SUPPLEMENTARY DATA

Tandem RNA binding sites induce self-association of the stress granule marker protein TIA-1

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Supplementary Figure 1. Sequence specific multisite RNA and DNA induces liquid-liquid phase separation of TIA-1

Supplementary Figure 2. Nucleic acid containing multiple TIA-1 binding sites induced fibril formation of TIA-1.

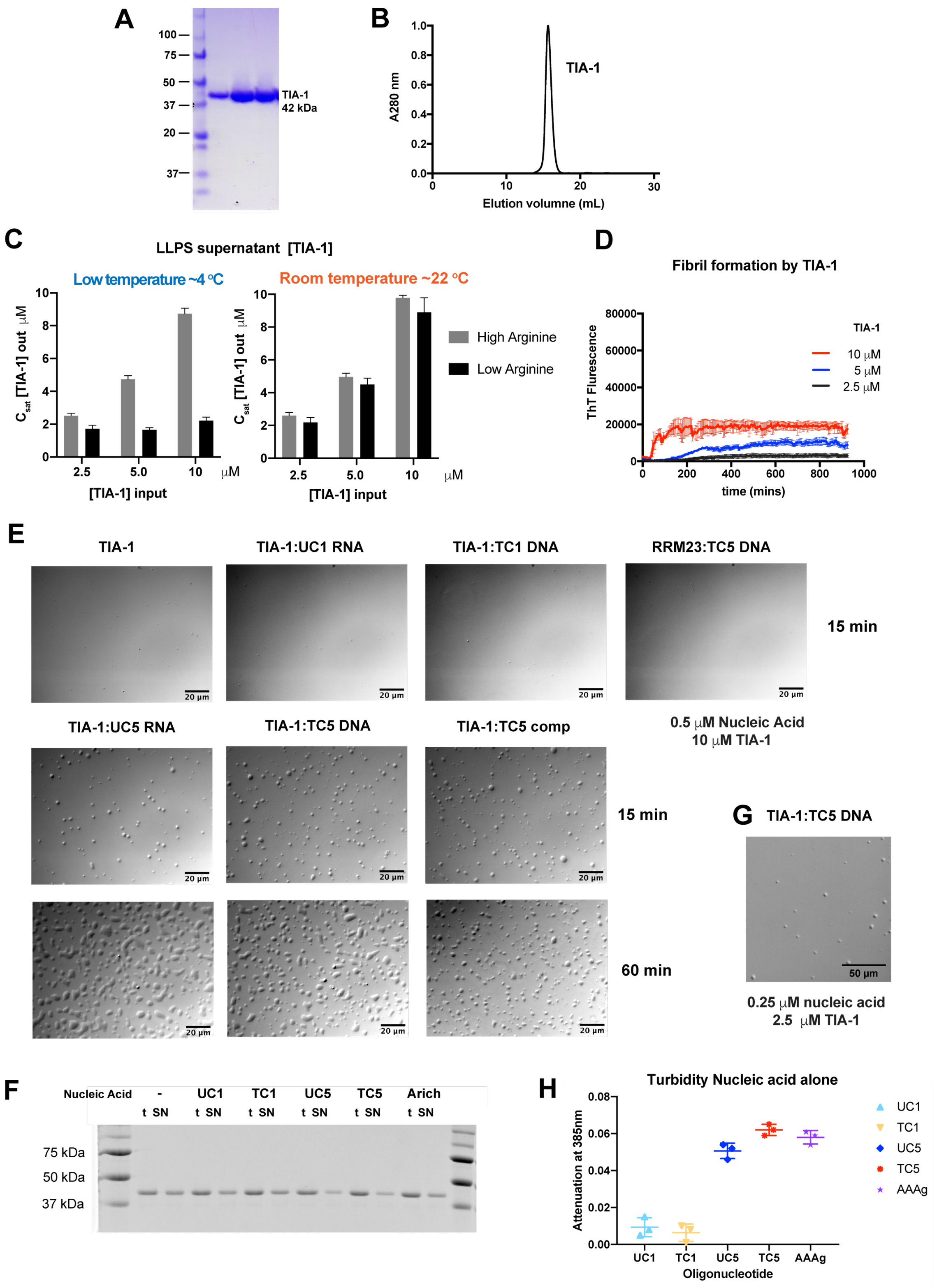
Supplementary Figure 3. SAXS analysis shows slight elongation of TIA-1 on binding ssRNA UC1 and ssDNA TC1 (Active voice caption)

Supplementary Figure 4. The ratio of TIA-1 protein:tandem binding sites finely tunes liquid-liquid phase separation and fibril formation.

