

Supplementary information

Regulating vitamin B12 biosynthesis via the *cbiMCbl* riboswitch in *Propionibacterium* strain UF1

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Supplemental material

Figures. S1 to S4

Table S1 to S2

Supplementary Figure Legends

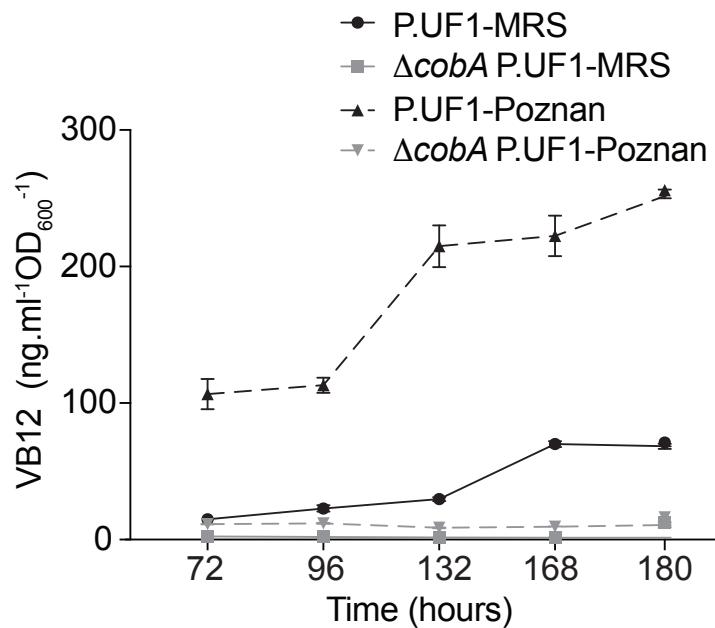


Figure S1 *cobA* is an essential gene for VB12 biosynthesis by P.UF1. Time-course analysis of VB12 production by P. UF1 and Δ cobA P. UF1 strains in MRS and Poznan medium. Data are representative of three independent experiments. Error bars indicate SEM.

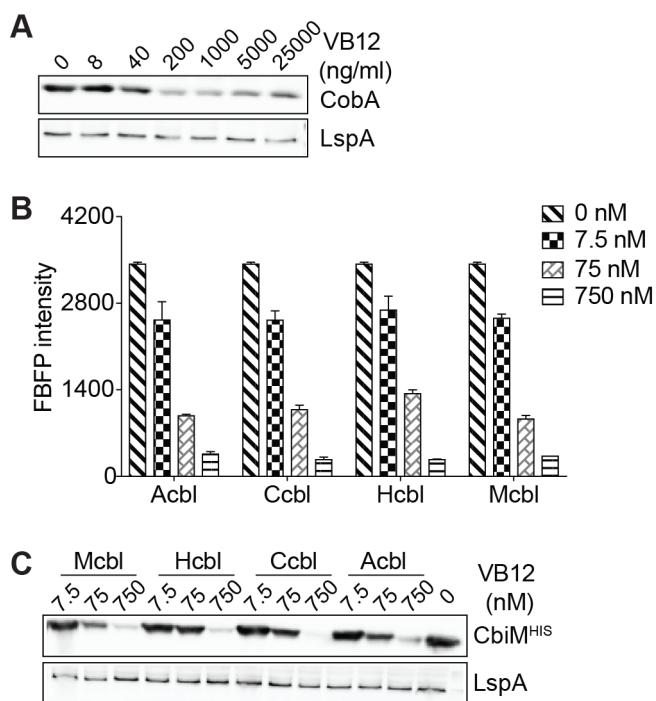


Figure S2 The *cobA* operon is feedback-regulated by VB12. (A) WB analysis of *cobA* expression of P. UF1 incubated with increasing VB12 (0-25000 ng/ml) using mouse serum antibodies against CobA. (B) FbFP fluorescence intensity of FbFP-Δ*cobA* P. UF1 strain incubated with different VB12 analogues. Acbl: adenosylcobalamin, Ccbl: Cyanocobalamin, Hcbl: Hydroxocobalamin, Mcbl: Methylcobalamin. (C) WB analysis of *cbiM* expression in CbiM-Δ*cobA* P. UF1 strain incubated with different VB12 analogues using anti-His antibody. Data are representative of three independent experiments (B). Error bars indicate SEM.

