Supplementary material

Engineering a Hybrid System of *Corynebacterium glutamicum* and Co-Immobilized Enzyme for Efficient Cadaverine Production from Glycerol

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Supplementary Figure S1-S8

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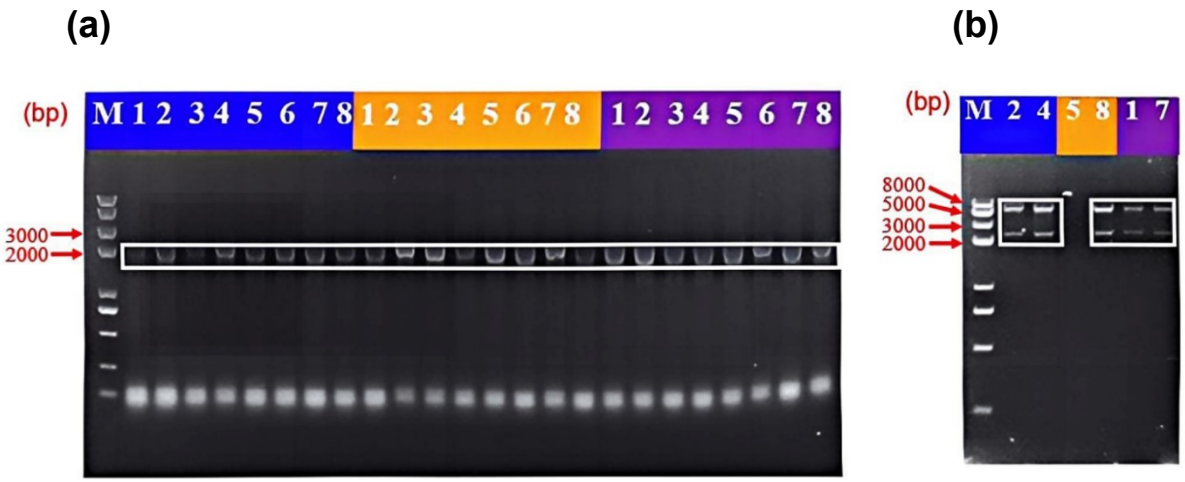

Fig. S1. The Colony PCR (a) and digestion verification (Digested by Bgl II and BamH I) (b) of N-10EK, B-10EK and C-10EK (Blue areas: N-10EK; Orange area: B-10EK; Purple area: C-10EK; the numbers indicated different samples)