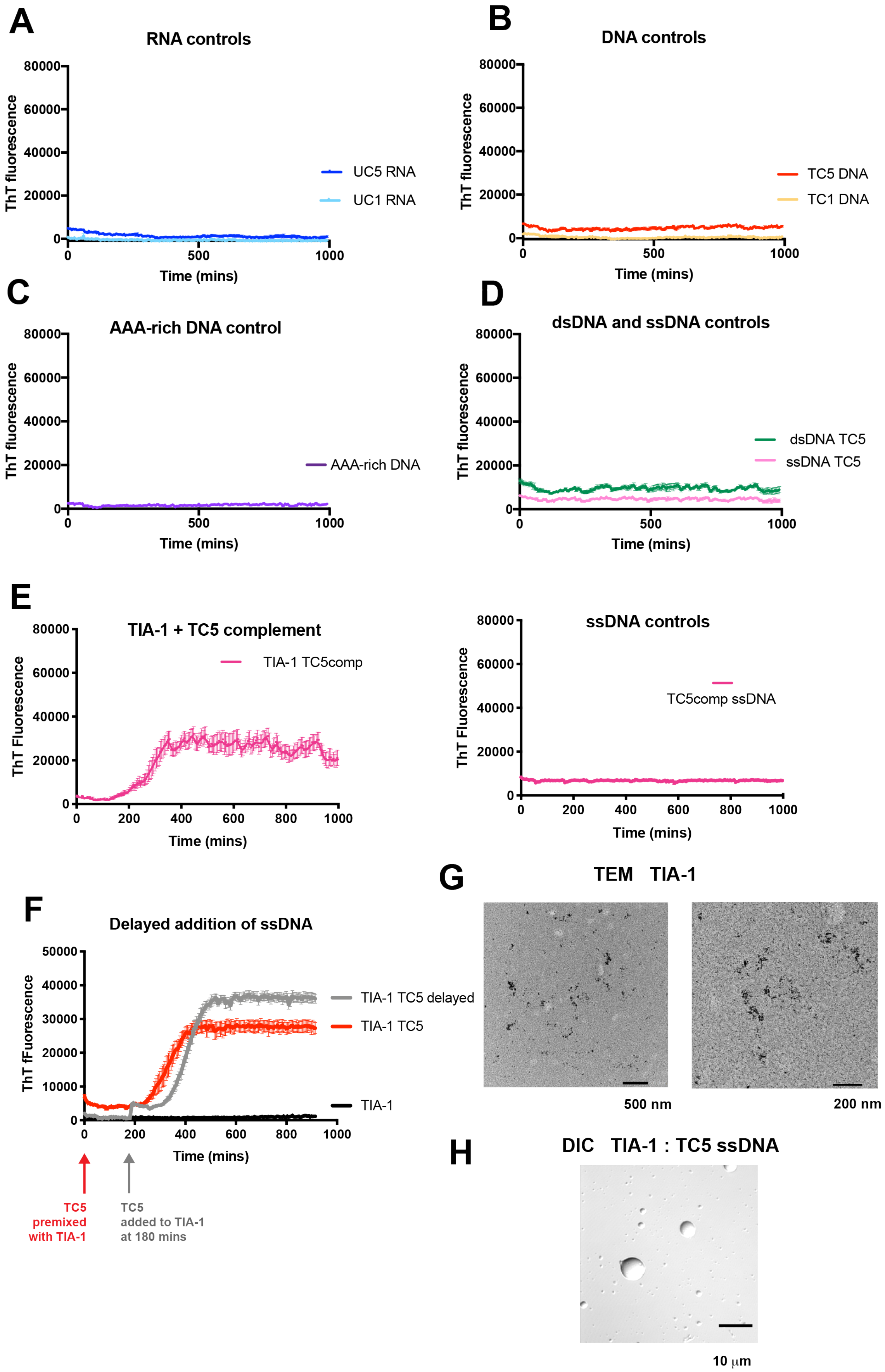
Supplementary Table 1. Nucleic acids used in this study

