

sp Q9UN86 G3BP2_HUMAN	MVMEKPSPLLVRGEFVRQYTTLLNKAPYLHRFYGRNSSYVHGGVDASGKPEAVYQND	60
sp Q9UN86-2 G3BP2_HUMAN	MVMEKPSPLLVRGEFVRQYTTLLNKAPYLHRFYGRNSSYVHGGVDASGKPEAVYQND	60
sp P97379 G3BP2_MOUSE	MVMEKPSPLLVRGEFVRQYTTLLNKAPYLHRFYGRNSSYVHGGVDASGKPEAVYQND	60
sp P97379-2 G3BP2_MOUSE	MVMEKPSPLLVRGEFVRQYTTLLNKAPYLHRFYGRNSSYVHGGVDASGKPEAVYQND	60
sp Q13283 G3BP1_HUMAN	MVMEKPSPLLVRGEFVRQYTTLLNQAPDMLHRFYGRNSSYVHGLDSNGKPADAVYQKE	60
sp P97855 G3BP1_MOUSE	MVMEKPSPLLVRGEFVRQYTTLLNQAPDMLHRFYGRNSSYVHGLDSNGKPADAVYQKE	60

sp Q9UN86 G3BP2_HUMAN	IHHKVLNLFSECHTKIRHVDHAHATLSDGVVVQVMGLLSNSGQPERKFMQTFVLAPEGSV	120
sp Q9UN86-2 G3BP2_HUMAN	IHHKVLNLFSECHTKIRHVDHAHATLSDGVVVQVMGLLSNSGQPERKFMQTFVLAPEGSV	120
sp P97379 G3BP2_MOUSE	IHHKVLNLFSECHTKIRHVDHAHATLSDGVVVQVMGLLSNSGQPERKFMQTFVLAPEGSV	120
sp P97379-2 G3BP2_MOUSE	IHHKVLNLFSECHTKIRHVDHAHATLSDGVVVQVMGLLSNSGQPERKFMQTFVLAPEGSV	120
sp Q13283 G3BP1_HUMAN	IHRKVMNQFTNCHTKIRHVDHAHATLNDGVVVQVMGLLSNNQALRRFMQTFVLAPEGSV	120
sp P97855 G3BP1_MOUSE	IHRKVMNQFTNCHTKIRHVDHAHATLNDGVVVQVMGLLSNNQALRRFMQTFVLAPEGSV	120

sp Q9UN86 G3BP2_HUMAN	PNKFYVHDMFRYDEVFVGDSEPELDEESEDEVEEEQEEERQPSPEPVQENANSYVYEAHP	180
sp Q9UN86-2 G3BP2_HUMAN	PNKFYVHDMFRYDEVFVGDSEPELDEESEDEVEEEQEEERQPSPEPVQENANSYVYEAHP	180
sp P97379 G3BP2_MOUSE	PNKFYVHDMFRYDEVFVGDSEPELDEESEDEVEEEQEEERQPSPEPVQENANSYVYDAHP	180
sp P97379-2 G3BP2_MOUSE	PNKFYVHDMFRYDEVFVGDSEPELDEESEDEVEEEQEEERQPSPEPVQENANSYVYDAHP	180
sp Q13283 G3BP1_HUMAN	ANKFYVHDMFRYDEVFVGDSEPELDEESEDEVEEEQEEERQPSPEPVQENANSYVYDAHP	178
sp P97855 G3BP1_MOUSE	ANKFYVHDMFRYDEVFVGDSEPELDEESEDEVEEEQEEERQPSPEPVQENANSYVYDAHP	177

sp Q9UN86 G3BP2_HUMAN	VTNGIEEPLLEESSHEPEPESETKTEELKQVVEEK-----LEELEEKSTTTPPAEP	233
sp Q9UN86-2 G3BP2_HUMAN	VTNGIEEPLLEESSHEPEPESETKTEELKQVVEEK-----LEELEEKSTTTPPAEP	233
sp P97379 G3BP2_MOUSE	VTNGIEEPLLEESSHEPEPESETKTEELKQVVEEK-----LEELEEKSTTTPPAEP	233
sp P97379-2 G3BP2_MOUSE	VTNGIEEPLLEESSHEPEPESETKTEELKQVVEEK-----LEELEEKSTTTPPAEP	233
sp Q13283 G3BP1_HUMAN	VSDNMEELLEEVVAEPEPDPPEPEPVEIQQEKKPEVLEETAPEDAQKSSSPAPADI	238
sp P97855 G3BP1_MOUSE	VSDNLEELLEEVVAEPEPEPEPEPEPVSQIQEDKPEAALEEAAPDQKSTSPAPADI	237

