NIR-Cyanine Dye Linker: a Promising Candidate for Isochronic Fluorescence Imaging in Molecular Cancer Diagnostics and Therapy Monitoring

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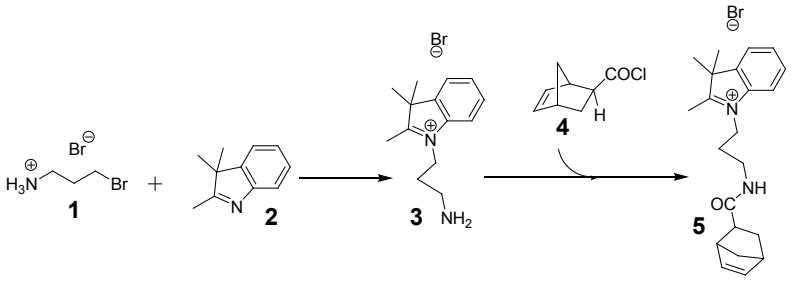
Supplementary information

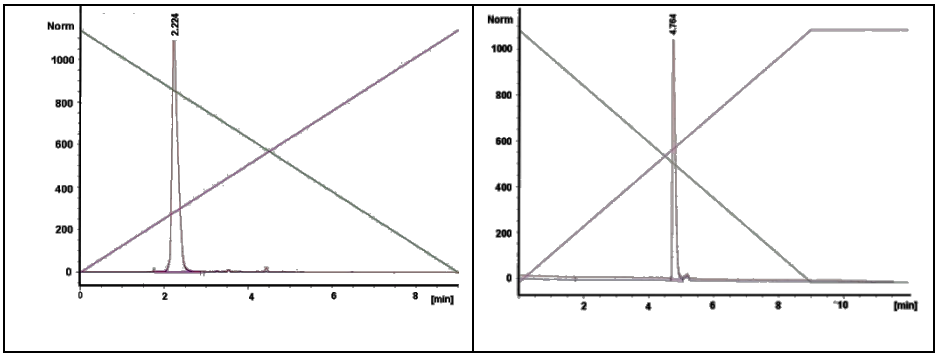
[Figure S1] Overview of molecule numbers and compound names

Molecule Number	
1	3-bromopropylamine-hydrobromide
2	2,2,3-trimethyl-3*H*-indolenine
3	aminopropyl-indolenium-bromide
4	norbornene-5-exo-carboxylic acid chloride
5	norbornenyl-aminopropyl-indolenium-bromide
6	2-chloro-1-formyl-3-(hydroxymethylene)-1-cyclohexene
7	chloro-cyclohexenyl-Cy7-bis-norbornenyl-bromide
8	3-mercapto-propionic acid
9	3-mercapto-propionic-cyclohexenyl-Cy7-bis-norbornenyl-bromide
10	*N*-(2-aminopropyl)-4-(6-(pyrimidine-2-yl)-1,2,4,5-tetrazine-3-yl)benzamide-TMZ
11	3-mercapto-propionic-cyclohexenyl-Cy7-bis-norbornenyl-bromide-CPP
12	3-mercapto-propionic-cyclohexenyl-Cy7-bis-TMZ-bromide-CPP

[Figure S2] Synthesis of the norbornenyl-aminopropyl-indolenium-bromide **(5)**

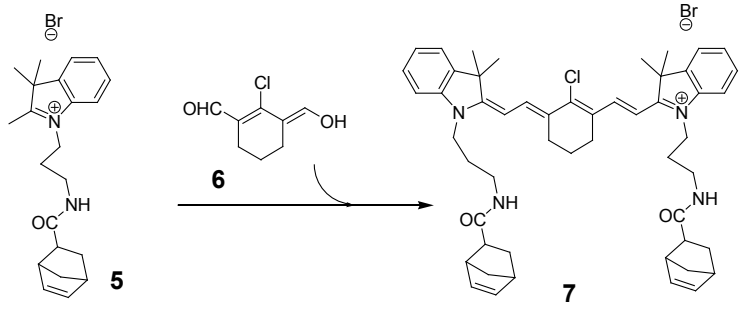


We carried out the chemical reaction steps of 3-bromopropylamine-hydrobromide **1** and 2,3,3-trimethyl-3*H*-indolenine **2** to the intermediate aminopropyl-indolenium-bromide **3** [27] reacting with the norbornene-5-exo-carboxylic chloride **4** [26] to the product norbornenyl aminopropyl-indolenium-bromide **5 .**

