Supplementary information

Supplementary method

Bioinformatics analysis

The Gene Ontology (GO) program Blast2GO (https://www.blast2go.com/) was used to annotate DEPs（differential expression proteins） to create histograms of GO annotations, including cell components, biological processes, and molecular functions.

For pathway analysis, the differentially proteins were mapped to the terms in the KEGG (Kyoto Encyclopedia of Genes and Genomes) database by using the KAAS program (http://www.genome.jp/kaas-bin/kaas_main).

Protein-protein interaction networks were analyzed using the publicly available program STRING (http://string-db.org/) and minimum required interaction score set 0.400. STRING is a database of known and predicted protein-protein interactions. The interactions include direct (physical) and indirect (functional) associations, and they are derived from four sources: the genomic context, high-throughput experiments, co-expression, and previous knowledge.

Supplementary figures

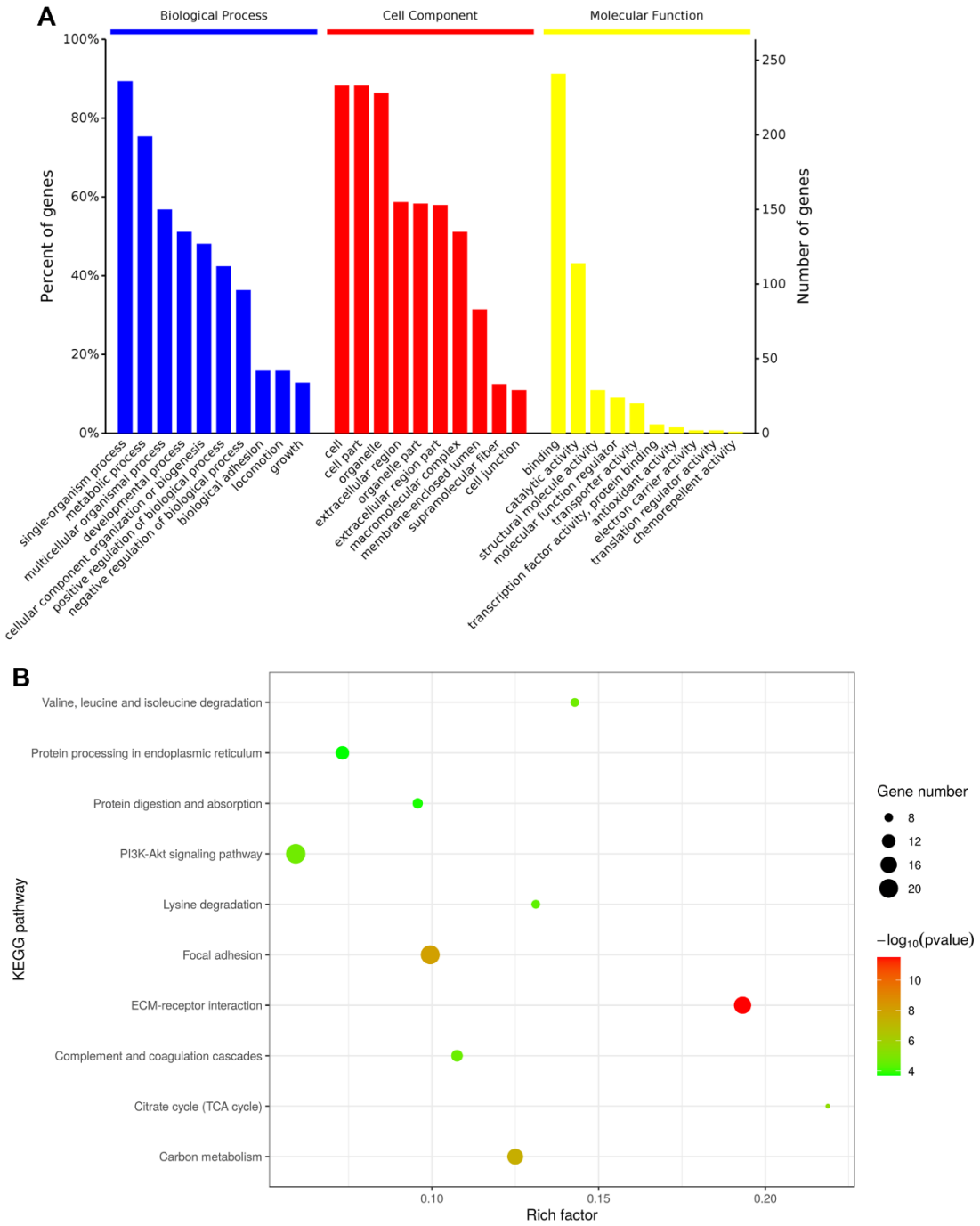


Figure S1. The GO analysis (A) and KEGG pathway annotation (B) for the differentially expressed protein comparisons in the young and newborn groups.

