SUPPLEMENTARY INFORMATION

Hypoxia strongly affects mitochondrial ribosomal proteins and translocases, as shown by quantitative proteomics of HeLa cells

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Supplementary Tables

Table S1. Proteins exhibiting altered regulation profiles in HeLa cells under hypoxic conditions, as identified by SILAC-based quantitative proteomic analyses (in alphabetical order). Three independent replicates were analyzed, and the normalized SILAC ratios of proteins and their corrected p-values are given (tested against the null hypothesis that there is no regulation). The variability was calculated from at least two log-transformed quantification values per protein.

| Protein name | Gene ontology/biological process | Uniprot accession (Gene name) | Ratio (H/L) | Corrected p-value | Variability |
|---------------|----------------------------------|-------------------------------|-------------|-------------------|-------------|
| **Down-regulated proteins** | | | | | |
| 3-hydroxyisobutyryl-CoA hydrolase, mitochondrial | lipid metabolism | Q6NVY1 (HIBCH) | 0.50 | 0.009 | 0.057 |
| Apolipoprotein B-100 | recognition signal for the cellular binding and internalization of LDL | P04114 (APOB) | 0.06 | <0.001 | 0.092 |
| Aspartate aminotransferase, mitochondrial | amino acid metabolism | P00505 (GOT2) | 0.58 | 0.064 | 0.063 |
| Bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase, mitochondrial | NAD-dependent bifunctional enzyme | P13995 (MTHFD2) | 0.47 | 0.004 | 0.067 |
| Carbamoyl-phosphate synthase [ammonia], mitochondrial | amino acid metabolism, nitrogen metabolism | P31327 (CPS1) | 0.59 | 0.069 | 0.082 |
| Carnitine O-palmitoyltransferase 1, liver isoform | fatty acid metabolism | P50416 (CPT1A) | 0.46 | 0.003 | 0.038 |
| Collagen alpha-1(XII) chain | cell adhesion, extracellular structural organization | Q99715 (COL12A1) | 0.32 | <0.001 | 0.102 |
| Complement component 1 Q subcomponent-binding protein, mitochondrial | complement binding | Q07021 (C1QBP) | 0.60 | 0.095 | 0.061 |
| Cytochrome c oxidase assembly factor 6 homolog | cytochrome-c oxidase activity | Q5TJ3 (C1orf31) | 0.60 | 0.095 | 0.040 |
| Dynamin-like 120 kDa protein, mitochondrial | microtubule-based process, intracellular transport | O60313 (OPA1) | 0.56 | 0.046 | 0.063 |
| Electron transfer flavoprotein subunit alpha, mitochondrial | generation of precursor metabolites and energy | P13804 (ETFA) | 0.59 | 0.077 | 0.036 |
| Ferritin, light polypeptide | ion binding, ion transport | P02792 (FTL) | 0.55 | 0.035 | 0.103 |
| Heat shock protein 75 kDa, mitochondrial | protein folding | Q12931 (TRAP1) | 0.55 | 0.036 | 0.037 |
| Hydroxysteroid dehydrogenase-like protein 2 | oxidation reduction | Q6YN16 (HSDL2) | 0.59 | 0.080 | 0.065 |
| Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial | citric acid cycle (TCA cycle) | P50213 (IDH3A) | 0.57 | 0.057 | 0.074 |
| Leucine-rich PPR-motif containing protein, mitochondrial | microtubule-based process, intracellular transport | P42704 (LRPPRC) | 0.58 | 0.057 | 0.178 |
| Medium-chain specific acyl-CoA | fatty acid metabolism | Q5HYG7 | 0.57 | 0.049 | 0.046 |
| Protein Name                                      | Function                          | Gene ID         | E-value | Bit-score | P-value |
|--------------------------------------------------|-----------------------------------|----------------|---------|-----------|---------|
| dehydrogenase, mitochondrial                     |                                   | (ACADM)        |         |           |         |
| Mimitin, mitochondrial                           | NADH dehydrogenase activity       | Q8N183 (NDUFAF2)| 0.58    | 0.065     | 0.059   |
| Mitochondrial import inner membrane translocase subunit TIMM16 | protein transport                 | Q9Y3D7 (TIMM16) | 0.60    | 0.095     | 0.049   |
| Mitochondrial import inner membrane translocase subunit TIMM23 | protein transport, protein localization | O14925 (TIMM23) | 0.56    | 0.039     | 0.051   |
| Mitochondrial import inner membrane translocase subunit TIMM44 | protein transport, protein localization | Q53G69 (TIMM44) | 0.60    | 0.084     | 0.027   |
| Mitochondrial import inner membrane translocase subunit TIMM50 homolog | protein transport, protein localization | Q9NS69 (TOMM22) | 0.46    | 0.003     | 0.066   |
| Mitochondrial import receptor subunit TOMM22 homolog | protein transport, protein localization | Q96008 (TOMM40) | 0.55    | 0.031     | 0.043   |
| Mitochondrial ribosomal protein L1               | RNA processing                    | Q9BYD6 (MRPL1)  | 0.60    | 0.095     | 0.086   |
