

## Supplementary Materials

### **Antiproliferative activity of berberine in HepG2 cells via inducing apoptosis and arresting cell cycle**

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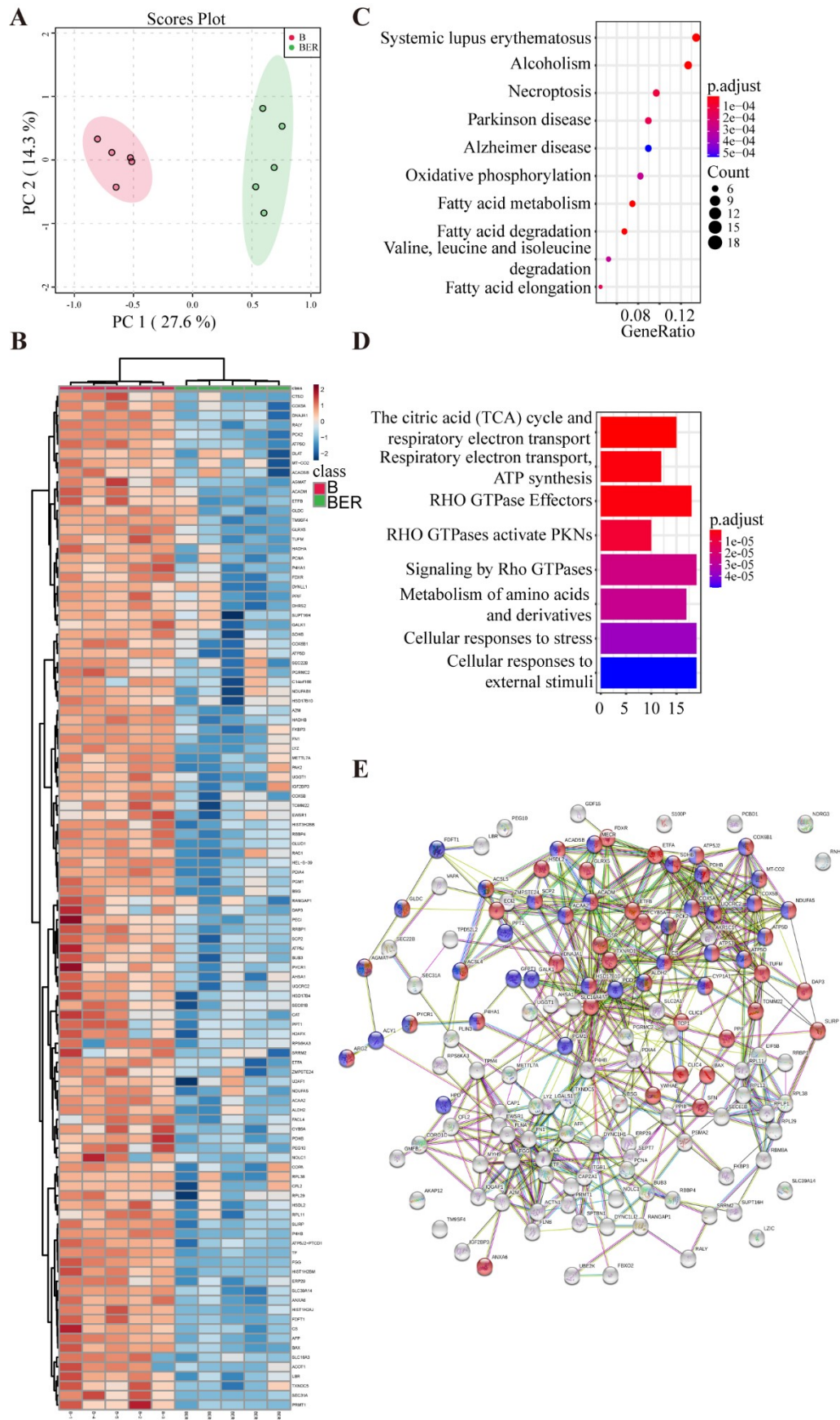
