Additional file 1

Proteomic Analysis

Frozen liver sections from 12 Lp(a) transgenic and 12 wildtype mice were homogenized in Tri-reagent (Progenz, Auckland, New Zealand) containing 2% protease inhibitor cocktail (Roche) on ice. Proteins were precipitated with isopropanol and subsequently washed with ethanol and contaminants removed using a 2D clean-up kit (GE Healthcare). Liver proteins were resolubilised in 7M urea, 2M thiourea, 2% protease inhibitor cocktail, 1% ASB-14, 1% DTT, and 0.5% carrier ampholytes pH 3-10. The concentration of solubilised proteins was determined using the Ettan 2D Quant kit (GE Healthcare). For 2-D PAGE, 100 µg of protein was applied to a 75 mm IEF vertical tube gel (pH gradient, 3.5-10, nonlinear). The gels were focused for 7.4kVh at 20°C in a Mini-PROTEAN 2D electrophoresis system (Bio-Rad Laboratories). Following isoelectric focusing, the tube gels were equilibrated and then mounted onto 12.5% polyacrylamide gels in a Mini-PROTEAN III electrophoresis system (Bio-Rad Laboratories). Electrophoresis in the second dimension was performed at 100V until the dye-front reached the bottom of the gel. Following electrophoresis, gels were fixed in 20% methanol, 1.3% orthophosphoric acid and proteins then visualised by staining with colloidal Coomassie brilliant blue [1]. Stained gels were scanned with a calibrated densitometer (ImageScanner, GE Healthcare). The raw 2-D PAGE images were analysed with ImageMaster 2D platinum software (GE Healthcare). Raw spot volumes were log transformed and normalized by zero-centering through median subtraction before being subject to statistical analysis [2]. Spots exhibiting a statistical difference ($p<0.05$) were excised for identification by mass spectrometry.

Mass Spectrometry

Excised protein spots were digested with modified trypsin (Roche) as previously described [3]. Peptides were recovered by sequential extraction in 5% TFA and 5% acetronitrile and desalted on Zip tips (Millipore). Samples were analysed on a 4800 MALDI-TOF/TOF MS analyzer (ABSciex, Foster City, Ca) in positive ion reflector

mode with alpha cyano-4-hydroxycinnamic acid (CHCA) as the matrix. The 15-20 strongest precursor ions of each spot were used for MS/MS collision-induced dissociation analysis. The resulting spectra were processed through the GPS Explorer software (ABSciex, Foster City, Ca) and searched against the SwissProt database using the MASCOT search engine. The search allowed for up to 4 missed cleavage sites, a mass tolerance threshold of 75 ppm and a maximum fragment mass error of 0.4 Da. Cysteine carbamidomethylation and methionine oxidation were selected as variable modifications. Individual MS/MS peptide ion scores with a confidence interval calculated by the GPS Explorer software of >95% were accepted as significant. In some cases, multiple identifications for one protein spot were gained due to the search identifying homologues of the mouse protein.

References

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2. Meleth S, Deshane J, Kim H: The case for well-conducted experiments to validate statistical protocols for 2D gels: different pre-processing = different lists of significant proteins. *BMC Biotechnol* 2005, 5:7.
3. Shevchenko A, Jensen ON, Podtelejnikov AV, Sagliocco F, Wilm M, Vorm O, Mortensen P, Boucherie H, Mann M: Linking genome and proteome by mass spectrometry: large-scale identification of yeast proteins from two dimensional gels. *Proc Natl Acad Sci U S A* 1996, 93(25):14440-14445.

Figure S1. Plasma lipid profiles of wildtype and Lp(a) mice. Plasma lipoproteins from wildtype and Lp(a) mice (n=20) were separated by gel permeation chromatography and the triglyceride content of each fraction measured. The Lp(a) mice had TG-rich LDL particles, whereas the plasma TG in the wildtype mice was largely all in the VLDL.

Figure S2. 2D-PAGE replicates of wildtype mouse liver

Figure S3. 2D-PAGE replicates of Lp(a) mouse liver

Table S1. Proteins identified by MALDI-TOF MS/MS from the livers of wildtype and Lp(a) mice on a normal chow diet

