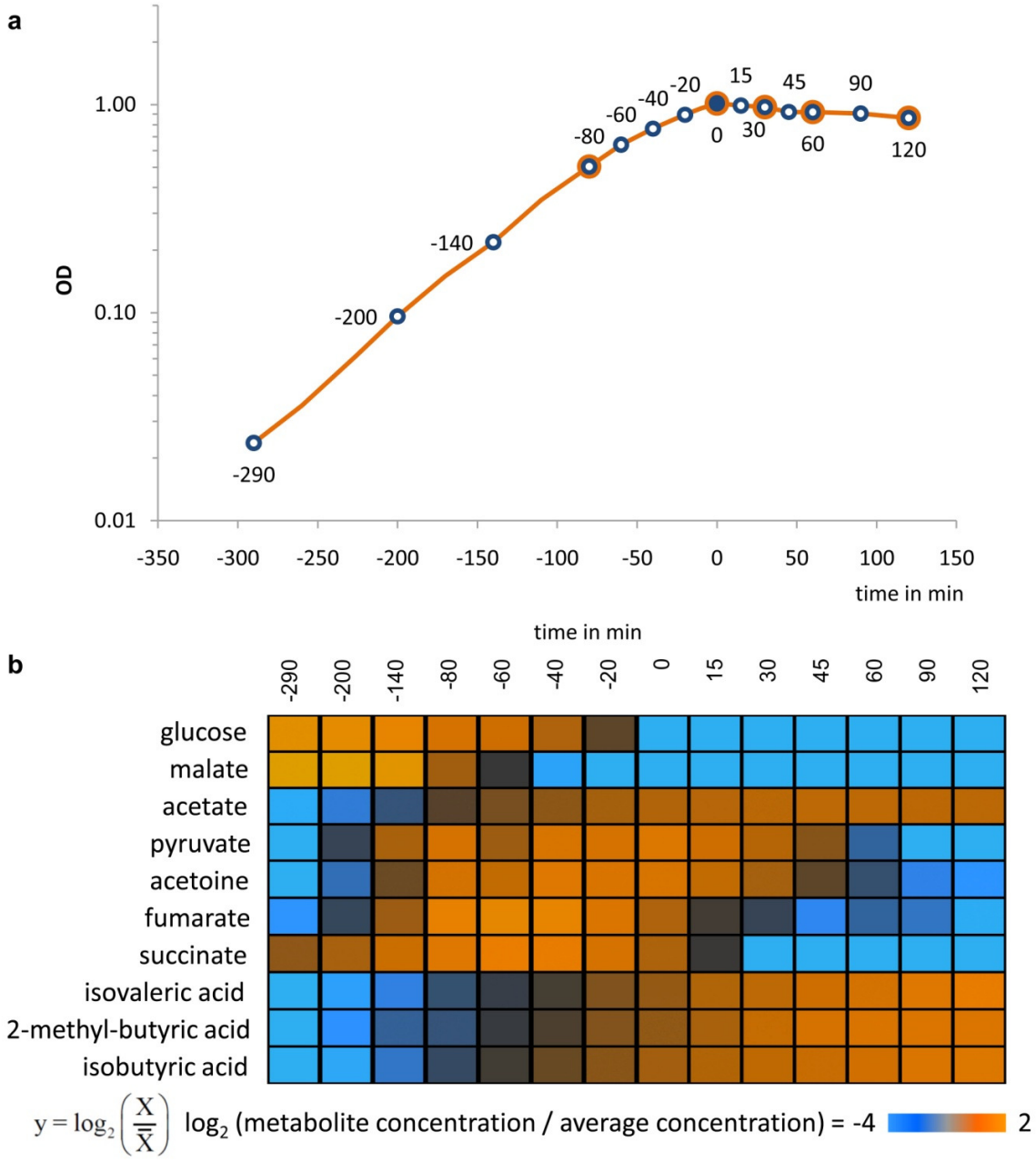
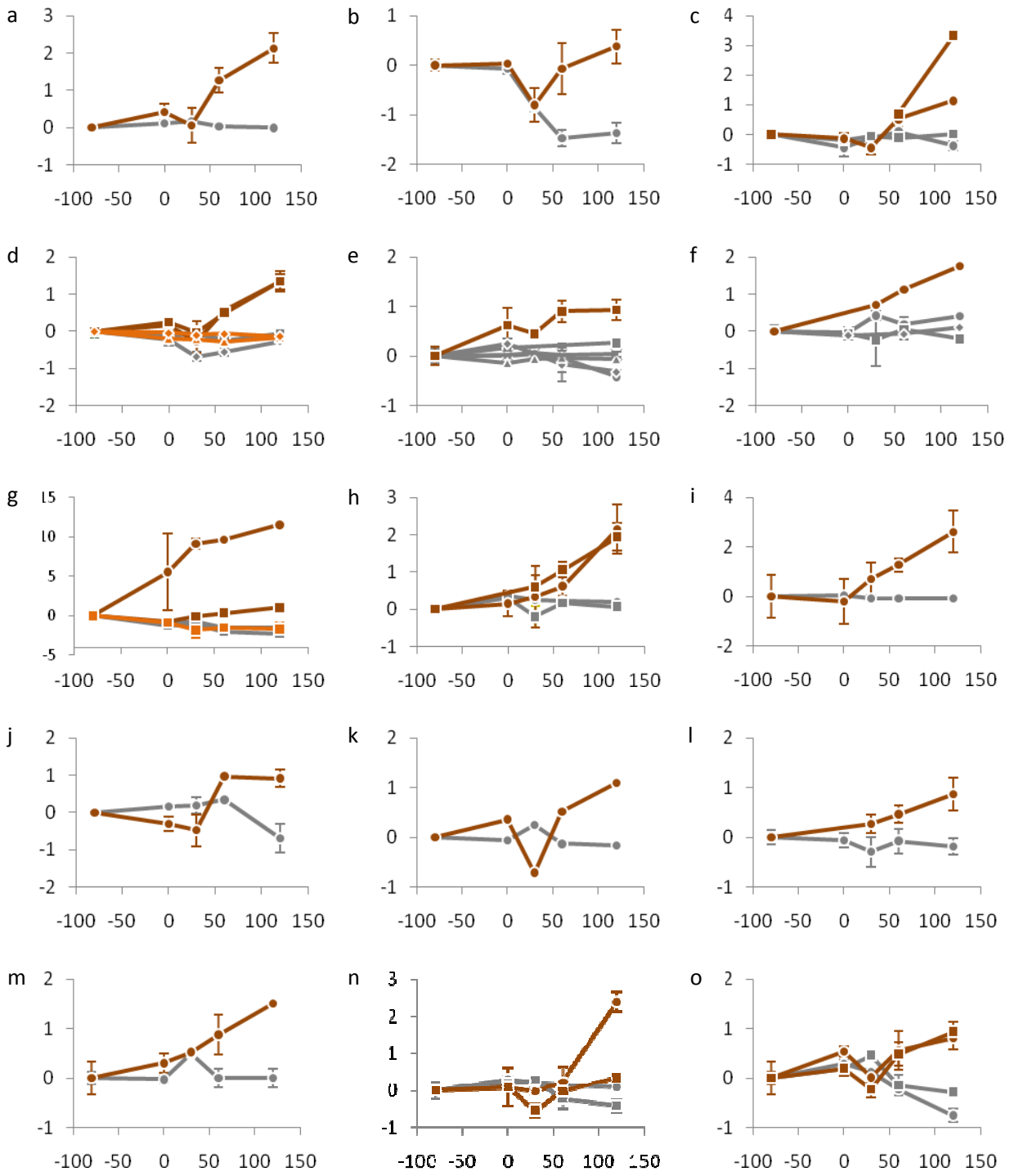
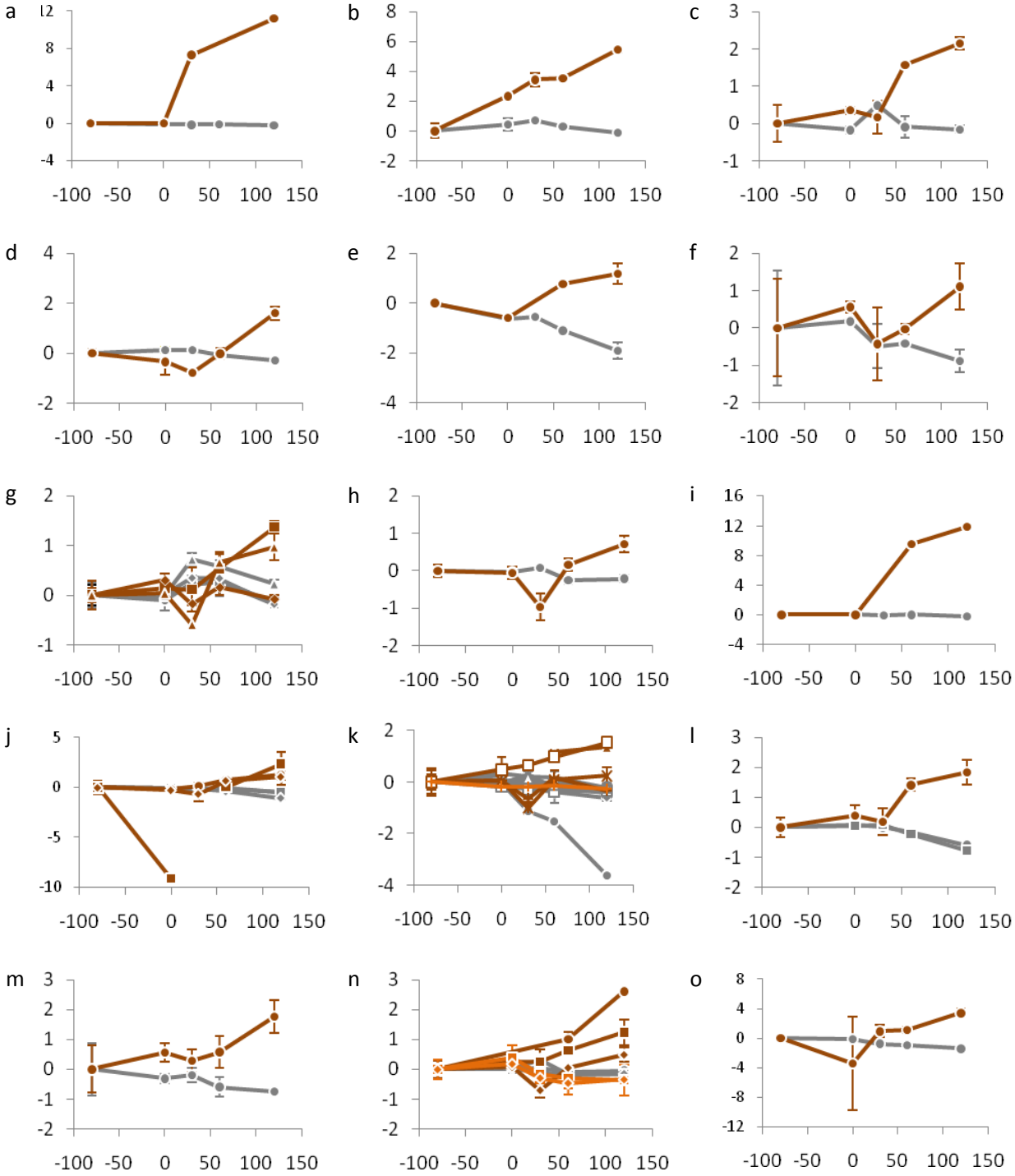


