Supporting Information

Translational targeted proteomics profiling of mitochondrial energy metabolic pathways in mouse and human samples

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Table S1. Demographics of human liver donors

	n=9
Age (years)	59 (46 - 65)
Gender	
Male	5 (56%)
Female	4 (44%)
Body mass index	24.7 (23.1 - 26.6)
Type of donor	
Donation after cardiac death (DCD)	9 (100%)
Cold ischemia time*	7.3 (6.8 - 8.9)
Warm ischemia time*	34 (29 - 52)
Cause of death	
Anoxia	4 (44%)
Cardiovascular accident	2 (22%)
Trauma	3 (33%)
Reason for rejection	
Moderate / severe steatosis	3 (33%)
DCD and age > 60 years	4 (44%)
Malignancy	1 (11%)
High hepatic injury markers	1 (11%)

Continuous variables are presented as median and interquartile range, categorical variables are presented as numbers and percentage.

* Cold ischemia time is the time between the donor aortic cold flush until the biopsy. Donor warm ischemia time is the time between withdrawal of life support until the aortic cold flush.

Table S2: Selected peptides per protein for the targeted LC-MS SRM analyses

Protein function	Peptide sequence	Protein mouse	Protein human	Protein rat	Comments
Concatemer 1 (QcC1)					
Antioxidant proteins	EFAAGYNVK	GPX4	GPX4		
	YAECGLR	GPX4	GPX4	GPX4	
	LNTIYQNNLTK	GSR			
	AAVVESHK	GSR	GSR		Not retained on the column
	TSLMIR	GSR	GSR	GSR	Peak shape inconsistent
	DFTPVCTTELGR	PRDX6/PRDX6B	PRDX6	PRDX6	
	NFDEILR	PRDX6/PRDX6B	PRDX6	PRDX6	Peak shape inconsistent
	AIWNVINWENVTER	SOD2	SOD2		
	NVRPDYLK	SOD2	SOD2	SOD2	Signal standard below LOD
Complex I (NADH dehydrogenase) proteins	ADANTAAIQAILYNR		MTND5		
	TISQHQISTSIITSTQK		MTND5		
	ANPYSSFSTLLGFFPSIIHR	MTND5			Signals near LOD
	HINFPLYTTTSIK	MTND5			
	FASEIAGVDDLGTTGR	NDUFS1	NDUFS1	NDUFS1	
	GLLTYTSWEDALSR	NDUFS1	NDUFS1	NDUFS1	
Complex II (Succinate dehydrogenase) proteins	GEGGILINSQGER	SDHA	SDHA	SDHA	
	LGANSLLDLVVFGR	SDHA	SDHA	SDHA	
	DLVPDLSNFYAQYK	SDHB	SDHB	SDHB	
	GLNPGK	SDHB	SDHB	SDHB	Not retained on the column
Complex III (coenzyme Q : cytochrome c oxidoreductase) proteins	LFDYFPKPYPNSEAAR		CYC1		Signal standard below LOD
	TPQAVALSSK		CYC1		Signal peptide
	LSDYFPKPYPNPEAAR	CYC1			Signal standard below LOD
	TPQAVSLSSK	CYC1		CYC1	Signal peptide
	LSVTATR	UQCRC2	UQCRC2	UQCRC2	
	NALANPLYCPDYR	UQCRC2	UQCRC2	UQCRC2	
Complex IV (Cytochrome c oxidase) proteins	TPGPAVAIQSVR		COX5A		Signal peptide
	GLLHPASAPSPAAAVCSIR	COX5A			Signal peptide
	IIDAALR	COX5A	COX5A	COX5A	
	FHFTSK	MTCO3/CERK		MTCO3	Peak shape inconsistent
	ESTYQGHHTPPVQK		MTCO3		Signals below LOD
	EGTYQGHHTPIVQK	MTCO3		MTCO3	
Complex V (ATP synthase) proteins	IPVGPETLGR	ATP5B	ATP5B	ATP5B	
	VVDLLAPYAK	ATP5B	ATP5B	ATP5B	
	NAAFLGPGVLQATR		ATP5F1		Signal peptide
	VVLSAAATAAPSLK		ATP5F1		Signal peptide
	NAAALGPGVLQATR	ATP5F1			Signal peptide

