**GO and KEGG functional enrichment analysis.**

For gene ontology (GO) function analysis (includes biological processes (BP) molecular functions (MF) cellular components (CC)) topGO software [1] was used to annotate and classify the functions. The functional enrichment for the target gene was listed and used Fisher's exact test to calculate the representative GO function set in the target gene list. The *P value* of whether the gene list is significantly enriched, and then the P value is corrected by Benjamini & Hochberg's multiple tests to get the false discovery rate (FDR)[2]. The KEGG pathway functional enrichment analysis[3] from the differentially expressed genes (DEGs) was similar to the GO functional enrichment analysis, and the significant enrichment pathways are screened according to *p value* ≤ 0.05.

1. Alexa, A.; Rahnenfuhrer, J., TOPGO: enrichment analysis for gene ontology 2.40. 0. *R package version* **2020,** 2, (0), 2010.

2. Benjamini, Y.; Hochberg, Y., Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B (Methodological)* **1995,** 57, (1), 289-300.

3. Kanehisa, M.; Goto, S.; Sato, Y.; Furumichi, M.; Tanabe, M., KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res* **2012,** 40, (Database issue), D109-14.

# −log(Pvalue)

**a**

20

15

10

GO Term

cell development cell differentiation generation of neurons

5

0

cellular developmental process

neuron differentiation nervous system development

neurogenesis anatomical structure development

cell morphogenesis involved in differentiation anatomical structure morphogenesis

system development

locomotion multicellular organismal development movement of cell or subcellular component

cell morphogenesis involved in neuron differentiat...

localization anatomical structure formation involved in morphog...

regulation of localization neuron development developmental process

cell projection cell projection part

synapse neuron part

plasma membrane part plasma membrane region

neuron projection synapse part cell junction postsynapse

cell leading edge membrane region whole membrane cell periphery plasma membrane dendritic spine neuron spine

leading edge membrane cell projection membrane

axon cytoskeletal protein binding

ion binding anion binding nucleotide binding

nucleoside phosphate binding carbohydrate derivative binding

gated channel activity ion gated channel activity ribonucleotide binding small molecule binding

purine ribonucleotide binding metal ion transmembrane transporter activity

purine nucleotide binding voltage−gated cation channel activity

purine nucleoside binding purine ribonucleoside binding ribonucleoside binding nucleoside binding

adenyl ribonucleotide binding adenyl nucleotide binding

BP CC MF

# −log(Pvalue)

30

**b**

20

10

GO Term

metabolic process cellular metabolic process

0

organic substance metabolic process cellular component organization or biogenesis

primary metabolic process cellular component organization nitrogen compound metabolic process

cellular nitrogen compound metabolic process

heterocycle metabolic process cellular aromatic compound metabolic process

biosynthetic process organic cyclic compound metabolic process organic substance biosynthetic process single−organism metabolic process

cellular macromolecule metabolic process

cellular biosynthetic process nucleobase−containing compound metabolic process

organelle organization single−organism organelle organization macromolecule metabolic process

intracellular part intracellular membrane−bounded organelle

intracellular membrane−bounded organelle

organelle intracellular organelle

cytoplasm organelle part

intracellular organelle part

cytoplasmic part intracellular organelle lumen

nuclear part membrane−enclosed lumen

organelle lumen nuclear lumen nucleoplasm

nucleus cytosol

macromolecular complex non−membrane−bounded organelle

binding ion binding protein binding catalytic activity

heterocyclic compound binding organic cyclic compound binding

transferase activity nucleoside phosphate binding

nucleotide binding ribonucleotide binding purine nucleotide binding

anion binding nucleoside binding

purine ribonucleotide binding purine nucleoside binding ribonucleoside binding

purine ribonucleoside binding carbohydrate derivative binding small molecule binding

purine ribonucleoside triphosphate binding

BP CC MF

# −log(Pvalue)

30

***c***

20

10

GO Term

metabolic process cellular metabolic process

0

organic substance metabolic process primary metabolic process

cellular component organization or biogenesis

cellular component organization nitrogen compound metabolic process

cellular nitrogen compound metabolic process organic cyclic compound metabolic process cellular aromatic compound metabolic process

nucleobase−containing compound metabolic process

heterocycle metabolic process cellular macromolecule metabolic process single−organism metabolic process macromolecule metabolic process organic substance biosynthetic process

