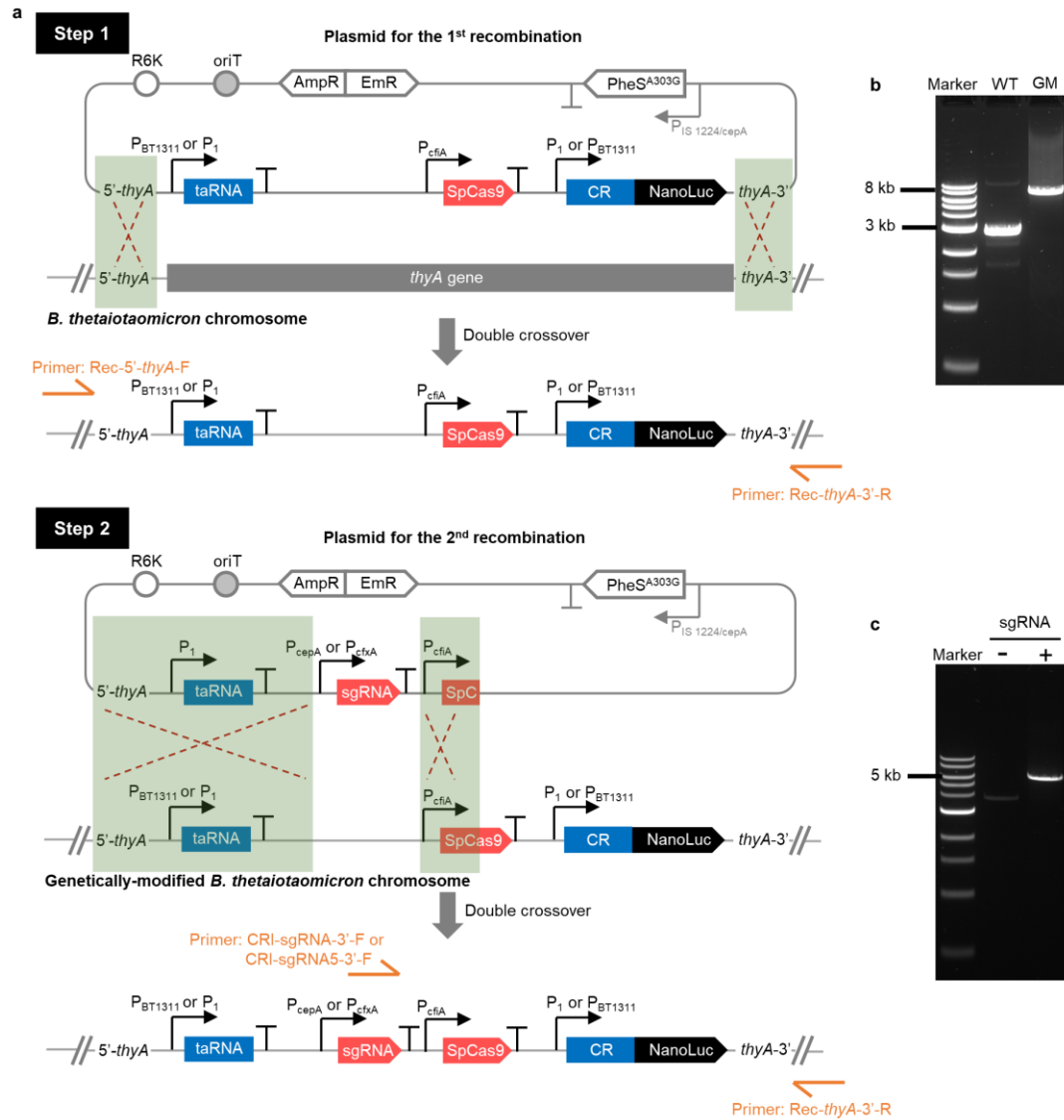
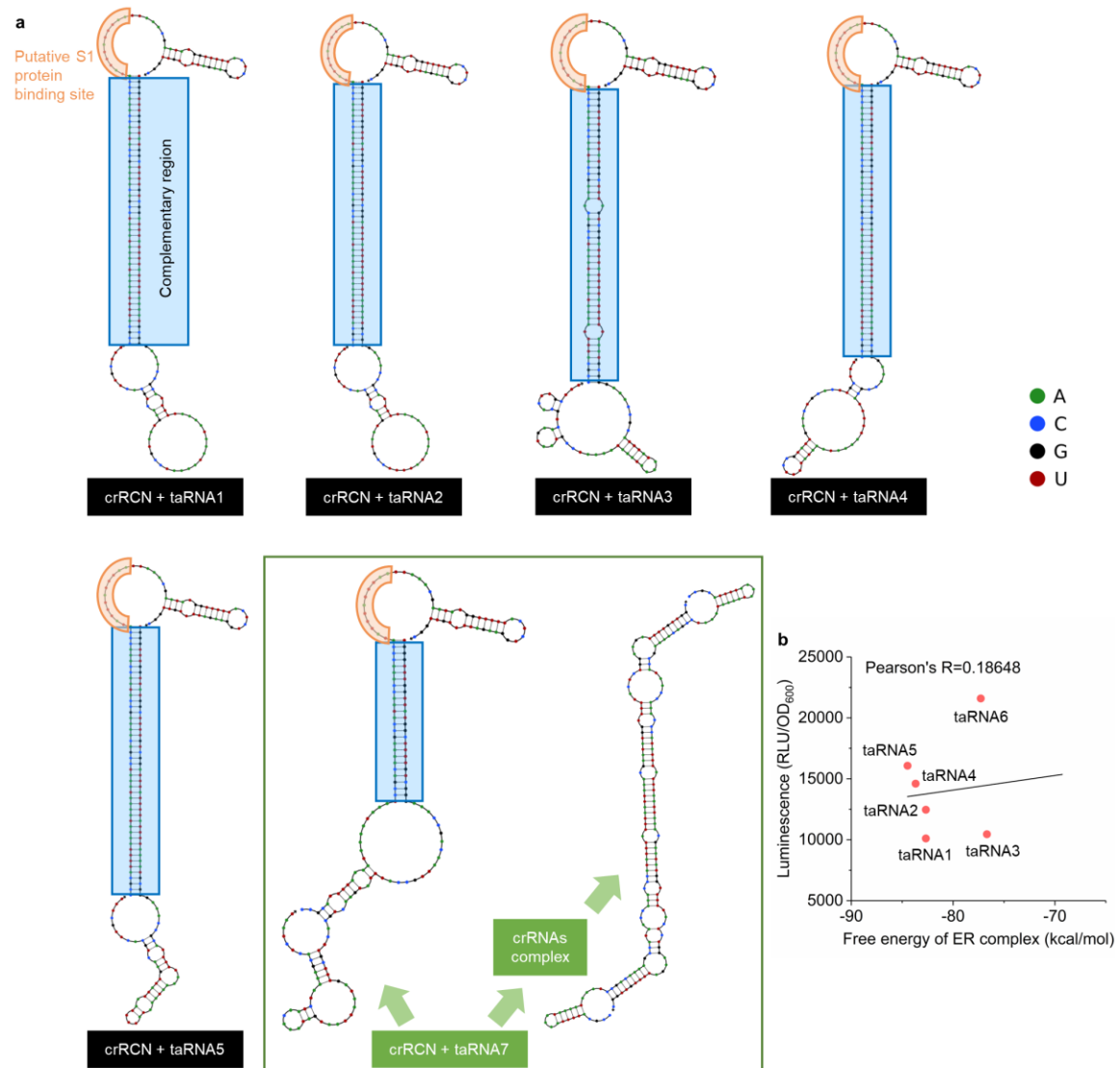


Supplementary Fig. 1 Genetic maps of representative plasmids used in this study. Left, pNH9188, used for the first recombination step to integrate the components of the Engineered Riboregulator (ER), i.e., the trans-activating RNA sequence (taRNA6) and the cis-repressive sequence (crRCN), as well as the SpCas9 gene, the NanoLuc gene, and their promoters into the chromosome of *Bacteroides thetaiotaomicron*. Right, pNH9196, used for the second recombination step, to integrate the single-guide RNA sequence (sgRNA1) and its promoter (P_{cepA}).

