RNA Binding Protein TAF15 Suppresses Toxicity in a Yeast Model of FUS Proteinopathy

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Research Article

Keywords: FUS, RNA binding protein, ALS, toxicity

Posted Date: April 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-437201/v1

RNA binding protein TAF15 suppresses toxicity in a yeast model of FUS proteinopathy

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Abstract

Mutations in Fused in Sarcoma (FUS), an RNA binding protein that functions in multiple steps in gene expression regulation and RNA processing, are known to cause familial amyotrophic lateral sclerosis (ALS). Since this discovery, mutations in several other RNA binding proteins (RBPs) have also been linked to ALS. Some of these ALS-associated RBPs have been shown to colocalize with ribonucleoprotein (RNP) granules such as stress granules and processing bodies (p-bodies). Characterization of ALS-associated proteins, their mis-localization, aggregation and toxicity in cellular and animal models have provided critical insights in disease. More and more evidence has emerged supporting a hypothesis that impaired clearance, inappropriate assembly, and dysregulation of RNP granules play a role in ALS. Through genome-scale overexpression screening of a yeast model of FUS toxicity, we found that TAF15, a human RBP with a similar protein domain structure and belonging to the same FET protein family as FUS, suppresses FUS toxicity. The suppressor effect of TAF15 is specific to FUS and not found in other yeast models of neurodegenerative disease-associated proteins. We showed that the RNA recognition motif (RRM) of TAF15 is required for its rescue of FUS toxicity. Furthermore, FUS and TAF15 physically interact, and the C-terminus of TAF15 is required for both the physical protein-protein interaction and its protection against FUS toxicity. Finally, while FUS induces and colocalizes with both stress granules and p-bodies, TAF15 only induces and colocalizes with p-bodies. Importantly, co-expression of FUS and TAF15 induces more p-bodies than individually expressing each gene alone, and FUS toxicity is exacerbated in yeast that is deficient in p-body formation. Overall, our findings suggest a role of p-body formation in the suppression of FUS toxicity by TAF15.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration of motor neurons resulting in paralysis and ultimately death within 2-5 years of symptom onset [1]. The precise mechanisms leading to motor neuron death are not known; however, RNA dysregulation has emerged as a prominent mechanism contributing to ALS pathogenesis [2, 3].

A pathologic hallmark of ALS is the presence of cytoplasmic protein inclusions within affected neuronal cells; in a vast majority of cases these inclusions contain the RNA binding protein TAR DNA Binding Protein (TDP-43) [4, 5]. Furthermore, mutations in the gene encoding TDP-43 cause familial ALS and rare sporadic cases of ALS [6]. Like TDP-43, another RNA-binding protein, FUS, is mutated in ALS and accumulates in pathologic cytoplasmic inclusions in ALS patient tissue [7, 8]. Over the last decade, mutations in several other genes encoding RNA-binding proteins have been found in ALS patients; including hnRNPA1, hnRNPA2B1 [9], Matrin 3[10], TAF15 [11, 12] and EWSR1[13]. Proposed mechanisms leading to neurodegeneration in ALS include misregulation of RNA metabolism through sequestration of RNA and RBPs in pathologic inclusions, splicing dysregulation, RNA transport and stability defects, and disruption of nonsense-mediated decay.

It has been established that when overexpressed in yeast cells, both TDP-43 and FUS form cytoplasmic inclusions and induce cytotoxicity [14-16]. Genetic screening using yeast models of neurodegenerative disease has been successful in identifying mechanisms of toxicity contributing to various neurological disorders as well as developing candidate therapeutic targets [15-21]. This screening pipeline relies on screening against a library of ~5,500 yeast genes and then identifying and testing the human homologs of the yeast suppressors for their effects on FUS toxicity [22]. Our lab and others have taken advantage of the yeast model system to perform genome wide screening to identify modifiers of ALS protein toxicity. We identified that a yeast gene encoding an RNA-binding protein, Ecm32, protects yeast cells against FUS induced toxicity [16]. The human homolog of Ecm32, hUPF1, is an ATP-dependent RNA helicase that associates with p-bodies, and is a key regulator of the nonsense-mediated mRNA decay pathway [23]. Subsequently, we found that expression of hUPF1 is protective against ALS protein toxicity in mammalian neurons as well as in a rat model of TDP-43 [24, 25], supporting the

hypothesis that there are conserved mechanisms underlying the rescue of FUS toxicity in yeast and mammalian cells.

Importantly, expression of hUPF1 in yeast protects cells from the toxicity induced by FUS and TDP-43 [16]. Based on these observations, we reasoned that human-gene modifiers might be directly identified in yeast models. Direct screening of human genes would provide greater coverage and allow the identification of suppressors without obvious yeast homologs. This is significant as the majority of human genes do not have homologs in yeast, and therefore could not be identified based on homology relationships with yeast suppressor genes. To achieve this goal, we developed a high-throughput screening platform by constructing a library consisting of 13,570 human genes individually cloned into a yeast expression vector. Using this human-gene library, we screened a yeast model of FUS toxicity. We identified 37 human genes that when overexpressed strongly suppress FUS toxicity [26]. The identified suppressor genes are enriched in genes encoding RNA-binding proteins, including TAF15.

TAF15 shares similar domain structures to two other RBPs, FUS and EWSR1. In fact, the three RBPs belong to the same protein family, known as the FET proteins. which participate in the regulation of transcription, mRNA processing, and nucleocytoplasmic transport [27, 28]. Like FUS, TAF15 accumulates into cytoplasmic stress granules under a variety of stress conditions [29, 30]. All three FET proteins, FUS, EWSR1 and TAF15, are present in the FUS positive cytoplasmic inclusions found in patients with ALS and Frontotemporal Lobar Degeneration (FTLD) [11, 12, 29, 31].

In this study, we characterized the suppressor effect of TAF15 on FUS toxicity. We found that TAF15 specifically suppressed the toxicity of FUS, while having no effect on cellular toxicity induced by a few other proteinopathies previously established in yeast. While TAF15 does not change cytoplasmic localization or protein level of FUS, the RNA recognition motif (RRM) in TAF15 is essential for its suppressor effect. We observed a physical protein-protein interaction between FUS and TAF15 that is also required for suppression of FUS toxicity. We show that TAF15 induces and localizes to p-bodies in yeast. Unlike FUS, TAF15 does not induce or associate with stress granules. When FUS and TAF15 are co-expressed, induction of p-bodies is stronger than with individual expression of each protein. Moreover, FUS toxicity is exacerbated in mutant yeast strains that are deficient in the formation of p-bodies. Taken

together, our data suggest a role of p-body induction in the suppression of FUS toxicity by TAF15.

Results

Expression of the RNA-binding protein TAF15 rescues FUS toxicity

To directly identify human suppressor genes of FUS induced toxicity, we performed a genome-scale genetic screen using a library containing 13,570 human genes individually cloned in an inducible yeast-expression vector [26]. Through this work, we identified TAF15 as a suppressor of FUS toxicity (Fig. 1A). TAF15, FUS and EWSR1 are three members of the FET protein family. EWSR1 was not included in the human-gene library used for the screen. Given the functional and structural similarities between the three FET proteins, we also tested the effect of EWSR1 on FUS toxicity. We individually cloned EWSR1 in the same expression vector and performed serial dilution growth assays in the yeast model of FUS. Unlike TAF15, expression of EWSR1 does not suppress the toxicity of FUS (Fig. 1A).

It has been established that toxicity of FUS is dose-dependent in yeast [16]. To test whether TAF15 is acting by decreasing FUS protein level, we performed western blot using antibodies against TAF15 and FUS. Upon six hour of induction, FUS protein levels were not altered by the expression of TAF15 (Fig. 1B). Additionally, we performed western blot for FUS protein at earlier and later time points in TAF15 expression, and FUS protein levels remained unchanged by TAF15 (Supplementary Fig. S1).

To investigate if the suppressor effect of TAF15 is specific to FUS-mediated protein toxicity, we expressed TAF15 in several other well-established yeast models of neurodegenerative disease proteotoxicity, including another ALS-linked protein, (TDP-43), a Huntington's disease associated protein (mutant huntingtin fragment HTT103Q) and a Parkinson's disease associated protein (α-synuclein) [14, 18, 32-35]. Toxicity caused by these proteins seems to occur largely through different mechanisms as genetic screening for modifiers of toxicity has uncovered different sets of modifier genes [15, 18, 20, 21, 36, 37]. Serial dilution growth assays of each of these yeast models showed that TAF15 only protects cells against FUS toxicity, while having no effect on the toxicity caused by TDP-43, HTT103Q or α-synuclein (Fig. 1C).

