Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Electronic Supplementary Information

Rational engineering of *Acinetobacter tandoii* glutamate dehydrogenase for asymmetric synthesis of L-homoalanine through biocatalytic cascades

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Table S1. Enzyme activities of dual cofactor dependent GluDHs towards 2-ketobutyric acid in laboratory.

Enzyme	Activity (U/mg)
Acinetobacter tandoii GluDH	57.3 ± 9.1
Cupriavidus necator GluDH	42.5 ± 3.7
Geobacillus thermocatenulatus GluDH	31.4 ± 1.2

Table S2. Kinetic paramrters of *At*GluDH towards NADH and NADPH.

	k_{cat} (s^{-1})	K_m (mM)	k_{cat} / K_m (mM^{-1} s^{-1})
NADH[a]	3673.1±121.9	0.52±0.08	7063.65
NADPH[b]	4013.8±208.5	3.87±0.14	1037.16

[a] Data for NADH and NADPH was measured with 200 mM NH_4Cl/NH_4OH buffer (pH 9.5), 50 mM 2-ketobutyric acid, 0.01-5 mM NADH or 0.01-30 mM NADPH and at 30°C.

Table S3. Primers used for site-directed mutagenesis of *At*GluDH.

Primer	Sequence (5'-3')
K76G-F	GGT**GGC**GGCGGTATTCGTTACCAT
K76G-R	TGGTCCACGCGACAAATTATGTTG
K76A-F	GGT**GCG**GGCGGTATTCGTTACCAT
K76A-R	TGGTCCACGCGACAAATTATGTTG
K76V-F	GGT**GTG**GGCGGTATTCGTTACCAT
K76V-R	TGGTCCACGCGACAAATTATGTTG
K76L-F	GGT**CTG**GGCGGTATTCGTTACCAT
K76L-R	TGGTCCACGCGACAAATTATGTTG
K76I-F	GGT**ATC**GGCGGTATTCGTTACCAT
K76I-R	TGGTCCACGCGACAAATTATGTTG
S355A-F	GTG**GCT**TACTTCGAGTGGGTTCAA
S355A-R	GGTTACACCGCCAGCATTACAGAG
S355V-F	GTG**GTG**TACTTCGAGTGGGTTCAA
S355V-R	GGTTACACCGCCAGCATTACAGAG
T180A-F	GTG**GCG**GGTAAACCTGTACATTTAGGT
T180A-R	TACACCTGTGACAGTATGACCCTT
T180V-F	GTG**GTG**GGTAAACCTGTACATTTAGGT
T180V-R	TACACCTGTGACAGTATGACCCTT
T180S-F	GTG**TCT**GGTAAACCTGTACATTTAGGT
T180S-R	TACACCTGTGACAGTATGACCCTT
T180C-F	GTG**TGC**GGTAAACCTGTACATTTAGGT
T180C-R	TACACCTGTGACAGTATGACCCTT

Table S4. Binding free energy analysis using the Molecular Mechanics Poisson Boltzmann (Generalized Born) surface area method.

Energy (kcal/mol)[a]	*At*GluDH-WT	*At*GluDH-K76L/T180C
VDW	-11.32±2.83	-14.18±2.97
EEL	-32.49±8.33	-10.71±8.46
GB	13.51±7.21	-5.77±7.14
GBSURF	-2.99±0.07	-3.09±0.005
GBGAS	-43.81±8.09	-24.90±7.90
GBSOLV	10.52±7.21	-8.87±7.13
GBTOT	**-33.30±3.06**	**-33.76±3.76**
PB	-20.64±9.44	-44.51±5.15
PBNPOLAR	-13.62±0.24	-13.74±0.15
PBDISPER	20.52±0.62	20.71±0.62
PBGAS	-43.81±8.09	-24.90±7.90
PBSOLV	-13.75±9.73	-37.54±5.21
PBTOT	**-57.56±10.16**	**-62.44±6.81**

[a] VDW: van der Waals energy. EEL: energy of electrostatic. GBTOT/PBTOT: final binding free energy.

