Kedersha et al., http://www.jcb.org/cgi/content/full/jcb.201508028/DC1

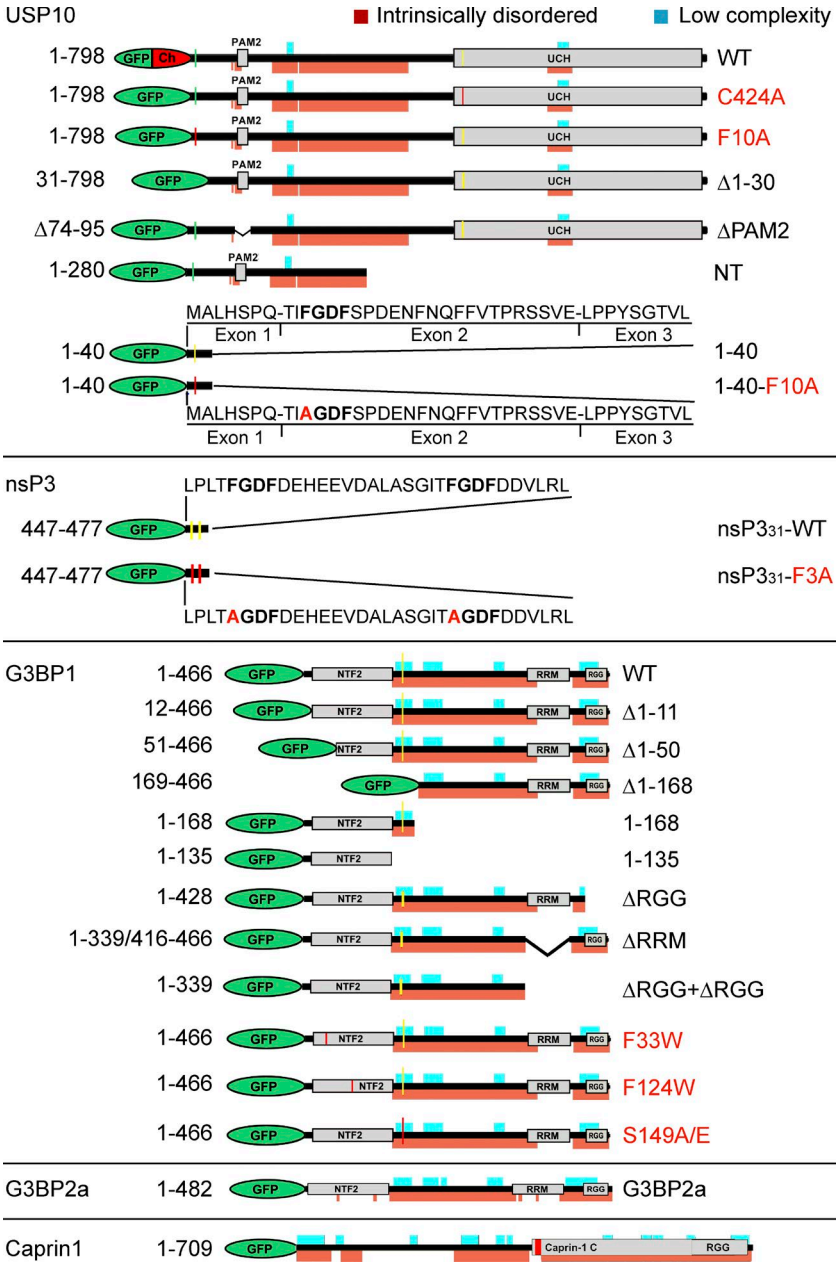


Figure S1. **Constructs used in this study.** Point mutants are indicated by red lines, and key residues are indicated by green or yellow lines. Gray boxes represent structured classical domains, and red areas indicate ≥50% predicted ID/IU regions, whereas aqua shading represent LC regions. aa residue numbers appear at the left.

