A Genetic Screen for Human Genes Suppressing FUS Induced Toxicity in Yeast

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ABSTRACT  FUS is a nucleic acid binding protein that, when mutated, cause a subset of familial amyotrophic lateral sclerosis (ALS). Expression of FUS in yeast recapitulates several pathological features of the disease-causing mutant proteins, including nuclear to cytoplasmic translocation, formation of cytoplasmic inclusions, and cytotoxicity. Genetic screens using the yeast model of FUS have identified yeast genes and their corresponding human homologs suppressing FUS induced toxicity in yeast, neurons and animal models. To expand the search for human suppressor genes of FUS induced toxicity, we carried out a genome-scale genetic screen using a newly constructed library containing 13570 human genes cloned in an inducible yeast-expression vector. Through multiple rounds of verification, we found 37 human genes that, when overexpressed, suppress FUS induced toxicity in yeast. Human genes with DNA or RNA binding functions are overrepresented among the identified suppressor genes, supporting that perturbations of RNA metabolism is a key underlying mechanism of FUS toxicity.

KEYWORDS  FUS, ALS, human gene suppressors, yeast genetic screen

ALS is a neurodegenerative disease characterized by the degeneration of upper and lower motor neurons in the brain and spinal cord leading to progressive paralysis and ultimately death within five years of symptom onset. FUS is a multifunctional RNA-binding protein involved in diverse RNA metabolic processes. Mutations in FUS cause an inherited form of ALS (Kwiatkowski et al. 2009). Several features associated with FUS pathology, including formation of cytoplasmic inclusions and cytotoxicity, have been recapitulated in the budding yeast Saccharomyces cerevisiae, enabling us and others to employ this powerful genetic system to rapidly identify genetic suppressors (Ju et al. 2011; Sun et al. 2011b). A critical resource used in such genetic screens is a library consisting of ~5,000 sequence verified protein-coding yeast genes (Hu et al. 2007). Screening against this library has identified yeast genes that, when overexpressed, reduce the toxicity of FUS (Ju et al. 2011; Sun et al. 2011b). Consistent with the existence of conserved cellular mechanisms underlying FUS induced toxicity from yeast to human, UPF1, the human homolog of a yeast suppressor gene, shows strong protective effect in yeast, neurons and animal models of ALS (Barmada et al. 2015; Jackson et al. 2015; Xu et al. 2019). UPF1 is an essential component of the nonsense mediated mRNA decay (NMD) pathway, which detects and directs mRNAs with premature stop codons for degradation thus preventing the accumulation of truncated proteins (He and Jacobson 2015). NMD plays a critical role in mRNA surveillance, and is conserved from yeast to human (He and Jacobson 2015). Convergent suppressor mechanisms of FUS induced toxicity in yeast and mammalian systems supports the use of yeast as a model to study FUS toxicity.

We reasoned that direct expression of human genes in yeast may expand the search for genetic modifiers of cytotoxicity induced by disease-associated proteins. The obstacle to set up such a genetic screen is the availability of the full collection of human protein-coding genes cloned in a yeast expression vector. With Gateway cloning technology and a collection of 13570 human genes as entry clones generously provided to us by Dr. Marc Vidal, we generated a library of human genes individually cloned on a yeast expression vector, pAG416-GAL-ccdB (Alberti et al. 2007). Using this library, we screened for human suppressor genes of toxicity mediated by ALS-associated protein FUS.

Overexpression genetic screen is typically done by individually transforming a library of plasmid clones into a yeast model of interest (Willingham et al. 2003; Cooper et al. 2006; Ju et al. 2011; Sun et al. 2011b). We developed a more efficient and cost-effective method for the screen that relies on highly efficient yeast mating rather than...
transformation (Hayden et al. 2018). Using this method, we introduced the arrayed library of human genes into the yeast model of FUS and screened for human suppressor genes. After several round of verifications, we confirmed 37 human genes that, when overexpressed, strongly reduce FUS induced toxicity. Gene ontology (GO) enrichment analysis revealed that FUS suppressors are enriched in genes with nucleic acid or RNA binding functions. Given that our lab and others have previously identified FUS suppressors genes involved in RNA metabolism (Ju et al. 2011; Sun et al. 2011b), expression of the suppressor human RNA-binding proteins in yeast may counter deleterious effects of FUS on RNA processing, transport or stability.

