

ANKRD22, a novel mitochondrial protein, induced by the tumor microenvironment promotes the metabolic reprogramming of colorectal cancer cells

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Figure S1

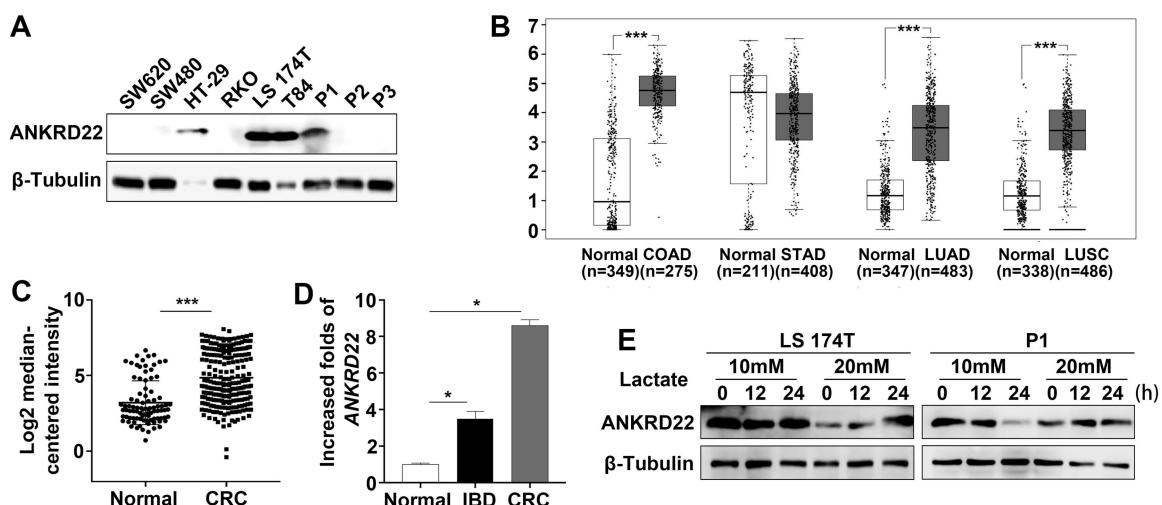


Figure S1 related to Figure 1. (A) Detection of the expression of ANKRD22 in different CRC cell lines by Western blot. (B) Expression of ANKRD22 in different tumors and corresponding normal tissues. Analysis was performed by GEPIA (<http://gepia.cancer-pku.cn>), of which RNA sequencing expression data were from the TCGA and the GTEx projects. COAD, Colon adenocarcinoma. STAD, Stomach adenocarcinoma. LUAD, Lung adenocarcinoma. LUSC, Lung squamous cell carcinoma. (C) Analysis of ANKRD22 expression in CRC (n=241) and normal colorectal tissues (n=90) in 4 datasets from the Oncomine database. Data were analyzed by Student's *t*-test, ****p*<0.001. (D) The relative expression of ANKRD22 in normal colorectum (n=3), IBD (n=3) and CRC (n=3) epithelia measured by RT-qPCR. (E) Detection of the effect of lactate stimulation on the expression of ANKRD22 in CRC cell by Western blot.

Figure S2

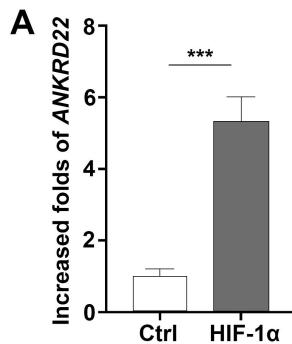


Figure S2 related to Figure 2. (A) Detection of the effect of HIF-1 α overexpression on ANKRD22 expression in SGC-7901 cells by RT-qPCR. Data were displayed as mean \pm SD and analyzed by Student's *t*-test, *** p <0.001.

Figure S3

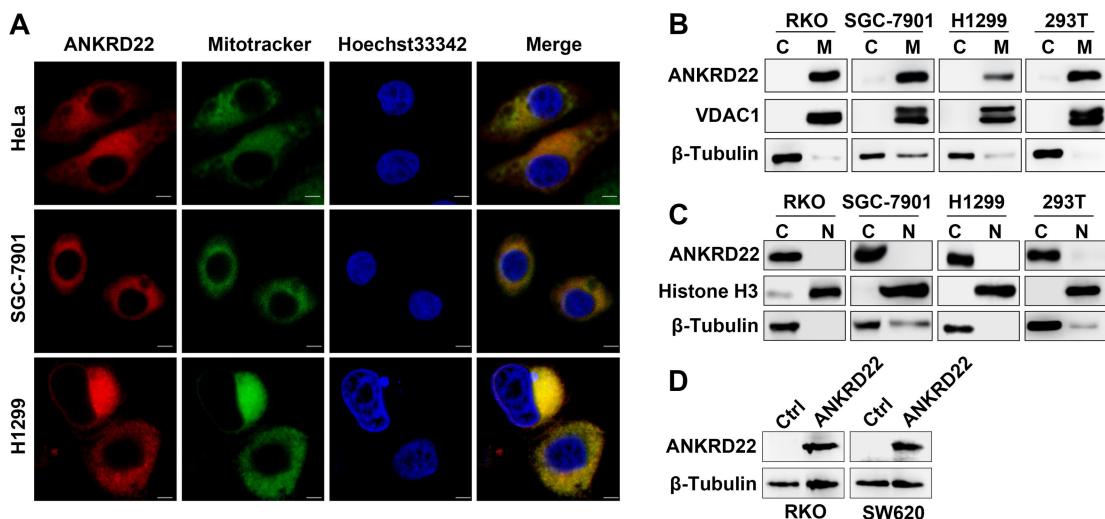


Figure S3 related to Figure 3. (A) Co-localization detection of exogenous-expressing ANKRD22 and mitochondria by confocal microscopy in HeLa, SGC-7901 and NCI-H1299 cells. Scale bar: 5 μ m. (B) Detection of exogenous-expressing ANKRD22 in the mitochondria (M) and residual cytoplasmic component (C) of cells by WB. β -Tubulin and VDAC1 were internal references for cytoplasmic and mitochondrial fractions, respectively. (C) Detection of exogenous-expressing ANKRD22 in the nucleus (N) and cytoplasm (C) of cells by WB. Histone 3 and β -Tubulin were internal references for nuclear and cytoplasmic fractions respectively. (D) Detection of ANKRD22 expression by WB in RKO and SW620 cells infected by lentivirus encoding Halo-ANKRD22 fusion protein. Cells infected by empty vector lentivirus were used as a control.