FUS contains a non-classical proline-tyrosine nuclear localization signal (PY-NLS) in its extreme C-terminus which allows FUS to shuttle between the nucleus and cytoplasm. FUS nuclear localization signal is nonfunctional in yeast, resulting in cytoplasmic localization and toxicity of FUS. Increasing the nuclear localization of FUS by adding a functional yeast NLS to the C-terminus of FUS suppressed its toxicity [16]. Promoting nuclear localization of FUS has been shown as a mechanism to reduce toxicity in several other model systems as well [38-40]. We therefore questioned whether TAF15 restores FUS localization to the nucleus. To test this possibility, we generated a yeast strain expressing YFP-tagged FUS, which exhibits similar toxicity as the non-tagged FUS model (Fig. S2). Upon co-expression of TAF15, FUS remained localized to cytoplasmic foci (Fig. 1D), indicating that TAF15 is not acting by promoting nuclear localization of FUS.

RNA and protein interaction domains of TAF15 are required for the rescue of FUS toxicity

Both FUS and TAF15 contain several conserved protein domains and share a similar domain structure. One of their most highly conserved domains is their RNA recognition motif (RRM). The RRM of FUS and TAF15 RRM are ~85% identical (Fig. 2A), while the RRM of FUS and EWSR1 are less similar (~60%). RRM alignment showed that TAF15 and FUS share the same conserved phenylalanine residues (Fig. 2A, highlighted in red). Conserved phenylalanine residues in FUS RRM are responsible for its direct stacking interactions with RNA bases [41]. Mutation of conserved phenylalanine residues to leucine in FUS disrupt its binding to RNA, and render FUS non-toxic in yeast and drosophila models [15, 42].

Given the similarity in RRMs between FUS and TAF15, we reasoned that these phenylalanine residues may be important for the RNA binding ability of TAF15 and its ability to rescue FUS toxicity. For example, TAF15 may compete for binding to a similar set of RNA targets in yeast as FUS. To test this possibility, we mutated four conserved phenylalanine residues within the RRM of TAF15 (Phe^{254}, Phe^{290}, Phe^{308} and Phe^{317}) to leucine. Unlike wild-type TAF15, the RRM mutant of TAF15 (Mut^{RRM}) fails to rescue FUS toxicity (Fig. 2B). Western blot demonstrated that the loss of rescue is not due to the abnormal expression of the mutant protein, as Mut^{RRM} was similarly expressed as wild type TAF15 (Fig. 2C). These data support our hypothesis that the rescue of FUS toxicity by TAF15 is dependent on its RRM.

It has previously been reported that the FET proteins interact with each other and are found in the same protein complex [43, 44]. Using recombinant proteins in pull-down experiments, the N-terminal domains of FUS and TAF15 were found to form homo and heterocomplexes with full length versions of themselves, as well as with each other. In addition, a second binding region in the C-terminus of TAF15 was also identified [44]. We wondered if the physical interaction between FUS and TAF15 is required for the observed rescue of FUS toxicity. To test this possibility, we generated TAF15 mutants lacking its N terminus (TAF15ΔN, lacking amino acids 1-150) and a C-terminal region (TAF15ΔC, lacking amino acids 385-592). Next, we tested whether these TAF15 mutants retain their ability to suppress FUS toxicity. Interestingly, TAF15ΔN but not TAF15ΔC rescues FUS toxicity (Fig. 2D). To confirm that the lack of rescue seen with TAF15ΔC is not due to abnormal expression of the mutant protein, western blot was performed. TAF15ΔC was similarly expressed as the full-length wild type protein (Fig. 2E). Finally, to test whether FUS and TAF15 are interacting partners in our yeast model, we used the yeast two-hybrid (Y2H) assay, with FUS expressed as a DB (Gal4 DNA-binding domain) fusion protein and TAF15 expressed as an AD (Gal4 activation domain) fusion protein. Based on the ability of yeast to grow on media lacking the amino acid histidine due to the activation of the HIS3 reporter gene, wild-type TAF15 and FUS physically interact (Fig. 2F). The same is true for TAF15ΔN and Mut^{RRM} mutants. The interaction between TAF15ΔC mutant and FUS is no longer detectable in the Y2H assay (Fig. 2F). These findings are consistent with our hypothesis that that the interaction between TAF15 and FUS is also required for TAF15 to act as a suppressor of FUS toxicity.

Both FUS and TAF15 induce and localize to p-bodies

Stress granules and p-bodies are conserved cytoplasmic ribonucleoprotein (RNP) granules that assemble via liquid-liquid phase separation in response to specific stress conditions [45, 46]. Stress granules contain translation initiation factors, small ribosomal subunits and RBP's and are thought to play a role in storage of mRNA that can be translated following resolution of the inducing stress. P-bodies function in the storage and degradation of mRNA, and contain proteins involved in decapping, 5'-3' mRNA decay, as well as nonsense mediated decay [47, 48]. FUS inclusions have been shown to colocalize with the stress granule markers PABP-1 and eIF4G in postmortem brain and spinal cord tissue from ALS patients with mutations in FUS [49]. Endogenous FUS localizes to stress granules following sorbitol stress, and ALS-linked mutant

FUS localizes to stress granules under a variety of stress conditions [50-52]. Many ALS-linked mutations occur in proteins that affect stress granules dynamics or in proteins that localize to stress granules, leading to the hypothesis that dysregulation of stress granules underlies ALS pathology [53, 54]. TAF15 was reported to form cytoplasmic foci in yeast, but whether these foci colocalize with stress granules or p-bodies has not been studied [12].

To test whether FUS and TAF15 localize to either or both of these RNP structures, we individually expressed FUS-YFP and ECFP-TAF15 in yeast containing mCherry tagged p-body marker (Edc3) and stress granule marker (Pub1). Overexpression of FUS and TAF15 both induce formation of p-bodies and both proteins colocalize with p-bodies (Fig. 3A-B). In contrast, FUS, but not TAF15, induces the formation of stress granules (Fig. 3C-D). FUS inclusions colocalize with Pub1-labeled stress granule structures (Fig. 3C), while Pub1 remains diffusely localized in the cytosol, when TAF15 is expressed alone (Fig. 3D). Similar results were obtained using another stress granule marker, Pab1 (Supplementary Fig. S3).

TAF15 enhances p-body formation when co-expressed with FUS

Considering that both RNA-binding and physical interaction with FUS are required for TAF15 to suppress FUS toxicity, we reasoned that p-body formation may be involved in the observed rescuing effect. We therefore examined the formation of p-bodies in yeast strains expressing both FUS and TAF15. We transformed yeast with both ECFP tagged TAF15 and YFP tagged FUS. Similar to the non-tagged TAF15 protein, ECFP tagged TAF15 rescues FUS toxicity (Fig. 4A). Similar to what we found in Fig. 3A and 3C, individual expression of FUS and TAF15 induced formation of p-bodies and both proteins co-localize with p-bodies (Fig. 4B). When FUS and TAF15 were co-expressed, a greater number of p-bodies (~50% increase) were detected compared to when there was individual expression of FUS and TAF15 (Fig. 4B-C). These data support a possible role of p-bodies in the rescue of FUS toxicity by TAF15.

To further explore the involvement of p-body in rescuing cells from FUS toxicity, we considered p-body associated yeast genes. Edc3, Dhh1 and Pat1 are important regulators of p-body assembly in yeast. Deletions of each of these three genes cause deficiency in the formation of p-bodies [55-57]. To test a role of p-body in FUS toxicity, we expressed FUS in three mutant yeast strains with *EDC3*, *DHH1*, or *PAT1* deleted. Deletion of each of the three genes alone does not elicit any growth defect. In contrast, FUS toxicity is markedly enhanced in these deletion

mutants. These data support the idea that formation of p-bodies is critical in mitigating cellular toxicity induced by FUS (Fig 4D).