	VVLSAAATAAPCLK	ATP5F1		ATP5F1	Signal peptide
Electron transport chain and oxidative phosphorylation proteins	TGQAAGFSYTDANK ADLIAYLK TGPNLHGLFGR	CYCS CYCS CYCS	 CYCS CYCS	CYCS CYCS CYCS	
Substrate transport proteins	FGFYEVFK TVEALYK	SLC25A3 SLC25A3	SLC25A3 SLC25A3	SLC25A3 SLC25A3	
	AAYFGVYDTAK EQGFLSFWR	SLC25A4 SLC25A4	SLC25A4/SLC25A6 SLC25A4	SLC25A4 SLC25A4	
	EQGVLSFWR AAYFGIYDTAK	SLC25A5 SLC25A5	SLC25A5/SLC25A6 SLC25A5	SLC25A5 SLC25A5	
	ATDVPPTATVK GTSPNVAR	UCP2 UCP2	UCP2 UCP2	UCP2 UCP2	 Not retained on the column
	IGLYDSVK SPYSGLVAGLHR MVAQEGPTAFYK	UCP2/UCP3 UCP3 UCP3	UCP2/UCP3 UCP3	UCP2/UCP3 UCP3 UCP3	
Concatemer 2 (QcC2)					
Connection between glycolysis and TCA cycle proteins	ILVPEGTR VPEANSSWMDTVIR	DLAT DLAT	DLAT DLAT	DLAT/M0RAP9 DLAT/M0RAP9	
	ALTGGIAHLFK VCHAHPTLSEAFR	DLD DLD	DLD DLD	DLD DLD	
	AHGFTFTR LPCIFICENNR	PDHA1 PDHA1	PDHA1 PDHA1	PDHA1/PDHA1L1 PDHA1/PDHA1L1	
	AVPLAGFGYGLPISR EISLLPDNLLR	PDK1 PDK1	PDK1 PDK1	PDK1 PDK1	
Substrate transport proteins	GLSSLLYGSIPK GTYQGLTATVLK	SLC25A1 SLC25A1	SLC25A1 SLC25A1	SLC25A1 SLC25A1	
	GALVTVGQLSCYDQAK WYFGGLASCGAACCTHPLDLLK	SLC25A10 SLC25A10	SLC25A10 SLC25A10	SLC25A10 SLC25A10	 Signals near LOD
	AVVVNAAQLASYSQSK GIYTGLSAGLLR	SLC25A11 SLC25A11	SLC25A11 SLC25A11	SLC25A11 SLC25A11	
	LAANDFFR NHGIAGLYK HEGPSAFLK	SLC25A22 SLC25A22 SLC25A22	SLC25A22/SLC25A18 SLC25A22	SLC25A22 SLC25A22	
TCA cycle proteins	NAVTQEFGPVPDTAR VAGILTVK	ACO2 ACO2	ACO2 ACO2	ACO2 ACO2	
	ALGVLAQLIWSR LVAQLYK	CS CS	CS CS	CS CS	
	DYIDISVAVATPR GLVVPVIR	DLST DLST	DLST DLST	DLST DLST	Broad peak shape
	IYELAAGGTAVGTGLNTR SGLGELILPENEPGSSIMPGK	FH1 FH1	FH1 FH1	FH1 FH1	
	LNEHFLNTTDFLDTIK	IDH2	IDH2	IDH2	