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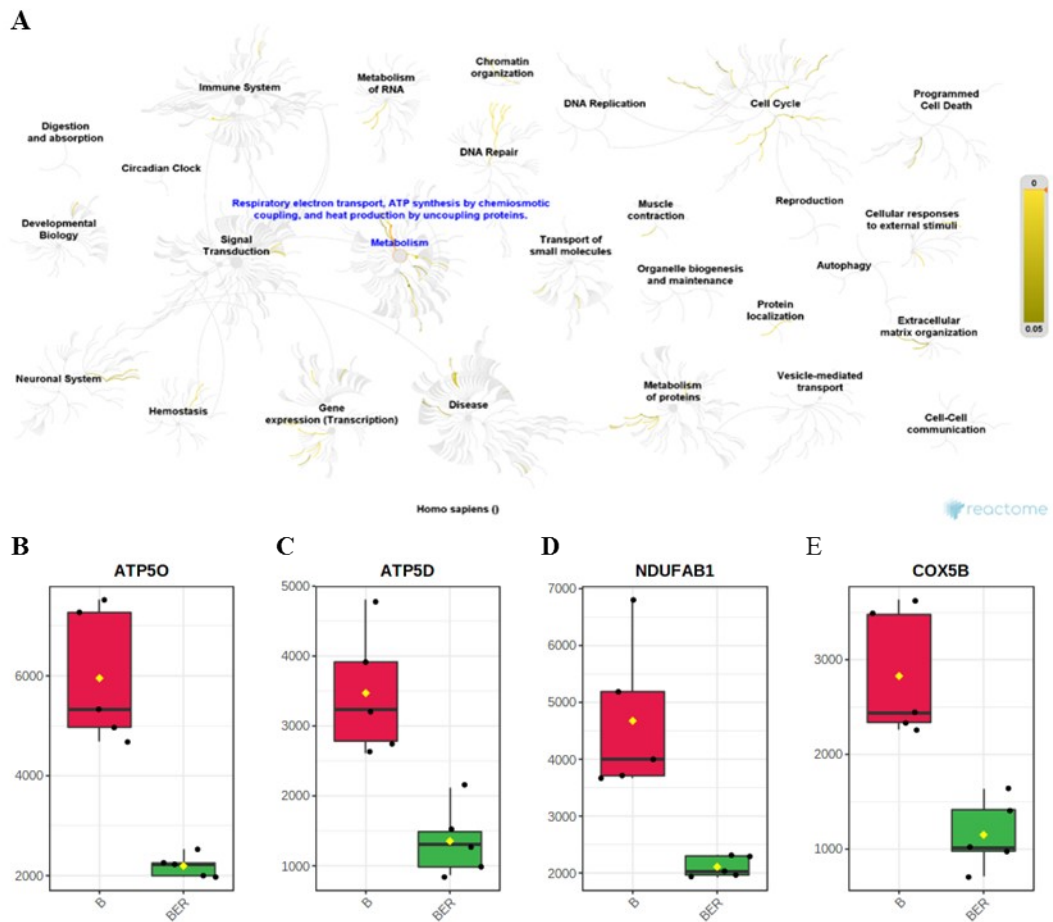
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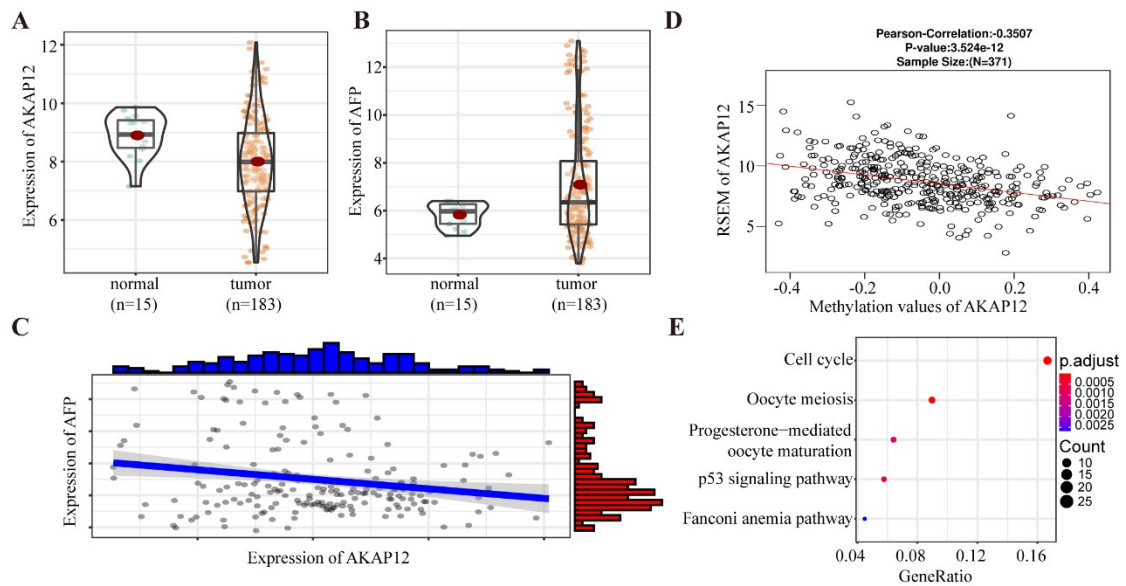


