

SERBP1 is a component of the Liver Receptor Homolog-1 transcriptional complex

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Supplemental Table 1: QPCR Primer Sequences. Sequences of the forward and reverse QPCR primers were used in this study.

Supplemental Figure 1: Overexpression of both LRH1 isoforms using HaloTag. HEK293T cells were transfected with HaloLRH1. After transfection (24hrs), cells were harvested and a sub-cellular enrichment strategy was used to separate the nuclear and insoluble fractions. Cells were then labeled with TMRDirect Halo Ligand following Promega's Technical Manual protocol Part # TM348. Proteins were separated by SDS-PAGE and the gel was scanned on a fluorescent scanner (Typhoon, GE Healthcare Biosciences).

Supplemental Figure 2: HaloLRH1 localizes to the nucleus. HEK293T cells were plated on 1-well chamber slides (3×10^5 cells/chamber) (BioExpress). The following day, cells were transfected with both HaloLRH1 isoforms and empty vector. After 24hrs, cells were labeled with HaloTag TMRDirect Ligand (1:200) following Promega's protocol Part# TM260. Cells were then fixed in 4% paraformaldehyde per manufacture's protocol and processed for immunocytochemistry. Images were analyzed by confocal microscopy and captured on an Olympus Fluoview 1000 with SIM scanner.

Supplemental Figure 3: Promoter:Reporter assay and QPCR in HEK293T cells confirm all plasmids are functional. A and B) Transient transfections were performed and luciferase levels were measured. Data was normalized as fold change over vector. Statistically significant differences are depicted by asterisks ($p < 0.05$) as analyzed by one-way ANOVA. C and D) HEK293T cells were transfected with AviLRH1 for 48hrs. RNA was isolated, cDNA was prepared from 2 μ g total RNA, and gene expression was analyzed by QPCR using GAPDH as the housekeeping gene. Data was normalized as fold change over vector. Statistically significant differences are depicted by asterisks ($p < 0.05$) as analyzed by one-way ANOVA.

Supplemental Figure 4: Detection of endogenous LRH1 isoform 1-specific peptide in the nuclear fraction by mass spectrometry-based proteomics analysis. LRH1 pull down samples were prepared as described in Figure 3 and the nuclear fraction was analyzed by LC MS/MS. The delta ppm between the theoretical and observed monoisotopic mass was determined to be <1ppm.

Supplemental Figure 5: Detection of endogenous LRH1 isoform 2-specific peptide in the insoluble fraction by mass spectrometry-based proteomics analysis. LRH1 pull down samples were prepared as described in Figure 3 and the insoluble fraction was analyzed by LC MS/MS. The delta ppm between the theoretical and observed monoisotopic mass was determined to be <1ppm.

Supplemental Figure 6: LC MS/MS analysis of endogenous LRH1 immunoprecipitation. Summary of the b and y ions from the MS2 data of the LRH1 v1-specific peptide detected in the nuclear fraction (A) and the LRH1 v2-specific peptide identified in the insoluble fraction (B).

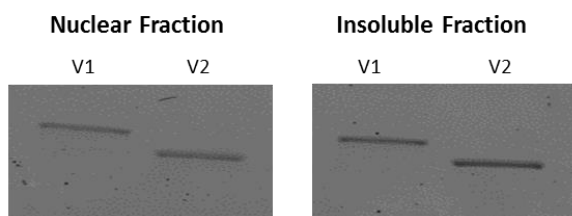
Supplemental Figure 7: LRH1 response element in the promoter region of SERBP1. The Genomatix genome analyzer v2.60912 (www.genomatix.de) was used for identification and extraction of the promoter sequence of SERBP1. Genomatix utilizes a large library of matrix descriptions for transcription factor binding sites. This software was used to search for LRH1 response elements in the promoter sequence, which resulted in identification of two transcription factor binding sites (V\$SF1.01,V\$FTF.01).

Supplemental Table 2: List of identified LRH1-specific proteins from overexpression studies.

