

Supplementary Information

A slight mismatch between a gene's codon usage and the cellular tRNA supply is beneficial

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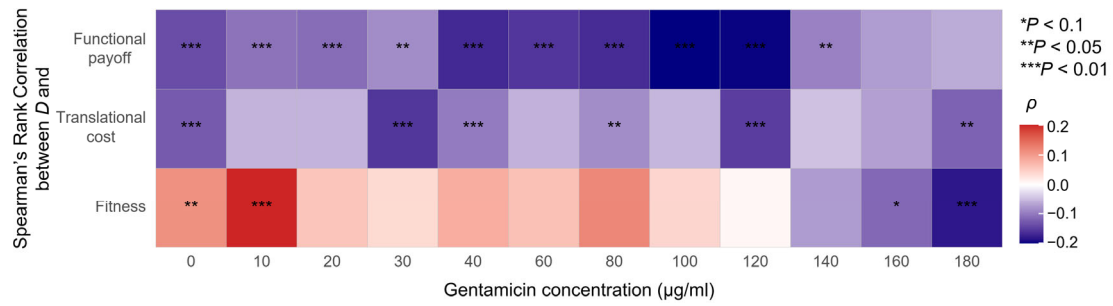
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These authors contributed equally to this work.

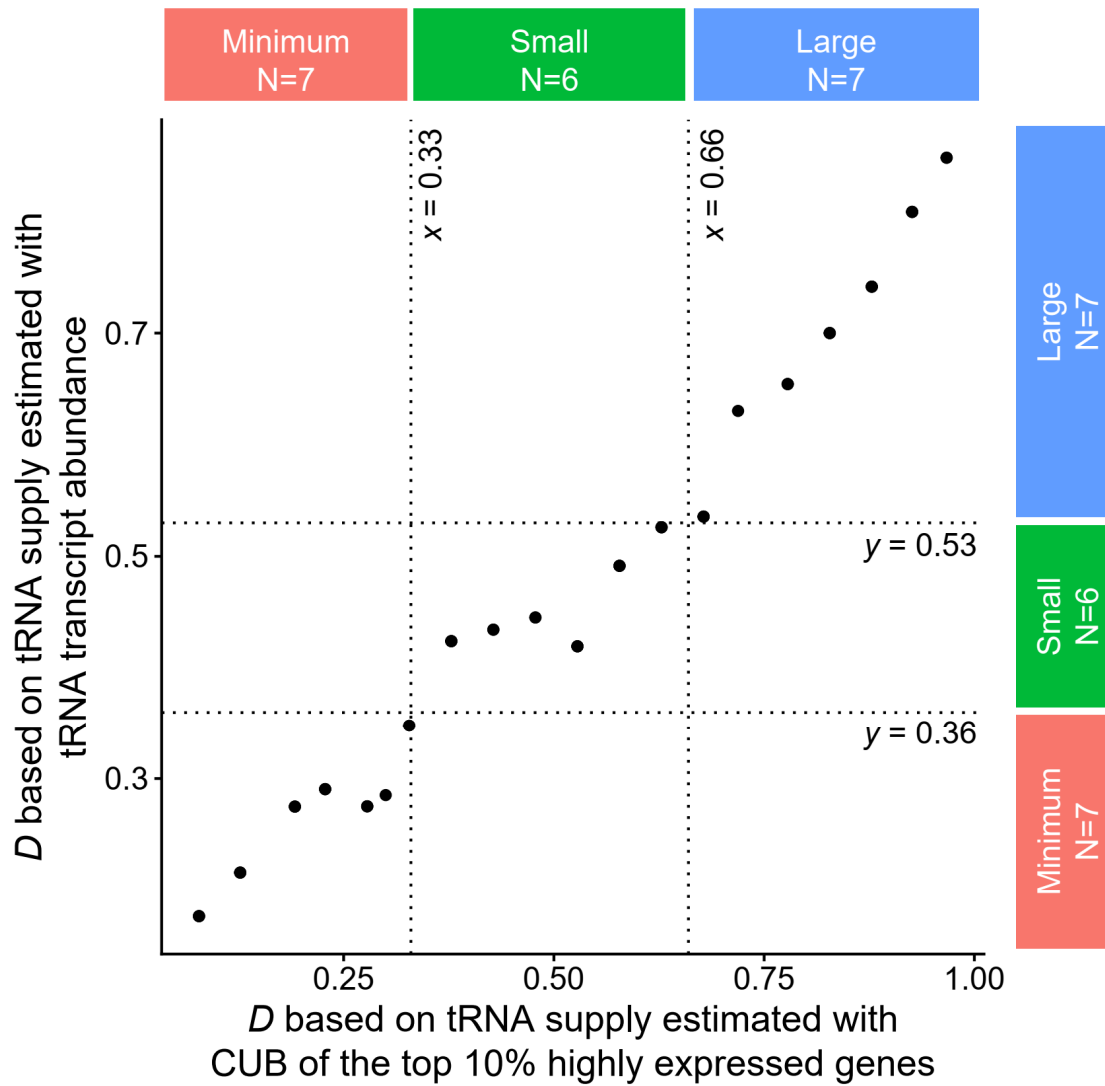
* Corresponding author:

E-mail: yangjianrong@mail.sysu.edu.cn (Yang JR)



