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pH-responsive Magnetic Nanospheres for Reversibly Selective Capture and Release of Glycoproteins

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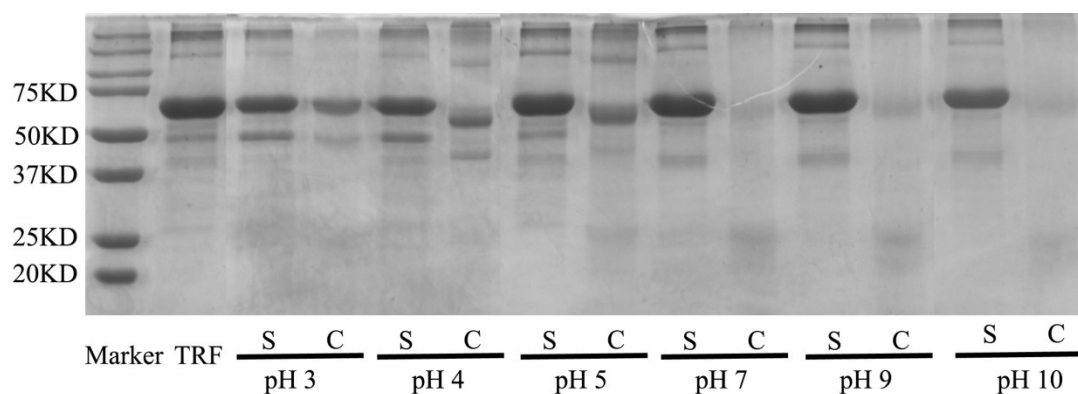


Fig. S1. SDS-PAGE analysis of the supernatant (S), protein-nanospheres composites (C) of pure TRF after treatment with $\text{Fe}_3\text{O}_4/\text{CMCS}/\text{PAAPBA}$ nanospheres at different pH for 1 h. Lane 1, marker; Lane 2, TRF before treatment; Lanes 3~14, supernatant and protein-nanospheres composites after treatment at different pH. ($C_{\text{Protein}} = 1 \text{ mg/ml}$, 300 μl protein solution, co-incubation for 1 h)

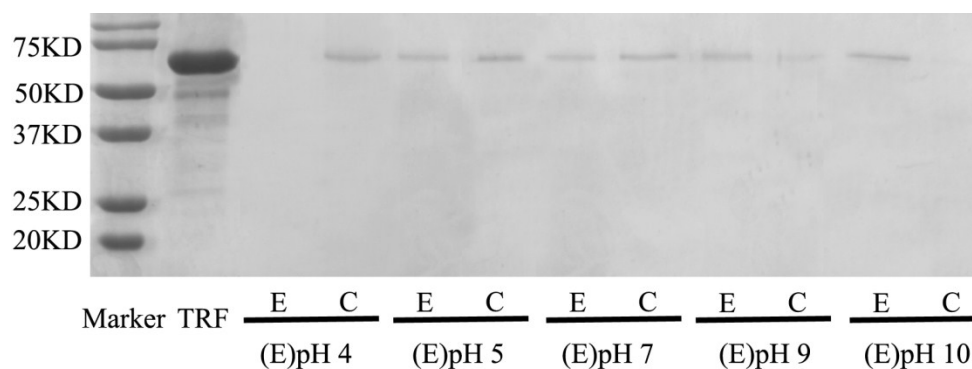


Fig. S2. SDS-PAGE analysis of the eluate (E), protein-nanospheres composites (C) of pure TRF after incubation with $\text{Fe}_3\text{O}_4/\text{CMCS}/\text{PAAPBA}$ nanospheres at pH 4 and elution at different pH. Lane 1, marker; Lane 2, TRF before treatment; Lanes 3~12, eluate and protein-nanospheres composites after elution at different pH. ($C_{\text{Protein}} = 0.5 \text{ mg/ml}$, 30 μl protein solution, incubation at pH 4, washing solution of 200 μl)

