Click-encoded rolling FISH for visualizing single-cell RNA polyadenylation and structures

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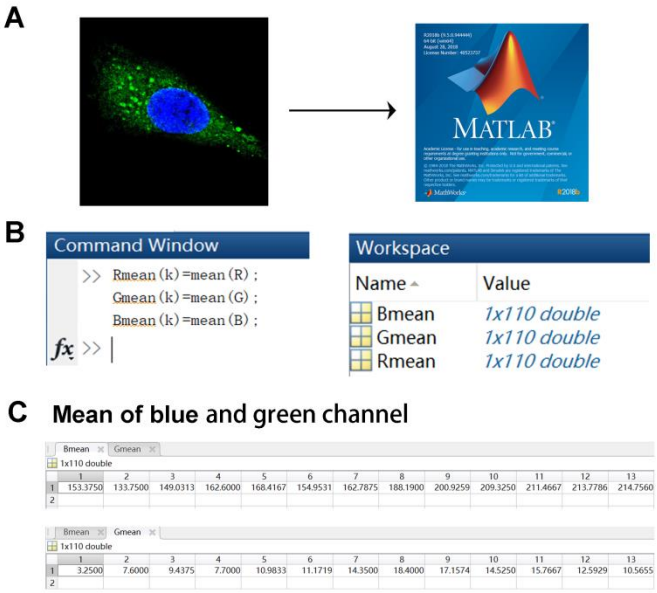
The codes and commands in MATLAB are shown below:



Note: (A) The single cell was opened by MATLAB. (B) The commands of average intensity within a series of annuli containing three color channels were entered in the command line window (R: red, G: green, and B: blue). (C) The form of mean of blue and green channel (green: RNA polyadenylation signal, blue: DAPI signal).

