Supplemental Information

Spatial organization of single mRNPs at different stages along the gene expression pathway

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Supplementary Figures

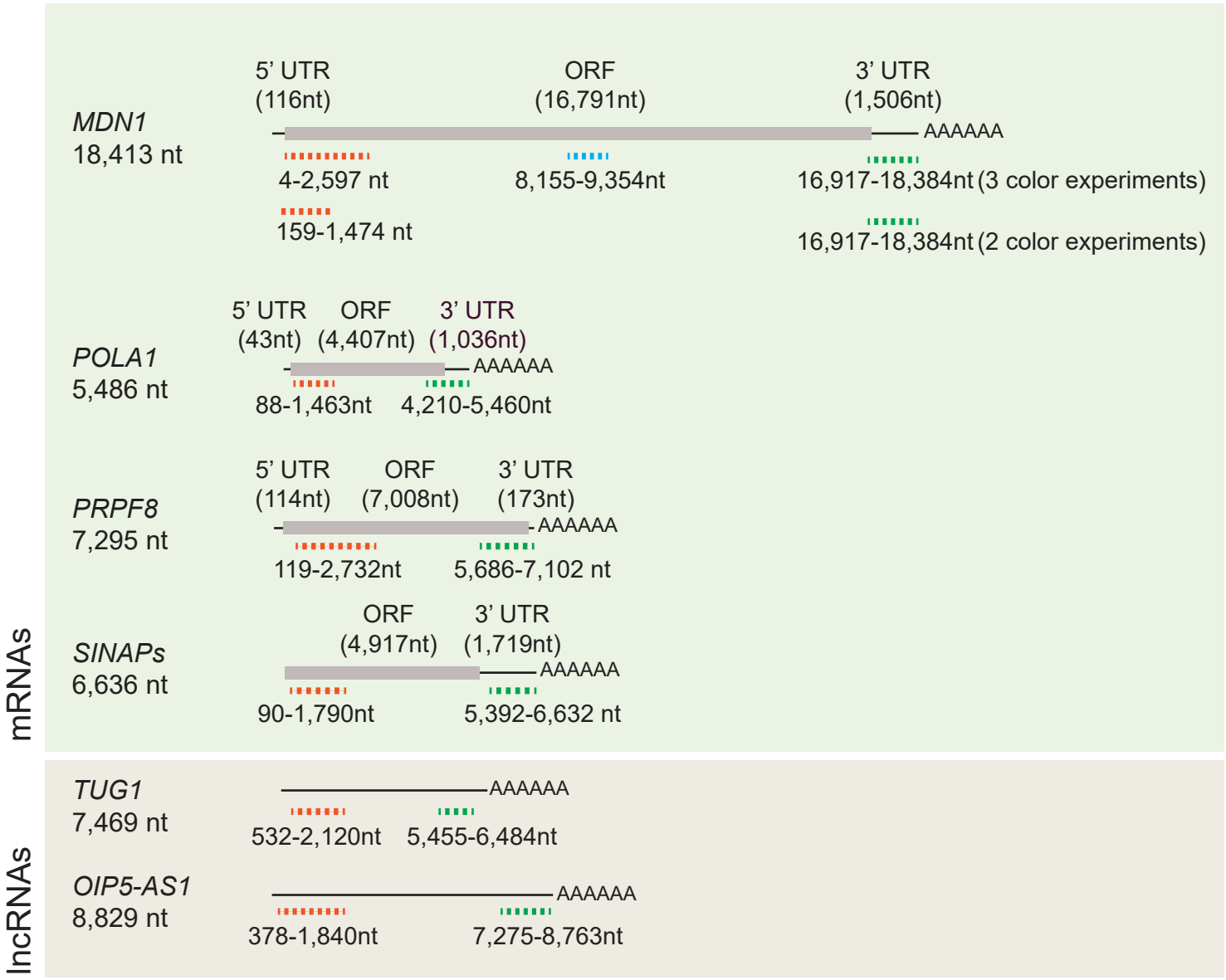
mRNAs
lncRNAs
MDN1
18,413 nt
5' UTR
(116nt)
ORF
(16,791nt)
3' UTR
(1,506nt)
AAAAAA
4-2,597 nt
8,155-9,354nt
16,917-18,384nt (3 color experiments)
159-1,474 nt
16,917-18,384nt (2 color experiments)
POLA1
5,486 nt
5' UTR
(43nt)
ORF
(4,407nt)
3' UTR
(1,036nt)
AAAAAA
88-1,463nt
4,210-5,460nt
PRPF8
7,295 nt
5' UTR
(114nt)
ORF
(7,008nt)
3' UTR
(173nt)
AAAAAA
119-2,732nt
5,686-7,102 nt
SINAPs
6,636 nt
ORF
(4,917nt)
3' UTR
(1,719nt)
AAAAAA
90-1,790nt
5,392-6,632 nt
TUG1
7,469 nt
AAAAAA
532-2,120nt
5,455-6,484nt
OIP5-AS1
8,829 nt
AAAAAA
378-1,840nt
7,275-8,763nt

Figure S1

Figure S1: Positions of smFISH probes used in this study. Cartoons illustrating the positions of the probes used for the different genes used. See Supplemental Table 1 for probe sequences. The transcripts sequences were obtained from ensembl – *MDN1* (ENST00000369393), *POLA1* (ENST00000379068) and *PRPF8* (ENST00000304992) mRNAs and *TUG1* (ENST00000644773) and *OIP5-AS1* (ENST00000500949) lncRNAs.

