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Supplementary Information for:

Building blocks are synthesized on demand during the yeast cell cycle

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Supplementary Information Text

Materials and Methods

Experimental Design

Yeast, strains and media

All chemicals, unless otherwise stated were purchased from Sigma Aldrich (Merck, St. Louis, MO, USA). The yeast *Saccharomyces cerevisiae* CEN.PK113-7D (MATa, MAL2–8c, SUC2 *⊿bar1::KANMX*) was used if not stated otherwise. This strain was created using BY4741 *⊿bar1::KANMX* as the genomic template, from the Yeast Knockout (YKO) Collection (1), the DNA region of *⊿bar1::KANMX* was amplified (Table S1), using the Phusion™ High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA) standard protocol. Positive transformants were selected on YPD plates (20 g/L peptone (Difco™, VWR, Radnor, PA, USA), 10 g/L yeast extract (Merck Millipore, Burlington, MA, USA), and 20 g/L glucose (VWR, Radnor, PA, USA)) containing 200 mg/L G418 (Formedium, Norfolk, UK) and verified by sequencing (Table S1).

Yeast was cultivated in Delft minimal medium (2), composed as follows: 7.5 g/L $(NH_4)_2SO_4$, 14.4 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, pH adjusted to 6, with 2 mL/L trace metals solution and 1 mL/L vitamins added after autoclavation. Trace metals solution contained: 3.0 g/L $FeSO_4 \cdot 7H_2O$, 4.5 g/L $ZnSO_4 \cdot 7H_2O$, 4.5 g/L $CaCl_2 \cdot 2H_2O$, 1 g/L $MnCl_2 \cdot 4H_2O$, 300 mg/L $CoCl_2 \cdot 6H_2O$, 300 mg/L $CuSO_4 \cdot 5H_2O$, 400 mg/L $Na_2MoO_4 \cdot 2H_2O$, 1 g/L H_3BO_3, 100 mg/L KI and 19 g/L $Na_2EDTA \cdot 2H_20$. Vitamins solution contained: 50 mg/L d-Biotin, 1.0 g/L D-Pantothenic acid hemicalcium salt, 1.0 g/L Thiamin-HCl, 1.0 g/L Pyridoxin-HCl, 1.0 g/L Nicotinic acid, 0.2 g/L 4-aminobenzoic acid, and 25 g/L myo-Inositol. 20 g/L glucose was used throughout the study as the carbon source.

As an internal standard for quantitative proteome analysis, *S. cerevisiae* CEN.PK113-7D *⊿lys1::KANMX* strain (3) was cultivated in Delft mineral medium supplemented with heavy labelled ^{15}N, ^{13}C-lysine (Cambridge Isotope Laboratories, Tewksbury, MA, USA). Fully labelled biomass (> 95% incorporation) was produced and harvested using fed-batch cultures of the lysine auxotrophic strain in DASGIP 1 L bioreactors with two exponential feeding rates (as performed in study currently being submitted for publication by Jianye Xia, Benjamin Sanchez, Yu Chen, K.C., S.K. and J.N.). These rates of exponential feeding were 0.1 h^{-1} and 0.35 h^{-1} with feeding being continued for at least one dilution volume (10 h for 0.1 h^{-1} and 2.86 h for 0.35 h^{-1}) before cells were harvested. Fully labelled biomass was harvested under respiratory and fermentative growth conditions to collect cell biomass with varying proteome compositions to ensure that a broad spectrum of labelled protein could be quantified against when measuring non-labelled samples cultured under varying conditions.

Culture synchronization and sample collection

Three individual colonies were inoculated respectively into 10 mL Delft minimal medium and grown 2 x overnight (200 rpm, 30°C). Cultures were then used to inoculate 200 mL Delft medium to an optical density measured at 600 nm (OD_{600}) of approx. 0.05. Once cells had reached an OD_{600} of approx. 0.2, alpha (α) factor was added to a final concentration of 15 ng/mL and incubated for 3 h (200 rpm, 30°C). After incubation with α-factor, cells were spun for 2 min at 4,000 g using a High-Performance Avanti J-26SXP Centrifuge equipped with a JLA-10.500 rotor (Beckman Coulter, Brea, CA, USA). Using 100 mL pre-warmed dH_2O, cells were washed twice to remove residual α-factor under the same centrifuge conditions. Cell pellets were then re-suspended in 500 mL pre-warmed Delft minimal medium in 2 L culture flasks and incubated at 30°C, 200 rpm for the duration of sample collection.

For transcriptomic samples, cells were added to ice- chilled 50 mL falcon tubes containing approx. 25 mL ice then immediately pelleted in a 4°C pre-chilled centrifuge for 3 min at 5,500 g. Proteomic cell pellets were collected similar to transcriptomic samples, except no ice was added to falcon tubes. After centrifugation, supernatants were discarded, and cell pellets were snap frozen in liquid nitrogen and stored at -80°C until further analysis. For metabolomic samples, cells were added to 50 mL falcon tubes containing 25 mL absolute methanol (100% v/v), pre-chilled on dry ice. Cells were immediately spun down for 5 min at 3,000 g in a pre-chilled (-9°C) centrifuge. Supernatant was discarded and pellets were snap frozen in liquid nitrogen and stored at -80°C until further analysis. For verification of cell cycle phase composition of sample populations, 500 μL of cultures during omic sample collection were added to 500 μL absolute methanol (100% v/v) at room temperature, containing 1 μg/mL 4′,6-diamidino-2-phenylindole (DAPI), to simultaneously fix and stain cells. Samples were spun down (2 min, 8,000 g) and washed twice with dH_2O then resuspended in 500 μL dH_2O and stored at 4°C until microscopy analysis.

Additional samples throughout time course were also collected to correspond OD_{600} with cell dry weight (CDW). Briefly, cells were collected in pre-chilled falcon tubes and pelleted (3 min, 5,500 g), supernatants were discarded, and pellets were snap frozen in liquid nitrogen and stored at -80°C until further analysis. To determine CDW, samples were placed on ice, and pellets were resuspended in 1 mL dH_2O. Samples were filtered and partially dried on pre-weighed membrane filters (Sartorius-Stedim Biotech, pore size 0.45 μm) using a water vacuum pump. Samples were then microwaved (15 min, 126W) and dried in a vacuum desiccator for at least 7 days before being re-weighed.

To determine the population composition for each sample, 3-4 μL of fixed cells were applied to a cover slide, coverslip was applied and cells were then imaged with a DMI4000B Leica wide-field fluorescence microscope using a 100x/1.40 oil objective, running LAS AF 6000 E Application Software (Leica Microsystems GmbH, Wetzlar, Germany). For each sample, $\geq$ 100 cells were imaged using the brightfield and DAPI channel respectively (DAPI filter cube, EX: 387/11, EM: 447/60).

RNA Sequencing

RNA from the biomass samples was extracted and purified using Qiagen RNeasy Mini Kit extraction and DNA degradation according to the user's manual (Qiagen, Hilden, Germany). Integrity of the product was verified using a 2100 Bioanalyzer instrument according to the user's manual (Agilent Technologies, Santa Clara, CA, USA). RNA concentration was determined by a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and cross-verified with Qubit (Life Technologies, Thermo Fisher Scientific, Carlsbad, CA, USA).

Quality control, normalization, pooling and sequencing of transcriptomic samples

The cDNA libraries were generated using Illumina TruSeq Stranded mRNA Library Prep (96 Samples) kit (Illumina Inc., San Diego, CA, USA) with the following modifications: Briefly, 2.5 ug of total undegraded RNA was diluted in 50 µL nuclease free water and mRNA was purified by oligo-dT selection by following the company's protocol. Purified mRNA was enzymatically fragmented for 8 min to approx. 250 base pair (bp) average fragment size. The adapter-modified cDNA fragments were then enriched by 10 cycles enrichment PCR. All the purification and size-selection steps during the protocol were conducted using Kapa Pure Beads (Roche Molecular Systems, Inc., Pleasanton, CA, USA). Nucleic acid concentrations were measured by Qubit® 2.0 Fluorimeter and Qubit RNA or dsDNA Broad range assays (Life Technologies, Thermo Fisher Scientific, Carlsbad, CA, USA). Size distribution profiles of each cDNA library were determined by using a Fragment Analyzer, running the DNF-473 Standard Sensitivity NGS Fragment Analysis Kit and were analysed by ProSize Software (Advanced Analytical Technologies, Agilent Technologies, Ankeny, IA, USA). Libraries were normalized and pooled in 10 mM Tris-Cl, pH 8.0, plus 0.05% Tween 20 to a final concentration of 4 nM. The pool of libraries was denaturated in 0.2 N NaOH and neutralized in 200 mM Tris-HCl, pH 7.0. 1.3 pM pool of libraries was spiked with a 1% PhiX control (Illumina Inc., San Diego, CA, USA) and loaded onto the flow cell provided in the NextSeq 500/550 Mid Output v2 Reagent kit (150 cycles; Illumina Inc., San Diego, CA, USA). Libraries were sequenced on the NextSeq 500 (Illumina Inc., San Diego, CA, USA) platform with paired-end reads protocol and read lengths of 2 x 75 nt.

