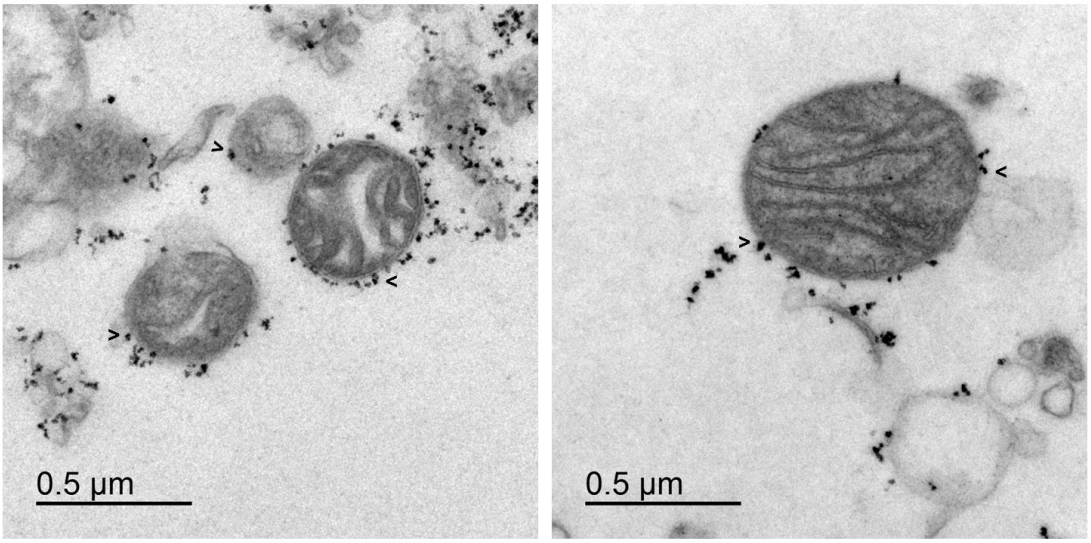
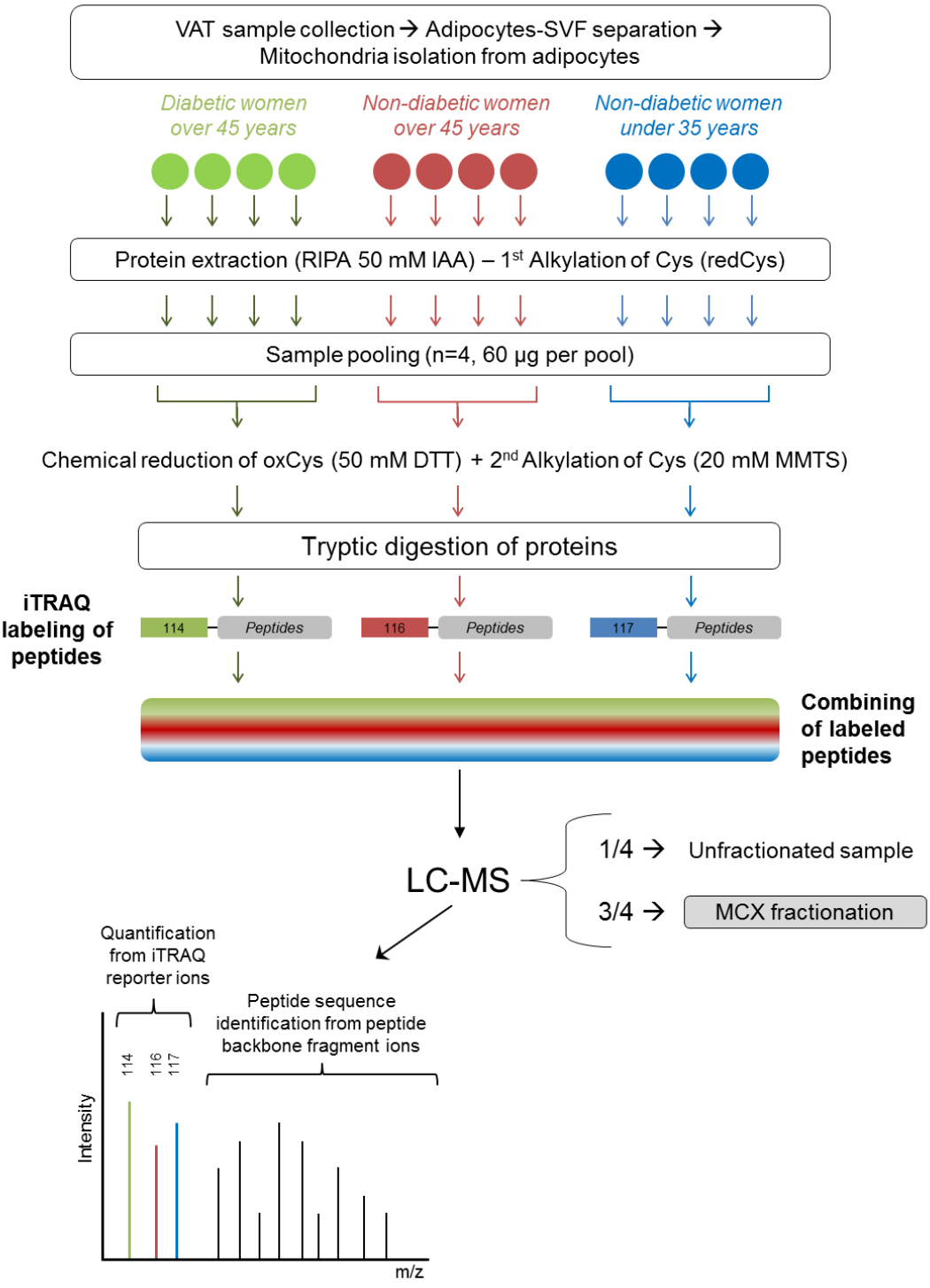
SUPPLEMENTARY INFORMATION