Figure S2. **Genotype of ΔG3BP1, ΔG3BP2, and ΔΔG3BP1/2 cells.** Gene sequences showing Cas9-induced deletions; initiator ATG appears blue. Predicted protein products of native gene are aligned above the mutant alleles, with predicted frameshifted aa and premature stop codons (asterisks) shown in red. Note that the ΔΔG3BP1/2 cells were made from the ΔG3BP1 cells; hence, the G3BP1 deletion is the same in both cell lines.

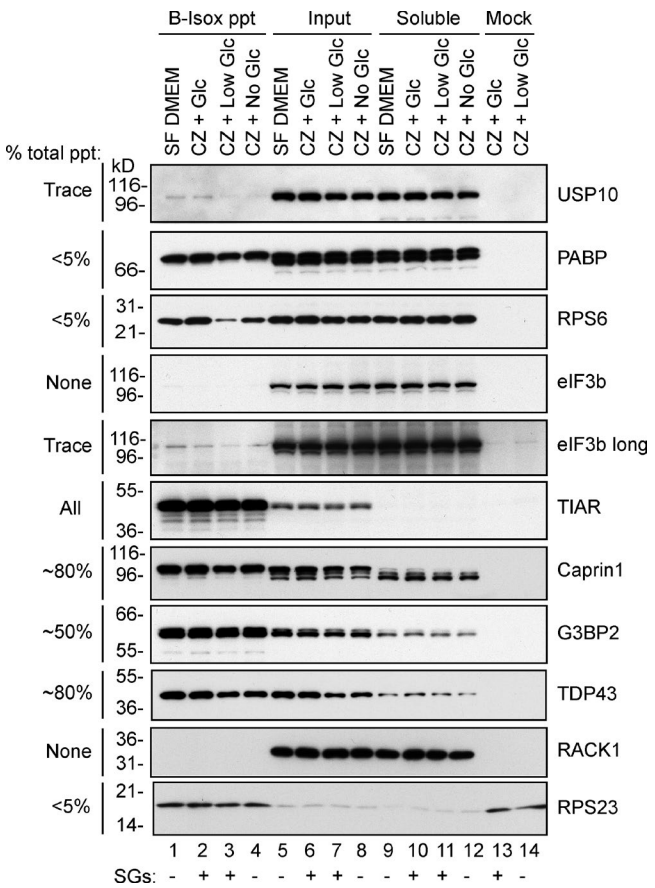


Figure S3. **B-isox solubility of different SG proteins with or without SG induction.** U2OS-WT cells, with or without SG-inducing treatment, were lysed in EE buffer and precipitated with B-isox. Insoluble (lanes 1–4), input (lanes 5–8), and soluble material (9–12) were subjected to Western blotting for the indicated proteins. "eIF3b long" indicates a long exposure. Values (soluble vs. input) were quantified by densitometry, and percent precipitated (ppt) was calculated. The presence of SGs induced by each treatment is indicated below. Treatmen\ts included serum-free media (lanes 1, 5, and 9) and 20 μM CZ (lanes 2, 6, 10, and 13) in serum-free media; CZ in serum-free media containing reduced (0.1 mM) glucose (lanes 3, 7, 11, and 14) or CZ in glucose-free media (lanes 4, 8, and 12) to induce energy starvation. Mock precipitates using vehicle alone are in lanes 13 and 14. M_r (kD) are shown.