Table S1. Sequence information for oligonucleotides used in this study**.**

Name	Sequences (5′-3′)
	Probes in ClickerFISH
N_3-barcode	**N_3**-GCTATGTTTCTTGAGGAGGGCAGCAA
Linker 1 (L1)	TTTCTTGAGGAGGGCAGCAA
Padlock 1 (P1)	**P**-CCTCAAGAAATCGACGCGTATAATAATGCTGGGCTCTAGTAGGTCTTGCTGCCCT
DBCO-barcode	**DBCO**-GGAGTGCAGCAAACGGGAAGAGTCTT
Linker 2 (L2)	CAGCAAACGGGAAGAGTCTT
Padlock 2 (P2)	**P**-CCGTTTGCTGTGGCCTGAGCCTTCCTCGGTACGGTCTGTAAGGTCAAGACTCTTC
NH_2-barcode	**NH_2**-ATCCTCGTAAATCCTCATCAATCATC
Linker 3 (L3)	GTAAATCCTCATCAATCATC
Padlock 3 (P3)	**P**-GAGGATTTACTCGACAGAGCTTACTCACAGCCAGCATCACAAGGTCGATGATTGAT
FAM-probe	GCGTATAATAATGCTGGGCTCTAGT-**FAM**
Cy5-probe	**Cy5**-TGAGCCTTCCTCGGTACGGTCTGTA
Cy3-probe	**Cy3**-AGAGCTTACTCACAGCCAGCATCACA
	Probes in smFISH for NUP43 mRNA
Guide-1	ACTAGAGCCCAGCATTATTATACGCCGCAGCAGGTACTGCAAAAA
Guide-2	ACTAGAGCCCAGCATTATTATACGCACTTCGCATAAATTTCCTCC
Guide-3	ACTAGAGCCCAGCATTATTATACGCTTTGCTGATTTTCTGGGACA
Guide-4	ACTAGAGCCCAGCATTATTATACGCAAGATCCTGTAGCGAACGTC
Guide-5	ACTAGAGCCCAGCATTATTATACGCATCACCATGGTGTCTGATAT
Guide-6	ACTAGAGCCCAGCATTATTATACGCCTGCTGGTTGACTGACAGAG
Guide-7	ACTAGAGCCCAGCATTATTATACGCCCTCTCCAACTGTAACGATT
Guide-8	ACTAGAGCCCAGCATTATTATACGCACAGCATGGAGTGTACTACT
Guide-9	ACTAGAGCCCAGCATTATTATACGCATCCCATATTTTCAACTGTC
Guide-10	ACTAGAGCCCAGCATTATTATACGCTATCAACACAGTGGAGTGGC
Guide-11	ACTAGAGCCCAGCATTATTATACGCAACATGCTGTTGGTTGGGAT
Guide-12	ACTAGAGCCCAGCATTATTATACGCCCATCTTCAGAGCAGGTAAA
Guide-13	ACTAGAGCCCAGCATTATTATACGCGCTTGTGATTTCAATTCGGT
Guide-14	ACTAGAGCCCAGCATTATTATACGCGTTCACAGACAGAGACCTAC
Guide-15	ACTAGAGCCCAGCATTATTATACGCGGCTAATTGCATGTTCTATC
Guide-16	ACTAGAGCCCAGCATTATTATACGCGGATTCTACAACTGTTTGGG
Guide-17	ACTAGAGCCCAGCATTATTATACGCCCACAAGCTGTCTGTCAATA
Guide-18	ACTAGAGCCCAGCATTATTATACGCCCATAACCACCATTGATGTG
Guide-19	ACTAGAGCCCAGCATTATTATACGCATTGTAGGAACCCATTGGTA
Guide-20	ACTAGAGCCCAGCATTATTATACGCAGGTGTATATGCCACATACT
Guide-21	ACTAGAGCCCAGCATTATTATACGCTTAACATTGGCAACTGCCAC
Guide-22	ACTAGAGCCCAGCATTATTATACGCAGCTGAAAGAGTTCTGCTCA
Guide-23	ACTAGAGCCCAGCATTATTATACGCATTGCACAATGTCCAGTTTG
Guide-24	ACTAGAGCCCAGCATTATTATACGCTGGTAACACTATAGGTGGCT
Guide-25	ACTAGAGCCCAGCATTATTATACGCTAGTTCATTATTATCCCTGC
Guide-26	ACTAGAGCCCAGCATTATTATACGCTGTCATTAAGGTCTCAGGTC
Guide-27	ACTAGAGCCCAGCATTATTATACGCGGCAAAACTTGTCAATTGGC
Guide-28	ACTAGAGCCCAGCATTATTATACGCCACTCTATTTGACAGTTTCA
Guide-29	ACTAGAGCCCAGCATTATTATACGCCTCACACACTAAAACCCTGC
Guide-30	ACTAGAGCCCAGCATTATTATACGCGGATTGAGTGTTTTGGATCC
Guide-31	ACTAGAGCCCAGCATTATTATACGCACAATAGGACTGTACCCTTT
Guide-32	ACTAGAGCCCAGCATTATTATACGCCTCACATCATCAGTGTGTTA
Guide-33	ACTAGAGCCCAGCATTATTATACGCAACAACTGGTGATTGGCTCA
Guide-34	ACTAGAGCCCAGCATTATTATACGCAAAGCGTCCCAACACAAAGC
Guide-35	ACTAGAGCCCAGCATTATTATACGCTAATGACAGACTGGTGAACA
Guide-36	ACTAGAGCCCAGCATTATTATACGCTGATTTTCTTCCACATGTTC
Guide-37	ACTAGAGCCCAGCATTATTATACGCTCATAGGAAATACTTGCAAT
Guide-38	ACTAGAGCCCAGCATTATTATACGCGTTCAAATGTGAAACTGTAT
FAM-probe	GCGTATAATAATGCTGGGCTCTAGT-**FAM**