| Mitochondrial ribosomal protein L11              | translation                       | Q9Y3B7 (MRPL11) | 0.52    | 0.015     | 0.065   |
| Mitochondrial ribosomal protein L13              | translation                       | Q9BYD1 (MRPL13) | 0.51    | 0.011     | 0.086   |
| Mitochondrial ribosomal protein L17              | translation                       | Q9NRX2 (MRPL17) | 0.46    | 0.003     | 0.052   |
| Mitochondrial ribosomal protein L21              | translation                       | Q7Z2W9 (MRPL21) | 0.52    | 0.015     | 0.026   |
| Mitochondrial ribosomal protein L22              | translation                       | Q9NWU5 (MRPL22) | 0.48    | 0.005     | 0.008   |
| Mitochondrial ribosomal protein L41              | translation                       | Q8IXM3 (MRPL41) | 0.50    | 0.008     | 0.040   |
| Mitochondrial ribosomal protein L43              | translation                       | Q8N9B3 (MRPL43) | 0.51    | 0.011     | 0.035   |
| Mitochondrial ribosomal protein L49              | translation                       | Q13405 (MRPL49) | 0.47    | 0.004     | 0.052   |
| Mitochondrial ribosomal protein L50              | unknown function                  | Q8N5N7 (MRPL50) | 0.52    | 0.015     | 0.037   |
| Mitochondrial ribosomal protein L9               | translation                       | Q9BYD2 (MRPL19) | 0.50    | 0.009     | 0.001   |
| Mitochondrial ribosomal protein S23              | unknown function                  | Q9Y3D9 (MRPS23) | 0.54    | 0.029     | 0.024   |
| Mitochondrial ribosomal protein S28              | unknown function                  | Q53G62 (MRPS28) | 0.52    | 0.014     | 0.062   |
| Mitochondrial ribosomal protein S29              | apoptosis                         | P51398 (DAP3)  | 0.49    | 0.008     | 0.073   |
| Mitochondrial ribosomal protein S34              | unknown function                  | P82930 (MRPS34) | 0.58    | 0.059     | 0.054   |
| Mitochondrial ribosomal protein S7               | translation                       | Q9Y2R9 (MRPS7)  | 0.41    | < 0.001   | 0.089   |
| Mitochondrial-processing peptidase subunit beta  | peptidase activity                | O75439 (PMPCB)  | 0.48    | 0.005     | 0.023   |
| Peptidyl-prolyl cis-trans isomerase F, mitochondrial | protein folding, mitochondrion organization | P30405 (PPIF) | 0.50    | 0.009     | 0.003   |
| Phosphatidyglycerophosphatase and                | phosphatase activity              | Q8WUK0         | 0.58    | 0.063     | 0.025   |
| Protein Name                                      | Function/Activity                                      | Q Value | FDR Value | Log2 Fold Change |
|--------------------------------------------------|--------------------------------------------------------|---------|-----------|-----------------|
| Protein-tyrosine phosphatase 1                   | (PTPMT1)                                               |         |           |                 |
| Polymerase delta-interacting protein 2           | unknown function                                       | 0.55    | 0.036     | 0.064           |
| Prostaglandin E synthase 2                       | oxidoreductase activity, fatty acid metabolic process  | 0.54    | 0.029     | 0.058           |
| Protein NOXP20                                   | neuronal cell development                               | 0.33    | <0.001    | 0.016           |
| Protein TBRG4                                    | nucleotide binding, cell cycle process                  | 0.58    | 0.061     | 0.015           |
| Putative ATP-dependent RNA helicase DHX30        | nucleotide binding                                     | 0.55    | 0.037     | 0.014           |
| Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondria | glycolysis/glucoseogenesis, citric acid cycle (TCA cycle) | 0.57    | 0.053     | 0.058           |
| Sequestosome-1                                   | intracellular signaling cascade, apoptosis              | 0.45    | 0.002     | 0.139           |
| SRA stem-loop-interacting RNA-binding protein, mitochondrial | regulation of transcription                               | 0.55    | 0.031     | 0.030           |
| Succinate dehydrogenase assembly factor 2, mitochondrial | mitochondrial electron transport                       | 0.50    | 0.010     | 0.023           |
| Transmembrane protein 126A                       | unknown function                                        | 0.54    | 0.024     | 0.075           |
| Up-regulated during skeletal muscle growth protein 5 | unknown function                                      | 0.60    | 0.094     | 0.049           |

