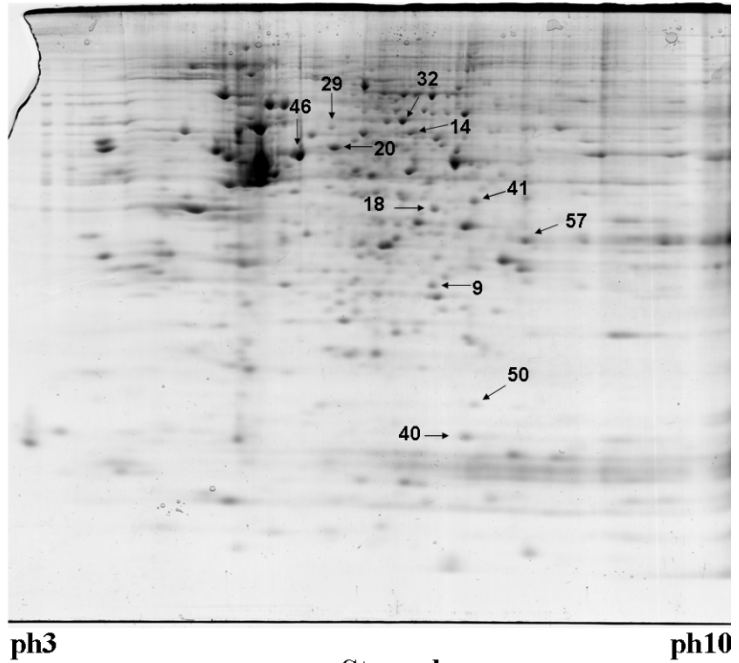
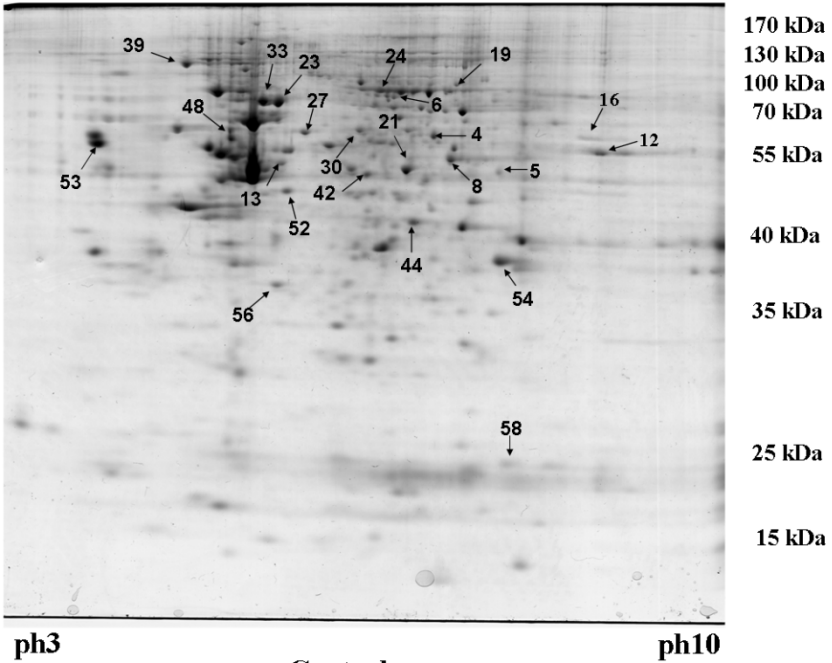


