

## **Supplementary S1. Supplementary Note of Materials and Methods**

### **2.1 Acquisition MCI-related and T2DM-related gene expression data**

The selection criteria of microarray datasets were as follows: 1. It met the diagnostic criteria of MCI and T2DM, and one data set included the diseased and the normal control groups; 2. The patient is older than 60 years old; 3. Considering that blood samples are more readily available than other tissue samples in clinical work, sample type we chose as the blood sample; 4. The research target of the data set was total RNA; 5. The validation datasets were derived from the same platform, and the patient's gender and race were consistent. All the extracted molecules that we included in the datasets were total RNA. Total RNA was extracted using Trizol reagent (Invitrogen). Total RNA from nucleated blood cells was following lysis of erythrocytes and removal of cell debris.

### **2.2 Construction of weighted gene co-expression network analysis (WGCNA)**

Weighted gene co-expression network analysis (WGCNA) is a common method to construct gene co-expression networks. The WGCNA analysis method aims to find the jointly expressed gene modules (module), and to explore the association relationship between the gene networks and the phenotypes of interest, the core genes in the network, as well as the enrichment analysis for each module gene. WGCNA is divided into two parts: expression quantity cluster analysis and phenotype association, which mainly includes gene co-expression network construction, module identification, module information extraction, module and trait association, and the regulatory relationship of genes within modules.

### **2.6 Conduction of Receiver Operating Characteristic (ROC) Curves**

Receiver Operating Characteristic Curve Analysis (ROC) curves were mainly used for the prediction accuracy of the X-axis on the Y-axis. Initially, the ROC curve was used in the military, but now more often in the medical field, to determine whether a particular factor is of diagnostic value for diagnosing a specific disease. The ROC plots are the curves reflecting the relationship between sensitivity and specificity. The Area Under Curve (AUC) is the area under the ROC curve with values between 0.5 and 1. The closer the AUC is to 1.0, the higher the authenticity of the detection method, which is equal to 0.5, then the authenticity is the lowest, and there is no application value.

### **2.9 Validation of the expression levels of hub genes by qRT-PCR**

#### **2.9.1 Patient Data**

This study was approved by the Medical Ethics Committee of our hospital. Inclusion criteria included meeting the diagnostic criteria for mild cognitive dysfunction and the diagnostic criteria for T2DM proposed in the China Clinical Guidelines for the Prevention and Treatment of T2DM (2020 edition). Exclusion criteria include patients with previous mental disorders such as major depression, schizophrenia and delusion, cognitive disorders caused by material poisoning and systemic diseases, patients with the acute phase of major diseases such as acute and critical diseases, and patients with malignant tumors; patients with recent chronic inflammation, metabolic diseases (Type 1 diabetes, latent autoimmune diabetes in adults, diabetic ketoacidosis, hyperosmolarity syndrome, hyperuricemia, severe obesity, thyroid disease), acute diseases, colds and infectious diseases. The patients and their families gave informed consent to the experiment, and they all signed the informed consent form.

