Supplementary Information

Transcriptomic Modulation by Exosomes Derived from Human Adipose Stem Cells in Neuronal Cells: Insights from mRNA Sequencing Analysis

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Supplementary Files 1 3D video on exosome uptake

Supplementary Files 2 Expression result

Supplementary Files 3 DEG analysis

Supplementary Files 4 Uncropped western blot images

Supplementary Table 1 List of mouse primers for qRT-PCR.

Target	Forward 5'→ 3'	Reverse 5'→ 3'
p21	AATCTGCGCTTGGAGTGATAG	CTTGTCGCTGTCTTGCACT
p16	TTGGCCCAAGAGCGGGGACA	GCGGGCTGAGGCCGGATTTA
NF-κB	ACCTTTGCTGGAAACACACC	ATGGCCTCGGAAGTTTCTTT
IL6	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
GDF15	AGCCGAGAGGACTCGAACTCAG	GGTTGACGCGGAGTAGCAGCT
GAPDH	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGA

Supplementary Table 2 Software list

Tools	Description	Linkages
HISAT2	A spliced read mapper for RNA-Seq	http://ccb.jhu.edu/software/hisat2/index.shtml
StringTie	Transcript assembly for RNA-Seq	https://ccb.jhu.edu/software/stringtie/index.shtml
ASprofile	ASprofile is a suite of programs for extracting, quantifying and comparing alternative splicing (AS) events from RNA-seq data	http://ccb.jhu.edu/software/ASprofile/
BLAST	Basic Local Alignment Search Tool	http://blast.ncbi.nlm.nih.gov/Blast.cgi
DESeq	An R package for RNA-Seq Differential Expression Analysis based on a model using the negative binomial distribution	http://www.bioconductor.org/packages/release/bioc/html/DESeq.html
EBSeq	An R package for RNA-Seq Differential Expression Analysis based on Bayesian approach	https://www.biostat.wisc.edu/~kendzior/EBSEQ/
Cytoscape	An open-source software platform for visualizing complex networks	http://www.cytoscape.org/
topGO	An R package for gene ontology enrichment analysis	The bioinformatic analysis software not given in the report
rMATs	MATS is a computational tool to detect differential alternative splicing events from RNA-Seq data.	http://rnaseq-mats.sourceforge.net/
TFBStools	An R package for the analysis and manipulation of transcription factor binding sites.	http://www.bioconductor.org/packages/release/bioc/html/TFBSTools.html

Supplementary Table 3 Database list

Database	Description	Homepage
NR	non-redundant protein sequence database	ftp://ftp.ncbi.nih.gov/blast/db/
Swiss-Prot	A manually annotated, non-redundant protein sequence database	http://www.uniprot.org/
GO	Gene Ontology database	http://www.geneontology.org/
COG	The database of Clusters of Orthologous Groups of proteins	http://www.ncbi.nlm.nih.gov/COG/
KOG	The database of Clusters of Protein homology	http://www.ncbi.nlm.nih.gov/KOG/
Pfam	The database of Homologous protein family	http://pfam.xfam.org/
KEGG	The database of Kyoto Encyclopedia of Genes and Genomes	http://www.genome.jp/kegg/
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins	http://www.string-db.org/
Ensembl	Database Sscrofa10.2 download from	http://asia.ensembl.org/index.html
Cosmic	COSMIC, is the world's largest and most comprehensive resource for exploring the impact of somatic mutations in human cancer.	https://cancer.sanger.ac.uk/cosmic
JASPAR	Database of transcription factor binding profiles	http://jaspar.genereg.net/

Supplementary Table 4 Description on annotation databases

Database name	Database description
NR database	Non-redundant protein database in NCBI, including Swissprot, PIR (Protein Information Resource), PRF (Protein Research Foundation), PDB (Protein Data Bank) protein database and CDS from GenBank and RefSeq
Swissprot database	A database maintained by EBI (European Bioinformatics Institute) containing a collated database of protein annotation information with relevant references and high credibility
COG database	A database for homologous classification of gene products. It is an early database for the identification of orthologous genes, which is obtained by comparing a large number of protein sequences of various organisms.
KOG database	For eukaryotes, homologous genes from different species are divided into different Ortholog clusters based on gene orthologous relationships and evolutionary relationships. Currently, KOG has 4852 classifications. Genes from the same Ortholog have the same function, so that functional annotations can be directly inherited to other members of the same KOG cluster.
Pfam database	The most comprehensive classification system for protein domain annotations. Proteins are composed of domains, and the protein sequences of each particular domain are somewhat conserved. Pfam divides the protein domain into different protein families, and establishes an HMM statistical model of the amino acid sequence of each family through alignment of protein sequences.
GO database	The internationally standardized gene function classification system provides a dynamically updated standard vocabulary to fully describe the functional properties of genes and gene products in organisms. There are three main categories of the database, namely molecular function, cellular component and biological process, each describing the molecular function that the gene product may perform, and the cellular environment and Participation in biological processes. The most basic concept in the GO database is Term, each entry has a Term name, such as "cell", "fibroblast growth factor receptor binding" or "signal transduction", with a unique number, like GO:nnnnnnn
KEGG database	A database that systematically analyzes the metabolic pathways of gene products in cells and the function of these gene products. It integrates data on genomics, chemical molecules, and biochemical systems, including PATHWAY, DRUG, DISEASE, GENES, and GENOME. Using this database helps to study the genes and their expressions as a whole network.

