Supplemental information

Quantitative high-confidence human mitochondrial proteome and its dynamics in cellular context

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Figure S1. Assessment of the Subcellular Fractionation Workflow and of Data Acquired in Subtractive Proteomics Experiments

Related to Figure 1

(A) Venn diagram representing unique proteins with mitochondrial annotation in different data repositories based on all entries (left) and entries with experimental evidence for mitochondrial protein localization (right). GO: Mito, GO-CC term 'mitochondrion'; IMPI, integrated mitochondrial protein index; HPA, human protein atlas.

(B) Outline of the subcellular fractionation protocol established in this study for the preparation of crude and gradient-purified mitochondrial fractions (highlighted in red) from human cell lines (HEK293T, HeLa). Samples highlighted in blue were used for spatial proteomics experiments. SEM, sucrose/EDTA/MOPS buffer; s/n, supernatant; P, pellet; S, supernatant; cM/pM, crude/pure mitochondria.

(C) Abundance profiles of organellar marker proteins across different subcellular fractions established by Western blot (left) and MS analysis (right). Error bars indicate SEM for n = 3 and the range for n = 2. Norm., normalized; Total, cell lysate; P0.8, nuclear fraction; cM/pM, crude/pure mitochondria; S100/P100, cytosolic/microsomal fraction; OM/IM, outer/inner mitochondrial membrane; PM, plasma membrane; Perox., peroxisome; ER, endoplasmic reticulum.

(D) Outline of the SILAC-based quantitative MS workflow employed in subtractive proteomics experiments. Equal amounts of crude and pure mitochondrial fractions prepared from differentially labeled HEK293T cells as depicted in (B) were mixed and proteins were digested with different proteases. Peptide mixtures were separated by high pH reversed-phase chromatography (pH 10 RP) into 32 fractions (fr.) per replicate (n = 4) and analyzed by LC-MS. Chymo., chymotrypsin.

(E) Reproducibility of protein quantification between four biological replicates. Numbers indicate the Pearson correlation between log2-transformed protein abundance ratios (pM/cM) of the indicated replicates, revealing a high correlation of SILAC ratios between replicates.

(F) Overlap of proteins quantified in four biological replicates. A total of 8,713 unique proteins were quantified with an average of 7,896 (±154) per replicate.
(G) Enrichment ($\log_2$ ratios $> 0$) or depletion ($\log_2$ ratios $< 0$) of mitochondrial (red) and other proteins (blue) from different subcellular compartments in pure compared to crude mitochondrial fractions. Protein ratios were filtered for $\geq 3$ valid values ($n=4$). MitoCoP refers to the high-confidence human mitochondrial proteome defined in this study (see Table S1). The 'Mito test set' contains known mitochondrial proteins according to the integrated mitochondrial protein index (IMPI). Non-mitochondrial subsets are based on GO-annotations excluding MitoCoP components. cM/pM, crude/pure mitochondria.

(H) GO-CC term overrepresentation analysis of classes defined in Figure 1B. Shown are selected overrepresented terms and the number of proteins assigned to a given term that are present in the respective class and, in parentheses, in the entire subtractive proteomics dataset. Numbers of proteins indicated in G and following subfigures include isoforms. RC, respiratory chain; PM, plasma membrane; ER, endoplasmic reticulum.

(I) Ratio-intensity plot as shown in Figure 1B illustrating the distribution of proteins of the mitochondrial (left) and non-mitochondrial assessment ('test') set (middle) as provided by MitoMiner/IMPI. The bar chart (right) shows the distribution of proteins of the subtractive proteomics dataset ('classification') and the mitochondrial and non-mitochondrial test sets across the classes defined in Figure 1B. Numbers indicate numbers of proteins present in the respective class.