RNA Seq analysis

RNA Seq data was processed using the NF-core best practice pipeline (https://github.com/nf-core/rnaseq). In short, Illumina adapters were removed using trim galore, then reads were aligned to the R64-1-1 genome using STAR. Reads mapping to genes were then counted using featureCounts and the corresponding ENSEMBL genome annotation.

Absolute mRNA quantitation

Absolute mRNA quantitation was performed using an approach adapted from Marguerat *et al.,* and Rueckert *et al.,* (4, 5). Briefly, 37 genes were selected from the RNA Seq data set, which had FPKM values that covered the dynamic range of mRNA expression and that also had low variability across samples (coefficient of variation (CV) $\leq$ 20%). Absolute copy number determination of these 37 genes was then performed via NanoString's nCounter platform (NanoString Technologies, Inc., Seattle, WA, USA), which provides a digital count for each mRNA target ID. To calibrate the digital counts against known concentrations of mRNA, synthetic mRNA standards were created matching 25 of the 37 selected genes. A synthetic plasmid was custom built by GenScript, by cloning 100mers of these 25 gene sequences, separated by ERCC (External RNA Controls Consortium) 50mer nonhomologous spacers, into a pUC57 vector. mRNA was transcribed from the plasmid DNA using a MEGAscript™ T7 Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA), and RNA concentration was determined by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and cross-verified with Qubit (Life Technologies, Thermo Fisher Scientific, Carlsbad, CA, USA) and was then prepared to 9 different concentrations (from 0.5 fM to 128 fM). Using the nCounter assay and the external synthetic standards a calibration curve was generated with a positive squared Pearson, $r^2 > 0.99$, to convert nCounter values (counts) to molecules of mRNA for the 25 genes.

To determine intracellular mRNA abundance of the 37 genes, sampling conditions that mimicked samples taken for RNA Seq was conducted. This included synchronization of cultures using α-factor arrest and release treatment, generating a total of 12 samples composed of four time points in biological triplicate. 200 ng of total RNA was extracted from samples using the same extraction, purification and quantification protocols as in *RNA Sequencing* and RNA was quantified using the nCounter assay.

All gene-expression profiling was performed using the nCounter® Analysis System with FLEX configuration. The hybridization reaction was performed according to procedures provided by NanoString Technologies (NanoString Technologies, Inc., Seattle, WA, USA). Samples were hybridized at 65°C for 24 h using a bench-top thermocycler with a heated lid set to 70°C. The nCounter Prep Station and Digital Analyzer were run according to manufacturer's specifications. The Digital Analyzer was set to scan at the maximum sensitivity setting defined as 555 FOV (fields of view).

The nCounts generated from the yeast derived mRNA were transformed to absolute mRNA copy numbers using the calibration curve generated by the synthetic standards. These values were used to construct a calibration curve between the absolute quantities of these 37 genes and their respective FPKM values, giving a positive squared Pearson (r^2) linear correlation of > 0.95. The resulting linear fit equation $y = mx+c$ between the molecules of mRNA and FPKM values of the 37 genes was then used to convert all remaining FPKM from RNA Seq to absolute quantities. Copy number was quantified per picogram of cell dry weight

(CDW) using the assumption that a haploid yeast cell is composed of 8% RNA (6). RNA Seq FPKM values and absolute mRNA abundances for the 37 genes can be found in Table S2.

Absolute quantitative proteomics

Sample preparation for absolute quantification of the total proteome and for phosphoproteome analysis

Cell pellets were suspended in a lysis buffer consisting of 6 M guanidine HCl, 100 mM Tris-HCl pH 8.0, 20 mM dithiothreitol (DTT), heated at 95°C for 10 min and sonicated with Bioruptor (Diagenode, Denville, NJ, USA) sonication (15 min, "*High"* setting). Samples were further homogenized by a FastPrep24 (MP Biomedicals, Santa Ana, CA, USA) bead beating device 2x at 4 m/s for 30 s with cooling between cycles. After removal of beads, the samples were further precleared with centrifugation at 17,000 g for 10 min at 4˚C. Aliquots of samples were precipitated overnight with 10% trichloroacetic acid (TCA) at 4°C and used for protein concentration measurement with a Micro BCA kit (Thermo Fisher Scientific, Waltham, MA, USA). For absolute quantification of the internal standard, 1.1 µg of Proteomics Dynamic Range Standard Set (UPS2) was mixed with 6 µg of heavy-labelled standard consisting of a 1:1 mix of yeast grown in Delft mineral medium supplemented with heavy labelled ^{15}N, ^{13}C-lysine (Cambridge Isotope Laboratories, Tewksbury, MA, USA). All other time-point samples were spiked in 1:1 ratio with the heavy standard. Next, samples were precipitated with 10% TCA and suspended in 7:2 M urea:thiourea, 100 mM ammonium bicarbonate (ABC) buffer. After reduction with 5 mM DTT and alkylation with 10 mM chloroacetamide, samples were digested for 4 h at room temperature with 1:50 (enzyme to protein) *Achromobacter lyticus* Lys-C (Wako Pure Chemical Industries, Osaka, Japan). Solutions were diluted 5x with 100 mM ABC and further digested overnight at room temperature. Peptides were desalted using in-house made C18 (3M Empore, Maplewood, MO, USA) tips and reconstituted in 0.5% trifluoroacetic acid (TFA).

For the phosphoproteome analysis, cells were lysed as described above, except samples were not mixed with the heavy standard and proteins were digested with dimethylated porcine trypsin instead of Lys-C. Sample preparation was carried out as described by the EasyPhos protocol (7). 500 µg of cellular protein was used as input for the enrichment. Final samples were reconstituted in 0.5% TFA.

Nano-LC/MS/MS analysis

2 µg of peptides (for phosphoenriched samples, the entire sample) were injected to an Ultimate 3000 RSLCnano system (Dionex, Sunnyvale, CA, USA) using a C18 cartridge trap-column in a backflush configuration and an in-house packed (3 µm C18 particles, Dr Maisch, Ammerbuch, Germany) analytical 50 cm x 75 µm emitter-column (New Objective, Woburn, MA, USA). Peptides were separated at 200 nL/min (for phosphopeptides: 250 nL/min) with a 5-40% B 240- and 480-min gradient for spiked time point and heavy standard samples, respectively. For the phosphopeptides, a 90 min two-step separating gradient was used, consisting of 5-15% 60 min and 15-30% 30 min steps. Buffer B was 80% acetonitrile + 0.1%

formic acid and buffer A: 0.1% formic acid. Eluted peptides were sprayed to a quadrupole-orbitrap Q Exactive Plus (Thermo Fisher Scientific, Waltham, MA, USA) tandem mass spectrometer (MS) using a nano-electrospray source and a spray voltage of 2.5 kV (liquid junction connection). The MS instrument was operated with a top-10 data dependent acquisition strategy. One 350-1400 m/z MS scan (at a resolution setting of 70,000 at 200 m/z) was followed by a MS/MS (R = 17,500 at 200 m/z) of the 10 most intense ions using higher-energy collisional dissociation fragmentation (normalized collision energies of 26 and 27 for normal and phosphopeptides, respectively). For total proteome analysis, the MS and MS/MS ion target and injection time values were $3x\ 10^6$ (50 ms) and $5x\ 10^4$ (50 ms), respectively. For phosphopeptides, the MS and MS/MS ion target and injection time values were $1x\ 10^6$ (60 ms) and $2x\ 10^4$ (60 ms), respectively. The dynamic exclusion time was limited to 45 s, 70 s and 110 s for phosphopeptide, spiked time point and heavy standard samples, respectively. Only charge states +2 to +6 were subjected to MS/MS and for phosphopeptides, the fixed first mass was set to 95 m/z. The heavy standard was analyzed with three technical replicates, all other samples were analyzed with a single technical replicate.

Mass-spectrometric raw data identification and quantification

Raw data were identified and quantified with the MaxQuant 1.4.0.8 software package (8). For heavy-spiked samples, the labelling state (multiplicity) was set to 2, and Lys8 was defined as the heavy label. Methionine oxidation, asparagine/glutamine deamidation and protein N-terminal acetylation were set as variable modifications and cysteine carbamidomethylation was defined as a fixed modification. For phosphoanalysis, serine/threonine phosphorylation was used as an additional variable modification. Search was performed against the UniProt (www.uniprot.org) *S. cerevisiae* S288C reference proteome database (version from July 2016), with this reference genome being selected over CEN.PK due to better annotation. This was performed using the LysC/P (trypsin/P for phosphoproteomics) digestion rule. Only protein identifications with a minimum of 1 peptide of 7 amino acids long were accepted, and transfer of peptide identifications between runs was enabled. Peptide-spectrum match and protein false discovery rate (FDR) were kept below 1% using a target-decoy approach.

