Table S1 σ^{54}-dependent promoters characterized in this study

Promoters	Genes	Gene functions
glnHp2	*glnH*	periplasmic binding protein of L-glutamine ABC transporter
ddpXp	*ddpX*	D-alanyl-D-alanine dipeptidase
glnKp	*glnK*	nitrogen regulator PII
astCp2	*astC*	succinylornithine transaminase
pabBp6	*pabB*	aminodeoxychorismate synthase subunit I
patAp	*patA*	putrescine aminotransferase
puuPp	*puuP*	proton dependent putrescine transporter
rutAp	*rutA*	pyrimidine oxygenase
yhdWp	*yhdW*	substrate binding protein of a putative polar amino acid transporter
argTp	*argT*	periplasmic binding protein of a basic amino acid ABC transporter
nacp	*nac*	DNA-binding transcriptional dual regulator
glnAp2	*glnA*	glutamine synthetase

Table S2 List of primers

Primers	Sequence 5'-3'
Primers for pathway construction	
pLM-F	cctttcgtcttcacctcgagacattcacatcgtggtgcagc
pLM-R	ctcgaggtgaagacgaaagg
pLM1-F	acgcgtgctagaggcatc
pLM1-R	ttaacccccagtttcgatttatc
pLM6-F	agatttcgctttatctttttgaattcattaaagaggagaaagg
pLM6-R	caccatgttggtgcaatgacctcgaggtgaagacgaaag
pLM8-F	ataagacctgcatgaaagagcgaattcattaaagaggaga
pLM8-R	aattgccctgaaacagggcactcgaggtgaagacgaaagg
avta-F	aatcgaaactgggggttaaacgcgtaagaaggagatataccatg
avta-R	ttgatgcctctagcacgcgtttagtgactttcagcccagg
glnAp2-F	gcccttcgtcttcacctcgaggtcattgcaccaacatggtg
glnAp2-R	ctttctcctctttaatgaattcaaaaagataaagcgaaatctgtgccaac
glnAp2-F2	gtcattgcaccaacatggtg
glnAp2-R2	aaaaagataaagcgaaatctgtgc
rrnBp-F	cctttcgtcttcacctcgagcaagtgctgccagagggaac
rrnBp-R	ttctcctctttaatgaattccccggcggcgtgtttgccgt
J23100-M1-F	tcagtcctaggtacagtgctagcgaattcattaaagaggagaaaggtacaatgttgacaaaag
J23100-97-F	tcagtcctaggtacagtgctagcgaattcattaaagaggagaaaggtaccatgaaaatct
J23100-R	actgtacctaggactgagctagccgtcaactcgaggtgaagacgaaagggcctc
Primers for promoter selection	
pLMg1-F	acgcgtgctagaggcatc
pLMg1-R	tgtacctttctcctctttaa
gfp-F	cattaaagaggagaaaggtacaatggtgagcaagggcgag
gfp-R	gatgcctctagcacgcgtttacttgtacagctcgtccatg
argTp-F	cctttcgtcttcacctcgagtgccctgtttcagggcaatt
argTp-R	ttctcctctttaatgaattcggctctttcatgcaggtctt
astCp-F	cctttcgtcttcacctcgagatgtcaacgatggcgcaaaa
astCp-R	ttctcctctttaatgaattcaaatgtagattgcagggttc
ddpXp-F	cctttcgtcttcacctcgagcttcccctcttgcaccaaaac
ddpXp-R	tctcctctttaatgaattccgctttccgcttatgcagatc
glnHp-F	cctttcgtcttcacctcgagaatcactgtgttgagtgcac
glnHp-R	ttctcctctttaatgaattcattcacatatatgaaaaaat
glnKp-F	cctttcgtcttcacctcgagatagcgcaatatttcatcgt
glnKp-R	ttctcctctttaatgaattcaagaaggtattgcaagcggt

