

1 **Supplementary Data**

2

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

5

6 Jinzhong Tian<sup>1,2</sup>, Gaohua Yang<sup>1,2</sup>, Yang Gu<sup>1</sup>, Xinqiang Sun<sup>3</sup>, Yinhua Lu<sup>4\*</sup>, Weihong  
7 Jiang<sup>1\*</sup>

8 1. Key Laboratory of Synthetic Biology, CAS Center for Excellence in Molecular Plant  
9 Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of  
10 Sciences (CAS), Shanghai 200032, China

11 2. University of Chinese Academy of Sciences, Beijing 100039, China

12 3. XinChang Pharmaceutical Factory, Zhejiang medicine LTD, Xinchang, Zhejiang  
13 Province, China

14 4. College of Life Sciences, Shanghai Normal University, Shanghai 200234, China

15 **\* Correspondence**

16 1. **Yinhua Lu**, College of Life Sciences, Shanghai Normal University, 100 Guilin Road,  
17 Shanghai 200234, China; **Email:** yhlu@shnu.edu.cn

18 2. **Weihong Jiang**, Key Laboratory of Synthetic Biology, CAS Center for Excellence  
19 in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology,  
20 Chinese Academy of Sciences (CAS), Shanghai 200032, China; **Email:**  
21 whjiang@sibs.ac.cn

22

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

Strains and plasmids	Genotype	References
<b>Strains</b>		
<i>E. coli</i> strains		
DH5 $\alpha$	F- $\Phi$ 80 <i>lacZ</i> $\Delta$ M15 $\Delta$ ( <i>lacZYA-argF</i> ) <i>U169 deoR recA1 endA1 hsdR17 supE44 <math>\lambda</math> thi-1 gyrA96 relA1</i>	Gicbo-BRL
ET12567/pUZ8002	<i>dam-13::Tn9 dcm-6 hsdM</i> ; harboring the non-transmissible RP4 derivative plasmid pUZ8002	(1)
<i>Streptomyces</i>		
<i>S. rapamycinicus</i> 2001	The parental strain derived from the wild-type <i>S. rapamycinicus</i> NRRL 5491	This study
2001/ <i>srbAp-dcas9</i>	2001 with the control plasmid pSET- <i>srbAp-dcas9</i>	This study
2001/ <i>srbAp-dcas9-3</i> $\times$ Flag	2001 with the plasmid pSET- <i>srbAp-dcas9-3</i> $\times$ Flag	This study
2001/ <i>ermEp*-dcas9</i>	2001 with the control plasmid pSET- <i>ermEp*-dcas9</i>	This study
2001/ <i>srbAp-dcas9-3</i> $\times$ Flag	2001 with the plasmid pSET- <i>ermEp*-dcas9-3</i> $\times$ Flag	This study
2001/ <i>srbA-3</i> $\times$ Flag	2001 with the 3 $\times$ Flag tag sequence inserted into the C-terminal of <i>srbA</i> before the stop codon	This study
2001/ <i>srbR-3</i> $\times$ Flag	2001 with the 3 $\times$ Flag tag sequence inserted into the C-terminal of <i>srbR</i> before the stop codon	This study
2001/ <i>sg-fabH1</i>	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg-fabH1</i>	This study
2001/ <i>sg-fabH2</i>	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg-fabH2</i>	This study
2001/ <i>sg-fabH3</i>	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg-fabH3</i>	This study
2001/ <i>ermEp*/sg-fabH1</i>	2001 with the <i>ermEp*</i> -driving CRISPRi plasmid pSET- <i>ermEp*-dcas9/sg-fabH1</i>	This study
2001/ <i>ermEp*/sg-fabH2</i>	2001 with the <i>ermEp*</i> -driving CRISPRi plasmid pSET- <i>ermEp*-dcas9/sg-fabH2</i>	This study
2001/ <i>ermEp*/sg-fabH3</i>	2001 with the <i>ermEp*</i> -driving CRISPRi plasmid pSET- <i>ermEp*-dcas9/sg-fabH3</i>	This study
2001/ <i>sg-gltA</i>	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg-gltA</i>	This study
2001/ <i>sg-gltA1</i>	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg-gltA1</i>	This study
2001/ <i>sg-gltA2</i>	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg-gltA2</i>	This study
2001/ <i>ermEp*/sg-gltA</i>	2001 with the <i>ermEp*</i> -driving CRISPRi plasmid pSET- <i>ermEp*-dcas9/sg-gltA</i>	This study
2001/ <i>ermEp*/sg-gltA1</i>	2001 with the <i>ermEp*</i> -driving CRISPRi plasmid pSET- <i>ermEp*-dcas9/sg-gltA1</i>	This study
2001/ <i>ermEp*/sg-gltA2</i>	2001 with the <i>ermEp*</i> -driving CRISPRi plasmid pSET- <i>ermEp*-dcas9/sg-gltA2</i>	This study
2001/ <i>sg-cm1</i>	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg-cm1</i>	This study
2001/ <i>sg-cm2</i>	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg-cm2</i>	This study
2001/ <i>sg-cm3</i>	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg-cm3</i>	This study
2001/ <i>sg-cm4</i>	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg-cm4</i>	This study
2001/ <i>ermEp*/sg-cm1</i>	2001 with the <i>ermEp*</i> -driving CRISPRi plasmid pSET- <i>srbAp-dcas9/sg-cm1</i>	This study

