



## SUPPLEMENTARY ONLINE DATA

# Fatty liver is associated with reduced SIRT3 activity and mitochondrial protein hyperacetylation

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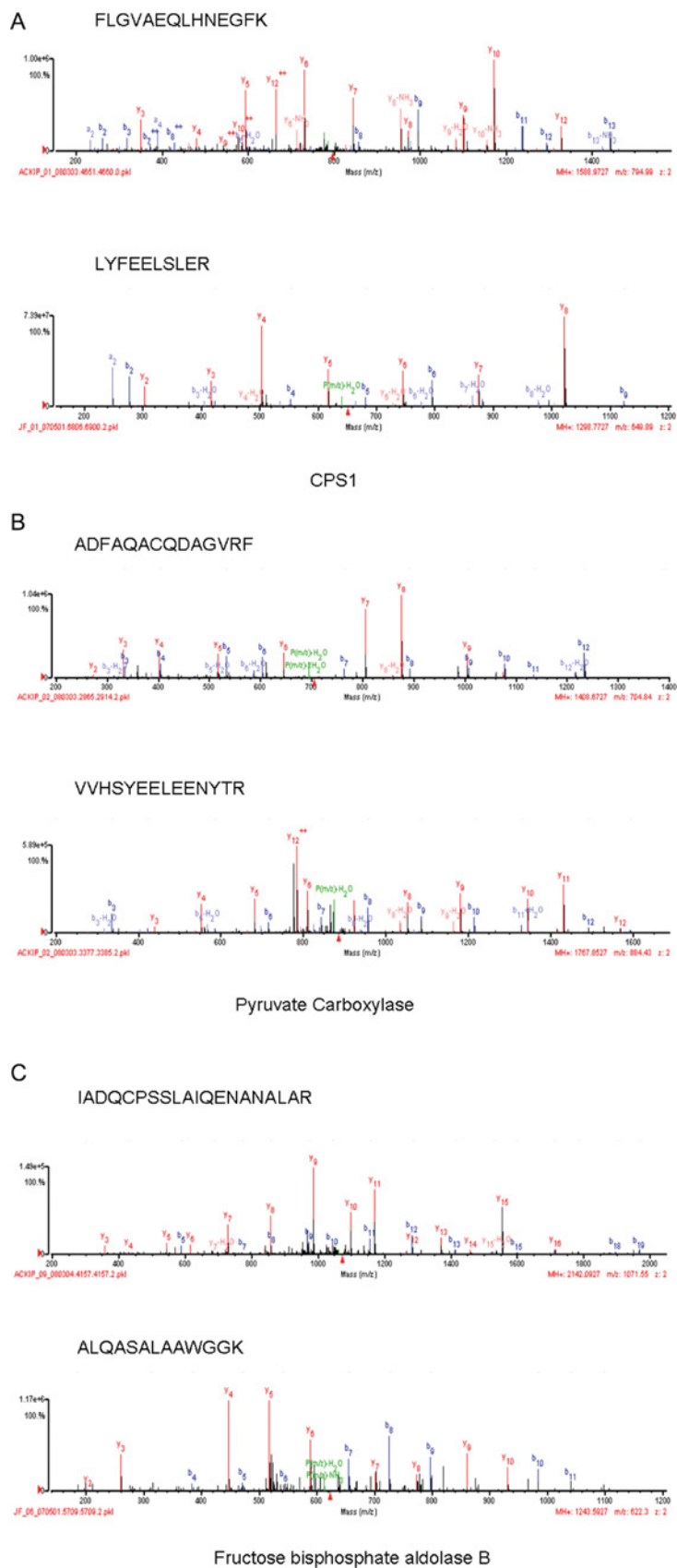
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See the pages that follow for Supplementary Figures S1–S3 and Supplementary Table S1.

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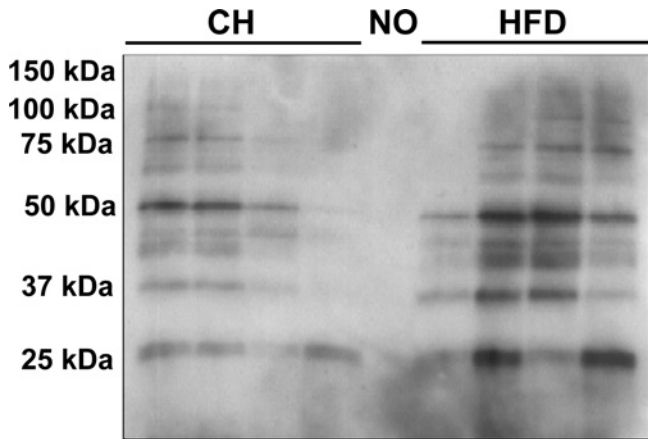
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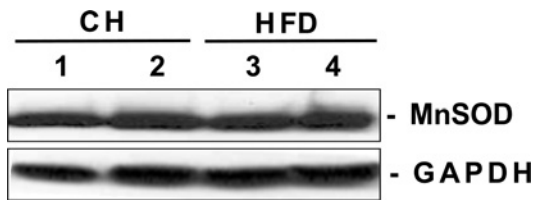
**Figure S1 Spectra used for validation of (A) CPS1, (B) PC and (C) aldase B**

