

# Potential Molecular Mechanism of the NPPB Gene in Post-Ischemic Heart Failure with and without T2DM

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## Research

**Keywords:** Co-expression genes, NPPB, Heart failure, Diabetes mellitus, Microarray dataset

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## Abstract

**Background:** This study aimed to investigate natriuretic peptide B (NPPB) co-expression genes and the pathways involved in post-ischemic heart failure (HF) among patients both with and without type 2 diabetes mellitus (T2DM).

**Methods:** The microarray dataset of GSE26887 was examined to detect the genes that co-expressed with NPPB from 19 post-ischemic HF patients' peripheral blood samples (7 with T2DM and 12 without T2DM). NPPB co-expression genes were then screened using the R packet. Further, using online analytical tools, we determined the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, Gene Ontology (GO) annotation, and protein-protein interaction (PPI) network of the co-expression genes. The modules and hub genes of the PPI network were then identified using the Cytoscape software.

**Results:** In patients with T2DM, a total of 41 biological processes (BP), 20 cellular components (CC), 13 molecular functions (MF), and 41 pathways were identified. Further, a total of 61 BPs, 16 CCs, 13 MFs, and 22 pathways in patients without T2DM were identified. In both groups of patients, 17 BPs, 10 CCs, 6 MFs, and 13 pathways were enriched. We also identified 173 intersectional co-expression genes (63 positive, 106 negative, and 4 differently co-expressed in patients with and without T2DM, respectively) in both types of patients, which enriched in 16 BPs, 8 CCs, 3 MFs, and 8 KEGG pathways. Moreover, the PPI network (contained 237 edges and 170 nodes) with the top module significantly enriched in 4 BPs (the tricarboxylic acid metabolic process, citrate metabolic process, tricarboxylic acid cycle, and aerobic respiration) and 3 pathways (the citrate cycle, malaria parasite metabolic pathway, and AGE-RAGE signaling pathway in diabetic complications) was constructed. *DEC1*, *BGN*, *TIMP1*, *VCAN* and *CTCF* are the top hub genes.

**Conclusions:** This study used genome-wide co-expression genes to identify the potential functions and mechanisms of the NPPB gene in post-ischemic HF with and without T2DM. Our findings may elucidate the functions and roles of NPPB in patients with post-ischemic HF and facilitate HF management. **Key words:** Co-expression genes, NPPB, Heart failure, Diabetes mellitus, Microarray dataset

## Background

Heart failure (HF) is a challenge for numerous cardiovascular specialists, as it affects both the health and quality of life of a tremendous number of patients. It is estimated that 26 million people worldwide suffer from HF, according to data from a prior survey [1]. Moreover, annual costs to treat and manage HF ranges from International Dollars (Int\$) 2,496.00 to Int\$ 84,434.00 per patient [2]. However, it is estimated that the in-hospital mortality ranges from 4–30%, and that the all-cause 1-year mortality rates among patients with acute HF and patients with chronic HF were 23.6% and 6.4%, respectively [3]. The etiology of heart failure involves coronary artery disease, rheumatic heart disease, cardiomyopathies, hyperthyroidism, and so on. Among these diseases, ischemic heart failure is common, especially when caused by ST-segment elevation myocardial infarction [4]. Although the numbers of chest-pain centers and cardiac care units (CCUs) have been increasing and thus more patients have received timely and effective interference, post-ischemic HF remains a challenge that cannot be neglected any further.

Type 2 diabetes mellitus (T2DM), which is an endocrine disease that mainly leads to vascular and nerve damage, is regarded as an equal-risk syndrome of coronary heart disease and accompanies patients for the rest of their lives [5]. It is reported that T2DM not only promotes the development of HF, but also increases the risk of cardiovascular disease (CVD) at 2–4 times level [6, 7]. It is estimated that more than 400 million persons are affected by T2DM worldwide, costing \$1.3 trillion annually [8]. In addition, coexistence of HF and T2DM is common, and in the populations range from 33 years to 84 years, the prevalence of HF in people with T2DM was 12% [9]. Moreover, T2DM and HF is mutual promotion to the development of each other, and HF patients with T2DM are more complex to treat [10].

Currently, diagnosis of HF is mainly based on clinical manifestations. Fortunately, serum BNP (encoded by the *NPPB* gene) and NT-proBNP levels have greatly contributed to the proper diagnosis of HF [11]. BNP is mainly secreted by atrial myocytes, and thus reflects the heart load. BNP can represent powerful biological effects, such as natriuresis, vasodilation, myocardial apoptosis inhibition, and modulation of immune and inflammatory responses of cardiac injury [12–14]. Some prior studies suggest that BNP can be used as a biomarker for prognosis in patients with HF [15], and it also participates in both occurrence and development of T2DM and ischemic cardiomyopathy [16]. What's more, in diabetic patients, BNP can be used for screening the absence of left ventricular dysfunction [17]. Therefore, BNP is associated with HF as well as T2DM. However, results from early researches show us that serum BNP levels are higher in the HF patients with diabetes than in HF patients without diabetes, while some others reports the opposite result [18, 19]. It is not yet clear that whether there are some shared and specific mechanisms of the *NPPB* gene in HF patients with and without T2DM is not yet clear.

In recent years, microarray sequencing technology has rapidly developed and has significantly assisted basic and clinical medicine. The Gene Expression Omnibus (GEO) database is a huge repository that stores a series of high-throughput microarray and next-generation sequence functional genomic datasets and is free for global researchers to use for mining purposes [20]. In this study, we aimed to further understand the function of *NPPB* in HF patients and better inform HF management by detecting the *NPPB* co-expression genes and pathways enriched in patients with post-ischemic HF either with or without T2DM.

## Methods

### Affymetrix Microarray Data

The microarray dataset GSE26887 was retrieved from the GEO database. This dataset contained 24 samples from 19 patients with post-ischemic heart failure (7 with T2DM and 12 without T2DM) and 5 from control non-failing hearts [21]. We recruited patients with post-ischemic heart failure either with T2DM (DHF group,  $n = 7$ ) or without T2DM (nDHF group,  $n = 12$ ) for analysis. The extracted data were normalized before further analysis in order to ensure the comparability of samples by the *limma* package that is available in the R platform [22] (Fig. 1).

## Identification of NPPB co-expression genes

A screening of co-expression genes for *NPPB* from the samples was performed by the `cor` function in R (version 3.6.1). Screening criteria were as follows:  $P < 0.05$ , and  $|\text{Pearson correlation coefficient}| \geq 0.4$ . The online analytical tool Draw Venn Diagram (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) was then used to determine the intersectional co-expression genes of both groups.

