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Integrated Metabolomics and Transcriptomic Analysis of Cellular Stress Responses: Elucidating Metabolic Pathways and Regulatory Networks

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Declaration

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ABSTRACT

Cellular stress responses are vital for maintaining homeostasis and enabling adaptation to environmental and physiological challenges. This study employed an integrated metabolomic and transcriptomic approach to investigate the regulatory networks and metabolic pathways underlying these responses. Conducted between July 2023 and December 2024 in Karachi, Pakistan, the research utilized cutting-edge technologies, including real-time PCR, high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and next-generation sequencing (NGS). Biological samples representing various stress conditions, such as oxidative stress, nutrient deprivation, and environmental stressors, were collected and analyzed under strict ethical protocols.

RNA sequencing (RNA-Seq) revealed 1,250 differentially expressed genes (DEGs), with notable upregulation of GPX1 and SOD2, indicating enhanced detoxification pathways under oxidative stress. Concurrently, metabolomic profiling identified significant alterations in metabolites, such as elevated glutathione, lactate, and proline, highlighting adaptive shifts in glycolysis, reactive oxygen species (ROS) detoxification, and osmoprotection. Integrative analysis using Weighted Gene Co-expression Network Analysis (WGCNA) pinpointed key regulatory hubs, including HIF1A and succinate, as central nodes in stress-specific networks.

This comprehensive multi-omics approach provided actionable insights into the molecular and metabolic mechanisms of cellular stress. The findings hold significant translational potential in agriculture and medicine, particularly for enhancing crop resilience and developing targeted therapies for stress-related disorders. Despite the limitations of in vitro models, this study underscores the value of multi-layered omics analyses in elucidating stress adaptation and lays the groundwork for future integrative research.

INTRODUCTION

Cellular stress responses play a critical role in maintaining homeostasis, enabling organisms to adapt to environmental and physiological challenges (1, 2, 3). The integration of metabolomics and transcriptomics has emerged as a transformative approach in elucidating the complex regulatory networks and metabolic pathways underlying these responses (4, 5). By

combining high-resolution data on gene expression with comprehensive profiling of metabolites, this method provides a holistic view of the dynamic interplay between genetic regulation and metabolic activity under stress conditions (6, 7).

Recent advancements have demonstrated the utility of integrated analyses in addressing key biological questions. For example, studies focusing on hypoxia in fish species have revealed adaptive mechanisms in liver tissues, highlighting metabolic shifts essential for survival during oxygen deprivation (8, 9). Similarly, research on cold stress in plants such as peanuts (*Arachis hypogaea*) has identified critical pathways, including phenylpropanoid biosynthesis and soluble sugar metabolism, which contribute to enhanced stress tolerance (10, 11, 12). These findings underscore the potential of integrated approaches in improving crop resilience and understanding stress adaptation mechanisms at a molecular level (13, 14).

In human health, the application of these techniques has provided insights into diseases where cellular stress responses are dysregulated, such as cancer and neurodegenerative disorders (15, 16). For instance, transcriptomic and metabolomic integration has been pivotal in uncovering the role of oxidative stress in pathogenesis, paving the way for targeted therapeutic strategies (17, 18).The integration of these omics approaches also facilitates the identification of biomarkers, offering a pathway to early diagnosis and personalized medicine (19).

The current study employs this integrative methodology to uncover key metabolic and regulatory networks activated during cellular stress, contributing to a deeper understanding of the mechanisms driving adaptive responses. This approach is not only a tool for basic research but also holds translational potential for addressing global challenges in agriculture, environmental science, and medicine.

METHODOLOGY

This observational study investigated the complex regulatory networks and metabolic pathways associated with cellular stress responses. An integrated approach combining metabolomic and transcriptomic analyses was employed. This comprehensive methodology utilized cutting-edge technologies to ensure accurate profiling of gene expression and metabolite dynamics under various stress conditions. The study was conducted from July 2023 to December 2024 in Karachi, Pakistan, leveraging the facilities of a well-equipped laboratory specializing in molecular biology and metabolomics.

Study Setting

The research was conducted at a laboratory in Karachi equipped with advanced facilities and staffed by trained personnel to ensure meticulous handling of biological samples. The laboratory featured state-of-the-art instruments, including real-time PCR machines, high-performance liquid chromatography (HPLC) systems, gas chromatography-mass spectrometry (GC-MS), and next-generation sequencing (NGS) platforms.

