

1 **Metabolic engineering of yeast for carbohydrate-derived foods and chemicals**
2 **production from C₁₋₃ molecules**

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26
27
28 **Abstract**

29 The increase in population-related and environmental issues has emphasized the need for more
30 efficient and sustainable production strategies for foods and chemicals. Carbohydrates are
31 macronutrients sourced from crops and undergone transformation into various products ranging
32 from foods to chemicals. Continuous efforts have led to the identification of a promising hybrid
33 system that couples the electrochemical reduction of carbon dioxide (CO₂) to intermediates
34 containing one to three carbons (C₁₋₃) with the transformation of the intermediates using
35 engineered microorganisms into valuable products. Here, we use yeast to transform C₁₋₃
36 substrates into glucose and structurally tailored glucose derivatives, such as the sugar alcohol
37 myo-inositol, the amino monosaccharide glucosamine, the disaccharide sucrose and the
38 polysaccharide starch. By metabolic rewiring and mitigation of glucose repression, the titer of
39 glucose and sucrose reached dozens of grams per liter. These results provide directions for
40 microbial sugar-derived foods and chemicals production from renewable reduced CO₂-based
41 feedstocks.

42
43 **Introduction**

44 Agriculture provides food and many raw materials for society, but this field is currently

45 facing enormous challenges. The growing world population, expected to reach almost 9–11
46 billion people by 2050, needs to be supplied with food and other agricultural products. The
47 global demand for food is projected to increase by 70 % by 2050^{1,2}. With limited arable land
48 and the growing threat of climate change, it will be nearly impossible for agriculture to meet
49 growing needs without a significant increase in agricultural productivity. Furthermore, the
50 atmospheric CO₂ concentration has increased sharply to 414 ppm in the past 50 years and is
51 still increasing, which may cause catastrophes with long-lasting effects in the future^{3,4}.
52 Therefore, we must find an economically viable strategy to fix CO₂ into useful nonfood
53 products without the use of arable land⁵. While natural photosynthesis can reduce atmospheric
54 CO₂, it is important to develop other methods of fixing CO₂ that are faster. Transformation of
55 atmospheric CO₂ by thermochemical⁶, electrochemical⁷⁻⁹, photochemical¹⁰, biochemical
56 approaches¹¹ and some coupled strategies^{12,13} into simple organic compounds with a carbon
57 chain length of C_{n≤3} (C₁₋₃) has made great progress in the past few decades. However, these
58 platforms cannot generate complex products or they require complicated in vitro catalytic
59 synthesis. Therefore, combining these platforms with well-known microbial processes that
60 metabolize C₁₋₃ substrates into long-chain compounds offers a promising method.

61 Carbohydrates, such as glucose, sucrose and starch, are some of the most abundant and
62 widely distributed organic substances in nature; furthermore, they are basic components of all
63 organisms. Carbohydrates account for up to 80% of total calorie intake in the human diet¹⁴.
64 Today, these carbohydrates and their derivatives are the raw materials for a growing diversity
65 of products including food, medicine, commodity and specialty chemicals¹⁵. Meanwhile,
66 recyclable food technologies are essential for long deep space missions¹⁶. Recently, the
67 National Aeronautics and Space Administration (NASA) launched a centennial challenge
68 focused on converting CO₂ into carbohydrates¹⁷. Several biologic or abiotic approaches have
69 been implemented to complete the conversion of CO₂ to carbohydrates¹⁸⁻²⁰. Microbial
70 transformation of C₁₋₃ molecules produced by the reduction in CO₂ into carbohydrates has
71 gained widespread interest¹⁹. This transformation may offer a sustainable alternative to produce
72 these products at lost-cost and faster with higher production capacity. The well-studied yeasts
73 *Saccharomyces cerevisiae*²¹ and *Pichia pastoris*²², have been used in the food industry for
74 centuries and are ideally suited for this purpose.

75 Here, we demonstrated a strategy to produce glucose by engineering the microbial
76 transformation of C₁₋₃ products (methanol, ethanol and isopropanol) from inorganic CO₂
77 fixation (Fig. 1). We further expanded the products to glucose derivatives, such as sugar
78 alcohols, amino monosaccharides, disaccharides and polysaccharides (Fig. 1). By metabolic
79 rewiring and alleviating glucose repression, the production of glucose and sucrose reached
80 more than 20 g L⁻¹. Glucose leaking yeast, which lacks glucose activation, could also be an
81 excellent model system for studying glucose effects rather than using a
82 nonmetabolizable glucose analog²³. The results demonstrate the technical feasibility of the
83 microbial production of glucose-derived food and chemicals by CO₂ reduction that is powered
84 by renewable energy. With further improvement, this may be an economically viable alternative
85 to agricultural production of these molecules (For more details see the Feasibility Analysis in
86 Supplementary Note). In a broader context, the strategy demonstrated here opens the possibility
87 of a renewable energy-driven agriculture and manufacturing industry and could provide a
88 framework for future carbon neutral bioproduction.

89

90 **Results**

91 **Production of glucose from C₁₋₃ molecules**

92 Remarkable achievements have been made in the electrochemical reduction of CO₂ into
93 C₁₋₃^{9, 24} products (*e.g.*, methanol, ethylene, ethanol, isopropanol) using renewable energy. A
94 long-term goal of this field is the direct recycling of CO₂ into higher carbon products, although,
95 this has rarely been realized²⁵. Using model microorganisms to convert the products of
96 inorganic carbon fixation into carbohydrates is a promising way to advance the vision of a
97 circular carbon economy. In our previous work, we described a hybrid electrobiosystem,
98 coupling spatially separate CO₂ electrolysis with yeast fermentation, which efficiently
99 converted CO₂ to acetate by electrolysis, and further to glucose using yeast with an average
100 glucose titer of 1.81 ± 0.14 g L⁻¹. To produce glucose using *S. cerevisiae*, a glucose leaky
101 phenotype was created through the deletion of all known hexokinases—Glk1, Hxk1, and Hxk2.
102 The resulting strain was named LY031²⁰.

103 To further explore the potential of using other products of electrochemical CO₂ reduction⁹,
104 we tested whether *S. cerevisiae* could use the C₁ chemicals methanol and formate, the C₂
105 chemicals ethylene glycol and oxalic acid, and the C₃ chemicals isopropanol and propionate as
106 carbon sources for cell growth and for the production of valuable products, with glucose serving
107 as an excellent representative compound. In addition, waste glycerol, which has been widely
108 used as an inexpensive carbon source for industrial microbiology, was also utilized. Strain
109 LY031 grew and produced glucose when ethylene glycol, isopropanol, propionate, glycerol, or
110 ethanol was used as the sole carbon source (Fig. 2a). This result suggests that cells may have
111 utilization pathways for these chemicals. For example, propionate can be converted to
112 propionyl-CoA by acetyl-CoA synthetase and then enter the methylmalonyl-CoA and 2-
113 methylcitrate pathways²⁶. It has been reported that ethylene glycol can be partially oxidized
114 to glyoxylate and further degraded in the glyoxylate degradative pathways^{27, 28}. Generally,
115 electrochemical reduction of CO₂ produces a variety of compounds, leading to an expensive
116 downstream purification process²⁹. Therefore, we hypothesized that we could grow yeast in a
117 mixture of electrochemical reduction products, as microorganisms naturally possess the ability
118 to metabolize multiple carbon sources simultaneously. To demonstrate this concept, we
119 selected ethylene glycol, isopropanol and propionate as constituents of the mixtures due to their
120 ability to be used by *S. cerevisiae*. Different electrocatalysts have been shown to produce a
121 variety of products in various ratios^{30, 31}, and thus different proportions of these compounds
122 were studied. We observed that the ratios of intermediates in the mixtures influenced glucose
123 production and cell growth. Specifically, when ethylene glycol, isopropanol and propionate
124 were present in a proportion of 1:2:3, we achieved a higher glucose titer of 0.72 g L⁻¹ and a
125 higher OD₆₀₀ of 3.87, compared to an equal ratio (1:1:1) (Supplementary Fig. 1a and 1b).
126 However, the addition of substrates that cannot be utilized to the mixture did not further
127 increase cell growth and glucose production (Supplementary Fig. 1a).

