

**Cell, Volume 181**

## **Supplemental Information**

### **RNA-Induced Conformational Switching**

### **and Clustering of G3BP Drive**

### **Stress Granule Assembly by Condensation**

**Jordina Guillén-Boixet, Andrii Kopach, Alex S. Holehouse, Sina Wittmann, Marcus Jahnel, Raimund Schlüßler, Kyoohyun Kim, Irmela R.E.A. Trussina, Jie Wang, Daniel Mateju, Ina Poser, Shovamayee Maharana, Martine Ruer-Gruß, Doris Richter, Xiaojie Zhang, Young-Tae Chang, Jochen Guck, Alf Honigmann, Julia Mahamid, Anthony A. Hyman, Rohit V. Pappu, Simon Alberti, and Titus M. Franzmann**

## Supplementary tables

**Table S1:** Summary of the biophysical characterization of G3BP1(WT) and variants. Related to **Figures 2 and 4**.

Variant	Fluorescence Correlation Spectroscopy		Static Light Scattering		Dynamic Light Scattering		Analytical Size Exclusion Chromatography	Oligomerization
	Mean rH (nm)	+/- (nm)	MW (kDa)	+/- (kDa)	Mean rH (nm)	+/- (nm)	Retention Time (min)	
WT	5.90	0.29	158	1	6.45	1.25	10.2	Dimer
ΔNTF2	3.96	0.21	70	1	4.55	1.38	11.7	Monomer
ΔRG	6.72	0.40	162	1	7.41	1.44	10.1	Dimer
ΔE1	6.28	0.37	172	1	n.d.	-	10.4	Dimer
ΔE2	6.16	0.33	163	2	n.d.	-	10.9	Dimer
ΔE1ΔE2	n.d.	-	220	5	n.d.	-	n.d.	Dimer
S149A	6.16	0.32	166	1	n.d.	-	10.3	Dimer
S149A S232A	6.17	0.31	188	-	n.d.	-	10.4	Dimer
S149E	n.d.	-	166	1	n.d.	-	10.3	Dimer

**Table S2:** Summary of the plasmids generated for this paper. Related to **STAR Methods**.

Protein purification					
Plasmid / ID	Gene	N-term tag	C-term tag	Mutation	Comments
pOCC189-G3BP1(WT) L-515	hG3BP1	HIS- monoGFP	MBP	N/A	Codon optimised for insect cells
pOCC189- G3BP1( $\Delta$ NTF2) L-546	hG3BP1	HIS- monoGFP	MBP	Deletion 1-133	Codon optimised for insect cells
pOCC189-G3BP1( $\Delta$ RG) TH0999	hG3BP1	HIS- monoGFP	MBP	Deletion 425-466	Codon optimised for insect cells
pOCC189- G3BP1(S149A) L-548	hG3BP1	HIS- monoGFP	MBP	Substitution Ser to Ala at position 149	Codon optimised for insect cells
pOCC189- G3BP1(S149E) L-549	hG3BP1	HIS- monoGFP	MBP	Substitution Ser to Glu at position 149	Codon optimised for insect cells
pOCC189- G3BP1(S149A/S232A) L-686	hG3BP1	HIS- monoGFP	MBP	Substitution Ser to Ala at positions 149 and 232	Codon optimised for insect cells
pOCC189-G3BP1( $\Delta$ E1) L-663	hG3BP1	HIS- monoGFP	MBP	Deletion 144-158 (insertion of Ser in the deleted region)	Codon optimised for insect cells
pOCC189-G3BP1( $\Delta$ E2) L-664	hG3BP1	HIS- monoGFP	MBP	Deletion 182-226 (insertion of Ala- Ser in the deleted region)	Codon optimised for insect cells
pOCC189- G3BP1( $\Delta$ E1 $\Delta$ E2) L-667	hG3BP1	HIS- monoGFP	MBP	Deletion 144-158 (insertion of Ser in the deleted region), combined with deletion 182- 226 (insertion of Ala-Ser in the deleted region)	Codon optimised for insect cells
pOCC171-G3BP1(WT) L-587	hG3BP1	HIS-MBP- SNAP	N/A	N/A	Codon optimised for insect cells
pOCC254-Caprin1 L-772	hCaprin1	HIS-MBP- mCherry	N/A	N/A	Codon optimised for insect cells
pOCC189-G3BP2(WT) TH1434	hG3BP2a	HIS- monoGFP	MBP	N/A	Codon optimised for insect cells
Cell expression					
pcDNA3.1-mCherry- G3BP1(WT) O-3507	hG3BP1	mCherry	N/A	N/A	N/A
pcDNA3.1-mCherry- G3BP1( $\Delta$ E1 $\Delta$ E2) O-3510	hG3BP1	mCherry	N/A	Deletion 144-158 (insertion of Gly in the deleted region), combined with deletion 182- 226 (insertion of Thr-Ser in the deleted region)	N/A
pcDNA3.1-mCherry- G3BP1( $\Delta$ RGG) O-3511	hG3BP1	mCherry	N/A	Deletion 425-466	N/A