Supplementary materials for

Design of a programmable biosensor-CRISPRi genetic circuits for dynamic and autonomous dual-control of metabolic flux in *Bacillus subtilis*

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Table S1. Plasmids used in this study

Plasmid	Characteristics	Source or reference
p7z6	ColE1 Amp^r, lox71-Zeo-lox66	(1)
pTSC	ColE1 Amp^r, RepF Em^r P_{spac}-cre, E. coli-B. subtilis shuttle vector	(1)
pHT01	ColE1 Amp^r, Cm^r, E. coli-B. subtilis shuttle vector	(2)
pSTOP1622	ColE1 Amp^r,RepB Tet^r, E. coli-B. subtilis shuttle vector	(3)
pP43-*GNA1*	ColE1 Amp^r, RepB Km^r, *E. coli-B. subtilis* shuttle vector, *GNA1* expressed from P43	(4)
pP43-*egfp*	ColE1 Amp^r, RepB Km^r, *E. coli-B. subtilis* shuttle vector, *egfp* expressed from P43	(5)
pLCx-dCas9	pMB1 $Spec^r$, Cm^r xylR-PxylA-dCas9 integrant expression at lacA locus of *B. subtilis*	(6)
psga	pMB1 $Spec^r$, Zeo^r P_{veg}-sgRNA integrant expression at *amyE* locus of *B. subtilis*	(6)
psga-GFP	psga derivate, contain sgRNA targeting to GFP	(6)
psga-*zwf*1-*pfkA*2-*glmM*2	psga derivate, contain sgRNAs targeting to front of *zwf*, middle of *pfkA*, and middle of *glmM*	(6)
pHTa0	pHT01 derivate, △P_{grac}::*egfp*	This study
pHTaga	pHTa0 derivate, P_{gamA}-*egfp*	This study
pHTave	pHTa0 derivate, P_{veg}-*egfp*	This study
pHTavg1	pHTa0 derivate, P_{vg1}-*egfp*	This study
pHTavg2	pHTa0 derivate, P_{vg2}-*egfp*	This study
pHTavg3	pHTa0 derivate, P_{vg3}-*egfp*	This study
pHTavg4	pHTa0 derivate, P_{vg4}-*egfp*	This study
pHTavg5	pHTa0 derivate, P_{vg5}-*egfp*	This study
pHTavg6	pHTa0 derivate, P_{vg6}-*egfp*	This study
pHTavg7	pHTa0 derivate, P_{vg7}-*egfp*	This study
pHTarg	pHTa0 derivate, P_{rg}-*egfp*	This study
pHTalg	pHTa0 derivate, P_{lg}-*egfp*	This study
pHTavg	pHTa0 derivate, P_{vg}-*egfp*	This study
pHTasg	pHTa0 derivate, P_{sg}-*egfp*	This study
pHTasg1	pHTa0 derivate, P_{sg1}-*egfp*	This study
pHTasg2	pHTa0 derivate, P_{sg2}-*egfp*	This study
pHTasg3	pHTa0 derivate, P_{sg3}-*egfp*	This study
pHTcvg6	pHTavg6 derivate, *gamR*-P_{vg6}-*egfp*	This study

pHTcga	pHTaga derivate, *gamR*-P_{gamA}-*egfp*	This study
pHTcsg2	pHTasg2 derivate, *gamR*-P_{sg2}-*egfp*	This study
pHTdsg2	pHTcsg2 derivate, *xylR*-P_{xylA}-*gamR*	This study
pHTcsg2m	pHTcsg2 derivate, P_{sg2}-*mcherry*	This study
pLCv-dCas9	pLCx-dCas9 derivate, △*xylR*-P_{xylA}::*gamR*-P_{vg6}	This study
pLCg-dCas9	pLCx-dCas9 derivate, △*xylR*-P_{xylA}::*gamR*-P_{gamA}	This study
pLCs-dCas9	pLCx-dCas9 derivate, △*xylR*-P_{xylA}::*gamR*-P_{sg2}	This study
pSTv-*GNA1*	pSTOP1622 derivate, △*xylR*-P_{xylA}::P_{vg6}-*GNA1*	This study
pSTg-*GNA1*	pSTOP1622 derivate, △*xylR*-P_{xylA}::P_{gamA}-*GNA1*	This study
pSTs-*GNA1*	pSTOP1622 derivate, △*xylR*-P_{xylA}::P_{sg2}-*GNA1*	This study
psga-*alsS1*	psga derivate, contain sgRNAs targeting to site1 of *alsS*	This study
psga-*alsS2*	psga derivate, contain sgRNAs targeting to site2 of *alsS*	This study
psga-*alsS3*	psga derivate, contain sgRNAs targeting to site3 of *alsS*	This study
psga-*alsS4*	psga derivate, contain sgRNAs targeting to site4 of *alsS*	This study
psga-*alsS5*	psga derivate, contain sgRNAs targeting to site5 of *alsS*	This study

Table S2. Primers used in this study

Primer	Sequence
Fluorescence plasmids construction	
pHT01-li-F	AACTGTACAAATAACTGCAGGTCGACGTCCCCGGGGC
pHT01-li-R	TTTTCTCGAGGGTACCGCTATCACTTGGATCCGTACCAAGCTAATTCCGGTGGAAACG
egfp-pHT01-F	TAGCGGTACCCTCGAGAAAAGGAGGAAAAAAAATGGGTAAGGGAGAAGAACTTTTCACT
egfp-pHT01-R	CTGCAGTTATTTGTACAGTTCATCCATGCCATGTGTAATCC
Pveg-pHTa0-F	AATTAGCTTGGTACGGATCCTTTTGTCAAAATAATTTTATTGACAACGTCTTATTAACG
Pveg-pHTa0-R	CTCCTTTTCTCGAGGGTACCCGACATTTATTGTACAACACGAGCCCATT
PgamA-pHTa0-F	AATTAGCTTGGTACGGATCCCTTTTATAACAAATTTTCAGAAATTAATATTGACAGTTT
PgamA-pHTa0-R	CTCCTTTTCTCGAGGGTACCGAGATAGACAAGCACCATATTTGTT
vgTOvg1-F	TAAATGTCGTAAATTGGTCATAACAAGGTACCCTCGAGAAAAGGAGGAAAAAAAATG
vgTOvg1-R	TCGAGGGTACCTTGTTATGACCAATTTACGACATTTATTGTACAACACGAGCCC
vgTOvg1-VF	AATGTCGTAAATTGGTCATAACAAGGTACC
vgTOvg2	GTATTGTTATGACCAATTTATGTCAAATAAAATTTAAATTATATCAACGTTAATAAGAC
vgTOvg2-F	TTTGACATAAATTGGTCATAACAATACAATAAATGTCGGGTACCCTC
vgTOvg2-VF	ATTTTATTTGACATAAATTGGTCATAACAATACAA
vgTOvg3-F	GTTGATAAATTGGTCATAACAATTGACAAAAATGGGCTCGTG
vgTOvg3-R	CCCATTTTTGTCAATTGTTATGACCAATTTATCAACGTTAATAAGACGTTGTCAATAAA
vgTOvg3-VF	CGTTGATAAATTGGTCATAACAATTGAC
vg1TOvg4-F	TTTATTTGACATAAATTGGTCATAACAATACAATAAATGTCGTAAATTGGTCATAACA
vg1TOvg4-R	GTATTGTTATGACCAATTTATGTCAAATAAAATTTAAATTATATCAACGTTAATAAGAC
vg1TOvg4-VF	TATTTGACATAAATTGGTCATAACAATACAATAAA
vg1TOvg5-F	AACGTTGATAAATTGGTCATAACAATTGACAAAAATGGGCTCGTG
vg1TOvg5-R	TTTTGTCAATTGTTATGACCAATTTATCAACGTTAATAAGACGTTGTCAATAA
vg1TOvg5-VF	CGTTGATAAATTGGTCATAACAATTGAC
vg2TOvg6-F	GTTGATAAATTGGTCATAACAATTGACATAAATTGGTCATAACAATACAATAAATG
vg2TOvg6-R	CCAATTTATGTCAATTGTTATGACCAATTTATCAACGTTAATAAGACGTTGTCAATAAA
vg2TOvg6-VF	CGTTGATAAATTGGTCATAACAATTGACATA