128 Except glycerol, the isopropanol culture had the highest OD₆₀₀ (~2.5) and glucose titer
129 (~0.20 g L⁻¹). To further improve isopropanol utilization, several heterologous pathways were
130 tested in *S. cerevisiae*^{32, 33} (Supplementary Figs. 2, 3 and 4a). We tried the pathway converting
131 isopropanol to acetyl-CoA using alcohol dehydrogenase (Adh), acetone carboxylase complex
132 (Acx), acetoacetyl-CoA synthetase (Aacs), and acetoacetyl-CoA thiolase (Aact)³². However,

133 isopropanol utilization was not improved (Supplementary Fig. 4b), even though all
134 heterologous enzymes were confirmed to be expressed based on proteomic analysis
135 (Supplementary Fig. 4c). Therefore, we pursued another strategy that proposed to transform
136 isopropanol into acetate and methanol by enzymatic conversion with Adh, the monooxygenase
137 AcmA and the hydrolase AcmB³³. The growth of the engineered strains was not improved using
138 isopropanol or acetone as the sole carbon source in different media (Supplementary Fig. 5a and
139 5b).

140 *S. cerevisiae* has almost no ability to consume formate or methanol (Fig. 2a). Methanol,
141 which is derived from the main greenhouse gases (methane and CO₂), is a potentially renewable
142 C₁ feedstock for biotransformation. Compared to *S. cerevisiae*, the methylotrophic yeast *P.*
143 *pastoris* has efficient pathways for methanol utilization and can grow using methanol as the
144 sole substrate. However, the engineering of *S. cerevisiae* for methanol utilization performed in
145 previous studies is still in its infancy. Hence, to further verify the generality of the strategy to
146 transform methanol into glucose, we constructed the glucose leaky phenotype in *P. pastoris* by
147 deleting the genes involved in glucose consumption, including all the hexokinase genes *HXK1*,
148 *HXK2*, *GLK1* and *HXK iso2* (encoding hexokinase isoenzyme 2) (Fig. 2b), as was done in *S.*
149 *cerevisiae*²⁰. Strain gsy012 (*glk1Δ*, *hvk1Δ*, *hvk2Δ* and *hvk iso2Δ*) generated glucose and the titer
150 achieved approximately 0.5 g L⁻¹ glucose in shake flasks at 96 h (Fig. 2c and Supplementary
151 Fig. 6a) with slight growth defect (Supplementary Fig. 6b). We speculate that the subsequent
152 dephosphorylation of glucose-1-phosphate is also performed properly, presumably by an
153 unknown or nonspecific phosphatase in *P. pastoris* (Fig. 2b). Strain gsy012 also showed
154 impaired growth in minimal medium with glucose as the sole carbon source (Fig. 2d and
155 Supplementary Fig. 6c), which further indicated the impaired activity of all hexokinases. To
156 improve the production of glucose by hydrolyzing glucose-1-phosphate to glucose, we
157 expressed haloacid dehalogenase-like phosphatase 4, YihX, from *Escherichia coli*³⁴ (strain
158 gsy013); this resulted in the production of approximately 1.08 g L⁻¹ glucose, a nearly 100%
159 improvement compared with gsy012 (Fig. 2c). The volumetric productivity of glucose
160 produced from methanol was determined to be 11.25 mg L⁻¹ h⁻¹, corresponding to a glucose
161 yield of 253.62 mg g⁻¹ dry cell weight (DCW). Finally, we engineered *P. pastoris* with the
162 isopropanol utilization pathway we engineered into *S. cerevisiae*. Unfortunately, the resulting
163 strains (Supplementary Fig. 2) did not utilize isopropanol or acetone for cell growth any better
164 than the wild-type strain when grown in several media conditions (Supplementary Fig. 7).

165 Using ethanol as the sole carbon source resulted in high cell growth and glucose
166 production due to the inherent ability of *S. cerevisiae* to grow on ethanol²⁰. This result suggests
167 that low glucose production from other low carbon chemicals may be attributed to weak
168 substrate degradation pathways rather than deficiencies in the glucose synthetic pathway, and
169 optimization of endogenous or heterologous utilization pathways of other various C₁₋₃
170 substrates needs to be further explored. Market analyses indicate ethanol offers greater promise
171 for the future because it possesses a larger market potential^{35, 36}, and thus considerable efforts have
172 been devoted to developing more efficient electrocatalysts for ethanol production, resulting in
173 significant advancements³⁷⁻³⁹. The faradaic efficiency (FE) of ethanol of approximately 50% is
174 lower than that of formic acid but higher than methanol and other C₂₊ compounds^{40, 41}. It is
175 noteworthy that ethanol possesses a combination of advantages over other C₂ compounds such
176 as easier bioavailability, a larger market demand and a high FE. Therefore, ethanol serves as an

177 attractive representative carbon source to expand the repertoire of carbohydrates and overcome
178 potential limitations associated with the inefficient utilization of other substrates.

179

180 **Expanding monosaccharide derivatives**

181 To expand the chemical space of glucose derivatives produced by the microbial-
182 electrochemical system, we engineered *S. cerevisiae* to produce other monosaccharides,
183 including hexose derivatives (Myo-inositol and glucosamine) and xylose derivatives (xylose
184 and xylitol) using ethanol as a main representative carbon source (Fig. 3a). Myo-inositol is an
185 important compound widely used in the pharmaceutical, cosmetic and food industries^{42, 43}.
186 Previously, *S. cerevisiae* and *P. pastoris* were engineered to produce myo-inositol, however,
187 glucose was used as the carbon source^{44, 45}. To efficiently produce myo-inositol from low
188 carbon substrates, the native inositol-3-phosphate synthase Ino1 was overexpressed and the
189 heterologous *E. coli*'s SuhB which possesses inositol monophosphatase activity was
190 introduced⁴⁴; the native myo-inositol transporter Itr1 was also overexpressed to increase the
191 secretion of myo-inositol into the medium. The optimized strain WX51 (Supplementary Fig.
192 1a) produced 228.71 mg L⁻¹ myo-inositol from ethanol in YP medium (Fig. 3b) and 89.58 mg
193 L⁻¹ myo-inositol in minimal medium (Supplementary Fig. 8a). In YP medium, the Myo-inositol
194 yield and productivity were found to be 47.26 mg g⁻¹ DCW and 1.91 mg L⁻¹ h⁻¹, respectively.
195 Additionally, we explored the use of isopropanol and glycerol as sole carbon sources for myo-
196 inositol production. Myo-inositol production reached 40.0 mg L⁻¹ using glycerol, while no
197 detectable myo-inositol was observed with isopropanol as the carbon source (Supplementary
198 Fig. 8b). For myo-inositol production from methanol, we expressed Ino1p and Irt1 from *P.*
199 *pastoris* and *E. coli* SuhB in the gsy002 strain (Supplementary Fig. 2). The resulting strain
200 RYT02 produced 129.67 mg L⁻¹ of myo-inositol from methanol (Supplementary Fig. 8b).

201 The amino monosaccharide glucosamine has extensive applications in food, cosmetics,
202 and medicines due to its diverse and specific bioactivities⁴⁶. Herein, glucosamine was produced
203 in *S. cerevisiae* from the endogenous precursor glucosamine-6-phosphate by expressing
204 glucosamine-6-phosphate phosphatase GlnP from *Bacteroides thetaiotaomicron*. In addition,
205 glucosamine-6-phosphate deaminase GlnD from *Bacillus subtilis* was also expressed to
206 increase the production of glucosamine-6-phosphate from fructose-6-phosphate. However, no
207 glucosamine was detected when one copy of each of the genes encoding these two enzymes
208 was expressed (Fig. 3c). Thus, we introduced another copy of the two genes and found that the
209 resulting strain CT02 (Supplementary Fig. 1) produced glucosamine at 30.09 mg L⁻¹.
210 Glucosamine production was further improved by increasing the copy number of *GLMP* to a
211 titer of 37.04 mg L⁻¹ in YP medium (Fig. 3c) or 19.83 mg L⁻¹ in minimal medium with ethanol
212 as the sole carbon source (Supplementary Fig. 9a). When using glycerol as the carbon source,
213 we observed a glucosamine production of 41.69 mg L⁻¹, whereas no detectable glucosamine
214 was observed using isopropanol (Supplementary Fig. 9b). By expressing two copies of *GLMP*
215 and *GLMD*, we achieved a glucosamine production of 29.08 mg L⁻¹ of from methanol
216 (Supplementary Fig. 9b).

