

# Pur-alpha participates in the progression of Alzheimer's disease through direct and indirect ways

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## Research article

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# Abstract

**Background:** Purine rich element binding protein A (Pur-alpha) encoded by the PURA gene is an important transcriptional regulator that binds to DNA and RNA and is involved in processes such as DNA replication and RNA translation. Pur-alpha plays an important role in the nervous system. Our previous research found that the regulatory effect of Pur-alpha on APP suggests that it may be involved in the production of beta-amyloids. Suggesting that Pur-alpha may play a role in Alzheimer's disease (AD), but the relevant evidence is insufficient.

**Methods:** We performed RNA-sequencing (RNA-seq) analysis of Pura-KO mouse hippocampal neuronal cell line (HT22) to analyze the effect of puralpha deletion on neuron expression profile. And then combined with ChIP-seq analysis to explore the mechanism of Pura on gene regulation.

**Results:** we found 656 differentially expressed genes between HT22 and Pura-KO HT22. A total of 62 overlapping genes were found by comparison with early brain protein expression profiles in mice, which may play important role in early neuronal development. We found 7 Alzheimer's disease (AD)-related genes (Lpl, Mapt, Mme, Ndufa3, Lrp1, Gapdh, mt-Co3) and 5 Ab clearance related genes (Mme, C3, Lrp1, Insr, Trem2) were regulated by Pur-alpha, suggesting that Pur-alpha plays an important role in AD. Through ChIP-seq analysis, we found that Pur-alpha binds directly to 47 genes and regulates their transcription, including Insr, Mapt, Vldlr, Jag1, etc. The direct regulation of Vldlr—the Reelin ligand—suggests that Pur-alpha may be involved in the process of synaptic plasticity. The direct regulation of Jag1 suggests that Pur-alpha may be involved in the Notch pathway.

**Conclusions:** Our study re-confirms the important role of Pur-alpha in neurodevelopment. The regulation of Pur-alpha on AD-related genes means that Pur-alpha plays an important role in the pathogenesis of AD.

## Background

Pura is a 322-amino acid protein encoded by PURA, presenting mainly in the nucleus and minor in cytoplasm and mitochondria [1]. It can bind to purine rich DNA or RNA, and promoter regions of some genes in order to form multimeric complexes, and can also interact with other transcription factors [2]. Pur-alpha's function is complex—it can promote transcription of some genes, such as TNF- $\alpha$  [3], myelin basic protein [4], placental lactogen [5], and PDGF- Protein A [6], etc. At the same time, Pur-alpha also inhibits the expression of some genes, such as fas [7],  $\alpha$ -actin [8], amyloid- $\beta$  protein precursor [9] and CD43 [10].

Pur-alpha is widely expressed in the cerebellum, adrenal glands, and other tissues [11], suggesting that it has a wide range of effects throughout the human body. Pur-alpha plays a major role in the development and maintenance of the nervous system [12]. Mice lacking the Pura gene developed severe tremors and spontaneous seizures 2 weeks after birth and died at about 4 weeks of age [13].

In the past few years, we have focused on the role of Pur-alpha in the nervous system, including repairing DNA damage in neurons [14] and influencing Alzheimer's disease pathogenesis [15]. Recent technological developments in the life sciences research have allowed new insights into the functions of Pur-alpha. In our

study, we performed RNA-seq and ChIP-seq analysis on Pura knockout cell lines constructed based on CRISPR/Cas9 gene editing technology. We hope to improve the understanding of Pur-alpha through these investigations.

## Methods

### Cell culture

The HT22 cell line was maintained in the laboratory. The Pura-KO cell line was independently constructed and validated by the laboratory, based on CRISPR/Cas9, and screened for single cell cultured monoclonal cell lines. The cells were cultured in an incubator at 37 °C. Nutrient composition: 89% DMEM (Bioind, USA), 10% FBS (Bioind, USA) and 1% penicillin-streptomycin (Solarbio, China).

### RNA-seq

Whole genome RNA was extracted using the TRIZOL method, RNA concentration was determined using NanoDrop 2000 (Thermo), and RNA integrity was detected using an Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). The sequencing library was built using the NEBNext Ultra™ RNA Library Preparation Kit for Illumina (NEB, USA) and library quality was assessed on an Agilent Bioanalyzer 2100 system. Clustering was performed using the TruSeq PE Cluster Kit v4-cBot-HS (Illumina), after which the library preparations were sequenced on an Illumina HiSeq Xten platform and the readings at the paired ends were generated.