(J, K) Same as in (I) highlighting proteins of the mitochondrial test set with amino-terminal presequence as predicted by MitoFates (Fukasawa et al., 2015) (J) and mitochondrial proteins of distinct submitochondrial localizations (K). The bar chart in (K) illustrates the distribution of proteins with indicated submitochondrial localization (based on GO-CC) across the classes defined in Figure 1B. Numbers indicate numbers of proteins present in the respective class. OM/IM, outer/inner mitochondrial membrane; IMS, intermembrane space.

(L) Distribution of components of the TIM22 complex, OXPHOS complexes, proteins involved in mitochondrial fission and fusion, components of mitochondrial ribosomes, mitochondrial proteins with dual localization and aminoacyl-tRNA synthetases in the subtractive proteomics dataset. Class 1 contains dual-localized proteins of predominantly mitochondrial localization, e.g. CHCHD2, COQ7 and HMGCL. Dually/multiply localized
proteins of minor abundance in mitochondria occur in class 2 such as CYB5R3, PARK7 and GARS. The log$_2$ SILAC ratio of $>0$ determined for these proteins points to the prevalence of the mitochondrial over the non-mitochondrial portion in the mitochondrial fractions analyzed in this experiment. loc., localized; AA, aminoacyl.

(H - L) Colors indicate classes as introduced in Figure 1B.

See also Tables S1 and S2.
Figure S2. Analysis of Spatial Proteomics Data
Related to Figure 1

(A) Number of proteins including isoforms present in the entire spatial proteomics dataset ('Combined') and identified in individual subcellular fractions generated as described in Figure S1B and analyzed by quantitative MS in a label-free approach (n = 3). Only proteins with MS intensity in ≥ 2/3 replicates per fraction were counted. Total, cell lysate; P0.8, nuclear fraction; cM/pM, crude/pure mitochondria; S100/P100, cytosolic/microsomal fraction.

(B) tSNE plots as shown in Figure 1D depicting the distribution of proteins of the mitochondrial (left) and non-mitochondrial test set (middle). Numbers in the bar chart (right) indicate the number of proteins assigned to cluster 1 (red) and cluster 2 (blue). Only 13 proteins (1.9%) of the non-mitochondrial test set were assigned to class 1 illustrating the high potential of the spatial proteomics approach to discriminate between mitochondrial and non-mitochondrial proteins. dim, dimension.

(C, D, F, G) Same as in (B) highlighting proteins of the mitochondrial test set with amino-terminal presequence as predicted by MitoFates (Fukasawa et al., 2015) (C), proteins of distinct submitochondrial (D) and subcellular localizations (F), and proteins of the TIM22 complex, OXPHOS complexes, proteins involved in mitochondrial fission and fusion, components of mitochondrial ribosomes, mitochondrial proteins with dual localization and aminoacyl-tRNA synthetases (G). All OXPHOS subunits highlighted in (G) were assigned to cluster 1; exceptions are MT-ND6 and ATP5MF, which exhibit limited or unfavorable cleavage sites for tryptic peptide-based identification and quantification. The bar chart in (D) indicates the number of proteins of distinct submitochondrial localizations assigned to cluster 1 (red) and cluster 2 (blue). Abundance profiles of selected proteins of different subcellular compartments are shown in (F). The localization of these proteins in the respective tSNE plots is indicated. ER, endoplasmic reticulum; OM/IM; outer/inner mitochondrial membrane; IMS, intermembrane space; PM, plasma membrane; Total, cell lysate; P0.8, nuclear fraction; cM/pM, crude/pure mitochondria; S100/P100, cytosolic/microsomal fraction; loc., localized; AA, aminoacyl.
(E) Left, same as in Figure 1D highlighting proteins of cluster 1 (i.e. the mitochondrial cluster) and different subclusters, which were defined by density-based clustering of the non-mitochondrial cluster 2. Right, GO-CC term overrepresentation analysis of subclusters 2a-2e. Shown are selected overrepresented terms including the number of proteins assigned to a given term that are present in the respective class and, in parentheses, in the entire subtractive proteomics dataset. Numbers of proteins indicated in G and following subfigures include isoforms. PM, plasma membrane; ER, endoplasmic reticulum.