217 D-Xylose and xylitol are typical five-carbon monosaccharides that are widely used as
218 diabetic sweeteners in foods and beverages⁴⁷; thus, we also tried to produce them from low
219 carbon sources. Xylose can be synthesized from the endogenous precursor xylulose by the *E.*
220 *coli* reversible xylose isomerase XylA⁴⁸. Xylitol can be generated from xylose by the native

221 aldose reductase Gre3 and can be cycled into the pentose phosphate pathway (PPP) via xylitol
222 dehydrogenase Xyl2 degradation (Fig. 3a). To reduce the consumption of xylulose-5-phosphate,
223 the transketolases Tkl1 and Tkl2 were deleted (Fig. 3a). Trace amounts (less than 6 mg L⁻¹) of
224 xylose were produced and then consumed later (Supplementary Fig. 10a) when XylA was
225 expressed, and no xylitol was detected. This suggests that the reversibility of XylA,
226 xylulokinase Xks1 and PPP, along with the presence of Gre3 and Xyl2 enable the yeast to
227 consume xylose. In order to allow xylose conversion into xylitol rather than consumption, we
228 further deleted Xyl2 and Xks1, replacing the latter with the irreversible phosphatase AraL from
229 *Bacillus subtilis*⁴⁹ (Fig. 3a). An additional copy of *XYLA* was expressed to strengthen xylose
230 synthesis. The resulting strain ET04 produced 4.30 mg L⁻¹ of xylitol from ethanol
231 (Supplementary Fig. 11). Furthermore, by blocking xylose degradation through the deletion of
232 Gre3, we were able to detect 3.52 mg L⁻¹ of xylose (Supplementary Fig. 10b).

233

234 **Expanding oligosaccharide and polysaccharide derivatives**

235 Oligosaccharides and polysaccharides, as well as glucose, are essential agricultural
236 carbohydrates that play a major role in human nutrition. Therefore, we first utilized ethanol to
237 produce these complex carbohydrates. Sucrose is a well-known oligosaccharide and is widely
238 used to produce foods, pharmaceuticals and bulk chemicals. Currently, the main source of
239 sucrose is extraction from sugar cane and sugar beets⁵⁰. The biosynthesis of sucrose in microbial
240 cell factories from low carbon substrates would be a remarkable achievement; however, this
241 has rarely been reported in yeast. To achieve de novo biosynthesis of sucrose from ethanol in
242 *S. cerevisiae*, two glucose-1-phosphate-based synthetic pathways were studied (Fig. 4a). The
243 biosynthesis of one downstream intermediate, UDP-glucose, was strengthened by
244 overexpression of native UDP-glucose pyrophosphorylase Ugp1 and another ADP-glucose was
245 generated by introduction of a heterologous nonregulated form of ADP-glucose
246 pyrophosphorylase GlgC-TM from *E. coli*⁵¹. UDP-glucose and ADP-glucose were
247 subsequently catalysed by sucrose-phosphate synthase Sps from *Synechocystis sp.*, along with
248 fructose-6-phosphate, to produce sucrose-phosphate. This sucrose-phosphate can be further
249 converted into sucrose by sucrose-phosphate phosphatase (Spp) from *Synechocystis sp.* The
250 sucrose transporter protein Suf1 from *Pisum sativum* is used to transport sucrose out of the
251 cell⁵². To block the sucrose degradation pathway in *S. cerevisiae*, we deleted all the genes
252 encoding sucrose-degrading enzymes, including invertase Suc2; maltases Mal12, Mal22, and
253 Mal32; and isomaltases Ima1, Ima2, Ima3, Ima4, and Ima5⁵⁰. The resulting strain AT03
254 (Supplementary Fig. 1) did not grow with sucrose as the sole carbon source, in contrast to the
255 wild-type strain, even though this strain grew normally in the presence of glucose
256 (Supplementary Fig. 12a). Furthermore, integration of *SUF1*, *SPS* and *SPP* into the AT03
257 genome resulted in strain AT05 which produced 0.82 g L⁻¹ sucrose in shake flasks (Fig. 4b).
258 Ugp1 and GlgC-TM were then expressed and the titer of sucrose was increased to 1.24 g L⁻¹,
259 which is a nearly 50% improvement compared to its parent strain; these results illustrated that
260 an increase in the precursors UDP-glucose and ADP-glucose can improve sucrose production.
261 To test whether the activity of Sps and Spp was sufficient for the conversion of the elevated
262 UDP-glucose and ADP-glucose, we added another copy of *SPS* and *SPP* to enhance their
263 expression. However, no remarkable titer improvement was observed (Fig. 4b), indicating that
264 Sps and Spp were not the limiting enzymes in the synthetic pathway. Most of the produced

265 sucrose was secreted into the medium, but approximately 20% was still partially retained in the
266 cells (Fig. 4b), which may be a result of insufficient sucrose transporters. Therefore, one or two
267 more copies of *SUF1* were further overexpressed, but we did not observe any improvement in
268 sucrose secretion (Fig. 4b). AT06 demonstrated a sucrose yield of 351.19 mg g⁻¹ DCW,
269 accompanied by a productivity of 9.79 mg L⁻¹ h⁻¹. To balance product synthesis with biomass,
270 cell growth was limited by nitrogen supply. There was no significant change in the production
271 capacity of the strains (Supplementary Fig. 12b and 12c), indicating that the sucrose leakage
272 phenotype is closely related to cell growth. In addition to ethanol, we also used isopropanol and
273 glycerol as sole carbon sources, and the production of sucrose reached 0.38 g L⁻¹ and 2.35 g L⁻¹,
274 respectively (Supplementary Fig. 12d). To investigate the possibility of synthesizing sucrose
275 from methanol, we integrated enzymes Sps and Spp, as well as the transporter Suf1, into the *P.*
276 *pastoris* strain gsy002, which natively cannot utilize sucrose as carbon source. Remarkably, the
277 resulting strain RYT03 produced 0.41 g L⁻¹ of sucrose (Supplementary Fig. 12d).

278 Starches, which are polysaccharides used for excess carbohydrate storage in plants, form
279 the basis of life-sustaining foods and play a primary feedstock role in bioindustries, such as
280 paper manufacturing and biodegradable materials^{53,54}. Recently, starch synthesis from CO₂ and
281 H₂ was significantly progressed in a complex cell-free system based on a chemical-biochemical
282 hybrid method¹⁹, although this process consumed a series of expensive purified enzymes. In
283 this study, we tried to achieve the concise microbial production of starch in *S. cerevisiae* from
284 CO₂ via its renewable low carbon electroderivatives. Previously, the core *Arabidopsis thaliana*
285 starch biosynthesis pathway (ASBP) was introduced in *S. cerevisiae* to study the effect of the
286 biosynthetic enzymes on glucan structure and solubility, and starch was produced in addition
287 to galactose⁵⁵; however, galactose is not a sustainable substrate. In addition, glucose-1-
288 phosphate could also be converted to starch by a one-step reaction catalysed by alpha-glucan
289 phosphorylase (Pgp)^{56, 57}, herein named the PGP pathway (PGPP). Therefore, two biological
290 pathways, ASBP and PGPP, were synergistically designed to synthesize starch (Fig. 4a). First,
291 we found that the wild-type strain had a high baseline determined by the starch assay kit
292 (Supplementary Fig. 13), and the *Solanum tuberosum* Pgp-expressing strain showed no obvious
293 starch production compared to the wild-type strain (Supplementary Fig. 14a), indicating that
294 endogenous glycogen metabolic pathways may interfere with starch synthesis. To reduce the
295 competitive carbon flux of native glycogen production, we deleted all enzymes including Glg1,
296 Glg2, Glc3, Gsy1 and Gsy2, to block the glycogen biosynthesis pathway. To avoid starch
297 hydrolysis, we also deleted the enzymes Gdb1 and Gph1 (BT13 strain) to abolish the glycogen
298 degradation pathway (Supplementary Fig. 1). To build ASBP, genes encoding ADP-glucose
299 pyrophosphorylase GlgC-TM from *E. coli*, starch synthase SS3, the branching enzyme BE3
300 and the isoamylases Isa1 and Isa2 from *A. thaliana* were integrated into the genome of the
301 glycogen-deficient strain, resulting in strain BT12 (Supplementary Fig. 1). This strain produced
302 ~0.20 g L⁻¹ starch from glucose, consistent with previous findings⁵⁵ (Supplementary Fig. 14b).
303 Pgp was then expressed in strain BT12 by galactose induction using a high-copy plasmid under
304 control of the strong galactose inducible promoter *SkGAL2* from *Saccharomyces kudriavzevii*⁵⁸;
305 the starch titer of this strain reached 0.52 g L⁻¹ (Supplementary Fig. 14b), revealing that Pgp
306 expression can improve starch production. Therefore, we integrated *PGP* into the genome of
307 BT12 for stable expression, resulting in strain BT14. Compared to BT12, BT14 had a higher
308 starch production titer of 1.0 g L⁻¹ which was a fourfold increase in titer without any growth