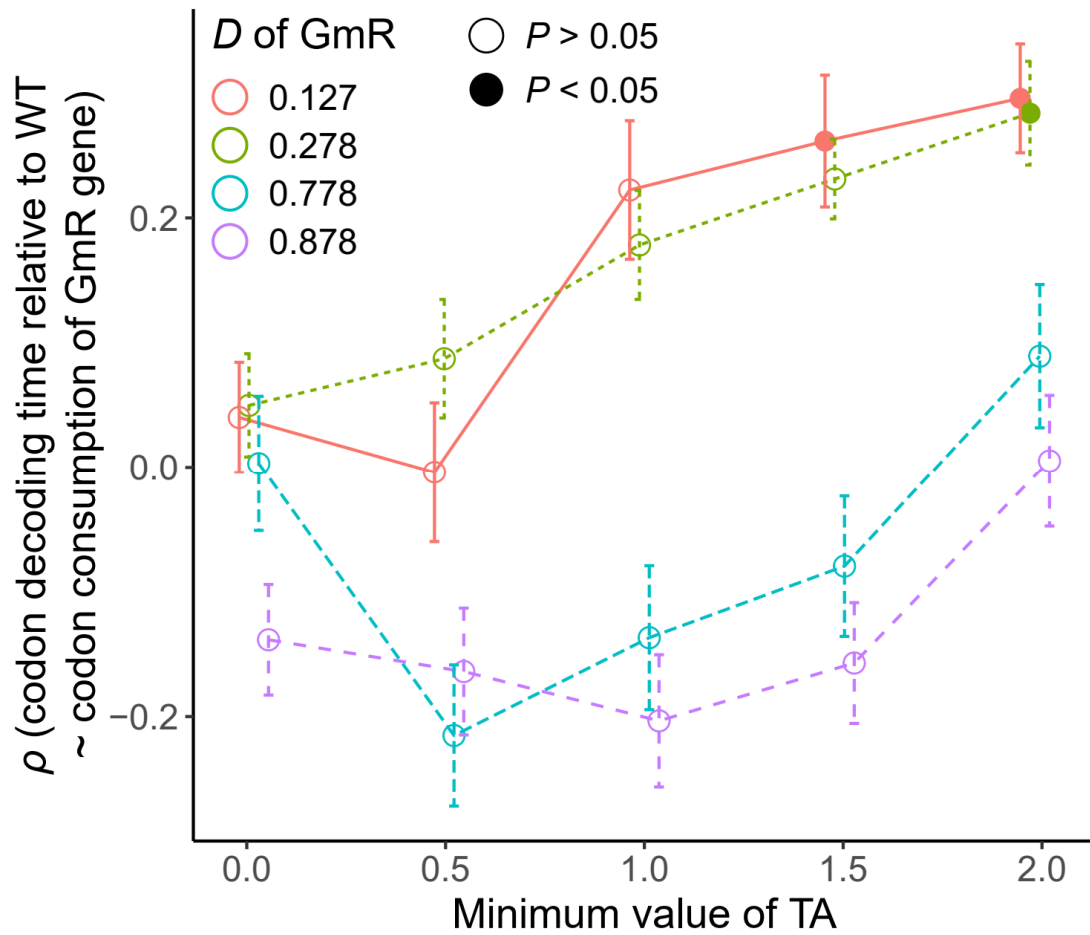
Supplementary Fig. 1. Correlations between D and functional payoff, translational cost or fitness

Using the same raw data as **Figure 3b, d** and **e**, the Spearman's Correlation Coefficient ρ (the color scale bar to the right) between D and the indicated metric (y axis) is calculated for data obtained in LB media supplemented with 0-180 $\mu\text{g/ml}$ gentamicin (x axis). The statistical significance for each tile is indicated as *: $P < 0.1$, **: $P < 0.05$, or ***: $P < 0.01$. The two-tailed Spearman's Correlation Coefficient ρ of the raw data (N=53), as well as the corresponding P value, are indicated. For each strain, we performed three biological and three technical replicates. Source data are provided as a Source Data file.



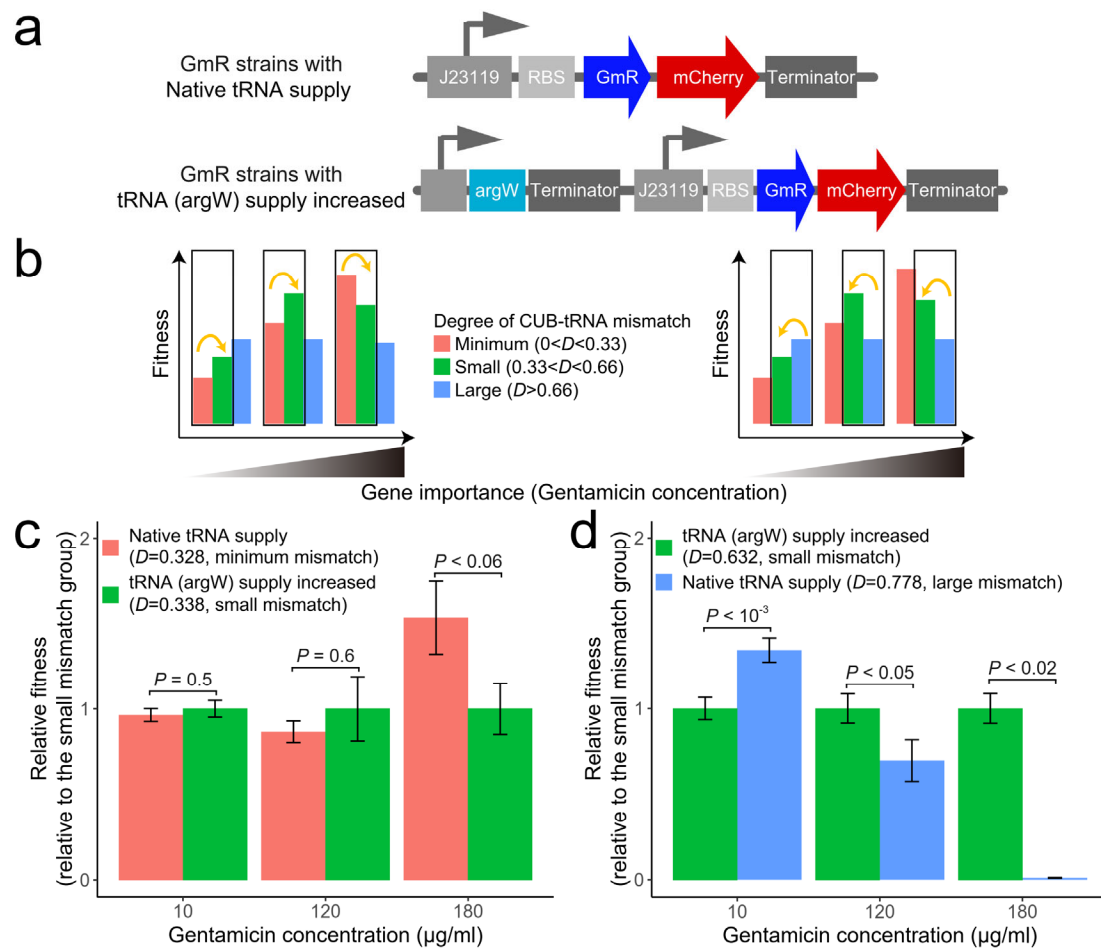
Supplementary Fig. 2. Grouping by D of *GmR* strains remains unchanged if high-throughput tRNA sequencing data are used to estimate tRNA supply

The D values of designed *GmR* synonymous variants were calculated with tRNA supply based on either transcriptomic codon usage of the top 10% highly expressed genes (x axis) or tRNA transcript abundance derived from high-throughput tRNA sequencing (y axis). The black lines indicate the grouping, which is identical for both types of D values. Please note that each dot has up to three unique sequences (see **Supplementary Data 1**). Source data are provided as a Source Data file.