**Fig. S1.** (A) Principal component analysis plot illustrating the sample distribution of

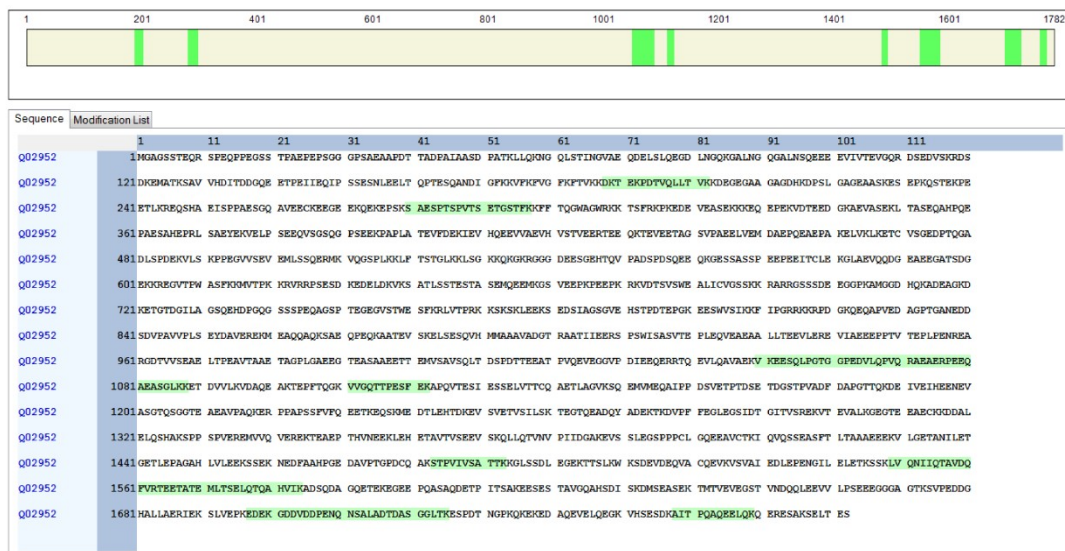
blank control (B, red) and berberine-treated (BER, green) group. (B) Heatmap of all downregulated proteins. (C) Top 10 significant enriched KEGG pathways and (D) top 8 enriched Reactome pathways. (E) Protein-protein interaction network of altered proteins assessed with the STRING database. Proteins labeled in red and blue color were enriched in the mitochondria and metabolism pathways.



