Supplemental figure legends for Kato et al., manuscript

Figure S1. Identified proteins in the b-isox-mediated precipitate. Related to Figure 1
(A) Mass spectrometry analyses of b-isox precipitated proteins from lysates prepared from mouse brain and testis tissue, and mouse embryonic stem cells (ES) and NIH 3T3 cells. As visualized in the Venn diagram, proteins precipitated from the four lysates revealed significant overlap. 162 proteins were found in common in all four datasets, and were defined as a "core" subset of precipitated proteins highlighted for subsequent analyses.

(B) 106 proteins that were found in the b-isox precipitates of all tissue and cell types are shown on left. 151 literature cited RNA granule proteins are shown on right. Proteins found on both lists are highlighted in yellow.

Figure S2. Sequences of the LC domains and statistical analyses of LC-domain containing proteins. Related to Figure 2
(A) Amino acid sequences of LC domains of all RNA binding proteins that were expressed, purified and studied in b-isox precipitation and hydrogel retention experiments. All sequences are derived from human proteins. The start and end residue numbers of the LC domain are provided in parentheses.

(B) Computational quantitation of presence of LC domains in the core set of 106 proteins precipitated by the b-isox chemical from all tissues and cell types, compared with all 151 literature cited RNA granule proteins, and the entire mouse proteome.

Figure S3. Additional results of hydrogel binding assays. Related to Figure 3
Hydrogel retention assays testing the ability of the GFP-linked LC domains of 10 RNA binding proteins to be trapped by mCherry:FUS LC (A) and mCherry:hnRNPA2 LC (B) hydrogel droplets. All GFP-LC domains were used at 1 μM in 1 ml gelation buffer. It should be noted that very strong binding resulted in concentrated GFP signal at a periphery of the hydrogel droplet as shown by GFP:hnRNPA2, GFP:hnRNPA1, and GFP:CIRBP with mCherry:hnRNPA2 hydrogel.

Figure S4. Sequences of the FUS LC mutants, statistical analyses of [G/S]Y[G/S]-motif containing proteins and co-localization of FUS and TIA1 in stress granules *in vivo*. Related to Figure 4
(A) Amino acid sequence of the low complexity (LC) domain of FUS highlighting [G/S]Y[G/S] tripeptide motifs, and sequences of S1, S2, S3 and all S mutants.

(B) Computational quantitation of presence of [G/S]Y[G/S] tripeptide motifs in the core set of 106 proteins precipitated by the b-isox chemical from all tissues and cell types, compared with all 151 literature cited RNA granule proteins, and the entire mouse proteome.

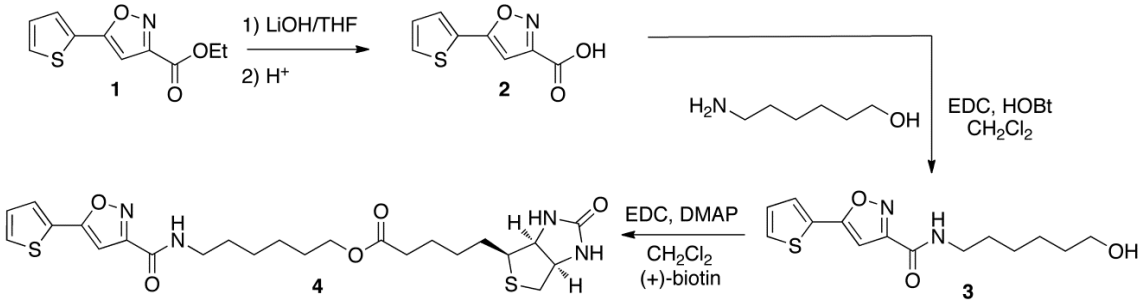
(C) Wild-type FUS lacking the C-terminal nuclear localization sequence was observed to enter cytoplasmic puncta, presumed to reflect stress granules, which was co-stained with antibodies specific to TIA1.

Figure S5. Additional examples of co-polymerization between two different LC domains. Related to Figure 7
Co-polymerization assays of mCherry:hnRNPA2 seeds incubated with soluble GFP:RBM3 (A), mCherry:hnRNPA2 seeds incubated with soluble GFP:hnRNPA1 (B), and mCherry:FUS seeds incubated with soluble GFP:CIRBP (C).

Supplementary Data, Experimental Procedures

Chemical synthesis of biotinylated isoxazole

To a solution of ethyl 5-(thiophen-2-yl)-isoxazole-3-carboxylate (**1**) (3.8g) in THF (20mL) was added an aqueous solution of LiOH (34mL of 1M). The reaction was heated to 60°C for 4 hours and then cooled to room temperature. The reaction was extracted twice with toluene and the remaining aqueous layer acidified with citric acid to pH 3. The aqueous layer was extracted with EtOAc and then concentrated to provide the crude acid product (**2**) as a white solid (2.06g, 62%). This product was combined with EDC (2.08g), HOBt (1.47g), and 6-amino-1-hexanol (2.26g) in dichloromethane (150mL). The reaction was stirred at room temperature for 3.5 hours, concentrated and then redissolved in EtOAc (25mL). This solution was washed with water, brine and dried over anhydrous $MgSO_4$. Concentration provided the crude product that was purified by recrystallization from methyl *tert*-butyl ether to give the 1.09g (35% yield) of the desired alcohol product (**3**).