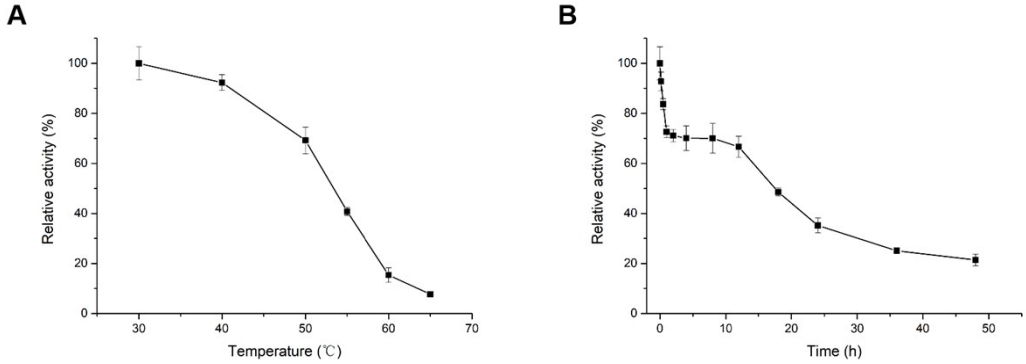

Figure S1. (A) Enzyme inactivation assay at different temperatures for 12 h. (B) Time courses of thermal inactivation at 50°C. Purified *At*GluDH-WT was incubated at a certain temperature for a set time, and then added into the reaction mixure. A 200 μL-scale reaction mixture containing a certain amount of purified *At*GluDH-WT, 2-ketobutyric acid (50 mM), 0.1 mM NADH, 200 mM NH_4Cl/NH_4OH buffer (pH 9.5). Then, the activity was measured by monitoring the absorbance at 340 nm (ε=6220 M^{-1} cm^{-1}) at 30°C.

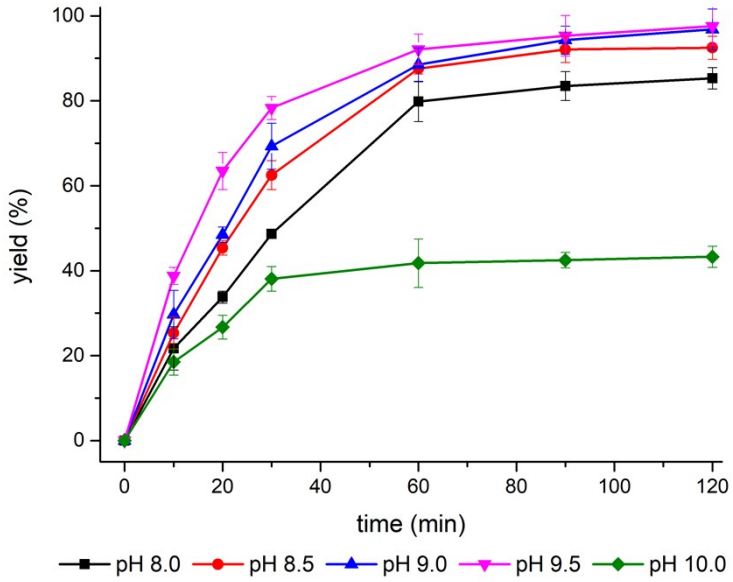

Figure S2. Reaction curve at different pH. A 20 mL-scale reaction mixture contained *Ec*TD (3 g/L), *At*GluDH-WT (10 g/L) and *Bm*GDH (3 g/L) which were prepared as lyophilized cells, 0.5 M L-threonine, 0.1 mM NADH, 0.6 M glucose, 200mM $(NH_4)_3PO_4$ buffer (pH 8.0-8.5) or 200 mM NH_4Cl/NH_4OH buffer (pH 9.0-10.0), and at 30°C.

