Supporting Information for

Engineering a bifunctional ComQXPA-P_{srfA} quorum-sensing circuit for dynamic control of gene expression in *Corynebacterium glutamicum*

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Table S1. Sequences of natural and engineered P_{srfA} promoters in this study.

Promoter	Sequence
P_{srfA}	**GTGATA**AAAACATTTTTTTCA**TTTAAACTGA**ACGGTAGAAA
P_{srfA} library	**TTGNCA**AAAACATTTTTTTCA**GNTANANTNG**ACGGTAGAAA
M26	**TTGTCA**AAAACATTTTTTTCA**GTTATCAGTGG**ACGGTAGAAA
M6	**TTGACA**AAAACATTTTTTTCA**GCTA - ACTCG**ACGG - AGAA -
M21	**TTGTCA**AAAACATTTTTTTCA**GTTATA - TCG**ACGGTAGAAA
M16	**- - G - C -** AAAACATTTTTTTCA**GTTACAATTG**ACGGTAGAAA
M2	**TTGA** - - - - AACATTTTTTTCA**GATAAAGTCG**ACGGTAGAAA
M3	**TTGGCA**AAAACATTTTTTTCA**GATAC - GTAG**A- - - - - - - -AA
M1	**TTG – TA**AAAACATTTTT - - CA**GCTACACTCG**ACGGTAGAAA
M10	**TTGCCA**AAAACATTTTTTTCA**GTTAATAGTAG**ACGGTAGAAA
M40	**TTGACA**AAAACATTTTTTTCA**GATA - - - - TG**ACGGTAGAAA
M18	C - **TGACA**AAAACATTTTTTTCA**GCTAGACTAG**ACGGTAGAAA
M11	- **TGCCA**AAAACATTTTTTTCA**GATAAAGTCG**ACGGTAGAAA
M22	- **TGGCA**AAAACATTTTTTTCA**GATAGACTGG**ACGGTAGAAA
M36	**TTGTCA**AGAACATTTTTTTCA**AGGTAAAGTTG**ACGGTAGAAA
M20	**TTGGCA**AAAACATTTTTTTCA**GATAGATTCG**ACGGTAGAAA
M29	**TTGTCA**AAAACATTTTTTTCA - **CTACAGTTG**ACGGTAGAAA
M28	**TTGCCA**AAAAACATTTTTTTCA**GATAGACTTG**ACGGTAGAAA
M35	**TTGTCA**AAAACATTTTTTTCA**GGTTAGAGTT**GAGGTAGAAA
M14	**TTGCCA**AAAACATTTTTTTCA**GATACAATAG**GAGGTAGAAA

The −35 region and −10 region of natural and engineered P_{srfA} promoters are in boldface in this table.

Table S2. Strains used in this study.

Strains	Relevant characteristics	Source
JM109	*E. coli* gene-cloning strain	Novagen
SN01	Ile-producing strain of *C. glutamicum* ssp. *lactofermentum*	CCTCC
B. subtilis 168	*rpC2*	Lab stock
MG1655	F-, λ-, *rph*$^{-1}$	Lab stock
QS1T0	SN01 harboring pS1 and pSYW	This study
RT1	SN01 harboring pT1	This study
RT2	SN01 harboring pT2	This study
QS1T2	SN01 harboring pS1and pT2	This study
RT3	SN01 harboring pT3	This study
RT4	SN01 harboring pT4	This study
RT5	SN01 harboring pT5	This study
QS1T5	SN01 harboring pS1 and pT5	This study
QS1T6	SN01 harboring pS1 and pT6	This study
QS1T7	SN01 harboring pS1 and pT7	This study
QS1T8	SN01 harboring pS1 and pT8	This study
QS2T6	SN01 harboring pS2 and pT6	This study
QS3T6	SN01 harboring pS3 and pT6	This study
QS4T6	SN01 harboring pS4 and pT6	This study
QS5T6	SN01 harboring pS5 and pT6	This study
QS2T9	SN01 harboring pS2 and pT9	This study
QS3T9	SN01 harboring pS3 and pT9	This study
QS4T9	SN01 harboring pS4 and pT9	This study
QS5T9	SN01 harboring pS5 and pT9	This study
QS2T10	SN01 harboring pS2 and pT10	This study
QS3T10	SN01 harboring pS3 and pT10	This study
QS4T10	SN01 harboring pS4 and pT10	This study
QS5T10	SN01 harboring pS5 and pT10	This study

