Supplementary information

Biosensor-based growth-coupling and spatial separation as an evolution strategy to improve small molecule production of Corynebacterium glutamicum

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Table S1

Overview of mutations in 15 L-valine producer mutants. Only high frequency mutations (>35%) are shown, unless no high frequency mutations were found (ND); *fs*: frameshift mutation, *sv*: structural variant, *ins*: insertion.

#	Strain	Experiment	Phenotype	Mutation	Occurence (%)
C1	P_{brnF}-*pfkA*	rbALE in liquid media	cheater	Lrp (M1I)	100
C2	P_{brnF}-*pfkA*	rbALE in liquid media	cheater	sv, duplication *pfkA*	18
C3	P_{brnF}-*pfkA*	rbALE in liquid media	cheater	Lrp (F139L)	100
C4	P_{brnF}-*hisD*	rbALE in liquid media	cheater	sv *lrp*-P_{brnF}	ND
C5	P_{brnF}-*hisD*	rbALE in liquid media	cheater	C to A *lrp*-P_{brnF}	100
C6	P_{brnF}-*hisD*	rbALE in liquid media	cheater	sv *lrp*-P_{brnF}	ND
V1	P_{brnF}-*pfkA*	FACS-based ALE	L-valine producer; 2.5 mM	*ilvN* sv	46
V2	P_{brnF}-*pfkA*	1st plate-based ALE, BHI plate	L-valine producer; 4.8 mM	IlvN (F29L) #4	50.9
V3	P_{brnF}-*pfkA*	1st plate-based ALE, CGXII plate large colony	L-valine producer; 10.0 mM	IlvN (F29L) #2	100
V4	P_{brnF}-*pfkA*	1st plate-based ALE, CGXII plate large colony	L-valine producer; 10.8 mM	IlvN (F29I)	100
V5	P_{brnF}-*hisD*	1st plate-based ALE, CGXII plate large colony	L-valine producer; 1.5 mM	IlvN fs (ins 442G)	96.4
				A391A in NCgl0375(-), NCgl0375, cation transport ATPase	100
V6	P_{brnF}-*hisD*	1st plate-based ALE, CGXII plate large colony	L-valine producer; 10.5 mM	IlvN (D17E)	34.2
V7	P_{brnF}-*hisD*	1st plate-based ALE, CGXII plate large colony	L-valine producer; 9.9 mM	IlvN (F29L) #3	100
V8	P_{brnF}-*hisD*	1st plate-based ALE, CGXII plate small colony	L-valine producer; 3.5 mM	*ilvN* ins (105CCTCGTGTC)	40
				A to T intergenic region of NCgl1020 (major facilitator superfamily permease) and NCgl1021 (transposase)	52.4
V9	P_{brnF}-*hisD*	1st plate-based ALE, CGXII plate small colony	L-valine producer; 7.0 mM	IlvN (I158M)	63.6
V10	P_{brnF}-*pfkA*	2nd HT plate-based ALE	L-valine producer; 5.8 mM	IlvB (D133G)	100
V11	P_{brnF}-*pfkA*	2nd HT plate-based ALE	L-valine producer; 12.0 mM	IlvB (R141G)	87.7
V12	P_{brnF}-*pfkA*	2nd HT plate-based ALE	L-valine producer; 15.0 mM	IlvN (S155F)	100
V13	P_{brnF}-*hisD*	2nd HT plate-based ALE	L-valine producer; 11.2 mM	IlvN (A42E)	100
V14	P_{brnF}-*hisD*	2nd HT plate-based ALE	L-valine producer; 13.1 mM	IlvN (I22M)	100
V15	P_{brnF}-*hisD*	2nd HT plate-based ALE	L-valine producer; 10.7 mM	IlvN (F29L) #1	100

Table S2

Amount of colonies, and size of colony, on agar plates CGXII 2% glucose or BHI media. ND, not determined.

strain	*plate media*	*dilution*	*normal colonies*	*large colonies*
P_{brnF}-*pfkA*	CGXII	10^6	87	0
P_{brnF}-*pfkA*	CGXII	10^5	1004	0
P_{brnF}-*pfkA*	CGXII	10^4	ND	2
P_{brnF}-*pfkA*	CGXII	10^3	ND	10
P_{brnF}-*pfkA*	BHI	10^6	151	ND
P_{brnF}-*pfkA*	BHI	10^5	924	ND
WT	CGXII	10^6	104	ND
WT	CGXII	10^5	828	ND
P_{brnF}-*hisD*	CGXII	10^6	267	0
P_{brnF}-*hisD*	CGXII	10^5	1376	1
P_{brnF}-*hisD*	CGXII	10^4	ND	10
P_{brnF}-*hisD*	CGXII	10^3	ND	>30
P_{brnF}-*hisD*	BHI	10^6	219	ND
P_{brnF}-*hisD*	BHI	10^5	2308	ND
WT	CGXII	10^6	210	ND
WT	CGXII	10^5	1296	ND

Table S3

Bacterial strains and plasmids used in this study.

Strain/plasmid	Genotype and relevant characteristic	Reference
E. coli DH5a	*supE44 ΔlacU169* (ϕ80*lacZ*DM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Invitrogen (Karlsruhe, Germany)
C. glutamicum ATCC13032	Biotin-auxotrophic wild type	(Kalinowski et al., 2003)
C. glutamicum:: *PbrnF-pfkA*	Integrated lrp sensor construct (terminator, *lrp*, *lrp-brnF* intergenic region and first 30 bp of *brnF* followed by a stopcodon, RBS and linker) upstream of *pfkA*	This study
C. glutamicum:: *PbrnF-hisD*	Integrated lrp sensor construct (terminator, *lrp*, *lrp-brnF* intergenic region and first 30 bp of *brnF* followed by a stopcodon, RBS and linker) upstream of *hisD*	This study
C. glutamicum:: *PbrnF-pfkA* **/** *pJC1-lrp-brnF'-eyfp*	*C. glutamicum PbrnF-pfkA* harboring pJC1-lrp-brnF'-eyfp	This study
C. glutamicum:: *PbrnF-hisD* **/** *pJC1-lrp-brnF'-eyfp*	*C. glutamicum PbrnF-hisD* harboring pJC1-lrp-brnF'-eyfp	This study
pJC1-*lrp-brnF'-eyfp*	Kan^R, Lrp sensor plasmids containing a terminator (term part), *lrp,* the *lrp-brnF* intergenic region, the first 30 bp of *brnF* followed by a stopcodon, RBS and linker and *eyfp*	(Mustafi et al., 2012)
pK19-*mobsacB*	Used for allelic exchange in *C. glutamicum*; $oriV_{E.c.}$., *sacB lacZα KanR*	(Schäfer et al., 1994)
pJC1-*venus*-term	Used to obtain term part, Kan^R	(Baumgart et al., 2013)
pK19-*mobsacB-pfkA-lrp-brnF'*	Vector for integration of the Lrp sensor construct upstream of *pfkA*	This Study
pK19-*mobsacB-hisD-lrp-brnF'*	Vector for integration of the Lrp sensor construct upstream of *hisD*	This Study

