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## **Protein-protein interaction analysis highlights the role of septins in membrane enclosed lumen and mRNA processing**

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### **Abstract**

Septins are a family of GTP-binding proteins that assemble into non-polar filaments which can be recruited to negatively charged membranes and serve as a scaffold to recruit cytosolic proteins and cytoskeletal elements such as microtubules and actin so that they can perform their important biological functions. Human septins consist of four groups, each with 13 members, and filaments formation usually involve members from each group in specific positions. However, little is known about the molecular mechanisms that drive the binding of septins to membranes and its importance to their biological functions. Here we have built a protein-protein interaction (PPI) network around human septins and highlighted the connections with 170 partners. Functional enrichment by inference of the network of septins and their partners revealed their participation in functions consistent with some of the roles described for septins, including cell cycle, cell division and cell shape, but we also identified septin partners in these functions that had not previously been described. Interestingly, we identified important and multiple connections between septins and mRNA processing and their export from the nucleus. Analysis of the enrichment of gene ontology cellular components highlighted some important interactions between molecules involved in the spliceosome with septin 2 and septin 7 in particular. RNA splicing regulates gene expression, and through it, cell fate, development and physiology. Mutations in components of the in the splicing machinery is linked to several diseases including cancer, thus taken together, the different analyses presented here open new perspectives to elucidate the pathobiological role of septins.

Keywords: septin, network, spliceosome, interactome, nucleus

## **I- Introduction**

Septins are members of an evolutionarily conserved GTPase family of proteins that were originally identified in *Saccharomyces cerevisiae* (Hartwell, 1971) as cell division control proteins (cdc proteins) involved in controlling the cell cycle and cytokinesis. These proteins were named septins because of their role in the septum (Sanders and Field, 1994), although they were subsequently described in many eukaryotes except for higher plants and their role was shown to extend largely beyond cytokinesis to include cell polarity, cell cortex compartmentalization, vesicle transport ciliogenesis, vesicular trafficking and lipid droplet formation (Akil et al., 2016; Dolat and Spiliotis, 2016; Field and Kellogg, 1999; Fung et al., 2014, 2014; Kartmann and Roth, 2001; Longtine et al., 2000; Oh and Bi, 2011; Pagliuso et al., 2016; Schink et al., 2016; Song et al., 2016; Trimble, 1999; Zhang et al., 1999). In addition, the expression of different septins is deregulated in several pathological conditions, including cancers and infectious diseases (Akil et al., 2016; Angelis et al., 2014; Dolat et al., 2014; Fung et al., 2014; Hall and Russell, 2004; Mostowy and Cossart, 2012). Thirteen septins have been identified in mammals (septins 1 to 12 and 14) and classified in four different groups (the septin 2, 3, 6 and 7 groups) (Peterson and Petty, 2010). Septins assemble into apolar complexes that are able to form high order structures such as filaments and rings. The filaments formed by septins are complex and usually involve members from each group in specific positions (Weirich et al., 2008). Septins assemble through membrane binding (Bridges et al., 2014) and act as diffusion barriers or scaffolds to recruit cytosolic proteins and other cytoskeletal elements such as microtubules or actins (Tanaka-Takiguchi et al., 2009; Fung et al., 2014; Bridges et al., 2016; Mostowy and Cossart, 2012).

Most of the eukaryotic cellular membrane consists of a bilayer of lipids containing various phospholipids and sterols in which different transmembrane and peripheral proteins are embedded. Biological membranes are fluid structures that require deformation to curve and shape them into tubules, sacs, cisternae or more complex structures to build the organelles and ensure their functions and the membrane trafficking between them. Membrane shaping thus ensures several biological processes such as cell growth, division, migration and polarization (McMahon and Gallop, 2005; Stachowiak et al., 2013).

The generation and maintenance of curvature can be modulated dynamically by changes to lipid composition and proteins including transmembrane proteins, cytoskeletal proteins or peripheral

membrane proteins which act as scaffolds and amphipathic helices that are inserted into the bilayer (McMahon and Gallop, 2005).

Septins preferentially bind to a curved membrane and have recently emerged as sensors of micron-scale membrane curvature in eukaryotes (Bridges et al., 2016). The membrane binding of septins is dependent on an amphipathic helix (AH) which is located near the N terminus and is responsible for the curvature sensing of septins (Cannon et al., 2019; McMurray, 2019; Omrane et al., 2019). Septins also associate with a variety of phosphoinositides (PIs) at different intracellular membranes. The binding of septins to PIs is mediated by a polybasic domain (PB1) located in close proximity to the G-domain (Akil et al., 2016; Tanaka-Takiguchi et al., 2009; Zhang et al., 1999). In a recent study we found that septin 9 has a second polybasic domain (PB2) conserved in the entire human septin family (Omrane et al., 2019). Like the first PB well-characterized (PB1), PB2 binds specifically to PIs, and both domains are critical for septin filament formation. Thus, the membrane binding of septins is AH-dependent, while PB domains control the specific association of septins to PI-containing membranes, which is critical to shaping and docking the protein on specific PI-enriched organelles (Omrane et al., 2019). Despite all this evidence regarding the crucial role of septin-membrane interactions in septin assemblies and functions, there are still several questions as to how septins assemble and shape the membrane and their related functions. Like myotubularin molecules, septins could interact with PIs and could form molecular complexes which allow these molecules to play an active role in several cellular functions conserved in the evolution (Raess et al., 2017). We propose that the multi-protein complexes recruited to the membrane by septins could play an active role in their large cellular functions. To assess these protein complexes and get new insights on the cellular function of septins, we build a protein-protein interaction (PPI) network of partners around the 13 human septins. The extraction of septin partners was performed across the InnateDB database of protein interactions (Breuer et al., 2013). The PPI network started with 13 seeds (13 septins) and enabled us to find 183 nodes (13 septins and 170 partners) and 255 connections between these molecules. In order to understand the global influence of the septins on their partners, functional inference was performed on the PPI network using Gene Ontology databases. The findings revealed new partners for septins and a new function in mRNA processing.

## **II- Role of septin/membrane interactions in cellular function**

Septins bind to a membrane and assemble in a high order structure to perform their diverse biological roles. To gain further insights into the molecular mechanisms involved, we built a protein-protein interaction (PPI) network of partners around the thirteen human septins. These septin partners were extracted from the Innate DB database of protein interactions (Breuer et al., 2013). The PPI network started with 13 seeds (13 septins) which enabled us to find 183 nodes (13 septins and 170 partners) and 255 connections between these molecules (Figure 1A). In order to understand global influence of the septins and their partners, functional inference was performed on the PPI network using Gene Ontology databases. Our findings revealed some important biological functions (Table 1). Surprisingly, the most significant connections were found with RNA splicing and processing which was unprecedented among the roles for septins described previously (Table 1 and Figure 1A). The main known functions of septins such as cell division, cell cycle, the regulation of cell shape and organelle localization were also revealed (Figure 2A). In this septin PPI network, the superimposition of a splicing enriched function did not harbor molecules common with the known functions of septins (the regulation of cell shape and organelle localization) except for the SON molecule sharing splicing and cell division functions (Figure 1B). The SON protein binds to RNA to promote the splicing of molecules with poor splice sites and is implicated in the cell cycle (Ahn et al., 2011). Among the partners of septins, important molecules were also associated with cellular component and macromolecular assemblies (Table 1) which also represent a known role for septins. A Venn diagram was constructed with the partner molecule involved in the cell division, cell shape and organelle localization functionalities (Figure 2B). The Myosin Heavy Chains MYH9 and MYH10 appear to play a central role in the septin network (particularly connected to SEPT7; Figure 2A) and in these functionalities (Figure 2B) but independently of the splicing process (Figure 1B). Both septin 7 and NM-IIA (MYH9) associate with SNAP23, a SNARE protein involved in GLUT4 storage vesicle docking and fusion with the plasma membrane (Wasik et al., 2017).