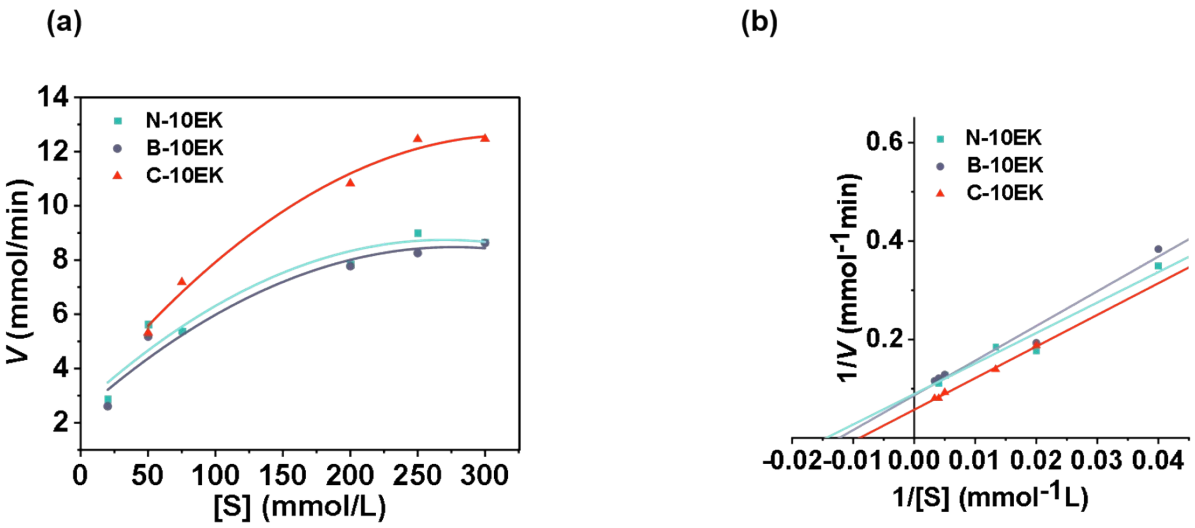

Fig. S2 The Michealis equation curve of N-10EK, B-10EK and C-10EK (a) Michaelis-Menten model (b) Lineweaver-Burk model

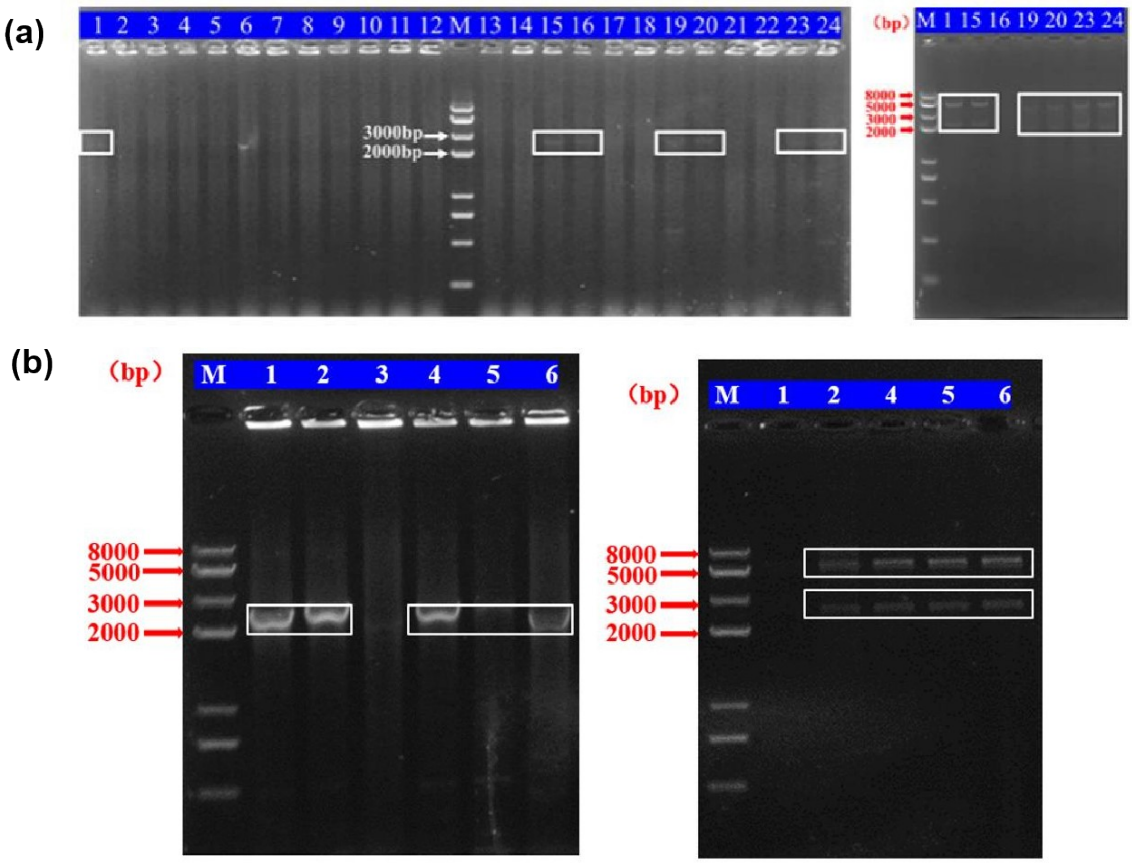

Fig. S3 The Colony PCR and digestion verification of C-30EK. (a) (Different numbers indicate different colony samples). (b) The Colony PCR and digestion verification of C-50EK (Digested by Bgl II and BamH I)

