Supporting Information

Inducible expression systems based on xenogeneic silencing and counter-silencing and design of a metabolic toggle switch

Johanna Wiechert[a], Cornelia Gätgens[a], Astrid Wirtz[a], and Julia Frunzke [a,*]

[a]Institut für Bio- und Geowissenschaften, IBG-1: Biotechnologie, Forschungszentrum Jülich, Jülich, Germany

* Address correspondence to Prof. Dr. Julia Frunzke, j.frunzke@fz-juelich.de;

ORCID 0000-0001-6209-7950

Supplemental figures

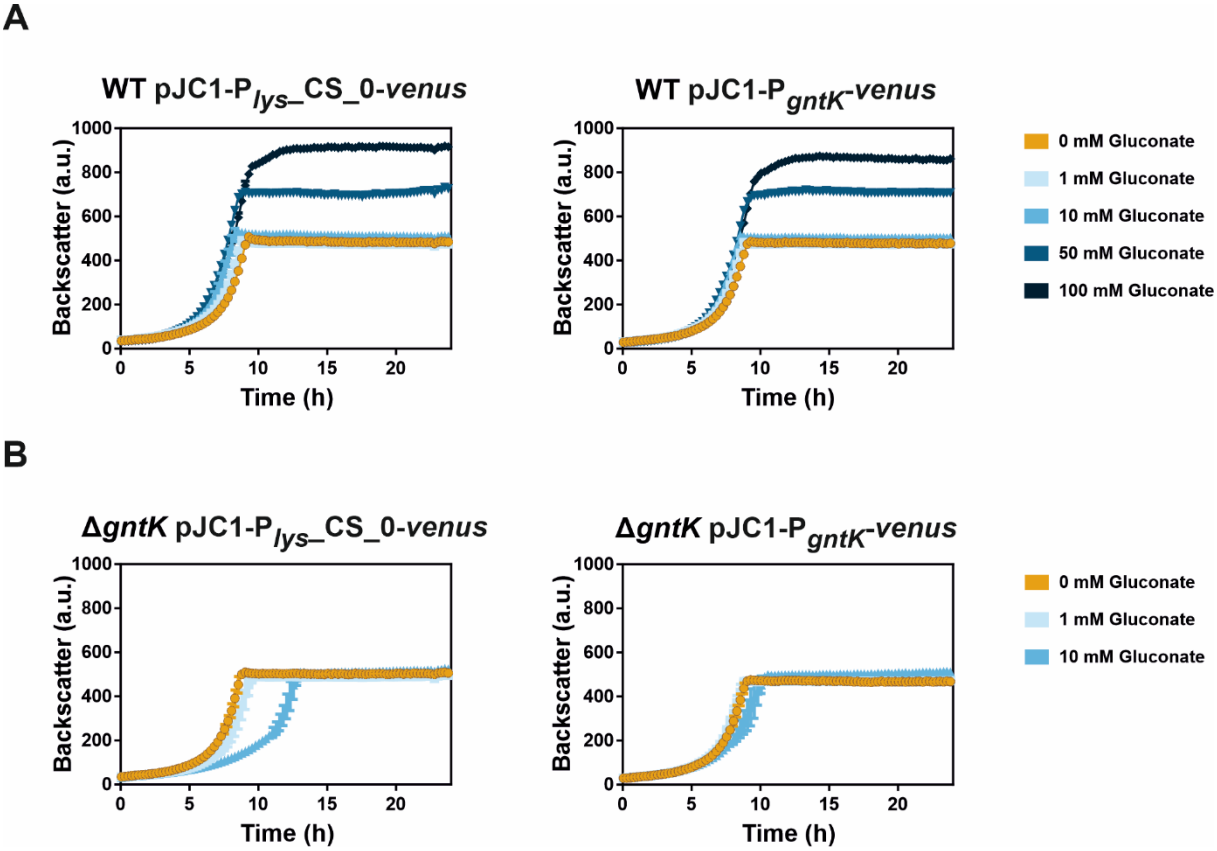

Figure S1: Growth curves corresponding to the analysis of tunability of the native GntR target promoter P_{gntK} and the synthetic counter-silencer promoter P_{lys}_CS_0 shown in Figure 4. Shown are backscatter values of *C. glutamicum* wild type cells (WT) **(A)** or *C. glutamicum* cells lacking the gene encoding the gluconate kinase (Δ*gntK*) **(B)** harbouring the plasmid-based constructs pJC1-P_{lys}_CS_0-*venus* or pJC1-P_{gntK}-*venus* during cultivation in the presence of different gluconate concentrations. Graphs show the mean and error bars the standard deviation of biological triplicates over time. Cells were cultivated in a microtiter cultivation system in CGXII medium supplemented with glucose (100 mM in analysis of pJC1-P_{gntK}-*venus* and 111 mM for characterization of pJC1-P_{lys}_CS_0-*venus*) and either no or varying amounts of gluconate as effector. Backscatter values were measured in 15 min intervals. The presented data show that varying amounts of gluconate affect the final backscatter values but did not significantly influence growth rates of wild-type cells. In contrast, ΔgntK cells displayed reduced growth rates upon addition of gluconate.