vg6TOvg7-F	AATGTCGTAAATTGGTCATAACAAGGTACCCTCGAGAAAAGGAG
vg6TOvg7-R	GAGGGTACCTTGTTATGACCAATTTACGACATTTATTGTATTGTTATGACCAATTT
vg6TOvg7-VF	AATGTCGTAAATTGGTCATAACAAGGTA
pHTaga-lin-F	TAAATTCGTAATGACAAGTATCATAAATTGGTCATAACAAATATGG
pHTaga-lin-R	GGATCCGTACCAAGCTAATTCCGG
PlepA-pHTaga-F	AATTAGCTTGGTACGGATCCTCAATATGTTTTCAAAAGCCGGAAAAGCG
PlepA-pHTaga-R	TACTTGTCATTACGAATTTATACACTAGCTTAGATTATATCAATAGGATTGTAAAGATTCAATGTAA
PrelA-pHTaga-F	AATTAGCTTGGTACGGATCCCGAGCTTTCTTACCTTGACGGC
PrelA-pHTaga-R	TACTTGTCATTACGAATTTACAAGAAAAAAACGAAAGATGTAAAGAGCAGAAGT
PsrfA-pHTaga-F	AATTAGCTTGGTACGGATCCAATGTTTAGTGGAAATGATTGCGGCAT
PsrfA-pHTaga-R	TACTTGTCATTACGAATTTAAATGAAAAAAATGTTTTTGTCAACGAAAAATGGGTGAAAAGTTTCA
Pveg-pHTaga-F	AATTAGCTTGGTACGGATCCTTTTGTCAAAATAATTTTATTGACAACGTCT
Pveg-pHTaga-R	TACTTGTCATTACGAATTTACAACACGAGCCCATTTTTG
pHTa-pHTc-liF	GGAATTAGCTTGGTACGGATCC
pHTa-pHTc-liR	GGCACTAGTATAAAAAACGCCC
gamR-pHTc-F	ATCCGTACCAAGCTAATTCCATTTCTGAAAATTTGTTATAAAAGAAGGATACAAATCTT
gamR-pHTc-R	GCGTTTTTTATACTAGTGCCTTATGAATGATATGACTGTTCTACGGTG
pHTc-pHTd-liF	GGAATTAGCTTGGTACGGATCC
pHTc-pHTd-liR	ATGACAGCTTTATATTCTGTTATCAAGTT
Pxyl-pHTd-F	ACAGAATATAAAGCTGTCATGGATCCCATTTCCCCCTTTGATTTTT
Pxyl-pHTd-R	ATCCGTACCAAGCTAATTCCCGTGAAAAAAGCCCGCTCATTAGGCGGGCTGAACGTCCCGGGGAGCTC
pHTcm-liF	TGGACGAGCTGTACAAGTAACTGCAGGTCGACGTCCCC
pHTcm-liR	CCAGTAGATTTCATAAACATTTTTTTTCCTCCTTTTCTCGAGGGTACC
mcherry-pHTc-F	ATGTTTATGAAATCTACTGGTATTGTACGTATGGTGAGCAAGGGCGAG
mcherry-pHTc-R	TTACTTGTACAGCTCGTCCATGCC
Gene knockout and overexpression	
nagB-L-F	ATATCCACGGCGGCTATG
nagB-L-R	GCCATGGCATGATCTGGTCAGCCTCCTTG
nagB-R-F	TCGACCTGCAGAAGGAACATGCTGACTTATGAATATCAA
nagB-R-R	TTATTTTGAATGTAACGTATATACCAATGAAGAG
nagB-Z-F	GCTGACCAGATCATGCCATGGCATGAGATTCT
nagB-Z-R	GCATGTTCCTTCTGCAGGTCGACGATTTACC
gamA-L-F	TGGCGGACATGGAATAAATCAC

gamA-L-R	TAGAGGATCCCCGCACAATAAAAGGAGACTGTCATGTTTAAAAAG
gamA-R-F	CGACCTGCAGTGACACCCCCTCAAAGAG
gamA-R-R	TCCCCACAGCACTTTTCCAT
gamA-Z-F	TCCTTTTATTGTGCGGGGATCCTCTAGAGATTGT
gamA-Z-R	GGGGTGTCACTGCAGGTCGACGATTCTACC
gamR-L-F	AATTCCTCCGCCTGAGAACG
gamR-L-R	GATCCCCGGGTACCATGTTTAAAAAGGCATTTCAAATTCTGCAG
gamR-R-F	TCGACCTGCAGAGCAATGTGTTTTAAGAAGGGAATGGT
gamR-R-R	AGCACGATCATAAATGTATAGCTCCTGG
gamR-Z-F	CCTTTTTAAACATGGTACCCGGGGATCCTCT
gamR-Z-R	AAACACATTGCTCTGCAGGTCGACGATTCTACC
alsSD-L-F	TCTTTGCGGTAAGCGATAACCCAT
alsSD-L-R	CTCGAATTCGTAACACCCTCACTCCTTATTATGCATTTTAAACGTAAAATTTTAAATAT
alsSD-R-F	GCACTGGCCGTCAAGAAAAAAAGAAAGCCCCTTTTAGCAGG
alsSD-R-R	TATTGTCGTATGCTGTGTCGAAACCTTACA
alsSD-Z-F	GGAGTGAGGGTGTTACGAATTCGAGCTCGGTACC
alsSD-Z-R	TTCTTTTTTTCTTGACGGCCAGTGCCA
ptsG-L-F	GAGCTGAGGATCGTGAATGG
ptsG-L-R	CTTCGTATAATGTATGCTATACGAACGGTAAAGAATTGACCTCCTCTTTTTACTAGT
ptsG-R-F	CGGTACCATTATAGGTAAGAGAGGAATGTACACATGTTTAAAGCATTATTCGGCGT
ptsG-R-R	GGACTGGAGCAACGAATAAGAA
ptsG-C-F	AATTCTTTACCGTTCGTATAGCATACATTATACGAAGTTATGCCATAGTGACTGGCGAT
ptsG-C-R	ATACCGTTCGTATAATGTATGCTATACGAAGTTATTTATTGGTATGACTGGTTTTAAGC
ptsG-P43-F	AATAACTTCGTATAGCATACATTATACGAACGGTATGATAGGTGGTATGTTTTCGC
ptsG-P43-R	GTGTACATTCCTCTCTTACCTATAATGG
pgi-L-F	GCGGAGGCAGTGTGATTGA
pgi-L-R	CGGTATCTCTAGAGGATCCCCGGTGCTTGTCCCTCCATAACG
pgi-R-F	CGGTACCATTATAGGTAAGAGAGGAATGTACACATGACGCATGTACGCTTT
pgi-R-R	GTTCAAGCCGTCAAGATCGTTA
pgi-Z-F	CCGGGGATCCTCTAGAGA
pgi-Z-R	CATACCACCTATCAGTCGACGATTTACCGTTCG
pgi-P43-F	GGTAAATCGTCGACTGATAGGTGGTATGTTTTCGCT
pgi-P43-R	GTGTACATTCCTCTCTTACCTATAATGG
CRISPRi	