sp Q9UN86 G3BP2_HUMAN	VSLPQEPKAFSASVTSKLNPPSGTVSSSGIPPHV-KAPVSPRVEAKPEVQSQPPRV	292
sp Q9UN86-2 G3BP2_HUMAN	VSLPQEPK-----PRVEAKPEVQSQPPRV	259
sp P97379 G3BP2_MOUSE	ASLPQEPKAFSASVTSKLNPPSGTVSSSGIPPHV-KAPVSPRVDKPEVQSQPPRV	292
sp P97379-2 G3BP2_MOUSE	ASLPQEPK-----PRVDKPEVQSQPPRV	259
sp Q13283 G3BP1_HUMAN	AQTVQEDLRTFSASVTSKLNPPSGAVPVTGIPPHVVKVPASQPRPESKPEVQSQPPRV	298
sp P97855 G3BP1_MOUSE	AP-AQEDLRTFSASVTSKLNPPSGAVPVTGIPPHVVKVPASQPRPESKPDVQSQPPRV	296

sp Q9UN86 G3BP2_HUMAN	E-QRP--RERPGFPPRGPRGRGDMQNDSDNRRIIRYPDSHQLFVGNLPHDIDENELKE	349
sp Q9UN86-2 G3BP2_HUMAN	E-QRP--RERPGFPPRGPRGRGDMQNDSDNRRIIRYPDSHQLFVGNLPHDIDENELKE	316
sp P97379 G3BP2_MOUSE	E-QRP--RERPGFPPRGPRGRGDMQNDSDNRRIIRYPDSHQLFVGNLPHDIDENELKE	349
sp P97379-2 G3BP2_MOUSE	E-QRP--RERPGFPPRGPRGRGDMQNDSDNRRIIRYPDSHQLFVGNLPHDIDENELKE	316
sp Q13283 G3BP1_HUMAN	RDQRVREQRINIPPRGPRPIREAGEQDIEPRRMVRRHPSHQLFVGNLPHDIDENELKE	358
sp P97855 G3BP1_MOUSE	RDQRVREQRINIPPRGPRPIREAGEQDIEPRRMVRRHPSHQLFVGNLPHDIDENELKE	356

sp Q9UN86 G3BP2_HUMAN	<u>FFMSFGNVVE</u> <u>LRINTKGVGGKLPNFGFVVDDESEPVQRI</u> <u>LI</u> AKPIMFRGVRNLVVEEKKT	409
sp Q9UN86-2 G3BP2_HUMAN	<u>FFMSFGNVVE</u> <u>LRINTKGVGGKLPNFGFVVDDESEPVQRI</u> <u>LI</u> AKPIMFRGVRNLVVEEKKT	376
sp P97379 G3BP2_MOUSE	<u>FFMSFGNVVE</u> <u>LRINTKGVGGKLPNFGFVVDDESEPVQRI</u> <u>LI</u> AKPIMFRGVRNLVVEEKKT	409
sp P97379-2 G3BP2_MOUSE	<u>FFMSFGNVVE</u> <u>LRINTKGVGGKLPNFGFVVDDESEPVQRI</u> <u>LI</u> AKPIMFRGVRNLVVEEKKT	376
sp Q13283 G3BP1_HUMAN	<u>FFMSFGNVVE</u> <u>LRINS</u> ---GGKLPNFGFVVDDESEPVQRLVSNRIPIMFRGVRNLVVEEKKT	415
sp P97855 G3BP1_MOUSE	<u>FFMSFGNVVE</u> <u>LRINS</u> ---GGKLPNFGFVVDDESEPVQRLVSNRIPIMFRGVRNLVVEEKKT	413