Supplementary Table 5 Sample information for mRNA-sequencing

#ID	read1_name	read2_name
CTL1	Unknown_CP734-001T0001_good_1.fq	Unknown_CP734-001T0001_good_2.fq
CTL2	Unknown_CP734-001T0002_good_1.fq	Unknown_CP734-001T0002_good_2.fq
CTL3	Unknown_CP734-001T0003_good_1.fq	Unknown_CP734-001T0003_good_2.fq
DG1	Unknown_CP734-001T0004_good_1.fq	Unknown_CP734-001T0004_good_2.fq
DG2	Unknown_CP734-001T0005_good_1.fq	Unknown_CP734-001T0005_good_2.fq
DG3	Unknown_CP734-001T0006_good_1.fq	Unknown_CP734-001T0006_good_2.fq
DGEx1	Unknown_CP734-001T0007_good_1.fq	Unknown_CP734-001T0007_good_2.fq
DGEx2	Unknown_CP734-002T0001_good_1.fq	Unknown_CP734-002T0001_good_2.fq
DGEx3	Unknown_CP734-001T0008_good_1.fq	Unknown_CP734-001T0008_good_2.fq

Note: #ID:sample name; read1_name & read2_name:Double-ended fq file name corresponding to the sample name.

Supplementary Table 6 Sequencing data Statistics

#SampleID	ReadSum	BaseSum	GC (%)	N (%)	Q20(%)	Q30(%)
CTL1	19829143	5.92E+09	49.79	0	99.85	98.76
CTL2	21755225	6.49E+09	49.61	0.02	98.79	94.99
CTL3	20372686	6.08E+09	50.05	0.01	98.89	95.36
DG1	21125385	6.3E+09	50.13	0.02	98.68	94.72
DG2	20400624	6.09E+09	49.45	0.01	98.86	95.38
DG3	21236873	6.34E+09	49.99	0.01	98.86	95.25
DGEx1	20293541	6.07E+09	51	0.01	98.86	95.34
DGEx2	22084086	6.6E+09	50.47	0.01	98.88	95.42
DGEx3	21632825	6.47E+09	50.54	0.02	98.76	94.88

Note:

(1) Samples: Sample name;

(2) Clean reads: Counts of clean PE reads;

(3) Clean bases: total base number of Clean Data;

(4) GC content: Percentage of G, C in clean data.

(5) ≥Q30%: Percentage of bases with Q-score no less than Q30.

Supplementary Table 7 Statistics on data mapping

Sample	Total Reads	Mapped Reads	Uniq Mapped Reads	Multiple Map Reads	Reads Map to '+'	Reads Map to '-'
CTL1	39658286.00	38,044,113 (95.93%)	34,888,310 (87.97%)	3,155,803 (7.96%)	21,010,023 (52.98%)	20,993,585 (52.94%)
CTL2	43510450.00	41,393,251 (95.13%)	37,893,988 (87.09%)	3,499,263 (8.04%)	22,871,461 (52.57%)	22,866,541 (52.55%)
CTL3	40745372.00	38,677,910 (94.93%)	35,553,675 (87.26%)	3,124,235 (7.67%)	21,295,490 (52.26%)	21,287,769 (52.25%)
DG1	42250770.00	39,691,063 (93.94%)	36,595,130 (86.61%)	3,095,933 (7.33%)	21,802,760 (51.60%)	21,777,413 (51.54%)
DG2	40801248.00	36,265,809 (88.88%)	33,538,161 (82.20%)	2,727,648 (6.69%)	19,846,596 (48.64%)	19,838,251 (48.62%)
DG3	42473746.00	40,196,814 (94.64%)	37,046,210 (87.22%)	3,150,604 (7.42%)	22,077,305 (51.98%)	22,054,631 (51.93%)
DGEx1	40587082.00	38,649,165 (95.23%)	36,097,856 (88.94%)	2,551,309 (6.29%)	20,979,844 (51.69%)	20,966,220 (51.66%)
DGEx2	44168172.00	42,202,397 (95.55%)	38,900,197 (88.07%)	3,302,200 (7.48%)	23,169,263 (52.46%)	23,155,199 (52.43%)
DGEx3	43265650.00	41,261,149 (95.37%)	38,326,028 (88.58%)	2,935,121 (6.78%)	22,508,378 (52.02%)	22,494,249 (51.99%)

Note:

Sample: sample ID in system;

Total Reads: Counts of Clean Reads, counted as single end;

Mapped Reads: Counts of mapped reads and the proportion of that in clean data;

Uniq Mapped Reads: Counts of reads mapped to a unique position on reference genome and proportion of that in clean data;

Multiple Mapped Reads: Counts of reads mapped to multiple positions on reference genome and proportion of that in clean data;

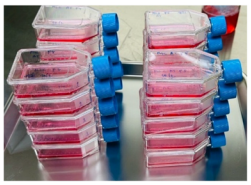
Reads Map to '+': Counts of reads mapped to the sense chain and the proportion of that in clean data;

Reads Map to '-': Counts of reads mapped to antisense chain and proportion of that in clean data.

Supplementary Figure 1

Schematic overview of experimental workflow for assessing the transcriptomic effects of hASC-derived exosomes on D-galactose–induced neuronal cells. Human adipose-derived stem cells (hASCs) were exposed to serum-free medium for 48 h, and the conditioned media (CM) were collected, centrifuged (2,000 g, 10 min), and filtered (0.22 μm) prior to storage at –80 °C. Exosomes were subsequently purified and concentrated from CM using a Macrosep 100K ultrafiltration device with repeated centrifugation (2,000 g, 15 min, 6 cycles). Purified hASC-derived exosomes were characterized by nanoparticle tracking analysis, cryo-electron microscopy (cryo-EM), Western blot, and PKH26 staining. For transcriptomic analysis, neuronal CAD cells were divided into three groups: untreated cells (A), D-galactose–treated cells (B), and D-galactose plus hASC-exosome–treated cels(C). Total RNA was extracted and subjected to Illumina sequencing, followed by bioinformatics analysis.

