

Supplementary Material:
Detecting the Critical States of Type 2 Diabetes
Mellitus Based on Degree Matrix Network Entropy by
Cross-tissue Analysis

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S1. Comparison of different parameters of CSN

According to the research of Dai et al. [1], the influence of box size and P value in different data sets on clustering effect is tested from the point of view of clustering. It is indicated that the optimum box size is about 0.1, and the optimum P-value is about 0.01 on average, which are set as the default parameters of CSN method. So we choose $0.1 \times k$, and we can adjust the parameters according to the specific needs. From the Figure 1, we can see some different data sets box size is stable and the final estimate of the value not much difference.

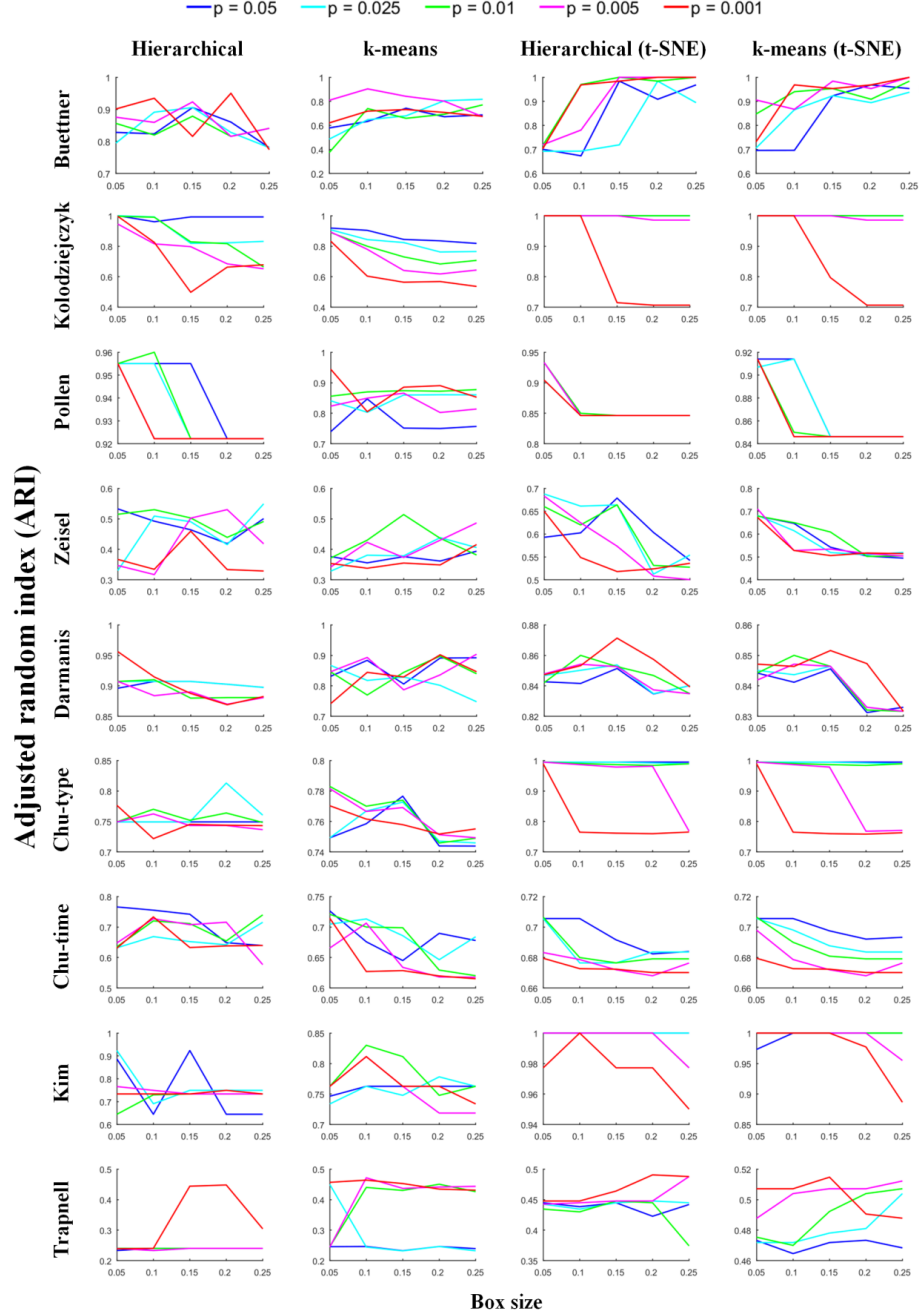


Figure S1. Comparison of different parameters of CSN. X-axis is box size and different colors represent different p-value. Y-axis is the ARI in clustering analysis. The results show that the optimum box size is about 0.1, and the optimum p-value is about 0.01, on average.

S2. The identification of ‘dark genes’ for T2DM with PAAD data

In clinical practice and scientific research, differentially expressed genes draw much attention in early diagnosis of disease, screening drug targets, treating diseases, and developing new drugs. However, some non-differential genes in the coding region of DNA are called “Dark Matter” [2]. Based on DMNE method, we find some ‘dark genes’ with-out differential expressions which are especially sensitive to DMNE score. Traditional analyses usually ignore them.

Pancreatic adenocarcinoma (PAAD) and T2DM are inter-related. The outcomes of this association are the topic of interest of a lot of prior research in this field. At present, due to the lack of corresponding prognostic data in the study of T2DM, the prognostic analysis of T2DM cannot be performed directly. Therefore, we use the data of PAAD to analyze the prognosis of T2DM, attempting to reveal the mechanism of the development of T2DM from another view.

PAAD survival data and the gene expression values in different samples are downloaded from the TCGA database. 178 samples are selected for analysis. The expression values are processed by DMNE to obtain the DMNE score. We analyze the prognosis of these ‘dark genes’ respectively based on gene expression and DMNE score by dividing the samples into two groups based on the median of genes expression or DMNE score. Group 1 is a group with higher value and Group 2 is a group with a lower value. Based on the result of prognosis, the ‘dark genes’ can be categorized into two types of molecules as a mutual biomarker for all samples. Those genes with high scores that cause poor prognosis are termed “negative dark genes”, and those genes with high scores that cause good prognosis termed “positive dark genes”. If “negative dark genes” appear in the DNBs, the sample’s prognosis will be more negative than that of other samples. Similarly, if “positive dark genes” appear in the DNBs, the sample’s prognosis will be more positive than others.

Further analysis shows that ‘dark genes’ are all strongly related to patients’ survival based on DMNE score but not expression levels. Figure.S4 shows the survival analysis of PCOLCE, HPX, BCKDHA, IFITM1, DEPDC1 and COL1A1 results with P-values < 0.05 based on some non-differential genes in DNBs of T2DM. As shown in Figure S4, the p values of survival curves obtained from gene expression values are all greater than 0.05 (PCOLCE:0.56, HPX:0.41, BCKDHA:0.89, IFITM1:0.19, DEPDC1:0.72, COL1A1:0.93), and the p values of survival curves obtained from DMNE score are all less than 0.05 (PCOLCE:0.034, HPX:0.036, BCKDHA:0.028, IFITM1:0.031, DEPDC1:0.0041, COL1A1:0.0071). P value <0.05 is statistically significant. A higher level of DMNE score in PCOLCE and HPX is significantly related to a good prognosis, i.e., positive ‘dark genes’. While a higher level of DMNE score in BCKDHA, IFITM1, DEPDC1 and COL1A1 is significantly related to poor prognosis, i.e., negative ‘dark genes’ (Figure 4). COL1A1 is linked to hypoglycemic activity [2]. This shows the effectiveness of the development of PAAD for the ‘dark genes’ in the DNBs from the side.

A large number of epidemiological studies have found that T2DM is positively associated with an increased risk of PAAD. Long-term type 2 diabetes increases the risk of PAAD by 1.5 to 2 times. Thirty to 40 percent of pancreatic cancer patients have

diabetes and 80 percent have abnormal glucose tolerance. Therefore, some ‘dark genes’ related with the prognosis of PAAD may play important role in the development of T2DM, such as COL1A1 is the most significant gene in the extracellular matrix receptor interaction pathway and is linked to hypoglycemic activity [3], which may affect the fluctuation of blood glucose and the prognosis of T2DM and the occurrence and development of chronic complications [4].