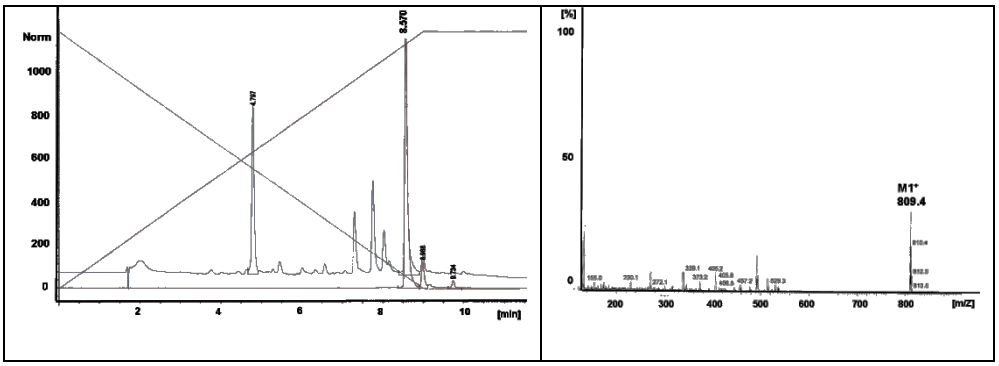


HPLC diagrams of **6** (left) and **7** (right). The green lines represent the solvent A (H_2O/0.1% TFA) and the magenta lines the solvent B (acetonitrile). The retention times were 2.224 (**6**) and 4.764 (**7**) min.

[Figure S3] Synthesis of the chloro-cyclohexenyl-Cy7-bis-norbornenyl-bromide **(7)**



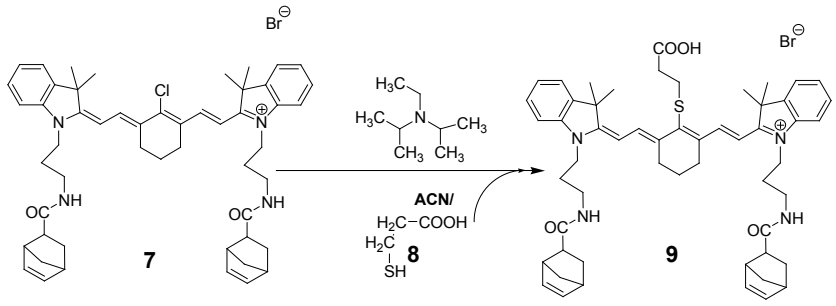
The chemical reaction of norbornenyl aminopropyl-indolenium-bromide **5** and 2-chloro-1-formyl-3-(hydroxymethylene)-1-cyclohexene **6** to the product chloro-cyclohexenyl-Cy7-bis-norbornenyl-bromide **7** is documented by Li [28].



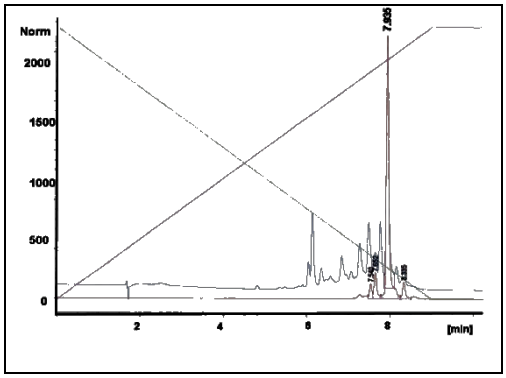
The left panel shows the HPLC diagram of **9**. The green line represents the solvent A (H_2O/0.1% TFA) and the magenta line the solvent B [acetonitrile (ACN)]. The relative amount of the peak area was 88.74%.

