Reprogramming the sulfur recycling network to improve L-cysteine production in *Corynebacterium glutamicum*

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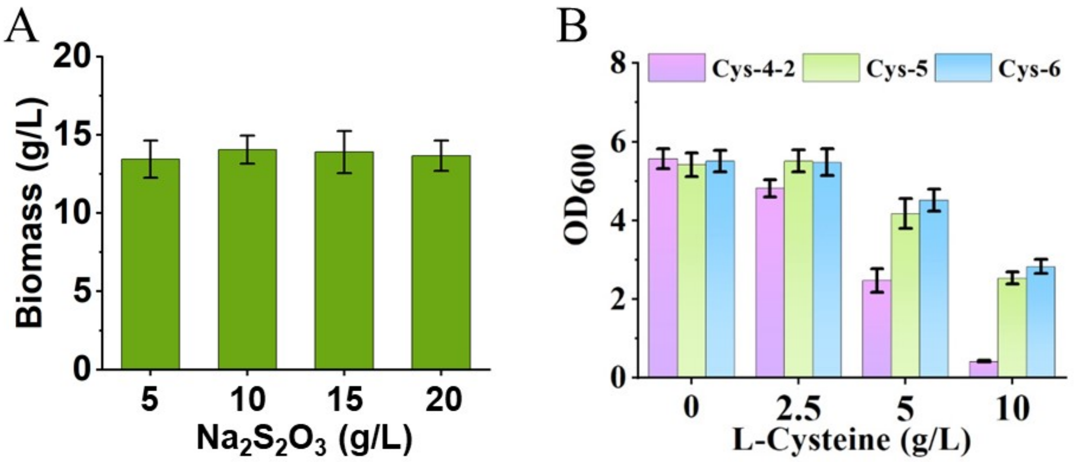

Fig. S1 (A) Measurement of the biomass of the engineered strain Cys-4-2 cultivated with different concentrations of $Na_2S_2O_3$. (B) Determination of the L-cysteine tolerance of the engineered strains. All data were the average of three independent experiments with standard deviations. Cys-4-2, Cys-4-1, *poxB* :: P_{H7}-*cysK*^{Ec}; Cys-5, Cys-4, *ldh* :: P_{H7}-*eamA*^{Ec}; Cys-6, Cys-5, △ *NCgl2463*.

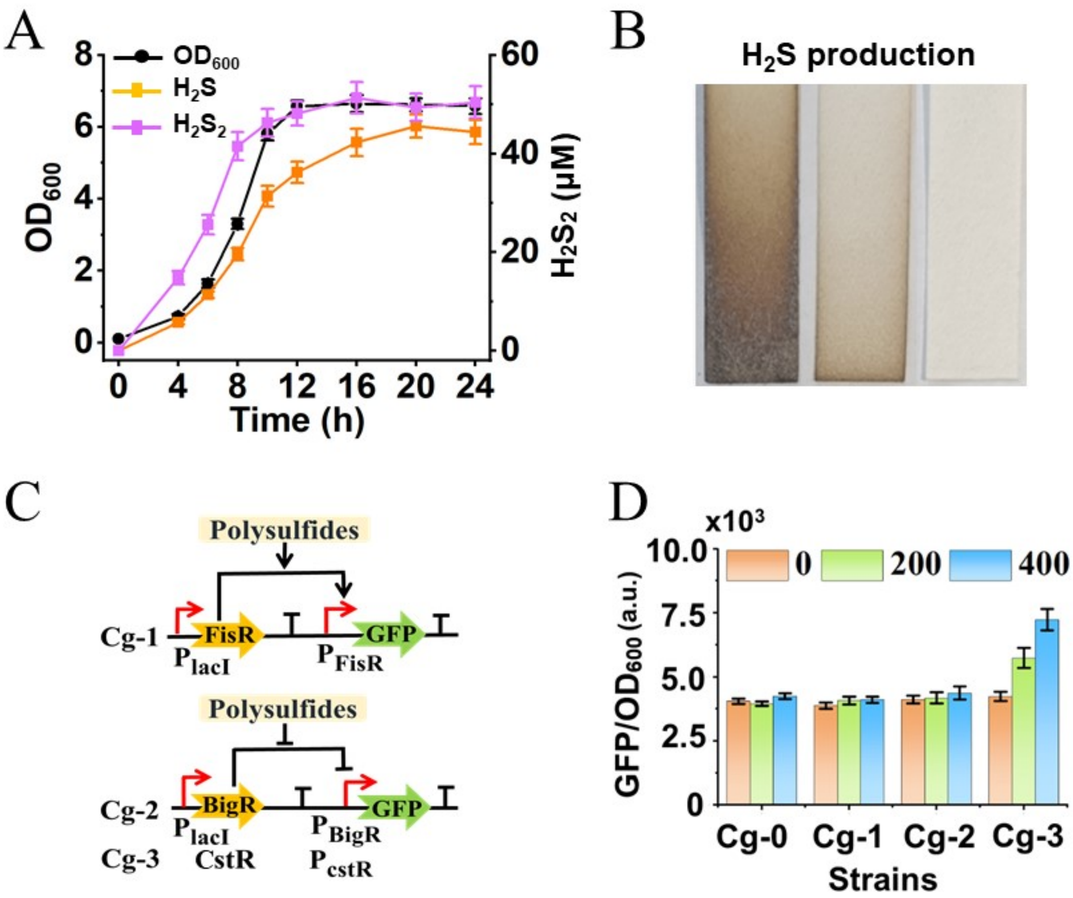

Fig. S2 Screening the H_2S_2-responsive transcription factor for the construction of genetic circuit in *C. glutamicum*. Determination of cell growth, intracellular H_2S and H_2S_2 levels (A) and the escaped H_2S (B) in *C. glutamicum* WT. (C) Schematic representation of testing plasmids containing FisR, CstR, and BigR. (D) Investigation of the function of three reporting systems in *C. glutamicum*. All data were the average of three independent experiments with standard deviations. Cg-0, *C. glutamicum* WT, containing pXMJ20; Cg-1, *C. glutamicum* WT, containing pFisR-P_{lac}-GFP; Cg-2, *C. glutamicum* WT, containing pBigR-P_{lac}-GFP; Cg-3, *C. glutamicum* WT, containing pcstR-P_{lac}-GFP.