Discussion

Mutations in a number of RBPs have been linked to neurodegenerative diseases such as ALS and FTD, and accumulating evidence suggests that dysregulation of RNA metabolic processes is an underlying mechanism contributing to disease pathogenesis[58-61]. Evidence from in-vitro and in-vivo models of FUS pathology point to cytoplasmic mislocalization and accumulation into stress granules as drivers of FUS toxicity through a variety of proposed mechanisms. These mechanisms include sequestration of essential mRNA and RNA binding proteins, disruption in mRNP dynamics and dysregulation of NMD, to name a few[15, 16, 51, 62-64]. Previous yeast genome-wide overexpression and deletion screens using a yeast model of FUS toxicity identified RBPs and proteins involved in RNA metabolism as modifiers of FUS toxicity [15, 16]. In a newly developed genome-scale genetic screen, we identified human genes that strongly suppress FUS toxicity [26]. The identified suppressors are enriched in genes encoding RBPs. Among them is TAF15, which belongs to the same FET protein family as FUS.

TAF15 has no yeast homologue, making it a unique suppressor that could not be identified by homologous relationship to yeast suppressor genes. TAF15 does contain conserved protein domains found in yeast proteins such as its prion-like domain, RNA recognition motif, and RGG motif, and may target RNA in yeast cells. Notably, while expression of FUS causes robust toxicity in yeast, TAF15 expression is well tolerated and does not lead to overt toxicity (data not shown). A yeast screen of 133 human RRM containing proteins identified 38 RNA binding proteins that cause toxicity to yeast, with only seven that cause a similar degree of toxicity as FUS [12]. Indeed, not all RBPs cause toxicity in yeast when overexpressed, and further exploration of the differences in properties of RBPs accounting for their toxicity would be relevant for understanding neurodegeneration in the context of disruption of cellular functions linked to specific RBPs.

FUS toxicity is dependent on RNA binding. Given the similarity in RNA binding profiles between FUS and TAF15, we speculate that TAF15 may be competing with FUS for binding RNA targets thus preventing FUS from mediating RNA binding dependent toxicity. Interestingly, EWSR1 had

no effect on FUS toxicity. Comparison of the RRM of FUS, TAF15 and EWRS1 revealed greater similarity between the RRM of FUS and TAF15 compared to EWSR1. This supports the idea that TAF15 may rescue the toxicity through competing with FUS for binding RNA. Consistent with this, mutation of conserved phenylalanine residues in the RRM of TAF15, which are important for RNA binding, eliminate the ability of TAF15 to rescue FUS toxicity (Fig. 2B).

Apart from RNA-binding, our data support that a protein-protein interaction between FUS and TAF15 is also required for the rescue. Deletion of the C-terminal region of TAF15 which is critical for its interaction with FUS eliminates its ability to rescue FUS toxicity (Fig. 2D-F). Levels of FUS protein, however, are unchanged in the presence of TAF15 (Fig. 1B). Moreover, FUS remains localized to cytoplasmic foci in the presence of TAF15 (Fig. 1D), indicating that TAF15 bypasses FUS toxicity without directly acting on the FUS protein. Given that deficiency of FUS in animals leads to a variety of issues including perinatal lethality, genomic instability and hippocampal vacuolation [65, 66] and that depletion of Cabeza, the Drosophila homolog of FUS, results in locomotor defects [67-69], therapeutic strategies aimed at knockdown of FUS may be problematic.

The rescue by TAF15 is specific to FUS as TAF15 has no effect on the toxicity of other neurodegenerative disease associated proteins. It is not surprising that TAF15 had no effect on toxicity of HTT103Q and α-syn yeast models given the lack in yeast modifier genes shared between FUS and these models. TDP-43 and FUS, however, are both RBPs containing prion-like domains as well as other shared domains. Like FUS, TDP-43 has been shown to colocalize with stress granules and p-bodies in yeast [36, 70] which is interesting considering that TAF15 had no effect on TDP-43 toxicity. In this regard, it is notable that using the same Y2H assay, we could detect interaction of TAF15 with FUS but not with TDP-43 (data not sown). The lack of physical interaction may account for the lack of rescue.

Pull down experiments with FUS and TAF15 have previously shown that incubation of the protein complex with DNase and/or RNase did not affect the binding between FUS and TAF15 [44], suggesting that RNA molecules bound to FUS and TAF15 are not necessary for maintaining protein-protein interaction between the two proteins. Consistent with this finding, no difference was observed in the interaction strength with FUS between the RRM mutant and wild-type TAF15 (Fig. 2F). Given that the RRM mutant of TAF15 fails to rescue FUS toxicity, it is likely

that protein-protein interaction alone is not sufficient for the rescue of FUS toxicity by TAF15 and that RNA-binding activity is also required.

Considering our observations that FUS localizes to both p-bodies and stress granules, while TAF15 exclusively localizes in p-bodies (Fig. 3), we speculate that formation of p-bodies may be protective. Consistent with this idea, overexpression of the yeast core p-body proteins Sbp1 and Edc3 were both found through genome wide screens to suppress FUS toxicity [15, 16]. To test if the induction of p-bodies may be protective, we quantified the number of p-bodies in yeast strains expressing FUS and TAF15 individually and together. Consistent with our hypothesis, yeast co-expressing FUS and TAF15 contained more p-bodies per cell as compared to yeast expressing each protein individually (Fig. 4B-C). It is possible that in the presence of TAF15, FUS is being targeted from toxic stress granules to protective p-bodies. This hypothesis is further supported by our finding that FUS toxicity is markedly enhanced in yeast deletion strains defective in p-body formation (Fig. 4D). Characterization of the effect of TAF15 on FUS toxicity and examining differential effects of TAF15 and FUS on RNPs in other model systems should provide deeper insights in FUS mediated protein toxicity in ALS.

Methods

Yeast strains, media and plasmids

The 1XFUS integration strain was generated in the haploid W303 yeast strain (*MAT*a can1-100, leu2-3,112, trp1-1, ura3-1, ade2-1, his3-11,15::pRS303Gal1FUS) as previously described [16]. To The FUS-YFP strain was generated by transforming pRS413Gal1FUS-YFP into the haploid W303 yeast strain (*MAT*a can1-100, leu2-3,112, trp1-1, ura3-1, ade2-1, his3-11,15). Yeast transformed with free (non-integrating) plasmids were grown in synthetic complete media containing all amino acids except for those used for selection of the plasmid. Media contained either 2% dextrose, galactose or raffinose as the carbon source. Yeast was grown at 30°C with shaking (200rpm) for liquid cultures.

An LR reaction was used to transfer genes from Gateway entry clones to Gateway compatible yeast destination vectors [71]. Products of the LR reaction was transformed into DH5α competent bacteria using standard bacterial transformation methods. Expression clones were extracted and confirmed with restriction enzyme digestion and sequencing.

To generate pRS413Gal1FUS-YFP plasmid, an BP reaction was used to transfer full length FUS gene from pRS426Gal-FUS-YFP (Addgene source item #29592)[15] onto Gateway entry clone pDONR223FUS. FUS was then transferred to the Gateway destination vector pRS413Gal1ccdB-YFP for making the yeast expression construct of C-terminal YFP tagged FUS. The expression plasmid was confirmed by DNA sequencing.

To generate TAF15 RRM mutant plasmids (TAF15 Mut^{RRM}), a DNA fragment containing TAF15's RRM domain with four phenylalanine mutations (F254L, F290L, F308L, & F317L) was synthesized and cloned into the Gateway entry vector pDONR223. The final product, pDONR223-TAF15 Mut^{RRM}, was confirmed by sequencing. A gateway LR reaction was then used to shuttle TAF15 Mut^{RRM} into various destination vectors.

Site-directed mutagenesis was used to generate the TAF15 N-terminal deletion mutant (TAF15ΔN) and C-terminal deletion mutant (TAF15ΔC). Mutagenesis was performed using the QuickChange II Site-Directed Mutagenesis Kit (Agilent) according to the manufacturer's instructions. Mutagenic primers were designed online using the QuickChange Primer Design Program available at www.agilent.com/genomics/qcpd. Mutations were verified by DNA sequencing.

Yeast Transformation

Yeast expression constructs were transformed using standard PEG/lithium acetate method [72]. Cells were then spread onto respective synthetic amino acid dropout plates selecting for presence of the plasmid and grown at 30°C for 3 to 4 days.

Serial Dilution Growth Assays

Yeast cultures were grown overnight in synthetic media containing raffinose as the carbon source. Cultures were normalized to an OD_{600}=1.0, 5X serially diluted, and spotted to their respective synthetic agar plates containing 2% glucose to shut down gene expression (Gene Off) or galactose (Gene On) to induce gene expression. Agar plates were incubated at 30°C for 4 days, and pictures of the plates were taken every 24 hours.