In heavy-spiked samples, to account for any H/L 1:1 mixing deviation, normalized H/L ratios (generated by shifting median peptide log H/L ratio to zero) were used in all down-stream quantitative analyses. Protein H/L values themselves were derived by using the median of a protein's peptide H/L ratios, and, requiring that at least one peptide ratio measurement was present for reporting quantitative values (*i.e.* the minimum ratio count was set to 1). Signal integration (re-quantification) of missing label channels was enabled. For enriched phosphoproteome samples, an in-house written R script based on median phosphopeptide intensity was used to normalize the phosphopeptide intensities.

Normalization of protein and protein phosphorylation abundance

The heavy spike-in standard used for deriving protein copy numbers was quantified using the iBAQ method as described by Scwanhausser *et al* (9). Protein intensities from UPS2 (a mix of 48 human proteins in varying concentrations) were divided by the number of theoretically observable peptides, log-transformed and plotted against log-transformed known protein amounts of the UPS2 proteins. This regression was then applied to derive all other protein absolute quantities (in femtomole) using each protein's iBAQ intensity. Using these fmol values and the molecular weight of each protein, absolute protein quantities by mass were then summed and normalized to equal 6 µg, the protein amount extrapolated by MaxQuant software when 1.1 µg of UPS is expected (with 6 µg of heavy labelled protein being mixed per 1.1 µg of UPS, and heavy and light samples being mixed 1:1). The average total protein content per picogram of cell dry weight (CDW) was then used to generate protein copy number/ pg CDW for each sample.

Median normalized phosphopeptide intensities were transformed to normalized protein phosphorylation sites/ molecule of protein/ pg CDW by dividing through all normalized intensity values by protein copy number/ pg CDW for each sample.

Metabolomics using the Metabolon platform

Relative metabolome quantification

For intracellular metabolomic analysis, frozen biomass pellets were delivered to Metabolon, Inc. (Durham, NC, USA) wherein non-targeted MS was performed. Briefly, metabolites were identified by matching their ion chromatographic retention index and mass spectral fragmentation signatures to the Metabolon reference library of chemical standards. Relative quantification of metabolite concentrations was then performed via peak area integration.

Sample preparation for metabolomic samples

All samples were maintained at -80°C until processed. Samples were prepared using the automated MicroLab STAR® system from Hamilton Company. Several recovery standards were added prior to the first step in the extraction process for QC purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Geno/Grinder® 2000, Glen Mills Inc., Clifton, NJ, USA) followed by centrifugation. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by hydrophilic interaction liquid chromatography (HILIC)/UPLC-MS/MS, with negative ion mode ESI, and one sample was reserved for backup. Samples were placed briefly on a TurboVap® (Zymark, Caliper Life Sciences, Hopkinton, MA, USA) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis.

Quality assurance (QA)/ quality control (QC)

Several types of controls were analyzed in concert with the experimental samples: (i) a pooled matrix sample generated by taking a small volume of each experimental sample, which served as a technical replicate throughout the data set; (ii) extracted water samples, which served as process blanks; and (iii) a cocktail of QC standards, which were carefully chosen not to interfere with the measurement of endogenous compounds, that were spiked into every analyzed sample allowing instrument performance monitoring as well as aiding towards chromatographic alignment. Instrument variability was determined by calculating the median relative standard deviation (RSD) for the standards that were added to each sample prior to injection into the mass spectrometers. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the pooled matrix samples. Experimental samples were randomized across the platform run with QC samples spaced evenly among the injections. Briefly, a small aliquot of each sample was pooled to create a CMTRX technical replicate sample, which was then injected periodically throughout the platform run (see Table S3 for more information on CMTRX samples**)**. Variability among consistently detected biochemicals was then used to calculate an estimate of overall process and platform variability.

Ultrahigh performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS)

All methods utilized a Waters ACQUITY UPLC and a Thermo Scientific Q-Exactive high resolution/ accurate MS, interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operating at 35,000 mass resolution. The sample extract was dried then reconstituted in solvents compatible to each of the four methods. Each reconstitution solvent contained a series of standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion conditions, chromatographically optimized for more hydrophilic compounds. In this method, the extract was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1x100 mm, 1.7 µm) using water and methanol, containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA). Another aliquot was also analyzed using acidic positive ion conditions, however it was chromatographically optimized for more hydrophobic compounds. In this method, the extract was gradient eluted from the same aforementioned C18 column using methanol, acetonitrile, water, 0.05% PFPA and 0.01% FA and was operated at an overall higher organic content. Another aliquot was analyzed using basic negative ion optimized conditions using a separate dedicated C18 column. The basic extracts were gradient eluted from the column using methanol and water, however with 6.5 mM ABC at pH 8. The fourth aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1x150 mm, 1.7 µm) using a gradient consisting of water and acetonitrile with 10 mM ammonium formate, pH 10.8. The MS analysis alternated between MS and data-dependent MSn scans using dynamic exclusion. The scan range varied slighted between methods but covered 70-1,000 m/z.

Data extraction and compound identification

Raw data was extracted, peak-identified and QC processed using Metabolon's hardware and software. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Metabolon maintains a library based on authenticated standards that contains the retention time/index (RI), mass to charge ratio (m/z), and chromatographic data (including MS/MS spectral data) on all molecules present in the library. Furthermore, biochemical identifications are based on three criteria: (i) retention index within a narrow RI window of the proposed identification, (ii) accurate mass match to the library +/- 10 ppm, and (iii) the MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores are based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum. While there may be similarities between these molecules based on one of these factors, the use of all three data points can be utilized to distinguish and differentiate biochemicals (metabolites). When metabolites were not identified based on a standard but for which there was confidence in their identify, they are annotated with '*'.

Metabolite quantification and data normalization

Peaks were quantified using area-under-the-curve (AUC). To correct for variation, which may have resulted from instrument inter-day tuning differences, each compound was corrected in run-day blocks by registering the medians to equal one (1.00) and normalizing each data point proportionately (termed the "block correction"). To account for differences in metabolite levels due to differences in the amount of material present in each sample, metabolite intensities were normalized by each sample's biomass (CDW), followed by total intensities being scaled to equal one for each sample.

Limma modelling

To identify periodic omic components, limma modelling was used (10).

Using limma, a model was fit where the expression of a gene was explained by Equation 1

$$y_{g,h} = G1_h g1_g + S_h s_g + G2M_h g2m_g + P_h p_g + \varepsilon \quad (1)$$

where

$y_{g,h}$ is the measured expression of gene *g* in sample *h*.

$G1_h, S_h, G2M_h$ are the fractions of cells in G1, S and G2/M phase respectively, in sample *h*.

$(G1_h, + S_h + G2M_h = 1)$.

$g1_g, s_g, g2m_g$ is the estimated expression of gene *g* in G1, S and G2/M phase respectively.

P_h is the pheromone effect on sample h (1 for samples in the first and second time point (n = 6), 0 for other samples (n = 24).

p_g is the estimated effect of pheromone on gene *g*.

ε is the residual expression, unaccounted for by the other coefficients in the model.

A gene was considered to be periodically expressed if 1) the F-test *P*-value for any differences between G1, S and G2/M phases was $\leq$ 0.001, $\leq$ 0.1 and $\leq$ 0.56 after false discovery rate (FDR) correction for transcriptomic, proteomic and phosphoproteomic data respectively, and 2) the expression difference between any two phases met or exceeded the minimum log fold change cut-off set for each omic data set. *P*-value cut offs were selected via cross-validation of results with previously identified periodic genes, using the 113 genes identified from small-scale studies, as a benchmark (11).

A gene was considered to be pheromone regulated if the *P*-value for the pheromone effect was 0.001 or lower after FDR correction, and the estimated expression change due to pheromone addition met or exceeded the minimum log fold change cut-off set for the omic data set. *N.B.,* a pheromone coefficient was only set for transcriptomic data due to increasing variability of omic data types.

Due to the increase in variability between samples, depending on the omics analyzed, different minimum log fold changes (min.log.fc) were applied. Transcriptomics, min.log.fc = 0.4, proteomics min.log.fc = 0.2, phosphoproteomics min.log.fc = 0.4. For the metabolomics data, due to the high variability in abundance between samples, limma modelling was not applied.

Throughout identification of periodic components, mRNA, protein and protein phosphorylated sites were labelled using their associated systematic gene names (e.g. YNL241C) for identification. Genes identified as having verified ORFs as well as those that were uncharacterized were used for further analysis only (n = 5,915, using list from https://yeastmine.yeastgenome.org/yeastmine/bag.do?subtab=view). From genes identified as periodic from the transcriptome, fifteen dubious genes were removed leaving 1,727/1,742 (99%); one was removed from the periodic proteomic data leaving 991/992 (99%), and none were removed from the phosphoproteomic data set.

Outliers in omic data

Following PCA analysis of all samples (n = 30) for each omic level respectively, Sample 27 from proteomic measurements, Sample 29 from phosphoproteomic measurements and Sample 26 from metabolomic measurements were removed.

