Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary Information

NMR analysis for budding yeast metabolomics: a rapid method for sample preparation

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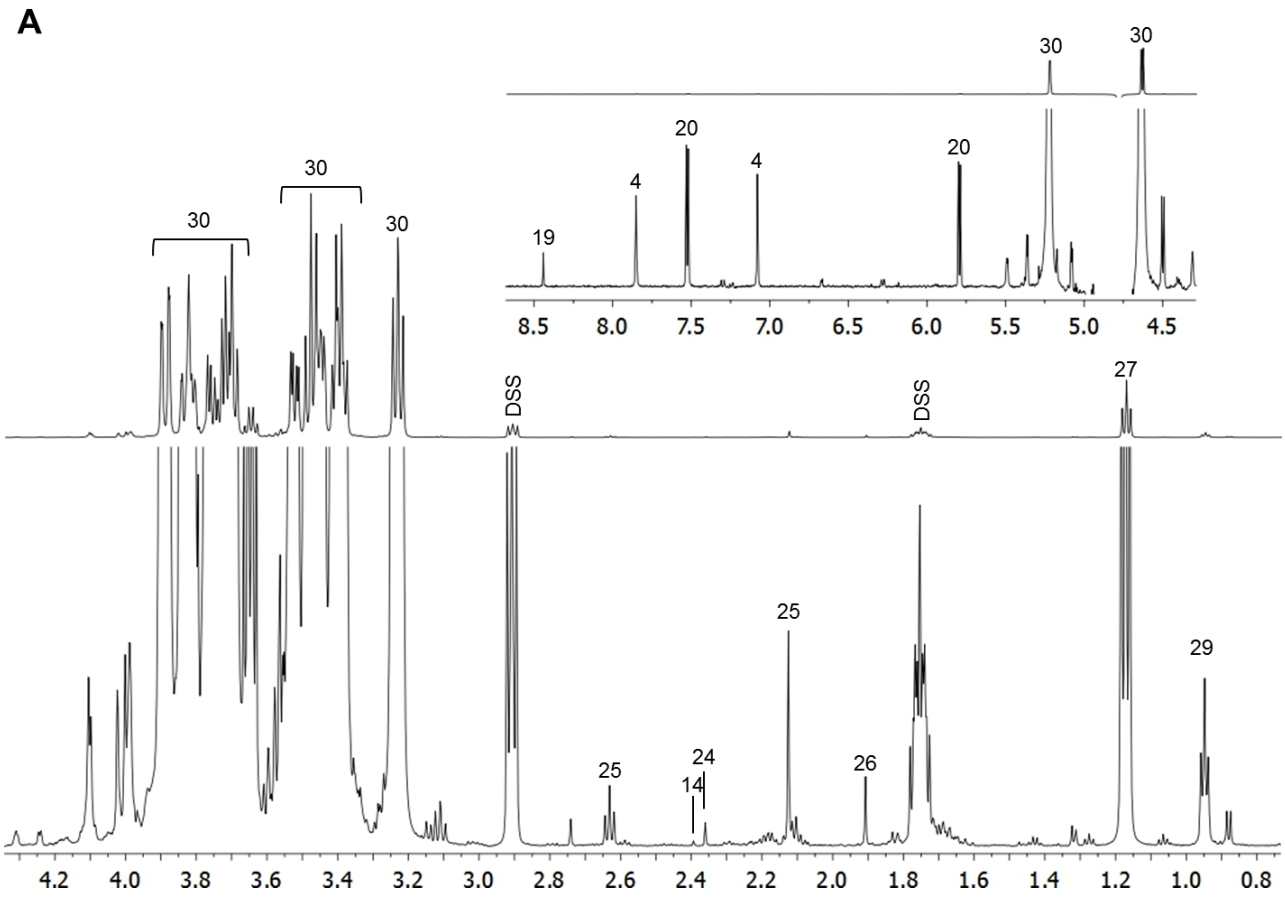
Supplementary figures