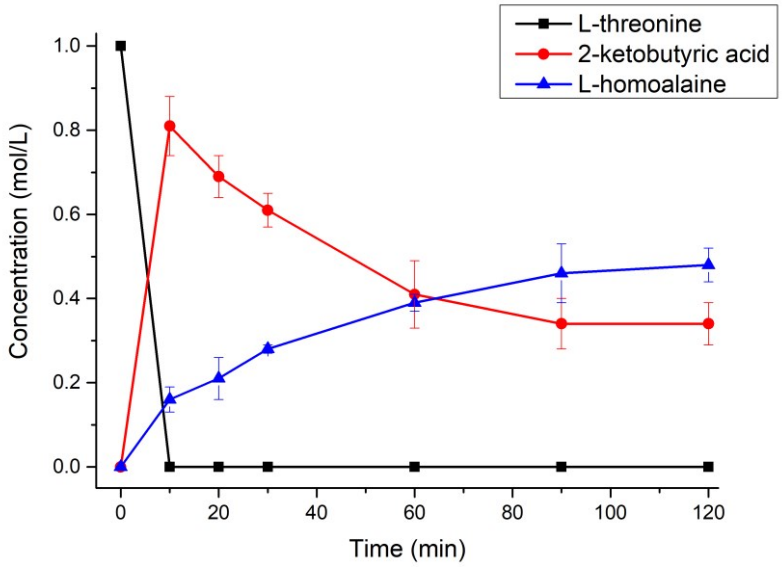

Figure S3. Cascade reaction curves with 1 M L-threonine as substrate. A 20 mL-scale reaction mixture contained *Ec*TD (3 g/L), *At*GluDH-WT (10 g/L) and *Bm*GDH (3 g/L) which were prepared as lyophilized cells, 1 M L-threonine, 0.1 mM NADH, 1.2 M glucose, 200 mM NH_4Cl/NH_4OH buffer (pH 9.5), and at 30°C. Error bars represent the standard deviation of three independent experiments.

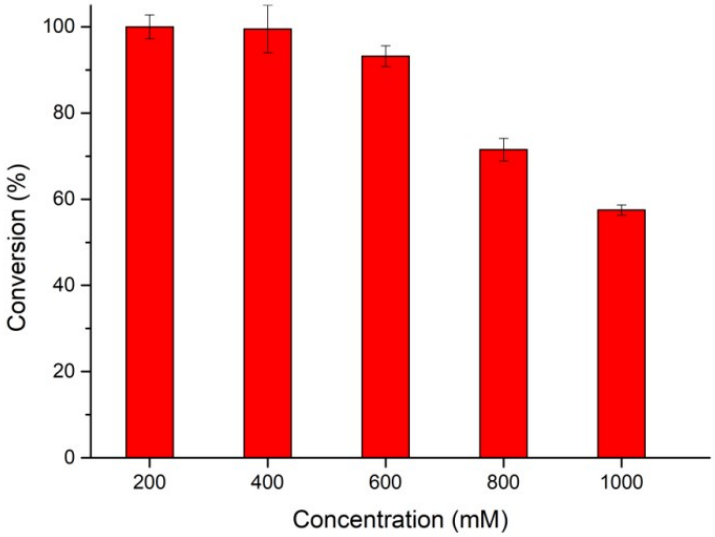

Figure S4. The conversion of the reductive amination of 2-ketobutyric acid by wild type *At*GluDH. A 1 mL-scale reaction mixture contained *At*GluDH-WT (10 g/L) and *Bm*GDH (3 g/L) which were prepared as lyophilized cells, 2-ketobutyric acid (200-1000 mM), 0.1 mM NADH, glucose (1.2 x substate concentration), 200 mM NH_4Cl/NH_4OH buffer (pH 9.5), and at 30°C within 24 h. Error bars represent the standard deviation of three independent experiments.

