

Supporting Information

Protein targets for carbonylation by 4-hydroxy-2-nonenal in rat liver mitochondria

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Supplementary Table S1. A list of HNE-modified tryptic peptides of primarily non-mitochondrial liver proteins identified after SPH enrichment and data-dependent MS/MS acquisition.

Protein name (<i>gene symbol</i>)	IPI accession number	HNE-modified peptides	Modification site(s)
Arginase-1 (<i>Arg1</i>) ^a	IPI00327518	DHGDLAFVDVPNDSPFQIVKNPR	50
Carbonic anhydrase 3 (<i>Ca3</i>) ^a	IPI00230788	DIRHDPSLQPWSVSYDPGSAK	40
Carboxylesterase 3 (<i>Ces3</i>) ^b	IPI00326972	AKEAAEESHWK	559
Fatty acid-binding protein (<i>Fabp1</i>) ^c	IPI00190790	GVSEIVHEGKK	43
78 kDa glucose-regulated protein (<i>Hspa5</i>) ^d	IPI00206624	VTHAVVTVPAYFNDAQR	167
Glutathione S-transferase alpha-2 (<i>Gsta2</i>) ^a	IPI00555178	SHGQDYLVGNR	143
Hydroxyacid oxidase 1 (<i>Hao1</i>) ^e	IPI00207601	HGVDGILVSNHGAR	260
Isoform 1 of Peroxisomal acyl-coenzyme A oxidase 1 (<i>Acox1</i>) ^e	IPI00211510	ASATFNPELITHILDGSPENTR	21
Peroxiredoxin 4 (<i>Prdx4</i>) ^a	IPI00208209	TRENECHFYAGGQVYPGEVSR	54
Phosphoglycerate mutase 1 (<i>Pgam1</i>) ^f	IPI00421428	HGEAQVK	107
Programmed cell death 5 (<i>Pdcd5</i>) ^g	IPI00193547	HGDPGDAAQQEAK	21

^a = cytosol

^b = endoplasmic reticulum (also in mitochondria)

^c = cytosol, nucleus and peroxisome

^d = endoplasmic reticulum

^e = peroxisome.

^f = cytosol and nucleus

^g = nucleus and cytoplasm

IPI00551812 (100%), 56,354.3 Da

ATP synthase subunit beta, mitochondrial

12 unique peptides, 16 unique spectra, 21 total spectra, 194/529 amino acids (37% coverage)

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MLSLVGRVAS ASASGALRGL NPLAALPQAH LLLRTAPAGV HPARDYAAQS SAAPKAGTAT
GQIVAVIGAV VDVQFDEGLP PILNALEVQG RESRLVLEVA QHLGESTVRT IAMDGTEGLV
RGQKVLDSGA PIKIPVGPET LGRI MNVIGE PIDERGPIKT KQFAPIHAEA PEFIEMSVEQ
EILVTGIKVV DLLAPYAKGG KIGLFGGAGV GKTVLMELI NNVAKAHGGY SVFAGVGERT
REGNDLYHEM IESGVINLKD ATSKVALVYG QMNEPPGARAR VALTGLTVA EYFRDQEGQD
VLLFIDNIFR FTQAGSEVSA LLGRIPSAVG YQPTLATDMG TMQERITTTK KGSITSVQAI
YVPADDLTDP APATTFAHLD ATTVLSRAIA ELGIYPAVDP LDSTSRIMDP NIVGSEHYDV
ARGVQKILQD YKSLQDI IAI LGMDELSEED KLTVSRARKI QRFLSQPFQV AEVFTGHMGK
LVPLKETIKG FQQILAGDYD HLPEQAFYMV GPIEEAVAKA DKLAEHGS
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Supplementary Fig. S1. ATP synthase β -subunit tryptic peptides identified (highlighted by yellow within the protein sequence, green highlights indicate modified residue, M = methionine oxidation) in the protein by in-gel digestion, data-dependent LC-MS/MS and protein database search from the 50 \pm 5 kDa SDS-PAGE band of liver mitochondrial proteins (Fig 2 (a)).