**Up-regulated proteins**

| Protein Name                                      | Function/Activity                                      | Q Value | FDR Value | Log2 Fold Change |
|--------------------------------------------------|--------------------------------------------------------|---------|-----------|-----------------|
| 1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase | amino acid and methionine biosynthesis                   | 1.67    | 0.034     | 0.023           |
| 1,4-alpha-glucan branching enzyme                  | carbohydrate transport and metabolism                   | 2.40    | <0.001    | 0.015           |
| 5'--nucleotidase                                  | nucleotide transport and metabolism                     | 3.47    | <0.001    | 0.063           |
| 6-phosphofructokinase type C                      | glycolysis/glucoseogenesis, pentose phosphate pathway  | 1.78    | 0.014     | 0.004           |
| 6-phosphofructokinase, liver type                 | glycolysis/glucoseogenesis, pentose phosphate pathway  | 2.27    | <0.001    | 0.021           |
| Adenylate kinase 4, mitochondrial                  | purine metabolism                                       | 3.15    | <0.001    | 0.075           |
| Alpha-enolase 1                                   | glycolysis/glucoseogenesis                              | 1.81    | 0.011     | 0.025           |
| Ankyrin repeat and zinc finger domain containing 1 | ion binding                                             | 3.19    | <0.001    | 0.014           |
| Annexin A8                                       | homeostasis, lipid binding, ion binding                 | 1.76    | 0.015     | 0.045           |
| Aspartyl-tRNA synthetase                          | aminoacyl tRNA biosynthesis                             | 1.62    | 0.051     | 0.003           |
| ATP-binding cassette, sub-family B member 6, mitochondrial | cellular homeostasis                                  | 1.76    | 0.015     | 0.156           |
| Autophagy-related protein 9A                      | cellular response to stress                             | 1.93    | 0.004     | 0.077           |
| Carbonic anhydrase IX                             | participate in pH regulation                            | 17.6    | <0.001    | 0.040           |
| Protein Name                          | Function                                      | Uniprot ID | Fold Change | p-value | q-value |
|--------------------------------------|-----------------------------------------------|------------|-------------|---------|---------|
| CD109 antigen                        | enzyme inhibitor activity                     | Q6YHK3     | 1.53        | 0.095   | 0.061   |
| CD63 antigen                         | regulation of endocytosis                     | P08962     | 1.74        | 0.019   | 0.042   |
| Cellular retinoic acid binding protein 2 | retinoic acid binding                       | P29373     | 2.58        | < 0.001 | 0.036   |
| Chloride intracellular channel 3     | ion transport                                 | QSSQ17     | 1.64        | 0.045   | 0.084   |
| Collagen alpha-1 (V) chain           | member of group I collagen                    | P20908     | 1.60        | 0.058   | 0.102   |
| Collagen alpha-1(VII) chain          | cell adhesion                                 | Q02388     | 1.57        | 0.073   | 0.089   |
| Collagen alpha-2(IV) chain           | anti-angiogenic and anti-tumor cell activity  | P08572     | 1.62        | 0.053   | 0.073   |
| Dihydropyrimidase-related protein 2  | dehydropyrimidase activity                   | Q16555     | 1.90        | 0.005   | 0.007   |
| Dihydropyrimidase dehydrogenase      | pyrimidine metabolism                         | Q12882     | 6.69        | < 0.001 | 0.030   |
| Dynein heavy chain 6, axonemal       | microtubule-based process                     | Q9C0G6     | 1.72        | 0.022   | 0.037   |
| EH domain binding protein 1-like protein 1 | generation of precursor metabolites and energy | Q6HE7     | 3.13        | < 0.001 | 0.035   |
| Fibrinectin                          | cell adhesion                                 | P02751     | 4.84        | < 0.001 | 0.030   |
