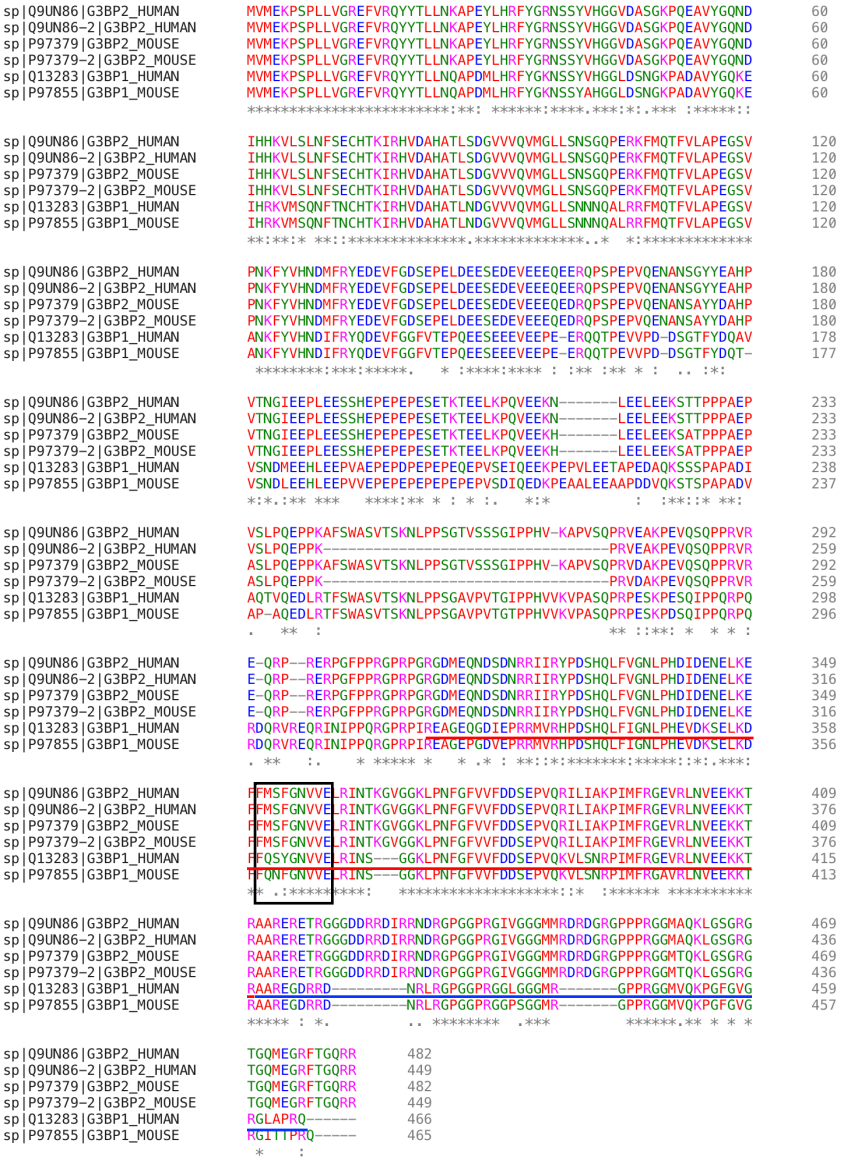



**Fig. S1. Amino acid alignment of human and mouse G3BP1 and G3BP2.** The alignment was generated by Clustal Omega. FLNA-binding site is indicated in a square. Red underline indicates domain 4 (320-416) and blue underline indicates domain 5 (417-466) in Fig. 4.