IPI00210644 (100%), 164,582.7 Da

Carbamoyl-phosphate synthase [ammonia], mitochondrial

47 unique peptides, 66 unique spectra, 90 total spectra, 540/1500 amino acids (36% coverage)

MTRILTACKV	VKTLKSGFGL	ANVTSKRQWD	FSRPGIRLLS	VK AQT AHIVL	EDGTKMKGYS
FGHPSSVAGE	VVFNTGLGGY	SEALTDPAYK	GQILT MANPI	IGNGGAPDTT	ARDELGLNKY
MESDGIK VAG	LLVLNYSHDY	NHWLATK SLG	QWLQEEKVPA	IYGVDRMLT	KIIRDKGTML
GK IEFEGQSV	DFVDPNK QNL	IAEVSTKDVK	VFGKGNPTKV	VAVDCGIKNN	VIRLLVKRGA
EVHLVPWNHD	FTQMDYDGLL	IAGGPGNPAL	AQPLIQNVKK	ILESDRK EPL	FGISTGNIIT
GLAAGAK SYK	MSMANR GQNQ	PVLNITNRQA	FITAQNHGYA	LDNTLPAGWK	PLFVNVNDQT
NEGIMHESKP	FFAVQFHPEV	SPGPTDTEYL	FDSFFSLIKK	GKGTITSVL	PKPALVASRV
EVSQVILIGS	GGLSIGQAGE	FDYSGSQAVK	AMKEENVKTV	LMNPNIASVQ	TNEVGLKQAD
AVYFLPITPQ	FVTEVIKAER	PDGLILGMGG	QTALNCGVEL	FKRGVLKEYG	VKVLGTSVES
IMATEDRQLF	SDKLNEINEK	IAPSFVAVESM	EDALKAADTI	GYPVMIRSAY	ALGGLGSGIC
PNKETLMDLG	TKAFAMTNQI	LVERSVTGWK	EIEYEVV RDA	DDNCVTV CNM	ENVDAMGVHT
GDSVVVAPAQ	TLSNAEFQML	RRTSINVVRH	LGIVGECNIQ	FALHPTSM EY	CIEV NARLS
RSSALASKAT	GYPLAFIAAK	IALGIPLPEI	KNVVSGK TSA	CFEPSLDY MV	TKIPRWDLDR
FHGTSSRIGS	SMK SVGEVMA	IGRTFEESFQ	KALRMCHPSV	DGFTPR LPMN	KEWPANLDR
KELSEPSSTR	IYAI AKALEN	NMSLDEIVKL	TSIDKWFLYK	MRDILNMDKT	LKGLNSESVT
EETLRQAKEI	GFSDKQISK C	LGLTEAQTRE	LRLKKN IHPW	VKQIDTLAAE	YPSVTNYLYV
TYNGQEHDIK	FDEHGIMVLG	CGPYHIGSSV	EFDWCAVSSI	RTLRLQLGKKT	VVNVNCPETV
STDFDECDKL	YFEELS LERI	LDIYHQEACN	GCIISVGGQI	PNNLAVPLYK	NGVK IMGTSP
LQIDRAEDRS	IFSAVLDELK	VAQAPWKAVN	TLNEALEFAN	SVGYPCLLRP	SYVLSGSAMN
VVFSEDEMKR	FLEEATR VSQ	EHPVVLTKFI	EGAREVEMDA	VGKEGRVISH	AISEHVEDAG
VHSGDATLML	PTQTI SQGAI	EKVKDATRKI	AKAFAISGPF	NVQFLVK GND	VLVIECNLRA
SRSFPFVSKT	LGVD FIDVAT	KVMIGESVDE	KHLPTLEQPI	IPSDYVAIKA	PMFSWPRLRD
ADPILRCEMA	STGEVACFGE	GIHTAFLKAM	LSTGFKIPQK	GILIGIQQSF	RPRFLGVAEQ
LHNEGFKLFA	TEATSDWLN A	NNVPATPVAW	PSQEGQNP SL	SSIRKLIRDG	SIDLVINLPN
NNTK FVHDNY	VIRRTAVDSG	IALLTNFQVT	KLFAEAVQKA	RTVDSKSLFH	YRQYSAGKAA

Supplementary Fig. S2. Carbamoyl-phosphate synthase tryptic peptides identified (highlighted by yellow within the protein sequence, green highlights indicate modified residue: M = methionine oxidation, C = cystein amidomethylation) by in-gel digestion, data-dependent LC-MS/MS and protein database search from the 150±25 kDa SDS-PAGE band of liver mitochondrial proteins (Fig 2 (a)).