Supplementary Figure S1
Extracellular metabolite concentration changes along the growth curve
(a) Growth curve representing three independent batch cultures of *B. subtilis* 168 undergoing glucose starvation at t = 0. Open blue circles representing sample points for extracellular metabolomics data and open orange circles representing sample points for proteome/ transcriptome data. **(b)** heatmap representing changes in metabolite concentration as $\log_2$ratio of metabolite concentration over average concentration.



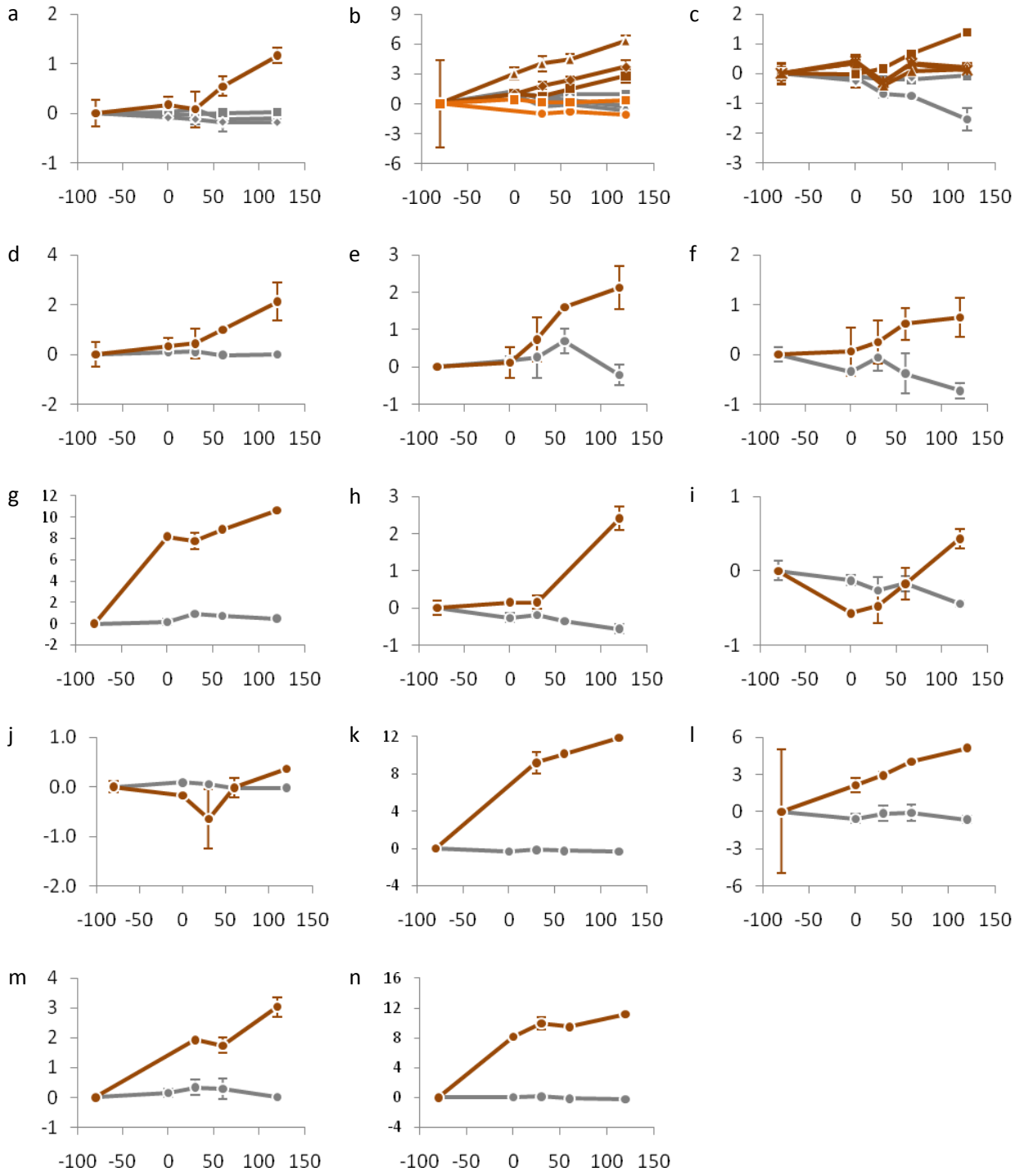
Supplementary Figure S2
Aggregation of cytosolic protein in the enriched membrane fraction
Different changes of protein amount for predicted intracellular proteins in the cytosolic fraction and the enriched membrane fraction (EMF). Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Error bars indicate standard deviation (SD) of the biological replicates (n=3). Differences of membrane shaving fraction (MSF) vs. EMF clarify aggregation only to be seen due to the experimental process. Changes in protein amount assigned to specific localizations: grey: cytosolic fraction; brown: EMF; orange: MSF. **(a)** AccC • **(b)** ArgG • **(c)** AroA • / AroF ■ **(d)** AtpA • / AtpD ■ / AtpF ◆ / AtpE ▲ **(e)** CysC • / CysE ■ / CysH ◆ / CysK ▲/ CysS x **(f)** DegS • / DegU ■ / DegV ◆ **(g)** DhbB • / DhbF ■ **(h)** FabG • / FabI ■ **(i)** FusA • **(j)** GapA • **(k)** GatA • **(l)** GtaB• **(m)** HemL • **(n)** IlvC • / IlvH ■ **(o)** LeuA • / LeuB ■