### ***Role in cell division***

As could be expected, the cell division function directly involved 12 of the 13 septins (SEPT3, SEPT2, SEPT11, SEPT4, SEPT6, SEPT1, SEPT14, SEPT9, SEPT12, SEPT10, SEPT5 and SEPT7), excluding only SEPT8 (Figure 2B and Table 1). A direct association between the different septins was found, as well as a link between some septins and molecules with known functions in the division process (Figure 2A and 2B).

Among the different septins represented, septin 7 was the most closely linked to molecules implicated in cell division. Indeed, septin 7 interacts with other septins (such as septins 2, 11, 9, 6) and with other molecules directly involved in division such as centrosome-associated protein E (CENPE) which is a kinesin-like motor protein that accumulates in the G2 phase of the cell cycle. This interaction has previously been reported (Zhu et al., 2008, p. 7). Two other genes encoding non-muscle myosin 2 (NM2) heavy chains (MYH10 and MYH9) (Wasik et al., 2017) and UBE2I, the gene for the SUMO conjugating enzyme UBC9, also interact with septin 7. The SON molecule which encodes the splicing factor involved in cell cycle progression was found to be linked to SEPT7 and SEPT2. RNF8 is a gene encoding E3 ubiquitin-protein ligase and was identified as a co-partner of SEPT7 and SEPT8.

Septin 1 is connected to important partners implicated in cell division such as RAD21, a gene coding for a protein involved in double strand DNA repair, and AURKB which codes for aurora kinase B. SEPT10 is individually linked to CUL3 which encodes for cullin 3, a member of the cullin protein family that plays a critical role in poly-ubiquitination. RB1 (the gene encoding retinoblastoma protein) is individually connected to SEPT4.

This analysis therefore revealed important new partners for septins in cell division and offers further opportunities to investigate this role for septins in cell division

### ***Role in the regulation of cell shape***

Septins control cytoskeleton organization and membrane curvature in order to shape cells and organelles. Five molecules were found to be directly involved in cell shaping and interacted with septins. Among them we identified the Borg family of proteins which are effectors of cdc42 and known partners of septins; they include Cdc42EP3 and Cdc42EP5 (Bahou et al., 1992; Joberty et al., 1999). Although these effectors have been shown to regulate and bind SEPT2, 6, and 7, their role on regulation of cell shape has not previously been suggested (Joberty et al., 2001). During the cell shaping process, we found an interaction between CDC42EP2 and CDC42EP3 and septins 7 and 6, but not septin 2 (Figure 1 and Table 1). This therefore suggested that the same effector might bind to different septins to achieve a different biological process.

SH3KBP1 (Human Src Family Kinase-Binding Protein 1) has also been described as a partner of septin 9 (Diesenberg et al., 2015). As presented in the PPI network (Figure network), SH3KBP1 interacted with seven septins in total: septins 10, 11, 6, 2, 9, 8 and 7. This revealed that the involvement of SH3KBP1 in regulating cell shape appeared to be strongly linked to the activities of different members of the septin family. As well as these partially known partners

of septins, FERMT2 (Fermitin Family Member 2), which is implicated in integrin-mediated cell adhesion to the extracellular matrix and cell spreading, has been found to interact with septins 9 and 11 (Kawamura et al., 2018, p. 2). FN1 (fibronectin 1), a glycoprotein present at the cell surface and in the extracellular matrix and implicated in migration and cell adhesion, has also been revealed as a co-partner of both septins 2 and 9 (Figure 2A and 2B). It could be considered as a potential target in the treatment of colorectal cancer (Cai et al., 2018, p. 1).

Taken together, the data from PPI revealed that most of the molecules that are co-partners with the septins that regulate cell shape are involved in cytoskeleton organization and the cell adhesion process.

### ***Role in organelle localization***

Eukaryotic cells are composed of morphologically and functionally distinct organelles which include the nuclei, ER, Golgi, mitochondria or lysosomes, as well as lipid droplets. These different features of organelles are maintained in dynamic processes that lead to changes to the number, size distribution and intracellular location of the different organelles (Cohen, 2018). Membrane curvature is one of the processes that regulate organelle morphogenesis and localization, so we analyzed the molecules identified by PPI around septins involved in organelle localization (Figure 2A and 2B). Several interactions were found between septins 2, 3, 4, 9 and 7 and molecules involved in organelle localization. The data revealed that each of the five septins has different and specific partners, except for COPA (the gene of the coatamer protein, COP), which is a co-partner of both septin 2 and septin 9. Furthermore, septin 2 is a partner of COPE encoding for coatamer subunit epsilon. COP proteins enable the formation of coated vesicles to ensure transport between endomembranes (Bonifacino and Lippincott-Schwartz, 2003). These findings are therefore in line with the known functions of septins in intracellular trafficking (Song et al., 2016). The other partners of septin 2 are PIK3R1 and PAK2 (serine/threonine kinase, p21-activated kinase 2), which is localized with E-cadherin at cell-cell contact in epithelial cells and mediates survival (Campbell et al., 2019; Frank et al., 2012). Septin 3 interacts with iEIF6 Eukaryotic translation initiation factor 6 (EIF6), also known as Integrin beta 4 binding protein (ITGB4BP), and is an important functional component of hemidesmosomes. Septin 4 is a partner of XIAP (X-linked inhibitor of apoptosis) and BIRC3, another inhibitor in the apoptosis family. Septin 7 is a direct partner of proteins involved in cell adhesion such as vitronectin (VTN) and integrin alpha 4 (ITGA4) which both are elements of the cell adhesion complex and participate in extracellular matrix assembly (Dejana et al., 1988; Hsia et al., 2005). Finally, the organelle localization partners of septin 9

are mainly involved in trafficking and include TMED9 (transmembrane p24 trafficking protein 9). TMED9 is a member of a family of genes that encode transport proteins located in the endoplasmic reticulum and the Golgi. TMED9 participates in COPI vesicle-mediated retrograde transport and supports the partnership between septin 9 and COPA described above in terms of controlling cell shape. Septin 9 is also a direct partner of the actin binding protein filamin B which is encoded by the FLNB gene (Nakamura et al., 2011).

Once again, our data highlighted the role of septins in cytoskeleton organization and cell adhesion.

### **III- Molecular mechanisms underlying septin/membrane interactions**

The PPI interaction networks built using 13 septins and their partners were further analyzed using the gene ontology cellular component database (figure GO cellular component), and among a total of 183 molecules in the PPI network, cellular components such as the cell cortex and intracellular organelles were found. Furthermore, several nuclear components such as the nuclear body, nucleoplasm and nuclear speckle were also determined. Interestingly, once again, the spliceosomal complex was identified (Figure 3A), thus reinforcing the data from gene ontology on cellular functions which revealed RNA splicing as a significant function involving septin molecular relationships (Table 1). Membrane-enclosed lumen compartments concerning several organelles were highlighted in blue on the network. Among the 72 molecules associated with membrane-enclosed lumens, septins 2 and 7 and their partners displayed important connections with the spliceosome. Septin 2 and 7 are not part of the same septin group, but they are involved in the hexameric complex of septins which form a core complex that assembles to form filament. Septins 2 and 7 have been found to be ubiquitously expressed during different analyses (Hall et al., 2005), especially using SAGE (Serial analysis of gene expression ) analysis in different human tissues (Cao et al., 2007). The spliceosome components shared by these two septins involved U2 Small Nuclear RNA Auxiliary Factors 1 and 2 (U2AF1, U2AF2), serine- and arginine-rich splicing factors 5, 7 (SRSF5, SRSF7) and Pre-mRNA Processing Factors 3 and 8 (PRPF3, PRPF8). As well as these common partners, septin 2 was specifically connected to the serine- and arginine-rich splicing factors 3 and 10 (SRSF3 and SRSF10) and to signaling molecules such as mTOR (mammalian target of rapamycin). It is known that cardiomyocyte-specific SRSF3 knockout mice have shown evidence of the alternative splicing of mTOR mRNA, generating a shorter protein isoform that lacks catalytic activity (Ortiz-Sánchez et al., 2019, p. 3). Also, the AKT key mediator of PI3K is able to regulate the SR protein through phosphorylation and modifying the splicing process (Blaustein et al., 2005).



