Linking TPP2 to the protein interaction and signalling networks

Jarmila Nahálková, *[a]

[a] - Biochemworld co., Snickar-Anders väg 17, 74394, Skyttorp, Uppsala County, Sweden; Mobile: + 46 73 7707700; E-mail: jarmila.nahalkova@biochemworld.net;

* - corresponding author

Abstract:

The present manuscript explores signalling and metabolic pathways which mediate functions of Tripeptidyl-peptidase 2 (TPP2) using the analysis of its protein-protein interaction network. The protein interaction data were retrieved from our previous experimental published work and the public databases BioGRID 3.5.169; STRING v.11 and Agilent Literature Search 3.1.1 using the increased threshold criteria. Totally 13 interacting proteins were obtained from the open public sources, and they were combined with TPP2 interacting proteins included in the interaction network PIN7, which involves seven interacting proteins with the pleiotropic biological functions. The interaction network of TPP2 was analysed by the pathway enrichment, the gene/protein function prediction and the gene/protein node prioritisation analysis by GeneMania and Cytohubba applications run under Cytoscape 3.7.0 environment. The results of the study were combined with deep literature mining. The most enriched signalling pathways were functionally linked to the regulation of the adaptive and innate immunity (ID, Kit Receptor, BCR, IL2, the regulation of NFκB), the aerobic glycolysis (ID and IL-2), tumorigenesis (TGFβ, p53, the high priority nodes MAPKs, and the control of mTOR), diabetes (Kit receptor, the top priority node GSK3β) and

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neurodegeneration (the regulation of mTOR and Aβ peptide degradation). The BioGRID database mining also showed the interaction with two lung cancer suppressors (DOK3, DENND2D), a protein involved in the increased risk of the lung cancer in smokers (CYP1A1) and a protein involved in asthmatic reactions (CHIA). The potentially unexplored functions of TPP2 in the lung pathologies are also discussed with regards to these interactions. The new exciting function might suggest the interaction with methyltransferase CARNMT1, which modifies di- and tripeptides and the xenobiotic processing enzyme CYP1A1.

Introduction:

TPP2 functions as a proteolytic enzyme downstream complementing the ubiquitin-proteasome system, which contributes to the recycling of the amino acid pool in the cells. Its vital role is to establish a balance between the protein degradation functions of the proteasome and the lysosomal activity, which is essential for the amino acid homeostasis in the cells (Lu et al., 2014). Besides this obviously house-keeping role it is also involved in the immune responses, senescence, apoptosis, cell cycle regulation, DNA damage responses and the key glycolytic enzymes in the mammalian cells (Huai et al., 2008), (Stepensky et al., 2014), (Lu et al., 2014), (Hilbi et al., 2000), (Stavropoulou et al., 2005), (Gavioli et al., 2001), (Huai et al., 2008), (Preta et al., 2009), (Preta et al., 2010). The inhibitors of TPP2 enzymatic activity are suggested as a tumour-suppressing agent; however, the side effects are not clearly defined (Huai et al., 2008).

The age-related function is suggested using TPP2 knockout mice, which have induced cell-type specific death programs expressed by early immunosenescence and shorter lifespan due to premature ageing of animals (Huai et al. 2008).

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TPP2 is a protein node of PIN7, a protein-protein interaction network of seven proteins with pleiotrophic functions (Nahálková, 2016). It is likely that these protein-protein interactions of TPP2 mediate the above mentioned biological events and therapeutically applicable effects (Nahálková and Tomkinson, 2014), (Nahálková, 2015) (Nahálková, 2016).

In the search for the signalling pathways involving TPP2, it was found that the enzyme contributes to the MAPK signalling in response to the oxidative and DNA damage agents (Preta et al., 2009). In Burkitt lymphoma cells, TPP2 knockdown by RNAi alters not only the expression levels of several members of the MAPK pathway but also TGF-β signalling pathway and Focal adhesion assemblies. Interestingly, MAPK8 and MAPK1 are the central protein nodes of TPP2 interaction network constructed based on the differential gene expression (Sompallae et al., 2008).

The present study utilises the pathway enrichment, gene/protein function prediction and the gene/protein node prioritisation methods for the analysis of TPP2 interaction network constructed based on the information retrieved from the public databases and our previous research. The study utilises the hypothesis that the proteins interacting directly via protein-protein interactions are with the high confidence participating in identical cellular and molecular functions. If they represent disease genes, the mutations in their genes lead to similar disease phenotypes (Oti, 2006).

The main aim of the analysis is to show new information about the signalling pathways and the critical gene and protein nodes driving the extended interaction network of TPP2. The results obtained by the interaction network analysis are further discussed based on their functions in the cellular amino acid homeostasis, the aerobic glycolysis, the adaptive and innate immunity, tumorigenesis, diabetes, neurodegeneration and their mutual relationships. Separately are discussed five TPP2 interaction partners with functions previously not related to TPP2.

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Material and Methods

The list of TPP2 interacting proteins (TIP) was created from the databases BioGRID (Biological General Repository for Interaction Datasets; version 3.5.169) (Stark, 2006); STRING version 11.0 (Franceschini et al., 2013; Szklarczyk et al., 2017) and Agilent Literature Search 3.1.1 (Cline et al., 2007) application. For the BioGRID database, the minimum of two experimental pieces of the evidence was applied as the threshold for including the protein on the TIP list (BioGRID database). The selected protein-protein interactions have high scores >0.9 derived from CompPASS, a software platform which uses unbiased metrics to assign the confidence measurements to express the probability of the interactions between parallel proteomic experiments (Sowa et al., 2009). Increased confidence level 0.7 was applied for the interacting proteins collected from *H. sapiens* STRING database, where the interactions from the databases, text mining and the experiments were applied during the selection. The Agilent Literature Search, a meta-analysis tool containing the interaction data collected from PubMed, OMIM and United States Patent and Trademark Office (USPTO) was used with the 'interaction lexicon limited' settings. Finally, the directly interacting proteins included in PIN7 were added to complete the interaction list (MYBBP1A, CDK2, SIRT7, p53) (Nahálková, 2016, 2015; Nahálková and Tomkinson, 2014).

The final TIP list contained the following proteins: ADCY10 C9ORF41 CDK2 CHIA CSE1L CYP1A1 DENND2D DOK3 ERAP2 MAPK1 MAPK3 MSH2 MYBBP1A p53 SIRT7 TIPRL TPP2

The analysis using Cytoscape (3.7.0) and its applications GeneMania (3.4.1) and Cytohubba (0.1): GeneMania (Warde-Farley et al., 2010) analysis was run under Cytoscape (3.7.0) environment using TIP list as a query against *H. sapiens* database and by including all types of the

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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. Fig. 1S shows the legend defining the types of the nodes and their interactions. The interaction networks were further analysed by Cytohubba, a Cytoscape application, which identifies the crucial regulatory hub protein nodes and pathways of the interaction network (Chin et al., 2009). The topological method Maximum click centrality (MCC) was used for the analysis, which provides the highest precision in the prediction of the essential nodes (Schneider and Laskowski, 1974). Further, the settings of the analysis were adjusted for the selection of the first-stage nodes and the display of the shortest path.

Results and discussion:

The interaction network analysis used in the present work benefits from the integration of the genomic and proteomic data with the information about the pharmacological targeting involved in GeneMania, which extends the provided interactions with other known gene/protein nodes. The involvement of TPP2 in the signalling pathways was analysed by the construction of the extended interaction network, which was further examined by the pathway enrichment, the gene and protein function prediction and the protein node prioritisation methods (Fig. 1, Tab. 1).