MATERIALS AND METHODS

Human ORF clones

The human ORF clones used to generate the yeast-expression plasmids are Gateway entry clones kindly provided by Dr. Marc Vidal (Center for Cancer Systems Biology at Dana-Farber Cancer Institute and Department of Genetics, Harvard Medical School). The collection of human ORF clones, including the clones from hORFeome version 8.1 (Yang et al. 2011)(http://horfdb.dci.harvard.edu/) and clones from the ORFeome collaboration (Collaboration 2016)(http://www.orfeomecollaboration.org), have been sequence verified previously. All ORF clones were cloned into the pAG416GAL-ccdB vector (Alberti et al. 2007) using Gateway LR recombination cloning (Hartley et al. 2000; Wallhout et al. 2000). The resulting expression clones of human genes were subsequently transformed into the haploid w303 yeast strain (MATa can1-100, leu2-3,112, trp1-1, ura3-1, ade2-1, his3-11,15) using a high-throughput yeast transformation protocol as previously described (Fleming and Gitler 2011; Hayden et al. 2018). Transformed yeast strains bearing the expression plasmids of human genes were arrayed in 96-well plates and stored as glycerol archives at -80°C.

Yeast strains and growth media

The 1xFUS integration strain was generated in the haploid W303 yeast strain (MATa can1-100, leu2-3,112, trp1-1, ura3-1, ade2-1, his3-11,15::pRS305Gal1FUS) as previously described (Ju et al. 2011). YPD media (1% yeast extract, 2% peptone and 2% glucose in distilled water) was used for mating. Synthetic dropout media lacking uracil (Ura-) was used for growing the haploid w303 yeast strains containing the expression plasmids of human genes. Synthetic dropout media lacking both uracil and histidine (Ura- His-) was used for growing the diploid yeast strains containing both FUS integration and the human-gene plasmids. Yeast media contained 2% glucose, galactose or raffinose as the carbon source.

Genetic screen and verification

Detailed methods of screening by mating have been previously described (Hayden et al. 2018). Briefly, the haploid 1xFUS integration strain was crossed individually with each of the archived haploid yeast strains containing the expression clones of the human genes. The resulted diploid yeast strains were selected in Ura- His- liquid media, and plated onto Ura- His- agar plates containing glucose and galactose respectively, using the Singer RotoR robotic equipment (Singer Instruments). Following incubation at 30°C, pictures of yeast colonies grown on the agar plates were taken daily until day four. Suppressors of FUS were identified by visually inspecting galactose agar plates. Yeast strains that grew clearly better than the background growth of the diploid FUS model on galactose plates were considered as screening hits. The corresponding human gene plasmids of all screening hits were isolated, transformed in the haploid w303 MATa yeast strain. Each transformants was crossed with the haploid 1xFUS integration strain. The resulting diploid strains were then grown to mid-log phase in Ura- His- liquid media containing Raffinose. Next, cell cultures were normalized to OD600 at 1.0, serially diluted by five folds and spotted onto the respective Ura- His- dropout agar plates containing 2% glucose and galactose respectively. Agar plates were incubated at 30°C for three days and images of yeast colonies were taken. The identity of each verified human suppressor gene was confirmed by Sanger end-read sequencing and alignment to the expected sequence of the ORF clone.

Go term enrichment analysis

Go term enrichment analysis was conducted using PANTHER over-representation test. The reference gene list used to run the analysis included the 13570 Entrez gene IDs corresponding to all the cloned human ORF clones in the library. The annotation datasets used for the analysis was GO molecular function, biological process and cellular component released on 12/09/2019. Statistical analyses include Fisher’s Exact Test followed by FDR correction. All GO terms with a fold enrichment greater than or equal to three and FDR of less than or equal to 0.05 were included in Table 2.

