RNA Name	Sequence	Source
A_{19}-Cy3	AAAAA AAAAA AAAAA AAAA-Cy3	IDT
C_{19}-Cy3	CCCCC CCCCC CCCCC CCCC-Cy3	IDT
G_5-Alexa488	GGGGG-Alexa488	IDT
U_{19}-Cy3	UUUUU UUUUU UUUUU UUUU-Cy3	IDT
PTR-Cy3	UCUCU AAAAA UCUCU AAAAA UCUCU AAAAA UCUCU AAAAA-Cy3	IDT (see Van Treeck et al., 2018)

Table S1. Sequences of fluorescent RNA oligonucleotides used in this study. *See "Fluorescent RNA nucleotides" in the Key Resources table.*

Probe Name	Source	Identifier/Reference
Oligo(dT)-Cy3	IDT	Khong et al., 2017a
Oligo(dT)-Cy5	IDT	Khong et al., 2017a
Human *POLR2A* Quasar 570 smFISH probe set	Biosearch Technologies	Khong et al., 2017a Stock number SMF-2003-1
Human *TFRC* Quasar 570 smFISH probe set	Biosearch Technologies	Khong et al., 2017a Stock number SMF-2006-1
Human *NORAD* Cy5 smFISH probe set	Biosearch Technologies (design), IDT (synthesis)	Khong et al., 2017a
Human *AHNAK* Cy5 smFISH probe set	Biosearch Technologies (design), IDT (synthesis)	Khong et al., 2017a; Khong and Parker, 2018
Human *DYNH1C1* Cy5 smFISH probe set	Biosearch Technologies (design), IDT (synthesis)	Khong et al., 2017a; Khong and Parker, 2018
Human *PEG3* Cy5 smFISH probe set	Biosearch Technologies (design), IDT (synthesis)	Khong et al., 2017a
Human *MCM2* Cy5 smFISH probe set	Biosearch Technologies (design), IDT (synthesis)	This study See Table S5 for probe sequences

Table S2. DNA probes for (single molecule) fluorescence in situ hybridization used in this study. *See "Fluorescent in situ hybridization probes" in the Key Resources table.*

RNA Name	Source	Identifier/Reference
polyA homopolymer RNA, potassium salt	Amersham Pharmacia Biotech	Van Treeck et al., 2018
polyC homopolymer RNA, potassium salt	Amersham Pharmacia Biotech	Van Treeck et al., 2018
polyU homopolymer RNA, potassium salt	Amersham Pharmacia Biotech	Van Treeck et al., 2018
polyA homopolymer RNA, potassium salt	Sigma-Aldrich	Stock number P9403
polyC homopolymer RNA, potassium salt	Sigma-Aldrich	Stock number P4903
polyU homopolymer RNA, potassium salt	Sigma-Aldrich	Stock number P9528

Table S3. Homopolymer RNA potassium salts used in this study. *See "Homopolymer RNA Potassium Salts" in the Key Resources table.*

Primer Name	Sequence	Source
EIF4A1 E183Q Forward Primer	CAAGATGTTTGTACTGGATCAAGCTGACGAAATGTTAAGC	IDT
EIF4A1 E183Q Reverse Primer	GCTTAACATTTCGTCAGCTTGATCCAGTACAAACATCTTG	IDT
EIF4A1 R362Q Forward Primer	CCAAACCGTCCACCTTGACCGATTCTGTGG	IDT
EIF4A1 R362Q Reverse Primer	CCACAGAATCGGTCAAGGTGGACGGTTTGG	IDT
EIF4A1 T158Q Forward Primer	CCACATCATCGTGGGTGACCCTGGCCGTGTGTTTG	IDT
EIF4A1 T158Q Reverse Primer	CAAACACACGGCCAGGGTCACCCACGATGATGTGG	IDT

Table S4. Sequences of primers used in this study. *See "Primers for eIF4A1 mutations" in the Key Resources table.*

FISH Probe Name	Sequence
MCM2_1	cgaccagaagggacacggag
MCM2_2	tacggaacgcgacgtcatga
MCM2_3	cgcgaaaagtggttcacgtg
MCM2_4	ctacagcaacaaccaggttt
MCM2_5	atagcagtaccacgatcctc
MCM2_6	gaaggattccgatgattccg
MCM2_7	gaggtgagggcatcagtacg
MCM2_8	atcctcaaatggtggaaggt
MCM2_9	tacgtcctcatcatccagag
MCM2_10	tcatcgctgtcatacaggag
MCM2_11	ttctcgatgctctcgatcat
MCM2_12	acagagtggcctttgagatc
MCM2_13	tgcgcaggaagttcttgaag
MCM2_14	tgatgcgctccttgaagacg
MCM2_15	cacggttctctttgcacatg
MCM2_16	catcaaagatctgcagcagc
MCM2_17	gtcgtacttggggtacatgg
MCM2_18	catggatgtggttggtgatg
MCM2_19	atcagctggttcagatgcag
MCM2_20	cacttgttgcagttgtactt
MCM2_21	cagaaaggacccaggacgaa
MCM2_22	actggcactcaggacaggag
MCM2_23	ctccatgttgacctcaaagg
MCM2_24	cgctggtagttctgatagat
MCM2_25	ttgtgatagatgccagtcag
MCM2_26	tgttgagggagccatcatag
MCM2_27	gcaaagacagggaagccatt
MCM2_28	gtggttggctaggatgacag
MCM2_29	caaccttgttgtccttcttg
MCM2_30	cttgatgtcttcatgaccat
MCM2_31	cgcacaagagcacgttgata
MCM2_32	gagaaactgcgacttcgctg
MCM2_33	ggctggacactttctcaata
MCM2_34	tcaatgagacacactcctcg
MCM2_35	ctggtcattcatcttgtcaa
MCM2_36	ttcgagatggagatgctctg
MCM2_37	tcagagaaagtcagcgaggg
MCM2_38	cacacaggatgtcaaagcgt
MCM2_39	cttggcgtagatgatgtact
MCM2_40	tcaggtcactgtacatcttg

MCM2_41	cggatcatggactcgatgtg
MCM2_42	tcgtcttcgatcacatagtc
MCM2_43	tgtgtgtctatgaagctctc
MCM2_44	caaaagtcttgcgcatgctg
MCM2_45	tcacgccggaatgaaaggta
MCM2_46	aaagcggttgcgctgatatg
MCM2_47	tggatgttgatctgacgagc
MCM2_48	acttgttcatcctgaagagc
MCM2_49	agaatcccaaggaatcctta
MCM2_50	cataaagcacagagggcact
MCM2_51	catcaagtgctctggttttg
MCM2_52	aacaaacagtcatgccaggt
MCM2_53	taagacacactggcaaggca
MCM2_54	gtggcaagatgttcagcaac
MCM2_55	agtggagacaaagcactcgg
MCM2_56	tgaactcagcagctctgatc
MCM2_57	aacacctaaaccacacgcag
MCM2_58	tctcctgacatccatgtaag
MCM2_59	aacatcttcaaccagctctc
MCM2_60	gataatccgcatgactcaca

Table S5. Sequences of pooled smFISH probes targeting human *MCM2* mRNA. Pooled probes were 3' end labeled. *See "Fluorescent in situ hybridization probes" in the Key Resources table.*