Table S4

Oligonucleotides used in this study

name	*sequence*
term_fw	TTTTGGCGGATGAGAGAAGA
term_rv	CAAAAGAGTTTGTAGAAACGCA
lrp_fw	GTTTCTACAAACTCTTTTGTCACACCTGGGGGCGAGC
lrp_rv	ATGATATCTCCTTCTTAAAGTTCAGCT
hisD_up_fw	CCTGCAGGTCGACTCTAGAGTCCGGTGTCGCTGAAGTTAA
hisD_up_fw	TCTTCTCTCATCCGCCAAAAACCTATTGTATTCCCCACGTAAC
hisD_down_fw	CTTTAAGAAGGAGATATCATATGTTGAATGTCACTGACCTGC
hisD_down_fw	TTGTAAAACGACGGCCAGTGGACAGCCCACACCTCATCAA
pfkA_up_fw	CCTGCAGGTCGACTCTAGAGAGAGTCGCCCCGATAAGTTT
pfkA_up_fw	TCTTCTCTCATCCGCCAAAATCTGACCATCTTATTTAATCGCCA
pfkA_down_fw	CTTTAAGAAGGAGATATCATATGCGAATTGCTACTCTCACG
pfkA_down_fw	TTGTAAAACGACGGCCAGTGATACCTGCGTGCAGAGCAAT

Supplementary data 1

Sequence of *lrp*-P_{brnF} integration upstream from *pfkA*, intergenic region upstream of *pfkA* is given, showing the first codon of *pfkA* (underlined), the terminator sequence (red), *lrp* (green), the first 30 bp of *brnF* (yellow) and the linker sequence (blue)

GGTGAGCCAGTCTAGAGACAAAATTTTTCCGCGGGGGTTTTCTTGATCTGATCCGACAACCCAATGGGGG
CAAAAATGTGTCCGACCAAAAATTGTGCAGCACACCACATGCCCGCTCGGACAATGTCGATTTGTTAATG
AAACTGCAGCTCTGGCGATTAAATAAGATGGTCAGATTTTGGCGGATGAGAGAAGATTTTCAGCCTGATA
CAGATTAAATCAGAACGCAGAAGCGGTCTGATAAAACAGAATTTGCCTGGCGGCAGTAGCGCGGTGGTCC
CACCTGACCCCATGCCGAACTCAGAAGTGAAACGCCGTAGCGCCGATGGTAGTGTGGGGTCTCCCCATGC
GAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTAT
CTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAAG
CAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCA
TCCTGACGGATGGCCTTTTTGCGTTTCTACAAACTCTTTTGTCACACCTGGGGGCGAGCTGGTTTCACCA
CTTTCATAGCAAAACGTGATGAGATCTTTGCAATTCCTGGCACGGTTTGAATGTGACTGGATAAAAATTG
CTCATACGCCTCCAAATCAGCAACGCCGATGCGGACAAAATAATCTGGCGAACCAAAAAGCCTGTGCAAC
TCCAGTACTTCATCATGCTGCGCAACGGAGCTTTCAAAATTGTCTACAGTGGAGCGGTCGAAGTTGCTGA
GAGTGACATCCACGGTCACCTCAAATCCACGATTCATCACCGCAGGGTGAATGTCCGCGCTGTAGCCCAA
AATGATTCCTTCGGCTTCCAAACGCTGCACCCTCCTCAAGCAAGGTCCCGGAGTGAGATGCACCTTGTCA
GCCAGTGCGAGATTTGAGATGCGCGCATTCGCGCTAAGCTCCGCAATAATTGCGCGATCAATGGAATCTA
GCTTCATATATTGCACAATAGCCTAGTTGAGGTGCGCAAACTGGCAACAAAACTACCCGGCAATTGTGTG
ATGATTGTAGTGTGCAAAAAACGCAAGAGATTCATTCAAGCTGAACTTTAAGAAGGAGATATCATATG

Supplementary data 2

Sequence of *lrp*-P_{brnF} integration upstream from *hisD*, intergenic region upstream of *hisD* is given, showing the first codon of *hisD*. (underlined), the terminator sequence (red), *lrp* (green), the first 30 bp of *brnF* (yellow) and the linker sequence (blue)

CATATGATATCTCCTTCTTAAAGTTCAGCTTGAATGAATCTCTTGCGTTTTTTGCACACTACAATCATCA
CACAATTGCCGGGTAGTTTTGTTGCCAGTTTGCGCACCTCAACTAGGCTATTGTGCAATATATGAAGCTA
GATTCCATTGATCGCGCAATTATTGCGGAGCTTAGCGCGAATGCGCGCATCTCAAATCTCGCACTGGCTG
ACAAGGTGCATCTCACTCCGGGACCTTGCTTGAGGAGGGTGCAGCGTTTGGAAGCCGAAGGAATCATTTT
GGGCTACAGCGCGGACATTCACCCTGCGGTGATGAATCGTGGATTTGAGGTGACCGTGGATGTCACTCTC
AGCAACTTCGACCGCTCCACTGTAGACAATTTTGAAAGCTCCGTTGCGCAGCATGATGAAGTACTGGAGT
TGCACAGGCTTTTTGGTTCGCCAGATTATTTTGTCCGCATCGGCGTTGCTGATTTGGAGGCGTATGAGCA
ATTTTTATCCAGTCACATTCAAACCGTGCCAGGAATTGCAAAGATCTCATCACGTTTTGCTATGAAAGTG
GTGAAACCAGCTCGCCCCCAGGTGTGACAAAAGAGTTTGTAGAAACGCAAAAAGGCCATCCGTCAGGATG
GCCTTCTGCTTAATTTGATGCCTGGCAGTTTATGGCGGGCGTCCTGCCCGCCACCCTCCGGGCCGTTGCT
TCGCAACGTTCAAATCCGCTCCCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGAT
AAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCAGTTCCCTACTCTCGC
ATGGGGAGACCCCACACTACCATCGGCGCTACGGCGTTTCACTTCTGAGTTCGGCATGGGGTCAGGTGGG
ACCACCGCGCTACTGCCGCCAGGCAAATTCTGTTTTATCAGACCGCTTCTGCGTTCTGATTTAATCTGTA
TCAGGCTGAAAATCTTCTCTCATCCGCCAAAAACCTATTGTATTCCCCACGTAACAAGTTTCTGATTTGG
GTACATCAGAGTTCATTTGAATTAGACTTAAAACTTAAAATGACCACCCCAGATTTACCTGAATTAAACC
CGCTTTCACCTTTGAGATACTGGAAGGA

Supplementary data 3

PDF export of the jupyter notebook containing the information on the rbALE simulation model.