nacp-F	cctttcgtcttcacctcgagtgaaccatcgtggtgcatac
nacp-R	ttctcctctttaatgaattcacaaccagattgcaagatgc
pabBp-F	cctttcgtcttcacctcgagcacgattcgctcgccggagt
pabBp-R	tctcctctttaatgaattccgggtagcatgaaataattag
patAp-F	cctttcgtcttcacctcgagcgcaccatgttgtgcggctg
patAp-R	ttctcctctttaatgaattcatttaagtattgcagggatt
puuPp-F	cctttcgtcttcacctcgagtgcagtttgagcgcctcatc
puuPp-R	ttctcctctttaatgaattcaacggaagcccgttgccgcc
rutAp-F	cctttcgtcttcacctcgagtgcactctcatcgcgcacag
rutAp-R	ttctcctctttaatgaattcgtattggcttgtttgcaaag
yhdWp-F	cctttcgtcttcacctcgagtgcgccaaaatgtggcgcat
yhdWp-R	ttctcctctttaatgaattctcctcttataagcaaaagta
glnAp2g-F	cctttcgtcttcacctcgagacattcacatcgtggtgcag
glnAp2g-R	ttctcctctttaatgaattcaaaaagataaagcgaaatct

To clone pLM1, primers pLM1-F and pLM1-R were used to amplify $P_{L}lacO_{1}$: *alsS-ilvC-ilvD* from pSA69, primers avta-F and avta-R were used to amplify *avtA* from the *E. coli* genomic DNA, the PCR products were assembled by Gibson Assembly.

To clone pLM2, primers rrnBp-F and rrnBp-R were used to amplify *rrnBp1* from the *E. coli* genomic DNA, primers pLM-F and pLM-R were used to amplify *alsS-ilvC-ilvD-avtA* from pLM1, the PCR products were assembled by Gibson Assembly.

To clone pLM3, primers rrnBp-F and rrnBp-R were used to amplify *rrnBp1* from the *E. coli* genomic DNA, primers pLM-F and pLM-R were used to amplify *leuDH-kivD-yqhD; lacI* from pYX97, the PCR products were assembled by Gibson Assembly.

To clone pLM4, primers J23100-M1-F and J23100-R were used to amplify J23100: *alsS-ilvC-ilvD-avtA* from pLM1, the PCR product was assembled by Gibson Assembly.

To clone pLM5, primers J23100-97-F and J23100-R were used to amplify J23100: *leuDH-kivD-yqhD; lacI* from pYX97, the PCR product was assembled by Gibson Assembly.

To clone pLM6, primers pLM6-F and pLM6-R were used to amplify *glnAp2* from the *E. coli* genomic DNA, primers pLM-F and pLM-R were used to amplify *alsS-ilvC-ilvD-avtA* from pLM1, the PCR products were assembled by Gibson Assembly.

To clone pLM7, primers pLM6-F and pLM6-R were used to amplify *glnAp2* from the *E. coli* genomic DNA, primers pLM-F and pLM-R were used to amplify *leuDH-kivD-yqhD; lacI* from pYX97, the PCR products were assembled by Gibson Assembly.

To clone pLM8, primers pLM8-F and pLM8-R were used to amplify *argTp* from the *E. coli* genomic DNA, primers pLM-F and pLM-R were used to amplify *alsS-ilvC-ilvD-avtA* from pLM1, the PCR products were assembled by Gibson Assembly.

To clone pLM9, primers pLM8-F and pLM8-R were used to amplify *argTp* from the *E. coli* genomic DNA, primers pLM-F and pLM-R were used to amplify *leuDH-kivD-yqhD; lacI* from pYX97, the PCR products were assembled by Gibson Assembly.

