

Multiple time points of transcriptome analysis revealed altered genes involved in maintaining hibernation in the hypothalamus of *Tamias sibiricus*

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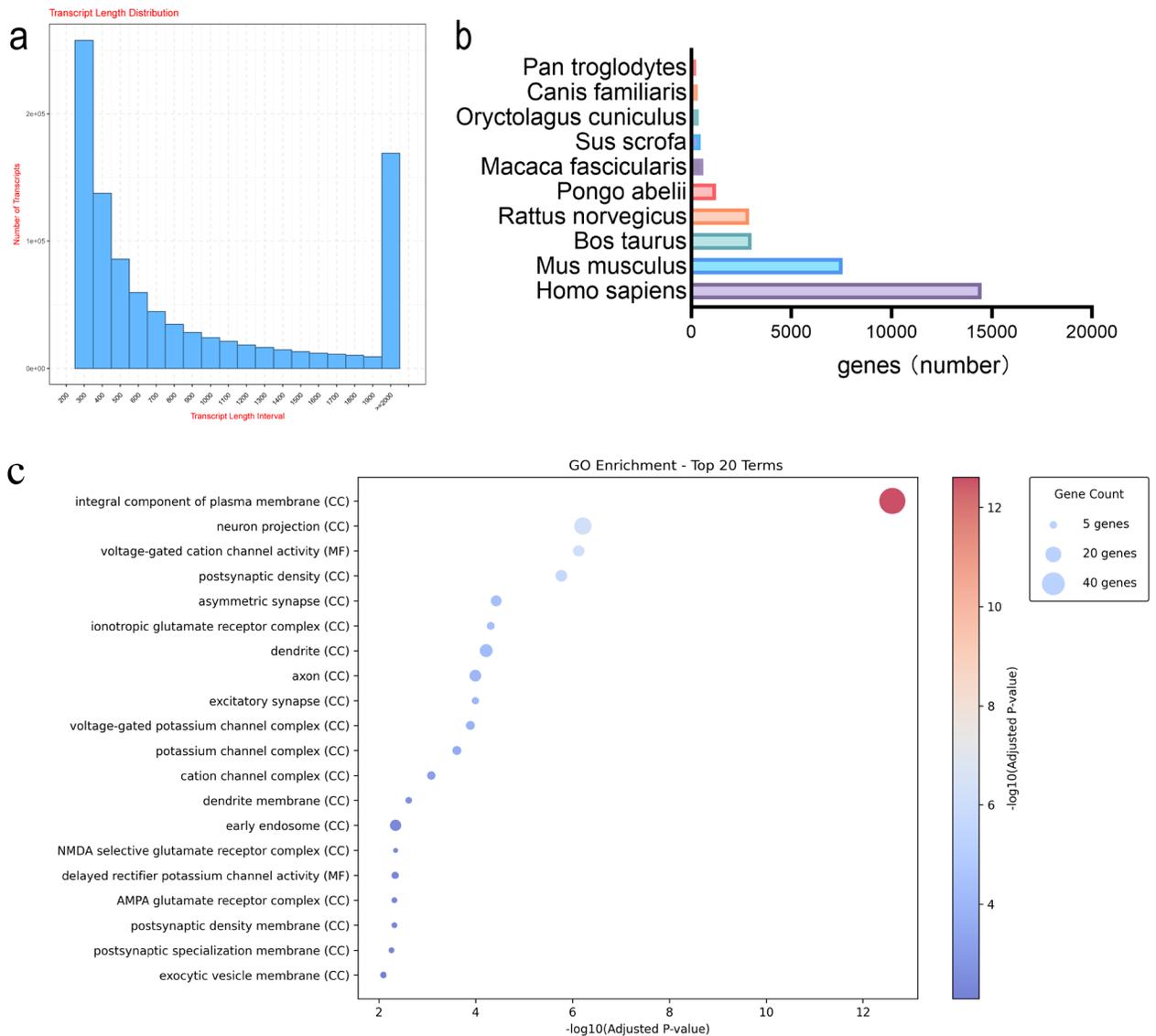
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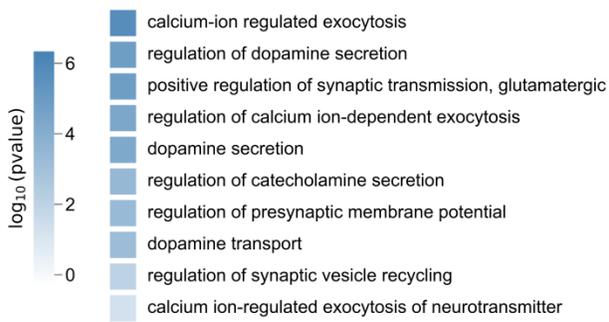
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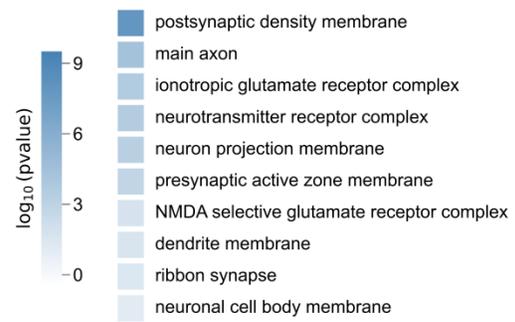
28 Figure S1. Quality control chart for transcriptomics de novo sequencing and species match chart of
 29 gene annotations from the Swissprot database. **a** Transcript length distribution. The x-axis represents
 30 the number of transcripts, and the y-axis represents the transcript length interval. **b** Gene function
 31 annotation results based on the Swissprot reference database. **c** Top 20 GO pathways enriched for
 32 downregulated differentially expressed genes (DEGs) between the ACT and M-EUT groups.
 33 Pathways are ranked by adjusted p-value. The x-axis represents the enrichment score, calculated as -
 34 log₁₀(p-value), and the y-axis lists the pathway names. This analysis integrates Biological Process
 35 (BP), Cellular Component (CC), and Molecular Function (MF) categories, with pathways ranked by
 36 significance across these GO categories. The color gradient indicates increasing significance,
 37 transitioning from blue to red as -log₁₀(adjusted p-value) increases. The size of the circles represents
 38 the number of genes enriched in each pathway.