Additional information for omic data

All abundance values for each omic component are expressed as being ‘per picogram of cell dry weight’ (pg CDW) to enable cell size-independent comparisons between time points. Values however can be transformed to being ‘per cell’, based on the assumption that a haploid cell is approximately 15 pg CDW (6).

Values for all measured components can be found in Dataset S1. As well as their systematic gene names (also referred to as 'ensembl gene id' or 'ORF'), gene symbols (also referred to as 'wikigene name') are given, as well as their gene descriptions when available. For proteins and protein phosphorylation sites, their UniProt protein ID (labelled as 'uniprotswissprot') is given. When more than one phosphorylation site is quantified for a protein, a unique identifier is provided for the systematic gene name and protein ID respectively (e.g. YDL225W.4 and Q07657.9). For protein phosphorylation sites, the amino acid phosphorylated as well as its position in the protein sequence is annotated (e.g. Thr285 indicates a phosphorylation on threonine at position 285 in the protein sequence). Periodic information on measured components can be found in Dataset S2, where the cell cycle phase, demonstrating peak expression of the given component is also included, alongside a significance value generated by the limma model.

Custom visualization of omic data

Abundance of all components can be visualized at https://www.sysbio.se/tools/cellcycle/ . The website can be used to browse the multi-omics data and to generate abundance plots for each component (mRNA, protein, phosphorylated protein and metabolite). The plots have time on the x-axis and abundance on the y-axis. Individual biological replicates are shown in each plot, with their colors reflecting the cell cycle phase that each time point has been assigned. Local fitting of points by the loess (locally estimated scatterplot smoothing) function from the ggplot2 package (R studio) has also been applied to each plot with span for fitting each local regression = 0.6 (12). Confidence interval for regression line = 95%.

Below the plots are four separate tabs for the mRNA, protein, phosphorylated protein and metabolite data. These can be browsed by scrolling and searching (by typing in the text box above the tables on the right). When a row in the tables is selected, the abundance for this mRNA/ protein/ phosphoprotein/ metabolite is displayed in the plots above. The plots currently shown on the screen can be saved as a pdf file by clicking the button "Save as pdf" in the top right corner.

The tables show the average abundance of each component for each phase in each cycle (e.g. 'Cycle1_G1' indicates average abundance for a component in G1 phase of the first cell cycle). Significance (F.pval) and adjusted significance (adj.F.pval) values are also indicated to help identify if a component is significantly periodic or not. 'MaxPhase' in the far-right column shows cell cycle phase where peak expression of a component is expected. This is assigned by the limma model, taking into account synchronization effects at the start of the time course as well as sample heterogeneity. Each table can be downloaded as an excel file, by clicking the link "Download table" in the top left corner above the table. *N.B.* these tables show all abundance values without significance cut-off as in Dataset S2 for periodic components.

Metabolite abundance can only be modified when selecting the 'Select metabolite' tab. Protein phosphorylation site abundance can only be searched for through the 'Select mRNA' or the 'Select protein' tab. Once a gene is selected (e.g. *STE20*), multiple phosphorylation site data for a given protein can be plotted in the 'Select phosphorylation site' tab.

Intergene distance analysis

Analysis of genomic locations was performed as in Kristell *et al*., (13). Briefly, genes were divided into five sets: 1) periodic genes with peak expression in G1 phase, 2) periodic genes with peak expression in S phase, 3) periodic genes with peak expression in G2/M phase, 4) all periodic genes and 5) a background set of all genes. For each set, all pairwise distances between gene pairs on the same chromosome were computed. These distances were used to compare the periodic gene sets to the background set, using a Wilcoxon's rank-sum test.

Mitochondrial volume analysis via fluorescence microscopy

The volume analysis of yeast mitochondria was conducted on cells chemically fixed using 500 µL of 4 g/L paraformaldehyde (PFA) in 3.6% sucrose solution and resuspended in 20 µL of Vectashield mounting medium (Vector Labs Inc., Burlingame, CA, USA). The samples were visualized using a Nikon A1 confocal fluorescent microscope (Nikon Instruments Inc, Melville, NY, USA), equipped with a CFI Apo λS 60x oil objective with nano-crystal coat and A1-F filter Cube (EX: 450/50; DM: 525/50; EM: 595/5), using 3.5 µL of sample applied to 10x poly-L-lysine coated slides. The fluorescence of the mitochondrial network was visualized by excitation at 488 nm (eGFP). To obtain volumetric data of the 50 cells considered, Z-stacks were acquired with a step of 0.125 µm. Pictures were all deconvolved using the automatic deconvolution tool of the NIS-Elements software (Version 4.11.0, Nikon Instruments Inc., Melville, NY, USA). Volume was determined for the mitochondrial network using the volume function in Nikon A1 dedicated software, NIS-Elements Confocal. Total cell volume was inferred using a representative Z-stack from bright field images acquired; cell area was then calculated in ImageJ and transformed to volume with the assumption that all cells were spherical.

Statistical tests

All statistical tests unless otherwise stated were performed using the Welch two sample t-test.

Code availability

All code is available upon reasonable request to nielsenj@chalmers.se .

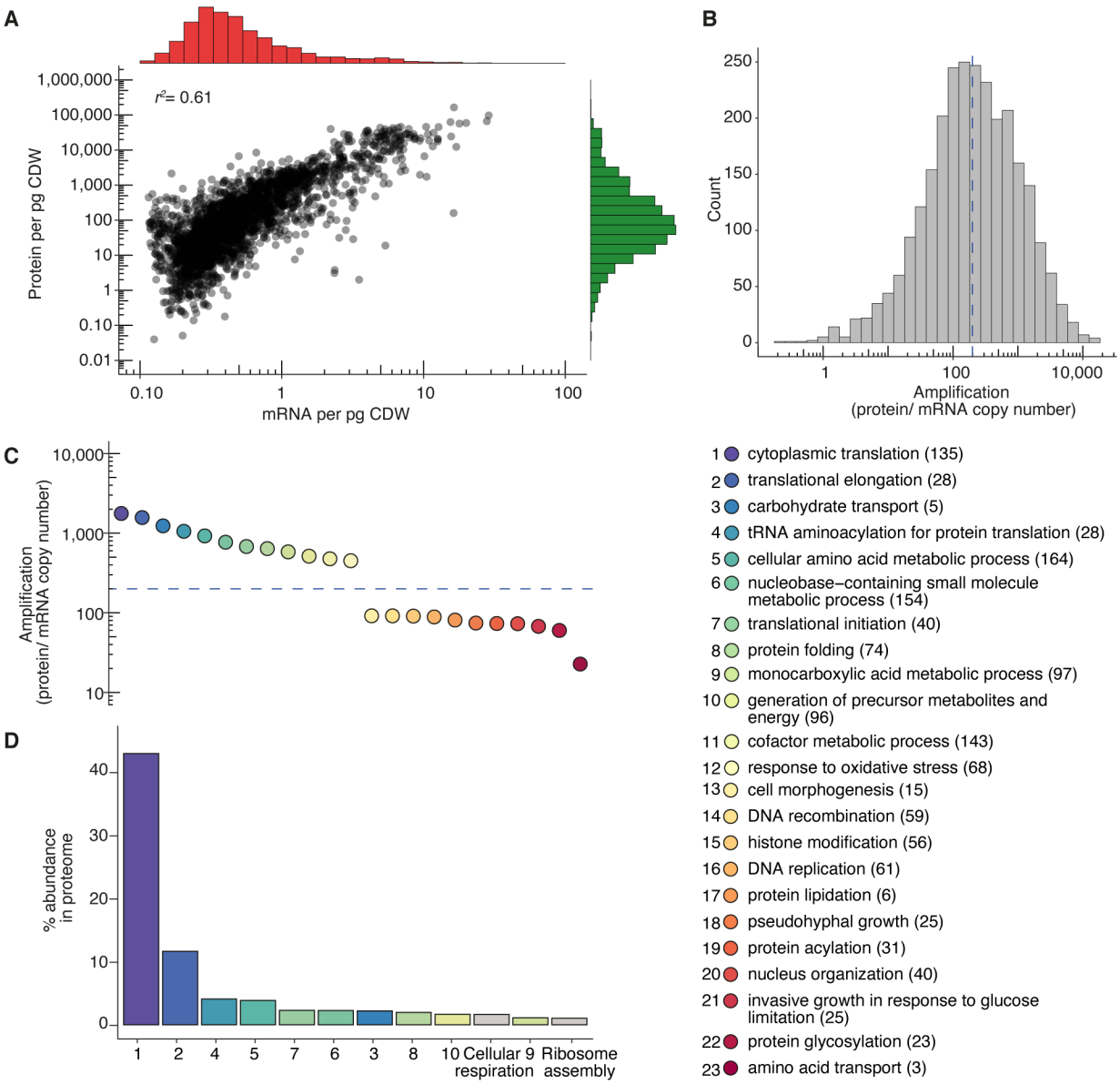

Fig. S1. Overview of quantified transcriptome and proteome. (**A)** Median absolute mRNA and protein abundances for each gene. (**B)** Median amplification of each mRNA gene product during translation to protein. Blue dashed line= median amplification across all genes measured. (**C)** Median amplifications for all genes binned into gene ontology (GO) processes. Total number of genes in each process are in parenthesis. Only GO processes which had a $\log_2$ fold change from median amplification $\geq$ 0.75 or $\leq$ -0.75 are plotted. Blue dashed line= median amplification. (**D)** Ranked GO processes according to the median abundance of all proteins within each GO process. Only GO processes that make up $\geq$ 1% of the proteome are plotted. Bars are coloured as in **(C).** All GO processes were taken from SGD database (https://www.yeastgenome.org/), using SGD Gene Ontology Slim Mapper and "Yeast GO-Slim: Process" as the filter.

