Supplemental Materials

Molecular Biology of the Cell

Huang *et al*.

Detecting protein-protein interaction during liquid-liquid phase separation using fluorogenic protein sensors

Yanan Huang[1], Junlin Chen[1], Chia-Heng Hsiung[1], Yulong Bai[1], Zizhu Tan[1], Songtao Ye[1] and Xin Zhang*[1,2,3]

[1]Department of Chemistry, Research Center for Industries of the Future, Westlake University, 600 Dunyu Road, Hangzhou 310030, Zhejiang, China;

[2]Institute of Natural Sciences, Westlake Institute for Advanced Study;

[3]Westlake Laboratory of Life Sciences and Biomedicine, 18 Shilongshan Road, Hangzhou 310024, Zhejiang Province, China

*Correspondence author: Xin Zhang, zhangxin@westlake.edu.cn

KEYWORDS: Protein-protein interaction; Liquid-liquid phase separation; Fluorescent proteins

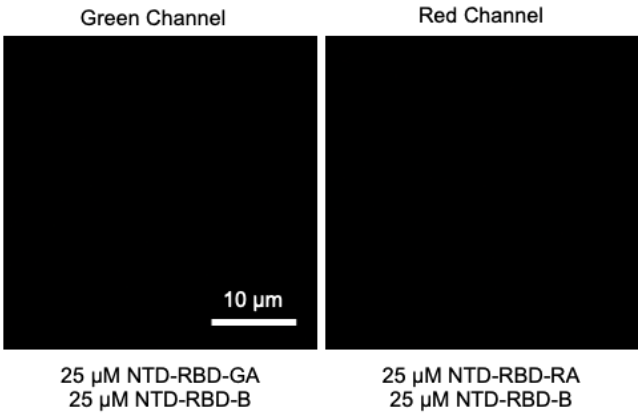
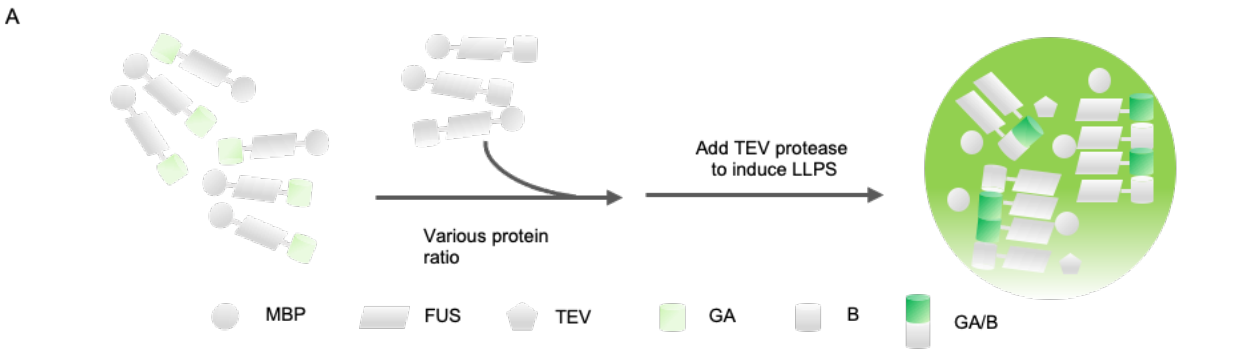
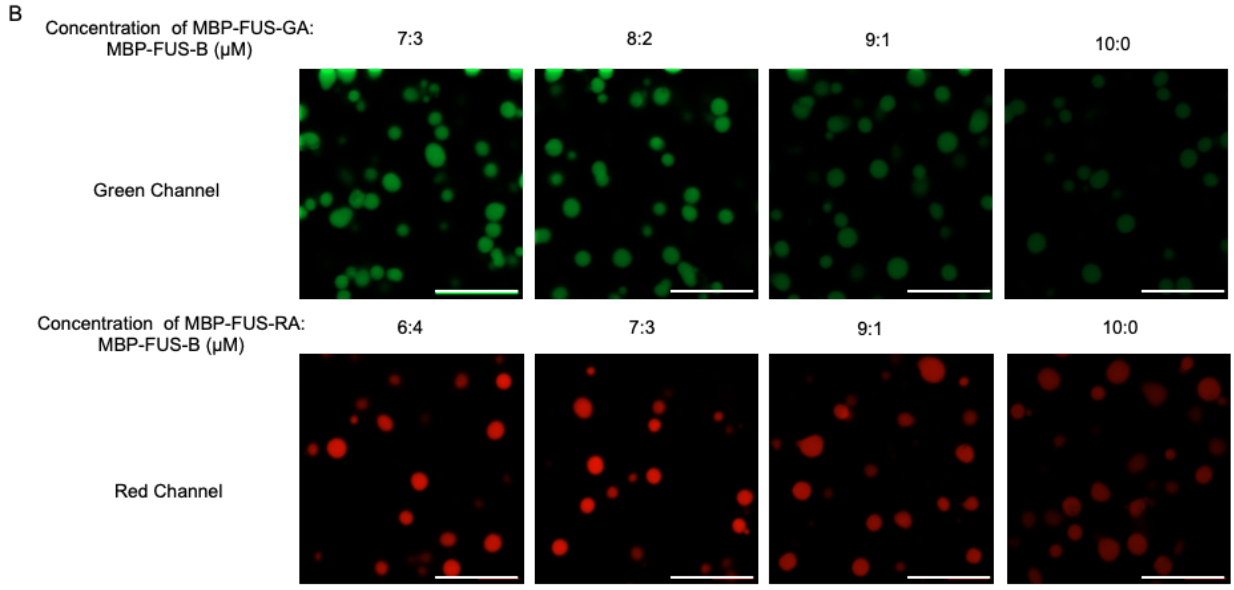