Yeast-two-hybrid (Y2H) assay

AD Y2H constructs were transformed into the yeast strain Y8800 (*MAT*a leu2-3,112 trp1-901 his3Δ200 ura3-52 gal4Δ gal80Δ GAL2::ADE2 GAL1::HIS3@LYS2 GAL7::lacZ@met2 cyh2R) and DB Y2H constructs were transformed into the yeast strain Y8930 (*MAT*α leu2-3,112 trp1-901 his3Δ200 ura3-52 gal4Δ gal80Δ GAL2::ADE2 GAL1::HIS3@LYS2 GAL7::lacZ@met2 cyh2R). DB and AD yeast strains of the opposite mating types were crossed on a YPD agar plate. Diploid yeast from YPD plates were selected on SD/-Leu/-Trp agar plates. For the Y2H interaction assay, 3ul of diploid yeast growing in SD/-Leu/-Trp media were spotted on SD/-Leu/-Trp (as control) and selective SD/-Leu/-Trp/-His plates. Growth in the absence of Histidine indicates a positive protein-protein interaction. Both the control and selective plates were incubated at 30°C for 5 days and pictures of the plates were taken every 24 hours.

Western Blotting

Yeast crude extract was prepared using a post-alkaline extraction method [73]. Protein was subjected to SDS/PAGE and transferred to a PVDF membrane (Millipore). The membrane was rinsed with water and then blocked with 5% nonfat dry milk in TBST for 1 hour. The membrane was then incubated with the primary antibody overnight at 4 C. After being washed, the membrane was incubated with an alkaline phosphatase conjugated secondary antibody for 1 hour. The membrane was washed again in TBST and then developed with one-step NBT/BCIP solution (Thermo Scientific). The anti-FUS (Abcam), anti-PGK1 (Invitrogen), anti-TAF15 (Abcam) antibodies, AP-conjugated anti-rabbit and anti-mouse secondary antibodies were used at a dilution of 1:10,000. The image was taken using the epi-illumination colorimetric capture option of Amersham Imager 600.

Fluorescence Microscopy

Yeast strains were grown in synthetic media containing raffinose to mid-log phase. Cultures were centrifuged, washed with sterile ddH_2O twice, and then resuspended in media containing 2% galactose to induce protein expression for 6 hours unless otherwise specified. Cultures were then harvested and placed on a microscope slide with a cover slip. Images were obtained with an Olympus IX83 inverted fluorescent microscope at either 40X or 100X (oil immersion) magnification using FITC, EYFP, ECFP and TxRed filter cubes (chroma 39002, 39003, 49001, 39010). For multi-color imaging experiments, bleed through experiments were first conducted

with each fluorescently tagged protein of interest individually to make sure that signal from the fluorescently tagged protein was only visible in its appropriate filter.

Quantification of P-bodies

Yeast strains were grown overnight at 30°C to mid-log phase (OD_{600}=~0.5) in Raffinose containing media. Cultures were induced with galactose for 6 hours as described above. Live yeast cells were imaged at 100X magnification using EYFP, ECFP and TxRed filter cubes (chroma 39003, 49001, 39010). P-bodies in the TxRed channel were detected by the count and measure function of Olympus cellSens Dimension software. The number of yeast cells in the image was manually counted using the bright field image.

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Acknowledgements

We thank Dr. Marc Vidal and Center for Cancer Systems Biology (CCSB) at Dana-Farber Cancer Institute and Harvard Medical School for sharing the human ORFeome entry clone collections. We thank the members of the Ju and Zhong groups for laboratory assistance and helpful discussions. This work was supported primarily by funding from the Fidelity Bioscience Research Initiative (Q.Z., S.J.). S.J. and Q.Z. are also supported by National Institutes of Health (NIH) grants R15NS109804 and R21AG067446.

Author contributions (names must be given as initials)

S.J., and Q.Z. conceived the project. E.H. performed the experiments with help from A.K., S.C., A.C., and C.X. Experimental results were analyzed by E.H., Q.Z. and S.J., and S.J. supervised the project. E.H., Q.Z. and S.J. wrote the manuscript. All authors read and approved the final manuscript.

Competing Interests

The author(s) declare no competing interests.

Figure legends

Figure 1. TAF15 rescues FUS toxicity (**A**) Serial dilution growth assay was performed using the 1XFUS model transformed with the pAG416Gal-ccdB vector control (-), TAF15 or EWSR1 expression plasmid. Wild-type yeast strain transformed with the vector (Top -) was used as a control strain showing no toxicity. (**B**) Expression of FUS protein was examined in the 1XFUS model transformed with the vector control (-) or the TAF15 expression plasmid. Yeast containing empty vectors was used as a control strain without FUS or TAF15 expression. Protein expression was induced with 2% galactose for 6 hours in the indicated strains. Western blot analysis was performed using an antibody against FUS, TAF15 and the control protein PGK1. Quantification was performed using Amersham Imager 600 analysis workflow. FUS protein levels were detected and normalized to the level of the PGK1, as indicated by the values beneath the gels. The cropped images were shown here for the purpose of clarity. The full-length blots are presented in Supplemental Figure S4. (**C**) Serial dilution growth assay using yeast models of TDP-43, HTT103Q, and α-synuclein (IntTox) [14, 32, 34] transformed with the vector control (-) or the TAF15 expression plasmid (+). Wild-type yeast transformed with two empty vectors serves as a no toxicity control. (**D**) Yeast expressing FUS-YFP and transformed with the vector control (-) or the TAF15 expression plasmid (+) were grown in media containing 2% galactose for 6 hours to induce protein expression, then fixed and stained with DAPI. A yeast strain expressing YFP was used as a control. Imaging was performed at 100X magnification. Scale bar – 2μm. Images above are representatives of three independent experiments.

Figure 2. RRM and the C-terminal interaction domain of TAF15 are required for rescuing FUS toxicity. (**A**) Alignment of the RRM of FUS (amino acids 285-371) and TAF15 (amino acids 234-320) was performed using the Uniport align function (https://www.uniprot.org/align/). Conserved Phenylalanine residues highlighted in red are important for RNA binding and toxicity of FUS[15, 41, 42]. (**B**) Serial dilution growth assay was performed using the 1XFUS model transformed with the vector control (-) or the expression plasmid containing wild-type TAF15 (+) or the RRM mutant (Mut^{RRM}). Yeast transformed with an empty vector was used as a control strain showing no toxicity. (**C**) All strains used in (**B**) were induced with 2% galactose for 6 hours and Western blot was performed using an antibody against FUS, TAF15 and the loading control protein PGK1. The cropped images of Western blots were shown here for the purpose of clarity.

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Figure 3. TAF15 induces and localizes to p-bodies. (**A**) Yeast expressing the p-body marker (Edc3-mCherry) were transformed with YFP (-) and FUS-YFP plasmids and induced with 2% galactose for 6 hours followed by microscopy. (**B**) Yeast expressing the p-body marker (Edc3-mCherry) were transformed with ECFP (-) and ECFP-TAF15 plasmids and induced with 2% galactose for 6 hours followed by microscopy. (**C**) Yeast expressing the stress granule marker (Pub1-mCherry) were transformed with YFP (-) and FUS-YFP plasmids and induced with 2% galactose for 6 hours followed by microscopy. (**D**) Yeast expressing the stress granule marker (Pub1-mCherry) were transformed with ECFP (-) and ECFP-TAF15 plasmids and induced with 2% galactose for 6 hours followed by microscopy. Scale bar – 2μm. Images are representatives of three independent experiments.

Figure 4. TAF15 increases the induction of P-bodies when co-expressed with FUS. (**A**) Serial dilution growth assay was performed using yeast strains expressing the p-body marker (Edc3-mCherry) and transformed with FUS-YFP, ECFP-TAF15, or both. Wild-type yeast strain

transformed with a YFP and ECFP plasmid was used as a control strain showing no toxicity. (**B**) Yeast strain expressing the p-body marker (Edc3-mCherry) was transformed with FUS-YFP, ECFP-TAF15, or both. The same yeast strain transformed with ECFP, and YFP were used as controls without the expression of FUS or TAF15. All yeast strains were induced with 2% galactose for 6 hours. Live yeast cells were observed at 100X magnification in the YFP, ECFP, TxRed and BF channels. The same exposure time and microscope settings were used to take pictures for each strain. Scale bar – 2μm. (**C**) Quantification of the number of p-bodies per cell after 6 hours of galactose induction. Data generated from three biological replicates with mean ± SEM shown. (D) Serial Dilution growth assay was performed using yeast strains with deletions in genes involved in p-body formation *Edc3* (Edc3Δ), *Dhh1* (Dhh1Δ), and *Pat1* (Pat1Δ) transformed with empty vector pRS413Gal1YFP (-) and FUS expression construct pRS413FUS-YFP (+). Isogenic wild type strain (WT) transformed with the empty vector (-) and FUS expression constructs (+) were used as controls. Images are representatives of three independent experiments.

