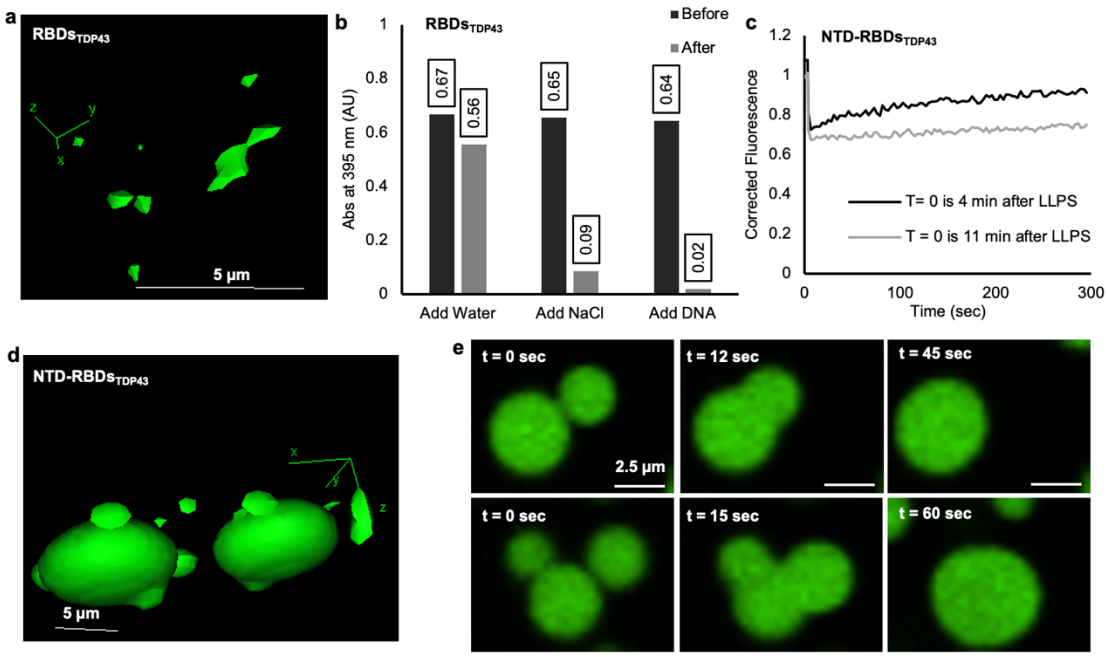
Supporting Information

N-terminal domain of TDP43 enhances liquid-liquid phase separation of globular proteins

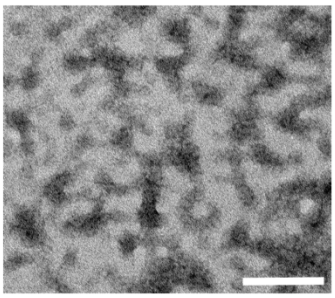
G. Campbell Carter[1,2], Chia-Heng Hsiung[1,2], Leman Simpson[1], Haopeng Yang[1], Xin Zhang*[1,2]

[1]Department of Chemistry, [2]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802, United States

