Supplementary Material for

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

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MATERIALS AND METHODS

Reagents Used, stock concentrations, working concentrations and treatment conditions: Puromycin dihydrochloride (Sigma P8833) – stock at 5 mg/ml in water, Cycloheximide (Sigma C7698-1G) – stock 5 mg/ml in ethanol, Sodium Arsenite (Sigma 35000-1L-R) – stock 50 mM in water. The drugs were diluted in warm media to get final working concentrations and cells were treated prior to fixation as follows: Puromycin (100 μg/ml for 10 min), Cycloheximide (100 μg/ml for 10 min) and Sodium Arsenite (2 mM for 1 hour).

Cell culture and drug treatment: HEK293 and U2OS osteosarcoma cell lines were maintained at 37°C and 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) (Wisent, 319-005-CL) supplemented with 10% fetal bovine serum (FBS) (Wisent, 080-150) and passaged every 2-3 days with Trypsin (Wisent 325-043-EL). Cells were plated on poly-L-Lysine (Sigma, P8920) coated coverslips the day before treatment and fixation. On the day of the experiment, media was replaced with fresh warm media containing drug in indicated concentrations and placed back in the incubator. After treatment, the cells were briefly washed with 1xPBS, fixed with 4% paraformaldehyde (pH 7.2) for 10 minutes at room temperature, washed three times with 1xPBS and stored overnight in 70% ethanol at -20^{o}C.

smRNA FISH: Custom DNA probe sets were designed using Stellaris® Probe Designer, synthetized by Biosearch Technologies containing 3' amine reactive group and labeled with far red dye Cy5 (GEPA25001), red dyes Cy3 (GEPA23001) from Sigma or Dylight 550 (Thermo Scientific 62263) or green dye Dy488 (Thermo Scientific 46403) as described in(*1*). Probe sequences are shown in Supplemental Table 1. smFISH was done as described in (*2*). Prior to hybridization, cells are rehydrated in 1xPBS, then washed with 10% formamide/2xSSC for 10

minutes at room temperature. The cells were hybridized with 10-20 ng of each probe mix plus 40 μg of ssDNA/tRNA resuspended in the hybridization solution (10% dextran sulfate/10% formamide/2xSSC/2 mM VRC/0.1 mg/ml BSA) for 3 hours in the dark at 37°C. Post hybridization washes (2x 30 min) were carried out at 37°C with 10% formamide/2xSSC. Samples were then rinsed with 1xPBS and mounted with ProLong Gold antifade reagent with DAPI (P36935, Invitrogen).

Image Acquisition and pixel shift correction: Images were acquired with a 63x NA 1.46 oil objective on a Zeiss Elyra PS.1 system equipped with an Andor EMCCD iXon3 DU-885 CSO VP461 camera (1004x1002 pixels), the following filter sets: DAPI: BP420-480 + LP750 (Zeiss SR cube 07), Cy2: BP495-590+LP750 (Zeiss SR cube 13), Cy3: LP570 (Zeiss SR cube 14), Cy5: LP655 (Zeiss SR cube 10) and the following lasers: 50 mW 405 nm HR diode, 100 mW 488 nm HR diode, 100 mW 561 nm HR DPSS, 150 mW 642 nm HR diode. Each image was acquired using 3 rotations and a grid size of 42 μm for all channels. The microscope was located in a temperature controlled room and samples were kept in the room for at least an hour before imaging to minimize thermal fluctuations. To correct for pixel shifts between channels, 0.1 μm TetraSpec beads (Invitrogen T-7279) were imaged in all channels and the channel shift values were calculated and corrected using the built-in channel alignment tool in ZEN 2012 SP5.

RNA spot detection, spot assignment and distance measurements: For image analysis, 3D datasets were reduced to 2D data using maximum projections in FiJi. Spot detection was done by 2D Gaussian fitting as described in (*3*, *4*). To separate cytoplasmic and nuclear mRNPs, masks were created in FiJi by manual segmentation using DAPI stained nuclei as reference, while ensuring that regions with overlapping spots within the same channel were not included. Assignment of the 5', 3' and/or the mid spots to either the cytoplasmic or the nuclear masks was

done using MATLAB. To measure distances between different regions of mRNPs, spots from different channels were first grouped to assign neighboring spots corresponding a single RNA. This was achieved by using spots from one channel as a reference, and finding spots from the other channels within a radius of 300nm using the coordinates from 2D Gaussian fitting using a custom MATLAB script. 300nm were chosen to limit assigning signals from neighboring RNAs. Groups with more than one spot from each channel, which could correspond to overlapping mRNPs or mRNPs close together in space, were discarded. For 2 color imaging, the 5' signal was taken as reference and for 3 color imaging, the middle was taken as reference. Switching references yielded comparable results (not shown). 2D distance between different regions of the mRNP were then calculated for each signal within a group.

