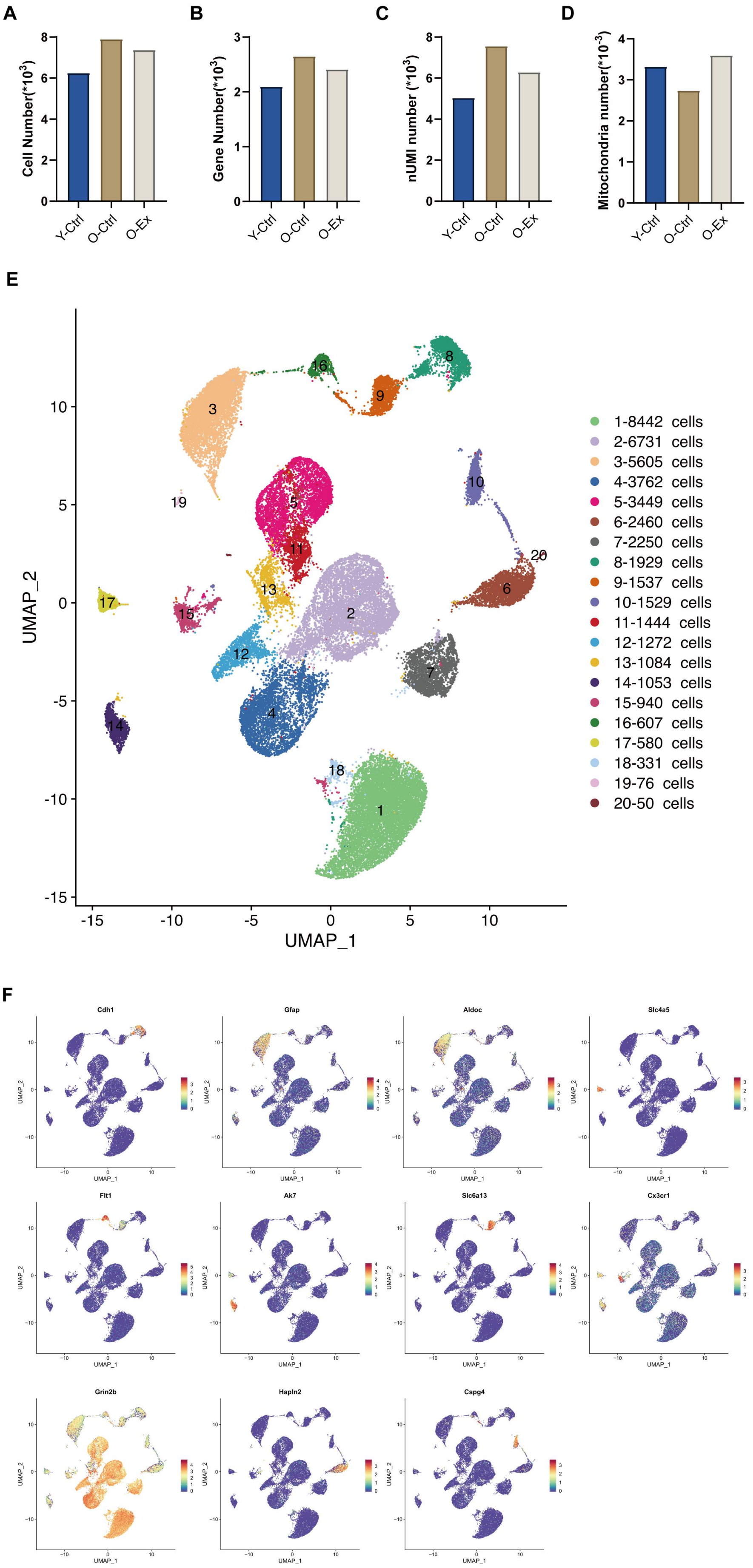
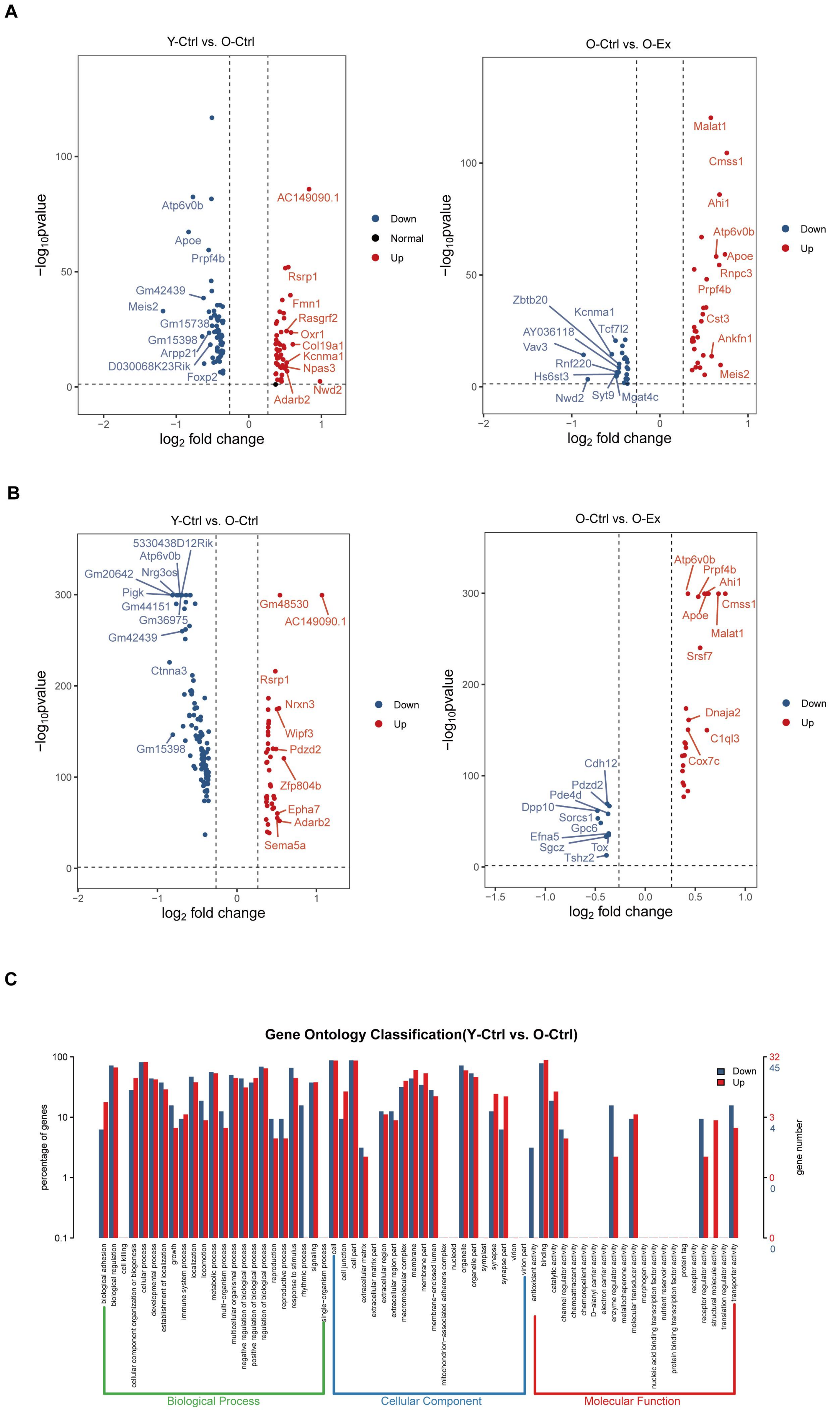