## Go And Kegg Pathway Enrichment Analyses

The online database DAVID (version 6.8) [23] was used for GO and KEGG enrichment analyses [24, 25].  $P$ -value of  $< 0.05$  was set as significance. The `ggplot2` package was used for visualization of the results in R (version 3.6.1).

## Integration Of The Ppi Network

The STRING (version 10.5) database was used for evaluating the interactions among the co-expression genes, and a combined interaction score of  $> 0.4$  was set as significant [26]. In addition, the top 10 hub genes were identified used Cytoscape plugin cytoHubba (version 0.1) with the degree ratio ranking method. Further, the MCODE and ClueGO apps in Cytoscape were used to identify the modules, namely the GO annotation and KEGG pathway enrichment analyses, respectively, of the PPI network [27].

## Results

### Identification of NPPB co-expression genes

A total of 577 negatively co-expressed genes and 457 positively co-expressed genes in the DHF group were identified, along with 666 negatively co-expressed genes and 422 positively co-expressed in the nDHF group. Figure 2 portrays 106 negatively and 63 positively co-expressed genes in both patient types, whereby 173 intersection co-expression genes were screened out. Interestingly, of these intersection co-expression genes, we found 3 genes (*CENPBD1P1*, *KHDRBS3*, and *PHOX2B*) that were positively co-expressed with *NPPB* in patients with T2DM, but negatively co-expressed in patients without T2DM, and 1 gene (*NQO1*) that was negatively co-expressed with *NPPB* in patients with T2DM, but positively co-expressed in patients without T2DM.

## Functional Go And Kegg Pathway Enrichment Analyses

GO analyses revealed 41 BPs, 20 CCs, and 13 MFs in the DHF group, and 61 BPs, 16 CCs, and 13 MFs in the nDHF group (details in Tables S1-S2). Due to the excessive number of enrichment analyses, the top seven BPs, CCs, and MFs were selected for visualization with  $P < 0.05$  (Fig. 3A and Fig. 3C). Further, there were 10 BPs (fatty acid beta-oxidation, oxidation-reduction process, metabolic process, mitochondrial respiratory chain complex I assembly, glyoxylate metabolic process, ubiquinone biosynthetic process, positive regulation of cell growth, tricarboxylic acid cycle, cell adhesion, and aerobic respiration), 8 CCs (mitochondrial inner membrane, extracellular space, mitochondrion, extracellular matrix, myelin sheath, extracellular exosome, Z disc, and mitochondrial matrix), and 3 MFs (growth factor activity, protein binding, and electron carrier activity) enriched in both patient groups. There were 41 identified pathways in patients with T2DM (Fig. 3B) and 22 in patients without T2DM (Fig. 3D) (details in Table S1-S2). Moreover, common pathways are shown in Table 1.

The share pathways enriched by the *NPPB*

| Pathway                     | DHF      |          |                                                                                                                                                                                      |
|-----------------------------|----------|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                             | Count(%) | P value  | Gene                                                                                                                                                                                 |
| Biosynthesis of antibiotics | 33(2.30) | 6.34E-07 | BCAT1/LDHB/EHHADH/ALDOC/OGDHL/P GAM2/ECHS1/OGDH/ACAT1/PDHB/CMBL/GOT2/GOT1/IDH3G/ATIC/RGN/PDHA1/NSDHL/DLST/ACO2/CS/IDH3B/PFKM/PYCR1/G6PD/HMGCS2/SDHC/DLD/PCYOX1/PCCB/MDH2/PRPS2/ACAA1 |

|                    |           |          |                                                                                                                                                                                                                            |
|--------------------|-----------|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Metabolic pathways | 116(8.07) | 3.08E-08 | LDHB/EHHADH/NDUFAB1/P GAM2/OGDH/PDHB/HIBADH/MTHFD1L/CMBL/GOT2/GOT1/IDH3G/ST3GAL5/ST3GAL4/RGN/PDHA1/BST1/LPCAT4/CD38/PYCR1/NNT/DLD/PCCB/PRPS2/MDH2/ACAA1/ACADSB/ME3/ALDOC/ACSBG2/ACAT1/B4GALT6/UPB1/MAOB/SPHK1/ACMSD/IDH3B/ |
|--------------------|-----------|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

| Pathway                                    | DHF      |          | P value | Gene List                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|--------------------------------------------|----------|----------|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                            | Count(%) |          |         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                                            |          |          |         | ACACB/NDUFV3/GGT5/HMGCS2/PLCG2/COX6A1/BCO1/PHYKPL/UQCRC2/ETNPPL/COX11/COX10/OGDHL/ACOT2/QARS/MTHFD1/MCCC2/ALAS1/NDUFS4/PLCB4/XYL11/MCEE/MCCC1/PIGC/AGPAT4/PTDSS1/NDUFS3/NDUFS2/NDUFS1/HYAL1/ACO2/ALDH5A1/NADSYN1/COX4I1/RIGS/PFKM/NDUFA10/PIGO/COQ6/ACADVL/COQ2/G6PD/CKM/PRDX6/PANK1/INPP4A/BCAT1/NDUFB6/ALDH18A1/NDUFB8/NDUFB9/NFS1/ECHS1/COMT/AZIN2/TYMS/ATIC/DHCR7/ALDH1A3/CKMT2/CYP26B1/GALNT16/BDH1/AGL/INPP5A/NSDHL/DLST/NDUFA2/NDUFA8/NDUFA9/NDUFA6/CS/MPI/PYGM/SDHC/AKR1B1/LIPG/DPM2/SCP2 |
| Citrate cycle (TCA cycle)                  | 12(0.83) | 5.08E-07 |         | DLST/IDH3G/ACO2/SDHC/DLD/OGDHL/CS/IDH3B/PDHA1/OGDH/MDH2/PDHB                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Malaria                                    | 10(0.70) | 1.14E-03 |         | VCAM1/CCL2/COMP/TGFB3/THBS1/THBS2/IL10/TGFB1/SDC2/TGFB2                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Valine, leucine and isoleucine degradation | 14(0.97) | 1.89E-06 |         | BCAT1/ACADSB/EHHADH/ECHS1/ACAT1/HIBADH/MCCC2/HMGCS2/MCCC1/MCEE/DLD/AACS/PCCB/ACAA1                                                                                                                                                                                                                                                                                                                                                                                                                |
| Butanoate metabolism                       | 7(0.49)  | 4.12E-03 |         | HMGCS2/ALDH5A1/EHHADH/ECHS1/AACS/BDH1/ACAT1                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Carbon metabolism                          | 26(1.81) | 5.38E-09 |         | ME3/EHHADH/ALDOC/OGDHL/ECHS1/PGAM2/OGDH/ACAT1/PDHB/GOT2/IDH3G/GOT1/MCEE/RGN/PDHA1/DLST/ACO2/CS/IDH3B/PFKM/G6PD/SDHC/DLD/PCCB/MDH2/PRPS2                                                                                                                                                                                                                                                                                                                                                           |
| HIF-1 signaling pathway                    | 14(0.97) | 3.89E-03 |         | CAMK2G/RPS6/PDHB/TIMP1/RBX1/CDKN1A/PLCG2/SERPINE1/TEK/PDHA1/PIK3R3/EGF/NPPA/AKT2                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Pyruvate metabolism                        | 9(0.63)  | 1.96E-03 |         | LDHB/ME3/DLD/LDHD/PDHA1/ACACB/ACAT1/MDH2/PDHB                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| TGF-beta signaling pathway                 | 11(0.77) | 2.50E-02 |         | LTBP1/GDF6/TGFB1/TGFB3/ID4/BMPR1B/THBS1/TGFB1/TGFB2/BMP8A/RBX1                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Calcium signaling pathway                  | 18(1.25) | 3.40E-02 |         | ORAI2/ADORA2B/ERBB4/CAMK2G/SPHK1/HTR4/PTGFR/VDAC3/VDAC1/CD38/PLCB4/ATP2A2/P2RX1/PDE1C/P2RX3/PLCG2/RYR2/F2R                                                                                                                                                                                                                                                                                                                                                                                        |
| Fatty acid degradation                     | 9(0.63)  | 2.71E-03 |         | CPT1C/ACADVL/ECI2/ACADSB/EHHADH/ACSBG2/ECHS1/ACAT1/ACAA1                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| 2-Oxocarboxylic acid metabolism            | 7(0.49)  | 2.85E-04 |         | GOT2/BCAT1/GOT1/IDH3G/ACO2/CS/IDH3B                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