biosynthetic process cellular biosynthetic process organelle organization

gene expression

intracellular intracellular part membrane−bounded organelle

organelle cytoplasm intracellular organelle

intracellular membrane−bounded organelle

organelle part intracellular organelle part

cytoplasmic part intracellular organelle lumen

nuclear part membrane−enclosed lumen

organelle lumen nucleoplasm nuclear lumen

cytosol nucleus

macromolecular complex organelle membrane

binding ion binding catalytic activity

heterocyclic compound binding organic cyclic compound binding

protein binding nucleoside phosphate binding

nucleotide binding purine nucleotide binding ribonucleotide binding

purine ribonucleotide binding

anion binding nucleoside binding

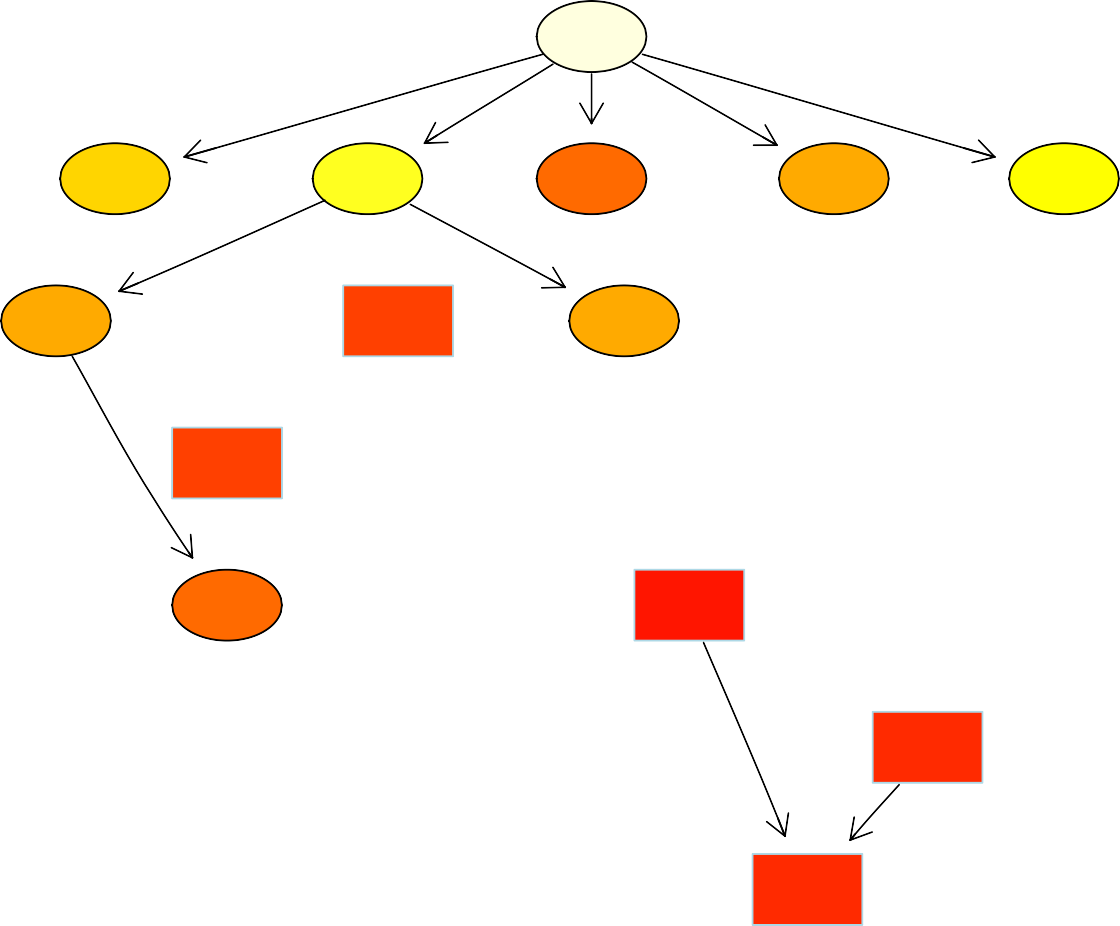
purine nucleoside binding ribonucleoside binding

purine ribonucleoside binding small molecule binding carbohydrate derivative binding

purine ribonucleoside triphosphate binding

adenyl nucleotide binding

BP CC MF



**d**

GO:0008150

biological\_process 1.00000

2762 / 16446

GO:0071840

cellular component o...

5.35e−07

1109 / 5928

GO:0009987

cellular process 0.00146

2628 / 15447

GO:0032502

developmental proces...

3.51e−12

1109 / 5662

GO:0044699

single−organism proc...

1.64e−08

2452 / 14056

GO:0032501

multicellular organi...

7.16e−05

1281 / 7086

GO:0016043

cellular component o...

1.92e−08

1095 / 5763

GO:0048856

anatomical structure...

4.74e−14

1002 / 4974

GO:0044763

single−organism cell...

1.42e−08

2323 / 13212

GO:0044767

single−organism deve...

4.93e−12

1095 / 5588

GO:0044707

single−multicellular...

7.83e−05

1242 / 6859

GO:0009653

anatomical structure...

1.11e−13

565 / 2578

GO:0048869

cellular development...

4.84e−15

802 / 3820

GO:0007275

multicellular organi...

3.40e−13

960 / 4770

GO:0032989

cellular component m...

2.40e−11

299 / 1257

GO:0030154

cell differentiation 3.27e−16

759 / 3546

GO:0048731

system development 1.32e−13

864 / 4215

GO:0000902

cell morphogenesis 2.29e−11

283 / 1176

GO:0048468

cell development 9.28e−17

472 / 2008

GO:0007399

nervous system devel...

6.70e−15

476 / 2075

GO:0000904

cell morphogenesis i...