pLCx-dCas9-liF	GAGAAAAGGAGGATGTACACATGGATAAGAAATACTCAATAGGCTTAGCTATCGG
pLCx-dCas9-liR	TCCGCTATCCTGCCAATCTGGCAATATAGCGGATTTCTGTTCTGAATTAACGGAATTCCGTACCGTT
Biosen-dCas9-F	CAGATTGGCAGGATAGCGGATTTTTCTTTTTCTACCTTATGCTTCAGAACGCTCGGTTG
Biosen-dCas9-R	GTGTACATCCTCCTTTTCTCGAGGGTACCG
sg-MF	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC
sg-alsS1-R	TTCTAGCTCTAAAACTTGTGAAAAACAGAGGGGCGACATTTATTGTACAACACGAGCCC
alsS1-VF	ATGTCGCCCCTCTGTTTTTCACAA
sg-alsS2-R	TTCTAGCTCTAAAACCTTTTGTGAGCTTTCCGCAAACATTTATTGTACAACACGAGCCC
sg-alsS2-VF	AAATGTTTGCGGAAAGCTCACAAAAG
sg-alsS3-R	TTCTAGCTCTAAAACGATCTTGAATTGATCGGTGAACATTTATTGTACAACACGAGCCC
sg-alsS3-VF	AATGTTCACCGATCAATTCAAGATC
sg-alsS4-R	TTCTAGCTCTAAAACGGAGAAAAAGTGGTTTCTGTACATTTATTGTACAACACGAGCCC
sg-alsS4-VF	AAATGTACAGAAACCACTTTTTCTCC
sg-alsS5-R	TTCTAGCTCTAAAACAAAGAATTCGGGGAACTCATACATTTATTGTACAACACGAGCCC
sg-alsS5-VF	ATGTATGAGTTCCCCGAATTCTTT
thrC-sgRNA-LF	GCTTCCTGACCATCATCCGC
thrC-sgRNA-LR	CCATGGCATGGACCGGGATCGCTAAAACAT
thrC-sgRNA-RF	CATGGATGAGCCGGAAATGCTGGAAACATCAC
thrC-sgRNA-RR	ACGACTACGTCAATGTCAGCATTCG
thrC-sgRNA-ZF	ATCCCGGTCCATGCCATGGCATGAGATTCTACC
thrC-sgRNA-ZR	CAGCATTTCCGGCTCATCCATGTCGACGGAATTCA
***GNA1* expression plasmids**	
pSTOP1622-li-F	TTCACCGGTCCAAGAATTGG
pSTOP1622-li-R	TTAAGTGAACGCAAAGGTTAGCA
Pbiosen-pST-F	TAACCTTTGCGTTCACTTAAGGAATTAGCTTGGTACGGATCCCT
PgamA-pST-R	ATAATGGTACCGCTATCACTGAGATAGACAAGCACCATATTTGTT
Psg2-pST-R	ATAATGGTACCGCTATCACTGAGATAGACAAGCACCATATTTGTTATG
PgamA-pST-R	ATAATGGTACCGCTATCACTCGACATTTATTGTATTGTTATGACCAATTT
GNA1-pST-F	AGTGATAGCGGTACCATTATAGG
GNA1-pST-R	CCAATTCTTGGACCGGTGAATTAAAAGCGCTGGGTCATAAAATTACAGTCA

RT-qPCR	
GNA1(qPCR)-F	CGATTTGAGGCGATGAGAACA
GNA1(qPCR)-R	TCAACACGACCCCTTGATCC
zwf(qPCR)-F	CAATGGAGCCGCCTATCAAA
zwf(qPCR)-R	CAGGAACCGGTACACCGTCA
pfkA(qPCR)-F	ACGCGGCAAGAAGCACAGTA
pfkA(qPCR)-R	GGTCAGCAGCACTCGGAGAA
glmM(qPCR)-F	TCATCCTTGGCGACACACCT
glmM(qPCR)-R	CCGAGATCCGCGTTTTTCTC
rpsj(qPCR)-F	ACAAATACAAAGATTCTCGTGAGC
rpsJ(qPCR)-R	CTAATCGCATAAGAGCATCAACAG

Underlined letters represent homologous sequences for fusion PCR, inverse PCR or Seamless Cloning.

Table S3. Sequences of genetic parts used in this study.

Part name	Type	Sequence
P_{43}	promoter	TGATAGGTGGTATGTTTTCGCTTGAACTTTTAAATACAGCCATTGAACATACGGTTGATTTAATAACTGA CAAACATCACCCTCTTGCTAAAGCGGCCAAGGACGCTGCCGCCGGGGCTGTTTGCGTTTTTGCCGTG ATTTCGTGTATCATTGGTTTACTTATTTTTTTGCCAAAGCTGTAATGGCTGAAAATTCTTACATTTATTTT ACATTTTTAGAAATGGGCGTGAAAAAAAGCGCGCGATTATGTAAAATATAA
P_{gamA}	promoter	CTTTTATAACAAATTTTCAGAAATTAATATTGACAGTTTGATAAGGGCGGTGC**TAAATTCGTAATGACA** **A**GTATCA**TAAATTGGTCATAACAA**ATATGGTGCTTGTCTATCTC
P_{veg}	promoter	TTTTGTCAAAATAATTTTATTGACAACGTCTTATTAACGTTGATATAATTTAAATTTTATTTGACAAAAATG GGCTCGTGTTGTACAATAAATGT
P_{vg1}	promoter	TTTTGTCAAAATAATTTTATTGACAACGTCTTATTAACGTTGATATAATTTAAATTTTATTTGACAAAAATG GGCTCGTGTTGTACAATAAATGTCG**TAAATTGGTCATAACAA**
P_{vg2}	promoter	TTTTGTCAAAATAATTTTATTGACAACGTCTTATTAACGTTGATATAATTTAAATTTTATTTGACA**TAAATT** **GGTCATAACAA**TACAATAAATGT
P_{vg3}	promoter	TTTTGTCAAAATAATTTTATTGACAACGTCTTATTAACGTTGA**TAAATTGGTCATAACAA**TTGACAAAAA TGGGCTCGTGTTGTACAATAAATGT
P_{vg4}	promoter	TTTTGTCAAAATAATTTTATTGACAACGTCTTATTAACGTTGATATAATTTAAATTTTATTTGACA**TAAATT** **GGTCATAACAA**TACAATAAATGTCG**TAAATTGGTCATAACAA**
P_{vg5}	promoter	TTTTGTCAAAATAATTTTATTGACAACGTCTTATTAACGTTGA**TAAATTGGTCATAACAA**TTGACAAAAA TGGGCTCGTGTTGTACAATAAATGTCG**TAAATTGGTCATAACAA**
P_{vg6}	promoter	TTTTGTCAAAATAATTTTATTGACAACGTCTTATTAACGTTGA**TAAATTGGTCATAACAA**TTGACA**TAAA** **TTGGTCATAACAA**TACAATAAATGT
P_{vg7}	promoter	TTTTGTCAAAATAATTTTATTGACAACGTCTTATTAACGTTGA**TAAATTGGTCATAACAA**TTGACA**TAAA** **TTGGTCATAACAA**TACAATAAATGTCG**TAAATTGGTCATAACAA**
P_{rg}	promoter	CGAGCTTTCTTACCTTGACGGCAGAAATAAGCTCGAAGATTACGACATCTTAACATTGATGAAATACTA AGGATAACCGCATAACGAAAAAGCACTCCATGTCAGGGTGCTTTTTTCCTATTGTTTTGCATTTATTTTA TATAATATTTGGCTATTTGAACTTCTGCTCTTTACATCTTTCGTTTTTTTCTTG**TAAATTCGTAATGACAA** GTATCA**TAAATTGGTCATAACAA**ATATGGTGCTTGTCTATCTC
P_{lg}	promoter	TCAATATGTTTTCAAAAGCCGGAAAAGCGCTTTCGGACACCGTAACCAATACTGCCCAGTCAATGTATG AATGGATACGGGATATGAATCAATAAGTACGTGAAAGAGAAAAGCAACCCAGATATGATAGGGAACTT TTCTCTTTCTTGTTTTACATTGAATCTTTACAATCCTATTGATATAATCTAAGCTAGTGTA**TAAATTCGTA** **ATGACAA**GTATCA**TAAATTGGTCATAACAA**ATATGGTGCTTGTCTATCTC
P_{vg}	promoter	TTTTGTCAAAATAATTTTATTGACAACGTCTTATTAACGTTGATATAATTTAAATTTTATTTGACAAAAATG GGCTCGTGTTG**TAAATTCGTAATGACAA**GTATCA**TAAATTGGTCATAACAA**ATATGGTGCTTGTCTAT CTC
P_{sg}	promoter	AATGTTTAGTGGAAATGATTGCGGCATCCCGCAAAAAATATTGCTGTAAATAAACTGGAATCTTTCGGC ATCCCGCATGAAACTTTTCACCCATTTTTCGTTGACAAAAACATTTTTTTCATT**TAAATTCGTAATGACA** **A**GTATCA**TAAATTGGTCATAACAA**ATATGGTGCTTGTCTATCTC
P_{sg1}	promoter	AATGTTTAGTGGAAATGATTGCGGCATCCCGCAAAAAATATTGCTGTAAATAAACTGGAATCTTTCGGC ATCCCGCATGAAACTTTTCACCCATTTTTCGTTGACA**TAAATTGGTCATAACAATAAATTCGTAATGAC**