Plotting: All measurements were made for at least 2 independent biological replicates and the data plotted are representative from one of the replicates. For each measurement, at least 5 different fields were imaged with each image containing a minimum of 10 cells to make a total of at least 50 cells. A minimum of 940 RNAs were considered for cytoplasmic plots and a minimum of 430 RNAs were considered for the nuclear plots, unless mentioned otherwise. The center of mass plots in Fig. 1G, 2D, 4D were made using R. The center of mass was calculated as the mean of the coordinates of the three regions. The different conformations were then aligned using their center of masses. For the 3-color scatter plot in Fig. 2E, 4E and S4, to get a pair of co-localization precision values, two values were chosen randomly from our data. These values were taken as the X and Y coordinates for the scatter plot. The values that served as the X and Y coordinates were used to get density plots in the same figure. The mean Radius of gyration ($<R_g>$) was calculated using:

$$< R_g >= \sqrt{\frac{1}{3}\sum_{k=1}^{3}(r_k - r_{mean})^2}$$

where k represents one of the three regions of the mRNP and r_k the position of the corresponding position in space as determined by 2D Gaussian fitting.

Supplementary Figures

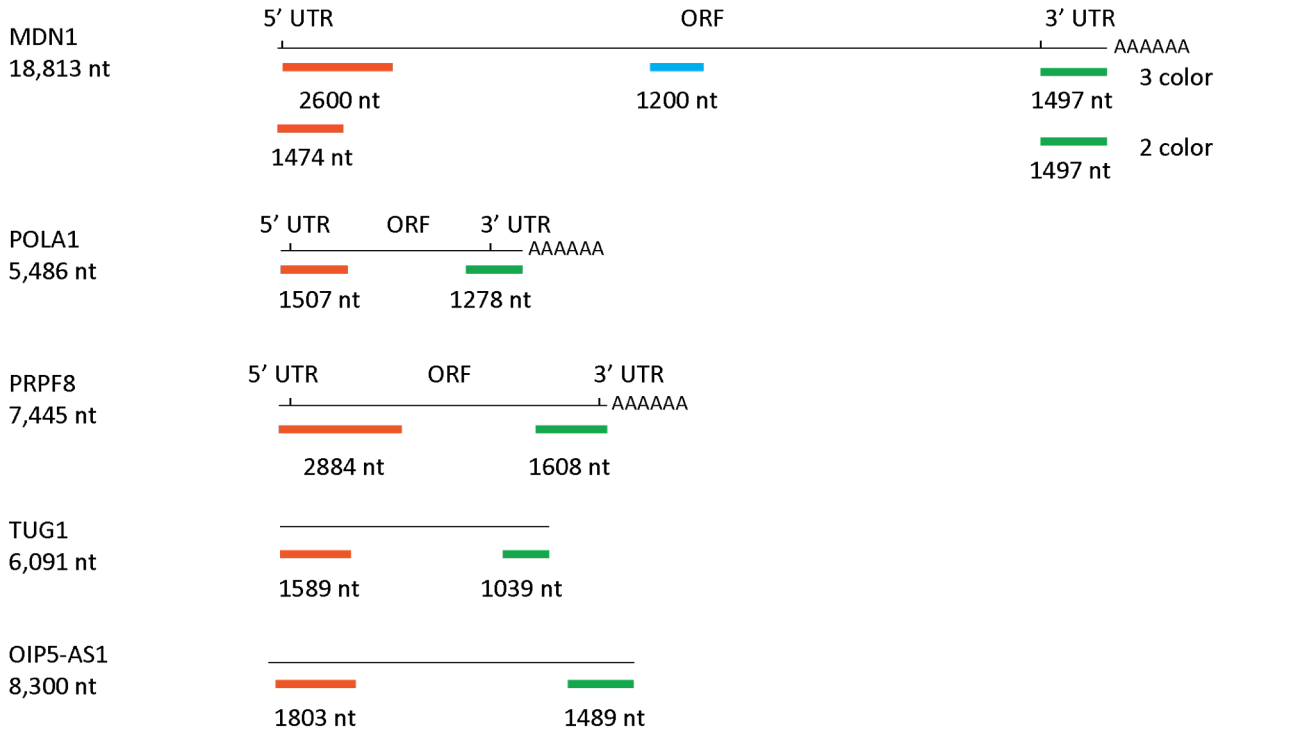
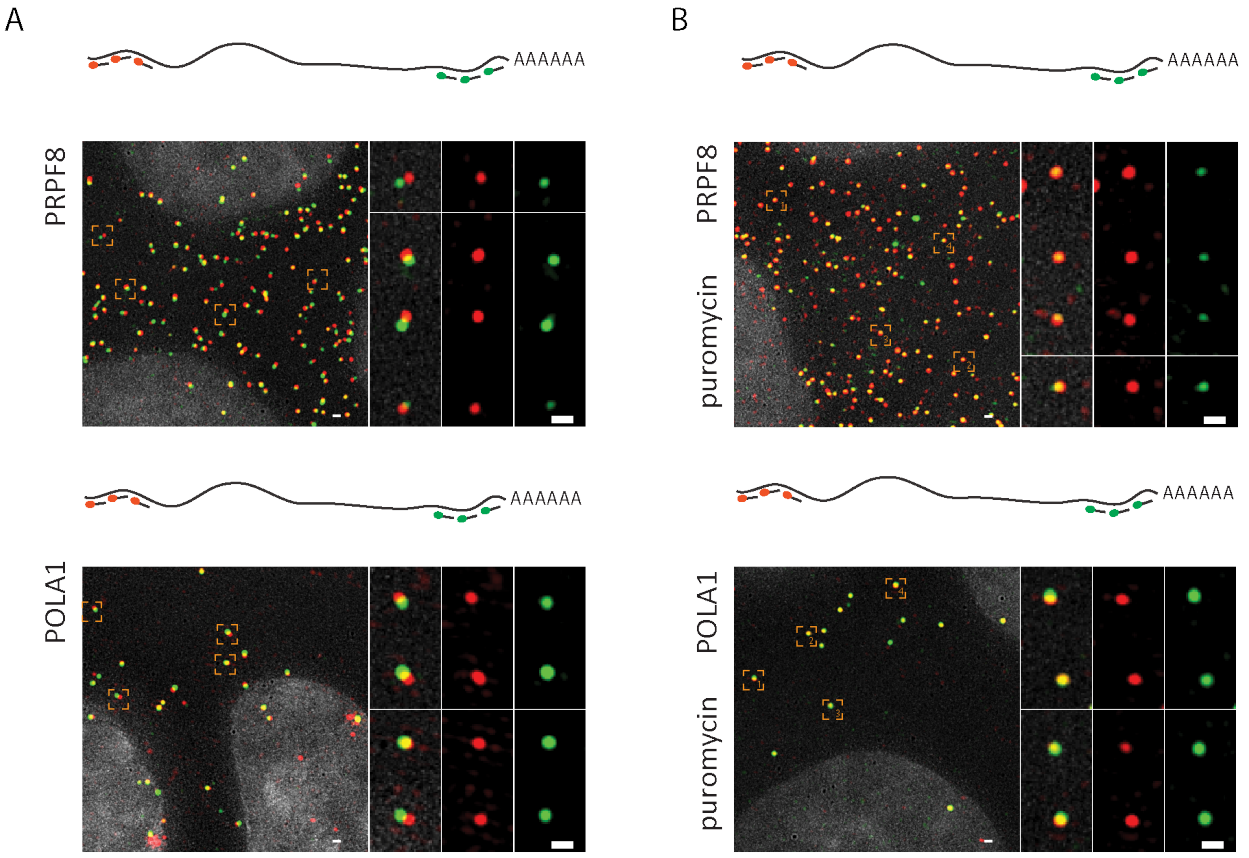