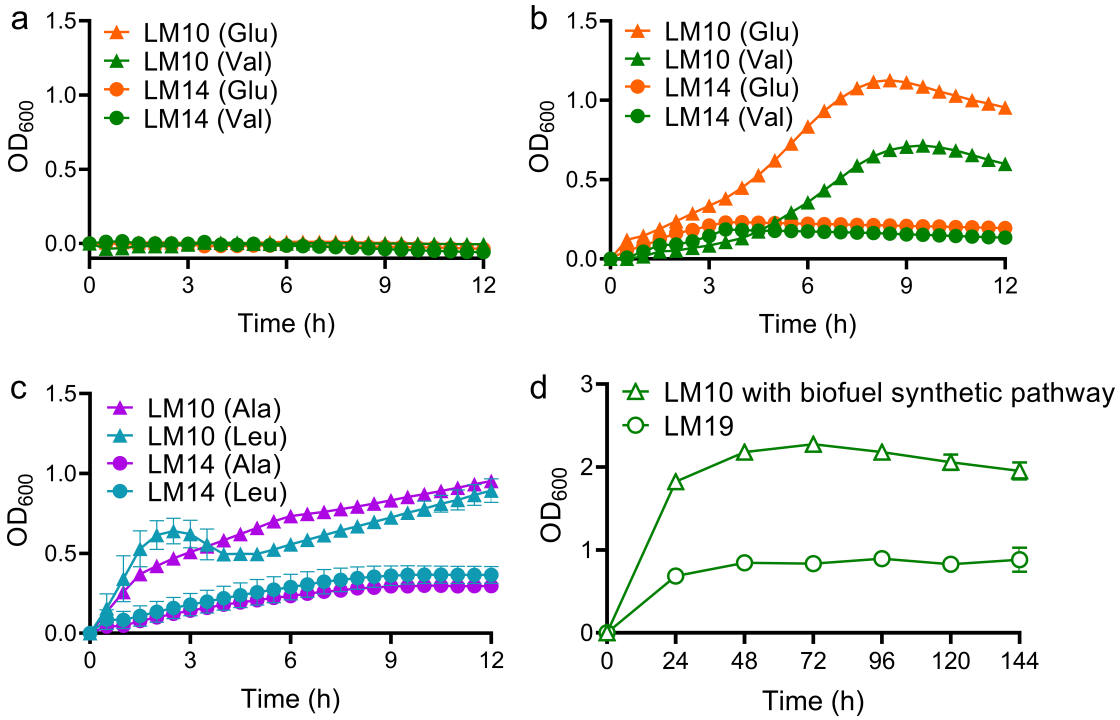

Figure S1 The growth curves of the parent strain and the ammonia-assimilation-pathway-deleted strain in defined media. **a** The growth curve of LM10 and LM14 in M9 medium with 15 mM valine (Val) or 15 mM glutamine (Glu) as the sole carbon and nitrogen sources. **b** The growth curve of LM10 and LM14 in M9 medium with 0.4% glucose as the carbon source and 15 mM Val or 15 mM Glu as the nitrogen source. **c** the growth curve of LM10 and LM14 in M9 medium with 0.5% yeast extract as the carbon source and 15 mM alanine (Ala) or 15 mM leucine (Leu) as the nitrogen source. **d** The growth curve of LM10 with isobutanol producing pathway and LM19 in M9 medium with 0.4% glucose as the carbon source and 15 mM glutamine as the nitrogen source.

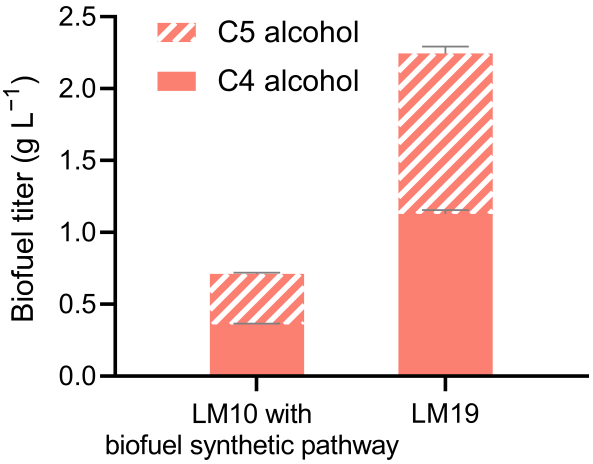

Figure S2 The biofuel titer for LM19 and LM10 with biofuel synthetic pathway in M9 medium with 40 g L^{-1} yeast extract as the carbon and nitrogen sources.

