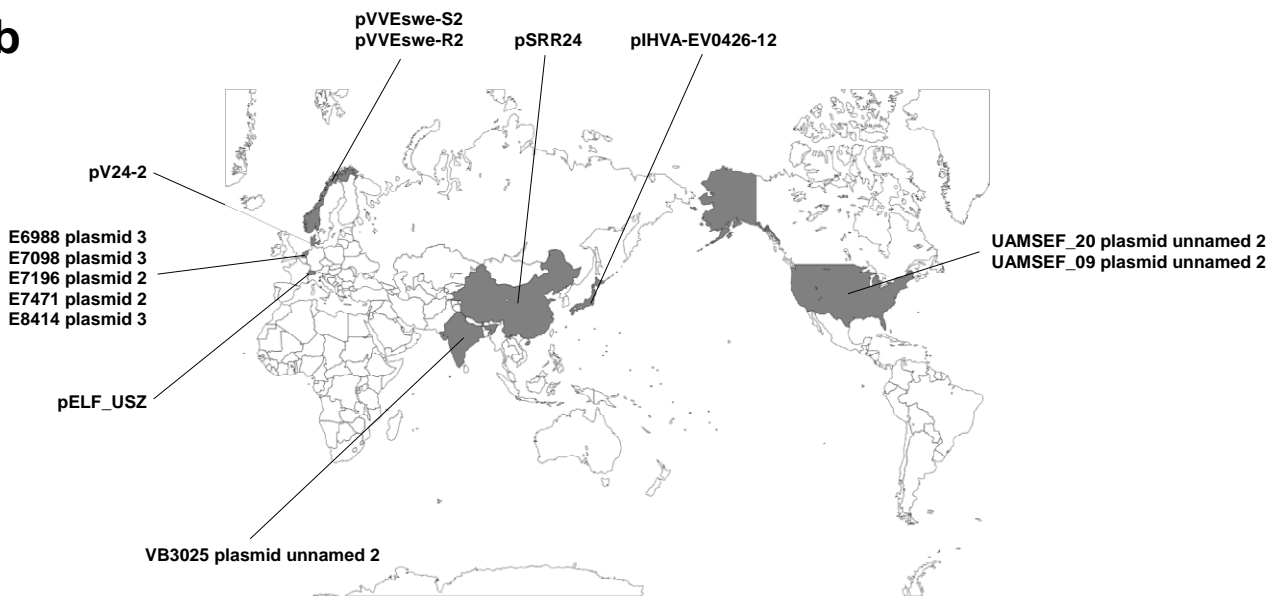


a

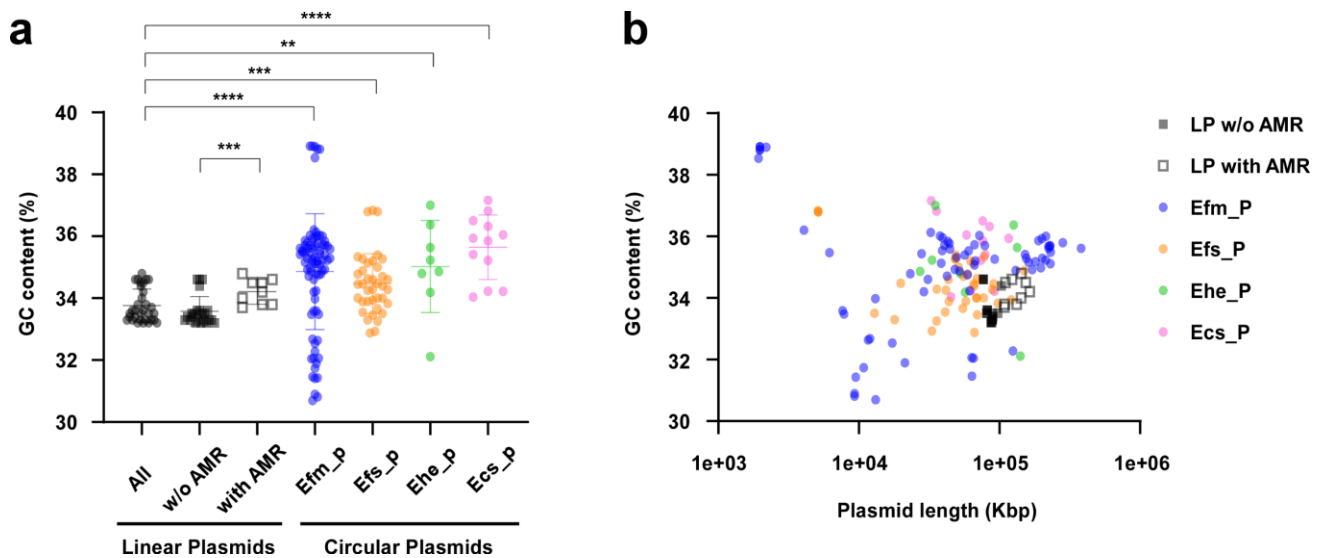


b



Supplementary Figure 1. Geographic areas of detection of enterococci harboring pELF1-like plasmids.

(a) Prefectures where enterococci harboring pELF1-like plasmids were isolated in Japan, are shown in black. ^{a,b} AA708 and KUHS13 were strains previously reported. Underlined strains indicate VSE strains. (b) The Countries where presumed pELF1-type plasmid harboring strains are detected are shown in black.

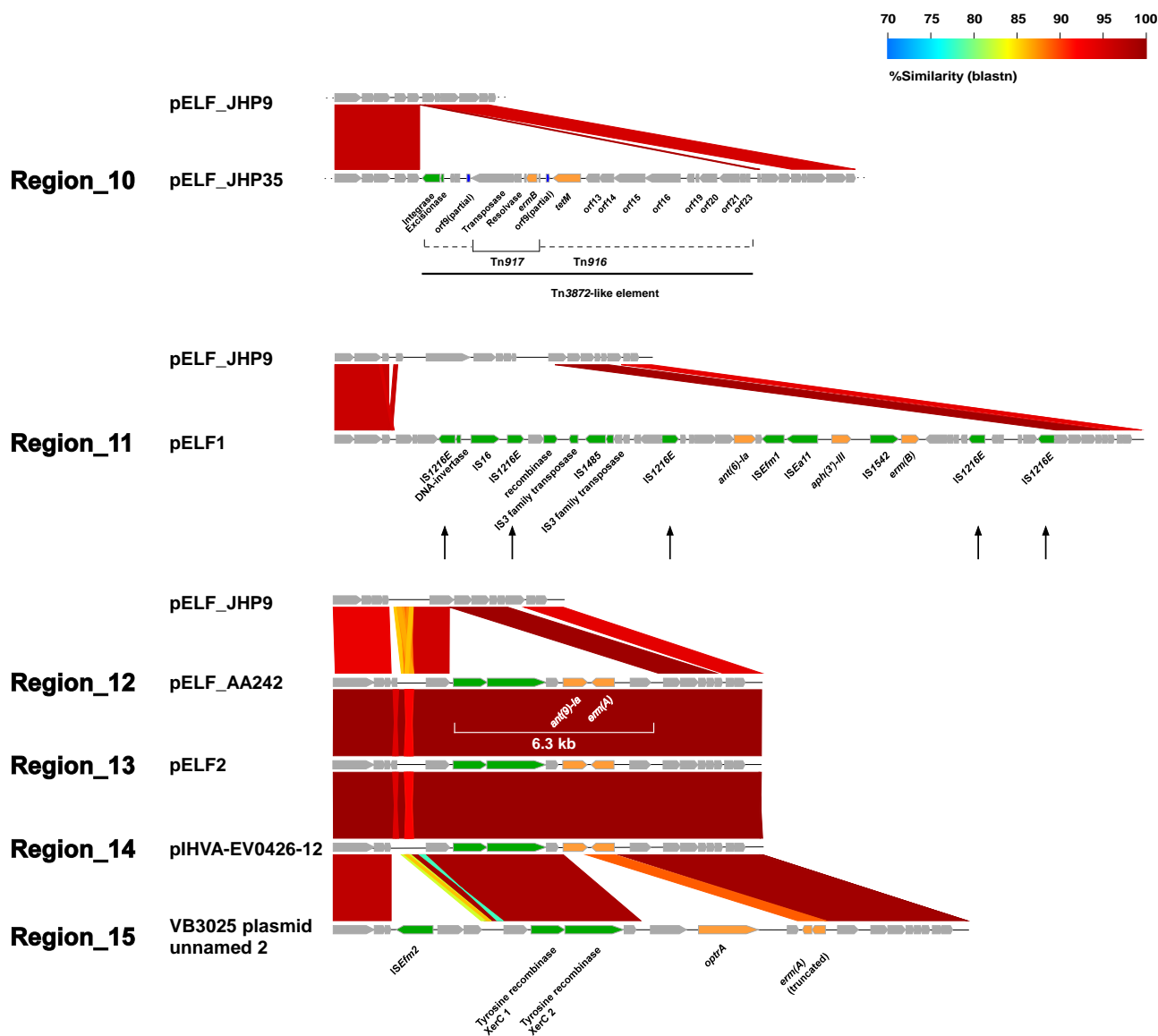


Supplementary Figure 3. Scatter plot of GC content and plasmid size of enterococcal circular plasmids along with 32 pELF1-like plasmids.

The GC content of 138 circular plasmids and 32 pELF1-like plasmids was analyzed. (a) The GC content of the plasmids possessed by each species and (b) the size and GC content of the plasmids are shown in the scatter plots. Black dots indicate all linear plasmids, Black squares indicate pELF1-like plasmids without AMR regions, white squares indicate linear plasmids with AMR regions, blue dots indicate circular plasmids of *E. faecium*, orange dots indicate circular plasmids of *E. faecalis*, green dots indicate circular plasmids of *E. hirae*, and pink dots indicate circular plasmids of *E. casseliflavus*. All circular plasmids were checked for possession of *rep* genes using PlasmidFinder (2.1). ****, $p < 0.0001$; ***, $p < 0.001$; **, $p < 0.01$ (Mann–Whitney test). LP stands for pELF1-like linear plasmids, Efm_p for circular plasmids possessed by *E. faecium*, Efs_p for circular plasmids possessed by *E. faecalis*, Ehe_p for circular plasmids possessed by *E. hirae*, and Ecs_p for circular plasmids possessed by *E. casseliflavus*.