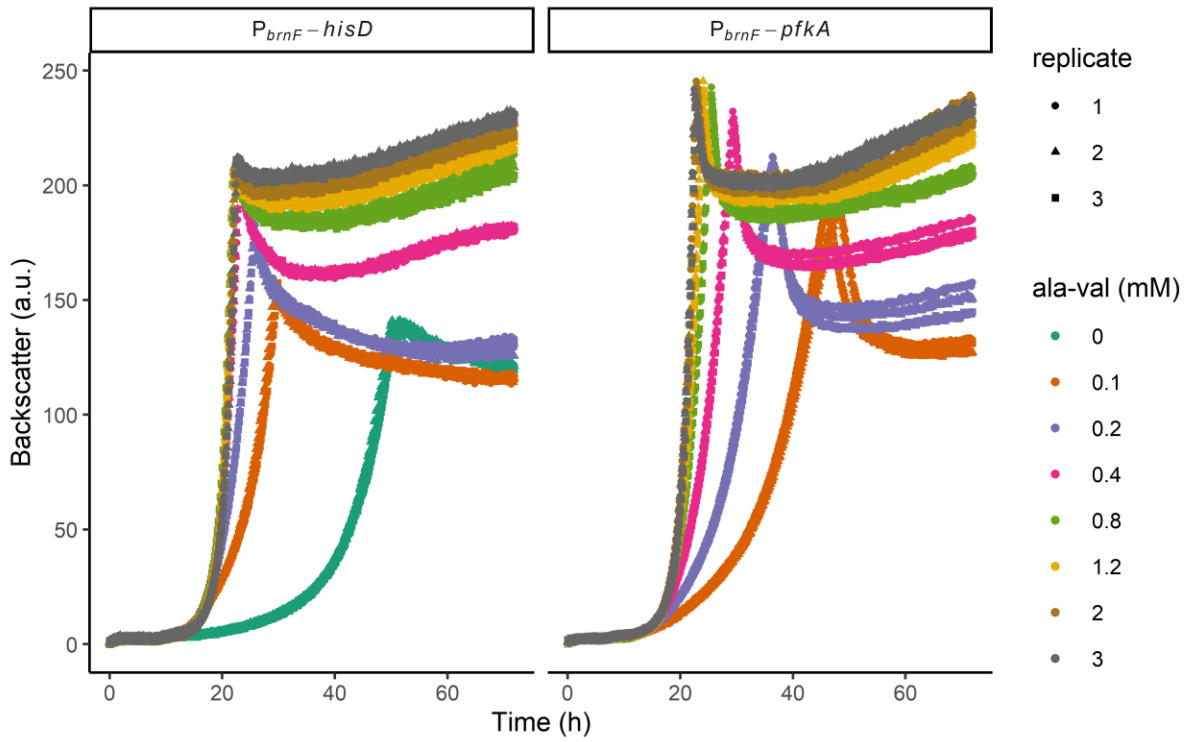

Figure S1

Microtiter growth of *C. glutamicum* growth-coupled strains P_{brnF}-*hisD* and P_{brnF}-*pfkA* supplemented with different amounts of ala-val dipeptide (0-3 mM), grown in CGXII 2% glucose. Supplementary information to the results shown in Figure 1C.