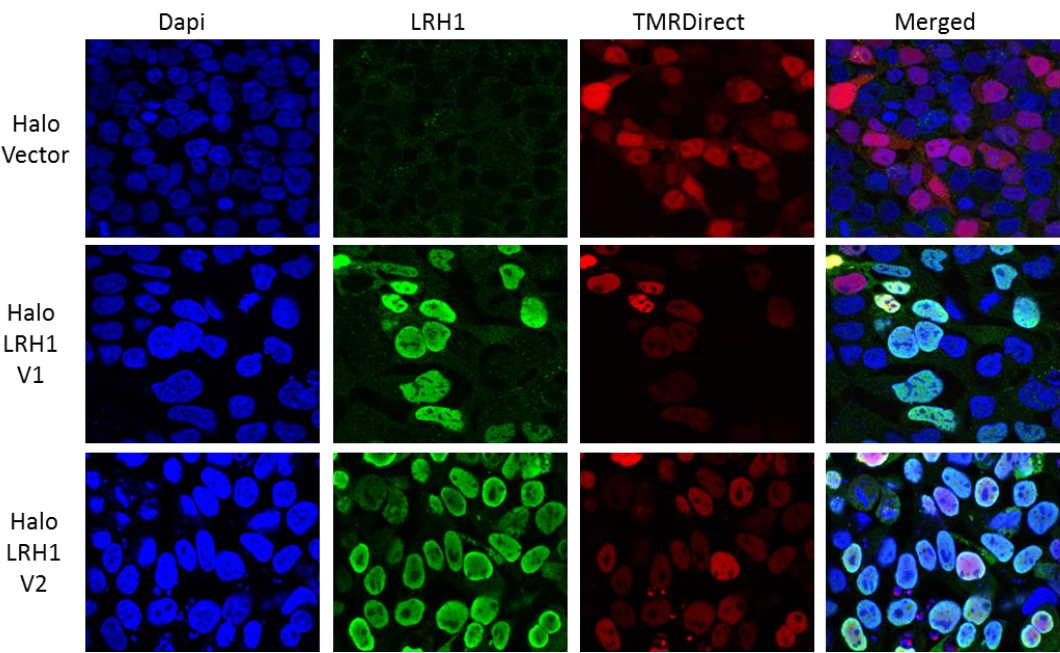
Supplemental Table 1: QPCR Primer Sequences

Gene	Forward	Reverse
hGAPDH	5'-GAAATCCCATCACCATCTTCCAGG-3'	5'-GAGCCCCAGCCTTCTCCATG-3'
hLRH1	5'-CTGATACTGGAACTTTGA-3'	5'-CTTCATTTGGTCATCAACCTT-3'
hSHP	5'-AAAGGGACCATCCTCTTCAAC-3'	5'-CTGGTCGGAATGGACTTGAG-3'
hSERBP1	5'-GTGACTGAGGAAACACCTGAA-3'	5'-AGCCTTCCACTCATCCAAAG-3'
hILF3	5'-CCAGAGGACGACAGTAAAGAAG-3'	5'-GTTGTGGGCTTCTCCTTACA-3'