Supplementary Figure S3

Aggregation of cytosolic protein in the enriched membrane fraction

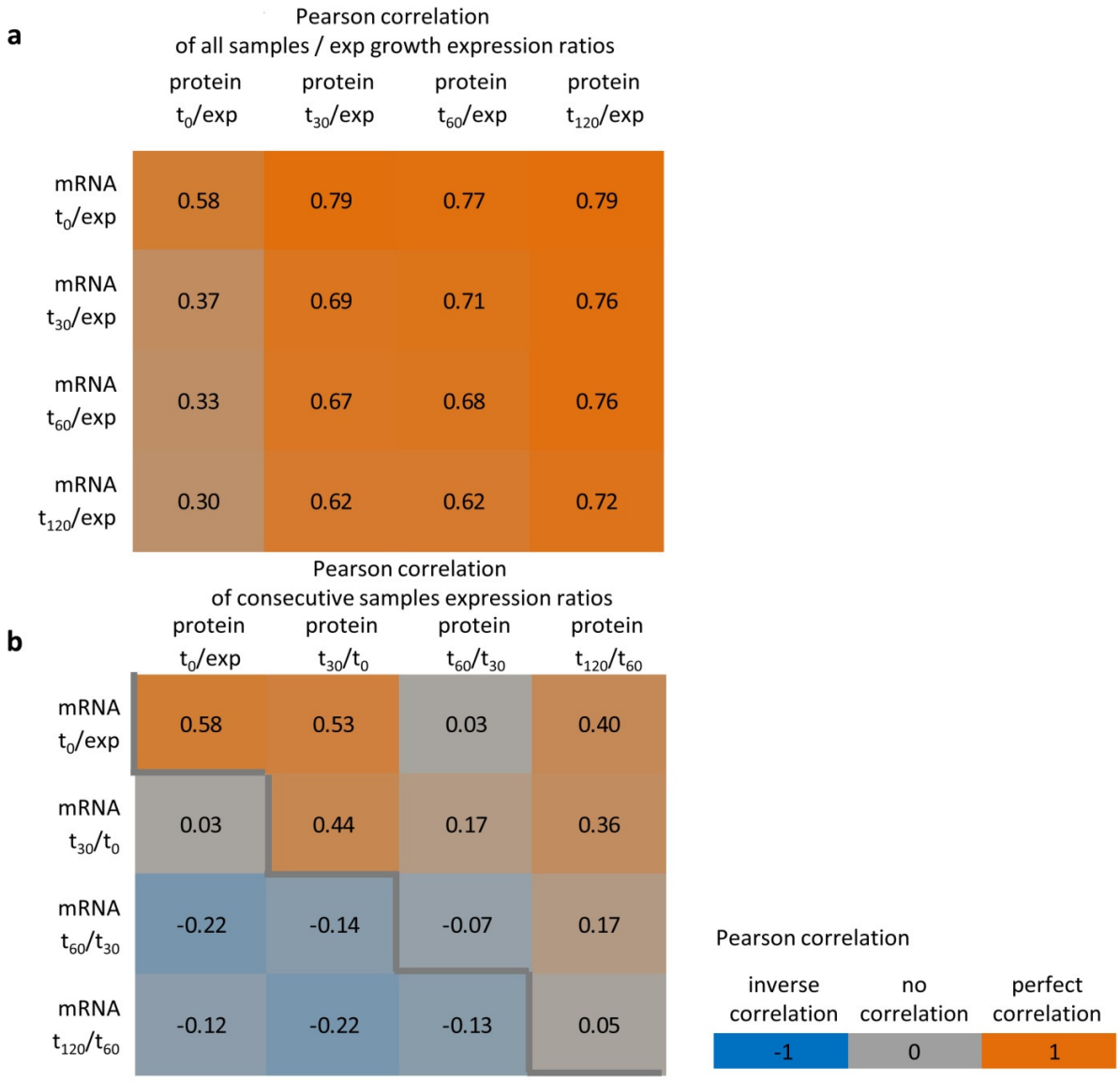
Different changes of protein amount for predicted intracellular proteins in the cytosolic fraction and the enriched membrane fraction (EMF). Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Error bars indicate standard deviation (SD) of the biological replicates (n=3). Differences of membrane shaving fraction (MSF) vs. EMF clarify aggregation only to be seen due to the experimental process. Changes in protein amount assigned to specific localizations: grey: cytosolic fraction; brown: EMF; orange: MSF. **(a)** MenB • **(b)** MetE • **(c)** MetK • **(d)** MtnA • **(e)** NadA • **(f)** Nin • **(g)** PdhA • / PdhB ■ / PdhC ◆ / PdhD ▲ **(h)** PdxS • **(i)** PtsI • **(j)** PurA • / PurB ■ / PurL ◆ **(k)** PyrB • / PyrC ■ / PyrD ◆ / PyrE ▲/ PyrF * / PyrG o / PyrH □ / PyrK ◇ / PyrR △ / PyrP + **(l)** PyrAA • / PyrAB ■ **(m)** RecA • **(n)** RpoA • / RpoB ■ / RpoC ◆ **(o)** Sat •