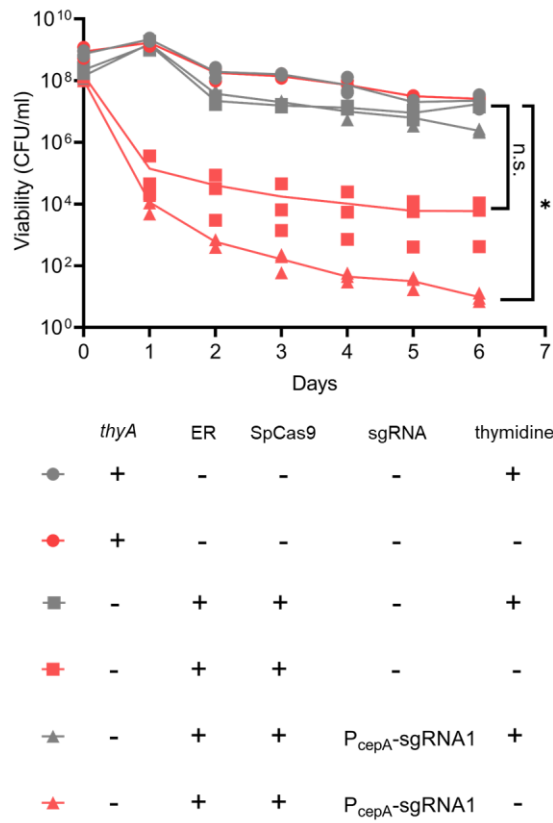


Supplementary Fig 2 Strategy for genetic modification. **a**, Overview of the recombination method used in this study. Step 1: Introduction of the taRNA, SpCas9, cis-repressive sequence (CR), and the NanoLuc gene onto the chromosome of wild-type (WT) *B. thetaiotaomicron*. Step 2: Introduction of sgRNA and its promoters. R6K, origin of replication; oriT, origin of transfer; AmpR, ampicillin resistance cassette; EmR, erythromycin resistance cassette; PheS^{A303G}, mutated α -subunit of phenylalanyl-tRNA synthetase gene. **b**, PCR identification of the integration of the gene cassette with primers

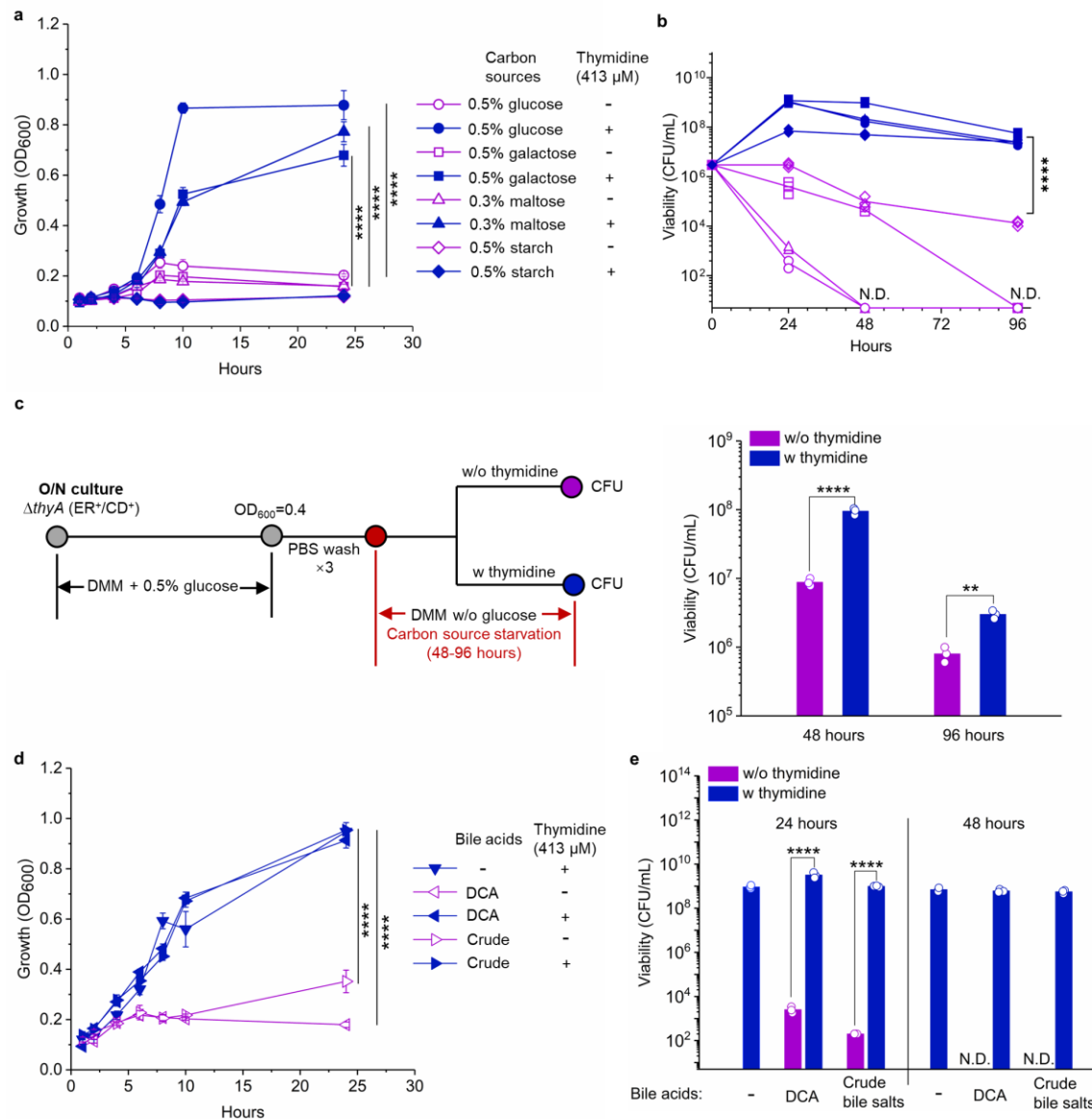
shown in **(a)**. **c**, PCR identification of the integration of sgRNA. Source data are provided as a Source Data file.



Supplementary Fig. 3 Analysis of crRNA (crRCN)/taRNA complexes with NUPACK web server. a, NUPACK-predicted structures of crRNA (crRCN)/taRNA complexes. **b**, Correlation between Minimum Free Energy (MRE), i.e., free energy of ER complex, and bioluminescent signal of each crRNA (crRCN)/taRNA complex: crRNA (crRCN)/taRNA1, crRNA (crRCN)/taRNA2, crRNA (crRCN)/taRNA3, crRNA (crRCN)/taRNA4, crRNA (crRCN)/taRNA5, and crRNA (crRCN)/taRNA6. Source data are provided as a Source Data file.



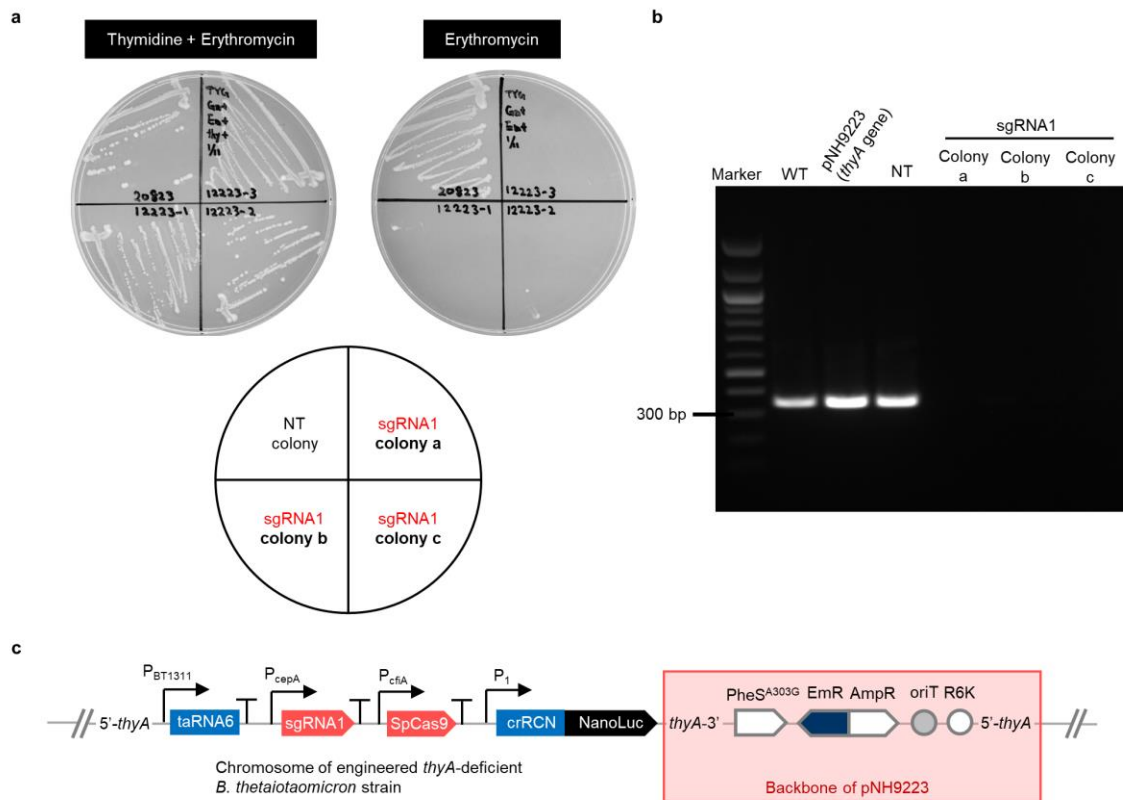
Supplementary Fig. 4 Death rate accelerated by CRISPR Device (CD). Cell viability of WT strain and genetically modified strain with and without P_{cepA}-sgRNA1, cultured anaerobically in the presence or absence of thymidine. Each dot is a biological replicate, and lines are the values of the mean of three biological replicates (Dunn's multiple comparisons test with Kruskal-Wallis test on log-transformed data at day6, *P<0.05). n.s. represents not significant. Source data are provided as a Source Data file.