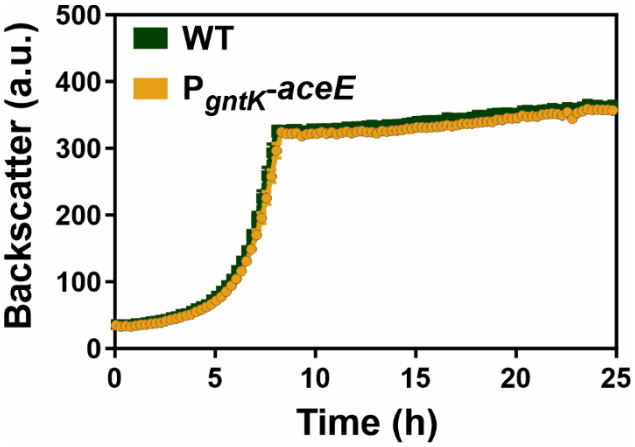

Figure S2: Growth of the strain with dynamically controlled *aceE* expression (P_{gntK}-*aceE*) in comparison to *C. glutamicum* wild type cells, both harbouring the plasmid pJC1-P_{lys}_CS_0-*venus*. Shown are backscatter values of both *C. glutamicum* strains during cultivation in a microtiter cultivation system in CGXII minimal medium with 100 mM gluconate and 25 µg/ml kanamycin. Strains have been pre-cultivated in CGXII containing 100 mM gluconate and 111 mM glucose. Graphs show the mean and error bars the standard deviation of biological triplicates over time. Backscatter values were measured in 15 min intervals. This experiment verified that growth of P_{gntK}-*aceE* is not impaired in comparison to wild-type cells in the presence of gluconate (100 mM).