	TIEAEAAHGTVTR	IDH2	IDH2	IDH2	
	SNVTAVHK	IDH3A	IDH3A	IDH3A	
	TPYTDVNIVTIR	IDH3A	IDH3A	IDH3A	
	VAVLGASGGIGQPLSLLLK	MDH2	MDH2	MDH2	
	VNVPVIGGHAGK	MDH2	MDH2	MDH2	
	HWLDSPWPGFFTLDGQPR	OGDH	OGDH	OGDH	Signals near LOD
	SSPYPTDVAR	OGDH/OGDHL	OGDH/OGDHL	OGDH/OGDHL	
	ALIADSGLK	SUCLA2	SUCLA2	SUCLA2	
	IVFSPEEAK	SUCLA2	SUCLA2	SUCLA2	
	LIGPNCPGVINPGECK	SUCLG1	SUCLG1		
	QGTFHSQQALEYGTK	SUCLG1	SUCLG1	SUCLG1	
	NQAADQITK	SUCLG2			
	IDATQVEVNPFGETPEGQVVCFDAK	SUCLG2	SUCLG2	SUCLG2	
	VPLVVR	SUCLG2	SUCLG2	SUCLG2	
Concatemer 3 (QcC3)					
Connection between beta-oxidation and electron transport chain proteins	LLYDLADQLHAAVGASR	ETFA	ETFA	ETFA	
	TIYAGNALCTVK	ETFA	ETFA	ETFA	
	LGPLQVAR		ETFB		
	LSVISVEDPPQR		ETFB		
	AGDLGVDLTSK	ETFB		ETFB	
	VSVISVEEPPQR	ETFB		ETFB	
	LQINAQNCVHCK	ETFDH	ETFDH	ETFDH	
	NLSIYDGPEQR	ETFDH	ETFDH	ETFDH	
Fatty acid beta-oxidation proteins	DFTATDLSEFAAK		ACAA2		
	VGVPTETGALTLNR	ACAA2		ACAA2	
	AALSAGK	ACAA2	ACAA2	ACAA2	
	AYVDAR	ACADL	ACADL	ACADL	Peak shape inconsistent
	THICVTR	ACADL	ACADL		
	ALDEATK	ACADM	ACADM	ACADM	
	ANWYFLLAR	ACADM	ACADM		
	ITEIYEGTSEIQR	ACADS	ACADS	ACADS	
	LVIAGHLLR	ACADS	ACADS	ACADS	
	IFEGANDILR	ACADVL			
	FFEEVNDPAK	ACADVL	ACADVL	ACADVL	
	YYTLNGSK	ACADVL	ACADVL	ACADVL	Variable results
	SLLHGR	CPT1A	CPT1A	CPT1A	Not retained on the column
	VWLYHDGR	CPT1A	CPT1A	CPT1A	
	ALADDVELYCFQFLPFGK	CPT1B	CPT1B	CPT1B	
	ALLHGNCYNR	CPT1B	CPT1B		
	QYGQTVATYESCSTAAFK	CPT2	CPT2	CPT2	
	SEYNDQLTR	CPT2	CPT2	CPT2	
	ATAEQISSQTGNK		DECR1		

	FFSFGTK	DECR1		DECR1	Signal peptide
	VAFITGGGTGLGK	DECR1	DECR1	DECR1	
	AQFGQPEILLGTIPGAGGTQR	ECHS1		ECHS1	
	ISAQDAK	ECHS1	ECHS1	ECHS1	
	QAGLVSK	ECHS1	ECHS1	ECHS1	Not retained on the column
	NPAHYAEYWK	ECI1		ECI1	Protein not present in human
	WLAIPDHSR	ECI1			
	LLVPYLIEAVR	HADH			Signals below LOD
	LGAGYPMGPFELLDYVGLDTTK	HADH	HADH	HADH	
	LVEVIK	HADH	HADH	HADH	
	DGPGFYTTR	HADHA	HADHA	HADHA	
	THINYGVK	HADHA	HADHA	HADHA	
	DQLLLGPTYATPK	HADHB	HADHB	HADHB	
	LAAAFAVSR	HADHB	HADHB	HADHB	
	GLASTLLR	SLC25A29	SLC25A29	SLC25A29	
	QESVLGLYK	SLC25A29	SLC25A29	SLC25A29	
Synthetic peptide					
Substrate transport protein	EQGFLSFWR	SLC25A4	SLC25A4	SLC25A4	