A portion of the alcohol product (**3**) (30mg) was stirred for 3 days with (+)-biotin (57mg), EDC (51mg) and DMAP (6mg) in dichloromethane (2mL). After concentration to dryness, the crude reaction was partitioned between EtOAc (5mL) and water (2mL). The resulting precipitate that formed was collected by vacuum filtration and then purified by silica gel column chromatography using 10% methanol in dichloromethane as eluent. Concentration of the appropriate fractions provided the desired product that was further purified to >98% purity by recrystallization from EtOAc to yield 14mg (26% yield) of the biotinylated isoxazole (**4**). The product was fully characterized by ^{1}H NMR and LC/MS (electrospray ionization).

Biotinylated isoxazole-mediated precipitation

Biotinylated isoxazole was resuspended in DMSO as 100x stock for use at 10, 30, or 100 uM final concentration. Cells or tissue samples were homogenized into a lysis buffer containing 20 mM Tris buffer with 150 mM NaCl, 5 mM $MgCl_2$, 20 mM β-mercaptoethanol (BME), 0.5% NP-40, 10% glycerol, 1:300 mammalian protease inhibitor (Sigma, St. Louis, MO), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 1:100 RNasin ribonuclease inhibitor (Promega, Madison, WI, USA), and 2 mM vanadyl ribonucleoside vanadyl complex (NEB, Ipswich, MA, USA). Lysates for precipitation were pre-cleared with a 14K spin for 15 minutes. Biotinylated isoxazole was added at 1:100 and rotated gently at 4°C for 1 hour. The

incubated reaction was then spun at 14K for 15 minutes to pellet th**e** precipitant. The pellet was washed twice in the lysis buffer and resuspended in 2x SDS loading buffer for protein analysis. For identification by mass spectrometry, proteins were precipitated from cell lysates and the resuspended pellets were resolved on 4-12% Tris-Glycine gradient gels (Invitrogen, USA). The entire lane was then excised into 10 or more fractions for mass spectrometry analysis.

Computation analysis of low complexity sequences and [GS]Y[GS] motifs

The mouse and human proteomes were downloaded from the NCBI refseq database. The fruit fly proteome was downloaded from the NCBI genome database. For each protein-coding gene, only one protein isoform (the longest one) was selected, giving rise to 19,752, 23,096 and 13,543 proteins for the human, mouse and fly proteomes respectively. Low complexity sequences were identified using the SEG program with default parameter settings (Wootton and Federhen, 1996). The length of the longest continuous low complexity segment was calculated for each protein. Proteins with such lengths no less than 35 residues were considered to have a long, continuous stretch of low complexity sequence. The cutoff value of 35 was selected by examining the distribution of such lengths among the human proteome. 2,345, 2,195 and 2,239 proteins were determined to have long, continuous low complexity regions for the human, mouse and fly proteomes respectively.

The [GS]Y[GS] tripeptide motif was counted for each protein in the human, mouse and fly proteomes. Proteins with four or more copies of this motif were considered to be enriched. 189, 192 and 150 proteins were determined to be enriched with the [GS]Y[GS] motif in the human, mouse and fly proteomes. The same methods used to score for both low complexity sequences and [GS]Y[GS] tripeptides in the human, mouse and fly proteomes were employed to interrogate protein samples precipitated by the b-isox compound both before and after RNase treatment, and to score for these domains in literature cited RNA granule proteins. Fisher's Exact test using "R" software (R Development Core Team, 2010) was used to determine if any set was enriched for proteins containing LC domains and [GS]Y[GS] motifs as compared to the statistics of the proteome.

Stress granule recruitment assay

A 10-cm confluent dish of U2OS cells was transfected with 10ug of plasmid with 10 uL of Lipofectamine 2000 (Invitrogen, USA) in Opti-MEM for 6 hours for each construct. Cells were split 24 hours after transfection on to glass coverslips in 24-well plates. Stress granules were induced with 0.5mM sodium arsenite for 1 hour 36 hours after transfection. Coverslips were fixed with 4% paraformaldehyde for 10 minutes, permeabilized in 1% Triton for 15 minutes, blocked in PBTA (5% BSA, 0.2% Tween in PBS) for 30 minutes, and incubated with 1:1000 αFlag antibody (Sigma, USA) in PBTA overnight at 4C. Primary antibody was developed with 1:1000 αmouse conjugated to AlexaFluor 488 (Invitrogen, USA) for 1 hour at room temperature. Coverslips were then mounted on to glass microscope slides with Vectashield and

DAPI (Jackson, Bar Harbor, Maine, USA). Flag-FUSΔ32 plasmids were constructed by amplifying the region encoding FUS214 with Y->S mutations from bacterial expression vectors with the Flag epitope encoded in the forward primer and fusing it to the truncated C-terminal fragment lacking the last 32 amino acids by bridging PCR. The Flag-FUSΔ32 PCR product was then cloned into the multiple cloning sites of the pcDNA3.1(+) vector for mammalian expression.

