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Spatial imaging of glycoRNA in single cells with ARPLA

In the format provided by the authors and unedited

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Supplementary Table 1. DNA sequences used in the experiments (5´ to 3´).

Oligonucleotide names	Oligonucleotide sequences
RNA binding probe for U1 glycoRNA - 1	**CTG GGA AAA CCA CCT TCG TGA TCA TGG TAT CTC CCC TGC CAG GTA AGT AT**A AAA AAA AAA *TAT GAC AGA ACT AGA CAC TCT T*
RNA binding probe for U1 glycoRNA - 2	**CGA ACG CAG TCC CCC ACT ACC ACA AAT TAT GCA GTC GAG TTT CCC ACA TT**A AAA AAA AAA *TAT GAC AGA ACT AGA CAC TCT T*
RNA binding probe for U3 glycoRNA - 1	**TCT TCC TCG TGG TTT TCG GTG CTC TAC ACG TT**A AAA AAA AAA *TAT GAC AGA ACT AGA CAC TCT T*
RNA binding probe for U3 glycoRNA - 2	**CTC CCC AAT ACG GAG AGA AG**A AAA AAA AAA *TAT GAC AGA ACT AGA CAC TCT T*
RNA binding probe for U8 glycoRNA	**GTT CTA ATC TGC CCT CCG GAG GAG GAA CAG GTA AGG ATT ATC CCA CC**A AAA AAA AAA *TAT GAC AGA ACT AGA CAC TCT T*
RNA binding probe for U35a glycoRNA - 1	**AGA CCA TCG TGA GAT AAG** AAA AAA AAA A*TA TGA CAG AAC TAG ACA CTC TT*
RNA binding probe	**CTC CTG GCA TCA GCT AAG** AAA AAA AAA A*TA TGA CAG AAC TAG ACA CTC TT*

for U35a glycoRNA - 2	
RNA binding probe for Y5 glycoRNA - 1	**GGG AGA CAA TGT TAA ATC** AAA AAA AAA A*TA TGA CAG AAC TAG ACA CTC TT*
RNA binding probe for Y5 glycoRNA - 2	**AAA ACA GCA AGC TAG TC**A AAA AAA AAA *TAT GAC AGA ACT AGA CAC TCT T*
RNA binding probe w/o RISH-R	A AAA AAA AAA *TAT GAC AGA ACT AGA CAC TCT T*
Glycan probe	**TAG GGA ATT CGT CGA CGG ATC CCG TGG CGT CTG CAA CGG AAA AGA ATT TAT CTT GTC CTG CAG GTC GAC GCA TGC GCC G**AA AAA AAA AA*G ACG CTA ATA GTT AAG ACG CTT rUrUrU*
Glycan probe w/o Aptamer-G	AA AAA AAA AA*G ACG CTA ATA GTT AAG ACG CTT rUrUrU*
Glycan probe using DNA with scrambled sequence	**CGG CGC ATG CGT CGA CCT GCA GGA CAA GAT AAA TTC TTT TCC GTT GCA GAC GCC ACG GGA TCC GTC GAC GAA TTC CCT A**AA AAA AAA AA*G ACG CTA ATA GTT AAG ACG CTT rUrUrU*