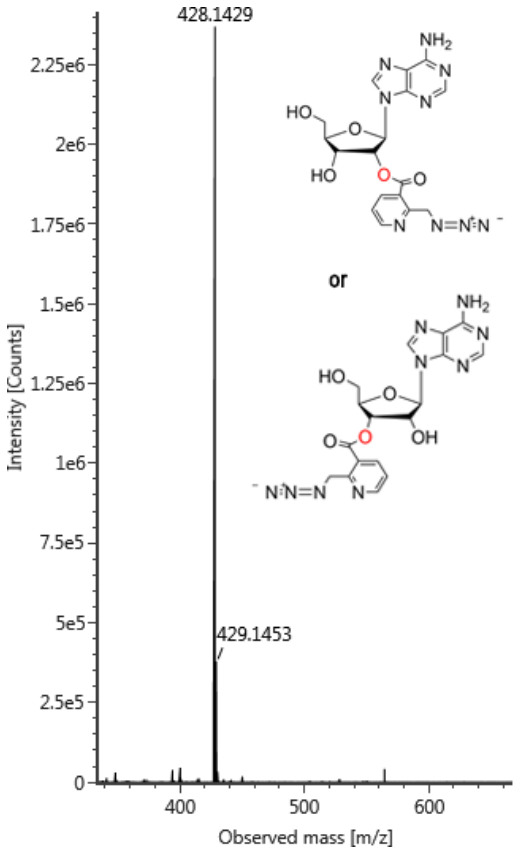


Figure S1. Mass spectrum of the acylation of ATP at 2′ or 3′ hydroxyl by NAI-N_3. Calculated mass of $[C_{17}H_{18}N_9O_5]^+$ =428.1425, found: 428.1429.

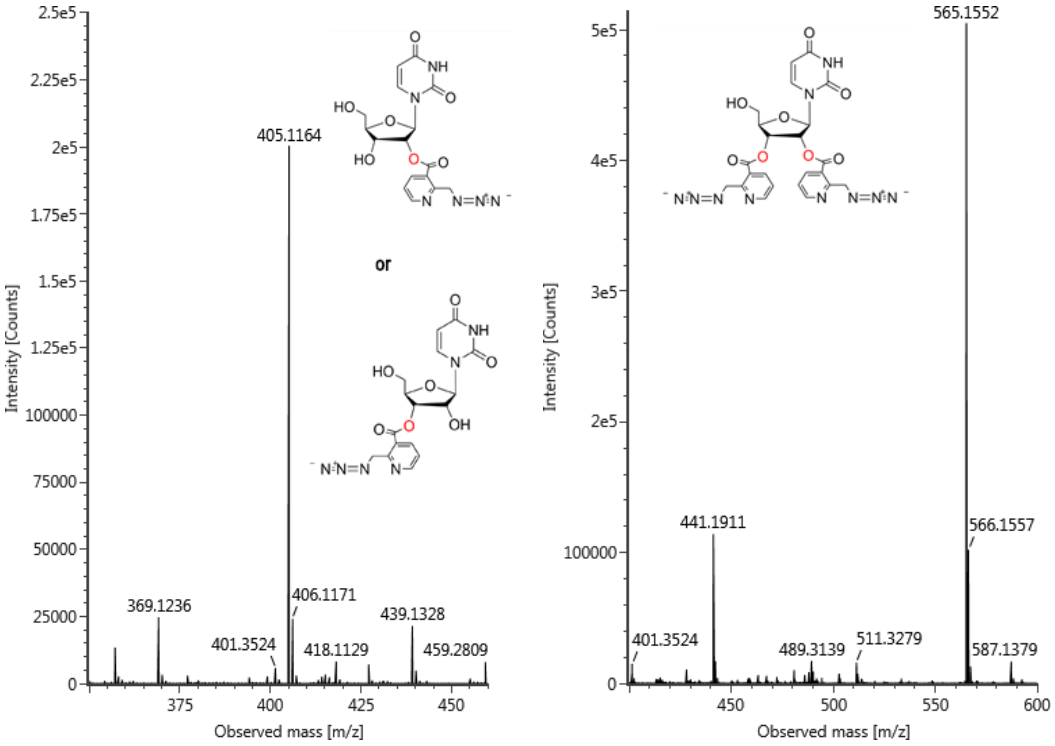


Figure S2. Mass spectrum of the acylation of UTP by NAI-N_3 (left, calculated mass of $[C_{16}H_{17}N_6O_7]^+$ =405.1153, found: 405.1164) and UTP-2NAI-N_3 (right, calculated mass of $[C_{23}H_{21}N_{10}O_8]^+$ =565.1538, found: 565.1552).

