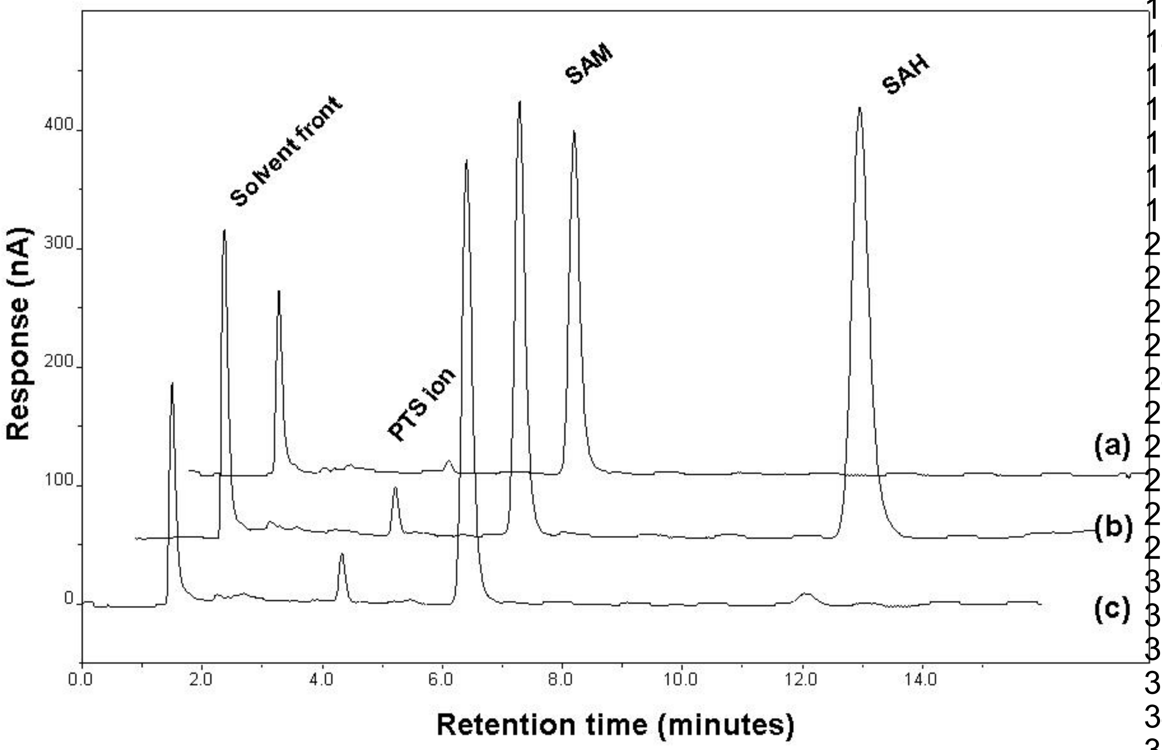
Supplemental Information Section for Andringa…Bailey. Analysis of the Liver Mitochondrial Proteome in Response to Ethanol and S-Adenosylmethionine Treatments: Novel Molecular Targets of Disease and Hepatoprotection.

SAM quality control. Figure shows a representative HPLC analysis of the SAM used in these feeding studies compared to purified SAM and SAH standards. As shown, the SAM used for these feeding studies was pure and contained no contaminating levels of SAH [compare chromatogram line a (SAM alone) with line b (SAM standard + SAH standard)]. This quality control experiment is important as studies have shown that SAH sensitizes liver to TNFα thereby enhancing alcohol hepatotoxicity (58).

Figure 1.



Supplement Figure 1. HPLC analysis of SAM. SAM used in feeding studies and standards was prepared and analyzed as discussed in methods section. Representative chromatograms are shown with line (a) 2 nmol SAM, used in feeding studies. Line (b) is a mixture of 2 nmol SAM standard and 100 µM SAH standard and line (c) is 2 nmol SAM standard. The first peak in each chromatogram at 1.52 min shows the solvent front. The second peak in each chromatogram at 4.32 min is the PTS ion (sulfate tosylate). The third peak in each chromatogram at 6.40 min is SAM. The fourth peak present in line (b) at 13.07 min is SAH. These results demonstrate no contaminating SAH in SAM sample used for animal feeding studies.