Data availability

Strains and plasmids are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables.

RESULTS AND DISCUSSION

Identification of human gene suppressing the toxicity of FUS in yeast

With the ease to carry out genome-wide genetic screens, yeast has served as a model for studying many human disease-associated proteins. One of such genetic screens, involving systematic over-expression of yeast genes, was used to screen yeast suppressor genes of FUS toxicity. Dosage-dependent genetic rescue of FUS toxicity has also been confirmed for the human homolog of a yeast suppressor gene, leading to the identification of conserved mechanisms underlying FUS pathology (Ju et al. 2011; Barmada et al. 2015; Jackson et al. 2015).

More than 50% of human genes, however, do not have readily detectable homologs in yeast. Human genes without yeast homologs cannot be identified using the current genetic screens in yeast. A lack of sequence similarity does not necessarily exclude the possibility of such non-conserved human genes to function in yeast. Non-conserved human proteins may interact directly with FUS, suppressing its cytotoxicity in yeast. Alternatively, non-conserved human proteins may form inter-species protein-protein interactions with yeast proteins (Zhong et al. 2016), indirectly modulating the toxicity of FUS. To expand the search for new human gene targets related to FUS toxicity in yeast, we developed a new genomic tool that allows systematic overexpression of human genes in yeast (Figure 1). To do so, we first obtained a collection of sequence verified human ORFs as Gateway entry clones (corresponding to 13570 genes) (Yang et al. 2011; Collaboration 2016). While the collection does not contain all human genes, it was developed through unbiased large-scale cloning efforts and represents a key resource for systematic unbiased functional studies of human genes. We individually amplified the plasmid DNA of each entry clone and carried out Gateway LR reaction to transfer each human ORF into a yeast expression vector, pRS416Gal1ccdB (Alberti et al. 2007). The human ORFs cloned in the pRS416Gal1ccdB vector...
are under the control of the GAL1 promoter, which is induced upon shifting to galactose containing media.

Previously, high-throughput yeast transformation was used to introduce the plasmids bearing the gene to be overexpressed. Preparation of the library of plasmids can be time consuming and costly. Considering that the human-gene library contains many more clones than the yeast-gene libraries, we tested a method that relies on highly efficient yeast mating instead of transformation to introduce the arrayed plasmid library of human ORF clones into the yeast model (Hayden et al. 2018). First, we verified that yeast suppressor genes previously identified in the haploid yeast model of FUS (Ju et al. 2011) also exhibited suppressor effects on FUS toxicity in diploid yeast (Figure 2). This result indicates that screening for suppressors of FUS toxicity may be carried out in a diploid strain background. Next, we introduced the human-gene library into the W303 MATα strain, the isogenic but opposite mating type of the haploid yeast model of FUS (Ju et al. 2011). We arrayed yeast transformants, each with one human-gene expression clone in microplates and archived them as glycerol stock.

For the screen, we revived the arrayed human-gene library strains and individually crossed to the yeast model of FUS in YEED. The resulting diploid yeast strains containing both the integrated FUS gene and the human genes from the library were selected. Diploid yeast cells were robotically pinned in quadruplicate onto agar plates containing either glucose or galactose as the carbon source. Since FUS expression is highly toxic to yeast, growth for most diploid strains was reduced on galactose as compare to glucose. In contrast, the growth for a subset of the strains was better, indicating the suppression of FUS toxicity by simultaneously expression of another human gene (Figure 3).

After screening the entire library, we cherrypicked the expression clones of all screening hits and re-transformed them into w303 MATα. We restested the suppressor effect of all new transformants using the mating method. Finally, we confirmed their suppressor phenotype by serial dilution spotting assay (Figure 4). In total, we identified 37 human ORF clones corresponding to 37 human genes (Table 1), that suppress FUS toxicity when overexpressed.