Figure S1: Positions of smFISH probes used in this study. Cartoons illustrating the positions of the probes used for the different genes used. See Supplemental Table 1 for probe sequences.



Adivarahan et al. Figure S2

Figure S2: Visualizing mRNP conformation of single POLA1 and PRPF8 mRNAs. **(A and B)** smFISH images using probes hybdirizing to the 5' and 3' ends of PRPF8 and POLA1 mRNAs in paraformaldehyde fixed HEK293 cells, either untreated (A) or treated with puromycin (10 min, 100 µg/ml) (B). 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.

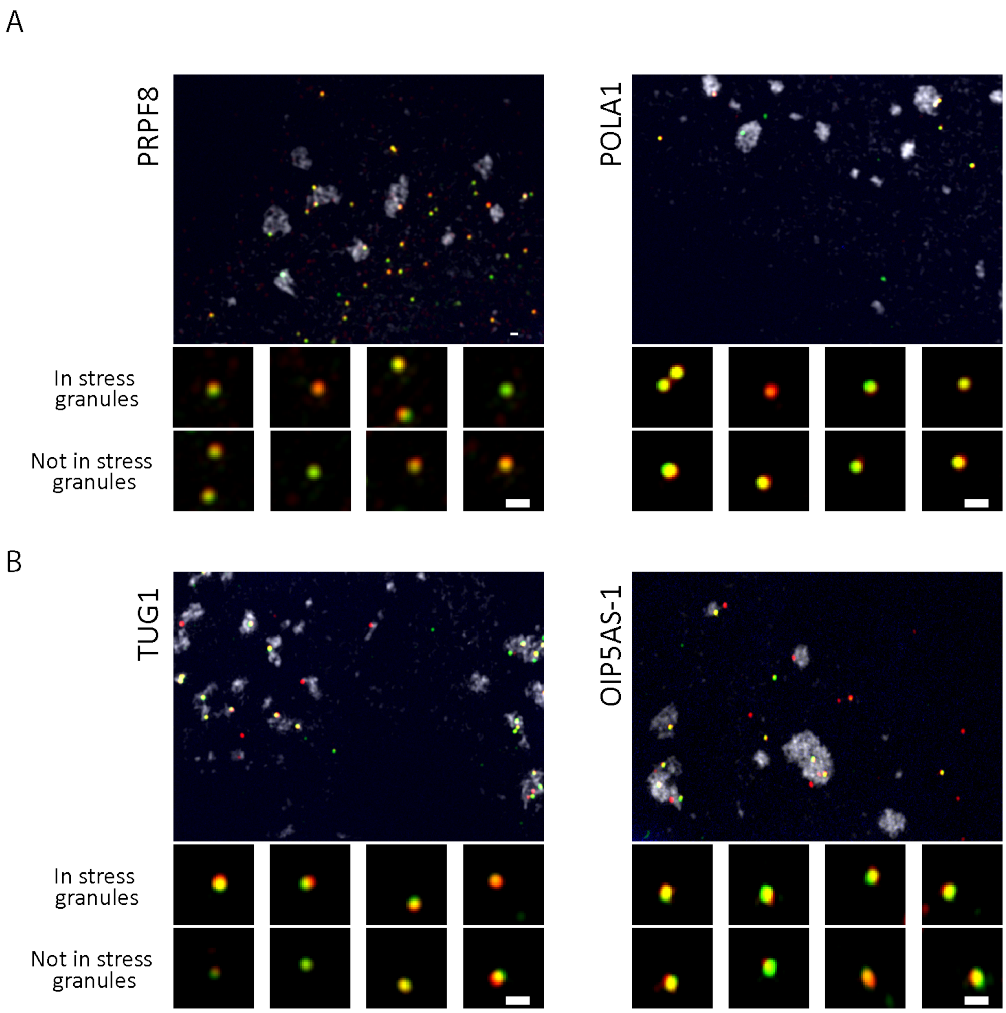
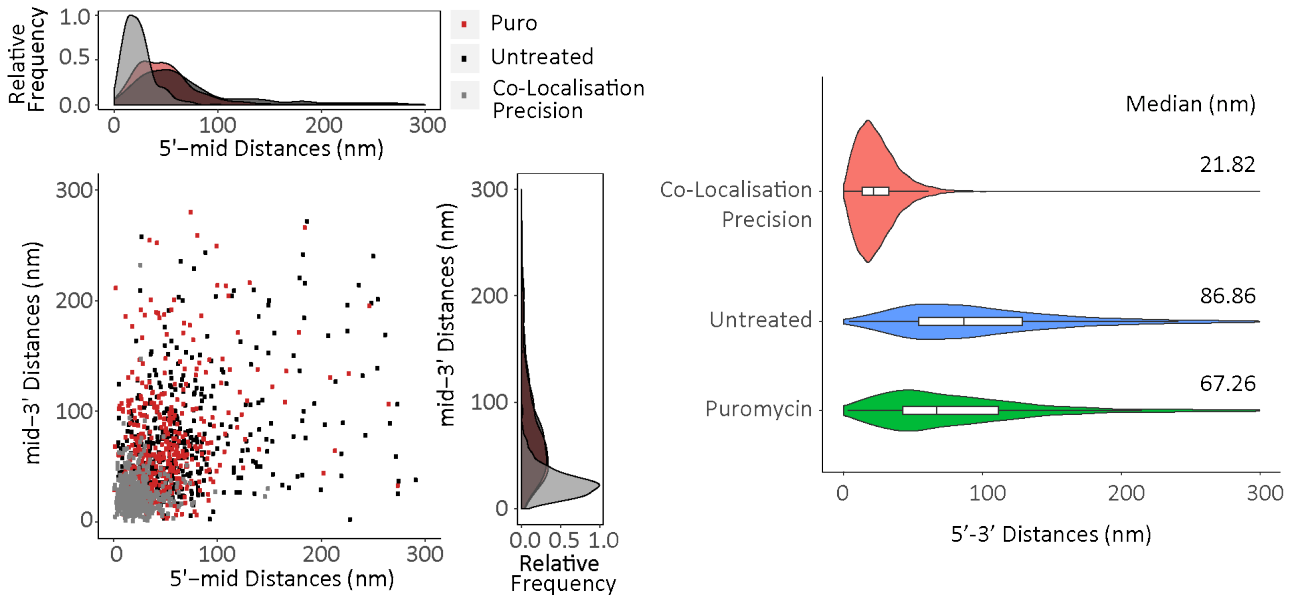


Figure S3: mRNA and lncRNA compaction and accumulation in stress granules. smFISH visualizing 5' and 3' ends of PRPF8 and POLA1 mRNAs (A) or TUG1 and OIP5-AS1 lncRNAs (B) in U2OS cells treated with arsenite (1 hour, 2 mM). Only a selected cytoplasmic region of cells is shown. Stress granules are visualized 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. For POLA1 and OIP5-AS1, not all magnified single RNAs shown in the bottom are from the corresponding image above. Scale bars, 500 nm.