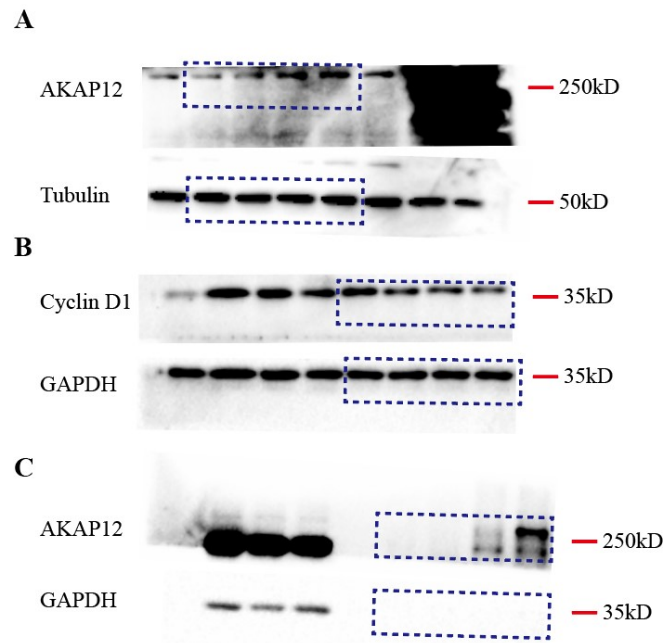
**Fig. S2.** (A) Overrepresented pathways from REACTOME pathway analysis of the altered proteins. Expression of mitochondrial dysfunction-mediated protein, including (B) ATP5O, (C) ATP5D, (D) NDUFAB1, (E) COX5B. ATP5O, ATP synthase subunit O; ATP5D, ATP synthase subunit delta; NDUFAB1, NADH dehydrogenase (ubiquinone) 1 beta subcomplex; COX5B, Cytochrome c oxidase subunit 5B.



**Fig. S3.** (A, B) Box plots of AKAP12 expression ( $p \leq 0.001$ ) and AFP expression ( $p \leq 0.001$ ) in normal and tumor samples from GSE112790 dataset. (C) Correlation analysis between expression levels of AKAP12 and AFP ( $p = 0.005$ ,  $r = -0.2$ ). (D) Spearman correlation analysis of the expression of AKAP12 and its methylation levels using LinkedOmics. (E) Dot plot of the enriched GO terms using the upregulated genes obtained from TCGA LIHC dataset.



**Fig. S4.** The protein-sequence coverage of AKAP12 observed using Proteome Discoverer software (version 2.3.0).



Images in support of the representative western blots data shown in Figure 6. (A, B) Western blot analysis of AKAP12 and Cyclin D1 expression. (C) Co-immunoprecipitation of AKAP12 and Cyclin D1 was visualized using immunoblots.

**Table S1.** The hub target proteins of berberine (ranked according to Clustering Coefficient).

name	Glax Cluster	Average Shortest Path Length	Betweenness Centrality	Closeness Centrality	Clustering Coefficient	Degree
SHC1	1	2.506	0.006	0.399	0.189	61
SMAD4	3	2.507	0.005	0.399	0.170	48
PTK2	1	2.521	0.006	0.397	0.156	47
RAF1	1	2.452	0.005	0.408	0.146	47
HDAC1	3	2.480	0.008	0.403	0.120	59
LCK	1	2.485	0.005	0.402	0.120	54
BRCA1	3	2.362	0.009	0.423	0.119	68
BCL2	5	2.523	0.006	0.396	0.119	39
PIK3R1	1	2.426	0.013	0.412	0.117	72
SMAD2	3	2.360	0.010	0.424	0.116	62
STAT3	3	2.382	0.010	0.420	0.115	64
JUN	3	2.452	0.008	0.408	0.114	73
XRCC6	3	2.520	0.007	0.397	0.114	33
KAT5	3	2.474	0.005	0.404	0.113	36
RELA	3	2.492	0.007	0.401	0.112	68
RXRA	3	2.496	0.007	0.401	0.112	56
SMAD3	3	2.288	0.016	0.437	0.109	79
GRB2	1	2.471	0.011	0.405	0.107	83
CAV1	1	2.458	0.012	0.407	0.105	45
PRKCD	1	2.462	0.007	0.406	0.102	58
CCND1	3	2.527	0.011	0.396	0.101	40