| Follistatin-like protein 4           | ion binding                                   | Q6MZW2     | 1.60        | 0.057   | 0.158   |
| Fructose-bisphosphate aldolase A     | glycolysis/gluconeogenesis, pentose phosphate pathway | P04075    | 2.00        | 0.002   | 0.013   |
| Fructose-bisphosphate aldolase C     | glycolysis/gluconeogenesis                    | P09972     | 2.78        | < 0.001 | 0.107   |
| Gamma-enolase                        | glycolysis/gluconeogenesis                    | P09104     | 3.94        | < 0.001 | 0.024   |
| Glucose phosphate isomerase          | glycolysis/gluconeogenesis, pentose phosphate pathway | P06744    | 2.46        | < 0.001 | 0.012   |
| Glycogen synthase 1                  | sucrose metabolism, insulin signaling pathway  | P13807     | 2.17        | < 0.001 | 0.016   |
| Hexokinase 2                         | glycolysis/gluconeogenesis, amino acid metabolism | P52789   | 3.27        | < 0.001 | 0.031   |
| Integrin alpha-2                     | response to hypoxia, regulation of actin cytoskeleton | P17301    | 2.09        | 0.001   | 0.161   |
| Integrin alpha-5                     | regulation of actin cytoskeleton, cell adhesion | P08648    | 3.05        | < 0.001 | 0.032   |
| Isocitrate dehydrogenase 2 (NADP), mitochondrial | citric acid cycle (TCA cycle)                | P48735     | 1.67        | 0.034   | 0.022   |
| Lactate dehydrogenase A              | glycolysis/gluconeogenesis, pyruvate metabolism | P00338    | 2.92        | < 0.001 | 0.009   |
| Leucin-rich repeat-containing protein 8A | involved in B-cell development               | Q81WT6     | 1.75        | 0.018   | 0.178   |
| Lysophosphatidylcholine acyltransferase | phospholipid metabolic                      | Q8NF37     | 2.49        | < 0.001 | 0.033   |
| Rank | Process                                                                 | Gene ID   | Fold Change | p-value   | q-value   |
|------|--------------------------------------------------------------------------|-----------|-------------|-----------|-----------|
| 1    | Macrophage migration inhibitory factor amino acid metabolism             | P14174 (MIF) | 1.93        | 0.004     | 0.017     |
| 2    | Monocarboxylate transporter, MCT4/5 (solute carrier family 16, member 3) | Q53C91 (SLC16A3) | 1.97        | 0.003     | 0.038     |
| 3    | Peptidylprolyl isomerase C protein folding                               | P45877 (PPIC) | 1.68        | 0.031     | 0.023     |
| 4    | Phosphoglucomutase-1 glycolysis/gluconeogenesis, pentose phosphate pathway | P36871 (PGM1) | 2.05        | 0.001     | 0.003     |
| 5    | Phosphoglycerate kinase 1 glycolysis/gluconeogenesis                      | P00558 (PGK1) | 3.48        | < 0.001   | 0.034     |
| 6    | Phosphoglycerate kinase 2 glycolysis/gluconeogenesis                      | P07205 (PGK2) | 3.24        | < 0.001   | 0.010     |
| 7    | Monocarboxylate transporter, MCT4/5 (solute carrier family 16, member 3) | Q53C91 (SLC16A3) | 1.97        | 0.003     | 0.038     |
| 8    | Plexin-D1 morphogenesis of a branching structure                          | Q9Y4D7 (PLXND1) | 1.90        | 0.005     | 0.027     |
| 9    | Procollagen galactosyltransferase 1 carbohydrate and lipid biosynthetic process | Q8NB15 (GLT25D1) | 1.63        | 0.049     | 0.016     |
| 10   | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 response to hypoxia, lysine degradation, oxidoreductase activity | Q5JK88 (PLOC1D1) | 2.80        | < 0.001   | 0.040     |
| 11   | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 response to hypoxia, lysine degradation, oxidoreductase activity | Q59ED2 (PLOC2D2) | 6.39        | < 0.001   | 0.034     |
| 12   | Prolyl 4-hydroxylase subunit alpha-1 amino acid metabolism, oxidoreductase activity | P13674 (P4HA1) | 5.30        | < 0.001   | 0.051     |
| 13   | Prolyl 4-hydroxylase subunit beta oxidoreductase activity                 | P07237 (P4HB) | 1.80        | 0.011     | 0.021     |
| 14   | Prolyl 4-hydroxylase, subunit alpha-2 amino acid metabolism, oxidoreductase activity | O15460 (P4HA2) | 3.41        | < 0.001   | 0.028     |
