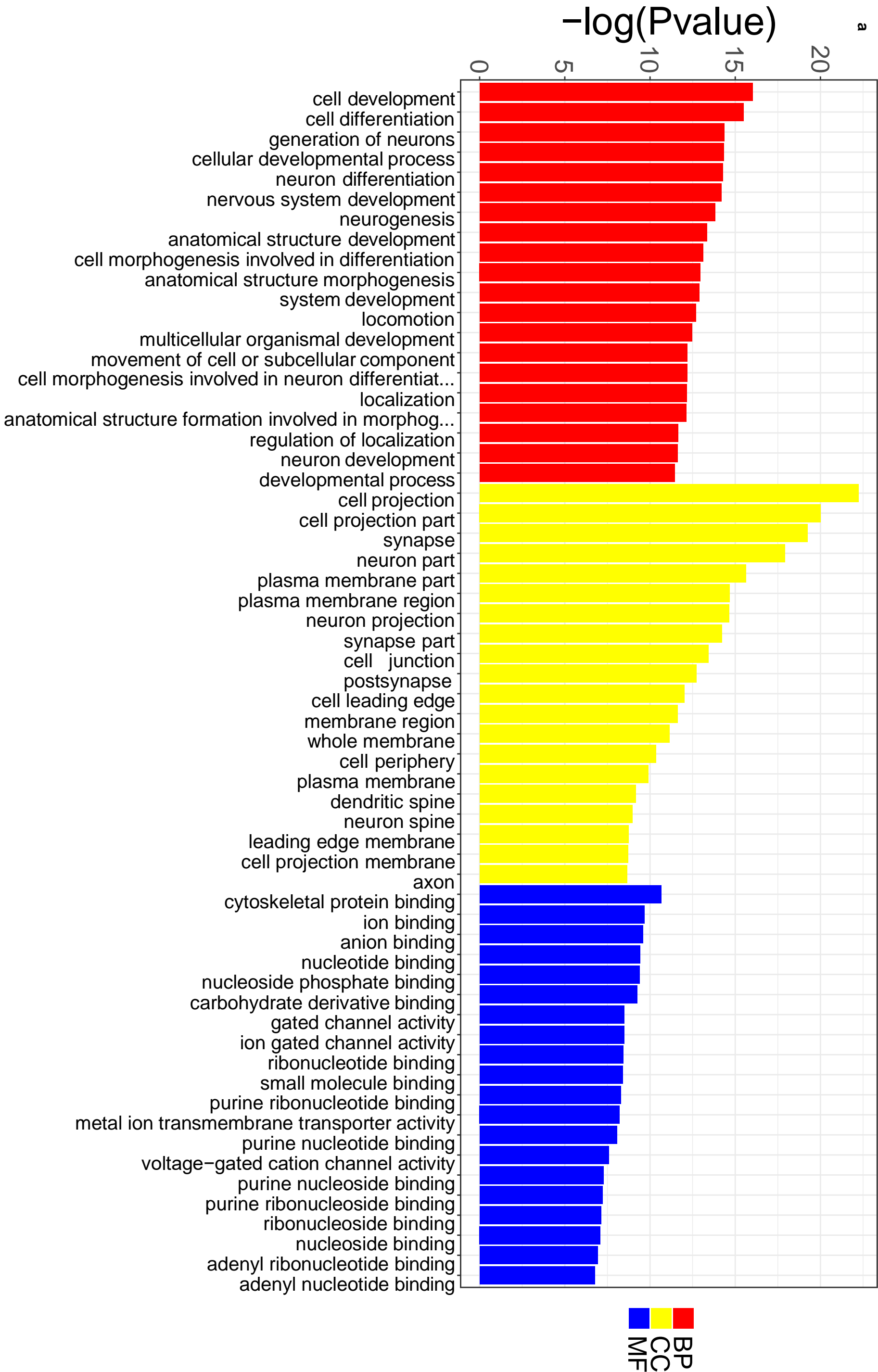


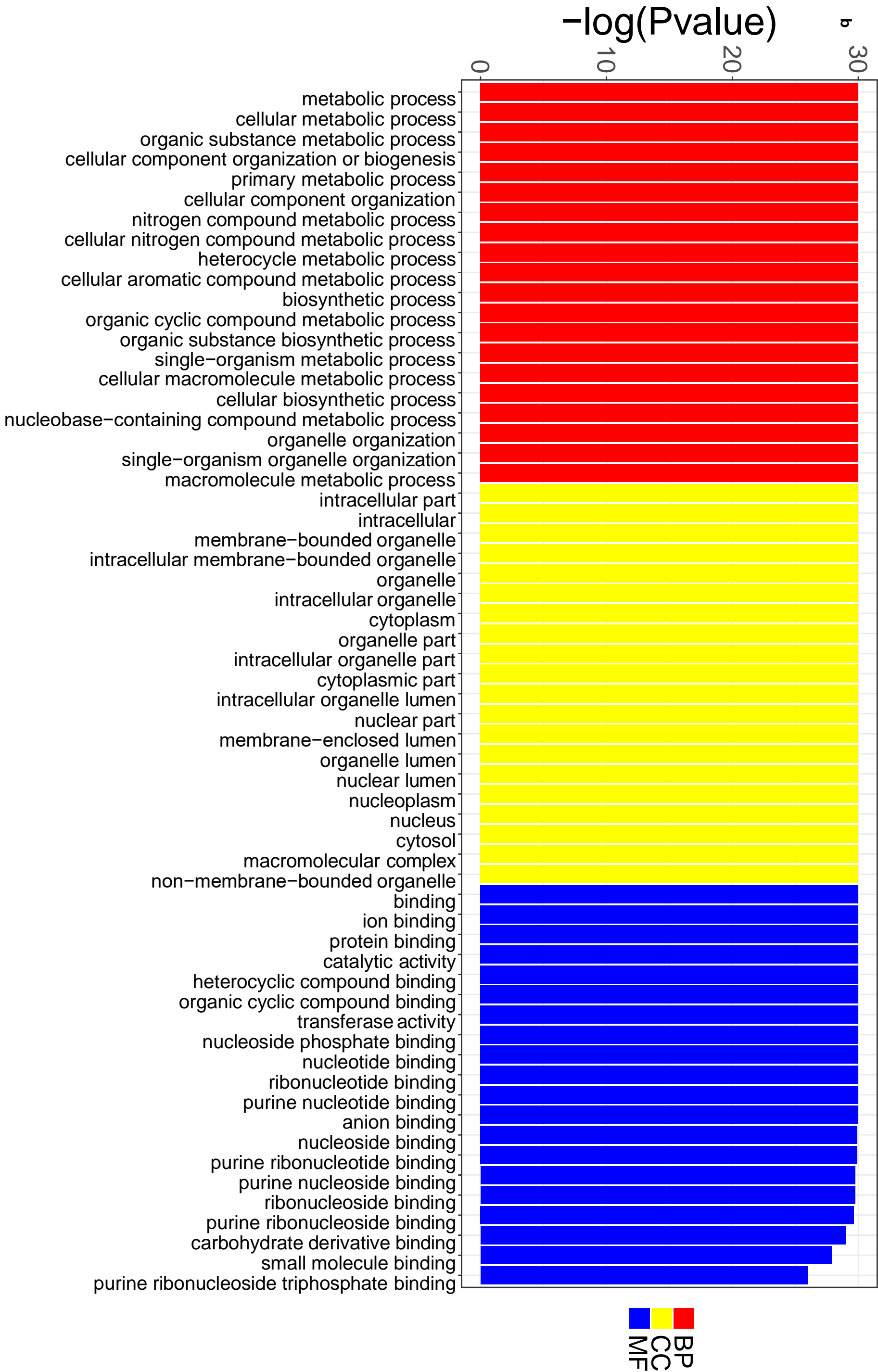
GO and KEGG functional enrichment analysis.