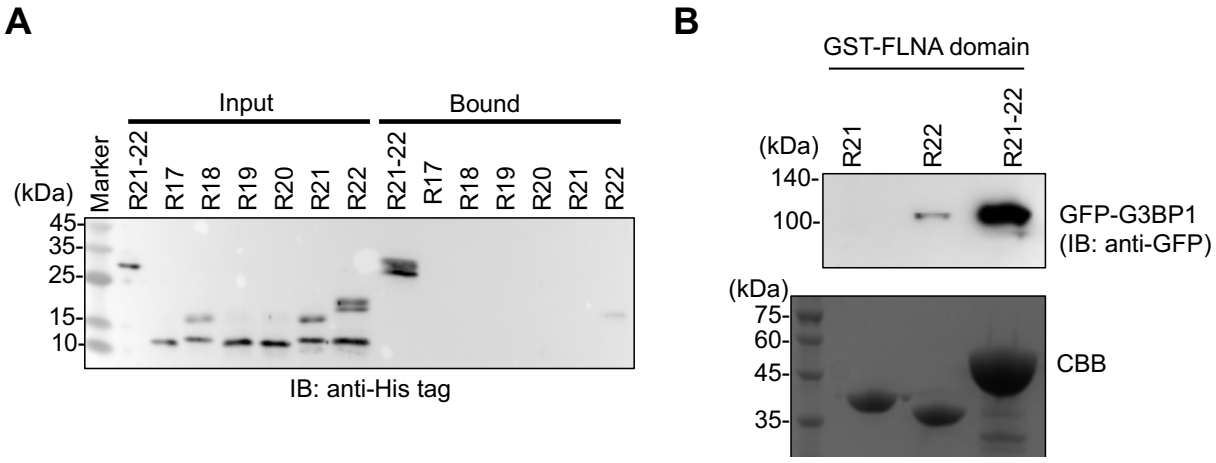



**Fig. S2. FLNA R22 is necessary for FLNA-G3BP1 interaction.**

**(A)** Purified His-tag FLNA repeats were incubated with GST-G3BP1 immobilized on glutathione beads. Bound protein was detected by western blotting against His-tag. Note that no bound protein was detected. **(B)** GFP-G3BP1 expressed in HEK 293 cells was pulled down with GST-FLNA R21, R22, and R21-22 immobilized on glutathione beads. Bound protein was detected by western blotting against GFP.

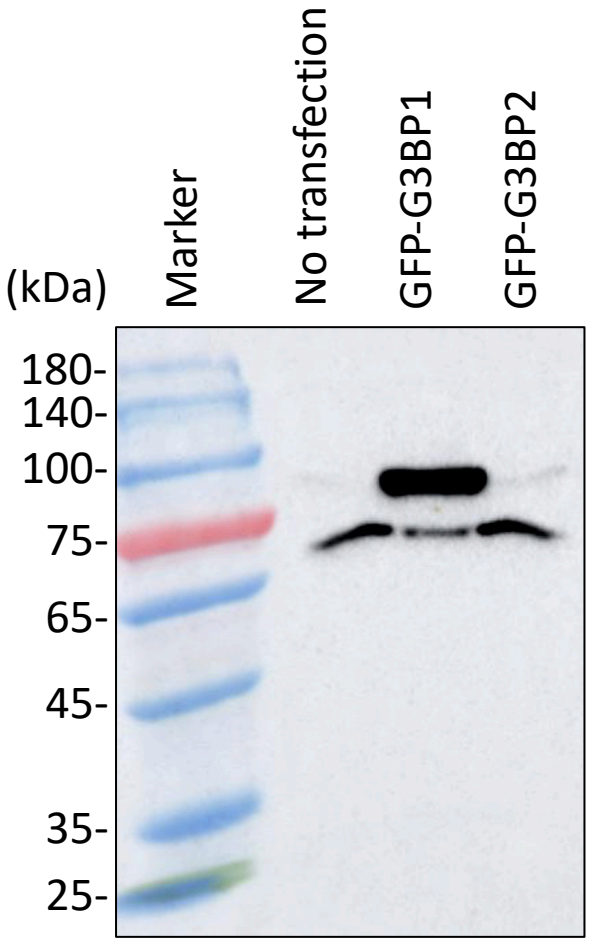



**Fig. S3. Mouse monoclonal anti-G3BP1 antibody specifically interacts with G3BP1.** GFP-G3BP1 and G3BP2 were expressed in HEK293 cells. The cell lysates were blotted using monoclonal anti-G3BP1 antibody (Proteintech, 66486-1-Ig). Note that anti-G3BP1 antibody recognizes endogenous G3BP1 and GFP-G3BP1 but not GFP-G3BP2.