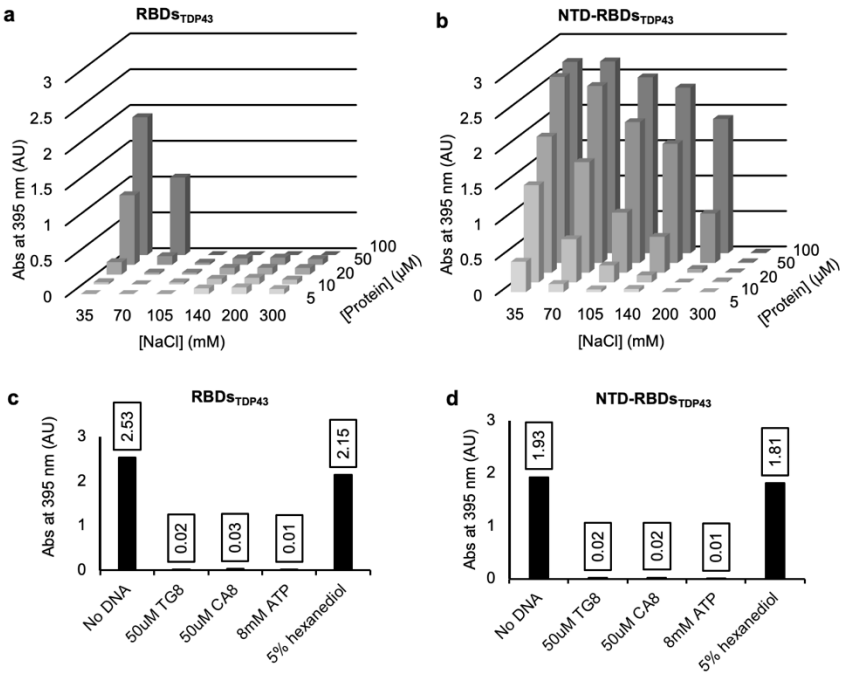
Corresponding author: Xin Zhang (xuz31@psu.edu)



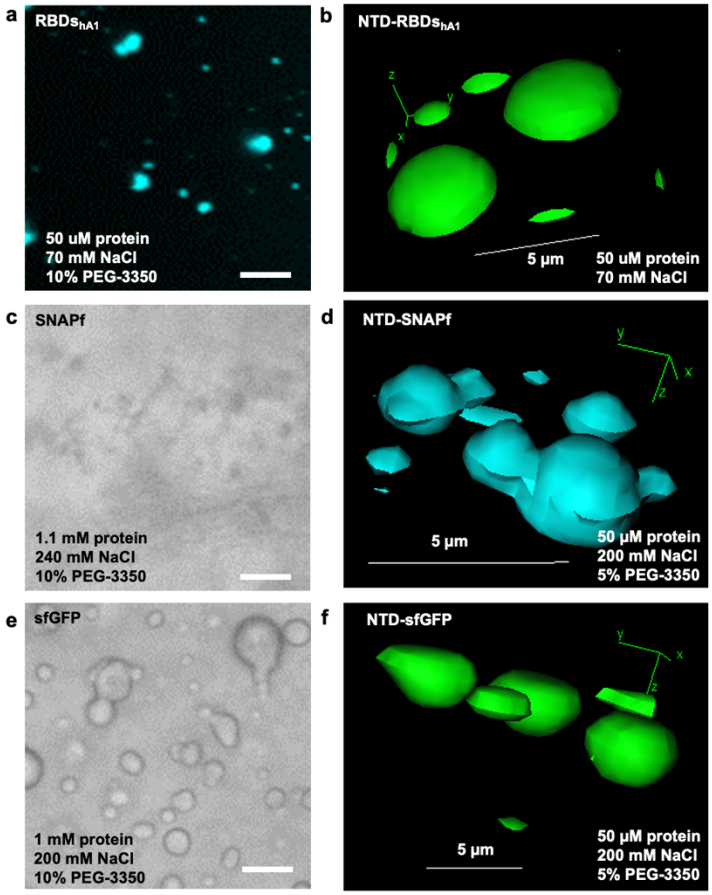
Supplementary Figure S1. (a), 3D reconstruction of 50 µM RBDs$_{TDP43}$ in 100 mM NaCl and 10% w/v PEG-3350. **(b)** Turbidity values of RBDs$_{TDP43}$ to assess LLPS reversibility. Samples initially contain 50 µM protein, 100 mM NaCl, and 10% PEG-3350. Additions are 20 µL water, 20 µL 5 M NaCl, or 10 µL 1 mM TG8, a DNA oligo with a sequence known to bind TDP43. **(c)** FRAP recovery of NTD-RBDs$_{TDP43}$ either 4 minutes or 11 minutes after initiating LLPS by dilution. Fluorescence values corrected to an unbleached droplet in the same frame. Samples contain 100 µM unlabeled protein, 5 µM protein labeled by fluorescein-maleimide, and 105 mM NaCl. **(d)** 3D reconstruction of 50 µM NTD-RBDs$_{TDP43}$ in 100 mM NaCl. 45 µM protein is unlabeled, 5 µM protein is labeled with fluorescein-maleimide. **(e)** Fusion of NTD-RBDs$_{TDP43}$ droplets in 100 µM protein, 100 mM NaCl. Frames selected from **Supplementary Movie S1**.