Supplementary Table 2. Absorption values in C_{sat} measurements from Figure 1

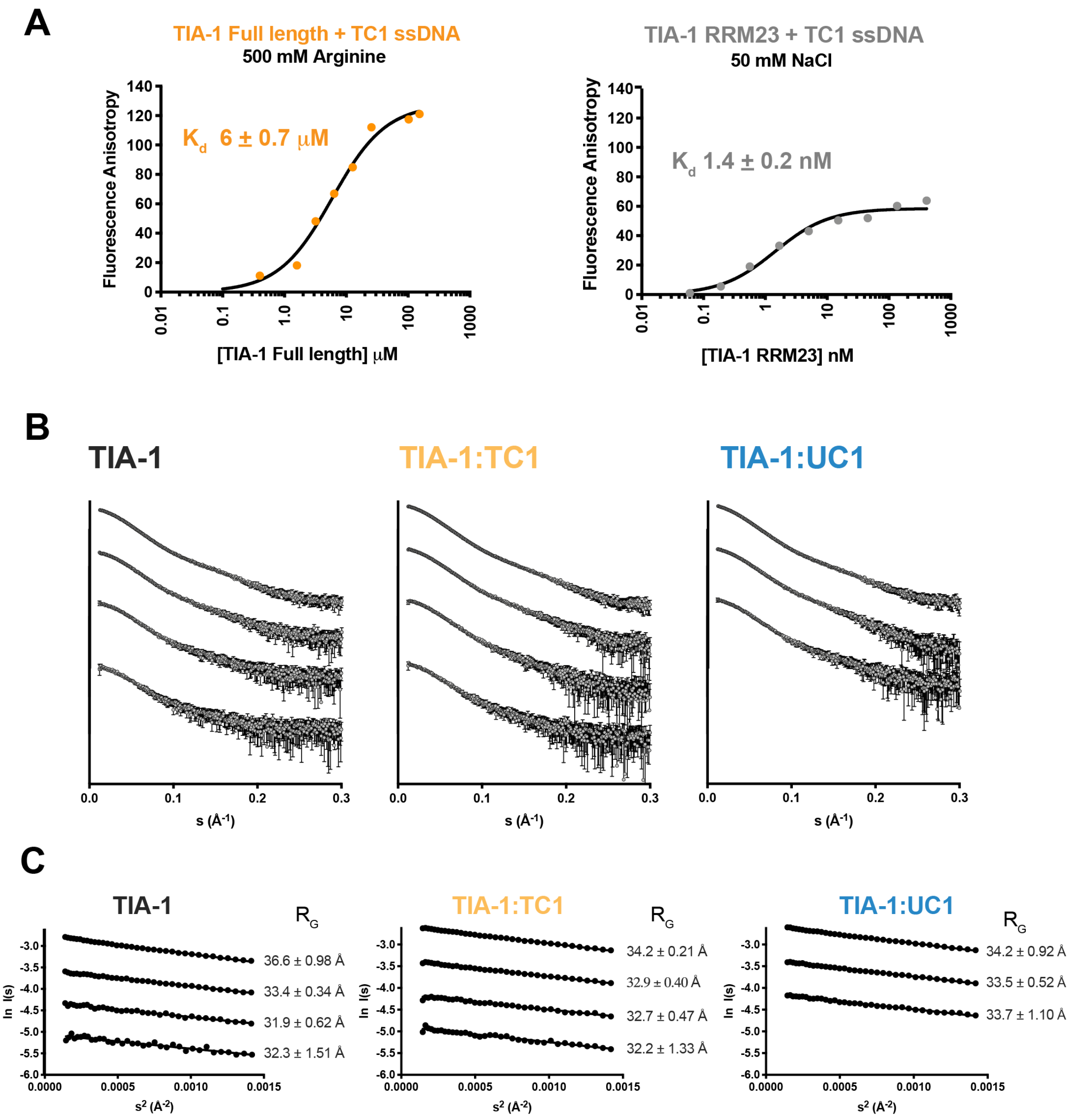
Supplementary Table 3. Absorption values in C_{sat} measurements from Figure 4