Figure 1

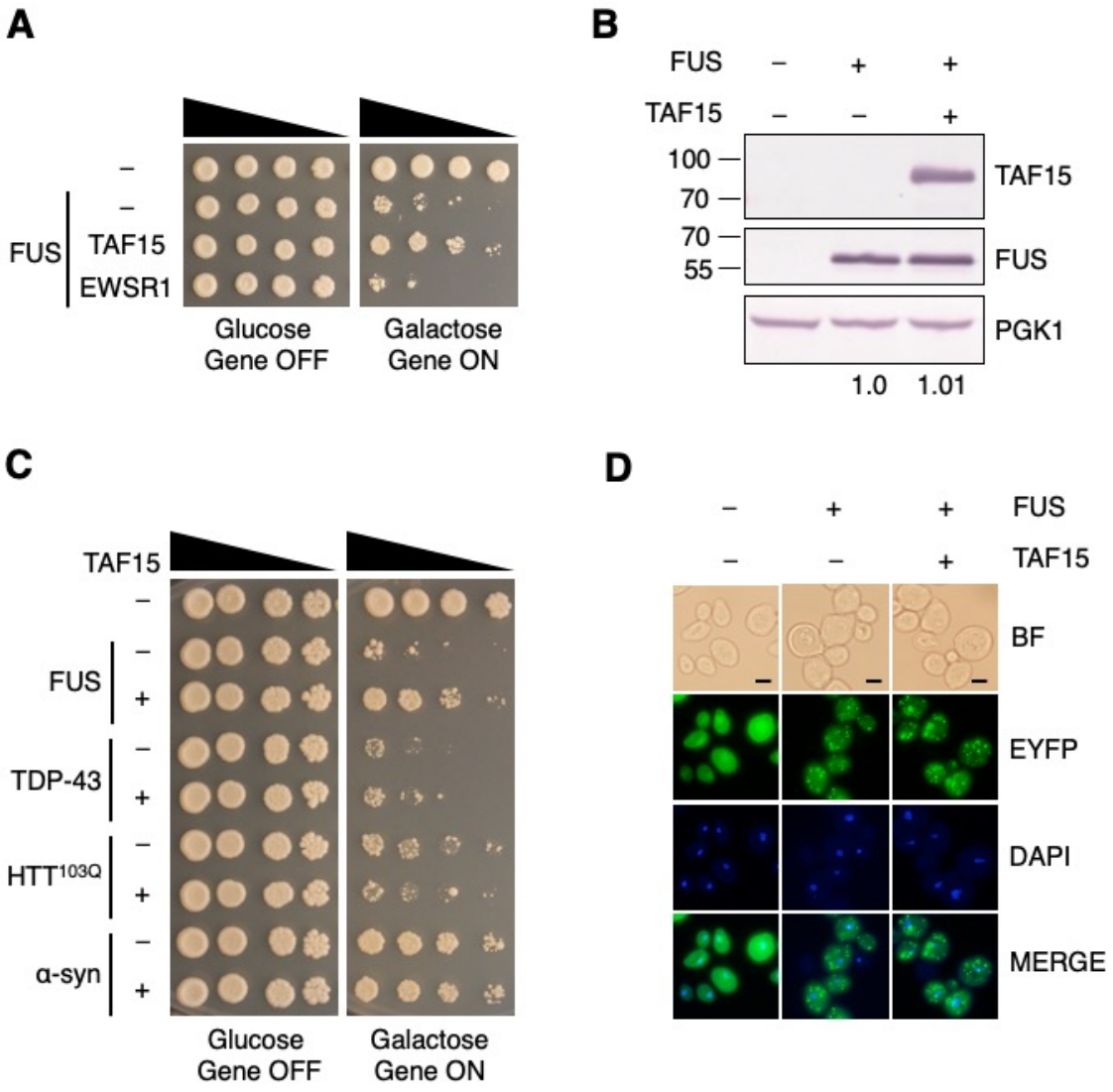
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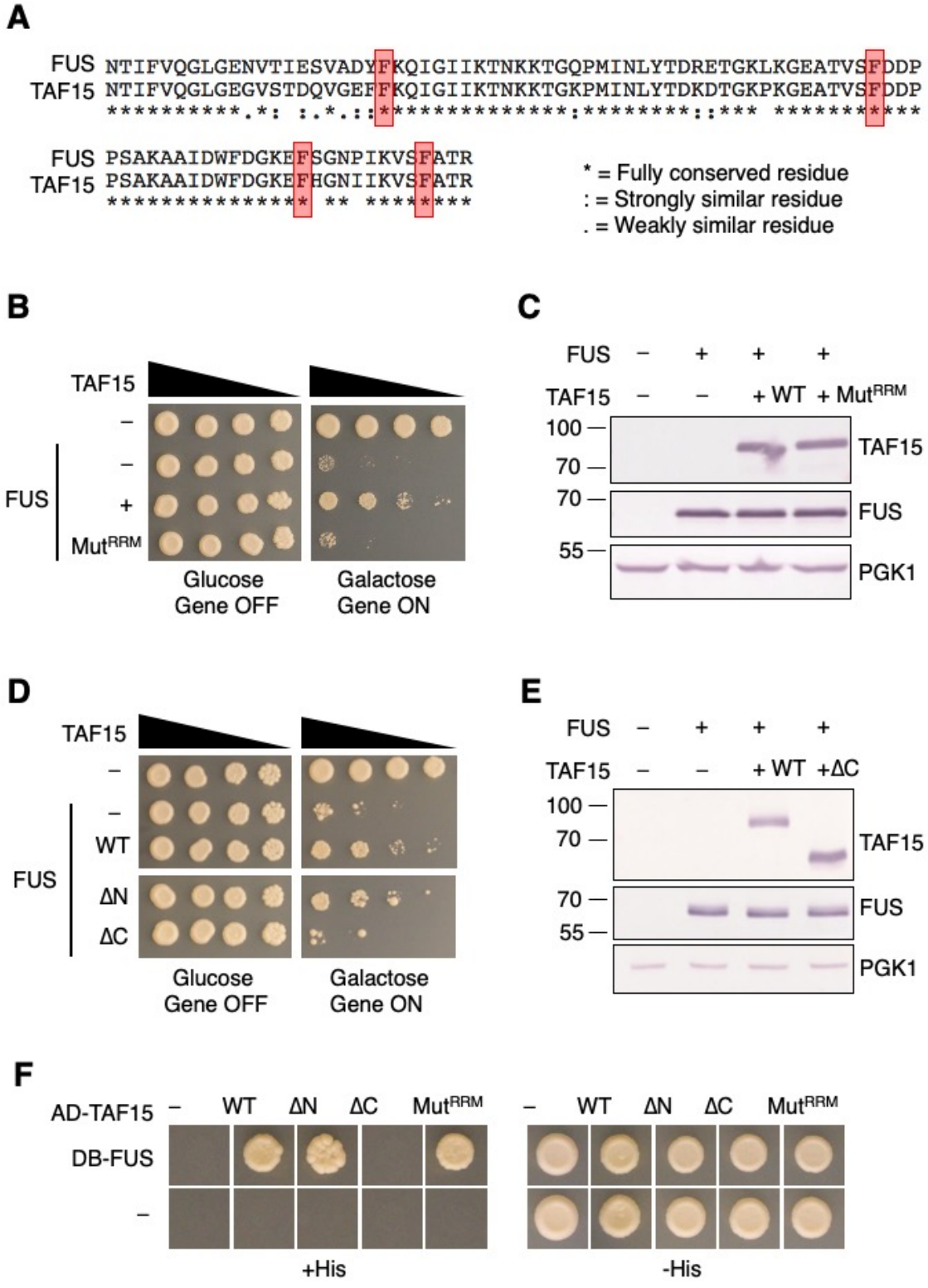
Figure 2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FUS PSAKAAIDWFDGKEFSGNPIKVSFATR
TAF15 PSAKAAIDWFDGKEFHGNIIKVSFATR
* = Fully conserved residue
: = Strongly similar residue
. = Weakly similar residue
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Figure 3