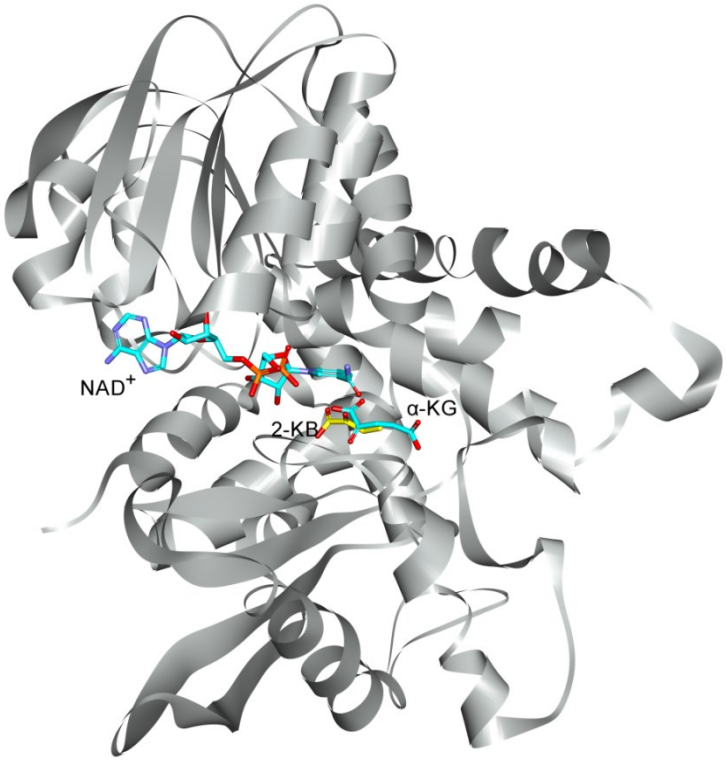

Figure S5. Homology model of *At*GluDH with NAD^+, α-ketoglutarate and 2-ketobutyric acid. NAD^+, α-ketoglutarate and 2-ketobutyric acid are shown as sticks.