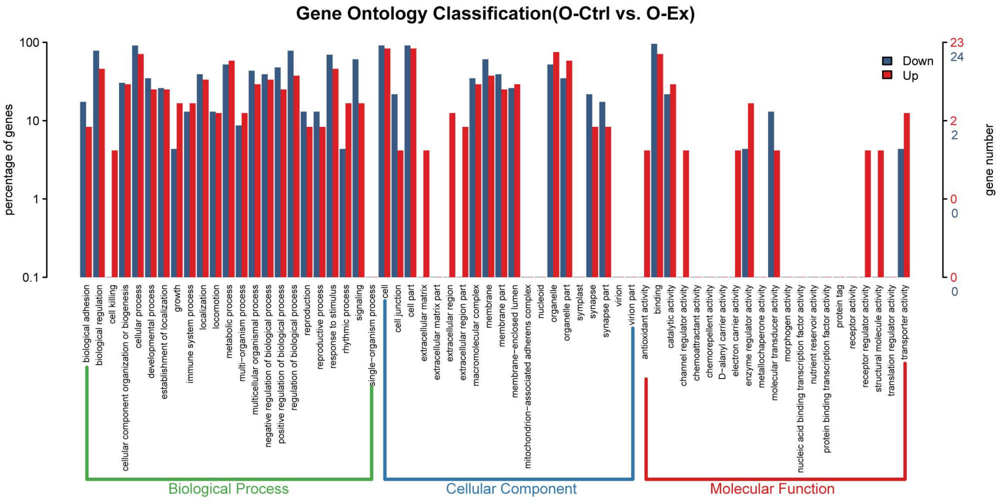
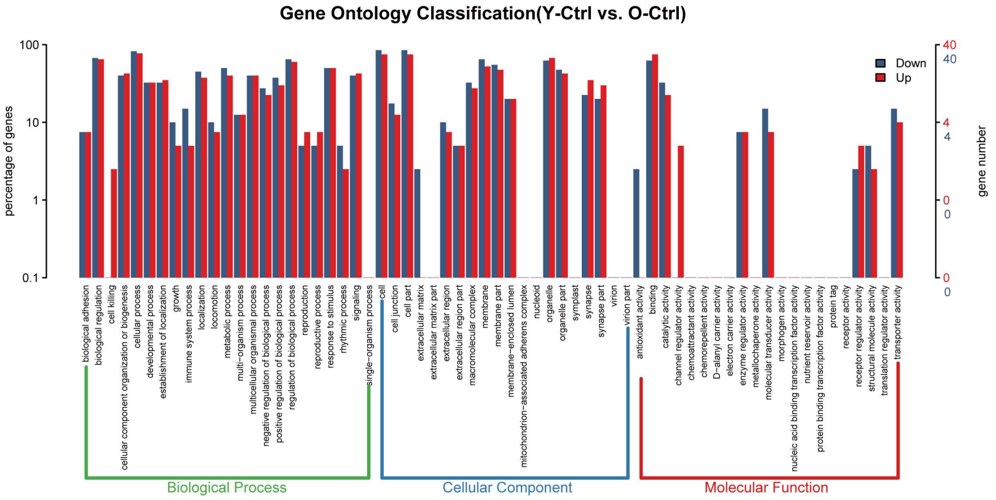