Other signaling molecules have been found to be connected to septin 2 and include IKBKE (inhibitor of nuclear factor kappa b kinase subunit epsilon) and the transcription factor VHL (Von Hippel-Lindau Tumor Suppressor). This relationship between septin 2 and VHL appears to be implicated in renal cell carcinoma (Craven et al., 2006).

Septin 7 has specific partners implicated in the cell cycle and DNA replication such as CDC5L, TOP2A and CENPE. Septins 9 and 11 are not connected to spliceosome components but both proteins are well represented in this cellular component network (Figure 3B). Their partners are specific to each septin and include SIN3 Transcription Regulator Family Member A (SIN3A) and also SMARCA2. The protein encoded by this gene is part of the large ATP-dependent chromatin remodeling complex SNF/SWI, which is required for the transcriptional activation of genes normally repressed by chromatin. The MSH2 gene that encodes for DNA repair interacts with septin 9. Taken together, these data provide evidence of a potentially crucial role for septins in changes to DNA and RNA structure, which are both poorly investigated functions of septins.

#### **IV- Septin/nuclear components**

The inference of functional enrichment of the septin PPI network with Gene Ontology Cellular Components also pinpointed the involvement of the nuclear compartment with the nucleoplasm and nuclear speck (respectively in green and orange; Figure 4A). Components of the nucleoplasm were found to be widely distributed around the septin PPI network (in green, Figure 4B) but tending to concentrate around septins 2 and 7. Concerning the nuclear speck, fewer components were found in the network when compared to the nucleoplasm, but they were mainly connected to septins 2 and 7 (in orange, Figure 4B). These relationships with septins 2 and 7 in the nucleoplasm and nuclear speck mainly implicated components of the spliceosome, such as SRSF5, SRSF7, U2AF1, U2AF2, RBM39, SON, PRPF3 and PRPF8.

Interestingly we also found in the nuclear compartment that some molecules shared by septins 2 and 7 and implicated in both chromatin dynamics and mRNA splicing included HNRPU, which plays a role in regulating interphase large-scale gene-rich chromatin organization through chromatin-associated RNAs in a transcription-dependent manner, and thereby maintains genomic stability (Fackelmayer et al., 1994; Nozawa et al., 2017; Romig et al., 1992); ACIN1 which act both in splicing and also the induction of chromatin condensation during apoptosis post casp3 activation (Sahara et al., 1999), and SMARC5, a Swi/SNF component that is an actin-dependent regulator of the chromatin (Santoro et al., 2002) especially at the level of the nucleolar remodeling complex, and mediates heterochromatin formation. Thus, in these

nuclear relationships in the PPI networks of septins, we highlighted links between chromatin partners and septins, coupled with links to splicing molecules. Chromatin has already been described as being link to splicing regulation (de Almeida and Carmo-Fonseca, 2014) and the importance of septin integrity at cell cycle arrest has recently been connected to nucleolar asymmetry (Rai et al., 2017). In this network, we showed that through their partner relationships, septins could participate in the interplay between the spliceosome and chromatin in the nuclear part of cells. Using the Human Protein Atlas (Thul et al., 2017), we confirmed that in A-431 epidermoid cells, septin 7 was expressed not only on actin filaments but also in the nuclear part of the cell, and particularly in the midbody and nucleoli (Figure 5A). Septin 2 was found to be expressed on actin filaments and also in the nucleus under speck conformation (Figure 5B); the SRSF5 splicing component that we found to be connected to both septins 2 and 7 in the nuclear part (Figure 4B), was also seen to be expressed in nuclear parts of the cell (Figure 5C) (the nucleus and nucleoli), thus covering the expression in nuclear regions of septins 2 and 7.

### **Conclusion/Discussion**

The PPI study presented here highlighted the key role that septins may play in membrane-enclosed lumens which include the multiple organelles of the endomembrane system, the nucleus and cilia (Rout and Field, 2017), which are all of different shapes.

The structural features of septin interactions with membranes have recently been investigated (Bridges and Gladfelter, 2015; Omrane et al., 2019). However, much still needs to be learnt about the septin partners involved in their association with membranes and the mechanisms that drive septin-dependent membrane shaping and related biological functions (organelle formation, localization and transport, cell division). Interestingly the PPI network presented here revealed new partners for septins in membrane dynamics and vesicle transport, including the COP proteins and TMED9; the latter, also called p25, belongs to the p24 family of type I transmembrane molecules which are found abundant in the endoplasmic reticulum and implicated in early secretory pathways (Dominguez et al., 1998; Wada et al., 1991). In yeast p24, family members have been proposed to act as cargo receptors for GPI anchored protein transport from the endoplasmic reticulum to the Golgi (Muñiz et al., 2001, 2000; Schimmöller et al., 1995). In *Caenorhabditis elegans*, p24 proteins ensure quality control regarding the retention of unfolded proteins (Wen and Greenwald, 1999). In particular, it has been suggested that TMED9 is involved in the formation of vesicular tubular clusters and endoplasmic reticulum exit sites (Lavoie et al., 1999) with COPI cargo molecules implicated in

vesicle-mediated retrograde transport. This molecule is necessary to maintain the stability of the endoplasmic reticulum Golgi intermediate compartment (ERGIC) and Golgi because its silencing reduces the number of ERGIC clusters and Golgi fragmentation without having any effect on anterograde transport (Mitrovic et al., 2008). TMED9 plays a critical role in retention of the p24 complex during early membrane biosynthesis, probably by anchoring the p24 oligomer in the COPI recycling pathway (Emery et al., 2003). TMED9 acts as a carrier in the endoplasmic reticulum TC48 form of PTPN2 phosphatase which regulates important cell functions such as growth, mitogenesis, motility, cell-cell interactions, metabolism, signal transduction and gene expression (Gupta and Swarup, 2006).

Unexpectedly, our gene ontology cellular component analysis revealed an association of septins with cellular compartments such as the nuclear body, nucleoplasm, nuclear speckle and nuclear lumen. In particular, we were able to highlight in this study the potential role of septins in RNA processing and their interaction with spliceosome components. The study notably revealed multiple interactions between septins 2 and 7 and the nuclear part of the spliceosome (Figure 4b) and with molecules also implicated in chromatin regulation. The SWI/SNF complex, initially described regarding its nucleosome remodeling activity at promoters, is also present within the coding regions of certain genes where it participates in regulating alternative splicing processes, and particularly with the BRM catalytic subunit (Batsché et al., 2006). SMARC5, a Swi/SNF component, was found to be shared between septins 2 and 7 with respect to spliceosome serine/arginine-rich splicing factors (SR proteins) such as SRSF5. Proteins in the spliceosome are commonly distributed diffusely in the nucleoplasm but concentrated in nuclear speckles, which represent a subnuclear compartment enriched in small nuclear ribonucleoprotein particles and other splicing factors (Schmidt-Zachmann et al., 1998). SWI/SNF subunits and SR proteins have been co-purified with RNA polymerase II (RNAPII), and the SWI/SNF complex participates in recruiting splicing factors to nascent RNA (Das et al., 2007). Furthermore, DNA remodeling proteins and transcription factors have been identified as partners of septin 9 and septin 11. Taken together, therefore, these findings strongly support a key role for septins in the nucleus. The localization and role of septins in the nucleus have been investigated to some extent; the nuclear localization of septin 9 isoforms has been reported but their potential functions were not described (Ge et al., 2019). The human protein atlas has described septin 2 as mainly being localized in actin filaments and the nucleus, while septin 7 has also been detected in actin filaments, nucleoli and the midbody. Septin 5 is present in the nucleoplasm but is mostly expressed in the brain. These observations support the important interactions between septin 2 and septin 7 and spliceosome complex proteins.