7.73e−14

207 / 759

GO:0022008

neurogenesis 1.49e−14

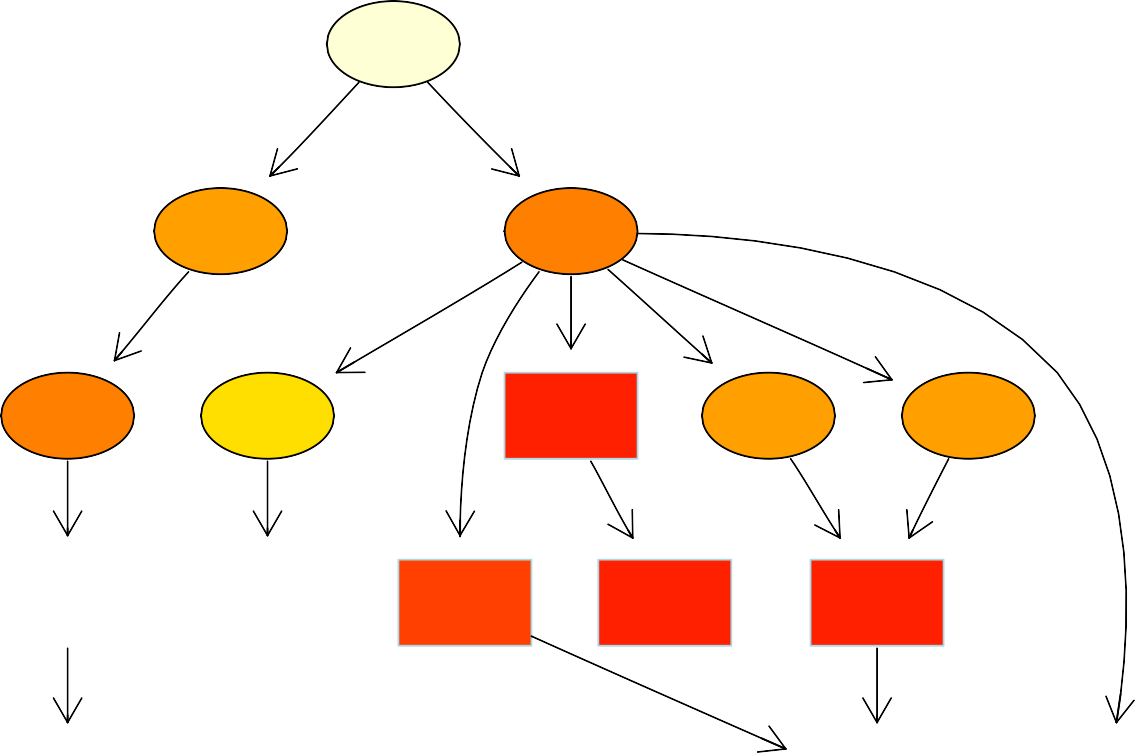
335 / 1365

GO:0048699

generation of neuron...

4.52e−15

319 / 1275



GO:0003674

molecular\_function 1.000000

2650 / 15780

GO:0005215

transporter activity 2.92e−06

312 / 1476

GO:0005488

binding 2.04e−07

2273 / 13004

GO:0022857

transmembrane transp...

2.34e−07

264 / 1185

GO:0005515

protein binding 0.000561

1550 / 8775

GO:0043167

ion binding 2.22e−10

1203 / 6302

GO:0097159

organic cyclic compo...

1.74e−06

1070 / 5741

GO:1901363

heterocyclic compoun...

3.58e−06

1053 / 5662

GO:0022803

passive transmembran...

1.34e−06

148 / 613

GO:0008092

cytoskeletal protein...

2.19e−11

214 / 836

GO:0036094

small molecule bindi...

3.97e−09

602 / 2941

GO:0043168

anion binding 2.57e−10

607 / 2922

GO:1901265

nucleoside phosphate...

4.15e−10

549 / 2613

GO:0015267

channel activity 1.98e−06

147 / 612

GO:0097367

carbohydrate derivat...

