Supplementary Data

Developing an endogenous quorum-sensing based CRISPRi circuit for autonomous and tunable dynamic regulation of multiple targets in *Streptomyces*

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23 Supplementary Table S1. Strains and plasmids used in this study

Strains and plasmids	**Genotype**	**References**
Strains		
E. coli strains		
DH5α	F-*Φ*80 *lacZ*ΔM15 Δ(*lacZYA-argF*) *U169 deoR recA1 endA1 hsdR17 supE44* λ^- *thi-1 gyrA96 relA1*	Gicbo-BRL
ET12567/pUZ8002	*dam-13*::Tn9 *dcm-6 hsdM*; harboring the non-transmissible RP4 derivative plasmid pUZ8002	(1)
Streptomyces		
S. rapamycinicus 2001	The parental strain derived from the wild-type *S. rapamycinicus* NRRL 5491	This study
2001/*srbAp-dcas9*	2001 with the control plasmid pSET-*srbAp-dcas9*	This study
2001/*srbAp-dcas9*-3×Flag	2001 with the plasmid pSET-*srbAp-dcas9*-3×Flag	This study
2001/*ermEp*-dcas9*	2001 with the control plasmid pSET-*ermEp*-dcas9*	This study
2001/*srbAp-dcas9*-3×Flag	2001 with the plasmid pSET-*ermEp*-dcas9*-3×Flag	This study
2001/*srbA*-3×Flag	2001 with the 3×Flag tag sequence inserted into the C-terminal of *srbA* before the stop codon	This study
2001/*srbR*-3×Flag	2001 with the 3×Flag tag sequence inserted into the C-terminal of *srbR* before the stop codon	This study
2001/*sg-fabH1*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-fabH1*	This study
2001/*sg-fabH2*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-fabH2*	This study
2001/*sg-fabH3*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-fabH3*	This study
2001/*ermEp*/sg-fabH1*	2001 with the *ermEp**-driving CRISPRi plasmid pSET-*ermEp*-dcas9/sg-fabH1*	This study
2001/*ermEp*/sg-fabH2*	2001 with the *ermEp**-driving CRISPRi plasmid pSET-*ermEp*-dcas9/sg-fabH2*	This study
2001/*ermEp*/sg-fabH3*	2001 with the *ermEp**-driving CRISPRi plasmid pSET-*ermEp*-dcas9/sg-fabH3*	This study
2001/*sg-gltA*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-gltA*	This study
2001/*sg-gltA1*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-gltA1*	This study
2001/*sg-gltA2*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-gltA2*	This study
2001/*ermEp*/sg-gltA*	2001 with the *ermEp**-driving CRISPRi plasmid pSET-*ermEp*-dcas9/sg-gltA*	This study
2001/*ermEp*/sg-gltA1*	2001 with the *ermEp**-driving CRISPRi plasmid pSET-*ermEp*-dcas9/sg-gltA1*	This study
2001/*ermEp*/sg-gltA2*	2001 with the *ermEp**-driving CRISPRi plasmid pSET-*ermEp*-dcas9/sg-gltA2*	This study
2001/*sg-cm1*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-cm1*	This study
2001/*sg-cm2*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-cm2*	This study
2001/*sg-cm3*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-cm3*	This study
2001/*sg-cm4*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-cm4*	This study
2001/*ermEp*/sg-cm1*	2001 with the *ermEp**-driving CRISPRi plasmid pSET-*srbAp-dcas9/sg-cm1*	This study

2001/*ermEp*/sg-cm2*	2001 with the *ermEp**-driving CRISPRi plasmid pSET-*srbAp-dcas9/sg-cm2*	This study
2001/*ermEp*/sg-cm3*	2001 with the *ermEp**-driving CRISPRi plasmid pSET-*srbAp-dcas9/sg-cm3*	This study
2001/*ermEp*/sg-cm4*	2001 with the *ermEp**-driving CRISPRi plasmid pSET-*srbAp-dcas9/sg-cm4*	This study
2001/*sg1-fabH3*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg1-fabH3*	This study
2001/*sg2-fabH3*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg2-fabH3*	This study
2001/*sg3-fabH3*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg3-fabH3*	This study
2001/*sg1-gltA2*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg1-gltA2*	This study
2001/*sg2-gltA2*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg2-gltA2*	This study
2001/*sg3-gltA2*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg3-gltA2*	This study
2001/*sg1-cm2*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg1-cm2*	This study
2001/*sg2-cm2*	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg2-cm2*	This study
2002	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-fabH3//*sg-*gltA2/sg-cm2*	This study
2003	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-fabH3/sg-gltA2/sg2-cm2*	This study
2004	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-fabH3/sg2-gltA2/sg-cm2*	This study
2005	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-fabH3/sg2-gltA2/sg2-cm2*	This study
2006	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-fabH3/sg3-gltA2/sg-cm2*	This study
2007	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg-fabH3/sg3-gltA2/sg2-cm2*	This study
2008	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg2-fabH3/sg-gltA2/sg-cm2*	This study
2009	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg2-fabH3/sg-gltA2/sg2-cm2*	This study
2010	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg2-fabH3/sg2-gltA2/sg-cm2*	This study
2011	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg2-fabH3/sg2-gltA2/sg2-cm2*	This study
2012	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg2-fabH3/sg3-gltA2/sg-cm2*	This study
2013	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg2-fabH3/sg3-gltA2/sg2-cm2*	This study
2014	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg3-fabH3/sg-gltA2/sg-cm2*	This study
2015	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg3-fabH3/sg-gltA2/sg2-cm2*	This study
2016	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg3-fabH3/sg2-gltA2/sg-cm2*	This study