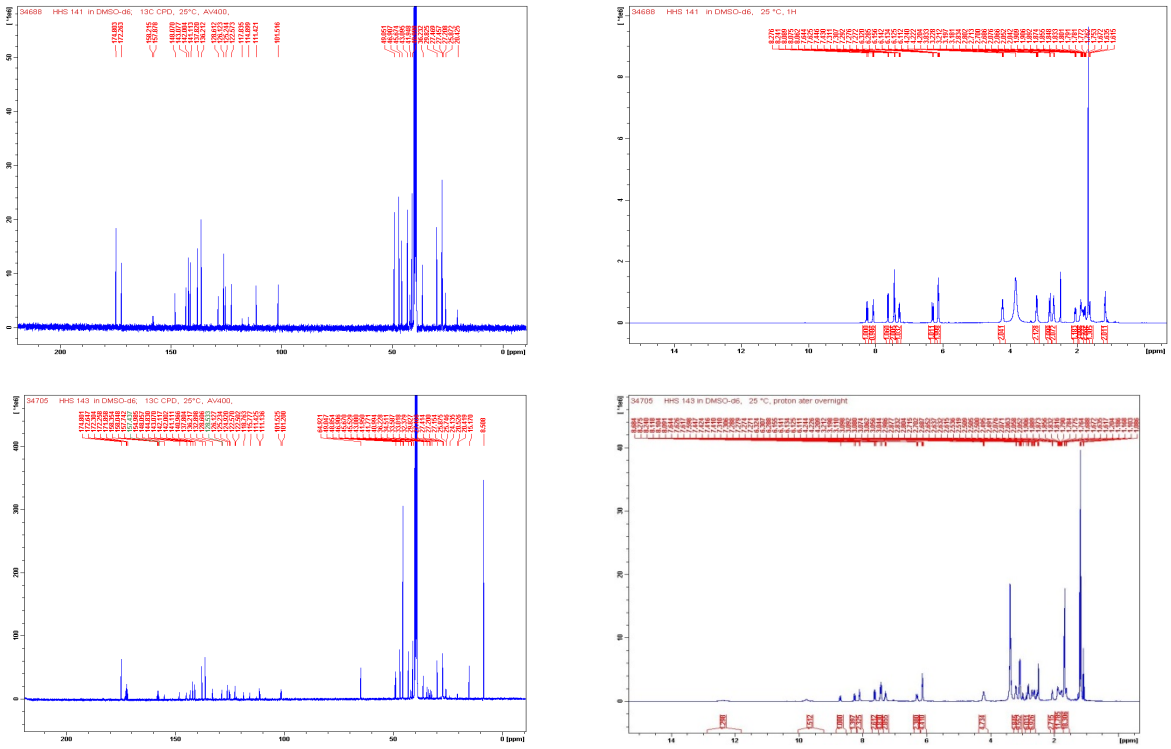
The right panel illustrates the TOF MS ES+ spectrometry diagram with the measured (in ACN/H_2O; 80% / 20%) mass of **7**.

[Figure S4] Synthesis of the 3-mercapto-propionic-cyclohexenyl-Cy7-bis-norbornenyl-bromide building block **(9)**



Chemical reaction step of **7** and **8** in ACN to the final product 3-mercapto-propionic acid-cyclohexenyl-Cy7-bis-norbornenyl-bromide **9** which is then ready for use as a building block for SPPS.





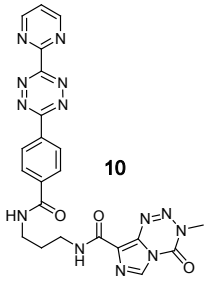
Upper panel: quantitative analysis in HPLC; the green line represents the solvent A (H_2O/0.1% TFA) and the magenta line the solvent B (ACN). The retention time was 7.935 min; the relative peak area was 91.4%.

Middle panel: the NMR diagrams of the carbon (left) and the proton (right) spectra of **7**.

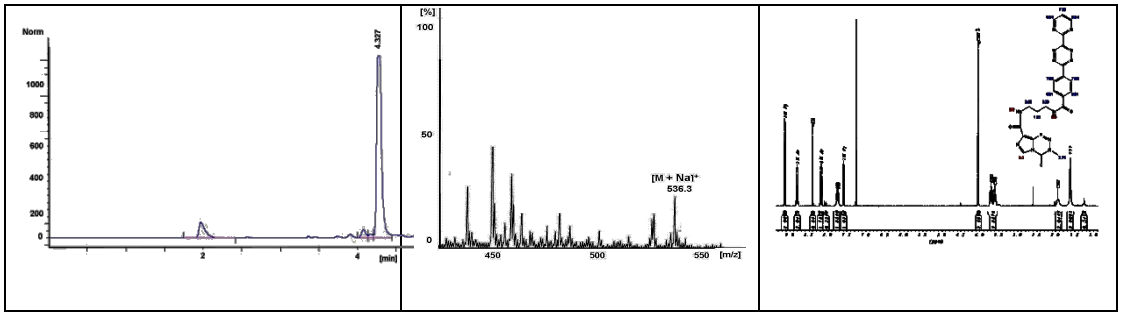
Lower panel: the NMR diagrams of the carbon (left) and the proton (right) spectra of **9**. The reaction step describes the substitution of chloride by S-CH_2-CH_2-COOH to **9**. The integrals

of diagrams suggest an interconversion, which results from the interference of the steric configuration of the norbornene groups by the 3-mercapto-propionic acid **8**.