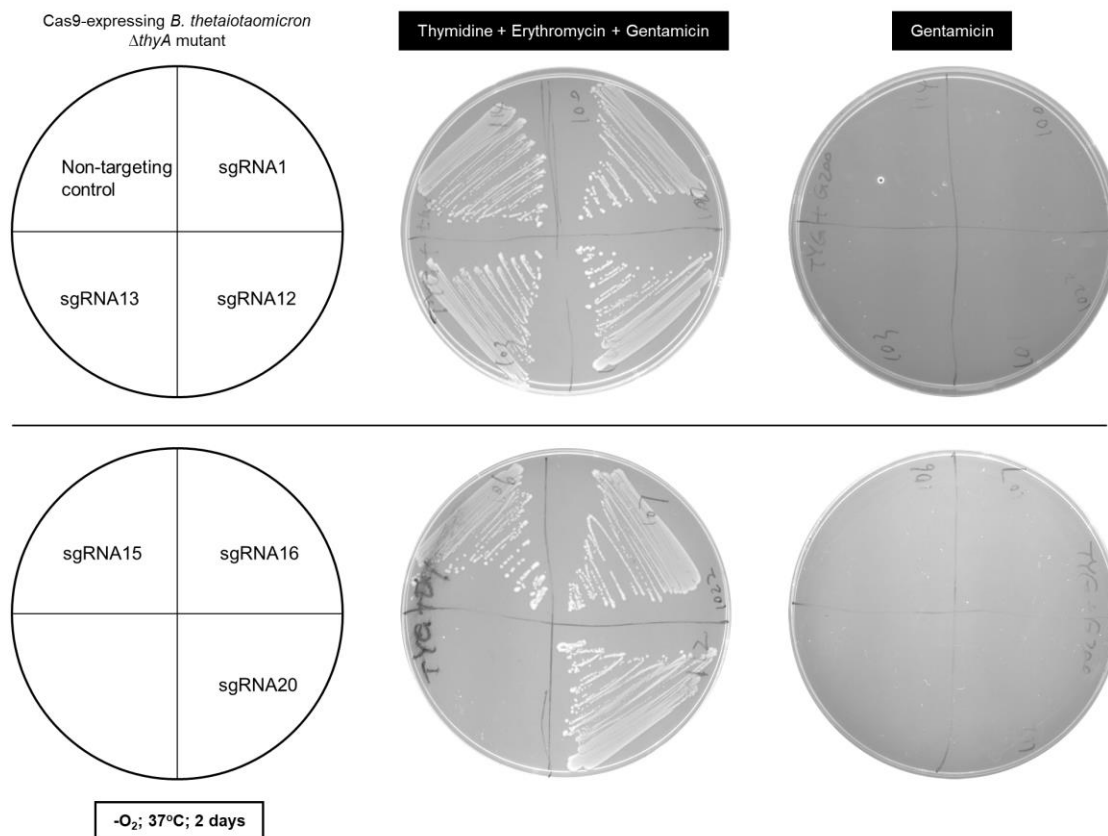
Supplementary Fig. 5 Thymidine auxotrophy of genetically modified *B. thetaiotaomicron* in various stress conditions. a, b, Growth curves (a) and cell viability (b) of *thyA*-deficient strain with P_{cepA}-sgRNA1 in defined minimal medium (DMM) with different carbon sources with (blue) or without (violet) thymidine. **c,** Schematic of carbon source starvation and cell viability of the engineered bacterial strain with (blue) or without (violet) thymidine. **d, e,** Growth curves (d) and cell viability (e) of *thyA*-deficient strain

with P_{cepA}-sgRNA1 in TYG medium with bile acids (62.5 uM DCA; 0.5% crude ox bile extracts) with (blue) or without (violet) thymidine.

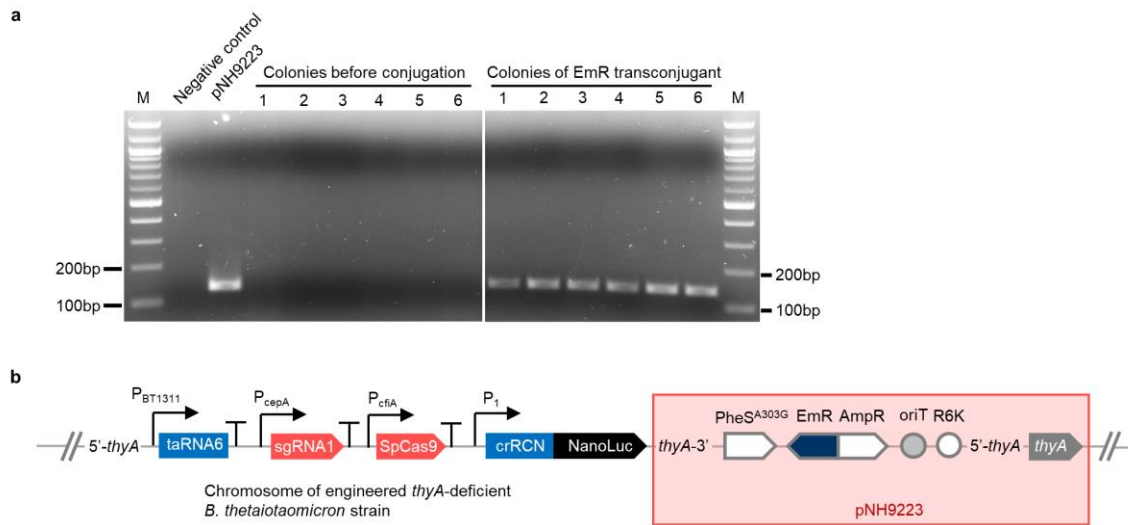
(a and d) Each dot is the value of the mean, and error bars represent the standard deviations of three biological replicates (Tukey's multiple comparisons test with one-way analysis of variance on data at 24hrs, **** P<0.0001). **(b)** Each dot is a biological replicate, and lines are the values of the mean (Tukey's multiple comparisons test with one-way analysis of variance on log-transformed data, **** P<0.0001). The detection limit is 5 CFU/ml. **(c and e)** Each dot is a biological replicate, and columns are the values of the mean (two-tailed unpaired Student's t-test on log-transformed data, ** P<0.01 **** P<0.0001). The detection limit is 5 CFU/ml. N.D. represents no detection. Source data are provided as a Source Data file.



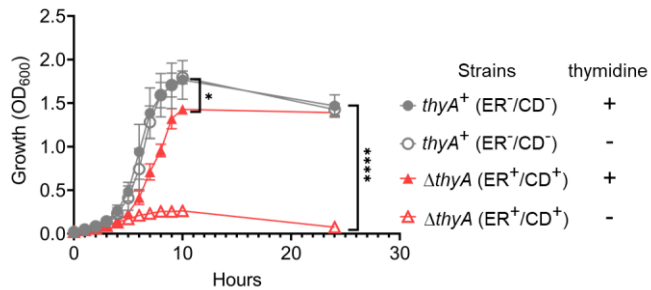
Supplementary Fig. 6 Prevention of acquisition of *thyA* gene by genetically engineered strain. **a**, Colonies streaked on TYG with and without thymidine in the presence of erythromycin and gentamicin. Viable *B. thetaiotaomicron* bearing sgRNA1 or NT, plated on BHIS agar plates with thymidine, erythromycin, and gentamicin after conjugation, were streaked to check thymidine auxotrophy. **b**, Amplicon patterns of transconjugants isolated from BHIS agar plates with thymidine, erythromycin, and gentamicin after conjugation. PCR was performed with primers flanking the sequence targeted by the CRISPR Device (CD) with sgRNA1 in order to detect the *thyA* gene. **c**, Schematic diagram showing integration of pNH9223 backbone into the chromosome of the engineered *thyA*-deficient *B. thetaiotaomicron* strain. Source data are provided as a Source Data file.



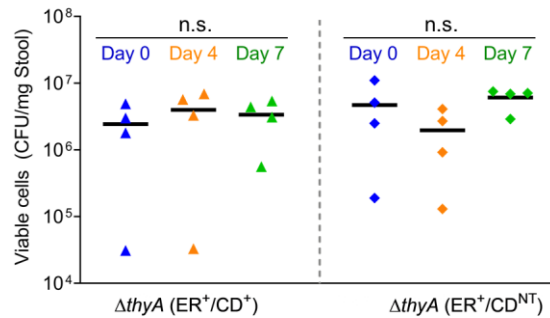
Supplementary Fig. 7 Thymidine auxotrophy of genetically engineered strains bearing the sgRNAs and their promoter constructed by pNBU2-based technology after the first recombination. The genetically engineered strains were streaked on gentamicin-containing TYG agar with both thymidine and erythromycin, or without either thymidine or erythromycin. Plates were incubated anaerobically at 37°C for 2 days.