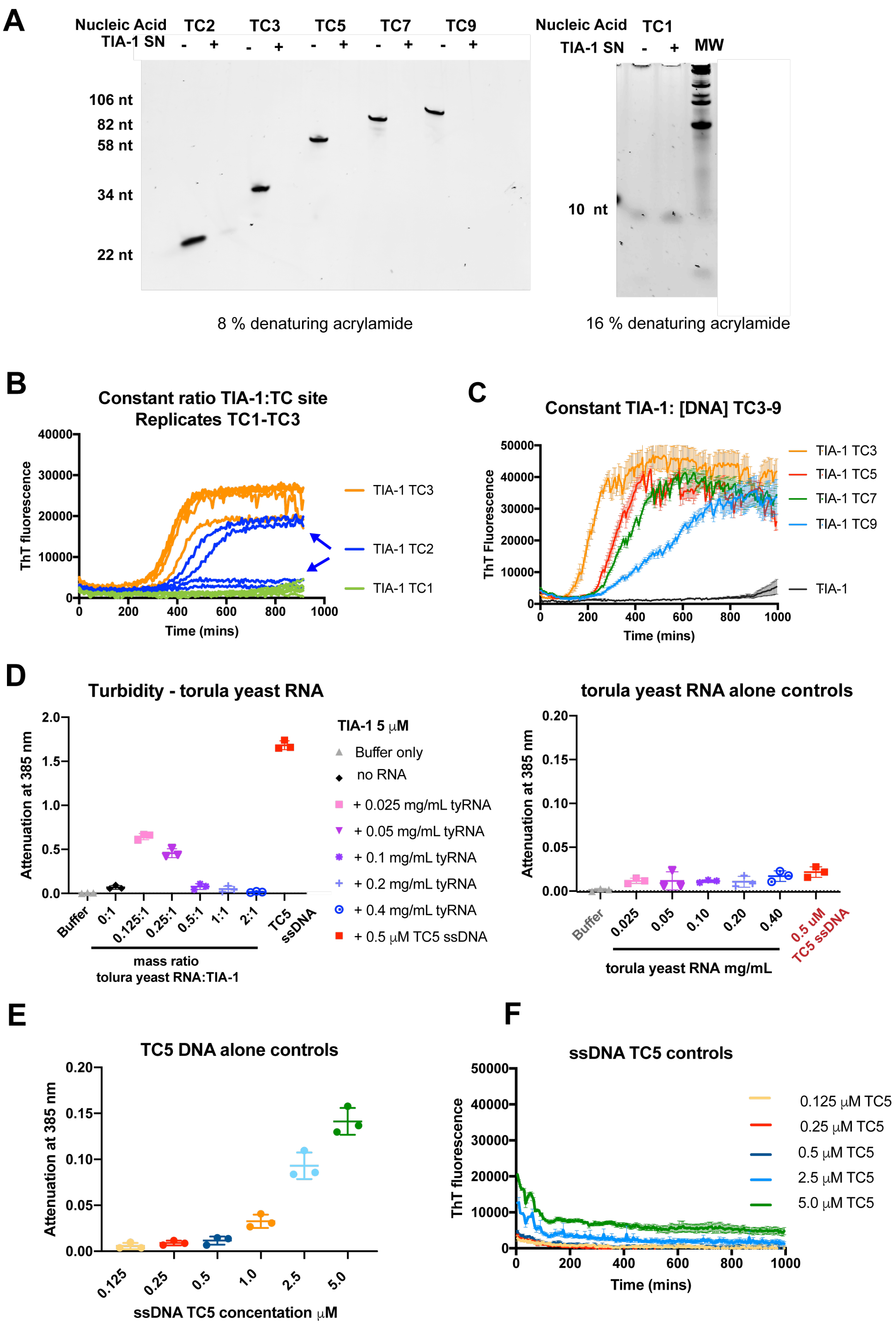
Supplementary Figure 1. Sequence specific multisite RNA and DNA induces liquid-liquid phase separation of TIA-1. A) SDS-PAGE of purified TIA-1, B) Size exclusion of TIA-1, C) The concentration of TIA-1 protein remaining dispersed in supernatant solution (C_{sat}) after phase separation at 4 °C or room temperature. D) Thioflavin T fluorescence monitoring aggregation of TIA-1 protein at 30 °C. E) DIC microscopy of TIA-1 (10 µM) alone or in the presence of nucleic acids (0.5 µM). F) SDS-PAGE analysis of TIA-1 protein dispersed in supernatant solution (t: total sample, SN: supernatant after spin) shows depletion of TIA-1 protein in supernatant. G) DIC microscopy of TIA-1 (2.5 μM) in the presence of TC5 (0.25 μM). H) Turbidity measurements of nucleic acid alone. Assays show 3 replicates and error bars representing S.D.