Protein expression and purification

DNA fragments encoding the LC domains of human FUS and other RNA-binding proteins were amplified by PCR using a cDNA library made from human U2OS cells as a template. The DNA fragments were inserted into the multiple coning sites of the pHis, pHis-GST, pHis-mCherry, pHis-GFP, or pHis-CFP parallel vectors (Sheffield et al., 1999). The latter three plasmids were made by cloning the mCherry, GFP or CFP coding DNA fragments at the NcoI site of the pHis-parallel vector. Tyrosine-to-serine mutations in the FUS LC domain were introduced by a multi-quick change site-directed mutagenesis technique. The sequence of the resulting vectors were confirmed by DNA sequencing. The amino acid sequences of the LC domains are shown in Supplementary Data Figures S3 and S5.

All proteins were over-expressed in *E. Coli* BL21(DE3) cells with 0.5 mM IPTG at 16 °C for overnight. Harvested cells were lysed with 0.4mg/mL lysozme in a lysis buffer containing 50mM Tris-HCl pH7.5, 500mM NaCl, 20mM BME, 1% Triton X-100 and protein inhibitor cocktail (Roche, USA) for 30 min on ice, and then sonicated. The cell lysate was centrifuged at 35,000 RPM for 1 hour. The supernatant was mixed for 30 min at 4^{o}C with either Ni-NTA resin (Qiagen, USA) for the His-tagged proteins, or glutathion resin for the GST-tagged proteins (GE Healthcare, USA). The Ni-NTA resin was packed in a glass column and washed with a buffer containing 20mM Tris-HCl pH7.5, 500mM NaCl, 20mM imidazole, 20mM BME, and 0.1mM PMSF. The bound proteins were eluted from the resin with a buffer containing 20mM Tris-HCl pH7.5, 500mM NaCl, 200mM imidazole, 20mM BME, and 0.1mM PMSF. The glutathione resin was washed with a buffer containing 20mM Tris-HCl pH7.5, 500mM NaCl, 20mM BME, and 0.1mM PMSF. The bound proteins were eluted with a buffer containing 20mM Tris-HCl pH7.5, 500mM NaCl, 20mM BME, 0.1mM PMSF, and 10mM glutathione. EDTA was added to a final concentration of 0.5mM to the eluted protein solutions. The purified proteins were concentrated with Amicon Ultra centrifugal filters (Millipore, USA), and then glycerol was added to the final concentration of 50%. The protein solutions were kept at -20 ^{o}C. The purities of the purified proteins were confirmed by SDS-PAGE, and the concentrations were determined by absorbance at UV_{280}.

Formation of FUS and hnRNPA2 hydrogels

The purified FUS LC domain linked to three different tags GST, mCherry, and GFP were dialyzed against a gelation buffer containing 20mM Tris-HCl pH7.5, 200mM NaCl, 20mM BME, 0.5mM EDTA and 0.1mM PMSF for overnight. The protein solutions were concentrated to roughly 60mg/ml. After concentration,

the protein solutions (~50 μl) were filled into 2-cm silicon tubes (3.6 mm diameter) followed by sealing the tube ends with parafilm. The tubes were incubated at 4˚C for 2 days. The formed hydrogels were carefully squeezed out from the tubes for photography. For hydrogel binding assays, the dialyzed mCherry:FUS and mCherry:hnRNPA2 protein solutions (~300 μl) were sonicated 10 sec at a 12% power level on a Fisher Scientific Sonic Dismembrater Model 500. After centrifugation, a 0.5μl droplet of the supernatant was deposited onto a glass-bottomed microscope dish (MatTek, MA, USA). The dish was sealed with parafilm and incubated for a couple of days at RT.

Hydrogel binding assays

Glycerol stocks of the purified GFP-fusion LC domains were diluted in 1mL of the gelation buffer at the final concentration of 1μM of the protein. This solution was poured into the hydrogel dish so as to soak the hydrogel droplets in the GFP solution. The hydrogel dish was incubated at 4 ˚C for up to a week. Horizontal sections of the soaked hydrogel droplets were scanned with both the mCherry and GFP excitation wavelengths on Zeiss LSM510 or Leica TCS SP5 confocal microscopes. The montage images were made by the program ImageJ (Rasband, 1997-2011). For signal decay assays, the GFP solution in the hydrogel dish was replaced with 1 ml of the fresh gelation buffer after taking an original GFP-signal image (0 min control). GFP fluorescent images were subsequently taken at the indicated time points. The GFP signal intensities at the different time points were integrated with ImageJ and the fractions of the retained GFP signals against the original intensity were plotted with the program Prism (Graphpad, USA). A one-phase exponential decay equation was applied to calculate half lives of the GFP signal retention.