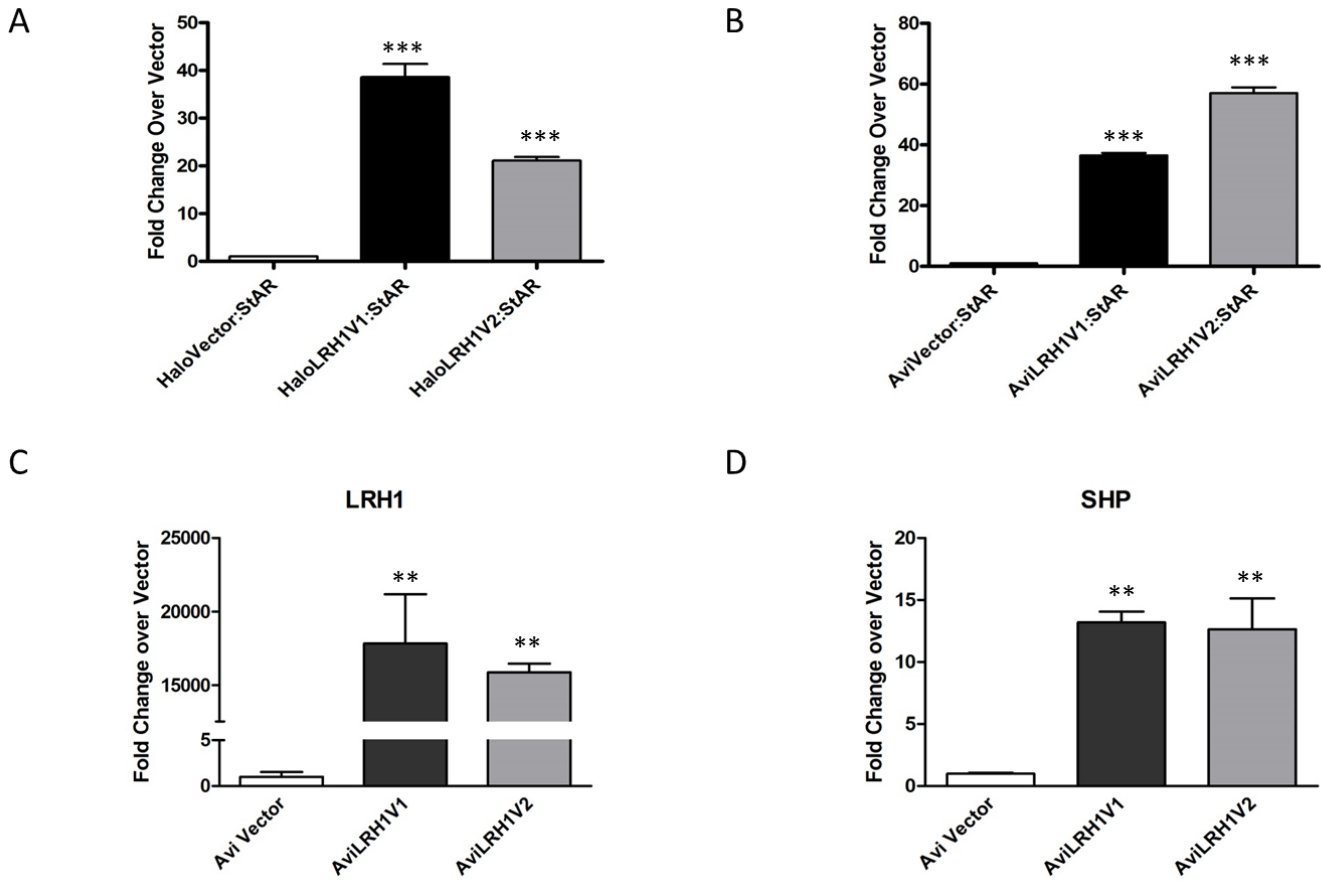
Supplemental Figure 1: Overexpression of both LRH1 isoforms using HaloTag



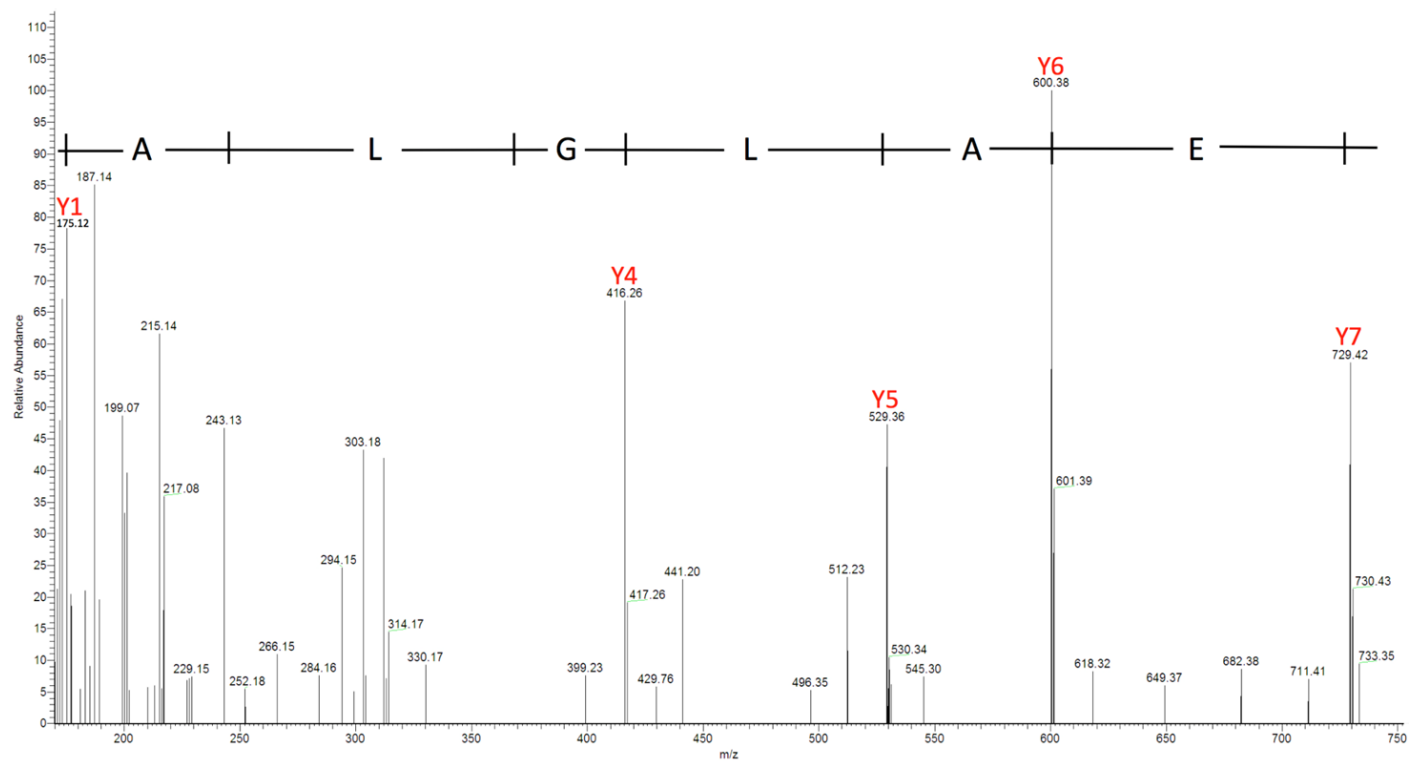
Supplemental Figure 2: HaloLRH1 localizes to the nucleus