A
RNA controls
ThT fluorescence
80000
60000
40000
20000
0
UC5 RNA
UC1 RNA
0
500
1000
Time (mins)
B
DNA controls
ThT fluorescence
80000
60000
40000
20000
0
TC5 DNA
TC1 DNA
0
500
1000
Time (mins)
C
AAA-rich DNA control
ThT fluorescence
80000
60000
40000
20000
0
AAA-rich DNA
0
500
1000
Time (mins)
D
dsDNA and ssDNA controls
ThT fluorescence
80000
60000
40000
20000
0
dsDNA TC5
ssDNA TC5
0
500
1000
Time (mins)
E
TIA-1 + TC5 complement
ThT Fluorescence
80000
60000
40000
20000
0
TIA-1 TC5comp
0
200
400
600
800
1000
Time (mins)
ssDNA controls
ThT Fluorescence
80000
60000
40000
20000
0
TC5comp ssDNA
0
200
400
600
800
1000
Time (mins)
F
Delayed addition of ssDNA
ThT fFuorescence
50000
40000
30000
20000
10000
0
TIA-1 TC5 delayed
TIA-1 TC5
TIA-1
0
200
400
600
800
1000
Time (mins)
TC5 premixed with TIA-1
TC5 added to TIA-1 at 180 mins
G
TEM TIA-1
500 nm
200 nm
H
DIC TIA-1 : TC5 ssDNA
10 μm

Supplementary Figure 2. Nucleic acid containing multiple TIA-1 binding sites induced fibril formation of TIA-1. A-D) ThT fluorescence measurements of nucleic acid alone used in Figure 1 A-D. A) ssRNA UC1 (*light blue*) and UC5 (*blue*), B) ssDNA TC1 (*yellow*) and TC5 (*red*), C) ss AAA-rich DNA (*purple*), D) dsDNA dsTC5 (*green*) and ssDNA TC5 (*pink*). E) ThT fluorescence measurement of TIA-1 in presence of ssDNA TC5 complement (left) and ssDNA TC5 complement alone (right). F) Comparison of TIA-1 pre-mixed with TC5 (*red*) or adding TC5 after 200 minutes (*grey*). G) Representative TEM images TIA-1 alone showing no fibril formation by TIA-1 at 2.5 μM in 20 mM HEPES pH 7.2, 50 mM NaCl, 40 mM Arginine. H) DIC microscopy of TIA-1 TC5 ssDNA from ThT assay after ~ 10 hours showing the presence of phase separated liquid droplets.



Supplementary Figure 3. SAXS analysis shows slight conformational change of TIA-1 on binding ssRNA UC1 and ssDNA TC1. A) Fluorescence anisotropy of TIA-1 full length binding to Fl-TC1 in size exclusion buffer (20 mM Sodium phosphate, 60 mM KCl, 0.5 M Arginine-HCl, 1mM $MgCl_2$, 2 mM DTT, 0.5 mM EDTA, pH 7) compared to TIA-1 RRM23 binding to the same oligonucleotide in aggregation buffer (20 mM HEPES, 50 mM NaCl 1 mM DTT). B) Experimental scattering profiles of TIA-1 alone and in complex with ssDNA TC1 and ssRNA UC1 obtained by plotting the log of scattering intensity [log I(s)] as a function of forward scattering vector in the units of Å $^{-1}$ with grey spheres and black error bars representing the ± SD. C) Gunier Plots of TIA-1 and nucleic acid complexes and Rg over concentrations ranges 0.37, 0.73, 1.5, 2.9 mg/mL