**Table S2.** Apoptosis and cell cycle-related enrichment pathways.

term ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in your network (labels)
GO:0042981	regulation of apoptotic process	32	1501	6.63E-23	ABL1,AKT1,AR,BCL2,BRCA1,CASP3,CAV1,CDK1,CDK5,CREBBP,CSNK2A1,CTNNB1,EGFR,ESR1,FYN,GSK3B,HDAC1,JUN,LCK,PIK3R1,PRKCA,PRKCD,PTK2,RAF1,RELA,SHC1,SMAD3,SRC,STAT3,TP53,YWHAG,YWHAZ
GO:0043066	negative regulation of apoptotic process	27	859	1.19E-22	ABL1,AKT1,AR,BCL2,BRCA1,CASP3,CAV1,CDK1,CSNK2A1,CTNNB1,EGFR,FYN,GSK3B,HDAC1,JUN,PIK3R1,PRKCA,PRKCD,PTK2,RAF1,RELA,SHC1,SMAD3,SRC,STAT3,TP53,YWHAZ
GO:2001233	regulation of apoptotic signaling pathway	18	388	2.53E-17	AKT1,AR,BCL2,BRCA1,CAV1,CSNK2A1,CTNNB1,FYN,GSK3B,LCK,PRKCD,RAF1,RELA,SMAD3,SRC,TP53,YWHAG,YWHAZ
GO:0006915	apoptotic process	22	915	7.26E-16	ABL1,AKT1,BCL2,BRCA1,CASP3,CAV1,CDK1,CDK5,CSNK2A1,EP300,GSK3B,JUN,MAPK1,MAPK3,NR3C1,PIK3R1,PRKCA,PRKCD,RAF1,RB1,SMAD3,TP53
GO:0097190	apoptotic signaling pathway	14	295	1.49E-13	ABL1,BCL2,BRCA1,CASP3,CAV1,EP300,GSK3B,JUN,PIK3R1,PRKCA,PRKCD,RAF1,SMAD3,TP53
GO:0043065	positive regulation of apoptotic process	16	604	6.72E-12	ABL1,AKT1,BCL2,CASP3,CAV1,CDK5,CTNNB1,GSK3B,JUN,LCK,PRKCD,SMAD3,SRC,TP53,YWHAG,YWHAZ
GO:2001234	negative regulation of apoptotic signaling pathway	10	218	1.29E-09	AKT1,AR,BCL2,BRCA1,CSNK2A1,CTNNB1,FYN,RAF1,RELA,SRC
GO:2001236	regulation of extrinsic apoptotic signaling pathway	9	158	1.87E-09	AKT1,AR,BCL2,BRCA1,CAV1,FYN,RAF1,RELA,SRC
GO:2001237	negative regulation of extrinsic apoptotic signaling pathway	8	104	2.17E-09	AKT1,AR,BCL2,BRCA1,FYN,RAF1,RELA,SRC
GO:0045930	negative regulation of mitotic cell cycle	11	243	1.79E-10	ABL1,BCL2,BRCA1,CCND1,CDK1,CTNNB1,EGFR,EP300,RB1,SMAD3,TP53
GO:1901990	regulation of mitotic cell cycle phase transition	12	351	4.23E-10	AKT1,BCL2,BRCA1,CCND1,CDK1,EGFR,EP300,HSP90AA1,PRKACA,RB1,TP53,YWHAG
GO:0010564	regulation of cell cycle process	15	684	4.55E-10	AKT1,BCL2,BRCA1,CCND1,CDK1,CDK5,CSNK2A1,CTNNB1,EGFR,EP300,HSP90AA1,PRKACA,RB1,TP53,YWHAG
GO:0045787	positive regulation of cell cycle	12	376	8.59E-10	ABL1,AKT1,BRCA1,CCND1,CDK1,EGFR,EP300,PRKACA,PRKCA,RB1,SRC,TP53
GO:0045786	negative regulation of cell cycle	13	517	2.04E-09	ABL1,BCL2,BRCA1,CASP3,CCND1,CDK1,CDK5,CTNNB1,EGFR,EP300,RB1,SMAD3,TP53
GO:2000045	regulation of G1/S transition of mitotic cell cycle	8	148	2.68E-08	AKT1,BCL2,CCND1,CDK1,EGFR,EP300,RB1,TP53
GO:0071156	regulation of cell cycle arrest	7	111	9.42E-08	BRCA1,CCND1,CDK1,CDK5,EP300,PRKACA,TP53
GO:0022402	cell cycle process	13	890	8.16E-07	ABL1,BRCA1,CCND1,CDK1,CTNNB1,EP300,HSP90AA1,PRKACA,PRKCA,RB1,SMAD3,TP53,YWHAG
GO:1901991	negative regulation of mitotic cell cycle phase transition	7	160	8.70E-07	BCL2,BRCA1,CCND1,CDK1,EP300,RB1,TP53
GO:2000134	negative regulation of G1/S transition of mitotic cell cycle	6	97	1.03E-06	BCL2,CCND1,CDK1,EP300,RB1,TP53
GO:0000278	mitotic cell cycle	11	628	1.49E-06	ABL1,BRCA1,CCND1,CDK1,EP300,HSP90AA1,PRKACA,PRKCA,RB1,TP53,YWHAG
hsa04210	Apoptosis	10	135	4.83E-12	AKT1,BCL2,CASP3,JUN,MAPK1,MAPK3,PIK3R1,RAF1,RELA,TP53