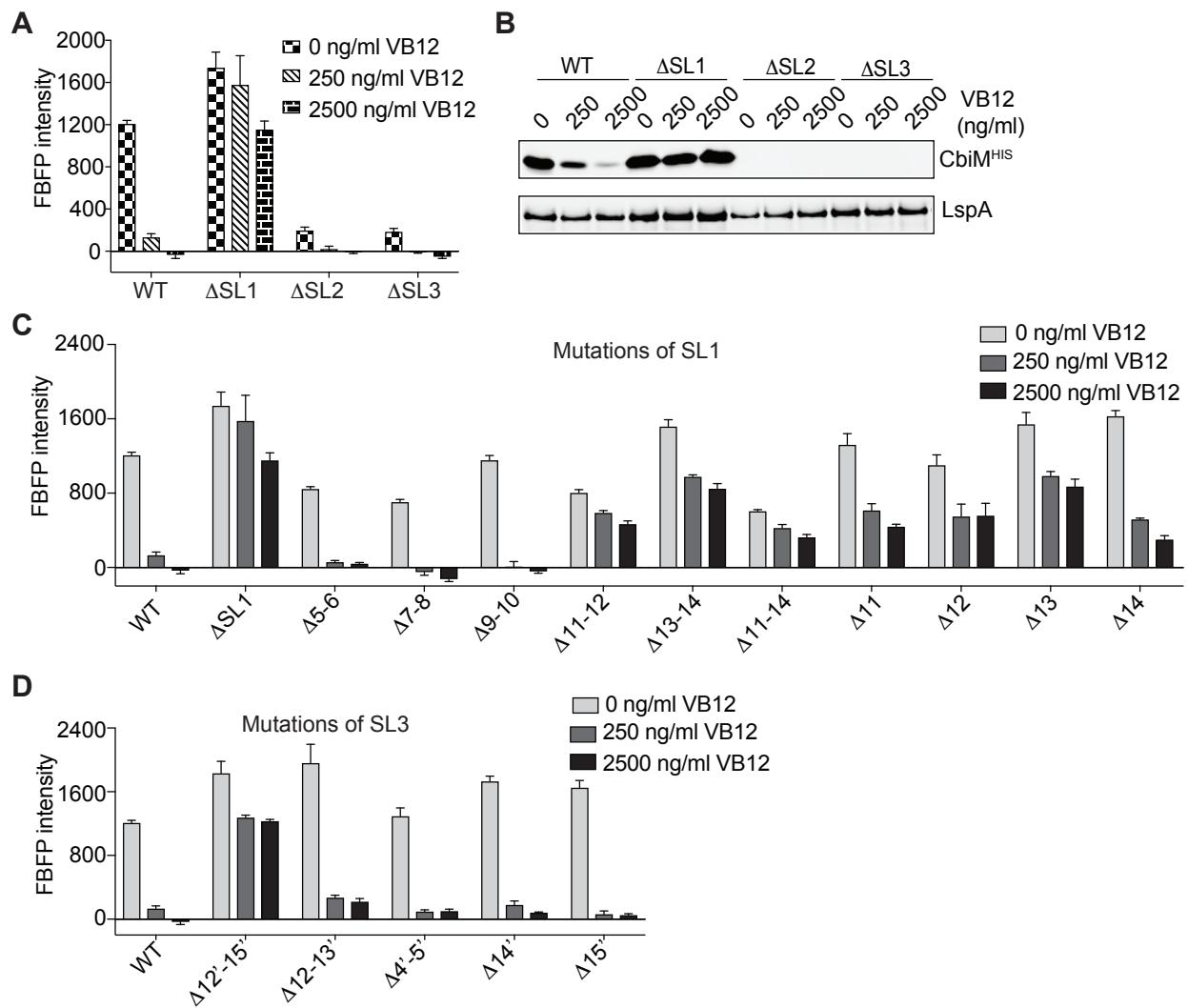


Figure S3 Key stem-loops of *cbiMCbl* riboswitch (A) FbFP fluorescence intensity of FbFP- Δ cobA P. UF1 strain incubated with various concentrations of VB12, wild-type, SL1, SL2 and SL3 deleted mutant *cbiMCbl* riboswitch. (B) WB analysis of *cbiM* expression of CbiM- Δ cobA P. UF1 strain incubated with various concentrations of VB12 using anti-His antibody, wild-type, SL1, SL2 and SL3 deleted mutant *cbiMCbl* riboswitch. (C) FbFP fluorescence intensity driven by SL1-mutated riboswitches in response to VB12 (0, 250, 2500 ng/ml). (D) FbFP fluorescence intensity directed by SL3-mutated riboswitches with various concentrations of VB12. Data are representative of three independent experiments (A, C and D).