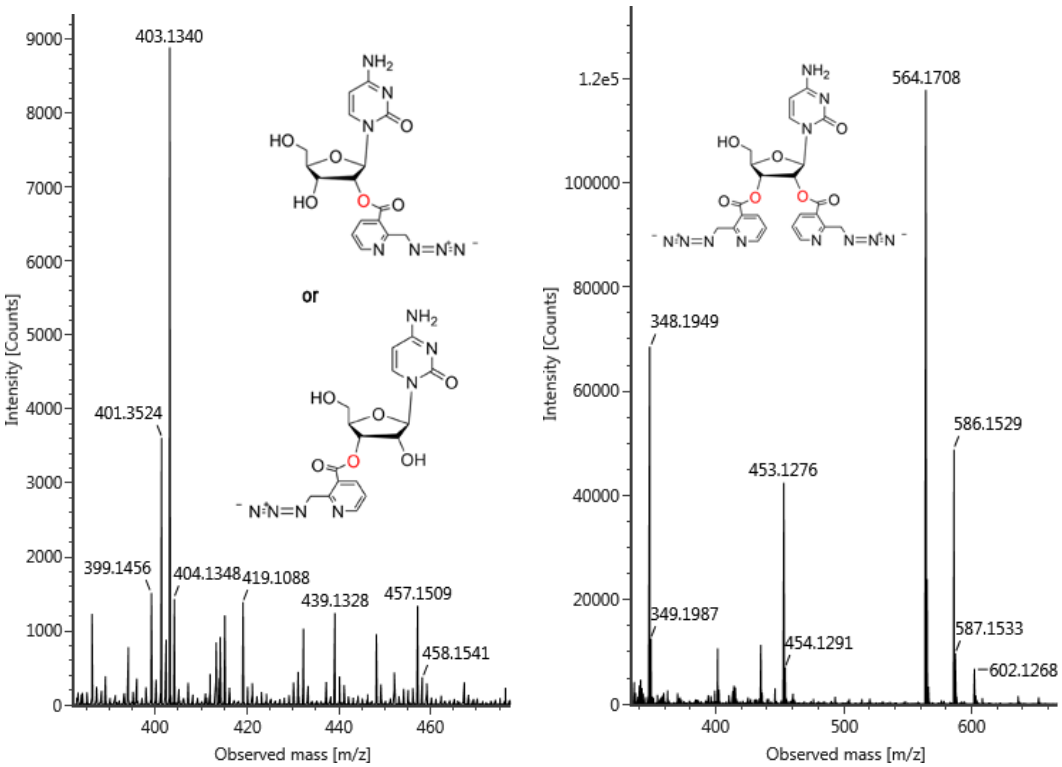


Figure S3. Mass spectrum of the acylation of CTP by NAI-N_3 (left, calculated mass of $[C_{16}H_{18}N_7O_6]^+$ =404.1313, found: 404.1348) and CTP-2NAI-N_3 (right, calculated mass of $[C_{23}H_{22}N_{11}O_7]^+$ =564.1698, found: 564.1708).