QS2T11	SN01 harboring pS2 and pT11	This study
QS3T11	SN01 harboring pS3 and pT11	This study
QS4T11	SN01 harboring pS4 and pT11	This study
QS5T11	SN01 harboring pS5 and pT11	This study
QS1T12	SN01 harboring pS1 and pT12	This study
QS1T13	SN01 harboring pS1 and pT13	This study
QS1T14	SN01 harboring pS1 and pT14	This study
QS1T15	SN01 harboring pS1 and pT15	This study
QS1T16	SN01 harboring pS1 and pT16	This study
QS1T17	SN01 harboring pS1 and pT17	This study
QS1T18	SN01 harboring pS1 and pT18	This study
QS1T19	SN01 harboring pS1 and pT19	This study
QS1T20	SN01 harboring pS1 and pT20	This study
QS1T21	SN01 harboring pS1 and pT21	This study
QS1T22	SN01 harboring pS1 and pT22	This study
QS1T23	SN01 harboring pS1 and pT23	This study
QS1T24	SN01 harboring pS1 and pT24	This study
QS1T25	SN01 harboring pS1 and pT25	This study
QS1T26	SN01 harboring pS1 and pT26	This study
QS1T27	SN01 harboring pS1 and pT27	This study
RT22	SN01 harboring pT22	This study
RT23	SN01 harboring pT23	This study
RT24	SN01 harboring pT24	This study
RT25	SN01 harboring pT25	This study
RT26	SN01 harboring pT26	This study
RT27	SN01 harboring pT27	This study

Table S3. Plasmids used in this study.

Plasmids	Relevant characteristics	Source
pDTW109	*cre*-expressing vector of *C. glutamicum*, temperature sensitive, Cm^R	[1]
pDTW107	pDTW109 without *cre*	This study
pIL-I	derivative of pJYW-4 in which P_{tacM} is replaced by *lrp* and P_{brnFE}-controlled *ido*	[2]
pS1	*comQXPA* operon under P_{brnFE} in pDTW107	This study
pS2	*mCherry* under M1 in pS1	This study
pS3	*mCherry* under M11 in pS1	This study
pS4	*mCherry* under M22 in pS1	This study
pS5	*mCherry* under P_{srfA} in pS1	This study
pJYW-4	constitutive expression vector of *C. glutamicum*, Km^R	[3]
pJYW-4-*gfp*	pJYW-4 harboring *gfp* under P_{tacM} promoter	[2]
pSYW	pJYW-4 without *alr*	[2]
pT1	*gfp* under P_{tacM} in pSYW	This study
pT2	*gfp* under P_{srfA} in pSYW	This study
pT3	*gfp* under P_{tacM} and *hfq* under P_{tacM} in pSYW	This study
pT4	*gfp* under P_{tacM} and sRNA targeting *gfp* under P_{tacM} in pSYW	This study
pT5	*gfp* under P_{tacM}, *hfq* under P_{tacM} and sRNA targeting *gfp* under P_{tacM} in pSYW	This study
pT6	*gfp* under P_{tacM}, *hfq* under P_{srfA} and sRNA targeting *gfp* under P_{tacM} in pSYW	This study
pT7	*gfp* under P_{tacM}, *hfq* under P_{tacM} and sRNA targeting *gfp* under P_{srfA} in pSYW	This study
pT8	*gfp* under P_{tacM}, *hfq* under P_{srfA} and sRNA targeting *gfp* under P_{srfA} in pSYW	This study
pT9	*gfp* under P_{tacM}, *hfq* under M1 and sRNA targeting *gfp* under P_{tacM} in pSYW	This study
pT10	*gfp* under P_{tacM}, *hfq* under M11 and sRNA targeting *gfp* under P_{tacM} in pSYW	This study
pT11	*gfp* under P_{tacM}, *hfq* under M22 and sRNA targeting *gfp* under P_{tacM} in pSYW	This study
pT12	*amyE* under M14 in pSYW	This study
pT13	*amyE* under M20 in pSYW	This study
pT14	*amyE* under M28 in pSYW	This study
pT15	*amyE* under P_{srfA} in pSYW	This study
pT16	*amyE* under M14, *hfq* under M1 and sRNA targeting *amyE* under P_{tacM} in pSYW	This study