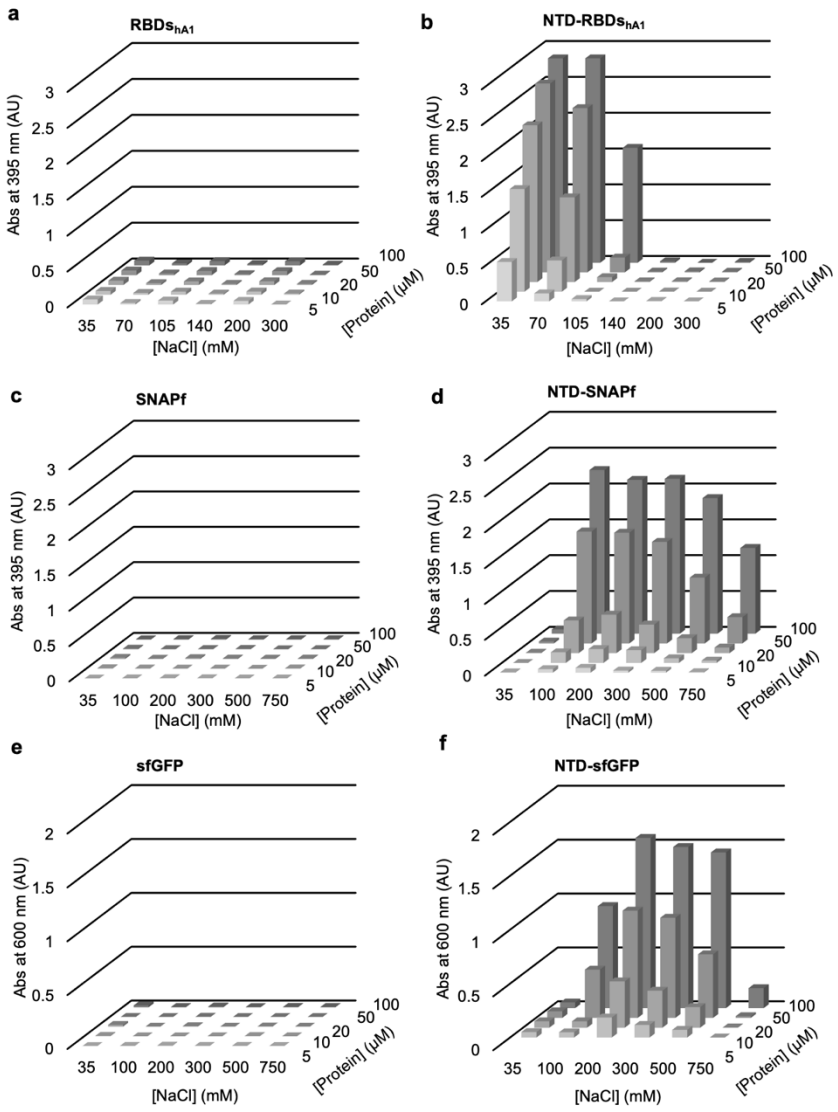
Supplementary Figure S2. TEM of 50 μM NTD in 35 mM NaCl. Scale bar is 200 nm.