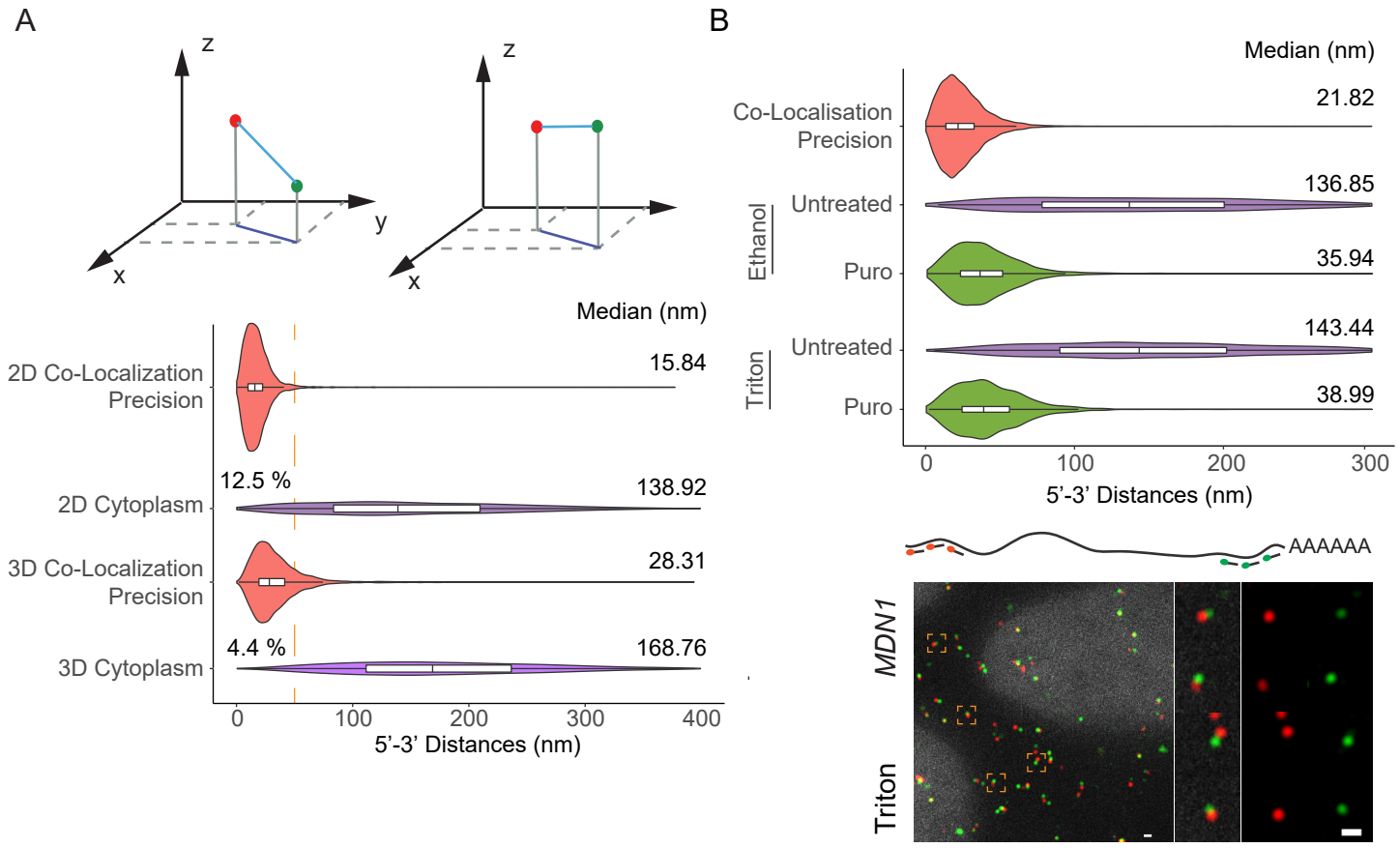
A
z
y
x
z
x
Median (nm)
2D Co-Localization Precision
15.84
12.5 %
2D Cytoplasm
138.92
3D Co-Localization Precision
28.31
4.4 %
3D Cytoplasm
168.76
0
100
200
300
400
5'-3' Distances (nm)
B
Median (nm)
Co-Localisation Precision
21.82
Ethanol
Untreated
136.85
Puro
35.94
Triton
Untreated
143.44
Puro
38.99
0
100
200
300
5'-3' Distances (nm)
AAAAAA
MDN1
Triton
C
AAAAAA
AAAAAA
PRPF8
POLA1