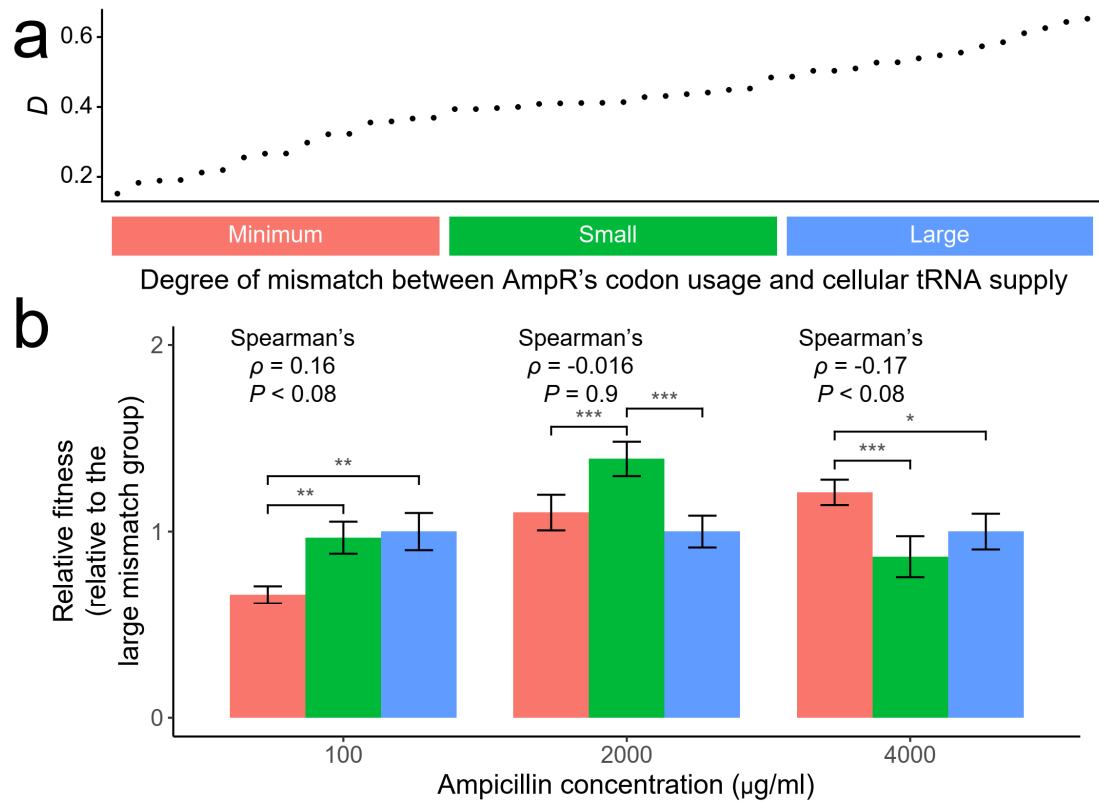
Supplementary Fig. 3. Slowed decoding as a result of *GmR* translation

The decoding time of each codon in endogenous *E. coli* genes was measured by Ribo-Seq in wildtype and four *E. coli* strains expressing *GmRs* of different *D* (see **Methods**). The two-tailed Spearman's correlation coefficient between *GmR*'s consumption of a codon and its relative decoding time as compared to the same codon in wildtype *E. coli* (*y* axis) is calculated for endogenous genes above a given minimum value of translational activity (*x* axis) (see **Methods**). The data are presented as mean $\pm$ standard deviation. Source data are provided as a Source Data file.



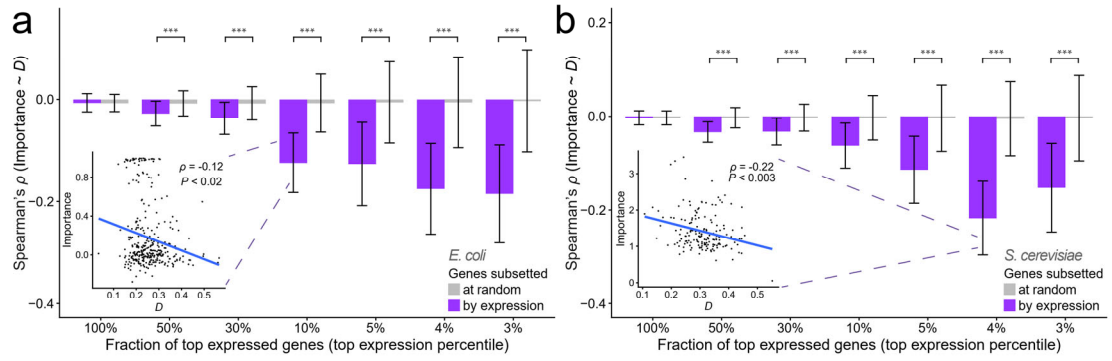
Supplementary Fig. 4. Fitness change upon manipulating tRNA supply

(a) A schematic illustration of the expression vector structure with (bottom) or without (top) the additional tRNA gene *argW*. (b) Our model's prediction for fitness changes following an increase in *argW* supply. This is essentially same as the bottom panel of **Figure 3a**. (c and d) The relative fitness of two *GmR* variants in cultures with 10, 120, and 180 $\mu\text{g/ml}$ gentamicin before and after *argW* tRNA overexpression, which resulted in D values change from 0.328 to 0.338 (c) and from 0.778 to 0.632 (d). The statistical significance of two-tailed Wilcoxon Rank Sum Tests are indicated. The rightmost bar in d indicates no observable growth for the native strain under 180 $\mu\text{g/ml}$ gentamicin. The data are presented as mean $\pm$ standard error. For each strain, we performed three biological and three technical replicates. Source data are provided as a Source Data file.