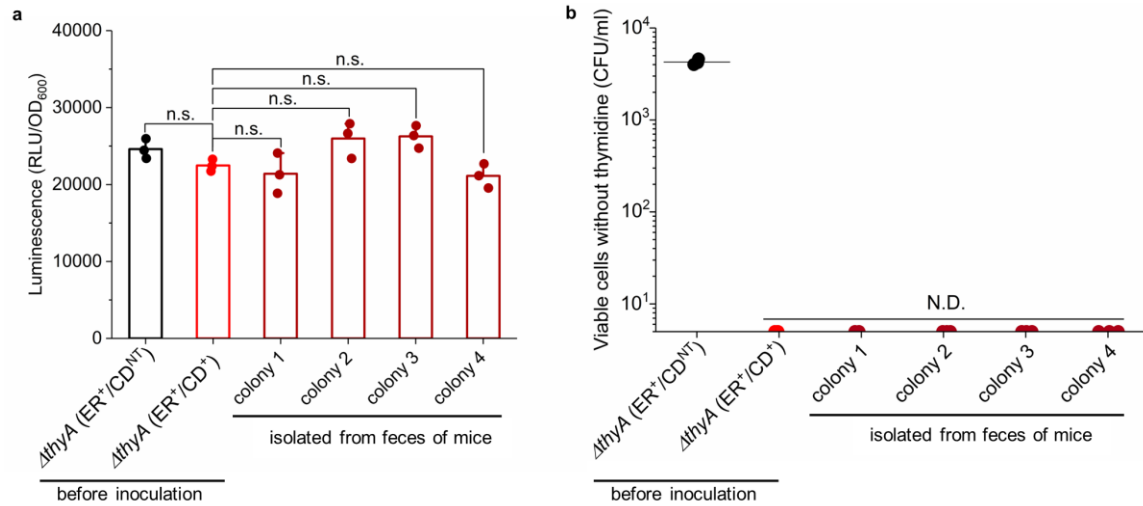
Supplementary Fig. 8 Integration of EmR and *thyA* genes in engineered *thyA*-deficient *B. thetaiotaomicron* strain after conjugation at day 21. a, PCR amplification of EmR gene fragment (144 bp) in engineered *B. thetaiotaomicron* strains before and after conjugation of pNH9223. **b**, Schematic diagram showing integration of pNH9223 plasmid into the chromosome of engineered *thyA*-deficient *B. thetaiotaomicron* strain. Source data are provided as a Source Data file.



Supplementary Fig. 9 Growth curves of *thyA*⁺ (ER⁻/CD⁻) and *ΔthyA* (ER⁺/CD⁺) strains in the presence and absence of thymidine. The cells were grown anaerobically at 37°C for 24 hrs. 300 μl of samples were withdrawn from the cultures every hour for 10 hrs and at 24 hrs to monitor growth (OD₆₀₀). Each dot is the value of the mean, and error bars represent the standard deviations of three biological replicates made on different days (Tukey's multiple comparisons test with one-way analysis of variance on data at 10hrs and 24hrs, * P<0.05, **** P<0.0001). Source data are provided as a Source Data file.



Supplementary Fig. 10 Viability in mice feces of genetically engineered thymidine-auxotrophic *B. thetaiotaomicron* with Engineered Riboregulator (ER) and CRISPR Device (CD). Fecal suspensions from mice gavaged with either of $\Delta thyA$ (ER⁺/CD⁺) or $\Delta thyA$ (ER⁺/CD^{NT}) strains but without the $thyA^+$ (ER⁻/CD⁻) strain were plated on selective agar plates with thymidine on the day when feces were retrieved, and after four and seven days of storage at ambient temperature in the presence of oxygen. Lines are the values of the mean of four biological replicates (Tukey's multiple comparisons test with one-way analysis of variance on log-transformed data). n.s. represents not significant. Source data are provided as a Source Data file.



Supplementary Fig. 11 *In vivo* genetic stability of the genetically engineered strain bearing the Engineered Riboregulator (ER) and CRISPR Device (CD). **a**, Outputs of NanoLuc in cells isolated from mouse feces, which were collected at day10 after administration of the genetically engineered *B. thetaiotaomicron*. Dots represent three biological replicates and columns represent the values of the mean. Error bars represent the standard deviations of the three biological replicates (Tukey's multiple comparisons test with one-way analysis of variance on data). **b**, Viable *B. thetaiotaomicron*: bacterial cells, isolated from mouse feces collected at day 10 after administration of the genetically engineered *B. thetaiotaomicron*, were conjugated with the *E. coli* donor strains which have an intact *thyA* gene on their plasmids on TYG agar plates with thymidine and then plated on TYG agar plates without thymidine. Individual dots represent three biological replicates of recipients. Black bars are the values of the mean. The detection limit is 5 CFU/ml. n.s.

represents not significant. N.D. represents no detection. Source data are provided as a Source Data file.

Supplementary Table 1. Cis-repressive sequences (CRs) used in Figure 2.

Name	Sequence
crRCN	ccgacaaaagccataaccaaacca
crR7N	tcgtttaattaaattcatattgggt
crR10N	tcgttaaataaaaatacatattgggt
crR12N	tcgtataaataaatacatattgggt

Supplementary Table 2. Trans-activating RNAs (taRNAs) used in Figure 2.

Name	Description	Sequence
Non Sense (NS)	Non-sense trans-activating RNA	taatacccaaataccaggaggtgattggtagtgtggtaatgaaaattaacttactactaccatatatctctaga
taRNA1	trans-activating RNA	tgggtttggttatggctttgtcggattatttatttt aaaatgctgggaaaccgcatttttaaataaat aaaatccgtaa
taRNA2	trans-activating RNA	tgggtttggttatggctttgtcggattatttatttt aaaatgctgggaaaccgcatttttaaataaat atttccgtaa
taRNA3	trans-activating RNA	tgggtttggttatggctttatcggattatttatttt taaatactgggaaaccgcatttataaataaat aaaatccgtaa
taRNA4	trans-activating RNA	tgggtttggttatggctttgtcggattatttatttt aaaatgctgggaaaccgcatttttaaataaat atttccgtat
taRNA5	trans-activating RNA	tgggtttggttatggctttgtcggattatttatttt aaaatgctgggaaaccgcatttttaaataaat attttaattat
taRNA6	trans-activating RNA	tgggtttggttatggctttatcggattatttatttt taaatactgggaaaccgcatttataaataaat attttaattat
taRNA7	trans-activating RNA	tgggtttggttatggctttgtcgggaaaccgcac aattgccattta

Sequences complementary to crRNA are highlighted in blue.

Supplementary Table 3. Single guide RNAs (sgRNAs) used in experiments.

Name	Description	Figure	Sequence	PAM
Non-targeting control (NT)	Non-targeting single guide RNA	4, 5, 7, S6, S7, S10, S11	attctctagactttacttga	None
sgRNA1	Single guide RNA	3, 4, 5, 6, 7, S1, S4, S5, S6, S7, S8, S9, S10, S11	acgcatctggaacgaatggg	CGG
sgRNA12	Single guide RNA	4, S7	aggcgctgacaatgatacgg	CGG
sgRNA13	Single guide RNA	4, S7	atgcgtttcaacctcgacga	GGG
sgRNA15	Single guide RNA	4, S7	actgagaaaagtgaccgtac	AGG
sgRNA16	Single guide RNA	4, S7	agttttacgtggcagacggt	CGG
sgRNA20	Single guide RNA	4, S7	gcctcaaataaaaattaatc	CGG

Supplementary Table 4. Genetic parts used in experiments.

Name	Description (Source)	Sequence
AmpR	Ampicillin-resistance cassette (Cell Syst. 2015; 1(1): 62-71.)	atgagtattcaacatttccgtgtcgcccttattccctttttgcggcattttgccttcc tgttttgctcaccagaaacgctgggtgaaagtaaagatgctgaagatcag ttgggtgcacgagtggttacatcgaactggatctcaacagcggtaagatcc ttgagagttttcgccccgaagaacggtttccaatgatgagcacttttaaagttct gctatgtggcgcggtattatcccgtattgacgcgggcaagagcaactcggg cgccgcatacactattctcagaatgacttggttgagtactaccagtcacaga aaagcatcttacggatggcatgacagtaagagaattatgcagtgtgcat aaccatgagtataacactgcggccaacttacttctgacaacgatcggagg accgaaggagctaaccgctttttgcacaacatgggggatcatgtaactcgc cttgatcgttgggaaccggagctgaatgaagccataccaaacgacgagcg tgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaaactattaac tggcgaactacttactctagcttcccggaacaataatagactggatggag gaggataaagttgcaggaccacttctgcgtcggccctccggctggctggt tattgtgataaatctggagccggtgagcgtgggtctcgcggtatcattgcag cactggggccagatggtgaagccctcccgatcgtagttatctacacgacggg gagtcaggcaactatggatgaacgaaatagacagatcgctgagatagggtg cctcactgattaagcattggttaa
EmR	Erythromycin-resistance cassette (Cell Syst. 2015; 1(1): 62-71)	atgaacaaagtaaataaaaagatagtcaaaattttattactcaaaatatca catagaaaaataatgaattgcataagtttagatgaaaaagataacatctt gaaatagggtgcagggaagggtcattttactgctggattggtaaagagatgta attttgtaacggcgatagaaattgattctaaattatgtgaggtaactcgtaata gctcttaaattatcctaactatcaaatagtaaatgatgatactgaaattaca ttcctagccacaatccatataaaaatttggcagcatacctacaacataag cacaataataatcgaaaaattgtttgaaagttcagccacaataagttattta atagtgaatatggtttgctaaaatgttattagatacaaacagatcactagca ttgctgttaatggcagaggtagatattctatattagcaaaaattcctaggatta ttccatccaaaacctaaagtggatagcacattaattgtattaaaaagaaag ccagcaaaaatggcatttaaaagagagaaaaaaatgaaacttttgaatg aaatgggttaacaaagagtacgaaaaactgtttacaaaaaatcaatttaata aagctttaaacaatgcgagaatatatgatataaacaatatttagtttgaacaa ttgtatcgctatttaatagttataaaaattttaacggctaa
R6K	Origin of replication (Cell Syst. 2015; 1(1): 62-71)	gatctgaagatcagcagttcaacctgttgatagtacgtactaagctctcatgtt cacgtactaagctctcatgtttaacgtactaagctctcatgtttaacgaactaa accctcatggctaacgtactaagctctcatggctaacgtactaagctctcatgt ttcacgtactaagctctcatgtttgaacaataaaaattaataaaatcagcaact aaatagcctctaagggttttaagttttataagaaaaaaaagaatatataaggct tttaaagcttttaagggttaacgggttggtgacaacaagccagggtgtaacgc actgagaagcccttagagcctctcaaagcaattttgagtgcacaggaaca cttaacggctgaca
RP4	Origin of transfer (Cell Syst. 2015; 1(1): 62-71)	ccggccagcctcgagagcaggattcccgttgagcaccgccagggtgcga ataagggacagtgaagaaggaaacaccgctcgcggtgggcctacttca cctatcctgccc

