Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

A novel strategy for facile serum exosome isolation based on specific interactions between phospholipid bilayers and TiO_2

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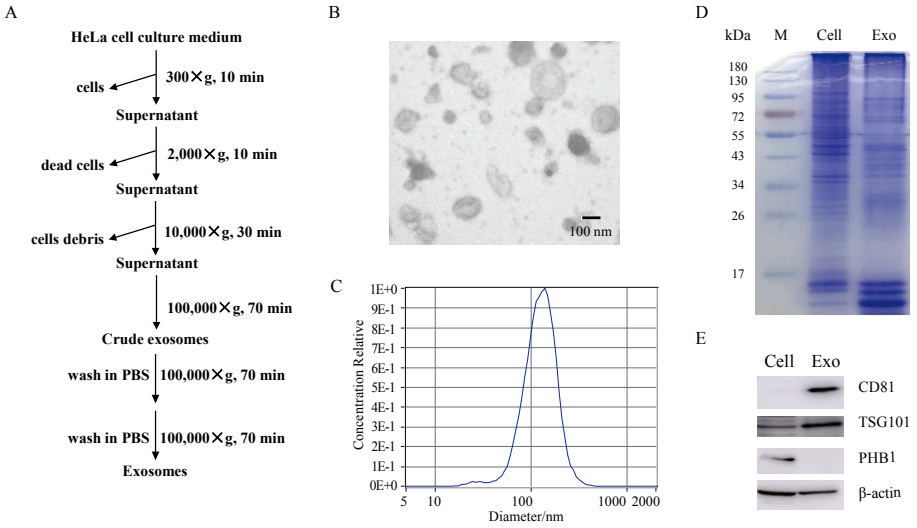
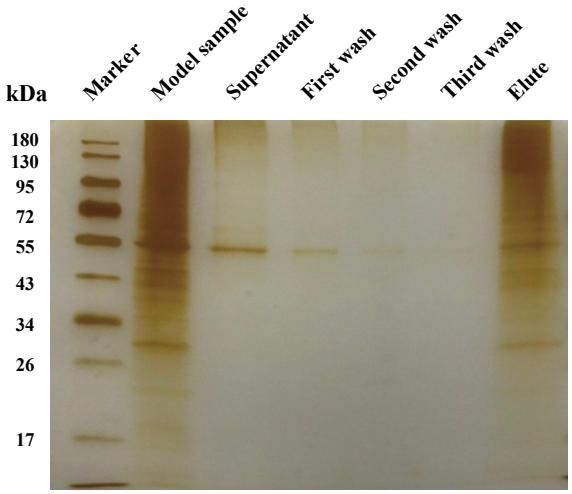
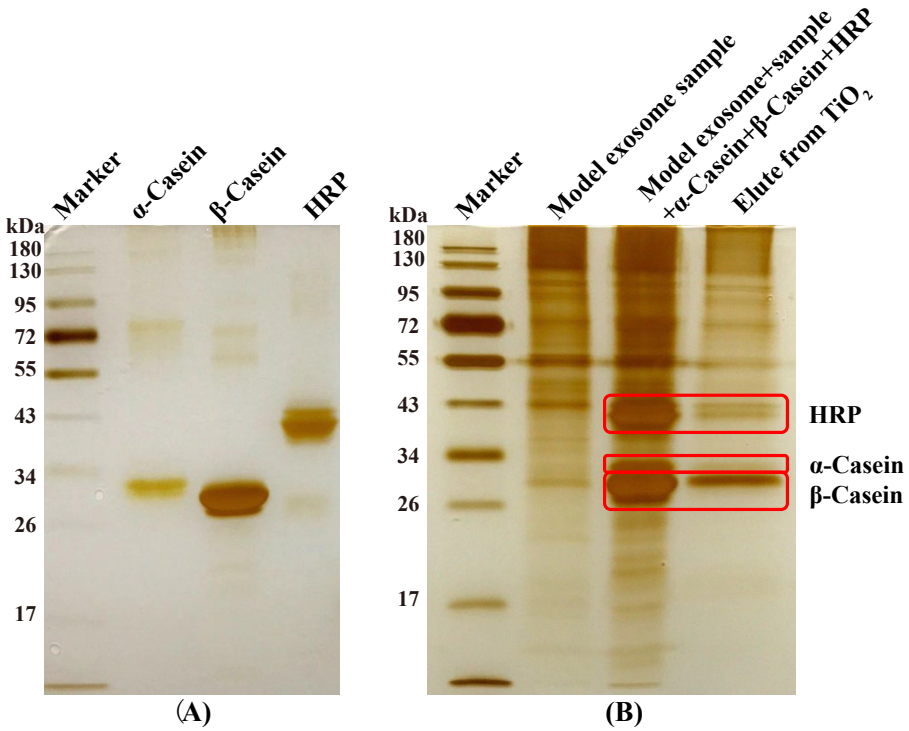