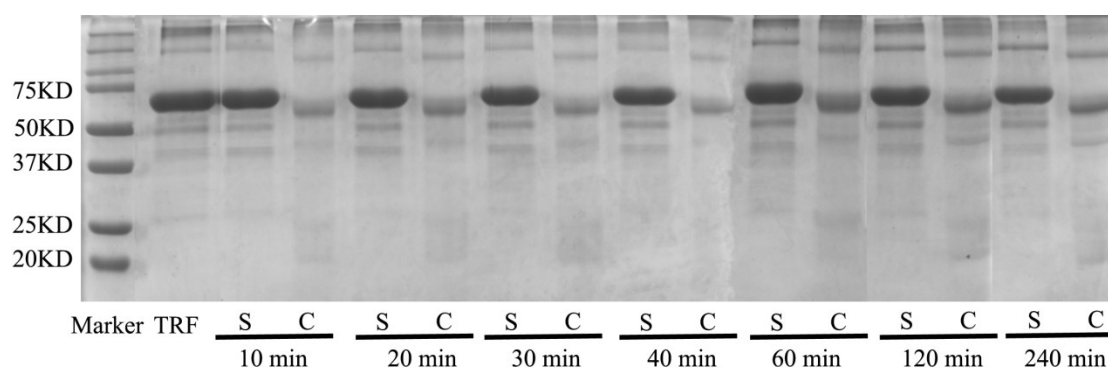


Fig. S3. SDS-PAGE analysis of the supernatant (S), protein-nanospheres composites (C) of pure TRF after treatment with Fe₃O₄/CMCS/PAAPBA nanospheres at pH 4 with different incubation time. Lane 1, marker; Lane 2, TRF before treatment; Lanes 3~16, supernatant and protein-nanospheres composites after treatment at pH 4 with different incubation time. (C_{Protein} =1 mg/ml, 300 μl protein solution, pH 4)

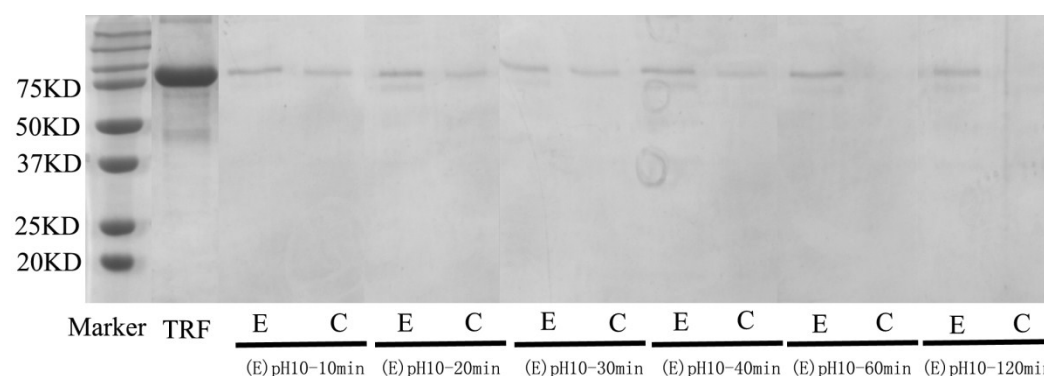


Fig. S4. SDS-PAGE analysis of the eluate (E), protein-nanospheres composites (C) of pure TRF after incubation with Fe₃O₄/CMCS/PAAPBA nanospheres at pH 4 and eluation at pH 10 with different elution time. Lane 1, marker; Lane 2, TRF before treatment; Lanes 3~14, eluate and protein-nanospheres composites after eluation at different pH. (C_{Protein} =0.5 mg/ml, 30 μl protein solution, incubation at pH 4, washing solution of 200 μl)