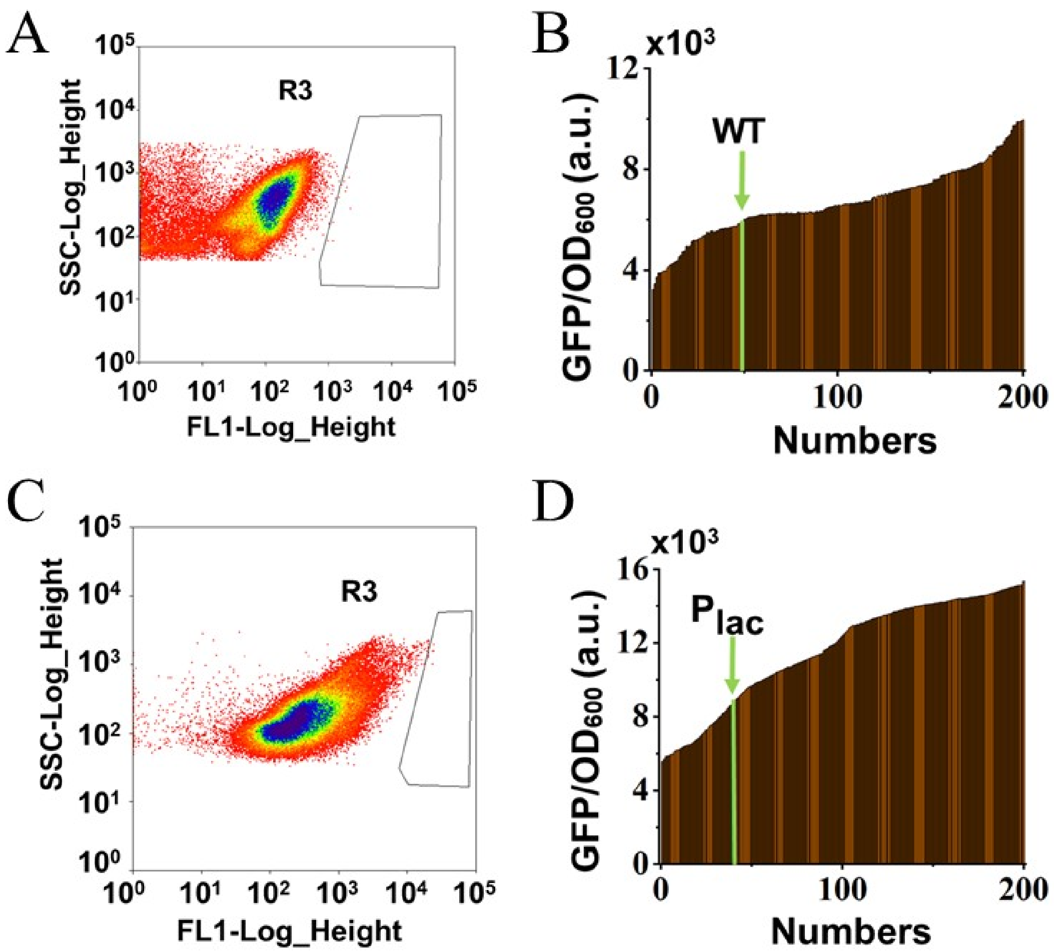

Fig. S3 Optimization of the H_2S_2-responsive genetic circuit in *C. glutamicum*. (A) FACS screening of the CstR variants. The black pentagon refers to the top 0.01% of all screened cells. (B) 96-well plate screening. The strains were screened using FACS, the wide-type CstR was used as the control, and the sorted strains were cultivated in 96-well plates. The fluorescence intensities were further analyzed by using a synergy neo2 multimode microplate reader. (C) FACS screening of the promoter library. The black pentagon refers to the top 0.01% of all screened cells. (D) 96-well plate screening. The strains were screened using FACS, the P_{lac} strain was used as the control, and the sorted strains were cultivated in 96-well plates. The fluorescence intensities were further analyzed by using a synergy neo2 multimode microplate reader.

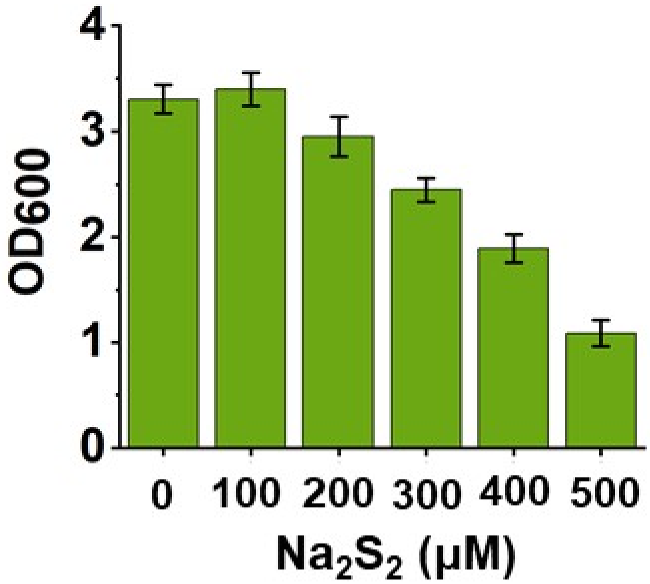

Fig. S4 Effects of different concentrations of Na_2S_2 on the biomass of *C.glutamicum* WT. All data were the average of three independent experiments with standard deviations.