Figure S1. The fluorescence of ddFP system rarely can be detected when proteins are soluble in solution. Samples are incubated in 20 mM HEPES, 300 mM NaCl, 5% PEG 3350. Scale bar = 10 µm.


A
Add TEV protease
to induce LLPS
Various protein
ratio
MBP
FUS
TEV
GA
B
GA/B


B
Concentration of MBP-FUS-GA:
MBP-FUS-B (μM)
7:3
8:2
9:1
10:0
Green Channel
Concentration of MBP-FUS-RA:
MBP-FUS-B (μM)
6:4
7:3
9:1
10:0
Red Channel

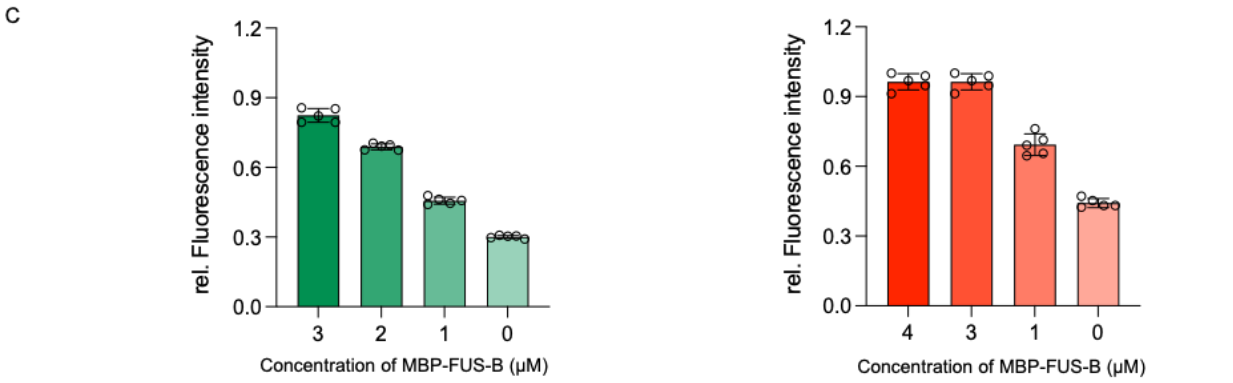

C
rel. Fluorescence intensity
1.2
0.9
0.6
0.3
0.0
3
2
1
0
Concentration of MBP-FUS-B (μM)
rel. Fluorescence intensity
1.2
0.9
0.6
0.3
0.0
4
3
1
0
Concentration of MBP-FUS-B (μM)

Figure S2. The ddFP system can detect protein interaction during *in vitro* LLPS. (A) The LLPS process of MBP-FUS-RA/B. (B) Fluorescence intensity is ddFP dimers concentration dependent. The total protein concentration is 10 μM, change the ratio of MBP-FUS-GA and MBP-FUS-B. Fluorescence intensity of droplets decrease with concentration of ddFP dimers. (C) Quantification of the fluorescence intensity of the droplets in B. LLPS of proteins is induced in 20 mM HEPES, 200 mM NaCl, 10 μM TEV, 5% PEG 3350. All measurements were conducted in triplicates and error bars are calculated as standard deviations. Scale bar = 10 μm.