pT17	*amyE* under M20, *hfq* under M1 and sRNA targeting *amyE* under P_{tacM} in pSYW	This study
pT18	*amyE* under M28, *hfq* under M1 and sRNA targeting *amyE* under P_{tacM} in pSYW	This study
pT19	*amyE* under M14, *hfq* under M11 and sRNA targeting *amyE* under P_{tacM} in pSYW	This study
pT20	*amyE* under M20, *hfq* under M11 and sRNA targeting *amyE* under P_{tacM} in pSYW	This study
pT21	*amyE* under M28, *hfq* under M11 and sRNA targeting *amyE* under P_{tacM} in pSYW	This study
pT22	*gfp* under M1 in pSYW	This study
pT23	*gfp* under M11 in pSYW	This study
pT24	*gfp* under M14 in pSYW	This study
pT25	*gfp* under M20 in pSYW	This study
pT26	*gfp* under M22 in pSYW	This study
pT27	*gfp* under M28 in pSYW	This study

Table S4. Primers used in this study.

Primer	Sequence	Plasmid construction
comQ-F	GAGAAGGAGTTATAGGATGAAGGAGATTGTGGAGC	pS1
comA-R	TGAGCTGCTCGAGATATTTAAAGTACACCGTCTGAT	pS1
lrp-P$_{brnFE}$-F	GTATACCTGCAGAGATTAATTTCACACCTGGGGGCGAGCT	pS1
pDTW107-R	CCTATAACTCCTTCTCTCCAG	pS1
srfA-F (*Kpn*I)	ATAGGTACCTAGTGGAAATGATTGCGGCAT	pT2, pT2 library
P$_{srfA}$-F (*hfq*)	TAGTGGAAATGATTGCGGCATC	pT6, pT8
srfA-R	CCTATACCTCCTTCTCAATATTTTTTATCTTTCTACCG	pT2, pT6, pT8
gfp-F (RBS)	AGAAGGAGGTATAGGATGAGTAAAGGAGAAGAAC	pT2
gfp-R (*Bam*HI)	ATAGGATCCCTATTTGTATAGTTCATCC	pT2, pT2 library
*srfA*M	CTCAATATTTTTTATCTTTCTACCGTCNANTNTANCTGAAAA	pT2 library
	AAATGTTT TTGNCAACGAAAAATGGGTG	
gfp (P$_{tacM}$)-F	CGCATATGTTTGGTACCCTATTTGTATAGTTCATCC	pT1
P$_{tacM}$ (sRNA)-F	CCGCATATGTTTGGTACCTGAGCTGTTGACAATTAATC	pT3–pT6, pT9–pT11
P$_{tacM}$ (RBS)-R	CCTATACCTCCTTCTAATTG	pT5, pT7
*rrnB*T1T2-R	GCCGCAATCATTTCCACTACGCCCAGGAGGTCGAAG	pT6, pT8–pT11, pT16–pT21
hfq (RBS)-F	AGAAGGAGGTATAGGATGGCTAAGGGGCAATCTT	pT5–pT11, pT16–pT21
hfq-R	GTTGACTATTTTACCTCTGGTTATTCGGTTTCTTCGCT	pT5–pT11
gfp (sRNA)-F	GAGGTAAAATAGTCAACCTATTTGTATAGTTCATCC	pT5–pT11
gfp (sRNA)-R	GAATTCGTCGACGGATCCTGAGCTGTTGACAATT	pT1, pT3–pT11
P$_{srfA}$ (sRNA)-F	TATTTCACACCGCATATTAGTGGAAATGATTGCG	pT7–pT21
P$_{srfA}$ (sRNA)-R	AAGGAGAAGAACTTTTCCAATATTTTTTATCTTTCTACCG	pT7, pT8
srfA-R2	CCTATACCTCCTTCTCAAT	pT9–pT21, pS2–pS5
sRNA (*gfp*)-F	GAAAAGTTCTTCTCCTTTACTCAT	pT7, pT8
rrnB T1T2 (*gfp*)-R	GGATGAACTATACAAATAGCGCCCAGGAGGTCGAAGC	pT4
gfp (*rrnB* T1T2)-F	GCTATTTGTATAGTTCATCC	pT4
P$_{srfA}$ (*mCherry*)-F	AGCTCGTATACCTGCATAGTGGAAATGATTGCG	pS2–pS5
mCherry-F (RBS)	AGAAGGAGGTATAGGATGGTGAGCAAGGGCGAG	pS2–pS5