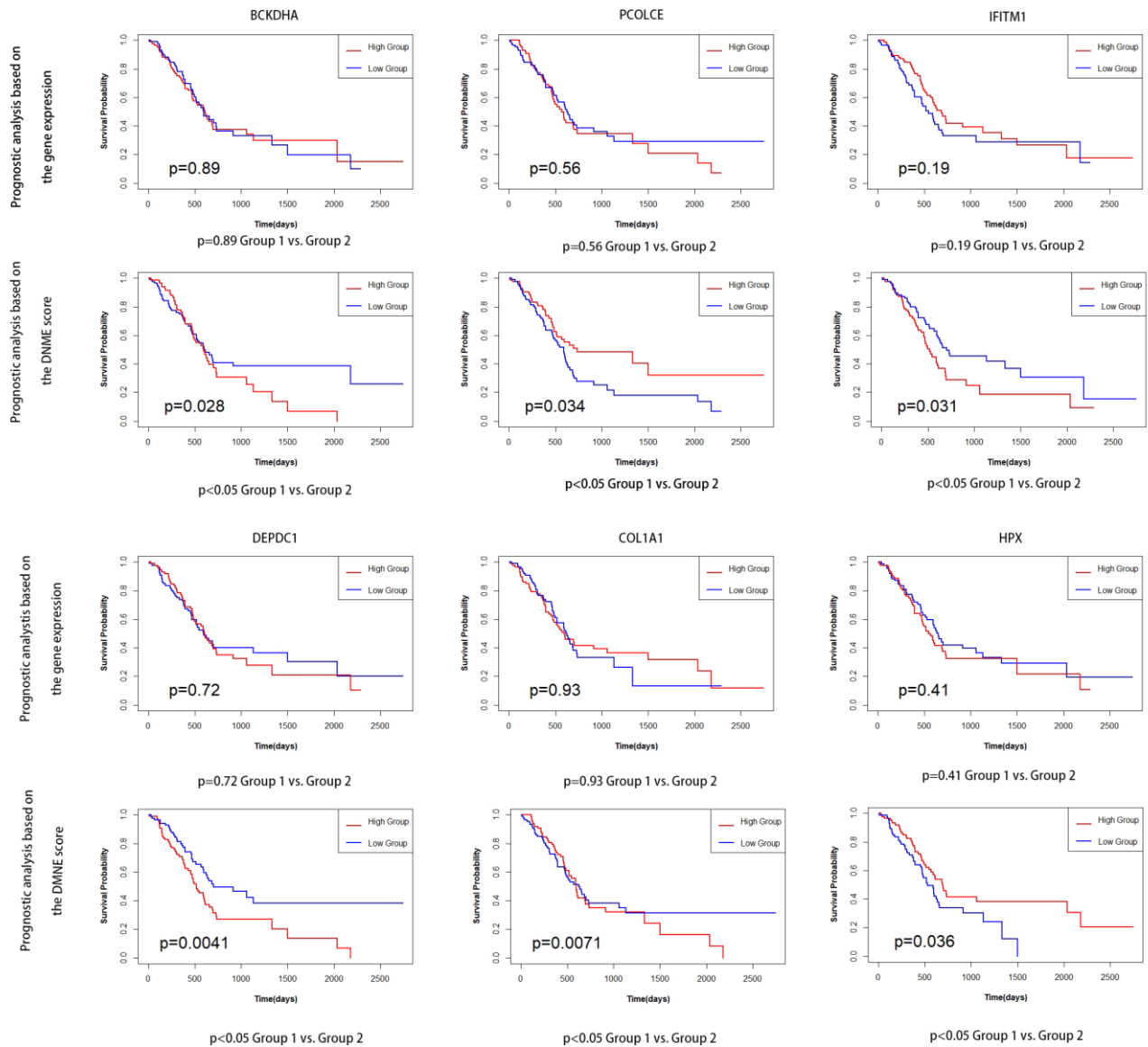
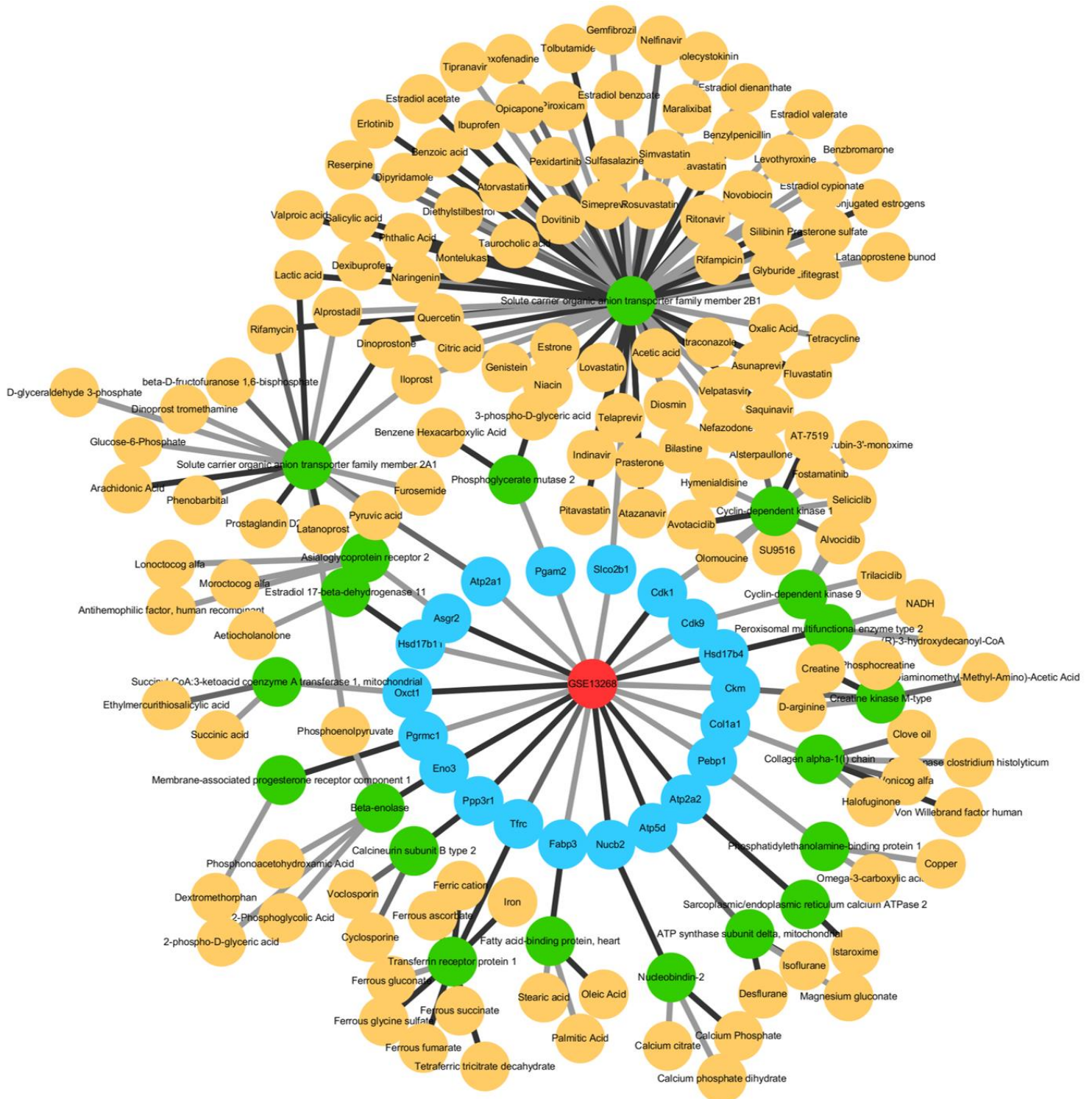


Figure S2. The comparison of prognosis analysis between gene expression and DMNE score of ‘dark genes’. For T2DM, BCKDHA, PCOLCE, IFITM1, DEPDC1, COL1A1 and HPX, are ‘dark genes’, whose DMNE scores are more sensitive to the early-warning signal of disease deterioration, and can predict prognosis better than gene expressions. The prognosis of these ‘dark genes’ show that there are significant differences between survival times of the two group samples, i.e., the high value group and the low value group, based on DMNE scores rather than gene expressions.