sp Q9UN86 G3BP2_HUMAN	RAAERETRGGGDRRDIRNRDGPGGPRIVGGGMRDRDGRPPRGGMAQKLGSGRG	469
sp Q9UN86-2 G3BP2_HUMAN	RAAERETRGGGDRRDIRNRDGPGGPRIVGGGMRDRDGRPPRGGMAQKLGSGRG	436
sp P97379 G3BP2_MOUSE	RAAERETRGGGDRRDIRNRDGPGGPRIVGGGMRDRDGRPPRGGMAQKLGSGRG	469
sp P97379-2 G3BP2_MOUSE	RAAERETRGGGDRRDIRNRDGPGGPRIVGGGMRDRDGRPPRGGMAQKLGSGRG	436
sp Q13283 G3BP1_HUMAN	RAAEGDRRD-----NRLRGPGGPRGGLGGMR-----GPPRGGVQKPGFVGV	459
sp P97855 G3BP1_MOUSE	RAAEGDRRD-----NRLRGPGGPRGPGSGMR-----GPPRGGVQKPGFVGV	457

sp Q9UN86 G3BP2_HUMAN	TGMEGRFTGQRR	482
sp Q9UN86-2 G3BP2_HUMAN	TGMEGRFTGQRR	449
sp P97379 G3BP2_MOUSE	TGMEGRFTGQRR	482
sp P97379-2 G3BP2_MOUSE	TGMEGRFTGQRR	449
sp Q13283 G3BP1_HUMAN	RLAPRQ-----	466
sp P97855 G3BP1_MOUSE	RLAPRQ-----	465

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.

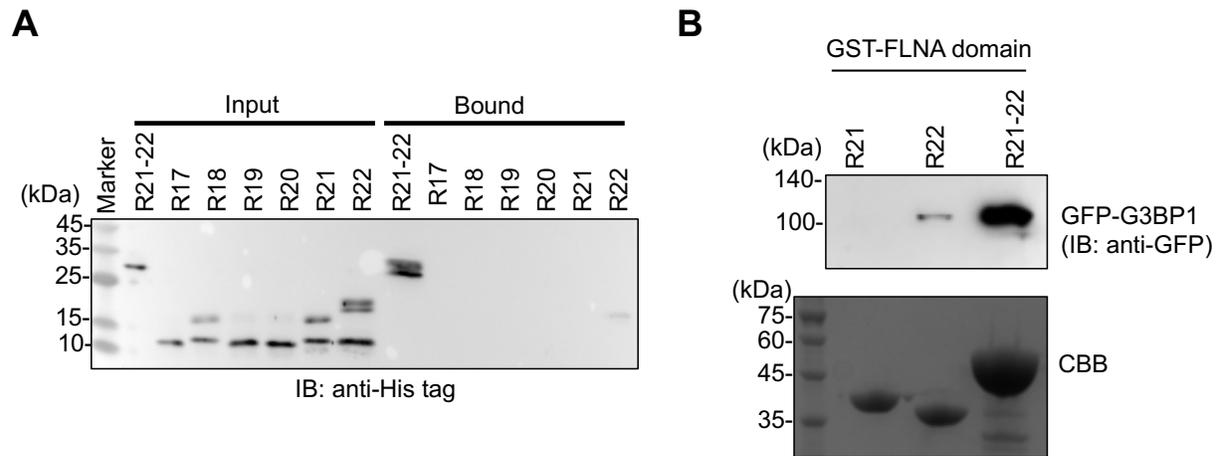


Fig. S2. FLNA R22 is necessary for FLNA-G3BP1 interaction.

(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.

