SUPPORTING INFORMATION

IS-SCP: Enhanced in single-cell proteomics using an in-situ simplified strategy

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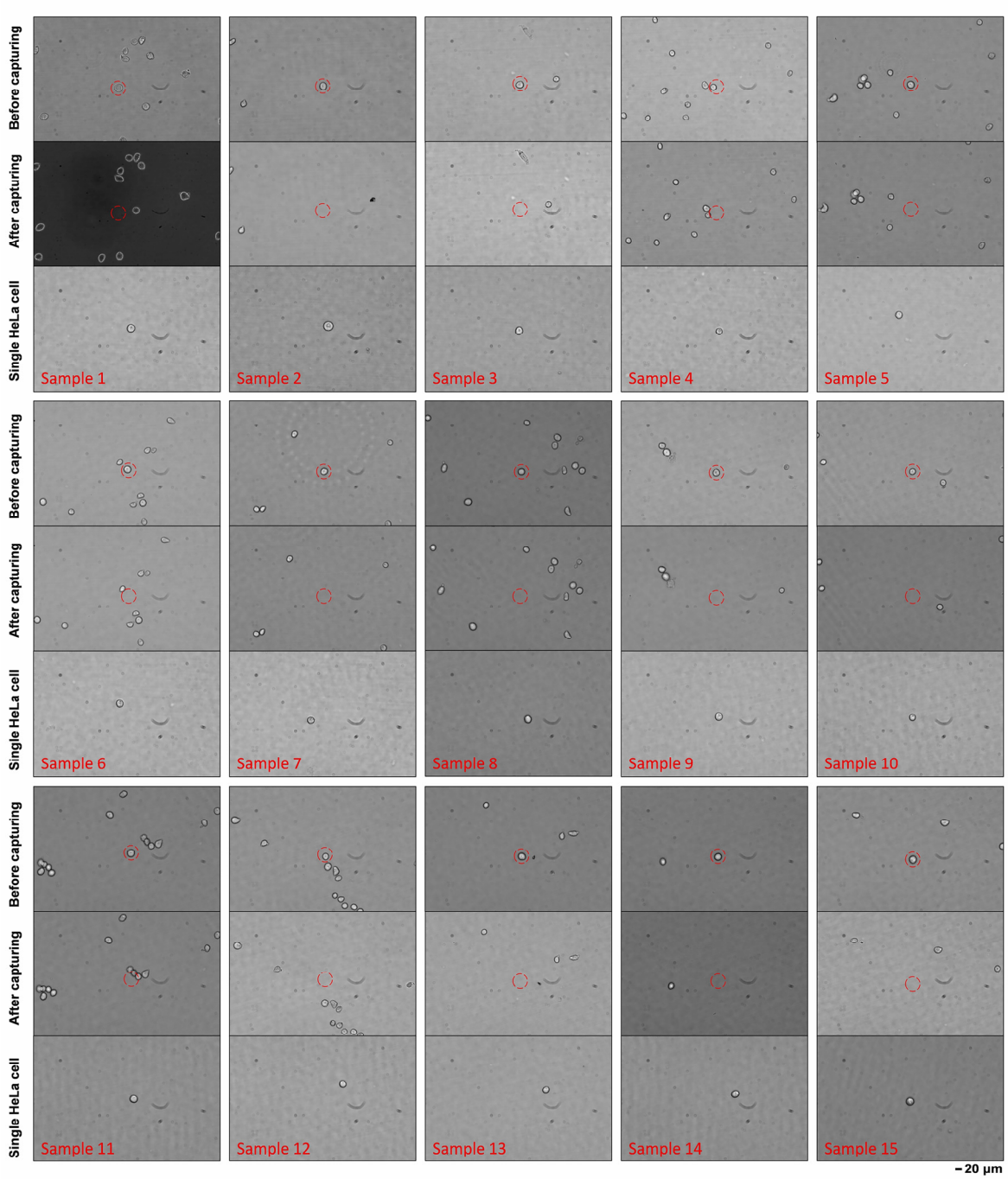

Fig. S1 A series of microscopic images of HeLa cells before and after single-cell capture in cell suspension in a petri dish, as well as the captured single cells in blank solution (n = 15).