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

2017	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg3-fabH3/sg2-gltA2/sg2-cm2</i>	This study
2018	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg3-fabH3/sg3-gltA2/sg-cm2</i>	This study
2019	2001 with the EQCi plasmid pSET- <i>srbAp-dcas9/sg3-fabH3/sg3-gltA2/sg2-cm2</i>	This study
<i>Streptomyces coelicolor</i> M145	Wild type; SCP1 <sup>-</sup> SCP2 <sup>-</sup> <i>pgl</i> <sup>+</sup>	(2)
M145/ <i>scbAp-dcas9</i>	M145 with the control EQCi plasmid pSET- <i>scbAp-dcas9</i>	This study
M145/ <i>sg-gltA(sco)</i>	M145 with the control EQCi plasmid pSET- <i>scbAp-dcas9/sg-gltA(sco)</i>	This study
<b>Plasmids</b>		
pSET152	An integrative plasmid, <i>acc(3)IV</i> , <i>oriTRK2</i> , $\Phi$ C31 integrase/ <i>attP</i>	(2)
<u>Reporter plasmids</u>		
pSET- <i>srbAp-lacZ</i>	A reporter plasmid, in which the codon optimized thermophilic <i>lacZ</i> gene under the control of the GBL-responsive promoter <i>srbAp</i> was cloned between <i>NdeI</i> and <i>XbaI</i>	This study
pSET- <i>ermEp*-lacZ</i>	A reporter plasmid, in which the codon optimized thermophilic <i>lacZ</i> gene under the control of the constitutive strong promoter <i>ermEp*</i> was cloned between <i>NdeI</i> and <i>XbaI</i>	This study
<u><i>ermEp*</i>-driving CRISPRi plasmids</u>		
pSET- <i>dcas9-actII4-NT-S1</i>	A pSET152-derived CRISPRi plasmid, in which dCas9 is under the control of <i>ermEp*</i> and the sgRNA <i>actII4-NT-S1</i> transcription cassette (targeting <i>actII-ORF4</i> , a pathway-specific activator for actinorhodin biosynthesis in <i>Streptomyces coelicolor</i> ) is under the control of <i>j23119p</i>	(3)
pSET- <i>ermEp*-dcas9</i>	A control CRISPRi plasmid, in which dCas9 is under the control of <i>ermEp*</i> and the sgRNA without N20 guide sequence is under the control of <i>j23119p</i>	(3)
pSET- <i>ermEp*-dcas9-3×Flag</i>	pSET- <i>ermEp*-dcas9</i> harboring the 3×Flag tag sequence inserted into the C-terminal of <i>dcas9</i> before the stop codon	This study
pSET- <i>ermEp*-dcas9/sg-fabH1</i>	pSET- <i>ermEp*-dcas9</i> harboring the expression cassette of the sgRNA <i>sg-fabH1</i> targeting the nucleotide position of 8-27 on the non-template strand of <i>fabH1</i>	This study
pSET- <i>ermEp*-dcas9/sg-fabH2</i>	pSET- <i>ermEp*-dcas9</i> harboring the expression cassette of the sgRNA <i>sg-fabH2</i> targeting the nucleotide position of 27-46 on the non-template strand of <i>fabH2</i>	This study
pSET- <i>ermEp*-dcas9/sg-fabH3</i>	pSET- <i>ermEp*-dcas9</i> harboring the expression cassette of the sgRNA <i>sg-fabH3</i> targeting the nucleotide position of 179-198 on the non-template strand of <i>fabH3</i>	This study
pSET- <i>ermEp*-dcas9/sg-gltA</i>	pSET- <i>ermEp*-dcas9</i> harboring the expression cassette of the sgRNA <i>sg-gltA</i> targeting the nucleotide position of 137-156	This study