Our protein identification validation strategy included manual assessment of at least two high-scoring peptide fragmentation mass spectra. The amino acid sequence of each peptide is provided and b- and y-type fragmentation products are labelled.



**Figure S2 HFD increases oxidized mitochondrial proteins in liver**

NO, empty lane. The molecular mass in kDa is shown of the left-hand side.



**Figure S3 Western blot showing equal amounts of MnSOD (manganese superoxide dismutase) and GAPDH in liver extracts from CH- and HFD-fed animals**

**Table S1 Summary of protein identification from three separate experiments**

Three different experiments in which livers from HFD- and CH-fed mice were harvested, proteins were extracted and enriched using the anti-(acetylated-lysine) antibody, and band densities were compared. Proteins marked with an asterisk were identified from whole-cell lysate preparations, whereas the other proteins were identified in high-salt-enriched fractions (see the Experimental section of the main paper). Protein identifications required a minimum of two peptide hits to each protein, a protein score minimum of 13, a peptide score minimum of 10 and an SPI of 85%. # OP, the total number of spectra assigned to the given protein; # DP, the number of distinct peptides assigned to the given protein. We required # DP to be at least two. Score, the summed score from the DP peptides. AA (%), the percentage of amino acids detected, considering the distinct peptides. Molecular masses and accession numbers are from the SwissProt database (UniProtKB/Swiss-Prot Release 57.6; 495880 entries). A total of 193 unique proteins were identified in these experiments. Band numbers listed were used in each experiment and do not necessarily compare between experiments. Although some effort was made to excise the same bands in different experiments performed on different days, additional bands were excised with numbers that do not correlate with previous experiments. Therefore protein identification was assessed separately for each band in each experiment. Within each group of identifications from every band, the molecular mass provided by the protein database was compared with the molecular mass observed on the gel. Proteins were only included if, for a given band, their molecular mass appeared within 1 S.D. of the average molecular mass for the band.