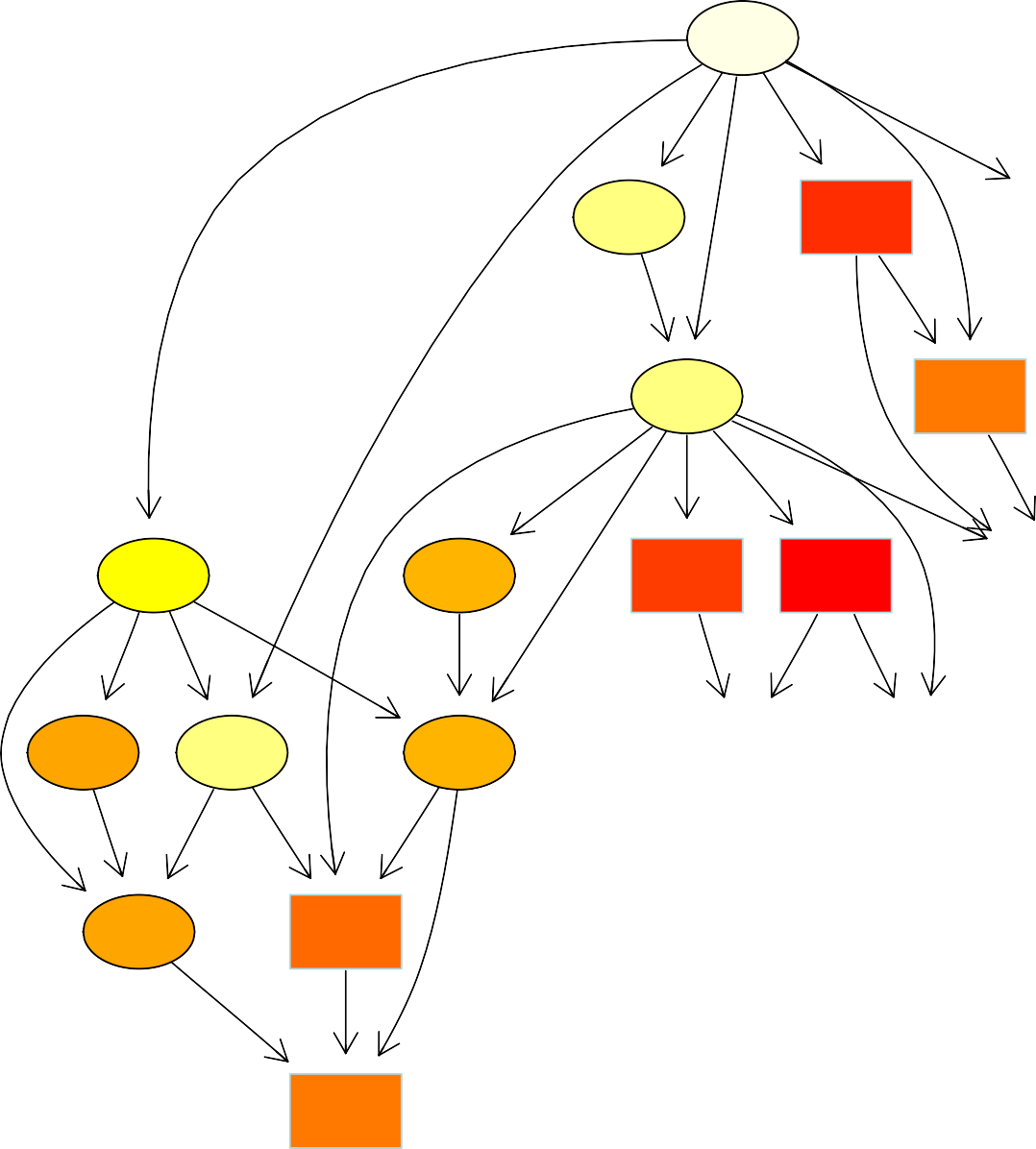
5.51e−10

527 / 2498

GO:0000166

nucleotide binding 3.90e−10

549 / 2612



GO:0005575

cellular\_component 1.00000

2929 / 17681

GO:0005623

cell 0.01353

2689 / 16040

GO:0045202

synapse 5.61e−20

203 / 664

GO:0030054

cell junction 3.83e−14

326 / 1345

GO:0044464

cell part 0.00687

2688 / 16011

GO:0044456

synapse part 6.19e−15

155 / 514

GO:0016020

membrane 9.38e−06

1590 / 8957

GO:0071944

cell periphery 4.42e−11

991 / 5092

GO:0097458

neuron part 1.15e−18

313 / 1193

GO:0042995

cell projection

< 1e−20 451 / 1792

GO:0098805

whole membrane 7.37e−12

439 / 1989

GO:0044425

membrane part 0.00505

1155 / 6597

GO:0005886

plasma membrane 1.30e−10

969 / 4987

GO:0098589

membrane region 2.40e−12

270 / 1103

GO:0044459

plasma membrane part 2.45e−16

571 / 2563

GO:0098590

plasma membrane regi...

2.19e−15

240 / 899

GO:0044463

cell projection part

< 1e−20 264 / 929

GO:0043005

neuron projection 2.32e−15

251 / 952

GO:0098794

postsynapse 1.91e−13

107 / 324

GO:0030182

neuron differentiati...

5.43e−15

295 / 1159

GO:0022836

gated channel activi...

3.19e−09

114 / 402

GO:0022839

ion gated channel ac...

3.19e−09

114 / 402

GO:0032553

ribonucleotide bindi...

3.73e−09

456 / 2144

GO:0006725

cellular aromatic co...

< 1e−20 6542 / 6851

GO:0006807

nitrogen compound me...

< 1e−20 7373 / 7723

GO:0044238

primary metabolic pr...

< 1e−20 10343 / 10922

GO:0009987

cellular process 0.000189

14273 / 15447

GO:0071840

cellular component o...

< 1e−20 5725 / 5928

GO:0008152

metabolic process

< 1e−20 11298 / 11916

GO:0016043

cellular component o...

< 1e−20 5565 / 5763

GO:0034641

cellular nitrogen co...

< 1e−20 7023 / 7356

GO:0071704

organic substance me...

< 1e−20 10653 / 11247

GO:0044237

cellular metabolic p...

< 1e−20 10310 / 10848

GO:0046483

heterocycle metaboli...

< 1e−20 6529 / 6836

GO:0000166

nucleotide binding

<1e−20 2543 / 2612

GO:0032553

ribonucleotide bindi...

<1e−20 2096 / 2144

GO:0036094

small molecule bindi...