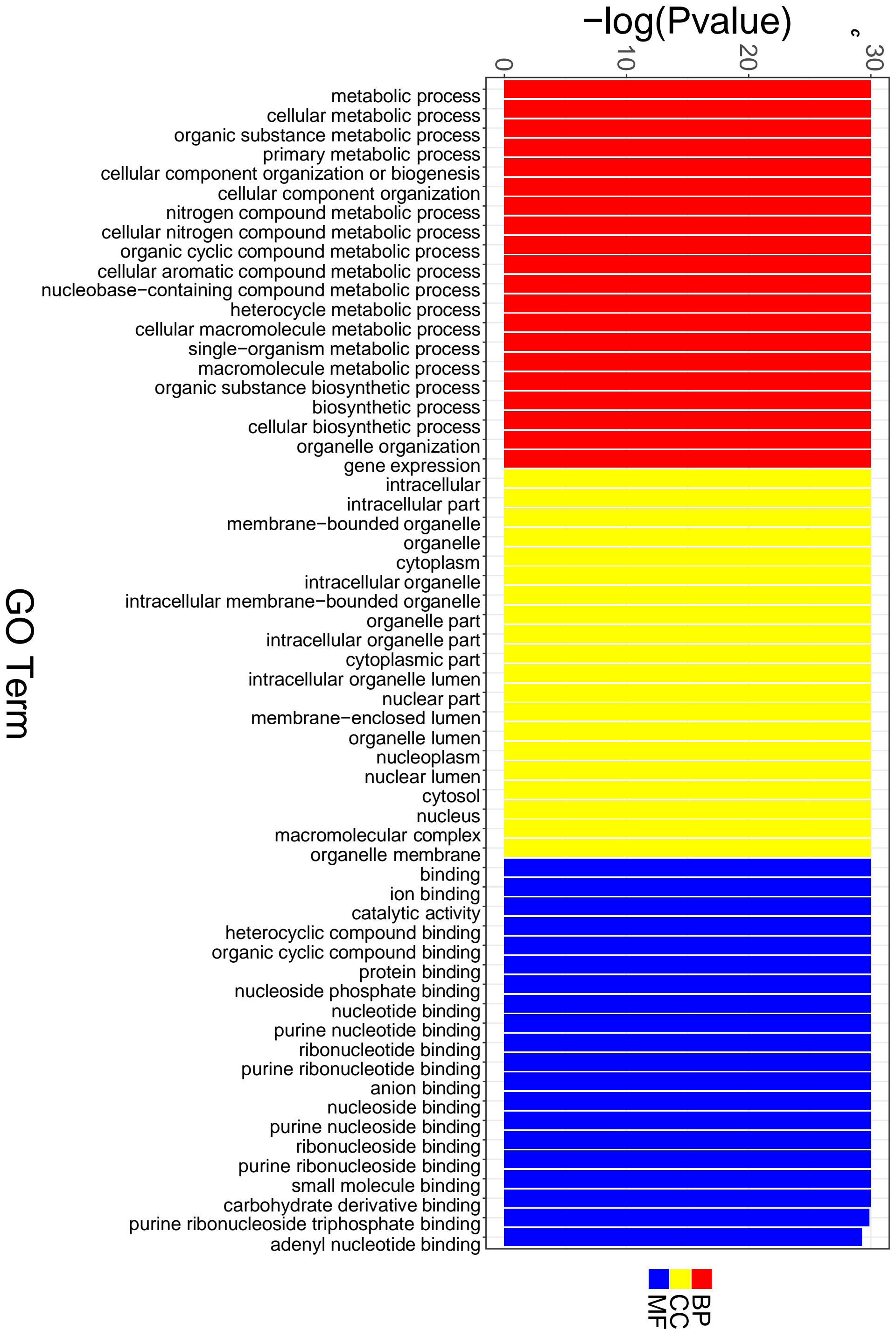
For gene ontology (GO) function analysis (includes biological processes (BP) molecular functions (MF) cellular components (CC)) topGO software¹ was used to annotate and classify the functions. The functional enrichment for the target gene was listed and used Fisher's exact test to calculate the representative 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 analysis³ 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 2*, 2010 (2020).
- 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)* **57**, 289-300 (1995).
- 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* **40**, D109-114, doi:10.1093/nar/gkr988 (2012).