Band number	# OP	# DP	Score	AA (%)	Molecular mass (Da)	Accession number	Protein name
Band 1							
	82	54	912	47	164619	Q8C196	Carbamoyl-phosphate synthase [ammonia], mitochondrial precursor
*	8	6	93.86	5	164619	Q8C196	Carbamoyl-phosphate synthase [ammonia], mitochondrial precursor
*	52	32	492.5	27	164619	Q8C196	Carbamoyl-phosphate synthase [ammonia], mitochondrial precursor
Band 2							
	10	8	125.5	17	61337	P26443	Glutamate dehydrogenase 1, mitochondrial precursor
	6	5	80.67	15	61120	Q8BWQ1	UDP-glucuronosyltransferase 2A3 precursor
	9	6	94.42	20	60721	Q9DBG1	Cytochrome P450 27, mitochondrial precursor
	4	3	52.17	7	60124	Q63886	UDP-glucuronosyltransferase 1-1 precursor
	26	17	277.9	37	59753	Q03265	ATP synthase subunit $\alpha$ , mitochondrial precursor
	5	5	81.83	12	59673	Q8JZZO	UDP-glucuronosyltransferase 3A2 precursor
	4	4	61.64	8	58720	O35728	Cytochrome P450 4A14 precursor
	8	7	111.3	20	57855	Q64459	Cytochrome P450 3A11
	3	3	44.19	10	57553	Q9CZS1	Aldehyde dehydrogenase X, mitochondrial precursor
	10	10	160.4	28	56976	Q8CIM7	Cytochrome P450 2D26
	15	13	209.9	27	56805	Q05421	Cytochrome P450 2E1
	21	15	241.6	45	56538	P47738	Aldehyde dehydrogenase, mitochondrial precursor
	9	6	108.4	18	56301	P56480	ATP synthase subunit $\beta$ , mitochondrial precursor
	2	2	34.92	5	54468	P24549	Retinal dehydrogenase 1
	8	5	83.24	14	53943	P47740	Fatty aldehyde dehydrogenase
	6	6	88.65	16	52610	Q63836	Selenium-binding protein 2
	5	4	58.42	15	50152	P05213	Tubulin $\alpha$ -1B chain
	10	7	112.5	25	49671	P99024	Tubulin $\beta$ -5 chain
*	19	12	192.3	15	129685	Q05920	Pyruvate carboxylase, mitochondrial precursor
Band 3							
	14	8	133.7	17	60856	P17717	UDP-glucuronosyltransferase 2B5 precursor
	14	11	170.1	28	59753	Q03265	ATP synthase subunit $\alpha$ , mitochondrial precursor
	3	2	34.35	7	58939	Q91XD4	Formimidoyltransferase-cyclodeaminase
	7	5	74.65	13	57960	Q9JMA7	Cytochrome P450 3A41
	3	3	41.59	6	57493	Q64464	Cytochrome P450 3A13
	4	4	67.87	9	57234	P24456	Cytochrome P450 2D10
	7	6	84.25	17	56976	Q8CIM7	Cytochrome P450 2D26
	3	3	41.01	8	56823	P54869	Hydroxymethylglutaryl-CoA synthase, mitochondrial precursor
	3	2	34.27	6	56805	Q05421	Cytochrome P450 2E1
	5	5	80.36	11	56741	P20852	Cytochrome P450 2A5
	23	17	278.4	46	56538	P47738	Aldehyde dehydrogenase, mitochondrial precursor
	28	19	335.7	55	56301	P56480	ATP synthase subunit $\beta$ , mitochondrial precursor
	7	6	89.3	17	56174	P56593	Cytochrome P450 2A12
	9	7	116.5	19	55780	P56657	Cytochrome P450 2C40
	18	14	230.4	41	55730	Q64458	Cytochrome P450 2C29
	11	9	157.5	25	55514	P56654	Cytochrome P450 2C37
	3	3	49.12	6	54566	P11679	Keratin, type II cytoskeletal 8
	2	2	31.22	9	53943	P47740	Fatty aldehyde dehydrogenase
	11	9	139.4	31	51929	P16331	Phenylalanine-4-hydroxylase
	3	3	46.06	9	51740	Q91Y10	Argininosuccinate lyase
	4	4	55.87	9	50114	P10126	Elongation factor 1- $\alpha$ 1
	4	3	45.95	11	49831	P68372	Tubulin $\beta$ -2C chain
	2	2	38.98	7	48995	Q9D2G2	Dihydropyridyllysine-residue succinyltransferase 2-oxoglutarate dehydrogenase
	7	6	102.2	24	48101	Q922R8	Protein disulfide-isomerase A6 precursor
	11	8	125	23	43509	Q91 × 83	S-Adenosylmethionine synthetase isoform type-1
*	13	5	98.79	21	45021	Q35490	Betaine-homocysteine S-methyltransferase 1
*	8	5	95.05	19	41858	Q8BWT1	3-Ketoacyl-CoA thiolase, mitochondrial
*	4	4	64.67	7	73529	P38647	Stress-70 protein, mitochondrial precursor
*	7	5	85.43	11	72422	P20029	Glucose-regulated protein precursor of 78 kDa
Band 4							
	4	4	61.96	10	61337	P26443	Glutamate dehydrogenase 1, mitochondrial precursor
	3	3	52.9	6	60124	Q63886	UDP-glucuronosyltransferase 1-1 precursor
	12	12	195	31	59753	Q03265	ATP synthase subunit $\alpha$ , mitochondrial precursor
	5	4	58.38	9	57234	P24456	Cytochrome P450 2D10

Table S1 (Continued)