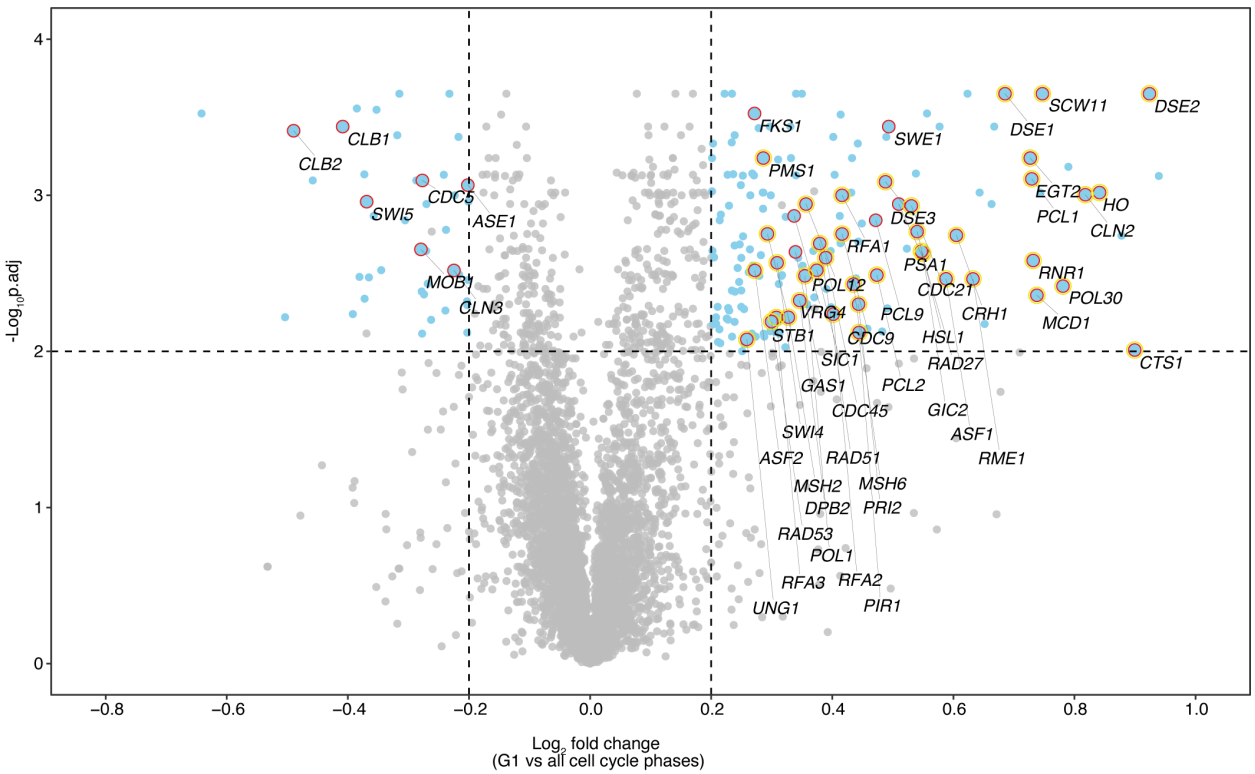

Fig. S2. Validation of limma modelling approach to identify periodic gene expression. Gene expression changes between G1 and average gene expression across all cell cycle phases. Horizontal dashed line designates adjusted *P*-value of 0.01, vertical dashed lines = $\log_2$ FC ± 0.2. Samples analyzed omit the first six time points, to exclude effects of pheromone addition required for cell cycle synchronization. Blue points = genes that were identified as periodic by our limma model within significance and size cut offs. Blue points with red border= genes identified by our limma model that have previously been confirmed as periodic in small scale studies (11). Blue points with both red and yellow border = same as the latter but also known to peak in expression in G1, as identified in multiple studies in Cyclebase 3.0 for *S. cerevisiae* (https://cyclebase.org/) (14).

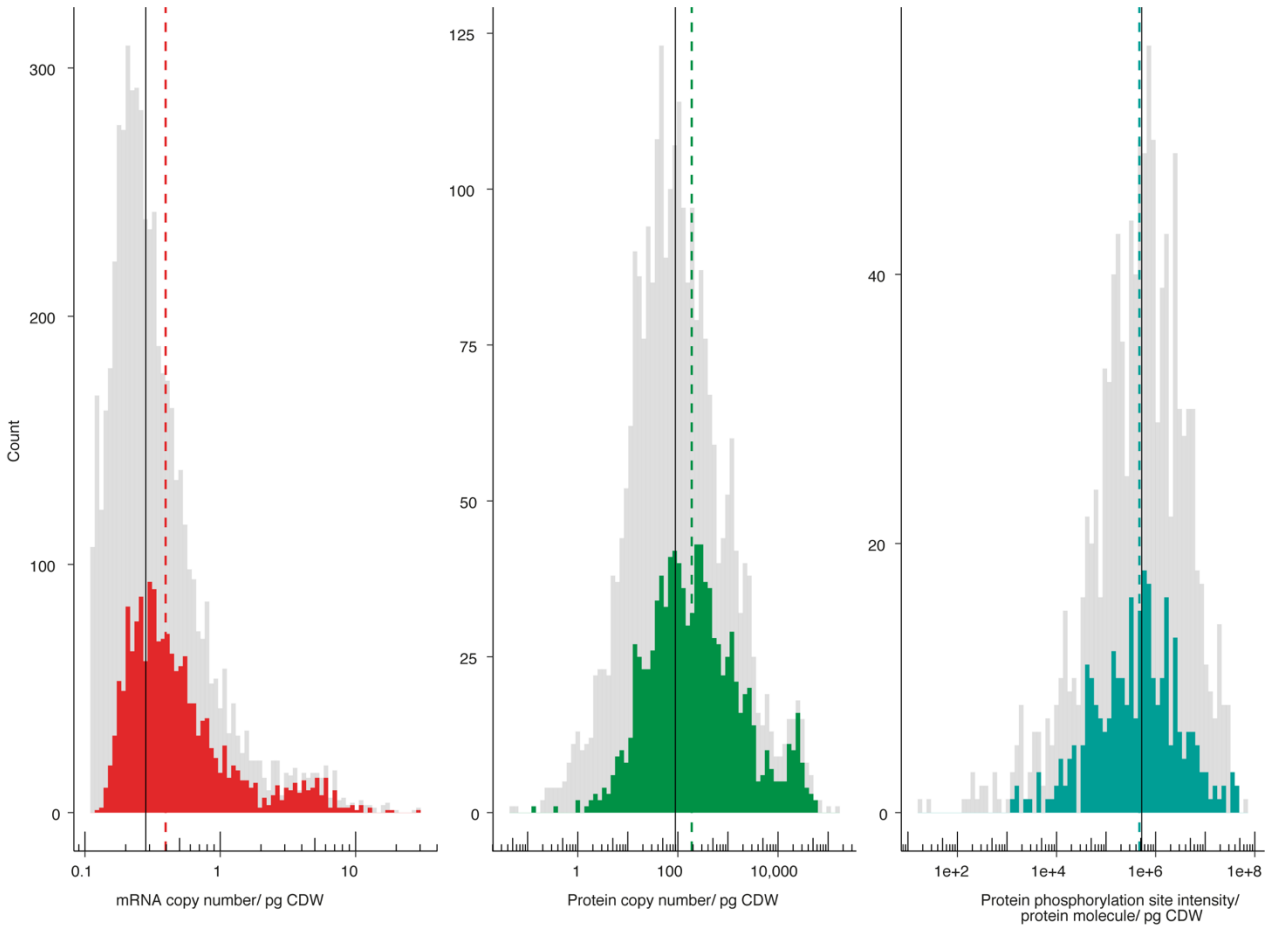

Fig. S3. Periodic multi-ome spans similar abundance range as background multi-ome. Histogram of abundance values for all measured components of the transcriptome (left), proteome (middle) and phosphoproteome (right) with periodic components highlighted in red, green and teal respectively. Solid and dashed vertical lines= median abundance for entire omic level and periodic components respectively.

