Supplementary Materials:

Table S1. Primers used in this study.

Primer	Sequence (5′->3′)
ecgadPcat1-F	GAAACAGCTATGACCGCGGCCGCGTAGACTTTAAGGATGGAACCTTTGA
ecgadPcat1-R	CTTATCCATAAAAACCACCCTTTCATAAATTATATAAA
ecgadB-F	GGGTGGTTTTTATGGATAAGAAGCAAGTAACGGATT
ecgadB-R	GACGTCGACTCTAGAGGATCCTCAGGTGTGTTTAAAGCTGTTCTGC
lcgadPcat1-F	GAAACAGCTATGACCGCGGCCGCGTAGACTTTAAGGATGGAACCTTTGA
lcgadPcat1-R	CGTATAACATAAAAACCACCCTTTCATAAATTATATAAA
lcgad-F	GGTGGTTTTTATGTTATACGGAAAAGAAAATCGCG
lcgad-R	GACGTCGACTCTAGAGGATCCTTAGTGAGTAAAGCCATAAGTTTTATTTTC
lpgadPcat1-F	GAAACAGCTATGACCGCGGCCGCGTAGACTTTAAGGATGGAACCTTTGA
lpgadPcat1-R	CGTATAACATAAAAACCACCCTTTCATAAATTATATAAA
lpgad-F	GGTGGTTTTTATGTTATACGGTAAACACAATCATGAAG
lpgad-R	GACGTCGACTCTAGAGGATCCTCAGTGTGTGAATCCGTATTTCTTAG
BS2Pcat1-F	GAAACAGCTATGACCGCGGCCGCGTAGACTTTAAGGATGGAACCTTTGA
BS2Pcat1-R	ACTTGCCATAAAAACCACCCTTTCATAAATTATATAAA
1BS2-F	GGGTGGTTTTTATGGCAAGTTTTCAAAGTTTTGG
1BS2-R	CCATGGACGCGTGACGTCGACTTATTCAAGAAGCTTTTCATATTCTTTT
BS2Pfla-F	GAAACAGCTATGACCGCGGCCGCTATTTAATAATTATTATTGAATAATTTTATTTTTGT
BS2Pfla-R	TTGAAAACTTGCCATTTTAACTCCTCCTCAACACTAAATAATTAT
2BS2-F	TGAGGAGGAGTTAAAATGGCAAGTTTTCAAAGTTTTGG
2BS2-R	CCATGGACGCGTGACGTCGACTTATTCAAGAAGCTTTTCATATTCTTTT
BS2Pcathl-F	GAAACAGCTATGACCGCGGCCGCTTTTTAACAAAATATATTGATAAAAATAATAATAGTGG
BS2Pcathl-R	TTGAAAACTTGCCATTCTAACTAACCTCCTAAATTTTGATACG
3BS2-F	AGGAGGTTAGTTAGAATGGCAAGTTTTCAAAGTTTTGG
3BS2-R	CCATGGACGCGTGACGTCGACTTATTCAAGAAGCTTTTCATATTCTTTT
grpE-QF	GATGTAGAAGAAATTCCTGCA
grpE-QR	ATTTGCCACTTTAACCATACTGT
dnaK-QF	AGGGGGTTAATCCAGATGAGTGTG
dnaK-QR	CAAGAGTAAGTGGTGTAACATCAAG
dnaJ-QF	GAAGCCTATCAGGTTTTATCGG
dnaJ-QR	GCTCCACCAGCACCACTAAAATCAG
groES-QF	GCTGCTAAAGAAAAACCACAGGAAG
groES-QR	CTCTACACCATCAATCTTTAC
groEL-QF	GGCAAAGAGCATTTTATTCGGTG
groEL-QR	CTTCCCTTTGGTCCAAGTGTAA
htpG-QF	AAGCATTTCAGGAGGATAAGG
htpG-QR	CATCATTGGCAAATTGCACTGG
GTP-binding gene-QF	CAATAATTGCCCATGTAGATCACGG
GTP-binding	GAGTCCATAACCCTCTCTTGAAC

gene-QR	
PRO-F	TGAAGTACATCACCGACGAGCAAG
PRO-R	TGCTGCAAGGCGATTAAGTTGGGT
Pcat1(groESL)-F	TAAGGATCCTCTAGAGTCGACGTAGACTTTAAGGATGGAACCTTTGA
Pcat1(groESL)-R	TTCATAAAAACCACCCTTTCATAAATTATATAAA
groESL-F	GAAAGGGTGGTTTTTATGAATATTAGACCACTTGGAGACAGA
groESL-R	CAGGCCTCGAGATCTCCATGGTTAATACATTCCTTCCATTCCGC

Table S2. Strains and plasmids used in this study.