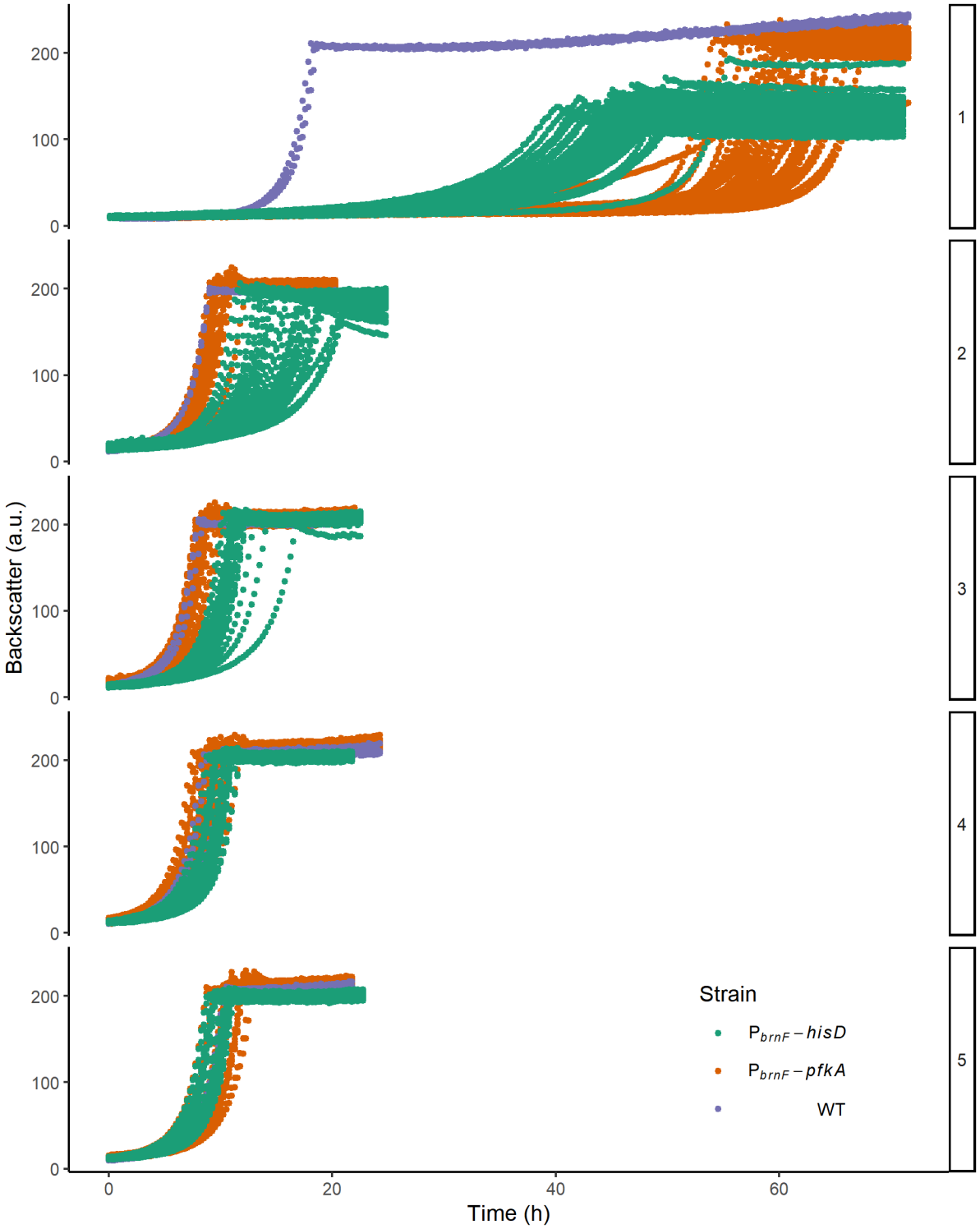

Figure S2

Growth of cultures after FACS-based selection. Backscatter values of five *C. glutamicum* repetitive batch cultivations in CGXII 2% glucose media after FACS sorting, covering 44 P_{brnF}-*pfkA* P_{brnF}-*pfkA* pJC1-*lrp-brnF'-eyfp* and P_{brnF}-*hisD* P_{brnF}-*pfkA* pJC1-*lrp-brnF'-eyfp* cultures and three *C. glutamicum* WT controls. Supplementary information to the results shown in Figure 4B.

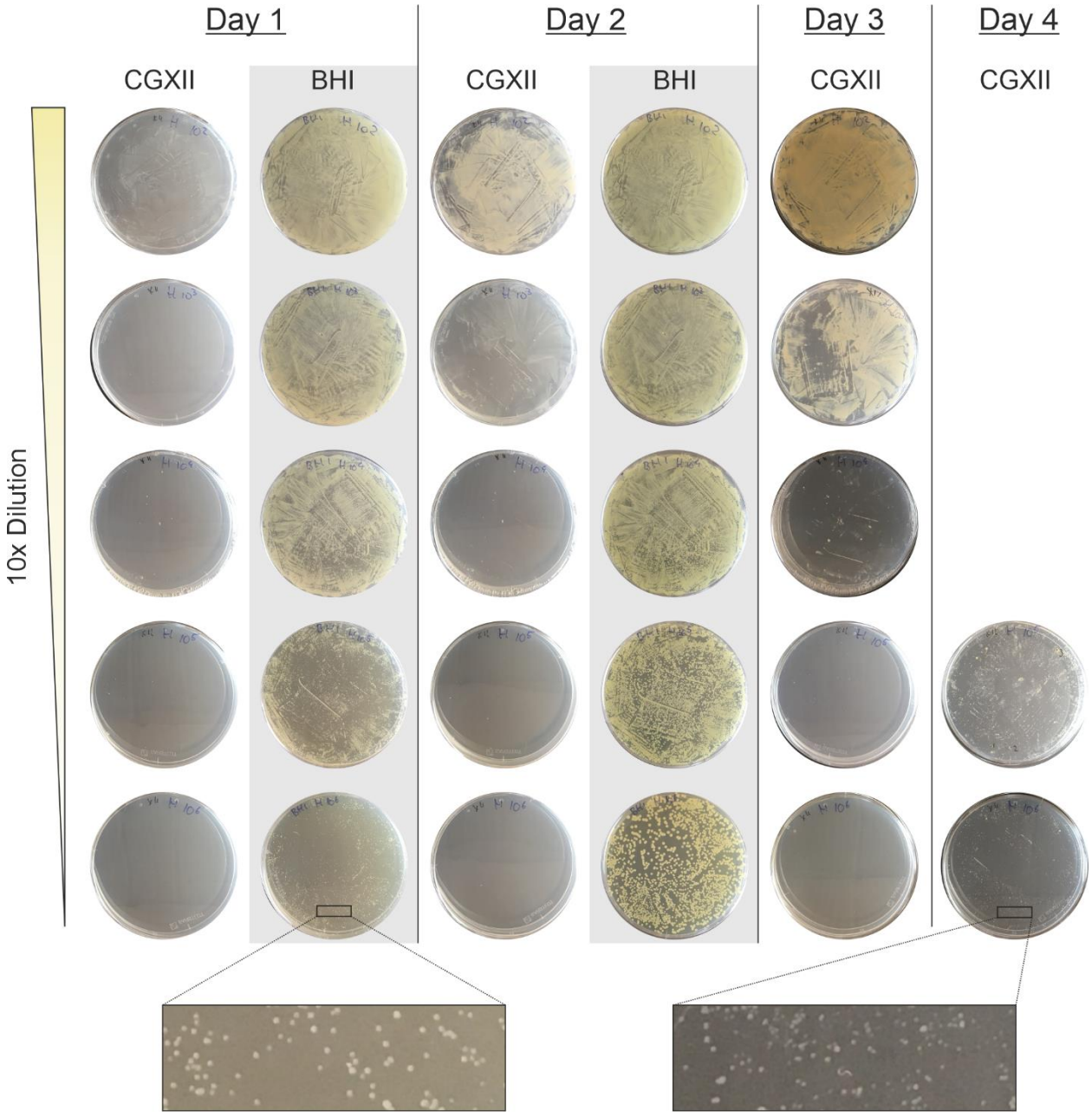

Figure S3

Growth of *C. glutamicum*::P_{brnF}-*hisD* on agar plates containing CGXII 2% glucose or BHI media. Different amounts of culture solutions were plated and photographs were taken after multiple days of incubation.