Supplementary Figure S4

Aggregation of cytosolic protein in the enriched membrane fraction

Different changes of protein amount for predicted intracellular proteins in the cytosolic fraction and the enriched membrane fraction (EMF). Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Error bars indicate standard deviation (SD) of the biological replicates (n=3). Differences of membrane shaving fraction (MSF) vs. EMF clarify aggregation only to be seen due to the experimental process. Changes in protein amount assigned to specific localizations: grey: cytosolic fraction; brown: EMF; orange: MSF. **(a)** SerA • / SerC ■ / SerS ◆ **(b)** SrfAA • / SrfAB ■ / SrfAC ◆ / SrfAD ▲ **(c)** TagD • / TagE ■ / TagF ◆ / TagG ▲/ TagH * **(d)** Tuf • **(e)** YfmT • **(f)** YjbV • **(g)** Yjcl • **(h)** YjlD • **(i)** YkaA • **(j)** YkqC • **(k)** YumC • **(l)** YurP • **(m)** YurY • **(n)** YurU •

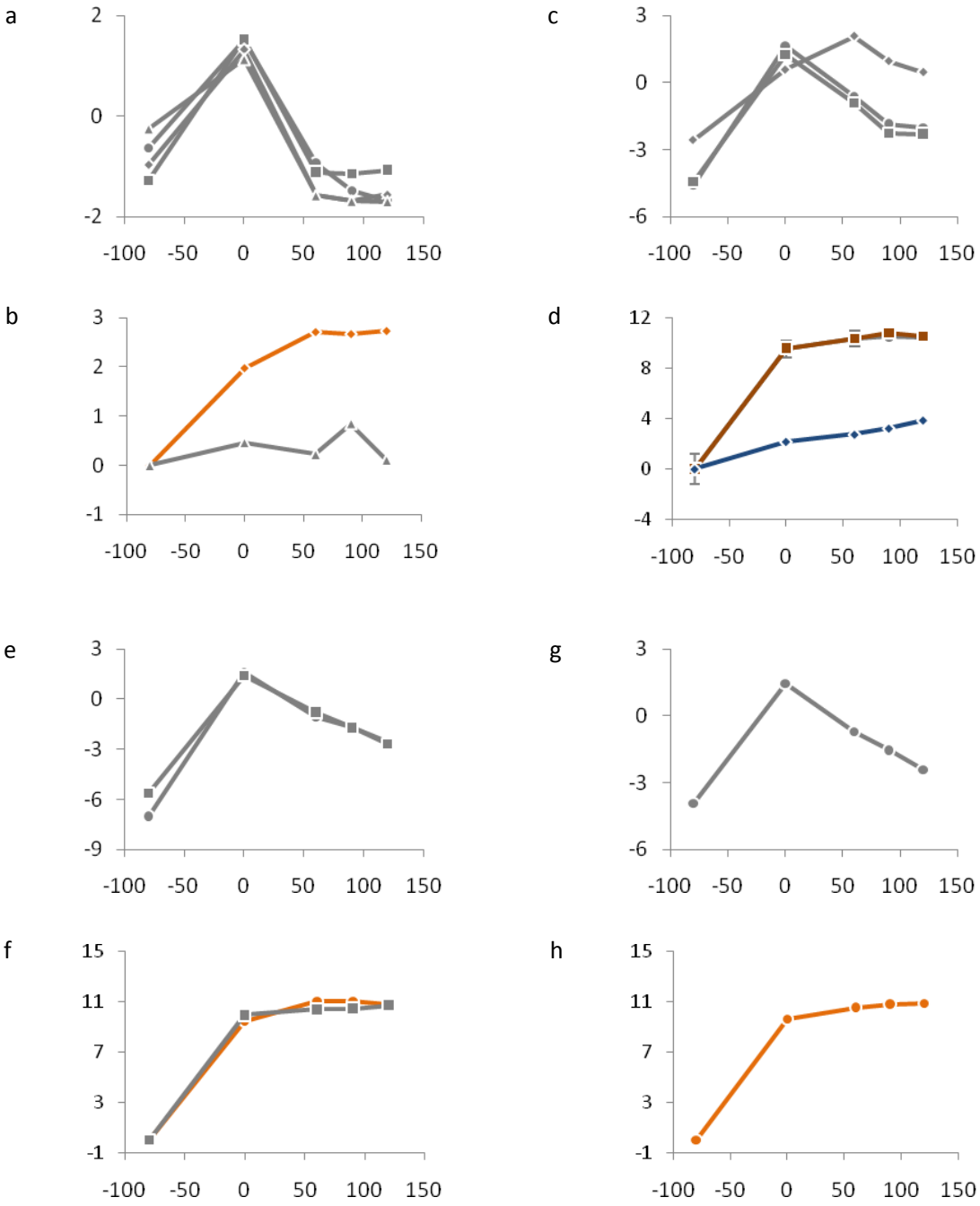


Supplementary Figure S5
Correlation analysis between changes in mRNA and protein amount
Changes in protein levels correlate only to a little extent to changes in mRNA amount (Pearson Correlation $\rho \leq 0.79$). Changes of expression values on mRNA and protein levels normalized to exponential growth phase have been subjected to a Pearson correlation analysis. **(a)** Most of the gene products decrease in response to entry into stationary phase on mRNA and slowly decrease on protein level. This dominates Pearson correlation analysis and results in positive values for all pair wise comparisons. The fact that the increase of protein amounts drag behind induction of mRNA expression is illustrated by the increase of correlation coefficients in later sample ratios (columns) compared to mRNA ratios (rows). Log change fold analysis of consecutive samples gives a more differentiated picture **(b)** Globally almost all protein changes are dragging behind or running simultaneously (t_0, t_{30}) to mRNA expression changes. Most cells on or right of the diagonal line show positive Pearson coefficients (t_0, t_{30}, t_{120}). As expected, lower left cells (correlations of late mRNA changes to early protein changes) show a cluster of non correlating comparisons.

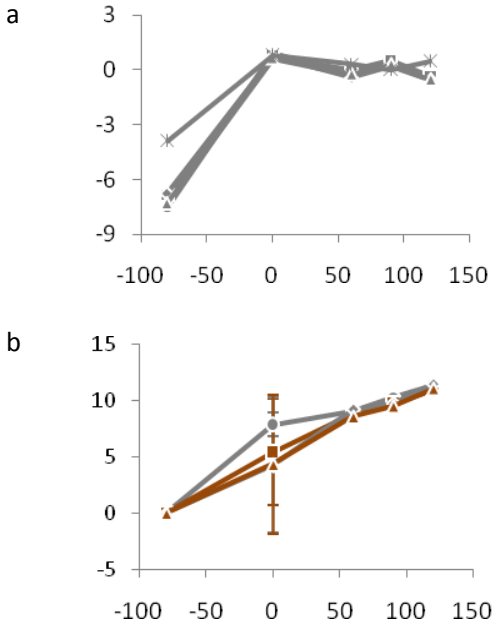
Supplementary Figure S6

Quantitative changes of members of the Fur regulon

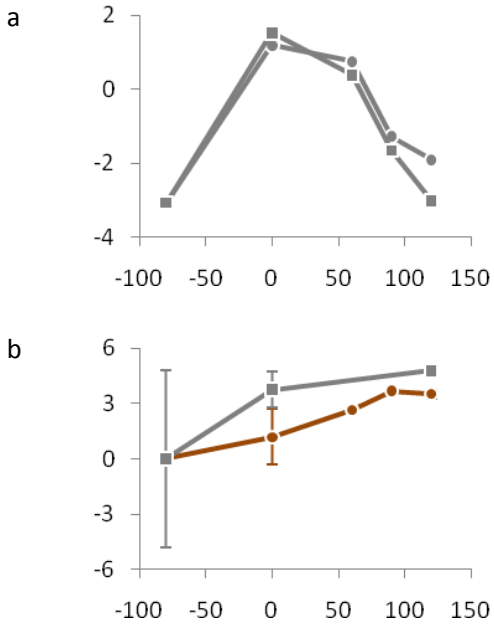
Quantitative changes of members of the Fur regulon are shown in this table according to the DBTBS database of genes depending on the transcriptional factor Fur (*http://dbtbs.hgc.jp/*). All regulated genes are negatively regulated by Fur. Members of the *nas*BCDEF and the *ybb*B operon were not detected (n.d.) in this study. Heatmap representing $\log_2$ratio changes of protein amount corrected for the first time point. EMF: enriched membrane fraction; MSF: membrane shaving fraction; BEF: biotinylation enriched fraction.



