Supplementary information

A synergistic network of interactions promotes the formation of *in vitro* processing bodies and protects mRNA against decapping.

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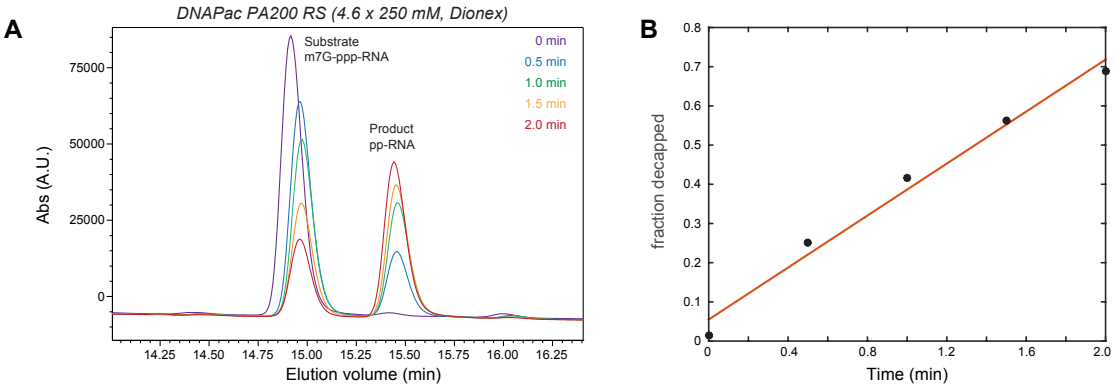
Supplementary Table 1. Protein constructs used in this study

