Supplementary information

Gene amplification on demand accelerates cellobiose utilization in engineered *Saccharomyces cerevisiae*

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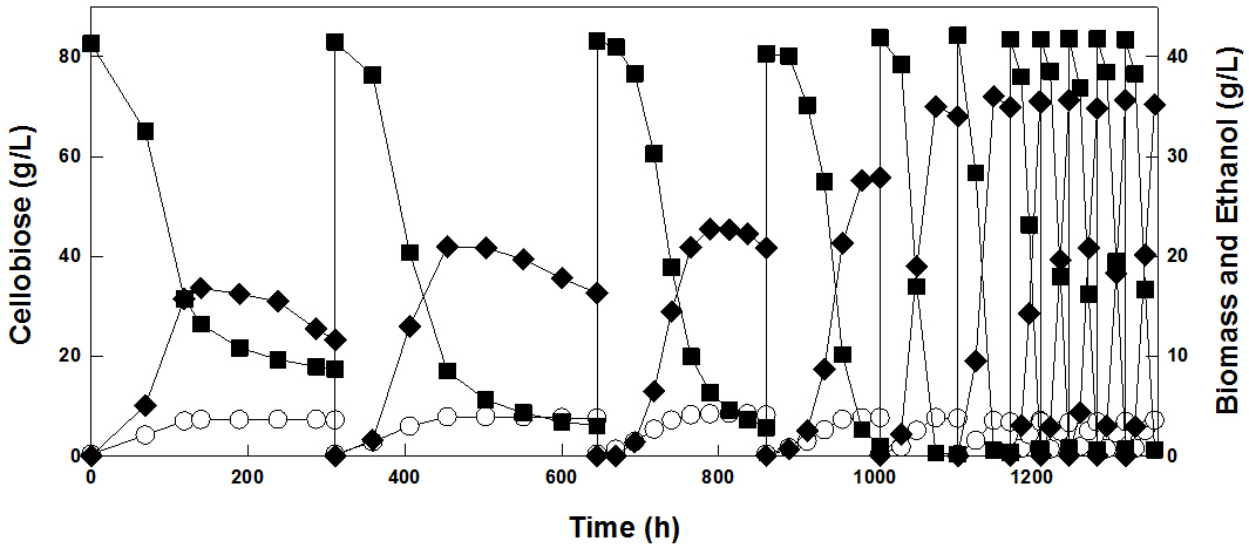

Figure S1. Cellobiose fermentation profiles by engineered *S. cerevisiae* EJ1 strain during serial subcultures. The subcultures were performed under oxygen-limited conditions (100 rpm) in YP medium with 80 g/L of cellobiose, and the initial cell density of each subculture was adjusted to 0.29 g/L (OD_{600} = 1). Symbols: biomass (open circle), cellobiose (solid square), and ethanol (solid diamond).

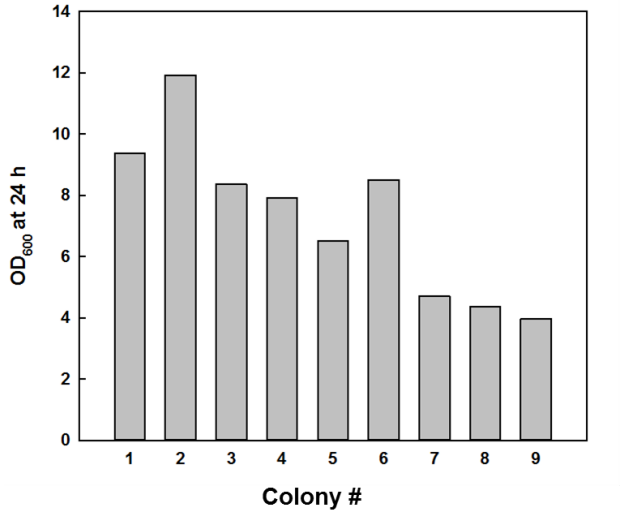

Figure S2. Comparison of cell growth of the nine colonies isolated from the last subculture of laboratory evolution. The cultures were performed under aerobic conditions (250 rpm) in YP medium with 80 g/L of cellobiose, and the initial cell density of each subculture was adjusted to 0.029 g/L (OD_{600} = 0.1).