Figure S4

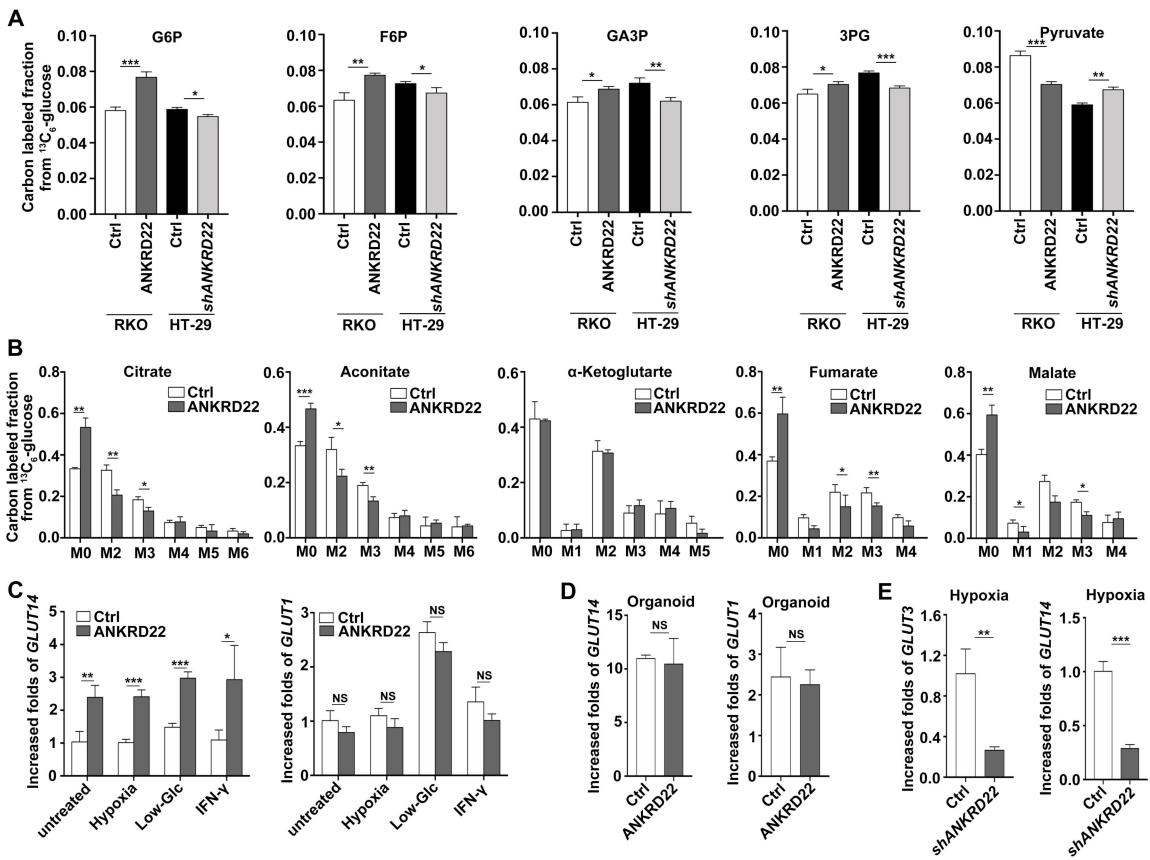


Figure S4 related to Figure 4. (A) The fraction of ^{13}C isotope-labeled metabolites in glycolysis, including G6P, F6P, GA3P, 3PG and pyruvate in ANKRD22-overexpressing RKO cells and control cells, ANKRD22-knockdown HT-29 cells and control cell. (B) The fraction of ^{13}C isotope-labeled metabolites in the TCA cycle, including citrate, aconitate, α -ketoglutarate, fumarate and malate in ANKRD22-overexpressing RKO cells and control cells. M0 to Mn isotopologues indicate the number of ^{13}C atoms present in each metabolite. (C) Determination of the effect of ANKRD22 overexpression on the expression of *GLUT14* and *GLUT1* under TMEs by RT-qPCR. (D) Determination of the effect of ANKRD22 overexpression on the expression of *GLUT14* and *GLUT1* in organoid-cultured RKO cells by RT-qPCR. Results in C and D were normalized to the values of control cells in the untreated group cultured under 2D condition. (E) Determination of the effect of ANKRD22 knockdown on the expression of *GLUT3* and *GLUT14* in HT-29 cells under hypoxia by RT-qPCR. Data in this figure were presented as mean \pm SD and analyzed by Student's *t*-test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS, not significant.

Figure S5

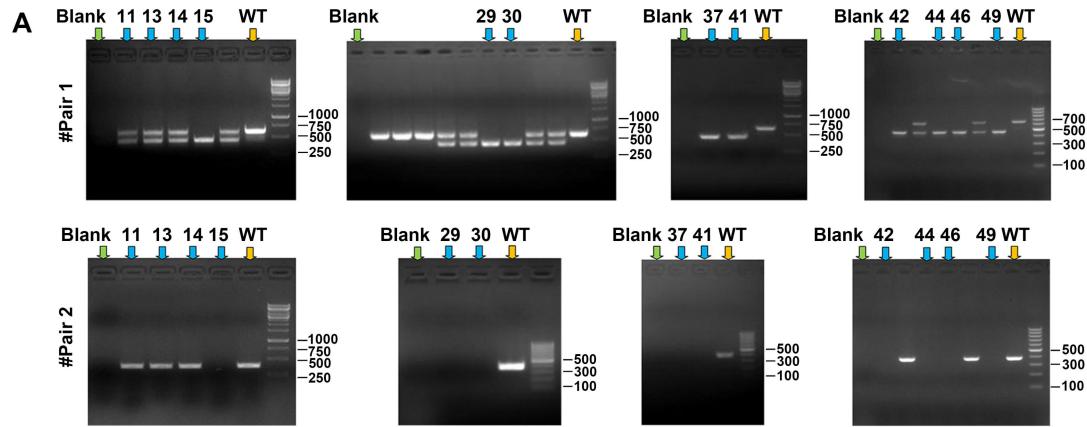


Figure S5 related to Figure 5. (A) The results of *Ankrd22* knockout of C57BL/6 mice were identified by PCR. Two different pairs of primers were used to amplify *Ankrd22*.