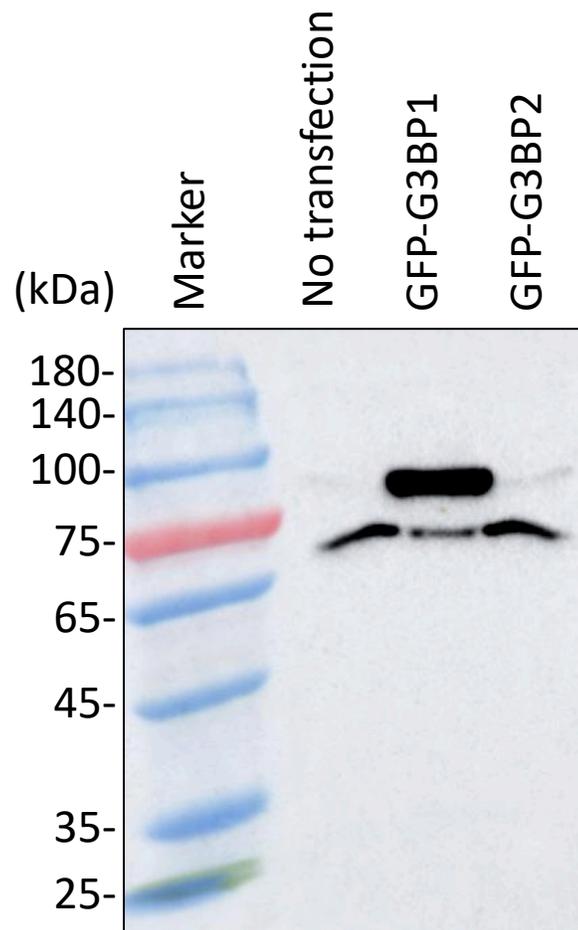


Fig. S3. Mouse monoclonal anti-G3BP1 antibody specifically interacts with G3BP1. GFP-G3BP1 and G3BP2 were expressed in HEK293 cells. The cell lysates were blotted using monoclonal anti-G3BP1 antibody (Proteintech, 66486-1-Ig). Note that anti-G3BP1 antibody recognizes endogenous G3BP1 and GFP-G3BP1 but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

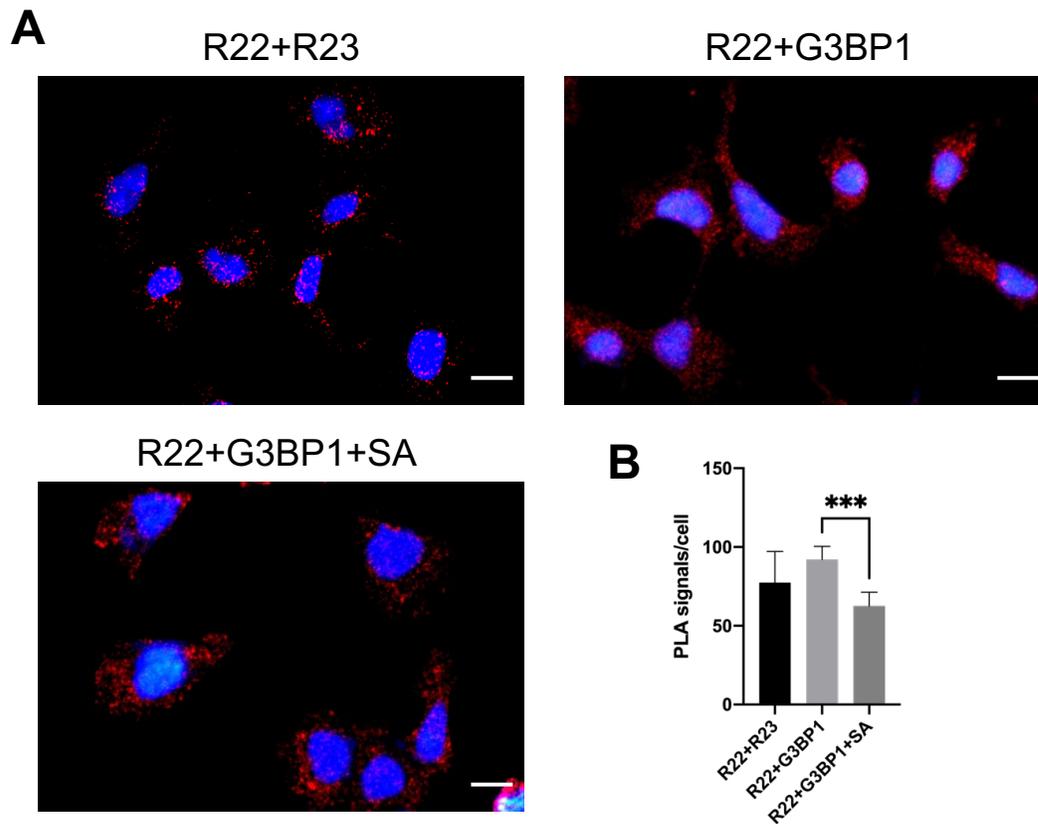


Fig. S4. PLA signal is significantly decreased when cells are treated with sodium arsenite. (A) Proximity ligation assay. Representative PLA images where the PLA signal (red) represents close proximity (<40nm) between two proteins. PLA signal is significantly decreased when cells are treated with 1.5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