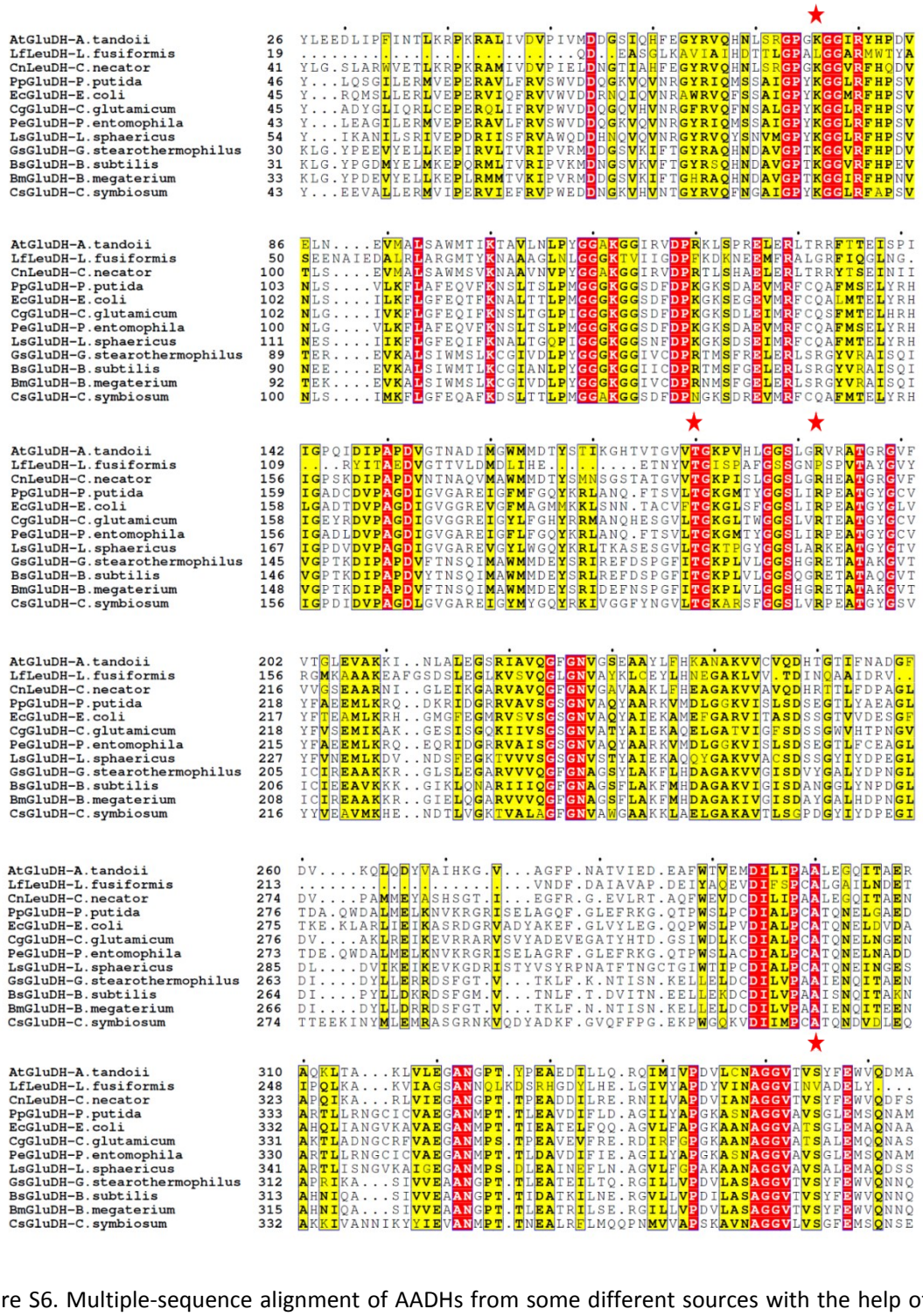

Figure S6. Multiple-sequence alignment of AADHs from some different sources with the help of T-coffee and Espript 3.

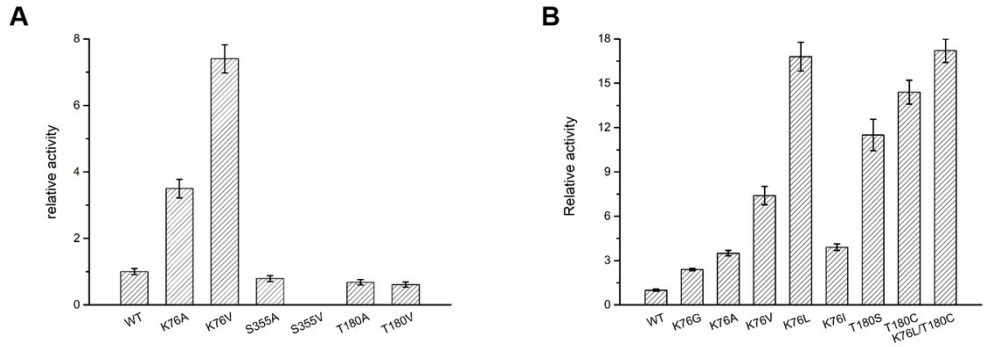

Figure S7. (A and B) Relative activity of the mutants of *At*GluDH compared to the wild-type protein. Reaction conditions: 200 μL final volume, 200 mM NH_4Cl/NH_4OH buffer (pH 9.5), 0.1 mM NADH, 50 mM 2-ketobutyric acid and at 30°C. Error bars represent the standard deviation of three independent experiments.