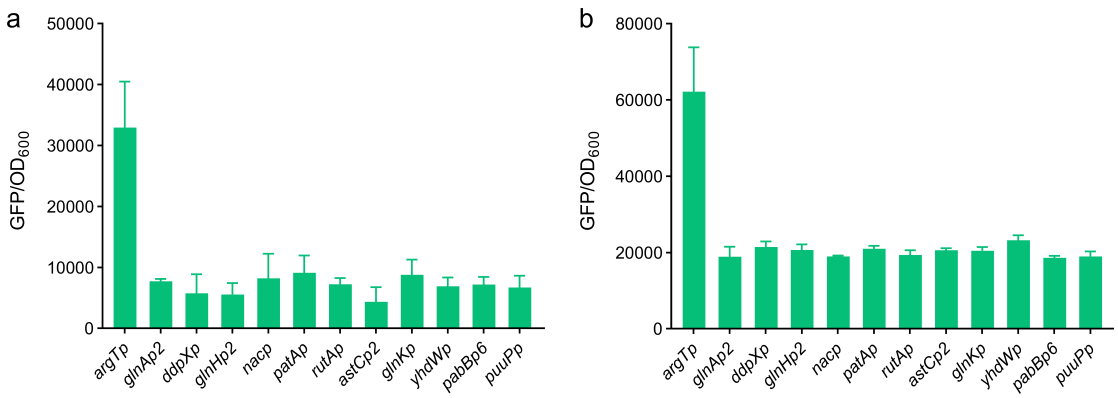

Figure S3 The fluorescence intensities for GFP expressed from different σ^{54}-dependent promoters. **a** Fluorescence intensities for cells in the early stationary phase. **b** Fluorescence intensities for cells in the late stationary phase. All the strains were cultured in a 96 deep-well (2 mL) plate and sampled at defined time points for measurement of fluorescence intensity. Values and error bars represent the mean and the s.d. (n = 3).

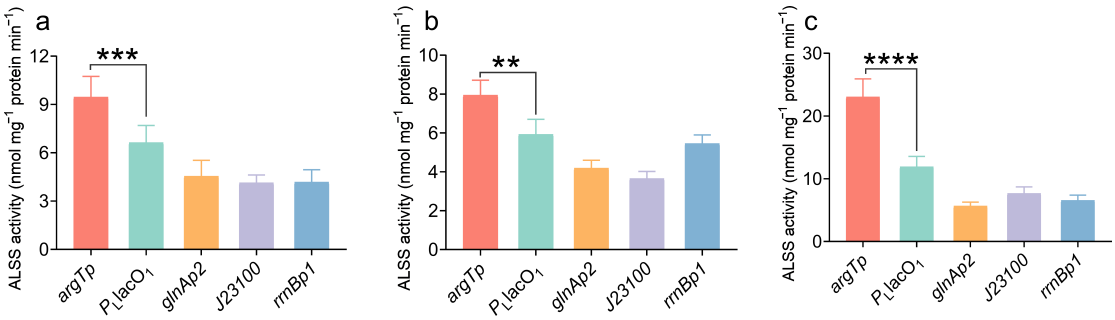

Figure S4 Activities of AlsS in the biofuel synthetic pathway driven by different promoters. **a** AlsS activities under optimal condition; **b** AlsS activities under osmotic stress (400 mM NaCl); **c** AlsS activities under acid stress (pH 5.0). Values and error bars represent the mean and the s.d. (n = 3). $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ as determined by two-tailed *t* test.

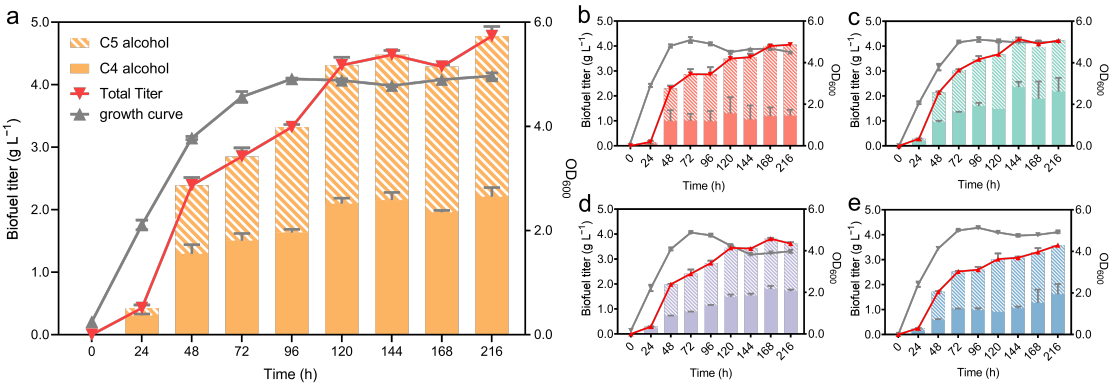

Figure S5 Biofuel production from pathway driven by **a** *glnAp2*, **b** *argTp*, **c** $P_{\mathrm{L}}\mathrm{lacO}_1$, **d** J23100 and **e** *rrnBp1* in batch fermentation.

