# Exploring the sirtuin functionality in aging through the human protein interaction networks

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# Exploring the sirtuin functionality in aging through the human protein interaction networks

**Abstract:** The sirtuin family contains seven proteins with the functions in multiple diseases of aging, which makes them an attractive subject for the development of therapies of age-related diseases and anti-aging treatments. The primary objective of the protein-interaction network analysis presented here is to identify the signaling pathways and protein nodes driving the functions of the sirtuins. For this purpose, the protein-protein interaction data were collected from the available public databases, which fulfilled the quality threshold and included at least one member of the sirtuin family. The databases provided 66 interactions validated by several experiments, which were further processed by the bioinformatic tools connected to the integrated genomic, proteomic, and pharmacologic data. The interactions were analyzed by the pathway enrichment, the gene function prediction analysis, and the protein node prioritization by use of Cytoscape applications GeneMania and Cytohubba. The constructed sirtuin protein interaction network (SPIN) contained after the extension 98 protein nodes. TGF$\beta$, PTK2, CARM1, Notch signaling and the pathways regulating androgen and estrogen levels, significantly scored in the pathway enrichment analysis of SPIN. The enriched signaling pathways mediating the pleiotropic effects of the sirtuin family, play the roles in several age-related diseases probably. The Cytohubba application has highlighted the function of HDAC1, EP300, SMAD4, MYC, SIN3A, RBBP4, HDAC, SIN3B, RBBP7 and SMAD3 as the high priority protein nodes driving the molecular functions of SPIN. The presented protein interaction study provide new understandings of the sirtuin functions in the longevity and diseases of aging including cancer, neurodegenerative and metabolic disorders.

Abbreviations: HDAC – histone deacetylase; SPIN – sirtuin protein interaction network; SPIL - sirtuin protein interaction list; RB – retinoblastoma

# 1. Introduction

The research of protein-protein interactions represents an essential tool for drug discovery since the interacting proteins significantly share the functions. A better understanding of the complex interaction networks can be achieved from available functional data of well-characterized protein nodes since the missing functional data of the targeted protein nodes can be extrapolated.

The idea to study the interaction networks is supported by a hypothesis, that the proteins interacting directly via protein-protein interactions are very probably participating in the identical cellular and molecular functions. If they represent disease genes, the mutations in their genes lead to similar disease phenotypes (1). The fundamental model of the interaction network medicine relies on the targeting of multiple essential proteins since the treatment development for the disease pathologies through just the single node appears not feasible. The multiple node targeting also has practical implications for the development of the therapeutical approaches. The most promising drug targeting strategies are based on the partial inhibition of several protein nodes within the interaction network instead of the complete inhibition of the single node (2), which is pharmaceutically very challenging.

The protein-protein interaction analysis benefits from the integration with the genomic and proteomic data and with the information about the pharmacological targeting. It provides the information about the protein function prediction and the pathway enrichment data; it ranks the protein nodes of the biological networks based on their priority, which suggests the critical hub proteins regulating the key molecular mechanisms.

Sirtuins are belonging to the class III of the histone deacetylases, enzymes defined by the ability to remove acetyl groups from ε-N-acetyl-lysine of the histone and other non-histone substrates and requiring NAD+ for their enzymatic activity. Their activity is an essential part of cellular metabolism, chromatin modification, and aging (3). Although they were defined as a protein deacetylases, their desuccinylase, demalonylase (4,5) or lipoamidase (6) activities are usually higher. The significant functions of the sirtuins in the mechanisms of aging and longevity attract many scientists (7), despite there were also raised doubts about their leading roles in longevity due to the results obtained in nematodes *C. elegans* and fruit flies *D.*

*melanogaster* (8). The recent research development confirmed that the sirtuins indeed are important protagonists playing essential functions affecting aging and age-related diseases. Brain-specific SIRT1 overexpressing mice exhibits not only significantly extended lifespan but also increased neuronal activity, which is another encouraging result for the research of the anti-aging effects of the sirtuins (9). Interestingly, SIRT3 also participates in the mechanisms of several diseases of aging such as cancer, cardiovascular disease, metabolic syndrome or neurodegenerative diseases. SIRT3 knockout in the mice causes either the spontaneous occurrence of these diseases or when they are crossed with the disease model (10). Furthermore, the SIRT6 deficient mice also exhibit the age-related degenerative changes, genomic instability, and a very short lifespan (11). The knockdown of SIRT6 using shRNA in human fibroblasts shows extremely short replicative lifespan, increased senescence, and the telomere dysfunction, which results in the premature senescence of SIRT6 deficient cells (12). Interestingly, SIRT6 overexpression causes male gender-specific life extension in the transgenic mice (13). Aging-related functions were also confirmed in SIRT7 knockout mice, which exhibit reduced lifespan accompanied by the signs of premature aging, subcutaneous fat loss, activated cell death programs, and the decreased resistance to oxidative and genotoxic stresses (14). The phenotype of SIRT7-/- HSCs closely resembles old cells by exhibiting increased mitochondrial protein folding stress, apoptosis, and the compromised regenerative ability. In overall, SIRT7 expression naturally decreases in aged human HSCs. On the other hand, the upregulation of SIRT7 in aged HSCs increases the rejuvenation capability. It confirms that the aging of the HSCs is the reversible process and it is promising news, which encourages future research (15).

The current work interprets the results of the pathway enrichment and gene function prediction analysis performed on the protein interaction data of the sirtuin family collected from the public databases and validated by several independent experiments. The major aim of the study is the identification of the signaling and metabolic pathways driving the interaction network and mediating the sirtuin functions in longevity, the mechanisms of aging and age-related diseases. The discussion further provides insights into the functional relationship of the sirtuins with the main regulatory hub proteins of the interaction network, which could be relevant for the pharmacological interventions.

# 2. Materials and Methods

The sirtuin protein interaction list (SPIL): The list of the sirtuin interacting proteins was created from the databases BioGRID (Biological General Repository for Interaction Datasets; version 3.4.154) (Stark, 2006); HPRD (Human Protein Reference Database; version 9) (Keshava Prasad et al., 2009) and MINT (The Molecular Interaction Database; update 2012) (16). The threshold for including of the protein on the sirtuin protein interaction list (SPIL) was the interactions with at least one sirtuin supported by minimum of three experimental pieces of the evidence. The interacting proteins were also collected from *H. sapiens* STRING database when the high confidence level 0.9 was applied for the selection (16). Additional interacting proteins supported by the high-quality experimental evidence were added through the literature mining as well. SPIL included the following proteins: AATF BOP1 BRCA1 CAPRIN1 CCAR2 CREBBP CLOCK DDB1 DNM2 ESR1 FAT4 FOXO1 FOXO3 GAPDH GLTSCR2 HDAC1-HDAC11 HIF1A HTT IDE JAK1 LRRK2 MAX MDM2 MYC NCOR1 NCOR2 NF1 EP300 PARK7 PARP1 PKM2 PPARA PPARGC1A PS1 PSMC2 PSMC5 PSMD1 PSMD2 PTEN RBBP4 RBBP7 RELA SENP1 SIN3B SIRT1-SIRT7 SMAD2 SMAD3 SMAD7 STAT3 STB2.