Supplemental Fig. S1 Isolation and characterization of exosomes from HeLa cells culture medium. (A) Workflow for isolation of exosomes using ultracentrifugation. (B) TEM image of exosomes. (C) NTA measurement of the size distribution of exosomes. (D) Coomassie Brilliant Blue stained 12% SDS-PAGE analysis of HeLa cell lysates and exosome proteins. (E) Western blotting analysis of common exosome markers (TSG101 and CD81), mitochondrial marker (PHB1) and internal control (β-actin).

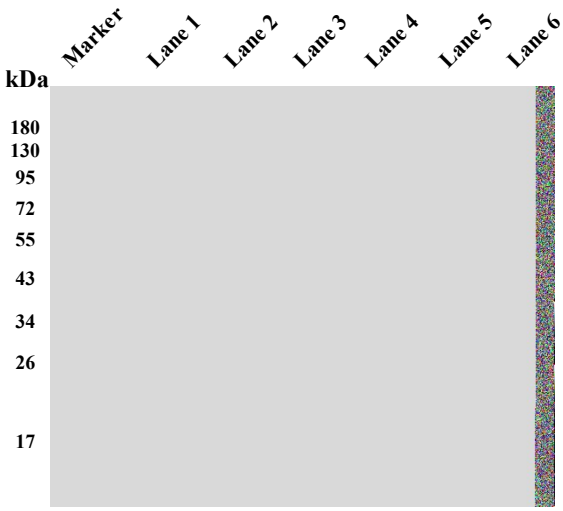
As shown in Fig. S1B, the exosomes exhibited typical cup-shaped vesicles structure. Nanoparticle tracking analysis (NTA) showed that the size distribution of the exosomes is from 45 nm to 225 nm and centered at 122 nm, which was consistent with the typical size range of exosomes (30-150 nm) (Fig. S1C). To further investigate the performance of model exosomes preparations, the proteins extracted from HeLa cell lysates and from model exosome samples were separated by SDS-PAGE and stained with Coomassie Brilliant Blue, respectively. The distinct protein profiles of the two types of samples were shown in Fig. S1D. Clearly visible differences could be observed between 43 kDa to 95 kDa and small molecular weight range (less than 17 kDa). Then, the expression of exosomal proteins in HeLa cell lysates and model exosome samples was examined by western blotting analysis, respectively. As shown in Fig. S1E, the commonly used exosomal marker proteins, TSG101 and CD81, were highly enriched in the model exosome samples compared with HeLa cell lysates. Moreover, the mitochondrial marker, prohibitin-1 (PHB1) was not detected in the exosome fraction. The results indicated that the isolated exosomes had high purity by avoiding the interference by organelles such as mitochondria.



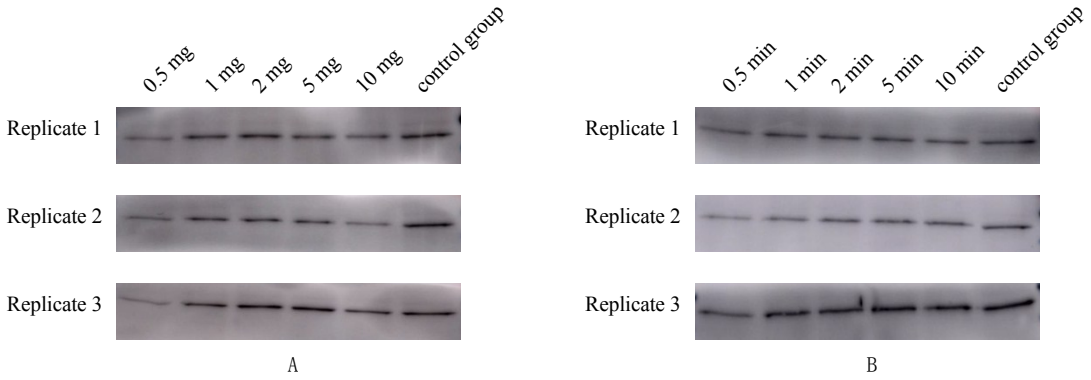
Supplemental Fig. S2 SDS-PAGE analysis (silver stained) of the proteins isolated from model sample by TiO_2 microspheres.