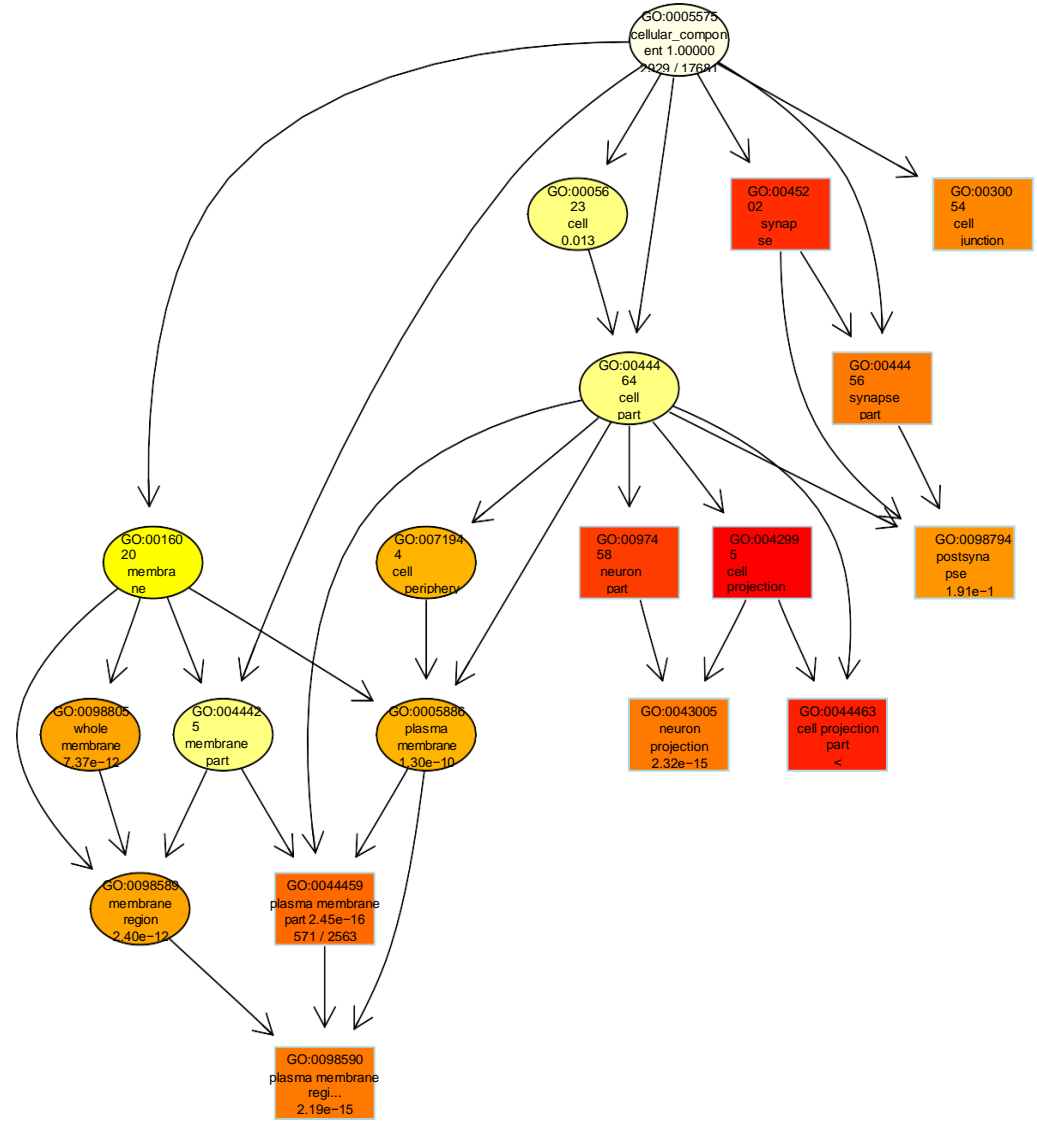
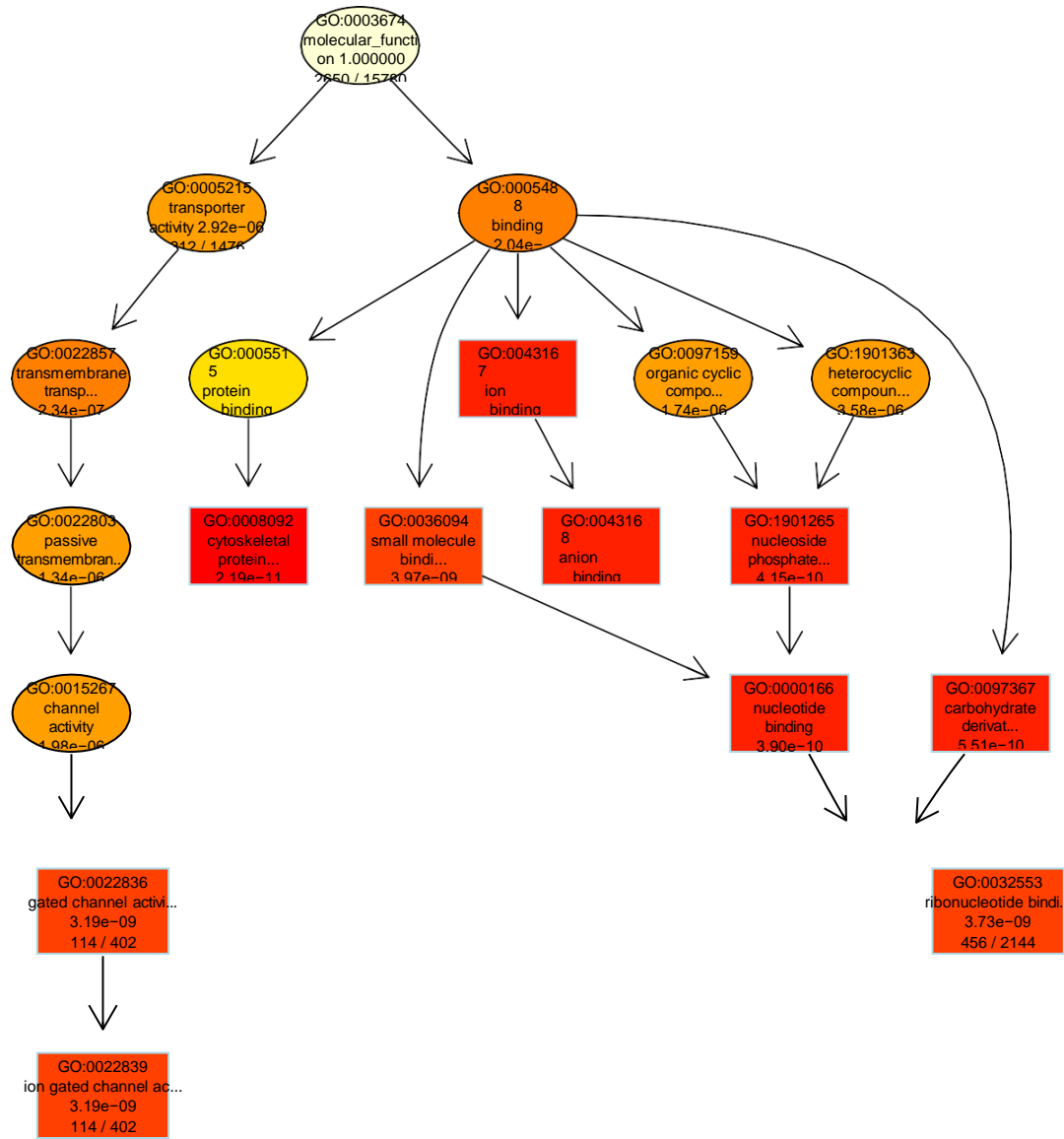
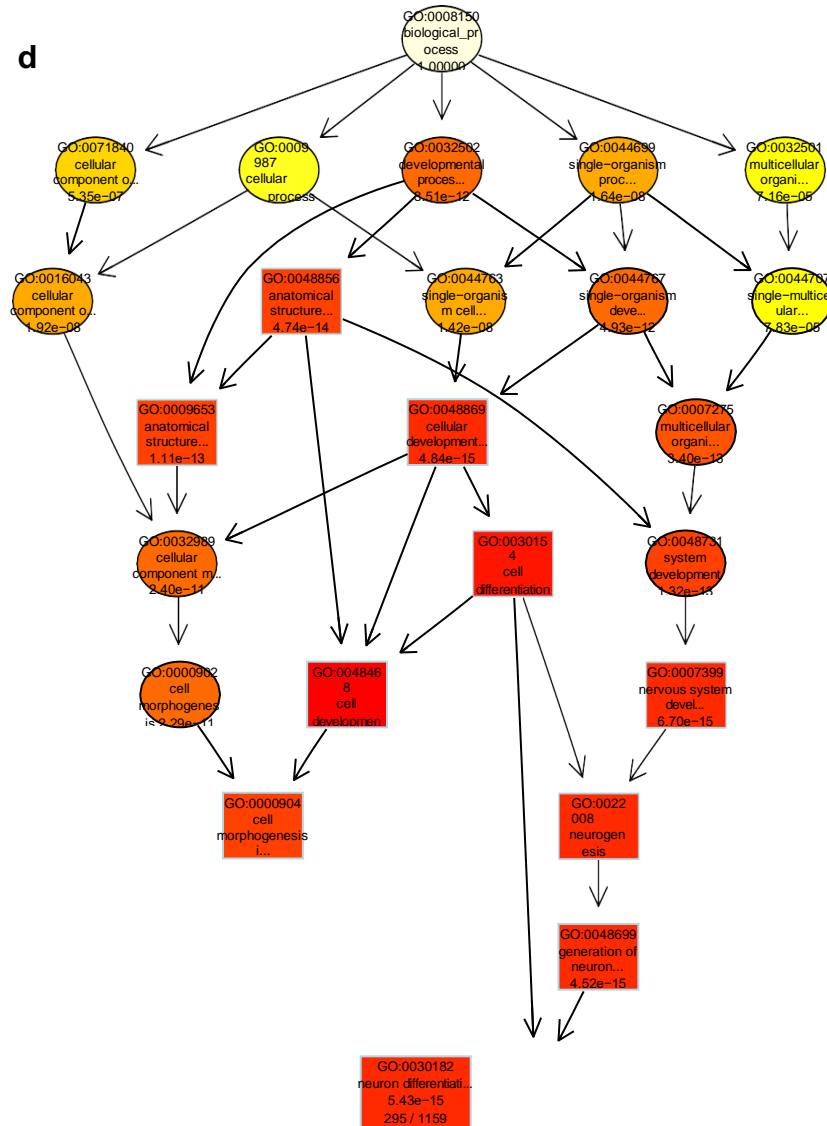