TPP2 interaction network constructed by GeneMania application was further analysed based on the priority of the protein nodes, which identified the critical hub protein nodes navigating the main molecular mechanisms (Fig. 2). The high priority protein nodes could be potentially applicable for the development of the multi-target therapeutics. As expected, several top priority protein nodes are involved in MAPK signalling (MAPK1, MAPK8 and MAPK3), which was

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previously reported to be affected by TPP2 (Preta et al., 2009). The priority nodes MAPK1, MAPK3, and MAPK8, are involved in several pathways, which are guiding the immune responses, the primary metabolism of the glucose and the molecular mechanisms of tumorigenesis. MAPK1 and MAPK3 are also directly interacting with TPP2, which supports the significance of the observations that MAPK-s are playing significant roles in the extended TPP2 interaction networks. MAPK8 and MAPK1 are also the central protein nodes of TPP2 interaction network constructed based on the differential gene expression (Sompallae et al., 2008). TPP2 regulates the phosphorylation level of MAPK1 and MAPK3, which has an impact on several downstream signalling pathways (Wiemhoefer et al., 2015). The targeting ERK1(MAPK3)/ERK2(MAPK1) is the promising strategy to overcome the cancer resistance to MEK and BRAF inhibitors (Liu et al., 2018), however, according to the recent reports only very few ERK inhibitors reached to the clinical trials (Liu et al., 2018). The combination strategy of use ERK inhibitors together with the compounds targeting upstream targets was suggested for increased efficacy of the treatments (Liu et al., 2018).

There exists the regulatory feedback loop between ERK1/ERK2 and TGF-β signalling (Ventura et al., 2017), which creates the functional link between the most enriched signalling pathway (Tab. 1) and the highest priority nodes of the extended TPP2 interaction network (Fig. 2). Additionally, MAPK-s also play the central roles in significantly enriched ID, BCR, and Kit receptor signalling pathways (Fig. 1, Tab. 1).

The second highest significance protein node of the extended TPP2 interaction network is GSK3B (Tab. 1); however, without any existing evidence of the mutual interaction with TPP2. The kinase, which is involved in many signalling pathways, is phosphorylating more than 100 known substrates. The search for the post-translational modifications of TPP2 yielded no reports in the

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literature. However, the search performed using PhosphoSitePlus® (Hornbeck et al., 2015) showed the presence of the phosphorylation, acetylation, monomethylation and ubiquitinations sites on TPP2 molecule, which were identified using proteomic discovery mass spectrometry https://www.phosphosite.org/proteinAction?id=10851&showAllSites=true. It shows that the phosphorylation by GSK3B is possible. TPP2 also directly interacts with several deacetylases/kinases such as SIRT7, MAPK1, MAPK3 and CDK2, but the potential role of the post-translational modifications and the identity of the modifying enzymes remains to be GSK3B discovered.

The results of the significantly scored pathway enrichment analysis are further functionally categorised and discussed based on their roles in the immune system, the aerobic glycolysis, tumorigenesis and their mutual interrelationships (Fig. 3). The protein-protein interactions with five proteins (DOK3, DENND2D, CYP1A1, CHIA, CARNMT1) with distinct functions are also discussed since their roles might reveal new functions for TPP2 as well.

The role of TPP2 in the immune system

It is not surprising that several highest score signalling pathways enriched by the GeneMania analysis are involved in the immune system function (Tab. 1), since the observations from the loss of TPP2 function mutations taking place in Evans syndrome (ES) support this role. The disease is demonstrated by the increased cellular immunosenescence of T and B cells, the defects in apoptosis and by the immunodeficiency combined with the raised susceptibility to viral and microbial infections. The premature senescent phenotype in ES patients and TPP-2 deficient mice is accompanied by the presence of anti-nuclear and anti-cytoplasmic antibodies (Stepensky et al.,

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158 2014) (Lu et al., 2014), which also occurs in the naturally aged human populations (Vadasz et al.,
159 2013). The reduced TPP2 level also contributes to the decreased adaptive and innate immunity
160 (Perez et al., 2012). Three signalling pathways (ID, BCR, and IL-2), which are involved in the
161 immune system functions might mediate the autoimmunity and immunodeficiency due to the
162 absence of TPP2. The potential functional relationships of TPP2, and these pathways are discussed
163 more in detail further.

164

165 **ID signalling pathway (priority nodes MAPK3, MAPK1, RAF1)**

166 ID-s (Inhibitors of differentiation) (Tab. 1) are proteins, which influence several essential
167 signalling pathways through their extremely versatile interactions with different transcription
168 factors. They are involved in the immune responses and the regulation of immunoglobulin
169 production.

170 ID1 plays a significant role in the inhibition of the tumour microenvironment caused by responses,
171 triggered among other factors also by TGF-β and by this way it promotes the tumour growth and
172 vascularisation (Papaspyridonos et al., 2015). ID2 suppresses the serum levels of IgE and prevents
173 allergic hypersensitive reactions (Katakai et al., 2002). As a part of the TPP2 interaction network,
174 ID signalling could be functionally linked to the immunodeficiency and altered innate and adaptive
175 immunity observed in ES patients and TPP2 knockout mice, (Stepensky et al., 2014) (Lu et al.,
176 2014).

177 Both ID-s and TPP2 play roles in the cellular senescence, and they act as inhibitors of the cell
178 cycle regulators. However, ID-s functions are linked to the replicative senescence (Hara et al.,
179 1994), while TPP2 deficiency is connected to the stress related senescence with no contribution to

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the replicative senescence (Stepensky et al., 2014). The peptide aptamers targeting ID1 and ID3 induces the cell cycle arrest and apoptosis (Mern et al., 2010), which is therapeutically interesting (Sharma et al., 2012). ID1 and ID3 silencing promote the cell cycle arrest at G1, and they play a role in prostate cancer development.

ID signalling network also contains direct interactions of TPP2 with the cell cycle regulatory protein nodes CDK2, MAPK3, and MAPK1 (Fig. 4). The TPP2 downregulation by RNAi results in the group of the differentially expressed genes involved in the control of the cell cycle. MAPK signalling is the primary pathway regulating the cell survival, proliferation, immune responses, ion transport, metabolism and genomic stability functionally associated with TPP2 (Sompallae et al., 2008). CDK2 downregulation activates G1/S checkpoint, increases cell apoptosis and DNA damage response (Neganova et al., 2011). Additional priority node RAF1 is RAS activated member of MAPK signalling involved in the phosphorylation of MEK followed by the activation of ERK. RAF1 fulfils the anti-apoptotic function in cells (Baccarini, 2005).

The results of the pathway enrichment analysis suggest that ID signalling might mediate the functions of TPP2 in immune responses, cellular senescence and the cell cycle. Further links to the potential roles of TPP2 and ID-s in aerobic glycolysis will be discussed later.

BCR signalling pathway (priority nodes MAPK3, GSK3B, MAPK8, MAPK1, PIK3R1, RAF1, AKT1)

The second pathway significantly enriched within the extended TPP2 interaction network with the function in the immune system is BCR (B-cell receptor) signalling (Fig. 1, Tab. 1, Fig. 5). The most (about 85 %) of the human immunodeficiency disorders are connected to the mutations of

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Bruton Agammaglobulinemia tyrosine kinase (BTK), which is essentially phosphorylated and activated through BCR pathway (Perez et al., 2012). The mutations affecting the activity of the protein node PIK3R1 (Fig. 5) encoding a regulatory p85α subunit of the phosphatidylinositol 3-kinase (PIK3) are causing the immunodeficiency in the patients (Deau et al., 2014). The lack of p85α subunit causes the entire absence of B cells (Perez et al., 2012). Conclusively, BCR signalling is another pathway worthy of further investigation, which might modulate the immunodeficiency caused by TPP2 deficiency.

IL-2 pathway (priority nodes MAPK3, MAPK8, MAPK1, MYC, PIK3R1, RAF1)

The activation of the IL-2 pathway, a part of TPP2 interaction network (Fig. 1, Tab. 1, Fig. 6) stimulates the anti-tumour activity of the immune system. The inhibition of TPP2 activity by butabindide decreases the IL-2 expression (Lu et al., 2014), which supports the existence of the functional link with the TPP2 interaction network. Interestingly, TPP2, IL-2 and ID pathway, fulfil the functions both in immunity and aerobic glycolysis (Fig. 3), which suggest that further study of the priority nodes of these interaction networks in the relationship with TPP2 might bring new motivating results.

The metabolism of glucose

Remarkably, TPP2 mediated immunodeficiency is linked to the impaired glycolysis, and the enzymatic activity of TPP2 indeed regulates several major glycolytic enzymes essential for the metabolism of cancer cells. The impaired glycolysis can be triggered by the downregulation of

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TPP2 expression (Lu et al., 2014), which affects the effector function of T cells requiring active aerobic glycolysis and the fast switch from oxidative phosphorylation (Chang et al., 2013).