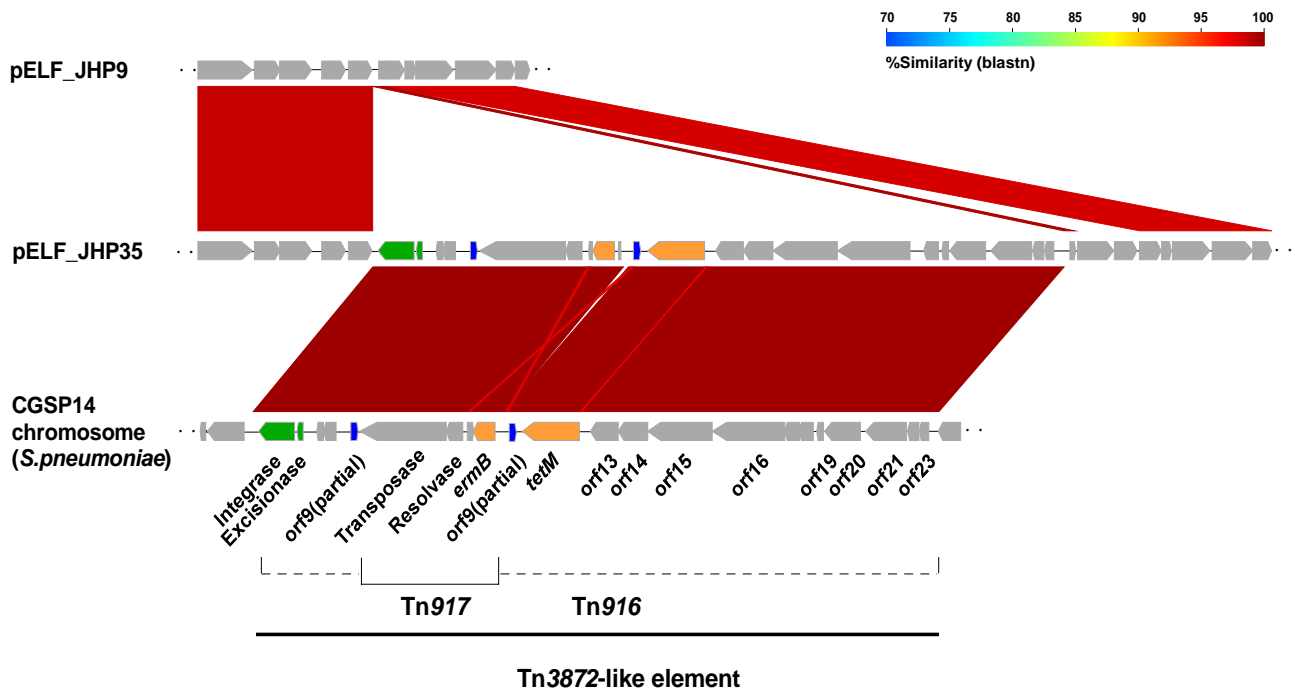
Supplementary Figure 4. Comparison of AMR regions containing vancomycin resistance genes in pELF1-like plasmids.

The nine AMR regions containing vancomycin resistance genes on the pELF1-like plasmid were compared. The numbers assigned to each AMR region are consistent with those in Figure 2. The panels show the genetic structures. The green panels represent mobile genetic element-related genes, and the orange panels represent AMR genes. The arrows pointing vertically to the panels indicate the position of *IS1216E*.



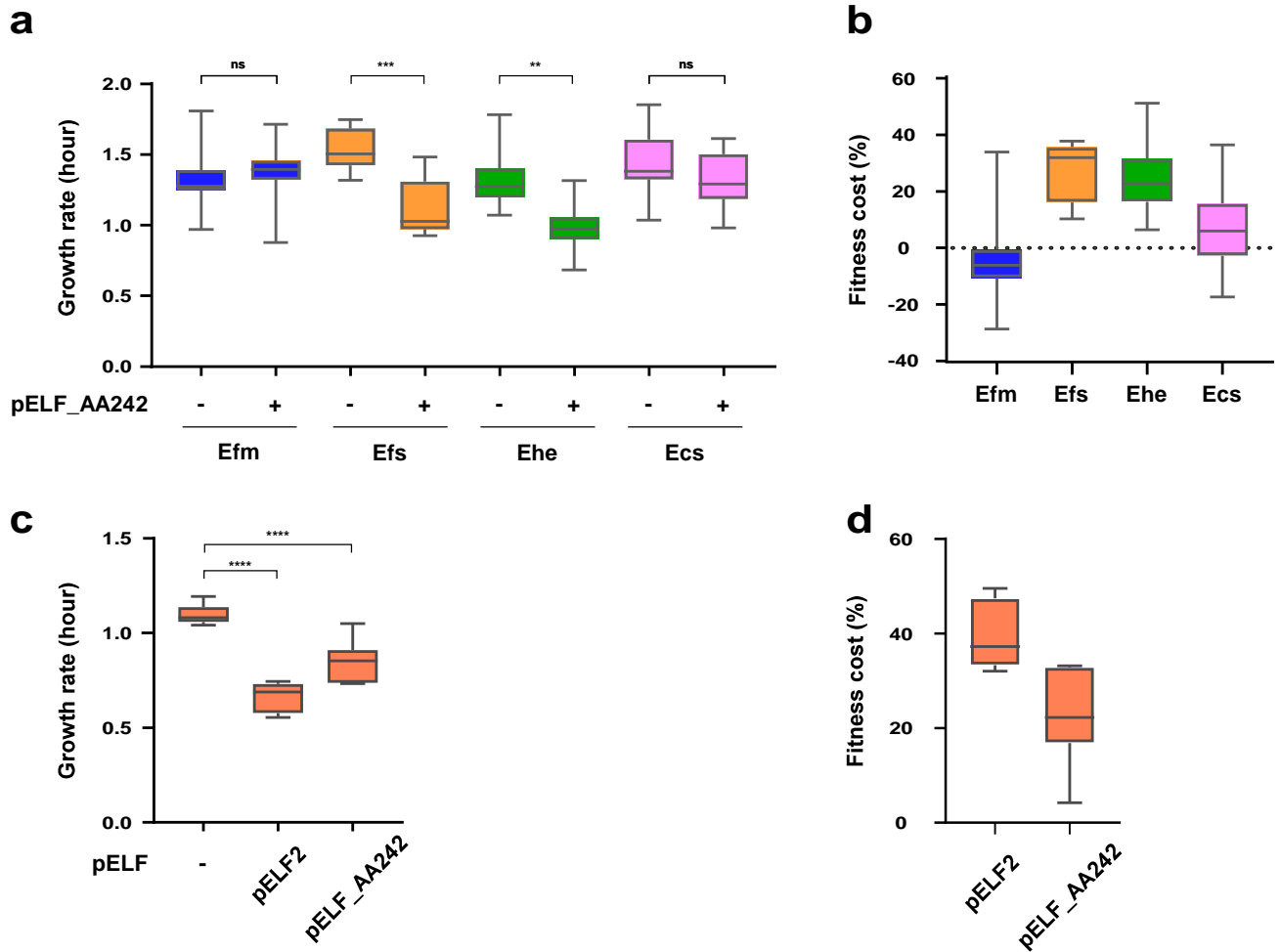
Supplementary Figure 5. Comparison of AMR regions without vancomycin resistance genes in pELF1-like plasmids.

The six AMR regions without vancomycin resistance genes on the pELF1-like plasmid were compared. The numbers assigned to each AMR region are consistent with those in Figure 2. The panel shows the genetic structure. The green panels represent mobile genetic element-related genes, and the orange panels represent AMR genes. The arrows pointing vertically to the panels indicate the position of *IS1216E*.



Supplementary Figure 6. Comparative analysis of Tn3872-like element in pELF_JHP35.

The structures of the *Tn3872*-like element of pELF_JHP35 and (B) the regions around *ant(9)-Ia* and *erm(A)* of pELF_AA242 were compared with those of pELF_JHP9. The blue panels represent partial *orf9* disrupted by Tn917, the orange panels represent AMR genes, and the green panels represent mobile genetic element-related genes.



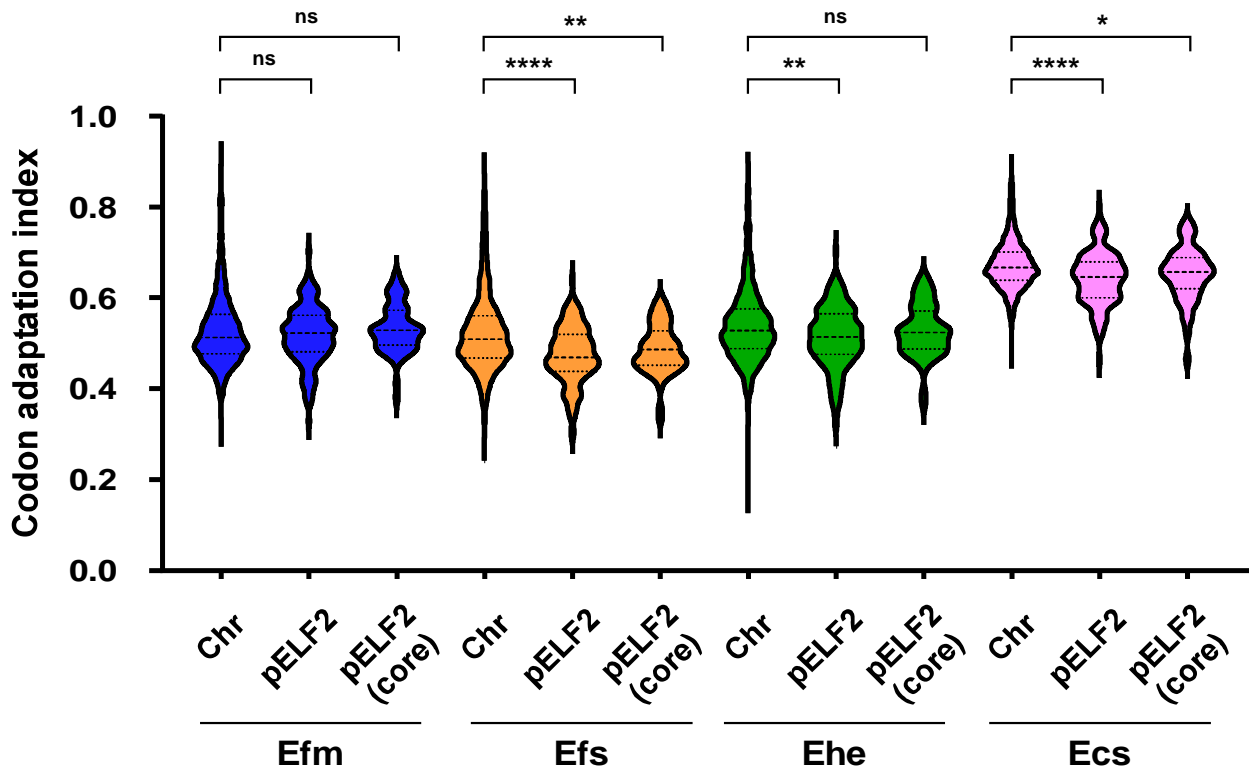
Supplementary Figure 7. Additional analysis of the effect of pELF1-like plasmids on the growth of enterococci.

The effects of possession of pELF1-like plasmid (pELF_AA242) on (a) the growth rate and (b) the fitness cost of four *Enterococcus* species are shown. Efm: *E. faecium* (BM4105RF), Efs: *E. faecalis* (FA2-2), Ehe: *E. hirae* (ATCC9790RF), Ecs: *E. casseliflavus* (KT06RF). The box-and-whiskers indicate the minimum to maximum values. The unpaired *t*-test was used to analyze the pELF1-like plasmid harboring and non- harboring strains in the growth rate ((a) $p = 0.6564$, $p = 0.0002$, $p = 0.0016$, and $p = 0.2744$, respectively, and (c) $p < 0.0001$, and $p < 0.0001$). The *E. faecalis* strain was changed to

OG1RF, and (c) the growth rates and (d) the fitness costs of pELF2 and pELF_AA242 were analyzed.