GO Term

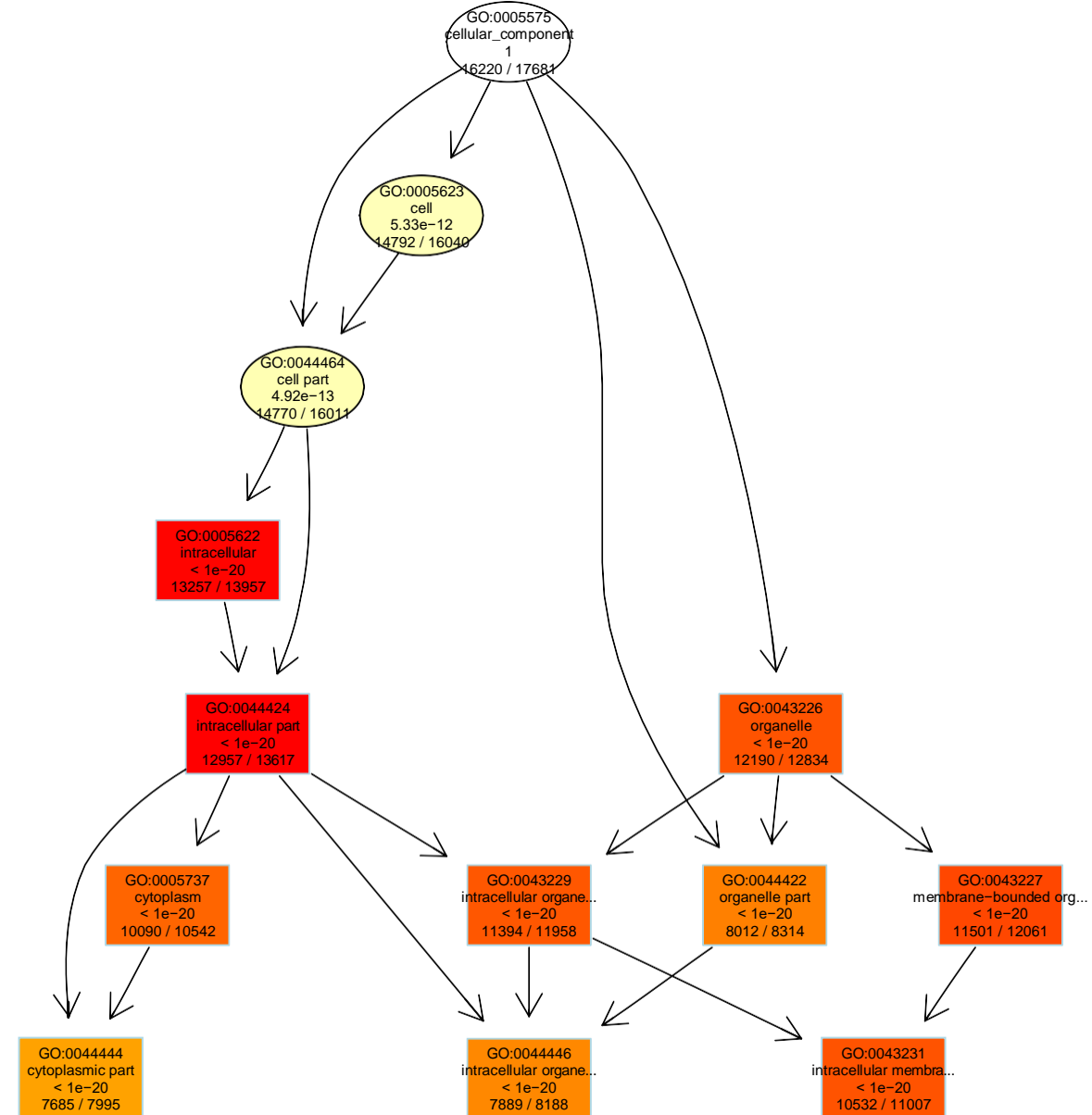