It has become evident that splicing has important physiological functions in developmental processes for many tissues and organs (Baralle and Giudice, 2017). Furthermore, recent developmental analysis revealed that alterations to the splicing process are observed in diseases such as cancer (David and Manley, 2010; Scotti and Swanson, 2016). Splicing factor genes have been reported to be frequently mutated in hematopoietic compartment of patients with myelodysplastic syndrome (MDS) (Armstrong et al., 2018). Principally, SF3B1 which has been shown to stabilize the U2 snRNP to the branch point sequence during pre-mRNA splicing. SF3B1 mutations in MDS are mainly found in low grade patients which harbored MDS with ring sideroblast phenotype. Also, U2AF1 and SFSF2, others splicing factors were found mutated in MDS but with an higher risk of evolution in acute myeloid leukemia or chronic myelomonocytic leukemia (Pellagatti and Boulwood, 2017). In MDS patients harboring splicing factor mutations there is also a frequent co-occurrence of mutations affecting epigenetic regulators, such as: DNMT3A, TET2, ASXL1 suggesting closed relations between splicing and epigenetics in the pathophysiology of these disorders. Interestingly, it was shown that septin 6 could harbor aberrant splicing by being a target gene of the mutated SF3B1 splicing factor in hematopoietic progenitors of MDS patients (Dolatshad et al., 2016).

Importantly a relevant role of septins in MDS has been reported in relation with their involvement as fusion partners of mixed lineage leukemia (MLL). These included septins 2, 5, 6, 9 and 11 (Cerveira et al., 2011; Kojima et al., 2004; Megonigal et al., 1998; Ono et al., 2002; Osaka et al., 1999; Taki et al., 1999) and thus reinforced the association of septins to MDS and spliceosome as revealed by our PPI data.

Important biological functions of septins are dependent of their binding to PIs (Akil et al., 2016; Tanaka-Takiguchi et al., 2009; Zhang et al., 1999). PIs and mainly PtdIns(4,5)P2 (PIP2) are enriched in a nuclear compartment called Nuclear lipid islets (NLIs) which are considered as determinant of spatial and functional diversification of nuclear structure. PIs are amphipathic molecules which could be distribute in the membrane-free nuclear environment through binding with splicing factor 1 protein suggesting their connection with pre-mRNA splicing cell functionality. Nuclear PIs are recognized as regulators of many nuclear processes including chromatin remodeling, splicing, transcription, DNA repair and epigenetic (Sztacho et al., 2019). Thus septin/PI interactions could be crucial for these molecules to control their wide nuclear functions and beyond. Together our work therefore opens important perspectives to study the pathobiological roles of septins.

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Tables

Pathway	Total molecules in function	Molecule hits in the network	p Value	FDR
RNA splicing via transesterification reactions	321	21	2.83e-10	2.32e-07
RNA splicing	478	25	5.78e-10	2.37e-07
MRNA processing	551	26	2.26e-09	6.18e-07
Cell division	507	24	9.4e-09	1.93e-06
MRNA metabolic process	817	30	3.86e-08	6.34e-06
Cytokinesis	120	10	1.83e-06	0.000249
Establishment of organelle localization	125	10	2.65e-06	0.00031
Cell cycle	1860	44	5.98e-06	0.000613
RNA processing	890	27	7.88e-06	0.000718
Neuron apoptotic process	169	10	3.79e-05	0.00309
Regulation of cell shape	106	8	4.15e-05	0.00309
RNA export from nucleus	86	7	7.83e-05	0.00501
Mitotic cell cycle checkpoint	149	9	7.94e-05	0.00501
Organelle localization	189	10	9.71e-05	0.00569
Protein modification by small protein conjugation	713	21	0.000134	0.00735
Cellular component assembly	1790	39	0.000143	0.00735
Macromolecular complex assembly	1120	28	0.000171	0.00826
Cellular protein complex assembly	343	13	0.000264	0.0109
Nucleobase-containing compound transport	175	9	0.000269	0.0109
Nuclear export	139	8	0.000277	0.0109

**Table 1: Functions found to be enriched after inference on septin networks using the Gene Ontology Biological Processes database:** the top 20 biological processes are presented with columns describing: the total number of genes implicated in the functions, number of hits of genes in the network and the corresponding function, the p-value of the hypergeometric test and corrected False Discovery Rate (FDR) q-values. Functions projected on the biological process network are highlighted with their respective color.

## Figure legends

**Figure 1: Splicing functionality inference on a protein-protein interaction network of human septins:** A/ Protein-protein interaction network of human septins with projections in orange projection regarding their implication in splicing; B/ Venn diagrams of molecules from the PPI network implicated in splicing and which cross-over with the three enriched biological processes: cell division, regulation of cell shape and organelle organization.

**Figure 2: Cell division, regulation of cell shape and organelle localization inference on a protein-protein interaction network of human septins:** A/ Protein-protein interaction network of human septins with projections regarding the biological process of cell division (in orange), the regulation of cell shape (green) and organelle localization (in blue); B/ Venn diagram of molecules from the PPI network which cross the three enriched biological processes: cell division, regulation of cell shape and organelle organization.

**Figure 3: The membrane-enclosed lumen is largely represented in the protein-protein interaction network of human septins:** A/ barplot of functional enrichment performed by inference on the septin protein-protein interaction (PPI) network using the gene ontology cellular component database. Membrane-enclosed lumen belonging to the membrane cellular compartment shown in blue; B/ PPI network of human septins with projection in blue of membrane-enclosed lumen enrichment