| 15   | Protein FAM162A may be involved in hypoxia-induced cell death             | Q96A26 (FAM162A) | 2.53        | < 0.001   | 0.020     |
| 16   | Protein NDRG1 cell activation                                            | Q92597 (NDRG1) | 11.51       | < 0.001   | 0.040     |
| 17   | Protein NOXP20 unknown function                                          | Q8IWE2 (FAM114A1) | 1.58        | 0.068     | 0.016     |
| 18   | Protein S100-A10 calcium ion binding                                     | P60903 (S100A10) | 2.23        | < 0.001   | 0.017     |
| 19   | Protein S100-A16 response to metal ion                                   | Q96FQ6 (S100A16) | 1.62        | 0.051     | 0.087     |
| 20   | Pyruvate kinase PKM glycolysis/gluconeogenesis, pyruvate metabolism      | P14618 (PKM2) | 1.79        | 0.012     | 0.031     |
| 21   | Receptor-type tyrosine-protein phosphatase F cell adhesion, insulin signaling pathway, phosphatase activity | P10586 (PTPRF) | 1.54        | 0.091     | 0.020     |
| 22   | RelA-associated protein inhibitor regulation of apoptosis                | Q8WUF5 (PPP1R13L) | 1.59        | 0.061     | 0.016     |
| 23   | SH3 domain binding glutamic acid-rich-like protein 3 cell redox homeostasis | Q9H299 (SH3BGLL3) | 1.79        | 0.013     | 0.050     |
| 24   | Solute carrier family 2 (facilitated glucose transporter), member 1 glucose transporter | Q59GX2 (SLC2A1) | 2.76        | < 0.001   | 0.025     |
| 25   | Solute carrier family 2 (facilitated glucose transporter) glucose transporter | P11169 | 5.08        | < 0.001   | 0.021     |
| Protein/Enzyme Description                                                                 | Function                                                                 | Gene ID  | p-Value | Adjusted p-Value |
|-------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|----------|---------|-----------------|
| transporter), member 3                                                                     |                                                                          | SLC2A3   | 1.94    | 0.004           |
| Solute carrier family 38, member 2                                                         | ion transport                                                            | Q96QD8   | (SLC38A2)|                 |
| Spermine synthase                                                                          | carbohydrate transport and metabolism                                     | P52788   | (SMS)   | 1.55            |
| Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial                    | citric acid cycle (TCA cycle), oxidative phosphorylation                  | P21912   | (SDHB)  | 1.88            |
| Supervillin                                                                                | cytoskeleton organization                                                 | Q569J5   | (SVIL)  | 1.68            |
| Thymidine kinase, cytosolic                                                                | translation                                                              | P04183   | (TK1)   | 1.67            |
| Transforming growth factor-beta-induced protein ig-h3                                       | cell adhesion, extracellular structural organization                      | Q15582   | (TGFBI) | 2.42            |
| Triosephosphate isomerase                                                                  | glycolysis/gluconeogenesis                                                | Q53HE2   | (TP11)  | 2.89            |
| Tubulin alpha-1A chain                                                                     | microtubule-based process                                                | Q71U36   | (TUBA1A)| 1.91            |
| Vimentin                                                                                   | cell motion                                                              | P08670   | (VIM)   | 1.54            |
Table S2. Pathway and functional annotation cluster analysis. (A) KEGG pathway and (B) Gene Ontology based analysis of regulated proteins in HeLa cells in hypoxic environments. Enrichment scores are used to rank the biological significance. GO, gene ontology.