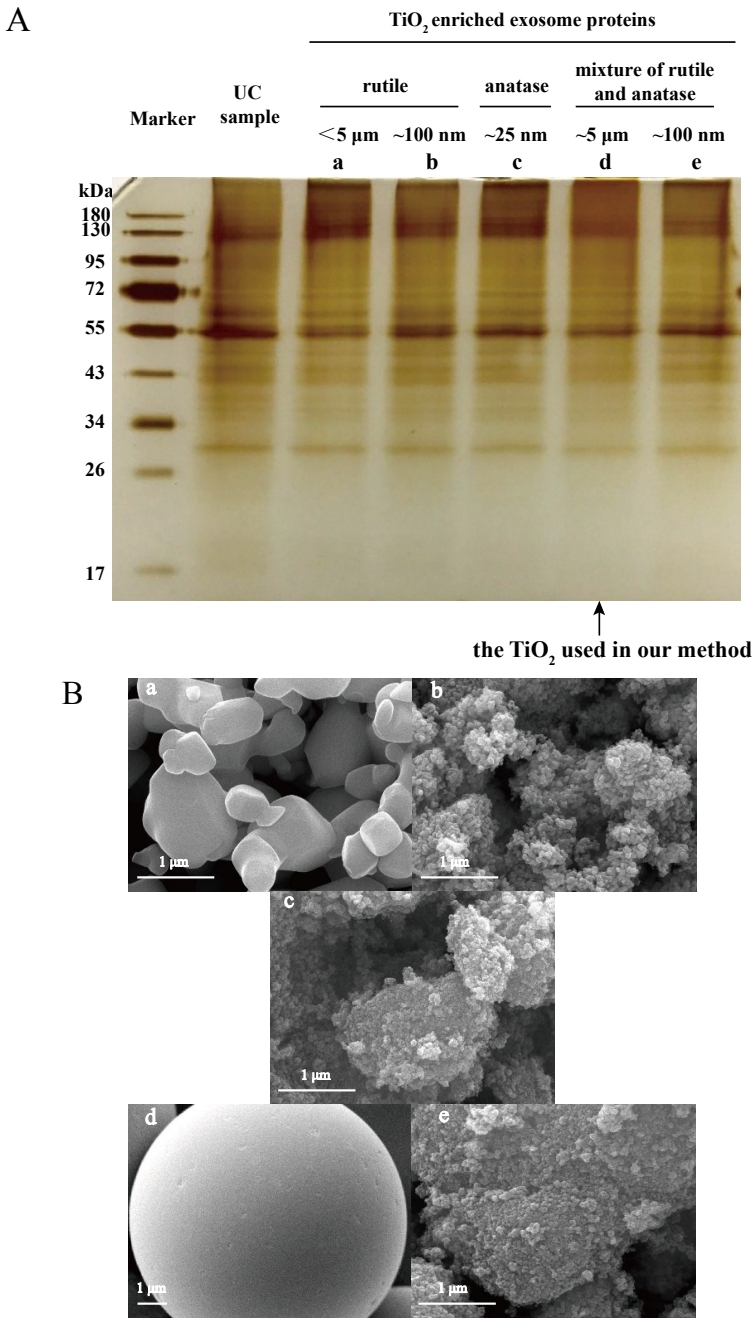
Supplemental Fig. S3 SDS-PAGE images of (A) α-casein (1 μg), β-casein (1 μg) and horseradish peroxidase (1 μg). (B) the model exosomal proteins (2 μg), mixture of the model exosomal proteins (2 μg)+α-casein (1 μg)+ β-casein (1 μg)+horseradish peroxidase (1 μg), and mixture of the model exosomal protein (2 μg)+α-casein (1 μg)+β-casein (1 μg)+horseradish peroxidase (1 μg) after isolated by TiO_2