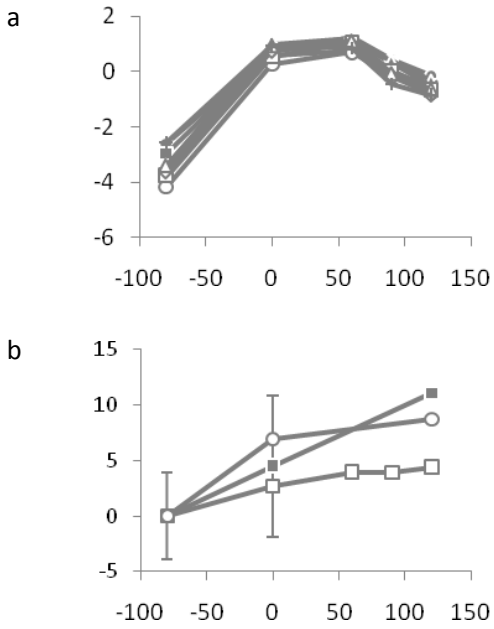
Supplementary Figure S7
Changes of gene expression and of protein amount for sugar transporters and associated cytosolic/extracellular proteins: group I glucuronate/ ß-glycosides/ lichenane / sucrose
Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Error bars indicate standard deviation (SD) of the biological replicates (n=3). Changes in protein amount assigned to specific localizations: grey: cytosolic fraction; brown: enriched membrane fraction (EMF); orange: membrane shaving fraction (MSF); blue: extracellular fraction. **(a)** changes in gene expression for glucuronate transporter genes. *gnt*R • / *gnt*K ■ / *gnt*P ◆ / *gnt*Z ▲ **(b)** changes in protein amount for proteins involved in glucuronate transport. GntP ◆ /GntZ ▲ **(c)** changes in gene expression for ß-glycoside transporter genes. *bgl*H • / *bgl*P ■ / *bgl*S ◆ **(d)** changes in protein amount for proteins involved in ß-glycoside transport. BglH • / BglP ■ / BglS ◆ **(e)** changes in gene expression for lichenane transporter genes. *lic*C • / *lic*B ■ **(f)** changes in protein amount for proteins involved in lichenane transport. LicC • / LicB ■ **(g)** changes in gene expression for sucrose transporter genes. *sac*P • **(h)** changes in protein amount for proteins involved in sucrose transport. SacP •



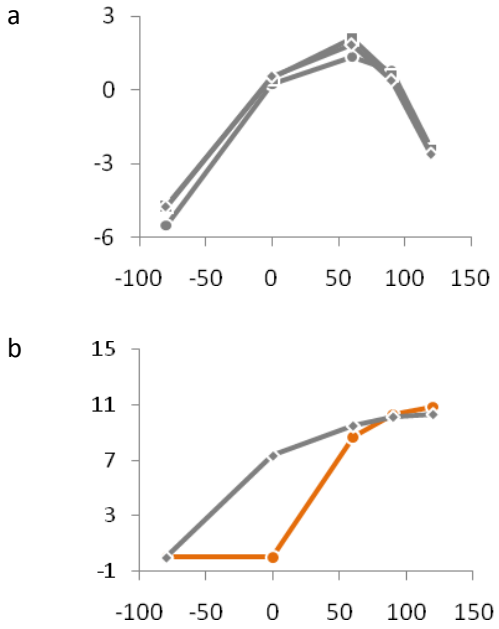
Supplementary Figure S8
Changes of gene expression and of protein amount for sugar transporters and associated cytosolic/extracellular proteins: group II ribose
Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Error bars indicate standard deviation (SD) of the biological replicates (n=3). Changes in protein amount assigned to specific localizations: grey: cytosolic fraction; brown: enriched membrane fraction (EMF) **(a)** changes in gene expression for ribose transporter genes. *rbs*A • / *rbs*B ■ / *rbs*D ◆ / *rbs*C ▲ / *msm*X * **(b)** changes in protein amount for proteins involved in ribose transport. RbsA • / RbsB ■ / RbsD ◆ / RbsC ▲



Supplementary Figure S9
Changes of gene expression and of protein amount for sugar transporters and associated cytosolic/extracellular proteins: group III ribose/ levane
Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Error bars indicate standard deviation (SD) of the biological replicates (n=3). Changes in protein amount assigned to specific localizations: grey: cytosolic fraction; brown: enriched membrane fraction (EMF) **(a)** changes in gene expression for ribose/ levane transporter genes. *lev*G • / *lev*E ■ **(b)** changes in protein amount for proteins involved in ribose/ levane transport . LevG • / LevE ■

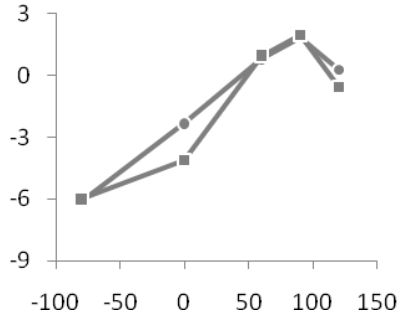


Supplementary Figure S10
Changes of gene expression and of protein amount for sugar transporters and associated cytosolic/extracellular proteins: group IV myo- inositol
Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Error bars indicate standard deviation (SD) of the biological replicates (n=3). Changes in protein amount assigned to specific localizations: grey: cytosolic fraction **(a)** changes in gene expression for myo-inositol transporter genes. *fba*B • / *iol*I ■ / *iol*H ◆ / *idh* ▲ / *iol*F * / *iol*E ○ / *iol*D ◘ / *iol*C ◊ / *iol*B △ / *msm*A + **(b)** changes in protein amount for proteins involved in myo-inositol transport . IolI ■ / IolD ◘ / IolC •



Supplementary Figure S11
Changes of gene expression and of protein amount for sugar transporters and associated cytosolic/extracellular proteins: group V mannose/ mannitol
Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Changes in protein amount assigned to specific localizations: grey: cytosolic fraction; orange: membrane shaving fraction (MSF) **(a)** changes in gene expression for mannose/ mannitol transporter genes. *man*P • / *mtl*D ■ / *mtl*A ◆ **(b)** changes in protein amount for proteins involved in mannose/ mannitol transport. ManP • / MtlD ■ / MtlA ◆

a



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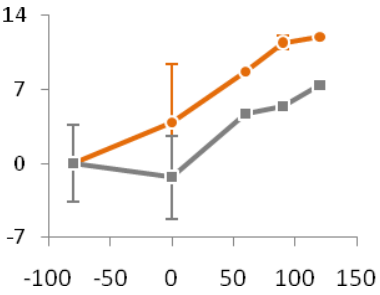
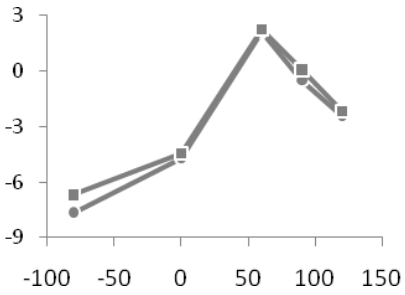