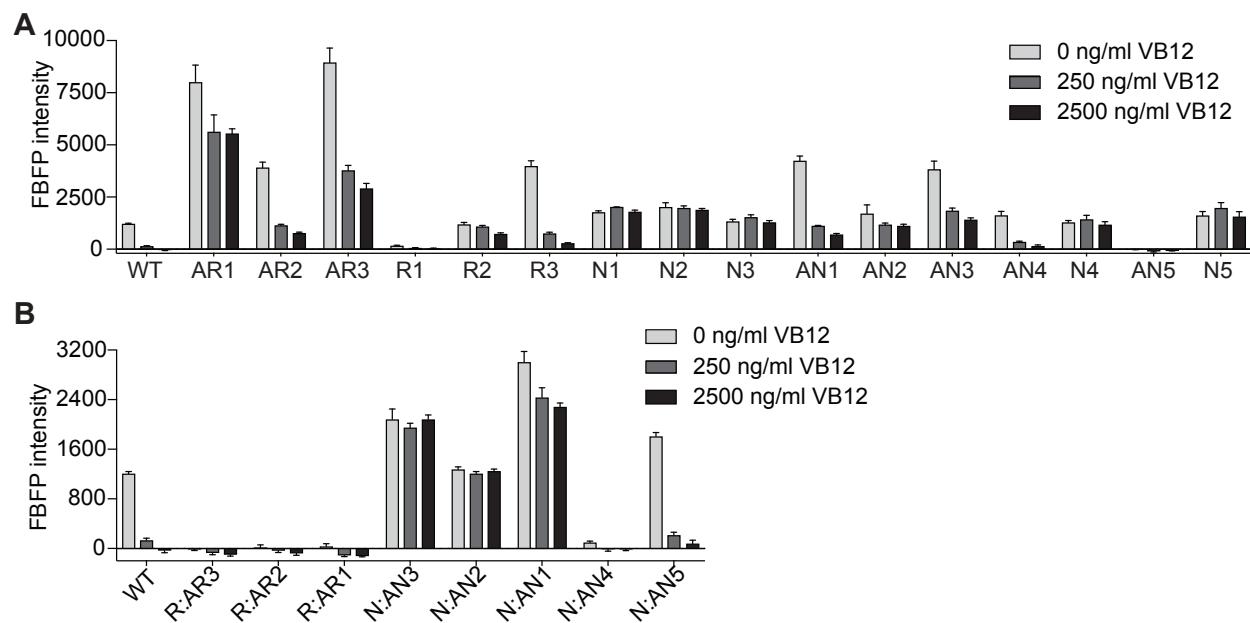


Figure S4 *cbiMCbl* riboswitch regulates *cbiM* expression by RBS-mediated base pairing. (A) FbFP fluorescence intensity by a series of riboswitches mutated in Pkn, Pkn', sequester, or antisequester. (B) FbFP fluorescence intensity by riboswitches with paired double mutations in Pkn and Pkn' or sequester and antisequester. Data are representative of three independent experiments (A and B). Error bars indicate SEM.

Table S1 P. UF1 derivative strains and plasmids used in this study

Plasmids/strains	Description
Plasmids	
pYMZ	Expressing vector in P. UF1, <i>hygB</i> ^R .
pUCC	Vector used for chromosomal insertion in P.UF1, derived of pUC19, <i>cm</i> ^R .
pUCH	Vector used for complementary expression in P.UF1, derived of pUC19, <i>hygB</i> ^R .
pET21b	Vector used for protein overexpression in <i>E. coli</i> , <i>Amp</i> ^R
Strains	
NEB 5-alpha	Competent <i>E. coli</i> used for gene cloning.
Rosetta <i>E. coli</i>	Protein overexpression
E-21CobA	Rosetta <i>E. coli</i> overexpressing CobA protein
P. UF1	Propionibacterium UF1 was isolated from human breast milk.
ΔcobA P. UF1	Deletion of <i>cobA</i> gene in P. UF1 using pUCC
C-ΔcobA P. UF1	Complementary expression of <i>cobA</i> gene in ΔcobA P. UF1 by the native promoter of <i>cobA</i> using pUCH.
FbFP-WT	Transforming pYMZ harboring <i>cbl</i> riboswitch and <i>FbFP</i> into P. UF1.
CbiM-WT	Transforming pYMZ harboring <i>cbl</i> riboswitch and <i>cbl</i> ^{HIS6} into P. UF1.
FbFP-ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch and <i>FbFP</i> into ΔcobA P. UF1.
CbiM-ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch and <i>cbl</i> ^{HIS6} into ΔcobA P. UF1.
CbiN-ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch and <i>cbl</i> ^{HIS6} into ΔcobA P. UF1.
CbiO-ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch and <i>cbl</i> ^{HIS6} into ΔcobA P. UF1.
CobA-ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch and <i>cobA</i> ^{HIS6} into ΔcobA P. UF1.
DSL1F-ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch deleting SL1 mutant and <i>FbFP</i> into ΔcobA P. UF1.
DSL1CM ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch deleting SL1 mutant and <i>cbl</i> ^{HIS6} into ΔcobA P. UF1.
DSL2F ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch deleting SL2 mutant and <i>FbFP</i> into ΔcobA P. UF1.
DSL2CM ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch deleting SL2 mutant and <i>cbl</i> ^{HIS6} into ΔcobA P. UF1.
DSL3F ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch deleting SL3 mutant and <i>FbFP</i> into ΔcobA P. UF1.
DSL3CM ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch deleting SL3 mutant and <i>cbl</i> ^{HIS6} into ΔcobA P. UF1.
D5-6F ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch D5-6 mutant and <i>FbFP</i> into ΔcobA P. UF1.
D5-6CM ΔcobA	Transforming pYMZ harboring <i>cbl</i> riboswitch D5-6 mutant and <i>cbl</i> ^{HIS6} into ΔcobA P. UF1.
D7-8FΔcobA	