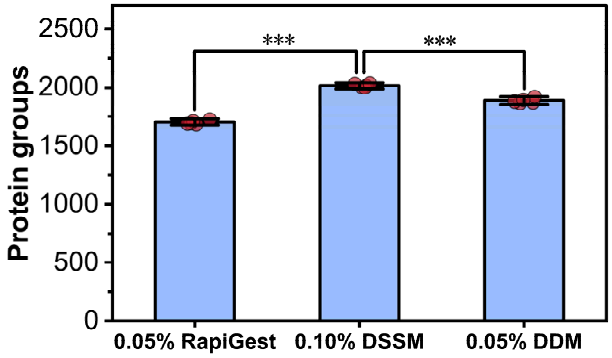

Fig. S2 Comparison of the identification numbers of protein groups in single HeLa cells using different surfactants including 0.05% RapiGest (n = 5), 0.10% DSSM (n = 5) or 0.05% DDM (n = 5). ***$p \leqslant$ 0.001.

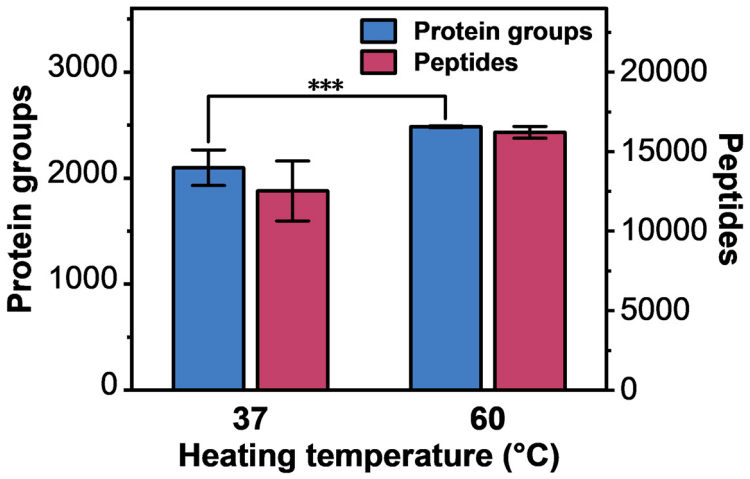

Fig. S3 Comparison of the effect of incubation at 37 °C without auxiliary heating treatments (n = 5) and with auxiliary heating treatments at 60 °C (n = 5) in quantified proteins in single HeLa cells. ***$p \leqslant$ 0.001.