		AAGTATCA**TAAATTGGTCATAACAA**ATATGGTGCTTGTCTATCTC
P_{sg2}	promoter	AATGTTTAGTGGAAATGATTGCGGCATCCCGCAAAAAATATTGCTGTAAATAAACTGGAATCTTTCGGCATCCCGCATGAAACTTTTCACCCATTTTTCG**TAAATTGGTCATAACAA**TTGACAAAAACATTTTTTTCATT**TAAATTCGTAATGACAA**GTATCA**TAAATTGGTCATAACAA**ATATGGTGCTTGTCTATCTC
P_{sg3}	promoter	AATGTTTAGTGGAAATGATTGCGGCATCCCGCAAAAAATATTGCTGTAAATAAACTGGAATCTTTCGGCATCCCGCATGAAACTTTTCACCCATTTTTCG**TAAATTGGTCATAACAA**TTGACA**TAAATTGGTCATAACAATAAATTCGTAATGACAA**GTATCA**TAAATTGGTCATAACAA**ATATGGTGCTTGTCTATCTC
sgRNA$_{zwf1}$-sgRNA$_{pfkA2}$-sgRNA$_{glmM2}$	sgRNA array	CGAAGATCCTCATTAGACCAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAATTCCGCCACCGTCGACCTGCAGGCTTATTAACGTTGATATAATTTAAATTTTATTTGACAAAAATGGGCTCGTGTTGTACAATAAATGTCCGATTGTAAAATCAGTGCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT
sgRNA$_{alss1}$	sgRNA	CGCCCCTCTGTTTTTCACAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT
sgRNA$_{alss2}$	sgRNA	TTGCGGAAAGCTCACAAAAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT
sgRNA$_{alss3}$	sgRNA	TCACCGATCAATTCAAGATCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT
sgRNA$_{alss4}$	sgRNA	ACAGAAACCACTTTTTCTCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT
sgRNA$_{alss5}$	sgRNA	ATGAGTTCCCCGAATTCTTTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT
dCas9	gene	ATGGATAAGAAATACTCAATAGGCTTAGCTATCGGCACAAATAGCGTCGGATGGGCGGTGATCACTGATGAATATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACAGTATCAAAAAAAATCTTATAGGGGCTCTTTTATTTGACAGTGGAGAGACAGCGGAAGCGACTCGTCTCAAACGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACAGGAGATTTTTTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGTCTTTTTTGGTGGAAGAAGACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGATGAAGTTGCTTATCATGAGAAATATCCAACTATCTATCATCTGCGAAAAAAATTGGTAGATTCTACTGATAAAGCGGATTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTGGTCATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATGTGGACAAACTATTTATCCAGTTGGTACAAACCTACAATCAATTATTTGAAGAAAACCCTATTAACGCAAGTGGAGTAGATGCTAAAGCGATTCTTTCTGCACGATTGAGTAAATCAAGACGATTAGAAAATCTCATTGCTCAGCTCCCCGGTGAGAAGAAAAATGGCTTATTTGGGAATCTCATTGCTTTGTCATTGGGTTTGACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGATACTTACGATGATGATTTAGATAATTTATTGGCGCAAATTGGAGATCAATATGCTGATTTGTTTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAGATATCCTAAGAGTAAATACTGAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAACGCTACGATGAACATCATCAAGACTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTATAAAGAAATCTTTTTTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGGAGCTAGCCAAGAAGAATTTTATAAATTTATCAAACCAATTTTAGAAAAAATGGATGGTACTGAGGAATTATTGGTGAAACTAAATCGTGAAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCCATCAAATTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAAAAGACAATCGTGAGAA

		GATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATTGGCGCGTGGCAATAGTCGTTTT
		GCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCATGGAATTTTGAAGAAGTTGTCGATAAAGG
		TGCTTCAGCTCAATCATTTATTGAACGCATGACAAACTTTGATAAAAATCTTCCAAATGAAAAAGTACTA
		CCAAAACATAGTTTGCTTTATGAGTATTTTACGGTTTATAACGAATTGACAAAGGTCAAATATGTTACTG
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GNA1	gene	ATGAGCCATATCTTCGACGCATCTGTACTGGCTCCACATATTCCTAGTAACCTTCCTGATAATTTCAAG GTGAGACCACTGGCAAAGGATGATTTTTCGAAGGGATATGTCGACCTGCTGTCACAATTGACGTCAGT TGGAAACCTTGACCAAGAAGCATTTGAGAAACGATTTGAGGCGATGAGAACAAGCGTACCGAATTATC ACATCGTAGTAATTGAGGATTCCAACAGCCAGAAAGTGGTGGCGTCTGCTAGTTTGGTTGTTGAAATG AAATTCATTCATGGGGCCGGATCAAGGGGTCGTGTTGAAGATGTTGTCGTCGATACAGAAATGCGCC GGCAAAAATTAGGTGCCGTGCTTTTAAAAACTTTGGTGTCACTTGGCAAATCTTTAGGCGTCTACAAAA TAAGCCTCGAATGCGTCCCGGAATTACTCCCGTTCTATTCCCAATTTGGCTTTCAGGATGACTGTAATT TTATGACCCAGCGCTTTTAA