Figure S6

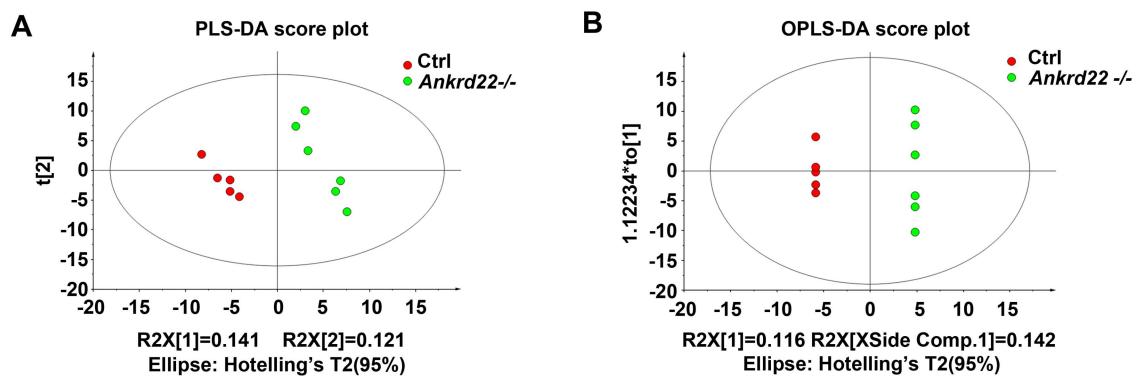


Figure S6 related to Figure 6. (A) PLS-DA score plot of *Ankrd22*^{-/-}(n=6) and Ctrl (n=5) group in lipidomics analysis. (B) OPLS-DA score plot of *Ankrd22*^{-/-}(n=6) and Ctrl (n=5) group in lipidomics analysis.

Figure S7

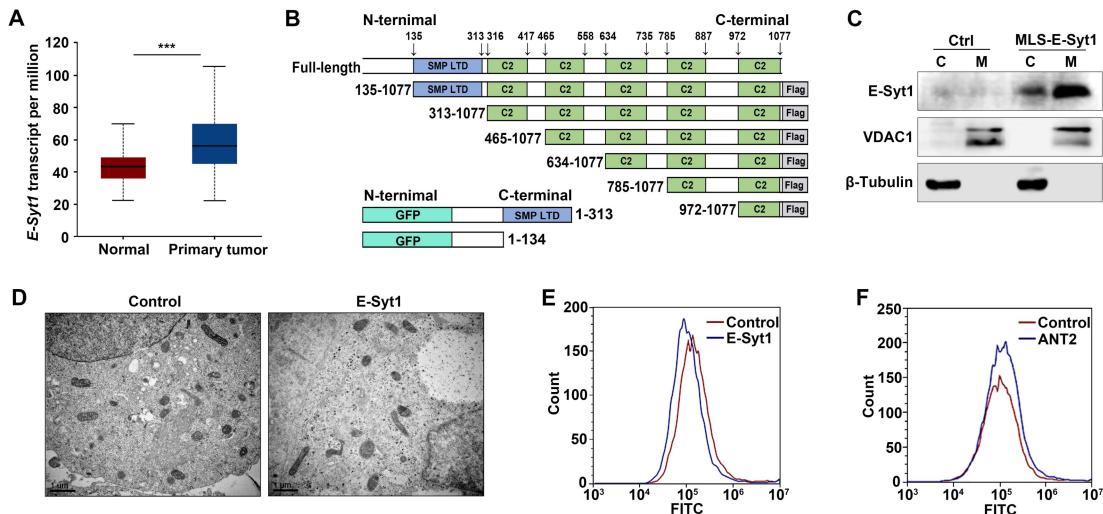


Figure S7 related to Figure 7. (A) *E-Syt1* expression in normal colorectal tissues (n=41) and CRC (n=286) analyzed by UALCAN website, *** $p<0.001$. (B) A schematic map of constructed eight different fragments of *E-Syt1* based on its domains. 6 fragments fused with Flag tag at the C-terminal while 2 fragments fused with GFP tag at the N-terminal. (C) Detection of *E-Syt1* expression in the mitochondria of 293T cells that were transfected with MLS-*E-Syt1* or empty vector (Ctrl). β -Tubulin and VDAC1 were internal references for cytoplasmic (C) and mitochondrial (M) fractions, respectively (D) Observation of mitochondria morphology of 293T cells that were transfected with wild *E-Syt1* or empty vector and observed under a transmission electron microscope. (E-F) Effect of *E-Syt1* or ANT2 on cytoplasmic Ca^{2+} level. Fluo-4 staining was used to detect the effect of *E-Syt1* or ANT2 on the cytoplasmic Ca^{2+} level of SGC-7901 cell. Fluorescence intensity of FITC was detected by FCM.

Figure S8

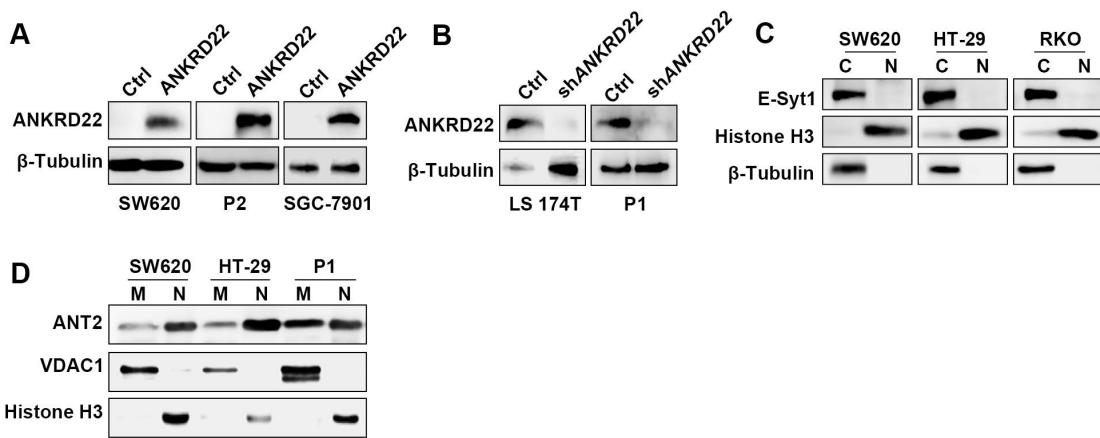


Figure S8 related to Figure 8. **(A)** Detection of ANKRD22 expression in gastrointestinal cells infected by lentivirus encoding ANKRD22 or empty vector lentivirus (Ctrl) by WB. **(B)** Detection of ANKRD22 expression in CRC cells infected by *ANKRD22* shRNA lentivirus or scrambled shRNA lentivirus (Ctrl) by WB. **(C)** Detection of E-Syt1 expression in the cytoplasm (C) and nucleus (N) of CRC cells by WB. Histone 3 and β -Tubulin were nuclear and cytoplasmic internal references respectively. **(D)** Detection of ANT2 expression in mitochondria (M) and nucleus (N) of CRC cells by WB. Histone 3 and VDAC1 were nuclear and mitochondrial internal references respectively.