Adivarahan et al. Figure S4

Figure S4: Compaction of nuclear MDN1 mRNA is not altered upon puromycin treatment. **(Left)** Scatter plot showing 5'- mid and mid-3' distances for individual nuclear MDN1 mRNAs from untreated cells (black) and cells treated with puromycin (red). Co-localization precision is shown in grey. Frequency distribution are shown on top and on the right. **(Right)** Violin plots showing distance distribution of co-localization precision and 5'-3' distances for MDN1 mRNAs determined by Gaussian fitting from untreated, puromycin or arsenite treated HEK293 cells. White box plot inside the violin plot shows first quartile, median and third quartile. Median distances are shown on the right.

Tables

Table S1: List of probes used

MDN1 5'	tcgttcttggctgcgattaa	POLA1 3'	tgtacagggacttgtcagaa
MDN1 5'	taaggtactcaggacacact	POLA1 3'	taccggtaaaagcacagctg
MDN1 5'	cacagtacagtccttatcca	POLA1 3'	gtgcacactccgcatcaaaa
MDN1 5'	agcaaatccaaaaggagagg	POLA1 3'	tctcatgatcggtagtaagt
MDN1 5'	ttgaaagactggggatgtgt	POLA1 3'	ctgtagtcctgcagaacttt
MDN1 5'	gcatctgaactctctaggaa	POLA1 3'	ctctgctgtgttcttgagtt
MDN1 5'	gtgctcttcattcatacagg	POLA1 3'	tagccacttcgggacaagaa
MDN1 5'	cctcaacctgaaatggatca	POLA1 3'	tttgctcagattcacttcgg
MDN1 5'	aaaaccaaggccttctccaa	POLA1 3'	ttaggatttcacggcacaac
MDN1 5'	aaagggagacttctggattg	POLA1 3'	cttggttactcctgggattc
MDN1 5'	tcagacgaaacaagatgtcc	POLA1 3'	ggaagctgggatttttcaac
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MDN1 5'	tcctattggtccttccaaca	POLA1 3'	ctcagaaaccgggtcttcag
MDN1 5'	cctgtcactgcagctaaata	POLA1 3'	gctactctcaatccaagtag
MDN1 5'	aagctggactttgagaagct	POLA1 3'	gcctggggtcacttacattc
MDN1 5'	acatctgtgcagcgatacat	POLA1 3'	cataggctaaaggccctgag
MDN1 5'	atatcctccagaaggatcca	POLA1 3'	ttcagtcaggctctgagaag
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MDN1 5'	aatggcttcggcattccttt	POLA1 3'	cagtgtgtgtctgttggact
MDN1 5'	gttcatgcagatcatggttg	POLA1 3'	atgtgagtgtaaaacacctg
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MDN1 5'	gacatcaggatggttaccaa	POLA1 3'	ccctacacatgttaatggat
MDN1 5'	aatatctcagggcaaacggg	POLA1 3'	tgaatacaacacagtgatcc
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MDN1 5'	acaattggctgtataccagc	POLA1 3'	agagaggaaagactgccata

MDN1 5'	acaccacaaacagctgtcac	POLA1 3'	cagcataattgtacaagggg
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MDN1 tiling	gttctaaacactgagcatgc	TUG1 5’	ggactcaaacagggcttcaa
MDN1 tiling	aaccaagtgagagtcctttg	TUG1 5’	agaaacagctcacatatccc
MDN1 tiling	ggcaaagggttccacattag	TUG1 5’	aaccccgaatatccattatg
MDN1 tiling	tccaaaacagacttgggtgc	TUG1 5’	aatagaagccaagcagggga