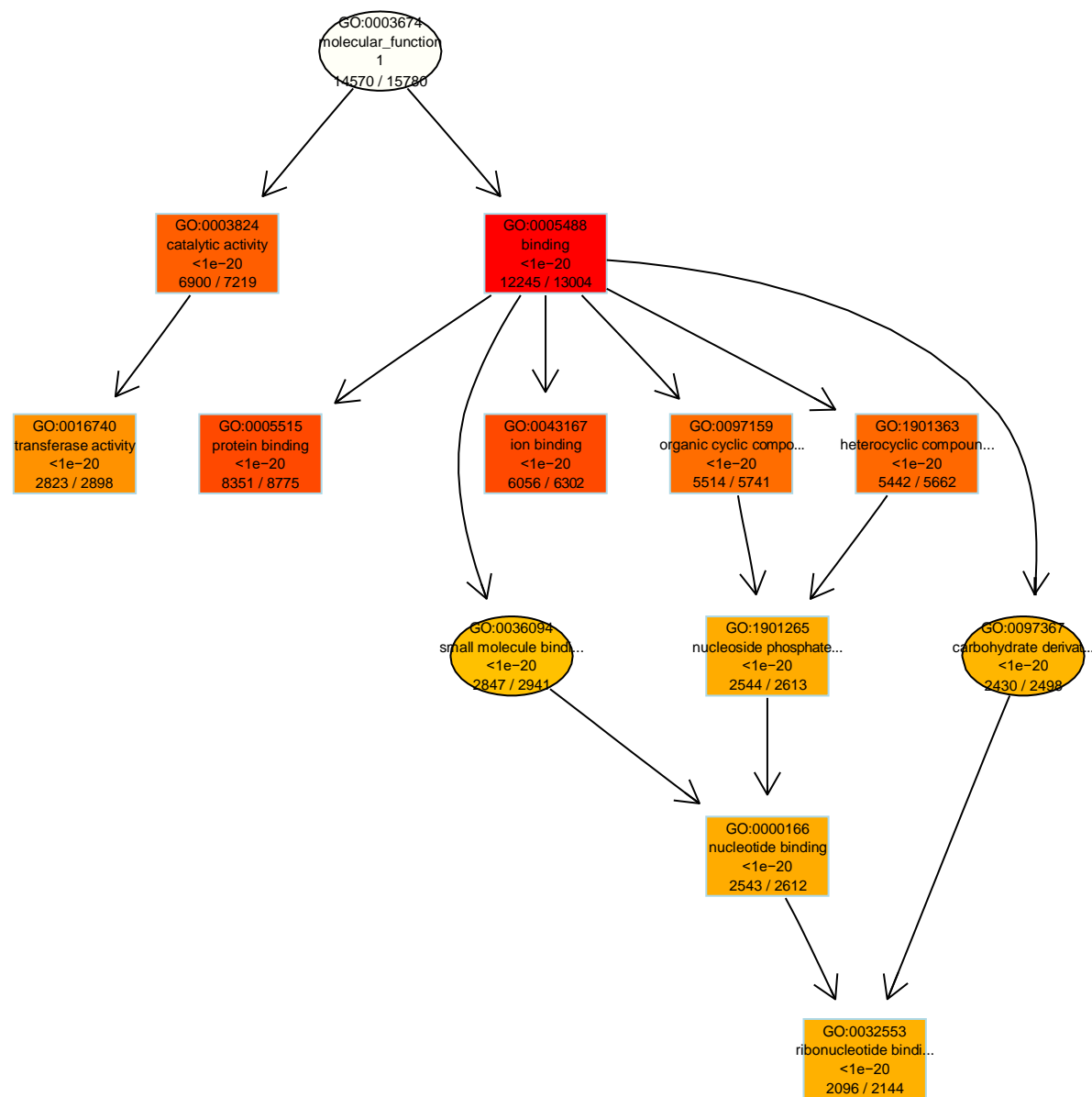
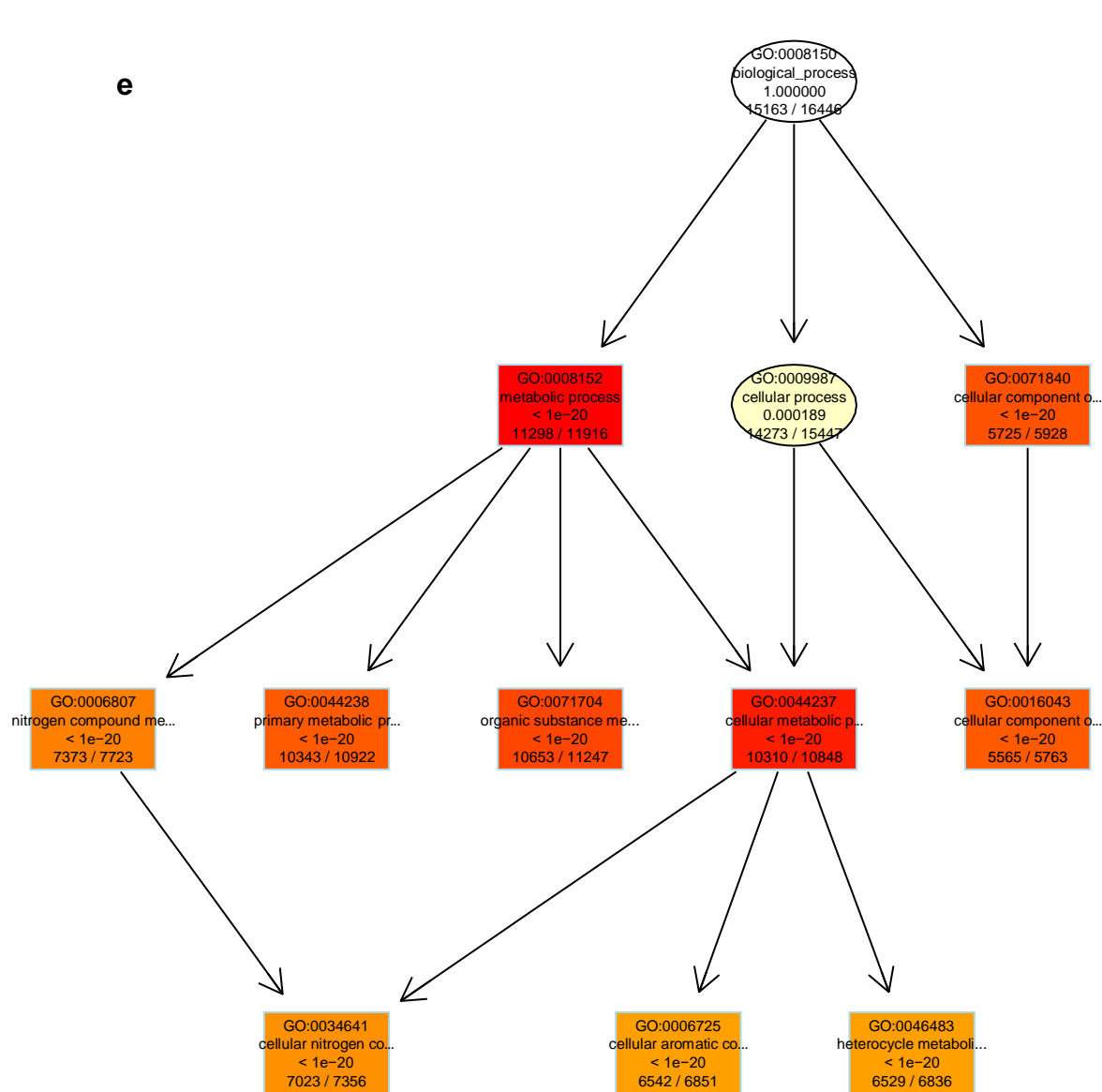