Supplement Table 1. Supplemental table listing all matched proteins from 2D IEF/SDS-PAGE gels.

Spot #	Spot Designation	Mass (kDa)	MOWSE Score	Matched Peptides	Accession Number
	Chaperones				
1	Protein disulfide isomerase/T3 binding protein	54.0	209	16	gi|202549
2	Hspd1 protein	60.9	163	16	gi|1334284
3	Heat shock 70 kDa protein 5	72.3	191	18	gi|38303969
4	Prohibitin	27.7	106	10	gi|62664759
5	Hspd1 protein	60.9	166	16	gi|1334284
6	Protein disulfide isomerase A3 precursor	56.6	92	11	gi|56905
7	Stress-70 protein, mitochondrial (GRP75)	73.8	107	12	gi|55584140
8	Glucose regulated protein, 58 kDa	56.5	217	24	gi|8393322
9	Stress-70 protein, mitochondrial precursor	73.8	178	19	gi|55584140
	Fatty Acid Metabolism				
10	Enoyl-CoA hydratase 1, peroxisomal	36.1	89	7	gi|38566122
11	Acetyl-CoA dehydrogenase, long-chain	47.8	141	13	gi|38541337
12	Acetyl-CoA dehydrogenase, long-chain	47.8	82	10	gi|38541337
13	Chain F, Enoyl-CoA hydratase in complex with hexadienoyl-CoA	28.2	94	6	gi|24159086
14	Chain B, Acyl-CoA dehydrogenase in complex with acetoacetyl-CoA	42.1	92	9	gi|20150495
15	Carnitine palmitoyltransferase 2	74.1	128	10	gi|6978705
16	Chain F, Enoyl-CoA hydratase in complex with hexadienoyl-CoA	28.2	90	7	gi|24159086
17	Electron transfer flavoprotein α	34.9	158	14	gi|57527204
18	Acetyl-CoA dehydrogenase, medium chain	46.5	119	11	gi|8392833
19	Electron transfer flavoprotein β	27.7	85	5	gi|51259425
20	Acetyl-CoA dehydrogenase, medium chain	46.5	140	12	gi|8392833
21	L-3-Hydroxyacyl-CoA dehydrogenase	34.4	104	14	gi|17105336
22	Acetyl-CoA acyltransferase 2	41.9	208	18	gi|55544
23	Hadh protein (trifunctional protein)	82.6	127	13	gi|60688124
	Ligases				
24	Suclg2 protein	46.5	97	8	gi|71681082

25	Suclg2 protein	46.5	171	16	gi|71681082
26	Unidentified	*			
27	Propionyl-CoA carboxylase alpha chain, mitochondrial precursor	77.7	91	13	gi|109502895
28	Carbamoyl phosphate synthetase 1, mitochondrial	164.5	189	21	gi|8393186
29	Carbamoyl phosphate synthetase 1, mitochondrial	164.5	266	36	gi|8393186
30	Pyruvate carboxylase	129.7	207	27	gi|55716041
31	Carbamoyl phosphate synthetase 1, mitochondrial	164.5	321	44	gi|8393186
32	Carbamoyl phosphate synthetase 1, mitochondrial	164.5	312	39	gi|8393186
	Oxidative Phosphorylation				
33	Ubiquinol-cytochrome *c* reductase core protein I	52.8	109	7	gi|51259340
34	ATP synthase, H^+ transporting F1 complex β subunit	56.4	163	17	gi|1374715
35	ATP synthase, H^+ transporting F1 complex β subunit	56.4	191	17	gi|1374715
36	Adenylate kinase 2, isoform b	25.5	101	8	gi|38511598
37	Chain A, Rat Liver F1-ATPase	55.3	148	14	gi|6729934
38	Chain A, Rat Liver F1-ATPase	55.3	199	18	gi|6729934
39	Ubiquinol-cytochrome *c* reductase, Rieske iron-sulfur polypeptide 1	29.3	75	5	gi|18044191
	Oxidoreductases				
40	Pyruvate dehydrogenase β subunit	39.0	106	7	gi|50925725
41	Mitochondrial aldehyde dehydrogenase	56.4	171	11	gi|25990263
42	Mitochondrial aldehyde dehydrogenase	56.4	205	16	gi|25990263
43	Sarcosine dehydrogenase	101.4	227	21	gi|25742657
44	3-Hydroxyisobutyrate dehydrogenase	35.3	128	9	gi|83977457
45	Isovaleryl CoA dehydrogenase	46.4	128	10	gi|6981112
46	Aldehyde dehydrogenase 1 family, member B1	57.6	102	10	gi|58865518
47	Dimethylglycine dehydrogenase	96.0	185	17	gi|2498527
48	Alcohol dehydrogenase, iron containing, 1	50.2	84	8	gi|67678029
49	Dihydrolipoamide dehydrogenase	54.0	143	10	gi|38303871
50	Glutamate dehydrogenase 1, mitochondrial precursor (GDH) isoform 2	61.4	144	15	gi|6980956
51	Dihydrolipoamide dehydrogenase	54.0	130	13	gi|38303871