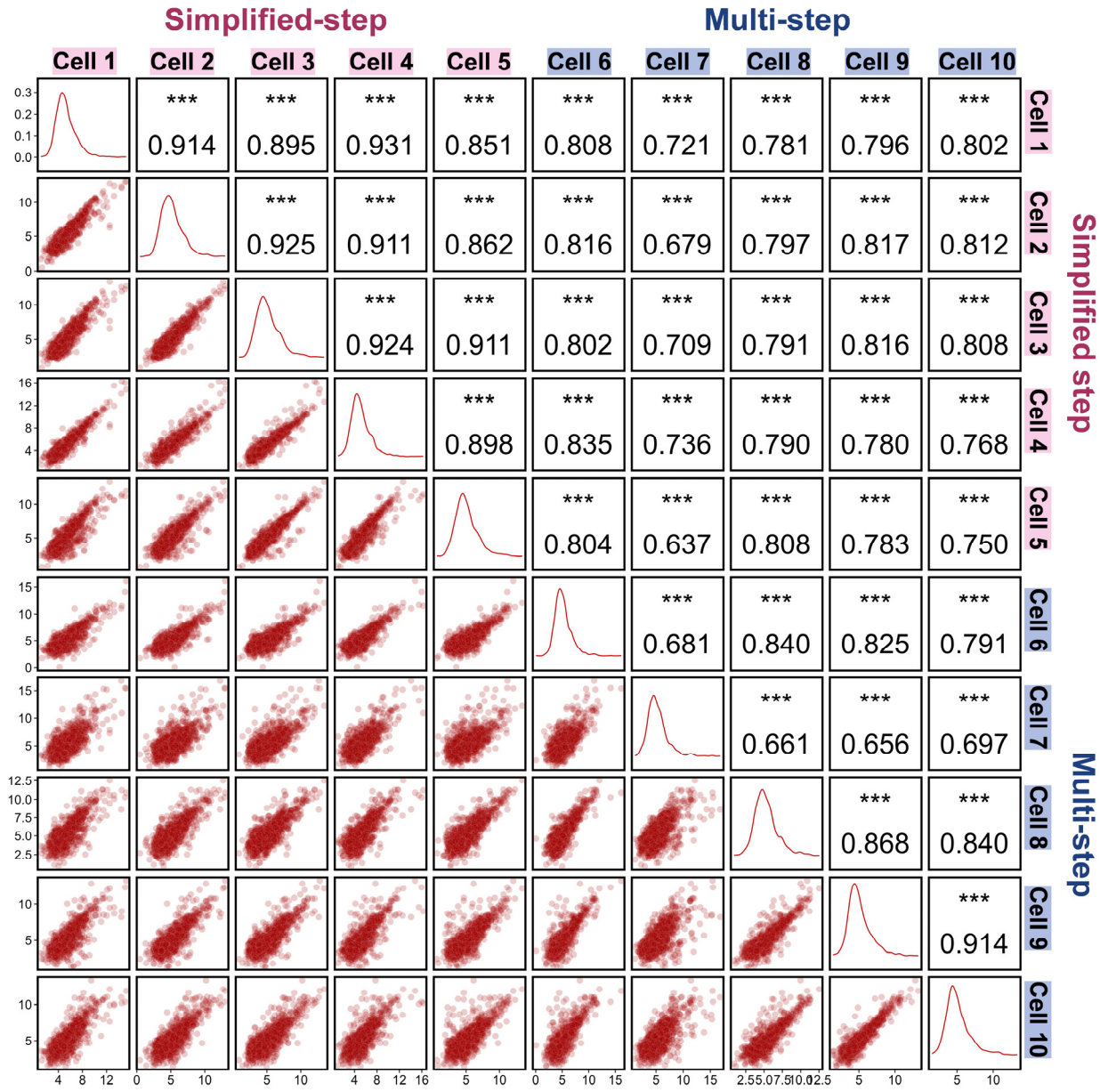

Fig. S4 Correlation analysis of protein expression in single HeLa cells using the simplified-step approach (n = 5) and the multi-step approach (n = 5). ***$p \leqslant 0.001$.

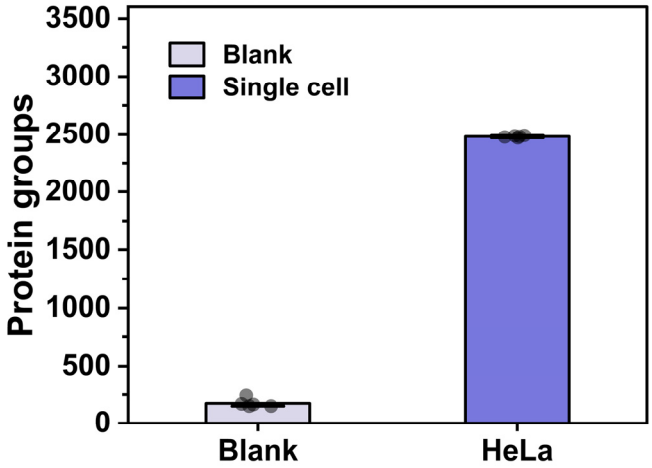

Fig. S5 Protein groups numbers identified in the blank samples (n = 5) and single-cell samples (n = 5).

Table S1 Accuracy and precision for 400 nL liquid manipulation.

Test No.	Mass before depositing 400 nL droplet (g)	Mass after depositing 400 nL droplet (g)	Δm (g)	Normalized to water volume (nL)
1	1.66707	1.66750	0.00043	430
2	1.66709	1.66752	0.00043	430
3	1.66703	1.66744	0.00041	410
4	1.66704	1.66742	0.00038	380
5	1.66703	1.66740	0.00037	370
6	1.66698	1.66740	0.00042	420
7	1.66701	1.66741	0.00040	400
8	1.66720	1.66758	0.00038	380
9	1.66701	1.66744	0.00043	430
10	1.66700	1.66743	0.00043	430
11	1.66695	1.66735	0.00040	400
12	1.66698	1.66736	0.00038	380
13	1.66700	1.66740	0.00040	400
14	1.66704	1.66744	0.00040	400
15	1.66703	1.66741	0.00038	380
Average	/	/	/	403
CV	/	/	/	5.3%

Table S2 is an independent Excel file that has been uploaded as Supporting Information with a file name of “Table S1. Identification data of proteins the single A549 and HeLa cells.”.