TEM imaging and X-ray diffraction of Hydrogel

Each of the mCherry:FUS and His-tagged FUS hydrogels were directly transferred to the surface of a TEM grid (LC305-Cu grid from Electron Microscopy Sciences, USA) coated with a 6-nm carbon film. The surface of the grid was washed with 10μl of distilled water to remove excess of hydrogel material. The grid was subsequently stained for a few seconds with a 10μl drop of 3% uranyl acetate. After the uranyl solution was blotted, the grid was dried in air. TEM images were obtained at 80kV on FEI Morgagni TEM. For X-ray diffraction experiments, 500μL each of pre-formed mCherry:FUS and mCherry:hnRNPA2 hydrogel was placed in a dialysis bag and dialyzed against 2L of distilled water overnight twice to eliminate salt and buffer materials. For the controls, 100μl of each of mCherry:FUS, mCherry:hnRNPA2, and mCherry in a solution state was also dialyzed. The samples were lyophilized. For each sample, a particle of the solid material was mounted on the tip of a nylon cryoloop. The tip of the loop was wetted with a 50% ethylene glycol solution then brought in contact with the sample to be mounted. A sample particle, approximately 150 microns in diameter stuck to the tip of the loop. To reduce background X-ray scattering, the sample was placed so that only the sample and no part of the nylon loop was in the path of the 100 micron diameter X-ray beam. The diffraction images were collected at 100 K using a Rigaku FR-E generator (copper anode, wavelength 1.5418 Å) equipped with R-AXIS HTC imaging plate detector

and nitrogen gas cryostream. The sample-to-detector distance was 300 mm. Each of the samples oscillated 1° during a 5 minute exposure.

Fiber extension assays

The mCherry:FUS protein solution (20μM) in the gelation buffer was sonicated to break down the pre-formed fibers in many short fiber seeds. Seeds (2 μl) were mixed with 50μl of the monomeric LC domains of GFP:FUS wild-type, S1, S2, S3 and allS (100 μM). After 2 hours at RT, the mixture was diluted 100-fold into the gelation buffer, and 10μl of the diluted solution was placed on a microscope cover slip (22mm x 55mm, 1.5 mm thickness). A second cover slip (22 mm x 22 mm) was placed on the droplet. This sample was analyzed on Olympus total internal reflection fluorescent (TIRF) microscope with a 100X objective lens. Fluorescent images for the mCherry and GFP signals were recorded with a Hamamatsu CCD camera. Montage images were generated with ImageJ. In the extension experiments testing for GFP:hnRNPA1 polymerization onto mCherry:FUS seeds, the concentration of the GFP solution was decreased to 10μM. After the samples were incubated at 4˚C, the mixture was diluted 1,000-fold in gelation buffer followed by TIRF microscopic analysis. Similar methods were used to monitor fiber extension from mCherry:FUS and mCherry:hnRNPA2 seeds exposed to GFP fusion proteins linked to the LC domains of hnRNPA1, RBM3 and CIRBP.

Assays of SDS sensitivity

mCherry:FUS and mCherry:hnRNPA2 were dialyzed against the gelation buffer overnight. Dialyzed samples were diluted to a final protein concentration of 10mg/ml and incubated at 4^{o}C for five days. At this protein concentration the proteins fully polymerized into fibers, but did not form a hydrogel. To determine the amount of monomeric protein remaining in solution, the fiber samples were passed through a 0.1um spin filter to remove fiber particles. UV absorbance was used to monitor the amount of monomeric protein that passed through the filter. The samples were also exposed to 2% SDS and incubated at 37^{o}C for 10 min to disassemble fibers into monomers followed by the same filtration and UV measurement. Under the latter conditions all UV detectable material was observed to pass through the filter. For SDD-AGE experiments, fiber solutions were briefly sonicated to break down long fibers to allow entry into the 1.5% agarose gel. Fiber samples (10ug of protein) were mixed with the indicated concentrations of SDS in the gelation buffer supplemented with 5% glycerol and bromophenol blue and incubated at 37^{o}C for 10 min. Fibers and monomers were separated by agarose gel electrophoresis in TAE buffer supplemented with 0.1% SDS. In parallel, the classically defined, SDS-resistant amyloid fibers from the yeast Sup35 protein were treated under the same conditions. Following SDD-AGE, the separated proteins were transferred to a nitrocellulose filter by capillary transfer and visualized by western blotting using a His-tag antibody for mCherry:FUS and mCherry:hnRNPA2, and an antibody specific for Sup35.

