

Title: Supplementary Data 1.

Legends: The designed sequences of neo-chromosomes and the identified variations of assembled neo-chromosomes.

Title: Supplementary Data 2.

Legends: Differentially expressed genes (DEG) in corresponding strains, including ptWT10-10KO, ptWT10U-10KO, yWT12, yWT25 and ySYN24. DESeq2 was used to calculate Benjamini-Hochberg adjusted p-values and fold change (FC) values. Differential expression in this study was defined as $|\log_2FC| > 1$ and $-\log_{10}(\text{Adjusted p-value}) > 4$. BY4742 was used as the control strain.

Title: Supplementary Data 3.

Legends: Sequences of reconstructed TUs, synthetic promoters and synthetic terminators.

Title: Supplementary Data 4.

Legends: Strains used in this study.

Title: Supplementary Data 5.

Legends: Oligonucleotides used in this study.