S3. Drug target analysis for GSE13268: Rat Adipose Tissue



a



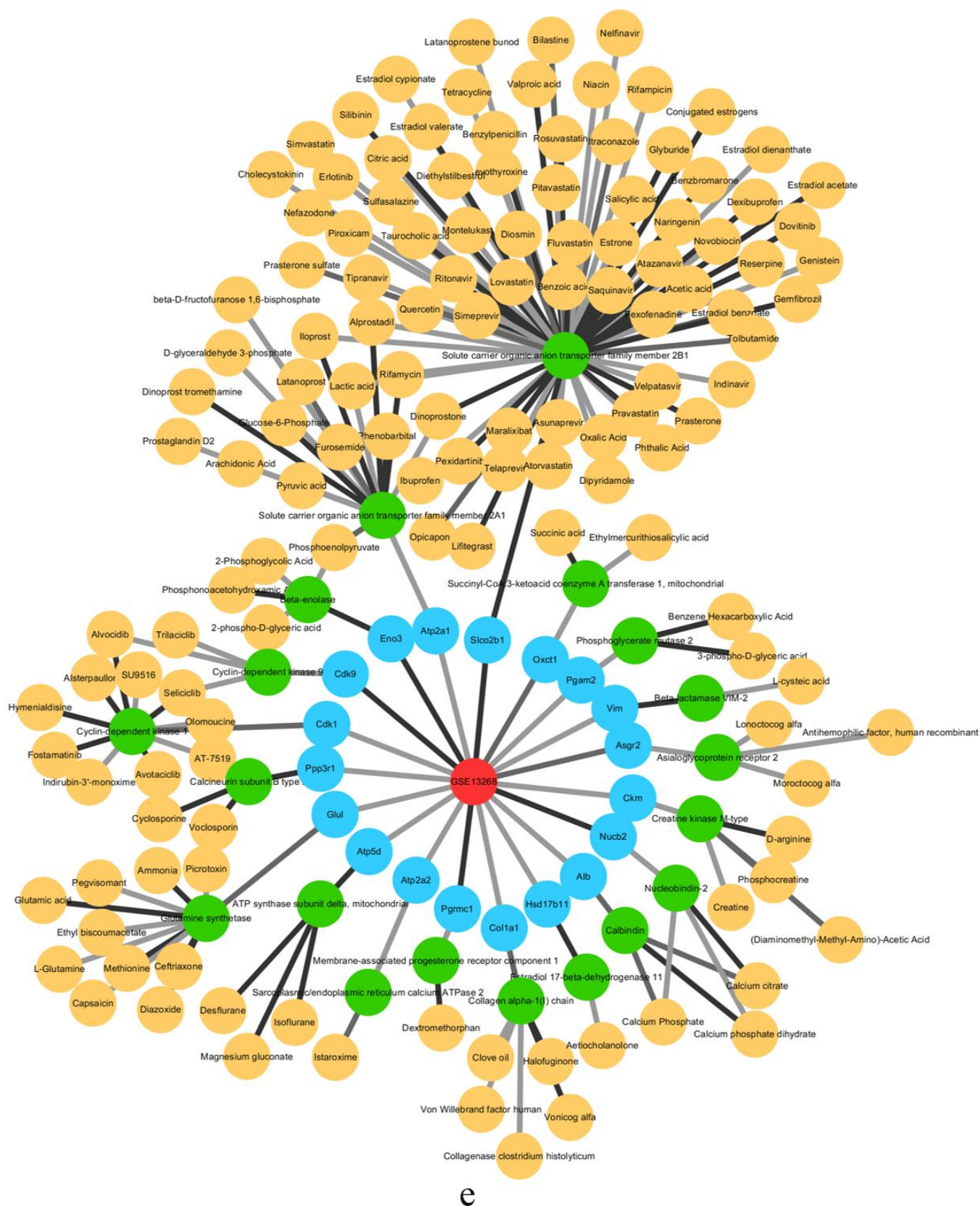
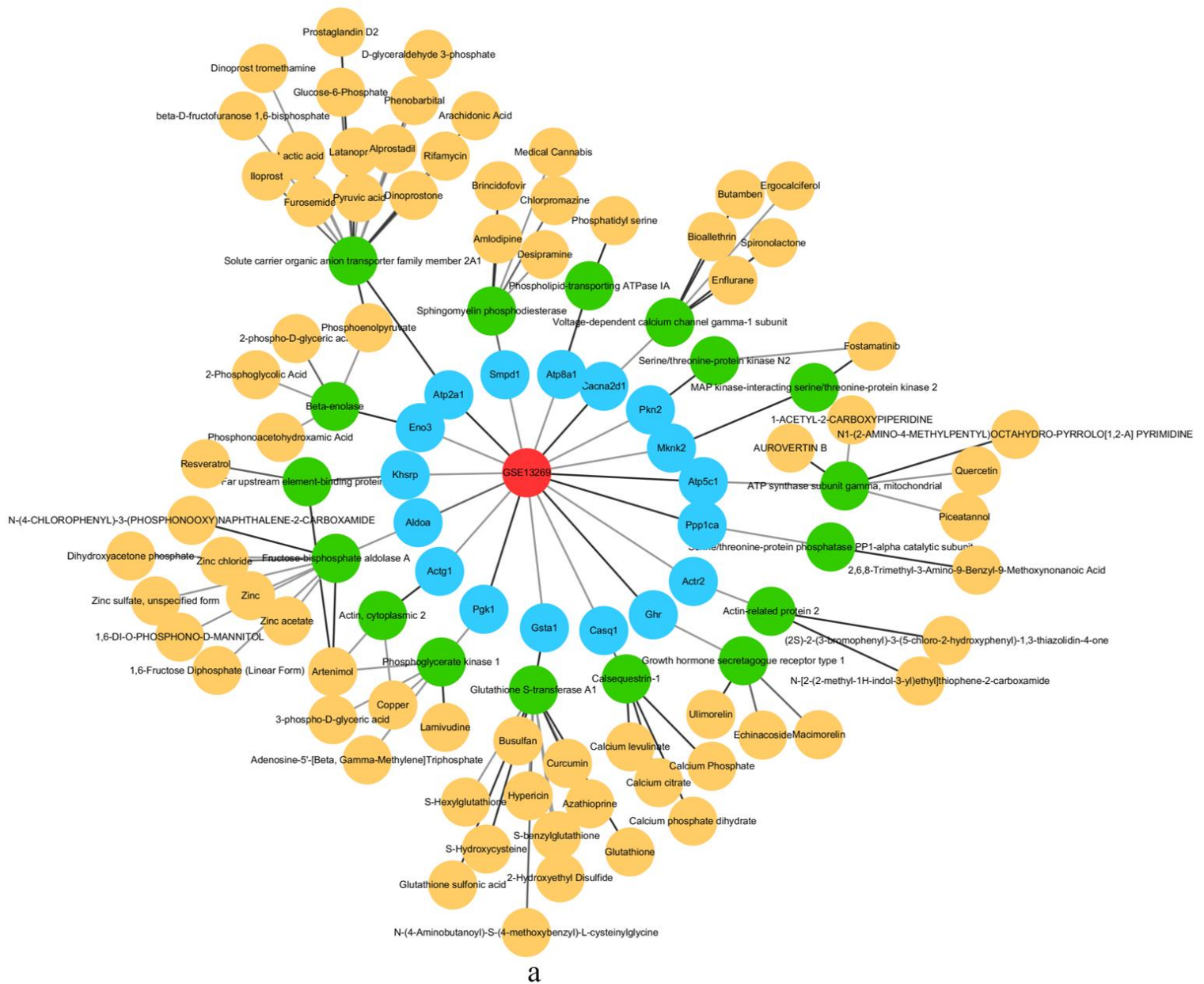