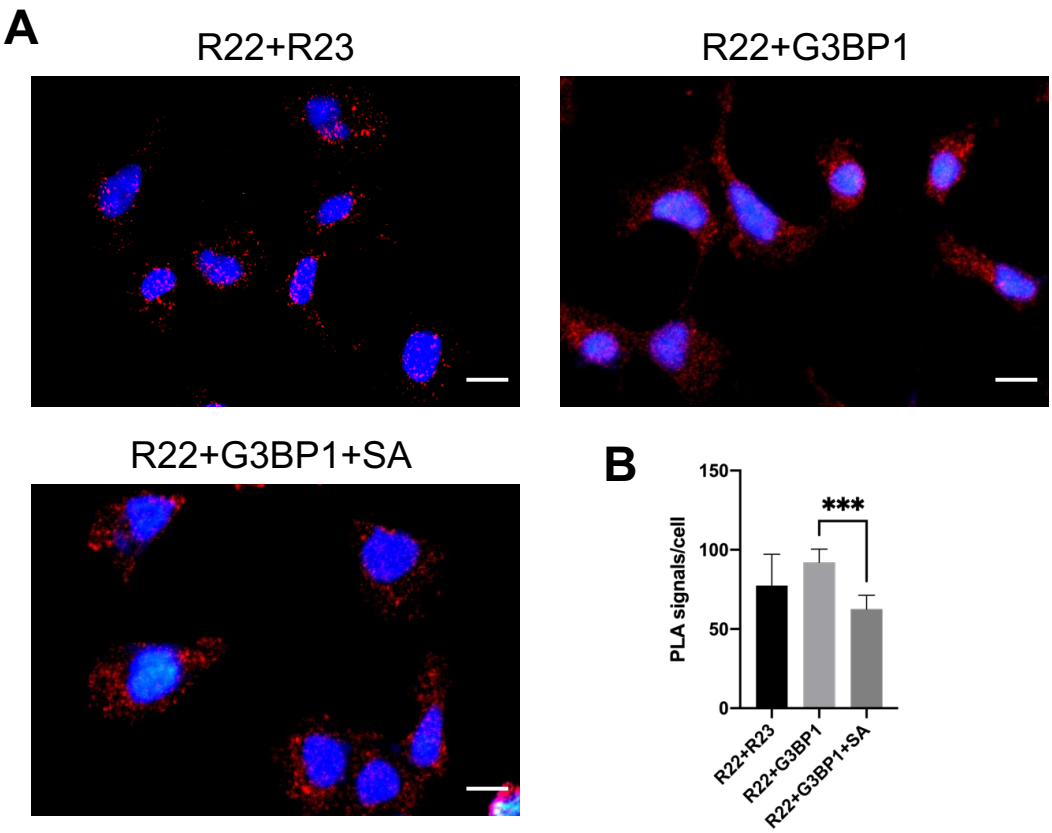
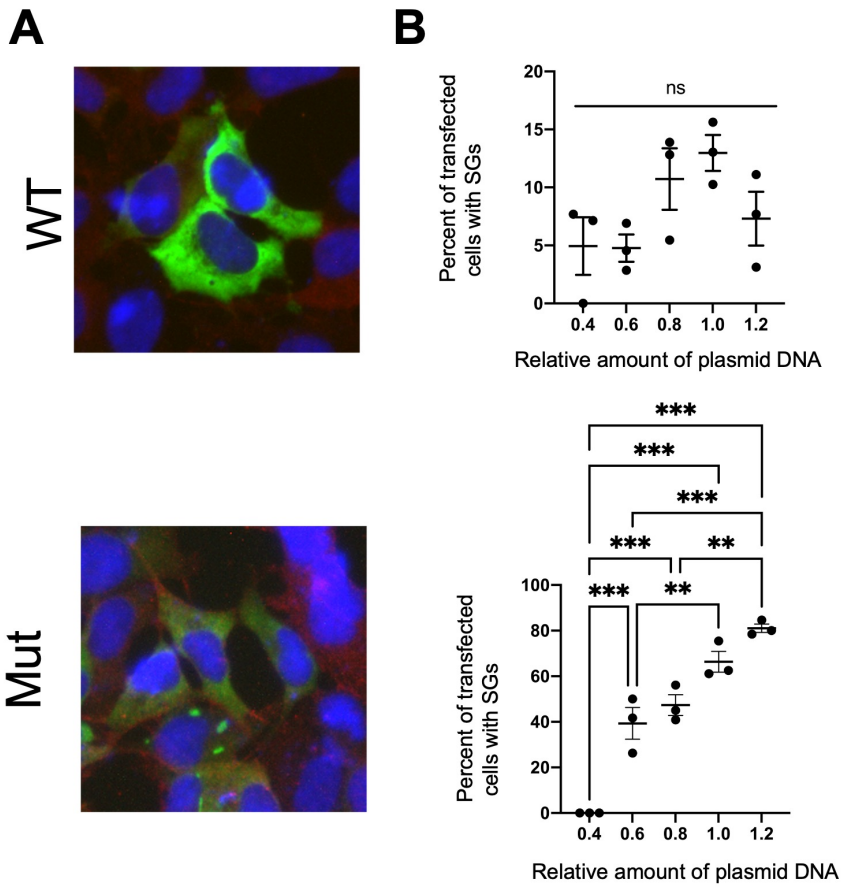