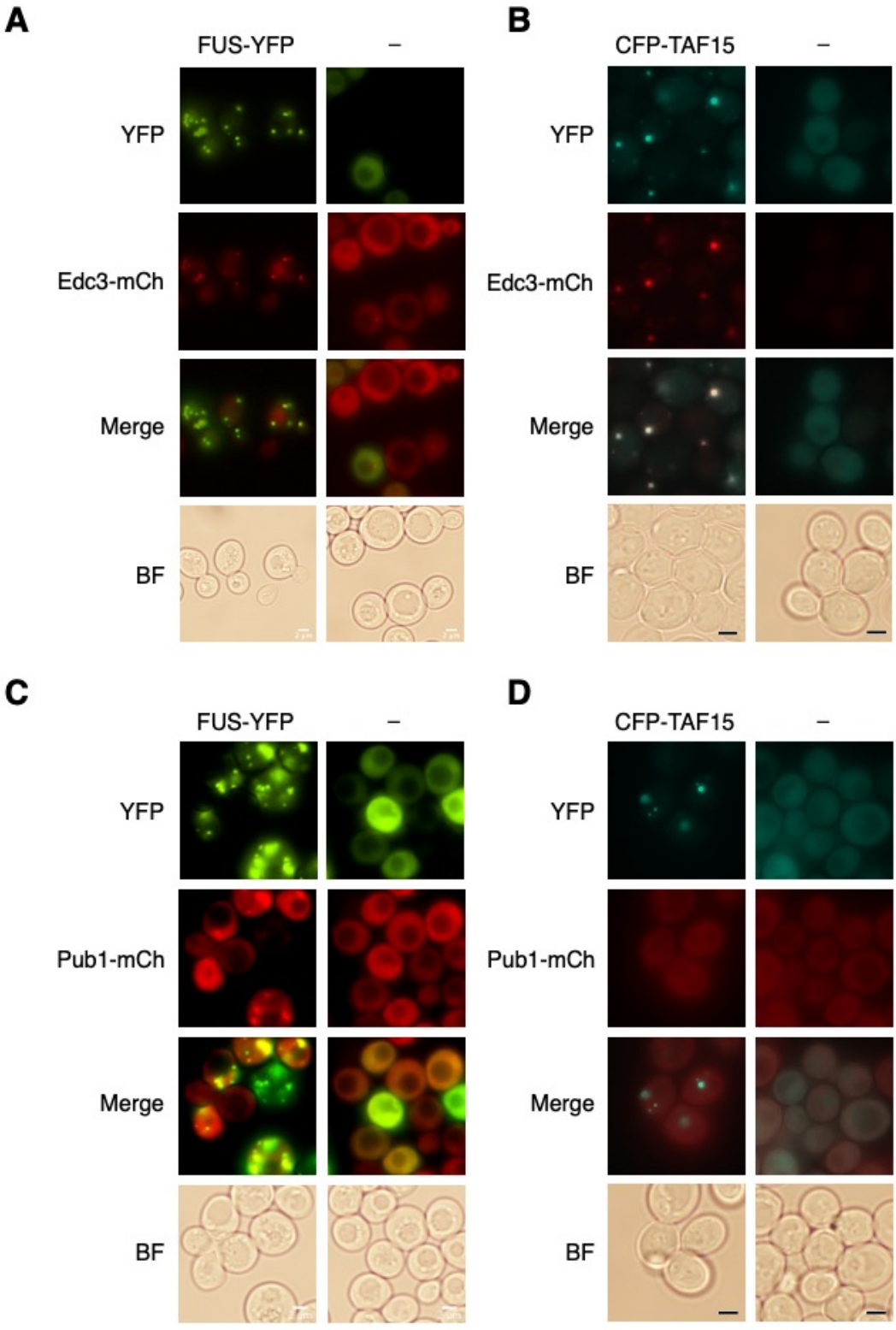
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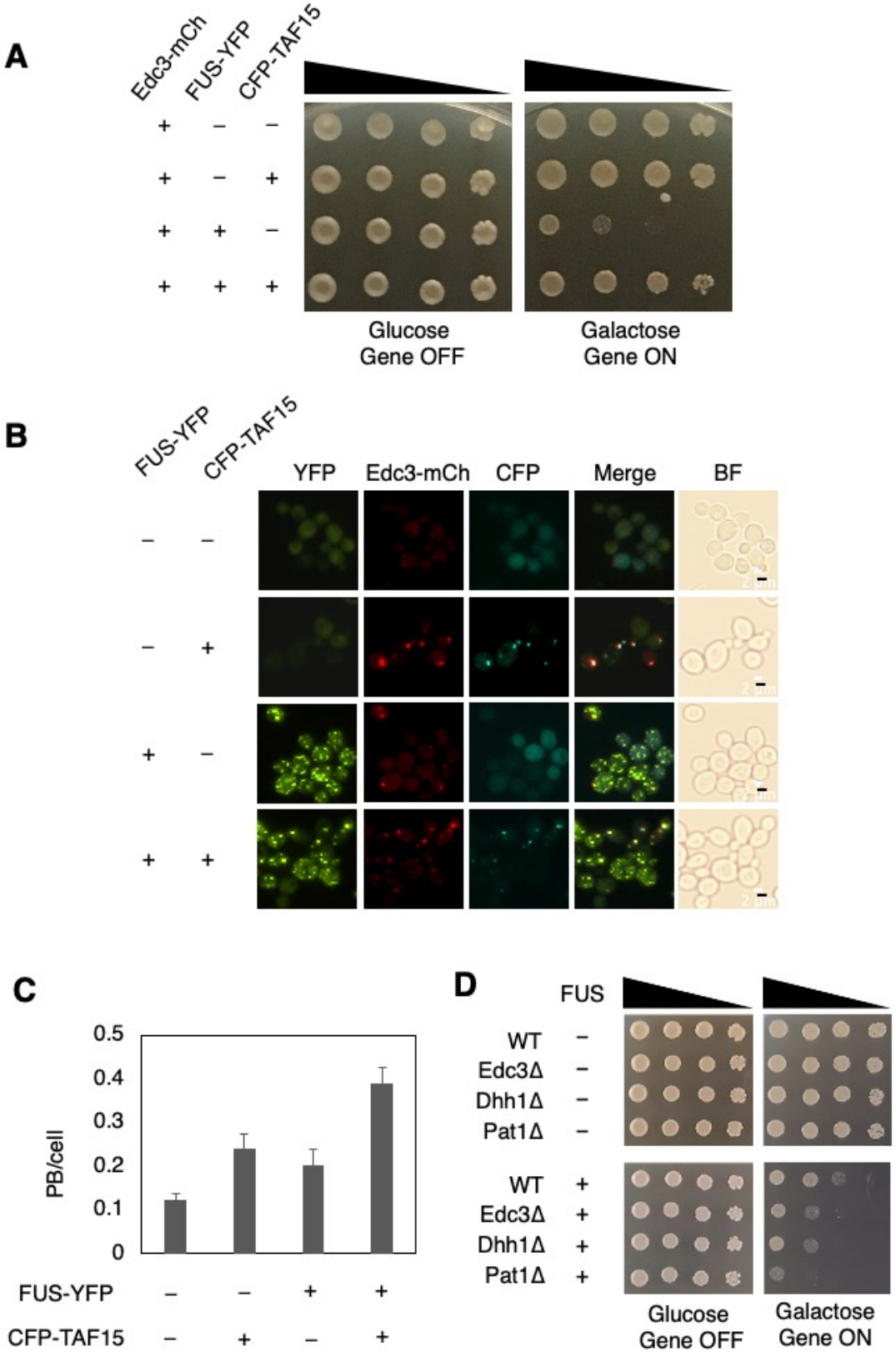
Figure 4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YFP
Edc3-mCh
CFP
Merge
BF
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+ –
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0.3
0.2
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D
FUS
WT –
Edc3Δ –
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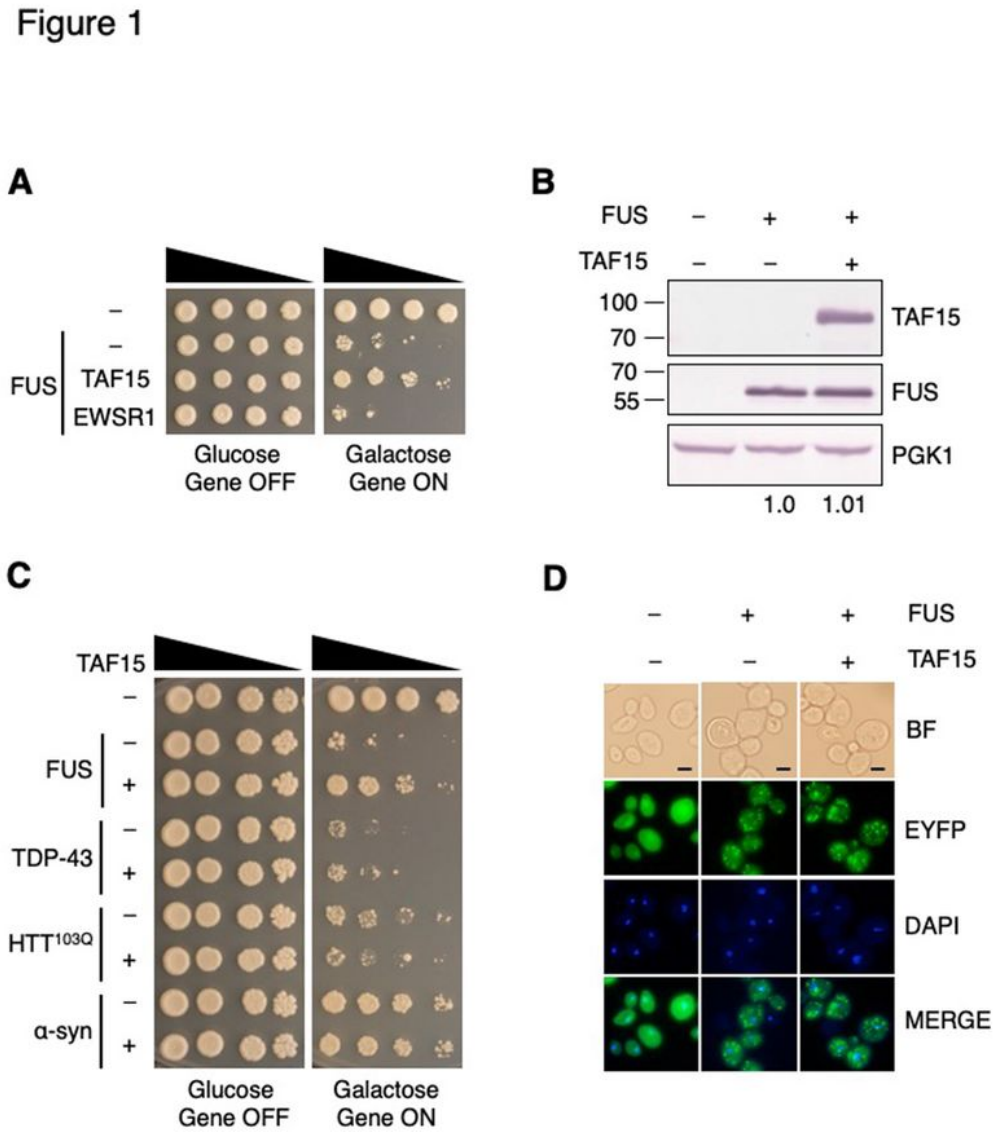
Figures