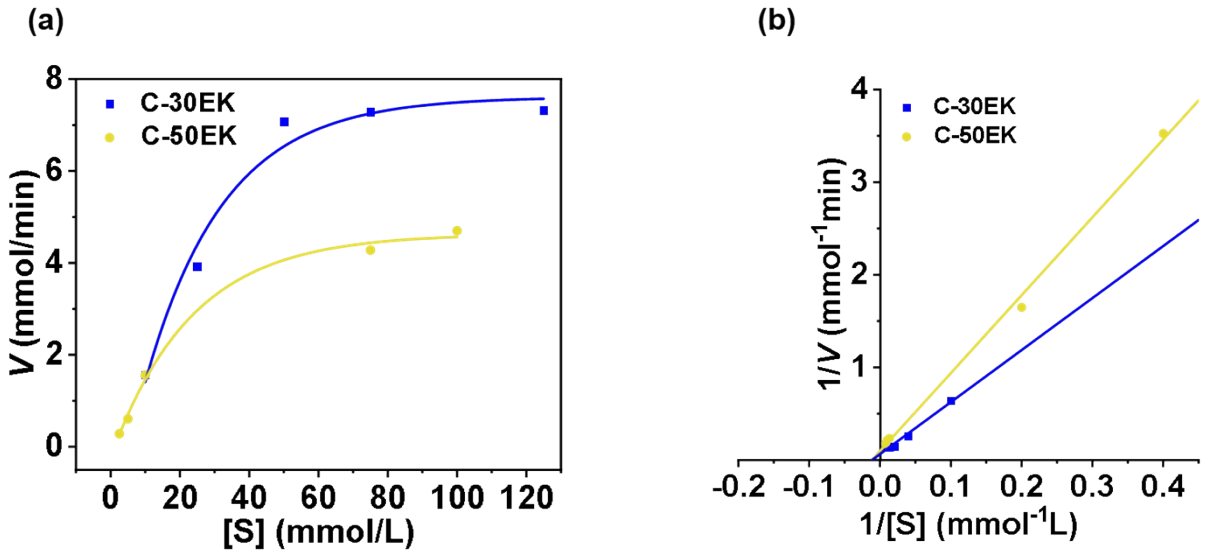

Fig. S4 The Michealis equation curve of C-30EK and C-50EK (a) Michaelis-Menten model (b) Lineweaver-Burk model