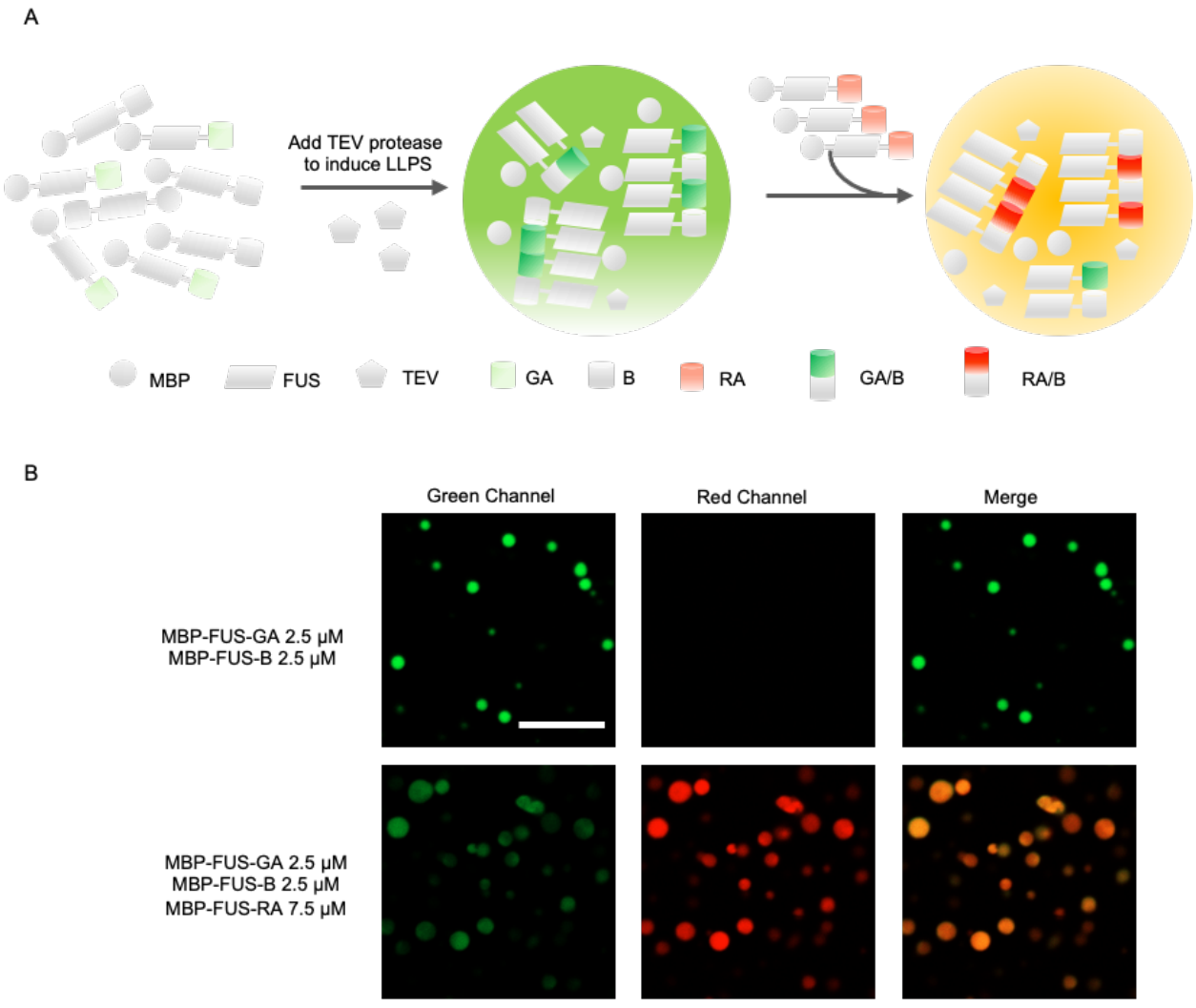


Figure S3. MBP-FUS-GA competed with MBP-FUS-RA to combine with MBP-FUS-B. (A) Process diagram of competition experiment. Abundant MBP-FUS-RA was added in the LLPS system to compete with MBP-FUS-GA. (B) After adding MBP-FUS-RA, red fluorescence appears and green fluorescent intensity decreases. TEV protease was added to induce LLPS. Scale bar = 10 μm.