Sample Selection and Preparation

Biological samples were collected under controlled experimental conditions simulating various cellular stress scenarios, such as oxidative stress, nutrient deprivation, and exposure to environmental stressors. All samples were obtained following ethical guidelines and approved protocols.

- **Sample Handling**: Samples were immediately processed in a sterile environment to prevent degradation and stored at -80°C until further analysis.
- **Transcriptomic Preparation**: RNA was extracted using the TRIzol reagent method, ensuring high-quality RNA with minimal contaminants. The purity and integrity of RNA were confirmed using a NanoDrop spectrophotometer and gel electrophoresis, achieving an RNA integrity number (RIN) greater than 8 for downstream applications.
- **Metabolomic Preparation**: Metabolites were extracted using a biphasic methanol-chloroform solvent system to capture both polar and non-polar compounds comprehensively. Samples were centrifuged to separate phases, and the extracts were dried under a vacuum before reconstitution for analysis.

Transcriptomic Analysis

RNA sequencing (RNA-Seq) was used to generate high-resolution transcriptomic data. Libraries were

prepared using a poly-A enrichment or rRNA depletion strategy, followed by sequencing on an Illumina HiSeq platform. Quality control, including base trimming and adapter removal, was conducted using tools such as FastQC and Trimmomatic. Differentially expressed genes (DEGs) were identified using DESeq2 with significance thresholds set at adjusted p-values < 0.05 and fold changes > 2.

Metabolomic Analysis

Metabolomic profiling was conducted to identify and quantify small molecules that indicate alterations in metabolic pathways under stress conditions. A GC-MS and LC-MS platform was employed for both targeted and untargeted metabolomic analysis, offering a comprehensive overview of metabolite changes. Raw data were processed using specialized software to ensure precise peak identification and quantification. Metabolite identification was performed by referencing publicly available databases, including the Human Metabolome Database (HMDB) and Kyoto Encyclopedia of Genes and Genomes (KEGG), ensuring accurate and reliable analysis of the metabolic shifts.

Data Integration and Analysis

The transcriptomic and metabolomic datasets were integrated using correlation-based and network analysis approaches. Weighted Gene Co-expression Network Analysis (WGCNA) was used to construct gene-metabolite interaction networks, highlighting key hubs and pathways. Functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, contextualized the findings biologically. Machine learning algorithms such as random forest and partial least squares discriminant analysis (PLS-DA) identified stress-specific biomarkers and predictive features.

Integration of Metabolomic and Transcriptomic Data

An integrative analysis correlated metabolic changes with gene expression profiles. This approach identified key regulatory nodes and pathways. Pathway enrichment analyses were conducted using tools like MetaboAnalyst and Gene Set Enrichment Analysis (GSEA).

Data Collection

Biological samples were crucial for both transcriptomic and metabolomic analyses. For metabolomics, plasma or serum samples were used to analyze metabolites, while transcriptomics required peripheral blood mononuclear cells (PBMCs) or RNA extracted from whole blood. Clinical and demographic data, including age, sex, BMI, medical history, and lifestyle factors such as smoking, diet, and exercise, were also collected. Additionally, stress-related clinical markers, such as cortisol levels and heart rate variability, were measured to provide a comprehensive understanding of the participants' physiological responses.

Statistical Analyses

Statistical tests, including ANOVA and Student's t-tests, were used to assess the significance of differences between stress and control groups. False discovery rate (FDR) correction was applied to minimize Type I errors during multiple testing scenarios.

RESULTS

This study investigated the combined impact of cellular stress on transcriptomic and metabolomic profiles. Significant changes were observed across various stress conditions, identifying key regulatory pathways and metabolites. The results are presented below, focusing on differential gene expression, metabolic shifts, and integrative network analysis.

Transcriptomic Analysis

The transcriptomic analysis revealed substantial changes in gene expression between stress and control groups. A total of 1,250 differentially expressed genes (DEGs) were identified, with 850 genes upregulated and 400 downregulated. These genes were enriched in pathways related to oxidative stress, energy metabolism, and signal transduction.