[Figure S5] Synthesis of the diaryl-tetrazine-TMZ **(10)**

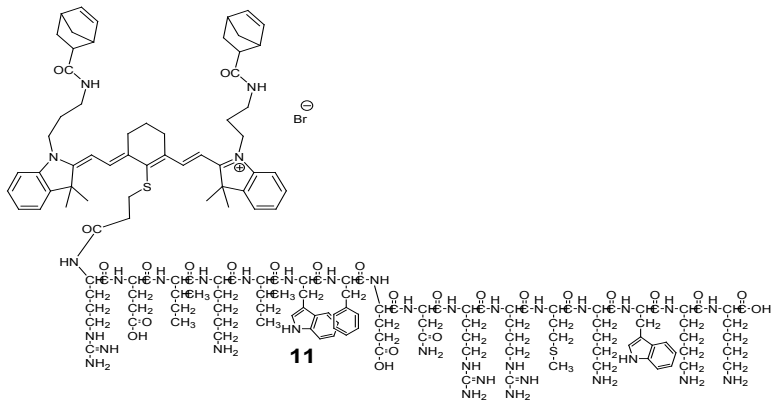


Structural formula of **10**.

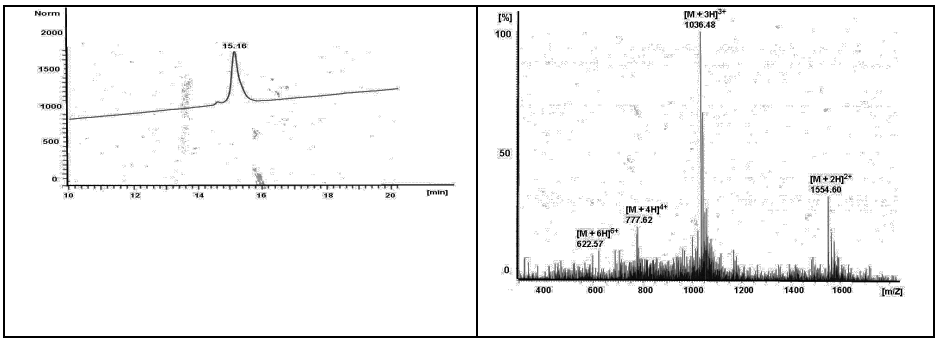


Quantitative analysis in HPLC (left) with the peak of the reaction product **12**. The retention time was 4.327 min. The relative amount of the peak area was 98%. The ESI-MS spectrograph demonstrates the identity of the reaction product **10**. The right graph describes the H-NMR-spectrum in $CDCl_3$. The structure illustrates the shift calculation for protons of **10** with ChemDraw Ultra 2004. (Numbers indicate the predicted shift of the signals in ppm; quality of estimation is indicated in color: blue = good, red = rough).

[Figure S**6**] SPPS of the 3-mercapto-propionic-cyclohexenyl-Cy7-bis-norbornenyl-bromide-CPP **(11)**



Structural formula of the product **11** harbouring the cell penetrating peptide sequence (CPP = CRQIKIWFQNRRMKKWKK) after SPPS, de-protection and cleavage.