The analyses further identified 16 BPs, 8 CCs, and 3 MFs that were enriched by intersectional co-expression genes in both patient groups (Fig. 3E), and these genes mainly clustered in the following 8 pathways: the citrate cycle (TCA cycle), carbon metabolism, biosynthesis of antibiotics, malaria, glyoxylate metabolism, dicarboxylate metabolism, cardiac muscle contraction, and African trypanosomiasis (Fig. 3F) (details in Table S3).

## Ppi Network Construction And Hub Gene Identification

As Fig. 4 shows, the interactions among intersectional co-expression genes were displayed by a PPI network with 273 edges and 170 nodes. This finding was saved in TSV format and then imported into Cytoscape for visualization. With a cutoff criterion of a degree that is > 5 and a K-core > 5, only one module with 4 BPs (the tricarboxylic acid metabolic process, citrate metabolic process, tricarboxylic acid cycle, and aerobic respiration) and 3 pathways (citrate cycle, malaria parasite metabolic pathway, and AGE-RAGE signaling pathway in diabetic complications) significantly enriched in was identified. With the degree ratio ranking method, the top 10 hub genes of this PPI network were also identified (*CS*, *DEC1*, *ACO2*, *BGN*, *TIMP1*, *CTGF*, *VCAN*, *SERPINE1*, *SDHC*, and *CCL2*). With the same cutoff criterion, a PPI network that consists of 953 nodes and 4,946 edges of *NPPB* co-expression genes in the DHF group, and a PPI network of 1,009 nodes and 4,245 edges in the nDHF group were also constructed. The top 10 hub genes of the former were *CYCS*, *FN1*, *CS*, *DEC1*, *ACO2*, *ATP5A1*, *NDUFAB1*, *EGF*, *ATP5H*, and *ATP5C1*, while the later were *CS*, *DEC1*, *BGN*, *TIMP1*, *ACO2*, *CTGF*, *VCAN*, *SERPINE1*, *CCL2*, and *SDHC* (Figs. 5 and 6, respectively).

## Verification Of Hub Genes

Another dataset GSE5406 was downloaded from GEO database to verify the hub genes. We selected the heart failure with advanced ischemic cardiomyopathy for *NPPB* co-expression gene analysis, with the same method as above. The correlation value of *NPPB* in the GSE5406 dataset and GSE26887 dataset are shown in Table 2. Expect for *CCL2*, other hub genes are co-expressed to *NPPB* with  $P < 0.05$ , and | Pearson correlation coefficient | > 0.2. Both the positive co-expressed relationship and negative co-expressed relationship are correspondence.

Table 2  
Verification of hub genes.

| Hub gene                             | Cor     | P      | Cor          | P      | Cor           | P      |
|--------------------------------------|---------|--------|--------------|--------|---------------|--------|
|                                      | GSE5406 |        | GSE26887 DHF |        | GSE26887 nDHF |        |
| <i>DECRT1</i>                        | -0.48   | < 0.01 | -0.83        | 0.02   | -0.74         | < 0.01 |
| <i>BGN</i>                           | 0.51    | < 0.01 | 0.87         | 0.01   | 0.59          | 0.04   |
| <i>TIMP1</i>                         | 0.44    | < 0.01 | 0.89         | < 0.01 | 0.76          | < 0.01 |
| <i>VCAN</i>                          | 0.50    | < 0.01 | 0.81         | 0.03   | 0.61          | 0.04   |
| <i>CTCF</i>                          | 0.50    | < 0.01 | 0.95         | < 0.01 | 0.79          | < 0.01 |
| <i>CS</i>                            | -0.35   | < 0.01 | -0.84        | 0.02   | -0.60         | 0.04   |
| <i>ACO2</i>                          | -0.34   | < 0.01 | -0.86        | 0.01   | -0.62         | 0.03   |
| <i>SERPINE1</i>                      | 0.38    | < 0.01 | 0.89         | < 0.01 | 0.68          | 0.01   |
| <i>SDHC</i>                          | -0.21   | 0.03   | -0.92        | < 0.01 | -0.64         | 0.03   |
| <i>CCL2</i>                          | 0.08    | 0.39   | 0.85         | 0.02   | 0.69          | 0.01   |
| Cor, Pearson correlation coefficient |         |        |              |        |               |        |

