## **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 (gene symbol)	IPI accession number	HNE-modified peptides	Modification site(s)
Arginase-1 (Arg1) <sup>a</sup>	IPI00327518	D <b>H</b> GDLAFVDVPNDSPFQIVKNPR	50
Carbonic anhydrase 3 (Ca3) <sup>a</sup>	IPI00230788	DIR <b>H</b> DPSLQPWSVSYDPGSAK	40
Carboxylesterase 3 (Ces3) <sup>b</sup>	IPI00326972	AKEAAEEPSHWK	559
Fatty acid-binding protein (Fabp1) c	IPI00190790	GVSEIV <b>H</b> EGKK	43
78 kDa glucose-regulated protein ( <i>Hspa5</i> ) <sup>d</sup>	IPI00206624	VT <b>H</b> AVVTVPAYFNDAQR	167
Glutathione S-transferase alpha-2 (Gsta2) <sup>a</sup>	IPI00555178	SHGQDYLVGNR	143
Hydroxyacid oxidase 1 ( <i>Hao1</i> ) <sup>e</sup>	IPI00207601	HGVDGILVSN <b>H</b> GAR	260
Isoform 1 of Peroxisomal acyl-coenzyme A oxidase $1 (Acox I)^e$	IPI00211510	ASATFNPELIT <b>H</b> ILDGSPENTR	21
Peroxiredoxin 4 ( <i>Prdx4</i> ) <sup>a</sup>	IPI00208209	TRENECHFYAGGQVYPGEVSR	54
Phosphoglycerate mutase 1 (Pgam1) <sup>f</sup>	IPI00421428	HGEAQVK	107
Programmed cell death 5 ( <i>Pdcd5</i> ) <sup>g</sup>	IPI00193547	<b>H</b> GDPGDAAQQEAK	21

a = cytosol

<sup>&</sup>lt;sup>b</sup> = endoplasmic reticulum (also in mitochondria)

<sup>&</sup>lt;sup>c</sup> = cytosol, nucleus and peroxisome

<sup>&</sup>lt;sup>d</sup> = endoplasmic reticulum

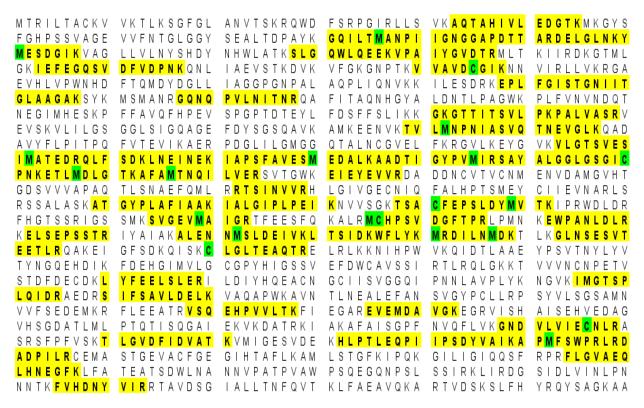
e = peroxisome.

f = cytosol and nucleus

g= nucleus and cytoplasm

MLSLVGRVAS	ASASGALRGL	NPLAALPQAH	LLLRTAPAGV	H P A R D Y A A Q S	SAAPKAGTAT
<u>G</u> QIV <u>AVIGAV</u>	VDVQFDEGLP	PILN <u>A</u> LEVQG	RESRLVLEVA	Q H L G E S T V R <mark>T</mark>	I A M D G T E G L V
R G Q K V L D S G A	P I K I P V G P E T	L G R I M N V I G E	<pre>PIDERGPIKT</pre>	<u>K Q F A P</u> I H A E A	PEFIEMSVEQ
<u>EILVTGIK<mark>VV</mark></u>	<b>DLLAPYAK</b> <u>G</u> G	K I G L F G G A G V	G K <mark>T V L I <mark>M</mark> E L I</mark>	<mark>N N V A K</mark> A H G G Y	<u>SVFA</u> GVGER <mark>T</mark>
R E G N D L Y H E M	I E S G V I N L K D	A T S K <mark>V A L V Y G</mark>	Q M N E P P G A R A	R <mark>V A L T G L T V A</mark>	EYFR DQEGQD
VLLFIDNIFR	FTQAGSEVSA	LLGRIPSAVG	Y Q P T L A T D M G	T M Q E R   T T T K	KGSITSVQAI
Y V P A D D L T D P	APATTFAHLD	ATTVLSR <mark>AIA</mark>	ELGIYPAVDP	L D S T S R I M D P	NIVGSEHYDV
<b>AR</b> GVQKILQD	YKSLQDIIAI	LGMDELSEED	K L T V S R A R K <u>I</u>	QRFLSQPFQV	AEVFTGHMGK
LVPLKETIKG	FQQILAGDYD	HLPEQAFYMV	GPIEEAVAK <mark>A</mark>	DKLAEEHGS	

**Supplementary Fig. S1.** ATP synthase  $\beta$ -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±5 kDa SDS-PAGE band of liver mitochondrial proteins (Fig 2 (a)).



**Supplementary Fig. S2.** Carbamoyl-phospate 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)).