**Supplementary S2. upregulated and down-regulated co-DEGs**

| Gene ID   | Gene symbol | Gene name   | Type |
|-----------|-------------|---|------|
| 900       | CCNG1       | cyclin G1   | up   |
| 89845     | ABCC10      | ATP binding cassette subfamily C member 10                  | up   |
| 23310     | NCAPD3      | non-SMC condensin II complex subunit D3                     | up   |
| 57448     | BIRC6       | baculoviral IAP repeat containing 6                         | up   |
| 3043      | HBB         | hemoglobin subunit beta                                     | up   |
| 7374      | UNG         | uracil DNA glycosylase                                      | up   |
| 3078      | CFHR1       | complement factor H related 1                               | up   |
| 26039     | SS18L1      | SS18L1 subunit of BAF chromatin remodeling complex          | up   |
| 7342      | UBP1        | upstream binding protein 1                                  | up   |
| 134430    | WDR36       | WD repeat domain 36   | up   |
| 121536    | AEBP2       | AE binding protein 2  | up   |
| 853734    | RRN3        | rDNA-binding RNA polymerase I transcriptional factor        | up   |
| 851099    | SMC6        | DNA repair protein SMC6                                     | up   |
| 26476     | OR10J1      | olfactory receptor family 10 subfamily J member 1           | up   |
| 55278     | QRSL1       | glutaminyl-tRNA amidotransferase subunit QRSL1              | up   |
| 124808    | CCDC43      | coiled-coil domain containing 43                            | up   |
| 222484    | LNX2        | ligand of numb-protein X 2                                  | up   |
| 140707    | BRI3BP      | BRI3 binding protein  | up   |
| 157567    | ANKRD46     | ankyrin repeat domain 46                                    | up   |
| 138065    | RNF183      | ring finger protein 183                                     | up   |
| 109448382 | MPP5        | membrane palmitoylated protein 5                            | up   |
| 390058    | OR51B6      | olfactory receptor family 51 subfamily B member 6           | up   |
| 23089     | PEG10       | paternally expressed 10                                     | up   |
| 2833      | CXCR3       | C-X-C motif chemokine receptor 3                            | up   |
| 3667      | IRS1        | insulin receptor substrate 1                                | up   |
| 6671      | SP4         | Sp4 transcription factor                                    | up   |
| 6421      | SFPQ        | splicing factor proline and glutamine rich                  | up   |
| 178       | AGL         | amylo-alpha-1, 6-glucosidase,<br>4-alpha-glucanotransferase | up   |
| 6945      | MLX         | MAX dimerization protein MLX                                | down |
| 27429     | HTRA2       | HtrA serine peptidase 2                                     | down |
| 19164     | PSEN1       | presenilin 1  | down |
| 4828      | NMB         | neuromedin B  | down |
| 7307      | U2AF1       | U2 small nuclear RNA auxiliary factor 1                     | down |
| 6707      | SPRR3       | small proline rich protein 3                                | down |
| 853566    | VPS25       | ESCRT-II subunit protein VPS25                              | down |
| 107057624 | C19orf43    | chromosome 19 open reading frame 43                         | down |
| 6452      | SH3BP2      | SH3 domain binding protein 2                                | down |
| 9144      | SYNGR2      | synaptogyrin 2  | down |
| 10955     | SERINC3     | serine incorporator 3                                       | down |
| 51295     | ECSIT       | ECSIT signaling integrator                                  | down |

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|--------|-----------|--|------|
| 12922  | CRHR2     | corticotropin releasing hormone receptor 2             | down |
| 23263  | MCF2L     | MCF.2 cell line derived transforming sequence like     | down |
| 1584   | CYP11B1   | cytochrome P450 family 11 subfamily B member 1         | down |
| 26090  | ABHD12    | abhydrolase domain containing 12, lysophospholipase    | down |
| 83787  | ARMC10    | armadillo repeat containing 10                         | down |
| 285596 | FAM153A   | family with sequence similarity 153 member A           | down |
| 13447  | DOC2B     | double C2, beta  | down |
| 5982   | RFC2      | replication factor C subunit 2                         | down |
| 3038   | HAS3      | hyaluronan synthase 3                                  | down |
| 27433  | TOR2A     | torsin family 2 member A                               | down |
| 6354   | CCL7      | C-C motif chemokine ligand 7                           | down |
| 4146   | MATN1     | matrilin 1   | down |
| 10011  | SRA1      | steroid receptor RNA activator 1                       | down |
| 12295  | CACNB1    | calcium channel, voltage-dependent, beta 1 subunit     | down |
| 51330  | TNFRSF12A | TNF receptor superfamily member 12A                    | down |
| 84985  | FAM83A    | family with sequence similarity 83 member A            | down |
| 14536  | NR6A1     | nuclear receptor subfamily 6, group A, member 1        | down |
| 148066 | ZNRF4     | zinc and ring finger 4                                 | down |
| 335524 | ODF3B     | outer dense fiber of sperm tails 3B                    | down |
| 3914   | LAMB3     | laminin subunit beta 3                                 | down |
| 18828  | PLSCR2    | phospholipid scramblase 2                              | down |
| 11012  | KLK11     | kallikrein related peptidase 11                        | down |
| 91977  | MYOZ3     | myozenin 3   | down |
| 26353  | HSPB8     | heat shock protein family B (small) member 8           | down |
| 79734  | KCTD17    | potassium channel tetramerization domain containing 17 | down |
| 10555  | AGPAT2    | 1-acylglycerol-3-phosphate O-acyltransferase 2         | down |
| 23743  | BHMT2     | betaine--homocysteine S-methyltransferase              | down |
| 7189   | TRAF6     | TNF receptor associated factor 6                       | down |
| 9362   | CPNE6     | copine 6   | down |
| 5545   | PRB4      | proline rich protein BstNI subfamily 4                 | down |
| 1544   | CYP1A2    | cytochrome P450 family 1 subfamily A member 2          | down |
| 163126 | EID2      | EP300 interacting inhibitor of differentiation 2       | down |
| 7018   | TF        | transferrin  | down |
| 12180  | SMYD1     | SET and MYND domain containing 1                       | down |
| 217    | ALDH2     | aldehyde dehydrogenase 2 family member                 | down |
| 18609  | PDX1      | pancreatic and duodenal homeobox 1                     | down |
| 26610  | ELP4      | elongator acetyltransferase complex subunit 4          | down |
| 69539  | TRNP1     | TMF1-regulated nuclear protein 1                       | down |
| 5331   | PLCB3     | phospholipase C beta 3                                 | down |
| 11610  | AGTRAP    | angiotensin II, type I receptor-associated protein     | down |
| 80774  | LIMD2     | LIM domain containing 2                                | down |
| 57474  | ZNF490    | zinc finger protein 490                                | down |
| 9758   | FRMPD4    | FERM and PDZ domain containing 4                       | down |
| 70237  | BHLHB9    | basic helix-loop-helix domain containing, class B9     | down |