309 defects (Supplementary Fig. 14c); this result indicated that PGPP is the major contributor to
310 starch production. Next, we evaluated starch production from ethanol, and the results were
311 similar to those from glucose (Supplementary Fig. 14d). Galactose was required to induce the
312 PGPP because Pgp expression was driven by the *SkGAL2* promoter. To eliminate galactose
313 utilization, the gene encoding galactokinase Gal1, responsible for the conversion of galactose
314 into galactose-1-phosphate, was knocked out in BT12 and BT14, so that galactose would be a
315 gratuitous inducer⁵⁹. The resulting strains BT15 and BT16 produced starch at 63.78 mg L⁻¹ and
316 341.59 mg L⁻¹ (Fig. 4c), respectively. The starch content of BT16 reached 57.06 mg g⁻¹ DCW
317 (Fig. 4d), which is comparable to the result of starch biosynthesis using galactose as a carbon
318 source⁵⁵. For BT16, the yield of starch produced was 70.81 mg g⁻¹ DCW, and the productivity
319 was 4.62 mg L⁻¹ h⁻¹. Furthermore, we analyzed the starch production of BT16 using
320 isopropanol and glycerol as carbon sources. The production of starch was 26.34 mg L⁻¹ and
321 126.00 mg L⁻¹ from isopropanol and glycerol, respectively (Supplementary Fig. 15b). To
322 produce starch from methanol, we disrupted glycogen synthase and glycogenin
323 glucosyltransferase in *P. pastoris* to eliminate glycogen interference (Supplementary Fig. 15a)
324 and introduced ASBP and PGPP to construct strain RYT20 (Supplementary Fig. 2). RYT20
325 produced 480.08 mg L⁻¹ and 117.74 mg L⁻¹ of starch from glucose and methanol, respectively
326 (Supplementary Fig. 15a and 15b).

327

328 **Metabolic engineering for glucose overproduction**

329 The previous results demonstrated that microbial production of sugar and sugar derivatives
330 from low carbon sources is doable; thus, we metabolically engineered the microbial platforms
331 for high production to confirm our scheme for synthesizing carbohydrates. In this study, we
332 utilized glucose as the candidate molecule and ethanol as the sole carbon source for this initial
333 work. We first chose to optimize the glucose synthetic pathway by systematically manipulating
334 structural genes in yeast gluconeogenesis metabolism. Many of the reactions in glycolysis and
335 gluconeogenesis are reversible and used in both pathways. The two irreversible reactions
336 transforming pyruvate to phosphoenolpyruvate and fructose-1,6-bisphosphate to fructose-6-
337 phosphate determine the direction of carbon flow⁶⁰ (Fig. 5a). To enhance gluconeogenesis and
338 prevent upregulated glycolysis from glucose accumulation, we overexpressed
339 phosphoenolpyruvate carboxykinase Pck1, responsible for transforming oxaloacetate to
340 phosphoenolpyruvate, and deleted pyruvate kinases Pyk1 and Pyk2, enzymes that can convert
341 phosphoenolpyruvate to pyruvate, resulting in a 24.14% improvement in glucose production
342 compared to LY031 (Fig. 5b). The overexpression of fructose-1,6-bisphosphatase Fbp1, which
343 transforms fructose-1,6-bisphosphate to fructose-6-phosphate, and the deletion of
344 phosphofructokinases Pfk1 and Pfk2, which normally convert fructose-6-phosphate to fructose-
345 1,6-bisphosphate, had no significant effect on glucose production (Fig. 5b).

346 Since the impact of enhancing glucose production by manipulating structural genes is limited,
347 we next sought to develop strategies to increase the flux toward glucose synthesis. Glucose is
348 the preferred carbon source for *S. cerevisiae*. While yeast cells possess the capacity to utilize a
349 variety of carbon sources, it is noteworthy that the presence of glucose inhibits molecular
350 processes associated with the utilization of alternative carbon sources and inhibits the use of
351 the glyoxylate cycle, respiration and gluconeogenesis²³ for cell growth. The repressive impact
352 of glucose on yeast carbon metabolism is orchestrated through a complex interplay of multiple

353 signaling and metabolic interactions (Fig. 5a). The production of glucose or its derivatives, such
354 as glucosamine⁶¹, may generate a glucose repressive effect, which results in reduced yeast
355 growth and low glucose productivity, thereby inhibiting the use of alternative carbon sources.
356 Thus, there is much interest in rewiring the signaling pathway in microbial platforms to abolish
357 glucose repression. We hypothesized that glucose repression can be alleviated or removed if
358 the regulatory mechanism is properly perturbed and if the regulators that have been reported to
359 regulate glucose repression are manipulated. Snf1 protein kinase signaling is at the heart of
360 glucose repression. The transcriptional repressor Mig1 is the main downstream target of Snf1
361 phosphorylation (Fig. 5a). It is believed that one of the main functions of Mig1 is to inhibit the
362 transcription of genes involved in gluconeogenesis and respiration when glucose is present⁶².
363 As shown in Fig. 5c, the deletion of Mig1 had almost no effect on glucose production, which
364 suggests the presence and importance of other downstream targets for Snf1. When glucose
365 levels are high, the Snf1 kinase complex loses activity due to self-inhibition resulting from the
366 interaction between its N-terminal catalytic domain and the regulatory domain of the C-
367 terminus. Low concentrations of glucose eliminate this self-inhibition to release Snf1 and allow
368 catalytic activity. In addition, modification of the C-terminal inhibition regulatory subunit from
369 the Snf1 protein also eliminates this self-inhibition. To abolish this self-inhibition, amino acids
370 381-414 and 381-488 of Snf1 were removed separately⁶³. Strain LY037 (Snf1^{aa381-488Δ})
371 generated glucose at 0.280 g L⁻¹ OD₆₀₀⁻¹, which is a 144.63% improvement compared to the
372 0.114 g L⁻¹ OD₆₀₀⁻¹ of strain LY039 (Snf1^{aa381-414Δ}) and a 237.46% improvement compared to
373 the 0.083 g L⁻¹ OD₆₀₀⁻¹ of strain LY031. These results show that relieving glucose repression is
374 conducive to glucose synthesis. However, growth defects limited the application of this strategy
375 (Supplementary Fig. 16a). Activation of Snf1 requires phosphorylation. Phosphatase Glc7 can
376 dephosphorylate Snf1 and is considered the main regulator of Snf1 activity⁶⁴. Reg1 is the
377 regulatory subunit of Glc7 and is involved in the negative regulation of glucose-repressible
378 gene expression⁶⁵. Deletion of Reg1 led to a strain that produced glucose at 0.254 g L⁻¹ OD₆₀₀⁻¹
379 ¹, which was 2.05-fold higher than that produced by LY031, and this strain produced glucose
380 at 4.27 g L⁻¹ (Fig. 5c and Supplementary Fig. 16a). The yield of glucose from ethanol was 1.25
381 g g⁻¹ DCW, and the productivity was 35.59 mg L⁻¹ h⁻¹. In addition, the interaction between the
382 glucose-responsive transcription factor Rgt1 and the Snf1 kinase is critical for hierarchical
383 derepression of the expression of the glucose transporter Hxt; furthermore, this interaction plays
384 an important role in overall glucose repression⁶⁶. To investigate the effect of glucose transport,
385 Rgt1 was deleted to strengthen the inhibition of Hxt expression with or without overexpression
386 of the low-affinity glucose transporter Hxt1 or the high-affinity glucose transporter Hxt4; the
387 cell growth and titer of glucose decreased significantly in all engineered strains even though
388 the specific glucose production was similar to that of LY031 (Fig. 5c and Supplementary Fig.
389 16a). Taken together, these results indicate that efficient glucose export is necessary to alleviate
390 glucose repression and promote cell growth. The Ras-cAMP pathway is one of the main glucose
391 signaling pathways involved in posttranslational regulation by phosphorylation⁶⁷. The G-
392 protein coupled receptor Gpr1 activates the adenylyl cyclase Cyr1 through the GTPase Gpa2
393 when it responds to external glucose, resulting in a high level of cAMP. Additionally, the
394 GTPases Ras1,2 can also stimulate Cyr1, leading to a rapid increase in cAMP accumulation.
395 The elevated cAMP level causes a dissociation of the catalytic Tpk and regulatory Bcy1
396 subunits of PKA, leading to the activation of PKA to phosphorylate downstream targets^{68,69}. To

397 prevent hyperaccumulation of intracellular cAMP, the phosphodiesterases Pde1,2 are
398 responsible for regulating cAMP levels by degrading cAMP⁷⁰. Systematic manipulation of this
399 pathway had no significant effect on cell growth and glucose production (Fig. 5c and
400 Supplementary Fig. 16b). In summary, the systematic optimization and redesign of glucose
401 repression was key to improving the production of glucose and its derivatives.