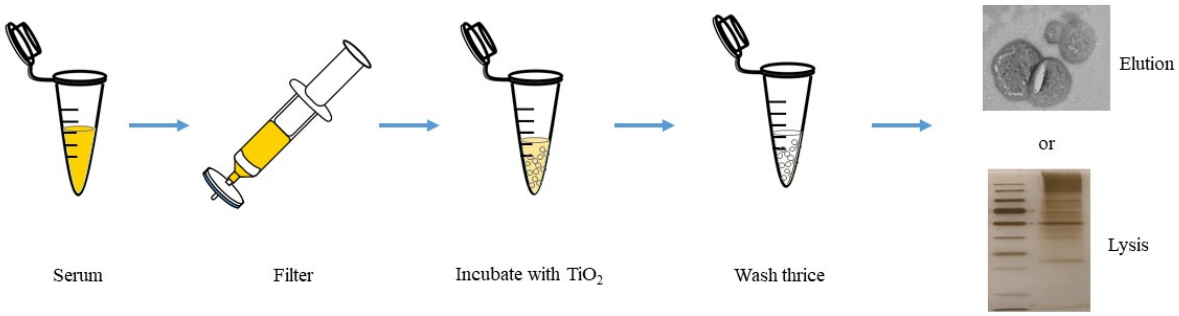
Supplemental Fig. S4 SDS-PAGE image of the proteins extracted from the exosomes isolated by TiO_2 in three replicates (Lane 1, 3 and 5) and the corresponding residual proteins on TiO_2 after exosome elution (Lane2, 4, 6).



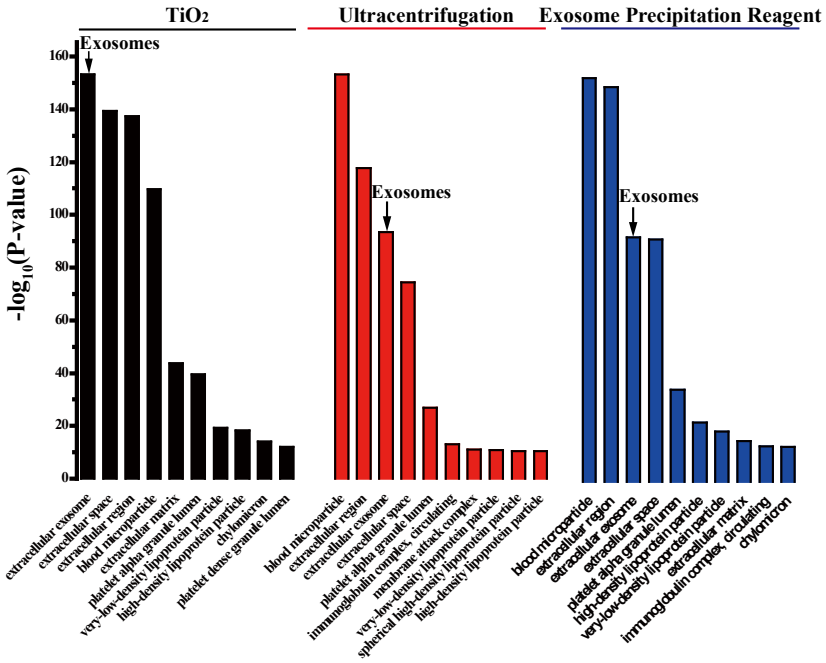
Supplemental Fig. S5 Exosome enrichment effect by using (A) different TiO_2 amounts and (B) different incubation time (evaluated by Western Blot analysis of TSG101).



Supplemental Fig. S6 Exosome isolation using different commercial titanium dioxide particles. (A) SDS-PAGE image of the proteins extracted from the exosomes isolated by different titanium dioxide particles, (B) SEM images of different titanium dioxide particles.



Supplemental Fig. S7 Schematic of the TiO_2-based method for exosomes isolation and downstream analyses



Supplemental Fig. S8 Gene Ontology analysis of cellular components of proteins identified in exosomes obtained by different methods

Supplemental Table S1. Brief Comparison of Various Exosome Isolation Methods[1]

Methods	Assay time	Exosome recovery, %
Ultracentrifugation	5-9 h	2-80% (blood, serum or plasma)
Density Gradient Centrifugation	16-90 h	10% (plasma or serum)
Size Exclusion Chromatograph	0.3 h	40-90% (plasma)
Ultrafiltration	0.5 h	10-80% (plasma)
Immuno-affinity	4-20 h	/
Precipitation based on polymer	0.25-12 h	90% (a mixture of EVs and serum proteins)
TiO_2-based method	5 min for incubation, 30 min for all steps (filtration needs less than 1 min, washing steps cost about 4 min and on microspheres exosome lysis takes about 20 min)	≈80% (serum) 93.4% (model exosomes)

Supplemental Table S2. Numbers of protein groups identified under different conditions.