2017	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg3-fabH3/sg2-gltA2/sg2-cm2*	This study
2018	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg3-fabH3/sg3-gltA2/sg-cm2*	This study
2019	2001 with the EQCi plasmid pSET-*srbAp-dcas9/sg3-fabH3/sg3-gltA2/sg2-cm2*	This study
Streptomyces coelicolor M145	Wild type; $SCP1^{-}$ $SCP2^{-}$ *pgl*$^{+}$	(2)
M145/*scbAp-dcas9*	M145 with the control EQCi plasmid pSET-*scbAp-dcas9*	This study
M145/*sg-gltA(sco)*	M145 with the control EQCi plasmid pSET-*scbAp-dcas9/sg-gltA(sco)*	This study
Plasmids		
pSET152	An integrative plasmid, *acc(3)IV*, *oriT* RK2 , ΦC31 integrase/*attP*	(2)
<u>Reporter plasmids</u>		
pSET-*srbAp-lacZ*	A reporter plasmid, in which the codon optimized thermophilic *lacZ* gene under the control of the GBL-responsive promoter *srbAp* was cloned between *Nde*I and *Xba*I	This study
pSET-*ermEp*-lacZ*	A reporter plasmid, in which the codon optimized thermophilic *lacZ* gene under the control of the constitutive strong promoter *ermEp** was cloned between *Nde*I and *Xba*I	This study
<u>*ermEp**-driving CRISPRi plasmids</u>		
pSET-*dcas9-actII4-NT-S1*	A pSET152-derived CRISPRi plasmid, in which dCas9 is under the control of *ermEp** and the sgRNA *actII4-NT-S1* transcription cassette (targeting *actII-ORF4*, a pathway-specific activator for actinorhodin biosynthesis in *Streptomyces coelicolor*) is under the control of *j23119p*	(3)
pSET-*ermEp*-dcas9*	A control CRISPRi plasmid, in which dCas9 is under the control of *ermEp** and the sgRNA without N20 guide sequence is under the control of *j23119p*	(3)
pSET-*ermEp*-dcas9*-3×Flag	pSET-*ermEp*-dcas9* harboring the 3×Flag tag sequence inserted into the C-terminal of *dcas9* before the stop codon	This study
pSET-*ermEp*-dcas9/sg-fabH1*	pSET-*ermEp*-dcas9* harboring the expression cassette of the sgRNA *sg-fabH1* targeting the nucleotide position of 8-27 on the non-template strand of *fabH1*	This study
pSET-*ermEp*-dcas9/sg-fabH2*	pSET-*ermEp*-dcas9* harboring the expression cassette of the sgRNA *sg-fabH2* targeting the nucleotide position of 27-46 on the non-template strand of *fabH2*	This study
pSET-*ermEp*-dcas9/sg-fabH3*	pSET-*ermEp*-dcas9* harboring the expression cassette of the sgRNA *sg-fabH3* targeting the nucleotide position of 179-198 on the non-template strand of *fabH3*	This study
pSET-*ermEp*-dcas9/sg-gltA*	pSET-*ermEp*-dcas9* harboring the expression cassette of the sgRNA *sg-gltA* targeting the nucleotide position of 137-156	This study

	on the non-template strand of *gltA*	
pSET-*ermEp*-dcas9/sg-gltA1*	pSET-*ermEp*-dcas9* harboring the expression cassette of the sgRNA *sg-gltA1* targeting the nucleotide position of 8-27 on the non-template strand of *gltA1*	This study
pSET-*ermEp*-dcas9/sg-gltA2*	pSET-*ermEp*-dcas9* harboring the expression cassette of the sgRNA *sg-gltA2* targeting the nucleotide position of 197-216 on the non-template strand of *gltA2*	This study
pSET-*ermEp*-dcas9/sg-cm1*	pSET-*ermEp*-dcas9* harboring the expression cassette of the sgRNA *sg-cm1* targeting the nucleotide position of 32-51 on the non-template strand of *cm1*	This study
pSET-*ermEp*-dcas9/sg-cm2*	pSET-*ermEp*-dcas9* harboring the expression cassette of the sgRNA *sg-cm2* targeting the nucleotide position of 149-168 on the non-template strand of *cm2*	This study
pSET-*ermEp*-dcas9/sg-cm3*	pSET-*ermEp*-dcas9* harboring the expression cassette of the sgRNA *sg-cm3* targeting the nucleotide position of 51-70 on the non-template strand of *cm3*	This study
pSET-*ermEp*-dcas9/sg-cm4*	pSET-*ermEp*-dcas9* harboring the expression cassette of the sgRNA *sg-cm4* targeting the nucleotide position of 22-41 on the non-template strand of *cm4*	This study
<u>EQCi plasmids</u>		
pSET-*srbAp-dcas9*	A control EQCi plasmid, in which dCas9 is under the control of *srbAp* and the transcription of the sgRNA without N20 guide sequence is under the control of *j23119p*	This study
pSET-*srbAp-dcas9*-3×Flag	pSET-*srbAp-dcas9* harboring the 3×Flag tag sequence inserted into the C-terminal of *dcas9* before the stop codon	This study
pSET-*srbAp-dcas9/sg-fabH1*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg-fabH1* targeting the nucleotide position of 8-27 on the non-template strand of *fabH1*	This study
pSET-*srbAp-dcas9/sg-fabH2*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg-fabH2* targeting the nucleotide position of 27-46 on the non-template strand of *fabH2*	This study
pSET-*srbAp-dcas9*/sg-*fabH3*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg-fabH3* targeting the nucleotide position of 179-198 on the non-template strand of *fabH3*	This study
pSET-*srbAp-dcas9/sg-gltA*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg-gltA* targeting the nucleotide position of 137-156 on the non-template strand of *gltA*	This study
pSET-*srbAp-dcas9/sg-gltA1*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg-gltA1* targeting the nucleotide position of 8-27 on the non-template strand of *gltA1*	This study
pSET-*srbAp-dcas9/sg-gltA2*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg-gltA2* targeting the nucleotide position of 197-216 on the non-template strand of *gltA2*	This study

pSET-*srbAp-dcas9/sg-cm1*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg-cm1* targeting the nucleotide position of 32-51 on the non-template strand of *cm1*	This study
pSET-*srbAp-dcas9/sg-cm2*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg-cm2* targeting the nucleotide position of 149-168 on the non-template strand of *cm2*	This study
pSET-*srbAp-dcas9/sg-cm3*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg-cm3* targeting the nucleotide position of 51-70 on the non-template strand of *cm3*	This study
pSET-*srbAp-dcas9/sg-cm4*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg-cm4* targeting the nucleotide (nt) position of 22-41 on the non-template strand of *cm4*	This study
pSET-*srbAp-dcas9/sg1-fabH3*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg1-fabH3* targeting the nucleotide (nt) position of 29-48 on the non-template strand of *fabH3*	This study
pSET-*srbAp-dcas9/sg2-fabH3*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg2-fabH3* targeting the nucleotide (nt) position of 419-438 on the non-template strand of *fabH3*	This study
pSET-*srbAp-dcas9/sg3-fabH3*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg3-fabH3* targeting the nucleotide (nt) position of 665-684 on the non-template strand of *fabH3*	This study
pSET-*srbAp-dcas9/sg1-gltA2*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg1-gltA2* targeting the nucleotide (nt) position of 8-27 on the non-template strand of *gltA2*	This study
pSET-*srbAp-dcas9/sg2-gltA2*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg2-gltA2* targeting the nucleotide (nt) position of 383-402 on the non-template strand of *gltA2*	This study
pSET-*srbAp-dcas9/sg3-gltA2*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg3-gltA2* targeting the nucleotide (nt) position of 593-612 on the non-template strand of *gltA2*	This study
pSET-*srbAp-dcas9/sg1-cm2*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg1-cm1* targeting the nucleotide (nt) position of 24-43 on the non-template strand of *cm1*	This study
pSET-*srbAp-dcas9/sg2-cm2*	pSET-*srbAp-dcas9* harboring the expression cassette of the sgRNA *sg2-cm2* targeting the nucleotide (nt) position of 290-309 on the non-template strand of *cm2*	This study
pSET-*srbAp-dcas9*/*sg-fabH3//sg-gltA2/sg-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of *sg-fabH3*, *sg-gltA2* and *sg-cm2*	This study
pSET-*srbAp-dcas9/sg-fabH3/sg-gltA2/sg2-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg-*fabH3*, sg-*gltA2* and *sg2-cm2*	This study
pSET-*srbAp-dcas9/sg-fabH3/sg2-gltA2/sg-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg-*fabH3*, sg2-*gltA2* and *sg-cm2*	This study
pSET-*srbAp-dcas9/sg-fabH3/sg2-fabH3/sg2-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg-*fabH3*, sg2-*fabH3* and *sg2-cm2*	This study