In vitro expansion of human ASCs



Serum-free media, 48 h

Condition media (CM) collection

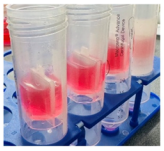
2,000 g, 10 min
0.22 µm filtered

Stored -80 ˚C

Exosome purification & characterization

Macrosep 100K ultrafiltration device

3,000 g
15 min
X 6 times



Concentrated hASC-exosomes

Characterization

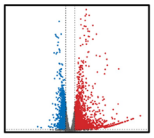
- ☑ Nanotracking particle analysis
- ☑ Cryo EM
- ☑ Western blot
- ☑ PHK26 staining

Gene expression profile in D-galactose-exposed neuronal cells

Neuronal CAD cells

D-galactose	-	+	+
hASC-exosomes	-	-	+

Illumina sequencing



Differentially expressed genes

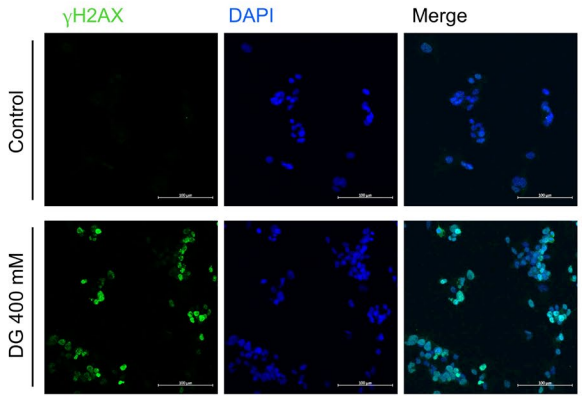
- DESeq2

Bioinformatics

- GOseq R packages
- KOBAS software
- STRING database

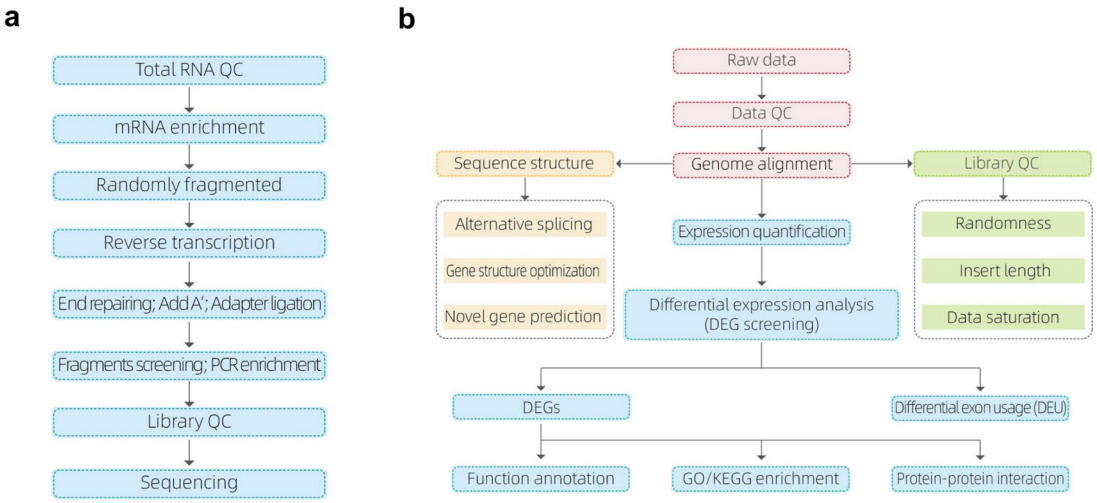
Supplementary Figure 2

D-galactose induced DNA-damage response in neuronal CAD cells. The CAD cells were exposed to D-galactose (DG, 400 mM) for 24. The immunocytochemistry stained for γ-H2AX, a marker of DNA damage showed the increased number of the γ-H2AX-positive cells after DG exposure. DAPI stained for nucleus. Scale bar is 100 μm.


γH2AX
DAPI
Merge
Control
DG 400 mM

Supplementary Figure 3

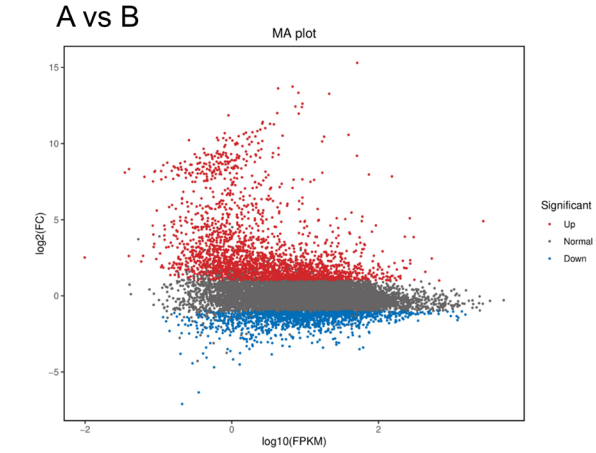
Diagram showing the workflow of bulk mRNA-sequencing and downstream analysis. (a) the sequential steps of mRNA isolation, enrichment, and preparation for mRNA-sequencing analysis. (b) The diagram showing the post-sequencing steps including data quality control (QC), genome alignment, expression quantification, differential expression analysis, and functional annotation.