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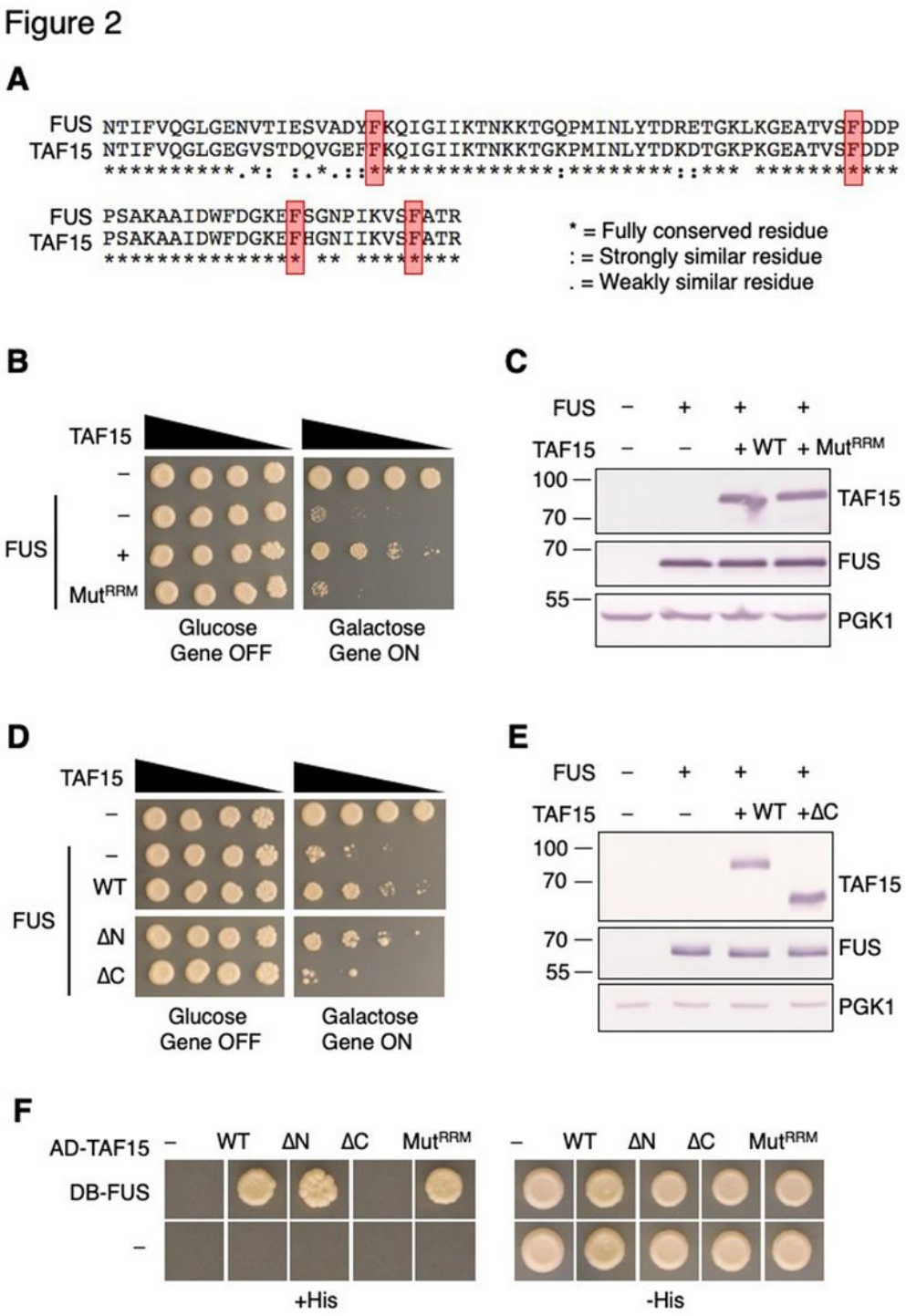