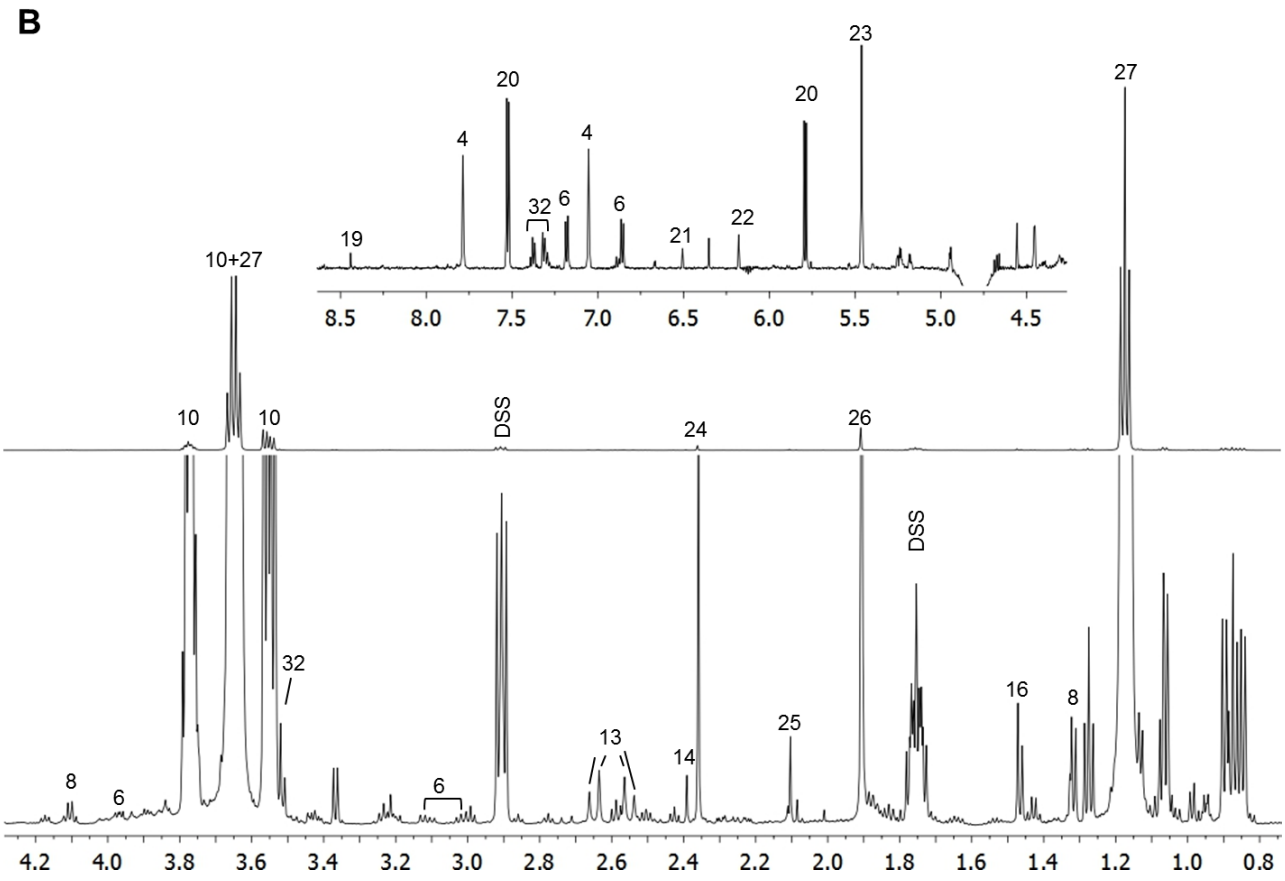


Fig. S1 Representative ^{1}H NMR spectra of media collected in exponential (**A**) and stationary (**B**) growth phase. Numbers correspond to metabolite assignments reported in Table 1 (main text).

Materials and Methods

Strains, media and culture conditions

Saccharomyces cerevisiae strains wt and *gpd2Δ* strains are isogenic to BY4741 (*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*) and were obtained from the Euroscarf collection (http://web.uni-frankfurt.de/fb15/mikro/euroscarf/). The auxotrophic wild-type strain GRF18c (*Matα*) was also tested. Cells were grown in synthetic complete dextrose (SCD) medium, prepared by assembling 2% glucose, 6.7 g/liter yeast nitrogen base (Difco), 50 mg/liter of required amino acids. Cells were grown until mid-exponential phase to 0.350±0.015 OD_{600}. For experiments in early stationary phase, cells were grown for two days and typically reached 3.5±0.1 OD_{600}.

Metabolite extraction procedure

100 OD of cells were collected by rapid filtration, washed with one volume of ice-cold water and immediately frozen at -80°C. Then the following extraction methods were tested: (A) Collected cells were washed three times with 10 ml ice-cold water. Then, pelleted cells were resuspended in 3 ml 75% EtOH and 2 ml glass beads; metabolites were extracted by vortexing tubes 12 times for 30 seconds with 30 seconds breaks in a +80°C water-bath; (B) Collected cells were washed three times with 10 ml ice-cold water. Then, pelleted cells were resuspended in 3 ml 75% EtOH and metabolites were extracted by vortexing tubes 12 times for 30 seconds with 30 seconds breaks in ice; (C) Collected cells were washed three times with 10 ml ice-cold water. Then, pelleted cells were resuspended in 3 ml 75% EtOH and 2 ml glass beads; metabolites were extracted by vortexing tubes 12 times for 30 seconds with 30 seconds breaks in ice; (D) As in (C) but omitting both washing during cells collection and the further three washes. Finally, samples were centrifuged 5 min at 2000 x g to separate cell residues and glass beads from the extract and equal volumes of the supernatant (1.6 ml) were evaporated under vacuum. Each sample was prepared at least in technical triplicate during each set of experiments.