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|--------|-----------|---|------|
| 388407 | C17orf82  | long intergenic non-protein coding RNA 2875                 | down |
| 3626   | INHBC     | inhibin subunit beta C                                      | down |
| 335    | APOA1     | apolipoprotein A1   | down |
| 5499   | PPP1CA    | protein phosphatase 1 catalytic subunit alpha               | down |
| 54828  | BCAS3     | BCAS3microtubule associated cell migration factor           | down |
| 283579 | C14orf178 | chromosome 14 open reading frame 178                        | down |
| 11945  | ATP4B     | ATPase, H+/K+ exchanging, beta polypeptide                  | down |
| 245    | ALOX12P2  | arachidonate 12-lipoxygenase pseudogene 2                   | down |
| 79946  | C10orf95  | chromosome 10 open reading frame 95                         | down |
| 91661  | ZNF765    | zinc finger protein 765                                     | down |
| 4852   | NPY       | neuropeptide Y  | down |
| 854376 | MRM1      | Mrm1p   | down |
| 4968   | OGG1      | 8-oxoguanine DNA glycosylase                                | down |
| 65268  | WNK2      | WNK lysine deficient protein kinase 2                       | down |
| 164592 | CCDC116   | coiled-coil domain containing 116                           | down |
| 10095  | ARPC1B    | actin related protein 2/3 complex subunit 1B                | down |
| 54209  | TREM2     | triggering receptor expressed on myeloid cells 2            | down |
| 7040   | TGFB1     | transforming growth factor beta 1                           | down |
| 185    | AGTR1     | angiotensin II receptor type 1                              | down |
| 84167  | C19orf44  | chromosome 19 open reading frame 44                         | down |
| 9462   | RASAL2    | RAS protein activator like 2                                | down |
| 401565 | FAM166A   | family with sequence similarity 166 member A                | down |
| 60495  | HPSE2     | heparanase 2 (inactive)                                     | down |
| 22905  | TRAK1     | trafficking kinesin protein 1                               | down |
| 9088   | PKMYT1    | protein kinase, membrane associated tyrosine/threonine<br>1 | down |
| 237987 | OTOP2     | otopetrin 2   | down |
| 255426 | RASGEF1C  | RasGEF domain family member 1C                              | down |
| 284379 | LOC284379 | solute carrier family 7 member 3 pseudogene                 | down |
| 19218  | PTGER3    | prostaglandin E receptor 3 (subtype EP3)                    | down |
| 84735  | CNDP1     | carnosine dipeptidase 1                                     | down |
| 5676   | PSG7      | pregnancy specific beta-1-glycoprotein 7                    | down |
| 4046   | LSP1      | lymphocyte specific protein 1                               | down |
| 10602  | CDC42EP3  | CDC42 effector protein 3                                    | down |
| 79758  | DHRS12    | dehydrogenase/reductase 12                                  | down |