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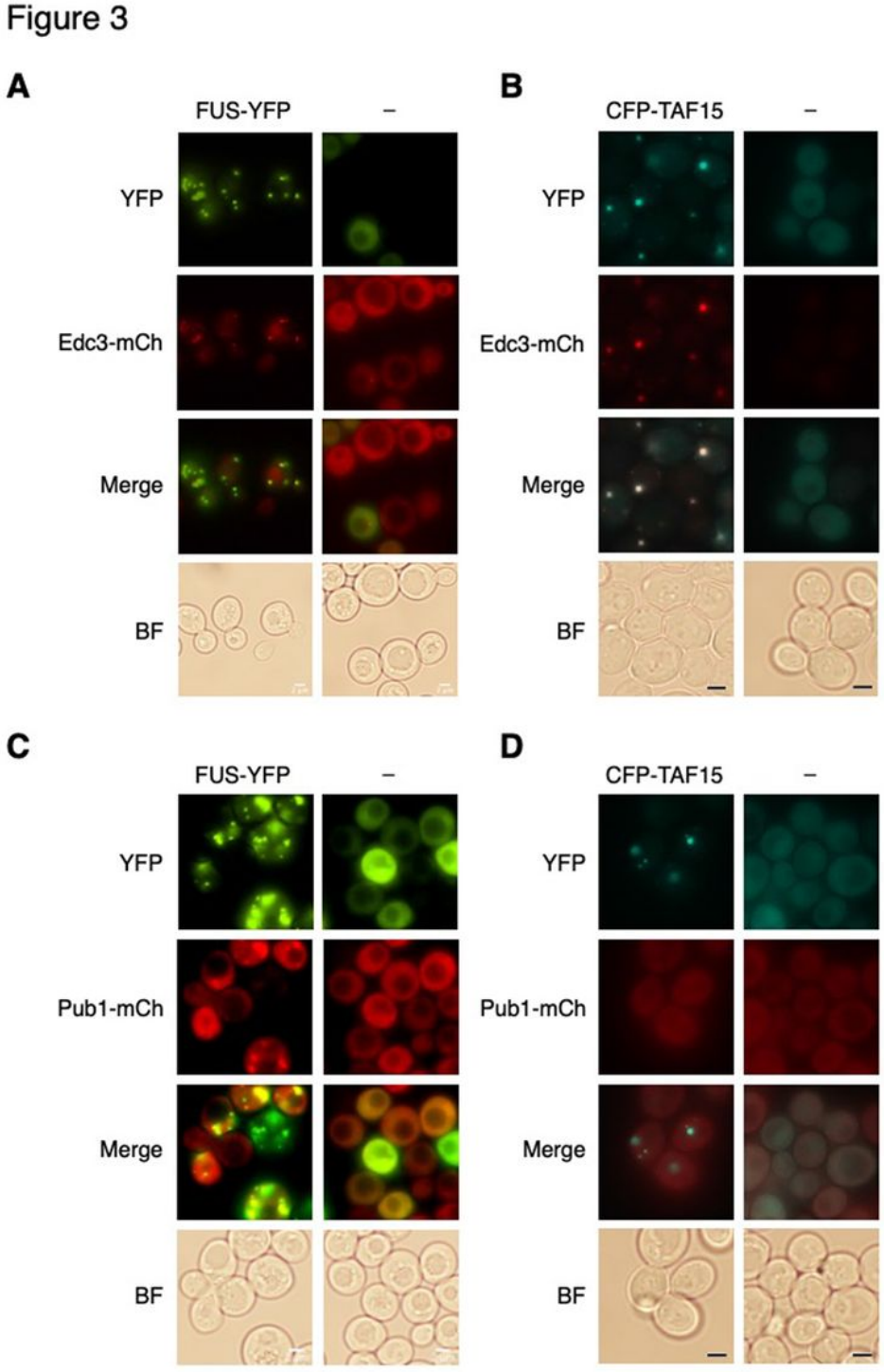

Figure 3

TAF15 induces and localizes to p-bodies. (A) Yeast expressing the p-body marker (Edc3-mCherry) were transformed with YFP (-) and FUS-YFP plasmids and induced with 2% galactose for 6 hours followed by microscopy. (B) Yeast expressing the p-body marker (Edc3-mCherry) were transformed with ECFP (-) and ECFP-TAF15 plasmids and induced with 2% galactose for 6 hours followed by microscopy. (C) Yeast expressing the stress granule marker (Pub1-mCherry) were transformed with YFP (-) and FUS-YFP

plasmids and induced with 2% galactose for 6 hours followed by microscopy. (D) Yeast expressing the stress granule marker (Pub1-mCherry) were transformed with ECFP (-) and ECFP-TAF15 plasmids and induced with 2% galactose for 6 hours followed by microscopy. Scale bar – 2µm. Images are representatives of three independent experiments.

Figure 4

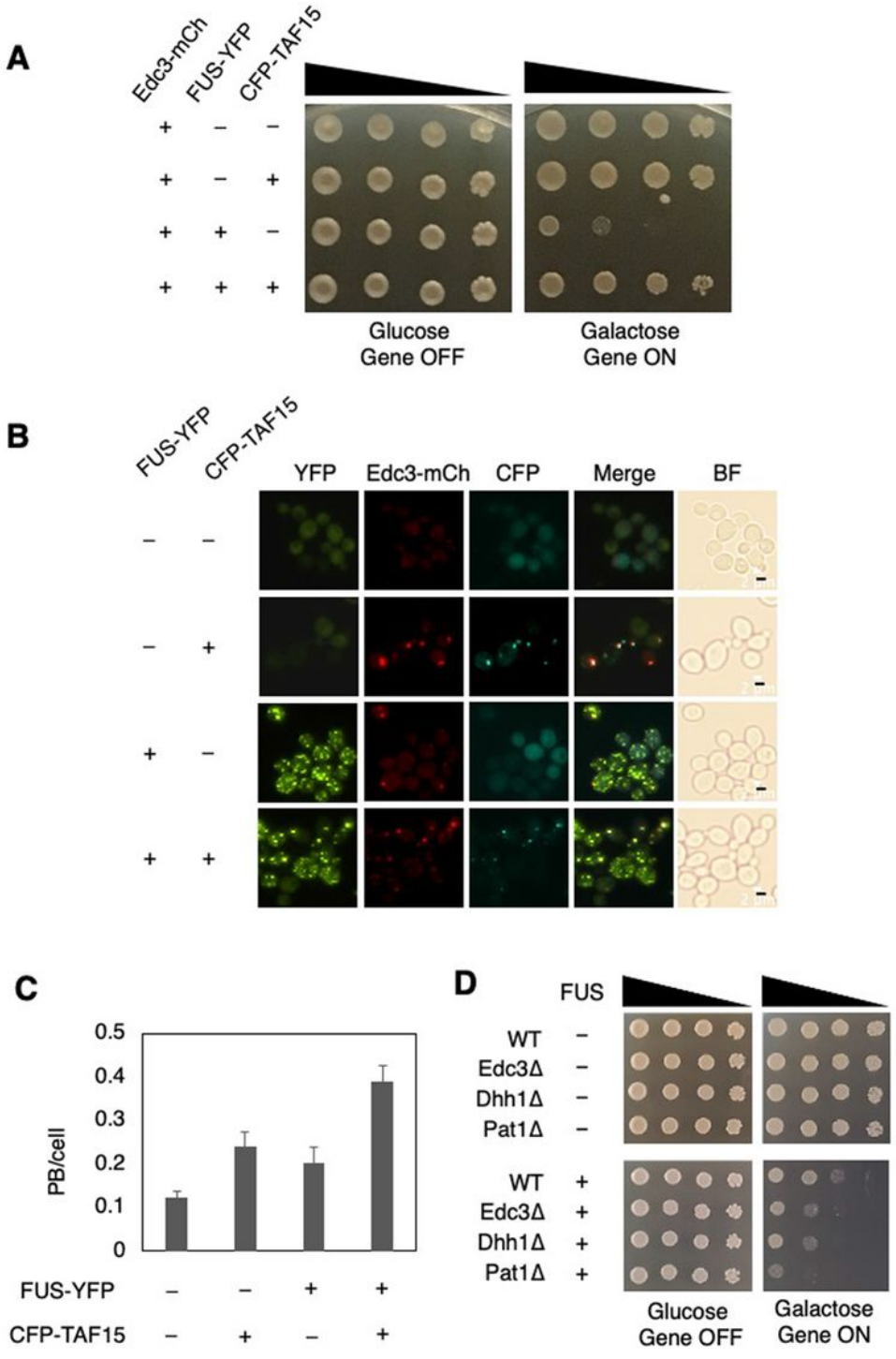

Figure 4

TAF15 increases the induction of P-bodies when co-expressed with FUS. (A) Serial dilution growth assay was performed using yeast strains expressing the p-body marker (Edc3-mCherry) and transformed with FUS-YFP, ECFP-TAF15, or both. Wild-type yeast strain transformed with a YFP and ECFP plasmid was used as a control strain showing no toxicity. (B) Yeast strain expressing the p-body marker (Edc3-mCherry) was transformed with FUS-YFP, ECFP-TAF15, or both. The same yeast strain transformed with ECFP, and YFP were used as controls without the expression of FUS or TAF15. All yeast strains were induced with 2% galactose for 6 hours. Live yeast cells were observed at 100X magnification in the YFP, ECFP, TxRed and BF channels. The same exposure time and microscope settings were used to take pictures for each strain. Scale bar – 2µm. (C) Quantification of the number of p-bodies per cell after 6 hours of galactose induction. Data generated from three biological replicates with mean ± SEM shown. (D) Serial Dilution growth assay was performed using yeast strains with deletions in genes involved in p-body formation Edc3 (Edc3D), Dhh1 (Dhh1D), and Pat1 (Pat1D) transformed with empty vector pRS413Gal1YFP (-) and FUS expression construct pRS413FUSYFP (+). Isogenic wild type strain (WT) transformed with the empty vector (-) and FUS expression constructs (+) were used as controls. Images are representatives of three independent experiments.

Supplementary Files

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- SupplementalInformationHayden.pdf