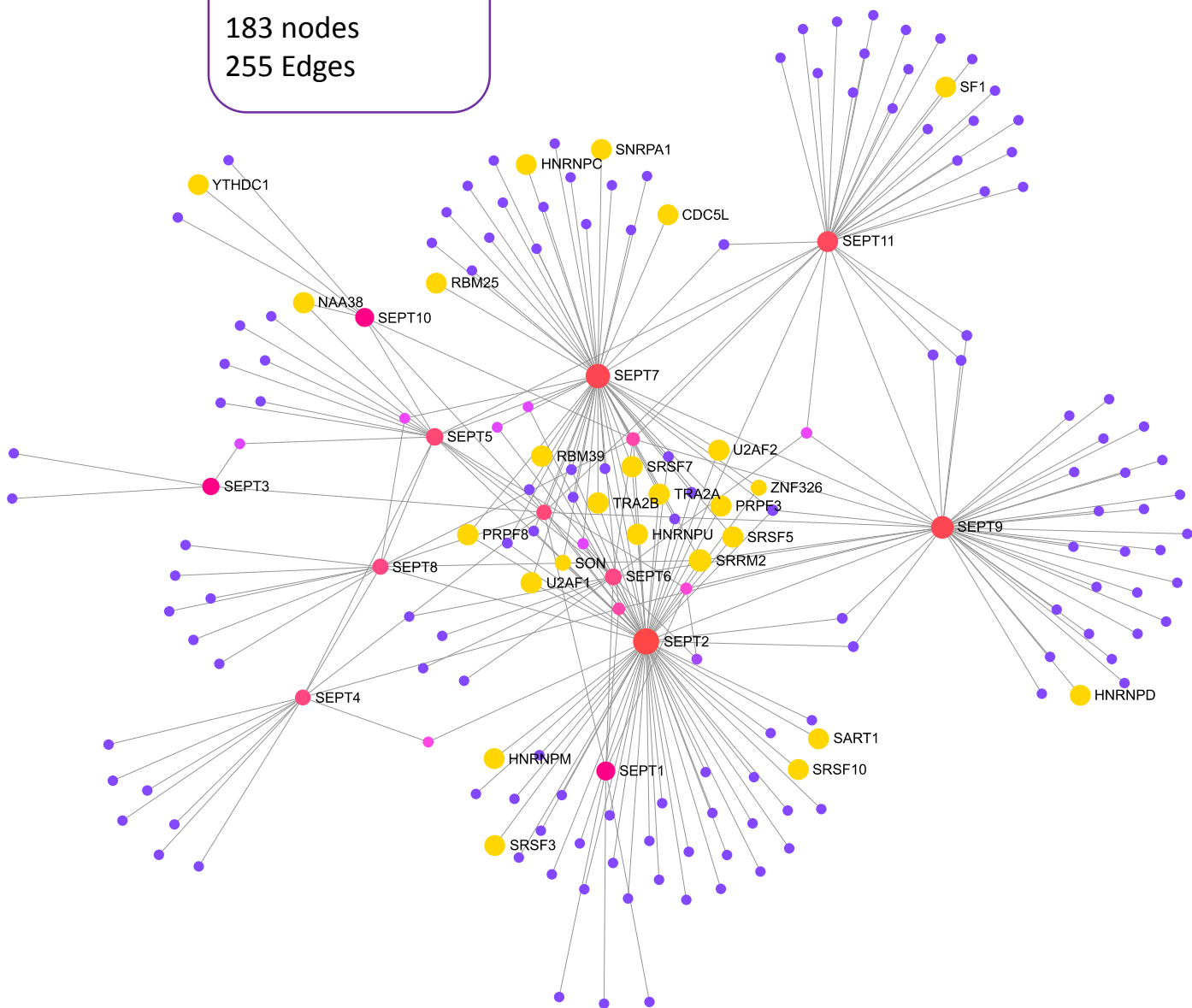
**Figure 4: Shared localization of molecule partners and septin members in the nucleoplasm and nuclear speck compartments:** A/ barplot highlighting the involvement of the nucleoplasm and nuclear speck compartments after enrichment performed on a protein-protein interaction (PPI) septin network; B/ PPI network of human septins with projections of nuclear speck enrichment (in orange) and of nucleoplasm enrichment (in green).

**Figure 5: Expression of septins 2 and 7 in A-431 epidermoid cells cover nuclear expression of the SRSF5 spliceosome component:** A/ Septin 7 is principally expressed with actin filaments, but also in the midbody and nucleoli of human A431 epidermoid cells; B/ Septin 2 is principally expressed with actin filaments, but also in the nucleus of human A431 epidermoid cells; C/ SRSF5 is principally expressed in the nucleus and nucleoli of human A431 epidermoid cells

A

NETWORK  
 13 septins seeds  
 InnateDB database  
 183 nodes  
 255 Edges

● splicing



B

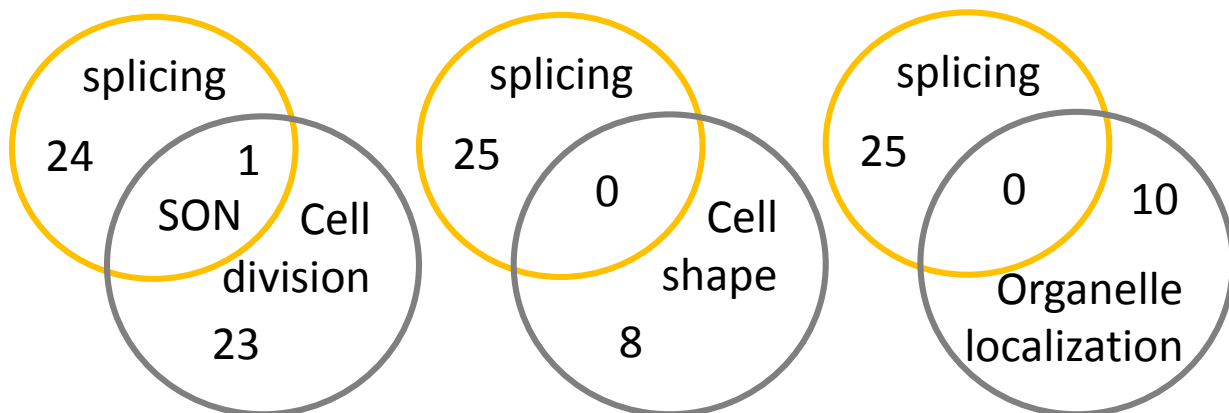
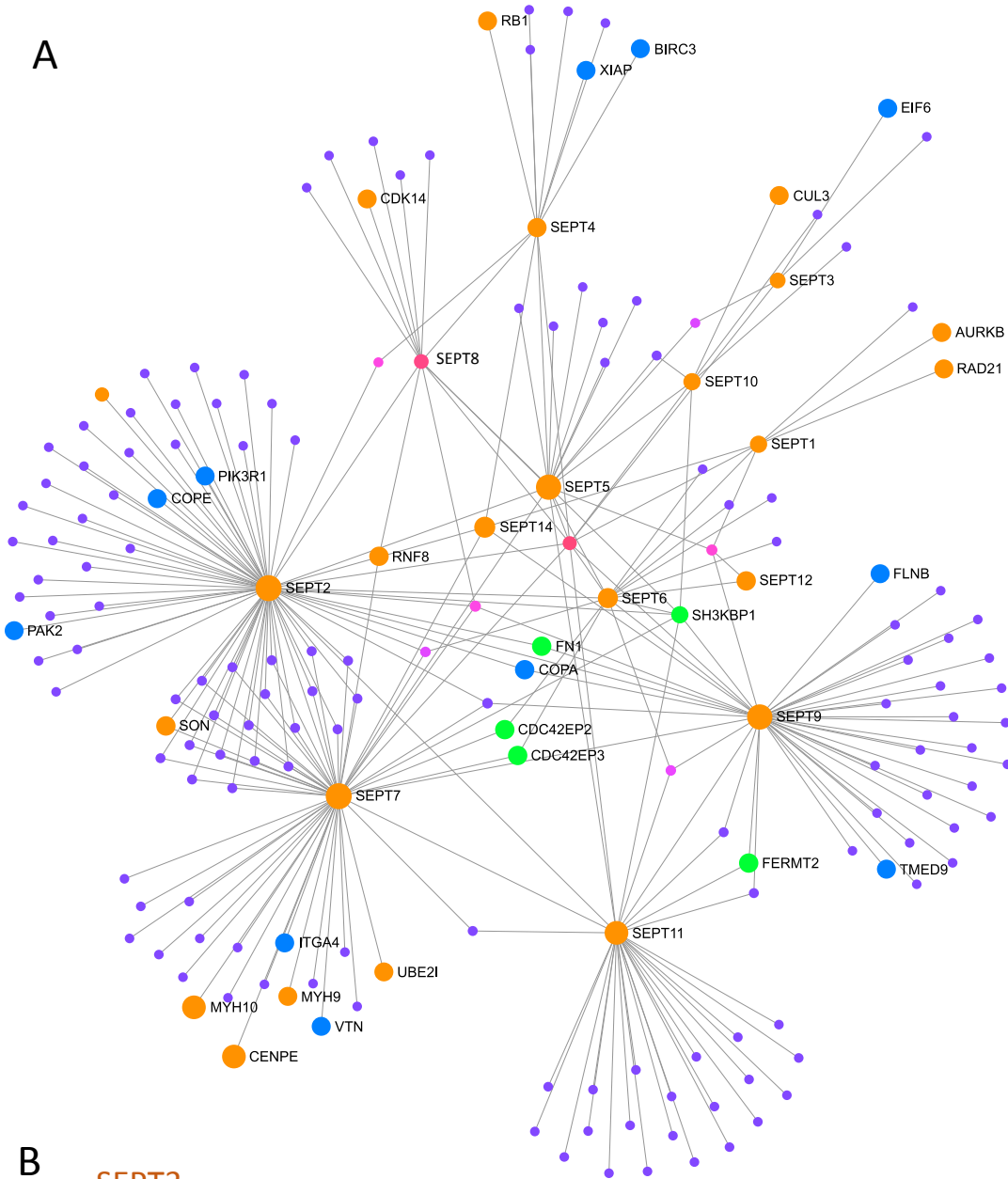