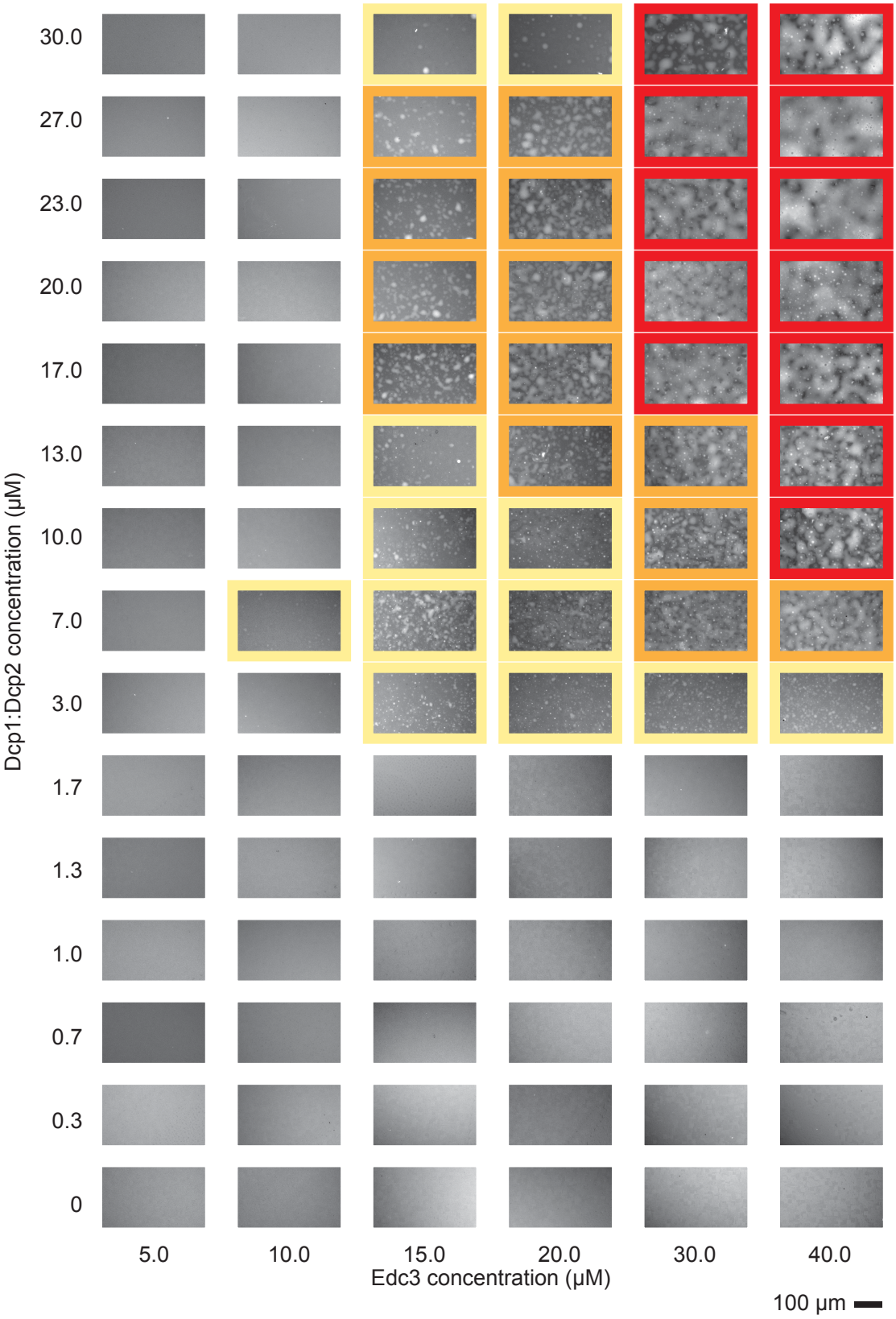
Protein / complex	Residues	Solubility / purification tag	Internal reference
Edc3	1-454	N-His_6-TEV	#180
Edc3	1-71 (LSm)	N-His_6-TEV	#1380
Edc3	72-194 (IDR)	N-His_6-GST-TEV	#1293
Edc3	195-454 (YjeF_N)	N-His_6-TEV	#196
Edc3	1-194 (ΔYjeF_N)	N-His_6-TEV	#244
Edc3	1-454 Δ72-194 (ΔIDR)	N-His_6-TEV	#1291
Edc3	72-454 (ΔLSm)	N-His_6-TEV	#1301
Edc3	72-194 Δ90-110 (IDRΔ1)	N-His_6-GST-TEV	#1514
Edc3	72-194 Δ158-171 (IDRΔ2)	N-His_6-GST-TEV	#1512
Edc3	72-194 Δ90-110 + Δ158-171 (IDRΔ12)	N-His_6-GST-TEV	#1516
Dcp1:Dcp2 ΔMid	1-127 (Dcp1), 1-741 Δ290-554 (Dcp2)	N-His_6-TEV on Dcp1	#264
Pdc1	1-105 (Pdc1) – GST – 880-1076 (Pdc1)	N-His_6-TEV	#928
Ste13	250-421	N-His_6-MBP-TEV	#205

Supplementary Table 2. RNA used in this study.

RNA	Properties	Sequence	Figure	Internal reference
15mer	GA-only	GGAGAAGAGAAGGAG	2A, 2B, 4C, 4D	#4 primer
21mer	GA-only	GGAAGGAGAGGAAGGAAAGGA	3B, S1	#40 plasmid
30U5mer	GA-only, but single U at position 5	GGAGUGAGAGGAAGGAAGGGAAGAAAGAAG	2A, 2B, 4A, 4D, 4E, 4F, S6	#8 primer
30U10mer	GA-only, but single U at position 10	GGAGGAGAGUGAAGGAAGGGAAGAAAGAAG	3A	#9 primer
30U15mer	GA-only, but single U at position 15	GGAGGAGAGGAAGGUAAGGGAAGAAAGAAG	2C	#10 primer
63mer	GA-only	GGAAGGAGAGGAAGGAAGGGAAGAAAGAAGAGGAGAGGAAGGAAGGGAAGAAAGAAGGGAAGA	2A, 2B, 4D	#5 primer
100U15mer	GA-only, but single U at position 15	GGGAAGGAAGGGAAUAAAGAAGGGAAGAGGAAGGAGAGGAGGGAAGAAAGAAGAGGAGAGGAAGGAAGGGAAGAAAGAAGAGGGAAGAGGAAGGAGAGGA	2A, 2B, 3C	#6 primer