Membrane integrity assay

The membrane integrity of yeast cells was assayed using propidium iodide (PI), which penetrate cells with damaged membrane. Cells were resuspended in 1 ml saline solution (NaCl, 0.9%) and then mixed with 0.5 ml propidium iodide solution (concentration 0.5 mg/ml). After incubation for 10 min at 0°C, the samples were inspected using fluorescence microscopy. Membrane integrity was checked before and after freezing at -80°C; cell pellets after metabolite extraction were also stained to compare lysis efficiencies of the different extraction methods.

Sample for intracellular metabolite analysis

The dried aqueous extracts were rehydrated in 560 ul D_2O, buffered in 20 mM sodium phosphate (pD 7.8) containing 0.1 mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) as an internal standard for both chemical shift and concentration.

Samples for extracellular metabolites analysis

1,5 ml of culture medium were centrifuged 2 min at 16000 x g to separate cells and 1 ml of medium was recovered and frozen at -20°C until the analysis. The starting medium was uses as a control to evaluate initial metabolite concentrations. 56 uL of D_2O containing DSS were added to 0.504 ml of each sample to reach a final DSS concentration of 3.5 mM; pH value was adjusted to 7.4. Each sample was prepared at least in technical duplicate during each set of experiments.

NMR spectroscopy

The NMR spectra were recorded at 25°C on a Bruker Avance III-600 MHz using a 5-mm QCI cryoprobe. Sample temperature was equilibrated inside the spectrometer for 5 min before data acquisition. For each sample *noesygppr1d* and Carr-Purcell-Meliboom-Gill T2 filter *cpmgpr1d* Bruker pulse sequences were acquired with 128 free induction decays (FIDs) and 64 k data points over a spectral width of 20 ppm. A 5-s relaxation delay was incorporated between FIDs. The FID values were multiplied by an exponential function with a 0.3 Hz line broadening factor. Spectra were automatically phased, corrected for baseline and referenced using the library Topspin AU program apk0.noe.

Metabolite identification and assignment were performed with the support of 2D NMR experiments, Metabominer[1] and the Biological Magnetic Resonance Data Bank.[2] Total correlation spectroscopy (TOCSY) and heteronuclear single quantum correlation (HSQC) were performed for representative samples. For all these experiments, 256–512 t1 increments were used, and 40–96 transients were collected. The relaxation delays were set at 1.5 s, and the experiments were acquired in the phase sensitive mode. The TOCSY spectra were recorded with a spin-lock of 60 ms.

Data analysis

The ^{1}H NMR spectra were normalised to the sum of total spectrum intensity to minimise the effect of the differences in sample concentration. The regions corresponding to water and DSS were excluded from normalisation.

For metabolites quantification we exploited the algorithm called GSD (global spectrum deconvolution), available in the Mnova software package of Mestrelab (MestReNova v 9.0, 2013 Mestrelab Research S.L.).[3] Overlapping regions were deconvolved and absolute quantification performed also for metabolites with resonances in crowded spectral areas.[4] For each compound, the mean value of the different assigned signals was determined.

Statistical analysis

Experiments were performed in triplicate. Results are expressed as mean±SD. Results were compared by using the two-sided Student's t-test. Differences were considered statistically significant at $p < 0.05$.

References

[1] Xia, J.; Bjorndahl, T. C.; Tang, P.; Wishart, D. S., MetaboMiner--semi-automated identification of metabolites from 2D NMR spectra of complex biofluids. BMC Bioinformatics 9, 507 (2008).
[2] http://www.bmrb.wisc.edu/
[3] Cobas, C.; Seoane, F.; Domínguez, S.; Sykora, S.; Davies, A. N. A new approach to improving automated analysis of proton NMRSpectrosc. Eur. 23, 26–30 (2010).
[4] Garcia-Manteiga JM, Mari S., Godejohann , Spraul M, Napoli C, Cenci S , Musco G and Sitia R, Metabolomics of B to plasma cell differentiation, J. Proteome Res. 10, 4165–4176 (2011).