Table S1**Pulldown proteins of ANKRD22 in 2D culture**

Link	Pulldown molecules of ANKRD22		Pulldown molecules of negative control		Sequence Header
	Relative Abundance	Number of Peptides	Relative Abundance	Number of Peptides	
P04792	0.032093149	10	0	0	>sp P04792 HSPB1_HUMAN Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=2
P05141	0.132670483	8	0	0	>sp P05141 ADT2_HUMAN ADP/ATP translocase 2 OS=Homo sapiens GN=SLC25A5 PE=1 SV=7
Q71U36	0.043188367	8	0	0	>sp Q71U36 TBA1A_HUMAN Tubulin alpha-1A chain OS=Homo sapiens GN=TUBA1A PE=1 SV=1
P18085	0.112377208	7	0	0	>sp P18085 ARF4_HUMAN ADP-ribosylation factor 4 OS=Homo sapiens GN=ARF4 PE=1 SV=3
P23396	0.039478857	7	0	0	>sp P23396 RS3_HUMAN 40S ribosomal protein S3 OS=Homo sapiens GN=RPS3 PE=1 SV=2
P07437	0.017000136	7	0	0	>sp P07437 TBB5_HUMAN Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2
P62269	0.023538118	5	0	0	>sp P62269 RS18_HUMAN 40S ribosomal protein S18 OS=Homo sapiens GN=RPS18 PE=1 SV=3
P62249	0.051934671	5	0	0	>sp P62249 RS16_HUMAN 40S ribosomal protein S16 OS=Homo sapiens GN=RPS16 PE=1 SV=2
P60709	0.008775214	4	0	0	>sp P60709 ACTB_HUMAN Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
O75947	0.027605297	4	0	0	>sp O75947 ATP5H_HUMAN ATP synthase subunit d, mitochondrial OS=Homo sapiens GN=ATP5H PE=1 SV=3
P24539	0.011735538	4	0	0	>sp P24539 AT5F1_HUMAN ATP synthase F(0) complex subunit B1, mitochondrial OS=Homo sapiens GN=ATP5F1 PE=1 SV=2
P51571	0.035619913	4	0	0	>sp P51571 SSRD_HUMAN Translocon-associated protein subunit

					delta OS=Homo sapiens GN=SSR4 PE=1 SV=1
Q01650	0.003162564	3	0	0	>sp Q01650 LAT1_HUMAN Large neutral amino acids transporter small subunit 1 OS=Homo sapiens GN=SLC7A5 PE=1 SV=2
P51149	0.014755368	3	0	0	>sp P51149 RAB7A_HUMAN Ras-related protein Rab-7a OS=Homo sapiens GN=RAB7A PE=1 SV=1
Q00325	0.016866797	3	0	0	>sp Q00325 MPCP_HUMAN Phosphate carrier protein, mitochondrial OS=Homo sapiens GN=SLC25A3 PE=1 SV=2
P36542	0.008280994	3	0	0	>sp P36542 ATPG_HUMAN ATP synthase subunit gamma, mitochondrial OS=Homo sapiens GN=ATP5C1 PE=1 SV=1
Q5VYY1	0.009275611	3	0	0	>sp Q5VYY1 ANR22_HUMAN Ankyrin repeat domain-containing protein 22 OS=Homo sapiens GN=ANKRD22 PE=2 SV=1
P62263	0.020528619	3	0	0	>sp P62263 RS14_HUMAN 40S ribosomal protein S14 OS=Homo sapiens GN=RPS14 PE=1 SV=3
Q8WY22	0.031777639	3	0	0	>sp Q8WY22 BRI3B_HUMAN BRI3-binding protein OS=Homo sapiens GN=BRI3BP PE=1 SV=1
P51148	0.018565728	3	0	0	>sp P51148 RAB5C_HUMAN Ras-related protein Rab-5C OS=Homo sapiens GN=RAB5C PE=1 SV=2
P48047	0.036047137	3	0	0	>sp P48047 ATPO_HUMAN ATP synthase subunit O, mitochondrial OS=Homo sapiens GN=ATP5O PE=1 SV=1
P62913	0.010767888	2	0	0	>sp P62913 RL11_HUMAN 60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2
O95816	0.00299205	2	0	0	>sp O95816 BAG2_HUMAN BAG family molecular chaperone regulator 2 OS=Homo sapiens GN=BAG2 PE=1 SV=1
P42677	0.040577173	2	0	0	>sp P42677 RS27_HUMAN 40S ribosomal protein S27 OS=Homo sapiens GN=RPS27 PE=1 SV=3

P51151	0.00527008	2	0	0	>sp P51151 RAB9A_HUMAN Ras-related protein Rab-9A OS=Homo sapiens GN=RAB9A PE=1 SV=1
P61026	0.009804593	2	0	0	>sp P61026 RAB10_HUMAN Ras-related protein Rab-10 OS=Homo sapiens GN=RAB10 PE=1 SV=1
Q9NR31	0.055170446	2	0	0	>sp Q9NR31 SAR1A_HUMAN GTP-binding protein SAR1a OS=Homo sapiens GN=SAR1A PE=1 SV=1
O60762	0.009630416	2	0	0	>sp O60762 DPM1_HUMAN Dolichol-phosphate mannosyltransferase subunit 1 OS=Homo sapiens GN=DPM1 PE=1 SV=1
Q969X5	0.004517752	2	0	0	>sp Q969X5 ERGI1_HUMAN Endoplasmic reticulum-Golgi intermediate compartment protein 1 OS=Homo sapiens GN=ERGIC1 PE=1 SV=1
Q15118	0.000472479	2	0	0	>sp Q15118 PDK1_HUMAN [Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 1, mitochondrial OS=Homo sapiens GN=PDK1 PE=1 SV=1
Q07021	0.012608879	2	0	0	>sp Q07021 C1QBP_HUMAN Complement component 1 Q subcomponent-binding protein, mitochondrial OS=Homo sapiens GN=C1QBP PE=1 SV=1
P61224	0.001925609	1	0	0	>sp P61224 RAP1B_HUMAN Ras-related protein Rap-1b OS=Homo sapiens GN=RAP1B PE=1 SV=1
P11441	0.002690838	1	0	0	>sp P11441 UBL4A_HUMAN Ubiquitin-like protein 4A OS=Homo sapiens GN=UBL4A PE=1 SV=1
P67812	0.001898596	1	0	0	>sp P67812 SEC11A_HUMAN Signal peptidase complex catalytic subunit SEC11A OS=Homo sapiens GN=SEC11A PE=1 SV=1
P62829	0.003130231	1	0	0	>sp P62829 RL23_HUMAN 60S ribosomal protein L23 OS=Homo sapiens GN=RPL23 PE=1 SV=1