Supplemental figures

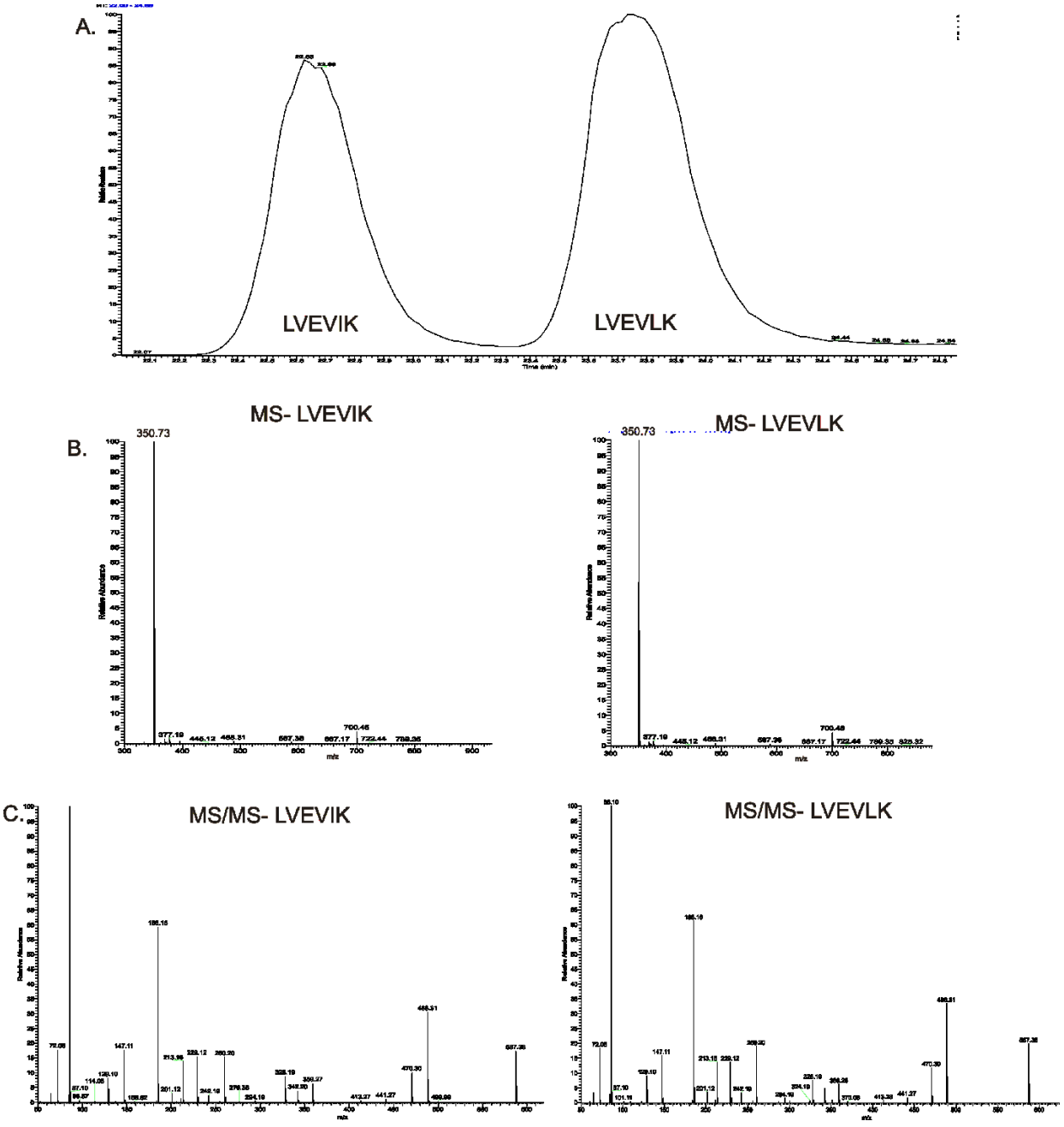

Figure S1. Uniqueness of leucine or isoleucine containing peptides. MS and MS/MS spectra of the LVEVIK and LVEVLK peptides (measured on a Q Exactive Plus instrument) showing that both peptides have different retention times on the LC column (A), although they cannot be distinguished by MS (B) or MS/MS (C).