Band number	# OP	# DP	Score	AA (%)	Molecular mass (Da)	Accession number	Protein name
	33	20	327.4	36	56823	P54869	Hydroxymethylglutaryl-CoA synthase, mitochondrial precursor
	11	9	139.7	29	56538	P47738	Aldehyde dehydrogenase, mitochondrial precursor
	9	8	130.4	23	56301	P56480	ATP synthase subunit $\beta$ , mitochondrial precursor
	4	2	34.55	7	55514	P56654	Cytochrome P450 2C37
	5	5	78.49	13	54566	P11679	Keratin, type II cytoskeletal 8
	9	7	115.7	24	54371	P97807	Fumarate hydratase, mitochondrial precursor
	3	3	45.2	11	53943	P47740	Fatty aldehyde dehydrogenase
	12	8	155.4	26	52769	Q9CZ13	Cytochrome <i>b-c</i> <sub>1</sub> complex subunit 1, mitochondrial precursor
	10	8	109.3	23	52576	Q9D379	Epoxide hydrolase 1
	18	14	242.3	37	51387	Q99JY0	Trifunctional enzyme subunit $\beta$ , mitochondrial precursor
	3	3	52.61	10	50537	P61598	Rab GDP dissociation inhibitor $\beta$
	10	6	90.73	22	50493	P58710	L-gulonolactone oxidase
	11	7	116.2	20	50114	P10126	Elongation factor 1- $\alpha$ 1
	4	3	51.6	8	50061	Q9D8N0	Elongation factor 1- $\gamma$
	5	5	74.41	15	49509	Q8BFR5	Elongation factor Tu, mitochondrial precursor
	3	3	50.23	7	49014	O54734	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 48 kDa subunit
	6	4	72.32	14	47572	Q9QZ85	Interferon-inducible GTPase 1
	17	14	243.1	48	47538	P05784	Keratin, type I cytoskeletal 18
	6	6	107.2	22	47141	P17182	$\alpha$ -Enolase
	12	10	165.6	35	46154	P60843	Eukaryotic initiation factor 4A-I
	4	4	60.76	15	45021	Q35490	Betaine-homocysteine S-methyltransferase 1
*	33	10	198.6	37	41858	Q8BWT1	3-Ketoacyl-CoA thiolase, mitochondrial
*	3	3	45.35	7	62516	P34914	Epoxide hydrolase 2
*	6	5	86.01	11	60956	P63038	60 kDa heat-shock protein, mitochondrial precursor
*	3	2	35.67	4	59766	P24270	Catalase
*	4	3	48.21	6	59126	P32020	Non-specific lipid-transfer protein
Band 5							
	11	10	162.6	26	56823	P54869	Hydroxymethylglutaryl-CoA synthase, mitochondrial precursor
	4	4	56.42	12	51387	Q99JY0	Trifunctional enzyme subunit $\beta$ , mitochondrial precursor
	10	8	130.3	28	50114	Q9Z2I9	Succinyl-CoA ligase [ADP-forming] $\beta$ -chain, mitochondrial precursor
	5	4	58.19	9	50114	P10126	Elongation factor 1- $\alpha$ 1
	9	8	142.5	30	48647	Q60759	Glutaryl-CoA dehydrogenase, mitochondrial precursor
	11	8	138.8	26	48235	Q9DB77	Cytochrome <i>b-c</i> <sub>1</sub> complex subunit 2, mitochondrial precursor
	4	4	64.42	8	47908	P51174	Long-chain specific acyl-CoA dehydrogenase, mitochondrial precursor
	8	6	108.8	25	47538	P05784	Keratin, type I cytoskeletal 18
	4	3	51.3	9	46840	Q9Z2I8	Succinyl-CoA ligase [GDP-forming] $\beta$ -chain, mitochondrial precursor
	35	17	298.8	49	46585	P16460	Argininosuccinate synthase
	4	4	70.31	12	46482	P45952	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial precursor
	4	4	58.88	15	46301	Q99J08	SEC14-like protein 2
	5	5	88.33	20	45250	Q99PG0	Arylacetylamide deacetylase
	4	4	54.31	14	45054	P49429	4-Hydroxyphenylpyruvate dioxygenase
	7	6	96.45	19	44816	Q8QZT1	Acetyl-CoA acetyltransferase, mitochondrial precursor
	4	4	60.9	14	44551	P09411	Phosphoglycerate kinase 1
	3	2	36.3	7	43509	Q91 $\times$ 83	S-Adenosylmethionine synthetase isoform type-1
	2	2	31.33	6	43232	P35486	Pyruvate dehydrogenase E1 component subunit $\alpha$ , somatic form
	5	4	78.88	18	42120	P15105	Glutamine synthetase
*	16	4	78.61	17	43996	Q8VCH0	3-Ketoacyl-CoA thiolase B, peroxisomal precursor
*	10	6	100.9	22	41858	Q8BWT1	3-Ketoacyl-CoA thiolase, mitochondrial
*	14	6	116.2	23	39772	P00329	Alcohol dehydrogenase 1
*	9	5	79.43	11	50114	P10126	Elongation factor 1- $\alpha$ 1
*	3	2	38.26	8	47538	P05784	Keratin, type I cytoskeletal 18
*	6	2	30.45	6	45021	Q35490	Betaine-homocysteine S-methyltransferase 1
*	5	2	39.36	7	43509	Q91 $\times$ 83	S-Adenosylmethionine synthetase isoform type-1
Band 6							
	9	7	116.4	28	42509	Q9DC69	NADH dehydrogenase [ubiquinone] 1 $\alpha$ subcomplex subunit 9
	2	2	35.53	7	41793	P63260	Actin, cytoplasmic 2
	8	5	89.38	19	39772	P00329	Alcohol dehydrogenase 1
	4	3	44.17	12	39507	Q91Y97	Fructose-bisphosphate aldolase B
	3	2	32.56	8	38937	Q9D051	Pyruvate dehydrogenase E1 component subunit $\beta$ , mitochondrial precursor
	6	5	70.82	12	38195	Q922Q1	MOSC domain-containing protein 2, mitochondrial precursor
	4	4	73.4	21	37979	Q9CW42	MOSC domain-containing protein 1, mitochondrial precursor
	14	9	143.2	40	37048	P70694	Oestradiol 17 $\beta$ -dehydrogenase 5
	11	6	96.26	30	35810	P16858	Glyceraldehyde-3-phosphate dehydrogenase
	5	4	53.51	16	35612	P08249	Malate dehydrogenase, mitochondrial precursor
	5	3	44.63	15	34995	Q9WUM5	Succinyl-CoA ligase [GDP-forming] subunit $\alpha$ , mitochondrial precursor
	5	4	79.53	25	34593	Q8VBT2	L-Serine dehydratase
	3	3	43.2	13	34401	P47962	60S ribosomal protein L5
	7	6	103.1	33	34217	P14869	60S acidic ribosomal protein P0
	2	2	31.58	9	34099	Q91XE0	Glycine N-acyltransferase