The metabolic switch towards aerobic glycolysis occurring in Warburg effect required for the growth of the cancer cells is supported by the specific protein expression of isoform pyruvate kinase M2 (embryonic form, PKM2) (Christofk et al., 2008). The replacement of PKM2 by the isoform PKM1 with higher enzymatic activity or the enzymatic activation of PKM2 by the low molecular weight compounds directly impairs tumorigenesis and the growth of tumour xenografts (Anastasiou et al., 2012). The expression of PKM2 is very interestingly regulated by the enzymatic activity of TPP2. The downregulation of the key glycolytic enzymes PKM2 and hexokinase 2 (HK2) and impaired glycolysis after butabindide treatment demonstrates the crucial metabolic regulatory effect of TPP2 in naive CD4T cells (Lu et al., 2014).

ID and IL2- signalling

As it was mentioned above, the significantly enriched pathways ID and IL-2, which are functionally linked to the adaptive and innate immunity, also take part in the primary glucose metabolism (Fig. 3). ID1 reprogrammes the metabolism of the cancer cells by altering the aerobic glycolysis and the metabolism of the glutamate. It is upregulated in several hepatocarcinoma cell lines, and its expression is regulated through the MAP/ERK pathway (Fig. 4) (Sharma et al., 2016), which is also the significant mediator of TPP2 functionality. The knockdown of ID1 at aerobic conditions alter several critical glycolytic enzymes, including PKM2 and HK2, which shifts the metabolism towards the oxidative phosphorylation (Sharma et al., 2016). It also significantly decreases glutaminolysis, which also represents the main metabolic adaptation of the cancer cells

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for their proliferation. Xenografted ID1-shRNA HEPA1-6 cells in nude mice cause significantly smaller tumour growth both based on the volume and weight (Sharma et al., 2016). At anaerobic conditions, when HIF-1 is strongly activated, the expression of ID1 is suppressed, which suggests that it does not affect the glycolysis at these conditions (Sharma et al.,2016).

Both TPP2 and ID1 exhibits a significant effect on the vital glycolytic enzymes PKM2 and HK2, which supports the result of the pathway enrichment analysis. Top rank signalling pathways ID and IL-2 play functions in the adaptive and innate immunity and participate in the primary glucose metabolism, which further supports their significance in the extended TPP2 interaction network. The detail interplay between TPP2, ID-s and ID signalling, however, remains unexplored.

Kit receptor (CD117, priority nodes MAPK3, GSK3B, MAPK8, MAPK1, PIK3R1, RAF1, AKT1)

Kit receptor signalling is involved in the regulation of anti-apoptosis, cell proliferation and as an intermediate in several signalling pathways. The pathways, which are activated by c-Kit signalling include PI3K/mTOR/Akt, Ras/ERK and Src family kinases (Fig. 7).

Kit receptor signalling is affected in many cancers by mutations, and the inhibitors of its kinase activity are considered as a promising target, however mostly as a part of the multi-targeting approach only (Zaman Huri et al., 2016). C-kit signalling is also involved in glucose homeostasis and B cell development. Its deficiency in the male mice is causing early signs of diabetes symptoms and the reduction of B cell mass. The females also develop the abnormal glucose metabolism as males, however with later onset (Krishnamurthy et al., 2007).

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The detail information about the functional connection of TPP2 with Kit receptor signalling pathway is generally missing in the literature. However, the common effects on mTOR and ERK signalling might provide the explanation, why it appeared among the significantly enriched pathways of the extended TPP2 interaction network.

Molecular mechanisms involved in tumorigenesis

TGF-*β* receptor pathway (priority nodes MAPK3, MAPK8, MAPK1, MYC, PI3KR1, RAF1, AKT1, p53)

TGF-*β* receptor pathway (Fig. 8) obtained the highest score in the present pathway enrichment analysis. In the earlier study, the downregulation of TPP2 also causes the changes in MAPK, TGF-*β,* and Focal adhesion pathways in the gene expression study performed in Namalwa cells, where MAPK1 and MAPK8 are the central hubs of the TPP2 interaction network. Interestingly, INHBE, a member of the TGF-*β* family, is the most down-regulated gene observed after the silencing of TPP2 by shRNA. (Sompallae et al., 2008).

Interestingly, there exists a functional relationship between TGF-*β* signalling pathway and the ERK1/2 signalling, which plays an essential role in the regulation of epithelial-mesenchymal transition (EMT) in non-small cell lung cancer cells (NSCLC). TGF-*β*1 plays a tumour suppressor role in the early stages of the tumour development; however later it promotes the growth and metastasis through EMT. TGF-*β*1 increases phosphorylation of ERK1 and increases its kinase activity, which is essential for TGF-*β*1 induced EMT *in vitro* (Xie et al., 2006).

TGF-*β* signalling also regulates PI3K/mTOR/Akt pathway, which is suggested to play the importance in TPP2 interaction network due to the PI3KR1 and AKT1 among the priority nodes.

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There exists direct evidence that TPP2 enzymatic activity affects mTOR since butabindide can decrease mTOR activity (Lu et al., 2014). There exists experimental evidence that butabindide reduces the cellular amino acid level leading towards the depletion of mTOR activity and increased expression of TFEB, which is the activator of the lysosomal biogenesis in the Jurkat T cells (Lu et al., 2014) (Fig. 3). The cell treatment with TGF-β enhances EMT, the formation of mTORC2 and it increases the phosphorylation of Akt and mTORC2 is required for TGF-β induced EMT (Lamouille et al., 2012).

TGF-β receptor pathway as a part of TGF-β signalling was shown here to be the most crucial pathway navigating the functions of the extended TPP2 interaction network. Its interaction network is functionally linked with ERK1/ERK2 and mTOR signalling, which also has an experimentally proven functional significance in the extended TPP2 interaction network.

P53 pathway (priority nodes GSK3B, AKT1, p53)

TPP2 interacts with p53 by the direct protein-protein interaction, which correlates with the third highest significance score for the p53 pathway within TPP2 interaction network. Together with another p53 interacting protein CDK2 and MYBBP1A, TPP2 forms the interaction network with multiple interactions, which would be interesting to characterise (Fig. 9) (Nahálková, 2015; Nahálková, 2016; Nahálková, 2019).

TPP2 regulates several crucial transcription factors besides p53 and NF-κB, which provides the experimental proof of the functional relationships (Huai et al., 2008). The possible explanation could give the interaction of TPP2 with MYBBP1A and p53, where MYBBP1A is an activator of the gene transcription of p53 through the direct binding. It acts as a co-repressor of NF-κB as well

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(Nahálková and Tomkinson, 2014; Nahálková, 2016). TPP2 overexpression also displays a downregulating effect on the gene expression of MYBBP1A during the cell detachment of HEK293 cells (Nahálková, 2015).

The p53 interaction network (Fig. 9) contains the priority node GSK3B (Fig. 2) directly interacting with p53, which, however, does not interact with TPP2 according to the present status of the experimental evidence. The interaction regulates DNA damage responses in the cells, which is also one of the main cellular functions of TPP2. The DNA damaging agents cause quick upregulation of both nuclear p53 and GSK3B, which increases their physical interaction and activates GSK3B (Watcharasit et al., 2002). GSK3B also regulates the DNA damage-induced p53 signalling by the feedback loop (Watcharasit et al., 2002). Several pathways are controlled by active GSK3B, which inhibits MDM2 mediated regulation of p53, leading to the DNA repair, cell cycle arrest and conditionally to the apoptosis (Jacobs et al., 2012).

Both the present analysis and the literature review supports the hypothesis that the p53 pathway mediates the effects of TPP2 in the cancer cells. Based on the current status of the knowledge, TPP2 is linked into p53 interaction network through several direct protein-protein interactions with MYBBP1A, CDK2 and p53. The interaction network analysis also emphasised the role of the priority node GSK3B, whose roles are mostly related to the p53 and other signalling pathways, however, without clear functional connection to TPP2.

ID signalling (MAPK3, MAPK1, RAF1)

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ID1 signalling regulates the tumour invasion by the promotion of the matrix metalloproteinase expression (MMP-s) (Ling et al., 2006). ID1 is required for the invasiveness of the tumour cells, and it is essential for angiogenesis (Volpert et al., 2002).