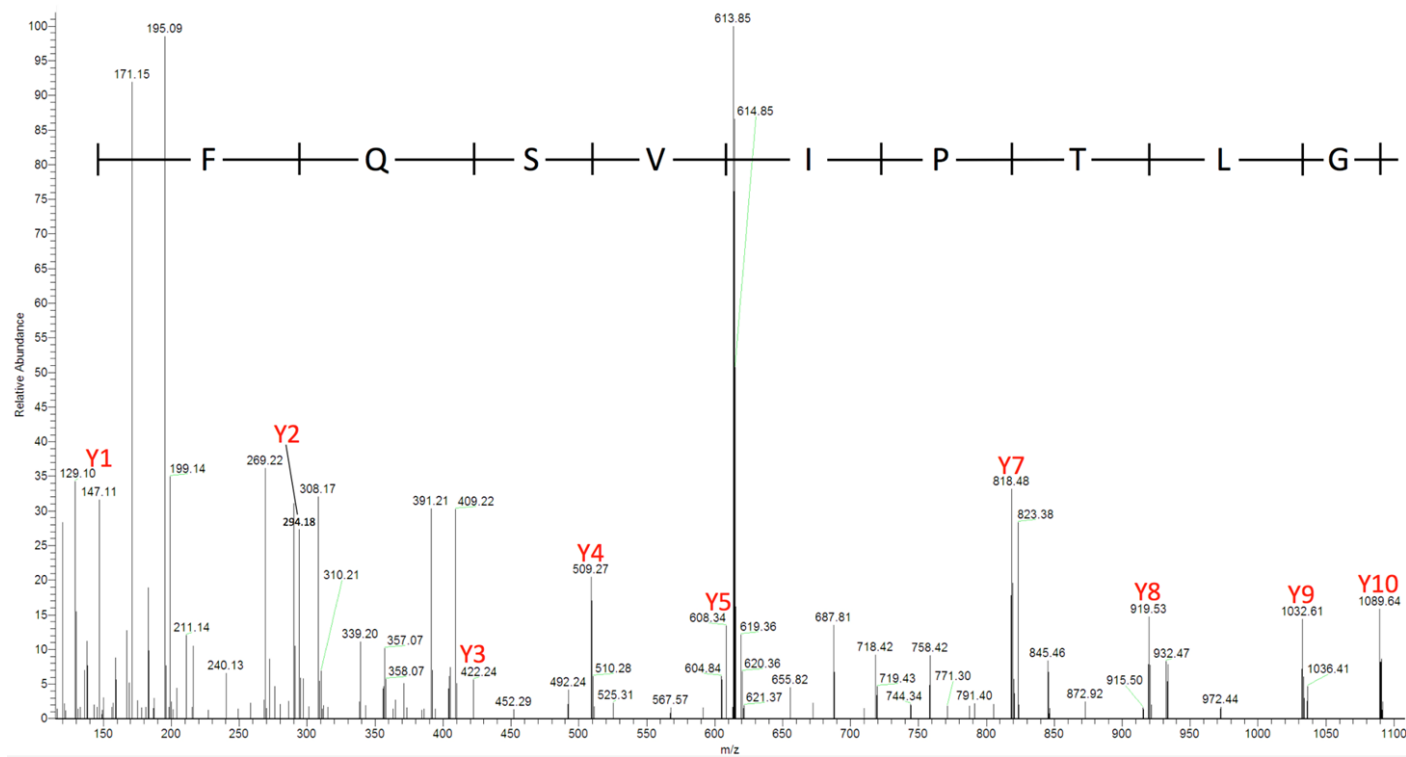
Supplemental Figure 3: Promoter:Reporter assay and QPCR in HEK293T cells confirm all plasmids are functional