Supplementary Figure 1. Representative mRNA decapping experiment.
20 µM capped RNA substrate were supplemented with 1 µM Dcp1:Dcp2. At different time-points during the decapping reaction a part of the sample was removed for analysis as described in the Methods section.
(A) HPLC trace (DNAPac PA200 RS column (4.6 x 250 mM, Dionex) of an mRNA decapping experiment. The substrate (capped RNA) and the product (uncapped RNA) are indicated.
(B) Time course of a decapping reaction, the best fit to the data is indicated. Note that the reaction is no longer linear after 2 minutes, due to the product inhibition of the decapping enzyme.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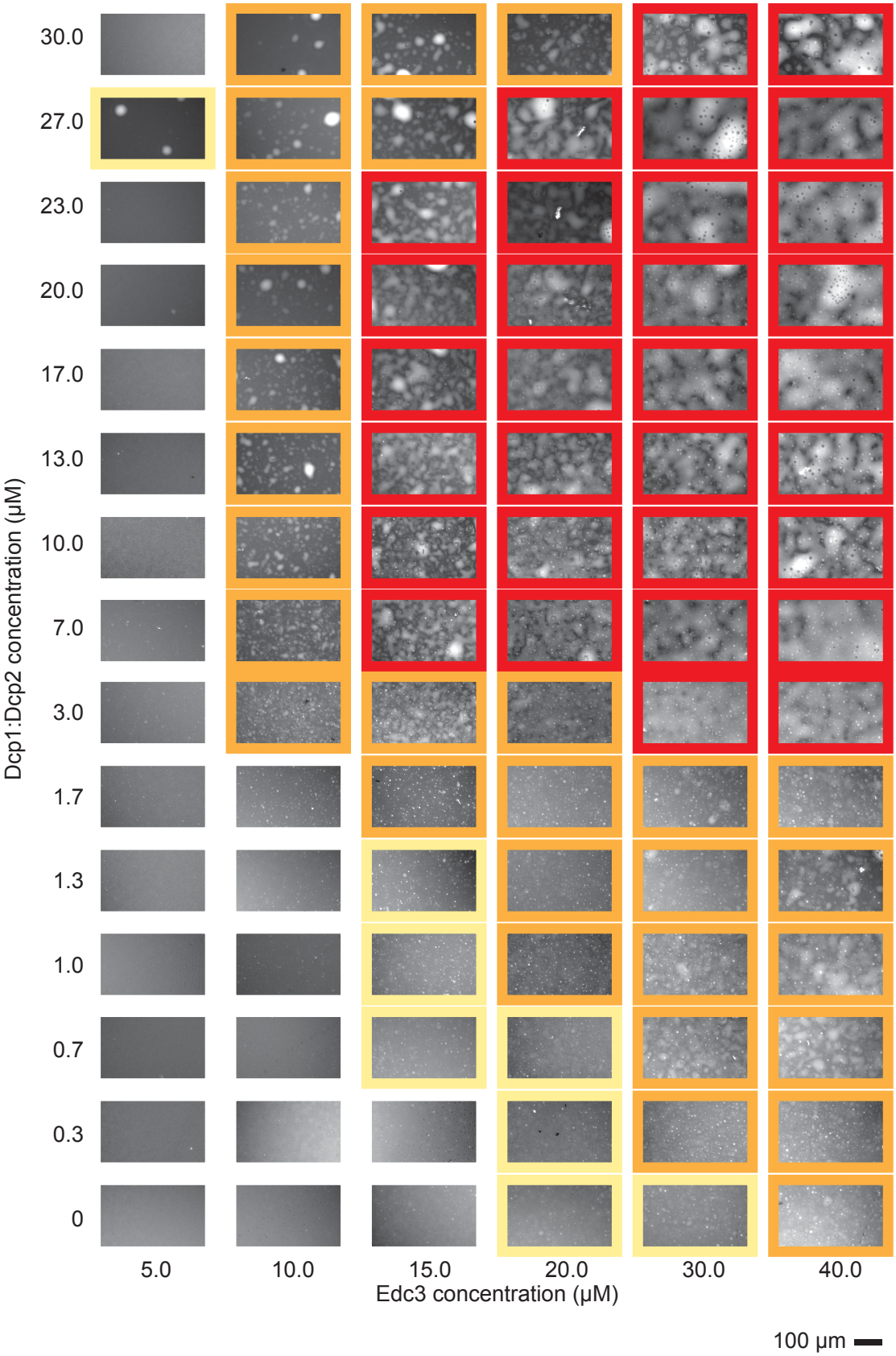
27.0
23.0
20.0
17.0
13.0
10.0
7.0
3.0
1.7
1.3
1.0
0.7
0.3
0
Dcp1:Dcp2 concentration (μM)
5.0
10.0
15.0
20.0
30.0
40.0
Edc3 concentration (μM)
100 μm