The similar function also has CD147, a pleiotropic transmembrane glycoprotein with diverse biological roles, which include its inducing effect on extracellular matrix metalloproteinases (MMPs), lymphocyte responsiveness, spermatogenesis, and the regulation of monocarboxylate transporters. As a part of the protein interaction network PIN7, CD147 interacts with TPP2 indirectly through the interaction with MYBBP1A (Nahálková, 2016), which might suggest interconnection with p53 signalling.

The ID proteins operate downstream of TGFβ, which represses ID2 and ID3 genes (Moustakas et al., 2004). The functional roles upstream of Rb and p53 signalling makes them promising targets for metastatic cancer and angiogenesis treatments (Lyden et al., 1999). ID1 activates RAF-1/MAPK pathway essential for the prostate cancer cells proliferation (Ling et al., 2006), where it promotes epithelial-mesenchymal transition (EMT) by the activation of the Akt pathway and the resistance to the anti-tumour drug-induced apoptosis (Zhang et al., 2007). ID1 is also a biomarker of the prostate cancer grade, which is the most crucial cancer type linked to the function of TPP2 interaction network (Sharma et al., 2012) (Tab. 1).

ID signalling represents another candidate pathway, which links TPP2 to the tumorigenesis. The regulatory effect of ID signalling on MMP-s is shared with the indirectly interacting protein CD147, and together, they promote cancer cell invasiveness.

Other significant protein-protein interactions of TPP2

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A special mention deserves the result of the data mining of BioGrid database, which surprisingly revealed several interactions with the proteins linked to the lung cancer pathology. The physical interaction of TPP2 with two lung cancer suppressors (DOK3, DENND2D), one protein linked to the increased lung cancer risk in smokers (CYP1A1) and an additional interacting protein with the significant function in asthma (CHIA). The interactions were proven by two experimental affinity capture experiments combined with MS identification (Huttlin et al., 2017, 2015), which provides increased confidence that they do not represent the experimental artefact. According to the Human Protein Atlas (https://www.proteinatlas.org/), TPP2 is expressed in the lung tissue on the medium level compared to other tissues, which is matching with its potential role.

Additional interacting protein is involved in the processing of drugs and the environmental xenobiotics (CYP1A1). TPP2 also physically interacts with the methyltransferase modifying di- and tripeptide substrates CARNMT1, while both proteins fulfil functions in the sperm.

The cellular and molecular functions of the mentioned five protein-protein interactions of TPP2 are merely unknown. However, they will be further discussed with regards to the common functions, which suggests an unexplored role of TPP2 in the lung tissue pathologies.

DOK3

Interesting finding among TPP2 interacting proteins is DOK3 (Docking Protein 3), a member of the DOK family of lung cancer suppressors. The genetic knockout of DOK1-3 in the experimental mice ultimately leads to lung adenocarcinoma development (Berger et al., 2010).

Within the extended TPP2 interaction network, DOK3 links TPP2 to Kit Receptor and BCR signalling and through the genetic interaction with SMAD3 also to TGF-β signalling (Fig. 1).

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374 DOK3 knockout mice exhibits significantly increased α-pERK1/ERK2 nuclear staining,
375 suggesting the role of DOK3 in MAPK signalling cascade, which is also the pathway regulated by
376 TPP2.

377 The detail characterisation of TPP2 and DOK3 interactions together with the common molecular
378 mechanisms controlling the MAPK, Kit Receptor, BCR signalling and TGF-β signalling is at the
379 moment waiting for further experimental exploration.

380

381 **DENND2D**

382 The lung tumour suppressor DENND2D, a TPP2 interacting protein triggers apoptosis in the cells
383 with DNA damage, and its down-regulation activates tumorigenesis. The lung tumour specimen,
384 precancerous lesions and immortalised lung cancer cell lines exhibit down-regulation of
385 DENND2D on mRNA level. Its over-expression in non-small cell lung cancer cells stops cell
386 proliferation by stimulating apoptosis (Ling et al., 2013; Kanda et al., 2014). The function of
387 DENND2D and TPP2 interaction would be undoubtedly worthy of further additional scientific
388 exploration.

389

390 **CYP1A1**

391 Prominent third interaction with the protein linked to the lung cancer pathology represents the
392 interaction of TPP2 with CYP1A1, a member of cytochrome p450 superfamily involved in the
393 processing of drugs and environmental xenobiotics.

394 Interesting is the correlation between CYP1A1 exon 7 valine polymorphism in the smokers, who
395 have more than 2-times higher DNA damage due to the exposure of polycyclic aromatic

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hydrocarbons (PAH) DNA adducts compared to the control, thus showing an increased risk of the lung cancer (Shields et al., 1993). The reason for it is the exposure of the cells by PAH included in the smoke exhalations such as benzo-α-pyrene (BaP), which increases the expression of CYP1A1 in dependence on p53 expression. PAH are processed into DNA-reactive intermediates causing mutations with carcinogenic effects (Wohak et al., 2016). CYP1A1 also mediate the detoxification of another xenobiotic aristolochic acid I (AAI) by the demethylation of the compound (Stiborová et al., 2014).

CHIA – acidic chitinase

CHIA is a chitin processing enzyme present in the crustacean shells and parasites with a function in the asthmatic reactions. It is highly differentially expressed in the lung of the asthma patients compared to the healthy controls (Zhu et al., 2004) and thus it represents another enzyme interacting with TPP2, which is functionally related to the lung pathology.

CARNMT1

CARNMT1 is methyltransferase, which can modify both di- and tripeptides containing C-terminal His, with high specificity to carnosine and Gly-Gly-His. According to the gene expression data collected from HPA, GTEx, and Fantom5 dataset projects (https://www.proteinatlas.org), TPP2 and CARNMT1 are highly overexpressed on mRNA level in human testis, where TPP2 fulfils a function in the sperm maturation (Zhou et al., 2013).

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Conclusions

TPP2 is a protein node of the interaction network PIN7 created by seven proteins with pleiotrophic functions related to the tumorigenesis, neurodegeneration and ageing (Nahálková, 2016; Nahálková 2019). The present study supplements it by the in-depth study of the extended TPP2 interaction network, which includes integrated genomic and proteomic data. It defines the signalling pathways navigating the effect of the interactions network, and it identifies the critical protein nodes driving it, which could be used for the multi-target drug development.

TPP2 regulates the amino acid homeostasis by its enzymatic activity and by the lysosomal biogenesis. It also regulates the activity of the main glycolytic enzymes, which together with the lysosomal activity, further impacts the growth of the tumour cells and adaptive, innate and autoimmunity. The public database and the literature mining surprisingly also revealed the interactions with the proteins involved in the lung pathologies and the xenobiotic detoxification, which would deserve more attention in the future experimental explorations.

The multitasking biological role of TPP2 suggested by the results of the present study has the potential to reveal impressive scientific knowledge in the future. The time will show which of TPP2 interactions will be possible to use for the regulation of the whole interaction network.