pSET-*srbAp-dcas9/sg-fabH3/sg3-gltA2/sg-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg-*fabH3*, sg3-*gltA2* and *sg-cm2*	This study
pSET-*srbAp-dcas9/sg-fabH3/sg3-gltA2/sg2-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg-*fabH3*, *sg3-gltA2* and *sg2-cm2*	This study
pSET-*srbAp-dcas9/sg2-fabH3/sg-gltA2/sg-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg2-*fabH3*, *sg-gltA2* and *sg-cm2*	This study
pSET-*srbAp-dcas9/sg2-fabH3/sg-gltA2/sg2-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg2-*fabH3*, *sg-gltA2* and *sg2-cm2*	This study
pSET-*srbAp-dcas9/sg2-fabH3/sg2-gltA2/sg-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg2-*fabH3*, *sg2-gltA2* and *sg-cm2*	This study
pSET-*srbAp-dcas9/sg2-fabH3/sg2-gltA2/sg2-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg2-*fabH3*, *sg2-gltA2* and *sg2-cm2*	This study
pSET-*srbAp-dcas9/sg2-fabH3/sg3-gltA2/sg-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg2-*fabH3*, *sg3-gltA2* and *sg-cm2*	This study
pSET-*srbAp-dcas9/sg2-fabH3/sg3-gltA2/sg2-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg2-*fabH3*, *sg3-gltA2* and *sg2-cm2*	This study
pSET-*srbAp-dcas9/sg3-fabH3/sg-gltA2/sg-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg3-*fabH3*, *sg-gltA2* and *sg-cm2*	This study
pSET-*srbAp-dcas9/sg3-fabH3/sg-gltA2/sg2-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg3-*fabH3*, *sg-gltA2* and *sg2-cm2*	This study
pSET-*srbAp-dcas9/sg3*-fabH3*/sg2-gltA2/sg-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg3-fabH3, *sg2-gltA2* and *sg-cm2*	This study
pSET-*srbAp*-dcas9-sg3-*fabH3/sg2-gltA2/sg2-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg3-*fabH3*, *sg2-gltA2* and *sg2-cm2*	This study
pSET-*srbAp-dcas9/sg3-fabH3/sg3-gltA2/sg-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of sg3-*fabH3*, *sg3-gltA2* and *sg-cm2*	This study
pSET-*srbAp-dcas9/sg3-fabH3/sg3-gltA2/sg2-cm2*	pSET-*srbAp-dcas9* harboring the expression cassettes of the sgRNA combination of *sg3-fabH3*, *sg3-gltA2* and *sg2-cm2*	This study
pSET-*scbAp-dcas9*	A control EQCi plasmid, in which dCas9 is under the control of *scbAp* (from *Streptomyces coelicolor*) and the transcription of the sgRNA without N20 guide sequence is under the control of *j23119p*	This study
pSET-*scbAp-dcas9/sg-gltA(sco)*	pSET-*scbAp-dcas9* harboring the expression cassette of the sgRNA *sg-gltA(sco)* targeting the nucleotide position of 105-124 on the non-template strand of *gltA* (*SCO2736*) in *S. coelicolor* M145	This study
<u>Plasmids for 3×Flag knock-in</u>		
pKC-*srbA*-up-3×Flag-down	The replication temperature-sensitive plasmid pKC1139 with the upstream and downstream arms (harboring the 3×Flag tag sequence) for the knock-in of 3×Flag into the *srbA* gene of 2001	
pKC-*srbR*-up-3×Flag-down	pKC1139 with the upstream and downstream arms (harboring the 3×Flag tag sequence) for for the knock-in of 3×Flag into the *srbR* gene of 2001	

Supplementary Table S2. Primers used in this study

Oligonucleiotide	Sequence (5′-3′)
Primers for the amplification of sgRNA expression cassettes	
sg-gltA-F	TAATACTAGT**CGCGGCGGTGTTGCCGTAAC**GTTTTAGAGCTAGAA
sg-gltA1-F	TAATACTAGT**TTCGAGTCCGGGTACGAAGT**GTTTTAGAGCTAGAA
sg-gltA2-F	TAATACTAGT**GGCCTCCAGCAACCGACCCT**GTTTTAGAGCTAGAA
sg-fabH1-F	TAATACTAGT**GAGCGCCAGTACGTGCGAGC**GTTTTAGAGCTAGAA
sg-fabH2-F	TAATACTAGT**AGCCGGGCTTTTGAGCACCC**GTTTTAGAGCTAGAA
sg-fabH3-F	TAATACTAGT**GTGGCGCAGTGCCTTGAGAC**GTTTTAGAGCTAGAA
sg-cm1-F	TAATACTAGT**CGCGGTGCCGATCACATGGG**GTTTTAGAGCTAGAA
sg-cm2-F	TAATACTAGT**CTGGGTGCATTTGAAGCGCT**GTTTTAGAGCTAGAA
sg-cm3-F	TAATACTAGT**ACCTGCTCATGCATGTGCTC**GTTTTAGAGCTAGAA
sg-cm4-F	TAATACTAGT**ACGGCGATCAGCGCATGGCG**GTTTTAGAGCTAGAA
sg1-gltA2-F	TAATACTAGT**GGCCTCCAGCAACCGACCCT**GTTTTAGAGCTAGAA
sg2-gltA2-F	TAATACTAGT**GGCCACGGCCTCCTCCAGCC**GTTTTAGAGCTAGAA
sg3-gltA2-F	TAATACTAGT**CTCCGCCAGCGCCCGCTCCA**GTTTTAGAGCTAGAA
sg1-fabH3-F	TAATACTAGT**GTGGCGCAGTGCCTTGAGAC**GTTTTAGAGCTAGAA
sg2-fabH3-F	TAATACTAGT**TTCGTCCGGGAACCGGGCAC**GTTTTAGAGCTAGAA
sg3-fabH3-F	TAATACTAGT**GGTCTCGATGCAGCGGTGGG**GTTTTAGAGCTAGAA
sg1-cm2-F	TAATACTAGT**CTGGGTGCATTTGAAGCGCT**GTTTTAGAGCTAGAA
sg2-cm2-F	TAATACTAGT**GATGAAGTTGAGCAGCTTCT**GTTTTAGAGCTAGAA
sg-scramble-1-F	TAATACTAGT**AGATCACTGAGAGTCAGTCA**GTTTTAGAGCTAGAA
sg-scramble-2-F	TAATACTAGT**TGCCATCCTCGCATCTGCTG**GTTTTAGAGCTAGAA
sg-gltA(sco)-F	TAATACTAGT**TCACCAGACCGGTCTGGGCG**GTTTTAGAGCTAGAA
NS-F	TAATACTAGTGTTTTAGAGCTAGAA
sgRNA-R	TTACGAATTCGGGTGTACATCCA
Primers for the amplification of QS elements	
srbAp-F	CGACTCTAGAGTGTTCGCCGTCCTTCCCCG
srbAp-R	TTCTTGTCCATATGACGATCACCTTAAAATACTAATAATGTT
scbAp-F	CGACTCTAGAGCCTGCCTCCTTGTTCATGT
scbAp-R	TGTCCATATGGGGTCCCCCCCAGGAAT
Primers used for RT-qPCR analysis	
hrdB-F1	TTCGCGCTCGACCTCTAGTA
hrdB-R1	GCACCTCTTGAACATCGGGA
QT gltA1-F	GACTCGAAGGAGTCGTCGC
QT gltA1-R	CGAAGGAGATATGGCCCACC
QT gltA2-F	CCACGTTCCGGGATTCGG
QT gltA2-R	GATGTTCATCGGCACGGGAC
QT gltA-F	CACAACCCCTTCGACGAGAA
QT gltA-R	ATATTGGCCTGCGAGGAACC
QT cm1-F	ATGAGCAGCAGCGTTTCGG
QT cm1-R	CAGCTCCAGCAGCGTCAT
QT cm2-F	CACAGCACGAGCGACC
QT cm2-R	GTTGTCGGAGCGTGCGAT