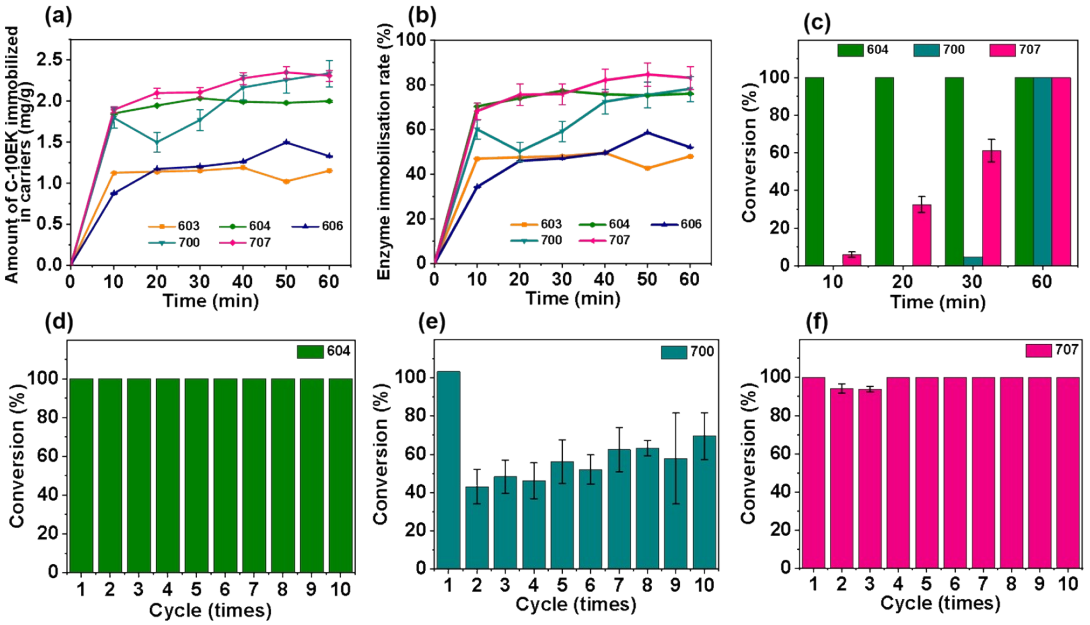

Fig. S5 Lysine decarboxylase immobilization amount (a) and immobilization rate (b) of five kinds of resins Catalytic efficiency (c) and cyclic stability (d, e, f) of the three immobilized enzymes

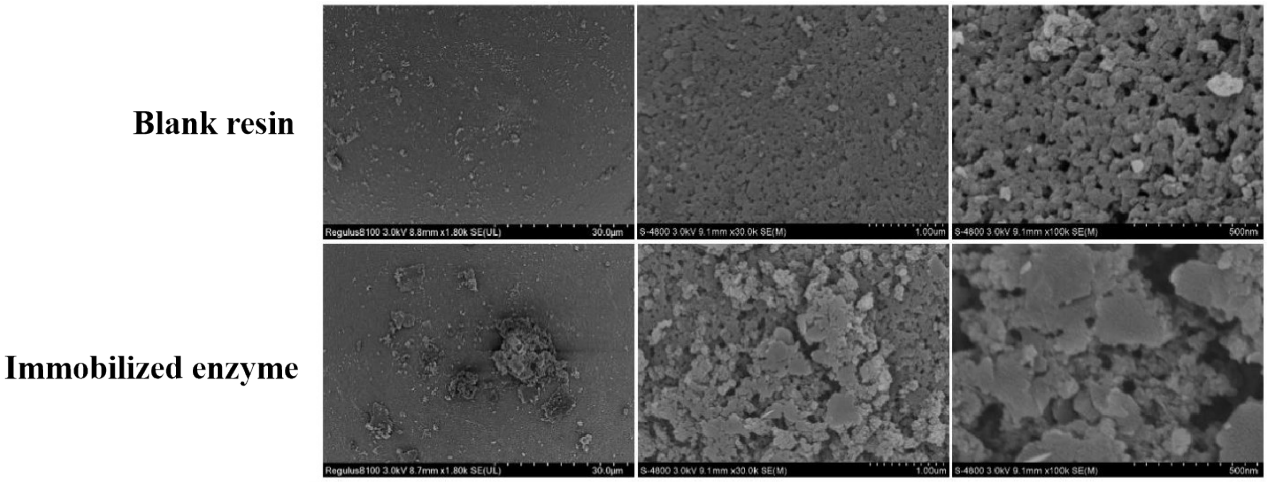

Fig. S6 SEM images of blank resin material, immobilized material and immobilized enzyme at different magnifications