X-ray structure determination of b-isox crystals

The b-isox-mediated precipitation from the lysate of human U2OS cells were prepared as describe above and deposited on microscope slides. Light microscope images were taken with differential interference contrast mode on a Deltavision fluorescent microscope. Ethidium bromide was added in the same reaction at the final concentration of 1ug/ml, and the precipitated materials were analyzed by fluorescence microscopy with an excitation wavelength of 488nm and emission collected between 550nm and 640nm. The b-isox micro-crytals were prepared with the same lysis buffer used for the culllar lysate preparation, and visualized with the similar method described above.
The b-isox chemical was crystallized at 4^{o}C by vapor diffusion under the following conditions: a compound drop (4ul) containing 0.75mM b-isox, 80% DMSO and 20% lysis buffer was diffused against a reservoir (1ml) consisting of 70% DMSO and 30% lysis buffer. Clusters of small plate-like crystals (10-20um) appeared after roughly one week. Crystal clusters were broken and isolated crystals were seeded in fresh crystallization drops. Many seeds grew as clusters again, but some exhibited a single-crystal part. After multiple trials to cut out single-crystal parts, well-diffracting crystals were obtained (80 X 50 X 10um). X-ray diffraction data were collected at Beamline 19ID of the Structural Biology Center at Argonne Photon Source (Argonne, Chicago IL). The crystals belonged to a space group $P2_1$ and the unit cell dimensions were a=18.53A, b=7.50A, c=19.68A, and β=111.69° with two b-isox molecules per asymmetric unit. The structure was solved by a direct method using SHELX program package (Sheldrick, 2008), and was refined to an R-factor of 10.6% with all atoms of the b-isox molecule including hydrogens. Statistics for X-ray data collection and structure refinement is shown in Supplementary Data, Table S2. The coordinate and reflection files were deposited at Cambridge Crystallographic Data Center (CCDC 873064).

References

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Supplementary Data Table S2

Table S2. Statistics for X-ray data collection and structure refinement of the b-isox crystal structure

Crystal data	
Chemical formula	$C_{24}H_{32}N_4O_5S_2$
Molecular weight	520.66
Unit cell	
a (Å)	18.53
b (Å)	7.50
c (Å)	19.68
β (°)	111.69
Space group	$P2_1$, Monoclinic
Z	4
Data collection	
X-ray beam	Beamline 19ID at APS
Detector	ADSC Quantum 315r CCD
Wavelength (Å)	0.9184
Temperature (K)	100
Number of observed reflections	19109 (50-0.93Å)
Number of unique reflections	3162
Number of reflections with I > 4σ(I)	2853
Completeness (%)	87.5 (47.0)[a]
R-merge (%)	5.2 (24.8)[a]
Average redundancy	3.4 (2.4)[a]
I/σ(I)	21.9 (4.2)[a]
Refinement	
Number of reflections used (I > 2σ(I))	3152
Number of parameters	644
R-factor (%) for reflections with I > 4σ(I)	10.4
Goodness of fit = S	1.47
$\Delta\rho_{max}$ (e/Å^{-3})	0.97
$\Delta\rho_{min}$ (e/Å^{-3})	-0.57

[a] Values in parentheses are statistics for the highest resolution shell (0.95-0.93Å).

Supplemental Figure S1
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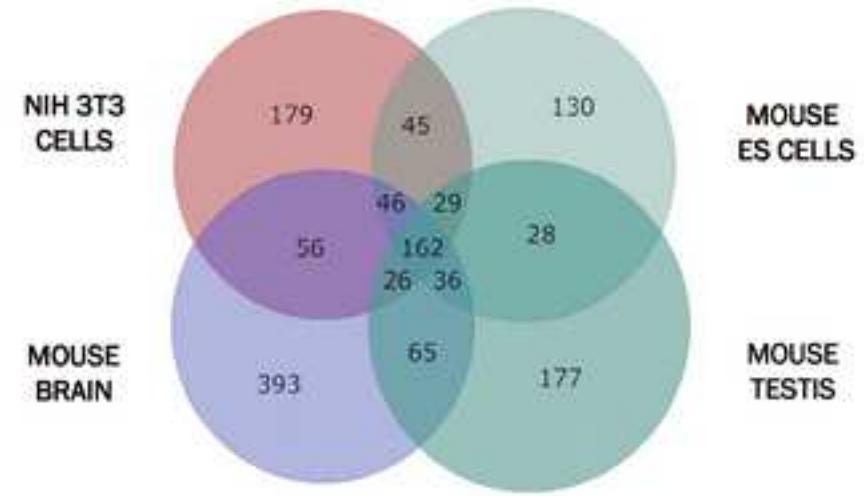
Supplementary Data Figure S1