mCherry-R	GGTGTGAAATTAATCTCTTACTTGTACAGCTCGTC	pS2–pS5
2070-F	TTGAGAAGGAGGTATAGGACAGCTAACTCGGTCAGC	pT12–pT15
2070-R	CGATTTGTTCGCCGTTTCTGCCTGTGCTGGGGAGG	pT12–pT15
amyE-U-F	GAAACGGCGAACAAATCGAATG	pT12–pT15
amyE-D-R	GAATTCGTCGACGGATCCTTAATGATGATGATGATGATGATG GGGAAGAGAACCGC	pT12–pT15
P_{tacM} (pSYW)-F	ATTCGTCGACGGATCCTGAGCTGTTGACAATTAATCAT	pT16–pT21
sRNA (*amyE*)-R	GAAACGGCGAACAAATCGAAAATTGTTATCCGCTCACAAT	pT16–pT21
sRNA (*amyE*)-F	TTCGATTTGTTCGCCGTTTCGTTATATGCCTTTATTGTC	pT16–pT21
hfq (6xHis)-R	CATCATCATCATCATTAATTATTCGGTTTCTTCGCTGTCC	pT16–pT21
16S rRNA-1	ACCTGGAGAAGAAGCACCG	
16S rRNA-2	TCAAGTTATGCCCGTATCG	
qPCR-*gfp*-F	GCGTTCAACTAGCAGACCATTATC	
qPCR-*gfp*-R	GTTCATCCATGCCATGTGTAATCC	

Table S5. Sequences of sRNAs in this study.

Target gene	24-mer target binding sequence of sRNA
gfp	GAAAAGTTCTTCTCCTTTACTCAT
amyE	CTCATTCGATTTGTTCGCCGTTTC

Figure S1. Regulatory mechanism of the ComQXPA-P_{srfA} QS system in *B. subtilis*. When the cells grow to a high density, ComQ inverts the precursor of ComX to the ComX pheromone and exports it to the extracellular environment. The membrane protein ComP monitors the secreted ComX pheromone to phosphorylate ComA. Phosphorylated ComA binds to the P_{srfA} promoter to activate the expression of the *srfA* operon, which is involved in surfactin biosynthesis.

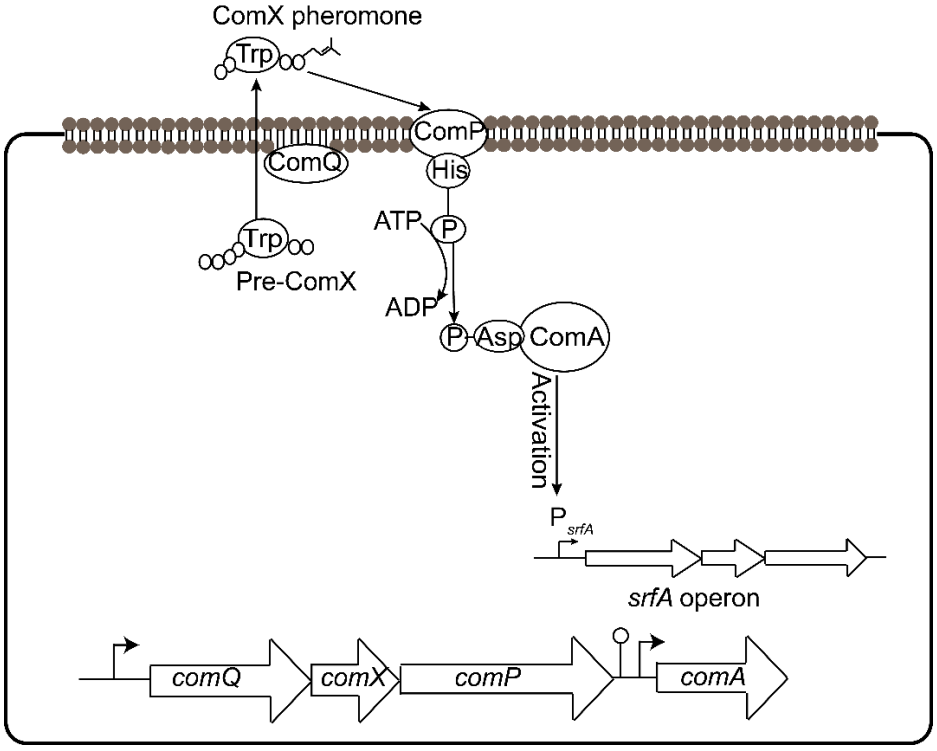


Figure S2 Fitted curve of strain QS1T2. QS1T2 carried pS1, which contained the P_{brnFE}-regulated *comQXPA* operon, and pT2, which contained P_{srfA}-controlled *gfp*.