**Human suppressor genes of FUS toxicity**

The identified suppressor genes appear to have a wide range of functions. GO enrichment analysis identified several functional groups of genes are overrepresented (Table 2), particularly those with RNA or DNA binding functions. FUS is a nucleic acid binding protein that has been implicated in transcription regulation, RNA splicing, RNA transport, and RNA stability (Polymenidou et al. 2012; Butti and Patten 2018). The identification of the group of suppressor genes with similar functions supports the idea that expression of FUS perturbs RNA metabolism in yeast, thus inducing cellular toxicity.

**RNA binding or splicing**

Cellular toxicity of FUS has been speculated to involve binding and sequestering of essential proteins and mRNAs into persistent intracellular inclusion bodies (Ciryam et al. 2017; Hayden et al. 2017). Consistent with this idea, previous studies using a yeast model of FUS have shown that FUS aggregation colocalizes with stress granules (Sun et al. 2011b). The identified human-gene suppressors in this group may compete to bind yeast mRNAs and protect them from being sequestered into FUS inclusions.

CPEB1 and CPEB2: Cytoplasmic polyadenylation element-binding protein 1 and 2 are member of the cytoplasmic polyadenylation element binding protein family, which promotes polyadenylation induced translation. CPEB proteins are involved in a wide range of cellular functions, including germ-cell development, cell division, synaptic plasticity, learning and memory (Richter 2007; Iwshina et al. 2014).

CPSF4 and CPSF6: Cleavage and polyadenylation specificity factor subunit 4 and 6 are essential components of the 3’ end processing machinery of pre-mRNAs. The cleavage factor complex plays a key role in pre-mRNAs 3’-cleavage and polyadenylation. Studies have found overexpression of CPSF4 in several types of cancer, such as lung, breast, and colorectal cancer (Zhang et al. 2016; Wu et al. 2019). Mutation studies indicate CPSF6 functions in regulating poly A site selection and preventing premature 3’-UTR cleavage (Sasado et al. 2017). It was reported that the interaction of
CPSF6 with HIV-1 capsid plays a critical role for targeting integration of HIV-1 to transcriptionally active chromatin (Sowd et al. 2016). CPSF6 is also implicated in breast cancer as a tumor promoting factor (Binothman et al. 2017).

PABPC5: Poly(A)-binding protein 5 is a protein that binds to the polyA tail of eukaryotic mRNAs. It plays a critical role in the regulation of mRNA transport and mRNA decay in the cytoplasm (Bhattacharjee and Bag 2012).

POLR2G: RNA polymerase II subunit B7 is a conserved protein that is part of the large 12-subunit RNA polymerase II. Together with another subunit B4, POLR2G not only is important for initiation of transcription, but also plays critical roles in diverse cellular processes, such as mRNA export, mRNA decay, DNA repair, protein translation, and stress response (Sharma and Kumari 2013; Kumar et al. 2019).

PYM1: Partner of Y14 and mago (PYM homolog 1) is an exon junction complex-associated factor that play an important role in exon-exon junction complex disassembly. It is also involved in the nonsense-mediated decay pathway and regulation of protein translation (Diem et al. 2007).

RBM14: RNA-binding motif protein 14 is an RNA binding protein that is involved in multiple important cellular processes, such as transcriptional regulation (Li et al. 2017), DNA damage response (Yuan et al. 2014; Simon et al. 2017), genome integrity maintenance (Shiratsuchi et al. 2015; Li et al. 2020), and maintaining the pluripotency of embryonic stem cells (Chen et al. 2018).

SF3A2: Splicing factor 3A subunit 2 is a subunit of the splicing factor 3A protein complex that includes subunits 1, 2 and 3 and is necessary for the assembly of spliceosome that plays a critical role in pre-mRNA splicing (Bennett and Reed 1993). Together with Prp31, it

Figure 3 A mating-based strategy to screen for human suppressor genes rescuing FUS toxicity upon overexpression. W303 MATa yeast containing the expression clones of human ORFs were revived from the glycerol stock and crossed with the FUS model generated in the isogenic yeast strain with the opposite mating type, W303 MATa. Diploid yeast strains were selected and spotted in quadruplicate, using the Singer RotoR robotic equipment, onto agar plates containing glucose (genes-off condition) and galactose (genes-on condition), respectively. On the galactose plates, most yeast had severely reduced growth due to the expression of FUS. The red square labels a screening hit of a human ORF clone that suppresses FUS toxicity allowing yeast to form much larger colonies.