## Supplemental Information

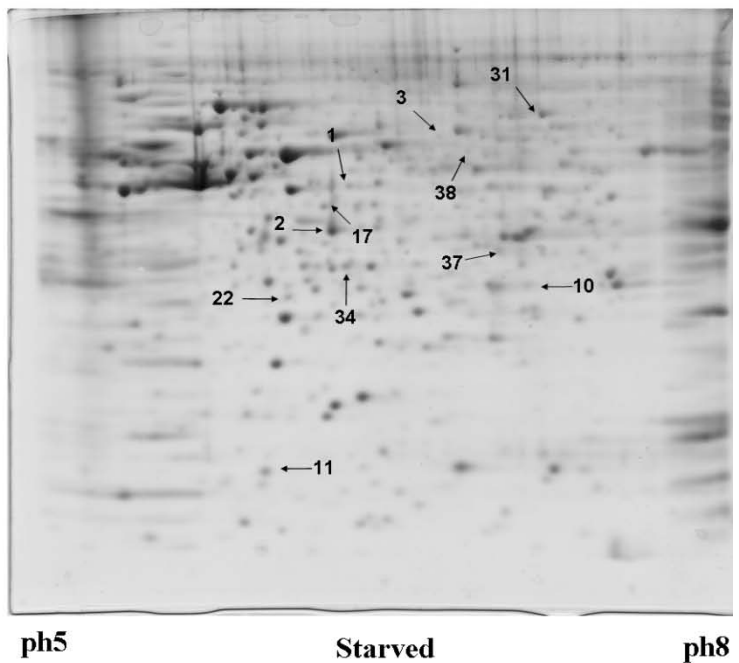
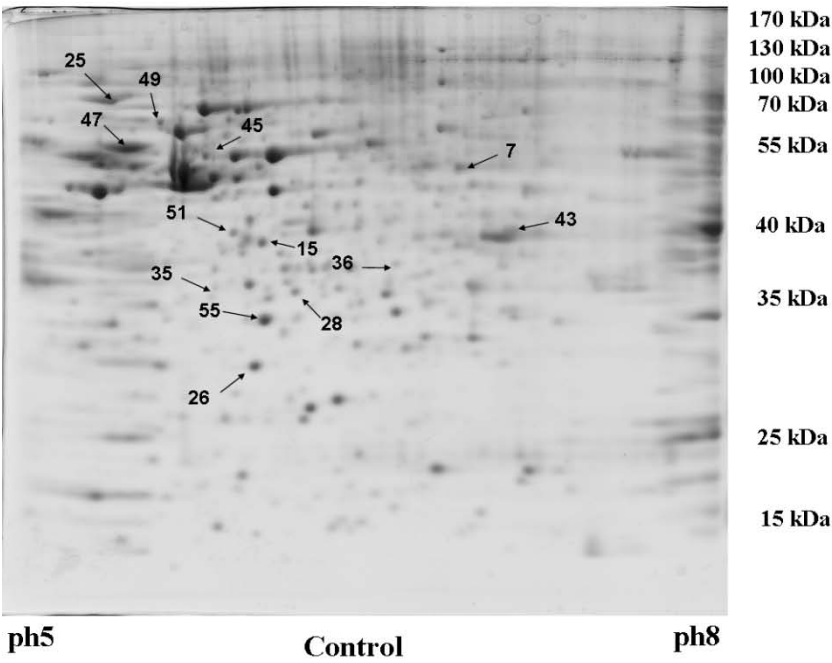
**Figure S1. The proteomic alterations of HCT116 following starvation.** HCT116 cells were incubated with complete medium (Control) or EBSS (Starved) for 2 h. Proteins from whole cell lysates were separated on a pH 3–10 IPG strip (A) or pH 5–8 IPG strip (B) in the first dimension and on a 12% SDS-PAGE gel in the second dimension. The gels were stained with Coomassie Blue. Protein spots significantly affected by starvation are marked by arrows. The numbers are corresponding to those listed in supplemental Table 1.