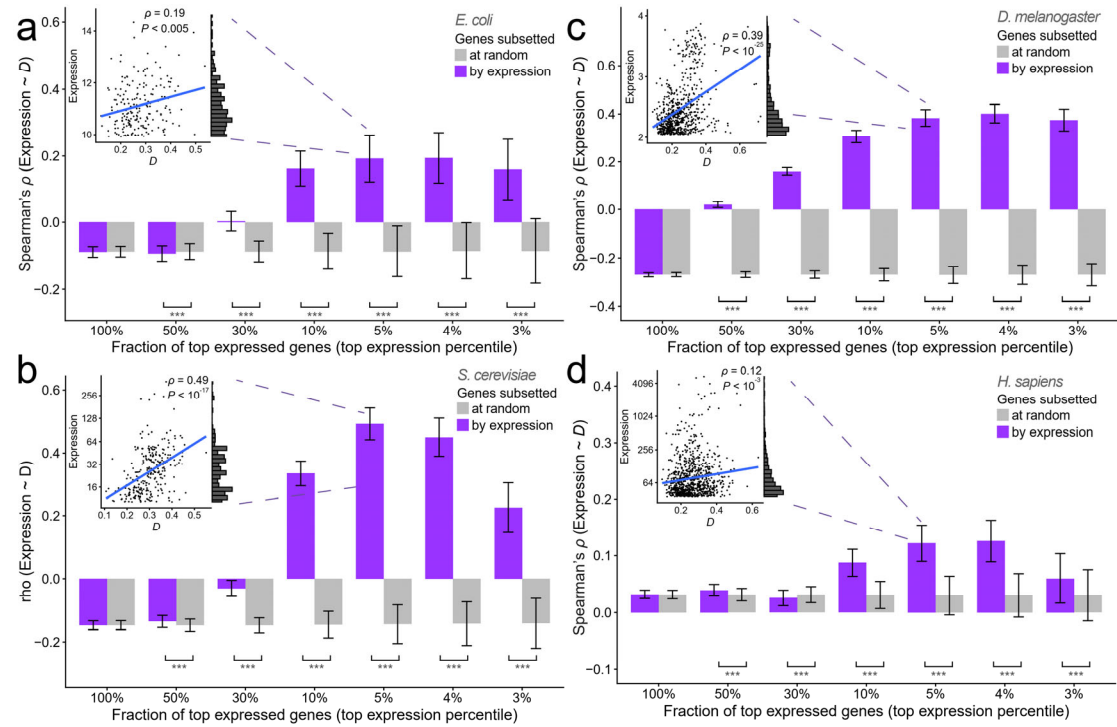
Supplementary Fig. 5. The optimal D for AmpR

(a) A total of 47 synonymous AmpR variants with different D values were designed, which were categorized into three equal-sized groups of minimum ($N=16$), small ($N=16$) and large ($N=15$) D . **(b)** The relative fitness of cells expressing different AmpR synonymous variants in LB media supplemented with 100, 2000, or 4000 µg/ml ampicillin. The statistical significance of two-tailed Wilcoxon Rank Sum Tests are indicated as *: $P < 0.1$, **: $P < 0.05$, or ***: $P < 0.01$. The data are presented as mean $\pm$ standard error. For each strain, we performed three biological and three technical replicates. Source data are provided as a Source Data file.



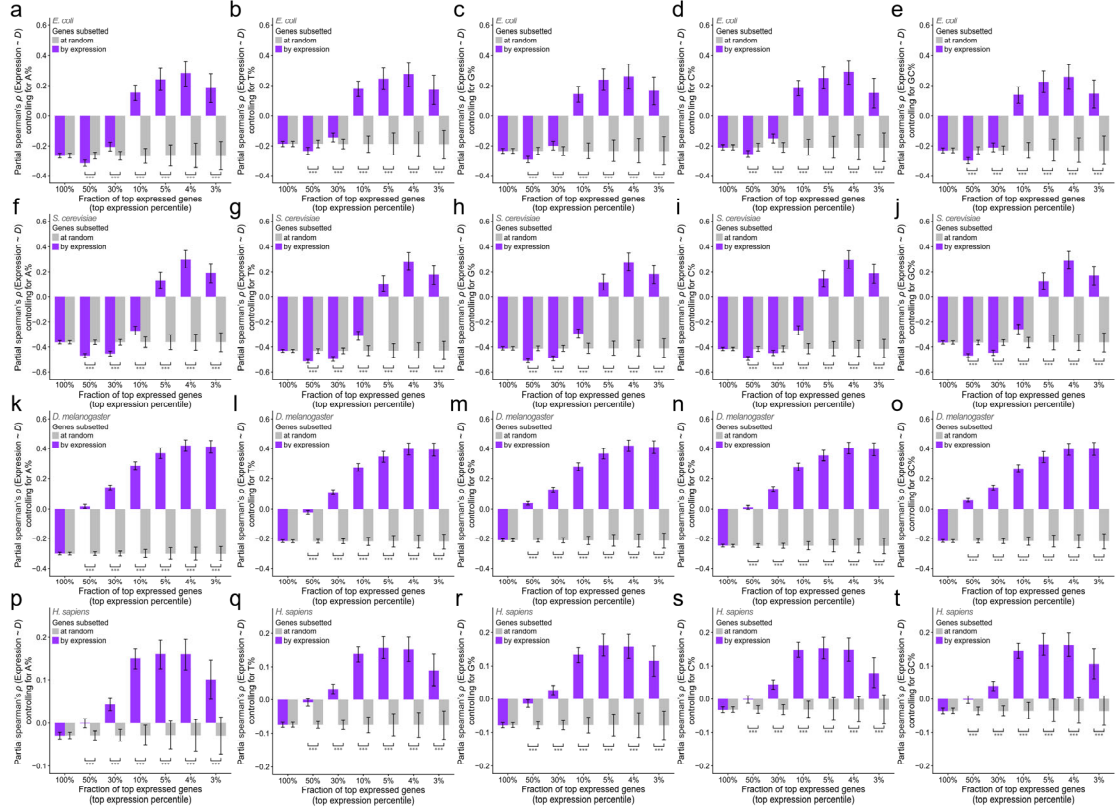
Supplementary Fig. 6. The effect of functional payoff on native codon usage of top expressed endogenous genes is recapitulated by tRNA expression-based D

These panels are similar to **Figure 4a-b**, except that the tRNA supply (X_{ij} in D) is estimated using tRNA expression level instead of the codon usage of the 10% most highly expressed genes. Source data are provided as a Source Data file.