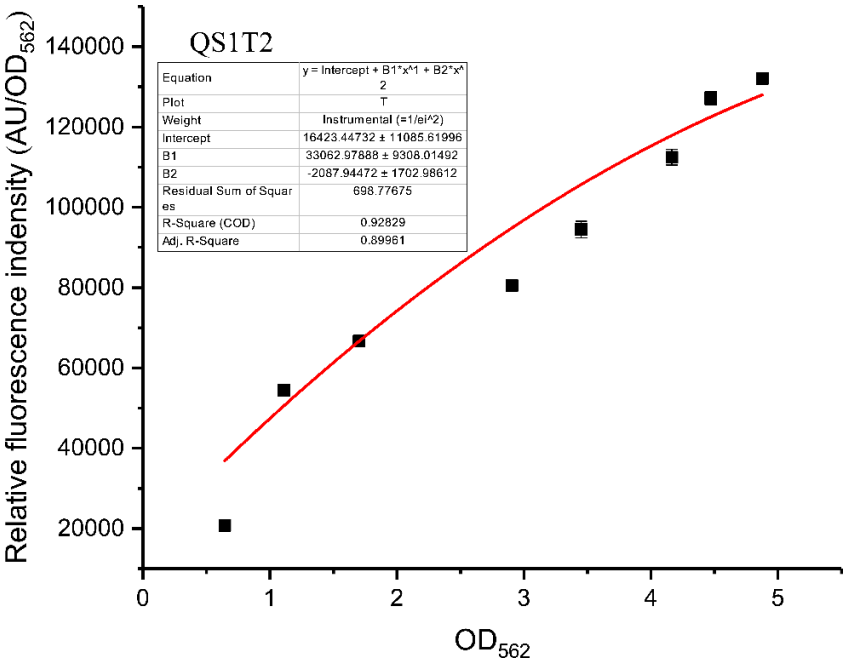


Figure S3. Architecture of the ComQXPA-P_{srfA} QS-based *hfq*-sRNA circuit. The sRNA platform for repression consists of a target sRNA, a MicC scaffold and a sRNA chaperone Hfq protein. The sRNA (target)-MicC scaffold or *hfq* is regulated by the P_{srfA} promoter, resulting in the repression of the target gene in a cell density-dependent manner via the ComQXPA-P_{srfA} QS-based *hfq*-sRNA circuit. In pT6, sRNA targeting *gfp* [sRNA(*gfp*)] is controlled by the P_{tacM} promoter, and *hfq* is controlled by the P_{srfA} promoter. In pT7, sRNA(*gfp*) is controlled by the P_{srfA} promoter, and *hfq* is controlled by the P_{tacM} promoter. In pT8, both sRNA(*gfp*) and *hfq* are controlled by the P_{srfA} promoter, while in pT5, both sRNA(*gfp*) and *hfq* are controlled by the P_{tacM} promoter. These plasmids were individually introduced into the *C. glutamicum* strain carrying the pS1 plasmid, resulting in QS1T5, QS1T6, QS1T7 and QS1T8.