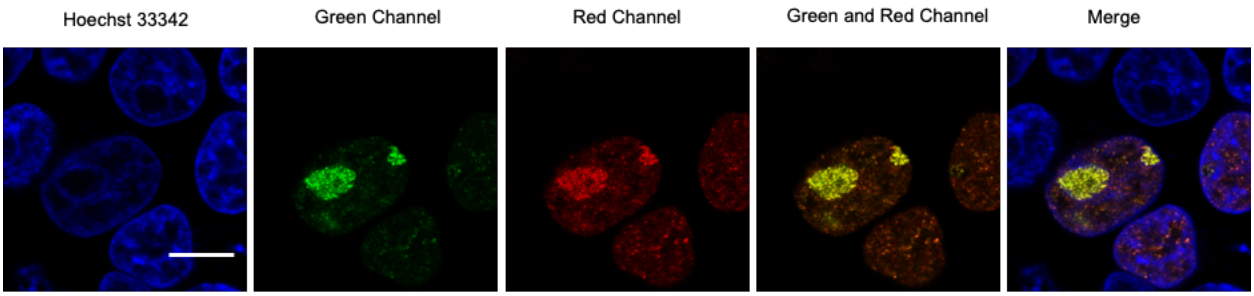


Figure S4. Cells transfected with the tandem plasmid that simultaneously expreses FUS-GA, FUS-B, and TDP-43-RA. Cells were treated with 25 μM AdOx for 24 h to induce LLPS of FUS. Scale bar = 10 μm.

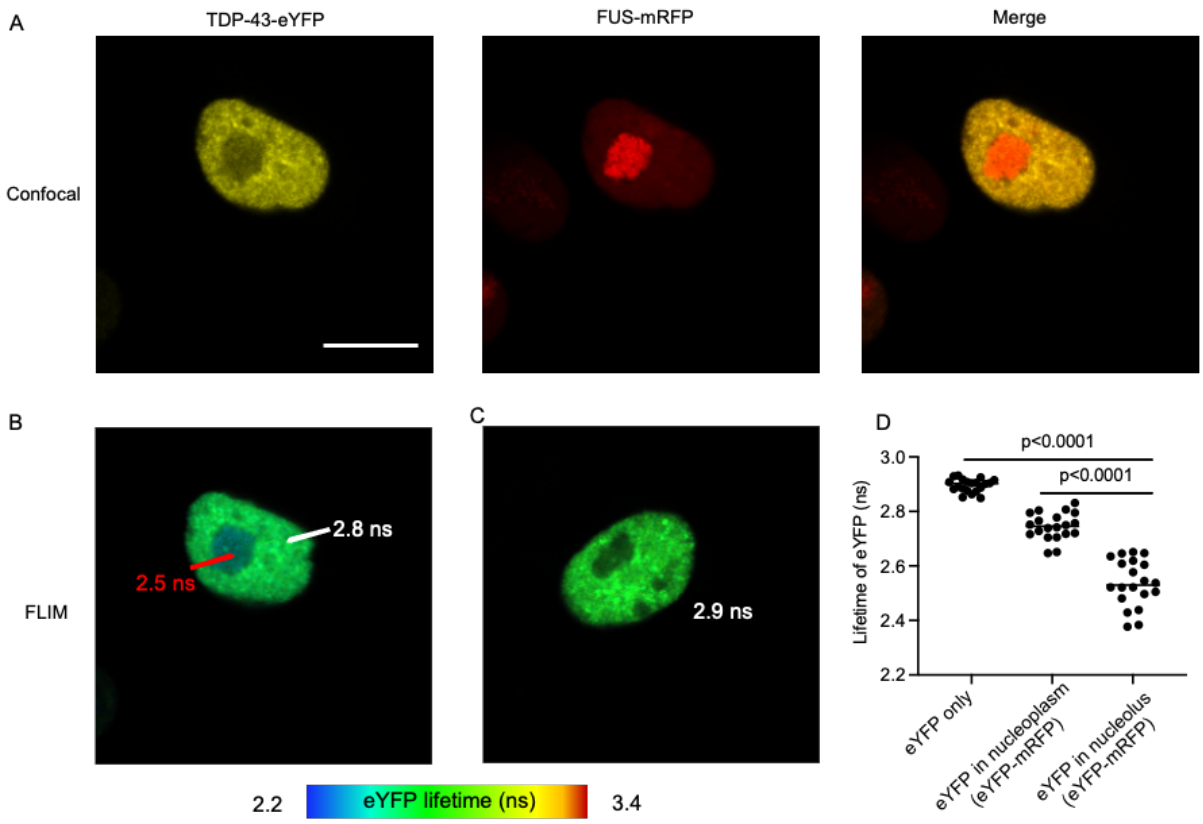


Figure S5. TDP-43 and FUS have interaction. (A) Confocal of cells transfected with TDP-43-eYFP and FUS-mRFP and treated with 25 µM AdOx for 24 h. TDP-43-eYFP and FUS-mRFP co-localize in nucleolus. (B) FLIM of cells in A. The lifetime of eYFP in nucleolus decrease due to interaction of FUS and TDP-43. (C) FLIM of cells transfected with TDP-43-eYFP only. (D) Quantification of lifetime of eYFP in different locations of nucleus, t test was used, n = 20 cells, $p < 0.0001$. Scale bar = 10 µm.