**Fig. S4. PLA signal is significantly decreased when cells are treated with sodium arsenite.** (**A**) Proximity ligation assay. Representative PLA images where the PLA signal (red) represents close proximity (<40nm) between two proteins. PLA signal is significantly decreased when cells are treated with 1.5 mM sodium arsenite (SA) for 1h. Scale bars: 20µm. (**B**) Graph shows quantification of PLA signals. (***, $P < 0.001$). Error bars represent S.D. from five independent counts.




C

| | | Relative amount of plasmid DNA | | | | | One-way ANOVA P | Two-way ANOVA P interaction |
|---|---|---|---|---|---|---|---|---|
| | | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | | |
| Percent of transfected cells with SGs | WT | 4.9±4.3 | 4.8±2.0 | 10.7±4.6 | 13.0±2.7 | 7.3±4.0 | 0.0764, ns | |
| | Mut | - | 39.3±12.0 | 47.3±7.9 | 66.4±7.9 | 81.0±3.2 | <0.001,*** | P<0.001 |
| | t-test P | 0.1167, ns | 0.0080, ** | 0.0022, ** | 0.0004, *** | <0.001, *** | | |

**Fig. S5. Expression of WT and F360A G3BP1 in HEK293A cells.**

(**A**) Merged images of HEK293A cells expressing WT or mutant G3BP1-HA. HA-tag, FLNA, and nucleus were stained in green, red, and blue, respectively. 100 × 100 μm. (**B**) Quantitation of cells exogenously expressing G3BP1 by transfection of different relative amount of plasmid DNA (0.4, 0.6, 0.8, 1.0, 1.2 times). P values are presented by * ($P \leqslant 0.05$), ** ($P < 0.01$) or *** ($P \leqslant 0.001$) on the graph, ns (not significant). Error bars represent S.D. from three independent counts. (**C**) Statistical analyses. Ten cells were counted for 0.4 (relative amount of plasmid DNA) and over 50 cells were counted for 0.6~1.2 (relative amount of plasmid DNA), from three independent replicates. The results shown represent the mean (± S.D.) for three independent experiments. t-Test values are given for comparison of results in WT v.s. Mut.