Strain/plasmid	Relevant characteristics	Reference/source
Strains		
C. tyrobutyricum	ATCC 25755	ATCC
Ct-pMA01	ATCC 25755 with pMA01	This study
Ct-pMA02	ATCC 25755 with pMA02	This study
Ct-pMB01	ATCC 25755 with pMB01	This study
Ct-pMAG	ATCC 25755 with pMAG	This study
E. coli CA434	E. coli HB101 with plasmid R702	[1]
Plasmids		
pMTL82151	ColE1 ori; Cm^R; pBP1 ori; Tarj	[2]
pMA01	From pMTL82151; Pcat1-*ecgadB*	This study
pMA02	From pMTL82151; Pcat1-*lcgadB*	This study
pMB01	From pMTL82151; Pcat1-*lpgadB*	This study
pMAG	From pMTL82151; Pcat1-*lcgadB*-Pcat1-*groESL*	This study

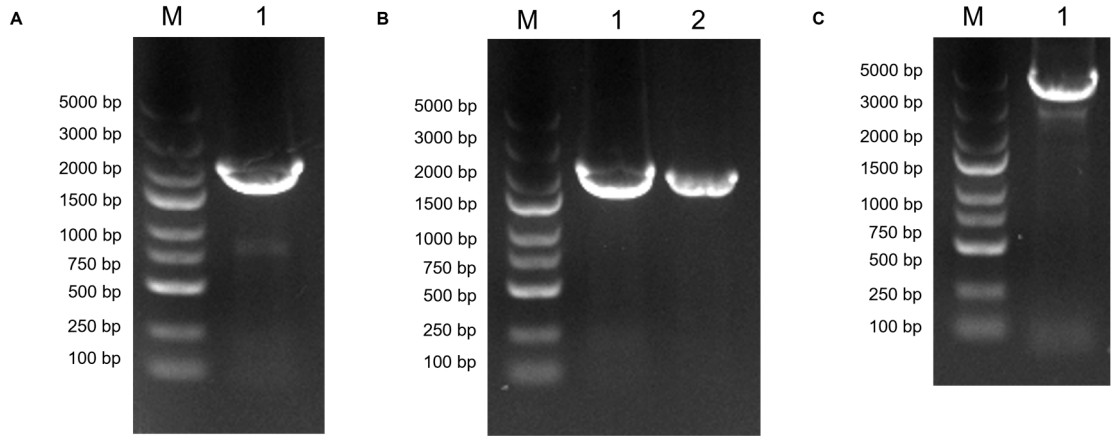

Figure S1. Electrophoresis results of colony PCR for recombinant strains. (**A**) M: DNA Marker; 1: Ct-pMA01; (**B**) M: DNA Marker; 1: Ct-pMA02; 2: Ct-pMB01; (**C**) M: DNA Marker; 1: Ct-pMAG

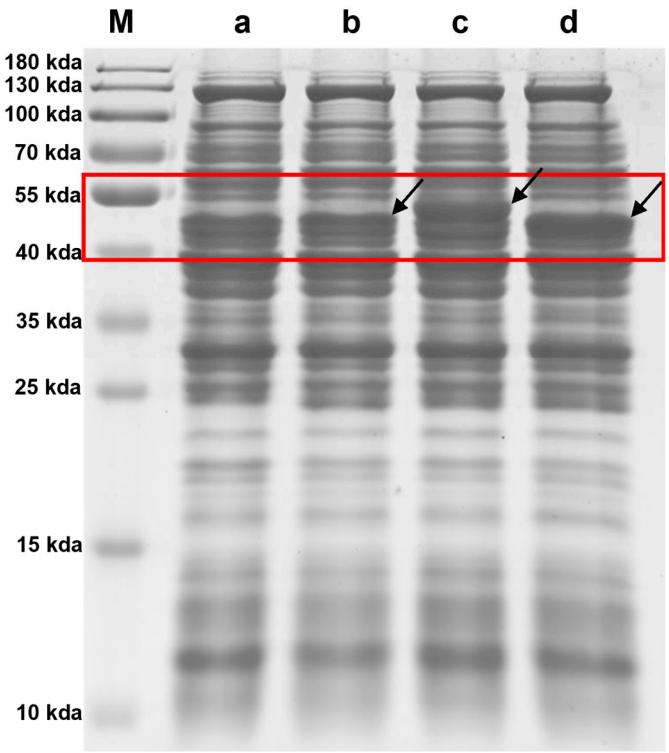

Figure S2. SDS-PAGE of cell extracts prepared by *C. tyrobutyricum* wild type and engineered strains. **M** contained molecular mass marker proteins. **a**, **b**, **c** and **d** were wild type, Ct-pMA01 (EcgadB 52.7 kDa), Ct-pMA02 (LcgadB, 54.0 kDa) and Ct-pMB01 (LpgadB, 53.4 kDa), respectively. Arrows and box indicated the location of the bands.