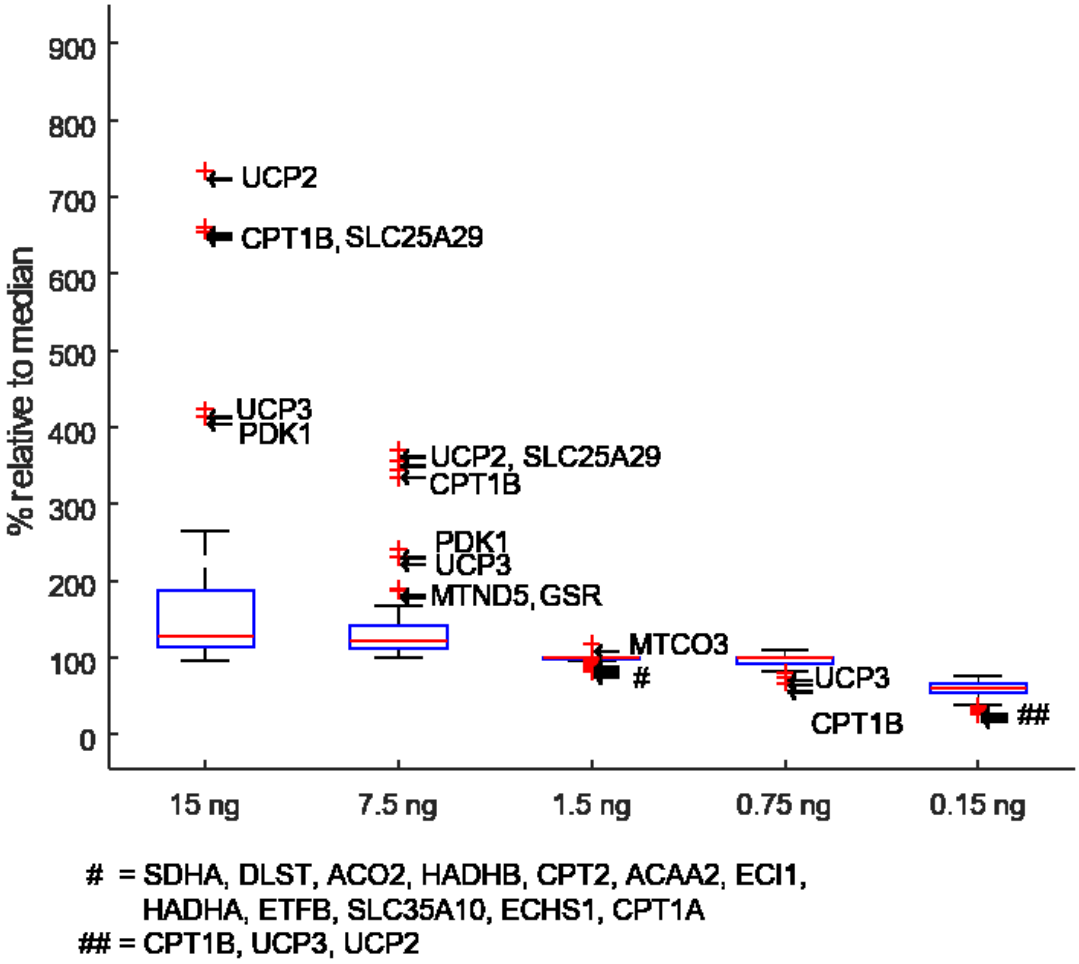

Figure S2. Protein variability expressed as the percentage (%) of the median after spiking with different concentrations of concatemers to the same amount of total mitochondrial protein digest (50 µg). Proteins were quantified in mouse liver mitochondria (n=3) for all concatemer targets after spiking 0.15 ng - 15 ng concatemer per µg total mitochondrial protein. The median of the determined endogenous protein concentrations using five spiked concatemer concentrations was calculated for each target and the variation from this median in percent is shown as a boxplot to represent to what extent the endogenous protein quantification was affected by the spiking level.