<1e−20 2847 / 2941

GO:1901265

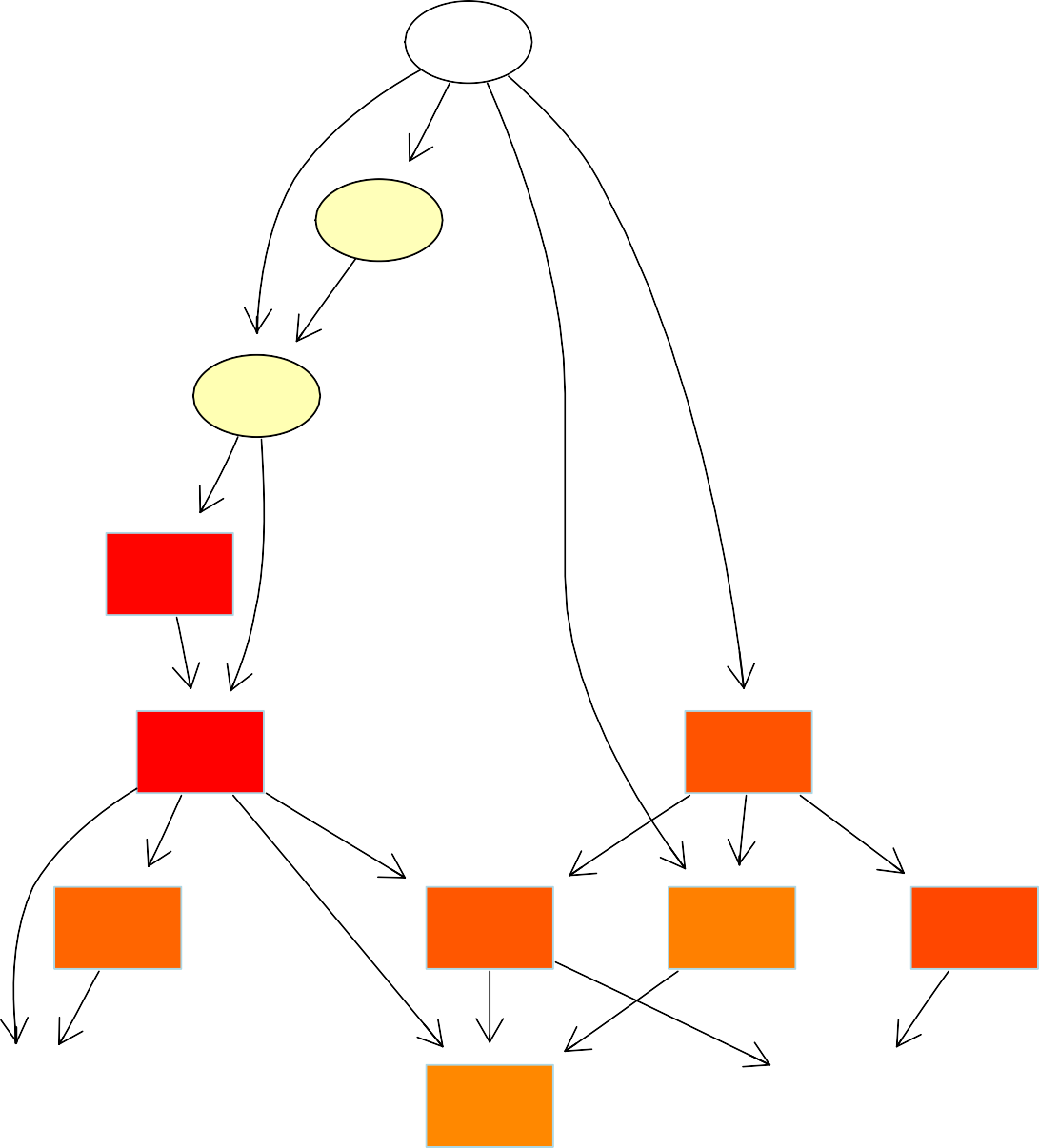
nucleoside phosphate...

<1e−20 2544 / 2613

GO:0097367

carbohydrate derivat...

<1e−20 2430 / 2498



GO:0005575

cellular\_component 1

16220 / 17681

GO:0005623

cell 5.33e−12

14792 / 16040

GO:0044464

cell part 4.92e−13

14770 / 16011

GO:0005622

intracellular

< 1e−20 13257 / 13957

GO:0044424

intracellular part

< 1e−20 12957 / 13617

GO:0043226

organelle

< 1e−20 12190 / 12834

GO:0005737

cytoplasm

< 1e−20 10090 / 10542

GO:0043229

intracellular organe...

< 1e−20 11394 / 11958

GO:0044422

organelle part

< 1e−20 8012 / 8314

GO:0043227

membrane−bounded org...

< 1e−20 11501 / 12061

GO:0044446

intracellular organe...

< 1e−20 7889 / 8188

GO:0043231

intracellular membra...

< 1e−20 10532 / 11007

GO:0044444

cytoplasmic part

< 1e−20 7685 / 7995

GO:0005515

protein binding

<1e−20 8351 / 8775

GO:0016740

transferase activity

<1e−20 2823 / 2898

**e**

GO:0008150

biological\_process 1.000000

15163 / 16446

GO:0003674

molecular\_function 1

14570 / 15780

GO:0003824

catalytic activity

<1e−20 6900 / 7219

GO:0005488

binding

<1e−20 12245 / 13004

GO:0097159

organic cyclic compo...

<1e−20 5514 / 5741

GO:1901363

heterocyclic compoun...

<1e−20 5442 / 5662

GO:0043167

ion binding

<1e−20 6056 / 6302

GO:0006725

cellular aromatic co...

< 1e−20 6407 / 6851

GO:0006807

nitrogen compound me...

< 1e−20 7214 / 7723

GO:0016043

cellular component o...

< 1e−20 5441 / 5763

GO:0034641

cellular nitrogen co...

< 1e−20 6876 / 7356

GO:0044238

primary metabolic pr...

< 1e−20 10106 / 10922

GO:0044237

cellular metabolic p...

< 1e−20 10070 / 10848

GO:0071704

organic substance me...

< 1e−20 10405 / 11247

GO:1901360

organic cyclic compo...