### ChIP-seq

ChIP immunoprecipitation was performed using a Pierce™ Agarose ChIP Kit (Catalog number: 26156, Thermo USA). The antibody used to capture Pur-alpha was purchased from SANTA CRUZ (sc-130397). HT22 cells were seeded in a 10 cm diameter petri dish and subjected to ChIP immunoprecipitation with cell growth to 95% confluence. The ChIP immunoprecipitation operation was carried out according to the instructions. The main processes included formaldehyde cross-linking, sonication, enrichment of the target protein with the magnetic beads coated with the antibody, de-crosslinking, and recovery of the DNA fragment. The library was constructed using the ChIP-seq Library Prep Master Mix Set for Illumina (NEB, USA), the main processes including end repair, add in A to the 3' end, ligate adaptation, gel purification and size selection, PCR amplification. Sequencing was performed using the Illumina HiSeq 2500 sequencing platform.

### Sequencing data quality control

Raw data in the fastq format (original reads) removed the purchase of ploy-N and low-quality reads to get clean data (clean reads). At the same time, Q20, Q30, GC content and sequence repeat levels of clean data were calculated (S5). All downstream analyses are based on high quality, clean data.

### Differential gene acquisition

The sequencing fastq data were compared to the mouse reference gene (GRCm38/mm10) using hisat2. The samtools tool was used to convert the obtained sam file to a bam file. The readings were counted using the htseq-count tool. The read matrices were analyzed using the edgeR tool to obtain differential genes with a fold multiple of  $\geq 2$  and FDR  $< 0.05$  as a screening criterion. Since no biological duplication was set in this study, CORNAS (overlying RNA-Seq) was used for differential gene screening. Alpha and FDR use default values (Alpha=99%, FDR=1.5). 1165 differential genes were obtained by edgeR analysis, and 676 differential genes were obtained by CORNAS analysis. The intersection of the two results was taken as the final differential gene, and a total of 656 genes were obtained (S1).

#### ChIP-seq data analysis

Clean reads were compared with reference genomic sequences to obtain alignment information of ChIP-seq DNA (bowtie2); alignment peak position and alignment intensity information (MACS) were found by comparing position information of reads on the genome. We use MEME-ChIP software to identify and annotate Motif, and use MEME and Dreme to detect the significant motif sequences in the peak sequence, and then use Tomtom software to compare the obtained motif sequences with known motif databases.

#### Other tools

Wayne maps and gene maps on chromosomes were constructed using TBtools (<https://github.com/CJ-Chen/TBtools>). GO, KEGG analysis was performed using the Omic Share tools, a free online platform for data analysis (<http://www.omicshare.com/tools>).

## Results And Discussion

In our experiments, RNA-seq analysis was first performed on HT22 cells knocked out of Pura using CRISPR/Cas9. Comparing the Pura-KO and HT22 expression profiles, we found a total of 656 differential genes (Fig. 1A). The down-regulated genes are predominant 488/656 (S1), suggesting Pur-alpha plays a major role in promoting gene expression. Pur-alpha is an important transcriptional activator, means that pur-alpha knockout has a considerable impact on many metabolic pathways involved in growth and development, such as Pathways in cancer, PI3K-Akt signaling, and cytokine-cytokine receptor interaction (Fig. 1B). In addition, we found Pura was involved in important functions such as neurotrophin signaling and Axon guidance pathways.

Pur-alpha plays an important role in the development of nerves

In order to explore the biological functions of differential genes, we performed GO annotations on the up-regulated and down-regulated genes. The results showed that the down-regulated genes were involved in neuronal structure, neuronal projection, response to oxygen, and positive regulation of cellular processes (Fig. 1C). These findings highlight the vital role of Pur-alpha in the growth and development of neurons.

Pur-alpha has long been considered an indispensable factor in neurodevelopment. In experiments performed by Khalili [13], Pura knockout mice developed severe tremors, spontaneous epilepsy and other

neurological problems at 2 weeks of age and died 4 weeks after birth. However, the specific mechanism of Pur-alpha in the early development of neurons is still unclear. In a comparative study of cerebral cortex-suspended mice at different stages after birth by Gonzalez-Lozano [16], total expression of brain proteins decreased after birth. To clarify the trend of Pur-alpha in postnatal mice, we found that Pur-alpha expression was reduced in 280-day-old mice (adults) compared to 9- and 15-day mice from Gonzalez-Lozano's study [16]. This result would suggest that the main function of Pur-alpha occurs in infancy and does not persist in adulthood. Unfortunately, although the overall difference was statistically significant, only one of the three replicates was statistically different. To explore the role of Pur-alpha in the early development of the brain, we compared the Pura-KO gene expression profile with the protein profile from Gonzalez-Lozano's study [16]. We noticed a duplication of 62 proteins (S2), suggesting that the cause of early death in Pura-ko mice may be included in these 62 proteins. We further functionalized 62 genes and found that these genes were involved in many functional and metabolic pathways (based on GO and KEGG analysis), including neuronal structure (GO:0097458), neuronal projections (GO:0043005), and nervous system. They were also involved in development (GO:0007399), neurotransmitter levels (GO:0001505), glycolysis/gluconeogenesis (mmu04066), HIF signaling pathway (mmu04066), carbon metabolism (mmu01200) (Fig. 2). As such, Pur-alpha is involved in the regulation of neuronal metabolism, the formation of synapses, and the establishment of projections between neurons. These changes caused by Pura knockout have a major impact on the formation of neuronal synapses and the establishment of a network of connections between neurons, so this may be the cause of premature death in Pura-ko mice.