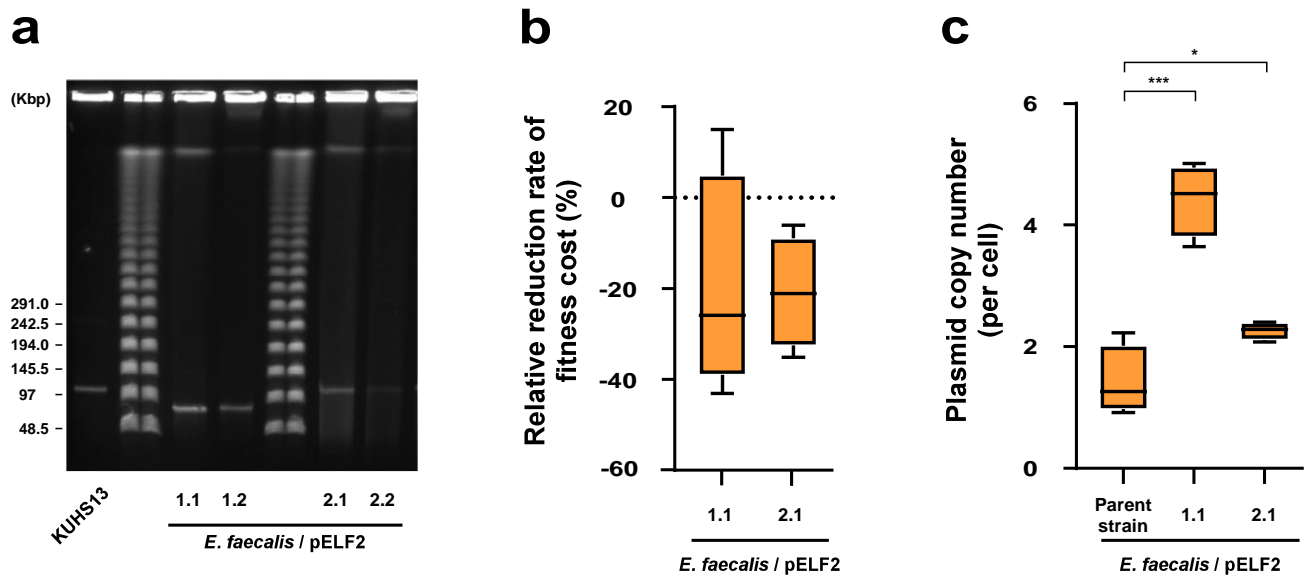
For this analysis of growth rate and fitness cost, at least six biological replicates were subject to the

analyses in duplicate. **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$; ns, not significant.



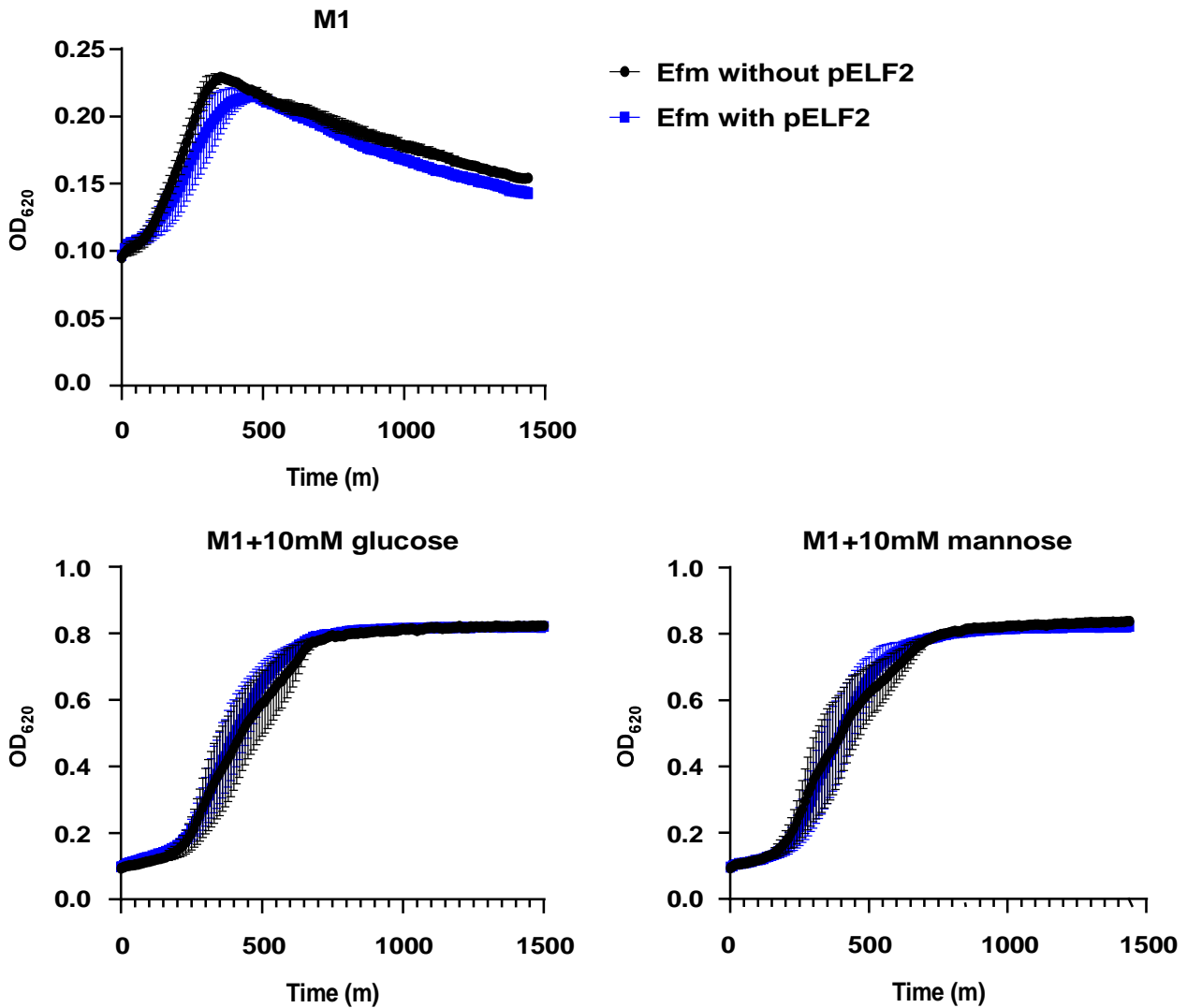
Supplementary Figure 8. Codon adaptation index analysis.

For the four enterococci species, the codon adaptation index for the chromosomal genes (Chr), the genes on pELF2, and the core genes on pELF2 were determined and illustrated in violin plots. *, $p \leq 0.05$; **, $p \leq 0.01$; ****, $p \leq 0.0001$ (Mann–Whitney test). Efm: *E. faecium* (BM4105RF), Efs: *E. faecalis* (FA2-2), Ehe: *E. hirae* (ATCC9790RF), Ecs: *E. casseliflavus* (KT06RF).



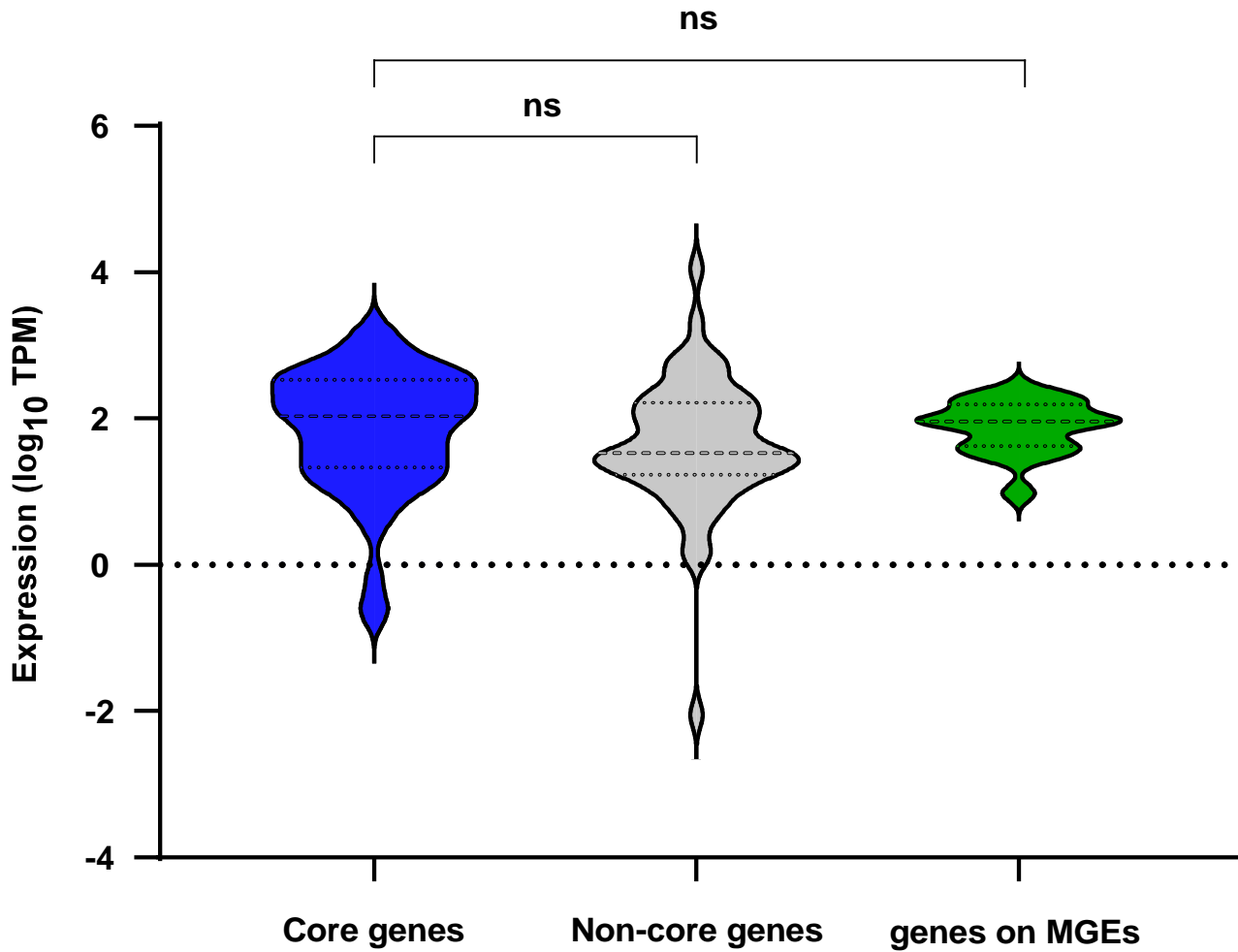
Supplementary Figure 9. Analysis of structure and stability of the pELF1-like plasmid in *E. faecalis*.