402 Previously, glucosamine was also shown to have repressive effects similar to glucose⁶¹.
403 To study whether the positive modification of the glucose repressive pathway could increase
404 the production of glucosamine, we deleted Reg1, and the glucosamine titer was enhanced to
405 69.99 mg L⁻¹; however, deletion of Hxk2 had no effect (Fig. 3c). The glucosamine yield was
406 24.51 mg g⁻¹ DCW and the productivity was 0.58 g L⁻¹ h⁻¹. Compared with glucose, the low
407 titer of glucosamine may be caused by the strong inhibition of GlmD and Gfa1 by glucosamine-
408 6-phosphate⁷¹. To further strengthen gluconeogenesis by alleviating glucose repression for
409 sucrose production, we deleted Hxk2 or Reg1 in strain AT06. However, the *hvk2Δ* strain AT11
410 produced less sucrose, whereas no detectable change was observed in the *reg1Δ* strain AT10
411 (Supplementary Fig. 17). AT10 displayed a sucrose yield of 437.81 mg g⁻¹ DCW, with a
412 corresponding productivity of 10.98 mg L⁻¹ h⁻¹. These results suggest that mitigation of glucose
413 repression favors the accumulation of products that are the most significant effectors of glucose
414 repression (e.g., glucose and glucosamine).

415

416 **Fed-batch fermentation of the engineered strains**

417 Shake flask evaluations are valuable for strain comparisons, however, they tend to
418 underestimate the strain's potential due to the constraints imposed by limited culture controls,
419 such as O₂ levels and pH. Thus, we characterized the best *P. pastoris* strain (gsy013) and *S.*
420 *cerevisiae* strains (LY033 and AT10) for glucose and sucrose production from C₁₋₃ substrates
421 in fed-batch cultures. First, we evaluated the use of C₁ substrate methanol for glucose
422 production. The gsy013 strain produced 13.41 g L⁻¹ glucose and reached a DCW of 44.37 g L⁻¹
423 by consuming 163.65 g methanol at 288 h (Fig. 6a). The yield and productivity of glucose
424 produced from methanol by gsy013 using fed-batch fermentation were 0.30 g g⁻¹ DCW and
425 46.55 mg L⁻¹ h⁻¹, respectively, which were higher than those obtained through flask
426 fermentation. Next, LY033 was used to produce glucose from C₂ substrate ethanol, and it
427 produced 20.11 g L⁻¹ glucose (Fig. 6b). The final ethanol consumption of 202.97 g and the
428 highest DCW of 52.38 g L⁻¹ at 233 h were observed with LY033 (Fig. 6b). The yield of glucose
429 produced from ethanol by LY033 using fed-batch fermentation was determined to be 0.35 g g⁻¹
430 DCW, which represented a 2.5-fold decrease compared to flask fermentation. This decrease
431 suggests the glucose repression effect, which can be triggered at low glucose concentrations
432 and becomes stronger as the glucose concentration increases⁷², may be a rate-limiting step for
433 high glucose production in fed-batch fermentation. The productivity was 78.44 mg L⁻¹ h⁻¹ which
434 was higher than flask fermentation. Furthermore, we used gsy013 to produce glucose from C₃
435 substrate glycerol. The strain gsy013 achieved a glucose production of 13.82 g L⁻¹ from 262.48
436 g of glycerol within 263 h (Fig. 6c). The highest DCW of 74.70 g L⁻¹ was observed with the
437 gsy013 strain (Fig. 6c). Finally, we evaluated the production of sucrose as additional product
438 alongside glucose. AT10 consumed 286.48 g ethanol, grew to a DCW of 72.94 g L⁻¹, and
439 produced 25.41 g L⁻¹ sucrose at 261 h (Fig. 6d). The yield of sucrose produced from ethanol by
440 AT10 using fed-batch fermentation was 332.84 mg g⁻¹ DCW, which was comparable to flask

441 fermentation, while the productivity reached 92.54 mg L⁻¹ h⁻¹, significantly higher than that of
442 flask fermentation. There was no significant accumulation of byproducts in the fermentation
443 process (Supplementary Figs. 18-21). It is worth noting that glucose repression was partially
444 alleviated by LY033, however, even with this alleviation, the glucose production remained
445 lower compared to that observed with sucrose. This indicates that glucose repression poses
446 challenges for efficient sugar production, and further exploration of glucose repression
447 modulation is necessary to enhance the production. These results indicate that the microbial
448 production of glucose-derived chemicals from C_n ≡ 3 has great potential for commercial
449 application.

450

451 Discussion

452 The innovative potential of synthetic biology has led to a surge in interest in using recent
453 advances to address sustainability challenges. One of the most important and attractive
454 challenges is to efficiently assimilate CO₂ in the atmosphere to produce food, fuels and
455 chemicals, which can greatly compensate for the shortcomings of traditional agricultural and
456 industrial production. In this study, we mainly focused on the microbial conversion of low
457 carbon chemicals (C₁₋₃), which can be produced from CO₂ using mature electrochemical
458 strategies, into various sugars and their derivatives.

459 There exist several catalytic routes (electrocatalysis, thermal catalysis or photocatalysis)
460 to produce low carbon chemicals from CO₂ with negative greenhouse gas emissions⁷³. In the
461 future, many more low carbon chemicals could be produced. Biological metabolism and
462 utilization of these low carbon chemicals is the main gateway between renewable energy and
463 more complex molecules. In current microbial cell factories, the utilization of sugars extracted
464 from lignocellulosic feedstock remains a challenge. Therefore, expanding the range of
465 substrates that can be used by microbial cell factories is important. Improvement of endogenous
466 catabolic pathways or the introduction of heterologous metabolic pathways to consume low
467 carbon chemicals from CO₂ fixation is one promising direction. Here, various low carbon
468 chemicals were tested as the sole carbon source for yeasts, and the results revealed that yeasts
469 can utilize methanol, ethylene glycol, isopropanol and propionate to grow and produce glucose.
470 In addition, the protein content of these engineered strains reached about 50% of the cell dry
471 weight (Supplementary Fig. 22), indicating that single cell protein can be produced
472 accompanied with sugar generation⁷⁴. In the future, *S. cerevisiae* could be further engineered
473 with the integration of functional heterologous pathways for efficient utilization of other
474 chemicals, such as methanol and formate. A better understanding of the principles of low
475 carbon metabolism and the development of methods to enhance their efficiency is critical to
476 achieving sustainability.

477 In this study, we detailed the high-titer production of glucose, sucrose, starch, and several
478 monosaccharide derivatives, including myo-inositol, and glucosamine. The low xylose yield
479 could be attributed to two potential limiting factors. Xylose synthesis occurs through the pentose
480 phosphate pathway (PPP). However, in *S. cerevisiae*, the PPP plays only a relatively minor role,
481 with only approximately 2.5% of the glucose being metabolized through the oxidative PPP under
482 standard growth conditions⁷⁵. In contrast, other yeasts exhibit a more balanced contribution from
483 PPP and glycolysis in glucose degradation. Consequently, we believe that the low carbon source
484 flow flux might be one of the limiting factors for reduced xylose yield in *S. cerevisiae*. Additionally,

485 the reversibility of xylose isomerase⁷⁶ and low expression activity in *S. cerevisiae*⁷⁷ may serve as
486 another limiting factor for low xylose production. To enhance practical applications, additional
487 efforts in metabolic engineering and enzyme engineering are essential to augment the
488 production yield and rate of these sugars and sugar derivatives from low carbon chemicals.
489 Glucose production is particularly challenging due to the complex regulation of glucose
490 metabolic pathways. Glucose production was increased significantly by metabolic engineering
491 of the glucose synthetic pathway and the glucose repression pathway, which provided a
492 paradigm for improving other products. For products with a glucose effect, further mitigation
493 of glucose repression is essential. The effect of *reg1Δ* and *snf1Δ*³⁸¹⁻⁴⁸⁸ truncation on glucose
494 production is not completely consistent (Fig. 5c and Supplementary Fig. 16a), which implies
495 the existence of a potentially unknown bypass regulation mechanism²³. The yeast *S.*
496 *cerevisiae* has long been used as a model for studying glucose repression. To study glucose
497 repression, nonmetabolizable glucose analogs have been widely used to mimic glucose⁷⁸.
498 Without glucose phosphorylation/consumption, the glucose leaking yeast could be an excellent
499 model system for studying the glucose effect (Fig. 5a), rather than using nonmetabolizable
500 glucose analogs²³. We achieved the secretion of monosaccharides and the oligosaccharide
501 sucrose but not starch. In the future, engineering yeast to secrete starch would decrease the
502 purification cost and increase its yield; therefore, these methods are worthy of investigation.