D7-8CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D7-8 mutant and <i>FbFP</i> into Δ cobA P. UF1.
D9-10F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D7-8 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D9-10CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D9-10 mutant and <i>FbFP</i> into Δ cobA P. UF1.
D11-12F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D9-10 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D11-12CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D11-12 mutant and <i>FbFP</i> into Δ cobA P. UF1.
D13-14F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D11-12 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D13-14CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D13-14 mutant and <i>FbFP</i> into Δ cobA P. UF1.
D11-14F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D13-14 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D11-14CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D11-14 mutant and <i>FbFP</i> into Δ cobA P. UF1.
D11F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D11-14 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D11CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D11 mutant and <i>FbFP</i> into Δ cobA P. UF1.
D12F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D11 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D12CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D12 mutant and <i>FbFP</i> into Δ cobA P. UF1.
D13F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D12 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D13CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D13 mutant and <i>FbFP</i> into Δ cobA P. UF1.
D14F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D13 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D14CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D14 mutant and <i>FbFP</i> into Δ cobA P. UF1.
D12'-15'F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D13 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D12'-15'CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D12'-15' mutant and <i>FbFP</i> into Δ cobA P. UF1.
D12'-13'F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D12'-15' mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D12'-13'CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D12'-13' mutant and <i>FbFP</i> into Δ cobA P. UF1.
D4'-5'F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D12'-13' mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D4'-5'CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D4'-5' mutant and <i>FbFP</i> into Δ cobA P. UF1.
D14'F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D4'-5' mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.

D14'CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D14' mutant and <i>FbFP</i> into Δ cobA P. UF1.
D15'F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D14' mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
D15'CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D15' mutant and <i>FbFP</i> into Δ cobA P. UF1.
N1F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch D15' mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
N1CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N1 mutant and <i>FbFP</i> into Δ cobA P. UF1.
N2F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N1 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
N2CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N2 mutant and <i>FbFP</i> into Δ cobA P. UF1.
N3F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N2 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
N3CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N3 mutant and <i>FbFP</i> into Δ cobA P. UF1.
N4F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N3 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
N4CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N4 mutant and <i>FbFP</i> into Δ cobA P. UF1.
N5F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N4 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
N5CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N5 mutant and <i>FbFP</i> into Δ cobA P. UF1.
AN1F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N5 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
AN1CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AN1 mutant and <i>FbFP</i> into Δ cobA P. UF1.
AN2F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AN1 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
AN2CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AN2 mutant and <i>FbFP</i> into Δ cobA P. UF1.
AN3F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AN2 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
AN3CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AN3 mutant and <i>FbFP</i> into Δ cobA P. UF1.
AN4F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AN3 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
AN4CM Δ cob	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AN4 mutant and <i>FbFP</i> into Δ cobA P. UF1.
AN5F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AN4 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
AN5CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AN5 mutant and <i>FbFP</i> into Δ cobA P. UF1.
N:AN1F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AN5 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
N:AN1CM Δ cobA	