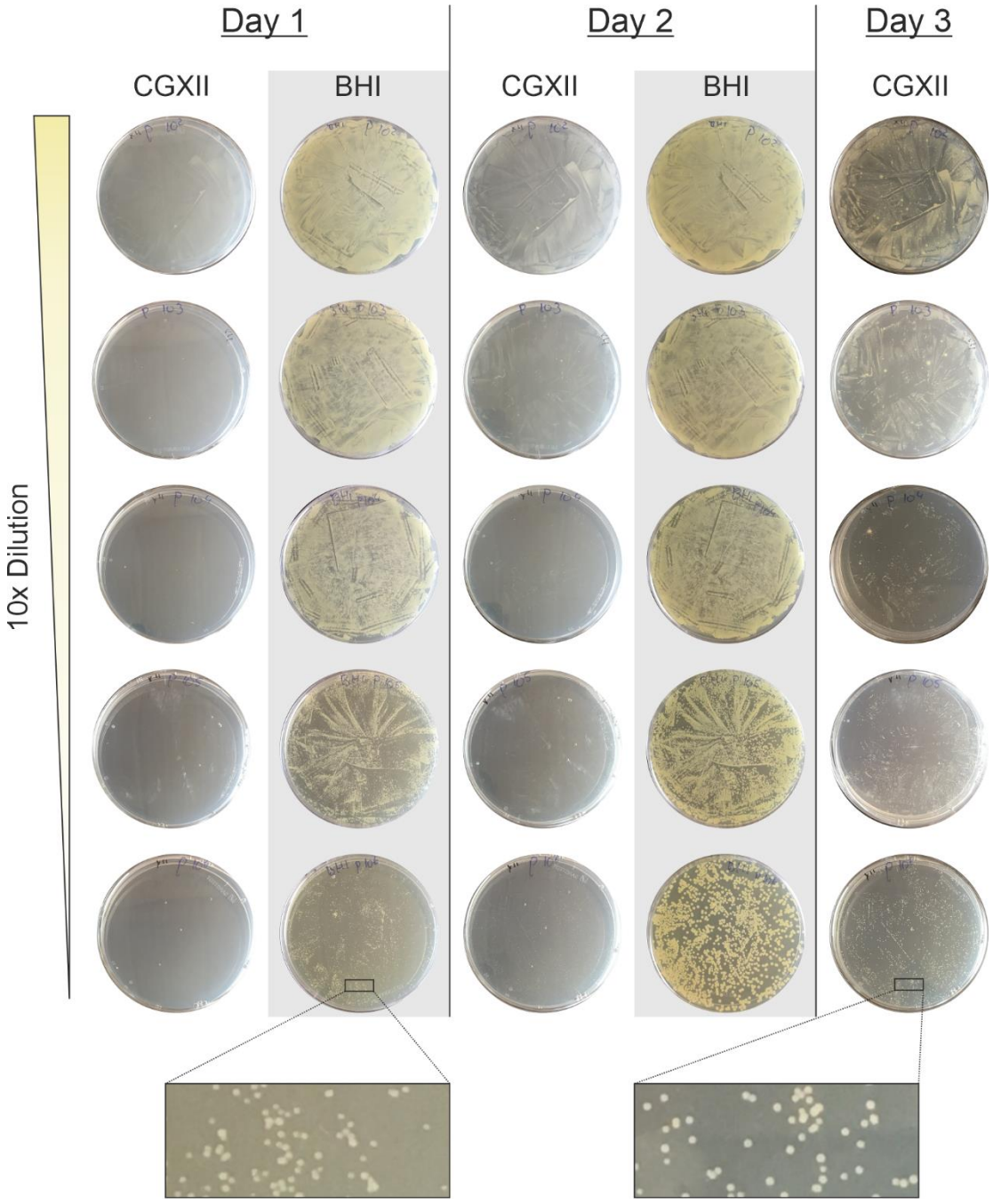

Figure S4

Growth of *C. glutamicum*::P_{brnF}-*pfkA* on agar plates containing CGXII 2% glucose or BHI media. Different amounts of culture solutions were plated and photographs were taken after multiple days of incubation.

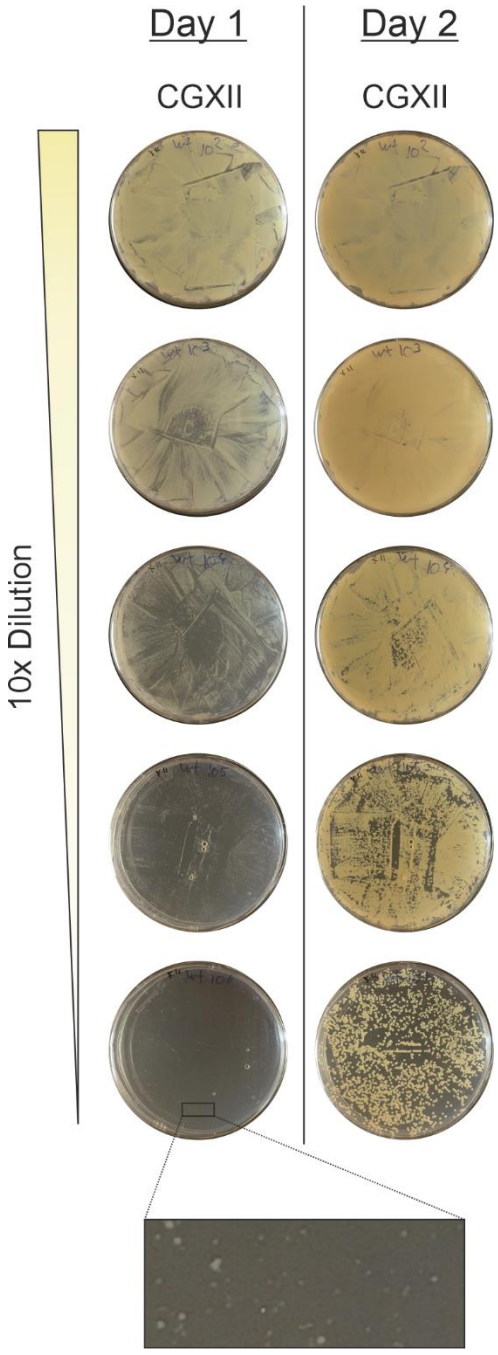

Figure S5

Growth of *C. glutamicum* WT on agar plates containing CGXII 2% glucose media. Different amounts of culture solutions were plated and photographs were taken after multiple days of incubation.