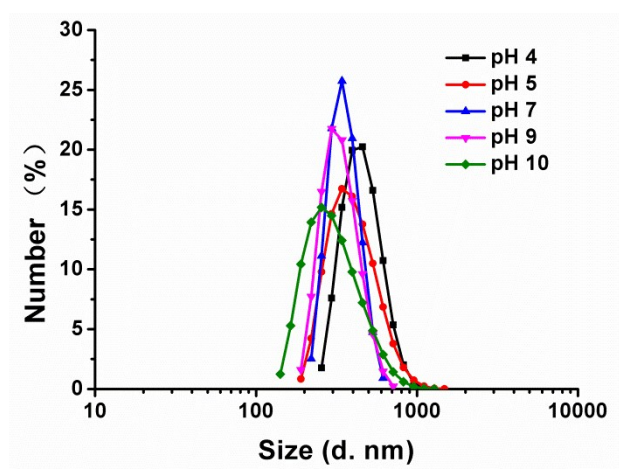


Fig. S5. Size distribution of the Fe₃O₄/CMCS/PAAPBA nanospheres at different pH media.

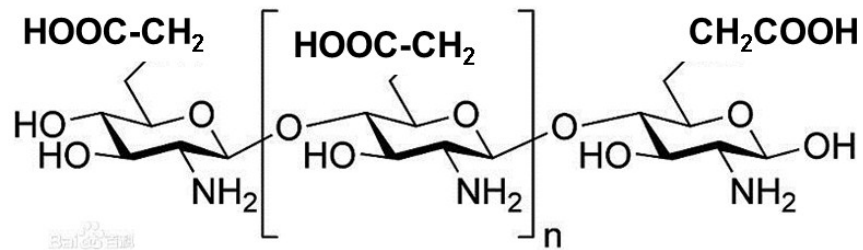


Fig. S6. The structure of CMCS.

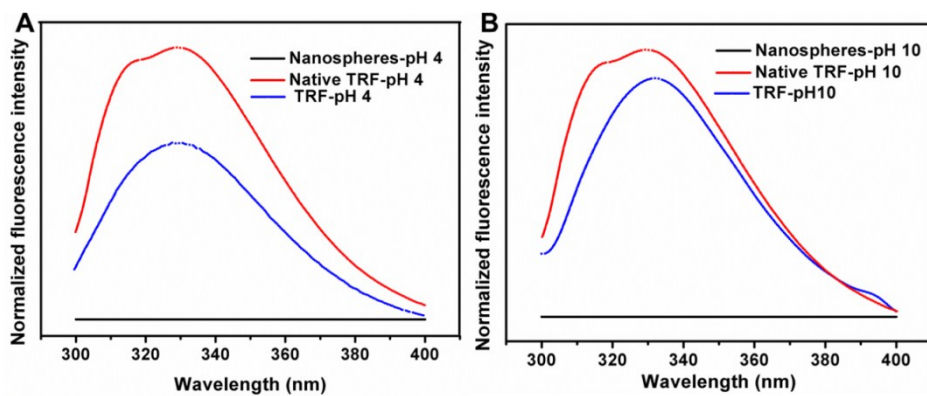


Fig. S7. The fluorescence spectra of the nanospheres, native TRF and adsorbed TRF at pH 4 (A) and at pH 10 (B).

Table S1. The glycoproteins in 10% FBS and the adsorbed glycoproteins by the nanospheres identified by mass spectrometry.

Glycoproteins in 10% FBS		Captured Glycoproteins by the Nanospheres	
Protein	Content (%)	Protein	Content (%)
Alpha-1-acid glycoprotein	1.19%	Alpha-1-acid glycoprotein	0.55%
Alpha-1B-glycoprotein	0.65%	Alpha-1B-glycoprotein	0.22%
Alpha-2-HS-glycoprotein	7.07%	Alpha-2-HS-glycoprotein	6.31%
Beta-2-glycoprotein	0.52%	Beta-2-glycoprotein	0.30%
Lactoferrin	0.03%	Lactoferrin	0.08%
Leucine-rich alpha-2-glycoprotein	0.20%	Leucine-rich alpha-2-glycoprotein	0.15%
Serotransferrin	6.94%	Serotransferrin	4.26%
		Alpha-fetoprotein	1.26%
		Galectin-3-binding protein	0.06%
		Histidine-rich glycoprotein	0.47%
		Lipopolysaccharide binding protein	0.11%
		Mannose-binding protein C	0.08%
		Phosphatidylinositol-glycan-specific phospholipase D	0.02%
		Platelet glycoprotein Ib alpha chain	0.01%
Total	16.61%	Total	13.89%

Table S2. The protein list of 10 % FBS identified by mass spectrometry (glycoproteins marked in red).