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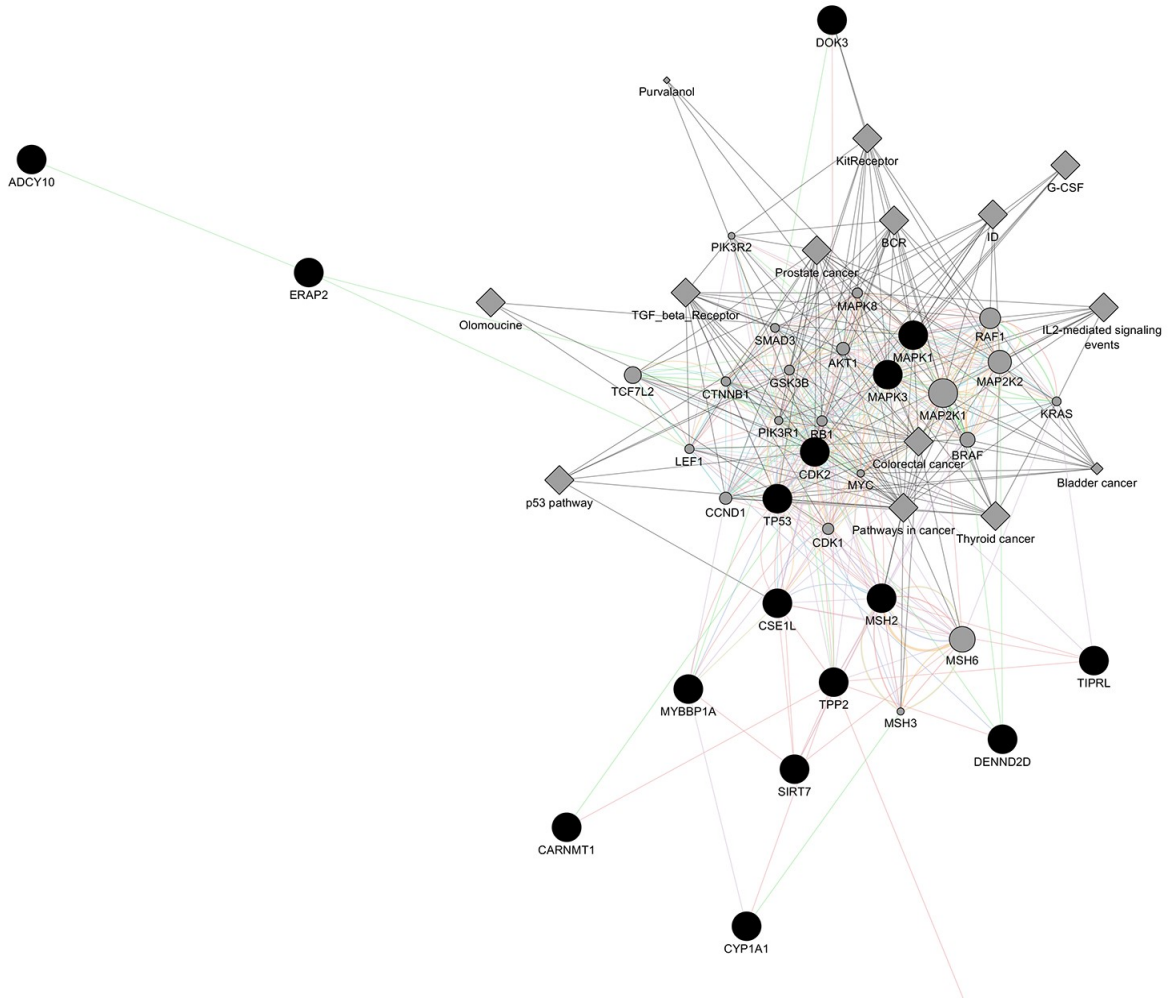

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616 Fig. 1 Pathway enrichment and the gene function prediction analysis of the TPP2 interaction
617 network constructed by GeneMania application (3.4.1).

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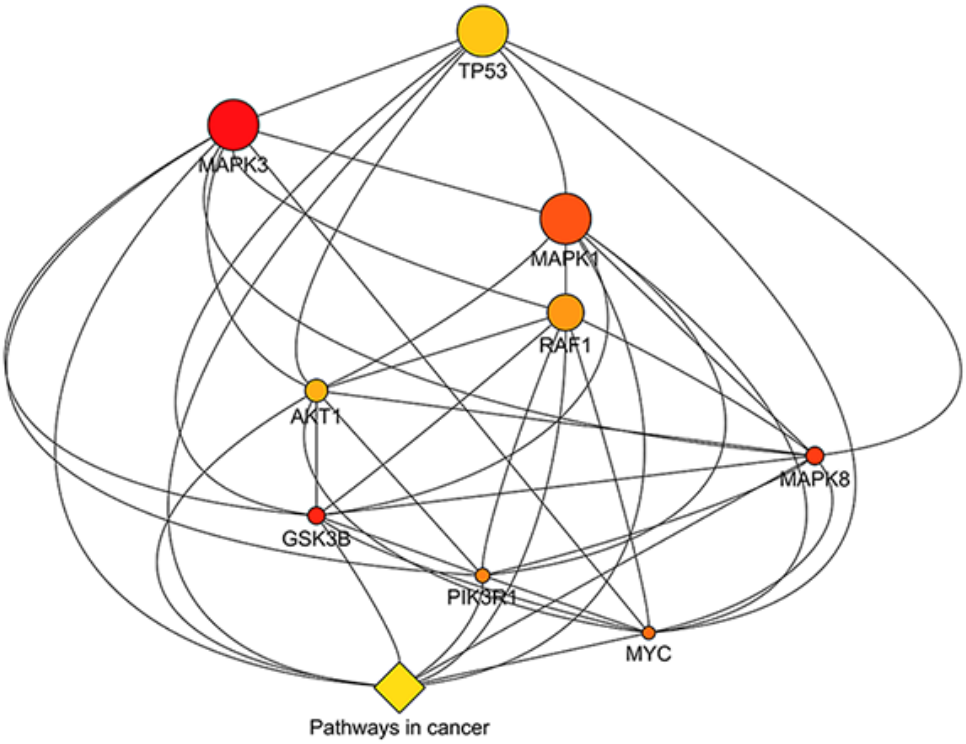

Top 10 protein nodes ranked by MCC method Rank	Name	Score
1	MAPK3	964957.0
2	GSK3B	797826.0
3	MAPK8	797040.0
4	MAPK1	775866.0
5	MYC	653448.0
6	PIK3R1	485800.0
7	RAF1	476664.0
8	AKT1	457560.0
9	TP53	423324.0
10	Pathways in cancer	411804.0

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621

622 Fig. 2 Top 10 highest rank protein nodes of the extended TPP2 interaction network analysed by

623 Cytohubba (0.1) application operating under Cytoscape (3.7.0) software.

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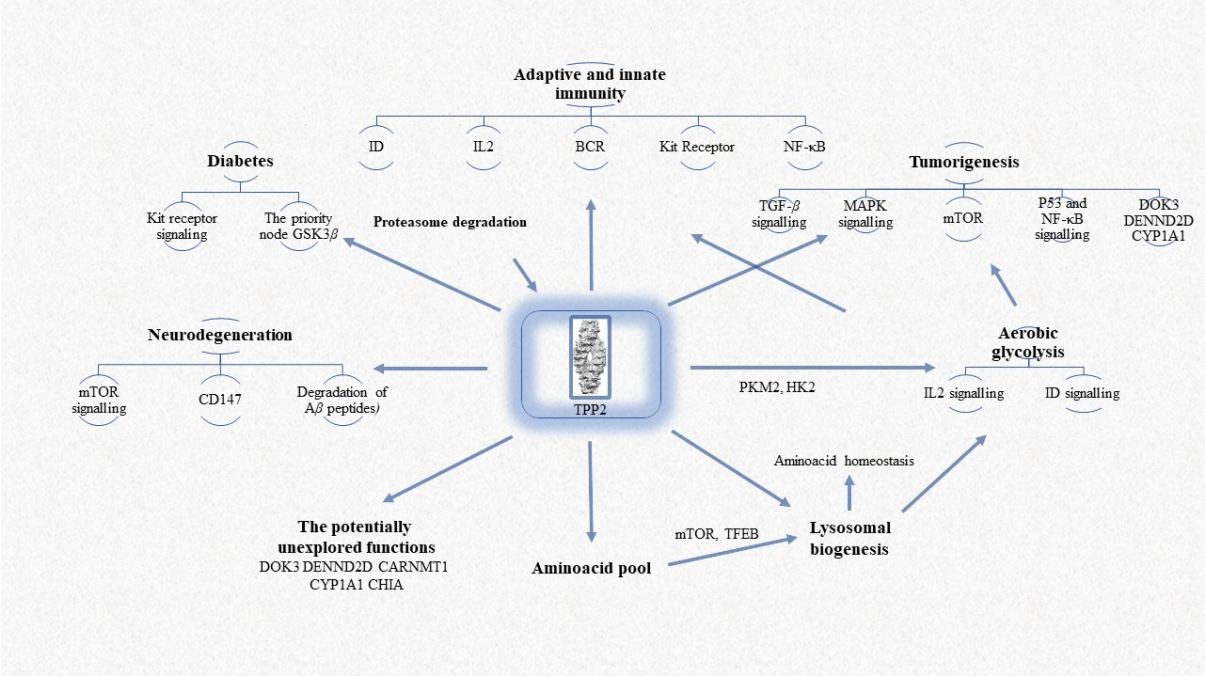

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627 Fig. 3 The overview of the biological functions of TPP2 showing the result of the pathway

628 enrichment study and the deep literature mining.