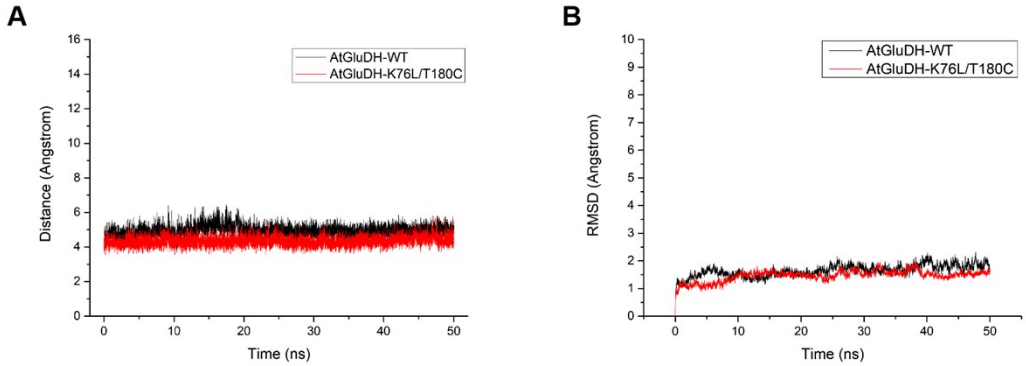

Figure S8. (A) Distance analysis between the reactive carbonyl carbon (C2) of the substrate and the hydride donating/accepting carbon (C4) of the nicotinamide group of the coenzyme in MD simulations. (B) RMSD analysis results.

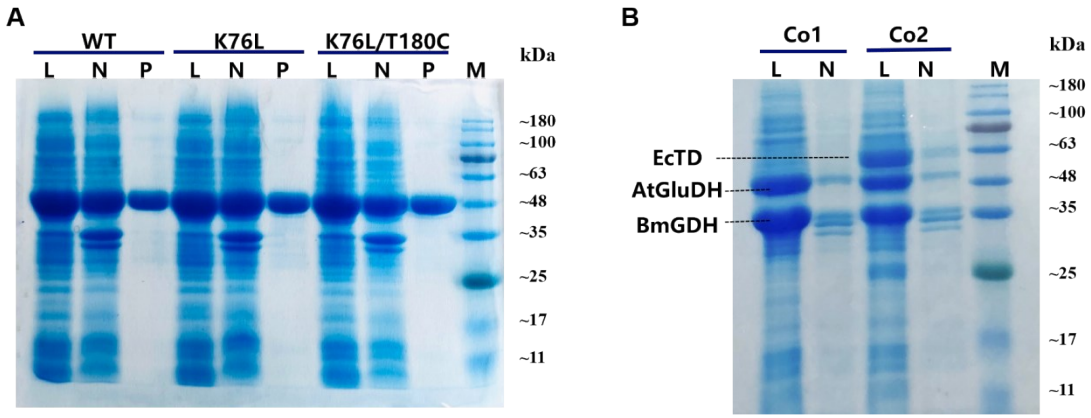

Figure S9. (A) SDS-PAGE of *At*GluDH and mutants. L: cell free extract, N: precipitation, P: pure protein elution fraction, M: marker. (B) SDS-PAGE of the co-expression systems. Co1: *E.coli* cells co-expressing *At*GluDH (K76L/T180C) and *Bm*GDH, Co2: *E.coli* cells co-expressing *At*GluDH (K76L/T180C), *Bm*GDH and *Ec*TD, L: cell free extract, N: precipitation, M: marker. Protein molecular weight: *At*GluDH (46.7 kDa), *Bm*GDH (28.2 kDa), *Ec*TD (56.2 kDa).