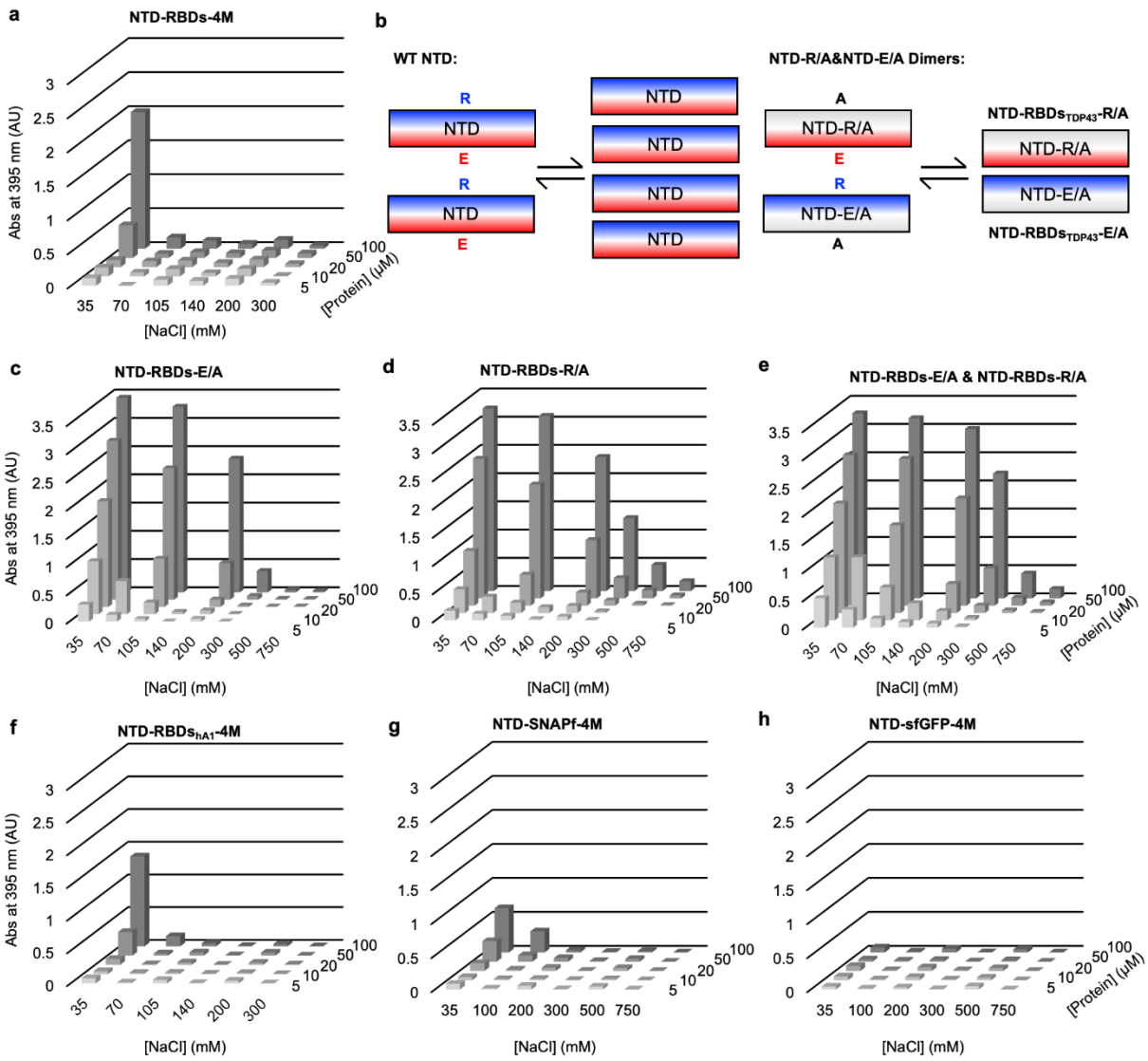
Supplementary Figure S3. a-b, Turbidity values for RBDs$_{TDP43}$ (**a**) and NTD-RBDs$_{TDP43}$ (**b**) used for Figure 1c. **c**, Turbidity of 50 μM RBDs$_{TDP43}$ in 35 mM NaCl, 10% w/v PEG-3350, and various additives known to affect LLPS. **d**, Turbidity of 50 μM NTD-RBDs$_{TDP43}$ in 100 mM NaCl and various additives known to affect LLPS.