Protein	Content (%)
Serum albumin	37.29%
BOVIN ALB protein	13.09%
Alpha-2-HS-glycoprotein	7.07%
Serotransferrin	6.94%
Alpha-1-antiproteinase	5.72%
Alpha-2-macroglobulin	5.68%
Vitamin D-binding protein	1.78%
Angiotensinogen	1.63%
Serpin A3-7	1.59%
Trypsin inhibitor	1.59%
Hemopexin	1.43%
Alpha-1-acid glycoprotein	1.19%
Serpin A3-3	1.14%
Antithrombin-III	0.81%
Serpin A3-4	0.78%
Fetuin-B	0.67%
Alpha-1B-glycoprotein	0.65%
Serpin A3-6	0.63%
Serpin A3-5	0.63%
Pancreatic elastase inhibitor	0.56%
Hemoglobin	0.55%
Beta-2-glycoprotein	0.52%
Ig gamma-1 chain C region	0.46%
Complement C3	0.43%
Pigment epithelium-derived factor	0.34%
Alpha-2-antiplasmin	0.33%
Thyroxine-binding globulin	0.32%
Kininogen-2	0.31%
Vitronectin	0.31%
Plasminogen	0.31%
Protein AMBP	0.29%
Kininogen-1	0.28%
KRT6A protein	0.24%
Complement factor B	0.23%
Plasma serine protease inhibitor	0.21%
Leucine-rich alpha-2-glycoprotein	0.20%
Lumican	0.20%
Apolipoprotein A-I	0.20%

Actin, cytoplasmic 2	0.19%
Gelsolin	0.15%
Serpin A3-8	0.15%
SERPIND1 protein	0.14%
Fibrinogen	0.14%
Sex hormone-binding globulin	0.13%
Pantetheinase	0.11%
Protein Z-dependent protease inhibitor	0.10%
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit	0.10%
Prothrombin	0.10%
Fibulin-1	0.08%
Complement component C9	0.07%
Inhibitor of carbonic anhydrase	0.07%
Complement factor I	0.06%
CPN2 protein	0.06%
Kinetochores-associated protein NSL1-like protein	0.05%
Fibroblast growth factor receptor type 1	0.05%
LOC617897 protein	0.05%
Apoptosis facilitator Bcl-2-like protein 14	0.05%
Complement component C7	0.05%
Uroplakin-2	0.04%
Complement factor H	0.04%
Regulator of G-protein signaling 10	0.04%
RNA polymerase-associated protein LEO1	0.04%
Alpha-S1-casein	0.04%
CD5L protein	0.03%
Lactoferrin	0.03%
Acidic mammalian chitinase	0.03%
Ectonucleotide pyrophosphatase/phosphodiesterase 5 (Putative function)-like	0.03%
Cadherin-5	0.03%
Spindle and kinetochores-associated protein 1	0.03%
Proteasome subunit alpha type (Fragment)	0.03%
Adipocyte plasma membrane-associated protein	0.03%
Zinc finger with UFM1-specific peptidase domain protein	0.03%
FAM84B protein	0.03%
Insulin-like growth factor binding protein acid labile subunit	0.03%
Olfactory receptor	0.03%
GRAM domain-containing protein 4	0.03%
C4b-binding protein alpha chain	0.03%
Protein RUFY3	0.02%
Plasma kallikrein	0.02%