Supplementary Figure S12
Changes of gene expression and of protein amount for sugar transporters and associated cytosolic/extracellular proteins: group VI maltose
Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Error bars indicate standard deviation (SD) of the biological replicates (n=3). Changes in protein amount assigned to specific localizations: grey: cytosolic fraction; orange: membrane shaving fraction (MSF) **(a)** changes in gene expression for maltose transporter genes. *glv*C • / *glv*A ■ **(b)** changes in protein amount for proteins involved in maltose transport. GlvC • / GlvA ■

a



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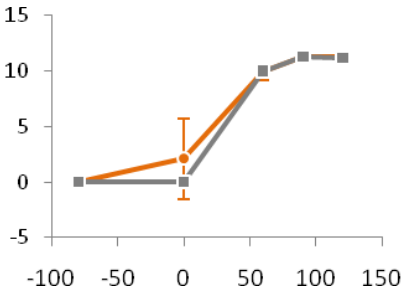
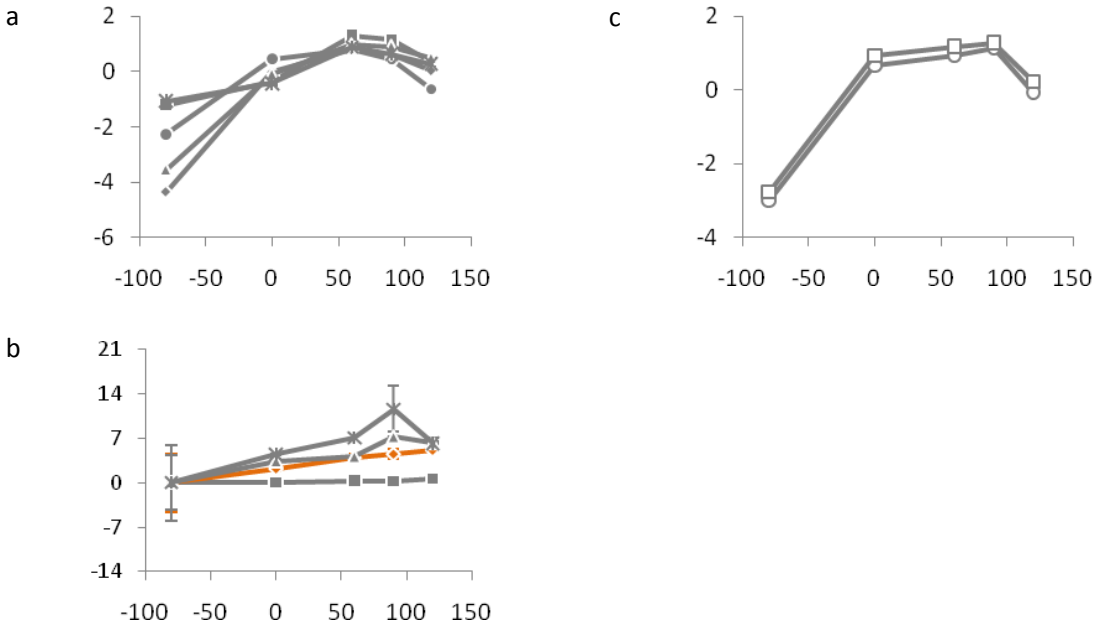
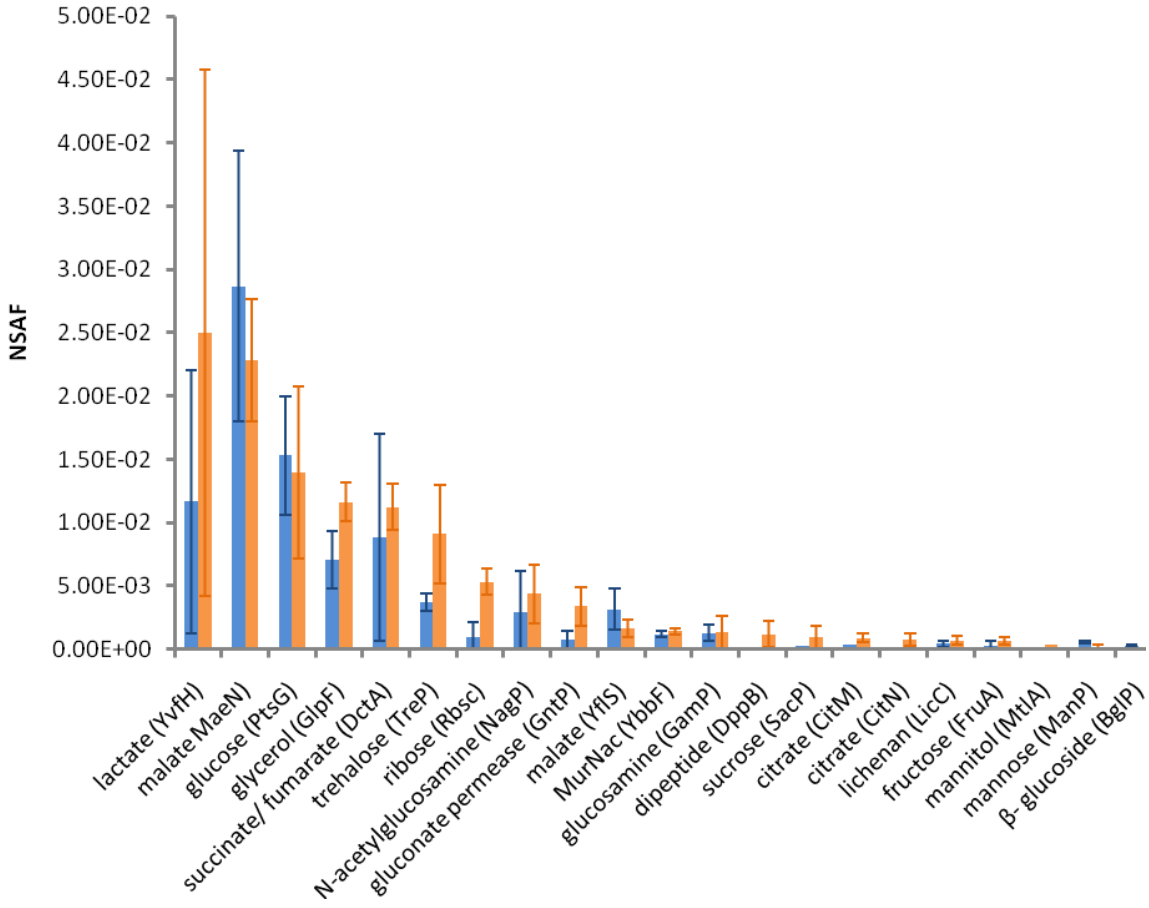


Supplementary Figure S13
Changes of gene expression and of protein amount for sugar transporters and associated cytosolic/extracellular proteins: group VII trehalose
Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Error bars indicate standard deviation (SD) of the biological replicates (n=3). Changes in protein amount assigned to specific localizations: grey: cytosolic fraction; orange: membrane shaving fraction (MSF) **(a)** changes in gene expression for trehalose transporter genes. *tre*P • / *tre*A ■ **(b)** changes in protein amount for proteins involved in trehalose transport. TreP • / TreA ■