< 1e−20 6596 / 7059

GO:0017076

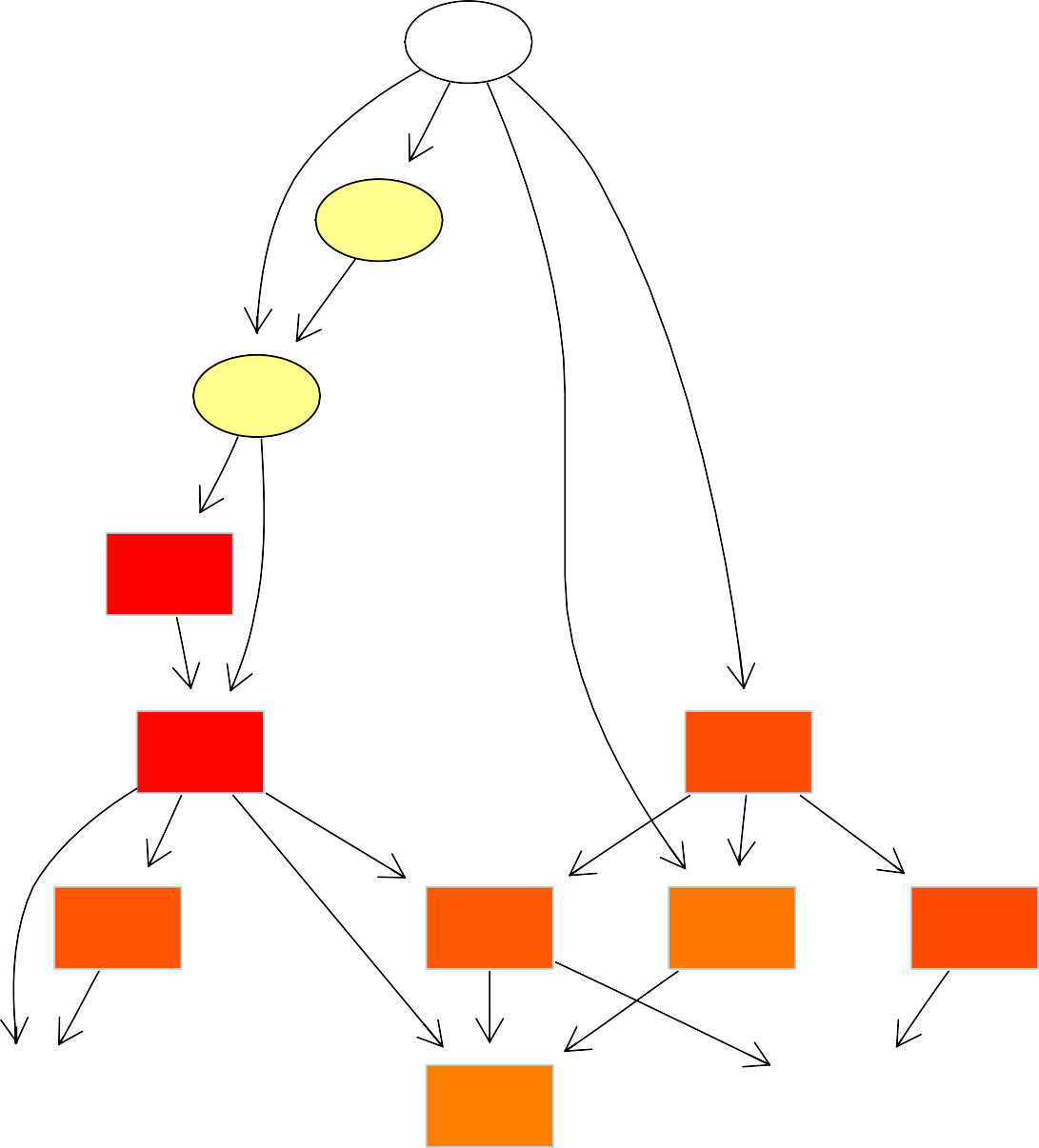
purine nucleotide bi...

<1e−20 2065 / 2141

GO:0032553

ribonucleotide bindi...

<1e−20 2067 / 2144



GO:0005575

cellular\_component 1

15710 / 17681

GO:0005623

cell 1.86e−18

14365 / 16040

GO:0044464

cell part 2.76e−19

14343 / 16011

GO:0005622

intracellular

< 1e−20 12915 / 13957

GO:0044424

intracellular part

< 1e−20 12621 / 13617

GO:0043226

organelle

< 1e−20 11881 / 12834

GO:0005737

cytoplasm

< 1e−20 9863 / 10542

GO:0043229

intracellular organe...

< 1e−20 11107 / 11958

GO:0044422

organelle part

< 1e−20 7837 / 8314

GO:0043227

membrane−bounded org...

< 1e−20 11213 / 12061

GO:0044446

intracellular organe...

< 1e−20 7718 / 8188

GO:0043231

intracellular membra...