Supplemental Figure 4: Detection of endogenous LRH1 isoform 1-specific peptide in the nuclear fraction by mass spectrometry-based proteomics analysis.



Supplemental Figure 5: Detection of endogenous LRH1 isoform 2-specific peptide in the insoluble fraction by mass spectrometry-based proteomics analysis.



Supplemental Figure 6: LC MS/MS analysis of endogenous LRH1 immunoprecipitation

A

Nuclear Fraction

LRH1 Variant 1 VETEALGLAR MH⁺²(mono) 529.7957

Identified y ions Full MS2 529.80

b				y
---	1	V	10	---
229.1	2	E	9	959.5
330.1	3	T	8	830.4
459.2	4	E	7	729.4
530.2	5	A	6	600.3
643.3	6	L	5	529.3
700.3	7	G	4	416.2
813.4	8	L	3	359.2
884.4	9	A	2	246.1
---	10	R	1	175.1

B

Insoluble Fraction

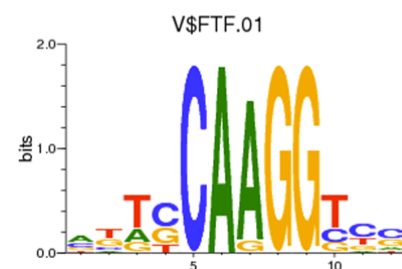
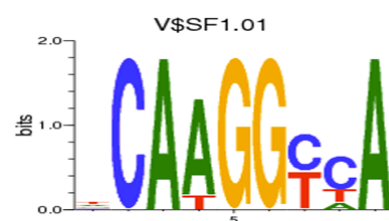
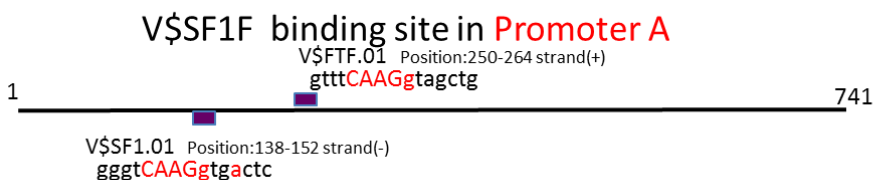
LRH1 Variant 2 HGLTPIVSQFK MH⁺²(mono) 613.8482

Identified b and y ions Full MS2 613.85

b				y
---	1	H	11	---
195.1	2	G	10	1089.6
308.2	3	L	9	1032.6
409.2	4	T	8	919.5
506.3	5	P	7	818.5
619.4	6	I	6	721.4
718.4	7	V	5	608.3
805.5	8	S	4	509.3
933.5	9	Q	3	422.2
1080.6	10	F	2	294.2
---	11	K	1	147.1