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

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

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

24 **Supplementary Table S2. Primers used in this study**

<b>Oligonucleotide</b>	<b>Sequence (5'-3')</b>
<b>Primers for the amplification of sgRNA expression cassettes</b>	
sg-gltA-F	TAATA <u>ACTAGT</u> CGCGGGCGGTGTTGCCGTAACGTTTTAGAGCTAGAA
sg-gltA1-F	TAATA <u>ACTAGT</u> TTTCGAGTCCGGGTACGAAGTGTTTTAGAGCTAGAA
sg-gltA2-F	TAATA <u>ACTAGT</u> GGCCTCCAGCAACCGACCCTGTTTTAGAGCTAGAA
sg-fabH1-F	TAATA <u>ACTAGT</u> GAGCGCCAGTACGTGCGAGCGTTTTAGAGCTAGAA
sg-fabH2-F	TAATA <u>ACTAGT</u> AGCCGGGCTTTTGGAGCACCCGTTTTAGAGCTAGAA
sg-fabH3-F	TAATA <u>ACTAGT</u> GTGGCGCAGTGCCTTGAGACGTTTTAGAGCTAGAA
sg-cm1-F	TAATA <u>ACTAGT</u> CGCGGTGCCGATCACATGGGGTTTTAGAGCTAGAA
sg-cm2-F	TAATA <u>ACTAGT</u> CTGGGTGCATTTGAAGCGCTGTTTTAGAGCTAGAA
sg-cm3-F	TAATA <u>ACTAGT</u> ACCTGCTCATGCATGTGCTCGTTTTAGAGCTAGAA
sg-cm4-F	TAATA <u>ACTAGT</u> ACGGCGATCAGCGCATGGCGGTTTTAGAGCTAGAA
sg1-gltA2-F	TAATA <u>ACTAGT</u> GGCCTCCAGCAACCGACCCTGTTTTAGAGCTAGAA
sg2-gltA2-F	TAATA <u>ACTAGT</u> GGCCACGGCCTCCTCCAGCCGTTTTAGAGCTAGAA
sg3-gltA2-F	TAATA <u>ACTAGT</u> CTCCGCCAGCGCCCGCTCCAGTTTTAGAGCTAGAA
sg1-fabH3-F	TAATA <u>ACTAGT</u> GTGGCGCAGTGCCTTGAGACGTTTTAGAGCTAGAA
sg2-fabH3-F	TAATA <u>ACTAGT</u> TTTCGTCCGGGAACCGGGCACGTTTTAGAGCTAGAA
sg3-fabH3-F	TAATA <u>ACTAGT</u> GGTCTCGATGCAGCGGTGGGGTTTTAGAGCTAGAA
sg1-cm2-F	TAATA <u>ACTAGT</u> CTGGGTGCATTTGAAGCGCTGTTTTAGAGCTAGAA
sg2-cm2-F	TAATA <u>ACTAGT</u> GATGAAGTTGAGCAGCTTCTGTTTTAGAGCTAGAA
sg-scramble-1-F	TAATA <u>ACTAGT</u> AGATCACTGAGAGTCAGTCAGTTTTAGAGCTAGAA
sg-scramble-2-F	TAATA <u>ACTAGT</u> TGCCATCCTCGCATCTGCTGGTTTTAGAGCTAGAA
sg-gltA(sco)-F	TAATA <u>ACTAGT</u> TCACCAGACCGGTCTGGGCGGTTTTAGAGCTAGAA
NS-F	TAATA <u>ACTAGT</u> GTTTTAGAGCTAGAA
sgRNA-R	TTACGAATTCCGGGTGTACATCCA
<b>Primers for the amplification of QS elements</b>	
srbAp-F	CGACTCTAGAGTGTTCCGCCGTCCTTCCCCG
srbAp-R	TTCTTGTC <u>CATATG</u> ACGATCACCTTAAAATACTAATAATGTT
scbAp-F	CGACTCTAGAGCCTGCCTCCTTGTTTCATGT
scbAp-R	TGTCCATATGGGGTCCCCCCCAGGAAT
<b>Primers used for RT-qPCR analysis</b>	
hrdB-F1	TTCGCGCTCGACCTCTAGTA
hrdB-R1	GCACCTCTTGAACATCGGGA
QT gltA1-F	GACTCGAAGGAGTCGTCGC
QT gltA1-R	CGAAGGAGATATGGCCCACC
QT gltA2-F	CCACGTTCCGGGATTCGG
QT gltA2-R	GATGTTTCATCGGCACGGGAC
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	AGATCAGCACTTGTTCCGGTG
QT fabH3-OT2-F	TCAGCGCGGTGCAGTACGT
QT fabH3-OT2-R	TCAGTTTCGGCGAAGGTGGAATA
QT gltA3-OT1-F	TCGACCGAGGAAATGCTCAA
QT gltA3-OT1-R	ACCAGGTTCCAGGATCATGAC
QT gltA3-OT2-F	TTCCAGCTGGGCCTGGACAT
QT gltA3-OT2-R	CTTGGTGCGCAGGAAGTGGT
QT cm2-OT1-F	ATGTCCGGACTGATCGACAC
QT cm2-OT1-R	TCATCACATGCTCCCAGCGG
QT cm2-OT2-F	ATGCATTTTGCCAGCTTCC
QT cm2-OT2-R	CTAGAGCACAAAGGCCCTTCG
QT cm2-OT3-F	ATGCTGCTGGTCTCGGGG
QT cm2-OT3-R	CCGTCACCGTGTCAACCA
QT cm2-OT4-F	TCGCTGTGGTGGTGGGCGTAT
QT cm2-OT4-R	AGCTCGGCCATCAGCCAGA
<b>Primers used for the construction of the engineered strains with 3×Flag tag sequence</b>	
srbA-3×Flag-Up-F	AACAGCTATGACATGATTACGAATTCTGTGCAGCCCCGAAGAAGGC
srbA-3×Flag-Up-R	CTTGTCGTCGTCGTCCTTGTAGTCGATGTCGTGGTCCTTGTAAATCGC CGTCGTGGTCCTTGTAGTCGAGGTACTGGAGCACGCCGA
srbA-3×Flag-down-F	ACAAGGACGACGACGACAAGTGAGCGGCGAACACGCCGTG
srbA-3×Flag-down-R	GTA AACGACGGCCAGTGCCAAGCTTGGGCTCGACGCGGCGAAGG
srbR-3×Flag-Up-F	AACAGCTATGACATGATTACGAATTCTGTTTCGTCGCACGGCGTGTTC GCCGCT
srbR-3×Flag-Up-R	CTTGTCGTCGTCGTCCTTGTAGTCGATGTCGTGGTCCTTGTAAATCGC CGTCGTGGTCCTTGTAGTCCTCTGACGGCGGCGCGGCGT
srbR-3×Flag-down-F	ACAAGGACGACGACGACAAGTGAGGCGCCCGCGCACATCA
srbR-3×Flag-down-R	GTA AACGACGGCCAGTGCCAAGCTTGAGCCGGTCCGGCTCTCGG A
dcas9-3×Flag-F	TCTCCCAGCTGGGCGGCGACGACTACAAGGACCACGACGGCGATTA CAAGGACCACGACATCGACT
dcas9-3×Flag-R	AGACGACAAAACCTTTAGATATTTAAATTCACCTTGTCGTCGTCGTCCT TGTAGTCGATGTCGTGGTCCTTG

---

25 Note: The restriction enzyme sites are underlined. The sequences written in boldface

26 letters indicate the specific N20 guide sequences of different genes.