### A

| GO-Term                                | Enrichment score | Count | Regulation |
|----------------------------------------|------------------|-------|------------|
| Glycolysis/ Gluconeogenesis             | 8.05             | 14    | Up         |
| Pentose phosphate pathway               | 8.05             | 6     | Up         |
| Fructose and mannose metabolism        | 8.05             | 6     | Up         |
| Starch and sucrose metabolism          | 3.19             | 5     | Up         |
| Galactose metabolism                   | 2.70             | 4     | Up         |

### B

| GO-Term                                | Enrichment score | Count | Regulation |
|----------------------------------------|------------------|-------|------------|
| Glycolysis                             | 8.05             | 14    | Up         |
| Mitochondrial part                     | 6.62             | 30    | Down       |
| Ion binding                            | 3.31             | 31    | Up         |
| Oxidoreductase activity                | 2.06             | 4     | Up         |
| Response to hypoxia                    | 2.00             | 5     | Up         |
Supplementary Figures

Figure S1. Workflow of a SILAC experiment. Two cell populations are labeled with either light or heavy amino acids, then combined and analyzed by LC-MS/MS. The differences in intensity in the signals between the light and heavy peptides are used to calculate the ratios between the samples.
Figure S2. Lactate (dark grey) and glucose (light grey) concentrations in cells cultivated under normoxic or hypoxic conditions.
**Figure S3.** Pie charts of PANTHER biological processes and molecular functions. (A, B) Biological processes and (C, D) Molecular functions. Panels A and C refer to up-regulated proteins, while panels B and D concern down-regulated proteins.
Figure S4. Representative Western blot analysis. Selected proteins are MRP-L17, Tim50 and CAIX. Shown are experiments from HeLa cells grown under normoxic (left lane) or hypoxic conditions (right lane). For comparison, the proteomics analysis yielded down-regulation for MRLP-L17 and Tim50 (H/L 0.46, p=0.003 for both proteins) and up-regulation for CA IX (H/L 17.6, p<0.001). The two bands for CA IX correspond to different CA IX isoforms. β-tubulin was used as a control, to demonstrate equal loading of the gels.
Supplementary Material and Methods