(B-D, F, G) Colors indicate clusters as depicted in Figure 1D.

(F) Error bars indicate SEM for n = 3 and the range for n = 2.

See also Tables S1 and S2.
Figure S3. Evaluation of Importomics and Multiple Cell Line Subtractive Proteomics Experiments

Related to Figure 2

(A) Representative growth curves of tomm40-shRNA cells treated with doxycycline (Dox) to induce tomm40 shRNA or mock-treated with DMSO (control). Dox was replenished every 48 to 72 hours to continue shRNA induction. Cells were treated with Dox or DMSO for 9 days. KD, shRNA-mediated knockdown.

(B) Overlap of proteins including isoforms quantified in four biological replicates.

(C) Reproducibility of protein abundance ratios determined in four biological replicates. Values indicate the Pearson correlation between log₂-transformed tomm40KD/mock ratios of replicates.

(D) Computation of significance thresholds for the definition of mitochondrial proteins based on the distribution of mitochondrial and non-mitochondrial test sets. F1 scores were determined for defined mean log₂ tomm40KD/mock ratios using the mitochondrial (left) and non-mitochondrial test set (right). The ratio with the highest F1 score best separates the test set tested from the opposite test set. t1 and t2 represent the mean log₂ ratios best separating the mitochondrial from the non-mitochondrial test set and vice versa in this dataset.

(E) Ratio-intensity plots illustrating the distribution of mitochondrial (left) and non-mitochondrial test proteins (right), each highlighted in dark grey.

(F) Volcano plots as depicted in Figure 2C showing the distribution of mitochondrial (left) and non-mitochondrial test proteins (right).

(G) Distribution of proteins of the TOMM40 importomics dataset ('Classification') and the mitochondrial and non-mitochondrial test set across the classes depicted in Figure 2C. The majority of mitochondrial test proteins (644) was present in class 1 and 106 proteins were grouped into classes 2 and 3. Only 9 proteins of the non-mitochondrial test set were found in class 1, while 517 non-mitochondrial proteins were members of class 4.

(H) Same as in (F) illustrating the distribution of proteins of the mitochondrial test set with amino-terminal presequence as predicted by MitoFates.

(I) Same as in (F) highlighting proteins of OXPHOS complexes.
(J) Assessment of a correlation between mitochondrial protein half-lives and protein abundance ratios determined in TOMM40 importomics experiments. The analysis is based on protein dynamics data generated in this study using HeLa cells (see Figures 7 and S7 and Table S6) for proteins of the high-confidence human mitochondrial proteome MitoCoP. Class I and class II proteins of the importomics dataset were grouped into distinct half-life bins as indicated. Boxplots show the distribution of KD/mock abundance ratios within a given bin. Numbers above boxplots indicate the number of proteins per bin. Volcano plots highlight the distribution of proteins with the indicated range of half-lives. Colors indicate classes as introduced in Figure 2C. KD, knockdown.

(K) Results of TOMM70 importomics experiments, performed as described in Figure 2B for TOMM40. Shown are representative growth curves of Dox- and mock-treated tomm70-shRNA cells treated as described in (A) for tomm40-shRNA cells (left); Western blot analysis of steady-state levels of TOMM70, selected other mitochondrial proteins and GAPDH (loading control) in whole cell lysates of Dox-treated (+) and untreated (-) tomm70-shRNA cells (middle); and the effect of tomm70 knockdown on the mitochondrial proteome (proteins depicted were quantified in ≥ 3/4 replicates) (right).

(L) Same as in (F) highlighting components of mitochondrial ribosomes and mitochondrial proteins with dual localization. The data confirm a mitochondrial localization of the dual-localized proteins COQ7, PARK7, and HMGCL (see Figures S1L and S2F). loc., localized.