Figure S2

Figure S2 ***Related to Figure 1*****: Visualizing mRNP conformations for** ***MDN1, POLA1 and PRPF8*** **mRNAs and measurement of 5'-3' distances for** ***MDN1*** **mRNAs in 2D, 3D and in cells permeabilized using either TritonX or Ethanol (A)** Cartoon illustrating how 2D projection alters 5'-3' distances measured (above) and violin plots showing distance distribution of co-localization precision and 5'-3' distances for *MDN1* mRNAs calculated in 2D and 3D. Dotted line delineates the percentage of *MDN1* mRNAs with 5'-3' distances less than 50nm. **(B)** Violin plots showing distance distribution of co-localization precision (Probe Set#1, Table S3) and 5'-3' distances for *MDN1* mRNAs (Probe Set#2, Table S3) in cells permeabilized with either Ethanol or TritonX-100 (above) and smFISH images using hybridizing to the 5' and 3' ends of *MDN1* mRNAs in cells permeabilized with TritonX-100 (below), **(C)**. smFISH images using probes hybridizing to the 5'(red) and 3'(green) ends of *PRPF8* (Probe Set#5, Table S3) and *POLA1*(Probe Set#6, Table S3) mRNAs in paraformaldehyde fixed HEK293 cells. Scale bars, 500 nm). Nuclei are visualized by DAPI staining (grey). Magnified images of individual RNAs marked by dashed squares are shown on the right. Schematic position of probes shown on top. White box plot inside the violin plot shows first quartile, median and third quartile. Median distances are shown on the right.

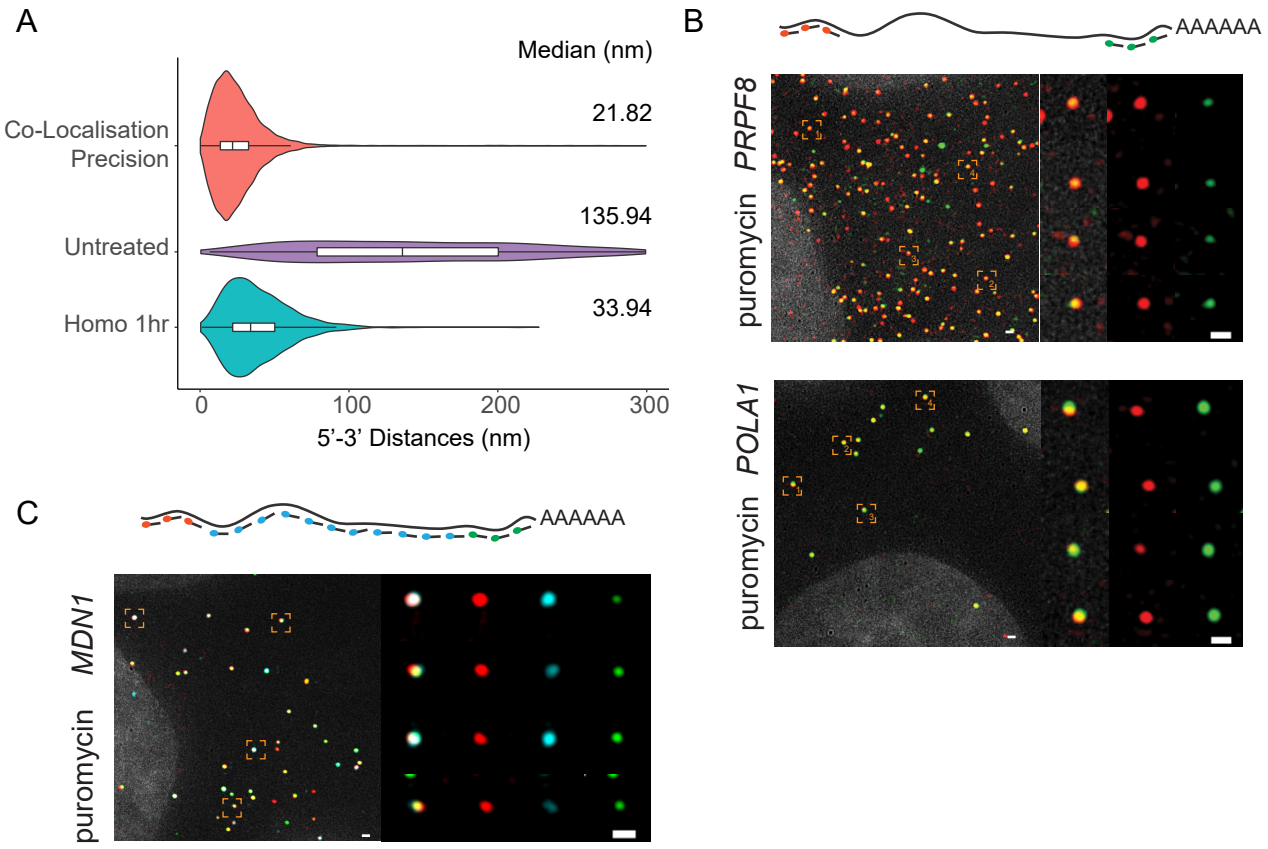
A
Median (nm)
Co-Localisation Precision
21.82
Untreated
135.94
Homo 1hr
33.94
0
100
200
300
5'-3' Distances (nm)
B
AAAAAA
PRPF8
puromycin
POLA1
puromycin
C
AAAAAA
MDN1
puromycin