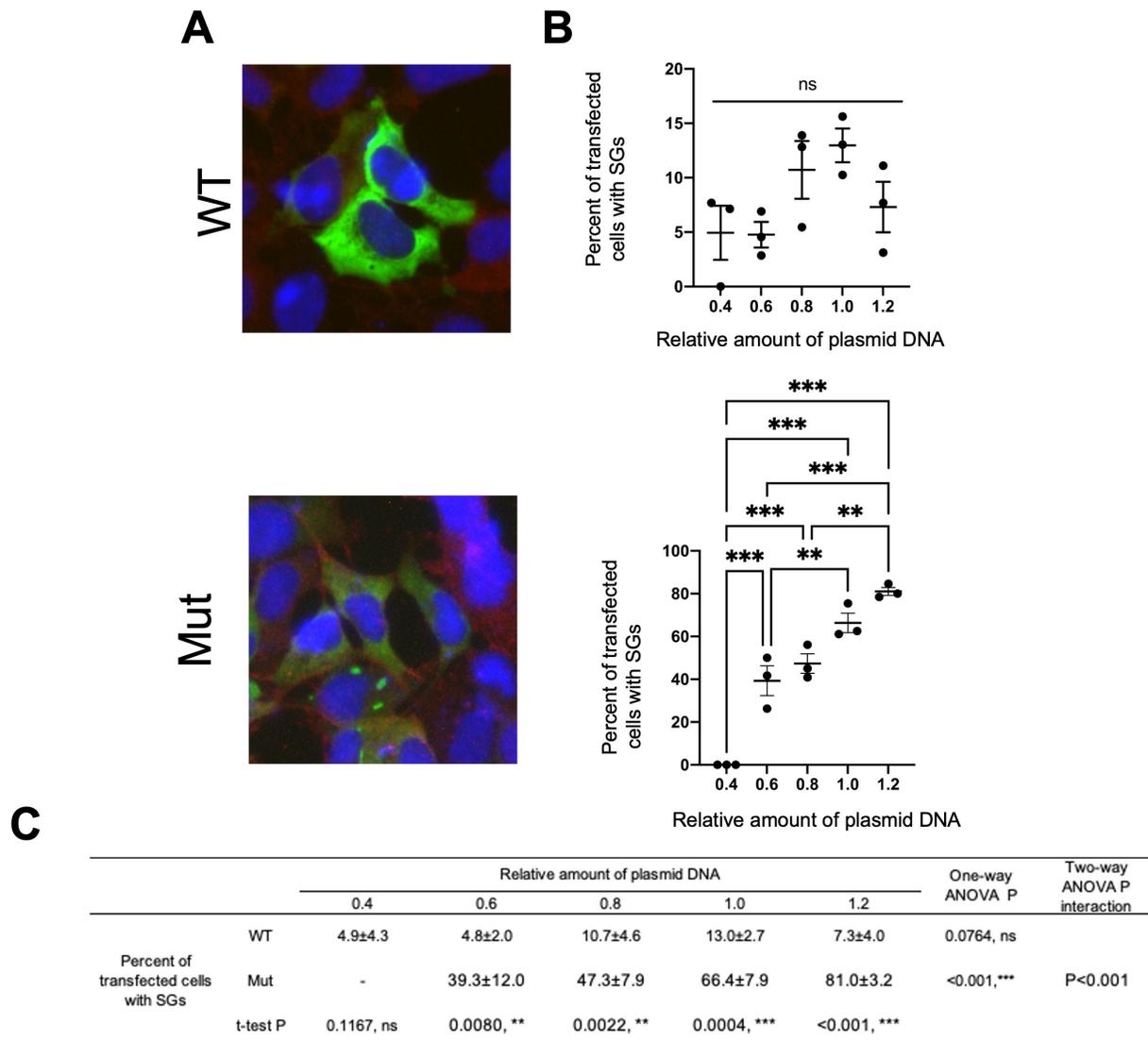


Fig. S5. Expression of WT and F360A G3BP1 in HEK293A cells.