(a) PFGE analysis was conducted to assess the size of the pELF1-like plasmid (pELF2) harbored by *E. faecalis* (FA2-2) on day 5 of the long-term passage assay (day 5-evolved strains) (Figure 3C). Colonies of day 5-evolved strains were randomly selected and subjected to PFGE (S1 nuclease untreated) along with the wild strain harboring pELF2 (KUHS13; accession number: SAMD00202474). (b) The percentage reduction in fitness cost relative to the parent strain (FA2-2/pELF2) was calculated in quadruplicate. (c) The plasmid copy number was measured using qPCR. Quadruplicate results were shown in the box-and-whisker diagram. *, $p \leq 0.05$, ***, $p \leq 0.001$ (unpaired *t*-test; $p = 0.0004$, and $p = 0.0286$).



Supplementary Figure 10. Effect of pELF1-like plasmid carriage on *E. faecium* growth in M1 medium.

The impact of pELF1-like plasmid (pELF2) on the growth of *E. faecium* (BM4105RF) under carbon source-limited culture conditions was analyzed. Black dots and lines indicate strains without pELF2, and blue dots and lines indicate strains with pELF2. The mean and standard error are plotted in four independent experiments. Individual culture conditions are shown at the top of each graph.



Supplementary Figure 11. Comparative analysis of transcript levels of genes on the pELF1-like plasmid.

Transcription levels (TPM) of genes on pELF2 were compared by classification of core genes, non-core genes, and accessory genes (MGE) and illustrated in violin plots. ns, not significant (Mann-Whitney test).

Supplementary Table 1. Primers used in this study.

Primer name	primer sequences (5' - 3')	Product size (bp)	Description	References
Left_end_F	CCAACCGCCAGATAAAAATG	552	For detecting the hairpin end of pELF1-like linear plasmids	This study
Left_end_R	GATGCGATTGAGAAGAAATAAGAA			
Right_end_F	ATGTCTGACGGATATTGAATAG	123	For detecting the invertron end of pELF1-like linear plasmids	This study
Right_end_R	GCAAGCGACTTGCTTAATG			
Linear_plasmid_2F	AGTGGCGAAATGGTTATTGG	122	For copy number and transcript level analyses (detecting replication protein gene of linear plasmids)	This study
Linear_plasmid_2R	AAATTCTTTCGCCTGCTC			
Efm_gyrB_F	GCTTCACITCCTGCTTCACC	115	For copy number and transcript level analyses (detecting <i>gyrB</i> of a <i>E. faecium</i> chromosome (internal control)	This study
Efm_gyrB_R	GGGCCATCATTGATTACTT			
Efs_gyrB_F	GAGTGTGCGATGATGCCATTG	101	For copy number and transcript level analyses (detecting <i>gyrB</i> of a <i>E. faecalis</i> chromosome (internal control)	This study
Efs_gyrB_R	CGTAATGGGCATTTCTTCG			
Ehe_gyrB_F	CAACGATGGACCCTAGCAAT	107	For copy number and transcript level analyses (detecting <i>gyrB</i> of a <i>E. hirae</i> chromosome (internal control)	This study
Ehe_gyrB_R	ACGAGGTTCACCTCGATCAC			
Ecs_gyrB_F	ACGGTTCAACGCTACAAAAGG	118	For copy number and transcript level analyses (detecting <i>gyrB</i> of a <i>E. casseliflavus</i> chromosome (internal control)	This study
Ecs_gyrB_R	CGGCTTCTATGGCATCATCT			
RepB_F	TGATCAAATCTTGAACGGAAC	136	For transcript level analysis (detecting <i>repB</i> of pELF1-like linear plasmids)	This study
RepB_R	ATCCCAATGAATCCGATCAA			

Supplementary Table 2. MIC information of the pELF1-like plasmids carrying strains.

Strain	Species	van genotype	Date of Isolation (Year)	Region	MIC (mg/L) ^a															
					VAN	TEC	LZD	AMP	GEN	KAN	STR	ERY	CHL	TET	MIN	CIP	CRO	CMZ	MEM	
JHP9	<i>E. faecium</i>	vanA	2000	Chiba	1024	256	2	256	512	>1024	2048	>1024	8	64	32	512	>1024	>1024	512	
JHP10	<i>E. faecium</i>	vanA	2000	Chiba	1024	128	2	512	1024	>1024	>2048	>1024	3	32	32	512	>1024	>1024	1024	
JHP35	<i>E. faecium</i>	vanA	2002	Aichi	512	16	2	512	>1024	>1024	16	>1024	8	64	32	4	>1024	>1024	>1024	
JHP36	<i>E. faecium</i>	vanA	2002	Aichi	1024	64	2	512	8	>1024	32	>1024	4	64	32	512	>1024	>1024	1024	
JHP38	<i>E. faecium</i>	vanA	2002	Aichi	1024	64	2	512	>1024	>1024	32	>1024	4	64	32	512	>1024	>1024	1024	
JHP80	<i>E. faecium</i>	vanB	2001-2002	Akita	32	1	2	128	8	>1024	>2048	>1024	4	1	2	32	>1024	>1024	512	
AA55	<i>E. faecium</i>	vanA	2010	Okinawa	1024	256	3	>1024	3	>1024	16-32	>1024	4	1	1	128	>1024	>1024	>1024	
AA94	<i>E. faecium</i>	vanB	2011	Okayama	32	1	3	512	>1024	>1024	256	>1024	4	128	32	64	>1024	>1024	>1024	
AA96	<i>E. faecium</i>	vanB	2011	Okayama	32	1	3	512	>1024	>1024	128	>1024	8	1	1	64	>1024	>1024	512	
AA242	<i>E. faecium</i>	vanA	2013	Tokyo	512	128	3	512	3	128	16-32	>1024	3	1	1	64	>1024	>1024	>1024	
AA290	<i>E. faecium</i>	vanA	2013	Chiba	512	32	3	>1024	>1024	>1024	2048	>1024	4	1	1	128	>1024	>1024	>1024	
AA610	<i>E. faecium</i>	vanB	2017	N. A.	32	1	2	512	4	>1024	16-32	>1024	4	1	1	32	>1024	>1024	>1024	
AA818	<i>E. faecium</i>	vanB	2019	Saitama	64	2	3	128	16	>1024	16	>1024	16	32	16	512	>1024	>1024	>1024	
GK923	<i>E. faecium</i>	-	2014	Gunma	1	1	2	512	8	>1024	32	>1024	3	3	1	256	>1024	>1024	>1024	
GK941	<i>E. faecium</i>	-	2015	Gunma	1	1	2	512	4-8	>1024	32	>1024	3	3	1	256	>1024	>1024	>1024	
GK961	<i>E. faecium</i>	-	2015	Gunma	1	1	2	64	3	512	32	>1024	3	2	1	256	>1024	>1024	>1024	

^aVAN; vancomycin, TEC; teicoplanin, LZD; linezolid, AMP; ampicillin, GEN; gentamicin, KAN; kanamycin, STR; streptomycin, ERY; erythromycin, CHL; chloramphenicol, TET; tetracycline, MIN; minocycline, CIP; ciprofloxacin, LVX; levofloxacin, CMZ; cefmetazole, CRO; ceftriaxone, MEM; meropenem, BAC; bacitracin

N. A.; not applicable

Supplementary Table 3. List of Enterococcal circular plasmids used for G+C content comparison