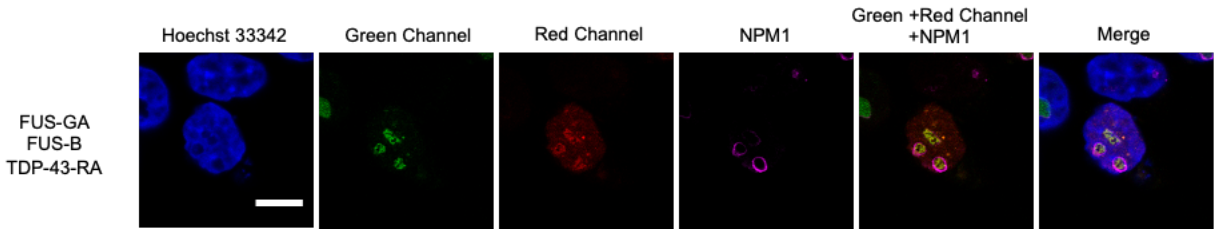


Figure S6. Under AdOx treatment, TDP-43 entered the nucleus due to interaction with FUS. Immunofluorescence staining assay exhibited that FUS was colocalized with the nucleolar marker protein NPM1. Scale bar = 10 μm.

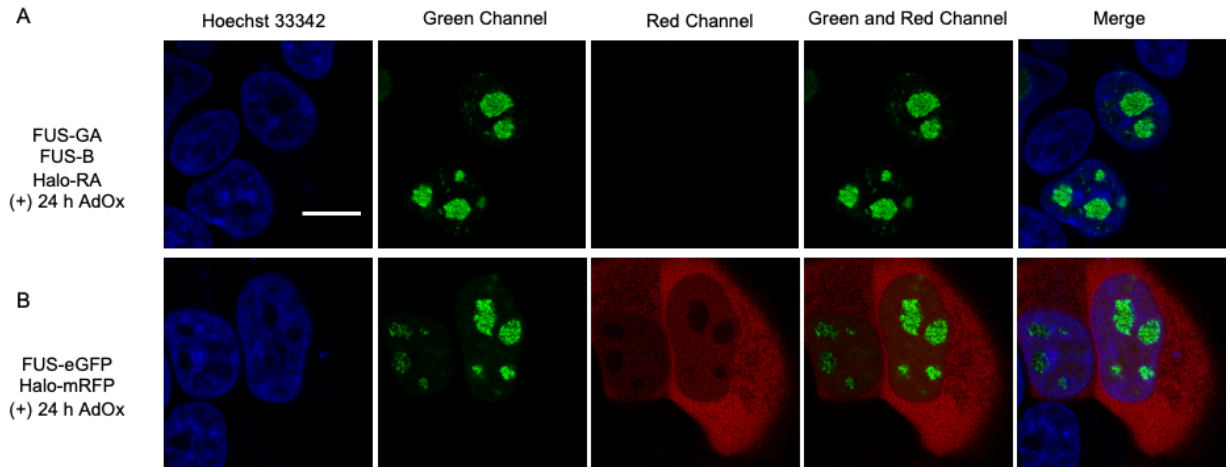


Figure S7. The innate affinity of the ddFP system is insufficient to induce binding between two proteins without affinity. (A) Halo-RA and FUS-B exhibited minimal red fluorescence due to the lack of interaction between FUS and Halo. (B) FUS-eGFP and Halo-mRFP locate in different cellular locations. Scale bar = 10 μm.