#### Pur-alpha plays an important role in the progression of Alzheimer's disease

In the past few years, we have been exploring the relationship between Pur-alpha and neurodegenerative diseases, especially Alzheimer's disease (AD) [15]. In previous studies, we noticed that Pur-alpha regulates the rejuvenation of APP proteins, but the study of subsequent Pur-alpha in AD seems to be interrupted. In our study we deliberately focused on the expression of APP after Pura knockout, but unfortunately the knockout of Pura seems to have no effect on APP or APP mRNA expression. This result highlights additional complexities in AD pathogenesis. Based on RNA-seq analysis, we found that 7 genes are enriched in Alzheimer disease (Table 1, Fig. 3), and 5 genes are enriched in amyloid-beta clearance (Table 2).

Table 1  
Differential gene enriched in Alzheimer's disease

gene ID	Gene Name	HT22 (count)	Pura-ko (count)	FDR	log2FC	regulated
ENSMUSG00000015568	Lpl	1138	554	4.20E-05	-1.075796463	down
ENSMUSG00000018411	Mapt	25	87	8.02E-05	1.756656016	up
ENSMUSG00000027820	Mme	361	86	7.48E-13	-2.105387765	down
ENSMUSG00000035674	Ndufa3	621	1178	0.00102346	0.886115743	up
ENSMUSG00000040249	Lrp1	7916	4904	0.006012088	-0.728224954	down
ENSMUSG00000057666	Gapdh	6112	3641	0.002800884	-0.784715839	down
ENSMUSG00000064358	mt-Co3	1381	778	0.001266429	-0.865192446	down

Polymorphisms in the LPL gene are thought to be associated with the risk of AD [17]. LPL is a key enzyme that regulates the hydrolysis of triglycerides. LPL deficiency or dysfunction can cause dyslipidemia, which may increase the risk of AD [18]. LPL binds to amyloid beta protein (A $\beta$ ) and promotes cell surface association and A $\beta$  uptake in mouse primary astrocytes [19] and BV2 microglia [20]. Studies after human brain death have shown that LPL is widely distributed throughout brain tissue. Compared with control groups, LPL in the dentate gyrus granule cells and CSF samples of the AD group are significantly reduced [21]. In our study, we found similar LPL changes in AD after knocking out Pura, suggesting that Pur-alpha can regulate LPL, which may be a potential mechanism for AD development. Ndufa3 and mt-Co3 are mitochondria-associated proteins, which are reported relatively less in AD and appear to be associated with mitochondrial dysfunction in AD [22].

The large accumulation of Tau protein is one of the characteristics of AD, and Mapt is the coding gene of tau [23]. A large number of studies have shown that there is a large accumulation of Tau protein in the brain tissue of AD patients. Therefore, hyperphosphorylation and deposition of Tau protein may be a cause of AD [24]. In our study we found that Tau expression was up-regulated after Pura knockout, implying a potential inhibitory effect of Pur-alpha on Tau. GAPDH is a key gene in sugar metabolism, but a large number of independent studies have shown that GAPDH has non-glycolytic activity and is involved in pathogenesis and death in neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease [25]. GAPDH is often present in the AD temporal cortex along with phosphorylated tau and Ab peptides [26]. Since GAPDH has a region that binds to Ab, some scholars believe that the aggregate of GAPDH provides seeds for the specificity of A $\beta$  [27]. Studies show that nitrosated GAPDH can enhance the degree of acetylation of Tau; in the presence of Ab, it can promote the aggregation of Tau into neurofibrillary tangles [28]. GAPDH and Tau appear to play highly intricate roles in the regulation of AD, so mRNA expression may not provide an adequate explanation for this phenomenon. At the same time, in our study, Tau was up-regulated and GAPDH was down-regulated.