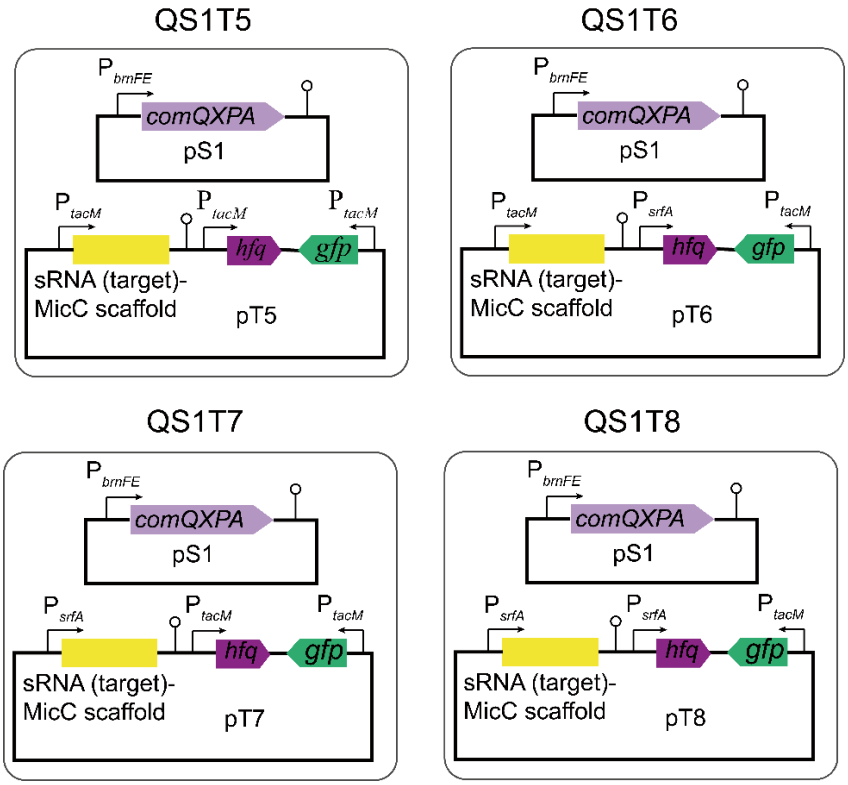


Figure S4. Fitted curves of strains QS1T22, QS1T23, QS1T24, QS1T25, QS1T26 and QS1T27. These strains carried different pT plasmids, i.e., pT22, pT23, pT24, pT25, pT26 and pT27 harbored M1-, M11-, M14-, M20-, M22- and M28-regulated *gfp*, respectively.