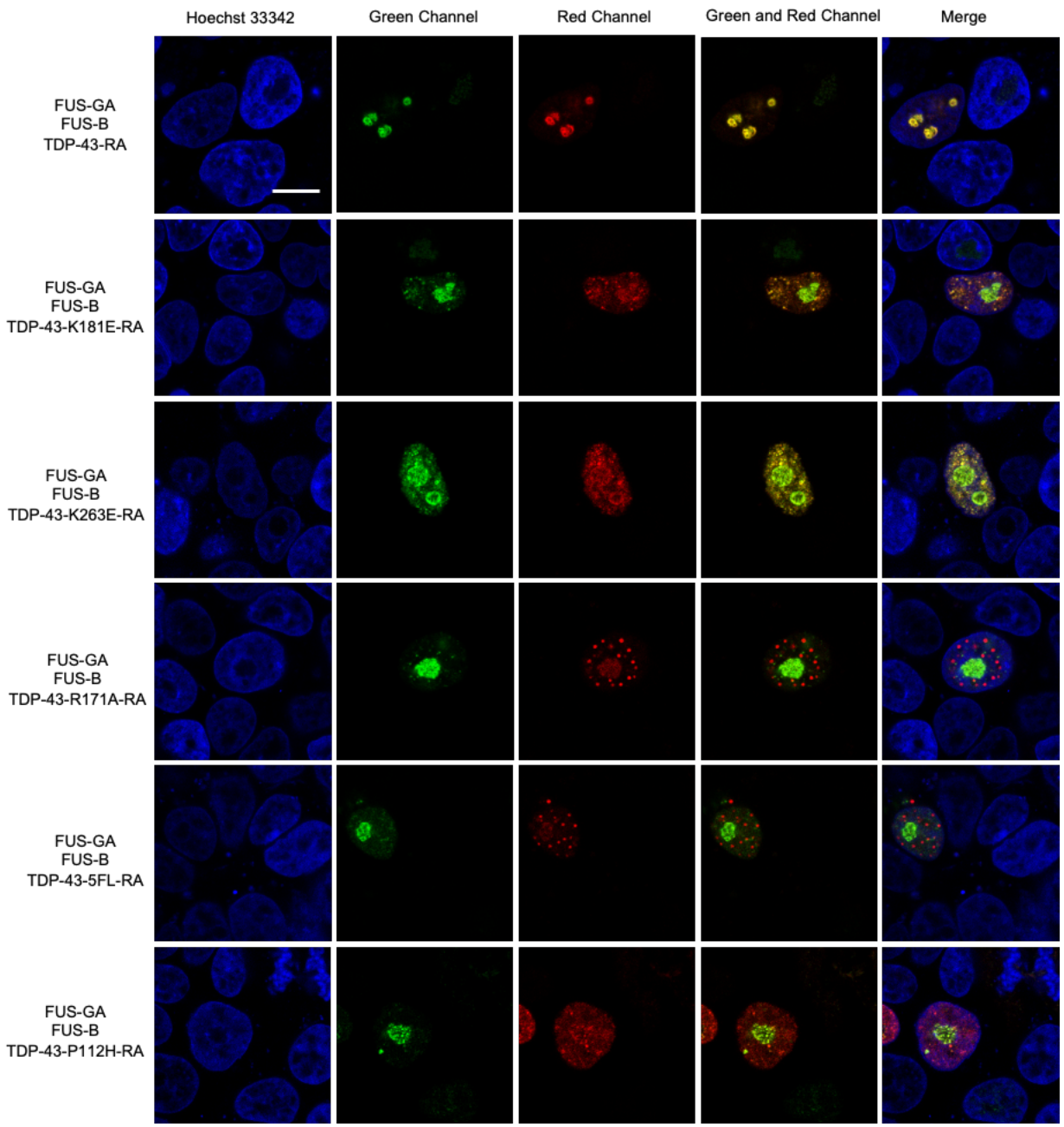


Figure S8. Cells transfected with FUS and TDP-43 variants. The interaction of FUS and TDP-43 decreased when TDP-43 RNA binding deficient mutants were induced. Cells were treated with 25 μM AdOx for 24 h to induce LLPS of FUS. Scale bar = 10 μm.