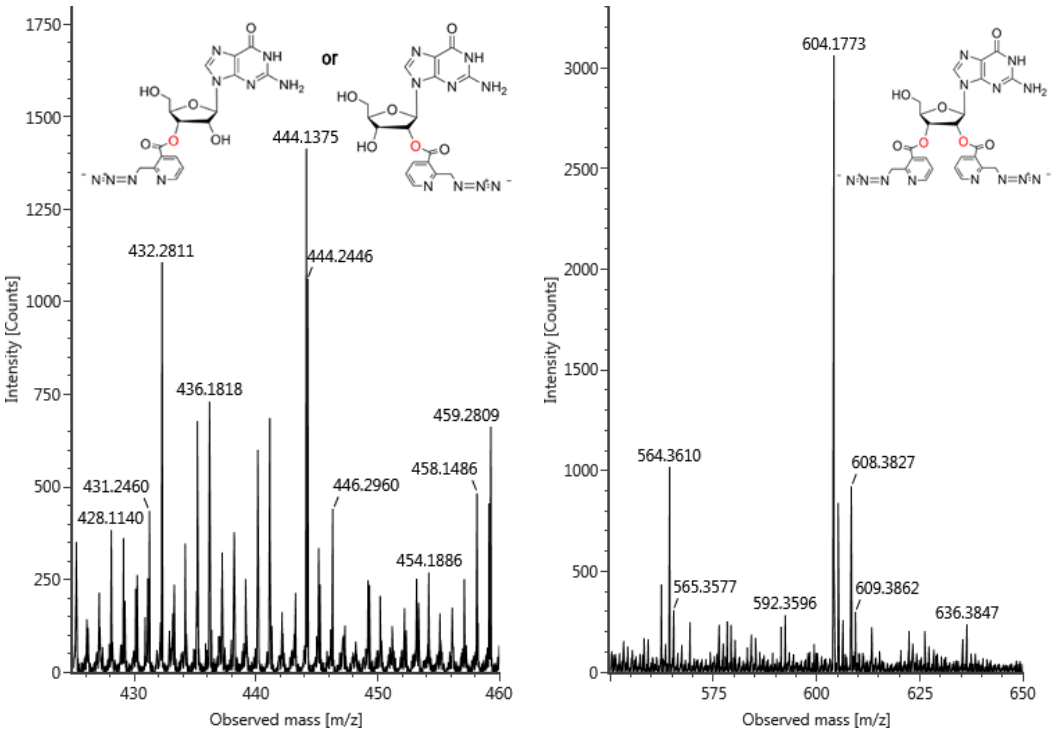


Figure S4. Mass spectrum of the acylation of GTP by NAI-N_3 (left, calculated mass of $[C_{17}H_{18}N_9O_6]^+$ =444.1375, found: 444.1375) and GTP-2NAI-N_3 (right, calculated mass of $[C_{24}H_{22}N_{13}O_7]^+$ =604.1760, found: 604.1773).

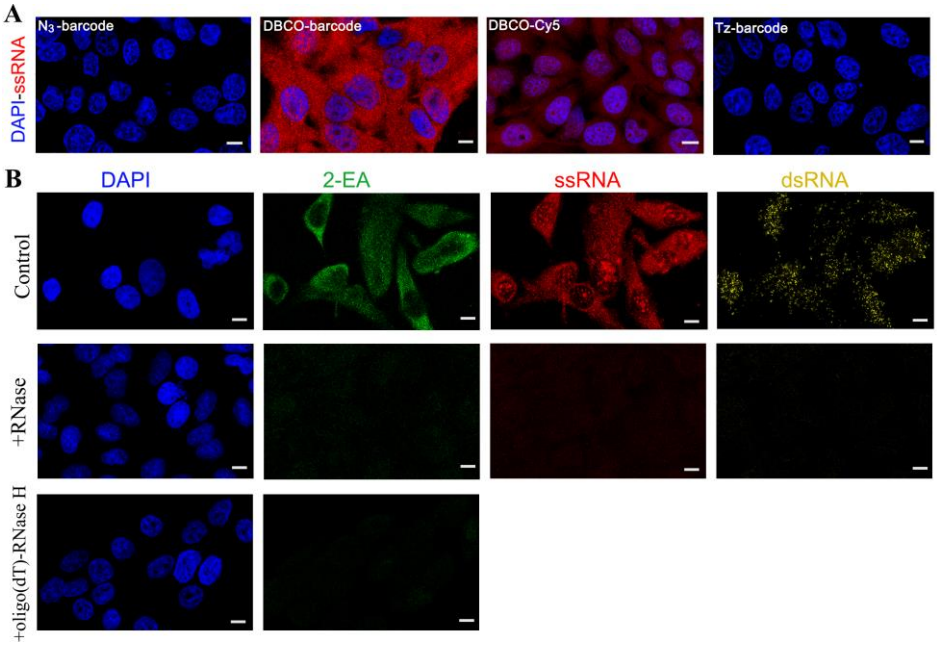


Figure S5. (A) Representative cell images by DBCO-barcode-specific ClickerFISH for mapping flexible or single-stranded RNA regions (red, RNA single-stranded regions; blue, DAPI). The sample treating with DBCO-cy5 is the control of non-amplifying system. Other two negative controls of N_3-barcode and Tz-barcode demonstrated the sequence specificity of ClickerFISH. The scale bars of all cell images in this work is 10 μm. (B) Negative ClickerFISH experiments of cell samples treated with RNase I or oligo(dT)-RNase H prior to ClickerFISH reactions.