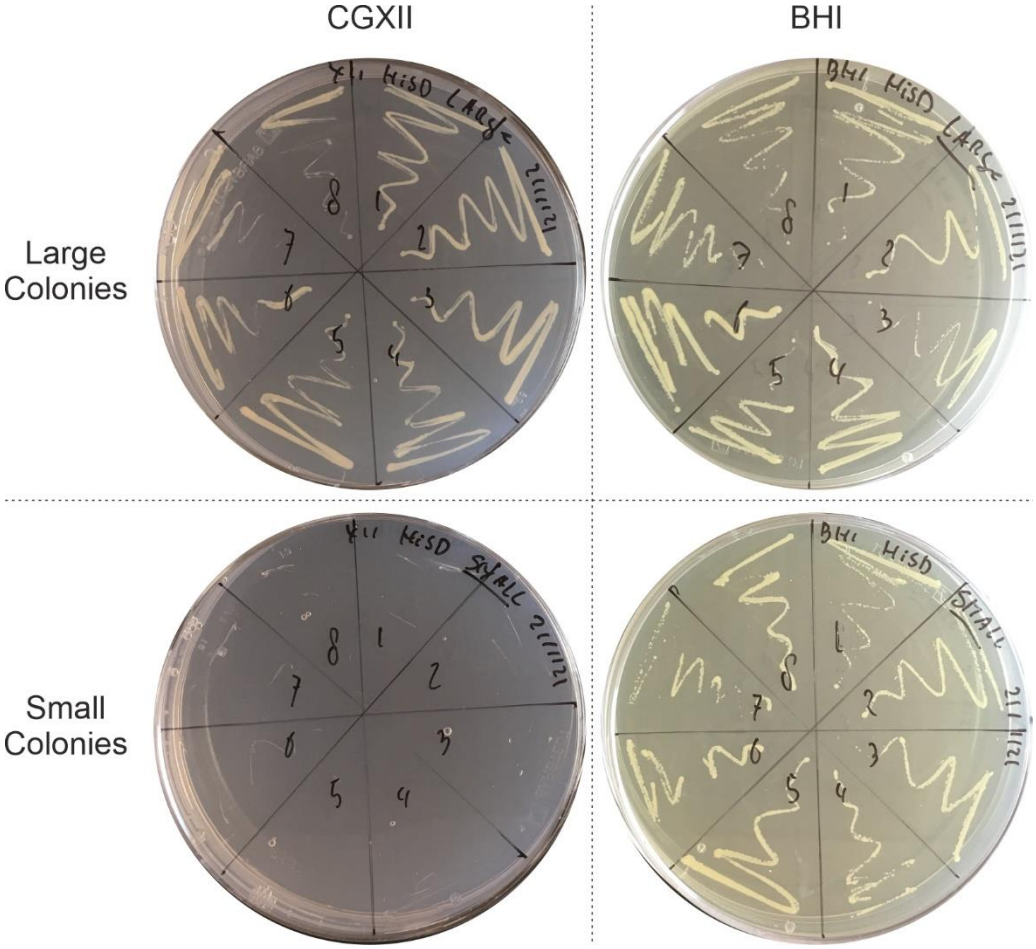

Figure S6

Growth of restreaked *C. glutamicum*::P$_{brnF}$-*hisD* colonies on agar plates containing CGXII 2% glucose or BHI media, after one day. Restreaking was done for 8 colonies, either large colonies or small colonies grown on CGXII 2% glucose media.

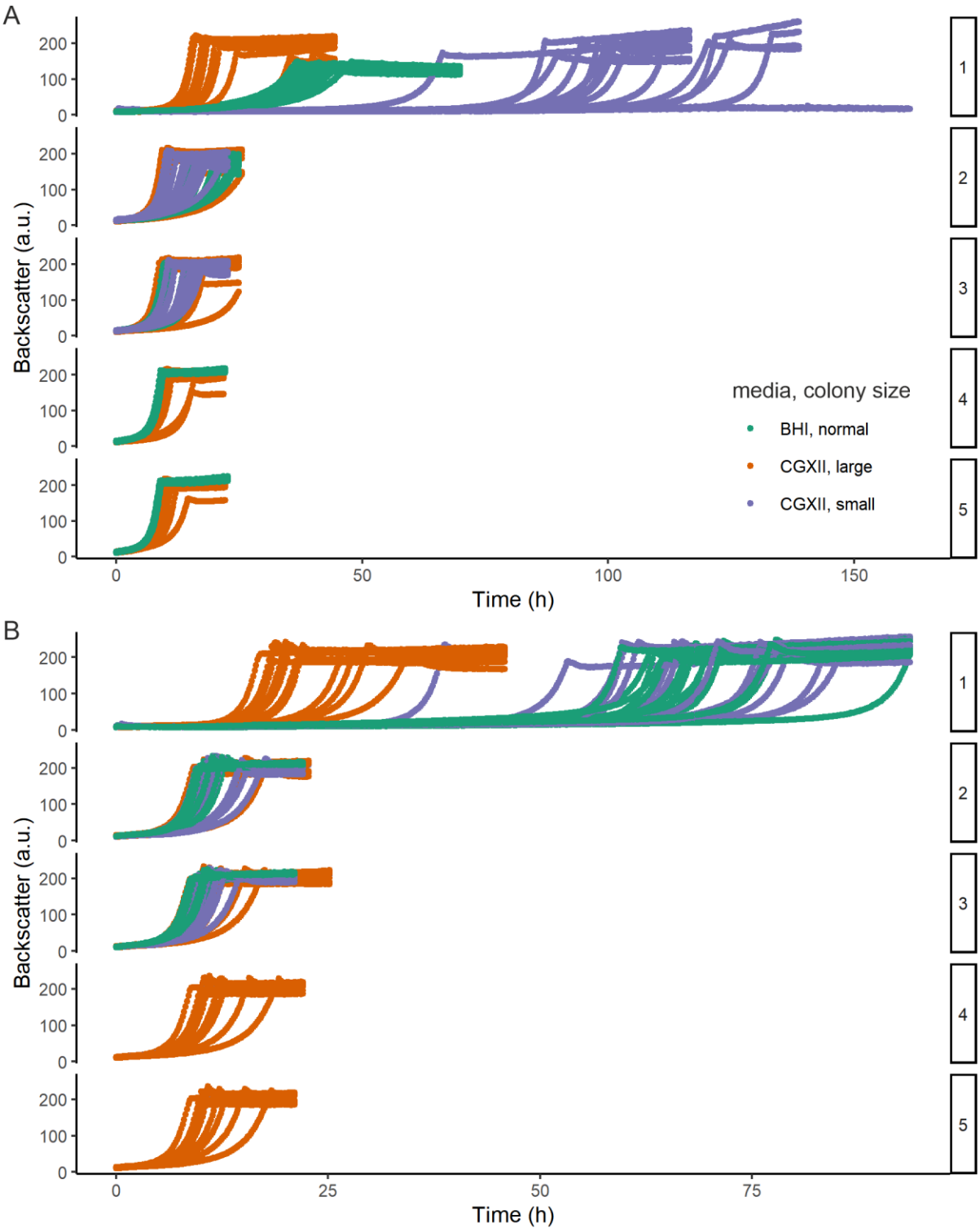

Figure S7

Backscatter values of *C. glutamicum*::P$_{brnF}$-*pfkA* and P$_{brnF}$-*hisD* cultures from plate-based evolutions, covering 15 cultures started from large colonies on CGXII plates, 15 cultures from small colonies on CGXII plates, and 15 cultures from normal colonies on BHI plates, for P$_{brnF}$-*hisD* (A) and P$_{brnF}$-*pfkA* (B). Supplementary information to the results shown in Figure 5B.