(M) Experimental setup of the multiple cell line subtractive proteomics approach. Cells of indicated cell lines were SILAC-labeled, followed by isolation of crude and pure mitochondrial fractions (n = 2). For each replicate, equal amounts of differentially labeled crude and pure mitochondrial fractions were mixed and proteolytically cleaved using trypsin. Tryptic peptides were fractionated and analyzed by LC-MS. cM/pM, crude/pure mitochondrial fraction.

(N) Determination of high-confidence mitochondrial proteins in multiple cell lines. Data acquired in the multiple cell line approach were analyzed together with the data of the large-scale multi-protease subtractive proteomics experiment of HEK293T cells ('Subtr. Prot.'; see Figures 1B, 1C, S1 and Table S2). Stringent filtering of proteins identified included (i) quantification in each replicate of the multiple cell line approach and
(ii) a sequence coverage of $\geq 10\%$ in each replicate per dataset. To define sets of mitochondrial proteins ('Mito-cluster'), data were subjected to a density-based clustering approach as detailed in STAR Methods. 'All proteins' refers to MitoCoP and non-MitoCoP proteins.

(O) Conformity of Mito-Cluster components as defined in (N) across cell lines. 'All proteins' corresponds to MitoCoP and non-MitoCoP proteins.

(F - I, L) Colors indicate classes as introduced in Figure 2C.

See also Tables S1, S2, S3 and S6.
**A**

| MitoCoP identified/validated |
|------------------------------|
| ABHD18                       |
| ACOT13                       |
| ADCK1                        |
| ADCK5                        |
| ADO                          |
| AKR1B1                       |
| AR10                         |
| AR14C                        |
| ATP23                        |
| BCL211                       |
| BPN1                         |
| C1orf40                      |
| C17orf80                     |
| C1orf53                      |
| C22orf39                     |
| CDK18                        |
| CHCHD7                       |
| CTPS2                        |
| CUTA                         |
| CXorf38                      |
| CXorf40A                     |
| CYP27C1                      |
| DTD1                         |
| DUSP11                       |
| FAM173A                      |
| FAM222B                      |
| FEM1B                        |
| FHL1                         |
| GDAP1L1                      |
| GK5                          |
| HSD17B7                      |
| HTATIP2                      |
| KIAA1111                     |
| KIF23                        |
| KIF3C                        |
| KYAT1                        |
| LYPLA1                       |
| LYPLAL1                      |
| LYRM9                        |
| MBLAC2                       |
| METTL9                       |
| MIGN1                        |
| MIGA2                        |
| MLLT11                       |
| NCBP2AS2                     |
| NIT2                         |
| NOCT                         |
| NR2F6                        |
| NSU3N2                       |
| NT5C3A                       |
| NTPCR                        |
| OVC4A                        |
| OXL1D                        |
| PCB12                        |
| PIBOS1                       |
| PPDPF                        |
| PRELI1D3A                    |
| PTHR1D1                      |
| RACGAP1                      |
| RFK                          |
| RMDN2                        |
| SDE2                         |
| SDR39U1                      |
| SLC25A4                      |
| SLC25A101                    |
| SLC25A26                     |
| SLC25A28                     |
| SLC25A35                     |
| SLC25A46                     |
| SLC25A47                     |
| SLC25A52                     |
| SLC25A56                     |
| SLC25A65                     |
| SLC25A66                     |
| SLC25A67                     |
| SLC25A70                     |
| SLC25A75                     |
| SLC25A80                     |
| SMIM10L1                     |
| SMIM26                       |
| TATDN1                       |
| TATDN3                       |
| TDRP                         |
| TJP1                         |
| TMEM160                      |
| TMEM242                      |
| TMEM256                      |
| TMEM69                       |
| TRMT1                        |
| TRUB1                        |
| TTC8                         |
| ZADH2                        |
| ZNF703                       |

**B**

- **o-FLAG**
- **Mitotracker**
- **Merge + DAPI**

Scale bar = 15 µm

**C**

| WT       | +Δψ | −Δψ | Prot. K | Lyse + K |
|----------|-----|-----|---------|----------|
| 1        | 3   | 10  | 30      | 30       |
| 1        | 3   | 10  | 30      | 30       |