P62987	0.008166933	1	0	0	>sp P62987 RL40_HUMAN Ubiquitin-60S ribosomal protein L40 OS=Homo sapiens GN=UBA52 PE=1 SV=2
P60866	0.004948391	1	0	0	>sp P60866 RS20_HUMAN 40S ribosomal protein S20 OS=Homo sapiens GN=RPS20 PE=1 SV=1
Q9GZT3	0.00483787	1	0	0	>sp Q9GZT3 SLIRP_HUMAN SRA stem-loop-interacting RNA-binding protein, mitochondrial OS=Homo sapiens GN=SLIRP PE=1 SV=1
P18859	0.005039536	1	0	0	>sp P18859 ATP5J_HUMAN ATP synthase-coupling factor 6, mitochondrial OS=Homo sapiens GN=ATP5J PE=1 SV=1
O75964	0.005431206	1	0	0	>sp O75964 ATP5L_HUMAN ATP synthase subunit g, mitochondrial OS=Homo sapiens GN=ATP5L PE=1 SV=3
P62805	0.004814516	1	0	0	>sp P62805 H4_HUMAN Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2
P02649	0.010311003	1	0.729752771	2	>sp P02649 APOE_HUMAN Apolipoprotein E OS=Homo sapiens GN=APOE PE=1 SV=1
P0DMV9	0.001435031	1	0	0	>sp P0DMV9 HS71B_HUMAN Heat shock 70 kDa protein 1B OS=Homo sapiens GN=HSPA1B PE=1 SV=1
P06576	0.00091116	1	0	0	>sp P06576 ATPB_HUMAN ATP synthase subunit beta, mitochondrial OS=Homo sapiens GN=ATP5B PE=1 SV=3
Q5VTE0	0.001489542	1	0	0	>sp Q5VTE0 EF1A3_HUMAN Putative elongation factor 1-alpha-like 3 OS=Homo sapiens GN=EEF1A1P5 PE=5 SV=1
P31689	0.000862834	1	0	0	>sp P31689 DNJA1_HUMAN DnaJ homolog subfamily A member 1 OS=Homo sapiens GN=DNAJA1 PE=1 SV=2
Q96G23	0.00068181	1	0	0	>sp Q96G23 CERS2_HUMAN Ceramide synthase 2 OS=Homo sapiens GN=CERS2 PE=1 SV=1

O14828	0.001694094	1	0	0	>sp O14828 SCAM3_HUMAN Secretory carrier-associated membrane protein 3 OS=Homo sapiens GN=SCAMP3 PE=1 SV=3
P59190	0.008557488	1	0	0	>sp P59190 RAB15_HUMAN Ras-related protein Rab-15 OS=Homo sapiens GN=RAB15 PE=1 SV=1
Q6IAN0	0.001042671	1	0	0	>sp Q6IAN0 DRS7B_HUMAN Dehydrogenase/reductase SDR family member 7B OS=Homo sapiens GN=DHRS7B PE=1 SV=2
Q9UNL2	0.050330095	1	0	0	>sp Q9UNL2 SSRG_HUMAN Translocon-associated protein subunit gamma OS=Homo sapiens GN=SSR3 PE=1 SV=1
Q02978	0.001982476	1	0	0	>sp Q02978 M2OM_HUMAN Mitochondrial 2-oxoglutarate/malate carrier protein OS=Homo sapiens GN=SLC25A11 PE=1 SV=3
Q9NZ01	0.004506917	1	0	0	>sp Q9NZ01 TECR_HUMAN Very-long-chain enoyl-CoA reductase OS=Homo sapiens GN=TECR PE=1 SV=1
Q9H082	0.007922216	1	0	0	>sp Q9H082 RB33B_HUMAN Ras-related protein Rab-33B OS=Homo sapiens GN=RAB33B PE=1 SV=1
Q6PJF5	0.003818444	1	0.270247229	2	>sp Q6PJF5 RHDF2_HUMAN Inactive rhomboid protein 2 OS=Homo sapiens GN=RHBDF2 PE=1 SV=2
O75396	0.001275752	1	0	0	>sp O75396 SC22B_HUMAN Vesicle-trafficking protein SEC22b OS=Homo sapiens GN=SEC22B PE=1 SV=4
P61019	0.007333705	1	0	0	>sp P61019 RAB2A_HUMAN Ras-related protein Rab-2A OS=Homo sapiens GN=RAB2A PE=1 SV=1
Q8TED1	0.002684051	1	0	0	>sp Q8TED1 GPX8_HUMAN Probable glutathione peroxidase 8 OS=Homo sapiens GN=GPX8 PE=1 SV=2
P04406	0.003187823	1	0	0	>sp P04406 G3P_HUMAN Glyceraldehyde-3-phosphate

					dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3
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Table S2
Pulldown proteins of ANKRD22 in organoid culture

Link	Pulldown molecules of ANKRD22		Pulldown molecules of negative control		Sequence Header
	Relative Abundance	Number of Peptides	Relative Abundance	Number of Peptides	
Q9BSJ8	17.05%	45	0	0	>sp Q9BSJ8 ESYT1_HUMAN Extended synaptotagmin-1 OS=Homo sapiens GN=ESYT1 PE=1 SV=1
P07437	12.08%	16	0	0	>sp P07437 TBB5_HUMAN Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2
P68363	14.08%	16	0	0	>sp P68363 TBA1B_HUMAN Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1
P25705	1.75%	13	0	0	>sp P25705 ATPA_HUMAN ATP synthase subunit alpha, mitochondrial OS=Homo sapiens GN=ATP5A1 PE=1 SV=1
P60709	10.73%	12	0	0	>sp P60709 ACTB_HUMAN Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
P06576	2.72%	6	0	0	>sp P06576 ATPB_HUMAN ATP synthase subunit beta, mitochondrial OS=Homo sapiens GN=ATP5B PE=1 SV=3
Q5VTE0	2.44%	5	0	0	>sp Q5VTE0 EF1A3_HUMAN Putative elongation factor 1-alpha-like 3 OS=Homo sapiens GN=EEF1A1P5 PE=5 SV=1
P23396	1.49%	4	0	0	>sp P23396 RS3_HUMAN 40S ribosomal protein S3 OS=Homo sapiens GN=RPS3 PE=1 SV=2
Q9H293	23.16%	4	0	0	>sp Q9H293 IL25_HUMAN Interleukin-25 OS=Homo sapiens GN=IL25 PE=1 SV=1
P05141	3.26%	4	0	0	>sp P05141 ADT2_HUMAN