MDN1 tiling	gaggtgagcaactggcagag	TUG1 5’	ccccatggtgaaagaaaagg
MDN1 tiling	ttcaccaacaaagtggtgca	TUG1 5’	attgtaccatatgcatcagc
MDN1 tiling	tttagaaggctctgggctac	TUG1 5’	tcctaattgtagctgcttta
MDN1 tiling	cggaactggtggtgtgactg	TUG1 5’	ggatgctacagaacacatcc
MDN1 tiling	acatgtctgaagagctgcag	TUG1 5’	gttgacgggccaaaggataa
MDN1 tiling	ttctcttgggctatgcgttc	TUG1 5’	accagtacaagcagcagata
MDN1 tiling	tagaagtcatagggcccatc	TUG1 5’	tatggtcaatgagagtcaga
MDN1 tiling	tgcttctggaacattgggat	TUG1 5’	atggatgacaaccatggtcg
MDN1 tiling	gcaacatcaaggtcatctgt	TUG1 5’	cctagacatgtaaagtagga
MDN1 tiling	aagtcgcacatggaactctc	TUG1 5’	ctattcaccaccaaccacac
MDN1 tiling	ctgacaccactctggaattg	TUG1 5’	ctggcagcaccatgtaaaaa
MDN1 tiling	tttggcaagcgacgcagaag	TUG1 5’	cgtgcaccattaattagctg
MDN1 tiling	gttgctagtgctgcgttaag	TUG1 5’	tgagcccgcttgctaaaagt
MDN1 tiling	aatgtgctgagaaccctctg	TUG1 5’	aattccatgccaggttcagt
MDN1 tiling	cagctgacttccaggtattg	TUG1 5’	aaaaaccccaaacatcttca
MDN1 tiling	caaaggtgatcctgtttccg	TUG1 5’	ctactgattttgaggttccg
MDN1 tiling	cataatcttcttgtgggctt	TUG1 5’	ttagctttgaatcacttcca
MDN1 tiling	ttagatgtcctgattgcagt	TUG1 5’	agaacacaaggagggccaag
MDN1 tiling	cccttctgaaatgtatcttc	TUG1 5’	tcctagtattacacttgcaa
MDN1 tiling	tcgcccttaatatcagattt	TUG1 5’	gcaaaaactggcatcttgga
MDN1 tiling	ccacctcattttcatcatag	TUG1 5’	tttgttggataatggcctga
MDN1 tiling	ttcctgattgccatggtaag	TUG1 5’	tgagcaccactccacaaaac
MDN1 tiling	ggccaactgggcaatgaaat	TUG1 5’	atgtgtttgtgttcacttgc
MDN1 tiling	ttttttcctggtgtgcttctg	TUG1 5’	agcataactggctaacatct
MDN1 tiling	gtaactctgccacatctcag	TUG1 5’	tctctgccctttaggaaaag
MDN1 tiling	gtgaaagaggcgctgttaag	TUG1 5’	gtcatttctctggaagtgag
MDN1 tiling	gagcagctgcaaacatgttg	TUG1 5’	accaacacttttttttctcc
MDN1 tiling	aactgatgttctgcgagagc	TUG1 5’	gaggtacatccggatttaat
MDN1 5’ additional	atgactgtttagcggtcgat	TUG1 5’	tcaccacagtcttaagtctt
MDN1 5’ additional	ccaggtgaattttggtccaa	TUG1 5’	ttctgctgaggaaagcatct
MDN1 5’ additional	cagttctctcttatccaggt	TUG1 3’	gagtgacagggtcagcagag
MDN1 5’ additional	gtttctctccagtaagttgg	TUG1 3’	gtttaggagtctatgctaca
MDN1 5’ additional	gaactatcactccaagagtg	TUG1 3’	cctcaatcagacttgaggtt
MDN1 5’ additional	aacttcttcaggtgcctgtt	TUG1 3’	actttcagctcaggagaagg
MDN1 5’ additional	ctcaagggttggtcttttgt	TUG1 3’	tttcttgctcatctgccaaa
MDN1 5’ additional	gggcaatcctattacaccaa	TUG1 3’	agtcaggtacatcagctttc
MDN1 5’ additional	ctcagaaagcattgctgtga	TUG1 3’	ggatacagttttctgatctt
MDN1 5’ additional	gcttccaataacttctgcca	TUG1 3’	ggttaatccatagggcttat
MDN1 5’ additional	tttgctgacacacactgcaa	TUG1 3’	gtttttactctgggtactca
MDN1 5’ additional	atggtagaggttttgccagt	TUG1 3’	ccagcagatcaataaggaag