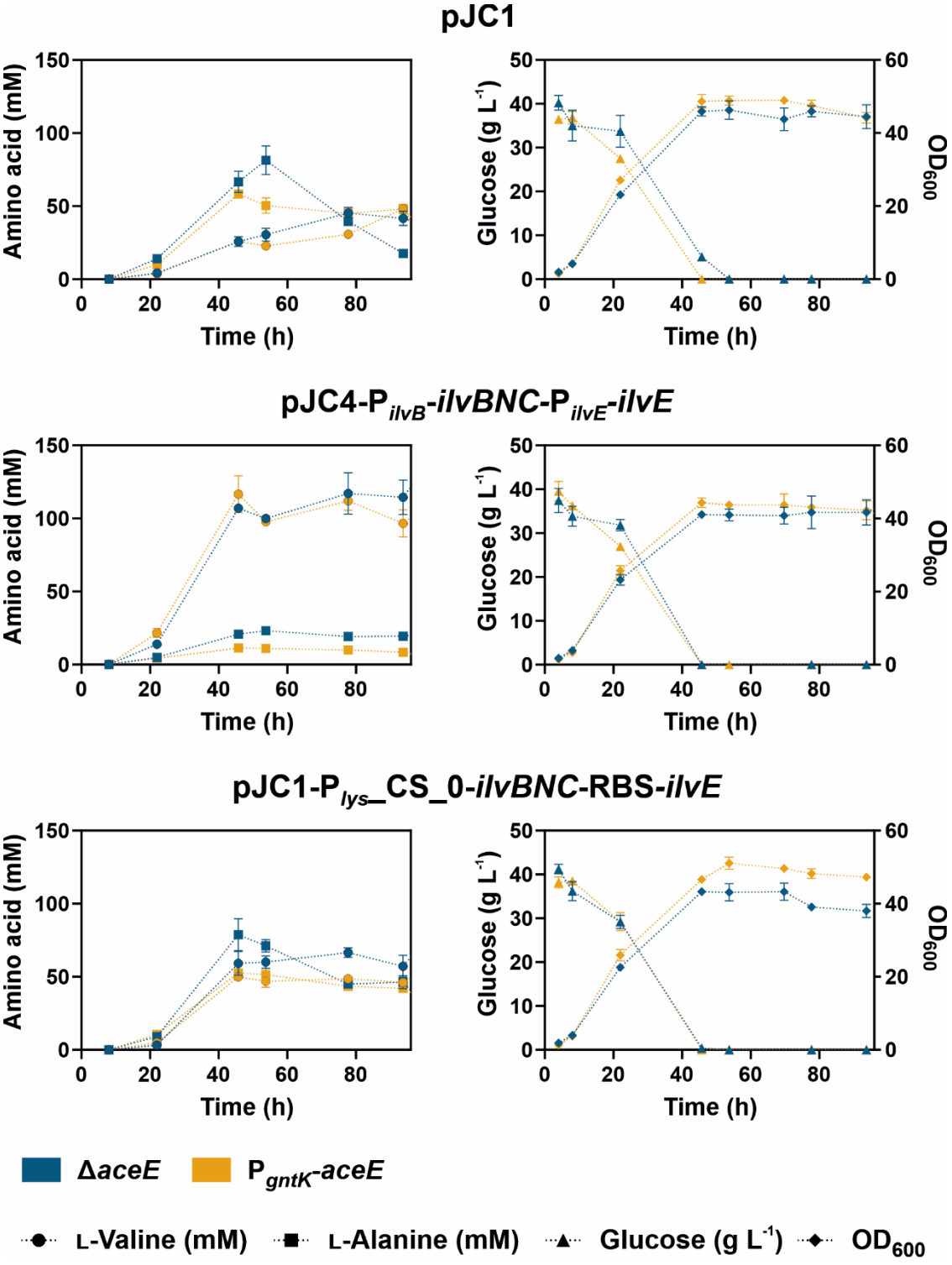

Figure S3: Growth, glucose consumption, product (L-valine) and by-product (L-alanine) formation during L-valine production. Cultivation of the strain with dynamically controlled *aceE* expression (P$_{gntK}$-*aceE*) in comparison to the previously established Δ*aceE* strain[1, 2] harbouring either the empty vector pJC1 (pJC1-*venus*-term), the synthetic L-valine biosynthesis operon controlled by the counter-silencer promoter (pJC1-P$_{lys}$_CS_0-*ilvBNC*-RBS-*ilvE*) or the native operon structure pJC4-P$_{ilvB}$-*ilvBNC*-P$_{ilvE}$-*ilvE* (pJC4-*ilvBNCE*)[3]. Cultivation was performed in CGXII supplemented with 25 µg/ml kanamycin, 222 mM glucose and 254 mM acetate. Graphs represent the values of biological triplicates and error bars the corresponding standard deviations after 8, 22, 46, 54, 78 and 94 h of cultivation. Measurements of glucose and L-valine in the supernatant over time revealed that glucose was completely consumed after 46 hours of cultivation and L-valine concentrations had mostly reached maximal values.

Supplemental tables

Table S1A: Overview of prices for glucose, gluconate and acetate provided by Sigma-Aldrich (December 2019, https://www.sigmaaldrich.com).

Carbon source (Product ID)	Purity (%)	Amount (kg)	Price (€)
D-Gluconic acid sodium salt (G9005)	≥99	1	49.80
D-(+)-Glucose (G8270)	≥99.5	1	48.50
Potassium acetate (P5708)	≥99	1	119

Considered were powders with minimal purity of 99% and an amount of 1 kg. Letter and numbers in brackets are the product numbers.

Table S1B: Overview of costs per litre CGXII minimal medium with different amounts of carbon sources.

Carbon source (Product ID)	Cultivation concentration (mM)	Molecular weight (g mol^{-1})	Cultivation concentration (g L^{-1})	Price per L (€)
D-Gluconic acid sodium salt	51	218.14l	11.13	0.55
D-(+)-Glucose	222	180.16	40.0	1.94
Potassium acetate	254	98.14	24.93	2.97
Potassium acetate + D-(+)-Glucose	254 + 222			4.91
D-Gluconic acid sodium salt + D-(+)-Glucose	51 +222			2.49

Calculations were performed based on the prices listed in Table S1a.

Table S2: Strains used in this study.

Strain	Relevant characteristics	Reference or source
E. coli		
DH5α	F^- Φ80*lacZ*ΔM15 Δ(*lacZYA-argF*) U169 *recA1 endA1 hsdR17*(r_k^-, m_k^+) *phoA supE44 thi-1 gyrA96 relA1* λ^-, strain used for cloning procedures	Invitrogen
C. glutamicum		
ATCC 13032	Biotin-auxotrophic wild type	[4]
Δ*aceE*	Derivate of ATCC 13032 with in-frame deletion of gene *aceE* (cg2466)	[1]
Δ*gntK*	Derivate of ATCC 13032 with in-frame deletion of gene *gntK* (cg2732)	This work
ΔP_{aceE}-*aceE*	Derivate of ATCC 13032 with in-frame deletion of gene *aceE* (cg2466) and its 300 bp upstream promoter region	This work
P_{gntK}-*aceE*	Derivate of Δ*aceE* with the re-integrated gene *aceE* (cg2466) under control of the promoter P_{gntK}. P_{gntK} (303 bp, P_{cg2732}). The first 30 bp of the coding sequence of *gntK* were fused to the *aceE* gene via a linker containing a stop codon and an artificial RBS.	This work

Table S3: Plasmids from other studies used in this work.