Complement C5a anaphylatoxin	0.02%
Transcription initiation protein SPT3-like protein	0.02%
NDE1 protein	0.02%
Aprataxin	0.02%
Cartilage oligomeric matrix protein	0.02%
Serpin B8	0.02%
Fibromodulin	0.02%
Thrombospondin-1	0.02%
Corticosteroid-binding globulin	0.02%
Rab effector MyRIP	0.02%
Coiled-coil domain-containing protein 121	0.02%
Alpha-enolase	0.02%
Vitamin K-dependent protein Z	0.02%
Glycerol-3-phosphate acyltransferase 3	0.02%
Clusterin	0.02%
Colony stimulating factor 1 receptor	0.02%
Dipeptidyl peptidase 1	0.02%
Coagulation factor IX	0.02%
MUM1 protein	0.02%
Coagulation factor XI	0.02%
Thrombospondin-4	0.02%
Ubiquitin carboxyl-terminal hydrolase	0.02%
F-box only protein 3	0.02%
Phospholipid transfer protein	0.02%
Alpha-amylase	0.02%
Tubulin-specific chaperone E	0.01%
Galectin-3-binding protein	0.01%
Tripeptidyl-peptidase 1	0.01%
Protein FAM38B	0.01%
Rhotekin	0.01%
METTL3 protein	0.01%
Acyl-coenzyme A oxidase	0.01%
Coagulation factor XII	0.01%
Fibronectin	0.01%
KLC1 protein	0.01%
tRNA-dihydrouridine(47) synthase [NAD(P)(+)]	0.01%
Succinate dehydrogenase [ubiquinone] flavoprotein subunit	0.01%
Ras and EF-hand domain-containing protein	0.01%
Ras and EF-hand domain-containing protein	0.01%
Acyl-CoA synthetase short-chain family member 3	0.01%
Vitamin K-dependent protein S	0.01%

Hepatocyte growth factor-like protein	0.01%
Cortactin-binding protein 2	0.01%
High affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8B	0.01%
Rho GTPase-activating protein RICH2	0.01%
Protein piccolo	0.01%
Kinesin-like protein KIF3C	0.01%
Ventricular zone-expressed PH domain-containing protein-like protein 1	0.01%
PDZ domain-containing protein 6	0.01%
Ionotropic glutamate receptor AMPA 1	0.01%
Hexokinase	0.01%
Putative RNA-binding protein 15	0.01%
LOC507082 protein	0.01%
Contactin-1	0.01%
Metalloendopeptidase	0.01%
Hydroxyindole O-methyltransferase	0.01%
Integrin alpha-2	0.01%
Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16	0.01%
E3 ubiquitin-protein ligase NEDD	0.01%
Regulator of nonsense transcripts 2	0.01%
Aldehyde oxidase	0.01%
ATP-dependent RNA helicase DHX29	0.01%
Microtubule-associated protein 1A	0.01%
Rap guanine nucleotide exchange factor 2	0.01%
WD repeat-containing protein 87	0.01%
Sperm flagella 2 protein	0.00%
Coagulation factor V	0.00%
Myosin-XVIIIb	0.00%
Collagen alpha-5(VI) chain	0.00%
Neurofibromin	0.00%
Sacsin	0.00%

Table S3. The list of the absorbed proteins on the Fe₃O₄/CMCS/PAAPBA nanospheres incubated with 10% FBS identified by mass spectrometry (glycoproteins marked in red).

Protein	Content (%)
Serum albumin	15.54%
Alpha-2-macroglobulin	9.31%
Alpha-2-HS-glycoprotein	6.31%
Apolipoprotein A-I,A-II,A-IV	6.10%
Alpha-1-antiproteinase	4.33%
Serotransferrin	4.26%
ALB protein	4.21%
Hemoglobin	4.11%
Plasminogen	2.04%
Inter-alpha-trypsin inhibitor heavy chain H1-H4	1.78%
Hemopexin	1.67%
Serum amyloid A protein	1.42%
Prothrombin	1.36%
Antithrombin-III	1.33%
Alpha-1-antitrypsin transcript variant 1	1.27%
Alpha-fetoprotein	1.26%
C4b-binding protein alpha chain	1.18%
Kininogen-1	1.08%
Factor XIIa inhibitor	0.84%
Ig lambda-1 chain C regions	0.83%
Complement C5	0.76%
Serum amyloid A-4 protein	0.70%
Complement C3	0.68%
Angiotensinogen	0.68%
Endopin 2	0.63%
SERPIND1 protein	0.61%
Ig gamma-1 chain C region	0.61%
Apolipoprotein E	0.57%
IGL@ protein	0.56%
Alpha-1-acid glycoprotein	0.55%
Pentaxin	0.53%
Transthyretin	0.52%
beta-globin	0.50%
Histidine-rich glycoprotein	0.47%
Complement component C4	0.47%
Serpin A3-1	0.45%
Serpin A3-4	0.42%

