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Supplemental Methods

Construction and complementation of markerless in-frame deletions

The *Δcg1360* mutant was constructed as follows: the *cg1360* upstream flanking region containing the first 14 codons of *cg1360* gene, and the *cg1360* downstream flanking region containing the last 9 codons of *cg1360* gene, were amplified with the Phusion high-fidelity DNA polymerase (Thermo Scientific, USA) using the primer pairs cg1360-1-For/cg1360-2-Rev and cg1360-3-For/cg1360-4-Rev, respectively. These two fragments were then fused by overlap extension PCR using the primer pairs cg1360-1-For/cg1360-4-Rev. The final PCR product was digested with *Bam*HI and *Xba*I, ligated into the same sites of pCRD206 vector, and directly transformed into *E. coli* DH5α host cells to yield the pCRD206-*cg1360* plasmid. The resulting plasmid was then transformed into *C. glutamicum* ATCC 13032 by the electroporation method. The *Δcg1360* deletion mutant was obtained through the first temperature selection and the second sucrose selection steps, and confirmed by colony PCR with the primer pairs cg1360-UF/cg1360-DR. The corrected *Δcg1360* deletion contains an in-frame deletion of 127 aa of open-reading frame (ORF). Additionally, the *Δcg1361* mutant containing an in-frame deletion of 207 base pairs of the coding region (Equivalent to 69 amino acids) was generated according to a similar strategy.

The *cg1360* complementation strain (shown as *Δcg1360*+*cg1360*) was constructed as follows: the *cg1360*comp upstream flanking region and the *cg1360*comp downstream flanking region were amplified with the Phusion high-fidelity DNA polymerase (Thermo Scientific, USA) using the primer pairs cg1360-1-

For/cg1360comp-Rev and cg1360comp-For/cg1360-4-Rev, respectively. These two fragments were then fused by overlap extension PCR using the primer pairs cg1360-1-For/cg1360-4-Rev to obtain the *cg1360* full-length complementation fragment with a synonymous point mutation at the Glu-4 site. The final PCR product was digested with *Bam*HI and *Xba*I, ligated into the same sites of pCRD206 vector, and directly transformed into *E. coli* DH5α host cells to yield the pCRD206-*cg1360comp* plasmid. The resulting plasmid was then transformed into the *Δcg1360* mutant by the electroporation method. The *cg1360* complementation strain was obtained through the first temperature selection and the second sucrose selection steps, and confirmed by colony PCR with the primer pairs cg1360-UF/cg1360-DR. The correct *cg1360* complementation construct contains a synonymous point mutation at the Glu-4 site (GAA to GAG) of *cg1360* gene, and confirmed by DNA sequencing. Additionally, the *cg1361* complementation strain (shown as *Δcg1361+cg1361*) containing a synonymous point mutation at the Leu-4 site (CTG to CTC) of *cg1361* gene was generated by a similar strategy.

Supplemental Figures:

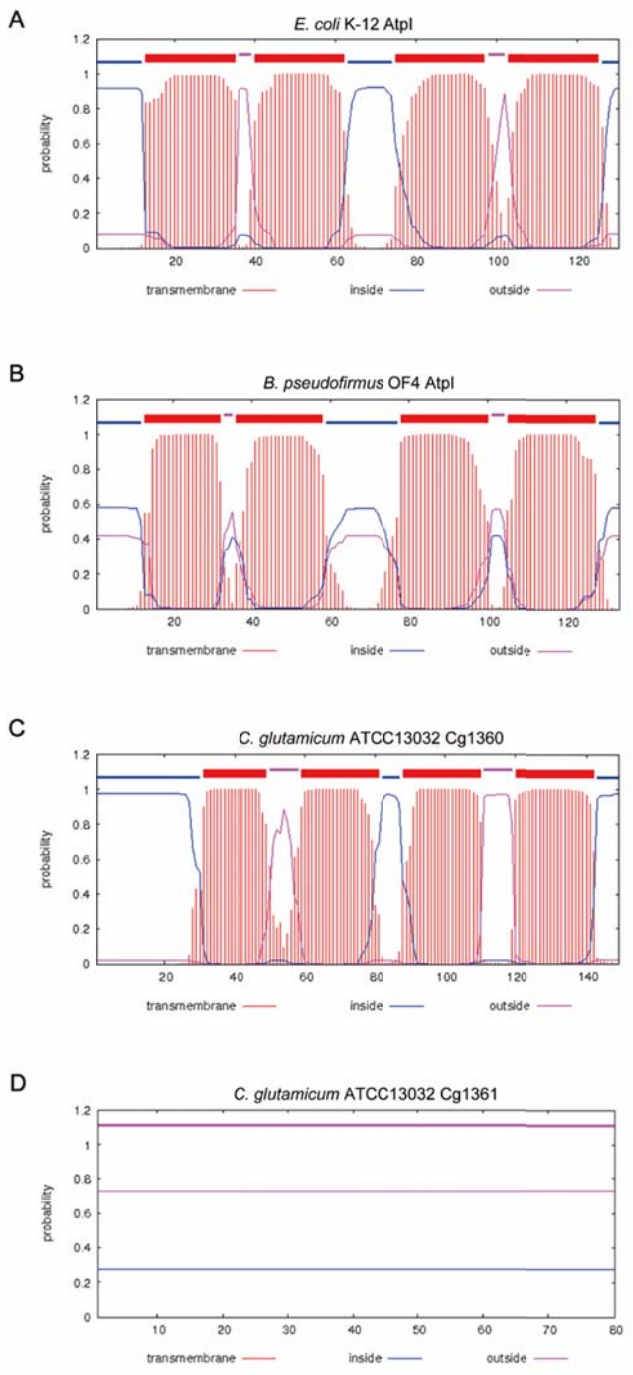

Fig. S1. Transmembrane region analysis of AtpI-like protein from *E. coli* K-12 (A), *B. pseudofirmus* OF4 (B) and *C. glutamicum* ATCC 13032 (C and D). TMHs were predicted by TMHMM version 2.0 server.

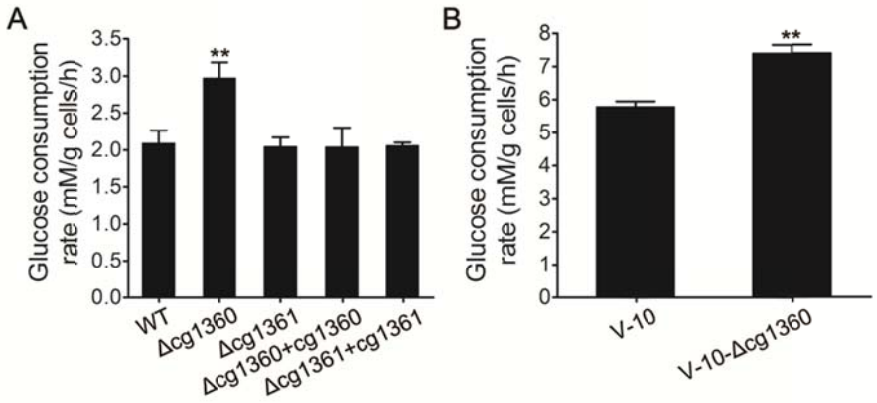

Fig. S2. Effects of *cg1360* or *cg1361* deletion on glucose consumption. (A) *C. glutamicum* WT and its mutant derivatives were grown in CGXII minimal medium containing 4% (w/v) glucose as a carbon source. Glucose consumption rate of cells during the exponential growth phase after 8 to 16 hours of incubation was determined. (B) *C. glutamicum* V-10 and its *cg1360* deletion mutant were grown in fermentation medium containing 125 g/l glucose as a carbon source. Glucose consumption rate of cells during the exponential growth phase after 18 to 30 hours of incubation was determined. Specific glucose consumption rate was calculated from the glucose consumption and biomass concentration using the formula: [decreased glucose mM] [increased dry cell (g/l)]$^{-1}$ h^{-1}. Asterisks indicate significant differences between *C. glutamicum* wild-type and its mutant derivatives according to a statistically Student's t test (**, $P < 0.01$).