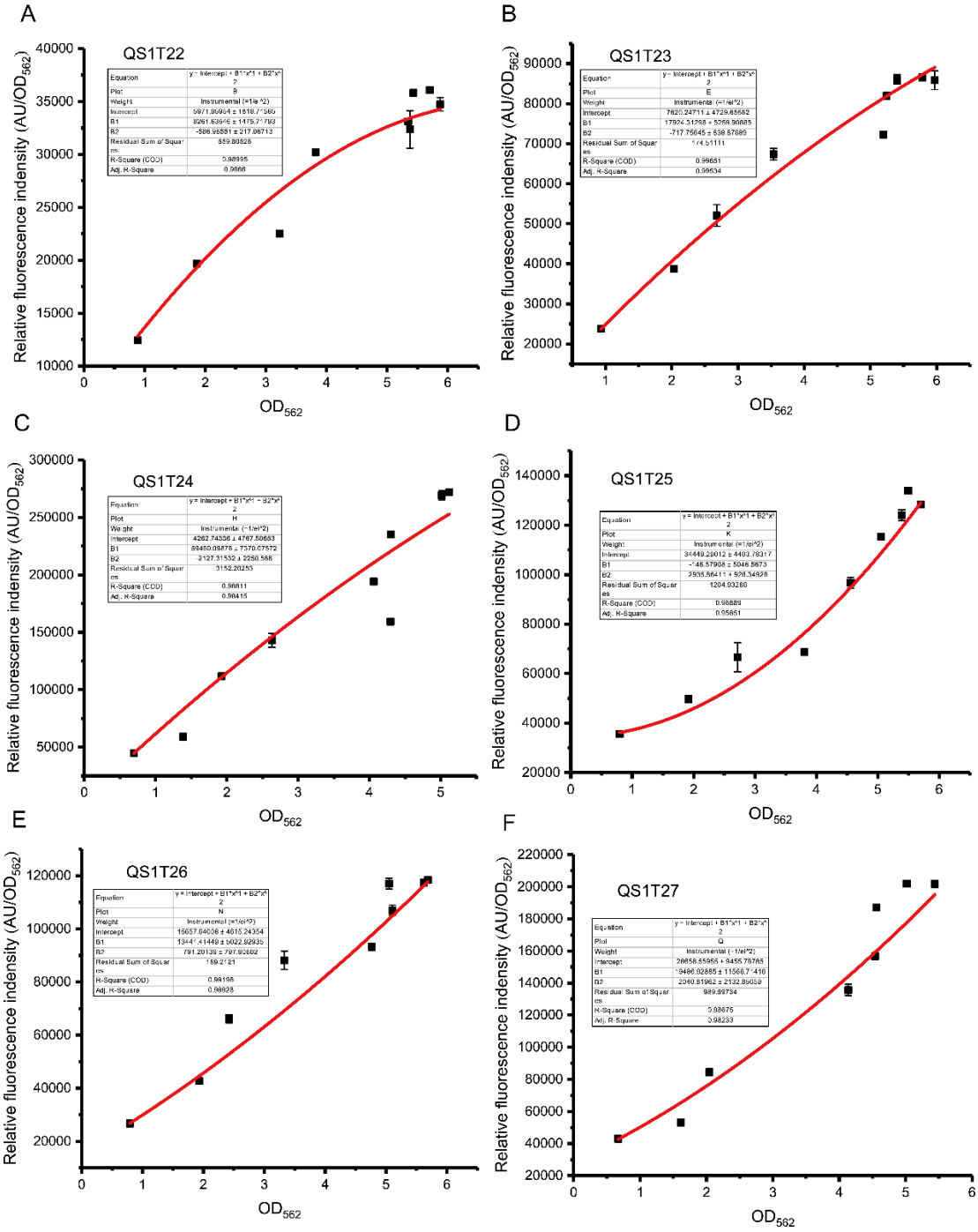


Figure S5. SDS-PAGE of different *amyE*-expressing strains regulated by ComQXPA-P_{srfA} QS circuits. *amyE* was upregulated by M14, M20, M28 and native P_{srfA} in strains QS1T12, QS1T13, QS1T14 and QS1T15, respectively. In addition to M14-, M20- or M28-regulated *amyE*, sRNA(*amyE*)-coupled *hfq* was also regulated by M1 or M11 in strains QS1T16–QS1T21. The culture supernatants of the corresponding *C. glutamicum* strains at 48 h were loaded. Red arrow indicated the amylase, 68 kDa. CM: culture supernatant of fresh medium. QS1T0: SN01 harboring pS1 and pSYW.