52	Glutamate dehydrogenase 1, mitochondrial precursor (GDH) isoform 2	61.4	83	10	gi|6980956
53	Aldehyde dehydrogenase family 6, subfamily A1	57.8	158	14	gi|13591997
54	Aldehyde dehydrogenase family 6, subfamily A1	57.8	107	8	gi|13591997
55	Chain D, 3-Hydroxyacyl-CoA deHase Binary Complex (NADH & Estradiol)	27.2	103	8	gi|14488727
56	3-Hydroxybutyrate dehydrogenase, type 1	38.2	83	7	gi|55250770
57	Malate dehydrogenase, mitochondrial precursor	35.6	216	16	gi|38648863
	Transferases				
58	3-Mercaptopyruvate sulfurtransferase	32.9	104	7	gi|55824737
59	Ornithine carbamoyltransferase	39.9	136	10	gi|205886
60	Thiosulfate sulfurtransferase	33.4	88	6	gi|57528682
61	Serine hydroxymethyl transferase 2 (mitochondrial)	55.7	98	14	gi|55154499
62	Thiosulfate sulfurtransferase	33.4	81	6	gi|57528682
63	Thiosulfate sulfurtransferase	33.4	93	8	gi|57528682
64	3-Hydroxy-3-methylglutaryl-CoA synthase 2	56.9	113	14	gi|27465521
65	Acetyl-CoA acetyltransferase 1	44.7	95	7	gi|8392836
66	Acetyl-CoA acetyltransferase 1	44.7	74	6	gi|8392836
67	Glutamate oxaloacetate transaminase 2	47.3	98	8	gi|38197424
68	Aspartate aminotransferase, mitochondrial precursor	47.4	87	7	gi|192050
	Alternate Activities				
69	Choline dehydrogenase	66.2	169	17	gi|34419913
70	Agmatinase	37.8	73	6	gi|60688189
71	Alpha-Methylacyl-CoA racemase	41.8	97	8	gi|6981184
72	Catalase	59.7	234	24	gi|51980301
73	Tu translation elongation factor, mitochondrial	49.5	127	10	gi|109462848
74	Tu translation elongation factor, mitochondrial	49.5	89	8	gi|109462848
75	Actin, cytoplasmic 2	41.8	154	10	gi|109507063
76	Catalase	59.7	163	23	gi|51980301
	Aldehyde dehydrogenase 4 family, member A1	61.7	163		gi|109475727

Mitochondrial proteins listed above were matched on all gels from the control, ethanol, control + SAM, and ethanol + SAM. These samples were broadly categorized into functional classes. Spot # is the same as those used to identify protein spots in the following location map. Mass is measured in kDa. MOWSE is an algorithmic calculation used to assign a statistical weight to each peptide match; therefore, the higher MOWSE score implies higher statistical likelihood of the match being correct. Accession number is the unique NCBI label given to proteins. Matched proteins are the number of peptides that were matched to allow for protein designation and MOWSE calculation.