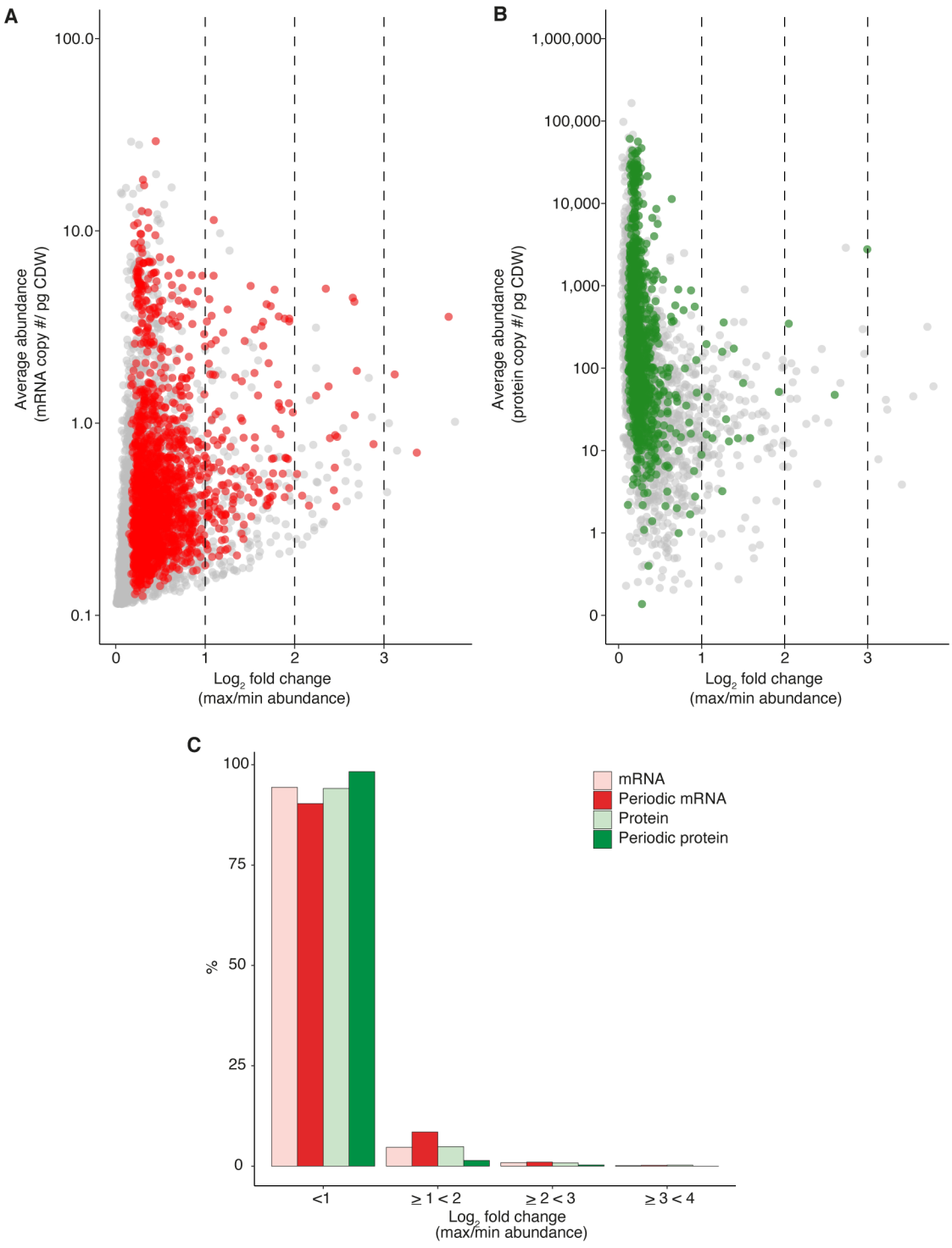

Fig. S4. Changes in mRNA and protein abundance mostly fall within a twofold range during cell division. Average abundance of mRNA **(A)** and protein **(B)** for each gene, against the $\log_2$ fold change between their highest and lowest measured values. **(C)** Percentage abundance of $\log_2$ fold change values in **(A)** and **(B)** after binning.

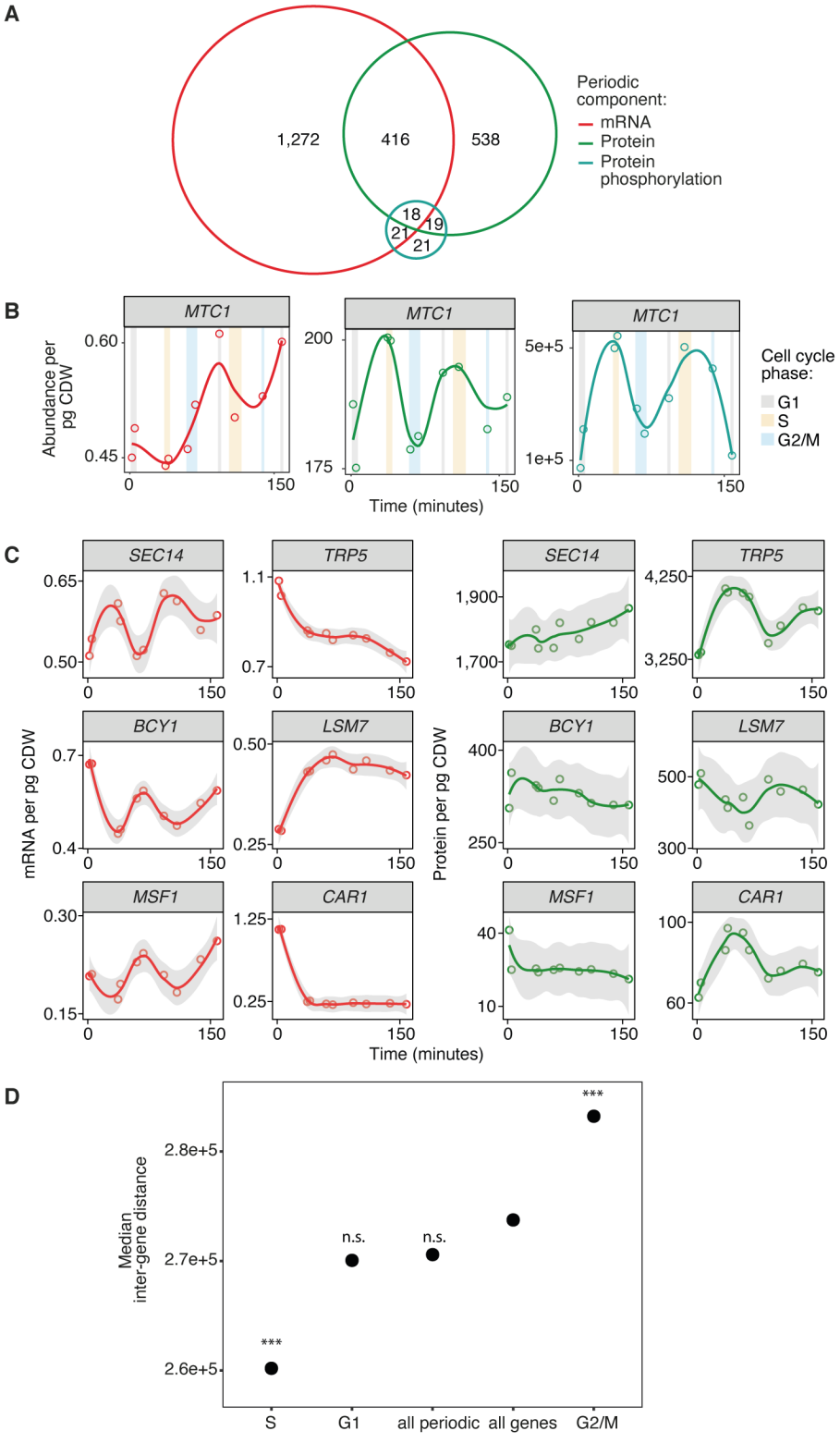
A
1,272
416
538
18
19
21
21
Periodic component:
mRNA
Protein
Protein phosphorylation
B
MTC1
MTC1
MTC1
Abundance per pg CDW
0.60
0.45
200
175
5e+5
1e+5
0
150
Time (minutes)
Cell cycle phase:
G1
S
G2/M
C
SEC14
TRP5
SEC14
TRP5
BCY1
LSM7
BCY1
LSM7
MSF1
CAR1
MSF1
CAR1
mRNA per pg CDW
Protein per pg CDW
Time (minutes)
D
Median inter-gene distance
2.8e+5
2.7e+5
2.6e+5
***
n.s.
n.s.
***
S
G1
all periodic
all genes
G2/M

Fig. S5. **Periodic genes across the different omic levels**. **(A)** Overlap of periodic genes across the transcriptome, proteome and phosphoproteome. **(B)** Periodic abundance of mRNA (red), protein (green) and protein phosphorylation (teal) for *MTC1*. Line represents a loess curve fitted to average abundance values of three biological replicates for each time point with span for fitting each local regression= 0.6 Vertical shading reflects cell cycle phase for the majority of cells at a given time point. **(C)** Genes showing periodic abundance only at the mRNA (red) or protein level (green), not both. Confidence interval for regression line= 95%. **(D)** Median inter-gene distance for all cell cycle affected genes. "Control" group includes all genes across the genome. *** = *P*-value $\leq$ 0.001, n.s. = not significant.