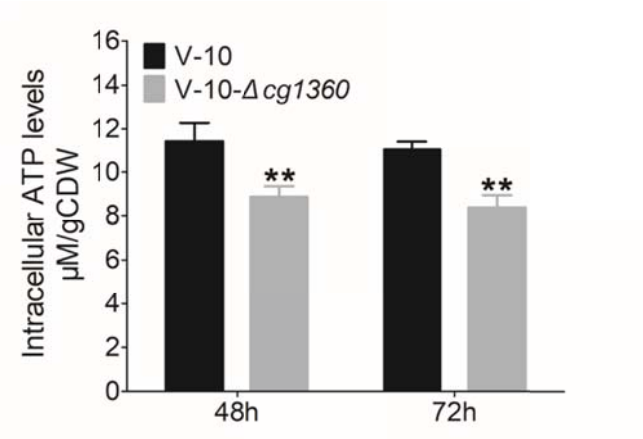

Fig. S3. The intracellular ATP levels of the valine-producing strains V-10 and its mutant with *cg1360* deletion after a 48 h or 72 h of fermentation.

Supplemental Tables:

Table S1 Plasmids and strains used in this study

Plasmid or strain	Description	Reference
Plasmids		
pCRD206	Temperature-sensitive replicon and *B. subtilis sacB* gene, Kan^R	[22]
pCRD206-*cg1360*	pCRD206 derivative; containing *C. glutamicum cg1360* flanking regions	This study
pCRD206-*cg1361*	pCRD206 derivative; containing *C. glutamicum cg1361* flanking regions	This study
pCRD206-*cg1360*comp	pCRD206 derivative; containing *C. glutamicum cg1360* E4E point mutation and its flanking regions	This study
pCRD206-*cg1361*comp	pCRD206 derivative; containing *C. glutamicum cg1361* L4L point mutation and its flanking regions	This study
pCRD206-*atpD*-6×His	pCRD206 derivative; containing a C-terminal 6×His-tagged *atpD gene* and its flanking regions	This study
Strains		
E. coli DH5α	*E. coli* derivative; competent cells for general cloning	Takara Bio, Shiga, Japan
C. glutamicum ATCC 13032	Wild-type *C. glutamicum* strain	[32]
C. glutamicum Δcg1360	*C. glutamicum* derivative; lacking the Cg1360 putative membrane protein	This study
C. glutamicum Δcg1361	*C. glutamicum* derivative; lacking the Cg1361 hypothetical protein	This study
C. glutamicum Δcg1360+cg1360	*C. glutamicum* derivative; containing a synonymous point mutation at the Glu-4 site of Cg1360 protein	This study
C. glutamicum Δcg1361+cg1361	*C. glutamicum* derivative; containing a synonymous point mutation at the Leu-4 site of Cg1361 protein	This study
C. glutamicum atpD-His-tag	*C. glutamicum* derivative; C-terminus of the ATPase β subunit with a 6×His-tag	This study
C. glutamicum Δcg1360 atpD-His-tag	*Δcg1360* derivative; C-terminus of the ATPase β subunit with a 6×His-tag	This study
C. glutamicum Δcg1361 atpD-His-tag	*Δcg1361* derivative; C-terminus of the ATPase β subunit with a 6×His-tag	This study
C. glutamicum CGMCC 1.586	The valine-producing parent strain	CGMCC, Beijing, China
V-10	*C. glutamicum* CGMCC 1.586 derivative; the valine producer obtained by multiple random mutagenesis	This study
V-10-*Δcg1360*	V-10 derivative; lacking the Cg1360 putative membrane protein	This study