Plasmid	Relevant characteristics	Reference
pJC1	*Kan*^{R}, *Amp*^{R}; *oriV*$_{C.g.}$, *oriV*$_{E.c.}$; *C. glutamicum*/*E. coli* shuttle vector	[5]
pJC1-*venus*-term	*Kan*^{R}, pJC1 derivative carrying the *venus* coding sequence followed by a terminator sequence of *Bacillus subtilis*	[6]
pJC1-P_{cg1897}::GntR_BS_pos0-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1897 (468 bp) with an inserted GntR BS directly upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg1936}::GntR_BS_pos0-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1936 (676 bp) with an inserted GntR BS directly upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the	[8]

	reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	
pJC1-P_{cg1940}::GntR_BS_pos0-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1940 (563 bp) with an inserted GntR BS directly upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg1955}::GntR_BS_pos0-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1955 (516 bp) with an inserted GntR BS directly upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg1977}::GntR_BS_pos0-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1977 (653 bp) with an inserted GntR BS directly upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg1999}::GntR_BS_pos-30-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1999 (448 bp) with an inserted GntR BS 30 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg1999}::GntR_BS_pos-20-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1999 (448 bp) with an inserted GntR BS 20 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg1999}::GntR_BS_pos-10-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1999 (448 bp) with an inserted GntR BS 10 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg1999}::GntR_BS_pos-5-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1999 (448 bp) with an inserted GntR	[8]

	BS 5 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	
pJC1-P_{cg1999}::GntR_BS_pos0-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1999 (448 bp) with an inserted GntR BS directly upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg1999}::GntR_BS_pos+5-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1999 (448 bp) with an inserted GntR BS 5 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg1999}::GntR_BS_pos+10-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg1999 (448 bp) with an inserted GntR BS 10 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg2014}::GntR_BS_pos0-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg2014 (545 bp) with an inserted GntR BS directly upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg2020}::GntR_BS_pos0-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg2020 (390 bp) with an inserted GntR BS directly upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{cg2022}::GntR_BS_pos0-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the gene cg2022 (309 bp) with an inserted GntR BS directly upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]

pJC1-P*lys*::GntR_BS_pos-100-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 100 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P*lys*::GntR_BS_pos-25-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 25 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P*lys*::GntR_BS_pos-20-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 20 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P*lys*::GntR_BS_pos-15-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 15 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P*lys*::GntR_BS_pos-10-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 10 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P*lys*::GntR_BS_pos-5-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 5 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P*lys*::GntR_BS_pos-4-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 4 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp	[8]

	of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	
pJC1-P$_{lys}$::GntR_BS_pos-3-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 3 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P$_{lys}$::GntR_BS_pos-2-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 2 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P$_{lys}$::GntR_BS_pos-1-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 1 bp upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P$_{lys}$_CS_0-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS directly upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P$_{lys}$::GntR_BS_pos+1-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 1 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P$_{lys}$::GntR_BS_pos+2-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 2 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P$_{lys}$::GntR_BS_pos+3-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the	[8]

	lys gene (cg1974) (444 bp) with an inserted GntR BS 3 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	
pJC1-P$_{lys}$::GntR_BS_pos+4-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 4 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P$_{lys}$::GntR_BS_pos+5-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 5 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P$_{lys}$::GntR_BS_pos+10-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 10 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P$_{lys}$::GntR_BS_pos+15-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 15 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P$_{lys}$::GntR_BS_pos+20-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 20 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P$_{lys}$::GntR_BS_pos+25-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 25 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the	[8]

	reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	
pJC1-P_{lys}::GntR_BS_pos+50-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of the *lys* gene (cg1974) (444 bp) with an inserted GntR BS 50 bp downstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{priP}_CS_0-*venus* (previously named pJC1-P_{priP}::GntR_BS_pos0-*venus*)	*Kan*^{R}; pJC1-*venus*-term derivative carrying the CgpS bound area of the promoter of P_{priP} (611 bp) with an inserted GntR BS directly upstream of the position of maximal CgpS binding [7] and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pJC1-P_{gntK}-*venus*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the P_{gntK} promoter (307 bp) (P_{cg2732}) and the first 30 bp of the coding sequence fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	[8]
pEC-P_{tetR}	*Cm*^{R}, pGA1 *ori*V_{Cg}, *ori*V_{Ec}, *tetR*, P_{tet}; *C. glutamicum*/*E. coli* shuttle vector	[9]
pEKEx2	*Kan*^{R}, pBL1 *ori*V_{Cg}, pUC18 *ori*V_{Ec}, *lacIq*, P_{tac}; *C. glutamicum*/*E. coli* shuttle vector	[10]
pK19-*mobsacB*	*Kan*^{R}, *oriT*, *ori*V_{Ec}, *sacB*, *lacZ*; plasmid for allelic exchange in *C. glutamicum*	[11]
pJC1-P_{lys}_CS_0-*venus*-T-P_{gntK}-*e2-crimson*	*Kan*^{R}; pJC1-*venus*-term derivative carrying the construct P_{lys}_CS_0-*venus* and the oppositely oriented P_{gntK}-*e2-crimson* construct.	[8]
pJC4-P_{ilvB}-*ilvBNC*-P_{ilvE}-*ilvE* (previously named pJC4-*ilvBNCE*)	*Kan*^{R}, P_{ilvB}-*ilvBNC*, P_{ilvE}-*ilvE* (genes encoding the L-valine biosynthesis enzymes acetohydroxyacid synthase, isomeroreductase and transaminase B under control of their native promoters)	[3]

Table S4: Plasmids constructed in this work.