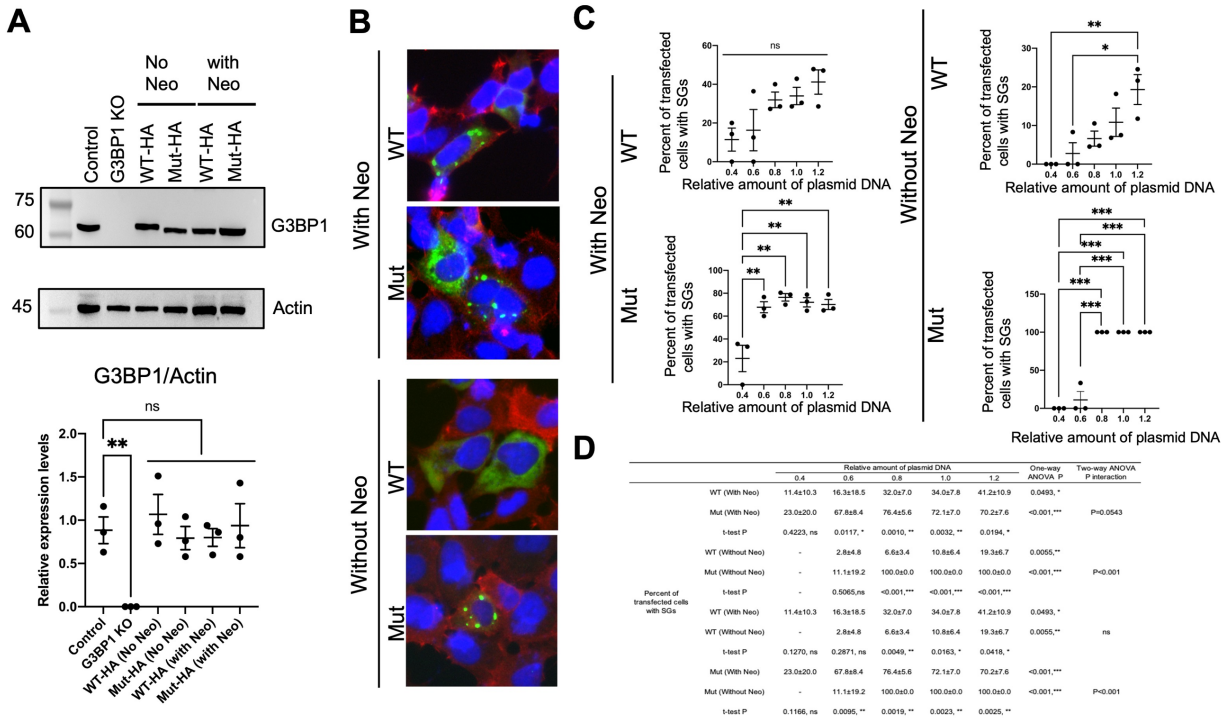



**Fig. S6. Expression of Neo$^R$ influences SG formation induced by exogenously expressed G3BP1.**

(**A**) Western blotting against G3BP1. G3BP1 knock-out (KO) HEK293A cells expressing HA-tagged WT and mutant G3BP1 with or without Neo$^R$. Qantitation of relative intensity of band (bottom). Error bars represent S.D. from three independent counts. (**B**) Merged images of G3BP1 KO HEK293A cells expressing WT or mutant G3BP1-HA. HA-tag, FLNA, and nucleus were stained in green, red, and blue. 100 × 100 μm. (**C**) Quantitation of cells exogenously expressing G3BP1 by transfection of different relative amount plasmid DNA (0.4, 0.6, 0.8, 1.0, 1.2 times). P values are presented by * ($P \leq 0.05$), ** ($P < 0.01$) or *** ($P \leq 0.001$) on the graph, and ns (not significant). Error bars represent S.D. from three independent counts. (**D**) Statistical analyses. Ten cells were counted for 0.4 (relative amount of plasmid DNA) and over 50 cells were counted for 0.6~1.2 (relative amount of plasmid DNA), from three independent replicates. The results shown represent the mean (± S.D.) for three independent experiments.

