Supplementary Files

Supplementary File 1. Composite view of all metabolomics profiles, including a table of targeted metabolites that passed quality control. See details in file.

Supplementary File 2. Excel file with primary metabolomics data. See details in file.

Supplementary File 3. Excel template for performing resection ddPCR calculations.

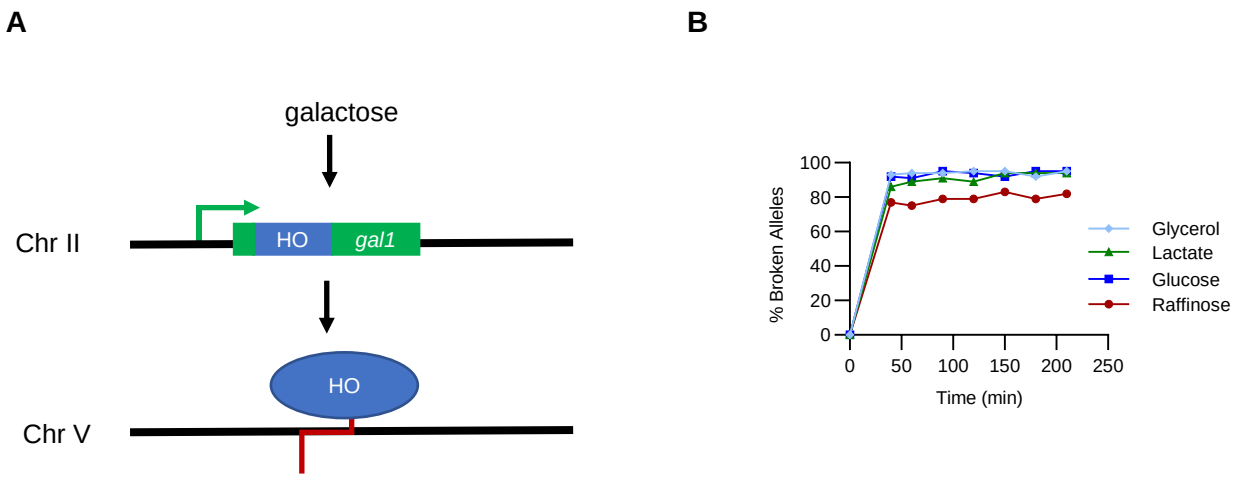
Supplementary File 4. R script that generated all metabolomics figures in the study. See comments in file.

Supplementary Table 1: Yeast strains used in this work.