QT cm3-F	GCGGTACGAGCGGTCC
QT cm3-R	GACATCGGACTTGGACAGGG
QT cm4-F	AGGATCTTCCGCGACCAGA
QT cm4-R	CCACGAGTTCGCCGTTGA
QT fabH1-F	GGCCAATCTGCGGATCATCG
QT fabH1-R	GAGAGGGCGAGCGGAATG
QT fabH2-F	GTGCTCGTACTCGTCGCTC
QT fabH2-R	GTGCCGTAGTGGATGAGGTC
QT fabH3-F	CGTCCTCGTCGTGAGTTTCG
QT fabH3-R	CCTTCTGTTTGTGAGCGACC
QT fabH3-OT1-F	ATGGCGCGCTGGAGCGCT
QT fabH3-OT1-R	AGATCAGCACTTGTTCGGTG
QT fabH3-OT2-F	TCAGCGCGGTGCAGTACGT
QT fabH3-OT2-R	TCAGTTTCGGCGAAGGTGGAATA
QT gltA3-OT1-F	TCGACCGAGGAAATGCTCAA
QT gltA3-OT1-R	ACCAGGTTCAGGATCATGAC
QT gltA3-OT2-F	TTCCAGCTGGGCCTGGACAT
QT gltA3-OT2-R	CTTGGTGCGCAGGAAGTGGT
QT cm2-OT1-F	ATGTCCGGACTGATCGACAC
QT cm2-OT1-R	TCATCACATGCTCCCAGCGG
QT cm2-OT2-F	ATGCATTTTGCCCAGCTTCC
QT cm2-OT2-R	CTAGAGCACAAAGGCCCCTTCG
QT cm2-OT3-F	ATGCTGCTGGTCTCGGGG
QT cm2-OT3-R	CCGTCACCGTGTCGAACCA
QT cm2-OT4-F	TCGCTGTGGTGGTGGGCGTAT
QT cm2-OT4-R	AGCTCGGCCATCAGCCAGA
Primers used for the construction of the engineered strains with 3×Flag tag sequence	
srbA-3×Flag-Up-F	AACAGCTATGACATGATTACGAATTCTGTGCAGCCCGAAGAAGGC
srbA-3×Flag-Up-R	CTTGTCGTCGTCGTCCTTGTAGTCGATGTCGTGGTCCTTGTAATCGCCGTCGTGGTCCTTGTAGTCGAGGTACTGGAGCACGCCGA
srbA-3×Flag-down-F	ACAAGGACGACGACGACAAGTGAGCGGCGAACACGCCGTG
srbA-3×Flag-down-R	GTAAAACGACGGCCAGTGCCAAGCTTGGGCTCGACGCGGCGAAGG
srbR-3×Flag-Up-F	AACAGCTATGACATGATTACGAATTCTGTTCGTCGCACGGCGTGTTCGCCGCT
srbR-3×Flag-Up-R	CTTGTCGTCGTCGTCCTTGTAGTCGATGTCGTGGTCCTTGTAATCGCCGTCGTGGTCCTTGTAGTCCTCTGACGGCGGCGCGGCGT
srbR-3×Flag-down-F	ACAAGGACGACGACGACAAGTGAGGCGCCCGCGCACATCA
srbR-3×Flag-down-R	GTAAAACGACGGCCAGTGCCAAGCTTGAGCCGGTCCGGCTCTCGGA
dcas9-3×Flag-F	TCTCCCAGCTGGGCGGCGACGACTACAAGGACCACGACGGCGATTACAAGGACCACGACATCGACT
dcas9-3×Flag-R	AGACGACAAAACTTTAGATATTTAAATTCACTTGTCGTCGTCGTCCTTGTAGTCGATGTCGTGGTCCTTG

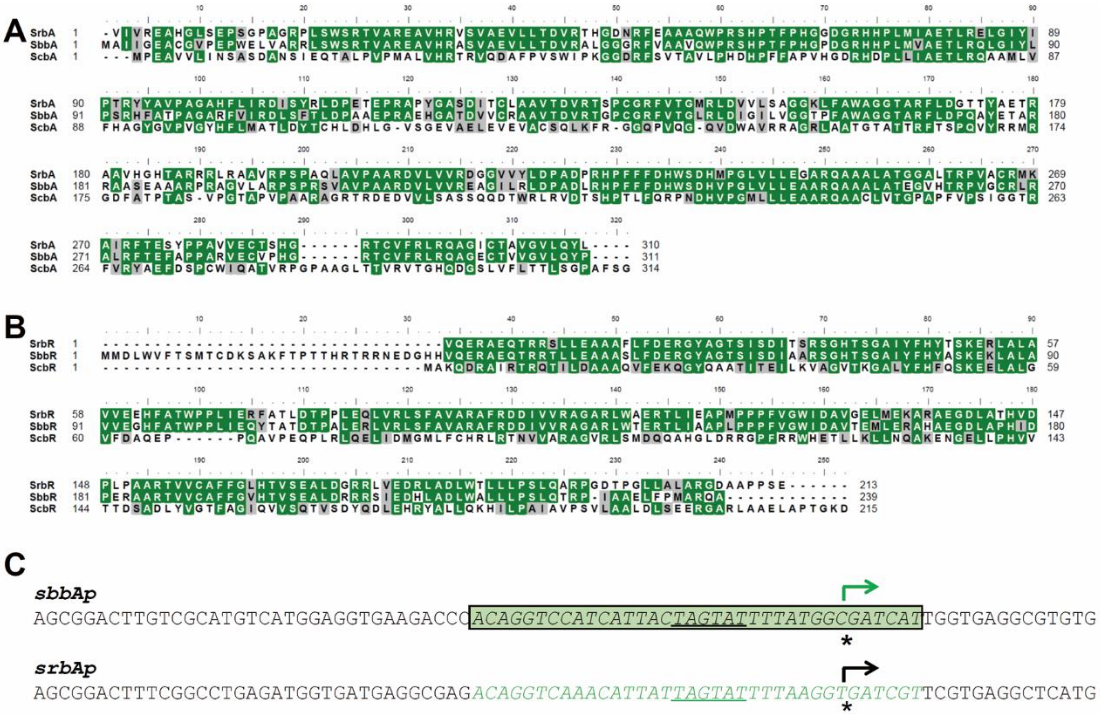
Note: The restriction enzyme sites are underlined. The sequences written in boldface letters indicate the specific N20 guide sequences of different genes.