- **Supplementary Figures and Legends**
    - **Supplementary Figure 1.** Electron microscopy of mitochondrial samples.
    - **Supplementary Figure 2.** Proteomic analysis.
    - **Supplementary Figure 3.** Analyses of mitochondrial proteins for orthogonal validation of proteomic signatures.
    - **Supplementary Figure 4.** Coordinated behavior of mitochondrial ETC complexes in the age and the T2DM comparisons.
    - **Supplementary Figure 5.** Protein changes in mtDNA-encoded proteins in the age and the T2DM comparisons.
    - **Supplementary Figure 6.** Correlation between protein abundance and oxidation levels.
- **Supplementary Tables**
    - **Supplementary Table 1.** Proteins and peptides identified in mitochondrial samples. [Excel File]
    - **Supplementary Table 2.** Mitochondrial proteins quantitated with the WSPP model (n=706). [Excel File]
    - **Supplementary Table 3.** Mitochondrial protein function alterations in the age and T2DM comparisons.
    - **Supplementary Table 4.** Quantitation of mitochondrial proteins and oxCys peptides thereof. [Excel File]
    - **Supplementary Table 5.** Enrichment analyses of mitochondrial proteins containing oxCys sites. [Excel File]

SUPPLEMENTARY FIGURES AND LEGENDS

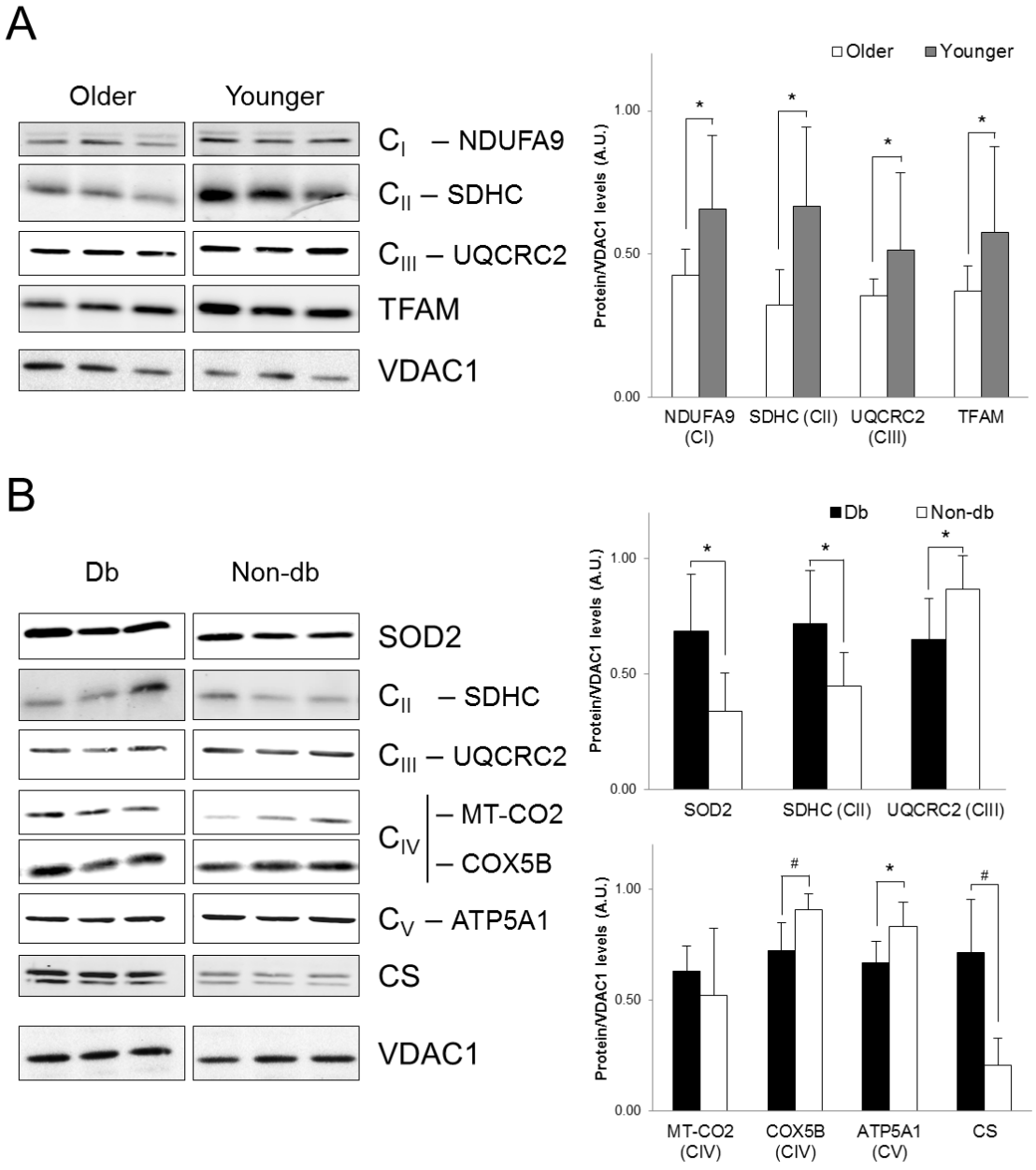


Supplementary Figure 1. Electron microscopy of mitochondrial samples. Mitochondria isolation by magnetic labeling was verified by transmission electron microscopy. Mitochondrial eluates were fixed in 2.5% glutaraldehyde and 2.5% paraformaldehyde in *Separation Buffer*, pH 7.4, for 1 h at room temperature. Mitochondrial pellets were then obtained by centrifugation and washed with phosphate buffer, post-fixed in 1% OsO_4 in H_2O for 60 min at 4 ºC, dehydrated in ethanol-acetone, and embedded in Durcupan resin. Thin sections were obtained with a Leica Reichert Ultracut S ultramicrotome (Wien, Austria), stained with uranyl acetate and lead citrate, and examined with a Jeol Jem1010 transmission electron microscope (Jeol, Tokyo, Japan). Magnetic beads of anti-TOM22 particles (arrowheads) could be recognized together with mitochondria. The figure shows representative images of four independent experiments.