(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 ≤ 0.05), ** (P < 0.01) or *** (P ≤ 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.

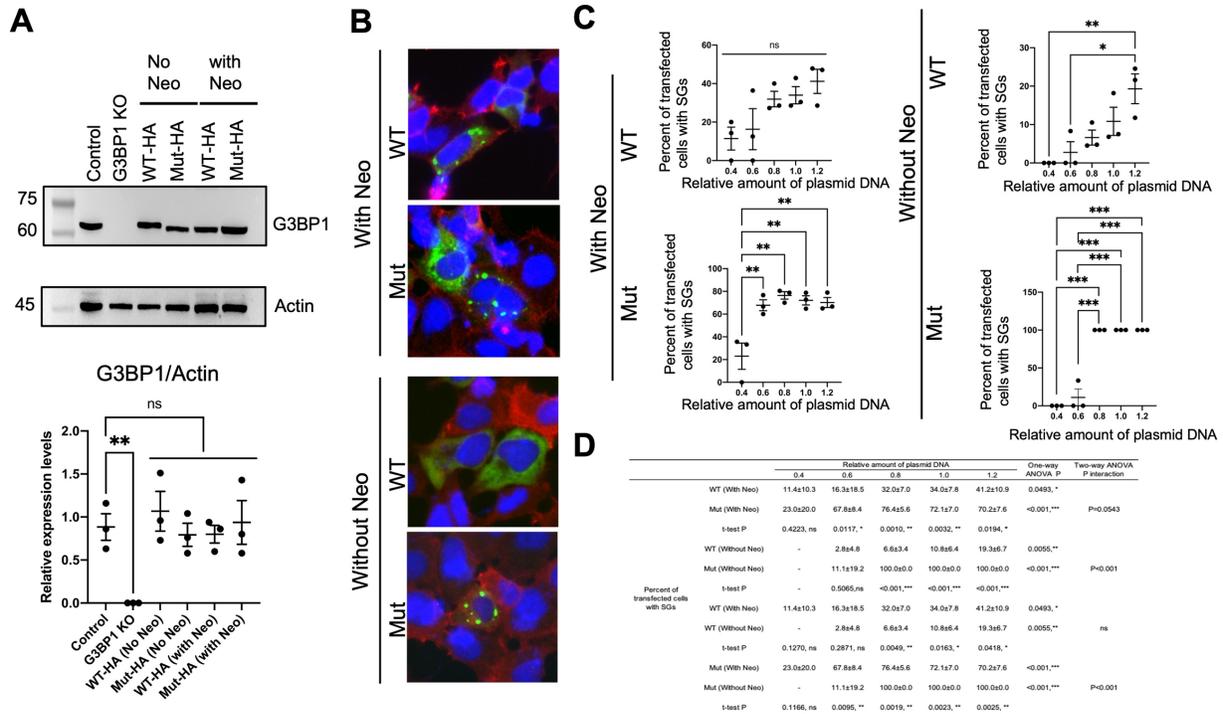


Fig. S6. Expression of Neo^R influences SG formation induced by exogenously expressed G3BP1.

(A) Western blotting against G3BP1. G3BP1 knock-out (KO) HEK293A cells expressing HA-tagged WT and mutant G3BP1 with or without Neo^R. Quantitation 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 ≤ 0.05), ** (P < 0.01) or *** (P ≤ 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.