a
Total RNA QC
mRNA enrichment
Randomly fragmented
Reverse transcription
End repairing; Add A'; Adapter ligation
Fragments screening; PCR enrichment
Library QC
Sequencing
b
Raw data
Data QC
Sequence structure
Genome alignment
Library QC
Alternative splicing
Gene structure optimization
Novel gene prediction
Expression quantification
Differential expression analysis (DEG screening)
Randomness
Insert length
Data saturation
DEGs
Differential exon usage (DEU)
Function annotation
GO/KEGG enrichment
Protein-protein interaction

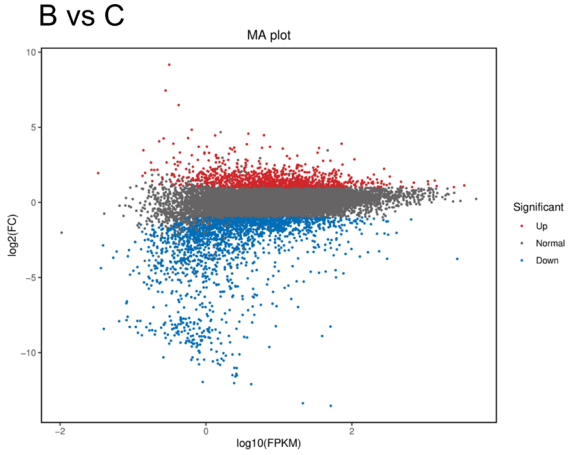
Supplementary Figure 4

MA plots illustrating the overall distribution of gene expression of the mRNA-seq data. MA plot of the gene expression and fold change of expression levels between (a) A vs B, and (b) B vs C samples. Note that each dot represents a single gene. X-axis represents values of log2 (FPKM). Y-axis represents values of log2(FC). The dots colored in red and blue stand for significant up-regulated and down-regulated genes respectively. Black dots stand for the genes without significant difference in expression between two samples. A: Untreated cells, B: D-galactose-induced cells, C: DG+ hASC-exosome treated cells.

a

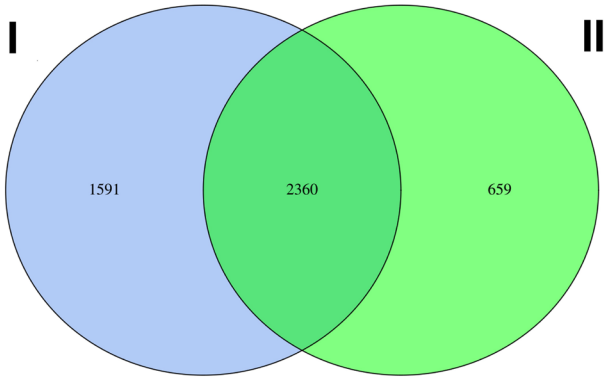

A vs B
MA plot
log2(FC)
log10(FPKM)
Significant
Up
Normal
Down

b


B vs C
MA plot
log2(FC)
log10(FPKM)
Significant
Up
Normal
Down

Supplementary Figure 5

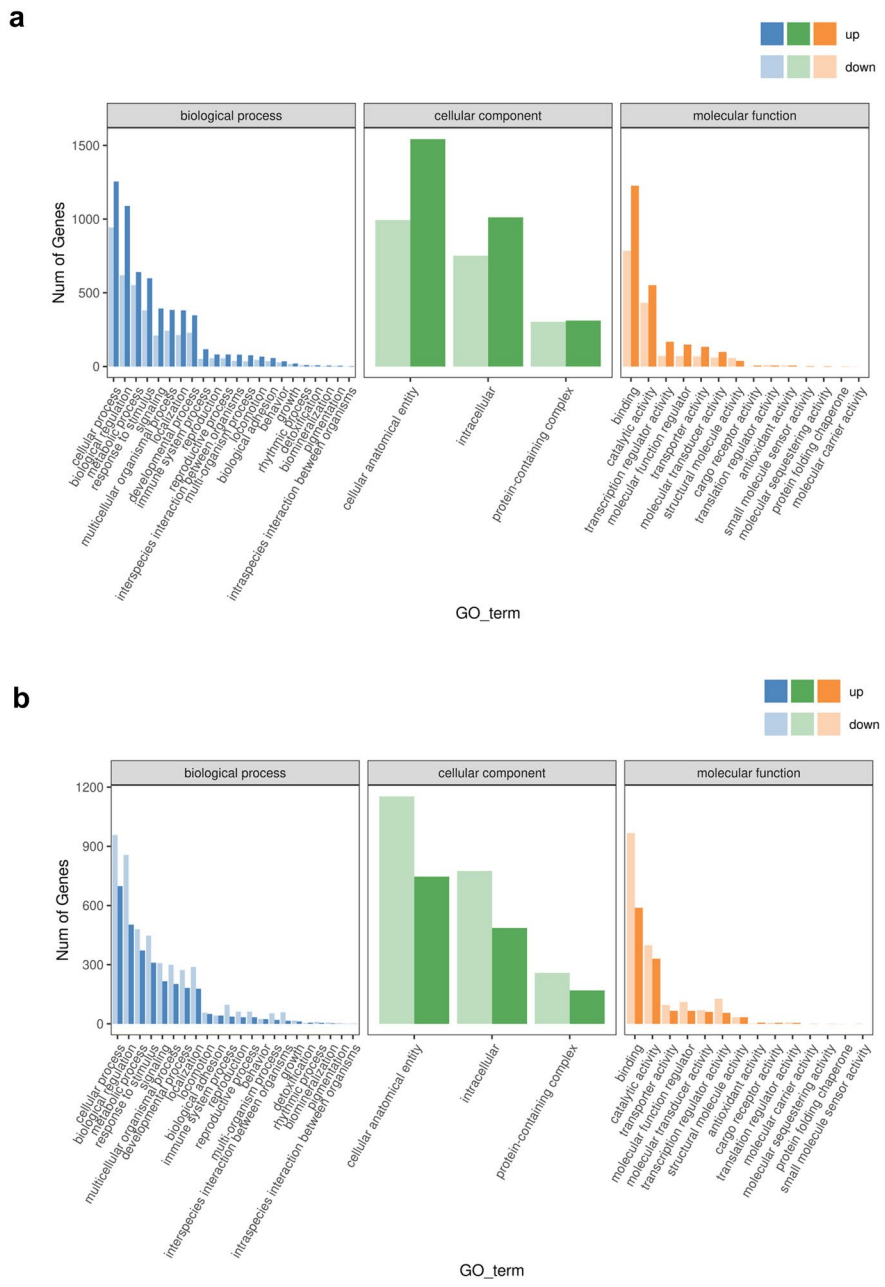
Venn Diagram showing the number of unique DEGS (1,597 and 659) between each comparing group and overlapping DEGs (2,360). I represents a total number of DEGS between A vs B, while II represents a total number of DEGs between B vs C. A: Untreated cells, B: D-galactose-induced cells, C: DG+ hASC-exosome treated cells.