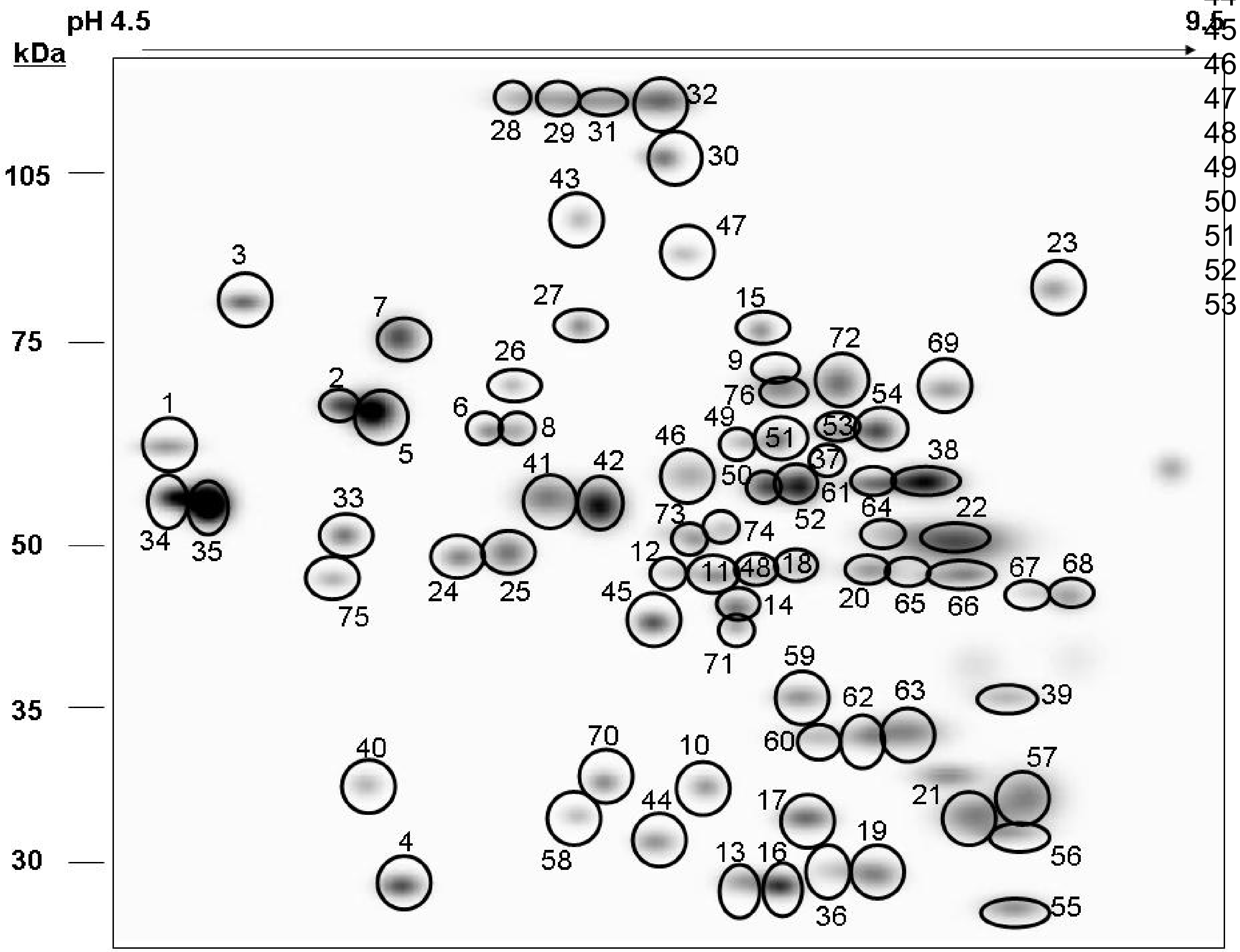


Figure 2. Master map of all proteins identified by mass spectrometry techniques. See supplemental Table 1 for protein ID's.