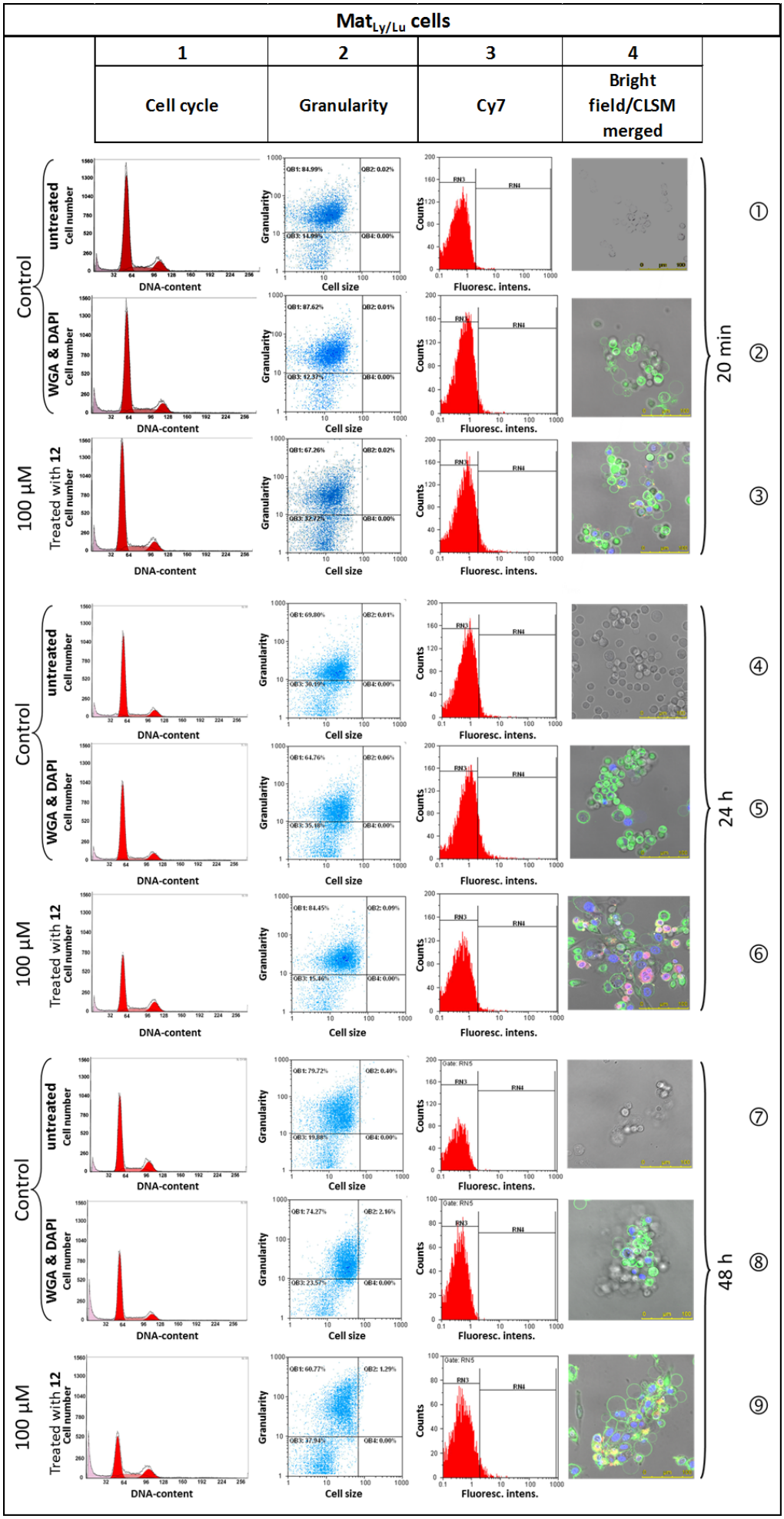
The left panel shows the HPLC diagram of **11**. The retention time was 15.16 min. The relative amount of the peak area was 98.29%.

The right panel illustrates the TOF MS ES+ spectrometry diagram with the measured mass of **11** (solvent: acetonitrile/H_2O; 80% / 20%).