A



B

Core Precipitated Proteins

Actb	hnRNPM	Ubap2l
Actg1	hnRNPR	Upf1
Ap2a2	hnRNPU	Ybx1
Ap2b1	hnRNPUl	Ythdf1
Atxn2	Hsp8	Ythdf2
Atxn2l	Hspa2	Ythdf3
Bat2d	ILF2	Zfr
Calm2	Khdrbs1	
Caprin1	Khsrp	
CW17R	Leng8	
Dazap1	Lsm12	
Ddx1	Matr3	
Ddx3x	mCBP	
Ddx5	mct-1	
Ddx6	Ncl	
Dhx9	Npm1	
Ddx17	Nufip2	
eIF2b	Nup214	
eIF3d	Pabpc1	
eIF3h	Pabpc4	
eIF3i	Pcbp1	
eIF4g3	Pcbp2	
EWS	Pcid2	
Fam98a	Polr2b	
FMR1	Purb	
Fubp1	Rbm3	
Fubp3	Rbms1	
FUS	Rbmxrt	
FXR1	Serbp1	
FXR2	Sfrs1	
G3bp1	Sfrs5	
G3bp2	Sfrs6	
GNB2L1	Sfrs7	
Hist1h2ai	Smap2	
Hist1h2bp	Snrnp70	
hnRNPAB	Srp20	
hnRNPA0	Ssb	
hnRNPA1	Stau1	
hnRNPA2	Syncrip	
hnRNPA3	TAF15	
hnRNPC	TDP-43	
hnRNPD	TIA1	
hnRNPDl	TIAl1	
hnRNPF	Tnrc6b	
hnRNPG	Tnrc6c	
hnRNPH1	Tra2b	
hnRNPH2	Tuba1b	
hnRNPK	Tubb5	
hnRNPL	Ubap2	

RNA Granule Proteins

Ago2	FXR2	Pan2/3	ZBP1
AKAP350	G3bp1	Pat1	
Apobec3g	G3bp2	Pcbp2	
Atxn2	Gbp2	PKP1/3	
Bat1a	Ge-1/Hedls	PMR1	
BRF1	GNB2L1	Pop2/Caf1	
Calreticulin	Grb7	Prkra	
Caprin1	GW182	Prohibitin 2	
CCAR1	hMex3A	PRTB	
Ccr4	hMex3B	Psd3	
Celf2	hnRNPA0	Pspc1	
Cirbp	hnRNPA1	Pum1	
Cpeb	hnRNPA3	Pum2	
CUG-BP1	hnRNPAB	Pura	
Cyfip2	hnRNPC	Purb	
Dcp1	hnRNPD	Rap55	
Dcp2	hnRNPH1	Rbm3	
Ddx1	hnRNPK	Rbm42	
Ddx3x	hnRNPL	RHAU	
Ddx5	hnRNPM	Roquin	
Ddx6	hnRNPR	Rpl3	
Dhx9	hnRNPU	Rpm2	
DiC1	Hrp1	RSK2	
DiS1	Hsp27	Rtcd1	
Eap1	Hspa8	Serbp1	
Ebs1	Htt	Sfpq	
Edc1-2	Igf2bp1	SGNP	
Edc3	ILF2	Smaug1	
eIF1a2	ILF3	SMN	
eIF2a	Importin-8	Stau1	
eIF2c2	IP5K	Syncrip	
eIF2s2	KHC/KLC	TAF15	
eIF2s3x	Khdrbs1	TDP-43	
eIF3	Khsrp	TDRD3	
eIF4a1	Lin28	Thoc4	
eIF4a2	LINE1	TIA1	
eIF4g1	Lsm1	TIAl1	
Elavl1	Matr3	Tnrc6b	
Elavl2	MBNL1	TRAF2	
Elavl3	MEX67	Trim2	
Elavl4	MLN51	Trim3	
eRF1	Musashi	TTP	
eRF3	Ncl	Upf1	
EWS	Nono	Upf2	
FAK	Npm1	Upf3	
FAST	Nrp1	Vbx1	
FMR1	NXF7	Xrn1	
FUS	p97/NAT1	Ybx1	
FXR1	Pabpc1	Ygr250c	

Supplemental Figure S2
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Supplementary Data Figure S2

A

hnRNPA1 (186-320)
MASASSSQRGRSGSGNFGGGRGGGFGGNDNFGRGGNFSGRGGFGGSRGGGGYGGSGDGYNGFGNDGS
NFGGGGSYNDFGNYNNQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGYGGSSSSSSYGSGRRF

hnRNPA2 (181-341)
MQEVQSSRSGRGGNFGFGDSRGGGGNFGPGPGSNFRGGSDGYGSGRGFGDGYNGYGGGPGGGNFGGS
PGYGGGRGGYGGGGPGYGNQGGGYGGGYDNYGGGNYGSGNYNDFGNYNQQPSNYGPMKSGNFGGSR
NMGGPYGGGNYGPGGSGGSGGYGGRSRY

TIA1 (280-375)
INPVQQQNQIGYPQPYGQWGQWYGNAQQIGQYMPNGWQVPAYGMYGQAWNQQGFNQTQSSAPW
MGPNYGVQPPQGQNGSMLPNQPSGYRVAGYETQ

CPEB2 (2-137)
PPPSPDSENGFYPGLPSSMNPAFFPSFSPVSPHGCTGLSVPTSGGGGGGFGGPFSATAVPPPPPPAMNIPQQ
QPPPPAAPQQPQSRRSPVSPQLQQQHQAAAAAFLQQRNSYNHHQPLLKQSPWSNHQSSGWGTGSM