Figure 4 Human suppressor genes rescue FUS toxicity upon overexpression. W303 MATa containing the empty vector, pRS416Gal1ccdB, or each of the identified human gene that suppress FUS toxicity were crossed with the FUS model generated in the isogenic strain with the opposite mating type, W303 MATa. Diploid yeast strains were selected, serially diluted and spotted onto agar plates containing glucose (genes-off condition) and galactose (genes-on condition), respectively. The picture, representing three independent experiments, was taken after growth at 30°C for three days. * indicating different ORF clones of the same gene.
also has a direct role in mitotic chromosome segregation (Pellacani et al. 2018).

TAF15: Mutations in TAF15 were found in familial form of ALS (Couthouis et al. 2011; Ticozzi et al. 2011). TAF15 encodes a TATA-binding protein-associated factor that functions in promoter recognition, transcription initiation complex assembly, and transcription activation (Kapeli et al. 2017).

THOC5: THO complex subunit 5 is a conserved protein in the THO complex that functions in processing and transport of mRNA (Scott et al. 2019). THOC5 is phosphorylated upon extracellular stimuli suggesting its role in DNA damage response (Ramachandran et al. 2011), growth factor/cytokine-mediated differentiation, and cancer development (Wang et al. 2013; Tran et al. 2016). New evidence also implicates this protein in synapse development and dopamine neuron survival (Maeder et al. 2018).

**DNA binding or transcription factors**

Overexpression of FUS in yeast may affect the expression of some yeast genes and subsequently disrupt their cellular functions and reduce cell fitness. Although the transcription factors identified from this screen may not directly target yeast genes and regulate their expression, it is possible that the suppressor proteins interact with FUS or with other yeast transcriptional factors, thus indirectly reverse the detrimental effect of FUS on yeast gene expression.

CDX2: Homeobox protein CDX-2 is a transcription factor that plays import roles in cell differentiation and proliferation. It is a well-known cancer risk factor. Abnormal CDX2 expression was indicated in metastasis of multiple cancers such as esophagus, stomach, colon, breast, ovarian, prostate cancer, and leukemia (Chawengsaksophak 2019).

DLX4: Homeobox protein DLX-4 is a transcription factor that belongs to the Distal-less (Dlx) family of proteins. DLX4 plays important functions in cell differentiation, proliferation, and migration. Abnormal DLX4 expression is associated with multiple cancers and preeclampsia (Sun et al. 2011a; Zhang et al. 2012). In addition, it was reported that DLX4, functionally replacing c-Myc, promotes generation of human induced pluripotent stem cells (Tamaoki et al. 2014).
EBF1 and EBF3: both genes encode highly conserved Collier/Olf/EBF (COE) family of transcription factors. EBF1 controls the expression of key proteins in B cell differentiation, signal transduction and function (Vilagos et al. 2012; Kong et al. 2016). EBF3 is thought to have functions in the nervous system, as mutations in EBF3 cause hypotonia, ataxia, and delayed development syndrome, a genetic neurodevelopmental syndrome (Chao et al. 2012; Kong et al. 2016). Expression of HOXC4 and HOXD4 was elevated in breast cancer, myeloid leukemias and uveal melanoma, suggesting its role as a potential oncogene (Bijl et al. 1997; Wu et al. 2020).

HLF: HLF gene encode a protein called hepatic leukemia factor. A Chimeric protein between HLF and E2A resulting from the leukemogenic translocation is responsible for a rare form of acute lymphoblastic leukemia. This arises from both impairment of normal function of E2A and TCF3 as a tumor suppressor in T lymphocytes, and activation of survival pathway triggered through HLF DNA binding domain (Seidel and Look 2001; Panagopoulos et al. 2012).