Figure S3

Figure S3 ***Related to Figure 2*****: 5'-3' distance measurements for** ***MDN1*** **mRNA upon treatment with homoharringtonine and visualizing mRNP conformation of single** ***POLA1, PRPF8*** **and** ***MDN1*** **mRNAs when treated with puromycin**. **(A)** Violin plots showing distance distribution of co-localization precision (Probe Set#1, Table S3) and 5'-3' distances for *MDN1* mRNAs (Probe Set#2, Table S3) determined by Gaussian fitting from untreated and homoharringtonine (1hr) treated cells **(B)** smFISH images using probes hybridizing to the 5' and 3' ends of *PRPF8* (Probe Set#5, Table S3) and *POLA1* (Probe Set#6, Table S3) mRNAs in paraformaldehyde fixed HEK293 cells treated with puromycin (10 min, 100 μg/ml) **(C)** smFISH using 5' (red), 3' (green), and tiling (cyan) for *MDN1* mRNA (Probe Set#3, Table S3) in paraformaldehyde fixed HEK293 cells treated with puromycin (10 min, 100 μg/ml). Nuclei are visualized by DAPI staining (grey). Magnified images of individual RNAs marked by dashed squares are shown on the right. Schematic position of probes shown on top. Scale bars, 500 nm. White box plot inside the violin plot shows first quartile, median and third quartile. Median distances are shown on the right.

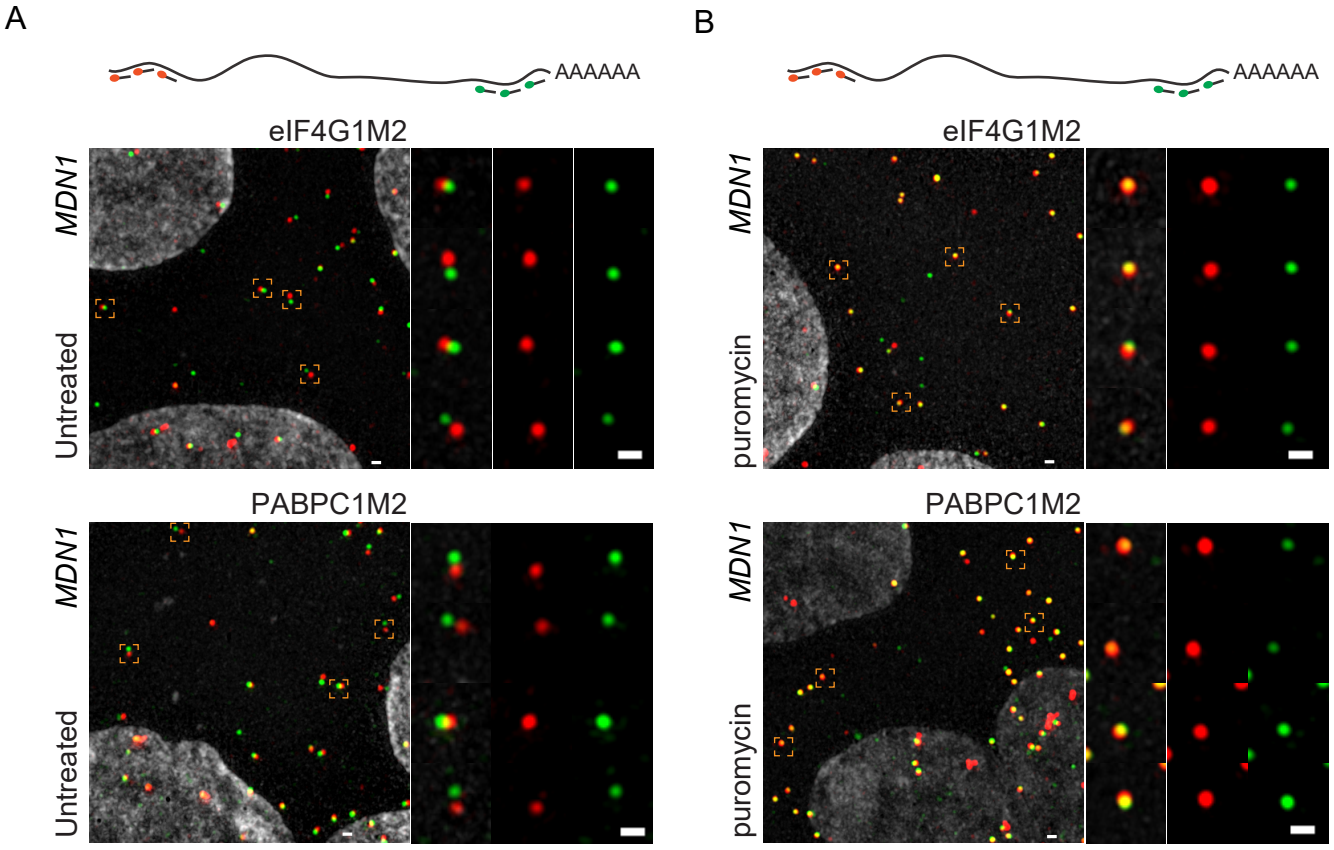
A
AAAAAA
eIF4G1M2
MDN1
Untreated
PABPC1M2
MDN1
Untreated
B
AAAAAA
eIF4G1M2
MDN1
puromycin
PABPC1M2
MDN1
puromycin

Figure S4

Figure S4 ***Related to Figure 3*****: Visualizing mRNP conformations in mutant cell lines of PABPC1 and eIF4G1. (A)** smFISH images using probes hybridizing to the 5' (red) and 3'(green) ends (Probe Set#2, Table S3) of *MDN1* mRNA in mutant cell lines of eIF4G1 and PABPC1 inparaformaldehyde fixed HEK 293 cells. **(B)** smFISH images using probes hybridizing to the 5' (red) and 3' (green) ends of *MDN1* mRNA in mutant cell lines of eIF4G1 and PABPC1 in HEK 293 cells treated with puromycin (10 min, 100 µg/ml). Nuclei are visualized by DAPI staining (grey). Magnified images of individual RNAs marked by dashed squares are shown on the right. Schematic position of probes shown on top. Scale bars, 500 nm.