Figure 1

A



B

- SEPT3
- SEPT2
- SEPT11
- SEPT4
- SEPT6
- SEPT1
- SEPT14
- SEPT9
- SEPT12
- CDK14
- RAD21
- BUB3
- SEPT10
- AURKB
- RNF8
- UBE21
- SON

Gene ontology biological process

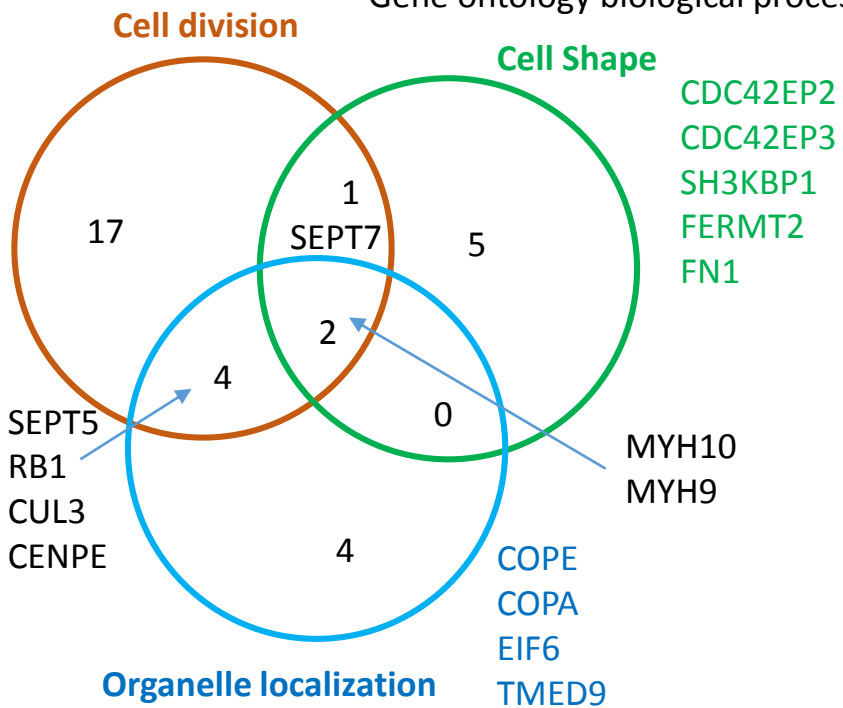
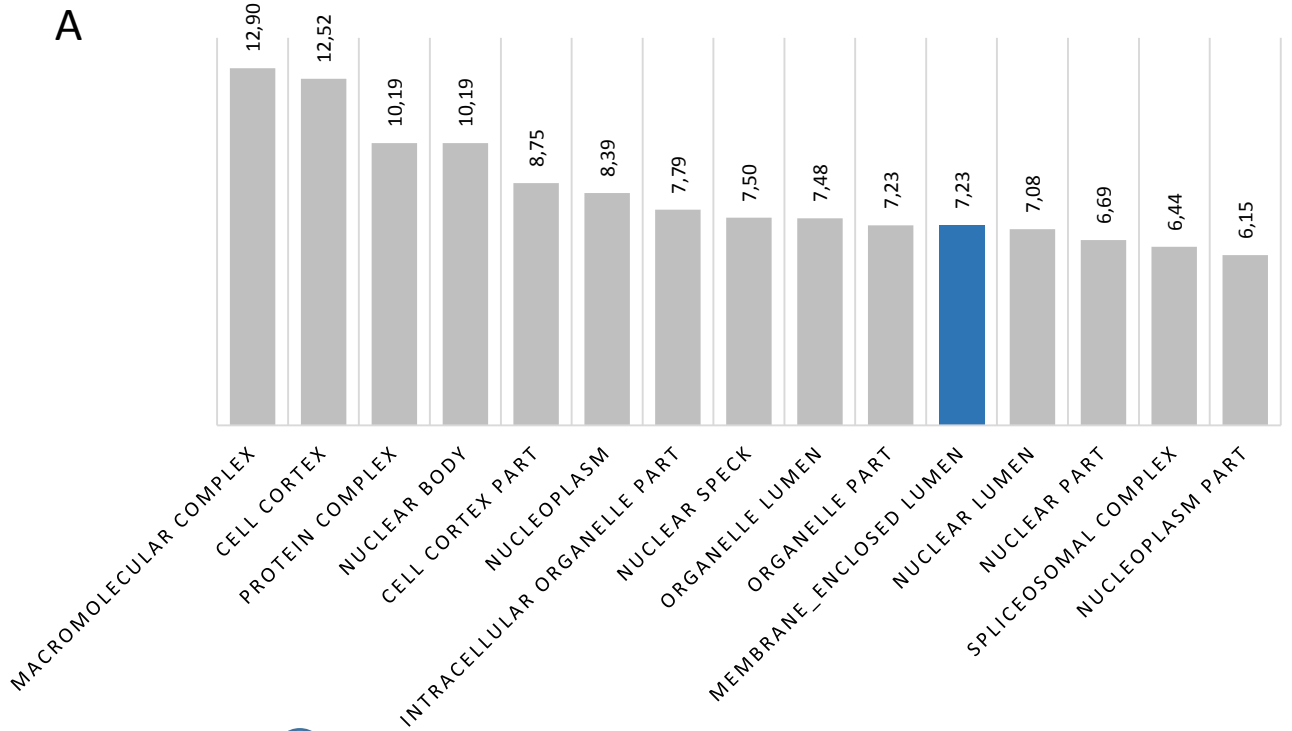