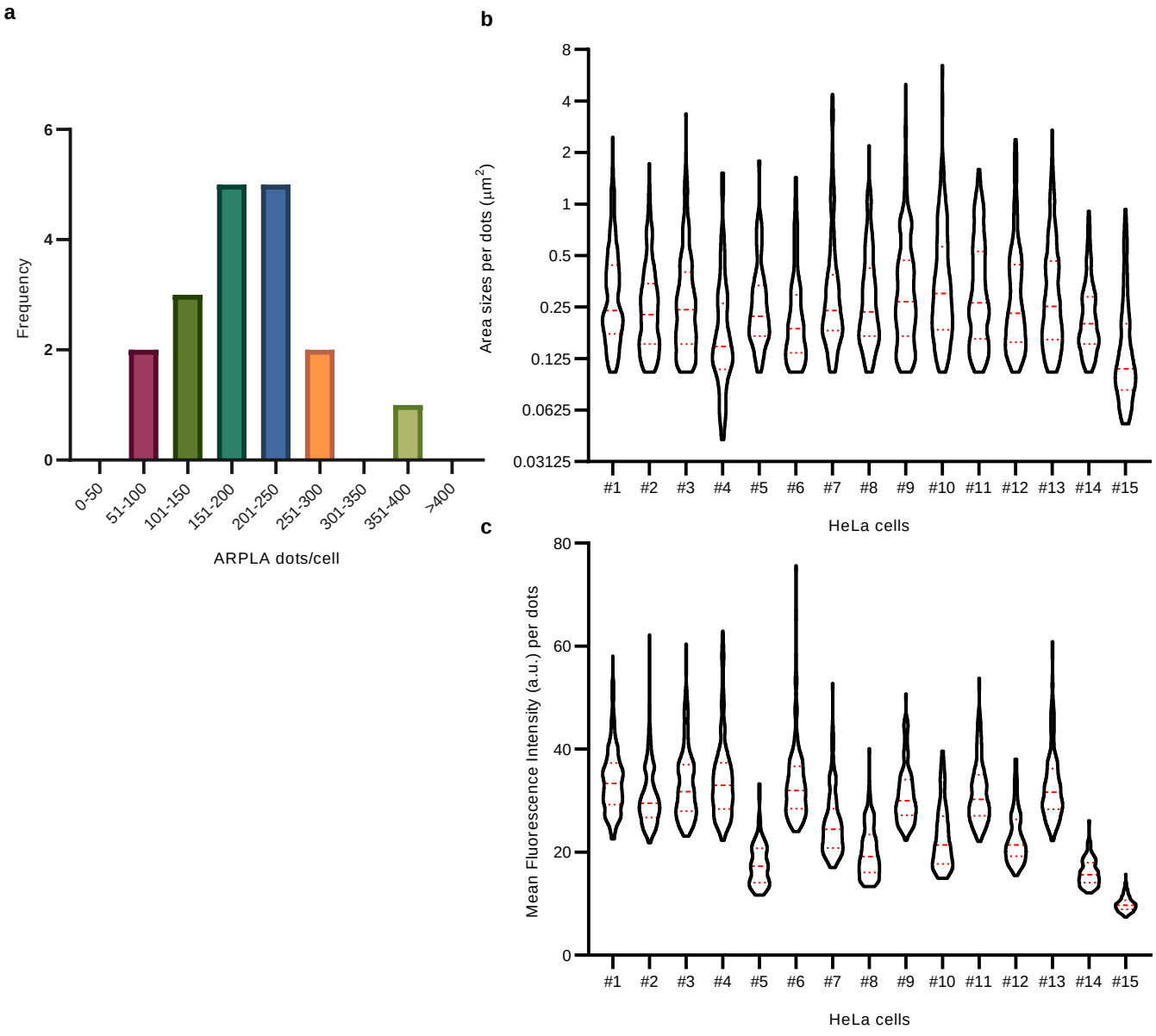
Glycan probe using Tn antigen aptamer	**GAG ACA AGA ATA AAC GCT CAA GGC TAT AGC ACA TGG GTA AAA CGA CTT CGA CAG GAG GCT CAC AAC AGG C**AA AAA AAA AA*G ACG CTA ATA GTT AAG ACG CTT rUrUrU*
Glycan probe using GalNAc aptamer	**GAG ACA AGA ATA AAC GCT CAA AAG GGA TGA CAG GAT ACG CCA AGC TTT CGA CAG GAG GCT CAC AAC AGG C**AA AAA AAA AA*G ACG CTA ATA GTT AAG ACG CTT rUrUrU*
Connector 1	Phosphate-CT ATT AGC GTC CAG TGA ATG CGA GTC CGT CTA AGA GAG TAG TAC AGC AGC CGT CAA GAG TGT CTA
Connector 2	Phosphate-GT TCT GTC ATA TTT AAG CGT CTT AA
poly T oligonucleotides	TTT TTT TTT TTT TTT TTT TTT TT
Reporter	Alexa 647-CA GTG AAT GCG AGT CCG TCT
Neu5Ac aptamer	TAG GGA ATT CGT CGA CGG ATC CCG TGG CGT CTG CAA CGG AAA AGA ATT TAT CTT GTC CTG CAG GTC GAC GCA TGC GCC G

Note:

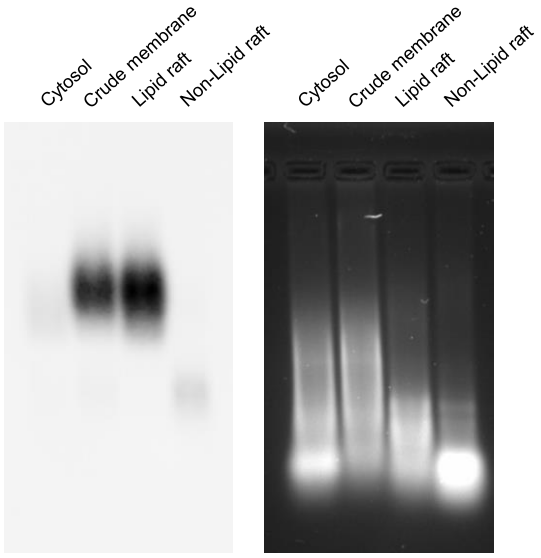
(1) glycan probe with 3 sections: a) Neu5Ac aptamer (Aptamer), bold; b) spacer sequence (Spacer), underline; c) DNA linker (Linker G), Italic.

(2) RNA binding probe with 3 sections: a) RNA *in situ* hybridization probe (RISH), bold; b) spacer sequence (Spacer), underline; c) DNA linker (Linker R), Italic.

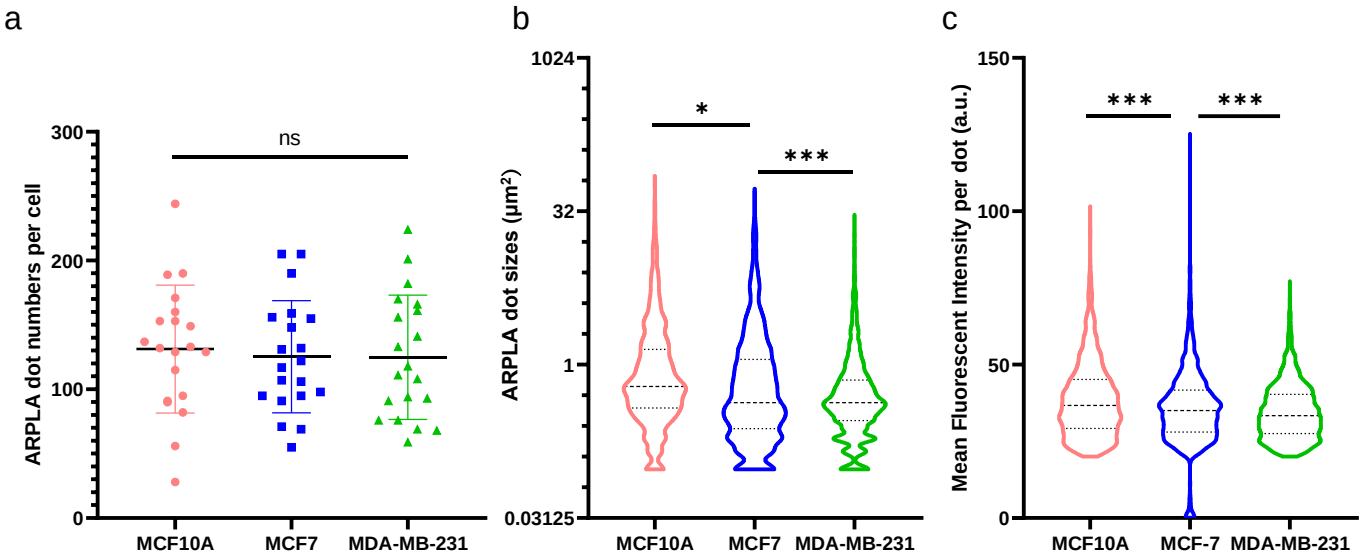
Supplementary Figure 1. Quantification of ARPLA signal dots for individual cells, using U1 glycoRNA in HeLa cells as an example. The amounts (a), sizes (b), and mean fluorescence intensities (c) of ARPLA signal dots from n=15 single cells in the same batch of an experiment. Violin plots in b and c were plotted from individual ARPLA dots n=359 (#1), n=231 (#2), n=228 (#3), n=210 (#4), n=71 (#5), n=155 (#6), n=103 (#7), n=162 (#8), n=259 (#9), n=129 (#10), n=161 (#11), n=156 (#12), n=255 (#13), n=97 (#14), and n=208 (#15). Violin plots lines at the median (dotted line) and quartiles (dashed lines).