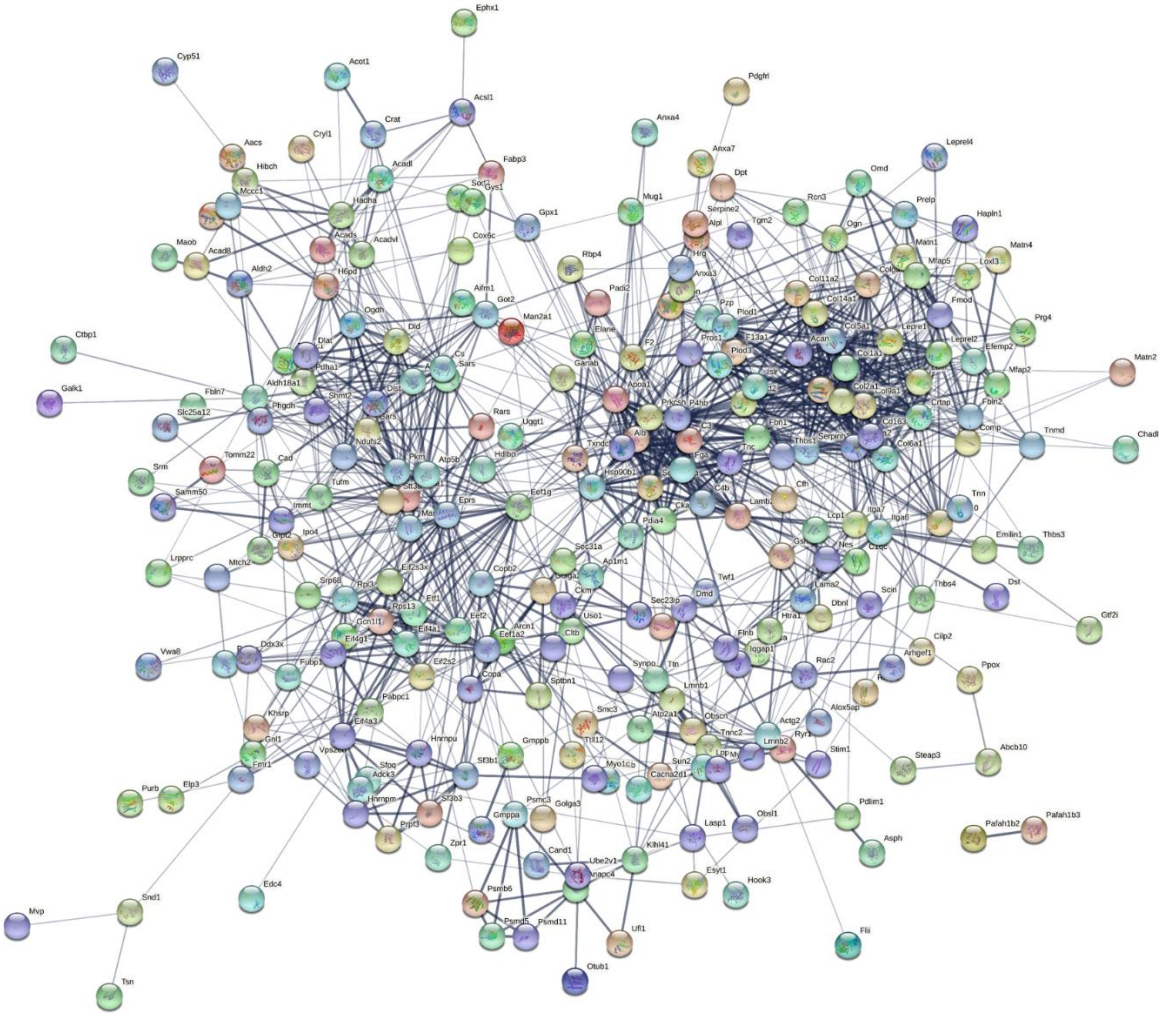
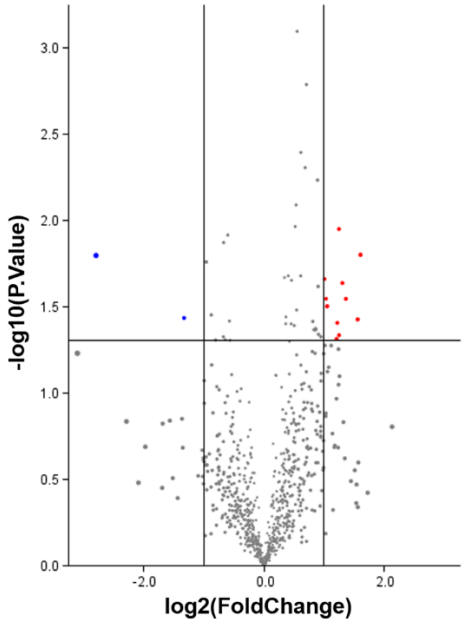