Underlined letters represent -10 and -35 region, and bold letters represent GamR binding site.

sgRNAs targeting sites on non-template strand of *alsS:*

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TTCGGGGAACTCATGAAAACGAAAGCTCTCTAG

Supplementary method: protocal of *B. subtilis* transforming

Protocal:

1) 2 ml SPI culture were inoculated overnight from a fresh colony at 37 °C in a 14 mL disposable tube, with 220 rpm shaking.
2) The following morning, 40μL of the culture was diluted with 2 ml fresh SPI and continued incubation at 37 °C with 220 rpm shaking for 4-5 h.
3) 200 μL of the culture was mixed with 2mL fresh SPII with continued incubation at 37 °C for additional 1.5-2 h.
4) 20μL EGTA (10mM) was added into the culture, and the competent cells was obtained after additional 10 min shake cultivation.
5) 500μL of the competent cells was divided into 1.5 mL sterile tubes, and then incubated at 37 °C for 1.5-2 h after adding 0.5-2 μg DNA into every tube.
6) After centrifuged (4500g) for 2 min, the cell pellet was gently resuspended in 150-200 μL of the saved supernatant and then plated onto LB agar plates with selective antibiotic(s).

Medias:

a) Every 2mL SPI contains 980 μL SPI-A, 980 μL SPI-B, 20 μL Glucose (50%), and 20 μL CAYE;
b) Every 2mL SPII contains 1960 μL SPI, 20 μL $CaCl_2$ (5 mM), and 20 μL $MgCl_2$ (250mM);
c) SPI-A contains 4 g/L $(NH_4)_2SO_4$, 28 g/L $K_2HPO_4·3H2O$, 12 g/L KH_2PO_4, and 2 g/L sodium citrate;
d) SPI-B was 0.4 g/L $Mg_2SO4·7H_2O$;
e) CAYE contains 20 g/L casamino acid and 100 g/L yeast extract;
f) SPI-A, SPI-B, 50% Glucose, CAYE, 5 mM $CaCl_2$, 250mM $MgCl_2$, and 10 mM EGTA (adjusting pH to 8.0 by NaOH) were sterilized at 121 °C for 15 min separately.

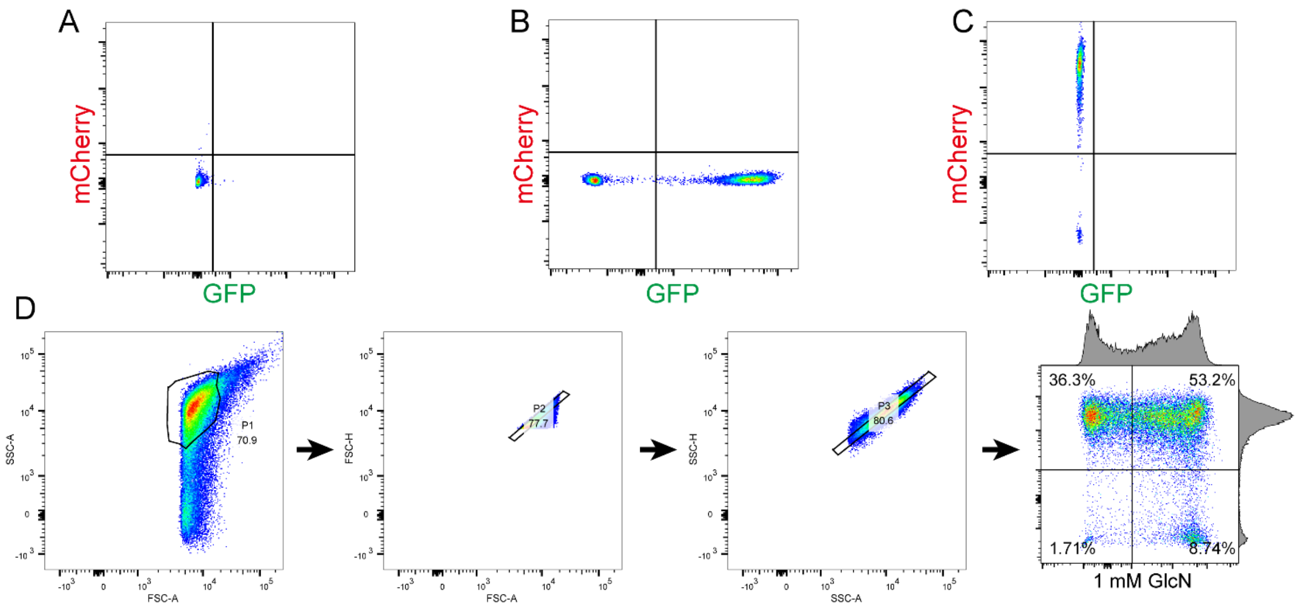

Figure S1. Flow cytometry analysis of negtive sample (A) and single positive samples of GFP (B) and mCherry (C). (D) The gates used in flow cytometry analysis to reduce false events.

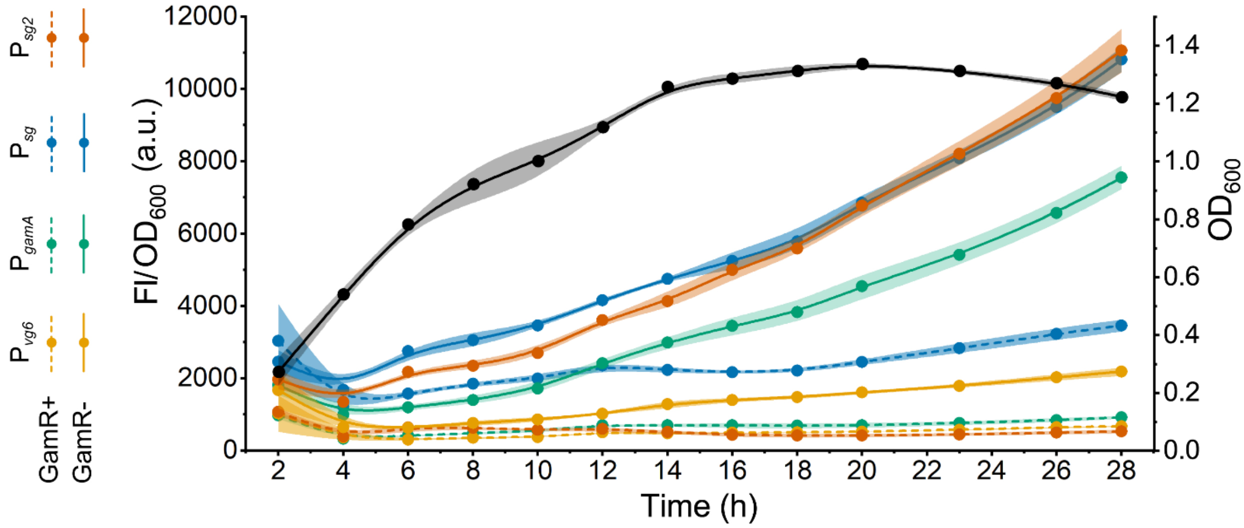

Figure S2. Expression properties of P_{vg6}, P_{gamA}, P_{sg}, and P_{sg2} over times.