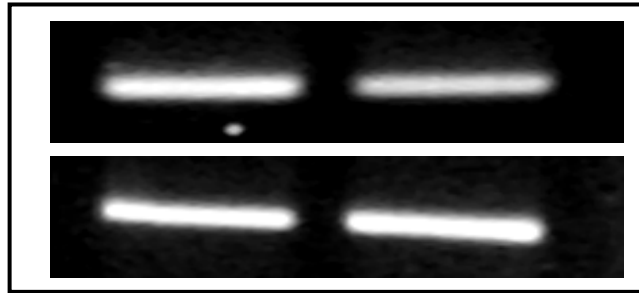
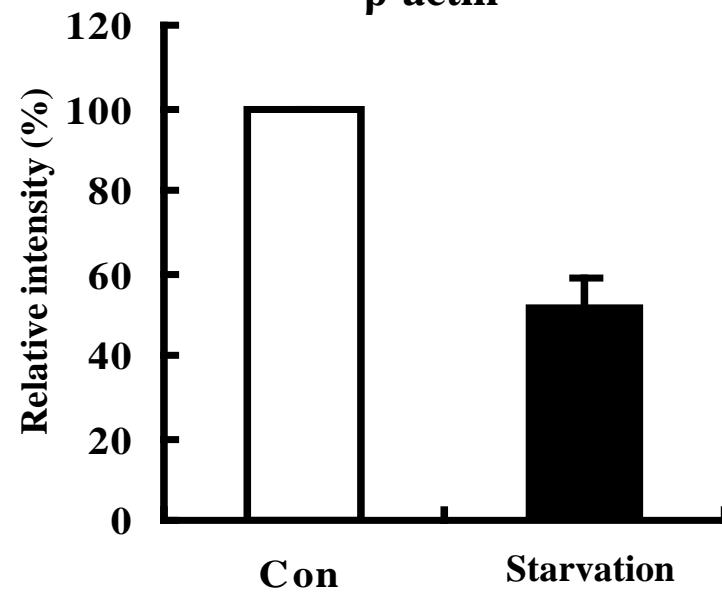
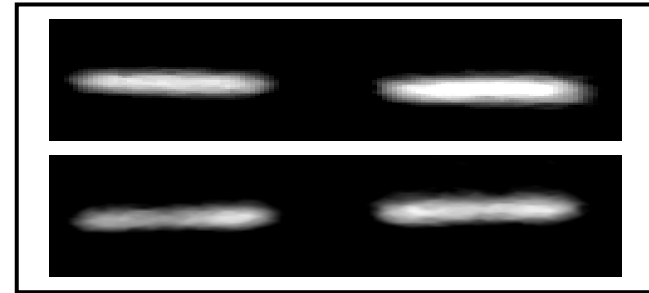
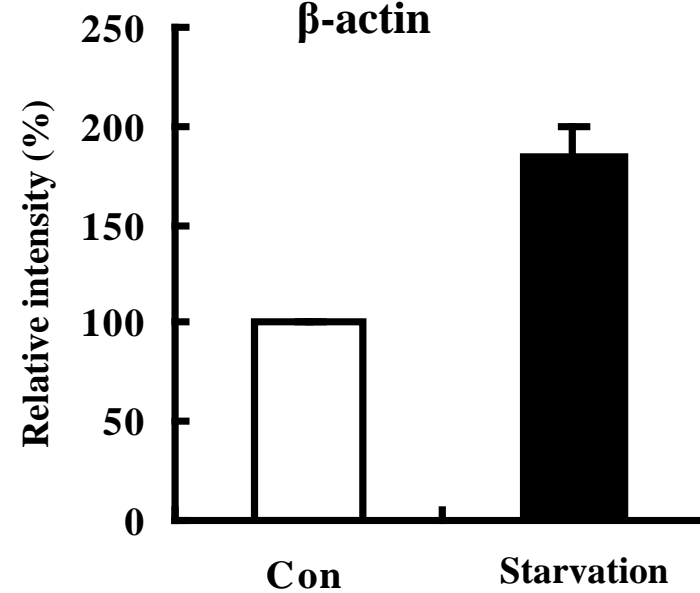
**Figure S21. Verification of the change of expression levels.** (A-D). The expression levels of prohibitin (A), phosphoglycerate mutase 1 (B), VDAC (C) and HSP70 (D) in HCT116 cells cultured in complete medium (Con) or EBSS (Starvation) were assessed by RT-PCR (A-B), or by Western blot (C-D).  $\beta$ -actin was used as the control. The intensity of the PCR products (A-B) or immunoreactive bands (C-D) were determined and expressed. Primer sets for prohibitin is GGA GGC GTG GTG AAC TCT G (forward), and CTG GCA CAT TAC GTG GTC GAG (reverse), for phosphoglycerate mutase 1 is GTG CAG AAG AGA GCG ATC CG (forward) and CGG TTA GAC CCC CAT AGT GC (reverse), for b-actin is AGA AAA TCT GGC ACC ACA CC (forward), and CTC CTT AAT GTC ACG CAC GA (reverse). (E). The reduced expression level of aldehyde dehydrogenase (circled) in starved cells as indicated by the Coomassie blue staining of the 2-D electrophoresis gel was confirmed by the enzymatic activity. Cells were sonicated in 50 mM potassium phosphate buffer (pH 9.0) and the supernatant was mixed with the reaction buffer (50mM potassium phosphate buffer, pH 9.0 containing 1mM EDTA, 500  $\mu$ M NAD<sup>+</sup> and 1mM propionaldehyde) at 27°C, which led to the formation of NADH, which was determined by the absorbance at 340 nm. One unit of enzyme activity was defined as the amount of enzyme required to reduce 1  $\mu$ mol of NAD<sup>+</sup> per min at 27 °C. The extinction coefficient for NADH was 6.22 mM<sup>-1</sup> cm<sup>-1</sup>.