					ADP/ATP translocase 2 OS=Homo sapiens GN=SLC25A5 PE=1 SV=7
P12235	1.78%	3	0	0	>sp P12235 ADT1_HUMAN ADP/ATP translocase 1 OS=Homo sapiens GN=SLC25A4 PE=1 SV=4
P04792	0.71%	3	0	0	>sp P04792 HSPB1_HUMAN Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=2
Q8NCF7	1.22%	3	0	0	>tr Q8NCF7 Q8NCF7_HUMAN cDNA FLJ90278 fis, clone NT2RP1000325, highly similar to Phosphate carrier protein, mitochondrialprecursor OS=Homo sapiens PE=2 SV=1
Q5VW36	0.40%	3	0	0	>sp Q5VW36 FOCAD_HUMAN Focadhesin OS=Homo sapiens GN=FOCAD PE=1 SV=1
P11142	0.33%	3	0	0	>sp P11142 HSP7C_HUMAN Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1
O15397	0.12%	3	0	0	>sp O15397 IPO8_HUMAN Importin-8 OS=Homo sapiens GN=IPO8 PE=1 SV=2
P35579	0.02%	2	0	0	>sp P35579 MYH9_HUMAN Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4
Q92616	0.01%	2	0	0	>sp Q92616 GCN1_HUMAN eIF-2-alpha kinase activator GCN1 OS=Homo sapiens GN=GCN1 PE=1 SV=6
Q9Y5L0	0.04%	2	0	0	>sp Q9Y5L0 TNPO3_HUMAN Transportin-3 OS=Homo sapiens GN=TNPO3 PE=1 SV=3
A8K401	0.89%	2	0	0	>tr A8K401 A8K401_HUMAN Prohibitin, isoform CRA_a OS=Homo sapiens GN=PHB PE=2 SV=1
P51159	2.25%	2	0	0	>sp P51159 RB27A_HUMAN Ras-related protein Rab-27A OS=Homo sapiens GN=RAB27A PE=1 SV=3
Q9H0U4	0.34%	1	0	0	>sp Q9H0U4 RAB1B_HUMAN Ras-related protein Rab-1B

					OS=Homo sapiens GN=RAB1B PE=1 SV=1
F8WE04	0.45%	1	0	0	>tr F8WE04 F8WE04_HUMAN Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=1
Q9UNL2	0.29%	1	0	0	>sp Q9UNL2 SSRG_HUMAN Translocon-associated protein subunit gamma OS=Homo sapiens GN=SSR3 PE=1 SV=1
P62917	0.16%	1	0	0	>sp P62917 RL8_HUMAN 60S ribosomal protein L8 OS=Homo sapiens GN=RPL8 PE=1 SV=2
P09493	0.15%	1	0	0	>sp P09493 TPM1_HUMAN Tropomyosin alpha-1 chain OS=Homo sapiens GN=TPM1 PE=1 SV=2
P43307	0.12%	1	0	0	>sp P43307 SSRA_HUMAN Translocon-associated protein subunit alpha OS=Homo sapiens GN=SSR1 PE=1 SV=3
Q15800	0.12%	1	0	0	>sp Q15800 MSMO1_HUMAN Methylsterol monooxygenase 1 OS=Homo sapiens GN=MSMO1 PE=1 SV=1
Q53GQ0	0.61%	1	0	0	>sp Q53GQ0 DHB12_HUMAN Very-long-chain 3-oxoacyl-CoA reductase OS=Homo sapiens GN=HSD17B12 PE=1 SV=2
P31943	0.14%	1	0	0	>sp P31943 HNRH1_HUMAN Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRNPH1 PE=1 SV=4
P81605	0.31%	1	0	0	>sp P81605 DCD_HUMAN Dermcidin OS=Homo sapiens GN=DCD PE=1 SV=2
O14980	0.01%	1	0	0	>sp O14980 XPO1_HUMAN Exportin-1 OS=Homo sapiens GN=XPO1 PE=1 SV=1
Q13535	0.06%	1	0	0	>sp Q13535 ATR_HUMAN Serine/threonine-protein kinase ATR OS=Homo sapiens GN=ATR PE=1 SV=3
P39656	0.03%	1	0	0	>sp P39656 OST48_HUMAN Dolichyl-diphosphooligosaccharide--

					protein glycosyltransferase 48 kDa subunit OS=Homo sapiens GN=DDOST PE=1 SV=4
P05091	0.07%	1	0	0	>sp P05091 ALDH2_HUMAN Aldehyde dehydrogenase, mitochondrial OS=Homo sapiens GN=ALDH2 PE=1 SV=2
Q8IYD1	0.27%	1	0	0	>sp Q8IYD1 ERF3B_HUMAN Eukaryotic peptide chain release factor GTP-binding subunit ERF3B OS=Homo sapiens GN=GSPT2 PE=1 SV=2
P0DMV9	0.01%	1	0	0	>sp P0DMV9 HS71B_HUMAN Heat shock 70 kDa protein 1B OS=Homo sapiens GN=HSPA1B PE=1 SV=1
Q16891	0.04%	1	0	0	>sp Q16891 MIC60_HUMAN MICOS complex subunit MIC60 OS=Homo sapiens GN=IMMT PE=1 SV=1
Q13200	0.01%	1	0	0	>sp Q13200 PSMD2_HUMAN 26S proteasome non-ATPase regulatory subunit 2 OS=Homo sapiens GN=PSMD2 PE=1 SV=3
Q9P035	0.27%	1	0	0	>sp Q9P035 HACD3_HUMAN Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 3 OS=Homo sapiens GN=HACD3 PE=1 SV=2

Table S3**Pulldown proteins of nuclear ANKRD22 in organoid culture**

Link	Number of peptides			Sequence Header
	Pulldown molecules of ANKRD22 in SW620	Pulldown molecules of ANKRD22 in RKO	Pulldown molecules of ANKRD22 in negative control	
Q15149	3	0	0	>sp Q15149 PLEC_HUMAN Plectin OS=Homo sapiens GN=PLEC PE=1 SV=3
Q9UFH2	3	0	0	>sp Q9UFH2 DYH17_HUMAN Dynein heavy chain 17, axonemal OS=Homo sapiens GN=DNAH17 PE=1 SV=2
P78527	6	0	0	>sp P78527 PRKDC_HUMAN DNA-dependent protein kinase catalytic subunit OS=Homo sapiens GN=PRKDC PE=1 SV=3
P15924	4	0	0	>sp P15924 DESP_HUMAN Desmoplakin OS=Homo sapiens GN=DSP PE=1 SV=3
Q13813	62	0	0	>sp Q13813 SPTN1_HUMAN Spectrin alpha chain, non-erythrocytic 1 OS=Homo sapiens GN=SPTAN1 PE=1 SV=3
O15020	33	0	0	>sp O15020 SPTN2_HUMAN Spectrin beta chain, non-erythrocytic 2 OS=Homo sapiens GN=SPTBN2 PE=1 SV=3
Q01082	17	0	0	>sp Q01082 SPTB2_HUMAN Spectrin beta chain, non-erythrocytic 1 OS=Homo sapiens GN=SPTBN1 PE=1 SV=2
Q13085	13	0	0	>sp Q13085 ACACA_HUMAN Acetyl-CoA carboxylase 1 OS=Homo sapiens GN=ACACA PE=1 SV=2
Q6P2Q9	2	0	0	>sp Q6P2Q9 PRP8_HUMAN Pre-mRNA-processing-splicing