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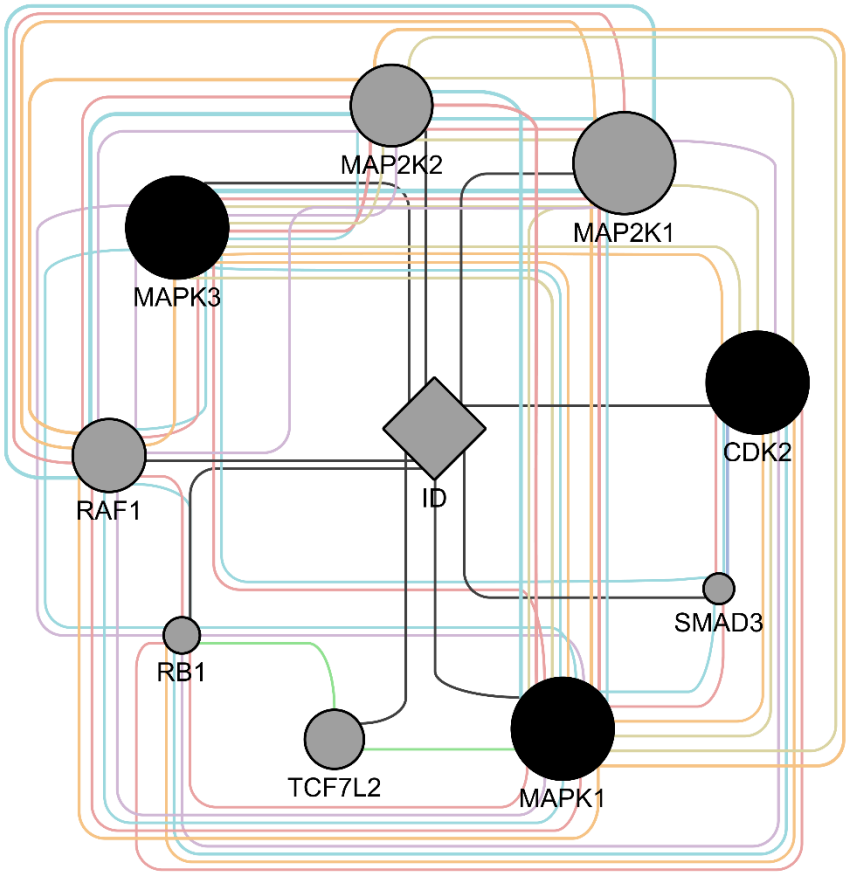

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632 Fig. 4 The protein interaction subnetwork ‘ID signalling’, a consolidated pathway cluster with

633 the third highest weight within the extended TPP2 interaction network.

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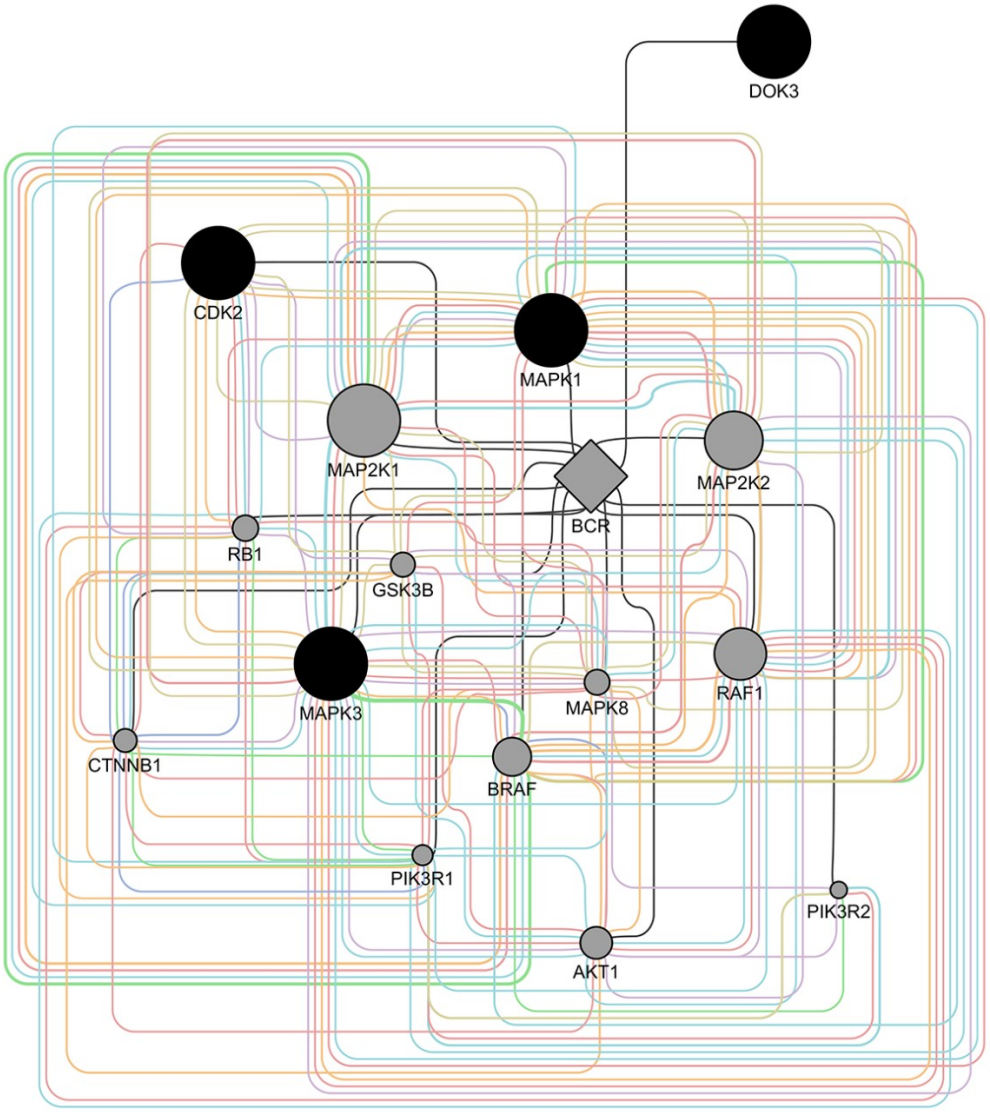

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637 Fig. 5 The protein interaction subnetwork 'BCR signalling', a consolidated pathway cluster with

638 a significant weight within the extended TPP2 interaction network.