Volumn (μL)	LC-MS condition	Data Searching softeware	Identified proteins groups in three replicate experiments
1	15 cm column, 78 min gradient	MaxQuant 1.5.2.8	308 (212, 248, 248)
100	15 cm column, 78 min gradient	MaxQuant 1.5.2.8	384 (294, 282, 319)
200	25 cm column, 120 min gradient	Proteome Discoverer 2.0	889 (549, 567, 588)

Supplemental Table S3. Significantly regulated serum exosomal proteins in pancreatic cancer.

No.	Gene.Symbol	Uniprot ID	Protein	Gene	logFC	adj.P.Val	Reported in Pancreatic Cancer (reference shown)
1	KV306_HUMAN	P01624	Immunoglobulin kappa variable 3-15	IGKV3-15	7.74	1.49E-05	
2	S10A9_HUMAN	P06702	Protein S100-A9	S100A9	7.55	4.50E-09	[2]
3	H2B1H_HUMAN	Q93079	Histone H2B type 1-H	HIST1H2BH	7.50	2.97E-05	
4	S10A6_HUMAN	P06703	Protein S100-A6	S100A6	7.14	1.49E-05	[2, 3]
5	PROF1_HUMAN	P07737	Profilin-1	PFN1	6.41	7.50E-06	[2, 4, 5]
6	LEAP2_HUMAN	Q969E1	Liver-expressed antimicrobial peptide 2	LEAP2	6.32	3.92E-06	
7	AATC2_HUMAN	Q8NHS2	Putative aspartate aminotransferase, cytoplasmic 2	GOT1L1	6.09	2.55E-07	
8	IGLL5_HUMAN	B9A064	Immunoglobulin lambda-like polypeptide 5	IGLL5	5.97	1.49E-05	
9	S10A7_HUMAN	P31151	Protein S100-A7	S100A7	5.44	2.93E-04	
10	RAN_HUMAN	P62826	GTP-binding nuclear protein Ran	RAN	5.44	3.43E-04	[6]
11	KPRP_HUMAN	Q5T749	Keratinocyte proline-rich protein	KPRP	5.18	1.63E-03	
12	CALL5_HUMAN	Q9NZT1	Calmodulin-like protein 5	CALML5	5.14	4.06E-03	[7]
13	SBSN_HUMAN	Q6UWP8	Suprabasin	SBSN	5.10	1.86E-04	
14	THIO_HUMAN	P10599	Thioredoxin	TXN	4.95	4.53E-03	[8]
15	SPR1B_HUMAN	P22528	Cornifin-B	SPRR1B	4.92	3.24E-03	
16	PRDX2_HUMAN	P32119	Peroxiredoxin-2	PRDX2	4.75	4.53E-03	[9]
17	LV301_HUMAN	P01714	Immunoglobulin lambda variable 3-19	IGLV3-19	4.66	7.62E-03	
18	FIBG_HUMAN	P02679	Fibrinogen gamma chain	FGG	4.59	4.57E-05	[4, 6]
19	CBG_HUMAN	P08185	Corticosteroid-binding globulin	SERPINA6	4.57	1.28E-03	
20	SHBG_HUMAN	P04278	Sex hormone-binding globulin	SHBG	4.49	2.00E-03	[10]
21	FKB1A_HUMAN	P62942	Peptidyl-prolyl cis-trans isomerase FKBP1A	FKBP1A	4.48	4.14E-03	
22	CYTA_HUMAN	P01040	Cystatin-A	CSTA	4.46	3.64E-03	
23	CSRP1_HUMAN	P21291	Cysteine and glycine-rich protein 1	CSRP1	4.45	9.88E-03	
24	S10A4_HUMAN	P26447	Protein S100-A4	S100A4	4.31	4.14E-03	[11]
25	PIP_HUMAN	P12273	Prolactin-inducible protein	PIP	4.29	1.08E-03	[12, 13]
26	MNDA_HUMAN	P41218	Myeloid cell nuclear differentiation antigen	MNDA	4.16	6.42E-03	
27	LCN1_HUMAN	P31025	Lipocalin-1	LCN1	4.15	2.76E-03	[14]
28	PIGR_HUMAN	P01833	Polymeric immunoglobulin receptor	PIGR	4.05	2.31E-03	[15, 16]
29	LDHB_HUMAN	P07195	L-lactate dehydrogenase B chain	LDHB	3.89	2.53E-04	[17]
30	FBX50_HUMAN	Q6ZVX7	F-box only protein 50	NCCRP1	3.81	1.39E-03	[18]
31	GPV_HUMAN	P40197	Platelet glycoprotein V	GP5	3.75	4.14E-03	[19]
32	APMAP_HUMAN	Q9HDC9	Adipocyte plasma membrane-associated protein	APMAP	3.67	9.07E-05	
33	DCD_HUMAN	P81605	Dermcidin	DCD	3.67	3.11E-06	[20]
34	FLNA_HUMAN	P21333	Filamin-A	FLNA	3.66	8.46E-03	[21]
35	BASP1_HUMAN	P80723	Brain acid soluble protein 1	BASP1	3.56	3.18E-03	
36	TGM3_HUMAN	Q08188	Protein-glutamine gamma-glutamyltransferase E	TGM3	3.55	1.38E-03	
37	CFAI_HUMAN	P05156	Complement factor I	CFI	3.53	1.85E-03	[22]
38	DMBT1_HUMAN	Q9UGM3	Deleted in malignant brain tumors 1 protein	DMBT1	3.52	4.09E-04	[23]
39	ELNE_HUMAN	P08246	Neutrophil elastase	ELANE	3.41	8.49E-03	[24]
40	F13B_HUMAN	P05160	Coagulation factor XIII B chain	F13B	3.37	1.63E-03	
41	K2C78_HUMAN	Q8N1N4	Keratin, type II cytoskeletal 78	KRT78	3.27	6.18E-03	
42	TCPG_HUMAN	P49368	T-complex protein 1 subunit gamma	CCT3	3.24	4.53E-03	[6]
43	HORN_HUMAN	Q86YZ3	Hornerin	HRNR	3.21	4.54E-03	[25]
44	DSC1_HUMAN	Q08554	Desmocollin-1	DSC1	3.21	2.85E-03	[26]
45	IF2A_HUMAN	P05198	Eukaryotic translation initiation factor 2 subunit 1	EIF2S1	3.17	1.63E-03	
46	IGJ_HUMAN	P01591	Immunoglobulin J chain	JCHAIN	3.14	4.38E-03	
47	MYH9_HUMAN	P35579	Myosin-9	MYH9	2.97	8.46E-03	[2]