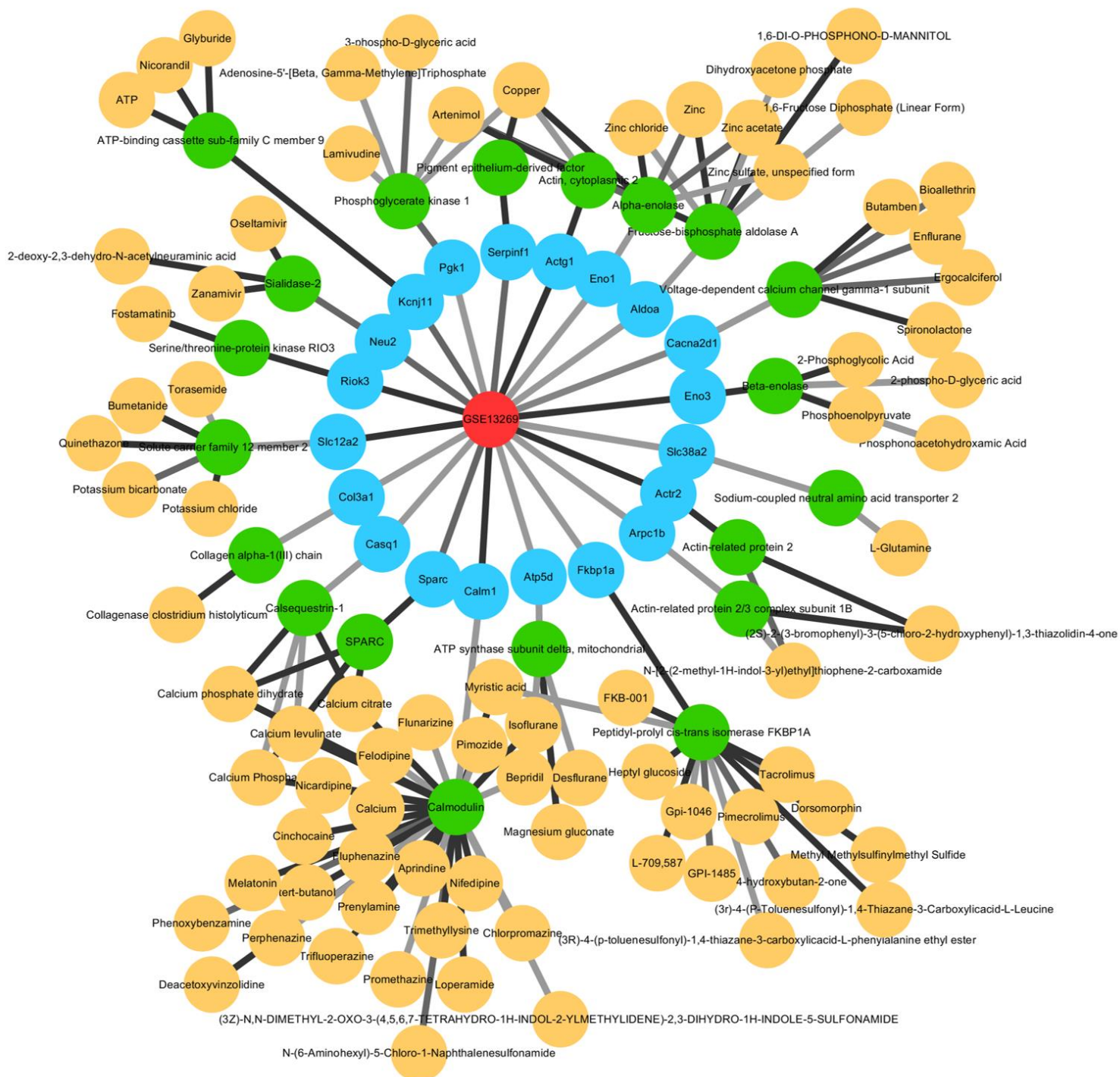
Figure S3: The drug target analysis for GSE13268, involving rat adipose tissue. (a) Drug targets, the corresponding proteins and targeted drugs at (a) stage I; (b) stage II; (c)

stage III; (d) stage IV; (e) stage V.

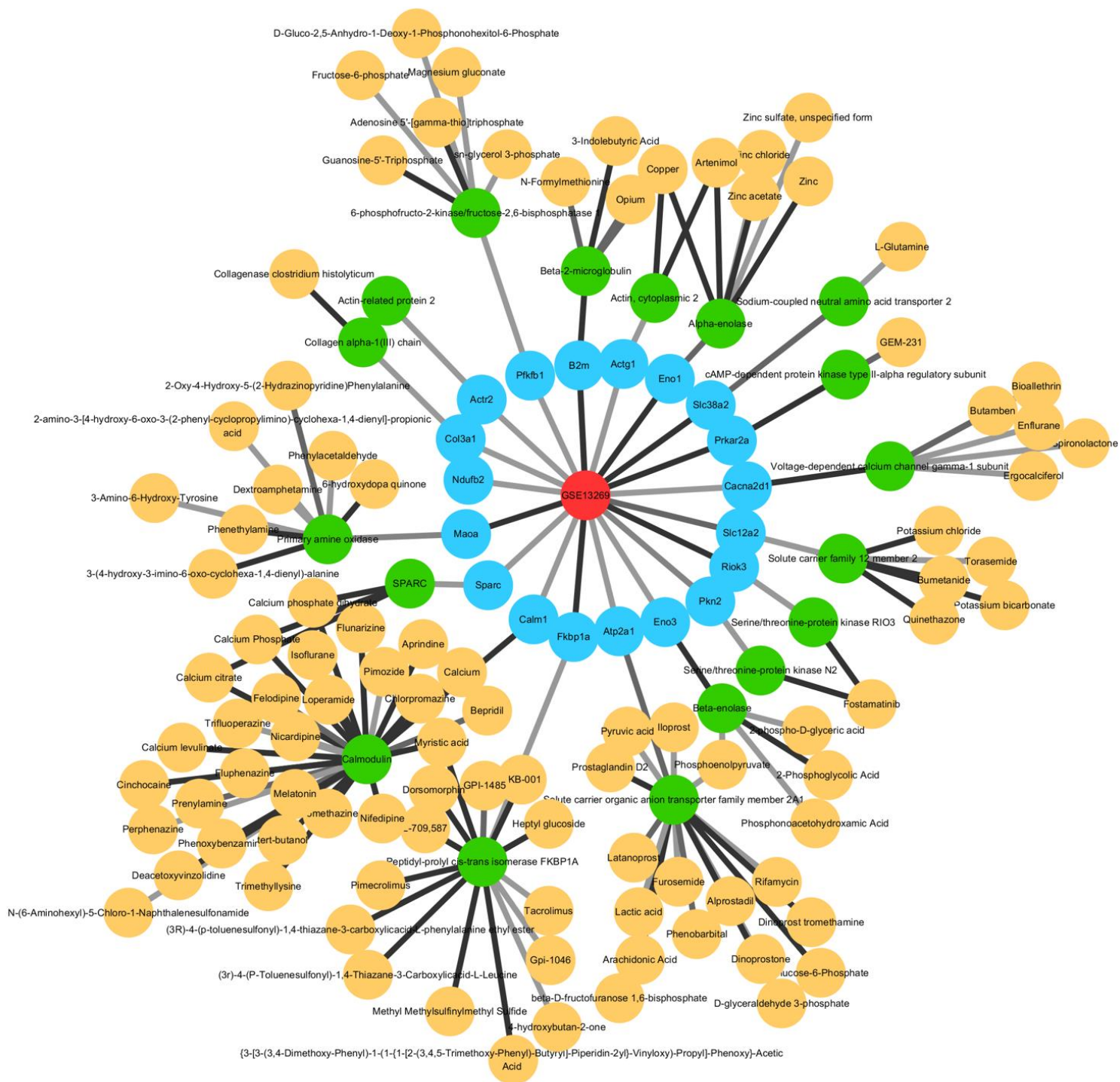
In the drug target of the second phase of GSE13268, CKM, or creatine kinase, is a target protein sought from mouse skeletal muscle and is associated with T2D-associated energy metabolism. CKM binds to 16:0/16:0-PA, with the highest affinity among PA binding proteins reported to date [5]. Col1a1 is the most important gene in extracellular matrix receptor interactions and has been identified to be associated with hypoglycemic activity. Therefore, in terms of alleviating T2D, Col1a1 can serve as a new potential therapeutic target [3].