Supplementary Table S3. The predicted off-target (OT) sites of *sg-fabH3*, *sg-gltA3* and *sg-cm2*

	Gene	ORF length	Off-target position (bp)	Function	Sequence(5′-3′)
OT1 of *sg-fabH3*	M271_24375	2178 bp	57-76	Alpha-galactosidase	GTGGCGCTGT GCCTGGGGGC
OT2 of *sg-fabH3*	M271_11125	2112 bp	1924-1943	Membrane protein	GTGGTGCTGT GCCTGGAGCC
OT1 of *sg-gltA3*	M271_22935	1065 bp	951-970	Phosphate ABC transporter permease	GGCCTCCTTC AACCGGGCCT
OT2 of *sg-gltA3*	M271_09330	2040 bp	747 -766	Short-chain dehydrogenase	GGCCTCCACC GACCGCCCCC
OT1 of *sg-cm2*	M271_28145	693 bp	323-342	DtxR family transcriptional regulator	CTGGGAGCAT GTGATGAGCG
OT2 of sg-cm2	M271_24960	354 bp	179-198	Sulfurtransferase	CCGGGTGCAT GTGATGTGCC
OT3 of *sg-cm2*	M271_32790	426 bp	106-125	Hypothetical protein	CTGGACGCCC TCGAAGCGCT
OT4 of *sg-cm2*	M271_44610	1581 bp	153-172	Apolipoprotein N-acyltransferase	CTGGCTGCTG CTGATGCGCT

Note: In the whole genome, no off-target sites of *sg-fabH3* and *sg-gltA3* with less than three mismatches were detected. For either sgRNAs, two off-target sites with four mismatches were found. For *sg-cm2*, no off-targets with less than four mismatches were detected. Only four off-targets with five mismatches were found. The red letters indicate the mismatches.

Supplementary Figures

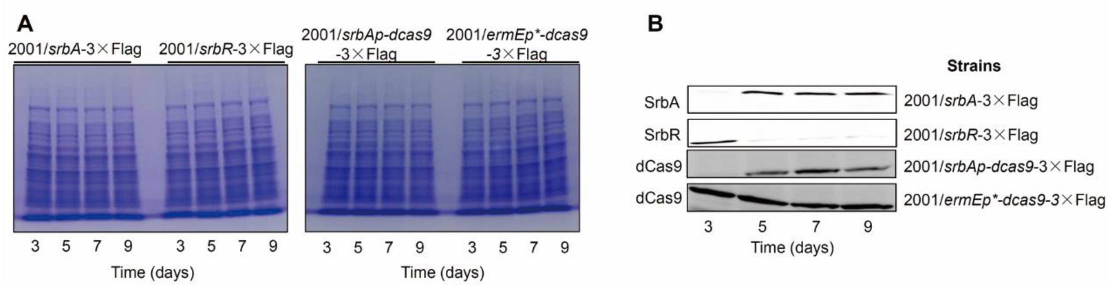


Supplementary Figure S1. Bioinformatics analysis of the QS system in *Streptomyces rapamycinicus*.

(A) Amino acid sequence alignment of the putative GBLs synthesis protein SrbA with other two identified GBLs synthesis proteins, SbbA from *Streptomyces bingchengensis* and ScbA from *Streptomyces coelicolor*.

(B) Amino acid sequence alignment of the putative GBLs receptor SrbR with other two identified GBLs receptors, SbbR from *S. bingchengensis* and ScbR from *S. coelicolor*.

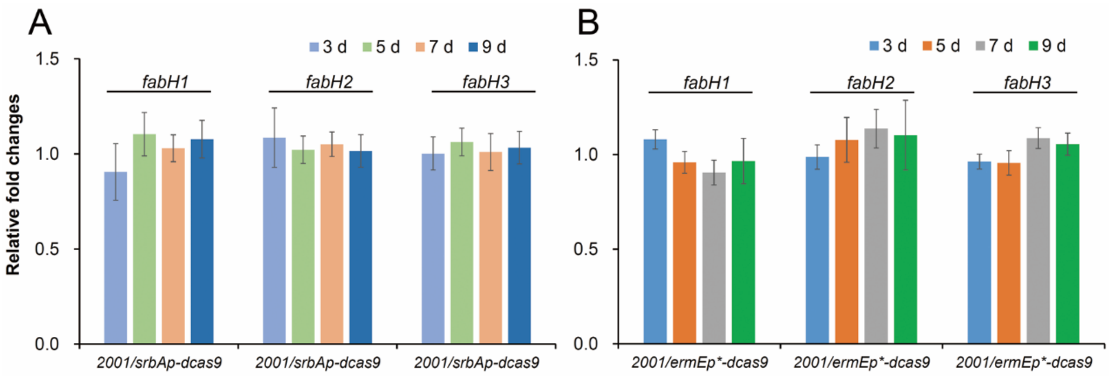
(C) Comparison of the promoter regions of *sbbA* from *S. bingchengensis* (*sbbAp*) and *srbA* from *S. rapamycinicus* (*srbAp*). The putative transcriptional start points (TSP) of *sbbAp* and *srbAp* are marked by bent arrows. The SbbR-binding sequences are boxed and the predicted SrbR-binding sequences are indicated in italicized blue letters. The putative –10 regions of *sbbAp* and *srbAp* are unlined.