Table 2  
Differential gene enriched in amyloid-beta clearance

gene ID	gene name	HT22 (count)	Pura-ko (count)	FDR	log2FC	regulated
ENSMUSG00000027820	Mme	361	86	7.48E-13	-2.1053878	down
ENSMUSG00000024164	C3	6635	1260	9.59E-26	-2.4339817	down
ENSMUSG00000040249	Lrp1	7916	4904	0.00601209	-0.728225	down
ENSMUSG00000005534	Insr	657	359	0.00126643	-0.9090988	down
ENSMUSG00000023992	Trem2	83	6	8.95E-12	-3.7995178	down

Pur-alpha may affect the progression of AD by regulating the expression of Ab-clearing related proteins

Decreased amyloid clearance is one of the main features of AD. In this study, we identified 5 genes involved in clearance of amyloid which may be affected by Pur-alpha. Coincidentally, the 5 genes were all down-regulated. The NEP protein encoded by the membrane metallo-endopeptidase (Mme) gene is one of the major contributors to brain Ab clearance and is directly involved in the degradation of Ab [29]. Studies have shown that NEP inhibitors can cause biochemical and pathological deposition of A $\beta$ <sub>1-42</sub> [30], and in vitro experiments show that NEP can rapidly degrade A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> [31], while exogenous supplementation of NEP can reduce the deposition of Ab in AD transgenic mice [32]. In our study, knocking out Pura resulted in a decrease in Mme expression, suggesting that the expression of NEP was dependent on Pur-alpha. Although the mechanism of this regulation is unclear, and ChIP-seq studies did not show evidence of Pur-alpha regulation of Mme, current research can still provide some insight. According to related studies, HIV-1 transactivator (tat) can reduce the expression and activity of NEP, thereby increasing the deposition of Ab, which is considered to be an important cause of HIV-related cognitive impairment [33]. At the same time, other studies have demonstrated the close relationship between Pur-alpha and tat. Pura promotes translation of HIV in vivo by binding to HIV-1 Tat and TAR RNA [34]. From the above studies we noticed that tat can bind to Pur-alpha, and this combination may have a similar repressive effect on Pur-alpha, causing Pur-alpha to fail to exert its normal physiological effects. The Pura knockout will result in a decrease in Mme, so the reduction in HIV-related NEP may be due to this relationship.

LRP1 is a member of the low-density lipoprotein receptor family [35], which has four extracellular ligand binding domains that bind to different ligands, including APP [36], apolipoprotein E. (Apolipoprotein E) [37], and  $\alpha$ 2 macroglobulin ( $\alpha$ 2M) [35]. LRP1 can be combined with APP before it is cut by furin [38], which slows APP movement [39], and promotes further processing of the protein [40]. LRP1 binds to APP to facilitate processing of APP, but this effect appears to increase the production of Ab [41]. Although LRP1 caused the production of Ab, we cannot ignore the fact that it promotes Ab transport. LRP1 can directly bind to Ab through the LRP1 N-terminal domain or by binding ApoE or  $\alpha$ 2M [42]. LRP1 can transport Ab to the blood-brain barrier by binding to Ab and releasing Ab into the blood, which is the main evidence that LRP1 is involved in Ab clearance [43–45]. In our study, Lrp1 decreased after Pura knockout, thus indicating that the

expression of Lrp1 requires the participation of Pur-alpha, indicating that Pur-alpha plays an important role in the processing of APP and the clearance of Ab.

Insulin receptor substrates have multiple functions, including enzyme binding activity, insulin binding activity, and binding activity of insulin receptor substrates [46]. There are few studies on the involvement of Insr in Ab regulation. In a 2009 study [47], it was shown that cells with normal Insr have the ability to reduce the reduction of Ab oligomers to Ab monomers, while the Insr mutation causes a loss of this ability, leading to the aggregation of Ab oligomers. This increase suggests that Insr has the ability to participate in Ab clearance.

Complement receptor 3 (C3) plays a central role in the activation of the complement system and participates in the human immune response [48]. In the brains of Alzheimer's patients, complement components were detected in the amyloid core of senile plaques [49], and an increase in CR3 was found in microglia [50]. C3 can be cleaved by C3 convertase to form C3b. On the one hand, C3b can bind to Ab to form an Ab-C3b complex, and can bind to CR3 and activate microglia to phagocytose Ab, thereby promoting Ab clearance [51]. In another study [52], the ability of C3-deficient N9 microglia to phagocytose fibrillar A $\beta$  was significantly reduced, further confirming that activation of the complement C3 system is an important factor in the phagocytosis of Ab by microglia. In our study, the decrease in Pur-alpha caused a decrease in C3, indicating that the synthesis of C3 was dependent on the presence of Pur-alpha. Therefore, while our study is based on neuronal cells, the reduction of Pur-alpha may also affect the ability of microglia.

