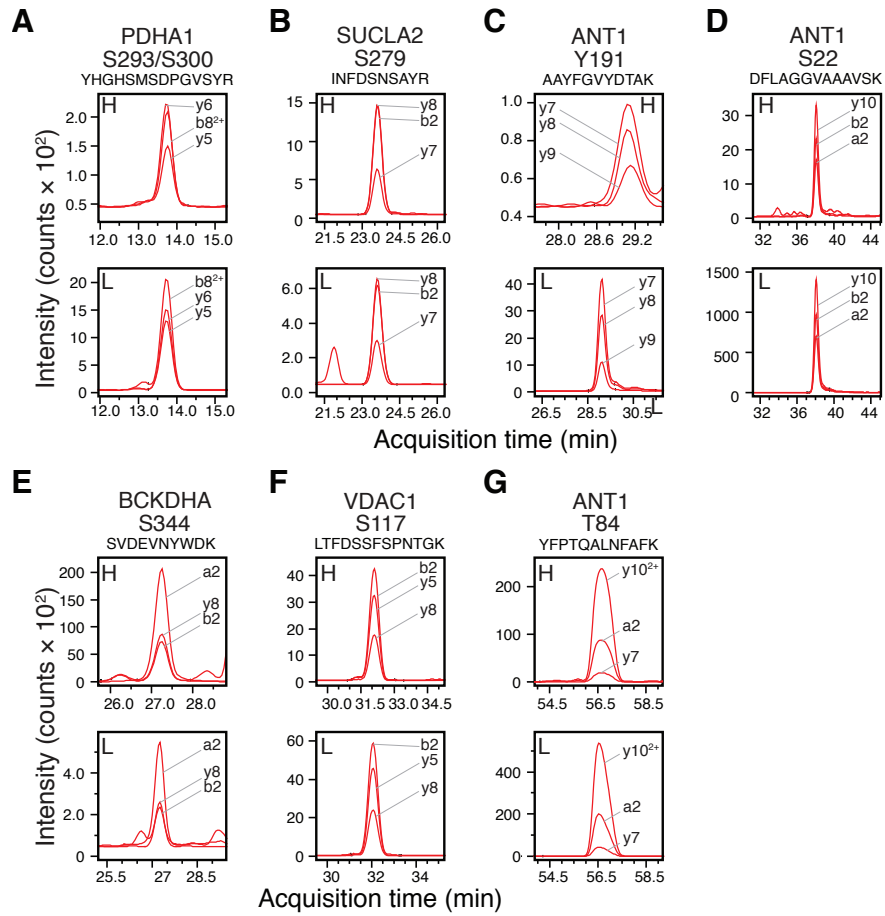


Supplementary materials

Supplementary Figure S1. MRM transitions and quantitative analysis of unmodified sequence counterparts of detected phosphopeptides detected in human heart mitochondrial samples. The new set of MRM assays were validated using heavy-isotope-labeled synthetic peptide standards. The unmodified sequences of human protein phosphorylation were detected by comparing the heavy-labeled standard (H) and unlabeled endogenous peptides (L). The MRM signals for a panel of transitions are shown and labeled; asterisks next to the fragment ions denote neutral loss of H_3PO_4 . **(A)** Pyruvate dehydrogenase E1 complex alpha subunit (PDHA1) unmodified peptide YHGHSMSDPGVS^{*}YR corresponding to the S293 and S300 sites. **(B)** Succinyl-CoA ligase [ADP-forming] subunit beta (SUCLA2) unmodified peptide INFDP^{*}SNSAYR corresponding to the S279 site. **(C)** Adenine nucleotide translocase 1 (ANT1) unmodified peptide AAYFGVYDTAK corresponding to the Y191 site. **(D)** ANT1 unmodified peptide DFLAGGVAAAVSK corresponding to the S22 site. **(E)** Branched-chain alpha-keto acid dehydrogenase subunit alpha (BCKDHA) unmodified peptide SVDEVNYW^{*}DK corresponding to the S344 site. **(F)** Voltage-dependent anion channel 1 (VDAC1) unmodified peptide LTFDSSFSPNTGK corresponding to the S114 site. **(G)** ANT1 unmodified peptide DFLAGGVAAAVSK corresponding to the T84 site. Amino acid residue numbering is based on mouse homolog sequence in Uniprot.

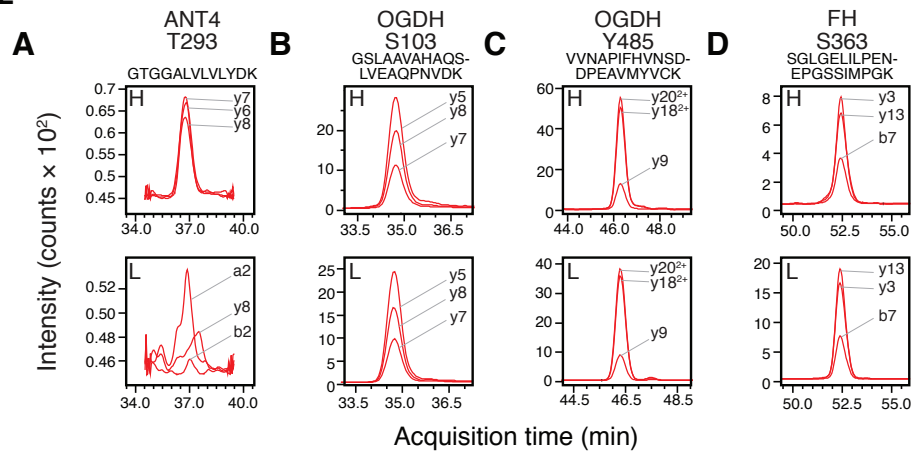
Supplementary Figure S2. MRM transitions and quantitative analysis of unmodified sequence counterparts of undetected phosphopeptides in human heart mitochondrial samples. Four of the 12 phosphopeptides corresponding to human protein sequences were below detection limit in the human heart samples analyzed. Their unmodified sequence counterparts were detected by comparing the heavy-labeled standard (H) and unlabeled endogenous peptides (L). **(A)** Adenine nucleotide translocase 4 (ANT4) unmodified peptide GTGGALV^{*}LVLYDK corresponding to the T293 site. **(B)** 2-Oxoglutarate dehydrogenase (OGDH) unmodified peptide GSLAAVAHAQSLVEAQPNV^{*}DK corresponding to the S103 site. **(C)** OGDH unmodified peptide VVNAPIFHVNSDDPEAVMYV^{*}CK corresponding to the Y485 site. **(D)** Fumarate hydratase (FH) unmodified peptide SGLGELILPENEPGSSIMPGK corresponding to the S363 site. Amino acid residue numbering is based on mouse homolog sequence in Uniprot.

Figure S1



Supplementary Figure S1

Figure S2



Supplementary Figure S2