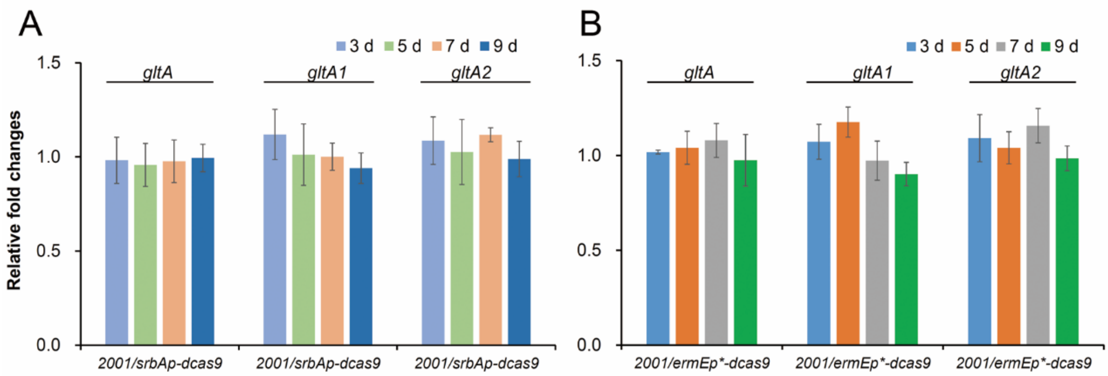
Supplementary Figure S2. Western-blot analysis of the protein levels of SrbA, SrbR and dCas9.

(A) SDS-PAGE analysis of the whole cell lysates. The same amount (20 μg) of cell lysates from the tested four strains collected at four time points (3, 5, 7 and 9 days) was subjected to SDS-PAGE analysis. 2001/*srbA*-3×Flag and 2001/*srbR*-3×Flag were constructed by inserting the 3×Flag tag sequence into the C-terminal of *srbA* and *srbR* of the parental strain 2001, respectively. 2001/*srbAp-dcas9*-3×Flag and 2001/*ermEp**-*dcas9*-3×Flag were generated by introducing the plasmids pSET-*srbAp-dcas9*-3×Flag (with the 3×Flag tag sequence inserted into the C-terminal of *dcas9* in pSET-*srbAp-dcas9*) and pSET-*ermEp*-dcas9*-3×Flag (with the 3×Flag sequence inserted into the C-terminal of *dcas9* in pSET-*ermEp*-dcas9*) into 2001, respectively.

(B) Western-blot analysis. SrbA and SrbR protein levels were checked in strains of 2001/*srbA*-3×Flag and 2001/*srbR*-3×Flag, respectively. The protein levels of dCas9 were tested in 2001/*srbAp-dcas9*-3×Flag and 2001/*ermEp*-dcas9*-3×Flag.

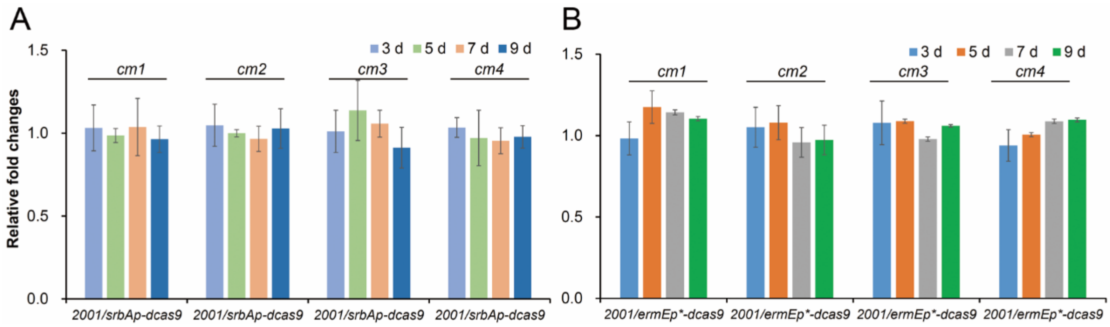


Supplementary Figure S3. Effects of the introduction of the control plasmids pSET-*srbAp-dcas9* and pSET-*ermEp*-dcas9* on the transcription of *fabH1-H3*. Transcription analysis of *fabH1-H3* by RT-qPCR in strains of 2001/*srbAp-dcas9* (2001 with the control plasmid pSET-*srbAp-dcas9*) **(A)** and 2001/*ermEp*-dcas9* (2001 with the control plasmid pSET-*ermEp*-dcas9*) **(B)**. RNA samples were isolated from the parental strain 2001, 2001/*srbAp-dcas9* and 2001/*ermEp*-dcas9* grown in fermentation medium for 3, 5, 7 and 9 days, respectively. The relative transcript levels of each tested gene were normalized to *hrdB* (*M271_14880*, an internal control). The relative fold changes of gene transcription (the tested strains vs. 2001) were determined by the $2^{-\Delta\Delta CT}$ method. Error bars represent the standard deviations (SD) from three biological replicates.



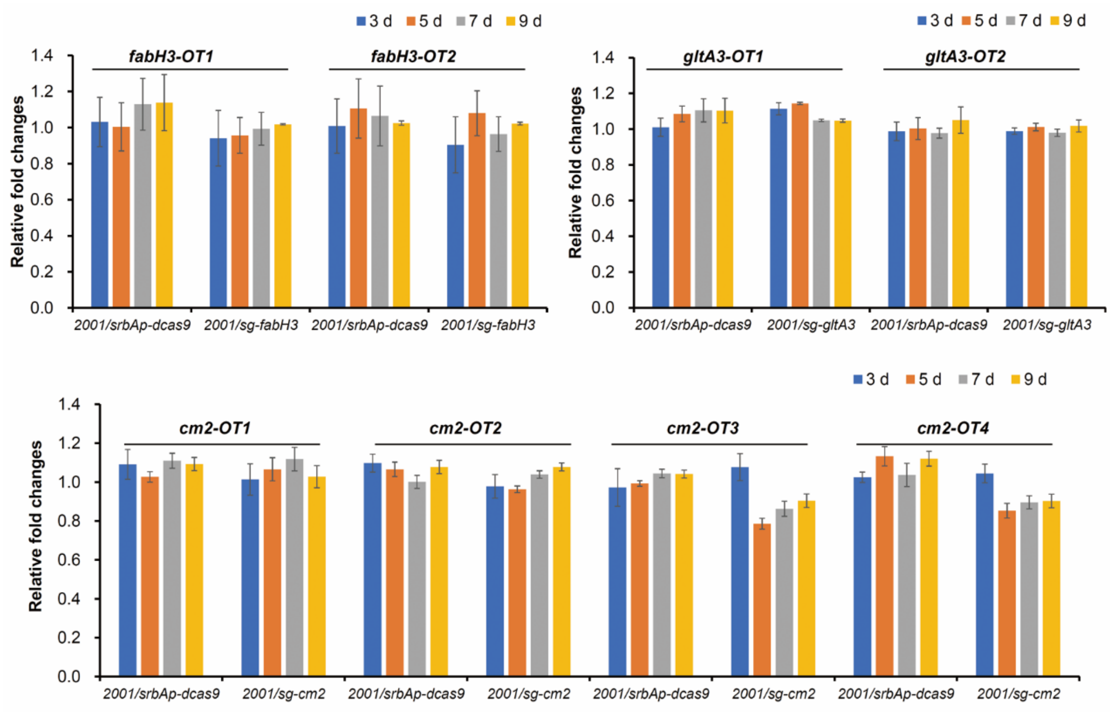
Supplementary Figure S4. Effects of the introduction of the control plasmids pSET-*srbAp-dcas9* and pSET-*ermEp-*dcas9* on the transcription of *gltA-A2*.**

