Label-free quantitative proteomics analysis

The proteins were extracted from heart tissues of mice by a mixed solution (4% sodium dodecyl sulphate, 100 mm Tris / HCl, 0.1 M DTT), and quantified by BCA method. Then, the filter aid preparation (FASP) method was used to hydrolyze an appropriate amount of protein by trypsin (Promega, USA).[20] The peptide was desalted on C18 cartridge, concentrated by vacuum centrifugation, redissolved with 40μl 0.1% formic acid solution, and quantified (od280).

LC-MS/MS analysis was performed on a Q exactive mass spectrometer (Thermo Scientific, USA) with Easy nLC (Proxeon Biosystems, Denmark). The detection mode was positive. The scanning range of precursor ion was 300-1800 m/Z, the measurement scanning was performed at 200m/z with resolution of 70,000, the automatic gain control target was 1e6, the maximum injection time was set to 50ms, and the dynamic exclusion time was 60s. The mass-to-charge ratios of polypeptide and polypeptide fragment were collected according to the following methods: 20 fragment maps (MS2 scan) were collected after each full scan, the MS2 activation type was HCD, the separation window was 2 m/Z, the resolution of secondary mass spectrometry was set to 17500 at 200 m/Z, the normalized collision energy was 30ev, and the underfill was defined as 1%.

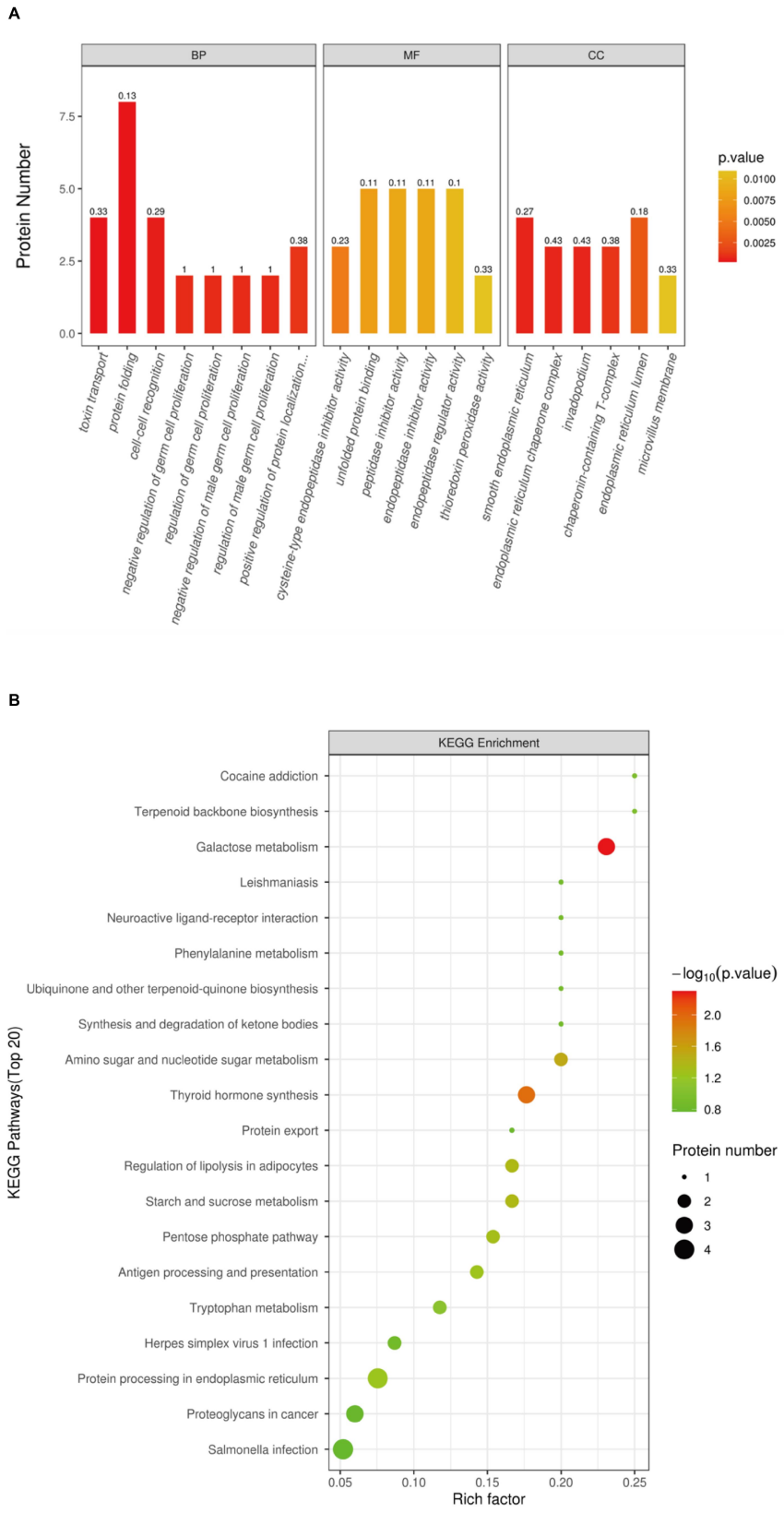
MaxQuant software (version No. 1.5.3.17) was used for identification and quantitative analysis. Differentially expressed proteins were annotated using blast2GO. Three ontologies (biological process, molecular function, and cellular component) of the identified proteins were further analyzed using information from Gene Ontology (GO) (http://www.geneontology.org/). Kaas (KEGG automatic annotation server) software was used to annotate the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway of the target protein set.

To further explore the influence of differentially expressed proteins on the physiological processes of cells and discover the internal connection among those differentially expressed proteins, Fisher's exact test was used to compare the GO enrichment or KEGG pathway with the entire quantitative protein annotation as the background data set. The significant change among the three groups was set as that the change trend of DCM group relative to the control group could be reversed by GLP-1RA treatment. A confidence value was set as $P \leq 0.05$.

Gene Ontology analysis and KEGG pathway of significant DEPs in myocardium of DCM mice after GLP-1RA treatment

Gene Ontology analysis was used to classify proteins according to their involvement in three main categories (biological process, molecular function, and cellular component). The top four biological processes identified were toxin transport, protein folding, cell-cell recognition and positive regulation of protein localization. The molecular function classification indicated that most of the differential proteins were associated with unfolded protein binding, peptidase inhibitor activity, endopeptidase inhibitor activity and endopeptidase regulator activity. The differential proteins in the main category of cellular component were mainly sub-categorized into smooth endoplasmic reticulum, endoplasmic reticulum chaperone complex, invadopodium, chaperonin-containing T-complex, and endoplasmic reticulum lumen (Supplemental Fig. 1A).

By annotating the KEGG pathway of differentially expressed proteins, our data demonstrated that galactose metabolism, thyroid hormone synthesis, amino sugar and nucleotide sugar metabolism, starch and sucrose metabolism, regulation of adipocyte lipolysis and other important pathways were all significantly changed (Supplemental Fig. 1B).



Supplementary Fig.1 Identification of significant differentially expressed proteins (DEPs) in myocardium of DCM mice after GLP-1RA treatment. A: Go function analysis of differentially expressed proteins. B: Enrichment analysis of KEGG pathway of differentially expressed proteins.