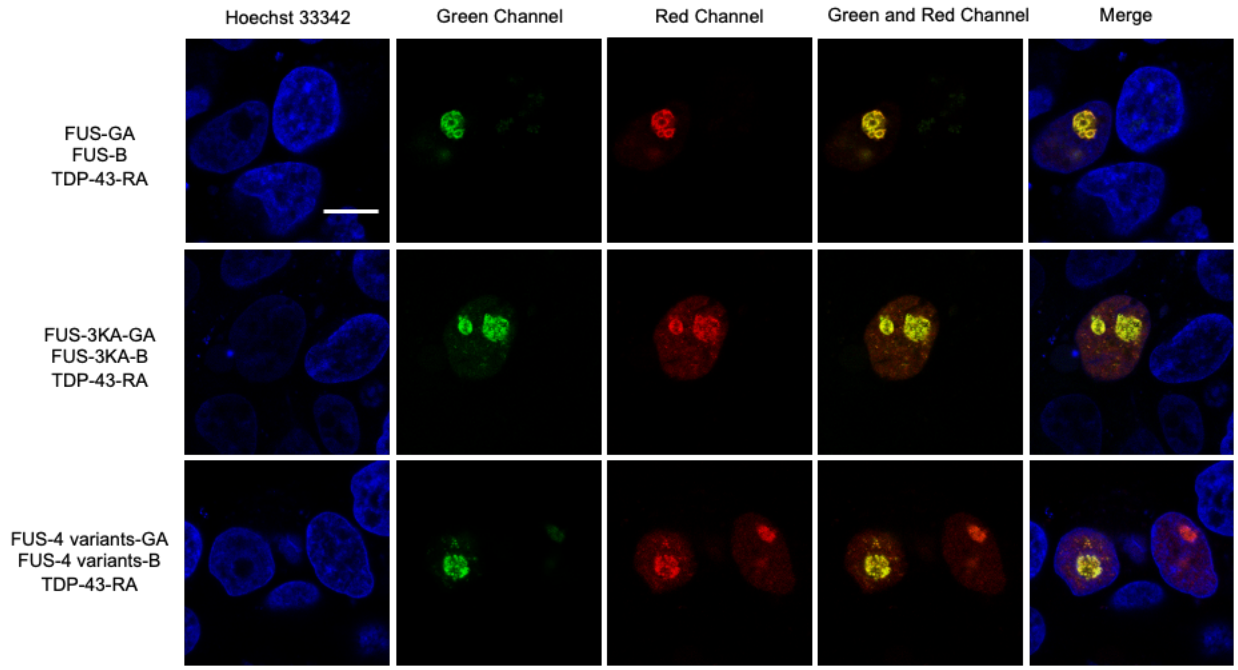


Figure S9. Cells transfected with TDP-43 and RNA-binding-deficient FUS variants. The interaction of FUS and TDP-43 decreased when FUS and RNA binding weakened. Cells were treated with 25 μM AdOx for 24 h to induce LLPS of FUS. Scale bar = 10 μm.