Transcription analysis of *gltA-A2* by RT-qPCR in strains of 2001/*srbAp-dcas9* (2001 with pSET-*srbAp-dcas9*) **(A)** and 2001/*ermEp**-*dcas9* (2001 with pSET-*ermEp**-*dcas9*) **(B)**. RNA samples were isolated from the parental strain 2001, 2001/*srbAp-dcas9* and 2001/*ermEp**-*dcas9* grown in fermentation medium for 3, 5, 7 and 9 days, respectively. The relative transcript levels of each tested gene were normalized to *hrdB* (*M271_14880*, an internal control). The relative fold changes of gene transcription (the tested strains vs. 2001) were determined by the $2^{-\Delta\Delta CT}$ method. Error bars represent the standard deviations (SD) from three biological replicates.



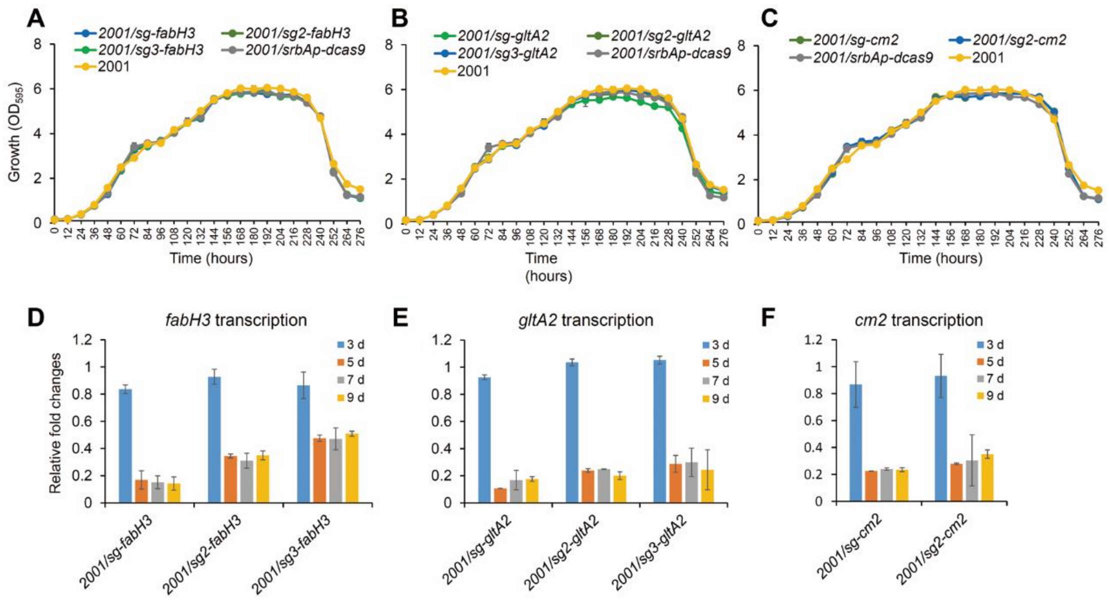
Supplementary Figure S5. Effects of the introduction of the control plasmids pSET-*srbAp-dcas9* and pSET-*ermEp*-dcas9* on the transcription of *cm1-4*.

Transcription analysis of *cm1-4* by RT-qPCR in strains of 2001/*srbAp-dcas9* (2001 with pSET-*srbAp-dcas9*) **(A)** and 2001/*ermEp*-dcas9* (2001 with pSET-*ermEp*-dcas9*) **(B)**. RNA samples were isolated from the parental strain 2001, 2001/*srbAp-dcas9* and 2001/*ermEp*-dcas9* grown in fermentation medium for 3, 5, 7 and 9 days, respectively. The relative transcript levels of each tested gene were normalized to *hrdB* (*M271_14880*, an internal control). The relative fold changes of gene transcription (the tested strains vs. 2001) were determined by the $2^{-\Delta\Delta CT}$ method. Error bars represent the standard deviations (SD) from three biological replicates.



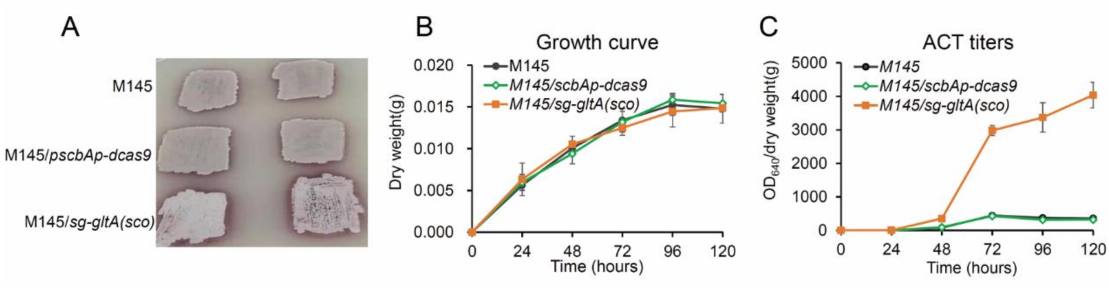
Supplementary Figure S6. Effects of the individual EQCi circuits harboring *sg-fabH3*, *sg-gltA3* and *sg-cm2* on the transcription of their potential off-targets.

RNA samples were isolated from 2001, 2001/*srbAp-dcas9* (2001 harboring the control plasmid pSET- *srbAp-dcas9*), 2001/*sg-fabH3* (2001 harboring the EQCi plasmid pSET-*srbAp-dcas9/sg-fabH3*), 2001/*sg-gltA3* (2001 harboring the EQCi plasmid pSET-*srbAp-dcas9/sg-gltA3*) and 2001/*sg-cm2* (2001 harboring the EQCi plasmid pSET-*srbAp-dcas9/sg-cm2*) grown in fermentation medium for 3, 5, 7 and 9 days, respectively. The relative transcript levels of each tested gene were normalized to *hrdB* (*M271_14880*, an internal control). The relative fold changes of gene transcription (the tested strains vs. 2001) were determined by the $2^{-\Delta\Delta CT}$ method. Error bars represent the standard deviations (SD) from three biological replicates. The detailed information of off-targets of the three sgRNAs (*sg-fabH3*, *sg-gltA3* and *sg-cm2*) is presented in Supplementary Table S3.