Plasmid	**Construction**	**Relevant characteristics**	**Primer used for sequencing**
pJC1-P_{priP}::GntR_BS_pos-100-*venus*	Gibson assembly: 200/280 (pJC1-P_{priP}-*venus*) and 279/116 (pJC1-P_{priP}-*venus*) into pJC1-*venus*-term *BamHI *BcuI	*Kan*^{R}; pJC1-P_{priP}-*venus* [8] derivative with an inserted GntR BS 100 bp upstream of the position of maximal CgpS binding [7].	R12, R13

pJC1-P_{priP}::GntR_BS_pos-50-*venus*	Gibson assembly: 200/278 (pJC1-P_{priP}-*venus*) and 277/116 (pJC1-P_{priP}-*venus*) into pJC1-*venus*-term *BamHI *BcuI	*Kan*^{R}; pJC1-P_{priP}-*venus* [8] derivative with an inserted GntR BS 50 bp upstream of the position of maximal CgpS binding [7].	R12, R13
pJC1-P_{priP}::GntR_BS_pos-10-*venus*	Gibson assembly: 200/276 (pJC1-P_{priP}-*venus*) and 275/116 (pJC1-P_{priP}-*venus*) into pJC1-*venus*-term *BamHI *BcuI	*Kan*^{R}; pJC1-P_{priP}-*venus* [8] derivative with an inserted GntR BS 10 bp upstream of the position of maximal CgpS binding [7].	R12, R13
pJC1-P_{priP}::GntR_BS_pos-5-*venus*	Gibson assembly: 200/288 (pJC1-P_{priP}-*venus*) and 287/116 (pJC1-P_{priP}-*venus*) into pJC1-*venus*-term *BamHI *BcuI	*Kan*^{R}; pJC1-P_{priP}-*venus* [8] derivative with an inserted GntR BS 5 bp upstream of the position of maximal CgpS binding [7].	R12, R13
pJC1-P_{priP}::GntR_BS_pos+5-*venus*	Gibson assembly: 200/290 (pJC1-P_{priP}-*venus*) and 289/116 (pJC1-P_{priP}-*venus*) into pJC1-*venus*-term *BamHI *BcuI	*Kan*^{R}; pJC1-P_{priP}-*venus* [8] derivative with an inserted GntR BS 5 bp downstream of the position of maximal CgpS binding [7].	R12, R13
pJC1-P_{priP}::GntR_BS_pos+10-*venus*	Gibson assembly: 200/282 (pJC1-P_{priP}-*venus*) and 281/116 (pJC1-P_{priP}-*venus*) into pJC1-*venus*-term *BamHI *BcuI	*Kan*^{R}; pJC1-P_{priP}-*venus* [8] derivative with an inserted GntR BS 10 bp downstream of the position of maximal CgpS binding [7].	R12, R13
pJC1-P_{priP}::GntR_BS_pos+50-*venus*	Gibson assembly: 200/284 (pJC1-P_{priP}-*venus*) and 283/116 (pJC1-P_{priP}-*venus*) into pJC1-*venus*-term *BamHI *BcuI	*Kan*^{R}; pJC1-P_{priP}-*venus* [8] derivative with an inserted GntR BS 50 bp downstream of the position of maximal CgpS binding [7].	R12, R13
pJC1-*tetR*-P_{tet}-*venus*	547/548 (pEC-P_{tetR}) and 115/116 (pJC1-*venus*-term) into pJC1-*venus*-term *BamHI *BcuI	*Kan*^{R}; pJC1-*venus*-term derivative carrying the *tetR* gene and the corresponding P_{tet} promoter fused to the reporter gene *venus* via a linker containing a stop codon and an artificial RBS.	R12, R13, 492
pJC1-*lacI*-P_{tac}-*venus*	Gibson assembly: 545/546 (pEKEx2) and 115/116 (pJC1-*venus*-term) into pJC1-*venus*-term *BamHI *BcuI	*Kan*^{R}; pJC1-*venus*-term derivative carrying the *lacI* gene and the corresponding P_{tac} promoter fused to the reporter gene *venus* via a linker containing a	R12, R13, 293, 399, 492, 546