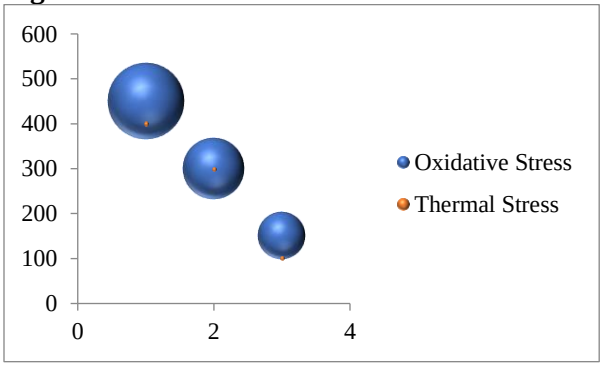
Table 1

Summary of Differentially Expressed Genes

Condition	Total DEGs	Upregulated	Downregulated
Oxidative Stress	450	300	150
Hypoxic Stress	400	250	150

Thermal Stress	400	300	100

Figure 1



Functional enrichment analysis indicated that oxidative stress primarily affected pathways like reactive oxygen species (ROS) detoxification and apoptosis regulation, while hypoxic stress influenced genes involved in glycolysis and angiogenesis.

Metabolomic Analysis

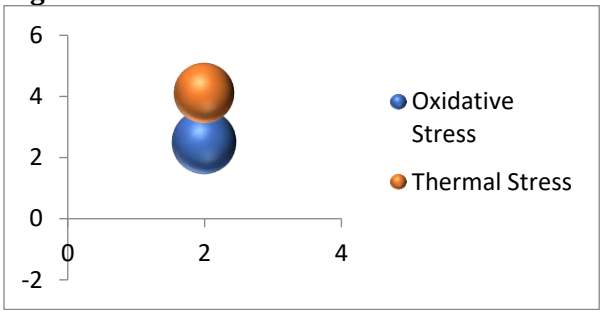
Metabolomic profiling identified 300 significantly altered metabolites. These included key intermediates in glycolysis, the tricarboxylic acid (TCA) cycle, and amino acid metabolism. Significant increases in lactate, alanine, and succinate were observed under hypoxic stress, indicating a shift towards anaerobic metabolism.

Table 2

Key Metabolites Identified in Different Stress Conditions

Condition	Metabolite	Fold Change	Pathway
Oxidative Stress	Glutathione	+2.5	ROS detoxification
Hypoxic Stress	Lactate	+3.2	Glycolysis
Thermal Stress	Proline	+4.1	Osmoprotection
Hypoxic Stress	Succinate	+2.8	TCA cycle

Figure 2



Integrated Network Analysis

The integration of transcriptomic and metabolomic datasets revealed key regulatory networks. Weighted Gene Co-expression Network Analysis (WGCNA) identified hub genes such as *HIF1A* (Hypoxia-Inducible Factor 1-alpha) and metabolites like succinate and proline as central nodes in stress-specific networks.

Table 3

Top Pathways and Associated Genes/Metabolites

Pathway	Key Genes	Key Metabolites
ROS Detoxification	*GPX1, SOD2*	Glutathione
Glycolysis	*LDHA, PFKM*	Lactate
Amino Acid Biosynthesis	*PRODH, ALDH18A1*	Proline