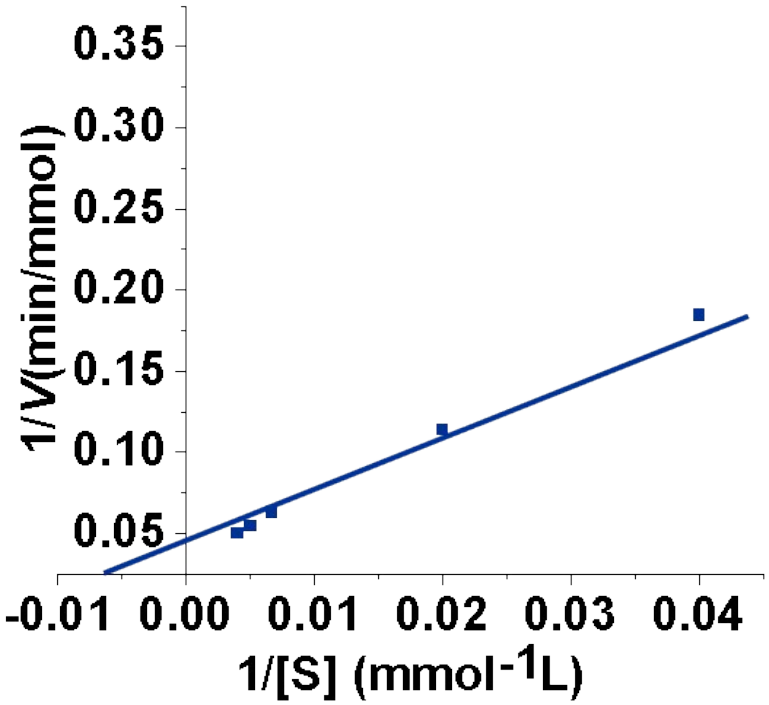

Fig. S7 The Michealis equation curve of ER604-10EK-PLP (Lineweaver-Burk model)

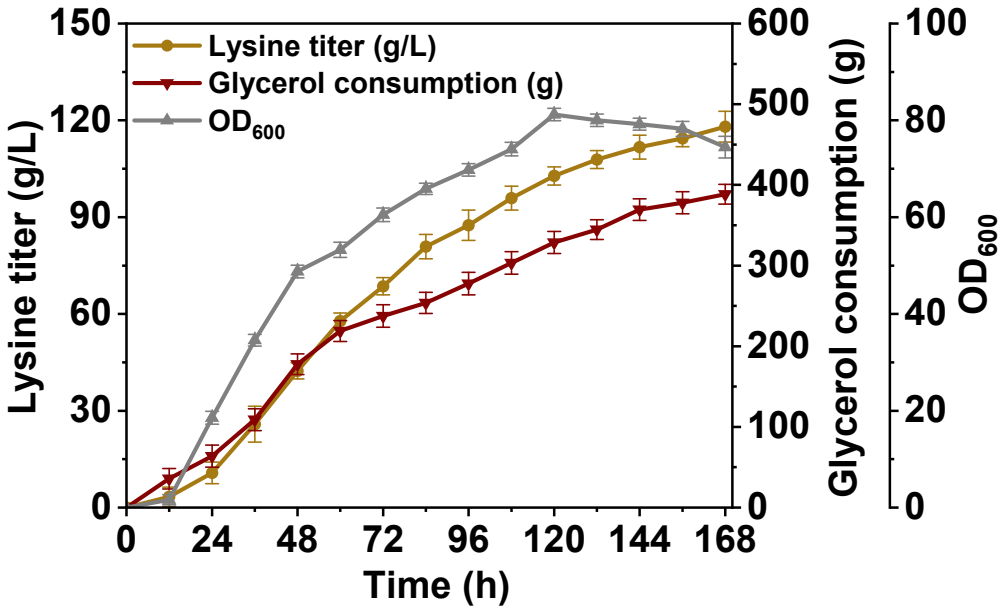

Fig. S8 Fed-batch fermentation profiles cgl-FDK-VIII in a 5 L bioreactor. Time profiles of OD_{600}, glycerol consumption, lysine yield were shown during the fed-batch cultivation. Values were shown as mean ± SD (n = 3)