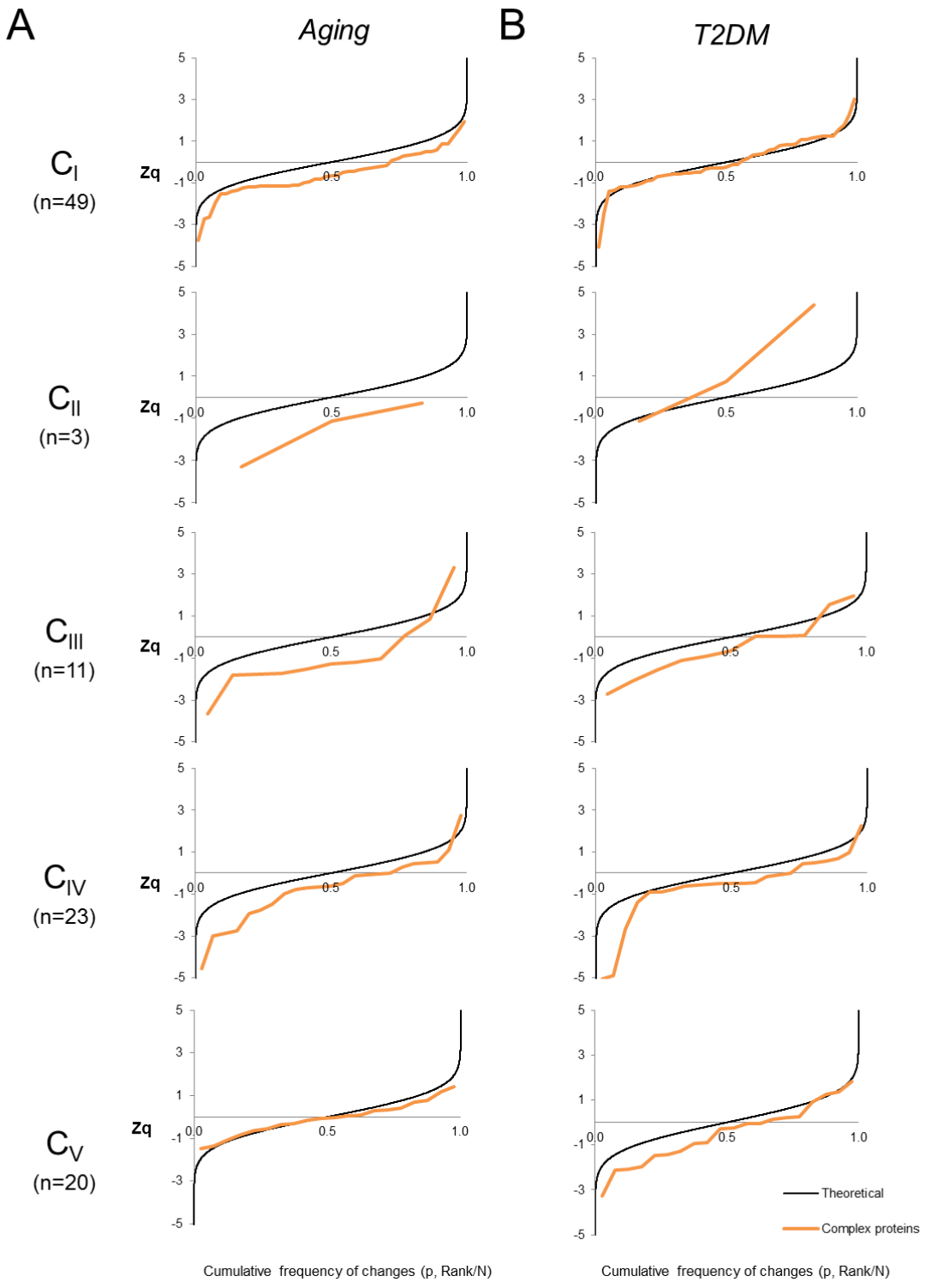


Supplementary Figure 2. Proteomic analysis. Samples from each patient (n=12) were individually processed during collection, separation of adipose fractions and mitochondrial isolation steps. During protein extraction, alkylation of free (reduced) Cys was achieved with 50 mM iodoacetamide (IAA). Protein extracts obtained from each patient sample were quantitated in triplicate to ensure proper sample pooling. To minimize differences among groups, chemical reduction of oxCys with dithiothreitol (DTT), second alkylation with methyl

methanethiosulfonate (MMTS), and protein digestion were carried out within protein pools. After tryptic digestion, a small amount of each peptide pool (~1 µg) was analyzed by LC-MS to check for comparable digestion across groups. Thereafter, the peptide pools were tagged with their corresponding iTRAQ labels and equally mixed. The so-obtained mixture of differentially labeled peptides was split into an unfractionated peptide sample (one-fourth) and three peptide samples obtained by mixed-mode cationic exchange (MCX) fractionation (three-fourths). LC-MS analyses allowed the identification of peptide sequences based on peptide backbone fragment ions, together with the quantification of their relative abundance across the compared groups based on the intensity of iTRAQ reporter ions showed in the low m/z region.