Figure S2. The protein-protein interaction network analysis of the differentially abundnat proteins in the young and newborn groups.



Lower abundance proteins (A has less)	FC
Trifunctional enzyme subunit alpha	0.144
Laminin subunit beta-1	0.398

- Higher abundance
- No changed
- Lower abundance

Higher abundance proteins (A has more)	FC
Fibulin-7	3.058
Serine protease HTRA4	2.958
Lamin-B1	2.581
Serine protease HTRA1	2.479
GTPase IMAP family member 4	2.385
Hyaluronan and proteoglycan link protein 1	2.338
Insulin-like growth factor-binding protein 3	2.318
Mucosa-associated lymphoid tissue lymphoma translocation protein 1 homolog	2.086
C-type lectin domain family 3 member A	2.069
Splicing factor 3B subunit 3	2.055
Phosphoenolpyruvate carboxykinase	2.018

Figure S3. The volcano plot of proteins in the adult and young groups. The blue dots represent the lower abundance proteins in the adult group compared with the young group. The red dots represent the higher abundance proteins in the adult group compared with the young group. The gray dots show the proteins with no difference between the two groups. The top 20 lower and top 20 higher proteins are listed (FC: fold change).

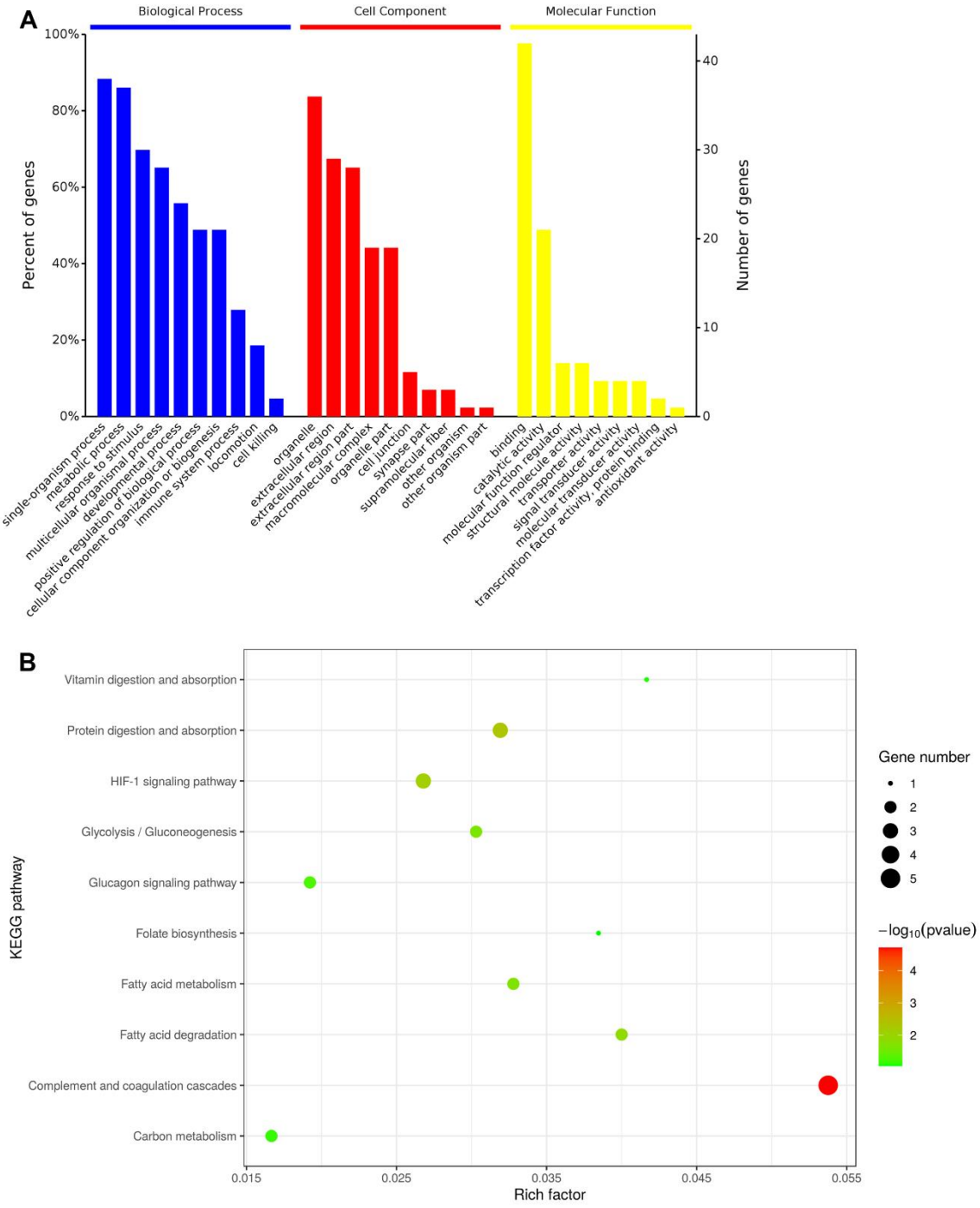


Figure S4. The GO analysis (A) and KEGG pathway annotation (B) for the differentially abundant protein comparison in the old and adult groups.

Supplementary tables

Table S1. Mass spectrometry data acquisition settings

	DDA	DIA
Injection Mode	5 µL partial loop pickup	5 µL partial loop pickup
Sample Loop	20 µL	20 µL
Stationary Phase	Easy Spray, PepMap RSLC C18 100 Å, 3 µm, 25 cm	Easy Spray PepMap RSLC C18 100 Å, 3 µm, 25 cm