Supplemental Figure S3

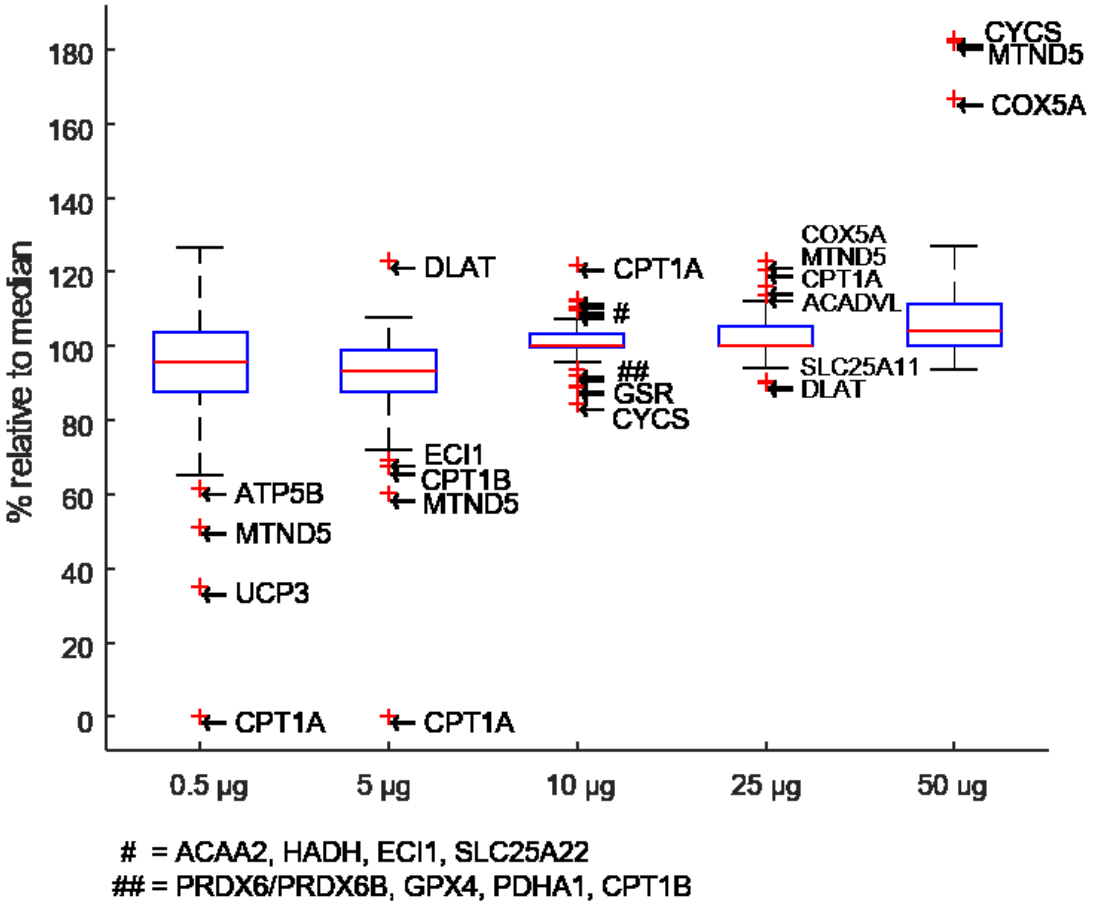

Figure S3. Protein variability expressed as the percentage (%) of the median starting with different amounts of liver mitochondria. Quantification of the proteins was done in mouse liver mitochondria ranging in protein levels from 0.5 to 50 µg total mitochondrial protein spiked with 1.5 ng concatemer per µg total mitochondrial protein. Analogous to Figure S2, the median and the variation from this median was used to represent whether endogenous protein quantification was affected by the amount of starting material.