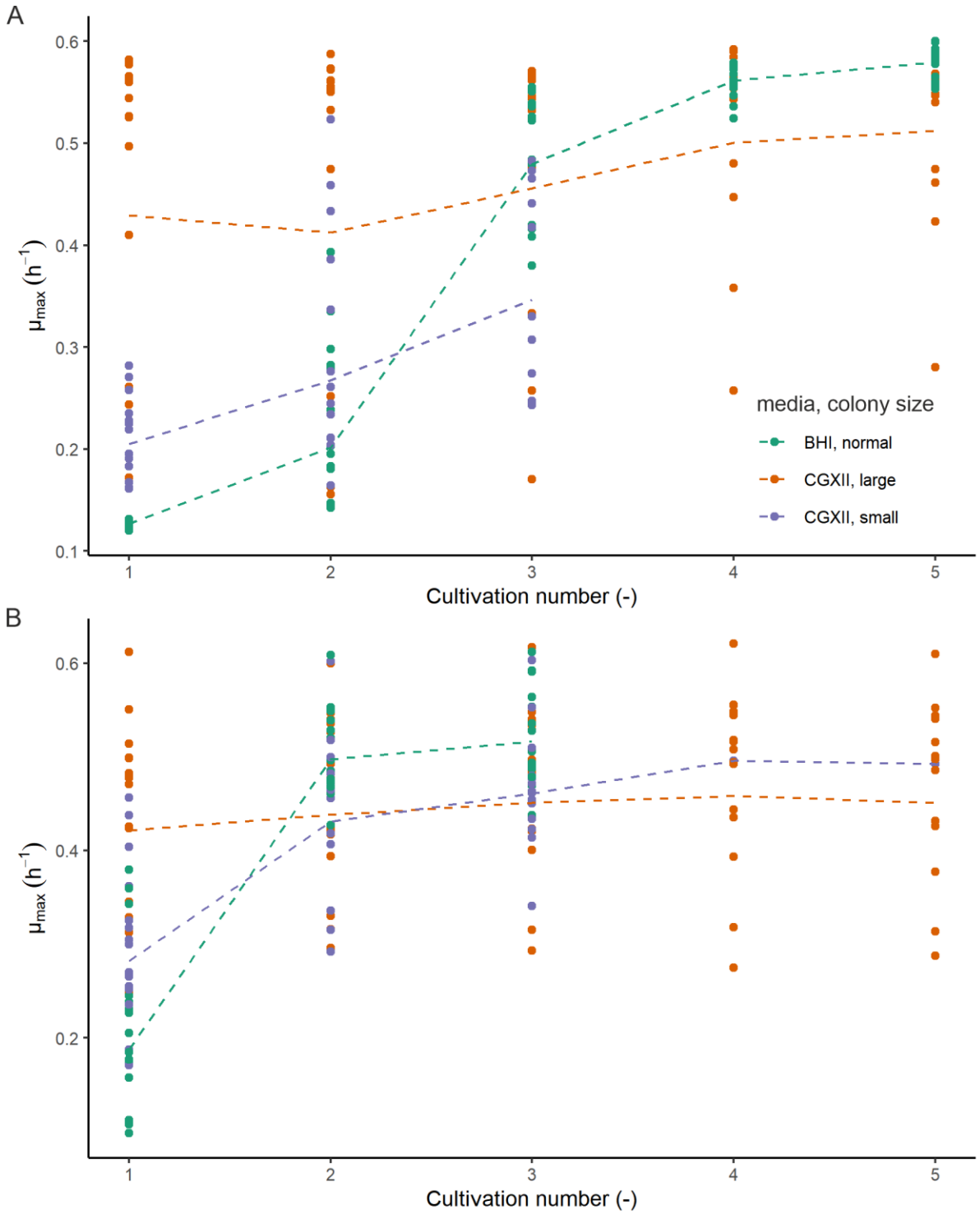

Figure S8

Growth rates of *C. glutamicum*::P_{brnF}-*pfkA* and P_{brnF}-*hisD* cultures from plate-based evolutions, covering 15 cultures started from large colonies on CGXII plates, 15 cultures from small colonies on CGXII plates, and 15 cultures from normal colonies on BHI plates, for P_{brnF}-*hisD* (A) and P_{brnF}-*pfkA* (B). Dots denote specific growth rates (μ_{max}) of each independent culture per repetitive batch, the line represents average per culture per strain. Supplementary information to the results shown in Figure 5B.