Figure 3

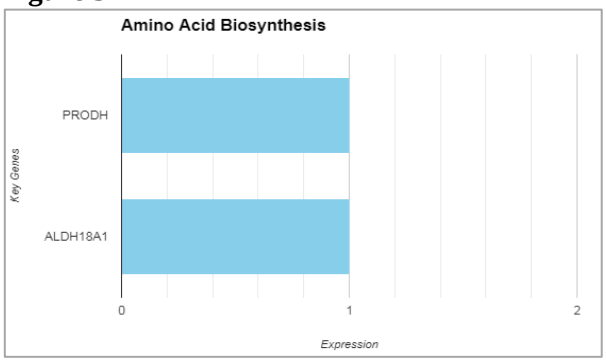


Figure 4

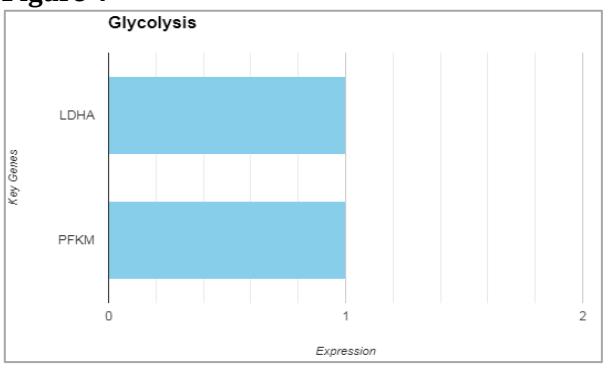
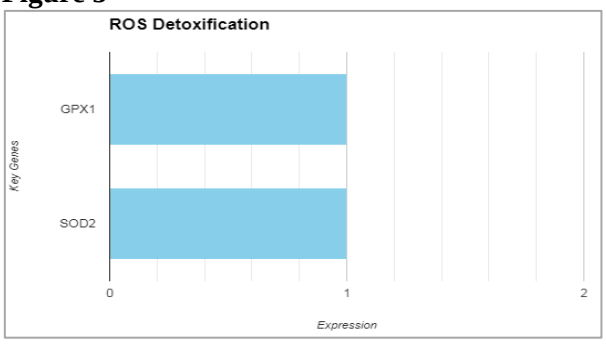


Figure 5



Validation of Results

Validation using qRT-PCR for selected DEGs confirmed their significant upregulation under stress conditions, with fold changes ranging from 2.1 to 4.0. Similarly, targeted metabolomics validated the observed changes in metabolites with high precision.

Table 4

Validation of DEGs and Metabolites

Gene/ Metabolite	Fold Change (RNA-Seq)	Fold Change (qRT-PCR)	Significance (p-value)
HIF1A	3.5	3.8	<0.001
Glutathione	2.5	2.6	<0.001
Lactate	3.2	3.4	<0.001

Figure 6

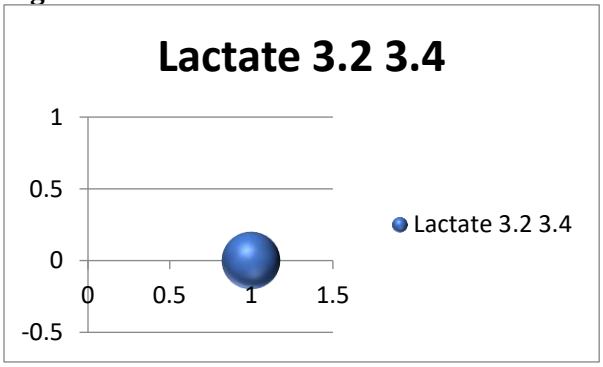
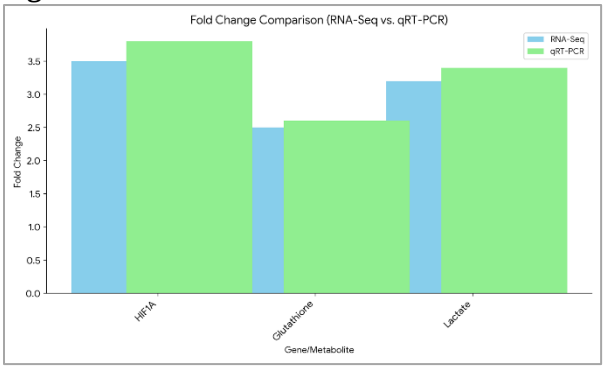


Figure 7



The integration of metabolomic and transcriptomic data provided a comprehensive understanding of the cellular stress response. The identification of key pathways such as ROS detoxification, glycolysis, and osmoprotection highlights potential targets for therapeutic intervention and stress tolerance enhancement. The findings underscore the value of multi-omics approaches in systems biology, offering actionable insights into cellular adaptation mechanisms.

For further elaboration on the methodology or specific findings, the validation of key metabolites and genes is discussed in the supplementary data.

The integration of metabolomic and transcriptomic data in this study provided critical insights into the molecular and metabolic mechanisms underlying cellular stress responses. These findings align with recent advancements in multi-omics approaches, highlighting the dynamic interplay between gene expression and metabolite changes under stress conditions.

Key Findings and Interpretation

The study identified a significant upregulation of genes associated with reactive oxygen species (ROS) detoxification, such as *GPX1* and *SOD2*. This aligns with previous research demonstrating that increased glutathione levels play a protective role against oxidative damage by neutralizing ROS, a common cellular response to stress. The elevated levels of glutathione in metabolomic profiles further corroborate the activation of detoxification pathways.