		stop codon and an artificial RBS.	
pJC1-P$_{lys}$_CS_0-*ilvBNC*-RBS-*ilvE*	Gibson assembly: 114/117 (pJC1-P$_{lys}$_CS_0-*venus*), 349/350 (pJC4-*ilvBNCE*) and 351/352 (pJC4-*ilvBNCE*) into pJC1-*venus*-term *BamHI *BcuI	*Kan^R*, pJC1-*venus*-term derivative carrying the operon *ilvBNC and* the gene *ilvE* (L-valine biosynthesis genes) which were combined in a synthetic operon controlled by the counter-silencer promoter P_CS_0.	R12, R182, R183, R215, 487, 488, 489, 490
pJC1-P$_{lys}$_CS_+1 bp down-*ilvBNC*-RBS-*ilvE*	Gibson assembly: 114/117 (pJC1-P$_{lys}$::GntR_BS_pos+1-*venus*), 349/350 (pJC4-*ilvBNCE*) and 351/352 (pJC4-*ilvBNCE*) into pJC1-*venus*-term *BamHI *BcuI	*Kan^R*, pJC1-*venus*-term derivative carrying the operon *ilvBNC and* the gene *ilvE* (L-valine biosynthesis genes) which were combined in a synthetic operon controlled by the counter-silencer promoter P_CS_+1 bp down (P$_{lys}$::GntR_BS_pos+1).	R12, R13, 490, 488, 489, 487, 491, 645, 831
pJC1-P$_{lys}$_CS_-5 bp up-*ilvBNC*-RBS-*ilvE*	Gibson assembly: 114/117 (pJC1-P$_{lys}$::GntR_BS_pos-5-*venus*), 349/350 (pJC4-*ilvBNCE*) and 351/352 (pJC4-*ilvBNCE*) into pJC1-*venus*-term *BamHI *BcuI	*Kan^R*, pJC1-*venus*-term derivative carrying the operon *ilvBNC and* the gene *ilvE* (L-valine biosynthesis genes) which were combined in a synthetic operon controlled by the counter-silencer promoter P_CS_-5 bp up (P$_{lys}$::GntR_BS_pos-5).	R12, R13, 490, 488, 489, 487, 491, 645, 831, 171
pK19-*mobsacB*-Δ*gntK*	Gibson assembly: 207/208 (*C. glutamicum* genome) and 209/210 (*C. glutamicum* genome) into pK19-*mobsacB* *BamHI *EcoRI	*Kan^R*, pK19-*mobsacB* derivative for the chromosomal deletion of the *gntK* gene (cg2732) (530 bp upstream and 524 bp downstream flanking regions).	M19, M20
pK19-*mobsacB*-ΔP$_{aceE}$-*aceE*	Gibson assembly: 62/112 (*C. glutamicum* genome) and 113/65 (*C. glutamicum* genome) into pK19-	*Kan^R*, pK19-*mobsacB* derivative for the chromosomal deletion of the *aceE* gene (cg2466) and its 300 bp upstream promoter region (501 bp	M19, M20

	mobsacB *BamHI *EcoRI	upstream and 500 bp downstream flanking regions).	
pK19-*mobsacB*-P_{gntK}-*aceE*	Gibson assembly: 62/63 (*C. glutamicum* genome), 97/92 (*C. glutamicum* genome), 70/71 (*C. glutamicum* genome) and 64/65 (*C. glutamicum* genome) into pK19-*mobsacB* *BamHI *EcoRI	Kan^R, pK19-*mobsacB* derivative for the chromosomal integration of the *aceE* gene (cg2466) und control of the P_{gntK} promoter (333 bp, P_{cg2732}) (501 bp upstream and 500 bp downstream flanking regions).	M19, M20, 93, 94, 95, 101, 102

Oligonucleotide pairs used for PCR are given as numbers (Table S5) with DNA templates indicated in brackets behind. The used backbones including the restriction enzymes used for linearization are listed behind (*). Used primers for sequencing are listed in Table S6.

Table S5: Oligonucleotides used for plasmid constructions.