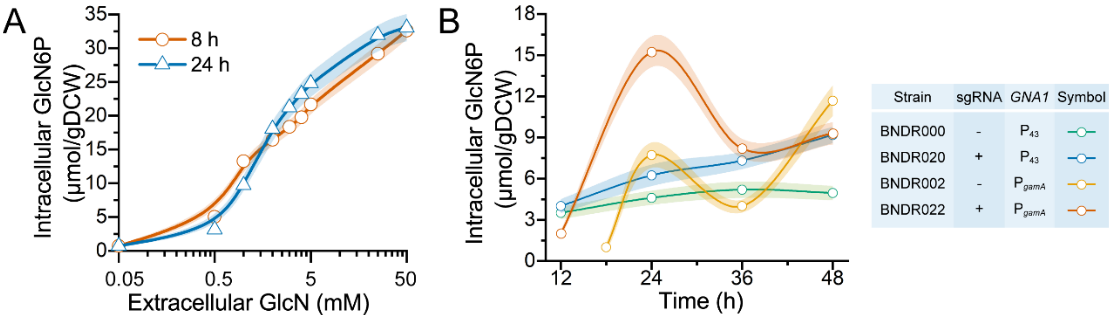

Figure S3. Intracellular GlcN6P concentration measured by LCMS. (A) Relationship between the extracellular GlcN added into the media and the intracellular GlcN6P. (B) The variation tendency of intracellular GlcN6P in the engineered strains. All data were the average of three independent studies with standard deviations.

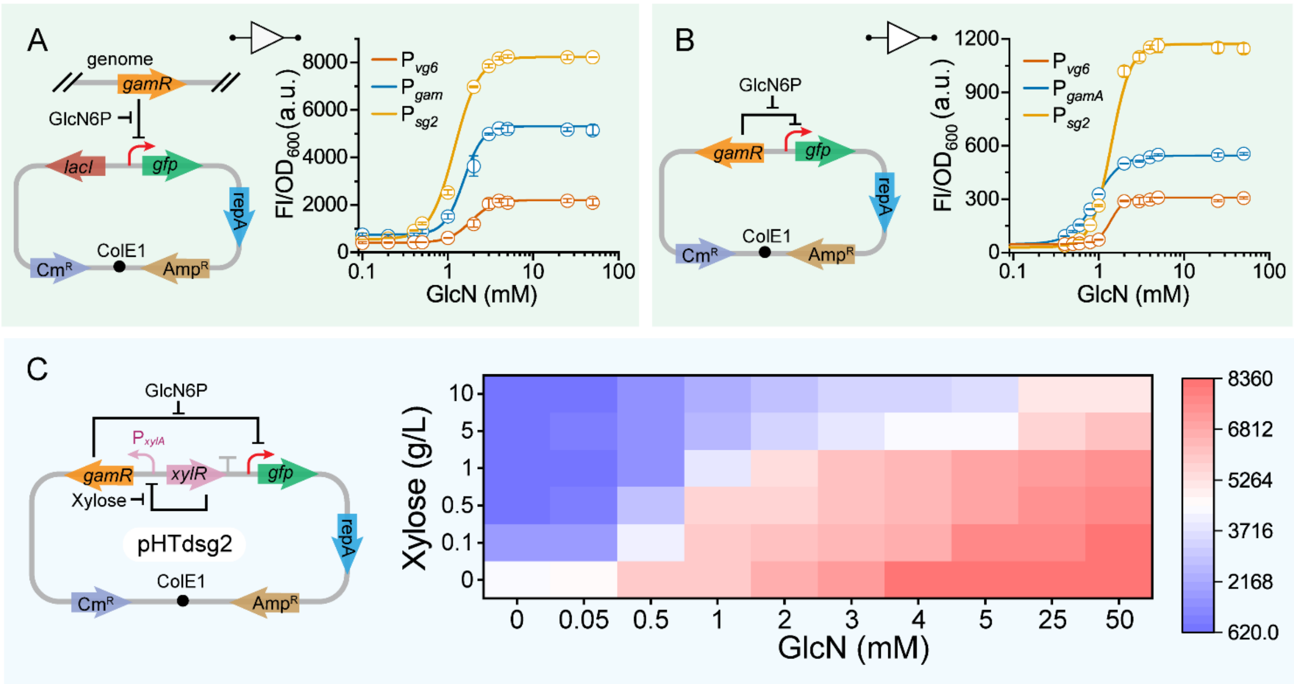

Figure S4. GlcN6P biosensors mediated transcription activation. (A) The dose-dependent property of the GlcN6P biosensors to GlcN6P in strain BS02 (*B. subtilis* 168Δ*nagB*Δ*gamA*). (B) The dose-dependent property of the GlcN6P biosensors in minimal media. (C) Effects of GamR expression on the biosensor response. All data were the average of three independent studies with standard deviations.

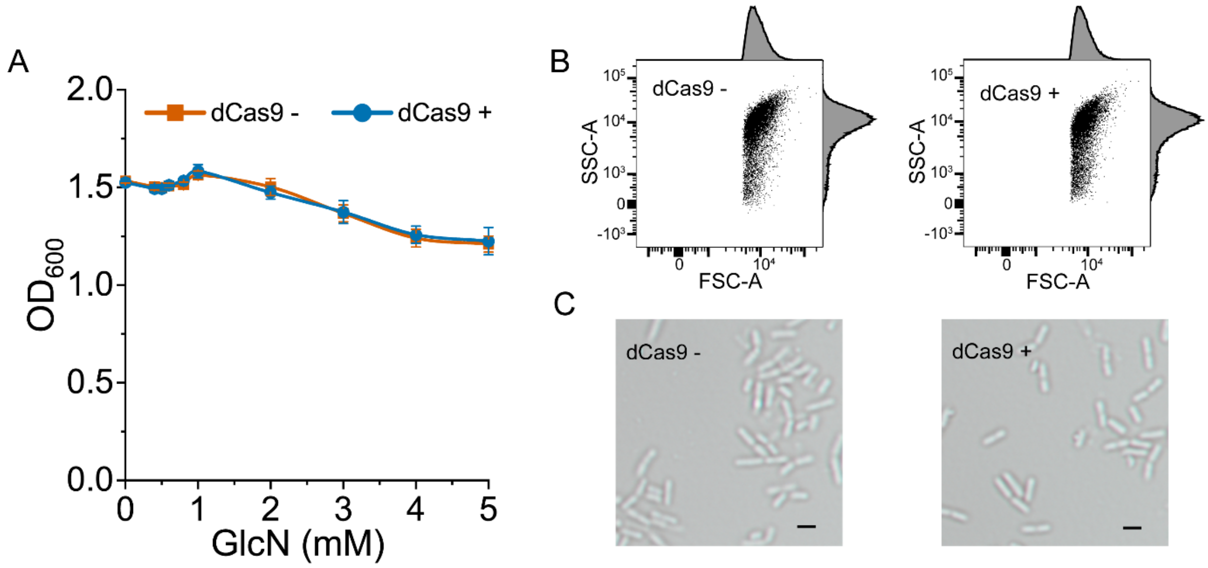

Figure S5. Evaluation the toxicity of dCas9 in the repression cascade. (A) Effect of dCas9 expression on cell growth. OD_{600} was measured at 20h. Intracellular accumulation of GlcN6P is weakly toxic to the cell, but there is no difference in growth between strain expressing dCas9 and a control without dCas9. (B) FSC-A/SSC-A distribution of strain expressing dCas9 and a control without dCas9 were measured by flow cytometry. (C) Microscopic images of strain expressing dCas9 and a control without dCas9. The scale bars are 2 μm.