PheS ^{A303G}	Mutated alpha subunit of Bacteroides phenylalanyl tRNA synthetase (Anaerobe. 2016; 42: 81-88.)	atgatagctaagattaatcaacttctgaagaggtgggggcttgaaagccg ccaatgccgaagaactcgaagttctgcgcatcaaataacctcagcaagaaa ggagccatcaatgacttgatggcagatttccgcaatgtggctgccgaacag aaaaaagaagtcggcatgaagctaacgagctgaaaacaaaagcaca agaaaaaatcaacgcactgaaagaacagttcgacaaccaggacaacgg acaggacgatctcgacctgacccgttcggcttatcccgtaggagctgggcac acgccatccgctttccattgtgcgaacgaaatcattgacatcttgcctgt gggattcaacattgccgaaggtccggaaatagaagatgactggcatgtgt ctccgcactcaacttgcgaagaccatccggcacgagacatgcaagaca cattcttcatcgaatcccacccgacgtactactgcgcacacacacctcatc gtacagagccgtgtgatggaggttcgcaaccacatccgtatcatctgtcc gggacgcgtttaccgtaacgaagctatcagctatcgtagcacactgtttctcca ccaggtagaagcgctgtacgttagaccgcaatgtatcttcaccgacctgaa acaagtgtgtactcttcgccaagagatgttcggtgccgatacgaagattc gctgcgtccgtctacttccggtcacagaaccagcgccgaaatggatat cagctgtaacatctgcggcggaaaaggctgccctctgcaaacacaccg gctgggtcgaaatctgggttcggaatgtagaccggaacgtactgtatgc caacggaatagacagcaaagtgtatagcggtatggactgggtatgggtat cgaaacgcatcacaacctgaaatatcaggtgaaagacctccgcatgttctc cgaaaacgatacacgttctgaaggagttcgaagcggcctattaa
P _{IS 1224/cepA} + RBS	Constitutive promoter + RBS (Anaerobe. 2016; 42: 81-88.)	ccttgaaaagagaagaagccgtgtgtgttcaaactgggtcaatgactcca ggaaaggacatcactcatcatcacggcaaacaaggcactcaccggtggc tggaacattggaggatgaagcggtcacagccgccttgcttgacaggctgc tctactgctgcgagattatcaggctcggaggaacaagctatcgcatgcaaa acaggaaaaaatttttagcaacaaaaacaggatagggacgtaaaa agagttaaggaaagtgaagcatcttcgatgctggagtgggatatactaaatt acctaaaaaagtggcgccaaaatttgcgcgccacaattattatcatacctt gtggaccgtattacaaagaacccaatcatat
P _{BT1311}	Constitutive promoter (Cell Syst. 2015; 1(1): 62-71)	tgatctggaagaagcaatgaaagctgctgtaagtctccgaatcaggtattgt tctgacagggtgattcccatccggtaaacgcggatacttgcagttgatctga ctcaggaataaattataaattaaggtaagaagattgtaggataagctaataga aatagaaaaaggatgccgtcacacaactgtcggcattctttttgtttattagt tgaaaatatagtgaaaaagttgcctaaatatgtatgtaacaaattattgtcgt aacttgcactc
P ₁	Constitutive promoter (Cell Syst. 2015; 1(1): 62-71)	gataaagtttgaagataaagctaaaagttcttatctttgcagt
P _{cftA} + RBS	Constitutive promoter + RBS (Cell Syst. 2015; 1(1): 62-71)	ggagtgcgcttctcgattttattgtattttgcatgacctgatgaggtttgttga ttattttttgcaacactaagttaagtgaatcctctgacatggcaaaatcctgag caacttttgtgtcagggtacttaaaaaaatattttataatagttgtcggaatt aaggtaaaagaataaa
P _{cfxA}	Constitutive promoter (Cell Syst. 2015; 1(1): 62-71)	tacaaagaaaattcgacaaactgtattttctatctattttgggtgggaaac ttagttatgtaccttgtcggc

P _{cepA}	Constitutive promoter (Cell Syst. 2015; 1(1): 62-71)	caaatttgcgcgccacaattattattcatacctttgtgg
rpil*	RBS (Cell Syst. 2015; 1(1): 62-71)	ccgcattttaaaataaaaataaattatttatttaattaaacgaat
5'- <i>thyA</i>	Upstream of <i>thyA</i> gene of <i>B. thetaiotaomicron</i> (This work)	tctatagccaatgcgctgttcgacctgctggccgaaaaagtgaagaaggc gtggaagtacgagccatgttcgatgcgttcggcaactggcgaacaataaa ccgctcaagaagagacatctcaaaaaatacagagagcaggggaatcgag attgtcaagtcgacccgttcacctttccctacatcaatcatgctggcacaccgt gatcaccgcaaaatagccgtcatcgacggtgaagtcgcctatacgggagg aatgaacatcgccgactactacatcaacggactccccaaaatcgggtacatg gcgtgatatgcacatgagaatagaaggcgatgccgtcaatgacctgcaag aaatatttcttaccatattggaacaaggagactaaacaaaattattggcggcg aagcatacttccccaaagcacaaggagcagtcggacagcaccaatgtagtc gtcgccatcgtagaccgcacaccgaagaaaaacagccgtatgctcagtc tgcttatgccatgtcgatctattcggcacagaaaaacgtgcacatcgtaacc cgtattttgtgccacatctccatcaacaaggcgcttcaacgcaccatcga acgagggtgtggatgtcacgattatggtttctctgcctccgacatcccgttactc cggacgcgcgcctctacaaacttcacaaactgatgaaaagggggggccac cgtctatatgtacaatggcgggttccaccattccaaaatcatgatggtcgacg atattttctgcacggttaggcaccgcaaacctgaacagccgcagcctccggt acgactacgagacgaacgcttcatctttaacaaggaaattacaggcgaac tgaacgaaatgtttcggaacgatatagaacactgtacgcagctaacaccgg aattctggaaaaagcgctcaccgtggaagaagttcgtcggtatggtttgcaa ttatttcacgccatttttgtaattttgcatcgccaccaactattggaaaa
<i>thyA</i> -3'	Downstream of <i>thyA</i> gene of <i>B. thetaiotaomicron</i> (This work)	ccacatattgccggaatagtagcgggtataaaacctagagtaaaatcaatt attgccgcgtagaccgccaatggcgatcggcttcagaataaaactgctttt ctggttgcccaacgacttgaaacgcttcaaggcactgactaccggaaacac catcataatgggaagaaaaaccttcgaatcgctgccgaaagggtgcgttgcc caatcgcagaaacggtgtgttatccaccgctccggatacgggtatgtccgggtg cggaagtctccgctcgctggaagtcgccctgcaaagctgcaaggaagat gagcacgtctatataataggtggcgcaagcgtctaccagcaggcacttctc ttgccgacgaactttgtctgacggaaataaatgacgttgctcccgaaagccga tgcttctttccggaagtatctccggctcaatggcacgaaaaaagcagaga agctcatcctgtggatgagaaacatctctgccgtagctttttagattacgtg aaacagtaagctattttattctgtacgattaatctctgcttcaatgtcgaccata atcggcacatcgccgcttatggcttcatggaaagagatcaatgtctccgtacg cgccaaccccaaaggttgcaatttatcatgtatgatactcagcaaatggtgat tgttcttagcgtaaatcttgataaacatatcatattaccagtagtgaaatgaca cttactacttccggaatggcttccaaagctctcggtacagaatcaaaggattc cggatctttcagatatataccaatataagcacaagtttcatacctatcttctca ggatcgatcacatattccgatcctttcaatattcccagattagtaacttctgaa tgcgctggtggatagccgccccggaaacgttacacgctcttgctacttccaa a
CR	Cis-Repressive sequence (This work)	See Supplementary Table 1