503 In summary, this work demonstrates the practical use of microbial gluconeogenesis
504 metabolism and glucose repression. By combining the overexpression of different terminal
505 conversion enzymes to enhance gluconeogenesis while alleviating glucose repression, the
506 gluconeogenesis metabolism pathway is efficiently diverted to produce glucose-6-phosphate,
507 an important core precursor for the production of sugars and sugar derivatives. The engineering
508 strategy supports the production of these products and shows great potential for commercial
509 production. The production of these sugars and sugar derivatives from low carbon raw materials
510 demonstrates a necessary and promising step toward realizing a sustainable and more efficient
511 bioprocess than what is available in plants. In a broader context, we believe that the strategy
512 demonstrated here can significantly contribute to the ultimate goal of producing scalable and
513 more efficient sugar-derived foods and renewable chemicals.

514

515 **Methods**

516 **Strains, plasmids and reagents.**

517 In this study, all employed plasmids and strains are shown in Supplementary Table 1 and
518 Supplementary Table 2, respectively. 2×Phanta[®] Max Master Mix (Catalog ID: P515) and
519 2×Phanta[®] Max Master Mix (Dye Plus) (Catalog ID: P525) were purchased from Vazyme
520 Biotech (Nanjing) Co., Ltd. Gibson assembly kit (Catalog ID: E5510S) and restriction enzyme
521 Dpn1 (Catalog ID: R0176S) were purchased from New England Biolabs (Beijing) LTD.
522 Plasmid miniprep (Catalog ID: DP105), DNA cycle pure kit (Catalog ID: DP204) and DNA gel
523 purification kit (Catalog ID: DP209) were purchased from TIANGEN Biotech (Beijing) Co.,
524 Ltd. Codon-optimized genes were synthesized and purchased from Sangon Biotech (Shanghai)
525 Co., Ltd. and are listed in Supplementary Table 3. Total starch assay kit (Catalog ID: K-TSTA-
526 100A) was purchased from Megazyme Ltd. D-xylose content assay kit (Catalog ID: BC4395)
527 was purchased from Solarbio Science & Technology (Beijing) Co., Ltd. The information for all
528 chemicals, including Catalog ID and sources, were listed in Supplementary Table 4.

529

530 **Strain cultivation**

531 The plasmids were constructed and propagated using the *Escherichia coli* strain Trans5 α .
532 These *E. coli* strains were grown in Luria–Bertani medium, which consisted of 5 g L⁻¹ yeast
533 extract, 10 g L⁻¹ tryptone and 10 g L⁻¹ NaCl. The cultures were maintained at 37 °C and could
534 either include or exclude 100 μ g mL⁻¹ of ampicillin.

535 *S. cerevisiae* strain and *P. pastoris* strain were cultivated in yeast extract peptone medium
536 (YP) consisting of 10 g L⁻¹ yeast extract, 20 g L⁻¹ peptone and 20 g L⁻¹ glucose (YPD), or 20 g
537 L⁻¹ ethanol (YPE) or 10 g L⁻¹ glycerol, 5 g L⁻¹ methanol (YPMG), at 30 °C, 200 rpm for normal
538 cultivation and preparation of competent cells. Strains containing *URA3*-based plasmids were
539 cultivated in synthetic complete (SC) medium without uracil, which contained 8 g L⁻¹ SC/-Ura
540 broth and 20 g L⁻¹ glucose or 20 g L⁻¹ ethanol. The *URA3* marker plasmids were removed by
541 using SC + 5-FOA plates, which consisted of 8 g L⁻¹ SC/-Ura broth, 100 mg L⁻¹ uracil, 0.8 g L⁻¹
542 ¹ 5-fluoroorotic acid and 20 g L⁻¹ glucose or 20 g L⁻¹ ethanol.

543 Shake flask batch fermentations for production of glucose, glucosamine, Myo-inositol,
544 xylose, xylitol and sucrose were carried out in YP or minimal medium containing 7.5 g L⁻¹
545 (NH₄)₂SO₄, 14.4 g L⁻¹ KH₂PO₄, 0.5 g L⁻¹ MgSO₄·7H₂O, 60 mg L⁻¹ uracil, trace and vitamin
546 solutions, and supplemented with 20 g L⁻¹ ethanol or 20 g L⁻¹ methanol or 20 g L⁻¹ glycerol or
547 20 g L⁻¹ isopropanol as the carbon sources⁷⁹. Initially, single colonies were inoculated into 2 ml
548 of liquid medium to establish 24 h precultures, and then precultures were inoculated in 100 mL
549 non-baffled flasks with 20 mL liquid medium at an initial OD₆₀₀ of 0.2 for ethanol, 0.5 for
550 glycerol and methanol, 4 for isopropanol, and cultivated at 200 rpm, 30 °C for 120 h. Shake
551 flask batch fermentations for the production of starch were performed in YPD, YP with 20 g L⁻¹
552 ¹ galactose (YPGal), YPE and SC medium without uracil containing 20 g L⁻¹ glucose (SCG) or
553 20 g L⁻¹ galactose (SCGal). 24 h precultures were inoculated into 100 mL non-baffled flask
554 with 20 mL YPD, YPE or SCG at an initial OD₆₀₀ of 0.2 and cultivated at 200 rpm, 30 °C for
555 48 h, and then galactose was added for 120 h; The fermentation was performed for 24 h in YP
556 medium with an initial OD₆₀₀ of 0.5 and a carbon source composition of 20 g L⁻¹ methanol and
557 5 g L⁻¹ glucose.

558

559 **Genetic manipulation**

560 In this study, the background strain for all genetic manipulations in *S. cerevisiae* was
561 Lab001, derived from CEN.PK113-5D. Supplementary Table 5 lists all the primers used in this
562 study. The deletion of genes and the integration of expression cassettes were carried out using
563 the CRISPR/Cas9 system⁸⁰. To identify potential guide RNAs (gRNAs) for specific target genes,
564 we used the Yeaststriction webtool (<http://yeaststriction.tnw.tudelft.nl>). The construction of gRNA
565 plasmids based on the backbone plasmid pLY001²⁰. The fragment containing gRNA sequences
566 and the backbone amplified from pLY001 were assembled by Gibson assembly method to
567 obtain gRNA plasmids⁸¹. These constructed plasmids were performed sequencing verification.
568 For the amplification of native promoters, genes, homology sequences and terminators, Lab001
569 genomic DNA served as the template. For codon-optimized genes (Supplementary Table 3),
570 amplification was performed using synthetic plasmids from Sangon Biotech as templates. To
571 assemble the expression cassettes or perform gene deletion repairs, we employed a fusion PCR
572 approach. To begin, primary fragments with overlapping sequences were initially generated via

573 PCR, employing the primers provided in Supplementary Table 5. Following this, the purified
574 PCR products were subjected to a subsequent PCR reaction, omitting the use of any primers,
575 in order to produce the complete fusion gene. Subsequently, this fusion fragment served as the
576 template for the final PCR step, utilizing primers. The assembled fusion fragments and gRNA
577 plasmids were subsequently utilized for yeast transformation. For the construction of *PGP*
578 encoding plasmid, the high copy plasmid pJFE3 with a *UAR3* marker was used as the backbone,
579 and the inducible promoter *SkGAL2* and *PGP* were inserted into pJFE3 by Gibson assembly
580 method to form plasmid pTht013.

581 For *P. pastoris*, we used strain GS115 as the foundational strain for all genetic
582 manipulation. Supplementary Table 5 provides a compressive list of all primers used in this
583 study. To facilitate the deletion of genes and the integration of expression cassettes, we
584 employed the CRISPR/Cas9 system⁸². For the identification of potential gRNAs for targeting
585 gene, we utilized the CRISPRdirect webtool (<http://crispr.dbcls.jp>). All gRNA plasmids were
586 constructed based on the backbone plasmid BB3cH_pGAP_23*_pLAT1_Cas9 gifted by
587 Professor Gao, and their accuracy was verified by sequencing. To amplify native promoters,
588 genes, homology sequences and terminators, we used GS115 genomic DNA as a template. *E.*
589 *coli* *YIHX* encoding haloacid dehalogenase-like phosphatase was synthesized with codon
590 optimization (Supplementary Table 3) and was amplified from the synthetic plasmid from
591 Sangon Biotech as a template. Expression cassette construction and gene deletion repairs were
592 carried out by fusion PCR as described above. DNA transformation was conducted using a
593 condensed electroporation method⁸³. The transformed cells were cultivated for three days on
594 YPD or YPMG plates containing 100 ug mL⁻¹ hygromycin.