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a GO:BP ACT vs M-EUT (Down-Regulated)

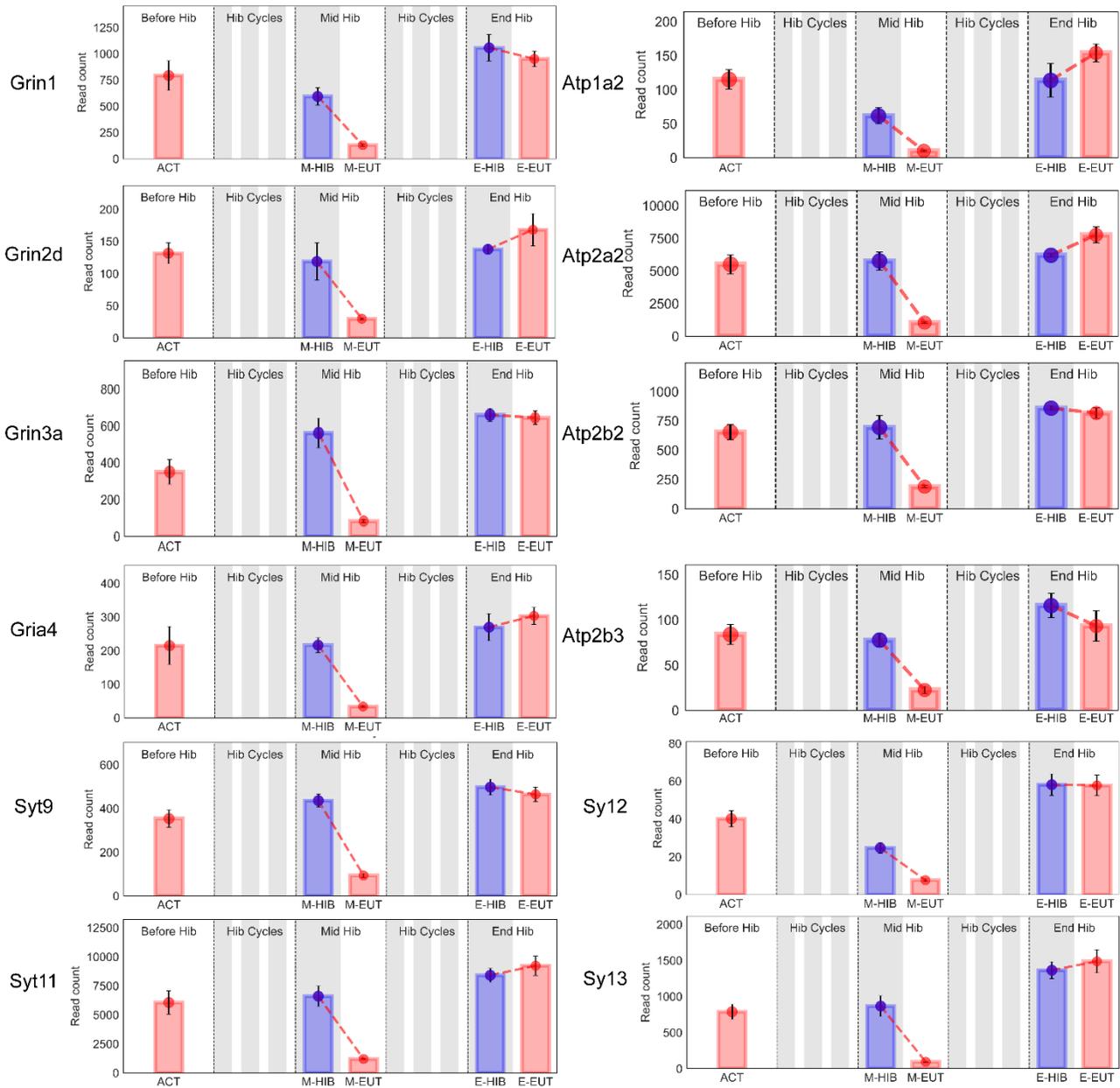


b GO:CC ACT vs M-EUT (Down-Regulated)