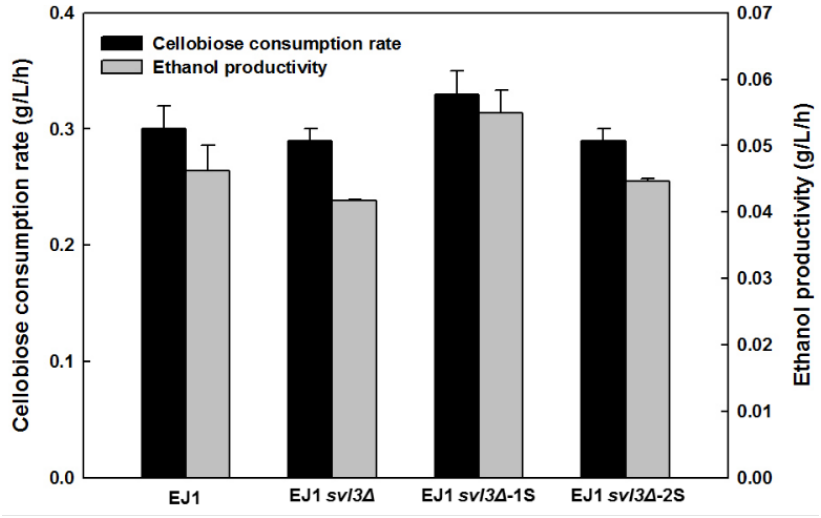

Figure S3. Effects of *SVL3* deletion on cellobiose fermentation. EJ1, D452-2 expressing low copies of cellodextrin transporter (*cdt-1*) and β-glucosidase (*gh1-1*); EJ1 *svl3Δ*, *SVL3* deletion in the EJ1 strain; EJ1 *svl3Δ*-1S, EJ1 *svl3Δ* with overexpression of *SVL3* cloned from the EJ1 strain (wild type *SVL3*); EJ1 *svl3Δ*-2S, EJ1 *svl3Δ* with overexpression of *SVL3* cloned from the EJ2 strain (mutant *SVL3*). Fermentations were performed in YP medium containing 80 g/L cellobiose under oxygen-limited conditions. The initial cell density was adjusted to 0.029 g/L (OD_{600} = 1). The results are the means of duplicate experiments and the error bars indicate standard deviations.

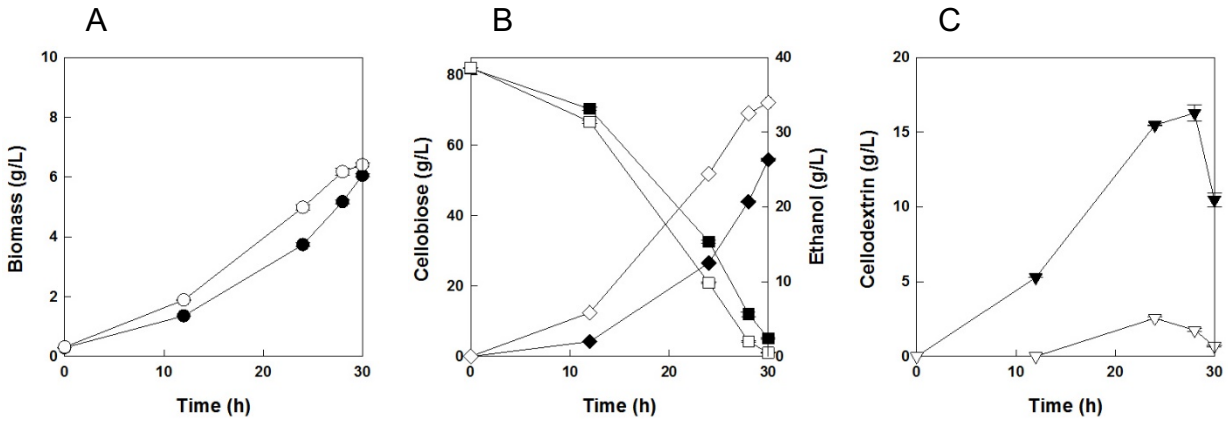

Figure S4. Fermentation profile comparisons between engineered *S. cerevisiae* DCDT-1 (solid symbols) and EJ2 (open symbols). (A) Cell biomass (circle). (B) Cellobiose (square) and ethanol (diamond). (C) Cellodextrin (triangle down). Fermentations were performed in YP medium containing 80 g/L cellobiose under oxygen-limited conditions. The initial cell density was adjusted to 0.29 g/L (OD_{600} = 1). The results are the means of duplicate experiments and the error bars indicate standard deviations.

Table S1. Primers used in this study. Restriction sites are underlined.

Name	Sequences
T3/T7 insert-F	ACGCCAAGCGCGCAATTAACC
T3/T7 insert-R	CGGCCAGTGAGCGCGCGTAAT
T3/T7 vector-F	GAGCTCCAGCTTTTGTTCCCT
T3/T7 vector-R	GGTACCCAATTCGCCCTATAG
SVL3 deletion-F	AACATGAAAATTTTTTATTTTTTTTCATTCGTTTATACC
SVL3 deletion-R	GATTTCTTTCTTTCTACGCTGGGATA
SVL3 cloning-F	GCCGGATCCAAAA ATGTCGTCTTCCTCACTTCGAGT
SVL3 cloning-R	GCCCTCGAG TTATTTCTTTGATTTATTTTTTTTTTGAATAGGCCAA
CDT1 qPCR-F	TCCAATATCAAGCCCTGGAG
CDT1 qPCR-R	GGACCAGTGTCACCAGTGTG
GH1-1 qPCR-F	CAAGCACTGGATCACCTTCA
GH1-1 qPCR-R	TGAGCGATGAGCAGGTTATG