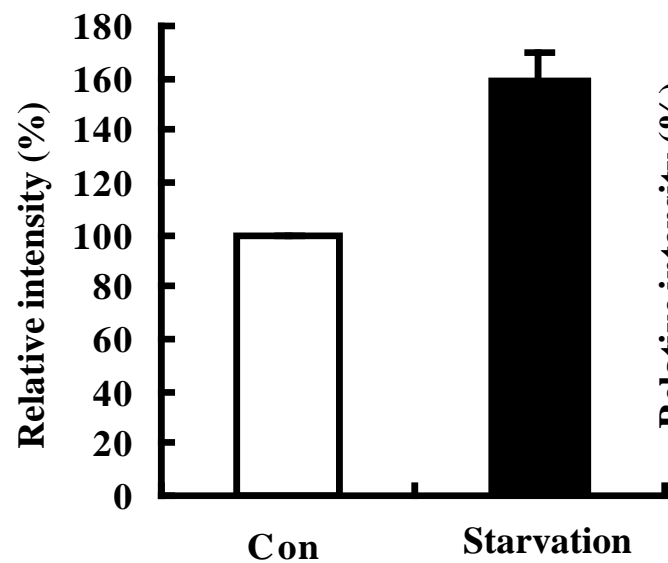
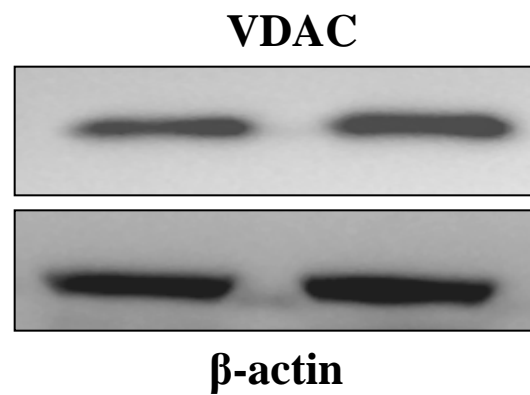
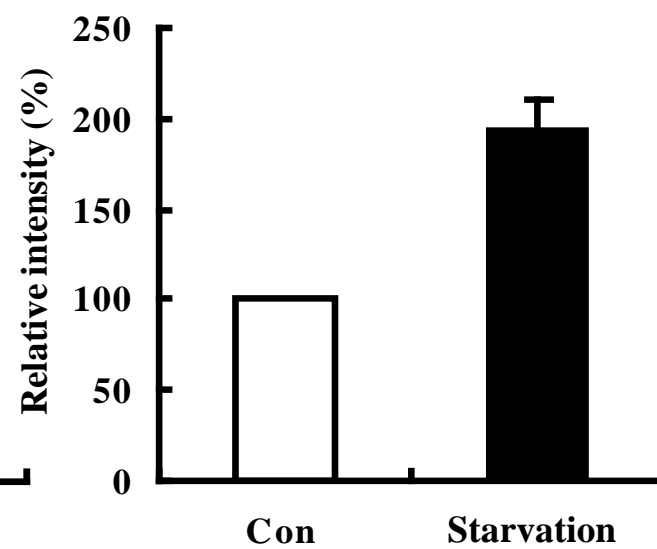
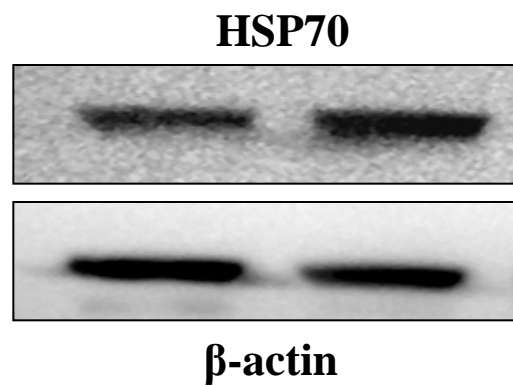
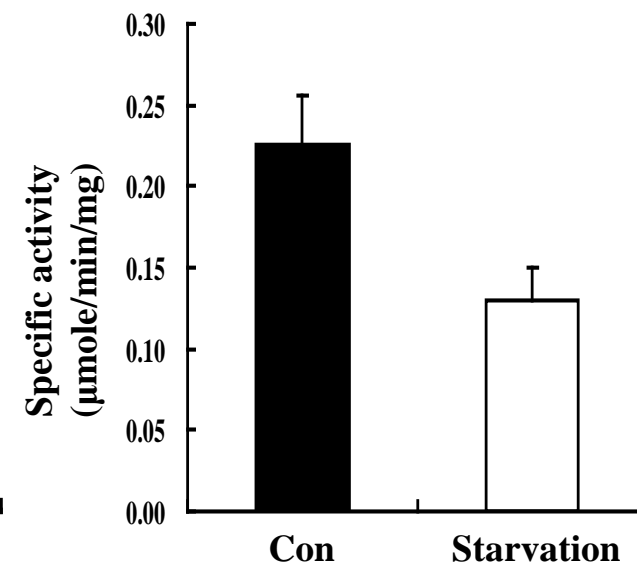
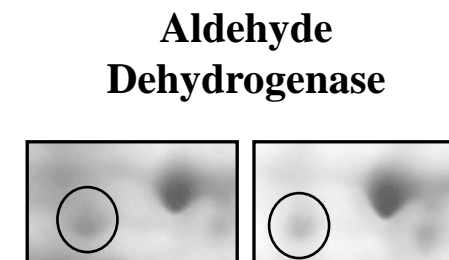
**A**