Supplementary Figure 2a. Interpretation of LLPS diagram based on fluorescence microscopy images.

Phase separation diagram of Dcp1:Dcp2 and Edc3. The degree of phase separation was visually classified as strong (red), intermediate (orange) or weak (yellow). See also Figure 1B in the main text.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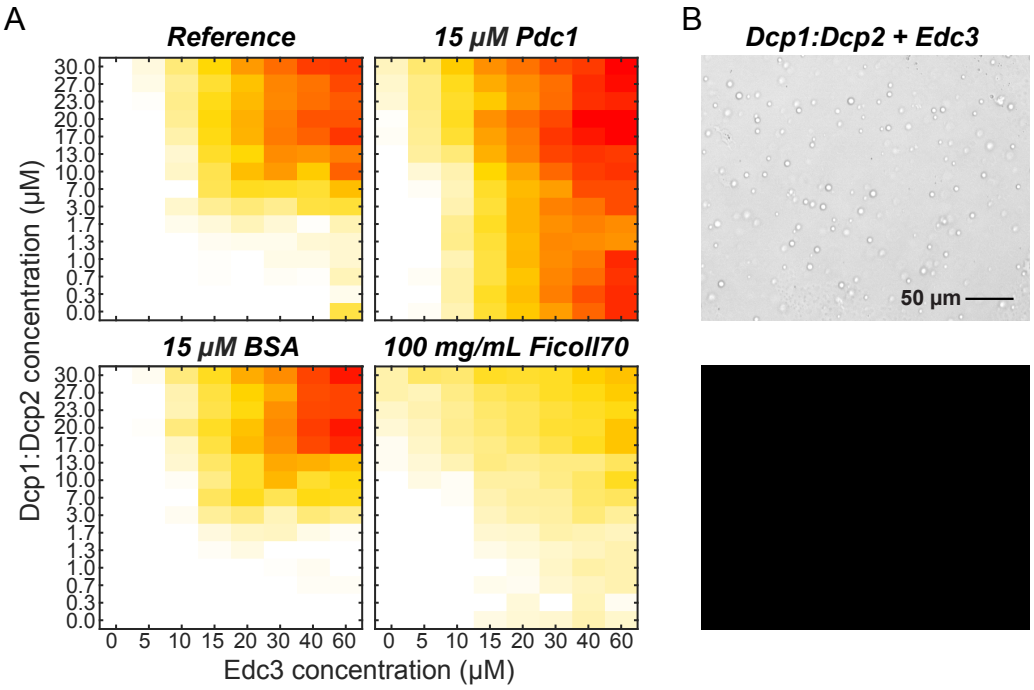
27.0
23.0
20.0
17.0
13.0
10.0
7.0
3.0
1.7
1.3
1.0
0.7
0.3
0
Dcp1:Dcp2 concentration (μM)
5.0
10.0
15.0
20.0
30.0
40.0
Edc3 concentration (μM)
100 μm

Supplementary Figure 2b. Interpretation of LLPS diagram based on fluorescence microscopy images.