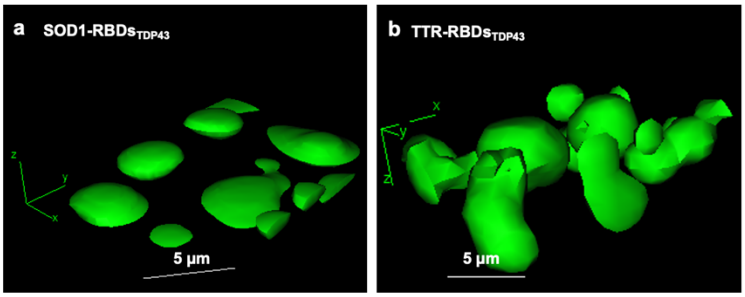
Supplementary Figure S4. a, Fluorescent confocal microscopy of 50 µM RBDs$_{hA1}$ in 100 mM NaCl and 10% w/v PEG-3350. Sample prepared with 45 µM unlabeled protein and 5 µM protein labeled with coumarin via maleimide conjugation. Scale bar is 5 µm. **b**, 3D reconstruction of 50 µM NTD-RBDs$_{hA1}$ in 70 mM NaCl. Sample prepared with 45 µM unlabeled protein and 5 µM protein labeled with fluorescein via maleimide conjugation. Scale bar is 5 µm. **c**, Brightfield image of SNAPf aggregates. Scale bar is 10 µm. **d**, 3D reconstruction of 50 µM NTD-SNAPf in 200 mM NaCl and 5% w/v PEG-3350. Sample labeled with 5 µM coumarin attached to SNAPf ligand benzyl-guanine. Scale bar is 5 µm. **e**, Brightfield image of sfGFP droplets. Scale bar is 10 µm. **f**, 3D reconstruction of 50 µM NTD-sfGFP in 200 mM NaCl and 5% w/v PEG-3350. Scale bar is 5 µm.



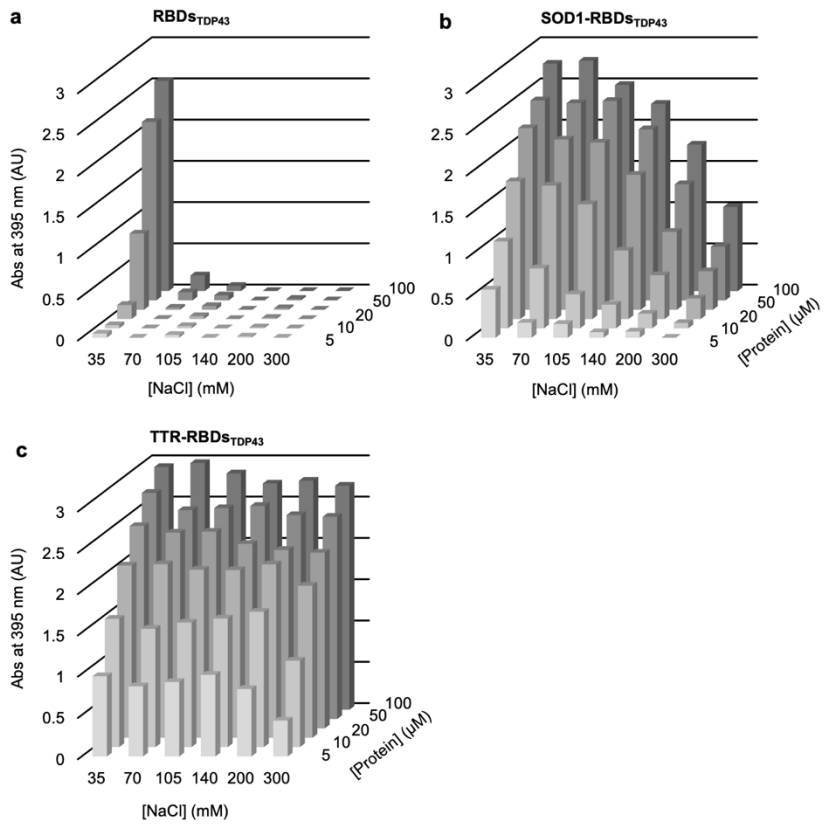
Supplementary Figure S5. **a-b,** Turbidity values for RBDs$_{hA1}$ (**a**) and NTD-RBDs$_{hA1}$ (**b**) used in Figure 2c-d. **c-d,** Turbidity values for SNAPf (**c**) and NTD-SNAPf (**d**) used in Figure 2f-g. Samples contain 5% w/v PEG-3350. **e-f,** Turbidity values for sfGFP (**e**) and NTD-sfGFP (**f**) used in Figure 2i-j. Samples contain 5% w/v PEG-3350.