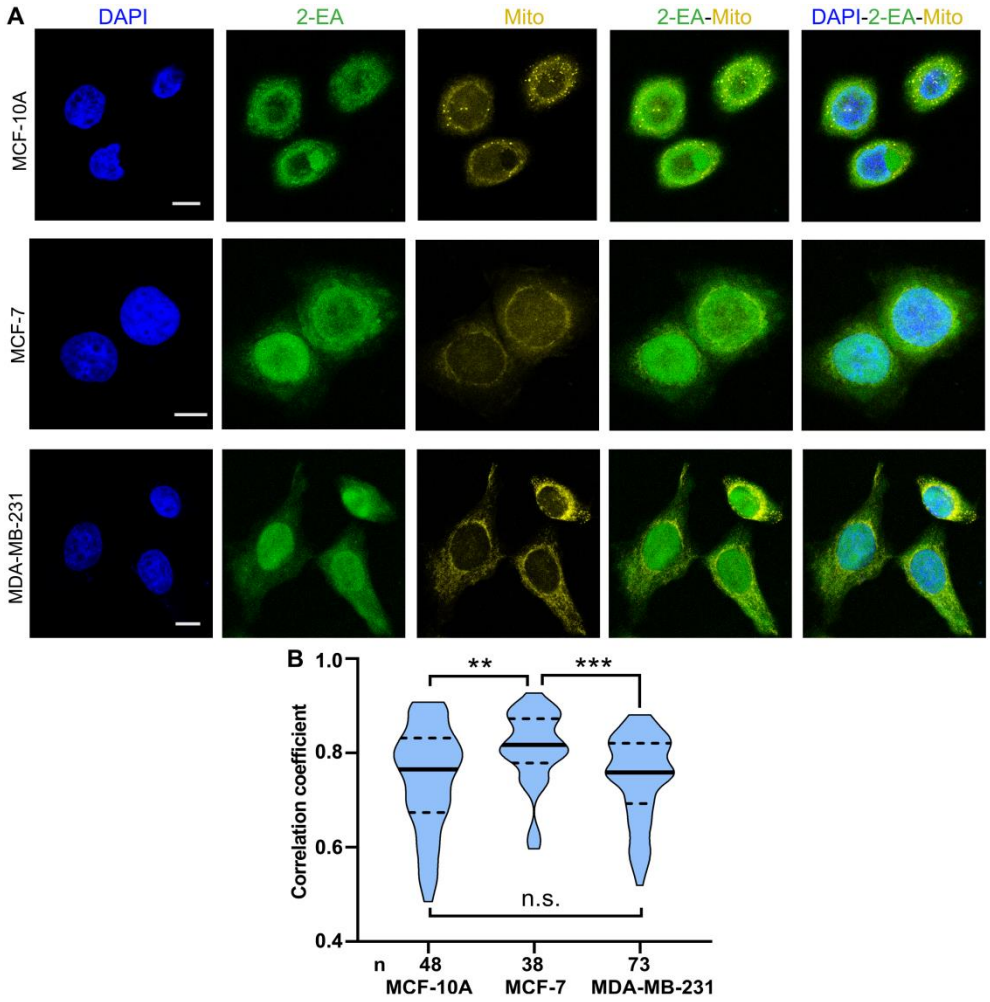


Figure S6. ClickerFISH for polyadenylation labeling with MitoTracker imaging to study mitochondrial RNA processing. (A) Representative cell images. (B) The violin plot represents of the correlation coefficients between RNA polyadenylation signal and MitoTracker signals. **(p<0.005), ****(*P*<0.0001), Student t-test.