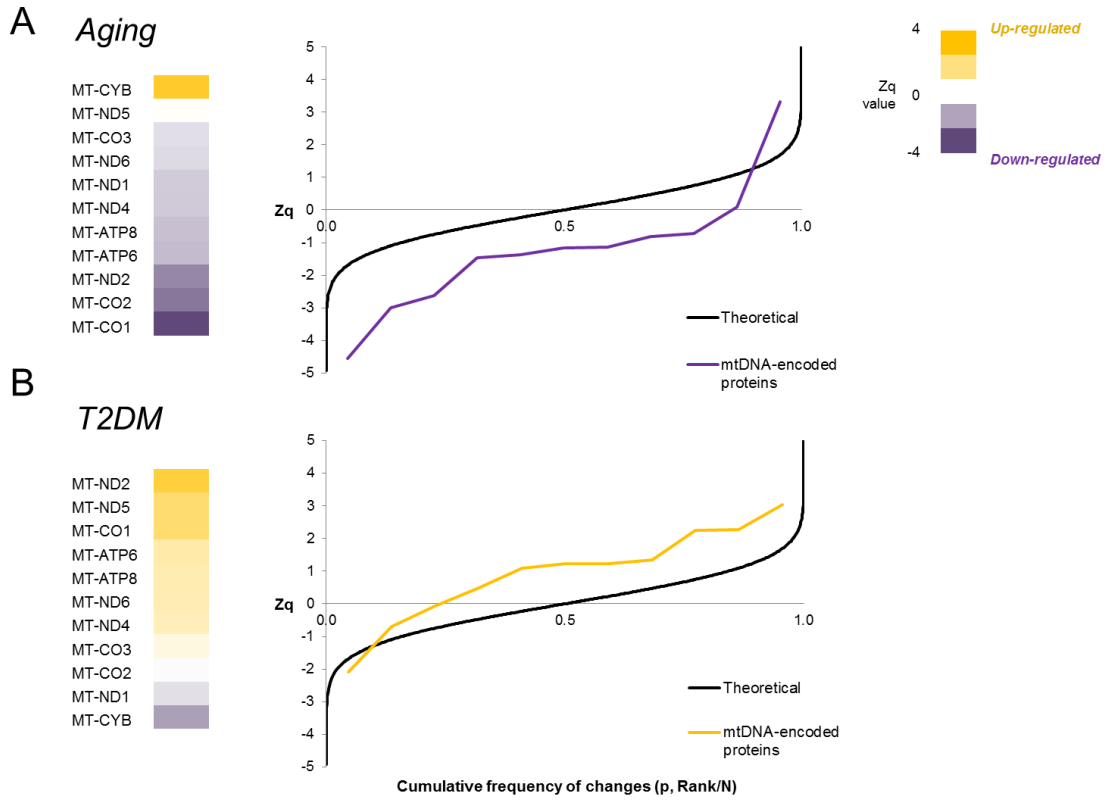


Supplementary Figure 3. Analyses of mitochondrial proteins for orthogonal validation of proteomic signatures. Representative WB images of selected markers using an independent set of mitochondria-enriched samples. For each comparison, all samples (10 µg) were loaded in the same gel. **(A)** WB analyses of older (n=7, mean-age 49 years) and younger (n=6, mean age 34 years) non-diabetic women. **(B)** WB analyses of diabetic, Db (n=7, mean-age 47 years), and non-diabetic women, Non-db (n=7, mean age 49 years). Results were normalized for VDAC1 density. The values for relative intensity obtained after densitometry of the bands are means ± SD. Statistical significance was set at $p < 0.05$; "*" and "#" mean $p < 0.05$ and $p < 0.01$, respectively.