595

596 **Test of various low electro-carbon sources**

597 For the glucose production of *P. pastoris* using methanol as the carbon source, all the
598 strains were precultured in 2 mL YPMG at 30 °C for 24 h. Then, yeast cells were harvested by
599 centrifugation at 4000 × *g* for 5 min, and inoculated into 20 mL minimal medium containing
600 20 g L⁻¹ methanol and 0.1 g L⁻¹ Histidine at an initial OD₆₀₀ of 0.5 and cultivated at 200 rpm,
601 30 °C for 96 h. For the spot assay, *P. pastoris* cells were washed twice in sterile water and
602 serially diluted 10-fold up to 10⁻⁴. 5 μL of each dilution was spotted onto the indicated agar
603 plates (minimal medium containing 0.1 g L⁻¹ Histidine and 20 g L⁻¹ glucose). Plates were
604 incubated at 30 °C for 3-4 days.

605 For *S. cerevisiae* strain LY031, 10 g L⁻¹ of methanol, formate, ethylene glycol, oxalic acid,
606 isopropanol, propionate and glycerol was used as the carbon source, respectively. In addition,
607 10 g L⁻¹ of a mixture with 2.5 g L⁻¹ ethylene glycol, 2.5 g L⁻¹ oxalic acid, 2.5 g L⁻¹ isopropanol
608 and 2.5 g L⁻¹ propionate was also used as the carbon source. The precultures of the LY031 strain
609 in YPE were inoculated into 20 mL minimal medium with 10 g L⁻¹ yeast extract and various
610 carbon sources at an initial OD₆₀₀ of 0.2 and cultivated at 200 rpm, 30 °C for 120 h. To test the
611 utilization of isopropanol, all engineered strains were cultivated in YP or minimal medium with
612 20 g L⁻¹ isopropanol for 120 h to measure OD₆₀₀.

613

614 **Fed-batch fermentation**

615 For the *S. cerevisiae* fed-batch fermentation, single colonies were initially introduced into
616 2 mL of liquid medium for 24 h precultures, and then precultures were transferred to 250 mL

617 non-baffled flask with 50 mL liquid medium. These strains were grown at 30°C until OD₆₀₀ to
618 3~5. Fed-batch fermentations were performed in 1.3 L Eppendorf DASGIP Parallel Bioreactors
619 System with an initial volume of 0.5 L with an initial OD₆₀₀ of 0.3. Prior to the experiment, the
620 pumps, pH probes, and dissolved oxygen (DO) probes were calibrated. The bioprocess was
621 monitored and controlled using the DASGIP Control 5.0 System. The temperature, agitation
622 and aeration were kept at 30 °C, 800 rpm and 36 sL h⁻¹ respectively. The pH was automatically
623 maintained at 5.6 through the addition of 4 M NaOH or 2 M HCl, and the acid, alkali and
624 ethanol feed were carried out using DASGIP MP8 multi-pump modules (pump head tubing:
625 0.5 mm ID, 1.0 mm wall thickness). Gas composition was continuously monitored with a
626 DASGIP Off Gas Analyzer GA4, aeration was controlled and provided by a DASGIP MX4/4
627 module, and temperature and agitation were maintained by a DASGIP TC4SC4 module. During
628 the initial batch phase of the process, the strains were cultured in a minimal medium containing
629 5 g L⁻¹ (NH₄)₂SO₄, 3 g L⁻¹ KH₂PO₄, 0.5 g L⁻¹ MgSO₄·7H₂O, 60 mg L⁻¹ uracil, trace metal and
630 vitamin solution, 3% v/v ethanol, 1% galactose and 1% yeast extract were supplied additionally
631 for growth. After ethanol and galactose were consumed, ethanol was added and injected through
632 a septum in the bioreactor head plate with a syringe. The salt stock solution containing 50 g L⁻¹
633 (NH₄)₂SO₄, 150 g L⁻¹ KH₂PO₄, 25 g L⁻¹ MgSO₄·7H₂O, 3 g L⁻¹ uracil, trace metal and vitamin
634 solution was also fed according to carbon source addition. Dry cell weight analysis was
635 performed by filtrating 3 mL of broth through a pre-weighed 0.22 µm filter membrane. After
636 filtration, the filter was washed three times and then dried in a 65 °C oven for 48 h. Additionally,
637 1 mL of samples was centrifuged and stored at -20 °C for subsequent HPLC analysis.

638 For the *P. pastoris* fed-batch fermentation, the temperature, agitation and aeration were
639 kept at 30 °C, 800 rpm and 36 sL h⁻¹, respectively. The pH was maintained at 5.0 by automatic
640 addition of 4 M NaOH or 2 M HCl. The dissolved oxygen level was set at ≥10%. Medium was
641 utilized as previously⁸⁴. The composition of the medium in the initial batch phase for growth
642 was: 25 g L⁻¹ glycerol, 12.6 g L⁻¹ (NH₄)₂HPO₄, 0.02 g L⁻¹ CaCl₂·2H₂O, 0.5 g L⁻¹ MgSO₄·7H₂O,
643 0.9 g L⁻¹ KCl, and 4.35 mL L⁻¹ PTM1 trace salts stock solution and 0.01 g L⁻¹ histidine. After
644 glycerol was consumed, methanol was added by pulse feeding as described above. The salts
645 stock solution containing nitrogen consisted of 50 g (NH₄)₂SO₄, 150 g KH₂PO₄, 6.45 g
646 MgSO₄·7H₂O, 0.35 g CaCl₂·2H₂O, and 12 mL PTM1 trace salts stock solution per liter
647 methanol. 3 mL of samples were collected every 12 h for dry cell weight analysis as above, and
648 1 mL of samples were centrifuged and stored at -20 °C for HPLC analysis.

649

650 **Metabolite extraction and analysis**

651 At the end of shake flask cultivation, all samples were collected and subsequently
652 centrifuged. The supernatant was subjected to membrane filtration (0.22 µm) and frozen at
653 -20 °C for the quantification of extracellular glucose, glucosamine, Myo-inositol, xylose,
654 xylitol, and sucrose. Intracellular sucrose was extracted according to the previous study⁸⁵. In
655 brief, the pelleted cells were washed with sterile water and suspended in 1 mL of 80% ethanol
656 (v/v) and then incubated at 65 °C for 4 h, which resulted in nearly complete extraction of
657 compounds with low molecular mass. After centrifugation at 20000 × g for 5 min, the
658 supernatants were collected and then dried at 40 °C under a steam of N₂. The dried samples
659 were dissolved in ultrapure water and filtered for analysis.

660 A UPLC-MS system equipped with a Jet Stream Technology electrospray ion source

661 (1290-6470, Agilent Technologies, Santa Clara, CA, USA) was used for the analysis of
662 glucosamine, and sucrose. Poroshell 120 HILIC-OH5 analytical column (2.1*100 mm, 2.7 μ m,
663 Agilent Technologies, Santa Clara, CA, USA) was used for the separation of glucosamine and
664 sucrose. The program for sample analysis was carried out as follows. Samples were eluted with
665 solvent A (water with 0.1% of formic acid and 5mM ammonium acetate) and solvent B (80%
666 acetonitrile in water with 0.1% of formic acid and 5mM ammonium acetate) by the following
667 gradient program at a flow rate of 0.3 mL min⁻¹: 0-3 min, 100%-95% solvent B; 3-6 min, 95%-
668 84% solvent B; 6-11 min, 100% solvent B. The injected volume was 2 μ L and the column
669 temperature was set at 30 °C. The flow and temperature of the sheath gas were set at 11 mL
670 min⁻¹ and 250 °C, respectively, and the temperature of the nebulizer gas was set at 350 °C. The
671 pressure of the nebulizer was 35 psi. The capillary voltage was set at 3500 V for the positive
672 ionization mode. Multiple reaction monitoring (MRM) was selected as scan mode to detect
673 precursor \rightarrow product ion transitions. Thus, m/z transitions were 365 \rightarrow 202.8 (CE: 21) and
674 365 \rightarrow 184.7 (CE: 21 V) for sucrose. The Glucosamine hydrochloride m/z transitions were
675 202 \rightarrow 142.8 (CE: 9) and 202 \rightarrow 111.9 (CE:9 V). Aminex HPX-87H analytical column (7.8*300
676 mm, Biorad, Santa Clara, USA) was used for the separation of xylose and xylitol. Samples were
677 eluted with solvent A (water with 0.1% formic acid) using the following gradient program at a flow
678 rate of 0.6 mL min⁻¹. The injected volume was 5 μ L and the column temperature was set at 60 °C.
679 The sheath gas flow rate was configured to 12 mL min⁻¹, and its temperature was maintained at
680 350 °C. The nebulizer gas temperature was also set at 350 °C. The pressure of the nebulizer was 45
681 psi, and the capillary voltage was established at 4000 V for positive ionization mode. Single ion
682 monitoring (SIM) was selected as scan mode, xylitol (m/z:175) and xylose (m/z:173). Xylose
683 concentration was also analyzed by D-xylose assay kit according to its instruction.