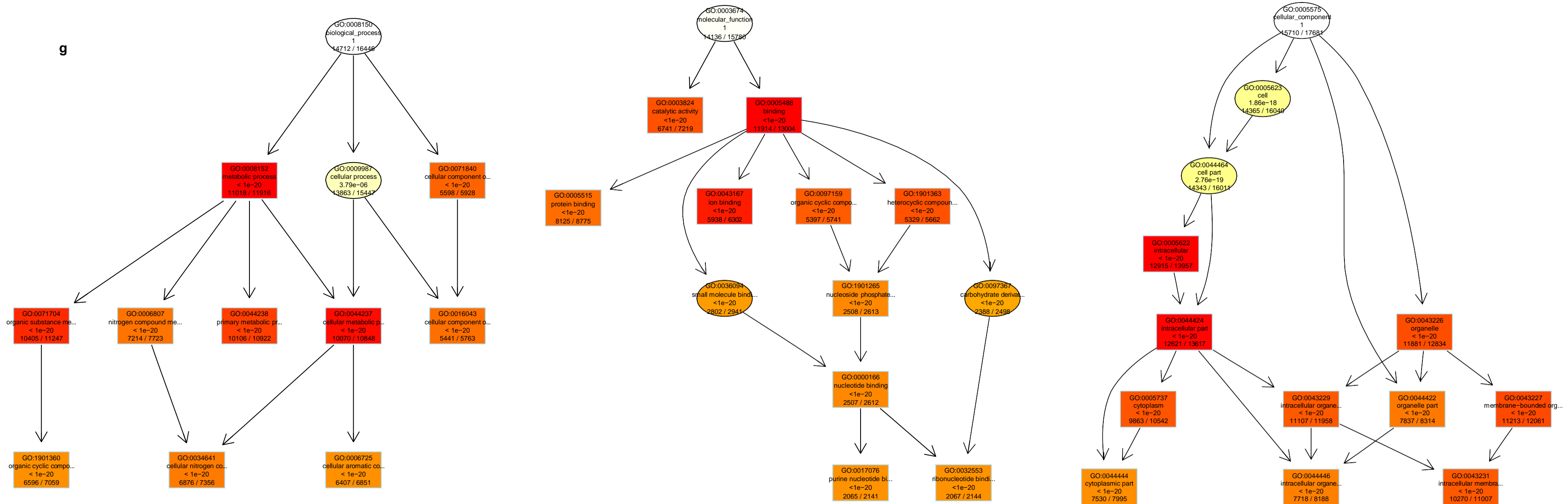
d



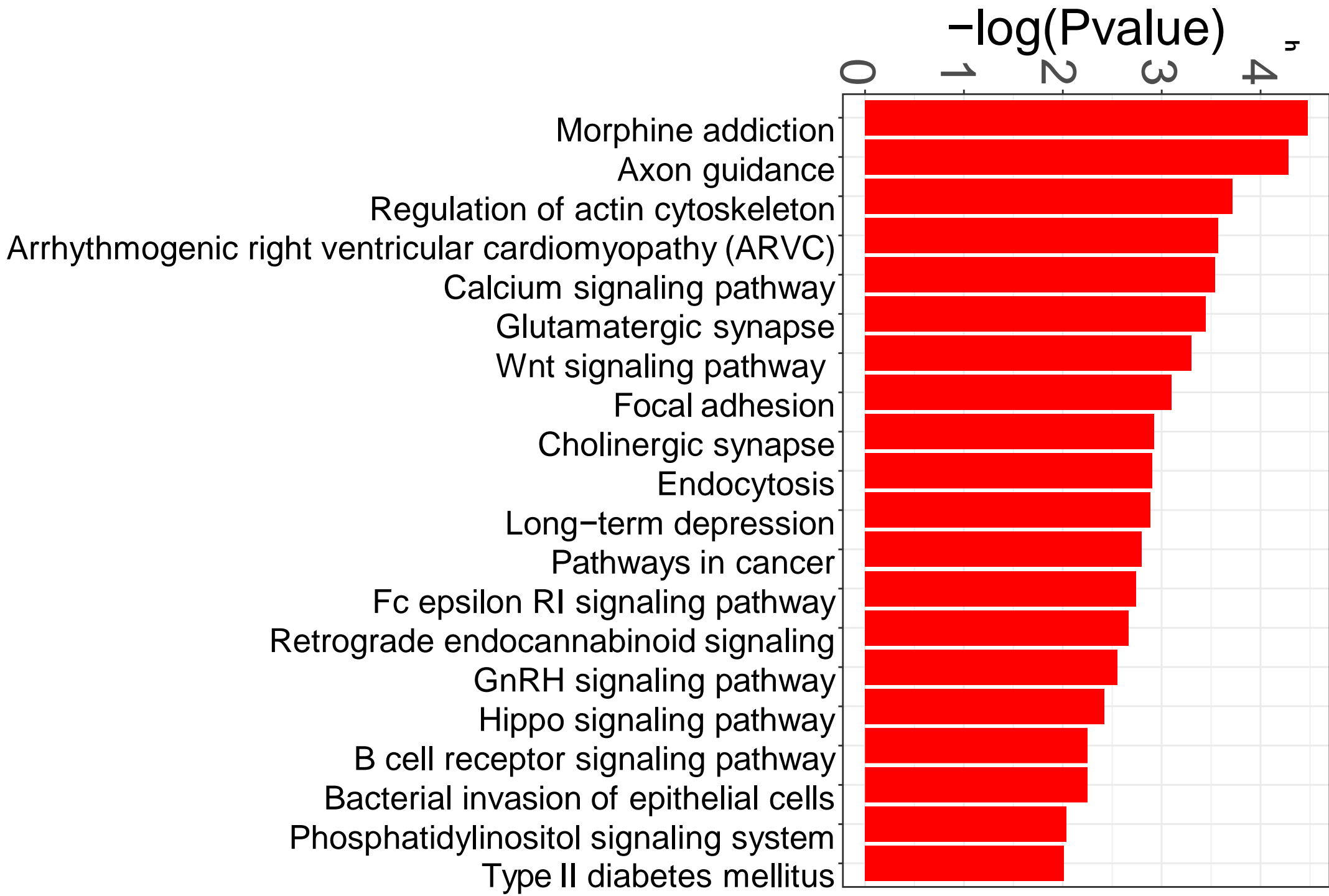
e