Supplemental Figure 7: LRH1 response elements in the promoter of SERBP1

>Promoter A | sym=SERBP1 | taxid=9606 | spec=Homo
 sapiens | chr=1 | ctg=NC_000001 | str=() | start=67895883 | end=67896623 | len=741 | comm=Promoter Region
 AGGCTGACGGAGGGCCACTCCAGCAACTGAGCAGCTCGGACTTGGCACAGGCAGGTCCAGCCTACCCGCGCCGGCGCG
 GCGCCTCAGCAACACCCCTCCCGGAAAGTTTGTGCTCTCAGATGTAAGGTCTGGGAGTCACCTTGACCCCTGGAG
 CGGTGGGGAAGTTAGCCCTGAGCCGAGGGGGCGTGAGTGAAACAAAGAGCACTTTCGCGAGTCAGTTACGCCGTAC
 GGTTTTTGGTTTCAAGGTAGCTGAAGACCTAGAGAGTTTAAGGGTGGCCAGAGCAGGTGGCAGGACTCCGCCACCACC
 ACGTTCCCTTCTTCAGCCCGCACGCTTCACTCCCTCATGGGGTCTCGCTCTCAGGCGCGCGGAAGAGTTGTGCG
 CGGTGCGTCTGGGAATTGTAGTCCGACGCGGAGAGTCGCTCAGGAGAAATGACTCTGGCCTACATACCCACAGTGC
 CTTGCGGCGCAGGCCGCTCCCGGATGTGTGCTGGCGCCGGAAGAGAAGACGCCCCCTCTCTCGGCCGGCCATCTTG
 TGGGAAGAGCTGAAGCAGGCGCTTTGGCTCGGCGCGGCCGCTGCAATCCGTGGAGGAACGCGCGCCGAGCCACCATC
 ATGCTGGGCACCTTACAGGAAGGCTTCGGCTGCGTGTCACCAACCGATTTCAGCAGTTATTTGACGACGAATCGGACCC
 CTTGAGGTGCTGAAGGCAGC



Supplemental Table 2: List of identified LRH1-specific proteins from overexpression studies