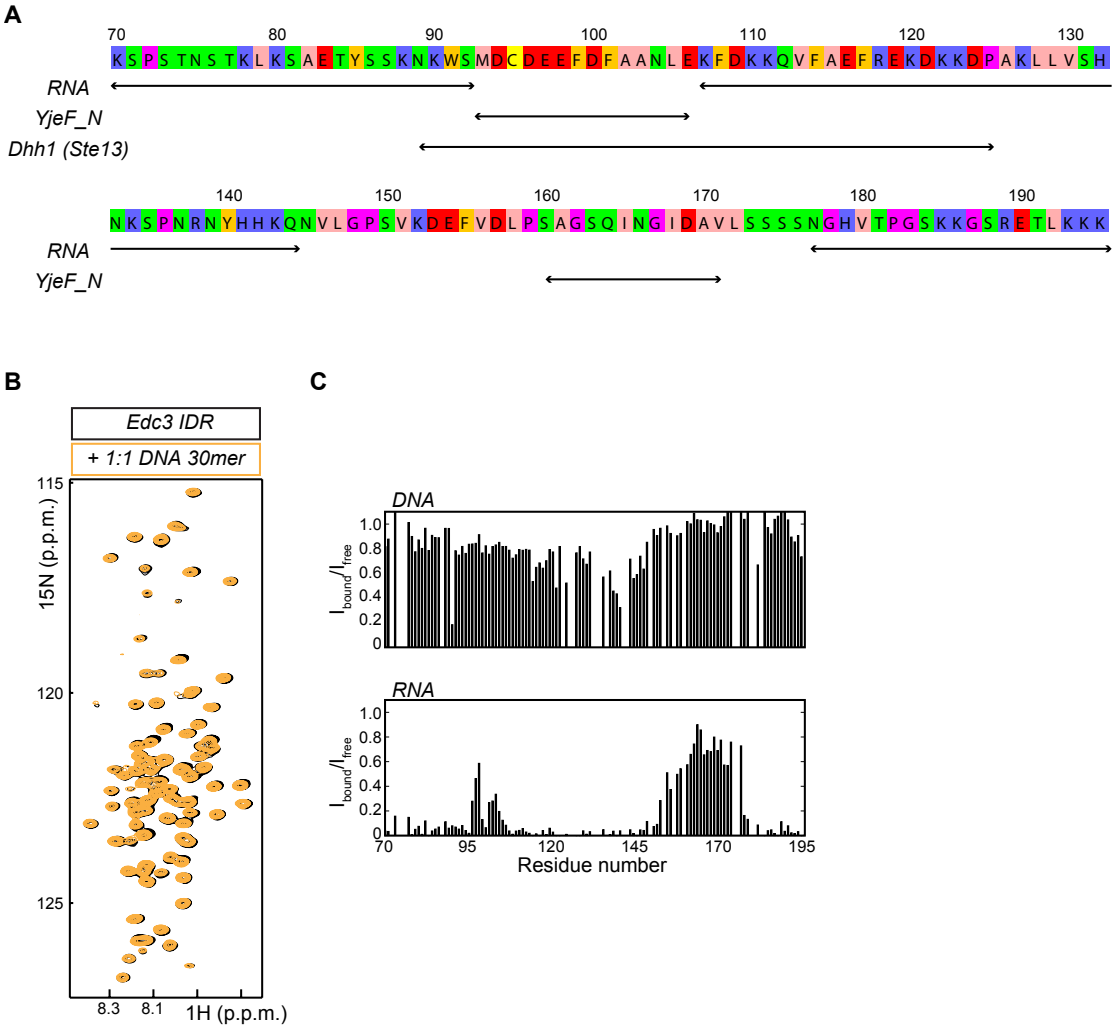
Phase separation diagram of Dcp1:Dcp2 and Edc3 in the presence of 15 μM Pdc1. The degree of phase separation was visually classified as strong (red), intermediate (orange) or weak (yellow). See also Figure 1B in the main text.



Supplementary Figure 3. Phase diagrams and RNA localization.

(A) Pdc1 has a strong effect on the phase separation diagram of Dcp1:Dcp2 and Edc3 (top row). This is a specific effect as BSA (bottom left) has no effect on the LLPS process. A very high amount of the crowding agent Ficoll70 only has a minor effect on the Dcp1:Dcp2:Edc3 phase separation process.