**The analysis using Cytoscape (3.7.0) and its applications GeneMania (3.4.1) and Cytohubba (0.1)**: GeneMania (3.4.1) (17) analysis was performed using SPIL as a query against *H. sapiens* database and by including all types of the interaction networks. The analysis was set up for the identification of the top 20 related genes and at the most 20 attributes using GO Molecular function weighting. The clusters from GeneMania consolidated pathway networks were merged using the in-built Cytoscape tool as specified in the text. Figure S1 shows the legend determining the types of the nodes and their interactions. The interaction networks were further analyzed by Cytohubba, a software application integrated into Cytoscape, which identifies the crucial regulatory hub protein nodes and pathways of the interaction network (18). The application was set up for the use of the topological method Maximum click centrality (MCC), which provides according to the developers the highest precision in the prediction of the essential nodes (19). Further, the settings of the analysis were adjusted for the selection of the first-stage nodes and the display of the shortest path.

# 3. The results and discussion

The sirtuin protein interaction network (SPIN) was constructed with the Cytoscape application GeneMania by the analysis of SPIL (Fig.1; Tab. 1), which extended the interaction network with the functionally related protein nodes (further called extended SPIN). The main identified consolidated pathways driving the interaction network are further discussed in the relationship with the central regulatory protein hubs defined by Cytohubba analysis and the sirtuins (Tab. 2). The further discussion provides explanations of the functional relationships between the identified signaling pathways and the molecular mechanisms of longevity, aging, and age-related diseases. Finally, the presented analysis raises several questions for the future directions of the research of healthy aging and age-related diseases.

## 3.1. Main identified consolidated pathways

### 3.1.1. TGF-*β* signaling pathway

Two elements of T*G*F-*β* signaling, the regulatory network of the nuclear SMAD2 and TGF-*β* receptor complex interaction network occurred among the most significantly enriched pathways (Fig. 1, Tab. 1). At the same time, the networks include 10/10 and 6/10 top rank protein nodes (HDAC1, HDAC2, MYC, SMAD4, SMAD3, EP300), which based on the Cytohubba analysis drive the functions of the whole extended SPIN (Fig. 2; Fig. 3; Tab. 2).

The subsequent scientific literature mining showed the regulatory roles of SIRT1, SIRT3, SIRT6, and SIRT7 on TGF-*β*/SMAD signaling occurring in cancer and other diseases. However, it appears that the detail investigations of the functional relationships between the sirtuins, SMAD2 and TG*F*-β receptor complex networks were not clarified yet.

Interestingly, the altering of SIRT1 expression or the enzymatic activity is pharmacologically sufficient for the regulation of TGF-*β* signaling (20)(21)(22). Further, SIRT1 physically interacts with SMAD3 through its deacetylation, which controls the pathological role of TGF-*β* signaling and produces the protective effect in chronic kidney disease (23) and TGF-*β*1 mediated tissue fibrosis (24). Reduced expression of TGF-*β* was also recorded in aortas of angiotensin infused vascular smooth muscle cell-specific SIRT1 transgenic mice, where the

overexpression of SIRT1 has an anti-hypertensive effect (25). SIRT1 also regulates TGF-$\beta$ signaling by affecting the ubiquitination mediated proteasome degradation of SMAD7 through its deacetylation, since SMAD7 expression controls TGF-$\beta$ signaling. Therefore, the stimulation of SIRT1 expression can be applied for the inhibition of TGF-$\beta$ induced apoptosis by the stimulation of SMAD7 degradation (26). Then, SIRT3 facilitates the age-dependent TGF-$\beta$1 mediated tissue fibrosis through deacetylation and activation of GSK3$\beta$, which directly blocks TGF-$\beta$ signaling (27). In human primary bronchial epithelial cells, the suppressive effect of SIRT6 expression efficiently controls TGF-$\beta$ induced senescence, while SIRT6 silencing is an inducer of the effect (28). SIRT6 functions at certain conditions as a tumor promoter, since it is required in human hepatocellular carcinoma (HCC) cells for the tumorigenesis triggered by TGF-$\beta$1 and reactive oxygen species (ROS). A suitable target for the inhibition of SIRT6 tumor growth-promoting activity is the ERK pathway, which decreases SIRT6 expression (29). SIRT7 also plays an essential role by phosphorylation and activation of SMAD2 and ERK, however SMAD2 signaling also appears essential for SIRT7 mediated cardiac fibrosis (30). Moreover, SIRT7 promotes the degradation of the hub protein SMAD4 through its deacetylation, modulates the TGF-$\beta$ activity, and acts as a breast tumor metastasis suppressor (31).

High priority protein nodes HDAC1 and HDAC2 physically interact with SIRT6 and with SIRT1, SIRT6, and SIRT7, respectively (Fig. 3). They also show a significant effect on TGF-$\beta$ signaling; however, it is not known if the interactions with the sirtuins represent any additional regulatory options on how to alter TGF-$\beta$ signaling and its components. Interestingly, the downregulation of HDAC1 but not HDAC2 blocks epithelial-to-mesenchymal transition (EMT) induced by TGF-$\beta$1 (32). The application of MS-275, a selective inhibitor of HDAC1 over HDAC3 and with no inhibitory activity towards HDAC8 effectively inhibits TGF-$\beta$ signaling in renal interstitial fibroblasts. The treatment completely abolishes the expression of TGF-$\beta$ receptor I and the phosphorylation of SMAD3, which suggests the efficient regulatory mechanism in several ways in fibrotic kidney diseases (33). However, the regulatory role of based on the SPIL analysis on HDAC2 has been observed in the pathology of induced renal injury as well. Among HDAC1-5 and HDAC8, only HDAC2 is upregulated in the rat kidney epithelial cells treated by TGF-$\beta$1. ROS (reactive oxygen

species) can induce the TGF-$\beta$1 mediated HDAC2 activation, which is reverted by the addition of antioxidant (34).

Here, the deep literature mining highlighted the significant controlling roles of SIRT1, SIRT3, SIRT6, and SIRT7 on TGF-$\beta$ signaling and its components. The critical effect on TGF-$\beta$ signaling also displays HDAC1 and HDAC2, which were identified as one of the top-ranked protein nodes within SPIN. The future research could determine the common effects of HDAC1, HDAC2, SIRT1, SIRT6, and SIRT7 interactions on TGF-$\beta$ signaling and its subnetworks and possibly also to include other sirtuins into the functional studies. Further studies of the functional links between the sirtuins and the regulation of the nuclear SMAD2 and TGF- $\beta$ receptor complex should aim to identify new and potentially interesting regulatory mechanisms.

### 3.1.2 Androgen Receptor Signaling (central hub proteins HDAC1, EP300, SMAD4, SIN3A, SMAD3)

GeneMania analysis suggested the Androgen Receptor (AR) signaling as a highly enriched pathway within the extended SPIN, however without any extensive previous research performed on the functional involvement of the sirtuins (Tab. 1). It is known that both the activities of HDAC class I and HDAC class III are essential for the antagonist mediated AR transcription activity, where SIRT1 directly interacts with AR by deacetylation at the functionally critical lysin and functions as an AR co-repressor (35). It substantially inhibits the AR-mediated growth of the prostate cancer cells and blocks both proliferation and the anchorage-independent growth of the cells (36).