I: A (untreated) vs B (D-galactose-treated)
II: B vs C (hASC-exosome and D-galactose treated)

Supplementary Figure 6

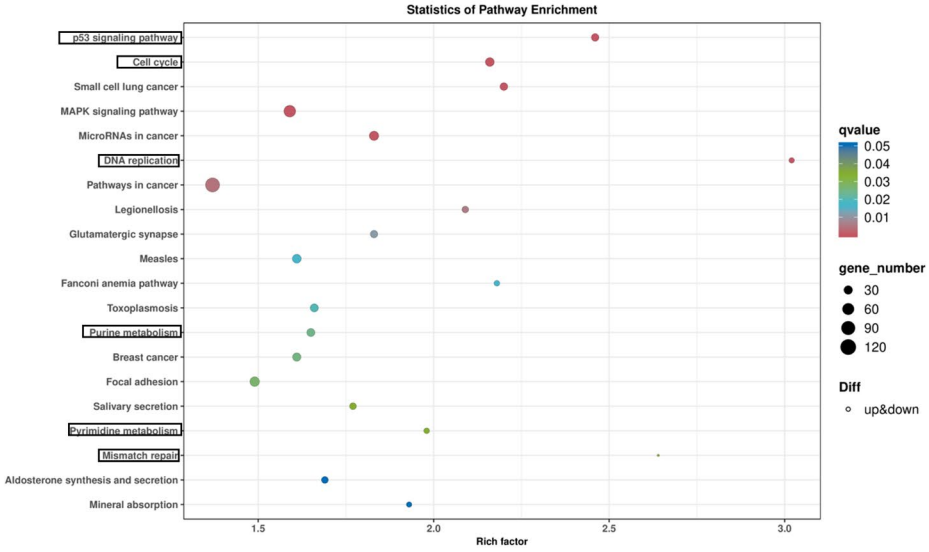
Gene Ontology (GO) classification of DEGs in each comparing group including (a) A vs B, and (b) B vs C. The bar graph illustrates the number of upregulated (dark color) and downregulated (lighter color) genes associated with each GO category, including Biological Process, Cellular Component, and Molecular Function.



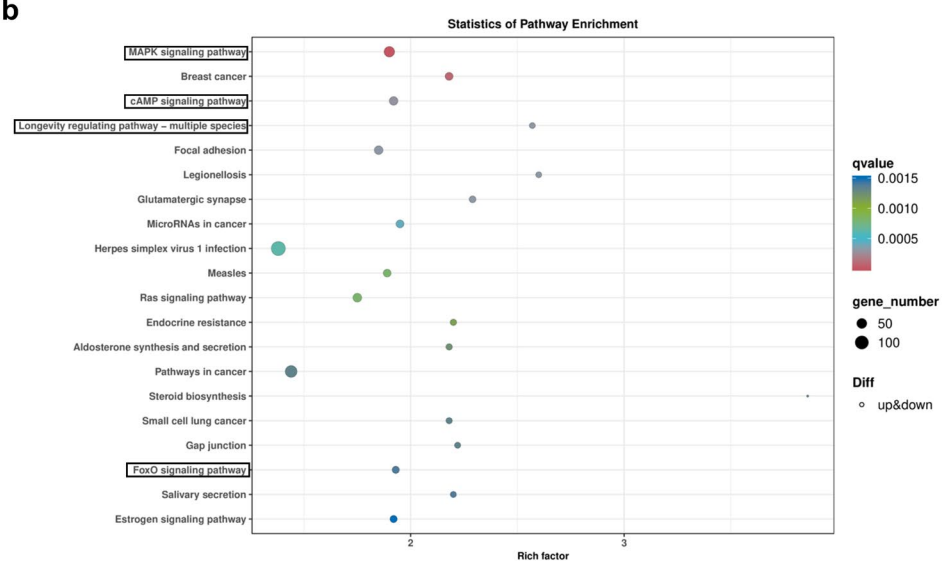
Supplementary Figure 7

KEGG pathway enrichment analysis of total DEGs identified from each comparing group. (a) KEGG pathway enrichment analysis of total DEGs from group B vs C. Key pathways such as p53 signaling pathway, Cell cycle, DNA replication, and Pyrimidine metabolism are highlighted. (b) KEGG pathway enrichment analysis of total DEGs from group B vs C. Key pathways such as MAPK signaling pathway, cAMP signaling pathway, Longevity regulating pathway, FoxO signaling pathway, and Estrogen signaling pathway are highlighted. The scatter plot displays enriched pathways based on the Rich factor (x-axis), with the number of genes involved represented by the size of each dot and the q-value indicated by color (from blue to red, where red denotes higher significance).