(B) Wide field (top) and fluorescence microscopy (bottom) images of Dcp1:Dcp2:Edc3 in the absence of fluorescently labelled RNA. The fluorescent signal observed in the images in Figure 2C is due to the fluorescently labelled RNA. The experimental conditions and the brightness and contrast of the images are the same as in Figure 2C. The size/ number of LLPS droplets in the images in this Figure is less than in Figure 2C due to the absence of RNA.

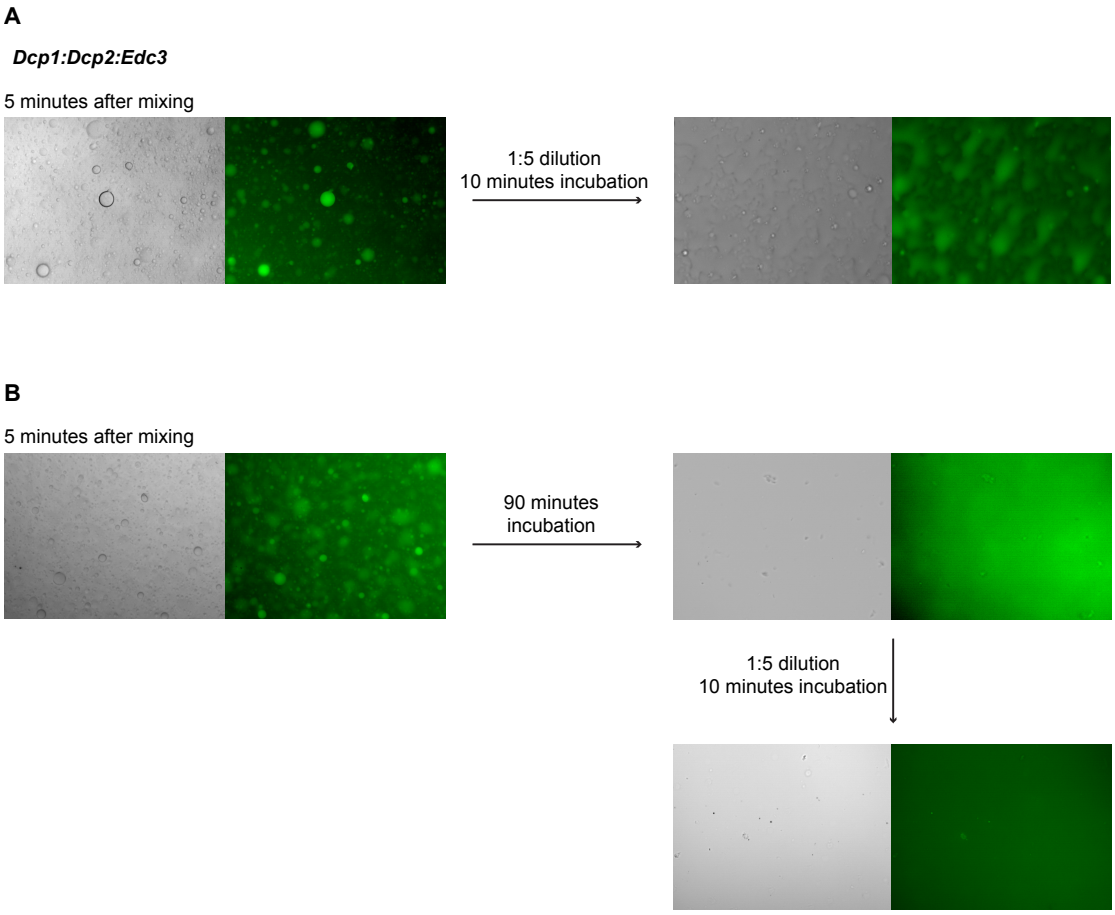


Supplementary Figure 4. DNA does not interact with the Edc3 IDR.