The role of other members of the sirtuin family and HDAC class I should be further investigated with regards to their regulatory functions on AR signaling, which is suitable for the hormonal therapies of the prostate cancer.

### 3.1.3 PTK2 signaling (HDAC1, EP300, SIN3A, RBBP4, HDAC2, SIN3B, RBBP7, SMAD3)

The constructed PTK2 (protein-tyrosine kinase; focal adhesion kinase) signaling pathway subnetwork (Tab. 1A, Fig. 4) is another highly scored subnetwork obtained by GeneMania analysis, which contains 8/10 top rank protein nodes. All members of HDAC class I (HDAC1-HDAC11) and HDAC class III (SIRT1-SIRT7) directly interconnect with the constructed PTK2 signaling network, which suggests common regulatory mechanisms. The results of the statistical analysis of the second-order sirtuin interaction network built from the data retrieved from the human genomic aging resource dataset (37) supported the presented data. It confirmed PTK2 and PTK2B among the proteins connecting the sirtuins to the mechanisms of aging (37).

The literature mining proved the regulatory effect of SIRT1 and SIRT3 on PTK2 gene expression, despite, the detail mechanisms of the mutual functionality are just anticipating the discovery. PTK2 is a substrate of SIRT1, which was observed using myeloid-specific SIRT1 knockout mice. The animals have specifically upregulated PTK2 both at mRNA and protein levels. Contrarywise, the upregulation of SIRT1 in HEK293 cells decreases the expression of PTK2 and its acetylation (38).

Additionally, PTK2 signaling is controlled by the sirtuins indirectly through the regulation of NF-κB signaling pathway. NF-κB1 directly binds to the PTK2 promoter and activates its transcriptional activity, while SIRT1 decreases NF-κB1 signaling through its deacetylation (39) (40). The oncogenic function of SIRT3 is beside other signaling pathways, also mediated through PTK2 (41). SIRT3 binds near to the transcription start site of PTK2 gene, which is followed by the deacetylation of H4K16 at PTK2 promoter (42). The overexpression of the nuclear SIRT3 reduces stress-induced activation of PTK2, and the stress-induced degradation of the nuclear SIRT3 activates the PTK2 transcriptional activity (41).

The present analysis suggests that PTK2 signaling contains direct protein-protein interactions of all sirtuins and HDAC1-HDAC11. However, more detail regulatory mechanisms were elucidated only for SIRT1 and SIRT3, which creates additional space for the discoveries how to control the PTK2 signaling.

### 3.1.4 CARM1 and ER Signaling (central hub nodes HDAC1, EP300, HDAC2)

Significantly enriched CARM1 (co-activator associated arginine methyltransferase) signaling is involved in the regulation of the protein activities by their methylation. Both CARM1 and the sirtuins use H3 and H4 as the main substrates, and they also regulate the transcriptional activities facilitated by the histones. Since the CARM1 interaction network (Fig. 5) contains directly interacting HDAC1-11, they could mediate the functions of the sirtuins. Merging of CARM1 and Estrogen Receptor (ER) signaling network with the functionally related methyltransferase activity cluster visualized the link of SIRT1 with the rest of the network through the interaction with HDAC2 (Fig. 5). Further literature mining showed that CARM1 methylation of HuR antigen R (HuR), a repressor of the replicative senescence, regulates the expression of SIRT1. The gene silencing of CARM1 decreases the methylation status of HuR, which is followed by the downregulation of SIRT1 expression. Inversely, its upregulation increases the methylation status of HuR, which stabilizes SIRT1 mRNA and increases its protein expression (43), (44).

The limited amount of the information about the regulatory relationship of ER signaling by HDAC class I, II and III showed that it occurs through direct interactions and the regulation of the downstream gene transcription. Both the inhibition of SIRT1 and the downregulation by gene knock-down can impair ERα signaling (45).

The present analysis suggested the existence of the functional relationships between CARM1, ER, and HDAC class I- III, however only little is known about according to the literature mining. The investigation of these effects would be more than impressive for the discoveries of common regulatory mechanisms of the protein methylation and acetylation.

### 3.1.5 NOTCH signaling (central hub nodes EP300, HDAC1, HDAC2, MYC)

Interestingly, the significantly enriched Notch signaling pathway subnetwork constructed by GeneMania application contained directly linked HDAC1-11. With the aim to visualize the interconnection with the sirtuins, the Notch signaling subnetwork was merged with the protein

and histone deacetylase clusters obtained by GO enrichment analysis. The resulting network visualized the protein-protein interactions of SIRT1 with HDAC2 and HDAC4; SIRT2 is associated with the network through HDAC6; SIRT6 and SIRT7 are connected through the direct physical interactions with HDAC2, and SIRT6 shows the additional interactions link with HDAC1 (Fig. 6).

SIRT1, SIRT3, and SIRT6 regulate Notch signaling according to several literature records; however, the molecular mechanisms of the common interplay with the interacting HDAC-s class I and II are still unknown. In lung cancer cells, SIRT1 negatively regulates the endothelial Notch by the deacetylation of its intracellular domain. It binds about 500 bp upstream of the transcriptional initiation site and controls the transcription of Notch1 (46). SIRT3 functions as a tumor suppressor in gastric cancer cell lines by downregulation of Notch (47). Finally, SIRT6 is an essential negative regulator of Notch signaling in podocytes potentially interesting for proteinuria treatment development (48).

Notch signaling pathway and the sirtuins play pleiotropic roles involved in the molecular mechanisms of tumorigenesis and neurodegeneration. The examination of these mechanisms should involve directly interacting proteins from HDAC class I-III, which could be used for the developments of the multitarget therapeutic strategies.

## 3.2. The sirtuin interaction network and the mechanisms of aging

The sirtuins have an impact on aging and longevity due to their roles in the calorie-restriction (CR) responses, nutrient sensing, starvation responses, fat storage mobilization, and ketone metabolism. To uncover new relationships of the sirtuins with the mechanisms of aging, they were functionally explored together with the central signaling pathways enriched by the analysis of SPIN and the molecular mechanisms of aging.