HMGB3 and HMGB20A: both genes encode members of the HMG-box superfamily of DNA-binding proteins. HMGB 3 encodes the high mobility group protein B3, which plays an important function in maintaining stem cell populations, and increased expression of HMGB3 is a risk factor for several type of cancer, possibly through its regulation on WNT/β-catenin pathway (Gao et al. 2015; Zhang et al. 2017; Lv et al. 2019). HMGB20A encodes high mobility group protein 20A, a close homolog HMGB20B that plays important roles in neuronal differentiation (Artegiani et al. 2010), and is required for SNAI1-mediated epithelial to mesenchymal transition (Rivero et al. 2015).

HOXC4 and HOXD4: Homeobox proteins C4 and D4 are a group of related transcription factors that play important roles in specifying regions of the body plan during development, ensuring that the structures form in the correct places (Rastegar et al. 2004; Nolte et al. 2006). Expression of HOXC4 and HOXD4 was elevated in breast cancer, myeloid leukemias and uveal melanoma, suggesting its role as a potential oncogene (Bijl et al. 1997; Wu et al. 2020).

OTX1: OTX1 is a member of the bicoid subfamily of homeo-domain-containing transcription factors that play critical roles in brain and sensory organ development (Simeone 1998; Zhang et al. 2015). Abnormal expression of OTX1 is also recently reported as a risk factor for various cancers, including neuroblastoma, breast, liver, gastric and colorectal cancer (Li et al. 2016; Qin et al. 2018; Li et al. 2019; Micheloni et al. 2019; Yang et al. 2019).

PLAG1: Pleomorphic adenoma gene 1 (PLAG1) encodes a zinc finger transcription factor, which is developmentally regulated. Study of PLAG1 knockout mice suggests it is an important regulator in postnatal growth and reproduction (Juma et al. 2016). Overexpression and knockdown of PLAG1 also indicate its role in regulating neuronal gene expression and neuronal differentiation of neocortical neural progenitor cells(Sakai et al. 2019).
TGIF1: TGIF1 belongs to the three-amino acid loop extension (TALE) superclass of atypical homeodomain proteins that function as transcription regulators. It plays an important function in normal brain development. Mutations in this gene are associated with holoprosencephaly type 4, which is a structural anomaly of the brain (Wotton and Taniguchi 2018; Zhu et al. 2018). Abnormal expression of TGIF1 has also been implicated in many cancers.

**Genes involved in cell signaling**

**DUSP10**: Dual specificity protein phosphatase 10 is an enzyme that inactivates stress-activated kinases, such as p38 and SAPK/JNK, by dephosphorylation. The gene is ubiquitously expressed, and its dephosphorylation. The gene is ubiquitously expressed, and its expression is highly induced upon stress stimuli. Studies indicated a clear role of DUSP10 in inflammation, immunity, and cancer (Jimenez-Martinez et al. 2019).

**RAPGEF4**: Rap guanine nucleotide exchange factor (GEF) 4 is a protein that is targeted by the second messenger cAMP, and functions as a guanine nucleotide exchange factor for the Ras-like small GT-Pase Rap upon cAMP stimulation. It is involved in various cellular processes such as integrin-mediated cell adhesion, cell-cell junction formation, cell proliferation and differentiation, cell survival, and neuronal signaling (Bos 2006; Roscioni et al. 2008; Kumar et al. 2018).

**RGL1**: Ras guanine nucleotide dissociation stimulator-like 1 is a small GTPase functioning in signal transduction (Soood et al. 2000). The Rap1-Rgl-Ral signaling network plays important role in regulating neuroblast cortical polarity and spindle orientation (Carmena et al. 2011).