N:AN2F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N:AN1mutant and <i>FbFP</i> into Δ cobA P. UF1.
N:AN2CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N:AN1 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
N:AN3F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N:AN2 mutant and <i>FbFP</i> into Δ cobA P. UF1.
N:AN3CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N:AN2 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
N:AN4F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N:AN3 mutant and <i>FbFP</i> into Δ cobA P. UF1.
N:AN4CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N:AN3 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
N:AN5F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N:AN4mutant and <i>FbFP</i> into Δ cobA P. UF1.
N:AN5CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N:AN4 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
R1F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch N:AN5 mutant and <i>FbFP</i> into Δ cobA P. UF1.
R1CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R1mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
R2F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R1mutant and <i>FbFP</i> into Δ cobA P. UF1.
R2CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R2mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
R3F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R2mutant and <i>FbFP</i> into Δ cobA P. UF1.
R3CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R3mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
AR1F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R3mutant and <i>FbFP</i> into Δ cobA P. UF1.
AR1CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AR1mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
AR2F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AR1mutant and <i>FbFP</i> into Δ cobA P. UF1.
AR2CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AR2mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
AR3F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AR2mutant and <i>FbFP</i> into Δ cobA P. UF1.
AR3CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AR3mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
R:AR1F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch AR3mutant and <i>FbFP</i> into Δ cobA P. UF1.
R:AR1CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R:AR1mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
R:AR2F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R:AR1mutant and <i>FbFP</i> into Δ cobA P. UF1.
R:AR2CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R:AR1mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.

R:AR3F Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R:AR2 mutant and <i>FbFP</i> into Δ cobA P. UF1.
R:AR3CM Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R:AR2 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
R:AR3CO Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R:AR3 mutant and <i>FbFP</i> into Δ cobA P. UF1.
R:tR Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R:AR3 mutant and <i>cbiM</i> ^{HIS6} into Δ cobA P. UF1.
R:AR3tR Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R:AR3 mutant and <i>cbiO</i> ^{HIS6} into Δ cobA P. UF1.
OW-operon Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch and <i>tetR</i> into Δ cobA P. UF1.
ODSL1-operon Δ cobA	Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch R:AR3 mutant and <i>tetR</i> into Δ cobA P. UF1. Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch and <i>cobA</i> operon into Δ cobA P. UF1. Transforming pYMZ harboring <i>cbiMCbl</i> riboswitch deleting SL1 mutant and <i>cobA</i> operon into Δ cobA P. UF1.