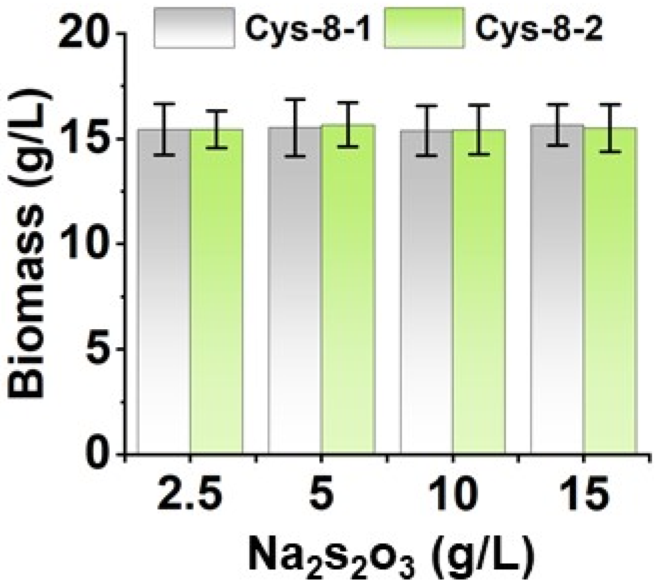

Fig. S5 Effects of different concentration of $Na_2S_2O_3$ on the biomass of BSGC and non BSGC strains. All data were the average of three independent experiments with standard deviations.Cys-8-1, Cys-6, containing pEC-mCh4 and pXMJ20; Cys-8-2, Cys-7, containing pEC-mCh4 and PcstR-cstB.

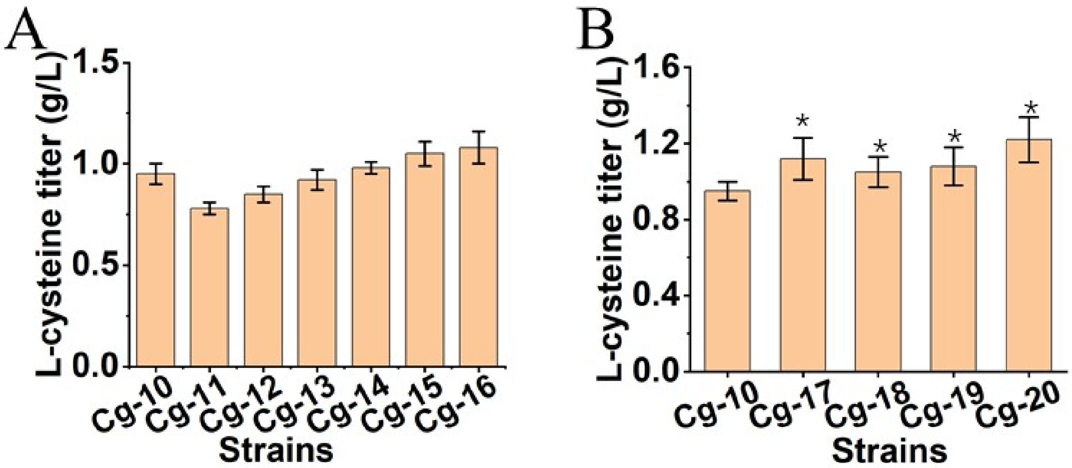

Fig. S6 Effects of the key genes of H_2S utilization and transportation module on L-cysteine production in *C. glutamicum*. (A) Determination of the role of different glutaredoxin and thioredoxin in L-cysteine production. (B) Measurement of the production of L-cysteine in different strains with overexpressing the H_2S utilization (thioredoxin, *cysM*, *cysK*) and transport module (*bcr*), respectively. All data were the average of three independent experiments with standard deviations. The * indicated the significant differences of $P \leq 0.01$ relative to the control. Cg-10, Cys-6 containing pXMJ20; Cg-11, Cys-6 containing pXMJ-cg0964; Cg-12, Cys-6 containing pXMJ-cg1244; Cg-13, Cys-6 containing pXMJ-cg2789; Cg-14, Cys-6 containing pXMJ-cg3299; Cg-15, Cys-6 containing pXMJ-cg3422; Cg-16, Cys-6 containing pXMJ-cg3423; Cg-17, Cys-6 containing pXMJ-Trx; Cg-18, Cys-6 containing pXMJ-cysM; Cg-19, Cys-6 containing pXMJ-cysK; Cg-20, Cys-6 containing pXMJ-bcr.