The idea that TGF-β/SMAD signaling is the central mechanism driving the sirtuin interaction network not only in tumorigenesis but also in aging is supported by the dominant role of T*GF*-β in aging cells. TGF-*β*/SMAD signaling obtained the highest ranking by the functional pathway enrichment of the comparative transcriptomics data derived from young and old

murine hematopoietic stem cells. Ingenuity pathway analysis (IPA) revealed the transcription factor TG*F*-β1 as the most differentially expressed (more than 5-times) compared to the random occurring control (49). TGF-*β*/SMAD signaling is also involved in the UV exposure responses in the young and aged skin (50), where UV induces the downregulation of TGF-*β* RII followed by the upregulation of SMAD7 and the inhibition of the TGF-*β* induced phosphorylation of SMAD2 (50). Further, it was shown, that the expression level of SMAD6, SMAD7, and TGF-*β* decreases in the mesenchymal/stromal stem cells from aged mice (51). CR compared to the regular diet fed animals causes 24 % decrease of TGF-*β* levels both in the serum and in the tumors, where it protects against metastasis (52). The idea about the neuroprotective role of TGF-*β i*n brain aging is supported by observed increasing of the expression levels in the brain of aging mice and several days after stroke (53). High expression levels of TGF-*β*, which might balance the effects of inflamm-aging were reported in the study identifying the explanations of longevity in centenarians (54). The significant role of TGF-*β* in anti-inflammatory, neuroprotective and antiproliferative response during the neurodegenerative changes in the brain was proposed already 25 years ago (55). TGF-*β* signaling in the brain provides the neuroprotective effect, maintain the mitochondrial potential and protects the neurons against the apoptosis (56). Early events in the development of Alzheimer´s disease (AD) are accompanied by the impaired TGF-*β*1 signaling, which contributes to the neuronal injuries due to extracellular accumulation A*β* plaques and neurofibrillary tangles (56) (57). AD brain is characteristic by decreased expression of TGF-*β* RII in neurons compared to the age-matched control, which does not occur in Parkinson disease, Pick's disease, Lewy body dementia or frontotemporal dementia (56). The TGF-*β* knockout mice are susceptible to excitotoxic insults compared to the wild-type, and they exhibit degenerative changes in their brain (58). Remarkably, the overexpression of SIRT1 has longevity, and anti-aging effect in the brain by controlling circadian clock  The brain-specific SIRT1 overexpressing mice exhibited a significant life extension, and old mice showed the anti-aging effect compared to the control (9). SIRT1 also regulates the circadian clock in the brain by controlling main transcription factors BMAL1 and CLOCK. By age-dependent SIRT1 downregulation, the circadian clock is also deteriorating and contributing to the aging of the organism (59).

The regulation of stress-induced premature senescence is the aging-related mechanism shared by both TGF-$\beta$ (60) and the sirtuins. The implantation of the senescent cells into young mice provided evidence that the senescence might drive the aging-related pathologies since it was sufficient to induce the aging-related dysfunction (61). Interestingly, the compounds preventing the cell senescence could revert the aging process (61), which provides evidence that the control of the aging process is feasible. The senescence has tumor suppressive functions in the young organisms, however, during the aging, it promotes tumorigenesis (62). One of the candidates for the development of the anti-aging therapies is SIRT6, which stabilizes telomere heterochromatin and prevents replicative senescence (63). Due to the essential roles of SIRT6 in the heterochromatin silencing, its decreased activity can significantly contribute to the deterioration of the organism during the aging and age-related diseases.

SIRT1 and SIRT6 are also involved in the laminopathy-based syndrome of premature aging as it was discussed earlier (64). Retinoblastoma (RB) signaling network is the regulator of the premature senescence in the cells and the expression of the stress-induced TGF-$\beta$ (60). The top rank proteins RBBP4 and RBBP7 are retinoblastoma protein (RB1) interacting proteins and fulfilling the functions of RB signaling network. The shared feature of RBBP4, RBBP7, and SIRT6 is the regulation of their functions by laminA/C and progerin, a primary factor of the premature aging syndrome Hutchinson-Gilford Progeria Syndrome (HGPS) (64).

The nutrient sensing, starvation responses, fat storage mobilization, and ketone metabolism are essential parts of the molecular mechanisms of aging, which includes the sirtuins. The responses to the Peroxisome proliferator-activated receptors (PPARs) have an essential metabolic role during both the starvations and fed stages. The expression levels of adipocyte-specific transcription factor PPAR-$\gamma$ tightly connects to the aging, and it increases in bone marrow with the aging (51). Fat mobilization starts after the starvation activated SIRT1 binds to PPAR-$\gamma$ (PPARGC1A) promoter and alters its target genes (65). Interestingly, SIRT1 and SIRT7 play antagonistic functions in adipose tissues, where SIRT7 increases the activity of SIRT1 by preventing the autodeacetylation (66). Additionally, SIRT7 also regulates the lipid metabolism in the liver and adipocytes, where it activates the genes participating in fatty acid uptake, and it controls the protein turnover. It seems the direct physical interaction exists

between SIRT7 and E3 ubiquitin ligase, where SIRT7 has a regulatory and essential function (67). Several proteasome 26S subunits are involved in the interaction network connecting sirtuins with the ketone metabolism and nutrient sensing (Fig. 7), which suggests close regulation of the metabolism of lipids and protein turnover.

The level of androgen and estrogen hormones (AR and ER signaling) is under the strong influence of aging. Then the hormonal decline is associated with muscular atrophy, weakening of the bone strength and shortening of the lifespan. The age-dependent decline in the hormonal activity can be at least partially restored by the anabolic effect of the intense and regular exercise and by very careful hormone replacement therapies (68). The regular exercise is fundamental for the increase in the activities of both SIRT1 and SIRT6, which are naturally declining during the aging of the animal models. The exercise stimulated raise of the activity of SIRT1 in the skeletal muscles of the aged experimental animals corresponds with the increased activity of nicotinamide phosphoribosyltransferase (NAMPT) involved in NAD+ biosynthesis required for sirtuin activity (69). The testosterone treatment reverses sarcopenia in aged mice by increasing Notch signaling (70), which suggests the interplay of AR and NOTCH signaling in the muscular atrophy during aging. The control of these signaling pathways together with the stimulation of SIRT1 and SIRT6 provides the promising base for the prevention of the sarcopenia during the aging.

Finally, PTK2 signaling recognized by GeneMania analysis as a priority pathway of SPIN was also identified as a hub protein node of Common signaling signature network (CSSN) of longevity and aging (71). PTK2 links the age-related disease genes to the longevity; however it is not considered as a direct longevity-associated protein (71). Another study, which involves PTK2 also supports the common role of the sirtuins and PTK2 and PTK2B in the mechanisms of aging (37).

# 4. Conclusions

The sirtuins continue to be promising targets for the anti-aging therapies and the attractive subject for further scientific studies on the field of aging and longevity. They effectively prevent premature cellular senescence and aging (72), which is a worthy subject of geriatrics.

Here, the pathway enrichment, gene function prediction analysis and protein node prioritization in the combination with the deep literature mining supports the common functions of HDAC class I-III and top rank proteins nodes in several signaling pathways (TGF-*β,* PTK2, CARM1, AR, ER, and Notch) functionally linked to the molecular mechanisms of aging. Mutually, the top rank proteins nodes and pathways manage the calorie restriction effect, longevity, cellular senescence, and fat storage mobilization. They also regulate the age-related diseases such as Alzheimer´s disease and cancer. The further investigation of their interplay with the sirtuins would bring new insights into the anti-aging treatments. The future direct targeting by low molecular modulators would be advantageous for the adjustment of their activities. New developments of the multitarget therapeutic strategies for aging and age-related diseases would bring the knowledge of how to accomplish better health and longevity.