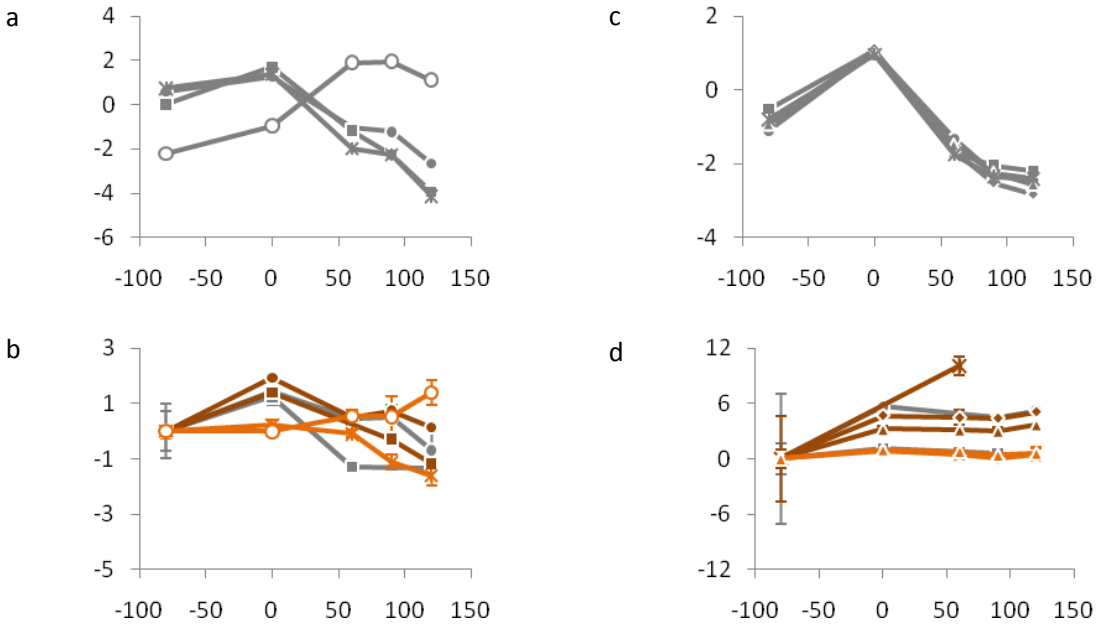
Supplementary Figure S14
Changes of gene expression and of protein amount for sugar transporters and associated cytosolic/extracellular proteins: group VIII glycerol / ß-xyloside
Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Error bars indicate standard deviation (SD) of the biological replicates (n=3). Changes in protein amount assigned to specific localizations: grey: cytosolic fraction; orange: membrane shaving fraction (MSF) **(a)** changes in gene expression for glycerol transporter genes. *glp*T • / *glp*P ■ / *glp*F ◆ / *glp*K ▲ / *glp*D * **(b)** changes in protein amount for proteins involved in glycerol transport. GlpP ■ / GlpF ◆ / GlpK ▲ / GlpD * **(c)** changes in gene expression for ß-xyloside transporter genes. *xyn*P • / *xyn*B ■. Proteins involved in ß-xyloside transport were not detected.



Supplementary Figure S15

Normalized Spectral Abundance Factors (NSAF) of selected transporters of growing and non-growing cells

The relative proportion of transporter proteins within the membrane shaving fraction (MSF)is visualized by calculation and plotting of the Normalized Spectral Abundance Factors (NSAF). NSAF were calculated according to Zybailov *et al.*[51].To obtain the NSAF for a specific protein, the number of spectral counts (*SpC*; the total number of MS/MS spectra accounting for this specific protein) is divided by the length *L* of the protein and divided by the sum of *SpC/L* for all proteins in the proteomic experiment. In this bar chart, NSAF for combined $^{14}N/^{15}N$ mass traces of selected transporter proteins identified in the membrane shaving fraction (MSF) are plotted for growing (logarithmic growth, blue bars) and non-growing cells (late stationary phase T120, orange bars). Error bars indicate standard deviation (SD) of the biological replicates (n=3).



Supplementary Figure S16
Changes of gene expression and of protein amount for members of the cannibalism operons

Y-axis: log_2ratios X-axis: time points -80, 0, 30, 60 and 120 minutes. Log_2ratios for changes in protein amount are corrected for the first time point. Error bars indicate standard deviation (SD) of the biological replicates (n=3). Changes in protein amount assigned to specific localizations: grey: cytosolic fraction; brown: enriched membrane fraction (EMF); orange: membrane shaving fraction (MSF) **(a)** changes in gene expression for the *sdp*- Operon. *sdp*A • / *sdp*C ■ / *sdp*B * / *sdp*I ○ **(b)** changes in protein amount for proteins belonging to the *sdp* operon. SdpA • / SdpC ■ / SdpB * / SdpI ○ **(c)** changes in gene expression for the *skf*- Operon. *skf*B • / *skf*G ■ / *skf*C ◆ / *skf*D ▲ / *skf*F * **(d)** changes in protein amount for proteins belonging to the *skf*- Operon. SkfB • / SkfG ■ / SkfC ◆ / SkfD ▲ / SkfF *.

Supplementary Methods

Proteome study

Proteomics measurement - extracellular fraction

The tryptic digested proteins obtained from the 1D SDS PAGE gel pieces were subjected to a reversed phase column chromatography (Waters BEH 1.7 µm, 100-µm i. D. x 100 mm, Waters Corporation, Milford, Mass., USA) operated on a nanoACQUITY-UPLC (Waters Corporation, Milford, Mass., USA). Peptides were first concentrated and desalted on a trapping column (Waters nanoACQUITY UPLC column, Symmetry C18, 5 µm, 180 µm x 20 mm, Waters Corporation, Milford, Mass., USA) for 3 min at a flow rate of 1ml/min with 99% buffer A (0.1% acetic acid). Subsequently the peptides were eluted and separated with a non-linear 80-min gradient from 5 –60 % ACN in 0.1 % acetic acid at a constant flow rate of 400 nl/min. MS and MS/MS data were acquired with the LTQ-Orbitrap mass spectrometer (Thermo Fisher, Bremen, Germany) equipped with a nanoelectrospray ion source. After a survey scan in the Orbitrap (r= 30,000) MS/MS data were recorded for the five most intensive precursor ions in the linear ion trap. Singly charged ions were not taken into account for MS/MS analysis.

Proteomics measurement - cytosolic fraction, BEF, EMF

The data were acquired as described in "extracellular fraction" except that the lock mass option was enabled in the Orbitrap survey scans.

Proteomics measurement - MSF

ProteinaseK/ Chymotryptic digests of the membrane shaving samples were loaded on a nanoACQUITY UPLC System (Waters Corporation, Milford, Mass., USA). The LC was equipped with an analytical column (nanoACQUITY UPLC column, BEH130 C18, 1.7 µm, 100

µm x 100 mm; Waters Corporation, Milford, Mass., USA) operated at 60°C at 400nl/min. Peptides were loaded directly on the column and after washing for 30 min with 99% buffer A (0.1% acetic acid) the peptides were eluted from the column in a 5 h linear gradient from 5- 90% buffer B (90% acetonitrile, 0.1% acetic acid).

MS/MS - data were acquired with the LTQ- Orbitrap mass spectrometer (Thermo Fisher, Bremen, Germany) equipped with a nanoelectrospray ion source. After a survey scan in the Orbitrap (r= 30,000) with the lock- mass option enabled. MS/MS data were recorded for the five most intensive precursor ions in the linear ion trap.