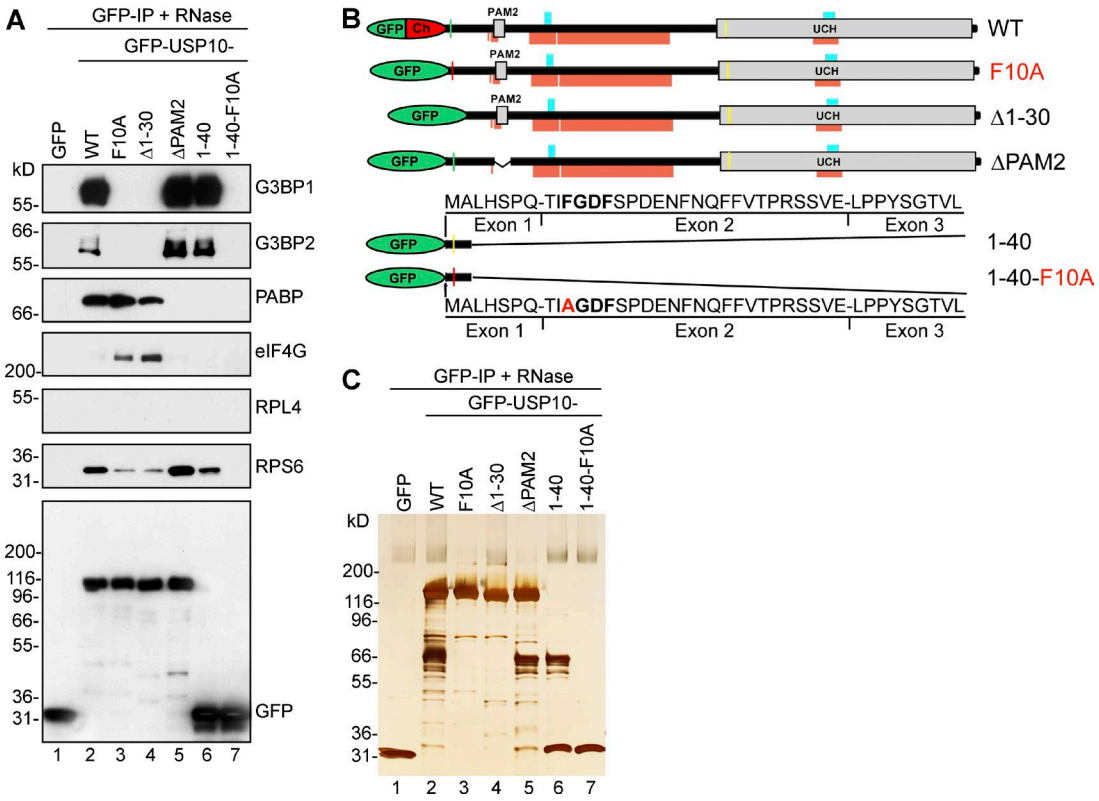


Figure S4. **USP10 mutants bind G3BP1/2 via the FGDF motif.** (A) IPs from transiently transfected COS7 cells prepared in EE buffer with RNase, resolved on SDS-PAGE, blotted and probed with as indicated. M_r (kD) are shown. (B) USP10 constructs depicted graphically. (C) Duplicate SDS-PAGE of A, silver stained. M_r (kD) are shown.

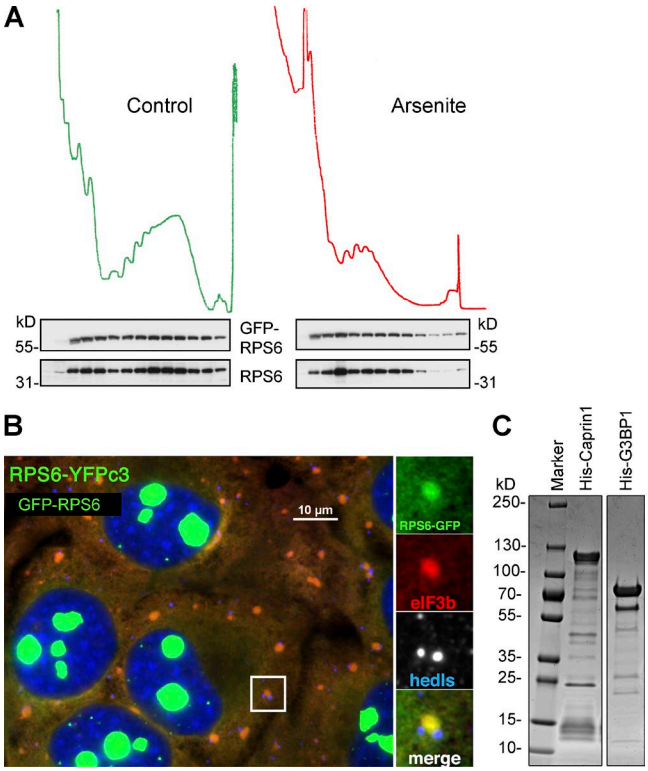
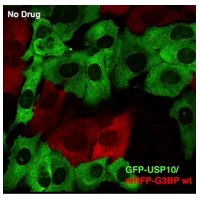
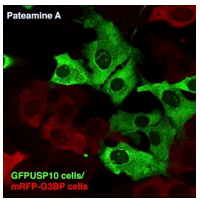