hsa04215	Apoptosis - multiple species	2	31	0.0037	BCL2,CASP3
hsa05200	Pathways in cancer	31	515	4.81E-35	ABL1,AKT1,AR,BCL2,CASP3,CCND1,CREBBP,CTNNB1,EGFR,EP300,ESR1,GRB2,GSK3B,HDAC1,HSP90AA1,JUN,MAPK1,MAPK3,PIK3R1,PRKACA,PRKCA,PTK2,RAF1,RB1,RELA,RXR,SMAD2,SMAD3,SMAD4,STAT3,TP53
hsa05225	Hepatocellular carcinoma	17	163	3.07E-22	AKT1,CCND1,CTNNB1,EGFR,GRB2,GSK3B,MAPK1,MAPK3,PIK3R1,PRKCA,RAF1,RB1,SHC1,SMAD2,SMAD3,SMAD4,TP53
hsa04110	Cell cycle	14	123	5.97E-19	ABL1,CCND1,CDK1,CREBBP,EP300,GSK3B,HDAC1,RB1,SMAD2,SMAD3,SMAD4,TP53,YWHAG,YWHAZ
hsa04217	Necroptosis	3	155	0.0081	BCL2,HSP90AA1,STAT3

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**Table S3.** Dysregulated proteins in berberine-treated group compared to control (ranked according to fold change).

No.	Unique peptides	Sequence coverage [%]	MS/MS count	Log Student's T-test p-value	Student's T-test Difference	Gene names
1	16	45.2	114	4.9	-4.7	AFP
2	9	32.6	30	5.8	-2.8	FGG
3	8	70.1	55	4.4	-2.6	SLIRP
4	6	6.5	28	3.9	-2.5	FN1
5	8	8.5	26	4.2	-2.3	A2M
6	24	44	199	6.6	-2.3	TF
7	3	31.8	37	3.6	-2.1	LYZ
8	8	26.7	52	2.9	-1.9	PEG10
9	6	25.7	60	3.5	-1.9	ACADM
10	4	15.2	96	3.8	-1.8	SLC39A14
11	6	15.1	16	3.3	-1.8	P4HA1
12	3	14.5	16	2.4	-1.8	HSDL2
13	4	24.7	25	1.8	-1.7	MT-CO2
14	2	15	25	3.1	-1.7	METTL7A
15	2	21	12	4.2	-1.7	GLRX5
16	5	23.7	34	1.6	-1.7	NDUFAB1
17	12	13.9	19	3.6	-1.7	SEC31A
18	4	10.1	90	1.8	-1.7	SLC16A3
19	3	12.9	9	2.5	-1.5	SDHB
20	4	8.2	10	1.4	-1.5	RPS6KA3
21	2	19.1	18	2.0	-1.5	PPT1
22	4	57	12	2.2	-1.5	COX6B1
23	3	53.1	47	2.7	-1.5	CYB5A
24	3	13.9	62	2.3	-1.5	AHSA1
25	2	9.3	17	2.3	-1.4	COX5B