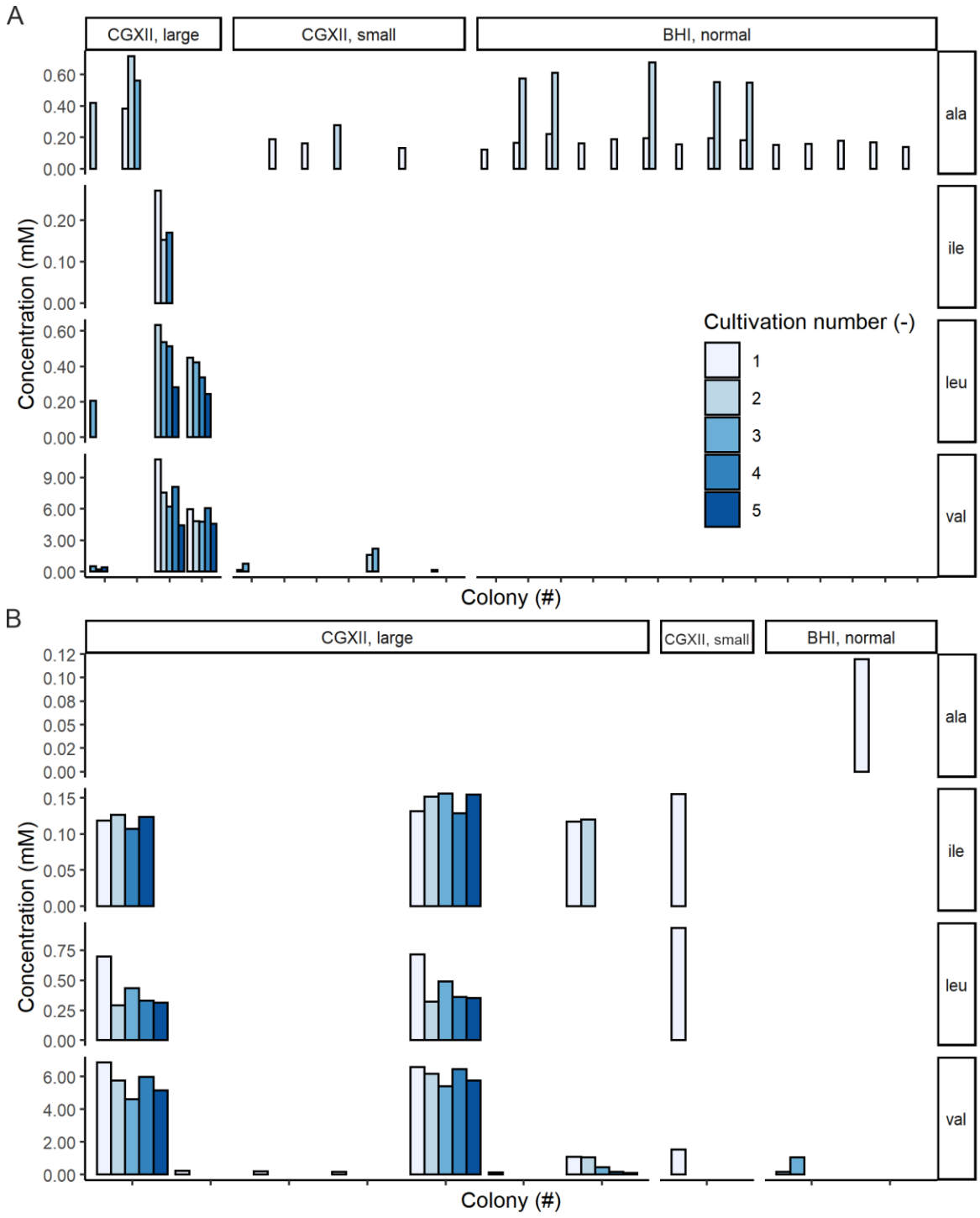

Figure S9

Amico acid production of *C. glutamicum*::P_{brnF}-*pfkA* and P_{brnF}-*hisD* cultures from plate-based evolutions, started from large colonies on CGXII plates, from small colonies on CGXII plates, and from normal colonies on BHI plates, for P_{brnF}-*hisD* (A) and P_{brnF}-*pfkA* (B). Fifteen colonies were picked for each plate-strain combination. Results are shown for clones that produced detectable amounts of amino acid (>0.1mM) in at least one repetitive culture. Color scales indicate results for a maximum of five repetitive cultures. Supplementary information to the results shown in Figure 5B.

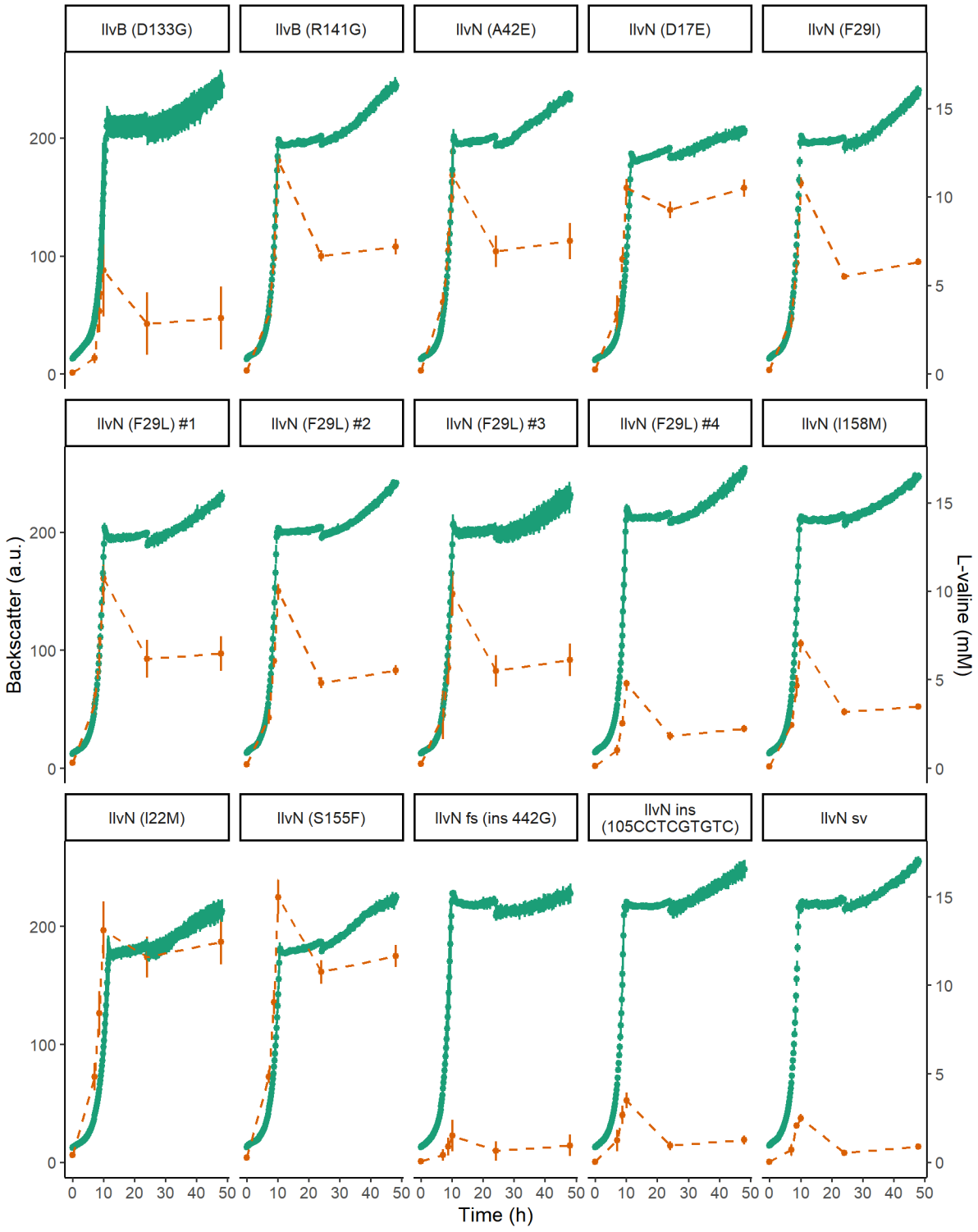

Figure S10

Growth and L-valine production of 15 L-valine producer mutants. Mean values and standard deviations of three biological replicates are shown; *fs*: frameshift mutation, *sv*: structural variant, *ins*: insertion. Supplementary information to the results shown in Figure 6C.

Literature

Baumgart, M., Luder, K., Grover, S., Gätgens, C., Besra, G.S., Frunzke, J., 2013. IpsA, a novel LacI-type regulator, is required for inositol-derived lipid formation in *Corynebacteria* and *Mycobacteria*. BMC Biol. 11, 1–14. https://doi.org/10.1186/1741-7007-11-122

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Schäfer, A., Tauch, A., Jäger, W., Kalinowski, J., Thierbach, G., 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. https://doi.org/10.1016/0378-1119(94)90324-7