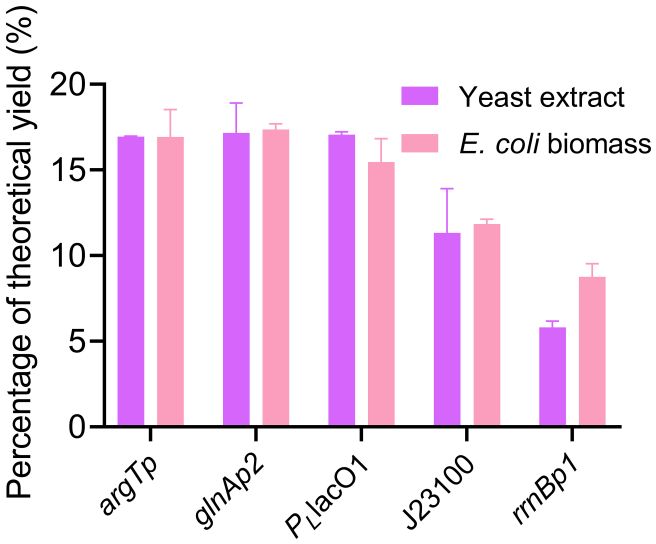

Figure S6 The percentage of theoretical yield [1] (g of product per g of consumed raw material) for biofuel produced from pathway driven by different promoters. Yeast extract and *E. coil* protein biomass were used as the feedstock.

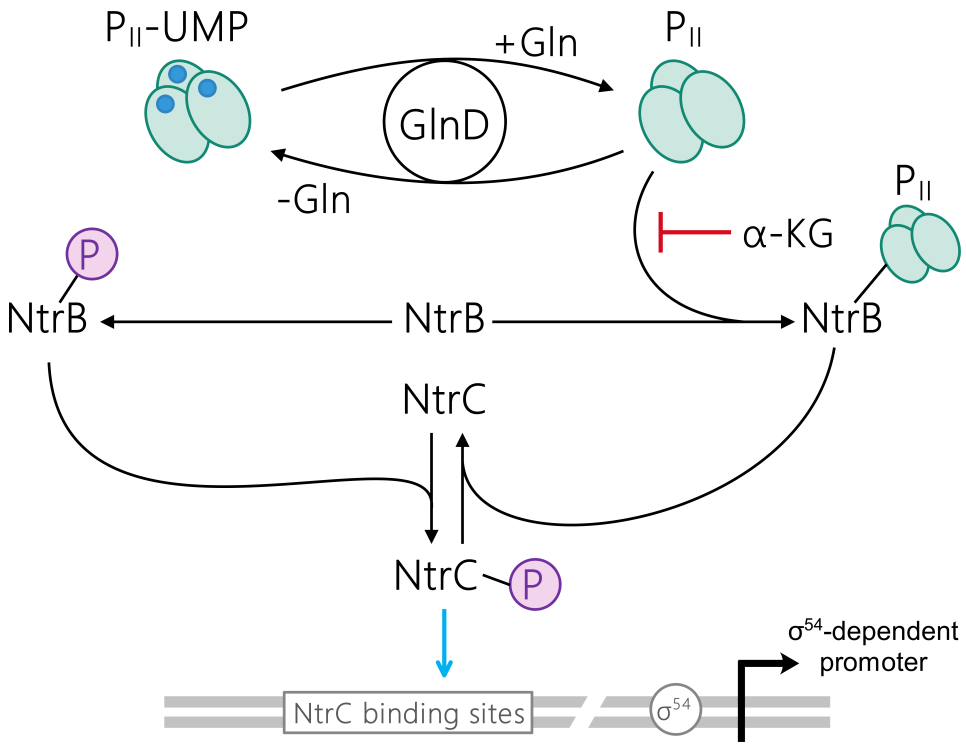

Figure S7 The regulatory cascade for nitrogen assimilation in *E. coli*. The nitrogen status is sensed by the GlnD and the P_{II} system. A low nitrogen availability promotes GlnD to uridylylate P_{II} protein (P_{II}-UMP) and inhibit its ability to bind NtrB, allowing free NtrB to undergo phosphorylation and subsequently transfer its phosphoryl group to NtrC. The phosphorylated NtrC (NtrC-P) then activates *glnAp2* and other targeted promoters [2]. The GlnD responds to intracellular glutamine and the PII is regulated by 2-oxoglutarate [3]. Accumulation of glutamine (Gln) promotes GlnD to deuridylylate PII-UMP and leads to the dephosphorylation of NtrC-P, which subsequently suppresses the NtrC-mediated transcription. Excessive α-ketoglutarate (α-KG) would bind with PII and promote the release of free NtrB. The increased level of NtrB available for phosphorylation would subsequently increase the level of NtrC-P and activate the targeted transcriptions.