< 1e−20 10270 / 11007

GO:0044444

cytoplasmic part

< 1e−20 7530 / 7995

GO:0005515

protein binding

<1e−20 8125 / 8775

**g**

GO:0008150

biological\_process 1

14712 / 16446

GO:0009987

cellular process 3.79e−06

13863 / 15447

GO:0071840

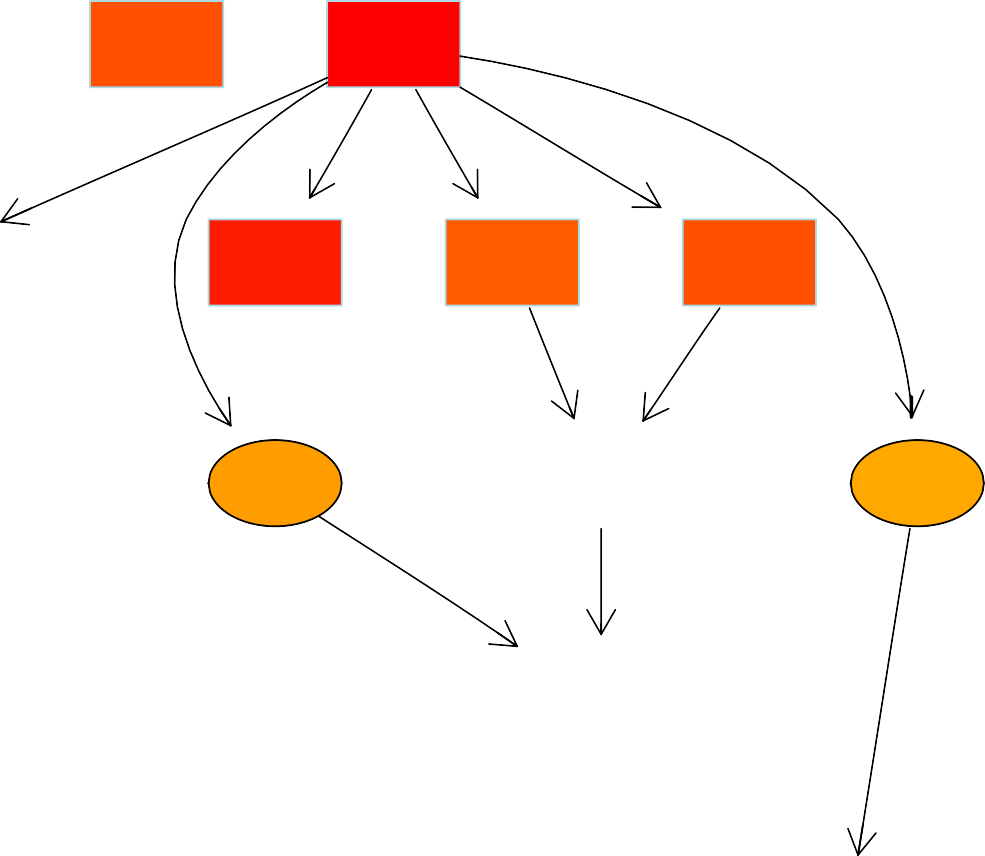
cellular component o...

< 1e−20 5598 / 5928

GO:0008152

metabolic process

< 1e−20 11018 / 11916



GO:0003824

catalytic activity

<1e−20 6741 / 7219

GO:0005488

binding

<1e−20 11914 / 13004

GO:0043167

ion binding

<1e−20 5938 / 6302

GO:0097159

organic cyclic compo...

<1e−20 5397 / 5741

GO:1901363

heterocyclic compoun...

<1e−20 5329 / 5662

GO:0036094

small molecule bindi...

<1e−20 2802 / 2941

GO:1901265

nucleoside phosphate...

<1e−20 2508 / 2613

GO:0097367

carbohydrate derivat...

<1e−20 2388 / 2498

GO:0000166

nucleotide binding

<1e−20 2507 / 2612

GO:0003674

molecular\_function 1

14136 / 15780

# −log(Pvalue)

0.39

●

**h**

4

3

2

1

0

GeneCount

10

KEGG Pathway

KEGG Pathway

## Morphine addiction Axon guidance

Regulation of actin cytoskeleton Arrhythmogenic right ventricular cardiomyopathy (ARVC)

## Calcium signaling pathway Glutamatergic synapse Wnt signaling pathway

Focal adhesion Cholinergic synapse

## Endocytosis Long−term depression Pathways in cancer

Fc epsilon RI signaling pathway Retrograde endocannabinoid signaling

## GnRH signaling pathway Hippo signaling pathway

B cell receptor signaling pathway Bacterial invasion of epithelial cells Phosphatidylinositol signaling system

Type II diabetes mellitus

# Rich factor

0.36

0.33

0.30

0.27

## ABC transporters Arrhythmogenic right ventricular cardiomyopathy (ARVC)

●

●

●

Axon guidance B cell receptor signaling pathway Bacterial invasion of epithelial cells

●

●●

## Cholinergic synapse Endocrine and other factor−regulated calcium reabsorption

●

●

Fc epsilon RI signaling pathway Glutamatergic synapse GnRH signaling pathway Hedgehog signaling pathway

●

●

●

●

## Inositol phosphate metabolism Long−term depression Morphine addiction Nicotine addiction

●

●

●

●

Phosphatidylinositol signaling system Retrograde endocannabinoid signaling

●

Steroid biosynthesis Type II diabetes mellitus Wnt signaling pathway

●

* 20
* 30
* 40

Minuslog10Pvalue

4.0

3.5

●

3.0

2.5

2.0

1.5

●



# −log(Pvalue)

1.50

GeneCount

**i**

50

9

6

3

0

KEGG Pathway

KEGG Pathway

## Metabolic pathways Focal adhesion Pathways in cancer

Ribosome Hippo signaling pathway

## Cell cycle Axon guidance Proteoglycans in cancer MAPK signaling pathway

Lysosome Circadian entrainment Neurotrophin signaling pathway Ubiquitin mediated proteolysis Insulin signaling pathway Glutamatergic synapse ECM−receptor interaction Small cell lung cancer