A
Nucleic Acid
TC2
TC3
TC5
TC7
TC9
TIA-1 SN
- + - + - + - + - +
106 nt
82 nt
58 nt
34 nt
22 nt
8 % denaturing acrylamide
Nucleic Acid
TC1
TIA-1 SN
- +
MW
10 nt
16 % denaturing acrylamide
B
Constant ratio TIA-1:TC site
Replicates TC1-TC3
ThT fluorescence
Time (mins)
TIA-1 TC3
TIA-1 TC2
TIA-1 TC1
C
Constant TIA-1: [DNA] TC3-9
ThT Fluorescence
Time (mins)
TIA-1 TC3
TIA-1 TC5
TIA-1 TC7
TIA-1 TC9
TIA-1
D
Turbidity - torula yeast RNA
Attenuation at 385 nm
Buffer
0:1
0.125:1
0.25:1
0.5:1
1:1
2:1
TC5 ssDNA
mass ratio
tolura yeast RNA:TIA-1
TIA-1 5 μM
Buffer only
no RNA
+ 0.025 mg/mL tyRNA
+ 0.05 mg/mL tyRNA
+ 0.1 mg/mL tyRNA
+ 0.2 mg/mL tyRNA
+ 0.4 mg/mL tyRNA
+ 0.5 μM TC5 ssDNA
torula yeast RNA alone controls
Attenuation at 385 nm
Buffer
0.025
0.05
0.10
0.20
0.40
0.5 uM TC5 ssDNA
torula yeast RNA mg/mL
E
TC5 DNA alone controls
Attenuation at 385 nm
0.125
0.25
0.5
1.0
2.5
5.0
ssDNA TC5 concentation μM
F
ssDNA TC5 controls
ThT fluorescence
Time (mins)
0.125 μM TC5
0.25 μM TC5
0.5 μM TC5
2.5 μM TC5
5.0 μM TC5

Supplementary Figure 4. The ratio of TIA-1 protein: binding sites finely tunes liquid-liquid phase separation and fibril formation. A) Denaturing PAGE gels showing ssDNA in input (-) and in supernatant (-) from C_{sat} experiments. B&C) ThT assay of TIA-1 with ssDNA at constant TC binding site concentrations: B) TC1-3 showing individual replicates. C) ThT assay of TIA-1 in presence of TC3-TC9 at the same molar ratio. D) Turbidity assay of TIA-1 (5 μM) with increasing amounts of tolura yeast RNA or TC5 ssDNA (0.5 μM) *left* and controls for nucleic acid alone, *right*. E&F) Control of ssDNA alone: E) ThT assay of ssDNA alone for increasing TC5 concentration. F) Turbidity of ssDNA alone for increasing TC5 concentration.

Supplementary Table 1. Nucleic acids used in this study

Nucleic acid	**nt**	**sequence**	**# TC sites**
		DNA	
TC1	10	TTTTTACTCC	1
TC1.5	15	TTTTTTTTTTACTCC	1
TC2	22	TTTTTACTCCAATTTTTACTCC	2
TC3	34	TTTTTACTCCAATTTTTACTCCAATTTTTACTCC	3
TC5	58	TTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCC	5
TC7	82	TTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCC	7
TC9	106	TTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCC	9
Arich		AAAAAAAAAAGAGAAAAAAAAGAGAAAAAAAAAAGAGAAAAAAAAAAGAGAAAAAAAA	0
dsTC5	58 x 2	TTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCCAATTTTTACTCC AAAAATGAGGTTAAAAATGAGGTTAAAAATGAGGTTAAAAATGAGGTTAAAAATGAGG	5
TC5 comp		AAAAATGAGGTTAAAAATGAGGTTAAAAATGAGGTTAAAAATGAGGTTAAAAATGAGG	
		RNA	
UC1	10	UUUUUACUCC	1
UC5	58	UUUUUACUCCAAUUUUUACUCCAAUUUUUACUCCAAUUUUUACUCCAAUUUUUACUCC	5
P53 3' UTR site 1	14	UUUUUUACCUUGUA	1
P53 3' UTR site 2	27	UUUUUUUUUUUUUUACCCCUUUUUAUA	2
P53 3' UTR wt	326	Gggucccuucugcugcc**uuuuuuaccuug**uagcuag ggcucagccccucucugaguagugguuccuggcccaaguuggggaauagguugauaguu gucaggucucugcuggcccagcgaaauucuauccagccaguuguuggacccuggcaccua caaugaaaucucacccuaccccacacccuguaagauucuaucuugggcccucauagggguc cauauccuccagggccuacuuuccuuccauucugcaaagccugucugcauuuauccaccc cccacccugucucccuc**uuuuuuuuuuuuuuaccccuuuuu**auauaucaa	
P53 3'UTR mut	326	Gggucccuucugcugcc**uauguaaccuag**uagcuag ggcucagccccucucugaguagugguuccuggcccaaguuggggaauagguugauaguu gucaggucucugcuggcccagcgaaauucuauccagccaguuguuggacccuggcaccua caaugaaaucucacccuaccccacacccuguaagauucuaucuugggcccucauagggguc cauauccuccagggccuacuuuccuuccauucugcaaagccugucugcauuuauccaccc cccacccugucucccuc**uauguauguauguaaccccugauu**auauaucaa	