27

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

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

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

35

36

37 **Supplementary Figures**



38

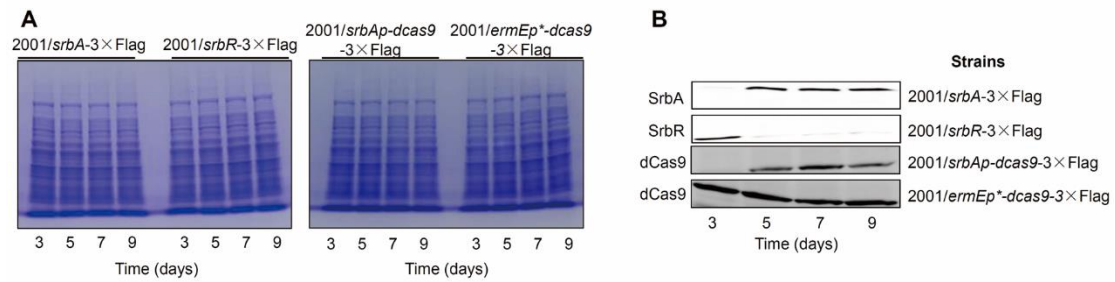
39 **Supplementary Figure S1. Bioinformatics analysis of the QS system in**  
 40 ***Streptomyces rapamycinicus*.**

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

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

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

51



52

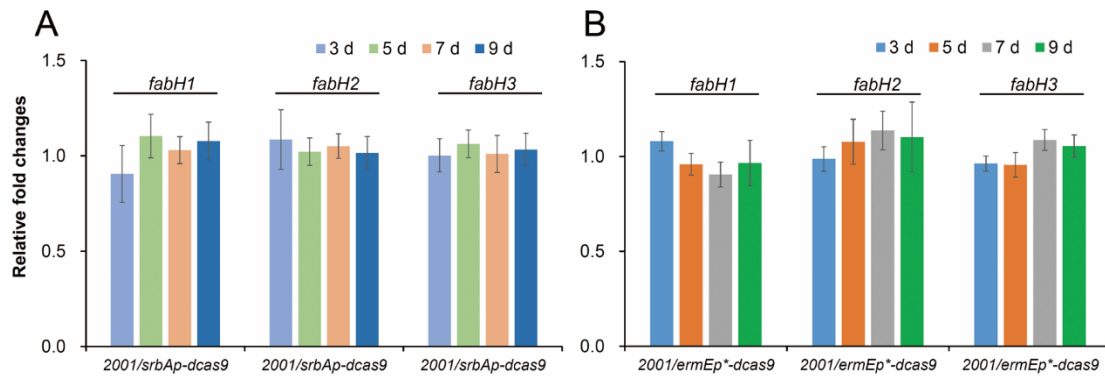
53

54 **Supplementary Figure S2. Western-blot analysis of the protein levels of Srba,**  
 55 **SrbR and dCas9.**

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

65 **(B)** Western-blot analysis. Srba and SrbR protein levels were checked in strains of  
 66 2001/*srbA*-3×Flag and 2001/*srbR*-3×Flag, respectively. The protein levels of dCas9  
 67 were tested in 2001/*srbAp-dcas9*-3×Flag and 2001/*ermEp\*-dcas9*-3×Flag.

68



70

71

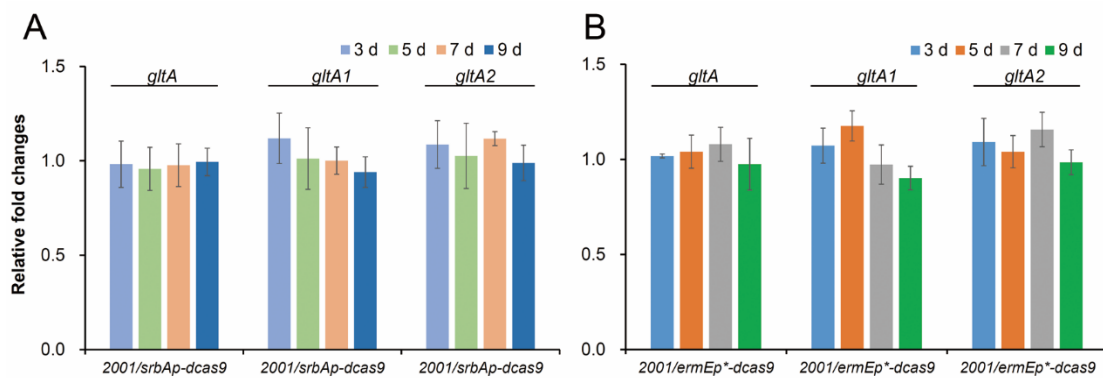
72 **Supplementary Figure S3. Effects of the introduction of the control plasmids**  
 73 **pSET-*srbAp-dcas9* and pSET-*ermEp\*-dcas9* on the transcription of *fabH1-H3*.**

74 Transcription analysis of *fabH1-H3* by RT-qPCR in strains of 2001/*srbAp-dcas9* (2001  
 75 with the control plasmid pSET-*srbAp-dcas9*) (A) and 2001/*ermEp\*-dcas9* (2001 with  
 76 the control plasmid pSET-*ermEp\*-dcas9*) (B). RNA samples were isolated from the  
 77 parental strain 2001, 2001/*srbAp-dcas9* and 2001/*ermEp\*-dcas9* grown in fermentation  
 78 medium for 3, 5, 7 and 9 days, respectively. The relative transcript levels of each tested  
 79 gene were normalized to *hrdB* (*M271\_14880*, an internal control). The relative fold  
 80 changes of gene transcription (the tested strains vs. 2001) were determined by the  $2^{-\Delta\Delta CT}$   
 81 method. Error bars represent the standard deviations (SD) from three biological  
 82 replicates.