Pigment epithelium-derived factor	0.42%
Complement factor H	0.42%
Alpha-2-antiplasmin	0.42%
Vitamin D-binding protein	0.41%
Serpin A3-7	0.41%
Complement component 5	0.40%
Gelsolin	0.39%
Complement C5a anaphylatoxin	0.39%
Complement factor B	0.38%
Protein AMBP	0.35%
Complement C4	0.35%
Plasma serine protease inhibitor	0.33%
Ceruloplasmin	0.33%
Beta-2-microglobulin	0.33%
Kininogen-2	0.32%
Fetal globin	0.32%
Carboxypeptidase	0.32%
Beta-2-glycoprotein	0.30%
Vitamin K-dependent protein	0.29%
Immunoglobulin light chain, lambda gene cluster	0.28%
Complement component C9	0.28%
Complement component C7	0.28%
Adiponectin	0.27%
Tetranectin	0.25%
Haptoglobin	0.25%
Similar to vimentin	0.24%
Protein Z-dependent protease inhibitor	0.24%
Fibulin-1	0.24%
LOC790886 protein	0.23%
KRT4 protein	0.22%
Inhibitor of carbonic anhydrase	0.22%
Apolipoprotein C-III	0.22%
Alpha-1B-glycoprotein	0.22%
Neutrophil gelatinase-associated lipocalin	0.21%
Myoglobin	0.21%
Insulin-like growth factor binding protein	0.19%
Complement component C8	0.19%
C-X-C motif chemokine	0.18%
Coagulation factor IX	0.18%
Ig gamma-3 chain C region	0.17%
Hepatocyte growth factor activator	0.17%

Fetuin-B	0.16%
Embryo-specific fibronectin 1 transcript variant	0.16%
ECM1 protein	0.16%
Corticosteroid-binding globulin	0.16%
Complement component C6	0.16%
Clusterin	0.16%
ApoN protein	0.16%
Apolipoprotein D	0.16%
SLAM family member 9	0.15%
Serpin A3-8	0.15%
Plasma kallikrein	0.15%
Leucine-rich alpha-2-glycoprotein	0.15%
Coagulation factor X	0.15%
Primary amine oxidase, liver isozyme	0.13%
Lysozyme	0.13%
Complement C1q subcomponent subunit C	0.13%
Complement C1q subcomponent subunit B	0.13%
Coagulation factor V	0.13%
C22H3ORF10 protein	0.13%
Ubiquinol-cytochrome-c reductase complex assembly factor 3	0.12%
L-lactate dehydrogenase	0.12%
Fibrinogen	0.12%
Alpha-amylase	0.12%
Thrombospondin	0.11%
Pyruvate kinase	0.11%
Protein HP-20-like protein	0.11%
Lipopolysaccharide binding protein	0.11%
Cartilage oligomeric matrix protein	0.11%
Beta-lactoglobulin	0.11%
Apolipoprotein C-II	0.11%
Thioredoxin	0.10%
Protein HP-25 homolog 1	0.10%
Olfactory receptor	0.10%
Histone H4	0.10%
Cornifin alpha	0.10%
Apolipoprotein M	0.10%
Alpha-S1-casein	0.10%
Mannose-binding protein C	0.08%
Lactoferrin	0.08%
Histone H2A	0.08%
Fructose-bisphosphate aldolase	0.08%