MDN1 5’ additional	cctgtaatgtgagccaagta	TUG1 3’	gccagaatatgatctggaag
MDN1 5’ additional	attgacaaccctcaaacggt	TUG1 3’	agaaaggccagccataccaa
MDN1 5’ additional	gcaagtctgcagtatcactt	TUG1 3’	aatagacaagcagggtacct
MDN1 5’ additional	atggtccaccggtttataac	TUG1 3’	aaaggcaggcaagagctgag
MDN1 5’ additional	gtaagggtagccaaataagc	TUG1 3’	gtctaattgcagcaacatgt
MDN1 5’ additional	aagagttcctcaaatgcctc	TUG1 3’	aattgactgtagtcctcacg
MDN1 5’ additional	gccccaagaacgtaaagttt	TUG1 3’	aaatccttgttgtattgggc
MDN1 5’ additional	ctgtctgtaacaggtctgaa	TUG1 3’	aggtgtgggttgttactatt
MDN1 5’ additional	ttagtctcaggagatcatgc	TUG1 3’	gaacattgagtcctagtggg
MDN1 5’ additional	agcagacttgtgtacatgct	TUG1 3’	aagttgctggtcaaggagta
MDN1 5’ additional	cactgtcttttccatccttg	TUG1 3’	acatacaggaatagaggcct
MDN1 5’ additional	ccaaatgcttcccatttctc	TUG1 3’	aggttccaggtgctgaaaat
MDN1 5’ additional	ttgggcatggttgagtctaa	TUG1 3’	aactgctgtattccttccag
PRPF8 5’	ctcgataaggaaacactccg	TUG1 3’	agagaaatggacgcggcttt
PRPF8 5’	caattgctgccattttcgag	TUG1 3’	actcatttctgcactactga
PRPF8 5’	acttccgcttttctgcatag	TUG1 3’	tctgtgtgtactggtgaatc
PRPF8 5’	tggtctcgaatgatcttcct	TUG1 3’	tgctaggttgaactggtaca
PRPF8 5’	aacttcctgttggtcatgtc	TUG1 3’	acagtggaaacttttcttct
PRPF8 5’	catgttctccaggagtttga	TUG1 3’	ctgtgaggcaatttgagtca
PRPF8 5’	attgacgaaggaaatggctc	TUG1 3’	acaactagccttctacatca
PRPF8 5’	gtagacaggttcaatgaccc	TUG1 3’	aactgtcctgctgatctgaa
PRPF8 5’	ttttctcggcgcatcataat	TUG1 3’	agtggtcatgagtctgagag
PRPF8 5’	ggaaaacgcatcctcttgaa	TUG1 3’	ccacagtttcaacacaagca
PRPF8 5’	cggctcctcatcatcaaaag	TUG1 3’	gtccaatagcatatgttgga
PRPF8 5’	ggatgttgtcagcatagtcc	TUG1 3’	cctcaagaagtctgtaatcc
PRPF8 5’	ggtcatagaaccagtccaac	TUG1 3’	atgagagataagtttgtcct
PRPF8 5’	tggtaagtggagccatttac	TUG1 3’	gtttcttccttggttataaa
PRPF8 5’	cggtagagagtcgacatcat	TUG1 3’	ttgaatggtaacagctggca
PRPF8 5’	agtctgtcaggagctgatta	TUG1 3’	agcttaatctctgcttaaga
PRPF8 5’	gacgtaaagaaggccttcaa	PRPF8 3’	gtgacaatgatctgcttggg
PRPF8 5’	caggaatggccatattgagt	PRPF8 3’	gtgggtccagcatgcccttc
PRPF8 5’	gtctcgaacaagaggttcaa	PRPF8 3’	gggaagtccagtaagtgcac
PRPF8 5’	ctgccggatgataatcttgt	PRPF8 3’	ctccgatcctttgatgacaa
PRPF8 5’	tcttgtactcagtgcggata	PRPF8 3’	cttgagacacgcctggaaag
PRPF8 5’	aagtagaaagctggcaagtc	PRPF8 3’	tgagatccccgaatttttcc
PRPF8 5’	gggagctcaaattcctcatc	PRPF8 3’	ctggggctcagtggctttaa
PRPF8 5’	ccattggctgtattgtctgt	PRPF8 3’	catagaggttgaagagaacc
PRPF8 5’	cttaagcagcttctggtagg	PRPF8 3’	tgaaatagtcttgagccagt
PRPF8 5’	cttcttttgagccttagggg	PRPF8 3’	cgcagaatcaggatgagacg
PRPF8 5’	cagctttgtggactgaaaga	PRPF8 3’	atcgttgttcacatgtaggg