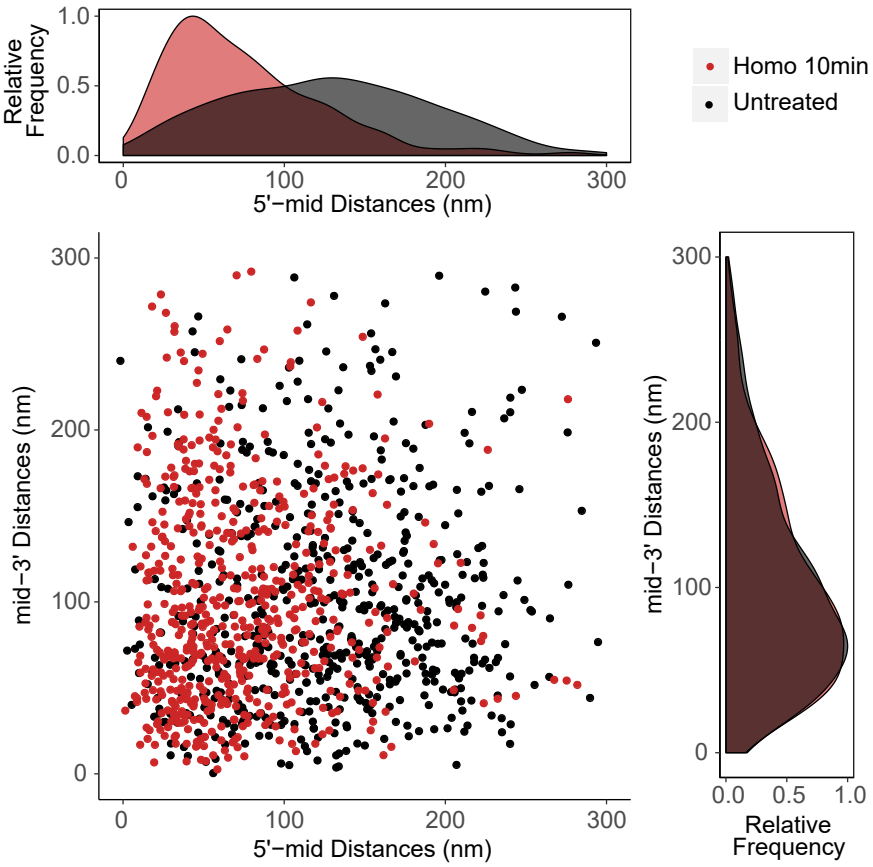
Relative Frequency
1.0
0.5
0.0
0
100
200
300
5'−mid Distances (nm)
Homo 10min
Untreated
mid−3' Distances (nm)
300
200
100
0
0.0
0.5
1.0
Relative Frequency

Figure S5

Figure S5 ***Related to Figure 4*****: Compaction of the 5' end is altered upon a pulsed homoharringtonine treatment for 10min.** Scatter plot showing 5'- mid and mid-3' distances for individual cytoplasmic *MDN1* mRNAs from untreated cells (black) and cells treated with homoharringtonine (100μg/ml, 10min) (red). Frequency distribution are shown on top and on the right.