Oligonucleotide number	Sequence (5´→3´)
279	CGCTCGATATGATAGTACCAATTCACGTGCAGCAGCACTCCC
280	GAATTGGTACTATCATATCGAGCGTTACGAACCATAACTG
277	GTGCACGTGTATGATAGTACCAATCAACTGTGCGCTAAATGCGTC
278	CACAGTTGATTGGTACTATCATACACGTGCACACATATGCGCG
275	GTTTTATGATAGTACCAATCTTTATTACTAAGCTTGTTTAAATTGAAAC
276	GTAATAAAGATTGGTACTATCATAAAACTCAACGGTTTATTAAGACGC
287	CTTTATATGATAGTACCAATTTACTAAGCTTGTTTAAATTGAAAC
288	GTAAATTGGTACTATCATATAAAGAAACTCAACGGTTTATTAAG
289	CTAAGCTTATGATAGTACCAATTGTTTAAATTGAAACTTCGTTATATTC
290	CAATTGGTACTATCATAAGCTTAGTAATAAAGAAACTCAACGG
281	GCTTGTTTTATGATAGTACCAATAAATTGAAACTTCGTTATATTCTG
282	GTTTCAATTTATTGGTACTATCATAAAACAAGCTTAGTAATAAAGAAAC
283	GAAAGTAAGTTATGATAGTACCAATAATTAAGTACTTCGGCTCCACG
284	CTTAATTATTGGTACTATCATAACTTACTTTCTTTAATCAGAATATAAC
285	CTTTATTACTATATGATAGTACCAATAGCTTGTTTAAATTGAAACTTCG
286	CAAGCTATTGGTACTATCATATAGTAATAAAGAAACTCAACGG
547	AGCGACGCCGCAGGGGGATCCTTAAGACCCACTTTCACATTTAAGTTGTTTTTC
548	ATGATATCTCCTTCTTAAAGTTCAGTGTATCAACAAGCTGGGGATCTTAAGC
115	TGAACTTTAAGAAGGAGATATCATATGGTGAGCAAGGGCGAGGAG
116	AAAACGACGGCCAGTACTAGTTACTTGTACAGCTCGTCCATGCC
545	AGCGACGCCGCAGGGGGATCCTCAAGCCTTCGTCACTGGTCCC
546	ATGATATCTCCTTCTTAAAGTTCAGGATCCTCTAGAGTCGACCTGC
114	TGATATCTCCTTCTTAAAGTTCAATTTTTCGGCATTGCGCCTTTAATCGC
117	AGCGACGCCGCAGGGGGATCCGCTCAAGGAAGAGTTCTTCATTGGTC

349	TGAACTTTAAGAAGGAGATATCATGTGAATGTGGCAGCTTCTCAAC
350	ATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGTTTAAGCGGTTTCTGCG CGAGC
351	GTTTAACTTTAAGAAGGAGATATACATGTGTATCTGTCAGGTAGCAGG
352	AAAACGACGGCCAGTACTAGTTAGCCAACCAGTGGGTAAAGC
207	CAGGTCGACTCTAGAGGATCATGGTGGCGTCATGCTCGGC
208	GTCTGTAACCGAGCATCTCTCCTAGACAATATGTAAGCCTTCGGCTG
209	GAGAGATGCTCGGTTACAGACGCAGAGTGGGTTCGCAACAAATAA
210	GTTGTAAAACGACGGCCAGTGAATTGCAGGTCGAGTTCTTCCCACAG
62	CAGGTCGACTCTAGAGGATCTCGATGGACTCGCTGATCAGC
112	GTCTGTAACCGAGCATCTCTCAAAGAATTATCGGGTAGTTTCCCGC
113	GAGAGATGCTCGGTTACAGACATCACCTCAAGGGACAGATAAATCC
65	GTTGTAAAACGACGGCCAGTGAATTCCGTGAGCAATTCAAGCAGGAAC
63	GGACGAGCTGTACAAGTAAACTAGTAAAGAATTATCGGGTAGTTTCCCGC
97	CTAGTTTACTTGTACAGCTCGTCCGAGGATCGTCTCCGCGAAGAG
92	CATTTCCACACCTCCTGTTGGGTCGACAATATGTAAGCCTTCGGCTGC
70	GACCCAACAGGAGGTGTGGAAATGGCCGATCAAGCAAAACTTGGTG
71	GGATTTATCTGTCCCTTGAGGTGATTTATTCCTCAGGAGCGTTTGGATC
64	GATCCAAACGCTCCTGAGGAATAAATCACCTCAAGGGACAGATAAATCC

Table S6: Oligonucleotides used for plasmid sequencing.

Oligonucleotide number	**Sequence (5´→3´)**
M19	CGCCAGGGTTTTCCCAGTCAC
M20	AGCGGATAACAATTTCACACAGGA
R12	CAGGGACAAGCCACCCGCACA
R13	GGAAGCTAGAGTAAGTAGTTCGC
R182	CGATTCCTATGGACCCTGCCACC
R183	GGTGGCAGGCTCCATAGGAATCG
R215	CTGCGTTCTGATTTAATCTGTATCAGG
93	CTCTGGCAGGTAGCCACCG
94	CTGCCAGAGCGTCGTGAGAAC
95	CACACCACGGGGACTGTGG
101	CTATGGAACCTGAATTCCCAGGC
102	CTTCAGGTGCCTCACGGTAGG
171	GATACTTATGATAGTACCAATAGAGTTTAATTTGTAGTATCCAGGGAAC
293	CGCCGCTTCCACTTTTTCCCG
399	CACCAAACGTTTCGGCGAGAAGC
487	GGTCAACGATGAGCTTGAGCTC
488	CTGCTGGAAACCACCAAGGC
489	CACTCGGATTGCGCCCATTC
490	GCCCACATTTGGTGCTGGCC
492	CTCGAACTTCACCTCGGCGC
546	ATGATATCTCCTTCTTAAAGTTCAGGATCCTCTAGAGTCGACCTGC
645	TGCCATACGCGCCGATTCCTATGGATCCTGCCACCACCGTATTCCACTAC
831	CATGAGGTCGCGGAGCTTAG

Table S7: Oligonucleotides used for sequencing of chromosomal modifications.