Table S1 The strains used in this study

Strains	Relevant characteristics/ genotype	Sources
E.coli DH5α	Host for construction of plasmids	This study
BL21（DE3）	F^- ompT hsdS (rB- , mB-) gal dcm (DE3)	This study
CadA-EK	BL21 with pET-32a(+)-cadA-EK	This study
C-10EK	BL21 with pET-28a(+)-cadA-EK	This study
N-10EK	BL21 with pET-28a(+)-EK-cadA	This study
B-10EK	BL21 with pET-28a(+)-EK-cadA-EK	This study
C-30EK	BL21 with pET-28a(+)-cadA-30EK	This study
C-50EK	BL21 with pET-28a(+)-cadA-50EK	This study
C. glutamicum ATCC 13032	Wild-type, the parent strain	ATCC
CgGly 2	Wild type containing optimized glycerol-utilization pathway	(Wei et al, 2022)[1]
LYS1	CgGly 2; P_{sod}-$lysC^{FBR}$	This study
LYS2	LYS1; Δldh::Ptac-ddh	This study
LYS3	LYS2; ΔalaT::Ptac-lysA	This study
LYS4	LYS3; P_{sod}-PYC	This study
LYS5	LYS4; P_{gltA}-Riboswitch-gltA	This study
LYS6	LYS5; Δpck::P_{tac}-SNgapN	This study
LYS7	LYS6; ΔpoxB::P_{tac}-PntAB	This study
LYS8	LYS7; ΔavtA::P_{sod}-TmASDH	This study
LYS9	LYS8; ΔavtA::P_{sod}-EcdapB	This study
LYS10	LYS9; ΔavtA::P_{sod}-$CgDdh^{Y11K/R36E}$	This study

Table S2 The plasmids used in this study

Plasmids	Characteristics	Sources
pEFDK	Kan^R, pEC-XK99E derivate with genes *glpF*,	(Wei et al,

	dhaD, *dhaK* overexpression	2022)[1]
pK18mobsacB	*Kan*^{R}, gene deletion plasmid	Lab Collection
pK18-lysC^{FBR}	pK18mobsacB; introducing the site mutant (T311I) to removing feedback inhibition	This study
PK18-P$_{sod}$-lysC	pK18mobsacB; replacing the strong Psod promoter and lysC^{FBR}	This study
PK18-Δldh-ddh	pK18mobsacB; expressing *ddh* gene using Ptac promoter at *ldh* locus	This study
pK18-ΔalaT-lysA	pK18mobsacB; expressing *lysA* gene using Ptac promoter at *alaT* locus	This study
pK18-P$_{sod}$-PYC	pK18mobsacB; replacing the strong P$_{sod}$ promoter	This study
pK18-ECRS-gltA	pK18mobsacB; inserting the lysine riboswitch from E. coli into the upstream of *gltA*	This study
pK18-Δpck-SmgapN	pK18mobsacB; expressing *gapN* from *S. mutans* using *Ptac* promoter at *pck* locus	This study
pK18-ΔpoxB-PntAB	pK18mobsacB; expressing *PntAB* from *E. coli* using *Ptac* promoter at *poxB* locus	This study
pK18-TmASDH	pK18mobsacB; expressing *asd* from *T. mobilis* using P$_{sod}$ promoter at *avtA* locus	This study
pK18-EcDapB	pK18mobsacB; expressing *DapB* from *E. coli* under the downstream of TmASDH	This study
pK18-CgDDHm	pK18mobsacB; expressing DDH$^{Y11K/R36E}$ gene from *C. glutamicum* using downstream of *EcdapB*	This study