taRNA	trans-activating RNA (This work)	See Supplementary Table 2
SpCas9	Cas9 gene of <i>Streptococcus pyogenes</i> modified to work in <i>B. thetaiotaomicron</i> (This work)	atggataagaaataactcaataggcttagatatcggcacaaatagcgtcggatggcggtgatcactgatgaatataaggttccgtctaaaaagtcaaggttctgggaaatacagaccgccacagtatcaaaaaaatcttataggggctctttattgacagtggagagacagcgggaagcgactcgtctcaaacggacagctcgtagaaggtatacacgtcgggaagaatcgtatttggtatctacaggagatttttcaaatgagatggcgaaagtagatgatagtttcttcatcgactgaagagctttttgggtgaagaagacaagaagcatgaacgcatcctattttggaaatatagtagatgaagttgcttatcatgagaaatatccaactatctatcatctcgcaaaaaattggtagattctactgataaagcggatttgcgcttaatctatttggccttagcgcatatgattaagttcgtggtcatttttgattgagggagattaaatcctgataatagtgtgtggacaaactatttaccagttggtacaaacctacaatcaattattgaagaaaacctattaacgcaagtggagtagatgctaaagcgattcttctgcacgattgagtaaataagacgattagaaaatctcattgctcagctccccggtgagaagaaaaatggcttatttgggaatctcattgcttgcattgggttgaccctaatttaaatcaaaatttgatttggcagaagatgctaaattacagctttcaaaagatactacgatgatgatttagataattattggcgcaaattggagatcaatattgctgatttgggttggcagctaagaatttatcagatgctattttacttccagataccaaagagtaaatactgaaataactaaggctcccctatcagcttcaatgattaaacgctacgatgaacatcatcaagacttgactctttaaaagctttagttcgacaa caactccagaaaagtataaagaatcttttgatcaatcaaaaaacggat atgcaggttatattgatggggagctagccaagaagaattttataaattatca aaccaatttagaaaaaatggatggtactgaggaattattggtgaaactaaa tctgtaagatttgcgcgcaagcaacggaccttgacaacggctctattcccc atcaaattcactgggtgagctgcatgctatttggagaagacaagaagactttt atccattttaaaagacaatcgtgagaagattgaaaaatcttgacttttcgaa ttcttattatgttggtccattggcgcgtggcaatagtcgttttgcattggatgactc ggaagtctgaagaaacaattaccccatggaatttgaagaagttgtcgataa aggtgctcagctcaatcatttattgaacgcatgacaaacttgataaaaaatctt ccaatgaaaaagtactaccaaacaatagtttgctttatgagattttacggttt ataacgaattgacaaaggtaaatatgttactgaaggaatgcgaaaacca gcatttcttcagggtgaacagaagaagccattgttgattactcttcaaaaca aatcgaaaagtaaccgttaagcaattaaaagaagattttcaaaaaaata gaattttgatagttgaaatttcaggagttgaagatagatttaattgcttcatt aggtacctaccatgatttgcataaaattataaagataaagatttttggataat gaagaaaatgaagatatcttagaggatattgttttaacattgacctatttgaa gatagggagatgattgaggaaagacttaaaacatatgctcacctcttgatg ataaggtgatgaacagcttaaacgtcgccgttatactggttggggacggttg tctcgaaaattgattaatggtattagggataagcaatctggcaaaacaatatt agatttttgaaatcagatggtttgccaatcgcaattttatgcagctgatccatg atgatagtttgacatttaagaagacattcaaaaagcacaagtgctggaca aggcgatagtttacatgaacatattgcaaatttagctggtagccctgctattaa aaaaggtattttacagactgtaaaagtgttgatgaattggtcaaagtaattgg ggcgcataagccagaaaatatcgttattgaaatggcacgtgaaaaatcag acaactcaaaagggccagaaaaattcgcgagagcgtatgaaacgaatcg aagaaggtatcaaagaattaggaagtcagattcttaaagagcatcctgtga aaatactcaattgcaaaatgaaaagctctatctctattatctccaaaatggaa gagacatgtatgtggaccaagaattagatattaatcgtttaagtattatgatg tcatcacattgtccacaaagtttcttaagacgattcaatagacaataag

		<p>gtcttaacgcgttctgataaaaaatcggtgtaaatcggaataacgttccaagtga agaagtagtcaaaaagatgaaaaactattggagacaacttcaaacgccca agttaatcactcaacgtaagtttgataatttaacgaaagctgaacgtggaggt ttgagtgaactgataaagctggttttatcaaacgccaatgtgtgaaactcgc caaatacctaagcatgtggcacaattttggatagtcgcatgaataactaaat acgatgaaaaatgataaacttattcgagagggttaaagtgattaccttaaaatct aaattagtttctgacttccgaaaagattccaattctataaagtacgtgagatta acaattaccatcatgcccattgatgcgtatctaaatgccgtcgttggaaactgctt tgattaagaaatatccaaaactgaatcggaagttgtctatggtgattataaagt ttatgatgttcgtaaaatgattgctaagtcgagcaagaaataggcaaagca accgcaaaaatatttctttactctaataatcatgaacttctcaaaacagaaatta cacttgcaaatggagagattcgcaaacgccctctaatacgaaactaatgggg aaactggagaaattgtctgggataaagggcgagattttgccacagtgcgca aagtattgtcatgccccagtgcaatattgtcaagaaaacagaagtacaga caggcggattctcaaggagtgcaattttacaaaaaagaaattcgggacaagc ttattgtctgtaaaaaagactgggatccaaaaaaatatggtggtttgatagtc caacggtagcttattcagtcctagtggttgctaaggtggaaaaagggaaatc gaagaagttaaaatccgttaaagagttactagggatcacaattatggaaag aagttccttgaaaaaaatccgattgacttttgaagctaaaggatataagg aagttaaaaaagacttaatacctaactacctaataatagtccttttgagttaga aaacggctgtaaacggatgctggctagtgccggagaattacaaaaagga aatgagctggctctgccaagcaaatatgtgaatttttatatttagctagtcatta tgaagagttgaagggtagtcagaagataacgaacaaaaacaattgtttgt ggagcagcataagcattatttagatgagattattgagcaaatcagtgaaatttc taagcgtgttattttagcagatgccaattagataaagttcttagtgcatataac aaacatagagacaaaccaatacgtgaacaagcagaaaatattattcattta ttacgttgacgaatcttgagctcccgtgcttttaaatattttgatacaacaatt gatcgtaaacgatatacgtctacaaaagaagtttagatgccactcttatccat caatccatcactggctttatgaaacacgcattgatttagctcagctaggaggt gac</p>
NanoLuc	NanoLuc gene (Cell Syst. 2015; 1(1): 62-71)	<p>atggttttactctggaagattttgttggcgattggcgctcagaccgcggttata atttgatcaagtcctggaacaggggtggcgtaagctctctgttccagaacctg ggtgtgagcgtgacgccgattcagcgcacgttctgtccggcgagaacggt ctgaaaattgatattcatgtgatcatccgtacgaaggcctgagcggtgacc aaatgggtcaaatcgagaaaatcttaaagtcgtctaccagttgacgatca ccactcaagggtatcttgattacggtacgctggtgattgatggtgtgacccc gaatatgattgactatttcggccgtccgtatgaaggcattgccgttttgacggt aaaaagatcacccgtacccgtaccctgtggaatggcaataagattattgac gagcgtctgattaaccggagcggcagcctgctgttccgctgaccatcaac ggtgtcacgggttggcgctgtgctgagcgcatcctggcataa</p>
sgRNA1- upstream	Upstream of sgRNA (This work)	<p>tctatagccaatgcgtgttcgacctgctggccgaaaaaagtgaagaagggc gtggaagtacgagccatgttcgatgcgttcggcaactggtcgaacaataaa ccgctcaagaagagacatctcaaaaaaatacgagagcaggggaatcgag attgtcaagttcgacctgtcacctttccctacatcaatcatgcggcacaccgt gatcacccgcaaaaatagccgtcatcgacggtgaagtcgcctatacgggagg aatgaacatcggcactactacatcaacggactccccaaaaatcggtagatg gcgtgatatgcacatgagaatagaaggcgatgccgtcaatgacctgcaag aaatatttcttaccatatggaacaaggagactaaacaaaatattggcggcg aagcatacttcccaagcacaggagcagtcggacagcaccaatgtagtc gtcgccatcgtagaccgcacaccgaagaaaaaacagccgtatgctcagtc</p>