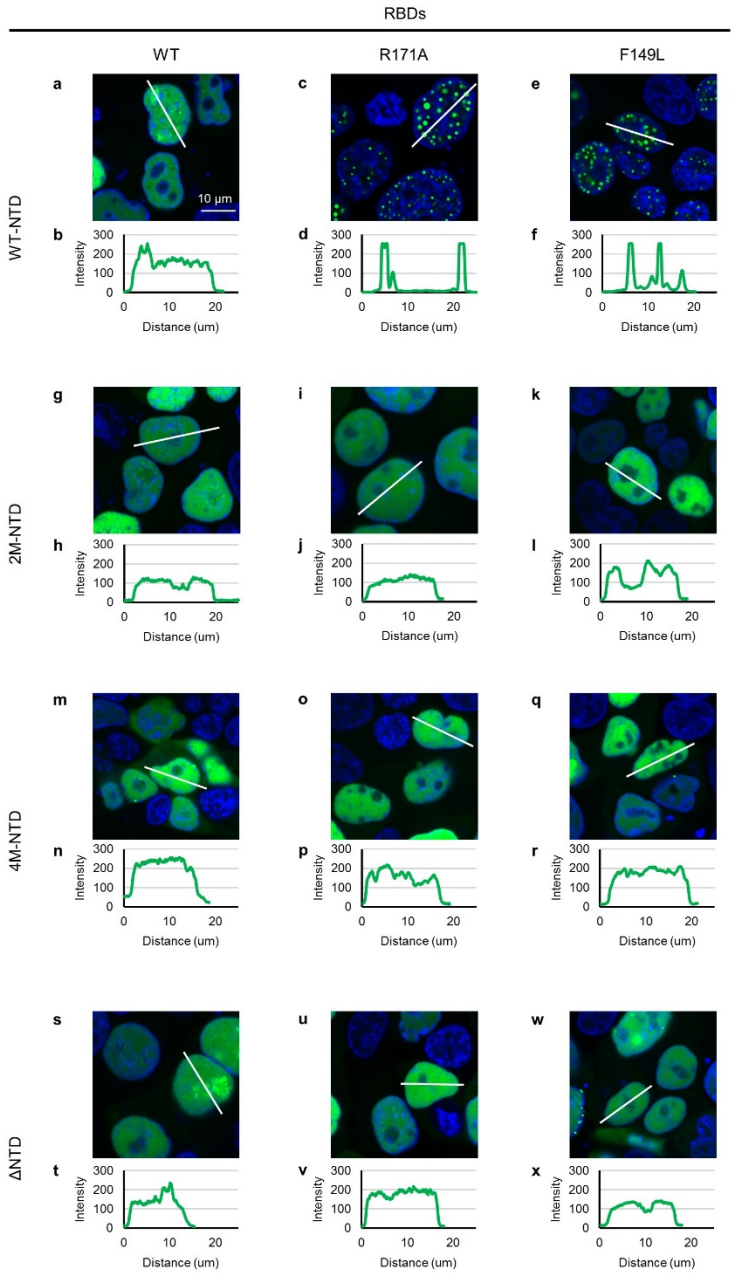
Supplementary Figure S6. **a**, Turbidity for NTD-RBDs$_{TDP43}$-4M (E17A/E31A/R52A/R55A) used in Figure 3b. **b,** Schematic for creating NTD-based dimers by mutating one or the other side of the oligomerization interface. **c-e**, Turbidity values for NTD-RBDs$_{TDP43}$ E/A (E17A/E31A) in **a**, NTD-RBDs$_{TDP43}$ R/A (R52A/R55A) in **b**, or NTD-RBDs$_{TDP43}$ E/A complexed with NTD-RBDs$_{TDP43}$ R/A in **c**. E/A + R/A sample consists of 50% E/A and 50% R/A. Samples contain 10% w/v PEG-3350. **f-h,** Turbidity values for oligomerization-deficient mutants of NTD-RBDs$_{hA1}$-4M (**f**), NTD-SNAPf-4M (**g**), and NTD-sfGFP-4M (**h**). NTD-SNAPf-4M and NTD-sfGFP-4M samples contain 5% w/v PEG-3350.