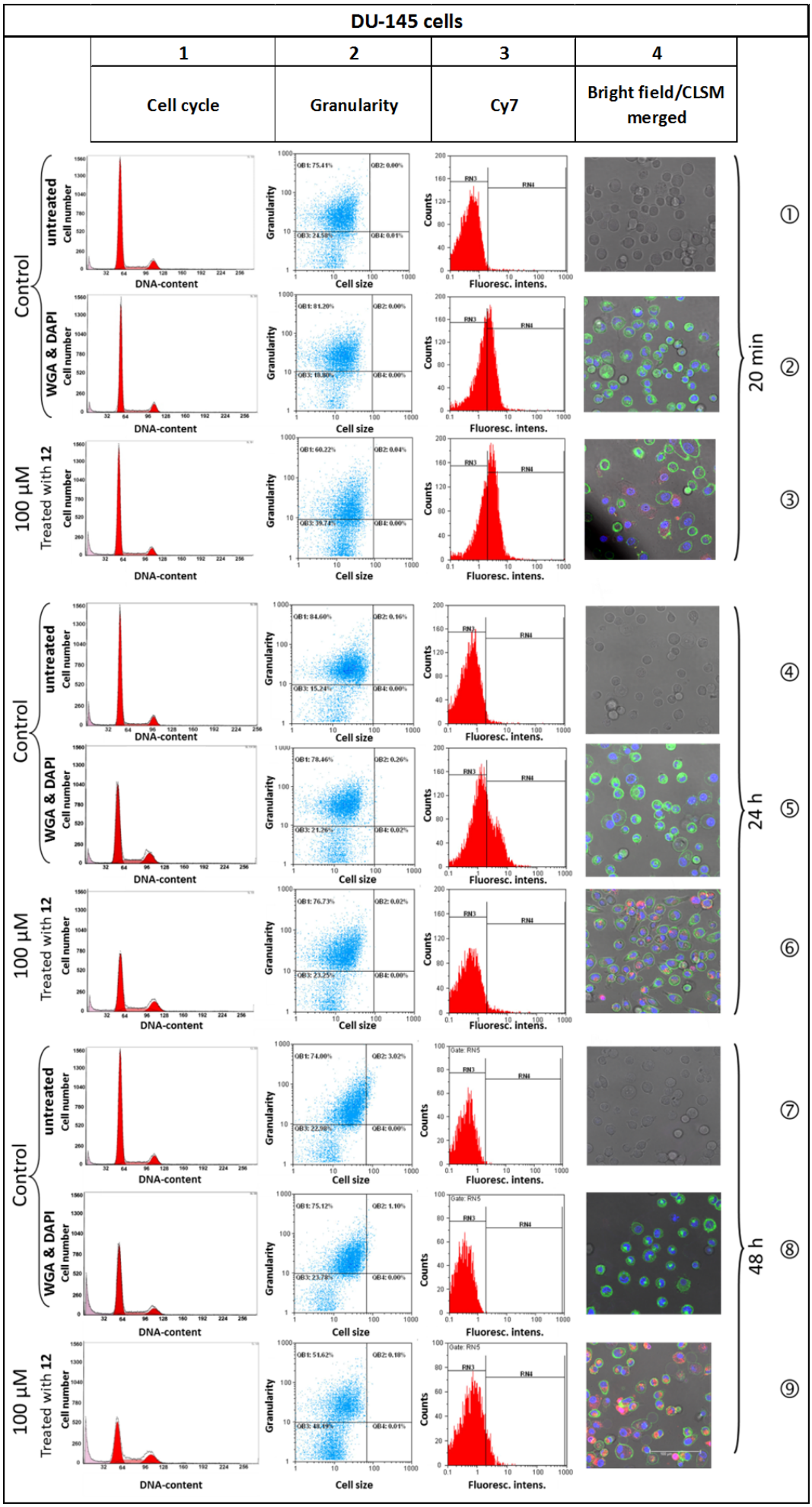
[Figure S7] Effect of **12** on the cell cycle distribution (column 1), granularity/cell size ratio (column 2), cell counts/fluorescence intensity ratio (column 3) and the CLSM-assessed

change of the cell phenotype (column 4) of $Mat_{Ly/Lu}$ [Figure S7A], DU-145 [Figure S7B] and MDA-MB-231 [Figure S7C] cells. Scale bar: 100 μm.