S4. Drug target analysis for GSE13269: Rat Gastrocnemius Muscle Tissue

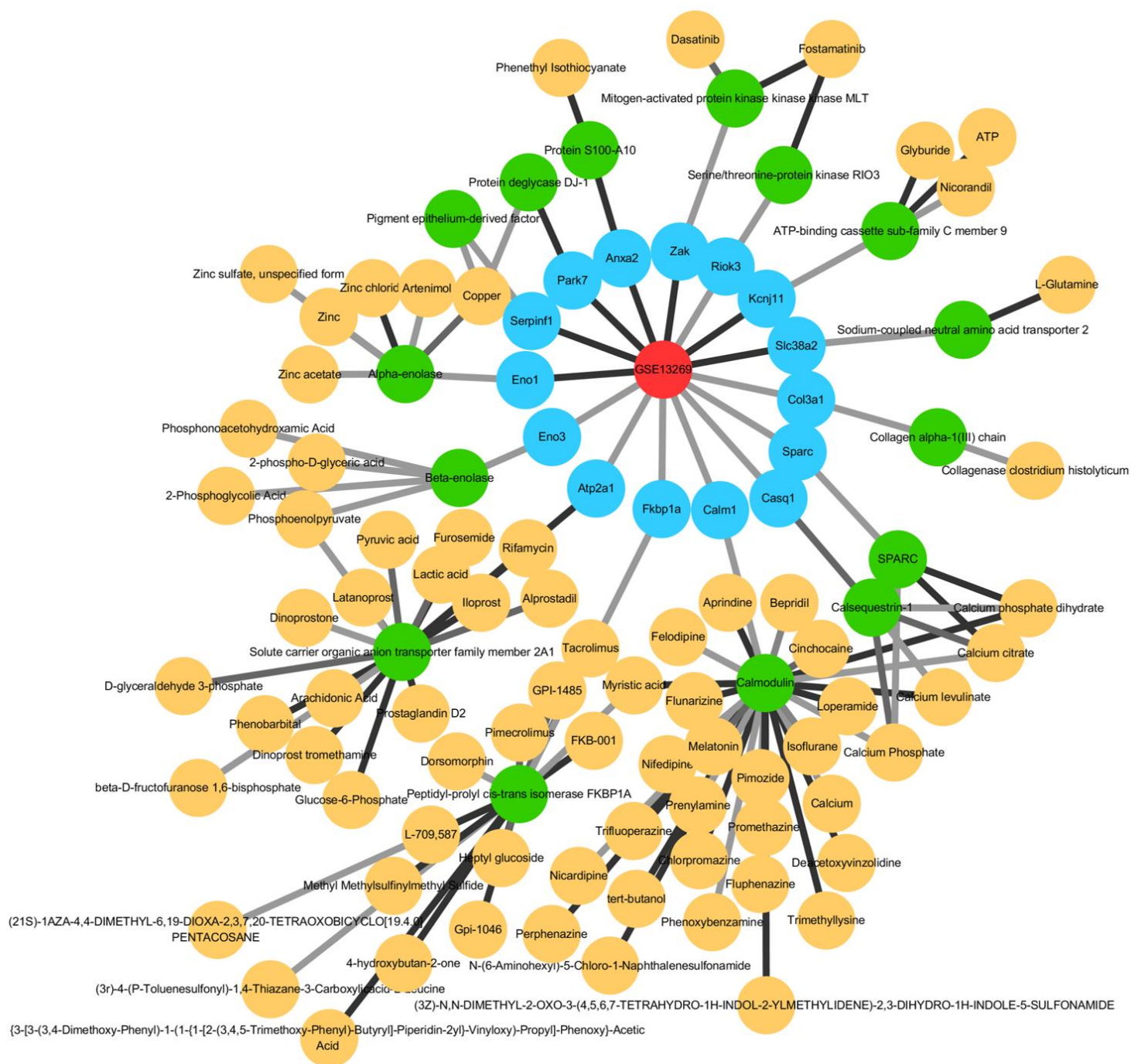




b



C

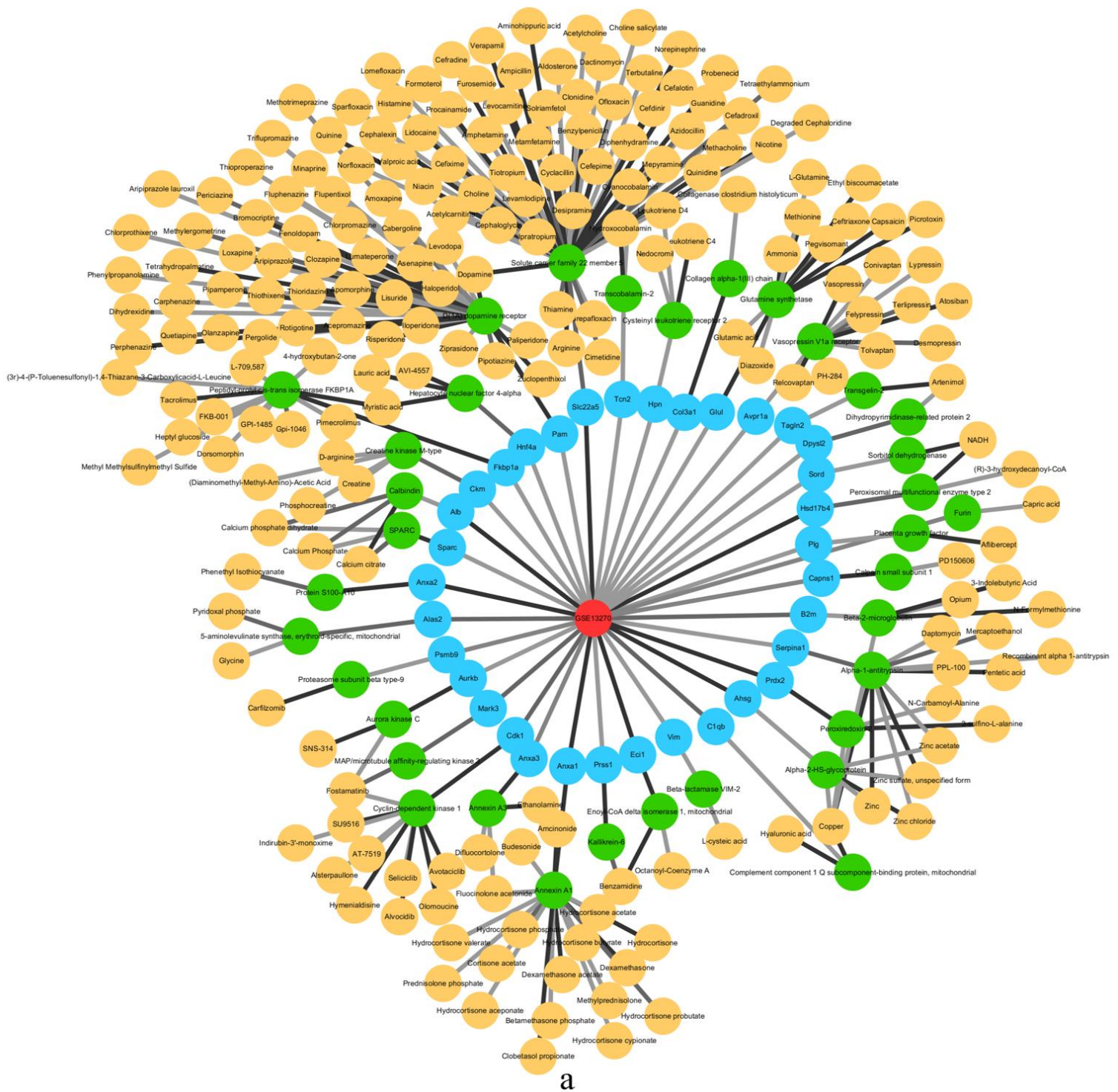


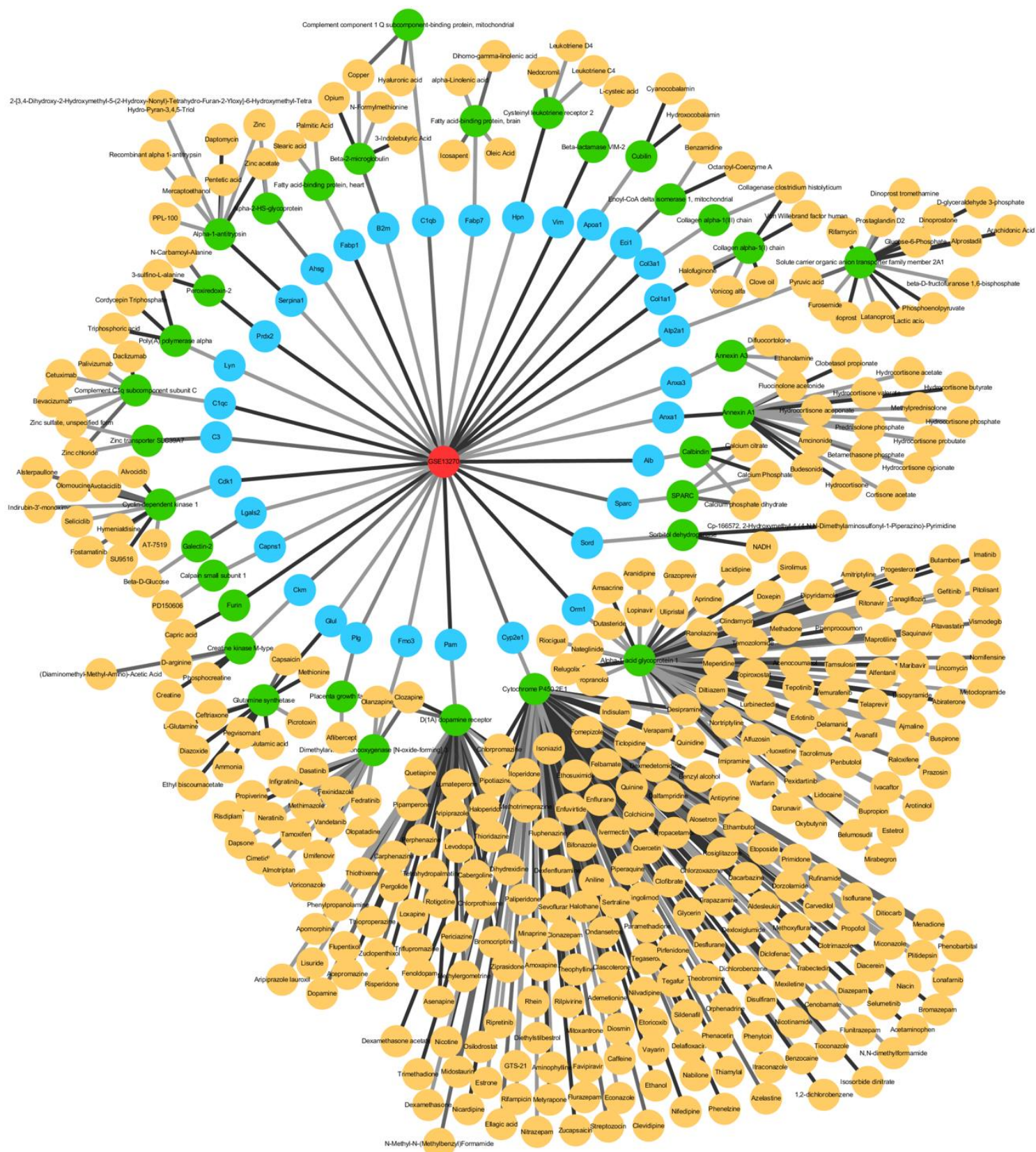
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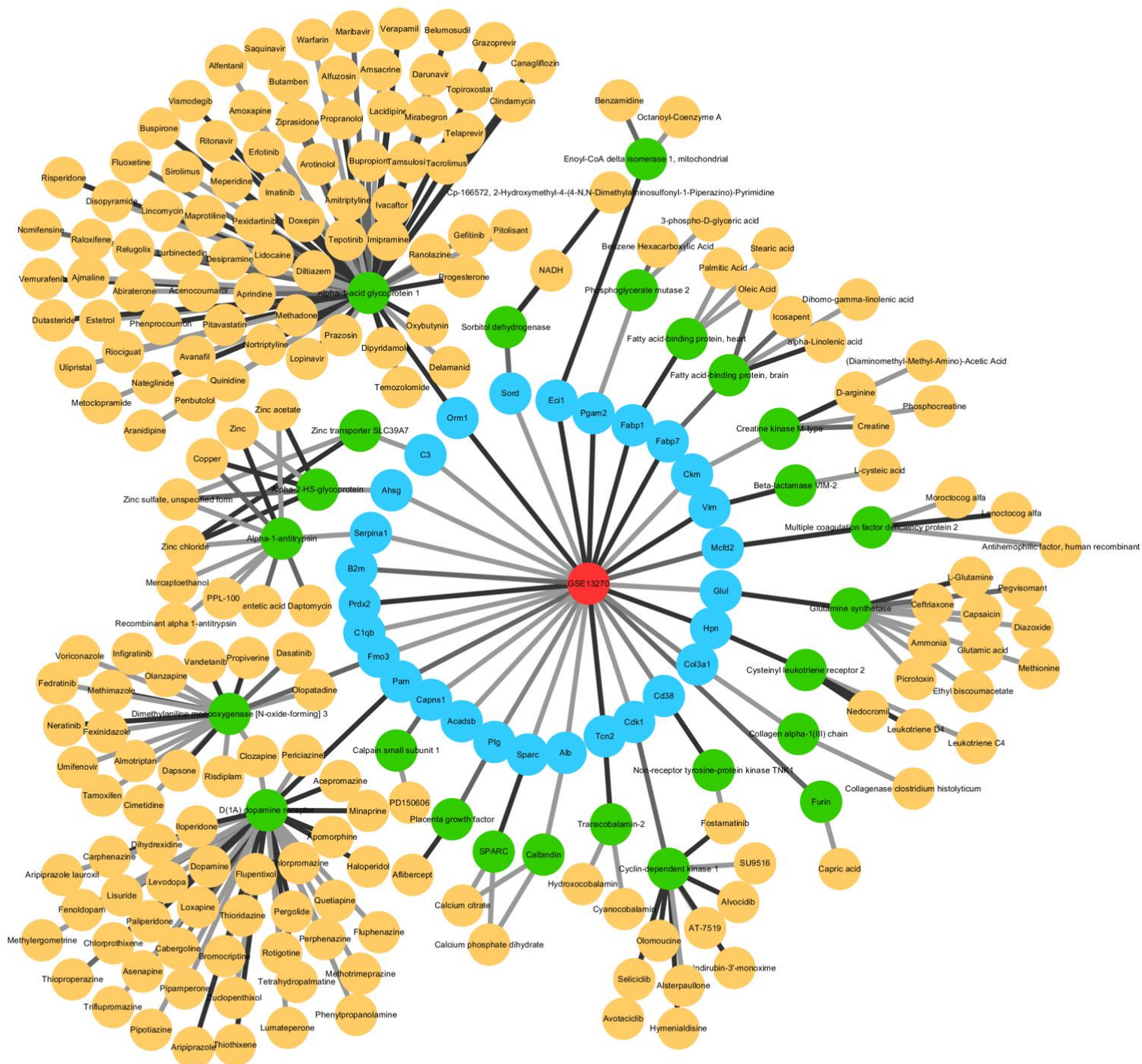
Figure S4: The drug target analysis for GSE13269, involving rat gastrocnemius muscle tissue. Drug targets, the corresponding proteins and targeted drugs at (a) stage I; (b) stage II; (c) stage III; (d) stage IV; (e) stage V.