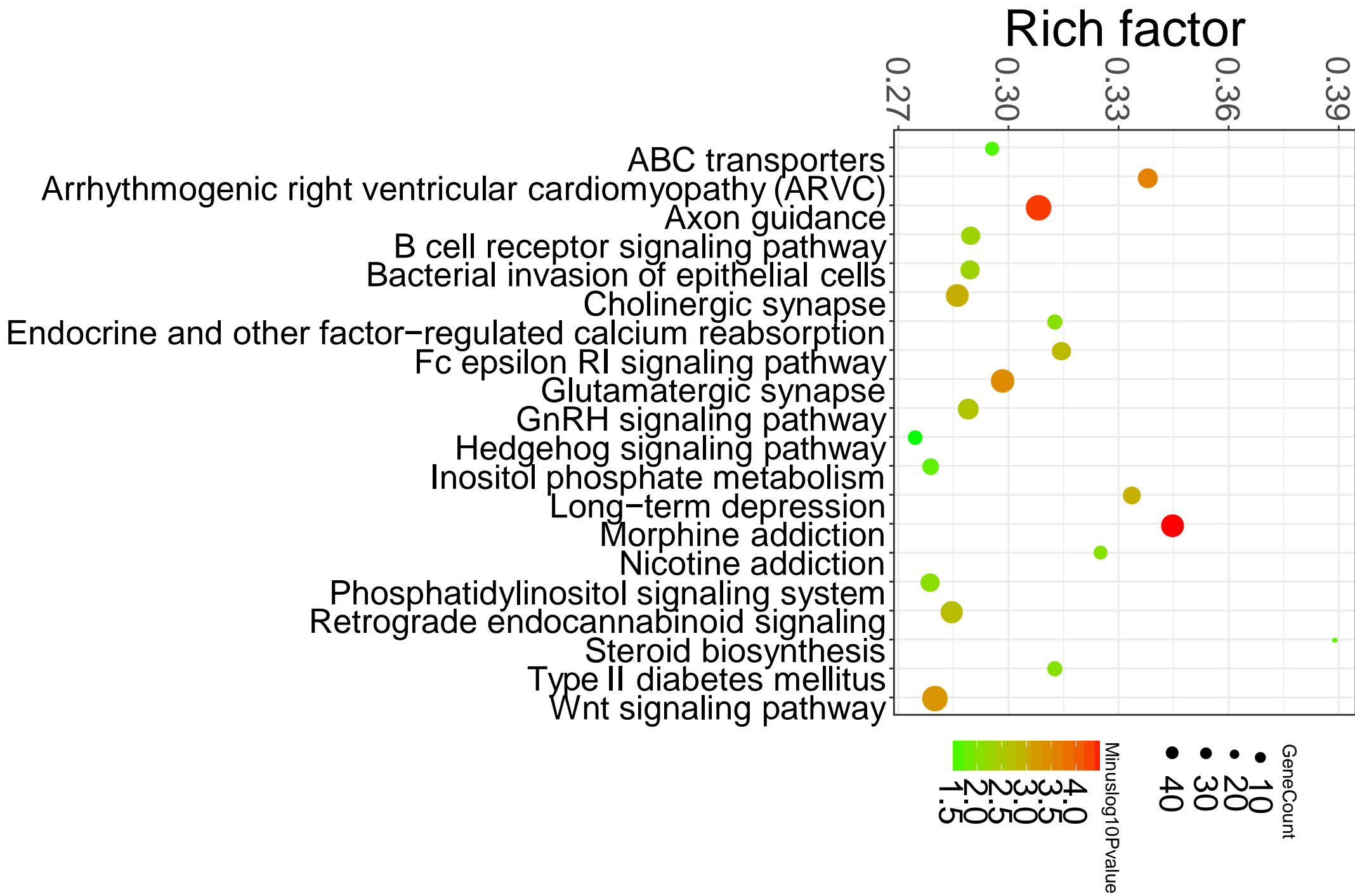
g

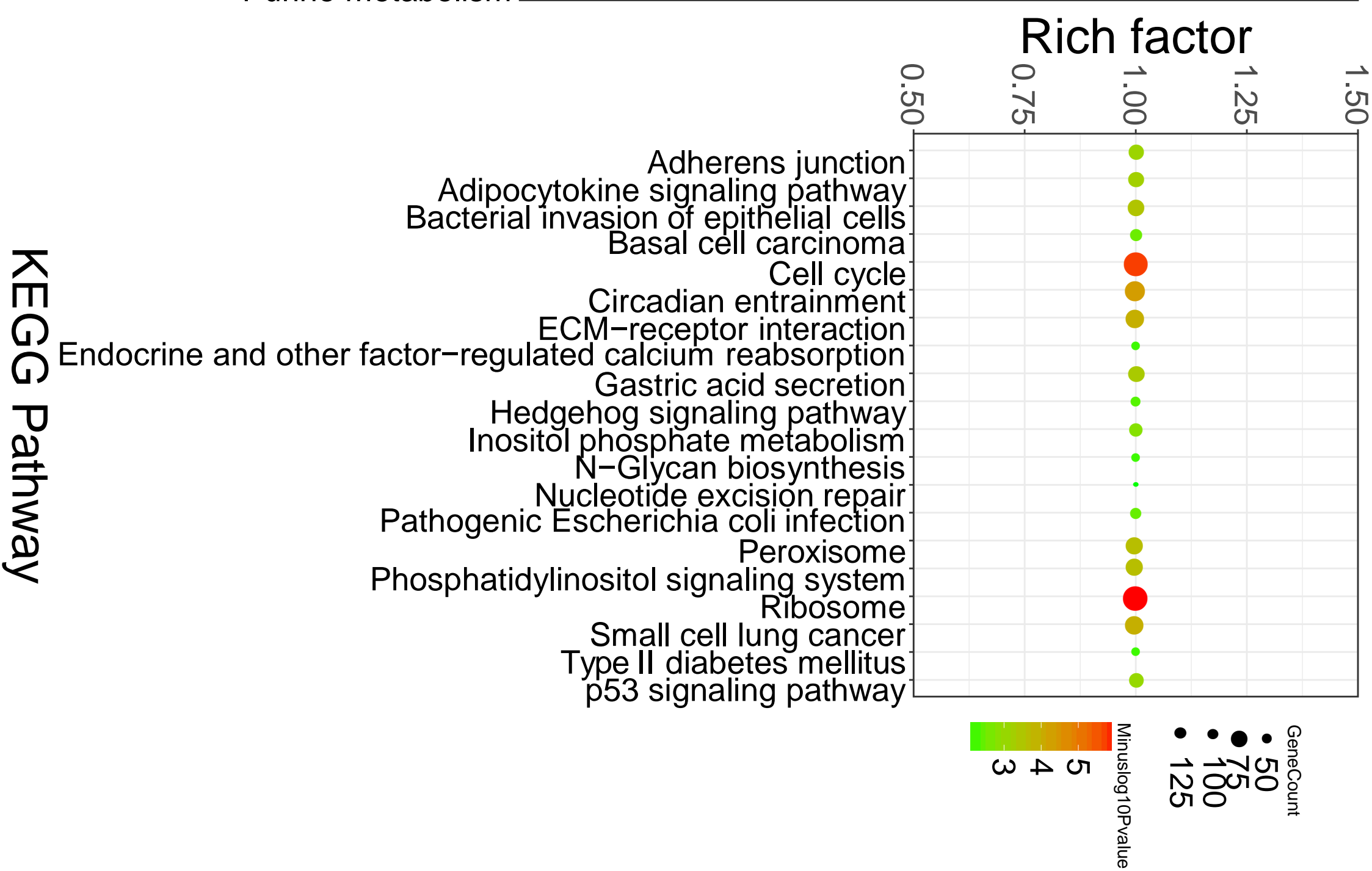
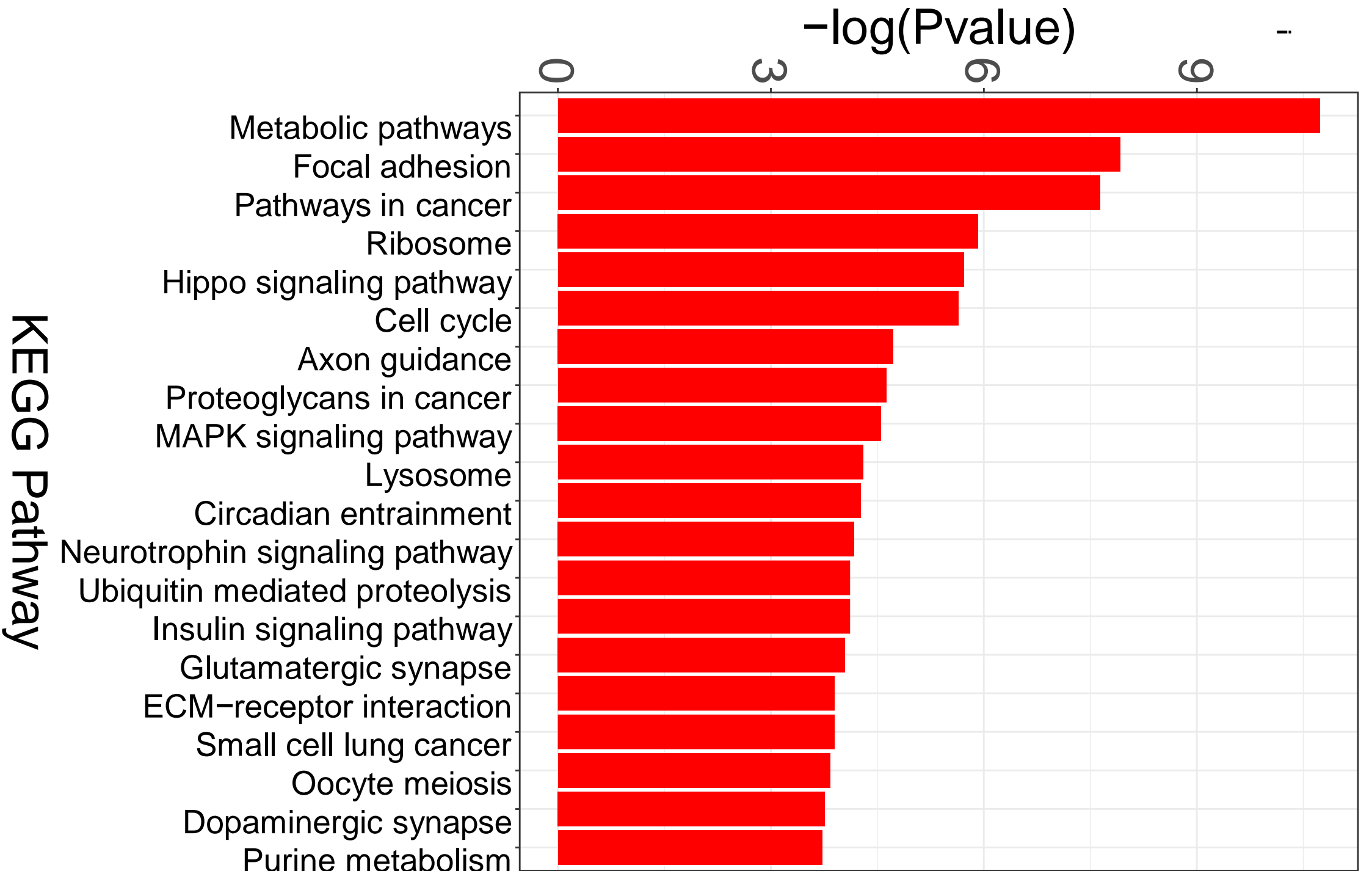