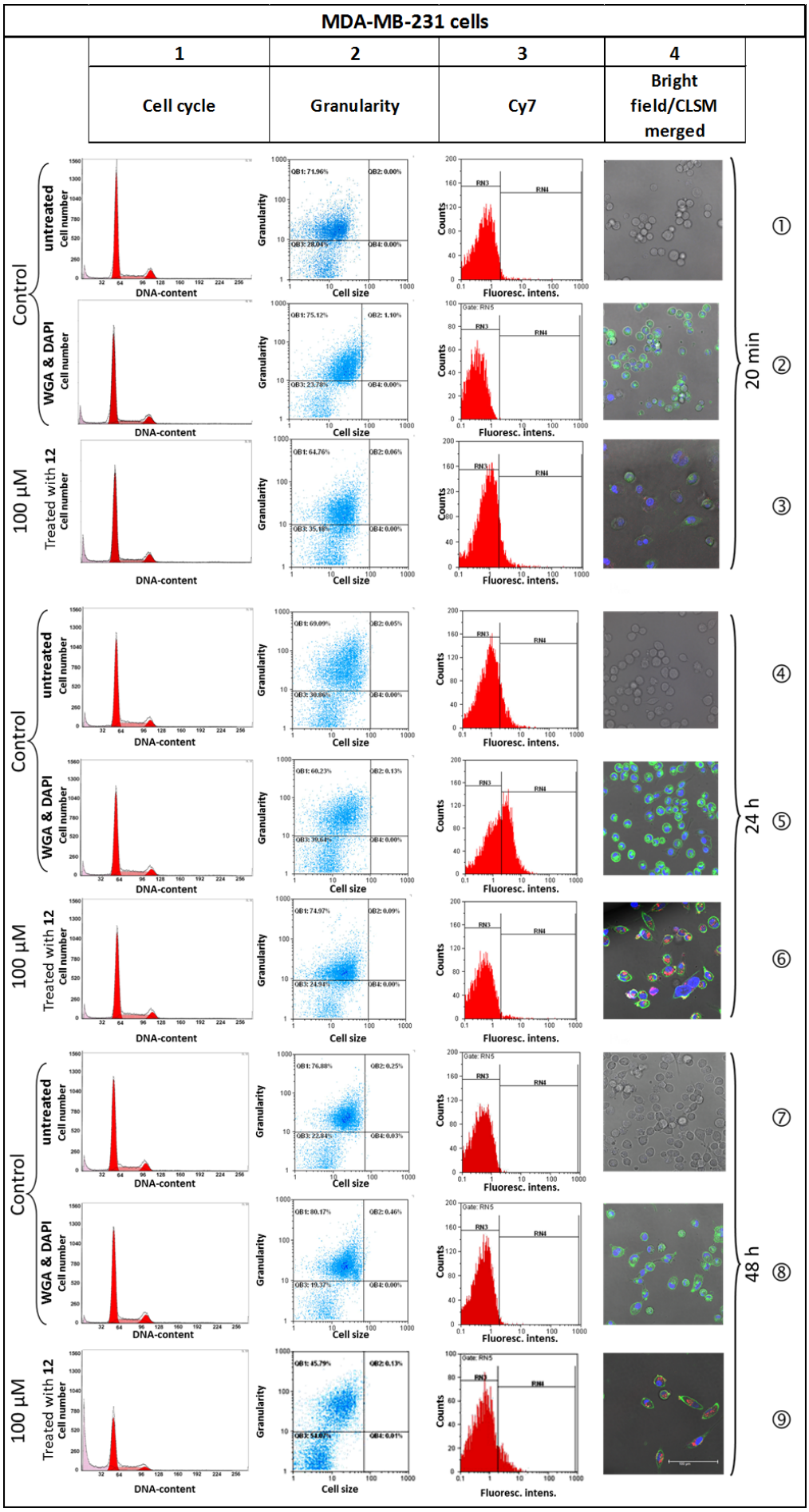
[Figure S7A]


Mat$_{Ly/Lu}$ cells
1
2
3
4
Cell cycle
Granularity
Cy7
Bright field/CLSM merged
Control
untreated
WGA & DAPI
100 µM
Treated with 12
Cell number
DNA-content
Granularity
Cell size
Counts
Fluoresc. intens.
20 min
24 h
48 h
①
②
③
④
⑤
⑥
⑦
⑧
⑨

[Figure S7B]


DU-145 cells
1
Cell cycle
2
Granularity
3
Cy7
4
Bright field/CLSM merged
Control
untreated
WGA & DAPI
100 µM
Treated with 12
Cell number
DNA-content
Granularity
Cell size
Counts
Fluoresc. intens.
20 min
24 h
48 h
①
②
③
④
⑤
⑥
⑦
⑧
⑨

[Figure S7C]


MDA-MB-231 cells
1
2
3
4
Cell cycle
Granularity
Cy7
Bright field/CLSM merged
Control
untreated
WGA & DAPI
100 µM
Treated with 12
Cell number
DNA-content
Granularity
Cell size
Counts
Fluoresc. intens.
RN3
RN4
20 min
24 h
48 h
①
②
③
④
⑤
⑥
⑦
⑧
⑨