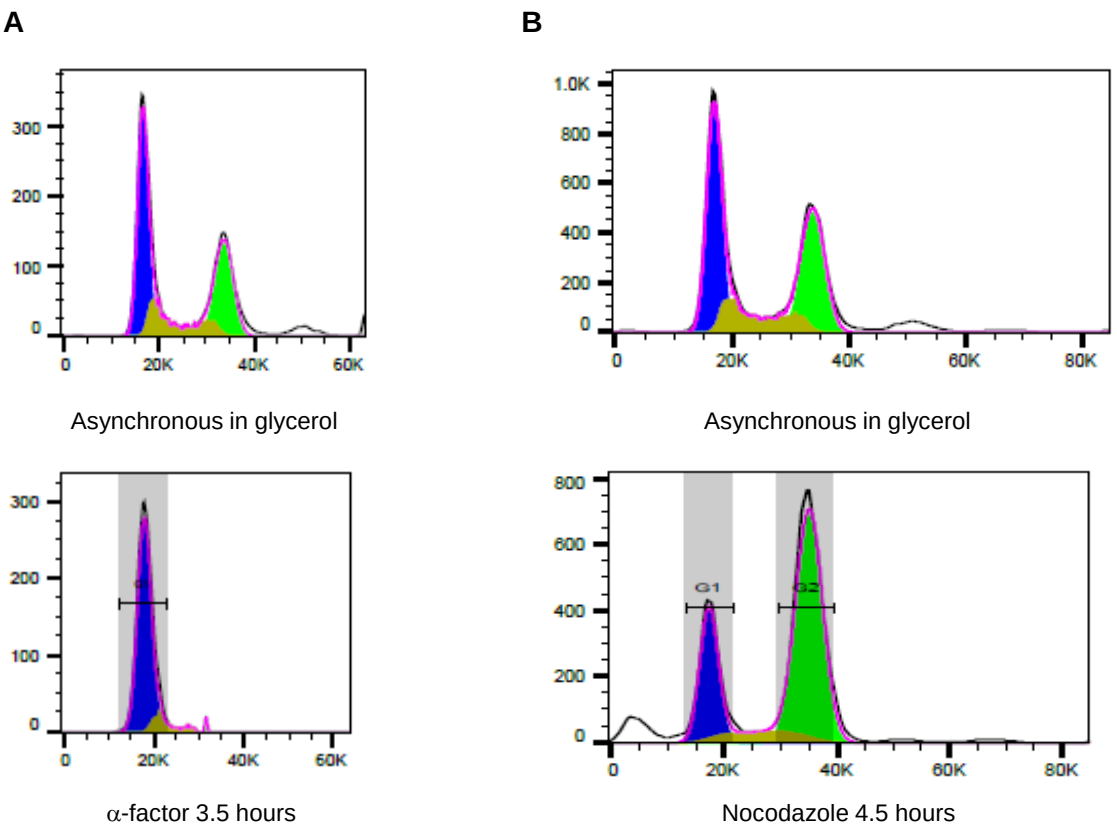
Name	Genotype
YW3104	*MAT*a-inc::*LEU2 can1*Δ::*ILV1*-QPCR *gal1*::HO *his3*Δ1 *ILV1*prm::HOcs *ILV1*Reg::Ori-His3MX6 *bar1*Δ *leu2*Δ0 *met15*Δ0 *ura3*Δ0 *dnl4*-K466A
YW3081	YW3104 *mig1*Δ
YW3178	YW3104 *sak1*Δ
YW3179	YW3104 *gal83*Δ
YW3225	YW3104 HOcs ACA deletion
YW3227	YW3104 *rph1*Δ
YW3278	YW3104 *tpk1*Δ
YW3279	YW3104 *tpk2*Δ
YW3280	YW3104 *tpk3*Δ
YW3284	YW3104 *hxk2*Δ

Supplementary Table 2: Primers and probes for resection ddPCR assay.

Name	Target	Sequence
OW3058	Forward primer *ACT1*	AGAGTTGCCCCAGAAGAACA
OW3059	Reverse primer *ACT1*	GGCTTGGATGGAAACGTAGA
OW4100	*ACT1* MGBLQ probe VIC	TGACTGAAGCTCCAATGAACCCT
OW3991	Forward primer HOcs	AATAAGAAGGGCAAAAAGAAAAAGC
OW3992	Reverse primer HOcs	AAAGCAGCAACAACAAAAGTTTTC
OW4221	HO cut site MGBNFQ probe FAM	CGCTTTTAGTTTCAGCTTTCCGCA
OW4182	Forward primer BglII 400 bp away	TCCCGTCTAAACACGAATGTCA
OW4183	Reverse primer BglII 400 bp away	GGTGTACAAACAGGCATAACGATA
OW4184	BglII site 400 bb MGNFQ probe FAM	ATCATGCCCAAGGTGTGGCC
OW4141	Forward primer BglII 1.2 kb away	TGGTGAAGGAAAGGAAGTCT
OW4142	Reverse primer BglII 1.2 kb away	GCTTGATTTCCTTTTCTCTGTCA
OW4140	BglII site 1.2 kb MGBNFQ probe FAM	ACCCGACGTCCCTGGTGCGTTCA



Supplementary Figure 1. DSB induction systems. (A) *gal1*-HO system, induced by galactose. **(B)** HO cutting efficiency of *gal1*-HO system in various carbon sources. The target HOcs and resulting DSB are in the native yeast *ILV1* promoter on ChrV.



Supplementary Figure 2. Flow cytometry DNA content histograms of G1 arrest and G2 arrest. (A) Asynchronous culture in glycerol (top) and then after addition of α-factor (bottom). **(B)** Asynchronous culture in glycerol (top) and then after the addition of nocodazole (bottom). Cells growing in glycerol (unlike glucose) never achieved complete G2/M arrest in nocodazole but the fraction of G1 cells dropped considerably from 62% to 18%.