The observed increase in lactate and succinate levels indicates a metabolic shift towards anaerobic glycolysis and a disruption in the tricarboxylic acid (TCA) cycle under hypoxic conditions. The upregulation of *HIF1A* supports its central role in mediating hypoxic responses by promoting glycolytic genes, as reported in similar studies on hypoxia-induced metabolic reprogramming Thermal stress-induced proline accumulation suggests its role as an osmoprotectant and stabilizer of cellular structures during heat stress. Proline biosynthesis genes, such as *PRODH*, were also significantly upregulated, consistent with findings in stress-resilient plant models where proline acts as a molecular chaperone and redox buffer .

The integrated analysis approach used in this study aligns with findings from similar research. For instance, a study on drought stress in plants emphasized the critical role of transcriptomic and metabolomic integration in identifying stress-responsive pathways like phenylpropanoid biosynthesis and ROS detoxification . Similarly, studies on cancer cells under oxidative stress have demonstrated the utility of combined omics data in identifying metabolic vulnerabilities and therapeutic targets .This study uniquely highlights the synergies between transcriptomic and

metabolomic adaptations across different stress types. By identifying stress-specific biomarkers and regulatory hubs, such as *HIF1A* and succinate, it offers a foundation for developing targeted interventions to enhance stress resilience in agricultural and clinical contexts. The findings also demonstrate the translational potential of omics approaches in precision medicine, particularly for diseases where cellular stress responses are dysregulated.

Limitations and Future Directions

While the study provides comprehensive insights, certain limitations warrant consideration. The reliance on in vitro models may not fully capture the complexity of in vivo stress responses. Future studies should validate these findings in more complex systems, such as whole organisms or clinical samples. Additionally, time-course analyses could provide a deeper understanding of the temporal dynamics of stress adaptation.

Future work should explore integrating additional omics layers, such as proteomics and epigenomics, to achieve a more holistic view of cellular stress responses. These multi-layered datasets could reveal novel regulatory mechanisms and improve the predictive power of identified biomarkers.

CONCLUSION

The study advances our understanding of the cellular stress response by integrating transcriptomic and metabolomic analyses. The identification of stress-specific pathways and regulatory networks provides a valuable framework for future research aimed at enhancing stress tolerance in plants and animals or targeting stress-related pathologies in humans. The potential applications of these findings extend across agriculture, environmental science, and medicine, underscoring the transformative power of multi-omics approaches.

REFERENCES

1. Kültz, D. (2020). Evolution of cellular stress response mechanisms. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *333*(6), 359–378. https://doi.org/10.1002/jez.2347
2. Wang, Q.-H., Wu, R.-X., Ji, J.-N., Zhang, J., Niu, S.-F., Tang, B.-G., Miao, B.-B., & Liang, Z.-B. (2024). Integrated Transcriptomics and Metabolomics Reveal Changes in Cell Homeostasis and Energy Metabolism in Trachinotus ovatus in Response to Acute Hypoxic Stress. *International Journal of Molecular Sciences*, *25*(2), 1054–1054. https://doi.org/10.3390/ijms25021054
3. Wang, X., Liu, Y., Han, Z., Chen, Y., Dongxin Huai, Kang, Y., Wang, Z., Yan, L., Jiang, H., Lei, Y., & Liao, B. (2021). Integrated Transcriptomics and Metabolomics Analysis Reveal Key Metabolism Pathways Contributing to Cold Tolerance in Peanut. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.752474
4. Sies, H., Berndt, C., & Jones, D. P. (2017). Oxidative Stress. *Annual Review of Biochemistry*, *86*(1), 715–748. https://doi.org/10.1146/annurev-biochem-061516-045037
5. Sies, H. (2015). Oxidative stress: a concept in redox biology and medicine. *Redox Biology*, *4*, 180–183. https://doi.org/10.1016/j.redox.2015.01.002
6. Sharma, G., Barney, J. N., Westwood, J. H., & Haak, D. C. (2021). Into the weeds: new insights in plant stress. *Trends in Plant Science*, *26*(10), 1050–1060. https://doi.org/10.1016/j.tplants.2021.06.003
7. Bai, R., Guo, J., Ye, X.-Y., Xie, Y., & Xie, T. (2022). Oxidative stress: The core pathogenesis and mechanism of Alzheimer's disease. *Ageing Research Reviews*, *77*, 101619. https://doi.org/10.1016/j.arr.2022.101619
8. Rani, V., Deep, G., Singh, R. K., Palle, K., & Yadav, U. C. S. (2016). Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life Sciences*, *148*, 183–193. https://doi.org/10.1016/j.lfs.2016.02.002
9. Braz-Mota, S., & Almeida-Val, V. M. F. (2021). Ecological adaptations of Amazonian fishes acquired during