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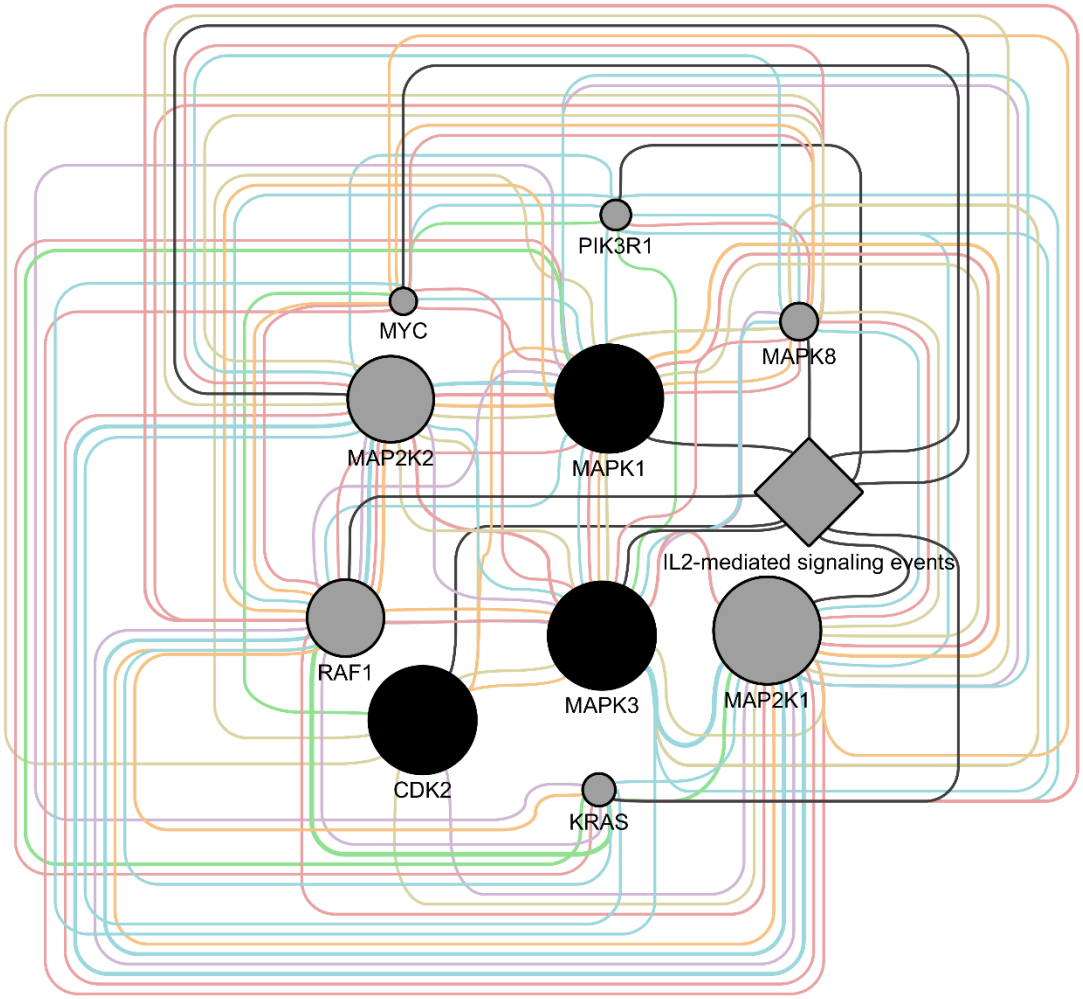

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642 Fig. 6 The protein interaction subnetwork ‘IL-2 signalling’, a consolidated pathway cluster with

643 a significant weight within the extended TPP2 interaction network.

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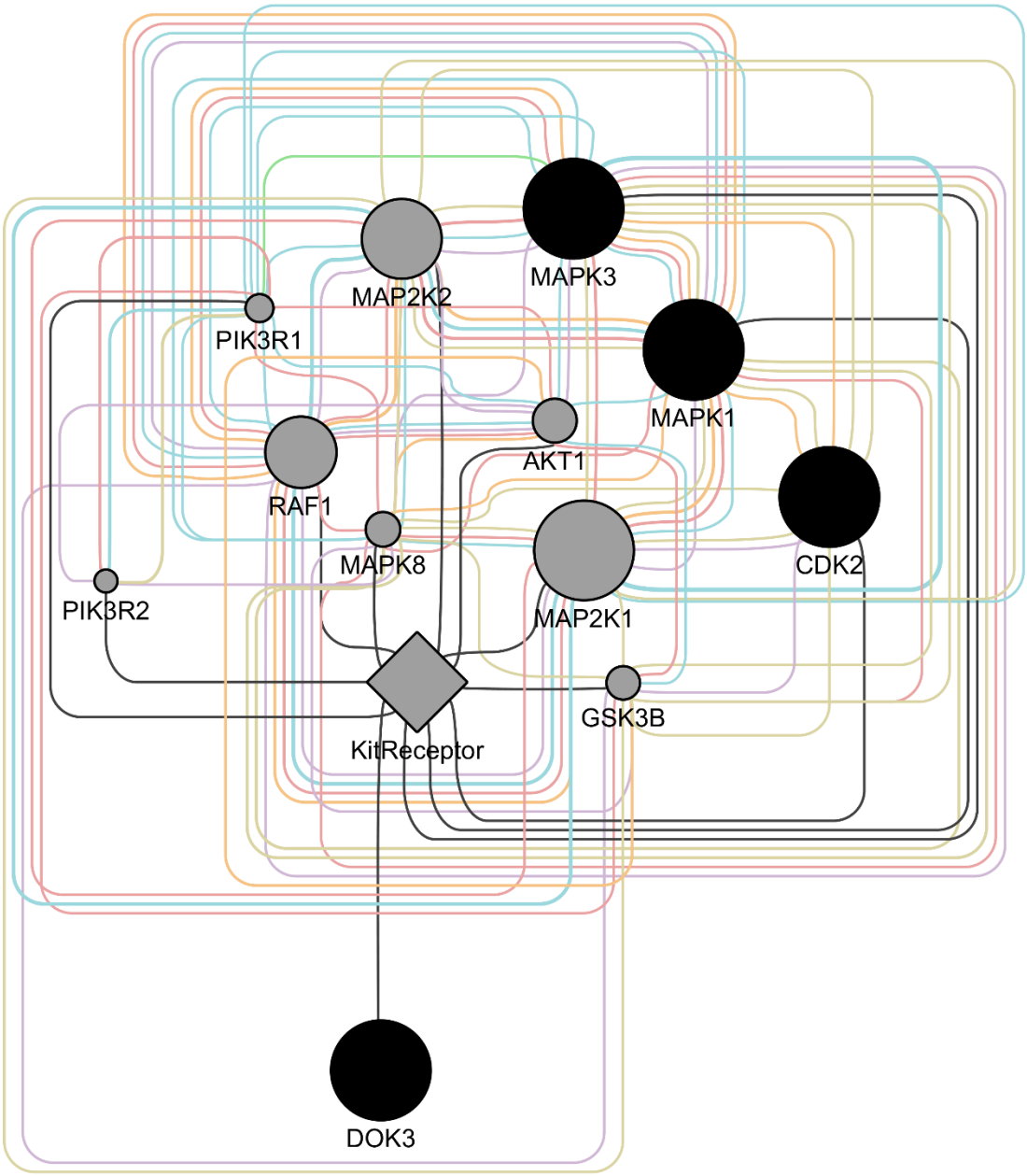

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646

647 Fig. 7 The protein interaction subnetwork ‘Kit receptor signalling’, a consolidated pathway

648 cluster with a significant weight within the extended TPP2 interaction network.

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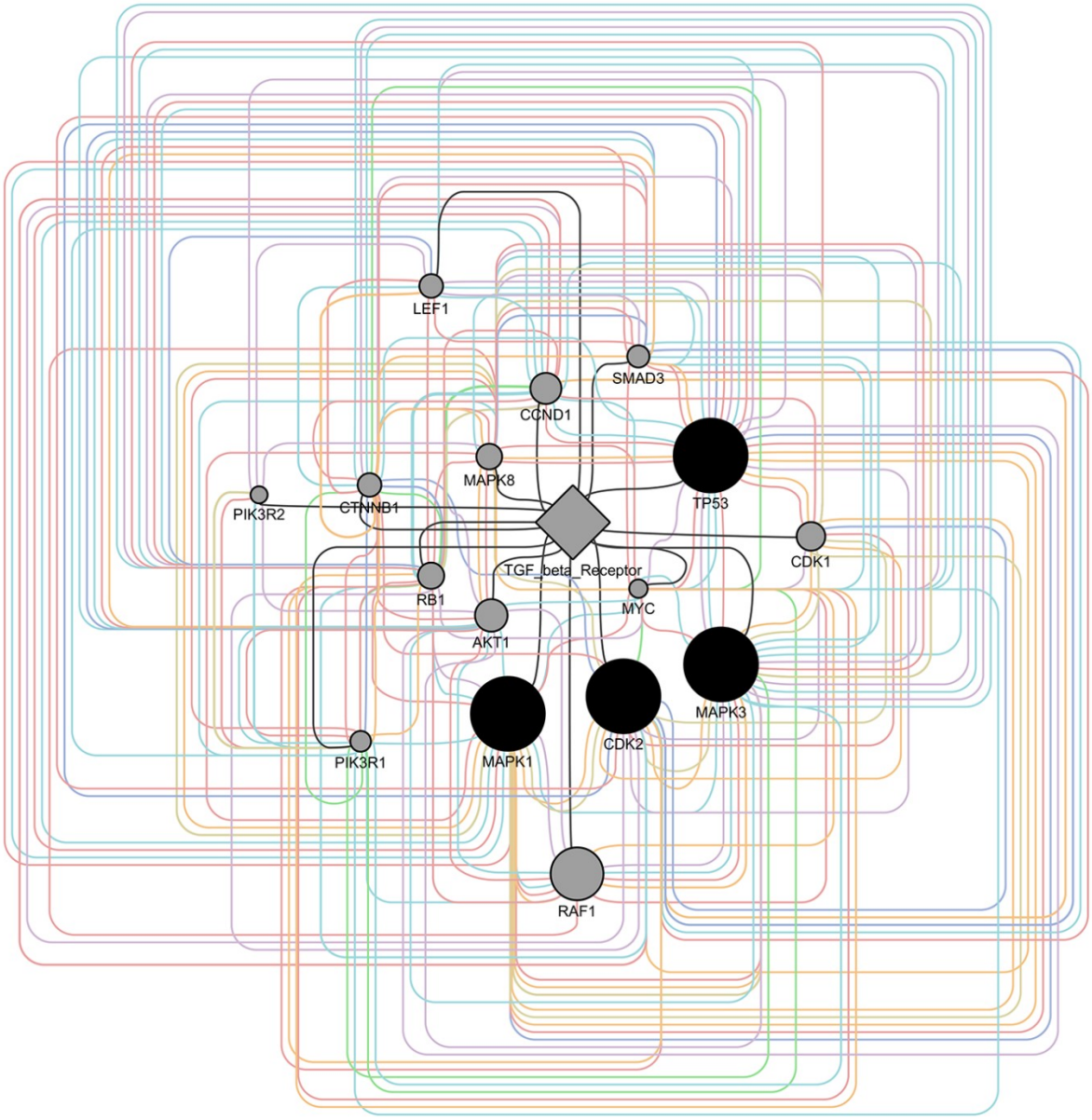