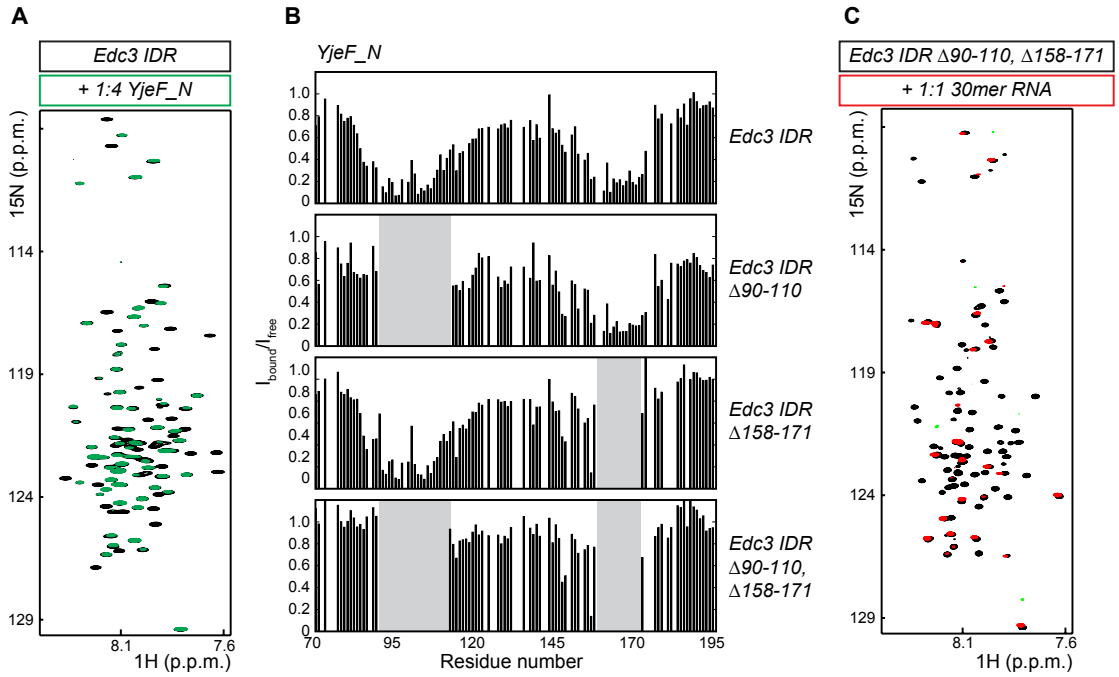
(A) Sequence of the Edc3 IDR. The residues are colored according to charge and hydrophobicity. The regions that interact with RNA (Figure 4), the Edc3 YjeF_N domain (Figure 5 and supplementary Figure 5) and the helicase Dhh1 (Ste13; Supplementary Figure 6) are indicated.

(B) ^{1}H-^{15}N NMR spectra of the Edc3 IDR region in the absence (black) and presence (orange) of DNA. The lack of clear CSPs reveals that the Edc3 IDR does not interact with single-stranded DNA. See also Figure 4C in the main text.

(C) The ratio of the intensities of the NMR signals in the presence and absence of DNA is close to unity for all residues. As a comparison, the intensity ratio of the NMR resonances in the absence and presence of a 30mer RNA is shown (See also Figure 4E in the main text).



Supplementary Figure 5. Matured LLPS phase is resistant against dilution.
Dcp1:Dcp2 and fluorescently labeled Edc3 were mixed.
(A) Left: Wide field and fluorescent images after 5-minute incubation. Right: images of the same condition, after 1:5 dilution and 10 minute incubation. The LLPS droplets largely disappear. A small degree of matured droplets are visible in the fluorescent images. These have formed during the incubation times.
(B) Left: Wide field and fluorescent images after a 5-minute incubation. Right: images of the same condition, after 90-minute incubation. The LLPS droplets have merged as a homogeneous layer on the bottom of the well. Bottom: a 1:5 dilution and 10 minute incubation does not result in the disappearance of the homogenous gel-like structure. This shows that the mature phase is resistant against dilution and that the intermolecular interactions in the early (A) and late (B) *in vitro* processing bodies are significantly different (see also Figure 5 in the main text).