KEGG Pathway

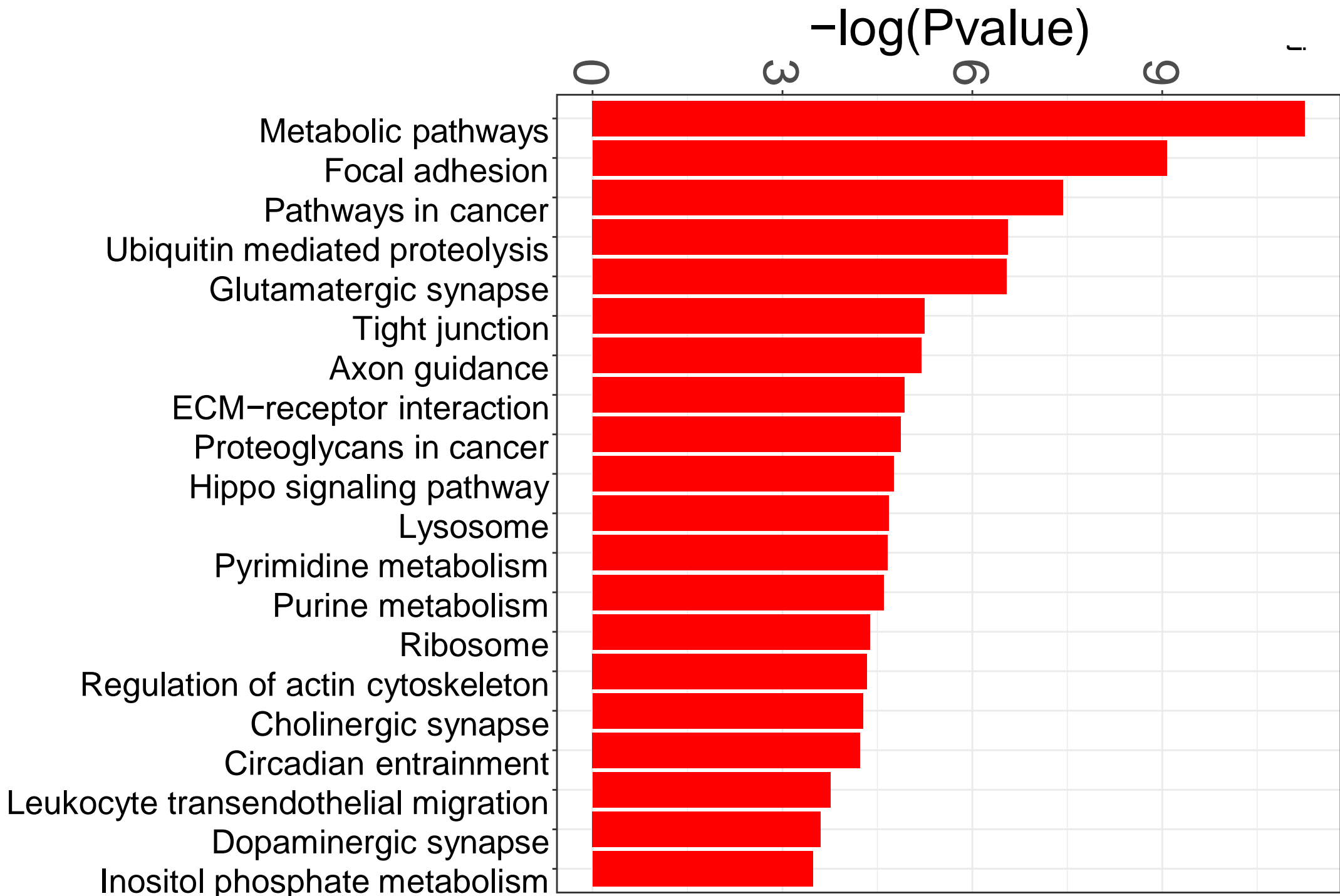


KEGG Pathway

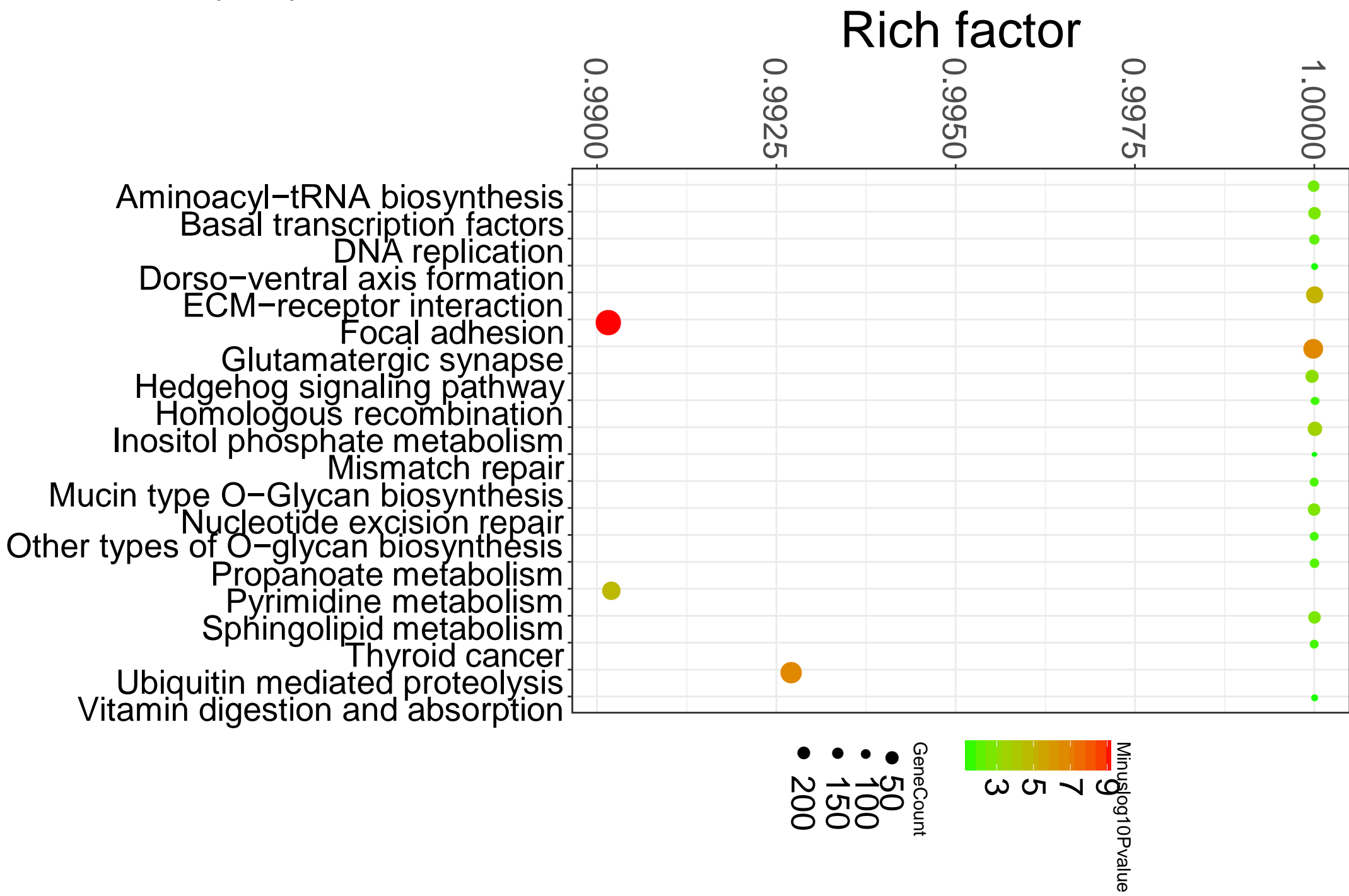




KEGG Pathway



KEGG Pathway



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

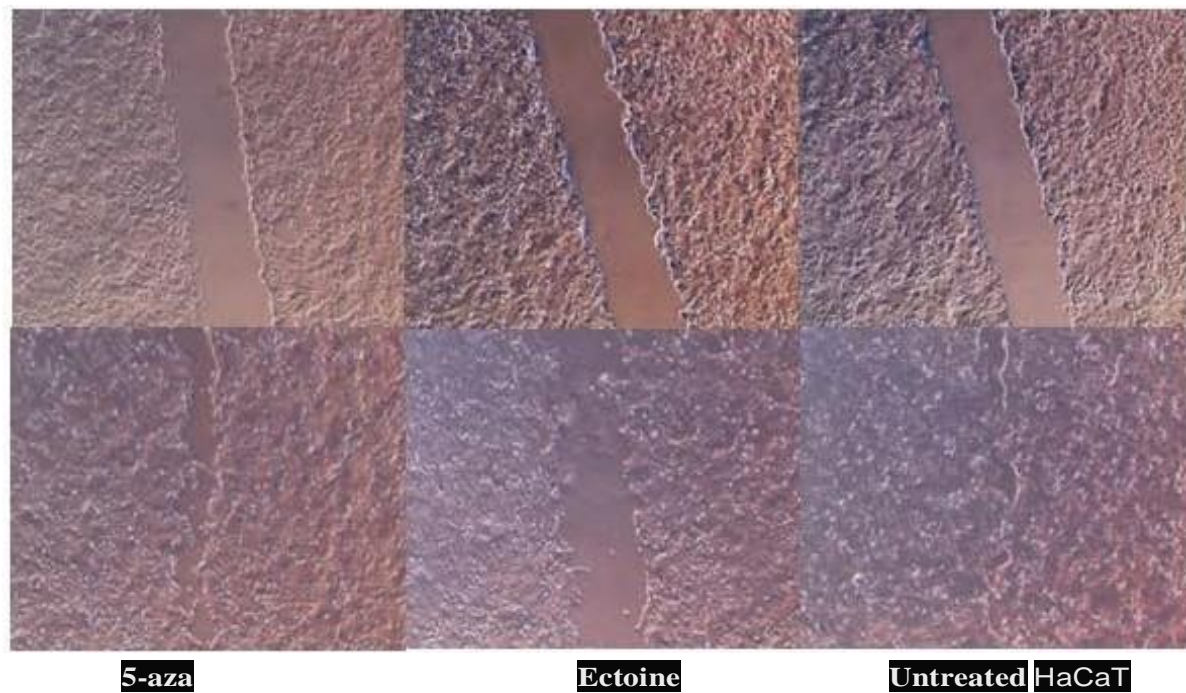
$-\log_{10}(p \text{ value})$, and the abscissa represents the names of significantly enriched GOs (up to the top 10 GOs for each category). Similarly, using treemap ectoine vs untreated (d) the ion binding, small molecule binding, nucleotide binding and ribonucleotide binding related DEGs were significantly 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 $-\log_{10}(p \text{ value})$. 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 $-\log_{10}(p \text{ value})$. According to the sorting information of the Rich factor, at most, the top 20 KEGG pathways are displayed.

a



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).