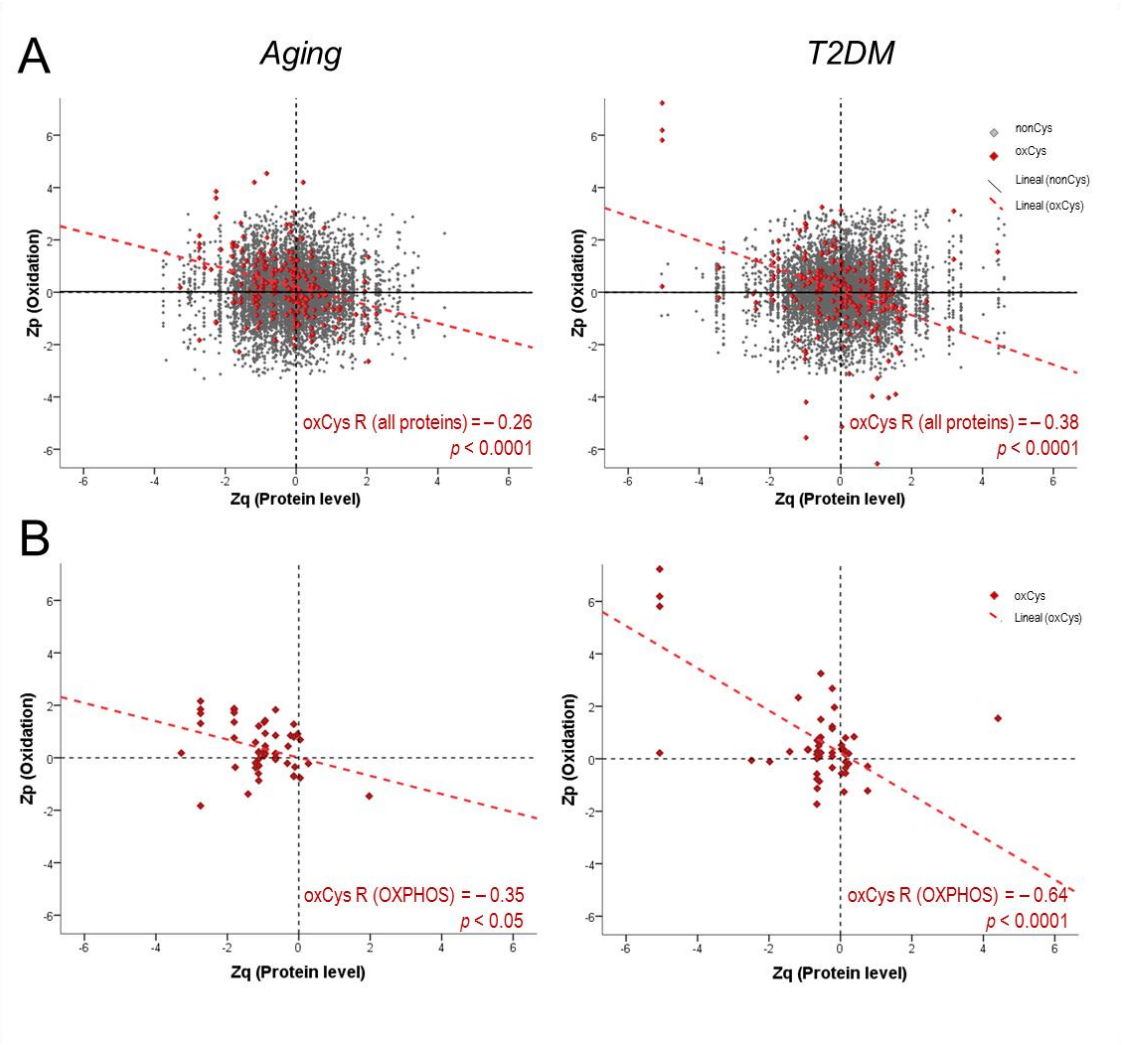


Supplementary Figure 4. Coordinated behavior of mitochondrial ETC complexes in the age and the T2DM comparisons. Sigmoidal curves represent the theoretical normal distribution of data (black lines) together with the cumulative frequency of the protein changes (orange lines, Zq values) of the mitochondrial complexes in aging **(A)** and T2DM **(B)** comparisons. The number of protein represented in each curve is enclosed in brackets, on the

left. Assembly factors and related proteins were also considered. An up- or down-wards shift from the theoretical curve means the global tendency of up- and down-regulation, respectively.



Supplementary Figure 5. Protein changes in mtDNA-encoded proteins in the age and the T2DM comparisons. Heat-maps (left) representing protein abundance changes in the age **(A)** and the T2DM **(B)** assessments. For each protein gene symbols are displayed together with the corresponding Zq (standardized log_2ratio) values in a color scale (yellow means up-regulated and purple down-regulated). On the right, sigmoidal curves represent the cumulative frequency of mtDNA-encoded protein changes in each comparison. MT-ND3 is not quantified given that its identification relies only on Cys-containing peptides.