83

84

85

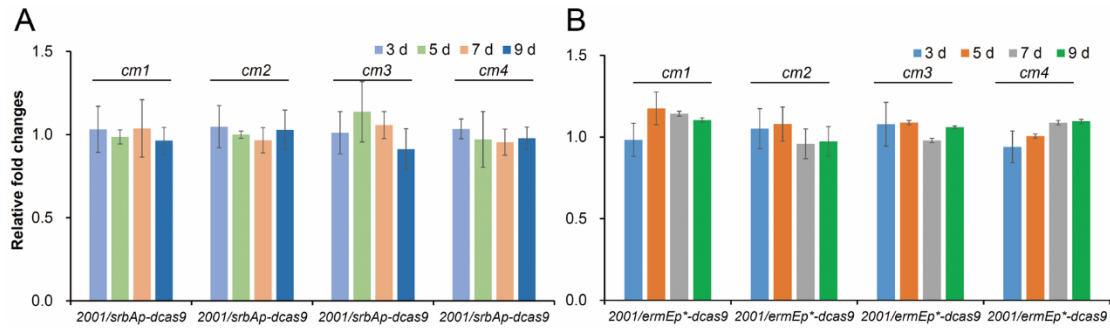


87

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

90 Transcription analysis of *gltA-A2* by RT-qPCR in strains of 2001/*srbAp-dcas9* (2001  
 91 with pSET-*srbAp-dcas9*) (A) and 2001/*ermEp\*-dcas9* (2001 with pSET-*ermEp\*-dcas9*)  
 92 (B). RNA samples were isolated from the parental strain 2001, 2001/*srbAp-dcas9* and  
 93 2001/*ermEp\*-dcas9* grown in fermentation medium for 3, 5, 7 and 9 days, respectively.  
 94 The relative transcript levels of each tested gene were normalized to *hrdB*  
 95 (*M271\_14880*, an internal control). The relative fold changes of gene transcription (the  
 96 tested strains vs. 2001) were determined by the  $2^{-\Delta\Delta CT}$  method. Error bars represent the  
 97 standard deviations (SD) from three biological replicates.

98



100

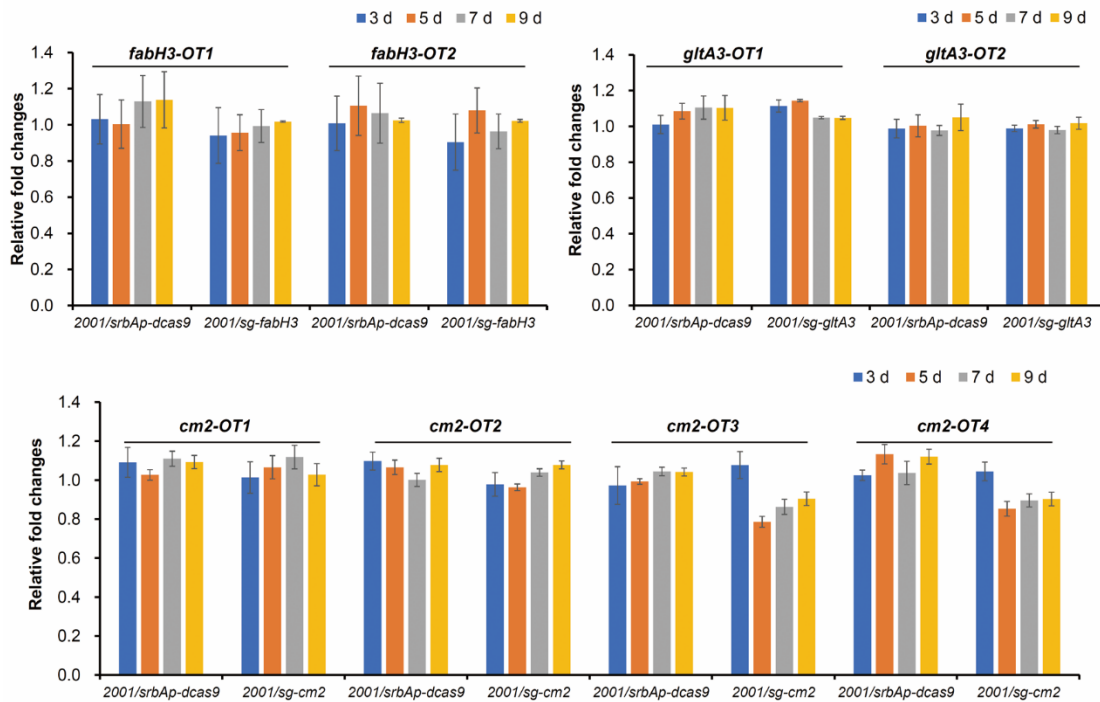
101

102 **Supplementary Figure S5. Effects of the introduction of the control plasmids**103 **pSET-*srbAp-dcas9* and pSET-*ermEp\*-dcas9* on the transcription of *cm1-4*.**104 Transcription analysis of *cm1-4* by RT-qPCR in strains of 2001/*srbAp-dcas9* (2001 with105 pSET-*srbAp-dcas9*) (A) and 2001/*ermEp\*-dcas9* (2001 with pSET-*ermEp\*-dcas9*)106 (B). RNA samples were isolated from the parental strain 2001, 2001/*srbAp-dcas9* and107 2001/*ermEp\*-dcas9* grown in fermentation medium for 3, 5, 7 and 9 days, respectively.108 The relative transcript levels of each tested gene were normalized to *hrdB*109 (*M271\_14880*, an internal control). The relative fold changes of gene transcription110 (the tested strains vs. 2001) were determined by the  $2^{-\Delta\Delta CT}$  method. Error bars

111 represent the standard deviations (SD) from three biological replicates.

112

113



115

116

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

119 RNA samples were isolated from 2001, 2001/*srbAp-dcas9* (2001 harboring the control  
 120 plasmid pSET-*srbAp-dcas9*), 2001/*sg-fabH3* (2001 harboring the EQCi plasmid pSET-  
 121 *srbAp-dcas9/sg-fabH3*), 2001/*sg-gltA3* (2001 harboring the EQCi plasmid pSET-  
 122 *srbAp-dcas9/sg-gltA3*) and 2001/*sg-cm2* (2001 harboring the EQCi plasmid pSET-  
 123 *srbAp-dcas9/sg-cm2*) grown in fermentation medium for 3, 5, 7 and 9 days, respectively.