a


Statistics of Pathway Enrichment
p53 signaling pathway
Cell cycle
Small cell lung cancer
MAPK signaling pathway
MicroRNAs in cancer
DNA replication
Pathways in cancer
Legionellosis
Glutamatergic synapse
Measles
Fanconi anemia pathway
Toxoplasmosis
Purine metabolism
Breast cancer
Focal adhesion
Salivary secretion
Pyrimidine metabolism
Mismatch repair
Aldosterone synthesis and secretion
Mineral absorption
1.5
2.0
2.5
3.0
Rich factor
qvalue
0.05
0.04
0.03
0.02
0.01
gene_number
30
60
90
120
Diff
up&down

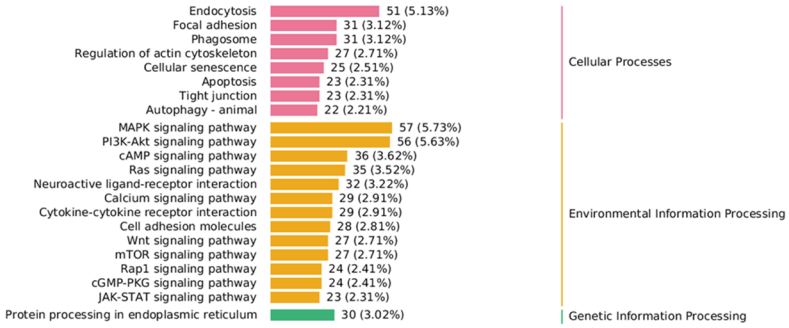
b


Statistics of Pathway Enrichment
MAPK signaling pathway
Breast cancer
cAMP signaling pathway
Longevity regulating pathway – multiple species
Focal adhesion
Legionellosis
Glutamatergic synapse
MicroRNAs in cancer
Herpes simplex virus 1 infection
Measles
Ras signaling pathway
Endocrine resistance
Aldosterone synthesis and secretion
Pathways in cancer
Steroid biosynthesis
Small cell lung cancer
Gap junction
FoxO signaling pathway
Salivary secretion
Estrogen signaling pathway
2
3
Rich factor
qvalue
0.0015
0.0010
0.0005
gene_number
50
100
Diff
up&down

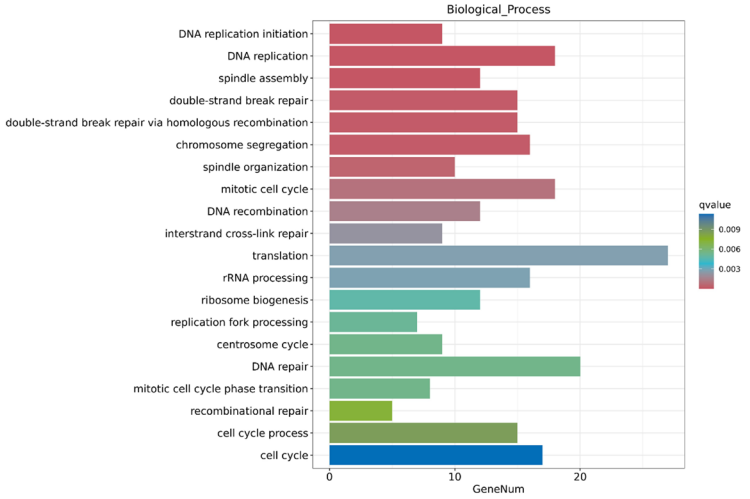
Supplementary Figure 8

Functional enrichment analysis of differentially expressed genes in A vs B. (a) KEGG pathway enrichment analysis of upregulated DEGs. The bar chart displays the percentage and number of annotated genes associated with significantly enriched pathways. (b) GO term enrichment analysis of downregulated DEGs. Bar chart showing significantly enriched Biological Process. The x-axis indicates the number of genes (GeneNum) associated with each term, and bar colors reflect statistical significance (q-values). (c) Network diagram illustrating gene-to-process associations of the downregulated DEGs, focusing on Cellular Component categories. Genes are connected to their respective biological processes, with color-coded categories. Fold change values are represented by shades of blue, suggesting key structural regions affected by gene downregulation under D-galactose exposure in neuronal cells.