Table S3 List of differentially abundant proteins identified between the A549 and HeLa cell lines.

ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
AKR1C3	3.936076	4.40476	6.799342	9.37E-07	4.00E-05	5.76793
ASS1	-3.65193	6.377783	-11.7192	9.51E-11	1.51E-07	14.74255
PCDHGC4	-2.43923	5.299937	-3.85558	0.000899	0.006051	-0.99916
ASPH	2.386268	3.980432	8.917927	1.23E-08	2.02E-06	10.03354
FLNC	2.224754	5.818796	4.516266	0.000184	0.001789	0.552866
PRR4	-2.21722	5.856755	-3.60919	0.00162	0.009242	-1.56954
PSMG1	2.216548	5.294284	11.34201	1.74E-10	1.51E-07	14.16483
ATP5IF1	2.085832	5.373809	9.189329	7.40E-09	1.58E-06	10.53443
GLO1	-2.08526	5.871674	-10.7889	4.34E-10	2.31E-07	13.28757
CSTA	-2.07262	8.693002	-4.93554	6.73E-05	0.000841	1.540324
RPS29	1.878011	6.348962	11.22067	2.12E-10	1.51E-07	13.9755
NDUFA5	-1.87625	3.960551	-5.06317	4.97E-05	0.00068	1.839684
TUBB3	1.851975	3.290839	8.026828	7.09E-08	5.53E-06	8.316583
PDLIM5	1.830812	3.468394	8.030069	7.04E-08	5.53E-06	8.32303
GLS	1.788904	5.35647	5.731825	1.04E-05	0.000212	3.389561
ZG16B	-1.76809	4.318763	-3.39518	0.002691	0.013256	-2.05796
KRT77	1.767644	10.66587	2.898709	0.008513	0.031643	-3.15358
SH3BGRL3	1.743631	6.039458	7.98105	7.78E-08	5.53E-06	8.225344
NQO1	1.729035	6.206747	6.724076	1.10E-06	4.36E-05	5.604891
PDLIM7	1.722413	4.866499	6.845488	8.47E-07	3.84E-05	5.867518
ARHGAP17	1.72165	4.234506	10.10266	1.41E-09	5.02E-07	12.14701
CPS1	-1.71454	7.464822	-5.18909	3.69E-05	0.000542	2.134132
MAP2K2	1.682704	4.161978	8.786844	1.59E-08	2.07E-06	9.787967
DPY30	-1.64184	4.195057	-7.04163	5.54E-07	2.78E-05	6.287594
ITGAV	1.614115	3.281244	8.117811	5.90E-08	5.47E-06	8.497031
CYB5B	-1.58084	5.27873	-6.73656	1.07E-06	4.33E-05	5.631997
ACTN4	1.578046	7.419805	9.934789	1.90E-09	5.79E-07	11.85889
SYNGR2	-1.57046	5.950734	-4.98256	6.02E-05	0.000773	1.650694
GSTM3	1.570417	6.227262	5.494879	1.80E-05	0.00033	2.844553
ANXA3	1.56861	5.400452	5.903031	6.98E-06	0.000162	3.779914
HPRT1	-1.56584	5.447339	-7.57793	1.78E-07	1.12E-05	7.409053
MCAM	1.548102	3.078354	6.068718	4.78E-06	0.000119	4.15468
TES	1.538312	4.807891	8.228615	4.73E-08	4.58E-06	8.715204
FABP5	-1.52507	9.515138	-3.39811	0.002673	0.013196	-2.05133
CKAP4	1.524625	5.030084	9.214097	7.06E-09	1.58E-06	10.57964
IFT25	-1.50613	3.2082	-4.30173	0.000308	0.002668	0.047056
PTGES3	-1.48917	5.692851	-8.9516	1.16E-08	2.02E-06	10.09624
FKBP9	1.480224	4.408221	6.00996	5.47E-06	0.000131	4.022127
TM9SF2	1.475876	3.920631	8.765354	1.65E-08	2.07E-06	9.74748
MARCKS	-1.46954	5.443119	-4.75358	0.000104	0.001205	1.112314
ACOT13	-1.46636	4.686799	-9.85814	2.18E-09	5.81E-07	11.72611
IL36G	-1.44951	5.16324	-2.65486	0.014715	0.04889	-3.66537