Plasmid description	Enterococcus spp.	Topology	Length (bp)	G+C content (%)	Rep type	Accession number
Enterococcus faecium Aus0085 plasmid p5, complete sequence.	<i>E. faecium</i>	circular	31,004	34.6	Inc18	CP006623
Enterococcus faecium strain 066 plasmid unnamed2, complete sequence.	<i>E. faecium</i>	circular	49,420	38.32	Inc18	CP013996
Enterococcus faecium strain ATCC 700221 plasmid unnamed2, complete sequence.	<i>E. faecium</i>	circular	63,692	31.46	Inc18	CP014451
Enterococcus faecium strain E745 plasmid p6, complete sequence.	<i>E. faecium</i>	circular	65,558	32.05	Inc18	CP014535
Enterococcus faecium strain E1 plasmid pE1_29, complete sequence.	<i>E. faecium</i>	circular	29,012	34.2	Inc18	CP018007
Enterococcus faecium strain HP954 plasmid pHP954-2, complete sequence.	<i>E. faecium</i>	circular	42,864	35.84	Inc18	CP083475
Enterococcus faecium strain SCI762-D plasmid p3, complete sequence.	<i>E. faecium</i>	circular	62,475	32.06	Inc18	CP085897
Enterococcus faecium strain SCI762-D plasmid p3, complete sequence.	<i>E. faecium</i>	circular	63,475	32.06	Inc18	CP085909
Enterococcus faecium strain P47 plasmid pP47-27, complete sequence.	<i>E. faecium</i>	circular	27,897	35.44	Inc18	CP091101
Enterococcus faecium strain P47 plasmid pP47-61, complete sequence.	<i>E. faecium</i>	circular	61,338	34.24	Inc18	CP091102
Enterococcus faecium strain UK040 plasmid pUK040_2, complete sequence.	<i>E. faecium</i>	circular	44,472	35.59	Inc18	CP091579
Enterococcus faecium strain E745 plasmid p2, complete sequence.	<i>E. faecium</i>	circular	32,423	36.13	Inc18	CP014531
Enterococcus faecium strain UK043 plasmid pUK043_2, complete sequence.	<i>E. faecium</i>	circular	66,324	35.07	Inc18, Inc18	CP0091303
Enterococcus faecium Aus0085 plasmid p5, complete sequence.	<i>E. faecium</i>	circular	4,072	36.2	Rep_trans	CP006625
Enterococcus faecium Aus0085 plasmid p5, complete sequence.	<i>E. faecium</i>	circular	2,189	38.9	Rep_trans	CP006626
Enterococcus faecium isolate V1225 plasmid pV1225-7, complete sequence.	<i>E. faecium</i>	circular	1,979	38.81	Rep_trans	CP083919
Enterococcus faecium isolate V1164 plasmid pV1164-9, complete sequence.	<i>E. faecium</i>	circular	1,979	38.81	Rep_trans	CP083920
Enterococcus faecium strain SCI762 plasmid p11, complete sequence.	<i>E. faecium</i>	circular	1,979	38.91	Rep_trans	CP085905
Enterococcus faecium strain SCI762-D plasmid p11, complete sequence.	<i>E. faecium</i>	circular	1,979	38.91	Rep_trans	CP085917
Enterococcus faecium strain NMVRE-001 plasmid p5, complete sequence.	<i>E. faecium</i>	circular	1,928	38.54	Rep_trans	CP089324
Enterococcus faecium isolate V1225 plasmid pV1225-2, complete sequence.	<i>E. faecium</i>	circular	48,157	34.93	Rep_trans, Inc18, Inc18	CP083914
Enterococcus faecium isolate V1164 plasmid pV1164-2, complete sequence.	<i>E. faecium</i>	circular	52,056	34.87	Rep_trans, Inc18, Inc18	CP083922
Enterococcus faecium strain 4995-20 plasmid p4995-2, complete sequence.	<i>E. faecium</i>	circular	42,382	34.71	Rep_trans, Rep3, Inc18	CP084180
Enterococcus faecium strain 4995-20 plasmid p4995-3, complete sequence.	<i>E. faecium</i>	circular	39,491	35.95	Rep3, Inc18	CP084181
Enterococcus faecium strain VRE001 plasmid unnamed2, complete sequence.	<i>E. faecium</i>	circular	59,226	34.75	Rep1, Rep3, Inc18, Inc18	CP018702
Enterococcus faecium strain UW8175 plasmid unnamed2, complete sequence.	<i>E. faecium</i>	circular	38,512	35.13	Rep1, Rep3, Inc18	CP018300
Enterococcus faecium AAG20 plasmid pAAG20-3 DNA, complete genome.	<i>E. faecium</i>	circular	9,507	31.43	Rep3	AP024834
Enterococcus faecium AAG22 plasmid pAAG22-3 DNA, complete genome.	<i>E. faecium</i>	circular	9,507	31.41	Rep3	AP024840
Enterococcus faecium Aus0085 plasmid p4, complete sequence.	<i>E. faecium</i>	circular	9,319	30.9	Rep3	CP006624
Enterococcus faecium strain E39 plasmid p5, complete sequence.	<i>E. faecium</i>	circular	13,141	30.7	Rep3	CP011285
Enterococcus faecium strain UW175 plasmid unnamed3, complete sequence.	<i>E. faecium</i>	circular	10,859	31.74	Rep3	CP011831
Enterococcus faecium strain E745 plasmid p5, complete sequence.	<i>E. faecium</i>	circular	9,210	30.81	Rep3	CP014532
Enterococcus faecium isolate V1225 plasmid pV1225-3, complete sequence.	<i>E. faecium</i>	circular	7,669	33.59	Rep3	CP083915
Enterococcus faecium isolate V1164 plasmid pV1164-4, complete sequence.	<i>E. faecium</i>	circular	7,669	33.59	Rep3	CP083924
Enterococcus faecium strain 4995-20 plasmid p4995-6, complete sequence.	<i>E. faecium</i>	circular	11,625	32.64	Rep3	CP084184
Enterococcus faecium strain SCI762 plasmid p5, complete sequence.	<i>E. faecium</i>	circular	7,838	33.48	Rep3	CP085899
Enterococcus faecium strain SCI762 plasmid p7, complete sequence.	<i>E. faecium</i>	circular	6,175	35.47	Rep3	CP085901
Enterococcus faecium strain SCI762-D plasmid p5, complete sequence.	<i>E. faecium</i>	circular	7,838	33.48	Rep3	CP085911
Enterococcus faecium strain SCI762-D plasmid p7, complete sequence.	<i>E. faecium</i>	circular	6,175	35.47	Rep3	CP085913
Enterococcus faecium strain NMVRE-001 plasmid p4, complete sequence.	<i>E. faecium</i>	circular	11,963	32.68	Rep3	CP089323
Enterococcus faecium strain E1 plasmid pE1_13, complete sequence.	<i>E. faecium</i>	circular	13,112	35.98	Rep3	CP018008
Enterococcus faecium strain UK040 plasmid pUK040_3, complete sequence.	<i>E. faecium</i>	circular	21,225	31.9	Rep3	CP091580
Enterococcus faecium AAG20 plasmid pAAG20-1 DNA, complete genome.	<i>E. faecium</i>	circular	92,522	35.26	RepA_N	AP024832
Enterococcus faecium AAG20 plasmid pAAG20-2 DNA, complete genome.	<i>E. faecium</i>	circular	71,966	36.03	RepA_N	AP024833
Enterococcus faecium AAG22 plasmid pAAG22-1 DNA, complete genome.	<i>E. faecium</i>	circular	155,411	35.29	RepA_N	AP024838
Enterococcus faecium AAG22 plasmid pAAG22-2 DNA, complete genome.	<i>E. faecium</i>	circular	71,967	36.03	RepA_N	AP024839
Enterococcus faecium T110 plasmid pT110, complete sequence.	<i>E. faecium</i>	circular	44,086	35.77	RepA_N	CP006031
Enterococcus faecium Aus0085 plasmid p1, complete sequence.	<i>E. faecium</i>	circular	130,716	34.9	RepA_N	CP006621
Enterococcus faecium Aus0085 plasmid p2, complete sequence.	<i>E. faecium</i>	circular	67,314	35.2	RepA_N	CP006622
Enterococcus faecium strain E39 plasmid p1, complete sequence.	<i>E. faecium</i>	circular	152,440	35.41	RepA_N	CP011282
Enterococcus faecium strain E39 plasmid p4, complete sequence.	<i>E. faecium</i>	circular	37,865	36.01	RepA_N	CP011284
Enterococcus faecium strain UW8175 plasmid unnamed1, complete sequence.	<i>E. faecium</i>	circular	229,882	35.68	RepA_N	CP011829
Enterococcus faecium strain UW76064-3 TC1 plasmid pWCF-TC1, complete sequence.	<i>E. faecium</i>	circular	66,496	35.73	RepA_N	CP013010
Enterococcus faecium strain ATCC 700221 plasmid unnamed3, complete sequence.	<i>E. faecium</i>	circular	39,138	35.54	RepA_N	CP014452
Enterococcus faecium strain E745 plasmid p1, complete sequence.	<i>E. faecium</i>	circular	223,688	35.68	RepA_N	CP014530
Enterococcus faecium strain E745 plasmid p4, complete sequence.	<i>E. faecium</i>	circular	17,244	32.54	RepA_N	CP014533
Enterococcus faecium strain E1 plasmid pE1_20, complete sequence.	<i>E. faecium</i>	circular	230,049	35.11	RepA_N	CP018006
Enterococcus faecium strain VRE001 plasmid unnamed1, complete sequence.	<i>E. faecium</i>	circular	78,226	35.7	RepA_N	CP018070
Enterococcus faecium strain VRE001 plasmid unnamed2, complete sequence.	<i>E. faecium</i>	circular	170,854	34.92	RepA_N	CP018073
Enterococcus faecium strain H7 plasmid unnamed, complete sequence.	<i>E. faecium</i>	circular	124,878	32.28	RepA_N	CP083180