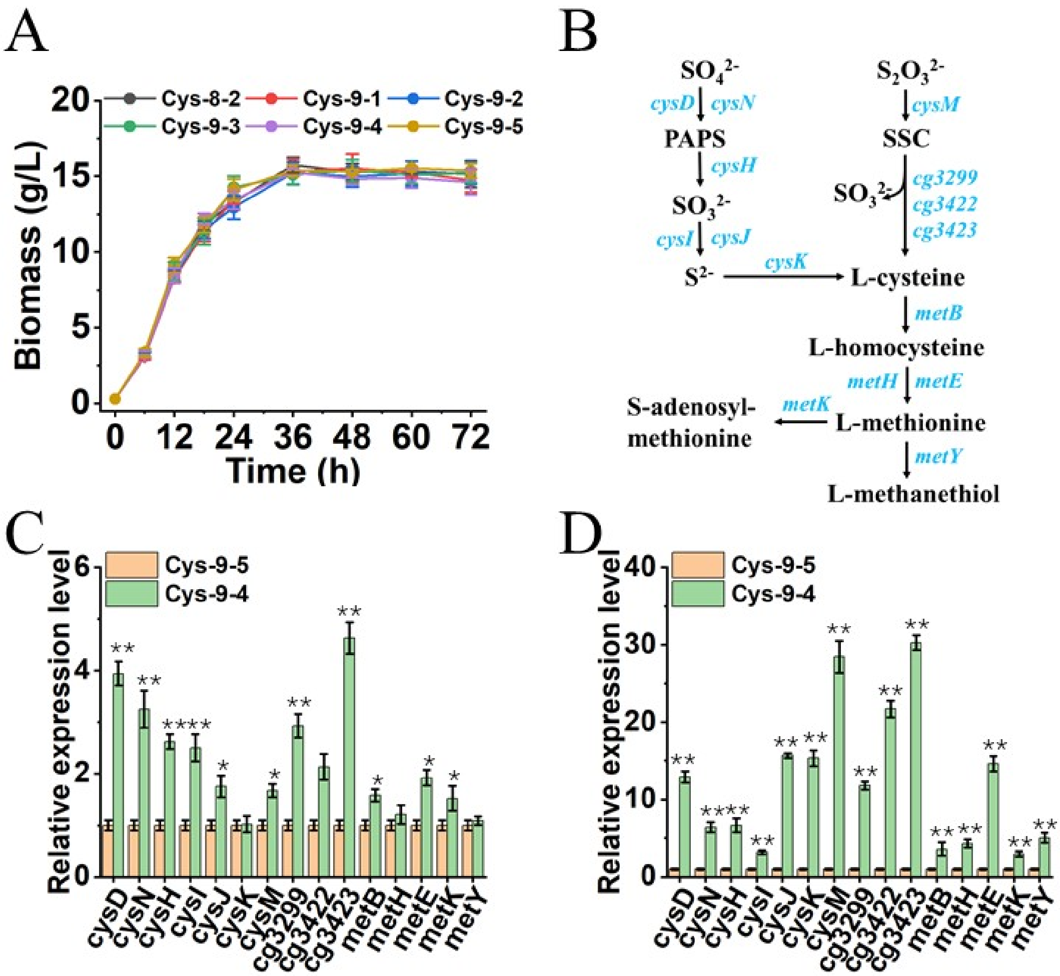

Fig. S7 (A) Determination of the cell growth in the L-cysteine biosynthetic network rewired strains. (B) The Schematic diagram of sulfur cycling network, including L-homocysteine, L-methionine, S-Adenosyl-L-methionine, Methanethiol pathways and sulfur assimilation pathway. (C and D) Measurement of the transcription levels of the key genes in L-homocysteine (*metB*), L-methionine (*metH* and *metE*), S-adenosyl-L-methionine (*metK*), methanethiol (*metY*) pathways and sulfur assimilation pathway (*cysD*, *cysN*, *cysH*, *cysI*, *cysJ*, *cysK*, *cysM*, *cg3299*, *cg3422* and *cg3423*) in the BSGC strain Cys-9-4 when cultivated at 24 h and 72 h, respectively. The static regulation strain Cys-9-5 was used as the control. All data were the average of three independent experiments with standard deviations. The * and ** indicated the significant differences of $P \leq 0.05$ and $P \leq 0.01$, respectively. cystathionine γ-synthase, *metB*; homocysteine methyltransferase, *metH* and *metE*; S-adenosylmethionine synthetase, *metK*; O-acetylhomoserine (thiol)-lyase, *metY*; sulfate adenyltransferase subunit 1,2, *cysD* and *cysN*; phosphoadenosine-phosphosulfate reductase, *cysH*; sulfite reductase, *cysI* and *cysJ*; cysteine synthase A, *cysK*; cysteine synthase B, *cysM*; thioredoxin-related genes, *cg3299*, *cg3422* and *cg3423*. Cys-8-2, Cys-7, containing pEC-mCh4 and PcstR-cstB; Cys-9-1, Cys-7, containing pEC-mCh4 and pXMJ-cstR-cysK; Cys-9-2, Cys-7, containing pEC-cys1 and pXMJ-cstR-cysK; Cys-9-3, Cys-7, containing pEC-cys1 and pXMJ-cstR-cys; Cys-9-4, Cys-7, containing pEC-cys2 and pXMJ-cstR-cys; Cys-9-5, Cys-6, containing pEC-mCh4 and pXMJ-cys-C.

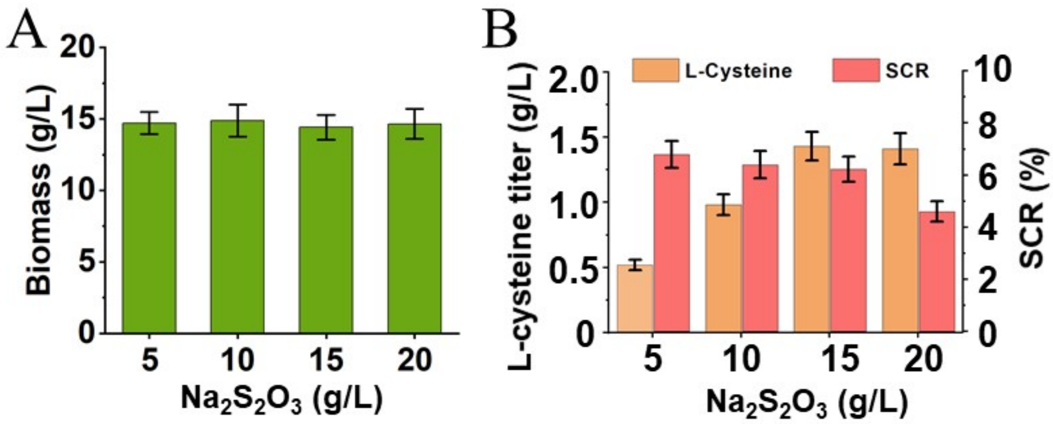

Fig. S8 Measurement of fermentation profiles in the static regulation strain Cys-9-5. Determination of biomass (A), L-cysteine production and SCR (B) under 5-20 g/L $Na_2S_2O_3$ supply. All data were the average of three independent experiments with standard deviations. Cys-9-5, Cys-6, containing pEC-mCh4 and pXMJ-cys-C