## Conflicts of interest

There is no conflict to declare

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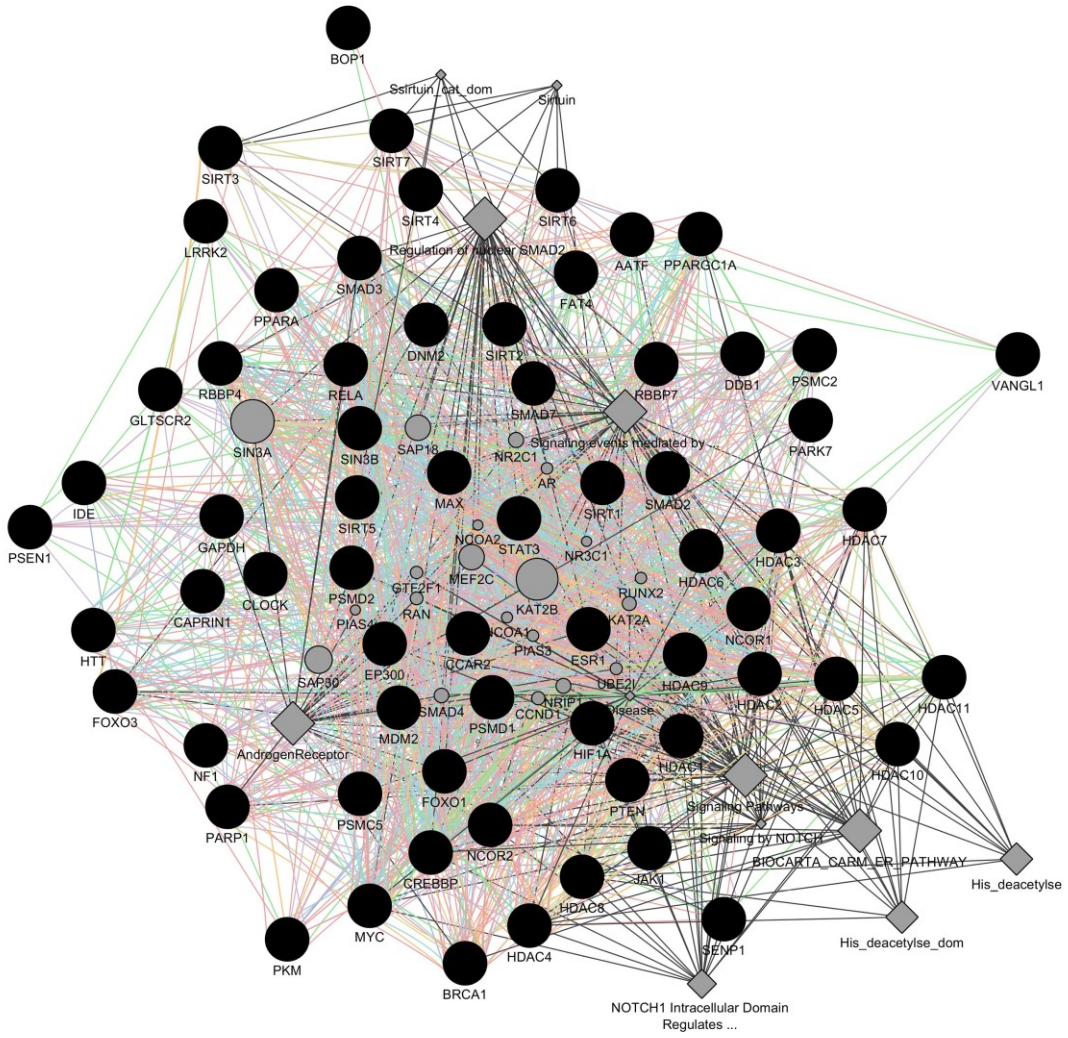



Fig. 1 Pathway enrichment and the gene function prediction analysis of the sirtuin protein interaction network (SPIN) constructed by GeneMania application (3.4.1).

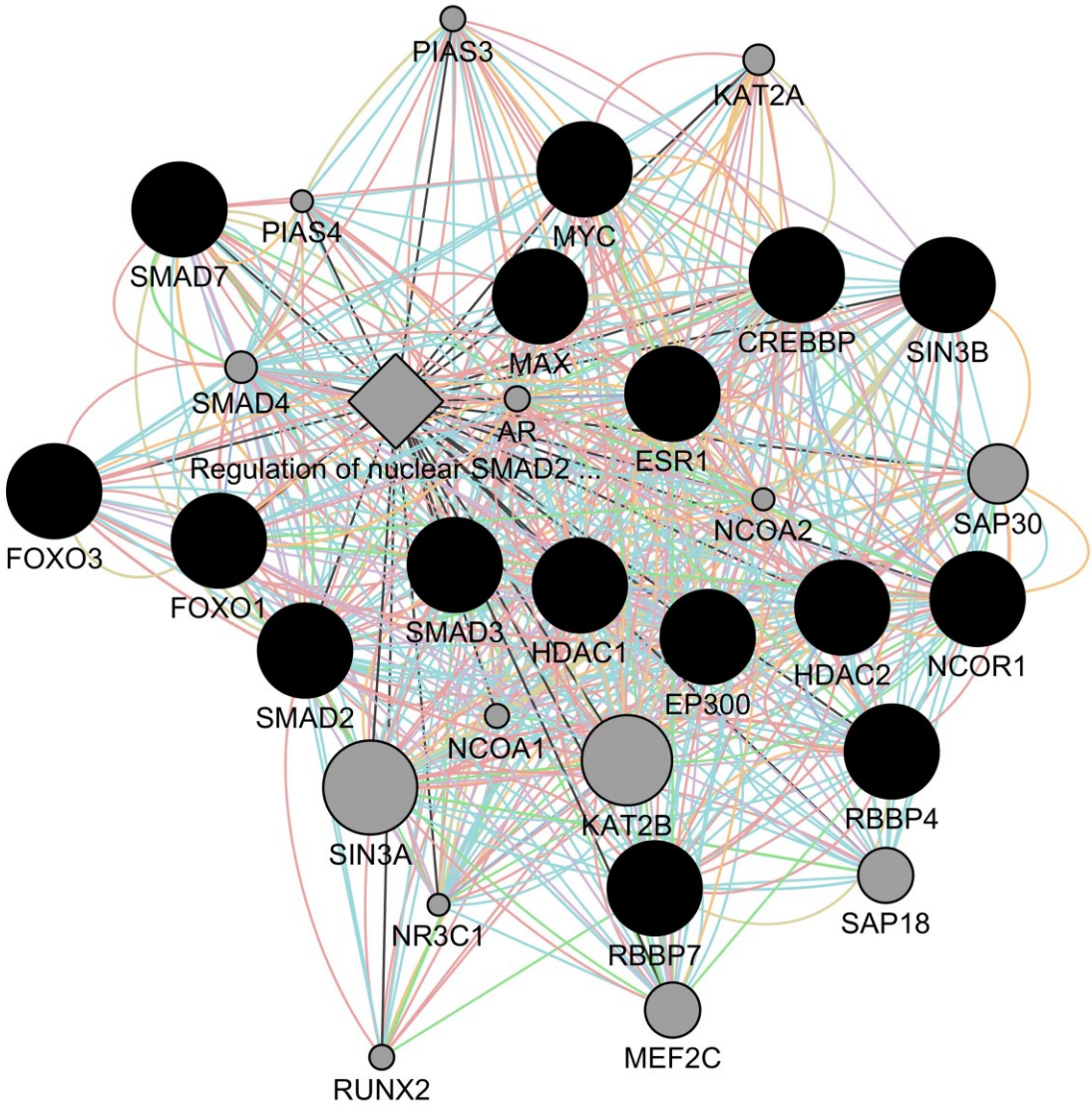



Fig. 2 The protein interaction subnetwork 'Regulation of nuclear SMAD2 signaling', a consolidated pathway cluster with the highest weight within the extended SPIN.