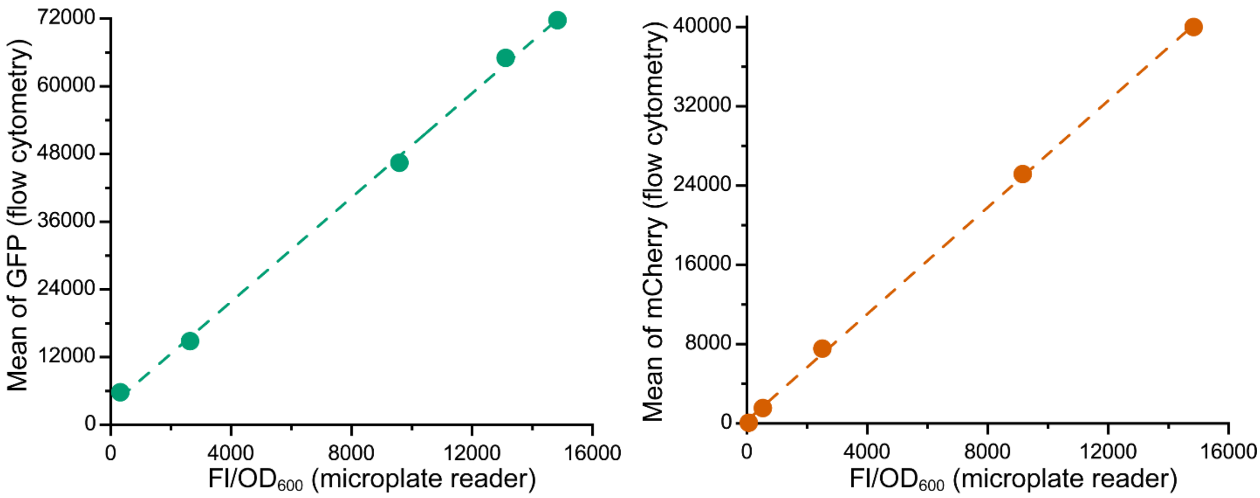

Figure S6. The relationships between fluorescence measured by flow cytometry and microplate reader.

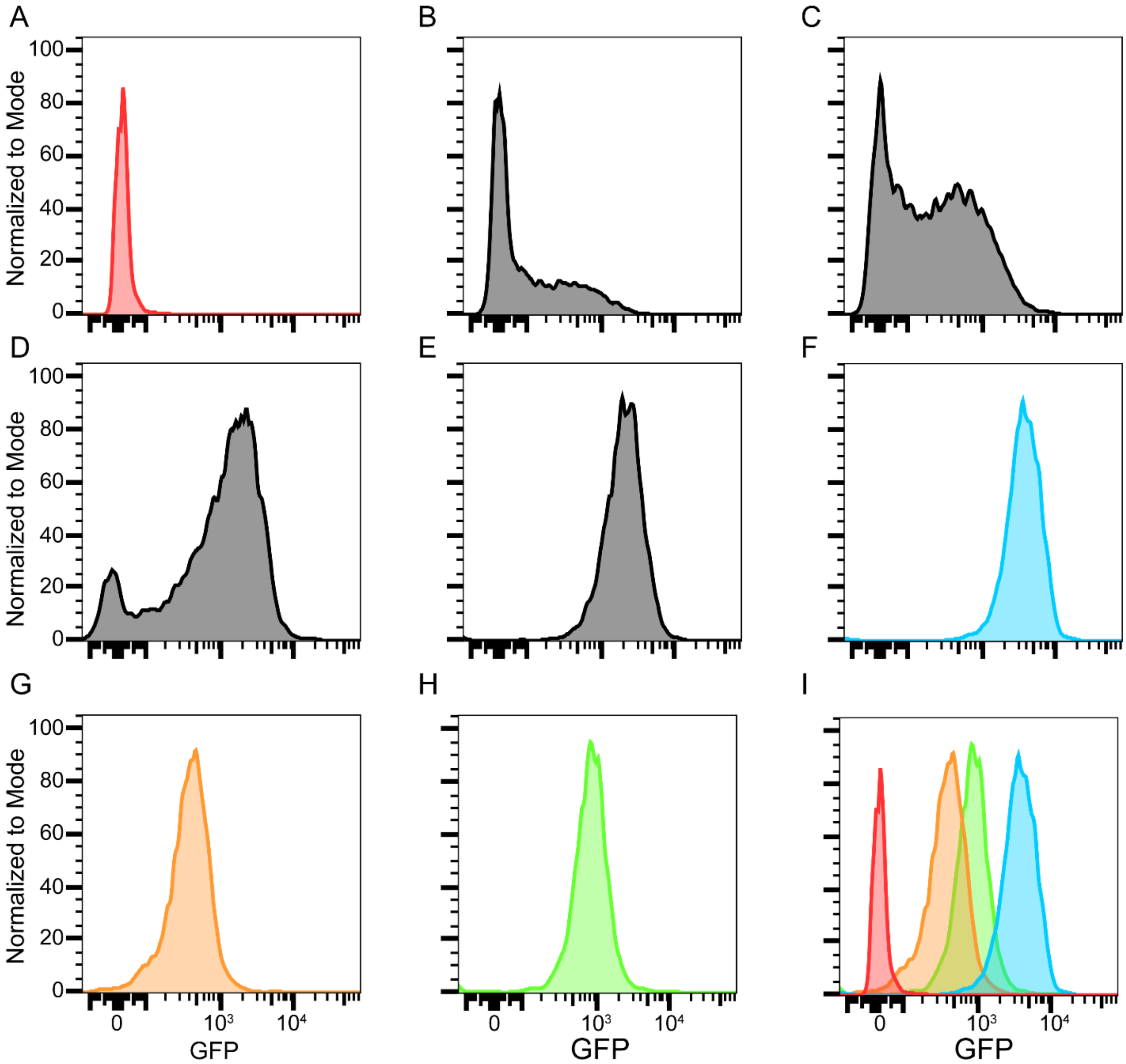

Figure S7. Flow cytometry analysis of pHTcsg2 in strain BS03 (A-F, LB media containing GlcN 0mM, 0.4mM, 0.5mM, 0.8 mM, 1 mM, and 4 mM, respectively), BNY0 (G, LB media without GlcN), and BNX0 (H, LB media without GlcN). (I) Overlays of A, F, G, and H.