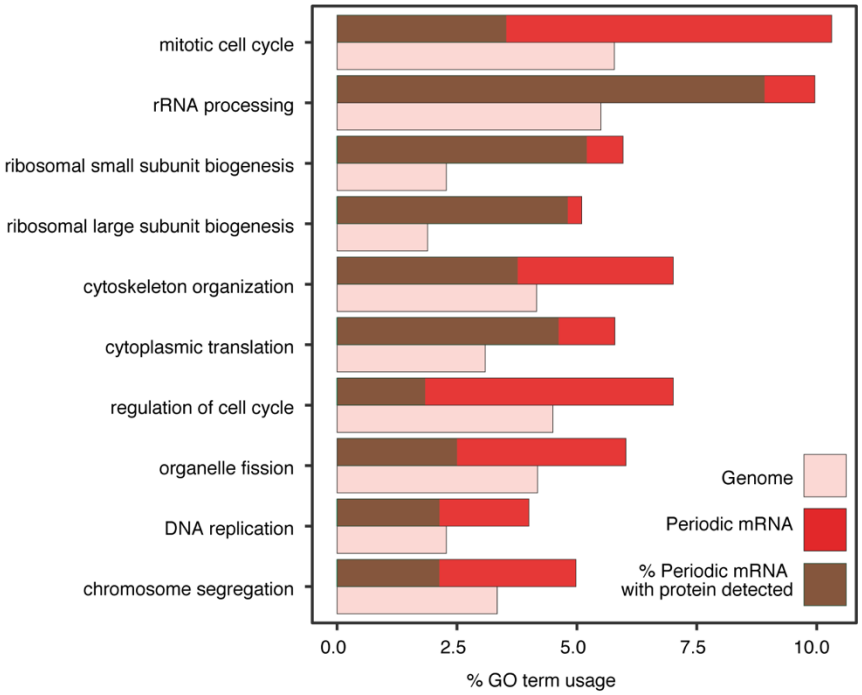

Fig. S6. Top ten biological process GO terms ranked by enrichment in the periodic transcriptome. Percentage periodic mRNA with protein detected by MS is also highlighted.

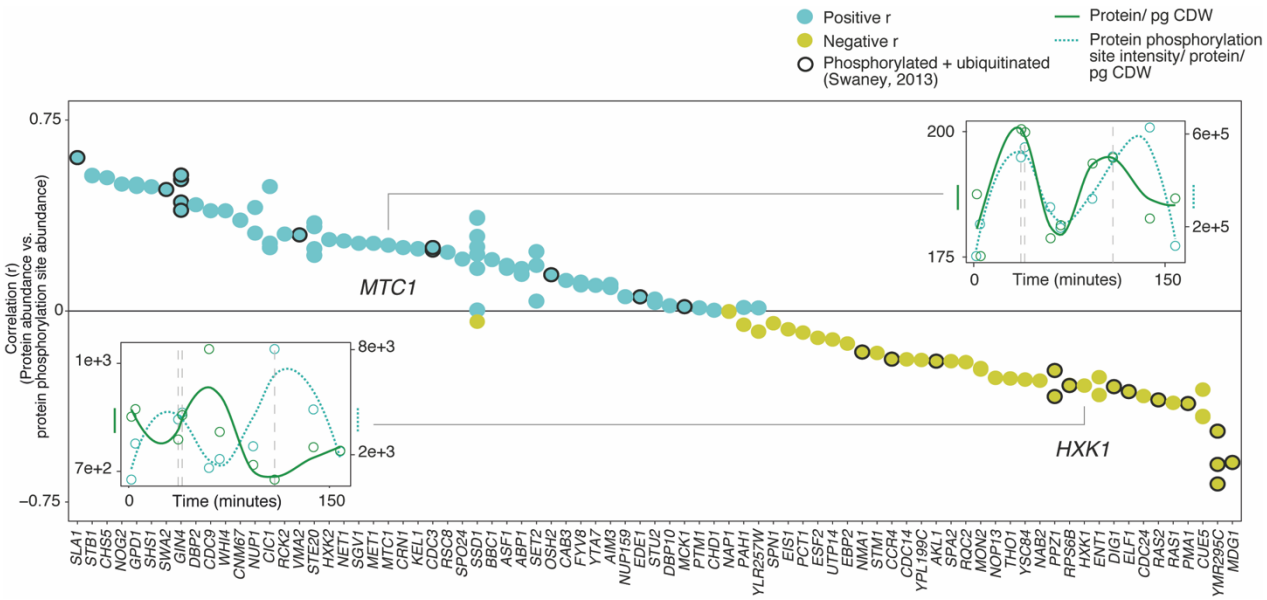

Fig. S7. Pearson *r* correlation for proteins with periodic phosphorylation. Correlation values which fall above and below 0 are coloured blue and yellow respectively. Proteins known to be simultaneously phosphorylated and ubiquitinated (15) are highlighted with a black border. Two proteins, *MTC1* and *HXK1*, reflecting positive and negative correlation between their protein (green) and phosphorylation abundance (dashed teal) respectively are shown inset, with dashed vertical grey line corresponding to S phase. Lines represent a loess curve fitted to the data points with span for fitting each local regression= 0.6. All points reflect the average abundance from biological triplicates.

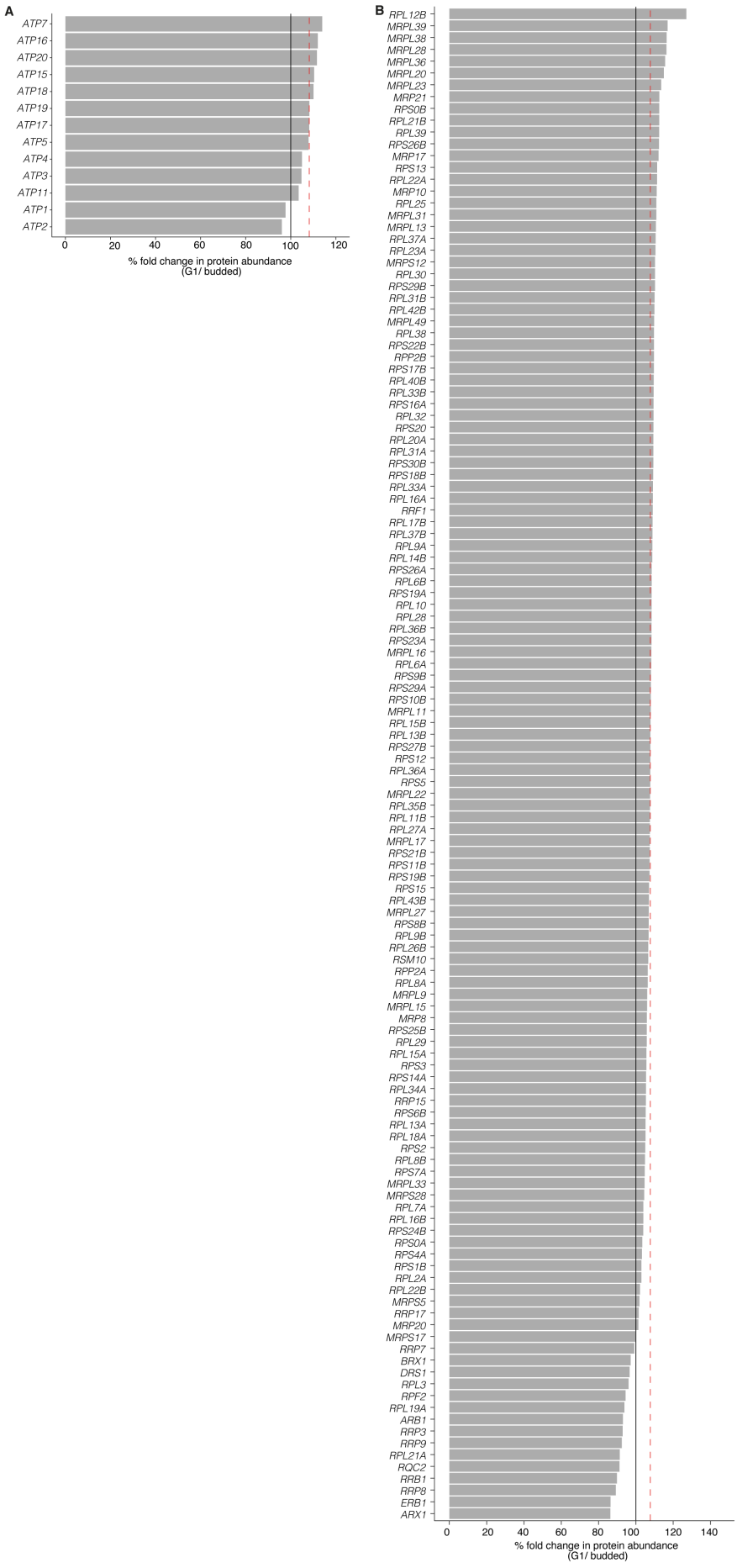
A
ATP7
ATP16
ATP20
ATP15
ATP18
ATP19
ATP17
ATP5
ATP4
ATP3
ATP11
ATP1
ATP2
0
20
40
60
80
100
120
% fold change in protein abundance
(G1/ budded)
B
RPL12B
MRPL39
MRPL38
MRPL28
MRPL36
MRPL20
MRPL23
MRP21
RPS0B
RPL21B
RPL39
RPS26B
MRP17
RPS13
RPL22A
MRP10
RPL25
MRPL31
MRPL13
RPL37A
RPL23A
MRPS12
RPL30
RPS29B
RPL31B
RPL42B
MRPL49
RPL38
RPS22B
RPP2B
RPS17B
RPL40B
RPL33B
RPS16A
RPL32
RPS20
RPL20A
RPL31A
RPS30B
RPS18B
RPL33A
RPL16A
RRF1
RPL17B
RPL37B
RPL9A
RPL14B
RPS26A
RPL6B
RPS19A
RPL10
RPL28
RPL36B
RPS23A
MRPL16
RPL6A
RPS9B
RPS29A
RPS10B
MRPL11
RPL15B
RPL13B
RPS27B
RPS12
RPL36A
RPS5
MRPL22
RPL35B
RPL11B
RPL27A
MRPL17
RPS21B
RPS11B
RPS19B
RPS15
RPL43B
MRPL27
RPS8B
RPL9B
RPL26B
RSM10
RPP2A
RPL8A
MRPL9
MRPL15
MRP8
RPS25B
RPL29
RPL15A
RPS3
RPS14A
RPL34A
RRP15
RPS6B
RPL13A
RPL18A
RPS2
RPL8B
RPS7A
MRPL33
MRPS28
RPL7A
RPL16B
RPS24B
RPS0A
RPS4A
RPS1B
RPL2A
RPL22B
MRPS5
RRP17
MRP20
MRPS17
RRP7
BRX1
DRS1
RPL3
RPF2
RPL19A
ARB1
RRP3
RRP9
RPL21A
RQC2
RRB1
RRP8
ERB1
ARX1
0
20
40
60
80
100
120
140
% fold change in protein abundance
(G1/ budded)