Table S2 Primers used in this study

Name	Sequence (5'-3')	Usage
cobAbam-F	CCCGGATCCctcgccggccggccggc	Deletion of <i>cobA</i> gene in P.
cobAxba-R	CCCTCTAGAgcggacgcacgtggcgccgtcca	UF1
P1	gtggcactcggtgatctt	For identifying <i>cobA</i> mutant
P2	GAGCGAGGAAGCGGAAGAG	and complementary strain
P3	ccccggccctacaaggacatcga	
P4	ccggccctctgtgatgttctgg	
Amy-cobA-1	CCCGGATCCtcagacgcccggcgccacc	For constructing strain for
Amy-cobA-2	cgtggggccgtcgtagtgggacgtcatgtgagcgactggcgacgaga	complementary expression of
Amy-cobA-3	tctcgccagtcgtcacatgcacgtcccactacgacggcccccacg	<i>cobA</i> gene in Δ cobA P.
Amy-cobA-4	gccggcaacagtgtgggtcatgacgcacgatcccggagaaaggaaatgcc	
Amy-cobA-5	ggcattccttcggatcgtgcgtcatgaccaccacactgtggccggc	
Amy-cobA-6	CCCTCTAGAtcagtggcgctggcgccgat	
21-cobA-F	CCCGGATCCatgaccaccacactgttgccggca	Overexpression of <i>cobA</i> in
21-cobA-R	GCGCTCGAGgtggcgctggcgccgtgg	<i>E.coli</i>
S-cobA-1	CCCGGATCCcgccactacgacggccc	For constructing FbFP-WT and CbiM-WT mutants
S-cobA-22	TGGTGGTGCACGAGCCCCATggcattttctcggtatcg	For constructing FbFP-WT
S-cobA-33	cacatcccggagaaaggaaatgccATGGGCTCGTCGCACCACCA	mutant
S-cobA-4	CCCTCTAGATCAGTGCTTGGCCTGGCCCT	For constructing FbFP-WT mutant
ScbiM-5	CCCTCTAGAtcaGTGGTGGTGGTGGTGGTggacatgtgccacctccggAACCT	For constructing W-CM mutant
DSL1-F	gttcacagtggcgccaccgtg cttcctgcaccacggcgagg	For constructing DSL1F/CM mutants
DSL1-R	cctcgcccggtggcgccggaaag cacggcgccactgtgaac	
DSL2-F	ggggcatttgctccggaaatgtt tcggctgaaccgacttctgcac	For constructing DSL2F/CM mutants
DSL2-R	gtgcaggaaagtgggtcaaggccgaa acatttcgagccaaatggccc	
DSL3-F	gtccgtggccaggcttactt gttcacagtggcgccaccgtg	For constructing DSL3F/CM mutants
DSL3-R	cacggcgccactgtgaac agagtccggaaactggccacggc	
SL1-5-6F	tgcggcggtgcaggaaagtggcGCcaaggccacccgtcgccactgtgaacatttc	For constructing D5-6F/CM mutants
SL1-5-6R	gaaatgttcacagtggcgccaccgtgtcgGCCCacttctgcaccacggcgA	
SL1-7-8F	TgcggcggtgcaggaaagtgggtTGagccgacacggcgccactgtgaacatttc	For constructing D7-8F/CM mutants
SL1-7-8R	gaaatgttcacagtggcgccaccgtgtcggtCAAaccgacttctgcaccacggcgA	
SL1-9-10F	TgcggcggtgcaggaaagtgggtcaCTccgacacggcgccactgtgaacatttc	For constructing D9-10F/CM mutants
SL1-9-10R	gaaatgttcacagtggcgccaccgtgtcgAGtgaaccgacttctgcaccacggcgA	
SL1-11-12F	TgcggcggtgcaggaaagtgggtcaagTAagacacggcgccactgtgaacatttc	For constructing D11-12F/CM mutants
SL1-11-12R	gaaatgttcacagtggcgccaccgtgtcTActtgaaccgacttctgcaccacggcgA	
SL1-13-14F	TgcggcggtgcaggaaagtgggtcaagccTGacggcgccactgtgaacatttc	For constructing D13-14F/CM mutants
SL1-13-14R	gaaatgttcacagtggcgccaccgtgCAGgtgaaccgacttctgcaccacggcgA	
SL1-11-14F	tgcggcggtgcaggaaagtgggtcaagATAGcacggcgccactgtgaacatttc	For constructing D11-14F/CM mutants
SL1-11-14R	gaaatgttcacagtggcgccaccgtgCTATcttgaaccgacttctgcaccacggcgA	
A9G-F	tgcggcggtgcaggaaagtgggtca GggcgcacacggcgccactgtgaaCA	For constructing D9F/CM mutants
A9G-R	TGttcacagtggcgccaccgtgtcgccCtgaaccgacttctgcaccacggcgA	
C11A-F	gcccggtgcaggaaagtgggtcaagAcgacacggcgccactgtgaacatt	For constructing D11F/CM mutants
C11A-R	aatgttcacagtggcgccaccgtgtcgTcttgaaccgacttctgcaccacggcg	
C12A-F	gcccggtgcaggaaagtgggtcaagcAgacacggcgccactgtgaacatt	For constructing D12F/CM mutants
C12A-R	aatgttcacagtggcgccaccgtgtcTgcttgaaccgacttctgcaccacggcg	
G10T-F	tgcggcggtgcaggaaagtgggtca aTccgacacggcgccactgtgaaCA	For constructing D10F/CM mutants
G10T-R	TGttcacagtggcgccaccgtgtcggttcaaccgtgtcgaccacggcgA	