In the first stage of GSE13269, the calsequestrin protein (CASQ) is involved in calcium chelation, storing and releasing calcium within cells, a process that has been shown to mediate the transport of glucose in muscles. CasQ' s gene CASQ1 is encoded in chromosome 1q21, a region that has been shown to be associated with type 2 diabetes in Amish people and several other populations [6]. The single-stranded nucleic acid-binding protein KHSRP (KH-type splicing regulatory protein) regulates the lifespan of RNA and its gene expression at different levels. KHSRP also controls important cellular functions such as proliferation, differentiation, metabolism, and response to infectious agents [7]. In the fourth stage of GSE13269, there is an *in vitro* protein that reduces the process of insulin-stimulated glucose uptake, glycogen incorporation and oxidation by increasing the signaling of the 5'-adenosine monophosphate-activated protein kinase AMPK, while stimulating fatty acid oxidation and incorporation into triglycerides, thereby reducing protein synthesis [8]. We also demonstrated *in vivo* function and effects on overall obesity with the common SERPINF1 variant rs12603825, where the secondary A allele represents plasma PEDF- and the risk of elevated body fat alleles [9].

S5. Drug target analysis for GSE13270: Rat Liver: Rat Liver Tissue







C

Figure S5: The drug target analysis for GSE13270, involving rat liver tissue. Drug targets, the corresponding proteins and targeted drugs at (a) stage II; (b) stage III; (c) stage V.

In addition, the stage I and the stage IV are critical stages, and the corresponding drug target map is put into the body of the paper and analyzed, so it will not be repeated here. The non-critical phase does not have to be analyzed.

S6. Enrichment Results and Analysis of GSE13269 (Rat Gastrocnemius Muscle Tissue) and GSE13270 (Rat Liver Tissue).

For GK rat muscle, the biological processes related in 4 weeks mainly include muscle system process, response to stimulus, response to glucose, heart process, cellular response to glucose stimulus and PI3K-Akt signaling pathway, which are provided in Table S7 and Figure S6 (a). The biological processes in 16 weeks mainly include regulation of peptidase activity, negative regulation of phosphorylation, regulation of necrotic cell death, carbohydrate metabolic process, glycolysis/gluconeogenesis, HIF-1 signaling pathway, PI3K-Akt signaling pathway, proteoglycans in cancer and response to lipid, which are also provided in Table S7 and Figure S6 (a). For PI3K-Akt signaling pathway, the gene *Sirt1* participates in diabetic myocardial injury and the occurrence and development of early diabetic cardemonopathy by negatively regulating PI3K/Akt/MTOR signaling pathway [10]. Previous studies through cell transfection and other experiments have shown that HIF-1 α /KIM1 signaling pathway can participate in the process of renal fibrosis in diabetic nephropathy by regulating the expression of KIM1 in renal tubular epithelial cells in high glucose environment. HIF-1 α is the key transcription factor of oxygen homeostasis regulation, which can make the body adapt to the external environment oxygen by regulating the expression of target genes. The expression levels of HIF-1 α , KIM1, COL-1mRNA and protein in kidney of diabetic rats are significantly increased, suggesting that diabetic nephropathy model is accompanied by changes of HIF-1 α /KIM1 pathway in kidney [11]. For some genes in DNB, PRDX2 has been found to participate in the oxidative stress process through a variety of signaling pathways. The hypoxia environment caused by it leads to the increase of reactive oxygen species. In order to survive in a high level of reactive oxygen species, cells need to increase antioxidants, so the expression of related factors, including PRDX2 protein, has changed. Previous studies suggest that overexpression of PRDX2 can protect pancreatic cell apoptosis induced by oxidative stress and re-duce the risk of diabetes [12]. Some studies [13,14] believe that SPARC is an autocrine and/or paracrine factor of adipose tissue, which can inhibit the fat formation. Other studies [15] show that insulin can increase the expression of SPARC, while SPARC can increase the phosphorylation of AKT and PI3-K, indicating that SPARC may be in-volved in insulin signaling pathway [16].

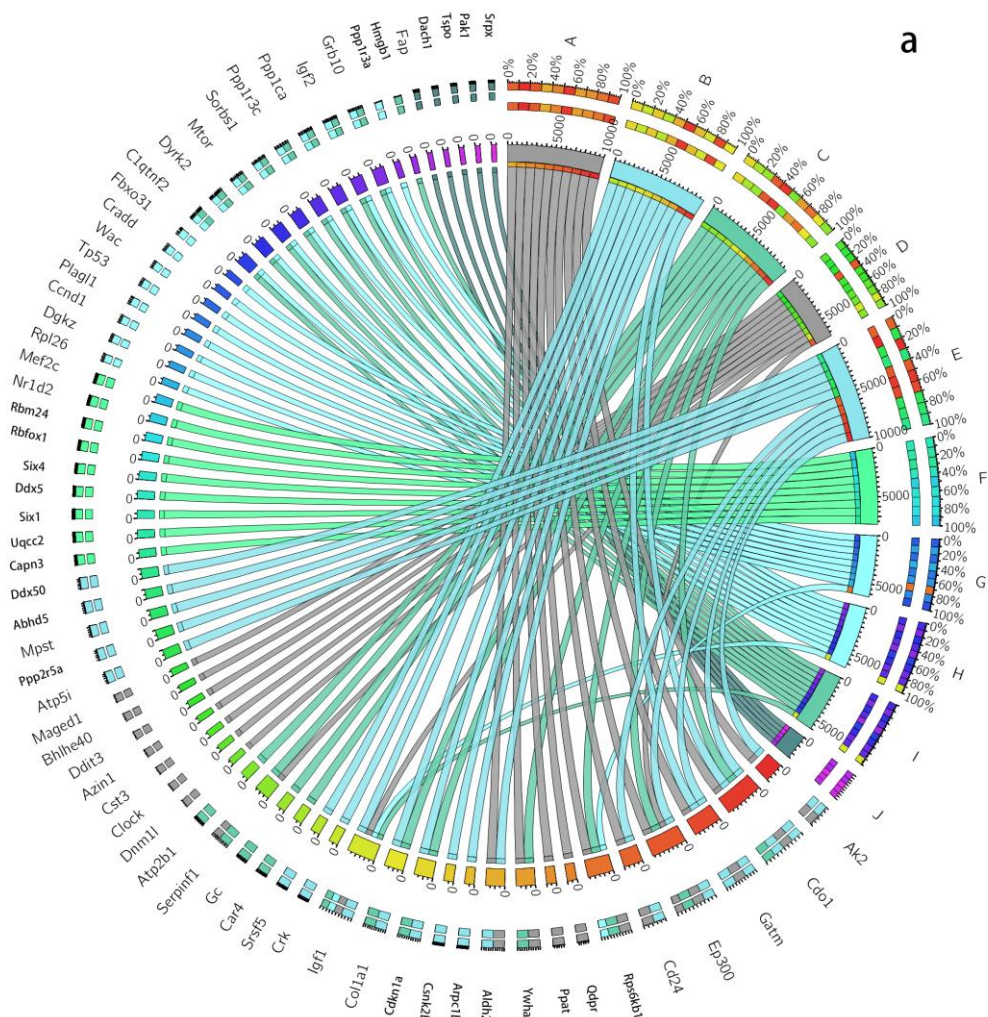
For GK rat liver, the biological processes in 4 weeks mainly include response to hormone, inflammatory response, response to interleukin-1, metabolism of xenobiotics by cytochrome P450, AGE-RAGE signaling pathway in diabetic complications and protein digestion and absorption, which are provided in Table S7 and Figure S6 (b). The biological processes in 16 weeks mainly include liver de-elopement, regulation of secretion, cellular lipid metabolic process, vitamin A metabolic process, regulation of cellular protein metabolic process, PPAR signaling pathway and AGE-RAGE signaling pathway in diabetic complications, which are also provided in Table S7 and Figure S6 (b). Vitamin A is required for PSC (a lipid storage cell, which is normally in a resting state) to maintain a resting state. Vitamin A and its metabolites can inhibit the expression of resting and activated pancreatic stellate cells α -SMA [17]. The peroxisome proliferator-activated receptors (PPARs) modulate several biological processes that are perturbed in obesity, including inflammation, lipid and glucose metabolism and overall energy homeostasis. And PPARs regulate the functions of adipose tissues, such as adipogenesis, lipid storage and adaptive thermogenesis [18]. For the AGE-RAGE signaling pathway, moderate intensity aerobic exercise inhibit-its AGE—RAGE axis and NF- κ B pathway, which may decrease oxidative stress and in-Flammarion, and so reduce tissue injure for the prevention and treatment of complications of the T2DM [19]. For some genes in DNB, CD74 receptor and other