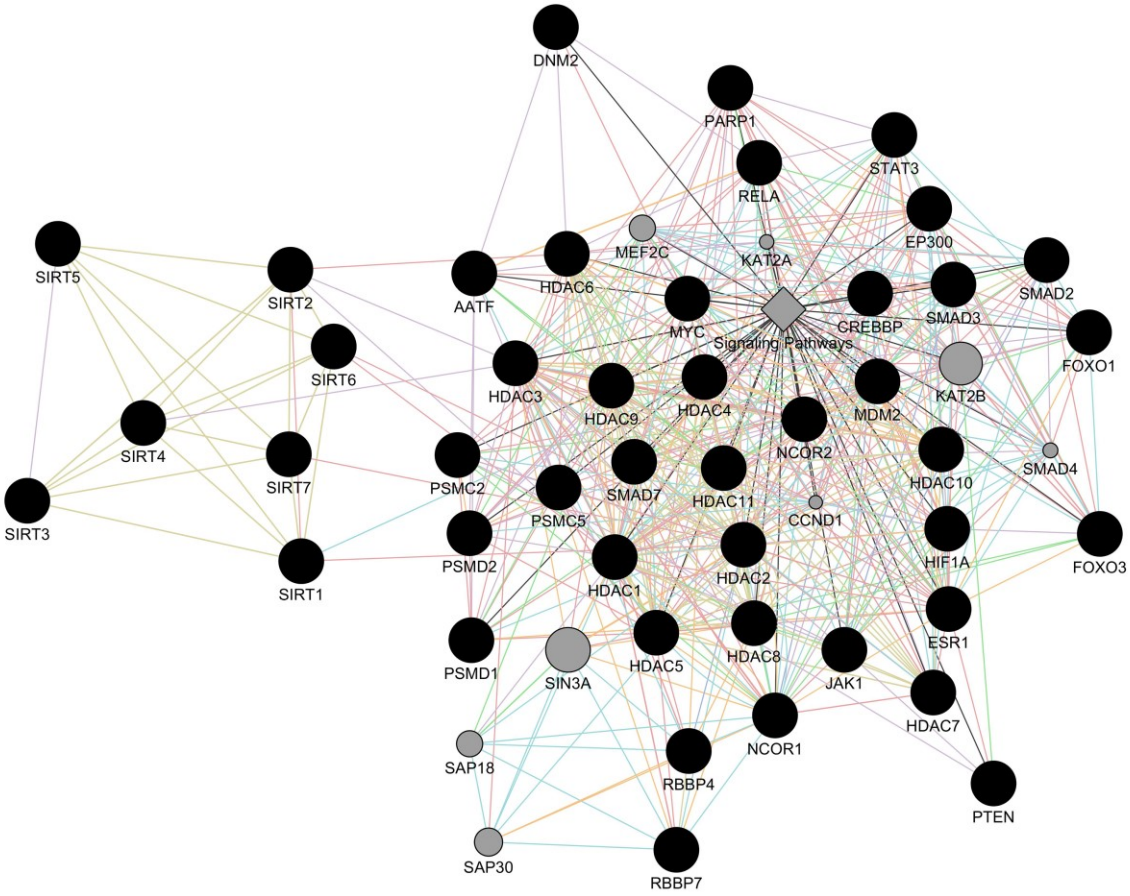
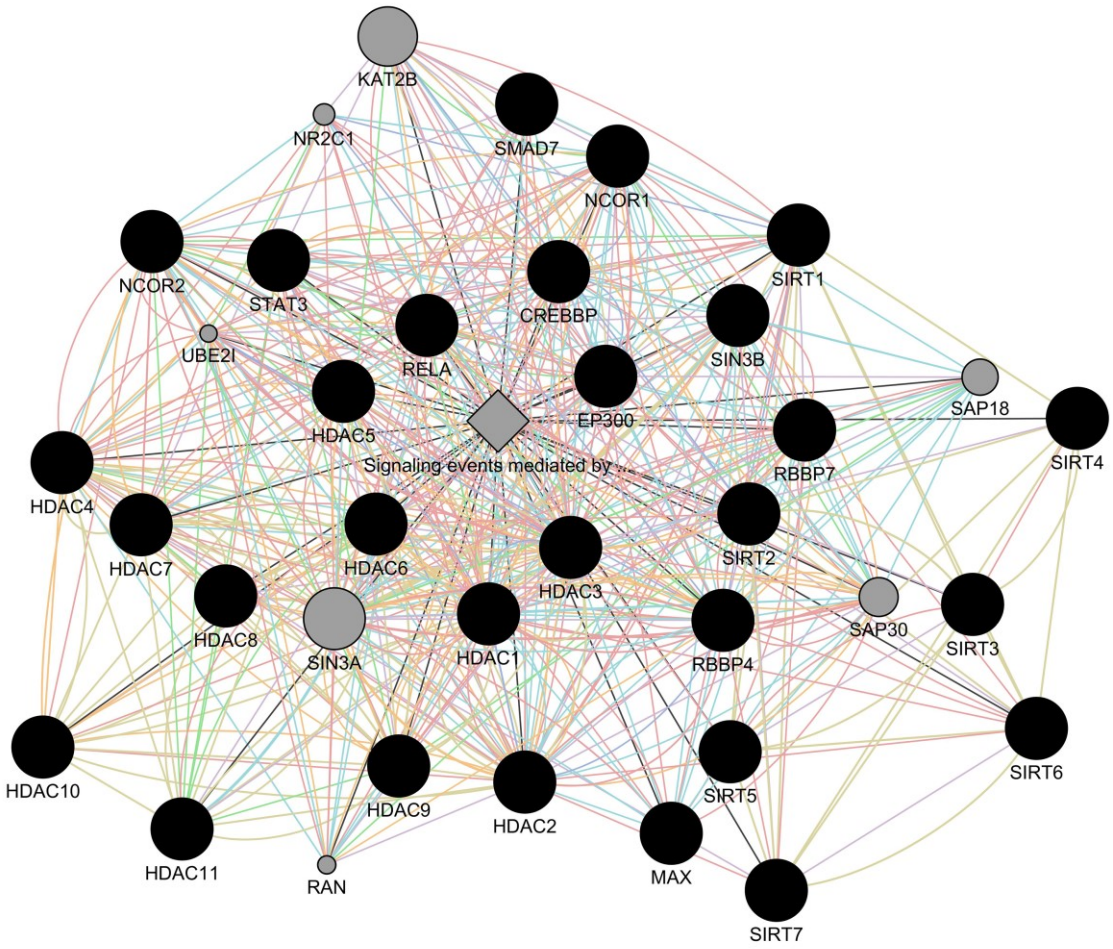



Fig. 3 The protein interaction subnetwork 'TGF$\beta$ receptor signaling' merged with the protein and histone deacetylase cluster obtained by GO enrichment analysis.