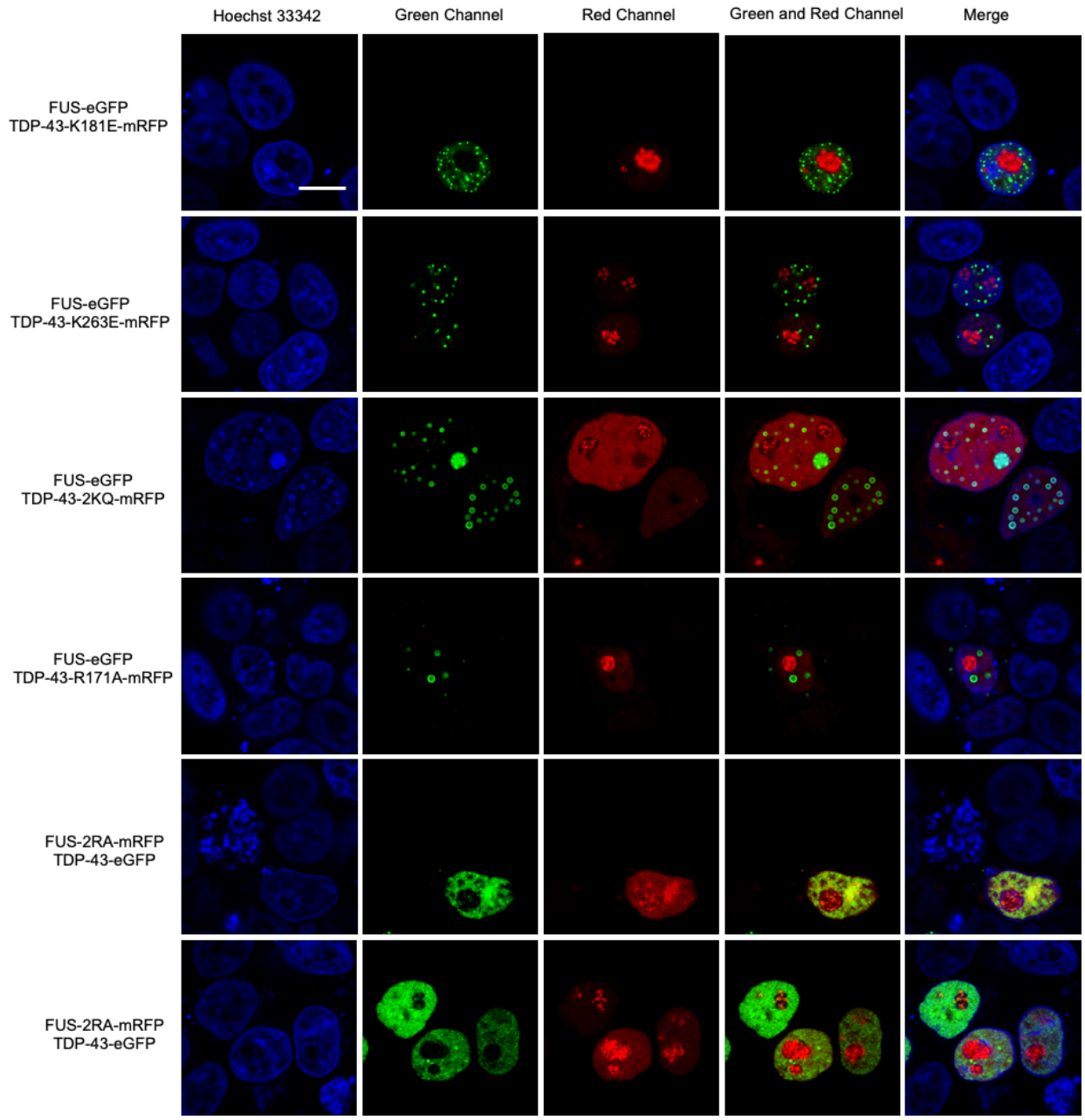


Figure S10. Co-localization of FUS and TDP-43 decrease due to RNA binding deficient mutation. The interaction of FUS and TDP-43 decrease. Cells were treated with 25 μM AdOx for 24 h to induce LLPS of FUS. Scale bar = 10 μm.

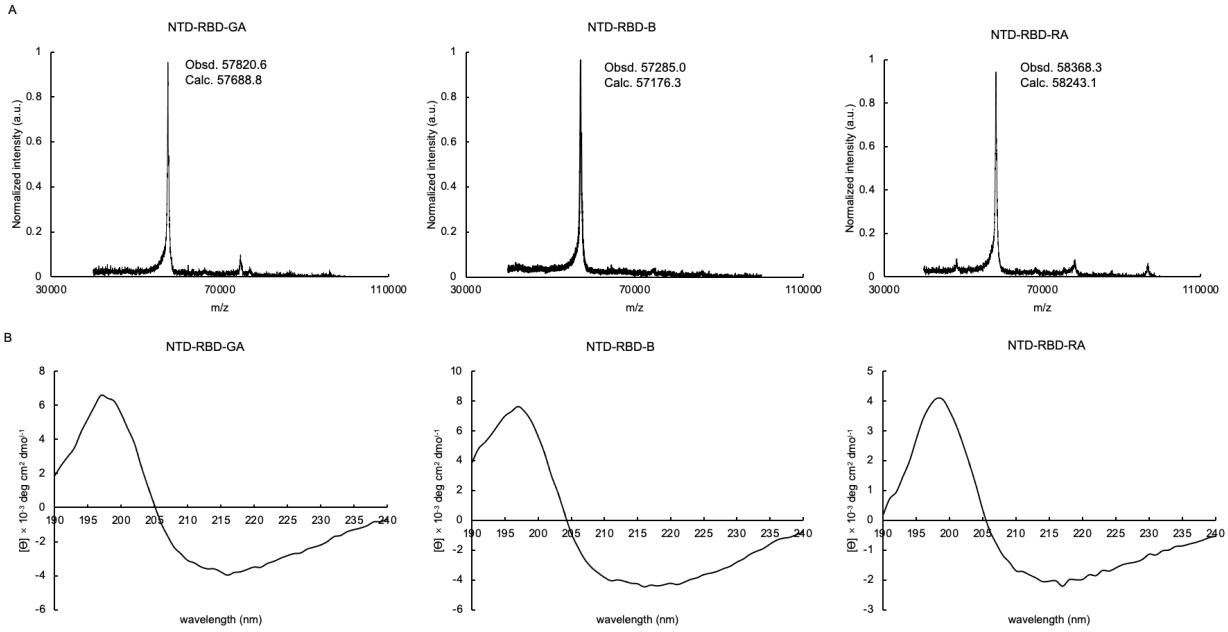


Figure S11. Purified proteins were characterized by MALDI-MS and Circular Dichroism. (A) MALDI mass spectra of NTD-RBD-GA (57820.6 Da), NTD-RBD-B (57285.0 Da), NTD-RBD-RA (58368.3 Da). (B) Circular Dichroism was performed to examine the second structure of proteins in A.