124 The relative transcript levels of each tested gene were normalized to *hrdB*  
 125 (*M271\_14880*, an internal control). The relative fold changes of gene transcription (the  
 126 tested strains vs. 2001) were determined by the  $2^{-\Delta\Delta CT}$  method. Error bars represent the

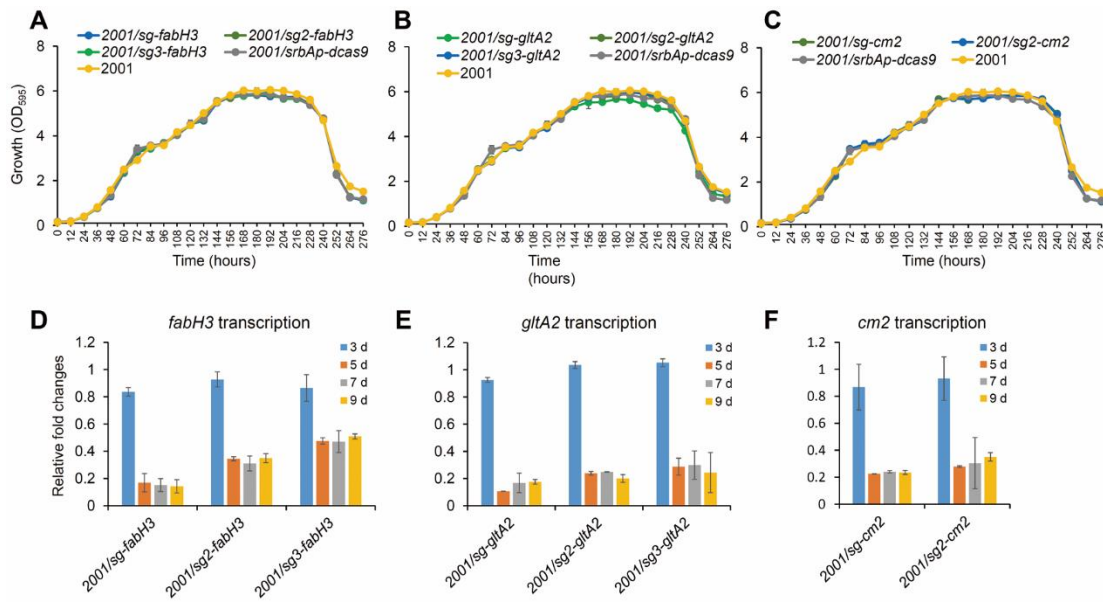
127 standard deviations (SD) from three biological replicates. The detailed information of

128 off-targets of the three sgRNAs (*sg-fabH3*, *sg-gltA3* and *sg-cm2*) is presented in

129 Supplementary Table S3.



130



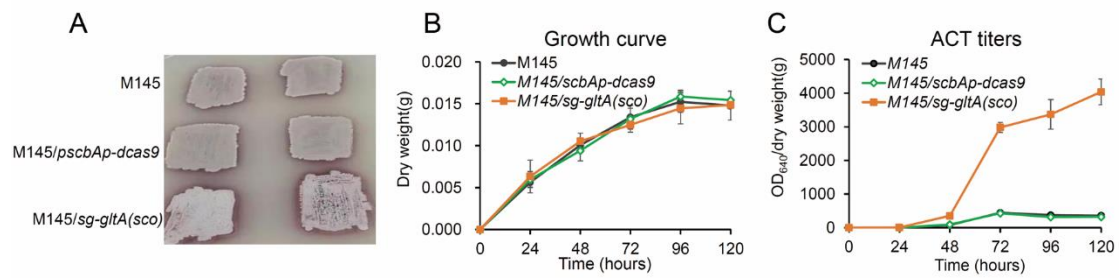
131

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

134 (A-C) Growth curves of strains with EQCi circuits containing sgRNAs targeting  
 135 different positions of *fabH3*, *gltA2* and *cm2*. Strains with the corresponding circuits  
 136 were as indicated. Samples were harvested from fermentation medium at the time points  
 137 as indicated and the interval time is 12 hours. The parental strain 2001 and 2001 with  
 138 the control plasmid pSET-*srbAp-dcas9* (2001/*srbAp-dcas9*) were used as controls. All  
 139 the engineered strains were fermented together. Therefore, the same growth curves of  
 140 2001 and 2001/*srbAp-dcas9* or 2001/*ermEp\*-dcas9* were used as in panel A, B and C.

141 (D-F) Transcript levels of target genes in strains with the EQCi circuits containing  
 142 sgRNAs targeting different positions of *fabH3*, *gltA2* and *cm2*. RNA samples were  
 143 isolated from *S. rapamycinicus* strains grown in fermentation medium for 3, 5, 7 and 9  
 144 days, respectively. The relative transcript levels of each tested gene were normalized to  
 145 *hrdB* (*M271\_14880*, an internal control). The relative fold changes of gene transcription  
 146 (the tested strains vs. 2001/*srbAp-dcas9*) were determined by the  $2^{-\Delta\Delta CT}$  method. Error  
 147 bars in (A-F) represent the standard deviations (SD) from three biological replicates.