Spot number	Identified Protein (Swiss-Prot Accession Number)	Masses Matched (C.I. > 95%)	Three Highest Ranked Peptide Sequences (C.I. > 95%)	Total Ion Score
1	*Park7* Protein DJ-1 (Q99LX0)	3	TQGPYDVVVLPGGNLGAQNLSESPMVK GPGTSFEFALAIVEALVGK GAEEMETVIPVDVMR	291
2	*Prdx6* Peroxiredoxin 6 (O08709)	5	FHDFLGDSWGILFSHPR LIALSIDSVEDHLAWSK LPFPIIDDK	488
3	*Prdx6* Peroxiredoxin 6 (O08709)	10	PGGLLLGDEAPNFEANTTIGR FHDFLGDSWGILFSHPR LIALSIDSVEDHLAWSK	798
4	*Prdx6* Peroxiredoxin 6 (O08709)	10	PGGLLLGDEAPNFEANTTIGR FHDFLGDSWGILFSHPR LIALSIDSVEDHLAWSK	733
5	*Gpx1* Glutathione peroxidase 1 (Q5RJH8)	5	YVRPGGGFEPNFTLFEK FLVGPDGVPVR NDIAWNFEK	305
6	*Sod1* Superoxide dismutase [Cu-Zn] (P08228)	6	VISLSGEHSIIGR GDGPVQGTIHFEQK HVGDLGNVTAGK	435
7	*Sod1* Superoxide dismutase [Cu-Zn] (P08228)	2	VISLSGEHSIIGR KHGGPADEER	169
8	*Acaa2* 3-ketoacyl-CoA thiolase, mitochondrial (Q8JZR8)	2	DGTVTAGNASGVSDGAGAVIIASEDAVKKHNFTPLAR TPFGAYGGLLKDFSATDLTEFAAR	229

9	*Echs1* Enoyl-CoA hydratase, mitochondrial (Q8BH95)	1	AQFGQPPEILLGTIPGAGGTQR	133
10	*Apoa4* Apolipoprotein A-IV (P06728)	1	ALVQQLEQFR	111
11	*Apoa1* Apolipoprotein A-I (Q00623)	4	LAELKSNPTLNEYHTR VAPLGAELQESAR WKEDVELYR	358
12	*Fabp4* Fatty acid-binding protein (P04117)	9	LVSSENFDDYMKEVGVGFATR SIITLDGGALVQVQK LGVEFDEITADDRK	922
13	*Serpinc1* Antithrombin III (P32261)	2	AFLEVNEEGSEAAASTSVVITGR LQPLDFKENPEQSR	104
14	*Ywhag* 14-3-3 protein gamma (P61982)	2	NVTELNEPLLSNEERNLLSVAYKNVVGAR NLLSVAYKNVVGAR	
15	*Pebp1* Phosphatidylethanolamine-binding protein 1 (P70296)	4	GNDISSGTVLSDYVGSGPPSGTGLHR VDYAGVTVDELGK LYTLVLTDPDAPSR	455
16	*Ca3* Carbonic anhydrase 3 (P16015)	5	YAAELHLVHWNPK QFHLHWGSSDDHGSEHTVDGVK VVFDDTYDR	367
17	*Glo1* Lactoylglutathione lyase (Q9CPU0)	6	GLAFIQDPDGYWIELNPNK FSLYFLAYEDKNDIPK DFLLQQTMLR	435

18	*Uqcrc1* Cytochrome b-c1 complex subunit 1, mitochondrial (Q00896)	3	EVESIGAHLNAYSTR MVLAAAGGVEHQQLLDLAQK TDLTDYLNR	239
19	*Cyb5a* Cytochrome b5 (P56395)	5	EQAGGDATENFEDVGHSTDAR FLEEHPGGEEVLR TYIIGELHPDDR	442
20	*Atp5h* ATP synthase subunit d, mitochondrial (Q9DCX2)	6	IPVPEDKYTALVDQEEKEDVK LASLSEKPPAIDWAYYR ANVAKPGLVDDFEKK	496
21	*Ppa1* Inorganic pyrophosphatase (Q9D819)	4	VIAINVDDPDAANYK DVFHMVVEVPR GQYISPFHDVPIYADK	370
22	*Haao* 3-hydroxy anthranilate 3,4-dioxygenase (Q78JT3)	8	DLGTQLAPIIQEFFHSEQYR AQSVALSTQDPAR FANTMGLVIER	555
23	*Pnpo* Pyridoxine-5'-phosphate oxidase (Q91XF0)	2	EAENYFHSRPK FFTNYESR	146
24	*Tpt1* Translationally-controlled tumour protein (P63028)	3	DLISHDELFSDIYK GKLEEQKPER EDGVTPFMIFFK	226
25	*Pdia3* Protein disulfide isomerase A3 (P27773)	8	FISDKDASVVGFFR ALEQFLQEYFDGNLK DLLTAYYDVDYEK	572
26	*Hspd1* 60 kDa heat shock protein, mitochondrial (P63038)	9	DMAIATGGAVFGEEGLNLNLEDVQAHDLGK LVQDVANNTNEEAGDGTTTATVLAR ALMLQGVDLLADAVAVTMGPK	904

27	*Pbld2* Phenazine biosynthesis-like domain-containing protein 2 (Q9CXN7)	5	GLILTVKGEPGGQTALYDFYSR KLQPTDDFTQSSR TLDEDAHQDIAR	432