48	CO4B_HUMAN	P0C0L5	Complement C4-B	C4B	2.86	3.23E-04	[5]
49	CO6_HUMAN	P13671	Complement component C6	C6	2.83	1.40E-04	[5]
50	DESP_HUMAN	P15924	Desmoplakin	DSP	2.76	6.71E-03	
51	H2A1J_HUMAN	Q99878	Histone H2A type 1-J	HIST1H2AJ	2.75	7.36E-03	
52	MOES_HUMAN	P26038	Moesin	MSN	2.71	6.18E-03	[27]
53	CO7_HUMAN	P10643	Complement component C7	C7	2.63	1.89E-05	
54	FILA_HUMAN	P20930	Filaggrin	FLG	2.56	3.72E-04	
55	CO8G_HUMAN	P07360	Complement component C8 gamma chain	C8G	2.48	9.07E-05	
56	PTN6_HUMAN	P29350	Tyrosine-protein phosphatase non-receptor type 6	PTPN6	2.46	6.78E-03	
57	C4BPB_HUMAN	P20851	C4b-binding protein beta chain	C4BPB	2.39	1.85E-03	
58	ACTB_HUMAN	P60709	Actin, cytoplasmic 1	ACTB	2.21	1.42E-03	[4]
59	CO5_HUMAN	P01031	Complement C5	C5	2.14	4.79E-05	[15, 22]
60	PLTP_HUMAN	P55058	Phospholipid transfer protein	PLTP	-2.04	2.27E-03	
61	PCYOX_HUMAN	Q9UHG3	Prenylcysteine oxidase 1	PCYOX1	-3.57	4.14E-03	
62	DNJC3_HUMAN	Q13217	DnaJ homolog subfamily C member 3	DNAJC3	-4.17	2.31E-04	
63	HV310_HUMAN	P01771	Immunoglobulin heavy variable 3-33	IGHV3-33	-4.25	5.57E-04	
64	SIAT1_HUMAN	P15907	Beta-galactoside alpha-2,6-sialyltransferase 1	ST6GAL1	-5.45	8.71E-04	
65	APOA5_HUMAN	Q6Q788	Apolipoprotein A-V	APOA5	-5.74	1.06E-04	

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Author Contributions

F. Gao and F. Jiao designed experiments and wrote the original draft; F. Gao, F. Jiao and C. Xia carried out experiments; Y. Zhao performed the data analyses; W. Ying and Y. Xie helped perform the data acquisition; X Guan and M Tao collected serum from patients; Y Zhang, W Qin and X. Qian reviewed and edited the manuscript. All authors read and approved the manuscript.