The protein encoded by TREM2 is part of the immunoglobulin and lectin-like superfamily and is part of the innate immune system. TREM2 is a surface receptor required for microglia to respond to neurodegeneration, including proliferation, survival, aggregation and phagocytosis. TREM2 mutations cause autophagy in microglia. Increasing cyclocreatine in the diet to supplement energy can reduce autophagy of microglia and reduce Ab deposition in TREM2-deficient mice, suggesting that TREM2 affects Ab clearance in microglia by affecting cell energy metabolism [53]. In addition, Ab 42 deposition in age-related macular degeneration also appears to be associated with a deficiency in TREM2 [54]; the TREM2 R47H variant also shows reduced TREM2 mRNA expression and increases the risk of AD development [55]. Studies have shown that TREM2 is involved in the formation of AD, and in our study, there was a significant decrease in Trem2 after Pura knockout, indicating that Pur-alpha may be involved in the important process of AD.

The regulation of Pura on AD-related genes and Ab-cleavage-related genes appears to be indirect.

We have enriched 656 differential genes that may be regulated by Pur-alpha base on RNA-seq. Not all genes are directly regulated by Pur-alpha. In order to clarify the regulatory mechanism of Pur-alpha on genes, we analyzed the DNA fragments that may be directly bound to Pur-alpha by ChIP-seq, and found that Pur-alpha can bind to 1389 genes (S3). To further analyze the regulation of Pur-alpha on genes, we combined ChIP-seq results with RNA-seq results, and we found that Pur-alpha can bind to 47 of them and cause a large number of changes (Fig. 4, S4). Therefore, it is believed that Pur-alpha can directly regulate

these 47 genes, and the emergence of other differential genes may be affected by these 47 genes. Among the genes mentioned earlier in relation to AD pathogenesis and Ab cleavage, only *Insr* is directly regulated by Pur-alpha. This means that Pur-alpha may rely on a deeper mechanism for the regulation of these genes.

We found some genes that may interact with APP and PSEN from the above 47 genes, based on string protein interaction analysis, such as *Vldlr*, *Igfbp7*, *Kng2*, *Pros1*, and *Jag1*. Among these results, APP, *Kng2*, and *Igfbp7* were simultaneously regulated by phosphorylation of Fam20C enzyme [56], and there was a weak co-expression relationship between APP and *Igfbp7* (co-expression score = 0.057).

*Vldlr* belongs to the low-density lipoprotein receptor family and binds to apolipoprotein E (ApoE), which is essential for Reelin pathway activation [57]. Activation of the Reelin pathway increases NMDA receptor activity by promoting tyrosine phosphorylation of the NR2 subunit, which is important in enhancing glutamatergic neurotransmission[58–61]. In addition, Reelin is involved in the transport and processing of APP, and is able to interact with A $\beta$  oligomers to antagonize its negative effects on synaptic function [62–64]. In this study, we found that Pura binds directly to *Vldlr* DNA and positively regulates it, which means that Pur-alpha enhances Reelin activity by promoting *Vldlr* expression. *Pros1* is a ligand for Mer tyrosine kinase (MerTK) and activation of MerTK is considered to be an important factor in amyloid-stimulated phagocytosis [65]. A decrease in *Pros1* means that the likelihood of activation of MerTK is diminished, which in turn may affect the phagocytosis of Ab. *Jag1* is a substrate for BACE1 and can be cleaved by Bace1[66]. At the same time, *Jag1* is a ligand of Notch that promotes the activation of Notch. Loss of BACE1 cleavage causes an increase in *Jag1*, which enhances the transmission of Notch signaling. This is thought to be a possible mechanism by which BACE1 is involved in the balance of neurogenesis and astrogenesis [66]. In this study, Pur-alpha was able to directly regulate *Jag1*, and the lack of Pur-alpha caused up-regulation of *Jag1*, indicating that Pura can participate in the regulation of BACE1 on neurons and astrocytes.

## Conclusions

In this study, we found a potential mechanism for Pur-alpha in neuronal development and maintenance of normal function, raising awareness of Pur-alpha. Pur-alpha's regulatory role in AD was unforeseen. Our research confirms that Pur-alpha can participate in the pathogenesis of AD by directly regulating the Tau and indirectly regulating A $\beta$  clearance and the regulation of AD-related genes. Of course, the occurrence of AD is a complex process. Pur-alpha plays a vital role in the occurrence of AD, participating in important aspects of pathogenesis.

## Abbreviations

AD: Alzheimer's disease; pur-alpha: Purine rich element binding protein A; HT22: Hippocampal neuronal cell line, HT22; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes;  $\alpha$ 2M:  $\alpha$ 2 macroglobulin; ApoE: apolipoprotein E; MerTK: Mer tyrosine kinase.

## Declarations

## **Ethical Approval and Consent to participate**

Not applicable

## **Consent for publication**

All authors have approved of the manuscript and agree with its submission.