FMRP (466-632)
GQGMGRGSRPYRNRGHGRRGPGYTSGTNSEASNASETESDHRDELSDWSLAPTEEERESFLRRGDGRRRG
GGGRGQGGRGRGGGFKGNDDHSRTDNRPRNPREAKGRTTDGSLQIRVDCNNERSVHTKTLQNTSSEGSRL
RTGKDRNQKKEKPDSVDGQQPLVNGVP

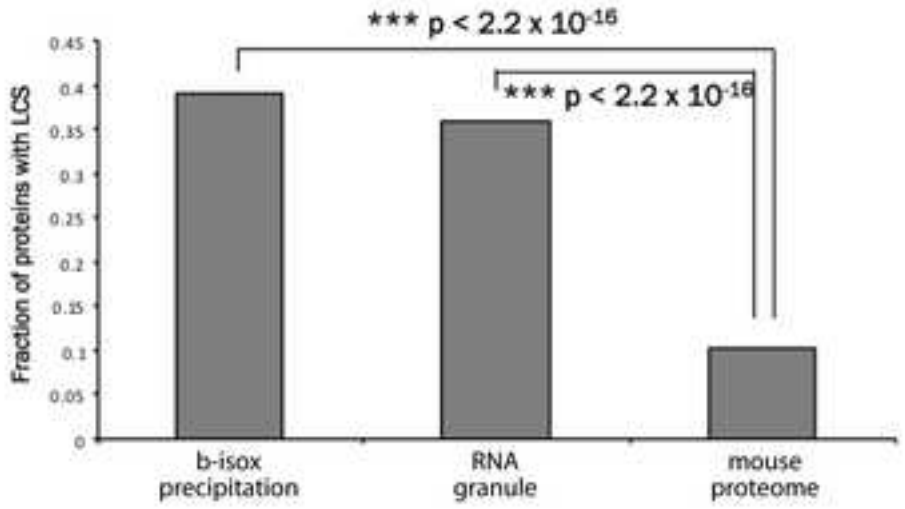
CIRBP (1-172)
MASDEGKLFVGGLSFDTNEQSLEQVFSKYGQISEVVVVKDRETQRSRGFGFVTFENIDDAKDAMMAMNGK
SVDGRQIRVDQAGKSSDNRSRGYRGGSAGGRGFFRGGRGRGRGFSRGGGDRGYGGNRFESRSGGYGGSR
DYYSSRSQSGGYSDRSSGGSYRDSYDSYATHNE

RBM3 (1-157)
MSSEEGKLFVGGLNFNTDEQALEDHFSSFGPISEVVVVKDRETQRSRGFGFITFTNPEHASVAMRAMNGESL
DGRQIRVDHAGKSARGTRGGGFGAHGRGRSYSRGGGDQGYGSGRYYDSRPGGYGYGYGRSRDYNGRNQG
GYDRYSGGNYRDNYDN

TDP43 (263-414)
KHNSNRQLERSGRFGGNPGGFGNQGGFGNSRGGGAGLGNNQGSNMGGGMNFGAFSINPAMMAAAQA
ALQSSWGMMGMLASQQNQSGPSGNNQNQGNMQREPNQAFGSGNNSYSGSNSGAAIGWGSASNAGS
GSGFNGGFGSSMDSKSSGWGM

Yeast Sup35 (2-134)
SDSNQGNNQQNYQQYSQNGNQQQGNNRYQGYQAYNAQAQPAGGYYQNYQGYSGYQQGGYQQYNPD
AGYQQQYNPQGGYQQYNPQGGYQQQFNPQGGRGNYKNFNYNNNLQGYQAGFQPQSQGMSLNDFQK
QQKQ