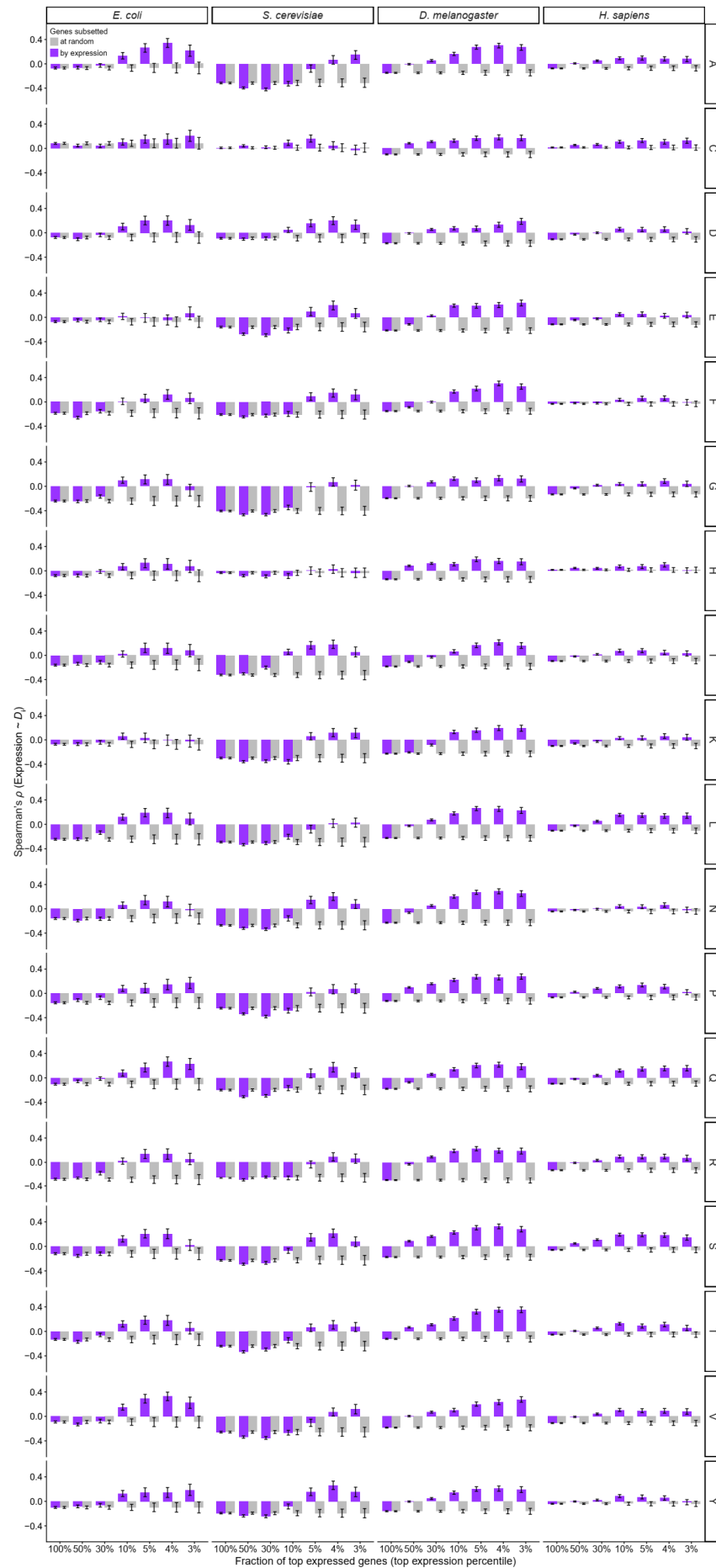
Supplementary Fig. 7. The correlation between D and expression for endogenous genes is recapitulated by tRNA expression-based D

These panels are similar to **Figure 5b-e**, except that the tRNA supply (X_{ij} in D) is estimated using tRNA expression level instead of the codon usage of the 10% most highly expressed genes. Source data are provided as a Source Data file.



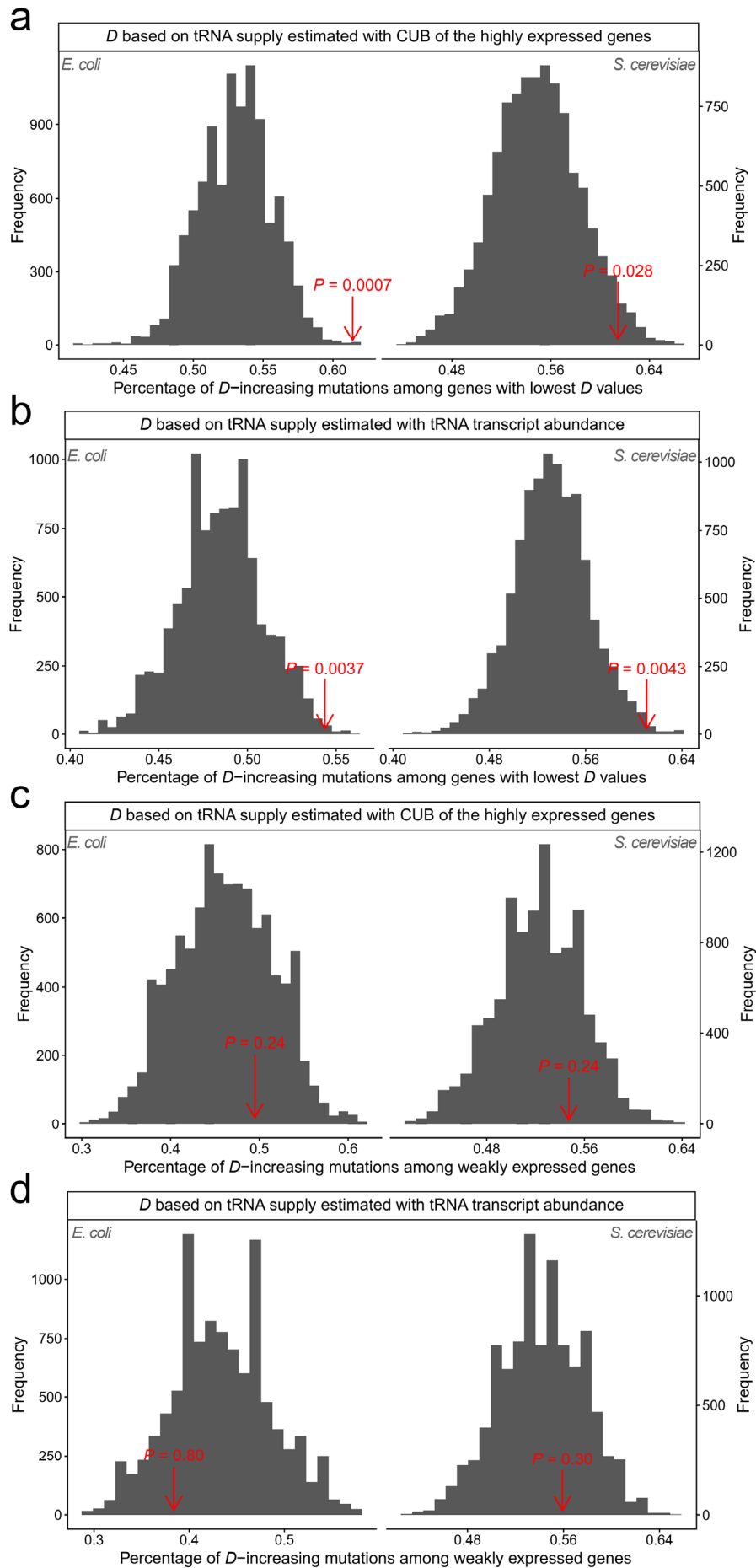
Supplementary Fig. 8. The correlation between D and expression for endogenous genes is not confounded by base composition bias