Strain	Oligonucleotide number	Sequence (5´→3´)
Δ*gntK*	313	GCCCACTGCTCAGCGATTTC
	314	CGGGGTCGAGTTCTTTGATCC
ΔP_{aceE}-*aceE*	104	CCAGGGCTCCTTCTTTACCAATG
	105	CGTTCTTCCCCGGCACTGTG
P_{gntK}-*aceE*	93	CTCTGGCAGGTAGCCACCG
	94	CTGCCAGAGCGTCGTGAGAAC
	95	CACACCACGGGGACTGTGG
	101	CTATGGAACCTGAATTCCCAGGC
	104	CCAGGGCTCCTTCTTTACCAATG
	105	CGTTCTTCCCCGGCACTGTG

Table S8: Oligonucleotides used for quantitative Real-time PCR (qRT-PCR).

Target gene	Oligo-nucleotide number	Sequence (5´→3´)
venus	554	GCGCACCATCTTCTTCAAGG
	555	CGGCGGTGATATAGACGTTGTG
ddh	558	AGCAGGTATGGAGCAACTTCG
	559	TGATTACCACCGGCGACAC

References

1. Schreiner, M. E., Fiur, D., Holátko, J., Pátek, M., and Eikmanns, B. J. (2005) E1 enzyme of the pyruvate dehydrogenase complex in *Corynebacterium glutamicum*: molecular analysis of the gene and phylogenetic aspects. *J. Bacteriol. 187*, 6005-6018.
2. Blombach, B., Schreiner, M. E., Holátko, J., Bartek, T., Oldiges, M., and Eikmanns, B. J. (2007) L-valine production with pyruvate dehydrogenase complex-deficient *Corynebacterium glutamicum*. *Appl. Environ. Microbiol. 73*, 2079-2084.
3. Radmacher, E., Vaitsikova, A., Burger, U., Krumbach, K., Sahm, H., and Eggeling, L. (2002) Linking central metabolism with increased pathway flux: L-valine accumulation by *Corynebacterium glutamicum*. *Appl. Environ. Microbiol. 68*, 2246-2250.
4. Kinoshita, S., Udaka, S., and Shimono, M. (1957) Studies on the amino acid fermentation. Part 1. Production of L-glutamic acid by various microorganisms. *J. Gen. Appl. Microbiol. 3*, 193-205.
5. Cremer, J., Eggeling, L., and Sahm, H. (1990) Cloning the *dapA dapB* cluster of the lysine-secreting bacterium *Corynebacterium glutamicum*. *Mol. Gen. Genet. 220*, 478-480.
6. Baumgart, M., Unthan, S., Rückert, C., Sivalingam, J., Grünberger, A., Kalinowski, J., Bott, M., Noack, S., and Frunzke, J. (2013) Construction of a prophage-free variant of *Corynebacterium glutamicum* ATCC 13032 for use as a platform strain for basic research and industrial biotechnology. *Appl. Environ. Microbiol. 79*, 6006-6015.
7. Pfeifer, E., Hünnefeld, M., Popa, O., Polen, T., Kohlheyer, D., Baumgart, M., and Frunzke, J. (2016) Silencing of cryptic prophages in *Corynebacterium glutamicum*. *Nucleic Acids Res. 44*, 10117-10131.
8. Wiechert, J., Filipchyk, A., Hünnefeld, M., Gätgens, C., Brehm, J., Heermann, R., and Frunzke, J. (2020) Deciphering the rules underlying xenogeneic silencing and counter-

silencing of Lsr2-like proteins using CgpS of *Corynebacterium glutamicum* as a model. *mbio 11*, e02273-02219.
9. Huber, I., Palmer, D. J., Ludwig, K. N., Brown, I. R., Warren, M. J., and Frunzke, J. (2017) Construction of recombinant Pdu metabolosome shells for small molecule production in *Corynebacterium glutamicum*. *ACS Synth. Biol. 6*, 2145-2156.
10. Eikmanns, B. J., Thum-Schmitz, N., Eggeling, L., Ludtke, K. U., and Sahm, H. (1994) Nucleotide sequence, expression and transcriptional analysis of the Corynebacterium glutamicum gltA gene encoding citrate synthase. *Microbiology (Reading, U. K.) 140*, 1817-1828.
11. Schäfer, A., Tauch, A., Jager, W., Kalinowski, J., Thierbach, G., and Pühler, A. (1994) Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli plasmids* pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene 145*, 69-73.