26	7	7.3	19	2.3	-1.4	UGGT1
27	2	11.9	22	1.6	-1.4	ACOT1
28	5	27.9	23	1.4	-1.3	C14orf166
29	3	5.8	8	2.3	-1.3	GLDC
30	6	18.8	41	1.9	-1.3	IGF2BP3
31	2	7.5	3	1.5	-1.3	DLAT
32	11	31.6	57	4.2	-1.3	HADHB
33	6	19.8	13	2.5	-1.3	PECI
34	2	47.6	74	3.1	-1.2	HIST3H2BB
35	2	17.2	7	1.8	-1.2	RAC1
36	2	11.6	19	2.7	-1.2	FKBP3
37	5	15.1	18	3.3	-1.2	PGM1
38	18	42.5	173	3.9	-1.2	PCK2
39	3	37.5	79	1.8	-1.2	ATP5D
40	8	54.5	71	4.0	-1.2	ATP5O
41	6	14.7	9	1.4	-1.2	RANGAP1
42	2	61.9	30	1.4	-1.2	RPL38
43	20	52.4	480	2.9	-1.1	TUFM
44	4	48.6	110	1.9	-1.1	TOMM22
45	16	20.4	30	1.7	-1.1	COPA
46	4	9.7	7	2.7	-1.1	TM9SF4
47	8	33.7	283	2.9	-1.1	CTSD
48	3	12.4	25	2.1	-1.1	PAK2
49	10	43	135	3.0	-1.1	UQCRC2
50	4	40.7	150	3.0	-1.0	COX5A
51	6	48.7	168	1.7	-1.0	HSD17B10
52	10	44.2	91	2.1	-1.0	PYCR1
53	0	55.2	152	1.3	-1.0	H2AFX
54	7	32.9	61	4.6	-1.0	RALY

55	8	33.5	52	4.1	-1.0	ACAA2
56	4	25.6	43	1.5	-1.0	SEC22B
57	3	57.4	48	3.7	-1.0	ATP5J2-PTCD1
58	3	44.8	18	2.3	-1.0	NDUFA5
59	0	66.2	1095	4.4	-1.0	HIST1H2AJ
60	3	44.4	56	3.4	-0.9	ATP5J
61	5	37.3	118	3.9	-0.9	RBBP4
62	4	4.9	16	1.6	-0.9	SUPT16H
63	3	61.9	1271	4.2	-0.9	GLUD1
64	2	41.6	55	1.3	-0.9	CFL2
65	11	29.6	69	2.4	-0.9	CAT
66	3	18.4	6	1.8	-0.9	PGRMC2
67	17	35.7	103	4.1	-0.9	ANXA6
68	14	56.7	735	4.0	-0.9	HEL-S-39
69	14	50.9	167	3.2	-0.9	ALDH2
70	7	45.4	198	3.2	-0.9	BSG
71	2	16.6	28	1.3	-0.9	RPL29
72	10	52.1	271	3.1	-0.9	ETFA
73	2	6.2	3	3.1	-0.9	LBR
74	3	30.9	276	2.3	-0.9	PPIF
75	8	37.9	257	1.9	-0.9	DHRS2
76	7	33.8	145	3.4	-0.9	DNAJA1
77	8	32.4	140	3.4	-0.9	AGMAT
78	2	47.6	1165	4.7	-0.8	HIST1H2BM
79	1	9.7	140	1.7	-0.8	NOLC1
80	16	47.3	170	2.6	-0.8	FDXR
81	6	44.4	240	2.2	-0.8	RPL11
82	3	13.7	11	1.6	-0.8	DAP3
83	5	20	94	1.3	-0.8	U2AF1