Enterococcus faecium strain HP954 plasmid pHP954-1, complete sequence.	<i>E. faecium</i>	circular	196,609	34.98	RepA_N	CP083474
Enterococcus faecium isolate V1225 plasmid pV1225-1, complete sequence.	<i>E. faecium</i>	circular	213,284	35.23	RepA_N	CP083913
Enterococcus faecium isolate V1164 plasmid pV1164-1, complete sequence.	<i>E. faecium</i>	circular	213,279	35.23	RepA_N	CP083921
Enterococcus faecium strain 4995-20 plasmid p4995-1, complete sequence.	<i>E. faecium</i>	circular	262,637	35.8	RepA_N	CP084179
Enterococcus faecium strain SCI762 plasmid p1, complete sequence.	<i>E. faecium</i>	circular	224,636	35.61	RepA_N	CP085896
Enterococcus faecium strain SCI762 plasmid p4, complete sequence.	<i>E. faecium</i>	circular	23,182	34.79	RepA_N	CP085898
Enterococcus faecium strain SCI762-D plasmid p1, complete sequence.	<i>E. faecium</i>	circular	224,638	35.61	RepA_N	CP085907
Enterococcus faecium strain SCI762-D plasmid p4, complete sequence.	<i>E. faecium</i>	circular	23,177	34.79	RepA_N	CP085910
Enterococcus faecium strain V13-21-E11-012-001 plasmid pK2IEFM001, complete sequence.	<i>E. faecium</i>	circular	201,226	35.29	RepA_N	CP089093
Enterococcus faecium strain NMVRE-001 plasmid p1, complete sequence.	<i>E. faecium</i>	circular	228,311	35.7	RepA_N	CP089320
Enterococcus faecium strain NMVRE-001 plasmid p3, complete sequence.	<i>E. faecium</i>	circular	40,605	35.71	RepA_N	CP089322
Enterococcus faecium strain UK043 plasmid pUK043_1, complete sequence.	<i>E. faecium</i>	circular	161,029	35.16	RepA_N	CP091302
Enterococcus faecium strain UK043 plasmid pUK043_3, complete sequence.	<i>E. faecium</i>	circular	63,306	35.53	RepA_N	CP091304
Enterococcus faecium strain UK040 plasmid pUK040_1, complete sequence.	<i>E. faecium</i>	circular	231,428	35.49	RepA_N	CP091578
Enterococcus faecium Aus0004 plasmid AUS0004_p1, complete sequence.	<i>E. faecium</i>	circular	56,520	35.4	RepA_N	NC_017032
Enterococcus faecium NRRL B-2354 plasmid pNB2354_1, complete sequence.	<i>E. faecium</i>	circular	214,319	36	RepA_N, Inc18	CP004064
Enterococcus faecium strain ATCC 700221 plasmid unnamed1, complete sequence.	<i>E. faecium</i>	circular	189,452	35.86	Inc18	CP014450
Enterococcus faecium strain E843GE-1-TC1 plasmid pE843-TC-200, complete sequence.	<i>E. faecium</i>	circular	200,566	35.93	RepA_N, Inc18, Inc18	CP081053
Enterococcus faecium strain P47 plasmid pP47_180, complete sequence.	<i>E. faecium</i>	circular	180,523	35.18	RepA_N, Inc18, Inc18	CP091103
Enterococcus faecium strain 066 plasmid unnamed1, complete sequence.	<i>E. faecium</i>	circular	381,524	35.61	RepA_N, RepA_N	CP013995
Enterococcus faecalis D32 plasmid pFD32A, complete sequence.	<i>E. faecalis</i>	circular	12,893	35.5	Inc18	CP003727
Enterococcus faecalis D32 plasmid pFD32B, complete sequence.	<i>E. faecalis</i>	circular	62,162	35.2	Inc18	CP003728
Enterococcus faecalis ATCC 29212 plasmid 1, complete sequence.	<i>E. faecalis</i>	circular	66,548	32.88	Inc18	CP008815
Enterococcus faecalis strain FFS36 plasmid pFFS36_2, complete sequence.	<i>E. faecalis</i>	circular	35,757	34.29	Inc18	CP085293
Enterococcus faecalis strain FFS108 plasmid pFFS108_3, complete sequence.	<i>E. faecalis</i>	circular	69,496	33.45	Inc18	CP085297
Enterococcus faecalis strain E512 plasmid pE512, complete sequence.	<i>E. faecalis</i>	circular	48,881	35.4	Inc18	CP086559
Enterococcus faecalis strain E512-TC1 plasmid pE512-TC1, complete sequence.	<i>E. faecalis</i>	circular	48,549	35.27	Inc18	CP086665
Enterococcus faecalis strain E512-TC2 plasmid pE512-TC2, complete sequence.	<i>E. faecalis</i>	circular	48,589	35.27	Inc18	CP086667
Enterococcus faecalis strain Fac74 plasmid pFac74_1, complete sequence.	<i>E. faecalis</i>	circular	93,245	34.63	Inc18	CP086893
Enterococcus faecalis strain UK045 plasmid pUK045_1, complete sequence.	<i>E. faecalis</i>	circular	90,994	34.46	Inc18	CP091197
Enterococcus faecalis V583 plasmid pV583, complete sequence.	<i>E. faecalis</i>	circular	17,963	33.3	Inc18	NC_004670
Enterococcus faecalis DORA_14 plasmid pEFLP_DORA_14 Q608_EFLP00001, whole genome shotgun sequence.	<i>E. faecalis</i>	circular	5,143	36.8	Rep3	AZL1Y000050
Enterococcus faecalis 62 plasmid pE62A, complete sequence.	<i>E. faecalis</i>	circular	5,143	36.8	Rep3	CP002492
Enterococcus faecalis strain E006:H2-2-TC1 plasmid pE006-TC-121, complete sequence.	<i>E. faecalis</i>	circular	121,520	33.94	Rep3	CP081506
Enterococcus faecalis strain E006 plasmid pE006-19, complete sequence.	<i>E. faecalis</i>	circular	19,832	34.48	Rep3	CP082233
Enterococcus faecalis strain FFS108 plasmid pFFS108_5, complete sequence.	<i>E. faecalis</i>	circular	5,120	36.84	Rep3	CP085299
Enterococcus faecalis 62 plasmid pE62B, complete sequence.	<i>E. faecalis</i>	circular	51,164	35	RepA_N	CP002493
Enterococcus faecalis 62 plasmid pE62C, complete sequence.	<i>E. faecalis</i>	circular	55,303	34.7	RepA_N	CP002494
Enterococcus faecalis ATCC 29212 plasmid 2, complete sequence.	<i>E. faecalis</i>	circular	41,610	34.25	RepA_N	CP008814
Enterococcus faecalis strain AR_0780 plasmid pAR_0780, complete sequence.	<i>E. faecalis</i>	circular	65,096	35.14	RepA_N	CP063981
Enterococcus faecalis strain FFS36 plasmid pFFS36_1, complete sequence.	<i>E. faecalis</i>	circular	43,405	34.6	RepA_N	CP085292
Enterococcus faecalis strain E509 plasmid pE509-2, complete sequence.	<i>E. faecalis</i>	circular	33,086	32.93	RepA_N	CP086562
Enterococcus faecalis strain UK045 plasmid pUK045_2, complete sequence.	<i>E. faecalis</i>	circular	41,202	33.55	RepA_N	CP091199
Enterococcus faecalis strain UK045 plasmid pUK045_3, complete sequence.	<i>E. faecalis</i>	circular	38,753	33.25	RepA_N	CP091200
Enterococcus faecalis strain 152 plasmid p1, complete sequence.	<i>E. faecalis</i>	circular	68,770	34	RepA_N	CP091885
Enterococcus faecalis strain 143-1 plasmid p1, complete sequence.	<i>E. faecalis</i>	circular	68,770	34	RepA_N	CP091887
Enterococcus faecalis strain 142-1 plasmid p1, complete sequence.	<i>E. faecalis</i>	circular	77,272	34	RepA_N	CP091890
Enterococcus faecalis strain 142-1 plasmid p2, complete sequence.	<i>E. faecalis</i>	circular	59,470	34.78	RepA_N	CP091891
Enterococcus faecalis strain 101-1 plasmid p1, complete sequence.	<i>E. faecalis</i>	circular	68,785	34.45	RepA_N	CP091900
Enterococcus faecalis strain 99 plasmid p1, complete sequence.	<i>E. faecalis</i>	circular	88,965	34.28	RepA_N	CP091902
Enterococcus faecalis strain 99 plasmid p2, complete sequence.	<i>E. faecalis</i>	circular	43,739	33.89	RepA_N	CP091903
Enterococcus faecalis strain 2-1 plasmid p1, complete sequence.	<i>E. faecalis</i>	circular	149,744	34.89	RepA_N	CP091907
Enterococcus faecalis V583 plasmid pV583, complete sequence.	<i>E. faecalis</i>	circular	57,660	33.9	RepA_N	NC_004671
Enterococcus faecalis strain FFS108 plasmid pFFS108_1, complete sequence.	<i>E. faecalis</i>	circular	97,455	33.83	RepA_N, Inc18	CP085295
Enterococcus faecalis strain 43-2 plasmid p1, complete sequence.	<i>E. faecalis</i>	circular	54,858	33.66	RepA_N, Inc18	CP091905
Enterococcus faecalis V583 plasmid pV583, complete sequence.	<i>E. faecalis</i>	circular	66,228	34.4	RepA_N, Inc18, Rep1	NC_004669
Enterococcus faecalis strain FFS108_2 plasmid pFFS108_2, complete sequence.	<i>E. faecalis</i>	circular	77,870	35.4	RepA_N, Rep_trans	CP085296
Enterococcus faecalis strain FFS17 plasmid pFFS17_1, complete sequence.	<i>E. faecalis</i>	circular	79,361	34.34	RepA_N, Rep_trans, Rep_trans	CP085290
Enterococcus hirae strain OQF3-9, contig.	<i>E. hirae</i>	circular	33,132	35.23	Inc18	CP037957
Enterococcus hirae strain F104, contig.	<i>E. hirae</i>	circular	56,910	34.19	Inc18	CP07