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652 Fig. 8 The protein interaction subnetwork 'TGF-β receptor signalling', a consolidated pathway

653 cluster with the highest weight within the extended TPP2 interaction network.

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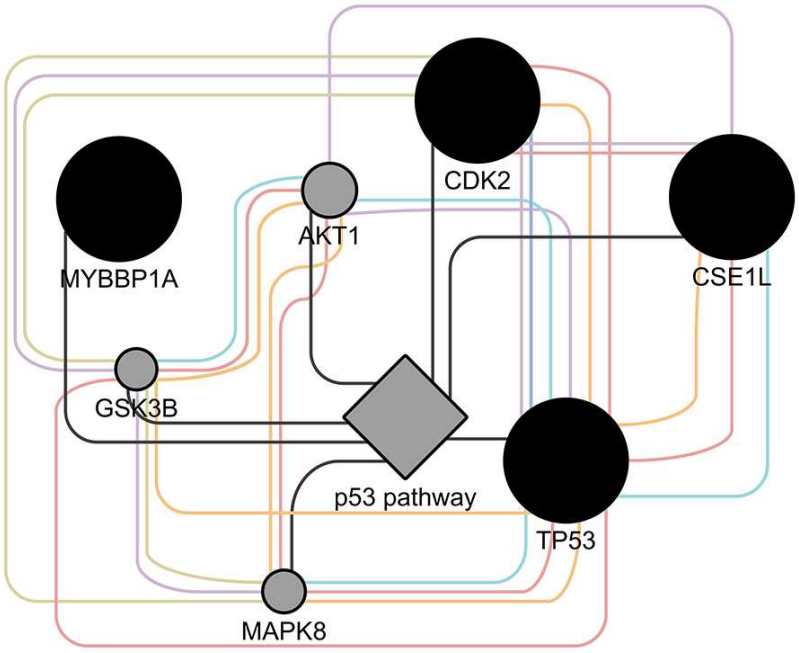

Fig. 9 The protein interaction subnetwork ‘p53 signalling’, a consolidated pathway cluster with a significant weight within the extended TPP2 interaction network.

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660 Tab. 1 The consolidated pathways with the highest scores in the pathway enrichment analysis
661 and the gene function prediction analysis of the extended TPP2 interaction network

Network	**Weight**	**Title**
	52.31	
TGF-β receptor	11.70	IOB TGF-β receptor
Pathways in cancer	9.14	KEGG hsa05200 Pathways in cancer
ID signalling	6.15	Netpath ID
Prostate cancer	5.22	KEGG hsa05215 Prostate cancer
p53 pathway	4.32	Pathway interaction database NCI-Nature curated data p53 pathway
Colorectal cancer	3.82	KEGG hsa05210 Colorectal cancer
Kit receptor signalling	2.96	NETPATH Kit Receptor
BCR signaling	2.74	NETPATH BCR
Thyroid cancer	2.65	KEGG hsa05216 Thyroid cancer
IL2-mediated signalling events	1.72	Pathway interaction database NCI-Nature curated data IL2-mediated signalling events
G-CSF	1.21	IOB G-CSF
Bladder cancer	0.68	KEGG hsa05219 Bladder cancer

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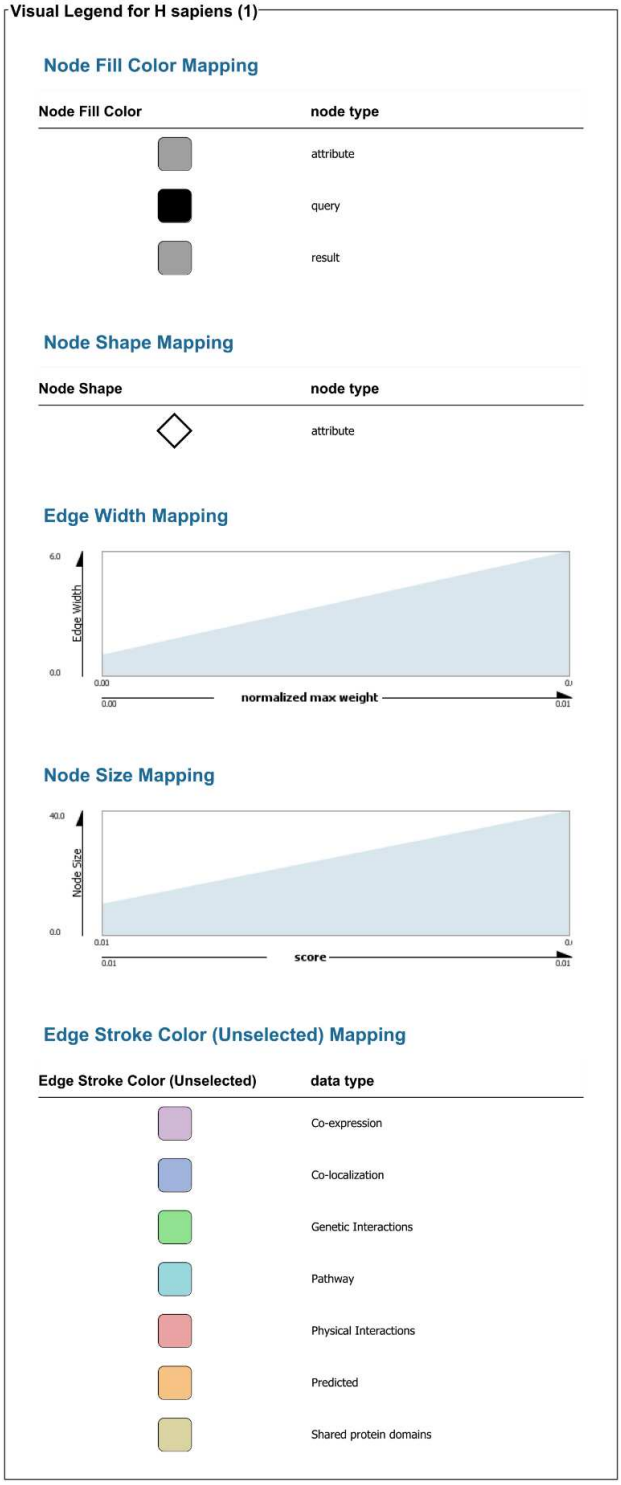

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667 Fig. S1 The definition of the types of interactions and nodes used in the protein interaction
668 networks.

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