TUBB6	1.436123	6.33021	8.612439	2.22E-08	2.63E-06	9.457517
ALDH1A3	1.417619	3.910359	4.710722	0.000115	0.001307	1.011356
RNPEP	-1.39811	4.733671	-6.65661	1.28E-06	4.71E-05	5.458117
FLNB	1.397668	7.601996	7.188401	4.04E-07	2.15E-05	6.598495
NXN	1.395413	3.182981	5.234355	3.31E-05	0.000507	2.239731
MSN	1.386377	7.152952	8.806538	1.53E-08	2.07E-06	9.825016
CHMP1B	1.386341	4.222936	7.177952	4.14E-07	2.15E-05	6.576459
LIG1	1.381722	3.541202	3.817235	0.000986	0.006443	-1.08838
DDB2	1.364678	4.142858	6.813088	9.09E-07	3.96E-05	5.797627
ABCC1	1.358452	3.453479	8.381115	3.49E-08	3.73E-06	9.012628
TMX3	1.356105	3.205873	6.923766	7.15E-07	3.39E-05	6.035793
LGALS1	1.356082	7.413005	5.419145	2.15E-05	0.000381	2.669289
MYH9	1.352009	6.996067	7.998301	7.51E-08	5.53E-06	8.259761
MIF	1.335499	6.488651	2.729236	0.012473	0.042432	-3.51148
TUBB4A	1.331601	5.756937	4.704148	0.000117	0.001321	0.995865
FERMT2	1.3312	4.624513	7.285589	3.29E-07	1.80E-05	6.802722
TMED4	-1.32598	3.882189	-4.41695	0.000233	0.002135	0.318627
TUBA4A	1.309625	7.38604	7.447483	2.34E-07	1.39E-05	7.139987
SLC7A5	1.297949	6.335751	5.837696	8.11E-06	0.000177	3.631309
PTMS	1.288337	7.704394	5.343837	2.56E-05	0.00043	2.494546
EEF1A2	1.276421	5.546804	3.517948	0.002013	0.010627	-1.77871
ITGB1	1.257503	5.479732	7.585868	1.75E-07	1.12E-05	7.425357
RSU1	1.252856	4.731904	7.359514	2.81E-07	1.62E-05	6.957183
TOMM6	1.242838	5.101543	6.687035	1.20E-06	4.65E-05	5.524379
UBE2S	1.242685	4.067822	3.946688	0.000723	0.005119	-0.7866
SERPINH1	1.241513	5.443466	3.931314	0.00075	0.00521	-0.82252
ALDH3A2	1.236768	3.781486	5.094887	4.61E-05	0.000643	1.913936
EFHD2	-1.2357	4.04059	-3.95363	0.000711	0.005071	-0.77037
DENR	1.232603	3.99586	6.372937	2.41E-06	7.89E-05	4.834513
UBE2H	1.230066	3.381712	3.203812	0.004215	0.018771	-2.48741
CMBL	-1.22922	5.766087	-4.33484	0.000284	0.002484	0.125054
KRT18	-1.22577	8.45795	-4.52192	0.000181	0.001773	0.566209
UGDH	1.220161	5.982577	6.530832	1.69E-06	6.01E-05	5.182882
PPA1	-1.21954	6.866053	-10.4808	7.32E-10	3.12E-07	12.78274
ALB	-1.21203	9.435385	-3.39325	0.002703	0.013286	-2.06233
LRPPRC	-1.20619	6.824484	-8.5085	2.72E-08	3.05E-06	9.258544
TXNRD1	1.200003	4.765637	5.109785	4.45E-05	0.000633	1.948795
ATP6	-1.19968	5.23491	-3.36551	0.002886	0.013958	-2.12504
TMED2	-1.19421	3.987412	-2.90582	0.008377	0.031453	-3.13836
PODXL	1.193437	4.954199	3.896934	0.000814	0.005532	-0.90277
COX7A2	-1.19311	5.603622	-3.14455	0.004839	0.021087	-2.61874
PPIC	1.184663	4.372707	5.243712	3.24E-05	0.000507	2.261544
ACTBL2	1.184368	11.14808	4.064738	0.000544	0.004206	-0.5102
GBE1	1.179578	4.278924	4.508882	0.000187	0.001813	0.535449
VAMP3	1.170018	2.851471	4.180933	0.000412	0.003376	-0.2373