Figure 2

# GO-CC NEGATIVE LOG FDR

A



● Gene Ontology Cellular Component : membrane enclosed lumen

B

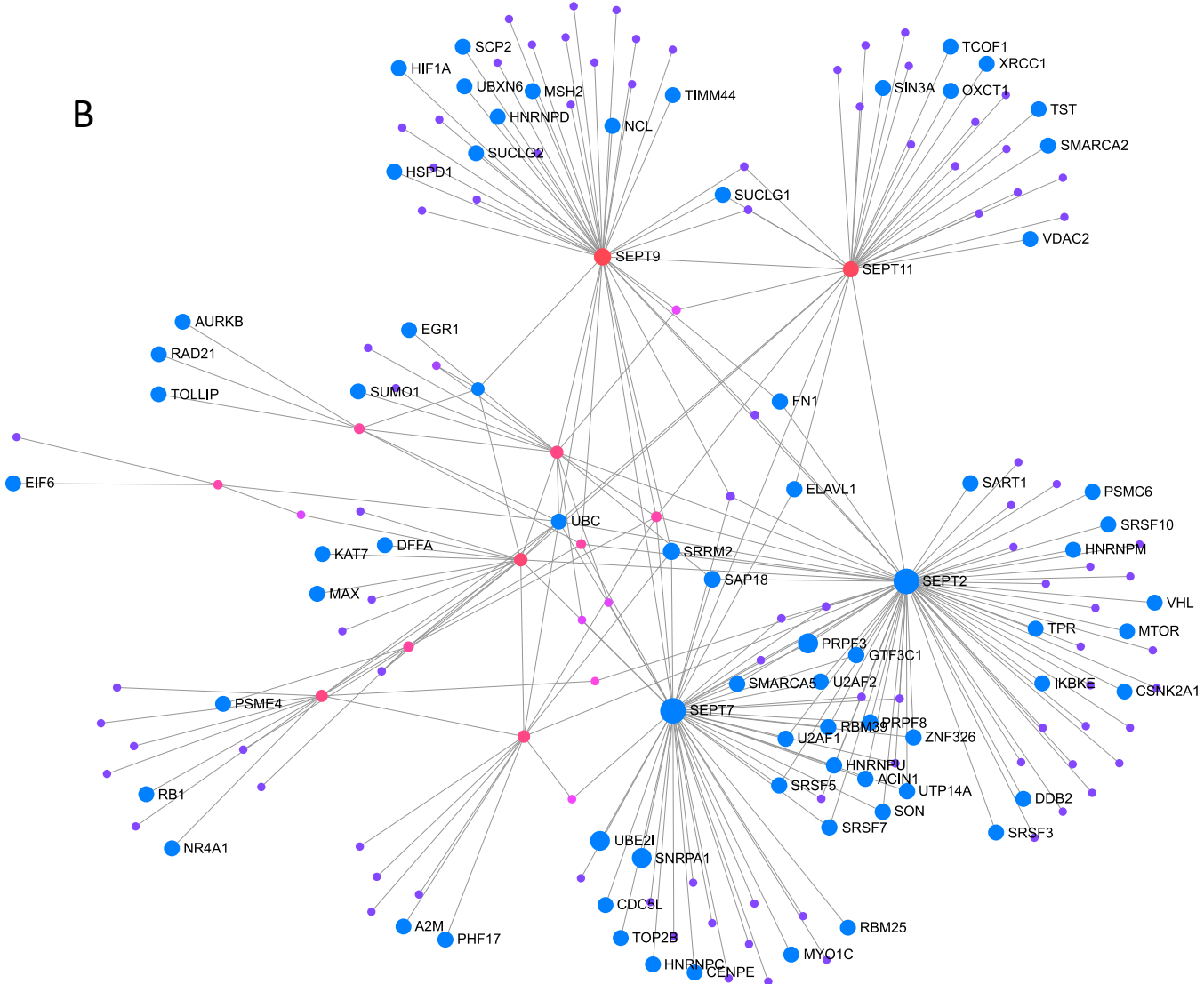
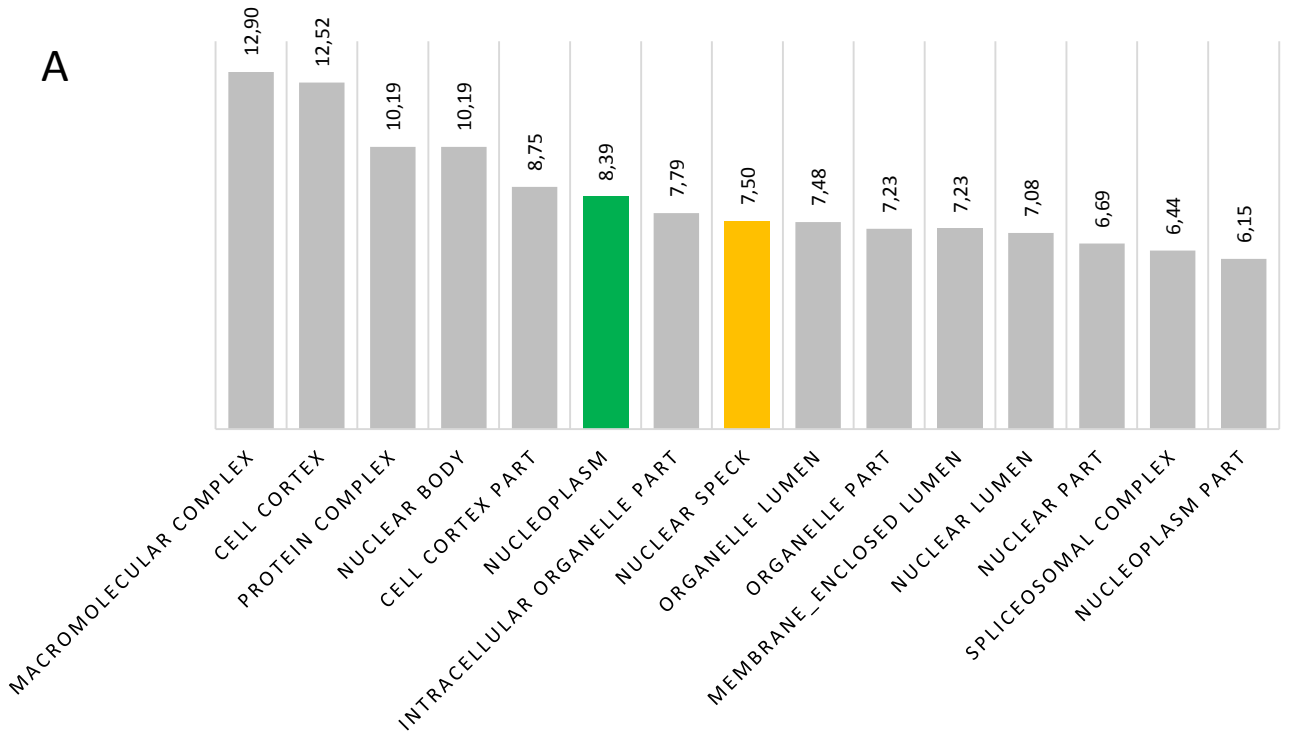