LC Solvent A	100% H_2O, 0.1% formic acid	100% H_2O, 0.1% formic acid
LC Solvent B	100% MeCN, 0.1% formic acid	100% MeCN, 0.1% formic acid
LC Gradient	2-25% 5-95 min 25-55% 95-98 min 55-85% 98-98.1 min	2-25% 5-95 min 25-55% 95-98 min 55-85% 98-98.1 min
LC Flow Rate	300 nL/min	300 nL/min
Mass Spectrometer	Thermo Orbitrap Exploris 480	Thermo Orbitrap Exploris 480
Method Type	Data dependent MS2, cycle time 3s	Data dependent MS2, cycle time 3s
Spray Voltage	2.0 kV	2.0 kV
Ion Transfer Temperature	275 °C	275 °C
RV lens	40%	40%
MS^1 Detector	Orbitrap	Orbitrap
MS^1 scan range	375-1800 m/z	380-985 m/z
MS^1 resolution	120,000	60,000
MS^1 AGC Target	300 %	100 %
MS^1 Maximum IT	100 ms	100 ms
MS^2 Detector	Orbitrap	Orbitrap

MS^2 resolution	15,000	15,000
Isolation Window	1.6 m/z	10 m/z
MS^2 AGC Target	50%	200%
MS^2 Maximum IT	40 ms	40 ms
Activation Type / Collision Energy	HCD 28%	HCD 28%
Intensity Threshold	5e3	NA
Dynamic Exclusion	36 s	NA
Charge State Inclusion	+2-5	NA
Window overlap	NA	1 m/z
Window placement optimization	NA	On
# windows	NA	59
Loop control	NA	N=30

Table S2. Data Analysis settings

Workflow	DIA library	DIA
Platform	Spectronaut 14	Spectronaut 14
Search Algorithm	Pulsar™	Spectral Matching
Validation	Kernel Density Estimator	Kernel Density Estimator
Database	SwissProt; Mouse; Trypsin, created 12/12/2019	SwissProt; Mouse; Trypsin, created 12/12/2019
Enzyme (semi/full)	Trypsin/P	Trypsin/P
Missed Cleavages	2	2
Precursor mass tolerance	Dynamic	Dynamic
Fragment mass tolerance	Dynamic	Dynamic
Static Modifications	Methylthio (C)	NA
Dynamic Modifications	Oxidation (M), Acetylation (N-term)	NA
Target FDR (Strict):	0.01	0.01
Target FDR (Relaxed):	na	na
Validation basis	q-value	q-value