## Discussion

Although living and medical standards have undergone remarkable progress, heart failure remains a worldwide challenge, which costs countries a tremendous amount of money and affects the quality of life for patients at different degrees. Ischemic cardiomyopathy is one of the most common causes of heart failure; moreover, a portion of these patients also suffer from other diseases, such as type 2 diabetes mellitus, which complicates the treatment interventions for heart failure. Angiotensin converting enzyme inhibitors, beta-blockers, diuretics, positive inotropic drugs, and cardiac resynchronization therapy (CRT) have been widely used in post-ischemic heart failure therapy, but quite a few patients inevitably go into end-stage heart failure for a variety of reasons [8]. Thus, they experience repeated hospitalizations, a severe decline in quality of life, complications in other organs, and even death. Serum BNP, encoded by *NPPB*, is secreted primarily by atria muscle cells and increases when the heart is overloaded. It has been applied in clinics as a diagnostic and prognostic biomarker of HF for a long time, which is a great achievement [28]. Besides, BNP is also reported associated with the development of T2DM, and in turn, diabetes affected its expression in patients with HF. Some early researches reveal that the serum BNP level in HF patients without diabetes is higher than in the one with diabetes, while the opposite reports. Up to now, the mechanism is still completely clear. In this study, *NPPB* co-expression genes and their GO and KEGG pathways were identified in post-ischemic HF with T2DM and without T2DM, respectively, in order to further understand the potential mechanism of *NPPB* in post-ischemic HF patients with and without T2DM.

Heart failure is the result of the contradiction between the supply and demand of oxygen, blood, and energy, and the tricarboxylic acid cycle (TCA cycle) and mitochondrial respiratory transport chain are important links in glycolysis. As screened by the Venn diagram, a total of 64 positively co-expression genes were identified. Carnitine palmitoyl transferase 1 (*CPT1*) encodes an important enzyme in the body, involved in fatty acid metabolism. As a subtype of *CPT1*, *CPT1C* can promote cell survival under metabolic stress conditions [29]. Further, HtrA serine peptidase 1 (*HTRA1*) encodes a protein that is suggested to be a cell growth regulator, and its loss impairs smooth muscle cell maturation [30]. In previous research, hypermethylation of *SOCS3* gene could be an underlying mechanism of intimal hyperplasia and restenosis. *SOCS3* can also regulate cavin-1 function by enhancing its stability and consequently maintaining expression levels of caveolin-1 and cell surface caveolae. Moreover, proteins encoded by cavin-1 are also believed to modify lipid metabolism and insulin-regulated gene expression [31, 32]. In terms of vascular function, *CCN1* not only functions as an inhibitory regulator of SMC muscle contractility through inhibiting actomyosin interactions but also regulates TNF- $\alpha$  induced vascular endothelial cell apoptosis [33]. The PDLIM7 gene product is involved in actin filament-associated complex assembly, which is essential for the transmission of ret/ptc2 mitogenic signaling. In addition, its expression is positively correlated to typical smooth muscle cell markers in atherosclerosis plaques, and PDLIM7 silencing in vitro led to downregulation of smooth muscle cell (SMC) markers, disruption of actin cytoskeleton, decreased cell spreading, and increased proliferation [34]. The data from Stine B Thomsen et al. suggested that, in patients with ischemic heart disease, increased plasma MGP levels are indicative of a progressing calcification process [35]. Moreover, protease-activated receptor 2 (*PAR2*) in microvascular endothelial cells is indispensable for vascular stability, and its deficiency attenuates atherosclerosis [36, 37]. The above-mentioned genes mainly play a role in energy supply and metabolism, cell proliferation and apoptosis, and vessel function and development, and have been reportedly associated with blood and oxygen supply and cardiac remodeling in patients with HF.

On the other hand, a Venn diagram allowed identifying 106 genes negatively co-expressed with *NPPB*. Coq8p and human COQ8A are related to CoQ biosynthesis and acute inhibition of Coq8p is sufficient to cause CoQ deficiency and respiratory dysfunction [38]. *NDUFS2* and *NDUFA9* encode compound I subunits in the mitochondrial membrane respiratory chain, while *SDHC* encodes compound II subunits. Also, *DECRT1* encodes an enzyme, referred to as NADPH, which provides H<sup>+</sup> ions for NAD<sup>+</sup> and then converts to NADH to participate in the respiratory chain. In addition to the respiratory chain, the TCA cycle also features several genes that are mainly active in its processes [39]. *PDHB* encodes a pyruvate dehydrogenase compound, which catalyzes the conversion of pyruvate into acetyl-coa and carbon dioxide for the TCA cycle. Citrate synthase, which is encoded by *CS*, catalyzes citric acid synthesis from oxaloacetic

acid and acetyl coa; further, citric acid synthesis by oxaloacetic acid and acetylcoa are catalyzed by cisaconitum, which is encoded by *ACO2*. *ALAS1* encodes mitochondrial enzymes that catalyze rate-limiting steps in the heme (iron protoporphyrin) biosynthesis pathway. In the context of cell proliferation and vascular function, Chen Yan reported that, in senescent vascular SMCs, *PDE1A* and *PDE1C* mRNA levels are significantly upregulated, and cellular senescent makers were reduced when *PDE1* was inhibited [40]. Data from Wilson LS et al. suggest that therapies specifically aimed at inhibiting the PDE3A isoform may lead to amelioration of excessive vascular SMC growth and decrease the atherosclerosis process [41]. Thus, the above-mentioned genes are mainly involved in the regulation of the tricarboxylic acid cycle and respiratory transport chain in terms of energy supply and maintain the normal function of vascular SMC. Finally, *CACNB2*, *KCNAB2*, and *TIMM22* encode subunits that participate in dysfunctional voltage-gated channels that may be associated with arrhythmia events rather than aggravated heart failure [42, 43]. Thus, these are factors that are associated with the development of heart failure.