G13T-F	cccggtgtcaggaagtccgttcaagcc Tacacggtcgcgccactgtaacattcg	For constructing D13'F/CM mutants
G13T-R	cgaaaatgttcacagtggcgccgaccgtgtAggcttaaccgacttcctgcaccacgg	
A14G-F	cccggtgtcaggaagtccgttcaagcc gGcacggtcgcgccactgtaacattcg	For constructing D14F/CM mutants
A14G-R	cgaaaatgttcacagtggcgccgaccgtgCccgcttaaccgacttcctgcaccacgg	
SL3-DF	gttccgtactctcatcgccccccgtccgaaatgttcacagtggcg	For constructing D12'-15'F/CM mutants
SL3-DR	gcccactgtgaacattcgagc gcccccgatgagagtcaagaa	
SL3-LSF	cgcgcactgtgaacattcgagcGGatgcccccgatgagagtcaagaa	For constructing D12'-13'F/CM mutants
SL3-LSR	ttctgactctcatcgccccccatCCcgctccgaaatgttcacagtggcg	
SL3-SSF	acacggtcgcgccactgtgaacatCCcgagcaaatgcccccgatga	For constructing D4'-5'F/CM mutants
SL3-SSR	catcgccccccatgtccgGGatgttacagtggcgaccgtgt	
3AGF	tgcgcactgtgaacattcgagcaaGtgcgcgcgtgagagtcaggaact	For constructing D14'F/CM mutants
3AGR	agttccgtactctcatcgcccccaCttgtccgaaatgttcacagtggcg	
4TCF	tgcgcactgtgaacattcgagcaaCgcgcgcgtgagagtcaggaact	For constructing D15'F/CM mutants
4TCR	agttccgtactctcatcgccccGttgtccgaaatgttcacagtggcg	
2-T-F	gaagtccgttcaagccgacacgt TATAT cactgtgaacattcgagcaaatgcccc	For constructing N3F/CM mutants
2-T-R	ggggcatttgctccgaaatgttcacagtgATATAaccgtgtcggttcaaccgacttc	
2-AT-F	aactggccacggacgagcccttcaa ATATAaggacgcacgatcccagaaaggaatg	For constructing N2F/CM mutants
2-AT-R	cattcccttcggatctgtcggttccTATATTtggaaaggctgtccgtggccagt	
3-T-F	gaagtccgttcaagccgacacgt GGGGC cactgtgaacattcgagcaaatgcccc	For constructing N1F/CM mutants
3-T-R	ggggcatttgctccgaaatgttcacagtgGCCCCaccgtgtcggttcaaccgacttc	
3-AT-F	aactggccacggacgagcccttcaa GCCCCCggacgcacgatcccagaaaggaatg	For constructing AN3F/CM and N-AN3F/CM mutants
4-AT-R	cattcccttcggatctgtcggttccGGGGCttgaaaggctgtccgtggccagt	
4-T-F	gaagtccgttcaagccgacacgt GGAGC cactgtgaacattcgagcaaatgcccc	For constructing AN2F/CM and N-AN2F/CM mutants
4-T-R	ggggcatttgctccgaaatgttcacagtgGCTCCaccgtgtcggttcaaccgacttc	
4-AT-F	aactggccacggacgagcccttcaa GCTCCggacgcacgatcccagaaaggaatg	For constructing AN1F/CM and N-AN1F/CM mutants
4-AT-R	cattcccttcggatctgtcggttccGGAGCttgaaaggctgtccgtggccagt	
SL2-DLF	aagtccgttcaagccgacacgt gctcgactgtgaacattcgagcaaatgcccc	For constructing N5F/CM mutants
SL2-DLR	ggcatttgctccgaaatgttcacagtgaccgtgtcggttcaaccgact	
ASL2-DLF	aactggccacggacgagcccttcaaggacgcacgatcccagaaaggaatg	For constructing AN5F/CM and N-AN5F/CM mutants
ASL2-DLR	cattcccttcggatctgtcggttccGAGCttgaaaggctgtccgtggccagt	
SL2-SLF	Aagtccgttcaagccgacacgt gctcgactgtgaacattcgagcaaatgcccc	For constructing N4F/CM mutants
SL2-SLR	ggcatttgctccgaaatgttcacagtgaccgtgtcggttcaaccgact	
ASL2-SLF	Aactggccacggacgagcccttcaacgcacgatcccagaaaggaatg	For constructing AN4F/CM and N-AN4F/CM mutants
ASL2-SLR	cattcccttcggatctgtcggttccGAGCttgaaaggctgtccgtggccagt	
A-site-F	GagtccgttcaacgcacgatcccagccTcaaggccggacgcacgatccc	For constructing AR2F/CM and N-AR2F/CM mutants
A-site-R	gggatctgtcggttcccgcttgaGaggctgtccgtggccagtccctactC	
A-region-F	gagtcaggaacttgccacggacggTTCCCTtcaaggccggacgcacgatccc	For constructing AR3F/CM and N-AR3F/CM mutants
A-region-R	gggatctgtcggttcccgcttgaAGGAAActgtccgtggccagtccctactC	
R-S-cbimF	aagcggggacgcacgatcccagaGaggaatgcccgtcatatgcagaaggcgt	For constructing AR2CM mutant
R-S-cbimR	acgccttcgtcgatatgcacggcattcctCtctcggtatctgtcggtcccgctt	
R-R-cbimF	caagcggggacgcacgatcccagaAGGAAatgcccgtcatatgcagaaggcgt	For constructing AR3CM mutant
R-R-cbimR	acgccttcgtcgatatgcacggcattTCCTtcggatctgtcggtcccgctt	
R-S-GFPF	caagcggggacgcacgatcccagaGaggaatgcccgtcatatgcagaaggcgt	For constructing AR2F mutant
R-S-GFPR	TGGTGGTGCACGAGCCCCATggcatttcctCtctcggtatctgtcggtcccgctt	
R-R-GFPF	caagcggggacgcacgatcccagaAGGAAatgcccgtcatatgcagaaggcgt	For constructing AR3F mutant
R-R-GFPR	TGGTGGTGCACGAGCCCCATggcattTCCTtcggatctgtcggtcccgctt	
NR-GPF-F	aagcggggacgcacgatcccacttaggaatgccATGGGCTCGTCGCCACCACCA	For constructing AR1F mutant
NR-GPF-R	TGGTGGTGCACGAGCCCCATggcatttcctAAGtcggatctgtcggtcccgctt	
NR-cbiM-F	aagcggggacgcacgatcccagaCTTaggaatgcccgtcatatgcagaaggcgt	For constructing AR1CM mutant
NR-cbiM-R	acgccttcgtcgatatgcacggcattcctAAGtcggatctgtcggtcccgctt	
ANR-F	aggaactggccacggacgcctAAGaagcggggacgcacgatcccaga	