Supplementary Figure 6. Correlation between protein abundance and oxidation levels. Dot-plots of nonCys (grey points) and oxCys (red points) containing peptides. Each point represents the peptide ratio change (Zp, Y-axis) with their corresponding protein ratio (Zq, X-axis). OxCys peptide abundance reflects the Cys oxidation levels for the whole set **(A)** and only OXPHOS **(B)** proteins in aging (left) and T2DM (right). The relationship of Zp and Zq variables was assessed through Pearson coefficient (R). Statistical significance was set at $p < 0.05$.

SUPPLEMENTARY TABLES

[Excel Files]

Supplementary Table 1. Proteins and peptides identified in mitochondrial samples.

Supplementary Table 1A. Proteins identified in mitochondrial samples at 1% FDR (n=3,542). UniProt accession codes, Fasta protein description and the corresponding gene names (N/A means "Not Assigned") are displayed. Unique peptides and spectral counts are also reported. The FDR for peptide identification was set at 1%.

Supplementary Table 1B. Peptides identified in mitochondrial samples at 1% FDR. Peptide sequences and their corresponding proteins are shown together with the peptide spectral counts. Peptide types are also displayed according to their different modifications. **M#,** Methionine oxidation (+ 15.9949 Da); **C@,** Cysteine carbamidomethylation (+ 57.0215 Da); **C***, Cysteine methylthio modification (+ 45.9877 Da); **K^**, Lysine iTRAQ 4-plex tag (+ 144.1021 Da). **nonCys**, non-cysteine-containing peptide; **redCys**, reduced-cysteine-containing peptide; **oxCys**, oxidized-cysteine-containing peptide; **redoxCys**, reduced and oxidized-cysteines-containing peptide; **freeCy**s, non-modified-cysteine-containing peptide.

