

**Carbon-economic biosynthesis of poly-2-hydrobutanedioic acid
driven by nonfermentable substrate ethanol**

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Fig. S1. Colony morphology of *A. pullulans* on plate cultures with different concentrations of carbon sources. A–E. 1%–5% (m/v) glucose plate culture colony morphology. F–J. 1%–5% (v/v) ethanol plate culture colony morphology.

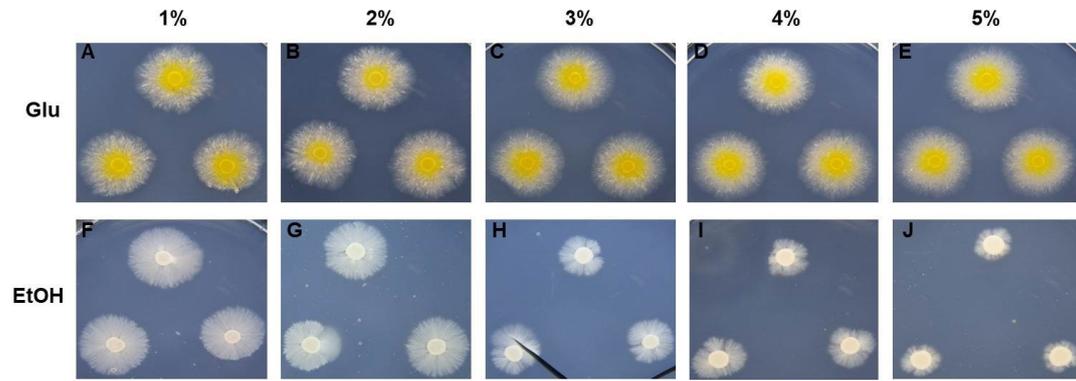


Fig. S3. Schematic showing homologous replacement of *cat8* by homologous donor DNA-mediated HDR via CRISPR-Cas9 system.

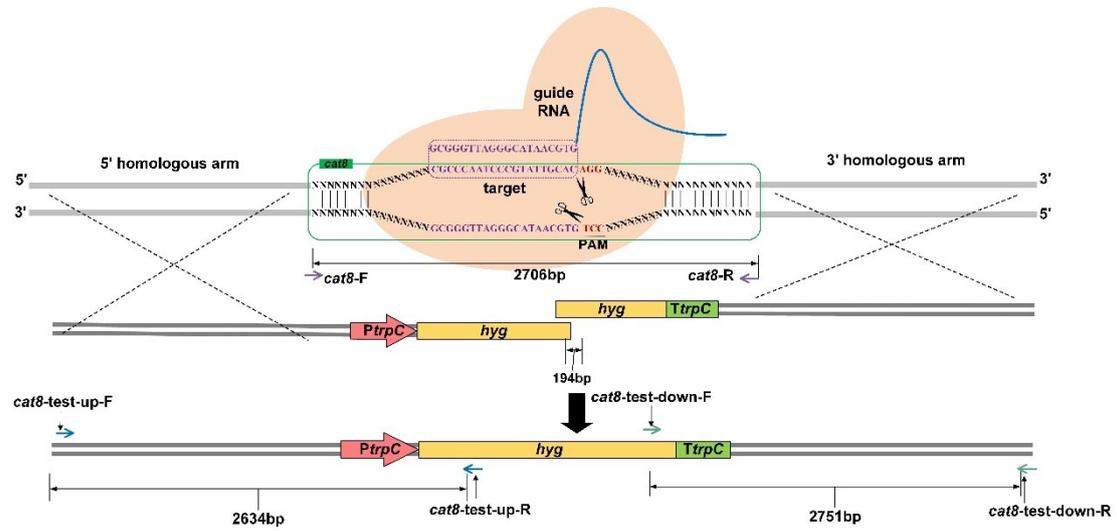


Fig. S4. Analysis of the relative mRNA levels of key genes in the glyoxylic acid shunt and the P2HBD synthesis. A. Relative mRNA levels of *icl1* in YJ-ICL1. B. Relative mRNA levels of *icl2* in YJ-ICL2. C. Relative mRNA levels of *mls* in YJ-MLS. D. Fermentation changes of different mutants in shake flask compared with WT. *: $0.01 < p < 0.05$, **: $0.001 < p < 0.01$, ***: $0.0001 < p < 0.001$.