## **Availability of supporting data**

RNA-seq and CHIP-seq data have been uploaded to the Sequence Read Archive (SRA):  
<https://www.ncbi.nlm.nih.gov/sra>. RNA-seq data: SRA:SUB6809189; CHIP data: SRA:SUB6906483.

## **Competing interests**

The authors declare that they have no competing interests.

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## **Authors' contributions**

Jianqi Cui, Qinwen Wang, Xiaoguang Shi and Tao Sun designed research; Xiaoguang Shi, Bingying Zhang, Shanshan Guo, Wenxin He performed research; Xiaoguang Shi, Chengmin Yuan and Xiaofan Yang analyzed data; and Jianqi Cui, Xiaoguang Shi and Kevin Ig-lzevbekhai wrote the paper.

All authors read and approved the final manuscript.

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## Figures

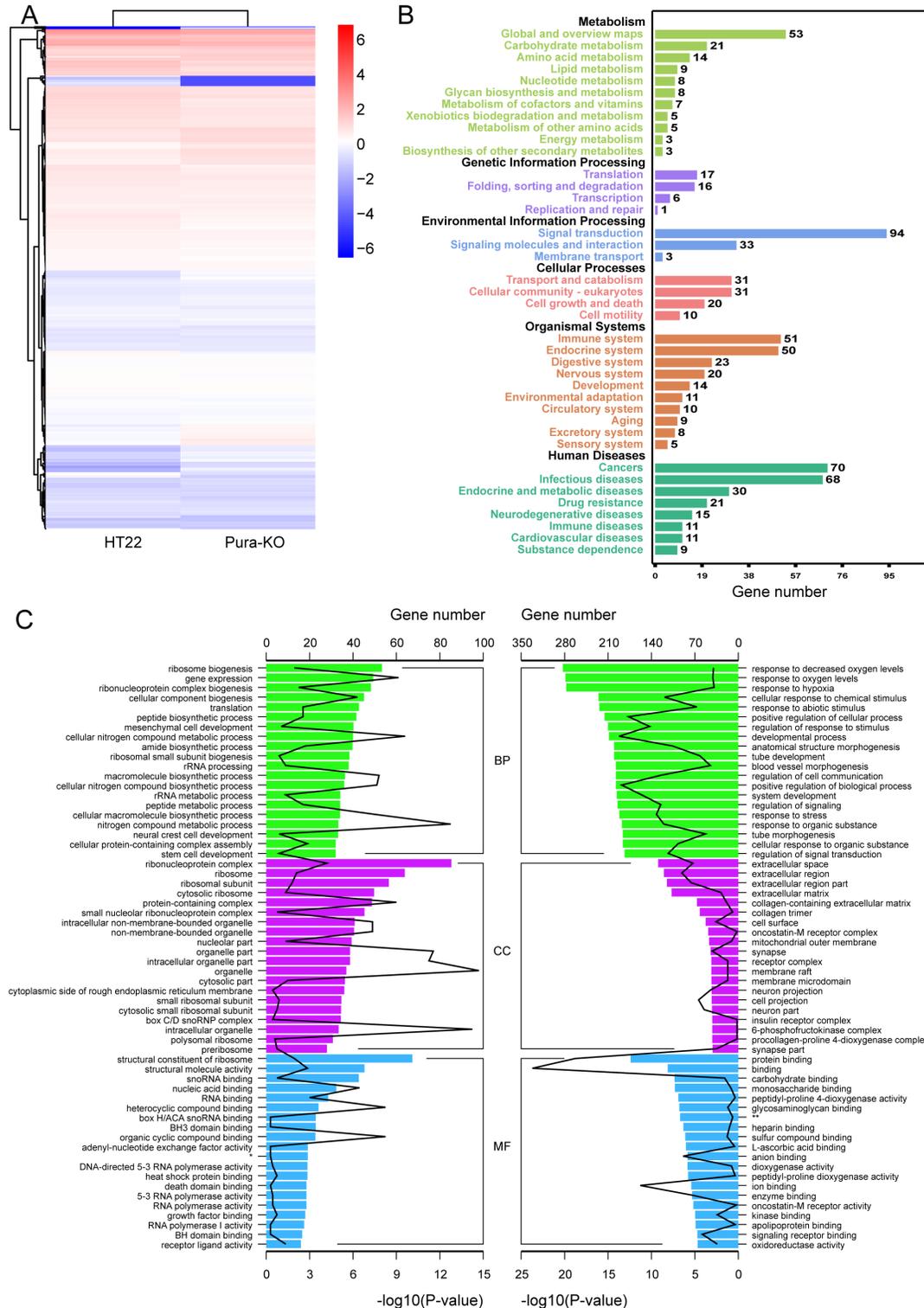
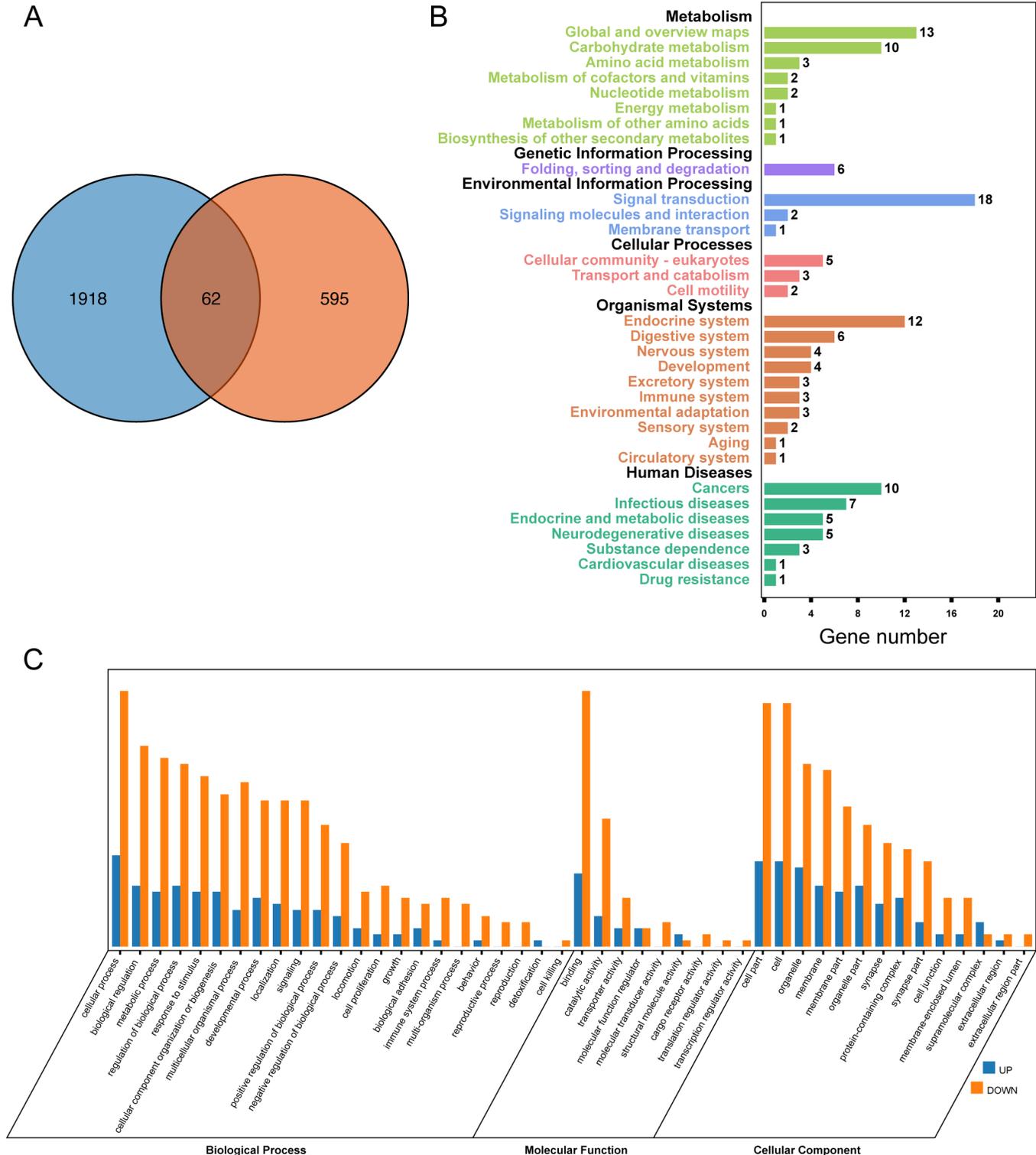