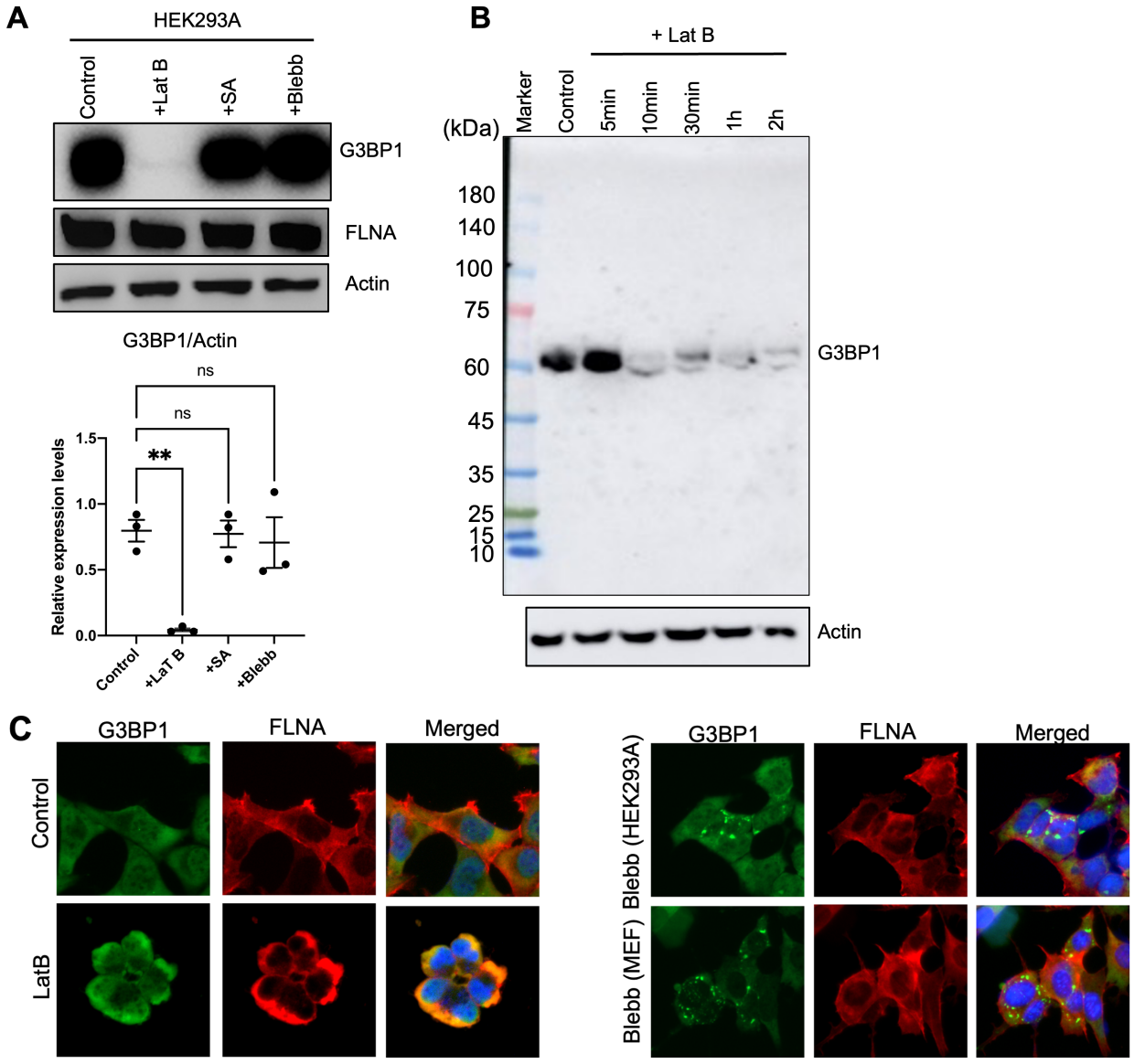



**Fig. S7. Effects of latrunculin B, sodium arsenite, and blebbistatin on expression of G3BP1 and SG formation.**

(**A**) Western blotting of cells treated with 5μM Latrunculin B (Lat B) for 2 hrs, 1.5 mM sodium arsenite (SA) for 1 hr, and 5μM blebbistatin (Blebb) for 2 hrs. The membrane was blotted with anti-G3BP1, anti-FLNA, and anti-actin. Relative intensities of the bands were quantitated on bottom panels. (n=3) (**B**) HEK293A cells were treated with 5μM Latrunculin B (Lat B) for various time as indicated and lysed with SDS sample buffer. Expression of G3BP1 and actin were detected by western blotting. (**C**) Immunofluorescent images of HEK293A cells treated with or without 5μM Latrunculin B (Lat B) or 5μM blebbistatin for 2 hrs. G3BP1, FLNA, and nucleus were stained in green, red, and blue. 100 × 100 μm.

**Table S1. Potential FLNA-binding proteins identified by SILAC-based quantitative proteomics.**

Click here to download Table S1

**Table S2. Antibodies used in this study**

| Antibodies | Source | Identifier | Dilution |
|---|---|---|---|
| Anti-Histone H3 Monoclonal Antibody | Invitrogen | 865R2 | 1:1000 (WB) |
| Anti-GFP Mouse Monoclonal Antibody | Thermo Fisher | MA5-15256 | 1:1000 (WB) |
| Anti-6x-His Tag Mouse Monoclonal Antibody | Thermo Fisher | MA1-21315 | 1:2000 (WB) |
| Anti-GFP Rabbit Polyclonal Antibodies | Abclonal (Wuhan, China) | AE011 | 1:2000 (WB) |