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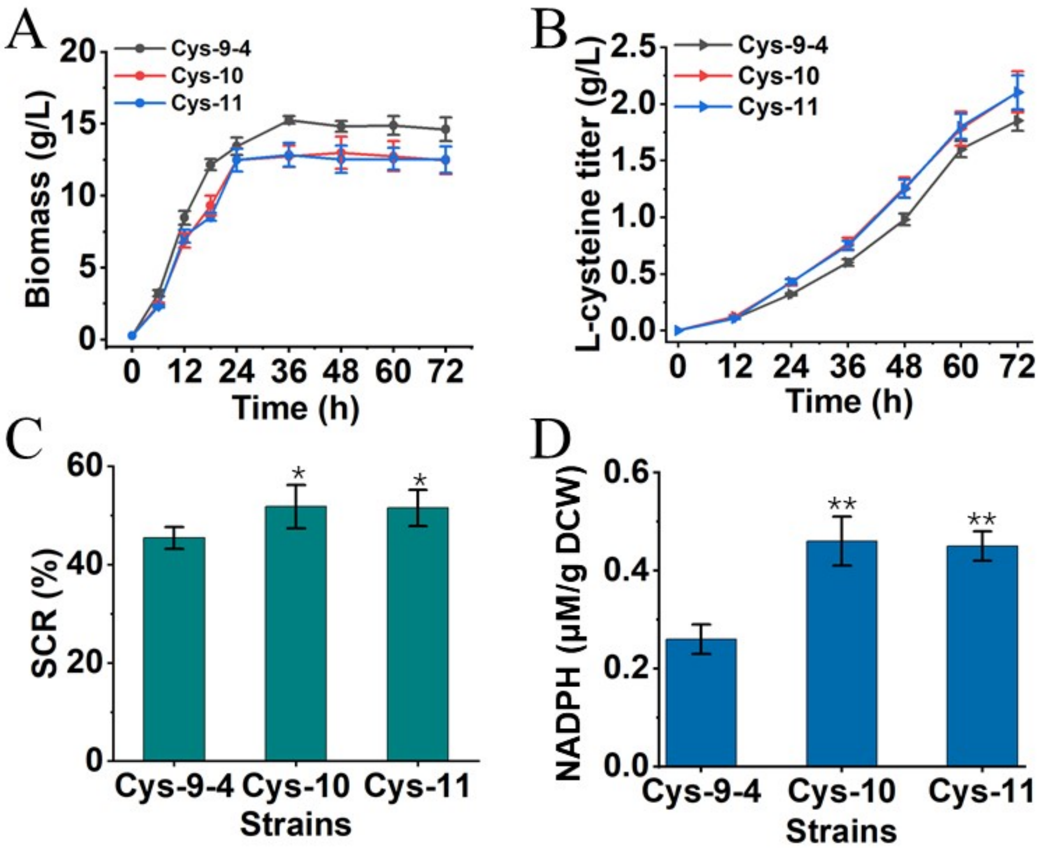

Fig. S9 Enhancing NADPH pool to promote L-cysteine production. Measurement of cell growth (A), L-cysteine production (B), SCR (C) and intracellular NADPH (D) in the NADPH-enhanced strains. All data were the average of three independent experiments with standard deviations. The * and ** indicated the significant differences of $P \leq 0.05$ and $P \leq 0.01$, respectively. Cys-9-4, Cys-7, containing pEC-cys2 and pXMJ-cstR-cys; Cys-10, Cys-9-4, P_{zwf} :: P_{H7}; Cys-11, *pck* :: P_{H7}-*pntAB*

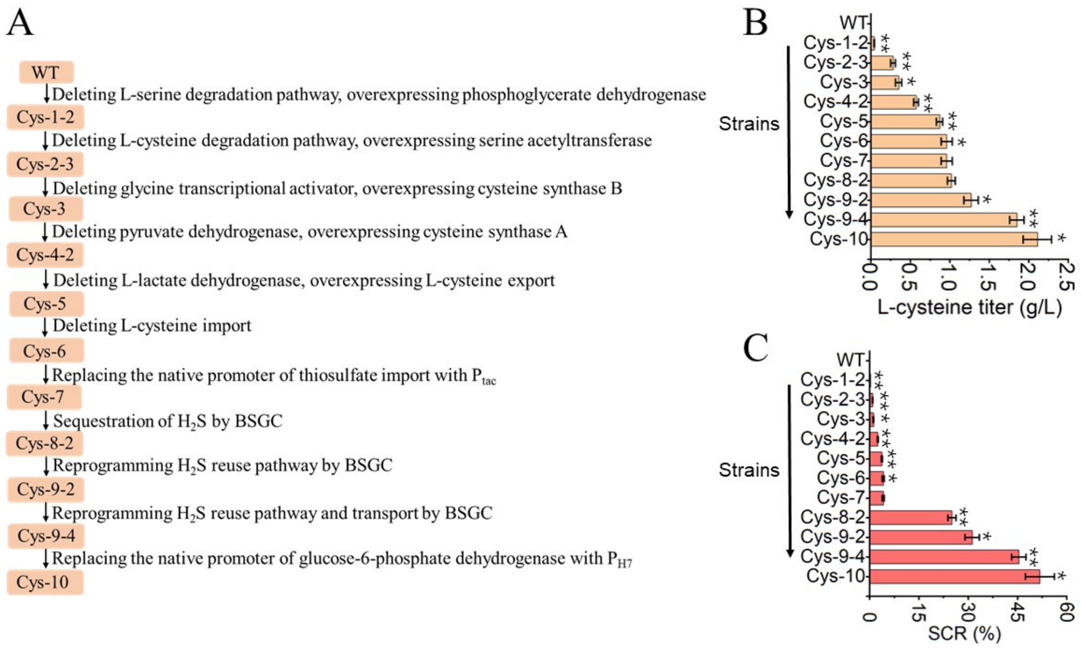

Fig. S10 (A) Flow chart of constructing an L-cysteine-producing strain in *C. gluamicum*. Determination of the L-cysteine production (B) and SCR (C) of the engineered strains in each step. All data were the average of three independent experiments with standard deviations. The * and ** indicated the significant differences of $P \leq 0.05$ and $P \leq 0.01$, respectively. WT, *C. glutamicum* WT; Cys-1-2, Cys-1-1, *sdaA* :: P_{H7}-*serA*$^{\triangle 197AA}$; Cys-2-3, Cys-2-1, *aecD* :: P_{H7}-*cysE*(M201R)-*cysE*(M201R); Cys-3, Cys-2-3, *glyR* :: P_{H7}-*cysM*^{Ec}; Cys-4-2, Cys-4-1, *poxB* :: P_{H7}-*cysK*^{Ec}; Cys-5, Cys-4, *ldh* :: P_{H7}-*eamA*^{Ec}; Cys-6, Cys-5, △ *NCgl2463*; Cys-7, Cys-6, P_{cysU} :: P_{tac}; Cys-8-2, Cys-7, containing pEC-mCh4 and PcstR-cstB; Cys-9-2, Cys-7, containing pEC-cys1 and pXMJ-cstR-cysK; Cys-9-4, Cys-7, containing pEC-cys2 and pXMJ-cstR-cys; Cys-10, Cys-9-4, P_{zwf} :: P_{H7}.