Each column of panels is similar to **Figure 5b-e**, except that the Spearman's correlation in **Figure 5b-e** is replaced with partial Spearman's correlation controlling for the percentage of Adenine (A%, in panels **a/f/k/p**), Thymine (T%, in panels **b/g/l/q**), Guanine (G%, in panels **c/h/m/r**), Cytosine (C%, in panels **d/i/n/s**) or G+C% (in panels **e/j/o/t**) in the sequences. Source data are provided as a Source Data file.



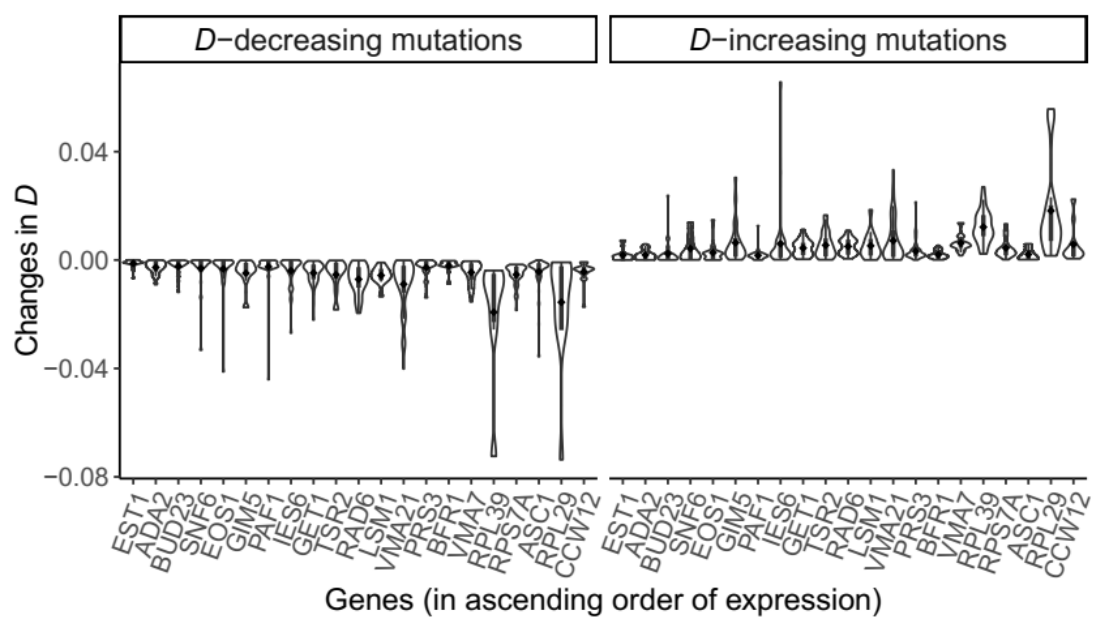
Supplementary Fig. 9. The correlation between D and expression for endogenous genes is consistent across amino acids

Each column of panels is similar to **Figure 5b-e**, except that D is replaced by D_i , i.e. the mismatch between synonymous codon usage and tRNA supply within one amino acid. There are therefore 18 columns, each for one amino acid encoded by >1 synonymous codons. Source data are provided as a Source Data file.



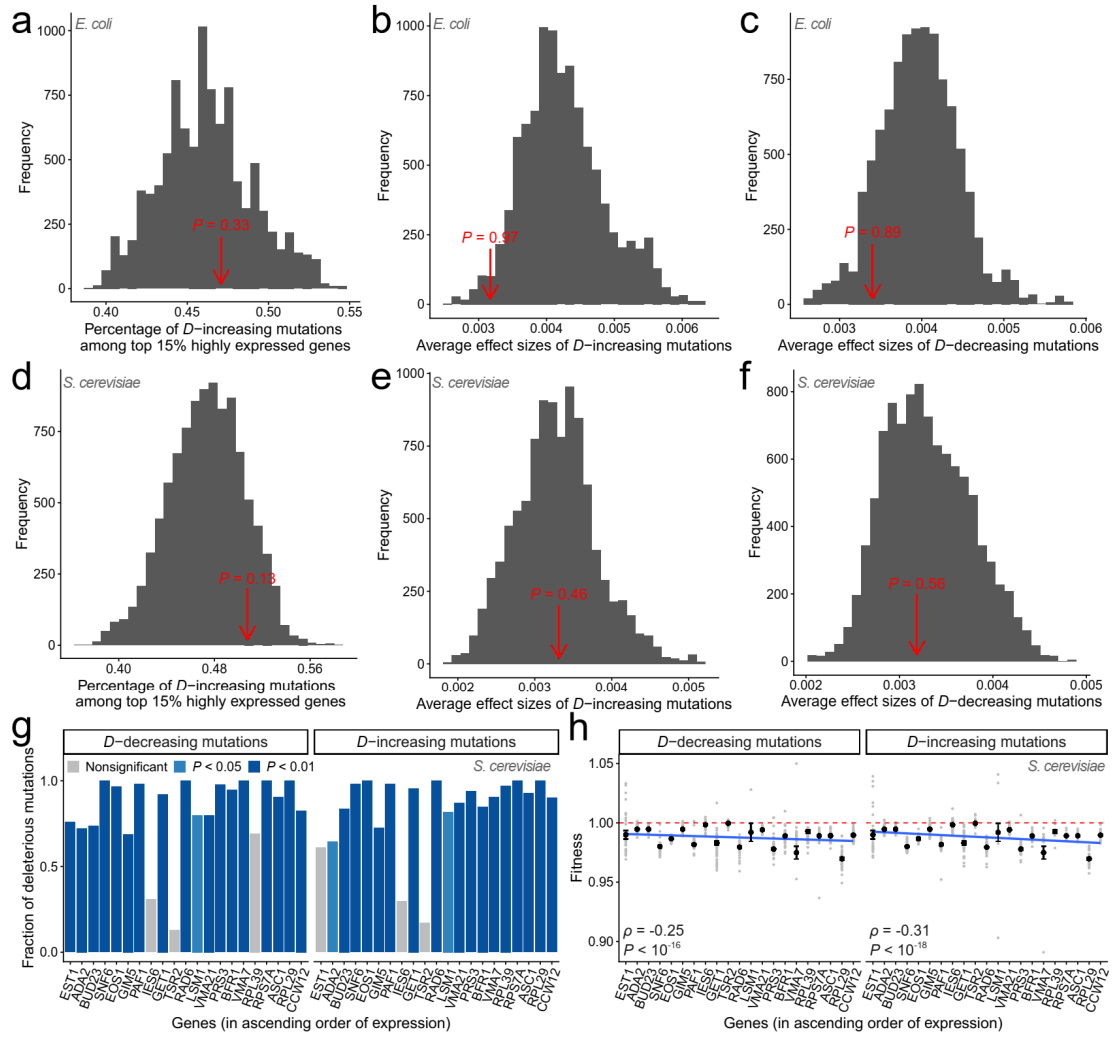
Supplementary Fig. 10. The fraction of *D*-increasing mutations is significantly higher than random expectation among genes with minimal *D*, but not among weakly expressed genes