Table S2 Primers used in this study

Primers	Sequences (5'–3') [a]
Used for gene disruption and synonymous point mutation	
cg1360-1-For	TGAC*GGATCC*GGCGATGTTCAGTGGATTTC
cg1360-2-Rev	CCCTAGAGGTTTAAACAACTTCAAATGGGGAATCTGTGT
cg1360-3-For	CCCATTTGAAGTTGTTTAAACCTCTAGGGTTACATACGT
cg1360-4-Rev	TCAG*TCTAGA*TACGTGAGTTGGAGGAGATG
cg1360-UF	GCGAAGTACTTGGAGTGTC
cg1360-DR	GGTGGAATGTTCGGAATCAC
cg1361-1-For	AGTC*GGATCC*TCACCCATCATCGTTGTTCTC
cg1361-2-Rev	TGAATTCGTCTTAAACAACGTTTCCCAGCACGGACACTC
cg1361-3-For	GCTGGGAAACGTTGTTTAAGACGAATTCACCTGTTAGTC
cg1361-4-Rev	TACG*TCTAGA*CTCAATGTATACAGCGACCAG
cg1361-UF	GCTAATTCCAACGTCACCAC
cg1361-DR	GTGCACCTGAAATGTTGTTGT
cg1360comp-For	GCC**GAG**AATACCGAGAAC
cg1360comp-Rev	GTATT**CTC**GGCGCTCACG
cg1361comp-For	TCCGTG**CTC**GGAAACTGG
cg1361comp-Rev	TTCC**GAG**CACGGACACTC
atpD-His_6-1-For	CGC*GGATCC*AACAGGCATCAAGGTTATCG
atpD-His_6-2-Rev	CTTAGTGGTGGTGGTGGTGGTGCTTTCCGGTCAGCTTCTTG
atpD-His_6-3-For	AAAGCACCACCACCACCACCACTAAGGTAGAGACACATGGC
atpD-His_6-4-Rev	CTAG*TCTAGA*GCATCTGGTGCTGCTTCC
atpD-His_6-UF	GGAAAGCACCACCACCAC
atpD-His_6-DR	TGGCTTATAGGCACGGTC
Used for qPCR analyses	
atpB-qPCR-5F	AGTCCGTAGCGATTACAGCGAG
atpB-qPCR-3R	CATTGCGAACCAACCATTAGCG
atpE-qPCR-5F	AACCGAGTCCACCATCACCG
atpE-qPCR-3R	AACAGGAAGCCAGCAACAAGG
atpH-qPCR-5F	ACTGGCAAAGGTTTCGTCCG
atpH-qPCR-3R	CGAGAACAGATGGGCTCACTTT
atpD-qPCR-5F	TTGAAGAGCAGAACGCACAGC
atpD-qPCR-3R	TGGTGCGGATGAGGTTGTCG
gyrB-qPCR-5F	ATGGTGGCGTTCAAGTTGTC
gyrB-qPCR-3R	AACGGCGTAAGAATCGGAGT
16S-5F	ACCTGGAGAAGAAGCACCG
16S-3R	TCAAGTTATGCCCGTATCG

[a] Restriction sites are shown in underlined italics, and the mutated sites are shown in bold.

Table S3 Homologues analyses of *C. glutamicum* Cg1360 protein

Protein	Size (aa)	Identity (%)	Species	Accession No.
Cg1360	149	100	*C. glutamicum* ATCC13032	CAF19908.1
ATP synthase protein I	133	16.07	*B. pseudofirmus* OF4	ADC49435.1
UncI polypeptide	130	23.28	*E. coli* K-12	AAA24730.1
ATP synthase protein I	135	19.83	*P. putida* KT2440	P0A103.1
AtpI	126	24.11	*S. boydii* Sb227	ABB68220.1
AtpI	122	16.82	*C. kluyveri* DSM 555	EDK35680.1
ATP synthase protein I	129	15.65	*V. alginolyticus*	P12983.1
ATP synthase protein I	127	22.12	*P. atrosepticum* SCRI 1043	CAG77414.1

Table S4 Homologues analyses of *C. glutamicum* Cg1361 protein

Protein	Size (aa)	Identity (%)	Species	Accession No.
Cg1361	80	100	*C. glutamicum* ATCC13032	CAF19909.1
ATP synthase protein I	133	22.41	*B. pseudofirmus* OF4	ADC49435.1
UncI polypeptide	130	16.67	*E. coli* K-12	AAA24730.1
ATP synthase protein I	135	22.81	*P. putida* KT2440	P0A103.1
AtpI	126	16.67	*S. boydii* Sb227	ABB68220.1
AtpI	122	13.73	*C. kluyveri* DSM 555	EDK35680.1
ATP synthase protein I	129	22.22	*V. alginolyticus*	P12983.1
ATP synthase protein I	127	16.36	*P. atrosepticum* SCRI 1043	CAG77414.1