**B**



**A****Prohibitin** $\beta$ -actin**B****Phosphoglycerate mutase 1** $\beta$ -actin

**C****D****E**

**Supplemental Table 1. Differentially expressed proteins in HCT 116 cells following starvation**

Number of Spot	Name	Accession No.	Function	Localization	Fold of Change	Obs. MW(kDa)	Obs. PI	Tho. MW(kDa)	Tho. PI	score	Sequence coverrang (%)
<b>Carbohydrate Metabolism</b>											
1	L-lactate dehydrogenase B chain	P07195	Glycolysis	Cytoplasm	0.6	42	6.3	36	5.7	6737	26.9
2	L-lactate dehydrogenase B chain	P07195	Glycolysis	Cytoplasm	0.59	36	6.25	36	5.7	1.88E+06	22.2
3	Aldehyde dehydrogenase	P30837	Carbohydrate metabolic	Mitochondrion	0.4	58	6.7	57	6.4	830211	31
4	Dihydrolipoyl dehydrogenase	P09622	Carbohydrate metabolic	Mitochondrion	0.6	54	7	54	7.6	721	14.1
5	Fumarate hydratase	P07954	Carbohydrate metabolic	Mitochondrion	0.59	44	7.4	54	8.8	29764	21.2
6	Glycerol-3-phosphate dehydrogenase	P43304	Glycerol metabolism	Mitochondrion	0.6	80	6.6	80	7.2	221515	18.3
7	Alpha-enolase	P06733	Glycolysis	Cytoplasm	1.89	49	6.4	47	7	7818	20.3
8	Alpha-enolase	P06733	Glycolysis	Cytoplasm	1.71	46	7	47	7	4.31E+05	28.8
9	Phosphoglycerate mutase 1	P18669	Glycolysis	Cytoplasm	2.63	29	6.8	28	6.7	9837	32.7
10	Triosephosphate isomerase	P60174	Carbohydrate metabolic	Cytoplasm	1.67	26	7.1	26	6.4	2348	50.6
<b>Other Metabolism</b>											
11	ATP synthase D chain	O75947	ATP synthesis	Mitochondrion	0.59	20	5.6	18	5.2	46606	72
12	ATP synthase subunit alpha	P25705	ATP synthesis	Mitochondrion	0.6	55	8.6	59	9.2	4.18E+04	20.1
13	Ubiquinol-cytochrome-c reductase complex core protein 1	P31930	Mitochondrial respiration	Mitochondrion	2.16	52	5.5	52	5.9	1.94E+03	27.5
14	Inosine-5'-monophosphate dehydrogenase 2	P12268	Purine metabolism	Cytoplasm	1.6	56	6.5	55	6.4	22045	22.4
15	Inorganic pyrophosphatase	Q15181	Phosphate metabolic	Cytoplasm	1.59	32	6	32	5.5	931	32.9
16	Serine hydroxymethyltransferase	P34897	Carbon metabolism	Mitochondrion	1.57	58	8.5	55	8.8	5356	15.9
<b>RNA processing</b>											
17	60S acidic ribosomal protein	P05388	Translation	Cytoplasm	0.58	34	6.2	34	5.7	81449	35.6

18	Poly(rC)-binding protein 1	Q15365	Transcription	Nucleus	1.83	38	6.9	37	6.7	5.92E+05	13.4
19	Far upstream element-binding protein	Q92945	Transcription	Nucleus	1.54	74	7.3	73	6.8	1.27E+04	14.1
20	Heterogeneous nuclear ribonucleoprotein H	P31943	Transcription	Nucleus	1.57	49	6	49	5.9	2.91E+03	15.4
21	mRNA turnover protein 4 homolog	Q9UKD2	Transcription	Nucleus	1.6	48	6.7	27	8.3	97	13.8

### Protein Folding and Stress Response

22	Heat shock protein beta-1	P04792	Protein folding	Cytoplasm	0.6	26	6.1	22	6	904	27.8
23	Stress-70 protein	P38646	Protein folding	Mitochondrion	0.57	70	5.4	73	5.9	2120	19.3
24	Heat shock protein 75 kDa	Q12931	Protein folding	Mitochondrion	0.58	80	6.6	80	8.3	1302	21
25	78 kDa glucose-regulated protein	P11021	Unfolded protein response	Endoplasmic reticulum	0.56	75	5.4	72	5.1	1137	15.7
26	Glutathione S-transferase P	P09211	Redox regulation	Cytoplasm	0.6	25	6.25	23	5.4	2912	26.2
27	Protein disulfide-isomerase A3	P30101	Redox regulation	Endoplasmic reticulum	0.6	53	5.8	56	6	73614	18
28	Peroxiredoxin-4	Q13162	Redox regulation	Cytoplasm	0.58	27	6.2	30	5.9	8474	28
29	T-complex protein 1 subunit alpha	P17987	Protein folding	Cytoplasm	5.33	63	6.1	60	5.8	681	13.8
30	T-complex protein 1 subunit beta	P78371	Protein folding	Cytoplasm.	1.5	54	6.2	57	6	1430	20.4
31	T-complex protein 1 subunit zeta	P40227	Protein folding	Cytoplasm	2	62	7.1	58	6.2	552	14.7
32	Stress-induced-phosphoprotein 1	P31948	Protein folding	Golgi apparatus	1.62	63	6.5	62	6.4	525	21
33	Heat shock cognate 71 kDa protein	P11142	Protein folding	Cytoplasm	1.82	70	5.3	70	7.4	20024	13.5