**Table S1 (Continued)**

Band number	# OP	# DP	Score	AA (%)	Molecular mass (Da)	Accession number	Protein name
	14	10	160.8	40	33466	P52196	Thiosulfate sulfurtransferase
	2	2	32.17	9	32952	Q9JMD3	PCTP-like protein
	3	3	55.76	11	32410	Q99MZ7	Peroxisomal trans-2-enoyl-CoA reductase
	4	4	59.86	15	32364	P50172	Corticosteroid 11 $\beta$ -dehydrogenase isozyme 1
*	4	2	40.21	8	41858	Q8BWT1	3-ketoacyl-CoA thiolase, mitochondrial
*	27	8	143.3	25	39772	P00329	Alcohol dehydrogenase 1
*	3	2	31.85	9	39507	Q91Y97	Fructose-bisphosphate aldolase B
*	20	7	126.2	31	45021	Q35490	Betaine-homocysteine S-methyltransferase 1
Band 7							
	6	5	81.76	25	28949	Q9CQQ7	ATP synthase B chain, mitochondrial precursor
	4	4	68.31	24	28049	Q9DD20	Methyltransferase-like protein 7B precursor
	10	7	115.1	45	27419	O08756	3-Hydroxyacyl-CoA dehydrogenase type-2
	7	5	81.99	19	27376	Q64471	Glutathione transferase theta 1
	5	4	75.85	26	25970	P10649	Glutathione transferase Mu 1
	5	5	82.59	26	25704	Q9DCM2	Glutathione transferase kappa 1
	5	3	52	22	25361	P30115	Glutathione transferase A3
	4	2	35.88	14	24871	O08709	Peroxioredoxin-6
	11	7	126.2	42	24275	Q9WVLO	Maleylacetoacetate isomerase
	2	2	31.26	14	24146	Q9CZM2	60S Ribosomal protein L15
	2	2	33.82	12	23897	Q91V41	Ras-related protein Rab-14
	2	2	28.29	10	23413	P35278	Ras-related protein Rab-5C
	4	3	47.31	16	23364	Q9DB20	ATP synthase subunit O, mitochondrial precursor
	3	2	30.6	11	22382	Q9CQC9	GTP-binding protein SAR1b
	6	6	82.85	29	22177	P35700	Peroxioredoxin-1
	6	6	102.5	33	21694	O55022	Membrane-associated progesterone receptor component 1
	2	2	37.64	13	21645	P35980	60S Ribosomal protein L18
*	4	3	44.52	10	39772	P00329	Alcohol dehydrogenase 1
*	5	2	34.37	9	35039	P25688	Uricase
*	4	2	33.51	12	41793	P63260	Actin, cytoplasmic 2
Band 8							
*	18	13	187.8	26	59753	Q03265	ATP synthase subunit $\alpha$ , mitochondrial precursor
Band 9							
*	2	2	32.77	9	39507	Q91Y97	Fructose-bisphosphate aldolase B
Band 10							
*	2	2	36.07	5	42120	P15105	Glutamine synthetase
*	4	4	49.1	18	22177	P35700	Peroxioredoxin-1

Received 27 May 2010/30 September 2010; accepted 2 November 2010  
 Published as BJ Immediate Publication 2 November 2010, doi:10.1042/BJ20100791