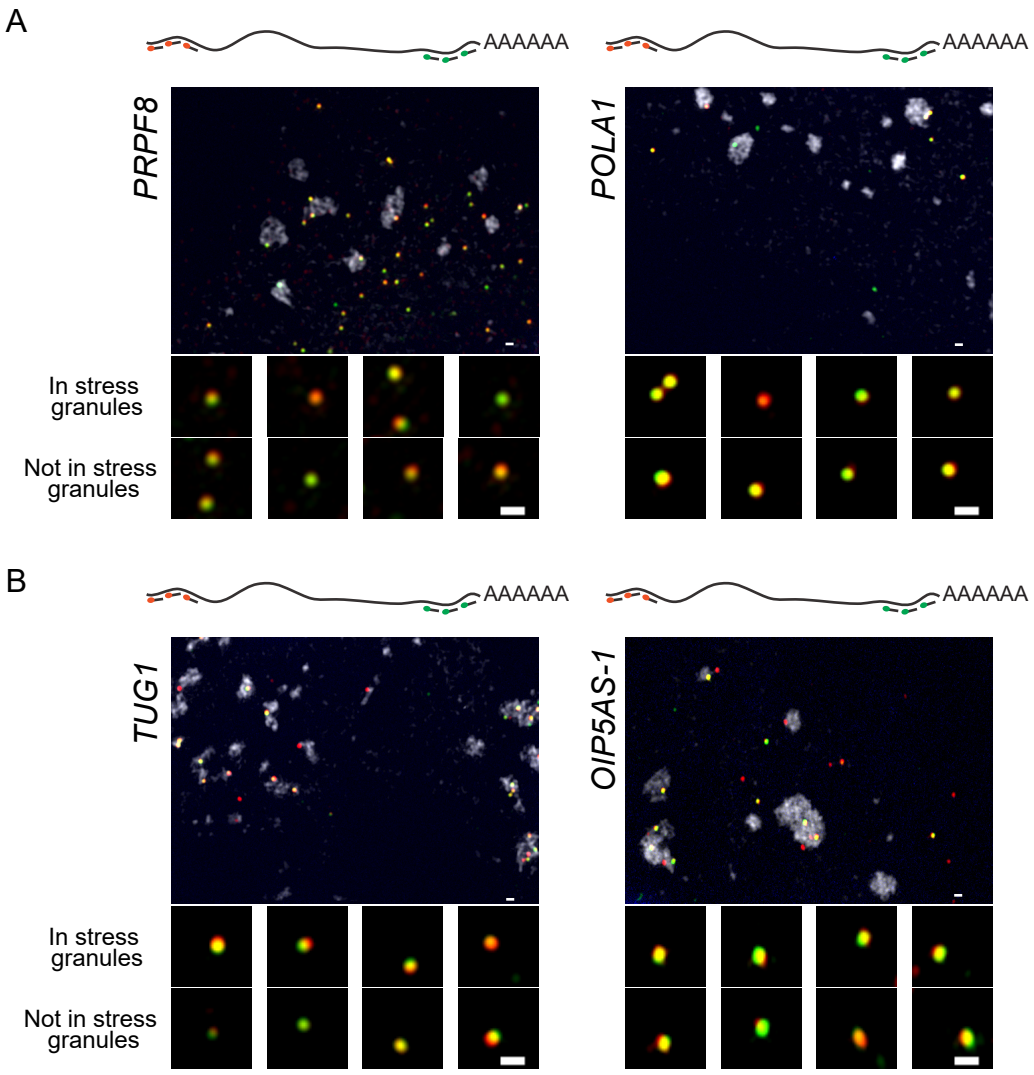
A
AAAAAA
AAAAAA
PRPF8
POLA1
In stress granules
Not in stress granules
B
AAAAAA
AAAAAA
TUG1
OIP5AS-1
In stress granules
Not in stress granules

Figure S6

Figure S6 *Related to Figure 5*: mRNA and lncRNA compaction and accumulation in stress granules. smFISH visualizing 5' and 3' ends of *PRPF8* (Probe Set#11, Table S3) and *POLA1* (Probe Set#12, Table S3) mRNAs (A) or *TUG1* (Probe Set#13, Table S3) and *OIP5-AS1*(Probe Set#14, Table S3) lncRNAs (B) in U2OS cells treated with arsenite (1-hour, 2 mM). Only a selected cytoplasmic region of cells is shown. Stress granules are visualized using an oligo dT probe (grey). Magnified images of individual RNAs localized inside or outside of stress granules are shown on the bottom of the images. Schematic position of probes shown on top. For *POLA1* mRNAs and *OIP5-AS1* lncRNAs, not all magnified single RNAs shown in the bottom are from the corresponding image above. Scale bars, 500 nm.