A
Cell Number(*10^3)
0
2
4
6
8
Y-Ctrl
O-Ctrl
O-Ex
B
Gene Number(*10^3)
0
1
2
3
Y-Ctrl
O-Ctrl
O-Ex
C
nUMI number (*10^3)
0
2
4
6
8
Y-Ctrl
O-Ctrl
O-Ex
D
Mitochondria number(*10^-3)
0
1
2
3
4
Y-Ctrl
O-Ctrl
O-Ex
E
UMAP_2
UMAP_1
-15
-10
-5
0
5
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
1-8442 cells
2-6731 cells
3-5605 cells
4-3762 cells
5-3449 cells
6-2460 cells
7-2250 cells
8-1929 cells
9-1537 cells
10-1529 cells
11-1444 cells
12-1272 cells
13-1084 cells
14-1053 cells
15-940 cells
16-607 cells
17-580 cells
18-331 cells
19-76 cells
20-50 cells
F
Cdh1
Gfap
Aldoc
Slc4a5
Flt1
Ak7
Slc6a13
Cx3cr1
Grin2b
Hapln2
Cspg4
UMAP_1
UMAP_2

Figure S1: Single-cell sequencing quality control (QC). (**A-D**) Bar graphs presenting key metrics of quality control in single-cell sequencing: total cell number, mean nGene, mean nUM, and mean mitochondria. Percent of cells post QC. (**E**) Visualization of dimensionality reduction using PCA (principal component analysis), and UMAP (Uniform manifold Approximation and Projection) for single-cell cluster clustering. The clustering algorithm employed shared nearest neighbor analysis to achieve optimal cell clusters. The figure displays the first and second principal components on the horizontal and vertical axes, respectively, with each poin representing an individual cell, distinct cell clusters are color-coded. (**F**) t-distributed Stochastic Neighbor Embedding(t-SNE) visualization highlighting major cell populations and expression patterns of representative well-known cell type-specific marker genes, with darker red color indicating higher expression of the respective gene.