(a) This is similar to **Figure 6c** and **f**, except that mutations among genes with 15% lowest *D* values were analyzed. This is a positive control for **Figure 6c** and **f**, demonstrating that the observation therein is not due to insufficient statistical power. See **Methods** for details. **(b)** This is similar to panel **a**, except that tRNA supply (X_{ij} in *D*) is estimated using tRNA expression level instead of the codon usage of the 10% most highly expressed genes. **(c-d)** These are similar to panel **a-b**, except that a group of lowly expressed genes (defined as 20% least expressed genes) that matched the highly expressed genes in **Figure 6c** and **f** were analyzed as a control. The number of genes, number of mutations of each type in a gene and GC content of a gene ($\pm 15\%$) were matched. Source data are provided as a Source Data file.



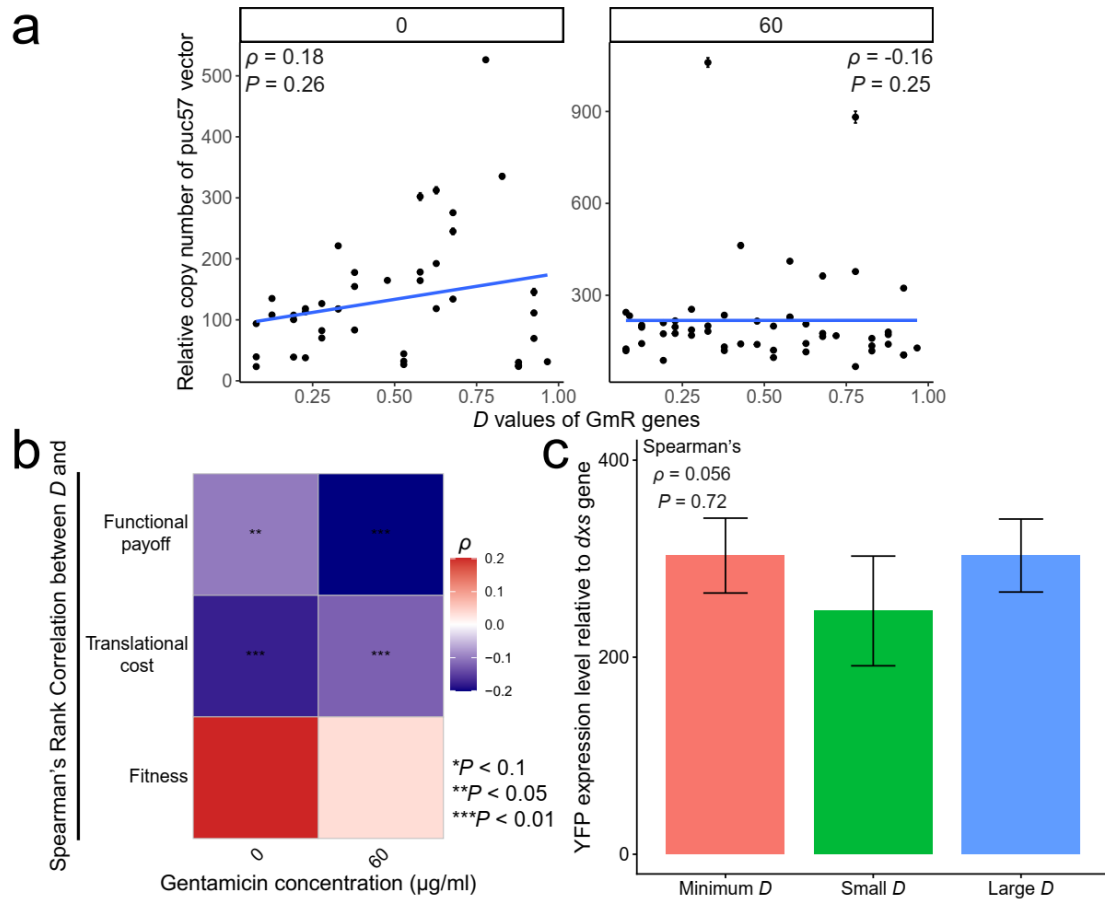
Supplementary Fig. 11. More results comparing D -decreasing and D -increasing mutations

Violin plots showing the changes in D due to the mutations listed in **Figure 6j**. The effect size of D -decreasing and D -increasing mutations are similar (Wilcoxon rank sum test $P = 0.10$). Source data are provided as a Source Data file.



Supplementary Fig. 12. Selective maintenance of the mismatch between endogenous gene's codon usage and cellular tRNA supply is recapitulated by tRNA expression-based *D*

These panels are similar to **Figure 6c-h**, except that the tRNA supply (X_{ij} in *D*) is estimated using tRNA expression level instead of the codon usage of the 10% most highly expressed genes. Source data are provided as a Source Data file.



Supplementary Fig. 13. Copy number of *GmR* genes identified by quantitative real-time PCR.