		<p>tgcttatgccatgtcgatctattcggcacagaaaaacgtgcacatcgtaacc cgtatttgtgccacatcctccatcaacaaggcgcttcaacgcaccatcga acgagggtgtggatgtcacgattatggtttctctgcctccgacatcccgttactc cggacgcgcctctacaaacttcacaaactgatgaaaaggggggcccac cgtctatatgtacaatggcgggttccaccattccaaaatcatgatggtcgacg atatttctgcacggtaggcaccgcaaacctgaacagccgcagcctccggt acgactacgagacgaacgcttcatctttaacaaggaaattacaggcgaac tgaacgaaatgttcggaacgatatagaacactgtacgcagctaacaccgg aattctggaaaaagcgctaccggtggaagaagttcgtcggtatggttgcaa tttattcacgccatttttgtaatttgcacgcaccaactattggaaaaagcacg tcgtctcagactgaggctactgagagtagctgatctggaagaagcaatgaa agctgctgttaagtctccgaatcaggatgttctctgacagggtatttccatcc ggtaaaccgcgatacttgcagttgatctgactcaggaataaattataaatta aggtaagaagattgtaggataagctaataagaaatagaaaaaggatgccgtc acacaactgtcggcattcttttgtttattagtgaataatagtgaaaaagtt gcctaaatatgtatgttaacaaattatttgcgtaacttgcactctgggttggt atggcttagtcggatttatttttaaatgcgggaaccgcattataaaata aatatttaattattgaactgcacttgccttgataattaatgataaacaatctaaa agcactctaatacgttatcgagtgcttttagattactaatcaattgcttacta attgcctatcttcagtgatggaacagcatttgtgcattggctgcaacaacgct aagatgctgg</p>
sgRNA1- downstream	Downstream of sgRNA (This work)	<p>ccacgtgctaaggagtgcgttctcgattttattgtattttgccatgcctgatg agggtttgttgattatttttgcacactaagttaagtgaatcctctgacatggc aaaatcctgagcaacttttgtgctcagggtacttaaaaaaatattttataata gtgttcggaattaaggtaaaagaataaaatggataagaaatactcaatag gcttagatatcggcacaatatagcgtcggtggcggtgatcactgatgaat ataagggtccgtctaaaaagttcaagggttctgggaaatacagaccgccaca gtatcaaaaaaatcttataggggtcttttatttgacagtggagagacagcg gaagcgactcgtctcaaacggacagctcgtagaaggatatacacgtcggaa gaatcgatttgttatctacaggagatttttcaaatagagatggcgaaagtagat gatagtttcttcatcgactgaagagcttttttggtggaagaagacaagaag catgaacgtcatcctatttttgaaatatagtatgaagttgcttatcatgaga aataccaactatctatcatctgcgaaaaaaattggttagattctactgataaa gcggatttgcgttaatactatttggccttagcgcatatgattaagttcgtggtcat ttttgattgaggagatttaaatcctgataatagtgatgtggacaaactatttat ccagttggtacaaacctacaatcaattattgaagaaaacctattaacgca agtggagtagatgctaaagcgattcttctgcacgattgagtaaatcaagac gattagaaaatctcattgctcagctccccggtgagaagaaaaatggcttatt gggaatctcattgttgcattgggttgaccttaattttaaatacaattttgatt tggcagaagatgctaaattacagcttcaaaagatacttacgatgatgattta gataatttattggcgcaaattggagatcaatatgctgattgttttggcagctaa gaatttatcagatgctattttactttcagatatcctaagagtaaaatactgaaata actaaggctcccctatcagcttcaatgattaaacgctacgatgaacatcatca agacttgactcttttaaaagcttttagttcgacaacaactccagaaaagtataa agaaaatcttttgatcaatcaaaaaacggatatgcagggtatattgatggggg agctagccaagaagaattttataaatttatcaaaccaattttagaaaaaatgg atggtagtgagggaattattggtgaaactaaatcgtgaagatttgcgtcgcaag c</p>
sgRNA	Single guide RNA (This work)	See Supplementary Table 3

gRNA scaffold	Guide RNA scaffold (Cell Syst. 2015; 1(1): 62-71)	gttttagagctagaatatagcaagttaaaataaggctagtcggttatcaacttg aaaaagtggcaccgagtcggtgc
<i>thyA</i>	<i>thyA</i> gene (Science. 2003; 299(5615): 2074-6.)	atgaaacaatatatttagattactcaatcgcgtattaactgaaggaactgagaa aagtgaccgtacaggaaccggaacgatcagtggttcggacatcagatgc gtttcaacctcgacgaggggttcccggtgtctgaccacaaaaaactgcatctg aaatcaatcatctacgagttactctggttctgcaagggtatcgaacgcgaa atatctgcaagaacacgggtgtacgcatctggaacgaatgggcgagcaga acgggtgatttagggcatatctatggctatcagtggttcgtggcccgactac gacggcggattcatcgaccagatcagcgaagcggtagagacgatcaagc acaatcccgaactcccgcgtatcattgtcagcgcctggaatgtagccgattta aagaatatgaacctgcctccctgtcatgccttctccagttttacgtggcagac ggtcggctgagcctgcaactttaccagcgcagcgcggatattttctcgag tcccggtcaatatcgcatcatatgcactgtctgctacaaatgatggcgcagggtg acaggactgaaagcgggtgaatttatccatacacttggcgtatgccatatct atctgaaccacttggatcaggtcaaattgcagcttagccgcgaaccacgcg cattgcctcaaatgaaaattaatccggatgtgaagagtatctatgacttccagt tcgaagacttcgaactgggtgaactacgacccgcatccacatattgccggaat agtagcgggtataa
Mutated <i>thyA</i>	Mutated <i>thyA</i> gene (This work)	atgaaacaatatatttagattactcaatcgcgtattaactgaaggaactgagaa aagtgaccgtacaggaaccggaacgatcagtggttcggacatcagatgc gtttcaacctcgacgaggggttcccggtgtctgaccacaaaaaactgcatctg aaatcaatcatctacgagttactctggttctgcaagggtatcgaacgcgaa atatctgcaagaacacgggtgtgctgatttgaatgagtgggcgagcagaa cgggtgatttagggcatatctatggctatcagtggttcgtggcccgactacg acggcggattcatcgaccagatcagcgaagcggtagagacgatcaagca caatcccgaactcccgcgtatcattgtcagcgcctggaatgtagccgatttaa agaatatgaacctgcctccctgtcatgccttctccagttttacgtggcagacg gtcggctgagcctgcaactttaccagcgcagcgcggatattttctcgagtc ccgttcaatatcgcatcatatgcactgtctgctacaaatgatggcgcagggtga caggactgaaagcgggtgaatttatccatacacttggcgtatgccatatctat ctgaaccacttggatcaggtcaaattgcagcttagccgcgaaccacgcgca ttgcctcaaatgaaaattaatccggatgtgaagagtatctatgacttccagttc gaagacttcgaactgggtgaactacgacccgcatccacatattgccggaata gtagcgggtataa
NBU2 integrase	<i>intN2</i> tyrosine integrase gene to integrate plasmid onto the genome of <i>B. thetaiotaomicron</i> . (Cell Syst. 2015; 1(1): 62-71)	atgaatatcaagcgcaacatcattttgcattggagagccggaaaaagaac gggtgtgccaatcgtagagaacgtacccatccgtatgctgtcatctttgccag ccaacgcatcgagttacaacgggtaccggattgacgtagccaaatggg atgcagataagcagcgggtaaagagcggatgtaccaacaagctaaagc aaagtgcagccgaaatcaatacggacttgcgtgaaatactatgccgaaatcc agaatatttcaaggaatttgaggtgcaggaggtcatgccaacgaccaac agttgaaggaagctttcaacatgagaatgaaagacaccagcgaagaaca gccggaagaagcccctgcagcttttgggaggtgttcgatgagttgtaaaa gagtgcggtaaccagaataactggacggcatccacatgaaaaatttgca gcagtgaggaaccacctaagaggttcaaggaggatgcaacgttcaacta ttcaacgagtttgattgaacgaatacgtcaacttctcgtgacaccaagg atatgagaacagcaccatcggcaagcaaatgggattcctcaaatggttcc tgcgctggagcttcaagaaaggacatcatcagaacattgcatacgatacgtt caaaccgaaactgaaaaccacctcgaaaaaagtaatttctcgtacttggg atgaactgaacaagctgaaagactaccagatacccaaggataagcaata