A
Y-Ctrl vs. O-Ctrl
$-\log_{10}$pvalue
$\log_2$ fold change
Atp6v0b
Apoe
Prpf4b
Gm42439
Meis2
Gm15738
Gm15398
Arpp21
D030068K23Rik
Foxp2
AC149090.1
Rsrp1
Fmn1
Rasgrf2
Oxr1
Col19a1
Kcnma1
Npas3
Nwd2
Adarb2
Down
Normal
Up
O-Ctrl vs. O-Ex
Malat1
Cmss1
Ahi1
Atp6v0b
Apoe
Rnpc3
Prpf4b
Cst3
Ankfn1
Meis2
Zbtb20
Kcnma1
Tcf7l2
AY036118
Vav3
Rnf220
Hs6st3
Nwd2
Syt9
Mgat4c
Down
Up
B
Y-Ctrl vs. O-Ctrl
5330438D12Rik
Atp6v0b
Nrg3os
Gm20642
Pigk
Gm44151
Gm36975
Gm42439
Ctnna3
Gm15398
Gm48530
AC149090.1
Rsrp1
Nrxn3
Wipf3
Pdzd2
Zfp804b
Epha7
Adarb2
Sema5a
Down
Up
O-Ctrl vs. O-Ex
Atp6v0b
Prpf4b
Ahi1
Cmss1
Apoe
Malat1
Srsf7
Dnaja2
C1ql3
Cox7c
Cdh12
Pdzd2
Pde4d
Dpp10
Sorcs1
Gpc6
Efna5
Sgcz
Tox
Tshz2
Down
Up
C
Gene Ontology Classification(Y-Ctrl vs. O-Ctrl)
percentage of genes
gene number
Down
Up
biological adhesion
biological regulation
cell killing
cellular component organization or biogenesis
cellular process
developmental process
establishment of localization
growth
immune system process
localization
locomotion
metabolic process
multi−organism process
multicellular organismal process
negative regulation of biological process
positive regulation of biological process
regulation of biological process
reproduction
reproductive process
response to stimulus
rhythmic process
signaling
single−organism process
cell
cell junction
cell part
extracellular matrix
extracellular matrix part
extracellular region
extracellular region part
macromolecular complex
membrane
membrane part
membrane−enclosed lumen
mitochondrion−associated adherens complex
nucleoid
organelle
organelle part
symplast
synapse
synapse part
virion
virion part
antioxidant activity
binding
catalytic activity
channel regulator activity
chemoattractant activity
chemorepellent activity
D−alanyl carrier activity
electron carrier activity
enzyme regulator activity
metallochaperone activity
molecular transducer activity
morphogen activity
nucleic acid binding transcription factor activity
nutrient reservoir activity
protein binding transcription factor activity
protein tag
receptor activity
receptor regulator activity
structural molecule activity
translation regulator activity
transporter activity
Biological Process
Cellular Component
Molecular Function


Gene Ontology Classification(O-Ctrl vs. O-Ex)
percentage of genes
100
10
1
0.1
gene number
23
24
2
2
0
0
0
0
Down
Up
biological adhesion
biological regulation
cell killing
cellular component organization or biogenesis
cellular process
developmental process
establishment of localization
growth
immune system process
localization
locomotion
metabolic process
multi−organism process
multicellular organismal process
negative regulation of biological process
positive regulation of biological process
regulation of biological process
reproduction
reproductive process
response to stimulus
rhythmic process
signaling
single−organism process
cell
cell junction
cell part
extracellular matrix
extracellular matrix part
extracellular region
extracellular region part
macromolecular complex
membrane
membrane part
membrane−enclosed lumen
mitochondrion−associated adherens complex
nucleoid
organelle
organelle part
symplast
synapse
synapse part
virion
virion part
antioxidant activity
binding
catalytic activity
channel regulator activity
chemoattractant activity
chemorepellent activity
D−alanyl carrier activity
electron carrier activity
enzyme regulator activity
metallochaperone activity
molecular transducer activity
morphogen activity
nucleic acid binding transcription factor activity
nutrient reservoir activity
protein binding transcription factor activity
protein tag
receptor activity
receptor regulator activity
structural molecule activity
translation regulator activity
transporter activity
Biological Process
Cellular Component
Molecular Function