**E**

- Log ratio (P/SN) (Carbonate assay)
- Log ratio (P/SN) (Sonication assay)

**F**

- Classification
- TCA cycle
- Fe-S biogenesis
- Carrier proteins
- Further carrier proteins

**Figure S4**
Figure S4. Analysis of Subcellular Localization and Membrane Association of MitoCoP Proteins

Related to Figure 3

(A) Protein correlation profiles for MitoCoP-A/B proteins established in spatial proteomics experiments. Shown are the mean of normalized MS intensities (top) and Western blot analyses (bottom) of the indicated fractions. Error bars indicate SEM for n = 3 and the range for n = 2. Total, cell lysate; P0.8, nuclear fraction; cM/pM, crude/pure mitochondria; S100/P100, cytosolic/microsomal fraction. Immunodecoration of cellular fractions by specific antibodies.

(B) Immunofluorescence microscopy of transiently transfected U2OS cells expressing TMEM256\textsubscript{FLAG}. TMEM256\textsubscript{FLAG} was visualized by anti-FLAG antibody, the mitochondrial network by Mitotracker red staining and the nucleus by DAPI. Scale bar, 15 μm.

(C) In organello import assays of radiolabeled precursor proteins into HEK293T mitochondria in the presence or absence of a membrane potential (ΔΨ) or mitochondria, followed by proteinase K (Prot. K) treatment. Lysate, in vitro synthesized radiolabeled precursor protein.

(D) Experimental strategy followed to assess the membrane association of MitoCoP proteins. Gradient-purified mitochondria were either treated with sodium carbonate pH 11.5 to separate integral membrane proteins from other proteins (i.e. carbonate assay) or sonified to separate integral and peripheral membrane proteins from soluble proteins (i.e. sonication assay). Proteins of soluble and insoluble fractions generated by centrifugation were digested with trypsin and the resulting peptides were labeled using stable isotope dimethyl labeling. Differentially labeled peptides from soluble and insoluble fractions of each replicate (n = 4) were mixed, fractionated by high pH reversed-phase chromatography (pH 10 RP) and analyzed by LC-MS. Carb., carbonate; Son., sonication; P, pellet/insoluble fraction; SN, supernatant/soluble fraction; fr., fractions.

(E) Reproducibility of protein quantification between four biological replicates of carbonate (left) and sonication assays (right). Numbers indicate the Pearson correlation between loge-transformed P/SN protein abundance ratios of the indicated replicates.
(F) Membrane association of MitoCoP proteins. Carbonate extraction and sonication of gradient-purified mitochondria as described in (C) results in the separation of the mitochondrial proteome into integral membrane proteins, peripheral membrane proteins and soluble proteins as reflected in this scatterplot showing the classification. Classes were defined by statistical data analysis based on values for '% associated' and '% integral'. 'Associated' and 'Integral' denote data of sonication and carbonate extraction experiments, respectively.

(G) Scatterplots as shown in (F) highlighting mitochondrial carrier proteins, proteins of the tricarboxylic acid cycle and proteins involved in iron-sulfur cluster biogenesis. Colors indicate classes as shown in (D). TCA, tricarboxylic acid; Fe-S, iron-sulfur.

See also Tables S1 and S3.
Figure S5. Analysis of MitoCoP Protein Copy Numbers and Functional Disease Gene Classification

Related to Figures 4 and 5

(A) Outline of the strategy for the estimation of cellular protein copy numbers and mito-copy numbers per cell using the data acquired in the spatial proteomics approach. Overall cellular protein copy numbers were calculated using the MS intensities determined for whole cell lysates (Total) following the total protein approach (TPA) (Wiśniewski et al., 2012). To determine mito-copy numbers per cell, iBAQ intensities determined in gradient-purified mitochondrial fractions (pM) were used. Mito-copy numbers per cell were inferred from copy numbers per cell as shown in (B). iBAQ, intensity-based absolute quantification.