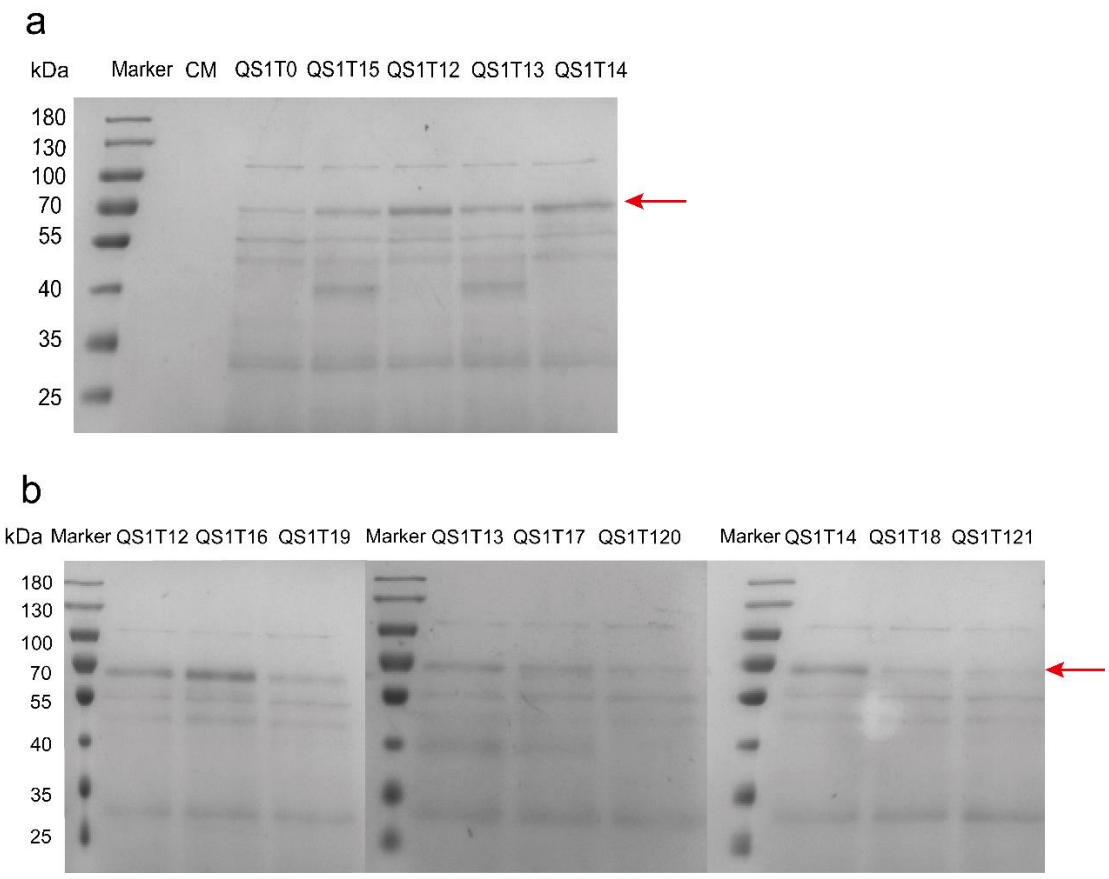
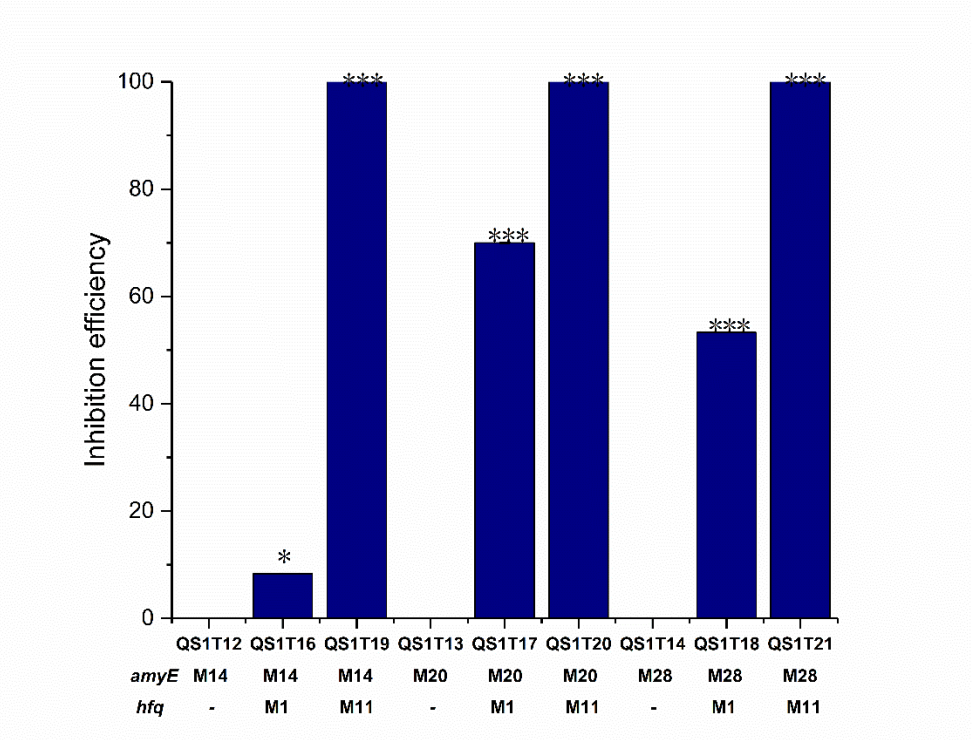


Figure S6. Inhibition efficiency of different P_{srfA}M-regulated *hfq*-sRNA cassettes targeting *amyE* in *C. glutamicum*. Compared to the ComQXPA-P_{srfA} QS-upregulated *amyE*-expressing strains QS1T12, QS1T13 and QS1T14, the M1-regulated *hfq*-sRNA cassette targeting *amyE* suppressed *amyE* expression by an efficiency of 8%–70% in strains QS1T16–QS1T18, while the M11-regulated *hfq*-sRNA(*amyE*) suppressed *amyE* expression by an efficiency of 100% in strains QS1T19–QS1T21. The data represent the means ± SD. * $p < 0.05$, *** $p < 0.001$ compared with the corresponding *amyE*-upregulated strains (QS1T12 or QS1T13 or QS1T14).



References

(1) Hu, J., Tan, Y., Li, Y., Hu, X., Xu, D. and Wang, X. (2013) Construction and application of an efficient multiple-gene-deletion system in *Corynebacterium glutamicum*. *Plasmid 70*, 303–313.

(2) Tan, S., Shi, F., Liu, H., Yu, X., Wei, S., Fan, Z., Li, Y. (2020) Dynamic control of 4-hydroxyisoleucine biosynthesis by modified l-isoleucine biosensor in recombinant *Corynebacterium glutamicum*. *ACS Synth. Biol. 9* (9), 2378–2389.

(3) Hu, J., Li, Y., Zhang, H., Tan, Y., and Wang, X. (2014) Construction of a novel expression system for use in *Corynebacterium glutamicum*. *Plasmid 75*, 18–26.