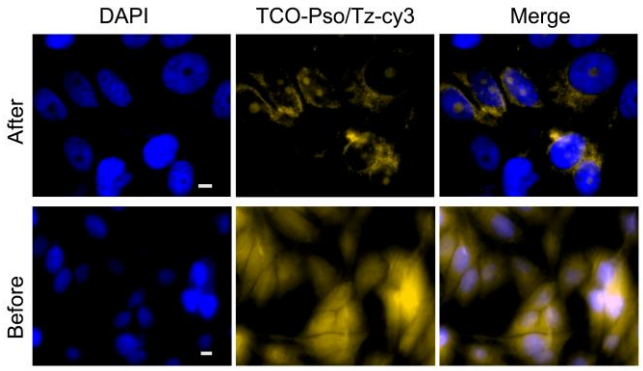


Figure S7. Images of cells treated with TCO-Pso and Tz-cy3 after or before DAPI staining. Above, the fixed cells were firstly incubated with 0.5 μg/mL DAPI for 20 min at room temperature. After three washes, these cells were incubated with 0.4 mM TCO-Pso in PBS for 10 min and then were irradiated with 365 nm UV radiation for 20 min. The click reaction was performed by incubating cells with 5 mM Tz-cy3 for 1 h at 37 ºC in the dark. Below, the fixed cells were firstly incubated with 0.4 mM TCO-Pso in PBS for 10 min and then were irradiated with 365 nm UV radiation for 20 min. The click reaction was performed by incubating cells with 5 mM Tz-cy3 for 1 h at 37 ºC in the dark. After three washes, the cells were stained with 0.5 μg/mL DAPI for 20 min at room temperature. Scale bar=10 μm. The fluorescence images were acquired using a fluorescent inverted microscope (Eclipse Ti, Nikon).

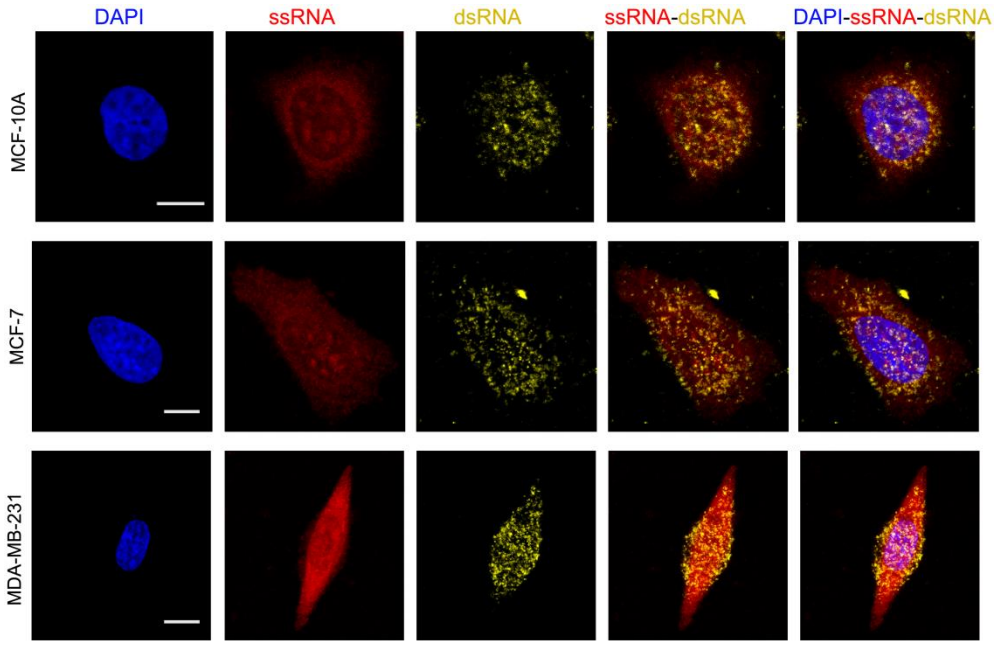


Figure S8. Representative cell images of RNA structures analysis by ClickerFISH.

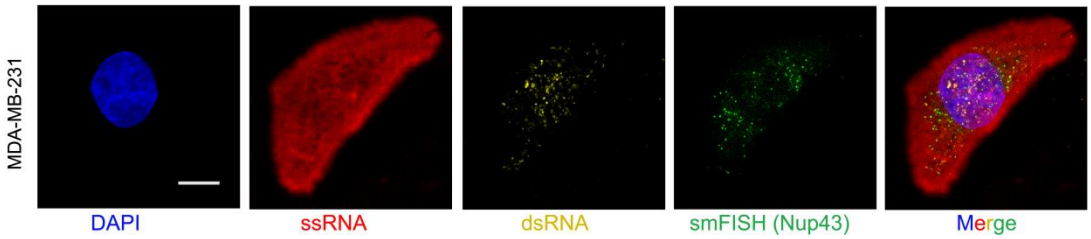


Figure S9. Representative cell images of combining ClickerFISH with smFISH of Nup43 mRNA from MDA-MB-231.