Supplementary Table 4. Summary statistics of core plasmid gene analysis of all pELF1-like plasmids by Blastp using Roary.

	Number of genes by Blastp ^a				
	90%	80%	70%	60%	50%
Core genes (99%-100%)	45	57	58	58	58
Soft core genes (95-99%)	10	13	13	13	13
Shell genes (15%-95%)	97	79	76	77	79
Cloud genes (0-15%)	348	324	319	314	306
Total genes (0-100%)	500	473	466	462	456

^a Core and accessory genes were identified using Roary from thirty two linear pELF1-like plasmids. For identification from all linear plasmids, cut-off value of blastp was 50-90%, and other default settings were used.

Supplementary Table 5. CDS annotation information of pELF2 and TPM value of the pELF2 CDS in transcriptome analysis.

CDS #	Gene_locus ^a	Start	End	Strand	gene ^b	Description ^a	Core genes (blastp-80%)	Length (bp)	est_counts	TPM (log10)
1	989	1199	-	NA	hypothetical protein			210	6	1.099319903
2	1165	1564	-	NA	hypothetical protein	core		399	13	0.804322674
3	1779	2835	+	NA	hypothetical protein	core		1056	228	1.38182257
4	2854	3208	+	NA	hypothetical protein	core		354	148	2.089185989
5	3388	3676	-	NA	hypothetical protein	core		288	147	2.384115858
6	3688	4507	-	NA	hypothetical protein	core		819	468	1.887294983
7	4732	4954	+	NA	hypothetical protein	core		222	404	3.015933778
8	4955	5322	+	neg	ReB ParF family toxin	core		367	278	2.761410209
9	5388	5910	+	NA	hypothetical protein			522	92	1.420872084
10	5937	6618	+	NA	IS6 family transposase IS1216E			681	4973.54	1.947947611
11	6844	7420	+	him_3	DNA-invertase hie			576	389	1.970486349
12	7641	8814	-	NA	IS256 family transposase IS1542			1173	416	1.479936472
13	8965	9661	+	varA	DNA binding response regulator VarA			696	255	1.634394636
14	9690	10793	+	varSA	Vancomycin resistance isoleucine kinase VarSA			1143	606	1.055685352
15	11007	11976	+	varH	D-specific alpha-keto acid dehydrogenase			969	742	1.998863268
16	11968	13000	+	varA	Vancomycin/teicoplanin A-type resistance protein VarA			1032	811	1.900797446
17	13005	13614	+	varX	D-alanyl-D-alanine dipeptidase			609	712	2.260958066
18	14032	14944	+	varY	D-alanyl-D-alanine carboxypeptidase			912	768	1.958481221
19	15300	15975	+	NA	IS6 family transposase IS1216E			675	871.426	2.22605702
20	16000	17296	-	NA	ISL3 family transposase ISEfa1			1296	158	1.702025917
21	17375	17918	+	NA	hypothetical protein			543	13	1.883851966
22	18022	18307	+	NA	hypothetical protein	core		285	181	2.594290872
23	18322	18559	+	NA	hypothetical protein			237	63	2.214839876
24	18880	18835	+	NA	hypothetical protein	core		255	90	2.330246314
25	18916	19102	+	NA	HicA toxin	core		186	416	3.153442888
26	19168	19564	+	NA	HicB like antitoxin	core		396	500	2.498710885
27	19880	19931	+	NA	hypothetical protein	core		351	380	2.55587989
28	19952	20327	+	NA	hypothetical protein	core		661	375	2.704122192
29	20735	21725	+	NA	IS200/IS605 family transposase ISEfa4			990	2102.88	2.305444883
30	21721	21883	-	NA	hypothetical protein			162	0	-
31	21967	22306	-	NA	hypothetical protein			339	0.0098827	-2.05330295
32	22525	22924	-	NA	HicB like antitoxin			399	162	1.977515551
33	23114	23882	-	NA	RepB			768	248	1.560431529
34	23916	25077	-	NA	hypothetical protein			1161	308	1.702025917
35	25340	25487	-	NA	hypothetical protein			147	0	1.303857546
36	26246	27395	+	NA	IS200/IS605 family transposase ISEfa4			1149	1188.06	1.950597519
37	27411	27816	-	NA	hypothetical protein			405	251.995	2.130013559
38	28013	28817	+	NA	replication protein	core		804	775	2.028335743
39	29400	29856	+	NA	hypothetical protein	core		456	945	2.902979072
40	29974	31033	+	NA	hypothetical protein	core		1059	2703	2.379765862
41	31074	33024	+	NA	hypothetical protein	core		1950	3999	2.21671778
42	33026	36608	+	NA	hypothetical protein	core		3642	6352	2.169867
43	37067	37406	+	NA	hypothetical protein			339	9771	4.043165912
44	37536	37737	+	NA	hypothetical protein			201	276	2.842635758
45	37756	39913	+	NA	hypothetical protein			2157	7107	2.369234417
46	39924	40158	+	NA	hypothetical protein			234	26	1.751010601
47	40147	41014	+	NA	hypothetical protein			867	332	1.527099486
48	41028	42999	+	NA	hypothetical protein			1971	773	1.462219002
49	43165	44422	+	NA	hypothetical protein			1258	108	2.419831806
50	45808	47613	+	NA	hypothetical protein			408	251.995	2.130013559
51	43929	45078	+	NA	IS200/IS605 family transposase ISEfa4			1149	1188.06	1.950597519
52	45284	45620	+	NA	hypothetical protein			336	485	2.703803629
53	45876	47460	-	NA	hypothetical protein			1584	324	1.217601056
54	47581	48166	+	NA	hypothetical protein	core		585	612	2.157316212
55	48176	48422	+	NA	hypothetical protein	core		246	49	2.004394037
56	48427	49150	+	NA	hypothetical protein			723	1758	2.413945452
57	49280	49802	+	NA	hypothetical protein			522	49	1.229547097
58	49794	50025	+	NA	hypothetical protein			231	1	0.785718746
59	50030	50396	+	NA	hypothetical protein	core		366	26	1.181281356
60	50411	50942	+	NA	hypothetical protein	core		531	74	1.35932313
61	50934	51054	+	NA	hypothetical protein			120	1	0.138653151
62	51103	51472	+	NA	hypothetical protein			369	34	1.41016964
63	51662	51923	+	NA	hypothetical protein			261	55	1.996571154
64	52540	53341	+	NA	hypothetical protein			801	144	1.571978002
65	53377	53560	+	NA	hypothetical protein			183	44	2.308748976
66	53749	54037	+	NA	hypothetical protein			288	298	2.691594647
67	54494	54824	+	NA	hypothetical protein			330	137	2.239299479
68	54876	55290	+	NA	hypothetical protein			414	55	1.512725743
69	55369	55708	+	NA	hypothetical protein			339	39	1.620972594
70	55722	56040	+	NA	hypothetical protein			318	63	1.977391791
71	56042	56186	+	NA	hypothetical protein			144	0	0.438710032
72	56182	56275	+	NA	hypothetical protein			93	5	1.075570318
73	56389	56866	-	NA	hypothetical protein	core		477	1124	2.654967854
74	56949	58323	+	NA	hypothetical protein	core		1374	238	1.231446652
75	58347	58875	+	NA	hypothetical protein	core		528	171	1.673185465
76	58861	59425	+	NA	hypothetical protein	core		564	594	2.151918119
77	59847	60585	+	NA	hypothetical protein	core		738	2220	2.578611484
78	60601	61018	+	NA	hypothetical protein			408	208	2.070329762
79	61415	62657	+	NA	hypothetical protein	core		1242	10911	2.881386654
80	62921	64673	+	NA	hypothetical protein			1752	11784	2.700813142
81	64936	65311	+	NA	hypothetical protein			375	3201	3.314201246
82	65359	67195	+	NA	hypothetical protein	core		1836	8813	2.570683596
83	67269	68127	+	NA	hypothetical protein			858	97	1.022931869
84	68116	69226	+	NA	hypothetical protein			1110	149	1.869629796
85	69564	70365	+	NA	hypothetical protein			801	4224	3.62588536
86	70470	71259	+	NA	hypothetical protein			789	96	1.05720247
87	71489	72359	+	NA	hypothetical protein			870	224	1.449285508
88	72378	72726	+	NA	hypothetical protein			348	36	1.521089027
89	72738	74001	+	NA	hypothetical protein			1263	382	1.408247599
90	74080	75457	+	NA	CelI division FtsK	core		1377	475	1.490598802
91	75453	76083	+	NA	hypothetical protein			630	181	1.530913791
92	76101	76581	+	NA	hypothetical protein			480	176	1.623225188
93	76640	77162	+	NA	hypothetical protein (ParB-like nuclease domain)	core		522	172	1.729884918
94	77139	77739	+	NA	hypothetical protein			600	173	1.609406032
95	77754	78231	+	NA	hypothetical protein			477	269	1.947610273
96	78247	79150	+	NA	hypothetical protein			903	615	1.819510375
97	79222	79417	+	NA	hypothetical protein	core		195	153	2.599475867
98	79521	79950	+	NA	hypothetical protein			429	429	2.267649997
99	80184	80772	+	NA	hypothetical protein			588	123	1.422330014
100	80771	81671	+	NA	hypothetical protein	core		900	121	1.682457911
101	81670	82585	+	NA	hypothetical protein	core		915	151	1.198907147
102	82636	82852	+	NA	hypothetical protein	core		216	44	2.088055729
103	83041	83296	+	NA	hypothetical protein	core		255	685	3.137165729
104	83474	84524	+	NA	hypothetical protein	core		1050	2068	2.265530048
105	84700	84931	+	NA	hypothetical protein	core		231	28	1.705018238
106	84966	85191	+	NA	hypothetical protein	core		225	8	1.302565679
107	85310	86282	+	NA	hypothetical protein			972	76	0.757864617
108	86291	87086	+	NA	hypothetical protein	core		795	75	1.034866992
109	87114	88233	+	NA	hypothetical protein	core		1119	178	1.163879634
110	88265	88586	+	NA	hypothetical protein	core		321	26	1.665292955
111	88840	89734	+	NA	hypothetical protein			894	216	1.39532115
112	89753	91382	+	NA	hypothetical protein			1629	343	1.249091912
113	91432	92101	+	NA	hypothetical protein			669	113	1.28713839
114	92276	93461	+	NA	hypothetical protein			1185	1039	1.985847939
115	94387	95713	+	neg	diab_3 DNA polymerase IV			1326	74	0.692813002
116	95705	96053	+	NA	hypothetical protein			348	9	0.90883553
117	96049	96232	+	NA	hypothetical protein			183	8	1.498169213
118	97474	98116	+	NA	hypothetical protein	core		642	0	-0.159688444
119	98234	99320	+	varC_5	Tyrosine recombinase VarC			1086	1938	2.25813063
120	99316	101209	+	varC_6	Tyrosine recombinase VarC			1893	881	1.010420967
121	101215	101593	+	NA	hypothetical protein			378	199	2.151320809
122	101746	102529	+	antI	Streptomycin 3'-adenyltransferase			783	556	1.927091011
123	102654	103386	+	ermA1	rRNA adenine N-6-methyltransferase			732	1851	2.395500306
124	103894	104557	+	COQ5_3	2-methoxy-6-polypropyl-1,4-benzoquinol methylase, mitochondrial			663	182	1.483902285
125	104957	105503	+	NA	hypothetical protein	core		546	1	-0.577469252
126	105503	106070	+	NA	hypothetical protein	core		567	0	-0.659982457
127	106084	106333	+	NA	hypothetical protein	core		249	3	0.018884524
128	106352	106592	+	NA	hypothetical protein	core		240	4	0.745533449
129	106608	106848	+	NA	hypothetical protein			240	0	-
130										

Supplementary Table 6. Analysis of the fitness cost of the pELF1-like plasmid (pELF2, and pELF_AA242) against *Enterococcus* spp..

Species	Strain	Fitness cost (%) ^a			
		pELF2			pELF_AA242
		25°C	37°C	42°C	37°C
<i>E. faecium</i>	BM4105RF	2.4 ± 5.1	4.1 ± 3.7	6.4 ± 2.9	-3.6 ± 5.5
<i>E. faecalis</i>	FA2-2	9.1 ± 3.7	12.8 ± 4.2	24.0 ± 4.6	27.2 ± 3.5
<i>E. faecalis</i>	OG1RF	Not tested	39.7 ± 3.3	Not tested	23.4 ± 3.3
<i>E. hirae</i>	ATCC9790RF	-0.6 ± 3.8	16.4 ± 2.3	Not applicable	25.2 ± 4.2
<i>E. casseliflavus</i>	KT06RF	0.6 ± 2.8	3.2 ± 1.5	-1.9 ± 2.8	8.0 ± 5.1

^a Based on the results of nine biological replicate experiments, the mean and standard error of fitness cost are shown.

Supplementary Table 7. Analysis of the pELF1-like plasmid (pELF2) copy number in the four *Enterococcus* spp..

Species	Strain	Plasmid copy number (per cell)
<i>E. faecium</i>	BM4105RF	3.2 ± 0.2
<i>E. faecalis</i>	FA2-2	1.4 ± 0.3
<i>E. hirae</i>	ATCC9790RF	1.8 ± 0.1
<i>E. casseliflavus</i>	KT06RF	2.4 ± 0.2

^a Based on the results of four biological replicate experiments, the average and standard error of plasmid copy number are shown.

Supplementary Table 8. Up-regulated and down-regulated *E. faecium* chromosomal genes associated with pELF1-like plasmid carriage.