		cctggaacgtgtgctgatgtttcctgttctgctgctttacgagtttgcggtattc ggatgttcgcaatctgaaaagaagcgatgtgaagtccgaccacatcgaaat aaccacagtcaagactgccgacagcctgacgattgaactgaacaaatac agcaaagccatactggacaaatacaaggacatccattcgagaattacatg gctctgcccgtcatcagcaaccagaagatgaacgattacctgaaagagct gggcgaactggcagaaatcaacgagcctgtacgggaaacctactacaag ggaaatgaacgtattgatgaagtcacacccaaatacgtttgctcagtacct atgcaggaagaaggacattcatctgcaatgcgctggctctcggaatcccg cacagggtgcatgaaatggacgggacacagcgactacaaagctatgaa accctacattgacatagcggatgatattaaggcaaatgccatgaacaagttt aatcaacta
<i>attN2</i>	Recombination site of pNBU2 plasmid with <i>attBT</i> sites on the genome of <i>B.</i> <i>thetaitaomicron</i> . (Cell Syst. 2015; 1(1): 62-71)	cctgtctctccgcaaaaaacgct
TcR	Tetracycline resistance gene (Genbank: NZ_CYYO01000 017)	gtgcgtttcgacaatgcacatctactgtagtattattgcttaatccaaatgaatat tataaatttaggaattctgtctcattgatgcaggaaaaactccgtaaccga gaatctgctgtttgccagtggagcaacggaaaagtgcggccgtgtggataa tggtagaccataacggactctatggatagagaaacgtagagggaattac tgtccgggctctacgacatctattatctggaatggagtgaatgcaatatcatt gacactccgggacacatggattttattgcggaagtggagcggacattcaaa atgcttgatggagcagtcctcatcttatccgcaaaggaaggcatacaagcg cagacaaaagttgctgttcagtactttacaaaagctgcaaatcccgacaattat atttatcaataagattgaccggtgccggtgtgaattggagcgtttgtatatggat ataaaaaacaaatctgtcgcaagatgtcctgtttatgcaaactgtgtcgatgg atcggtttatccggttgctcccaaacatataaaaggaagaatacaaaagaa ttgtatgaaccatgacgacgatatattagaacgataatttggcggatagcga aatttcaccggctgattattggaatacgaataatcgctctgttggaagcca aagtcataccggtgctacatggatcagcaatgttcaatatcggtatcaatgagt tgttgacgccatttctctttatacttctccggcatcagtcctcaaacagacttt cagcttatctataagatagagcatgaccccaaagggcataaaagaagttt tcttaaaataattgacggaagtctgagacttcgagacgttgtaagaatcaac gattcggaaaaaattcatcaagattaaaaatctaaagactatttatcagggca gagagataaatgttgatgaagtgggtgccaatgatatcgcgattgtagaag atatagaagattttcgaatcggagattatttaggtgctaaacctgttgattcaa ggattatctcatcagcatcccgtctcaaatcctccgtccggccaaataagcc cgaagagagaagcaaggtgatatccgctctgaatacattgtggattgaaga cccgtcttgcctttccataaactcataatagtgatgaattgaaatctcgttata tggtttgacccaaaaggaaatcacacagacattgctggaagaacgattttcc gtaaaggtccattttgatgagatcaagactatctacaaagaacgacctataa aaaaggtcaataagattattcagatcgaagtaccaccaacccttactggg ccacaatagggctgactctgaacccttaccgttaggggcagggttgcaaat cgaaagtgcacatctcctatggttatctgaaccattctttcaaaatgccgttttg aagggttcgtatgtcttgccaatctggtttacatggatgggaagtgcagat ctgaaagtaacttttactcaagccgagtattatagcccgttaagtacacctgc tgatttcagacagctgaccccttatgtcttcaggctggctttgcaacagtcagg tgtggacattctgaaccgatgctctgttttgagttgcagataccccaagtagc

		<p>gagttccaaagctattacagattgcaaaaactgatgtctgagattgaagac atcagttgtaataatgagtgggtcatattaaagggaaagtccattaaataca agtaaagactatgcctcagaagtaagttcgtacactaagggcttaggcatttt tatggtaagccatgtgggtatcaaataacaaaagacggttattctgataatat ccgcatgaacgaaaaagataaaactttattcatgttccaaaatcaatgcatt aaaataa</p>
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Supplementary Table 5. Plasmids used in experiments.

Name	Description	Purpose	Figure
pNH9104	pNBU2-phes-P ₁ -NS-cas9-P _{BT1311} -rpiL [*] -nanoluc	First recombination	2, S2
pNH9040	pNBU2-phes-P ₁ -NS-cas9-P _{BT1311} -crRCN-rpiL [*] -nanoluc	First recombination	2, S2
pNH9058	pNBU2-phes-P ₁ -NS-cas9-P _{BT1311} -crR7N-rpiL [*] -nanoluc	First recombination	2, S2
pNH9059	pNBU2-phes-P ₁ -NS-cas9-P _{BT1311} -crR10N-rpiL [*] -nanoluc	First recombination	2, S2
pNH9060	pNBU2-phes-P ₁ -NS-cas9-P _{BT1311} -crR12N-rpiL [*] -nanoluc	First recombination	2, S2
pNH9106	pNBU2-phes-P ₁ -taRNA1-cas9-P _{BT1311} -crRCN-rpiL [*] -nanoluc	First recombination	2, S2, S3
pNH9107	pNBU2-phes-P ₁ -taRNA2-cas9-P _{BT1311} -crRCN-rpiL [*] -nanoluc	First recombination	2, S2, S3
pNH9108	pNBU2-phes-P ₁ -taRNA7-cas9-P _{BT1311} -crRCN-rpiL [*] -nanoluc	First recombination	2, S2, S3
pNH9109	pNBU2-phes-P ₁ -taRNA3-cas9-P _{BT1311} -crRCN-rpiL [*] -nanoluc	First recombination	2, S2, S3
pNH9142	pNBU2-phes-P ₁ -taRNA4-cas9-P _{BT1311} -crRCN-rpiL [*] -nanoluc	First recombination	2, S2, S3
pNH9143	pNBU2-phes-P ₁ -taRNA5-cas9-P _{BT1311} -crRCN-rpiL [*] -nanoluc	First recombination	2, S2, S3
pNH9144-2	pNBU2-phes-P ₁ -taRNA6-cas9-P _{BT1311} -crRCN-rpiL [*] -nanoluc	First recombination	2, S2, S3
pNH9169	pNBU2-phes-P _{BT1311} -NS-cas9-P ₁ -rpiL [*] -nanoluc	First recombination	2, 4, S2
pNH9168	pNBU2-phes-P _{BT1311} -NS-cas9-P ₁ -crRCN-rpiL [*] -nanoluc	First recombination	2, S2
pNH9188	pNBU2-phes-P _{BT1311} -taRNA6-cas9-P ₁ -crRCN-rpiL [*] -nanoluc	First recombination, Evaluation of HGT	2-7, S1-S11
pNH9195	pNBU2-phes-P _{cfxA} -sgRNA1	Second recombination	3, S2
pNH9196	pNBU2-phes-P _{cepA} -sgRNA1	Second recombination	3, 4, 6, 7, S1, S2, S4, S5, S6, S8, S9, S10, S11
pNH9198	pNBU2-phes-P _{cepA} -NT	Second recombination	4, 7 S2, S6,

			S10, S11
pYL100	pNBU2-NBU2 integrase- <i>attN</i> -P _{cepA} -sgRNA1-emR	Integration of a plasmid	4, S7
pYL114	pNBU2-NBU2 integrase- <i>attN</i> -P _{cepA} -NT-emR	Integration of a plasmid	4, S7
pYL101	pNBU2-NBU2 integrase- <i>attN</i> -P _{cepA} -sgRNA12-emR	Integration of a plasmid	4, S7
pYL103	pNBU2-NBU2 integrase- <i>attN</i> -P _{cepA} -sgRNA13-emR	Integration of a plasmid	4, S7
pYL106	pNBU2-NBU2 integrase- <i>attN</i> -P _{cepA} -sgRNA15-emR	Integration of a plasmid	4, S7
pYL107	pNBU2-NBU2 integrase- <i>attN</i> -P _{cepA} -sgRNA16-emR	Integration of a plasmid	4, S7
pYL112	pNBU2-NBU2 integrase- <i>attN</i> -P _{cepA} -sgRNA20-emR	Integration of a plasmid	4, S7
pNH9223	pNBU2-phes- <i>thyA</i> -emR	Evaluation of HGT	4, 6, 7 S6, S8, S11
pNH9233	pNBU2-phes-Mutated <i>thyA</i> -emR	Evaluation of HGT	4, 6, S6
pNH9225	pNBU2-phes-P _{BT1311} -taRNA6-P _{cfxA} -sgRNA1-cas9-P ₁ -crRCN-rpiL*-nanoluc	Evaluation of HGT	5
pNH9226	pNBU2-phes-P _{BT1311} -taRNA6-P _{cepA} -sgRNA1-cas9-P ₁ -crRCN-rpiL*-nanoluc	Evaluation of HGT	5, 7
pNH9227	pNBU2-phes-P _{BT1311} -taRNA6-P _{cepA} -NT-cas9-P ₁ -crRCN-rpiL*-nanoluc	Evaluation of HGT	5
pNH9216 (Cell Syst. 2015; 1(1): 62-71)	pNBU2-NBU2 integrase- <i>attN</i> -emR	Integration of a plasmid	7, S9, S10
pNH9230-2	pNBU2-NBU2 integrase- <i>attN</i> -tcR	Integration of a plasmid	7, S9

Supplementary Table 6. Primers used in experiments.

Name	Description	Figure	Sequence
Seq- <i>thyA</i> -F	PCR identification of horizontal gene transfer of the synthetic gene cassettes.	5	cactgtacgcagctaacac
mmD663	PCR identification of horizontal gene transfer of the synthetic gene cassettes.	5	atcaccagcgtaccgtaatg
qPCR-EmR-F	PCR identification of horizontal gene transfer of the EmR gene.	5, S8	aacagcaatgctagtgatctg
qPCR-EmR-R	PCR identification of horizontal gene transfer of the EmR gene.	5, S8	attggcagcataccttacaac
Rec-5'- <i>thyA</i> -F	PCR identification of integration of the synthetic gene cassettes.	S2	attcattgatttattcagcgccatc
Rec- <i>thyA</i> -3'-R	PCR identification of integration of the synthetic gene cassettes and the sgRNA1.	S2	agatgctttagatgagcaaattctg
CRI-sgRNA-3'-F	PCR identification of integration of the sgRNA1.	S2	ggaacgaatgggggttttagag
CRI-sgRNA5-3'-F	PCR identification of integration of the sgRNA (Non-targeting control).	S2	gtggattctctagactttacttgag
<i>thyA</i> -F	PCR identification of horizontal gene transfer of the <i>thyA</i> gene.	S6	tggtcggacatcagatgcgtttc
<i>thyA</i> -R	PCR identification of horizontal gene transfer of the <i>thyA</i> gene.	S6	ggaagaaggcatgacagggag