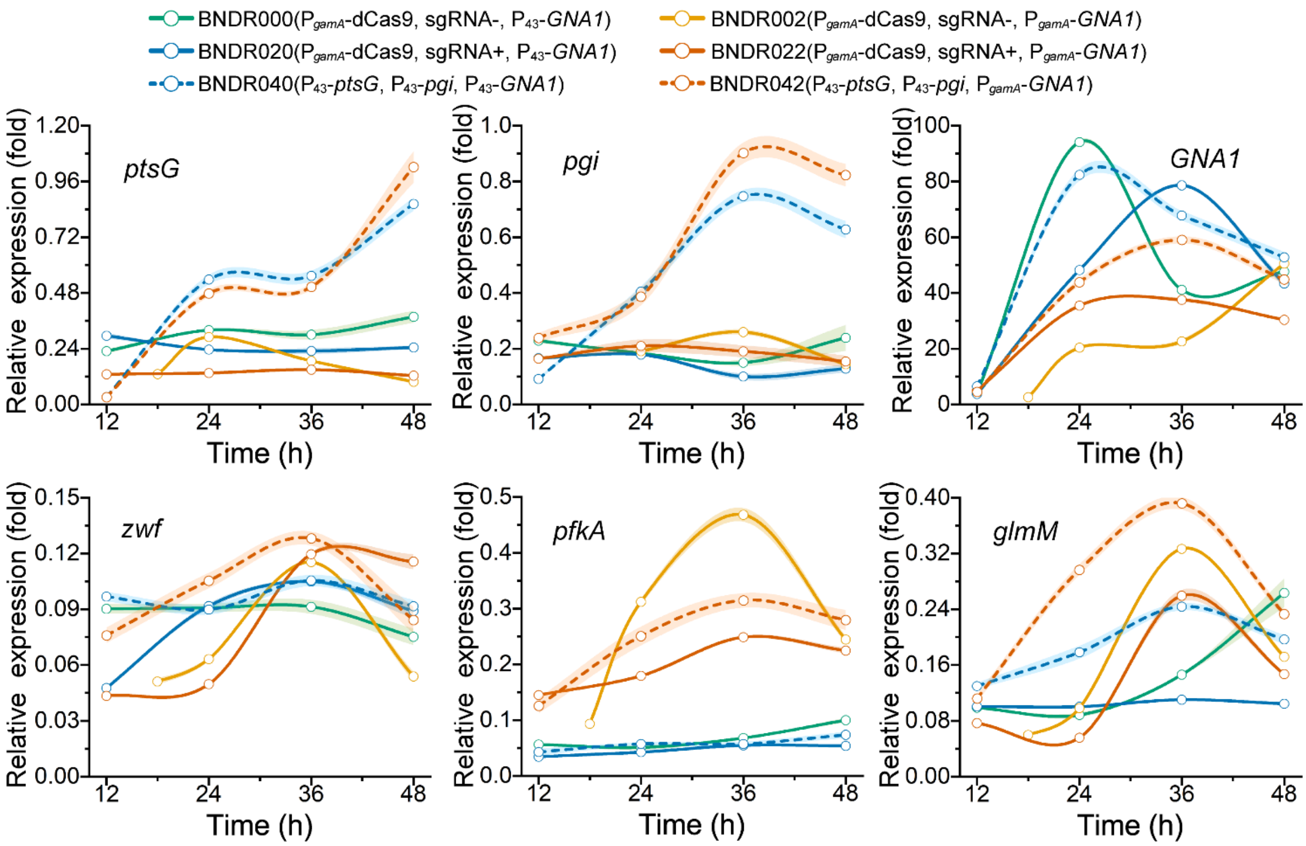

Figure S8. Relative transcription expression levels of the regulated genes to the reference gene *rpsJ*. All data were the average of three independent studies with standard deviations.

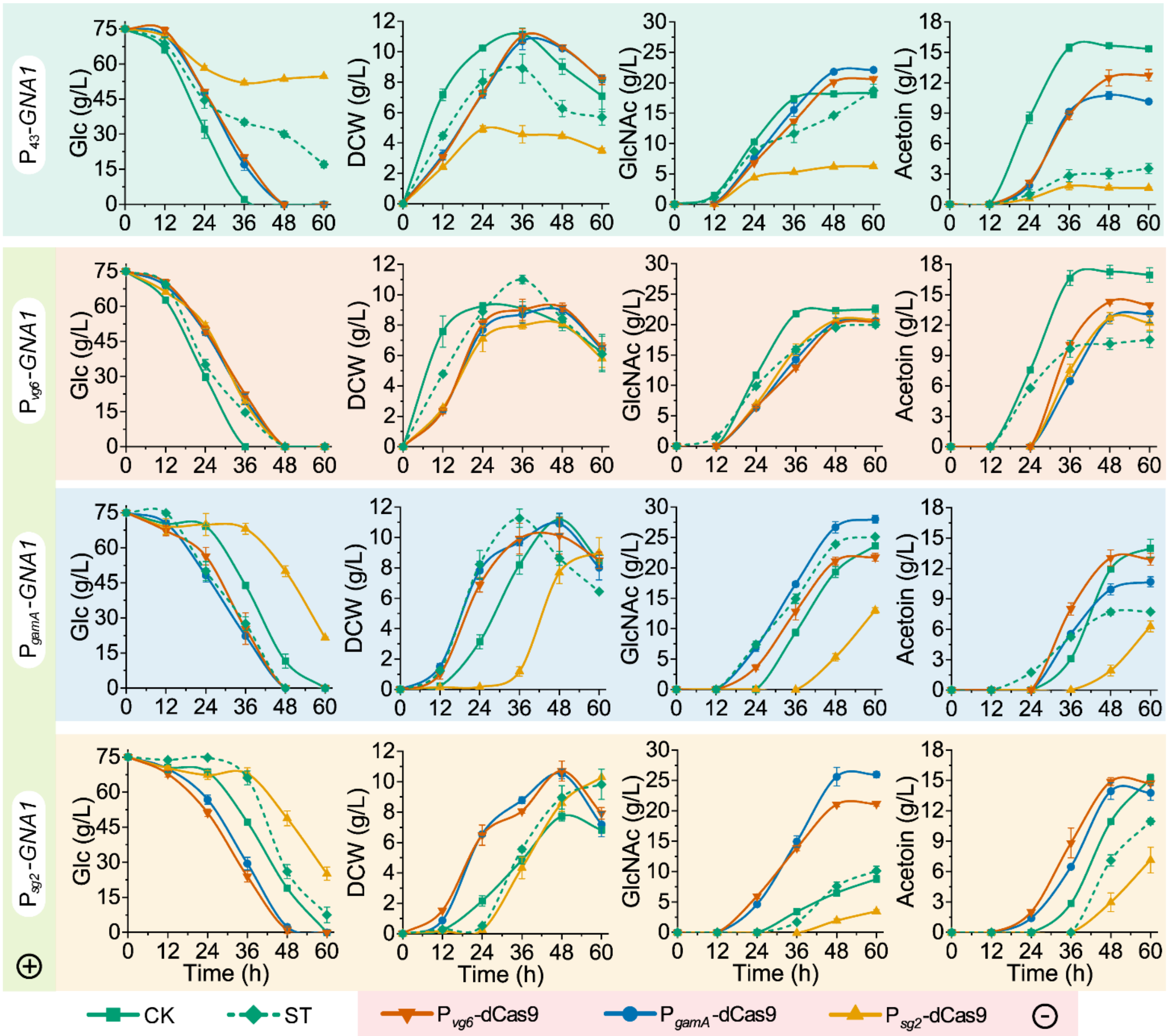

Figure S9. Dynamic regulation of GlcNAc synthesis by the GlcN6P biosensors. CK: control strain that only possessed dCas9 without sgRNAs. ST: statically upregulating *ptsG* and *pgi* (The promoters of *ptsG* and *pgi* in strain BNY0 were both replaced with P_{43}, and *GNA1* was expressed by P_{43}, P_{vg6}, P_{gamA}, and P_{sg2}, respectively.) All data were the average of three independent studies with standard deviations.

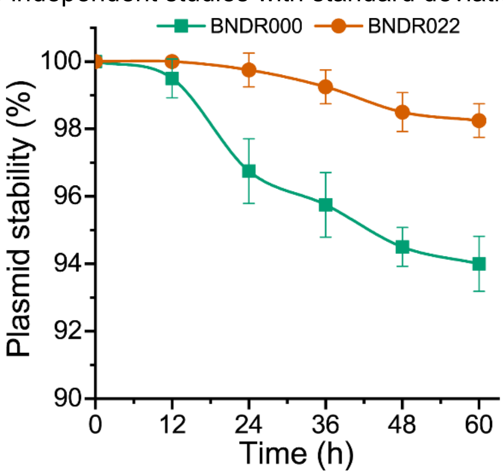

Figure S10. Plasmid stability of cultures over time span in strains BNDR000 and BNDR022.

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