84	4	35.4	10	4.9	-0.8	BAX
85	13	32.7	163	2.8	-0.8	FACL4
86	4	21.7	16	1.8	-0.8	ZMPSTE24
87	24	45.6	362	3.5	-0.8	HADHA
88	8	31.3	133	3.5	-0.7	CS
89	9	41.8	426	2.9	-0.7	ERP29
90	4	19.4	32	2.7	-0.7	BUB3
91	13	33.3	109	2.1	-0.7	HSD17B4
92	3	13.6	10	1.5	-0.7	GALK1
93	36	75.6	3914	6.8	-0.7	P4HB
94	3	49.4	29	1.4	-0.7	DYNLL1
95	33	56.3	853	3.6	-0.7	PDIA4
96	33	28.5	511	4.0	-0.7	RRBP1
97	7	22.1	54	5.0	-0.7	FDFT1
98	3	51	8	2.1	-0.7	SEC61B
99	13	44.2	199	2.2	-0.7	TXNDC5
100	4	5.9	29	3.1	-0.7	SCP2
101	9	33.1	296	1.5	-0.6	ACADSB
102	8	34.5	86	2.5	-0.6	ETFB
103	10	55.2	494	2.4	-0.6	PCNA
104	6	23.2	24	3.8	-0.6	PDHB
105	4	11.7	131	1.5	-0.6	EWSR1
106	5	22.1	12	3.0	-0.6	PRMT1
107	3	1.6	15	1.4	-0.6	SRRM2
108	43	25	503	3.6	0.6	FLNA
109	20	56.6	260	3.7	0.6	ACTN1
110	4	20.4	109	1.6	0.6	RPL13
111	4	4.8	5	1.4	0.6	IQGAP1
112	4	31.2	233	2.2	0.6	CLIC4

113	15	48.4	176	2.0	0.6	GSR
114	17	5.7	38	1.4	0.6	DYNC1H1
115	18	43.9	208	2.9	0.6	YARS
116	4	28.5	28	1.5	0.6	VAPA
117	24	60.1	464	1.9	0.7	TCP1
118	4	26.1	168	2.2	0.7	PSMA2
119	14	58.1	159	2.3	0.7	RNH1
120	17	83	1150	4.2	0.7	CLIC1
121	3	7.6	10	1.4	0.7	ACY1
122	10	42.2	176	2.3	0.7	PLIN3
123	20	62.9	1003	2.7	0.7	CAP1
124	8	13.1	31	2.5	0.7	ITGB1
125	14	62.7	764	1.6	0.7	YWHAE
126	23	62.5	768	4.4	0.7	PGD
127	1	80.5	2996	2.7	0.7	AKR1C1
128	4	94.7	1507	1.4	0.7	RPLP1
129	8	51.4	144	1.7	0.8	CAPZA1
130	53	35.5	728	5.4	0.8	FLNB
131	4	11.7	4	2.0	0.8	SEPT7
132	2	10.6	12	2.8	0.9	NDRG3
133	4	33	23	1.5	0.9	UBE2K
134	22	42.7	270	6.1	0.9	GFPT1
135	101	58.3	1779	7.7	0.9	SPTBN1
136	6	49.5	325	1.9	0.9	TPD52L2
137	9	17.1	482	4.7	0.9	SLC2A1
138	23	23.9	252	2.2	0.9	MYH9
139	5	18.1	28	2.5	0.9	CORO1C
140	4	6.2	5	1.4	0.9	EIF5B
141	7	48.8	159	2.9	1.0	TPM4

142	3	27.3	5	1.3	1.0	GMFB
143	2	11.5	11	2.4	1.2	FBXO2
144	3	9.7	12	1.8	1.2	HPD
145	3	32.2	12	2.8	1.2	RBM8A
146	9	14.9	30	1.8	1.2	VCL
147	4	45.2	37	2.1	1.3	PCBD
148	13	60.5	761	2.8	1.3	SFN
149	4	33.8	81	2.5	1.4	CHCHD2
150	2	22.3	16	1.4	1.5	LZIC
151	6	58.5	121	4.2	1.5	LGALS1
152	5	71.6	624	3.4	1.5	S100P
153	4	64.8	47	3.8	1.8	S100A11
154	4	20.9	18	4.0	1.8	ARG2
155	17	28.6	164	5.9	2.1	ACSL5
156	9	40.2	184	6.1	2.6	SQSTM1
157	14	41.3	171	2.8	2.6	TXNRD1
158	12	45.1	215	6.3	3.5	GDF15
159	7	22.6	116	6.1	3.8	CYP1A1
160	28	30.7	174	6.5	4.4	AKAP12

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