mechanisms are stimulated by multi effect cytokine MIF to promote inflammatory response in glomerular podocytes. MIF can activate CD74 on the surface of local glomerular podocytes, resulting in the phosphorylation of extracellular signal regulated kinase 1/2 (ERK1/2) and p38MAPK [20]. SPARC is an autocrine and paracrine factor of adipose tissue, which can inhibit adipogenesis. Insulin can increase SPARC expression, while SPARC can increase Akt and P13-K phosphorylation, indicating that SPARC may be involved in insulin signaling pathway [21]. The overexpression of PRDX2 can protect pancreatic β cell apoptosis induced by oxidative stress and reduce the risk of diabetes. The changes of oxidative stress in diabetic kidney disease and other pathological processes are related to the expression of PRDX2 and related pathways. For example, PRDX2 can participate in the regulation of tumor development and oxidative stress in treatment through P13 AKT-resistant pathway. PRDX2 can regulate the oxidative stress of colon cancer cells through Wnt/B-catenin signaling pathway and influence the oxidative stress of tumor by regulating some microRNA [22].

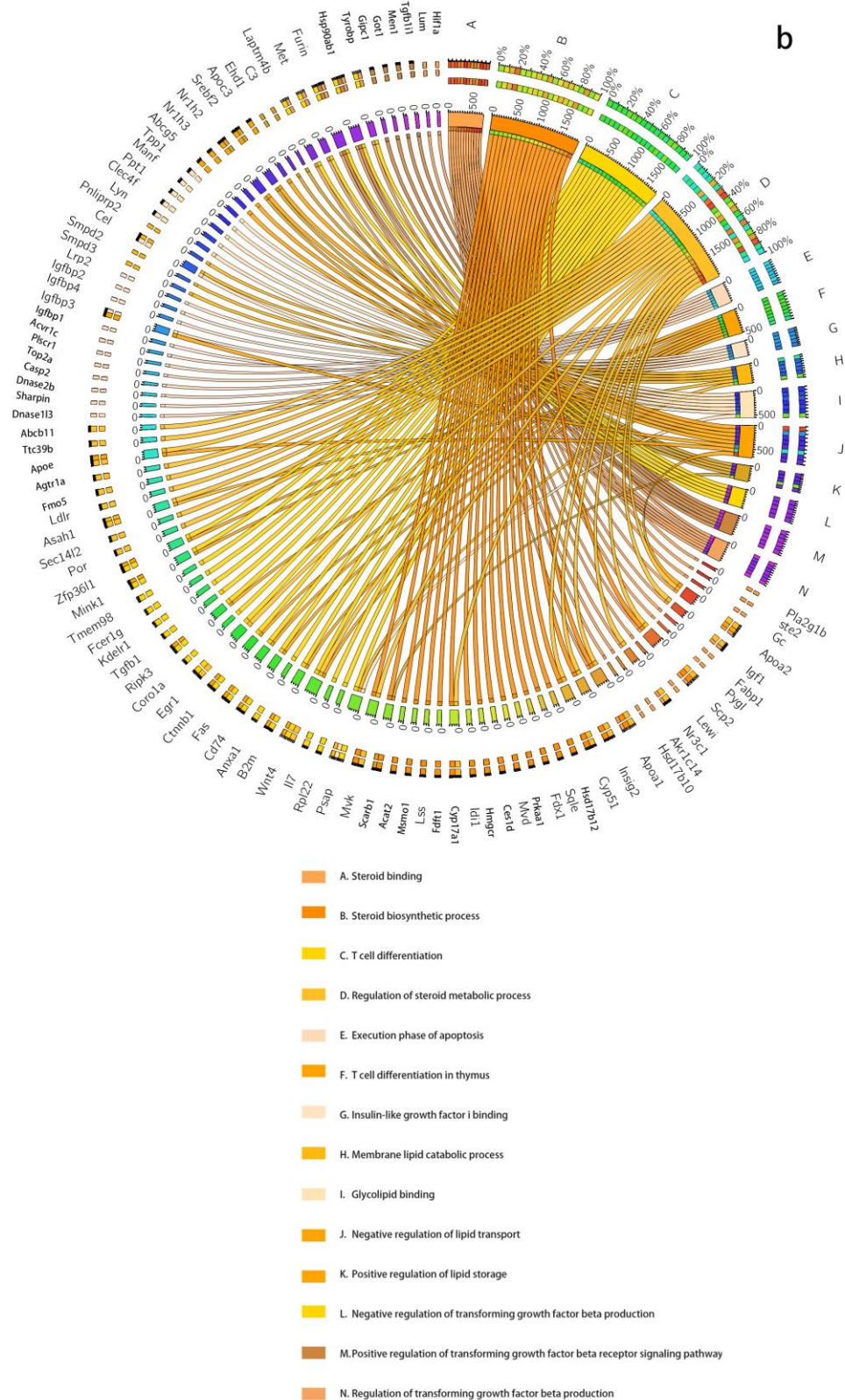
Table S7. Enrichment results for DNBs of GSE13269 (rat gastrocnemius muscle tissue) and GSE13270 (rat liver tissue) datasets.

Tissue	Case	Term	P-value	Term name
Muscle	Muscle 4 weeks	GO:0003012	1.05×10^{-5}	Muscle system process
		GO:0050896	0.0067	Response to stimulus
		GO:0009749	0.0121	Response to glucose
		GO:0003015	0.0334	Heart process
		GO:0071333	0.0351	Cellular response to glucose stimulus
		GO:0042593	0.0458	Glucose homeostasis
		rno04260	3.72×10^{-8}	Cardiac muscle contraction
		rno00190	1.95×10^{-7}	PI3K-Akt signaling pathway
	Muscle 16 weeks	GO:0052547	0.0048	Regulation of peptidase activity
		GO:0042326	0.0117	Negative regulation of phosphorylation
		GO:0010939	0.0176	Regulation of necrotic cell death
		GO:0005975	0.0239	Carbohydrate metabolic process
		rno00010	0.00045	Glycolysis / Gluconeogenesis
		rno04066	0.0075	HIF-1 signaling pathway
		rno04151	0.0092	PI3K-Akt signaling pathway
		rno05205	0.0213	Proteoglycans in cancer
Liver	Liver 4 weeks	GO:0033993	1.63×10^{-11}	Response to lipid
		GO:0009725	5.36×10^{-9}	Response to hormone
		GO:0006954	0.0002	Inflammatory response

Liver 16 weeks	GO:0002443	0.00033	Leukocyte mediated immunity
	GO:0070555	0.0292	Response to interleukin-1
	rno00980	0.0172	Metabolism of xenobiotics by cytochrome P450
	rno04933	0.0369	AGE-RAGE signaling pathway in diabetic complications
	rno04974	0.0438	Protein digestion and absorption
	GO:0001889	0.00075	Liver development
	GO:0051046	0.0056	Regulation of secretion
	GO:0044255	0.0208	Cellular lipid metabolic process
	GO:0006776	0.0336	Vitamin a metabolic process
	GO:0032268	0.0473	Regulation of cellular protein metabolic process
	rno03320	0.0039	PPAR signaling pathway
	rno04933	0.0077	AGE-RAGE signaling pathway in diabetic complications
	rno00982	0.0098	Drug metabolism - cytochrome P450



- A. Response to hormone
- B. Response to peptide hormone
- C. Response to steroid hormone
- D. Rhythmic process
- E. Metabolic process
- F. Regulation of skeletal muscle cell differentiation
- G. G1 DNA damage checkpoint
- H. Regulation of glycogen biosynthetic process
- I. Regulation of glycogen metabolic process
- J. Contact inhibition



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