Fig. S8. Relative increase in protein subunit abundance for complexes related to protein synthesis in G1. (A) ATP synthase subunit and **(B)** ribosome subunit protein abundance changes between G1 (when cells are unbudded), versus budded cells. Solid line= same abundance between budded and unbudded, red dashed line = median % increase between budded and unbudded cells.

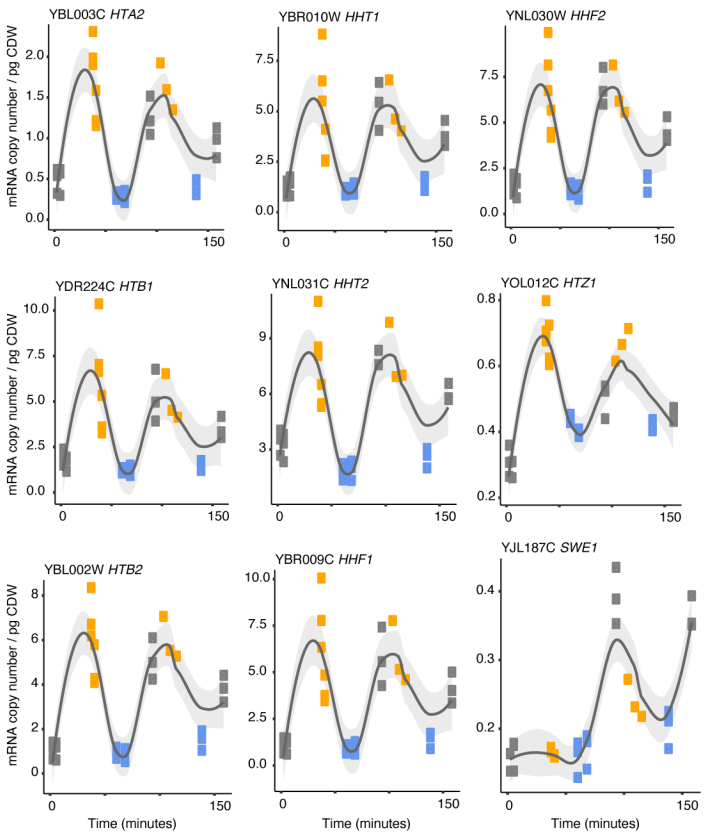

Fig. S9. mRNA abundance for *S. cerevisiae* genes with periodic *Sc. pombe* orthologs. Significant (*P*< 0.05) periodic abundance of mRNA for genes with *Sc. pombe* orthologs, which also demonstrate periodic mRNA abundance during their cell cycle (see Fig. 3 in (4)). Points reflect mRNA abundance for individual replicates, point color reflects the cell cycle phase that the majority of the cell population are in at each time point (grey= G1 phase, orange= S phase, blue= G2/M phase). Black line represents a loess curve fitted to replicate abundance values for each time point with span for fitting each local regression= 0.6 Confidence interval for regression line= 95%.

Table S1. Primers used to generate and verify CEN.PK113-7D (MATa, MAL2–8c, SUC2 *Δbar1::KANMX*)

Name	**Sequence**
bar1KanMX_fw	5'-TGGAACATTGGAAGAGCA-3'
bar1KanMX _rv	5'-TATTCCAACATTCCGACAC-3'
verification_ bar1KanMX _fw	5'-AAGGAAATTACATGGCGAG-3'
verification_ bar1KanMX _rv	5'-TGATATCAGTAAAACTCCCC-3'

Table S2. mRNA in FPKM and absolute abundance for 37 genes measured by nCounter

#	Ensembl gene id	Wikigene name	FPKM	mRNA molecules/ pg CDW
1	YJL084C	*ALY2*	21.330	0.151
2	YJR058C	*APS2*	76.878	0.298
3	YPR176C	*BET2*	84.621	0.169
4	YCR032W	*BPH1*	6.571	0.119
5	YDL126C	*CDC48*	450.643	2.889
6	YDR155C	*CPR1*	2699.834	7.900
7	YKR054C	*DYN1*	4.402	0.135
8	YBL047C	*EDE1*	61.026	0.734
9	YBR008C	*FLR1*	23.085	0.058
10	YJR140C	*HIR3*	9.971	0.166
11	YOR189W	*IES4*	121.535	0.309
12	YER092W	*IES5*	245.167	0.634
13	YER038C	*KRE29*	15.556	0.063
14	YNL029C	*KTR5*	28.038	0.101
15	YDR062W	*LCB2*	112.960	0.741
16	YBR136W	*MEC1*	3.558	0.101
17	YBR193C	*MED8*	52.414	0.174
18	YCR026C	*NPP1*	45.898	0.093
19	YDL188C	*PPH22*	93.888	0.438
20	YGR135W	*PRE9*	361.753	1.286
21	YPL151C	*PRP46*	42.344	0.194
22	YGL058W	*RAD6*	162.080	0.407
23	YPL009C	*RQC2*	57.210	0.105
24	YGR263C	*SAY1*	30.272	0.108

25	YOR329C	*SCD5*	49.374	0.196
26	YOR367W	*SCP1*	73.055	0.216
27	YOR007C	*SGT2*	623.321	2.068
28	YDR422C	*SIP1*	32.883	0.169
29	YOR154W	*SLP1*	38.719	0.148
30	YGL131C	*SNT2*	12.461	0.154
31	YOR353C	*SOG2*	35.495	0.150
32	YBL075C	*SSA3*	25.949	0.101
33	YDR293C	*SSD1*	106.399	0.860
34	YNL121C	*TOM70*	219.936	0.627
35	YMR022W	*UBC7*	141.516	0.296
36	YGL095C	*VPS45*	65.827	0.261
37	YAL002W	*VPS8*	17.896	0.097

Table S3. Description of Metabolon quality control (QC) samples

Type	Description	Purpose
MTRX	Large pool of human plasma maintained by Metabolon that has been characterized extensively.	Assure that all aspects of the Metabolon process are operating within specifications.
CMTRX	Pool created by taking a small aliquot from every sample.	Assess the effect of a non-plasma matrix on the Metabolon process and distinguish biological variability from process variability.
PRCS	Aliquot of ultra-pure water	Process Blank used to assess the contribution to compound signals from the process.
SOLV	Aliquot of solvents used in extraction.	Solvent Blank used to segregate contamination sources in the extraction.

Dataset S1 (separate file). Abundance data for transcriptome, proteome, phosphoproteome and metabolome.

Dataset S2 (separate file). Data for the periodic transcriptome, proteome and phosphoproteome.

Dataset S3 (separate file). List of essential genes compiled by Liu *et al.,* (16).

Dataset S4 (separate file). List of conserved genes compiled by Kachroo *et al.,* (17).

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