### Proteolysis

34	Proteasome activator complex subunit 1	Q06323	proteolysis	Proteasome	0.6	28	6,2	28	5.8	82581	42.6
35	Proteasome activator complex subunit 2	Q9UL46	proteolysis	Proteasome	0.35	29	5.8	27	5.4	229436	42.3
36	Proteasome subunit alpha type-1	P25786	proteolysis	Proteasome	0.6	32	6.75	29	6.1	1826	27
37	Proteasome subunit alpha type-1	P25786	proteolysis	Proteasome	2.56	28	7	29	6.1	1731	28.9
38	26S proteasome non-ATPase regulatory subunit 11	O00231	proteolysis	Proteasome	1.5	46	6.4	47	6.1	6199	24.6

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**Transport and Trafficking**

39	Endoplasmic precursor	P14625	Transport	endoplasmic reticulum	0.59	100	4.6	92	4.8	1.56E+03	9.8
40	Golgi SNAP receptor complex member 1	O95249	Transport	Golgi apparatus	2.13	12	7.2	28	9.5	5.93	19.6
41	Probable mitochondrial import receptor subunit TOM40	O96008	Transport	Mitochondrion	1.59	36	7.3	37	6.8	1136	18.6
42	Voltage-dependent anion-selective channel protein 2	P45880	Transport	Mitochondrion	1.6	30	7.6	31	7.5	4.71E+06	45.6
43	Annexin A1	P04083	Trafficking	Cytoplasm	3.67	33	7.2	38	6.6	68970	41.3
44	Annexin A1	P04083	Trafficking	Cytoplasm	3.46	33	6.5	38	6.6	8172	25.4

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**Cytoskeleton-related**

45	Cytokeratin-8	P05787	Structural constituent	Cytoplasm	0.58	54	5.7	53	5.5	1305	31
46	Cytokeratin-8	P05787	Structural constituent	Cytoplasm	1.7	52	5.5	53	5.5	7.51E+03	10.8
47	Tubulin alpha-1A chain	Q71U36	Structural constituent	Cytoskeleton	0.59	55	5.6	50	4.9	4516	15.7
48	Tubulin alpha-1A chain	Q71U36	Structural constituent	Cytoskeleton	1.65	52	4.9	50	4.9	1.42E+05	20.2
49	Lamin-B1	P20700	Structural constituent	Nucleus	0.52	77	5.6	66	5.1	5942	25.4
50	Cofilin-1	P23528	Structural constituent	Cytoplasm	1.5	15	7	18	8.2	392	30.7
51	F-actin-capping protein subunit alpha-1	P52907	Structural constituent	Cytoskeleton	4.3	33	5.9	32	5.4	107884	39.5

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**Cell signaling**

52	Protein phosphatase 1A	P35813	Cell cycle	Cytoplasm	0.38	40	5.5	42	5.2	61	3.7
53	Tripartite motif-containing protein 55	Q9BYV6	Signal transduction	Cytoplasm	0.54	55	4	60	4.7	1.10E+01	9.9
54	Proliferation-associated protein	Q9UQ80	Cell cycle	Nucleus	1.53	45	6.3	43	6.1	3580	24.4
55	Prohibitin	P35232	Cell growth	Mitochondrion	0.55	27	6	29	5.6	1.25e+8 16	58
56	Prohibitin	P35232	Cell growth	Mitochondrion	0.6	27	5.5	29	5.6	7609	37.5
57	Annexin A2	P07355	Cell cycle	Membrane	0.57	32	7.7	38	7.6	2923	18.9

The numbers of spots are corresponding to those indicated in supplemental Figure 1.

Theo: theoretical. Obs.: experimental. Identification was performed using the softwares MS-Fit (<http://prospector.ucsf.edu/propector/4.272>) against respectively the NCBI (2007.10.10) and Swiss-Prot (Swissport. 2007.10.10) databases. Assignments were made according to UniProtKB/TrEMBL Release 39.0 of 22-Jul-2008: 6070084 entries and to UniProtKB/Swiss-Prot Release 56.0 of 22-Jul-2008: 392667 entries. Fold change is calculated by the ratio of mean of normalized volume of spot from amino acid starvation cells/mean of normalized volume of spot from control cells.