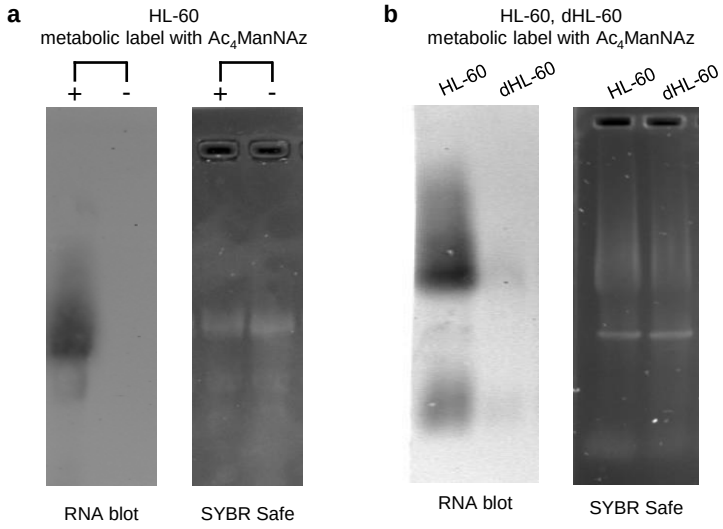
Supplementary Figure 2. GlycoRNA gel blot in HeLa cell subfractions. Blotting of RNA from the cytosol, crude membrane, lipid rafts, and non-lipid rafts membranes in HeLa cells after metabolic labeling with Ac4ManNAz. Similar results were obtained from 3 independent biological replicates.



Supplementary Figure 3. Single cell analysis of ARPLA dots in breast cell lines. For U1 glycoRNA, the ARPLA punctate numbers (a), sizes (b), and mean fluorescence intensities (c) in MCF-10A, MCF-7, and MDA-MB-231 were analyzed and compared. 20 single cells were quantified for each cell line. All three cell lines showed similar numbers of ARPLA signal dots of U1 glycoRNA in individual cells (a). Whereas MCF-10A cells showed the largest size (b) and highest intensity (c) in ARPLA signals, followed by MCF-7 cells, while the MDA-MB-231 have the smallest size and lowest intensity in signals. Dot plot (a) is presented as mean values ±SD. The statistical significance is determined by One-way ANOVA as (ns) p=0.8913, n=20 cells. Violin plots (b, c) describing the individual dot sizes and intensities were calculated from n=2017 dots (MCF10A), n=2506 dots (MCF7), and n=2497 dots (MDA-MB-231). Violin plots lines at the median (dotted line) and quartiles (dashed lines). Statistic assays are performed with the two-tailed unpaired t-test. In (b), * p=0.0106, *** p<0.0001; In (c), both p<0.0001.



Supplementary Figure 4. Investigation of total glycoRNA level during HL-60 differentiation by RNA blot. (a) Blotting of total RNA from HL-60 cells after metabolic labeling with Ac_4ManNAz, or HL-60 cells without metabolic labeling; (b) The total glycoRNA level during HL-60 differentiation, tested by RNA blot. Agarose gel electrophoresis image of total RNA from HL-60, dHL-60. These cells were treated with Ac_4ManNAz for 48 h before RNA extraction.



Supplementary Figure 5. Estimation of the limit of resolution. U1 glycoRNA ARPLA of HeLa image was analyzed and four typical pairs of signal dots were picked. The intensity of dots along the lines (red, with arrow) were measured and fitted with Gaussians fitting (i, ii, iii, iv). As shown in (i), the distance over 600 nm can be distinguished easily. The distances in (ii) and (iii) are around 320-360 nm, at this range ARPLA can still resolve two individual dots, whereas the signals started to merge and became difficult to fully distinguish. Furthermore, in (iv), when the two dots are about 300 nm apart, they were fully merged and cannot be resolved by Gaussians fitting. The limit of resolution was calculated based on the distances between resolved peaks. The estimated limit of resolution of ARPLA is ~ 300 nm under the following conditions: a) ZEISS 710 confocal microscope; b) Objective: Plan-Apo 63 × (oil); NA = 1.4, WD = 0.19 mm; c) Fluorophore of ARPLA is Alexa 647; d) Laser: 633 nm, detection wavelength: 638-720 nm; e) Pinhole:1.0 AU. Scale bar: 5 µm.