In addition, Table 1 shows us the shared pathway that occurs in both post-ischemic HF with or without T2DM. Most of the pathways are related to metabolism, such as the following: the citrate cycle (TCA cycle); butanoate, carbon, pyruvate, and 2-oxocarboxylic acid metabolism; and valine, leucine, isoleucine, and fatty acid degradation. Figure 4 shows that it is similar to the pathways of the intersection of co-expression genes and the genes of the module that it is enriched in within the PPI network. Further, the HIF-1 signaling pathway is a hot topic that researchers focus on. In M1 macrophages, HIF-1 $\alpha$  activates the expression of the iNOS gene, increasing nitric oxide synthesis, which expands the blood vessels. As such, in hypoxia macrophages, the HIF-1 $\alpha$  - pyruvate dehydrogenase kinase (PDK1) axis can induce active glycolysis [44]. In addition, an investigation from Ya-Fang Chen *et al.* [45] suggests that HIF-1 $\alpha$  and FoxO3a show synergistic effects of cardiomyocyte apoptosis under hypoxia, as well as elevated glucose levels. Another pathway, the TGF- $\beta$  signaling pathway, is also a popular hot topic. TGF- $\beta$  is a multifunctional cytokine, which can regulate the macrophage phenotype, promote T<sub>reg</sub> cell activation, and reduce adhesion molecule synthesis by endothelial cells that lend a powerful anti-inflammatory effect [46]. Data from the study by Jooyeon Kim shows us that the TGF- $\beta$  signaling pathway plays an important role in the regulation of cardiac fibrosis [47]. Lastly, as a classical pathway, the calcium signaling pathway was also found in both the DHF and nDHF patient groups. Ca<sup>2+</sup> participates in excitation-contraction coupling, regulating myocardial contraction and diastole. In addition, it also takes part in the regulation of the cardiomyocyte action potential, which plays an essential role in managing heart rhythm [48, 49]. Thus, regulation disorders of the calcium signaling pathway will lead to heart rate disorders, myocardial contraction, and adrenal dysfunction. The above-mentioned pathways affect patients with post-ischemic heart failure in terms of energy supply, metabolism, inflammation, and myocardial fibrosis.

Compared to the HF patients without T2DM, the *NPPB* co-expression genes enriched in several other pathways, such as arrhythmogenic right ventricular cardiomyopathy (ARVC), dilated cardiomyopathy, hypertrophic cardiomyopathy (HCM), cardiac muscle contraction, alcoholism, PI3K-Akt signaling pathway and so on. The former three are different types of cardiomyopathy, mainly affect the morphology and function of ventricular muscle cells, result in deterioration of cardiac function [50]. Alcohol abuse may increase 2 times risk of chronic HF than the one who do not has alcohol abuse [51], and the BNP level would increase markedly [34]. In context to PI3K-Akt signaling pathway, it has been revealed involved in the expression level of BNP and the cardio-protection afforded by BNP infusion [52, 53]. Thus, these pathways and the genes enriched in would affect the level of BNP and the development of HF.

Although we use the micro-array dataset to help us identify the *NPPB* co-expression genes and pathways they enriched in in post-ischemic HF patients, either with T2DM or without T2DM, the occurrence and development of HF is complex, and variety aspects should be taken in consideration of the management of HF. We hope our finding could give a hand to a deeper understanding of the role and function of *NPPB* gene in HF patients and provide aspects for the research and management of HF in the future.

## Conclusions

The *NPPB* co-expression genes were used to identify the potential molecular mechanisms of the *NPPB* gene in DHF and nDHF patients in this study. Our findings may help elucidate the roles of *NPPB* and its co-expression genes in post-ischemic heart failure, and serve as a clinical reference for future HF management. However, the role of these co-expression genes and pathways remain required further researches to validate.

## Declarations

### Availability Of Data And Materials

The datasets used and/or analyzed during the current study are available from the Gene Expression Omnibus repository (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE26887>; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5406>).

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

Y.-Z.G. conceived the study, participated in the design, performed the statistical analyses, and drafted the manuscript. R.-X.Y. conceived the study, participated in the design and helped to draft the manuscript. G.-X.D. and P.-F.Z. contributed in formal analysis. C.-X.L. and B.-L.W helped to draft the manuscript. All authors read and approved the final manuscript.

### Acknowledgements

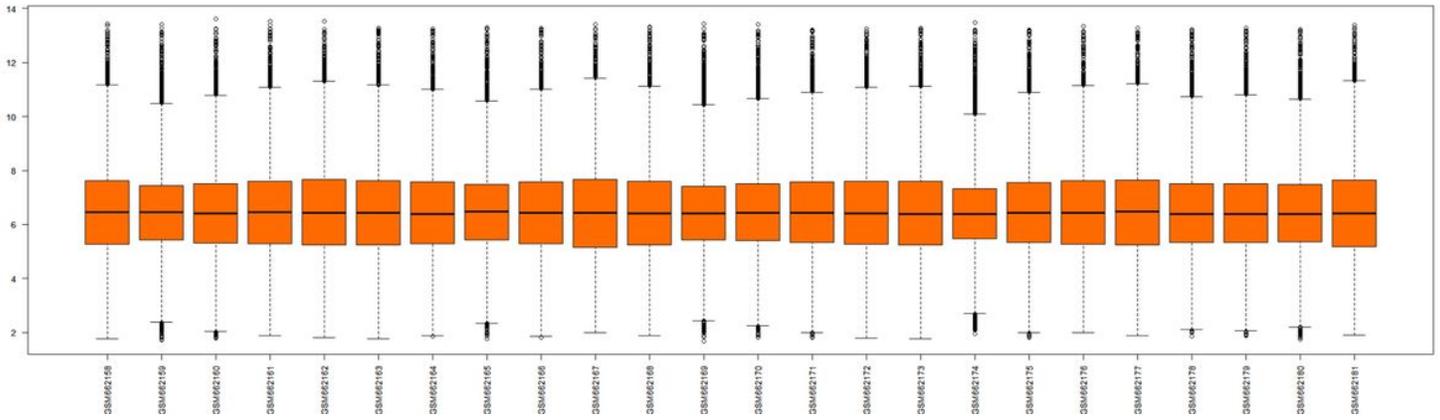
Not applicable.