Oocyte meiosis Dopaminergic synapse Purine metabolism

# Rich factor

1.25

1.00

0.75

0.50

## Adherens junction Adipocytokine signaling pathway Bacterial invasion of epithelial cells

●

●

●

●

Basal cell carcinoma

●● ●

## Cell cycle Circadian entrainment ECM−receptor interaction

●

Endocrine and other factor−regulated calcium reabsorption

●

## Gastric acid secretion Hedgehog signaling pathway Inositol phosphate metabolism N−Glycan biosynthesis Nucleotide excision repair

●

●

●

●

●

Pathogenic Escherichia coli infection

●● ●●

## Peroxisome Phosphatidylinositol signaling system

Ribosome Small cell lung cancer Type II diabetes mellitus p53 signaling pathway

●

75

●

* 100
* 125

Minuslog10Pvalue

●

●

5

4

3



# −log(Pvalue)

1.0000

● ●

●

●

●

●

●

●

●

●

●

●

●

●

●

●

●

**j**

9

6

3

0

KEGG Pathway

KEGG Pathway

## Metabolic pathways Focal adhesion Pathways in cancer

Ubiquitin mediated proteolysis Glutamatergic synapse

## Tight junction Axon guidance ECM−receptor interaction Proteoglycans in cancer Hippo signaling pathway

Lysosome Pyrimidine metabolism Purine metabolism

## Ribosome Regulation of actin cytoskeleton

Cholinergic synapse Circadian entrainment

## Leukocyte transendothelial migration

Dopaminergic synapse Inositol phosphate metabolism

# Rich factor

0.9975

0.9950

0.9925

0.9900

## Aminoacyl−tRNA biosynthesis Basal transcription factors

DNA replication Dorso−ventral axis formation ECM−receptor interaction

●

## Focal adhesion Glutamatergic synapse Hedgehog signaling pathway Homologous recombination Inositol phosphate metabolism

Mismatch repair Mucin type O−Glycan biosynthesis Nucleotide excision repair

## Other types of O−glycan biosynthesis

Propanoate metabolism Pyrimidine metabolism Sphingolipid metabolism

●

## Thyroid cancer Ubiquitin mediated proteolysis Vitamin digestion and absorption

Minuslog10Pvalue

9

7

5

3

GeneCount

50

●

* 100
* 150
* 200

●



**Supplementary figure1**:***Effects of ectoine treatment on skin cell’s pathway and functions****.*

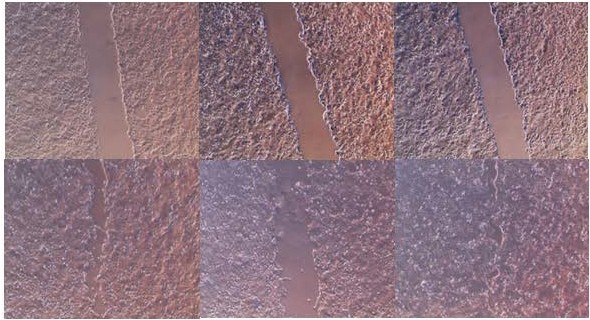
Histogram represents the GO enrichment functional analysis to the difference between functional pathways between ectoine/or 5-aza-2-deoxycytidine treated cells and untreated cells. The ectoine vs untreated showed lower expression in the Biological Processes (BP) Molecular Functions (MF) Cellular Components (CC) (a) compared to 5-aza vs untreated (b) and 5-aza vs ectoine (c). Note: The ordinate represents

-log10 (*p value*), and the abscissa represents the names of significantly enriched GOs (up to the top 10 GOs for each category). Similarly, using treemap ectoine vs untreated (d) the ion binding, small molecule binding, nucleotide binding and ribonucleotide binding related DEGs were significantly increased compared to 5-aza vs untreated (e) and 5-aza vs ectoine (f). Moreover, ectoine improved cell’s biological processes such as cell morphogenesis,

**Note:** There are three subtrees in total, Biological\_process subtree, molecular\_function subtree, and cellular\_component subtree, the highlighted box represents significant enrichment GO.

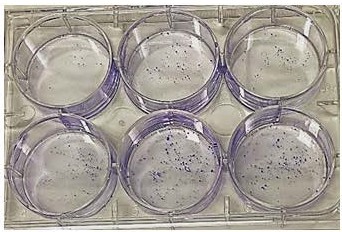
KEEG functional analysis showed that ectoine vs untreated (g) reduced the overall expression of genes compared to 5-aza vs untreated (h) and 5-aza vs ectoine (i). ectoine induced the expression of morphine addiction.

Note: The abscissa represents the name of the significantly enriched KEGG pathway, and the ordinate represents -log10*(p value*). Red bars indicate significant pathways and blue bars indicate insignificant pathways. Display up to the top 20 KEGG pathways. The ordinate represents the Rich factor (the ratio of the total number of genes belonging to this KEGG pathway to the total number of genes belonging to this KEGG pathway). The larger the Rich factor, the greater the degree of enrichment, and the abscissa represents -log10 (*p value*). According to the sorting information of the Rich factor, at most, the top 20 KEGG pathways are displayed.

**a**

**5-aza Ectoine Untreated** HaCaT

**b**



Ectoine

Control

**Suppl. figure 2: *Ectoine suppressed skin cells proliferation.*** Wound healing assay in A-431 cell lines treated with ectoine or 5-aza (A) and colonogenic assay for HaCaT cell line treated with ectoine (B).