Supplementary Figure S7. Effects of EQCi-mediated dynamic regulation with varying repression strength on cell growth and gene transcription

(A-C) Growth curves of strains with EQCi circuits containing sgRNAs targeting different positions of *fabH3, gltA2* and *cm2*. Strains with the corresponding circuits were as indicated. Samples were harvested from fermentation medium at the time points as indicated and the interval time is 12 hours. The parental strain 2001 and 2001 with the control plasmid pSET-*srbAp-dcas9* (2001/*srbAp-dcas9*) were used as controls. All the engineered strains were fermented together. Therefore, the same growth curves of 2001 and 2001/*srbAp-dcas9* or 2001/*ermEp*-dcas9* were used as in panel A, B and C. **(D-F)** Transcript levels of target genes in strains with the EQCi circuits containing sgRNAs targeting different positions of *fabH3, gltA2* and *cm2*. RNA samples were isolated from *S. rapamycinicus* strains grown in fermentation medium for 3, 5, 7 and 9 days, respectively. The relative transcript levels of each tested gene were normalized to *hrdB* (*M271_14880*, an internal control). The relative fold changes of gene transcription (the tested strains vs. 2001/*srbAp-dcas9*) were determined by the $2^{-\Delta\Delta CT}$ method. Error bars in **(A-F)** represent the standard deviations (SD) from three biological replicates.



Supplementary Figure S8. Effects of EQCi-mediated repression of the *gltA* gene (*sco2736*) in the TCA cycle on cell growth (A) and actinorhodin production (B) in *Streptomyces coelicolor*. Three strains, namely, the parental strain M145, M145/*scbAp-dcas9* (M145 carrying the control EQCi plasmid, pSET-*scbAp-dcas9*) and M145/*sg-gltA(sco)* [M145 carrying the EQCi plasmid, pSET-*scbAp-dcas9/sg-gltA(sco)*, harboring the sgRNA targeting *gltA*], were tested. The image was photographed after growth on YM agar medium at 30°C for 72 hours (A). For quantitative analysis of ACT production (B), cultures were taken at five time points as indicated. Actinorhodin titers were calculated as OD_{640}/g (dry weight). Error bars denote the standard deviations (SD) of three biological replicates.

DNA sequences of different combinations of three sgRNA expression cassettes.

1. *sg-fabH3*/sg-*gltA2/sg-cm2* (in strain 2002)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GTGGCGCAGTGCCTTGAGAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCTCCAGCAACCGACCCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTGGGTGCATTTGAAGCGCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

2. *sg-fabH3*/sg-*gltA2/sg2-cm2* (in strain 2003)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GTGGCGCAGTGCCTTGAGAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCACGGCCTCCTCCAGCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GATGAAGTTGAGCAGCTTCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC

CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

3. *sg-fabH3*/sg2-*gltA2/sg-cm2* (in strain 2004)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GTGGCGCAGTGCCTTGAGAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCACGGCCTCCTCCAGCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTGGGTGCATTTGAAGCGCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

4. *sg-fabH3*/sg2-*gltA2/sg2-cm2* (in strain 2005)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GTGGCGCAGTGCCTTGAGAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCACGGCCTCCTCCAGCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GATGAAGTTGAGCAGCTTCT**GTT

TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

5. *sg-fabH3*/sg3-*gltA2/sg-cm2* (in strain 2006)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GTGGCGCAGTGCCTTGAGAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTCCGCCAGCGCCCGCTCCA**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTGGGTGCATTTGAAGCGCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

6. *sg-fabH3/sg3-gltA2/sg2-cm2* (in strain 2007)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GTGGCGCAGTGCCTTGAGAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCACGGCCTCCTCCAGCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC

TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GATGAAGTTGAGCAGCTTCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

7. *sg2-fabH3/sg-gltA2/sg-cm2* (in strain 2008)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**TTCGTCCGGGAACCGGGCAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCTCCAGCAACCGACCCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTGGGTGCATTTGAAGCGCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

8. *sg2-fabH3/sg-gltA2/sg2-cm2* (in strain 2009)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**TTCGTCCGGGAACCGGGCAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCTCCAGCAACCGACCCT**GTTTTAGAGCTAGAAATAGCA

AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GATGAAGTTGAGCAGCTTCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

9. *sg2-fabH3/sg2-gltA2/sg-cm2* (in strain 2010)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**TTCGTCCGGGAACCGGGCAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCACGGCCTCCTCCAGCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTGGGTGCATTTGAAGCGCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

10. *sg2-fabH3/sg2-gltA2/sg2-cm2* (in strain 2011)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**TTCGTCCGGGAACCGGGCAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA

GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCACGGCCTCCTCCAGCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GATGAAGTTGAGCAGCTTCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

11. *sg2-fabH3/sg3-gltA2/sg-cm2* (in strain 2012)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**TTCGTCCGGGAACCGGGCAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTCCGCCAGCGCCCGCTCCA**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTGGGTGCATTTGAAGCGCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

12. *sg2-fabH3/sg3-gltA2/sg2-cm2* (in strain 2013)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**TTCGTCCGGGAACCGGGCAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC

CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTCCGCCAGCGCCCGCTCCA**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GATGAAGTTGAGCAGCTTCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

13. *sg3-fabH3/sg-gltA2/sg-cm2* (in strain 2014)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGTCTCGATGCAGCGGTGGG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCTCCAGCAACCGACCCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTGGGTGCATTTGAAGCGCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

14. *sg3-fabH3/sg-gltA2/sg2-cm2* (in strain 2015)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGTCTCGATGCAGCGGTGGG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCTCCAGCAACCGACCCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GATGAAGTTGAGCAGCTTCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

15. *sg3-fabH3/sg2-gltA2/sg-cm2* (in strain 2016)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGTCTCGATGCAGCGGTGGG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCACGGCCTCCTCCAGCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTGGGTGCATTTGAAGCGCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

16. *sg3-fabH3/sg2-gltA2/sg2-cm2* (in strain 2017)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGTCTCGATGCAGCGGTGGG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGCCACGGCCTCCTCCAGCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GATGAAGTTGAGCAGCTTCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA

17. *sg3-fabH3/sg3-gltA2/sg-cm2* (in strain 2018)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGTCTCGATGCAGCGGTGGG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTCCGCCAGCGCCCGCTCCA**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**CTGGGTGCATTTGAAGCGCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC

CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT
TACTGGA

18. *sg3-fabH3/sg3-gltA2/sg2-cm2* (in strain 2019)

GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GGTCTCGATG**
CAGCGGTGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC
CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC
AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA
GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT
AATACTAGT**CTCCGCCAGCGCCCGCTCCA**GTTTTAGAGCTAGAAATAGCA
AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG
GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC
TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAG
CTAGCTCAGTCCTAGGTATAATACTAGT**GATGAAGTTGAGCAGCTTCT**GTT
TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGA
AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC
CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT
TACTGGA

Note:

Green letters: the *j23119* promoter. Red letters: the specific N20 guide sequences of different genes. Black letters: sgRNA scaffold. Grey letters: T0 terminators.

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