B



Supplemental Figure S3
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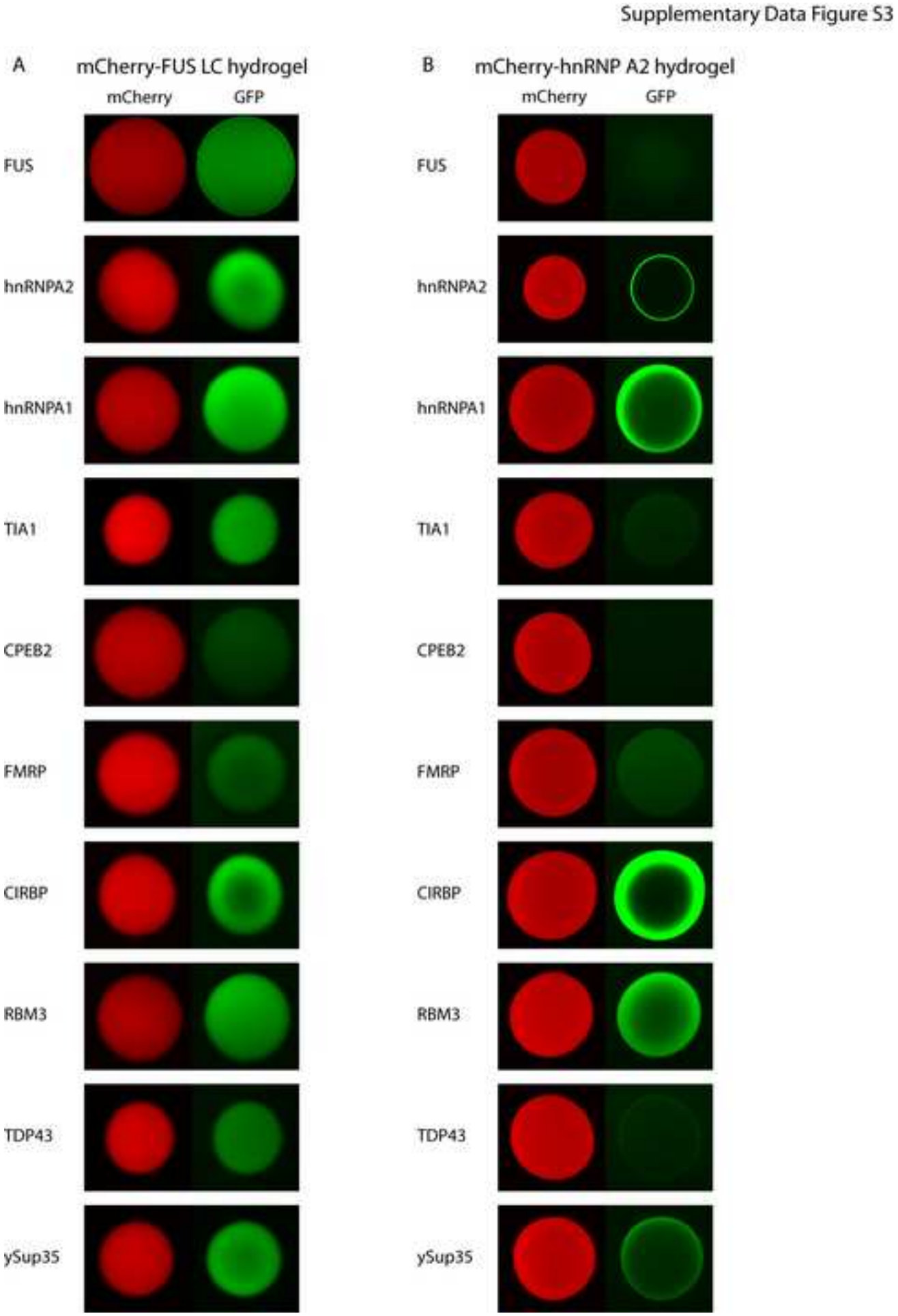

Supplemental Figure S4
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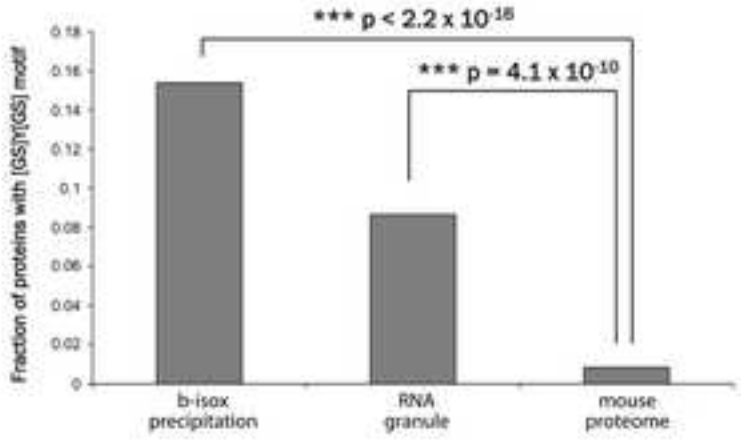
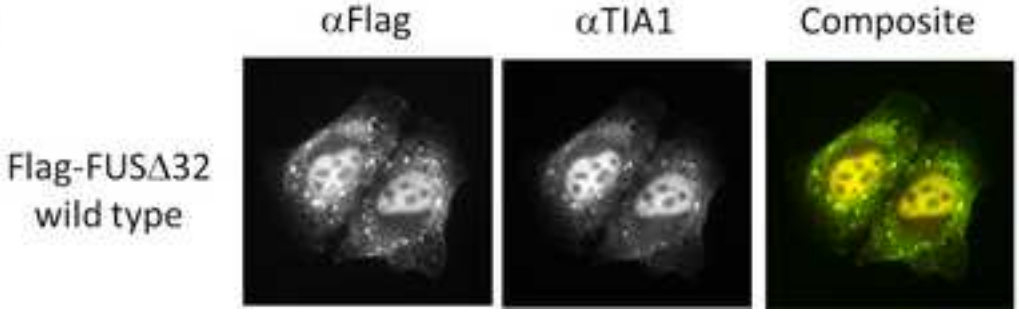
Supplementary Data Figure S4

A

B



C



Supplemental Figure S5
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Supplementary Data Figure S5