(B) Correlation between log_{10} copy numbers per cell and log_{10} iBAQ values obtained in the pM fraction determined for all proteins (left) and for bona fide mitochondrial proteins, defined as class 1 proteins of the spatial proteomics dataset after filtering (right). Shown are the data for replicate 1. R, Pearson correlation coefficient; rep, replicate.

(C) Reproducibility of the line-fit determined based on bona fide mitochondrial proteins (left) and of mito-copy numbers per cell (right) of all three biological replicates. corr., correlation; pM, pure mitochondria; rep, replicate.

(D) Enrichment of functional classes as defined in Figure 4A in the indicated quartiles (depicted in Figure 4B) with regard to the overall dataset, which comprises 1,016 mitochondrial protein groups for which mito-copy numbers per cell were calculated. Numbers indicate how many times a functional term occurs in the indicated quartile. Only terms with a fold enrichment of ≥ 2 are shown. Coloring refers to colors introduced in Figure 4A. TCA, tricarboxylic acid; Q, quartile.

(E) Summed protein copy numbers determined for the cellular proteome (CP, 8,436 proteins; including proteins of the MitoCoP) and for the mitochondrial proteome (MitoCoP, 1,016 proteins). Error bars indicate SEM.

(F) Detailed functional classification of the MitoCoP disease genes related to Figure 5B.

(G) Number of disease-related observations per MitoCoP gene.

See also Tables S1 and S4.
Figure S6. Complexome Profiling of Human MitoCoP Proteins

Related to Figure 6

(A) Purified mitochondria isolated from human HEK293T cells were lysed with digitonin and separated by blue native (BN) PAGE (n = 2). Image shows the BN gel of replicate (rep) 1.

(B) Complexome profiles of purified mitochondria. Complexome #1 (Rep1, left) is based on LC-MS analysis of 116 slices cut from the whole BN gel shown in (A) and hierarchical clustering. For complexome #2 (Rep2, right), the BN gel was cut into 114 slices.

(C) Cluster profiles from complexome #1 (B), the rolling mean over three relative intensity values is depicted in red.

(D) Correlation of protein migration profiles between the replicates of the complexome analysis shown in (B). Comparison of 896 protein profiles resulted in a median correlation of 0.75.

(E) Sequence alignment highlighting the homology of C3orf33 with the prohibitin-stomatin protein family.

(F, G) Comparison of BN-profiles from prohibitins and C3orf33 (F) and subunits of the ATP synthase and C15orf61 (G).

(H) Immunodecorations of mitochondrial protein complexes resolved by BN-PAGE after lysis with digitonin or n-Dodecyl-β-D-maltoside (DDM).

(I) Label-free comparative complexome profiling of mitochondrial fractions isolated from HEK293T LYRM9KO and control cells. Mitochondria were lysed with digitonin and separated by BN-PAGE. Lanes of both samples were cut into 45 slices and analyzed via LC-MS. Shown is the difference between normalized intensities of LYRM9KO and control cells for the indicated proteins. Intensities were normalized by dividing each intensity by the sum of the respective row’s intensity. Thus, the sum over all slices for an individual protein is identical for LYRM9KO and control cells and set to 100% for the following examples. For the depicted proteins, the maximum intensity for an individual slice is 17.2% for control and 13.9% for LYRM9KO cells. E.g. the 12.2% intensity difference in slice 2 for mt-ND2 between control (17.2%) and LYRM9KO (5.0%) cells indicates a reduction to less than 30% mt-ND2 containing supercomplex. Conversely, the 5.0% difference in slice 20
for TMEM126B between LYRM9KO (9.0%) and control cells (4.0%) indicates a ~225% increase of TMEM126B containing complex I assembly intermediate.

(J) Complex I activity of isolated C22orf39KO, LYRM9KO and control mitochondria. Box plot (n = 3) with whiskers extending to minimum and maximum.