Table S3 Primer used in this study

Primer	sequence (5′ →3′)

pK18-F	ggatccccgggtaccgagctcgaattcg
pK18-R	tctagagtcgacctgcaggcatgcaagcttg
lysC1-F	caagcttgcatgcctgcaggtcgactctagacccaaactgaaggcaacaattgg
lysC1-R	cccgctcaactctacctttataaactg
Psod-lysC-F	cagtttataaaggtagagttgagcgggaagccttatgcccttcaaccctac
Psod-lysC-R	gggtaaaaaatcctttcgtaggtttccgc
lysC2-F	gcggaaacctacgaaaggatttttttacccatggccctggtcgtacagaaatatg
lysC2-R	cgaattcgagctcggtacccggggatccgccaacgcacggaaaaccttcgcagc
lysCm1-F	caagcttgcatgcctgcaggtcgactctagaatatggcggttcctcgcttgagagtg
lysCm1-R	tgatgtcggtggtgccgtcttctacag
lysCm2-F	ctgtagaagacggcaccaccgacatcatcttcacctgccctcgttccgacg
lysCm2-R	cgaattcgagctcggtacccggggatcctctgctcttcatcggtttcgaaggtgc
ldh-1F	caagcttgcatgcctgcaggtcgactctagagcgcagggctcccgtgcggcgcatttc
ldh-1R	cacacattatacgagccgatgattaattgtcaacagctcatttcagaatatttgccagaaccg gcaagagcttgtgcccggattcctttg
ddh-F	tgacaattaatcatcggctcgtataatgtgtggaattgtgtttaactttaagaaggagatataca tatgaccaacatccgcgtagctatc
ddh-R	ttagacgtcgcgtgcgatcagatcgtccaag
ldh-2F	cttggacgatctgatcgcacgcgacgtctaacattccgcaaataccctgcgcgaaattcag
ldh-2R	cgaattcgagctcggtacccggggatcctgccgtagacccgggagcggtcgtag
alaT-1F	caagcttgcatgcctgcaggtcgactctagaacacctcatccggtcagtaccacctc
alaT-1R	cacacattatacgagccgatgattaattgtcaacagctcatttcagaatatttgccagaaccg ctagctctaaaagggtgtaatcccacg
lysA-F	aattaatcatcggctcgtataatgtgtggaattgtgtttaactttaagaaggagatatacatatg gctacagttgaaaatttcaatgaac
lysA-R	ttatgcctctagtgagaggatgtcgtcgagcg
alaT-2F	gctcgacgacatcctctcactagaggcataagccacatcacgatcacttccgagtggtcac
alaT-2R	cgaattcgagctcggtacccggggatccatcgtgtgccttgtcctacgactttgatttc
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pyc-1R	tagagtaattattcctttcaacaagagac
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glta-2F	ctgaaaatggatcccctgacacgaggtagttatgtttgaaagggatatcgtggctac
glta-2R	cgaattcgagctcggtacccggggatccgcgtgggcggttgatcttgttgcctgc
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PCK-1R	acacattatacgagccgatgattaattgtcaacagctcatttcagaatatttctattttgggggt ggtttcaagaattaacc
SmgapN-F	aattaatcatcggctcgtataatgtgtggaattgaactttaagaaggagatatacatatgacaa

	aacaatataaaaattatgtcaatggc
SmgapN-R	ttatttgatatcaaatacgacggatttaacagttgtc
PCK-2F	ctgttaaatccgtcgtatttgatatcaaataagtcaaggaagcactgaccgctcctg
PCK-2R	cgaattcgagctcggtacccggggatccccaccattttgcgatggatgaacggc
POXB-1F	caagcttgcatgcctgcaggtcgactctagagattccttgatcgtgatgatcaac
POXB-1R	taaagttcaattccacacattatacgagccgatgattaattgtcaacagctcatttcagaatatt tgtactcggtaccaaacagggatgc
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PntAB-R	ttacagagctttcaggattgcatccacgctgcagctttgtatagttcatccatgccatgtg
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avtA-2R	cgaattcgagctcggtacccggggatcccatgagacgaatggtgtgggacag
TmASD-1F	caagcttgcatgcctgcaggtcgactctagaatgcgcatcggcatcgttggagcc
TmASD-1R	ttataccaaaagctctgcgatctgcacggtgttggtttgcagctttgtatagttcatccatgcca tgtg
EcDapB-F	caacaccgtgcagatcgcagagcttttggtataactagagaaaggaggacaaccatgcat gatgcaaacatccgcgttgc
EcDapB-R	ttacaaattattgagatcaagtacatctcgcatatc
avtA-3F	gatatgcgagatgtacttgatctcaataatttgtaagtggctgataaaaagaagatcgcaaac gtcctg
ECdapB-1F	caagcttgcatgcctgcaggtcgactctagaatgcatgatgcaaacatccgcgttg
ECdapB-1R	ttacaaattattgagatcaagtacatctcgc
Cgddh-F	gatatgcgagatgtacttgatctcaataatttgtaaatgaccaacatccgcgtagctatcgtg ggcaagggaaacctgggacgcag
Cgddh-R	ttagacgtcgcgtgcgatcagatcgtc
avtA-4F	ggacgatctgatcgcacgcgacgtctaagtggctgataaaaagaagatcgcaaacg
Ddh-R36E-1R	gactggcgtctttgtgtcgagggtggcccgctccgagaagattcctac
Ddh-R36E-2F	gagcgggccaccctcgacacaaagacgccagtctttg