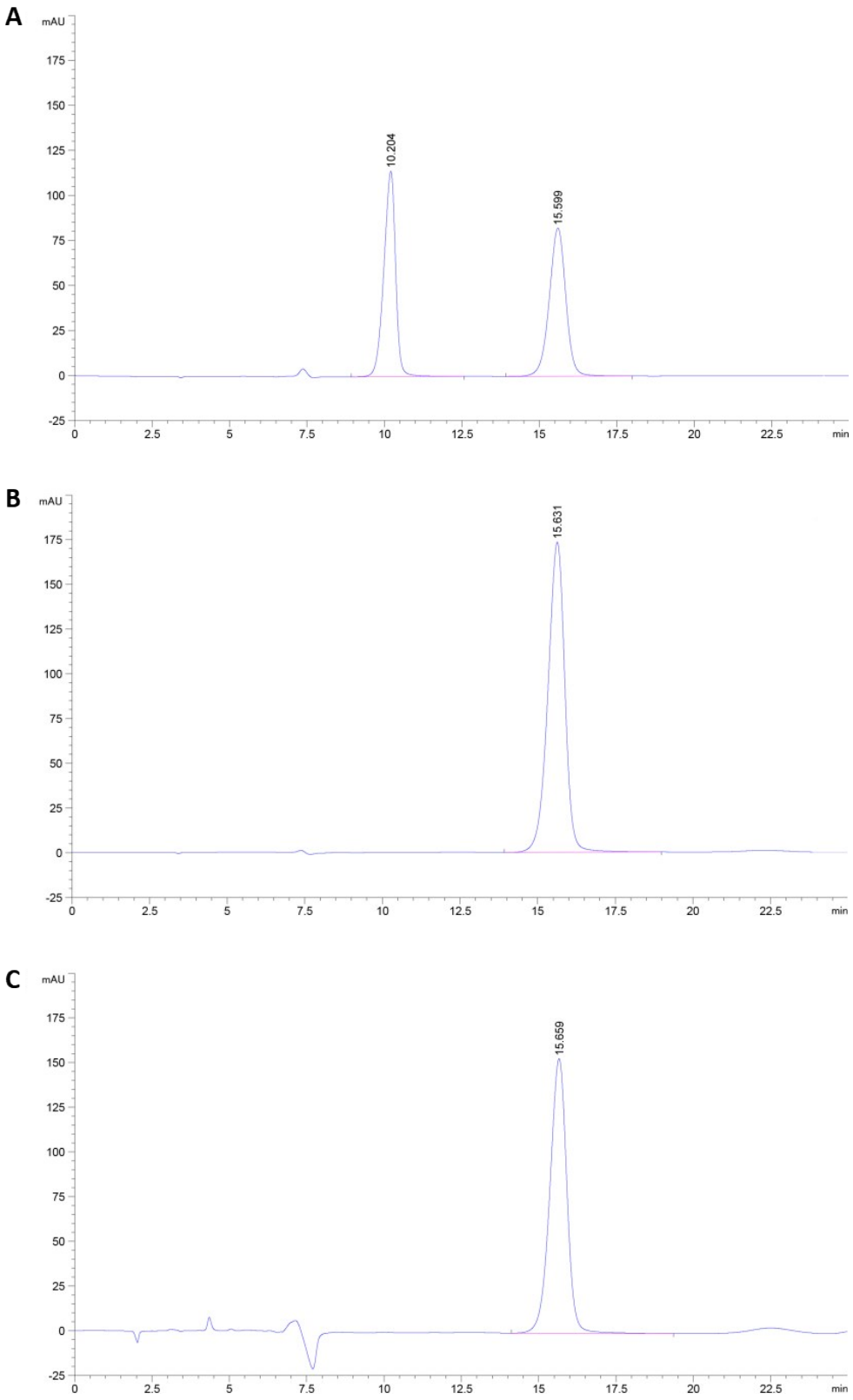

Figure S10. (A) HPLC chromatogram of racemic 2-aminobutyric acid. (B) HPLC chromatogram of L-2-aminobutyric acid. (C) HPLC chromatogram of product L-2-aminobutyric acid yielded by *At*GluDH.

Figure S11. NMR spectra of the purified L-homoalanine. (A) ^{1}H NMR; (B) ^{13}C NMR. ^{1}H NMR (600 MHz, D_2O): δ = 3.71 (td, *J* = 5.8, 2.8 Hz, 1H), 1.89 (m, 2H), 0.97 (td, *J* = 7.6, 1.7 Hz, 3H); ^{13}C NMR (150 MHz, D_2O): δ = 174.9, 55.8, 23.7, 8.5.