◆ - the symbol for TGF$\beta$ receptor signaling pathway




1

2

3

4 Fig. 4 The protein interaction subnetwork ‘PTK2 signaling pathway’ which contains all
5 HDAC class I-III.

- the symbol for PTK2 signaling pathway

7

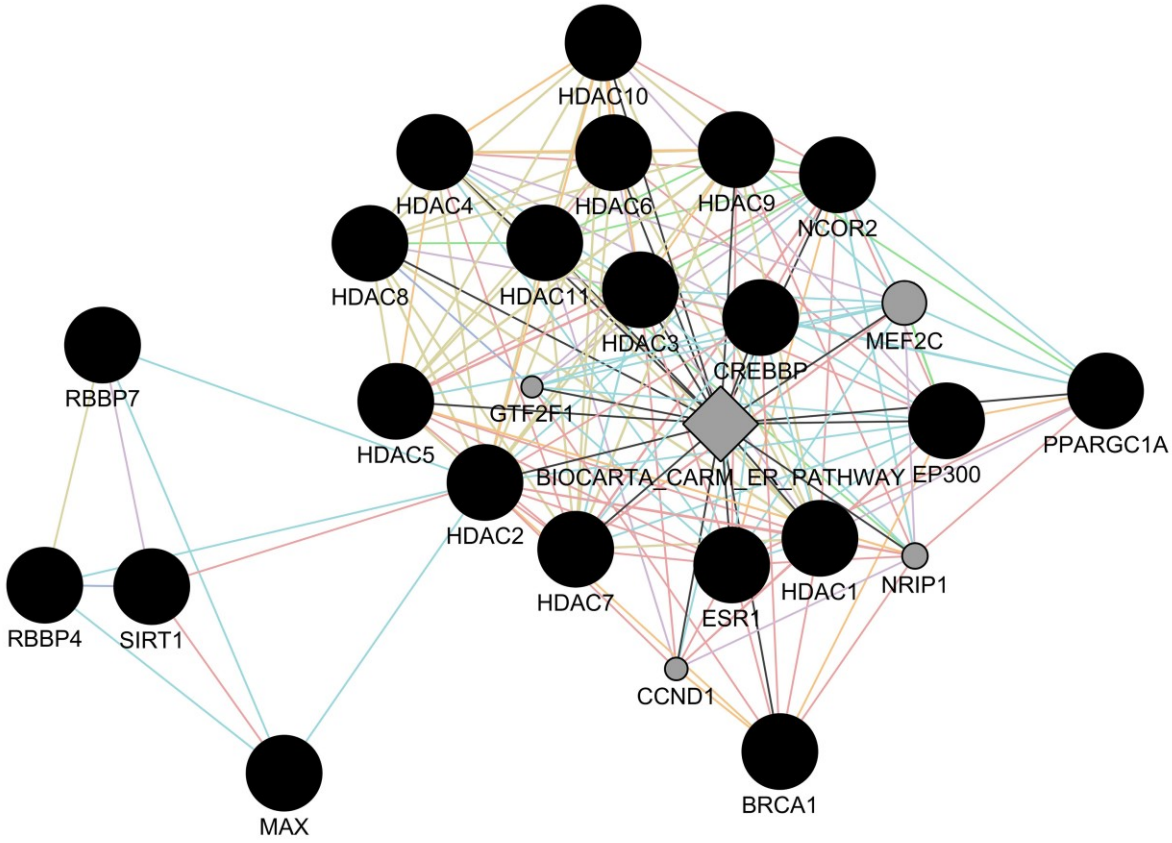



Fig. 5 The protein interaction subnetwork 'CARM1 and ER signaling pathway' merged with the methyltransferase cluster obtained by GO enrichment analysis.

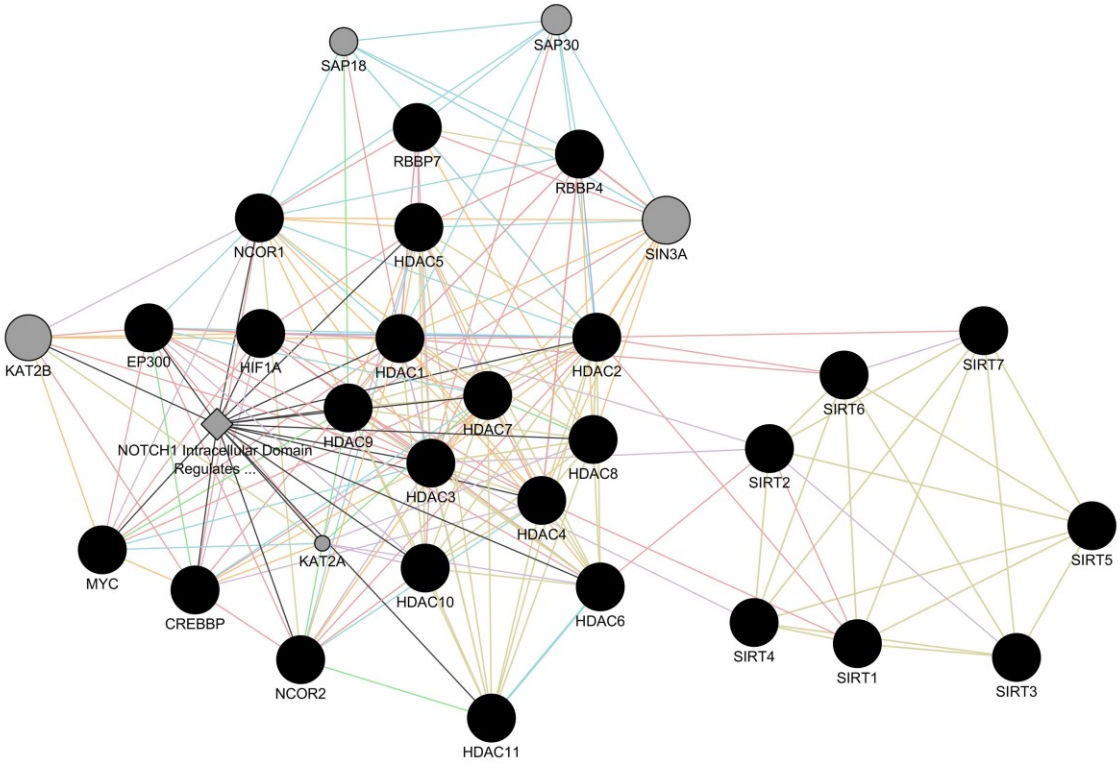



Fig. 6 The union of 'Notch signaling regulatory network' and 'histone and protein deacetylase' interaction networks constructed by merging of the corresponding clusters obtained by GeneMania (3.4.1) analysis and GO enrichment analysis.