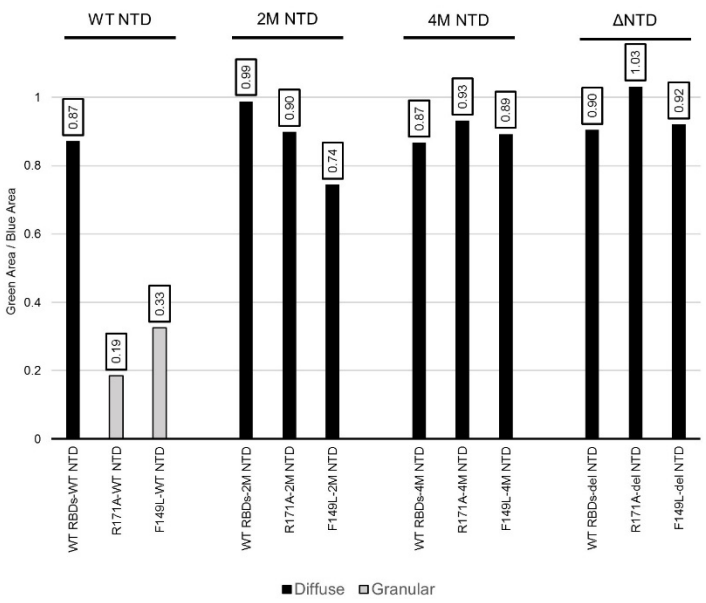
Supplementary Figure S7. a, 3D reconstruction of 50 µM SOD1-RBDs$_{TDP43}$ in 100 mM NaCl and 10% w/v PEG-3350. Protein is 45 µM unlabeled and 5 µM labeled by fluorescein conjugated via maleimide. Scale bar is 5 µm. **b,** 3D reconstruction of 50 µM TTR-RBDs$_{TDP43}$ in 100 mM NaCl and 10% w/v PEG-3350. Protein is 45 µM unlabeled and 5 µM labeled by fluorescein conjugated via maleimide. Scale bar is 5 µm.