Table S1 Characteristics of strains used in this study

Strains	Characteristics	Source
E. coli DH5α	Host for plasmids construction	Invitrogen
E. coli MG1655	Host for genes cloning	Laboratory stock
C. glutamicum WT	Wild-type, the parent strain	Laboratory stock
Cys-1-1	*C. glutamicum* WT, P_{serA} :: P_{H7}	This study
Cys-1-2	Cys-1-1, *sdaA* :: P_{H7}-*serA*$^{\triangle 197AA}$	This study
Cys-1-3	Cys-1-1, *sdaA* :: P_{H7}-*serA*$^{\triangle 197AA}$-*serA*$^{\triangle 197AA}$	This study
Cys-2-1	Cys-1-2, P_{cysE} :: P_{H7}	This study
Cys-2-2	Cys-2-1, *aecD* :: P_{H7}-*cysE*(M201R)	This study
Cys-2-3	Cys-2-1, *aecD* :: P_{H7}-*cysE*(M201R)-*cysE*(M201R)	This study
Cys-2-4	Cys-2-3, *glyR* :: P_{H7}-*cysE*(M201R)	This study
Cys-3	Cys-2-3, *glyR* :: P_{H7}-*cysM*^{Ec}	This study
Cys-4-1	Cys-3, P_{cysK} :: P_{H7}	This study
Cys-4-2	Cys-4-1, *poxB* :: P_{H7}-*cysK*^{Ec}	This study
Cys-4-3	Cys-4-1, *poxB* :: P_{H7}-*cysK*^{Ec}-*cysK*^{Ec}	This study
Cys-5	Cys-4-2, *ldh* :: P_{H7}-*eamA*^{Ec}	This study
Cys-6	Cys-5, △ *NCgl2463*	This study
Cys-7	Cys-6, P_{cysU} :: P_{tac}	This study
Cys-8-1	Cys-6, containing pEC-mCh4 and pXMJ20	This study
Cys-8-2	Cys-7, containing pEC-mCh4 and PcstR-cstB	This study
Cys-9-1	Cys-7, containing pEC-mCh4 and pXMJ-cstR-cysK	This study
Cys-9-2	Cys-7, containing pEC-cys1 and pXMJ-cstR-cysK	This study
Cys-9-3	Cys-7, containing pEC-cys1 and pXMJ-cstR-cys	This study
Cys-9-4	Cys-7, containing pEC-cys2 and pXMJ-cstR-cys	This study
Cys-9-5	Cys-6, containing pEC-mCh4 and pXMJ-cys-C	This study
Cys-10	Cys-9-4, P_{zwf} :: P_{H7}	This study
Cys-11	Cys-9-4, *pck* :: P_{H7}-*pntAB*^{Ec}	This study
Strains used to verify gene function in this study		
Cg-0	*C. glutamicum* WT, containing pXMJ20	This study
Cg-1	*C. glutamicum* WT, containing pFisR-P_{lac}-GFP	This study
Cg-2	*C. glutamicum* WT, containing pBigR-P_{lac}-GFP	This study
Cg-3	*C. glutamicum* WT, containing pcstR-P_{lac}-GFP	This study
Cg-4	*C. glutamicum* WT, containing pXMJ-sqr^{Cp}	This study
Cg-5	*C. glutamicum* WT, containing pXMJ-sqr^{Ss}	This study
Cg-6	*C. glutamicum* WT, containing pXMJ-cstA	This study
Cg-7	*C. glutamicum* WT, containing pXMJ-cstB	This study

Cg-8	*C. glutamicum* WT, containing pXMJ-pdo	This study
Cg-9	*C. glutamicum* WT, containing PcstR-sqr and pEC-mCh4	This study
Cg-10	Cys-6 containing pXMJ20	This study
Cg-11	Cys-6 containing pXMJ-cg0964	This study
Cg-12	Cys-6 containing pXMJ-cg1244	This study
Cg-13	Cys-6 containing pXMJ-cg2789	This study
Cg-14	Cys-6 containing pXMJ-cg3299	This study
Cg-15	Cys-6 containing pXMJ-cg3422	This study
Cg-16	Cys-6 containing pXMJ-cg3423	This study
Cg-17	Cys-6 containing pXMJ-Trx	This study
Cg-18	Cys-6 containing pXMJ-cysM	This study
Cg-19	Cys-6 containing pXMJ-cysK	This study
Cg-20	Cys-6 containing pXMJ-bcr	This study