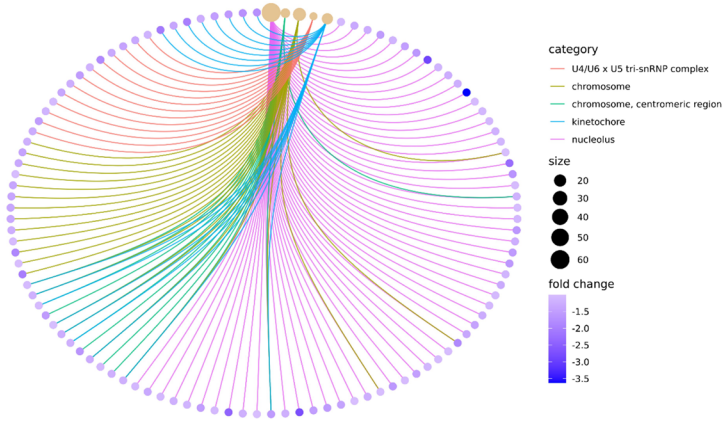
a


Endocytosis 51 (5.13%)
Focal adhesion 31 (3.12%)
Phagosome 31 (3.12%)
Regulation of actin cytoskeleton 27 (2.71%)
Cellular senescence 25 (2.51%)
Apoptosis 23 (2.31%)
Tight junction 23 (2.31%)
Autophagy - animal 22 (2.21%)
MAPK signaling pathway 57 (5.73%)
PI3K-Akt signaling pathway 56 (5.63%)
cAMP signaling pathway 36 (3.62%)
Ras signaling pathway 35 (3.52%)
Neuroactive ligand-receptor interaction 32 (3.22%)
Calcium signaling pathway 29 (2.91%)
Cytokine-cytokine receptor interaction 29 (2.91%)
Cell adhesion molecules 28 (2.81%)
Wnt signaling pathway 27 (2.71%)
mTOR signaling pathway 27 (2.71%)
Rap1 signaling pathway 24 (2.41%)
cGMP-PKG signaling pathway 24 (2.41%)
JAK-STAT signaling pathway 23 (2.31%)
Protein processing in endoplasmic reticulum 30 (3.02%)
Cellular Processes
Environmental Information Processing
Genetic Information Processing

b


Biological_Process
DNA replication initiation
DNA replication
spindle assembly
double-strand break repair
double-strand break repair via homologous recombination
chromosome segregation
spindle organization
mitotic cell cycle
DNA recombination
interstrand cross-link repair
translation
rRNA processing
ribosome biogenesis
replication fork processing
centrosome cycle
DNA repair
mitotic cell cycle phase transition
recombinational repair
cell cycle process
cell cycle
0
10
20
GeneNum
qvalue
0.009
0.006
0.003

c

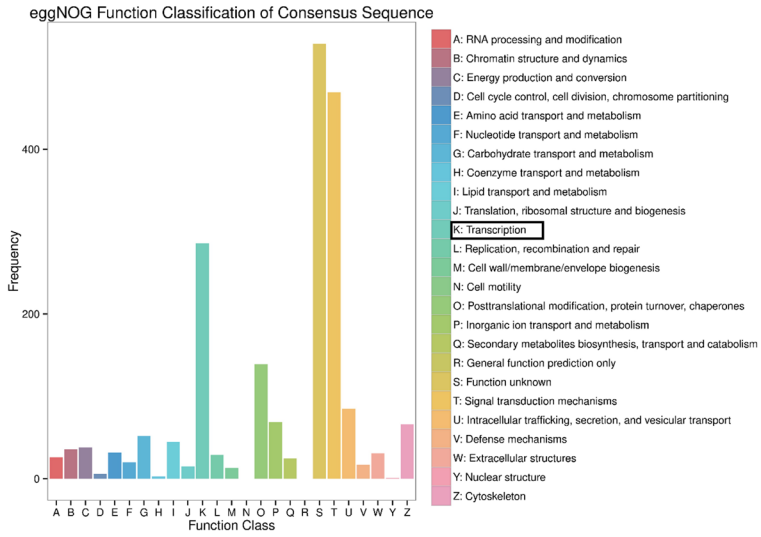

category
U4/U6 x U5 tri-snRNP complex
chromosome
chromosome, centromeric region
kinetochore
nucleolus
size
20
30
40
50
60
fold change
-1.5
-2.0
-2.5
-3.0
-3.5

Supplementary Figure 9

The eggNOG functional classification of consensus sequences of DEGs. The bar chart displays the frequency of sequences assigned to various functional categories (A–Z) based on eggNOG annotations. (a) eggNOG functional classification of consensus sequences of upregulated DEGs from group A vs B. The upregulated DEGs from group A vs B are enriched in transcription-related functions. (b) eggNOG functional classification of consensus sequences derived from downregulated DEGs between group B and group C. Notably, category K (Transcription) is highlighted, indicating a marked reduction in transcription-related functions. This suggests a shutdown of transcriptional activity, consistent with a broader suppression of gene expression in group B compared to group C.