Table S4 The specific enzyme activities of dehydrogenases in the engineered strains

		Specific enzyme activities (mU/mg protein)			
	Cofactor	LYS7	LYS8	LYS9	LYS10
ASDH	NADPH	189.7 ± 12.52	257.5 ± 21.3	242.5 ± 22.4	235.9 ± 18.5
	NADH	25.8 ± 2.45	103.2 ± 5.78	98.4 ± 5.22	95.2 ± 4.43
DHDPR	NADPH	121.5 ± 13.56	118.7 ± 10.32	168.2 ± 8.75	177.4 ± 12.12
	NADH	18.8 ± 2.45	15.5 ± 1.33	85.8 ± 3.87	77.6 ± 1.35
DDH	NADPH	2359.8 ± 189.6	2238.8 ± 257.9	2278.9 ± 123.2	2956.7 ± 215.7
	NADH	35.7 ± 2.56	28.5 ± 3.12	32.6 ± 3.58	249.9 ± 15.49

Table S5 Summary of Microbial Fermentation Methods and Yield for Cadaverine Production

Product	Strains	Carbon source	Titer /g·L^{-1}	Yield /g·g^{-1}	Productivity / g (L^{-1}·h^{-1})	Scale	Ref.
1,5-DAP	*E.coli* XQ56 (p15CadA)	Glucose	9.61	0.12	0.32	5L bioreactor	2
	E.coli XQ56 (p15CadA) anti murE	Glucose	12.6	-	0.18	5L bioreactor	3
	C.glutamicum TM45	Glucose	2.6	0.14	0.05	Shake flask	4
	C.glutamicum DAP-3c	Glucose	30.6	-	-	Shake flask	5
	C.glutamicum DAP-16	Glucose	88	0.29	2.2	5L bioreactor	6
	C.glutamicum CDV-2	Glucose	2.7	-	0.07	Shake flask	7
	C.glutamicum KCTC 1857/ pCES208H30ECLdcC	Glucose	40.91	0.21	0.62	5L bioreactor	8
	C.glutamicum G-H30	Glucose	103.7	0.30	1.47	5L bioreactor	9
	C.glutamicum Δ*ald*Δ*fadH*	Glucose	1.5	-	-	Shake flask	10
	C. glutamicum DAP-Xyl1	xylose	1.42	0.11	0.044	Shake flask	11
	C. glutamicum DAP-Xyl2	xylose	103	0.218	1.37	5L bioreactor	6
	C. glutamicum Δ*ald*Δ*fadH*	Methanol	1.5	-	-	Shake flask	10
	C. glutamicum cgl-FDK and E.coli BL-ABST-Spy	Glycerol	9.3	0.2	0.07	5L bioreactor	12
	C.glutamicum LYS10 with ER604-10EK-PLP	Glycerol	90.7	0.2	0.54	5L bioreactor	This study

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