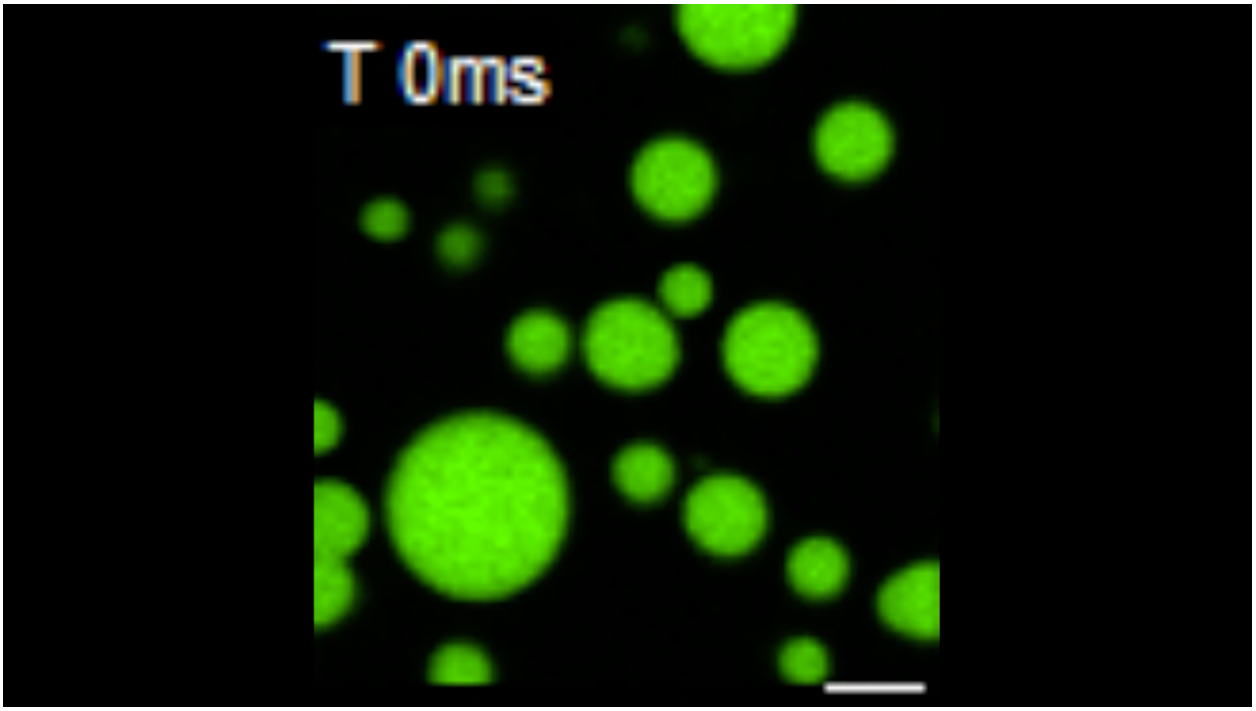
Supplementary Figure S8. a, Turbidity values of RBDs$_{TDP43}$ at varying salt and protein concentrations. All samples contain 10% w/v PEG-3350. **b**, Turbidity values of SOD1-RBDs$_{TDP43}$ at varying salt and protein concentrations. All samples contain 10% w/v PEG-3350. **c**, Turbidity values of TTR-RBDs$_{TDP43}$ at varying salt and protein concentrations. All samples contain 10% w/v PEG-3350.



Supplementary Figure S9. Intensity profile of GFP fluorescence corresponding to marked cross-sections (white line) of images from Figure 5. Images with cross-sections and corresponding intensity profiles for WT, R171A, and F149L RBDs constructs respectively, with either WT NTD (**a**+**b**, **c**+**d**, **e**+**f**), 2M-NTD (**g**+**h**, **i**+**j**, **k**+**l**), 4M-NTD (**m**+**n**, **o**+**p**, **q**+**r**), or ΔNTD (**s**+**t**, **u**+**v**, **w**+**x**).



Supplementary Figure S10. Quantification of distribution of EGFP-TDP43 compared to nuclear stain. From expanded images used in Figure 5, surface area of EGFP-TDP43 fluorescence (green) was compared to nuclear stain area (blue). Values depicted are green area/blue area (unitless). Black bars: diffuse signal. Grey bars: granular signals.



Supplementary Movie S1. Fusion of NTD-RBD$_{STDP43}$ droplets in 100 μM protein, 100 mM NaCl.