Supplemental Table 2. Supplemental table listing all matched proteins from 2D BN Page gels.

Spot #	Spot Designation	Mass (kDa)	MOWSE Score	Matched Peptides	Accession Number
1	Propionyl-CoA carboxylase α chain	79.8	272	37	gi66773933
	NADH dehydrogenase Fe-S Protein 1	79.7	272	37	gi74147040
2	NADH dehydrogenase flavoprotein 1	50.8	176	27	gi19526814
	ATP synthase, H+ transporting F1 complex β subunit	56.3	176	27	gi89574015
3	NADH dehydrogenase Fe-S protein 2	53.3	171	19	gi23346461
4	NADH dehydrogenase 1 α subcomplex 10	40.6	160	12	gi26337977
5	NADH dehydrogenase flavoprotein 2	27.3	80	7	gi110625954
6	NADH dehydrogeanse 1 α subcomplex 8	20.0	102	7	gi21312012
7	NADH dehydrogenase1 α subcomplex subunit 12	17.1	108	9	gi47117166
8	NADH dehydrogenase 1 β subcomplex 4	15.1	94	7	gi21314826
9	NADH dehydrogenase Fe-S protein 6	13.0	105	7	gi56711244
10	ATP synthase, H+ transporting F1 complex α subunit	59.7	167	25	gi6680748
	ATP synthase H+ transporting F1 complex β subunit	56.3	167	25	gi74198645
11	ATP synthase γ chain	32.9	106	8	gi21263432
12	ATP synthase H+ transporting F0 complex subunit b isoform1	28.9	189	11	gi78214312
13	ATP synthase H+ transporting F0 complex subunit d	18.7	102	8	gi21313679
14	Cytochrome *c* oxidase subunit IV isoform1	19.5	74	7	gi6753498
15	ATP synthase H+ transportingF0 complex subunit g	11.4	81	7	gi31980744
16	Mitochondrial trifunctional protein α subunit	82.6	93	17	gi33859811
17	60 kDa heat shock protein, mitochondrial	61.0	99	13	gi129378
18	Ubiquinol-cytochrome *c* reductase, complex core protein 1	52.7	94	12	gi14548301
19	Ubiquinol-cytochrome *c* reductase, core protein 2	48.2	98	10	gi22267442
20	Cytochrome *c*1	35.3	114	8	gi74213069
21	Ubiquinol-cytochrome *c* reductase, Rieske Fe-S polypeptide 1	29.3	86	6	gi13385168
22	Ubiquinol-cytochrome *c* reductase, subunit 7 (14 kDa)	13.5	104	9	gi17380333
23	Ubiquinol-cytochrome *c* reductase, subunit 8	9.8	84	5	gi21539585
24	Ubiquinol-cytochrome c reductase, subunit protein 9	7.4	89	4	gi37574048
25	2-Hydroxyacyl-CoA lyase	63.6	96	18	gi31560355
26	Electron transferring flavoprotein β polypeptide	27.6	154	12	gi12832367

27	Cytochrome *c* oxidase, subunit II	25.8	90	10	gi1706015
28	Unidentified	*			
29	Cytochrome *c* oxidase, subunit IV isoform 1	19.5	140	13	gi6753498
30	Cytochrome *c* oxidase, subunit VA	16.0	95	6	gi117099
31	Cytochrome *c* oxidase, subunit VIa	12.3	88	5	gi1352173
32	Cytochrome *c* oxidase, subunit VIb	10.1	97	6	gi13385090
33	Unidentified	*			

Mitochondrial proteins listed above were matched on all gels from the control, ethanol, control + SAM, and ethanol + SAM. These samples were categorized to the location on the gel in relation to OxPhos complexes. Spot # is the same as those used to identify protein spots in Figure 6A. Mass is measured in kDa. MOWSE is an algorithmic calculation used to assign a statistical weight to each peptide match; therefore, the higher MOWSE score implies higher statistical likelihood of the match being correct. Accession number is the unique NCBI label given to proteins. Matched proteins are the number of peptides that were matched to allow for protein designation and MOWSE calculation.

*Unidentified proteins