Ec: *Escherichia coli*

Sa: *Staphylococcus aureus*

Cp: *Cupriavidus pinatubonensis* JMP134

Ss: *Synechococcus* sp. strain PCC7002

Table S2 Plasmids used in this study

Plasmids	Relevant characteristics	Sources
pCRD206		
pCRD206	Kan^R, gene deletion plasmid	(32)
pCRD-P_{H7}-serA	pCRD206 derivate, replacing native promoter of *serA* by the promoter P_{H7}	This study
pCRD-sdaA-P_{H7}-serA	pCRD206 derivate, deleting *sdaA* gene，overexpressing $serA^{\triangle 197AA}$	This study
pCRD-sdaA-P_{H7}-serA-serA	pCRD206 derivate, deleting *sdaA* gene, overexpressing two copies of $serA^{\triangle 197AA}$	This study
pCRD-P_{H7}-cysE	pCRD206 derivate, replacing native promoter of *cysE* by the promoter P_{H7}	This study
pCRD-aecD-P_{H7}-cysE	pCRD206 derivate, deleting *aecD* gene，overexpressing *cysE* (M201R)	This study
pCRD-aecD-P_{H7}-cysE-cysE	pCRD206 derivate, deleting *sdaA* gene, overexpressing two copies of *cysE* (M201R)	This study
pCRD-glyR-P_{H7}-cysE	pCRD206 derivate, deleting *sdaA* gene, overexpressing *cysE* (M201R)	This study
pCRD-glyR-P_{H7}-cysM	pCRD206 derivate, deleting *glyR* gene，overexpressing $cysM^{Ec}$	This study
pCRD-P_{H7}-cysK	pCRD206 derivate, replacing native promoter of *cysK* by the promoter P_{H7}	This study
pCRD-poxB-P_{H7}-cysK	pCRD206 derivate, deleting *poxB* gene，overexpressing $cysK^{Ec}$	This study
pCRD-poxB-P_{H7}-cysK-cysK	pCRD206 derivate, deleting *poxB* gene，overexpressing two copies of $cysK^{Ec}$	This study
pCRD-ldh-P_{H7}-eamA	pCRD206 derivate, deleting *ldh* gene，overexpressing $eamA^{Ec}$	This study
pCRD-NCgl2463	pCRD206 derivate, deleting *NCgl2463* gene	This study
pCRD-P_{tac}-cysU	pCRD206 derivate, replacing native promoter of *cysU* by the promoter P_{tac}	This study
pCRD-P_{H7}-zwf	pCRD206 derivate, replacing native promoter of *zwf* by the promoter P_{H7}	This study
pCRD-pck-P_{H7}-pntAB	pCRD206 derivate, deleting *pck* gene，overexpressing $pntAB^{Ec}$	This study
pXMJ19		
pXMJ19	Cm^R, *C. glutamicum*-*E. coli* shuttle vector	Invitrogen

pXMJ20	pXMJ19 derivate, removing *BsaI* recognition site	(33)
pXMJ21	pXMJ 20 derivate, removing *lacIq* and P_{tac} promoter and inserting *GFP*	(33)
pXMJ-P_{lac}-GFP	pXMJ21 derivate, P_{lac}-GFP	This study
pcstR-P_{lac}-GFP	pXMJ-P_{lac}-GFP derivate, fused with *cstR*^{Sa}-P_{cstR} at the C-terminus of GFP	This study
pFisR-P_{lac}-GFP	pXMJ-P_{lac}-GFP derivate, fused with *fisR*-P_{FisR} at the C-terminus of GFP	This study
pBigR-P_{lac}-GFP	pXMJ-P_{lac}-GFP derivate, fused with *bigR*-P_{BigR} at the C-terminus of GFP	This study
PcstR^{E27G}	pcstR-P_{lac}-GFP derivate, CstR mutation site E27G	This study
PcstR^{S41G}	pcstR-P_{lac}-GFP derivate, CstR mutation site S41G	This study
PcstR^{V83A}	pcstR-P_{lac}-GFP derivate, CstR mutation siteV83A	This study
PcstR$^{E27G/S41G}$	pcstR-P_{lac}-GFP derivate, CstR mutation site E27G/S41G	This study
PcstR$^{E27G/V83A}$	pcstR-P_{lac}-GFP derivate, CstR mutation site E27G/V83A	This study
PcstR$^{S41G/V83A}$	pcstR-P_{lac}-GFP derivate, CstR mutation site S41G/V83A	This study
PcstR$^{E27G/S41G/A64D}$	pcstR-P_{lac}-GFP derivate, CstR mutation site E27G/S41G/A64D	This study
PcstR$^{E27G/S41G/I45T}$	pcstR-P_{lac}-GFP derivate, CstR mutation site E27G/S41G/I45T	This study
PcstR$^{E27G/S41G/A64D/I45T}$	pcstR-P_{lac}-GFP derivate, CstR mutation site E27G/S41G/A64D/I45T	This study
PcstR-P1	PcstR$^{E27G/S41G/A64D}$ derivate, E56 (P_{R1})	This study
PcstR-P2	PcstR$^{E27G/S41G/A64D}$ derivate, G28 (P_{R2})	This study
PcstR-P3	PcstR$^{E27G/S41G/A64D}$ derivate, K72 (P_{R3})	This study
PcstR-sqr	PcstR-P3 derivate, fused with P_{H7}-*sqr*^{Cp} at the N-terminus of GFP	This study
PcstR-cstB	PcstR-sqr derivate, replacing *GFP* by *cstB*	This study
pXMJ-sqr^{Cp}	pXMJ 20 derivate, inserting *sqr*^{Cp} at MCS	This study
pXMJ-sqr^{Ss}	pXMJ 20 derivate, inserting *sqr*^{Ss} at MCS	This study
pXMJ-cstA	pXMJ 20 derivate, inserting *cstA* at MCS	This study
pXMJ-cstB	pXMJ 20 derivate, inserting *cstB* at MCS	This study
pXMJ-pdo	pXMJ 20 derivate, inserting *pdo* at MCS	This study
pXMJ-cg0964	pXMJ 20 derivate, inserting *cg0964* at	This study