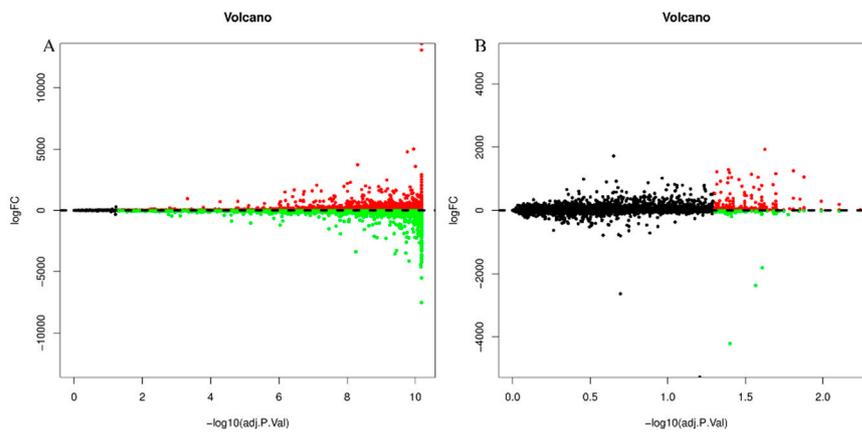
**Table S1. Primers used for quantitative PCR**

| gene  | Primer sequences                     |
|-------|--------------------------------------|
| LNX2  | Forward primer: CCCAGCCTGGTAACACCATT |
|       | Reverse primer: AACGGTCATGCCAAGGGATT |
| BIRC6 | Forward primer: CTGTGAGTTCCTTCGGGGTT |
|       | Reverse primer: TCTCTTGCAGCACCTGTAGC |

|        |  |
|--------|--|
| ANKRD4 | Forward primer: CGTTCGAGTCGCAGATCCTT     |
| 6      | Reverse primer: TGGGTCAAAGCCACTTTCCA     |
| IRS1   | Forward primer: AGAGGACCGTCAGTAGCTCA     |
|        | Reverse primer: ACTGAAATGGATGCATCGTACC   |
| TGFB1  | Forward primer: GGAAATTGAGGGCTTTCGCC     |
|        | Reverse primer: GAGGTCCTTGCGGAAGTCAA     |
| APOA1  | Forward primer: AGAGACTGCGAGAAGGAGGT     |
|        | Reverse primer: TCTCTGCCGCTGTCTTTGAG     |
| PSEN1  | Forward primer: GGAAGCGTATACCTAATCTGGGAG |
|        | Reverse primer: ACGTACAGTATTGCTCAGGTGG   |
| NPY    | Forward primer: CGCTGCGACACTACATCAAC     |
|        | Reverse primer: AGGCCAGAGAGCAAGTCTCA     |
| ALDH2  | Forward primer: GGAAGATGTGGACAAGGCAGT    |
|        | Reverse primer: GGGATGGTTTTCCCGTGGTA     |

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**Figure S1.** Volcano maps of (A) AD; (B) T2DM



**Figure S2.** ROC curve of co-DEGs in MCI, T2DM. (A) LNX2 in MCI; (B) BIRC6 in MCI; (C) ANKRD46 in MCI; (D) IRS1 in MCI; (E) TGFB1 in MCI; (F) APOA1 in MCI; (G) PSEN1 in MCI; (H) NPY in MCI; (I) ALDH2 in MCI; (J) LNX2 in T2DM; (K) BIRC6 in T2DM; (L) ANKRD46 in T2DM; (M) IRS1 in T2DM; (N) TGFB1 in T2DM; (O) APOA1 in T2DM; (P) PSEN1 in T2DM; (Q) NPY in T2DM; (R) ALDH2 in T2DM.