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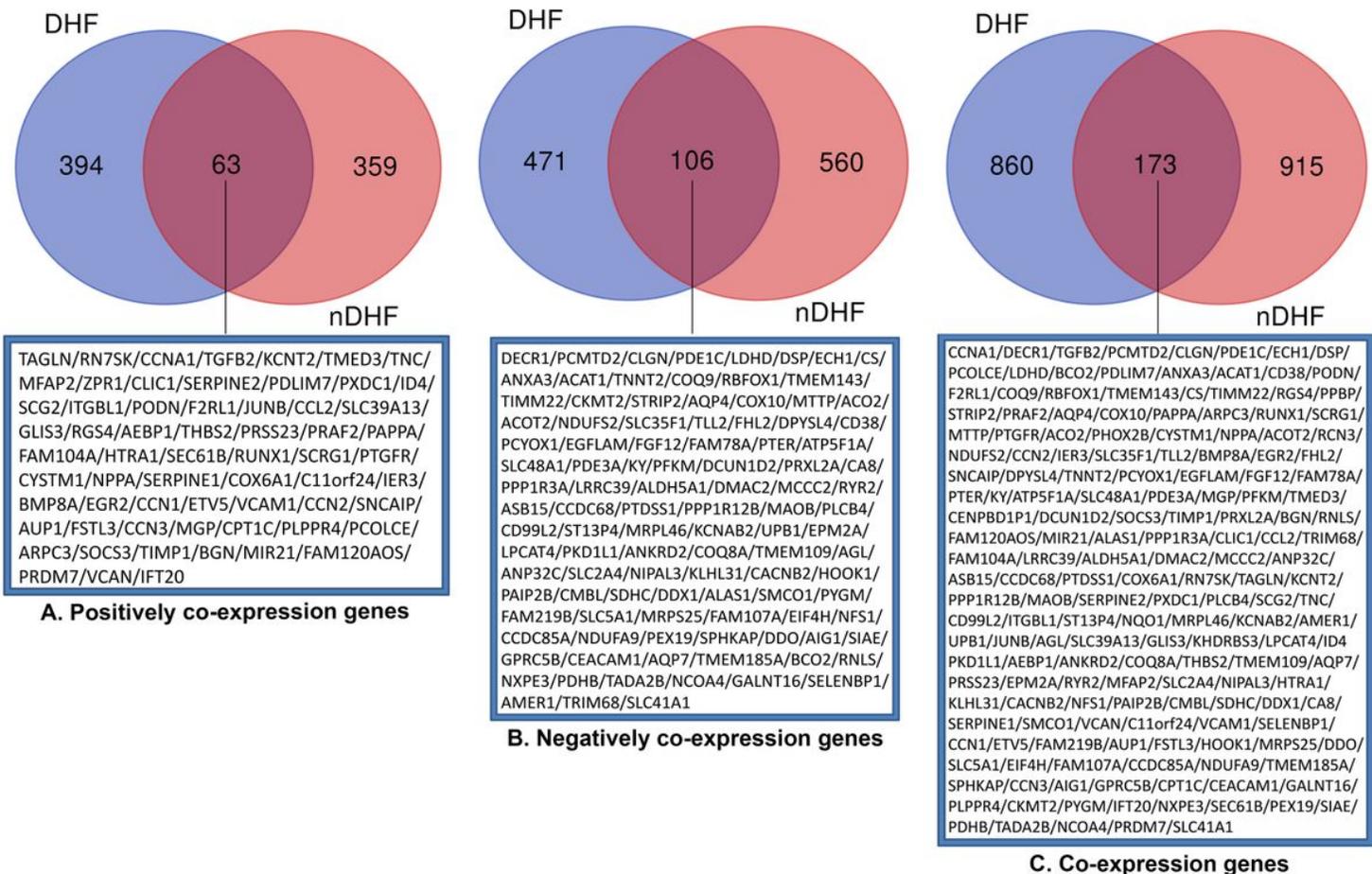
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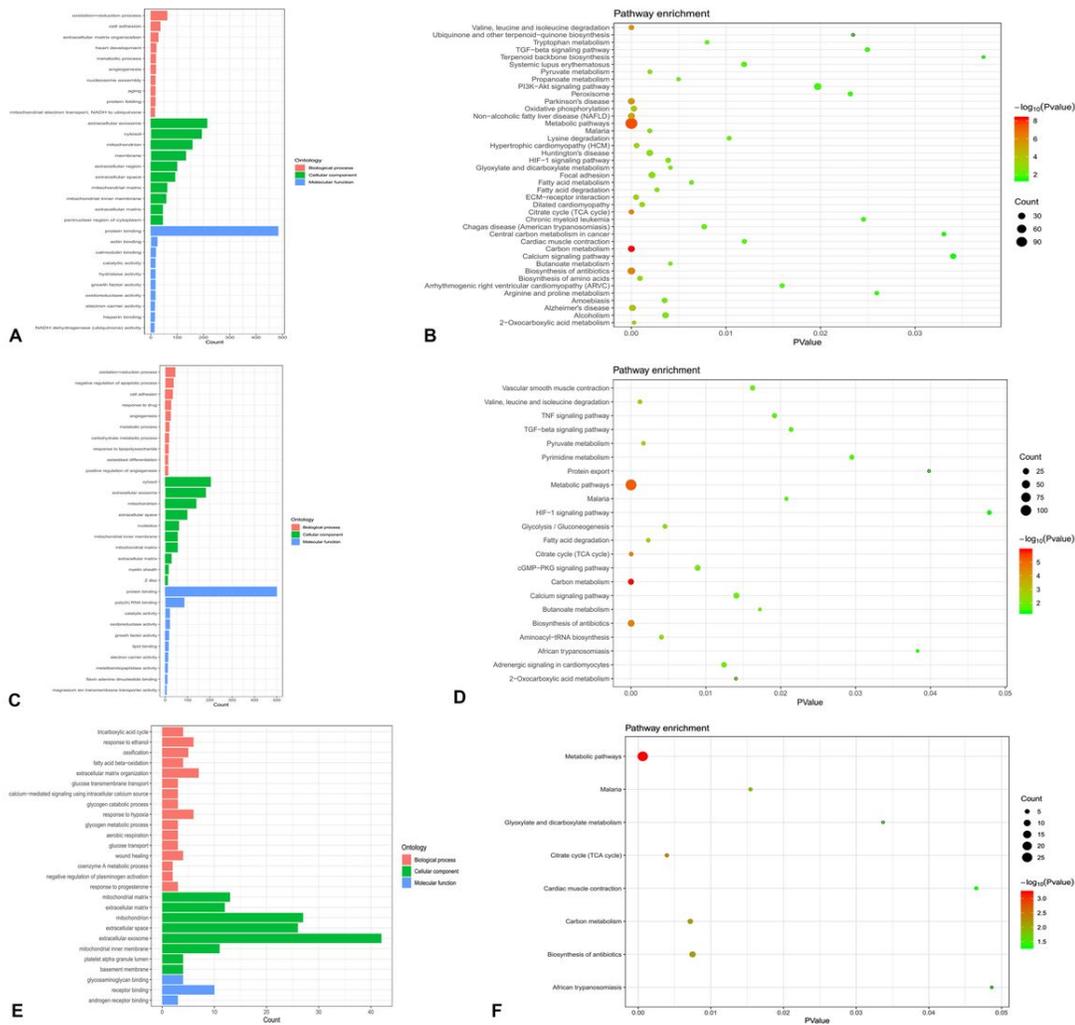
## Figures