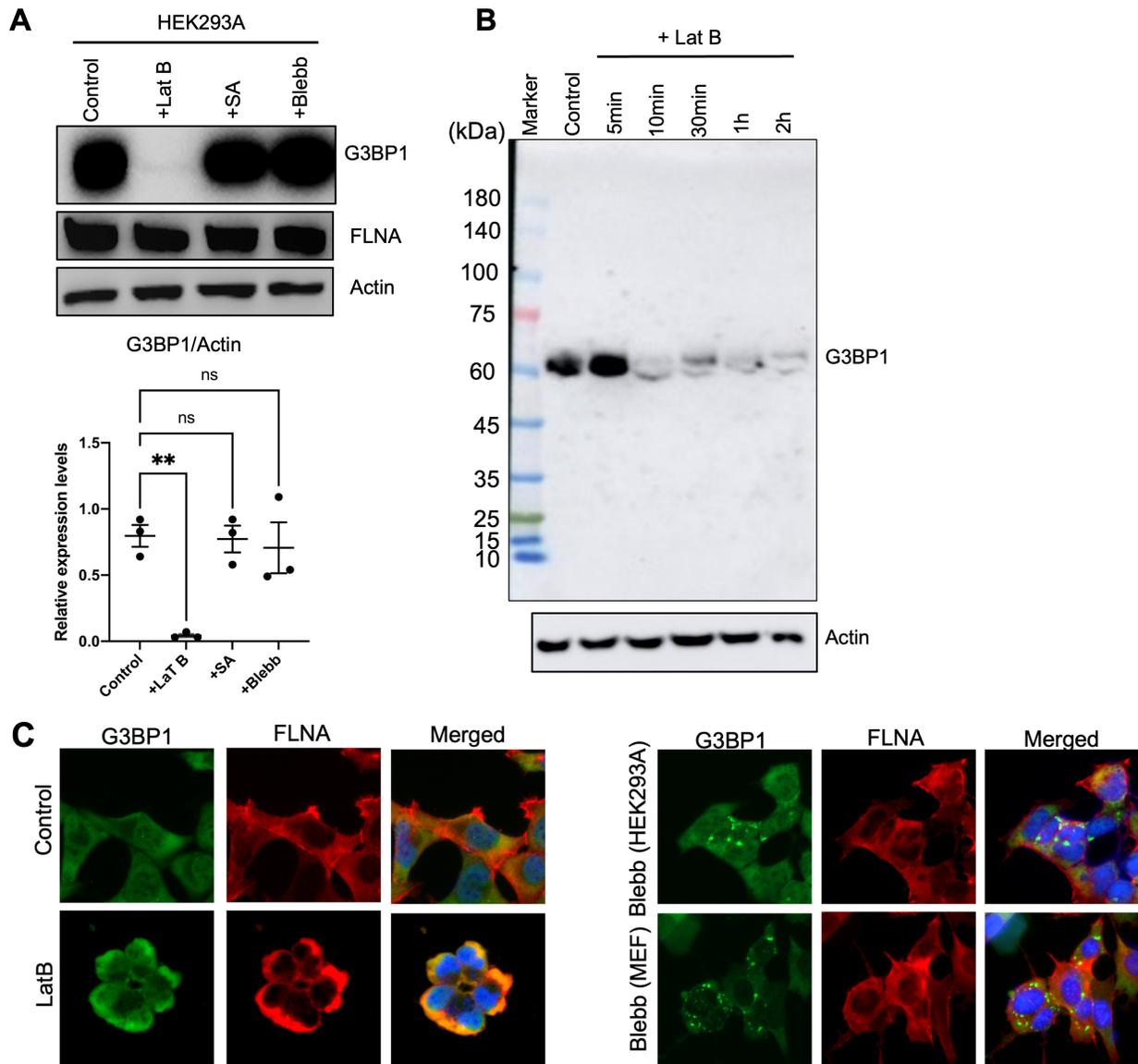


Fig. S7. Effects of latrunculin B, sodium arsenite, and blebbistatin on expression of G3BP1 and SG formation.

(A) Western blotting of cells treated with 5 μ M Latrunculin B (Lat B) for 2 hrs, 1.5 mM sodium arsenite (SA) for 1 hr, and 5 μ M 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 5 μ M 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 5 μ M Latrunculin B (Lat B) or 5 μ M blebbistatin for 2 hrs. G3BP1, FLNA, and nucleus were stained in green, red, and blue. 100 \times 100 μ m.

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) 1:200 (IF)
Anti-HA Mouse monoclonal antibody	Proteintech (China)	66006-1-Ig	1:2000 (WB) 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 – AAAAAAAAAAATATGACAGAAGCTAGACACTCTT
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)