D


Gene Ontology Classification(Y-Ctrl vs. O-Ctrl)
percentage of genes
100
10
1
0.1
gene number
40
40
4
4
0
0
0
0
Down
Up
biological adhesion
biological regulation
cell killing
cellular component organization or biogenesis
cellular process
developmental process
establishment of localization
growth
immune system process
localization
locomotion
metabolic process
multi−organism process
multicellular organismal process
negative regulation of biological process
positive regulation of biological process
regulation of biological process
reproduction
reproductive process
response to stimulus
rhythmic process
signaling
single−organism process
cell
cell junction
cell part
extracellular matrix
extracellular matrix part
extracellular region
extracellular region part
macromolecular complex
membrane
membrane part
membrane−enclosed lumen
mitochondrion−associated adherens complex
nucleoid
organelle
organelle part
symplast
synapse
synapse part
virion
virion part
antioxidant activity
binding
catalytic activity
channel regulator activity
chemoattractant activity
chemorepellent activity
D−alanyl carrier activity
electron carrier activity
enzyme regulator activity
metallochaperone activity
molecular transducer activity
morphogen activity
nucleic acid binding transcription factor activity
nutrient reservoir activity
protein binding transcription factor activity
protein tag
receptor activity
receptor regulator activity
structural molecule activity
translation regulator activity
transporter activity
Biological Process
Cellular Component
Molecular Function

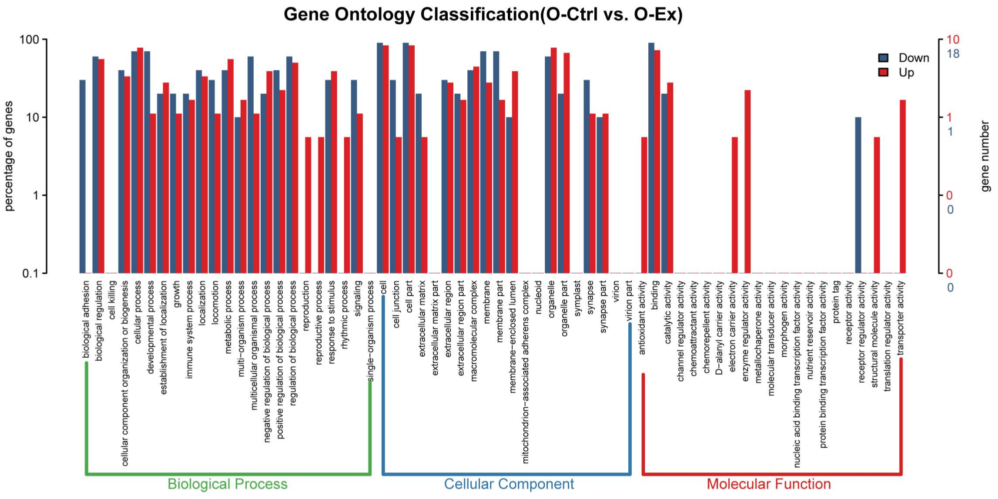

Gene Ontology Classification(O-Ctrl vs. O-Ex)
percentage of genes
100
10
1
0.1
gene number
10
18
1
1
0
0
0
0
Down
Up
biological adhesion
biological regulation
cell killing
cellular component organization or biogenesis
cellular process
developmental process
establishment of localization
growth
immune system process
localization
locomotion
metabolic process
multi−organism process
multicellular organismal process
negative regulation of biological process
positive regulation of biological process
regulation of biological process
reproduction
reproductive process
response to stimulus
rhythmic process
signaling
single−organism process
cell
cell junction
cell part
extracellular matrix
extracellular matrix part
extracellular region
extracellular region part
macromolecular complex
membrane
membrane part
membrane−enclosed lumen
mitochondrion−associated adherens complex
nucleoid
organelle
organelle part
symplast
synapse
synapse part
virion
virion part
antioxidant activity
binding
catalytic activity
channel regulator activity
chemoattractant activity
chemorepellent activity
D−alanyl carrier activity
electron carrier activity
enzyme regulator activity
metallochaperone activity
molecular transducer activity
morphogen activity
nucleic acid binding transcription factor activity
nutrient reservoir activity
protein binding transcription factor activity
protein tag
receptor activity
receptor regulator activity
structural molecule activity
translation regulator activity
transporter activity
Biological Process
Cellular Component
Molecular Function

Figure S2: Differential Gene Expression (DGE) and Gene Ontology(GO) analysis reveal exercise-related changes associated with aging in GABA and GLUTs. (A) Volcano plot illustrating exercise-related DGEs associated with aging in GABA (FC>1.2). (B) Volcano plot illustrating exercise-related DGEs associated with aging in in GLUT (FC>1.2). (C) The bar chart after gene set enrichment analysis (GSEA) highlights enriched terms specific to GABA. (D) The bar chart after gene set enrichment analysis (GSEA) highlights enriched terms specific to GLUT.