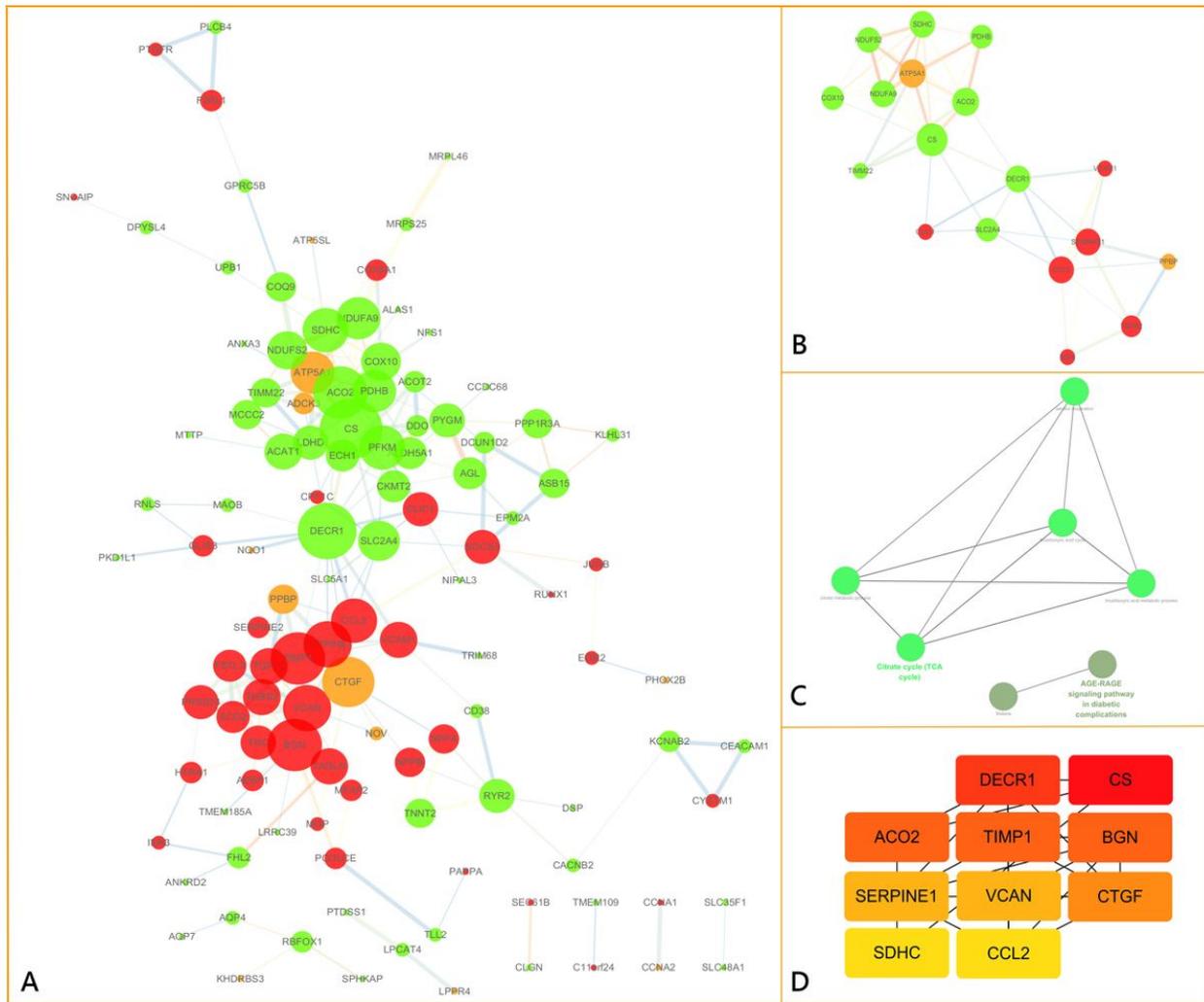
**Figure 1**  
 1 Normalization of gene expression. The orange box represents the expression of genes, and the black line in the box represents the median. The x-axis represents the sample name and the y-axis represents the expression level.



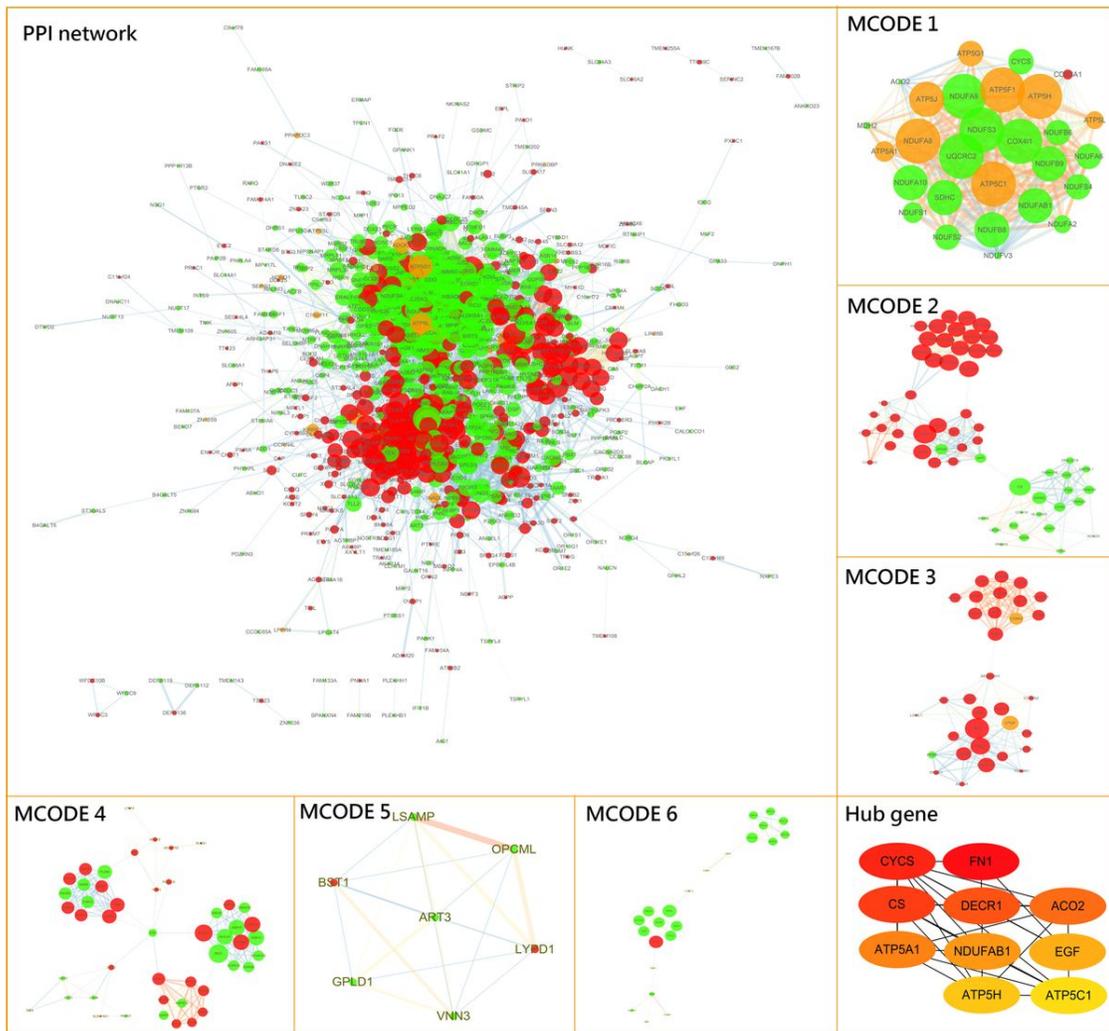
**Figure 2**  
 Venn diagram of co-expression genes. A. Venn diagram of NPPB positively co-expression genes. B. Venn diagram of negatively NPPB co-expression genes. C. Venn diagram of NPPB co-expression genes. DHF, post-ischemic patients with T2DM. nDHF, post-ischemic patients without T2DM