	MCS	
pXMJ-cg1244	pXMJ 20 derivate, inserting *cg1244* at MCS	This study
pXMJ-cg2789	pXMJ 20 derivate, inserting *cg2789* at MCS	This study
pXMJ-cg3299	pXMJ 20 derivate, inserting *cg3299* at MCS	This study
pXMJ-cg3422	pXMJ 20 derivate, inserting *cg3422* at MCS	This study
pXMJ-cg3423	pXMJ 20 derivate, inserting cg3423 at MCS	This study
pXMJ-Trx	pXMJ 20 derivate, inserting *cg3299-3422-3423* at MCS	This study
pXMJ-cysM	pXMJ 20 derivate, inserting *cysM* at MCS	This study
pXMJ-cysK	pXMJ 20 derivate, inserting *cysK* at MCS	This study
pXMJ-bcr	pXMJ 20 derivate, inserting *bcr* at MCS	This study
pXMJ-cstR-cysK	PcstR-cstB derivate, fused with *cysK* at the C-terminus of *cstB*	This study
pXMJ-cys-C	pXMJ 20 derivate, inserting *cysM-cg3299-3422-3423-cysK-bcr* at MCS	This study
pXMJ-cstR-cys	PcstR-cstB derivate, fused with *cysK-bcr* at the C-terminus of *cstB*	This study
pEC-XK99E		
pEC-XK99E	Kan^R, *C. glutamicum-E. coli* shuttle vector	Invitrogen
pEC-mCh1	pEC-XK99E derivate, inserting mCherry before MCS	This study
pEC-mCh2	pEC-mCh1 derivate, replacing native promoter of *LacI* by the promoter P_{R3}	This study
pEC-mCh3	pEC-mCh2 derivate, replacing P_{trc} of mCherry by the promoter P_{lac}	This study
pEC-mCh4	pEC-mCh2 derivate, replacing P_{trc} of mCherry by the promoter P_{tac}	This study
pEC-cys1	pEC-mCh4 derivate, replacing mCherry by P_{R3}-*cysM-Trx*	This study
pEC-cys2	pEC-cys1 derivate, fused with DAS+8 tag at the N-terminus of *lacI*	This study

Table S3 Primers used in this study

Name	Sequence (5’-3’)
Primers for the pCRD206	
pCRD206-F	tctagagtcgacctgcaggcatgcaagct
pCRD206-R	ggatccccgggtaccgagctcgaattc

H7-serA-1F	cgaattcgagctcggtacccggggatccgatcaaatcaataccgatcacctggaac
H7-serA-1R	gcagaatcaatagatcactcgagcaagttactcctggaaaaactagga
H7-serA-2F	tcctagtttttccaggagtaacttgctcgagtgatctattgattctgc
H7-serA-2R	ctaccggacggccattctggctcattatcttcctttccagatcaatag
H7-serA-3F	ctattgatctggaaaggaagataatgagccagaatggccgtccggtag
H7-serA-3R	caagcttgcatgcctgcaggtcgactctagattagcgagccagatccatccacaca
sdaA-serA-1F	acgaattcgagctcggtacccggggatccgtccaccactcgctccgtggcggatcaa
sdaA-serA-1R	agaatcaatagatcactcgagcggctgagggtgggccttttctatggtggtgtac
sdaA-serA-2F	gaaaaggcccaccctcagccgctcgagtgatctattgattctgcg
sdaA-serA-2R	accggacggccattctggctcattatcttcctttccagatcaatagagtt
sdaA-serA-3F	gaaaggaagataatgagccagaatggccgtccggta
sdaA-serA-3R	cgtgttaaagccgtaccgctaattagcgagccagatccatccacac
sdaA-serA-4F	gtgtggatggatctggctcgctaattagcggtacggctttaacacg
sdaA-serA-4R	aagcttgcatgcctgcaggtcgactctagaaggtgaatgcccctgtggtgatca
serA-serA-1F	acgaattcgagctcggtacccggggatccgtccaccactcgctccgtggcggatcaa
serA-serA-1R	agaatcaatagatcactcgagcggctgagggtgggccttttctatggtggtgtac
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Primers for the pXMJ19	
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pXMJ-R	tacgacgataccgaagacagctcatgtt
lacI-GFP-F	catgagctgtcttcggtatcgtcgtatatatggcgagcgcaatgaccatt
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Primers for the pEC-XK99E	
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pEC-mCh4-2R	tgacgccagaagcattggtgcacaccacagcttccgatggctgcct
pEC-cys1-1F	gtggaaaaactagagaagcacctctaggaattcgagctcggtacccggggatcc
pEC-cys1-1R	tattgtttgttctaatgtactcataataacctcctgttaaatacctat
pEC-cys1-2F	ataggtatttaacaggaggttattatgagtacattagaacaaacaata
pEC-cys1-2R	ggatccccgggtaccgagctcgaattcctagaggtgcttctctagtttttccac
pEC-cys2-F	ctacgctgatgcttcctaagctgcaaacgacgaaaactacaactacgctgatgcttcct aagcgcaacgcaattaatgtgagttag
pEC-cys2-R	gtagttttcgtcgtttgcagcttaggaagcatcagcgtagttttcgtcgtttgcagcctgcc cgctttccagtcgggaaacctgt
Primers for the RT-qPCR	
cysD-F	aagtccaggactggatcgat
cysD-R	gcttcagtccagttggagat
cysN-F	atcaccatcgacgttgccta
cysN-R	ctgtgacatcaagtgcagat
cysH-F	catgctgctgcgttgtatga
cysH-R	cttgaagtggtaaccggtgt
cysI-F	ccattgcaccggatgacatt
cysI-R	tacgaatccagtgcagctga
cysJ-F	acgcatcaagggcatcgtta
cysJ-R	gtcgtagaagccaacgaact
cysK-F	aactcgctgactatcggtca

cysK-R	gctacataggccagtgcaat
cysM-F	ctggtgaagttgcagcgaat
cysM-R	tgttgtcgggcatcagcaat
cg3299-F	aaccatcgatgtaaccgaa
cg3299-R	catcagagttgggatggact
cg3422-F	tggctataccgcagcagtat
cg3422-R	gacaagctccatgtgcatgt
cg3423-F	caagccagtcatcgttgact
cg3423-R	gaccgacaaattcctcgact
metB-F	atgagccagacgactactac
metB-R	gatgatgcggaacaggatgt
metH-F	cctgatgtgttgaggcagat
metH-R	gcaacgatcagcgatgtcat
metE-F	gcgaagcgtgaactgaagtt
metE-R	atcgagcattgcgtcgtagt
metK-F	accagcgcttacgtagagat
metK-R	actgctcaccgatggatact
metY-F	caattccaatgctgaccagt
metY-R	ctagatcctcaagtgcgaa
16S-F	acctggagaagaagcaccg
16S-R	tcaagttatgcccgtatcg