Complement C1s subcomponent	0.08%
Annexin	0.08%
Active regulator of SIRT1	0.08%
14-3-3 protein zeta/delta	0.08%
Ubiquitin-60S ribosomal protein L40	0.07%
Tumor necrosis factor	0.07%
Tectonic-3	0.07%
Histone H3	0.07%
Cystatin	0.07%
Collectin-43	0.07%
Beta-enolase	0.07%
5'-nucleotidase, cytosolic III-like	0.07%
Immunoglobulin J chain	0.06%
Hepatocyte growth factor-like protein	0.06%
Galectin-3-binding protein	0.06%
Cathelicidin	0.06%
Arginase-1	0.06%
Triosephosphate isomerase	0.05%
Selenoprotein P	0.05%
PRKR-interacting protein 1	0.05%
Peroxiredoxin-1	0.05%
Periostin	0.05%
Gamma-glutamylcyclotransferase	0.05%
Elongation factor 1-alpha	0.05%
Creatine kinase	0.05%
Complement component 1	0.05%
Coagulation factor XII	0.05%
Coagulation factor XI	0.05%
40S ribosomal protein S3	0.05%
Thyroglobulin	0.04%
SUMO-protein ligase NSE2	0.04%
Sulfhydryl oxidase	0.04%
SLA protein	0.04%
Secreted, acidic, cysteine-rich protein	0.04%
Reticulocalbin 2, EF-hand calcium binding domain	0.04%
Poly [ADP-ribose] polymerase	0.04%
Junction plakoglobin	0.04%
Ig kappa chain C region	0.04%
Hyaluronan-binding protein 2	0.04%
Heat shock protein	0.04%
Heat shock cognate 71 kDa protein	0.04%

Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-3	0.04%
Cadherin-5	0.04%
VPS33B-interacting protein	0.02%
Vitamin D3 receptor	0.02%
UPF0544 protein C5orf45 homolog	0.02%
Sperm-associated antigen 16 protein	0.02%
Similar to Protein C11orf33	0.02%
SERPINA11 protein	0.02%
Serpin H1	0.02%
Regulator of G-protein-signaling 9	0.02%
Phosphatidylinositol-glycan-specific phospholipase D	0.02%
Peripherin	0.02%
Pancreatic elastase inhibitor	0.02%
Osteomodulin	0.02%
Myocyte enhancer factor 2D	0.02%
Mitogen-activated protein kinase	0.02%
Interleukin-2 receptor	0.02%
EGF-containing fibulin-like extracellular matrix protein 1	0.02%
Desmoglein-1	0.02%
Cytochrome P450 20A1	0.02%
CCDC90A protein	0.02%
Caspase 8	0.02%
cAMP-dependent protein kinase type I-alpha regulatory subunit	0.02%
ATP synthase subunit beta	0.02%
Ventricular zone-expressed PH domain-containing protein-like protein 1	0.01%
WD repeat-containing protein 48	0.01%
Transmembrane protein 63C	0.01%
Transforming growth factor-beta-induced protein ig-h3	0.01%
TFIIH basal transcription factor complex helicase XPB subunit	0.01%
Schlafen family member 12	0.01%
Ribosomal RNA processing protein 1-like protein B	0.01%
Protein Shroom2	0.01%
Platelet glycoprotein Ib alpha chain	0.01%
Phosphatidate phosphatase LPIN2	0.01%
Leucine-rich repeat flightless-interacting protein 1	0.01%
Hepatocyte growth factor receptor	0.01%
General vesicular transport factor p115	0.01%
FMR1-interacting protein 1	0.01%
Fanconi anemia group A protein	0.01%
E3 ubiquitin-protein ligase Topors	0.01%
Disintegrin and metalloproteinase domain-containing protein 10	0.01%

Cytochrome b-245 heavy chain	0.01%
Collagen, type III, alpha 1	0.01%
Coagulation factor XIII, B polypeptide	0.01%
AT-rich interactive domain-containing protein 5A	0.01%
Acyl-CoA synthetase long-chain family member 5	0.01%
Centrosomal protein of 152 kDa	0.01%
DNA polymerase	0.00%
Pre-mRNA-processing-splicing factor 8	0.00%
Alpha-1A subunit calcium channel long splice variant	0.00%
Piezo-type mechanosensitive ion channel component	0.00%
Fibrillin-1	0.00%
WD repeat-containing protein 87	0.00%
Protein furry-like protein	0.00%
Cytoplasmic dynein 2 heavy chain 1	0.00%