148



149

150

151 **Supplementary Figure S8. Effects of EQCi-mediated repression of the *gltA* gene**

152 **(*sco2736*) in the TCA cycle on cell growth (A) and actinorhodin production (B) in**

153 ***Streptomyces coelicolor*. Three strains, namely, the parental strain M145, M145/*scbAp-***

154 ***dcas9* (M145 carrying the control EQCi plasmid, pSET-*scbAp-dcas9*) and M145/*sg-***

155 ***gltA(sco)* [M145 carrying the EQCi plasmid, pSET-*scbAp-dcas9/sg-gltA(sco)*,**

156 **harboring the sgRNA targeting *gltA*], were tested. The image was photographed after**

157 **growth on YM agar medium at 30°C for 72 hours (A). For quantitative analysis of ACT**

158 **production (B), cultures were taken at five time points as indicated. Actinorhodin titers**

159 **were calculated as OD<sub>640</sub>/g (dry weight). Error bars denote the standard deviations (SD)**

160 **of three biological replicates.**

161

162

163 **DNA sequences of different combinations of three sgRNA expression cassettes.**

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

165 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GTGGCGCAGT**  
166 **GCCTTGAGAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
167 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
168 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
169 GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
170 AATACTAGT**GGCCTCCAGCAACCGACCCT**GTTTTAGAGCTAGAAATAGCA  
171 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
172 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
173 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA**GGATCCTTGACAG**  
174 **CTAGCTCAGTCCTAGGTATAATACTAGTCTGGGTGCATTTGAAGCGCTGT**  
175 TTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGA  
176 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
177 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
178 TACTGGA

179

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

181 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT**GTGGCGCAGT**  
182 **GCCTTGAGAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
183 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
184 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
185 GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
186 AATACTAGT**GGCCACGGCCTCCTCCAGCC**GTTTTAGAGCTAGAAATAGCA  
187 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
188 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
189 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA**GGATCCTTGACAG**  
190 **CTAGCTCAGTCCTAGGTATAATACTAGTGATGAAGTTGAGCAGCTTCTGTT**  
191 TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGA  
192 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC

193 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
194 TACTGGA  
195  
196 3. *sg-fabH3/sg2-gltA2/sg-cm2* (in strain 2004)  
197 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTGTGGCGCAGT  
198 GCCTTGAGACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
199 CGTTATCAACTTGAAAAAGTGGCACCCGAGTCGGTGCTTTTTTTGAGTCACC  
200 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
201 GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
202 AATACTAGTGGCCACGGCCTCCTCCAGCCGTTTTAGAGCTAGAAATAGCA  
203 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCCGAGTCG  
204 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
205 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAG  
206 CTAGCTCAGTCCTAGGTATAATACTAGTCTGGGTGCATTTGAAGCGCTGT  
207 TTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGA  
208 AAAAGTGGCACCCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
209 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
210 TACTGGA  
211  
212 4. *sg-fabH3/sg2-gltA2/sg2-cm2* (in strain 2005)  
213 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTGTGGCGCAGT  
214 GCCTTGAGACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
215 CGTTATCAACTTGAAAAAGTGGCACCCGAGTCGGTGCTTTTTTTGAGTCACC  
216 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
217 GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
218 AATACTAGTGGCCACGGCCTCCTCCAGCCGTTTTAGAGCTAGAAATAGCA  
219 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCCGAGTCG  
220 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
221 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAG  
222 CTAGCTCAGTCCTAGGTATAATACTAGTGATGAAGTTGAGCAGCTTCTGTT

223 TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
224 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
225 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
226 TACTGGA

227

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

229 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTGTGGCGCAGT  
230 GCCTTGAGACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
231 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
232 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
233 GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
234 AATACTAGTCTCCGCCAGCGCCCGCTCCAAGTTTTAGAGCTAGAAATAGCA  
235 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
236 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
237 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAG  
238 CTAGCTCAGTCCTAGGTATAATACTAGTCTGGGTGCATTTGAAGCGCTGT  
239 TTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
240 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
241 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
242 TACTGGA

243

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

245 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTGTGGCGCAGT  
246 GCCTTGAGACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
247 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
248 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
249 GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
250 AATACTAGTGGCCACGGCCTCCTCCAGCCGTTTTAGAGCTAGAAATAGCA  
251 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
252 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC

253 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA**GGATCCTTGACAG**  
254 **CTAGCTCAGTCCTAGGTATAATACTAGTGATGAAGTTGAGCAGCTTCTGTT**  
255 TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
256 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
257 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
258 TACTGGA

259

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

261 **GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTTTCGTCCGGG**  
262 **AACCGGGCAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
263 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
264 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
265 GTTCTGAGGTCATTACTGGAG**GGATCCTTGACAGCTAGCTCAGTCCTAGGTAT**  
266 **AATACTAGTGGCCTCCAGCAACCGACCCT**GTTTTAGAGCTAGAAATAGCA  
267 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
268 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
269 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA**GGATCCTTGACAG**  
270 **CTAGCTCAGTCCTAGGTATAATACTAGTCTGGGTGCATTTGAAGCGCTGT**  
271 TTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
272 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
273 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
274 TACTGGA

275

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

277 **GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTTTCGTCCGGG**  
278 **AACCGGGCAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
279 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
280 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
281 GTTCTGAGGTCATTACTGGAG**GGATCCTTGACAGCTAGCTCAGTCCTAGGTAT**  
282 **AATACTAGTGGCCTCCAGCAACCGACCCT**GTTTTAGAGCTAGAAATAGCA

283 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
284 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
285 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA**GGATCCTTGACAG**  
286 **CTAGCTCAGTCCTAGGTATAATACTAGTGATGAAGTTGAGCAGCTTCT**GTT  
287 TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGA  
288 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
289 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
290 TACTGGA

291

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

293 **GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTTTCGTCCGGG**  
294 **AACCGGGCAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
295 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
296 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
297 GTTCTGAGGTCATTACTGGA**GGATCCTTGACAGCTAGCTCAGTCCTAGGTAT**  
298 **AATACTAGTGGCCACGGCCTCCTCCAGCC**GTTTTAGAGCTAGAAATAGCA  
299 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
300 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
301 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA**GGATCCTTGACAG**  
302 **CTAGCTCAGTCCTAGGTATAATACTAGTCTGGGTGCATTTGAAGCGCT**GT  
303 TTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGA  
304 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
305 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
306 TACTGGA

307

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

309 **GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTTTCGTCCGGG**  
310 **AACCGGGCAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
311 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
312 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA

313 GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
314 AATACTAGTGGCCACGGCCTCCTCCAGCCGTTTTAGAGCTAGAAATAGCA  
315 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
316 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
317 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAG  
318 CTAGCTCAGTCCTAGGTATAATACTAGTGATGAAGTTGAGCAGCTTCTGTT  
319 TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
320 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
321 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
322 TACTGGA

323

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

325 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTTTCGTCCGGG  
326 AACCGGGCACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
327 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
328 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
329 GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
330 AATACTAGTCTCCGCCAGCGCCCGCTCCA GTTTTAGAGCTAGAAATAGCA  
331 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
332 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
333 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAG  
334 CTAGCTCAGTCCTAGGTATAATACTAGTCTGGGTGCATTTGAAGCGCTGT  
335 TTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
336 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
337 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
338 TACTGGA

339

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

341 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTTTCGTCCGGG  
342 AACCGGGCACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC



343 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
344 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
345 GTTCTGAGGTCATTA CTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
346 AATACTAGTCTCCGCCAGCGCCCGCTCCA GTTTTAGAGCTAGAAATAGCA  
347 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
348 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
349 TGAACAAATCCAGATGGAGTTCTGAGGTCATTA CTGGAGGATCCTTGACAG  
350 CTAGCTCAGTCCTAGGTATAATACTAGTGATGAAGTTGAGCAGCTTCTGTT  
351 TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
352 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
353 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
354 TACTGGA

355

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

357 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTGGTCTCGATG  
358 CAGCGGTGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
359 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
360 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
361 GTTCTGAGGTCATTA CTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
362 AATACTAGTGGCCTCCAGCAACCGACCCT GTTTTAGAGCTAGAAATAGCA  
363 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
364 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
365 TGAACAAATCCAGATGGAGTTCTGAGGTCATTA CTGGAGGATCCTTGACAG  
366 CTAGCTCAGTCCTAGGTATAATACTAGTCTGGGTGCATTTGAAGCGCTGT  
367 TTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
368 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
369 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
370 TACTGGA

371

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

373 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTGGTCTCGATG  
374 CAGCGGTGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
375 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
376 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
377 GTTCTGAGGTCATTA CTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
378 AATACTAGTGGCCTCCAGCAACCGACCCTGTTTTAGAGCTAGAAATAGCA  
379 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
380 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
381 TGAACAAATCCAGATGGAGTTCTGAGGTCATTA CTGGAGGATCCTTGACAG  
382 CTAGCTCAGTCCTAGGTATAATACTAGTGATGAAGTTGAGCAGCTTCTGTT  
383 TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
384 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
385 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
386 TACTGGA

387

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

389 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTGGTCTCGATG  
390 CAGCGGTGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
391 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
392 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
393 GTTCTGAGGTCATTA CTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
394 AATACTAGTGGCCACGGCCTCCTCCAGCCGTTTTAGAGCTAGAAATAGCA  
395 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
396 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
397 TGAACAAATCCAGATGGAGTTCTGAGGTCATTA CTGGAGGATCCTTGACAG  
398 CTAGCTCAGTCCTAGGTATAATACTAGTCTGGGTGCATTTGAAGCGCTGT  
399 TTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
400 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
401 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
402 TACTGGA

403

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

405 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTGGTCTCGATG  
406 CAGCGGTGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
407 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
408 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
409 GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
410 AATACTAGTGGCCACGGCCTCCTCAGCCGTTTTAGAGCTAGAAATAGCA  
411 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
412 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
413 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA GGATCCTTGACAG  
414 CTAGCTCAGTCCTAGGTATAATACTAGTGATGAAGTTGAGCAGCTTCTGTT  
415 TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
416 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
417 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
418 TACTGGA

419

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

421 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTGGTCTCGATG  
422 CAGCGGTGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
423 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
424 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
425 GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
426 AATACTAGTCTCCGCCAGCGCCCGCTCCA GTTTTAGAGCTAGAAATAGCA  
427 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
428 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
429 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGA GGATCCTTGACAG  
430 CTAGCTCAGTCCTAGGTATAATACTAGTCTGGGTGCATTTGAAGCGCTGT  
431 TTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTGA  
432 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC

433 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
434 TACTGGA  
435  
436 18. *sg3-fabH3/sg3-gltA2/sg2-cm2* (in strain 2019)  
437 GGATCCTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTGGTCTCGATG  
438 CAGCGGTGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC  
439 CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACC  
440 AATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGA  
441 GTTCTGAGGTCATTACTGGAGGATCCTTGACAGCTAGCTCAGTCCTAGGTAT  
442 AATACTAGTCTCCGCCAGCGCCCGCTCCAAGTTTTAGAGCTAGAAATAGCA  
443 AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG  
444 GTGCTTTTTTTGAGTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTC  
445 TGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAGGATCCTTGACAG  
446 CTAGCTCAGTCCTAGGTATAATACTAGTGATGAAGTTGAGCAGCTTCTGTT  
447 TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGA  
448 AAAAGTGGCACCGAGTCGGTGCTTTTTTTGAGTCACCAATAAAAAACGCC  
449 CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT  
450 TACTGGA

451 **Note:**

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

454 .

455

456 **REFERENCES**

- 457 1. Gust, B., Challis, G.L., Fowler, K., Kieser, T., and Chater, K.F. (2003) PCR-targeted  
458 *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis  
459 of the sesquiterpene soil odor geosmin. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1541-1546.  
460 2. Kieser, T., Bibb, M.J., Buttner, M.J., and Chater, K.F. (2000) Practical *Streptomyces*  
461 genetics. John Innes Foundation, Norwich, England.

462 3. Zhao, Y., Li, L., Zheng, G., Jiang, W., Deng, Z., Wang, Z. and Lu, Y. (2018)  
463 CRISPR/dCas9-mediated multiplex gene repression in *Streptomyces*. *Biotechnol. J.*,  
464 **13**, 1800121.