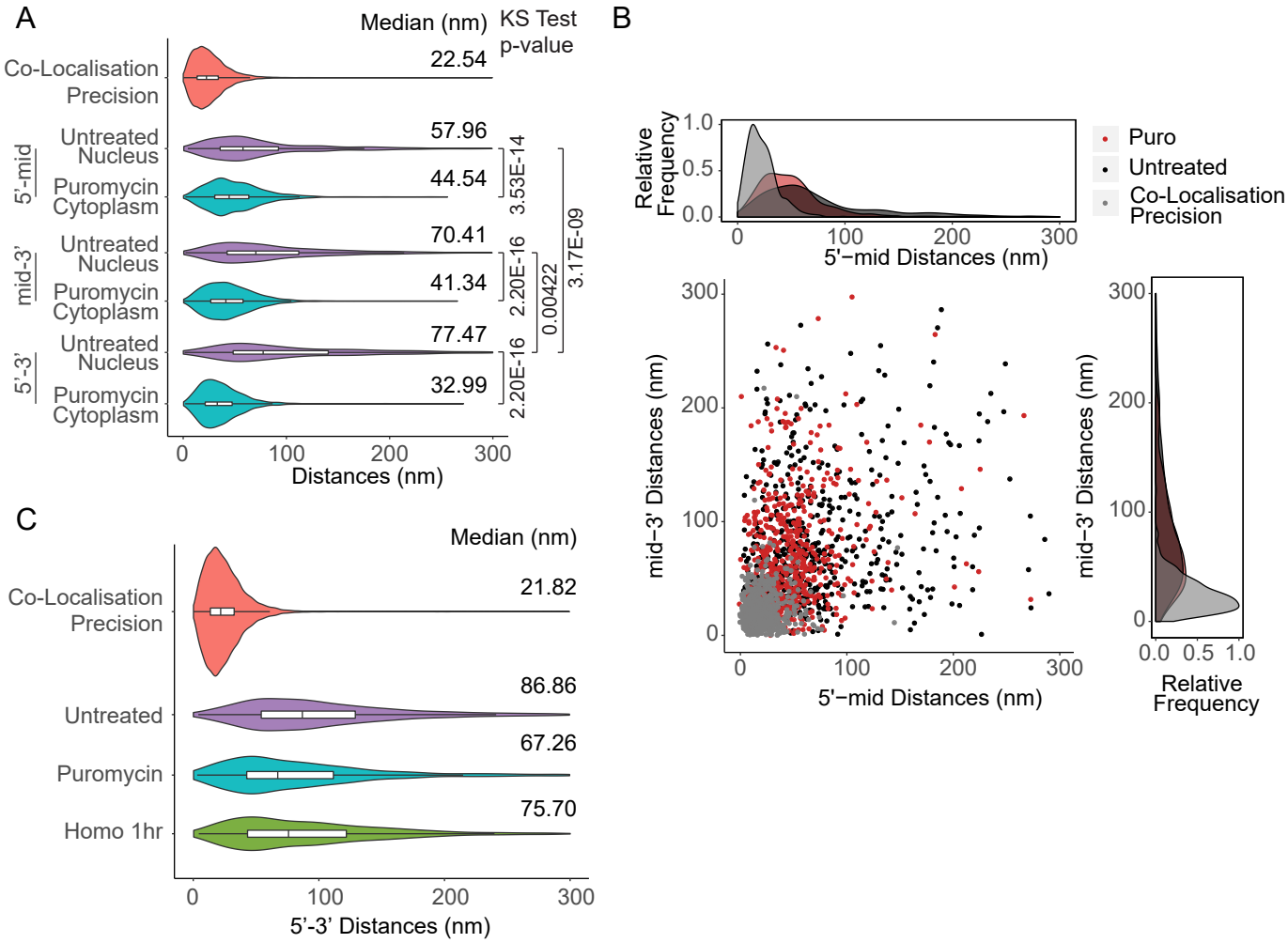
A
Median (nm)
KS Test p-value
Co-Localisation Precision
22.54
5'-mid
Untreated Nucleus
57.96
Puromycin Cytoplasm
44.54
3.53E-14
mid-3'
Untreated Nucleus
70.41
Puromycin Cytoplasm
41.34
2.20E-16
0.00422
3.17E-09
5'-3'
Untreated Nucleus
77.47
Puromycin Cytoplasm
32.99
2.20E-16
0
100
200
300
Distances (nm)
B
Relative Frequency
1.0
0.5
0.0
0
100
200
300
5'−mid Distances (nm)
Puro
Untreated
Co-Localisation Precision
mid−3' Distances (nm)
300
200
100
0
0
100
200
300
5'−mid Distances (nm)
mid−3' Distances (nm)
300
200
100
0
0.0 0.5 1.0
Relative Frequency
C
Median (nm)
Co-Localisation Precision
21.82
Untreated
86.86
Puromycin
67.26
Homo 1hr
75.70
0
100
200
300
5'-3' Distances (nm)

Figure S7

Figure S7 ***Related to Figure 6*****: Compaction of nuclear MDN1 mRNA upon puromycin or homoharringtonine treatment. (A)** Violin plots showing distance distribution of co-localization precision (Probe Set#1, Table S3) and 5'-3' distances for *MDN1* mRNAs (Probe Set#2, Table S3) determined by Gaussian fitting from nuclear mRNAs in untreated cells and cytoplasmic mRNAs in cells treated with puromycin (100μg/ml, 10 min) (Probe Set#4, Table S3). **(B)** Scatter plot showing 5'- mid and mid-3' distances for individual nuclear *MDN1* mRNAs from untreated cells (black) and cells treated with puromycin (100μg/ml, 10min) (red). Co-localization precision is shown in grey. Frequency distribution are shown on top and on the right. **(C)** Violin plots showing distance distribution of co-localization precision (Probe Set#1, Table S3) and 5'-3' distances for *MDN1* (Probe Set#2, Table S3) mRNAs determined by Gaussian fitting from untreated, puromycin (100μg/ml, 10min) or homoharringtonine (100μg/ml, 1 hour) treated HEK293 cells. White box plot inside the violin plot shows first quartile, median and third quartile. Median distances and p-values calculated using Kolmogorov-Smirnov test are shown on the right.

Supplementary Tables

Table S1 ***Related to Figure 3*****:** Primers used for making CRISPR/Cas9 cells lines