Figure S5. **Verification of RPS6 incorporated into 40S subunits and purity of His-Caprin1 and His-G3BP1.** (A) Polysome profiles from RPS6-GFP cells, without (green) or with (red) pretreatment with AS. RPS6-GFP migrates with endogenous RPS6 in both untreated and arsenite-treated cells. (B) AS-treated RPS6-GFP cells (green), stained for SGs (eIF3b, red) or P-bodies (Hedls, blue). Bar, 10 µm. Insets zoomed 2.4× with separated colors. (C) Coomasie staining of SDS-PAGE of purified recombinant His-Caprin1 and His-G3BP1. M_r (kD) are shown.



Video 1. **U2OS cells expressing GFP-USP10 resist AS-induced SG assembly.** U2OS cells stably expressing mRFP-G3BP (red) or GFP-USP10 (green), were cocultured for 24 h before image collection. Images were acquired using confocal microscopy (Z-stacks) at 1-min intervals and volume and time rendered. Cocultured cells monitored before and after addition of 100 µM AS, as indicated in the video.



Video 2. **U2OS cells expressing GFP-USP10 resist Pat A–induced SG assembly.** U2OS cells stably expressing mRFP-G3BP (red) or GFP-USP10 (green), were cocultured for 24 h before image collection. Images were acquired using confocal microscopy (Z-stacks) at 1-min intervals and volume and time rendered. Cocultured cells monitored before and after addition of 50 nM Pat A, as indicated in the video.

Table S1. **Oligos used in this study**

CRISPR/Cas 9 KO	**gDNA**	**Sequence**
G3BP1	1	5′-GGAGAAGCCTAGTCCCCTGC-3′
	2	5′-AAGCCTAGTCCCCTGCTGGT-3′
	3	5′-AGCCTAGTCCCCTGCTGGTC-3′
	4	5′-CTAGTCCCCTGCTGGTCGGG-3′
G3BP2	1	5′-CGCCCTACAAGCAGCGGACT-3′
	3	5′-CCGCCCTACAAGCAGCGGAC-3′
siRNA KD	**Number**	**Sequence**
G3BP1	1	5′-UAACAGUGGUGGGAAAUUA-3′
	2	5′-UGACAUGGAAGAACAUUUA-3′
	3	5′-GAAGGCGACCGACGAGAUA-3′
	4	5′-GUGCGAGAACAACGAAUAA-3′
G3BP2	1	5′-GAAUAAAGCUCCGGAAUAU-3′
	2	5′-GGAAGUACGUUUAAAUGUG-3′
	3	5′-UGAAGGAUCUGUUCCAAAU-3′
	4	5′-GAUGAUCGCAGGGAUAUUA-3′
USP10	1	5′-CCAUAAAGAUUGCAGAGUU-3′
	2	5′-CAAACAAGAGGUUGAGAUA-3′
	3	5′-CCACAUAUAUUUACAGACU-3′
	4	5′-GAGUUGCACACCACGGAAA-3′
Caprin1	1	5′-AGGGUAAGCUUGAUGAUUA-3′
	2	5′-GCACGUCGGGAGCAGCUUA-3′
	3	5′-GGAAAUUGUUGAGCGUGUU-3′
	4	5′-UAGUCAGCCUCACCAAGUA-3′
Control (GFP)		5′-GGCTACGTCCAGGAGCGUA-3′
Plasmid	**Forward primer**	**Reverse primer**
GFP-USP10 F10A	5′-GCCGGAGATTTTAGCCCTGATGAATTC-3′	5′-AATATACTGCGGGCTGTGGAG-3′
GFP-USP10 Δ1–30	5′-CTTCCTCCATACAGTGGAACAG-3′	5′-AGCTCGAGATCTGAGTCCG-3′
GFP-USP10 ΔPAM2	5′-GCTTCCAAAATAACCCCTGATG-3′	5′-GTAGCTGGGGGTTCTCGG-3′
GFP-G3BP1 Δ1–11	5′-GGGCGGGAATTTGTGAGACA-3′	5′-AGATCTGAGTCCGGACTTGTAC-3′
GFP-G3BP1 Δ1–50	5′-GCAGATGCAGTCTACGGACA-3′	5′-AGATCTGAGTCCGGACTTGTAC-3′
GFP-G3BP1 1–135	5′-TAAGAATTCTGCAGTCGACGGTA-3′C	5′-ATCTTGGTATCTGAAGATATCATTG-3′
GFP-G3BP1 1–168	5′-TAATCAGCCATACCACATTTGTAG-3′	5′-ATCAGGTACCACCTCAGGTGT-3′
GFP-G3BP1 169–466	5′-GATTCTGGAACTTTCTATGATCAG-3′	5′-CTTGTACAGCTCGTCCATGCC-3′
GFP-G3BP1 ΔRRM	5Phos/5′-ACTGTCAGGGTGTCTCACCATTCTTC-3′	5Phos/5′-CGAGCTGCCAGGGAAGGC-3′
GFP-G3BP1 ΔRGG	5Phos/5′-AAGGCGATTATCTCGTCGGTCGC-3′	5Phos/5′-TAAGAATTCTGCAGTCGACGGTACCGC-3′
GFP-G3BP1 ΔRRM+ΔRGG	5Phos/5′-ACTGTCAGGGTGTCTCACCATTCTTC-3′	5Phos/5′-TAAGAATTCTGCAGTCGACGGTACCGC-3′