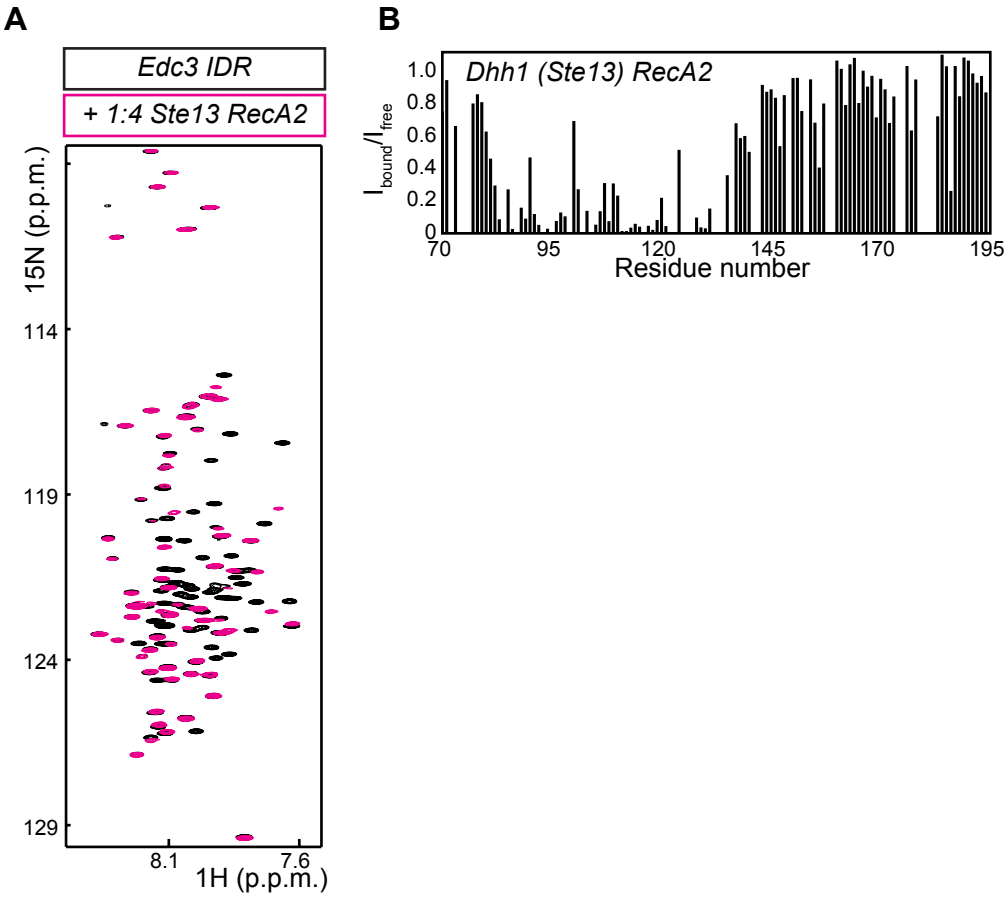


Supplementary Figure 6. Two regions in the Edc3 IDR interact independently with the Edc3 YjeF_N domain.

(A) ^{1}H-^{15}N NMR spectra of the Edc3 IDR region in the absence (black) and presence (green) of the Edc3 YjeF_N domain.

(B) Ratio of the intensities of the ^{1}H-^{15}N resonances of the Edc3 IDR in the presence and absence of the Edc3 YjeF_N domain. Two regions in the Edc3 IDR are prominently influenced by the interaction with the C-terminal domain of Edc3: a region around residues 100 and a region around residue 165. Removal of one these regions does not influence the interaction with the other region (middle two panels, the deleted region is indicated in gray). Removal of both regions (lower panel) abolishes the interaction between the Edc3 IDR and the Edc3 YjeF_N domain.

(C) The Edc3 IDR that is no longer able to interact with the YjeF_N domain still interacts with RNA.



Supplementary Figure 7. The second RecA domain of the helicase Dhh1 (Ste13) interacts with the Edc3 IDR.

(A) ^{1}H-^{15}N NMR spectra of the Edc3 IDR region in the absence (black) and presence (pink) of the second RecA domain of Dhh1 (Ste13).

(B) Ratio of the intensities of the ^{1}H-^{15}N resonances of the Edc3 IDR in the presence and absence of the Dhh1. The region in the Edc3 IDR that is affected is around the FDF motif. The binding sites of Dhh1 and the Edc3 YjeF_N on the Edc3 IDR thus partially overlap.