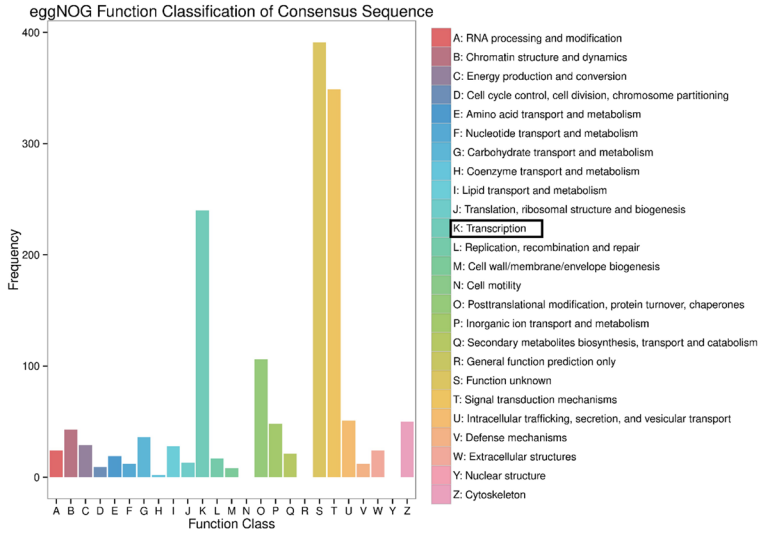
a

Upregulated genes in A vs B



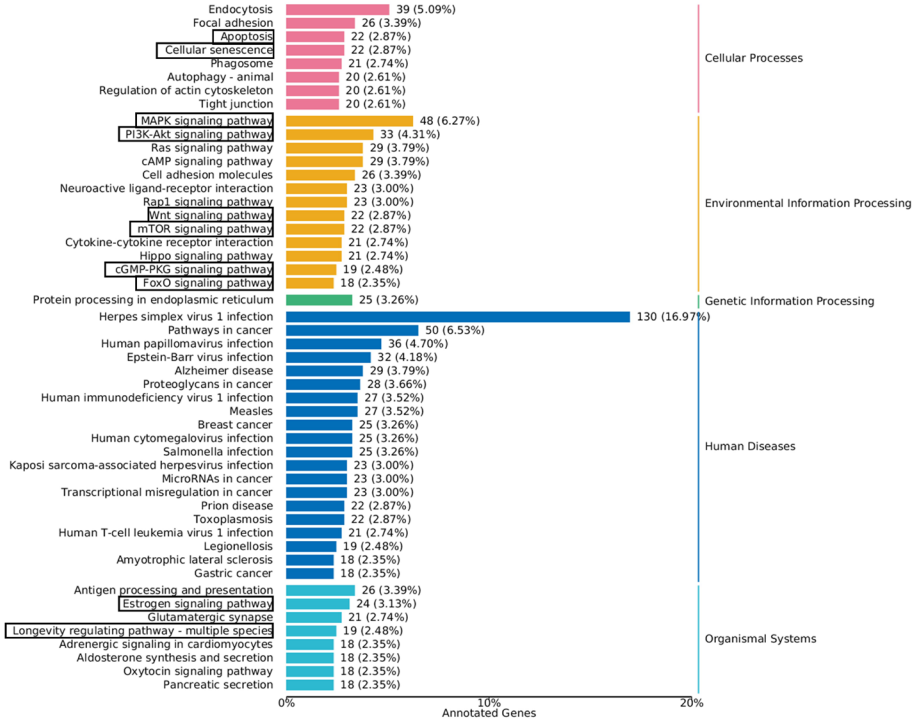
b

Downregulated genes in B vs C



Supplementary Figure 10

KEGG pathway enrichment analysis of downregulated DEGs between group B vs C. The bar chart displays the number and percentage of genes annotated to significantly enriched pathways across multiple functional categories, including apoptosis and cellular senescence. Highlighted cellular processes (square) are apoptosis and cellular senescence and age-implicated pathways


Endocytosis 39 (5.09%)
Focal adhesion 26 (3.39%)
Apoptosis 22 (2.87%)
Cellular senescence 22 (2.87%)
Phagosome 21 (2.74%)
Autophagy - animal 20 (2.61%)
Regulation of actin cytoskeleton 20 (2.61%)
Tight junction 20 (2.61%)
Cellular Processes
MAPK signaling pathway 48 (6.27%)
PI3K-Akt signaling pathway 33 (4.31%)
Ras signaling pathway 29 (3.79%)
cAMP signaling pathway 29 (3.79%)
Cell adhesion molecules 26 (3.39%)
Neuroactive ligand-receptor interaction 23 (3.00%)
Rap1 signaling pathway 23 (3.00%)
Wnt signaling pathway 22 (2.87%)
mTOR signaling pathway 22 (2.87%)
Cytokine-cytokine receptor interaction 21 (2.74%)
Hippo signaling pathway 21 (2.74%)
cGMP-PKG signaling pathway 19 (2.48%)
FoxO signaling pathway 18 (2.35%)
Environmental Information Processing
Protein processing in endoplasmic reticulum 25 (3.26%)
Genetic Information Processing
Herpes simplex virus 1 infection 130 (16.97%)
Pathways in cancer 50 (6.53%)
Human papillomavirus infection 36 (4.70%)
Epstein-Barr virus infection 32 (4.18%)
Alzheimer disease 29 (3.79%)
Proteoglycans in cancer 28 (3.66%)
Human immunodeficiency virus 1 infection 27 (3.52%)
Measles 27 (3.52%)
Breast cancer 25 (3.26%)
Human cytomegalovirus infection 25 (3.26%)
Salmonella infection 25 (3.26%)
Kaposi sarcoma-associated herpesvirus infection 23 (3.00%)
MicroRNAs in cancer 23 (3.00%)
Transcriptional misregulation in cancer 23 (3.00%)
Prion disease 22 (2.87%)
Toxoplasmosis 22 (2.87%)
Human T-cell leukemia virus 1 infection 21 (2.74%)
Legionellosis 19 (2.48%)
Amyotrophic lateral sclerosis 18 (2.35%)
Gastric cancer 18 (2.35%)
Human Diseases
Antigen processing and presentation 26 (3.39%)
Estrogen signaling pathway 24 (3.13%)
Glutamatergic synapse 21 (2.74%)
Longevity regulating pathway - multiple species 19 (2.48%)
Adrenergic signaling in cardiomyocytes 18 (2.35%)
Aldosterone synthesis and secretion 18 (2.35%)
Oxytocin signaling pathway 18 (2.35%)
Pancreatic secretion 18 (2.35%)
Organismal Systems
0%
10%
20%
Annotated Genes