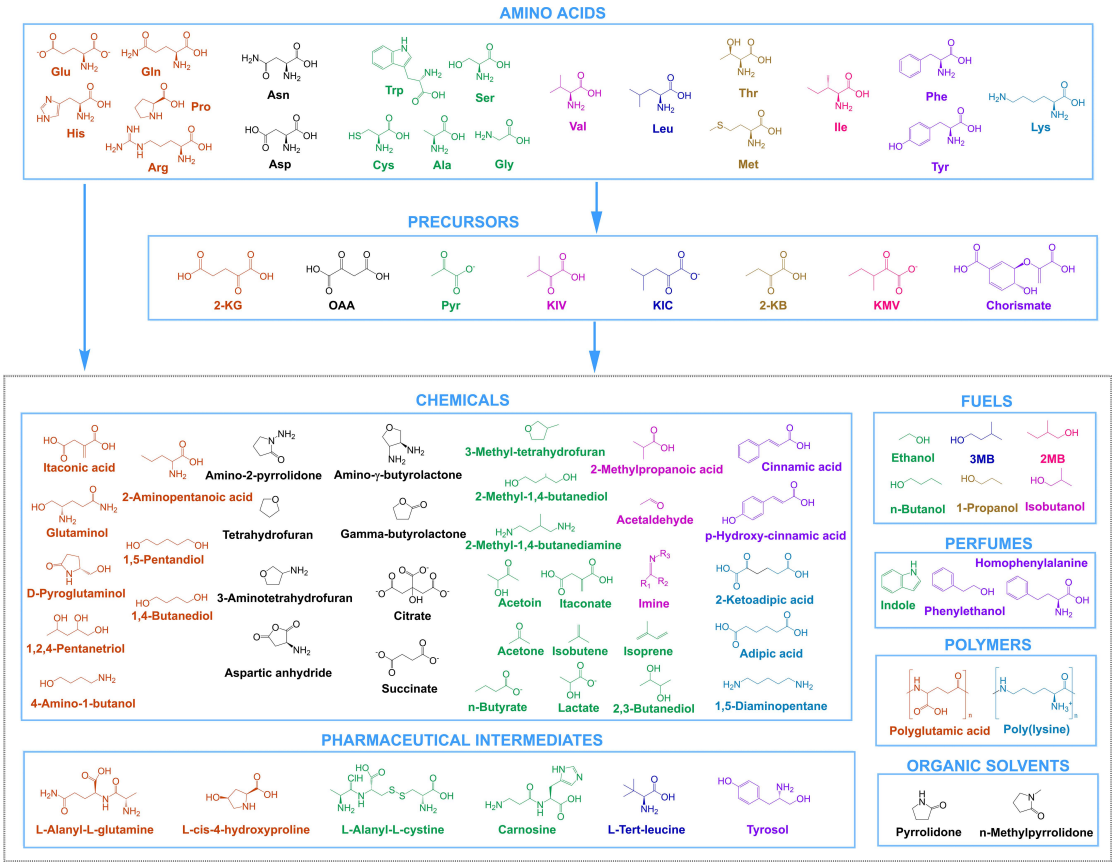

Figure S8 Precursors of the 20 amino acids and their value-added derivatives. The amino acids and their corresponding precursors and derivatives are of the same color. 2-KG: 2-ketoglutarate, OAA: oxaloacetic acid, PYR: pyruvic acid, KIV: 2-ketoisovaleric acid, KIC: 2-ketoisocaproic acid, 2-KB: α-ketobutyrate, KMV: 2-ketomethylvaleric acid. The derivatives include fuels (e.g. ethanol [4], 2-methyl-1-butanal (2MB), 3-methyl-1-butanol (3MB), isobutanol [1], n-butanol [5] and 1-propanol [6]), perfumes (e.g., indole [7], phenylethanol [8] and homophenylalanine [9]), polymers (e.g., polyglutamic acid [10] and polylysine [11]), organic solvents (e.g., pyrrolidone [12] and n-methylpyrrolidone [13]), pharmaceutical intermediates (e.g., L-alanyl-L-glutamine [14], L-cis-4-hydroxyproline, L-alanyl-L-cystine, carnosine [15], L-tert-leucine [16], and tyrosol [18]), and other chemicals (e.g., itaconic acid [19], 1,5-diaminopentane [20], 1,5-pentandiol [21], 4-amino-1-butanol [22], D-pyroglutaminol [21], 2-aminopentanoic acid [23], 1,2,4-pentanetriol [24], glutaminol [25], amino-γ-butyrolactone [27], amino-2-pyrrolidone [12], tetrahydrofuran [1], γ-butyrolactone [21], 3-aminotetrahydrofuran [21], aspartic anhydride [27], citrate [28], 1,4-butanediol [29], succinate [30], 2,3-butanediol [31], isoprene [32], n-butyrate [33], acetoin [31], acetone