ANR-R	tcgggatcggtcccgccgttCTTaggctcgccgtggccagttcct	For constructing AR1F/CM
w-cbiOF	gacgcacgatcccgagaaaggaaatgcCatgagccctgctggccgccccac	and N-AR1F/CM mutants
w-cbiOR	gtgggcggccagcaggcgctcatGgcattcccttcgggatcgatcg	For constructing CbiO- Δ cobA
w-tetRF	gacgcacgatcccgagaaaggaaatgcCatGTCCCGCCTCGACAAGTCCAAGGT	
w-tetRR	ACCTTGGACTTGTGAGGCAGGACATGgcattcccttcgggatcgatcg	For constructing tetR- Δ cobA
tetR-HidR	GGCAAGCTTTAGGAGGCCGGACTCGCACTTGAGCTG	
cbiN-hid	CCCAAGCTTcaGTGGTGGTgggggtggggcgatccggggggccgtcggtc	For constructing R-AR3tR and tetR-WT
cbiQ-hid	CCCAAGCTTcaGTGGTGGTGGTGGTgggtcgccaccaccaggctgatcg	For constructing CbiN- Δ cobA mutant
cbiO-hid	CCCAAGCTTcaGTGGTGGTGGTGGTGGTgggtcgccaccaccaggctgatcg	For constructing CbiQ- Δ cobA mutant
cobA-hid	CCCAAGCTTcaGTGGTGGTGGTGGTGGTgggtcgctggcgccgatgg	For constructing CbiO- Δ cobA mutant
m-cbiOF	gacgcacgatcccgagagagaaatgcCatgagccctgctggccgccccac	For constructing CobA- Δ cobA mutant
m-cbiOR	gtgggcggccagcaggcgctcatGgcattcccttcgggatcgatcg	
m-tetRF	gacgcacgatcccgagagagaaatgcCatGTCCCGCCTCGACAAGTCCAAGGT	For constructing R-AR3CO mutant
m-tetRR	ACCTTGGACTTGTGAGGCAGGACATGgcattcccttcgggatcgatcg	For constructing R-AR3tR mutant
GroL2-RT-F	CAATGTCGTGTTGGAGAAG	qPCR
GroL2-RT-R	CGCCGATCTTGTGGTAGG	qPCR
qcobiM-F	ctcatcggtcgatcttcca	qPCR
qcobiM-R	GAGCTTCTTGTGAGCACATAG	qPCR
qcobiN-F	GGTTCAGCCGCTGTT	qPCR
qcobiN-R	CCCAGGCAGTAGAACATGATG	qPCR
qcobiQ-F	CCATCGTGGCTTCGAGAC	qPCR
qcobiQ-R	CCACCAGGCTGATCGAC	qPCR
qcobiO-F	TGCACCAGATGCGTGAC	qPCR
qcobiO-R	TCACAGACGATAGCGACCT	qPCR
qcobA-F	CCAGGAGGAGATCAACCAAC	qPCR
qcobA-R	GCCCCGAAGACGAACGAG	qPCR
cbop-hid-R	GGCAAGCTTtcagtggtcgctggcgccgat	qPCR
cbop-sbf-F	GCGCCTGCAGGcgcccactacgacggccccacgg	For constructing OW-operon/ODSL1-operon mutants