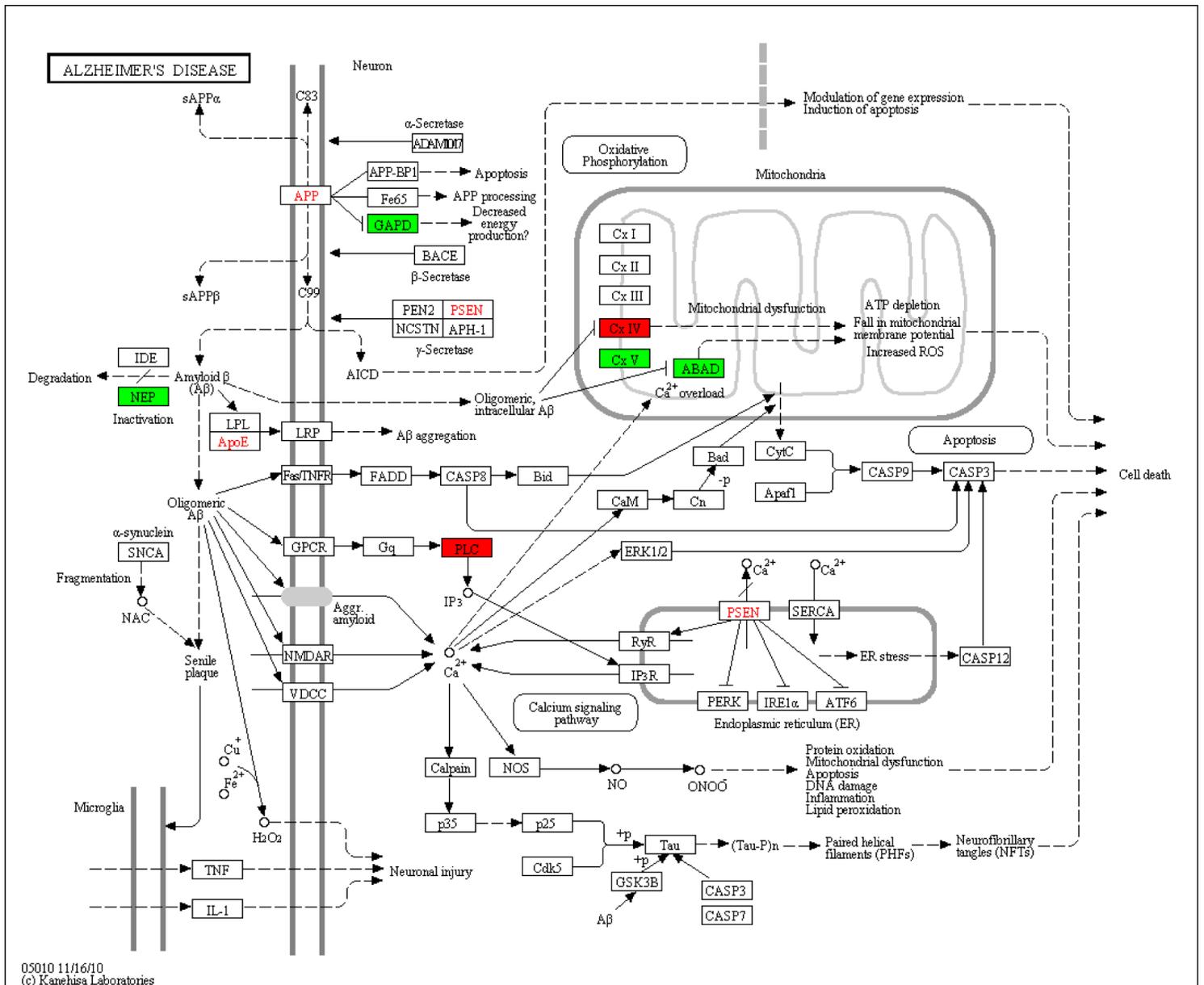
Figure 1

Differential gene enrichment analysis. (A) The difference between the differential genes in the HT22 group and the Pura-KO group is shown in the form of a heat map. (B) Histogram results of KEGG enrichment analysis of differential genes. The abscissa is the number of genes and the ordinate is the enrichment result. (C) GO enrichment of differential genes. The left aspect is the result of up-regulated gene enrichment and the right represents down-regulation. \* RNA polymerase II sequence-specific DNA-binding transcription factor binding. \*\* oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors.



**Figure 2**

Comparison of early brain protein expression profiles with mice. (A) Compared with the brain protein expression profiles of mice from the 9-day postnatal experiment from the Gonzalez-Lozano study (blue), it was found that expression of a total of 62 genes appeared in the differential gene results of Pura-KO (Orange). These 62 genes may be key regulators of Pura in early brain development. (B) Histogram results of KEGG enrichment analysis of differential genes. The abscissa is the number of genes and the ordinate is the enrichment item. (C) GO enrichment of differential genes. Orange indicates genes that are up-regulated and blue indicates down-regulation.



**Figure 3**

Distribution of differential genes in Alzheimer disease pathway. Through KEGG annotation analysis, we found 7 genes related to the Alzheimer disease pathway. There are 4 core factors in Alzheimer disease, which are APP, PSEN, ApoE, and Tau, shown in red font. The seven genes enriched were Lpl (LPL), Mapt



alpha may bind, and the logos are sorted in order. (D) The position and expression of the 47 genes obtained from A on the chromosome. The blue represents gene down-regulation and the red represents up-regulation.

## Supplementary Files

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