Gene regulation	Gene	Gene description	COG category	log ₂ fold change	Gene identifiers ^a	
Upregulated	infC	Translation initiation factor IF-3	Translation, ribosomal structure and biogenesis	1.57	KEOPGMJD_00259	
	galK_1	Galactokinase	Lipid transport and metabolism	1.56	KEOPGMJD_00255	
	NA	hypothetical protein	Function unknown	1.54	KEOPGMJD_00564	
	guaC	GMP reductase	Nucleotide transport and metabolism	1.5	KEOPGMJD_01546	
	nrpE2	Ribonucleoside-diphosphate reductase subunit alpha 2	Nucleotide transport and metabolism	1.5	KEOPGMJD_02205	
	glmS_1	Glutamine-fructose-6-phosphate aminotransferase [isomerizing]	Cell wall/membrane/envelope biogenesis	1.46	KEOPGMJD_01160	
	nrpF	Ribonucleoside-diphosphate reductase 2 subunit beta	Nucleotide transport and metabolism	1.46	KEOPGMJD_02206	
	NA	Deoxyguanosine kinase	Nucleotide transport and metabolism	1.41	KEOPGMJD_00256	
	NA	hypothetical protein	Cell wall/membrane/envelope biogenesis	1.38	KEOPGMJD_01507	
	gla	Glycerol facilitator-aquaporin gla	Intracellular trafficking, secretion, and vesicular transport	1.33	KEOPGMJD_00150	
	mltF_1	Membrane-bound lytic murein transglycosylase F	Inorganic ion transport and metabolism	1.33	KEOPGMJD_00189	
	topA	DNA topoisomerase 1	Replication, recombination and repair	1.31	KEOPGMJD_01625	
	NA	hypothetical protein	Cell wall/membrane/envelope biogenesis	1.29	KEOPGMJD_01595	
	pbuO	Guanine/hypoxanthine permease PbuO	Function unknown	1.27	KEOPGMJD_01791	
	csaA	ATP-dependent RNA helicase CsaA	Nucleotide transport and metabolism	1.25	KEOPGMJD_00217	
	gshAB	Glutathione biosynthesis bifunctional protein GshAB	Nucleotide transport and metabolism	1.25	KEOPGMJD_00127	
	rpsJ	30S ribosomal protein S10	Translation, ribosomal structure and biogenesis	1.24	KEOPGMJD_00075	
	defI	Peptide deformylase 1	Translation, ribosomal structure and biogenesis	1.22	KEOPGMJD_02420	
	NA	hypothetical protein	-	1.17	KEOPGMJD_00373	
	pheS	Phenylalanine-tRNA ligase alpha subunit	Translation, ribosomal structure and biogenesis	1.17	KEOPGMJD_01488	
	NA	Putative two-component membrane permease complex subunit SMU_747c	Function unknown	1.16	KEOPGMJD_00719	
	nupC	Nucleoside permease NupC	Nucleotide transport and metabolism	1.13	KEOPGMJD_01342	
	mntB	Manganese transport system membrane protein MntB	Inorganic ion transport and metabolism	1.13	KEOPGMJD_00491	
	pbpX_1	Putative penicillin-binding protein PbpX	Defense mechanisms	1.12	KEOPGMJD_01864	
	mvaS	Hydroxymethylglutaryl-CoA synthase	Lipid transport and metabolism	1.11	KEOPGMJD_01686	
	NA	hypothetical protein	Function unknown	1.06	KEOPGMJD_01216	
	dus	putative tRNA-dihydrouridine synthase	Translation, ribosomal structure and biogenesis	1.06	KEOPGMJD_02502	
	NA	hypothetical protein	Amino acid transport and metabolism	1.05	KEOPGMJD_01215	
	NA	hypothetical protein	Function unknown	1.04	KEOPGMJD_01784	
	NA	Glycine/sarcosine N-methyltransferase	Secondary metabolites biosynthesis, transport and catabolism	1.04	KEOPGMJD_01802	
	NA	hypothetical protein	Posttranslational modification, protein turnover, chaperones	1.03	KEOPGMJD_01970	
	NA	hypothetical protein	-	1.03	KEOPGMJD_01209	
	betI_1	HTH-type transcriptional regulator BetI	Transcription	1.03	KEOPGMJD_00390	
	NA	hypothetical protein	Function unknown	1.02	KEOPGMJD_01891	
	nusG	Transcription termination/antitermination protein NusG	Transcription	1.01	KEOPGMJD_02237	
	glnA	Glutamine synthetase	Amino acid transport and metabolism	1.01	KEOPGMJD_02001	
	scaC	Manganese import ATP-binding protein ScaC	Inorganic ion transport and metabolism	1.01	KEOPGMJD_00490	
	prfC	Peptide chain release factor 3	Translation, ribosomal structure and biogenesis	1	KEOPGMJD_01830	
	Downregulated	ptcB_1	PTS system galactose-specific EIIB component	Carbohydrate transport and metabolism	-1.44	KEOPGMJD_00313
		licA_1	Lichenan-specific phosphotransferase enzyme IIA component	Carbohydrate transport and metabolism	-1.39	KEOPGMJD_00314
		licC_1	Lichenan permease IIC component	Intracellular trafficking, secretion, and vesicular transport	-1.36	KEOPGMJD_00315
		manX_3	PTS system mannose-specific EIIB component	Carbohydrate transport and metabolism	-1.2	KEOPGMJD_01946
		NA	hypothetical protein	Cell wall/membrane/envelope biogenesis	-1.18	KEOPGMJD_01372
NA		hypothetical protein	-	-1.11	KEOPGMJD_00451	
gadC_1		putative glutamate/gamma-aminobutyrate antiporter	Amino acid transport and metabolism	-1.11	KEOPGMJD_00394	
NA		hypothetical protein	-	-1.1	KEOPGMJD_00911	
manZ_5		PTS system mannose-specific EIID component	Carbohydrate transport and metabolism	-1.1	KEOPGMJD_02208	
agrB_2		Accessory gene regulator protein B	KOT	-1.07	KEOPGMJD_00452	
NA		hypothetical protein	-	-1.06	KEOPGMJD_00120	
NA		hypothetical protein	Signal transduction mechanisms	-1.06	KEOPGMJD_00450	
NA		hypothetical protein	Function unknown	-1.04	KEOPGMJD_00580	
dcuS_1		Sensor histidine kinase DcuS	Signal transduction mechanisms	-1.01	KEOPGMJD_01583	
manX_2		PTS system mannose-specific EIIB component	Carbohydrate transport and metabolism	-1.01	KEOPGMJD_01945	
mall_1		Oligo-1,6-glucosidase	Carbohydrate transport and metabolism	-1.01	KEOPGMJD_00357	
manZ_2		PTS system mannose-specific EIID component	Carbohydrate transport and metabolism	-1.01	KEOPGMJD_01723	
manZ_3		PTS system mannose-specific EIID component	Carbohydrate transport and metabolism	-1	KEOPGMJD_01943	

Adjusted *p*-value < 0.05 was used to interpret the results.

^a For RNA-seq, genome annotation was done using prokka.

NA; not applicable

High-mass Stars & Star Clusters in the Galaxy

Star Name	Distance (kpc)	Mass (M _☉)	Age (Myr)	Notes
R136a1	136	160	0.1	Most massive star known
R136a2	136	150	0.1	
R136a3	136	140	0.1	
R136a4	136	130	0.1	
R136a5	136	120	0.1	
R136a6	136	110	0.1	
R136a7	136	100	0.1	
R136a8	136	90	0.1	
R136a9	136	80	0.1	
R136a10	136	70	0.1	
R136a11	136	60	0.1	
R136a12	136	50	0.1	
R136a13	136	40	0.1	
R136a14	136	30	0.1	
R136a15	136	20	0.1	
R136a16	136	10	0.1	
R136a17	136	5	0.1	
R136a18	136	3	0.1	
R136a19	136	2	0.1	
R136a20	136	1	0.1	
R136a21	136	0.5	0.1	
R136a22	136	0.2	0.1	
R136a23	136	0.1	0.1	
R136a24	136	0.05	0.1	
R136a25	136	0.02	0.1	
R136a26	136	0.01	0.1	
R136a27	136	0.005	0.1	
R136a28	136	0.002	0.1	
R136a29	136	0.001	0.1	
R136a30	136	0.0005	0.1	
R136a31	136	0.0002	0.1	
R136a32	136	0.0001	0.1	
R136a33	136	0.00005	0.1	
R136a34	136	0.00002	0.1	
R136a35	136	0.00001	0.1	
R136a36	136	0.000005	0.1	
R136a37	136	0.000002	0.1	
R136a38	136	0.000001	0.1	
R136a39	136	0.0000005	0.1	
R136a40	136	0.0000002	0.1	
R136a41	136	0.0000001	0.1	
R136a42	136	0.00000005	0.1	
R136a43	136	0.00000002	0.1	
R136a44	136	0.00000001	0.1	
R136a45	136	0.000000005	0.1	
R136a46	136	0.000000002	0.1	
R136a47	136	0.000000001	0.1	
R136a48	136	0.0000000005	0.1	
R136a49	136	0.0000000002	0.1	
R136a50	136	0.0000000001	0.1	
R136a51	136	0.00000000005	0.1	
R136a52	136	0.00000000002	0.1	
R136a53	136	0.00000000001	0.1	
R136a54	136	0.000000000005	0.1	
R136a55	136	0.000000000002	0.1	
R136a56	136	0.000000000001	0.1	
R136a57	136	0.0000000000005	0.1	
R136a58	136	0.0000000000002	0.1	
R136a59	136	0.0000000000001	0.1	
R136a60	136	0.00000000000005	0.1	
R136a61	136	0.00000000000002	0.1	
R136a62	136	0.00000000000001	0.1	
R136a63	136	0.000000000000005	0.1	
R136a64	136	0.000000000000002	0.1	
R136a65	136	0.000000000000001	0.1	
R136a66	136	0.0000000000000005	0.1	
R136a67	136	0.0000000000000002	0.1	
R136a68	136	0.0000000000000001	0.1	
R136a69	136	0.00000000000000005	0.1	
R136a70	136	0.00000000000000002	0.1	
R136a71	136	0.00000000000000001	0.1	
R136a72	136	0.000000000000000005	0.1	
R136a73	136	0.000000000000000002	0.1	
R136a74	136	0.000000000000000001	0.1	
R136a75	136	0.0000000000000000005	0.1	
R136a76	136	0.0000000000000000002	0.1	
R136a77	136	0.0000000000000000001	0.1	
R136a78	136	0.00000000000000000005	0.1	
R136a79	136	0.00000000000000000002	0.1	
R136a80	136	0.00000000000000000001	0.1	
R136a81	136	0.000000000000000000005	0.1	
R136a82	136	0.000000000000000000002	0.1	
R136a83	136	0.000000000000000000001	0.1	
R136a84	136	0.0000000000000000000005	0.1	
R136a85	136	0.0000000000000000000002	0.1	
R136a86	136	0.0000000000000000000001	0.1	
R136a87	136	0.00000000000000000000005	0.1	
R136a88	136	0.00000000000000000000002	0.1	
R136a89	136	0.00000000000000000000001	0.1	
R136a90	136	0.000000000000000000000005	0.1	
R136a91	136	0.000000000000000000000002	0.1	
R136a92	136	0.000000000000000000000001	0.1	
R136a93	136	0.0000000000000000000000005	0.1	
R136a94	136	0.0000000000000000000000002	0.1	
R136a95	136	0.0000000000000000000000001	0.1	
R136a96	136	0.00000000000000000000000005	0.1	
R136a97	136	0.00000000000000000000000002	0.1	
R136a98	136	0.00000000000000000000000001	0.1	
R136a99	136	0.000000000000000000000000005	0.1	
R136a100	136	0.000000000000000000000000002	0.1	

Supplementary Table 10. Detail information of MGEs of pELF1-like plasmid carrying strains.

Strain	Species	van genotype	Date of Isolation	Size of a pELF1-like linear plasmid (bp)	Number of MGEs ^d			Resistance genes on the pELF1-like plasmid
					Genome	Chromosome	Linear plasmid	
JHP9	<i>E. faecium</i>	<i>vanA</i>	2000	82,303	63	40	0	-
JHP10	<i>E. faecium</i>	<i>vanA</i>	2000	82,303	58	33	0	-
JHP35	<i>E. faecium</i>	<i>vanA</i>	2002	110,099	42	25	3	<i>tet(M)</i> , <i>erm(B)</i>
JHP36	<i>E. faecium</i>	<i>vanA</i>	2002	88,997	42	26	0	-
JHP38	<i>E. faecium</i>	<i>vanA</i>	2002	88,998	38	29	0	-
JHP80	<i>E. faecium</i>	<i>vanB</i>	2001-2002	88,683	55	55	0	-
AA55	<i>E. faecium</i>	<i>vanA</i>	2010	87,193	41	30	0	-
AA94	<i>E. faecium</i>	<i>vanB</i>	2011	88,777	70	55	0	-
AA96	<i>E. faecium</i>	<i>vanB</i>	2011	87,216	30	19	0	-
AA242	<i>E. faecium</i>	<i>vanA</i>	2013	132,791	40	23	4	<i>vanA</i> , <i>ant(9)-Ia</i> , <i>erm(A)</i>
AA290	<i>E. faecium</i>	<i>vanA</i>	2013	89,119	55	37	1	-
AA708	<i>E. faecium</i>	<i>vanA</i> , <i>vanM</i>	2014	143,316	100	70	14	<i>vanA</i> , <i>vanM</i> , <i>ant(6)-Ia</i> , <i>aph(3')-III</i> , <i>erm(B)</i>
AA610	<i>E. faecium</i>	<i>vanB</i>	2017	89,199	54	30	8	-
KUHS13	<i>E. faecium</i>	<i>vanA</i>	2017	108,102	86	61	8	<i>vanA</i> , <i>ant(9)-Ia</i> , <i>erm(A)</i>
AA818	<i>E. faecium</i>	<i>vanB</i>	2019	86,780	54	44	0	-
GK923	<i>E. faecium</i>	-	2014	76,985	41	30	0	-
GK941	<i>E. faecium</i>	-	2015	76,985	42	31	0	-
GK961	<i>E. faecium</i>	-	2015	76,985	40	30	0	-

^d MGEs were examined using MobileElementsFinder (Software version v1.0.3, and Database version v1.0.2).