[34], itaconate [35], isobutene [36], lactate [33], 3-methyl-tetrahydrofuran [37], 2-methyl-1,4-butanediol [38], 2-methyl-1,4-butanediamine [39], 2-methylpropanoic acid [40], acetaldehyde [41], imine [42], cinnamic acid [43], p-hydroxy-cinnamic acid [44], 2-ketoadipic acid [42] and adipic acid [45]).

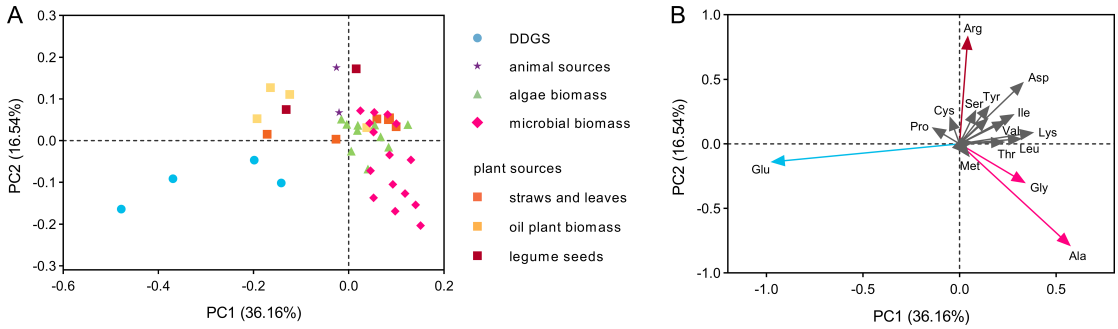

Figure S9 Amino acid composition of different protein sources. **a** Principle component analysis (PCA) of the amino acid composition of different raw materials, the proportion of variance accounted for by the two axes are presented in the parenthesis. **b** The distribution of individual amino acids in the corresponding PCA space. When projected onto the vectors, protein sources with projections located in the positive direction of a vector have higher abundance of the corresponding amino acid than that of other materials. For instance, the distillers dried grains with solubles (DDGS) is rich in glutamic acid and the bacterial biomass has a relatively high abundance of alanine and glycine. The DDGS used for analysis are sorghum DDGS [46], maize DDGS, wheat DDGS, and sugarbeet vinasse [47]; the animal sources of proteins are poultry feathers [47] and eggs [48]; the algae biomass include *Gracilaria* sp.[49], *Dromogomphus armatus* [50], *Chlorella* sp., *Spirulina* sp. [47], *Aphanizomenon* sp., *Chlorella vulgaris*, *Dunaliella bardawil*, *Scenedesmus obliquus*, *Arthrospira maxima*, and *pirulina platensis* [48]; the microbial biomass include *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *Saccharomyces uvarum*, *Saccharomycodes ludwigii* [51], *Brevibacterium methylicum* [52], *Bacillus subtilis* [52], *Rhodocyclus gelatinosus* [53], *Rhodopseudomonas capsulate* [53], *Corynebacterium glutamicum* [54], *Staphylococcus aureus* [55], *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus plantarum*, and *Streptococcus thermophilus* [56]; the straws and leaves protein sources are sugarbeet leaves [57], sugarcane leaves [58], cassava leaves [59], ryegrass meal [60], wheat straw and alfalfa hay [47]; the oil plant biomass are from jatropha meal [61], sunflower seed meal, rapeseed, and palmoil meal [47]; the legume seed biomass are soybeen [62] and carob germ [63].

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