				factor 8 OS=Homo sapiens GN=PRPF8 PE=1 SV=2
A0A0U4 BW16	268	0	0	>tr A0A0U4BW16 A0A0U4BW16_ HUMAN Non-muscle myosin heavy chain 9 OS=Homo sapiens GN=MYH9 PE=2 SV=1
P35579	306	1	0	>sp P35579 MYH9_HUMAN Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4
P13533	3	0	4	>sp P13533 MYH6_HUMAN Myosin-6 OS=Homo sapiens GN=MYH6 PE=1 SV=5
Q86UU0	3	0	1	>sp Q86UU0 BCL9L_HUMAN B-cell CLL/lymphoma 9-like protein OS=Homo sapiens GN=BCL9L PE=1 SV=1
Q9BZF9	2	0	0	>sp Q9BZF9 UACA_HUMAN Uveal autoantigen with coiled-coil domains and ankyrin repeats OS=Homo sapiens GN=UACA PE=1 SV=2
Q9UM54	4	0	0	>sp Q9UM54 MYO6_HUMAN Unconventional myosin-VI OS=Homo sapiens GN=MYO6 PE=1 SV=4
A6NGG8	1	0	0	>sp A6NGG8 CB071_HUMAN Uncharacterized protein C2orf71 OS=Homo sapiens GN=C2orf71 PE=1 SV=1
Q12770	1	0	0	>sp Q12770 SCAP_HUMAN Sterol regulatory element-binding protein cleavage-activating protein OS=Homo sapiens GN=SCAP PE=1 SV=4
Q08211	38	0	0	>sp Q08211 DHX9_HUMAN ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4
Q13045	3	0	0	>sp Q13045 FLII_HUMAN Protein flightless-1 homolog OS=Homo sapiens GN=FLII PE=1 SV=2
Q86SQ0	3	0	0	>sp Q86SQ0 PHLB2_HUMAN Pleckstrin homology-like domain family B member 2 OS=Homo sapiens GN=PHLDB2 PE=1 SV=2

Q27J81	1	0	0	>sp Q27J81 INF2_HUMAN Inverted formin-2 OS=Homo sapiens GN=INF2 PE=1 SV=2
P55265	2	0	0	>sp P55265 DSRAD_HUMAN Double-stranded RNA-specific adenosine deaminase OS=Homo sapiens GN=ADAR PE=1 SV=4
A0A0D9SF60	2	0	0	>tr A0A0D9SF60 A0A0D9SF60_HU MAN Plakophilin-4 OS=Homo sapiens GN=PKP4 PE=1 SV=1
Q9P2M7	2	0	0	>sp Q9P2M7 CING_HUMAN Cingulin OS=Homo sapiens GN=CGN PE=1 SV=2
P11498	1	0	0	>sp P11498 PYC_HUMAN Pyruvate carboxylase, mitochondrial OS=Homo sapiens GN=PC PE=1 SV=2
O00159	16	0	0	>sp O00159 MYO1C_HUMAN Unconventional myosin-Ic OS=Homo sapiens GN=MYO1C PE=1 SV=4
Q7L014	1	0	0	>sp Q7L014 DDX46_HUMAN Probable ATP-dependent RNA helicase DDX46 OS=Homo sapiens GN=DDX46 PE=1 SV=2
O14974	3	0	0	>sp O14974 MYPT1_HUMAN Protein phosphatase 1 regulatory subunit 12A OS=Homo sapiens GN=PPP1R12A PE=1 SV=1
Q9H2U1	2	0	0	>sp Q9H2U1 DHX36_HUMAN ATP-dependent RNA helicase DHX36 OS=Homo sapiens GN=DHX36 PE=1 SV=2
O94832	4	0	0	>sp O94832 MYO1D_HUMAN Unconventional myosin-Id OS=Homo sapiens GN=MYO1D PE=1 SV=2
Q9Y2W1	4	0	0	>sp Q9Y2W1 TR150_HUMAN Thyroid hormone receptor-associated protein 3 OS=Homo sapiens GN=THRAP3 PE=1 SV=2
Q9NYF8	4	0	0	>sp Q9NYF8 BCLF1_HUMAN Bcl-2-associated transcription factor 1 OS=Homo sapiens GN=BCLAF1

				PE=1 SV=2
Q08043	1	0	0	>sp Q08043 ACTN3_HUMAN Alpha-actinin-3 OS=Homo sapiens GN=ACTN3 PE=1 SV=2
P43243	1	0	0	>sp P43243 MATR3_HUMAN Matrin-3 OS=Homo sapiens GN=MATR3 PE=1 SV=2
Q00839	5	0	0	>sp Q00839 HNRPU_HUMAN Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=6
P60709	32	10	5	>sp P60709 ACTB_HUMAN Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
Q4KMQ1	1	0	0	>sp Q4KMQ1 TPRN_HUMAN Taperin OS=Homo sapiens GN=TPRN PE=1 SV=2
Q14444	11	0	0	>sp Q14444 CAPR1_HUMAN Caprin-1 OS=Homo sapiens GN=CAPRIN1 PE=1 SV=2
Q86VG2	3	0	0	>tr Q86VG2 Q86VG2_HUMAN Splicing factor proline/glutamine-rich (Polypyrimidine tract binding protein associated) OS=Homo sapiens GN=SFPQ PE=2 SV=1
O95447	3	0	0	>sp O95447 LCA5L_HUMAN Lebercilin-like protein OS=Homo sapiens GN=LCA5L PE=1 SV=1
Q16643	2	0	0	>sp Q16643 DREB_HUMAN Drebrin OS=Homo sapiens GN=DBN1 PE=1 SV=4
Q9Y2M5	3	0	0	>sp Q9Y2M5 KLH20_HUMAN Kelch-like protein 20 OS=Homo sapiens GN=KLHL20 PE=1 SV=4
B7Z344	1	0	0	>tr B7Z344 B7Z344_HUMAN cDNA FLJ59625, highly similar to Rho guanine nucleotide exchange factor 7 OS=Homo sapiens PE=2 SV=1
Q59EI8	2	0	0	>tr Q59EI8 Q59EI8_HUMAN Dual specificity phosphatase 5 variant (Fragment) OS=Homo sapiens PE=2 SV=1
Q99419	1	0	0	>tr Q99419 Q99419_HUMAN