Number	Identified Proteins	Accession Number	Molecular Weight
1	Ubiquitin-60S ribosomal protein L40 OS=Homo sapiens GN=UBA52 PE=1 SV=2	RL40_HUMAN (+3)	15 kDa
2	Chromobox protein homolog 3 OS=Homo sapiens GN=CBX3 PE=1 SV=4	CBX3_HUMAN	21 kDa
3	FACT complex subunit SSRP1 OS=Homo sapiens GN=SSRP1 PE=1 SV=1	SSRP1_HUMAN	81 kDa
4	Regulator of chromosome condensation OS=Homo sapiens GN=RCC1 PE=1 SV=1	RCC1_HUMAN	45 kDa
5	Plasminogen activator inhibitor 1 RNA-binding protein OS=Homo sapiens GN=SERBP1 PE=1 SV=2	PAIRB_HUMAN	45 kDa
6	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5	HSP71_HUMAN	70 kDa
7	Interleukin enhancer-binding factor 2 OS=Homo sapiens GN=ILF2 PE=1 SV=2	ILF2_HUMAN	43 kDa
8	GTP-binding nuclear protein Ran OS=Homo sapiens GN=RAN PE=1 SV=3	RAN_HUMAN	24 kDa
9	Coiled-coil domain-containing protein 86 OS=Homo sapiens GN=CCDC86 PE=1 SV=1	CCD86_HUMAN	40 kDa
10	Heterogeneous nuclear ribonucleoprotein A3 OS=Homo sapiens GN=HNRNPA3 PE=1 SV=2	ROA3_HUMAN	40 kDa
11	Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens GN=DDX17 PE=1 SV=2	DDX17_HUMAN	80 kDa
12	Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1	DDX5_HUMAN	69 kDa
13	Heat shock 70 kDa protein 6 OS=Homo sapiens GN=HSPA6 PE=1 SV=2	HSP76_HUMAN (+1)	71 kDa
14	Putative high mobility group protein B1-like 1 OS=Homo sapiens GN=HMGB1P1 PE=5 SV=1	HGB1A_HUMAN (+1)	24 kDa
15	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1	HSP7C_HUMAN	71 kDa
16	ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4	DHX9_HUMAN	141 kDa
17	Cluster of Core histone macro-H2A.1 OS=Homo sapiens GN=H2AFY PE=1 SV=4 (H2AY_HUMAN)	H2AY_HUMAN	40 kDa
18	Interleukin enhancer-binding factor 3 OS=Homo sapiens GN=ILF3 PE=1 SV=3	ILF3_HUMAN	95 kDa
19	ATP synthase subunit alpha, mitochondrial OS=Homo sapiens GN=ATPSA1 PE=1 SV=1	ATPA_HUMAN	60 kDa
20	High mobility group protein HMG-I/HMG-Y OS=Homo sapiens GN=HMGA1 PE=1 SV=3	HMGA1_HUMAN	12 kDa
21	Poly [ADP-ribose] polymerase 1 OS=Homo sapiens GN=PARP1 PE=1 SV=4	PARP1_HUMAN	113 kDa
22	ATPase inhibitor, mitochondrial OS=Homo sapiens GN=ATPIF1 PE=1 SV=1	ATIF1_HUMAN	12 kDa
23	Drebrin OS=Homo sapiens GN=DBN1 PE=1 SV=4	DREB_HUMAN	71 kDa
24	Insulin-like growth factor 2 mRNA-binding protein 1 OS=Homo sapiens GN=IGF2BP1 PE=1 SV=2	IF2B1_HUMAN	63 kDa
25	Cluster of Zinc finger protein 280C OS=Homo sapiens GN=ZNF280C PE=1 SV=1 (Z280C_HUMAN)	Z280C_HUMAN	83 kDa
26	Heme oxygenase 1 OS=Homo sapiens GN=HMOX1 PE=1 SV=1	HMOX1_HUMAN	33 kDa
27	DnaI homolog subfamily C member 9 OS=Homo sapiens GN=DNAJC9 PE=1 SV=1	DNJC9_HUMAN	30 kDa
28	DNA mismatch repair protein Msh6 OS=Homo sapiens GN=MSH6 PE=1 SV=2	MSH6_HUMAN	153 kDa
29	Histone acetyltransferase KAT7 OS=Homo sapiens GN=KAT7 PE=1 SV=1	KAT7_HUMAN	71 kDa
30	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial OS=Homo sapiens GN=PDHB PE=1 SV=3	ODPB_HUMAN	39 kDa
31	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 OS=Homo sapiens GN=ATP2A2 PE=1 SV=1	AT2A2_HUMAN	115 kDa
32	RNA-binding protein Musashi homolog 2 OS=Homo sapiens GN=MSI2 PE=1 SV=1	MSI2H_HUMAN	35 kDa
33	Telomere-associated protein RIF1 OS=Homo sapiens GN=RIF1 PE=1 SV=2	RIF1_HUMAN	274 kDa
34	Nuclear pore complex protein Nup85 OS=Homo sapiens GN=NUP85 PE=1 SV=1	NUP85_HUMAN	75 kDa
35	Alpha-globin transcription factor CP2 OS=Homo sapiens GN=TFCP2 PE=1 SV=2	TFCP2_HUMAN	57 kDa
36	Cell division control protein 42 homolog OS=Homo sapiens GN=CDC42 PE=1 SV=2	CDC42_HUMAN	21 kDa
37	Sister chromatid cohesion protein PDS5 homolog A OS=Homo sapiens GN=PDS5A PE=1 SV=1	PDS5A_HUMAN	151 kDa
38	Transcription termination factor 2 OS=Homo sapiens GN=TTF2 PE=1 SV=2	TTF2_HUMAN	130 kDa
39	Glycerol-3-phosphate dehydrogenase, mitochondrial OS=Homo sapiens GN=GPD2 PE=1 SV=3	GPDM_HUMAN	81 kDa
40	Importin subunit alpha-2 OS=Homo sapiens GN=KPNA2 PE=1 SV=1	IMA2_HUMAN	58 kDa
41	Catenin beta-1 OS=Homo sapiens GN=CTNNB1 PE=1 SV=1	CTNB1_HUMAN	85 kDa
42	CUGBP Elav-like family member 1 OS=Homo sapiens GN=CELF1 PE=1 SV=2	CELF1_HUMAN	52 kDa
43	Multiple myeloma tumor-associated protein 2 OS=Homo sapiens GN=MMTAG2 PE=1 SV=1	MMTA2_HUMAN	29 kDa
44	40S ribosomal protein S16 OS=Homo sapiens GN=RPS16 PE=1 SV=2	RS16_HUMAN	16 kDa
45	Histone acetyltransferase KAT6A OS=Homo sapiens GN=KAT6A PE=1 SV=2	KAT6A_HUMAN	225 kDa
46	Bromodomain adjacent to zinc finger domain protein 2A OS=Homo sapiens GN=BAZ2A PE=1 SV=4	BAZ2A_HUMAN	211 kDa
47	MOSC domain-containing protein 1, mitochondrial OS=Homo sapiens GN=MARC1 PE=1 SV=1	MOSC1_HUMAN	38 kDa
48	60S ribosomal protein L36a OS=Homo sapiens GN=RPL36A PE=1 SV=2	RL36A_HUMAN (+1)	12 kDa