Supplementary Table 2. Mitochondrial proteins quantitated with the WSPP model (n=706). X'q is calculated as a weighted average of the peptides used to quantify the protein corrected by the grand mean (calculated as a weighted average of the total protein values). **Wq** refers to the statistical weight associated with the protein, calculated from the corresponding peptide weights and the protein variance (the statistical weights are the inverses of variances). **Zq** corresponds to the standardized $\log_2$ ratio, which is defined at the protein level as the mean-corrected $\log_2$ ratio expressed in units of standard deviation. A color scale for up- (yellow) and down-regulated (purple) proteins has been added. **FDRq** refers to the False Discovery Rate at the protein level. Number **(1)** refers to the aging comparison and number **(2)** refers to the T2DM comparison.

Supplementary Table 3. Mitochondrial protein function alterations in the age and T2DM comparisons. Functional categories were regarded as differentially modulated when n ≥ 5 and FDR < 0.05. "n" represents the number of proteins within each category; **Zc** is the standardized $\log_2$ ratio at the category level. A color scale for up- (yellow) and down-regulated (purple) categories has been added. The least redundant functional categories for each cluster are highlighted in grey (see Figures 2C and D).

Cluster	Category	n (proteins)	Zc	FDR
Age differences				
1	P04396:Vitamin D metabolism and pathway	5	4.58	0.00
2	GO:0006122_mitochondrial electron transport, ubiquinol to cytochrome c	5	-3.69	0.02
	hsa04260:Cardiac muscle contraction	26	-4.56	0.00
	GO:0042773_ATP synthesis coupled electron transport	53	-4.14	0.01
	GO:0022904_respiratory electron transport chain	60	-3.92	0.01
	GO:0070469_respiratory chain	70	-4.17	0.01
	hsa05010:Alzheimer's disease	79	-3.73	0.02
	hsa00190:Oxidative phosphorylation	88	-3.82	0.01
	GO:0022900_electron transport chain	77	-3.68	0.02
	hsa05016:Huntington's disease	90	-4.07	0.01
	hsa05012:Parkinson's disease	91	-4.11	0.01
T2DM differences				
1	GO:0034614_cellular response to reactive oxygen species	6	3.52	0.02
	GO:0043523_regulation of neuron apoptosis	7	3.47	0.03
	GO:0034599_cellular response to oxidative stress	7	3.80	0.01
	GO:0001666_response to hypoxia	7	3.99	0.01
	GO:0009611_response to wounding	7	4.72	0.00
	GO:0070482_response to oxygen levels	11	4.32	0.00
	GO:0055093_response to hyperoxia	5	4.13	0.00
2	GO:0006575_cellular amino acid derivative metabolic process	17	3.35	0.04
3	GO:0007242_intracellular signaling cascade	14	-4.28	0.00

[Excel Files]

Supplementary Table 4. Quantitation of mitochondrial proteins and oxCys peptides thereof. Peptide sequences and the symbols for the corresponding proteins are shown. The position of the oxidized Cys residue in the protein sequence is also reported. **Zp** is the standardized $\log_2$ ratio at the peptide level expressed in units of standard deviation. A color scale for up- (red) and down-regulated (blue) peptides has been added. **FDRp** refers to FDR at the peptide level. **Zq** is the standardized $\log_2$ ratio at the protein level. A color scale for up- (yellow) and down-regulated (purple) proteins has been added. Number **(1)** refers to the age comparison and number **(2)** refers to the T2DM comparison. **M#**, Methionine oxidation; **C***, Cysteine methylthio modification (referring to the oxidation site); **K^**, Lysine iTRAQ 4-plex tag.

Supplementary Table 5. Enrichment analyses of mitochondrial proteins containing oxCys sites. Enrichment analyses of oxCys proteins (n=116) were performed by DAVID software. Only categories with *p* value < 0.01 (5% FDR) are shown.