**RASA3**: Ras GTPase-activating protein 3, a member of the GAP1 subfamily, is an inositol 1,3,4,5-tetrakisphosphate-binding protein (Schurmans et al. 2015). The protein enhances the weak intrinsic GT-Pase activity of RAS proteins resulting in the inactive GDP-bound form of RAS (Cullen et al. 1995). It plays important roles in erythropoiesis, megakaryopoiesis, megakaryocyte adhesion and migration as well as integrin signaling (Blanc et al. 2012).

**INPP5A**: The protein encoded by this gene is inositol polyphosphate 5-phosphatase A, a membrane-associated enzyme. It hydrolyzes inositol polyphosphate, which regulates calcium release from intracellular stores and acts as a second messenger regulating cell proliferation and survival. Deletion of INPP5A causes progressive and permanent loss of cerebellar Purkinje cells in mice, suggesting its crucial role in Purkinje cell survival (Ooms et al. 2009; Yang et al. 2015).

**IRAK4**: Interleukin-1 receptor-associated kinase 4 is one of the four members of the IRAK family that plays an important role in signaling innate immune responses from Toll-like receptors. Accumulated evidence indicates its abnormal expression in inflammatory autoimmune disorders (Su et al. 2020). Interestingly, targeted degradation of IRAK4 was used for the treatment of cancer, neurodegenerative and cardiovascular diseases (Kargbo 2019a; Kargbo 2019b).

**Additional genes**

**ASB7**: Ankyrin repeat and SOCS box protein 7 is an E3 ubiquitin ligase. It plays a crucial role in regulating spindle dynamics and genome integrity through targeting DDA3 for proteasomal degradation (Uematsu et al. 2016). A recent study also indicated the expression of ASB7 was elevated upon activation of the unfolded protein response pathway under endoplasmic reticulum stress (Anasa et al. 2018).

**DENND11**: DENN domain containing 11 protein is highly conserved in a wide range of animal species. Studies indicated its important role in neurogenesis and neuronal recovery in the hippocampus following transient cerebral ischemia (Zhang et al. 2007).

**NKAPD1**: NKAPD1 encodes an uncharacterized protein C11orf57, which is also called NKAP Domain Containing 1.

**SNTG1**: SNTG1 encodes gamma-1 syntrophin, a neuronal cell specific protein. Syntrophins are scaffold cytoplasmic membrane proteins that bind signaling molecules, such as gamma-enzolase for its neurotrophic activity (Hogan et al. 2001; Hafner et al. 2010). Gamma-1 syntrophin associates directly with dystrophin, a protein involved in the Duchenne muscular dystrophy (Bashir et al. 2004).

**SS18**: SS18 gene encodes a protein called synovial sarcoma translocated to X chromosome. Disruption of SS18 gene in mouse results in embryonic death due to placental failure. Fusion SS18-SXX1 is believed to underlie the pathogenesis of synovial sarcoma through elevated expression of the key Wnt target AXIN2 (De Bruijn et al. 2006; Cironi et al. 2016).

**TEX11**: TEX11, a male germ cell specific X-linked gene, encodes a testis expressed sequence 11 protein, which is essential for meiosis and male fertility in animals (Yatsenko et al. 2015).

**UGP2**: UGP2 gene encodes UTP-glucose-1-phosphate uridylyltransferase, an enzyme conserved from bacteria to human as a key player in glycogenesis and cell wall synthesis. In yeast, its expression contributes to oxidative stress response and long-term cell survival through production of storage carbohydrates. In higher animals, it is highly active in the liver and muscles (Smith and Rutter 2007; Yi and Huh 2015).

**ZMYND12**: Zinc finger MYND domain-containing protein 12 is conserved among higher eukaryotes. Its function is unknown.

In summary, we screened a previously established yeast model of FUS against a new collection of 13570 human genes and identified 37 suppressor genes of FUS induced toxicity in yeast. Although the identified suppressors have a wide range of cellular functions, genes encoding proteins involved in RNA and DNA binding are significantly overrepresented, suggesting a strong relationship between the toxicity of FUS in yeast and dysregulation in RNA metabolism, which is a widely considered major contributor to ALS pathogenesis (Polymeridou et al. 2012; Butti and Patten 2018).

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