Filtering protein identification lists

Protein identification data were cured with an in-house java script to ensure that each protein hit relies on at least two different peptides as judged by the amino acid sequences.

Determination of protein coverage

Proteins that were identified by two unique peptides in at least one sample ($^{14}N/ ^{15}N$ combined) were added to yield the protein identification list (Supplementary Data 1).

Data analysis relative quantitation

The cured search results served as the base for the software Census[21] to obtain quantitative data of ^{14}N peaks (sample) and ^{15}N peaks (pooled reference[20]). Peptide ratios were determined by Census and subsequently exported (R^2 values bigger than 0.7 and only unique peptides; proteins failing to be relatively quantified were checked manually in the graphical user interface for on/off proteins). Proteins relatively quantified with at least 2 peptides were taken into account for the subsequent analysis. Raw quantitation results including number of peptides taken into account for protein quantitation and standard deviation (SD) of peptide quantitation values are found in Supplementary Data 2-6.

ANOVA Analysis

The ANOVA analysis was carried out using TMEV[53] (version 4.4) on the quantitative data from "Data analysis and relative quantitation". The median-centered log_2ratio data was adjusted by the standard deviation (SD) per row in the software and ANOVA was carried out with the following settings: number of groups 5; p- values based on permutation (1000) resulting in p- values <0.01 as significance threshold. ANOVA results are found in Supplementary Data 2-6.

Compilation of quantitation tables

All quantitation results of a complete GeLC-MS run were median centered and ratios were log_2-transformed. Averaging was carried over the biological replicates. Combined quantitation results consisting of averaged quantitation result, standard deviation (SD) and number of replicates quantified per protein are found in Supplementary Data 2-6. Time course data for each protein was adjusted to the first time point as reference point; proteins without quantitative information for the first time point were not taken into account for a detailed discussion. Error bars for figures are based on the standard deviation for doubly/ triply determined quantitation values of the averaged quantitation results from the biological replicates.

Prediction of protein localization

Predictions of protein localization in the cell were assigned according to the LocateP algorithm[52].

Transcriptome study

RNA preparation

Total RNA was isolated by the method described by Eymann et al.[54] with some modifications. Briefly, bacterial cells were harvested by mixing with ½ volume of frozen killing buffer (20 mM Tris/HCl [pH 7.5], 5 mM $MgCl_2$, 20 mM NaN_3) and subsequent centrifugation for 3 min at 4 °C. After discarding the supernatant, cell pellets were frozen in liquid nitrogen and stored at -80 °C.

For mechanical disruption, the pellets were resuspended in 200 µl of ice-cold killing buffer, immediately dropped into a Teflon disruption vessel (precooled and filled with liquid nitrogen), and then disrupted in a Mikro-Dismembrator S (Sartorius) (2 min at 2,600 rpm). The resulting frozen powder was resuspended in 4 ml of prewarmed lysis solution (4 M guanidine thiocyanate, 25 mM sodium acetate [pH 5.2], 0.5% N-laurylsarcosinate [wt/vol], 50 °C) by repeated pipetting. Afterwards, 1 ml aliquots of the lysate were transferred to microcentrifuge tubes and immediately frozen in liquid nitrogen.

Total RNA was isolated by acid-phenol extraction. The samples were extracted twice with an equal volume of acid phenol/chloroform/isoamyl alcohol (25:24:1, [pH 4.5]) and once with chloroform/isoamyl alcohol (24:1). After adding 1/10 volume of 3 M sodium acetate (pH 5.2), RNA was precipitated with isopropanol, washed with 70 % ethanol and dissolved in 100 µl of RNase free water. For transcriptome analysis, 35 µg RNA were DNase-treated using the RNase-Free DNase Set (Qiagen) and purified using the RNA Clean-Up and Concentration Micro Kit (Norgen). The quality of the RNA preparations was assessed by means of the Agilent 2100 Bioanalyzer according to the manufacturer's instructions. For two-color hybridizations, a reference pool containing equal amounts of RNA from each sample was prepared.

Synthesis of fluorescently labeled cDNA, array hybridization and scanning

Synthesis and purification of fluorescently labeled cDNA were carried out according to Charbonnier et al.[55] with minor modifications. In detail, 10 µg of total RNA were mixed with random primers (Promega) and spike-ins (Two-Color RNA Spike-In Kit, Agilent Technologies). The RNA/primer mixture was incubated at 70 °C for 10 min followed by 5 min incubation on ice. Then, the following reagents were added: 10 µl of 5x First Strand Buffer (Invitrogen), 5 µl of 0.1 M DTT (Invitrogen), 0.5 µl of a dNTP mix (10 mM dATP, dGTP, and dTTP, 2.5 mM dCTP),

1.25 µl of Cy3-dCTP or Cy5-dCTP (GE Healthcare) and 2µl of SuperScript II reverse transcriptase (Invitrogen). The reaction mixture was incubated at 42 °C for 60 min and then heated to 70 °C for 10 min. After 5 min on ice, the RNA was degraded by incubation with 2 units of RNaseH (Invitrogen) at room temperature for 30 min. Labeled cDNA was then purified using the CyScribe GFX Purification Kit (GE Healthcare). The individual samples were labeled with Cy5, whereas the reference pool was labeled with Cy3. 500 ng of Cy5-labeled cDNA and 500 ng of Cy3-labeled cDNA were hybridized together to the microarray following Agilent's hybridization, washing and scanning protocol (Two-Color Microarray-based Gene Expression Analysis, version 5.5).

Data analysis transcriptome data

Data were extracted and processed using the Feature Extraction software (version 9.5, Agilent Technologies). For each gene on a microarray, the error-weighted average of the log ratio values of the individual probes was calculated using the Rosetta Resolver software (version 7.2.1, Rosetta Biosoftware). As the analysis was performed by hybridizing the individual samples against a common reference, the ratio values represent relative gene expression levels at a given time point. For the determination of maximum transcript intensities over the time course (construction of figure 7), intensity values of each gene were calculated as the average of background subtracted Cy5 intensities of the corresponding probes.

Extracellular metabolome study

Extracellular metabolite sampling and measurement by ^{1}H-NMR

2.5 mL cell culture was sterile filtrated rapidly into a 2 mL tube. The filter were discarded, while the supernatant was stored on ice and afterwards frozen and stored at -20°C as described previously[56].

400 µL of the extracellular sample was buffered to pH 7.0 by addition of 200 µL of a sodium hydrogen phosphate buffer (0.2 mM [pH 7.0], including 1mM sodium 3-trimethylsilyl-[2,2,3,3-D4]-1-propionic acid (TSP)) made up with 25% D_2O to provide a nuclear magnetic resonance (NMR)-lock signal. The sample was transferred into 5-mm NMR-glass tubes (length, 7 in.). Spectral referencing was done relative to 1 mM TSP in phosphatebuffer. All NMR spectra are obtained at 600.27 MHz at a nominal temperature of 310 K using a Bruker AVANCE-II 600 NMR spectrometer operated by TOPSPIN 2.1 software (both from Bruker Biospin GmbH, Rheinstetten, Germany). A modified 1D-NOESY pulse sequence was used with presaturation on the residual HDO signal during both the relaxation delay and the mixing time. A total of 32 free induction decays (FID scans) were collected using a spectral width of 30 ppm for a one-dimensional spectrum[57].

Supplementary References

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