Figure 3



# GO-CC NEGATIVE LOG FDR

A



B

- Nucleoplasm
- Nuclear speck

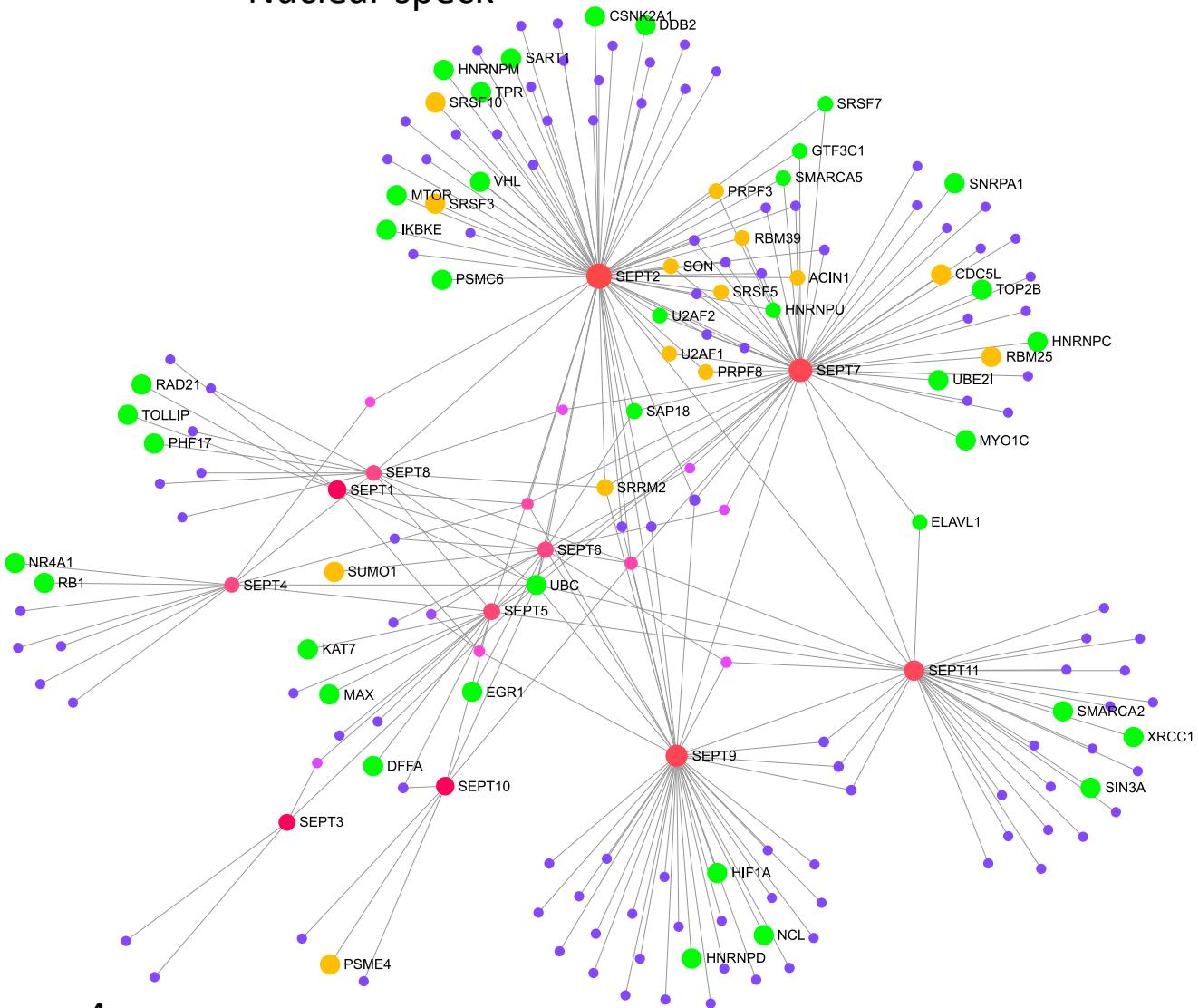
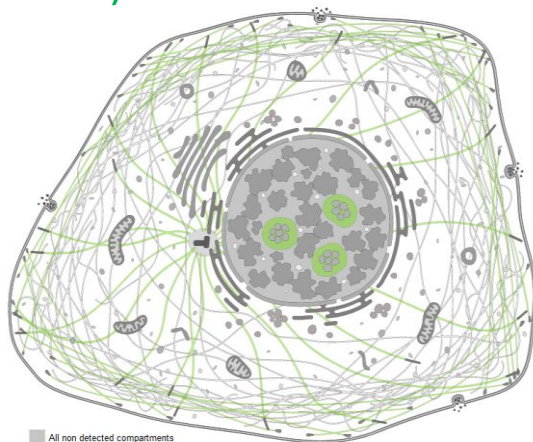
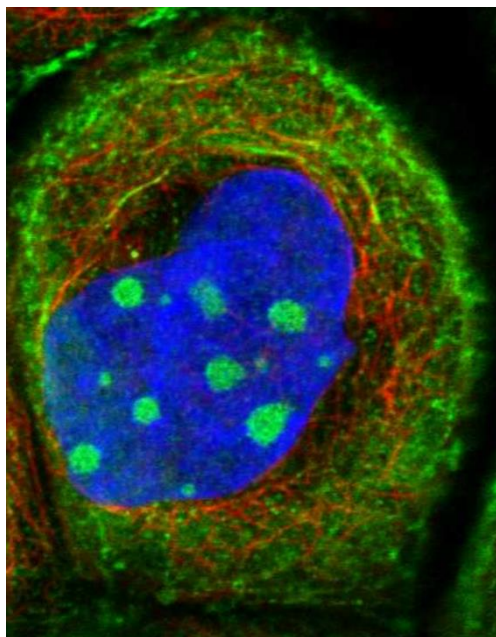
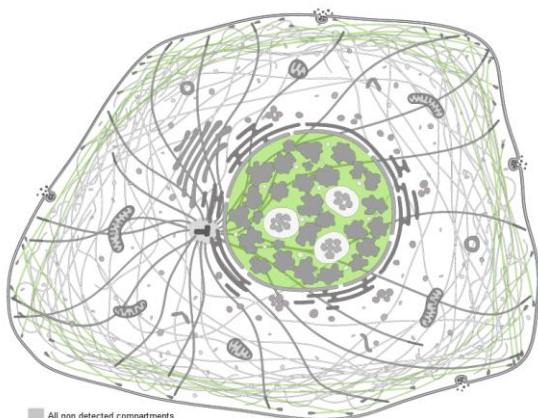


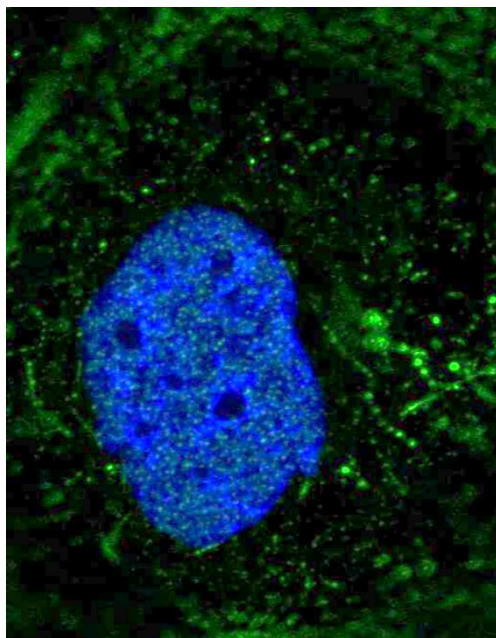
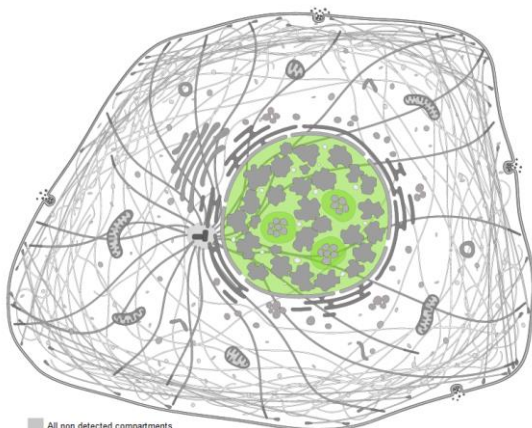
Figure 4

**A****SEPT7 (actin filaments, midbody, nucleoli)**

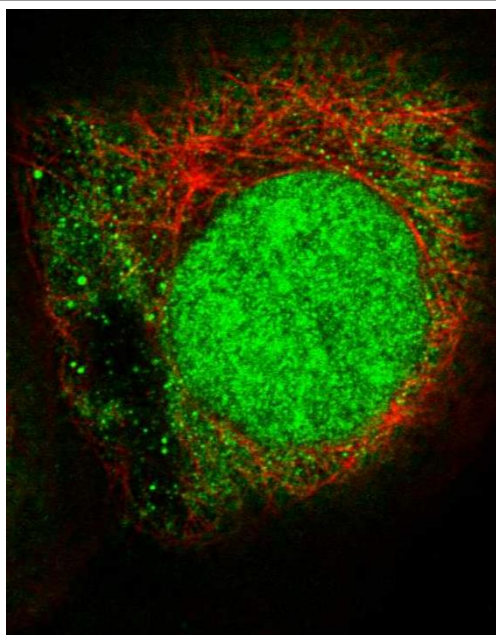
■ All non detected compartments  
 ■ SEPT7 detected in Actin filaments, Nucleoli and Midbody

**B****SEPT2 (actin filaments, nucleus)**

■ All non detected compartments  
 ■ SEPT2 detected in Actin filaments and Nucleus

**C****SRSF5 (nucleus and nucleoli)**

■ All non detected compartments  
 ■ SRSF5 detected in Nucleus and Nucleoli

**Figure 5**