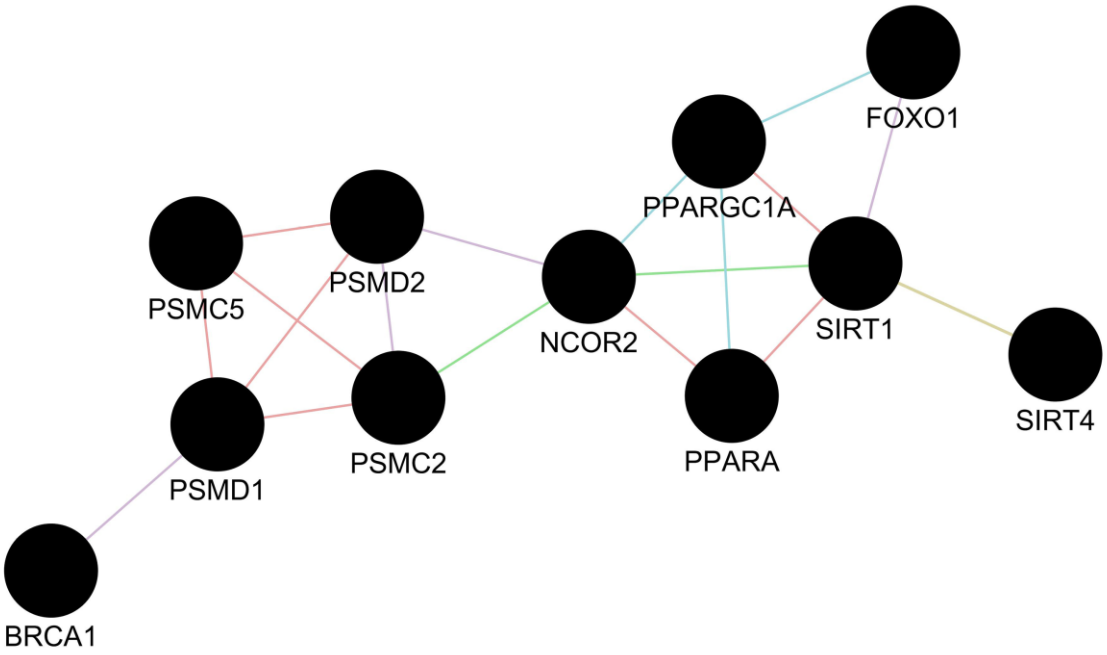



Fig. 7 The interaction network 'Ketone metabolism and the response to the nutrient levels' constructed by merging of the subnetworks of the interacting proteins involved in the regulation of the ketone metabolic processes and the cellular responses to the nutrients obtained by GO Enrichment analysis.

Tab.1 The consolidated pathways with the highest scores in the pathway enrichment analysis and the gene function prediction analysis of the extended SPIN.

| Network | Weight | Source |
|---|---|---|
| | 47.50 | |
| Regulation of nuclear SMAD2 signaling | 11.41 | Pathway Interaction Database NCI-NATURE - Regulation of nuclear SMAD2 signaling |
| Androgen Receptor signaling | 9.40 | IOB Androgen Receptor |
| Signaling by TGF-$\beta$ receptor complex | 9.34 | Reactome React_111102.4 Signaling Pathways: Signaling by TGF-$\beta$ Receptor Complex |
| Signaling events mediated by Focal Adhesion Kinase | 5.52 | Pathway Interaction Database NCI-Nature - Signaling events mediated by Focal Adhesion Kinase |
| Biocarta_CARM_ER_Pathway | 5.44 | MSIGDB_C2 Biocarta_CARM_ER_Pathway |
| Notch1 Intracellular Domain Regulates Transcription | 3.68 | Reactome React_118780.2 NOTCH1 Intracellular Domain Regulates Transcription |
| Disease | 1.87 | Reactome React_116125.4 Disease |
| Signaling by Notch | 0.84 | Reactome React_299.5 Signaling by Notch |

Tab. 2 Top 10 highest rank protein nodes of the extended SPIN analyzed by Cytohubba (0.1) application operating under Cytoscape (3.7.0) software.

| Rank | Name | Score |
|---|---|---|
| 1 | HDAC1 | 1.48498739399E12 |
| 2 | EP300 | 1.484736769938E12 |
| 3 | SMAD4 | 1.484366098632E12 |
| 4 | MYC | 1.484202246194E12 |
| 5 | SIN3A | 1.484168389614E12 |
| 6 | RBBP4 | 1.484127164634E12 |
| 7 | HDAC2 | 1.484019931764E12 |
| 8 | SIN3B | 1.483968130458E12 |
| 9 | RBBP7 | 1.483343159574E12 |
| 10 | SMAD3 | 1.483282808622E12 |

# Supporting information

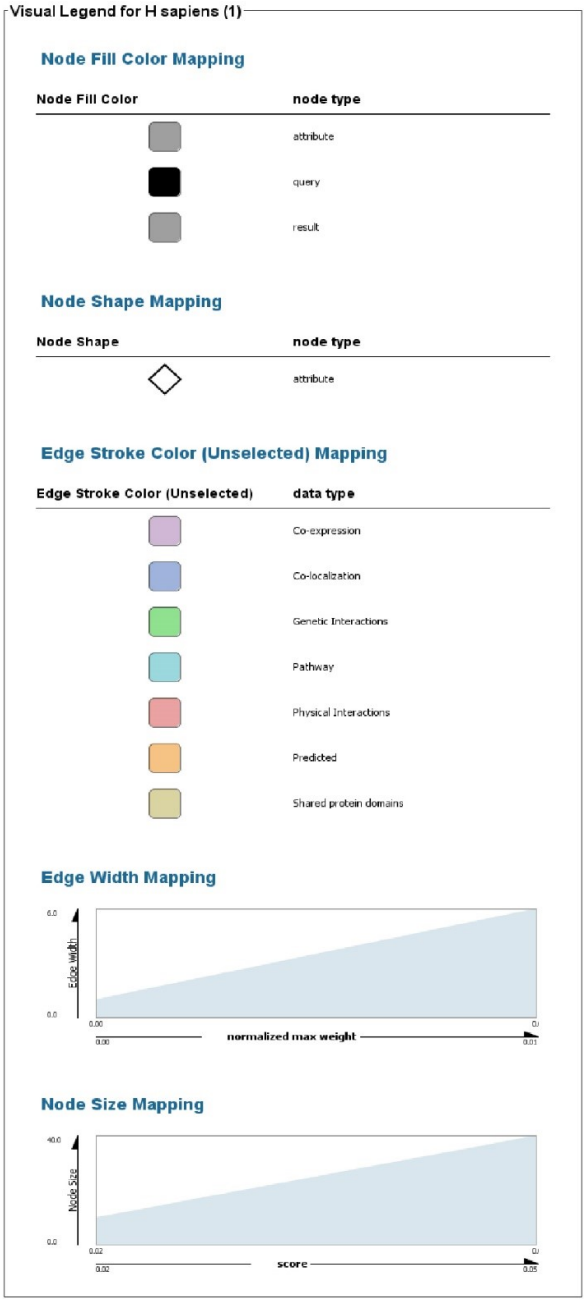



Fig. S1 The definition of the types of interactions and nodes used in the protein interaction networks.