(K, L) Comparison of BN-profiles from TIM23 core subunits and TMEM256 (K) and of proteins clustered to the TIM23 accessory subunits PAM and TIMM21 (L).

(M) NCBP2-AS2MYC was transfected transiently into HEK293 T cells. Isolated mitochondria were lysed with digitonin, protein complexes were separated by BN-PAGE, followed by a 2nd dimension SDS-PAGE and Western blot analysis. HMM, high molecular mass.

(N) NCBP2-AS2Myc was transiently transfected in TIM23FLAG expressing cells. FLAG-immunoprecipitations eluates of whole cells lysed with digitonin were analyzed by SDS-PAGE. (Load = 2%, Eluate = 100%).

(O) HEK293T cells were depleted for TMEM256 using siRNA molecules. After mitochondrial isolation, the [35S]COX4I precursor was imported for indicated time points, followed by proteinase K treatment. Samples were analyzed by SDS-PAGE and digital autoradiography. Error bars, SEM (n = 3). Lysate, synthesized precursor; NT, not treated; p, precursor; m, mature.

See also Table S5.
Figure S7. Evaluation of Pulsed SILAC Data for Human MitoCoP Half-life Analysis Related to Figure 7

(A) Outline of the pulsed SILAC (pSILAC) approach applied to determine half-lives of MitoCoP proteins, shown for replicate 1. HeLa cells were grown in cell culture medium containing light (L, control) or medium-heavy (MH) amino acids. 48 h, 24 h, 12 h, 6 h, 3 h, and 1 h prior to harvesting, MH-labeled cells were shifted to medium containing heavy (H) amino acids. Control cells were maintained in light medium for the entire duration of the experiment. Equal numbers of differentially SILAC-labeled pulsed and control cells were mixed, crude mitochondrial fractions were prepared and proteins were analyzed by LC-MS following digestion with trypsin and peptide fractionation using high pH reversed-phase chromatography. The calculation of protein synthesis and degradation rates was based on MH/L and H/L peptide/protein abundance ratios, respectively (this applies to replicate 1; for replicates 2 and 3, the respective label-switch has to be taken into account).

(B) Protein degradation (blue) and synthesis (green), exemplarily shown for selected proteins of the presequence translocase import motor system TIM23-PAM with fast and slow turnover rates determined by fitting exponential curves (lines) prior to correcting the data for cell doubling time. T_{1/2}, corresponding protein half-life.

(C) Range of half-lives determined for human MitoCoP constituents from HeLa cells.

(D, E) Half-lives determined for MitoCoP-A/B proteins (D) and proteins involved in iron-sulfur cluster biogenesis (E, top) and coenzyme Q biosynthesis (E, bottom).

(F) Outline of the pSILAC approach applied to determine half-lives of MitoCoP proteins from Huh7 cells. Following growth in cell culture medium containing light (L) amino acids, Huh7 cells were shifted to heavy (H) amino acids for 0 h, 5 h, 10 h, 24 h or 48 h prior to harvesting. Isolation of crude mitochondrial fractions was followed by peptide fractionation using high pH reversed-phase chromatography and LC-MS analysis (n = 2 for each time point). The calculation of protein synthesis and degradation rates was based on H/L peptide ratios. cM, crude mitochondrial fraction.
(G) Correlation of half-life values determined from pSILAC analysis of Huh7 and HeLa cells for proteins with half-lives in the range of 2 hours to 7.5 days (indicated via dashed black line) and above 7.5 days. r, Pearson correlation.

(H) Range of half-lives determined for human MitoCoP constituents from Huh7 cells.

(I) Comparison of half-lives of MitoCoP proteins determined from HeLa and Huh7 cells. Shown are 748 proteins for which protein half-lives were calculated from both cell lines.

(J) Western blotting of cell extracts grown for the indicated times in the presence of cycloheximide (CHX) compared to determined protein half-lives.

(D, E, H, J, I) color code as in (C).

See also Table S6.