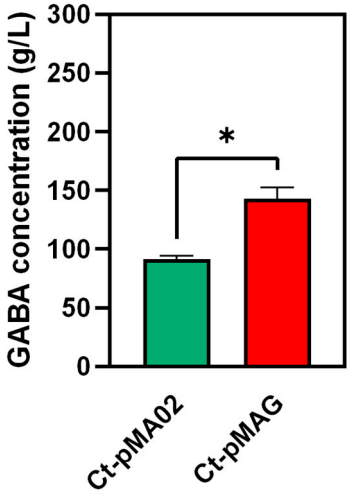

Figure S3. Profiles of L-Glu bioconversion by Ct-pMA02 and Ct-pMAG. Cells were harvested by centrifugation at 12,000 rpm for 1 min. The cell pellets were washed by PBS and then resuspended to

OD_{600}=5 in ddH_2O in the flash tank with a working volume of 50 mL. Bioconversion was carried out at 37 °C, 150 rpm with 3 mol/L L-Glu. GABA production was determined by collecting samples at 12 h. (*p < 0.05, Student's t-test).

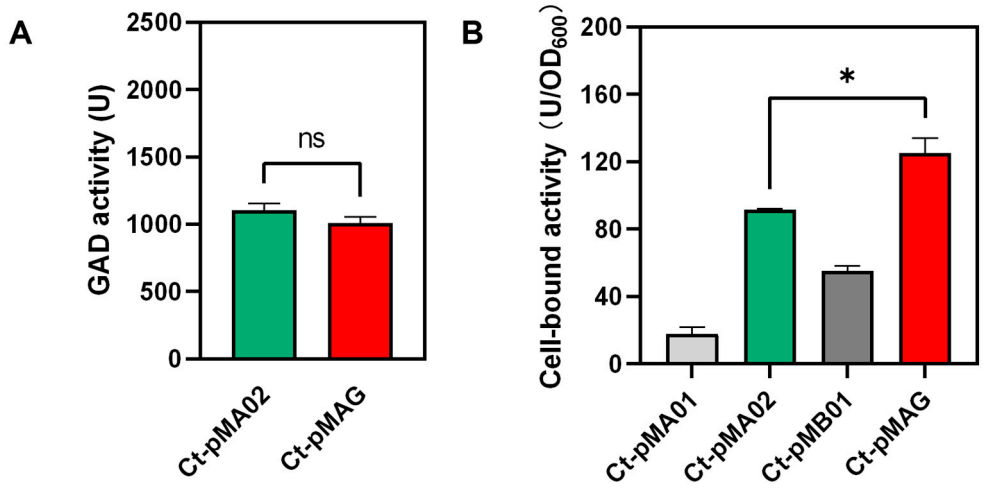

Figure S4. (**A**) Comparison of GAD activity between Ct-pMA02 and Ct-pMAG. (**B**) Profiles of Cell-bound activity of different engineered *C. tyrobutyricum*. GAD activity and Cell-bound activity were determined by collecting samples at stationary phase. Statistically significant differences (ns indicated no significance, *p < 0.05, Student's t-test) were indicated by asterisks.

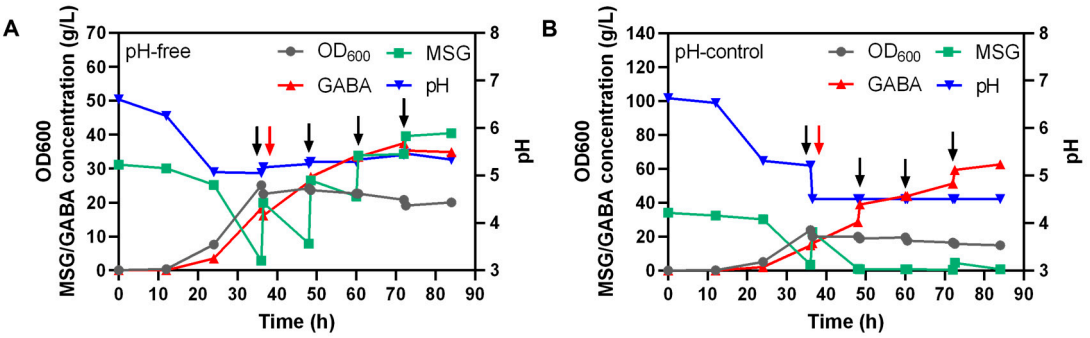

Figure S5. Fed-batch fermentation of Ct-pMAG in a 5 L bioreactor at 37 °C for 84 h. MSG and glucose were supplied during the period. (**A**) Fermentation using pH-free strategy, with 4 times 50 mL MSG aliquot (600 g/L) added. (**B**) Fermentation using pH-control strategy, with 4 times 50 mL MSG aliquot (600 g/L) added. Black arrow indicated MSG feeding moment and red arrow indicated 60 g/L glucose feeding moment.

1. Williams, D.R.; Young, D.I.; Young, M. Conjugative plasmid transfer from Escherichia coli to Clostridium acetobutylicum. *Journal of general microbiology* **1990**, *136*, 819-826, doi:10.1099/00221287-136-5-819.
2. Heap, J.T.; Pennington, O.J.; Cartman, S.T.; Minton, N.P. A modular system for Clostridium shuttle plasmids. *Journal of Microbiological Methods* **2009**, *78*, 79-85, doi:https://doi.org/10.1016/j.mimet.2009.05.004.