| Anti-G3BP1 Mouse monoclonal antibody | Proteintech (China) | 66486-1-Ig | 1:1000 (WB)<br>1:200 (IF) |
| Anti-HA Mouse monoclonal antibody | Proteintech (China) | 66006-1-Ig | 1:2000 (WB)<br>1:500 (IF) |
| Anti-FLNA antibody | anti-FLNA was outsourced (Pacific immunology, CA) using human FLNA repeat 1 and affinity purified using the antigen immobilized on NHS-Sepharose (GE Healthcare) as an affinity ligand | | 1:400 (IF) |
| Goat anti-mouse IgG (H+L)-horseradish peroxidase (HRP) | BioRad | 172-1011 | 1:3000 (WB) |
| Goat anti-rabbit IgG (H+L)-HRP | BioRad | 172-1019 | 1:3000 (WB) |
| Anti-Mouse IgG-Alexa Fluor Plus 488 | invitrogen | A32723 | 1:400 (IF) |
| Anti-Mouse IgG-Alexa Fluor Plus 594 | invitrogen | A32744 | 1:400 (IF) |
| Anti-Rabbit IgG-Alexa Fluor Plus 488 | invitrogen | A32731 | 1:400 (IF) |
| Anti-Rabbit IgG-Alexa Fluor Plus 594 | invitrogen | A32754 | 1:400 (IF) |

**Table S3. Oligonucleotide sequences used for PLA assay.**

| Name | Sequence |
|---|---|
| Probe-1 | 5′ azide – AAAAAAAAAATATGACAGAACTAGACACTCTT |
| Probe-2 | 5′ azide – AAAAAAAAAAGACGCTAATAGTTAAGACGCTT - 3 × 2′ O-methyl RNA uracil (UUU) |
| Circularization oligonucleotide 1 | 5′ phosphate – GTTCTGTCATATTTAAGCGTCTTAA |
| Circularization oligonucleotide 2 | 5′ phosphate – CTATTAGCGTCCAGTGAATGCGAGTCCGTCTAAGAGA GTAGTACAGCAGCCGTCAAGAGTGTCTA |
| Detection oligonucleotide | Cy3-CAGTGAATGCGAGTCCGTCT – 3 × 2′ O-methyl RNA uracil (UUU) |