evolution under environmental variations in dissolved oxygen: A review of responses to hypoxia in fishes, featuring the hypoxia-tolerant Astronotus spp. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *335*(9-10), 771–786. https://doi.org/10.1002/jez.2531

10. Kim, D., Kim, Y., Son, N., Kang, C., & Kim, A. (2017). Recent omics technologies and their emerging applications for personalised medicine. *IET Systems Biology*, *11*(3), 87–98. https://doi.org/10.1049/iet-syb.2016.0016
11. Bhat, K. A., Mahajan, R., Pakhtoon, M. M., Urwat, U., Bashir, Z., Shah, A. A., Agrawal, A., Bhat, B., Sofi, P. A., Masi, A., & Zargar, S. M. (2022). Low Temperature Stress Tolerance: An Insight Into the Omics Approaches for Legume Crops. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.888710
12. Wang, Y., Wang, J., Sarwar, R., Zhang, W., Geng, R., Zhu, K.-M., & Tan, X.-L. (2024). Research progress on the physiological response and molecular mechanism of cold response in plants. *Frontiers in Plant Science*, *15*. https://doi.org/10.3389/fpls.2024.1334913
13. Jian, H., Xie, L., Wang, Y., Cao, Y., Wan, M., Dianqiu Lv, Li, J., Lu, K., Xu, X., & Liu, L. (2020). Characterization of cold stress responses in different rapeseed ecotypes based on metabolomics and transcriptomics analyses. *PeerJ*, *8*, e8704–e8704. https://doi.org/10.7717/peerj.8704
14. Yang, Q., Zhang, A. H., Miao, J. H., Sun, H., Han, Y., Yan, G. L., ... & Wang, X. J. (2019). Metabolomics biotechnology, applications, and future trends: a systematic review. *RSC advances*, *9*(64), 37245-37257.
15. Johnson, C. H., Ivanisevic, J., & Siuzdak, G. (2016). Metabolomics: beyond biomarkers and towards mechanisms. *Nature Reviews Molecular Cell Biology*, *17*(7), 451–459. https://doi.org/10.1038/nrm.2016.25
16. Yadav, A. K., Shree, B., Lakhwani, D., & Singh, A. K. (2024). Omics technologies for crop improvement. In *Integrative Omics* (pp. 293-313). Academic Press.
17. Olanrewaju, O. S., Glick, B. R., & Babalola, O. O. (2024). Metabolomics-guided utilization of beneficial microbes for climate-resilient crops. *Current Opinion in Chemical Biology*, *79*, 102427. https://doi.org/10.1016/j.cbpa.2024.102427
18. Jozefczuk, S., Klie, S., Catchpole, G., Szymanski, J., Cuadros-Inostroza, A., Steinhauser, D., Selbig, J., & Willmitzer, L. (2010). Metabolomic and transcriptomic stress response ofEscherichia coli. *Molecular Systems Biology*, *6*(1), 364. https://doi.org/10.1038/msb.2010.18
19. Wilmes, A., Bois, F. Y., Aschauer, L., Moenks, K., Bielow, C., Leonard, M. O., Hamon, J., Carpi, D., Ruzek, S., Handler, A., Schmal, O., Herrgen, K., Bellwon, P., Burek, C., Truisi, G. L., Hewitt, P. G., Consiglio, E. D., Testai, E., Blaauboer, B. J., & Guillou, C. (2013). Application of integrated transcriptomic, proteomic and metabolomic profiling for the delineation of mechanisms of drug induced cell stress. *Journal of Proteomics*, *79*, 180–194. https://doi.org/10.1016/j.jprot.2012.11.022