SUB1	1.168369	7.64908	6.402281	2.25E-06	7.51E-05	4.899492
PSME1	-1.16074	5.599829	-6.26944	3.03E-06	9.21E-05	4.604466
WDR1	1.158773	7.402086	6.070254	4.76E-06	0.000119	4.15814
SNX12	1.155004	4.179857	6.452347	2.01E-06	7.04E-05	5.01011
HINT2	1.154992	3.502355	4.53442	0.000176	0.00174	0.595684
POFUT1	1.154129	3.509096	5.933114	6.52E-06	0.000154	3.848183
NEK7	1.153984	3.381982	4.640389	0.000136	0.001447	0.845581
TCEA1	1.150671	4.859589	6.895162	7.60E-07	3.53E-05	5.974398
EPHX1	1.147861	4.087401	3.634967	0.001524	0.008833	-1.51023
MTHFD2	-1.1354	4.527142	-4.24689	0.000351	0.00296	-0.08211
KYNU	-1.13393	5.127757	-6.55671	1.60E-06	5.78E-05	5.239679
NPM1	-1.12746	8.249948	-5.35451	2.50E-05	0.000429	2.519349
ATP1B1	1.126127	3.758781	5.862274	7.67E-06	0.00017	3.687265
C8orf33	1.125818	3.66517	6.110965	4.34E-06	0.000114	4.249743
S100A6	1.122886	10.50379	6.292481	2.88E-06	9.04E-05	4.655802
CTSB	-1.11923	5.110845	-3.10325	0.005325	0.022624	-2.70975
VIM	1.117774	9.566289	5.913648	6.81E-06	0.00016	3.804018
PGRMC2	1.114397	5.093689	6.200001	3.55E-06	9.58E-05	4.44941
EHD2	1.111192	3.910149	4.641258	0.000136	0.001447	0.847629
SLC2A1	1.106946	5.343138	3.791534	0.001048	0.006696	-1.1481
NDUFB10	-1.10308	4.76248	-4.59202	0.000153	0.001586	0.731529
TRIM16	1.101452	4.115901	4.551911	0.000169	0.001688	0.636937
EHD4	1.090832	3.483415	6.416064	2.18E-06	7.40E-05	4.929976
UBAP2	1.078606	3.538836	5.766187	9.57E-06	0.000202	3.468149
TKFC	-1.05458	2.848502	-3.11323	0.005203	0.022152	-2.68779
DSTN	1.049481	6.252502	5.373585	2.39E-05	0.000417	2.563627
PAFAH1B2	1.049284	3.82466	3.027178	0.006346	0.025932	-2.8762
CTTN	1.046432	6.350559	8.253214	4.50E-08	4.57E-06	8.763401
PPP3CA	1.045847	4.425186	8.059471	6.64E-08	5.53E-06	8.38146
GALNT2	1.044185	5.086139	5.647026	1.26E-05	0.00024	3.195119
NUCB1	1.041343	4.803993	6.039987	5.10E-06	0.000125	4.089913
PDCD6	1.030009	5.932162	9.016141	1.02E-08	1.99E-06	10.21598
DDT	1.02858	5.902511	4.847547	8.30E-05	0.001006	1.333498
OAT	-1.02708	5.079414	-5.64928	1.25E-05	0.00024	3.2003
IGBP1	1.023544	3.699434	6.833263	8.70E-07	3.87E-05	5.841163
SOAT1	-1.01925	4.171562	-3.42194	0.002526	0.01274	-1.99733
COMT	1.016318	3.699755	4.115217	0.000482	0.003817	-0.39173
GMFB	1.015712	3.865412	5.667655	1.20E-05	0.000233	3.242486
RCN1	-1.01566	6.660164	-7.58241	1.76E-07	1.12E-05	7.418257
FERMT1	1.008596	3.802657	3.77342	0.001095	0.006869	-1.19015
TAX1BP3	1.004987	3.002768	2.769422	0.0114	0.039475	-3.4275
CNN2	1.003968	5.417961	4.175951	0.000416	0.003404	-0.24901
WASF2	1.003763	3.242017	5.172599	3.83E-05	0.00056	2.095626