		DNA for preparing 3' UTR RNA templates	
P53 3'UTR wt DNA template		GCTTAATACGACTCACTATAGggtcccttctgctgcc**ttttttaccttg**tagctag ggctcagcccctctctgagtagtggttcctggcccaagttggggaataggttgatagtt gtcaggtctctgctggcccagcgaaattctatccagccagttgttggaccctggcaccta caatgaaatctcaccctaccccacaccctgtaagattctatcttgggccctcatagggtc catatcctccagggcctactttccttccattctgcaaagcctgtctgcatttatccaccc cccaccctgtctccctc	
P53 3' UTR mut DNA template		GCTTAATACGACTCACTATAGggtcccttctgctgcc**tatgtaacctag**tagctag ggctcagcccctctctgagtagtggttcctggcccaagttggggaataggttgatagtt gtcaggtctctgctggcccagcgaaattctatccagccagttgttggaccctggcaccta caatgaaatctcaccctaccccacaccctgtaagattctatcttgggccctcatagggtc catatcctccagggcctactttccttccattctgcaaagcctgtctgcatttatccaccc cccaccctgtctccctc**tatgtatgtatgtaacccctgatt**atatatcaa	
P53 Fwd primer		GCTTAATACGACTCACTATAG	
P53 wt Rev primer		ttgatatataaaaaggggtaaaaaaaaaaaaaagagggagacagggtggg	
P53 mut Rev primer		ttgatatataatcaggggttac	

Supplementary Table 2. Absorption values in C_{sat} measurements from Figure 1

Sample	**A_{280}**	**$A_{260/280}$**	**Corrected A_{280}**
TIA-1	0.69 0.66 0.80	0.64	
TIA-1 + UC1	0.68 0.62 0.69	0.68	
TIA-1 + TC1	0.64 0.64 0.63	0.67	
TIA-1 + UC5	0.17 0.14 0.17	0.70	
TIA-1 + TC5	0.18 0.21 0.19	0.68	
TIA-1 + AAg*	0.73 0.80 0.72	1.08	0.54 0.51 0.51
TIA-1 + dsTC5*	0.77 0.71. 0.77	1.03	0.59 0.61 0.58
TIA-1 + TC5compl	0.29 0.29 0.33	0.76	

* Corrected for contribution from nucleic acid remaining in the supernatant

Supplementary Table 3. Absorption values in C_{sat} measurements from Figure 4

Sample	**A_{280}**	**$A_{260/280}$**	**Corrected A_{280}**
TIA-1	0.70 0.76. 0.73	0.62	
TIA-1 + TC1*	0.87 0.88. 0.88	0.72	0.71 0.72 0.72
TIA-1 + TC2	0.38 0.41 0.46	0.67	
TIA-1 + TC3	0.36 0.38 0.38	0.68	
TIA-1 + TC5	0.44 0.45 0.49	0.63	
TIA-1 + TC7	0.37 0.37. 0.41	0.68	
TIA-1 + TC9	0.39 0.43. 0.39	0.65	

* Corrected for contribution from nucleic acid remaining in the supernatant