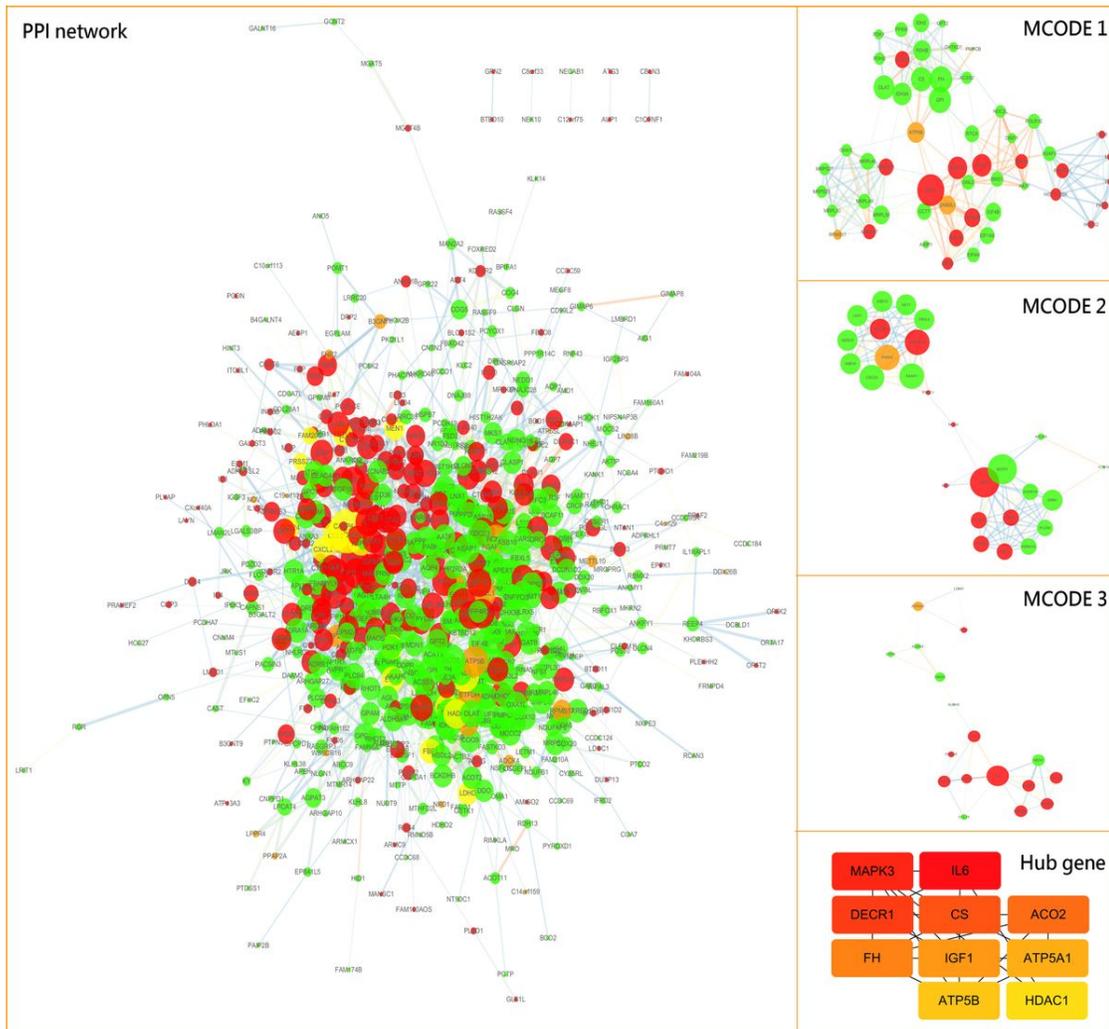
**Figure 3**  
 GO annotation and KEGG pathways of NPPB co-expression genes in post-ischemic heart failure patients. a-b. In post-ischemic patients with T2DM. c-d. In post-ischemic patients without T2DM. e-f. Based on the intersectional co-expression genes of the two type patients.



**Figure 4**  
 Protein-protein interaction (PPI) network of intersectional NPPB co-expression genes. a. PPI network based on the intersectional NPPB co-expression genes of two type patients. The red ball represents the positive co-expression while green ball represents the negative co-expression. The thickness of the line represents the strength of the correlation b. Module identified with a cutoff criterion of MCODE score  $\geq 5$ . c. Biological process and KEGG pathways t enriched in the module. d. Top 10 hub genes. The color depth represents the ranking of hub genes. The sequence of colors is red-orange-yellow from high ranking to low ranking.



**Figure 5**  
 Protein-protein interaction (PPI) network of NPPB co-expression genes in DHF group. The red ball represents the positive co-expression while green ball represents the negative co-expression. The thickness of the line represents the strength of the correlation. The modules were identified with a cutoff criterion of MCODE score  $\geq 5$ . In the box at the bottom right, the color depth represents the ranking of hub genes. The sequence of colors is red-orange-yellow from high ranking to low ranking.



**Figure 6**  
 Protein-protein interaction (PPI) network of NPPB co-expression genes in nDHF group. a. PPI network. The red ball represents the positive co-expression while green ball represents the negative co-expression. The thickness of the line represents the strength of the correlation. b. The modules were identified with a cutoff criterion of MCODE score  $\geq 5$ . In the box at the bottom right, the color depth represents the ranking of hub genes. The sequence of colors is red-orange-yellow from high ranking to low ranking.

## Supplementary Files

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