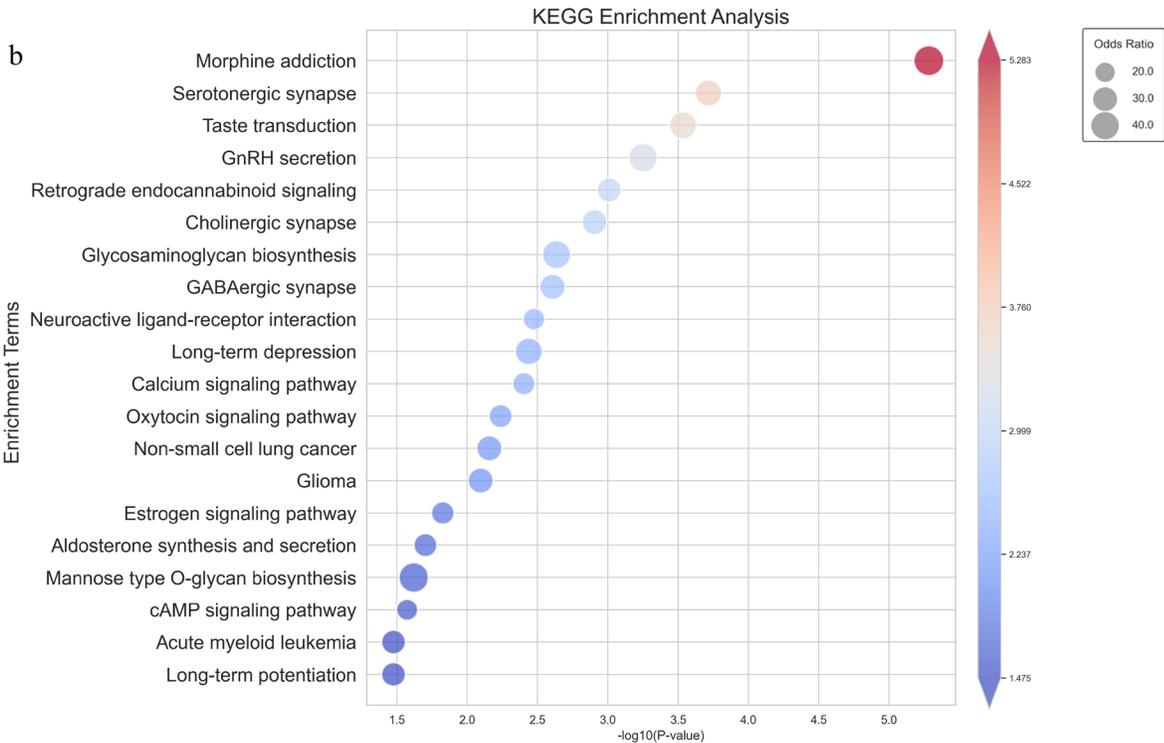
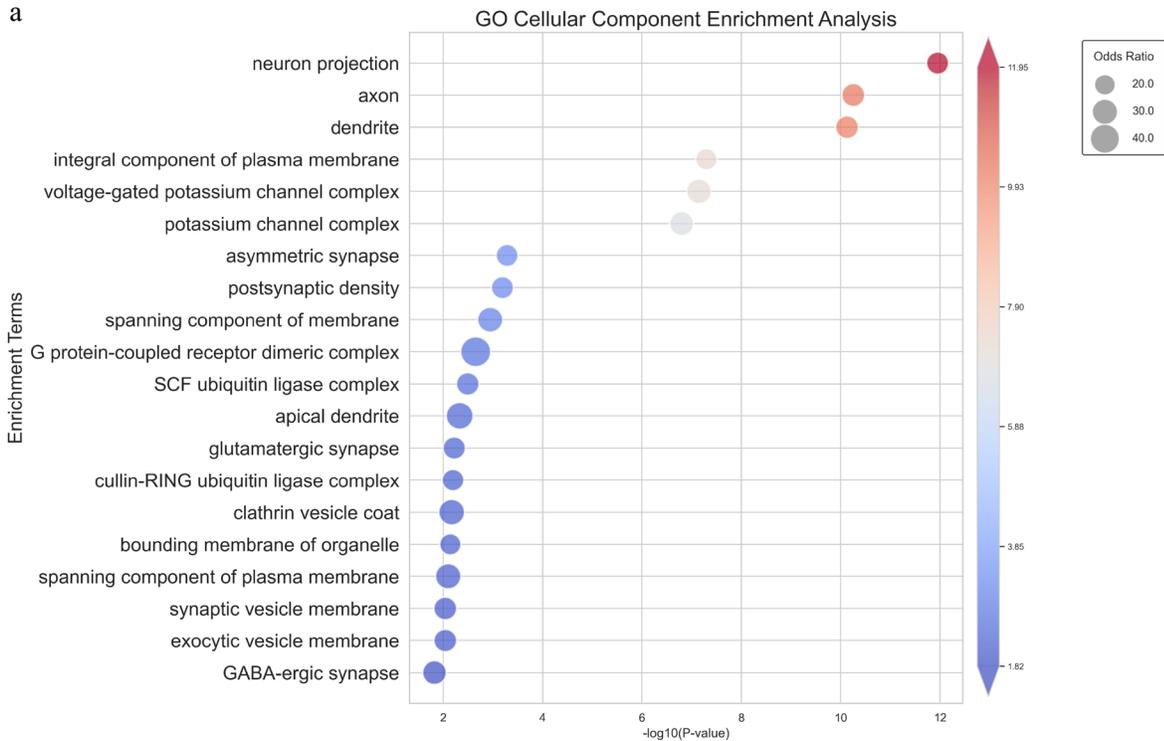
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41 Figure S2. Enrichment plots for the complementary sets of ACT vs. M-EUT and M-DS vs. M-EUT.
42 **a** Gene Ontology-Biological Process (GO-BP) enrichment pathway chart for the genes in the
43 intersection of the complementary sets of ACT vs. M-EUT and M-DS vs. M-EUT. **b** GO-BP
44 enrichment pathway chart for the genes at the intersection of the complementary sets of ACT vs. M-
45 EUT and M-DS vs. M-EUT. Color intensity represents log₁₀ (p-value), with darker colors indicating
46 larger log₁₀ (p-value) magnitudes.



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48 Figure S3. Bar graphs of Grins, Atps, and Syts gene expression in the hypothalamus of *T. sibiricus* at
 49 different time points. Each graph, from left to right, represents the following stages: pre-hibernation
 50 (Before Hib), hibernation cycles (Hib Cycles), mid-hibernation (Mid Hib), and end-of-hibernation
 51 (End Hib). The color of the sample represents the sleep/arousal state: red for awake and blue for
 52 sleeping. Time points are ordered from left to right as ACT, M-DS, M-EUT, E-DS, and E-EUT.



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54 Figure S4. Enrichment analysis of intersected genes in KEGG and GO Cellular Component
 55 (GO_CC) pathways. **a** Bubble chart showing the top 20 significantly enriched KEGG pathways,
 56 where the size of each bubble represents the odds ratio and the color reflects the $-\log_{10}(P\text{-value})$. **b**
 57 Bubble chart showing the top 20 significantly enriched GO Cellular Component (GO_CC) pathways,
 58 where the size of each bubble represents the odds ratio and the color reflects the $-\log_{10}(P\text{-value})$.