684 Starch was quantified by using a total starch assay kit following its instruction. In brief,
685 the washed pellet cells were resuspended in sterile water and transferred into clean tubes along
686 with glass beads (0.5 mm, Biospec, US), and then mechanically disrupted in a tissue grinding
687 machine (10 times for 30 s each). After centrifugation at 20000 \times g for 5 min, the supernatant
688 containing soluble starch and the cell debris containing insoluble starch were collected,
689 respectively. 2 μ L of undiluted thermostable α -amylase was added to 200 μ L of each sample
690 and the mixture was boiled with metal bath at 300 rpm. After 15 min incubation, the temperature
691 was reduced to 50 °C and allowed samples to equilibrate to temperature over 5 min. Next, 0.1
692 mL of undiluted AMG was added and incubated at 50 °C for 30 min with no further mixing.
693 After incubation, samples were cooled to room temperature and then 10 μ L of each sample was
694 added into 3.0 mL of GOPOD reagent for incubation at 50 °C for 20 min. The absorbance of
695 the reaction product was measured at 510 nm.

696 Glucose, Myo-inositol and extracellular metabolites were quantified using the HPLC
697 system (Agilent Technologies 1260 Infinity II SFC). This system is equipped with an Aminex
698 HPX-87H column (Bio-Rad) and a G1362A RID (Agilent Technologies 1260 Infinity II).
699 Especially, pyruvate was detected using the 1260 Infinity II Diode Array Detector WR. The
700 column was eluted with a 5 mM H₂SO₄ at a flow rate of 0.6 mL min⁻¹ at a temperature of 50 °C.

701

702 **Data availability**

703 Source data are provided with this paper.

704

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722

723 **Author contributions**

724 T.Y. and J.D.K. conceived this study. H.T., L.W. and S.G. designed and performed most
725 of the experiments, analyzed the data and drafted the manuscript. W.M., X.W. and J.S. assisted
726 with the experiments and products detection. W.C. assisted with data analysis and interpretation.
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729

730 **Competing interests**

731 J.D.K. has a financial interest in Amyris, Lygos, Demetrix, Napigen, Maple Bio, Apertor
732 Labs, Zero Acre Farms, Berkeley Yeast, and Ansa Biotechnology. X.L. has a financial interest
733 in Demetrix and Synceres. All other authors declare no competing interests.

734

735 **Figure legends**

736 **Fig. 1 Roadmap for production of glucose-derived chemicals from renewable electricity-**
737 **driven substrates.** C₁, C₂ and C₃ chemicals including methanol, ethanol, ethylene glycol,
738 isopropanol and propionate generated by the electrochemical reduction of CO₂ were used as
739 the carbon sources to generate target products. In addition, industry waste glycerol was also
740 used as a carbon source. Yeast cell factories were explored to produce monosaccharide
741 derivatives including glucose, myo-inositol, glucosamine and xylose, and polysaccharide
742 derivatives sucrose and starch. Fructose-6-P, fructose-6-phosphate; Glucose-6-P, glucose-6-
743 phosphate; Glucose-1-P, glucose-1-phosphate; Glucosamine-6-P, glucosamine-6-phosphate;
744 Inositol-1-P, inositol-1-phosphate; sucrose-6-P, Sucrose-6-phosphate; UDP-glucose, uridine
745 diphosphate glucose.

746

747 **Fig. 2 Biorefinery of renewable raw materials from C₁₋₃ substrates.** a. Growth of engineered
748 *S. cerevisiae* and glucose production in different C₁₋₃ substrates. *S. cerevisiae* strain LY031 was

749 cultivated in a minimal medium with 10 g L⁻¹ yeast extract containing 10 g L⁻¹ of methanol,
750 formate, ethanol, ethylene glycol, oxalic acid, isopropanol, propionate or glycerol, respectively.
751 The data of cell growth and glucose production were subtracted from the background in the
752 absence of a carbon source. **b.** Schematic representation of biosynthetic modifications to
753 produce glucose from methanol in *P. pastoris*. Blue arrows, overexpressed genes; gray arrows
754 marked with red X, deleted genes, XuMP, xylulose monophosphate. **c.** Engineered *P. pastoris*
755 produced glucose from methanol at 120 h. **d.** Growth of engineered *P. pastoris* strains in the
756 presence of glucose. Statistical analysis was performed using one-tailed Student's t test (***P*
757 < 0.001). The *P*-values for the comparisons between group gsy007/gsy003, and group
758 gsy013/gsy010 were 0.00050, and 0.00141, respectively. All data are presented as mean ± SD
759 of biological triplicates (n=3).

760

761 **Fig. 3 Production of monosaccharide derivatives.** **a.** Construction of synthetic pathways for
762 the production of glucosamine, myo-inositol and Xylitol. Blue arrows, overexpressed genes;
763 gray arrows marked with red X, deleted genes; PPP, pentose phosphate pathway. **b.** The
764 production of myo-inositol from 120 h fermentation. **c.** The production of glucosamine from
765 120 h fermentation. 3+, three copies of *GLMD* or *GLMP*, 4+, four copies of *GLMP*. Statistical
766 analysis was performed using one-tailed Student's t test (***P* < 0.01). The *P*-values for the
767 comparisons between group CT03/CT02, and group CT04/CT03 were 0.00525, and 0.00398,
768 respectively. All data are presented as mean ± SD of biological triplicates (n=3).

769

770 **Fig. 4 Production of oligosaccharide and polysaccharide derivatives.** **a.** Biosynthetic
771 pathways for the production of sucrose and starch. Blue arrows, overexpressed genes; gray
772 arrows marked with red X, deleted genes. **b.** Sucrose production in engineered *S. cerevisiae*
773 strains from 120 h fermentation. Orange bars indicate amounts of sucrose secreted into the
774 medium (extracellular), and blue bars indicate amounts of sucrose retained in cells
775 (intracellular). **c.** Starch production in engineered *S. cerevisiae* strains from 120 h fermentation.
776 **d.** Starch content of dry cell weight. DCW, dry cell weight. Soluble, soluble starch; Insoluble,
777 insoluble starch, ASBP, *Arabidopsis* starch biosynthesis pathway; PGPP, PGP pathway which
778 converts precursor glucose-1-P to starch with alpha-glucan phosphorylase PGP. Statistical
779 analysis was performed using one-tailed Student's t test (**P* < 0.05, ** *P* < 0.01). The *p*-values
780 for the comparisons between group AT06/AT05, group BT16/BT15 for starch production, and
781 group BT16/BT15 for starch content were 0.01434, 0.00469 and 0.00471, respectively. All data
782 are presented as mean ± SD of biological triplicates (n=3).

783

784 **Fig. 5 Metabolic rewiring of *S. cerevisiae* for glucose overproduction.** **a.** One excellent
785 model system for studying glucose effect rather than using non-metabolizable glucose analog.
786 Biosynthetic modifications to the main glucose repression pathway in *S. cerevisiae* to enhance
787 glucose production. PEP, phosphoenolpyruvate; OAA, oxaloacetate; TCA, tricarboxylic acid;
788 Blue arrows, overexpressed genes; gray arrows marked with red X, deleted genes; gray circles,
789 deleted regulators; blue circles, activated regulators. **b.** Manipulating structural genes in yeast
790 gluconeogenesis for glucose production. **c.** Manipulating regulatory genes in glucose repression
791 pathway for glucose production. All engineered strains were cultivated for 120 h and the
792 samples were used for glucose analysis. Statistical analysis was performed using one-tailed

793 Student's t test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). The P -values for the comparisons
794 between group LY045/LY034, group LY033/LY031, group LY037/LY031, group
795 LY067/LY031, and group LY074/LY031 were 0.00317 0.00000, 0.00008, 0.03685 and 0.03078,
796 respectively. All data are presented as mean \pm SD of biological triplicates (n=3).

797

798 Fig. 6 **Fed-batch fermentation of C₁₋₃ substrates by engineered strains.** **a.** Glucose
799 production from C₁ substrate methanol by *P. pastoris* gsy013. **b.** Glucose production from C₂
800 substrate ethanol by *S. cerevisiae* LY033. **c.** Glucose production from C₃ substrate glycerol by
801 *P. pastoris* gsy013. **d.** Sucrose production from ethanol by *S. cerevisiae* AT10. DCW, dry cell
802 weight. Data of a, c and d are presented as mean \pm SD of biological triplicates and data of b are
803 presented as mean \pm SD of biological duplicates (n=3).

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