				ICSAT transcription factor (Fragment) OS=Homo sapiens PE=2 SV=1
A8K617	1	0	0	>tr A8K617 A8K617_HUMAN cDNA FLJ78611 OS=Homo sapiens PE=2 SV=1
Q6RFH5	1	0	0	>sp Q6RFH5 WDR74_HUMAN WD repeat-containing protein 74 OS=Homo sapiens GN=WDR74 PE=1 SV=1
Q6PJV4	3	0	0	>tr Q6PJV4 Q6PJV4_HUMAN THRAP3 protein (Fragment) OS=Homo sapiens GN=THRAP3 PE=2 SV=1
Q96HJ3	3	0	0	>sp Q96HJ3 CCD34_HUMAN Coiled-coil domain-containing protein 34 OS=Homo sapiens GN=CCDC34 PE=1 SV=2
Q9H293	3	0	6	>sp Q9H293 IL25_HUMAN Interleukin-25 OS=Homo sapiens GN=IL25 PE=1 SV=1
Q562R4	2	1	1	>tr Q562R4 Q562R4_HUMAN Actin-like protein (Fragment) OS=Homo sapiens GN=ACT PE=4 SV=1
I3L1Z6	2	0	0	>tr I3L1Z6 I3L1Z6_HUMAN Multidrug resistance-associated protein 6 OS=Homo sapiens GN=ABCC6 PE=4 SV=1
A0N4V7	15	0	0	>tr A0N4V7 A0N4V7_HUMAN HCG2039797 (Fragment) OS=Homo sapiens GN=Tcr-alpha PE=4 SV=1
Q8TF72	0	0	2	>sp Q8TF72 SHRM3_HUMAN Protein Shroom3 OS=Homo sapiens GN=SHROOM3 PE=1 SV=2
Q9H0J4	0	0	2	>sp Q9H0J4 QRIC2_HUMAN Glutamine-rich protein 2 OS=Homo sapiens GN=QRICH2 PE=1 SV=1
Q15058	0	0	2	>sp Q15058 KIF14_HUMAN Kinesin-like protein KIF14 OS=Homo sapiens GN=KIF14 PE=1 SV=1
O75037	0	0	2	>sp O75037 KI21B_HUMAN Kinesin-like protein KIF21B

				OS=Homo sapiens GN=KIF21B PE=1 SV=2
Q5SXM2	0	0	1	>sp Q5SXM2 SNPC4_HUMAN snRNA-activating protein complex subunit 4 OS=Homo sapiens GN=SNAPC4 PE=1 SV=1
Q99490	0	0	2	>sp Q99490 AGAP2_HUMAN Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 2 OS=Homo sapiens GN=AGAP2 PE=1 SV=2
Q9NQW1	0	0	2	>sp Q9NQW1 SC31B_HUMAN Protein transport protein Sec31B OS=Homo sapiens GN=SEC31B PE=1 SV=1
Q4VCS5	0	0	2	>sp Q4VCS5 AMOT_HUMAN Angiomotin OS=Homo sapiens GN=AMOT PE=1 SV=1
O15083	0	0	4	>sp O15083 ERC2_HUMAN ERC protein 2 OS=Homo sapiens GN=ERC2 PE=1 SV=3
O95503	0	0	2	>sp O95503 CBX6_HUMAN Chromobox protein homolog 6 OS=Homo sapiens GN=CBX6 PE=1 SV=1
Q9UJZ1	0	0	2	>sp Q9UJZ1 STML2_HUMAN Stomatin-like protein 2, mitochondrial OS=Homo sapiens GN=STOML2 PE=1 SV=1
U3KPU4	0	0	1	>tr U3KPU4 U3KPU4_HUMAN GRIP and coiled-coil domain-containing protein 2 (Fragment) OS=Homo sapiens GN=GCC2 PE=1 SV=1
Q71U36	0	1	0	>sp Q71U36 TBA1A_HUMAN Tubulin alpha-1A chain OS=Homo sapiens GN=TUBA1A PE=1 SV=1
P31943	0	1	0	>sp P31943 HNRH1_HUMAN Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRNPH1 PE=1 SV=4
Q13885	0	1	0	>sp Q13885 TBB2A_HUMAN Tubulin beta-2A chain OS=Homo sapiens GN=TUBB2A PE=1 SV=1

Q9HD23	0	1	0	>sp Q9HD23 MRS2_HUMAN Magnesium transporter MRS2 homolog, mitochondrial OS=Homo sapiens GN=MRS2 PE=1 SV=1
Q9P035	0	1	0	>sp Q9P035 HACD3_HUMAN Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 3 OS=Homo sapiens GN=HACD3 PE=1 SV=2
P63244	0	1	0	>sp P63244 RACK1_HUMAN Receptor of activated protein C kinase 1 OS=Homo sapiens GN=RACK1 PE=1 SV=3
Q99623	0	2	0	>sp Q99623 PHB2_HUMAN Prohibitin-2 OS=Homo sapiens GN=PHB2 PE=1 SV=2
P09493	0	1	0	>sp P09493 TPM1_HUMAN Tropomyosin alpha-1 chain OS=Homo sapiens GN=TPM1 PE=1 SV=2
P35232	0	3	0	>sp P35232 PHB_HUMAN Prohibitin OS=Homo sapiens GN=PHB PE=1 SV=1

Table S4**Primers and probes used in RT-qPCR**

Name	Sequence	Strand	Modification
<i>ACCI</i>	5'-TTCCGCAGAAAGGATCTGGT-3'	forward	
	5'-CAACTCCTCCGCTCAGTTG-3'	reverse	
	5'-ACCATGCGTCGGTGGACCCA-3'	Probe	5'Fam - 3'Tamra
<i>FASN</i>	5'-ACAGTGTCCCTGGAGGTACG-3'	forward	
	5'-TGGTACACCTTCCCACTCAC-3'	reverse	
	5'-CCTGGAGGCCACCGGTGCCT-3'	Probe	5'Fam - 3'Tamra
<i>ACLY</i>	5'-CTGATGCCATGAGGAAGCAC-3'	forward	
	5'-TCAGCTATGATGGCGATGGT-3'	reverse	
	5'-TGCCTCTCTCCGCTCTGCCT-3'	Probe	5'Fam - 3'Tamra
<i>BBOX1</i>	5'-ATGGCCGACGTAGCTATGAA-3'	forward	
	5'-GCCTTGACATGACCACATCC-3'	reverse	
	5'-CCCGCCATCTAGAAGGAGCTTATGCT-3'	Probe	5'Fam - 3'Tamra
<i>PGC1A</i>	5'-TGACTTTGACCTGCTTCCA-3'	forward	
	5'-AGGTATTGCCATCCCTCTG-3'	reverse	
	5'-ACCTGCGCAAGCTTCTTGAGC-3'	Probe	5'Fam - 3'Tamra
<i>CPT1A</i>	5'-TGGACCGGGAGGAAATCAA-3'	forward	
	5'-CATGTGCTGGATGGTGTCTG-3'	reverse	
	5'-CCGCTCCCACTGAGCGGAGCA-3'	Probe	5'Fam - 3'Tamra
<i>ANKRD22</i>	5'- CCAGCTTGGACTTCTAGGGA-3'	forward	
	5'-GGCAGATGGCCTCAGAGTAT-3'	reverse	
	5'-TCCCATGCTGGCCTCACAGG-3'	Probe	5'Fam - 3'Tamra
<i>TUBB</i>	5'-AGGAGGTCGATGAGCAGATG-3'	forward	
	5'-TTGCCAATGAAGGTGACTGC-3'	reverse	
	5'-TGTGACATCCCACCTCGTGGCC-3'	Probe	5'Fam - 3'Tamra