(a) The dots and error bars represent the average and standard error of the copy number of *GmR* genes relative to *dxs* as measured by qPCR. The strains growing in LB medium supplemented with 0 $\mu\text{g/ml}$ (left panel) or 60 $\mu\text{g/ml}$ (right panel) gentamicin is analyzed. The Spearman's Rank Correlation Coefficient (ρ) and corresponding P value are shown at the top left corner. For each strain, we performed three technical replicates. (b) The partial Spearman's correlation between D and functional payoff/translational cost/fitness controlling for the measured plasmid copy number. (c) The abundance of *YFP* mRNA in different strains, as determined by quantitative RT-PCR (see **Methods**), is not correlated with codon usage of *GmR*. The Spearman's Correlation Coefficient ρ of the raw data, as well as the corresponding P value, are indicated. For each strain, we performed three technical replicates. Source data are provided as a Source Data file.

Supplementary Table 1. Designed sequence of template reporter cassette

> PUC57-GmR vector expression system: forward (J23119)
 (promoter-RBS-GmR-linker-mCherry-terminator) – insulator – inverse (J23114)
 (promoter-RBS-YFP-terminator) – selection marker (AmpR)

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> PUC57-argW-GmR vector expression system: forward (argW promoter-argW-argW
 terminator)-forward (J23119 promoter-RBS-GmR-linker-mCherry-terminator) – insulator – inverse
 (J23114 promoter-RBS-YFP-terminator) – selection marker (AmpR)

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> PUC57-AmpR vector expression system: forward (J23119)
 promoter-RBS-AmpR-linker-mCherry-terminator) – insulator – inverse (J23114)
 promoter-RBS-YFP-terminator) – selection marker (MCR1)

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Supplementary Table 2. MIC of GmR strains

Strain	<i>D</i> of GmR	MIC (µg/mL)
0.967_3	0.967	20
0.300_1	0.300	80
0.528_3	0.528	80
0.719_1	0.719	80
0.828_2	0.828	80
0.127_1	0.127	160
0.192_3	0.192	160
0.278_3	0.278	160
0.328_3	0.328	160
0.378_2	0.378	160
0.428_3	0.428	160
0.478_2	0.478	160
0.478_3	0.478	160
0.628_2	0.628	160
0.678_1	0.678	160
0.678_2	0.678	160
0.778_1	0.778	160
0.778_2	0.778	160
0.828_3	0.828	160
0.967_2	0.967	160
0.078_1	0.078	>160
0.078_2	0.078	>160
0.078_3	0.078	>160
0.127_2	0.127	>160
0.127_3	0.127	>160
0.192_1	0.192	>160
0.192_2	0.192	>160
0.228_1	0.228	>160
0.228_2	0.228	>160
0.228_3	0.228	>160
0.278_1	0.278	>160
0.278_2	0.278	>160
0.328_1	0.328	>160
0.328_2	0.328	>160
0.378_1	0.378	>160
0.378_3	0.378	>160
0.428_2	0.428	>160
0.528_1	0.528	>160
0.528_2	0.528	>160
0.578_1	0.578	>160

0.578_2	0.578	>160
0.578_3	0.578	>160
0.628_1	0.628	>160
0.628_3	0.628	>160
0.678_3	0.678	>160
0.778_3	0.778	>160
0.828_1	0.828	>160
0.878_1	0.878	>160
0.878_2	0.878	>160
0.878_3	0.878	>160
0.926_1	0.926	>160
0.926_2	0.926	>160
0.926_3	0.926	>160

Supplementary Table 3. MIC of AmpR strains

<i>D</i> of AmpR	MIC (µg/mL)
0.4412127	>100
0.3939022	>2000
0.3939386	>2000
0.4867892	>2000
0.6256856	>2000
0.6432589	>2000
0.1520398	>4000
0.1830521	>4000
0.1891276	>4000
0.1910427	>4000
0.2123167	>4000
0.2195083	>4000
0.2554561	>4000
0.2663515	>4000
0.2668172	>4000
0.2979173	>4000
0.3219447	>4000
0.3231296	>4000
0.3555402	>4000
0.3586054	>4000
0.3668593	>4000
0.3690677	>4000
0.3968076	>4000
0.3999948	>4000
0.4086403	>4000
0.4103535	>4000
0.4113387	>4000
0.4118842	>4000
0.4138739	>4000
0.4283306	>4000
0.4313274	>4000
0.4366902	>4000
0.4489568	>4000
0.452766	>4000
0.4843855	>4000
0.5033768	>4000
0.5035456	>4000
0.5101515	>4000
0.5269234	>4000
0.5278113	>4000

0.5390567	>4000
0.5476983	>4000
0.5555651	>4000
0.5735878	>4000
0.5852674	>4000
0.6110103	>4000
0.6523893	>4000

Supplementary Table 4. Primers used

N O.	Names	Sequence	Details
1	GmRn-F	AGTCGCCCTAAAACAAAGTT	Used for construction of GmR strains
2	GmRn-R	TGGATTGGAAGTACAGGTTT	
3	PUC-F	AAACCTGTACTTCCAATCCA	
4	PUC-R	AACTTTGTTTTAGGGCGACT	
1	MCR1-F	CATGAGACAATAACCCTGATAAATGCTTCAAT AATATTGAAAAAGGAAGAGTatggtccagcatactcct gtg	Used for construction of AmpR strains
2	MCR1-R	GAGTAAACTTGGTCTGACAGtcagcggatgaatgcgg t	
3	PUC-F2	tcagggttattgtctcatg	
4	PUC-R2	ctgtcagaccaagttactc	
5	AMP-F	ctcatcctccaacggcagcagcggatccatgagtattcaacatttccgtgt cgcccttattccctttttgcggcattttgccttc	
6	Amp-R	GATTGGAAGTACAGGTTTTCCC	
7	PUC-F3	GAAAACCTGTACTTCCAATCCA	
8	PUC-R3	ctgccgttgaggatgag	
1	argW-F	gcgtttcggatgacggtgaCATAAATGGCGAGGGTTT AAG	Used for construction of argW-GmR strains
2	argW-R	tacaatctgctctgatgccgTAAGTTCTTGAGCAGTCAG G	
3	PUC-F4	cggcatcagagcagattgta	
4	PUC-R4	tcaccgtcatcaccgaaac	
1	Bla-F	CTACGATACGGGAGGGCTTA	Used for determining the copy number of GmR genes
2	Bla-R	ATAAATCTGGAGCCGGTGAG	
3	Dxs-F	CGAGAAACTGGCGATCCTTA	
4	Dxs-R	CTTCATCAAGCGGTTTCACA	
1	YFP-F	CCATGATTGCTTGGTCAC	Used for determining the expression level of YFP genes
2	YFP-R	CTTCATCAAGCGGTTTCACA	
3	Dxs-F	CGAGAAACTGGCGATCCTTA	
4	Dxs-R	CTTCATCAAGCGGTTTCACA	