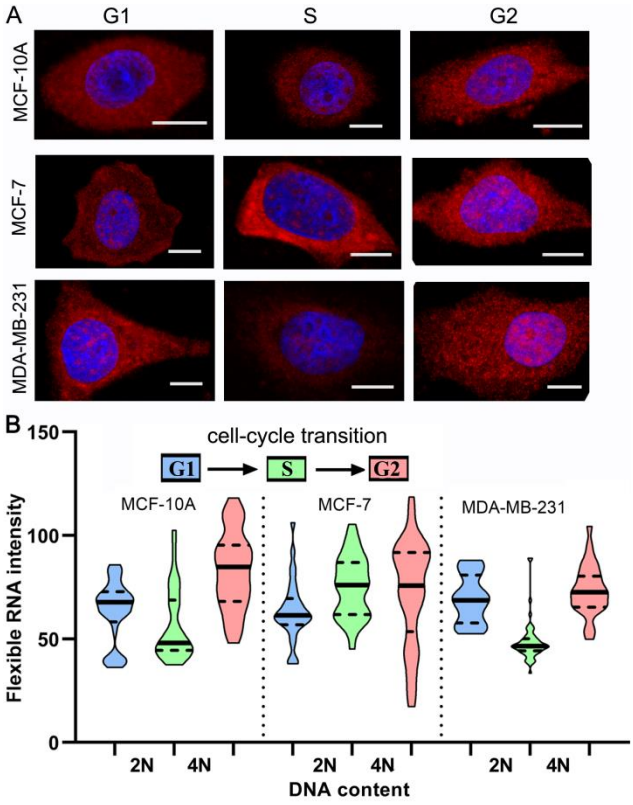


Figure S10. Cell-cycle-dependent RNA flexible regions. (A) Representative cell images (red, fluorescence of flexible regions; blue, DAPI; scale bar, 10 μm). (B) The violin plot (G1, blue; S, green; G2, pink) depicting RNA flexible regions intensity distribution. The cell numbers are 43, 49, 39, 30, 33, 50, 27, 57 and 28, respectively (from left to right).

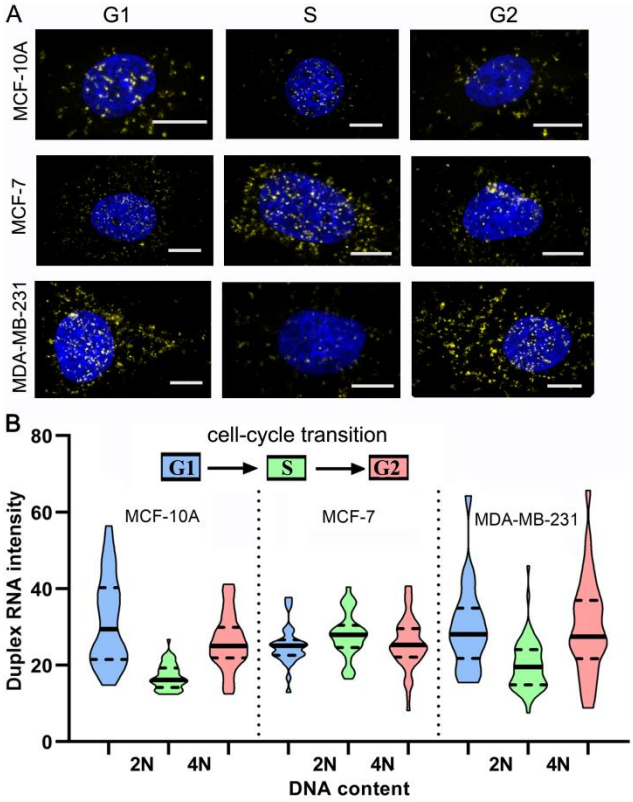


Figure S11. Cell-cycle-dependent RNA duplex structures. (A) Representative cell images (yellow, fluorescence of duplex structures; blue, DAPI; scale bar, 10 μm). (B) The violin plot (G1, blue; S, green; G2, pink) depicting RNA duplex structures intensity distribution. The cell numbers are 43, 49, 39, 30, 33, 50, 27, 57 and 28, respectively (from left to right).