Name	Sequence (5'-3')	Purpose
PABPC1 M161A gRNA 1 Fw	CACCGAATCTGTTAGCCATCTAACC	Guide RNA targeting PABPC1; annealed and ligated into pX330
PABPC1 M161A gRNA 1 Rev	AAACGGTTAGATGGCTAACAGATTC	
PABPC1 region Fw NotI	GCGCACTAGCGGCCGCGAGGAAGCGTTCAACTGTGA	To amplify 5' arm region of PABPC1
PABPC1 Not I 5' arm Rev guide 1 XhoI	GCGCACTAGCGGCCGCCTCGAGACCTGGATATTTGTGAAATAAAG	
PABPC1 BamHI 3' arm Fw guide 1	CGCGGATCCTAGATGGCTAACAGATTGTCTCTC	To amplify 3' arm region of PABPC1
PABPC1 region Rev BamHI	CGCGGATCCTTGGTCAGGCTGGTCTCAAA	
PABPC1 mutation Fw	GAAAGAGCTATTGAAAAAATGAATGGAGCGCTCCTAAATGATCGCAAAGTATTTGTTGG	Internal primers for stitch PCR to make PABPC1 mutation
PABPC1 mutation Rev	CCAACAAATACTTTGCGATCATTTAGGAGCGCTCCATTCATTTTTTCAATAGCTCTTTC	
PABPC1 region Fw	GGCGAGAGATTGCGTCAAGAA	Screening for puromycin cassette insertion/excision
PABPC1 region Rev	CCCTGGTAACAGGCATTTGTGAG	
PABP seq Fw1	GCAATATGGAATTCTTTTATATG	To sequence PCR products from PABPC1 mutant cell line screening
PABP seq Fw2	GGAACTGTGCAGTAATGGATATC	
PABP seq Rev1	CAATCTTGTCGCCCAGACTGG	
eIF4G1 mutant gRNA 1 Fw	CACCGTGCTGCTGGGACATTGTGC	Guide RNA targeting eIF4G1; annealed and ligated into pX330
eIF4G1 mutant gRNA 1 Rev	AAACGCACAATGTCCCAGCAGCAC	
eIF4G1 5' HR Arm Fw NotI	GCGCACTAGCGGCCGC GAGACAGGAACTAGACTCAAG	To amplify 5' arm region of eIF4G1
eIF4G1 5' HR Arm Rev NotI XhoI	GCGCACTAGCGGCCGC CTCGAG CAATGTCCCAGCAGCACCTGACC	
eIF4G1 3' HR Arm Fw BamHI	CGC GGATCC TGCCGGAAAGAGCAGTGACTTG	To amplify 3' arm region of eIF4G1
eIF4G1 3' HR Arm Rev BamHI	CGC GGATCC GGCACCCTATTCTGGGCACC	
eIF4G1 mutation Fw	TGCAGCCGCTGCCGCGGGAGCAATCTGGGGTGGCTGGTTC	Internal primers for stitch PCR to make eIF4G1 mutation
eIF4G1 mutation Rev	CGCCAGTGGGAAACTGCTGCACCCCTTGGGCTGGATAGTAGG	

eIF4G1 region Fw	GTAGTCGCACAGTCTTGGCTC	Screening for puromycin cassette insertion/excision
eIF4G1 region Rev	GAGTCCAGGGCAGAACAGAC	
4G seq Rev1	CACCCCTCGTAGGCAGGCACTC	To sequence PCR products from eIF4G mutant cell line screening
4G seq Fw 1	CAGAGTATGTGTGTACATGTTG	
4G seq Rev2	CTTCCTCGCTAGGCACTTCAG	
4G seq Rev 3	CCAGCAGTCCCCAAGTCAGTGG	

Table S2 ***Related to Figure S1*****:** List of smFISH probes used (see attached excel file)

Table S3 ***Related to Figure S1 and Table S2*****:** Probe and antibody combinations used

Probe Set #	Experiment	Combination of probes/antibodies and dyes used (From Table S2)
1	MDN1 middle alternating probes	MDN1 middle odd – Cy5 MDN1 middle even – Cy3
2	MDN1 5'-3'	MDN1 5' -Cy5 MDN1 3'- Dy550
3	MDN1 5'-tiling-3'	MDN1 5'+ MDN1 5' additional – Dy488 MDN1 tiling – Cy5 MDN 3' – Dy550
4	MDN1 5'-middle-3'	MDN1 5'+ MDN1 5' additional – Dy488 MDN1 middle – Cy5 MDN 3' – Dy550
5	PRPF8 5'-3'	PRPF8 5' -Cy5 PRPF8 3'- Cy3
6	POLA1 5'-3'	POLA1 5' -Cy3 POLA1 3'- Cy5
7	TUG1 5'-3'	TUG1 5' -Cy5 TUG1 3'- Cy3
8	OIP5-AS1 5'-3'	OIP5-AS1 5' -Cy5 OIP5-AS1 3'- Cy3
9	MDN1 5'-3'-dT	MDN1 5' -Cy5 MDN1 3'- Dy550 dT – Cy2
10	MDN1 tiling-3'-dT	MDN1 tiling + MDN1 tiling additional– Cy5 MDN1 3'– Dy550 dT- Cy2
11	PRPF8 5'-3'-dT	PRPF8 5' -Cy5 PRPF8 3'- Cy3 dT – Cy2
12	POLA1 5'-3'-dT	POLA1 5' -Cy3 POLA1 3'- Cy5 dT – Cy2
13	TUG1 5'-3'-dT	TUG1 5' -Cy5 TUG1 3'- Cy3 dT – Cy2
14	OIP5-AS1 5'-3'-dT	OIP5-AS1 5' -Cy5 OIP5-AS1 3'- Cy3 dT – Cy2
15	SINAPs 5'-3'	SuntagV4 5' – Quasar 570 MS2v5 3' – Cy5 Chicken anti-GFP Antibody Goat-Anti Chicken Alexa 488