PRPF8 5’	ttttcacaggcttgaggttg	PRPF8 3’	gcttcaggatcacttttgcc
PRPF8 5’	gtggaaagcattcccaaaac	PRPF8 3’	cgaccttgatccattcttcg
PRPF8 5’	cagcttagtcaaacgcagaa	PRPF8 3’	caagatcagatccttgagct
PRPF8 5’	atactgcacgtgactatcca	PRPF8 3’	tgtttttcttgccgtagtca
PRPF8 5’	gcccaacatgggcaaatata	PRPF8 3’	gtcagtgatgccacgttcac
PRPF8 5’	tatcgatacatgcccgtcaa	PRPF8 3’	ggatgatgtctcgaatttct
PRPF8 5’	ctgtcgcatcagcttgtatt	PRPF8 3’	ggtgccgagatctccatacc
PRPF8 5’	atgaaaaagagccagactcg	PRPF8 3’	gatctgctgccgctgctgtg
PRPF8 5’	gccatcgctctaataaaggg	PRPF8 3’	ttggtctgcttctcgatctc
PRPF8 5’	ttgttactgtctttgccacc	PRPF8 3’	gttgccgtcagctgcgattg
PRPF8 5’	ggtcaaaatgtgactccact	PRPF8 3’	cttgttgacagtgcgagtct
PRPF8 5’	atatcatgcatcacagctgc	PRPF8 3’	gaggtgatgatctcatcgcc
PRPF8 5’	catattctctatgggcgtcg	PRPF8 3’	tctgggtctcatagttgctg
PRPF8 5’	aacagtcttgtccacagtgg	PRPF8 3’	tccactcagtcttggatgag
PRPF8 5’	tcctgttctgccttcagata	PRPF8 3’	tgattggtccttaggtgcag
PRPF8 5’	ggtatatactgccactgctt	PRPF8 3’	tgatgtcgtcagatgaaaca
PRPF8 5’	tttccaaccaatgcactgtg	PRPF8 3’	atgtaggtgtagccagtctc
PRPF8 5’	aagatgagcaacttggtgtc	PRPF8 3’	tcttaagcacattcttggga
PRPF8 5’	ctataagcttccttgagccg	PRPF8 3’	cgaaggtcagatatgcagat
PRPF8 5’	ctggttcaaccgagacttca	PRPF8 3’	ccccatataggtatcctgca
PRPF8 5’	ttatcgtaggcctgctcgat	PRPF8 3’	cggatctccttcacctgggg
dT	ttttttttttttttttttttttttttttttttttttttttttttttt	PRPF8 3’	actgcggcaccatcacaatg
MDN1 tiling additional	tccagcaagaagtgctccat	PRPF8 3’	caacctaagggttccatctc
MDN1 tiling additional	cgttcttggctgcgattaac	PRPF8 3’	gttgtcagccatgatcttgg
MDN1 tiling additional	tgaaggctgcttccattagg	PRPF8 3’	ataatggtcttctcgccatc
MDN1 tiling additional	aaggtgtcatggcttctgag	PRPF8 3’	cttgtaggccgtcagtgtac
MDN1 tiling additional	cagcattctgagaagcaacc	PRPF8 3’	cccattcgtagccactgggg
MDN1 tiling additional	cctattggtccttccaacaa	PRPF8 3’	ttgcccttgtctgtgttctg
MDN1 tiling additional	aagcaggtgatcaaccactg	PRPF8 3’	ctctcatagtgtgaaggcag
MDN1 tiling additional	ccaacagaactatcactcca	PRPF8 3’	gtccgacagcagcatctgca
MDN1 tiling additional	tgagaacagaggacggccgt	PRPF8 3’	ccatgaagaagccaaggaaa
MDN1 tiling additional	acacacactgcaagctgctc	PRPF8 3’	gaagttgtagttccacgagg
		PRPF8 3’	agctcatatttcatgttggg
		PRPF8 3’	ctctttggggttcgccagct
		PRPF8 3’	gcctgtgcacctcgtggtag
		PRPF8 3’	ctgcaggagagcaaagttga
		PRPF8 3’	gatccgcagagtaaacctcc

Table S2: Probe combinations used

Experiment	Combination of Probes and dyes used (From Table S1)
MDN1 5'-3'	MDN1 5' -Cy5 MDN1 3'- Dy550
MDN1 5'-tiling-3'	MDN1 5'+ MDN1 5' additional – Dy488 MDN1 tiling – Cy5 MDN 3' – Dy550
MDN1 5'-middle-3'	MDN1 5'+ MDN1 5' additional – Dy488 MDN1 middle – Cy5 MDN 3' – Dy550
PRPF8 5'-3'	PRPF8 5' -Cy5 PRPF8 3'- Cy3
POLA1 5'-3'	POLA1 5' -Cy3 POLA1 3'- Cy5
TUG1 5'-3'	TUG1 5' -Cy5 TUG1 3'- Cy3
OIP5-AS1 5'-3'	OIP5-AS1 5' -Cy5 OIP5-AS1 3'- Cy3
MDN1 tiling-3'	MDN1 tiling + MDN1 tiling additional– Cy5 MDN1 3'– Dy550
MDN1 5'-3'-dT	MDN1 5' -Cy5 MDN1 3'- Dy550 dT – Cy2
MDN1 tiling-3'-dT	MDN1 tiling + MDN1 tiling additional– Cy5 MDN1 3'– Dy550 dT- Cy2
PRPF8 5'-3'-dT	PRPF8 5' -Cy5 PRPF8 3'- Cy3 dT – Cy2
POLA1 5'-3'-dT	POLA1 5' -Cy3 POLA1 3'- Cy5 dT – Cy2
TUG1 5'-3'-dT	TUG1 5' -Cy5 TUG1 3'- Cy3 dT – Cy2
OIP5-AS1 5'-3'-dT	OIP5-AS1 5' -Cy5 OIP5-AS1 3'- Cy3 dT – Cy2

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