Table S2. **List of antibodies used in this study**

Antigen	Species	Catalog number	Source
Caprin1	Rabbit	15112-1-AP	Proteintech
Caprin1	Rabbit	HPA018126	Sigma-Aldrich
eIF3b	Goat	sc-16377	Santa Cruz Biotechnology, Inc.
eIF3f	Rabbit	A303-005A	Bethyl Laboratories
eIF4G	Rabbit	sc-11373	Santa Cruz Biotechnology, Inc.
FMRP	Mouse	sc-101048	Santa Cruz Biotechnology, Inc.
FXR1	Goat	sc-10554	Santa Cruz Biotechnology, Inc.
G3BP1	Mouse	sc-81940	Santa Cruz Biotechnology, Inc.
G3BP1	Mouse	611126	BD Transduction Labs
G3BP1	Rabbit	A302-034A	Bethyl Laboratories
G3BP1	Rabbit	A302-033A	Bethyl Laboratories
G3BP2	Rabbit	A302-040A	Bethyl Laboratories
G3BP2	Rabbit	C18193	Assay Biotechnology
GFP	Chicken	G160	Abm
hedls/S6K	Mouse	sc-8418	Santa Cruz Biotechnology, Inc.
P0	Human	HPO-0100	Immunovision
PABP	Mouse	sc-32318	Santa Cruz Biotechnology, Inc.
p-eIF2α	Rabbit	ab32157	Abcam
Puromycin	Mouse	MABE343	EMD Millipore
RACK1	Mouse	sc-17754	Santa Cruz Biotechnology, Inc.
RPL4	Rabbit	11302-1-AP	Proteintech
RPL7A	Rabbit	2403	Cell Signaling
RPS23	Mouse	sc-100837	Santa Cruz Biotechnology, Inc.
RPS6	Mouse	2317S	Cell Signaling
TDP43	Rabbit	10782-2-AP	Proteintech
TIA-1	Goat	sc-1751	Santa Cruz Biotechnology, Inc.
TIAR	Goat	sc-1749	Santa Cruz Biotechnology, Inc.
USP10	Rabbit	A300-900A	Bethyl Laboratories
USP10	Rabbit	A300-901A	Bethyl Laboratories
USP10	Mouse	ab119418	Abcam