Oxygen sensing
Pericellular oxygen concentration and cellular respiration/consumption were performed as described [1]. Briefly, an oxygen sensor (Unisense, Denmark) fixed to a motor driver was mounted on an inverted microscope (Nikon TS100, Japan) with a Paxcam3 camera (MIS, IL, USA). The sensor (25 µm in diameter) was positioned through the open flask neck, allowing the measurement of the oxygen level inside the culture flask at the same time as image capturing (after the end point of the sensor tip was carefully positioned at the flask bottom close to the attached cells). Measurement of the oxygen concentration started above the surface of medium, and proceeded in steps of 100 µm through the medium until the bottom of the flask was reached. This procedure was automatized using the integrated measuring and motor control software Sensor Trace Profiler (Unisense, Denmark) and repeated every 2 hours during the experiment. Readings from the last point in each profile gave the real pericellular oxygen concentration. The oxygen concentration gradient along the depth of medium was used to calculate oxygen flux in real time. Combined with cell density data obtained from photographs taken at the same time points, the consumption of oxygen per cell was calculated. The microscope and oxygen measuring system were placed inside an InVivo2 400 hypoxia incubator box (Ruskinn Technology, UK) and pictures were taken every 24 hours. The oxygen level inside the box was set to 1% for hypoxia and 19% for normoxic conditions.

NanoLC-LTQ Orbitrap mass spectrometry
The cell lysates from each labeling, heavy and light, were mixed 1:1 and subjected to SDS-PAGE. Each Coomassie G-250 stained SDS-PAGE gel lane was cut into 12 slices, which were in-gel digested using 0.1 µg of trypsin in 25 µl of 50 mM ammonium bicarbonate, pH 7.8. After micropurification using µ-C18 ZipTips (Millipore, Oslo, Norway), the peptides were dried in a SpeedVac and dissolved in 10 µl 1% formic acid, 5% acetonitrile in water. Half of the volume was injected into
an Ultimate 3000 nanoLC system (Dionex, Sunnyvale CA, USA) connected to a linear quadrupole ion trap-orbitrap (LTQ-Orbitrap XL) mass spectrometer (ThermoScientific, Bremen, Germany) equipped with a nanoelectrospray ion source. For liquid chromatography separation, an Acclaim PepMap 100 column (C18, 3 µm beads, 100 Å, 75 µm inner diameter) (Dionex, Sunnyvale CA, USA) capillary of 50 cm bed length was used. The flow rate was 0.3 µL/ min, with a solvent gradient of 7% B to 35% B in 110 minutes. Solvent A was aqueous 0.1% formic acid, whereas solvent B was aqueous 90% acetonitrile in 0.1% formic acid. The mass spectrometer was operated in the data-dependent mode to automatically switch between Orbitrap-MS and LTQ-MS/MS acquisition. Survey full scan MS spectra (from m/z 300 to 2,000) were acquired in the Orbitrap with the resolution R = 60,000 at m/z 400. The method used allowed the sequential isolation of up to the seven most intense ions for fragmentation on the linear ion trap using collision induced dissociation (CID) at a target value of 10,000 charges. Target ions already selected for MS/MS were dynamically excluded for 60 seconds. The lock mass option was enabled in MS mode for internal recalibration during the analysis. Other instrument parameters were set as previously described [2].

**Protein identification and quantification**

Trypsin was selected for cleavage. For the analysis, two missed cleavage sites were allowed, and tolerance levels for identification were set to 10 ppm and 0.5 Da for MS and fragment MS/MS scans, respectively. In addition to $^{13}$C isotopes of arginine and lysine, methionine oxidation, deamidation of asparagines and glutamines, N-terminal protein acetylation and conversion of N-terminal peptide glutamine to pyro-glutamic acid were selected as variable modifications.
Supplementary References

1. Pettersen EO, Larsen LH, Ramsing NB, Ebbesen P. Pericellular oxygen depletion during ordinary tissue culturing, measured with oxygen microsensors. Cell Prolif. 2005;38(4):257-67. Epub 2005/08/16.

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